GROWTH RESPONSE OF NARRA (*PTEROCARPUS INDICUS* WILLD.) SEEDLINGS TO MICROBIAL BIOFERTILIZER APPLICATIONS IN MINED-OUT SOILS OF PLACER AND CLAVER, SURIGAO DEL NORTE, PHILIPPINES

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Abstract. Two concurrent nursery experiments were conducted following a randomized complete block design (RCBD) aimed at screening the effectiveness of arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing bacteria (NFB) in promoting growth and survival of narra seedlings grown in Ni and Au mined-out soils. Seedlings were inoculated with either MYKORICH®, MYKOVAM®, newMycorrhiza (newMYC), newNitrogen-fixing bacteria (newNFB) and combined newMYC and newNFB or not inoculated (control). Narra seedlings were inoculated with uniform amounts of microbial biofertilizers and were grown for a month to ensure root infection. After 30 days, the uninoculated (control) and inoculated seedlings were transferred into polybags filled with mined-out soils mixed with compost (1:1; v%/v%) and were applied with 10 g complete (14:14:14NPK) fertilizer per seedling. After six months of observation, results demonstrated notable and significant effects to the incremental growth in height, stem diameter, plant dry weight and root-to-shoot ratio of narra seedlings in response to the applied microbial biofertilizers. MYKORICH®, newMYC, newNFB and the combined newMYC and newNFB are recommended microbial biofertilizers for narra seedlings used for rehabilitating Ni and Au mined-out sites. Furthermore, combined newMYC + newNFB and MYKORICH® yielded the highest quality seedling index while MYKORICH® gave the highest biovolume index, for seedlings grown in both Ni and Au mined-out soils. The assumption that adding NFB to the already NFB-loving narra species enhances its capability to absorb more nutrients and adapt in heavy metal contaminated soils was proven in this study which makes it a potential host-plant in the bioremediation and rehabilitation of Ni and Au mined-out areas in Claver and Placer (Surigao del Norte), respectively.

Keywords: *arbuscular mycorrhizal fungi (AMF), nitrogen fixing bacteria (NFB), bioremediation, quality seedling index, mining area rehabilitation*

Introduction

Rehabilitating polluted and mined-out areas involves different physical and chemical methods making it a very costly process, which should be carefully planned and performed (Grant, et al., 2016; Mavrommatis and Menegaki, 2017; Mayes et al., 2009). In some cases, mining companies abandon their mined-out areas because of the highly restrictive operational costs of their rehabilitation efforts. Yet, government regulations in the Philippines and worldwide compel them be compliant at whatever costs possible (Clemente et al., 2018). In the Philippines, the Department of Environment and Natural Resources Administrative Order (DAO) 2000-99 contains the rules and regulations on

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the implementation of the Social Development and Management Programs (SDMP) for mining projects. In the study of Raborar and Recio (2019), the Social Development and Management Program (SDMP) needs to be implemented by all active mining/quarrying companies to support the educational, health, social, cultural, and economic aspects of the host and affected community. Thus, mining companies are monitored by the law and required to have concrete and viable plans to successfully implement their mining rehabilitation program.

In the study of RoyChowdhury et al. (2018), mining rehabilitation includes physical methods such as soil replacement, soil washing, and vitrification which are very expensive, time-consuming, and chemical techniques such as extraction of heavy metals involving the use of different chemical agents which makes it ineffective because of the possible formation of toxic organic vapors that are both very expensive and often require the disposal of large amounts of hazardous waste and can destroy the local ecosystems. The issues on responsible mining, environmental stewardship, and sustainability are constant reminders of mining companies to step up their rehabilitation efforts. Mining companies face major challenges in rehabilitating mined-out soils because of their negative properties to include; heavy metal contamination, acidification, unfavorable pH value for plant growth, salinization and alkalization, poor physical structure, and inadequate nutrition (Sun et al., 2018). To achieve successful mining rehabilitation programs, mined-out soils must be properly and suitably amended and remediated. A method called bioremediation is a much better option that transforms harmful contaminants into innocuous substances using natural biological activities. Techniques in bioremediation involve biological systems where microorganisms and plants are used which are known to be sustainable and cost-effective options in converting the pollutants into harmless forms through natural biological processes (Arora, 2018).

By definition, bioremediation is a natural biological process that uses beneficial microorganisms, plants, or microbial or plant enzymes to detoxify contaminants in the soil and other environments (Das and Dash, 2014). The strategy of bioremediation is favorable since it uses naturally-occurring microorganisms together with plants in order to reduce the problems of metal contamination and promotes restoration of degraded soils. According to Aggangan and Cortes (2018), beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) can be of great use in rehabilitating mine tailings because of their sensitivity towards a range of soil pollutants. They are capable of enhancing the tolerance of plants to heavy metal contamination because they are able to fix, transfer, and decompose the pollutants in the soil (Lara et al., 2020: Meier et al., 2012; Park et al., 2016; Song et al., 2020). In comparison to the potential use of microorganisms in metal accumulation in polluted waters which received much attention, the potential use of microorganism for metal mobility in the soil considerably still received much less attention (Ledin, 2000). The focus in this study is the use of beneficial microorganisms which were inoculated to a plant species grown in mined-out areas. Specifically, this study screened potential microbial biofertilizers inoculated on narra seedlings by establishing nursery trials using the mined-out soils collected from the Taganito Mining Corporation (TMC) and Manila Mining Corporation (MMC), in Claver and Placer, Surigao del Norte, respectively.

Narra is endemic to the Philippines and is listed as endangered in the IUCN Red List due to severe exploitation (Barstow, 2018). As mentioned in Aggangan and Morong (2019), narra is one of the indigenous species in the Philippines and it is highly

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encouraged for it to be conserved making it a priority species for restoration. A better rehabilitation strategy involves beneficial microbes along with well-suited tree species (Karthikeyan et al., 2012). Narra (*Pterocarpus indicus*) as part of the legume family has the ability to form an association with arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing bacteria (Aggangan et al., 2019). In this regard, narra can be considered as one of the valuable tree species for rehabilitation purposes but only scanty information of their use as host plant in bioremediation has been reported (Aggangan and Cortes, 2018).

This study used narra seedlings as host species in screening five (5) biofertilizers applied on seedling production and grown in the mined-out soils in TMC and MMC. The purpose of this study were to expose and acclimatize the inoculated nursery raised seedlings in good soils used by nurseryman or foresters to grow seedlings in the nursery prior to outplanting in mined-out soil, thereby increasing the chance of higher survival and better growth in the field. Results from the study of Aggangan et al. (2019) showed that the survival rate of plants with microbial biofertilizers and soil amendments ranged from 92-95% while the survival rate of the plants in soil without any amendments was 15% only after 18 months in the field. The results of this study provided more evidence on the most appropriate biofertilizer treatment and provide some recommendations on the development of site-based protocol to be used in rehabilitating Ni and Au mined-out areas in Claver and Placer, Surigao del Norte, respectively.

Materials and methods

Establishment of nursery trials

(a) Experimental design

The nursery experiments followed the randomized complete block design (RCBD) with ten (10) replicates (per replicate is represented by 6 × 5 block) and raised in the experimental nursery located at Forest and Wetlands Research and Development Extension Center (FWRDEC) Satellite Office, Butuan City. The six (6) treatments were: control (no microbial inoculant), MYKORICH® (from UPLB-BIOTECH), MYKOVAM® (from UPLB-BIOTECH), nitrogen-fixing bacteria (NFB from Marinduque), Mycorrhiza (MYC from Marinduque), and combined Nitrogen Fixing Bacteria and Mycorrhiza (1:1 ratio). For one (1) block, each treatment was represented with five (5) narra seedlings randomly arranged (*Fig. 1*). Since there are six (6) treatments, a total of 30 narra seedlings were planted and arranged randomly in the 6 × 5 (6 treatments; 5 seedlings) block. The 6 × 5 blocks were then replicated 10 times for each site.

(b) Preparation of narra seedlings

A total of 800 narra seedlings were prepared for the nursery experiments but only 600 were used. The seedlings of the same height were obtained from the nursery of the Provincial Environment of Natural Resources Office, Zamboanga del Norte (DENR-PENRO ZDN). The seedlings were already 3 months old prior to biofertilizer inoculation. A total of ten (10) 6 × 5 blocks per site was prepared with 30 narra seedlings. A total of 300 narra seedlings were potted for each soil. Hence, a total of 600 narra seedlings were treated for this study for possible outplanting into the two mining sites and the remaining 200 seedlings served as buffer seedlings for mortality replacement.

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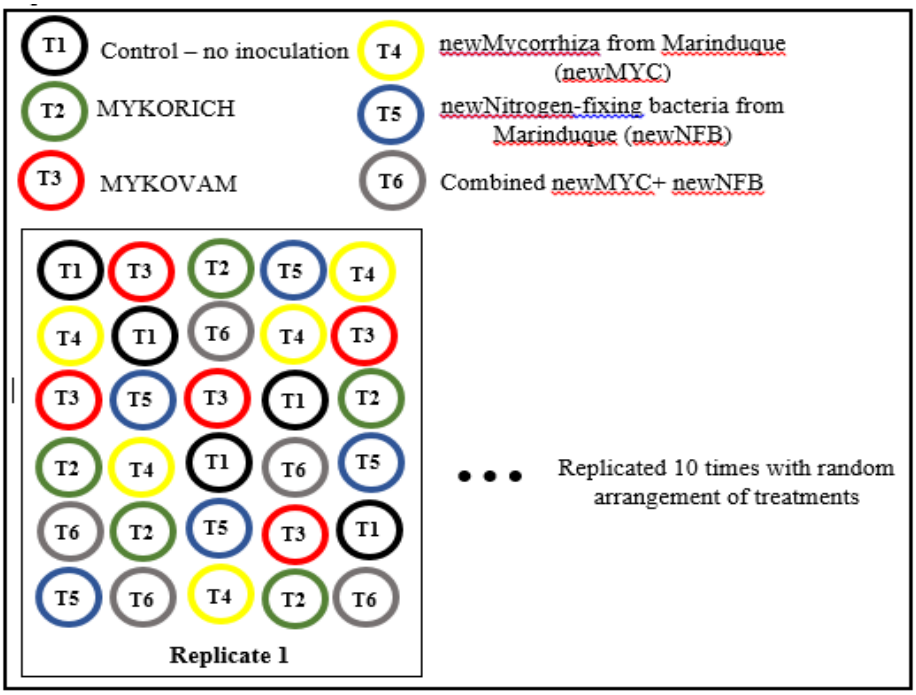

Figure 1. *Nursery experimental lay-out using RCBD*

(c) Collection of degraded mined-out soils

Taganito Mining Corporation (Soil 1). The soil sample collection was done in Taga 1 rehabilitation area of the mining site. A 100 m × 100 m quadrat (1 ha) was laid out in the area. Another 10 m × 10 m (100 grids) was selected and ten (10) sampling points were randomly selected for the collection of the soil samples. An estimated of 20 to 30 kg of soils per sampling points were collected. An estimated of 400 to 600 kg of mined-out soil were collected for site 1 and were brought to FWERDEC Satellite Office, Butuan City for further processing (*Fig. 2*).

