



Potential of Vetiver (*Chrysopogon zizanioides* L.), Inoculated With Arbuscular Mycorrhizal Fungi, To Improve Soil Quality in Degraded Soil

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ABSTRACT

Alterations of healthy soil properties can result from land degradation. However, application of some grass species that are colonized with arbuscular mycorrhizal fungi (AMF), such as vetiver (*Chrysopogon zizanioides* L.), can mitigate the negative impacts of soil degradation. The present study evaluated the ability of AMF-colonized vetiver to improve the quality of degraded soil in pine and tea plantations, as well as in adjacent secondary forest. Applications of organic manure and biochar were also tested in conjunction with AMF to examine their comparative effects on soil rehabilitation. The different treatments were significantly different ($p \leq 0.05$) in their effects on soil pH, soil microbial activity, bacterial count, fungal count, and percentage of AMF colonization. Vetiver application, with and without the additional soil amendments, improved the degraded chemical and biological soil properties to comparative levels with the healthy soil of a nearby mountain region. Therefore, vetiver application, with the added AMF, biochar, and organic manure, can be effectively used in soil quality amelioration.

Keywords: arbuscular mycorrhizal fungi, *Chrysopogon zizanioides* L., pine plantation, soil degradation, soil quality improvement

1. INTRODUCTION

Soil degradation is the decline in productivity or functional capacity of the soil which adversely changes biological, chemical, physical, and hydrological properties of soil due to deforestation, overgrazing, agricultural practices, overexploitation of the vegetative cover, and industrial activities [1,2]. Soil degradation is becoming a severe environmental issue in this era owing to its impact on world food security and the quality of the environment. Soil

quality is defined as the potential of soil within an ecosystem to influence sustainable biological productivity, maintain environmental quality, and promote plant and animal health including human health [3]. Exploited lands used for long-term continuous cropping and excessive use of chemical fertilizers have caused a serious decline in soil quality [4-6].

Sri Lanka is covered with manmade forest plantations and agro-cultivation areas which consist of even-aged monocultures that include exotic species such as Caribbean pine (*Pinus caribaea*) and tea (*Camellia sinensis*) [7, 8]. Studies have shown that several negative consequences associated with pine and tea cultivated mountain slopes can lead to soil degradation [9]. Groundwater depletion, forest fires, soil erosion, nutrient loss, and other negative impacts on forest regeneration and wildlife are characteristic issues associated with pine plantations [10, 11]. The organic carbon content of the soil is also low in pine plantations due to the slow decomposition rate and the dry litter layer. In addition, heavy layers of needle litter inhibit the germination of seedlings of some species [12, 13], and contributes to high acidity in the soil to the high level of phenolic compounds and other accumulated allelochemicals [8]. Cultivated lands also have low organic matter content due to the removal of shade trees and surface leaf litter. As a result, the water holding capacity of the soil is hampered, the water table is lowered and the moisture level in the surface layers of the soil is reduced. During times of high precipitation, the soil is then eroded, causing soil infertility. Due to this infertility, synthetic fertilizers containing nitrogen (N) [9], phosphorous (P), potassium (K), and magnesium (Mg) must be applied to crops in large quantities each year, resulting in a negative impact on the soil. Additionally, the application of synthetic inorganic fertilizers such as ammonium sulfate has reduced the soil pH [14], and excessive use of these fertilizers increases chemical leaching, causing groundwater pollution [15].

Soil rehabilitation has potential to mitigate these negative impacts and restore biological functions of soil, while also increasing the ecosystem processes, services, and productivity. Application of some grasses and plant growth-promoting, such as arbuscular mycorrhizal fungi (AMF), can help accomplish this mitigation and aid in rehabilitating ecosystems [16, 17].

Vetiver, *Chrysopogon zizanioides*, is an important aromatic plant that belongs to the Family Poaceae, which can tolerate extreme climatic variations such as prolonged drought, flood, submergence [18], and extreme temperatures ranging from -22°C to 60°C . It has a high tolerance to a wide pH range from 3 to 10.5, is tolerant to heavy metals, and has the potential to establish under constant shade, even for decades [19]. These properties of vetiver make it an eco-friendly grass useful for growing in degraded lands [20]. In 1986, the World Bank introduced the vetiver system technology (VST) for soil and water conservation in developing countries worldwide [21]. Due to its unique morphological and physiological characteristics, vetiver can play a special role in phytoremediation, phytostabilization, phytoextraction, and phytofiltration [20, 22]. Soil and water conservation, erosion control, slope stabilization, absorption of heavy metals, and wastewater treatment are some examples of its beneficial effects [18, 22-24]. Practical applications of harvested vetiver grass include uses in mulching, composting, insect repellants, pesticides, antimicrobial agents, flavoring agents, and fragrance agents [25, 26]. In addition, vetiver grass is colonized extensively by arbuscular mycorrhizal fungi [27], which enhances its potential for soil rehabilitation [28, 29].

