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The Effect of AMF-Cacao Association on Varying Physicochemical, Nutrient, and Biological Soil Parameters in an Agroforest System

Diana P. Paguntalan[1,*], **Nelly S. Aggangan**[2], **and Inocencio E. Buot Jr.**[3]

[1]Division of Biological Sciences College of Arts and Sciences University of the Philippines Visayas Miagao, Iloilo, Philippines
[2]National Institute of Molecular Biology and Biotechnology 3Institute of Biological Sciences University of the Philippines Los Baños College, Laguna, Philippines

[*]Author for correspondence: Email: dppaguntalan@up.edu.ph

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The increasing interest in sustainable methods in agroforestry directs the attention of many to arbuscular mycorrhizal fungi (AMF) for their role in maintaining or improving overall plant health and nutrition of many plants including cacao (*Theobroma cacao* L.) trees. The effects of different treatments of AMF and other soil amendments applied singly or in combination on physicochemical properties, nutrient levels, spore count, and rhizosphere microbial counts, were investigated and compared across different sampling periods of varying climatological conditions in an agroforest system. Results showed that there was a significant effect on the N content using the different treatments. The treatment using AMF from MR (MYKORICH) alone has the highest N level (%) (0.587 ± 0.024, $p < 0.001$). Levels of P (ppm) and K ($cmol_c/kg$) and soil physicochemical properties were not significantly affected by AMF applications. N levels were highest in January (1.772 ± 0.018, $p = 0.004$) which was the same month recorded with the lowest P content (0.0163 ± 2.228, $p < 0.0001$). Soil physicochemical properties did not significantly vary between sampling periods except for organic matter (OM) which was highest in October (2.883 ± 0.151, $p = < 0.001$), and with water-holding capacity (WHC) in March (80.429 ± 1.775, $p < 0.001$). Mycorrhizal spore counts were significantly higher in treatments with AMF ($p > 0.0001$) and between the sampling months ($p < 0.0001$). Results showed that the effect of season on spore count was not the same for each of the treatments used and vice versa ($p = 0.001$). Microbial counts on nitrogen-fixing bacteria and phosphate-solubilizing bacteria showed no variability between treatments ($p = 0.134$). These findings guide cacao farmers in directing future interventions for improving soil conditions and yield in cacao agroforests.

Keywords: arbuscular mycorrhizal fungi, cacao, agroforest system, soil NPK, spore count, phosphate-solubilizing bacteria, nitrogen-fixing bacteria

INTRODUCTION

Unsustainable agricultural practices such as the use of chemical fertilizers and pesticides often leave unmeasured costs. This hampers the ability of natural ecosystems to operate and provide goods and services resulting in the degradation of water and soil quality (Tilman et al. 2002). In response, a variety of practices and innovations are currently being explored to mitigate the adverse and environmental impacts of the use of unsustainable practices. One of these is the use of arbuscular mycorrhizal fungi (AMF) as biofertilizers. AMF are a group of fungi under the phylum *Glomeromycota* (Pagano et al. 2016) and are so named for the formation of arbuscules that serve as the key element of nutrient exchange between the 2 symbiotic partners (Bonfante.and Genre 2010). In the natural environment, mycorrhizal associations are widespread. They are one of the most abundant organisms found in all types of ecosystems (Meliani et al. 2012) making them readily available for studies and the development of suitable inocula for commercial propagation and use. Many studies have explained the role of AMF on several different crops such as corn (Garcés-Ruiz 2017), tomato (Balliu et al. 2015), wheat (Al-Karaki 2004), rice (Rajeshkannan et al. 2009), and one of the most important crops worldwide—cacao (Isaac et al. 2005; Snoeck et al. 2010; Tchameni et al. 2012; Aggangan, Cortes, Opulencia et al. 2019; Aggangan, Cortes, Reaño 2019; Aggangan and Jomao-as 2019; Aggangan and Victoria 2019).

Cacao is one of the most important crops traded worldwide for its most prized role as the sole source of raw material in making chocolates and other chocolate-based goods and was valued at approximately US$9 billion in 2015 (DTI 2017). In terms of production, the Philippines can grow several cacao varieties due to its suitable climate conditions. However, the challenges press on in increasing production to meet the increasing demand in the years to come due to several factors that cause declines, including pest and disease infestation and climate change impacts.

The recent attention gained by AMF as biofertilizers carries along with it a demand to explore the complexities of its association with other plants in terms of soil dynamics and fertility (Snoeck et al. 2010), diversity (Ngonkeu 2003), stress tolerance (Begum et al. 2019), plant yield and quality (Bona et al. 2017), and many others. Among these, one important aspect of AMF association is mineral nutrition which relates most, if not all, to the efficient uptake of nitrogen (N), phosphorus (P), and potassium (K). However, laboratory or greenhouse experiments largely dominate the studies that have been done on AMF associations. This creates a need to conduct actual field experiments and monitor the impacts of AMF associations, especially in established communities as their adaptation to varying environmental conditions.

Moreover, AMF associations are known to sustain unfertilized cacao agroforests due to the temporal dynamism of vesicular-arbuscular mycorrhiza (VAM) fungi that affect organic matter and major nutrients in the soil (Snoeck et al. 2010). Several studies have shown the density and diversity of VAM fungi (later named AMF) to be directly correlated to the changes in soil fertility and overall soil biological dynamics at different stages of cacao agroforests (Isaac et al. 2005; Snoeck et al. 2010) including the influence on the rhizosphere development of fungal and bacterial communities across plant groups (Solís-Domínguez et al. 2011; Aggangan, Cortes, Reaño 2019). Most importantly, AMF is known to exhibit interactions with other soil microorganisms to enhance soil fertility (Gui et al. 2017; Nanjundappa et al. 2019). This is an important aspect of crop growth as soil microorganisms drive the impact of nutrients and their availability in the soil (Lu et al. 2016). Aside from AMF, other soil amendments such as biochar (pyrolyzed and carbon-rich product) and optimization of nitrogen fixation through biological soil amendments with nitrogen-fixing bacteria (NFB) have been reported to affect change in soil microbial abundance and are known to improve soil fertility, soil structure, and crop production (Ducey et al. 2015; Soumare et al. 2020). AMF is also known to interact synergistically with phosphate-solubilizing bacteria (PSB) to make phosphorous available for AMF absorption and transport to the host (Nacoon et al. 2020). In addition to the abovementioned factors, AMF abundance in terms of spore count is another area that contributes to the knowledge of the impacts of AMF on cacao field soils. Several studies have shown the density and diversity of VAM fungi to be directly correlated to the changes in soil fertility and overall soil biological dynamics at different stages of cacao agroforests (Isaac et al. 2005; Snoeck et al. 2010).

In this study, the effects of 2 different sources of AMF inocula applied singly or in combination with other amendments in affecting the concentrations of several mineral elements, soil physicochemical properties, and biological soil properties from a cacao agroforest were investigated. Specifically, soil analysis was carried out to determine whether levels of N, P, K, cation exchange capacity (CEC), soil organic matter (OM), water holding capacity (WHC), pH, mycorrhizal spore count, and PSB and NFB colony-forming units vary across different sampling periods with varying agroclimatological conditions.

MATERIALS AND METHODS

Field Site. The study site was an agroforest area cultivated with 3-year-old cacao seedlings, located in Barangay Mabacan, Calauan, Laguna. Calauan is a municipality in the central part of Laguna, comprising 7 458.6629 ha land (PhilAtlas n.d.). The study site was divided into 2 major areas, 1 of which was used in this study. There were 7 plots with UF18 cacao trees that were intercropped with banana, coconut, lanzones, papaya, durian, and rambutan. Mabacan is known to be of rolling to flat topography, with an average annual temperature of 27°C and precipitation of 1995 mm (Climate-Data n.d.).

