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Enhanced Growth Performance of *Bauhinia purpurea* L. and Rhizosphere Soil Microbial Communities by Inoculation of Beneficial Microbes

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Bauhinia purpurea **(belonging to Family Fabaceae-Leguminosae) is a small to medium-sized deciduous fast-growing tree that is very important in reforestation and agroforestry, and also used as fodder for livestock. This study investigated the influence of mycorrhizal inoculant with varying nitrogen-fixing bacteria (NFB) amendment levels on the growth performance of *B. purpurea*, the buildup of microbial communities in its rhizosphere soil, and root colonization in screenhouse conditions with UV plastic roofing, following a two-factor randomized complete block design. Factor 1 was the type of mycorrhizal inoculants applied on containerized seedlings grown in garden soil: AMF1 (MYKOCAP® or MCAP) and AMF2 (MYKORICH® or MRICH). Factor 2 was the rate of NFB (BioN™) amendment (0 g, 5 g per seedling). At 90 d, MCAP + BioN treated plants were 37% taller than the MRICH + BioN treated ones (34 ± 0.50 cm). Seedlings with MCAP + BioN or MRICH + BioN had higher diameter increment; partitioned biomass from stems, lateral roots, and primary roots; root-shoot ratio; and root colonization than those without BioN and the control. Likewise, the NFB population was 276 and 126%, respectively higher with MCAP + BioN and MRICH + BioN than their counterparts without BioN. Contrarily, partitioned biomass from leaves, roots, and shoots, and the arbuscular mycorrhizal fungi (AMF) spore density were higher with AMF inoculants alone. Spore count was strongly positively correlated with primary root biomass (p = 0.013, r = 0.573), NFB buildup with height increment (p = 0.001, r = 0.708), and root colonization with diameter increment (p = 0.001, r = 0.805), total biomass (p = 0.023, r = 0.532), stem biomass (p = 0.001, r = 0.692), shoot biomass (p = 0.001, r = 0.698), primary root biomass (p = 0.029, r = 0.514), and root biomass (p = 0.005, r = 0.628). AMF and NFB symbiosis was inferred to have been developed, thereby promoting overall plant growth with an increased mycorrhizal root infection and NFB buildup, while the increased spore density contributed to enhanced primary root growth. Field verification trials must be conducted to determine the microbial fertilizer's efficacy under abiotic and biotic stresses.**

Keywords: biomass, microbial fertilizers, mycorrhizal inoculation, root-shoot ratio, soil degradation

INTRODUCTION

One-fifth of the world's soil is degraded and continuously degrading at a rate of 5–10 billion hectares annually (Bateman and Muñoz-Rojas 2019) and threatens the welfare and food security of the poor farmers in the most densely populated marginal lands (Scherr 2019) in the countries in Asia and Africa, where agriculture is the "engine of growth."

Ill effects of anthropogenic activities have resulted worldwide in unparalleled loss of soil nutrients, organic matter, and acidification, among other soil degradation processes, making agricultural lands unproductive. Soil

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degradation is one of the major research priorities in the Philippines (Castro 2013) along with identifying sustainable farming techniques such as agroforestry and the use of microbes to address both environmental and socio-economic concerns.

Beneficial soil microorganisms are essential for keeping a healthy environment and in the cycling of elements (Jeffries and Barea 1994). The microbial technology that employs beneficial microbes to achieve sustainable agri-food systems and optimize crop productivity, although a traditional practice, was still understudied (Chanway 1997), particularly about the mechanisms of plant-microbe association, as well as diversity and persistence of microbes at various locations, soils, and hosts. Under abiotic and biotic stressed environments, with limited nutrients, or during bioremediation, other PGR microbes work well, whereas arbuscular mycorrhizal fungi (AMF) induced root growth for greater nutrient absorption.

Another valuable technology is agroforestry. The World Agroforestry Center (ICRAF 2021) defines agroforestry as "agriculture with trees." Thus, it bridges the gap between agriculture and forestry, whereby agricultural landscapes and agricultural use of trees are dealt with, along with the interaction of various tree species with annual crops, perennials, livestock, wildlife, and humans. Traditionally, the tree species planted included *Sesbania*, *Gliricidia*, *Moringa*, mahogany, *Acacia*, *Leucaena*, and *Jatropha*, among others. However, there are other lesser-known, multi-purpose forest tree species that can potentially uplift the agricultural economy and reduce, if not prevent, soil degradation.

Bauhinia is a lesser-known tree species with multiple uses – as a side dish, flavors, fodder, and a source of fiber, tannin, or dyestuff, medicine, lipid, gum or resin, fuel, and timber. *Bauhinia purpurea* is one of the species belonging to *Bauhinia* (under the Family Fabaceae - Leguminosae), naturalized in the Philippines and native in Bangladesh, Bhutan, China, India, Indonesia, Japan, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka, Taiwan, and Thailand. *B. purpurea* is an agroforestry species growing up to 10 m, an important cover crop to protect the soil and reduce surface erosion and a source of fodder for livestock production in Nepal (Kala 2007) and also a source of fuel, timber, and medicinal compounds for astringent, or as a cure to dysentery in Tehri District of India (Bijalwan 2013).

In the sloping areas in Nepal, Shrestha *et al.* (2008) revealed an improved AMF and bacterial biomass when amended with organic matter, and so, in the composition of soil microorganisms. Further, microbial biomass buildup was observed to be higher in the wet season than in the dry season. Tamilarasi *et al.* (2007) also found an association between and among *B. purpurea* bacteria and fungi.

B. purpurea (commonly called "fringon pula") is rarely used in the Philippines as a fodder crop. Instead, it is popular for landscaping due to its purple, showy flowers and resistance against extreme environments. Its ability to coppice right after the occurrence of typhoons or forest fires makes it suitable for reforestation, bioremediation, and slope stabilization due to its deep-penetrating root system and high root-to-shoot ratio (Singh *et al.* 2000) that provides better anchorage, thus lessening soil erosion. Finally, because it is a legume, it is a good nitrogen fixer.

Grasslands in the tropics are highly acidic, have low organic matter and nutrient contents, have a shallow solum, contain toxic substances, and are compact (Asio *et al.* 2009). Because of these characteristics, crop growth in tropical grasslands is usually constrained. With the characteristics suited for both reforestation and agroforestry, *B. purpurea* could be a promising plant species to rehabilitate some of the Philippines' grasslands and grazelands (Mendoza 1985). Furthermore, applying microbial inoculants to *B. purpurea* could significantly improve tree biomass production, which will later on translate to rural livelihood and development among tree farming communities. With the use of AMF and nitrogen-fixing bacteria (NFB), *B. purpurea* was inferred to produce a safer, greener, and greater supply of foliage as fodder for livestock and is more resistant and effective in restoring critically degraded areas.

Thus, the authors aimed to test the effectiveness of UPLB (University of the Philippines Los Baños) developed inoculants, specifically the MYKOCAP®, MYKORICH®, and BioN™ to: (1) determine the effects of these inoculants in improving the growth of *B. purpurea* in terms of height and diameter, biomass, and root/shoot ratio in nursery condition, (2) compare the effects of AMF and NFB inoculants on soil microbial characteristics as manifested by the mycorrhizal sporulation, build-up of NFB and root colonization; and (3) determine which among these parameters can best explain the variation in plant growth in response to inoculation treatments.

MATERIALS AND METHODS

The Study Site

This study was conducted in the screenhouse of Mycorrhiza Laboratory, of the National Institute of Molecular Biology and Biotechnology (UPLB-BIOTECH), Laguna, Philippines geographically located at 14°8'55" N, 121°15'43" E, from August to November 2021.

