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Article

Growth and Nitrogen Uptake by Potato and Cassava Crops Can Be Improved by *Azospirillum brasilense* Inoculation and Nitrogen Fertilization

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Abstract: Nitrogen (N) is the nutrient most taken up by potato and cassava crops and *Azospirillum brasilense* may contribute to the growth of these crops. Pot experiments evaluated *A. brasilense* and mineral N application on leaf N concentration, plant growth, and N uptake by potato and cassava grown under natural and disinfected soil. The rates of 2.8×10^8 colony-forming units mL^{-1} of *A. brasilense* combined with 0, 75, 150, and 300 mg dm^{-3} N or 0, 50, 100, and 200 mg dm^{-3} N were used for potato or cassava grown. At low N supply in natural soil, *A. brasilense* inoculation increased N concentration in potato leaves by 23–38%, without benefits to plant growth or N uptake. At unfertilized N treatments of both soils, *A. brasilense* inoculation increased cassava leaf N concentration by 25–33%, but an 11–32% increase in shoot biomass occurred in treatments inoculated and N supplied. Potato crops responded positively to mineral N supply, but cassava responded to fertilization only in disinfected soil. In disinfected soil fertilized with N, *A. brasilense* inoculation increased cassava N uptake by 27–40%. In contrast, in natural soil, *A. brasilense* minimized the negative effect of N excess on the tuber development of cassava. These results show that the use of *A. brasilense* is a more interesting alternative to improve N status and growth in cassava than in potatoes.

Keywords: tuber crops; growth-promoting bacteria; nitrogen fixation

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1. Introduction

Potato (*Solanum tuberosum* L.) and cassava (*Manihot esculenta* Crantz) are the two most important tuber crops grown in Brazil. The area cultivated annually with potatoes in Brazil is approximately 116.4 thousand hectares, while cassava has an annual cultivation area of 1.20 million hectares [1]. Both crops take up large amounts of nutrients from the soil, with an emphasis on nitrogen (N), which is one of the key nutrients influencing tuber production [2,3].

As a crop, potato responds positively to N fertilization [4,5]; thus, the rates applied in this crop are normally high (>100 kg ha^{-1}) [2,5]. In contrast, cassava does not often respond to N fertilization [6,7]; therefore, although the natural supply of N in tropical soils is limited, in Brazilian conditions, most farmers still do not use this nutrient in cassava cultivation [8]. Moreover, unlike potatoes, cassava takes up large amounts of N (>110 kg ha^{-1}) [3], even with a low mineral N supply via fertilizer.

Because excess N can cause environmental problems, such as air pollution, greenhouse gas emissions, global warming, and water contamination, strategies for integrated N management that reduce the amount of N fertilizer to be applied to crops should be encouraged [9]. The use of N-fixing *Azospirillum* species in agriculture increased after its rediscovery in 1976, and the species *Azospirillum brasilense* and *A. lipoferum* are the most studied and best described [10]. Initially, it was believed that *Azospirillum* spp. were associated mainly with cereals and grasses [11], but subsequently, it has been observed that, although *Azospirillum* is not specific to cereals in terms of gender and species, there is an affinity between strains and plant genotypes [12].

Bacteria of the *Azospirillum* genus can stimulate plant growth by several mechanisms; however, the two main mechanisms are free N_2 fixation and the synthesis of phytohormones such as abscisic acid, auxins, cytokinins, ethylene, gibberellins, and polyamines [13,14]. Despite the ability to fix N_2, the more significant contribution of inoculation with *A. brasilense* (especially strains Ab-V5 and Ab-V6) has been attributed to plant growth-promoting effects [15]. *Azospirulum* associates with the rhizosphere of plants, that is, the bacteria adhere to the root growth zones, producing and secreting phytohormones (especially auxins) in the rhizosphere region, which stimulates meristematic activity and root development of the host plant [15–17]. Thus, plants inoculated with *Azospirillum*, in addition to fixing some N_2 (7.4–19.4% of the plant demand) [15] can also increase their root growth [14,18], as well as be able to explore a larger soil volume, improve their water and nutrient uptake [14], and promote better adaptation to environmental stresses, such as drought and salinity [19].

Despite the positive benefits of *A. brasilense* inoculation for several agricultural crops (including corn, legumes, pasture grass, rice, sugarcane, and wheat) [15], its effects on biological N fixation and growth promotion of crops such as potato and cassava are still poorly understood. However, the use of certain strains ("Sp245" and "SR80") of *A. brasilense* has improved the survival of micropropagated potato plants under in vitro conditions [20]. The inoculation of *A. brasilense* strain "Sp245" in potatoes grown in an aeroponic system increased plant growth and mini tuber yield and quality [21]. Another strain ("TN10") of *Azospirillum* has also shown the potential to increase plant growth and N uptake in potatoes grown under controlled conditions [22]. In cassava, bacteria of the genus *Azospirillum* and native mycorrhizae from the soil are naturally associated with plant roots [23]. Both *A. brasilense* and *A. lipoferum* species are associated with cassava plants [23,24], and the presence of the bacteria increases the mycorrhizal colonization of cassava roots [25,26]. The combined inoculation of *Azospirillum* spp. and *Trichoderma* also benefited the cassava tuber yield under field conditions [27]. However, there are still no commercial inoculants based on *Azospirillum* indicated for use in potato and cassava crops.

In Brazil, the strains "Ab-V4", "Ab-V5", "Ab-V6", and "Ab-V7" of *A. brasilense* are recommended for corn crops, and liquid inoculants containing a combination of the "Ab-V5" and "Ab-V6" strains have been effective in both corn and wheat crops [28]. However, the affinity of the *A. brasilense* strains "Ab-V5" and "Ab-V6" for potato and cassava crops and whether these strains can benefit the development of these tuber crops is still unknown. Thus, we hypothesized that inoculation with *A. brasilense* alone or in combination with a low N supply can improve the growth and N uptake in potato and cassava crops, thereby reducing the environmental risks caused by using mineral N.

Therefore, in this study, we evaluated plant growth, N status in the leaves, and accumulation in potato and cassava plants inoculated with *A. brasilense* strains "Ab-V5" and "Ab-V6" under different amounts of mineral N in natural and disinfected soils.

