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Impact of conservation agriculture on humic acid quality and clay humus complexation under maize (*Zea mays*)-wheat (*Triticum aestivum*) and pigeon pea (*Cajanus cajan*)-wheat cropping systems

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ABSTRACT

An attempt was made to study the humic acid (HA) quality and clay humus complex in order to generate valuable information regarding soil carbon (C) and recalcitrant carbon variations under conservation agriculture (CA) practices. It is worthwhile to mention that CA has got wider acceptance among researchers and farmers nowadays. A field experiment was conducted in an Inceptisol with three treatments, namely conventional tillage (CT), zero tillage (ZT) without residue and zero tillage with residue (ZT+R) in a maize (*Zea mays* L.)-wheat (*Triticum aestivum* L.) (M-W) and pigeon pea (*Cajanus cajan* L.)-wheat (P-W) cropping system at ICAR-Indian Agricultural Research Institute, New Delhi, with a view to characterize the HA by E4/E6 ratio and total acidity, and to specify the functional groups of clay humus complex. In ZT+R based treatments, lower E4/E6 ratio and total acidity of extracted HA showed higher degree of humification and stability of humic acid carbon (HA-C). The FTIR spectroscopy of the clay-humus complex (as extracted from soil) displayed the presence of a large number of functional groups in ZT+R treatment followed by ZT and CT. It was also observed that the yield of crops was also significantly higher in ZT+R than CT in both the cropping systems except in wheat crops in the M-W system. Therefore, it can be concluded that ZT+R has the potential to enrich the organic carbon (C) quality in soil and increase the aromaticity of HA, leading to carbon stabilization in soils.

Keywords: Aromaticity, Conservation agriculture, Clay humus, Humic acid, Total acidity

Soil health is a crucial factor for sustainable agriculture, as it directly impacts crop productivity, nutrient cycling, and overall ecosystem functioning. Soil is involved in C sequestration and C stabilization (Ndzelu *et al.* 2021). Soil organic matter (SOM) acts as a vital tool to maintain physical, chemical and biological processes in soil (Reddy *et al.* 2014). Soil humic substances that hold the largest recalcitrant pool of C are a natural product and classified as humin (HN), humic acid (HA) and fulvic acid (FA) based upon the base solubility and all the fractions are sensitive to management practices (Galantini *et al.* 2014). Humic substances help in regulation of soil C stabilization (Stevenson *et al.* 1976). The chemical composition, structure and stability of these humic substances are influenced by active and passive factors of soil formation (Katyal *et al.* 2000) besides changes in management practices such as zero tillage, retention of crop residue which can give rise to the C content of the soils (Kubar *et al.* 2019). Humus C contents have been physically protected against decomposition by soil aggregates (Dou *et al.* 2020).

Conservation agriculture (CA) is an important tool to increase soil organic carbon (SOC) in soils. CA relies upon a set of management principles namely, minimum tillage, retention of crop residues, and crop rotation (Naorem *et al.* 2023). Residue retention and crop rotation in CA also influence soil health through the build-up of SOC. The chemical composition of residues affects the storage and oxidation rates of organic C compounds. Crop residues in soils enrich the soil with amino and aliphatic, methoxyl, carboxyl, hydroxyl, groups (Zhang *et al.* 2020). Several researchers reported that no tillage or zero tillage simplify the humic molecular structure and enhance the aliphatic C in HA, which influence the stabilization of soil C and regeneration of HA. It was also found that residue incorporation enhanced aromatic C and decreased aliphatic C groups into the subsurface, leading to an increase soil C stability. Understanding the structural characteristics of humic acid (HA) and its relationship with soil management practices is crucial for optimizing soil health and fertility.

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Fourier transform infrared (FTIR) spectroscopy is a tool revealing insight into the clay humus and humic acid (HA) structure. FTIR spectroscopy of HA in the rice-wheat system shows that continuous organic manuring imparted higher stability to SOC due to strong humification (Purakayastha *et al.* 2020). Similar kind of observations were also found in long term manuring and fertilization where residue incorporation along with vermicompost and chemical fertilizer improved C stability (Yadav *et al.* 2023). Soil properties that are influenced by CA-based practices affect functional groups of humic substances and improve SOC stability (Datta *et al.* 2022). However, very few studies reported about the influence of CA on clay humus and C stabilization. Thus, this study was aimed to determine CA's impact on clay humus structure and humic acid composition in an Inceptisol. Specifically, our goal was to find (1) the functional groups present in clay humus and (2) evaluate changes in the characteristics of humic acid under CA practices.

