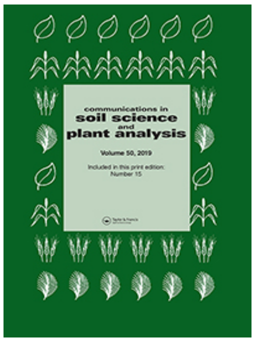

Communications in Soil Science and Plant Analysis

ISSN: 0010-3624 (Print) 1532-2416 (Online) Journal homepage: https://www.tandfonline.com/loi/lcss20

Biomass Yield, Nitrogen Accumulation and Nutritive Value of Mavuno Grass Inoculated with Plant Growth-promoting Bacteria

G. C. R. Sá, C. L. M. Carvalho, A. Moreira, M. Hungria, M.A. Nogueira, R. Heinrichs & C. V. Soares Filho

To cite this article: G. C. R. Sá, C. L. M. Carvalho, A. Moreira, M. Hungria, M.A. Nogueira, R. Heinrichs & C. V. Soares Filho (2019) Biomass Yield, Nitrogen Accumulation and Nutritive Value of Mavuno Grass Inoculated with Plant Growth-promoting Bacteria, Communications in Soil Science and Plant Analysis, 50:15, 1931-1942, DOI: 10.1080/00103624.2019.1648498

To link to this article: https://doi.org/10.1080/00103624.2019.1648498

Published online: 02 Aug 2019.

COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS
2019, VOL. 50, NO. 15, 1931–1942
https://doi.org/10.1080/00103624.2019.1648498

Taylor & Francis
Taylor & Francis Group

Check for updates

Biomass Yield, Nitrogen Accumulation and Nutritive Value of Mavuno Grass Inoculated with Plant Growth-promoting Bacteria

G. C. R. Sá[a], C. L. M. Carvalho[a], A. Moreira [b], M. Hungria[b], M.A. Nogueira[b], R. Heinrichs[c], and C. V. Soares Filho[a]

[a]Department of Production and Animal Health, São Paulo State University (UNESP), Araçatuba, Brazil; [b]Departament of Soil Science and Plant Nutrition, Embrapa Soja, Londrina, Brazil; [c]Department of Crop Science, São Paulo State University (UNESP), Dracena, Brazil

ABSTRACT

The objective of this study was to evaluate the effects of strains of *Azospirillum brasilense, Pseudomonas fluorescens and Rhizobium tropici* on the shoot dry weight (SDW) and root dry weight (RDW) yield, N uptake and nutritive value of 'Mavuno' grass inoculated with plant growth-promoting bacteria. We evaluated the effects of inoculation with the Ab-V5 and Ab-V6 strains of *Azospirillum brasilense* and *Pseudomonas fluorescens* or co-inoculation with *Rhizobium tropici* and Ab-V6, with and without nitrogen (N) application, as well as re-inoculations. The growth promoting bacteria + N promoted increases in SDW and RDW yield, tillers dry weight, relative chlorophyll index and N uptake. There were no effects of re-inoculation by *Azospirillum brasilense, Pseudomonas fluorescens and Rhizobium tropici* on nutrition, nutritive value and SDW and RDW yield, demonstrating that this technique still needs further studies with 'Mavuno' grass in the form and the correct period to be performed.

ARTICLE HISTORY
Received 3 April 2019
Accepted 16 July 2019

KEYWORDS
Urochloa spp; Azospirillum brasilense; Pseudomonas fluorescens; Rhizobium tropici; biological nitrogen fixation

Introduction

Brazilian livestock needs to ally productivity and sustainability, very divergent purposes that are being demanded by the population increase, climatic changes and behavior of consumers. In this context, the discovery and use of rational technologies are essential, since the increase in the meat and milk production are only achieved with large quantities of chemical fertilizers and openings in new areas (Teixeira et al. 2019).

Among the nutrients to increase pasture productivity, nitrogen (N) is essential, especially in tropical regions (Guimarães et al. 2016; Hungria, Nogueira, and Araújo 2016). It is estimated that N fertilization generates a total cost of 40% of yield. In addition, this type fertilizer has great potential pollutant, being able to cause serious problems to environment. In this sense, using sustainable alternatives for plant nutrition, such as the exploration of microorganisms with potential of biological N fixation (BNF), becomes fundamental to increase the productivity of grasses (Hungria, Nogueira, and Araújo 2016).

The use of these microorganisms in forage plants is innovative because they can help in the sustainability of the production system by reducing the probability of degradation of pastures, besides contributing to the increase in carbon sequestration and, consequently, decreasing of greenhouse gases (Hungria, Nogueira, and Araújo 2016). BNF occurs from the transformation of atmospheric N_2 into combined forms of N, which are then assimilated by plants to form organic molecules (Hungria, Mendes, and Mercante 2013a). The benefits of bacteria in plant growth have

CONTACT A. Moreira adonismoreira66@gmail.com Departament of Soil Science and Plant Nutrition, Embrapa Soja, Londrina, Brazil

been attributed to a variety of unique or combined mechanisms that act in a cumulative or cascading manner (Bashan and De-Bashan 2010), including increased nutrient and water uptake (Ardakani et al. 2011); the production and secretion of phytohormones and other signaling molecules, such as auxins (Spaepen and Vanderleyden 2015), cytokinins (Tien, Gaskins, and Hubbell 1979), gibberellins (Bottini et al. 1989), salicylic acid (Sahoo et al. 2014), and phosphate solubilization (Rodriguez et al. 2004).

As an example, in a study with 'Marandu' grass (*Urochloa brizantha*) the use of PGPB resulted in higher productivity, confirming the positive effects of inoculation on forage grasses (Oliveira, Oliveira, and Barioni 2007). In another study, inoculation with *Azospirillum brasilense* in different grass species [maize (*Zea mays*), *Urochloa* and sugarcane (*Saccharum* spp.)] allowed a lower N fertilizers application without reducing forage yield (Vogel, Martinkoski, and Ruzicki 2014). The association between 40 kg ha^{-1} N and inoculation with *A. brasilense* strains Ab-V5 and Ab-V6 in three distinct regions of Brazil resulted in higher yields in the biomass of *Urochloa* spp. (Hungria, Nogueira, and Araújo 2016).

