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ICAR-NASF Funded Project on
Comparative study on carbon dynamics and functional rhizosphere microbial biomass of agroforestry systems in dry- and wet-tropical climatic situations

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Foreword

Agroforestry is one of the most important land use systems for deriving multiple benefits from its multiple components, in which woody perennials are deliberately integrated with crops on the same piece of land. In this context, the integration of high-value timber species, such as teak (*Tectona grandis*), and nutrient-rich fruit trees, such as aonla (*Phyllanthus emblica*), holds considerable promise for the development of sustainable and climate-resilient agroecosystems. Agroforestry holds significant potential for addressing climate change by facilitating long-term carbon sequestration in both tree biomass and soil. This dual capability makes it one of the most impactful strategies for mitigating the effects of a changing climate. Carbon dynamics within agroforestry system are an essential topic in recent research, particularly for understanding below-ground processes. The rhizosphere microbiome associated with trees and crops plays a crucial role in regulating soil carbon transformations, nutrient cycling, and overall soil health. A diverse and functionally active microbial community in the rhizosphere can enhance carbon stabilization, nutrient recycling, and system resilience, contributing to sustainable development goals and net-zero emissions in agroecosystems. Therefore, exploring carbon dynamics and the role of the rhizosphere microbiome is vital for maximizing carbon sequestration potential and leveraging microbiome benefits to improve long term productivity and sustainability. The composition of the belowground microbiome plays a crucial role in determining the fate of carbon in the soil. Gaining a thorough understanding of the rhizosphere microbiome associated with agroforestry trees is essential. This knowledge not only addresses current research gaps but also aids in developing targeted research strategies to help fulfil the multiple objectives expected of agroforestry in the future.

I am pleased to learn that the authors have been working on the cutting edge science of carbon dynamics and rhizosphere microbiome in agroforestry under an ongoing project funded by National Agricultural Science Fund (NASF), and the ICAR-Central Agroforestry Research Institute, Jhansi is publishing this technical bulletin entitled *"Carbon dynamics and functional rhizosphere microbiome in teak and aonla based agroforestry systems"*, which represents a commendable effort to document important scientific observations and research findings. I am confident that the insights shared in the publication will serve the true purpose of making agroforestry more convincing, acceptable, and popular among stakeholders, including farmers. Such knowledge will be instrumental in addressing the emerging challenges of climate change, livelihood improvement, and overall food, nutritional, and environmental security from a national and global perspective.

(M. Mohanty)

Preface

Agricultural systems are facing many challenges to increase food security amidst devastating impact of climate change, growing global population, threat of land degradation and responsibility to safeguard environmental security as well. Among many of the provisions obtained from agroforestry, carbon sequestration and soil health improvement are major parameters related to the mechanisms for checking environmental degradation. As carbon dynamics plays crucial role in harnessing the potential of carbon sequestration from the agroforestry systems, role of rhizosphere microbiome is very important to be unravelled. Thus, simultaneous evaluation of carbon dynamics and the contribution of rhizosphere microbiome in agroforestry systems hold very serious significance, which was felt to be investigated on top priority. In this direction, the opportunity for the research emerged through the ongoing project funded by the National Agricultural Science Fund (NASF), ICAR.

Being an important work on carbon dynamics and microbiome in agroforestry, it was felt essential to bring a document on **"Carbon dynamics and functional rhizosphere microbiome in teak and aonla based agroforestry systems"** in form of a Technical Bulletin, which will be very useful for streamlining the scientific studies on the present topic and to document the salient results and observations achieved so far through the course of such investigations in teak (*Tectona grandis*) and aonla (*Phyllanthus emblica*) based agroforestry systems. Significance of carbon stock in soil and tree biomass in agroforestry system is widely known, but the role of microbial communities in the rhizosphere for carbon dynamics and especially in comparison to the non-rhizosphere, as in sole crop land yet to be delineated in greater details. This work aims to bring the approaches together for evaluating the soil carbon dynamics, rhizosphere microbial diversity through culture, quantification and metagenome analysis along with their differential role, contribution of carbon from biomass components (tree, litter fall and crop residue) in bridging the gap between ecosystem-scale observations and molecular level biological functions. This will provide an avenue for the great network of rhizosphere interface, microbes focusing on bacteria, fungi and actinomycetes in soil-crop-tree-environment for enhancing the potential of agroforestry for climate resilience and sustainability.

This Technical Bulletin will serve many meaningful purposes for researchers, farmers and environmental policymakers for sustainable land management involving agroforestry. We hope that the work presented here will not only facilitate research but also help to build strategic approaches for the potential stake-holders of agroforestry and land managers functioning on combating the impact of changing climate across agro-ecosystem through improved land use planning.

- Authors

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1 Background

Comprehensive insights in carbon dynamics in agroforestry systems are very much essential for climate change mitigation perspectives. Greater focus and advanced research to harness the multiple potentials of agroforestry systems in this direction are highly desirable. There are many research gaps for understanding the intricacies of carbon dynamics in vegetation of the agroforestry systems (Newaj *et al.* 2012). Agroforestry has received much attention from researchers, policymakers and other stakeholders in India for its perceived ability to contribute significantly to economic growth, poverty alleviation and ecosystem services including carbon credit/trading opportunities (Chavan *et al.* 2020). A large number of variabilities may exist in case studies of India when compared with abroad in any agro-ecosystem aspects including the role of agroforestry systems for carbon sequestration over time and seasons in conjunction with understorey crop components in contrasting climatic situations. Agroforestry and its role in response to changing climate is important towards the assessment of various aspects as land use systems or as in tree-crop combinations in the context of climate change for its wider scope including the challenges and scope for carbon dynamics (Kellermann and Riper III, 2015; Athanasiou *et al.* 2010; Alam *et al.* 2018; Cida et al 2018). There are very scanty information on how carbon dynamics will be modulated in agroforestry systems in the changing climate scenarios. More importantly, rhizosphere microbiomes and their association with carbon dynamics are not yet clearly unraveled in agroforestry systems in broader perspectives.

Recent characterization of rhizosphere microbiomes with the use of omics tools has made progress in many tree species (Yu *et al.*, 2021; Yuan *et al.*, 2022; Jiang *et al.*, 2017; Zambounis *et al.*, 2019; Pervaiz *et al.*, 2020, Effendi *et al.*, 2020). Few studies clearly indicated that agroforestry drives the composition and diversity of plant microbiome at various spheres to impact plant growth and health as evidenced from Canadian silvipastoral, hedgerow and shelterbelt agroforestry systems (Banerjee *et al.*, 2016); agroforestry systems (Falkowski *et al.*, 2016); moso bamboo (Zhang *et al.*, 2019). In India, conventional agricultural crops like cereals, pulses, and vegetables have been in target to explore the rhizosphere microbial diversity and function. Cyanobacterial diversity in rice rhizosphere soils (Prasanna *et al.*, 2009); actinomycetes diversity in different medicinal plants rhizosphere (Thangapandian *et al.*, 2007) and diversity of N-acyl-homoserine lactone producing Gamma proteobacteria associated with southern mangrove rhizosphere (Viswanath *et al.*, 2015) thoroughly investigated.

Molecular diversity of 395 *Bacilli* associated with wheat from six agro-ecological zones of India through 16s rDNA gene sequencing was studied by Verma *et al.* (2016). Fungal identification and development of rhizosphere-microbiome derived microbial consortia for stress amelioration activities reported in solanaceous crops (Goswami *et al.* 2019) and pea (Chaudhari *et al.* 2020). In India, the complete characterization of rhizosphere microbiome using advanced genomics tools started of late in many agricultural crops such as rice (Bhattacharyya *et al.* 2016); wheat (Srivastava *et al.* 2020); cotton (Singh *et al.* 2020); lentil (Pramanik *et al.* 2020) and tea (Dutta and Thakur, 2021). In contrast, very few studies in this area on tree species exist in India such as in mangroves (Sakhia *et al.*, 2016), karira (Sharma *et al.*, 2008), and sisham (Joshi *et al.* 2021). A recent study in shiwalik hills emphasized the greater association of soil microflora and enzymatic activities with grassland and agroforestry land use systems (Bhowmik *et al.* 2019). Therefore, studies related to the rhizosphere microbial communities under agroforestry systems and their contribution in carbon dynamics are warranted.

Concept and Significance

Multipurpose tree species (MPTS) cultivated under diverse agroforestry systems across the varied agro-climatic zones of India remain largely underexplored in terms of their rhizosphere microbiome diversity and community composition. The rhizosphere, being a hotspot of biological activity, plays a crucial role in regulating soil processes, nutrient cycling, and carbon stabilization. Agroforestry systems, characterized by the integration of perennial trees with annual crops, offer a unique and dynamic platform to investigate the interactions between short-term (crop-associated) and long-term (tree-associated) microbiomes and their collective influence on soil carbon dynamics. Understanding these complex web of interactions is essential for generating comparative insights into carbon cycling pathways, differential carbon sequestration potential, and the functional roles of microbial communities across different agroforestry configurations. Such knowledge can help identify key microbial taxa and processes that enhance soil organic carbon storage, ecosystem services, and overall resilience. Moreover, tree rhizosphere-derived microbial inoculants, including beneficial bacteria and fungi, hold immense potential as biofertilizers. These microbial resources can improve nutrient use efficiency, stimulate plant growth, and enhance crop tolerance to abiotic stresses such as drought and temperature extremes. They can also be used for soil fertility restoration of the degraded landscapes to improve the productivity of such agroecosystems across the country.

