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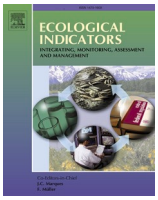

Inoculation and amendment strategies influence switchgrass establishment in degraded soil

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ABSTRACT

Bioenergy feedstock production on degraded land can serve as a means for modulating land competition for food versus energy. Due to little or no agricultural value of degraded soil, fortification of the soil with an organic amendment or inoculum will improve biomass productivity. However, as farmers struggle to rejuvenate their degraded land, there is a need for a quick screening strategy to select the best method of enhancing cellulosic (switchgrass, SG) biomass production in degraded soil. The goal of this study is to evaluate the effects of soil amendment and inoculation strategies on biomass productivities of SG in a reclaimed surface-mined soil (RMS). Experiments were conducted in the greenhouse using moisture replacement microcosms (MRM) to screen strategies for enhancing biomass productivities of SG in a RMS. Strategies included soil amendment with organic by-products (poultry litter, paper mill sludge, and vermicompost), inorganic nutrients (nitrogen and phosphorus fertilizers), or a commercial preparation of endomycorrhizae fungi (AMF, BioVam). Experiments were implemented with ten (10) treatments with six replicates for each treatment. After eight weeks of incubation in MRM systems, inoculation of RMS with AMF produced the highest aboveground and total biomass (0.9 g and 1.77 g per microcosm container) at $p < 0.05$. The total biomass of commercial AMF significantly ($p < 0.05$) outperformed all other treatments in the order of AMF > AMF + VC > PMS + N > VC = PMS = PL > PMS + AMF > N + P > ASL > Control. This microcosm screening experiment served as a quick screening to establish that soil enhancement and inoculation strategies can enhance biomass productivities of SG in degraded soil.

1. Introduction

A growing concern on how to reduce pollution and make our environment safer brought great attention to biofuel production. Biofuel, an environmental friendlier alternative to fossil fuel, has the ability to reduce carbon dioxide (CO_2) and greenhouse gas (GHG) emission (Qin et al., 2011; Skevas et al., 2014). Bioenergy biomass production on degraded land has been suggested to free arable land for the production of food, fodder, and fiber (Lehmann and Rillig, 2015). It is vital to improve grassland cropping systems on degraded land by using conservative agricultural practices to reduce agricultural runoff of soil and nutrients, increase carbon sequestration (Mbuthia et al., 2015; Mehmood et al., 2016), and improve potential to rejuvenate the degraded land.

Degraded land often results from soil contamination or deficiencies in plant nutrition (Lehmann & Rillig, 2015). To overcome this challenge preharvest soil fertilization is often carried out to enhance whole plant tissues for increased biomass yield (Sikström, 2001). The addition of fertilizer has been reported to effect changes in soil quality (Simmons & Coleman, 2008). An integral part of soil quality that is affected by fertilization includes carbon (C), nitrogen (N), microbial biomass and community of the soil (Acosta-Martinez et al., 2011; Acosta-Martínez, Zobeck, & Allen, 2004). Soil management practices, therefore, become critical since the application of fertilizer effect changes in structural and biochemical characteristics of soil microbial community (Al-Kaisi et al., 2005; Mbuthia et al., 2015).

An alternative approach to inorganic fertilizer is the application of beneficial or symbiotic soil microbes (especially arbuscular mycorrhizal fungi, AMF) that can form an integral section of the root to improve soil quality and enhance plant health. AMF are ubiquitous symbionts forming arbuscular mycorrhizas in the roots of most land plants. AMF can enhance biomass production of perennial grasses in arable soil (Adeleke & Dzantor, 2017). In addition, inoculation of consortium of plant growth promoting microbes will increase microbial diversity and high microbial diversity have been reported to enhance soil ecosystem functioning (Maron et al., 2018), although this is still debated (Chapin

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et al., 1992; Cox et al., 2001; Setälä & McLean, 2004) as enormous diversity as a result of compositional shift may result in functional redundancy or equivalence that may not affect the ecosystem processes (Allison & Martiny, 2008). Although functional redundancy is difficult to achieve it may result from two situations, the first, when the taxa that makes up a newly derived community performs the similar function to the previous taxa in the old community and the second is when the new taxa may have different function but the effect of the function is the same when profiled at community level (Allison et al., 2014; Allison & Martiny, 2008). The increase in carbon source can reduce functional redundancy through combination of soil diversity with change in carbon cycling. It has been suggested that functional redundancy may be reduced with increasing carbon source and that coupling of soil diversity with carbon cycling may change consequently (Maron et al., 2018).

Another alternative approach to improve soil fertility is by addition of allochthonous nutrients (referred to organic material that are not naturally present but anthropogenically added to the soil) with high carbon source. The application of organic residues has been well reported to improve soil quality (Acosta-Martinez et al., 2011; Li et al., 2015; Moland et al., 2018) while the microbial communities undergo rapid shift in response to the modification of environmental conditions (Tardy et al., 2015). Although the initial addition of high carbon sources may favorably increase the dominance of the copiotrophs over other populations, this dominance is later offset by resilience of oligotrophs and fungi (Tardy et al., 2015). The addition of organic allochthonous nutrients to degraded soil may therefore initiate a tripartite association between the organic amendment, plant and the stress-tolerant microbe in the soil (Vimal et al., 2017). The influence of organic nutrient on aboveground biomass enhancement in degraded soil with *in situ* spatial response of root system is not well documented. In this study we have decided to select switchgrass (SG) (*Pannicum virgatum* L.) as our crop of interest because of its importance as a model bioenergy crop.

SG is a native warm season grass that has been designated as a model cellulosic bioenergy crop by the US (McLaughlin & Adams Kszos, 2005; Mitchell et al., 2016; Sanderson et al., 2006). SG is a herbaceous perennial species that forms extensive root systems in its rhizomes-that possess the nutrient storage potential at the end of the growing season. The emergence and early growth of shoot at the onset of a new growing (spring) season (Youngs and Somerville, 2012) is supported by the nutrient stored in the rhizome from previous season. This also allows SG to make only little investments into root biomass once the crop is successfully established unlike in annual crops. SG has two types of roots, the seminal roots that emerges directly from the seed embryo to form deep fibrous roots and the nodal roots that emerges from the lower tiller nodes to form rhizomatous roots. The depth of SG root can be 3.05 m deep but majority of the root in is in the top 12 in. region of the soil profile (Mitchell et al., 2016). Most SG will be grown on degraded soil to avoid competition with food crops for arable land (Jiang et al., 2012; Liu et al., 2015). Therefore, it is imperative to evaluate on a case by case study the establishment of SG root in the degraded soil. In addition to the role of SG root in establishment, the root also play important role in plant-microbe association (Wagg et al., 2014). Association of plant growth microbe with the is crucial for plants survival in contaminated soil (Khan, 2006; Tahat & Sijam, 2012; Yang et al., 2015, 2016; Lenoir et al., 2016; Wang, 2017). Therefore, assessment of root development of SG is critical for plant establishment. The stress tolerant adaptive trait related to root physiology and morphology have been reported in SG (Barney et al., 2009; Meyer et al., 2014). Although, phenotyping root systems is integral to the discovery of genes responsible for root system architecture (RSA) traits, the constraints of studying root in soil and on the field remains germane (de Dorlodot et al., 2007; Ingram et al., 2012; Zhu et al., 2011). RSA can be defined as the spatial and temporal arrangement of the entire root system in the soil (Chen et al., 2018; Kochian, 2016; Zhu et al., 2011). Since, there is a large knowledge gap in SG RSA in degraded soil, we evaluated its root morphology and how it responds to soil amendment and inoculation strategies.

Therefore, our objective is to evaluate the effects of soil amendment and inoculation strategies on biomass productivities of SG in a reclaimed surface-mined soil (RMS). We achieved this by conducting a greenhouse experiment where we used a moisture replacement microcosm (MRM) to screen strategies for enhancing biomass productivities of SG in a RMS.

2. Materials and methods

2.1. Soil

RMS site was identified with the assistance of Tennessee Natural Resource Conservation Service (NRCS) in Eastern Tennessee (Latitude 35°59′32.6″N Longitude 84°39′52.2″W). RMS is a reclaimed site where surface mining was historical conducted until the late 70′s. The mining was stopped in 1978 and thereafter, the land was reclaimed by reconstruction of the top layer that was stripped off during mining. During growing season, the RMS site has a very sparse population of grasses that been majorly dominated by *Andropogon virginicus* (broomsedge). RMS demonstrated low biomass production (less than 0.5 kg per hectare) compared to arable land. Bulk soils were collected at the beginning of the growing season (April 2016) at 0-12 in. deep from the RMS site as the degraded soil. Specific permission was not required to collect the soil sample since the landowner and NRCS collaborated with this study and transportation of the soil samples was within the state. Armour silt loam (ASL), was collected as an arable soil comparison to degraded soil from Tennessee State University (TSU) Agricultural Research and Education Center, Nashville, TN (Latitude 36°10′42.9″N Longitude 86°49′32.1″W).

Plant material Seeds of 'Alamo' variety of SG, a lowland ecotype, were obtained from Star Seed Inc., Osborne, KS.

2.2. Commercial mycorrhiza

Bag (11.25lbs) of commercial mycorrhiza - BioVam (containing Endomycorrhizae - approx. 80 spores g^{-1}, Ectomycorrhizae - approx. 100 spores g^{-1}, Bacteria: *Arthrobacter globiformis*, *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Bacillus subtilis*, *Pseudomonas alcaligenes*, *Pseudomonas fluorescens*, *Pseudomonas pseudoalcaligenes* and *Pseudomonas putida* with estimated minimum viable cells of 20,000 g^{-1}, and other Fungi: *Trichoderma harzianum* and *Trichoderma koningii* with estimated minimum cells of 10,000 g^{-1}) was obtained from T&J Enterprises, Spokane, WA.

Vermicompost (VC) served as soil amendment and was purchased from Worm Power, Avon, NY. Pelletized poultry litter (PL) was purchased from natural organic warehouse (NOW), Andover, KS. N-P-K analysis of the product was reported as 2–4-2. Paper mill sludge (PMS) was obtained from resolute forest products, Catawba, SC by gratis.

Urea, N (46 0 0) and Phosphorus P (0 45 0) were purchased from Tennessee Farmers' Cooperative, LaVergne, TN.

2.3. Preparation of soil samples

Bulk soils were sieved through 2 mm (#10) soil test sieve. The sieved soils were kept in the greenhouse at 12 °C and stored up in linear low-density polyethylene bag that is then placed in 5 US gallon bucket with lid cover to avoid drying or significant moisture loss. The gravimetric soil water content measured using HB43 halogen moisture analyzer (Mettler Toledo, Columbus, OH) was averagely 15.8% during storage.

