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ORIGINAL PAPER

Influence of Clay Mineralogy on Soil Organic Carbon Stabilization under Tropical Climate, India

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Abstract

Mechanisms of soil organic carbon (SOC)stabilization has received much focus recently due to its relevance incontrolling the global carbon (C) cycle. Clay minerals are knownto stabilize SOC through mechanisms such as, ligand exchange, polyvalent cationbridging, electrostatic attraction, H-bonding, and van der Waals forces. Most studies focused on clayorganic interactions derived from geological deposits. However, the effect ofpedogenic clay on SOC stability is still lacking especially in tropicalconditions like India. Therefore,the impact of clay with different mineralogy such as smectite, 2:1interstratified minerals, illite, kaolinite on soil C mineralization, and labileC fractions in four distinct soils under natural conditions was evaluated. The results indicated that the cumulative Cmineralization (CO_2-C_{cum}) was the highest in Mollisol (0.97 gC kg^{-1}) and Vertisol (0.96g C kg^{-1}), which was dominatedby kaolinite+illite+chlorite–interstratified minerals and smectite/2:1 interstratifiedminerals, respectively followed by Alfisol and Inceptisol, which was dominatedby kaolinite and illite. The percentage of SOC loss showed opposite trend wherethe highest SOC loss was accounted in Inceptisol (10.1%) and Alfisol (9.02%) whereasMollisol and Vertisol lost lowest amount of SOC. Labile C fractions anddehydrogenase activity were significantly higher in Mollisol and Vertisol over Alfisoland Inceptisol. Specific surface area (SSA) ($r = 0.65$, $P \leq 0.05$) and cationexchange capacity (CEC) ($r = 0.62$, $P \leq 0.05$) positively correlated with Cmineralization and labile C fractions and negatively correlated with percentageSOC loss. Principal component analysis confirmed that varying mineralogy significantlyinfluenced the sequestration of labile C in soil under natural conditions. This study highlighted the positive influence of 2:1expanding/limited expanding clay mineralogy in sequestering and stabilizinglabile C in soil.

Keywords Soil carbon stabilization · Clay mineralogy · Carbon mineralization · Labile carbon fractions · Enzyme activity

Abbreviations

C	Carbon
OC	Organic carbon
SOC	Soil organic carbon
OM	Organic matter
Pg	Peta gram
DOC	Dissolved organic carbon
SSA	Specific surface area
CEC	Cation exchange capacity
MBC	Microbial biomass carbon
DHA	Dehydrogenase activity
XRD	X-ray diffraction
WBC	Walkley black carbon
APD	Automated powder diffractometer
POM	Particulate organic matter
COM	Complex organic matter
PCA	Principal component analysis
HIV	Hydroxy interlayered vermiculite
CO_2-C_{cum}	Cumulative carbon mineralization
EGME	Ethylene glycol monoethyl ether

1 Introduction

In terrestrial ecosystem, protection of soil organic carbon (SOC) plays a central role in carbon (C) sequestration. Understanding the process of SOC turnover can provide insights on how to tackle global warming in this changing climate. The underlying mechanisms governing the storage and protection of SOC in soil are influenced by natural

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as well as management-induced changes (Dhaliwal et al. 2020; Piazza et al. 2020). In terrestrial system, the soil stores around 1550 Pg of C which is roughly two times its concentration in atmosphere i.e., 780 Pg (Lal 2008), 80% of which is actively involved in global C cycle (Singh et al. 2017). The organic C (OC) present in soil ranges from easily decomposable simple carbohydrates to complex aromatic hydrocarbons. Based on their mean residence period and turnover rates, the soil C could be defined as labile and recalcitrant. Mineralization of stored SOC with time is a function of prevailing climate (Tisdall 1996), soil temperature and moisture (Singh et al. 2017), soil texture (Baldock 2007), organic residue (Roychand and Marschner 2013), composition and strength of organo-mineral associations (Sarkar et al. 2018; Singh et al. 2016), and land use patterns (Hassink 1994). Among these factors, the organo-mineral associations are thought to be the strongest and most significant (Singh et al. 2018).

SOC loss via heterotrophic respiration is one of the major contributors for increasing the atmospheric CO_2 concentration (Lal 2008). Even though mineralization is necessary for nutrient cycling in soil, which provides nutrition to crops, uncontrolled mineralization is causing global temperature to rise at an alarming rate. Much research has previously focused on how environmental factors, such as temperature and moisture affect heterotrophic soil respiration (Dan et al. 2016; Suseela et al. 2012; Larionova et al. 2017) but mineral composition also plays an important role in soil C stabilization. It is assumed that coarse textured soil may have high susceptibility to soil C respiration than their fine textured counterpart (Conant et al. 2008; Lal 2007; Nguyen and Marschner 2014). Clay minerals in soils offer an essential restriction to OC mineralization based on their composition, content (Feng et al. 2005; Kahle et al. 2003), and other surface properties (Singh et al. 2018). With difference in clay mineralogy the labile C fractions like dissolved organic carbon (DOC) showed different tenacity and binding (Singh et al. 2016). Clay minerals having higher specific surface areas (SSA) and cation exchange capacities (CEC) and iron/aluminium (Fe/Al) oxides are reported to protect C in soil by reducing C mineralization (Singh et al. 2018). Soils with a similar texture but a higher concentration of smectitic and allophanic clays retained more C than soils with kaolinite or vermiculite minerals (Saggar et al. 1996, 1999). The fundamental difference in mineralogical composition may influence how these minerals behave in different natural environment in terms of soil C retention. Smectite minerals are expanding in nature having 2:1 layer structure, a higher CEC, SSA, and permanent negative charge whereas kaolinitic clays are non-expanding having 1:1 layer structure with lower CEC, SSA, and pH-dependant charge. In terms of surface properties, illitic clays are intermediate between smectite and kaolinite with non/limited expansion capabilities. Apart from these, the presence of sesquioxides in soil clay fractions has a significant impact on clay surface properties due to their higher SSA (Saidy et al. 2013; Singh et al. 2018). Mechanism through which minerals interact with organic matter (OM) can also be different. Ligand exchange and cation bridging can result in strong bonding between clay minerals and OC moieties whereas hydrogen bonding, van der Waals forces and proton interactions could also establish some weak interactions (Rashad et al. 2010; Singh et al. 2018).

Despite many previous studies focused on how clay organic interaction influences the retention of OC in soils, they were based on refined clay minerals from geological deposits (Feng et al. 2005; Rashad et al. 2010; Saidy et al. 2013). Pedogenic clays, on the other hand, behave quite differently. Clay behavior may differ dynamically under the influence of natural environment as opposed to a controlled scenario. They undergo intense weathering process, changing their particle size. Having OM or sesquioxides coatings may influence their surface properties like SSA and CEC drastically (Churchman and Lowe 2012). The presence of cations in natural and processed clay environments may differ significantly which might influence their interactions. Large areas of land in India are covered under the soil orders namely Vertisol, Mollisol, Inceptisol and Alfisol. Under tropical conditions, these soils undergo continuous hydrothermal changes resulting in widely different clay mineralogy and varying cementing agent contents such as OM and sesquioxides which ultimately affect the SOC retention and mineralization (Chatterjee et al. 2013). As SOC is approximately three times that of atmospheric and terrestrial pools (Schmidt et al. 2011), even minor changes in soil C stock will result in a significant change in atmospheric C concentration. Temperature and climate affect SOC mineralization on macro scale; however, on microscale, the influence of mineralogy cannot be overlooked. Barré et al. (2014) previously reviewed the impact of phyllosilicates on SOC stabilization, but there is little information about the stabilization potential of other minerals. Furthermore, intensive crop management gradually alters clay characteristics, affecting soil C storage capacity (Das et al. 2019a, b), so assessing clays of natural origin can help us evaluate them from a common reference. Keeping this in view, this study was conducted to know the effect of varying clay mineralogy on SOC dynamics in four different natural soils with the understanding that natural pedogenic clays will behave differently than processed clays. It was conceived with the following objectives (i) to examine SOC mineralization as influenced by mineralogy, (ii) to compare the effects of different clay mineralogy on labile C fractions, and (iii) to establish relationship between SOC mineralization and labile C fractions with different surface properties of clays like SSA, CEC, and clay content.