However, in spite of the benefits promoted by plant growth promoting bacteria (PGPB) in agricultural crops, its effects on forage plants are still restricted and need studies, as already evidenced by Hungria, Nogueira, and Araújo (2013b). Aware of the concern to produce meat in a sustainable manner we observed the potential of the use of growth promoting bacteria in forage plants, since the pastures comprise the main source of feed of the Brazilian herd.

The effects of these growth-promoting bacteria (PGPB) in forage plants is fundamental for economic and sustainable exploitation. The objective of this study was to evaluate the effects of inoculation of PGPB on SDW and RDW yield and N concentration and uptake and nutritive value [crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF)] concentration in 'Mavuno' grass hybrids (*Urochloa* spp.).

Materials and methods

Local site and experimental design

The experiments were conducted during summer under greenhouse conditions (average temperature of 29°C and photoperiod of 14/10 h, day/night) in eight-liter plastic pots. The forage 'Mavuno' grass (*Urochloa ruziziensis* × *U. brizantha* cv. Marandu × *U. brizantha* apomictic) was cultivated at São Paulo State University (UNESP) in Araçatuba County, São Paulo State, Brazil (21°8′ LS, 50°25′ LW, 415 m a.s.l.).

We used a Typic Ultisol (Santos et al. 2018) collected at 0–0.2 m depth with the following chemical attributes: phosphorus (P, resin) = 23 mg dm^{-3}, soil organic matter (SOM) = 26 g dm^{-3}, pH in $CaCl_2$ = 5.2, potassium (K^+) = 2.9 $mmol_c$ dm^{-3}, calcium (Ca^{2+}) = 25 $mmol_c$ dm^{-3}, magnesium (Mg^{2+}) = 17 $mmol_c$ dm^{-3}, potential acidity (H^++Al^{3+}) = 28 $mmol_c$ dm^{-3}, cation exchange capacity (CEC) = 72.9 $mmol_c$ dm^{-3} and base saturation (V%) = 62. Using an NFb culture in a semi-solid form, we estimated the population of diazotrophic microorganisms in the soil to be 9.5×10^4 bacteria g^{-1} of soil by the technique described by Döbereiner, Marriel, and Nery (1976).

The treatments were applied in a randomized complete block design with five replicates arranged in subdivided plots with repeated measures over time (three growth cycles). The treatments were determined based on the inoculation of plant growth promoting bacterial (PGPB) strains, including (1) *Azospirillum brasilense* CNPSo 2083 (Ab-V5) and CNPSo 2084 (Ab-V6), (2) *Pseudomonas fluorescens* CNPSo 2719 and (3) co-inoculation with *Rhizobium tropici* CIAT 899 and *Azospirillum brasilense* CNPSo 2084 (Ab-V6), each with or without the N application. In addition to the three treatments, we evaluated the effect of re-inoculation after each round of cutting, as well as two control treatments, one with the application N (positive control) and one without N fertilization and without inoculation (negative control), totaling eleven treatments. The inoculants with the concentration of each bacterial inoculant adjusted to 2×10^8 cells per mL. The bacteria were obtained from CNPSo PGPB (Ab-V5 and Ab-V6) were used (Hungria et al. 2010).′The soil from each pot following nutrient addition consisted of the following: $Ca(H_2PO_4)_2$–200 mg dm^{-3} P, K_2SO_4 – 150 mg dm^{-3} K and 61 mg dm^{-3} S, H_3BO_3 – 0.5 mg dm^{-3} B, $CuSO_4$ – 1.0 mg dm^{-3} Cu, H_2MoO_4 – 0.1 mg dm^{-3} Mo, $MnSO_4$ – 5.0 mg dm^{-3} Mn, and $ZnSO_4$ – 2.0 mg dm^{-3} Zn. After four days, the 'Mavuno' grass was sowed. The bacterial inoculant concentrations were also estimated by counting colonies on solid NFb medium plates (Döbereiner, Marriel, and Nery 1976; Hungria and Araujo 1994).

We used 50 mL of each inoculant for each kilogram of seed before sowing. Seeds were dried for approximately 30 min in a cool and sun-sheltered location, after which they were seeded at 15 seeds per pot. The plants were thinned when they presented three fully expanded leaves, with five uniform plants maintained per pot. The plants were reinoculated by spraying (300 mL) after the first and second cuts, at which time the leaves began to develop again. 4.0 mL concentration of diluted inoculants in 296 mL water also was used. Spraying was performed directly onto the plant leaves, with the sprayer filled to a volume sufficient to reinoculated all five pots corresponding to each treatment. N fertilization occurred via a solution from a graduated pipette four days before the forage was sown, for a total of 100 mg N dm^{-3} (NH_4NO_3). Two weeks after the emergence of 'Mavuno' grass, thinning was performed to keep five uniform plants per pot and deionized water was used for irrigation.

Plant harvest and measurements of productive and nutritional parameters

The grass was evaluated when it reached an average height of 0.5 m, and the plants were harvested down to 0.1 m above the surface of the ground. Three growth cycles with four-week intervals were evaluated. After each harvest, the material was identified, weighed and oven dried at approximately 65°C until it reached a constant weight. The material was subsequently weighed on a precision balance to quantify the shoot dry weight (SDW) yield. After drying, the samples were ground in a Wiley type mill. N concentration was determined using a micro-Kjeldahl method, a modification of the aluminum block digestion technique described by Gallaher, Weldon, and Futral (1975). Crude protein (CP) was determined by multiplying N concentration by 6.25. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentration was determined according to Barnes (1980).

On the day of each cutting took place, plant height readings were taken with a millimeter ruler. Immediately before each harvest, the relative chlorophyll index (RCI) was determined using a SPAD-502 Plus chlorophyll meter. The RCI values were obtained by the average of 10 readings performed in the middle third of newly expanded leaves (diagnostic leaves) of each experimental unit (Lavres Junior, Santos Junior, and Monteiro 2010). The number of tillers per pot were also counted. The collected plant material was first separated into tillers and main plants, and later the tiller mass per pot was determined. The material was then collected, and a second separation was performed on the grass leaves and stems to determine the mass of each component. The roots were collected at the end of the experiment and washed in running water using 2-mm mesh sieves until all soil was removed. To determine the RDW yield the samples were properly identified, bagged, and the material was dried as described above. After drying in forced ventilation at approximately 60°C to a constant mass, all plant material collected during harvesting was weighed on a precision balance to quantify the RDW yield.

Statistical analysis

The data were tested for error normality and homogeneity of variances. The results were assessed using analysis of variance (ANOVA) with the F test ($p \leq 0.05$) and compared using the Scott-Knott test with a 5% significance. When we identified an interaction effect between factors, the responses were compared within each cutting.

