Carbon, nitrogen, and phosphorus stoichiometry mediate sensitivity of carbon stabilization mechanisms along with surface layers of a Mollisol after long-term fertilization in Northeast China

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Abstract

Purpose Soil organic carbon (SOC) is an important parameter determining soil fertility and sustaining soil health. How C, N, and P contents and their stoichiometric ratios (C/N/P) regulate the nutrient availability, and SOC stabilization mechanisms have not been comprehensively explored, especially in response to long-term fertilization. The present study aimed to determine how the long-term mineral and manure fertilization influenced soil C/N/P ratios and various protection mechanisms underlying the stabilization of OC along with profile in a cropland soil.

Materials and methods The soil was sampled from five depths, viz., 0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm, from plots comprising wheat-maize-soybean rotation system subjected to the long-term (35 years) manure and mineral fertilizer applications.

Results and discussion Results revealed that the soil C, N, P stoichiometry and their contents in topsoil depths (0–20 and 20–40 cm) and subsoil depths (40–60, 60–80, and 80–100 cm) varied significantly ($p < 0.01$) among the soil layers. Compared with CK, the C, N, and P contents were significantly higher ($p < 0.05$) in NPKM in the topsoil layers, while M alone increased these contents throughout the subsoil. Overall, the C, N, and P contents and their stoichiometry decreased with the increase in depth. Regression analysis showed that C/N, C/P, and N/P ratios associated significantly with the OC fractions in the topsoil layers only. These negative correlations indicated that these ratios significantly influence the C stabilization in the surface layers. However, the results warrant further investigations to study the relationship between soil and microbial stoichiometry and SOC at various depths.

Conclusions Long-term manure applications improved the C sequestration not only in the topsoil but also in the deep layers; hence, these facts can be considered relevant for fertilizer recommendations in cropping systems across China.

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1 Introduction

A number of sustainable development goals (SDGs) such as Goal 2 (zero hunger), 6 (clean water and sanitation), 13 (climate action), 14 (life below water), and 15 (life on land) are interrelated and need immediate and integrated actions. Increased population, urbanization, land-use changes, and deforestation have led to intensive agriculture with an increased rate of nitrogenous fertilizers and are major challenges to these SDGs. Sustainable increase in food production is often linked to sustainable soil management that is necessary for collective climate actions, as soils are a major source of C sequestration.

Soil organic carbon (SOC) is an indicator of soil health and quality as it regulates soil fertility. Any management practice/s or factor/s to increase SOC will sustain its productivity and sequester more carbon. In addition to carbon, nitrogen (N) and phosphorus (P) contents in the soil and their ratios (C/N/P) are also vital indicators of soil fertility status (Tian et al. 2010). The stoichiometric ratios of (C/N/P) are broadly influenced by biogeochemical cycling of nutrients not only at soil level (Xia et al. 2014) but also at the whole ecosystem level (Yuan and Chen 2009; Xu et al. 2013). Furthermore, the variations in C, N, and P are closely interrelated, and their stoichiometric ratios are often governed by climatic conditions, soil moisture, temperature, soil-forming processes, vegetation, soil microbes, and anthropogenic activities (Lambers et al. 2010).

The provision of nutrients, specifically N, P, and K, from mineral or manure to the soil influences the availability of other nutrients and input of one vital nutrient alters biogeochemical soil cycling (Blair et al. 2006; Gong et al. 2009). Unlike other nutrients, N-based fertilization in agriculture has caused a large-scale increment in available N inputs into global ecosystems over the last few decades (Sutton et al. 2013) (Sutton et al. 2013). It has altered the C/N/P stoichiometry and intensifies the limitation of P relative to N that ultimately disturbs the ecological stoichiometric flexibility of nutrients those related to C, N, and P and their cycles (Vitousek et al. 2010; Crowley et al. 2012; Peñuelas et al. 2013). Fertilization augments the plant productivity, influences elemental concentrations of plants and soil, and actively manipulates soil nutrient consortium and affects soil microbes (Ludwig et al. 2007; Shahid et al. 2013; Ashraf et al. 2020).

The alteration of soil elemental stoichiometry may simulate a change in nutrient balance/limitation, thus giving an insight to understand the nutrient off-limit phenomenon in soil systems (Cleveland and Liptzin 2007). Ecological stoichiometry is broadly applicable in disciplines of soil chemistry, ecology, and biochemistry in the contemporary era, which has provided new perspectives on the soil-plant relationship and nutrient cycles

mainly of C, N, and P (Elser et al. 2000; Güsewell 2004; Sardans and Peñuelas 2012; Zhang et al. 2013; Ye et al. 2018). It is, therefore, indispensable to understand soil C/N/P stoichiometry with varying climate, soil types, and conditions of management.

Chemical fertilization alters the soil nutrient status, which indirectly affects soil carbon balance, whereas organic fertilization is believed to replenish soil carbon balance by direct addition of organic carbon into the soil (Zhang et al. 2019). Soil organic carbon (SOC) has a strong influence on soil health, mitigation of climate change, and crop productivity; however, its concentration can be improved after long-term management practices. The mechanisms of soil organic carbon protection must, therefore, be determined for a better understanding of the processes involved in its storage and to ensure sustainability. Fertilization is often considered the most reliable management practice that improves soil quality and enhances SOC content in intensively managed agricultural settings (Majumder et al. 2007; Shang et al. 2014). The bulk SOC can be categorized into four fractions based on the degree of protection mechanisms such as non-protection, and physical, chemical, and biochemical protection (Six et al. 2002a). These SOC fractions are characterized by the varying rate of decomposability and turnover and hence provide a clear image of the consequences of management practices that would otherwise not be achieved by studying total SOC in bulk soil (He et al. 2015). Vitally, evidence suggests that individual SOC fractions are more robust indices of the influence caused by management practices, i.e., fertilization and land-use change relative to the total SOC (Six et al. 2002b; Fazhu et al. 2015). Recently, we reported that long-term addition of manure and mineral fertilizers improved SOC contents and their various fractions and stabilization mechanisms (Abrar et al. 2020) in a Mollisol.

Black soils (Mollisols) in China are known for their higher crop productivity (Qing et al. 2011); its total area is about 6 million, out of which about 4.39 million ha area is under agriculture (Liu et al. 2003) (Liu et al. 2003). Moreover, this region is famous for the major production of crops, especially maize and soybean in China (Liu et al. 2003; Xing et al. 2004).

Many studies have explored the effects of management practices (e.g., fertilization) on SOC dynamics in topsoil, and only a few have pinpointed the deep soil layers (Ogle et al. 2005; Gregory et al. 2014). Nutrient stoichiometric ratios have a definite role in SOC stabilization and decomposition and have been explored in topsoils (Falkowski et al. 2000; Mooshammer et al. 2012) and their contribution in the subsoil has been remained relatively neglected. Furthermore, these findings have only assessed variations in SOC in relation to soil stoichiometry and have been inadequately described the

phenomenon responsive for the SOC protection in soils. Moreover, how soil C/N/P stoichiometric ratios respond to long-term mineral and mineral plus manure fertilizer application in a Chinese cultivated Mollisol needs to be explored. Additionally, the relationship between soil nutrients stoichiometry and variously protected SOC fractions, viz., unprotected, physically, chemically, and biochemically protected, has not been investigated so far, which in the present study constitutes the further novelty of this work. It is, therefore, significant to clarify the stoichiometric elemental ratios of soil and their interactions with SOC fractions in the whole soil profile to understand better the processes involved in C and N cycling under current long-term management practices. We hypothesized that long-term fertilization influences the relationship of elemental stoichiometry and contents and their association with the protection mechanisms of organic carbon (OC) throughout the soil profile. The objectives of this study were to (a) analyze the C, N, and P contents of bulk soil; (b) assess the comparison of the soil C/N, C/P, and N/P stoichiometry in top- vs subsoil subjected to the long-term mineral and organic fertilizers additions; and (c) identify the influences of long-term mineral and organic fertilization on the relationship between C, N, P stoichiometry and OC protection mechanisms throughout the soil profile.

2 Materials and methods

2.1 Study site description

A long-term field experiment for the evaluation of fertility and fertilizer efficiency was established (1979) in Hailun county (126° 51′ E, 45° 50′ N) of Harbin, Heilongjiang province, at an altitude of 151 m. This area belongs to the second terrace of Songhua River; the terrain is flat, and the parent material is diluvial loess clay. More information about the climate variables, climate normals, and physico-chemical properties of soil in this area is given by Abrar et al. (2020).

2.2 Experimental design and soil sampling

The area of the experimental field was 168 m^2 (30 × 5.6 m) and arranged in a complete randomized design. No irrigation was applied, and the crops were solely dependent on the rainfall. The crops such as wheat, maize, and soybean were used as crop rotation. The plowing depth was 20 cm every year. The treatments selected for the experiment and relative rates of different mineral and manure fertilizer application are given in Supplementary materials (Table S3).

Each treatment plot was replicated three times and the soil samples were randomly collected from every plot at five depths (with 20 cm increment) along with whole profile with the help of an auger (5 cm diameter) at the beginning of October 2014. Crop stubble was picked from the field carefully. From each experimental plot, 12 soil samples were randomly picked, and the soil from four random samples was mixed to make one sample; resultantly, fifteen samples were prepared for five treatments in each soil layer. The clods were mildly disrupted manually till the soil could be sieved through a 2-mm sieve. The subsamples were kept on the brown paper for drying at room temperature. Then these samples were put in plastic bags and stored for further analyses.

2.3 Soil chemical properties analyses

The initial soil properties at the start of the experiment (1979) are reported by Abrar et al. (2020). The soil samples were analyzed for total organic carbon (TOC) and total nitrogen (TN) with a CHNS analyzer–Eurovector Elemental analyzer (Milan, Italy). In contrast, the TP content of bulk soil samples was analyzed by digesting samples with H_2SO_4-$HClO_4$ (Nelson and Sommers 1982).

2.4 Fractionation procedure

The bulk soil carbon was fractionated into different constituent fractions based on the protection mechanisms by utilizing a method (with slight modifications) given by Six et al. (2002c), comprised of physical-chemical and density fractionation procedures. It includes three steps: the 1st step encompassed physical fractionation of 20 g soil sample after partial dispersion based on size. The 1st step yielded four fractions, namely, cPOC (> 250 μm), microaggregate (> 53 μm), dSilt, and dClay (53–250 μm). The second step was the further isolation of fPOC, iPOC, μSilt, and μClay. The last step was the acid hydrolysis of the easily dispersible silt (dSilt) and clay (dClay) and microaggregate-derived silt (μSilt) and clay (μClay) fractions recovered from the 1st and 2nd steps, respectively (Plante et al. 2006). More details about the fractionation scheme and their contents along the soil profile have already been reported in our previous study (Abrar et al. 2020) and also been given as supplementary (Table S1).

2.5 Statistical analysis

Microsoft Excel (for Windows 10) was employed to process the preliminary data. Each element (C, N, and P) percentages were used to determine bulk contents based on dry soil weight. Soil stoichiometric ratios such as C/N, C/P, and N/P were expressed as mass ratios. Pearson correlation coefficients (R) were used to find correlations among different parameters. All determinants were triplicated, and values were expressed as means on oven-dried soil mass basis (105 °C). Statistical significance of bulk soil sample distributions along different soil profile depths, C/N, C/P, and N/P ratios, and organic C, total N, and total P contents of the soil under different fertilization strategies were analyzed using one-way analysis of variance (ANOVA) using SPSS 23.0

statistical software package (SPSS Inc., Chicago, IL, USA), followed by post hoc multiple comparisons using Duncan's multiple comparisons test ($p \leq 0.05$). Graphical presentations were produced by Sigmaplot v. 14.0 (Systat Software Inc. USA), and the data presented as means ± standard errors.

3 Results

3.1 Soil organic carbon, total nitrogen, and total phosphorus

Balanced fertilization in combination with organic manure (NPKM) considerably improved soil organic carbon (OC) contents of bulk soil in the topsoil (0–40 cm) in comparison to control (CK) ($p < 0.05$; Table 1). Total stabilized SOC in the 0–20 cm was improved by 22% in NPKM treatment, whereas no increase was noticed in SOC throughout the subsoil. However, manure application (M) improved (> 130%) SOC in each layer of subsoil (40–100 cm) compared with CK.

Similar to total SOC, NPKM increased total N (TN) significantly ($p < 0.05$) in 0–20 and 20–40 cm layers by 34% and 42%, respectively, compared with the CK (Table 1). In all subsoil layers, manure application (M) notably ($p < 0.05$) increased TN content compared with other fertilizer treatments and control. Similarly, balanced fertilization with manure (NPKM) compared with CK increased the total phosphorus (TP) content by 122%, 145% in both 0–20 and 20–40 cm, respectively, and 158% in the 40–60 cm. At the same time, no significant difference was observed among all the fertilized treatments in the 60–100 cm soil layers.