Experimental Design, Treatment, and Inoculation

The experiment followed a two-factor randomized complete block design (RCBD) in three blocks. Factor

1 was the type of mycorrhizal inoculants used: AMF1 (MYKOCAP® or MCAP) and AMF2 (MYKORICH® or MRICH) plus a control. Factor 2 was the rate of NFB amendment per seedling: [0 g (– BioN™) and 5 g (+ BioN™)]. Treatment inoculations with AMF were done during transplanting from the germination box into individual polybags (4" x 6") filled with 500-g sterilized sand and garden soil mixture (1:1, v/v). At this time, half of the total number of seedlings was amended with 5 g BioN™ per seedling. A total of 60 vigorous seedlings – having the first set of true leaves, of uniform height (41.80–44.85 cm), and a well-established root system – were selected at random from the pre-germinated seedlings. The rate of inoculation followed the procedure indicated on the label of the packaging (5 g per plant for MYKOCAP® and BioN™), whereas two capsules per plant for MYKORICH® were placed directly beneath the roots of the seedlings so the microbes can immediately infect the roots and promote early plant growth and survival.

The MYKOCAP® and MYKORICH® used in this study contain 15 and 12 AMF species, respectively – belonging to the genera *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora*, and *Entrophospora* collected and isolated from stressed sites such as grasslands and abandoned mine tailings. On the other hand, Bio-N™ contains *Azospirilum* sp. that promotes nitrogen fixation. These are all commercial biofertilizers produced by UPLB-BIOTECH and proven effective in promoting plant growth, survival, and mineral uptake, as well as reducing heavy metal pollution in the environment. These are applied either in forestry plantations, agroforestry, reforestation, or bioremediation of mined-out areas (Moon and Aggangan 2019; Victoria and Aggangan 2019; Aggangan and Anarna 2019).

Specifically, Moon and Aggangan (2019) showed that mycorrhizal inoculation promotes plant growth and mineral uptake of nursery-grown *Eucalyptus pellita*. They also showed that MYCOGROE™+MYKORICH®, a mixture of ectomycorrhizal fungi (ECM) and arbuscular mycorrhizal fungi (AMF), effected the best growth for *E. pellita*, as it induced more primary root growth and colonization, as well as more and broader leaves than the ECM, AMF alone, and the control. For seedlings of *Pterocarpus indicus* planted on mine tailings in a mined-out area in Mogpog, Marinduque, Philippines, BioN™ and MYKORICH® produced the highest increments in height and stem diameter, respectively (Aggangan and Anarna 2019).

The inoculants, MYKOVAM®, MYKORICH®, and BioN™ had also been found to promote the growth of cacao seedlings and eventually enhance their yield performance in an acidic agroforest ecosystem in selected areas in Laguna and Batangas (Victoria and Aggangan 2019).

Production of Planting Materials

The seeds of *B. purpurea* used in the pot experiment were collected in May 2021 from a healthy mother tree growing in the vicinity of the UPLB-BIOTECH. *B. purpurea* seeds were extracted from each pod and cleaned of impurities. Seed sorting by floating method was carried out to separate good from poor seeds. Extracted seeds were poured into tap water and allowed to settle for 30 min. Those that sank or the viable ones were sown in the germination boxes, whereas the floating ones or the dead seeds were discarded. It was observed that *B. purpurea* seeds can germinate in 3 wk when soaked in tap water for 30 min.

Soil medium was sterilized thrice for three consecutive days in a pressure cooker using the standard protocol (15 min each time at 121 °C and 15 psi) to eliminate soil-borne microorganisms and pathogens that may affect plant growth and treatment effects. Daily watering of plants was done at a rate of 500 mL/seedling.

Description of the Experimental Area

Table 1 presents the prevailing environmental conditions in the greenhouse throughout the course of the experiment. The maximum temperature ranged from 30.98–32.05 °C, whereas the minimum temperature ranged from 24.10–24.33 °C. Precipitation ranged from 91.10–321.70 mm.

Table 1. Average of daily climatological parameters between sampling periods.

Parameters	Aug 2021	Sep 2021	Oct 2021	Nov 2021
Max. temperature (°C)	32.05	31.53	30.98	31.20
Min. temperature (°C)	24.21	24.10	24.28	24.33
Precipitation (mm d^{-1})	172.80	321.70	249.70	91.10
Rel. humidity (%)	82.74	85.00	85.55	84.93
Evapotranspiration	129.20	94.70	93.70	94.90

Source: Agrometeorology, Bio-structures, and Environment Engineering Division (ABSEED)
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Relative humidity ranged from 82.74–85.55%, whereas evapotranspiration ranged from 93.70–129.20. The benches inside the screenhouse used in the pot experiment were made of G.I. flat bars and steel matting and are elevated to about 0.5 m from the ground.

Plant Growth Monitoring

Plant height and stem diameter measurements were taken 15 d after inoculation, then once every 30 d, for over 90 d. Plant height was measured at the soil surface up to the tip of the main stem using a meter stick. On the other hand, the stem diameter was measured 1 in above the soil surface using a digital Vernier caliper (Jomao-as and Aggangan 2019).

Determination of Plant Dry Weight and Root-Shoot Ratio

Three random plant samples from each treatment were harvested for the determination of partitioned biomass from the shoot (leaves and stems separated) and root (primary roots and lateral roots separated), 90 d after inoculation. The stems were cut 1 in from the soil surface, above the root collar. Shoot and root samples were cleaned of dirt and soil under running water, air-dried before wrapping with a paper towel, and oven-dried at 60 °C for 3 d, dry weight was determined one hr after taking it out from a desiccator.

Assessment of Soil Health

Mycorrhizal spore population. Mycorrhizal spore count was assessed from three samples of 50 g rhizosphere soil each, taken at random per treatment and from each factor. Spores extraction was done through wet sieving and centrifugation technique (Brundrett *et al.* 1996). Soil samples suspended in water were decanted three times and poured into a series of steel sieves with pore openings of 0.5, 100, and 325 µm. After the first centrifugation, the supernatant was discarded, and the pellet was re-suspended in 60% sucrose. After centrifugation, spores in the sucrose solution were collected in a fine-mesh sieve (325 µm), washed several times with water, and then placed in Petri dishes with grid lines. Mycorrhizal spores were counted in 10 field views under a stereomicroscope and expressed as spores/ 50 g soil.

Microbial population. Ten (10) g rhizosphere soil samples were simultaneously taken with the 50-g samples to assess the microbial population. NFB was determined using a nitrogen-free malate medium (Day and Döbereiner 1976). Before pouring the medium onto the plate, it is added with 1 mL/L (v/v) antifungal to prevent contamination. NFB were counted and the estimated colony-forming unit (CFU) 10 g^{-1} of soil was calculated to determine the population count of culturable bacteria.

Root colonization. Root colonization was determined from root samples of harvested plants. Fine (< 1 mm diameter) roots with 0.1-g fresh weight were cut into 2–3 mm lengths and suspended in 50% ethanol. It was then placed in test tubes with 10% KOH (w/w), cleared in a water bath at 75 °C, stained with 0.05% trypan blue in lactic acid: glycerol water (1:1:1) solution, and finally destained with lactic acid: glycerol water solution (Brundrett *et al.* 1996). The pigmented roots were bleached with 3% hydrogen peroxide and 2% hydrogen chloride solution for 5 min before staining. The presence of hyphae, arbuscules, spores, and vesicles was scored as AMF-mycorrhiza-infected roots when they crossed the grid lines under the microscope. Percent root colonization was expressed as the total number of infected roots over the total number of roots counted multiplied by 100.

Data Analysis

All data gathered were subjected to two-way ANOVA using the MSTATC program. Treatment means were compared using LSD at $p < 0.05$. Correlation and regression analyses were done using Microsoft Excel to explain the interrelations between each variable. To find out the extent of variations in plant growth, biomass, and root-shoot ratio due to treatment effects, regression weights used the AMF spore count, microbial population, and root colonization taken at 90 d as independent variables, with all others as the dependent variables.

RESULTS

Plant Growth Performance

Height increment. Table 2 shows that BioN-treated plants were significantly taller than the rest of the treated and untreated plants at 15 DAI but outgrown by those with MRICH alone (38.50 ± 0.50 cm) at 30 DAI, by MCAP alone (44.00 ± 3.46 cm) at 60 DAI, and then by MCAP + BioN (46.50 ± 2.29 cm) at 90 DAI. Amendment of BioN with either of the AMF inoculants could either increase or decrease its height increment as compared to its counterparts without BioN amendment. Overall, the effects of interaction between AMF and NFB on the height increment of *B. purpurea* (Table 3) were significant at 15 DAI ($p = 0.001$), 30 DAI ($p = 0.01$), 60 DAI ($p = 0.002$), and 90 DAI ($p = 0.000$).