2. Materials and Methods

2.1. Site, Soil, Experimental Design, and Treatments

Four pot experiments were conducted in a greenhouse at the Center of Tropical Roots and Starches (CERAT) at the São Paulo State University (UNESP), in Botucatu, São Paulo, Brazil (22°50′ S, 48°25′ W, and 773 m above sea level). The experiments were carried out under the following conditions: potato grown in natural soil, potato grown in disinfected soil, cassava grown in natural soil, and cassava grown in disinfected soil. Pots of 38 and 25 dm^{-3} were used for potato and cassava, respectively. The soil used was a clayey-textured Typic Rhodudalf soil previously corrected and fertilized (Table 1). For experiments with disinfected soil, the soil was disinfected in an autoclave for 45 min at 1 atm.

Table 1. Soil chemical characteristics at the soils after correction and fertilization.

Soil Characteristic	Values	
	Potato	Cassava
pH($CaCl_2$)	6.0	5.6
Content of oxidizable carbon (Cox) (%)	0.30	0.73
$P_{resin\text{-}extractable}$ (mg dm^{-3})	161.0	141.0
H+Al ($mmol_c$ dm^{-3})	23.0	25.0
K ($mmol_c$ dm^{-3})	3.7	3.8
Ca ($mmol_c$ dm^{-3})	40.0	45.0
Mg ($mmol_c$ dm^{-3})	20.0	14.0
Cation exchange capacity ($mmol_c$ dm^{-3})	86.7	87.8
Base saturation (%)	73.5	71.5
B (mg dm^{-3})	0.6	1.6
Cu (mg dm^{-3})	4.6	3.5
Fe (mg dm^{-3})	14.0	10.0
Mn (mg dm^{-3})	4.0	2.8
Zn (mg dm^{-3})	0.8	1.7

The four experiments were carried out using a randomized block design in a 2 × 4 factorial scheme, with four replications. For the potatoes, treatments were composed of the inoculation (or not) of *A. brasilense* strains "Ab-V5" and "Ab-V6" with four N rates (0, 75, 150, and 300 mg dm^{-3} N). For the cassava, treatments were represented by the inoculation (or not) of *A. brasilense* strains "Ab-V5" and "Ab-V6" with four N rates (0, 50, 100, and 200 mg dm^{-3} N). The N source in all experiments was urea (45% N). Each plot consisted of one pot with a single potato or cassava plant. For the potatoes, the N rates were split-applied with one-third at planting, one-third at 15 days after emergence (DAE), and one-third at 40 DAE. For the cassava, N rates were split-applied with half at 15 DAE and the remainder at 40 DAE. Inoculation with *A. brasilense* was at a dose of 20 mL of the inoculant (GRAP NOD A®) containing 2.0×10^8 CFU (colony-forming units) mL^{-1} of the *A. brasilense* strains "Ab-V5" and "Ab-V6".

2.2. Planting and Management of Potato and Cassava

The soil was collected between the depths of 0.0 to 0.20 m, sieved, analyzed, and corrected with dolomitic limestone to increase the base saturation to 70%. For potato experiments, the soil was previously fertilized with 150 mg dm^{-3} P and 150 mg dm^{-3} K, whereas for cassava experiments, fertilization was carried out with 150, 150, 0.5, and 0.8 mg dm^{-3} P, K, B, and Zn, respectively. The soil's chemical properties after correction and fertilization are listed above in Table 1.

The potato cultivar "Agata" and cassava cultivar "IAC 576-70" were used. The cultivar Agata is the most widely grown in Brazil [5], whereas the cultivar IAC 576-70 is the main sweet cassava cultivar grown in São Paulo State, Brazil [29]. For planting, holes were opened 0.10 m deep in the soil of pots. Planting-N fertilization for the potatoes was applied to the hole and incorporated into the hole soil. For planting, potato seed tubers type III (approximately 41 g in mass, with diameters of between 30 and 40 mm) were used, and 0.15 m-long cassava stems were taken from the middle third of 12-month-old plants. Before planting, the seed tubers and cassava stems were disinfected using the methodology adapted from Buensanteai et al. [30]. The seed tubers and cassava stems were immersed first in an ethanol solution (70%) for 2 min and then in a 20%-bleach solution (2.5% sodium hypochlorite) for 10 min. After treatment, the seed tubers and cassava stems were washed five times in deionized water to remove excess bleach.

After the treatment, seed tubers or cassava stems were planted in each pot hole. In the treatments with diazotrophic bacteria inoculation, inoculants containing *A. brasilense* were applied to the seed tubers or cassava stems, and then the holes were closed manually with the soil of the pots.

Irrigation was performed with deionized and sterile water according to the technical recommendations for both crops. The irrigation water was treated using an ion exchange resin deionizer coupled to a 1000 L water box, equipped with a UV lamp system. Pest and disease control in both crops was carried out according to need and technical recommendations. The potatoes and cassava were harvested at maturation, that is, 90 and 240 days after planting (DAP), respectively.

2.3. Sampling and Analyses

In the potato, the third expanded leaf was collected from the apex at 30 DAE, and in the cassava, the fully expanded leaf blade was collected from the apex at 4 months after planting [3,5]. Leaf samples were dried in a forced air circulation oven at 65 °C for 72 h, ground, and the N concentration in the plant tissues was obtained by the semi-micro-Kjeldahl method.

At 90 DAP for the potatoes and 240 DAP for the cassava, the plants were harvested and separated into shoots, roots, tubers, and planted cuttings (only in the cassava). The plant parts were washed and dried in a forced air circulation oven at 65 °C for 96 h. The dry samples were weighed to obtain dry matter (DM) accumulation in each plant part. The whole plant DM was obtained by the sum of the amounts of DM accumulated in all plant parts.

The samples of each plant part were ground to pass through a 40-mesh stainless steel, and the N concentration in each plant part was determined using the semi-micro-Kjeldahl method. The amount of N accumulated in each plant part was calculated by multiplying the amount of DM accumulated by the N concentration in each plant part. The amount of N accumulated in the whole plant was calculated as the sum of the amounts of N accumulated in each plant part.

The N-uptake efficiency was calculated by dividing the amount of N accumulated in the whole plant by the root DM. The N-use efficiency was obtained by dividing the whole plant DM by the amount of N accumulated in the whole plant.

2.4. Statistical Analyses

Data were analyzed separately by experiment using the Sisvar statistical software package [31]. Blocks and block interactions were considered random effects, whereas the effects of *A. brasilense* inoculation and N rate were considered fixed effects. The effects of the N rate were evaluated by regression analysis, whereas the effects of *A. brasilense* inoculation were compared using an LSD test at a probability level of 0.05. The graphics were plotted using SigmaPlot 10.0 software (Systat Software, Inc., San Jose, CA, USA).

