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Research Article

Response of Soil Microbial Populations and Biomass under Five Agroforestry Systems in the Sub-humid Tropics

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Abstract

Background and Objective: Soil biological activity has been influenced by several biotic and abiotic factors prevailing in the region. For instance, variation in species composition, management practices and climatic conditions has had their reflections on the microbial population vis-à-vis biomass in the agroforestry systems. The present study was designed to understand the seasonal dynamics of microbial population and microbial biomass under the agroforestry systems of sub-humid Gujarat, India. **Materials and Methods:** Seasonally collected soil samples (0-15 and 15-30 cm) have been used for analyzing their physicochemical properties. Moist samples were used for soil biological studies. Bacterial and fungal counts were made following serial dilution methods. Microbial biomass (C, N and P) were estimated following the chloroform-fumigation extraction method. **Results:** Agri-silvicultural and home garden systems registered greater bacterial ($27.20 \times 10^4 \text{ g}^{-1}$) and fungal ($75.86 \times 10^2 \text{ g}^{-1}$) counts in the topsoil (0-15 cm). More or less similar trend was also observed in microbial biomass carbon. However, microbial biomass nitrogen ($50.71 \mu\text{g } \mu^{-1}$) and phosphorus ($5.83 \mu\text{g } \mu^{-1}$) were highest in the home gardens. Seasonally, microbial counts and biomass (C, N and P) in the soil were maximum during spring and minimum during the rainy season. **Conclusion:** Soil microbial population (bacteria and fungi) and microbial biomass C, N and P were significantly ($p < 0.05$) influenced by different typologies of agroforestry practices in the sub-humid tropics. Overall, our study concludes that the home garden system performed better in influencing the biological health in tree-based farming systems.

Key words: Agroforestry, carbon, soil nutrients, microbial biomass, home gardens, sub-humid tropics, seasonal variation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The existence of perennial trees in agricultural systems offers a stabilizing effect on the soil ecosystem through organic matter dynamics discharging its functional regime to address the challenges of climate change and the sustainability of land use. The role of Soil Organic Matter (SOM) in maintaining the quality, fertility and biological health of the soil is well accepted and prerequisite for the overall productivity of the soil¹. Interestingly, around 95% of the total soil biomass is estimated to be constituted by soil microorganisms², which in turn played an important role in maintaining the soil ecosystem services and functions like soil formation processes, SOM dynamics and cycling of nutrients^{3,4}. Notwithstanding, it has been reported that the litter composition and rooting depth of the perennial crops do create a sensitive and dynamic ecosystem for the soil microbial turnover that eventually affects the soil biochemical processes⁵. For example, the Microbial Biomass Carbon (MBC) plays a crucial role in biogeochemical cycles of the ecosystem and is reportedly considered as sensitive and an early indicator of Soil Organic Carbon (SOC) changes vis-à-vis cycling of SOM^{6,7}. A few studies have established such concepts through field and laboratory studies^{8,9}, but most of them are in India are confined to the high rainfall areas of north-east India.

The present study was aimed to characterize the temporal and spatial variations in soil microbial population and biomass, influenced by soil physicochemical properties. This would help in understanding the role of microbial biomass in soil organic matter and nutrient dynamics in the tree-based farming systems and would provide ample insights into the influence of management practices in different practices influencing soil health per se.

MATERIALS AND METHODS

Study area: This research was conducted from June, 2015-May, 2017 in five dominant agroforestry systems (Table 1) managed by the farmers of Navsari district of Gujarat (20°45' to 21°00' N latitude and 72°45' to 73°15' E longitude). The

sites were selected by adopting the criteria given by Nair¹⁰. The study area experiences moderate rainfall of about 1400 mm annually with a hot summer from April to June months and rainfall from July to August, followed by brief autumn and cool and dry winter.

Soil sampling: Ten soil samples were collected at two different depths (0-15 and 15-30 cm) by using a power auger from these sites using a randomized block design method during July-August, October-November, December-January and February-March representing rainy, autumn, winter and spring seasons, respectively. The composite soil samples from each of the sites were transported to the laboratory in polyethylene bags and stored at 4°C until analysis. The subsamples of air-dried soil were used for analyzing the different physicochemical parameters¹¹. The analytical works were done at the Soil and Plant Analysis Laboratory of College of Forestry, Navsari Agricultural University, Navsari (Gujarat), India.

