

## In-furrow application of arbuscular mycorrhizal fungi propagules enhanced yields, plant vigor, and biomass accumulation of soybeans (*Glycine max*) and maize (*Zea mays*)

Rafael Munhoz Pedroso<sup>1</sup>, Carlos Antônio Medeiros<sup>2</sup>, Alexandre Ometto<sup>2</sup>, Viviane Paes<sup>2</sup>, Eduardo Figueiredo de Andrade<sup>2</sup>, Scott Inman<sup>3</sup>, Jefferson Rangel da Silva<sup>2</sup>, Gabriel Munhoz Pedroso<sup>2\*</sup>

<sup>1</sup>University of Sao Paulo, “Luiz de Queiroz” College of Agriculture (ESALQ/USP), Piracicaba, SP, Brazil

<sup>2</sup>Sumitomo Chemical do Brasil, São Paulo, SP, Brazil

<sup>3</sup>Mycorrhizal Applications, Grants Pass, Oregon, USA

\* Corresponding author: [scott.inman@mycorrhizae.com](mailto:scott.inman@mycorrhizae.com)

### Abstract

Highly weathered soils are commonly found in most crops cultivation sites of Brazil, such as maize and soybean. They impose many challenges for plant growth, such as reduced nutrient availability. Hyphal networks, resulting from mutualistic symbiotic associations between plants and arbuscular mycorrhizal fungi (AMF), could be a viable alternative to improve plant growth and nutrition by enhancing root access to a significantly larger soil surface area, increasing nutrient availability and uptake. Thus, we aimed at evaluating in-furrow applications of propagules from four AMF species to enhance soybeans and maize yields, plant vigor, plant height, and biomass accumulation. Thereby, four soybeans and six maize field trials were conducted in the 2018/2019 growing season in Brazil in different environments. Two EndoMaxx SC rates (150 or 300 ml ha<sup>-1</sup>) were tested, with or without P fertilization during sowing. Results indicated that both rates positively impacted all variables analyzed, regardless of crop species and P fertilization. Even in the absence of P fertilization, AMF-colonized soybeans and maize treatments were either significantly higher or similar to the untreated control. Moreover, crop yield was 2.6-9.1% more when the highest rate of AMF was applied in combination with P fertilization compared to the P-fertilized untreated control. In-furrow EndoMaxx SC applications are an alternative to increase the sustainability of soybean and maize production, especially in a scenario, in which yield must be maximized in the coming decades to supply food for the exponentially growing population without expanding cultivations to new sites.

**Keywords:** symbiosis; crop productivity; phosphorus; *Rhizophagus irregularis*; *Claroideoglomus luteum*; *Claroideoglomus etunicatum*; *Claroideoglomus claroideum*.

**Abbreviations:** AMF\_arbuscular mycorrhizal fungi; DAS\_days after sowing; DAB\_dry aboveground biomass; TGW\_thousand grain weight (TGW).

### Introduction

Soils in tropical and subtropical humid regions commonly display an overall low diversity of mineral species, as well as advanced pedogenetic developmental stages (e.g. highly weathered soils), representing the most evolved soils on Earth (Allen and Hajek, 1989). Highly weathered soils (such as latosols, which account for roughly 30% of Brazil's territory) are commonly found in several soybeans (*Glycine max* (L.) Merr.) and maize (*Zea mays* L.) cultivation sites in Brazil, one of the most important grain producers of the world (Lepsch, 2002). Despite displaying desirable physical properties (such as depth and porosity), weathered soils present reduced fertility, which is one of the major obstacle's farmers need to overcome to achieve high yields (Fink et al., 2014). Indeed, the availability

of essential nutrients, such as P, can be low due to its high adsorption into soil colloids and acidic conditions. The latter is also widespread in these regions and is known to severely damage soybeans and maize growth due to high exchangeable Al<sup>3+</sup> concentrations (Embrapa, 1997).

