

## Effects of different roostertree (*Calotropis procera*) amounts and spatial arrangements on the performance of the beet-cowpea intercropping system

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### Abstract

Intercropping of beet with cowpea-vegetable is increasing in the State of Rio Grande do Norte, Brazil. However, its management requires scientific information and technologies pertaining to space arrangement and adequate fertilization with roostertree. The present study evaluated the agronomic/biological performance of the beet and cowpea-vegetable intercropping system with different amounts of roostertree and spatial arrangements of the component cultures. We established a 4 × 3 factorial completely randomized design with four blocks. The first factor was the amount of roostertree biomass incorporated into the soil (20, 35, 50, and 65 t ha<sup>-1</sup> in dry matter), while the second was the spatial arrangement of beet rows alternated with cowpea rows (2:2, 3:3, and 4:4). We evaluated the total, commercial, and root yields of beet, as well as the number of green pods per m<sup>2</sup>, green and dry pod yields, the number of green grains per pod, the weight of 100 green grains, and green and dry grain yields of cowpea. The agronomic efficiency of the intercropping system was evaluated by the land equivalent ratio, yield efficiency index, and z-score. The results showed no significant interactions among the amounts of roostertree biomass and spatial arrangements for any evaluated trait or agronomic index. The highest agronomic efficiency of the intercropping system was obtained with 65 t ha<sup>-1</sup> of roostertree biomass and the 2:2 spatial arrangement.

**Keywords:** *Beta vulgaris* L.; *Calotropis procera* (Ait.) R. Br.; green manure; intercropping system; *Vigna unguiculata* (L.) Walp.

**Abbreviations:** Ca, calcium; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; N, nitrogen; Na, sodium; P, phosphorus; Zn, zinc.

### Introduction

Beet (*Beta vulgaris* L.) is one of the main vegetable crops in Brazil, as it contains elements of excellent nutritional value, such as iron (Fe), sodium (Na), potassium (K), vitamin A, and B complex (Tivelli et al., 2011). It is produced in approximately 100,000 rural properties, occupying an area of approximately 10,000 ha with a total annual yield of approximately 300,000 tons (Matos et al., 2012). In 2009, Brazil had 21,937 beet producing units, of which 2,693 were located in the northeastern region of the country and 32 in Rio Grande do Norte (IBGE, 2009).

Cowpea (*Vigna unguiculata* [L.] Walp), also known as the macassar bean or string bean, is one the main sources of income and food for the population in Northeastern Brazil (Oliveira et al., 2009), where it occupies 60% of the cultivated area. The producers are usually small-scale farmers (Andrade Júnior et al., 2007), as cowpea (green pods

or green beans) production, especially harvest and threshing, requires manual labor (Freire Filho et al., 2011). Both beet and cowpea are mainly produced by employing conventional agronomic practices, including the application of mineral fertilizers, which favors soil erosion and accelerates environmental degradation (Oliveira et al., 2010). Therefore, the development of effective management strategies is considered essential to minimize or eliminate the use of mineral fertilizers. The use of green manure crops can improve the physical, chemical, and biological attributes of the soil (Batista et al., 2016a).

Several plants can be potentially used as green manure crops, including hairy woodrose (*Merremia aegyptia*), one-leaf senna (*Senna uniflora*), and roostertree (*Calotropis procera*), as they have known agro-economic advantages in vegetable cultivation in Northeastern Brazil (Bezerra Neto et

al., 2014). Among these, roostertree has been shown to increase the productivity of commercial carrot (Silva et al., 2013) and beet (Batista et al., 2016a), revealing its high potential as a green manure crop.

Intercropping is known to improve ecological balance, resource utilization, and reduce pest and weed damages to crops by increasing the diversity of the agricultural ecosystem. Consequently, it promotes crop productivity and agricultural sustainability (Tavella et al., 2010). Various intercropping systems have been studied in terms of ecological interactions, plant population, crop diversification, and factors affecting agronomic management, such as fertilizers and spatial arrangement (Batista et al., 2016b).