MATERIALS AND METHODS

Experimental site details: A long-term zero tillage and residue retention-based field study on maize (*Zea mays* L.)-wheat (*Triticum aestivum* L.) (M-W) and pigeon pea (*Cajanus cajan* L.)-wheat (P-W) cropping system was started in the year 2010 at the experimental farm in ICAR-Indian Agricultural Research Institute, New Delhi. The experimental field receives maximum and minimum temperatures of 40.5–44°C and 3–8°C, respectively. It receives around 700 mm of rainfall annually. The soil is sandy clay loam in texture under Typic Haplustept with *p*H 8.04–8.18, Walkley and Black C 5.26–6.28 g/kg and available N 75.7–96 g/kg. The present experiment was conducted in 2021 with three treatments, zero tillage with residue (ZT+R), zero tillage without residue (ZT) and conventional tillage (CT) in a completely randomized block design (RBD) to study the effect of ZT and residue retention on HA properties and clay humus functional groups. The plot size was 220 m^2. The recommended doses of fertilizers were applied to maize, wheat and pigeon pea crops. In CT and ZT, the crops were harvested manually by following farmers' practice and in zero tilled plot (ZT+R) where about 40% of maize stover, wheat straw and pigeon pea straw were retained as residue.

Collection of soil sample: Triplicate samples of both surface (0–5 cm) and sub-surface (5–10, 10–20 cm) soil layers were collected from all treatments using a core sampler and processed for further analysis.

Extraction of clay humus and FTIR spectroscopy and isolation and purification of HA: Clay humus extraction from bulk soil was performed using the method of Datta *et al.* (2015). 50 g soil along with 500 ml distilled water was taken in a 1000 ml plastic bottle and was shaken in a mechanical shaker for 30 min and after the dispersion, soil sample was transferred to a 2.5 litre bottle and filled with the distilled water up to the neck. After 16 h, the upper 20 cm suspension, containing clay humus was siphoned out (Jackson 1985), and then it was concentrated and dried for further use. The functional groups of clay humus were characterized by FTIR spectroscopy. Images were analyzed using Origin software. The HA was extracted using a 0.1 mol/litre NaOH solution and purification was carried out using hydrochloric acid and hydrofluoric acid and dialyzed against distilled water as per the standard procedure. E4/E6 ratio was measured following the method of Chen *et al.* (1977). Total acidity in HA was measured by $Ba(OH)_2$ method (Schnitzer and Gupta 1965). 50 mg HA sample was reacted with an excess of $Ba(OH)_2$ and the remaining $Ba(OH)_2$ was measured by back titration with 0.5 N HCl.

Grain yield: Crops were harvested manually from each plot for recording the grain yield. Grain yield was recorded in the year 2019–20 and 2020–21 at standard moisture content.

Statistical analysis: The data were processed to test the significance of treatments using one-way ANOVA pertinent to completely randomized block design. Treatment means were separated by Duncan multiple range test at 5% level of significance ($P<0.05$) using R software.

RESULTS AND DISCUSSION

Tillage and residue management for more than 10 years helps in improving the elemental composition of HA. Elemental composition of HA showed that ZT+R had 50% higher C% in M-W and 52% in P-W system in 0–5 cm depth and N% was nearly 4.6% higher in M-W and 4.78% higher in P-W system.

Grain yield: Grain yield was significantly affected due to tillage and residue management. Maize crops showed significantly higher yield in ZT+R and ZT over CT in the M-W system in both years while no significant difference was observed in wheat crop among treatments in both years. Similar results were observed in the P-W system, yield of pigeon pea showed that all the treatments were significantly different and followed the order ZT+R>ZT>CT in both years, while in wheat ZT+R showed significantly higher yield over CT (Table 1).

