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Inoculation with *Azospirillum brasiliense* increases maize yield

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Abstract

Background: Maize plants have a high nitrogen (N) demand, and thus, the fertilizers' sustainable management and the inoculation with N_2-fixing bacteria are the alternatives to reduce fertilization cost and the environmental impacts with N fertilizers. Under no tillage system, the N_2-fixing inoculant *Azospirillum brasiliense* effects were compared with mineral N fertilization for maize crop.

Results: The N fertilization treatments influenced all variables in the 2014/2015 season and the cob insertion, plant height, yield, and N concentration in leaves in the 2015/2016 season, suggesting that the N supply, either N mineral from *A. brasiliense* increased growth and yield of maize plants. The maize cultivars influenced all variables in the two seasons. There was no interaction effect of cultivars × N fertilization on the variables. Thus, the maize cultivars had similar performance regardless treatments.

Conclusions: The inoculation with *A. brasiliense* increased maize yield compared with non-N-fertilized plants and the inoculation with *Azospirillum* at sowing + mineral N at topdress provided similar grain yield as mineral N at sowing and topdress for both cultivars.

Keywords: *Zea mays* L., Inoculation, Grain yield, Mineral fertilization

Background

The maize (*Zea mays* L.), the main cereal crop in Brazil, was grown near 16 million hectares during the 2015/2016 harvest, at mean yield of 4181 kg ha^{-1}, despite its increasing global demand [1]. This low yield is explained by the high cost of inputs and low level of technology adopted by farmers. In Amazonas state, the maize yield is around only 2515 kg ha^{-1} [1].

The photosynthetic rate of maize is high, but is easily influenced by environmental stress, as the low soil N level [2, 3] is an essential element for plant cell and fundamental to increase crop yield [4]. In addition, most of tropical soils have low N availability, requiring the N fertilization as an indispensable practice [5]. In this context, inorganic fertilizers are the main N source to increase maize plant development and yield [6]. However, the inadequate management of N fertilizers has negative environmental impacts, as water body eutrophication and nitrous oxide emission [7].

The reduction of N fertilizers and their negative impacts requires urgent alternative strategies. For instance, supplementing partially the plant needs by natural processes, as biological N_2 fixation and organic phosphate solubilization [8, 9]. The commercial use of *Bradyrhizobium* is successfully implemented in Brazil and USA [10], leading together the annual N_2 fixation by soybean [11].

The beneficial effects of biofertilizers include promotion of plant growth, yield quality, nutrient mobilization, soil health, and reduced susceptibility to disease caused by environmental changes [12]. Previous studies evaluated the positive effects of diazotrophic bacteria *Azospirillum* on increasing growth, root length, yield, dry matter, and N accumulation of maize plants [6, 13–15].

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Besides these benefits, it has a potential to reduce the production costs [16].

In this context, we evaluated the effect of *A. brasiliense* inoculation on maize cultivated under no tillage system.

Methods

Study area

The experiment was performed in an Oxisol (Latossolo Amarelo, distrófico, muito argiloso [17]; Xanthic Hapludox [18]; Xantic Ferralsol, [19]) on an upland area on two harvests—2014/2015 and 2015/2016—under no tillage system.

The experimental area was located at km 29 of Highway AM010, in the State of Amazonas, (02°53′25″S, 59°58′06″W, 102 m a.s.l.). The climate, according to the Köppen classification, is the rainy tropical type AF, with an average temperature of 26.2 °C, and annual rainfall of 2 mm [20].

The maize was sown in December 2014 and December 2015, on millet straw desiccated with glyphosate (900 g ha^{-1} of active ingredient). The potassium and phosphorus rates used for maize crop were based in the chemical and physical properties of 0–20 cm soil layer (Table 1).

Experimental design

The experimental design was four randomized blocks in a 2 × 4 factorial combination (two cultivars of maize × four treatments with N fertilization). Five seeds were sowed per meter on 0.9-m spaced lanes. Each experimental plot size was 5.4 m (length) per 10 m (width), summing 54 m^2.