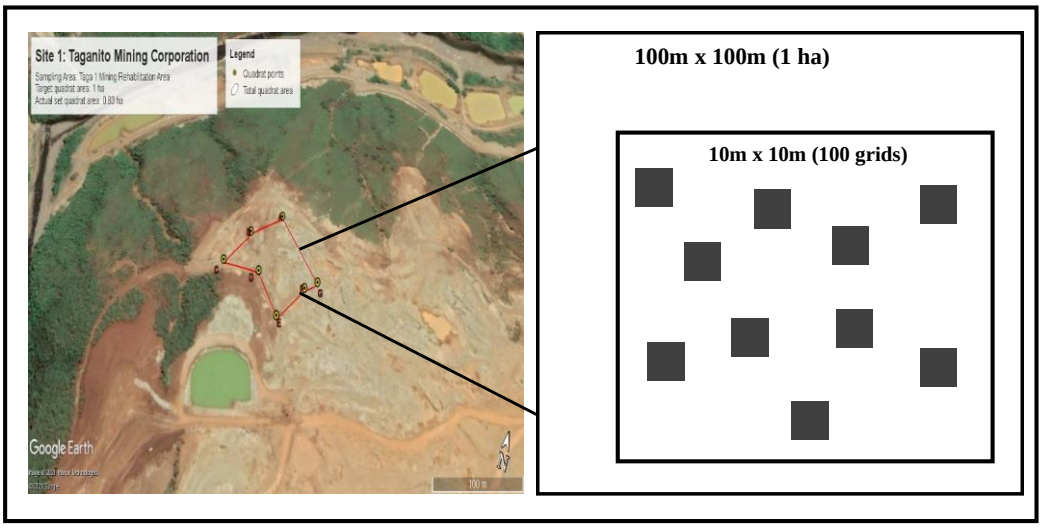

Figure 2. *Field lay-out for soil sampling in Site 1*

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Prior to the experiment, mined-out soils were initially characterized to know the chemical properties of the soil. TMC soils have deficient to low nutrient availability (%organic matter, total nitrogen, available P and exchangeable K) and have high nickel (7,166 ppm) content which is an order of magnitude higher than the Dutch standard intervention value of 210 ppm.

Manila Mining Corporation (Soil 2). The sampling area is located beside the runoff pathway of the mining area. Site 2 is different from the nickel mined-out area since in MMC, underground mining was being used and some open pit mined-out areas are already rehabilitated in the previous years. Thus, some areas beside the runoff pathways have little to no vegetation, so these areas were selected for the collection of soil samples. The same soil amount was collected, rough estimate of 400 to 600 kg of soils were collected. All the collected soil samples were then brought to the FWRDEC Satellite Office in Butuan City for further processing and nursery establishment. MMC soils have low to moderate nutrient availability and a pH value of 6.14. Additionally, MMC soils have high concentration of molybdenum and copper with 39, 400 ppm and 449.2 ppm, respectively which extremely exceeded the Dutch standard intervention value of 200 ppm (molybdenum) and 190 ppm (copper).

Processing of soil samples. The collected soils from TMC and MMC were independently pooled together, air-dried, and sieved using a 0.5' × 0.5' metal wire mesh. This is to remove unwanted debris and to collect finer soil particles. The sieved soils were homogenized by mixing thoroughly by hand. After the sieving process, the soils were mixed with compost to a 1:1 ratio. The composite soils were then potted to a 6' × 10' polyethylene bags which were used in the repotting, one month after inoculation. A total of 600 potted soils in 6 × 10 polyethylene bags were prepared, 300 potted polybags for each mined-out soil.

(d) Microbial inoculation on narra seedlings

The collected and prepared 800 narra seedlings were allowed to grow naturally in a shaded area for 2 weeks to recover from stress during transport from DENR-PENRO nursery in Zamboanga del Norte to Ecosystems Research and Development Bureau (ERDB)-FWRDEC Satellite Office in Butuan, Agusan del Norte. After 2 weeks, 600 seedlings were inoculated by making two holes (2-3 inches depth) in the pot using a barbecue stick. Inoculants were then placed in the holes where: 4 capsules were inserted for MYKORICH, 10 g of MYKOVAM, 10 g of newMYC, 10 g of newNFB and 10 g of combined newMYC + newNFB (1:1 ratio). For the control treatment, no inoculation was done. The treated narra seedlings were then grown for another month in the established nursery located at FWRDEC Satellite Office, Butuan City. This is to allow the microbes to infect the narra roots prior to transfer into the poly bags filled with composite soils (mined-out soil mixed with compost). Proper handling of the experimental seedlings was done to avoid root disturbance while transferring into the composite soils. After a week, 10 g of NPK were applied to all seedlings by placing it around the pot away from the roots and one inch below the soil surface.

The experimental set-up was then observed for the next six (6) months in the nursery with black net as the cover to allow natural light to pass through. Plants were watered using mist to avoid draining of the fertilizers. Soil properties already includes biofertilizers, compost and mined-out soils.

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Determination of growth response

Seedling height and stem diameter were measured once a month for 6 months (1 month after inoculation and 5 months in the treated composite soils). Incremental growth (difference between the current and initial measurement) in height and diameter were computed and recorded. Height was measured one inch above the soil surface up to the main shoot tip of the seedling using a ruler. Stem diameter was also measured similarly (one inch above the soil surface) using a Vernier caliper.

After six months, destructive sampling was done on three narra seedling per treatment in TMC and MMC mined-out soils. A total of 18 plant samples were harvested by cutting the polybags and carefully separated the entire plant from the bulk soil. Each stem was cut one in the root collar, and the leaves were separated from the stem. Each stem was then cut into pieces about 1 to 2 inch length. During harvest, the bulk soil was carefully separated from the roots in order not to lose the fine roots and nodules. Excess soil particles were removed by washing the roots in running water. The fresh weight of stem, leaves and roots were measured using Azuki® top-loading balance before wrapping the plant parts separately into a foil and oven dried at 65 °C for 72 h. The oven-dried weights were recorded using the same top-loading balance.

Seedling quality index and bio-volume index

Seedling quality indices and bio-volume indices were calculated using the data gathered from the measured parameters: plant height, diameter and dry weight. Quality index is a measure to assess the quality of seedling based on the height, stem diameter and dry biomass using the following formulae while bio-volume index is a non-destructive quick method to calculate the aboveground portion of the seedling using the formula introduced by Hatchell (Kumar et al., 2015):

$$\textbf{Quality index} = \frac{seedling\ dry\ biomass\ (g)}{\frac{height\ (mm)}{diameter\ (mm)} + Top\ dry\ biomass\ (g)} \quad \text{(Eq.1)}$$

$$\textbf{Bio - volume index} = plant\ height\ (cm) x\ stem\ diameter\ (mm) \quad \text{(Eq.2)}$$

Root-to-shoot ratio determination

Additional growth response parameter is the root-to-shoot ratio. It is usually given as the ratio of the weight of the roots to the weight of the shoot. This parameter indicates that with a higher proportion of roots (ratio is > 1), the plant can absorb more nutrients ensuring the plant's survival.

Statistical analysis

Test for normality and homogeneity of variance were carried to all data sets out using Shapiro-Wilks and Levene's test, respectively. All data were analyzed using Analysis of Variance (ANOVA) in IBM SPSS Statistics 22®. Treatment means were computed and compared using the Tukey's Honest Significant Difference (HSD) test for Multiple Comparison and Least Significant Difference at the 0.05 level of significance.

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Results and discussion

Height and diameter increments in Ni mined-out soil

The general appearance of Narra seedlings due to microbial inoculation treatments and grown in Ni mined-out soil is shown *Fig. 3.* A one-way ANOVA test results showed significant ($p < 0.05$) differences on Narra's height and diameter for the last six months observation period. MYKORICH® and MYKOVAM®-inoculated seedlings are significantly different from other treatments in terms of promoting height of Narra (*Fig. 4*). The MYKORICH®-inoculated seedlings significantly ($p < 0.05$) had the highest (36.35 cm) height growth after 90 days of inoculation while the uninoculated one gave the lowest (23.8 cm). Inoculation with MYKORICH® increased height growth by 153% relative to the uninoculated control. For the last 180 days, treatments 1 (control), 2 (MYKORICH®) and 3 (MYKOVAM®) significantly yielded the highest height (91.22 cm, 94.2 cm and 88.28 cm) incremental growth in height while the combined treatments (treatment 6) gave the lowest (71.7 cm). These results are different from Aggangan et al. (2019) where the dual inoculation promoted the higher height growth of narra seedlings after four months in copper mined-out soil (*Fig. 3*).

Figure 3. *Appearance of narra seedlings grown in TMC (nickel) mined-out soil after 6 months under nursery conditions*

In terms of stem diameter increment, MYKORICH®-inoculated seedlings consistently yielded ($p < 0.05$) the highest stem diameter increment throughout the six (6) months observation and the lowest was in newMYC (*Fig. 5*). Inoculation with MYKORICH® increased diameter by 118% relative to the newMYC. This result is similar with Aggangan et al. (2019) where the addition of MYKORICH® improved the stem diameter when applied alone.