2.4. Plant incubation in moisture replacement microcosm system

Seeds of SG were germinated in AMF-free nursery potting substrate within germination trays. Two seedlings of SG at three-leaf stage (approx. 4 weeks old) were transplanted into 50 ml conical tubes containing appropriate soil treatments and incubated in MRM system as described by Le et al. (2011). Briefly, MRM system is a unit made up of

expanded polystyrene (EPS) foam boxes (Fig. 1) with 62.5 × 30 × 20 cm dimension that had been divided into two-halves (upper and lower compartment). The upper compartment had 36 holes drilled into it with approximately 3.5 cm × 2.5 cm separating each hole from one another. Each hole had 50 ml conical centrifuge tubes (ID: 27.7 mm × OD: 29.1 mm × Length: 114.4 mm × Cap Diam.: 35.2 mm; Corning, Inc, Corning, NY) that held soil inserted into it with a black O-ring (ID:33 mm × OD:40 mm × TH:3.5 mm) hole sealing gasket washer fitted snugly on the conical tubes as a support. Insulated wicks (assembled from 3 mm × 150 mm replacement oil lamp wick, Amazon Inc, Bellevue, WA and ID:3mm × OD:4.8 mm black nylon tubing, Grainger, Lake Forest, IL) were inserted into the bottom end of the conical tubes (with hole of ~ 5 mm Diam.) such that it extended into one-third of the tubes and the remaining two-third extended into the reservoir. The lower compartment of the MRM system served as the reservoir which held approximately 9 L of distilled water. The wick served as a conduit that

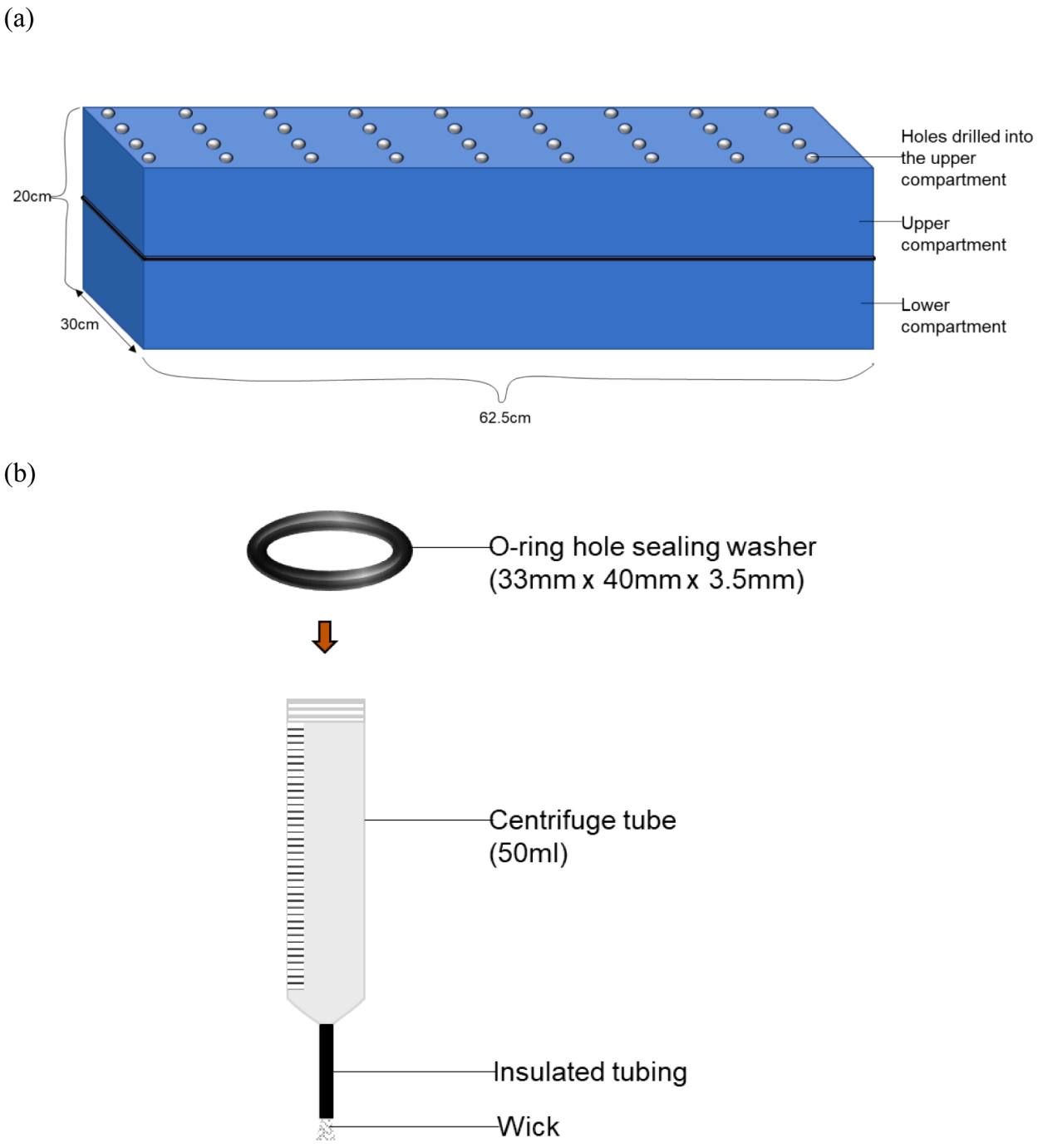

Fig. 1. Moisture replacement microcosm (MRM) system. (a) Illustrates the EPS box with upper and lower compartments. The upper compartment which is about 10 cm in height fits tightly on the lower compartment. The upper compartment also had 36 holes (with each hole with 3.5 cm diameter with approximately 3.5 cm × 2.5 cm spacing) drilled into it. The lower compartment (10 cm high) is the section of the EPS box that serves as the reservoir for the distilled water. (b) Illustrates the assembling of the conical centrifuge tube that is inserted into the hole of the EPS foam boxes. The centrifuge tube is inserted into the O-ring which fits snuggly on it and the wick is inserted into the bottom of the centrifuge tube up to a third of the section. After labeling and filling the centrifuge tubes with appropriate soil treatment, each assembled tube was inserted into the hole of the upper compartment and the remaining two-third of the wick extended into the water reservoir of the EPS foam box.

supplied distilled water via hydraulic gradient to the soil held in the conical tubes thereby creating an average soil moisture content of approximately 70% during plant incubation. The lower compartment is also laminated with a black plastic tarp to prevent leaching from the EPS foam boxes. The level of the water was checked and maintained at the same level (9 L) by topping the distilled water every 2 days as needed and refilling up to half the entire content every two weeks. This MRM system allowed economical amount of resource – degraded soil and water to be screened for biomass enhancement using amendment and inoculation strategies, thereby determining the right estimation of amendment or inoculum needed for rejuvenation of degraded soil.

2.5. Optimization of commercial mycorrhiza inoculum experiment

The optimum inoculum of the commercial mycorrhiza (AMF) used was determined prior to inoculation in MRM system. Four different inoculum ratios of commercial AMF 0, 3, 10, and 15% of total volume of ASL soil (50 ml ods) in the centrifuge tube was examined. Summarily, *Sorghum bicolor* seeds were surface sterilized by washing seeds with distilled water three times and the distilled water was decanted with the floating seeds. The remaining seeds (that submerged in the distilled water) were washed with 70% ethanol for 5 s and the solution was then decanted. The seeds were then soaked with 1% sodium hypochlorite solution plus 0.2 ml of Tween 20 for 15 min in a 250 ml beaker under a biosafety hood. The solution was strained off and the seeds washed again with 70% ethanol and the ethanol was decanted. The remaining seeds were then rinsed thoroughly with pre-sterilized distilled water. Average of ten seeds were placed on sterilized filter papers in a petri dish. The petri dishes were incubated in the dark at 25 °C for four days. After four days, sorghum seeds that had a radicle length of about 0.5 cm was used in this experiment. Each treatment was replicated three (3) times with two seedling per replicate. Sorghum seeds were used for the optimization experiment based on previous studies (unpublished) that we conducted.

2.6. Experimental design

The experiment consisted of ten (10) treatments that were replicated six(6) times and arranged in completely randomized design in MRM system (Fig. S1). The treatments included single or combined amendments with or without AMF inoculation of the RMS soil. Two (2) mm-sieved soil were mixed with or without appropriate amendment and/or inoculum. Treatments consisted of urea and phosphorus amendment (N + P); poultry litter amendment (PL); vermicompost amendment (VC); paper mill sludge alone (PMS); BioVam commercial mycorrhiza alone (AMF); combination of paper mill sludge and BioVam commercial mycorrhiza (PMS + AMF); combination of paper mill sludge and urea (PMS + N); combination of BioVam commercial mycorrhiza and vermicompost (AMF + VC); and the controls consisted of ASL and RMS without amendment or inoculum. The N and PL were applied to appropriately designated portions of soil to stimulate a nitrogen rate addition of 75mgN/Kg while P were applied to the appropriate amount of soil at a rate of 25mgP/Kg (50lbs/ac).

2.7. Soil physicochemical analyses

The initial $pH_{(water)}$, percentage of organic matter content, phosphorus, potassium, calcium, and magnesium of the RMS, ASL and PMS were determined. Several sub-samples of soils and amendment were taken and mixed thoroughly in a sterile whirl-pak bags (Nasco, Fort Atkinson, WI). Six different aliquots were thereafter taken from the soil consolidation and shipped for analysis to the Soil, Plant and Pest Laboratory of University of Tennessee, at Ellington Agricultural Center, Nashville, TN. The summary of the limited physicochemical analyses of the degraded and arable soils used is summarized in Table 1.

2.8. Determination of aboveground biomass

After 8 weeks of incubation in the MRM system, the SG plant height was measured according to Andariese & Covington (1986) method. Summarily, each SG height was measured from the plant tiller base to the extended length of the longest blade. The plant height measurement was recorded to the nearest 0.25 cm using a metric scale (Fig. S2 A-B) and average height was determined for each treatment. The SG plant was harvested by clipping the entire above soil biomass as representative of the shoot. Shoot from each replicate was bagged separately and oven-dried to constant weight at 70 °C for 4 days as a measure of shoot dry weight. The belowground biomass (BGB) was extracted from the soil and washed under gentle stream of water as representative of the root. Thereafter, washed roots were dried with Kimberly-Clark wypall – X60 (Kimberly Clark Coop., Roswell, GA) and transported to the lab for root image analysis.

2.9. Quantification of mycorrhizal colonization of root

Fine roots from the harvested SG roots were subsampled from each plant and used as representative for quantification of mycorrhizal colonization of the roots. The subsampled roots from each plant was cut into an average of 5 cm-long segments and placed into tissue processing cassettes. Each treatment had at least 100 samples of small root pieces that were then stained for quantification using modified method of trypan blue staining (Bernaola et al., 2018a, 2018b; Koske & Gemma, 1989). Summarily, the appropriately labelled cassettes containing the pieces of roots subsampled from each plant was cleaned by rinsing in distilled water four times while straining the water out to avoid loss of roots. The clearing of the washed root samples was carried out in 2.5% KOH (w/v) at 90 °C for 20 min in a water bath. Cleared root samples were rinsed with distilled water to remove KOH and the samples were acidified for staining by immersion of cassettes holding the root samples in 2% HCl (v/v) at room temperature for 15 min. The cassettes containing roots were then transferred into 0.05% trypan blue solution and incubated at 90 °C for 15 min in a water bath and then transferred into beaker containing lactoglycerol (1:1:1 of lactic acid, glycerin, and water (HPLC-high grade)) at 4 °C for 48 h for the de-staining of the roots. The roots were mounted on microscopic slide using lactoglycerol solution and covered with glass slips. Excess solution was strained from the slide and three microscopic slides containing randomly selected root fragments were examined under compound microscope at 40× magnification to affirm the amount of AMF that colonized each root fragment. Images of root observation were taken with camera attached to the microscope. The roots were observed for the presence of blue-stained hyphae, arbuscules or vesicles as indication of colonization by AMF from commercial inoculant or native soils.

2.10. Root morphology

Harvested SG roots were cleaned as described above and transported to root imaging lab. Six replicates per treatment with each replicate comprising of two plants given a total of 12 plants per treatment. The entire RSA parameter of each SG sample was acquired using an Epson Perfection V39 – flatbed scanner (Epson Inc., Long Beach, CA). Root

Table 1
Soil chemistry associated with soil and PMS used prior to amendment and inoculation. Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) are presented as parts per million (ppm). Percent organic matter (OM) and pH. ND – not detected.

Samples	$pH_{(water)}$	Organic matter (%)	P	K	Ca	Mg
RMS	5.8	3.5	9.5	105	898	193
ASL	5.6	2.4	39.5	48	812	188
PMS	7.5	ND	96.6	ND	–	–

image processing of the acquired scanned root was analyzed using RootSnap software (CID Bioscience Inc., Camas, WA) and eleven root traits (root counts, total root length – TRL, total root volume – TRV, total root area – TRA, average root diameter – ARD, average root length – ARL, average root area – ARA, average root volume – ARV, average root angle – ARAngle, average start tip angle – ASTAngle, average end tip angle – AETAngle) were measured. After root imaging, the labelled bags of root were transported to the greenhouse support facility and oven-dried to constant weight at 70 °C for 4 days as a measure of root dry weight. All oven-dried shoot and root samples were weighed to the nearest 0.01 g.