Treatments and cultivation

We used the varieties BRS Caimbé and double-cross hybrid AG 1051 in four treatments to evaluate N fertilization: (1) control (without inoculant and mineral N); (2) seeds inoculated with *A. brasiliense* bacterium; (3) N fertilization with urea (20 kg ha^{-1} at sowing and 100 kg ha^{-1} at topdress band); and (4) seeds inoculation with *A. brasiliense* + N fertilization with urea at topdress (100 kg ha^{-1} of N). Seeds were inoculated with a commercial product containing Ab-V5 and Ab-V6 strains at concentration of 10^8 cell ml^{-1}, applied at rate of 100 ml on 60,000 seeds. The inoculation was performed by adding the inoculant using an automatic pipette directly on the seeds, which were shaken in plastic bags for uniform product distribution.

The sowing was carried 1 h after inoculation. At sowing, were applied 80 kg ha^{-1} of P_2O_5 triple superphosphate and 60 kg ha^{-1} of K_2O as potassium chloride.

To control weeds, we applied nicosulfuron (50 g ha^{-1} of active ingredient) at 25 days after plant emergence, and to control armyworm [*Spodoptera frugiperda* (J.E. Smith)], we applied deltamethrin (5 g ha^{-1} of active ingredient) at 40 days after plant emergence.

Data analysis

The dependent variables were measured considering 10 central plants on each experimental plot: first cob insertion height; plant height; and total N concentration in leaves and grains. For the N concentration in leaves, plants were sampled during the flowering season and 100 grains were sampled after harvest for the N concentration determination. A 36 m^2 central area on each experimental plot was used to evaluate the variable yield of grains at 13% of moisture. All variables were subjected to analysis of variance (ANOVA), and the Tukey ($p < 0.05$) test compared the means. The data analysis was performed using SAS 9.1 statistical package.

Results

The N fertilization treatments influenced ($p < 0.01$) all variables in the 2014/2015 season and the cob insertion, plant height, yield, and N concentration in leaves in the 2015/2016 season (Table 2). The maize cultivars influenced ($p < 0.01$) all variables in the two seasons (Table 2). There was no interaction effect ($p > 0.05$) of cultivars × N fertilization on the variables. Thus, the maize cultivars had similar performance regardless treatments (Table 2).

The AG 1051 hybrid had higher cob insertion and plant height than the BRS Caimbé variety in both seasons (Figs. 1a, b, 2a, b). Considering the N fertilization treatments, only the control treatment—without N—showed lower cob insertion and plant height comparing with other treatments in both seasons (Figs. 3a, b , 4a, b), suggesting that the N supply, either N mineral from *A. brasiliense* increased growth of maize plants.

Table 1 Chemical and physical soil properties (0–20 cm) before maize sow of 2014/2015 and 2015/2016

Season	pHH_2O	SOM	N	P	K	Ca	Mg	Al	C.E.C	B.S
		g kg^{-1}		mg dm^{-3}		$cmol_c$ dm^{-3}				%
2014/2015	5.62	20.02	1.98	78	61	3.51	1.17	0	7.08	44.77
2015/2016	6.13	24.33	3.23	98	61	3.16	0.95	0	8.01	53.46

SOM soil organic matter, *N* KCl extractable nitrogen, *P and K* Mehlich-1 available phosphorus and potassium, *Ca, Mg and Al* KCl extractable calcium, magnesium and aluminum, *C.E.C* cation exchange capacity, *B.S* basis saturation

Table 2 Analysis of variance considering the influence of different N fertilization treatments, different cultivars AG 1051 e BRS Caimbé in two seasons of evaluation on first cob insertion height (FCIH), plant height (PH), grains yield (GY), N leaves concentration (NL), and N grains concentration (NG)

