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Efficacy of alginate- and clay-encapsulated microorganisms on the growth of Araçá-Boi seedlings (*Eugenia stipitata*)

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ABSTRACT. The present work aimed to evaluate the effects of encapsulated microorganisms on seedlings of *Eugenia stipitata*, popularly known as araçá-boi, to evaluate the interaction between the inoculum and encapsulating agents such as clay and alginate. The experiment was carried out in a completely randomized design using a 3×2 factorial scheme. The treatments were control, inoculum, clay without microbial inoculum, clay with microbial inoculum, alginate without microbial inoculum, and alginate with microbial inoculum. The seedlings were grown under nursery conditions over a period of 3 months. No treatment increased the height, stem diameter, shoot dry matter or root dry matter of the araçá-boi seedlings. The use of alginate increased the ammonium content compared to the clay and control treatments. Alginate and clay increased the nitrate content in relation to the control. Alginate increased the total number of bacteria in relation to the clay and control treatments. The application of inoculum combined with alginate increased the nitrate content only in relation to the clay and control treatments. Although the application of inoculum promoted an increase in the nitrate content compared to the uninoculated treatments, there was no effect for the other parameters analyzed. The results suggest that clay and alginate encapsulating agents with the presence or absence of microorganisms may improve some soil parameters.

Keywords: araçá-boi; encapsulating; microbial inoculum; plant growth-promoting agents.

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Introduction

The use of rhizobacterial plant growth promoters in agriculture is a great strategy to decrease environmental impacts resulting from the continued use of chemical fertilizers, herbicides, and insecticides (Meena et al., 2017). The term is used to describe soil bacteria that colonize the rhizosphere, growing within and surrounding plant tissues and stimulating their growth through several mechanisms, such as phosphorus solubilization, nitrogen fixation, phytohormone synthesis, and siderophore synthesis (Pérez-Montaño et al., 2014; Souza, Ambrosini, & Passaglia, 2015).

The use of plant growth promoting rhizobacteria (PGPR) in agriculture has some challenges, such as the poor colonization of inoculated bacteria in the rhizosphere due to the presence of soil microbiota and poor plant associations. The difficulties in bacterial colonization cause inefficiency in plant growth promotion. A strategy to decrease these losses would be encapsulation. Inoculum encapsulation has been used to enhance the effectiveness of bacteria by supplying nutrients and providing protection n against desiccation, which increase the consistency of inoculated microorganisms in the soil (Bashan & Holguin, 2002; Amavizca, Bashan, Ryu, Farag, Bebout, & de-Bashan, 2017). The success of microbial inocula introduced to the soil requires the effective survival of the inoculated bacteria and their delivery to a proper habitat, which can keep them alive (Heijnen & Veen, 1991; Bulgarelli et al., 2015;). The main aims of microbial encapsulation are to protect the bacteria being introduced to the soil until their acclimation to the new conditions (John, Tyagi, Brar, Surampalli, & Prévost, 2011; Schoebitz, López & Roldán, 2012; Przyklenk, Vemmer, Hanitzsch, & Patel, 2017) and to ensure their gradual release into the soil (Bashan & Holguin, 2002; Amavizca et al., 2017).

The present study used seedlings of *Eugenia stipitata*, known as araçá-boi, a medium-sized tree native to the Peruvian Amazon that produces yellow fruits that are used in the preparation of ice creams, creams and jellies. The production of goods using these fruits is a profitable alternative to agricultural production for small farmers in the Amazon region (Canuto, Xavier, Neves, & Benassi, 2010; Virgolin, Seixas, & Janzantti, 2017). Moreover, it guarantees that this plant species does not become endangered with extinction.

The aim of this study was to verify the growth-promoting effect of a mixture of microorganisms on araçá-boi seedlings using two encapsulating agents, clay and alginate, and to evaluate the microbe-induced changes in some soil chemical parameters under nursery conditions.

