**Resilience capacity of limestone mine soils by adopting arbuscular mycorrhizal fungi assisted remediation in maize-cowpea intercropping system of Mawsmai, Meghalaya.**

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**Key Words**: limestone, mawsmai, meghalaya, mine spoils, phytological, plant species

**Abstract**

The present study aims to elucidate the resilience capacity of limestone mine spoils through maize-cowpea intercropping in Mawsmai village of Cherrapunjee, Meghalaya which has been witnessing open cast mining for several decades. The field experiment was conducted by following standard agronomic practices of cultivation during *kharif* season in limestone mine spoils of less than 8 years.

**Introduction**

Limestone mine spoils are physically, chemically, nutritionally and microbiologically impoverished habitats inhibiting the establishment and growth of plant species (Singh, 2012). The vegetation development and succession process are slow on mine spoils under natural conditions (Puschel *et al*., 2007). After mining it is desirable to develop vegetation on overburden dumps for the purpose of reclamation and environmental stability of the area (Roberts *et al*., 1981; Singh and Jha, 1992). One of the most important symbiotic microorganisms known as arbuscular mycorrhizal fungi are reported to reduce the detrimental effects of soil-associated plant stresses, such as lack of nutrients, organic matter, high salinity or high pH (Sylvia and Williams, 1992; Entry *et al*., 2002). The mycorrhizal symbiosis therefore is an important potential strategy for phytorestoration schemes (Dodd *et al*., 2002; Renker *et al*., 2004). In mine spoils, nitrogen is a major limiting nutrient and regular addition of fertilizer nitrogen may be required to maintain healthy growth and persistence of vegetation (Yang *et al*., 2003; Song *et al*., 2004). An alternative approach might be to introduce legumes and other nitrogen-fixing species. Intercropping maize (*Zea mays* L.) with various legumes such as cowpea(*Vigna unguiculata* L.) has been widely encouraged in sustainable agriculture because it has the potential to improve the yield significantly and allow plants to use soil N more efficiently ([Eaglesham](https://www.frontiersin.org/articles/10.3389/fpls.2015.00339/full" \l "B7) *[et al](https://www.frontiersin.org/articles/10.3389/fpls.2015.00339/full" \l "B7)*[., 1981](https://www.frontiersin.org/articles/10.3389/fpls.2015.00339/full" \l "B7); [Li *et al*., 2001](https://www.frontiersin.org/articles/10.3389/fpls.2015.00339/full#B27); [Hauggaard-Nielsen *et al*., 2009](https://www.frontiersin.org/articles/10.3389/fpls.2015.00339/full#B14); [Gao](https://www.frontiersin.org/articles/10.3389/fpls.2015.00339/full" \l "B12) *[et al](https://www.frontiersin.org/articles/10.3389/fpls.2015.00339/full" \l "B12)*[., 2014](https://www.frontiersin.org/articles/10.3389/fpls.2015.00339/full" \l "B12)). The role of AMF in restoration of disturbed soils has been the subject of interest to scientists over the decades. Though there are many success stories of ecorestoration around the world (Cunningham and Berti, 1993; Mendez and Maier, 2008; Gonzalez and Gonzalez-Chavez, 2006; Wong, 2003) and in different parts of India (Tiwary, 2001; Ghose, 2004; Maiti, 2007; Juwarkar and Jambulkar, 2008), till date no attempt is being made to reclaimed limestone mine spoils in NE region of India particularly in Meghalaya by using native AMF in combination with different inorganic fertilizers through cereal legume intercropping.

**Materials and methods:**

The field experiment was conducted by following standard agronomic practices of cultivation during *kharif* season in limestone mining area of Mawsmai, Sohra (Cherrapunjee) Meghalaya. The study area lies in the south-western part at 25°15’N latitude, 91°43’E longitude and elevation of the study site varies between 1221-1263 m above the mean sea level (Figure 1).