Sources	DF	Mean square				
		FCIH	PH	GY	NL	NG
Growing season 2014/2015						
Block	3	31.85^{ns}	149.91^{ns}	476,496.1^{ns}	2.72^{ns}	1.57^{ns}
Cultivar (C)	1	3606.13**	1199.28**	31,621,724.6**	88.05**	186.58**
Nitrogen (N)	3	227.86**	686.25**	20,983,449.3**	144.44**	13.26**
C × N	3	158.19^{ns}	482.69^{ns}	993,154.7^{ns}	2.05^{ns}	3.13^{ns}
Error	21	58.47	168.17	326617.5	7.47	0.92
CV (%)		7.3	6.4	14.0	9.7	6.1
Growing season 2015/2016						
Block	3	36.53^{ns}	6.91^{ns}	75,302.1^{ns}	4.92^{ns}	3.59^{ns}
Cultivar (C)	1	2397.58**	1928.21**	12,329,709.0**	65.35**	50.07**
Nitrogen (N)	3	214.96**	871.44**	27,503,747.9**	191.12**	5.45^{ns}
C × N	3	40.77^{ns}	107.19^{ns}	625,594.3^{ns}	4.54^{ns}	1.57^{ns}
Error	21	21.92	51.99	257,929.3	3.91	3.52
CV (%)		4.1	3.3	11.4	7.7	12.3

DF degrees of freedom

** Significant at *F* test, $p < 0.01$

^{ns} Not significant at *F* test ($p > 0.05$)

The AG 1051 hybrid had higher yield than the BRS Caimbé for both seasons (Figs. 1c, 2c). The treatments mineral N fertilization at sowing + mineral N fertilization at topdress, and the seed inoculation + mineral N at topdress had similar yield in both seasons. These treatments showed yield grain higher than the other N fertilization treatments. In addition, the yield of inoculation with *A. brasiliense* treatment was higher than control treatment in both seasons (Figs. 3c, 4c).

Considering the N concentration in leaves and grains, the AG 1051 hybrid showed higher concentration than BRS Caimbé variety in both seasons (Figs. 1d, e, 2d, e). The treatments mineral N at sowing + mineral N at topdress and seed inoculation + mineral N at topdress had the same response and were superior to the other treatments, considering the N concentration in leaves (Figs. 3d, 4d), as well as the N concentration in grains had this same behavior in 2014/2015 season (Fig. 3e), while in 2015/2016 season, the N concentration in grains was not different among the N fertilization treatments (Fig. 4e).

Discussion

We demonstrated that the *Azospirillum* seed inoculation at sowing increased N concentration in plants and growing variables compared with control treatment. Moreover, the reported yield was higher than the average of yield maize at Amazonas state [15] confirmed that *Azospirillum* seed inoculation build up plants' height. As well known, the positive effects of N on plant development are due to its function on the roots and shoots growth, composing protein, enzymes, coenzymes, nucleic acids, phytochromes, photosynthetic pigments, etc. Consequently, increasing the photosynthetically active area of plants and photoassimilates synthesis translocated to the grains [21] providing a higher yield. The demonstrated benefits of *Azospirillum* are explained by its successful association with grasses [15, 16, 22]. These microorganisms called diazotrophics reduce the atmospheric N_2 to ammonia (NH_3)-breaking triple bonds by the nitrogenase enzyme, process with high demand of energy as ATP. After this reduction, the NH_3 is converted to ammonium (NH_4^+), assimilated as glutamine by the vegetal cells [23]. Besides the N_2-fixing, these bacteria also (i) synthesize phytohormones [24]; (ii) solubilize inorganic phosphate [25, 26]; (iii) stimulate root system development [27], and (iv) increase resistance of plants by stress and their own N biological fixation [26].

Moreover, the inoculation with *Azospirillum* at sowing + mineral N at topdress provided similar grain yield as mineral N at sowing and topdress for both maize cultivars. Previous studies generally reported the benefits of *Azospirillum.* The combination of 200 ml ha^{-1} of *A. brasiliense* with 200 kg ha^{-1} of N increased the physiological response of the maize crop [6]. Gains on maize grains yield with this inoculation are reported from 7% [28] to 30% [29, 30]. Lana et al. [28] reported that inoculated plants' yield was equivalent to those topdressed with 100 kg ha^{-1} of mineral N.