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Height increment (Ni mined-out soil)

Figure 4 shows the periodic height increment of narra seedlings in Ni mined-out soil. Combined treatments not having the highest increase in narra's diameter growth in comparison to the study of Aggangan et al. (2019) where mycorrhizal fungi applied in combination with NFB could promote better plant growth in diameter might be affected with some factors. As stated in the study of Giassi et al. (2016), mixed bacterial isolates come with varying mechanisms of action where they are applied. Moreover, some nitrogen-fixing bacteria are non-nodulating or also termed as DMI (does not make infection) were also found to be blocked in colonization with AMFs (Franche et al., 2008). However, the result of this study indicated that MYKORICH® and newNFB promoted the incremental growth in height and diameter, respectively, of narra seedlings exposed in Ni mined-out soil. Moreover, in the study of Algabre et al. (2019), *Acacia mangium* gave the highest height when applied with MYKORICH® as well as a positive increase of height and diameter of NFB-inoculated *A. mangium* and *P. indicus.*

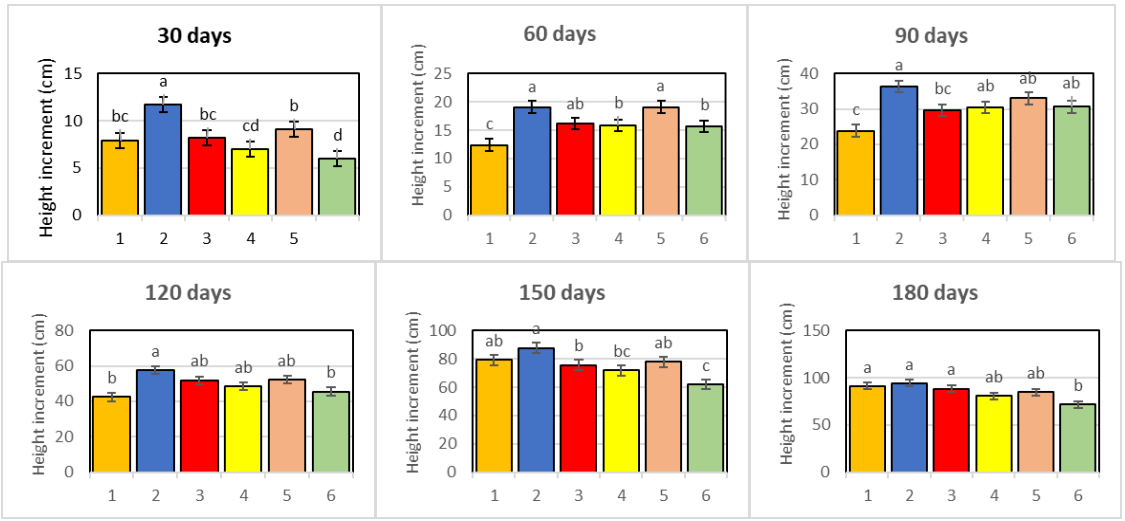
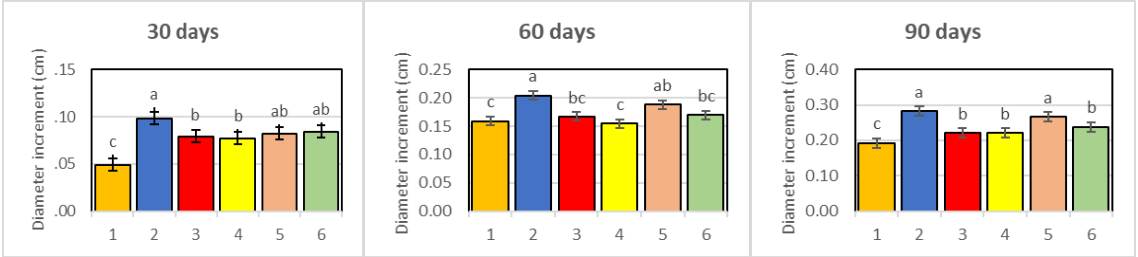


Figure 4. *Periodic height growth increment of inoculated and control narra seedlings grown for 6 months in Ni mined-out soil. 1–control (uninoculated); 2-MYKORICH®; 3–MYKOVAM®; 4 – newMYC; 5 – newNFB; 6-combined newMYC + newNFB. n = 50. Bars with the same letters are not significantly different from each other using Tukey's test at 5% level of significance (Error bars represent the standard deviation)*

Diameter increment (Ni mined-out soil)

Figure 5 shows the periodic diameter increment of narra seedlings in Ni mined-out soil.



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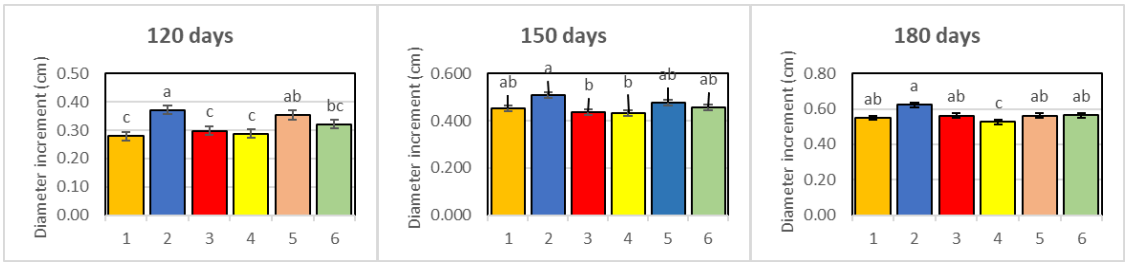

Figure 5. *Periodic diameter growth increment of inoculated and control narra seedlings grown for 6 months in Ni mined-out soil. 1–control (uninoculated); 2-MYKORICH®; 3–MYKOVAM®; 4 – newMYC; 5 – newNFB; 6-combined newMYC + newNFB. n = 50. Bars with the same letters are not significantly different from each other using Tukey's test at 5% level of significance (Error bars represent the standard deviation)*

The repeated measures ANOVA with a Greenhouse-Geisser correction revealed a significant effect on plant height and diameter in between time points in response to the different treatments application, respectively. Post hoc analysis with a Bonferroni adjustment revealed that the effect of the treatments on narra seedlings are statistically significant throughout the six (6) months observation period for both parameters. This implies that the applied microbial biofertilizers to narra seedlings enhances and hastens its ability to grow faster in terms of height and diameter as compared to the uninoculated control (*Fig. 6*).

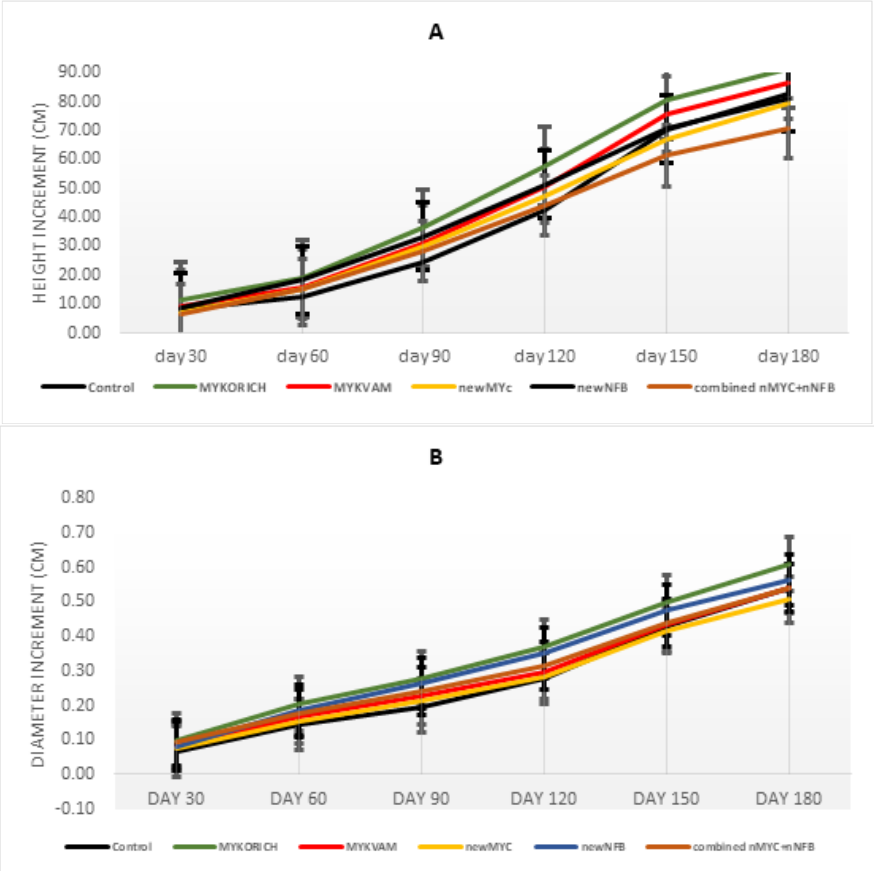

Figure 6. *One-way ANOVA repeated measures for height (A) and diameter (B) increments for 180 days in Ni mined-out soil. (Error bars represent the standard deviation)*

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Height and diameter increments in Au mined-out soil

The observed results of the height and diameter of seedlings grown in Au mined-out soils are shown in *Figures 8* and *9*. A one-way ANOVA revealed that there was a statistically significant differences on the height of inoculated and uninoculated narra seedlings ($p > 0.05$) up to 4 months of the observation period (*Fig. 10*). Tukey's HSD Test for multiple comparisons found that the mean values of inoculated seedlings was significantly different from the control ($p < 0.05$), 30 and 60 days upon inoculation. On day 90, Tukey's test revealed that MYKORICH® ($p = 0.00$) and MYKOVAM® ($p = 0.044$) are statistically different from the control (no inoculation). After 120 days, only MYKORICH®-inoculated seedlings gave significant difference ($p < 0.05$) from the control (no inoculation). However, one-way ANOVA revealed no significant differences ($p > 0.05$) on the height in response to the treatments and the control after six (6) months of observation period. Although statistically not significant, after 6 months from inoculation, MYKORICH®, combined nMYC + nNFB and MYKOVAM®-inoculated seedlings gave height increase of 116%, 104% and 103% relative to the uninoculated control narra seedlings (*Fig. 7*).

Figure 7. *Appearance of narra seedlings grown in MMC (gold) mined-out soil after six (6) months under nursery conditions*

These results implies that the effect of the microbial biofertilizers might be limited through time and might be attributed to some factors. In the study of Ronsheim (2012) the effect of mycorrhiza on plant growth, reproduction and developmental stages, where the results of the presence of mycorrhizae shows no significant effect. It was also indicated that spatial variation in nutrient availability in the field has the potential to shift the overall effect of mycorrhizae from beneficial to neutral. In the case of this study, the source of the soil substrate may cause the nonsignificant effect of AM to stem height of narra grown in gold mined-out soil. The physico-chemical status of gold mined-out soil as well as its contamination with the presence of metals

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embedded in the mined-out soil might cause some neutralization. Thus, further results on the metal concentration in soil of inoculated narra seedlings is highly recommended.