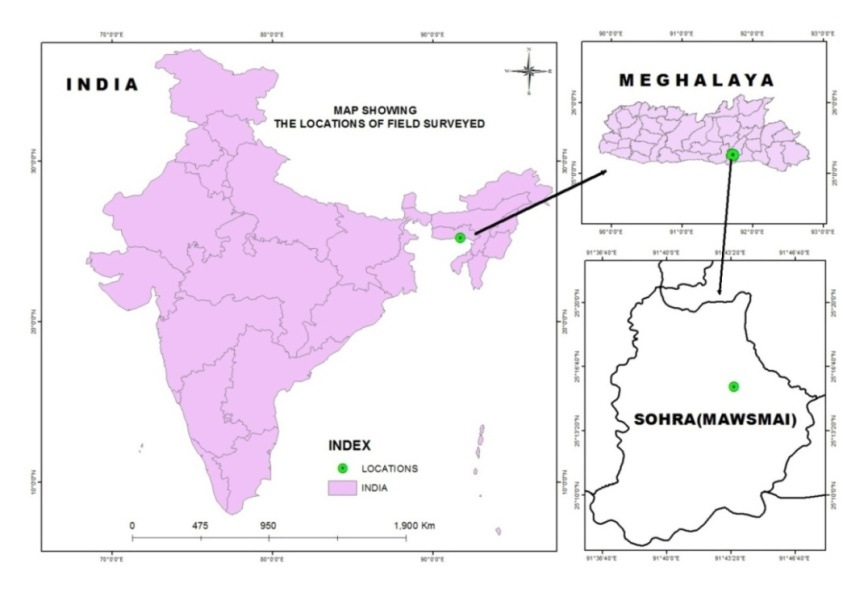


Figure 1 Map showing location of the study area

Maize (*Zea mays* L.) RCM-76 variety intercropped with cowpea (*Vigna unguiculata* L.) Khasi Kanchan variety were chosen as model crops for field trial experiment as these two agricultural crops are excellent and compatible performance crops for rejuvenation of the disturbed ecosystem. Maize plant was chosen as a symbiotic partner, because of its high mycorrhizal dependency, high germination percentage, early susceptibility to mycorrhizal colonization and abundant root production (Liu and Wang, 2003). Cowpea is also an excellent leguminous crop for nitrogen fixation, recent research has shown that the association of cereals and legumes can increase yields and improve N and P uptake via the biological fixation of N2 and chemical changes within the root zone (Betencourt *et al*., 2012; Latati *et al*., 2013). These two crops are also commonly cultivated by farmers of Mawsmai village in agricultural fields as a source of livelihood.

**Climate and weather condition during experimental period**

The village of Mawsmai, Sohra (Cherrapunjee) experienced a sub-tropical type of climate with high rainfalls and cold winters. Cherrapunjee and Mawsynram, located in the southern part receive the highest rainfall in the world with an average annual rainfall of 11,820 mm. There is a marked seasonal variation in rainfall distribution as more than 80% share of annual rainfall is precipitated in summers (May-September). The total rainfall received during the study period was 7189.20 mm. The maximum rainfall of 2053.60 mm was recorded during the month of July. The maximum and minimum temperature recorded during the cropping season ranged from 24.6°C and 18.5°C respectively. Relative humidity ranged from 78% to 95% during the experimental period (Figure 2).

Figure 2 Climatogram showing mean monthly variation of air temperature, relative humidity and rainfall of the study area during the year 2018 (Source Indian Meteorological Department)

**Land and crop history**

The experimental land was remaining fallow and no agricultural crop has been cultivated since mining activities started in the area. The configuration of the plots used for the experiment was a mid-hill bench terrace. The land was leveled before starting the field experiment. The experiment was conducted in limestone mine spoils of less than 8 years (LMS8) site considering the optimum vegetational successional stage and edaphic factors taken into consideration for early management intervention of the abandoned mine spoils for rejuvenation and restoration of the degraded ecosystem (Figure 3).

|  |  |
| --- | --- |
| F:\Gary Photos\IMG_20170708_165104.jpg  Figure 3 (a, b) Land preparation for field experimental trial with isolated native AMF species in limestone mine spoils  **a** | IMG_20170708_165111.jpg  **b** |

**Experimental details**

The experiment was carried out using Randomized Block Design (RBD) with 4 replications for each treatment. The treatment blocks were perpendicular to the slope/altitude of the experimental site. There were 44 plots with an individual size of (4 x 3) m2. The treatment layout of the experiment of the various plots and the pattern of planting of the crops in the experimental field is indicated in Figure 4. Maize intercropped with cowpea was sown in the prepared plots on the 27th April, 2018.