Material and methods

This study was carried out at *Ripado de fruticultura*, which belongs to the Department of Plant Production, School of Agrarian and Veterinary Sciences of Jaboticabal, located at coordinates 21º14'33"S and 48º17'02"W, with altitude of 563 meters a.s.l., from March to June 2015. The climate according to Köppen is CWA (subtropical with a dry winter and rainy summer), and the average minimum and maximum temperatures were 17 and 28ºC, respectively, throughout the experiment.

Araçá-boi seedlings were kindly donated by the Fruit sector of the Department of Plant Production and were randomly chosen from the nursery for the experiment. The seedlings were two months old, with an initial shoot dry mass (SDM) of 9.55 g and root dry mass (RDM) of 4.60 g, and were grown in 2-L plastic bags with an average height of 46 cm and a diameter of 5.90 cm. Throughout the experiment, all seedlings were grown under nursery growing conditions and were irrigated daily using an automatic sprinkler.

The experimental design was completely randomized with five replicates in a 3×2 factorial scheme and was carried out to evaluate the effects of the encapsulating material (control, clay, and alginate) and the presence of microbial inoculum (with or without inoculum).

The treatments were as follows: T1 = control, T2 = microbial inoculum, T3 = clay, T4 = clay + microbial inoculum, T5 = alginate, and T6 =alginate + microbial inoculum.

Inoculum and encapsulating agent preparation

The microbial inoculum used in this study contained the following microorganisms: *Azospirillum brasilense*, *Burkholderia cepacia*, *Bacillus thuringiensis*, *B. megaterium*, *B. cereus*, *B. subtilis*, *B. subtilis* strain 1411 and *Trichoderma* sp. All microorganisms were obtained from the Laboratory of Microbiology stock collection. The microbes were grown separately in Erlenmeyer flasks containing nutrient both for seven days at 25ºC, then centrifuged for 10 min at 10,000 rcf, and the supernatant was discarded. This procedure was repeated many times to concentrate the culture. All inoculum treatments contained 8×10^8 colony-forming units (CFU) of each microorganism. The application of the treatments was conducted near the roots in the rhizosphere region.

The treatments involving alginate as the encapsulating agent was prepared by adding the microbial inoculum to 1% alginate solution, mixing the inoculum and alginate solution together, and dripping the resultant solution into a 0.1 M calcium chloride solution (Bashan, Hernandez, Leyva, & Bacilio, 2002). Each drop of the solution formed a sphere. Each ml of the microbial alginate solution generated 12 spheres. These spheres were cleaned in distilled water with the aid of a sieve, and the spheres from the different microorganisms were stored in separate beakers. Each container received a total of 96 spheres, 12 from each of the eight different microorganisms. The control treatment included the same number of spheres but with no inoculum. For the clay treatments, clay was added to water at a ratio of 1:3. For the treatment including the microbial inoculum and clay, 8 ml of a clay suspension was added to 8 mL of the microbial inoculum. The application of the treatment was conducted with the aid of a syringe near the root.

The experiment was carried out from March to June, and the assessment of plants was performed twice, at the initial and final stages of the experiment. At the final stage, all the plastic bag were dismounted, and the soil and plants were separated. The soil samples were kept in a refrigerator for future analysis. The shoots and roots were separated, cleaned with water, kept in paper bags and dried in a drying oven at 60ºC for 48 hours. Then, the dry weight of the plant samples was recorded.

Plant biometric assessment

Assessments of plant height, stem diameter, shoot dry matter and root dry matter were conducted. For the growth assessment, the differences between the values obtained during the final evaluation and initial evaluation of each parameter were calculated.

Biomass carbon

Biomass carbon was determined following the irradiation method and with extraction according to Mendonça and Matos (2005). This method consists of the use of electromagnetic power (microwaves), which causes cell lysis and the release of intracellular compounds, which are further extracted and quantified. The results are obtained by subtracting the values from the nonirradiated soil samples from those of the irradiated soil samples. The extraction was conducted using 0.5 M potassium sulfate, and the extract was rust colored with 0.066 M potassium dichromate in the acid medium. Quantification was performed by titration of an aliquot with 0.03 M ammonium ferrous sulfate.