Stem diameter increment. At 15 days after inoculation (DAI), all test plants with BioN alone had a significantly larger diameter than those without BioN (Table 4). Those with BioN alone had 29% larger than the control, MCAP + BioN treated plants gave 38% bigger stem diameter than those with MCAP (1.57 ± 0.12 mm) alone, whereas MRICH

Table 2. Height increment (cm) of *B. purpurea* untreated or treated with MYKOCAP® (MCAP) and MYKORICH® (MRICH) and with/without BioN™ amendment at 15–90 d after inoculation (DAI).

Treatments		Height increment (cm)			
AMF inoculation	NFB amendment	15 DAI	30 DAI	60 DAI	90 DAI
CONTROL	– BioN	22.17 ± 0.29 c	34.00 ± 0.50 bc	36.17 ± 0.76 cd	37.00 ± 0.87 b
	+ BioN	31.00 ± 0.50 a	32.67 ± 0.29 cd	34.67 ± 1.04 d	37.67 ± 0.76 b
MCAP	– BioN	25.33 ± 1.15 b	35.67 ± 2.08 b	44.00 ± 3.46 a	36.17 ± 1.26 bc
	+ BioN	25.67 ± 2.75 b	33.50 ± 0.87 c	38.00 ± 0.87 bc	46.50 ± 2.29 a
MRICH	– BioN	22.67 ± 0.76 c	38.50 ± 0.50 a	33.33 ± 0.29 d	37.83 ± 2.08 b
	+ BioN	25.17 ± 1.04 b	32.17 ± 1.61 c	41.00 ± 1.32 ab	34.00 ± 0.50 c

Values in a column followed by identical letter/s have no significant difference (LSD at $p < 0.05$).

Table 3. Effects of mycorrhizal inoculations, NFB amendment, and its interaction on height increment of *B. purpurea* at 15–90 d after inoculation (DAI).

Source of variation	Height increment (cm)			
Mycorrhizal inoculation (A)	15 DAI	30 DAI	60 DAI	90 DAI
CONTROL	**26.58***	33.33	35.42	37.33
MCAP	25.50	34.58	**41.00****	**41.33*****
MRICH	23.92	**35.33***	37.17	35.92
NFB amendment (B)				
0 g BioN	23.39	**36.06*****	37.83 ns	37.00
5 g BioN	**27.28*****	32.78	37.89 ns	**39.39****
A x B	**0.001**	**0.01**	**0.002**	**0.000**
Coef. of variation (%)	5.69	3.47	4.74	4.12

Values in boldface are significant at *p*-value <.05*, .01**, .001***

Table 4. Diameter increment (cm) of *B. purpurea* untreated or treated with MYKOCAP® (MCAP) and MYKORICH® (MRICH) and with/without BioN™ amendment at 15–90 d after inoculation (DAI).

Treatments		Diameter increment (mm)			
AMF inoculation	NFB amendment	15 DAI	30 DAI	60 DAI	90 DAI
CONTROL	– BioN	1.73 ± 0.06 c	2.73 ± 0.12 bc	4.37 ± 0.29 ab	4.57 ± 0.06 c
	+ BioN	2.23 ± 0.06 a	2.67 ± 0.06 cd	4.07 ± 0.06 bc	4.70 ± 0.17 bc
MCAP	– BioN	1.57 ± 0.12 c	3.30 ± 0.10 a	4.50 ± 0.36 a	5.47 ± 0.31 a
	+ BioN	2.10 ± 0.17 ab	2.57 ± 0.12 d	3.87 ± 0.10 c	5.47 ± 0.06 a
MRICH	– BioN	1.53 ± 0.23 c	2.83 ± 0.06 b	4.43 ± 0.21 ab	4.87 ± 0.06 b
	+ BioN	1.97 ± 0.06 b	2.77 ± 0.06 bc	4.23 ± 0.06 abc	5.53 ± 0.12 a

Values in a column followed by identical letter/s have no significant difference (LSD at $p < 0.05$).

+ BioN treated plants were 29% larger than those with MRICH (1.53 ± 0.23 mm) alone. However, this effect was not evident at 30 and 60 DAI. During these times, MCAP alone (3.30 ± 0.10 and 4.50 ± 0.36 mm, respectively) consistently effected the widest stem diameter in test plants. The difference was more pronounced at 30 DAI than at 60 DAI. Notably, at 90 DAI, MCAP alone (5.47 ± 0.31 mm), MCAP with BioN (5.47 ± 0.06 mm), and MRICH with BioN (5.53 ± 0.12 mm) had comparatively wider stem diameters, which in turn were significantly different from the control (4.57 ± 0.06 mm) and the MRICH without BioN (4.87 ± 0.06 mm). Table 5 shows that the effects of the interaction of AMF and NFB on the diameter increment of *B. purpurea* at 15 DAI and 60 DAI were not significant, although its interaction was noted to be significant at 30 DAI ($p = 0.0001$) and 90 DAI ($p = 0.0165$).

Table 5. Effects of mycorrhizal inoculations, NFB amendment, and its interaction on diameter increment of *B. purpurea* at 15–90 d after inoculation (DAI).

Source of variation	Diameter increment (mm)			
Mycorrhizal inoculation (A)	15 DAI	30 DAI	60 DAI	90 DAI
CONTROL	**1.98***	2.70	4.22 ns	4.63
MCAP	1.83	**2.93***	4.18 ns	**5.47 *****
MRICH	1.75	2.80	4.33 ns	5.20
NFB amendment (B)				
0 g BioN	1.61	**2.96*****	**4.43****	4.97
5 g BioN	**2.10*****	2.67	4.06	**5.23****
A x B	ns	**0.0001**	ns	**0.0165**
Coef. of variation (%)	7.2	3.09	5.37	3.36

Values in boldface are significant at *p*-value <.05*, .01**, .001***

Table 6. Total plant biomass from root and shoot of *B. purpurea* untreated or treated with MYKOCAP® (MCAP) and MYKORICH® (MRICH) and with/without BioN™ amendment at 90 d after inoculation (DAI).

Treatments		Biomass (g plant^{-1})		
AMF inoculation	NFB amendment	Root	Shoot	Total
CONTROL	– BioN	6.03 ± 0.43c	12.10 ± 0.48c	18.90 ± 2.74c
	+ BioN	5.68 ± 0.30c	12.30 ± 0.60bc	19.43 ± 1.20bc
MCAP	– BioN	7.01 ± 0.38a	12.99 ± 0.27b	22.48 ± 3.79a
	+ BioN	5.20 ± 0.33d	13.92 ± 0.41b	21.46 ± 1.96ab
MRICH	– BioN	6.66 ± 0.11b	15.90 ± 0.41a	23.33 ± 2.32a
	+ BioN	5.46 ± 0.53c	13.60 ± 0.40b	19.89 ± 0.61bc

Values in a column followed by identical letter/s have no significant difference (LSD at $p < 0.05$).

Plant Biomass

Root and shoot biomass. Table 6 shows that the root biomass of plants inoculated with MCAP alone (7.01 ± 0.38 g plant^{-1}) was 5.26% heavier than with MRICH alone (6.66 ± 0.11 g plant^{-1}). Amendment of BioN with either MCAP or MRICH rather reduced their respective root biomass by 35 and 22%, respectively. For shoot biomass, MRICH alone had the highest which was 22.4% higher than MCAP alone (12.99 ± 0.27 g plant^{-1}). Combining BioN with MCAP gave no significant effect on shoot biomass but significantly reduced by 17% when combined with MRICH. Table 7 shows the overall significant effects of the interaction between AMF and NFB on root biomass ($p = 0.0031$) and shoot biomass ($p = 0.0000$) but not on the total plant biomass.

