

Soil organic carbon fractions, carbon stocks and microbial biomass carbon in different agroforestry systems of the Indo-Gangetic Plains in Bihar, India

Nongmaithem Raju Singh^{1,4,*}, A. Raizada², K. K. Rao¹, Kirti Saurabh¹, Kumari Shubha¹, Rachana Dubey¹, L. Netajit Singh³, Ashish Singh⁴ and A. Arunachalam⁵

¹ICAR Research Complex for Eastern Region, Patna 800 014, India

²ICAR-Mahatma Gandhi Integrated Farming Research Institute, Motihari 845 429, India

³College of Agriculture University, Jodhpur 342 304, India

⁴ICAR Research Complex for North Eastern Hill Region, Umiam 793 103, India

⁵ICAR-Central Agroforestry Research Institute, Jhansi 284 003, India

A study was undertaken in the Vaishali district of Bihar, India, in 2020 to assess the effect of various agroforestry systems (AFS) on the distribution of different pools of soil organic carbon (fraction I – very labile, fraction II – labile, fraction III – less labile and fraction IV – non-labile), carbon stocking and soil microbial activity. The mean (0–45 cm) total organic carbon (TOC) in different AFS ranged from 5.55 to 6.64 Mg C ha⁻¹, with the highest under poplar-based AFS (PB-AFS). Across the AFS studied, the C stocks (0–45 cm) varied from 36.24 (mango-based AFS) to 41.43 Mg C ha⁻¹ (PB-AFS). Overall, the magnitude of C fractions showed the order: fraction I > fraction IV > fraction III > fraction II. Significantly higher soil microbial biomass carbon was recorded under PB-AFS (219.36 µg g⁻¹) in 0–15 cm depth. Basal respiration was also the highest under PB-AFS (0.54 µg CO₂-C g⁻¹ h⁻¹), followed by TB-AFS (0.50 µg CO₂-C g⁻¹ h⁻¹) in 0–15 cm depth. Principal component analysis result showed that PC 1 and PC 2 represented about 97% of the total variation. TOC and active carbon pool had the maximum loading in PC 1, while microbial metabolic quotient and bulk density had the maximum value in PC 2.

Keywords: Agroforestry system, basal respiration, principal component analysis, soil microbial activity, total organic carbon.

THE protection and enhancement of soil organic matter (SOM) content is essential in achieving sustainable agricultural production. However, continued intensive cultivation has led to its depletion leading to a decline in soil fertility, crop productivity and increased atmospheric carbon dioxide concentration¹. The combination of labile fraction (LF) and recalcitrant fraction (RF) of carbon in the soil denotes the total organic carbon (TOC) of the soil. How-

ever, the measurement of TOC alone does not provide a clear picture of carbon dynamics in any cropping system since the status of LF can be easily affected by land management practices while RF in the soil cannot be changed easily due to alteration in land-use practices². Soil microbial biomass carbon (SMBC) influences major nutrient cycling patterns in the ecosystem vis-à-vis cycling of SOM³. In light of the present climate change scenarios, there is a need to identify efficient and sustainable land-use systems that could lead to long-term storage and sequestration of carbon.

Agroforestry, a practice of cultivating trees along with agricultural crops or animal husbandry, is considered one of the viable land-use systems while delivering the services of crop diversification, natural conservation as well as sequestering the atmospheric carbon. Considering the importance of agroforestry practices in terms of carbon sequestration, the Intergovernmental Panel on Climate Change (IPCC) and other prominent organizations, viz. Consultative Group on International Agricultural Research (CGIAR) have recognized the services of agroforestry and incorporated this land-use practice in many of its programmes. Although preliminary studies on dry-matter dynamics of poplar-based agroforestry system (PB-AFS) in Indo-Gangetic Plains of Bihar (Samastipur), India have been studied⁴, information regarding soil organic fractions and carbon stocks across different AFS in Bihar is limited. Therefore, the present study was undertaken to evaluate the effect of various AFS on the distribution of different soil organic carbon (SOC) fractions, carbon stocking and soil microbial activity.