Quantification of ammonium (NH_4^+) and nitrate (NO_3^-) levels

The ammonium and nitrate concentrations were measured according to Keeney and Nelson (1982). Briefly, 1 M potassium chloride solution was added to 5 g of dry soil sample under agitation. After decantation and extraction, an aliquot was collected from the extract, and ammonium was determined by adding a boric acid indicator solution. Then, an aliquot of magnesium oxide was added and titrated using 0.0025 M sulfuric acid until the light green color turned to light pink.

Bicarbonate-soluble phosphorus

The phosphorus concentration in the soil was measured according to Watanabe and Olsen (1965). For this measurement, a 0.6 g soil sample was collected and added to a 0.5 M sodium bicarbonate solution under agitation and filtered after decantation. The phosphorus content was determined using an aliquot of extract from the 0.5 M bicarbonate solution. Then, 5 N sulfuric acid was added, and the solution was agitated and incubated in a water bath at 45ºC for 20 min. Phosphorus determination was colorimetrically performed at Abs 820 nm, and the results were expressed as µg of Pi g^{-1} of dry soil.

Dehydrogenase activity

The dehydrogenase activity was measured according to Casida (1977) using 3 g of dry soil. Calcium carbonate and an aqueous solution of 3% triphenyl tetrazolium sodium chloride (TPF) were added. Then, distilled water was added to form a film on the samples. The samples were incubated in a water bath at 37ºC for 24 hours. Then, the soil samples were diluted with methanol to extract of all TPF formed. Then, the samples were filtered and spectrophotometrically read at Abs 485 nm. The activity was calculated as the mixture volume versus the standard curve of TPF versus the methanol volume used/the weight of the dry sample. The results were expressed as µg TPF g^{-1} of dry soil / 24 hours.

Analysis of competition among microbes in the inoculum

The microbial inoculum was evaluated to determine whether any competition could occur among them in the soil. For this analysis, each microorganism was grown separately in nutrient agar, and *Trichoderma* sp. was grown in PDA. Then, all microorganisms at a concentration of 8×10^8 CFU, encapsulated or not encapsulated, were inoculated together into the soil in vases. The soil in the vases that received the encapsulated microorganisms received 96 spheres. The experiment was conducted with five replicates. The soil in the vases was irrigated daily for 20 days. At the end of this period, the soil samples were collected, and the total bacterial numbers were counted. The values of total microorganisms were log10 transformed.

Total number of bacteria (colony forming units)

The total number of bacteria from the dry soil was measured according to Wollum (1982) through serial dilutions of the soil suspension, and the colonies that formed after 24 hours were plated on Petri dishes containing nutrient agar.

Statistical analysis

The data were analyzed based on Tukey's test at 5% probability using AgroEstat software (Barbosa & Maldonado Júnior, 2010).

Results and discussion

Figure 1 shows the total number of bacteria isolated from the soil samples inoculated with encapsulated and nonencapsulated microbial inocula. As the total numbers of bacteria were higher in the soils that received the microbial inoculum compared to the soil that did not receive the inoculum, these results show that the microbial inoculum was able to establish and increase in the soil at least in 20 days after inoculation, suggesting that this mixture could be used to promote plant growth.

Some studies have shown benefits in terms of the encapsulation of microbial inocula, increasing the survival time and establishment of microbes in the soil (Marcelino, Milani, Mali, Santos, & Oliveira, 2016). It is interesting that there were no significant differences in the total number of bacteria isolated from the soil that received the encapsulated microbial inoculum and the soil that received the nonencapsulated microbial inoculum (Figure 1). It is likely that this result occurred because the microbes in the soil were quantified only 20 days after inoculation, and this time is not sufficient for the encapsulated microorganisms to grow more than the nonencapsulated microorganisms in the soil because the encapsulating agent gradually releases the microorganisms into the soil.