Total plant biomass. At 90 DAI, MCAP (22.48 ± 3.79 g plant^{-1}) and MRICH (23.33 ± 2.32 g plant^{-1}) alone gave comparable total plant biomass. Co-amendment of MCAP with BioN reduced the total plant dry weight by 4.75%, although the reduction was not significant and its weight was still comparable with MCAP alone. On the other hand, for MRICH, amendment with BioN significantly reduced the total plant dry weight by 17.3%, which is comparable with BioN alone (19.43 ± 1.2 g plant^{-1}). The untreated control (18.90 ± 2.74 g plant^{-1}) had the lowest. Table 7 shows that mycorrhizal inoculation alone had a significant effect ($p < 0.01$) on total plant biomass but not the NFB inoculation or their interaction in the co-amended treatments ($p = 0.0968$) at 90 DAI.

Partitioned biomass. Table 8 shows the partitioned biomass from the stem, leaves, and lateral and primary root at 90 d. A pattern is not immediately discernible, although MCAP + BioN and MRICH alone seem to have favorable effects on biomass, especially for stems (10.58 ± 0.38 g plant^{-1} and 10.72 ± 0.32 g plant^{-1}), MRICH alone for leaves (5.10 ± 0.10 g plant^{-1}), and MRICH + BioN for lateral roots (4.08 ± 0.32a g plant^{-1}). For primary roots, MCAP (both alone or with BioN, 4.83 ± 0.23 g plant^{-1} and 4.42 ± 0.32 g plant^{-1}, respectively) promoted the highest biomass. Generally, AMF and NFB interaction resulted in a significant difference in increases in partitioned biomass from stems ($p = 0.001$), leaves ($p = 0.003$), and lateral root ($p = 0.001$), but not on the primary root ($p > 0.05$) (Table 9).

Table 7. Effects of mycorrhizal inoculations, NFB amendment, and its interaction on total plant biomass of *B. purpurea* at 90 d after inoculation (DAI).

Source of variation	**Total biomass (g plant^{-1})**		
Mycorrhizal inoculation (A)	Root biomass	Shoot biomass	Total plant biomass
CONTROL	5.96	12.14	19.17**
MCAP	**7.22*****	14.10	21.97
MRICH	6.64	**15.12*****	21.61
NFB amendment (B)			
0 g BioN	6.61 ns	13.64	21.57 ns
5 g BioN	6.60 ns	**13.94***	20.26 ns
A x B	**0.0031**	**0.0000**	0.0968
Coef. of variation (%)	4.6	1.49	6.79

Values in boldface are significant at *p*-value <.05*, .01**, .001***

Table 8. Partitioned biomass from stem, leaves, lateral roots, and primary root of *B. purpurea* untreated or treated with MYKOCAP® (MCAP) and MYKORICH® (MRICH) and with/without BioN™ amendment at 90 d after inoculation (DAI).

Treatments		**Partitioned biomass (g plant^{-1})**			
AMF inoculation	NFB amendment	Stem	Leaves	Lateral root	Primary root
CONTROL	– BioN	7.68 ± 0.40d	4.42 ± 0.08d	2.35 ± 0.31c	3.69 ± 0.12cd
	+ BioN	7.43 ± 0.40d	4.75 ± 0.21bc	2.11 ± 0.14cd	3.77 ± 0.16cd
MCAP	– BioN	8.31 ± 0.16c	4.68 ± 0.12c	2.19 ± 0.15c	4.83 ± 0.23a
	+ BioN	10.58 ± 0.38a	4.62 ± 0.03c	1.85 ± 0.01d	4.42 ± 0.32b
MRICH	– BioN	10.72 ± 0.32a	5.10 ± 0.10a	2.85 ± 0.09b	3.94 ± 0.02c
	+ BioN	9.53 ± 0.34b	4.89 ± 0.06b	4.08 ± 0.32a	3.57 ± 0.21d

Values in a column followed by identical letter/s have no significant difference (LSD at $p < 0.05$).

Table 9. Effects of mycorrhizal inoculations, NFB amendment, and its interaction on partitioned biomass from stem, leaf, lateral roots, and primary root of *B. purpurea* at 90 d after inoculation (DAI).

Source of variation	**Partitioned biomass (g plant^{-1})**			
Mycorrhizal inoculation (A)	Stem	Leaf	Lateral root	Primary root
CONTROL	7.55	4.59	2.23	3.73
MCAP	**9.45*****	4.65	2.02	**4.62*****
MRICH	**10.13 *****	**5.0 *****	**3.47*****	3.76
NFB amendment (B)				
0 g BioN	8.90	4.74 ns	2.46	**4.15***
5 g BioN	**9.18 ***	4.75 ns	**2.68***	3.92
A x B	**0.0000**	**0.0034**	**0.0000**	ns
Coef. of variation (%)	3.0	2.17	7.02	4.55

Values in boldface are significant at *p*-value < .05*, .01**, .001***

The general appearance of root systems of inoculated and uninoculated *B. purpurea* is presented in Figure 1. A more expansive and thicker root system was evident among the treatments that received BioN than those without BioN.

Root-shoot ratio. Plants with BioN alone (0.58 ± 0.04), MCAP alone (0.58 ± 0.01), and also in MCAP co-amended with BioN (0.55 ± 0.03) gave higher root-shoot ratio than the untreated control (0.47 ± 0.08) or with MRICH with BioN (0.458 ± 0.02) or without BioN (0.51+0.05). The root-shoot ratio from plants treated with MRICH + BioN

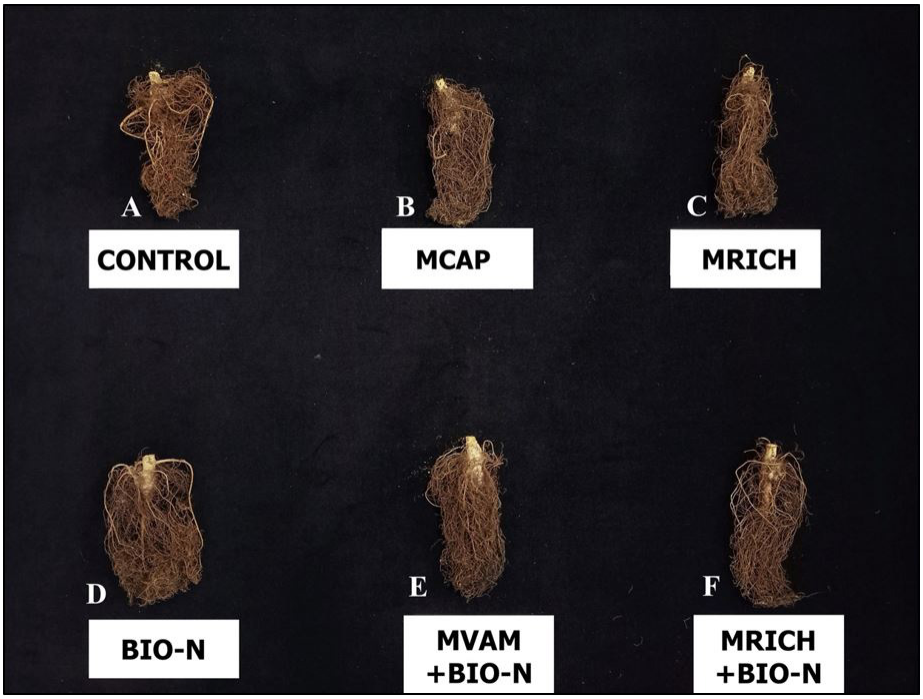

Figure 1. The morphological appearance of untreated Control (A) and treated (B–F) *B. purpurea* root system grown under nursery condition on sterilized soil + sand medium (1:1 v/v), 90 d after inoculation (DAI). Commercial biofertilizers used: [MCAP] MYKOCAP®, [MRICH] MYKORICH®, and [BIO-N] BioN™.