Plant morphometrics taken were used to estimate mycorrhizal dependency (MD) and growth enhancement (GE) response to the soil treatment. The MD of plant is defined as the degree of plant growth change associated with mycorrhizal colonization (Tawaraya, 2003). The MD was calculated according to method of Awoyemi & Dzantor (2017) using the formula (1):

$$\mathrm{MD}(\%) = ((\mathrm{DW_i} - -\mathrm{DW_u})/\mathrm{DW_i}) \times 100 \quad (1)$$

DW_i is the dry weight of plants in mycorrhizal inoculated soil and DW_u is the dry weight of plants in mycorrhizal uninoculated soil.

The GE of plant is defined as the growth response of plant associated with amendment applied to the soil. It is the degree of plant growth change associated with soil amendment expressed in percentages (%). GE was calculated using the formula (2):

$$\mathrm{GE}(\%) = ((\mathrm{AL_a} - \mathrm{AL_u})/\mathrm{AL_u}) \times 100 \quad (2)$$

AL_a is the average length of plant tissue (shoot or root) in amended soil and AL_u is the average length of the of plant tissue (shoot or root) in unamended soil.

For the root morphometrics only, the specific root length (SRL), root tissue density (RTD), and root production (RP) were computed using the Eqs. (3), (4) and (5) according to Pérez-Jaramillo et al. (2017).

$$\mathrm{SRL} = \text{Total root length}(m)/\text{Root dry weight}(g) \quad (3)$$

$$\mathrm{RTD} = \text{root dry weight}(g)/\text{total root volume}\left(cm^3\right) \quad (4)$$

$$\mathrm{RP} = \text{Total root length}(m)/\text{soil weight}(g, oven-dry-soil\ equivalent) \quad (5)$$

2.11. Statistical analysis

The results obtained for the optimization of AMF, plant height and biomass data were expressed as mean values, and the Pearson correlation between biomass and plant height was expressed in graphs. The mean values obtained were subjected to one-way analysis of variance (ANOVA) and the outcome compared by Tukey's range test. All statistical analyses were performed using IBM SPSS Statistics for Windows, v25.0 (IBM Corp., NY, USA) and R software version 3.5.1 (R Core Team, 2018) in R studio (RStudio Team, 2016).

Root phenotypic traits measured with open root software was analyzed. Data were tested for normal distribution with Shapiro–Wilk test and inspected visually using density-plot. The homogeneity of variance was also tested using Levene test. ANOVA analysis was applied to the mean values for each trait and outcome was compared by Tukey HSD to determine significance between treatments. The obtained values were compared to the corresponding value of the untreated control (RMS) treatments.

Comparison between the phenotypic traits measured was carried out for statistical relationships using spearman rank correlation coefficients (ρ) between traits. The tests for the degree of association between traits were calculated and reordered according to angular order of eigenvector (AOE) using R *corrplot* package. The AOE was calculated from the order of the angles a_i,

$$a_i = \{tan^{-1}(e_{i2}/e_{i1}), if e_{i1} > 0;\ tan^{-1}(e_{i2}/e_{i1}) + , otherwise.$$

where e_1 and e_2 are the largest two eigenvalues of the correlation matrix (Friendly, 2002).

Also, the correlation matrix was further arranged to depict the significant correlation coefficient at 95% confidence interval.

Multivariate statistical analysis of different phenotypic root traits was computed using R principal component analysis (PCA) and hierarchical cluster analysis (HCA). The HCA was carried out based on euclidean distances and complete linkage hierarchical clustering method of standardized sample data. The output of the HCA was visualized as heatmap (a false colored image) and dendrogram using *heatmap* function in *stats* package and the dendrogram enhanced with *dendextend* package (Fig. 9). Further exploratory data analysis of the measured traits for all the treatments (dataset) were visualized in a graphical output as PC1 (first principal component) vs PC2 (second principal component) according to the variation in the dataset using R *FactoMineR* package (Lê, Josse, & Husson, 2008) and visualized with *factoextra* package (Kassambara & Mundt, 2017) as shown in Fig. 10A,. Prior to PCA analysis, the variables were standardized due to the difference in scale of measurements (millimeters vs degree of angle) using the formula:

$$(x_i - mean(x))/SD(x)$$

where mean(x) is the mean of x values, and SD(x) is the standard deviation (SD). The PCA plot of variables was classified using *kmeans* clustering algorithm which showed three clusters (Fig. 10B) of the measured traits. The clusters were further visualized using *fviz_pca_var* function in *factoextra* package. The individual observations (biological replicates) were grouped according to the treatments applied.

The height, dry weight of shoot (ShootDW) and root (RootDW) were used as supplementary variable that was projected on the PCA variable plot (Fig. S3).

3. Results

3.1. Optimal AMF inoculum for biomass productivity

Six plants per treatment level of AMF inoculum was monitored for *S. bicolor* above-ground biomass (AGB) enhancement. At the end of 8 weeks, the dry weight of the harvested shoot showed that addition of 3% AMF inoculum to RMS increased the AGB by 12.5% and further increased the AGB of 10% AMF inoculum by 137.5% (from 1.04 g to 2.47 g) compared to the control. However, the increase of the AMF inoculum size to 15% did not increase the AGB compared to 10% but increased the biomass by 133% when compared to the control as shown in Fig. 2.

3.2. Vermicompost and commercial AMF influenced plant height

Twelve plants per treatment was examined for SG height measurement. The measurement of plant height after 8 weeks showed incremental difference between various treatments versus the untreated degraded soil (RMS). The order of increment was Control (RMS) < ASL = N + P = PMS + AMF < PL < PMS < AMF + VC < PMS + N < VC < AMF (as shown in Fig. 3). The arable soil (ASL), N + P, and PMS + AMF soil treatments were able to increase (by about 26%) the SG plant height more than the degraded soil. The other treatments like PL, PMS, AMF + VC, and PMS + N also increased the SG plant height compared to RMS soil by 33, 48, 52 and 59% respectively. Although all these amendments showed increased plant height in SG, only the treatments VC and AMF significantly ($p < 0.05$) increased the SG height compare to RMS by 67 and 93% respectively as shown in Fig. 3.

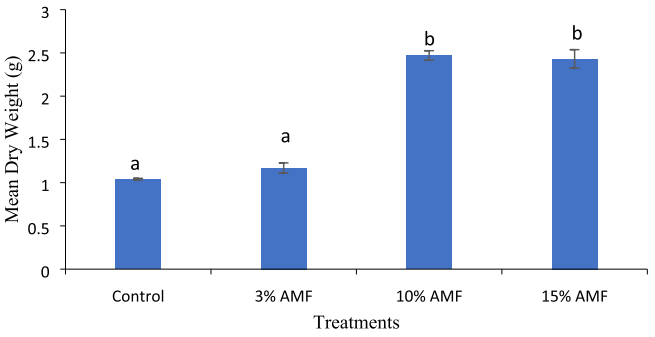

Fig. 2. Screening RMS for the optimum BioVam commercial AMF inoculum ratio (percentage). 10% AMF inoculum showed the highest dry weight(g) of biomass and it was significantly ($p < 0.05$) different from control and 3%. Each value is mean of triplicates and each replicate consisted of 2 plant samples and the bars with different alphabet on it is significantly different at $p < 0.05$ as determined by Tukey HSD. The error bar = ± 1 SD.

3.3. Biomass characterization

Twelve plants (six replicates) per treatment was analyzed for biomass characterization. Determination of dry weight (shoot, root and total) biomass after harvesting SG plants showed incremental differences for all soil amendments compared to the control (Fig. 4). The dry weight of the root biomass in AMF-amended soil (0.87 g/50 g $_{ods}$) was significantly higher than that of unamended control soil – RMS (0.25), ASL (0.35), N + P (0.38), PMS + AMF (0.4), and VC (0.52) as shown in Fig. 4C.

Similarly, AMF also significantly enhanced the dry weight of the shoot biomass compared to the RMS (Control), ASL, N + P, PMS + AMF, and PMS (0.9 g/50 g $_{ods}$ compared to 0.28, 0.38, 0.4, 0.45, and 0.57 respectively) while the amendment of soil with PMS + N and AMF + VC also significantly enhanced the dry weight of SG shoot compared to the control (Fig. 4B).

The dry weight of the total biomass showed similar trends as in the shoot and root biomass with the AMF-amended soil significantly enhanced more than the control, ASL, N + P, PMS + AMF, and PMS as shown in Fig. 4A.

3.4. Correlation between height and dry weight

Based on sixty (n = 60) observations for both plant morphometrics, the height and the dry weight of the SG shoot showed significant and positive correlation amongst all treatments applied ($r = 0.63$, $P < 0.001$). This correlation suggests that the aboveground biomass increase as the height of the SG increases as shown in Fig. 5.

3.5. Root colonization by AMF

The microscopic analyses of the randomly selected root fragments that are representatives of the 10 treatments studied confirmed the colonization of SG roots by commercial AMF. The observation of some selected root as a representative of the entire plant root was important as the entire root could not be stained. The AMF and AMF + VC treatments showed the highest percentage of SG roots colonized by inoculated AMF compared to that of the (control) degraded soil (Table 2). However, some uninoculated roots showed colonization by AMF which could have been as a result of colonization by the native mycorrhizae in the soil. The significant increase in shoot biomass of AMF inoculated treatment can be attributed to enhancement by the AMF that colonized the root.

3.6. Root phenotypic variation between treatments

3.6.1. Phenotypic means

The means of each phenotypic trait examined represent an average of six (6) replicates. All the phenotypic root traits (root counts, TRL, TRV, TRA, ARD, ARL, ARA, ARV, ARAngle, ASTAngle, AETAngle) of RSA

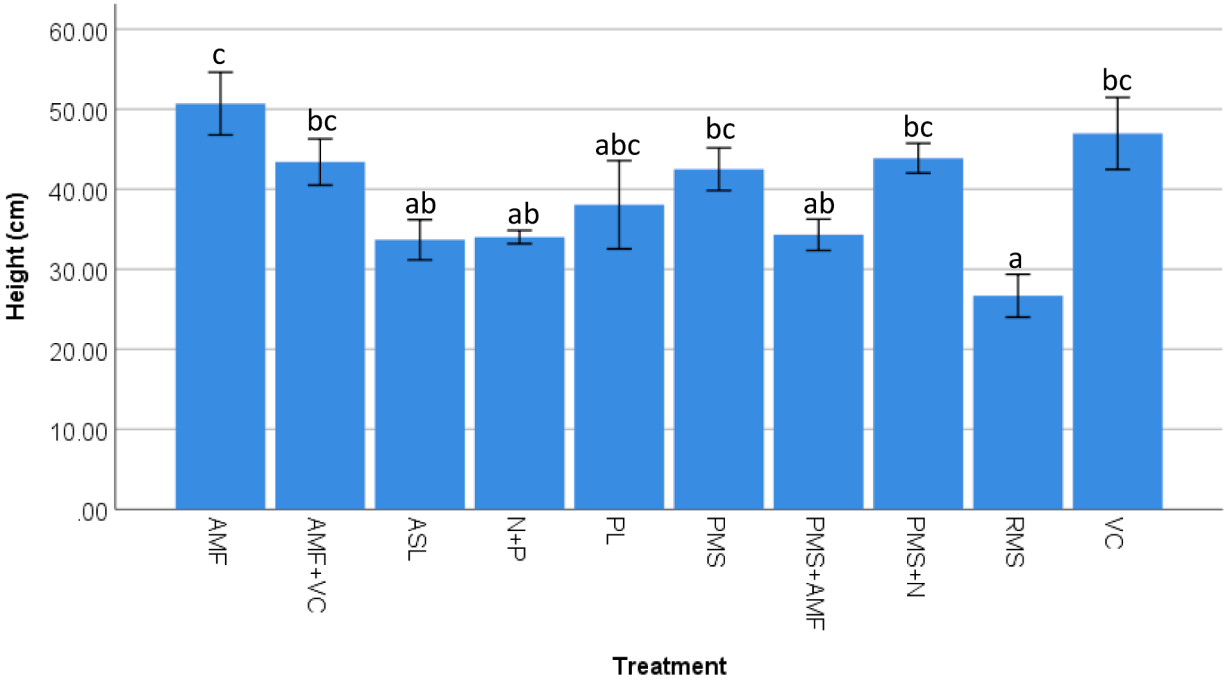

Fig. 3. Measurement of SG height after 8 weeks of incubation in MRM. Single treatments of commercial mycorrhiza and vermicompost were significantly ($p < 0.05$) higher than the plant height of the RMS (control soil). Treatment (TrtType) columns that do not share alphabets indicate significant differences in height between the ten treatments ($\alpha = 0.05$). The error bar = ± 1 SE. (Abbreviations: AMF – commercial mycorrhizal fungi, AMF + VC - commercial mycorrhizal fungi and vermicompost, ASL – amour silt loam, N + P – nitrogen and phosphorus, PL – poultry litter, PMS – paper mill sludge, PMS + AMF - paper mill sludge and commercial mycorrhizal fungi, PMS + N - paper mill sludge and nitrogen, RMS – untreated and uninoculated soil, VC – vermicompost).

