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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

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ABSTRACT

Estimates are that in Brazil there are about 180 million hectares of pasturelands, 70% with some degree of degradation. Reclamation of such areas demands re-establishment of soil fertility, plant growth and forage production, and microbial inoculants might help in these processes. We evaluated the ability of two strains of *Azospirillum brasilense* to promote the growth of two genotypes of *Brachiaria* spp. (=*Urochloa* spp.). The experiments were set up at three different sites in Brazil, and forage production estimated for 26 cuts in two years. On average, increases of 5.4% and 22.1% in response to N-fertilizer alone and to N-fertilizer in combination with *Azospirillum*, respectively, were observed over the non-inoculated and non-N-fertilized control treatment. Increase in N accumulation in the biomass in response to *Azospirillum* was equivalent to a second application of 40 kg of N-fertilizer ha^{-1}. Estimates attributed to the inoculation were of gains of 0.103 Mg C ha^{-1}, corresponding to 0.309 Mg CO_2-eq ha^{-1}. Inoculation with *Azospirillum* may represent a key component of programs to reclaim degraded pastures and help sequestration of CO_2 from the atmosphere.

1. Introduction

Pastures constitute the most economical and practical means to feed cattle and, in Brazil, extremely favorable climatic conditions increase the potentiality of animal husbandry activities (Corrêa et al., 2003). However, animal performance (fresh weight gain) and support capacity of pastures (number of animals per area unit), which are key factors for the success of extensive cattle raising (Boin, 1986), are directly affected by pasture quality and its capacity to produce fodder dry matter.

Estimates indicate that Brazil holds about 180 million hectares of pasturelands, of which 70% show some degree of degradation (Embrapa, 2012; Dias-Filho, 2014). The reclamation of such areas, in the great majority grown with *Brachiaria* spp. (=*Urochloa* spp.) is necessary to guarantee the sustainability of bovine cattle raising in the country, and would contribute to avoid the deforestation of new areas to be incorporated into livestock and agricultural activities.

Various factors contribute to pasture degradation, including the unsuitability of the forage species to local conditions, poor establishment of the plants before grazing is initiated, improper management of the area, and especially the loss of soil fertility due to excessive nutrient extraction in animal carcasses, soil erosion and fixation of nutrients to soil particles (Kichel et al., 1997). Therefore, the maintenance of pasture productivity becomes a great challenge to the sustainable management of tropical environments all over the world (Fonte et al., 2014).

The reduction of nitrogen (N) availability, among all nutrients, is considered the most limiting factor for tropical pastures (Werner, 1994), resulting in severe losses of animal support capacity and animal weight gain with time. The N cycle seems to be particularly important, and the balance between N export and reposition via litter decomposition, deposition in excretes and remobilization is important for the maintenance of the nutritional status of tropical pastures (Fisher et al., 1997). It is estimated that about 90% of all N taken in by the animals are returned to the soil surface as urine and faeces, but most of it is lost by leaching or volatilization of the ammonia in the excretes (Ferreira et al., 1995, 2000). In addition,

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overgrazing also contributes to nutritional imbalance and pasture depletion (Boddey et al., 1995).

Other nutritional deficiencies, such as phosphorus (P), along with N deficiency, may be associated with the process of pasture degradation and low animal weight gains. P is the second element most required by plants and very often a limiting factor in tropical soils. In addition, P availability is restricted due to the element's low solubility in the soil solution and to its fixation to the soil minerals (Sharma et al., 2013), producing low-quality forage that, in turn, exerts a negative influence on the reproductive performance of bovines (Fisher et al., 1992).

The reclamation of degraded pastures is a complex process that starts with a diagnostic evaluation to identify the causes of degradation, followed by a decision about the need to execute repair, renewal or recovery of the area (Costa et al., 2006). In any case, it will be necessary to address the issue of soil fertility (Oliveira et al., 2003), and nutrients will have to be replaced in order to re-establish plant growth and forage production. The inclusion of plant growth-promoting bacteria (PGPB), along with phosphate solubilizers and nitrogen fixers in reclamation programs may represent a viable alternative to reduce costs and environmental impacts.

The association between plants and PGPB can be highly beneficial, especially under low soil fertility conditions (e.g., Hungria et al., 2010; Carvalhais et al., 2013; Hungria et al., 2013a,b). PGPB are believed to benefit plant growth by an array of mechanisms that can act simultaneously or in a cascade (Bashan and de-Bashan 2010). The development of triticale (*X Triticosecale*, Witmark) seedlings, for example, improved when mycorrhizal fungi and *Azospirillum* bacteria were employed as bioactivators, producing forage with high nutritional quality (Lestingi et al., 2007). PGPB have also been successfully employed by Dominguez-Nuñez et al. (2012) to inoculate mesquite (*Prosopis juliflora*) used for feeding cattle. In addition, inoculation of different forage species with *Azospirillum brasilense* made it possible to reduce the amounts of N-fertilizers applied without compromising important yield components such as dry matter accumulation and plant height (Vogel et al., 2014).