**N**

**W**

**S**

**E**

**MCT10**

**MCT4**

**MCT6**

**MCT7**

**MCT0**

**MCT5**

**MCT9**

**MCT8**

**MCT3**

**MCT6**

**MCT1**

**MCT10**

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**MCT8**

Figure 4 Randomized block design of the experimental site

**Table 1. Treatment with native Arbuscular Mycorrhizal Fungi (AMF) in combination with inorganic fertilizers and top soil from community protected forest**

|  |  |  |
| --- | --- | --- |
| **LEGEND** | |  |
| Maize + Cowpea | | MC |
| **Treatment** | |  |
| i. | Control (no inputs) | T0 |
| ii. | *Funneliformis mosseae* AMF inoculum | T1 |
| iii. | *Rhizophagus intraradices* AMF inoculum | T2 |
| iv. | *Septoglomus constrictum*. AMF inoculum | T3 |
| v. | *F. mosseae* + *R. intraradices+ S. constrictum* inocula | T4 |
| vi. | 50% RDF + *F. mosseae* | T5 |
| vii. | 50% RDF + *R.intraradices* | T6 |
| viii. | 50% RDF + *S. constrictum* | T7 |
| ix. | 50% RDF + *F. mosseae* + *R. intraradices* + *S. constrictum* | T8 |
| x. | Recommended dose of inorganic fertilizers (NPK) 100% | T9 |
| xi. | Top soil from community protected Forest | T10 |

**Table 2. Inputs and cultural practices followed for field experiment trial**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl. No.** | **Cultural operations** | **Crops** | **Date of operation** |
| 1 | Field preparation | Maize | 25th& 26th March, 2018 |
| Cowpea | ……..do……. |
| 2 | Field layout | Maize | 7th, April, 2018 |
| Cowpea | ……..do……. |
| 3 | FYM applications (tons ha-1) | Maize | 10 tons ha-1  12th, April, 2018 |
| Cowpea | ……..do……. |
| 4 | Fertilizer dose  (N: P2O5: K2O in kg ha-1) | Maize | 80: 60: 30 |
| Cowpea | 20: 40: 30 |
| 5 | Variety sowed | Maize | RCM-76 |
| Cowpea | Khasi kanchan |
| 6 | Date of sowing | Maize | 27th, April, 2018 |
| Cowpea | ……..do……. |
| 7 | Spacing (cm) | Maize | 50 x 20 cm r-r and p-p |
| Cowpea | 30 x 50 cm r-r and p-p |
| 8 | Date of harvesting | Maize | 23rd, Sept., 2018 |
| Cowpea | ……..do……. |

**Soil physico-chemical and biological characteristics**

Soil physico-chemical properties and biological properties *viz.,* soil pH, electrical conductivity, moisture content, bulk density, maximum water holding capacity, soil mean weight diameter, exchangeable Ca + Mg, available nitrogen, available phosphorous, organic carbon, microbial biomass carbon, total glomalin, AMF spore density and AMF root colonization were determined as per standard methods given in Table

**Table 3. Methods followed for estimation of soil quality attributes**

|  |  |  |
| --- | --- | --- |
| Sl. No. | Particulars | Method adopted |
| 1. Physical Properties | | |
| 1. | Moisture Content (%) | Gravimetric Method |
| 2. | Bulk Density (g cc-1) | Clod method (Black, 1965) |
| 3. | Maximum Water Holding Capacity (%) | K.R. Box Method (Piper, 1966) |
| 4. | Soil mean weight diameter (mm) | Yoder Wet Sieving Method (Black, 1965 and Russel, 1949). |
| 1. Chemical Properties | | |
| 5. | pH (1:2.5 soil water suspension) | Glass Electrode (Jackson, 1973) |
| 6. | EC (µS cm-1) at 25°C | Conductometry (Jackson, 1973) |
| 7. | Organic Carbon (%) | Dichromate Wet Oxidation (Walkey and Black, 1934) |
| 8. | Soil Available Nitrogen (kg ha-1) | Alkaline Permanganate (Subbiah and Asija, 1956) |
| 9. | Soil Available Phosphorous (kg ha-1) | Olsen’s Method (Olsen *et al*., 1954) |
| 10. | Soil Available Potassium (kg ha-1) | Flame Photometry Method (Hanway and Heidel, 1952). |
| 11. | Exchangeable Ca+Mg  (meq 100-1 g soil) | EDTA Titration (Black, 1965). |
| 1. Biological properties | | |
| 12. | Microbial Biomass Carbon (μg g-1) | Chloroform Fumigation Extraction Method (Brookes and Joergensen, 2006). |
| 13.. | Total Glomalin Related Soil Protein (mg g-1) | Bradford Method (Wright and Upadhyaya, 1998) |
| 1. Arbuscular mycorrhizal profile | | |
| 14. | AMF spore density (100 g soil) |  |
| 15. | AMF root colonization (%) |  |

**Statistical analysis**

The data was analysed by one-way analysis of variance (ANOVA) incorporating Duncan’s multiple range test (DMRT) at 5% level of significance for pair wise comparison between means. Statistical analysis was performed using SPSS v21 (IBM, SPSS, 2012).

