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Inoculation with Plant Growth-Promoting Bacteria Improves the Sustainability of Tropical Pastures with *Megathyrsus maximus*

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Abstract: Brazil is the second-largest producer and the first exporter of beef, with herds mainly raised in extensive pastures, where *Megathyrsus maximus* occupies over 30 Mha. About 70% of the pastures are under degradation, and using plant growth-promoting bacteria (PGPB) may contribute to reversing this scenario. We investigated the effects of PGPB on the growth of six cultivars of *M. maximus*—Tanzania-1, Massai, BRS Zuri, Mombaça, BRS Tamani, and BRS Quênia—under greenhouse conditions. Plants were inoculated, or not, with the elite strains of *Azospirillum brasilense* CNPSo 2083 + CNPSo 2084, *Bacillus subtilis* CNPSo 2657, *Pseudomonas fluorescens* CNPSo 2719, or *Rhizobium tropici* CNPSo 103. At 35 days after emergence, plants were evaluated for ten root growth traits, shoot dry weight, and the levels of macro and micronutrients accumulated in shoots. Several root traits were increased due to inoculation in all genotypes, impacting plant growth and nutrient uptake. Despite the differences in effectiveness, all genotypes benefited from PGPB to some degree, but Mombaça and BRS Zuri were more responsive. Scanning electron microscopy indicated that bacterial species differed in their capacity to colonize seeds and rootlets. The results show that inoculation with elite PGPB strains may represent an important strategy for the sustainability of *M. maximus* pastures.

Keywords: *Azospirillum brasilense*; *Bacillus subtilis*; *Pseudomonas fluorescens*; *Rhizobium tropici*; inoculation

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1. Introduction

Livestock farming is one of the most important economic activities in Brazil, which currently owns the world's second-largest herd, totaling an estimated 218.15 million bovine heads, as well as being the first-ranked beef-exporting country. With a revenue of USD 70.72 billion in 2022, livestock is highly relevant to the Brazilian economy [1]. According to the Association for the Promotion of Research on the Improvement of Tropical Forages [2], the Brazilian livestock sector occupies 173 million hectares (Mha), equivalent to about 2.8 times the area occupied by grain crops, with 126 Mha consisting of cultivated pastures, and the remaining area represented by native pastures.

Based on the area used for forage seed production, 25.7% of the planted pastures in Brazil are occupied by the species *Megathyrsus maximus* (Jacq.) B.K. Simon and S.W.L. Jacobs (syn. *Panicum maximum* Jacq.), while 72.2% are occupied by by *Urochloa* spp. (syn. *Brachiaria* spp.) (Associação para Fomento à Pesquisa de Melhoramento de Forrageiras-UNIPASTO, unpublished data). *Megathyrsus maximus* has been increasingly used in Brazil, showing good adaptation to all edaphoclimatic conditions, as well as a high biomass production with good nutritional value and palatability [3].

Due to lower production costs, 93% of the cattle herd in Brazil has been raised in extensive pastures. In this system, animals are raised in pastures as a primary source of food, with lower inputs and labor costs, but across larger areas [4]. However, extensive growth is majorly associated with poor fertilizers and soil conservation investments, leading to pasture degradation and a decreased capacity of cattle support, altogether increasing the pressure to move to new areas of native vegetation [5]. As a result, about 70% of the pasture areas in Brazil are at some stage of degradation, mainly in the Central region, which is responsible for more than 55% of beef production [6]. The decrease in soil fertility due to inadequate management is among the main factors responsible for pasture degradation, leading to the progressive reduction in vigor and low productive and recovery capacities [7,8].

Nitrogen (N) is the most limiting nutrient required for pasture growth, followed by phosphorus (P). The low availability of N and P in tropical soils impairs forage production and quality, decreasing animal weight gain and reproductive performance. Besides its important role as a component of amino acids and proteins, N participates in photosynthesis, affecting light capture and favoring a greater production of proteins [9]. Phosphorus plays an important role in main metabolic processes such as photosynthesis, energy transference, signal transduction, macromolecule biosynthesis, and respiration [10].

Challenges to provide the needs of an ever-growing population, which is, globally, estimated to total 9.7 billion people by 2050, and concerns about the environmental impacts caused by livestock require new strategies and technologies in order to mitigate the impacts resulting from animal protein production [11]. In this context, several genera of plant growth-promoting bacteria (PGPB), mainly *Azospirillum*, *Pseudomonas*, *Bacillus,* and *Rhizobium*, have been studied and used as inoculants on grasses [10–18]. The main reported bacterial mechanisms of growth promotion in plants include the synthesis of phytohormones, such as indole-3-acetic acid (IAA), cytokinins, gibberellins, and ethylene, biological nitrogen fixation, the synthesis of enzymes such as ACC (1-aminocyclopropane-1-carboxylic acid) deaminase, nutrient mineralization, phosphate solubilization, and an increased tolerance to abiotic and biotic stresses and other benefits associated with a variety of other molecules [12,13,17–22]. However, the adoption of inoculants carrying PGPB requires increased knowledge of the interaction between the microorganisms and the host plant, as well as the development of good inoculation practices, including the adjustment of doses and methods of application [23,24]; currently, there are very few studies focusing on grass pastures, with modest information on *M. maximus*.

We aimed to evaluate the effects of the sole inoculation of four different species of PGPB, in six cultivars of *M. maximus,* on root parameters, biomass production, and total nutrient contents, in the shoots of plants grown under controlled conditions. Following this, the seed and root bacterial colonization in contrasting pairs of host x bacterium was evaluated with scanning electron microscopy, aiming to investigate the possible relationships between colonization and plant-growth-promotion performance.

2. Material and Methods

2.1. Biological Material

The bacterial strains used in the study are deposited at the "Diazotrophic and Plant-Growth-Promoting Bacteria Culture Collection of Embrapa Soja" (Collections WFCC #1213, WDCM #1054). *Azospirillum brasilense* strains CNPSo 2083 (=Ab-V5) and CNPSo 2084 (=Ab-V6) have been used in commercial inoculants in Brazil for maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), *Brachiaria* (*Urochloa* spp.), and in co-inoculation with the rhizobia of the common-bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* (L.) Merr.) [17]. Each strain was grown separately in dextrose yeast glucose sucrose (DYGS) medium [25] at 28 °C for 96 h, and then mixed, which resulted in a final concentration of 5×10^8 colony-forming units (CFU) mL^{-1}. Strains of *A. brasilense* were mixed, because in Brazil, the commercial inoculants carry both strains [17]. *Bacillus subtilis* strain CNPSo 2657 (=PRBS-1, =A3-5), previously selected as a growth promoter for soybean [26], was grown

in trypticase soy broth (TSB) medium at 28 °C for 48 h. *Rhizobium tropici* strain CNPSo 103 (=CIAT 899, =SEMIA 4077), a main symbiont of the common bean and other legumes [27], was grown in tryptone yeast extract (TY) medium at 28 °C for 48 h. *Pseudomonas fluorescens* strain CNPSo 2719 (=CCTB 03), first recommended for maize [28], and then for *Urochloa* spp. [29], was grown in King's B broth medium at 28 °C for 48 h. As for *A. brasilense*, the final concentrations of *B. subtilis*, *R. tropici* and *P. fluorescens* were adjusted to 5×10^8 CFU mL^{-1}.

The seeds of *M. maximus* cultivars BRS Tamani, Mombaça, Tanzânia-1, BRS Quênia, Massai, and BRS Zuri, broadly used as foraging plants in Brazilian pastures, were provided by Embrapa Gado de Corte, Campo Grande, Mato Grosso do Sul State, Brazil. From here, BRS Tamani, BRS Quênia and BRS Zuri will be nominated only as Tamani, Quênia and Zuri.