Results and discussion

Shoot and roots dry weight yields

There was a significant effect of the treatments on the SDW and RDW yield, considering the sum of the three cuttings. For the SDW yield, samples that received treatments with N fertilization had statistically higher yields relative to those in which N was not applied. The values varied between 9.9 and 27.3 g for the control treatment without N or inoculation and the co-inoculation of *R. tropici* and *A. brasilense* (Ab-V6) with N, respectively (Figure 1(a)). Plants that were inoculated with *P. fluorescens* only displayed performance that was statistically similar to the negative control treatment, however, the accumulated SDW yield were 12.6% higher (Figure 1(a)).

For most of the evaluated parameters, only treatments inoculated with PGPB had a lower performance than those that received a combination of PGPB inoculation and N fertilization. However, the use of PGPB increased the values evaluated relative to the positive control. The results showed that PGPB alone cannot replace N fertilizers in grasses, but that they do promote greater N uptake and utilization (Roesch et al. 2005; Saubidet, Fatta, and Barneix 2002), resulting in a synergistic effect between PGPB inoculation and N fertilization (Lana et al. 2012).

In general, there was no significant difference between inoculation with bacteria and the control treatments for most of the variables analyzed. This fact can be related to the use of a suitable soil in terms of fertility, reducing the chances for more significant contrasting effects. However, PGPB promoted increases in yields when compared to the treatments without inoculation, as we observed positive effects of PGPB inoculation on the RDW and SDW yield, tiller yield, RCI and overall height of 'Mavuno' grass plants. By contrast, PGPB inoculation presented similar results to the control treatments for leaf blade percentage (Figure 1(a–f)).

PGPB have the ability to promote growth and the availability of plant nutrients for BNF, to solubilize phosphate, to increase resistance, and to produce essential metabolites for growth (Shweta et al. 2014; Yang et al. 2014). One of the main phytonutrients synthesized by bacteria is indole-3-acetic acid (IAA), which promotes plant growth and increases the nutrients uptake, ensuring the efficient use of these resources (Hungria et al. 2010).

Brown (1972) found that the metabolites produced by PGPB, such as auxins, gibberellins and their precursors, influenced plant growth, as these substances are responsible for several physiological events that result in plant growth. Auxins and gibberellins act on the growth and elongation of stalks, leaves and roots, and induce changes in the expansion, division and cellular stretching of the meristematic regions, where plant growth occurs (Taiz and Zeiger 2013).

The main action mechanisms of the genus *Pseudomonas* are the solubilization of phosphate, the increase in water absorption and the promotion of phytohormones (Muleta et al. 2013). Criollo et al. (2012) studied the effect of inoculation with *P. fluorescens* on *Pennisetum clandestinum* during the winter and verified higher shoot green weight yield by the plant compared to plants receiving only N fertilization and emphasized that such increases were the result of the release of phytohormones.

The co-inoculation of *Azospirillum* and *Rhizobium* is a technique widely used in legumes, especially common beans (*Phaseolus vulgaris* L.) and soybeans, consisting of the combination of different microorganisms that produce a synergistic effect, which tends to surpass the productive results obtained when these organisms are used in an isolated form (Hungria, Nogueira, and Araújo 2013b). In Gramineae, strains of *Azospirillum* (Ab-V5 and Ab-V6) contribute as plant growth promoters (Hungria et al. 2010) to the yield and secretion of substances that promote better establishment and development of plants, including to the synthesis of IAA (Fukami et al. 2017a). By contrast, *Rhizobium* participates in BNF and phytohormone yields in non-legumes (García-Fraile et al. 2012). Certainly, this increase in yield is due to a series of products produced by the bacteria that are highly beneficial to the plant.

The results obtained here agree with those obtained by Aguirre et al. (2018) in which, when evaluating the yield of Croastcross-1 grass (*Cynodon dactylon*) inoculated with *Azospirillum* Ab-V5