3.2 Distribution of soil C, N, P stoichiometry in various profile layers

3.2.1 C/N ratio

Generally, the C/N ratio of the bulk soil samples was increased with increasing depth in all treatments. The lowest C/N ratio among all treatments was observed in M along with profile; the

Table 1 C, N, P contents and C/N/P stoichiometry of soil under different fertilization along the soil profile

	Depth (cm)	Treatments	C, N, and P contents			Soil elemental stoichiometry			
			C	N	P	C/N	C/P	N/P	C/N/P
Topsoil layers	0–20	CK	16.19d	0.74c	0.45c	21.8a	36.9a	1.70a	37:2:1
		PK	17.05c	0.77c	0.82b	21.9a	20.5b	0.94b	21:1:1
		NPK	17.85b	0.91b	0.83b	19.6c	21.5b	1.09b	22:1:1
		M	17.84b	0.88b	0.79b	20.4ab	22.6b	1.11b	23:1:1
		NPKM	18.85a	1.00a	0.99a	18.9c	19.0b	1.01b	19:1:1
	20–40	CK	14.23d	0.60c	0.37c	23.6a	36.4a	1.65a	36:2:1
		PK	15.77c	0.67b	0.72b	23.4a	21.9b	0.93c	22:1:1
		NPK	15.87ab	0.74bc	0.73b	21.4b	21.6b	1.03bc	22:1:1
		M	15.56bc	0.79bc	0.69b	19.7b	21.6b	1.15b	22:1:1
		NPKM	16.59a	0.86a	0.90a	19.4b	18.3b	0.95bc	18:1:1
Subsoil layers	40–60	CK	9.43c	0.38e	0.30c	25.2a	32.0a	1.27a	32:1:1
		PK	11.75b	0.59b	0.63b	19.9b	18.7bc	0.95b	19:1:1
		NPK	11.77b	0.47c	0.64b	24.9a	18.4bc	0.74bc	18:1:1
		M	14.68a	0.76a	0.63b	19.3b	23.3b	1.21a	23:1:1
		NPKM	11.79b	0.54f	0.77a	23.9a	16.67c	0.70c	17:1:1
	60–80	CK	7.31c	0.34c	0.26b	21.7b	29.6a	1.40a	30:1:1
		PK	8.31b	0.37b	0.56a	22.7b	15.0c	0.66bc	15:1:1
		NPK	7.26c	0.27c	0.57a	26.9b	12.1c	0.48c	12:1:1
		M	12.71a	0.60a	0.58a	21.1bc	21.2b	1.03b	21:0:0
		NPKM	8.33b	0.26c	0.63a	32.8a	12.4c	0.41b	12:0:0
	80–100	CK	7.15b	0.30b	0.23b	24.7b	33.0a	1.32a	33:1:1
		PK	7.34b	0.29b	0.50a	25.5ab	15.0b	0.60c	15:1:1
		NPK	7.06b	0.30b	0.52a	26.0ab	14.5b	0.55c	15:1:1
		M	9.31a	0.51a	0.55a	18.6b	17.1b	0.94b	17:1:1
		NPKM	7.47b	0.22b	0.59a	33.5a	12.9b	0.37c	13:0:0

Different letters indicate significant differences in the mean ± SE of all the nutrient contents and stoichiometric ratios at $p = 0.05$

mean values of the C/N ratio were 20.4, 19.7, 19.3, 20.6, and 18.6 in 0–20, 20–40, 40–60, 60–80, and 80–100 cm layers, respectively. Application of fertilizer N (NPK and NPKM) significantly reduced the C/N ratio in both 0–20 and 20–40 cm layers compared with CK and PK receiving no N. The lower layer 40–60 cm did not show any difference for C/N among various treatments receiving mineral fertilizers and CK, except the treatment receiving manure (M) (Table 1). Interestingly in both lower layers, i.e., 60–80 and 80–100 cm, the trend was opposite as C/N ratio was higher in NPKM and NPK than CK, where it ranged from 26 to 33.5. In both 0–20 and 20–40 cm layers, C/N ratio in CK and PK did not change significantly ($p > 0.05$). Similarly, among CK, NPK, and NPKM, no meaningful difference was observed in the 40–60 cm layer.

3.2.2 C/P ratio

The carbon to phosphorus (C/P) ratio throughout the soil profile was significantly higher in control (CK) receiving no fertilizer and/or manure, compared with all of the other treatments (Table 1). In all other treatments throughout the soil profile, the C/P ratio did not differ significantly among various fertilization treatments.

In all subsoil layers (40–100 cm), C/P ratio in NPKM and CK did not differ significantly with each other. C/P ratio in CK was significantly different ($p < 0.05$) among all the treatments throughout the soil layers. The highest C/P ratio was observed in CK, while the minimum C/P ratio was found in NPKM. In 0–20 and 20–40 cm, the average C/P ratio in CK was 36.9 and 36.4, respectively, while the average values of the C/P ratio for CK were 32, 29, and 33 in respective subsoil layers.

3.2.3 N/P ratio

Likewise, C/P ratio and N/P ratio are also significantly different in CK among all treatments as a result of long-term fertilization in the 0–20 and 20–40, and 40–60, 60–80, and 80–100 cm layers which were ranged from 1.33 to 1.97, 1.42 to 1.50, 1.06 to 1.46, 0.56 to 1.07 and 1.79 to 2.22, respectively. N/P ratio was considerably higher in CK among all the treatments throughout the soil layers except 40–60 cm (Table 1). N/P ratio followed the same pattern as the C/P ratio along with the soil profile. The N/P ratio in CK differed significantly from other treatments throughout the soil profile. In the 0–20 cm layer, the highest N/P ratio (1.70) was noted in CK, whereas the lowest N/P ratio (0.94) was found in PK. In all subsoil layers, minimum average N/P ratios 0.38, 0.38, and 0.29 were observed in the 40–60, 60–80, and 80–100 cm in NPKM.

3.3 Relationships between the SOC, TN, and TP contents along with the profile

As shown in Table 2, the contents of C, N, and P in the bulk soil had a strong positive association among each other in the topsoil and subsoil layers ($p < 0.01$). Moreover, C and N contents had a strong positive linear relationship, and the variations in SOC explained 92% of changes in the TN along with the whole profile. The relationship between SOC and TP and between TN and TP were also significant and positively correlated with each other, whereas the variability in TP explained by SOC and TN was 44% and 42%, respectively (Fig. 1 b, c; $p < 0.01$).

3.4 Association between C/N/P stoichiometry and C, N, P contents

In most cases, negative correlations were found between C/N, C/P, and N/P ratios and their contents along with the profile layers.

3.4.1 Correlation between C/N ratio and TOC, TN, and TP

Highly significant negative correlations ($R = 0.816$) were found between C/N and TOC contents in both topsoil layers. Conversely, no meaningful relationship was observed in the lower layers. The relationship between C/N and TN was negatively correlated in all soil profile layers ($p < 0.0001$; Table 2). The association between C/N ratio and TP was only meaningful ($p < 0.05$) in both the topsoil layers, while no other statistically significant correlations were found throughout the subsoil ($p > 0.05$).

3.4.2 Correlation between C/P ratio and TOC, TN, and TP

In both topsoil layers, a strong negative relationship was observed between the C/P ratio and TOC in 0–20 ($p < 0.05$) and 20–40 cm ($p < 0.01$) soil layers, while no significant relationship ($p > 0.05$) was found in all subsoil layers (Table 2). A similar pattern of results was noticed between the C/P ratio and TN in the top- as well as subsoil layers.

A significant negative association was recorded between C/P ratio and TP throughout the soil profile ($p < 0.0001$), with the highest ($R^2 = 0.938$) found in the 20–40 cm layer. The correlation coefficient was gradually decreased, with an increase in depth, and $R^2 = 0.699$ was noted in the 60–80 cm layer (Table 2).

3.4.3 Correlation between N/P ratio and TOC, TN, and TP

In both topsoil layers, the N/P ratio was negatively correlated with total SOC content in 0–20 cm and 20–40 cm layers, ($R = 0.579$ and 0.692, respectively).

Table 2 Relationship between soil C, N, P stoichiometry and total C, N, and P contents along various profile layers

	Depth (cm)	Regression	Model type	Slope	Coefficient of correlation (R)	Coefficient of regression (R^2)	Significance
Topsoil layers	0–20	C/N vs SOC	Linear	− 0.480	0.816	0.666	**$p < 0.001$*****
		C/N vs TN	Linear	− 0.058	0.948	0.898	**$p < 0.0001$******
		C/N vs TP	Linear	− 0.073	0.602	0.362	**$p < 0.05$***
		C/P vs SOC	Linear	− 0.092	0.706	0.499	**$p < 0.001$****
		C/P vs TN	Linear	− 0.008	0.584	0.341	**$p < 0.05$***
		C/P vs TP	Linear	− 0.026	0.951	0.905	**$p < 0.0001$******
		N/P vs SOC	Linear	− 1.743	0.579	0.336	**$p < 0.05$***
		N/P vs TN	Linear	− 0.133	0.420	0.177	$p > 0.05^{NS}$
		N/P vs TP	Linear	− 0.555	0.896	0.803	**$p < 0.0001$******
	20–40	C/N vs SOC	Linear	− 0.265	0.562	0.316	**$p < 0.05$***
		C/N vs TN	Linear	− 0.046	0.944	0.891	**$p < 0.0001$******
		C/N vs TP	Linear	− 0.062	0.655	0.429	**$p < 0.01$****
		C/P vs SOC	Linear	− 0.089	0.742	0.551	**$p < 0.01$****
		C/P vs TN	Linear	− 0.009	0.724	0.524	**$p < 0.01$****
		C/P vs TP	Linear	− 0.023	0.969	0.938	**$p < 0.0001$******
		N/P vs SOC	Linear	− 2.217	0.692	0.478	**$p < 0.01$****
		N/P vs TN	Linear	− 0.181	0.538	0.289	**$p < 0.05$***
		N/P vs TP	Linear	− 0.594	0.929	0.863	**$p < 0.0001$*****
Subsoil layers	40–60	C/N vs SOC	Linear	− 0.323	0.497	0.247	$p > 0.05^{NS}$
		C/N vs TN	Linear	− 0.039	0.844	0.712	**$p < 0.0001$******
		C/N vs TP	Linear	− 0.015	0.265	0.070	$p > 0.05^{NS}$
		C/P vs SOC	Linear	− 0.130	0.419	0.176	$p > 0.05^{NS}$
		C/P vs TN	Linear	− 0.007	0.305	0.093	$p > 0.05^{NS}$
		C/P vs TP	Linear	− 0.025	0.919	0.846	**$p < 0.0001$******
		N/P vs SOC	Linear	− 0.967	0.134	0.018	$p > 0.05^{NS}$
		N/P vs TN	Linear	0.0747	0.146	0.021	$p > 0.05^{NS}$
		N/P vs TP	Linear	− 0.479	0.756	0.571	**$p < 0.001$****
	60–80	C/N vs SOC	Linear	− 0.129	0.298	0.088	$p > 0.05^{NS}$
		C/N vs TN	Linear	− 0.018	0.677	0.458	**$p < 0.01$****
		C/N vs TP	Linear	0.0122	0.398	0.159	$p > 0.05^{NS}$
		C/P vs SOC	Linear	0.0410	0.138	0.019	$p > 0.05^{NS}$
		C/P vs TN	Linear	0.0072	0.394	0.155	$p > 0.05^{NS}$
		C/P vs TP	Linear	− 0.018	0.836	0.699	**$p < 0.0001$******
		N/P vs SOC	Linear	0.783	0.157	0.025	$p > 0.05^{NS}$
		N/P vs TN	Linear	0.143	0.469	0.220	$p > 0.05^{NS}$
		N/P vs TP	Linear	− 0.279	0.789	0.623	**$p < 0.001$*****
	80–100	C/N vs SOC	Linear	− 0.069	0.483	0.234	$p > 0.05^{NS}$
		C/N vs TN	Linear	− 0.016	0.888	0.789	**$p < 0.0001$******
		C/N vs TP	Linear	0.007	0.287	0.083	$p > 0.05^{NS}$
		C/P vs SOC	Linear	− 0.008	0.074	0.006	$p > 0.05^{NS}$
		C/P vs TN	Linear	0.0005	0.037	0.001	$p > 0.05^{NS}$
		C/P vs TP	Linear	− 0.016	0.911	0.832	**$p < 0.0001$******
		N/P vs SOC	Linear	0.4179	0.175	0.031	$p > 0.05^{NS}$
		N/P vs TN	Linear	0.1381	0.459	0.211	$p > 0.05^{NS}$
		N/P vs TP	Linear	− 0.325	0.843	0.711	**$p < 0.0001$******