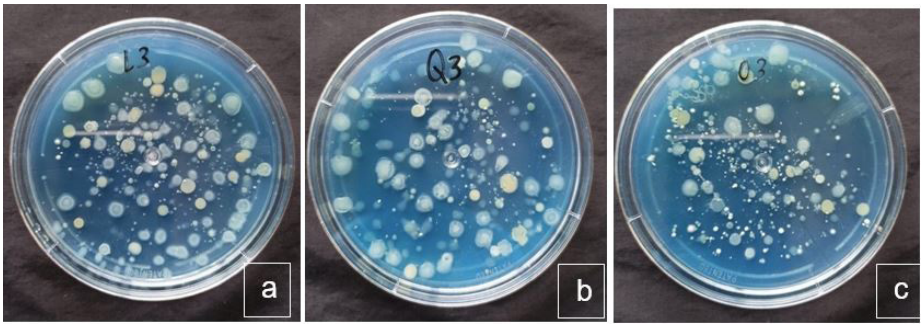

Figure 2. Culturable nitrogen-fixing bacteria grown on a Döbereiner medium with the highest colony forming units (CFU) observed on BioN (a), followed by MRICH + BioN (b) and lowest on MCAP + BioN (c).

(0.51 ± 0.05) was not significantly different from either the highest or the lowest ratios. Table 11 shows that AMF and NFB interactions had significantly affected ($p = 0.03$) the increase in the root-shoot ratio of plants that received MCAP and BioN amendment.

Microbial Characteristics

Mycorrhizal spore count. Mycorrhizal spore counts in rhizosphere soils taken from plants applied with MCAP (379.5 ± 3.5 spg) and MRICH (340 ± 28 spg) were 107.38 and 353.33%, respectively, higher than their BioN amended counterparts and the untreated control (Table 10). Table 11 also supported these findings, with a significant ($p = 0.0000$) increase in spore count in the mycorrhizal treated plants without BioN amendment.

Microbial buildup. The highest NFB population was observed from MCAP + BioN treated plants (117.16 ± 30 CFU), whereas the lowest was observed from those with MRICH alone (13.07 ± 0.36 CFU), which was 416% lower than the untreated control (Table 10). The interaction of AMF and NFB had significantly increased ($p = 0.0024$) the NFB population among the BioN amended MCAP and MRICH, although those with MCAP + BioN had 296% higher NFB population than those with MRICH + BioN (Table 11).

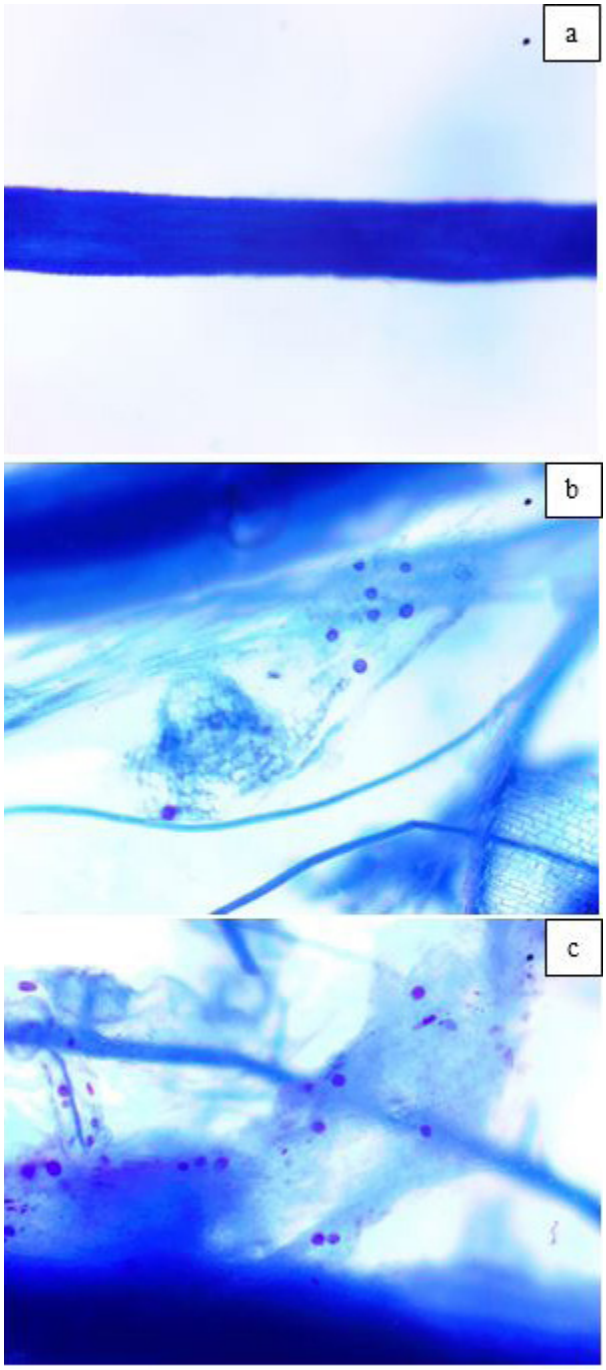

Figure 3. Mycorrhizal root infection in *B. purpurea* at 90 d: control plants not infected (a) and infected roots of MCAP-treated (b) and MCAP + BioN-treated plant (c).

Root colonization. Mycorrhizal root infections were comparable among plants that received mycorrhizal inoculants applied singly (39.67 ± 4.16 with MCAP and 31.67 ± 4.51 with MRICH) or in combination with BioN (38 ± 9.54 with MCAP + BioN and 36 ± 8.19 with MRICH + BioN) yet were significantly higher than with BioN alone (2.67 ± 2.31). There was no mycorrhizal infection at all from the fine roots of the uninoculated control plants (Table 10). Table 11 further shows that MCAP alone promoted a comparatively higher root infection in *B. purpurea* with MCAP or MRICH + BioN, or with MRICH alone. This means that BioN amendment with the mycorrhizal inoculants did not provide any significant difference ($p > 0.05$) in root colonization among the treated plants, but both the mycorrhizal inoculants applied singly had effected the increased root colonization as compared to the untreated plants.

Correlation and Regression Analysis

Table 12 shows the minimum parameters taken from the inoculated and uninoculated *B. purpurea* seedlings grown aseptically in sterilized soil + sand medium for 90 d. Among these parameters, spore count (Table 12a), microbial buildup (Table 12b), and root colonization (Table 12c) served as the independent variables inferred to have influenced *B. purpurea*'s growth, biomass, and root-to-shoot ratio.

Our investigation shows that mycorrhizal spore count was significantly correlated with only two of the 10 variables (Table 12a). Specifically, a high positive correlation between the spore count and primary root biomass (p = 0.01, r^2 = 0.329, r = 0.573) was revealed, contrary to the high negative correlation between the spore count and lateral root biomass (p = 0.045, r^2 = 0.229, r = –0.478). Specifically, the regression model (y = 0.003x + 3.44) can explain the 33% variation in the primary root biomass due to treatment inoculation. It is concluded here that the primary roots of *B. purpurea* got bigger among the treated plants with the increase in the mycorrhizal populations that supported the needed phosphorous to the plant, whereas induced lateral root growth was due to successful root colonization.

A significant (p = 0.001) correlation (r = 0.708) between the increase in height (r^2 = 0.5015) and increase in microbial buildup was also noted at 90 DAI (Table 12b).

Root colonization (Table 12c), on the other hand, was significantly correlated with six out of 10 variables – namely, stem diameter (p = 0.015, r = 0.56), total plant biomass (p = 0.02, r = 0.53), stem biomass (p = 0.001, r = 0.69), shoot biomass (p = 0.001, r = 0.70), primary roots (p = 0.03, r = 0.51) and root biomass (p = 0.01, r = 0.63). Specifically, the regression model can explain the 65, 28, 48, 49, 26, and 39% variation in the plants' stem diameter and dry weights obtained from total plant biomass, stem biomass, shoot biomass, primary roots, and root biomass, respectively.

Although the spore count, NFB buildup, and root infection can serve as predictors of changes in most of the investigated variables, effects of other factors such as the

Table 10. Root-shoot ratio, spore count, NFB population, and root colonization of *B. purpurea* untreated or treated with MYKOCAP® (MCAP) and MYKORICH® (MRICH) and with/without BioN™ amendment at 90 d after inoculation (DAI).