Arbuscular mycorrhizae have the potential to enhance productivity in low fertility soil [30] by increasing the availability of ions and nutrients such as NO_3^- , PO_4^{3-} , P, Zn, and Cu [31, 32], and by enhancing plant tolerance of abiotic and biotic stressors such as salinity [33, 34] and heavy metal contamination [35, 36]. Studies have revealed that AMF have the ability to increase soil quality, soil biodiversity, seedling survival, plant growth, and productivity [31, 32].

Despite the urgent demand for sustainable agriculture, the re-vegetation and rehabilitation of areas degraded by human activities remain challenging. Currently, vetiver grass is being used to rehabilitate and reclaim degraded systems such

as mines and coastal areas as well as in wastewater treatment systems. Thus, the current study was conducted to evaluate the ability of vetiver grass to ameliorate degraded soil in conjunction with the additional soil amendments of biochar, organic manure, and AMF. The findings of the study may contribute towards the application of vetiver grass towards the rehabilitation of agricultural fields and forest plantations, such as pine plantations, by mitigating negative effects and restoring degraded soil.

2. MATERIALS AND METHODS

2.1 Study Area and Weather Conditions

The research was conducted from January 2018 to July 2018 at Panwila, Kandy, Sri Lanka (7 20' 496" North, 80 38' 512" East). Laboratory analyses were done at the Faculty of Applied Sciences of Rajarata University of Sri Lanka (8 21' 0" North, 80 30' 0" East). The average environmental temperature was 24.0 °C - 30 °C and the average precipitation was around 440 mm during the pot experiment and soil sample collection at Panwila.

2.2 Sample Collection and Preparation of Amendments

Soil samples were collected from three different plantation types, pine plantation (7 22' 206" North, 80 42' 560" East), adjacent secondary forest (7 22' 228" North, 80 42' 563" East), and tea (*Camellia sinensis*) plantation (7 22' 230" North, 80 42' 568" East), in Panwila, Kandy, Central province of Sri Lanka. Some of the dominant tree species of adjacent secondary forest vegetation are *Semecarpus nigro-viridis*, *Alstonia macrophylla*, *Terminalia bellirica*, and *Elaeocarpus serratus*. A composite soil from each vegetation was gathered by 20 randomly selected points using 1m × 1 m quadrates, and each quadrate was sampled for two depths of the soil, surface 0-15 cm and 15-25 cm depths. Uniformly weight grass clumps from the grass bush of vetiver (*Chrysopogon zizanioides* L.), was selected for the present study. Prior to pot experiments, AMF colonization percentage was

verified for randomly selected vetiver plant roots by using the modified grid intersect method [37]. Dried and ground cow dung and *Gliricidia* sp. leaves were mixed in a 1:1 (w/w) ratio for organic nutrition supplement.

Biochar was prepared by pyrolysis of wood using a double barrel method. Two metal barrels, one larger and the other smaller were used. A small air inlet was prepared in the larger barrel. Then the small barrel was filled with the forest woods as tight as possible. The filled vessel was placed on a pair of bricks. Then it was covered with the larger barrel and wood was burnt. After about one hour when pyrolysis of the biomass had aborted, charcoal was collected.

The trap culture technique was used to prepare the arbuscular mycorrhizal fungal inoculum. Maize (*Zea mays*) was used as the bait plant and seeds and roots collected from home gardens around Panwila area. Maize seeds were soaked in water and well aerated for two days and were planted in the pots with sieved soil collected from the natural forest edges in Panwila, Kandy. Eleven pots were transplanted with seeds and 30-45 maize seeds per pot. After two weeks maize plants were uprooted and roots were separated. Then the roots were cut into pieces about 2 inches and mixed with the trap cultured soil.