The N leaves' concentrations on plants treated with inoculant at sowing + mineral N fertilization were higher

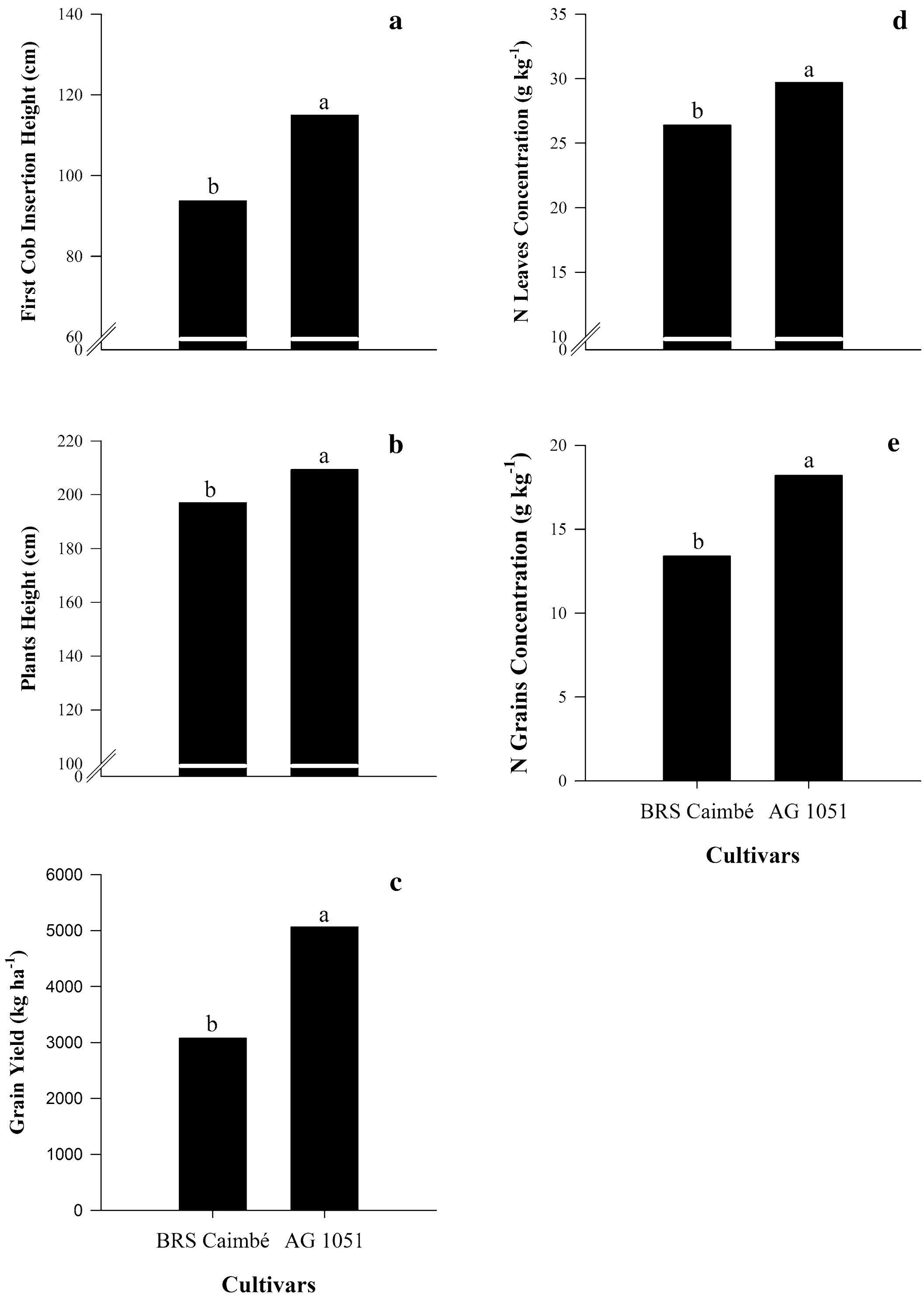

Fig. 1 Effects of BRS Caimbé and AG 1051 maize cultivars on first cob insertion height (FCIH) (**a**), plants height (PH) (**b**), grain yield (GY) (**c**), N leaves concentration (NL) (**d**), and N grains concentration (NG) (**e**) in 2014/2015 season. Different letters indicate differences ($p < 0.05$) between maize cultivars by Tukey test