Soil bacterial count estimation: Soil bacterial count was estimated according to Waksman¹² using nutrient agar medium (Himedia Laboratories, Mumbai, India) at 10⁴ dilutions and the fungal population¹³ in Martin's rose bengal agar medium (Himedia Laboratories, Mumbai, India) at 10² dilutions under laminar flow chamber (Daihan Labtech India Pvt. Ltd., Rajasthan, India). The prepared media was then inoculated at 21°C. The samples of each system with three replications were prepared following the serial dilution method¹⁴. The inoculated Petri plates (Borosil Glass Works Ltd., Mumbai, India) were kept in incubator (Equitron Medica Pvt. Ltd., Mumbai, India) at 30±1°C for bacteria for 24 hrs. The incubation time for fungi was 120 hrs at 25±1°C. The colonies developed were counted within 1 and 3 days for bacteria and fungi, respectively using a digital colony counter (Systronics India Ltd., Ahmedabad, India).

Microbial biomass carbon estimation: Microbial Biomass Carbon (MBC) was estimated after chloroform fumigation in Borosil desiccators, followed by extraction given by

Table 1: Dominant agroforestry systems practiced in Navsari district, Gujarat

Agroforestry systems	Tree-crop combination
Agri-horticultural (AH)	<i>Mangifera indica</i> +agricultural crops (Agricultural crops grown were <i>Oryza sativa</i> , <i>Dolichos lablab</i> and <i>Cicer arietinum</i>)
Agri-horti-silvicultural (AHS)	<i>Mangifera indica</i> + <i>Tectona grandis</i> +agricultural crops (Agricultural crops grown were <i>Abelmoschus esculentus</i> , <i>Solanum melongena</i> and <i>Solanum lycopersicum</i>)
Agri-silvicultural (AS)	<i>Tectona grandis</i> + <i>Saccharum officinarum</i>
Horti-pastoral (HP)	<i>Manilkara zapota</i> + <i>Sorghum</i> spp.
Home garden (HG)	Mixtures of trees and agricultural crops

Voroney *et al.*¹⁵ using the formula, $MBC (\mu g g^{-1} \text{ soil}) = (C_F - C_{UF}) / K_{EC}$, where $K_{EC} = 0.35$ that represents the efficiency of microbial biomass C^{16,17}. Microbial biomass nitrogen (MBN) was estimated following chloroform fumigation-extraction procedure¹⁸ and was calculated as the flush of Total N (K_2SO_4 -extractable N_F)-total N (K_2SO_4 -extractable N_{UF})/ K_N , where $K_N = 0.50$ as given by Joergensen and Mueller¹⁹. Microbial biomass phosphorus (MBP) was calculated as $b-a/(0.40)$, where, 'a' is the amount of inorganic P ($mg g^{-1}$) extracted from un-fumigated soil 'b', the inorganic P ($mg g^{-1}$) extracted from the fumigated soil and 0.40 is the fraction of biomass P mineralized and extracted using 0.5 M $NaHCO_3$ in accordance with Brookes *et al.*²⁰.

Statistical analysis: The temporal and spatial data collected were subjected to Duncan's multiple range tests (DMRT) and LSD at $p < 0.05$ using SPSS 17.0 (SPSS Inc., Chicago, USA) windows version package were derived. Correlation analysis²¹ was also worked out to understand the relationship between the different variables under study.

RESULTS AND DISCUSSION

Physico-chemical properties of soil: The soil was clayey in all the agroforestry systems studied (Table 2). The agri-horticultural system had the highest bulk density. Spatially, the topsoil (0-15 cm) recorded lower bulk densities than the subsoil (15-30 cm). The water holding capacity (WHC) of the topsoil (0-15 cm) layer was always greater as compared to lower soil depth in all the sites. The presence of soil organic carbon across the systems was found to be varied and ranged from 0.52 to 0.78% within 0-30 cm depth. The highest organic carbon content was recorded in home gardens and least in the agri-silvicultural system. Soil total available nitrogen was maximum in the home garden system and minimum in the agri-silvicultural system. Likewise, a greater concentration of