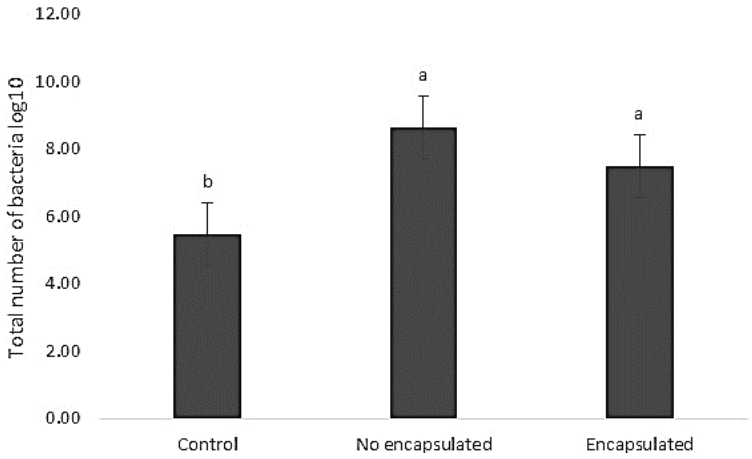

Figure 1. Total number of bacteria isolated from the soil in vases inoculated with encapsulated and nonencapsulated inoculum.

Table 1 shows the average biometric values obtained from the fruits. There were no significant differences among the evaluated treatments ($p > 0.05$). However, other authors have had success in the promotion of fruiting plant growth using microorganisms. Pedraza et al. (2010) reported that *Azospirillum* sp. isolated from strawberry and sugarcane promoted higher growth in papaya and guava. Rahman et al. (2018) reported growth using *Bacillus* spp. in strawberry. The same bacterium promoted growth in passion fruit (Vitorazi Filho, Lima, Freitas, Martins, & Olivares, 2012).

Table 1. Averages of different plant growth parameters measured 150 days after germination.

Treatments	Height (cm)	Diameter (cm)	SDM (g)	RDM (g)
Control	2.33 a	0.35 a	2.72 a	2.37 a
Inoculum	6.00 a	0.32 a	2.56 a	2.23 a
Clay	1.00 a	0.35 a	2.15 a	1.81 a
Clay + Inoculum	1.00 a	0.35 a	3.04 a	2.70 a
Alginate	3.67 a	0.27 a	1.71 a	1.44 a
Alginate + Inoculum	1.50 a	0.34 a	2.14 a	1.80 a
VC (%)	119.75	18.90	24.67	44.96

Means followed by the same letter within a column did not differ statistically with one another; SDM = shoot dry matter; RDM = root dry matter; VC = variation coefficient.

Azospirillum brasilense and *B. subtilis* have been used in both previous studies and in the present study and have been shown to have the ability to produce phytohormones, such as indole acetic acid (IAA), which promotes root and shoot development. Consequently, the plants become more efficient in taking up water and nutrients, thereby increasing their growth. Phytohormones are organic substances that can promote, inhibit, or modify the growth and development of plants at low concentrations (Damam, Kaloori, Gaddam, & Kausar, 2016). Phytohormones promote root cell proliferation by causing the overproduction of lateral

roots and root hairs with a concomitant increase in nutrient and water uptake (Sureshbabu, Amaresan, & Kumar, 2016).

In the present study, the encapsulated microbes agents did not enhance araçá fruit development compared to the control. Unlike Bashan and Holguin (2002) and Bashan, de-Bashan, Prabhu, and Hernandez (2014), who observed plant growth using encapsulated bacteria compared to nonencapsulated bacteria, we were not successful in promoting plant growth in this study.

The reasons for the lack of success in the growth of the plants might be that three-month-old araçá seedlings from plant nursery conditions were used in this study, and they were planted in plastic bags. The ineffectiveness of the growth-promoting agent likely occurred because the plants were grown in plastic bags for a long time. These growing conditions might have harmed or inhibited plant growth and eventually masked the bacterial growth-promoting activity. Although we verified the ability of this mixture of microorganisms to grow together in the soil, the microbial mixture used contained seven bacterial species and one fungal species, and it is possible that there may have been some competition among these microorganisms and therefore a decrease in the microbial count and their establishment in the rhizosphere. Although using a mixture of microorganisms can be beneficial by combining the abilities of microorganisms, such mixtures can sometimes also promote competition among the microorganisms (Bashan & Holguin, 2002; Gouda, Kerry, Das, Paramithiotis, Shin, & Patra, 2018). On the other hand, some studies showed that a mixture of microorganisms including *Bacillus*, *Azospirillum,* and *Burkholderia* species promoted plant growth in fruit crops such as papaya, coconut, pineapple and banana (Baldotto, Baldotto, Olivares, Viana, & Bressan-Smith, 2010; Mia, Shamsuddin, Wahab, & Marziah, 2010; Abraham-Juarez et al., 2018).