3. Results

3.1. Nitrogen Status in the Leaves of Potato and Cassava

In the natural soil, N concentration in the potato leaves increased up to 258 mg dm^{-3} N in the inoculated plants and up to 229 mg dm^{-3} N in the non-inoculated plants (Figure 1a). When no N was applied, *A. brasilense* inoculation increased N concentration in the potato leaves by 38%, but this value reduced by 23% when 75 mg dm^{-3} N was applied, and any *A. brasilense* effect occurred at higher N rates. However, in the disinfected soil, treatments did not alter the N concentration in the potato leaves (Figure 1b).

Nitrogen fertilization did not alter the N concentration in cassava leaves of the inoculated plants in either soil; however, in the non-inoculated plants, this variable increased linearly with the N rates in the disinfected soil and up to 139 mg dm^{-3} N in the natural soil (Figure 1c,d). In both soils, N concentration in the cassava leaves of the inoculated plants grown in the absence of mineral N was 25–33% higher than that in the non-inoculated plants. Only in the disinfected soil conditions, *A. brasilense* inoculation significantly increased the N concentration of cassava leaves by 13% when 100 mg dm^{-3} N was applied.

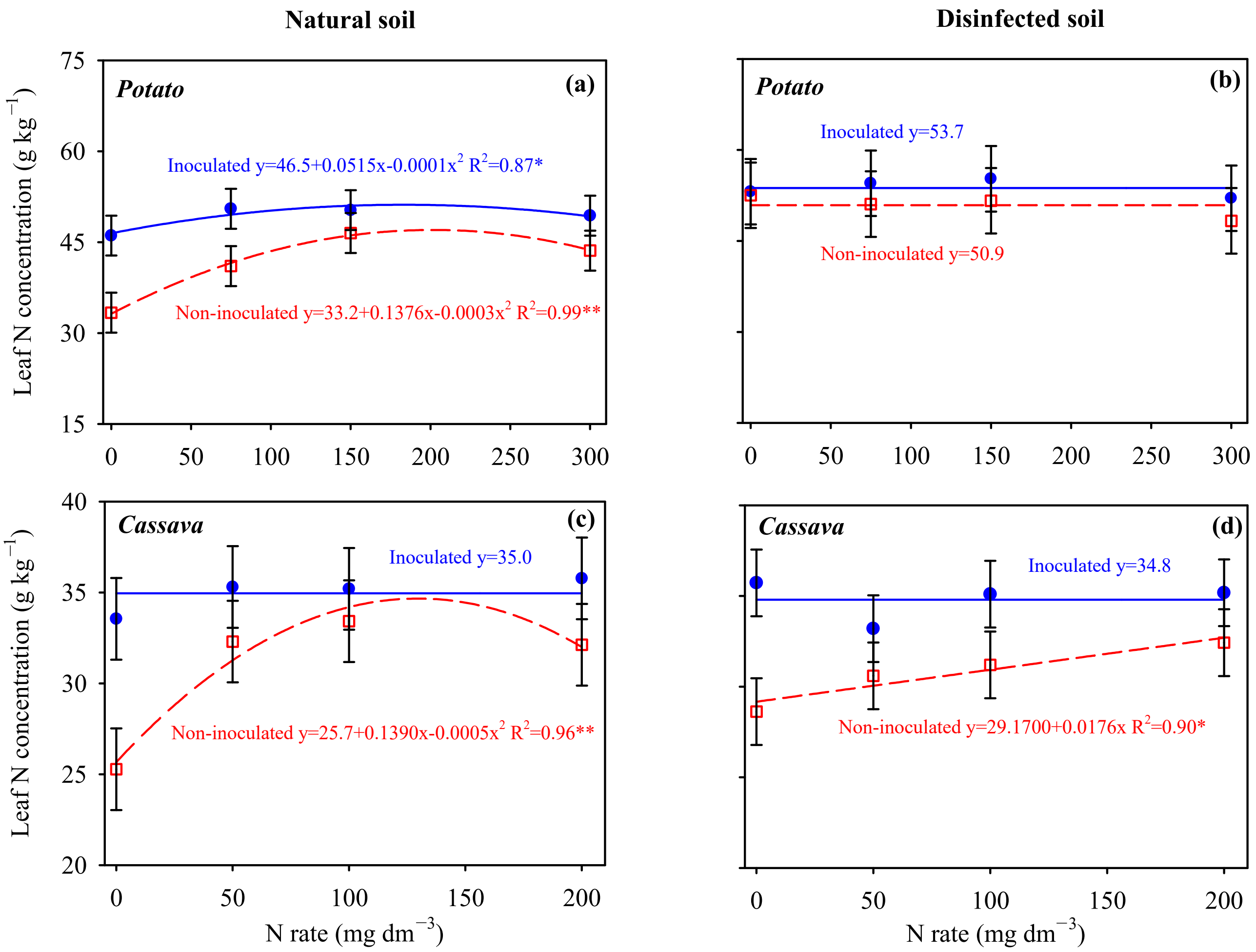


Figure 1. Leaf nitrogen (N) concentration of potato (**a**,**b**) and cassava (**c**,**d**) in response to N application rates and *A. brasilense* inoculation in natural and disinfected soil. Bars represent the least significant difference between *A. brasilense* inoculation in the same N rate at $p < 0.05$ according to the LSD test. * $p < 0.05$; ** $p < 0.01$.

3.2. Biomass Accumulation in Potato and Cassava Plants

The treatments did not influence the shoot DM of the potatoes in the natural soil (Figure 2a). In the disinfected soil, shoot DM of the potatoes decreased linearly with N fertilization, with no effect of *A. brasilense* inoculation (Figure 2b). In both soils, N fertilization reduced the absorbent root DM of the potatoes without the influence of *A. brasilense* inoculation on this variable (Figure 2c,d).

In natural soil, the *A. brasilense* inoculation did not influence the tuber or whole plant DM of potato, as these variables increased by N fertilization up to rates between 177 and 179 mg dm^{-3} N (Figure 2e,g). In the disinfected soil, N fertilization did not interfere with tuber and whole plant DM of non-inoculated potato plants; however, in inoculated plants, tuber and whole plant DM increased quadratically up to 134 and 48 mg dm^{-3} N, respectively (Figure 2f,h). Notably, at a rate of 300 mg dm^{-3} N, tuber and whole plant DM of the inoculated potato plants were 21–26% lower than that in the non-inoculated plants.