Roughly 90% of plant species establish mutualistic associations with microorganisms. Associations between plants and obligate biotrophic fungi of the phylum Glomeromycota form interdependent connections known as arbuscular mycorrhiza, which in turn might significantly enhance nutrient extraction from the soil and uptake by the host plant (Smith and Smith, 2012; Ahanger et al., 2014). Upon physical contact with plant roots, arbuscular mycorrhizal fungi (AMF) develop an infection

structure called an appressorium so that its penetration into the host is facilitated by the presence of plant cutin (Bonfante and Genre, 2010; Wang et al., 2012). Once inside cortex cells, hyphae structures, known as platoon, and arbuscules are formed (Harrison, 2005; Parniske, 2008).

In exchange for the carbohydrates photosynthetically produced by plants, hyphal networks resulting from mutualistic symbiotic associations between plants and AMF provide improvements to plant growth and nutrition (Rouphael et al., 2015; Bowles et al., 2016). Indeed, improved root access to a significantly larger soil surface area, increases nutrient availability and translocation within the host species, since arbuscules act as haustoria and form a far-reaching interface for nutrient exchange in combination with the periarbuscular membrane (Parniske, 2008; Bonfante and Genre, 2010). Moreover, enhanced  $H_2PO_4^-$  absorption by plants, as a result of overexpression of key transporters by AMF, is a key physiological process by which AMF improve plant growth (Bucher, 2007). These transporters can directly absorb ions from the soil and release them into the arbuscular mycorrhiza interface with the plant, where specific plant transporters interact with such nutrients and ultimately transfer these to the plant cell cytoplasm (Guether et al., 2009; Vergara et al., 2018). Enhanced absorption by transporters and transfer of nitrogen to the periarbuscular space are also key mechanisms by which AMF promote plant growth (López-Pedrosa et al., 2006; Smith and Smith, 2011). Once released into the periarbuscular mycorrhiza interface, specific plant transporters transfer nitrogen ions to the cytosol of plant cells (Guether et al., 2009).

Baslam et al. (2011) reported larger concentrations of mineral nutrients and plant protection compounds, such as carotenoids, tocopherols, and soluble phenolics, resulting from successful mycorrhizal symbiosis, whereas enhanced strawberry plant growth was observed due to improved antioxidant properties (Castellanos-Morales et al., 2010). Reports from AMF-enrichment field trials suggested a large potential for yield improvements in maize (Sabia et al. 2015), potatoes (*Solanum tuberosum* L.) (Hijri, 2016), and yam (*Dioscorea alata* L.) (Lu et al., 2015). Other benefits arising from plant-AMF associations include more vigorous plant growth in response to higher photosynthetic activity and water uptake rates (Birhane et al., 2012); abiotic stress mitigation due to the maintenance of soil pH (Rouphael et al., 2015), expedited soil organic matter decomposition (Paterson et al., 2016), amplified resistance to biotic stress, such as salinity and drought (Rodríguez et al., 2008; Ahanger et al., 2014; Salam et al., 2017), as well as causing soil structure and texture modifications, incurring in better plant health due to greater soil quality (Zou et al., 2016; Thirkell et al., 2017).

Thereby, plant-AMF beneficial mutualistic associations present a unique opportunity for improving crop growth and yields. Despite the large inherent potential arising from the utilization of AMF as soil conditioners, selecting the best microorganism species, as well as their propagule concentration and viability, represent a complex process. Given the widespread cultivation of soybeans and maize under suboptimal soil conditions, the present work aimed to assess whether a mixture of four AMF species can enhance soybeans and maize yields and plant growth due to mycorrhizal symbiosis.

## Results and Discussion

Supplementary Table S1 presents weather and other key information concerning four soybeans and six maize field trials designed to study AMF-enrichment effects onto these crops, respectively. Soybean and maize-growing sites presented edaphoclimatic conditions to which such crops are commonly exposed in Brazil, as well as displayed P levels in the soil (Supplementary Table S1 and Fig S1). The list of treatments can be observed in Table 1. Moreover, information about cultivar, sowing dates, target plant density, and row spacing is found in Table 2. A full description of treatments is presented under Material and methods.

