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Enrichment of organic compost with beneficial microorganisms and yield performance of corn and wheat[1]

Enriquecimento de composto orgânico com microrganismos benéficos e desempenho produtivo do milho e trigo

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HIGHLIGHTS:

A mature compost was enriched with free-living N-fixing bacteria and arbuscular mycorrhizal fungus using brachiaria as host. The enrichment changed the N dynamics in the compost, either increasing or decreasing ammonium and nitrate concentrations. In the field, the compost increased mycorrhizal colonization and yield of corn and wheat depending on the microorganism.

ABSTRACT: Enrichment with beneficial microorganisms may increase the benefits of organic compost. The aim of this study was to evaluate the enrichment of a mature compost with plant growth-promoting bacteria and arbuscular mycorrhizal fungus (*Rhizophagus clarus*), using brachiaria (*Urochloa brizantha*) as host plant, totaling seven treatments: control (compost with no bacteria, brachiaria or mycorrhizal fungus); compost + brachiaria; compost + brachiaria + mycorrhizal fungus; compost + brachiaria + mycorrhizal fungus + *Azorhizobium* sp.; compost + brachiaria + mycorrhizal fungus + *Azoarcus* sp.; compost + brachiaria + mycorrhizal fungus + *Bacillus subtilis*; and compost + brachiaria + mycorrhizal fungus + *Azotobacter* sp., in a completely randomized design with three replicates. Brachiaria shoot biomass, N and P concentrations, mycorrhizal colonization, and chemical characteristics of the compost were assessed five times over 183 days. *B. subtilis* and *Azotobacter* increased ammonium-N concentration in the compost in two and three sampling dates, respectively. In contrast, *Azotobacter* and *Azoarcus* decreased the concentrations of nitrate-N in at least one sampling. Despite high P availability in the compost (951-2927 mg kg^{-1}), mycorrhizal colonization reached up to 53%. In a field trial with the produced composts, in a randomized block design with six repetitions, the composts with brachiaria doubled the mycorrhizal colonization of corn (*Zea mays*) and wheat (*Triticum aestivum*), independent of the growth-promoting bacteria and, depending on the associated bacteria, increased grain yields.

Key words: composting, arbuscular mycorrhizal fungus, *Rhizophagus clarus*, *Urochloa brizantha*

RESUMO: O enriquecimento com microrganismos benéficos pode aumentar os benefícios do composto orgânico. Este estudo teve o objetivo de avaliar o enriquecimento de um composto maturado com quatro bactérias promotoras de crescimento de plantas e fungo micorrízico (*Rhizophagus clarus*) usando braquiária (*Urochloa brizantha*) como planta hospedeira, compreendendo sete tratamentos: controle (composto sem braquiária, bactérias ou fungo micorrízico); composto + braquiária; composto + braquiária + fungo micorrízico; composto + braquiária + fungo micorrízico + *Azorhizobium* sp.; composto + braquiária + fungo micorrízico + *Azoarcus* sp.; composto + braquiária + fungo micorrízico + *Bacillus subtilis*; e composto + braquiária + fungo micorrízico + *Azotobacter* sp., em delineamento inteiramente casualizado com três repetições. A biomassa aérea da braquiária, teores de P e N, colonização micorrízica e características químicas do composto foram monitoradas cinco vezes por 183 dias. *Bacillus subtilis* e *Azotobacter* aumentaram o teor de N-amônio no composto em duas e três avaliações, respectivamente, enquanto *Azotobacter* e *Azoarcus* diminuíram os teores de N-nitrato em pelo menos uma avaliação. Apesar da alta disponibilidade de P (951 a 2927 mg kg^{-1}), a colonização micorrízica chegou a 53%. Em experimento de campo com os compostos produzidos, no delineamento em blocos ao acaso com seis repetições, os compostos com braquiária dobraram a colonização micorrízica do milho (*Zea mays*) e do trigo (*Triticum eaestivum*), independente da bactéria promotora de crescimento e, dependendo da bactéria associada, aumentou a produtividade de grãos.

Palavras-chave: compostagem, fungo micorrízico arbuscular, *Rhizophagus clarus*, *Urochloa brizantha*

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Introduction

Composting allows the biological stabilization of organic residues of different origins, which can be used as source of nutrients for crops (Andrade et al., 2018). In addition to the beneficial effects of organic compost in improving soil fertility (Heck et al., 2013; Mota et al., 2019), enrichment with beneficial organisms like plant growth-promoting microorganisms may further increase the agricultural and environmental benefits of organic compost (Sousa et al., 2018).

Plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) may interact synergistically (Pereira et al., 2013) and promote plant growth by several mechanisms including P-solubilization, P-mineralization, biological nitrogen fixation (BNF), nutrient cycling, synthesis of siderophores, and phytohormones (Rodrigues et al., 2012). Moreover, some PGPB, also called "mycorrhiza-helper bacteria", may stimulate root mycorrhizal colonization (Choudhary et al., 2017). Some PGPB may perform free-living nitrogen fixation and enrich the compost with N (Sousa et al., 2018), whereas AMF may reach microsites with their external hyphae that are inaccessible to root hairs, thus increasing the uptake of low-mobility nutrients such as P (Brito et al., 2017). The tripartite interaction among the plant, AMF, and PGPB may result in a synergistic effect on plant performance (Pereira et al., 2013). Thus, enriching organic compost with beneficial microorganisms may yield even better results than the commonly used composting process.

The aim of this study was to evaluate the enrichment of a mature organic compost with free-living N-fixing plant growth-promoting bacteria and AMF using brachiaria (*Urochloa brizantha*) as host plant, follow the dynamics of nutrients in the compost during the enrichment process, and assess its effect on corn (*Zea mays*) and wheat (*Triticum aestivum*) mycorrhizal colonization, P and N nutritional status, and yield in the field.

Material and Methods

The first part of this study was carried out in a greenhouse (23° 11' 28.78" S; 51° 11' 1.55" W, altitude 620 m) between August 2016 and March 2017. A mature compost was obtained by composting organic solid residues (food scraps, meals, peels of vegetables and fruits, plant residues such as soybean grains, tree pruning, wheat, and soybean straw) at Embrapa Soybean, Londrina, PR, Brazil (Andrade et al., 2018).

Four PGPB isolates were obtained from the "Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja" (WFCC Collection #1213, WDCM Collection #1054). *Azorhizobium* sp. (CNPSo 1168), *Azoarcus* sp. (CNPSo 2541), and *Bacillus subtilis* (CNPSo 2723) were grown in 500 mL of TY broth, whereas *Azotobacter* sp. (CNPSo 3151) was grown in LG + $CaCO_3$ medium (Baldani et al., 2014). The bacterial cultures were shaken at 100 rpm at 28 °C in the dark for 7 days, and the concentrations of each isolate were adjusted to 1×10^8 cells mL^{-1}.

Trays (18 × 40 × 60 cm) were filled with 40 dm^3 of screened mature compost (4 mm), and the moisture was adjusted to 60% of the water holding capacity (WHC, which was determined gravimetrically after soaking a sample by capillarity for 24 hours and oven-drying at 105 °C) with distilled water. Each bacterial growth was mixed in the compost to provide 1.25×10^9 cells dm^{-3}, in three replicates, and kept for 30 days in the greenhouse, in addition to non-inoculated controls, with moisture adjusted to 60% of the WHC whenever necessary. During this period, an AMF pre-inoculum was simultaneously prepared as follows.

As AMF are obligate biotrophs, the production of propagules requires a living host. Thus, brachiaria (*Urochloa brizantha*) was used as host plant for multiplication of AMF (Zangaro et al., 2018). Seeds were rinsed with distilled water and incubated on moistened Germitest® paper rolls at 25 °C and 95% relative air humidity for seven days. Then, two 1.5-2 cm long plantlets were transplanted to 35-mL cells nursery trays filled with autoclave-sterilized sand + crushed charcoal (1:1, v v^{-1}). Every cell received 1 g of inoculum containing colonized root fragments, external hyphae, and spores (>50 g^{-1}) of *Rhizophagus clarus*. Non-inoculated plants were also grown as non-mycorrhizal controls. Plantlets were grown in the greenhouse for 30 days weekly receiving a modified nutrient solution (reduced to 1/5 of P as KH_2PO_4 and 1/3 of N as KNO_3) (Broughton & Dilworth, 1971).

Thirty days after the compost was inoculated with the respective bacterial isolate, nine 30-days old brachiaria plantlets were transplanted to the compost, resulting in the following treatments: T1: control (compost with no bacteria, mycorrhizal fungus - AMF or brachiaria); T2: compost + non-inoculated brachiaria; T3: compost + AMF brachiaria; T4: compost + AMF brachiaria + *Azorhizobium* sp.; T5: compost + AMF brachiaria + *Azoarcus* sp.; T6: compost + AMF brachiaria + *B. subtilis*; and T7: compost + AMF brachiaria + *Azotobacter* sp., in an entirely randomized design with three repetitions.

The experiment was monitored for another 153 days and received distilled water three times a week to replace 60% of the WHC. The average day/night temperature in the greenhouse was 33.2/21.5 °C and relative air humidity was 42/81.4%, respectively.