2 Materials and Methods

2.1 Soil Collection, Processing, and Determination of Initial Properties

Soil samples were collected from four different regions, each representing a different soil order according to the US soil taxonomy (Fig. 1). The soils collected from Jabalpur, Madhya Pradesh were formed under dry and sub-humid climate, belonged to Typic Haplusterts with clay texture and neutral reaction. This region experiences annual rainfall of 1386 mm with an average temperature of 25°C (Koppen climate classification, Csa) (Peel et al. 2007). Second soil sample was collected from Pantnagar, Uttarakhand, that belonged to Aquic Hapludoll with silty loam texture having neutral reaction. Climatically, the area is sub-humid subtropical with hot humid summers and severe cold winters with an average annual rainfall measuring around 1383 mm and relative humidity of 90–95% during the rainy season (Koppen climate classification, Cfa) (Peel et al. 2007). Soils of New Delhi belonged to Typic Ustocherpt with sandy loam texture having neutral reaction, with average annual temperature of 29°C and rainfall of 790 mm. New Delhi has a semi-arid subtropical climate with hot dry summers and cool winters (Koppen climate classification, BSh) (Peel et al. 2007). Ranchi (Jharkhand) soils formed under sub-humid climate with severe hot, dry summer, and cool winter, belonged to Typic Haplustalf with clay loam texture and acidic reaction. It experiences average annual temperature of 24°C with average rainfall of 1450 mm (Koppen climate classification, Cwa) (Peel et al. 2007). In each location, soil samples were collected to a depth of 0–20 cm from five randomly selected spots under natural vegetation in 2018. One part of the freshly collected sample were pooled together and kept in in a refrigerator at 4°C for analysis of microbial biomass carbon (MBC), dehydrogenase activity (DHA), and C mineralization study whereas the other part was air dried, ground, mixed thoroughly, and passed through 2mm sieve to obtain a homogeneous composite sample through quartering process for analysis of initial soil parameters, soil C, and its fractions. The texture analysis was done by modified dispersion and sedimentation method (Kettler et al. 2001). Chemical attributes of soil, viz., pH and electrical conductivity (Page et al. 1982), easily oxidizable organic

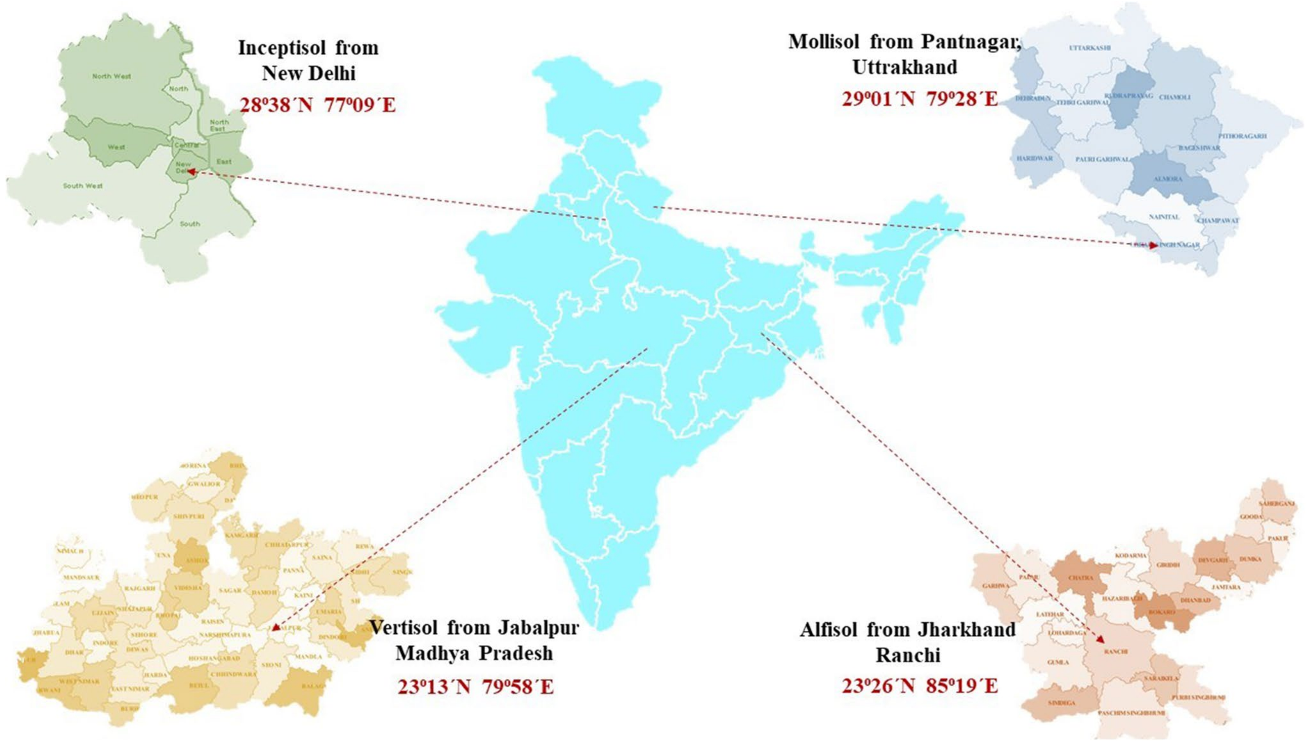


Fig. 1 Location map of soil sample collection from four different places across India

Table 1 Details of initial soil parameters

Properties	Vertisol, Jabalpur	Mollisol, Pantnagar	Inceptisol, Delhi	Alfisol, Ranchi
Sand (%)	5.8	17.5	57.5	40
Silt (%)	35.8	61.1	23.5	32.4
Clay (%)	58.4	21.4	19	27.6
Texture	Clay	Silty loam	Sandy loam	Clay loam
pH	7.85	7.84	8.32	5.74
EC (dS m^{-1})	0.17	0.29	0.23	0.16
Organic C (g kg^{-1})	6.68	9.52	4.42	5.24
Available N (kg ha^{-1})	179	180	119	141
Available P (kg ha^{-1})	18.7	95.8	15.8	14.2
Available K (kg ha^{-1})	324	165	334	185
Soil classification	Typic Haplusterts	Aquic Hapludoll	Typic Ustochrept	Typic Haplustalf

C (WBC) (Walkley and Black 1934), available nitrogen (Subbiah and Asija 1956), available phosphorus (Bray and Kurtz 1945; Olsen et al. 1954), and available potassium (Page et al. 1982) were determined (Table 1).