In the present study, we aimed to evaluate the effects of four different amounts of roostertree biomass incorporated into the soil in combination with three spatial arrangements on the agro-economic performance of the beet-cowpea intercropping system.

## Results and Discussion

### *Effects of treatments on the agronomic performance of beet*

There was no significant interaction among the amounts of roostertree biomass (20, 35, 50, and 65 t ha<sup>-1</sup> in dry matter) and spatial arrangements (2:2, 3:3, and 4:4) for any of the traits evaluated in beet (Figs 1 and 2; Tables 1 and 2).

The total and commercial yield of beet roots increased with the increase in the amount of roostertree biomass with the highest values of 19.93 and 16.18 t ha<sup>-1</sup>, respectively, with 65 t ha<sup>-1</sup> of roostertree biomass (Figs 1A and 1B). The total and commercial yields of beet increased partially due to the increased availability of nutrients, mainly N, P, and K, released by the green manure and also due to the improved chemical, physical, and biological attributes of the soil (Batista et al., 2016a). Studies have shown that, under optimal conditions, the soil microbiota gradually release the nutrients from the soil for the plants to absorb, thus preventing leaching losses.

In the present study, significant differences were observed in the total and commercial yields among the spatial arrangements with the highest values in the 2:2 arrangement (Table 1). This might be attributed to the low interspecific competition between beet and cowpea (Zanine and Santos, 2004), which allows both the crops to utilize the available resources more effectively (Grangeiro et al., 2011). Therefore, less competition and better soil utilization are expected in an intercropping system composed of plant species with roots that exploit the soil at different depths (Teixeira et al., 2005).

The yield of extra A, extra AA, and big roots increased with increase in the amount of roostertree biomass with the highest values of 5.24, 3.28, and 3.20 t ha<sup>-1</sup>, respectively, with 65 t ha<sup>-1</sup> of roostertree biomass (Figs 2B, 2C, and 2D). This might be attributed to the high amount of nutrients released by the green manure, which is also an important N supplier, thus favoring nutrient recycling. It has been reported that N increases the yield of beet by promoting leaf expansion and mass accumulation (Tivelli et al., 2011).

The yield of extra and scrap roots decreased with increase in the amount of roostertree biomass with the highest values of 6.44 and 4.09 t ha<sup>-1</sup> with 20 t ha<sup>-1</sup> of roostertree biomass (Figs 2A and 2E). These results might be due to increase in the number of large roots, which have a commercial value. These results are in agreement with those reported previously in other crops. Silva et al. (2013) reported that the percent of short roots in carrot decreases with the increase in the amount of roostertree biomass incorporated into the soil. Batista et al. (2013) also suggested that the percent of scrap roots in radish decreases with the increase in the amount of biomass of different green manure species, such as hairy woodrose, one-leaf senna, and roostertree, with roostertree exhibiting the strongest effect.

The results of the present study revealed significant differences in the yield of extra fine roots among the spatial arrangements with the highest values in the 2:2 arrangement (Table 2). This indicates that crop competition due to spatial arrangements does not interfere with the productivity of the beet-cowpea intercropping system.

### *Effects of treatments on the agronomic performance of cowpea*

There were no significant interactions among the amounts of roostertree biomass and spatial arrangements for any of the traits evaluated in cowpea (Figs 3 and 4; Tables 3 and 4). The number of green pods per m and weight of 100 grains initially increased with increase in the amount of roostertree biomass with the highest values of 79.57 pods m<sup>-2</sup> and 62.52 g, respectively, with 54 t ha<sup>-1</sup> of roostertree biomass, and then decreased with 65 t ha<sup>-1</sup> of roostertree biomass (Figs 3A and 3C). This suggests that the supply of adequate amounts of N into the soil by the green manure favors the growth, vegetative development, expansion of photosynthetic area, and yield potential of cowpea (Filgueira, 2008).