available phosphorus was recorded in horti-pastoral and agri-horticultural systems. The greatest bulk density under the agri-horticultural system may be attributed to the production of a lesser amount of litter²² and eventually influence on the soil structure via lesser microbial activity²³. The topsoil (0-15 cm) possesses lesser bulk density than lower depth (15-30 cm) and this might be due to restricted root activity in this layer as compared to the topsoil where the organic matter flux is presumably higher as evident through the recorded soil pH (Table 2). Variation in organic matter content in the different system was may be attributed to differences in plant species composition²⁴. In the present study, the home garden systems recorded greater species richness (Table 1) that may have enabled diverse litter accumulation on the floor leading to greater SOM or SOC, as compared to the other forms of agroforestry practices (Table 2). Similarly, total available nitrogen was also higher in the home gardens. Conforming to this contention, the type of land management and species compositions affects the soil nutrient availability²⁵. Nonetheless, available phosphorus content in the soil was found highest in horti-pastoral and agri-horticultural systems. Dorman²⁶ suggested that uptake of P from the subsoil by plants and add to the surface is an important component of the P recycling process in soil profile. Most of the P assimilation takes place in the agroforestry floor and upper mineral horizon, while P is returned mainly to surface in the form of leaf litter²⁷. Eventually, available phosphorus was greater in the topsoil than in the sub-surface soil.

Microbial population (Bacteria and Fungi): The data presented in Fig. 1 revealed that the practices of different agroforestry systems significantly ($p < 0.05$) influenced bacteria microbial populations (Fig. 1a,b) and fungi microbial populations (Fig. 1c,d). It is seen that topsoil layer (0-15 cm) had a higher microbial population than the sub surface layer (15-30 cm). Overall, in this present study, the populations

Table 2: Soil physico-chemical characteristics in different agroforestry system

Agroforestry systems	Depth (cm)	Texture (%)			WH (%)	BD ($Mg m^{-3}$)	pH	SOC (%)	Avail. N ($kg ha^{-1}$)	Avail. P ($kg ha^{-1}$)
		Sand	Silt	Clay						
Agri-horticultural (AH)	0-15	12.28	17.16	70.57	52.07	1.41	7.25	0.65	266.96	88.17
	15-30	11.14	16.89	71.98	48.68	1.53	7.46	0.53	232.35	59.02
Agri-horti-silvicultural (AHS)	0-15	12.28	13.31	70.07	58.20	1.38	7.63	0.70	284.01	47.00
	15-30	11.14	13.66	70.48	62.92	1.50	7.76	0.60	249.17	28.91
Agri-silvicultural (AS)	0-15	24.21	17.66	56.30	51.31	1.39	8.17	0.59	258.55	36.08
	15-30	24.04	18.39	53.99	57.43	1.48	8.30	0.52	232.53	22.02
Horti-pastoral (HP)	0-15	27.61	19.50	59.09	52.44	1.38	7.91	0.65	266.18	91.00
	15-30	26.02	21.98	60.32	52.54	1.50	8.04	0.58	242.59	77.32
Homegarden (HG)	0-15	26.89	20.83	52.29	51.66	1.36	7.38	0.78	315.23	67.46
	15-30	26.31	19.90	53.80	52.12	1.43	7.51	0.64	276.74	52.63

WH: Water holding, BD: Bulk density, SOC: Soil organic carbon, Avail. N: Available nitrogen, Avail. P: Available phosphorus