Results (in bold) are significant at 0.05, 0.01, 0.001, and 0.0001 denoted by *, **, ***, and ****, respectively

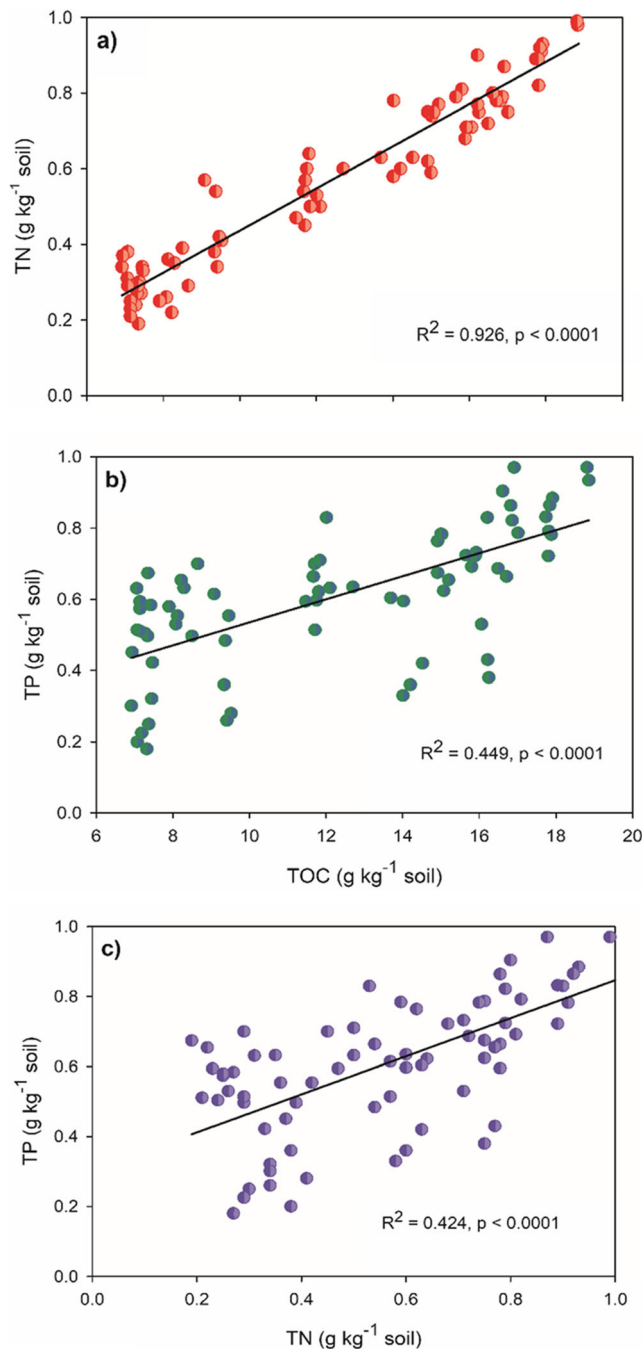Non-significant results ($p > 0.05$) are denoted by letters NS

Fig. 1 Relationship between total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) in the soil after long-term fertilization with manure and mineral fertilizers ($N = 75$)

However, no significant correlation was found between the N/P ratio and SOC content in all subsoil layers ($p > 0.05$; Table 2). No significant association between the N/P ratio and TN in all layers of profile except 20–40 cm layer where a strong negative correlation was observed ($p < 0.05$). N/P ratio and TP were strongly associated with each other throughout the soil profile.

3.5 SOC fractions throughout the soil profile and their association with C/N/P stoichiometry

Long-term fertilization with manure and mineral fertilizers significantly improved the SOC throughout the soil profile and its stabilization mechanisms. The results regarding various unprotected, physically, chemically, physico-chemically, physico-biochemically protected fractions of SOC along the soil profile have been provided and discussed in our preliminary publication (Abrar et al. 2020). The data is also reproduced as a supplementary Table S1. The correlation among these fractions and the CNP stoichiometry is produced as follows.

3.5.1 Association of C/N/P stoichiometry with unprotected and physically protected SOC fractions

A significant negative correlation ($p < 0.001$) of the C/N ratio with cPOC was observed in 0–60 cm layers. However, no other statistically significant associations were found in the rest of the subsoil layers (Fig. 2a). Similarly, no meaningful relationship was recorded between C/N ratio and fPOC in both the topsoil and the last subsoil (80–100 cm) layer; however, a positive relationship with a smaller tendency was noted in the 40–60 and 60–80 cm layers (Fig. 2b).

In the 0–40 cm, a significant negative relationship was recorded between C/N ratio and μagg fraction, whereas no association was noted in all the subsoil layers. C/N ratio significantly negatively correlated with iPOC in all layers from 0 to 60 cm, with the tendency decreased with depth increment (Fig. 2c, d).

A strong negative correlation was noted between the C/P ratio and cPOC in both topsoil layers, i.e., 0–40 cm. At the same time, no clear link was found between the C/P ratio and cPOC in the rest of the layers. Similar to the C/N ratio, no significant correlation ($p > 0.05$; Fig. 2b) was observed between the C/P ratio and fPOC throughout the profile. A meaningful negative relationship of the C/P ratio with μagg was found only in both topsoil layers. A similar relationship of the C/P ratio was noted with iPOC. However, no significant association was observed in all the subsoil layers (Fig. 4c, d).

Regression analysis revealed that N/P ratio correlated negatively with the non-protected cPOC in both layers of the topsoil. However, no significant ($p > 0.05$) relationship was noted in all the subsoil layers. Likewise, no significant association was recorded between the N/P ratio and fPOC throughout the profile. A significant negative correlation was found between the N/P ratio and physically protected μagg in the 2nd topsoil layer. However, N/P ratio negatively associated with iPOC in both topsoil layers (Fig. 6c).
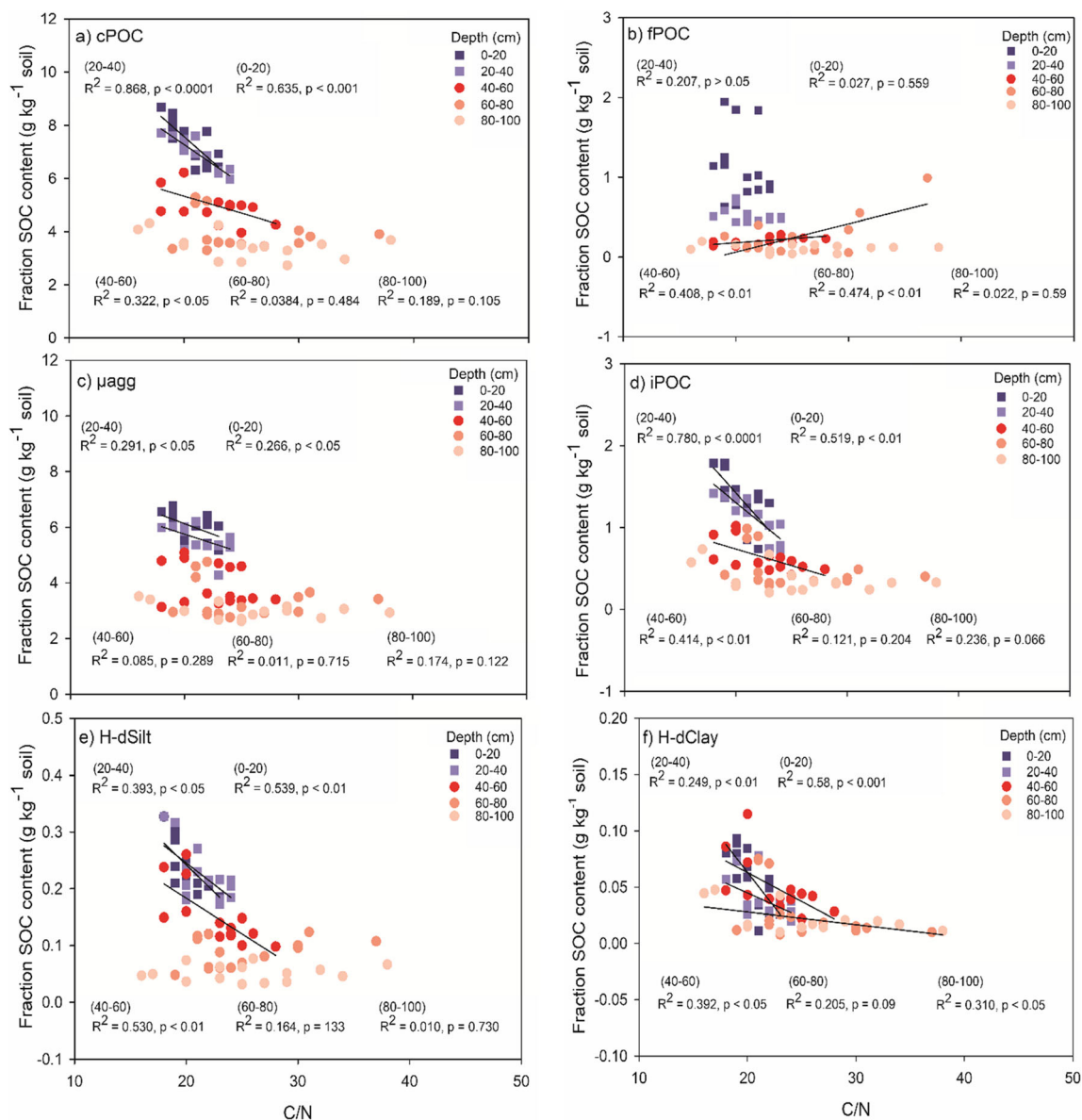
Fig. 2 Relationship between C/N ratio and various OC content of fractions along with soil profile layers after long-term fertilization with manure and mineral fertilizers ($N = 75$ and $n = 15$)

3.5.2 Association of C/N/P stoichiometry with chemically and biochemically protected SOC fractions

Similar to physically protected iPOC, a significant negative relationship was found between C/N ratio and hydrolyzable silt (H-dSilt) in 0–40 cm and with non-hydrolyzable silt (NH-dSilt) in 0–20 and 40–60 cm layers; however, the tendency was decreased gradually with the increase in depth (Figs. 2e, 3a). A strong negative correlation between the C/N ratio and H-dClay was observed throughout the soil profile except for the subsoil layer 60–80 cm. (Fig. 2f).

A significant association (but with smaller tendency, i.e., y = − 0.0034) was found between the C/P ratio and H-dSilt in the 20–40 cm layer, and almost similar kind of