Compost core samples were taken from the 0-5 cm layer in five points per tray using a steel auger (2.5 cm diameter) for analysis at 0, 30, 60, 102, 151, and 183 days after the bacterial inoculation. Brachiaria roots were assessed for mycorrhizal colonization based on the gridline plate method (McGonigle et al., 1990) after staining with 0.05% trypan blue (Brundrett et al., 1996) in four sampling dates (60, 102, 151, and 183 days). The shoots of brachiaria were cut at 5 cm above the ground to assess dry weight and N and P concentrations (EMBRAPA, 2009) on days 60, 102, and 183. Successive cuttings of brachiaria shoots also aimed to stimulate AMF sporulation and enrich the compost with AMF propagules.

From freshly taken, moist compost samples, mineral-N was extracted using 2 mol L^{-1} KCl (Schuster & Schroder, 1990). Ammonium-N (NH_4^+-N) was quantified in the extracts by the salicylate green method (Searle, 1984), and nitrate (NO_3^--N) was quantified using the Griess reagent method (Miranda et al., 2001). Both readings were taken in triplicate in a microplate reader Asys UVM 340 (Asys Hitech GMBH, Eugendorf,

Austria). Available P was determined on days 0 and 183 in Mehlich-I extract using molecular absorption spectrometry (EMBRAPA, 2011).

For determining the total concentrations of nutrients in the compost, samples were oven-dried at 60 °C for 48 hours and milled (<1 mm). Aliquots of approximately 2 g were crushed in a crucible to determine the total organic carbon (TOC) using the dry combustion method in a TOC device analyzer Elementar, model Vario TOC Cube (Elementar, Langenselbold, Germany) (Carmo & Silva, 2012). The total concentrations of P, K, Ca, Mg, S, B, Cu, Mn, and Zn were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) after microwave-assisted nitric-perchloric acid digestion of the samples. Total N was determined by Kjeldahl distillation after sulfuric acid digestion (EMBRAPA, 2009).

In the second part of this study, experiments with corn and wheat were carried out in the Experimental Farm at Embrapa Soybean, Londrina, PR, Brazil (23° 11' 2.75" S and 51° 10' 29.71" W, altitude 620 m) between March and August 2017. According to Köppen's classification, the climate of the region is Cfa (Humid subtropical); the meteorological data during the experimental period are presented in Figure 1. The soil is clay textured, classified as Rhodic Eutrudox, whose chemical characteristics and granulometric fractions are presented in Table 1.

The corn hybrid BM 709 PRO2 was sown on March 15, 2017, in plots consisting of five rows 0.9 m apart and 5.5 m long sown to a density of 5 plants m^{-1}, and fertilized with 300 kg ha^{-1} N-P-K (08-20-20) and 67.5 kg ha^{-1} N (urea) as topdressing at 30 days after sowing (DAS). Wheat BRS Gralha Azul was sown on May 9, 2017, in plots consisting of 19 rows spaced 0.17 m apart and 6.5 m long sown to a final density of 65-70 plants m^{-1}, and fertilized with 250 kg ha^{-1} N-P-K (08-20-20) and 30 kg ha^{-1} N (urea) as topdressing at 25 DAS. The composts were applied in the sowing rows at ~810 kg ha^{-1} for corn, and on the soil surface at ~860 kg ha^{-1} for wheat, to provide ~4000 AMF propagules m^{-2} for both crops.

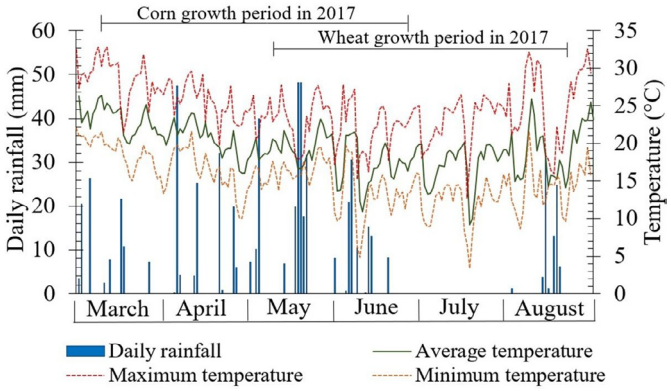


Figure 1. Daily climatic data on rainfall, minimum, maximum, and average air temperatures between March and August 2017 during the corn and wheat cropping

Both crops were sown under a no-till system, on an area previously grown with soybean (*Glycine max*). The experimental design was a randomized block with eight treatments (the seven composts previously produced and the control without compost) and six replicates.

To determine the shoot dry weight (SDW) at the vegetative stage, five plants of corn per plot and all the wheat plants in 1 m row per plot were sampled at 35 and 30 DAS, respectively, and oven-dried at 65 °C until a constant weight was achieved.

During the full flowering stage, the medium third part of 15 +3 leaves of corn without nervures, and 30 flag leaves of wheat were sampled per plot. After drying at 48 °C until constant weight, the samples were milled (<1 mm) and analyzed for N and P, as above described. Simultaneously, fine root samples were taken from the 0-10 cm of soil layer and 5 cm from the sowing rows and processed for AMF colonization, as above described. Corn was harvested on June 29, 2017, whereas wheat was harvested on August 21, 2017, to estimate the grain yield with moisture adjusted to 13%.