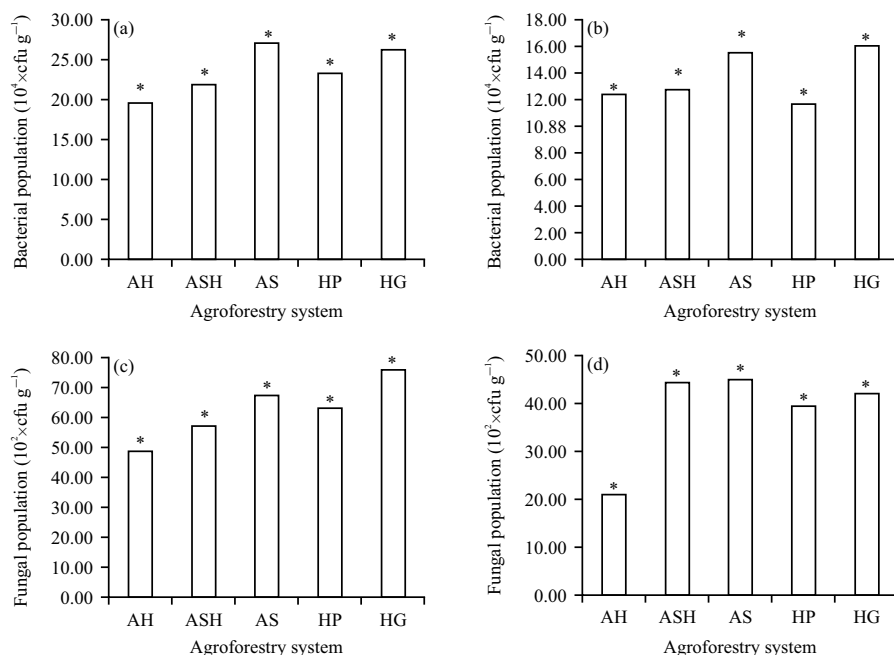


Fig. 1(a-d): Bacterial population (a) 0-15 cm, (b) 15-30 cm and Fungal population (c) 0-15 cm and (d) 15-30 cm, in the soil at two depths of different agroforestry systems

Means not sharing a letter in common differ significantly ($p < 0.05$) between the same soil layers of different agroforestry systems. *Means sharing in common differ significantly ($p < 0.05$) between soil layers of the same system

of fungi outweigh the bacterial counts in the entire site. Amongst the agroforestry system studied, the agri-silvicultural system had the highest bacterial population followed by home garden in 0-15 cm soil depth. However, in 15-30 cm depth, home gardens registered the greater bacterial population as compared to other systems. Nonetheless, the agri-horticultural and horti-pastoral systems registered the lowest bacterial population in the 0-15 cm and 15-30 cm soil layers, respectively. In the case of fungal counts, home garden produced the maximum count followed by an agri-silvicultural system for 0-15 cm soil depth. In the 15-30 cm soil layer, the agri-silvicultural system yielded the highest fungal count. The agri-horticultural system produced the lowest fungal count in both soil layers, however. Soil microbial populations varied seasonally across the agroforestry systems. Irrespective of agroforestry systems, higher bacterial ($31.33 \times 10^4 \text{ g}^{-1}$) (Fig. 2a,b) and fungal ($88.78 \times 10^2 \text{ g}^{-1}$) (Fig. 2c,d) populations in the topsoil (0-15 cm) layer during the spring season was observed in all the systems. Similar trend was also observed in the lower (15-30 cm) depth of the soil. The surface soil generally characterized by the presence of higher organic matter and thus favors the decomposition activities in the soil²⁸. Maithani *et al.*²⁹ had earlier advocated that the upper soil depth up to 10 cm has predominantly engaged in free gaseous exchange coupled with availability of adequate

moisture level would favor the condition for easy reproduction of fungal growth. Nonetheless, the presence of the microbial population in the soil is influenced by several factors viz., pH, moisture level, organic matter level, etc³⁰. The decline of microbial count in the sub-surface layer was reported by several researchers under different conditions such as upland grassland³⁰, during forest regrowth period³¹, mostar pit³², agricultural landscape³³, traditional agroforestry system³⁴. Variations in bacterial population among the system indicated that difference in plant composition leading to changes in bacterial community structure. In this context, Radhakrishnan and Varadharajan³⁵ also claimed that the input of organic matter, plant composition and their associated nutrient cycling have the major responsibility for variation of microbial population structure in a different agroforestry system.