2.2. Plant-Growth-Promotion Evaluation under Greenhouse Conditions

2.2.1. Treatments and Growth Conditions

One experiment was carried out in the greenhouse at Embrapa Soja, in Londrina, Paraná State, southern Brazil (23°11′30.7″ S, 51°11′00.8″ W), in modified Leonard jars [30] of 500 cm^3 capacity, containing a sterile substrate composed of a mix of sand and milled coal (3:1, *v*/*v*).

The treatments consisted of (i) a non-inoculated group (control); (ii) inoculation with *A. brasilense* CNPSo 2083 + CNPSo 2084; (iii) inoculation with *B. subtilis* CNPSo 2657; (iv) inoculation with *P. fluorescens* CNPSo 2719; (v) inoculation with *R. tropici* CNPSo 103. The experimental design was completely randomized, with six replicates.

Seeds were surface-sterilized as described before [29], and six seeds were sown per jar, receiving 1 mL of each inoculant adjusted to 5×10^5 CFU $seed^{-1}$ at the sowing hole, according to the respective treatment. A week after the emergence, seedlings were thinned to one plantlet per jar. Plants received sterile nutrient solution of Hoagland and Arnon [31] with the N supply corresponding to 60 kg ha^{-1} of N.

2.2.2. Evaluation of Root Growth Traits

Thirty-five days after emergence (DAE), the plants were removed from the jars and roots and shoots were separated. The root system was washed with tap water, weighed, and the root volume (RV) was estimated by the displacement of water in a graduated cylinder. Total root length (TRL) was determined by the modified line-intersection method [32]. Basically, about 1 g of fresh roots was randomly arranged on plates with 1 × 1 cm grid squares, and the intersections with the vertical and horizontal grid lines were counted; TRL was calculated with the formula TRL = N × 0.7857, where N is the number of intersections and 0.7857 is the conversion factor [32].

Root mean diameter (RMD) was calculated by the formula $2[(RV/TRL)\pi]^{0.5}$ [33]. Root area (RA) was estimated by the formula $\pi \times RMD \times TRL$. Subsamples of approximately 0.15 g of thin roots of each plant were stored in formaldehyde–acetic acid–ethanol-70% solution (FAA) (5%:5%:90%), for the determination of root-hair length (RHL), root-hair incidence (RHI), and the total number of root branches (TNB). RHL was determined by the average of 100 root hairs in at least 20 thin root fragments per sample, using a microscope at ×100 magnification equipped with an ocular micrometer. RHI was determined by the presence or absence of root hairs on at least 100 fine-root intersections using the gridline method [34]. TNB was determined, in a stereomicroscope at ×30 magnification, by counting 120–150 ramifications in a root fraction, and using the formula [(RW × NB)/FW], where RW is the root system fresh weight, NB the number of branches in the root fraction, and FW the fresh weight of the root fraction [33].

After these measurements, roots were oven-dried at 60 °C for 72 h until a constant weight, in order to obtain the root dry weight (RDW). The specific root length (SRL) was calculated by the ratio between the total length and RDW. Root-tissue density (RTD) was determined by the ratio between RDW and RV [33].

2.2.3. Biomass Production and Accumulation of Nutrients in Shoots

After being collected, shoots of each replicate were put in paper bags and oven-dried at 60 °C for 72 h until reaching a constant weight. After weighing, tissues were ground in a Wiley mill and submitted to digestion (sulfuric acid for N; nitro-perchloric acid for P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn; incineration for B), in order to determine the concentrations of macro- (N, P, K, Ca, Mg, and S) and micronutrients (B, Cu, Fe, Mn, and Zn) in the leaves, as described before [35].

2.2.4. Statistical Analysis

The data were first submitted to the analyses of normality, by the Shapiro–Wilk's test, and homoscedasticity, by Levene's test. When necessary, transformation to the square root, or the use of formulae provided by the software AgroEstat, were applied [36]. Root-hair incidence data were transformed to arcsine $(x/100)^{0.5}$ before analysis. Means were submitted to ANOVA and the Duncan's test at 5% significance. All analyses were performed with STATISTICA v.12.0 (Statsoft Inc., Tulsa, OK, USA).

2.3. Evaluation of Seed and Root Colonization with Scanning Electron Microscopy

2.3.1. Treatments, Plant Growth Conditions

A second experiment was performed, also under controlled conditions, with two contrasting cultivars identified in the first experiment, Zuri and Massai, which had the highest and the lowest responses to inoculation, respectively.

The same five inoculation and control treatments described in Section 2.2.1. were applied to both cultivars. Seeds of each cultivar were treated with the respective bacterial inoculant, in order to provide 5×10^5 CFU seed^{-1} per seed. Seeds were placed on a Petri dish containing germination paper and moistened with sterilized distilled water, and were incubated in a germination chamber at 25 °C and 70% relative humidity for 7 days.

2.3.2. Evaluation of Seed and Root Colonization

After seven days of growth, four germinated seeds from each treatment were prepared for scanning electron microscopic (SEM) analysis. Samples were fixed with glutaraldehyde 2.5% and sodium cacodylate buffer at 0.1 M for 24 h, and then were dehydrated with increasing concentrations of ethanol for 15 min at each concentration (30, 50, 70, 90, and 100%). The samples were then dried to a critical point with CO_2 (BALTEC CPD 030 Critical Point Dryer), and were then attached to a metal stub and covered with gold (BALTEC SDC 050 Sputter Coater) for SEM visualization (FEI Quanta 200, FEI Company, Hillsboro, Oregon, EUA).

3. Results

3.1. Plant Growth Promotion

3.1.1. Root Traits

The results for ten root growth traits, including all combinations of plant genotypes and bacterial inoculation treatments, are shown in Table 1, and differences were observed among plant genotypes and bacterial species.

Table 1. Root growth traits of six *Megathyrsus maximus* cultivars (Tamani, Mombaça, Tanzânia-1, Quênia, Massai, and Zuri) either inoculated with *Azospirillum brasilense* (CNPSo 2083 + CNPSo 2084), *Bacillus subtillis* (CNPSo 2657), *Pseudomonas fluorescens* (CNPSo 2719), or *Rhizobium tropici* (CNPSo 103), or remaining non-inoculated (control). The experiment was carried out under greenhouse conditions in a sterile substrate, and plants were sampled 35 days after emergence.