The normality and homoscedasticity of the datasets were analyzed with Shapiro-Wilk and Hartley's tests, respectively. Once the prerequisites were fulfilled, a one-way analysis of variance was applied at $p \leq 0.05$. For the greenhouse study, means were compared with Tukey's test, whereas Duncan's test was applied for the field data, both at $p \leq 0.05$.

Results and Discussion

Shoot biomass accumulation of brachiaria was incremental during the days after transplanting, but the effect of enrichment with microorganisms was observed only at the 60th day after transplanting. Greater values of shoot biomass were observed in plants solely inoculated with AMF (T3) than in the non-inoculated plants (T2), even with non-perceivable mycorrhizal colonization at that sampling (Table 2). Enrichment with PGPB + AMF (T4-T7) did not affect the shoot biomass production compared with T2 and T3.

Mycorrhizal colonization of brachiaria roots started slowly and was detected only after day 102 (Table 2). Costa et al. (2012) recorded colonization varying between 40 and 50% at 60 days after inoculation of *U. brizantha* with *Rhizophagus* spp. The colonization rate may have been slow in this study because of the high P availability in the compost at the beginning of

Table 1. Soil chemical characteristics and granulometric fractions at 0-20 and 20-40 cm layers at the experimental sites cultivated with corn and wheat in the autumn-winter 2017 cropping season before sowing

Layer (cm)	pH	Clay	Silt	Sand	CEC	Ca	Mg	K	Na	P	Cu	Fe	Mn	Zn
		(%)			($cmol_c\ dm^{-3}$)					(mg dm^{-3})				
Corn														
0-20	5.3	74.2	19.0	6.8	12.3	5.63	2.47	0.41	0.03	19.7	7.8	112.8	108.7	0.9
20-40	5.4	77.7	16.2	6.1	10.9	4.09	1.78	0.36	0.01	5.1	12.6	107.0	442.8	2.4
Wheat														
0-20	5.1	74.2	16.5	9.4	12.3	4.16	1.78	0.90	0.01	10.0	14.2	100.9	522.7	2.6
20-40	5.2	77.0	15.6	7.4	10.9	4.05	1.53	0.56	0.01	5.7	12.7	103.3	452.2	1.5

pH in 0.01 mol L^{-1} $CaCl_2$; Ca, Mg, K and Na in Mehlich-III by ICP-OES; Available P in Mehlich-I (EMBRAPA, 2009)

Table 2. Accumulated shoot dry biomass, mycorrhizal root colonization, and concentrations of N and P in the shoots of brachiaria (*Urochloa brizantha*) during the days after bacterial inoculation in the compost for enrichment with beneficial microorganisms

Treatments	Days after bacterial inoculation in the compost					
	60	81	102	122	151	183
	Accumulated shoot dry biomass (g tray^{-1})					
T1 - Compost (C)	-	-	-	-	-	-
T2 - C + non-AMF brachiaria	66.7 b	172 a	292 a	384 a	452 a	515 a
T3 - C + AMF brachiaria	84.4 a	195 a	311 a	407 a	470 a	532 a
T4 - C + AMF brachiaria + *Azorhizobium*	79.3 ab	183 a	297 a	392 a	451 a	511 a
T5 - C + AMF brachiaria + *Azoarcus*	74.3 ab	179 a	300 a	387 a	436 a	495 a
T6 - C + AMF brachiaria + *B. subtilis*	80.4 ab	180 a	302 a	389 a	439 a	493 a
T7 - C + AMF brachiaria + *Azotobacter*	81.8 ab	174 a	294 a	387 a	438 a	493 a
Average	77.8	180	299	391	448	507
CV (%)	7.09	6.00	5.64	4.69	4.90	5.06
	Mycorrhizal colonization (%)					
T1 - Compost (C)	-	-	-	-	-	-
T2 - C + non-AMF brachiaria	0	-	7 ab	-	8 b	7 b
T3 - C + AMF brachiaria	0	-	13 a	-	30 a	39 a
T4 - C + AMF brachiaria + *Azorhizobium*	0	-	8 ab	-	35 a	47 a
T5 - C + AMF brachiaria + *Azoarcus*	0	-	9 ab	-	39 a	43 a
T6 - C + AMF brachiaria + *B. subtilis*	0	-	6 b	-	40 a	50 a
T7 - C + AMF brachiaria + *Azotobacter*	0	-	10 ab	-	52 a	53 a
Average	-	-	16.9	-	34.7	37.9
CV (%)	-	-	13.8	-	17.9	19.5
	Shoot N concentration (g kg^{-1})					
T1 - Compost (C)	-	-	-	-	-	-
T2 - C + non-AMF brachiaria	28.4 a	-	22.9 a	-	-	10.9 a
T3 - C + AMF brachiaria	27.0 a	-	21.1 ab	-	-	10.9 a
T4 - C + AMF brachiaria + *Azorhizobium*	26.0 a	-	16.0 ab	-	-	10.1 ab
T5 - C + AMF brachiaria + *Azoarcus*	27.5 a	-	15.0 ab	-	-	9.26 b
T6 - C + AMF brachiaria + *B. subtilis*	27.2 a	-	14.6 b	-	-	9.50 ab
T7 - C + AMF brachiaria + *Azotobacter*	27.3 a	-	15.9 ab	-	-	9.57 ab
Average	27.2	-	17.6	-	-	10.0
CV (%)	4.62	-	16.5	-	-	5.31
	Shoot P concentration (g kg^{-1})					
T1 - Compost (C)	-	-	-	-	-	-
T2 - C + non-AMF brachiaria	3.84 a	-	3.78 a	-	-	3.08 a
T3 - C + AMF brachiaria	3.79 a	-	3.80 a	-	-	3.11 a
T4 - C + AMF brachiaria + *Azorhizobium*	3.54 a	-	4.08 a	-	-	3.72 a
T5 - C + AMF brachiaria + *Azoarcus*	3.65 a	-	3.62 a	-	-	3.03 a
T6 - C + AMF brachiaria + *B. subtilis*	3.32 a	-	3.54 a	-	-	3.54 a
T7 - C + AMF brachiaria + *Azotobacter*	3.32 a	-	3.70 a	-	-	3.21 a
Average	3.61	-	3.75	-	-	3.28
CV (%)	9.41	-	10.3	-	-	10.4

