Azospirillum Brasilense And Zinc Rates Affecting Fungal Root Colonization and Yield of Cereal Crops in Succession Under Brazilian Cerrado Conditions

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Abstract

Soil and plant beneficial microbes capitalize plant nutrition through successful colonization in roots rhizosphere which could sustainably increase cereal production. Zinc (Zn) is intensively reported a deficient nutrient for maize and wheat production in Brazilian Cerrado, which could be sustainably managed by beneficial microorganisms and their symbiotic association with other microorganisms like arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE). The objective of this study was to evaluate the effect of *Azospirillum brasilense* inoculation and residual Zn rates on root colonization and grain yield of maize and wheat in succession under Brazilian Cerrado conditions. These experiments were conducted in a randomized block design with four replications and arranged in a 5x2 factorial scheme. The treatments were consisted of five Zn rates (0, 2, 4, 6 and 8 kg ha$^{-1}$) applied from zinc sulfate in maize and residual on wheat, and without and with seed inoculation of *A. brasilense*. Both crops were evaluated for root colonization of AMF and DSE, number of spores of AMF, quantification of CO$_2$-C and grain yield. Colonization by AMF and DSE were significantly increased with interaction of Zn rates and inoculation treatments. The inoculation of *A. brasilense* favored root AMF and DSE colonization while increasing Zn rates by 4 kg ha$^{-1}$ for maize and while 2 and 4 kg ha$^{-1}$ Zn in residual for wheat reduced these colonizations. The inoculation did not influence spore production and CO$_2$-C in both crops while maize-wheat yield were increased with Zn rates up to 4 kg ha$^{-1}$ in edaphoclimatic condition of Brazilian Cerrado.

Introduction

Brazilian crop production system is dominated by soybean and maize cropping where maize (*Zea mays* L.) is the second large produced crop. Wheat (*Triticum aestivum* L.) yield is still low beyond consumption, therefore imported in a huge amount (Conab 2020). The cereal production system of Cerrado savannah is interrupted by several factors like soil-borne diseases, soil erosion and low nutrient and water use efficiency, rhizosphere microbiome and functional of soil existing microorganisms (Hatfield et al. 2017; Yang et al. 2020). However, cereal cultivation could be treated as a matter of food security due to their short life cycle. Cereals could also contribute to carbon (C) sequestration and soil organic matter which can improve nutrients pools, soil efficiency and productivity (Cherubin et al. 2018).

The intense weathering of tropical soils have devastating effects on organic matter and nutrients use efficiency especially Zn and therefore, can harm cereal productivity and soil fertility (Galindo et al. 2021). Zinc deficiency is a worldwide recognized micronutrient deficiency that limit crop growth and productivity (Jalal et al. 2020b). The low availability of Zn has drastic effects enzymatic activities and protein synthesis. Zinc is one of the fundamental constituents in the synthesis of tryptophan, indole acetic acid (IAA), proteinases, peptidases, dehydrogenases and phosphohydrolases which could improve soil and plant health (Marschner 2012; Castillo-González et al. 2018). The deficit or excess of Zn may have harsh consequences on plant health and yield therefore, proper Zn management is a wise agronomic strategy to improve soil health and crop productivity (Cakmak and Kutman 2017; Jalal et al. 2020a).
The interaction of soil-plant-microorganisms is alternative strategy that could contribute to soil-plant health and productivity (Jalal et al. 2021). The PGPBs especially the genus *Azospirillum* has the ability to fight for colonization site in above and below soil parts of several cereal crops and synthesis of phytohormones (Fukami et al. 2018a; Galindo et al. 2021; Karimi et al. 2021). The combination of *Azospirillum brasilense* and tryptophan as a precursor of Zn can increase IAA production and other hormones involved in plant growth (Housh et al. 2021). These beneficial activities of PGPBs can alter soil microbiota by root colonization as result of symbiosis between plants and certain fungi (Frey-Klett et al. 2007; Berta et al. 2014). These symbiotic fungi may be obligated biotrophs, arbuscular mycorrhizal (AMF) and facultative biotrophs, melanized septate endophytic (dark septate endophytic - DSE) that colonize in plant root system by spreading hyphae in soil to absorb water and nutrients, contributing to plant performance (Brundrett and Tedersoo 2018). These fungi (AMF and DSE) are coexisting in cortical region of plant to produce soluble metabolites that can increase germination, growth and implications of AMF hyphae (Vergara et al. 2018). These symbionts can indirectly influence host establishment by providing tolerance to biotic and abiotic stresses (Santos et al. 2017; He et al. 2019).