Another possible reason for the failure in plant growth may be the lack of specificity between the microorganisms and the plants. When there is no association between a plant and microorganisms, the colonization of the rhizosphere by the introduced organism could possibly be restricted by the native microbiota (Bashan & Holguin, 2002; Bulgarelli et al., 2015).

Plant growth-promoting microorganisms have many abilities that promote plant growth beyond the previously mentioned characteristics. Such microorganisms have the ability to fix nitrogen. Nitrogen is the most important nutrient used by all organisms, and it is also the most common nutrient in the atmosphere. However, few microorganisms have the ability to obtain this nutrient and transform it to make it available for plants and other organisms. Some nitrogen-fixing microorganisms are able to transform nitrogen and make it available to other organisms. Other characteristics of plant growth-promoting microorganisms include the ability to control phytopathogens and solubilize phosphorus, transforming unavailable phosphorus in the soil into a form that is available to plants (Pérez-Montaño et al., 2014). The microorganisms used in this study have the above-mentioned abilities to promote plant growth, which is why they were used. However, the use of microorganisms having plant growth-promoting characteristics does not guarantee that they will have an effect on plant growth, and some reasons for such results are mentioned above.

Table 2 shows the dehydrogenase activity, the ammonium, nitrate, and soluble phosphorus concentrations, the biomass carbon and the total bacteria number from the treatments that received encapsulating agents with or without microbial inocula and the control treatments. There were no differences among treatments ($p > 0.05$) in terms of the dehydrogenase activity when comparing the treatments that received the encapsulating agents with or without inocula and the control treatments. The dehydrogenase enzyme plays an important role in the electron transport chain, which is involved in cellular respiration. High dehydrogenase activity suggests high microbial activity due to the redox process, which breaks down energetic molecules with water and releases CO_2 (Arif et al., 2018).

When the soil microbial activity is increased, the microbial respiration and dehydrogenase activity also increase (Arif et al., 2018). No change in the dehydrogenase activity indicates that the soil microbial activity was unaffected by any of the applied treatments.

A higher dehydrogenase activity was found in the control that received inoculum (388.54 TPF g^{-1} dry soil / 24h) compared to the treatment that did not receive microbial inoculum (289.81 TPF g^{-1} dry soil / 24h). However, there were no statistical differences among the treatments.

Table 2. Dehydrogenase activity, ammonium, nitrate, and soluble phosphorus concentrations, microbial carbon biomass and total number of bacteria in soil subjected to the different treatments.

Treatments	Dehydrogenase μg TFF g^{-1} SS 24h	$N–NH_4^+$ μg g^{-1} DS	$N\text{-}NO_3^-$ μg g^{-1} DS	P μg g^{-1}DS	MCB μg C g^{-1} DS	CFU g^{-1} DS
Encapsulated						
Control	339.17 a	72.33 b	49.58 b	6.74 a	22.25 a	$1.69x10^6$ b
Clay	292.86 a	79.33 ab	59.50 a	6.52 a	17.56 a	1.79×10^6 b
Alginate	315.48 a	87.05 a	59.50 a	8.01 a	19.47 a	$3.71 x10^6$ a
F Test	1.19^{ns}	5.29*	8.26**	1.06^{ns}	0.50^{ns}	8.00**
SD (5%)	79.94	12.45	7.52	2.94	12.56	$1.52. 10^6$
Inoculum						
Inoculum	334.32 a	81.67 a	59.11 a	7.58 a	19.30 a	2.31×10^6 a
No inoculum	297.37 a	77.78 a	53.28 b	6.59 a	20.22 a	2.18×10^6 a
F test	2.28^{ns}	1.04^{ns}	6.43*	1.21^{ns}	0.06^{ns}	0.13^{ns}
SD (5%)	53.30	8.30	5.01	1.96	8.37	1.01×10^6
VC (%)	16.43	10.14	8.68	26.96	41.26	41.14