Further, the number of green grains per pod increased with increase in the amount of roostertree biomass with the highest value of 6.88 grains per pod with 65 t ha<sup>-1</sup> of roostertree biomass (Fig 3B). These results are in agreement with those reported by Pereira et al. (2016). They studied the effects of different amounts of roostertree biomass on the performance of a radish-cowpea intercropping system.

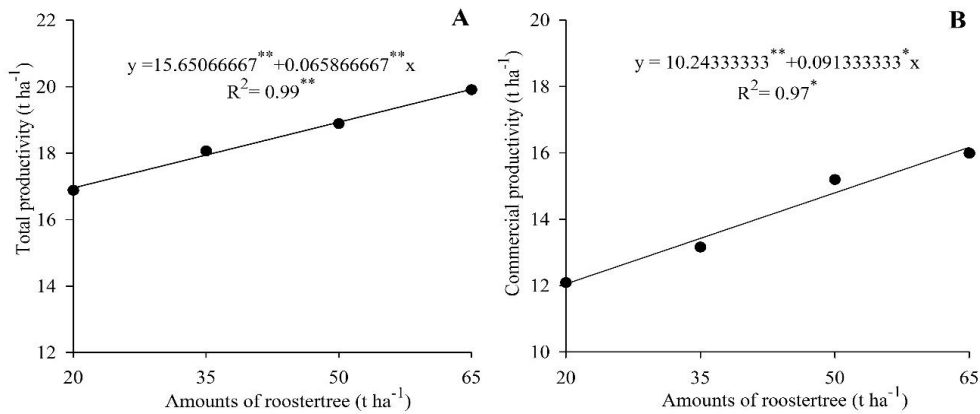
The number of green grains per pod and green pods per m<sup>2</sup> did not differ among the different spatial arrangements. However, the weight of 100 green grains was significantly high in the 4:4 arrangement (Table 3). These results indicate that the yield of cowpea in the 4:4 arrangement fulfills the requirements of the market, weighing more than 20 g per 100 grains (Freire Filho et al., 2011).

The green grain and dry grain yields initially increased with the increase in the amount of roostertree biomass with the highest values of 2.78, 0.88, and 0.46 t ha<sup>-1</sup> with 50.60, 53.00, and 31.83 t ha<sup>-1</sup> of roostertree biomass, respectively, and then decreased with 65 t ha<sup>-1</sup> of roostertree biomass (Figs 4A, 4B, and 4D). These results might be attributed to the increased availability of nutrients released by the green manure and to the synchronization in element release and plant absorption (Bezerra Neto et al., 2014). It has been reported that the efficient and balanced fertilization methods promote

**Table 1.** Mean values of total (TP) and commercial (CP) productivity of beet roots intercropped with cowpea-vegetable as a function of spatial arrangements of the component cultures.

Spatial Arrangements	TP (t ha <sup>-1</sup> )	CP (t ha <sup>-1</sup> )
2:2	20.91 a	15.44 a
3:3	17.20 b	13.97 ab
4:4	15.74 b	12.96 b

\*Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test at 5% probability.