Since these symbionts are considered sensitive indicators and can exhibit behavioral alterations under different environmental or nutritional changes therefore, it is essential to adopt Zn management and biotechnology to minimize environmental damage and increase agricultural production in Brazilian Cerrado soils. The hypothesis of this research was whether Zn residual fertilization in association with inoculation of diazotrophic bacteria could benefit soil microbiota and population of AMF and DSE, thus increasing maize and wheat productivity. Therefore, this study aimed to evaluate the effect of *Azospirillum brasilense* inoculation and residual Zn rates on root colonization and grain yield of maize and wheat in succession under Brazilian Cerrado conditions.

**Material And Methods**

**Site description**

The study was conducted under field conditions in Selvíria (Brazilian Cerrado region), State of Mato Grosso do Sul, Brazil (20°22'S and 51°22'W, 335 m above sea level (Fig. 1) during 2013/14 (maize) and 2014 (wheat).

The soil was classified as Rhodic Haplustox (clayey Oxisol) according to Soil Survey Staff (2014). Twenty random soil samples were collected from the entire experimental site with a soil core type cup auger (0.10 m × 0.40 m - cup diameter and length respectively) from 0.00-0.20 m depth before initiation of field trial. The soil samples collected from each depth were homogenised, air-dried, sieved (2 mm), and stored at room temperature. The soil chemical attributes (Raij et al. 2001) and granulometry characterization (Teixeira et al. 2017) are summarized in Table 1.
Table 1
Soil chemical attributes in 0-0.20 m layer before field trial beginning.

| Soil chemical attributes          | 0-0.20m layer |
|-----------------------------------|--------------|
| P (resin)                         | 13.0 mg dm\(^{-3}\) |
| S (SO\(_4\))                      | 6.0 mg dm\(^{-3}\) |
| Organic matter                    | 23.0 g dm\(^{-3}\) |
| pH (CaCl\(_2\))                   | 4.8          |
| K                                 | 2.6 mmol\(_c\) dm\(^{-3}\) |
| Ca                                | 13.0 mmol\(_c\) dm\(^{-3}\) |
| Mg                                | 8.0 mmol\(_c\) dm\(^{-3}\) |
| H + Al                            | 42.0 mmol\(_c\) dm\(^{-3}\) |
| Al                                | 2.0 mmol\(_c\) dm\(^{-3}\) |
| B (hot water)                     | 0.24 mg dm\(^{-3}\) |
| Cu (DTPA)                         | 5.9 mg dm\(^{-3}\) |
| Fe (DTPA)                         | 30.0 mg dm\(^{-3}\) |
| Mn (DTPA)                         | 93.5 mg dm\(^{-3}\) |
| Zn (DTPA)                         | 0.5 mg dm\(^{-3}\) |
| Cation exchange capacity (pH 7.0) | 65.6 mmol\(_c\) dm\(^{-3}\) |
| Bases saturation                  | 36%          |
| Soil granulometry                 | 0-0.20 m layer |
| Sand                              | 438 g kg\(^{-1}\) |
| Silt                              | 90 g kg\(^{-1}\) |
| Clay                              | 472 g kg\(^{-1}\) |
| *Azospirillum* sp. most probably number | 1.65×10\(^4\) CFU g\(^{-1}\) soil |

\(n = 20\), DTPA = diethylenetriaminepentaacetic acid
The experimental area had been cultivated with annual leguminous and cereal crops for over 28 years. In addition, the area has been under no-tillage cultivation system for the last 13 years. The crop sequence prior to field trial was fallow until 2013 and black oats (Avena strigosa Schreb.) in 2013. Maximum, average and minimum temperatures and rainfall observed during the field trial are presented in Fig. 2.