E4/E6 ratio of humic acid: The E4/E6 ratio which is related to the degree of aromaticity is considered as an important property to characterize HA (Datta *et al.* 2022). The E4/E6 ratio is based on the assumption that HA extract at 465 nm directs the early stages of humification and at 665 nm reflects the organic material at highly condensed humified stage (Zbytniewski and Buszewski 2005). The highest E4/E6 value was observed in HA extracted from CT (6.17, 5.76 and 5.09 in 0–5, 5–10 and 10–20 cm, respectively in M-W and 6.23, 5.75 and 5.19 in 0–5, 5–10 and 10–20 cm, respectively in P-W), and in ZT (the values were 5.89, 5.17 and 4.79 in 0–5, 5–10 and 10–20 cm in M-W and 5.9, 5.23 and 4.75 in 0–5, 5–10 and 10–20 cm in P-W) and ZT+R showed significantly lower values (5.71, 5.05 and 4.55 in 0–5, 5–10 and 10–20 cm in M-W and 5.85, 5 and 4. 57 in 0–5, 5–10 and 10–20 cm in P-W) (Table 2). In both the systems, at 0–5 cm depth all the treatments were statistically at par. Lower value of E4/E6 ratio in ZT+R indicated a greater degree of aromaticity and humification of humic

Table 1 Grain yield from different treatments

Treatment	Yield (q/ha)							
	2019–2020				2020–21			
	Maize	Wheat	Pigeonpea	Wheat	Maize	Wheat	Pigeonpea	Wheat
CT	3.78b	5.52a	1.28c	4.33b	3.97b	5.81a	1.33c	5.14b
ZT	4.36a	6.11a	1.44b	4.84a	5.18a	6.22a	1.74b	5.43ab
ZT+R	5.14a	6.34a	1.59a	5.18a	5.52	6.47a	1.93a	5.7a

Different letters indicate statistically significant differences (P<0.05) among treatments (Duncan multiple range tests for separation of mean). Treatment details are given under Materials and Methods.

Table 2 E4/E6 ratio of humic acid extracted from different treatments

Treatment	E4/E6 ratio					
	Maize-Wheat			Pigeonpea-Wheat		
	Soil depth					
	0–5 cm	5–10 cm	10–20 cm	0–5 cm	5–10 cm	10–20 cm
CT	6.17a	5.76a	5.09a	6.23a	5.75a	5.19a
ZT	5.89a	5.17ab	4.79ab	5.90a	5.23ab	4.75ab
ZT+R	5.71a	5.05b	4.55b	5.85a	5.00ab	4.57b

Different letters indicate statistically significant differences (P<0.05) among treatments (Duncan multiple range tests for separation of mean). Treatment details are given under Materials and Methods.

acids. Higher E4/E6 ratio of humic acid in CT indicated the degree of condensation of aromatic compounds was lower (Chen *et al.* 1977). Retention of C through residue retention and zero tillage system could be the reason behind higher aromaticity in ZT+R. Further it can be concluded that CA helped in formation of more condensed HA via conversion of crop residue carbon to soil organic carbon (Jat *et al.* 2019). Chanda *et al.* (2021) mentioned that E4/E6 ratio was affected by humic materials and SOM rather than HA concentration. Kobierski *et al.* (2018) stated that zero tillage and residue retention might help in the formation of humic acid-calcium-clay complexes to form condensed humic molecules. Zalba *et al.* (2016) reported that lower E4/E6 of HA molecules directed the manifestation of a profound conversion and humification of the SOM.

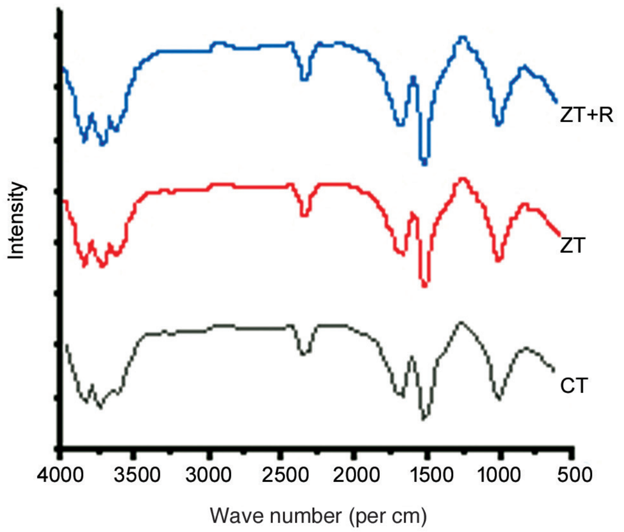
Total acidity of humic acid under zero tillage and residue management: The potentiometric titration of humic acid solutions represents the characteristic nature of a weak polybasic acid. Strong carboxylic groups are neutralized earlier than the phenolic groups (Stevenson 1976). In 0–5, 5–10 and 10–20 cm soil depth, ZT+R shows maximum total acidity (8.76, 8.46 and 7.48 meq/g in M-W and 8.79, 8.53 and 7.39 meq/g in P-W system) followed by ZT (8.17, 8.09 and 7.36 meq/g in M-W and 8.2, 8.2 and 7.3 meq/g in P-W system) followed by CT (7.48, 7.36 and 6.44 meq/g in M-W and 7.67, 7.75 and 7.1 meq/g in P-W system) (Table 3). Decrease in extent of oxidation due to CA results in the increase of total acidity in ZT+R with increase in molecular weight, i.e. increase in the concentration and quality of HA (Li *et al.* 2011). The humic acid took high *p*H and a longer time to reach stability, compared to the fulvic acid samples. This is due to the highly polymerized structure of humic acid, which contains phenolic -OH and -COOH groups.