Treatments	Dry Weight (RDW) (g)	Volume (RV) (cm^3)	Total Length (TRL) (m)	Specific Length (SRL) (m g^{-1})	Area (RA) (cm^2)	Mean Diameter (RMD) (μm)	Tissue Density (RTD) (g cm^{-3})	Hair Incidence (RHI) (%)	Hair Length (RHL) (μm)	Total Number of Root Branches (TNB)
					Tamani					
Non-inoculated	0.97 ± 0.12b [1]	7.75 ± 0.84b	94 ± 16.6bc	98 ± 14.7 [n.s.]	94 ±126bc	333 ± 23.3 [n.s.]	0.13 ± 0.01 [n.s.]	54 ± 6.89 [n.s.]	204 ± 17.4 [n.s.]	45,762 ± 9587c
A. brasilense	1.17 ± 0.07a	9.46 ± 0.30a	115 ± 7.05ab	99 ± 7.12	1167 ± 45.2a	325 ± 9.67	0.12 ± 0.01	40 ± 5.82	199 ± 22.3	53,689 ± 3874bc
B. subtilis	0.93 ± 0.05b	8.10 ± 0.23ab	122 ± 6.92a	133 ± 7.66	1117 ± 40.8ab	290 ± 7.81	0.11 ± 0	31 ± 6.15	189 ± 7.24	12,608 ± 16,874a
P. fluorescens	0.79 ± 0.03bc	7.56 ± 0.39b	85 ± 6.33bc	109 ± 6.06	902 ± 55.6c	336 ± 5.69	0.10 ± 0	37 ± 7.16	182 ± 10.9	62,497 ± 6155bc
R. tropici	0.68 ± 0.02c	6.18 ± 0.23c	72 ± 6.23c	106 ± 9.1	748 ± 34.8c	332 ± 17.3	0.11 ± 0	31 ± 6.78	186 ± 16.5	87,428 ± 19,830ab
p value	<0.001	<0.001	0.007	0.117	0.002	0.167	0.122	0.136	0.857	0.002
					Mombaça					
Non-inoculated	0.77 ± 0.08b	6.58 ± 0.64	82 ± 7.65 [n.s.]	109 ± 8.11 [n.s.]	881 ± 49.9 [n.s.]	315 ± 9.26 [n.s.]	0.12 ± 0.008b	34 ± 6.28 [n.s.]	205 ± 3.20a	45,135 ± 5986b
A. brasilense	0.94 ± 0.08ab	6.48 ± 0.6	101 ± 9.23	101 ± 1.62	1006 ± 42.5	294 ± 5.09	0.15 ± 0.006a	51 ± 6.84	188 ± 11.3ab	74,025 ± 3922a
B. subtilis	1.02 ± 0.13ab	7.63 ± 0.8	114 ± 19.2	99 ± 8.45	928 ± 76.6	317 ± 12.3	0.13 ± 0.002ab	32 ± 3.08	166 ± 5.77b	85,098 ± 7690a
P. fluorescens	1.15 ± 0.06a	8.63 ± 0.69	111 ± 7.41	106 ± 6.50	1093 ± 53.5	315 ± 17.7	0.14 ± 0.008ab	48 ± 3.79	199 ± 7.07a	76,837 ± 9638a
R. tropici	1.11 ± 0.09a	7.98 ± 0.65	108 ± 13.5	103 ± 7.08	1035 ± 93.3	311 ± 15.5	0.14 ± 0.005ab	32 ± 4.97	182 ± 10.6ab	69,579 ± 6913a
p value	0.001	0.152	0.36	0.42	0.192	0.705	0.04	0.077	0.025	0.004
					Tanzânia-1					
Non-inoculated	0.76 ± 0.08c	6.99 ± 0.66b	84 ± 5.19c	127 ± 16,9 [n.s.]	856 ± 3.5c	324 ± 15.9 [n.s.]	0.12 ± 0a [n.s.]	33 ± 8.46b	196 ± 15.9ab	39,241 ± 13,906b
A. brasilense	1.07 ± 0.11ab	10.61 ± 1.11a	135 ± 17.5ab	117 ± 8.22	1342 ± 152ab	318 ± 1.6	0.11 ± 0.01ab	55 ± 7.70a	221 ± 14.2a	82,433 ± 23,123a
B. subtilis	1.00 ± 0.07bc	9.92 ± 0.53a	139 ± 10.5ab	131 ± 7.12	1318 ± 80.3ab	302 ± 7.03	0.10 ± 0.01b	36 ± 6.41b	161 ± 5.48b	117,811 ± 5857a
P. fluorescens	1.43 ± 0.16a	11.46 ± 1.12a	159 ± 11.1a	103 ± 6.28	150 ± 119a	302 ± 11.2	0.12 ± 0a	37 ± 5.20b	181 ± 16.9b	10,348 ± 3523a
R. tropici	1.06 ± 0.11bc	9.01 ± 0.73ab	108 ± 8.9bc	109 ± 5.78	1105 ± 89.6bc	326 ± 4.20	0.12 ± 0a	27 ± 6.52b	178 ± 1.95b	105,178 ± 8801a
p value	0.011	0.012	<0.001	0.07	<0.001	0.32	0.001	0.012	0.032	0.002
					Quênia					
Non-inoculated	0.75 ± 0.03b	5.95 ± 0.17b	86 ± 2.04bc	116 ± 6.36ab	781 ± 32.7b	309 ± 7.81 [n.s.]	0.13 ± 0.005 [n.s.]	38 ± 3.11 [n.s.]	175 ± 7.57 [n.s.]	38,981 ± 1091b
A. brasilense	1.11 ± 0.10a	8.42 ± 0.63a	99 ± 4.08a	97 ± 6.54b	1084 ± 79.3a	296 ± 4.14	0.13 ± 0.008	47 ± 8.18	192 ± 17.7	52,194 ± 7614b
B. subtilis	0.78 ± 0.05b	6.58 ± 0.31b	81 ± 5.43c	104 ± 4.18b	819 ± 43.4b	317 ± 8.37	0.12 ± 0.004	40 ± 3.84	174 ± 11.7	75,650 ± 8898a
P. fluorescens	0.81 ± 0.09b	6.16 ± 0.54b	90 ± 2.37abc	127 ± 8.42a	850 ± 63.6b	299 ± 9.16	0.12 ± 0.012	44 ± 6.31	186 ± 3.64	41,344 ± 6967b
R. tropici	0.95 ± 0.06ab	6.60 ± 0.06b	96 ± 1.86ab	102 ± 3.4b	877 ± 35.7b	302 ± 3.31	0.13 ± 0.004	48 ± 7.45	184 ± 11.3	47,528 ± 1528b
p value	0.012	0.003	0.006	0.003	0.006	0.228	0.459	0.72	0.77	0.003

Table 1. *Cont.*

Treatments	Dry Weight (RDW) (g)	Volume (RV) (cm^3)	Total Length (TRL) (m)	Specific Length (SRL) (m g^{-1})	Area (RA) (cm^2)	Mean Diameter (RMD) (µm)	Tissue Density (RTD) (g cm^{-3})	Hair Incidence (RHI) (%)	Hair Length (RHL) (µm)	Total Number of Root Branches (TNB)
					Massai					
Non-inoculated	0.86 ± 0.06 $^{n.s.}$	6.56 ± 0.06bc	92 ± 4.35b	115 ± 8.84ab	889 ± 61.1bc	291 ± 14.3bc	0.12 ± 0.003 $^{n.s.}$	26 ± 1.31c	204 ± 13.9 $^{n.s.}$	65,717 ± 7486 $^{n.s.}$
A. brasilense	0.80 ± 0.08	5.91 ± 0.48c	80 ± 2.13c	89 ± 3.56c	734 ± 25.5d	330 ± 8.71a	0.13 ± 0.003	54 ± 5.26a	218 ± 16.6	55,375 ± 4692
B. subtilis	0.71 ± 0.06	5.54 ± 0.38c	90 ± 9.15b	137 ± 12.7a	815 ± 58.6cd	273 ± 13.7c	0.13 ± 0.002	28 ± 3.97c	207 ± 13.5	60,767 ± 4086
P. fluorescens	0.99 ± 0.08	8.25 ± 0.36a	126 ± 7.36a	122 ± 6.05ab	1112 ± 56.4a	297 ± 4.70abc	0.12 ± 0.006	42 ± 0.76ab	203 ± 20.6	65,631 ± 1807
R. tropici	0.93 ± 0.06	7.80 ± 0.56ab	97 ± 2.19b	106 ± 5.62bc	972 ± 41.9ab	319 ± 10.5ab	0.14 ± 0.002	31 ± 4.20bc	204 ± 6.86	64,013 ± 4834
p value	0.069	0.004	<0.001	0.006	<0.001	0.010	0.11	<0.001	0.956	0.534
					Zuri					
Non-inoculated	0.98 ± 0.24 $^{n.s.}$	6.16 ± 0.84b	76 ± 6.75b	83 ± 14.1 $^{n.s.}$	786 ± 41.1b	305 ± 5.99 $^{n.s.}$	0.112 ± 0b	55 ± 3.29b	201 ± 3.29 $^{n.s.}$	47,365 ± 6011d
A. brasilense	1.29 ± 0.11	10.94 ± 1.29a	130 ± 10a	102 ± 7.98	1330 ± 116.9a	326 ± 16.6	0.120 ± 0ab	73 ± 1.59a	226 ± 6.62	68,403 ± 7693bc
B. subtilis	1.40 ± 0.17	9.06 ± 0.63a	123 ± 10.5a	92 ± 10.4	1181 ± 80.4a	307 ± 10.3	0.128 ± 0a	47 ± 5.76b	195 ± 4.43	10,3847 ± 8402a
P. fluorescens	1.20 ± 0.07	10.14 ± 0.2a	120 ± 9a	100 ± 7.74	1235 ± 56.7a	330 ± 11.4	0.125 ± 0a	55 ± 3.32b	195 ± 12.3	62,285 ± 1919cd
R. tropici	1.13 ± 0.10	8.88 ± 0.63a	122 ± 14.8a	102 ± 2.51	1232 ± 77a	300 ± 5.6	0.131 ± 0a	53 ± 9.05b	197 ± 11.2	88,855 ± 10,595ab
p value	0.267	0.002	0.005	0.416	<0.001	0.235	0.006	0.041	0.069	<0.001
CV (%)	16.58	14.75	14.06	12.32	11.63	4.34	4.29	9.05	5.65	17.97

[1] Means (±SEM—standard error) (n = 6) followed by different letters differ from each other by the Duncan's test at $p \leq 0.05$; n.s., non-significant. When letters are not shown in a trait, there was lack of statistical difference.