Materials and methods

Selection of agroforestry system

Selection and identification of AFS were carried out following a preliminary reconnaissance survey in Vaishali district,

*For correspondence. (e-mail: rajuforestry@gmail.com)

Table 1. Details of different agroforestry systems (AFS) studied

AFS	Mean DBH* (cm)	Mean height (m)	Spacing (m)	Age of tree (yrs)
Teak-based (TB-AFS)	12.64	7.80	4 m × 4 m (boundary plantation)	8–10
Poplar-based (PB-AFS)	24.96	18.15	5 m × 5 m (boundary plantation)	5–6
Sissoo-based (SB-AFS)	6.74	3.15	4 m × 4 m	5–6
Mango-based (MB-AFS)	6.02	4.55	8 m × 8 m	5–6

*DBH, Diameter at breast height (1.3 m).

Bihar, and these were classified according to Nair⁵. In general, two types of AFS, viz. agri-silvicultural and agri-horticultural are widely practised by farmers of this region (25°41'–25°68'N lat. and 85°13'–85°22'E long.). Teak (*Tectona grandis*) + agricultural crops, poplar (*Populus* spp. + agricultural crops and sissoo (*Dalbergia sissoo*) + agricultural crops represented the agri-silvicultural system, while mango (*Mangifera indica*) + agricultural crops denoted the agri-horticultural system (Table 1). Under the teak-based agroforestry system (TB-AFS), farmers of this region generally grow mustard (*Brassica* spp.) and rice (*Oryza sativa*). Wheat (*Triticum aestivum*) and rice are commonly grown as intercrops in PB-AFS. Similarly, under the Sissoo-based agroforestry system (SB-AFS), farmers favour the cultivation of mustard, potato (*Solanum tuberosum*), maize (*Zea mays*), etc. while mustard, moong (*Phaseolus vulgaris*) and potato are commonly cultivated under mango-based agroforestry system (MB-AFS). Vaishali district of Bihar is a part of Indo-Gangetic Plains, where the average rainfall recorded is about 1400 mm. The study area belongs to the tropical region having the characteristics of hot summers from April to June followed by a brief autumn season and a mild, dry winter. Soil texture of this region is mainly sandy, loam and light clayey in nature.

Soil sampling and analysis

During January 2020, ten soil samples were taken from each AFS at three depths (0–15, 15–30 and 30–45 cm) using power augers. Next, composite soil samples were prepared by combining samples from each agroforestry system, transported to the laboratory in polyethylene bags and stored at 4°C until analysis. Soil bulk density was measured by the core method⁶. The subsamples of air-dried soil were used for analysing TOC and different fractions of carbon (very liable, liable, less liable and non-liable). TOC was analysed following Haenes⁷. Different fractions of carbon were also analysed according to the method suggested by Chan *et al.*⁸.

SOC stock (t ha⁻¹) at different depths in different AFS was estimated using the following formula:

$$\text{SOC stock (Mg ha}^{-1}\text{)} = \text{SOC (g kg}^{-1}\text{)} \\ \times \text{bulk density (Mg m}^{-3}\text{)} \times \text{soil depth (m)} \times 10.$$

Active pool of organic carbon was calculated by summing up fractions I and II, while passive pool was derived by

adding fractions III and IV. SMBC was estimated using the method suggested by Nunan *et al.*⁹, with certain changes according to Parihar *et al.*¹⁰. Carbon dioxide evolution in terms of basal respiration was determined following the alkali absorption method¹¹. Microbial quotient (MQ) and microbial metabolic quotient (qCO₂) were also estimated using the equation proposed by Anderson and Domsch¹².