Treatments		Root-shoot ratio and soil health parameters			
AMF inoculation	NFB amendment	Root-shoot ratio	Spore count 50 g^{-1} soil	NFB CFU x 10^5 10 g^{-1} soil	Root colonization
CONTROL	– BioN	0.47 ± 0.08b	155.5 ± 19.5c	67.5 ± 13b	0.00
	+ BioN	0.58 ± 0.04a	280 ± 38b	39 ± 19c	2.67 ± 2.31b
MCAP	– BioN	0.58 ± 0.01a	379.5 ± 3.5a	31.13 ± 24c	39.67 ± 4.16a
	+ BioN	0.55 ± 0.03a	183 ± 6c	117.16 ± 30a	38 ± 9.54a
MRICH	– BioN	0.458 ± 0.02b	340 ± 28a	13.07 ± 0.36d	31.67 ± 4.51a
	+ BioN	0.51 ± 0.05ab	75 ± 8d	29.61 ± 12c	36 ± 8.19a

Values in a column followed by identical letter/s have no significant difference (LSD at $p < 0.05$).

Table 11. Effects of mycorrhizal inoculations and NFB amendment level on root-shoot ratio, mycorrhizal spore count, NFB buildup, and root colonization in *B. purpurea* at 90 d after inoculation (DAI).

Source of variation	Root-shoot ratio and soil health parameters			
Treatment (A)	Root-shoot ratio	Spore count 50 g^{-1} soil	NFB CFU x 10^5 10 g^{-1} soil	Root colonization
CONTROL	0.52	217.75	53.25	1.33
MCAP	**0.57***	**281.25*****	**74.14****	**38.83*****
MRICH	0.49	207.50	21.34	33.83
NFB amendment (B)				
0 g BioN	0.50	**291.67*****	37.23	23.78 ns
5 g BioN	**0.55***	179.33	**61.92***	25.56 ns
A x B	**0.0313**	**0.0000**	**0.0024**	ns
Coef. of variation (%)	7.55	9.89	41.62	25.01

Values in boldface are significant at *p*-value < .05*, .01**, .001***

soil physico-chemical properties must also be investigated for a more accurate estimate of growth and biomass of *B. purpurea*.

Table 13 presents the regression equations derived from the DAI (independent variable) and the recorded diameter (dependent variable) at the time of investigation using the different treatments. Apparently, the DAI has a very strong correlation ($p < 0.001$, $r = 0.922$–0.969) with the diameter up to 90 d since transplanting, irrespective of treatments used. However, the highest $r = 0.969$ was obtained in the MCAP + BioN and the lowest $r = 0.922$ in MCAP. Among the different biofertilizer treatments, MCAP + BioN had brought the highest r^2 (94%), followed by MRICH + BioN (93%), BioN (91%), MRICH (88%), control (87%), and finally, MCAP (85%). The high r^2 shows goodness of fit at each model.

If the regression equations derived per treatment were to be used to predict the diameter increase of *B. purpurea* up to 120 d (Figure 4), MCAP + BioN (335.54%) would still have the highest diameter growth rate. However, MRICH + BioN would be outgrown by those with MCAP alone (324.29%), MRICH and control would have a comparable diameter (268.66%), whereas BioN would have the lowest diameter growth rate (264.87%). Results of regression analyses would be a helpful tool in making future management interventions prior to the conduct of field experiments, for example, in choosing the most effective inoculation treatment/s.

DISCUSSIONS

Importance of Microbial Inoculation at Nursery Stage

Soils, especially degraded ones, do not always have enough nutrients and minerals to sustain plant growth. Good soil has an abundant community of beneficial microorganisms that can boost plant growth, survival, and yield. In agriculture, cultured soil microorganisms

Table 12. Summary of correlation and regression analysis for the independent variables: mycorrhizal spore count (a), microbial buildup (b), and root colonization (c) as predictors of the dependent variables: growth performance (diameter and height) and biomass accumulation in roots and shoots of *B. purpurea* due to mycorrhizal inoculation applied alone or in combination with BioN.

Regression	Height	Diameter	Biomass	Root-shoot ratio	Stem biomass	Leaves biomass	Shoot biomass	Lateral root biomass	Primary root biomass	Root biomass
a. Spore count (x)										
Coefficient	–0.001	-0.001	0.011	0.000	–0.001	0.005	0.000	–0.003	0.003	–0.001
Intercept	38.41	5.23	18.37	0.49	9.19	4.61	13.82	3.36	3.44	6.80
r^2	0.001	0.020	0.218	0.064	0.003	0.171	0.000	0.229	0.329	0.018
T-stat	–0.10	–0.58	2.11	1.04	-0.20	1.82	–0.04	–2.18	2.80	–0.55
F-value	0.01	0.33	4.45	1.09	0.04	3.30	0.00	4.74	7.83	0.30
p-value	0.924	0.572	0.051	0.312	0.843	0.088	0.969	**0.045***	**0.013***	0.592
r	–0.024	–0.23	0.467	0.25	–0.05	0.23	–0.01	–0.478	0.573	–0.135
Equation			*y = 0.011x + 18.37*					*y = –0.003x + 3.36*	*y = 0.003x + 3.44*	
b. Microbial buildup (x)										
Coefficient	0.077	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Intercept	34.36	5.11	21.28	0.52	9.32	4.72	14.04	2.49	4.15	6.64
r^2	0.5015	0.002	0.068	0.036	0.136	0.043	0.093	0.032	0.181	0.009
T-stat	4.012	–0.16	–1.08	0.77	–1.59	0.85	–1.28	0.73	–1.88	–0.39
F-value	16.09	0.03	1.16	0.60	2.52	0.72	1.64	0.53	3.52	0.15
p-value	**0.001*****	0.874	0.297	0.450	0.132	0.408	0.218	0.476	0.079	0.701
r	0.708	–0.18	–0.26	0.19	–0.37	0.21	–0.31	0.18	–0.42	–0.10
c. Root colonization (x)										
Coefficient	0.05	0.02	0.08	0.00	0.05	0.01	0.06	0.01	0.01	0.02
Intercept	37.08	4.62	19.02	0.52	7.72	4.61	12.33	2.32	3.69	6.01
r^2	0.037	0.647	0.283	0.014	0.479	0.171	0.487	0.055	0.265	0.394
T-stat	0.78	5.42	2.51	0.47	3.83	1.82	3.90	0.96	2.40	3.23
F-value	0.61	29.38	6.31	0.22	14.68	3.30	15.20	0.93	5.75	10.42
p-value	0.446	**0.000*****	**0.023***	0.644	**0.001*****	0.088	**0.001*****	0.350	**0.029***	**0.005****
r	-0.129	0.805	0.532	0.117	0.692	0.413	0.698	0.234	0.514	0.628
Equation		*y = 0.02x + 4.62*	*y = 0.08x + 19.02*		*y = 0.05x + 7.72*		*y = 0.06x + 12.33*		*y = 0.01x + 3.69*	*y = 0.02x + 6.01*

Values in boldface are significant at *p*-value < .05*, .01**, .001**

Table 13. Summary of regression analysis for plant growth in terms of stem diameter (y) as predicted by the number of days after inoculation (x) with biofertilizers (MYKOCAP® or MCAP, MYKORICH® or MRICH, BioN™ or BION, and its combination).