For the cultivar Tamani, *A. brasilense* increased the root dry weight (RDW), root volume (RV), and root area (RA); *B. subtilis* increased the total root length (TRL) and the total number of branches (TNB), whereas *R. tropici* increased TNB (Table 1). For Mombaça, *A. brasilense* increased the root tissue density (RTD), *P. fluorescens* and *R. tropici* increased RDW, and all strains increased TNB. Inoculation of *A. brasilense* in Tanzania-1 increased RDW, RV, TRL, RA, and TNB, in addition to root hair incidence (RHI) by 67%, when compared with the non-inoculated control. Still, in Tanzania-1, *B. subtilis* increased RV, TRL, RA, SRL and TNB values, and *P. fluorescens* showed similar effects to *A. brasilense,* in addition to an increase in RDW and a lack of significant response in RHI, while *R. tropici* only increased TNB. For cultivar Quênia, inoculation with *A. brasilense* increased RDW, RV, TRL, and RA values; *B. subtilis* increased TNB, but no effects were observed for inoculation with *R. tropici* or *P. fluorescens*. In Massai, *A. brasilense* increased the RHI and root mean diameter (RMD). The best results in Massai were achieved with *P. fluorescens*, which enhanced RV, TRL, RA, and RHI values; there were no effects observed when inoculating *B. subtilis* or *R. tropici.* Finally, all strains showed increased RV, RTL, and RA values in Zuri; in addition, inoculation with *B. subtilis*, *P. fluorescens* or *R. tropici* increased RTD, inoculation with *A. brasilense* increased RHI, whereas inoculation with *B. subtilis*, *A. brasilense*, and *R. tropici* increased TNB (Table 1).

Despite differences among plant genotypes and bacterial strains, all inoculation treatments enhanced root growth traits in all cultivars of *M. maximus* (Table 1). However, some cultivars, such as Mombaça, Tanzânia-1, and Zuri, were more responsive to inoculation, irrespective of the bacterial species. In contrast, others, such as Quênia and Massai, were more specific, each one responding to only two bacterial species. Differences were also observed among bacteria, with *A. brasilense* affecting more root morphological traits across all genotypes.

3.1.2. Shoot Biomass Production and Nutrient Accumulation

At 35 DAE, there were no improvements in shoot dry weight (SDW) due to inoculation with any of the bacterial strains in the cultivar Tamani (Figure 1A). Conversely, all inoculated strains increased the SDW of Mombaça when compared with the non-inoculated control (Figure 1B), while Tanzânia-1 responded only to inoculation with *P. fluorescens* (Figure 1C). No statistical differences were observed for Quênia (Figure 1D) and Massai (Figure 1E) with any of the bacterial strains. Zuri presented higher shoot dry biomass (SDW) when inoculated with *A. brasilense*, *B. subtilis*, or *P. fluorescens* (Figure 1F).

Considering the bacterial species, an improvement in shoot growth due to inoculation was observed in two genotypes for *A. brasilense*, two for *B. subtilis*, three for *P. fluorescens*, and one for *R. tropici* (Figure 1).

Although no statistical differences were observed in the shoot growth of Tamani, Quênia and Massai (Figure 1), all bacterial strains showed increased Cu contents in the shoots of Tamani, especially *R. tropici* (196%) and *B. subtilis* (262%) (Table 2).

Inoculation affected the total content of 9 out of 11 nutrients in Mombaça, with at least one bacterium increasing the accumulation of P, Mg, S, B, Fe, Mn, and Zn in shoots (Table 2). All bacteria, except *P. fluorescens,* increased N contents, and all except for *A. brasilense* increased Ca. Inoculation with *A. brasilense* or *P. fluorescens* increased P, K, and Fe contents compared with the other treatments, whereas Ca, was also increased by *B. subtilis*, and Cu by *P. fluorescens*.

For Quênia, eight nutrients increased in tissues with at least one of the bacteria evaluated. All strains increased Mg and Zn, the latter by an average of 2.5 times. Boron was increased by 90% with *A. brasilense, P. fluorescens,* or *R. tropici* inoculation, whereas inoculation with *R. tropici* or *B. subtilis* increased N and P contents. Moreover, *A. brasilense* also increased Ca and Mn contents, *P. fluorescens* increased Mn, *R. tropici* increased Ca and S, and *B. subtilis* increased Mn contents (Table 2). For Massai, inoculation with *P. fluorescens* or *R. tropici* increased Mg; *B. subtilis* or *R. tropici* increased Mn, whereas all bacteria increased

Zn accumulation in shoots (Table 2). Finally, in Zuri, significant increases were observed for N, P, K, S, and B.