Experimental Design. The study used a Randomized Complete Block Design (RCBD) with 7 treatments replicated 3 times with a total of 21 samples in 3 blocks using the UF18 cacao variety. The trees used in the study were previously subjected to varying treatments utilizing AMF inocula obtained from the 2 commercial products MYKORICH® (MR) and MYKOVAM® (MV), which were applied alone or in combination with others such as BioN (containing nitrogen-fixing bacteria) and biochar (a carbon-rich organic material). The treatments used in the study were as follows: MR only, MV only, MR + BioN + BB, MR + BioN, MV+ BioN + BB, MV + BioN, and the control (without adding soil amendments). During field planting, vermicompost was applied to all seedlings while biochar, a carbon-rich organic soil amendment from pyrolyzed bamboo trimmings, was added to the designated treated seedlings (Table 1).

Inoculation and Raising of Experimental Cacao. Cacao seeds were germinated and seedlings were transferred to polybags with different treatments. After 6 mo at the National Institute of Molecular Biology and Biotechnology-University of the Philippines Los Baños (BIOTECH-UPLB) nursery, they were cultivated in the experimental field in Barangay Mabacan, Calauan, Laguna. During field planting, vermicompost was applied to all seedlings while biochar, a carbon-rich organic soil amendment from pyrolyzed bamboo trimmings, was added to the designated treated seedlings.

Sources of AMF Inocula. Two commercially available products (i.e., MYKOVAM® and MYKORICH®) were used in this study as sources of mycorrhizal fungi inoculants. MYKOVAM® is a biofertilizer that contains spores, infected roots, and propagules of beneficial VAM fungi that improve water and nutrient absorption and is applied to crops and most forest trees (BIOTECH-UPLB n.d.) while MYKORICH® comes in a capsule form containing spores and propagules of 12 species of arbuscular mycorrhizal fungi (AMF) from genera *Glomus, Gigaspora, Entrophospora,* and *Acaulospora* that can be used in almost all kinds of plants.

Data Gathering on Local Climatological Parameters. Before the sample collection, environmental factors such as ambient temperature, soil temperature, humidity, and light intensity were measured and recorded per block per setup. An indoor-outdoor digital hygrometer and thermometer (Brifit: temperature accuracy ± 1°C, humidity accuracy ± 5%) were hung from a tree branch 1 m from the ground and read for humidity and ambient temperature after 1 min. Light intensity was recorded using a light meter handheld 1 m at 3 angles in front of the tree under the canopy (URCERI Digital Light Meter: accuracy ± 3% rdg ± 8 dgts at < 10,000 Lux), ± 4% rdg ± 10 dgts at > 10,000 Lux). Soil temperature was determined using a digital soil thermometer (Rapitest) inserted to a minimum depth of 5 – 8 cm and making readings after 60 s. Secondary data on daily humidity and temperature were accessed from a NASA website containing and providing meteorological and solar-related data that can be directly downloaded after providing the coordinates of the specified location.

Soil Collection and Processing. Four sampling periods of the soil collection for nutrient and physicochemical analyses were carried out in October 2019, January 2020, March 2020, and July 2020 in the 3 yr old established cacao experimental plots. Soil samples were obtained from randomly chosen trees per treatment in each block. At least 500 g of soil was collected using a soil probe to dig up to 6 – 8 in down the surface of the ground, and 10 in from the tree base, which was randomly chosen by rolling dice in each block. A total of 21 samples from 21 trees were gathered for the experiment. The soil samples were placed in clean Ziplocs and properly labeled before transport. Samples were immediately brought to the laboratory, air-dried, and sieved through a 5 mm screen (Aggangan and Jomao-as 2019) and were subjected to the following analysis: pH, organic matter (% OM), available phosphorus (% P_2O_5), exchangeable potassium (% K_2O_5), total nitrogen (% N), cation exchange capacity (cmol$_c$/kg soil CEC) and water-holding capacity (% WHC). Soil analyses were performed at the Agricultural Systems Institute, UPLB. Soil pH was measured with a compound electrode using a soil-to-water ratio of 1:1 (Black et al. 1965). Soil N and P contents were determined by the Kjeldahl (Black et al. 1965) and Bray (Bray and Kurtz 1945) methods, respectively. Soil CEC was determined by ammonium acetate distillation (Black et al. 1965) while exchangeable K was analyzed by ammonium displacement of the exchangeable cations (Peech 1945).

AMF Spore Count. AMF spore count was done on a 50 g subsample from the 500 g rhizosphere soil sample collected from each of the 3 randomly selected cacao trees. Spores were separated from the soil using the wet sieving and centrifugation technique by Brundrett et al. (1995), decanted thrice using steel sieves of 0.5 mm, 100 µm, and 50 µm pore openings after the soils were mixed thoroughly and suspended in water. The decanted liquid was centrifuged and the remaining pellet was resuspended in 60% sucrose after the supernatant was discarded. Following another centrifugation, the supernatant containing the spores was poured into a fine mesh and rinsed several times with water (Aggangan and Jomao-as 2019). The final rinse was poured on a Petri plate with grids and spores were counted in 10 field views under a stereomicroscope. The average spore count in 10 field views (equivalent to 10 square grids) was multiplied by the number of square grids in the Petri plate.

NFB and PSB Population Count. Soil microbial count was carried out in 2 sampling periods, March and July 2020. NFB and PSB colony-forming units were quantified using the protocol described by Estrada-De Los Santos et al. (2001) and that of Nautiyal (1999), respectively. Modifications were applied following the work of Aggangan, Cortes, Opulencia et al. (2019) in the preparations of Dobereiner's and Pikovskaya's media for NFB and PSB, respectively. Briefly, a 10 g rhizosphere sample was diluted and mixed in 90 mL of sterile distilled water, making the first dilution. A 10-fold dilution of the soil sample was carried out by transferring 1 mL of suspension from the 1st dilution into 9 mL dilution tubes and was further serially diluted up to 10 dilutions. From each dilution tube, 0.1 mL was pipetted and dropped on the media plates for spread plating. This was done in duplicates and incubated for 5 – 10 d for bacterial growth at 30°C. Bacterial colonies were counted once when colonies had already formed and indicated as TNTC (too numerous to count) if the count exceeded 200 colonies and TFTC (too few to count) if the colonies were fewer than 30.

Statistical Analyses. Data gathered were subjected to analysis of variance (ANOVA) using STATA 12 and SPSS 25. Univariate analysis was used to determine significant differences in response variables among treatments from individual sampling periods for AMF spore and PSB and NFB microbial count. Mixed ANOVA was used to test for mean differences between 2 or more independent groups while subjecting samples to repeated measures and to determine whether an interaction effect exists between the time points of soil collection (in months) and treatments on soil physicochemical properties, nutrient levels, and AMF spore count. Appropriate effect analysis and Tukey's tests were carried out as follow-up tests to explore the difference between multiple group means.