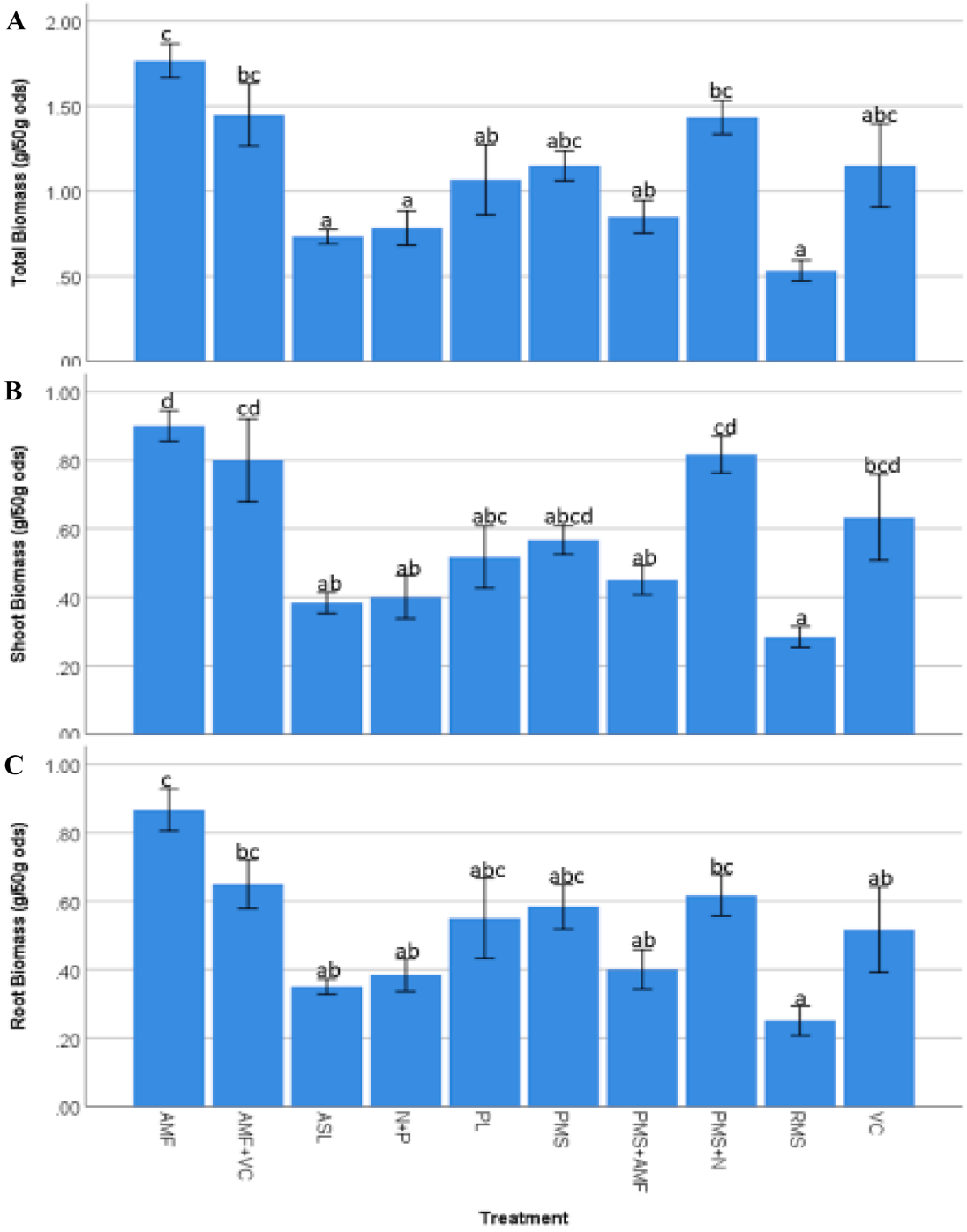

Fig. 4. Measurements of SG total biomass (A), shoot biomass (B), and root biomass (C) after 8 weeks of incubation in MRM. The treatments presented in columns are mean values ± standard errors (n = 12 for total biomass and n = 6 for shoot and root biomass). Treatment columns that do not share alphabets indicate significant differences between the ten treatments (α = 0.05). SG was grown in either untreated soil (ASL and RMS-Control), single amended soil (PL, VC, PMS, AMF) or combined amended soil (N + P, PMS + AMF, PMS + N, AMF + VC). The error bar = ± 1 SE. (Abbreviations: AMF – commercial mycorrhizal fungi, AMF + VC - commercial mycorrhizal fungi and vermicompost, ASL – amour silt loam, N + P – nitrogen and phosphorus, PL – poultry litter, PMS – paper mill sludge, PMS + AMF - paper mill sludge and commercial mycorrhizal fungi, PMS + N - paper mill sludge and nitrogen, RMS – untreated and uninoculated soil, VC – vermicompost).

evaluated showed significant differences at $p < 0.05$.

Root Count. The mean of total root counted ranged from 7.33 (±0.42, SE) to 36.17 (±1.1, SE) for all SG grown on control, amended and/or inoculated treatments. Compared with the control, the SG grown in AMF + VC and AMF soil treatments significantly increased root count by 306 and 393% respectively, and root count for both treatments (AMF + VC and AMF) were significantly more than the root counted for all other soil amendments (Fig. S4A). The root count of AMF + VC and AMF may have increased relative to other soil treatments because of the colonization of arbuscular mycorrhizae that led to early establishment of SG and subsequently enhanced the fine roots production.

Total root length (TRL). The total root length is the addition of all individual root length (seminal and lateral roots) that was quantified. The mean of total root length ranged from 1390.23 (±90, SE) to 7980.28 mm (±448, SE) for all SG grown on control, amended and/or inoculated treatments. Compared with control, the SG grown in PL,

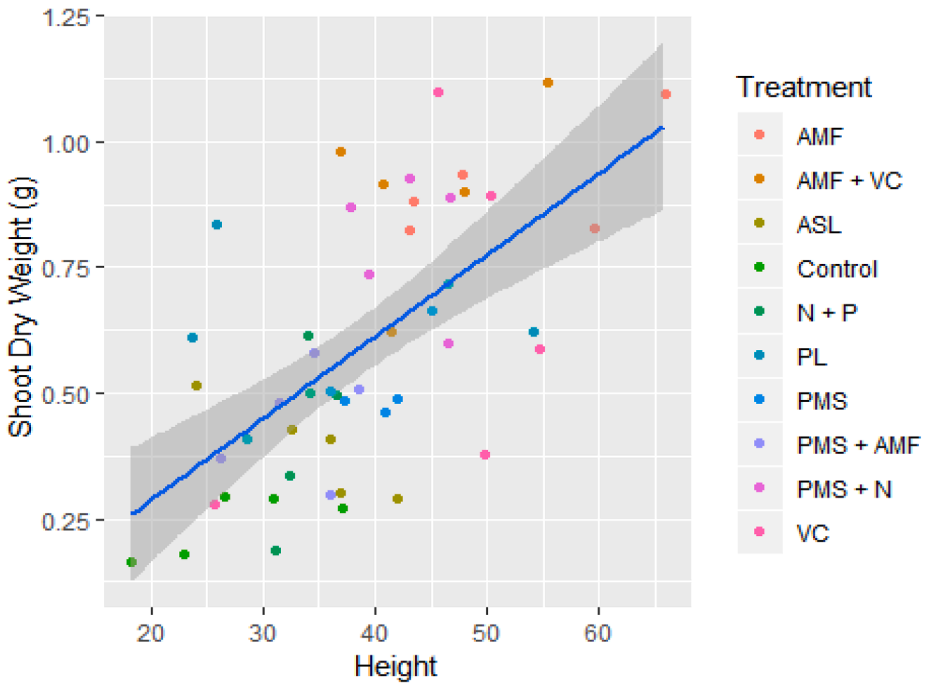

Fig. 5. Correlation between height and dry weight of shoot biomass. Blue line and the shaded area represent best line of fit and its standard error, respectively. Based on n = 60 observations, the dry weight and height showed a statistically significant ($p < 0.001$) positive linear relationship (Pearson's $r = 0.63$).

PMS + AMF, PMS, AMF + VC, VC, and AMF soil treatments increased the total root length by 67, 72, 77, 220, 234, and 474% respectively, while TRL of AMF + VC, VC, and AMF treatments were significantly longer that of all other treatments (Fig. S4B). The resultant increase of TRL in AMF + VC, VC and AMF relative to other soil treatments may have resulted from the single effect of VC amendment or AMF inoculation and combined effect of AMF + VC treatment on enhancement of root length.

Total root area (TRA). The total surface area was measured as a polygonal shape of the area occupied by the 2D image of the root system. The mean of the total root area ranged from 2277.01 (±117, SE) to 16751.76 mm^2 (±722, SE) for all SG grown on control, amended and/or inoculated treatments. Compared with the control, the total root area of SG grown in AMF treatment increased by 338% (Fig. S4C). The total surface area of the AMF inoculated soil increased significantly compared to the control, this is consistent with the increased TRL and root count.

Total root volume (TRV). The mean of the total root volume ranged from 331.46 (±59, SE) to 6436.34 mm^3 (±230, SE) for all SG grown on control, amended and/or inoculated treatments. Compared with control, the SG grown in N + P, AMF + VC, and AMF soil treatments increased the total root volume by 338, 363, and 956% respectively and these treatments were significantly larger than the TRV of all other treatments (Fig. S4D).

Average root diameter (ARD). The mean of the root diameter ranged from 0.5 (±0.06, SE) to 1.38 mm (±0.04, SE) for all SG grown on control, amended and/or inoculated treatments. Compared with control, the ARD of SG grown in AMF, and N + P soil treatments increased by 50, and 125% respectively (Fig. S4E).

Average root length (ARL). The mean of the seminal root length ranged from 158.24 (±6, SE) to 553.83 mm (±15, SE) for all SG grown on control, amended and/or inoculated treatments. Compared with control, the ARL of SG grown in PL, PMS, PMS + AMF, VC, AMF + VC, and AMF showed increased root length of 65, 93, 120, 140, 207, and 233% respectively (Fig. S4F).

Average root area (ARA). The mean of the root area ranged from 255.36 (±7, SE) to 941.74 mm^2 (±8, SE) for all SG grown on control, amended and/or inoculated treatments. Compared with control, the ARA of SG grown in VC, PMS, PMS + AMF, PL, N + P, AMF + VC, and AMF soil treatments increased by 66, 69, 80, 84, 103, 110, and 269% respectively (Fig. S4G).

Average root volume (ARV). The mean of the average root volume ranged from 51.24 (±2, SE) to 259.52 mm^3 (±11 SE) for all SG grown on control, amended and/or inoculated treatments. Compared with control, the ARV of SG grown in AMF + VC, N + P, and AMF soil treatments increased by 78, 83, and 206% respectively (Fig. S4H).