**Experimental design and treatments**

The experimental was designed in a randomized complete block with four replicates, arranged in a $5 \times 2$ factorial scheme. The treatments were consisted of five Zn rates (0, 2, 4, 6 and 8 kg Zn ha$^{-1}$) applied from zinc sulphate (20% Zn and 10% S) and two seed inoculation with *A. brasilense* (without or with). The total area of each experimental plot was 13.5 m$^2$, comprised of six maize rows of five meters long at row space of 0.45 m. Wheat were cultivated in twelve rows of five meters and 0.17 m apart with a plot total size of 10.2 m$^2$. The useful area of maize-wheat plot were central rows (10 m$^2$).

The seeds of maize and wheat were treated with insecticide and fungicide before inoculation. The seeds of both crops were treated with fungicides [carbendazim + thiram at an active ingredient (a.i.) of 45 g + 105 g per 100 kg seed] and insecticides [imidacloprid + thiodicarb at (a.i.) of 45 g + 135 g per 100 kg seed] before inoculation. This is a general practice used by cereals growing farmers however, the influence of chemical seed treatment on inoculation efficiency of PGPB is still controversial (Munareto et al. 2018; Silva et al. 2018; Cardillo et al. 2019). The inoculation with *A. brasilense* of maize or wheat seeds was carried out by mixing and coating inoculant manually in plastic bags before an hour of plantation.

The *A. brasilense* strains Ab-V5 and Ab-V6 [CNPSo 2083 and CNPSo 2084 respectively, guarantee of $2 \times 10^8$ colony forming unity (CFU) mL$^{-1}$] were applied to maize seeds (24 kg) at a rate of 200 mL liquid inoculant ha$^{-1}$ and 300 mL ha$^{-1}$ to 150 kg of wheat seeds. These strains under similar conditions (specifically Brazilian Cerrado) showed positive results on maize and wheat development (Galindo et al. 2016, 2020a, b; Alves et al. 2017; Martins et al. 2018). The draft genome sequences of *A. brasilense* strains Ab-V5 and Ab-V6 carry similar *fix* and *nif* genes which are linked to biological N fixation (Hungria et al. 2018). These strains have different hormones production capacity however, sharing the same gene for auxin production (Fukami et al. 2017, 2018a). In addition, Ab-V5 and Ab-V6 have the capacity to induce expression of genes associated to abiotic and biotic stress tolerance in plants (Fukami et al. 2017; 2018c).

Zinc rates (0, 2, 4, 6 and 8 kg ha$^{-1}$) were manually applied to soil surface at even distribution in maize crop. The calculated amount of fertilizer (zinc sulphate) per plot was applied in between rows at V$^2$ stage of maize (with two leaves completely unfolded). The experimental area was irrigated with central pivot irrigation system (14 mm) soon after side-dress Zn application to incorporate fertilizer in soil. The Zn fertilizer was not applied in wheat crop in order to analyse residual effect of treatments.

**Field management**

**Maize**
The area was broadcast applied with limestone (composed of 28% CaO and 20% MgO with an effective neutralizing power of 88%) at the rate of 2.5 Mg ha\(^{-1}\), 65 days before maize sowing. The amount of lime applied was based on initial soil analysis and base saturation to 70%, following Eq. 1.

\[
\text{LN} = \frac{\text{CEC} \times (V_2 - V_1)}{10 \times \text{ENP}} \tag{Equation 1}
\]

Where LN = Limestone required in Mg ha\(^{-1}\), CEC = cation exchange capacity, V2 = bases saturation to be achieved, V1 = current based saturation and ENP = effective neutralization power.