**Results and Discussion**

**Soil moisture content**

The mine spoiled soils had averaged soil moisture content (MC) of 6.20%, however on cultivating maize-cowpea (T0) for single season, soil MC increased by 1.74%. On replacement of mine spoiled soil by top soil of community protected forest (T10) soil MC further increased drastically by another 21.23%. We also observed an increased (10.14 to 2.06%) in soil MC of mine spoiled soils by other management interventions like adopting inorganic fertilizers (NPK @100 RDF) (T9) and 50% RDF + *F. mosseae* + *R. intraradices* + *S. constrictum* (T8) (Figure 7.5.). The pair wise comparison between treatments mean for soil MC was statistically non significant (*p* > 0.05) irrespective of all treatments (Table 7.4.).

**Soil bulk density**

The mine spoiled soils had averaged soil bulk density (BD) of 1.10g cc-1, however on cultivating maize-cowpea (T0) for single season, soil BD decreased by 0.91%. On addition of inorganic fertilizers (NPK @100 RDF) (T9) and replacement of mine spoiled soil by top soil of community protected forest (T10) soil BD further decreased by 8.25% respectively. We also observed a reduction (5.50 to 1.83%) in soil BD of mine spoiled soils by other management interventions (Figure 7.6.). The pair wise comparison between treatments mean for soil BD was statistically non significant (*p* > 0.05) irrespective of all treatments (Table 7.4).

**Soil water holding capacity**

The mine spoiled soils had averaged soil water holding capacity (WHC) of 40.54%, however on cultivating maize-cowpea (T0) for single season, soil WHC increased by 2.68%. On replacement of mine spoiled soil by top soil of community protected forest (T10) soil WHC further increased by 20.85%. We also observed an increased (17.09 to 3.57%) in soil WHC of mine spoiled soils by other management interventions (Figure 7.7.). The pair wise comparison between treatments mean for soil WHC was statistically non significant (*p* > 0.05) irrespective of all treatments (Table 7.4.).

**Soil mean weight diameter**

The mine spoiled soils had averaged soil mean weight diameter (MWD) of 2.15 mm, however on cultivating maize-cowpea (T0) for single season, soil MWD increased by 1.82%. On addition of inorganic fertilizers (NPK @100 RDF) (T9) soil MWD further increased by 5.93%. We also observed an increased (5.47 to 2.28%) in soil MWD of mine spoiled soils by other management interventions (Figure 7.8.). The pair wise comparison between treatments mean for soil MWD was statistically non-significant (*p* > 0.05) irrespective of all treatments (Table 7.4.).

**Soil pH**

The mine spoiled soils had averaged soil pH of 8.01, however on cultivating maize-cowpea (T0) for single season, soil pH decreased by 0.25 units. On addition of inorganic fertilizers (NPK @100 RDF) in maize-cowpea system (T9) soil pH further reduced drastically by another 0.75 unit. We also observed a reduction (0.56 to 0.36 unit) in soil pH of mine spoiled soils by other management interventions (Figure 7.3.). The pair wise comparison between treatments mean for soil pH was statistically significant (*p* < 0.05) in T9 and T5 (Table 7.4.). The observations substantiated the findings of Magdof *et al*. (1997), Barak *et al*. (1997), Eghball (2002) and Guo *et al*. (2010) who reported that NPK fertilizer treatment caused soil acidification because urea used by plants as NH4+ and H+ is released into the soil which thereby decreased the soil pH.