2.2 Clay Mineralogy and Surface Properties of Clays

As described in previous section, in each location soil samples from five randomly selected spots were pooled together, air dried and thoroughly mixed to study clay mineralogy. The clay sized particles (< 2 μm) of soil were separated by the procedure outlined by Jackson (1985). Extracted clays underwent four treatments, viz., (a) Mg-saturation and air drying, (b) Mg-saturation and glycerol solvation, (c) K-saturation and air drying, and (d) K-saturation followed by heating at 550 °C and arranged basally for X-ray diffraction (XRD) analysis. The XRD plots of the basally oriented samples were obtained in a Phillips X-ray diffractometer (PW 1710 diffractometer control, PW 1729 X-ray generator) with automated powder diffractometer (APD) software using Ni-filtered Cu-Kα radiation (λ, 0.154184 nm) at a scanning speed of 1.5°2θ min^{-1}. Semi-quantification of the clay minerals was done from the diffractograms of Mg-saturated and glycerol-solvated samples by the “peak area measurement” options of the APD software as per Gjems (1967) and Datta et al. (2015) (Fig. 2). Clay minerals were identified and categorized based on the observations of earlier researchers (Barré et al. 2008; Datta et al. 2015; Das et al. 2019a, b, 2022; Moore and Reynolds 1989). Peaks with C-axis spacing of ~1.8 nm correspond to smectite, one or more peaks observed adjacent to smectite peaks which might signify the presence of smectite interstratified with vermiculite, hydroxyl interlayer minerals (HIM), and other 2:1 minerals (Das et al. 2019a, b, 2022) was broadly categorized as 2:1 interstratified minerals. The presence of chlorite in Mollisol, Inceptisol, and Vertisol was confirmed after heating the K saturated clays at 550 °C where only the peak with c-axis spacing ~1.4 nm was reinforced and categorized as chlorite and chlorite-interstratified minerals. Peaks with C-axis spacing of ~1.01 and ~0.72 nm correspond to illite and kaolinite, respectively. Some broad peaks adjacent to illite were considered as illite-interstratified minerals. SSA of clays isolated from soils were determined by ethylene glycol monoethyl ether (EGME) method (Carter et al. 1965) and CEC of isolated clays were determined by N-ammonium acetate (pH 7) method outlined by Jackson (1985).

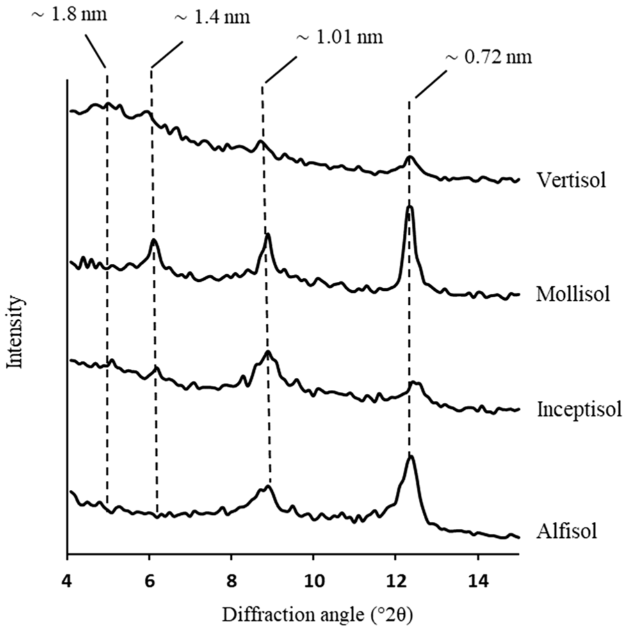


Fig. 2 X-ray diffractograms of Mg-saturated and glycerol-solvated clay samples of different soils

2.3 Carbon Mineralization Study

Carbon mineralization in the form of soil respiration was measured periodically by alkali trap method (Anderson 1982). Fresh soils were preincubated at 25°C for 24 h prior to incubation to acclimatize microbes to laboratory incubation conditions. In brief, 20 g soil was placed in a 500 mL mason glass jar and incubated at a constant temperature (25°C) and water content (60% of water holding capacity). C mineralization was measured using a set of 12 tightly capped mason jars containing four different soils with three replications. The moisture content of the soil in the jars was kept constant by measuring weight loss periodically and adding deionized water as needed. To trap CO_2, 5 mL of 1 N sodium hydroxide (NaOH) solution were kept inside small vials (10 mL, with no lids) within the jar. The CO_2 trapped NaOH solutions were taken out of the jar at each sampling day and was back titrated with 0.5 N hydrochloric acid (HCl) in presence of barium chloride ($BaCl_2$) using phenolphthalein indicator. After 3, 7, 14, 21, and 28 days of incubation, small NaOH vials were removed and replaced with a fresh set of NaOH vials, and jars were returned to the incubator. The quantity of C mineralized at each sampling day was summed over 28-day incubation period to express as cumulative CO_2-C mineralization (CO_2-C_{cum}).

C mineralization was computed by the following formula:

CO_2- C mineralized (mg g^{-1} soil) $= \frac{(B-S)\times N\times 6}{W}$ where B is the volume of HCl used in blank sample (mL), S is the volume of HCl used in soil sample (mL), N is the normality of HCl, and W is the weight of soil sample (g).

2.4 *Labile Carbon Fractions*

2.4.1 Soil Organic Carbon (SOC)

Total soil carbon (TC) was measured by CHNS analyser (Euro Vector make, EuroEA3000 model), while total OC or SOC was calculated by subtracting inorganic C (measured as per methods outlined by Snyder and Trofymow 1984) from TC.

2.4.2 Microbial Biomass Carbon (MBC)

Soil MBC was estimated by the substrate induced respiration method (Bailey et al. 2007) using a gas chromatograph (Make Agilent, Model GC-4890) and the following equation of Anderson and Domsch (1978):

$$x = 40.04y + 0.37$$

where x = microbial biomass C (mg kg^{-1}) and y = rate of CO_2 evolution (mL CO_2 kg^{-1} soil h^{-1})

2.4.3 Dissolved Organic Carbon (DOC)

The DOC was extracted by the methods outlined by Jones and Willett (2006). Here, 5g of dry soil was shaken (200 rpm) in a reciprocal shaker for 1 h with 25 mL ultra-pure water and then centrifuged at 13,000 rpm for 30 min at 4°C. The supernatant was passed through a 0.45 μm glass fiber filter and the C content in the supernatant was estimated following the procedure given by Synder and Trofymow (1984).

2.4.4 Potassium Permanganate Oxidizable Carbon ($KMnO_4$-C)

Active C oxidizable by $KMnO_4$ was determined by modified Blair method as outlined by Weil et al. (2003). In this method, dilute and slightly alkaline $KMnO_4$ reacted with the most readily oxidizable forms of soil C, converting Mn (VII) to Mn (II), and the absorbance was measured by a colorimeter at 550 nm. Oxidizable C was computed using following equation:

$KMnO_4$ oxidizable C (mg kg^{-1}) = [0.02mol/L − (a + b ×absorbance)] × (9000mg C/mol) × (0.02 L solution/0.005 kg soil).

where 0.02 mol/L is the initial solution concentration, a is the intercept and b is the slope of the standard curve, 9000 is the mg C (0.75 mol) oxidized by 1 mol of MnO_4 changing from ($Mn^{7+} \rightarrow Mn^{2+}$), 0.02 L is the volume of $KMnO_4$ solution reacted, and 0.005 is the kg of soil used.

2.4.5 Particulate Organic Matter Carbon (POM-C)

The particulate organic matter (POM) was extracted from the soil following the procedure outlined by Cambardella and Elliott (1992) and C content in POM was determined by dry combustion method in a CHNS analyser (Euro Vector make, EuroEA3000 model).

2.4.6 Complex Organic Matter Carbon (COM-C)

The content of complexed organic matter carbon (COM-C) was determined from the difference between the SOC pool and the POM-C pool.