Different letters in the columns indicate statistical differences among treatments by the Tukey's test at $p \leq 0.05$; AMF - Arbuscular mycorrhizal fungus; CV - Coefficient of variation; "-" - Not assessed

the experiment (951 mg kg^{-1}), which increased three times by the end of the experiment (2927 mg kg^{-1}) owing to the mineralization of organic P (Andrade et al., 2018; Sousa et al., 2018). Nevertheless, the mycorrhizal colonization increased by 183rd day and reached ~50% in plants of T6 (AMF + *B. subtilis*) and T7 (AMF + *Azotobacter*) treatments. This level of colonization suggests an enrichment of the compost with AMF propagules, not only by internal hyphae in the roots but also by external hyphae and spores. The previous inoculation of the compost with PGPB did not affect the brachiaria AMF colonization (Table 2), suggesting that none of the bacterial isolates worked as mycorrhiza helpers at this stage (Choudhary et al., 2017). The non-AMF control plants (T2) showed slight AMF root colonization, indicating the presence of AMF propagules in the compost, as the composting pile was in contact with the soil during the composting process. Even so, colonization was ~7% on day 183, in contrast with the colonization up to ~50% in plants inoculated with *R. clarus* (Table 2), showing that the introduced AMF propagules were more effective than the native propagules from the soil (Samarão et al., 2011).

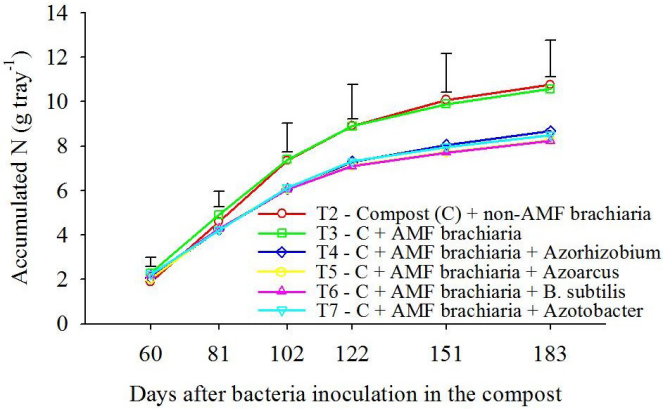
A pH close to neutral is considered adequate for *Rhizophagus* (Brito et al., 2017). Nevertheless, P availability is one of the main factors affecting mycorrhizal colonization (Costa et al., 2012), which is usually inversely proportional to P availability (Schiavo et al., 2010). Although AMF may induce growth depression in the host plant under high P availability owing to drain of photoassimilates (Zangaro et al., 2018), no negative effect was observed in accumulated shoot biomass compared with the non-mycorrhizal plants (Table 2). Physic nut (*Jatropha curcas* L.) also showed a low response to *R. clarus*, and, depending on the dose of compost, there was a negative effect on plant growth (Schiavo et al., 2010). Mycorrhizal colonization caused growth depression in *Urochloa* plants in a high-fertility soil; conversely, mycorrhizal plants grown in a low-fertility soil grew twice as much the non-mycorrhizal ones (Zangaro et al., 2018).