Soil microbial population varied seasonally across the agroforestry systems with the peak season during the spring season in all the sites. The result confirmed with the remark of the Jha *et al.*³⁶ where the maximum microbial count was found during spring and post-rainy seasons. Contrarily, the minimum population during rainy in the present study sites may be due to high rainfall and waterlogging conditions which are otherwise critical for the growth and activity of microbes. Climatically, the study sites are typically tropical humid/sub-humid, where most of the rains are concentrating during

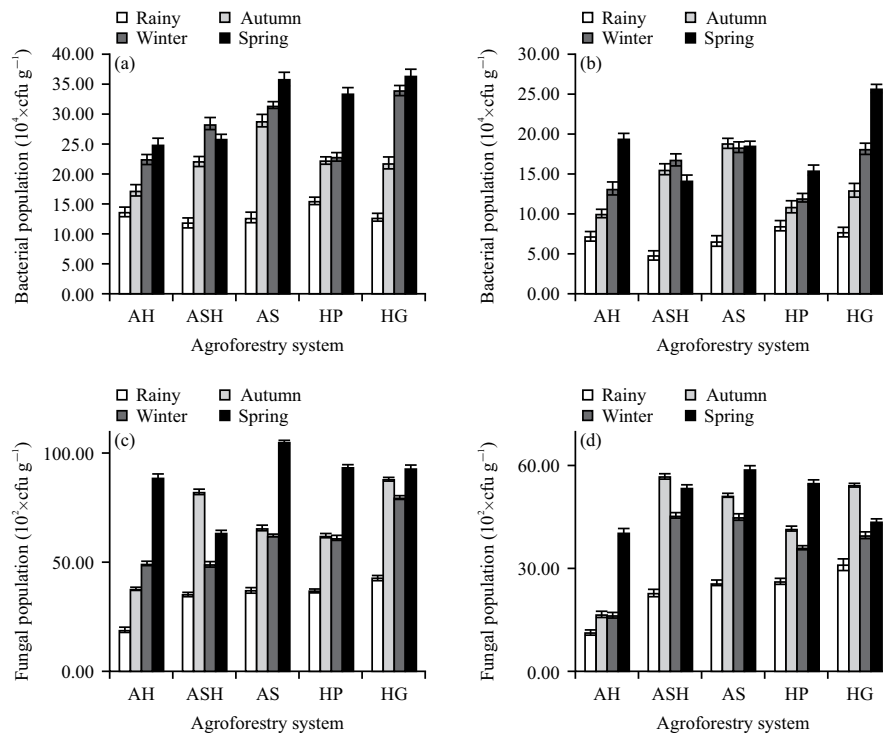


Fig. 2(a-d): Seasonal variation of bacteria microbial population (a) 0-15 cm, (b) 15-30 cm and fungi microbial population (c) 0-15 cm and (d) 15-30 cm in different agroforestry system

Table 3: Microbial biomass C, N and P in a different agroforestry system (Values are means of four seasons across one year)

Agroforestry systems	MBC ($\mu\text{g } \mu^{-1}$)			MBN ($\mu\text{g } \mu^{-1}$)			MBP ($\mu\text{g } \mu^{-1}$)		
	0-15	15-30	Between layers	0-15	15-30	Between layers	0-15	15-30	Between layers
Agri-horticultural (AH)	420.72 ^a	280.03 ^a	*	50.02 ^b	34.05 ^a	*	5.69 ^b	3.23 ^a	*
Agri-horti-silvicultural (AHS)	491.38 ^b	390.40 ^b	*	48.56 ^{ab}	37.96 ^b	ns	5.01 ^{ab}	4.01 ^{ab}	*
Agri-silvicultural (AS)	439.79 ^a	320.21 ^{ab}	*	41.73 ^{ab}	32.40 ^a	*	4.93 ^{ab}	4.23 ^b	ns
Horti-pastoral (HP)	412.14 ^a	312.14 ^a	*	39.12 ^a	31.45 ^a	*	4.56 ^a	3.69 ^{ab}	ns
Homegarden (HG)	490.48 ^b	381.03 ^b	*	50.71 ^b	37.62 ^b	*	5.83 ^b	4.28 ^b	*
General mean	450.90	336.76		46.03	34.70		5.21	3.89	

MBC: Microbial biomass carbon, MBN: Microbial biomass nitrogen, MBP: Microbial biomass phosphorus, Values followed by different alphabets in same column are significantly different at $p < 0.05$ based on Duncan's multiple range test (DMRT). *: Denotes the significance between soil layers of the system at $p < 0.05$ based on LSD; ns: Non-significant

July-August. It was evident that the total rainfall received during the study period (2016-2017) was about 1409 mm in which almost 50% (693 mm) was concentrated during the sampling time of the rainy season. After a brief spell of autumn, winter season commences with moderately cool and dry winter where average air temperature stays around 20-22°C. Following this the average air temperature slightly shoots up to 25-27°C with the initiation of February and this condition lasted till March. The changes in the climatic variables may be partly associated with variation in the microbial count in this present study. Seasonal variation in the microbial count has been reported in different land-use systems³⁷⁻³⁹.