### Soybeans trials

ANOVA results indicated that treatments significantly affected plant height, vigor, and yields at the 5% of probability ( $p < 0.05$ ) (Supplementary Table S2). Treatment effects on dry aboveground biomass (DAB) were only significant at the 10% significance level ( $p = 0.053$ ), whereas no significant effects were observed for thousand-grain weight (TGW) ( $p = 0.252$ ) (Supplementary Table S2). Furthermore, treatment effects on both plant vigor and yield varied significantly across locations (Supplementary Table S2). Since a significant *Treatment x Location* interaction indicates that the actual treatment effect varied according to each experimental site, plant vigor and yields were also analyzed separately for each location (Supplementary Table S2). Conversely, data from other variables found to be impacted by treatments (i.e. plant height and DAB) were pooled for discussion, since they were not significantly affected by the experimental site ( $p > 0.05$ ).

Results indicated a general trend towards slightly taller plants with the inoculation of arbuscular mycorrhizal fungi (AMF) propagules (Table 3), although none of these treatments differed significantly from their respective control (treatments 1 and 4) with or without P fertilization at crop sowing (Table 3). On the other hand, plant vigor showed a more significant increase in response to the addition of AMF propagules, even in the absence of P fertilization (Treatments 2 and 3), while the greatest vigor was scored at the highest product rate in combination with P fertilization (Treatment 6). Interestingly, treatments that only received AMF propagule applications without P fertilization (Treatments 2 and 3) presented a tendency towards higher average vigor scores than the untreated control + P fertilized counterpart (Treatment 4) (Table 3), suggesting AMF propagule applications might enhance the plants' nutrient uptake ability, as previously demonstrated by many reports in the literature (Ahanger et al., 2014; Bowles et al., 2016; Rouphael et al., 2015; Smith and Smith, 2012). Similarly, DAB was increased with the application of AMF propagules, but large data variability within each treatment affected mean separation negatively and impaired its ability to detect differences among treatments (Table 3).

TGW was not significantly affected by treatments (AMF and/or P fertilization). (Table 3). However, soybean yields were significantly larger in most treatments receiving in-furrow AMF propagule applications relative to the respective untreated control treatments in each category (e.g. with or without P fertilization) (Table 3). When P fertilization was not performed, both product rates tested at the present work (150 and 300 ml  $ha^{-1}$ ) increased soybean yields significantly, increasing

productivity by 9.1% and 8.9%, respectively, relative to the untreated control counterpart (Table 3). Moreover, when P fertilization was performed, yield increases of 2.6% and 7% were recorded at each product rate compared to the P-fertilized untreated control treatment. However, only the highest product rate incurred significantly larger yields when compared to the untreated control (Table 3).

Soybean yields and plant vigor were also analyzed separately according to data collected in each experimental site, given that treatment effects onto these variables varied across locations (as indicated by a significant *Treatment x Location* interaction for those variables on Supplementary Table S2). Importantly, a statistically significant increase was noticed across all sites on both variables (Fig 1). However, the actual intensity (or percent increase) of these parameters varied across sites. The largest increases in plant vigor were noticed at Uberlândia/MG and Planaltina/DF – sites with the lowest soil P values in this study (Supplementary Table S1), whereas soybean yield increases were the greatest at Ponta Grossa/PR and Lavras/MG experimental sites, despite Ponta Grossa/PR displaying the largest P availability among the locations (Supplementary Table S1, Fig 1). Such more pronounced yield increases reported in experimental sites with lower P availability can be related to benefits arising from functional AMF symbiosis, since the resulting larger soil surface and enhanced P absorption (Bucher, 2007; Guether et al., 2009; Vergara et al., 2018) would be expected to play a major role in supplying P to crop plants to offset natural low P availability conditions.

#### **Maize trials**

ANOVA results indicated that all treatments with EndoMaxx SC significantly affected plant height, vigor, TGW, and maize yields at the 5% level ( $p < 0.05$ ) (Supplementary Table S3), whereas no significant effects were observed for DAB ( $p = 0.465$ ). Treatment effects onto plant vigor and DAB varied significantly across locations. Since a significant *Treatment x Location* interaction indicates that the actual treatment effect varied across experimental sites, plant vigor and maize DBA values were analyzed separately at each experimental site. Conversely, plant heights, TGW, and maize yields data were pooled for discussion, as these were not significantly affected by location ( $p > 0.05$ ).