2.5 Statistical Analysis

The data obtained from the above measured parameters were statistically analyzed by one-way analysis of variance (ANOVA). For statistical significance, the treatment means were differentiated following a posthoc Tukey's test with honest significant difference (HSD) at 95% confidence value ($p < 0.05$) using the SPSS (version 16.0) software by taking clay mineralogy (soil orders) as a factor. Pearson's correlation among the C fractions and clay parameters were performed using the "corr_coef" function and the correlation plot constructed using the "corr_plot" function of "metan" package (Olivoto and Lúcio 2020) in R studio. Principal component analysis (PCA) was done to find out the traits or parameters

Table 2 Mineralogical make up of soil clay fractions (SCFs)

Major minerals	Percent distribution (%)			
	Vertisol	Mollisol	Inceptisol	Alfisol
Smectite	25.4	1.61	1.7	-
2:1 interstratified minerals	41.5	-	-	-
Chlorite and chlorite-interstratified mineral	-	16.1	2.45	2.1
Illite	12.1	20.9	1.95	17.2
Illite-interstratified mineral	-	6.89	58.7	3.8
Kaolinite	21	54.5	35.2	76.9

explaining more variation in the system and the relationship of individual parameters with soil orders. PCA was performed in R studio using packages "FactoMineR" (Lê et al. 2008) and "factoextra" (Kassambara and Mundt 2017). All the figures were drawn using Microsoft excel (2016).

3 Results

3.1 Mineralogy of Different Soil Samples

The mineralogical composition of different soil samples (Table 2) showed that Vertisol contained smectite and 2:1 interstratified minerals as major minerals constituting 25.4 and 41.5 percent (%), respectively. Overall, 67% of minerals present in Vertisol belonged to smectite/2:1 interstratified minerals. Kaolinite dominated the mineralogical composition (54.5%) in Mollisol but other minerals such as chlorite and chlorite-interstratified mineral, illite, illite-interstratified mineral, and smectite were also present with 16.1, 20.9, 6.89, and 1.61%, respectively accounting for 45.5% of the total mineralogical composition. Inceptisol mineralogical make up showed the presence of illite rich interstratified minerals as the major fraction followed by kaolinite contributing 58.7 and 35.2%, respectively. In Alfisol, kaolinite dominated the mineralogical make up having 76.9% followed by illite having 17.2%, respectively.

3.2 Effect of Clay Mineralogy on Surface Properties of Different Clays Isolated from Soil Samples

SSA and CEC of soil clays showed significant variations among different clays (Table 3). Vertisol clays showed the highest SSA of 506 (m^2 g^{-1} clay) followed by Mollisol, Inceptisol, and Alfisol clays having 149, 50.9, and 38.2 (m^2 g^{-1} clay), respectively. For CEC, a similar trend was followed where clays isolated from Vertisol showed significantly high amount of CEC i.e., 81.3 (m eq 100 g^{-1}) whereas Alfisol clays showed the lowest CEC of 33 (m eq 100 g^{-1}). Clay content was found to be highest in Vertisol having 58.4% clay followed by Alfisol, Mollisol, and Inceptisol.

3.3 Effect of Clay Mineralogy on SOC Mineralization

Carbon mineralization was found to be significantly affected by clay mineralogy. Respiration rates were invariably higher in Mollisol and Vertisol soils throughout the incubation period (Fig. 3). After 28 days of incubation, Mollisol and Vertisol showed CO_2-C_{cum} of 0.97 and 0.96 g C kg^{-1}, respectively being statistically at par (Fig. 4a). Inceptisol showed the lowest CO_2-C_{cum} of 0.84 g C kg^{-1}. The percentage of SOC lost as CO_2 (%) showed the opposite trend compared to the total C mineralization from soils (Fig. 4b). Mollisol and Vertisol showed significantly lower percentage loss of C lost as CO_2 from total SOC compared to other soils. Inceptisol showed the highest loss of 10.1% as CO_2 from SOC.

3.4 Effect of Clay Mineralogy on Labile Carbon Fractions and Enzymatic Activities

Difference in clay mineralogy significantly affected the labile C fractions in soils as well (Table 3). With respect to fractions like MBC, DOC, $KMnO_4$-C, and SOC, Mollisol soils showed the highest amount followed by Vertisol, whereas Alfisol and Inceptisol showed the lowest amount. In WBC, Mollisol showed the highest amount followed by Vertisol, Inceptisol, and Alfisol. SOC was partitioned into POM-C and COM-C (Fig. 5). POM-C was observed to be highest in Mollisol, which was significantly higher than

Table 3 Effect of different soil clay mineralogy (Soil order) on different labile carbon fractions and clay parameters

Soil orders	MBC (mg Kg^{-1})	DOC (mg Kg^{-1})	$KMnO_4$-C (mg Kg^{-1})	WBC (g Kg^{-1})	SOC (g Kg^{-1})	DHA (µg TPF g^{-1} day^{-1})	SSA (m^2 g^{-1} clay)	CEC (meq/100 g)	Clay content (%age)
Vertisol	705(±11.6) b	106(±8.72) a	288(±9.07) b	6.68(±0.10) b	18.4(±1.48) a	145(±8.81) a	506(±2.16) a	81.3(±2.40) a	58.4(±2.46) a
Mollisol	778(±13) a	112(±17.1) a	338(±4.46) a	9.52(±0.17) a	22.4(±1.57) a	165(±6.47) a	149(±2.90) b	62.7(±1.54) b	21.4(±1.15) bc
Inceptisol	623(±9.73) c	52(±8.72) b	258(±11.3) b	4.42(±0.13) d	8.3(±0.12) b	104(±6.78) b	50.9(±2.74) c	54.1(±2.14) b	19(±1.14) c
Alfisol	678(±4.71) b	34(±7.21) b	260(±5.37) b	5.24(±0.13) c	9.7(±0.16) b	132(±9.59) ab	38.2(±2.48) d	33(±2.82) c	27.6(±1.61) b

The values are shown as the mean ($n=3$) with standard error of mean given in parenthesis. Similar lowercase letters within each column are not significantly different at $P<0.05$ according to Tukey's HSD test

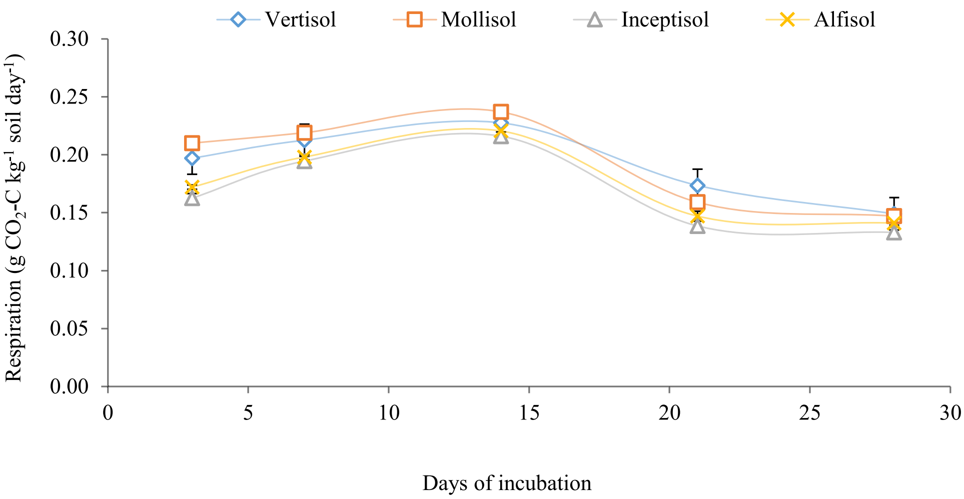


Fig. 3 Respiration rates of four different soils with varying mineralogy. Error bars indicate standard error of mean ($n = 3$)

Vertisol, Inceptisol and Alfisol. With respect to COM-C, Mollisol and Vertisol were statistically at par, whereas lowest amount was found in Inceptisol. For evaluation of overall enzymatic conditions of soil, DHA was determined. Mollisol and Vertisol exhibited the highest DHA whereas Inceptisol showed the lowest activity (Table 3).