**Soil electrical conductivity**

The mine spoiled soils had averaged soil electrical conductivity (EC) of 438 µS/cm, however on cultivating maize-cowpea (T0) for single season, soil EC decreased by 11.13%. On addition of inorganic fertilizers (NPK @100 RDF) in maize-cowpea system (T9) soil EC further significantly reduced by another 35.32%. We also observed a reduction (34.04 to 12.13%) in soil EC of mine spoiled soils by other management interventions (Figure 7.4.). The pair wise comparison between treatments mean for soil EC was statistically significant (*p* < 0.05) in T9, T6 and T3 (Table 7.4.). The decreased in soil EC may be attributed to the improvement of the soil physical conditions brought about by the microbial activity and root growth that stabilizes soil aggregates and created channels for downward movement of water and hence leaching of salts which is in agreement with the findings of Abdalla (1989).

**Soil organic carbon**

The mine spoiled soils had averaged soil organic carbon (SOC) of 0.35%, however on cultivating maize-cowpea (T0) for single season, SOC increased by 5.40%. On replacement of mine spoiled soil by top soil of community protected forest (T10), it increased significantly by 62.16%. We also observed an increased (56.75 to 37.83%) in SOC of mine spoiled soils by other management interventions (Figure 7.13.). The pair wise comparison between treatments mean for SOC was statistically significant (*p* < 0.05) only in T10 (Table 7.5.). The increased in SOC may be attributed due to application of forest organic matter which can improve soil aggregation, soil water retention, reduce bulk density, promoting crop growth and return more root residues to the soil (Hyvonen *et al*., 2008).

**Soil available nitrogen**

The mine spoiled soils had average soil available nitrogen (Av-N) of 214.35kg ha-1, however on cultivating maize-cowpea (T0) for single season, soil Av-N increased by 6.07%. On addition of inorganic fertilizers (NPK @100 RDF) (T9) it further significantly increased by 47.05%. We also observed significant increased (40.36 to 10.46 %) by other management interventions (Figure 7.10.). The pair wise comparison between treatments mean for soil Av-N was statistically significant (*p* < 0.05) in T9, T10 and T8 (Table 7.4). The increased in soil Av-N can be adduced to the fixation of atmospheric nitrogen by rhizobium bacteria in root nodules of cowpea, mineralization of N from organic residues and also the residual effect of applied chemical fertilizer (NPK) to respective crops based on recommendation dose. Same results were also reported by Padhi and Panigrahi (2006), Dahmardeh *et al*.(2010), Girijesh *et al*.(2015) and Kaushal *et al*.(2015).

**Soil available phosphorous**

The mine spoiled soils had averaged soil available phosphorous (Av-P) of 7.97 kg ha-1, however on cultivating maize-cowpea (T0) for single season, soil Av-P increased by 7.21%. On addition of inorganic fertilizers (NPK @100 RDF) (T9) it further increased significantly by 61.23%. We also observed an increased (56.11 to 18.50%) in soil Av-P of mine spoiled soils by other management interventions (Figure 7.11.). The pair wise comparison between treatments mean for soil Av-P was statistically significant (*p* < 0.05) in T9 and T10 (Table 7.4.). The increased in Av-P in the rhizosphere of intercropped maize cowpea can be attributed to the acidification of the legume rhizosphere in P deficient soils which could contribute to the increased in P availability. The observations corroborated the findings of Alkama *et al*. (2009, 2012) and Betencourt *et al*. (2012). Significant increase in available P content was also observed with the application of fertiliser in combination with FYM (Das *et al*., 1991).

**Soil available potassium**

The mine spoiled soils had averaged soil available potassium (Av-K) of 118.65 kg ha-1, however on cultivating maize-cowpea (T0) for single season, soil Av-K increased by 6.02%. On addition of inorganic fertilizers (NPK @100 RDF) (T9) it further increased significantly by 37.09%. We also observed an increased (35.02 to 12.90%) in soil Av-K of mine spoiled soils by other management interventions like replacement of mine spoiled soil by top soil of community protected forest (T10) and adoption of 50% RDF + *F. mosseae* + *R. intraradices* + *S. constrictum* (T8) (Figure 7.12.). The pair wise comparison between treatments mean for soil Av-K was statistically significant (*p* < 0.05) in T9 and T10 (Table 7.4). The buildup of Av-K in soil was due to the beneficial effect of fertilizers and organic content in forest soil in releasing potassium due to the interaction of organic matter with clay and direct addition of potassium to the available pool of soil. Similar beneficial effects of organic manures on Av-K content of soil were reported by Mishra *et al*. (1993) and Mathan *et al*. (1994).