RESULTS

Soil Physicochemical Properties and Nutrient Levels Compared Between Treatments in Individual Months

N level alone varied between treatments and differed statistically in January and March with $p = 0.017$ and $p = 0.034$, respectively (Fig. 1). For January, it is notable that the combination of MR and BioN promoted higher N levels in the soil when compared to the other treatments. Interestingly, in March, higher levels of N were significantly observed from combined MV and BioN, and MR alone. Other soil physicochemical properties such as CEC, WHC, OM, and pH did not vary statistically between treatments and across the different sampling periods (except OM and WHC) whereas K values also showed significant differences between treatments (Table 3). P contents were also found to be significantly comparable among treatments. Noteworthy, however, is a pattern of higher soil P content among treatments with amendments (13.244 (MR + BioN + BB); 9.338 (MR); 6.235 (MV + BioN + BB); 6.236 (MV)) than the control (5.970). Between the sampling months, remarkably twice higher P levels in treatments with combined MR + BioN + BB which were 10.9, 15.97, 19.07, and 22.83% compared to the control with 7.47, 7.47, 8.07, and 8.20% for October, January, March, and July, respectively were observed. After 3 yr in the field, MR + BioN + BB increased soil P by 46% in October 2019 relative to the control (7.47%). In July 2020, soil P was increased by 178% relative to the control (8.20%). From October 2019 to July 2020, MR + BioN + BB increased soil P by 109% while the control had an increase of 9.8% only (Table 3).

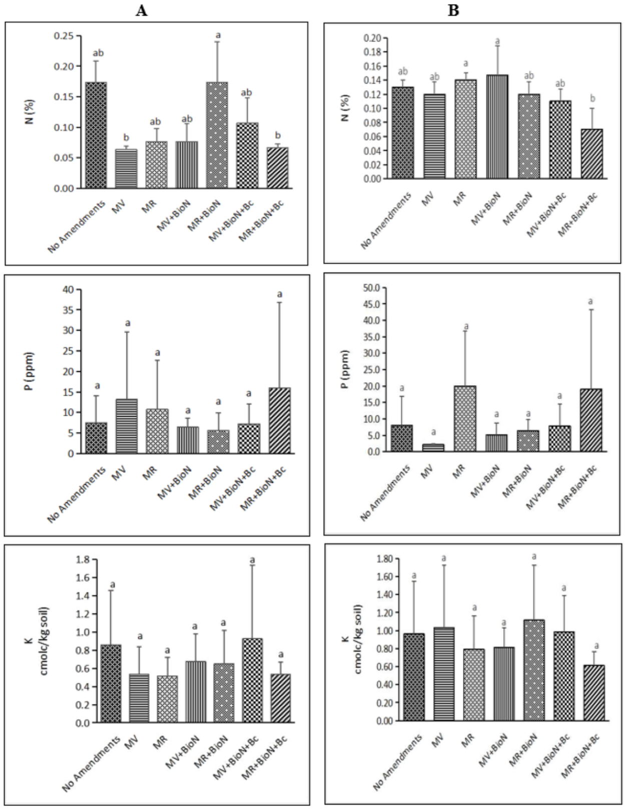

Fig. 1. NPK soil content for the month of A) January and B) March as affected by the different treatments. Boxplots with different letters indicate significant difference using Tukey's HSD test at $p < 0.05$; N = 21.

Soil Physicochemical Properties and Nutrient Levels Compared Across Time of Collection

There was a statistically significant main effect in time of collection on N content ($p < 0.05$) (Table 3). A posthoc analysis revealed a statistically significant difference in combined N content between soils collected in January 2020 versus the other months of soil collection ($p < 0.005$). N content in January 2020 was higher (1.772) than those collected in March 2020 (0.100), July 2020 (0.120), and October 2019 (0.155). Different treatments as well had a statistically significant main effect on N content ($p < 0.05$).

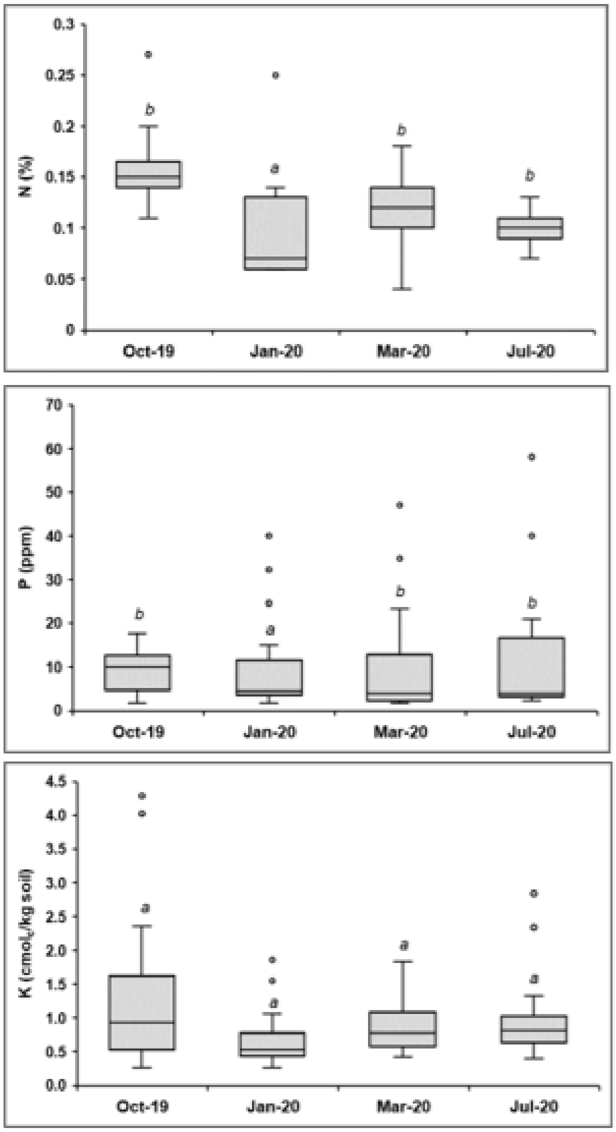

Fig. 2. Soil NPK contents as affected by the time of collection (sampling period). Boxplots with different letters indicate significant difference using Tukey's HSD test at *p* < 0.05; N = 21.

However, it was found that there was no statistically significant interaction effect between months and treatments (*p* > 0.05) (Fig. 2). This would mean that the effect of treatments on N content is the same across time. The same goes for the effect of time on N content which is the same across all treatments.

The results also show a statistically significant main effect in time of collection on P content (*p* < 0.05). A posthoc analysis showed a statistically significant difference in P content between soils collected in January as compared to other months involved (*p* < 0.005). Soils collected in January were associated with P content which was significantly lower than those collected in March, July, and October (Fig. 2). As for the treatments used, there was no statistically significant main effect on P content (*p* > 0.05). Moreover, there was no significant interaction between treatments and time of collection on P and K (Table 3) (*p* > 0.05). Likewise, when the 2 factors were analyzed separately, there were no significant main effects found in time as well as the treatments in terms of the K content in soils (*p* > 0.05). This indicated that the effect of time on P and K contents is the same for each of the treatments used.

OM and WHC showed statistical differences (*p* < 0.0001) between soils collected in different sampling months. Soils collected in October 2019 had significantly higher (2.883) OM content than those collected in January 2020 (1.997) and July 2020 (2.29) and were comparatively close to that of March 2020 (2.544). March 2020 soil collection had a 30% higher WHC (80.429) than that in January 2020 (58.857) and 10% higher than that in July (73.00) (Table 3).

Monthly AMF Spore Count Compared Between Treatments

The summary of statistics between means of spore counts and that all sampling periods had varying counts across treatments (*p* < 0.0001) was presented in Table 4. Post-hoc analysis of combined values of spore counts between treatments showed that there was a statistically significant difference in spore counts from soils amended with biofertilizer compared to its unamended counterpart for all sampling periods (Table 4). Looking at individual sampling months, in October, the statistically highest spore count was recorded in MV alone (113.13 ± 9.047) and comparably higher than the control (16.92 ± 9.04). In January, almost all treatments showed statistically high counts of spores except for the control and treatment of mycorrhiza in combination with BioN and biochar (*p* = 0.012). The same trend was observed for March wherein spore counts appeared comparably close in every treatment (*p* = 0.031). In July, spore counts were statistically unvaried between treatments, yet the highest count was still recorded in the treatment with MV alone and the lowest in the unamended treatment (*p* = 0.660) (Fig. 2).