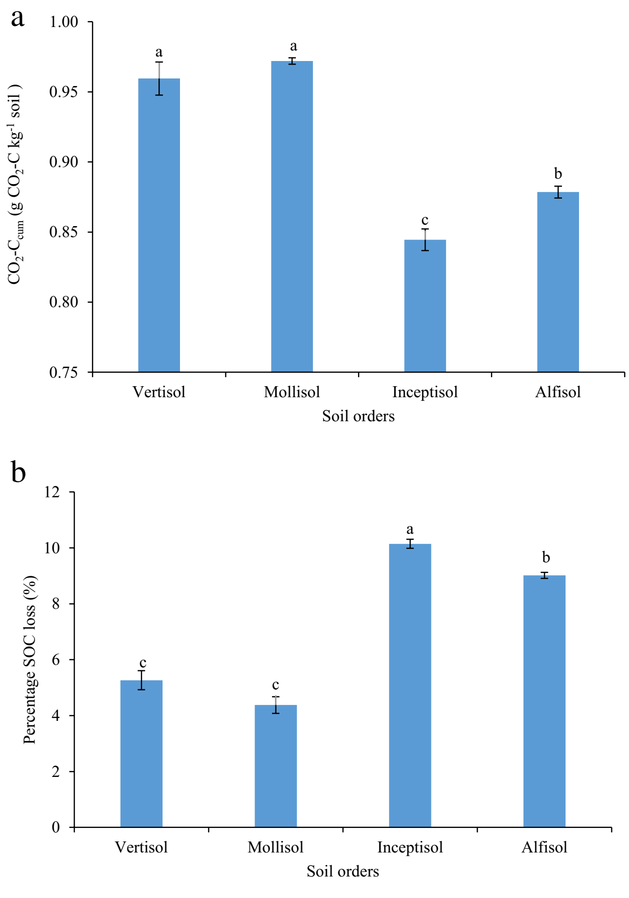


Fig. 4 Cumulative carbon mineralization after 28 days of incubation (**a**) and percentage of SOC loss (**b**) of different soils varying in clay mineralogy. Error bars indicate standard error of mean ($n = 3$). Vertical bars with similar lowercase letters are not significantly different at $P < 0.05$ according to Tukey's HSD test

3.5 Relationship Between Soil Clay Characteristics with SOC Fractions and Mineralization

Correlation studies indicated that all the soil chemical and biological properties exhibited a highly positive correlation ($p<0.05$ and $p<0.01$) among each other and CO_2-C_{cum} (Fig. 6). C mineralization showed strong and significant correlation with labile fractions, such as MBC, $KMnO_4$-C, POM-C, WBC, DOC, SOC, and COM-C. Irrespective of soil clay composition, SOC, POM-C, COM-C, and WBC exhibited significant positive linear relations with CO_2-C_{cum} (mg CO_2-C kg^{-1} soil), where the R^2 values were in order of SOC followed by COM-C, WBC, and POM-C (Fig. 7). Clay characteristics like SSA, CEC, and clay content showed significant correlation among themselves. SSA had significant positive correlation with CO_2-C_{cum}, COM-C, and DOC, whereas CEC showed significant positive correlation with COM-C, CO_2-C_{cum}, DOC, and SOC. Both SSA and CEC had significant negative correlation with percent C loss.

3.6 Principal Component Analysis

Principal component analysis (PCA) in form of two dimensional bi-plot (loading and score plots) revealed that up to 90.9% of cumulative variability in soil physico-chemical and clay properties due to difference in mineralogy can be explained by two principal components (Table 4). As shown in the biplot, the parameters clustered distinctly under two dominant principal components, PC1 and PC2, which explained 73.4% and 17.5% of the total variability with an eigenvalue >1 (Table 4, Fig. 8). The factor loading values in component matrix (Table 5) showed that PC1 had large positive loadings on SOC followed by CO_2-C_{cum}, COM-C, POM-C, and WBC,

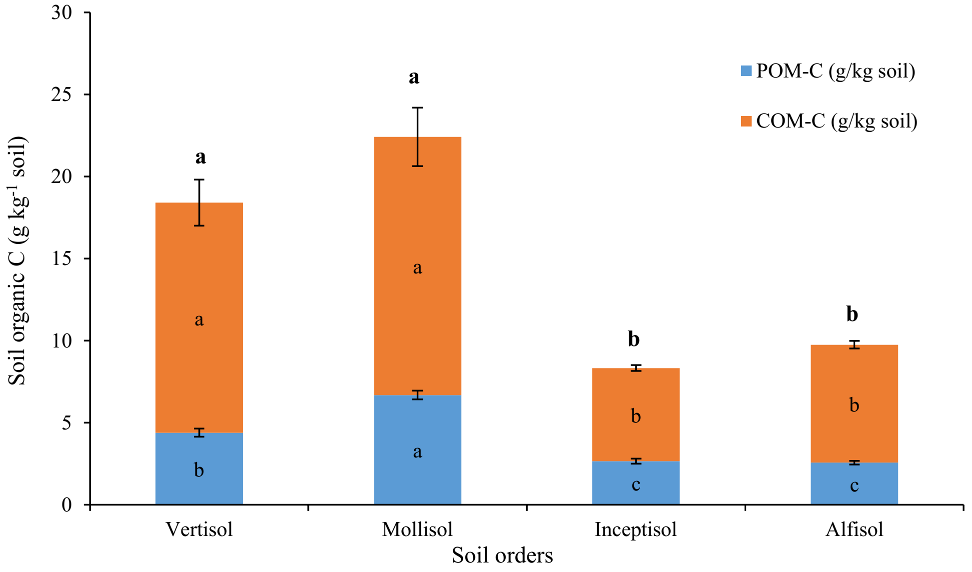
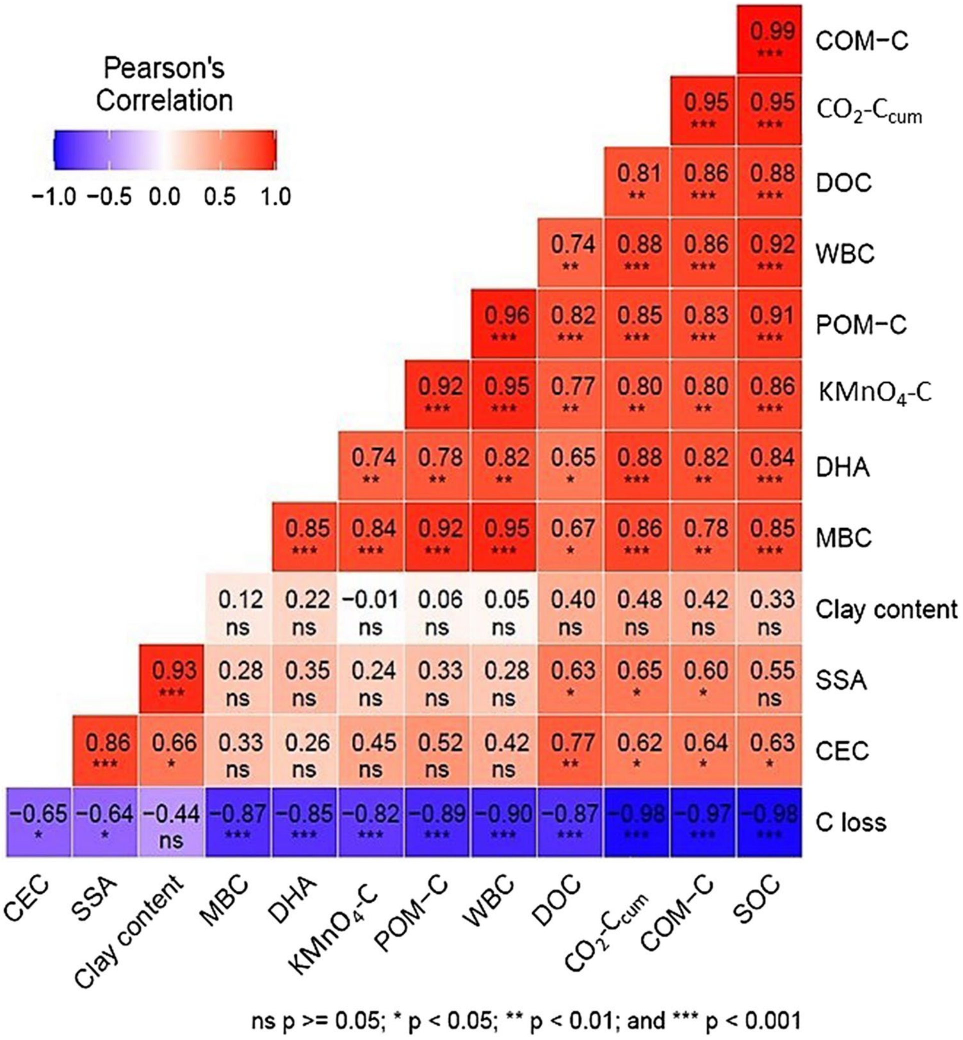