**Soil exchangeable Ca2+ + Mg2+**

The mine spoiled soils had averaged soil ex. Ca2+ + Mg2+ of 11.20 meq 100-1 g, however on cultivating maize-cowpea (T0) for single season, soil ex. Ca2+ + Mg2+reduced by 6.67%. On addition of inorganic fertilizers (NPK @100 RDF) (T9), it further reduced significantly by 22.76%. We also observed a reduction (20.09 to 14.57%) in soil ex. Ca2++ Mg2+ of mine spoiled soils by by other management interventions like replacement of mine spoiled soil by top soil of community protected forest (T10) and 50% RDF + *F. mosseae* + *R. intraradices* + *S. constrictum* (T8) (Figure 7.9). The pair wise comparison between treatments mean for soil ex. Ca2+ + Mg2+ was statistically significant (*p* < 0.05) only in T9 (Table 7.4.). The decrease in exchangeable bases is attributed to the soil acidification because of addition of fertilizers and top soil. As pH increases, the supply of available calcium and magnesium increases. The observation corroborated the findings of Bationo *et al*. (2007) who observed a significant decrease in exchangeable bases due to increase in soil acidification.

**Soil microbial biomass carbon**

The mine spoiled soils had averaged soil microbial biomass carbon (SMBC) of 150.50 µg g-1, however on cultivating maize-cowpea (T0) for single season, SMBC increased by 7.83%. On replacement of mine spoiled soil by top soil of community protected forest (T10) SMBC further increased significantly by 23.22%. We also observed an increased (18.79 to 9.97%) in SMBC of mine spoiled soils by other management interventions like adopting inorganic fertilizers (NPK @100 RDF) (T9) and *F. mosseae* + *R. intraradices* + *S. constrictum* inocula (T4) (Figure 7.14.). The pair wise comparison between treatments mean for SMBC was statistically significant (*p* < 0.05) only in T10 (Table 7.5.).The increased in SMBC is attributed to the activation of microorganisms through carbon source inputs from the forest soil which is abundant in organic residues and subsequently increases the soil organic matter which provides substrates for the microorganisms. The observation is in tune with the findings of Marschner *et al*. (2003), Logah (2009) and Chakraborty *et al*. (2011).

**Total glomalin related soil protein**

The mine spoiled soils had averaged total glomalin related soil protein (TGRSP) of 2.03 mg g-1, however on cultivating maize-cowpea (T0) for single season, TGRSP increased by 3.79%. On replacement of mine spoiled soil by top soil of community protected forest (T10) TGRSP further increased significantly by 27.48%. We also observed an increased by (21.32 to 9.47%) in TGRSP of mine spoiled soils by other management interventions like adopting *F. mosseae* + *R. intraradices* + *S. constrictum* inocula (T4) and 50% RDF + *F. mosseae* + *R. intraradices* + *S. constrictum* (T8) (Figure 7.15.). The pair wise comparison between treatments mean for TGRSP was statistically significant (*p* < 0.05) in T10 and T4 (Table 7.5.). This can be attributed due to the increased in organic matter content in the soil, furthermore, bacterial communities that promote spore germination and increase the rate of AMF colonization (Johansson *et al*., 2004) are favoured by the addition of organic fertilizers (Crecchio *et al*., 2001). The improvement in soil structure also contributes to AMF mycelia development because it reduces the mechanical resistance to hyphal growth.

**AMF spore density**

In maize cowpea intercropping condition (T0) the mine spoiled soils had averaged AMF spore density of 210.75 spores per 100 g soil, however on replacement of mine spoiled soil by top soil of community protected forest (T10), AMF spore density significantly increased by 40.77%. We also observed an increased by (21.58 to 9.40%) in AMF spore density of mine spoiled soils by other management interventions (Figure 7.16.). The pair wise comparison between treatments mean for AMF spore density was statistically significant (*p* < 0.05) only in T10 (Table 7.5.). This could be attributed to the influence of nutrients concentration on AMF symbiosis (Martin *et al*., 2002; Smith and Read, 2008; Talbot *et al*., 2008; Cheeke *et al*., 2011; Johnson *et al*., 2003).