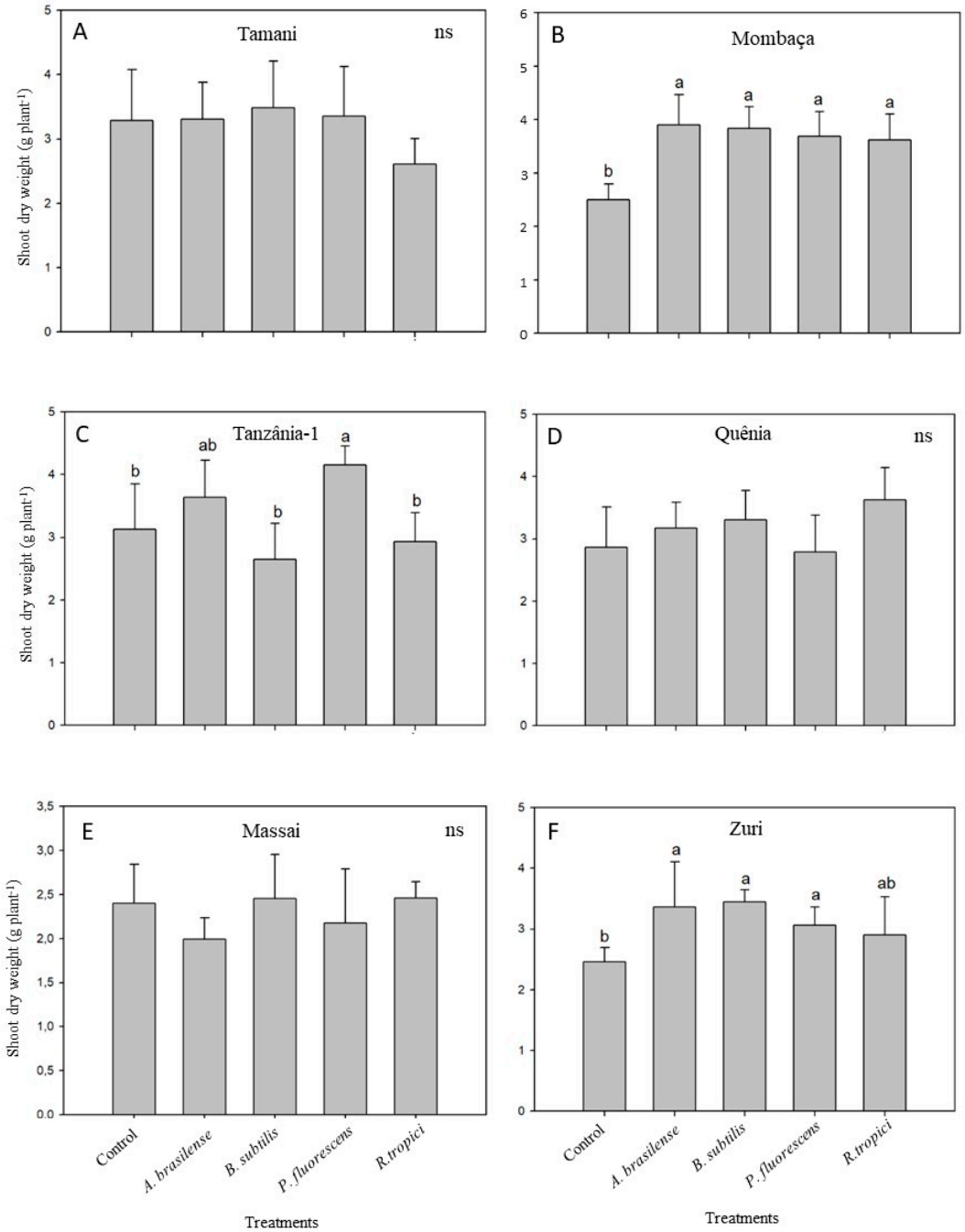

Figure 1. Shoot dry weight of six *Megathyrsus maximus* cultivars [Tamani (**A**), Mombaça (**B**), Tanzânia-1 (**C**), Quênia (**D**), Massai (**E**), and Zuri (**F**)] either inoculated with *Azospirillum brasilense* (CNPSo 2083 + CNPSo 2084), *Bacillus subtillis* (CNPSo 2657), *Pseudomonas fluorescens* (CNPSo 2719), or *Rhizobium tropici* (CNPSo 103), or remaining non-inoculated (Control). The experiment was carried out under greenhouse conditions in a sterile substrate and plants were sampled 35 days after emergence. Means (n = 6) followed by different letters differ from each other by the Duncan's test at $p \leq 0.05$.

Table 2. Total nutrient contents in the shoots of six *Megathyrsus maximus* cultivars (Tamani, Mombaça, Tanzânia-1, Quênia, Massai, and Zuri) either inoculated with *Azospirillum brasilense* (CNPSo 2083 + CNPSo 2084), *Bacillus subtillis* (CNPSo 2657), *Pseudomonas fluorescens* (CNPSo 2719), or *Rhizobium tropici* (CNPSo 103), or remaining non-inoculated (control). The experiment was carried out under greenhouse conditions in a sterile substrate and plants were sampled 35 days after emergence.

Treatments	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(µg)	(µg)	(µg)	(µg)	(µg)
Tamani											
Non-inoculated	24.68 ± 3.46$^{n.s.,1}$	11.59 ± 1.4 $^{n.s.}$	60.26 ± 9.72 $^{n.s.}$	7.01 ± 0.90 $^{n.s.}$	6.46 ± 0.81 $^{n.s.}$	3.25 ± 0.35 $^{n.s.}$	51.5 ± 4.52 $^{n.s.}$	7.65 ± 0.70b	190 ± 26.0 $^{n.s.}$	1142 ± 202 $^{n.s.}$	154.00 ± 4.0 $^{n.s.}$
A. brasilense	24.75 ± 2.59	10.52 ± 1.71	46.37 ± 7.28	5.92 ± 0.49	4.68 ± 0.39	3.12 ± 0.26	50.6 ± 2.58	18.48 ± 2.55a	173 ± 18.7	1030 ± 31.2	146.93 ± 19.5
B. subtilis	21.94 ± 1.77	12.03 ± 1.48	66.30 ± 7.07	6.99 ± 0.82	5.42 ± 0.66	3.37 ± 0.31	65.2 ± 11.8	20.07 ± 2.12a	173 ± 18.0	904 ± 83.2	113.85 ± 21.8
P. fluorescens	25.77 ± 6.19	11.36 ± 2.15	51.02 ± 8.32	6.41 ± 0.74	4.99 ± 0.63	3.65 ± 1.02	64.1 ± 11.9	17.79 ± 2.95a	209 ± 64.6	686 ± 134.9	117.80 ± 9.13
R. tropici	18.87 ± 2	8.26 ± 0.84	45.80 ± 3.88	5.53 ± 0.35	4.50 ± 0.35	2.65 ± 0.20	52.3 ± 4.49	15.02 ± 0.47a	150 ± 46	711 ± 88.6	23.31 ± 13.7
p value	0.67	0.22	0.29	0.56	0.24	0.79	0.91	<0.001	0.87	0.06	0.84
Mombaça											
Non-inoculated	18.88 ± 1.06c	7.82 ± 0.39b	44.54 ± 4.64 $^{n.s.}$	3.93 ± 0.18c	3.45 ± 0.17b	2.82 ± 0.17c	41.2 ± 1.76c	9.81 ± 0.98 $^{n.s.}$	98 ± 3.66b	620 ± 28.5b	10.34 ± 0.60c
A. brasilense	24.28 ± 1.75ab	11.11 ± 0.44a	48.48 ± 2.92	4.70 ± 0.32bc	5.37 ± 0.51a	3.70 ± 0.06ab	94.0 ± 7.52a	9.24 ± 0.65	174 ± 12.1a	941 ± 70.5a	84.78 ± 5.13a
B. subtilis	26.97 ± 1.52a	11.71 ± 0.53a	56.15 ± 1.91	6.68 ± 0.44a	5.92 ± 0.23a	3.52 ± 0.16b	68.4 ± 2.66b	10.77 ± 1.45	220 ± 19.3a	987 ± 36.6a	67.72 ± 2.79b
P. fluorescens	21.46 ± 0.88bc	12.07 ± 0.37a	55.85 ± 6.77	4.99 ± 0.377b	5.65 ± 0.57a	4.02 ± 0.02a	89.4 ± 5.45a	11.20 ± 1.39	189 ± 20.9a	981 ± 50a	77.27 ± 5.48ab
R. tropici	23.72 ± 1.52ab	11.01 ± 0.58a	52.59 ± 3.17	5.17 ± 0.06b	5.36 ± 0.07a	3.44 ± 0.2b	74.1 ± 7.02b	11.64 ± 1.50	180 ± 11.3a	1037 ± 29.1a	89.34 ± 3.68a
p value	0.044	<0.001	0.25	<0.001	<0.001	<0.001	<0.001	0.70	<0.001	<0.001	<0.001
Tanzânia-1											
Non-inoculated	24.82 ± 3.62ab	9.48 ± 0.83b	49.80 ± 3.11b	3.99 ± 0.36b	7.79 ± 1.23 $^{n.s.}$	3.27 ± 0.38ab	100.3 ± 5.52a	6.26 ± 0.59b	117 ± 5.53b	1036 ± 156 $^{n.s.}$	60 ± 15.4 $^{n.s.}$
A. brasilense	23.85 ± 2.75ab	12.90 ± 1.07a	76.12 ± 6.65a	8.67 ± 0.63a	7.72 ± 1.03	3.51 ± 0.23ab	52.4 ± 4.86b	7.28 ± 0.48b	177 ± 10.8a	982 ± 115	120 ± 6.39
B. subtilis	16.31 ± 1.66c	11.65 ± 0.25ab	42. ± 3.97b	5.05 ± 0.79b	6.33 ± 0.69	2.84 ± 0.24b	38.5 ± 1.50c	6.43 ± 0.90b	143 ± 13.4ab	1035 ± 81.3	107 ± 10.9
P. fluorescens	28.70 ± 1.21a	13.90 ± 1.25a	65.93 ± 2.80a	9.32 ± 0.96a	8.87 ± 1.35	3.90 ± 0.13a	41.3 ± 1.89bc	10.37 ± 0.93a	189 ± 26.3a	1211 ± 256	83 ± 26.6
R. tropici	19.58 ± 0.77bc	11.43 ± ab	47.61 ± 1.30b	7.03 ± 0.69a	7.40 ± 0.91	3.06 ± 0.08b	28.9 ± 4.83 d	7.20 ± 0.52 b	158 ± 19.6ab	1126 ± 138	96 ± 12.9
p value	0.004	0.045	<0.001	<0.001	0.61	0.039	<0.001	0.004	0.048	0.94	0.08
Quênia											
Non-inoculated	17.88 ± 2.14b	8.39 ± 0.57b	47.49 ± 7.19 $^{n.s.}$	2.45 ± 0.11c	3.74 ± 0.36c	2.93 ± 0.31b	78.6 ± 10.2c	12.03 ± 1.13 $^{n.s.}$	149 ± 15.1 $^{n.s.}$	811 ± 33.6c	17.03 ± 1.06c
A. brasilense	19.70 ± 0.55ab	9.62 ± 0.25ab	53.99 ± 5.79	3.23 ± 0.19b	5.51 ± 0.32b	3.18 ± 0.08ab	167.3 ± 18.6a	13.28 ± 2.68	230 ± 31.5	1037 ± 20.3a	54.38 ± 2.7a
B. subtilis	24.16 ± 2.22a	11.36 ± 0.66a	47.70 ± 3.81	3.13 ± 0.33bc	5.45 ± 0.30b	3.56 ± 0.27ab	104.3 ± 8.72bc	9.50 ± 1.34	198 ± 19.1	1092 ± 60.5a	36.15 ± 3.96b
P. fluorescens	20.30 ± 1.89ab	9.68 ± 0.89ab	49.93 ± 4.27	2.98 ± 0.13bc	5.06 ± 0.38b	2.87 ± 0.24ab	128.7 ± 11.5ab	8.87 ± 0.41	211 ± 30.4	986 ± 43.4ab	41.90 ± 2.95b
R. tropici	25.10 ± 0.54a	11.02 ± 0.68a	57.37 ± 2.17	3.97 ± 0.28a	6.29 ± 0.09a	3.81 ± 0.21a	154.5 ± 14.7a	13.53 ± 2.06	205 ± 17.4	877 ± 88.7bc	40.54 ± 3.16b
p value	0.02	0.023	0.53	0.002	<0.001	0.044	<0.001	0.25	0.19	0.006	<0.001