The average shoot N concentrations decreased from 27.2 to 10 mg kg^{-1} from days 60 to 183 (Table 2). Plants in treatments

T6 (AMF + *B. subtilis*) and T5 (AMF + *Azoarcus*) showed lower N concentrations than the non-mycorrhizal brachiaria (T2) at days 102 and 183, respectively. There was no effect of treatments on shoot P concentrations, which remained constant throughout the experiment (Table 2). The effect of AMF inoculation on leaf P concentration was not expected because of the high concentration of available P in the compost, which was high enough for plants to reach optimum P levels without the aid of AMF (Brito et al., 2017).

The treatments affected shoot N accumulation from day 102 onwards (Figure 2) when non-inoculated plants (T2) and plants inoculated solely with AMF (T3) had higher N accumulation than plants inoculated with AMF + PGPB. Inoculation of PGPB in the compost (T4-T7) may have affected the mineral-N dynamics, affecting N concentration and accumulation in plants.

Brachiaria plants absorbed and exported part of the N in its biomass (Figure 2), as evidenced by a decrease in nitrate-N concentrations in the compost, compared with the control (T1) between day 60 and the end of the trial (Table 3). In addition, PGPB affected both ammonium-N and nitrate-N concentrations in the substrate, which increased or decreased, depending on the inoculated PGPB.



Vertical bars represent the least significant difference of Tukey test at $p \leq 0.05$

Figure 2. Accumulated N in the shoots of brachiaria (*Urochloa brizantha*) during the days after bacterial inoculation in the compost for enrichment with beneficial microorganisms

On day 60, nitrate-N concentrations decreased in the compost that received mycorrhizal plants and was inoculated with PGPB (T4-T7) compared with the compost that received non-inoculated plants (T2), especially in the *Azotobacter* (T7) treatment (Table 3). On day 102, the lowest concentrations of nitrate-N were found in the compost inoculated with *B. subtilis* (T6) and *Azotobacter* (T7). On days 151 and 183, the compost inoculated with *Azotobacter* (T7) still had lower nitrate-N concentrations. This decrease in nitrate-N did not arise from plant uptake, since N concentrations (Table 2) and accumulations in the biomass (Figure 2) were similar among treatments that received PGPB. This suggests that some PGPB may have led to N losses via denitrification, especially *Azotobacter* (Furina et al., 2002) and *Azoarcus* (Lee et al., 2014), which are N-reducing bacteria.

The highest ammonium-N concentrations in the compost were observed in *B. subtilis* (T6) treatment on days 30 and 102, whereas in *Azotobacter* (T7) treatment, the highest ammonium-N concentrations were observed on day 183 (Table 3). These are known as free-living N-fixing microorganisms and may have transiently increased the ammonium-N concentration in the substrate. Inoculation of free-living N-fixing bacteria has been used as strategy to enrich organic compost with N (Sousa et al., 2018).

The chemical characteristics of the compost, such as C:N ratio (10.7-11.2), slightly alkaline pH (7.2-7.4), and high amounts of total N (15.4-16.3 g kg^{-1}) may have been favorable to N mineralization followed by denitrification (Table 4). pH values close to neutral are favorable for the oxidation of ammonium-N to nitrate-N by nitrifying microorganisms, which expose nitrate-N to losses via denitrification (Schuster & Schroder, 1990), and help to explain the decreases in

Table 3. Concentrations of nitrate-N and ammonium-N in the mature compost during the days after bacterial inoculation in the compost for enrichment with beneficial microorganisms

Treatments	Days after bacterial inoculation in the compost					
	0	30	60	102	151	183
			Nitrate-N (mg kg^{-1})			
T1 - Compost (C)	279.8 a	371.1 a	748.2 a	449.1 a	978.1 a	1193 a
T2 - C + non-AMF brachiaria	254.2 a	387.7 a	419.4 b	76.1 b	15.2 bc	13.3 bc
T3 - C + AMF brachiaria	214.7 a	350.6 a	278.3 cd	60.2 bc	16.4 bc	12.1 bc
T4 - C + AMF brachiaria + *Azorhizobium*	211.4 a	344.2 a	315.8 c	54.0 bc	23.7 b	18.1 b
T5 - C + AMF brachiaria + *Azoarcus*	247.2 a	367.5 a	203.5 de	45.7 bc	21.4 bc	17.4 b
T6 - C + AMF brachiaria + *B. subtilis*	225.1 a	392.9 a	285.0 cd	25.6 c	12.5 bc	8.9 bc
T7 - C + AMF brachiaria + *Azotobacter*	260.3 a	278.4 a	168.7 e	28.4 c	10.7 c	5.2 c
Average	241.8	356.0	345.5	105.6	154.0	182.1
CV (%)	12.3	14.7	9.6	12.8	2.91	2.34
			Ammonium-N (mg kg^{-1})			
T1 - Compost (C)	15.3 a	70.7 c	18.3 a	28.4 c	12.8 a	7.90 c
T2 - C + non-AMF brachiaria	25.3 a	71.0 bc	25.3 a	33.4 c	1.81 b	7.48 c
T3 - C + AMF brachiaria	24.9 a	77.9 bc	19.1 a	34.9 c	1.84 b	10.2 b
T4 - C + AMF brachiaria + *Azorhizobium*	20.0 a	77.0 bc	16.6 a	42.5 c	2.75 b	5.38 d
T5 - C + AMF brachiaria + *Azoarcus*	26.8 a	78.8 bc	21.2 a	39.2 c	1.79 b	4.32 d
T6 - C + AMF brachiaria + *B. subtilis*	18.1 a	94.6 a	20.7 a	116.4 a	3.30 b	4.23 d
T7 - C + AMF brachiaria + *Azotobacter*	30.5 a	80.0 b	25.6 a	85.8 b	3.04 b	12.3 a
Average	23.0	78.4	21.0	54.4	3.91	7.40
CV (%)	30.5	3.7	17.3	16.3	28.7	8.5