Carbon management index

The carbon management index (CMI) was calculated using the formula proposed by Blair *et al.*¹³.

$$\text{CMI} = \text{Carbon pool index (CPI)} \\ \times \text{liability index (LI)} \times 100.$$

CPI was calculated as follows:

$$\text{CPI} = \frac{\text{Sample TOC}}{\text{Reference TOC}}.$$

LI was calculated as follows:

$$\text{LI} = \frac{\text{Fraction I}}{\text{TOC}} \times 3 + \frac{\text{Fraction II}}{\text{TOC}} \times 2 + \frac{\text{Fraction III}}{\text{TOC}} \times 1.$$

Statistical analysis

One-way analysis of variance (ANOVA) was used to elucidate the effect of different AFS and soil depths on different soil parameters. Tukey test was utilized for multiple comparisons among the treatments at $P < 0.05$ using the Indian NARS Statistical Computing Portal (<http://stat.iasri.res.in/sscnarsportal>). Principal component analysis (PCA) biplot was prepared using open software R.

Results and discussion

Total organic carbon

TOC content was significantly ($P \leq 0.05$) influenced by both AFS and soil depth (Table 2). On average, TOC in different AFS ranged from 5.55 to 6.64 Mg C ha⁻¹. Among the AFS, the topsoil (0–15 cm) in PB-AFS had the

Table 2. Distribution of soil total organic carbon (TOC) and TOC stocks (Mg C ha⁻¹), carbon fractions (Mg C ha⁻¹), soil microbial biomass carbon (SMBC) (µg g⁻¹) and basal respiration (µg CO₂-C g⁻¹ h⁻¹) at three depths in different AFS

Soil depth (cm)	TOC	C stock	F I (very labile carbon)	F II (labile carbon)	F III (less labile carbon)	F IV (non labile carbon)	SMBC	Basal respiration
TB-AFS								
0–15	8.43 ^a	17.27 ^a	3.13 ^a	1.40 ^a	1.44 ^a	2.46 ^a	192.70 ^a	0.50 ^a
15–30	6.06 ^b	13.11 ^b	2.05 ^b	1.07 ^a	1.13 ^b	1.81 ^b	111.64 ^b	0.40 ^b
30–45	4.29 ^c	9.69 ^c	1.20 ^c	0.81 ^b	1.01 ^c	1.27 ^c	49.65 ^c	0.33 ^c
Mean	6.26 ^B	13.36 ^A	2.13 ^A	1.09 ^B	1.19 ^A	1.85 ^B	118.00 ^B	0.41 ^A
PB-AFS								
0–15	9.06 ^a	18.18 ^a	3.28 ^a	1.59 ^a	1.38 ^a	2.81 ^a	219.36 ^a	0.54 ^a
15–30	6.51 ^b	13.71 ^b	2.04 ^b	1.22 ^b	1.08 ^b	2.17 ^b	112.26 ^b	0.47 ^b
30–45	4.34 ^c	9.54 ^c	1.35 ^c	0.79 ^c	0.93 ^c	1.27 ^c	63.32 ^c	0.18 ^c
Mean	6.64 ^A	13.81 ^A	2.22 ^A	1.20 ^A	1.13 ^{AB}	2.08 ^A	131.65 ^A	0.40 ^A
SB-AFS								
0–15	7.85 ^a	16.21 ^a	2.81 ^a	1.43 ^a	1.39 ^a	2.22 ^a	170.62 ^a	0.45 ^a
15–30	5.68 ^b	12.20 ^b	1.71 ^b	1.29 ^a	1.14 ^b	1.54 ^b	94.91 ^b	0.41 ^a
30–45	4.01 ^c	8.87 ^c	1.11 ^c	0.79 ^b	0.83 ^c	1.28 ^c	46.06 ^c	0.25 ^b
Mean	5.85 ^C	12.43 ^B	1.88 ^B	1.17 ^A	1.12 ^{BC}	1.68 ^C	103.87 ^C	0.37 ^B
MB-AFS								
0–15	7.44 ^a	15.74 ^a	2.92 ^a	1.13 ^a	1.25 ^a	2.14 ^a	161.23 ^a	0.34 ^a
15–30	5.40 ^b	11.93 ^b	1.64 ^b	1.03 ^a	1.08 ^b	1.65 ^b	101.46 ^b	0.34 ^a
30–45	3.81 ^c	8.57 ^c	1.03 ^c	0.71 ^b	0.84 ^c	1.23 ^c	46.55 ^c	0.23 ^b
Mean	5.55 ^C	12.08 ^B	1.86 ^B	0.96 ^C	1.06 ^C	1.67 ^C	103.08 ^C	0.30 ^C