In the context of climate change and sustainable land management, systematic studies on agroforestry microbiomes are critically needed to harness their ecological and agronomic benefits. Quantification of carbon sequestration potential in selected agroforestry systems across contrasting climatic regions will provide robust, location-specific evidence to guide policy and practice. Such assessments will not only support the development of climate-resilient agroecosystems but also contribute to emerging carbon credit and trading frameworks. Ultimately, this approach will broaden the scope for leveraging agroforestry as a viable strategy for carbon mitigation, biodiversity conservation, and long-term sustainability of natural resources.

3 Methodology

Description of land use systems

This study was conducted on two well-established agroforestry systems comprising 28-year-old teak (*Tectona grandis* L.) and 27-year-old aonla (*Phyllanthus emblica* L.) as tree components spread across 1.0 and 0.5 ha area, respectively at ICAR-Central Agroforestry Research Institute, Jhansi. Canopy cover for teak-based and aonla-based agroforestry systems were about 23% and 25%, respectively. These tree species are the predominant multipurpose tree species (MPTS) for agroforestry systems in the central India, which involved strategic plantation of tree-rows (teak: 4 × 4 m; aonla: 8 × 8 m spacing), creating alleys for growing crops. Understory crops namely black gram (*Vigna mungo* cv. Azad-2) and mustard (*Brassica juncea* cv. Giriraj) were grown in sequence in the *kharif* (rainy) and subsequently in *rabi* (winter) season, respectively in the year 2024 where sowing of black gram was done in the month of July and the matured crop was harvested in the month of November. Subsequently, mustard was sown in November and harvested in March 2025. Cultivation was done following the minimum tillage practices for Bundelkhand region where black gram and mustard was cultivated under rainfed and irrigated condition, respectively. For comparison, there was a sole cropland (without trees, which served as control) in an area of 0.5 ha where similar cropping sequence was followed. The crop management practice in the agroforestry systems and in the conventional sole crop-land was similar and uniform.

Baseline data and information from the selected fields prior to beginning the experiments with kharif crop has been collected as per the approved technical plan for the project in the selected agroforestry systems (teak based and aonla based agroforestry systems) and in sole cropland in open field for comparison (Figs. 1 and 2).

Fig. 1. Glance of experimental fields with black gram as understory crop [A. Teak based; B. Aonla based agroforestry systems and C. Sole crop-land]

Fig. 2. Glance of experimental fields with mustard as understory crop [A. Teak based; B. Aonla based agroforestry systems and C. Sole crop land]

Carbon dynamics

Soil sampling and processing

Across the studied agroecosystems, soil was sampled at specific intervals that represent key crop phenology stages: pre-sowing stage of black gram (Baseline) in July (2024); flowering stage (100% flowering) of black gram (Flowering BG) in September (2024) and maturity stage of black gram (Post-harvest BG) in November (2024). Similar techniques were followed for flowering stage of mustard (Flowering MT) in February (2025), and maturity stage of mustard (Post-harvest MT) in March (2025). Apart from this, another sampling was done in the second cycle of black gram cropping and soil was sampled in maturity stage of black gram (Post-harvest BG) in November (2025). For sampling, a conventional zigzag pattern was followed and the soils were collected from two depths (0–30 and 30–60 cm) in quadruplets using a soil auger from the agroforestry systems and sole cropland. This represented 24 soil samples (3 systems × 2 depths × 4 replications) collected in each crop phenology and 144 soil samples collected during the entire study (Fig. 3) After removal of plant roots and other visible debris, samples were quickly transported to the laboratory in sterile and clean zippered polyethylene bags and a portion was stored at 4°C for analysis of soil biological properties (e.g. soil microbial biomass carbon, basal soil respiration, dehydrogenase enzyme assay). The other portion of samples were air dried at room temperature, ground and passed through a 2.0 mm sieve, and analyzed for SOC.

Soil carbon dynamics refers to the continuous transformation, stabilization, and loss of carbon in soil through biological, chemical, and physical processes. It involves the balance between carbon entering the soil through plant residues, root exudates, and organic amendments, and carbon leaving the soil through microbial decomposition and respiration as CO_2. During decomposition, microorganisms transform organic matter into microbial biomass and more stable humified compounds, some of which become physically protected within soil aggregates or chemically associated with

minerals. The rate and direction of these processes are regulated by climate, soil texture and mineralogy, vegetation type, and land management practices, ultimately determining soil fertility, carbon sequestration potential, and contributions to atmospheric carbon fluxes. In agroforestry systems, increased biomass inputs and reduced disturbance often enhance carbon stabilization and long-term soil carbon storage. The following carbon-related components are required for evaluation of carbon dynamics of a given ecosystem:

Fig. 3. Representative images of soil sampling in the studied land use systems

Soil organic carbon (SOC)

Soil organic carbon (SOC) content was determined by wet oxidation method (Walkley and Black, 1934). In this method, 1.0 g of soil was oxidized with a mixture of 1.0 N $K_2Cr_2O_7$ and concentrated sulphuric acid utilizing the heat of dilution of sulphuric acid. 200 mL of distilled water and 10 mL of orthophosphoric acid (85%) was added to the conical flask. Unconsumed $K_2Cr_2O_7$ was back-titrated using 0.5 N ferrous ammonium sulphate (FAS) in presence of diphenylamine indicator (Fig. 4). SOC content was quantified using the following equation:

$$\text{Soil organic carbon (g kg}^{-1}) = [(B-S)/B \times N \times 3 \times 10]/w$$

Where,

B = Volume (ml) of 0.5 N FAS solution used for blank titration

S = Volume (ml) of 0.5 N FAS solution used for soil sample titration

N = Normality of $K_2Cr_2O_7$

w= Weight (g) of soil sample

Total organic carbon (TOC)

Total organic carbon was measured using the following equation: Total organic carbon (g kg^{-1}) = SOC × 1.3

Fig. 4. Estimation of total organic carbon through Walkley-Black method

Soil microbial biomass carbon (SMBC)

The determination of soil microbial biomass carbon typically involves the modified fumigation-extraction method (Vance *et al.*, 1987). In this process, two soil samples of equal weight were prepared. One sample was fumigated with chloroform to kill the microbial cells, while the other sample remains unfumigated as a control (Fig. 5A). To determine microbial biomass carbon, field-moist sample was taken in duplicate (to give approximately 10 g oven-dry weight) in 50 mL glass beakers. One set was placed into a glass vacuum desiccator with ethanol-free chloroform ($CHCl_3$); apply a vacuum briefly to vaporize the $CHCl_3$, then seal and incubate at room temperature for 24-48 h in dark. After fumigation and incubation, the excess $CHCl_3$ vapour was released from the desiccator by repeated back suction and allow the soil to ventilate for 30 min. The fumigated and non-fumigated soils were extracted with 25 mL of 0.5 M K_2SO_4 solution (1:2.5 soil: extractant ratio) for 30 min and filtered with Whatman No. 1 filter paper.

A 5 mL extract of the fumigated and non-fumigated soils was transferred to a wet-oxidation diffusion tube, and digested in the presence of a mixture (25 mL) of concentrated sulphuric-phosphoric (85%) acid (3:2) and excess $K_2Cr_2O_7$ oxidant (1 g) in a digestion block at 120°C for 2 h (Fig. 5B). The tubes were allowed to cool at room temperature for about 12 h. The amount of CO_2-C that evolved was trapped in a shell vial containing 1 M NaOH kept over the indentation inside the diffusion tube and the unspent alkali was titrated against 0.5 M HCl using phenolphthalein as an indicator (Snyder and Trofymow, 1984). Two blanks per 30 tube digestion batch was used and the blank digests containing double distilled water was treated exactly as the sample digests. The calculation of CO_2-C trapped required subtracting the equivalents of acid used in titrating a sample from the equivalents used to titrate a blank. To calculate SMBC by comparing titration values between samples and blanks, the following equation was used:

Fig. 5. Experimental units representing analysis of soil microbial biomass carbon. (A) Chloroform-fumigation unit; (B) Wet-oxidation diffusion unit with samples.

$$\text{Soil microbial biomass carbon} = (OC_f - OC_{uf})/K_{EC}$$

where, OC_f and OC_{uf} are organic carbon extracted from fumigated and non-fumigated soil respectively (expressed on oven dry basis), and K_{EC} the efficiency of extraction. A K_{EC} of 0.36 for microbial extraction efficiency was used for the calculation (Vance *et al.*, 1987).

Basal soil respiration (BSR)

It was quantified as microbial CO_2 evolution method (Alef, 1995). A 100 g field-moist soil sample was placed in an airtight glass bottle (500 ml) for each experimental unit where the soil moisture was adjusted to 60% of maximum water holding capacity (MWHC) before start of the incubation experiment to maintain microbial activity (Fig. 6). The units were pre-incubated for 3 days at 28°C. Afterwards, the lids of the units were opened and kept at room temperature for 1 h for natural gas exchange and placed back in the incubator where the units were incubated for 15 days at 28°C. Following the pre-incubation period, glass vials containing 10 ml of 1 N NaOH to capture CO_2 evolved were placed in the glass bottles. The evolved CO_2 was measured every three days interval by titrating with 1 N HCl in presence of excess 1 M $BaCl_2$. To discern the temporal dynamics of BSR across the crop phenology we have expressed it in two scales: firstly, the basal soil respiration rate (R_{BSR}) throughout 3-day incubation intervals onward 15 days in mg CO_2-C 100 g^{-1} soil d^{-1} and secondly, cumulative CO_2 evolved over a 15-day period (C_{BSR}) in mg CO_2-C 100 g^{-1} soil.