FTIR spectra of clay humus: The extracted clay humus from bulk soil in M-W and P-W systems showed variation in recorded bands of different functional groups across the treatments (Fig 1). A higher number of functional groups were observed in FTIR of clay humus samples under ZT+R and ZT over CT in both cropping systems and in all the soil depths. Retention of crop residue causes increased SOC that

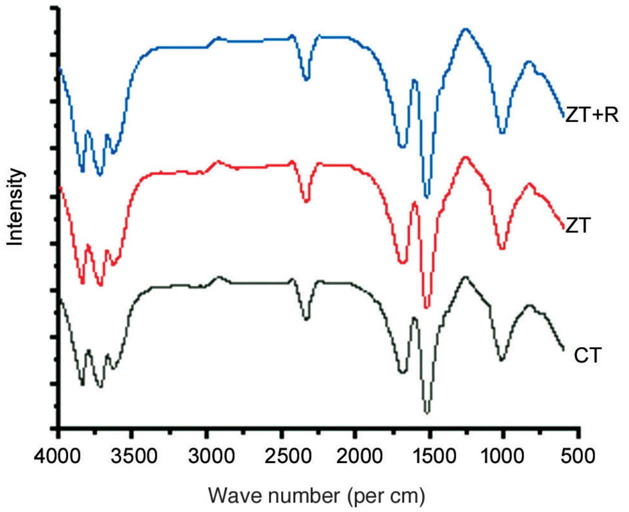
Table 3 Total acidity of humic acid extracted from different treatments

Treatment	Total acidity (meq/g)					
	Maize-Wheat			Pigeonpea-Wheat		
	Soil depth					
	0–5 cm	5–10 cm	10–20 cm	0–5 cm	5–10 cm	10–20 cm
ZT+R	8.76a	8.46a	7.48a	8.79a	8.53a	7.39a
ZT	8.17ab	8.09ab	7.36a	8.47ab	8.16ab	7.18a
CT	7.46b	7.36b	6.44b	7.61b	7.48b	6.13b

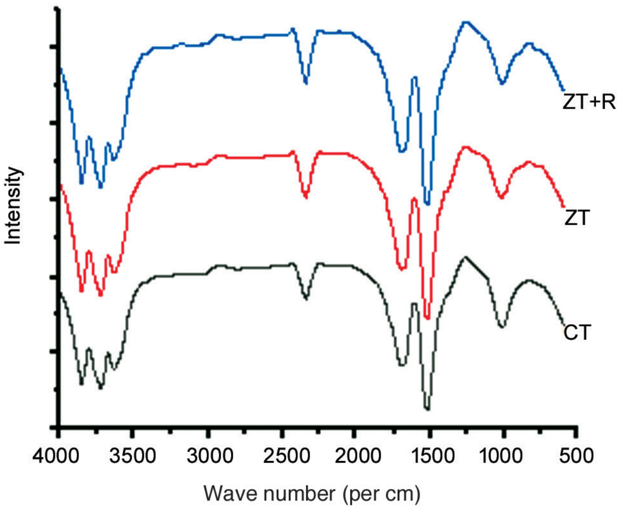
Different letters indicate statistically significant differences (P<0.05) among treatments (Duncan multiple range tests for separation of mean). Treatment details are given under Materials and Methods.



