Soil Nutrient Status and Microbial Communities as Influenced by Bio-Fuel Trees Plantation

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Soil Nutrient Status and Microbial Communities as Influenced by Bio-Fuel Trees Plantation

Agnes Imoya P. O., Prasanna K. T., Anantha K., Habimana Sylvestre

Abstract   Due to depletion of fossil fuel reserves that increased environmental problems presently the world has been confronted with an energy crisis. The field study on soil nutrient status and microbial communities as influenced by bio-fuel trees plantation was carried out in Bio-Fuel Park, Madenur, Hassan District of Karnataka state, India during pre and post-monsoon of 2017-2018. The objective of this study was to assess the soil nutrient status and microbial communities under the 4 trees species namely *Simarouba glauca, Madhuca latifolia, Azadirachta indica, Calophyllum inophyllum,* in their root zone depths such as 0-15 cm, 15-30 cm and 30-45cm depths during pre and post-monsoon seasons. The results revealed that in post-monsoon season recorded significantly higher in soil pH (5.96) and EC (dSm^{-1}) (0.11) but in soil OC (0.72%) and available N (335 kg ha^{-1}), P_2O_5 (30.43 kg ha^{-1}), K_2O (236.9 kg ha^{-1}) was recorded higher in pre-monsoon. Among bio-fuel species recorded higher significantly, *C. inophyllum* had strong influence on soil pH (6.53) and available P_2O_5 (36.04 kg ha^{-1}). The *S. glauca* record higher in the following EC (dSm^{-1}) (0.14), OC (0.81%),available N (350 kg ha^{-1}), K_2O (293.8 kg ha^{-1}). Influences among soil depth was highly recorded in depths of 15-30 in available K_2O (224.7 kg ha^{-1}), P_2O_5 (37.09 kg ha^{-1}), N (323 kg ha^{-1} each) and in depths 0-15 recorded higher in organic-C (0.73%, EC (dSm^{-1}) (0.14), but in soil pH significantly higher at depth of 30-45 cm (5.86). Correlation matrix between microbial characters and chemical characters was significantly negative, correlation between the total bacteria and soil pH (r^2 = -0.494) and EC (r^2 = 0.558). The ph supported the growth of *Azotobacte*r (r^2 = 0.593). PO_4-solubilizers microorganisms (MO) significantly correlated with organic-C and available-N. Study provides evidence that bio-fuel trees species are contributing positive impact to soil microbial and soil nutrient in particular trees species in different manner. Pre-monsoon was highly supporting the growth of microbial and supporting the availability of the soil nutrients compared to post-monsoon. And environmental factors are also influencing the growth of microbial communities, infinite conclusively indicate most of the microbiological characters weak to very strong relationship in maintaining good soil health at landscape level. So *Calophyllum inophyllum* and *Simarouba glauca* followed by *Azadirachta indica* and the least was *Madhuca latifolia,* the first two bio-fuel trees are the initiative plants for the restoration of barren and weakland.

Keywords Bio-fuel, Bacteria, Fungi, Soil nutrient.

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Introduction

Presently the world has been confronted with an energy crisis due to depletion of fossil fuel reserves and increased environmental problems. Bio-fuel is an alternative energy source to meet the demand gap producing in an eco-friendly manner. The bio-fuel species viz., Pongamia, Jatropha, Neem, Simarouba, were promising bio-fuel yielders by creating optimal microclimate environment for the beneficiaries of flora and fauna. India has about 40% of degraded land (Chopra 1996) which can be reclaimed by growing bio-fuel plant species. The bio-fuel plants not only yield bio-fuel also produces enormous amount of biomass, a major source of organic matter. The organic matter replenishes nutrients, the driving force for microbial activity, production of various organic acids and helps in enhancing soil health.

Terrestrial plants experiences complex interactions with soil and microorganisms that are surrounding the root region of the rhizosphere. Its proved in perennial plants where inter annual climatic variability and extensive long-lived root systems that invade and occupy large volumes of soil, may increase the complexity of rhizosphere interactions (Shakya et al. 2013). The research hypothesis is that the promising bio-fuel tree species, through differences in their leaf-fall, nutrient concentration, root proliferation, biomass generation, plants canopy, litter decomposition rate and influence on soil abiotic properties intend to affect the soil biota and their influence on soil health.