Fig. 6. Experimental unit showing incubation study related to basal soil respiration

Bulk density (BD)

Bulk density is defined as the mass of dry soil per unit volume including the pore spaces (Blake and Hartge, 1986). To assess the bulk density of soil, samples were collected using the core method from two distinct depth layers: 0–30 cm and 30–60 cm (Fig. 7). This technique ensures that the soil structure remains undisturbed during sampling, allowing for accurate measurement of soil mass per unit volume. Each soil core was carefully extracted and immediately placed in a zippered polythene bag to avoid moisture loss during handling. The collected soil samples were then oven-dried at 105°C for 72 h to remove all moisture content. This drying period is essential to obtain the true dry weight of the soil without any water interference. After cooling, the dry weight of the soil was recorded. Bulk density was then calculated by dividing the mass of the oven-dried soil by the internal volume of the core used for sampling using the following equation:

$$\text{Bulk density (Mg m}^{-3}) = \text{Dry mass of soil (Mg)/Volume of core (m}^{-3})$$

Fig. 7. Sample collection by soil core method to assess bulk density

Total organic carbon stock (TOCS)

The stock was computed from the soil thickness and bulk density using the following equation:

$$\text{Total organic carbon stock (Mg ha}^{-1}\text{)} = \text{TOC} \times \text{BD} \times \text{D} \times 10$$

where TOC = total organic carbon content (g kg^{-1}); BD = bulk density (Mg m^{-3}); D = soil thickness (m). Total organic carbon stock onward 0.6 m depth was computed by adding the carbon stock per soil layer.

Dehydrogenase enzyme assay (DHA)

The determination of dehydrogenase activity (Klein *et al.*, 1971) in soil involves assessing the activity of soil microorganisms by measuring the reduction of a substrate to a formazan product. This method used employs triphenyl tetrazolium chloride (TTC) as the substrate. In this process, soil samples were incubated with TTC, which acts as an electron acceptor and was reduced to triphenyl formazan (TPF) by dehydrogenase enzymes present in active microbial cells. One g of soil was mixed with 0.2 ml of a 3% TTC solution and 0.5 ml of 1% dextrose solution in a tube sealed with plastic stoppers. The mixture was incubated at 28°C for 24 h in the dark to allow the reaction to proceed. After incubation, the reaction was stopped by adding 10 ml methanol to extract the TPF from the soil. The soil suspension was then filtered, and the intensity of the red color of the TPF extract was measured on spectrophotometrically at 485 nm (Fig. 8). The amount of TPF produced was directly proportional to the dehydrogenase activity, providing an indication of microbial activity and soil health and expressed in mg TPF g^{-1} soil 24 h^{-1}.

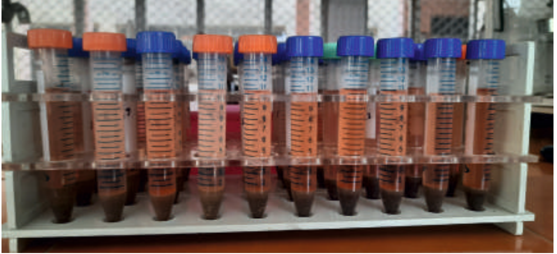


Fig. 8. Colour development during dehydrogenase enzyme assay

Biomass components

Tree Biomass

Non-destructive method for estimation of above ground (AG) tree biomass was followed using widely used allometric equation taking the tree trunk diameter at breast height (DBH) at 1.37 m at the ground level as the input parameter for teak trees (Negi *et al.*, 1995). The below ground biomass of teak tree was estimated taking the well referred fraction of 0.26 as the root to shoot ratio (Ravindranath and Ostwald, 2008). The allometric equation used for teak is given below:

Total above ground tree biomass (kg/tree) = $0.0758(DBH)^{2.6135}$ ....(T1)

Total below ground tree biomass (kg/tree) = T1(0.26) ....(T2)

Total tree biomass (ABG+BG) (kg/tree) = T1+T2 ....(T3)

Similarly, non-destructive method for estimation of tree biomass was followed using widely used allometric equation taking the collar diameter (CD) at 15 cm height at the ground level as the input parameter for aonla trees (Newaj *et al.* 2016). The allometric equations used for aonla are given below:

Total tree biomass (above ground + below ground) (kg/tree) = $2.994(CD)^{1.285}$ .....(A1)

Root (below ground biomass) (kg/tree) = $0.622(CD)^{1.313}$ .....(A2)

Above ground biomass (ABG) (kg/tree) = A1-A2 .....(A3)

Litter fall and leaf litter decomposition

Litter fall of trees was collected in agroforestry systems at monthly intervals using litter traps (0.5x0.5 m) (Fig. 9). The collection of litter fall continued for full one year covering the two consecutive crop seasons (*kharif* and *rabi*). The monthly litter fall data was pooled for calculation for annual litter fall quantification.

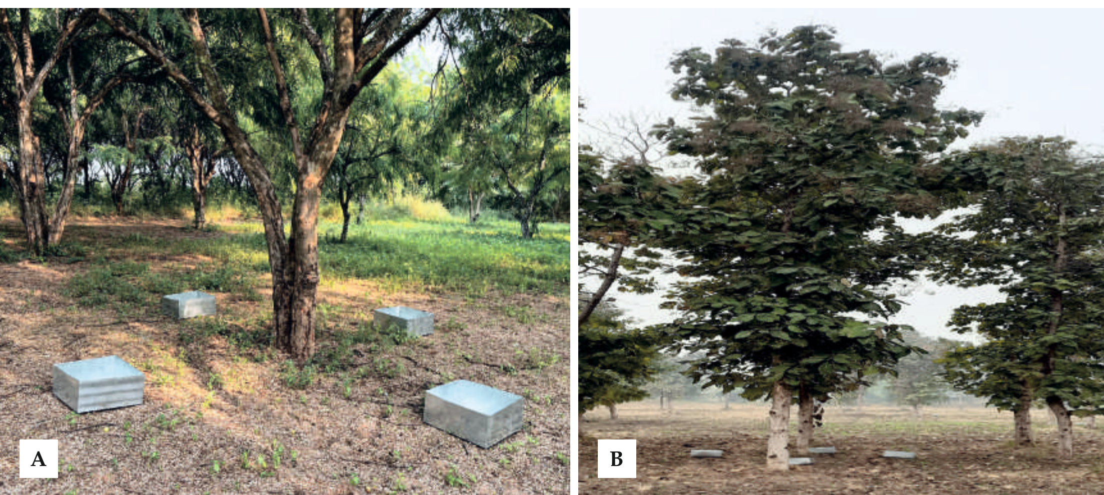


Fig. 9. A glance of litter fall estimation through litter traps placed in agroforestry systems. (A: Aonla based ; B: Teak based)

Nylon leaf litter bag (20 x 20 cm) having pore size of about 1 mm stitched with nylon thread was used for keeping a known weight (20 g) of leaf litter and placed into the soil (0-15 cm depth) in respective agroforestry systems (Fig. 10). Sixty leaf litter bags filled with leaf litter were placed in five places having twelve bags in each place in each respective agroforestry system. One bag from each of the five places (five bags/month) from the respective agroforestry system was pulled and carefully washed to remove the dirt before placing them for air-drying at room temperature. After drying, the remaining litter in the bag was weighed (g). Thus, every monthly interval, leaf litter remaining in the bag was weighed till the complete decaying of the litter in the bag. The progressive loss of the leaf litter data was used for calculation of leaf litter decomposition rate in the respective agroforestry systems and suitable equation will be fit for calculation decay-rate constant in due course.

Fig. 10. A glance of leaf litter decomposition study through leaf litter bags being placed in agroforestry systems. (A: Teak based ; B: Aonla based)

Crop growth, yield, crop biomass and crop residue biomass

Plants produced per square meter of ground surface were collected by placing a thin iron (square-shaped 1m x 1m) hollow block under each land use systems (Alam *et al.*, 2018). Thus, for dry biomass estimation, the plants in 1 m^2 area were collected by reaping the plants at ground level (Fig. 11). Then the plant parts were separated (straw & seed) and dried in a hot air oven at 70°C for 48 to 72 h to a constant weight. Dried samples were considered for biomass estimation. Similarly, for grain yield estimation, whole plants were harvested. Pods and seeds were separated per plant

basis. Following the aforesaid procedures, biomass and grain yield of understory crops were estimated in replications. Similarly, the crop residue biomass (both above ground and below ground separately) were estimated after the harvest of the crop. Crop biomass, yield and crop residue biomass were then expressed in kg/ha.

Fig. 11. A glance of quadrate method for estimation of crop yield and crop biomass in agroforestry systems and sole crop field. (A: Black-gram; B: Mustard)

Total carbon

Different components namely tree, crop residue and soil under agroforestry systems were considered for estimation of total carbon. The technique followed for estimation of the total carbon in the system is depicted in tabular form below for better clarity. The factor of 0.5 was used for converting biomass into carbon following the widely used technique.

Land use systems	**Tree biomass (t/ha)**			**Litter biomass (t/ha)**	**Crop residue biomass (t/ha)**	**Total biomass in the system (t/ha)**	**Total system biomass carbon stock (t/ha)**	**Soil carbon (t/ha)**	**Total carbon stock in the system (t/ha)**
	AG(a)	BG(b)	Total (c) (c=a+b)	d	e	f (c+d+e)	g (f*0.5)	h	g+h
Agro-forestry system									
Sole crop									

AG = Aboveground; BG = Belowground

Net carbon balance

Estimation of net-carbon sequestration in relation to microbial respiration was adopted (Alef, 1995). Total estimated net-carbon sequestration in the agroforestry system = [(Net carbon sequestration in the agroforestry system from biomass - soil respiration rate (from microbial studies) + SOC]

Rhizosphere soil microbiome

Agroforestry rhizosphere microbiome: An in-depth analysis

Rhizosphere being a complex niche for microbial adaptation provides diverse community composition to thrive in unique environment (Ashajyothi *et al.* 2022). The rhizosphere microbiome diversity depends upon the plant species, age, nutrient availability, climate and related stress conditions. Knowing the rhizosphere diversity helps in understanding the underlying activities of the system for ecological benefit. To study the total microbiome diversity of a given ecosystem as mentioned earlier, culturable and meta-genomics tools such as shotgun metagenome sequencing with the help of 'Next Generation Sequencing' technologies can be exploited. In present study, both the techniques have been adapted to study beneficial microflora as well as total microbiome diversity of tree-rhizospheres from the studied agroforestry systems and from sole crop land as control.