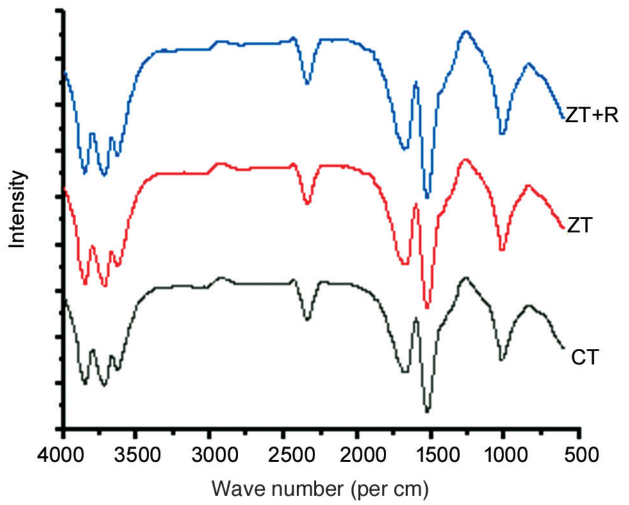
A) FTIR spectra of clay humus complexes in M-W system in 0–5 cm depth



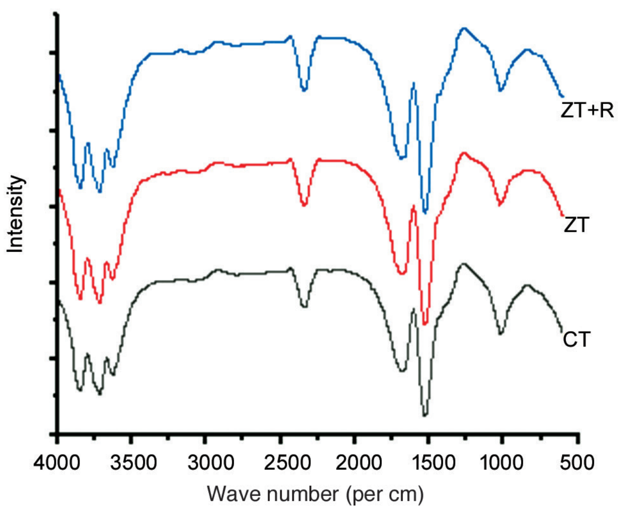
B) FTIR spectra of clay humus complexes in P-W system in 0–5 cm depth



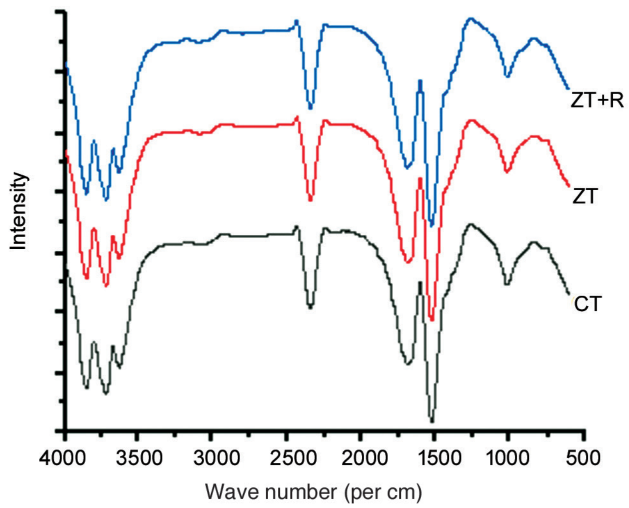
C) FTIR spectra of clay humus complexes in M-W system in 5–10 cm depth



D) FTIR spectra of clay humus complexes in P-W system in 5–10 cm depth



E) FTIR spectra of clay humus complexes in M-W system in 10–20 cm depth



F) FTIR spectra of clay humus complexes in P-W system in 10–20 cm depth

Fig 1 FTIR spectra of clay humus extracted from soil in 0–5 cm (A, B); 5–10 cm (C, D); 10–20 cm (E, F) depth.