Average root angle (ARAngle). The mean of the average root angle ranged from 23 (±1, SE) to 67° (±2, SE) for all SG grown on control, amended and/or inoculated treatments. Compared with control, the ARAngle of all SG grown in amended and/or inoculated treatments decreased in the order of RMS > PMS = ASL > PMS + N > PL > VC > AMF + VC > PMS + AMF > N + P > AMF, with AMF showing the largest decrease by 66% (Fig. S4I).

Average starting tip angle (ASTAngle). The mean of the average starting tip angle ranged from 29 (±1, SE) to 67° (±2, SE) for all SG grown on control, amended and/or inoculated treatments. Compared with control, the ASTAngle of SG grown in PMS soil treatments increased by 30% while ASTAngle of other soil treatments, VC, AMF + VC, PMS + N, AMF, N + P, and PMS + AMF decreased by 19, 20, 25, 35, 35, and 44% respectively (Fig. S4J).

Average ending tip angle (AETAngle). The mean of the average ending tip angle ranged from 30 (±2, SE) to 71° (±2, SE) for all SG grown on control, amended and/or inoculated treatments. Compared with

Table 2
Root colonization (RC) of SG 8 weeks after incubation in degraded soil.

Treatment	AMF + VC	AMF	N + P	PL	PMS + AMF	PMS + N	PMS	VC	ASL	Control
RC (%)	55	68	15	20	45	40	30	25	20	5

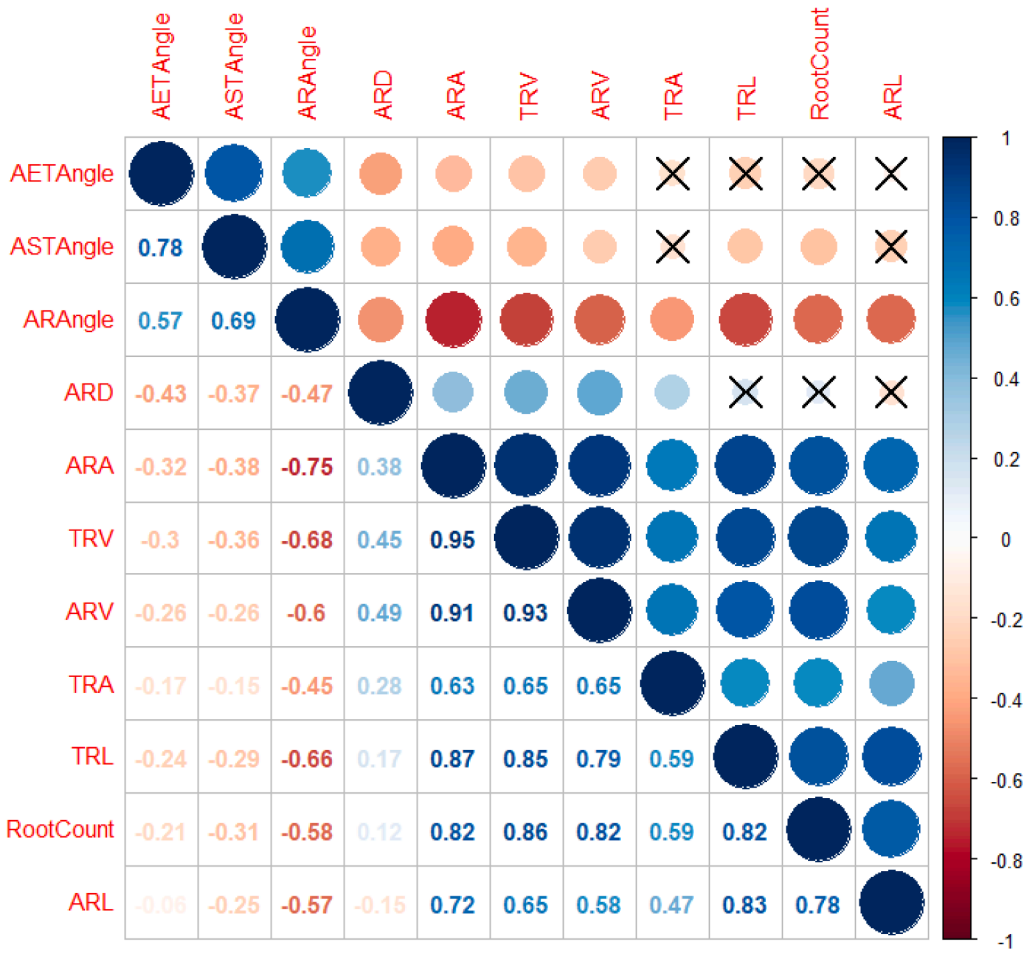

Fig. 6. Correlation plot of root morphology parameters at confidence level = 0.95 and significance level = 0.05. The upper triangular part of the matrix plot displayed in circle represents the correlation value that ranged between −1 (red and negative correlations) and + 1 (blue and positive correlations) while the lower triangular part displayed the correlation in numbers. In addition to the correlogram, the cross (X) are added to the plot with no significant coefficient, that is, correlations with p-value > 0.05 that are considered as insignificant.

control, the AETAngle of SG grown in ASL and PMS soil treatments increased by 31 and 62% respectively (Fig. S4K).

3.6.2. *Correlation of phenotypic parameters*

Phenotypic correlations of the root morphological traits appeared to cluster into three categories according to their AOE and associations between them: (a) the root angle traits; (b) the root diameter; and (c) the root lengths, the root areas, the root volumes, and root count (Fig. 6). The root angle traits (AETAngle, ASTAngle and ARAngle) were all negatively correlated with other phenotypic traits varying from highly negatively correlated to slightly negatively correlated or neutral association (ρ varied between −0.75 and −0.06). However, the negative correlation was not significant for association between AETAngle vs TRA, AETAngle vs TRL, AETAngle vs RootCount, AETAngle vs ARL, ASTAngle vs TRA, and ASTAngle vs ARL. Meanwhile, the intra-relationships between the root angle traits were significant and highly positive correlation, AETAngle vs ASTAngle ($\rho_{AETAngle,\ ASTAngle} = 0.78$), AETAngle vs ARAngle ($\rho_{AETAngle,\ ARAngle} = 0.57$) and ASTAngle vs ARAngle ($\rho_{AETAngle,\ ASTAngle} = 0.69$).

The ARD showed slightly negatively correlated association with ASTAngle ($\rho_{ARD,\ ASTAngle} = -0.37$), AETAngle ($\rho_{ARD,\ AETAngle} = -0.43$), and ARAngle ($\rho_{ARD,\ ARAngle} = -0.47$). However, ARD showed significantly and slightly positively correlated association with TRA ($\rho_{ARD,\ TRA} = 0.28$), ARA ($\rho_{ARD,\ ARA} = 0.38$), TRV ($\rho_{ARD,\ TRV} = 0.45$), and ARV ($\rho_{ARD,\ ARV} = 0.49$). The association between ARD vs TRL, RootCount and ARL were not significant.

All the remaining root traits showed significant relationship varying from highly positively correlated to slightly positively correlated association (ρ varied between 0.47 and 0.95). The ARA showed significantly and highly positively correlated association with TRA ($\rho_{ARA,\ TRA} = 0.63$), ARL ($\rho_{ARA,\ ARL} = 0.72$), RootCount ($\rho_{ARA,\ RootCouunt} = 0.82$), TRL ($\rho_{ARA,\ TRL} = 0.87$), ARV ($\rho_{ARA,\ ARV} = 0.91$), and TRV ($\rho_{ARA,\ TRV} = 0.95$). The TRV showed significantly and highly positively correlated association with ARL ($\rho_{TRV,\ ARL} = 0.65$), TRA ($\rho_{TRV,\ TRA} = 0.65$), TRL ($\rho_{TRV,\ TRL} = 0.85$), RootCount ($\rho_{TRV,\ RootCount} = 0.86$), and ARV ($\rho_{TRV,\ ARV} = 0.93$). The ARV showed significantly and highly positively correlated association with TRA ($\rho_{ARV,\ TRA} = 0.65$), TRL ($\rho_{ARV,\ TRL} = 0.79$), RootCount ($\rho_{ARV,\ RootCount} = 0.82$), and ARL ($\rho_{ARV,\ ARL} = 0.58$). The TRA showed significantly and highly positively correlated association with TRL ($\rho_{TRA,\ TRL} = 0.59$), and RootCount ($\rho_{TRA,\ RootCount} = 0.59$) except ARL ($\rho_{TRA,\ ARL} = 0.47$) that was slightly positively correlated association. The TRL showed significantly and highly positively correlated association with RootCount ($\rho_{TRL,\ RootCount} = 0.82$), and ARL ($\rho_{TRL,\ ARL} = 0.83$) while the RootCount showed significant and highly positively correlated association with ARL ($\rho_{RootCount,\ ARL} = 0.78$).

Correlations between root phenotypic traits and aboveground plant morphometrics varied from slightly negatively correlated to positively correlated association (ρ varied between −0.39 and 0.56). The height showed slightly negatively correlated with root angle traits (AETAngle, $\rho_{Height,\ AETAngle} = -0.07$; ASTAngle, $\rho_{Height,\ ASTAngle} = -0.01$; and ARAngle $\rho_{Height,\ ARAngle} = -0.39$) and ARD ($\rho_{Height,\ ARD} = -0.07$) while been positively correlated to other root traits TRA ($\rho_{Height,\ TRA} = -0.07$), ARV ($\rho_{Height,\ ARV} = 0.28$), TRV ($\rho_{Height,\ TRV} = 0.35$), ARA ($\rho_{Height,\ TRV} = 0.42$), TRL ($\rho_{Height,\ TRL} = 0.56$), RP ($\rho_{Height,\ SRP} = 0.56$), RootCount ($\rho_{Height,\ RootCount} = 0.37$), and ARL ($\rho_{Height,\ ARL} = 0.52$) as shown in Fig. S5. The correlation of other aboveground morphometrics (ShootDW and TotalDW) vs root phenotypic traits showed similar trend as those observed with the height measurements. These trends from ShootDW

Table 3
Mycorrhizal dependency (MD) and growth enhancement (GE) of SG 8 weeks after incubation in degraded soil.

Treatment	AMF + VC	AMF	N + P	PL	PMS + AMF	PMS + N	PMS	VC
MD (%)	20.8	68.5	–	–	−25.9	0	–	0
GE_r (%)	220.4	474.0	55.1	67.5	72.2	25.1	77.5	234.0
GE_s (%)	62.6	90.0	27.5	42.6	28.5	64.4	59.3	76.0