Similar to soybeans, results indicated a general trend towards slightly taller, more vigorous maize plants with in-furrow EndoMaxx SC inoculation (Table 4). Interestingly, average plant height in treatments only receiving AMF (treatments 2 and 3) equaled values recorded at treatments that did receive P fertilization during crop sowing (treatments 4-6) (Table 4). Moreover, plant vigor also presented an increase with the addition of AMF propagules, regardless of P fertilization status (with and without P) (Table 4). Both EndoMaxx SC rates displayed similar plant vigor scores, which indicates that increasing product rates from 150 to 300 ml ha<sup>-1</sup> did not change plant vigor. Interestingly, once again treatments that only received AMF propagule applications - but no P - (treatments 2 and 3) showed higher average vigor scores than P-fertilized, untreated control counterparts, strongly suggesting that AMF propagule applications enhance the plants' nutrient uptake ability (Table 4). Lastly, mean

separation results did not indicate a significant treatment effect on dry aboveground biomass (DAB) results (Table 4).

Unlike results presented for soybeans, treatments affected maize's TGW means significantly (Table 4). Moreover, TGW data collected from treatments 2 and 3 were similar to those of P-fertilized counterparts (treatments 5 and 6), while no significant difference between product rates was observed (Table 4). Maize yields were also significantly increased by in-furrow AMF propagule applications relative to both untreated control treatments (Table 4). Without P fertilization, both product rates increased maize yields significantly by nearly 4% relative to the untreated control counterpart. Even when P fertilization was performed, yield increases of 4% and 7.3% were observed for the rates of 150 and 300 ml ha<sup>-1</sup>, respectively, in comparison to the untreated control + P, closely resembling soybean yield results. Both product rates were found to allow for significantly larger maize yields compared to both untreated control - P and Untreated control + P (Table 4). These results agree with Sabia et al. (2015), who reported an 18% gain in dry matter yields, as well as increased P content in the whole plant when maize seeds were inoculated with AMF. Yield increases could be related to many complex processes. For instance, enhanced ammonium (NH<sub>4</sub><sup>+</sup>) absorption is expected to occur due to the expression of high- and low-affinity NH<sub>4</sub><sup>+</sup> transporters such as GintAMT1, GintAMT2, and GintAMT3 in *Rhizophagus irregularis* – one of four AMF species included in EndoMaxx SC, among other benefits (Calabrese et al., 2016). Moreover, AMF-inoculated maize has been shown to display improved osmoregulation capacity, which was correlated to its higher concentration of soluble sugars and electrolytes (Feng et al., 2002). Both have clear implications for maize grain production potential.

Maize plant vigor and DBA data were also analyzed separately according to location, given that treatment effects onto these variables varied across locations (as indicated by a significant *treatment x location* interaction for those variables on Supplementary Table S3). A significant increase in plant vigor was observed across all sites (Fig 2). However, the actual intensity (or percent of increase) set forth by treatments varied across sites. The largest increases in plant vigor were noticed at Ponta Grossa/PR, Lavras/MG, and Planaltina/DF despite contrasting soil P values in these sites (Supplementary Table S1). Furthermore, DBA values were significantly affected by treatments in three out of six locations (Itaara/RS, Ponta Grossa/PR, and Uberlândia/MG), as level of increase observed was reduced in the remaining locations (Fig 2).