**AMF root infection**

In maize cowpea intercropping condition (T0) the mine spoils had averaged AMF root infection of 60.59%, however on replacement of mine spoiled soil by top soil of community protected forest (T10) AMF root infection increased significantly by 49.78%. We also observed an increased by (41.11 to 15.33%) in AMF root infection of mine spoiled soils by other management interventions (Figure 7.17.). The pair wise comparison between treatments mean for AMF root infection was statistically significant (*p* < 0.05) only in T10 (Table 7.5.). Organic materials which are present in the forest soil increased the amount of carbon, water retention capacity and fertility status of the soil. Therefore, there are more AMF communities in soils containing organic inputs which can lead to higher percentage of mycorrhizal root colonization. The observations corroborated the findings of Arihara and Karasawa (2000) and Mariela *et al*. (2016) on AMF formation and growth of maize.

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| Fig. | |

**Table Analysis of variance (ANOVA) on the effect of maize-cowpea intercropping system on soil quality attributes under different treatments**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatments** | **Moisture Content**  **(%)** | **Bulk Density**  **(gcc-1)** | **Water Holding Capacity (%)** | **Soil mean weight diameter (mm)** | **Soil pH** |
| To | 6.31±0.19a | 1.09±0.01a | 41.66±0.41a | 2.19±0.04a | 7.76 ±0.01g |
| T1 | 6.60±0.15a | 1.06±0.01a | 43.15±0.51ab | 2.26±0.06a | 7.30±0.03d |
| T2 | 6.50±0.18a | 1.04±0.04a | 43.32±0.96abc | 2.27±0.04a | 7.32±0.01d |
| T3 | 6.45±0.14a | 1.05±0.07a | 43.60±0.74bc | 2.24±0.03a | 7.37±0.01e |
| T4 | 6.44±0.24a | 1.05±0.04a | 43.34±0.83abc | 2.28±0.06a | 7.36±0.03e |
| T5 | 6.63±0.43a | 1.07±0.07a | 44.45±0.53cd | 2.25±0.03a | 7.40±0.03f |
| T6 | 6.64±0.34a | 1.07±0.08a | 45.98±0.74d | 2.27±0.02a | 7.26±0.03c |
| T7 | 6.79±0.27ab | 1.05±0.07a | 45.55±0.42d | 2.25±0.02a | 7.29±0.01d |
| T8 | 6.93±0.34ab | 1.03±0.04a | 47.99±0.35e | 2.27±0.02a | 7.20±0.03b |
| T9 | 6.95±0.47ab | 1.00±0.02a | 48.78±0.31ef | 2.32±0.01a | 7.01±0.03a |
| T10 | 7.65±0.26b | 1.00±0.02a | 50.35±0.32f | 2.31±0.03a | 7.24±0.01bc |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatments** | **Electrical Conductivity (µS/cm)** | **Organic Carbon (%)** | **Av. N**  **(kgha-1)** | **Av. P**  **(kgha-1)** | **Av. K**  **(kgha-1)** |
| To | 389.25±1.31h | 0.37±0.01a | 228.22±3.42a | 8.59±0.03a | 126.26±1.28a |
| T1 | 270.25±3.20c | 0.53±0.02b | 261.75±0.34b | 10.30±0.45b | 142.56±0.89b |
| T2 | 318.50±2.99d | 0.57±0.01bc | 258.40±0.67b | 10.38±0.08b | 142.89±0.90b |
| T3 | 328.25±3.33e | 0.51±0.02b | 252.10±0.46b | 10.36±0.10b | 143.56±1.45b |
| T4 | 342.00±3.58g | 0.58±0.04bc | 260.55±0.48b | 10.18±0.05b | 150.35±1.29c |
| T5 | 338.75±5.25g | 0.51±0.01b | 285.75±0.72c | 12.29±0.31c | 153.65±1.40cd |
| T6 | 335.75±4.25f | 0.51±0.01b | 290.40±0.49c | 12.49±0.08c | 158.01±1.82d |
| T7 | 320.75±1.25d | 0.53±0.04b | 295.20±0.70c | 13.05±0.14d | 161.71±3.05de |
| T8 | 256.75±3.99b | 0.57±0.04bc | 314.50±0.52d | 13.41±0.10d | 168.77±1.93e |
| T9 | 251.75±2.84a | 0.55±0.01b | 335.60±0.52f | 13.85±0.33f | 173.09±1.15g |
| T10 | 265.25±2.63bc | 0.60±0.02d | 320.33±0.46e | 13.35±0.08e | 170.48±0.64f |