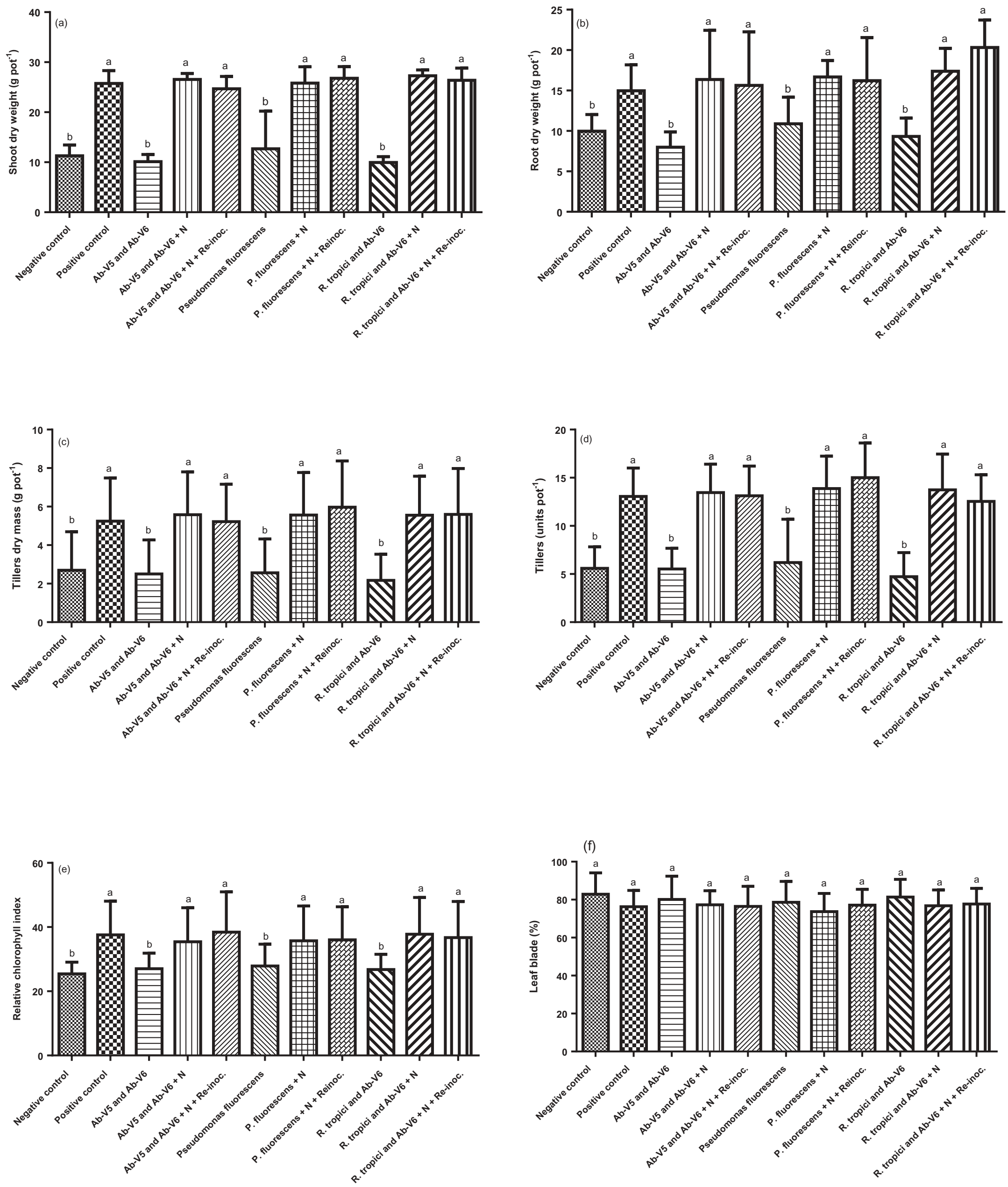

Figure 1. Shoot dry weight (SDW) (g per pot) and root dry weight (RDW) yield (g per pot) (b), tiller dry weight (g per pot) (c), number of tillers (d), relative chlorophyll index (e), and leaf blade percentage (f) in 'Mavuno' grass hybrids inoculated with PGPB. Error bars represent the mean standard error (n = 15). Means followed by letters differ for treatments, as determined by the Scott-Knott test ($p \leq .05$).

and Ab-V6 and fertilized with a 100 kg N ha^{-1}, they observed positive effects on SDW yield when compared to uninoculated controls. Hungria, Nogueira, and Araújo (2016) also observed beneficial effects on *Urochloa* spp. yield when combining strains of *Azospirillum* Ab-V5 and Ab-V6 and 40 kg ha^{-1} of N, with the bacteria promoting increases from 17.4- to 29.6%.

For the RDW yield, N fertilized treatments displayed statistically higher yields ($P \leq 0.05$) than those without N fertilizers. The highest productivity was with the co-inoculation of *R. tropici* and Ab-V6 with re-inoculation after each cutting. These plants had RDW yield of 20.3 g per pot, which was 35.8% higher than the positive control (Figure 1(b)). Although they were statistically similar, treatments inoculated with *P. fluorescens* displayed RDW yield increase of 9.2%, relative to the negative control (Figure 1(b)).

Increases in RDW are also due to inoculation with PGPB. Cardenas et al. (2012) found that PGPB inoculation favored plant growth and nutrition and that these effects were due to a greater proliferation of root villi. Hernandez et al. (2001) justified that the adequate performance of *A. brasilense* in increasing root density and size was due to the greater availability of phytohormones, which result in a greater uptake of water and nutrients. Itzigsohn et al. (2000) found that the inoculation of *Azospirillum* spp. in pastures has a beneficial potential, especially in regions with hydric deficits and low soil fertility, due to the larger root biomasses that result in a higher soil exploration capacity (Malik et al. 1997). This justifies the absence of significant results by the bacteria in the present study, since the soil used was chemically adequate and water was not a limiting factor.

Therefore, positive effects of PGPB inoculation on RDW and SDW yield of 'Mavuno' grass were observed, which promoted increases in yield when compared to treatments without inoculation, since the bacteria secrete substances which increase root growth, plants have greater support and conditions for productivity and growth. The conduction of the pot experiment with sifted soil do not impose limits for the growth and development of the roots.

Increases in leaf and stem yield of forage plants results in higher SDW yield and, consequently, higher amounts of carbon (C) are hijacked to increase the productivity and for storage in the soil via the roots. Well-managed forage plants with high biomass production can sequester a considerable amount of C (Cerri et al. 2010). Hungria, Nogueira, and Araújo (2016) reported the sequestration of 9.27 Mt e-CO_2 in pasture areas inoculated with *Azospirillum* and destined for forage biomass yields.

Fukami et al. (2017b) evaluated the effects of inoculation with *A. brasilense* strains (Ab-V5 and Ab-V6) or the co-inoculation of *R. tropici* and Ab-V6 in corn (*Zea mays* L.), and found increases in the height and SDW yield of the plants relative to the control treatments without inoculation. The combination of bacterial species should be considered as an interesting alternative to combat saline stress in corn plants.

For the tiller dry weights and units tillers, the highest productivity in the cuttings was in the plants inoculated with *P. fluorescens* together with N application (Figure 1(c,d)). The dry weight was 5.9 g per pot, which represented an increase of 13.7% relative to the positive control, despite being statistically similar. By contrast, the plants inoculated with *P. fluorescens* or *R. tropici* and Ab-V6 displayed tiller yield similar, relative to the negative control. The plants inoculated with PGPB and fertilized with N had higher RCI values relative to the unfertilized plants. According to Larcher (2000), the photosynthetic capacity is optimized with a higher availability of N, main constituent of the chlorophyll molecule (Taiz and Zeiger 2013). Thus, the RCI can be used to predict the nutritional status of N in plants by reading the amount of green pigments in the forage leaves. Guimarães et al. (2016) using the chlorophyll apparatus for RCI readings in *Urochloa brizantha* cv. 'Marandu' inoculated with PGPB along three cuts, showed similar characteristics to the present study. They found that the first cutting presented the highest values, followed by the third and second cutting, with an average value for the *A. brasilense* inoculated treatment group of 27.4 RCI.

The foliar re-inoculation did not present significant results for shoot and roots yields. These results are in agreement with Aguirre et al. (2018), who concluded that the re-inoculation of *A. brasilense* in 'Croastcross-1′ grass after the first year of cultivation was not necessary. Evaluating the agronomic response of triticale culture to different forms of *Azospirillum* strains application, Sipione et al. (2017) did not verify positive effects of leaf inoculation for height, stem dry weight and SDW. Galindo et al. (2015) evaluating the RCI of the irrigated wheat crop due to different times of application of strains of *A. brasilense* via foliar, also did not find significant difference between the foliar inoculation and the control treatment.