Interaction Effect Between Time and Treatments on Spore Count

Separate analysis of the factors shows significant main effects found in time as well as in the treatments in terms of spore count (*p* < 0.0001). It can be seen further from post-hoc results that spore counts differed between individual sampling months, with January having the highest count (138.095 ± 6.839) and March having the lowest (25.048 ± 6.839). A statistically significant interaction between the time of collection and treatments in terms of spore count in the soil (*p* = 0.001) was also observed. This implied that the effect of time of collection on spore count was not the same for all treatments used and vice versa. From this result, it was apparent that regardless of season, untreated trees had the lowest spore counts. It was also evident that in January, the treatments MV, MR, MR + BioN, and MV + BioN had comparably high results which were almost the same as in October for the same treatments (Table 4).

Effects of Treatments on NFB and PSB Microbial Count

The mean microbial counts of NFB and PSB between treatments across 2 sampling periods was summarized in Table 5. The NFB and PSB population varied in different soil samples where some treatments improved the bacterial counts across 2 sampling periods —for example, in March where NFB count was higher for unamended soil compared to the other treatments except for MR applied alone and in combination with BioN and biochar. However, there were no established significant variations between NFB and PSB in both sampling months (*p* = 0.134) and between treatments (*p* = 0.321).

DISCUSSION

Soil Physicochemical and Nutrient Parameters

The overall positive outcomes from AMF applications in plant production attributed to enhanced nutritional benefits, both in controlled and open-field conditions, have been fairly established (Berruti et al. 2016), however, very little is known to the establishment of AMF as they adapt to varying environmental conditions (Johnson et al. 2013). The investigation of major elements such as N, P, and K in cacao-planted soils and how these affect the dynamics of AMF response are needed. The results of this study showed that it can be observed that N levels varied between treatments of AMF applications whereas P and K levels did not, both in individual sampling months and in the combined analysis. Phosphorus level is a major factor on which AMF-associated plants depend, but nitrogen level is another factor to consider that determines responses and intensities of symbiotic associations (Nouri et al. 2014) and it is the nutrient needed in the largest quantity for cacao. Specifically, the N level was highest in the treatments with combined AMF and BioN for individual months with indicated significant differences (Table 3). This should be a logical outcome since BioN contains N-fixing bacteria, thus reinforcing available N in the soil. Very few studies have looked at levels of N in cacao-planted soils. This must be because AMF hyphae are known to be generally less participative in directly improving N nutrition (Hodge 2017), especially since N uptakes by plants are not limited by the mobility of inorganic N forms (Marschner 1995). It is therefore important to relate soil conditions and soil N content to make accurate observations on N uptake in plants and properly attribute this to AMF associations. For instance, it was observed that when mineral N is low in the soil, non-mycorrhizal plants take up more N due to the absence of competition with AMF (Püschel et al. 2016). Furthermore, the combined analysis in Table 3 only showed distinct differences in total N level between the 2 sources of AMF—MV and MR—but comparable to the other treatments including the non-AMF treatment. Despite this seemingly close observation of levels of N among treatments, important indirect effects of AM symbioses may depend on other aspects such as environmental factors (Corrêa et al. 2015). This study found that N contents were directly related to the sampling periods, of which the month of January 2020 yielded the highest result. Seasonal fluctuations on N levels have long been investigated for several plants but none so far has been reported in the cacao-AMF context.

Even though P levels appeared high from the treatments with combined amendments of MR, BioN, and biochar when compared with the unamended treatment, the data gave no significant difference between means. However, it was noteworthy that P levels in the treated soils ranged from 6.235 to 9.338 ppm, relatively lower than the published P level requirement by Ritung et al. (2007) of cacao plants but remarkably higher than the surveyed P levels in the soils of selected cacao agrosystems in Davao, Philippines. Among 8 surveyed orchards, only 2 had favorable (above-indicated level) soil conditions in terms of P levels. The rest of the surveyed plantations had P levels ranging only from 1.71 to 6.52 ppm (Villason and Olguera 2020). This implies the added boost on P levels when AMF is applied to soils based on the results of this study.

Relatedly, the study by Aggangan, Cortes, and Reaño (2019) investigated the effect of AMF application on several aspects of plant growth response between sterilized and unsterilized soil and reported higher P levels in non-mycorrhizal cacao for both setups. These differences in results were likely to be related to the effect of adding other amendments such as BioN and biochar in this study, while only biochar was added in the latter. The differences presented by using experimental setups in greenhouses compared to actual natural field conditions can also be taken into consideration. Moreover, in this study, P levels varied significantly across sampling periods, with January being remarkably low as compared to the other sampling months (Fig. 1). Some studies have reported the importance of P levels in the soil and how they direct the impacts of AMF association especially on diversity (Collins and Foster 2009) and in overall ecosystem stability (Yang et al. 2014). The same sampling month—January—was recorded to have the highest available soil N. This study raises the possibility that the high levels of N allowed for ample utilization by AMF hyphae, satisfying the N demand of the fungus and resulting in efficient P-uptake responses by the cacao plant (Hodge et al. 2010). This was however, inconclusive since P-uptake was not specifically computed for this study. The month of January is considered a cool dry season characterized by average soil temperatures of 26.71°C (Table 2), the lowest recorded soil temperature among the sampling periods. This finding can be related to the study by Ylivainio and Peltovuori (2012) on barley that demonstrated the increase in P movement by diffusion with increased soil temperature as they emphasized the value of identifying suboptimal soil temperatures for specific crops to make the addition of P fertilizers effective. Thus, it seems possible that another reason for the low level of P in January is the slow diffusion of P in the soil for this month.

In terms of other physicochemical properties investigated, levels of pH and CEC did not vary between treatments and between sampling periods. The absence of a significant effect of the treatments on soil physicochemical properties is the same as that of the findings of Aggangan, Cortes, and Reaño (2019). Interestingly, pH levels did not vary between months despite expected variations due to changes in local climatic conditions (Table 2). This urges the assumption that the agroforest soil has gained the ability to resist changes in pH, which can be translated to increased buffer capacity—a good indication of resilience in an agrosystem (Speranza 2013).

Soil OM is considered one of the important determinants of soil biochemical properties, making them important indicators of soil fertility and productivity (Wang et al. 2016). The current study found that OM levels varied across sampling periods and were recorded highest in October which is noted with high relative humidity and soil temperature (Table 3). Many studies reported the dependence of soil organic matter levels on soil temperature and moisture (Thongjoo et al. 2005; Wang et al. 2016). Another possible explanation for this might be the fresh additions of plant residues from leaf litters and decaying fruit materials or dried leaves that have fallen off from the cacao trees during this month and, consequently, increased microbial biomass—a significant factor in increased soil OM (Stolt and Lindbo 2010).

Table 1. Initial chemical properties of sampled soil and bamboo biochar.