2.3 Experimental Design

The study was carried out as a factorial experiment in randomized complete block design. Vetiver plants were grown under each treatment including control with four replicates. The control contained non treated pure soil collected from the plantations of pine, tea, and adjacent secondary forest. The treatment combinations are soil with 5% biochar, soil with 5% organic manure, soil with native AMF, and soil with 2.5% biochar, 2.5% organic manure, and native AMF.

2.4 Data Collection and Sample Analysis

The soil was sampled initially just before the vetiver was introduced and end of the study

into sterile containers prior to the soil chemical and biological properties analyses. End of the six months the vetiver plants were uprooted and stored prior to analyzing the plant growth parameters.

Soil pH (Soil suspension of 10 g soil: 25 ml deionized water was measured using the HANNA pH meter) [38]. Soil microbial activity was measured by absorbing CO_2 by NaOH and titrated with HCl after the addition of BaCl_2 to precipitate NaOH. Microbial count and diversity were measured following standard serial dilution and plate count method. Total organic carbon (TOC%) was measured following standard methods [39] Percentage of AMF colonization of vetiver roots was determined using the modified grid transect method [37].

The soil from the forest patch of the Knuckles mountain range ($80^\circ 48'$ East, $7^\circ 24'$ North) which is subjected to similar climatic conditions as Panwila, was tested for soil parameters and was used as the reference for healthy soil in statistical analysis.

2.5 Statistical Analysis of Data

Data were analyzed using mean differences of initial and final data in MINITAB 17.1.0. Tukey's method and 95.0% Confidence were used to test the differences among treatment means at a significance level of $p \leq 0.05$.

Deviation of initial and final data with respect to the reference data was calculated for

each parameter for three soil type and used in statistical analysis to determine the p values in paired T-test whereas difference among initial and final deviation of each parameter was used in Turkey's method to evaluate the treatment combinations.

3. RESULTS AND DISCUSSION

3.1 Chemical and Biological Measures of Reference Soil

The composite soil sample of 200 g was collected from the Knuckles mountain range which is a natural forest in the same climatic conditions as in Panwila area and used as the reference healthy soil (Table 1). It has been reported that the soil pH 6.0-7.5 is most productive [38].

3.2 Soil pH

The surface soil of pine plantation was shown the highest treatment effect while 15-25 cm soil of tea plantation with native AMF inoculum showed the lowest effect (Figure 1). Contrary, a drastic pH deviation away from the reference was observed in 15-25 cm depth tea plantation soil with biochar.

In consonance with several studies, vetiver grass has the ability to increase the soil pH by root exudation of anions such as hydroxyl to balance the cell charge during the uptake of nitrates and phosphates [34] and alkalinity of biochar can remediate the soil acidity [40].

Table 1. Soil parameters of the Knuckles mountain range.

Parameter	Soil depths (cm)	
	0-10	15-25
pH	7.14	7.07
Microbial activity (mg CO_2 /gmin)	3.2291	3.1324
Bacterial count (CFU/g)	5×10^8	3×10^8
Fungal count (CFU/g)	2×10^8	1×10^8
TOC (%)	6.5432	6.3254