In terms of the stem diameter of narra seedlings grown in Au mined-out soil, one-way ANOVA revealed that there was a significant difference on the treatments ($p < 0.05$) throughout the six months of observation period. Further, Tukey's HSD test for multiple comparison revealed that one month after inoculation, MYKORICH®, MYKOVAM®, newMYC and newNFB are significantly different from treatments 1 (control) and 6 (newMYC + newNFB) with p-values less than 0.05. On day 60, which was the second month after inoculation, Tukey's test results showed that MYKORICH®, MYKOVAM® and newMYC are significantly different from other treatments ($p < 0.05$). Day 90, 120 and 150 days after the inoculation, MYKORICH®-inoculated seedlings gave the highest diameter increase and yielded a significant difference in all other treatments ($p < 0.05$). Moreover, six (6) months after the observation period, Tukey's test revealed that MYKORICH® and newNFB promoted the diameter height and have a significant difference ($p < 0.05$) from the rest of the treatments. Narra seedlings inoculated with MYKORICH® and newNFB gave the diameter increase by 113% relative to the uninoculated control.

These results showed promising effects of MYKORICH® in promoting the plant's height and diameter. On the other hand, newNFB also showed significant effect on the plant's diameter. This result is supported with the study of Aggangan and Cortes (2018), where narra can grow very well in a much stressed environment especially when inoculated with NFB and other mycorrhizal fungi.

Height increment in Au mined-out soil

Figure 8 shows the periodic height increment of narra seedlings in Au mined-out soil.

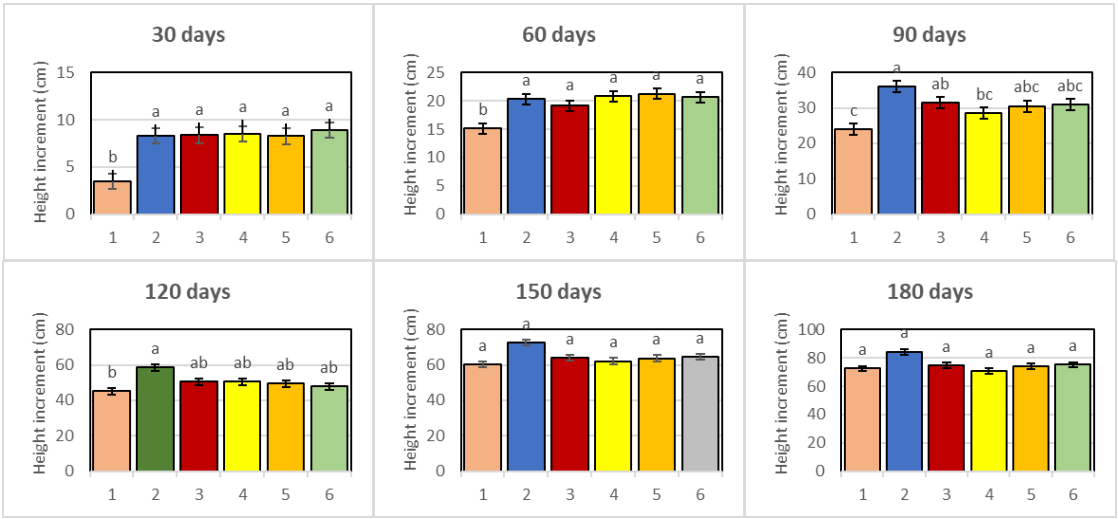

Figure 8. *Periodic height growth increment of inoculated and control narra seedlings grown for 6 months in Au mined-out soil. 1–control (uninoculated); 2-MYKORICH®; 3–MYKOVAM®; 4 – newMYC; 5 – newNFB; 6-combined newMYC + newNFB. n = 50. Bars with the same letters are not significantly different from each other using Tukey's test at 5% level of significance. (Error bars represent the standard deviation)*

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Diameter increment in Au mined-out soil

Figure 9 shows the periodic diameter increment of narra seedlings in Au mined-out soil.

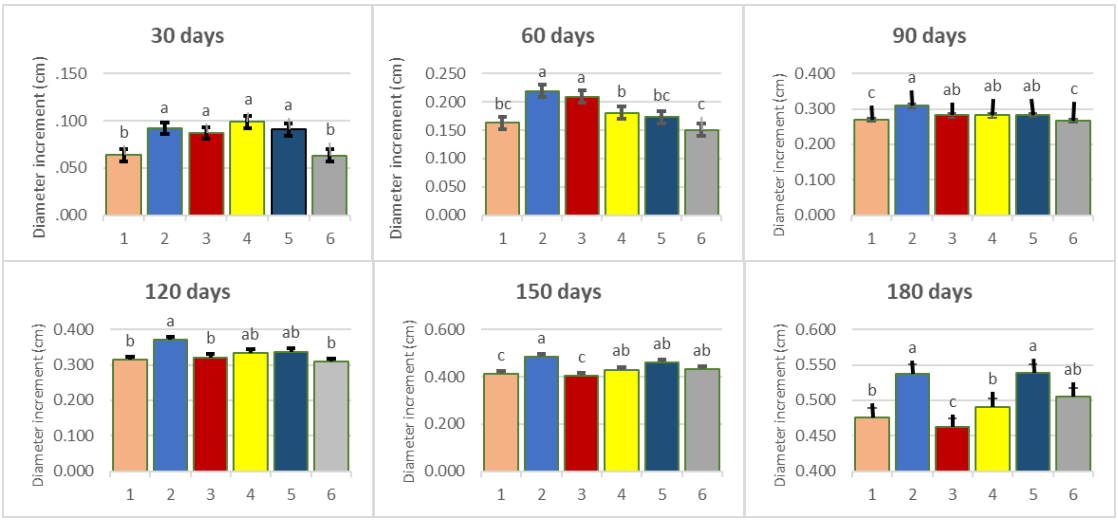
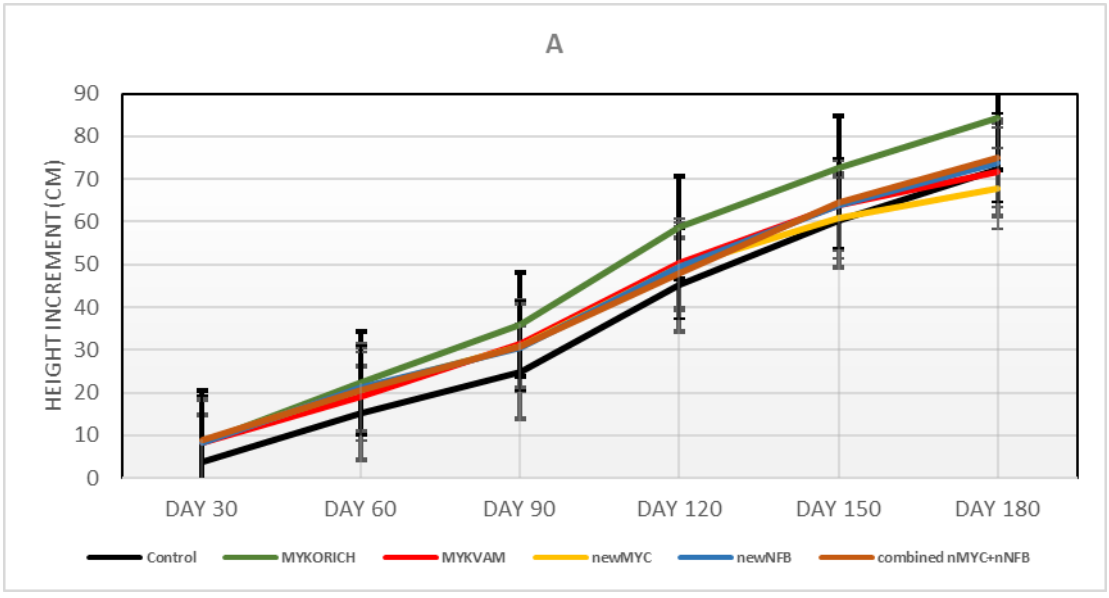


Figure 9. *Periodic diameter growth increment of inoculated and control narra seedlings grown for 6 months in Au mined-out soil. 1–control (uninoculated); 2-MYKORICH®; 3–MYKOVAM®; 4 – newMYC; 5 – newNFB; 6-combined newMYC + newNFB. n = 50. Bars with the same letters are not significantly different from each other using Tukey's test at 5% level of significance (Error bars represent the standard deviation)*

In Au mined-out soils, the repeated measures ANOVA with a Greenhouse-Geisser correction revealed significant effects on the height and diameter between time points in response to the different treatments application, respectively. Post hoc analysis with a Bonferroni adjustment revealed that the effect of the treatments on narra seedlings are statistically significant all throughout the six (6) months observation period for both parameters (*Fig. 10*).



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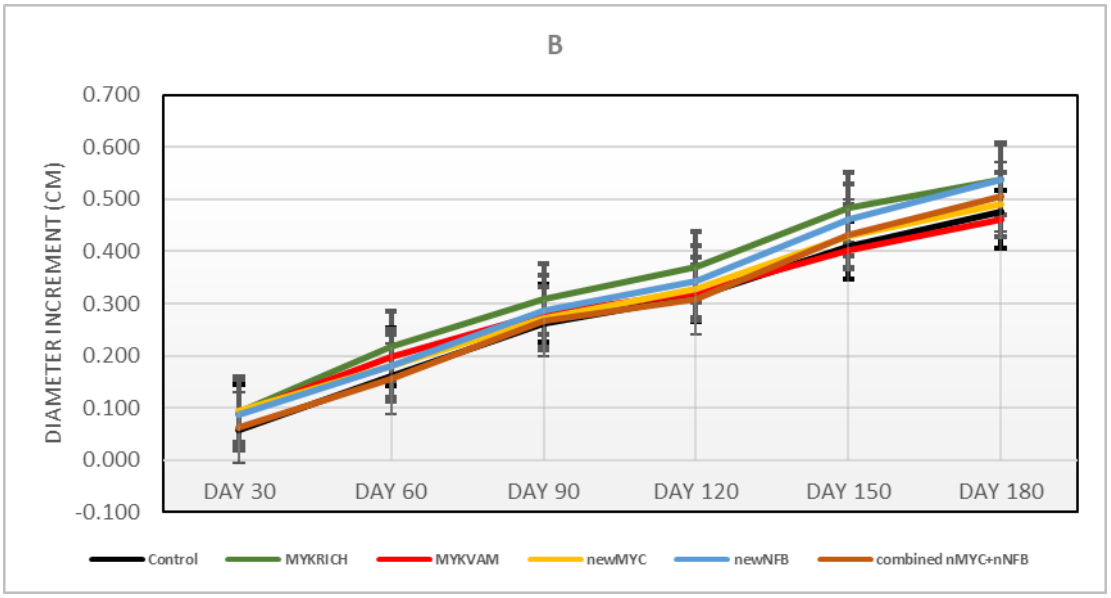