Characteristics	Calauan Acidic Soil	Bamboo Biochar
pH	4.72 ± 0.26	8.21 ± 0.01
OM%	3.16 ± 0.58	9.6 ± 0.02
Total N %	0.18 ± 0.01	0.48 ± 0.00
Available P (ppm)	12.58 ± 5.70	-
Total P_2O_5 %	-	0.30 ± 0.00
Exchangeable K (me/100g soil)	1.69 ± 0.17	-
Total K_2O %	-	1.15 ± 0.01
Exchangeable Ca (me/100g soil)	7.07 ± 0.91	1,675 ± 11
Exchangeable Mg (me/100g soil)	6.02 ± 0.77	1,126 ± 16
CEC (me/100g soil)	37.72 ± 3.71	17.29 ± 1.3
Fe (ppm)	138 ± 7.68	3,684 ± 139
Zn (ppm)	2.2 ± 0.45	48.64 ± 1.67
Cu (ppm)	-	11.71 ± 0.45
Mn (ppm)	-	-

* Excerpt from "Effectiveness of Multiple Inoculation of Biofertilizer with Biochar on Growth of Cacao (*Theobroma cacao* L.) Seedlings Planted under Agroforest Ecosystem" by Aggangan and Victoria (2019)

Table 2. Average of daily climatological parameters between sampling periods.

Parameters	Oct-19	Jan-20	March 2020	July 2020
Precipitation (mm day^{-1})	5.84*	4.60*	15.60*	8.23*
Relative humidity (%)	84.99*	84.08	83.97	84.35*
Temperature (°C)	26.29*	24.42	25.33	27.01*
Infrared radiative flux (MJ/m^2/day)	17.24*	14.46*	20.76*	19.06*
Soil temperature (°C)	-	26.71	30.11	-

Soil Biological Parameters

There was a very little information on how microbial communities, especially those directly involved in nutrient acquisition such as PSB and NFB, change over long periods in the field, especially in cacao agroforest systems. This study found that AMF spore count in the soil was largely affected by the treatments during the 3 yr field experiment on cacao plants. In accord with this is the study of Aggangan, Cortes, Opulencia et al. (2019) that previously reported increased root colonization and spore count in AMF-treated soils. This was generally a logical result as it was expected to observe high spore counts in soils with amended sources of mycorrhizal spores. The distinct role and association of AMF spores in several plant groups such as some legumes (Alori et al. 2020), corn (Emmanuel et al. 2012), and many others

Table 3. Interaction effect analysis of time of collection (months) and treatments on soil nutrient contents and chemical properties.

	Months			Treatments			Interaction* (Months x Treatment)
	Months	**Mean ± SE****	***p*-value***	**Treatment**	**Mean ± SE**	***p*-value***	***p*-value**
				NA	0.557 ± 0.024^{cd}		
N content	January	1.772 ± 0.018^{a}		MV	0.479 ± 0.024^{c}		
	March	0.100 ± 0.018^{b}	0.0001	MR	0.587 ± 0.024^{d}	0.031	0.1350
	July	0.120 ± 0.018^{b}		MV + BN	0.543 ± 0.024^{cd}		
	October	0.155 ± 0.018^{b}		MR + BN	0.523 ± 0.024^{cd}		
				MV+BN+BB	0.571 ± 0.024^{cd}		
				MR+BN+BB	0.498 ± 0.024^{cd}		
				NA	5.970 ± 2.948^{ns}		
P content	January	0.163 ± 2.228^{a}		MV	6.236 ± 2.228		
	March	9.770 ± 2.228^{b}	0.0040	MR	9.338 ± 2.228	0.456	0.9400
	July	10.924 ± 2.228^{b}		MV + BN	5.622 ± 2.228		
	October	8.800 ± 2.228^{b}		MR + BN	5.255 ± 2.228		
				MV+BN+BB	6.235 ± 2.228		
				MR+BN+BB	13.244 ± 2.228		
				NA	0.867 ± 0.208^{ns}		
K content	January	1.196 ± 0.157^{ns}		MV	1.256 ± 0.208		
	March	0.901 ± 0.157	0.3530	MR	0.969 ± 0.208	0.887	0.8640
	July	0.990 ± 0.157		MV + BN	1.125 ± 0.208		
	October	1.250 ± 0.157		MR + BN	1.118 ± 0.208		
				MV+BN+BB	1.090 ± 0.208		
				MR+BN+BB	1.167 ± 0.208		
				NA	26.41 ± 1.076^{ns}		
	October	26.296 ± 0.81^{ns}		MV	28.197 ±1.076		
CEC	January	28.199 ± 0.813	0.3570	MR	26.814 ±1.076	0.857	0.9480
	March	27.740 ± 0.813		MV + BN	27.983 ± 1.076		
	July	26.88 ± 0.813		MR + BN	26.516 ± 1.076		
				MV+BN+BB	27.581 ± 1.076		
				MR+BN+BB	27.449 ± 1.076		
				NA	2.318 ± 0.231^{ns}		

(Ghorbani et al. 2012) have been studied and are related to soil fertility and as a guide to fertilizer use, which is why spore abundance is an important aspect of investigating the overall impact of amending soils with AMF. Also, an interaction effect between the time of collection and the treatments used was found in this study, indicating that the effect of treatments on spore count varied across time (Fig. 2). In this study, the highest (138.095) spore count was recorded in January 2020, which was characterized by cool and dry climate conditions. This result reflects those of Vieira et al. (2020) who also found that spore density is highest during the dry season as they investigated the mycorrhizal fungi dynamics in major vegetation in Brazil. They related this result to the known importance of AMF in water absorption and tolerance to periods of drought. However, in this study, March—a hot dry season in the country—gave the lowest spore count. Beccera et al. (2011) reported that specific species of AMF are abundant for specific seasons. The identification of AMF spores was not carried out in this study but since the commercially available sources of AMF used were predominantly of the genera *Glomus, Gigaspora, Entrophospora,* and *Acaulospora,* they might be nonsporulating at this period which may explain the fewer counts in March with the seasonal sporulation of AMF that has been reported (Oehl et al. 2009), which is determined by several factors such as variation of environmental conditions and host phenology (Pringle and Bever 2002). Soumaila et al. (2012) studied the population dynamics of AMF in the cacao fields in the region of Yamoussoukro (Ivory Coast) and found that spores were widespread among study sites but were of different densities, attributing such differences to environmental factors. This was the first report in the Philippines on the seasonal variability of AMF spores in

Table 3. Continuation...

	Months			Treatments			Interaction* (Months x Treatment)
	Months	**Mean ± SE****	***p*-value***	**Treatment**	**Mean ± SE**	***p*-value***	***p*-value**
OM	October	2.883 ± 0.151^{a}		MV	2.094 ± 0.231		
	January-March	1.997 ± 0.151^{c}	< 0.001	MR	2.863 ± 0.231	0.807	0.272
	July	2.544 ± 0.15^{ab}		MV + BN	2.457 ± 0.231		
		2.29 ± 0.15^{bc}		MR + BN	2.446 ± 0.231		
				MV+BN+BB	2.421 ± 0.231		
				MR+BN+BB	2.408 ± 0.231		
				NA	4.933 ± 0.115^{ns}		
pH	October	4.824 ± 0.087^{ns}		MV	4.683 ± 0.115		
	January	4.671 ± 0.087		MR	4.650 ± 0.115	0.16	0.86
	March	4.562 ± 0.087	0.16	MV + BN	4.583 ± 0.115		
	July	4.78 ± 0.087		MR + BN	4.558 ± 0.115		
				MV+BN+BB	4.658 ± 0.115		
				MR+BN+BB	4.892 ± 0.115		
				NA	68.556 ± 2.711^{ns}		
WHC	January	58.857 ± 1.775^{c}		MV	71.556 ± 2.711		
	March	80.429 ± 1.775^{b}	< 0.001	MR	69.500 ± 2.711	0.715	0.764
	July	73.00 ± 1.837^{a}		MV + BN	73.111 ± 2.711		
				MR + BN	73.556 ± 2.711		
				MV+BN+BB	69.111 ± 2.711		
				MR+BN+BB	69.125 ± 2.711		

* Analyzed using two-way ANOVA with 95% confidence level (boldface indicates significance with 0.05 α level);

**Analyzed using two-way ANOVA main effects and Tukey's post-hoc test (levels that do not share superscripts differ by < 0.05 according to Tukey's significant difference)

terms of density for cacao in a biofertilizer-amended agroforest soil. The distinct variations in spore count between sampling months imply a major influence of the seasons on spore density and that while treatments of AMF and other amendments were found to cause changes in spore count differently for specific periods, the mechanism by which soil amendments and seasonal variations interact was still very scarcely understood.