Different small letters denote significant difference ($P \leq 0.05$) among soil depths within the AFS. Different capital letters denote significant difference ($P \leq 0.05$) between the AFS.

highest (9.06 Mg C ha⁻¹) TOC followed by TB-AFS (8.43 Mg C ha⁻¹), while MB-AFS recorded the lowest (7.44 Mg C ha⁻¹) TOC content in the soil. A variation in TOC under different AFS was strongly linked with the species characteristics and their litter input patterns¹⁴. Carbon dynamics in any system can also be related to the adoption of different management practices along with their past cropping patterns¹⁵. Das and Chaturvedi⁴ reported that the annual litterfall accumulation by poplar trees under poplar + wheat-based agroforestry system in Samastipur, Bihar, ranged from 2.46 (3 years) to 10.63 (9 years) Mg ha⁻¹ yr⁻¹. On the contrary, Rathore *et al.*¹⁶ reported that a ten-yr-old agri-horticultural system (mango + cowpea-toria) accumulated around 1.46 t ha⁻¹ of dry leaf biomass on the floor. This amount of litter biomass is far less than the litterfall added by poplar (2.46–10.63 Mg ha⁻¹ yr⁻¹), as reported earlier by Das and Chaturvedi⁴. Irrespective of AFS, TOC content in the surface soil (0–15 cm) was significantly higher than the bottom layers (15–45 cm), registering a decrease of ~28% and ~50% in 15–30 and 30–45 cm soil layers respectively. This might be due to the higher input of litter and dry biomass in the surface soil compared to the subsurface layer¹⁵.

Total organic carbon stock in soil

TOC stocks varied from 12.08 to 13.81 Mg C ha⁻¹ across different AFS. In 0–15 cm soil profile, PB-AFS had the highest (18.18 Mg C ha⁻¹) TOC stocks, followed by TB-AFS (17.27 Mg/ha), while MB-AFS recorded the minimum

(15.74 Mg C ha⁻¹) TOC stock. The significant variation in TOC stocks across different AFS is correlated with TOC accumulation in the soil. Singh *et al.*¹⁷ have reported that spatial and temporal admixture of various components in different AFS and their resulting *in situ* interface with the abiotic components favoured carbon storage. Significantly higher TOC content was recorded in the upper than lower soil layers. On average, the surface layer (0–15 cm) had 35% higher carbon stocks than the lower layers (15–45 cm) of soil. The rhizodeposition effects due to the varied nature of different tree species also significantly influenced the distribution of SOC across soil depth¹⁸. The mean stratification ratio (SR) (ratio of C stocks in 0–15 and 15–30 cm), as proposed by Franzluebbers¹⁹, in the study sites of different AFS was 1.33, which is comparable with the findings of Ramesh *et al.*¹⁴.

Distribution of different carbon fractions

Significant variations in different carbon fractions were observed across the different AFS throughout the depth (0–45 cm) of the soil profile (Table 2). Results revealed that the very labile carbon fraction (F I) represented the highest proportion, followed by the recalcitrant fraction (F IV) in all AFS. The pattern of distribution of different carbon fractions (different oxidability levels) determines the permanency of SOC status of the soil, and this characteristic is largely influenced by the adoption of several management activities²⁰. The soil of PB-AFS consistently had the highest carbon fractions of different oxidability than the other