In the natural soil, the shoot DM of the cassava increased quadratically up to 123 mg dm^{-3} N in the non-inoculated plants, and up to 126 mg dm^{-3} N in the inoculated plants (Figure 3a). Only when N rates between 50 and 200 mg dm^{-3} were applied, *A. brasilense* inoculation increased the shoot DM of the cassava between 11–13% compared to non-inoculated plants. In the disinfected soil, N linearly increased the shoot DM of the cassava, and for all N rates, the shoot DM of the inoculated plants was 19–32% higher than in the non-inoculated plants (Figure 3b).

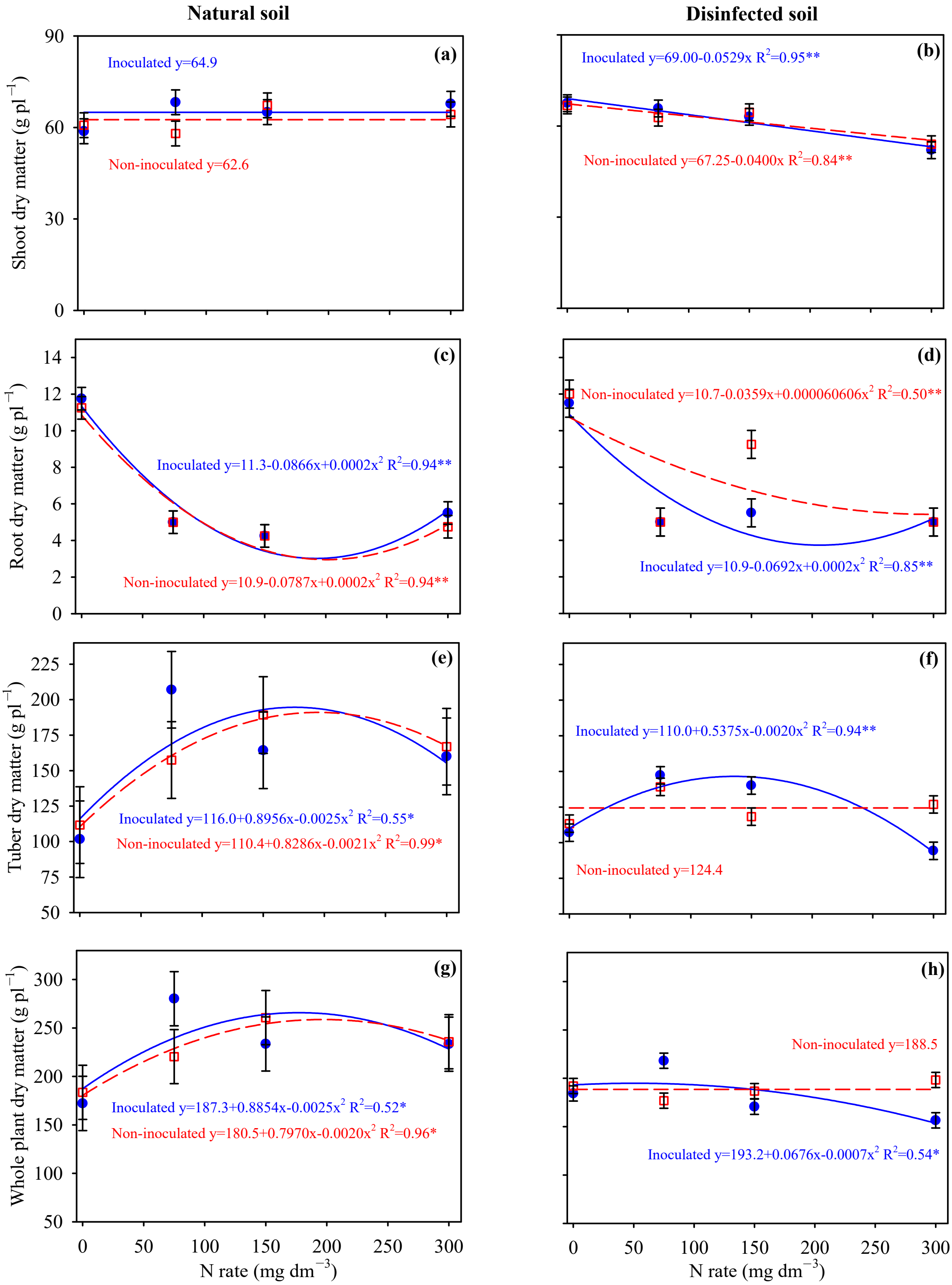


Figure 2. Dry matter accumulation in the shoot (**a**,**b**), root (**c**,**d**), tuber (**e**,**f**), and whole plant (**g**,**h**) of potato in response to N application rates and *A. brasilense* inoculation in natural and disinfected soil. Bars represent the least significant difference between *A. brasilense* inoculation in the same N rate at $p < 0.05$ according to the LSD test. * $p < 0.05$; ** $p < 0.01$.