Another variable investigated was the colony counts of PSB and NFB present in the rhizosphere soil of cacao. The comparably similar counts between these microbes in this study partially reflect the findings of Aggangan, Cortes, and Reaño (2019) who reported an increase in PSB in amended soils of cacao seedlings but which did not affect the NFB population. This is relatable to the statistically unvaried results on PSB and NFB counts from this study and the occasional higher PSB and NFB counts in the unamended treatment compared to the amended ones (Table 5). Wang et al. (2016) investigated the long-term effects of bioorganic fertilizer on microbial communities in an apple orchard and found that the application of bioorganic fertilizers stimulated soil productivity by enhancing bacterial community composition and improving soil enzyme activity. Thus, the results on microbial count seen in this investigation

Table 4. Interaction effect analysis of time of collection (months) and treatments on spore count; Univariate analysis of soil spore count in different time of collection (months) and treatments.

	Months			Treatments			Interaction* (Months x Treatment)
	Months	Mean ± SE	p-value*	Treatment	Mean ± SE	p-value*	p-value
Spore count	October	102.690 ± 6.839^{a}		NA	16.92 ± 9.047^{c}		
	January	138.095 ± 6.839^{b}	< 0.0001	MV	113.13 ± 9.047^{a}	< 0.0001	0.001
	March	25.048 ± 6.839^{c}		MR	99.50 ± 9.047^{ab}		
	July	58.048 ± 6.839^{d}		MV + BN	97.63 ± 9.047^{ab}		
				MR + BN	79.25 ± 9.047^{ab}		
				MV+ BN + BB	92.42 ± 9.047^{ab}		
				MR + BN + BB	67.96 ± 9.047^{b}		

*Analyzed using two-way ANOVA with 95% confidence level (boldface indicates significance with 0.05 α level);

**Analyzed using two-way ANOVA main effects and Tukey's post-hoc test (levels that do not share superscripts differ by < 0.05 according to Tukey's significant difference)

Table 5. Microbial count of PSB and NFB among treatments across two sampling periods.

Treatments	Microbial Count							
	NFB (CFU x 10^5 g^{-}/soil)				PSB (CFU x 10^5 g^{-}/soil)			
	January	*p*-value	March	p-value	January	*p*-value	March	*p*-value
MV	0.21^{ns}		0.51^{ns}		2.86^{ns}		1.92^{ns}	
MR	0.72		51.61		5.38		0.46	
MV+BioN	1.77		0.39		4.17		0.43	
MR+BioN	1.36	0.117	0.31	0.473	6.13	0.735	0.64	0.415
MV+BioN+Bc	0.71		0.33		1.22		0.60	
MR+BioN+Bc	0.33		14.18		12.17		0.48	
No Amendments	0.49		1.74		1.25		0.51	

*Analyzed using one-way ANOVA with 95% confidence level (boldface indicates significance with 0.05 α level);

**Tukey's post-hoc test (levels that do not share superscripts differ by < 0.05 according to Tukey's significant difference)

must be a function of an indirect effect of AMF association with cacao trees and their soil environment.

CONCLUSION

Distinct seasonal trends were recorded in N, P, water-holding capacity (WHC), and organic matter (OM) soil levels. Although differences occurred between sampling months in soil N and P contents, there was no clear relationship between the time of collection and the treatments used in the present study. Taken together, this implies no direct link between the treatments and the sampling periods in the absence of established interaction effects. The research also showed that seasonal variabilities did not influence the effect of arbuscular mycorrhizal fungi (AMF) on cacao trees in field conditions despite numerous studies that have established the direct link between environmental factors and mycorrhizal impacts for many different crops. Moreover, it was found that the addition of AMF and other soil amendments had a profound effect on AMF spore density from the soil rhizosphere of a three-year-old established cacao agroforest. Combined spore count showed that the treatment with MYKOVAM® (MV) alone yielded the highest spore count among treatments. Taken individually between months, high spore counts were generally recorded in amended soils compared to the unamended counterpart. Seasonal variations in spore density were found. The effects of these treatments on spore count differed across the sampling periods due to established interaction effects from statistical results. Interestingly, microbial counts did not vary across treatments between the sampling periods, which implies an indirect AMF effect on the microbial density in cacao in field conditions. Thus, the findings of this research provided insights for establishing the long-term effects of AMF application specifically in cacao trees, as this relationship can be intricate and dynamic, especially in being able to keep soil nutrients sustainable in the soil. The generality of these results, however, was subject to limitations set by unprecedented calamities that may have directly affected the local climate during the sampling period (such as the eruption and intense ashfall of Taal Volcano in January 2020).

RECOMMENDATIONS

Findings from this study can be used to supplement and interpret data on large spatial scales as well as create logical strategies in the application of biofertilizers alone or in combination with other amendments or fertilizers to maximize the health status and soil nutrient levels in cacao agroforests. This investigation allowed researchers to further understand how different seasons may affect the physiological demands of cacao trees as indicated in their soil physicochemical and nutrient contents. This was a useful tool in guiding agricultural practices such as field nutrient monitoring and fertilizer application. However, long-term field experiments were still needed to predict nutritional limitations in agroforest ecosystems, which were made up of complex processes. Also, further attention to studying arbuscular mycorrhizal fungi (AMF) communities or microbial communities associated with cacao and the processes involved in existing interactions in varying conditions will provide a venue to acquire more knowledge on plant-community interactions. Large randomized controlled trials could provide more definitive evidence on the long-term effects of the application of AMF under varying climatic conditions. Samples should be expanded and subsampling methods should be employed to assess variability more accurately. Lastly, the experiment was designed to account for the effects of adjacent intercropped plants on the cacao trees. This could have implications in determining the success of invasions of biotic components such as AMF in plants.

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REFERENCES CITED

AGGANGAN NS, CORTES AD, OPULENCIA RB, JOMAO-AS JG, YECYEC RP. 2019. Effects of mycorrhizal fungi and bamboo biochar on the rhizosphere bacterial population and nutrient uptake of cacao (*Theobroma cacao* L.) seedlings. Philipp J Crop Sci. 44(1):1–9. https://www.researchgate.net/publication/332230710_Effects_of_Mycorrhizal_Fungi_and_Bamboo_Biochar_on_the_Rhizosphere_Bacterial_Population_and_Nutrient_Uptake_of_Cacao_Theobroma_cacao_L_Seedlings.

AGGANGAN NS, CORTES AD, REAÑO CE. 2019. Growth response of cacao (*Theobroma cacao* L.) plant as affected by bamboo o biochar and arbuscular mycorrhizal fungi in sterilized and unsterilized soil. Biocatal Agric Biotechnol. 22:1013467. doi:10.1016/j.bcab.2019.101347.

AGGANGAN NS, JOMAO-AS JG. 2019. Biochar from sugarcane bagasse and arbuscular mycorrhizal fungi on growth and nutrient status of cacao (*Theobroma cacao* L.) seedlings under nursery conditions. Philipp J Sci. 148(4):647–657. https://www.ukdr.uplb.edu.ph/journal-articles/560.