Past studies measured the associated physical, chemical, spatial and temporal factors that may affect the soil and microbial communities and also several researchers revealed the existence of a close interaction between plants and soils and shown that perennial trees are ecosystem engineers able to generate species-specific effects on soil properties and soil communities that could potentially lead to soil rhizosphere (Gomez-Aparicio and Canham 2008, Vesterdal et al. 2008, Mitchell et al. 2012, Vesterdal et al. 2012, Prescott and Grayston 2013). Keeping this in view the study was conducted to compare the influence of bio-fuel species on soil nutrients comparison with microbial communities during pre-monsoon and post-monsoon in surface and subsurface soil.

Materials and Methods

The study was conducted at Bio-Fuel Park, Madenur, Hassan, Karnataka state, South India, located at $12^0 58´25´´$ N to $12^0 58´50´´$ N $75^0 15´50´´$ E to $75^0 16´20´´$ E with 951 m MSL. The mean annual rainfall was 806 mm with the mean daily temperature of 26^0C. The Red soils are dominated derived from Granite-Granite Gneiss parent material with a texture of loamy sand to sandy loam in upland and sandy clay loam to sandy clay in mid land areas. The experimental site in 0-15 cm depth was with neutral soil reaction pH (6.23), EC (dSm^{-1}) (0.07), OC (0.52%) with soil nutrients status as follows : Available Nitrogen (285.0 kg ha^{-1}), available P_2O_5 (8.10 kg ha^{-1}), available K_2O (84.80 kg ha^{-1}).

The representative composite soil samples were collected from the rhizosphere of bio-fuel plant species *Simarouba glauca, Madhuca latifolia, Azadirachta indica, Calophyllum inophyllum* during Feb-Mar (pre-monsoon) and Oct-Nov (post-monsoon) during the year 2017-2018 at depths of 0-15 cm, 15-30 cm and 30-45 cm. The collected soil samples were processed by applying quaternary technique, shade dried, sieved and used for soil physic-chemical properties.

The soil pH was determined with 1:2.5 soil water suspension using combined glass electrode and the electrical conductivity using conductivity bridge (Jackson 1973). The organic carbon was determined by the modified Walkley-Black method (partial oxidation) as outlined by Anderson and Ingram (1989), available nitrogen by alkaline potassium permanganate method (Subbaiah and Asija 1956), the available-P and available potassium contents in soil samples were estimated as per the procedure outlined by Jackson (1973). Total bacteria, *Azotobacter,* PO$_4$-solubilizing microorganisms, cellulose degrading microor-
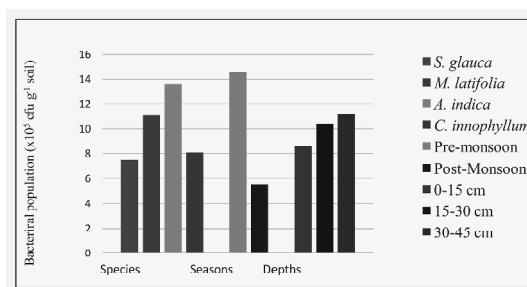
Table 1. Soil pH analysis in the root region soils of bio-fuel tree species.

Tree species	Pre-monsoon			Post-monsoon		
	Depth (cm)					
	0-15	15-30	30-45	0-15	15-30	30-45
Simarouba glauca	5.75	6.43	5.78	5.94	6.21	6.08
Madhuca latifolia	5.09	5.23	4.97	5.26	5.18	5.48
Azadirachta indica	5.05	5.29	5.14	5.37	5.33	5.82
Calophyllum inophyllum	6.38	6.01	5.90	6.80	6.53	7.57
CD at 5%			0.35			

Tree species	Mean	Season	Mean	Depth (cm)	Mean
Simarouba glauca	6.03	Pre-monsoon	5.59	0-15	5.70
Madhuca latifolia	5.20			15-30	5.76
Azadirachta indica	5.33	Post-monsoon	5.96		
Calophyllum inophyllum	6.53			30-45	5.86
CD at 5%	0.14		0.10		0.13

Fig. 1. Population of bacteria in the root region soils of bio-fuel tree species (× 10^5 cfu g^-1 oil).

Results and Discussion

This study conducted on the impact of bio-fuel plantations on soil nutrients status in different season and depths with correlationship with microbial communities in Hassan district of Karnataka state, India, the results of this study are presented here down.