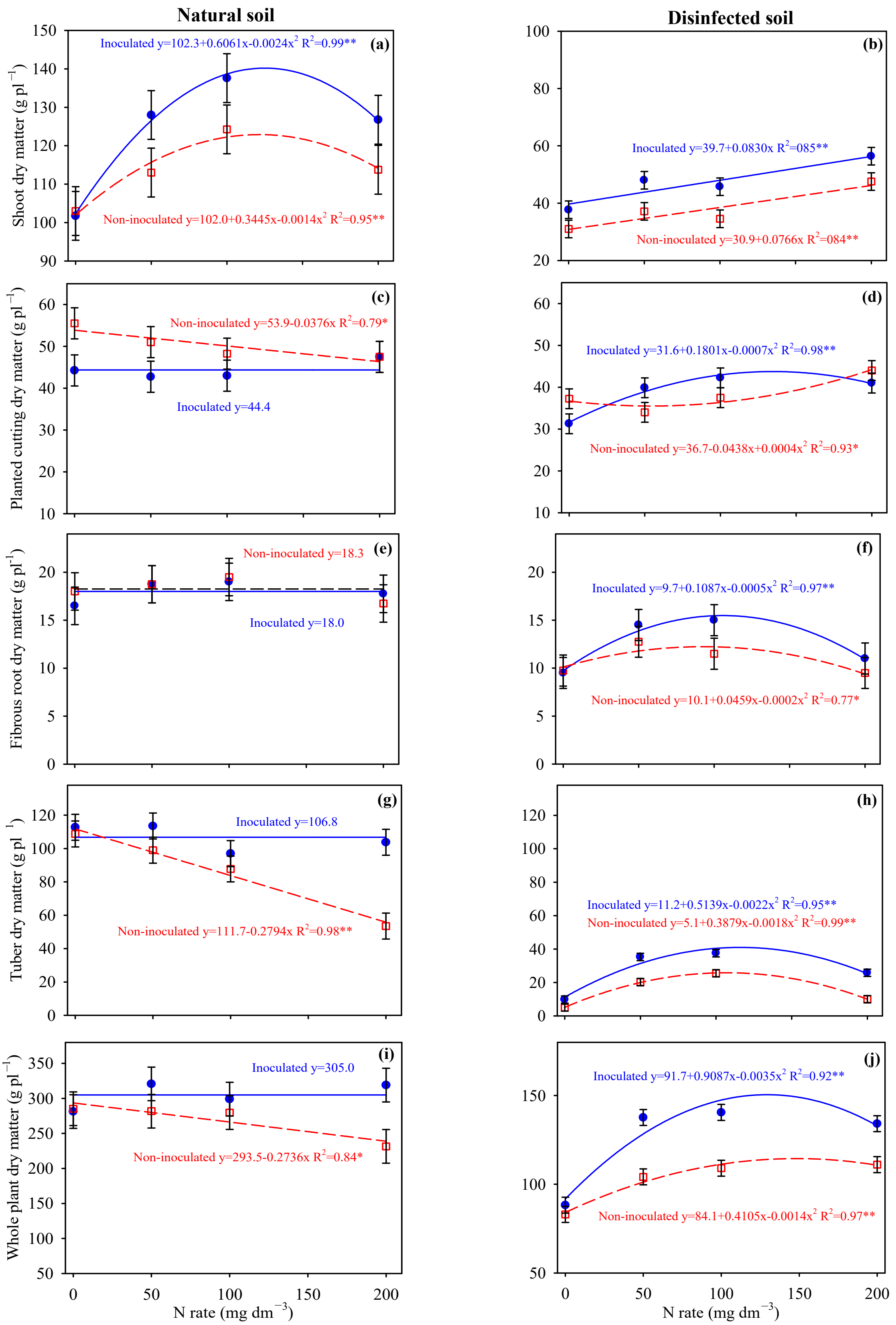


Figure 3. Dry matter accumulation in the shoot (**a**,**b**), planted cutting (**c**,**d**), fibrous root (**e**,**f**), tuber (**g**,**h**), and whole plant (**i**,**j**) of cassava in response to N application rates and *A. brasilense* inoculation in natural and disinfected soil. Bars represent the least significant difference between *A. brasilense* inoculation in the same N rate at $p < 0.05$ according to the LSD test. * $p < 0.05$; ** $p < 0.01$.

In the natural soil, the planted cutting DM of the inoculated cassava plants was not affected by N rates, but in the non-inoculated plants, the planted cutting DM reduced linearly with the N rates (Figure 3c). The application of *A. brasilense* combined with zero or 50 mg dm^{-3} N reduced the DM of cassava planted cuttings by 16–20%. In the disinfected soil, N rates increased the DM of the cassava planted cuttings in the inoculated plants up to 128 mg dm^{-3} N, whereas in the non-inoculated plants, there was a reduction in the biomass of planted cuttings up to 54 mg dm^{-3} N (Figure 3d).

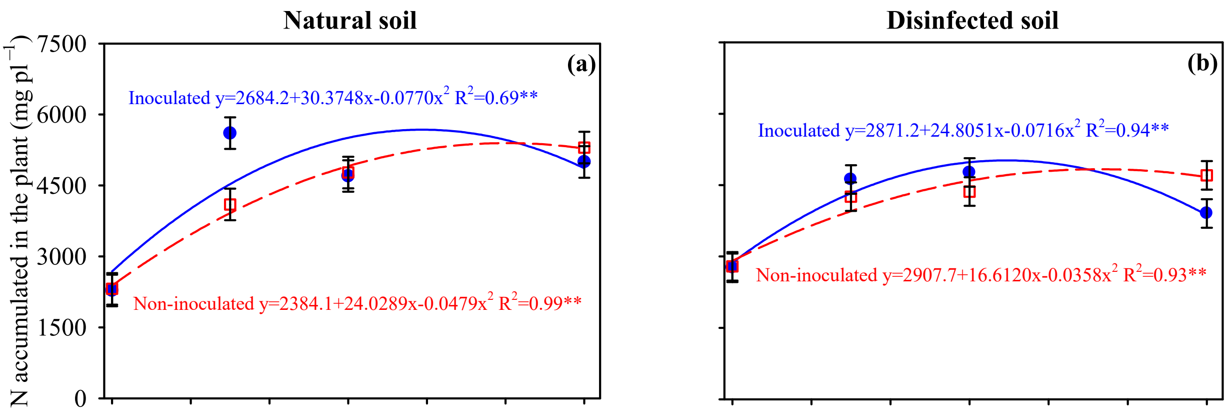
The N supply and *A. brasilense* inoculation did not influence the DM of cassava absorbent roots in the natural soil (Figure 3e). However, in the disinfected soil, N fertilization increased the absorbent root DM, and the inoculated plants showed a DM of absorbent roots 2.6% higher than that of the non-inoculated plants at the rate of 100 mg dm^{-3} (Figure 3f).

In natural soil, N fertilization linearly reduced the tuber and whole plant DM of the non-inoculated cassava plants; however, in the inoculated cassava plants N supply did not alter the tuber and whole plant DM (Figure 3g,i). Thus, when 200 mg dm^{-3} N was applied, *A. brasilense* inoculation resulted in cassava plants with tuber and whole plant DM being 38–94% higher than of the non-inoculated plants. In disinfected soil, N fertilization quadratically increased the DM of cassava tubers and whole plants, regardless of *A. brasilense* inoculation (Figure 3h,j). However, when mineral N was applied, inoculated plants of cassava showed a DM of tubers 47–158% higher than that of non-inoculated plants, while the DM of whole plant inoculated with *A. brasilense* was 21–32% higher than that of the non-inoculated plants.

3.3. Nitrogen Uptake and N-Use Efficiency by Potato and Cassava Plants

In the natural soil, the amount of N accumulated by the potato plants and the efficiency of N uptake at the rate of 50 mg dm^{-3} N in the inoculated treatments was 36–37% higher than in the non-inoculated treatments, while the N-use efficiency was not altered by *A. brasilense* inoculation (Figure 4a,c,e). Nitrogen fertilization increased the amount of N accumulated by the potato plants and the N-uptake efficiency up to rates between 185 and 251 mg dm^{-3} N, while the N-use efficiency was reduced up to N rates between 232 and 294 mg dm^{-3} N.