Different letters in the column indicate statistical difference among treatments by the Tukey's test at $p \leq 0.05$; AMF - Arbuscular mycorrhizal fungus; CV - Coefficient of variation

Table 4. Chemical characteristics (in the average of the composts, n = 21) and total concentrations of macro- and micronutrients in the mature compost during the days after bacterial inoculation in the compost for enrichment with beneficial microorganisms

Chemical characteristic	Days after bacterial inoculation in the compost					
	0	30	60	102	151	183
pH (H_2O)	7.3	7.2	7.3	7.3	7.4	7.4
Total C (g kg^{-1})	17.3	17.4	17.4	16.7	17.2	17.8
Total N (g kg^{-1})	16.1	16.3	16.3	15.7	15.4	15.9
C:N	10.9	10.8	10.8	10.7	11.2	11.2
P (g kg^{-1})	7.25	7.13	7.40	7.17	7.14	7.16
K (g kg^{-1})	3.74	3.81	3.30	2.65	2.10	2.31
Ca (g kg^{-1})	17.2	17.4	18.1	19.0	18.8	19.0
Mg (g kg^{-1})	5.44	5.31	5.45	6.04	6.11	6.26
S (g kg^{-1})	1.86	1.83	1.91	1.53	1.61	1.70
B (mg kg^{-1})	303	282	289	322	325	319
Cu (mg kg^{-1})	154	119	119	126	122	131
Mn (mg kg^{-1})	1246	1215	1244	1326	1332	1325
Zn (mg kg^{-1})	154	138	142	139	144	144

nitrate-N especially in the treatments with *B. subtilis* (T6) and *Azotobacter* (T7) (Table 3).

The chemical analysis of the compost at each sampling time showed slight increases in the relative concentrations of some nutrients (e.g., Ca and Mg) that can be consequence of mineralization of the organic fraction (Andrade et al., 2018; Sousa et al., 2018), or decreases (e.g., K and S) resulting from absorption and exportation in the plant biomass. On day 183, the final compost contained significant amounts of macro and micronutrients that may contribute to plant nutrition. These concentrations fulfill the minimal requirements for legal framing as organic fertilizer to be applied in the soil, based on the Normative Instruction 25/2009 of the Ministry of Agriculture, Livestock and Food Supply (Brasil, 2009). Thus, urban and rural organic residues from several human activities can be useful as sources of nutrients to crops (Mota et al., 2019).

The use of organic compost in the field with corn crop did not increase the SDW at 35 DAS (Table 5), but the compost enriched with PGPB (T4-T7) increased the percentage of AMF colonization compared with that in the non-enriched composts (T1 and T2). However, increase in mycorrhizal colonization did not affect leaf N and P concentrations (Table 5). Mycorrhizal colonization observed in plants that received non-enriched compost was owing to the native AMF occurring in the soil.

The national and state corn yields averaged 5564 and 5456 kg ha^{-1}, respectively (CONAB, 2018), whereas the yields in the present experiment averaged 4904 kg ha^{-1}. The yield of plants fertilized with compost enriched only with AMF (T3) averaged 23% over the control without compost (NC), T5 (AMF + *Azoarcus*), and T6 (AMF + *B. subtilis*). These results show that the enrichment only with AMF can be beneficial for corn yield.

The enriched organic compost did not increase the SDW of wheat at 30 DAS (Table 5), but enriching the compost with AMF increased root colonization of the plants in the field, independent of PGPB. No effect was observed on leaf N concentration, but leaf P concentration increased in plants that received compost enriched with AMF and PGPB (T4, *Azorhizobium* and T5, *Azoarcus*) compared with those that received compost with non-AMF brachiaria (T2), although it did not differ from the controls NC and T1 (sole compost). The average grain yield in this experiment was 3084 kg ha^{-1}, whereas the average national and state yields were 2225 and 2308 kg ha^{-1}, respectively (CONAB, 2018). Grain yield increased in the treatment using compost enriched with AMF+PGPB, compared with that in the AMF + brachiaria (T3) or only brachiaria (T2) treatment.