relationship was noted in the 80–100 cm layer, while a negative correlation between the C/P ratio and H-dClay was found in both topsoil layers. A moderate negative correlation was recorded in the 80–100 cm of subsoil (Fig. 4e, f). In both topsoil layers, a strong negative relationship was observed between the C/P ratio and NH-dSilt and NH-dClay (Fig. 5a, b). Interestingly, only in the 80–100 cm, a weak negative correlation ($p < 0.05$; Fig. 6e, f) was found between chemically protected H-dSilt and N/P ratio and the first two layers of topsoil between H-dClay and N/P ratio. In contrast, no significant relationship was noted in all the layers up to 0–80 cm. In both topsoil layers (up to 0–40 cm), a significant negative association was noticed between the N/P ratio and NH-dSilt.
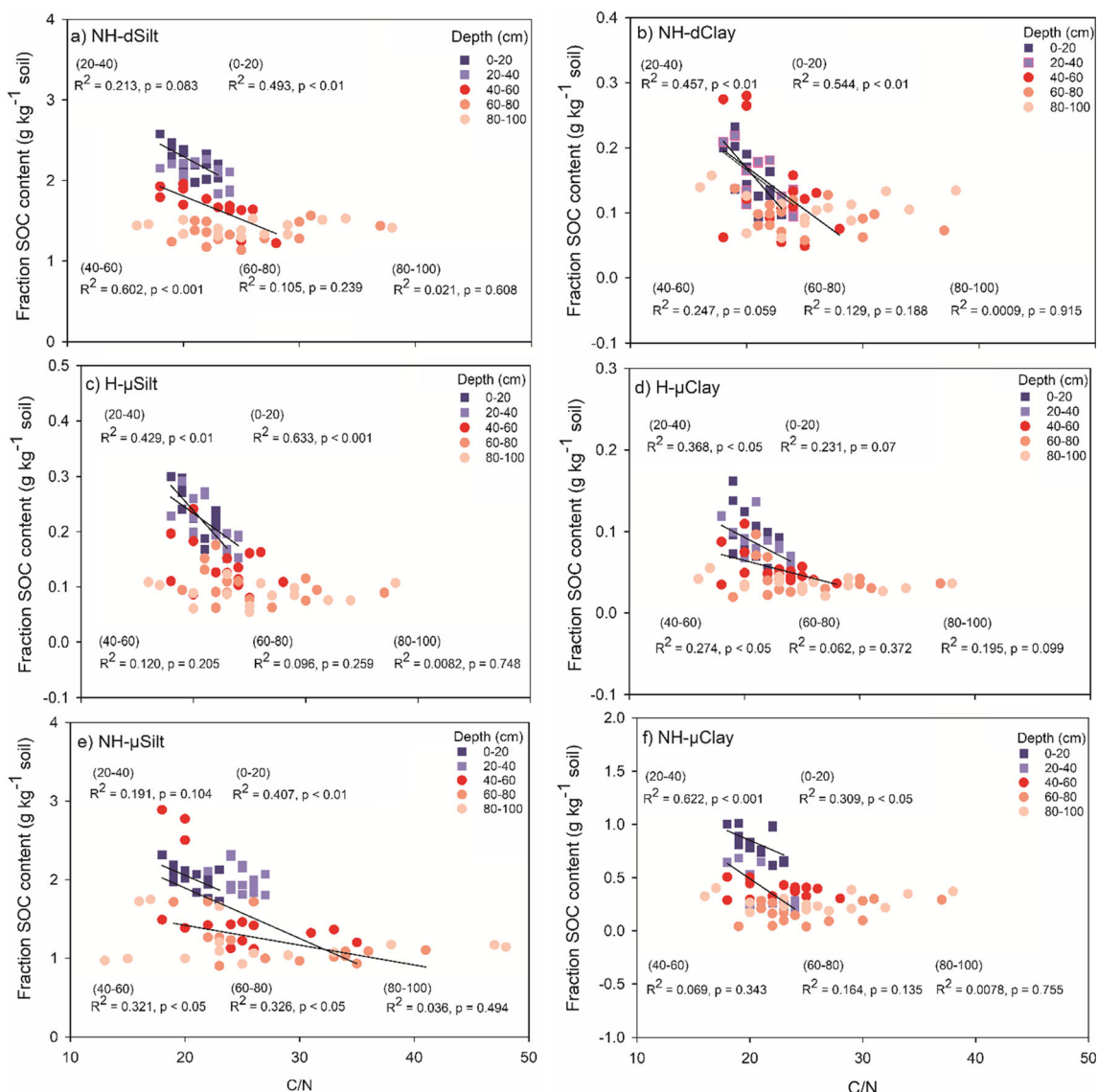
Fig. 3 Relationship between C/N ratio and various OC content of fractions along with soil profile layers after long-term fertilization with manure and mineral fertilizers ($N = 75$ and $n = 15$)

3.5.3 Association of C/N/P stoichiometry with physico-chemically and physico-biochemically protected SOC fractions

Chemically protected H-μSilt was negatively correlated with the C/N ratio but only in both topsoil layers, and the greater tendency was noted in the 0–20 cm layer compared with the 20–40 cm layer. Similarly, the C/N ratio and H-μClay correlated significantly with each other in 20–40 and 40–60 cm layers (Fig. 3c, d). A strong negative relationship between the C/N ratio and NH-μSilt was observed in the 1st layer of the topsoil and 1st two layers of subsoil. At the same time, no significant correlation was noticed in other layers. In contrast, C/N ratio correlated strongly with NH-μClay only in the topsoil layers (Fig. 3e, f). A moderate significant correlation ($R^2 =$

0.343) was found in the 20–40 cm layer of topsoil, whereas no meaningful relationship was observed in all the other layers. A notable ($p < 0.05$) association was recorded in the 0–20 cm, and a strong relationship in 20–40 cm was found between C/P ratio and H-μClay. A strong significant correlation between C/P ratio and NH-μSilt in the 0–20 cm and a moderate relationship was found in the 20–40 cm layer. A similar association was noted in both topsoil layers between the C/P ratio and NH-μClay.

No significant correlation was found in the whole profile between the N/P ratio and H-μSilt. In contrast, a significant negative correlation was found only in the 2nd layer of topsoil (20–40 cm). Interestingly, a positive association of N/P ratio with NH-μSilt was noticed in the 40–60 cm layer (Fig. 7e). In 0–20 cm layer, N/P ratio had a meaningful correlation ($R = 0.541$) with NH-μClay (Fig. 7f).
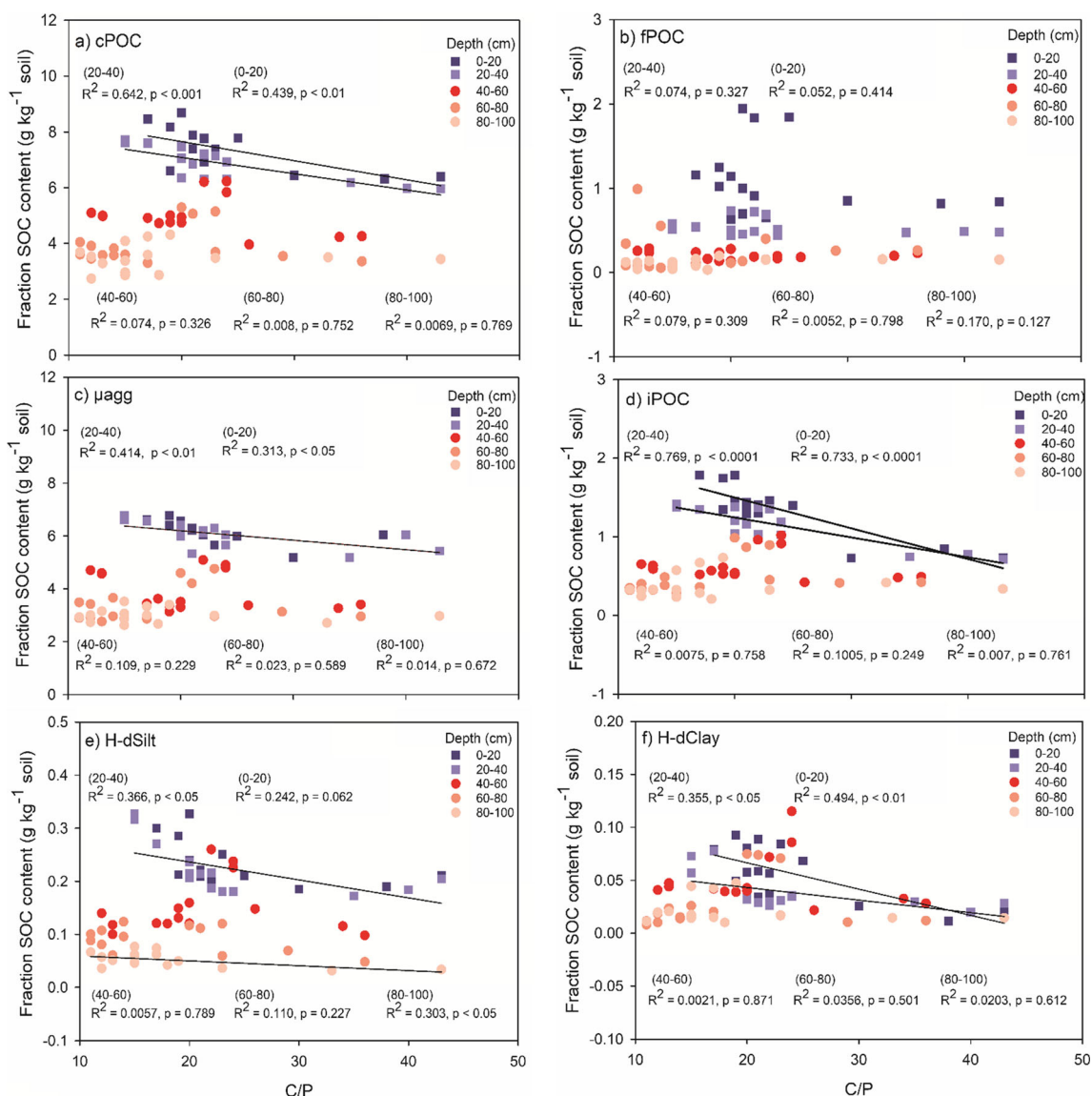
Fig. 4 Relationship between C/P ratio and various OC content of fractions along with soil profile layers after long-term fertilization with manure and mineral fertilizers ($N = 75$ and $n = 15$)

4 Discussion

4.1 C, N, P contents and stoichiometries affected with mineral and manure fertilization across profile

Soil organic carbon (SOC) is an important parameter determining soil health and fertility status; hence, efforts to improve its content are aimed to sustain crop production and ensure food security. However, significant changes in SOC can only be visualized by long-term land management practices, e.g., fertilization and manure application (Gerzabek et al. 2001; Abrar et al. 2020; Ashraf et al. 2020) as fresh input of manure significantly changes the C and N mineralization within the soil (Luo et al. 2016; Butterly et al. 2019) inducing

priming effect, i.e., positive or negative (Kuzyakov et al. 2000; Zhu et al. 2018).

Relative input and output quantities of SOC and N determine the soil C and N contents (Trumbore et al. 1996), and accumulation of both of these can be attributed to the slow degradation of indigenous SOC (Qiu et al. 2018). Long-term application of fertilizers and manure considerably increases SOC and N accumulation, soil microbial biomass (Kundu et al. 2007; Wang et al. 2019; Ashraf et al. 2020), and higher soil N availability which further enhances the microbial activity, enhancing soil N mineralization (Liu et al. 2019). Additionally, we have reported wide variations among the protection mechanisms of SOC as influenced by
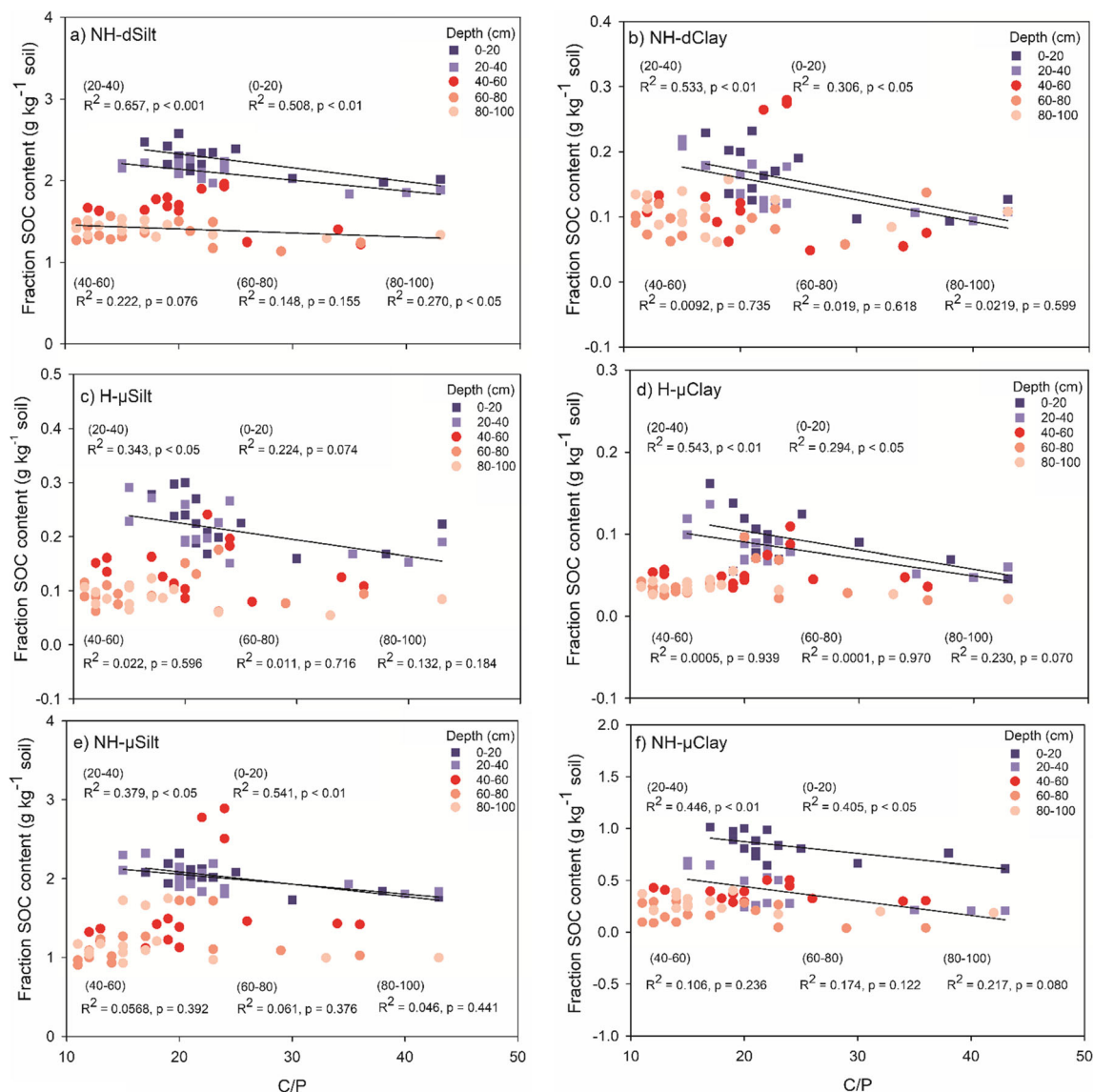
Fig. 5 Relationship between C/P ratio and various OC content of fractions along with soil profile layers after long-term fertilization with manure and mineral fertilizers ($N = 75$ and $n = 15$)

long-term application of manure combined with mineral fertilizers (Abrar et al. 2020).