In disinfected soil, inoculated potato plants that received 300 mg dm^{-3} N rate accumulated 17% less N than non-inoculated plants (Figure 4b). The N-uptake efficiency of the inoculated potato plants at a rate of 150 mg dm^{-3} N was 75% higher than that in the non-inoculated plants, while *A. brasilense* inoculation did not influence the N-use efficiency (Figure 4d,f). Nitrogen fertilization increased the amount of N accumulated in the whole plants and the N-uptake efficiency of the potato crops, regardless of *A. brasilense* inoculation (Figure 4b,d). The N-use efficiency by potato plants was reduced up to N rates of 195 and 200 mg dm^{-3} N (Figure 4f).



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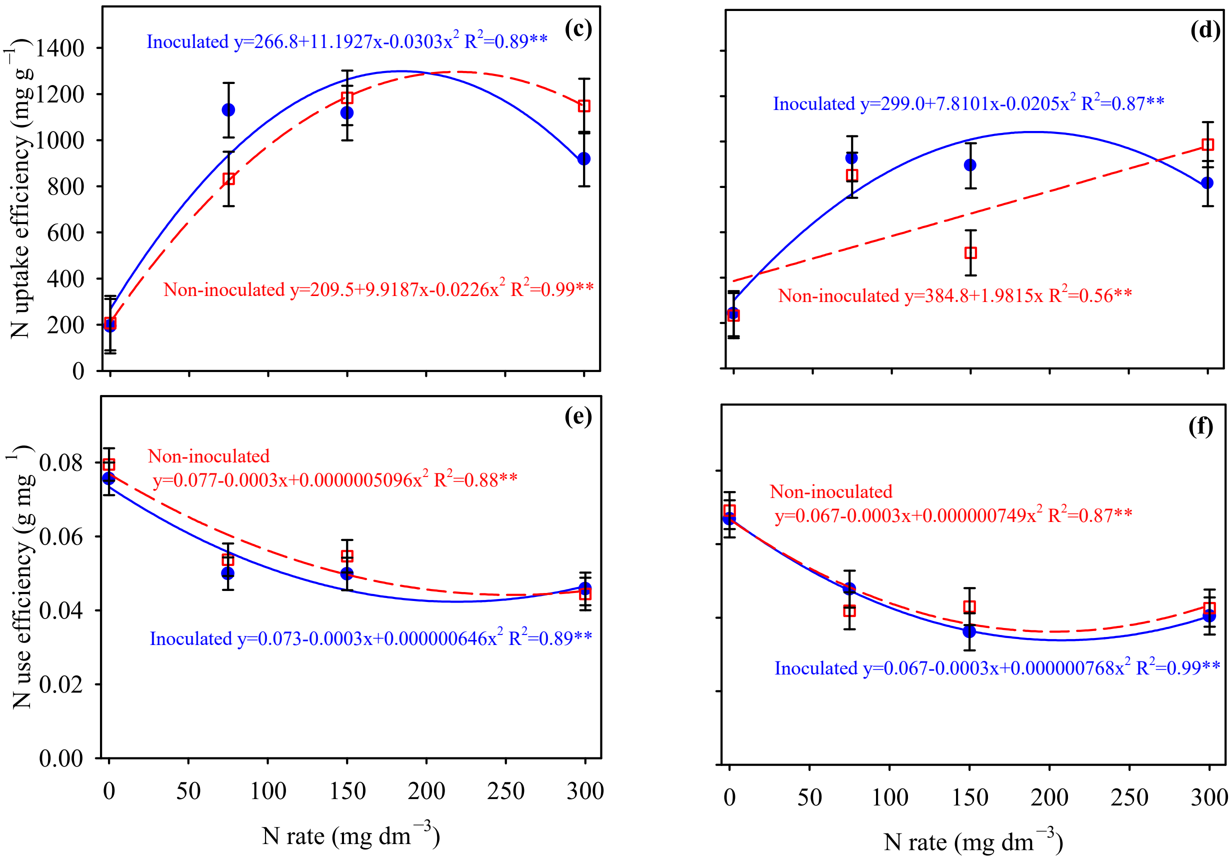
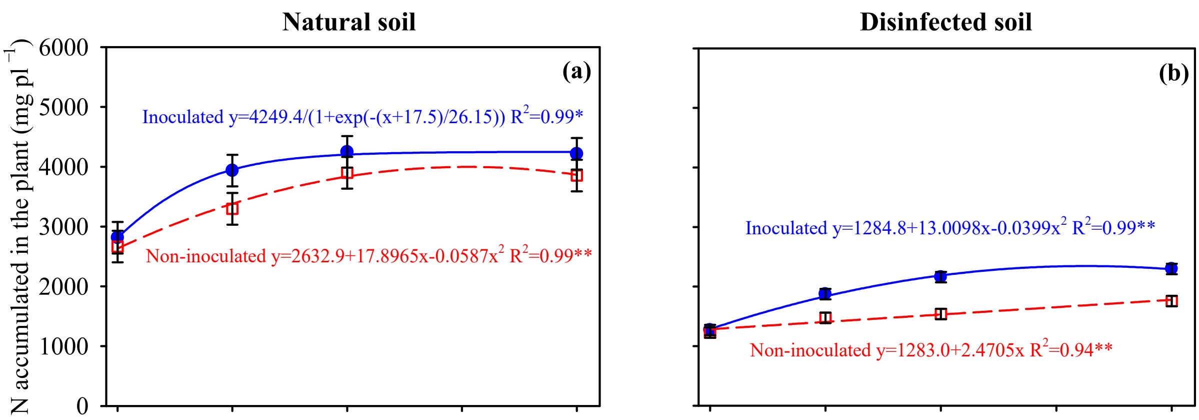


Figure 4. Amount of N accumulated in the whole plant (**a**,**b**), N-uptake efficiency (**c**,**d**), and N-use efficiency (**e**,**f**) of potato in response to N application rates and *A. brasilense* inoculation in natural and disinfected soil. Bars represent the least significant difference between *A. brasilense* inoculation in the same N rate at $p < 0.05$ according to the LSD test. ** $p < 0.01$.

The amount of N accumulated in the cassava plants in the natural soil increased significantly up to 50 mg dm^{-3} N in the inoculated plants, and up to 152 mg dm^{-3} N in the non-inoculated plants (Figure 5a). At a rate of 50 mg dm^{-3} N, the inoculated cassava plants accumulated 19% more N than the non-inoculated plants. In disinfected soil, the amount of N accumulated by cassava plants increased linearly with N fertilization in the non-inoculated plants, and up to an estimated rate of 163 mg dm^{-3} N in the inoculated plants (Figure 5b). In the disinfected soil, *A. brasilense* inoculation increased the amount of N accumulated by plants by 27–40% in all treatments with mineral N supply.