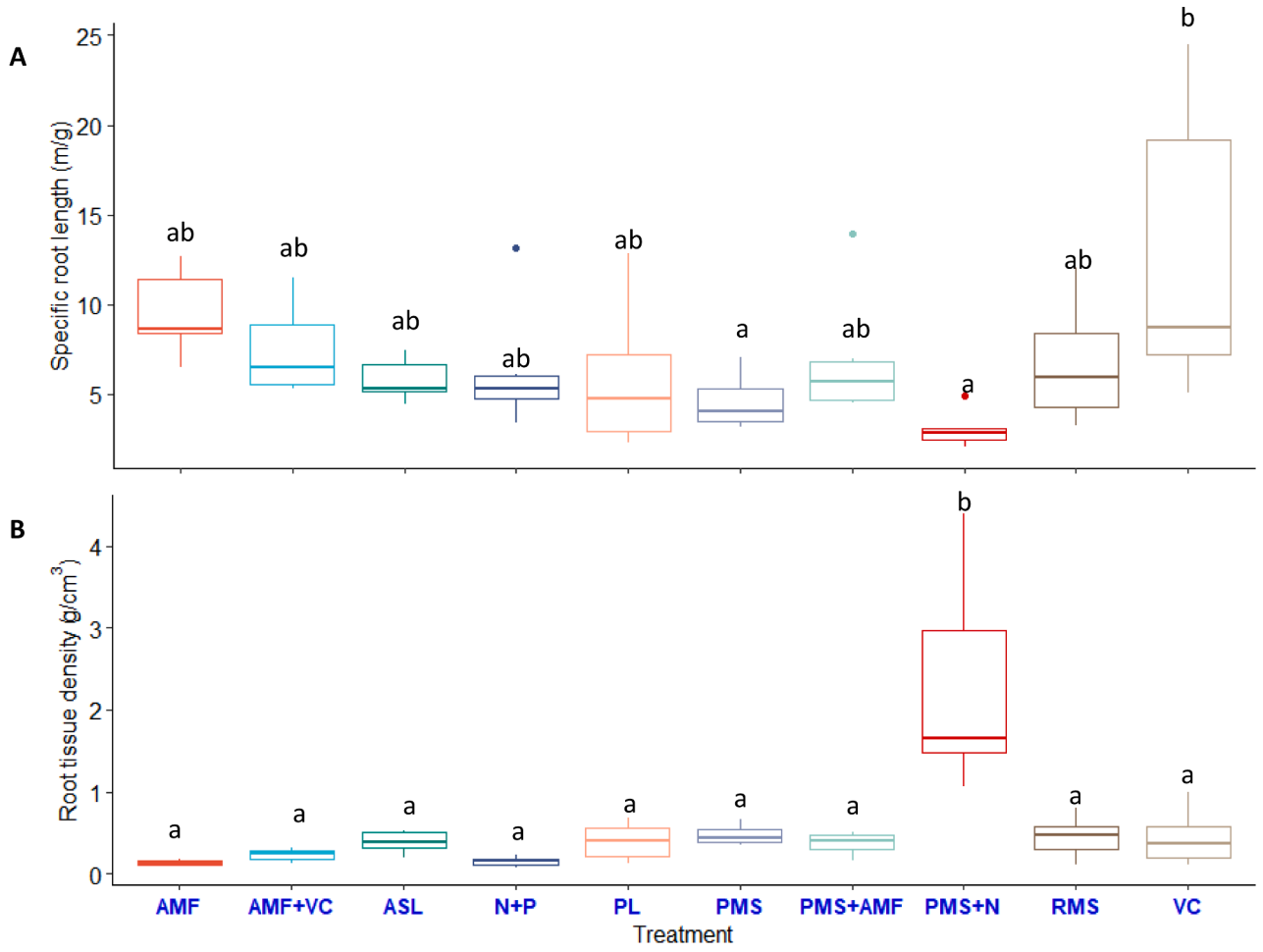

Fig. 7. Boxplot of computed root morphometrics of SG grown in different soil treatments. (A). The specific root length (SRL) is the ratio of total root length to the root dry weight. (B). The root tissue density (RTD) is the ratio of the root dry weight to the total root volume calculated as g/cm^3. The total root length and total root volume were measured using RootSnap software. Boxplot with different alphabets indicate statistically significant differences (Tukey HSD test). Statistically significant differences were determined by one-way ANOVA ($p < 0.05$) (Abbreviations: AMF – commercial mycorrhizal fungi, AMF + VC - commercial mycorrhizal fungi and vermicompost, ASL – amour silt loam, N + P – nitrogen and phosphorus, PL – poultry litter, PMS – paper mill sludge, PMS + AMF - paper mill sludge and commercial mycorrhizal fungi, PMS + N - paper mill sludge and nitrogen, RMS – untreated and uninoculated soil, VC – vermicompost)..

and TotalDW were slightly negatively correlated with root angle and positively correlated to other root traits (Fig. S5).

3.7. Mycorrhizal dependency and growth enhancement response of SG in degraded soil

The MD and GE of SG grown in degraded soil using different inoculation and amendment strategies are shown in Table 3. The result showed that MD increased in AMF inoculated treatments except for treatment where AMF and PMS were combined. The GE_r (root) increased with the inoculation of AMF and all amended soil treatments. The GE_s (shoot) followed similar trend with the root (as AMF and AMF + VC enhanced shoot biomass the most) except for PMS + N that had an inverted trend as it performed the better among amendment strategies for shoot enhancement (Table 3).

3.8. Specific root length, root tissue density and root length production

The specific root length (SRL, is the ratio of the root length to the dry weight of the root, m/g) of the of SG grown in VC soil treatment was different from other soil treatments (Fig. 7A). The amendment of soil with VC increased the SRL of SG grown by 90% when compared to the control. Increased SRL and reduced diameter depicts, increased thinner roots that may have improve water uptake. The root tissue density (RTD, is the ratio of the root dry weight to the total root volume) of the SG grown in PMS + N treatment was different from other soil treatments as shown in Fig. 7B. The amendment of soil with PMS + N increased RTD of the SG by 400% more than the control soil treatment. The root length production (RP, is the ratio of the total root length to the oven-dry weight volume of the soil, m/cm^3) of the SG grown in all the soil treatments were similar to the trend of the total root length. The values

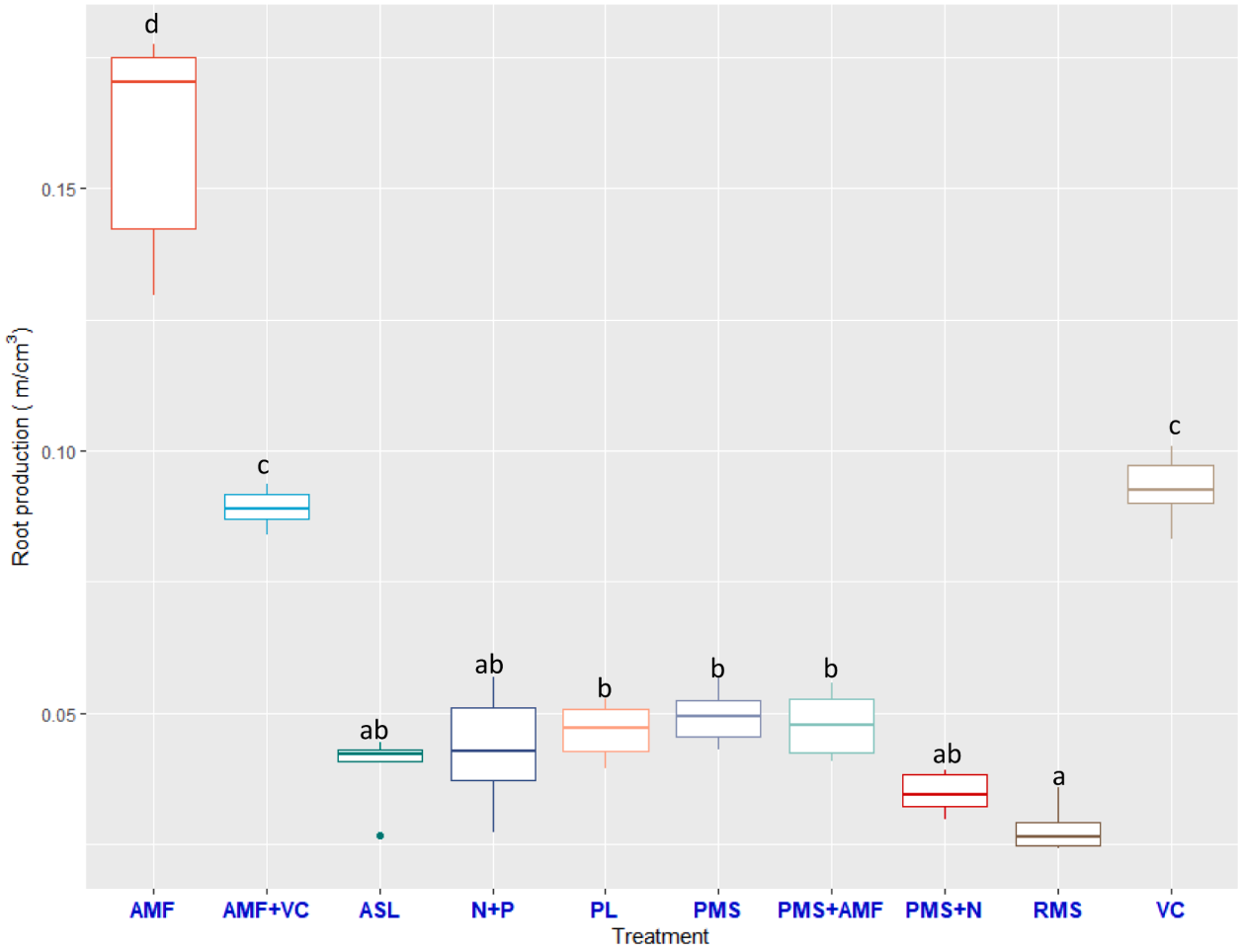

Fig. 8. Boxplot of root length production parameter of SG grown in different soil treatments. The root length production is the ratio of the total root length to the soil calculated as m/cm^3. Boxplot with different alphabets indicate statistically significant differences (Tukey HSD test). Statistically significant differences were determined by one-way ANOVA ($p < 0.05$) (Abbreviations: AMF – commercial mycorrhizal fungi, AMF + VC - commercial mycorrhizal fungi and vermicompost, ASL – amour silt loam, N + P – nitrogen and phosphorus, PL – poultry litter, PMS – paper mill sludge, PMS + AMF - paper mill sludge and commercial mycorrhizal fungi, PMS + N - paper mill sludge and nitrogen, RMS – untreated and uninoculated soil, VC – vermicompost).

of the result can be used to also extrapolate the production rate (in km. m^{-3}. yr^{-1}) by predicting from the time (t) used for the experiment which is approximately 0.15 years. Compared with control treatment, the soil treatment amended and/or inoculated with AMF, VC, AMF + VC, PMS, PMS + AMF, and PL increased the RP by 474%, 234%, 220%, 78%, 72%, and 67% respectively as shown in Fig. 8.

3.9. Assessment of root responses to amendment and inoculation strategies by PCA and HCA methods

PCA and HCA was performed on root phenotypic data acquired 8 weeks after transplanting SG into degraded soil, in order to assess the contributions of each trait to the response of amendment and inoculation strategies.

Based on the HCA, the variable can be clustered to two main groups that consisted of the root count; all the root length, area, and volume; and the root diameter traits in a group (blue) and the other main group (orange) consisting of all the root angle traits. The blue group (made up of traits TRV, ARA, ARV, Root Count, TRA, TRL, ARL and ARD) showed similarities among the samples and clear contrast to the orange group (made up of ASTAngle, AETAngle, and ARAngle). The clustering of the individual samples along the row revealed three main groups shown as purple (AMF), light blue (AMF + VC, PMS, ASL, and Control) and green (N + P, PL, PMS + N, VC, and PMS + AMF). The green cluster is the most diversified group based on the soil enhancement strategies. The light blue group is the next diversified with the PMS, ASL, and control treatment showing a strong differentiation between the blue and orange variables. The purple group is the only cluster with a single treatment (AMF) and also showed strong difference between the blue and orange variables (Fig. 9). To have a more resolved difference between generated clusters we proceed on using PCA which will allow differentiate between enhancement strategies while also highlighting the major clusters among the traits. A major reason for taking this step forward is depicted in the heatmap where the most distinct cluster (purple group) had a sample from the AMF + VC treatment. Visualizing this kind of overlap prompted for further classification of the cluster using PCA.