Quantification of culturable microbes

Isolation: The soil samples were processed inside the laboratory under aseptic conditions. To characterize the soil microbial population contributing to carbon dynamics the experiments have been initiated in the selected agroforestry systems in *kharif* season. For baseline data, soil samples have been collected before sowing for estimation of the total bacteria count, total fungi count and total actinomycetes count.

Total bacteria count: One gram of soil sample was vigorously mixed in 9 ml sterile distilled water in test tubes. Serial dilutions were made sequentially using 9 ml sterile distilled water in test tubes to give a ten-fold dilution from 10^{-4} to 10^{-6} for each soil sample. 1 ml of each diluted sample was added to each sterile petri plate and 20 ml of sterilized luke warm 'Nutrient Agar' media was added to each Petri plate and gently spread in all directions. Petri plates were kept for incubation at 28±1°C for 48 h. The total bacteria count for each soil sample was recorded and calculated as cfu/g soil.

Total actinomycetes count: One gram of soil sample was vigorously mixed in 9 ml sterile distilled water in test tubes. Serial dilutions were made sequentially using 9 ml sterile distilled water in test tubes to give a ten-fold dilution from 10^{-4} to 10^{-6} for each soil sample. 'Actinomycetes Isolation Agar (AIA)' for actinomycetes isolation was prepared, sterilized and plates were prepared. 100 µl of each diluted sample was spreaded on each media plate. Petri plates were kept for incubation at 28±1°C for 14 days. The total actinomycetes count for each soil sample was recorded and calculated as cfu/g soil.

Total fungi count: One gram of soil sample was vigorously mixed in 9 ml sterile distilled water in test tubes. Serial dilutions were made sequentially using 9 ml sterile distilled water in test tubes to give a ten-fold dilution from 10^{-3} to 10^{-5} for each soil sample. 'Potato Dextrose Agar' media for fungi isolation was prepared, sterilized and plates were prepared. 100 µl of each diluted sample was spreaded on each plate of Potato Dextrose media. Petri plates were kept for incubation at 28±1°C for 5-7 days. The total fungi count for each soil sample was recorded and calculated as cfu/g soil.

Isolation and screening of beneficial microbes

i. Isolation of nitrogen fixing bacteria (Azotobacter, Azospirillum and Rhizobium)

Isolation of nitrogen fixing bacteria was performed following the serial dilution method and pour plating. 1g of soil sample was taken and serially diluted using sterile distilled water up-to 10^{-8} dilutions. 1 ml of each diluted sample was added to each plate and about 20 ml of sterilized Burk's medium was added to each petri plate and mixed gently in all directions for isolation of *Azotobacter*. For isolation of *Rhizobium*, 1 ml of each diluted sample was added to each plate and about 20 ml of sterilized Yeast Mannitol agar medium was added to each petri plate and mixed gently in all directions. Similarly, for *Azospirillum* isolation *Azospirillum* semi solid media plates were used for pour plating. All the plates kept for incubation at 28±2°C for 7 days. Well-separated individual colonies were marked, picked up with a sterile loop, and transferred to specific media plates for sub culturing. Isolates were classified on the basis of colony characteristics such as size, color, shape, and texture for further pure culture preparation.

ii. Isolation of phosphate solubilizing microbes

For the isolation of phosphate solubilizing microbes serial dilution was performed with 1g of soil sample up to 10^{-5} dilutions. 1 ml of each diluted sample was added to each plate and about 20 ml of sterilized Pikovskaya's agar was added to each petri plate and spread gently in all directions and kept for incubation at 27±2°C for 2-5 days. Well-separated individual colonies of bacteria, actinomycetes and fungi were marked, picked up with a sterile loop, and transferred to Nutrient Agar, Actinomycetes Isolation Agar and Potato Dextrose Agar media plates for sub culturing as per the standard protocols, respectively. Isolates were classified on the basis of colony characteristics such as size, colour, shape, and texture for further pure culture preparation. Six bacteria, six actinomycetes and five fungi isolates were positive for phosphate solubilization. Cultural and morphological characteristics of phosphate solubilizing microorganisms have also been studied.

For preservation all the isolated microbial cultures were grown and purified by the streaking/inoculation of single colony/fungal disc on specific media slants/broth and allowed to grow for 24 - 48 h. Glycerol stocks were prepared for bacteria grown in broth and all pure cultures were preserved at -20 °C for further studies.

iii. Microbial characterization

All the isolated microbes were subjected to microscopy aided morphological characterization by considering the bacterial colony characteristics such as size, colour, shape, and texture; fungal mycelia, conidial characters. The best performing microbial isolates were selected for further molecular characterization by DNA isolation, target amplification of the universal house-keeping marker genes such as 16S rRNA for bacteria and ITS (Internal Transcribed Spacer) for fungi by 'Polymerase Chain Reaction', sequencing, phylogenetic analysis for further accessioning of the microbes from NCBI GenBank.

Whole genome metagenome studies

Total 12 soil samples were collected from two agroforestry systems along with sole crop land as a control at varying depths (0-30 cm and 30-60 cm) during post-harvest stage of *kharif* crop in the year 2024. Shotgun metagenomic sequencing that leveraging the high-throughput power of Illumina NovaSeq with a 2 x 150 bp paired-end chemistry, enables an unprecedented deep dive into the taxonomic and functional complexity of soil microbiome has been used to study the agroforestry soils. Shotgun metagenomic sequencing involves four major steps: DNA extraction, library preparation, sequencing, and bioinformatic analysis (Fig. 12). The initial stage, DNA extraction, and critical quality checks performed since all subsequent analyses in the shotgun metagenomics workflow are dependent on the quality of input DNA. Next, library preparation using the kits as per the instructions and preceded for the sequencing. Prior to sequencing in shotgun metagenomics, DNA must be fragmented (sheared) into smaller bits. NGS library preparation also includes adding index sequences to DNA molecules so that they can be sequenced simultaneously (multiplexed sequencing). Finally, once sequencing is completed, the reads processed or demultiplexed for curation that involved adapter removal, trimming, alignment, merging and final bioinformatic evaluation for further downstream analysis.

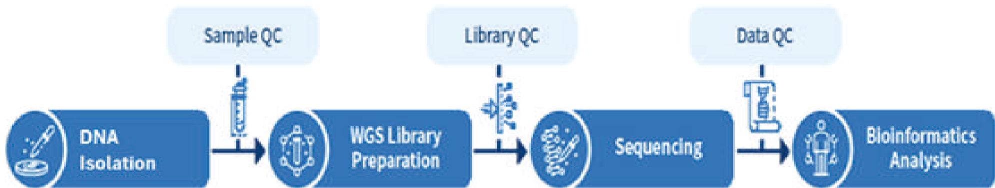


Fig. 12. Outline of shotgun metagenomic sequencing

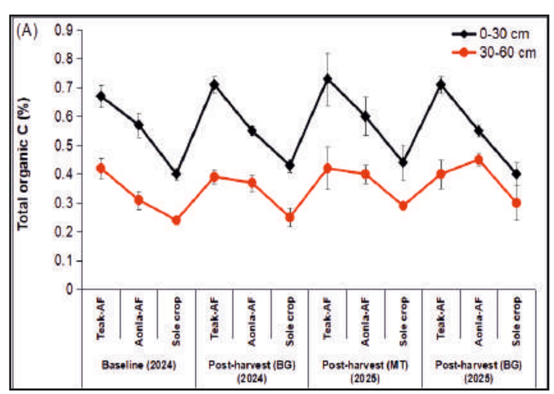
The raw data was subjected to curation for adapter removal and filtering of low-quality reads. Host genome mapping and merging were performed to generate contigs. The contigs were used for further downstream analysis

4 Salient Observations

Carbon dynamics

The total organic carbon (TOC) dynamics across the studied land-use systems revealed a clear and consistent influence of agroforestry on soil carbon sequestration over time and depth (Fig. 13A). Teak-based agroforestry exhibited the most pronounced TOC enrichment, particularly in the surface layer (0–30 cm), where TOC increased from 0.67% at baseline (2024) to a peak of 0.73% post-rabi (2025), indicating sustained carbon inputs and retention. Aonla-based agroforestry showed moderate TOC dynamics, with surface TOC maintained between 0.55 and 0.60% and a progressive increase in sub-surface TOC (30–60 cm), suggesting downward carbon translocation and improved profile-level storage. In contrast, the sole crop system displayed comparatively weak TOC dynamics, with consistently lower TOC concentrations (0.40–0.44% at 0–30 cm and 0.24–0.30% at 30–60 cm) and only marginal seasonal fluctuations. Across all systems, TOC remained higher in the surface soil than in the sub-surface layer. Overall, the observed TOC dynamics highlight the superior capacity of agroforestry systems, particularly teak-based system, to enhance and stabilize soil carbon stocks in the semiarid *Bundelkhand* region (Fig. 13B).