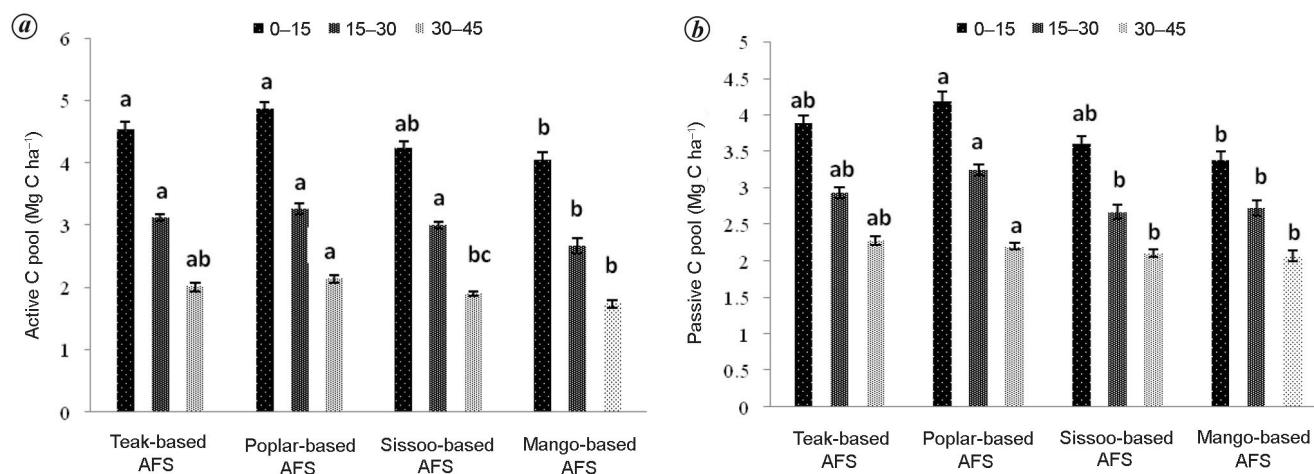


Figure 1. (a) Active and (b) passive C pools in the soil at three depths in different agroforestry systems (AFS). Each bar represents the mean and standard error ($n = 3$). Means not sharing a letter in common differ significantly ($P \leq 0.05$) between the same soil layers of different AFS.

counterparts. On the other hand, MB-AFS had the lowest carbon fractions of different oxidability levels. MBS-AFS and SB-AFS recorded the minimum C pools. This could be partly linked to the intercrops grown under these systems, like maize and potato, which are nutrient-exhaustive crops, leading to more consumption of organic carbon from the soil²¹. In this study, the order of allocating different carbon fractions (fraction I > fraction IV > fraction III > fraction II) was similar across AFS. The result is consistent with the findings of Benbi *et al.*²², where LF was the most dominating (56–60%) under PB-AFS in Punjab, India.

The percentage-wise distribution of different carbon fractions indicated that the labile form (fractions I and II) represented 51.50. The active and passive carbon pools also showed significant differences due to variations in the distribution of different forms of carbon fractions across AFS (Figure 1). The continuous addition of leaf litter and dry matter in the agroforestry floor enabled the build-up of more labile carbon fraction across different AFS, thus necessitating the continuation of the current land-use system in order to protect SOM²³. Moreover, proper management of these systems by the farmers through incorporation of an adequate amount of farmyard manure as nutrient supplement for crop growth and development will significantly affect the formation of very labile carbon fraction in the soil²⁴.

Soil microbial biomass carbon and basal respiration

Overall the SMBC in different AFS ranged from 103.08 and 131.65 $\mu\text{g g}^{-1}$ (Table 2). Significantly higher value of SMBC was recorded in the surface layer than in subsurface layers in all AFS, which can be attributed to the higher availability of substrate and easily hydrolysable carbon in the upper soil layer²⁵. In the 0–15 cm depth, the significantly highest SMBC was recorded in PB-AFS (219.36 $\mu\text{g g}^{-1}$),

followed by TB-AFS (192.70 $\mu\text{g g}^{-1}$), while SMB-AFS had the lowest (161.23 $\mu\text{g g}^{-1}$) SMBC. Similar trends were also observed in both subsurface layers (15–30 cm and 30–45 cm). Since TOC content in the soil was also the highest in PB-AFS, this indicates that the level of TOC in the soil is highly correlated with SMBC²⁶. The overall result on basal respiration (BR) across different AFS showed that TB-AFS (0.41 $\mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$) registered the maximum BR, but was statistically at par with PB-AFS (0.40 $\mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$). However, in the upper soil depth (0–15 cm), PB-AFS recorded the highest (0.54 $\mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$) BR, followed by TB-AFS (0.50 $\mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$) and the minimum under MB-AFS (0.34 $\mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$). This indicates higher biomass in PB-AFS and TB-AFS, followed by high mineralization of litter (including dry biomass) and root biomass, leading to the production of large CO_2 flux rates. Surface soil will have more CO_2 evolution rate than subsurface soil due to higher inputs of litter and plant residues followed by a better mineralization process²⁷.