Based on the PCA, the first five components (Dimension 1 to 5) explained 94.2% of the variance among the traits. However, the majority of this variance is explained by the first two principal components (Dimensions 1 and 2), that explained 77% of the total variation among the root traits (Fig. S6). Dim 1, the first principal component, explained 59.4% of the total variation and the traits that contributed majorly to this component were ARA, TRV, ARV, TRL, RootCount, and ARAngle. Dim 2, the second principal component, explained 17.6% of the total variation with the traits AETAngle, ASTAngle, and ARD contributing majorly to the component (Fig. S7). The individual observations on the principal components generated coordinates that were plotted as the PCA plot. These coordinates were then grouped according to the treatment applied to the degraded soil while ellipses were added with a 95% confidence level added as the center of the ellipses. The observations can

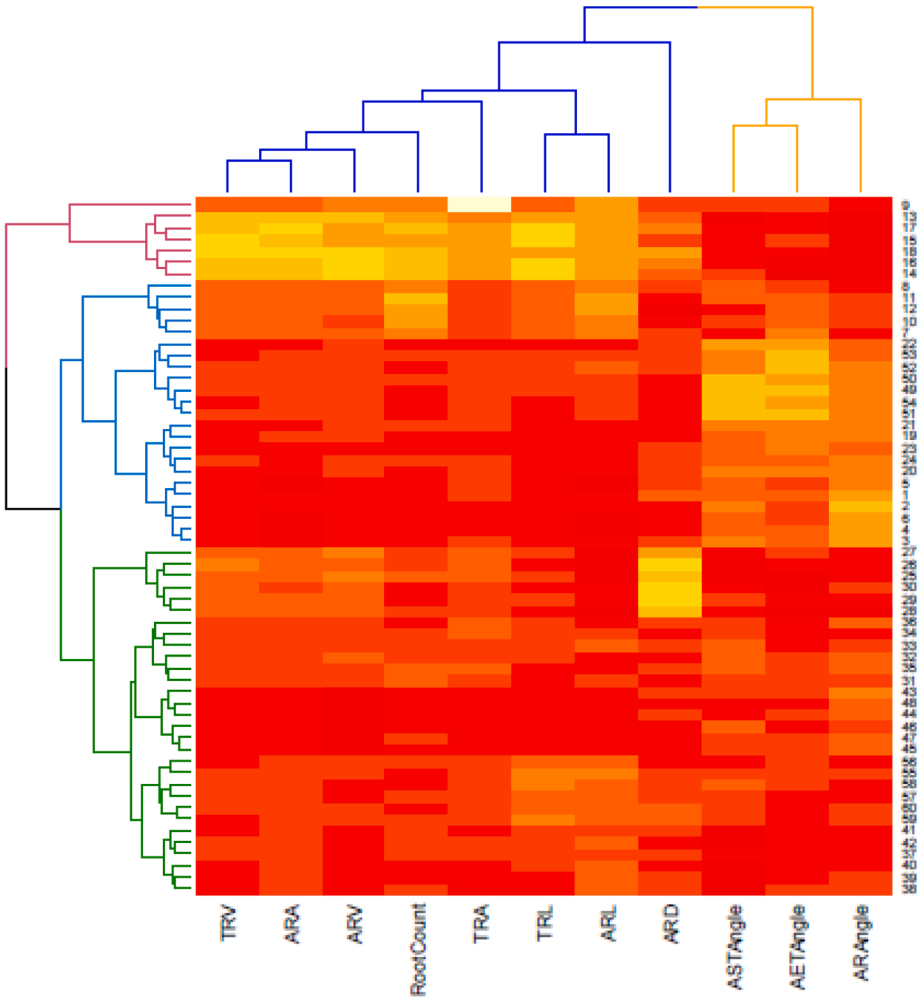

Fig. 9. Hierarchical clustering and heatmap of root traits measured 8 weeks after inoculation and amendment of the degraded soil (control). The chart represents the variables (in columns) vs individual sample (in rows). The data of each sample plotted for the heat map was standardized to generate a zero mean and unit variance. These scaled values were plotted in orange-yellow color scale using *stats* and *dendextend* package for the heatmap and clustering respectively. The orange color represents low value while the yellow color represents high value. The clustering analysis of the variables revealed two major groups shown as blue (TRV, ARA, ARV, Root Count, TRA, TRL, ARL and ARD), and orange (ASTAngle, AETAngle, and ARAngle). The clustering of the individual samples revealed three main groups shown as purple (AMF), light blue (AMF + VC, PMS, ASL, and Control) and green (N + P, PL, PMS + N, VC, and PMS + AMF).

be classified into 3 groups based on the PCA scores and ellipse overlap. The first group (those that have approximately −2 or lower on Dim1) as the soil treatments that showed low enhancement; the second group (those that ranges from approximately 0 to + 2 on Dim1) as those soil treatments that had moderate enhancement; and the third group (those have approximately + 4 or above on Dim1) as the soil treatment that showed high enhancement of SG root (Fig. 10A).

The graph of all variables (Fig. 10B) showed similar variable point to the same section of the plot while the negatively correlated variable point to the opposite side of the plot. The classification of the variables based on their *k mean* resulted in 3 clusters. Cluster 1 is the ARD, cluster 2 included TRV, ARA, ARV, Root Count, TRA, TRL, and ARL; and cluster 3 is comprised of the root angle traits, ASTAngle, AETAngle, and ARAngle. The variables are also plotted such that the closer the arrow is to the outside circle the higher the correlation or contribution to the principal components.

After plotting the variables that allowed for better classification of the traits into separate clusters it was of interest to use the data previously generated by the PCA to predict the coordinates of supplementary variables (height, root dry weight and shoot dry weight). The variables were first standardized and then the coordinates computed before plotting them on the variable plot (Fig. S3). The coordinates of the test variables were calculated as correlation between the variables and the principal components (the product of the loadings and standard deviation of the principal component) using *factoextra* package in R.

4. Discussion

Rejuvenation of degraded land proffer a solution that can restore poor soil to arable state and mitigate adverse effect of contaminated soil on human or environmental health. The use of organic amendments to improve physical properties of poor soil has been reported in a number of studies (Hornick and Parr, 1987; Mbuthia et al., 2015; Karimuna et al., 2016) but have not been broadly investigated in combination with AMF inoculant. More so, the screening of amendments and inoculum that is needed to enrich the soil will help against over fertilization, leaching of nutrient into the soil (Cui, et al., 2010; Fernández-Escobar et al., 2006; Geng et al., 2019; Song et al., 2016) while also allowing selection of the best treatment for soil restoration.

The commercial mycorrhiza (AMF) product used in this experiment improved biomass production for both aboveground and belowground biomass. This phenomenon has been investigated in different type of growth system (Berdeni et al., 2018; Cobb et al., 2018; Hahl et al., 2017; Moland et al., 2018; Ren et al., 2017). However, in this study, we screened amendment and inoculation strategies for enhancing biomass productivities of SG in RMS using a unique system that allowed us to investigate the impact of single strategy (inoculation or amendment) and combined strategy (inoculation and amendment) all within the same MRM system. Specifically, for the amendments we used three organic amendments - VC, PMS, and PL- and one inorganic amendment - N + P, while we used a commercial preparation of AMF that contains various species of endomycorrhizae. This experiment was carried out in an MRM system that allowed enough moisture delivery (approximately

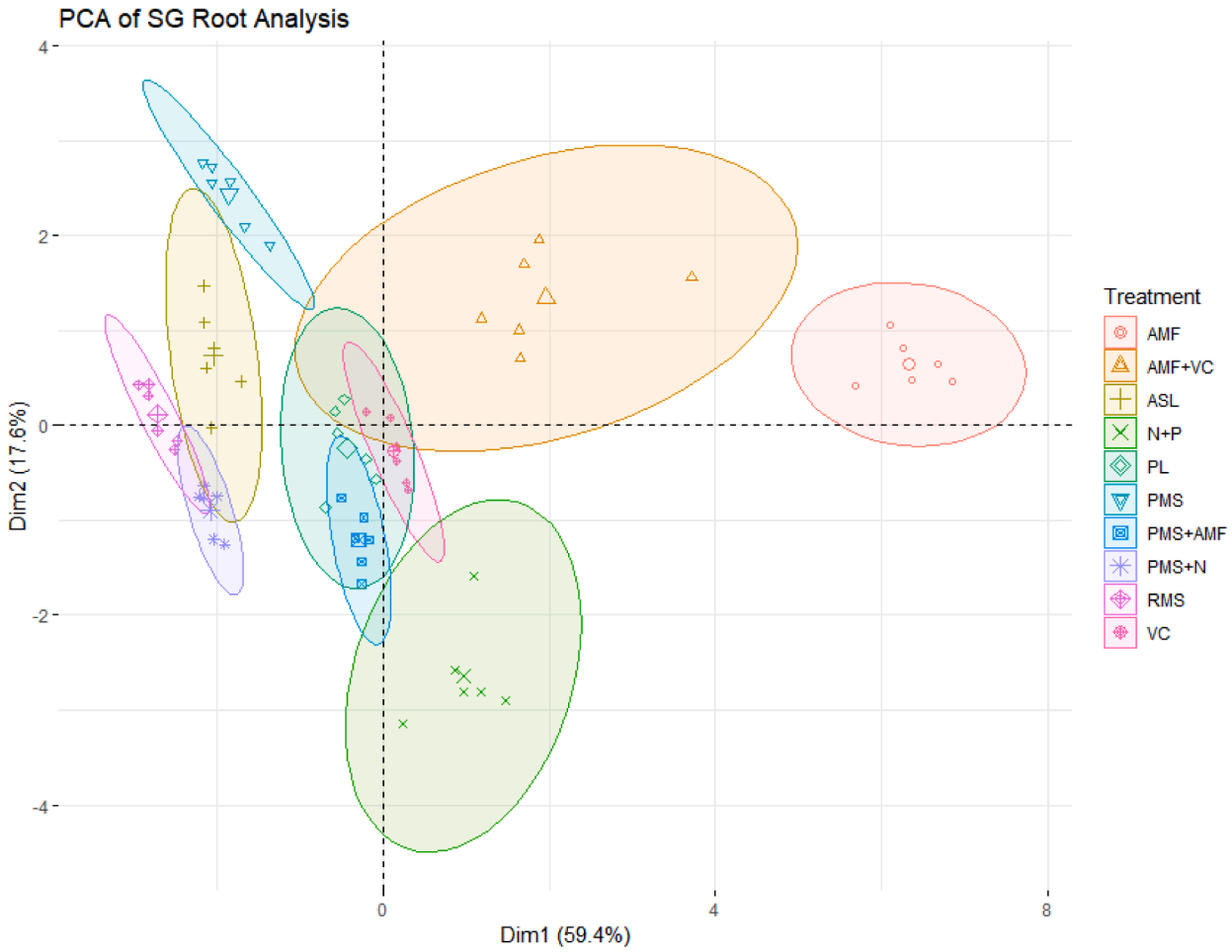

Fig. 10A. PCA plot of the entire SG root traits. Eclipse grouping of the entire data set by treatments. PCA plot of individuals with added ellipses having a 95% confidence level (0.95) at the center of the ellipses. The individual values are grouped together on the plot based on similarity of the reading. The coordinates of each group of eclipse is calculated as the mean coordinates of each individual reading within the group and this is represented as the bigger shape in the center of the eclipse.

70%) to the soil without necessarily causing flooding that will inhibit the establishment of AMF (Hartmond, et al., 1987; Miller & Sharitz, 2000; Ma et al., 2014) in SG roots.

Previous studies have reported that colonization rate does not always translate to enhancement of biomass by different strains of arbuscular mycorrhizae fungi colonized (Corkidi et al., 2005). As different strains may contribute different effect towards plant growth (Allen et al., 2003; Linderman & Davis, 2004; Corkidi et al., 2005) so there is need to correlate the colonization of plant root with enhancement. In this study, the commercial AMF used at the optimum inoculum increased the AGB of *S. bicolor* by 137.5% compared to the uninoculated (or native mycorrhizae) control soil. Tae et al. (2002), Eo & Eom (2009), and Kim et al. (2017), all reported positive growth increase in the AGB of *S. bicolor* inoculated by AMF.

The use of organic amendment increased the height of SG compared to the control soil. The amendment of degraded soil with VC, PMS + N, PMS, PL, N + P increased the height of SG compared to the control soil. In addition, inoculation of poor soil with AMF and the combination of the AMF with VC significantly improved the height of the SG. This is in agreement with the studies of Corkidi et al. (2005) that reported increase in height and leaves (DW) of plant inoculated with commercial mycorrhizae compared to their control. Furthermore, the height and the dry weight of the AGB showed significantly positive correlation.