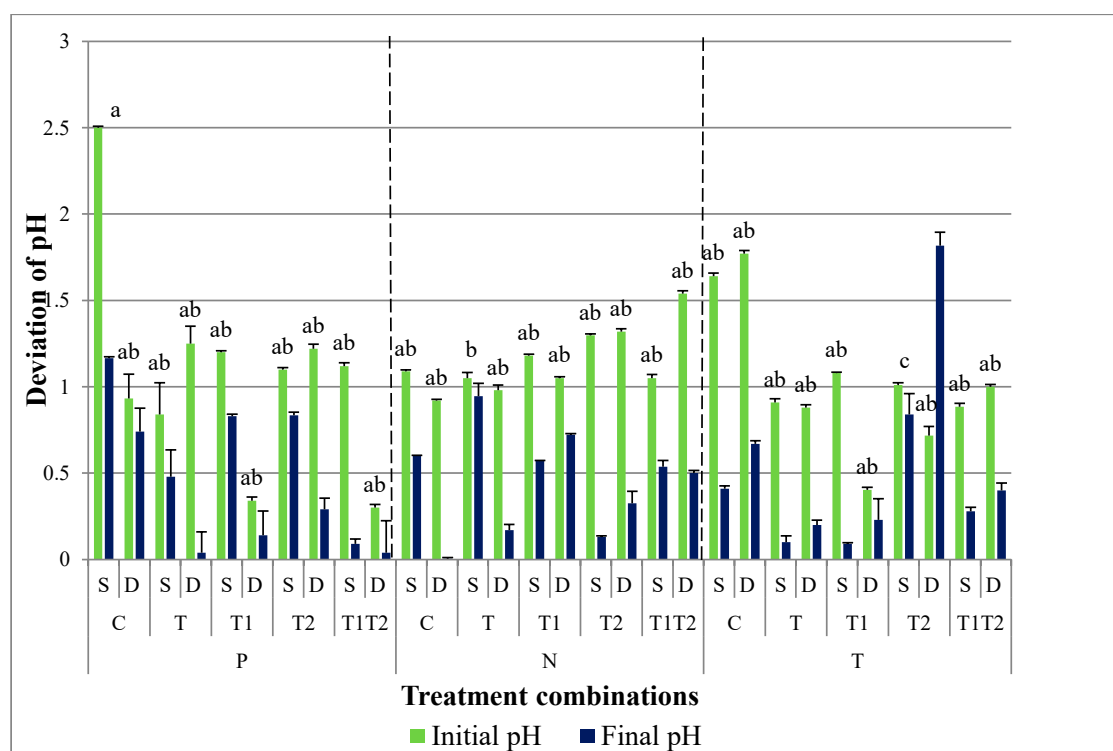


Figure 1. Deviation of pH with respect to the reference with different treatments. Means that do not share a same letter are significantly different ($p < 0.05$).

[P: *Pinus* plantation, N: Adjacent -forest to the *Pinus* plantation, T: Tea plantation, S: 0-10 cm depth of soil, D: 15-25 cm depth of soil, C: Control/Soil, T: Soil with organic manure, T1: Soil with native AMF inoculum, T2: soil with biochar, and T1T2: soil with native AMF inoculum and biochar].

3.3 Soil Microbial Activity

The highest treatment effect was claimed by adjacent secondary forest, surface soil with organic manure, while 15-25 cm soil of pine plantation was the lowest (Figure 2).

According to the studies, appropriate utilization of manures on crops within management systems can increase levels of plant nutrients and enhance soil microbial biomass, activity, and diversity [41]. Increases in the microbial population have been linked with organic management as well as various organic amendments, such as livestock manure, crop residue, and green manure [42, 43] which increase the microbial activity. The activity of

microbial populations is higher in the rhizosphere of vetiver grass. The soil microorganisms associated with vetiver root are nitrogen fixing bacteria, phosphate-solubilizing microbes, mycorrhizal fungi, and cellulolytic microorganisms [44]. The substances in the exudates of vetiver root served as nutrients and energy sources for the growth of microorganisms in the rhizosphere [45, 46]. Hence the highest effect may be caused by both vetiver grass and the organic manure associatively.

3.4 Soil Fungi and Bacteria Population Count

Results from the present experiment demonstrated that the soil microbial population