Figure 10. *One-way ANOVA repeated measures for height (A) and diameter (B) increments for 180 days in Au mined-out soils. (Error bars represent the standard deviation)*

Plant dry weight

One-way ANOVA results showed that there is a significant difference ($p < 0.05$) on the total plant dry weight of narra seedlings grown in Ni mined-out soils in response to the applied microbial biofertilizers. Tukey's HSD test for multiple comparison revealed that MYKORICH®, newMYC and combined newMYC + newNFB are significantly different from other treatments in the total plant dry weights of narra seedlings. MYKORICH®-inoculated seedlings yielded the heaviest total plant dry weight which is 296% relative to the uninoculated control. However, in the gold mined-out soils, one-way ANOVA revealed that there is no significant difference on the total plant dry weights of all treatments ($p > 0.05$). Despite of having no significant differences, MYKORICH®-inoculated seedlings gave the heaviest total plant dry weight of narra seedlings grown in Au mined-out soils. In the study of Algabre et al. (2019), *A. mangium* inoculated with mycorrhiza alone gave a 113% increase in the production of biomass and in *P. indicus*, seedlings with Mycorrhiza + NFB also produced higher biomass (17.03%) as compared to the control (15.32 mg ha^{-1}). Moreover, *Figure 11* shows that all inoculated seedlings in Ni and Au mined-out soils yielded heavier plant dry weight (15-30 g/plant and 10-25 g/plant, respectively) compared to the uninoculated control (10 g/plant and 8 g/plant, respectively) seedlings. These results are similar to the study of Aggangan et al. (2019a) where all the inoculated plants have heavier stem, root, leaves and nodule dry weight compare to the control. Also in the study of Wei et al. (2009), the biomass of *C. scoparius* when inoculated with *Bradyrhizobium* was twice that of the uninoculated plant. Microbial biofertilizers especially the arbuscular mycorrhizal fungi (AMF) are ubiquitous in the rhizosphere that significantly improve plant nutrient uptake and provide resistance to several stress factors (Sun et al., 2018). It was also highlighted in the study of Aggangan et al. (2019b) that AMFs infect plant roots enhancing its ability to take up water and nutrients.

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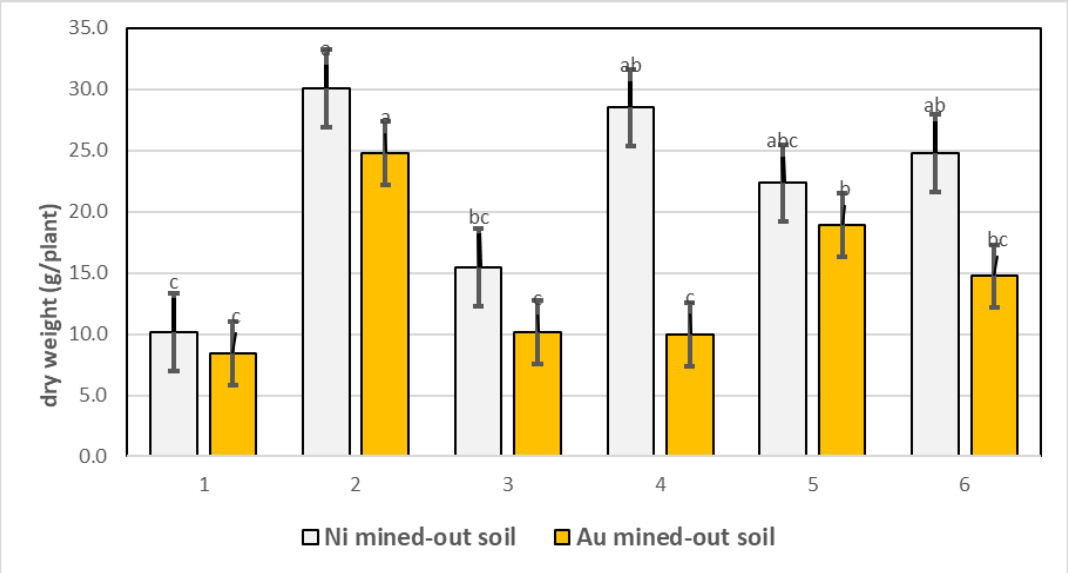

Figure 11. *Total dry weights of inoculated and control narra seedlings grown in nickel mined-out soil (site 1) and gold mined-out soil (site 2), 6-months observation period. 1–control (uninoculated); 2-MYKORICH®; 3–MYKOVAM®; 4 – newMYC; 5 – newNFB; 6-combined newMYC + newNFB. n = 18. Bars with the same letters are not significantly different from each other using Tukey's test at 5% level of significance (Error bars represent the standard deviation)*

Root dry weights

Figure 12 shows the root dry weights while *Fig. 13* shows the root architecture, morphology and structure of narra seedlings as affected by microbial inoculation and grown for six in Ni and Au mined out soils. In both sites, all the applied microbial biofertilizers improved the root dry weights of narra relative to the uninoculated control seedlings. For instance, for site 1, newMYC and the combined newMYC + newNFB gave the heaviest root dry weight by 309% and 320%, respectively, relative to the control seedlings (uninoculated). In site 2, MYKORICH® and newNFB gave the heaviest root dry weight increase by 190% and 173%, respectively, relative to the uninoculated control seedlings.

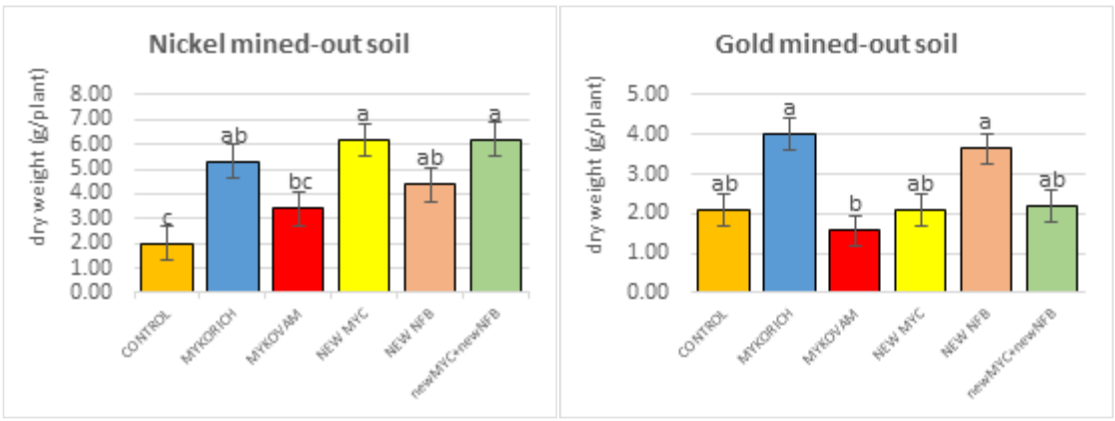

Figure 12. *Root dry weights of narra seedlings after 6 months of observation. n = 3. (Error bars represent the standard deviation)*

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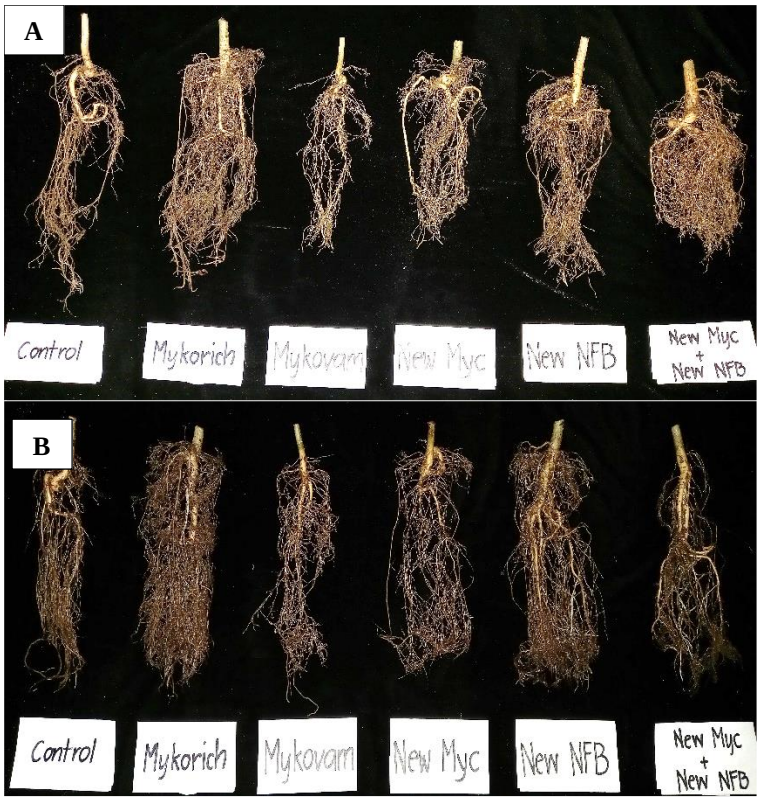

Figure 13. *Roots of 6-month-old narra seedlings grown in nickel (A) and gold (B) mined-out soils under nursery conditions*

Narra species belongs to the legume family. In the study of Scheublin et al. (2006), many legumes can form symbiotic associations with rhizobia and arbuscular mycorrhizal fungi (AMF). For the rhizobia, a nitrogen fixing bacteria, they accumulate in the root nodules and aids the plant in nitrogen fixation process. On the other hand, the mode of association of AMFs to the plant roots is by colonization in which they can also provide important nutrients to the plants. Looking closely from the results, newMYC and the combined newMYC + newNFB gave the heaviest root dry weight for site 1 (nickel mined-out soil). However, in terms of its height and diameter, the same treatments gave the lowest height and diameter increments (see *Figs. 4* and *5*). As stated in the study Scheublin and van der Heijden (2006), there are studies showed some interactions between AMF and rhizobia inside root nodules, however, AMF in the nodules were not active implying that AMF cannot fix nitrogen. Also, AMFs can limit or inhibit the N fixation when AMF are present in low soil nutrient availability. This suggests that the performance of these microbial biofertilizers might be affected by the soil nutrient status of the nickel mined-out soils.