Pre-experiment weeds were controlled by spraying 2, 4-D (670 g ha\(^{-1}\) a.i.) and glyphosate (1800 g ha\(^{-1}\) a.i.). A maize triple hybrid DKB 350 VT PRO was sown on 4th December, 2013 by placing 3.3 seeds per meter. The NPK 08-28-16 (32, 112 and 64 kg ha\(^{-1}\) of N, P\(_2\)O\(_5\) and K\(_2\)O respectively) was applied at a dose 400 kg ha\(^{-1}\) in plantation, based on soil analysis. The crop was irrigated with a center pivot irrigation system at 14 mm water supply. Seeds were emerged five days after sowing in both growing seasons. The recommended N (150 kg N ha\(^{-1}\) as ammonium sulfate, which contains 21% N and 23% S) was applied manually in side-dress on V6 growth stage. The post emergence weeds during crop development were controlled by the application of atrazine (1000 g ha\(^{-1}\) a.i.) and tembotrione (84 g ha\(^{-1}\) a.i.) along with vegetable oil adjuvant (720 g ha\(^{-1}\) a.i.). In addition, triflumuron (24 g ha\(^{-1}\) a.i.) and methomyl (215 g ha\(^{-1}\) a.i.) were used for controlling insects. The plants were harvested manually at 108 DAE (27th March, 2014).

**Wheat**

Wheat planting was carried out on exact area of preceding crop (maize) to analyze residual effect of Zn applied treatments in wheat (successor crop). The wheat genotype CD 116 was sown (80 seeds per meter) on 16th May, 2014. A basal fertilization of 350 kg ha\(^{-1}\) of N-P-K (08-28-16) applied at plantation was equivalent to 28, 98 and 56 kg ha\(^{-1}\) of N, P\(_2\)O\(_5\) and K\(_2\)O. Seedling were emerged five days after sowing. The area was irrigated with a center pivot irrigation system adjusted to 14 mm water depth at the intervals of 72 h approximately. Nitrogen (120 kg N ha\(^{-1}\) as ammonium sulfate, which contains 21% N and 23% S) was manually applied at a growth stage GS21 (Zadoks et al. 1974) in an even distribution of fertilizer to all treatments on soil surface. The post-emergence weeds were controlled by metsulfuron-methyl (3 g ha\(^{-1}\) a.i.). The crop was manually harvested at 110 DAE (9th September, 2014).

**Measurements**

The microbiological evaluations were performed by collecting four soil samples with maize or wheat roots at the depth of 0.00-0.10 m in each experimental plot. The collected roots were washed and stored in a 50% alcohol solution. One gram of root per plot was clarified in KOH 10% and HCl 1% solution, stained with trypan blue 0.05% and stored in lactoglycerol to assess root AMF and DSE colonization
(Phillips and Hayman 1970). Root colonization was determined by evaluating 100 segments of fine roots per plot.

The soil samples were homogenised and respiratory activity were determined by quantifying carbon released as CO$_2$-C in 100 g fresh soil per plot, following the methodology of Anderson and Domsch (1993).

The remaining of collected soil was air-dried, sieved (2 mm) and stored at room temperature. The number of AMF spores were determined from 100 g dry soil sample per plot. The spores were separated from the soil according to methods of decantation and wet sieving (Gerdemann and Nicolson 1963), centrifugation and sucrose flotation (Jenkins 1964). Acrylic plate with concentric rings were used to count the spores under a stereoscopic microscope (40x).

Grain yield was determined by spikes collection in useful lines of each maize and wheat plots. The grains were quantified after mechanical threshing and the data processed in kg ha$^{-1}$ to 13% (humidity).

**Statistical analysis**

All data were initially tested for normality using Shapiro and Wilk (1965) test and Levene's homoscedasticity test ($p < 0.05$) which showed the data to be normally distributed ($W \geq 0.90$). The data was then analyzed by ANOVA (F test) in a 2-way factorial design with Zn application rates and *A. brasilense* inoculation, their interaction was considered fixed effects in the model while block was considered a random variable. Mean separation was done for significant of main or interaction effects using Tukey test. Regression analysis was also performed to assess whether there is a linear or non-linear response to Zn rates using R software (R Development Core Team, 2015).