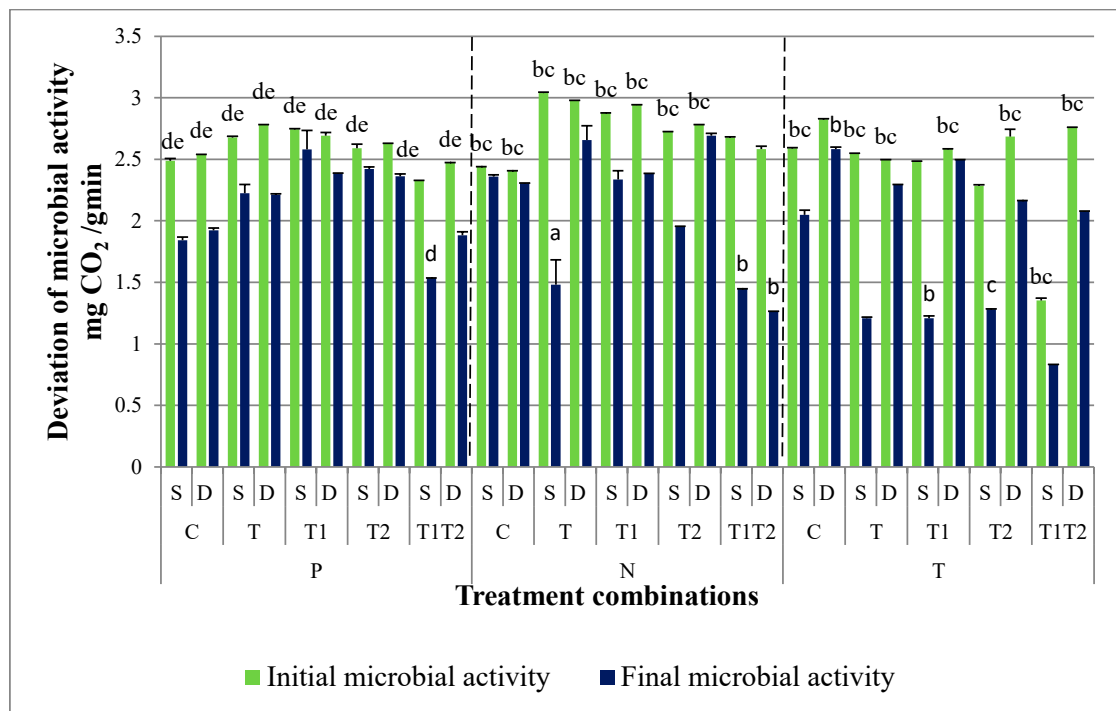


Figure 2. Deviation of microbial activity with respect to the reference with different treatments. Means that do not share a same letter are significantly different ($p \leq 0.05$).

[P: *Pinus* plantation, N: Adjacent -forest to the *Pinus* plantation, T: Tea plantation, S: 0-10 cm depth of soil, D: 15-25 cm depth of soil, C: Control/Soil, T: Soil with organic manure, T1: Soil with native AMF inoculum, T2: soil with biochar, and T1T2: soil with native AMF inoculum and biochar].

has increased with all the treatments. Although the analysis was carried out with concerning to the reference forest Knuckles mountain forest soil, microbial populations have deviated away from the reference which means treatments have increased the soil microbial population than the reference soil which is good for the soil fertility.

Contrary, the surface soil of pine forest, with added organic manure was shown the highest soil fungal population while tea plantation surface soil with added native AMF and biochar has the lowest population of fungi. However, non-treated tea plantation surface soil and adjacent secondary forest surface 15-25 cm depth soil with native AMF possessed the highest fungal populations

on tea plantations and adjacent secondary forest, respectively (Table 2).

However, the highest treatment effect was observed for the bacterial population in non-treated surface soil of tea plantations while the lowest effect in pine plantation surface soil treated with native AMF and biochar. Yet, adjacent forest 15-25 cm depth soil with native AMF inoculum and biochar and pine plantation surface soil with native AMF inoculum was shown the highest effect on bacterial populations on adjacent secondary forest and pine plantations (Table 2).

A higher fungi/bacteria ratio can reflect the relative abundance of the microbial population which is an important indicator of a stronger soil

Table 2. Soil microbial count with different treatments.