Impact on soil pH

The results revealed that *C. inophyllum* had strong influence on soil pH (6.53) it further decreased with the increase in soil depth, *M. latifolia* showed least influence on soil pH (5.20), significantly higher pH was recorded at 30-45 cm (5.86) soil depth followed by 15-20 cm soil depth (5.76) and the least pH was recorded in 0-15 cm soil depth (5.70). Among the season post-monsoon (5.96) was higher compared to pre-monsoon (5.59). In general pre-monsoon season

ganism was enumerated by using serial dilution plate count technique. Suitable dilutions were plated using respective media. Total bacteria by using Soil extract agar medium (Allen 1957) and functional groups such as *Azotobacter* (N$_2$-fixer) by using Waksman's medium-77 (Subba Rao 1983), PO$_4$-solubilizing microorganisms by using Sperber's hydroxyl apatite medium (Sperber 1957), cellulose degraders by using Dubo scellulose medium (Subba Rao 1983). The plates were incubated at 30^{0}C in a bacteriological incubator for 3-5 days. The colony forming units (cfu) appearing on the media was recorded and cfu g^{-1} was calculated by using the following formula.

Colony forming units (cfu) g^{-1} dry soil = cfu × dilution factor (Oven dry weight of soil)

Statistical analysis was done by three way factorial analysis by Web Based Agricultural Statistical Package (WASP). Correlation studies were made between micro-flora and soil physic-chemical properties. The values of correlation co-efficient (r) was calculated as per the procedure outlined by Snedekar and Cocoharan (1967).
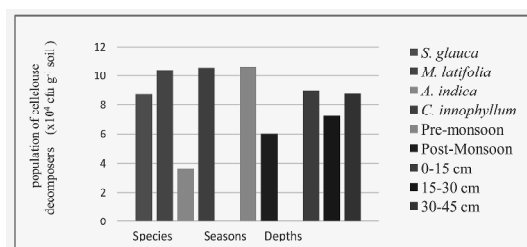

Fig. 2. Population of cellulose decomposers microorganisms in the root region soils of bio-fuel tree species (× 10^4 cfu g^-1 soil).
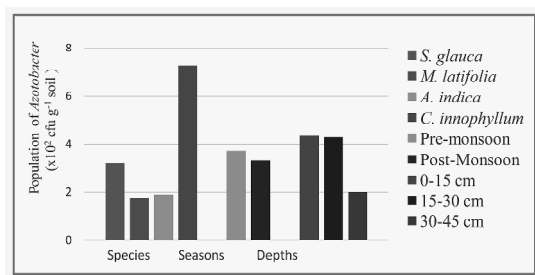
Table 3. Population of *Azotobacter* in the root region soils of bio-fuel tree species (× 10^2 cfu g^{-1} soil).

Table 4. Population of PO$_4$-solubilizing microorganisms in the root region soils of bio-fuel tree species (×10^4 cfu g^{-1} soil).

sample was higher compared to post monsoon due to extensive leaching of basic cations. In this study it was quite reverse and contradictory, this could be due to differences in root exudates in different plant species or environmental factors. These were no significant differences in pH due to different bio-fuel tree species and soil depths (Table 1).

In the microbial communities data correlationship which are present in Figs. 1–4. Soil pH shows the strong significant correlation with *Azotobactor* and normal significant correlation with cellulose degraded and PO$_4$-solubilizer but with bacteria it shows strong correlation (Joana et al. 2018).

Impact on electrical conductivity (d.Sm^{-1})

The soil EC was high in post-monsoon (0.11) compared to pre-monsoon (0.09). There were no significant differences in EC at any of the soil depths. Significant differences in FC of the root zone soils at different soil depths in different bio-fuel trees species were not recorded except with *S. glauca* and *C. inophyllum* in pre-as well as in post-monsoon seasons. Soil acidification changes in low pH and is negatively related with soil EC, this is a natural process that is generally accelerated by agriculture with increased concentration of hydrogen ions accompanied by the removal of bases and indicated by low soil pH. It is crucial to know soil pH status as it is a predictor of various chemical activities and a rough indicator of plant available nutrients. The EC also showed an inverse relationship to that of soil pH as expected for any soil (Table 2). While the microbial
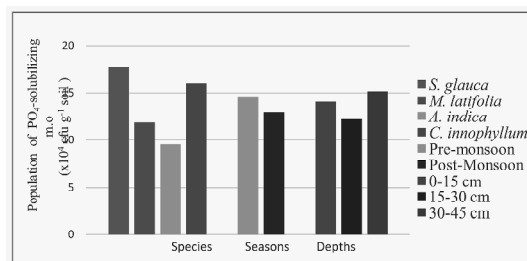
communities data correlationship are present in Figs. 1—4. The electrical conductivity values were found to be very low shows significant correlation with *Azotobactor*, Cellulose degraded and PO$_4$-solubilizer but with bacteria it show negative correlation (Dennis and Kevin 2018).