A gradual decrease in SOC and TN contents with an increase in depth (Table 2) has also been reported previously (Drahorad et al. 2013). Both accumulation and decomposition determine the N in the soil and are closely associated with the SOC; hence SOC and N contents demonstrate consistent distribution patterns along with profile (Tian et al. 2010). Similarly, a strong association ($R^2 = 0.926$) between SOC and TN (Fig. 1a; $p < 0.0001$) was observed along with the soil profile.

As expected, the addition of manure in combination with mineral fertilizers (NPKM) improved the SOC, TN, and TP contents mainly in surface horizons, i.e., first two layers (0–40 cm) except for TP, which showed a significant increase

throughout the soil profile. Interestingly, the sub-surface soil layers (40–60, 60–80, and 80–100 cm) showed the highest SOC and TN contents in the case of manure application alone (M) (Table 1).

Microbial activity regulated SOC mineralization in addition to soil nutrient status (N and P) and their stoichiometry (Kirkby et al. 2013; Chen et al. 2014a) and this can be a regulatory factor influencing the C accrual (Sistla and Schimel 2012). As microbial demand for these nutrients arises, microbes performed their activity depending on the stoichiometric decomposition theory (Sinsabaugh et al. 2009; Heuck et al. 2015). Thus, nutrient constraint might suppress microbial activity and limit SOC degradation (Chen et al. 2014b; Zhu et al. 2018) and vice versa. Long-term application of manure can significantly influence the microbial
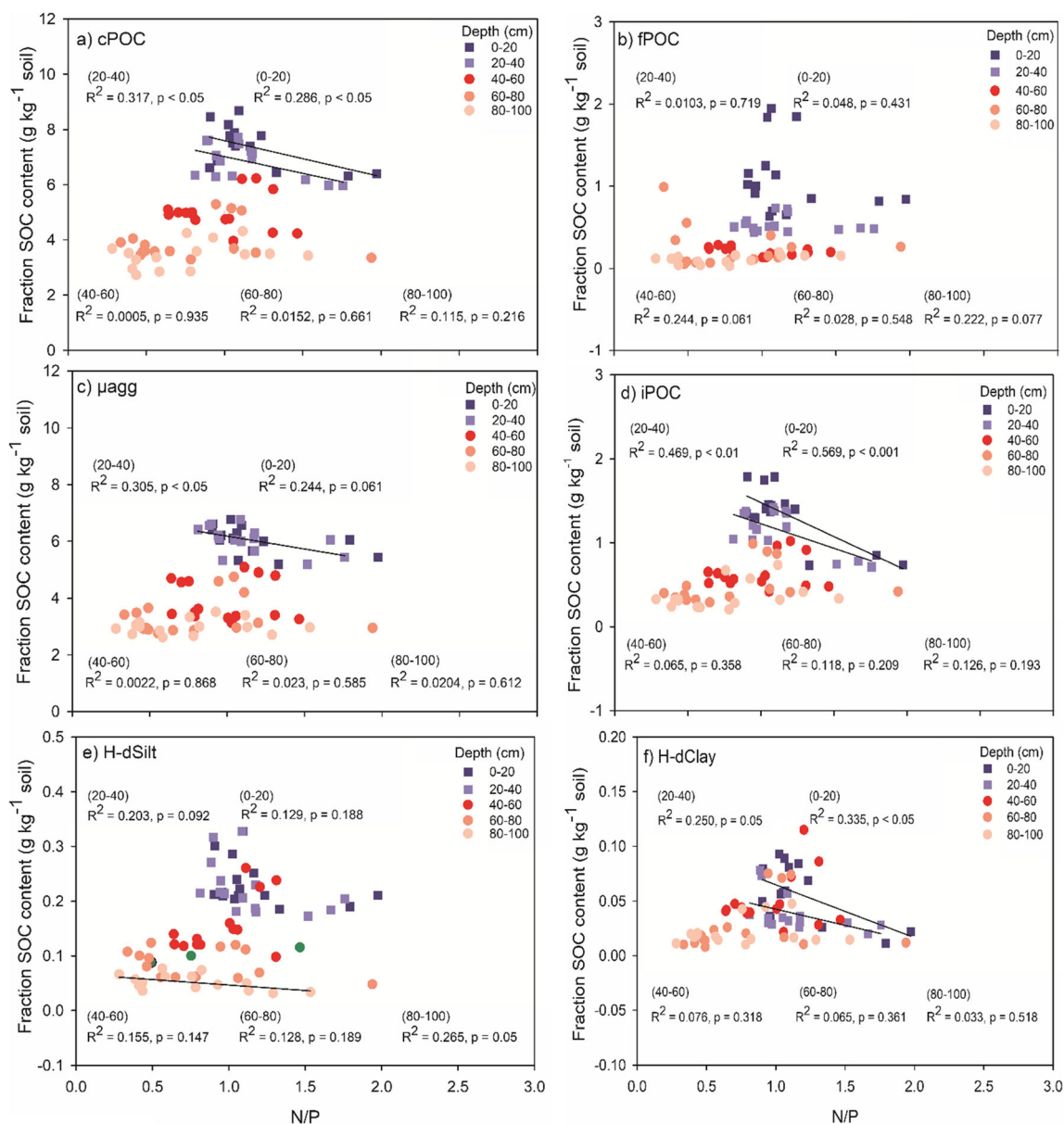
Fig. 6 Relationship between N/P ratio and various OC content of fractions along with soil profile layers after long-term fertilization with manure and mineral fertilizers ($N = 75$ and $n = 15$)

community and microbial C/N/P stoichiometry resultantly the SOC and N mineralization (Ashraf et al. 2020) by priming effects (Blagodatskaya and Kuzyakov 2008). The phenomenon is evident in topsoil layers as CN ratio was decreased in the top two layers, while the C/N ratio was higher in lower soil layers (60–80 and 80–100). The increased mineralization of N in topsoil because of high SOC and microbial activity may be one of the possible reasons for lower CN ratios (Table 1) in these layers. Contrarily, the CP ratio was decreased throughout the soil profile in treatments receiving mineral fertilizers relative to those receiving alone manure (M) and control (CK). The increased TP concentration along with the profile is the possible reason for decreased CP ratio as C concentration did not decrease; instead, it was increased in topsoil layers.

Reduction in C/N ratios and increased N/P ratio in soil have also been reported in northern and central Europe and North America, because of N deposition (Bobbink et al. 2010).

Cycling of C occurs in combination with N and P, both of which undergo significant alterations in biogeochemical cycling (Falkowski et al. 2000). The primary sources of organic matter input into the subsoil are roots and root exudates, dissolved organic matter, and topsoil SOC translocated by bioturbation (Rumpel and Kögel-Knabner 2011). Hence the contents of C, N, and P are lower in subsoil layers than in the topsoil (Table 1), where the addition of organic manure and crop roots directly add these elements (Abrar et al. 2020). The rate of mineralization of C and N is also high (Ashraf et al. 2020). The differences in the mineralization of SOC and N
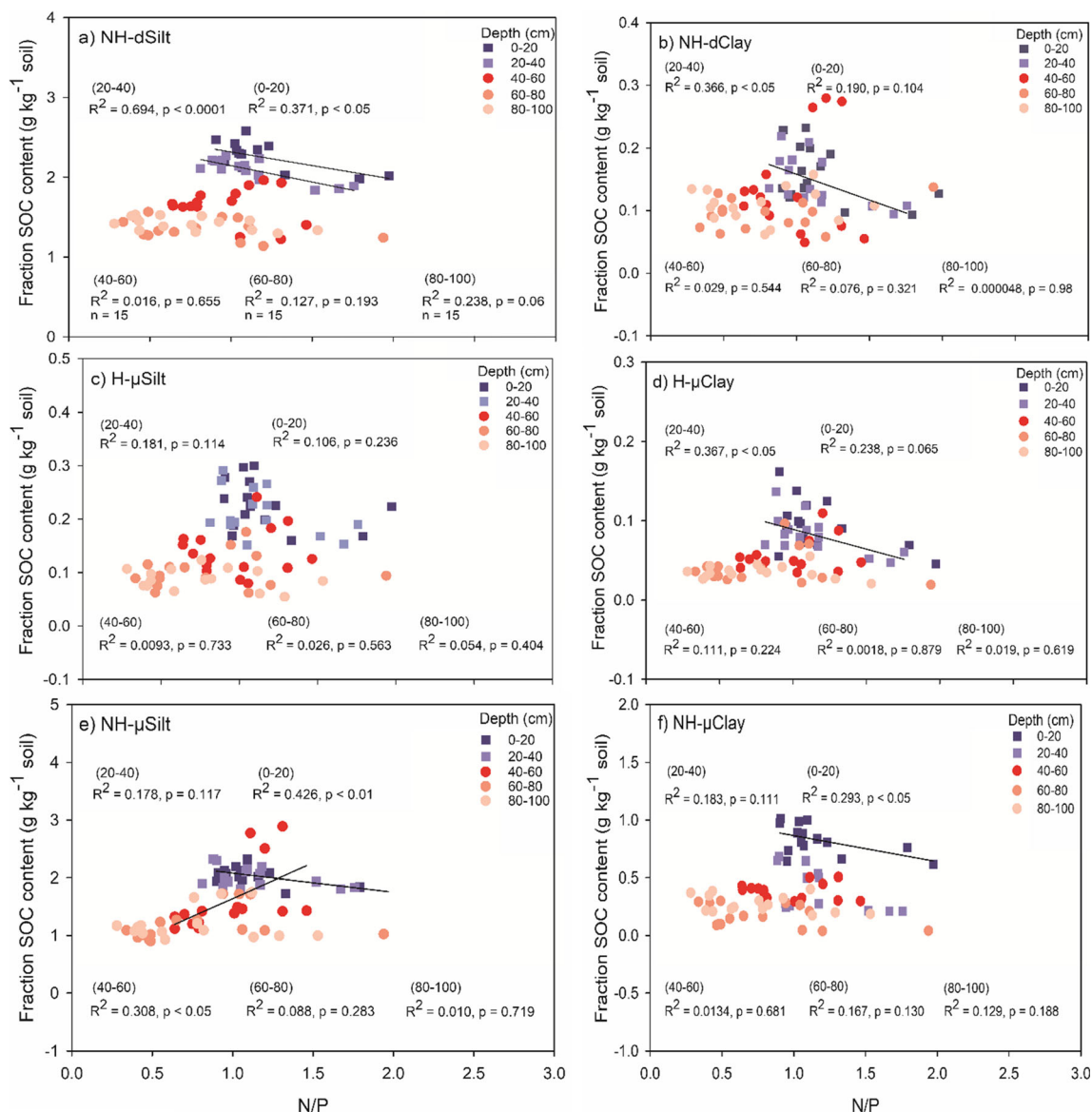
Fig. 7 Relationship between N/P ratio and various OC content of fractions along with soil profile layers after long-term fertilization with manure and mineral fertilizers ($N = 75$ and $n = 15$) Note: ($N = 75$, $n = 15$ where N is the total number of observations in the whole profile while n is the total number of observations in each soil profile layer from 0 to 100 cm)

after long-term fertilizer application was principally due to the SOC stabilization through different protection mechanisms (Wang et al. 2019; Xu et al. 2019). The SOC and N degradation was controlled by the DOC, Olsen-P, and N availability (Mooshammer et al. 2014), which was actively enhanced by NPKM fertilizer application (Ashraf et al. 2020). These variations affect the C, N, and P stocks in the soil and, in most cases, lead to a considerable decline of these stocks; however, the total stoichiometric ratios in soils seem to be somewhat controlled, at least in an ecosystem (Cleveland and Liptzin 2007; Xu et al. 2013; Khan et al. 2016).