Means followed by the same letter in each column did not differ statistically according to the Tukey (5%) test; significant at 0.01 (**) not significant (ns). DS = dry soil; TPF = triphenilformazan; MCB = microbial carbon biomass; CFU= colony-forming units; SD = significant difference; VC = variation coefficient.

There were no differences ($p > 0.05$) in the dehydrogenase activity (Table 3) among the treatments that included clay or alginate with or without inoculum. When the microorganisms were inoculated into the soil without encapsulation, the inoculum was deposited immediately upon soil contact. This process differs from what occurs when microorganisms are inoculated together with encapsulating agents, in which the release of microorganisms is slow and gradual. However, the increase in dehydrogenase activity suggests that the quick release of microbial inoculum would be more effective in promoting changes to the soil than when the microorganisms are not encapsulated. In contrast, microorganisms are immobilized in the encapsulating agents. There was a significant difference in dehydrogenase activity between the control and treatments that received microbial inoculum compared to the treatments that did not receive inoculum (Table 3). In addition, there was also a difference between the treatments that received different encapsulating agents. It is likely that the microbial activity was increased by the deposition of encapsulating agents and by microbial inoculation (Table 3). As dehydrogenase activity is related to the cycling of nutrients, such as carbon and nitrogen, the deposition of encapsulating agents, which contain such nutrients in their formula, might have promoted an increase in the concentration of this enzyme (Chodak & Niklińska, 2010; Choudhary et al., 2018).

Table 3. Unfolding the interactions between encapsulating agents and inoculation in terms of dehydrogenase activity.

Treatments	Dehydrogenase (μg TPF g^{-1} DS / 24h)		
	Control	Clay	Alginate
Inoculum	388.54 a	301.67 a	318.22 a
No inoculum	289.81 b	284.07 a	321.74 a
F test	5.43*	0.17^{ns}	0.02^{ns}
SD (5%)	92.33		

Means followed by the same letter in each column did not differ statistically according to the Tukey (5%) test; significant at 0.01 (**) not significant (ns). DS = dry soil; TPF = triphenilformazan; SD = significant difference.

The ammonium concentration was higher in the treatments including alginate (87.05 μg $N\text{-}NH_4^+$) compared to the clay (79.33 μg N-NH4$^+$) and control (72.33 μg $N\text{-}NH_4^+$) ($p < 0.01$) treatments. However, there was no difference in ammonium concentration when the two alginate treatments with or without microbial inocula were compared ($p > 0.01$). This result indicates that the alginate promoted an increase in the ammonium concentration and that this increase was not due to the microbial inoculum. Similarly, alginate favored an increase in the action of denitrifying microbes that are involved in ammonium synthesis in the soil (data not shown). A higher total number of bacteria was recorded for the alginate treatment (3.71×10^6 CFU g^{-1} dry soil) in comparison to the clay (1.79×10^6 CFU g^{-1} dry soil) and control (1.69×10^6 CFU g^{-1} dry soil) treatments. However, there was no difference ($p > 0.05$) in the total number of bacteria between soils that did or did not receive the microbial inoculum (Table 2). As alginate in the absence of the microbial inoculum promoted an increase of the total number of bacteria, the alginate likely served as a nutrient source for the soil microorganisms, thereby increasing their populations. This result is supported by the

increased ammonium concentration in the soil that received alginate. Alginate is a polysaccharide and may serve as a carbon and energy source for heterotrophic soil bacteria, which need energy from plants (Marcelino et al., 2016). The alginate with or without inoculum did not increase the total number of bacteria in the soil (Table 2). Alginate is a slow-release encapsulating material that releases microorganisms into the soil environment, making it difficult for alginate to rapidly alter the total number of bacteria in the soil. However, the total number of bacteria could have increased if the alginate had released bacteria, and these bacteria had gradually become established in the soil. This result suggests that the released inoculum failed to establish in the given time. On the other hand, when a microbial inoculum is added to the soil, recombination among the soil microbial communities occurs. Some microbial populations could increase, while other microbial populations could decrease, which would cause the final total number of bacteria to remain unchanged.