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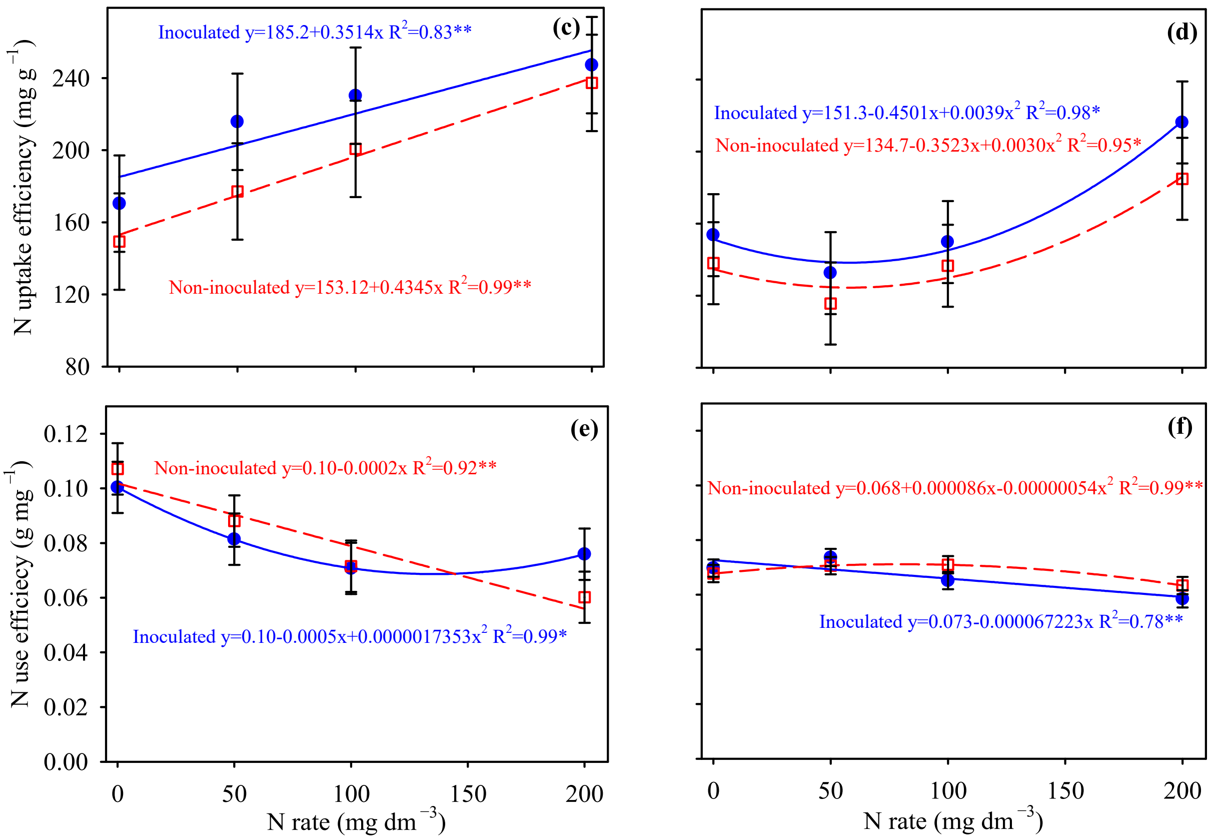

Figure 5. Amount of N accumulated in the whole plant (**a**,**b**), N-uptake efficiency (**c**,**d**), and N-use efficiency (**e**,**f**) of cassava in response to N application rates and *A. brasilense* inoculation in natural and disinfected soil. Bars represent the least significant difference between *A. brasilense* inoculation in the same N rate at $p < 0.05$ according to the LSD test. * $p < 0.05$; ** $p < 0.01$.

Azospirillum brasilense inoculation did not significantly affect the efficiencies of uptake and use of N by cassava in either soil (Figure 5c–f). Although there was no significant difference, the N-uptake efficiency in inoculated cassava plants was 14–22% higher than in non-inoculated plants when less than 100 mg dm^{-3} was applied. In natural soil, N fertilization linearly increased the N-uptake efficiency, and the increase in N supply reduced the N-use efficiency (Figure 5c,e). In disinfected soil, there was an increase in the N-uptake efficiency only when more than 100 mg dm^{-3} N was supplied, and the N-use efficiency had small changes due to the increase in the N supply (Figure 5d,f).

4. Discussion

Potatoes take up large amounts of N from the soil [2,32] and the application of mineral N increases tuber yield [4,5]. However, the process of disinfecting the soil kills most microorganisms and increases the availability of inorganic N in the soil [33]. As a result, it was shown in this study that, in the disinfected soil, both the *A. brasilense* inoculation and N supply did not alter the N concentration in the potato leaves, and even without any supply of N, leaf N concentrations were not deficient (i.e., lower than 40 g kg^{-1} N) [3]. In the disinfected soil, *A. brasilense* inoculation did not benefit plant growth or the amount of N accumulated by the potato plant. In addition, in the disinfected soil, the supply of mineral N unnecessarily increased the uptake of N because it did not benefit plant growth.