There was no significant difference between the inoculation by the bacteria and the control treatments for most of the analyzed attributes. This fact can be related to the use of a suitable soil in terms of fertility and to the fact that the pots are watered daily, not presenting water limitation for plant growth, reducing the chances of more significant contrasting effects.

The results show that only PGPB do not substitute N fertilizers in grasses, but when associated, they promote greater N uptake and utilization (Saubidet, Fatta, and Barneix 2002). Lana et al. (2012) also reported synergism effect between inoculation and N fertilization.

Shoot total N concentration and uptake

The total N concentration and uptake in the shoots were significant as determined by an analysis of variance for the treatments (Figure 2(a,b)). As well as the morphological and productive components, the total N uptake in the shoots showed positive effects when the plants were inoculated with the PGPB, this is explained by the increase in the root volume and consequently the greater uptake of water and nutrients.

The nutrient that had the highest concentration and accumulations were total N, result is due to the fact that nutrients are more absorbed and accumulated in the plant tissue of forages (Boer et al. 2007; Torres et al. 2005). N being an important structural nutrient in the functions of plants, participating in the biosynthesis of proteins and chlorophyll (Taiz and Zeiger 2013).The increases in total N concentration and uptake had beneficial effects that were mainly due to the inoculation with the *Azospirillum* and *Pseudomonas* strains because of the organic compounds production that benefit root growth and enhance the uptake of water and nutrients by plants (Gupta, Dey, and Gupta 2013). In addition, the genus *Azospirillum* also carries out BFN, thus contributing to the better assimilation of available N. Bacteria of the genus *Azospirillum* can influence the activity of glutamine synthetase in grass roots, and this compound is extremely important in the N incorporation process and is essential for plants to express their full potential (Machado et al. 1998; Unno et al. 2006).

When evaluating the effects of inoculation with *A. brasilense* strains on *U. brizantha* and *U. ruzizienses* cultivars, Hungria, Nogueira, and Araújo (2016) found significant effects of inoculation combined with N fertilization when compared to control treatments with only N fertilization. These data are similar to those found in the present study, although in spite of not having significant effects, the inoculation increased up to 6.0% the N accumulation.

The N fertilization together with inoculation was statistically similar to the positive control. Total N concentration and uptake in the shoots, although not significantly different from the negative control treatment, the plants inoculated with *A. brasilense* and *P. fluorescens* had 2.4% increased total N concentration and 14.7% total N uptake (Figure 2(a,b)). There was no difference between the plants that were reinoculated after the cuttings. It should be noted that in the present study the N accumulation decreased over time with the cuttings, which is justified because the SDW yield of the forage plants also decreased over time with the cuttings, which consequently resulted in less uptake of nutrients. In this sense, the re-inoculation after the first and second cutting of the plants did not have significant effects on the nutrient uptake of the forage plants.

Inoculation with plant growth-promoting bacteria was not able to replace N fertilization. However, when combined with inoculation with N fertilization, bacteria promoted increases in productivity, the relative chlorophyll index, shoot total N concentration and the uptake. This result indicates that PGPB can be a sustainable alternative for reducing the use of N fertilizers. There were no effects of re-inoculation with *Azospirillum brasilense, Pseudomonas fluorescens* or *Rhizobium tropici* on the nutrition or yield, demonstrating that these techniques still require further study to determine the correct form and period in which they should be performed.

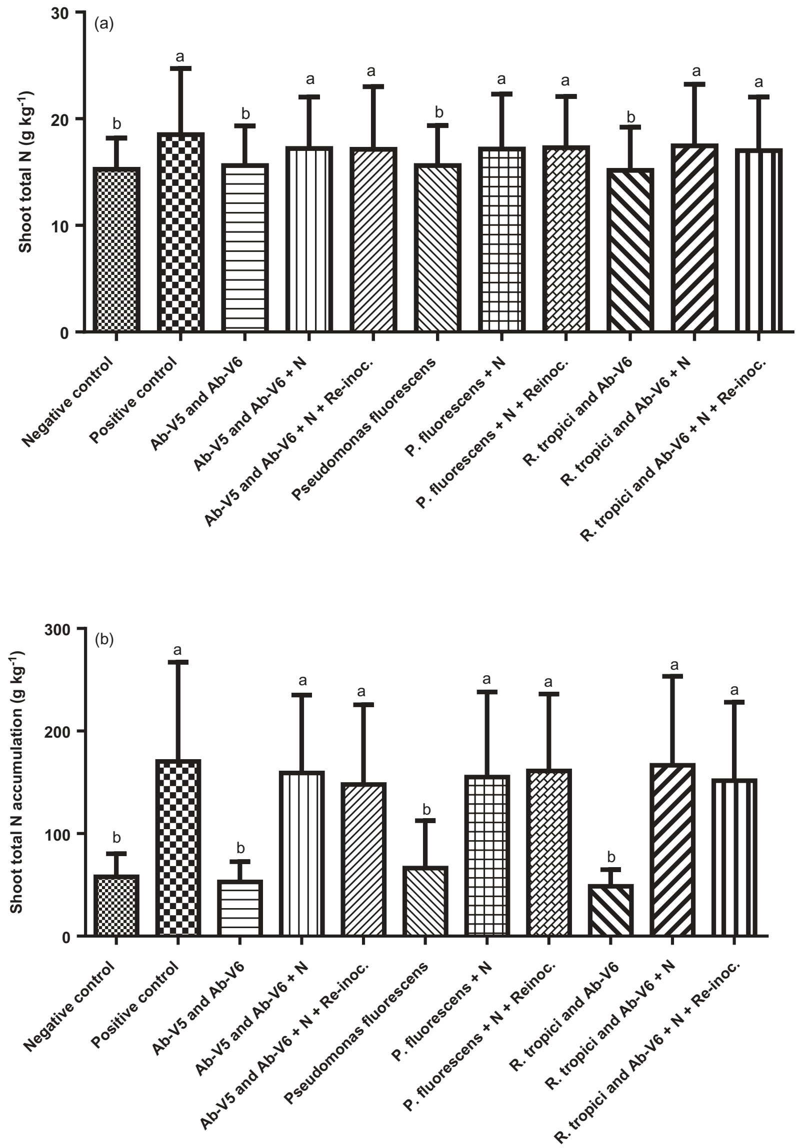

Figure 2. Shoot total N concentration (g kg^{-1}) (a) and shoot total N uptake (g kg^{-1}) (b), in 'Mavuno' grass hybrids inoculated with PGPB. Error bars represent the mean standard error (n = 15). Means followed by letters differ for treatments, as determined by the Scott-Knott test ($p \leq .05$).