Fig. 5 Partitioning of SOC into particulate organic matter-carbon (POM-C) and complex organic matter-carbon (COM-C) of four soils varying in clay mineralogy. Error bars indicate standard error of mean ($n = 3$; $P < 0.05$). Vertical bars with similar lowercase letters are not significantly different at $P < 0.05$ according to Tukey's HSD test



	CEC	SSA	Clay content	MBC	DHA	KMnO$_4$-C	POM−C	WBC	DOC	CO$_2$-C$_{cum}$	COM−C	SOC
COM−C												0.99 ***
CO$_2$-C$_{cum}$											0.95 ***	0.95 ***
DOC										0.81 **	0.86 ***	0.88 ***
WBC									0.74 **	0.88 ***	0.86 ***	0.92 ***
POM−C								0.96 ***	0.82 ***	0.85 ***	0.83 ***	0.91 ***
KMnO$_4$-C							0.92 ***	0.95 ***	0.77 **	0.80 **	0.80 **	0.86 ***
DHA						0.74 **	0.78 **	0.82 **	0.65 *	0.88 ***	0.82 **	0.84 ***
MBC					0.85 ***	0.84 ***	0.92 ***	0.95 ***	0.67 *	0.86 ***	0.78 **	0.85 ***
Clay content				0.12 ns	0.22 ns	−0.01 ns	0.06 ns	0.05 ns	0.40 ns	0.48 ns	0.42 ns	0.33 ns
SSA			0.93 ***	0.28 ns	0.35 ns	0.24 ns	0.33 ns	0.28 ns	0.63 *	0.65 *	0.60 *	0.55 ns
CEC		0.86 ***	0.66 *	0.33 ns	0.26 ns	0.45 ns	0.52 ns	0.42 ns	0.77 **	0.62 *	0.64 *	0.63 *
C loss	−0.65 *	−0.64 *	−0.44 ns	−0.87 ***	−0.85 ***	−0.82 ***	−0.89 ***	−0.90 ***	−0.87 ***	−0.98 ***	−0.97 ***	−0.98 ***

ns p >= 0.05; * p < 0.05; ** p < 0.01; and *** p < 0.001

Fig. 6 Pearson's correlation matrix showing the relationship among different soil parameters. ns: Not significant; *, **, and *** next to R^2 values indicate significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. CO$_2$-C$_{cum}$: cumulative C mineralization; MBC: microbial biomass carbon; DOC: dissolved organic carbon; KMnO$_4$-C: permanganate oxidizable C; SOC: soil organic carbon; POM-C: particulate organic matter carbon; COM-C: complex organic matter carbon; DHA: dehydrogenase activity; WBC: Walkley black carbon; SSA: specific surface area; CEC: cation exchange capacity, clay content (%), C loss (%): carbon lost as CO$_2$ from initial SOC

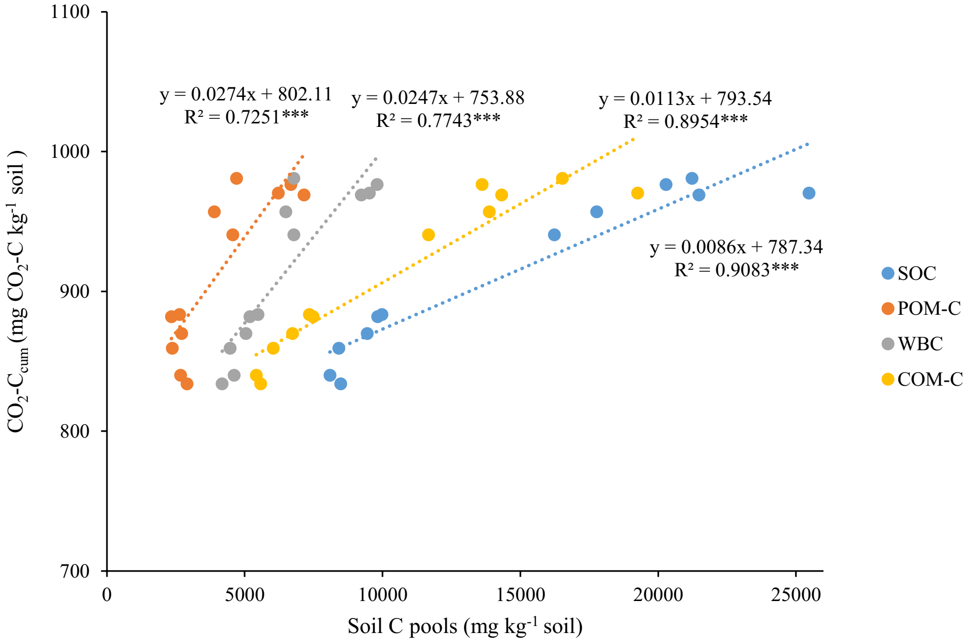


Fig. 7 Relationship of cumulative C mineralized (CO_2-C_{cum}) with soil organic carbon (SOC), particulate organic matter carbon (POM-C), Walkley black carbon (WBC), and complex organic matter carbon (COM-C). *, **, and *** next to R^2 values indicate significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

respectively, and exhibited a highly positive correlation with each other. DOC, MBC, $KMnO_4$-C, and DHA had also higher loading on PC1. The C loss (%) showed negative loadings on PC1. Remarkably, clay content, SSA and CEC had strong loadings on PC2 as evident in Table 5 and Fig. 8. In the biplot, Mollisol showed distinct and close affinity to all the labile C fractions and microbial properties, whereas Vertisol showed more affinity towards surface properties of clays. Alfisol and Inceptisol formed a distinct group showing close proximity towards C loss (%).

Table 4 Principal components with their corresponding eigenvalues, percentage of variance and cumulative percentage of variance for each principal component

Principal components	Eigenvalues	% of Variance	Cumulative variance (%)
1	9.54	73.4	73.4
2	2.27	17.5	90.9
3	0.63	4.85	95.7
4	0.22	1.69	97.4
5	0.13	1.00	98.4
6	0.10	0.77	99.2
7	0.08	0.60	99.8
8	0.01	0.11	99.9
9	0.01	0.07	100
10	0.01	0.04	100
11	2.936E-05	0.00	100
12	1.227E-16	9.440E-16	100
13	−8.877E-17	−6.828E-16	100