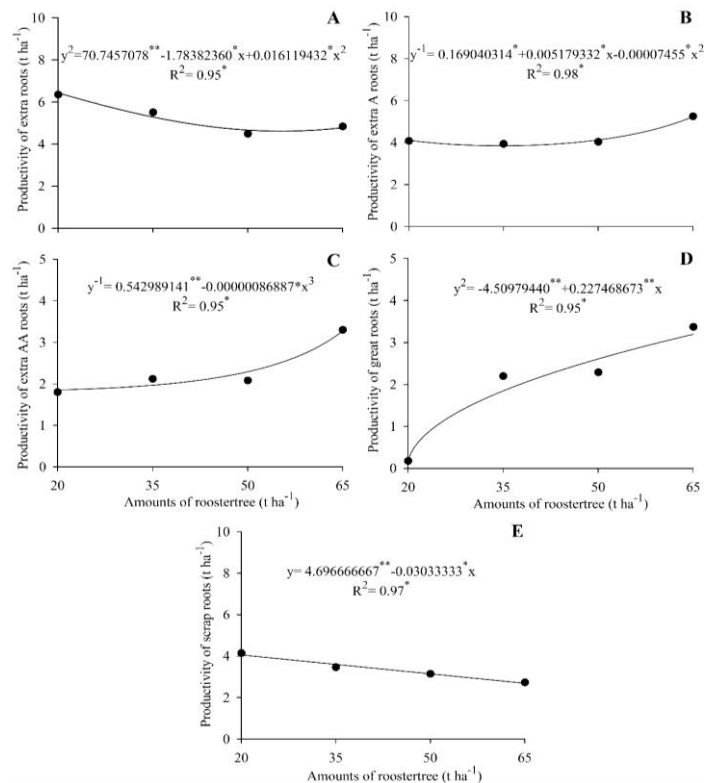


**Fig 1.** Total (A) and commercial (B) productivity of beet roots intercropped with cowpea-vegetable as a function of amounts of roostertree biomass incorporated into the soil.

**Table 2.** Average productivity values of extra roots (E), extra A (EA), extra AA (EAA), great (G), and scrap (ScR) of beet intercropped with cowpea-vegetable as a function of spatial arrangements of the component cultures.

Spatial Arrangements	E (t ha <sup>-1</sup> )	EA (t ha <sup>-1</sup> )	EAA (t ha <sup>-1</sup> )	G (t ha <sup>-1</sup> )	ScR (t ha <sup>-1</sup> )
2:2	6.98 a	4.39 a	2.68 a	2.37 a	3.19 a
3:3	4.95 b	4.68 a	1.81 a	1.72 a	3.73 a
4:4	4.02 b	3.94 a	2.51 a	1.97 a	3.30 a

\*Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test at 5% probability.

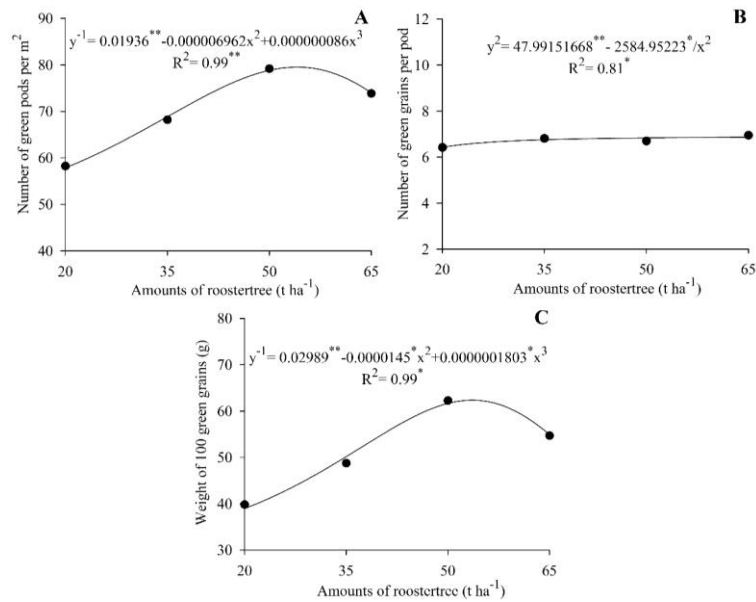


**Fig 2.** Productivity of extra roots (A), extra A (B), extra AA (C), great (D) and scrap (E) of beet intercropped with cowpea-vegetable as a function of amounts of roostertree incorporated into the soil.

**Table 3.** Average values of the number of green pods per m<sup>2</sup> (NGPm<sup>2</sup>), number of green grains per pod (NGGP) and weight of 100 green grains (W100GG) of cowpea-vegetable intercropped with beet as a function of spatial arrangements of the component cultures.

Spatial Arrangements	NGPm <sup>2</sup>	NGGP	W100GG (g)
2:2	70.50 a	6.67 a	12.45 c
3:3	69.62 a	6.69 a	20.53 b
4:4	69.69 a	6.84 a	28.94 a

\*Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test at 5% probability.