In natural soil, *A. brasilense* inoculation improved the leaf N concentration of the potato plants by 23–38% when the N supply was low (<50 mg dm^{-3} N). The potato plants not supplied with N and inoculated with *A. brasilense* showed leaf N concentration within the sufficiency range (40–50 g kg^{-1}) [5], while the non-inoculated potato plants were N deficient when no mineral N was applied. The inoculation of *A. brasilense* strains "Ab-V5" and "Ab-V6" has been found to improve the N nutritional status of wheat crops [9]; however, some studies have indicated that the main benefits of *A. brasilense* inoculation to the crops are most likely related with the promotion of plant growth rather than biological N fixing [15]. Nonetheless, in this study, in natural soil, *A. brasilense* inoculation did not have a consistent effect on the growth or amount of N accumulated in potato plants grown under low N supply. Moreover, *A. brasilense* inoculation did not increase the root system development of potato plants, as has occurred in other crops [15,18,19]. Thus, the greater amounts of N accumulated in the potato plants inoculated with *A. brasilense* and supplied with 50 mg dm^{-3} N cannot be attributed to a greater capacity of the root system to explore the soil volume and uptake of inorganic N from the soil; this is similar to results observed in one study with wheat [33]. However, under field conditions, the benefit of *A. brasilense* inoculation for root development and resource acquisition by potatoes may be greater, since in the field, there is no volume limitation for root growth, as occurs under pot conditions. In the natural soil, there was a limited fixation of atmospheric N_2 by the bacteria when only 50 mg dm^{-3} mineral N was supplied, but *A. brasilense* also increased the N partition to potato leaves, since the N status in the leaves increased by up to 38% when mineral N supply was absent or low. Another study has shown that *A. brasilense* does not make a high contribution to biological N_2 fixation (7.4–19.4% of the plant demand), but it does induce several other beneficial mechanisms for plant growth [15]. Potato seedlings grown in vitro and in aeroponic systems have been shown to benefit from inoculation with *A. brasilense* strain "Sp245" [20,23]. However, in the present study, the strains "Ab-V5" and "Ab-V6" of *A. brasilense* did not promote the growth of potato plants. This shows that although *Azospirilla* is not cereal-specific at the genus and species levels [12], the strains "Ab-V5" and "Ab-V6" have no high affinity for the potato cultivar used in this study in a pot condition. In contrast, under natural soil conditions, mineral N supply increased the growth and amount of N accumulated by potato plants, confirming that the potato crop has a high N requirement [2,32].

Cassava can take up large amounts of N from the soil [3,34], but unlike potatoes, cassava does not often respond to N fertilization in tropical soils [9]. An excess of N in cassava can reduce tuber yield due to excessive vegetative top growth [8,35]. However, in this study, this occurred only when the plants were not inoculated with *A. brasilense* in the natural soil, i.e., *A. brasilense* inoculation minimized the negative effect of N excess on tuber development. Under stress conditions, *A. brasilense* can benefit plant development by inducing changes in cell wall elasticity, osmotic adjustment, and the release of beneficial substances for plants [10,36,37]. In field conditions, when half of the recommended NPK fertilizer rate was applied, *Azospirillum* spp. and *Trichoderma* inoculation increased the cassava tuber yield [27]. However, in the present study, inoculation of *A. brasilense* strains "Ab-V5" and "Ab-V6" in natural soil conditions did not benefit the development of cassava tubers supplied with no or low mineral N.

Both *A. brasilense* [24] and *A. lipoferum* [23] have been associated with cassava plants. In the current study, the inoculation of *A. brasilense* strains "Ab-V5" and "Ab-V6" increased the N concentration in cassava leaves by 25–33% under low N supply and increased shoot growth by 11–32%, regardless of whether the soil was disinfected or not. *Azospirillum brasilense* fixes atmospheric N_2 [11], improves the N nutrition of cereals such as wheat [9], and promotes plant growth due to the production of hormones, mainly auxin, cytokinin, and gibberellin [10,13–15], as well as increases the root growth of cereal and non-cereal crops [18,19]. *Azospirillum brasilense* inoculation also increases the uptake of inorganic N from the soil by the roots [33] and *A. brasilense* can solubilize P from the soil [38] and favor its uptake by plants. However, in this study, *A. brasilense* inoculation had little or no effect on the cassava fibrous root biomass in both soils and the N-uptake efficiency was not statically changed by the bacteria, although N-use efficiency was increased by 14–22%. Thus, the benefits of *A. brasilense* inoculation on shoot growth and N status of cassava in both soils and the amount of N accumulated in the cassava plants from disinfected soil may be related to alterations in the root system morphology. Recent studies have shown that inoculation of *A. brasilense* can alter the carbon partition to the roots, and increase the root branches, specific root length, and root-hair length, which are morphological traits that improve soil resource acquisition [14,15]. As these morphological traits of the cassava root system were not evaluated in this study, it may be that the evaluation of root biomass alone was not able to detect these variations in the root morphology that benefitted the cassava plant growth and N uptake. Therefore, under field conditions with a greater volume of soil to be explored by the fibrous roots, the cassava response to *A. brasilense* inoculation may be even greater than that observed in this study.

The cassava crop is considered to have one of the highest dependencies on soil microorganism association. Under natural conditions, cassava is associated with diazotrophic bacteria [23,24] and native-mycorrhizae from soil [34]; thus, inoculation of *A. lipoferum* under controlled conditions tends to increase mycorrhizal colonization in cassava roots [25,26]. As soil disinfection kills most microorganisms [33], the disinfected soil in this study contained no mycorrhizae; therefore, the cassava plant biomass was, on average, 2.5-fold less than in the natural soil. This confirms that cassava development is highly dependent on the mycorrhizal association [35]. Only when the soil was disinfected, the mineral N supply increased the biomass of cassava plants, which did not occur in the natural soil. Thus, in natural soil (non-disinfected) cassava is naturally associated with diazotrophic bacteria and soil mycorrhizae [23,34], which favors plant growth, and therefore, the cassava plant tends to respond negatively to a high mineral N supply, as was observed in the current study. Thus, in natural soil conditions, inoculation with strains “Ab-V5” and “Ab-V6” of *A. brasilense* in cassava can favor cassava shoot growth and minimize the negative effects of N stress on tuber development, possibly by inducing stress resistance tolerance mechanisms, as has been observed in previous studies [14,39].

5. Conclusions

In this study, *A. brasilense* inoculation was found to increase the leaf N concentration of potato plants by 23–38% natural soil when low or no mineral N was applied, but did not benefit the plant growth or N uptake by potato. Further, the N supply increased the growth of the tuber, whole plant, and N uptake by the potato, regardless of *A. brasilense* inoculation. In both natural and disinfected soils, *A. brasilense* inoculation improved the cassava leaf N status by 25–33% when low mineral N was supplied. Under natural soil conditions, excess mineral N increased cassava shoot growth to the detriment of tuber development when *A. brasilense* was not inoculated. *Azospirillum brasilense* inoculation under natural soil conditions minimized the negative effect of a high N rate on cassava tuber development and increased cassava shoot biomass by 11–13%. In disinfected soil, N supply increased the growth of shoots and tubers of cassava, and *A. brasilense* inoculation together with N supply increased plant biomass by 21–32% and N uptake by 27–40% without significantly affecting the efficiency of N uptake or use. Therefore, this study shows that potato is a more responsive crop to N fertilizer than cassava. However, *A. brasilense* inoculation showed a more interesting alternative to improve the N status and plant growth of cassava than potato. Thus, studies aimed at improving *A. brasilense* inoculation techniques in cassava crops should be encouraged.

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