#### **Final considerations**

Altogether, soybeans and maize results suggest that field inoculation with AMF propagules can increase crop yields, among other benefits. This is corroborated by reports in the literature, as AMF root colonization in several crops (including maize and soybeans) resulted in average yield increases of 37% (McGonigle, 1988). AMF field inoculation could potentially aid Brazilian soybeans and maize growers who are currently adopting a practice referred to as systems fertilization, at which nutrients, such as P and K, are broadcast applied right after harvest of the preceding crop. Since phosphate can be highly adsorbed onto mineral surfaces in tropical and subtropical soils – especially given the widespread occurrence of 1:1 clay minerals (Fink et al., 2014), such association with

Table 1. List of treatments applied in soybeans and maize.			
Treatment ID	Treatment description	P fertilization	Application type
1. Untreated control - P	No AMF <sup>1</sup> and no P added (-AMF; -P)	No	--
2. EndoMaxx SC <sup>2</sup> (150 mL ha <sup>-1</sup> ) - P	Low rate AMF mixture (0.5x AMF; -P)	No	In-furrow
3. EndoMaxx SC (300 mL ha <sup>-1</sup> ) - P	High rate AMF mixture (1x AMF; -P)	No	In-furrow
4. Untreated control + P	No AMF; only P added (-AMF; +P)	Yes	--
5. EndoMaxx SC (150 mL ha <sup>-1</sup> ) + P	Low rate AMF mixture with P added (0.5x AMF; +P)	Yes	In-furrow
6. EndoMaxx SC (300 mL ha <sup>-1</sup> ) + P	High rate AMF mixture with P added (1x AMF; +P)	Yes	In-furrow

<sup>1</sup>Arbuscular mycorrhizal fungi; <sup>2</sup>Product consisting of a concentrated solution containing 1,522 AMF propagules mL<sup>-1</sup>.

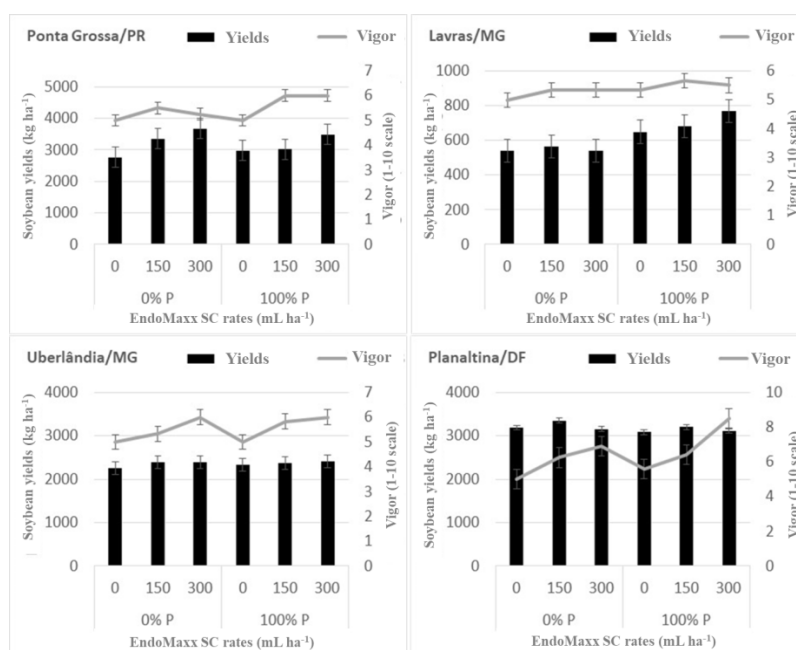
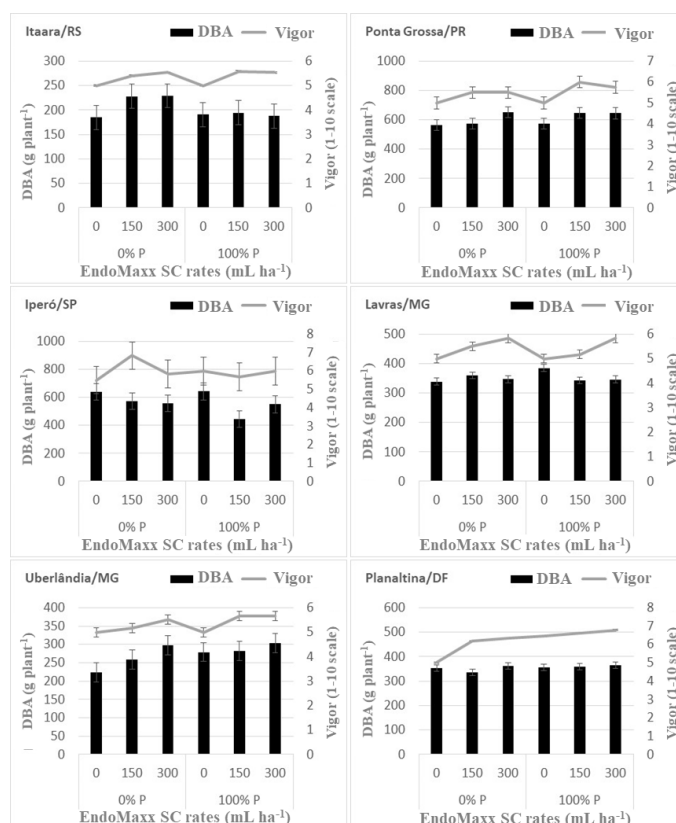