A higher ammonium concentration occurred in the treatment that received alginate plus microbial inoculum (93.33 µg $N\text{-}NH_4^+$ g^{-1} dry soil) compared to the treatments that did not receive the inoculum. There were no differences in $N\text{-}NH_4^+$ among the treatments containing the inoculum (Table 4). These values reinforce the suspicion that the alginate had been used by the microorganisms as a nutrient source. The soil microbiota might be influenced by several physical factors, such as temperature, aeration, minerals, nutrient availability and organic substrates. These factors could also affect the nitrogen immobilization and the nitrate level in the soil (Chen, Xu, Fan, Yu, & Ding, 2017; Don, Böhme, Dohrmann, Poeplau, & Tebbe, 2017).

Table 4. Effects of encapsulating agents and inoculum on soil NH_4^+ concentrations

Treatments	$N\text{–}NH_4^+$ (µg g^{-1} DS)	
	Without Inoculum	With Inoculum
Control	72.33 a	73.33 b
Clay	79.33 a	79.33 ab
Alginate	81.67 a	93.33 a
F test	1.08^{ns}	5.25*
DS (5%)	17.61	

Means followed by the same letter in each column did not differ statistically by the Tukey test (5%); significant at 0.01 probability (**), not significant (ns). DS = dry soil; SD= significant difference.

Higher nitrate concentrations were found in association with the treatments containing the microbial inoculum compared to those that were not inoculated. However, there were no differences ($p > 0.05$) among the treatments that received the inoculum. Among the treatments with or without microbial inocula, higher nitrate concentrations were recorded in association with the alginate and clay (57.17 µg $N\text{-}NO_3^-$) treatments compared to the control (45.50 µg $N\text{-}NO_3^-$) ($p > 0.05$) (Table 5).

Nitrogen can enter the soil as ammonium, nitrate and nitrite. Interestingly, the nitrate levels were increased by the presence of both clay and alginate. These results suggest an increase in biological nitrogen fixation activity caused by diazotrophic bacteria or an increase in nutrient cycling.

Table 5. NO_3^- concentrations associated with alginate, clay and control treatments with or without microbial inocula.

Treatments	$N\text{-}NO_3^-$ (µg g^{-1} DS)	
	Without Inoculum	With Inoculum
Control	45.50 b	53.67 a
Clay	57.17 a	61.83 a
Alginate	57.17 a	61.83 a
Test F	5.71*	2.80^{ns}
DMS (5%)	10.63	

Means followed by the same letters in each column did not differ statistically by the Tukey test (5%); significant at 0.01 probability (**), not significant (ns). DS = dry soil; SD = significant difference.

In general, most plants absorb nitrate and ammonium to undergo rapid development in nitrate-rich environments (Sun, Yu, & Hu, 2017; Yu, Ma, Sun, Zou, Lin, Fu, & Fu, 2018). However, some plant species require large amounts of nitrate in the initial phases of their development (Vitorazi Filho et al., 2012), which drastically reduces the ammonium concentration in the soil.

Among the control treatments, a higher level of soluble phosphorus ($p > 0.05$) was recorded in that containing the microbial inoculum (8.98 µg P g^{-1} dry soil) compared to the uninoculated control (4.98 µg P g^{-1} dry soil). However, there was no difference in the soluble phosphorus concentration between the clay and alginate treatments (Table 6). The microbial mixture had several PO_4^- solubilizing microorganisms,

which may be the reason for the highest phosphorus levels associated with the treatments including the nonencapsulated microbial inoculum. The quick release of a large amount of microorganisms might have intensified the PO_4^- solubilization process in the nonencapsulated microbial treatment, unlike the encapsulated microbial treatment, in which the release is slow and gradual. Fenice, Selbman, Federici and Vassilev (2000) studied *Penicillium variabile* encapsulated in alginate and reported great phosphorus solubilizing efficiency that attained up to a 36% increase in the total phosphate levels. These results were most likely achieved due to the larger period over which that study was conducted and the absence of microbial competition, as these authors worked with only one microorganism.