The improvement in nutrient accession with a regular manure application was found in recent experiments (Sadeghpour et al. 2017; Mi et al. 2016; Abrar et al. 2020), and this accretion might be associated with the input by indigenous nutrient reserves of OM-enriched soils (Chen et al. 2017).

In the 40–100 cm layers, in most cases, no significant associations were noticed between C/N/P stoichiometry and protection mechanisms. It implies that the relative proportion of slow recycling OC was also increased with the increase in depth (Jobbágy and Jackson 2000), which may attribute to the slower/insignificant mineralization of the OC and hence more variations in the C/N ratios. Moreover, the labile OC proportion decreases, whereas that of recalcitrant OC increases with the increment in soil depth (Fang and Moncrieff 2005).

Furthermore, the influence of external environmental factors is becoming less significant in the deep soil layers due to soil buffering capacity in comparison to topsoil layers. For example, temperature and moisture content in subsoil layers are relatively very less compared with the topsoil layers (Luo et al. 2010). Consequently, the associations between SOC content and environmental factors tend to be weaker with increasing soil depth. Moreover, the SOC stabilization in the subsoil was dictated mainly by the fresh C inputs from the topsoil (Fontaine et al. 2007; Abrar et al. 2020).

The input of combined mineral and organic manure (NPKM) fertilization markedly increased the SOC and TN content in the topsoil. In contrast, in the subsoil, M alone increased the SOC and TN content. In this study, C, N, and P contents increased considerably with additions of manure which could be ascribed to the continuous inputs of organic materials for 35 years further to the higher biomass production. Similar positive impacts of manure application on C, N, and P were also reported in other studies based on long-term experiments (Rochette and Gregorich 1998; Kanchikerimath and Singh 2001; Nayak et al. 2007).

Through assessment of C/P and N/P ratios, although we noted significant differences in C and N contents, low soil P content always resulted in greater C/P and N/P ratios. This pattern shows, as reported by Walker and Adams (1958), i.e., N/P ratio was primarily regulated by C/P ratio in the soil. Despite the lack of controlled stoichiometry of C/N/P in the subsoil layers, the P distribution along with the profile still provides insight to understand biotic regulations of soil nutrients. Also independent of locality of parent material and vertically downward translocation of P leaching, the microbes are still capable of affecting P gradient via uptake and translocating P from P-rich subsoil horizons to the topsoil to fulfill their nutrient constrictions (Zhang et al. 2005). In NPKM, a low C/P ratio was an indicator of high P availability throughout the profile. Moreover, the decrease in C/P and N/P ratios with the increase in C and N contents after application of manure and combination of mineral plus manure showed a high P content corresponding to C and N in the soils.

4.2 Relationship of C/N/P stoichiometry with non-protection and physical protection

The discrepancies in the OC and N mineralization subjected to the long-term mineral and organic fertilization (Ashraf et al. 2020) were primarily due to the SOC stabilization attributed to different protection mechanisms (Wang et al. 2019; Xu et al. 2019). Any factor influencing the protection mechanisms and various fractions of SOC would significantly affect the SOC stabilization.

Significant negative correlation ($R = 0.797$ and 0.932) was found between C/N ratio and cPOC in both layers of topsoil, and the 1st layer of the subsoil with the maximum tendency

($y = -0.38$) was noted in the surface soil layer indicating that C/N ratio affected cPOC only in the topsoil layers. This can be explained as the sole/combined application of manure with NPK increased unprotected OC, i.e., cPOC and fPOC in the topsoil layers (Supplementary Table; Abrar et al. 2020). The SOC and N mineralization is generally higher in the topsoil due to more disturbance by management practices, i.e., fertilization application, tillage operations, and cultivation (Ashraf et al. 2020), and relatively lesser or insignificant in the subsoil layers from 40 to 100 cm layers. Alternatively, our study convinced with the findings by Abrar et al. (2020) that more OC stabilization was found in the subsoil layers.

Assessment of C/N ratio and fPOC yielded no significant correlations ($p > 0.05$) in both the topsoil layers; however, a strong positive association between C/N ratio and fPOC with least tendencies were found in the 40–80 cm layers. This may be attributed to negative associations between TOC and fPOC that eventually resulted in the positive priming, i.e., the microbes utilized the C of fPOC as a vital energy source after fertilizer addition (Blagodatskaya and Kuzyakov 2008); thereby, the OC of fPOC may be related positively to the soil productivity (Lehmann and Kleber 2015). Consistent with this finding, Marzi et al. (2020) reported a negative relationship between N mineralization and C/N ratio, indicating that the mineral N thus produced was taken up by microbes (i.e., as immobilization) and resultantly showed reductions in mineral N in some treatments (Marzi et al. 2020). Physical protection underlies the mechanisms such as occlusion within microaggregates, and adsorption to the mineral surfaces has been proposed to stabilize OC adequately and thereby affects OC turnover (Torn et al. 1997; Marschner et al. 2008).

Six et al. (2000b) stated that macro- and microaggregates were susceptible to plowing practices, and the intensive plowing may lead to a more considerable disruption and hence limits the process of aggregation. Moreover, tillage decreased the microaggregate and silt plus clay-associated OC content (Song et al. 2016). Organic matter provides the gluing and cementation agents for the formation of macroaggregates, which are the key repositories of OC (Six et al. 2000a). Macroaggregates preserve the labile C pools, i.e., cPOC and fPOC, by encapsulation from microbes. In return, labile C helps to improve aggregate stabilization (Six et al. 2002c). Hence, decreased physical protection due to disruption of management practices e.g., tillage may cause detrimental effects on the active C pools which ultimately leads to C decline (Somasundaram et al. 2018), because of their vulnerability to oxidation (Luo et al. 2010). The soil N/P ratio comprises an essential foundation for assessing the change in nutrient limitation status of terrestrial ecosystems after N application (Bui and Henderson 2013; Schreeg et al. 2014). Physically protected SOC was also higher in topsoil layers (Supplementary Table, Abrar et al. 2020) and was improved significantly in treatments receiving manure with NPK

compared to CK. This finding has further been supported by our results as a significant negative correlation ($R = 0.516$ and 0.539) was observed in topsoil layers (0–40 cm) between C/N ratio and μagg fraction and iPOC. Similar to the reduction in the SOC along with depth, the relationship was weaker in the subsoil layers. These relationships indicated the role of stoichiometric ratios in soil and also of the microbial population. Similarly, C/P ratio was negatively correlated with the unprotected (cPOC) and physically protected SOC (μagg and iPOC) in topsoil layers. At the same time, no or very weak correlation was observed in the subsoil layers, indicating that the long-term fertilization influenced the nutrient stoichiometry as well as the protection mechanisms.

In the present study, the physically protected microaggregate (μagg) and iPOC correlated negatively with the increment in C/P and N/P ratios in both topsoil layers, whereas no significant association was noted between these variables in the deep soil layers (up to 100 cm). This may be attributed to the deficiency of P in macroaggregates which limits the ATP (adenosine triphosphate) formation (Elser et al. 2003), which may hamper the microbial growth and limit the OC mineralization (Blanco-Canqui and Lal 2004), thus enhancing OC stock (Van Groenigen et al. 2006; Hontoria et al. 2016). Conversely, in microaggregates and silt plus clay fractions, smaller C/P and N/P ratios suggest that microbial-derived organic compounds might provide a higher capacity for P release (Zechmeister-Boltenstern et al. 2015; Cao and Chen 2017). Moreover, Wei et al. (2014) indicated that greater phosphatase activity in microaggregates increased mineralization, which yielded large amounts of orthophosphate (PO_4^-) to promote microbial growth and release of C. Hence, compared with C/N ratio, C/P and N/P ratios can contribute more useful knowledge about the capacity of aggregates to stabilize C. Regression analysis revealed that N/P ratio correlated negatively with cPOC both layers of the topsoil. However, no significant ($p > 0.05$) relationship was noted in all the subsoil layers. Likewise, no meaningful relationship was recorded between NP ratio and fPOC throughout the profile. A close association between the N/P ratio and physically protected μagg may be due to the good aggregation in the topsoil owing to the sufficient amount N provided by mineral and NPKM fertilization.

In our study, the SOC fractions, i.e., cPOC and iPOC, were most sensitive to the C/N ratio (0–40 cm), which is in agreement with the total SOC content changes. This sensitivity may be attributed to the less stabilization degree and short turnover time (Lützow et al. 2006; Poeplau and Don 2013) as well as the mineralization of SOC and N (Ashraf et al. 2020). The non-protected fraction, i.e., cPOC, was observed to be most responsive to the change in C/N/P ratios in the topsoil layers (0–40 cm) (Figs. 2a, 4a, 6a).

Our study demonstrated that the OC fractions were noted to be most responsive in the topsoil layers (0–40 cm); the

variability in the C fractions in the subsoil layers under manure fertilization was significantly greater compared with the rest of treatments due to the lack of soil disturbances, i.e., tillage and other management operations, and hence caused aggregation in the subsoil layers, therefore influencing SOC stabilization. Moreover, Six et al. (2002d) reported the conversion of till to no-till enhanced aggregation and hence affecting SOC. Furthermore, the substrate and moisture availability in different depths determine the shifts in microbial community composition, i.e., bacteria, fungi, etc. (Fierer et al. 2003; Cleveland and Liptzin 2007).

4.3 Relationship of C/N/P stoichiometry with chemical and biochemical protection

A strong negative correlation between chemical and biochemically protected OC fractions (H-dSilt, H-dClay, NH-dSilt, and NH-dClay) with the C/N ratio in topsoil layers (Fig. 4) indicated their importance in the C stabilization within topsoil after long-term manure application (Abrar et al. 2020). Similarly, the C/P ratio was also negatively correlated with chemical and biochemically protected fractions, indicating the importance of P availability within topsoil layers as well as subsoil layers and its role in C stabilization.

Interestingly, the N/P ratio did not influence the physically protected OC fractions both in silt and clay size fractions throughout the soil profile, except a strong correlation was observed between H-dSilt and N/P ratio in the last subsoil layer (Fig. 6e). This implies that C/N and C/P ratio are more important than the N/P ratio in various C stabilization or decomposition mechanisms. However, the N/P ratio significantly influenced the chemical and biochemically protected SOC fractions in topsoil layers. The complex chemical nature and make-up of organic compounds determine the biochemical protection of SOC (Six et al. 2002c) that may be related to the regular additions of fresh OC in the deep soil layers (Fontaine et al. 2007). Further to the plant and microbial-associated nature, the OC comprises of charcoal or black C (Roth et al. 2012), which is a biochemically rigid material and is independent of saturation behavior due to lack of significant relation with silt or clay fractions (Six et al. 2002c; Stewart et al. 2008).

4.4 Relationship of C/N/P stoichiometry with physico-chemical and physico-biochemical protection

Low mineralization in silt and clay-sized (< 0.053) fractions was mainly owing to the physico-chemical protection of OC associated with silt plus clay fractions (limited microbial access) for stabilization (Sarker et al. 2018). The relative amount of aggregates enhanced from top to subsoil layers. Hence, the discrepancy in aggregate size distribution is the critical indicator of the variability in mineralization among the soil layers.

Consistent with these studies, our results demonstrate that the relationship between SOC and C/N and C/P was significant in the topsoil layers and this relationship became weaker with depth and it can be associated with the reduced microbial activity with no-tillage (Raiesi and Asadi 2006; Sarker et al. 2018) in the subsoil layers.

Long-term manure and fertilization application significantly influenced both the chemical and physico-chemical protection mechanisms of SOC, which may be attributed to changes in mineralization of SOC and N (Ashraf et al. 2020). Furthermore, Conant et al. (2011) perceived the OC resistance to decomposition was either because of chemical or physico-chemical protection, and it supports the conception that the physical protection due to aggregation determines both chemical and physico-chemical protection mechanisms. Additionally, the SOC characteristics, enzyme-associated kinetics, and microbial adaptations are crucial for SOC mineralization and degradation owing to temperature sensitivity (Billings and Ballantyne 2013). Physical and physico-chemical protection mechanisms play a vital role in C stabilization (Abrar et al. 2020).

The mechanisms such as the barrier formation between aggregates and microbes (i.e., physical protection) within aggregate involve physico-chemical interactions and are responsible for the maximum SOC stabilization and hence sequestration capacity (Six et al. 2002c). Increment in the total P content resulted in lower C/P and N/P ratios due to the addition of inorganic P related to higher P adsorption by the soil in comparison to N (Tiessen et al. 1982; Tischer et al. 2014).

The association between fraction C contents and soil C, N, and P ratios can be influenced by the availability of N and P in the soil and also by the management practices as already reported by Abrar et al. (2020) that microbial stoichiometry also influences the C and N mineralization. Similar findings were reported in previous studies that microbial C/P ratio was a useful indicator for anticipating nutrient availability since P immobilization leads to a decline in the C/P ratio due to the limited supply of active C fractions (Alvarez et al. 1998; Kwabiah et al. 2003).