Fig 1. Soybean yields and plant vigor means in each experimental site. Error bars represent confidence intervals. Non-overlapping confidence intervals indicate significant differences across treatments according to Fisher's LSD test ( $p \leq 0.05$ ).

Table 2. Cultivar, sowing dates, target plant density, and distance between planting rows of four soybeans and six maize field trials.				
Soybeans				
Trial ID (City/State)	Soybean cultivar	Sowing date	Target plant density (plants ha <sup>-1</sup> )	Distance between planting rows (m)
1. Ponta Grossa/PR	NA 5909 RG	12/14/2018	300	0.50
2. Uberlândia/MG	RK 6719 IPRO2	01/15/2019	300	0.50
3. Lavras/MG	AS 3610 IPRO	02/19/2019	300	0.50
4. Planaltina/DF	BMX Bônus IPRO	01/08/2019	100	0.50
Maize				
Trial ID (City/State)	Maize hybrid	Sowing date	Target plant density (plants ha <sup>-1</sup> )	Distance between planting rows (m)
1. Itaara/RS	27D28	03/02/2019	64.000	0.50
2. Ponta Grossa/PR	2A401PW	01/15/2019	90.000	0.50
3. Iperó/SP	30F53R	01/28/2019	160.000	0.50
4. Uberlândia/MG	RK 9110 PRO 2	01/15/2019	80.000	0.50
5. Lavras/MG	BM207	04/16/2019	93.331	0.75
6. Planaltina/DF	P3898	01/08/2019	80.000	0.50



**Fig 2.** Maize dry aboveground biomass (DBA) and plant vigor means in each experimental site. Error bars represent confidence intervals. Non-overlapping confidence intervals indicate significant differences across treatments according to Fisher's LSD test ( $p \leq 0.05$ ).

**Table 3.** Average values and mean separation results regarding the dependent variables plant heights, vigor, dry aboveground biomass (DBA), thousand grain weight (TGW), and yields gathered from four soybean field trials.

Treatment	Height <sup>1</sup> (cm)	Vigor <sup>2</sup> (1-10 scale)	DBA <sup>3</sup> (g/5 plants)
1. Untreated control - P	46.6	b <sup>6</sup>	67.7
2. EndoMaxx SC (150 mL ha <sup>-1</sup> ) - P	47.5	ab	83.9
3. EndoMaxx SC (300 mL ha <sup>-1</sup> ) - P	47.8	ab	70.0
4. Untreated control + P	47.2	ab	68.2
5. EndoMaxx SC (150 mL ha <sup>-1</sup> ) + P	48.2	ab	79.2
6. EndoMaxx SC (300 mL ha <sup>-1</sup> ) + P	49.1	a	78.1
Treatment	TGW <sup>4</sup> (g)	Soybean Yields (kg ha <sup>-1</sup> )	Efficiency <sup>5</sup> (%)
1. Untreated control - P	148	a	2137
2. EndoMaxx SC (150 mL ha <sup>-1</sup> ) - P	149	a	2331
3. EndoMaxx SC (300 mL ha <sup>-1</sup> ) - P	148	a	2327
4. Untreated control + P	146	a	2195
5. EndoMaxx SC (150 mL ha <sup>-1</sup> ) + P	150	a	2251
6. EndoMaxx SC (300 mL ha <sup>-1</sup> ) + P	151	a	2348