Microbial biomass C, N and P: The perusal of data from Table 3 revealed that there was significance ($p < 0.05$) difference of microbial biomass (C, N and P) in a different agroforestry system. The study also indicated that surface layer (0-15 cm) had significantly higher microbial biomass (C, N and P) than sub-surface (15-30 cm) layer, recording an increase of 25.31, 32.65 and 25.33% in 0-15 cm soil layer over 15-30 cm irrespective of systems for microbial biomass C, N and P, respectively. In 0-15 cm soil layer, higher microbial C was found in agri-horti-silvicultural (491.38 $\mu\text{g } \mu^{-1}$), followed by the home garden system (490.48 $\mu\text{g } \mu^{-1}$). The home gardens soils recorded highest microbial biomass N (50.71 $\mu\text{g } \mu^{-1}$) and P (5.83 $\mu\text{g } \mu^{-1}$) than the other systems with

Table 4: Correlation coefficients (r) of the microbial biomass with soil physical and chemical properties

Parameters	Bacteria (cfu × 10 ⁴ g ⁻¹)	Fungi (cfu × 10 ² g ⁻¹)	MBC (μg g ⁻¹)	MBN (μg g ⁻¹)	MBP (μg g ⁻¹)	SOC (%)	pH	Available N (kg ha ⁻¹)
Fungal (cfu × 10 ² g ⁻¹)	0.89**							
MBC (° μg g ⁻¹)	0.90**	0.88**						
MBN (μg g ⁻¹)	0.78**	0.69**	0.86**					
MBP (μg g ⁻¹)	0.69**	0.71**	0.79**	0.77**				
SOC (%)	0.70**	0.69**	0.84**	0.80**	0.74**			
pH	-0.26	-0.40	-0.32	-0.59**	-0.38	-0.50*		
Available N (kg ha ⁻¹)	0.74**	0.74**	0.82**	0.81**	0.69**	0.92**	-0.50*	
Available P (kg ha ⁻¹)	0.20	0.16	0.17	0.28	0.35	0.40	-0.45*	0.310

**significant at 1% level of significance; *Significant at 5% level of significance, MBC: Microbial biomass carbon, MBN: Microbial biomass nitrogen, MBP: Microbial biomass phosphorus; SOC: Soil organic carbon; N: Nitrogen; P: Phosphorus

the lowest value for microbial biomass N (39.12 μg μ⁻¹) and P (4.56 μg μ⁻¹) in horti-pastoral system. In this present study it was also observed that variation of microbial biomass C, N and P across the season (Table 3). Contrarily, the home garden system being present the highest species diversity, the maximum microbial C was found in the agri-horti-silvicultural system. This might be due to the higher application of fertilizers in the agri-horti-silvicultural system, as the home garden system was purely managed by farmyard manure generated from the household and its proximities. It is shown that temporal fluctuations in soil microbial C under different agroforestry systems is largely contributed due to difference in management within each site, soil texture, organic content level, etc.⁴⁰. However, the microbial biomass phosphorus was recorded highest in home garden and least in horti-pastoral system. This is maybe attributed to higher nutrient accumulation in the home garden *via* litterfall and decomposition activity²² while in the case of horti-pastoral system there might be high nutrient demand for *Sorghum*, especially during the rainy season. Generally, in this system the intercrop (*Sorghum*) was only grown during kharif season and the rest of the period remained as fallow without proper management.