4 Discussion

In this study, mineralogy of the soil reaffirms the influence of parent material, climate and topography. It was evident that mineralogy of Vertisol was dominated by smectite, 2:1 interstratified minerals. Previous studies have highlighted that in humid tropics, continuous supply of bases from Ca-rich zeolites helped in stabilizing these smectite in Indian soil (Bhattacharyya et al. 1993). In majority of Indian Vertisol found in semi-arid and sub-humid regions, the presence of smectite and kaolinite is quite common (Bhattacharyya et al. 1993; Pal 2003; Pillai et al. 1996). In Mollisol, chlorite, illite, and illite-interstratified minerals were present in significant amount even though highest was kaolinite. Usually, 2:1 phyllosilicates are predominantly found in Mollisol because of their formation in drier climate compared to Ultisol or Alfisol. However, there is a possibility of Mollisol having kaolinitic minerals in tropical climate (Allen and Fanning 1983). Previously, kaolinite and illite minerals were found in appreciable amount in the soils of this area (Samra 1982). Some studies have identified kaolinite as dominant mineral followed by interstratified mineralogy with traces of chlorite in soils developed on granite in Shivalik regions (Surya et al. 2015). In sub-humid subtropical climate of this region where summer temperature crosses > 40 °C having good drainage and higher OM accumulation, higher moisture/rock ratio, might lower the Si^{4+} concentration along with other basic cations hastening chemical weathering process. This process could have hastened the formation of the kaolinite directly from the silicate minerals (Grim 1968). The presence of chlorite also highlighted the intermediate stage of weathering of

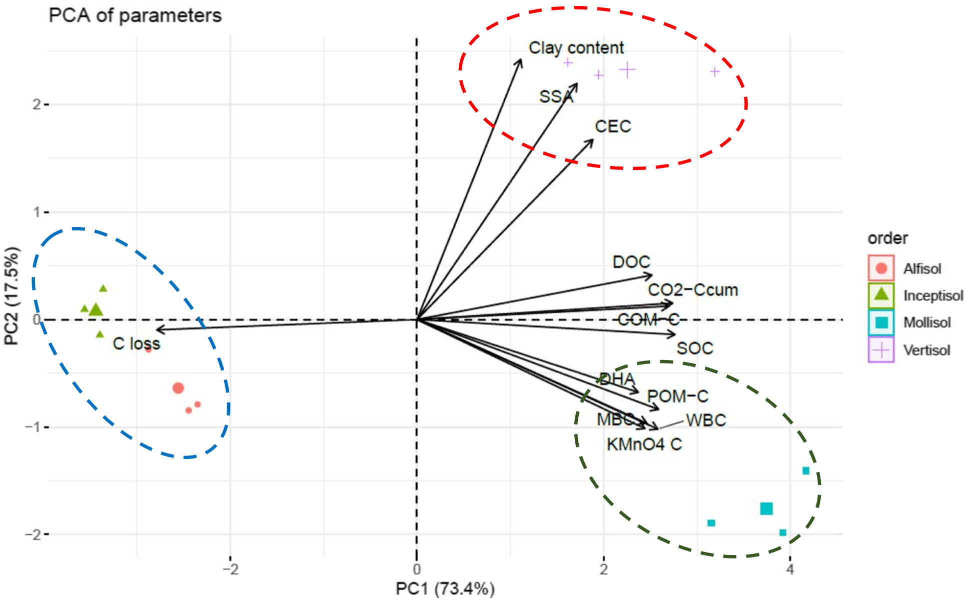


Fig. 8 Two-dimensional biplot of principal component analysis (PCA) showing loading and score plot made through first two principal components: PC1 and PC2 using soil variables and soil orders (having varying mineralogy) for explaining variability in the data-set. CO_2-C_{cum}: Cumulative C mineralization; MBC: Microbial biomass carbon; DOC: dissolved organic carbon; $KMnO_4$-C: permanganate oxidizable C; SOC: soil organic carbon; POM-C: particulate organic matter carbon; COM-C: complex organic matter carbon; DHA: dehydrogenase activity; WBC: Walkley black carbon; SSA: specific surface area; CEC: cation exchange capacity, clay content (%), C loss (%): carbon lost as CO_2 from initial SOC

the soil. The role of vegetation on alteration and neo-synthesis of minerals is also important. The presence of high OM, wet moisture regime with high leaching rates and high above and below ground root biomass with rich biodiversity has a definite role to play in shaping the alteration of mica into chlorite and/or kaolinite (Sharma 1982; Singhal and Sharma 1983). In Inceptisol, majority of mineral suite belonged to illite/illite-interstratified mineral. Inceptisol are predominantly found in regions subjected to moderately intensive pedogenic processes (Allen and Fanning 1983). This mineralogy indicated relative immaturity and presence of weatherable minerals (Pal 2017) as the parent materials are devoid of products of advanced stages of weathering (Allen and Fanning 1983). Some studies have found kaolinite as dominant mineral in Inceptisol of eastern India (Das et al. 2018). However, our finding corroborated with previous findings (Das et al. 2018, 2019a, b) where mica was found to be the dominant mineral fraction. Kaolinite dominated the mineralogical composition of Alfisol. Similar mineralogical composition was also previously found (Das et al. 2019a, b; Pal et al. 2003, 2017). Under the influence of humid sub-tropical climate, due to availability of high temperature and moisture, the parent material might have been transformed with time into illite/interstratified mica followed by vermiculite, smectite and finally into kaolinite by chemical weathering (Allen and Fanning 1983). Difference in mineralogy influenced the surface properties of clays significantly. Smectitic clays having 2:1 expanding silicate structure possess permanent negative charge which imparts higher SSA and CEC whereas kaolinite clays have 1:1 non expanding layer structure having small amount of pH dependent charge surface possessing low SSA and CEC. Illitic clays have limited expanding properties that expresses SSA and CEC higher than kaolinite but lower than smectite (Churchman and Lowe 2012). We employed EGME to determine the SSA, which has characteristics to penetrate internal surfaces of minerals to measure both internal and

Table 5 Component matrix showing factor loading in principal component 1 and 2

Factors	PC 1	PC 2
CO_2-C_{cum}	0.98	0.05
MBC	0.88	−0.35
DOC	0.90	0.15
$KMnO_4$ C	0.87	−0.36
POM-C	0.92	−0.30
COM-C	0.96	0.05
SOC	0.98	−0.05
WBC	0.92	−0.36
SSA	0.61	0.78
CEC	0.67	0.60
Clay content	0.40	0.86
C loss	−0.99	−0.03
DHA	0.84	−0.24

external surface areas (Ugochukwu 2017). In our study, we found Vertisol clays had higher amount of smectite and 2:1 interstratified minerals, which collectively expressed higher amount of SSA and CEC. The surface area, as well as CEC, decreased with increase in low activity, non/limited expanding clays like illite and kaolinite. Mollisol having relatively good amount of mixture of illite, chlorite and interstratified minerals showed SSA and CEC higher than Inceptisol and Alfisol clays. The trend of SSA and CEC was smectitic + 2:1 interstratified minerals > kaolinite + illite + chlorite mixed clay minerals > illite dominating minerals > kaolinitic clay minerals which is in conformity with several previous reports (Saidy et al. 2013; Singh et al. 2016, 2018; Ugochukwu 2017).