On the contrary, in site 2 (gold mined-out soil), MYKORICH® and newNFB-inoculated seedlings gave the heaviest root dry weight as well as high increase in height and diameter growth (see *Figs. 6* and *8*). In the study of Zaefarian et al. (2016), mycorrhizal fungi colonized plant roots and boost the plant's ability to uptake water and nutrients. They can also affect the morphology and physiology of the plants under different conditions including stressed environments. The NFB-legume association can form emphatic nitrogen fixation even under salt, heat, acid stresses and even in heavy

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metal contamination (Zahran, 1999). This implies that MYKORICH® and newNFB gave the best performance in promoting the growth of narra seedlings grown in gold mined-out soils.

Root-to-shoot ratio is a sensitive indicator of plant stress induced by chemical or physical agents as defined in the study of Agathokleous et al. (2019). A one-way ANOVA result revealed that there is no significant differences ($p > 0.05$) on the root-to-shoot ratio to narra seedlings in response to the different treatments, these results are the same in both nickel and gold mined-out soils. Root to shoot (R/S) ratio appears to be dependent on the identity of the plant species and plant growth stage (Ledig and Perry, 1966). As stated by Thomson (2006), the initial growth of narra is directed toward shoot elongation. As seen in *Table 1,* shoot dry weights or the aboveground mass are much higher compared to belowground mass/root dry weights. The study of Veresoglou et al. (2011), affirms that AMF inoculation would result in decreases in the R/S ratio through ameliorating plant species nutrient status and that this would occur irrespective of the fitness outcome of the symbiosis for the plant. Also, further hypothesized that the identity of both the plant host and the AM fungus would be key regulators of such a decrease in the R/S ratio. Also, heavy metal stressed plants tend to have a lower R/S ratio (Roosens et al., 2003), this further suggest to have a heavy metal concentration analysis to narra seedlings in response to the microbial biofertilizers.

Table 1. *Root and shoot dry weights and root-to-shoot ratio*

SITE 1				SITE 2			
Treatment	**Root DW (g)**	**Shoot DW (g)**	**R/S ratio**	**Treatment**	**Root DW (g)**	**Shoot DW (g)**	**R/S ratio**
1	2.00	8.17	0.245	1	2.10	6.33	0.332
2	5.30	24.77	0.214	2	4.00	20.77	0.193
3	3.40	12.07	0.282	3	1.57	8.57	0.183
4	6.17	22.33	0.276	4	2.10	7.87	0.267
5	4.37	17.97	0.243	5	3.63	15.27	0.238
6	6.20	18.60	0.333	6	2.20	12.53	0.176

In general, leguminous species like narra form symbiotic relationships with NFB as previously mentioned in the study. In a normal or contaminated environment, narra can typically adapt and survive along with the naturally-occurring nitrogen-fixing microbes. According to Wang et al. (2018), legumes form symbiotic relationship with nitrogen-fixing soil bacteria called rhizobia. This symbiosis is a nodule-forming plant-microbe relationship in which the bacteria can convert atmospheric nitrogen into ammonia that can be used by the plant for growth and development. The nitrogen fixation is done by the bacteria and the product they produce is absorbed by the plant, (Flynn and Idowu, 2015). The addition of nitrogen-fixing bacteria which is one of the treatments in this study on the narra seedlings may have resulted to the buildup of NFB community in the rhizosphere which further enhanced their capability to absorb more nutrients and further improved its adaptability and survivability when exposed in a heavy metal contaminated environment. The increase in the population of NFB in the rhizosphere may have enhanced the nitrogen fixation process producing substantial pool of fixed nitrogen. However, this species of nitrogen is not readily available to plants but need to be converted first by microorganisms using the nitrogenase enzyme to convert N_2 to NH_3

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which then become readily used by the plants for their metabolism, growth and development (Aasfar et al., 2021; Cooper and Scherer, 2012; Wagner, 2011).

Only few studies have noted that narra is a remarkable plant species that can be use in rehabilitating degraded soils and mined-out areas. Likewise, a dearth of information is available on the use of narra species as host plant in bioremediation (Aggangan and Cortes, 2018). The results of this study provide additional scientific evidence that narra is very much capable and is suitable in rehabilitating mined-out areas most especially as host plant in bioremediation. It can further be asserted that narra is a member of a Leguminaceae family and has the natural ability to form associations with AMFs and NFBs (Aggangan and Anarna, 2019b). This unique relationship make narra more resilient, adaptable and a good host plant species for rehabilitating mined-out areas.

Seedling quality index

One-way ANOVA revealed that there was a statistical difference ($p < 0.05$) in the quality index of narra seedlings as influenced by the different treatments applied after six (6) months of observation for both mined-out soils. *Table 2* shows that Tukey's HSD Test for multiple comparisons for Ni mined-out soil found that the mean value of quality index was significantly different between the combined newMYC + newNFB and the control (no microbial inoculation) ($p < 0.05$). As also observed, the quality index was the highest (0.1668) for seedlings treated with newMYC and the lowest quality index (0.0567) was recorded for the control seedlings which has no application of any microbial biofertilizers (*Table 2*). Furthermore, the Tukey's HSD Test for multiple comparison for Au mined-out soil showed no significant differences on any of the treatments applied on narra seedlings ($p > 0.05$). However, although statistically not significant, highest quality index was obtained with seedlings treated with MYKORICH® and the recorded lowest quality index was in the control (no inoculation) seedlings.

Table 2. *Mean quality index of narra seedlings as influenced by different biofertilizer treatments grown in Ni and Au mined-out soils after six (6) months of observation*

Ni mined-out soil			**Au mined-out soil**		
Treatments	**Quality index***	***p - value***	**Treatments**	**Quality index***	***p - value***
1 – Control	0.0567[b]	0.068	1 – Control	.0524[a]	0.321
2 – MYKORICH®	0.1606[b]	0.068	2 – MYKORICH®	.1256[a]	0.321
3 – MYKOVAM®	0.0894[b]	0.926	3 – MYKOVAM®	.0539[a]	1.000
4 – newMYC	0.1668[b]	0.061	4 – newMYC	.0559[a]	1.000
5 – newNFB	0.1234[b]	0.358	5 – newNFB	.1101[a]	0.593
6 – nMYC + nNFB	0.101[a]	0.039	6 – nMYC + nNFB	.0947[a]	0.845

*Values with the same letters are not significant from each other at the 5% level of significance

Seedling quality indices were obtained in order to know the effect of the microbial biofertilizers on the growth of narra seedlings grown in nickel and gold mined-out soils under nursery conditions. The computation of the seedling quality indices was based on the study of Kumar and Jjjeesh (2015) where they estimate the effect of chemical treatments on the mahogany (*Swietenia macrophylla*) seedling performance at a nursery

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level. To ensure that the needed seedlings for outplanting is able to survive, they must be strong and healthy seedlings. Seedlings raised in nurseries despite having good appearance but cannot survive and grow after planting out are worthless. Thus, it is important to know their quality indices in response to the treatments applied, to be able to know if the seedlings are well-suited and healthy enough to be introduced for outplanting in the field. The combined newMYC + newNFB and MYKORICH®-inoculated seedlings gave the highest seedling quality indices for TMC and MMC soils, respectively. This implies that these narra seedlings treated with combined newMYC + newNFB and MYKORICH® can survive and adapt when outplanted in nickel and gold mined-out contaminated areas. This contention needs further validation as these treated seedlings which have been grown in the nursery find their way to the field for outplanting.

Bio-volume index

Figure 14 shows the mean bio-volume index of narra seedlings.

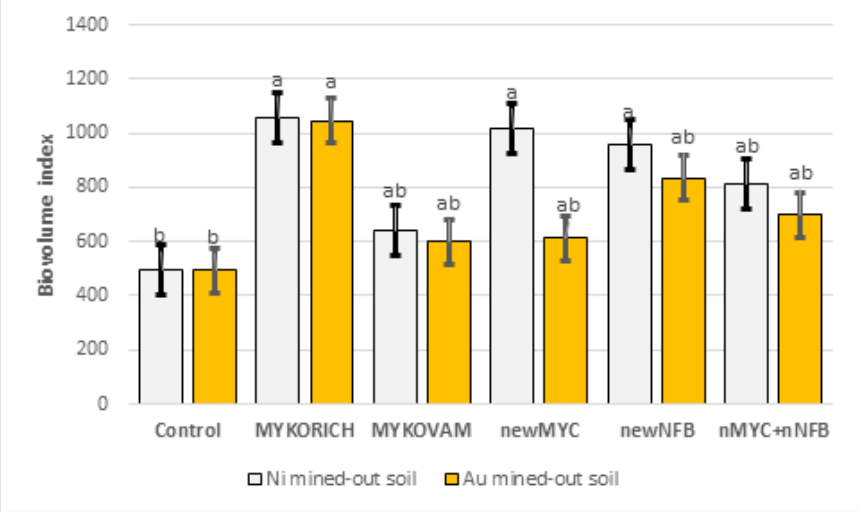

Figure14. *Mean bio-volume index of narra seedlings as influenced by different treatments grown in Ni and Au mined-out soils at 6th month. Values with the same letters are not significant from each other at the 5% level of significance. (Error bars represent the standard deviation)*

One-way ANOVA revealed that the treatments have statistical effect on the bio-volume index in Ni ($p < 0.05$) mined-out soils but not in Au ($p > 0.05$) mined-out soils after 6 months of observation. Tukey's HSD Test for multiple comparisons for Ni mined-out soil found that the mean value of bio-volume index was significantly different between the control and MYKORICH® ($p < 0.05$), control and newMYC ($p < 0.05$), and the control and newNFB ($p < 0.05$). The mean highest bio-volume index was found in MYKORICH®, newMYC and newNFB treated seedlings, respectively, in Ni mined-out soil. MYKORICH®, newMYC and newNFB were 215%, 206% and 194% higher than the uninoculated seedlings. Meanwhile, although results were not significant in Au mined-out soil, the mean highest bio-volume index was recorded in MYKORICH® treated seedlings which recorded 212% higher than the control seedlings.