Nutritive value

The percentages of CP, NDF, and ADF presented significance in the analysis of variance for treatments (Figure 3(a–c)). The percentages of CP ranged from 9.5- to 11.6%, and treatments with N fertilization were statistically superior to non-fertilizers (Figure 3(a)). The NDF concentration ranged from 67.9- to 72.0% and ADF ranged from 33.8 – to 36.3%, and treatments without N fertilization were higher than the treatments that received N, except for the ADF concentration, the positive control did not differ statistically from the treatments too much.

Evaluating the effect of inoculation by *Azospirillum brasilense* on seeds of *Urochloa brizantha* cv. Marandú associated with the use of N, Hanisch, Balbinot, and Vogt (2017) did not find significant

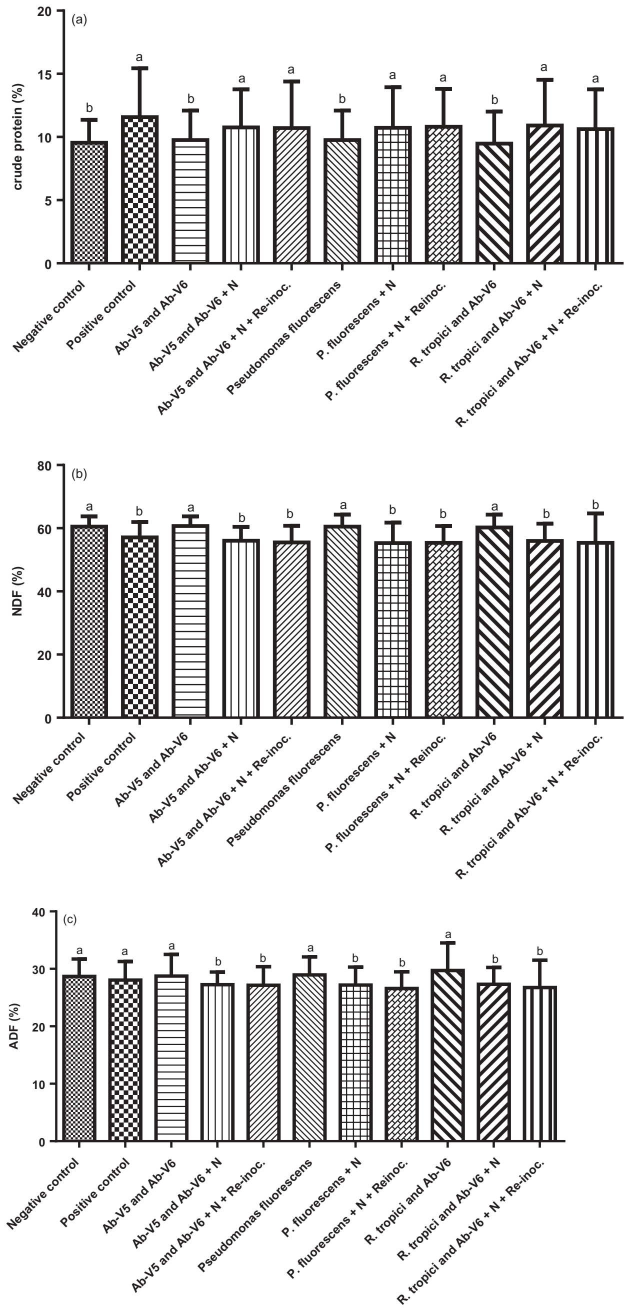

Figure 3. Crude protein (a), neutral detergent fiber (NDF) (b) and acid detergent fiber (ADF) (c) in 'Mavuno' grass inoculated with PGPB. Error bars represent the mean standard error (n = 15). Means followed by letters differ for treatments, as determined by the Scott-Knott test ($p \leq .05$).

effects of inoculation for the CP and NDF, presenting results similar to those of the present study. Bernd et al. (2014), when evaluating the effect of inoculation with *Pseudomonas fluorescens* and N levels on the CP in maize, also did not verify significant effects of the inoculation, whether they were combined with N fertilization or not.

Conclusion

The growth promoting bacteria (PGPB) and N fertilization promoted increases in shoot dry weight (SDW) and root dry weight (RDW) yield, tillers dry weight, relative chlorophyll index and total N uptake in 'Mavuno' grass. There were no effects of re-inoculation by *Azospirillum brasilense, Pseudomonas fluorescens and Rhizobium tropici* on nutrition, nutritive value and biomass yields, demonstrating that this technique still needs further studies in the form and the correct period to be performed.

Acknowledgments

We would like to thank the Foundation for Research Support of the State of São Paulo Process FAPESP Grant #2017/17573-4 for financial aid to support this research for the support in conducting this study and for stock exchange financing (Process Grant #2017/26364-0).

ORCID

A. Moreira http://orcid.org/0000-0003-4023-5990

References

Aguirre, P. F., C. J. Olivo, P. F. Rodrigues, D. R. Falk, C. B. Adams, and H. P. Schiafino. 2018. Forage yield of Coastcross-1 pastures inoculated with Azospirillum brasilense. *Acta Scientuarum, Animal Science* 40:e36392. doi:10.4025/actascianimsci.v40i0.36392.

Ardakani, M. R., D. Mazaheri, S. Mafakheri, and A. Moghaddam. 2011. Absorption efficiency of N, P, K through triple inoculation of wheat (*Triticum aestivum* L.) by *Azospirillum brasilense, Streptomyces* sp., *Glomus intraradices* and manure application. *Physiology and Molecular Biology of Plants* 17:181–92. doi:10.1007/s12298-011-0065-7.

Barnes, R. F. 1980. Infra-red reflectance spectroscopy for evaluating forages. In *Forage evaluation: Concepts and techniques*, ed. J. R. Wheeler and R. D. Mochrie, 89–102. Netley: Griffin Press Limited.

Bashan, Y., and L. E. De-Bashan. 2010. How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—A critical assessment. *Advances in Agronomy* 108:77–136.