Annual fluxes of CO_2 are higher in pastures than in forest soils (Salimon et al., 2004), and the inadequate management and overgrazing of pastures by livestock contributes to soil C and N depletion (Fisher et al., 2007). It has been estimated that cattle raising is responsible for one-half of all Brazilian emissions of greenhouse gases—GHG (Bustamante et al., 2012). Therefore, any practices that contribute to replenish soil C and N stocks and to reduce GHG emissions are highly desirable and necessary for the sustainability of agricultural and livestock activities in Brazil (Lal, 2005; Sá et al., 2015), and the utilization of beneficial microbial inoculants stands out as a viable alternative (Hungria et al., 2010; Hungria et al., 2013a,b). In addition, improved plant growth and dry matter accumulation would represent higher rates of CO_2 sequestration from the atmosphere and its fixation into high-quality forage.

It is known that the natural vegetation and the plant species introduced in an area affect the microbial community in the soil (Garrido et al., 2010; Viana et al., 2011), and that different species of *Brachiaria* spp. may influence the establishment and performance of PGPB in their rhizospheres (Reis Júnior et al., 2004). In this study, we evaluated the effects of selected strains of *Azospirillum* on the development of two genotypes of *Brachiaria* spp. for reclamation of degraded pastures, and estimated the contribution of the technology to sequester atmospheric CO_2.

2. Material and methods

Three field experiments were conducted at different geographic regions of Brazil, representative of extensive *Brachiaria* spp. pasturelands, during the years of 2011 and 2012. Geographic coordinates, altitudes and climatic conditions at each experimental site are presented in Table 1.

Prior to sowing, samples from the 0–20 cm layer of the soils were collected for chemical analysis and determination of soil granulometry, as described before (Hungria et al., 2015) and the results are shown in Table 2. In addition, the abundance of diazotrophic microorganisms in the soils was estimated by the most probable number (MPN) technique, in semi-solid NFb culture medium, according to Döbereiner et al. (1976) and Hungria and Araujo (1994).

Populations in all sites ranged from 10^5 to 10^6 cells g^{-1} soil.

All experiments were set up in a completely randomized block design with four replicates. Treatments consisted of: (i) negative control-no N-fertilizer, no inoculation; (ii) positive control—recommended rate of N-fertilizer (40 kg of N ha^{-1}, no inoculation, and (iii) the combination of N-fertilizer (40 kg of N ha^{-1}) and inoculation with *Azospirillum brasilense* strains CNPSo 2083 (=Ab-V5) and CNPSo 2084 (=Ab-V6). The treatment containing only inoculation, without N-fertilizer was not included because it has been determined before that the main benefits obtained with these two strains of *A. brasilense* are on plant growth promotion and not biological N_2 fixation (Hungria et al., 2010). Therefore, the objective of the experiments was to evaluate the ability of the bacteria to promote plant growth in the presence of N-fertilizer, and not their capacity to fix atmospheric nitrogen (N_2). In addition, we took into account that in all programs to recover degraded pastures, some level of N fertilization is accomplished, even if at low rates, as the soil conditions are of a great depletion of N.

The experimental plots measured 4 m × 5 m, with planting rows located 0.5 m apart. Sowing was performed at a density of 8 kg seeds ha^{-1}, with seeds of 60% cultural value of the species *Brachiaria brizanta* (cv. Marandu) and *Brachiaria ruziziensis* (common). All plots received a basal fertilization with 400 kg ha^{-1} of the 00-28-20 (N-P-K) formulation. Plots that were also fertilized

Table 1
Geographic coordinates, altitudes and climatic characteristics of the sites where experiments were conducted.

Site	State	Coordinates[a]	Altitude (m)[b]	Climatic Type[c]	Average annual low temperature (°C)	Average annual high temperature (°C)	Average annual rainfall (mm)
Londrina	Paraná	23°11′S, 51°11′W	620	Cfa	13.3	28.5	1651
Ponta Grossa	Paraná	25°13′S, 50°10′W	880	Cfb	8.4	25.9	1507
Três Lagoas	Mato Grosso do Sul	24°45′S, 51°40′W	310	Aw	19.4	26.4	1500

[a] Latitude, longitude.
[b] Above sea level.
[c] According to Köppen–Geiger classification classification: *Cfa* – humid, subtropical; *Cfb* – temperate, with mild summers; *Aw* – tropical, with dry winters.