Rhizophagus was more effective than *Acaulospora* in increasing the accumulation of N and P in wheat plants that

Table 5. Shoot dry weight (SDW), root colonization of arbuscular mycorrhizal fungi (AMF colonization), N and P leaf concentrations, and grain yields of corn and wheat fertilized with organic compost enriched with AMF and plant growth-promoting bacteria, Londrina, PR, Brazil, 2017, autumn-winter cropping season

Treatments	SDW (g $plant^{-1}$ or m^{-1})	AMF colonization (%)	Leaf N (g kg^{-1})	Leaf P (g kg^{-1})	Yield (kg ha^{-1})
			Corn		
NC - No compost	14.8 a	-	29.8 a	3.02 a	4585 b
T1 - Compost (C)	13.8 a	27 c	30.2 a	2.98 a	5111 ab
T2 - C + non-AMF brachiaria	14.6 a	33 bc	29.5 a	2.96 a	5103 ab
T3 - C + AMF brachiaria	15.6 a	44 ab	28.6 a	2.83 a	5576 a
T4 - C + AMF brachiaria + *Azorhizobium*	13.9 a	55 a	28.2 a	2.95 a	4820 ab
T5 - C + AMF brachiaria + *Azoarcus*	12.5 a	54 a	28.6 a	3.03 a	4366 b
T6 - C + AMF brachiaria + *B. subtilis*	12.4 a	51 a	28.1 a	2.92 a	4633 b
T7 - C + AMF brachiaria + *Azotobacter*	12.1 a	56 a	29.3 a	2.98 a	5035 ab
Average	13.7	46	29.0	2.96	4904
CV (%)	23.5	15.0	5.78	4.64	11.7
			Wheat		
NC - No compost	58.7 a	-	38.5 a	2.21 abc	3043 ab
T1 - Compost (C)	58.7 a	26 b	35.5 a	2.28 a	3161 a
T2 - C + non-AMF brachiaria	63.5 a	33 b	36.2 a	2.10 c	2873 b
T3 - C + AMF brachiaria	56.3 a	50 a	34.7 a	2.11 bc	2926 b
T4 - C + AMF brachiaria + *Azorhizobium*	59.4 a	51 a	34.0 a	2.33 a	3161 a
T5 - C + AMF brachiaria + *Azoarcus*	58.7 a	50 a	32.3 a	2.26 ab	3132 a
T6 - C + AMF brachiaria + *B. subtilis*	52.0 a	49 a	33.8 a	2.24 abc	3195 a
T7 - C + AMF brachiaria + *Azotobacter*	52.6 a	52 a	35.7 a	2.19 abc	3181 a
Average	57.5	44	31.1	2.21	3084
CV (%)	26.8	13.8	9.57	5.27	7.34

Different letters in the column indicate statistical difference among treatments by the Duncan's test at $p \leq 0.05$; AMF - Arbuscular mycorrhizal fungus; CV - Coefficient of variation; "-" - Not assessed

were co-inoculated with PGPB (Sala et al., 2007). Despite no effect on the initial plant growth and no clear effect on corn grain yield, the consistent increase in AMF colonization in both crops may improve plant performance under adverse environmental conditions, such as moderate drought (Wu et al., 2013). P availability in the soil was not limiting (Table 1), and, consequently, the likelihood of plant yield response to AMF under regular climatic conditions is low. In addition, competitiveness with native microorganisms in the soil, including AMF, may limit the plant response to selected beneficial microorganisms (Samarão et al., 2011). The observed responses on leaf P concentrations, root colonization, and grain yield when using compost enriched with mycorrhizal fungus compared with those using non-enriched compost instigates further studies on mycorrhizal inoculation in the field.

Conclusions

1. The enrichment with plant growth-promoting bacteria changed the mineral-N dynamics in the compost. Depending on the sampling date, an increase in ammonium-N concentration was observed when inoculated with *Bacillus subtilis* or *Azotobacter* and a decrease in nitrate-N concentration was observed when inoculated with *Azoarcus*, *B. subtilis*, or *Azotobacter*.

2. *Urochloa brizantha* grown in the compost multiplied the mycorrhizal fungus *Rhizophagus clarus* in their roots and allowed enrichment of the compost with propagules that increased mycorrhizal colonization of corn and wheat and the yield of wheat in the field.

3. The compost enriched with beneficial microorganisms showed sufficient total concentrations of macro- and micronutrients that qualify its use as an organic fertilizer according to Brazilian legislation.

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