Bernd, L. P., T. M. Souza, M. A. Oliveira, E. Y. S. Ono, C. Zucareli, and E. Y. Hirooka. 2014. Inoculation of Pseudomonas fluorescens and NPK fertilizationon chemical composition and fungal-fumonisincontamination of maize. *Revista Brasileira De Engenharia Agrícola E Ambiental* 18:1274–80. doi:10.1590/1807-1929/agriambi.v18n12p1274-1280.

Boer, C. A., R. L. Assis, G. P. Silva, A. J. Braz, A. L. Barroso, A. Cargnelutti Filho, and F. R. Pires. 2007. Nutrient cycling in off-season cover crops on a Brazilian savanna soil. *Pesquisa Agropecuária Brasileira* 42:1269–76. doi:10.1590/S0100-204X2007000900008.

Bottini, R., M. Fulchieri, D. Pearce, and R. P. Pharis. 1989. Identification of gibberellins A1, A3, and iso-A3 in cultures of *Azospirillum lipoferum*. *Plant Physiology* 90:45–47. doi:10.1104/pp.90.1.45.

Brown, M. E. 1972. Plant growth substances produced by microorganisms of soil and rhizosphere. *Journal of Applied | bacteriology* 35:443–51. doi:10.1111/j.1365-2672.1972.tb03721.x.

Cardenas, A., R. L. Rodriguez, V. Pizarro, L. F. Cadavid, and C. Arevalo-Ferro. 2012. Shifts in bacterial communities of two Caribbean reef-building coral species affected by white plague disease. *ISME Journal* 6:502–12. doi:10.1038/ismej.2012.11.

Cerri, C. C., M. Bernoux, S. M. F. Maia, C. E. P. Cerri, C. Costa Junior, B. J. Feigl, L. A. Frazão, F. F. C. Mello, M. V. Galdos, C. S. Moreira, et al. 2010. Greenhouse gas mitigation options in Brazil for land-use change, livestock and agriculture. *Scientia Agricola* 67:102–16. doi:10.1590/S0103-90162010000100015.

Criollo, P., M. Obando, L. Sánchez, and R. Bonilla. 2012. Efecto de bacterias promotoras del crecimiento vegetal (PGPR) asociadas a *Pennisetum clandestinum* en el altiplano cundiboyacensel. *Revista Corpoica – Ciencia Y Tecnologia Agropecuaria* 13:189–95. doi:10.21930/rcta.vol13_num2_art:254.

Döbereiner, J., I. Marriel, and M. Nery. 1976. Ecological distribution of *Spirillum lipoferum*. *Canadian Journal of Microbiology* 22:1464–73. doi:10.1139/m76-217.

Fukami, J., F. J. Ollero, M. Megías, and M. Hungria. 2017a. Phytohormones and induction of plant-stress tolerance and defense genes by seed and foliar inoculation with *Azospirillum brasilense* cells and metabolites promote maize growth. *AMB Express* 7:153–66. doi:10.1186/s13568-017-0453-7.

Fukami, J., C. Osa, F. J. Ollero, M. Megías, and M. Hungria. 2017b. Co-inoculation of maize with *Azospirillum brasilense* and *Rhizobium tropici* as a strategy to mitigate salinity stress. *Functional Plant Biology* 45:328–39. doi:10.1071/FP17167.

Galindo, F. S., M. G. Z. Ludkiewicz, J. L. M. Bellote, J. M. K. Santini, C. J. Alves, and M. C. M. Teixeira Filho. 2015. Effect of leaf application times of *Azospirillum brasilense* in the nutrients level of wheat irrigated. *Revista Tecnologia E Ciência Agropecuária* 9:37–42.

Gallaher, R. N., C. O. Weldon, and J. G. Futral. 1975. An aluminum block digester for plant and soil analysis. *Soil Science Society of America Journal* 39:803–06. doi:10.2136/sssaj1975.03615995003900040052x.

García-Fraile, P., L. Carro, M. Robledo, M. H. Ramírez-Bahena, J. D. Flores-Félix, M. T. Fernández, P. F. Mateos, R. Rivas, J. M. Igual, E. Martínez-Molina, et al. 2012. *Rhizobium* promotes non-legumes growth and quality in several production steps: Towards a biofertilization of edible raw vegetables healthy for humans. *PLoS One* 7: e38122. doi:10.1371/journal.pone.0038122.

Guimarães, S. L., C. S. A. Santos, E. M. Bonfim-Silva, A. C. Polizel, and E. R. Batista. 2016. Nutritional characteristics of marandu grass (*Brachiaria brizantha* cv. marandu) subjected to inoculation with associative diazotrophic bacteria. *African Journal of Microbiology Research* 10:873–82. doi:10.5897/AJMR2016.7951.

Gupta, K., A. Dey, and B. Gupta. 2013. Plant polyamines in abiotic stress responses. *Acta Physiologiae Plantarum* 35 (7):2015–36. doi:10.1007/s11738-013-1239-4.

Hanisch, A. L., A. A. Balbinot Jr, and G. A. Vogt. 2017. Productive performance of Urochloa brizantha cv. Marandu as a function of inoculation with *Azospirillum* and nitrogen doses. *Revista Agroambiente* 11:200–08.

Hernandez, J. A., M. A. Ferrer, A. Jimenez, A. R. Barcelo, and F. Sevilla. 2001. Antioxidant systems and O_2/H_2O_2 production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. *Plant Physiology* 27:827–31.

Hungria, M., and R. S. Araujo. 1994. *Manual of methods used in agricultural microbiology studies*. Brasília, Brazil: Embrapa.

Hungria, M., R. J. Campo, E. M. Souza, and F. O. Pedrosa. 2010. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant and Soil* 331:413–25. doi:10.1007/s11104-009-0262-0.

Hungria, M., I. C. Mendes, and F. M. Mercante. 2013a. *Biological nitrogen fixation as low carbon technology for common bean and soybean crops*. Londrina, Brazil: Embrapa Soja.

Hungria, M., M. A. Nogueira, and R. S. Araújo. 2013b. Co-inoculation of soybeans and common beans with rhizobia and azospirilla: Strategies to improve sustainability. *Biology Fertility Soils* 49:791–801. doi:10.1007/s00374-012-0771-5.

Hungria, M., M. A. Nogueira, and R. S. Araújo. 2016. Inoculation of *Brachiaria spp*. with the plant growth-promoting bacterium *Azospirillum brasilense*: An environment-friendly component in the reclamation of degraded pastures in the tropics. *Agriculture, Ecosystems and Environment* 221:125–31. doi:10.1016/j.agee.2016.01.024.