Soil microbial biomass carbon (SMBC) increased steadily from the baseline (2024) through post-*kharif* (2024) and reached its maximum during post-*rabi* (2025), indicating cumulative stimulation of microbial biomass with advancing crop phenology, residue return, and root turnover (Fig. 14). This temporal enrichment was more pronounced in the surface soil (0–30 cm), highlighting dynamic microbial responsiveness to fresh carbon inputs and favorable microclimatic conditions near the soil surface, while the subsoil (30–60 cm)



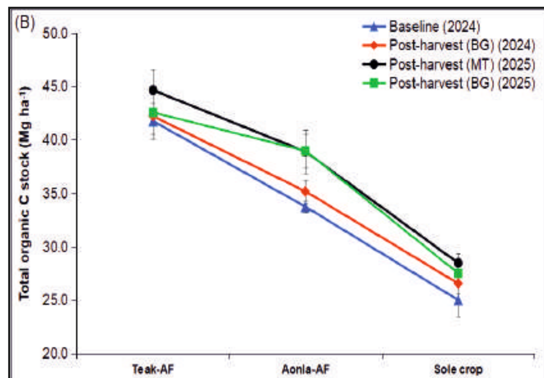


Fig. 13. Dynamics of total organic carbon in agroforestry systems and sole crop land. Bars on the markers indicate standard error (n= 4). (A) Total organic carbon; (B) Total organic carbon stock

showed a slower but consistent upward trend. Agroforestry systems exhibited stronger SMBC dynamics than sole cropping, reflecting sustained organic matter inputs from tree litter and rhizosphere interactions. Overall, the observed patterns underscore that SMBC dynamics are governed by the interaction of seasonal progression, soil depth, and land-use–driven carbon inputs, with agroforestry systems enhancing microbial biomass buildup and soil biological resilience.

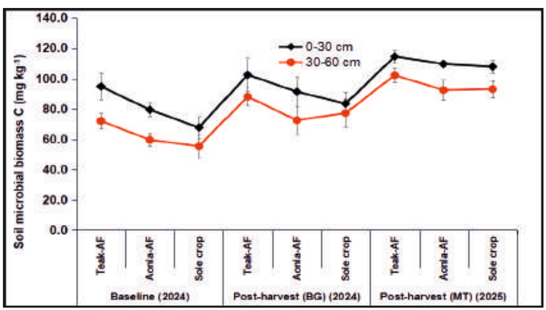
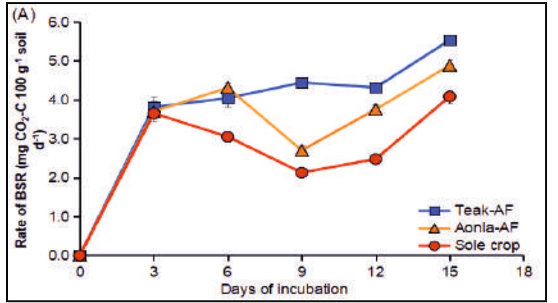


Fig. 14 Dynamics of soil microbial biomass carbon in agroforestry systems and sole crop land. Bars on the markers indicate standard error (n= 4).

Agroforestry and sole cropping systems significantly impacted on the temporal dynamics of basal soil respiration (BSR) rate across the soil depths throughout the incubation intervals (Fig. 15). At baseline, teak-based system showed higher rate with 4.81 and 3.55 mg CO_2-C 100 g^{-1} soil d^{-1} at 0-30 cm and 30-60 cm, respectively and the rate and cumulative BSR in teak-based system was higher than aonla-based system and sole cropland. From baseline, basal soil respiration rate and cumulative BSR increased to flowering stage of the intercrops followed by a decrease at maturity (Fig. 16). With respect to soil depth, the highest cumulative BSR (66.46 and 55.69 mg CO_2-C 100 g^{-1} soil) at 0-30 and 30-60 cm, respectively was observed at flowering stage of black gram used as understory crop. Comparatively higher rate and cumulative BSR was observed during black gram as understory crop than mustard across all the crop phenological stages and surface soil showed relatively higher values than the sub-surface soil. Across agroforestry and crop phenology, surface soil showed relatively higher cumulative BSR over a 15-day incubation period that ranged from 6.14 to 66.46 mg CO_2-C 100 g^{-1} soil over the subsurface soil where it ranged from 4.27 to 55.69 mg CO_2-C 100 g^{-1} soil. Moreover, relatively much higher basal soil respiration was observed in agroforestry systems than in the sole cropland.



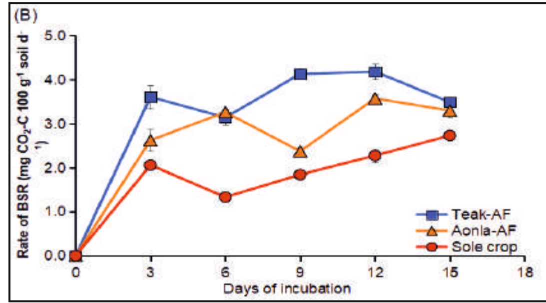


Fig. 15. Dynamics of basal soil respiration rate observed during 3-days incubation intervals at flowering stage of black gram as understory crop in agroforestry systems and sole crop land. (A) 0-30 cm; (B) 30-60 cm. Bars on the markers indicate standard error (n= 4)

Across all sampling stages, dehydrogenase enzyme activity was consistently higher in the surface soil (0–30 cm) than in the subsoil (30–60 cm), indicating greater microbial activity near the surface. Among land-use systems, agroforestry systems generally exhibited higher dehydrogenase activity than the sole cropping system at both depths (Fig. 17). From the baseline (2024) to post-*kharif* (2024), a moderate increase in activity was observed, followed by a pronounced increase by post-*rabi* (2025), reflecting cumulative effects of crop growth, residue inputs, and favorable soil biological conditions over time. Teak-based systems recorded the highest enzyme activity at the surface soil during post-*rabi* (8.59 µg TPF g^{-1} soil 24 h^{-1}), while aonla systems showed comparatively higher activity than teak at subsoil. Overall, the data indicate that agroforestry systems enhance soil biological functioning over time, with stronger effects in surface layers compared to sole cropping.

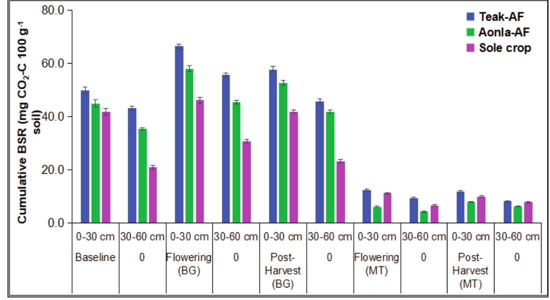


Fig. 16 Dynamics of cumulative basal soil respiration over a 15-day incubation period in agroforestry systems and sole crop land. Bars on the markers indicate standard error (n= 4). BG- Black gram; MT- Mustard

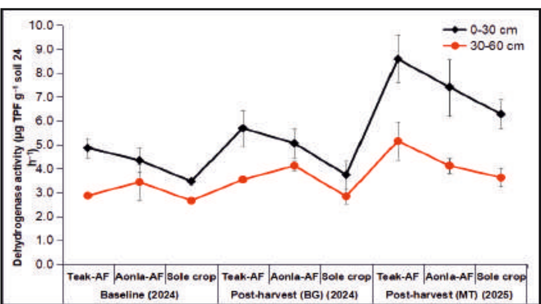
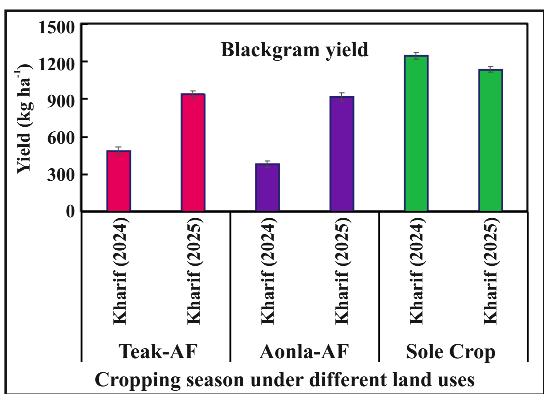


Fig. 17 Dynamics of dehydrogenase activity in agroforestry systems and sole crop land. Bars on the markers indicate standard error (n= 4).

Crop growth, yield, crop biomass, crop residue biomass, and litter fall

Differential responses in crop yield, crop residue biomass, and litter fall have been observed depending upon the systems and season as well (Figs. 18 and 19). These data can be used for accounting carbon dynamics and net carbon balance.



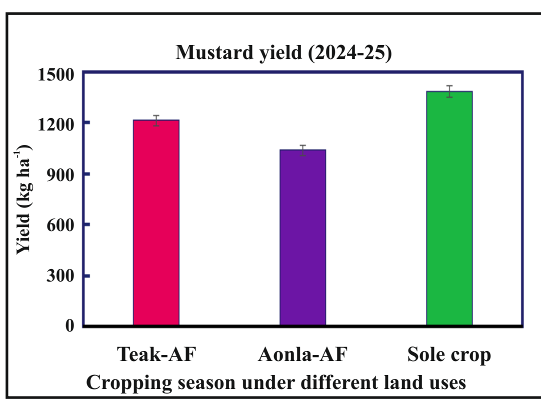
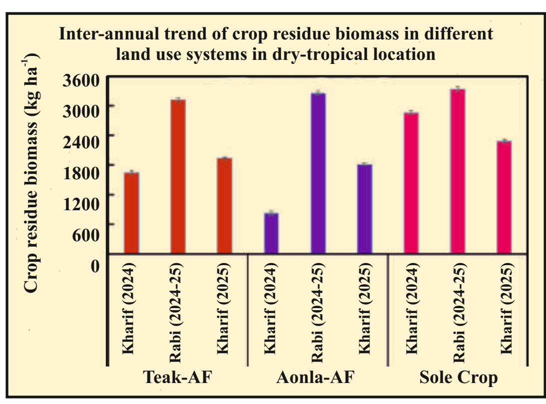


Fig. 18. Yield of blackgram and mustard under different land use systems (AF=agroforestry)