The effect of mineralogy on SOC mineralization and labile C sequestration were significant. It was observed that greater percentage of SOC is lost from Inceptisol, which is at odds with CO_2-C_{cum}, which were greater in Mollisol and Vertisol after 28 days of incubation (Fig. 4). The higher amount of C mineralization in Mollisol could be due to the higher amount of easily oxidizable C present initially in this soil that showed higher accumulation of fresh OM contributed to higher mineralization (Haynes 2005). The rapid decay of C in Mollisol and Vertisol might be due to the higher porosity in these soils because of fine texture, which maintained more water (Baldock and Skjemstad 2000; Chen et al. 2014). Better texture, porosity, higher microbial activity along with higher amount of available C in soil may have facilitated greater rate of mineralization than other soils (Singh et al. 2017; Six et al. 2002). However, the trends for C mineralization expressed in percentage loss of initial amounts of SOC followed illite dominated clays (Inceptisol) > kaolinite dominated clays (Alfisol) > smectitic+vermiculite clay minerals (Vertisol) > kaolinite+illite mixed clay minerals (Mollisol) although Vertisol and Mollisol showed no significant difference. Mollisol and Vertisol stabilized more SOC than other soils. These results highlighted the effect of mineralogy in protecting the C in soil. The effect of surface properties of clays was evident from the significant negative correlation among surface properties of clays and C lost as CO_2 (Fig. 6). Higher SSA and CEC in smectite-dominated soil resulted in stronger adsorption of OC by ligand exchange or cation bridging and protected SOC from mineralization (Sarkar et al. 2018; Singh et al. 2016, 2018). In this study, Vertisol and Mollisol expressed higher labile C in terms of MBC, DOC, $KMnO_4$-C and WBC, respectively, while Inceptisol and Alfisol showed the lowest amount (Table 3). The association of OM with mineral particles are affected by the mineralogy because of the difference in SSA and charge characteristics (Feng et al. 2013). Smectitic minerals due to their small size and high charge density could have helped in binding of OM strongly via mechanisms like ligand exchange, cation bridging, hydrophobic interaction, or van der Waals forces (Churchman and Lowe 2012; Singh et al. 2018). Smectite help in formation of micro-aggregates in fine textured soil where OM may get entrapped and remain protected as slowly available C pool for a long period whereas soils having 1:1/ non-expanding clays with lower SSA and CEC does not offer any protection to SOM (Baldock and Skjemstad 2000; Six et al. 2002). Many experiments have shown that "sorption" mechanism significantly affects the retention of OM in soil by protecting it from biodegradation (Arnarson and Keil 2000; Kalbitz et al. 2005; Singh et al. 2016), yet it can release them into solution in a systematic manner through particle associated OM (Kaiser and Guggenberger 2003). Our findings confirmed the effect of clay minerals in enrichment of labile C in soils, which are in line with previous findings (Nguyen and Marschner 2014; Saidy et al. 2012; Singh et al. 2016). Vertisol and Mollisol soils were richer in clay content as well as dominant in 2:1 type fine smectitic minerals (Gupta et al. 1999) than either Inceptisol and Alfisol which are poorer in clay content and dominated by 1:1 type clay minerals like illite and kaolinite. This could also play a role in SOC stabilization, which in turn enhanced the SOC to a greater extent (Nguyen and Marschner 2014). However, in temperate climate, Mayes et al. (2012) found that under low DOC concentrations, Ultisol and Alfisol exerted high adsorption capacity compared to Mollisol and a major role was played by textural clay and Fe oxide content. Similarly, a study consisting of 52 temperate mineral soil samples from podzol, volcanic as well as A and B horizons in Canada observed a dominant relationship between non-crystalline Al and Fe oxides and DOC adsorption rather than clay content (Kothawala et al. 2009). These researchers noted that the coatings of Fe and Al oxides masked the effect of clay content. However, in our case, clay content, SSA, and CEC influenced the accumulation and stabilization of SOC. Higher WBC in Mollisol than Vertisol could be attributed to the difference in average annual temperature and texture that exist between these two soils which might have impacted significantly the differential build-up of SOC in these soils (Surya et al. 2015). Due to higher availability of labile substrates and higher microbial biomass, the enzyme activity also followed the similar trend. The POM-C was higher in Mollisol and Vertisol compared to other soils. POM-C are essentially the plant−derived remains that are predominant in macro aggregates fraction due to their strong association with mineral particles (Six et al. 1998; Wright et al. 1999). In our study, Vertisol and Mollisol had fine texture soil along with 2:1 phyllosilicate mineralogy, which provided perfect microsites for microbial growth. Due to higher microbial secretions along with better soil aggregation, OM could have occluded into aggregates, which in turn sequestered more POM-C in soil compared to coarse textured soils having 1:1 type of mineralogy (Cotrufo et al. 2015; Schmidt et al. 2011; Schweizer et al. 2021; Steffens et al. 2017). The results of COM-C also highlighted the importance of 2:1 mineralogy of Vertisol in preferentially accumulating the OM of complex nature, viz., aromatic and phenolic compounds (Leinweber et al. 1999; Wattel-Koekkoek et al. 2001).

Correlation study among the labile C fractions and surface clay properties highlighted their strong interconnection among each other (Fig. 6). SOC showed a strong and significant relationship with CO_2-C_{cum}, MBC, DOC, $KMnO_4$-C, POM-C, COM-C, WBC, and DHA suggesting that change in SOC storage greatly depends upon these pools. Similar results were previously reported (Bongiorno et al. 2019; Rudrappa et al. 2006; Souza et al. 2016). Due to strong correlation, any of the above fractions could be used as potential indicators of SOC build up in the studied area. As the interplay of SOC with other fraction is very dynamic and responsive, therefore a strong positive influence of SOC, POM-C, COM-C, and WBC on C mineralization was observed (Fig. 7). Strong correlation between CO_2-C_{cum} and DHA showed that the microbial populations are actively contributing to respiration due to higher substrate availability. The higher correlation coefficient between SOC and POM-C than SOC and MBC suggested that the newer organic materials contributed to the POM largely. SSA and CEC showed significant correlation with SOC, CO_2-C_{cum}, and DOC highlighting the importance of clay surface characteristics in C accumulation. Higher surface areas and CEC helped in accumulating more OC through mechanisms like ligand exchange, cation bridging, and van der Waals interaction which has been previously reported (Dontsova and Bigham 2005; Saidy et al. 2013). Singh et al. (2016) highlighted that SSA was the most crucial factor in adsorption and stabilization DOC in clays. In addition to correlation studies, PCA analysis revealed that Mollisol accumulated higher amount of labile C fractions (Fig. 8). The prevalence of temperate subhumid climate helped in accumulation of fresh OM in terai regions of Pantnagar, which is why the WBC and SOC were quite higher in this soil. However, Vertisol had a higher affinity for SSA, CEC, and clay content indicating that clay properties significantly influenced SOC enrichment in this soil. Interestingly, Inceptisol and Alfisol formed a distinct group showing higher percentage of SOC loss. This similarity in behavior of both these soils despite their occurrence in two different sets of climatic conditions could be attributed to the clay characteristics. Both soils contained limited to non-expanding clays such as illite and kaolinite, which behaved similarly in terms of surface properties. Therefore, they sequestered less labile C and a higher percentage of C was lost as CO_2.

5 Conclusions

This study inferred that variation in pedogenic clay had a significant effect on stabilization of soil organic carbon. Higher cumulative mineralization and labile C fractions such as microbial biomass carbon, dissolved organic carbon, $KMnO_4$-C, particulate organic matter carbon, complex organic matter carbon along with dehydrogenase activity, and lower percentage of soil organic carbon loss were found in Mollisol and Vertisol soils than Inceptisol and Alfisol. Variation in CO_2 evolution through mineralization could be largely explained by soil organic carbon and complex organic matter carbon. Hence, under natural conditions, inclusion of these parameters will better reflect the process of soil respiration in future study. This study validated the clear influence of 2:1 mineralogy in terms of specific surface area and cation exchange capacity on labile C enrichment in Vertisol, whereas the presence of inherently higher amounts of soil organic carbon could be the predominant factor governing higher carbon sequestration in Mollisol. Stronger correlation between labile C fractions and clay properties revealed that greater specific surface area and cation exchange capacity would result in greater C sequestration and stabilization in soil. This study found that presence of limited to non-expanding clay mineralogy in Inceptisol and Alfisol is more important for soil C sequestration and stabilization than climate. This preliminary information will help future researchers to consider clay characteristics while performing soil carbon studies. As the ability of soil to store organic carbon varies according to climate-soil-management interactions, it will be worthwhile to confirm these findings by studying other soils from different agro-climatic regions.

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Data availability Data may be available on request to corresponding author.

Declarations

Conflict of Interest The authors declare no competing interests.

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