Table 2. *Cont.*

Treatments	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)	(µg)	(µg)	(µg)	(µg)	(µg)
						Massai					
Non-inoculated	20.56 ± 0.73a	8.56 ± 0.92 $^{n.s.}$	37.65 ± 1.84 $^{n.s.}$	6.04 ± 0.49 $^{n.s.}$	3.28 ± 0.26b	2.91 ± 0.06a	39.57 ± 2.76a	15.32 ± 1.54	164 ± 31.4 $^{n.s.}$	429 ± 23.5b	39 ± 2.85b
A. brasilense	13.79 ± 0.30c	9.31 ± 0.27	34.71 ± 0.83	5.48 ± 0.36	4.35 ± 0.06ab	2.01 ± 0.07b	26.14 ± 1.87b	15.76 ± 0.91	203 ± 38.2	483 ± 17.5ab	113 ± 5.31a
B. subtilis	17.91 ± 1.69ab	10.35 ± 0.65	33.68 ± 4.66	5.28 ± 0.30	3.30 ± 0.33b	2.78 ± 0.24a	29.43 ± 2.45b	12.66 ± 0.88	174 ± 46	664 ± 70.9a	165 ± 18.1a
P. fluorescens	15.53 ± 1.80bc	9.92 ± 1.41	36.36 ± 4.36	6.44 ± 0.71	4.77 ± 0.45a	2.53 ± 0.28ab	31.60 ± 2.57b	13.48 ± 2.12	164 ± 9.28	563 ± 57.9ab	177 ± 32.6a
R. tropici	18.22 ± 0.36ab	10.69 ± 0.62	35.21 ± 1.02	6.09 ± 0.58	5.05 ± 0.60a	2.76 ± 0.09a	30.09 ± 1.57b	5.00 ± 0.78	226 ± 16.4	667 ± 91.6a	45 ± 35.8a
p value	0.006	0.41	0.88	0.54	0.005	0.011	0.006	0.41	0.53	0.02	< 0.001
						Zuri					
Non-inoculated	15.9 ± 0.18bc	7.4 ± 0.32c	36.1 ± 3.49b	3.9 ± 1.0 $^{n.s.}$	5.1 ± 0.85 $^{n.s.}$	2.4 ± 0.04c	89.9 ± 2.31c	7.0 ± 1.01 $^{n.s.}$	131.1 ± 5.48a	1054 ± 123$^{n.s.}$	51.7 ± 8.18b
A. brasilense	19.6 ± 2.05ab	9.2 ± 0.80ab	64.0 ± 6.04a	5.5 ± 0.59	7.4 ± 0.67	3.1 ± 0.13ab	141.0 ± 12.3a	10.2 ± 1.64	132.8 ± 1.26a	1002 ± 140	76.3 ± 3.99a
B. subtilis	20.0 ± 0.22a	10.4 ± 0.54a	59.6 ± 3.00a	4.9 ± 0.24	7.2 ± 0.50	3.3 ± 0.07a	130.5 ± 3.61ab	7.0 ± 0.14	110.2 ± 2.73ab	853 ± 92.2	70.8 ± 6.27ab
P. fluorescens	17.0 ± 0.78abc	9.0 ± 0.28ab	52.5 ± 4.35a	4.5 ± 0.32	5.5 ± 0.19	2.9 ± 0.09ab	119.1 ± 4.90ab	7.7 ± 0.95	90.9 ± 5.46b	999 ± 34.6	79.9 ± 3.32a
R. tropici	15.6 ± 1.11c	8.1 ± 0.44bc	50.1 ± 5.34a	3.8 ± 0.87	6.2 ± 0.91	2.9 ± 0.26b	113.4 ± 12.56bc	6.9 ± 0.61	95.3 ± 8.50b	1031 ± 100	83.2 ± 5.62a
p value	0.009	0.004	0.001	0.28	0.089	0.002	0.002	0.38	0.005	0.68	0.015
CV (%)	0.84	8.85	13.88	21.26	15.09	7.59	10.88	5.17	11.95	12.88	19.96

[1] Means (±SEM—standard error) (n = 6) followed by different letters differ from each other by the Duncan's test at $p \leq 0.05$; n.s. = non-significant. When letters are not shown in a trait, there was lack of statistical difference.

3.2. Seed and Root Colonization

The colonization of seeds and rootlets of two contrasting genotypes, Massai (Figure 2) and Zuri (Figure 3), inoculated singularly with the four species *A. brasilense*, *B. subtilis*, *P. fluorescens* and *R. tropici*, or remaining non-inoculated, was evaluated using SEM. On both photomicrographs, the first line indicates seeds after seven days of growth, and the second line shows the rootlets. On both photomicrographs, a and b are negative controls (non-inoculated), c and d indicate Inoculation with *A. brasilense*, e and f indicate inoculation with *B. subtilis*, g and h indicate inoculation with *P. fluorescens*, and i and j indicate inoculation with *R. tropici*.