Impact on organic carbon

Soil OC was high in soil of *S. glauca* (0.81%) and the

Table 2. Electrical conductivity (dSm^{-1}) in the root region soils of bio-fuel tree species.

Tree species	Pre-monsoon Depth (cm)			Post-monsoon Depth (cm)		
	0-15	15-30	30-45	0-15	15-30	30-45
Simarouba glauca	0.14	0.13	0.12	0.15	0.13	0.17
Madhuca latifolia	0.07	0.07	0.06	0.12	0.04	0.06
Azadirachta indica	0.06	0.05	0.06	0.05	0.08	0.08
Calophyllum inophyllum	0.10	0.09	0.09	0.14	0.12	0.14
CD at 5%			0.4			

Tree species	Mean	Season	Mean	Depth (cm)	Mean
Simarouba glauca	0.14	Pre-monsoon	0.09	0-15	0.10
Madhuca latifolia	0.07			15-30	0.09
Azadirachta indica	0.06	Post-monsoon	0.11		
Calophyllum inophyllum	0.11			30-45	0.10
CD at 5%	0.05		0.01		0.013

Table 3. Organic carbon (%) in the root region soils of bio-fuel tree species.

Tree species	Pre-monsoon			Post-monsoon		
	Depth (cm)					
	0-15	15-30	30-45	0.-15	15-30	30-45
Simarouba glauca	0.90	0.81	0.77	0.83	0.80	0.74
Madhuca latifolia	0.73	0.69	0.59	0.62	0.52	0.52
Azadirachta indica	0.69	0.65	0.64	0.70	0.68	0.65
Calophyllum inophyllum	0.72	0.72	0.71	0.68	0.66	0.65
CD 5%			0.10			

Tree species	Mean	Season	Mean	Depth (cm)	Mean
Simarouba glauca	0.81	Pre-monsoon	0.72	0-15	0.73
Madhuca latifolia	0.61			15-30	0.69
Azadirachta indica	0.67	Post-monsoon	0.62		
Calophyllum inophyllum	0.69			30-45	0.66
CD at 5%	0.04		0.03		0.04

Table 4. Available nitrogen (kg ha^{-1}) in the root region soils of bio-fuel tree species.

Tree species	Pre-monsoon			Post-monsoon		
	Depth (cm)					
	0-15	15-30	30-45	0-15	15-30	30-45
Simarouba glauca	371	349	374	376	369	262
Madhuca latifolia	359	352	322	263	274	268
Azadirachta indica	364	286	263	278	296	297
Calophyllum inophyllum	310	331	333	268	326	328
CD at 5%			50.65			

Tree species	Mean	Season	Mean	Depth (cm)	Mean
Simarouba glauca	350	Pre-monsoon	335	0-15	323
Madhuca latifolia	306			15-30	323
Azadirachta indica	297	Post-monsoon	300		
Calophyllum inophyllum	316			30-45	306
CD at 5%	20.68		14.62		17.901

least in *M. latifolia* (0.61%). However, significant reduction in OC was recorded at 30-45 cm soil depth in both the seasons. Soil depths 0-15 cm recorded the highest organic-C (0.73%) and least 30-45 cm (0.66%). There were significant differences in different tree species under study but no significant differences in the root zone soils at 0-15 cm and 15-30 cm soil depths with different bio-fuel tree species, both in pre and post-monsoon seasons (Table 3). In the microbial communities data correlationship which are present in Figs. 1—4. Soil OC (%) show the significant correlation with *Azotobactor,* cellulose degraded and PO$_4$-solobizer but with bacteria it showed negative correlation. In the present study, there were differences in soil organic-C in soils of different plantations of tree species with different soil organic-C contents at different soil depths. This could be due to the fact that the tree species control the soil organic matter levels. Eugenio et al. (2011) reported that there were differences in SOC stocks between tree species mainly due to the particulate organic matter. Singh and Sharma (1992) also reported that soil organic carbon and nutrient content decreased with increase in soil depth irrespective of tree species.