**Results**

**Treatments effect on maize**

Maize root AMF colonization was higher in plants treated with inoculation of *A. brasilense* and 4 kg Zn ha$^{-1}$ as compared to non-inoculated plants (Fig. 3A). The Root AMF colonization responded non-linearly to increasing Zn rates in inoculated (up to 3.5 kg Zn ha$^{-1}$) and non-inoculated plants (up to 4.4 kg Zn ha$^{-1}$) (Fig. 3A). In contrast, root DSE colonization was higher in non-inoculated treatment with 2 and 6 kg Zn ha$^{-1}$ and in inoculated plants with 8 kg Zn ha$^{-1}$ (Fig. 3B). Root DSE colonization was linearly adjusted to increasing Zn rates in inoculated treatments and non-linearly (up to 4.3 kg Zn ha$^{-1}$) in non-inoculated treatments (Fig. 3B). Number of AMF spores and released CO$_2$-C were not affected by Zn rates or inoculation (Fig. 3C and F).

The maize grain yield was significantly influenced by Zn rates and *A. brasilense* inoculation (Sup. Table 2), showing a non-linearly response (up to 3.8 kg Zn ha$^{-1}$) with increasing Zn rates (Fig. 3G). Also, it was
observed that inoculated treatments provided greater grain yield (8286 kg ha$^{-1}$) compared to non-inoculated treatments (7943 kg ha$^{-1}$). Inoculated plots were noted with an increase of 4.3% (Fig. 3H).

**Treatments effect on wheat**

Wheat root AMF colonization was higher in treatments with *A. brasilense* inoculation and absence of residual Zn application or with of 2 and 4 kg Zn ha$^{-1}$ as compared to non-inoculated treatments (Fig. 4A). Root AMF colonization was linearly decreased in inoculated treatments as Zn rates increased (Fig. 4A). While, root DSE colonization was higher in non-inoculated and without Zn residual treatments or with 2 and 6 kg Zn ha$^{-1}$ (Fig. 4B). Root DSE colonization responded linearly to increasing Zn rates regardless of inoculation (Fig. 4B). Number of AMF spores decreased linearly to increasing residual Zn rates (Fig. 4C). It was also observed that AMF sporulation increased (38 x 100 g dry soil) with *A. brasilense* inoculation as compared to non-inoculated treatments (34 x 100 g dry soil), an increase of 11.8% was observed (Fig. 4D). Released CO$_2$-C responded non-linearly to increasing Zn residual rates (up to 3.6 kg Zn ha$^{-1}$) (Fig. 4E). In addition, released CO$_2$-C was higher with *A. brasilense* inoculation (13.5 µg g$^{-1}$ soil) compared to non-inoculated treatments (12.7 µg g$^{-1}$ soil) with an increase of 6.3% (Fig. 4F). Wheat grain yield responded non-linearly to increasing residual Zn rates (up to 4.7 kg Zn ha$^{-1}$) (Fig. 4G), but was not affected by inoculation of *A. brasilense* (Fig. 4H).

**Discussion**

In the present study, the root AMF colonization was increased with 4 kg Zn ha$^{-1}$ in maize while 2 and 4 kg Zn ha$^{-1}$ in residual form for wheat in succession along with inoculation of *A. brasilense* (Fig. 3A and 4A). Plants mainly absorb Zn in divalent form (Zn$^{2+}$) (Fernandes et al. 2018) which can also be required for several enzymatic activities, among them synthesis of indole acetic acid (IAA) via tryptophan route (Taiz and Zeiger 2013). Martínez-de la Cruz et al. (2015) reported that auxin has positive impact on branching and volume of plant root system. Our results indicated that Zn rates in association with bacteria has ability to produce IAA and enhance plant growth. In addition, this hormone promoted root system which can indirectly stimulate symbiotic relationship between AMF population and their hosts (Liu et al. 2018).