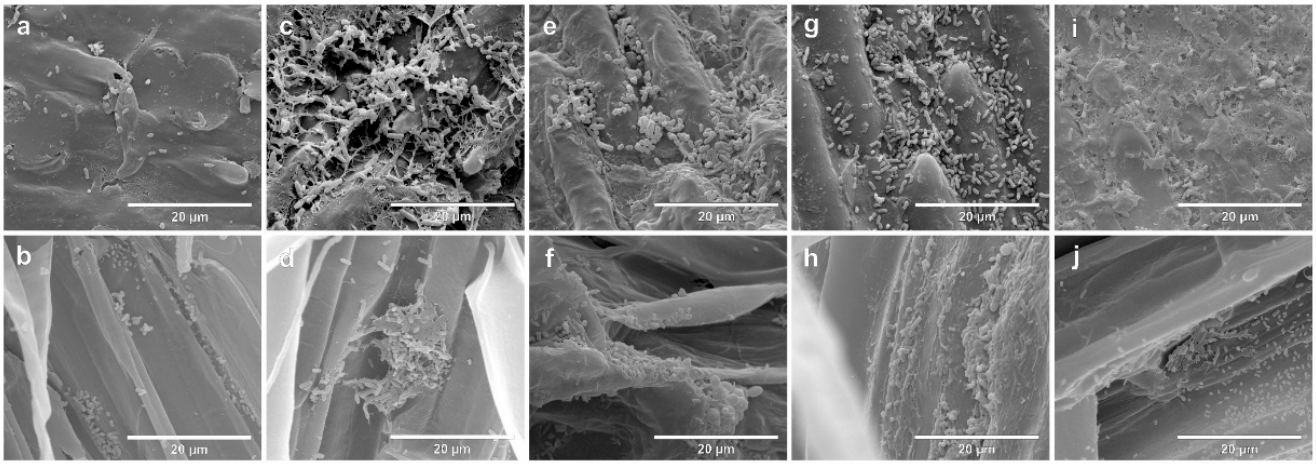

Figure 2. Scanning electron micrographs of the seeds and rootlets of *Megathyrsus maximus* cultivar Massai, colonized by *Azospirillum brasilense* (CNPSo 2083 + CNPSo 2084), *Bacillus subtillis* (CNPSo 2657), *Pseudomonas fluorescens* (CNPSo 2719), or *Rhizobium tropici* (CNPSo 103), after seven days of growth. Non-inoculated seeds (**a**) and rootlets (**b**); seeds (**c**) and rootlets (**d**) of plants inoculated with *A. brasilense*; seeds (**e**) and rootlets (**f**) of plants inoculated with *B. subtilis*; seeds (**g**) and rootlets (**h**) of plants inoculated with *P. fluorescens*; seeds (**i**) and rootlets (**j**) of plants inoculated with *R. tropici*.

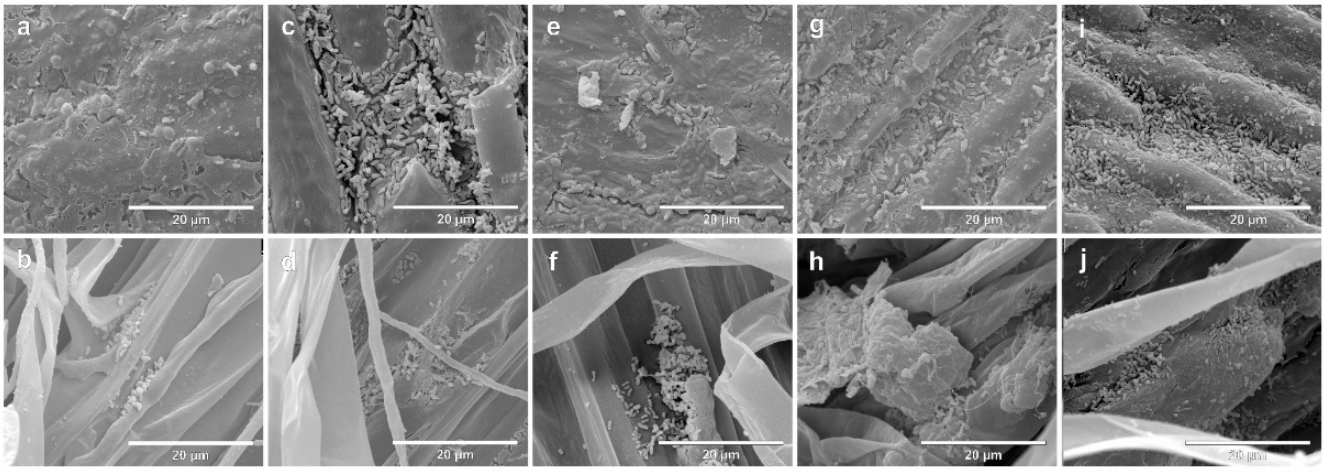

Figure 3. Scanning electron micrographs of seeds and rootlets of *Megathyrsus maximus* cultivar Zuri, colonized by *Azospirillum brasilense* (CNPSo 2083 + CNPSo 2084), *Bacillus subtillis* (CNPSo 2657), *Pseudomonas fluorescens* (CNPSo 2719), or *Rhizobium tropici* (CNPSo 103), after 7 days of growth. Non-inoculated seeds (**a**) and rootlets (**b**); seeds (**c**) and rootlets (**d**) of plants inoculated with *A. brasilense*; seeds (**e**) and rootlets (**f**) of plants inoculated with *B. subtilis*; seeds (**g**) and rootlets (**h**) of plants inoculated with *P. fluorescens*; seeds (**i**) and rootlets (**j**) of plants inoculated with *R. tropici*.

After seven days, considering that the seeds in this experiment were not surface-disinfected, it was possible to observe a community of native microorganisms in the tegument that were colonizing the rootlets in the non-inoculated control for Massai seeds (Figure 2a) and rootlets (Figure 2b), as well as Zuri seeds (Figure 3a) and rootlets (Figure 3b). Seeds and rootlet sections that were bacterized were examined, and we confirmed that cells of all strains were consistently distributed on the surface of seed coats and rootlets in Massai (Figure 2c–j) and Zuri (Figure 3c–j).

A biofilm consisting of bacterial cells, and a net-like material, suggesting extracellular matrix formation, were observed in both Massai (Figure 2) and Zuri (Figure 3), being more abundant, in both genotypes, with *A. brasilense* (c,d) and *R. tropici* (i,j), and less abundant with *P. fluorescens* (g,h) inoculation. A lower adherence of bacterial cells was observed for *B. subtilis* (e,f) in the sections studied.

Observations in the most responsive cultivar Zuri have shown strong biofilm formation and roots colonized by dense biofilm development when inoculated with *A. brasilense*, *R. tropici*, and *P. fluorescens* when compared to *B. subtilis* (Figure 3).

4. Discussion

The importance of plant roots has been increasingly highlighted, especially in view of global climate changes, with increasing periods of drought, and the fertilizer crisis, with a shortage in the supply of nutrients. Enhancement in root growth implies in higher efficiency in the uptake of water and nutrients, and PGPB can help in achieving this goal. All four species were able to colonize seed coats and rootlets, but differences were observed, e.g., with higher colonization by *A. brasilense*. Despite differences among plant genotypes and bacterial strains, inoculation with PGPB increased root growth traits, including RDW, RV, TRL, RMD, RTD, RHI, RHL, and TNB. Improvements in root systems result in a higher efficiency in water and nutrient uptake, and more exudation that favors interactions with other beneficial microorganisms [37]. Therefore, our results indicate that PGPB may represent an important strategy for the management of grass pastures. It is worth mentioning that the search for elite strains within the native population of each site is highly recommended, as the probability of success in the adaptation is higher. In our study, all bacteria used are elite strains selected from native populations [17,26,28], except for *R. tropici* CNPSo 103, which is native to Colombia, but in this case, the strain has shown excellent adaptations to a variety of edaphoclimatic conditions in the tropics [16], including after more than 20 years of experimentation with the common bean crop in Brazil. Benefits in root growth traits can mostly be attributed to the synthesis of phytohormones, mainly auxins, as reported for *A. brasilense* strains CNPSo 2083 and CNPSo 2084 [25]. The phytohormones synthesized by these bacteria [38–41], with an emphasis on IAA and cytokinins, play important roles in root development. The auxin/cytokinin balance regulates the cell division and formation of new tissues, affecting shoot and root growth [42]