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| **Treatments** | **Ex. Ca+ Mg**  **(meq100g-1)** | **Microbial biomass carbon**  **(μg g-1)** | **Total Glomalin**  **(mg g-1)** | **AMF Spores**  **(100 g soil)** | **Root Infection**  **(%)** |
| To | 10.50±0.34d | 163.30±1.02a | 2.11±0.04a | 220.75±2.17a | 60.59±1.32a |
| T1 | 8.85±0.08bc | 179.59±1.62b | 2.31±0.04ab | 243.75±2.32b | 73.25±1.89c |
| T2 | 8.82±0.11bc | 183.70±0.84cd | 2.35±0.05bc | 252.50±2.96c | 69.88±0.82b |
| T3 | 8.77±0.21bc | 180.29±0.79bc | 2.31±0.02ab | 241.50±2.63b | 72.50±1.27bc |
| sT4 | 8.89±0.08bc | 192.42±1.43e | 2.56±0.08d | 268.40±1.75e | 85.50±1.88e |
| T5 | 8.97±0.02c | 181.46±0.41bcd | 2.35±0.04bc | 260.75±3.47d | 81.00±1.75d |
| T6 | 8.72±0.20bc | 182.69±0.95bcd | 2.31±0.06ab | 265.40±2.84e | 84.25±2.39e |
| T7 | 8.66±0.28d | 182.08±0.76bcd | 2.34±0.05b | 260.70±2.87d | 83.00±1.39e |
| T8 | 8.47±0.17bc | 183.82±1.69d | 2.39±0.06bc | 257.75±3.35cd | 80.75±1.76d |
| T9 | 8.11±0.08a | 193.99±0.74e | 2.36±0.03bc | 250.50±2.60c | 78.23±0.79cd |
| T10 | 8.36±0.09b | 201.23±0.86f | 2.69±0.17e | 310.75±3.07f | 90.75±1.75f |

Values are mean for four replicates ± standard error mean; within a column values that differed significantly are followed by different letters as determined by One-way ANOVA incorporating Duncan’s Multiple Range Test (DMRT at *p* < 0.05) for pair-wise comparison between means. \*Significant at *p* < 0.05, ns: Non-significant at *p* > 0.05

|  |  |
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| F:\FRI-Final Submission\Plates\New folder\IMG_20190604_132018.jpg  **Plate 7.3.** (a-b) Luxuriant growth of maize plants through management interventions in limestone mine spoils | F:\FRI-Final Submission\Plates\New folder\IMG_20190604_132049.jpg |
| F:\AMF Photographs-final\Gary Suting (Colonization Pictures)\Treatments\Maize\T4-M-Ves-011.jpg | F:\AMF Photographs-final\Gary Suting (Colonization Pictures)\Treatments\Maize\T9-M-Ar-006.jpg |
| F:\AMF Photographs-final\Gary Suting (Colonization Pictures)\Treatments\Maize\T10-M-Hy-009.jpg  **Plate 7.5.** (a-f) AMF root colonization in maize (*Zea mays* L.) under different treatments (a) Vesicles colonization in 50 % RDF + *S. constrictum* treatment (T7) (b) Arbuscles colonization in50% RDF + *F. mosseae* + *R. intraradices* + *S. constrictum* treatment (T8) (c) Hyphae colonization inorganic fertilizers (NPK) 100% treatment (T9) (d) Arbuscles colonization from top soil of community protected forest treatment (T10) (e-f) Hyphae colonization in control, no inputs (T0) **Scale bars:** a- f = 50µm  **Scale bars:** a- f = 50µm | F:\AMF Photographs-final\Gary Suting (Colonization Pictures)\Treatments\Maize\T8M-Ar-003.jpg |

**Conclusion**

It was observed and experimentally tested that replacement of mined spoiled soil by top soil from adjacent un-mined community protected forest followed by addition of inorganic fertilizers (NPK@100 RDF) and adoption of 50 % RDF + *F. mosseae* + *R. intraradices* + *S. constrictum* were found to be the most suitable management interventions for rehabilitation and rejuvenation of the fragile mining ecosystem. The reclamation strategy can be applied for the successful restoration of limestone mine for faster recovery of plant species diversity and microbial populations.

**Acknowledgement**

The authors would like to thank Botanical Survey of India, Eastern Regional Centre, Shillong and Centre for Advanced Studies in Botany, North Eastern Hill University, Shillong for taxonomic identification of the plant species.

**References**

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