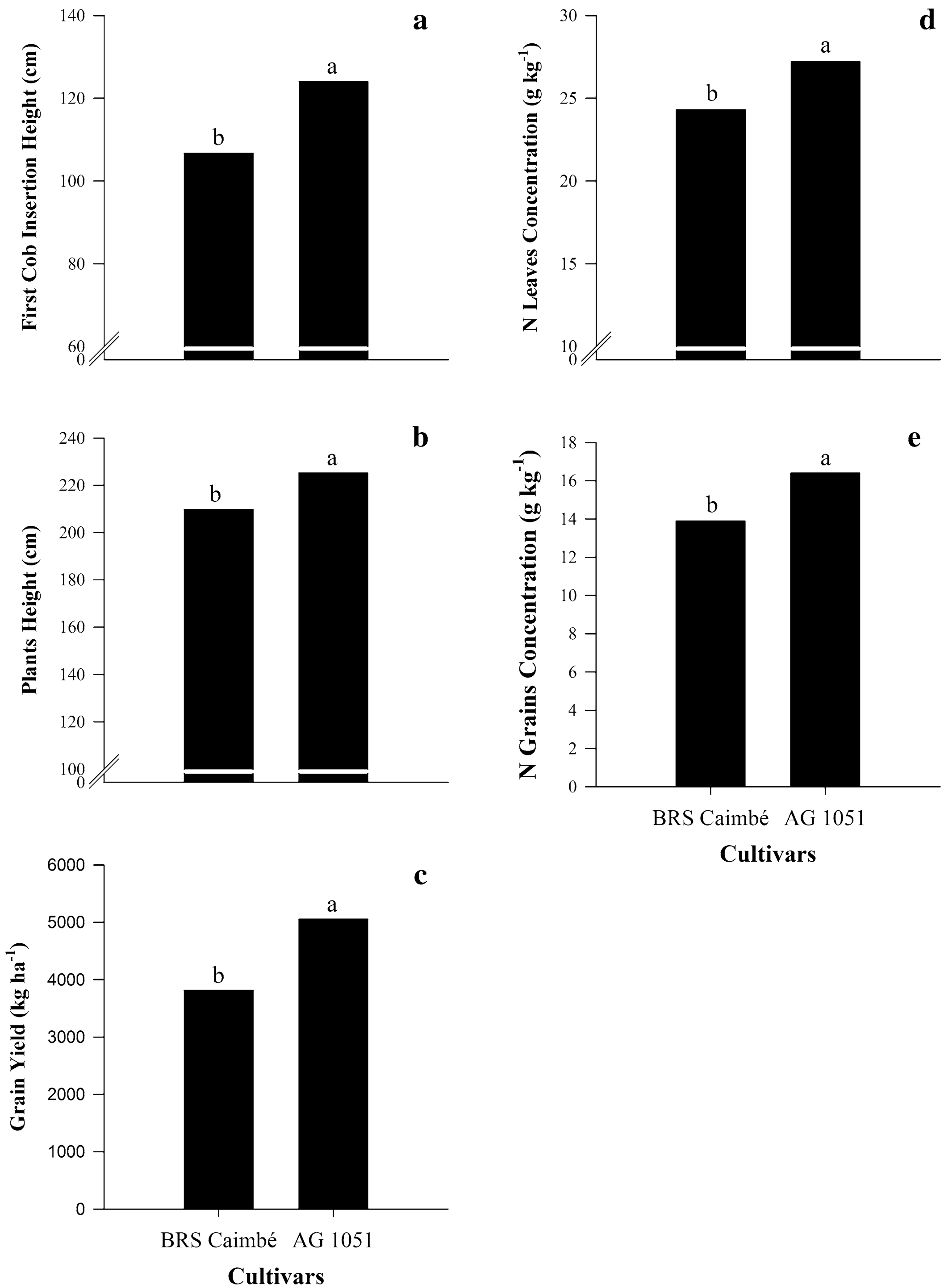

Fig. 2 Effects of BRS Caimbé and AG 1051 maize cultivars on first cob insertion height (FCIH) (**a**), plants height (PH) (**b**), grain yield (GY) (**c**), N leaves concentration (NL) (**d**), and N grains concentration (NG) (**e**) in 2015/2016 season. Different letters indicate differences ($p < 0.05$) between maize cultivars by Tukey test