<sup>1</sup>Plant height data were collected at early flowering stages (R1 soybean growth stage); <sup>2</sup>Plant vigor data were collected at the R3 growth stage (beginning pod); <sup>3</sup>Dry aboveground biomass was measured at the R5.1 growth stage (early seed development); <sup>4</sup>Thousand grain weight; <sup>5</sup>Efficiency (% increase) reflects actual treatment gains relative to the untreated control treatment in each level of P fertilization. Treatments 2 and 3 were compared to treatment 1 (which lacks P fertilization), whereas treatments 5 and 6 were compared to the P-fertilized untreated control; <sup>6</sup>Significantly different means, according to Fisher's LSD test ( $p \leq 0.05$ ), are indicated by different lowercase letters within a column.

**Table 4.** Average values and mean separation results regarding the dependent variables plant heights, vigor, dry aboveground biomass (DBA), thousand grain weight (TGW), and yields gathered from six maize field trials.

Treatment	Height <sup>1</sup> (cm)	Vigor <sup>2</sup> (1-10 scale)	DBA <sup>3</sup> (g/5 plants)
1. Untreated control - P	208	b <sup>6</sup>	5.09
2. EndoMaxx SC (150 mL ha <sup>-1</sup> ) - P	215	a	5.78
3. EndoMaxx SC (300 mL ha <sup>-1</sup> ) - P	216	a	5.77
4. Untreated control + P	215	a	5.43
5. EndoMaxx SC (150 mL ha <sup>-1</sup> ) + P	216	a	5.77
6. EndoMaxx SC (300 mL ha <sup>-1</sup> ) + P	219	a	5.94
Treatment	TGW <sup>4</sup> (g)	Maize Yields(kg ha <sup>-1</sup> )	Efficiency <sup>5</sup> (%)
1. Untreated control - P	221	b	6654
2. EndoMaxx SC (150 mL ha <sup>-1</sup> ) - P	230	a	6916
3. EndoMaxx SC (300 mL ha <sup>-1</sup> ) - P	228	a	6922
4. Untreated control + P	224	b	6769
5. EndoMaxx SC (150 mL ha <sup>-1</sup> ) + P	228	a	7044
6. EndoMaxx SC (300 mL ha <sup>-1</sup> ) + P	234	a	7261

<sup>1</sup>Plant height data were collected at early flowering stages (R1 maize growth stage); <sup>2</sup>Plant vigor also scored at the R1 growth stage; <sup>3</sup>Dry aboveground biomass measured at the R3 growth stage (kernel milk stage); <sup>4</sup>Thousand grain weight; <sup>5</sup>Efficiency (% increase) reflects actual treatment gains relative to the untreated control treatment in each level of P fertilization. Treatments 2 and 3 were compared to treatment 1 (which lacks P fertilization), whereas treatments 5 and 6 were compared to the P-fertilized untreated control; <sup>6</sup>Significantly different means. according to Fisher's LSD test (p≤0.05), are indicated by different lowercase letters within a column.

AMF might help to mitigate deleterious effects of broadcast P fertilization performed in this system. In-furrow EndoMaxx SC applications can be a viable alternative for more sustainable soybean and maize production to maximize food production to supply the increasing demands of the exponentially growing global human population, without expanding the cultivation area.

## Materials and Methods

### Field trials, plant material, application of treatments, and fertilization

Field trials were conducted in multiple locations during the 2018/2019 growing season in Brazil (Supplementary Table S1 and Fig S1). A similar protocol, consisting of six treatments (Table 1), was conducted across all research sites, in both maize and soybean, as described in Supplementary Table S1.