helps in the formation of humus (Cui *et al.* 2017). Treatments showed the vibration bands in the range 3500–3200 per cm that represented O-H stretching and N-H stretching, C–H asymmetrical and symmetrical stretching were observed at 3150–3000 per cm. Aromatic C=C and C=O in amide, ketone and quinone group stretching were observed at 1650–1626 per cm. The region 1690–1640 per cm represents C=N stretching vibration; COO– or ortho-di-substituted aromatic rings at 1459–1360 per cm and the band at 1027–1030 per cm was assigned to an aliphatic amine. ZT+R and ZT treatments showed sharp absorption peaks over control at aliphatic amine stretching vibrations and COO^- stretching. The absorption peaks were proportional to the abundance of these functional groups. These peaks differed in relative intensity. Extracted clay humus in M-W and P-W cropping systems indicated the presence of the H−bonded OH groups in alcohol and phenols stretched at 3500–3200 per cm. A strong band intensity was observed between 1485–1340 per cm irrespective of the treatments in the M-W and P-W cropping system, besides the bands had more intensity in ZT+R. It also indicated that CH deformation of CH_3 increases in CA. ZT+R and ZT showed changes in functional groups that occurred due to residue retention in clay humus extracted from different treatments of CA were C-H bending, asymmetric COO- stretching and C– C stretching within the ring (1465–1440, 1450–1360 and 1450–1419 per cm). The stretching vibrations at 2922−2850 per cm indicated CH_2 and CH_3 groups might be present in both the cropping systems in all the depths except CT in M-W (0–5 cm) depth and CT in P-W (5–10 and 10–20 cm depth). Aliphatic compound was formed due to residue retention that might have helped in the reduction of oxidation and storage of more organic matter (Lorenz and Lal 2005), but at the same time humification induced aromatic compound formation (Zhang *et al.* 2020). However, no-tillage and addition of fresh residues on the soil surface exceed the metabolizing needs of microorganisms and the soil will be lower in aromatic compounds and rich in aliphatic compounds (Bayer *et al.* 2002). Baumann *et al.* (2009) reported the C:N ratio of residues, polyphenol and lignin contents, and microbes also influence the humification process. It was also observed that among two cropping systems, P-W shows more functional groups and peaks with more intensity.

We observed that total acidity and the values of E4/E6 ratio of the humic acid extracted from soil were higher in CT followed by ZT and ZT+R in both M-W and P-W cropping systems, indicating residue retention increases aromaticity and reduces total acidity of humic acid. Besides, the aromaticity of the humic acid increased with the increasing soil depth, while total acidity of humic acid decreased with depth of soil. The FTIR spectra of the clay-humus complex revealed that the functional groups under ZT+R based treatments were more complex in nature over ZT and CT based treatments. Further, the clay-humus complex under the P-W system had more functional groups than that under the M-W system. Thus, it can be concluded that conservation agriculture has the ability to capture and preserve organic carbon in the clay-humus complex leading to sequestration of organic carbon in soil.

REFERENCES

Baumann K, Marschner P, Smernik R J and Baldock J A. 2009. Residue chemistry and microbial community structure during decomposition of eucalypt, wheat and vetch residues. *Soil Biology and Biochemistry* **41**: 1966–75.

Bayer C, Martin-Neto L, Mielniczuk J, Saab S C, Bagnato V S and Milori D M P. 2002. Tillage and cropping system effects on soil humic acid characteristics as determined by electron spin resonance and fluorescence spectroscopies. *Geoderma* **105**: 81–92.

Chanda M, Jha S, Mukhopadhyay D and Pandey M. 2021. Characterization of humic acid and fulvic acid extracted from soil samples of cultivated areas of North Bengal and Sikkim states, India. *Asian Journal of Chemistry* **33**: 62–66.

Chen Y, Senesi N and Schnitzer M. 1977. Information provided on humic substances by E4/E6 ratios. *Soil Science Society of America Journal* **41**: 352–58.

Cui T, Li Z and Wang S. 2017. Effects of *in-situ* straw decomposition on composition of humus and structure of humic acid at different soil depths. *Journal of Soils and Sediments* **17**: 2391–99.

Datta A, Choudhury M, Sharma P C, Jat H S, Jat M L and Kar S. 2022. Stability of humic acid carbon under conservation agriculture practices. *Soil and Tillage Research* **216**: 105240.

Datta S C, Takkar P N and Verma U K. 2015. Assessing stability of humus in soils from continuous rice-wheat and maize-wheat cropping systems using kinetics of humus desorption. *Communications in Soil Science and Plant Analysis* **46**: 2888–900.