	[T1] control	[T2] MCAP	[T3] MRICH	[T4] BioN	[T5] MCAP + BioN	[T6] MRICH + BioN
Regression equation (age, *x vs.* diameter, *y*)	y = 0.064x + 3.5	y = 0.064x + 3.32	y = 0.068x + 3.42	y = 0.061x + 3.38	y = 0.065x + 3.23	y = 0.68x + 3.30
T-stat	18.28	16.46	19.08	21.48	27.06	24.82
F-value	334.33	270.88	363.88	461.50	732.50	616.00
p-value	***0.001*****	***0.001*****	***0.001*****	***0.001*****	***0.001*****	***0.00*****
r	*0.935*	*0.922*	*0.940*	*0.952*	*0.969*	*0.963*
r^2	*0.87*	*0.85*	*0.88*	*0.91*	*0.94*	*0.93*
Predicted diameter at 120 d (%)	268.66	324.29	268.66	264.87	335.54	290.78

Values in boldface are significant at *p*-value < .05*, .01**, .001***

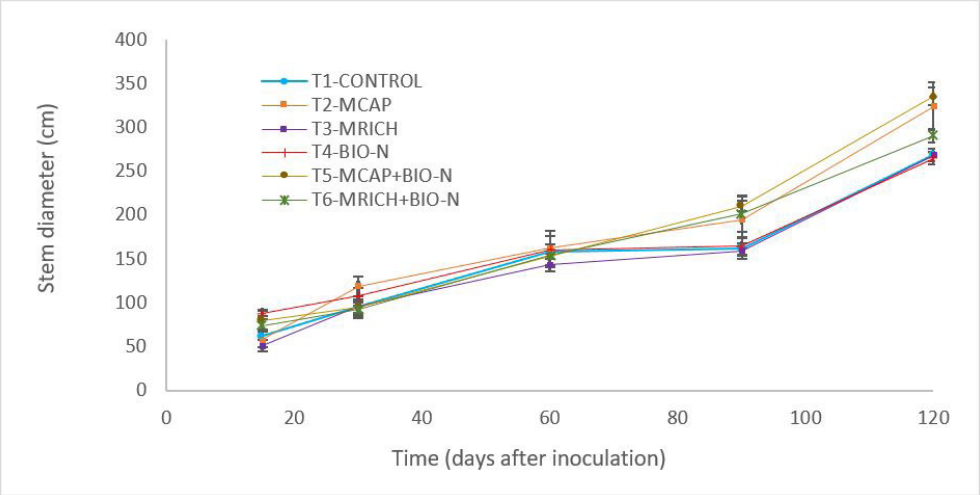

Figure 4. Diameter growth curve of B. purpurea in response to different treatments since inoculation at 15–90 d, and the predicted change in diameter at 120 d favored among the MCAP + BioN, MCAP, and MRICH + BioN inoculated ones. Commercial biofertilizers used: [MCAP] MYKOCAP®, [MRICH] MYKORICH®, and [BIO-N] BioN™.

have been used as bioinoculants to improve the soil aggregate stability, suppress plant pathogens and promote plant growth. These effects are possible through various mechanisms including improved nutrient uptake, biological control of pathogens, and production of plant growth regulators (Chanway 1997).

MYKOCAP® and MYKORICH® contain 15 and 12 species of AMF, respectively. Mycorrhizal fungi can co-exist with plants freely in the soil or can form symbioses with plants by entering the roots and heading through the vascular system. Once in the vascular system, AMF provides phosphates, nutrients, and water in exchange for carbohydrates produced by plants during photosynthesis (Chanway 1997). Hyphae or mycelia develop that allow greater water and nutrient absorption for the plants, as well as the production of chitin protective barrier in the roots that increase plant resistance against soil-borne pathogens and structural damages in roots and improves plant resistance against drought or heavy metal toxicity (Chanway 1997). Mycorrhizal association with plants has a direct benefit to plants by enhancing their growth, survival, and productivity (Chanway 1997).

BioN™, on the other hand, contains the commonly-known bacterium *Azospirilum* sp. Fukami *et al.* (2018) mentioned that the benefits to plants by inoculation with *Azospirillum* have been primarily attributed to its capacity to fix atmospheric nitrogen but also to its capacity to synthesize phytohormones. They also reported that the bacterium has an important role in conferring to plants tolerance to abiotic and biotic stresses.

Earlier, Muthukumar and Udaiyan (2006) obtained better growth on nursery-grown bamboo [*Dendrocalamus strictus* (Roxb.) Nees.] 180 DAI with *Glomus aggregatum*, *Bacillus polymixa*, and *Azospirillum brasilense* in two soil types (alfisol, vertisol). Gush and Gupta (2011) cited several studies wherein microbial application at the nursery stage was found to be useful in enhancing the productivity of some forest trees like *Albizzia*, *Acacia*, and *Dalbergia* (Rahangdale and Gupta 1998; Sahgal *et al.* 2004).

Effects of AMF, NFB, and Its Interaction in the Growth Performance of *B. purpurea*

In this study, the growth performance of *B. purpurea* was measured in various different ways: from the increment in height and diameter at each monitoring period, plant biomass obtained from the total/partitioned dry weight of plants at 90 d, and the root-to-shoot ratio derived from the mean dry weight of the roots over the mean dry weight of the shoots per treatment.

In almost all instances, *B. purpurea* had a significant improvement in height ($p < 0.01$–0.000) at 15–90 DAI and in stem diameter ($p = 0.0001$ at 15 DAI at $p = 0.0165$ at 90 DAI) with mycorrhizal alone or in combination with NFB as compared with the untreated control plants (Tables 2–5). The symbiosis of AMF and NFB effected the 1.34 and 29% increase in height of plants with MCAP + BioN at 15 DAI and at 90 DAI, respectively, relative to those with MCAP alone. Likewise, MCAP + BioN gave 13 and 23% much greater increments relative to those with MRICH alone at 15 DAI and 90 DAI, or even taller by 2 and 37% than the MRICH + BioN treated ones at 15 DAI and 90 DAI.

Contrarily, the amendment of NFB in both mycorrhizal inoculants did not significantly improve the partitioned root and shoot biomass and its totality among the MCAP + BioN and MRICH + BioN treated plants (Table 6). The increase in plant biomass was rather evidently promoted with MCAP alone or with MRICH alone (Table 7).

Apparently, symbiosis of AMF and NFB was evident in the increased stem biomass among the MCAP + BioN treated plants by 27.31% compared to those with MCAP alone. The highest leaf biomass (5.10 ± 0.10 g plant^{-1}) was rather promoted with MRICH alone, followed by its BioN (4.89 ± 0.06 g plant^{-1}) amended counterpart – whereas for primary root, the highest was obtained with MCAP alone. However, in the co-amended treatment dry weights of the lateral root and primary root were reduced among the MCAP + BioN treated plants. Moreover, the co-amendment of AMF and NFB in the MRICH + BioN treated plants did not improve the stem biomass, leaf biomass, and primary root biomass, except for the lateral root biomass that increased by 43.16%.

The increases in root and shoot biomass and leaf biomass of *B. purpurea* due to the mycorrhizal inoculant alone (without NFB amendment) were consistent with the findings of Rupnawar and Navale (2000), verifying that mycorrhizal inoculated pomegranate has bigger height, root length, number of leaves, dry weight of shoot and roots, and mycorrhizal dependency percentage than non-mycorrhizal plants and had increased nutrient uptake.

These studies emphasized the importance of having a large root system in a plant, as this sets the foundation for a healthy productive plant, increases water uptake and overall plant size, and also increases the foliage density that functions in the manufacture of the food for the plant.

Grayston *et al*. (1997) discussed thoroughly the mechanisms behind plant growth performance in response to plant-microbe interactions – how the plant indirectly influences soil microbial activity and, in return, how the microbes help in rendering soil nutrients available for the plant.

Endophytic bacteria and fungi can establish a close association with plants and could be more successful in plant growth promotion (Grayston *et al.* 1997). The rhizobacteria break the carbon molecule down into carbon dioxide, water, and positively charged hydrogen ions, thereby emitting heat and acids plus exudates.

Grayston *et al.* (1997) further that the production of hydrogen ions, on the other hand, triggers the release of nutrients tied up in the soil, by replacing the positively charged soil interlocked with the negatively charged soil particles, thus making them available for plants. The newly released nutrients, together with the acids and exudates, can now be translocated from the roots upwards to build the upper part of the plant, producing leaves, stem, and bloom.