There were reports for seasonal variation of microbial biomass under different agroforestry systems across the world. For instance in the babassu palm agroforestry system in Brazil⁴¹, subabul, eucalyptus and poplar based agroforestry system in Haryana⁴², cocoa agroforestry system in Bolivia⁴³. Seasonal variation in microbial biomass may be largely linked with the process of mineralization and immobilization phase, which is attributed by several biotic and biotic factors such as humidity and temperature⁴⁴, soil moisture⁴⁵, availability of nutrient reserves⁴⁶. In this study, it was noticed that the microbial biomass was found to be higher during the spring season and less during winter in all the sites. Our result confirmed with previous results reported by Chen *et al.*⁴⁵ and Diaz-Ravina *et al.*⁴⁷. The relatively higher amount of microbial biomass present during spring may be attributed due to the

abrupt rise of available substrate regenerated via higher root growth (exudates) and the turnover rate which results in the condition for the proliferation of microbes during spring season⁴⁸. Nevertheless, there are reports that microbial biomass peaks during winter in tropical soils^{45,49}; summer in moderately alkaline soil⁴², the rainy season in sandy loam⁵⁰. On the other hand, during the rainy season, most of the crops have peak growth period and the demand for nutrients have been drastically increasing thereby reducing the microbial biomass⁴². Whereas, the reason for the reduction in microbial biomass during the rainy season may be accredited due to loss of microbial propagules⁵¹; rapid mineralization⁵². The seasonal trends observed in the microbial biomass indicate that microbial biomass conserves the nutrients when they are in excess and simultaneously, releases them when the requirements of plants are high.

Correlation studies: The correlation studies carried out between different soil properties revealed a very strong positive correlation ($p < 0.01$) between the bacterial and fungal populations (Table 4). Similarly, it is seen from the microbial populations that have a positive significant ($p < 0.01$) relationship between microbial biomass C, N and P. However, the microbial populations had a negative relationship with the soil pH. Contrarily, Radhakrishnan and Varadharajan³⁵ found that bacterial count have a positive correlation with soil pH and consider pH as a good indicator for assessing microbial community. There was negative correlation between soil pH and MBC and this reflects the changes in soil pH and microbial biomass associated with different agroforestry systems under study. Wardle⁵³ had claimed that alterations in soil pH could bring the variation in microbial biomass. Further, Acosta-Martinez and Tabatabai⁵⁴ suggested that maximum activities of soil microbial biomass occur at pH values of about 6.5. There was a negative correlation between soil pH and MBP and this reflects the changes in soil pH and microbial biomass associated with different agroforestry systems under study. It was also observed that the existence of a significant

relationship between the soil organic carbon, nitrogen and microbial biomass C, N and P. This result indicated that the continuous development of microbial biomass over time without being interrupted by biotic factor⁵⁵⁻⁵⁷.

Overall from this study, it is perceived that species composition and associated management practices has significantly influenced on the soil nutrient flux and dynamics in agroforestry systems. This is also reflected in the microbial activity dependence on system complexity and self-regulating potential of different agroforestry systems under study. Additionally, the climatic variables during the investigation period were more or less similarity across the systems and indicating variation in microbial activity i.e. population dynamics and microbial biomass is largely attributed due to the availability and source of nutrient reserves in the system. Nonetheless, the variation in the microbial count and biomass due to the differences in abiotic factors such as soil temperature, moisture level cannot be completely ruled out. In-depth analysis of enzymatic processes in the soil would help further confirm our conclusions further.

CONCLUSION

The integration of trees in the agricultural systems has been playing a significant role in maintaining and improving soil productivity, thus ensuring the sustainability of the production systems. In this study, it was evident that the combination of tree-crop species in a system influenced the flux and dynamics of soil nutrients and also soil microorganisms. Also, the variability in climatic conditions and soil processes has had their reflections on the microbial population vis-à-vis biomass in the agroforestry systems. The study confirmed that the home gardens with greater species composition and monitored cultural practices have helped in the better soil health, thereby registering greater microbial population and biomass that had close correlations to the soil organic matter.

SIGNIFICANCE STATEMENT

This study discovers the influences of species diversity and its associated soil properties on soil bacterial and fungal populations (fungal populations' exhibit higher number) and microbial biomass under the same climatic condition. The understanding of different management practices and their impact on soil organic matter buildup vis-à-vis soil microbial dynamics shall help to evolve strategies for sustainable management of agroforestry systems *per se*.

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