Vegetation type	Treatments	Depth of the sampling (cm)	Average of bacterial count CFU/ g $\times 10^6$		Average of fungal count CFU/ g $\times 10^6$	
			Initial	Final	Initial	Final
Pine plantation	Control/Soil	0-10	3.00 \pm 1.24	100.00 \pm	0.02 \pm 0.008	2.00 \pm 1.24
		15-25	3.00 \pm 0.08	275 \pm 50	0.03 \pm 0.0002	2.00 \pm 0.45 ^c
	Soil with organic supplement mixture	0-10	14.00 \pm 2.58	100 \pm 0.56	2.25 \pm 1.50	200 \pm 21 ^a
		15-25	16.75 \pm 2.36	100 \pm 24	4.00 \pm 0.02	100 \pm 7
	Soil with AMF	0-10	2.50 \pm 3.00 ^a	375 \pm 50	4.00 \pm 0.81	100 \pm 12
		15-25	16.00 \pm 1.82	400 \pm 35	2.00 \pm 0.015	75 \pm 50
	Soil with biochar	0-10	10.00 \pm 1.82	10000 \pm 28	3.00 \pm 1.02	100 \pm 32
		15-25	1.00 \pm 0.05	100 \pm 4	1.00 \pm 0.008	150 \pm 173
	Soil with AMF and biochar	0-10	6.00 \pm 1.63	30000 \pm 45	2.00 \pm 1.23	10000 \pm 541
		15-25	3.25 \pm 1.25	300 \pm 41	2.00 \pm 0.021	200 \pm 120
Adjacent secondary forest of pine plantation	Control/Soil	0-10	2.75 \pm 0.50	750 \pm 500	1.00 \pm 0.31	100 \pm 38
		15-25	0.42 \pm 0.0095	100 \pm 12	0.02 \pm 0.0004	1.50 \pm 1.00
	Soil with organic supplement mixture	0-10	7.00 \pm 0.65	100 \pm 0.5	1.00 \pm 0.08	100 \pm 10
		15-25	1.00 \pm 0.002	100 \pm 2	1.00 \pm 0.008	100 \pm 0.8
	Soil with AMF	0-10	1.00 \pm 0.81	200 \pm 54	1.00 \pm 0.01	100 \pm 14
		15-25	1.00 \pm 0.05	100 \pm 32	1.00 \pm 0.003	100 \pm 16 ^b
	Soil with biochar	0-10	3.00 \pm 1.41	100 \pm 21	3.00 \pm 1.21	100 \pm 0.9
		15-25	2.25 \pm 1.70	100 \pm 21	2.00 \pm 1.08	200 \pm 78
	Soil with AMF and biochar	0-10	100.00 \pm 4.03	10000 \pm 38	1.50 \pm 1.00	10000 \pm 52
		15-25	3.00 \pm 0.81	300 \pm 110 ^b	2.00 \pm 0.08	200 \pm 71
Tea plantation	Control/Soil	0-10	18 \pm 1.82	600 \pm 81.64 ^a	0.03 \pm 0.002	100 \pm 45 ^b
		15-25	0.75 \pm 0.5	200 \pm 52	0.01 \pm 0.0007	1.50 \pm 0.014
	Soil with organic supplement mixture	0-10	27.00 \pm 2.94	1.00 \pm 0.003	0.007 \pm 0.005	10000 \pm 1258
		15-25	13.75 \pm 4.11	100 \pm 25	0.015 \pm 0.017	1.00 \pm 0.005
	Soil with AMF	0-10	20.00 \pm 2.58	10000 \pm 54	1.00 \pm 0.0001	100 \pm 15
		15-25	14.25 \pm 2.50	225 \pm 150	1.00 \pm 0.02	100 \pm 0.4
	Soil with biochar	0-10	22.00 \pm 2.44	20000 \pm 126	1.00 \pm 0.004	10000 \pm 452
		15-25	15.75 \pm 1.70	100 \pm 57	1.00 \pm 0.031	100 \pm 45
	Soil with AMF and biochar	0-10	10.00 \pm 11.80	10000 \pm 165	1.00 \pm 0.1	20000 \pm 72
		15-25	7.00 \pm 2.94	300 \pm 15	1.00 \pm 0.78	200 \pm 14