Bidondo et al. (2011) indicated that bacteria can interact with root microbial community by mycorrhizosphere, inside spores or mycelia as observed in-vitro study. It was also reported that diazotrophic bacteria promoted AMF colonization and spores number that lead to higher acquisition of nutrients especially P and Zn with improved rhizospheric environment (Jangra et al. 2019). Several strains of *Azospirillum* spp. and *Pseudomonas* spp. had been reported in degradation of biopolymers (Turrini et al. 2018) and along with Zn application increased root development, formation of new points of infection and auxin synthesis that could lead to higher spores production in successive wheat cultivation (Fig. 4D) and signal AMF colonization (Ludwig-Müller and Güther 2007).
The root architecture system of wheat has low root mycorrhization as observed in the present study (Sup. Table 2). The colonization of AM in wheat roots can be change during growth stages which may have influence on nutritional demand (Ma et al. 2019). In addition, an increase in root colonization was observed from seedling to maturity stage with an increase uptake of 20 g kg$^{-1}$ of P and 2 mg kg$^{-1}$ of Zn in wheat plants. Such increases were not observed in the present study that probably resulting from already exist optimal levels of soil fertility.

The colonization of DSE in root system of wheat or maize and its interaction with other endophytic microorganisms is still need to be addressed. The DSE colonization occur simultaneously with AMF in plant roots (Ranelli et al. 2015) to deal with biotic and abiotic factors in optimum levels of soil fertility or even increasing doses of zinc that can affect colonization of this fungal group (Lugo et al. 2018). A previous study reported that Zn application may alter DSE (*Exophiala pisciphila*) colonization in maize root system (Li et al. 2011). The low concentration of Zn provided a non-mutualistic relationship between plant and DSE which may result in low biomass production regardless of inoculation. Although, higher doses of Zn fertilizer increased root colonization and biomass production which indicated that alteration in DSE behavior may be due to increasing Zn rates which is verified in the present study (Fig. 3A, B and Fig. 4A, B).

The bacterium *A. brasilense* has the ability to produce siderophores and other molecules like salicylic acid that may decrease mycelial growth (Kumar et al. 2018). The present study also showed a decrease in DSE colonization in roots of wheat (Fig. 3B and 4B). However, there has not been reported any antagonistic effect between *A. brasilense* and DSE in root/ soil system (Newsham 2011). Despite this, Santos et al. (2017) reported a mutualistic association between these microorganisms in most situations but can also develop a pathogenic characteristic in others. The mycelia growth can be inhibited by bacteria action, generating colonization inhibitory effects as observed in the present study for wheat. However, the interactions are still unclear and there is need for further studies on the influence of molecules produced by *A. brasilense* in root DSE colonization.

The CO$_2$-C released in maize root did not show significant effect with inoculation or Zn fertilization (Fig. 4E, F). While, inoculation of *A. brasilense* under residual Zn rates increased respiratory activity of wheat and thus increasing CO$_2$-C (Fig. 4E, F). The reason might be due to influence of crop and soil management on microorganisms activities. The respiratory activities of soil microorganisms (bacteria, fungi and other) are responsible for the release of CO$_2$-C and being act as sensitive indicators for soil quality (Saurich et al. 2019). In addition, different crop practices or even external inoculation of microorganism can generate changes in soil microbial activities. Bera et al. (2018) observed an increasing trend in microbial respiratory activities in wheat succession to rice under no-tillage. The respiratory activity was higher from the time of sowing to flowering while decreasing in later maturity. The present results reflected similar behavior for wheat succession to maize at 110 days after emergence. In addition, there was no significant differences in CO$_2$-C release at wheat maturity in succession to maize during 24 hours observation (Li et al. 2019). These results corroborate with the low values of CO$_2$-C as
verified in present study (Fig. 3F and 4F), which could lead to stabilized environment where a higher carbon as microorganism biomass is incorporated into soil and a low value of CO$_2$-C is released lost to atmosphere.