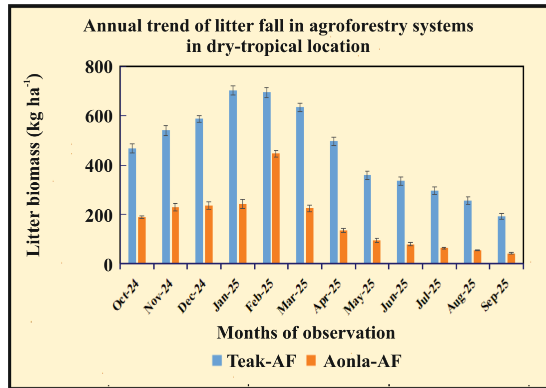


Fig. 19 Inter-annual trend of crop residue biomass and litter fall in different land use systems (AF=agroforestry)

Table 1. Carbon stock at baseline (2024) in selected agroforestry systems

Land use systems	Tree biomass (t/ha)			Litter biomass (t/ha)	Crop residue biomass (t/ha)	Total biomass in the system (t/ha)	Total system biomass carbon stock (t/ha)	Soil carbon (t/ha)	Total carbon stock in the system (t/ha)
	AG(a)	BG(b)	Total (c=a+b)	d	e	f (c+d+e)	g (f*0.5)	h	g+h
Teak based agro-forestry system	189.503	49.271	238.774	5.57	2.387	246.731	123.366	41.770	165.136
Aonla based agro-forestry system	23.801	7.041	30.842	2.04	2.050	34.932	17.466	33.730	51.196
Sole cropland					3.103	3.103	1.552	25.00	26.552

AG = Aboveground; BG = Belowground

Baseline data showed that total carbon stock was higher in agroforestry systems than in sole crop land where teak based agroforestry system had relatively higher carbon stock (Table 1).

Rhizosphere soil microbiome

Soil microbial populations were quantified under different land-use systems, including agroforestry systems (teak- and aonla-based) and sole cropping systems. Sampling was conducted during the baseline (pre-sowing) and post-*kharif* season. Soil samples were collected from two depths (0–30 cm and 30–60 cm) to evaluate the vertical distribution of microbial communities. Across all land-use systems, microbial abundance was

consistently higher in surface soils than in subsurface layers (Fig. 20). A pronounced seasonal effect was observed, with total microbial populations increasing during the post-kharif period relative to the pre-sowing stage.

Microbial counts increased at post-*kharif* compared to the pre sowing stage across the land use systems (Fig. 20). Total microbial counts (bacteria, fungi, actinomycetes) were higher in agroforestry systems compared to sole crop. A depth wise variation was observed with higher microbial populations in surface soil compared to sub-surface soil (Fig. 21). Fungal populations exhibited a similar pattern across land-use systems. In contrast, actinomycete populations were enhanced under agroforestry systems, with the highest counts recorded in teak-based systems (31.33×10^5 cfu g^{-1} soil), followed by aonla-based systems, while sole cropping systems showed comparatively lower populations (25.33×10^5 cfu g^{-1} soil).

Beneficial soil microorganisms, including *Rhizobium*, *Azotobacter*, *Azospirillum*, and phosphate-solubilizing microorganisms, were isolated, purified, screened, and preserved. Cultural and morphological characterization enabled the preliminary identification of seventy *Rhizobium*, seventeen *Azotobacter*, five *Azospirillum*, and seventeen phosphate-solubilizing isolates. Maximum phosphate solubilization efficiency (PSE) was shown by PSB-4 (90.48%), PSA-3 (94.44%) and PSF-2 (58.37%) among all the bacterial, actinomycetes and fungal isolates (Figs. 22 and 23; Tables 2-4).

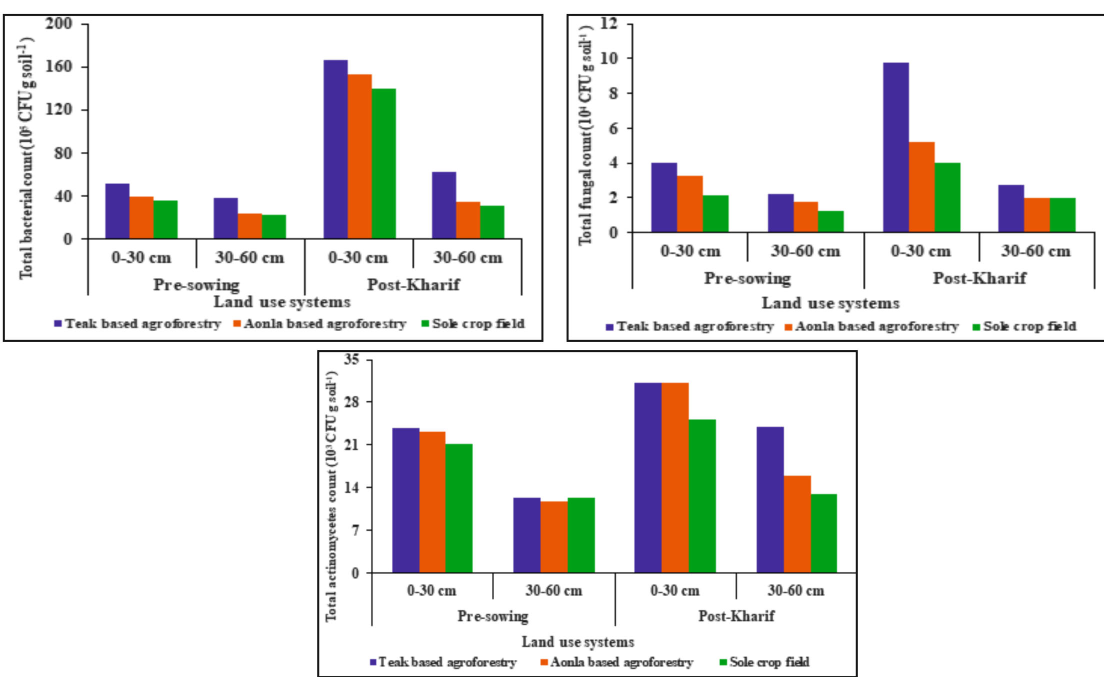


Fig. 20. Quantification of culturable microorganisms (bacteria, fungi and actinomycetes) across the land use systems

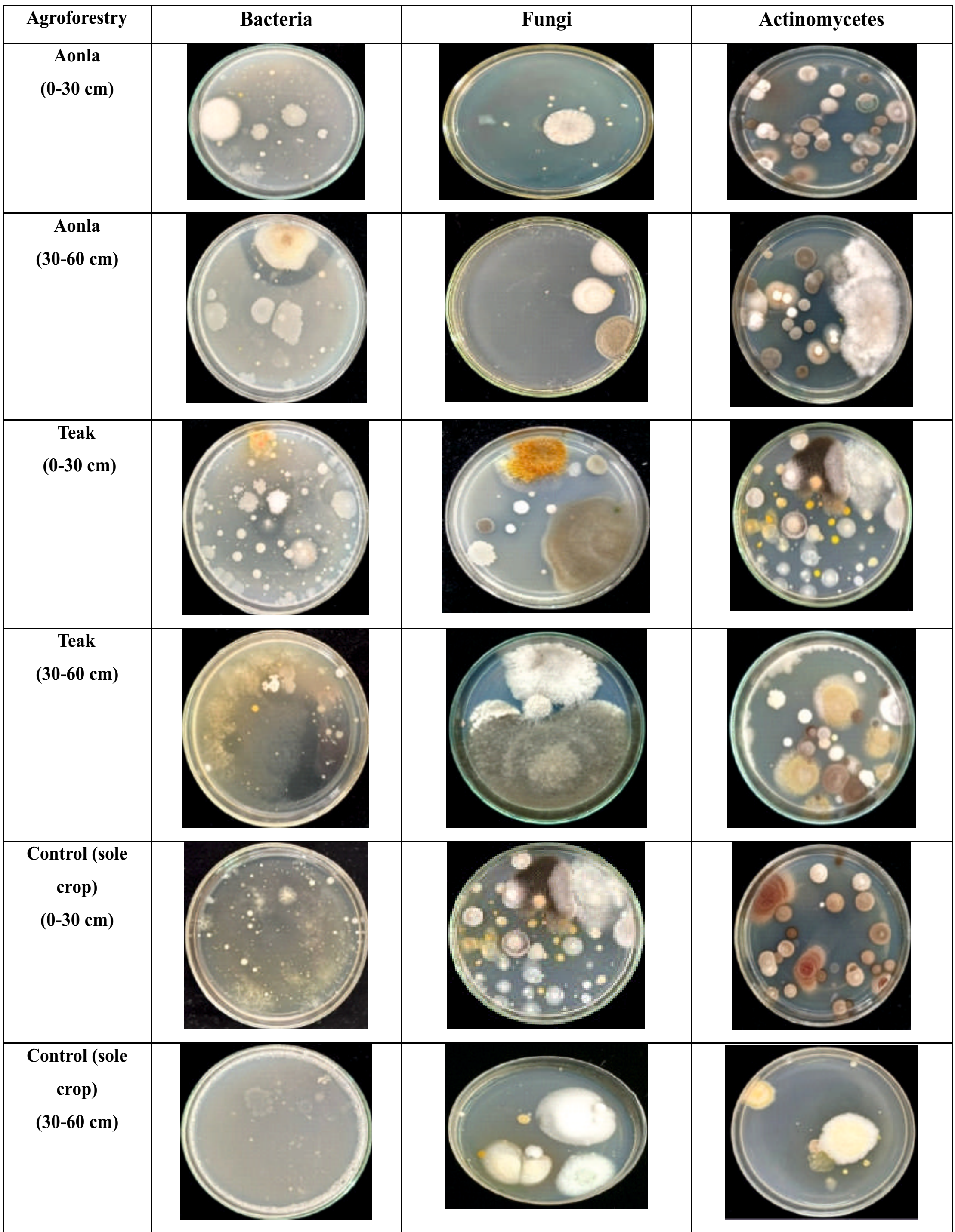


Fig. 21. A glance of isolation of soil bacteria, fungi, and actinomycetes from aonla and teak based agroforestry systems and sole crop land