Table 6. Phosphorus concentrations (µg g^{-1} dry soil) associated with alginate, clay and control treatments with or without microbial inocula.

Treatments	Soluble P (µg g^{-1} D)		
	Control	Clay	Alginate
With inoculum	8.98 a	6.50 a	7.27 a
Without inoculum	4.50 b	6.54 a	8.74 a
F test	8.26*	0.00^{ns}	0.89^{ns}
SD (5%)	3.39		

Means followed by the same letters in columns did not differ statistically by the Tukey test (5%); significant at 0.01 probability (**), not significant (ns). DS = dry soil; SD = significant difference.

A higher carbon biomass was found in association with the treatment of alginate without inoculum (27.12 µg C g^{-1} dry soil) compared to the treatment of alginate plus inoculum (11.83 µg C g^{-1} dry soil). The carbon biomass values did not differ among the other treatments (Table 7). The carbon biomass is directly related to the total number of microorganisms.

Alginate decomposition might have increased the nutrient values through microbial activity, promoting improved soil quality (Hernandez-Montiel, Chiquito-Contreras, Murillo-Amador, Vidal-Hernandez, Quinones-Aguilar, & Chiquito-Contreras, 2017; Patel, Bajpai, Bajpai, Saini, & Acharya, 2017; Baskaran, Kumari, & Van Staden, 2018).

These findings show that the application of alginate without inoculum promoted an increase in the total number of soil bacteria and thereby increased the microbial carbon biomass. The presence of inoculum along with alginate decreased or did not change the parameters analyzed. This result suggests that competition might have occurred among the inoculated microorganisms and native soil microbiota, thereby reducing the microbial growth when compared to other treatments (Tables 7 and 8).

Table 7. Microbial carbon biomass (MCB) in soil receiving alginate, clay and control treatments with or without microbial inocula.

Treatments	MBC (µg C g^{-1} DS)		
	Control	Clay	Alginate
With inoculum	24.98 a	21.08 a	11.83 b
Without inoculum	19.53 a	14.03 a	27.12 a
F test	0.67^{ns}	1.12^{ns}	5.28*
SD (5%)	14.50		

Means followed by the same letter in each column did not differ statistically by the Tukey test (5%); significant at 0.01 probability (**), not significant (ns). DS = dry soil; SD = significant difference. MCB = microbial carbon biomass

Table 8. Number of microbial colony forming units (CFU) in soil receiving different treatments.

Treatments	CFU (g^{-1} DS)	
	Without inoculum	With inoculum
Control	1.71 $x10^6$ b	1.67 x 10^6 a
Clay	1.60 $x10^6$ b	1.98 x 10^6 a
Alginate	4.13 $x10^6$ a	3.29 x 10^6 a
F test	6.30*	2.29^{ns}
SD (5%)	2.15 $x10^6$	

Means followed by the same letters in each column did not differ statistically by the Tukey test (5%); significant at 0.01 probability (**), not significant (ns). DS = dry soil; SD = significant difference. CFU = colony-forming units.

Some microbial genera and species in the soil are determined by the power with which the ecosystem acts (Parton et al., 1993), and environmental factors may sometimes influence the microbial soil balance, but the exact relationships among microbial communities are difficult to define (Wardle & Parkinson, 1990).

Conclusion

The findings of this study suggest that the encapsulating agents with or without microbial inocula positively modified the analyzed soil chemical and microbial parameters, even though there were no significant growth-promoting effects on the araçá seedlings. Therefore, further studies should be carried out to optimize both the amount and composition of the inoculant to improve its efficacy and promote the successful use of this inoculum in various crops.

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