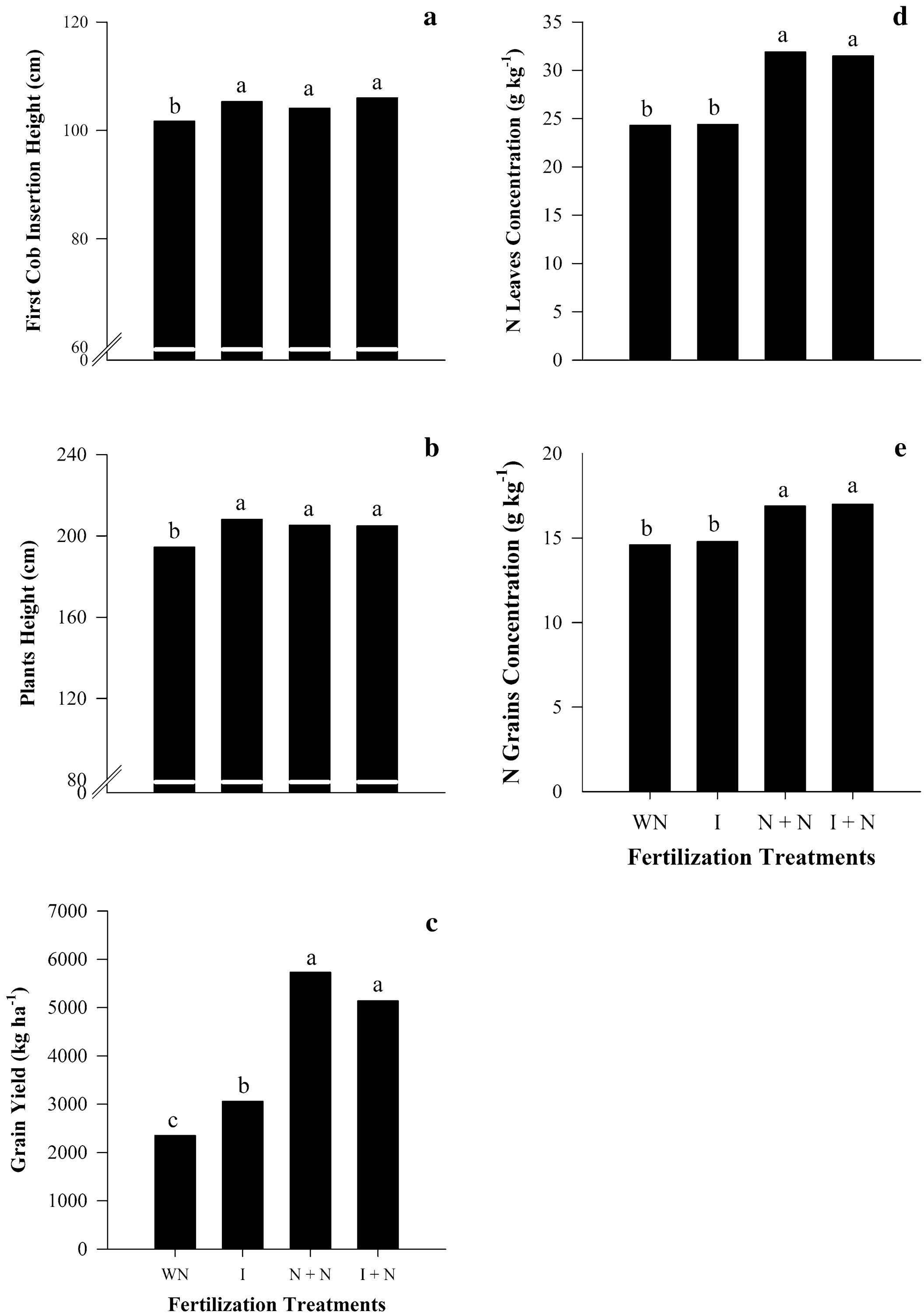

Fig. 3 Effects of N fertilization treatments without N (W N), inoculation with *Azospirillum brasiliense* (I), N fertilization as urea at sowing and at top-dress (N + N), and seeds inoculation + N fertilization at sowing (I + N) on first cob insertion height (FCIH) (**a**), plants height (PH) (**b**), grain yield (GY) (**c**), N leaves concentration (NL) (**d**), and N grains concentration (NG) (**e**) in 2014/2015 season. Different letters indicate differences ($p < 0.05$) among N fertilization treatments by Tukey test