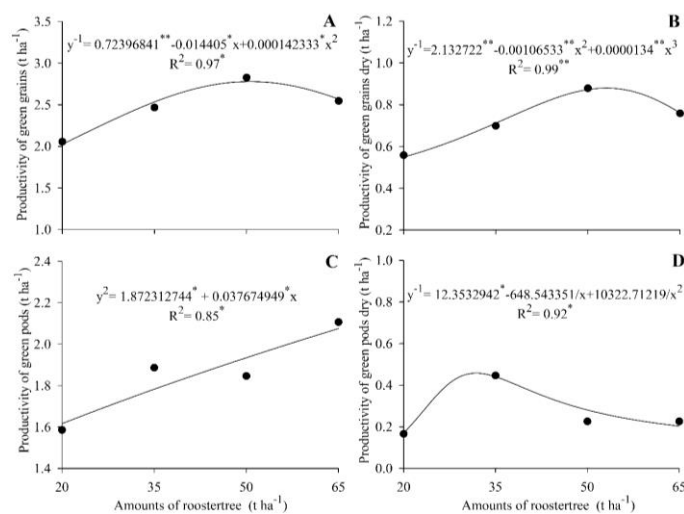


**Fig 3.** Number of green pods per m<sup>2</sup> (A), number of green grains per pod (B), and weight of 100 green grains (C) of cowpea-vegetable intercropped with beet as a function of amounts of roostertree biomass incorporated into the soil.

**Table 4.** Average values of green grain productivity (GGP), dry green grains (DGGP), green pods (GPP) and dry green pods (DGPP) of cowpea-vegetable intercropped with beet as a function of spatial arrangements of the component cultures.

Spatial Arrangements	GGP (t ha <sup>-1</sup> )	DGGP (t ha <sup>-1</sup> )	GPP (t ha <sup>-1</sup> )	DGPP (t ha <sup>-1</sup> )
2:2	2.98 a	0.73 a	2.14 a	0.77 a
3:3	2.64 a	0.74 a	1.77 b	0.35 a
4:4	1.81 b	0.71 a	1.68 b	0.31 a

\*Means followed by the same lowercase letter in the column do not differ from each other by the Tukey test at 5% probability.

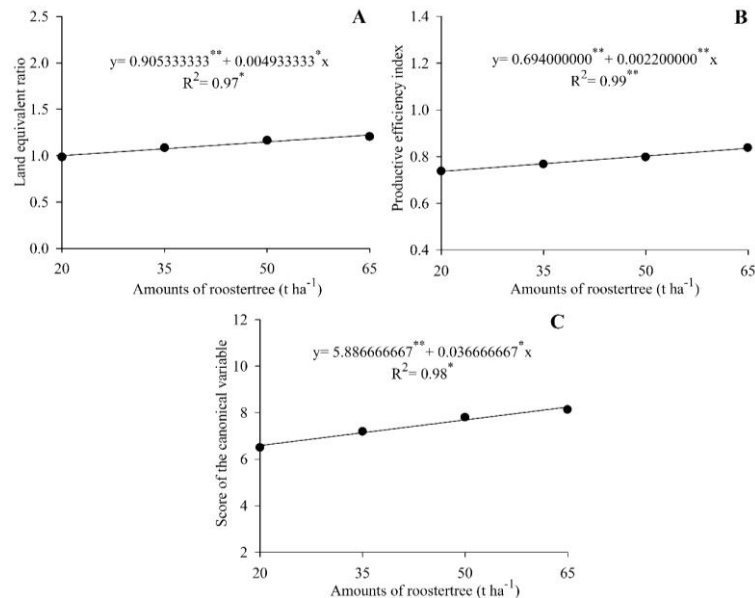


**Fig 4.** Productivity of green grains (A), dry green grains (B), green pods (C) and dry green pods (D) of cowpea-vegetable intercropped with beet as a function of amounts of roostertree biomass incorporated into the soil.