Sr. no.	Isolate code	Culture growth on YMA media	Culture growth on NA media	Close shot (Culture growth on NA media)
1	A3			
2	A7			

Fig. 22. A glance of isolation, purification and screening of *Rhizobium* bacteria on Yeast mannitol agar (YMA) and Nutrient agar (NA) medium

Organism	Halo zone formation	Pure culture	Phosphate solubilization efficiency (%)
Bacteria (Isolate: PSB-4A)			90.48
Actinomycetes (Isolate: PSA-3T)			94.44
Fungi (Isolate: PSF-1A)			55.27

Fig. 23. Isolation, purification and screening of phosphate solubilizing organisms on Pikovskaya's Agar and their maximum phosphate solubilization efficiency

Table 2. Cultural and morphological characteristics of selected phosphate solubilizing bacteria

S.No.	Isolates	Pigmentation	Surface	Elevation	Size	Cell shape	Colonies	Growth	Opacity	Color
1	PSB-1A	Creamy white to off-white	Smooth and glistening	Raised to slightly convex	3.5 mm	Predominantly circular to irregular or spreading margins	Smooth to mucoid, undulate to lobate	Moderate to rapid	Opaque to translucent	Off-white to creamy
2	PSB-2A	Non-pigmented	Smooth and glistening	Convex	2.5 mm	Predominantly circular	Entire or slightly undulate	Discrete, well-separated colonies	Opaque	Pale cream to light beige
3	PSB-3A	Non-pigmented	Smooth and glistening	Convex to raised	3 mm	Predominantly circular	Entire (smooth)	Discrete, scattered colonies	Opaque	Creamy white
4	PSB-4A	Grayish-white	Smooth, Rough and dry-looking	Flat or slightly raised	3.8 mm	Irregular, possibly filamentous or lobate	Irregular, possibly filamentous or lobate	Mixed - multiple discrete colonies	Opaque	Cream to off-white
5	PSB-5T	Non-pigmented	Smooth and moist	Slightly raised	3 mm	Rod-shaped	Circular colonies	Medium	Opaque	Off-white or cream-colored
6	PSB-6T	White	Rough/ dry	Raised	4 mm	Likely rod-shaped or filamentous	Irregular, spreading	Large	Opaque	White

Table 3. Cultural and morphological characteristics of selected phosphate solubilizing actinomycetes

S.No.	Isolates	Texture	Elevation	Colonies	Growth	Color	Margin
1	PSA-1T	Chalky, powdery	Slightly raised	Dry, rough surface with aerial mycelium	Moderate	Greyish-white aerial with pale substrate	Irregular
2	PSA-2T	Leathery, dry	Flat	Compact, wrinkled colonies	Slow	Pale brown to creamy	Entire
3	PSA-3T	Velvety	Raised	Spreading with dense aerial growth	Fast	White aerial mycelium with yellowish base	Lobed
4	PSA-4A	Powdery	Umbonate	Concentric wrinkled rings, tough colonies	Moderate	Gray aerial mycelium, reddish pigment	Lobed
5	PSA-5A	Crumbly, chalky	Convex	Dense with irregular folds	Moderate	Light grey with pale yellow reverse	Irregular
6	PSA-6T	Dry, tough	Slightly raised	Firm, leathery colonies	Slow	Creamy-white to light brown	Entire

Table 4. Cultural and morphological characteristics of selected phosphate solubilizing fungi

S.No.	Isolates	Texture	Elevation	Colonies	Growth	Color	Margin
1	PSF-1A	Powdery texture	Raised	Colony surface dark brown to black	Fast-growing on PDA	Dark brown with concentric rings	Smooth and regular
2	PSF-2A	Velvety	Flat to raised	White to grayish colony	Rapid growth	White to grey	Lobed
3	PSF-3A	Fluffy to granular	Slightly raised	Yellowish colony, circular with regular margin	Rapid growth on PDA	Yellowish green with creamy white border	Irregular
4	PSF-4T	Compact and leathery	Raised	Reddish-brown colony	Slow to moderate growth	Pink to reddish-orange	Entire
5	PSF-5T	Cottony	Slightly raised	Creamy white colony	Rapid radial spread	White initially, turning grayish	Wavy

Concise results on initial Metagenomics-based analysis

Metagenomics-based analysis of the agroforestry rhizosphere microbiome was initiated to assess microbial diversity. Soil samples collected during the post-*kharif* season (2024) from aonla- and teak-based agroforestry systems, along with a sole-crop control, at depths of 0–30 cm and 30–60 cm, were subjected to shotgun metagenomic sequencing using the Illumina NovaSeq platform (2 × 150 bp paired-end). Preliminary results indicated that dry-tropical soils (Jhansi) were dominated by Actinobacteria and Firmicutes whereas, among fungal communities, Ascomycota predominated followed by Basidiomycota and Mucoromycota. According to the Shanon index (Fig. 24), bacterial alpha diversity was higher in agroforestry systems compared to sole cropping system. However, the highest number of bacteria was recorded in sole cropping system (Fig. 25). Among the land use systems, teak based agroforestry system showed the higher alpha diversity. A depth wise variation was observed, with higher diversity in surface soil compared to sub-surface soil.

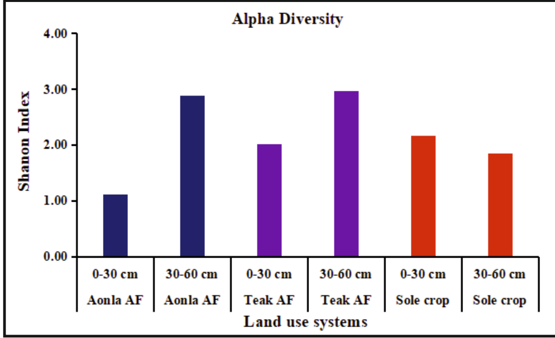


Fig. 24. Initial results as obtained from metagenome data on Alpha diversity (bacteria) from post-*kharif* season

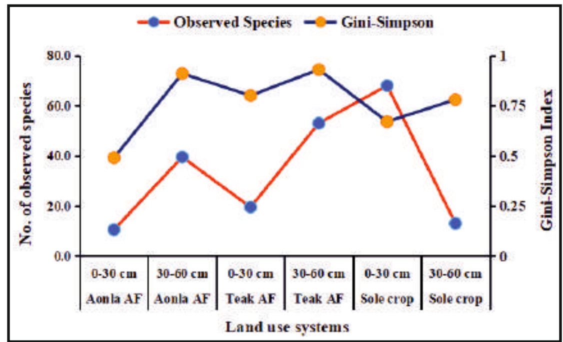


Fig. 25. Initial results as obtained from metagenome data on average number of observed bacteria at different depths of soils from post-*kharif* season

Future Perspectives

The efforts for the given topic on the carbon dynamics and rhizosphere microbiome in selected agroforestry systems will surely lead to facilitate advanced research considering many more aspects, which could be further strengthened as deemed necessary for augmenting potential of agroforestry for preferred land use systems. The focus, in general, so far used to be for below ground carbon stock, but of late, it is being shifted towards the microbial diversity especially the rhizosphere microbiome in agroforestry. Apart from the characterization and identification of the microbial communities from diversity point of views, emphasis on functional efficiencies at biochemical and molecular gene level are to be targeted in much greater details. Diversified actions for unravelling the riddles of harnessing fullest scope of rhizosphere microbiome from all types of agroforestry systems across widely distributed climatic zones of our country need priority. As agroforestry systems foster diverse group of microorganisms, so complete delineation of the microbiome for their definite contribution in nutrient cycling in addition to higher carbon use efficiency or litter decomposition leading more carbon accretion in the soil needs due importance for further advanced research. Diverse microbial community in tree-rhizosphere in agroforestry will strengthen as a more robust and better equipped system which will give opportunities to handle environmental constraints well in addition to high carbon storage.

Selected Bibliography

Alam, B., Singh, R., Uthappa, A.R., Chaturvedi, M., Singh, A.K., Newaj, R., Handa, A.K. and Chaturvedi, O.P. 2018. Different genotypes of *Dalbergia sissoo* trees modified microclimate dynamics differently on understory crop cowpea (*Vigna unguiculata)* as assessed through ecophysiological and spectral traits in agroforestry system. *Agricultural and Forest Meteorology,* 249: 138-148. https://doi.org/10.1016/j.agrformet.2017.11.031

Alef, K. 1995. Soil Respiration. In: Alef, K. and Nannipieri, P., Eds., Methods in Soil Microbiology and Biochemistry, Academic Press Inc., San Diego. Pp. 214-215.

Ashajyothi, M., Charishma, K., Patel, A., Paul, S., Venkatesh, Y.N., Prakash, I. and Tilgam, J. 2022. Rhizosphere microbiome: significance in sustainable crop protection. In *Rhizosphere microbes: biotic stress management* (pp. 283-309). Singapore: Springer Nature Singapore.