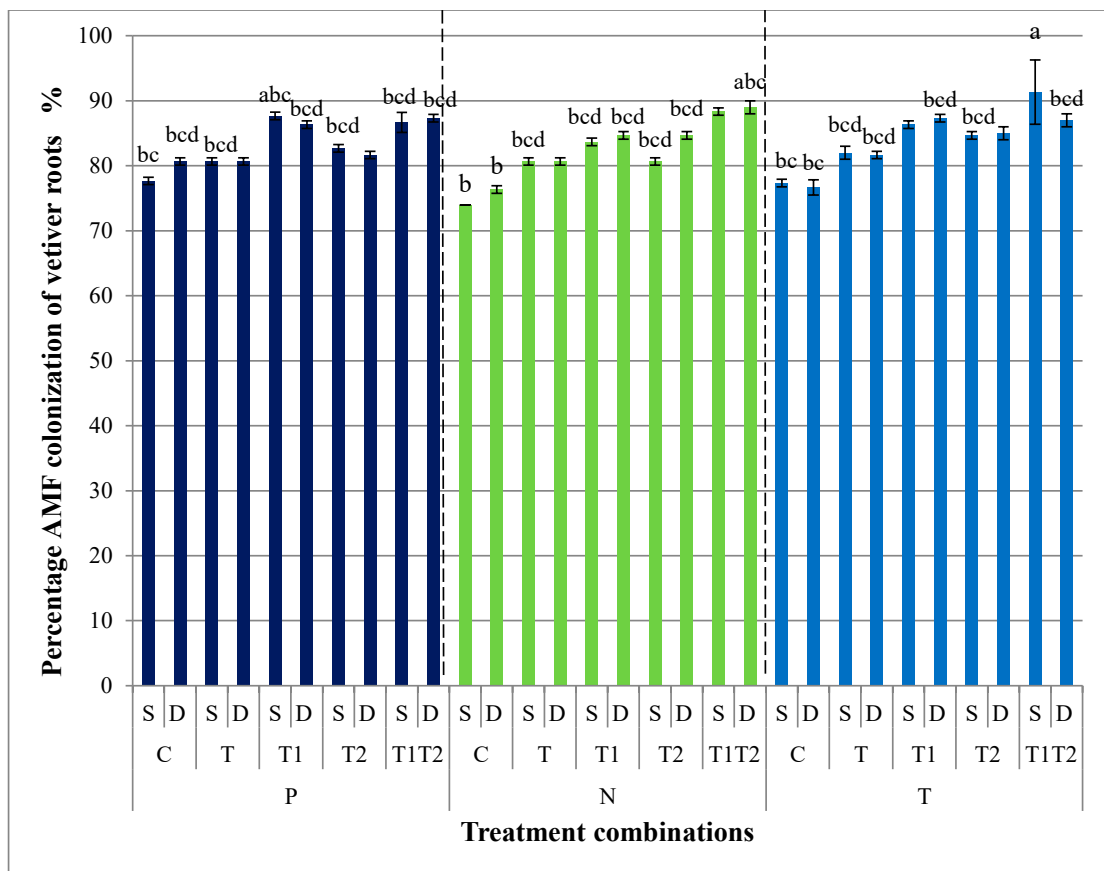


Figure 3. Percentage AMF colonization of vetiver roots with different treatments. Means that do not share a same letter are significantly different ($p \leq 0.05$).

[P: *Pinus* plantation, N: Adjacent -forest to the *Pinus* plantation, T: Tea plantation, S: 0-10 cm depth of soil, D: 15-25 cm depth of soil, C: Control/Soil, T: Soil with organic manure, T1: Soil with native AMF inoculum, T2: soil with biochar, and T1T2: soil with native AMF inoculum and biochar].

ecosystem buffering capacity and more sustainable land [47]. The root exudates of the vetiver secrete into the rhizosphere and it consists of some organic substances such as soluble carbohydrates, organic acids, amino acids, and growth hormones which influence the soil microbial growth [45, 46]. The population of specific groups of microorganisms as cellulose hydrolyzing microorganisms especially non-symbiotic nitrogen fixing bacteria and phosphate solubilizing microorganisms is being

increased by vetiver grass [44]. Organic manure application enhances the soil enzyme activity and soil microbial biomass [48]. The mycorrhizal hyphae promote soil aggregate formation and stability via biological, physical, and biochemical mechanisms which increase soil aeration and water infiltration, which together improve the soil quality. Studies revealed that AMF symbiosis influences the composition of the microbial community in the rhizospheric soil and its activity [49].

Mycorrhizal fungi can enhance the number and activity of beneficial soil organisms like nitrogen fixers and phosphate solubilizers leading to the improvement of plant growth [50], and enhance in the number of aerobic bacteria in the rhizosphere [51]. Particularly, biochar amendment can change soil biological community composition and its abundance [52] and enhance resistance against pathogens and diseases [53].

3.5 Percentage Colonization of AMF

The initial percentage of AMF colonization of the bait plant roots were 74.26% and 80.14% for vetiver grass before the pot experiment. According to the statistical analysis, treatments were significantly different for the percentage of AMF colonization of vetiver roots ($p=0.001$) (Figure 3). The highest effect was obtained by the treatments with added native AMF inoculum. Tea plantation surface soil with native AMF inoculum and biochar has the highest treatment effect while adjacent secondary forest surface soil without any amendments has the lowest effect.

The success of inoculation, persistence in soil with the compatibility to target environment

and the completion of other soil organisms in the target host are responsible for the effective colonization of AMF [54].

3.6 Soil TOC % and Microbial Diversity

Statistical analyses revealed that treatments were not significantly different for soil TOC % (Table 3) and microbial diversity (Table 3) ($p \geq 0.05$). Yet, all the treatments were observed to enhance the TOC % towards the reference.

4. CONCLUSIONS

Chrysopogon zizanioides colonized with AMF have the potential to improve soil quality by enhancing chemical and microbiological properties of the tested degraded soil towards the representative healthy soil in the Knuckles mountain range. Furthermore, this soil amelioration by *Chrysopogon zizanioides* can be enhanced with some amendments such as AMF and biochar. Therefore, the proper application of vetiver system technology, with the AMF and organic amendments can be successfully used for the soil health improvement of the degraded lands.