5 Conclusion

The pool sizes of OC, TN, and TP and the OC protection mechanisms were influenced by the long-term mineral and manure fertilizer application. The manure input enhanced soil microbial activity and elemental recycling, which changed the SOC protection in various fractions. Overall, it is widely accepted that manure inputs can improve soil quality. The C/N/P ratios had a strong relationship with SOC and its various fractions throughout the soil profile, though with varied strength. The association was more robust in topsoil layers, with the

least effective in the deep subsoil layers. It is recommended that manure should be used in combination with mineral fertilizer for improved SOC and its stabilization for healthy soil and better production.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent No informed or ethical consent was required for this research

References

Abrar MM, Xu M, Shah SAA, Aslam MW, Aziz T, Mustafa A, Ashraf MN, Zhou B, Ma X (2020) Variations in the profile distribution and protection mechanisms of organic carbon under long-term fertilization in a Chinese Mollisol. Sci Total Environ 723:138181. https://doi.org/10.1016/j.scitotenv.2020.138181

Alvarez CR, Alvarez R, Grigera MS, Lavado RS (1998) Associations between organic matter fractions and the active soil microbial biomass. Soil Biol Biochem 30:767–773. https://doi.org/10.1016/S0038-0717(97)00168-5

Ashraf MN, Hu C, Wu L, Duan Y, Zhang W, Aziz T, Cai A, Abrar MM, Xu M (2020) Soil and microbial biomass stoichiometry regulate soil organic carbon and nitrogen mineralization in rice-wheat rotation subjected to long-term fertilization. J Soils Sediments 20:3103–3113. https://doi.org/10.1007/s11368-020-02642-y

Billings SA, Ballantyne F (2013) How interactions between microbial resource demands, soil organic matter stoichiometry, and substrate reactivity determine the direction and magnitude of soil respiratory responses to warming. Glob Chang Biol 19:90–102

Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol Fertil Soils 45:115–131. https://doi.org/10.1007/s00374-008-0334-y

Blair N, Faulkner RD, Till AR, Poulton PR (2006) Long-term management impacts on soil C, N and physical fertility. Part I: Broadbalk experiment. Soil Tillage Res 91:30–38. https://doi.org/10.1016/j.still.2005.11.002

Blanco-Canqui H, Lal R (2004) Mechanisms of carbon sequestration in soil aggregates. CRC Crit Rev Plant Sci 23:481–504

Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman JW, Fenn M, Gilliam F, Nordin A, Pardo L, de Vries W (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecol Appl 20:30–59. https://doi.org/10.1890/08-1140.1@10.1002/(ISSN)1939-5582(CAT)SPECIALCOLLECTION(VI)VIRTUALISSUE

Bui EN, Henderson BL (2013) C:N:P stoichiometry in Australian soils with respect to vegetation and environmental factors. Plant Soil 373: 553–568. https://doi.org/10.1007/s11104-013-1823-9

Butterly CR, Armstrong RD, Chen D, Tang C (2019) Residue decomposition and soil carbon priming in three contrasting soils previously exposed to elevated CO 2. Biol Fertil Soils 55:17–29. https://doi.org/10.1007/s00374-018-1321-6

Cao Y, Chen Y (2017) Coupling of plant and soil C:N:P stoichiometry in black locust (Robinia pseudoacacia) plantations on the loess plateau, China. Trees - Struct Funct 31:1559–1570. https://doi.org/10.1007/s00468-017-1569-8

Chen C, Dynes JJ, Wang J, Sparks DL (2014a) Properties of Fe-organic matter associations via coprecipitation versus adsorption. Environ Sci Technol 48:13751–13759. https://doi.org/10.1021/es503669u

Chen R, Senbayram M, Blagodatsky S, Myachina O, Dittert K, Lin X, Blagodatskaya E, Kuzyakov Y (2014b) Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Glob Chang Biol 20:2356–2367. https://doi.org/10.1111/gcb.12475

Chen D, Yuan L, Liu Y, Ji J, Hou H (2017) Long-term application of manures plus chemical fertilizers sustained high rice yield and improved soil chemical and bacterial properties. Eur J Agron 90:34–42. https://doi.org/10.1016/j.eja.2017.07.007

Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85: 235–252. https://doi.org/10.1007/s10533-007-9132-0

Conant RT, Ryan MG, Ågren GI, Birge HE, Davidson EA, Eliasson PE, Evans SE, Frey SD, Giardina CP, Hopkins FM, Hyvönen R, Kirschbaum MUF, Lavallee JM, Leifeld J, Parton WJ, Megan Steinweg J, Wallenstein MD, Martin Wetterstedt JÅ, Bradford MA (2011) Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. Glob Chang Biol 17:3392–3404

Crowley KF, McNeil BE, Lovett GM et al (2012) Do nutrient limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the northeastern United States? Ecosystems 15:940–957. https://doi.org/10.1007/s10021-012-9550-2

Drahorad S, Felix-Henningsen P, Eckhardt KU, Leinweber P (2013) Spatial carbon and nitrogen distribution and organic matter characteristics of biological soil crusts in the Negev desert (Israel) along a rainfall gradient. J Arid Environ 94:18–26. https://doi.org/10.1016/j.jaridenv.2013.02.006

Elser JJ, Sterner RW, Gorokhova E, Fagan WF, Markow TA, Cotner JB, Harrison JF, Hobbie SE, Odell GM, Weider LW (2000) Biological stoichiometry from genes to ecosystems. Ecol Lett 3:540–550

Elser JJ, Acharya K, Kyle M, Cotner J, Makino W, Markow T, Watts T, Hobbie S, Fagan W, Schade J, Hood J, Sterner RW (2003) Growth rate-stoichiometry couplings in diverse biota. Ecol Lett 6:936–943. https://doi.org/10.1046/j.1461-0248.2003.00518.x

Falkowski P, Scholes RJ, Boyle E et al (2000) The global carbon cycle: a test of our knowledge of earth as a system. Science (80-) 290:291–296

Fang C, Moncrieff JB (2005) The variation of soil microbial respiration with depth in relation to soil carbon composition. Plant Soil 268: 243–253. https://doi.org/10.1007/s11104-004-0278-4

Fazhu Z, Jiao S, Chengjie R, di K, Jian D, Xinhui H, Gaihe Y, Yongzhong F, Guangxin R (2015) Land use change influences soil C, N, and P stoichiometry under "Grain-to-Green Program" in China. Sci Rep 5:1–10. https://doi.org/10.1038/srep10195

Fierer N, Allen AS, Schimel JP, Holden PA (2003) Controls on microbial CO2 production: a comparison of surface and subsurface soil horizons. Glob Chang Biol 9:1322–1332. https://doi.org/10.1046/j.1365-2486.2003.00663.x

Fontaine S, Barot S, Barré P, Bdioui N, Mary B, Rumpel C (2007) Stability of organic carbon in deep soil layers controlled by fresh

carbon supply. Nature 450:277–280. https://doi.org/10.1038/nature06275

Gerzabek MH, Haberhauer G, Kirchmann H (2001) Soil organic matter pools and carbon-13 natural abundances in particle-size fractions of a long-term agricultural field experiment receiving organic amendments. Soil Sci Soc Am J 65:352–358. https://doi.org/10.2136/sssaj2001.652352x

Gong W, Yan X, Wang J, Hu T, Gong Y (2009) Long-term manure and fertilizer effects on soil organic matter fractions and microbes under a wheat-maize cropping system in northern China. Geoderma 149: 318–324. https://doi.org/10.1016/j.geoderma.2008.12.010

Gregory AS, Kirk GJD, Keay CA, Rawlins BG, Wallace P, Whitmore AP (2014) An assessment of subsoil organic carbon stocks in England and Wales. Soil Use Manag 30:10–22. https://doi.org/10.1111/sum.12085

Güsewell S (2004) N:P ratios in terrestrial plants: variation and functional significance. New Phytol 164:243–266

He YT, Zhang WJ, Xu MG, Tong XG, Sun FX, Wang JZ, Huang SM, Zhu P, He XH (2015) Long-term combined chemical and manure fertilizations increase soil organic carbon and total nitrogen in aggregate fractions at three typical cropland soils in China. Sci Total Environ 532:635–644. https://doi.org/10.1016/j.scitotenv.2015.06.011

Heuck C, Weig A, Spohn M (2015) Soil microbial biomass C: N: P stoichiometry and microbial use of organic phosphorus. Soil Biol Biochem 85:119–129. https://doi.org/10.1016/j.soilbio.2015.02.029

Hontoria C, Gómez-Paccard C, Mariscal-Sancho I, Benito M, Pérez J, Espejo R (2016) Aggregate size distribution and associated organic C and N under different tillage systems and Ca-amendment in a degraded Ultisol. Soil Tillage Res 160:42–52. https://doi.org/10.1016/j.still.2016.01.003

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol Appl 10:423–436. https://doi.org/10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2

Kanchikerimath M, Singh D (2001) Soil organic matter and biological properties after 26 years of maize-wheat-cowpea cropping as affected by manure and fertilization in a Cambisol in semiarid region of India. Agric Ecosyst Environ 86:155–162. https://doi.org/10.1016/S0167-8809(00)00280-2

Khan KS, Mack R, Castillo X, Kaiser M, Joergensen RG (2016) Microbial biomass, fungal and bacterial residues, and their relationships to the soil organic matter C/N/P/S ratios. Geoderma 271:115–123. https://doi.org/10.1016/j.geoderma.2016.02.019

Kirkby CA, Richardson AE, Wade LJ, Batten GD, Blanchard C, Kirkegaard JA (2013) Carbon-nutrient stoichiometry to increase soil carbon sequestration. Soil Biol Biochem 60:77–86. https://doi.org/10.1016/j.soilbio.2013.01.011

Kundu S, Bhattacharyya R, Prakash V, et al (2007) Carbon sequestration and relationship between carbon addition and storage under rainfed soybean–wheat rotation in a sandy loam soil of the Indian Himalayas. Elsevier

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biol Biochem 32:1485–1498

Kwabiah AB, Palm CA, Stoskopf NC, Voroney RP (2003) Response of soil microbial biomass dynamics to quality of plant materials with emphasis on P availability. Soil Biol Biochem 35:207–216. https://doi.org/10.1016/S0038-0717(02)00253-5

Lambers H, Brundrett MC, Raven JA, Hopper SD (2010) Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. Plant Soil 334:11–31. https://doi.org/10.1007/s11104-010-0444-9

Lehmann J, Kleber M (2015) The contentious nature of soil organic matter. Nature 528:60–68. https://doi.org/10.1038/nature16069

Liu X, Han X, Song C, Herbert SJ, Xing B (2003) Soil organic carbon dynamics in black soils of China under different agricultural

management systems. Commun Soil Sci Plant Anal 34:973–984. https://doi.org/10.1081/CSS-120019103

Liu Y, Ge T, Ye J, Liu S, Shibistova O, Wang P, Wang J, Li Y, Guggenberger G, Kuzyakov Y, Wu J (2019) Initial utilization of rhizodeposits with rice growth in paddy soils: rhizosphere and N fertilization effects. Geoderma 338:30–39. https://doi.org/10.1016/j.geoderma.2018.11.040

Ludwig B, Schulz E, Rethemeyer J, Merbach I, Flessa H (2007) Predictive modelling of C dynamics in the long-term fertilization experiment at Bad Lauchstädt with the Rothamsted Carbon Model. Eur J Soil Sci 58:1155–1163. https://doi.org/10.1111/j.1365-2389.2007.00907.x

Luo Z, Wang E, Sun OJ (2010) Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agric Ecosyst Environ 139:224–231. https://doi.org/10.1016/j.agee.2010.08.006

Luo Z, Wang E, Sun OJ (2016) A meta-analysis of the temporal dynamics of priming soil carbon decomposition by fresh carbon inputs across ecosystems. Soil Biol Biochem 101:96–103. https://doi.org/10.1016/j.soilbio.2016.07.011

Majumder B, Mandal B, Bandyopadhyay PK, Chaudhury J (2007) Soil organic carbon pools and productivity relationships for a 34 year old rice-wheat-jute agroecosystem under different fertilizer treatments. Plant Soil 297:53–67. https://doi.org/10.1007/s11104-007-9319-0

Marschner B, Brodowski S, Dreves A, Gleixner G, Gude A, Grootes PM, Hamer U, Heim A, Jandl G, Ji R, Kaiser K, Kalbitz K, Kramer C, Leinweber P, Rethemeyer J, Schäffer A, Schmidt MWI, Schwark L, Wiesenberg GLB (2008) How relevant is recalcitrance for the stabilization of organic matter in soils? J Plant Nutr Soil Sci 171:91–110