AGGANGAN NS, VICTORIA KS. 2019. Effectiveness of multiple inoculation of biofertilizer with biochar on growth of cacao (*Theobroma cacao* L.) seedlings planted under agroforest ecosystem. Philipp J Crop Sci. 44:89–97. https://ovcre.uplb.edu.ph/journals-uplb/index.php/PJCS/article/view/363.

AL-KARAKI G, MCMICHAEL B, ZAK J. 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263–269. doi:10.1007/s00572-003-0265-2.

ALORI ET, FAWOLE OB, AKANJI MO. 2020. Occurrence of arbuscular mycorrhizal fungi spores in soils of some legumes and their response to varying concentrations of phosphorus application. Open Agr. 5(1):539–547. doi:10.1515/opag-2020-0052.

BALLIU A, SALLAKU G, REWALD B. 2015. AMF inoculation enhances growth and improves the nutrient uptake rates of transplanted, salt-stressed tomato seedlings. Sustainability. 7(12):15967–15981. doi:10.3390/su71215799.

BECERRA AG, CABELLO MN, BARTOLONI NJ. 2011. Native arbuscular mycorrhizal fungi in the Yungas forests, Argentina. Mycologia. 103(2):273–279. doi:10.3852/10-193.

BEGUM N, QIN C, AHANGER MA, RAZA S, KHAN MI, ASHRAF M, AHMED N, ZHANG L. 2019. Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front Plant Sci. 10:1068. doi:10.3389/fpls.2019.01068.

BERRUTI A, LUMINI E, BALESTRINI R, BIANCIOTTO V. 2016. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Front Microbiol. 6:1559. doi:10.3389/fmicb.2015.01559.

[BIOTECH-UPLB] National Institute of Molecular Biology and Biotechnology - University of the Philippines Los Baños. n.d. Mykovam. Laguna, Philippines: University of the Philippines Los Baños. https://biotech.uplb.edu.ph/products-and-technologies/products/mykovam/.

BLACK CA. 1965. Methods of soil analysis part 1: physical and mineralogical properties, including statistics of measurement and sampling. Madison (WI): American Society of Agronomy. 770 p.

BONA E, CANTAMESSA S, MASSA N, MANASSERO P, MARSANO F, COPETTA A, LINGUA G, D'AGOSTINO G, GAMALERO E, BERTA G. 2017. Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve yield, quality and nutritional value of tomato: a field study. Mycorrhiza. 27(1):1–11. doi:10.1007/s00572-016-0727-y.

BONFANTE P, GENRE, A. 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun. 1:48. doi:10.1038/ncomms1046.

BRAY RH, KURTZ LT. 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59:39–45. doi:10.1097/00010694-194501000-00006.

BRUNDRETT M, BOUGHER N, DELL B, GROVE T, MALAJCZUK N. 1995. Working with mycorrhizas in forestry and agriculture. Canberra, Australia: Pirie Printers.

CLIMATE-DATA. n.d. Mabacan climate. https://en.climate-data.org/asia/philippines/laguna/mabacan-1066841/.

COLLINS CD, FOSTER BL. 2009. Community-level consequences of mycorrhizae depend on phosphorus availability. Ecology. 90(9):2567–2576. doi:10.1890/08-1560.1.

CORRÊA A, CRUZ C, FERROL N. 2015. Nitrogen and carbon/nitrogen dynamics in arbuscular mycorrhiza: the great unknown. Mycorrhiza. 25:499–515. doi:10.1007/s00572-015-0627-6.

[DTI] Department of Trade and Industry. The Philippines in the cocoa-chocolate global value chain 2017. DTI Policy Briefs. Series No. 2017-09. https://innovate.dti.gov.ph/about/btipr-services/policy-brief/policy-brief-2017-09/#:~:text=The%20Philippines'%20current%20involvement%20in,share%20of%20less%20than%200.01%25.

DUCEY TF, NOVAK JM, JOHNSON MG. 2015. Effects of biochar blends on microbial community composition in two coastal plain soils. Agriculture. 5(4):1060–1075. doi:10.3390/agriculture5041060.

EMMANUEL B, FAGBOLA O, OSONUBI O. 2012. Influence of fertilizer application on the occurrence and colonisation of arbuscular mycorrhizal fungi (AMF) under maize/Centrosema and sole maize systems. Soil Res. 50(1):76–81. doi:10.1071/SR11254.

ESTRADA-DE LOS SANTOS P, BUSTILLOS-CRISTALES R, CABALLERO-MELLADO J. 2001. Burkholderia, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. Appl Environ Microbiol. 67(6):2790–2798. doi:10.1128/AEM.67.6.2790-2798.2001.

GARCÉS-RUIZ M, CALONNE-SALMON M, PLOUZNIKOFF K, MISSON C, NAVARRETE-MIER M, CRANENBROUCK S, DECLERCK S. 2017. Dynamics of short-term phosphorus uptake by intact mycorrhizal and non-mycorrhizal maize plants grown in a circulatory semi-hydroponic cultivation system. Front Plant Sci. 8:1471. doi:10.3389/fpls.2017.01471.

GHORBANI M, KHARA J, ABASPOOR N. 2012. Vesicular-arbuscular mycorrhizal symbioses in some plants and its relationship with soil factors and seasons. Iran J Plant Physiol. 3(1):589–594. https://ijpp.saveh.iau.ir/article_540667_ea6cf6e2b86a6b45e7f7b51af91f7243.pdf

GUI H, HYDE K, XU J, MORTIMER P. 2017. Arbuscular mycorrhiza enhance the rate of litter decomposition while inhibiting soil microbial community development. Sci Rep. 7:42184. doi:10.1038/srep42184.

HODGE A. 2017. Chapter 8 - accessibility of inorganic and organic nutrients for mycorrhizas. In: Johnson NC, Gehring C, Jansa J, editors. Mycorrhizal mediation of soils: fertility, structure, and carbon storage. Amsterdam: Elsevier. p. 129–148.

HODGE A, HELGASON T, FITTER AH. 2010. Nutritional ecology of arbuscular mycorrhizal fungi. Fungal Ecol. 3(4):267–273. doi:10.1016/j.funeco.2010.02.002.

ISAAC ME, GORDON AM, THEVATHASAN N, OPPONG SK, QUASHIE-SAM J. 2005. Temporal changes in soil carbon and nitrogen in west African multistrata agroforestry systems: a chronosequence of pools and fluxes. Agroforest Syst. 65:23–31. doi:10.1007/s10457-004-4187-6.

JOHNSON NC, ANGELARD C, SANDERS IR, KIERS ET. 2013. Predicting community and ecosystem outcomes of mycorrhizal responses to global change. Ecol Lett. 16(Suppl 1):140–153. doi:10.1111/ele.12085.

LU Y, RAO S, HUANG F, CAI Y, WANG G, CAI K. 2016. Effects of biochar amendment on tomato bacterial wilt resistance and soil microbial amount and activity. Int J Agron. 2016:2938282. doi:10.1155/2016/2938282.

MARSCHNER H. 1995. Mineral nutrition of higher plants. London: Academic Press.

MELIANI A, BENSOLTANE A, MEDERBEL K. 2012. Microbial diversity and abundance in soil: related to plant and soil type. Am J Plant Nutr and Fertil Technol. 2(1):10–18. doi:10.3923/ajpnft.2012.10.18.