**Table 5.** Mean values of the land equivalent ratio (LER), productive efficiency index (PEI) and score of the canonical variable (Z) of the beet intercropped with cowpea-vegetable as a function of spatial arrangements of the component cultures.

Spatial Arrangement	LER	PEI	Z
2:2	1.17 a	0.83 a	7.89 a
3:3	1.07 a	0.76 a	7.21 a
4:4	1.11 a	0.77 a	7.24 a

\*Means followed by the same lowercase letter in the column do not differ from each other by Tukey's test of 5% probability.



**Fig 5.** Land equivalent ratio (A), productive efficiency index (B) and score of the canonical variable (C) of the beet intercropped with cowpea-vegetable as a function of amounts of roostertree biomass incorporated into the soil.

plant yield, quality, and resistance to biotic and abiotic stresses (Zucareli et al., 2011).

The green pod yield increased with the increase in the amount of roostertree biomass with the highest value of 2.08 t ha<sup>-1</sup> with 65 t ha<sup>-1</sup> of roostertree biomass (Fig 4C). Pereira et al. (2016) also observed an increase in the green pod yield with increase in the amount of roostertree biomass incorporated into the soil in a radish-cowpea intercropping system. These results suggest that roostertree efficiently supplies essential nutrients for the development and growth of cowpea (Batista et al., 2016a).

Significant differences between the spatial arrangements were observed only for green grain and green pod yields, with the highest values in the 2:2 arrangement (Table 4). This might be due to the relatively high nutrient concentration, space, and sunlight availability. Teixeira et al. (2005) reported that the effect of solar radiation on plants is determined by their height, arrangement, and efficiency in intercepting and absorbing the sunlight.

#### Agronomic efficiency indices

There were no significant interactions among the amounts of roostertree biomass and spatial arrangements for any of the evaluated agronomic indices (Fig 5 and Table 5).

The land equivalent ratio, yield efficiency index, and z-score increased with the increase in the amount of roostertree biomass incorporated into the soil, with the highest values of 1.23, 0.84, and 8.27, respectively, with 65 t ha<sup>-1</sup> of

roostertree biomass (Figs 5A, 5B, and 5C). These results are in agreement with those reported by Cecílio Filho et al. (2015). The study suggested that the performance of an intercropping system is highly related to the complementation of the included species as differences in plant architecture favor the efficient use of available resources. Therefore, the complementarity of beet and cowpea increased the yield potential of the intercropping system and decreased the losses due to stresses (Teixeira et al., 2005).

There were no significant differences in the land equivalent ratio, yield efficiency index, and z-score among the different spatial arrangements (Table 5). However, the highest values were observed in the 2:2 arrangement, probably due to the relatively low interspecific competition (Zanine and Santos, 2004). Furthermore, the land equivalent ratio was higher than 1 in all treatment combinations. This suggests that the intercropping system favored the growth and improved the yield of both species, promoting the efficient utilization of environmental resources (Rezende et al., 2010).

#### Materials and Methods

##### Experimental area

The present study was conducted under field conditions at the Rafael Fernandes Experimental Farm, Federal Rural Semiarid University (UFERSA), Alagoinha District (5°11'S and 37°20'W, 18 m altitude), Mossoró-RN, Brazil from June to

December 2014. Per Thornthwaite classification, the climate of the area is classified as semi-arid, whereas according to the Koppen classification, it is BShw, dry, and very hot with two climatic seasons (dry, June–January; wet, February–May) (Alvares et al., 2014).