The dry weight of the AGB, BGB and total biomass followed similar trend as the height with the AMF alone and AMF + VC treatments significantly enhancing the SG biomass 8 weeks after transplanting. The addition of commercial AMF improved the plant growth despite the low phosphorus and high copper presence in the degraded soil. Mycorrhizal have been reported to be more active in soil with low phosphorus content (Hetrick, et al., 1996; Shaheen & Tsadilas, 2013). Also endomycorrhizae is reported to alleviate heavy metal toxicity (Cicatelli et al., 2012; Schneider et al., 2013; Aghababaei et al., 2014; Berruti et al., 2015; Awoyemi & Adeleke, 2017; Awoyemi et al., 2019) by inducing heavy metal transporter ATPase 5 (HMA5) in root for copper efflux (Andrés-Colás et al., 2006; Kobayashi et al., 2008; Palmer and Guerinot, 2009) or chelation of heavy metal in rhizospheric soil leading to metal dilution in plant tissue (Begum et al., 2019).

The colonization of the root by the endomycorrhizae from the commercial AMF was significant in the AMF and AMF + VC treatment while there was low colonization in other inoculated treatment. The colonization of roots that led to significant biomass increase within 8 weeks is in line with the results of Corkidi et al., (2005) and Faye et al., (2013) who both stated 6 weeks was enough to establish good mycorrhizal colonization of root.

The enhancement of the dry weight of BGB 8 weeks after transplanting changed the root phenotypic trait in the SG plant. The positive influence of inoculation with commercial AMF and addition of soil amendment changed the root system architecture of the AMF and AMF + VC treatment. However, the root system architecture of the AMF treatment showed the most apparent change as the length and shape phenotypic traits- root count, TRL, TRA, TRV, ARD, ARL, ARA and ARV- all significantly increased while the angular phenotypic traits- ARAngle,

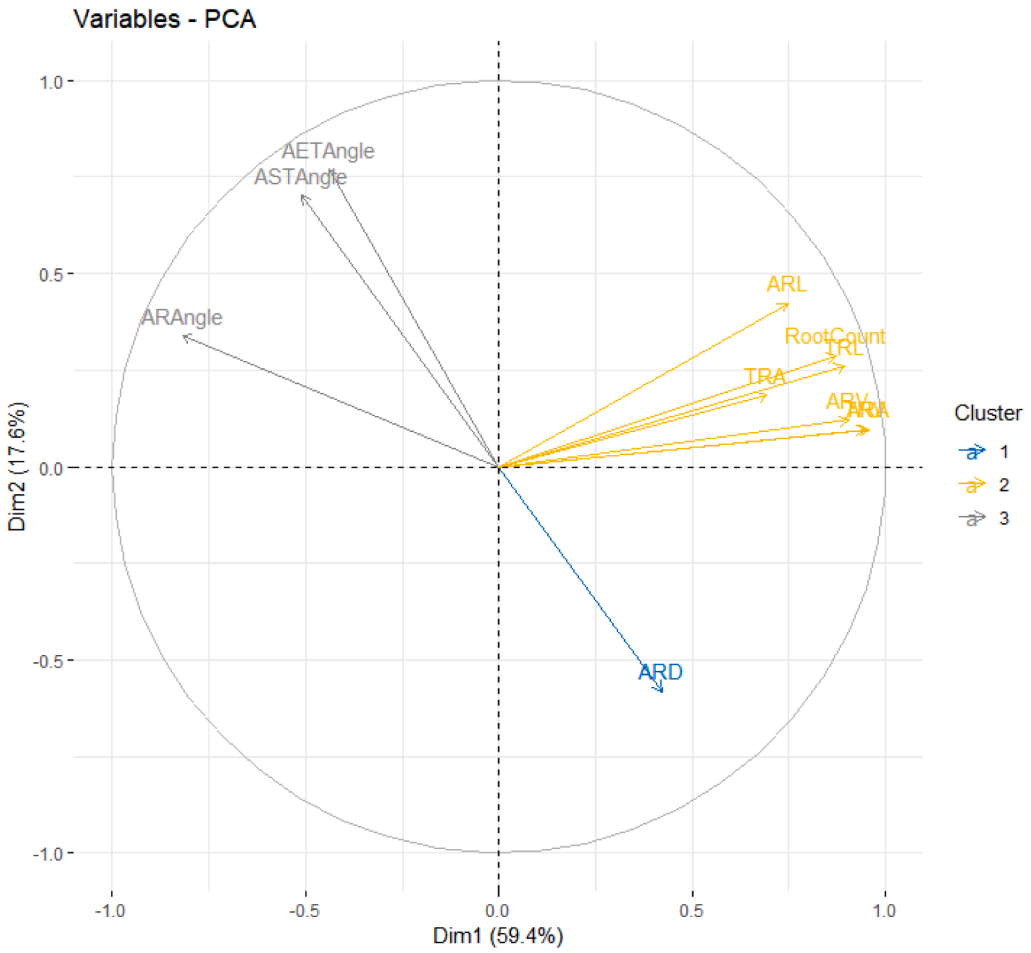

Fig. 10B. Dim1 represents the principal component (PC) 1 and Dim2 is the PC2. The PC1 was able to explain 59.4% of the total variable while PC2 was able to explain 17.6% of the total variable. The three clusters in the graph were determined using the *kmeans* clustering function in R. The first cluster is made up of the ARD; the second cluster is RootCount, TRV, TRA, TRL, ARV, ARA, and the ARL; and the third cluster is AETAngle, ASTAngle, and the ARAngle.

ASTAngle and AETAngle- were significantly lower than the root traits of the SG in uninoculated/unamended treatment. There were a lot of influence and variability with other treatments on phenotypic traits which is in line with the results of Eo & Eom (2009) and Hetrick et al., (1996) who reported that root morphology, RSA and soil nutrients influence mycorrhizal responsiveness or symbiose in their host plant (gramineae). SG belong to the family gramineae that have been reported as an important group of plants that form symbiotic association with AMF (Gutjahr, et al., 2009, 2015). In addition, Gutjahr et al. (2015) and Bernaola et al. (2018) studies on rice root system reported that fine roots are not susceptible to colonization and that large lateral roots are more susceptible to colonization than the fine lateral roots that may even never be colonized which agrees with our findings that ARD played important role in mycorrhizal responsiveness in AMF-only treatment but was not significant in other treatment except in the N + P treatment which was the only inorganic amendment applied to the degraded soil. The role of AMF in improving the root lengths traits and reducing the root angle traits singled out the treatment as a suitable candidate for enhancing drought tolerance while concomitantly using the AGB as biofuel feedstock or forage purposes. However, the unresolved question in this experiment is whether the enhancement of SG is only as a result of mycorrhizal, and if so, which species of the AMF or possibly beneficial bacteria in our commercial AMF product was responsible. Although it has been reported that combinations of AMF have different effects (Bernaola et al., 2018a; Berruti et al., 2015; Cruz & Ishii, 2012; Porras-Soriano, et al., 2009; Roger et al., 2013) and that role of colonized AMF species extends beyond plant growth enhancement (Ramasamy et al., 2011).

Plant functional traits have been reported to improve soil properties and it has also been considered as a crucial regulator of important ecosystem function and services – like soil nutrient dynamics and availability (Faucon et al., 2017). Our findings are in agreement with this report as soil amendments and inoculation has influenced root traits that are important drivers of nutrient dynamics and soil fertility. Physiological root traits are the functional traits that are involved in nutrient dynamics and availability by modifying nutrient speciation and influencing amendment decomposition for nutrient release (Faucon et al., 2017, 2015; Hobbie, 2015; Roumet et al., 2016). Assimilation of nutrient released as protons or carboxylates to solubilize the inorganic and organic phosphorus in the rhizosphere can be enhanced by the presence of AMF. The combined effects of AMF and fine roots have been reported as a key force that drives soil aggregation (Bardgett et al., 2014) as glomalin released by AMF serve as a binding agent. Furthermore, the role of improving AGB of SG by the combined effects of AMF and fine roots makes it a suitable combined treatment for high biomass yield that can result in initial net energy value (Schmer et al., 2008) that will result in higher ethanol yield.

The phenotypic correlation among root traits allowed us to cluster the traits into 3 groups – root length and shape group, diameter group, and the angle group- based on their association and this cluster is similar to what we also found with the PCA variable plot that showed the same group. The correlation between the root length and shape group was very strong (Fig. 10B). This association contributed to majority of the first principal component of the PCA result while the diameter and angle

group contributed to the second principal component of the PCA result. The combination of HCA and PCA to explore the individual sample allowed to cluster the variability in our result to three major groups of high, medium and low enhanced root phenotypes. The highly enhanced root samples composed of AMF treatment only, reaffirm the benefit of AMF in establishment of seedling and early growth in poor soil, this is in line with our previous findings in fly ash contaminated soil (Awoyemi et al., 2019). The medium-enhanced root was topped by AMF + VC which showed synergistic association between AMF and VC, this is in agreement with the findings of Ramasamy et al. (2011) who reported the synergistic effects of AMF and plant growth promoting rhizobacteria play in the role of improving soil fertility and plant health. The lowly-enhanced roots were those that showed very little enhancement compared to the unamended/uninoculated soil. They hardly showed any enhancement in the combination of both inoculation and amendment strategies 8 weeks after transplanting. Using PCA predictive model for supplementary variables of height, dry weight of shoot and root also showed that the predicted variables are strongly correlated to the length and shape group. However, their contribution to the first principal component was very low.

The SRL and RTD that explained accumulation of root biomass per meter and root biomass per volume respectively showed significant increase in the VC and PMS + N treatment but was not able to contribute significant information to explain biomass accumulation in the root further. RP is the amount of root length that is produced per volume of soil per 8 weeks after transplanting. This provided valuable information, with similar trend to the total root length and height. The production of root length showed AMF, VC, and AMF + VC were significantly different from others.

Finally, GE_r and GE_s percentage increase affirm that AMF, VC and AMF + VC were the best treatment for biomass enhancement in the RMS soil. In addition, the mycorrhizal dependency showed that AMF had the highest dependency followed by AMF + VC which is in agreement with the studies of Beltrano et al. (2013) which reported a higher mycorrhizal dependency and enhancement of plant shoot and root biomass in mycorrhizal plant than non-mycorrhizal plant. However, paper mill sludge had an inhibitory effect on colonization of mycorrhizal in plant root at the early stages which is also responsible for the negative effect of the mycorrhizal dependency in PMS + AMF treatment. The high carbon content of PMS may have been responsible for anti-synergistic effect on AMF by offsetting the balance of the soil C-N ratio therefore allowing the dominance of copiotrophic population and other fungi like *Trichoderma* sp. Although this treatment may have hindered early establishment of the SG, a long-term study may provide better insight as the copiotroph dominance wane into equilibrium. However, growth inhibition and stunted growth was also noted in all the PMS amendment within the first 3 weeks which may be due to increased water holding capacity of this treatment resulting in excessive moisture or nitrogen immobilization due to high C/N ratio created by PMS addition. Therefore, there is need to exercise caution in application of PMS to degraded soil.

A major limitation of our experiment is the inability to understudy the amendment and inoculation strategy on a long-term study. Transferability of our result to field study also require caution as other environmental conditions may influence repeatability in the field. The application of AMF treatment at field scale may also pose a challenge especially when scaling up our application rate. Therefore, exploring the native AMF of each degraded soil for future studies may make a significant difference especially when combined with organic amendment like VC or PMS.

5. Conclusions

This study reveals that there is strong correlation between the height and dry weight of AGB that allowed us to select four treatments that distinctly showed enhancement by both inoculation and amendment strategies - AMF, AMF + VC, VC, PMS + N - for further investigation in mesocosm or field study. Although the use of commercial AMF only is not feasible in a large-scale field trial, the combination of AMF + VC would be more suitable approach for biomass establishment, enhancement and soil health improvement in mesocosm and field trials.

The diversity observed in shoot and root dry weight and other root traits showed that combination of inoculation and amendment strategies require prior screening before application in degraded soil in order to select the best treatment for enhancing plant biomass and improving soil health. Also, the diversity observed among the root traits require further investigation to determine nutrient accumulation as well as heavy metal content in the plant root.

Future studies about accumulation of biomass during its enhancement would be beneficial by determining the relative growth rate of the SG when the experiment is carried out on a longer term or more than one harvest.

CRediT authorship contribution statement

E. Adeleke: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **E. Dzantor:** Project administration, Resources, Software, Supervision, Validation. **A. Taheri:** Project administration, Resources, Software, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.107068.

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