MQ was the highest under PB-AFS, while TB-AFS recorded the highest $q\text{CO}_2$ (Figures 2 and 3). The MQ value of different AFS varied from 1.67% to 1.87%, and these results are comparable with the findings of Ramesh *et al.*¹⁴. The maximum MQ under PB-AFS indicates higher steadiness of organic matter as MQ is generally related to the availability of C source for microbial activities. In this study, PB-AFS showed the lowest $q\text{CO}_2$ indicating better substrate utilization efficiency than the other counterparts. The higher $q\text{CO}_2$ under TB-AFS and SB-AFS might be associated with the higher demand and consumption of SOC. Bastida *et al.*²⁸ also stressed that the disturbance caused by several factors, primarily through the intervention of different cultural practices, is likely to affect the abundance of organic carbon in the system, which is also responsible for producing varied $q\text{CO}_2$ under different land-use systems.

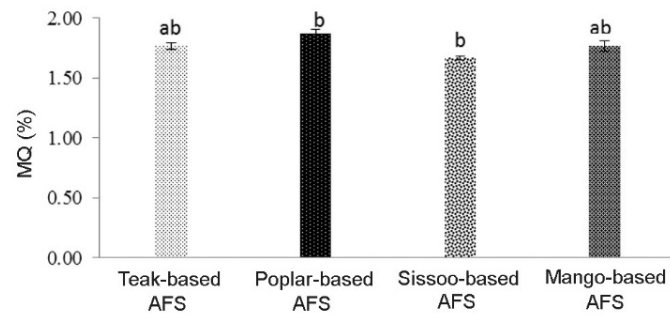


Figure 2. Microbial quotient (MQ) of different AFS.

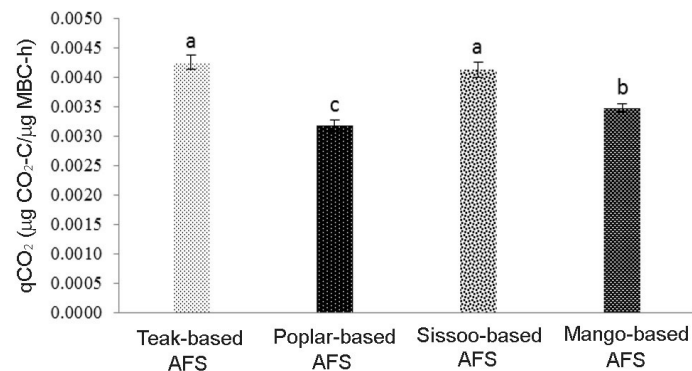


Figure 3. Microbial metabolic quotient (qCO₂) of different AFS.

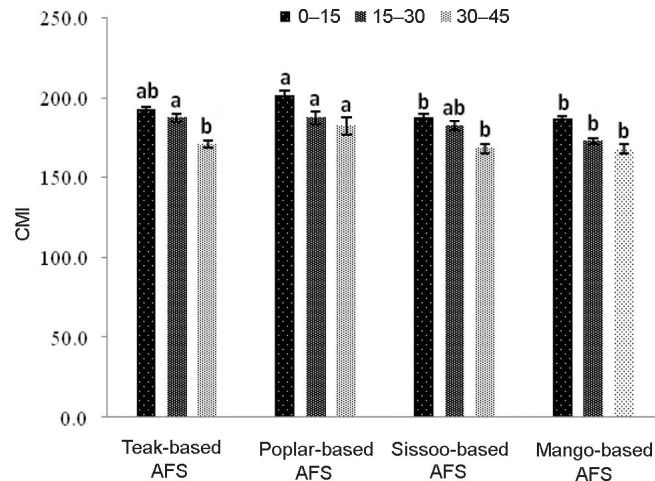


Figure 4. Carbon management index (CMI) of different AFS.