Impact on available nitrogen

In the root region soils of bio-fuel tree species at different depths in different seasons is presented in Table 4. Avail-N was significantly high in soil of *S. glauca* (350 kg ha^{-1}) and least was in *A. indica* (297 kg ha^{-1}), among the soil depths 0-15 cm and 15-30 cm (323 kg ha^{-1} each) are at par and the least was in 30-45 cm (306 kg ha^{-1}). Significant, differences in available-N were not recorded in the root zone soils at all the depths with a few exceptions. Significantly, less available-N with *A. indica* at 15-30 cm (286 kg ha^{-1}) and 30-45 cm (263 kg ha^{-1}) soil depths in the pre-monsoon and at 30-45 cm (262 kg ha^{-1}) with *S. glauca* and 0-15 cm (268 kg ha^{-1}) with *A.indica* in the post-monsoon. In regarding the correlationship in the microbial communities data correlationship in Figs. 1—4. Available-N show the significant correlation with cellulose degraded and PO$_4$-solubilizer bacteria but it showed negative correlation with *Azotobactor* (Xiaofeng et al. 2017).

Table 5. Available phosphorus (kg ha⁻¹) in the root region soils of bio-fuel tree species.

Tree species	Pre-monsoon Depth (cm)			Post-monsoon		
	0-15	15-30	30-45	0-15	15-30	30-45
Simarouba glauca	21.3	27.0	21.3	19.3	26.5	20.0
Madhuca latifolia	23.2	51.3	42.7	21.5	39.7	24.8
Azardirachta indica	20.0	23.2	24.5	22.5	43.5	33.3
Calophyllum inophyllum	41.4	43.3	26.0	45.3	42.3	18.0
CD at 5%			10.93			

Tree species	Mean	Season	Mean	Depth (cm)	Mean
Simarouba glauca	22.54	Pre-monsoon	30.43	0-15	26.80
Madhuca latifolia	33.86			15-30	37.09
Azardirachta indica	27.83	Post-monsoon	29.71		
Calophyllum inophyllum	36.04			30-45	26.32
CD at %	4.46		3.15		3.86

Table 6. Available potassium (kg ha-1) in the root region soils of bio-fuel tree species.

Tree species	Pre-monsoon Depth (cm)			Post-monsoon		
	0-15	15-30	30-45	0-15	15-30	30-45
Simarouba glauca	327.7	456.3	325.9	186.3	266.5	199.9
Madhuca latifolia	195.1	209.5	192.8	153.0	135.5	193.3
Azardirachta indica	124.0	242.8	251.6	204.0	171.6	248.8
Calophyllum inophyllum	199.5	160.5	158.2	119.8	154.9	204.7
CD at 5%			66.73			

Tree species	Mean	Season	Mean	Depth (cm)	Mean
Simarouba glauca	293.8	Pre-monsoon	236.9	0-15	188.7
Madhuca latifolia	179.8			15-30	224.7
Azardirachta indica	207.2	Post-monsoon	186.5		
Calophyllum inophyllum	166.3			30-45	221.8
CD at 5%	27.24		19.26		23.59

Impact on available phosphorus

The seasons did not have any influence on available P_2O_5 in soils both being at par with each other (30.43 and 29.71 kg ha⁻¹) P_2O_5 significantly, increased in the soils of *M. latifolia* at 15-30 (51 .3 kg ha⁻¹) and 30-45 cm depths (42.7 kg ha⁻¹) in the pre-monsoon and 39.7 kg ha⁻¹ in the post-monsoon. Among the plant species, the influence of *C. inophyllum* on available P_2O_5 was the highest (36.04 kg ha⁻¹) and the least influence with *S. glauca* (22.54 kg ha⁻¹). Among the depths 15-30 cm recorded high available P_2O_5 in soils (37.09 kg ha⁻¹). While in case of *C. inophyllum,* the available P_2O_5 was significantly decreased at 30-45 cm depth both in pre and post-monsoons. Significant, differences in available-P_2O_5 were not recorded in the root zone soils at different soil depths incase of S. glauca either in the pre or post- monsoon (Table 5). Correlationship in Figs 1—4. Microbial communities data soil P_2O_5 show the significant correlation with *Azotobactor,* cellulose degraded and PO_4- solubilizer but with bacteria it showed negative correlation (Dey et al. 2017).