Auxins are involved in the activation of meristem, cell elongation, cell differentiation, and lateral root development, while cytokinins act towards the regulation of cell division and the induction of new tissues [43]. Barbieri and Galli [44], comparing a wild strain of *A. brasilense* with a mutant having a lower auxin production, found a decrease in the number of lateral roots colonized by *Triticum durum* var. Appula for the mutant strain, while Ortíz-Castro and collaborators [45] reported that the cytokinins produced by a strain of *Bacillus megaterium* (syn. *Priesta megaterium*) stimulated the production of lateral roots by *Arabidopsis*. *Azospirillum brasilense* also synthesizes nitric oxide, which acts as a signaling molecule in the pathway mediated by auxins, inducing the formation of branches and indirectly stimulating the formation of lateral roots [46]. *Pseudomonas fluorescens*, on the other hand, synthesizes cyclodipeptides, which regulate genes that are responsive to auxins in roots, making them key players in the modulation of root growth traits [47]. Indeed, *A. brasilense* favors the increase of root hair incidences in grasses [48,49], which was also evidenced in *Arabidopsis* inoculated with *P. fluorescens* [47], and in *Urochloa brizantha* and *U. decumbens* inoculated with *A. brasilense* [29].

Altogether, these mechanisms related to PGPB may explain the increase of favorable root traits in all six genotypes of *M. maximus*. However, despite improvements in root traits, these effects occurred to different degrees, depending on the interaction between the cultivar and strain. Three grass genotypes, Mombaça, Tanzânia-1, and Zuri, had at least one root trait that was significantly improved in response to all four bacterial species, *A. brasilense*, *B. subtilis*, *P. fluorescens*, and *R. tropici*. Tanzânia-1 and Zuri were also outstanding, showing improvements in six out of 10 traits. On the other hand, Quênia and Massai significantly increased the expression of five root traits, but only when inoculated with *A. brasilense* and either *B subtilis* for Quênia, or *P. fluorescens* for Massai. In relation to the bacteria, all grass genotypes responded to the inoculation with *A. brasilense*, five responded to *B. subtillis*, three responded to *P. fluorescens*, and three responded to *R. tropici*.

This increase in the root system of inoculated plants may also have been responsible for the higher accumulation of one or more nutrients in shoots. A higher uptake of water and nutrients is essential for the production of forage biomass, especially in tropical soils, where such factors are limited [50,51]. These results are in agreement with previous field trials in three geographic regions, showing that the inoculation of *U. brizanta* cv. Marandu with the strains Ab-V5 and Ab-V6 of *A. brasilense* increased the shoot dry mass production, ranging from 13 to 29.5%, as well as increased the accumulation of N from 2.9 to 11.2% in comparison with the non-inoculated plants [13].

Improvements in root traits are not always correlated with shoot biomass. We hypothesize that the lack of response could be due to the short time of plant growth and the optimized growth conditions in the greenhouse. For example, *Pennisetum clandestinum* inoculated with *Pseudomonas* sp. and *Strenotrophomonas* sp. had a lower shoot dry mass at 70 days when compared with the non-inoculated control, but, after 130 days, the control plants were outperformed by inoculated plants by 30% [52]. In our study, Tamani, Quênia, and Zuri did not show any improvement in shoot growth, and the latter two showed very specific interactions, responding only to *A. brasilense* and *B. subtilis* in terms of root growth traits.

Significant effects of inoculation on shoot growth were observed mainly in Mombaça, with the four bacterial species, and in Zuri, except with *R. tropici*, whereas Tanzâni-1 responded only to *P. fluorescens*. Higher biomass productivity is important to improve pasture recovery after grazing, allowing cattle to return earlier and more often, leading to a greater gain of animal protein without need to open new areas for pastures [29], which can be considered to be a land-saving technology [53]. Our results indicate that increased biomass due to the inoculation with PGPB can help in the process of the improvement of cattle raising.

Pasture reclamation is very important to maintain the viability of livestock activity, as well as to improve the efficiency of land use, soil and water conservation, and carbon sequestration [13]. Low soil fertility is one of the main causes of pasture degradation [8]. Outstanding results were observed in the accumulation of nutrients in shoots due to inoculation, even when no effects were observed for shoot growth. Increased nutrient accumulation in shoots, including macro and micronutrients (N, P, K, Ca. Mg, S, B, Cu, Fe, Mn, Zn) occurred in at least one bacterium–genotype inoculation. Differences among genotypes were once again observed, with the lowest performance for Tamani and Massai, with an increased uptake of only one and three micronutrients, respectively. Conversely, Mombaça, Quênia, Zuri, and Tanzânia-1 had an increased accumulation of nine, eight, six, and five macro- and micronutrients, respectively. Considering the bacterial species, although effects were observed according to the plant genotype, responsive genotypes, such as Mombaça, had enhanced nutrient contents in the shoots, irrespective of the inoculated bacteria. Therefore, the improved performance of plants inoculated with these four elite strains may represent an important strategy to reduce or avoid pasture degradation, increase forage longevity and nutritional quality, and minimize water and nutritional stresses [54,55].

Even though the experiment was carried under axenic conditions in the greenhouse and plants were supplied with ample nutrient solution, the improvement in root traits contributed to a higher accumulation of nutrients in plant biomass. We hypothesize that, under field conditions, plant responses to inoculation will be of higher magnitude, e.g., as reported for pastures inoculated with *Azospirillum* spp. under water restriction and low-fertility soil conditions [56], as well as for *U. brizantha* cv. Marandu grown in low-fertility soil [57].

While for the genus *Brachiaria* (*Urochloa* spp.), which occupies about 70% of the cultivated pastures in Brazil, no differences were observed among genotypes considering the response to inoculation [13,18,29], apparently, for *M. maximus* there are prominent differences. However, one must consider that our study aimed to perform a detailed analysis of parameters and performances that required axenic and controlled growth conditions. Previously, Mombaça and Zuri were described as having higher growth rates, which demands more nutrients compared with the slower growth rates of Tamani and Massai [58,59]. This difference among genotypes was also observed in a study on P uptake (M.C.M. Macedo, data unpublished). Therefore, responses in root traits, shoot growth and nutrient accumulation in Mombaça and Zuri could be related to their higher growth rates. Interestingly, Tamani-1 and Massai are known to accumulate sodium (Na) (M.C.M. Macedo, data unpublished), which might affect interactions with the bacteria. Plant–microbe interactions depend on recognition at a molecular level between partners [60], and the identification of differences in these molecules among genotypes represents an interesting subject for further studies. Furthermore, there may have differences in the bacterial ability to colonize roots and/or internal tissues, which would interfere with the plant-growth-promotion capacity [61]. Studies on the colonization of plant genotypes by plant-growth-promoting bacteria can help to clarify these points and maximize the benefits of inoculation, contributing for the sustainability of planted pastures.

5. Concluding Remarks

Globally, pastures occupy far more land than crops, and the same occurs in Brazil. Grasslands comprise the great majority of pastures worldwide, but, unfortunately, a significant percentage of them are in some stage of degradation. We investigated the performance of six genotypes of *M. maximus* when inoculated with elite strains of four PGPB species. All species were able to colonize seeds and rootlets, but differences were observed, with a higher capacity of *A. brasilense*. Improvements were observed in terms of the root traits, shoot biomass, and accumulation of nutrients in shoots. However, differences among genotypes were observed, with the best performance of Zuri and Mombaça, and a lower responsiveness of Tamani and Massai. Our results have shown the feasibility of improving biomass, as well as the quality of pastures containing *M. maximus*, by inoculation with PGPB. However, our results also indicate the need to search for the best plant genotype host x bacterium combinations. It will also be interesting to evaluate the consortia of bacterial species, as they can contribute with different biological processes. In all cases, preference should be given to the search for elite strains identified within the native population.

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