The grain yield of maize was significantly different with Zn fertilization and inoculation and adjusted to a non-linear function with increasing Zn rates up to 3.8 kg ha$^{-1}$. The initial soil Zn content medium (Table 1) which could meet plant needs and also explain the decreasing trend in productivity with further increase in Zn rates (Fig. 3G). In addition, inoculation with A. brasilense showed an increase in grain production (Fig. 3H). It is possible that A. brasilense favored the development of root system with higher absorption of nutrients and water that has a positive influence on nutritional status of plant (Gómez-Godínez et al. 2019; Galindo et al. 2021).

The assimilation of water and nutrients to spike and shoot are directly related to plant nutritional status (Galindo et al. 2019) and therefore leading to higher grain productivity. Our results showed that grain yield of wheat was not statistically influenced by inoculation of A. brasilense (Sup. Table 1; Fig. 4G, H) but still higher (1637 kg ha$^{-1}$ in Table 1) than average wheat production (900 kg ha$^{-1}$) of State of Mato Grosso do Sul (Conab 2014). Kazi et al. (2016) stated that inoculation of A. brasilense did not significantly influence grain yield of different wheat genotypes. The reinoculation of these microorganisms was reported an essential management to increase their population and colonize in environment and can compete with less efficient native species (Cassán and Diaz-Zorita 2016).

**Conclusion**

Root colonization by AMF and DSE was positively increased with interaction of Zn rates and A. brasilense inoculation via maize seeds. The inoculation of A. brasilense favored colonization by AMF and reduced by DSE at 4 kg Zn ha$^{-1}$ in maize, while 2 and 4 kg ha$^{-1}$ of residual Zn in wheat in succession. It was also concluded that A. brasilense inoculation increased maize grain yield and number of AMF spores and CO$_2$-C released in wheat in succession.

**Declarations**

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**Conflicts of Interest**

The authors declare no conflict of interest.

**Authors' contributions**
Conceptualization: F.S.G., M.C.M.T.F.; Methodology: P.S.T.S., F.S.G., A.M.R.C.; Investigation: P.S.T.S.; Writing – original draft: P.S.T.S., F.S.G.; Writing – review and editing: A.M.R.C.; F.S.G., A.J., M.C.M.T.F.; Supervision: A.M.R.C., M.C.M.T.F.

Ethics declarations / Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

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**Supplemental Data**

Supplemental Table 2 is not available with this version.

**Figures**

![Figure 1](image_url)

**Figure 1**

Study area at the Selvíria, State of Mato Grosso do Sul, Brazil (20o22′S, 51o22′W, the altitude of 335 m above sea level). Map created by using QGIS software and Google Earth program. QGIS Development Team (2019). QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org. Image obtained in Google Earth program. Google company (2020).
Figure 2

Rainfall, maximum, average and minimum temperatures obtained from the weather station located in the Education and Research Farm of UNESP during the maize (A) and wheat (B) cultivation.
Figure 3

Interaction between Zn rates and inoculation or not with Azospirillum brasilense in maize root colonization (COL AM) (A) and root colonization by endophytic fungi with septated, melanized hyphae ("dark septate" - COL D) (B), number of spores of arbuscular mycorrhizal fungi (NSP), released carbon from CO2 (CO2-C) and maize grain yield as a function of single effect of Zn rates (C, E and G) and inoculation or not with Azospirillum brasilense (D, F, H).
Figure 4

Interaction between Zn rates and inoculation or not with Azospirillum brasilense in wheat root colonization (COL AM) (A) and root colonization by endophytic fungi with septated, melanized hyphae ("dark septate" - COL D) (B), number of spores of arbuscular mycorrhizal fungi (NSP), released carbon from CO2 (CO2-C) and maize grain yield as a function of single effect of Zn rates (C, E and G) and inoculation or not with Azospirillum brasilense (D, F, H).
Supplementary Files

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