Dou S, Shan J, Song X, Cao R, Wu M, Li C and Guan S. 2020. Are humic substances soil microbial residues or unique synthesized compounds. *Pedosphere* **30**(2).

Galantini J A, Duval M E, Iglesias J O and Kruger H. 2014. Continuous wheat in semiarid regions: Long-term effects on stock and quality of soil organic carbon. *Soil Science* **179**(6): 284–92.

Jackson M L. 1985. *Soil Chemical Analysis: Advanced Course*, 2nd edn. University of Wisconsin, Madison, US.

Jat H S, Datta A, Choudhary M, Sharma P C, Yadav A K, Choudhary V, Gathala M K, Sharma D K, Jat M L and McDonald A. 2019a. Climate smart agriculture practices improve soil organic carbon pools, biological properties and crop productivity in cereal-based systems of North-West India. *Catena* 181: 104059.

Katyal J C. 2000. Organic matter maintenance: Mainstay of soil quality. *Journal of the Indian Society of Soil Science* **48**(4): 704–16.

Kobierski M, Kondratowicz-Maciejewska K, Banach-Szott M, Wojewodzki P and Castejon J M P. 2018. Humic substances and aggregate stability in rhizospheric and non-rhizospheric soil. *Journal of Soils and Sediments* **18**: 2777–89.

Kubar K A, Huang L, Lu J, Li X, Xue B and Yin Z. 2019. Long-term tillage and straw returning effects on organic C fractions and chemical composition of SOC in rice-rape cropping system. *Archives of Agronomy and Soil Science* **65**(1): 125–37.

Li X, Xing M, Yang J and Huang Z. 2011. Compositional and functional features of humic acid-like fractions from vermicomposting of sewage sludge and cow dung. *Journal of Hazardous Materials* **185**(23): 740–48.

Lorenz K and Ratan L. 2005. The depth distribution of soil organic carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons. *Advances in Agronomy* **88**: 35–66.

Naorem A, Jayaraman S, Sinha N K, Mohanty M, Chaudhary R S, Hati K M and Lal R. 2023. Eight-year impacts of conservation agriculture on soil quality, carbon storage and carbon emission footprint. *Soil and Tillage Research* **232**: 105748.

Ndzelu B S, Dou S, Zhang X, Zhang Y, Ma R and Liu X. 2021. Tillage effects on humus composition and humic acid structural characteristics in soil aggregate-size fractions. *Soil and Tillage Research* **213**: 105090.

Purakayastha T J, Das R, Kumari S, Shivay Y S, Biswas S, Kumar D and Chakrabarti B. 2020. Impact of continuous organic manuring on mechanisms and processes of the stabilization of soil organic C under rice–wheat cropping system. *Soil Research* **58**: 73–83.

Reddy S, Nagaraja M S, Raj T P, Patil A P and Dumgond P. 2014. Elemental analysis, E4/E6 ratio and total acidity of soil humic and fulvic acids from different land use systems. *Annals of Plant and Soil Research* **16**(2): 89–92.

Schnitzer M and Gupta U C. 1965. Determination of acidity in soil organic matter. *Soil Science Society of America Proceedings* **29**: 274–77.

Stevenson F J. 1976. Stability constants of Cu^{2+}, Pb^{2+} and Cd^{2+} complexes with humic acids. *Soil Science Society of America Journal* **40**(5): 665–72.

Yadav R K, Purakayastha T J, Das R and Khan A. 2023. Impact of long-term manuring and cropping system on stability of humus associated with clay-organic complex. *Archives of Agronomy and Soil Science* **69(**12): 2356–69.

Zalba P, Amiotti N M, Galantini J A and Pistola S. 2016. Soil humic and fulvic acids from different land-use systems evaluated by E4/E6 ratios. *Communications in Soil Science and Plant Analysis* **47**(13–14): 1675–79.

Zbytniewski R and Buszewski B. 2005. Characterization of natural organic matter (NOM) derived from sewage sludge compost. Part 1: Chemical and spectroscopic properties. *Bioresource Technology* **96**(4): 471–78.

Zhang X, Dou S, Ndzelu B S, Guan X W, Zhang B Y and Bai Y. 2020. Effects of different corn straw amendments on humus composition and structural characteristics of humic acid in black soil. *Communications in Soil Science and Plant Analysis* **51**(1): 107–17.