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Although results showed that seedlings treated with microbial biofertilizers yielded high bio-volume indices compared to the uninoculated ones, MYKORICH®-treated seedlings resulted in highest bio-volume index both in Ni and Au mined-out soils. In the study of Srinivasan et al. (2012), AMFs are well-known to improve plant growth mainly through enhanced nutrition. The application of AMFs to seedlings that will be used in outplanting to mined-out soils can be of great help in ensuring high success rate of rehabilitation results.

The study of Parkash et al. (2011) discovered that the biovolume index and seedling quality index of *Ruta graveolens* was high in all inoculated treatments than the non-inoculated control treatments. In the study of Rhagu et al. (2020a), the field growth of inoculated *Acacia auriculiformis* in terms of biovolume index was 52% higher than that of the uninoculated trees proving that selected microbial consortium enhances nursery quality and field growth of *Acacia auriculiformis* plantations on dry wasteland. In addition, the biovolume index of inoculated *Tectona grandis* was 289% more than the uninoculated plants 73 months after outplanting (Rhagu et al., 2020b). The enhanced and improved seedling quality and biovolume index as it was aided with microbial biofertilizers gave an assurance that seedlings needed for outplanting to a toxic environment is able to survive and will have a successful mining rehabilitation. It can also be used to restore other degraded lands and can aid in biodiversity conservation.

Conclusion and recommendations

This study screened five (5) microbial fertilizers inoculated on narra seedlings and grown in Ni and Au mined-out soils collected from TMC and MMC in Surigao del Norte, respectively. In general, the results clearly show that the application of microbial fertilizers demonstrated notable and significant effects on the plant height, stem diameter, dry weights and root-to-shoot ratios. Moreover, MYKORICH®, newMYC, and newNFB promoted narra's tallest height and biggest diameter. NewMYC and the combined treatments (newMYC + newNFB) gave significant increases in the roots dry weight of narra grown in Ni mined-out soil. Furthermore, combined newMYC + newNFB gave the highest quality seedling index which means that seedlings treated with the combined microbial biofertilizers, although have the lowest height and diameter, still have the ability to survive when outplanted in nickel mined-out areas. For Au mined-out soil, MYKORICH® performed best in promoting growth in height, stem diameter, dry weight and root-to-shoot ratio. Additionally, MYKORICH® and newNFB gave the heaviest root dry weight. Lastly, MYKORICH®-inoculated seedlings yielded the highest quality seedling index and bio-volume index which implies that these seedlings treated with MYKORICH® will most likely to survive when outplanted in gold mined-out areas.

The assumption of adding NFB to the already NFB-loving narra species enhances its capability to absorb more nutrients and adapt in heavy metal contaminated sites was clearly demonstrated in this study. Furthermore, results of the study showed the enhanced growth of narra seedlings when inoculated with NFB as compared to the control. It was further observed that mycorrhizal fungi strongly enhanced the growth of narra grown in mined-out soils. In order to have high success rate in rehabilitating mined-out and other polluted areas, the soil and plants must be thoroughly amended with compost, organic fertilizers and microbial biofertilizers.

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The results of this study also provide scientific evidence that narra is suitable and holds so much potential in rehabilitating mined-out areas most especially as host plant in phytoremediation. It further shows how the narra plant species was able to perform best when applied with microbial fertilizers in mined-out soils. The ability of narra species to adapt and survive in degraded and polluted soil and its enhanced mechanism when applied with biofertilizers makes it a candidate species to be used as host plant in bioremediation and rehabilitating mined-out areas in Claver and Placer, Surigao del Norte, respectively.

It is highly recommended that measurements and monitoring of heavy metal concentrations initially in the soil and later on in the roots, stem and leaves of narra seedlings and in field studies will further enhance its potential for bioremediation of contaminated soils. Monitoring and understanding the mechanism of metallothionein**s** (MT) production in roots and leaves of narra and other species for bioremediation in nursery and field trials are likewise recommended. The purpose is to determine the potential of these metal-chelating protein in plants to reduce the toxic effects of heavy metals in mined-out soils.

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APPENDIX

ANOVA tables

(1) Soil 1_Height

Tests of between-subjects effects

Dependent variable: height

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	812009.951[a]	5	162401.990	5.042	.000
Intercept	72078086.662	1	72078086.662	2237.744	.000
Treatment	812009.951	5	162401.990	5.042	.000
Error	9373156.722	291	32210.161		
Total	82286934.000	297			
Corrected total	10185166.673	296			

a. R squared = .080 (adjusted R squared = .064)

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Height

	Treatment	N	Subset		
			1	2	3
Tukey HSD[a,b,c]	1	50	423.8200		
	6	50	440.6400		
	4	48	476.4375	476.4375	
	3	49	518.8980	518.8980	
	5	50	519.0000	519.0000	
	2	50		577.3600	
	Sig.		.092	.061	

(2) Soil 1_Diameter

Tests of between-subjects effects

Dependent variable: diameter

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	40.649[a]	14	2.904	6.831	.000
Intercept	2958.876	1	2958.876	6961.263	.000
Treatment	31.894	5	6.379	15.007	.000
Replicate	8.031	9	.892	2.099	.030
Error	116.463	274	.425		
Total	3111.355	289			
Corrected total	157.113	288			

Diameter

Tukey HSD[a,b,c]

Treatment	N	Subset			
		1	2	3	4
1	50	2.7970			
4	48	2.9625	2.9625		
3	50	2.9810	2.9810		
6	49		3.2020	3.2020	
5	49			3.5776	3.5776
2	43				3.7372
Sig.		.737	.467	.057	.837

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(3) Soil 2_Height

Tests of between-subjects effects

Dependent variable: height

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	1691644.180[a]	14	120831.727	3.236	.000
Intercept	75421590.803	1	75421590.803	2019.604	.000
Treatment	515907.817	5	103181.563	2.763	.019
Replicate	1175736.363	9	130637.374	3.498	.000
Error	10643254.017	285	37344.751		
Total	87756489.000	300			
Corrected total	12334898.197	299			

Height

Tukey HSD[a,b]

Treatment	**N**	**Subset**	
		1	**2**
1	50	452.1600	
6	50	478.3400	478.3400
5	50	493.4600	493.4600
4	50	493.7400	493.7400
3	50	504.2800	504.2800
2	50		586.4400
Sig.		.758	.061

(4) Soil 2_Diameter

Tests of between-subjects effects

Dependent variable: diameter

Source	**Type III sum of squares**	**df**	**Mean square**	**F**	**Sig.**
Corrected model	16.824[a]	14	1.202	2.602	.002
Intercept	3120.240	1	3120.240	6756.063	.000
Treatment	12.803	5	2.561	5.545	.000
Replicate	3.964	9	.440	.954	.479
Error	125.621	272	.462		
Total	3289.363	287			
Corrected total	142.446	286			

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Diameter

Tukey HSD[a,b,c]

Treatment	N	Subset	
		1	2
6	49	3.0633	
1	48	3.1396	
3	43	3.2105	
4	47	3.3415	3.3415
5	50	3.3860	3.3860
2	50		3.7030
Sig.		.190	.101

(5) Soil 1_repeated measures for height

Tests of between-subjects effects

Measure: height

Transformed variable: average

Source	Type III sum of squares	df	Mean square	F	Sig.	Partial eta squared
Intercept	317243282.107	1	317243282.107	2452.148	.000	.892
Error	38553341.893	298	129373.631			

Pairwise comparisons

Measure: height

(I) time	(J) time	Mean difference (I-J)	Std. error	Sig.[b]	95% confidence interval for difference[b]	
					Lower bound	Upper bound
1	2	-75.910*	2.628	.000	-83.685	-68.134
	3	-218.365*	6.458	.000	-237.473	-199.256
	4	-402.535*	10.206	.000	-432.734	-372.336
	5	-607.401*	14.821	.000	-651.256	-563.547
	6	-711.548*	16.488	.000	-760.336	-662.761
2	1	75.910*	2.628	.000	68.134	83.685
	3	-142.455*	4.919	.000	-157.009	-127.901
	4	-326.625*	8.791	.000	-352.638	-300.613
	5	-531.492*	13.841	.000	-572.447	-490.536
	6	-635.639*	15.521	.000	-681.566	-589.712
3	1	218.365*	6.458	.000	199.256	237.473
	2	142.455*	4.919	.000	127.901	157.009
	4	-184.171*	5.802	.000	-201.340	-167.002
	5	-389.037*	11.831	.000	-424.045	-354.028
	6	-493.184*	13.533	.000	-533.227	-453.140

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4	1	402.535*	10.206	.000	372.336	432.734
	2	326.625*	8.791	.000	300.613	352.638
	3	184.171*	5.802	.000	167.002	201.340
	5	-204.866*	9.217	.000	-232.139	-177.593
	6	-309.013*	11.375	.000	-342.674	-275.353
5	1	607.401*	14.821	.000	563.547	651.256
	2	531.492*	13.841	.000	490.536	572.447
	3	389.037*	11.831	.000	354.028	424.045
	4	204.866*	9.217	.000	177.593	232.139
	6	-104.147*	6.386	.000	-123.043	-85.252
6	1	711.548*	16.488	.000	662.761	760.336
	2	635.639*	15.521	.000	589.712	681.566
	3	493.184*	13.533	.000	453.140	533.227
	4	309.013*	11.375	.000	275.353	342.674
	5	104.147*	6.386	.000	85.252	123.043

(6) Soil 1_repeated measures for diameter

Tests of between-subjects effects

Measure: diameter

Transformed variable: average

Source	Type III sum of squares	df	Mean square	F	Sig.	Partial eta squared
Intercept	16290.727	1	16290.727	4696.964	.000	.940
Error	1037.037	299	3.468			

Pairwise comparisons

Measure: diameter

(I) time	(J) time	Mean difference (I-J)	Std. Error	Sig.[b]	95% confidence interval for difference[b]	
					Lower bound	Upper bound
1	2	-.899*	.023	.000	-.968	-.831
	3	-1.536*	.033	.000	-1.634	-1.438
	4	-2.346*	.044	.000	-2.475	-2.216
	5	-3.671*	.079	.000	-3.904	-3.438
	6	-4.658*	.100	.000	-4.953	-4.362
2	1	.899*	.023	.000	.831	.968
	3	-.637*	.028	.000	-.721	-.553
	4	-1.447*	.040	.000	-1.565	-1.328
	5	-2.771*	.075	.000	-2.995	-2.548
	6	-3.758*	.097	.000	-4.045	-3.472
3	1	1.536*	.033	.000	1.438	1.634
	2	.637*	.028	.000	.553	.721