NACOON S, JOGLOY S, RIDDECH N, MONGKOLTHANARUK W, KUYPER TW, BOONLUE S. 2020. Interaction between phosphate solubilizing bacteria and arbuscular mycorrhizal fungi on growth promotion and tuber inulin content of *Helianthus tuberosus* L. Sci Rep. 10:4916. doi:10.1038/s41598-020-61846-x.

NANJUNDAPPA A, BAGYARAJ DJ, SAXENA AK, KUMAR M, CHAKDAR H. 2019. Interaction between arbuscular mycorrhizal fungi and *Bacillus* spp. in soil enhancing growth of crop plants. Fungal Biol Biotechnol. 6:23. doi:10.1186/s40694-019-0086-5.

NAUTIYAL CS. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett. 170(1):265–270. doi:10.1111/j.1574-6968.1999.tb13383.x.

NGONKEU MEL. 2003. Biodiversité et potentiel des mycorhizes à arbuscules de certaines zones agro-écologiques du Cameroun [dissertation]. Yaoundé, Cameroon: University of Yaoundé I.

NOURI E, BREUILLIN-SESSOMS F, FELLER U, REINHARDT D. 2014. Phosphorus and nitrogen regulate arbuscular mycorrhizal symbiosis in Petunia hybrida. PLoS One. 9:e90841. doi:10.1371/journal.pone.0090841.

OEHL F, SIEVERDING E, INEICHEN K, MÄDER P, WIEMKEN A, BOLLER T. 2009. Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. Agric Ecosyst Environ. 134(3–4):257–268. doi:10.1016/j.agee.2009.07.008.

PAGANO MC, OEHL F, SILVA GA, MAIA LC, SILVA DK, CABELLO MN. 2016. Advances in arbuscular mycorrhizal taxonomy. In: Pagano MC, editor. Recent advances on mycorrhizal fungi. p. 15–21.

PEECH M. 1945. Determination of exchangeable cations and exchange capacity of soils—rapid micromethods utilizing centrifuge and spectrophotometer. Soil Sci. 59(1):25–38. https://journals.lww.com/soilsci/Citation/1945/01000/Determination_of_Exchangeable_Cations_and_Exchange.5.aspx.

PHILATLAS. n.d. Calauan: Province of Laguna. https://www.philatlas.com/luzon/r04a/laguna/calauan.html

PRINGLE A, BEVER JD. 2002. Divergent phenologies may facilitate the coexistence of arbuscular mycorrhizal fungi in a North Carolina grassland. Am J Bot. 89(9):1439–1446. doi:10.3732/ajb.89.9.1439.

PÜSCHEL D, JANOUŠKOVÁ M, HUJSLOVÁ M, SLAVÍKOVÁ R, GRYNDLEROVÁ H, JANSA J. 2016. Plant-fungus competition for nitrogen erases mycorrhizal growth benefits of Andropogon gerardii under limited nitrogen supply. Ecol Evol. 6(13):4332–4346. doi:10.1002/ece3.2207.

RAJESHKANNAN V, SUMATHI CS, MANIAN S. 2009. Arbuscular mycorrhizal fungi colonization in upland rice as influenced by agrochemical application. Rice Sci. 16(4):307–313. doi:10.1016/S1672-6308(08)60095-5.

RITUNG SW, WAHYUNTO, AGUS F, HIDAYAT H. 2007. Land suitability evaluation with a case map of Aceh Barat District. Bogor, Indonesia: Indonesian Soil Research Institute and World Agroforestry Centre. 33 p.

SNOECK D, ABOLO D, JAGORET P. 2010. Temporal changes in VAM fungi in the cocoa agroforestry systems of central Cameroon. Agroforest Syst. 78:323–328. doi:10.1007/s10457-009-9254-6.

SOLÍS-DOMÍNGUEZ FA, VALENTÍN-VARGAS A, CHOROVER J, MAIER RM. 2011. Effect of arbuscular mycorrhizal fungi on plant biomass and the rhizosphere microbial community structure of mesquite grown in acidic lead/zinc mine tailings. Sci Total Environ. 409(6):1009–1016. doi:10.1016/j.scitotenv.2010.11.020.

SOUMAILA ZBIM, TRA TB, NOEL ZG, GHISLAINE KZC, ROMAIN FK, ZEZE A. 2012. Arbuscular mycorrizal fungi associated with *Theobroma cacao* L. in the region of Yamoussoukro (Cote d'Ivoire). Afr J Agric Res. 7(6):993–1001. doi:10.5897/Ajar11.2057.

SOUMARE A, DIEDHIOU AG, THUITA M, HAFIDI M, OUHDOUCH Y, GOPALAKRISHNAN S, KOUISNI L. 2020. Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. Plants. 9(8):1011. doi:10.3390/plants9081011.

SPARKS AH. 2018. nasapower: A NASA POWER global meteorology, surface solar energy and climatology data client for R. J Open Source Softw. 3(30):1035. doi:10.21105/joss.01035.

SPERANZA CI. 2013. Buffer capacity: capturing a dimension of resilience to climate change in African smallholder agriculture. Reg Environ Change. 13:521–535. doi 10.1007/s10113-012-0391-5.

STOLT MH, LINDBO DL. 2010. Soil organic matter. In: Stoops G, Marcelino V, Mees F, editors. Interpretation of micromorphological features of soils and regoliths. Elsevier Science. p. 369–396.

TCHAMENI SN, NWAGA D, WAKAM LN, NGONKEU ELM, FOKOM R, KUATÉ J, ETOA FX. 2012. Growth enhancement, amino acid synthesis and reduction in susceptibility towards Phytophthora megakarya by arbuscular mycorrhizal fungi inoculation in cocoa plants. J Phytopathol. 160(5):220–228. doi:10.1111/j.1439-0434.2012.01888.x.

THONGJOO C, MIYAGAWA S, KAWAKUBO N. 2005. Effects of soil moisture and temperature on decomposition rates of some waste materials from agriculture and agro-industry. Plant Prod Sci. 8(4):475 -481. doi:10.1626/pps.8.475.

TILMAN D, CASSMAN KG, MATSON PA, NAYLOR R, POLASKY S. 2002. Agricultural sustainability and intensive production practices. Nature. 418:671–677. doi:10.1038/nature01014.

VIEIRA WG JR, MOURA JBD, SOUZA RFD, BRAGA APM, MATOS DJDC, BRITO GHM, SANTOS JMD, MOREIRA RM, DUTRA ESS. 2020. Seasonal variation in mycorrhizal community of different Cerrado phytophysiomies. Front Microbiol. 11:576764. doi:10.3389/fmicb.2020.576764.

VILLASON NA, OLGUERA DT. 2020. Physico-chemical characteristics of soils under cacao production system in Davao de Oro, Philippines. Southeast Philipp J Res Dev. 25(1):115–134. doi:10.53899/spjrd.v25i1.75.

WANG L, YANG F, YAOYAO E, YUAN J, RAZA W, HUANG Q, SHEN Q. 2016. Long-term application of bioorganic fertilizers improved soil biochemical properties and microbial communities of an apple orchard soil. 7:01893. doi:10.3389/fmicb.2016.01893.

YANG G, LIU N, LU W, WANG S, KAN H, ZHANG Y, XU L, CHEN Y. 2014. The interaction between arbuscular mycorrhizal fungi and soil phosphorus availability influences plant community productivity and ecosystem stability. J Ecol. 102(4):1072–1082. doi:10.1111/1365-2745.12249.

YLIVAINIO K, PELTOVUORI T. 2012. Phosphorus acquisition by barley (*Hordeum vulgare* L.) at suboptimal soil temperature. Agric Food Sci. 21 (4):453–461. doi:10.23986/afsci.6389.