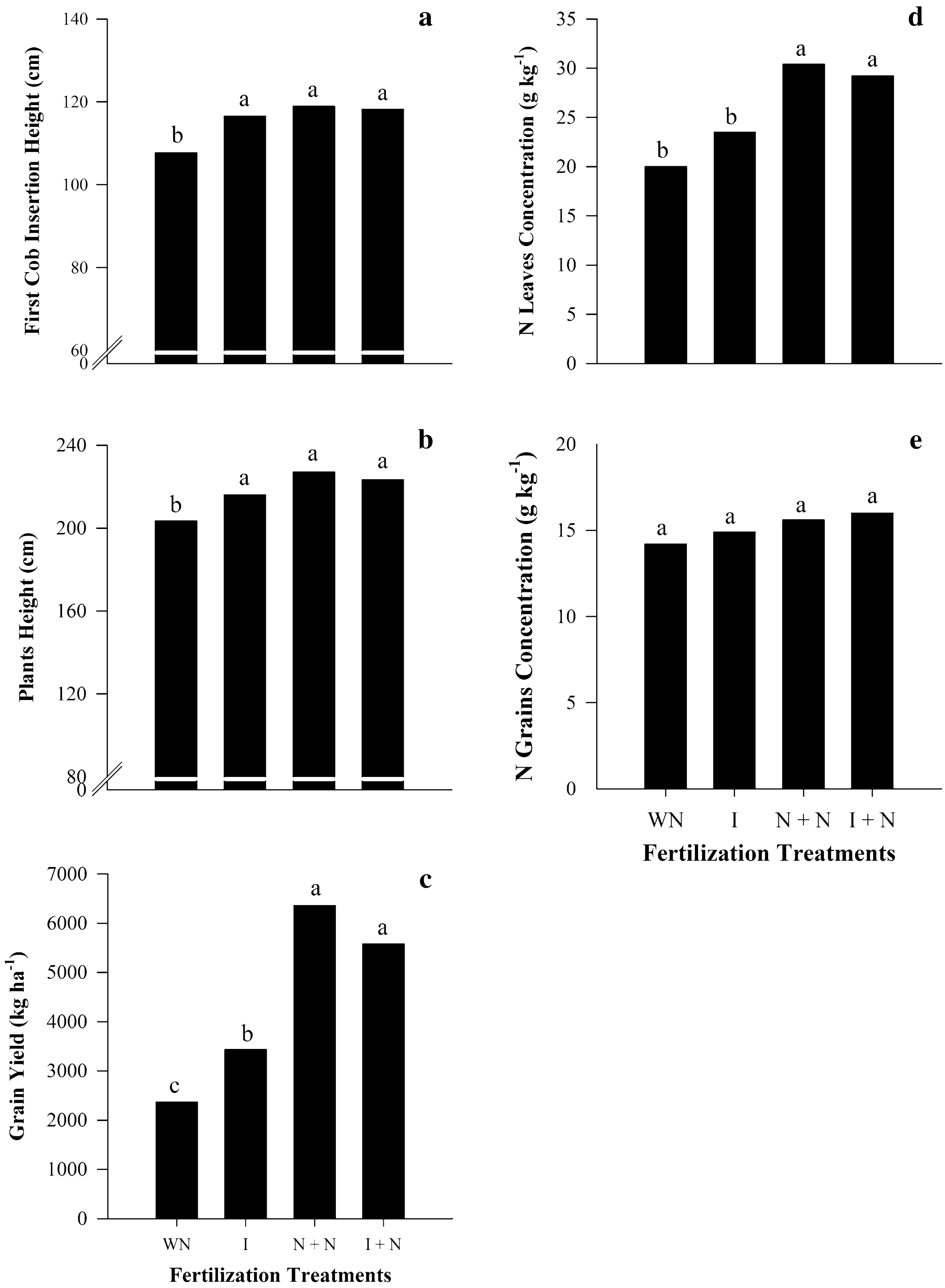

Fig. 4 Effects of N fertilization treatments without N (W N), inoculation with *Azospirillum brasiliense* (I), N fertilization as urea at sowing and at top-dress (N + N) and seeds inoculation + N fertilization at sowing (I + N) on first cob insertion height (FCIH) (**a**), plants height (PH) (**b**), grain yield (GY) (**c**), N leaves concentration (NL) (**d**), and N grains concentration (NG) (**e**) in 2015/2016 season. Different letters indicate differences ($p < 0.05$) among N fertilization treatments by Tukey test

than the sufficiency range for maize crop, from 27.5 to 32.5 g kg^{-1} [31], confirming the positive effects of the inoculant on the plant nutrition.

Considering the high economical and environmental costs of the industrial N fixation and the increasing demand for food, new technologies are essential to the best management practices of N fertilization. In this context, an alternative is associate diazotrophic bacteria N_2-fixing with crops of economical importance, increasing the vegetative growth, development, and yield [32]. Besides, it is an option to reduce and potentiate the effect of N fertilizers in the maize crop, since they are diazotrophic and thus can complement the quantity of the nutrient required by the plants via biological fixation of the atmospheric N (BNF) [15].

The high cost of chemical fertilizers and concerns for a sustainable agriculture are hightening the interest on inoculant bacteria [30]. We reported an alternative to cultivate maize without mineral fertilization at sowing, saving 20 kg ha^{-1} of