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	4	-.810*	.032	.000	-.904	-.716
	5	-2.135*	.070	.000	-2.343	-1.926
	6	-3.122*	.092	.000	-3.393	-2.850
4	1	2.346*	.044	.000	2.216	2.475
	2	1.447*	.040	.000	1.328	1.565
	3	.810*	.032	.000	.716	.904
	5	-1.325*	.056	.000	-1.489	-1.160
	6	-2.312*	.079	.000	-2.547	-2.077
5	1	3.671*	.079	.000	3.438	3.904
	2	2.771*	.075	.000	2.548	2.995
	3	2.135*	.070	.000	1.926	2.343
	4	1.325*	.056	.000	1.160	1.489
	6	-.987*	.043	.000	-1.115	-.859
6	1	4.658*	.100	.000	4.362	4.953
	2	3.758*	.097	.000	3.472	4.045
	3	3.122*	.092	.000	2.850	3.393
	4	2.312*	.079	.000	2.077	2.547
	5	.987*	.043	.000	.859	1.115

(7) Soil 2_repeated measures for height

Tests of between-subjects effects

Measure: height

Transformed variable: average

Source	Type III sum of squares	df	Mean square	F	Sig.	Partial eta squared
Intercept	304986546.134	1	304986546.134	2314.556	.000	.886
Error	39398914.033	299	131768.943			

Pairwise comparisons

Measure: height

(I) time	(J) time	Mean difference (I-J)	Std. error	Sig.[b]	95% confidence interval for difference[b]	
					Lower bound	Upper bound
1	2	-121.793*	3.518	.000	-132.203	-111.383
	3	-230.913*	7.425	.000	-252.885	-208.942
	4	-424.563*	11.328	.000	-458.083	-391.044
	5	-566.427*	14.233	.000	-608.541	-524.312
	6	-665.027*	15.785	.000	-711.733	-618.320
2	1	121.793*	3.518	.000	111.383	132.203
	3	-109.120*	5.259	.000	-124.681	-93.559
	4	-302.770*	9.020	.000	-329.460	-276.080

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	5	-444.633*	12.211	.000	-480.766	-408.501
	6	-543.233*	13.959	.000	-584.537	-501.929
3	1	230.913*	7.425	.000	208.942	252.885
	2	109.120*	5.259	.000	93.559	124.681
	4	-193.650*	6.362	.000	-212.476	-174.824
	5	-335.513*	9.911	.000	-364.841	-306.186
	6	-434.113*	11.955	.000	-469.487	-398.740
4	1	424.563*	11.328	.000	391.044	458.083
	2	302.770*	9.020	.000	276.080	329.460
	3	193.650*	6.362	.000	174.824	212.476
	5	-141.863*	6.488	.000	-161.063	-122.664
	6	-240.463*	8.935	.000	-266.901	-214.026
5	1	566.427*	14.233	.000	524.312	608.541
	2	444.633*	12.211	.000	408.501	480.766
	3	335.513*	9.911	.000	306.186	364.841
	4	141.863*	6.488	.000	122.664	161.063
	6	-98.600*	5.146	.000	-113.828	-83.372
6	1	665.027*	15.785	.000	618.320	711.733
	2	543.233*	13.959	.000	501.929	584.537
	3	434.113*	11.955	.000	398.740	469.487
	4	240.463*	8.935	.000	214.026	266.901
	5	98.600*	5.146	.000	83.372	113.828

(8) Soil 2_repeated measures for diameter

Tests of between-subjects effects

Measure: diameter

Transformed variable: average

Source	Type III sum of squares	df	Mean square	F	Sig.	Partial eta squared
Intercept	16255.548	1	16255.548	5179.374	.000	.945
Error	938.416	299	3.139			

Pairwise comparisons

Measure: diameter

(I) time	(J) time	Mean difference (I-J)	Std. error	Sig.[b]	95% confidence interval for difference[b]	
					Lower bound	Upper bound
1	2	-1.013*	.024	.000	-1.086	-.941
	3	-1.988*	.034	.000	-2.089	-1.887
	4	-2.497*	.042	.000	-2.620	-2.374
	5	-3.540*	.069	.000	-3.743	-3.337
	6	-4.143*	.087	.000	-4.401	-3.885

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2	1	1.013*	.024	.000	.941	1.086
	3	-.975*	.029	.000	-1.060	-.890
	4	-1.484*	.038	.000	-1.597	-1.370
	5	-2.527*	.067	.000	-2.725	-2.329
	6	-3.130*	.086	.000	-3.386	-2.874
3	1	1.988*	.034	.000	1.887	2.089
	2	.975*	.029	.000	.890	1.060
	4	-.509*	.027	.000	-.590	-.428
	5	-1.552*	.056	.000	-1.717	-1.387
	6	-2.155*	.075	.000	-2.375	-1.934
4	1	2.497*	.042	.000	2.374	2.620
	2	1.484*	.038	.000	1.370	1.597
	3	.509*	.027	.000	.428	.590
	5	-1.043*	.046	.000	-1.178	-.908
	6	-1.646*	.067	.000	-1.844	-1.448
5	1	3.540*	.069	.000	3.337	3.743
	2	2.527*	.067	.000	2.329	2.725
	3	1.552*	.056	.000	1.387	1.717
	4	1.043*	.046	.000	.908	1.178
	6	-.603*	.037	.000	-.712	-.494
6	1	4.143*	.087	.000	3.885	4.401
	2	3.130*	.086	.000	2.874	3.386
	3	2.155*	.075	.000	1.934	2.375
	4	1.646*	.067	.000	1.448	1.844
	5	.603*	.037	.000	.494	.712

(9) Soil 1_Plant dry weight

ANOVA

S1_totalDW

	Sum of squares	df	Mean square	F	Sig.
Between groups	893.731	5	178.746	7.515	.002
Within groups	285.407	12	23.784		
Total	1179.138	17			

S1_totalDW

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	3	10.1667		
	3.00	3	15.4667	15.4667	
	5.00	3	22.3333	22.3333	22.3333
	6.00	3		24.8000	24.8000
	4.00	3		28.5000	28.5000
	2.00	3			30.0667
	Sig.		.083	.058	.425

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(10) Soil 2_Plant dry weight

ANOVA

S2_totalDW

	Sum of squares	df	Mean square	F	Sig.
Between groups	603.724	5	120.745	2.388	.101
Within groups	606.753	12	50.563		
Total	1210.478	17			

S2_totalDW

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	1.00	3	8.4333
	4.00	3	9.9667
	3.00	3	10.1333
	6.00	3	14.7333
	5.00	3	18.9000
	2.00	3	24.7667
	Sig.		.122

(11) Soil 1_Root dry weight

ANOVA

DW_Root

	Sum of squares	df	Mean square	F	Sig.
Between groups	41.263	5	8.253	2.349	.105
Within groups	42.153	12	3.513		
Total	83.416	17			

DW_Root

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	1.00	3	2.0000
	3.00	3	3.4000
	5.00	3	4.3667
	2.00	3	5.3000
	4.00	3	6.1667
	6.00	3	6.2000
	Sig.		.136

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(12) Soil 2_Root dry weight

ANOVA

DW_Root2

	Sum of squares	df	Mean square	F	Sig.
Between groups	14.267	5	2.853	2.430	.096
Within groups	14.093	12	1.174		
Total	28.360	17			

DW_Root2

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	3.00	3	1.5667
	1.00	3	2.1000
	4.00	3	2.1000
	6.00	3	2.2000
	5.00	3	3.6333
	2.00	3	4.0000
	Sig.		.135

(13) Soil 1_ Seedling Quality Index

Tests of between-subjects effects

Dependent variable: QI

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	.043[a]	5	.009	4.070	.022
Intercept	.391	1	.391	186.530	.000
Treatment	.043	5	.009	4.070	.022
Error	.025	12	.002		
Total	.458	18			
Corrected total	.068	17			

QI

	Treatment	N	Subset	
			1	2
Tukey HSD[a,b]	1.00	3	.0667	
	3.00	3	.1020	.1020
	5.00	3	.1443	.1443
	2.00	3	.1853	.1853
	4.00	3	.1877	.1877
	6.00	3		.1980
	Sig.		.061	.179

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(14) Soil 2_Seedling Quality Index

Tests of between-subjects effects

Dependent variable: QI_2

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	.019[a]	5	.004	1.822	.183
Intercept	.157	1	.157	74.635	.000
Treatment	.019	5	.004	1.822	.183
Error	.025	12	.002		
Total	.202	18			
Corrected total	.045	17			

QI_2

	Treatment	N	Subset
			1
Tukey HSD[a,b]	1.00	3	.0623
	4.00	3	.0627
	3.00	3	.0630
	6.00	3	.1060
	5.00	3	.1237
	2.00	3	.1433
	Sig.		.321

(15) Soil 1_Biovolume Index

Tests of between-subjects effects

Dependent variable: BioVol1

Source	Type III sum of squares	df	Mean square	F	Sig.	Partial eta squared
Corrected model	761921.257[a]	5	152384.251	6.098	.005	.718
Intercept	12396931.675	1	12396931.675	496.113	.000	.976
Treatment	761921.257	5	152384.251	6.098	.005	.718
Error	299857.679	12	24988.140			
Total	13458710.612	18				
Corrected total	1061778.937	17				

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BioVol1

	Treatment	N	Subset	
			1	2
Tukey HSD[a,b]	1.00	3	493.6557	
	3.00	3	639.0720	639.0720
	6.00	3	812.1533	812.1533
	5.00	3		957.5000
	4.00	3		1017.3967
	2.00	3		1059.5660
	Sig.		.208	.059

(16) Soil 2_Biovolume Index

Tests of between-subjects effects

Dependent variable: BioVol2

Source	Type III sum of squares	df	Mean square	F	Sig.	Partial eta squared
Corrected model	594536.142[a]	5	118907.228	3.000	.055	.556
Intercept	9175963.864	1	9175963.864	231.536	.000	.951
Treatment	594536.142	5	118907.228	3.000	.055	.556
Error	475569.688	12	39630.807			
Total	10246069.693	18				
Corrected total	1070105.830	17				

BioVol2

	Treatment	N	Subset	
			1	2
Tukey HSD[a,b]	1.00	3	493.2727	
	3.00	3	599.6493	599.6493
	4.00	3	611.1673	611.1673
	6.00	3	697.9503	697.9503
	5.00	3	834.7573	834.7573
	2.00	3		1047.1180
	Sig.		.348	.135

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