The area soil is classified as Eutrophic Yellow Red Ultisol (Santos et al., 2006). Prior to the experiment, soil samples collected from the 0–20 cm layer were sent to the Laboratory of Water, Soil, and Plant Analysis, Department of Environmental Sciences, UFERSA, for chemical analysis, and the results were as follows: pH 6.12; phosphorus (P), 3.75 mg dm<sup>-3</sup>; K, 70.82 mg dm<sup>-3</sup>; calcium (Ca), 1.98 cmol<sub>c</sub> dm<sup>-3</sup>; magnesium (Mg), 0.68 cmol<sub>c</sub> dm<sup>-3</sup>; Na, 7.8 mg dm<sup>-3</sup>; electrical conductivity, 0.18 dS m<sup>-1</sup>; organic matter, 7.82 g kg<sup>-1</sup>; sum of bases, 2.88 cmol<sub>c</sub> dm<sup>-3</sup>; cation exchange capacity, 3.48 cmol<sub>c</sub> dm<sup>-3</sup>; exchangeable Na, 1.0%; effective cation exchange capacity, 2.88 cmol<sub>c</sub> dm<sup>-3</sup>; and base saturation, 83%.

### Experimental design

The experiment was a 4 × 3 factorial randomized complete block design with four blocks. The first factor was the amount of roostertree biomass incorporated into the soil (20, 35, 50, and 65 t ha<sup>-1</sup> on a dry basis) chosen based on the study by Bezerra Neto et al. (2013). The second factor was the spatial arrangement of the two crops (2:2, 3:3, and 4:4), which corresponds to rows of beet alternated with rows of cowpea. The roostertree biomass was estimated as described by Silva et al. (2011). The spacing adopted was 0.25 m × 0.04 m for beet and 0.25 m × 0.10 m for cowpea with 25 beet plants per m and 10 cowpea plants per m. The total area of the 2:2, 3:3, and 4:4 arrangements was 2.40, 3.00, and 3.60 m<sup>2</sup>, respectively, with a harvest area of 1.00, 1.50, and 2.00 m<sup>2</sup>, respectively.

To determine the efficiency indices of intercropping, the monocrop plots of beet and cowpea were planted in each block, with a total area of 1.44 and 3.60 m<sup>2</sup>, respectively. The harvest area of beet was 0.80 m<sup>2</sup> with a spacing 0.20 m × 0.10 m, resulting in a population of 500,000 plants ha<sup>-1</sup> (Silva et al., 2011), whereas the harvest area of cowpea was 2 m<sup>2</sup> with a spacing 0.50 m × 0.10 m, resulting in a population of 200,000 plants ha<sup>-1</sup> (Santos, 2011).

To acquire the green manure, roostertree plants were collected from a natural population near the city of Mossoró, Rio Grande do Norte as described by Silva et al. (2013). The plant samples were sent to the Laboratory of Soil Fertility and Plant Nutrition, UFERSA for chemical analysis, and the results were as follows: N, 7.43 g kg<sup>-1</sup>; P, 0.91 g kg<sup>-1</sup>; K, 16.46 g kg<sup>-1</sup>; Ca, 8.2 g kg<sup>-1</sup>; Mg, 7.35 g kg<sup>-1</sup>; Fe, 100.5 mg kg<sup>-1</sup>; Mn, 21.75 mg kg<sup>-1</sup>; Zn, 37.88 mg kg<sup>-1</sup>; Cu, 3.85 mg kg<sup>-1</sup>; and Na, 6,085 mg kg<sup>-1</sup>.

### Agonomic practices

The soil was prepared by mechanical cleaning using a tractor with a coupled plow, followed by soil bed harrowing and lifting. The soil beds were solarized for 45 days using 30-µm Vulcabrilho Bril Fles plastic in order to reduce the soil phytopathogen population, especially *Meloidogyne* spp. (Silva et al., 2006). After solarization, the intercropped plots were fertilized with roostertree; 50% of the amount was applied 20 days prior to planting in the 0–20 cm soil layer,

while the remaining 50% of the amount was applied on the soil surface 45 days after planting (Carvalho, 2011). The monocropped plots of beet and cowpea were fertilized with 39 t ha<sup>-1</sup> (Andrade Filho, 2012) and 59 t ha<sup>-1</sup> (Vieira, 2014) of roostertree, respectively.