Carbon management index

The CMI of different AFS ranged from 201.54 to 186.47 in 0–15 cm soil depth (Figure 4). Significantly higher CMI value was recorded under PB-AFS but was statistically at par with TB-AFS and SB-AFS, while the least CMI was recorded under MB-AFS. PB-AFS recorded the maximum CMI value compared to the rest of AFS, indicating a large potential to have more carbon-stock. The dissimilarity in CMI val-

ues across AFS can be mainly attributed to the level of litter accumulation as well as root decomposition activities exhibited by different tree species. However, the effect of management practices cannot be ruled out. The continuous addition of annual litterfall helps in building SOC under AFS compared to treeless farming. This is indicated by a CMI value of more than 100 in the present study. Higher value (>100) of CMI in agroforestry practices compared with treeless farming has been reported by several workers^{29,30}.

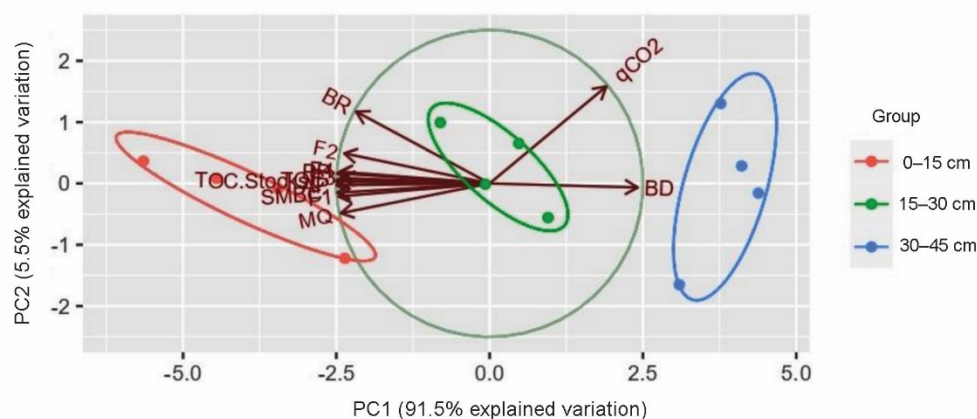


Figure 5. Principal component analysis representing different soil parameters at three depths.

Principal component analysis

PCA result revealed that the first two principal components, viz. PC 1 and PC 2 represented 91.5% and 5.5% respectively, of the total variation (Figure 5). In PC 1, TOC followed by active carbon pool was found to be the most sensitive factors, while qCO_2 and BD showed the most influence in PC 2. Across soil depths, it was found that all the studied soil parameters, except qCO_2 and BD, were higher in the surface layer (0–15 cm). So, all soil variables are positively correlated to each other, except qCO_2 and BD, as these two variables are negatively correlated with the remaining soil variables. Also, qCO_2 and BD were found to be the most influential factors in the 30–45 cm soil depth. In the present study, TOC was the most influential factor in PC1 as well in the soil group of 0–15 cm. TOC is heterogeneous, containing both liable and recalcitrant carbon forms. As a result, changes in different fractions of organic carbon will impact TOC in the soil. More precisely, the liable form of carbon can be easily influenced by changes in land management practices³¹. The greater qCO_2 values in the subsurface layers indicate that the substrate utilization efficiency of microorganisms decreases as soil depth increases.

Conclusion

PB-AFS had higher concentrations and a greater amount of carbon as compared to its other counterparts. The choice of intercrops (mustard, rice, wheat, potato, maize, moong, etc.), perennial components as well as management activities in different AFS largely depend on the farmer's interest. This has led to variation in the distribution and stability of SOC and its various forms in different AFS. Subsequently, it has shown a significant impact on microbial activity (biomass and BR) and thus has a profound effect on the overall nutrient flux and dynamics of the system. This study concludes that a tree-based farming system, especially PB-AFS, could be considered as one of the viable options not

only for carbon mitigation but also for increasing the profitability of farmers belonging to the Indo-Gangetic Plains of Bihar.

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