A novel product (EndoMaxx<sup>®</sup> SC) was applied in-furrow during sowing at two rates (150 or 300 ml ha<sup>-1</sup>), either with or without P fertilization (0 and 100%, respectively, and accordingly to each location requirements). EndoMaxx<sup>®</sup> SC contains a combined total of 1,522 AMF propagules mL<sup>-1</sup> from four AMF species - *Rhizophagus irregularis*; *Claroideoglossum luteum*; *Claroideoglossum etunicatum*; and *Claroideoglossum claroideum*. Sowing was performed using a plot seeder. The product was applied on seed furrows right after sowing using a CO<sub>2</sub>-pressurized backpack sprayer equipped with one Teejet XR 110.01 spray nozzle and calibrated to a flow rate of 50 L ha<sup>-1</sup>. Treatments were randomly distributed in blocks and consisted of: 1: Untreated control without phosphorus fertilization (Untreated control – P); 2: 150 mL ha<sup>-1</sup> of EndoMaxx SC applied at seed treatment and without phosphorus fertilization [EndoMaxx SC (150 ml ha<sup>-1</sup>) – P]; 3: 300 mL ha<sup>-1</sup> of EndoMaxx SC applied at seed treatment and without phosphorus fertilization [EndoMaxx SC (300 ml ha<sup>-1</sup>) – P]; 4: Untreated control with phosphorus fertilization (Untreated control + P); 5: 150 mL ha<sup>-1</sup> of EndoMaxx SC applied at seed treatment and

with phosphorus fertilization [EndoMaxx SC (150 ml ha<sup>-1</sup>) + P]; 6: 300 mL ha<sup>-1</sup> of EndoMaxx SC applied at seed treatment and with phosphorus fertilization [EndoMaxx SC (300 ml ha<sup>-1</sup>) + P]. Fertilization was applied according to local recommendations. All nutrients were applied equally to all treatments, except for P, which was applied at the rates of 53.9±14.8 (corn trials) and 68.3±13.5 kg ha<sup>-1</sup> (soybean trials) of P only in treatments with P fertilization (100% of P).

### Evaluation

Treatments effects on soybean growth and productivity were evaluated by assessing plant height at early flowering stages (i.e. R1 soybean growth stage), plant vigor at the R3 growth stage (beginning pod), and dry aboveground biomass at the R5.1 growth stage (early seed development) by randomly sampling five plants per plot. Accordingly, maize plant height and vigor were assessed at the beginning of flowering (R1 growth stage), followed by dry aboveground biomass at R3 (kernel milk stage). Crop yields and thousand-grain weight (TGW) were assessed for both crops by harvesting 3–6 m<sup>2</sup> within each plot, and values corrected to 13% humidity as needed.

### Experimental design and Statistical analysis

Detailed information concerning crop sowing dates, row spacing, and target plant density at each experimental site can be found in Table 2. Experimental units consisted of 12.5-24 m<sup>2</sup> plots. The number of replications varied from 4 to 6 depending on the location (Table 1). Data were submitted to assumption tests and subjected to analysis of variance (ANOVA) using JAMOVI v 1.2 for Windows (JAMOVI Project, 2020) for unbalanced designs. No data transformation was required. For the ANOVA, Location, Treatment, Block, and Treatment x Location interaction were considered as fixed, independent variables. The results were analyzed across Locations when the interaction between Treatment x Location was not significant

( $p > 0.05$ ). Means were compared using Fisher's LSD test when appropriate.

## Conclusions

In-furrow applications of AMF propagules in EndoMaxx SC positively impacted soybean and maize plant vigor and heights, dry aboveground biomass, and grain yields at both test rates (150 and 300 ml ha<sup>-1</sup>), regardless of P fertilization during crop sowing, which is likely related to successful plant root-mycorrhizal fungi associations. Treatments containing only AMF propagule (but no P during sowing) equaled or outperformed the untreated P-fertilized control treatment. Soybeans and maize yields were 2.6-9.1% larger when the highest product rate was applied in combination with P fertilization relative to untreated control + P. Such results constitute useful information, given that P fertilization during crop sowing is commonplace in Brazil.

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