The micro-sprinkling irrigation was performed in the morning and afternoon, providing approximately 8.0 mm of water per day (Lima et al., 2010) to promote the microbial activity of the soil, and thus the decomposition of the incorporated biomass. The crop management included manual weeding and beet heaping.

The planting was carried out on November 8, 2014 in holes of depth approximately 3 cm using four seeds of beet (Early Wonder; Tivelli et al., 2011) and two seeds of cowpea (BRS Itaim; Freire Filho et al., 2011) per hole. Cowpea is one of the most consumed legumes in the North and Northeast Brazil, representing an important source of protein, energy, fibers and minerals. Furthermore, its cultivation generates employment and income. We call it cowpea-vegetable because it is consumed in the form of green grains (Goméz, 2004).

Plant thinning was carried out 15 d after planting for beet and 7 d after planting for cowpea, leaving just one plant per hole.

### Harvest and evaluation

The beet plants were harvested 76 d after planting, and the following traits were estimated: total root yield (fresh weight of roots in the harvest area, t ha<sup>-1</sup>); commercial root yield (fresh weight of roots free of cracks, bifurcations, nematodes, and mechanical damages, t ha<sup>-1</sup>), and yield of different root classes (extra fine roots, 4 cm < root diameter [RD] < 5 cm; extra A roots, 5 cm ≤ RD < 6 cm; extra AA roots, 6 cm ≤ RD < 7 cm; big roots, RD > 7 cm; and scrap roots, all damaged, cracked, bifurcated roots, as well as those with an RD < 4 cm) (Horta et al., 2001).

The harvest of cowpea started on November 3, 2014 and four collections were carried out. One week prior to the harvest, five cowpea plants were randomly selected per row (20 plants per experimental plot), and the following traits were recorded: number of green pods per m<sup>2</sup>; number of green grains per pod; weight of 100 green grains (average weight of four random samples of 100 green grains, g); green grain yield (weight of green grains of each plot, t ha<sup>-1</sup>); dry grain yield (weight of four samples of 100 green grains that were dried in a forced-air circulation oven at 65 °C until reaching a constant mass, t ha<sup>-1</sup>); green pod yield (weight of all harvested pods, expressed in t ha<sup>-1</sup>), and dry pod yield (weight of all harvested pods that were dried in a forced-air circulation oven at 65 °C until reaching a constant mass, t ha<sup>-1</sup>).

The efficiency of the intercropping system was evaluated by the land equivalent ratio (LER) that is calculated using the formula:

$$LER = (Y_{becv}/Y_{be}) + (Y_{cvbe}/Y_{cv})$$

Where,

Y<sub>becv</sub> = commercial beet root yield in intercropping with cowpea

Y<sub>be</sub> = commercial beet root yield in monocropping

Y<sub>cvbe</sub> = green cowpea grain yield in intercropping with beet

Y<sub>cv</sub> = green cowpea grain yield in monocropping

The yield efficiency of each treatment was estimated using the yield efficiency index model described by Charnes et al. (1978). The z-scores were obtained by the multivariate analysis of variance of beet and cowpea yields.

### Statistical analysis

The univariate analysis of variance was performed to evaluate crop traits and efficiency indices using SISVAR (Ferreira, 2014). The multivariate analysis of variance was performed to evaluate crop yield in relation to roostertree biomass amounts using the Wilks criterion. The curve fitting analysis was carried out using the Table Curve 2D (Jandel Scientific, 1991). The means were separated by Tukey's post-hoc test at  $P < 0.05$ .

### Conclusions

The present study provided information related to the agronomic performance of the beet-cowpea intercropping system in relation to different amounts of roostertree biomass incorporated into the soil and different spatial arrangements. The highest agronomic efficiency of the intercropping system was recorded with 65 t ha<sup>-1</sup> of roostertree and the 2:2 arrangement. These results can be used to develop new, efficient management strategies in order to maximize the performance of the beet-cowpea intercropping system in Northeastern Brazil.

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