Table 3. Deviation of TOC% and microbial diversity with different treatments.

Vegetation type	Treatments	Depth of the sampling (cm)	Average of soil TOC %		Average of microbial diversity	
			Initial	Final	Initial	Final
Pine plantation	Control/Soil	0-10	2.060 ± 0.04	2.92 ± 0.02	6 ± 0.02	6 ± 0.02
		15-25	0.15 ± 0.02	0.95 ± 0.01	4 ± 0.02	4 ± 0.02
	Soil with organic supplement mixture	0-10	2.01 ± 0.01	2.10 ± 0.01	2 ± 0.02	2 ± 0.02
		15-25	0.98 ± 0.01	1.03 ± 0.14	2 ± 0.01	2 ± 0.01
	Soil with AMF	0-10	1.93 ± 0.04	2.16 ± 0.21	4 ± 0.01	4 ± 0.01
		15-25	0.95 ± 0.03	1.17 ± 0.02	4 ± 0.02	4 ± 0.02
	Soil with biochar	0-10	1.97 ± 0.02	2.00 ± 0.01	2 ± 0.33	2 ± 0.33
		15-25	0.98 ± 0.01	1.13 ± 0.01	4 ± 0.03	4 ± 0.03
	Soil with AMF and biochar	0-10	2.83 ± 0.01	2.90 ± 0.03	6 ± 1.71	7 ± 1.71
		15-25	1.12 ± 0.04	1.23 ± 0.03	6 ± 0.02	6 ± 0.02

Table 3. (Continued).

Vegetation type	Treatments	Depth of the sampling (cm)	Average of soil TOC %		Average of microbial diversity	
			Initial	Final	Initial	Final
Adjacent secondary forest of pine plantation	Control/Soil	0-10	1.17 ± 0.03	1.31 ± 0.02	2 ± 0.02	3 ± 0.02
		15-25	0.18 ± 0.02	0.77 ± 0.01	2 ± 0.01	2 ± 0.01
	Soil with organic supplement mixture	0-10	1.00 ± 0.01	1.21 ± 0.01	2 ± 0.01	2 ± 0.01
		15-25	1.03 ± 0.01	1.32 ± 0.01	2 ± 0.01	2 ± 0.01
	Soil with AMF	0-10	1.20 ± 0.02	1.30 ± 0.01	2 ± 0.02	2 ± 0.02
		15-25	1.03 ± 0.02	1.45 ± 0.01	2 ± 0.02	2 ± 0.02
	Soil with biochar	0-10	1.15 ± 0.03	1.27 ± 0.32	2 ± 0.01	2 ± 0.01
		15-25	0.98 ± 0.01	1.06 ± 0.02	2 ± 0.02	2 ± 0.02
	Soil with AMF and biochar	0-10	1.50 ± 0.01	2.00 ± 0.01	2 ± 0.02	2 ± 0.02
		15-25	1.33 ± 0.01	1.58 ± 0.02	4 ± 0.08	4 ± 0.08
Tea plantation	Control/Soil	0-10	1.28 ± 0.01	2.08 ± 0.12	3 ± 0.02	5 ± 0.02
		15-25	0.98 ± 0.04	1.15 ± 0.01	2 ± 0.01	2 ± 0.01
	Soil with organic supplement mixture	0-10	1.28 ± 0.02	2.07 ± 0.01	2 ± 0.005	2 ± 0.005
		15-25	1.01 ± 0.01	1.01 ± 0.02	2 ± 0.01	2 ± 0.01
	Soil with AMF	0-10	1.43 ± 0.01	2.18 ± 0.02	2 ± 0.02	2 ± 0.02
		15-25	1.03 ± 0.11	1.27 ± 0.02	4 ± 0.02	4 ± 0.02
	Soil with biochar	0-10	1.36 ± 0.1	2.01 ± 0.05	2 ± 0.01	2 ± 0.01
		15-25	0.98 ± 0.2	1.10 ± 0.13	2 ± 0.02	2 ± 0.02
	Soil with AMF and biochar	0-10	1.87 ± 0.2	2.27 ± 0.01	2 ± 0.02	2 ± 0.02
		15-25	1.10 ± 0.23	1.37 ± 0.02	4 ± 0.01	4 ± 0.01

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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