Banerjee, S., Baah-Acheamfour, M., Carlyle, C.N., Bissett, A., Richardson, A.E., Siddique, T., Bork, E.W., and Chang, S.X. 2016. Determinants of bacterial communities in Canadian agroforestry systems. *Environmental Microbiology* 18, 1805–1816. https://doi.org/ 10.1111/1462-2920.12986

Bhattacharyya, P., Roy, K.S., Das, M., Ray, S., Balachandar, D., Karthikeyan, S., Nayak, A.K., Mohapatra, T. 2016. Elucidation of rice rhizosphere metagenome in relation to methane and nitrogen metabolism under elevated carbon dioxide and temperature using whole genome metagenomic approach. *Sci. Total Environ*, 542: 886-898. https://doi.org/ 10.1016/j.scitotenv.2015.10.154

Bhowmik, A., Kukal, S.S., Saha, D., Sharma, H., Kalia, A., Sharma, S. 2019. Potential indicators of soil health degradation in different land use-based ecosystems in the Shiwaliks of Northwestern India. *Sustainability* ,11: 3908. https://doi.org/10.3390/su11143908

Blake, GR., Hartge, K.H. 1986. Bulk Density. In Klute A. (Ed.), Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods (2nd ed.), Madison, WI: *American Society of Agronomy*, *Soil Science Society of America*, pp. 363–375.

Chaudhari, D., Rangappa, K., Das, A., Layek, J., Basavaraj, S., Kandpal, B.K., Shouche, Y., Rahi, P. 2020. Pea (*Pisum sativum* L.) plant shapes its rhizosphere microbiome for nutrient uptake and stress amelioration in acidic soils of the North-East Region of India. *Front. Microbiol*. 11: 968. https://doi.org/10.3389/fmicb.2020.00968

Chavan, S.B., Newaj, R., Rizvi, R.H., Ajit, Prasad, R**.**, Alam, B., Handa, A.K., Dhyani, S.K., Jain, A. and Tripathi, D. 2021. Reduction of global warming potential vis-a-vis greenhouse gases through traditional agroforestry systems in Rajasthan, India. *Environment, Development and Sustainability*, 23: 4573–4593. https://doi.org/10.1007/s10668-020-00788-w

Cindy, U.S., Hazandy, A.H., Ahmad-Ainnuddin, N. and Mohd-Kamil, I. 2018. Soil CO_2efflux in relation to soil temperature and relative humidity in Gmelina, Mahogany and Pine stands in Malaysia. *J. Tropical Forest Science,* 30(2):207-15.

Dutta, J., Thakur, D. 2021. Diversity of culturable bacteria endowed with antifungal metabolites biosynthetic characteristics associated with tea rhizosphere soil of Assam, India. *BMC Microbiol*. 21: 216. https://doi.org/10.1186/s12866-021-02278-z

Effendi, Y., Aini, N., Pambudi, A., Sasaerila, H.Y. 2020. Metagenomics analysis of soil microbial communities in plant agroforestry system rubber tree (*Hevea brasiliensis*) – Ganyong (*Canna* sp.). IOP Conf. Series: *Earth Environ. Sci.* 468: 012045. doi:10.1088/1755-1315/468/1/012045

Falkowski, T.B., Diemont, S.A.W., Chankin, A., and Douterlungne, D. 2016. Lacandon Maya traditional ecological knowledge and rainforest restoration: Soil fertility beneath six agroforestry system trees. *Ecological Engineering* 92, 210-217. https://doi.org/10.1016/ j.ecoleng.2016.03.002

Goswami, S.K., Kashyap, P.L., Awasthi, S. 2019. Deciphering rhizosphere microbiome for the development of novel bacterial consortium and its evaluation for salt stress management in solanaceous crops in India. *Indian Phytopath*. https://doi.org/10.1007/s42360-019-00174-1

Jiang, J., Song, Z., Yang, X., Mao, Z., Nie, X., Guo, H., Peng, X. 2017. Microbial community analysis of apple rhizosphere around Bohai Gulf. *Sci. Rep.* 7: 8918. https://doi.org/ 10.1038/s41598-017-08398-9

Joshi, S., Jaggi, V., Gangola, S., Singh, A., Sah, V.K., Sahgal, M. 2021. Contrasting rhizosphere bacterial communities of healthy and wilted *Dalbergia sissoo* Roxb. *Forests. Rhizosphere* 17: 100295. https://doi.org/10.1016/j.rhisph. 2020.100295

Klein, D.A., Loh, T.C., Goulding, R.L. 1971. A rapid procedure to evaluate the dehydrogenase activity of soils low in organic matter. *Soil Biology and Biochemistry*, 3(4): 385-387.

Negi, M.S., Tandon, V.N. and Rawat, H.S. 1995. Biomass and nutrient distribution in young teak (*Tectona grandis* Linn. F) plantations in Tarai region of Uttar Pradesh. *Indian Forester*. Pp.455-464.

Newaj, R., Chavan, S.B., Alam, B. and Dhyani, S.K. 2016. Biomass and carbon storage in trees grown under different agroforestry systems in semi-arid region of Central India. *Indian Forester*, 142 (7):642-648.

Newaj, R., Dhyani, S.K., Alam, B., Prasad, R., Rizvi, R.H., Ajit, Handa, A.K. and Singh, R. 2012. Role of agroforestry for mitigating climate change-some research initiatives. *Technical Bulletin* 5/2012, NRCAF, Jhansi.

Pervaiz, Z.H., Contreras, J., Hupp, B.M., Linderberger, J.H., Chen, D., Zhang, Q., Wang, C., Twigg, P., Saleem, M. Root microbiome changes with root branching order and root chemistry in peach rhizosphere soil. *Rhizosphere*, 16: 100249. https://doi.org/ 10.1016/j.rhisph.2020.100249

Pramanik, K., Das, A., Banerjee, J., Das, A., Chatterjee, S., Sharma, R., Kumar, S., Gupta, S. 2020. Metagenomic insights into rhizospheric microbiome profiling in lentil cultivars unveils differential microbial nitrogen and phosphorus metabolism under rice-fallow ecology. *Int. J. Mol. Sci.* 21: 8895. https://doi.org/10.3390/ijms21238895

Prasanna, R., Jaiswal, P., Nayak, S., Sood, A., Kaushik, B.D. (2009). Cyanobacterial diversity in the rhizosphere of rice and its ecological significance. *Indian Journal of Microbiology* 49, 89–97 https://doi.org/10.1007/s12088-009-0009-x

Ravindranath, N.H., and Ostwald, M. 2008. Carbon inventory methods: Handbook for greenhouse gas inventory, carbon mitigation, and roundwood production projects (*Advances in Global Change Research* 29). Springer, Dordrecht, Netherlands, 113-147.

Sakhia, N., Prajapati, S., Shetty, V., Bhatt, S. and Bhadalkar, A. 2016. Study of bacterial diversity of mangroves rhizosphere. *Open J. Marine Sci.* 6: 23:31. http://dx.doi.org/ 10.4236/ojms.2016.61003

Singh, R.P., Johri, A.K., Dua, M. 2020. Metagenomic analysis of microbial diversity in cotton rhizosphere soil in Alwar, India. *Microbiol. Resour. Announc.* 9: e00987-20. https://doi.org/10.1128/MRA.00987-20.

Snyder, J.D., Trofymow, J.A. 1984. A rapid accurate wet oxidation diffusion procedure for determining organic and inorganic carbon in plant and soil samples. Communications in *Soil Science and Plant Analysis*, 15(5): 587–597.

Srivastava, R., Srivastava, A.K., Ramteke, P.W., Gupta, V.K., Srivastava, A.K. 2020. Metagenome dataset of wheat rhizosphere from Ghazipur region of Eastern Uttar Pradesh. *Data in Brief*, 28: 105094. https://doi.org/10.1016/j.dib.2019.105094

Thangapandian, V., Ponmurugan, P., Ponmurugan, K. (2007) Actinomycetes diversity in the rhizosphere soils of different medicinal plants in Kolly Hills-Tamilnadu, India, for secondary metabolite production. *Asian Journal of Plant Science* 6, 66-70. https://doi.org/10.3923/ajps.2007.66.70

Vance, E.D., Brookes, P.C., Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, 19(6): 703–707.

Verma, P., Yadav, A.N., Khannam, K.S., Kumar, S., Saxena, A.K., Suman, A. 2016. Molecular diversity and multifarious plant growth promoting attributes of *Bacilli* associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. *J. Basic Microbiol.* 56: 44-58. https://doi.org/10.1002/jobm.201500459.

Viswanath, G., Jegan, S., Baskaran, V., Kathiravan, R., Prabavathy, V.R. (2015) Diversity and N-acyl-homoserine lactone production by *Gammaproteobacteria* associated with *Avicennia marina* rhizosphere of South Indian mangroves. *Systematic and Applied Microbiology* 38, 340-345. https://doi.org/10.1016/j.syapm.2015.03.008

Walkley, A., Black, I. A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 37: 29-38.

Yu, Z., Liang, K., Huang, G., Wang, X., Lin, M., Chen, Y., Zhou, Z. 2021. Soil bacterial community shifts are driven by soil nutrient availability along a teak plantation chronosequence in tropical forests in China. *Biol.* 10: 1329. https://doi.org/10.3390/biology10121329

Yuan, Z.S., Liu, F., He, S.B., Zhou, L.L., Pan, H. 2022. Community structure and diversity characteristics of rhizosphere and root endophytic bacterial community in different Acacia species. *PLoS One*, 17: e0262909. https://doi.org/10.1371/journal.pone.0262909

Zambounis, A., Osathanunkul, M., Medesis, P. 2019. Metagenome data of bacterial diversity in pear (*Pyrus communis* L.) rhizospheres associated with *Phytophthora* infection and amino acid treatment. *Data in Brief*, 26: 104396. https://doi.org/10.1016/j.dib.2019.104396

Zhang, X., Gao, G., Wu, Z., Wen, X., Zhong, H., Zhong, Z., Bian, F., Gai, X. (2019) Agroforestry alters the rhizosphere soil bacterial and fungal communities of moso bamboo plantations in subtropical China. *Applied Soil Ecology* 143, 192-200. https://doi.org/10.1016/ j.apsoil.2019.07.019

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