The enhanced available nutrients mentioned above may have been stimulated in *B. purpurea* seedlings that received microbial inoculants. Hence, its greater height, diameter, root biomass, and shoot biomass. Grayston *et al.* (1997) point especially to carbon and nutrient flows, although carbon flow is the prime determinant in improved plant growth. Apparently, more carbon energy sent to the roots results in more acids and exudates produced in the root zones, which leads to more bacterial growth and higher nutrient availability, thus increasing the plant's potential. Carbon accounts for 45% of plant mass, whereas nutrients only account for 4% – focusing on the upper plant growth, such as foliage growth and fruit production. With balanced nutrient levels, the plant is healthier and fruits are heavier and more nutritious (Grayston *et al.* 1997).

Tilman (1988) showed that seedling growth and root-shoot ratio are influenced either by the plant's genetic makeup, ontogenetic stage, and seed source or by the prevailing environmental conditions or abiotic stresses. In nutrient-rich environments where above-ground competition for light is strong, plants distribute higher proportions of biomass into leaves and stems. On the other hand, in nutrient-poor environments – where below-ground competition prevails – they allocate a higher proportion to roots.

Genetically, *B. purpurea* plant has a high root-shoot ratio, making it a good slope stabilizer. Amendment of NFB with the mycorrhizal inoculants significantly improved the root-shoot ratio of MRICH + BioN treated *B. purpurea* by 8.51–11.35% as compared with control and with MRICH alone, respectively. Having a much higher root-shoot ratio is advantageous in the environmental condition where water is limited in the soil, as the high value of the root-shoot ratio in plants indicates high absorption and storage capacity for water (Beikircher and Mayr 2009). The value of the root-shoot ratio also shows the balance between the transpiration rate in the shoot and the water-absorbing capacity of the root to compensate for the loss of water in plants (Beikircher and Mayr 2009). The root-shoot ratio can, therefore, be related to the adaptive response developed by plants to avoid environmental stress. Another factor influencing the root-shoot ratio is the allocation of relative carbon to either shoot or root growth, whichever is experiencing environmental resource limitation (Kozlowski and Pallardy 2002).

Effects of AMF, NFB, and Its Interaction on Soil Microbial Community

Present findings showed that the inoculation treatments have also contributed to an enhanced soil health status, particularly to an increased mycorrhizal ($p = 0.0000$) and

NFB community (p = 0.0024), particularly among those with MCAP and MRICH alone or with MCAP + BioN, respectively (Tables 10 and 11). All these parameters can influence the efficiency of inoculation to obtain success in improving plant growth and productivity.

Syibli *et al.* (2013) pointed out that the abundance of AMF spores from the rhizosphere of *Tithonia diversifolia* indicates the chemical, biological, and physical fertility of the soil. This study also confirmed that AMF has a high correlation with organic carbon, organic matter, total phosphorus, cation exchange capacity, water level, soil fungi, and soil bacteria.

Domínguez-Núñez *et al.* (2015) explained that the AM fungi interact with *Azospirillum* directly by providing a niche and/or habitat or indirectly by modifying host plant morphophysiology. As with most interactions of beneficial soil microorganisms, this interaction in the soil can be beneficial for both the microorganisms and the host plant. Apparently, the *Azospirillum* inoculation is more successful and more profitable when other microorganisms are co-inoculated with *Azospirillum*. As they stress, "the inoculation consortia apparently work better when VAM fungi are incorporated."

In the study conducted by Vázquez *et al.* (2000), mycorrhizal inoculants containing *Glomus mosseae*, *Glomus deserticola*, and natural AMF from the test soil have acted symbiotically with other microbial inoculants *(Azospirillum*, *Pseudomonas*, and *Trichoderma*) and resulted to a quantitative and qualitative change in microbial communities in rhizospheres with maize plants.

Effects of AMF, NFB, and Its Interaction on Mycorrhizal Colonization

Remarkably, *B. purpurea* seedlings that received either of the mycorrhizal inoculant (MCAP and MRICH) – with or without BioN amendment – have comparable percent root colonization, whereas the control plants were not colonized at all by AMF. Successful colonization by mycorrhizal fungi is especially important in degraded soils where nutrient availability is low (Shrestha *et al.* 2008). AM fungi improve soil structure because they produce extraradical hyphal networks and their hyphae contain and release glomalin, a putative homolog of heat-shock protein (Gadkar and Rillig 2006). The concentration of glomalin-related protein in the soil is usually correlated with aggregate stability (Rillig 2004). Improved soil structure increases water infiltration and can reduce soil erosion (Tisdall and Oades 1982).

Further, mycorrhizal colonization induced qualitative changes in the bacterial population depending on the inoculant combination involved (Vázquez *et al.* 2000). Esterase activity was particularly increased by *G. mosseae* (256%), phosphatase activity by natural AMF (166%), chitinase by *G. mosseae* (197%), *G. deserticola* (152%) and natural AMF (151%), and trehalase by *G. deserticola* (444%).

In this study, root colonization is a much better indicator of the efficiency of inoculation as indicated by its significantly high correlation with all the stem diameter, stem biomass, shoot biomass, primary root biomass, and root biomass as compared to spore count that correlates with lateral root and primary root biomass only, or to NFB build up that correlates to height increment only. This notion was consistent with Douds (1994), who discussed that spore count is not the best method for studying AMF populations because not all AMF species sporulate.

SUMMARY AND CONCLUSIONS

The mycorrhizal inoculants, MCAP and MRICH, with or without BioN amendment, were tested effective to promote the growth of *B. purpurea* and its associated soil microbial communities in nursery condition. Effects of interaction between the AMF and NFB have significantly improved the height and diameter increment of the MCAP + BioN treated plants and the stem biomass, whereas the lateral root biomass had significantly increased among the MRICH + BioN treated ones. In almost all instances, said parameters were significantly lowest in the untreated control plants. Generally, AMF and NFB interaction had significant effects on the increase in partitioned biomass from stems (p = 0.0000), leaves (p = 0.0034), and lateral root (p = 0.0000) but not on the primary root ($p > 0.05$).

Leaf biomass was heaviest with MRICH alone compared to its BioN co-amended counterpart or to the control. Root biomass was 35% heavier with MCAP alone than its BioN co-amended counterpart, whereas shoot biomass with MRICH alone was 31.4% heavier than the control.

For the treatment effects on soil health status, spore count was comparatively higher in MCAP and MRICH than its BioN co-amended counterpart and in the control. Contrary, a higher microbial buildup was observed among the BioN-treated plants and comparatively higher root colonization in all treated plants with or without BioN and zero root colonization in the control plants.

Results showed that spore count, NFB buildup, and root colonization were significant predictors of growth changes in *B. purpurea.* Specifically, spore count has positive correlations with the total plant biomass (p = 0.05, r = 0.467) and primary root biomass (p = 0.013, r = 0.573), and it can predict about 21.8 and 32.9% change in the total plant biomass and primary root biomass, respectively, of *B. purpurea*, yet negatively correlated with lateral root

biomass (p = 0.045, r = -0.478), while NFB buildup can explain the 50.15% significant (p = 0.001, r = 0.708) variation in height increment of *B. purpurea.* With the successful root colonization in all treated plants, root colonization was inferred to be a significant predictor in the variation of six out of 10 variables – namely, stem diameter (p = 0.015, r = 0.56, r^2 = 65%), total plant biomass (p = 0.02, r = 0.53, r^2 = 28%), stem biomass (p = 0.001, r = 0.69, r^2 = 48%), shoot biomass (p = 0.001, r = 0.70, r^2 = 49%), primary roots (p = 0.03, r = 0.51, r^2 = 26%) and root biomass (p = 0.01, r = 0.63, r^2 = 39%). Among these three soil parameters, root colonization predicts the highest increase in diameter and plant biomass and, therefore, justifies the application of the microbial technology earliest at the nursery stage. These findings also support our claims that microbial technology and the use of leguminous, multi-purpose tree species like *B. purpurea* are, therefore, an effective strategy to ensure higher growth performance of seedlings after being field planted. Microbial technology and the use of leguminous tree species are therefore a better option in agroforestry and in the rehabilitation of critically degraded agricultural lands and forestlands not only in the Philippines but in other tropical ecosystems as well.

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