Impact on available potassium

No significant differences in Available-K^2O in root zone soils of different bio-fuel plants at different soil depths either in the pre or post- monsoon except in *S. glauca* which recorded significantly high at 15-30 cm (456.3 and 266.5 kg ha⁻¹) in both the seasons. Pre-monsoon recorded the highest K_2O (236.9 kg ha⁻¹) and post-monsoon recorded the least (186.5 kg ha⁻¹) *S. glauca* recorded the highest available-K_2O (293.8 kg ha⁻¹) and the least influence with *C. inophyllum* (166.3 kg ha⁻¹). among the depths 15-30 cm recorded high available -K_2O in soils (224.7 kg ha⁻¹ and the least was recorded in 0-15 cm depth 188.7 kg ha⁻¹ (Table 6). Correlationship in Figs 1—4. Microbial communities data available- K_2O showed negative correlation with *Azotobactor* and cellulose degraded but significant postive correlation with PO_4-solubilizer and bacteria.

So the results of this study have indicated significant differences in available-N, P_2O_5 and K_2O with

Table 7. Correlation matrix between microbial characters and chemical characters at landscape level at Bio-Fuel Park, Madenur, Hassan. **Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed), (-) No Correlation.

Parameters	pH	EC	Org-C	Avail-N	Avail-P_2O_5	Avail K_2O
Total bacteria	-0.494*	-0.558**	-0.125	0.012	-0.003	0.241
Cellulose degraders	0.008	0.075	0.230	0.405	0.104	-0.077
PO_4-solubulizers	0.396	0.365	0.484*	0.460*	0.043	0.377
Azotobacter	0.593**	0.358	0.247	-0.044	0.341	-0.015

different bio-fuel tree species. But, Majumdar et al. (2017) reported that their existed spatial distributions of 36-51% of tree species at these sites show strong associations to soil nutrient distributions.These results indicate that belowground resource availability plays an important role in the assembly of tropical tree communities at local scales. Further, our studies have clearly shown that there did not exist clear differences in available-N, P_2O_5 and K_2O at different depths except in a few cases. Literature regarding the effect of plant species seasons and soil depths on soil available-N, P_2O_5 and K_2O is lacking except that (Michael 1980) in a review reported that 46 Costa Rican forest sites indicated a negative correlation between soil nutrient availability and tree species richness. P, K, Ca, Na, total bases, base saturation and cation exchange capacity showed significant (p < 0.01) correlations, while available-N, total N, OM, Mn and Mg were not significantly correlated with species richness.

Correlation studies between microbiological and chemical characteristics at landscape

The correlation matrix between microbial characters and chemical characters at landscape level at Bio-Fuel Park, Madenur, Hassan is presented in the Table 7. There was a significant negative correlation between the total bacteria and soil pH (r^2 = -0.494) and EC (r^2 =0.558). This suggests that the soil pH did not favor the growth of bacteria. However, it is surprising that the pH of soil significantly supported the growth of *Azotobacter* (r^2=0.593). PO_4-solubilizing microorganisms were significantly correlated with organic-C and available-N indicating that their multiplication in soil was highly significant. The above results conclusively indicate most of the microbiological characters week to very strong relationship in maintaining good soil health at landscape level Snedekar and Cocoharan (1967).

Conclusion

This study provided evidence that bio-fuel trees species are contributing positive impact to soil microbial and soil nutrient in particular trees species in different manner. Pre-monsoon was highly supporting the growth of microbial and supporting the availability of the soil nutrients compared to post-monsoon. The environmental factors were also influencing the growth of microbial communities, infinite conclusively indicated most of the microbiological characters weak to very strong relationship in maintaining good soil health at landscape lavel. It could be concluded that *Calophyllum inophyllum* and *Simarouba glauca* can be utilized as the initiative plants for the restoration of barren and weak land. The high nutrient uptake is effective means by which the nutrient loss is minimized by plants growing in nutrient deficient habitats. Further, the difference in microbial communities and soil nutrients between bio-fuel plantations clearly suggests a strong selection for mitrients resorption between these bio-fuel plants. For implementing viable strategies of producing bio-fuel feedstock, soil and agronomic research is needed for developing site specific management practices of different bio-fuel species.

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