Marzi M, Shahbazi K, Kharazi N, Rezaei M (2020) The influence of organic amendment source on carbon and nitrogen mineralization in different soils. J Soil Sci Plant Nutr 20:177–191. https://doi.org/10.1007/s42729-019-00116-w

Mi W, Wu L, Brookes PC, Liu Y, Zhang X, Yang X (2016) Changes in soil organic carbon fractions under integrated management systems in a low-productivity paddy soil given different organic amendments and chemical fertilizers. Soil Tillage Res 163:64–70. https://doi.org/10.1016/j.still.2016.05.009

Mooshammer M, Wanek W, Schnecker J, Wild B, Leitner S, Hofhansl F, Blöchl A, Hämmerle I, Frank AH, Fuchslueger L, Keiblinger KM, Zechmeister-Boltenstern S, Richter A (2012) Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. Ecology 93:770–782. https://doi.org/10.1890/11-0721.1

Mooshammer M, Wanek W, Zechmeister-Boltenstern S, Richter A (2014) Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Front Microbiol 5:22

Nayak DR, Babu YJ, Adhya TK (2007) Long-term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition. Soil Biol Biochem 39:1897–1906. https://doi.org/10.1016/j.soilbio.2007.02.003

Nelson DW, Sommers LE (1982) Methods of soil analysis part 3 chemical methods. In: Chemical Methods Soil Science Society of America Book Series

Ogle SM, Breidt FJ, Paustian K (2005) Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. Biogeochemistry 72:87–121. https://doi.org/10.1007/s10533-004-0360-2

Peñuelas J, Poulter B, Sardans J, Ciais P, van der Velde M, Bopp L, Boucher O, Godderis Y, Hinsinger P, Llusia J, Nardin E, Vicca S, Obersteiner M, Janssens IA (2013) Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. Nat Commun 4:1–10. https://doi.org/10.1038/ncomms3934

Plante AF, Conant RT, Paul EA, Paustian K, Six J (2006) Acid hydrolysis of easily dispersed and microaggregate-derived silt- and clay-sized fractions to isolate resistant soil organic matter. Eur J Soil Sci 57:456–467. https://doi.org/10.1111/j.1365-2389.2006.00792.x

Qing H, Huajun T, Wenbin W et al (2011) Remote sensing based dynamic changes analysis of crop distribution pattern -taking Northeast China as an example. Acta Ecol Sin

Qiu H, Ge T, Liu J, Chen X, Hu Y, Wu J, Su Y, Kuzyakov Y (2018) Effects of biotic and abiotic factors on soil organic matter mineralization: experiments and structural modeling analysis. Eur J Soil Biol 84:27–34. https://doi.org/10.1016/j.ejsobi.2017.12.003

Raiesi F, Asadi E (2006) Soil microbial activity and litter turnover in native grazed and ungrazed rangelands in a semiarid ecosystem. Biol Fertil Soils 43:76–82. https://doi.org/10.1007/s00374-005-0066-1

Rochette P, Gregorich EG (1998) Dynamics of soil microbial biomass C, soluble organic C and CO2 evolution after three years of manure application. Can J Soil Sci 78:283–290. https://doi.org/10.4141/S97-066

Roth PJ, Lehndorff E, Brodowski S, Bornemann L, Sanchez-García L, Gustafsson Ö, Amelung W (2012) Differentiation of charcoal, soot and diagenetic carbon in soil: method comparison and perspectives. Org Geochem 46:66–75. https://doi.org/10.1016/j.orggeochem.2012.01.012

Rumpel C, Kögel-Knabner I (2011) Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant Soil 338:143–158. https://doi.org/10.1007/s11104-010-0391-5

Sadeghpour A, Ketterings QM, Godwin GS, Czymmek KJ (2017) Shifting from N-based to P-based manure management maintains soil test phosphorus dynamics in a long-term corn and alfalfa rotation. Springer. https://doi.org/10.1007/s13593-017-0416-z

Sardans J, Peñuelas J (2012) The role of plants in the effects of global change on nutrient availability and stoichiometry in the plant-soil system. Plant Physiol 160:1741–1761. https://doi.org/10.1104/pp.112.208785

Sarker JR, Singh BP, Dougherty WJ, Fang Y, Badgery W, Hoyle FC, Dalal RC, Cowie AL (2018) Impact of agricultural management practices on the nutrient supply potential of soil organic matter under long-term farming systems. Soil Tillage Res 175:71–81. https://doi.org/10.1016/j.still.2017.08.005

Schreeg LA, Santiago LS, Wright SJ, Turner BL (2014) Stem, root, and older leaf N:P ratios are more responsive indicators of soil nutrient availability than new foliage. Ecology 95:2062–2068. https://doi.org/10.1890/13-1671.1

Shahid M, Nayak AK, Shukla AK, Tripathi R, Kumar A, Mohanty S, Bhattacharyya P, Raja R, Panda BB (2013) Long-term effects of fertilizer and manure applications on soil quality and yields in a sub-humid tropical rice-rice system. Soil Use Manag 29:322–332. https://doi.org/10.1111/sum.12050

Shang Q, Ling N, Feng X, Yang X, Wu P, Zou J, Shen Q, Guo S (2014) Soil fertility and its significance to crop productivity and sustainability in typical agroecosystem: a summary of long-term fertilizer experiments in China. Plant Soil 381:13–23. https://doi.org/10.1007/s11104-014-2089-6

Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462:795–798. https://doi.org/10.1038/nature08632

Sistla SA, Schimel JP (2012) Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. New Phytol 196:68–78

Six J, Elliott ET, Paustian K (2000a) Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol Biochem 32:2099–2103. https://doi.org/10.1016/S0038-0717(00)00179-6

Six J, Paustian ET, Combrink C (2000b) Soil structure and organic matter: I. Distribution of aggregate-aize classes. Soil Sci Soc Am J 64: 681–689. https://doi.org/10.2136/sssaj2000.642681x

Six J, Conant RT, Paul EA, Paustian K (2002a) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241:155–176. https://doi.org/10.1023/A:1016125726789

Six J, Conant RT, Paul EA, Paustian K (2002b) Stabilisation mechanisms for carbon. 155–176

Six J, Feller C, Denef K, et al (2002c) Soil organic matter, biota and aggregation in temperate and tropical soils - effects of no-tillage. In: Agronomie

Somasundaram J, Chaudhary RS, Awanish Kumar D, Biswas AK, Sinha NK, Mohanty M, Hati KM, Jha P, Sankar M, Patra AK, Dalal R, Chaudhari SK (2018) Effect of contrasting tillage and cropping systems on soil aggregation, carbon pools and aggregate-associated carbon in rainfed Vertisols. Eur J Soil Sci 69:879–891. https://doi.org/10.1111/ejss.12692

Song K, Yang J, Xue Y, Lv W, Zheng X, Pan J (2016) Influence of tillage practices and straw incorporation on soil aggregates, organic carbon, and crop yields in a rice-wheat rotation system. Sci Rep 6:1–12. https://doi.org/10.1038/srep36602

Stewart CE, Plante AF, Paustian K, Conant RT, Six J (2008) Soil carbon saturation: linking concept and measurable carbon pools. Soil Sci Soc Am J 72:379–392. https://doi.org/10.2136/sssaj2007.0104

Sutton MA, Bleeker A, Howard CM, et al (2013) Our nutrient world: the challenge to produce more food and energy with less pollution

Tian H, Chen G, Zhang C, Melillo JM, Hall CAS (2010) Pattern and variation of C:N:P ratios in China's soils: a synthesis of observational data. Biogeochemistry 98:139–151. https://doi.org/10.1007/s10533-009-9382-0

Tiessen H, Stewart JWB, Bettany JR (1982) Cultivation effects on the amounts and concentration of carbon, nitrogen, and phosphorus in grassland soils 1. Agron J 74:831–835. https://doi.org/10.2134/agronj1982.00021962007400050015x

Tischer A, Potthast K, Hamer U (2014) Land-use and soil depth affect resource and microbial stoichiometry in a tropical mountain rainforest region of southern Ecuador. Oecologia 175:375–393. https://doi.org/10.1007/s00442-014-2894-x

Torn MS, Trumbore SE, Chadwick OA, Vitousek PM, Hendricks DM (1997) Mineral control of soil organic carbon storage and turnover. Nature 389:170–173. https://doi.org/10.1038/38260

Trumbore SE, Chadwick OA, Amundson R (1996) Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. Science (80-) 272:393–396. https://doi.org/10.1126/science.272.5260.393

Van Groenigen KJ, Six J, Hungate BA et al (2006) Element interactions limit soil carbon storage. Proc Natl Acad Sci U S A 103:6571–6574. https://doi.org/10.1073/pnas.0509038103

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol Appl 20:5–15

Walker TW, Adams AFR (1958) Studies on soil organic matter: I. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. Soil Sci 85:307–318. https://doi.org/10.1097/00010694-195806000-00004

Wang J, Chen G, Zou G, Song X, Liu F (2019) Comparative on plant stoichiometry response to agricultural non-point source pollution in different types of ecological ditches. Environ Sci Pollut Res 26:647–658. https://doi.org/10.1007/s11356-018-3567-9

Wei K, Chen Z, Zhu A, Zhang J, Chen L (2014) Application of 31P NMR spectroscopy in determining phosphatase activities and P composition in soil aggregates influenced by tillage and residue management practices. Soil Tillage Res 138:35–43. https://doi.org/10.1016/j.still.2014.01.001

Xing B, Liu X, Liu J, Han X (2004) Physical and chemical characteristics of a typical Mollisol in China. Commun Soil Sci Plant Anal 35: 1829–1838. https://doi.org/10.1081/LCSS-200026802

Xu X, Thornton PE, Post WM (2013) A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Glob Ecol Biogeogr 22:737–749. https://doi.org/10.1111/geb.12029

Xu Y, Ding F, Gao X, Wang Y, Li M, Wang J (2019) Mineralization of plant residues and native soil carbon as affected by soil fertility and residue type. J Soils Sediments 19:1407–1415. https://doi.org/10.1007/s11368-018-2152-7

Ye C, Chen D, Hall SJ, Pan S, Yan X, Bai T, Guo H, Zhang Y, Bai Y, Hu S (2018) Reconciling multiple impacts of nitrogen enrichment on soil carbon: plant, microbial and geochemical controls. Ecol Lett 21: 1162–1173. https://doi.org/10.1111/ele.13083

Yuan ZY, Chen HYH (2009) Global-scale patterns of nutrient resorption associated with latitude, temperature and precipitation. Glob Ecol Biogeogr 18:11–18. https://doi.org/10.1111/j.1466-8238.2008.00425.x

Zechmeister-Boltenstern S, Keiblinger KM, Mooshammer M, Peñuelas J, Richter A, Sardans J, Wanek W (2015) The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. Ecol Monogr 85:133–155

Zhang C, Tian H, Liu J, Wang S, Liu M, Pan S, Shi X (2005) Pools and distributions of soil phosphorus in China. Glob Biogeochem Cycles 19:1–8. https://doi.org/10.1029/2004GB002296

Zhang ZS, Song XL, Lu XG, Xue ZS (2013) Ecological stoichiometry of carbon, nitrogen, and phosphorus in estuarine wetland soils: influences of vegetation coverage, plant communities, geomorphology, and seawalls. J Soils Sediments 13:1043–1051. https://doi.org/10.1007/s11368-013-0693-3

Zhang K, Su Y, Yang R (2019) Variation of soil organic carbon, nitrogen, and phosphorus stoichiometry and biogeographic factors across the desert ecosystem of Hexi Corridor, northwestern China. J Soils Sediments 19:49–57. https://doi.org/10.1007/s11368-018-2007-2

Zhu Z, Ge T, Luo Y, Liu S, Xu X, Tong C, Shibistova O, Guggenberger G, Wu J (2018) Microbial stoichiometric flexibility regulates rice straw mineralization and its priming effect in paddy soil. Soil Biol Biochem 121:67–76. https://doi.org/10.1016/j.soilbio.2018.03.003

Xia C, Yu D, Wang Z, Xie D (2014) Stoichiometry patterns of leaf carbon, nitrogen and phosphorous in aquatic macrophytes in eastern China. Ecol Eng. https://doi.org/10.1016/j.ecoleng.2014.06.018

Lützow MV, Kögel-Knabner I, Ekschmitt K et al (2006) Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions - A review. Eur J Soil Sci 57:426–445. https://doi.org/10.1111/j.1365-2389.2006.00809.x

Poeplau C, Don A (2013) Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe. Geoderma 192:189–201. https://doi.org/10.1016/j.geoderma.2012.08.003