N. Considering cultivated 11,600 ha $year^{-1}$ of maize in Amazonas state [1], a reduction of 232 Mg of N shall occur, or 515 Mg of fertilizer as urea, saving yearly US$ 339,680.00, considering the local costs in Manaus—AM. Moreover, the contribution of *A. brasiliense* would represent a gain of 0.103 Mg C ha^{-1}, corresponding to 0.309 Mg CO_2-eq ha^{-1} [22].

Comparing the AG 1051 hybrid cultivar advantages over BRS Caimbé variety in all studied variables including yield was previously reported [33, 34]. Hybrids can be synthesized crossing lines with complementary genetic pools, allowing best exploitation of heterosis, as suggested by [35].

Conclusions

The inoculation with *A. brasiliense* increased the maize yield compared with non-N-fertilized plants, and the inoculation with *Azospirillum* at sowing + mineral N at topdress provided similar grain yield as mineral N at sowing and topdress for both cultivars of maize. Therefore, *A. brasiliense* can be a sustainable alternative to the N supply for the maize crop of tropical regions, in addition to saving costs on maize production, especially with chemical N fertilizers.

Authors' contributions

All authors have contributed equally to the work. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data were presented in the manuscript.

Consent for publication

All authors agreed to the publication.

Ethics approval and consent to participate

The manuscript is an original work that has not been published in other journals. The authors declare no experiments involving humans and animals.

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