Itzigsohn, R., S. Burdman, Y. Okon, E. Zaady, R. Yonatan, and A. Perevolotsky. 2000. Plant-growth promotion in natural pastures by inoculation with *Azospirillum brasilense* under suboptimal growth conditions. *Arid Soil Research* 13:151–58. doi:10.1080/089030600263076.

Lana, M. C., J. Dartora, D. Marini, and J. E. Hann. 2012. Inoculation with *Azospirillum*, associated with nitrogen fertilization in maize. *Revista Ceres* 59:399–405. doi:10.1590/S0034-737X2012000300016.

Larcher, W. 2000. *Plant Ecophysiology*. São Carlos, Brazil: Editora Rima.

Lavres Junior, J., D. G. Santos Junior, and F. A. Monteiro. 2010. Nitrate reductase activity and SPAD readings in leaf tissues of guinea grass submitted to nitrogen and potassium rates. *Revista Brasileira De Ciência Do Solo* 34:801–09. doi:10.1590/S0100-06832010000300022.

Machado, A. T., L. Sodek, J. Döbereiner, and V. M. Reis. 1998. Evaluation of nitrogen fertilizer and inoculation with diazotrophic bacteria on the biochemical behavior of the maize cultivar nitroflint. *Pesquisa Agropecuária Brasileira* 33:961–70.

Malik, K. A., R. Bilal, S. Mehnaz, G. Rasul, M. S. Mirsa, and M. S. Ali. 1997. Association of nitrogen-fixing, plant-growth-promoting rhizobacteria (PGPR) with kallar grass and rice. *Plant and Soil* 194:37–44. doi:10.1023/A:1004295714181.

Muleta, D., F. Assefa, E. Börjesson, and U. Granhall. 2013. Phosphate-solubilising rhizobacteria associated with *Coffea arabica* L. in natural coffee forests of southwestern Ethiopia. *Journal of the Saudi Society of Agricultural Sciences* 12:73–84. doi:10.1016/j.jssas.2012.07.002.

Oliveira, P. P. A., W. S. Oliveira, and W. J. Barioni. 2007. *Forage yield and quality of Brachiaria brizantha cv. Marandu with Azospirillum brasilense and fertilized with nitrogen*. São Carlos, Brazil: Embrapa Pecuária Sudeste.

Rodriguez, H., T. Gonzalez, I. Goire, and Y. Bashan. 2004. Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. *Naturwissenschaften* 91:552–55. doi:10.1007/s00114-004-0566-0.

Roesch, L. F., F. O. Camargo, P. A. Selbach, and E. S. Sá. 2005. Reinoculation of diazotrophic bacteria improving growth of wheat. *Ciência Rural* 35:1201–04. doi:10.1590/S0103-84782005000500035.

Sahoo, R. K., M. W. Ansari, M. Pradhan, T. K. Dangar, S. Mohanty, and N. Tuteja. 2014. Phenotypic and molecular characterization of native *Azospirillum* strains from rice fields to improve crop productivity. *Protoplasma* 251:943–53. doi:10.1007/s00709-013-0607-7.

Santos, H. G., P. K. T. Jacomine, L. H. C. Anjos, V. A. Oliveira, J. F. Lumbreras, M. R. Coelho, J. A. Almeida, J. C. Araujo Filho, J. B. Oliveira, and T. J. F. Cunha. 2018. *Brazilian system of soil classification*. Brasília, Brazil: Embrapa.

Saubidet, M. I., N. Fatta, and A. J. Barneix. 2002. The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. *Plant and Soil* 245:215–22. doi:10.1023/A:1020469603941.

Shweta, S., M. B. Shivanna, B. R. Gurumurthy, R. Uma Shaanker, T. R. Santhosh Kumar, and G. Ravikanthet. 2014. Inhibition of fungal endophytes by camptothecine produced by their host plant, *Nothapodytes nimmoniana* (Grahm) Mabb. (Icacinaceae). *Current Science* 107:994–1000.

Sipione, M. S., A. C. Limede, C. E. S. Oliveira, A. Zoz, C. S. Silva, and T. Zoz. 2017. Ways of inoculation of *Azospirillum brasilense* in the initial growth of triticale. *Revista Scientia Agraria* 18:86–94. doi:10.5380/rsa.v18i4.50714.

Spaepen, S., and J. Vanderleyden. 2015. Auxin signaling in *Azospirillum brasilense*: A proteome analysis. In *Biological nitrogen fixation*, ed. F. J. Brujin, 937–340. Hoboken: Wiley.

Taiz, L., and E. Zeiger. 2013. *Plant physiology*. Porto Alegre, Brazil: Artemed.

Teixeira, N. M., R. Heinrichs, C. S. B. Bonini, J. Afzal, G. C. Meirelles, C. V. Soares Filho, and A. Moreira. 2019. Chelated zinc leaf application on nutrients concentration and yield of Mombasa grass. *Journal of Plant Nutrition* 42:89–98. doi:10.1080/01904167.2018.1549673.

Tien, T. M., M. H. Gaskins, and D. H. Hubbell. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of Pearl Millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology* 37:1016–24.

Torres, J. L., M. G. Pereira, I. Andrioli, J. C. Polidoro, and A. J. Fabian. 2005. Cover crops residue decomposition and nitrogen release in a Cerrado soil. *Revista Brasileira De Ciência Do Solo* 29:609–18. doi:10.1590/S0100-06832005000400013.

Unno, H., T. Uchida, H. Sugawara, L. Kurisu, T. Sugyiama, G. Kurisu, T. Sugiyama, T. Yamaya, H. Sakakibara, T. Hase, et al. 2006. Atomic structure of plant glutamine synthetase. *The Journal of Biological Chemistry* 281:29287–96. doi:10.1074/jbc.M601497200.

Vogel, G. F., L. Martinkoski, and M. Ruzicki. 2014. Effects of *Azospirillum brasilense* in use of forage grasses: Amounts and results. *Agropecuária Científica No Semiárido* 10:1–6.

Yang, Y., H. Zhao, R. A. Barrero, B. Zhang, G. Sun, I. W. Wilson, F. Xie, K. D. Walker, J. W. Parks, R. Bruce, et al. 2014. Genome sequencing and analysis of the paclitaxel-producing endophytic fungus Penicillium aurantiogriseum NRRL 62431. *BMC Genomics* 15:197. doi:10.1186/1471-2164-15-69.