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Long-term effects of nitrogen and phosphorus fertilization on soil aggregate stability and aggregate-associated carbon and nitrogen in the North China Plain

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Abstract

Soil aggregates and carbon storage are important in soil conservation, nutrient supply, and climate change mitigation. The long-term responses of aggregate-associated organic C (OC) and nitrogen (N) in surface versus subsoil to N and P fertilization remain unclear. We examined the effects of different N and P fertilization rates on aggregate stability and the associated soil OC (SOC) and N in the North China Plain through a 35-yr double-crop field experiment, hypothesizing that higher rates of mineral fertilizer would promote aggregate stability and increase C/N ratios in large macroaggregates. The OC and N were highest in the silt + clay fraction, accounting for 34 to 48% of bulk SOC and 28 to 47% of bulk soil N at 0 to 20 cm, and 38 to 62% of bulk SOC and 40 to 62% of bulk soil N at 20 to 40 cm. After 35 yr, N + P fertilization increased SOC (16–35%) and N (21–27%), especially the 540 kg N + 67.5 kg P treatment; the N + P fertilizer also increased the OC and N of the macroaggregate, microaggregate, and silt + clay fractions at 0 to 20 cm. The N + P fertilizer treatments increased bulk soil C/N ratios; the C/N ratios of large macroaggregates decreased. Nitrogen and P fertilizer did not affect aggregate stability. Long-term N and P fertilization increased SOC concentrations, and altered OC and N distributions in aggregates. Quantifying the impacts of long-term fertilization strategies on organic matter sequestration and soil stabilization is important.

1 | INTRODUCTION

Two of the most important components of soil are soil organic carbon (SOC) and N, which play a key role in alleviating land degradation, maintaining soil fertility and quality, and enhancing crop yield (Dou, He, Zhu, & Zhou, 2016; Manna, Swarup, Wanjari, & Mishra, 2007). Both SOC and N concentrations can be significantly affected by agricultural fertilization management (Hai, Li, Li, Suo, & Guggenbergerc, 2010; Malhi et al., 2011). However, improper agricultural management, especially excessive N and P fertilization, may increase organic C (OC) and N loss in soil, through, for example, priming effects (Dai et al., 2016). Excessive mineral fertilizer increases the production of root exudates and litter, and

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Abbreviations: MWD, mean weight diameter; N_1, treatment with 270 kg N ha^{-1} yr^{-1}; N_1P_1, treatment with 270 kg N ha^{-1} yr^{-1} + 67.5 kg P ha^{-1} yr^{-1}; N2P1, treatment with 540 kg N ha^{-1} yr^{-1} + 67.5 kg P ha^{-1} yr^{-1}; N2P2, treatment with 540 kg N ha^{-1} yr^{-1} + 150 kg P ha^{-1} yr^{-1}; N_2, treatment with 540 kg N ha^{-1} yr^{-1}; OC, organic C; P_1, treatment with 67.5 kg P ha^{-1} yr^{-1}; P_2, treatment with 150 kg P ha^{-1} yr^{-1}; SOC, soil organic C.

also increases microbial populations and decomposition processes (Hoffmann, Hoffmann, Jurasinski, Glatzel, & Kuhn, 2014). Although excessive N and P fertilization can increase crop yields (Poffenbarger, Barke., Helmers, & Miguez, 2017; Yang, Chen, & Yang, 2019), it can also have negative environmental impacts, such as soil acidification (Guo et al., 2010) and increased nutrient runoff and pollution of waterways (Li, Liu, Huang, Zhang, & Yang, 2016; Wakida & Lerner, 2005). Several studies have found that excessive use of chemical fertilizers resulted in the loss of 7 to 11% of SOC and 30 to 35% of N in the soil (Bedada, Karltun, Lemenih, & Tolera, 2014; Yan et al., 2014; Zhang et al., 2016). Therefore, minimizing the tradeoff between soil fertility and environmental quality requires the efficient use of N and P fertilizer inputs (Poffenbarger et al., 2018). Improved N and P fertilization management could maintain or enhance long-term agricultural soil fertility (Niu, Hao, Zhang, & Niu, 2011).

Long-term fertilization with only N or P affects not only the soil's quality and physical structure (Niu, Hao, Zhang, & Niu, 2009), but also the biogeochemical cycle of C and N in the soil (Chivenge, Vanlauwe, Gentile, & Six, 2011). The effects of long-term application of N + P fertilization have been extensively studied, and the effects may be positive (Gong, Yan, Wang, Hu, & Gong, 2009; Purakayastha, Rudrappa, Singh, Swarup, & Bhadraray, 2008) or negative (Bedada et al., 2014; Yan et al., 2014; Zhang et al., 2016), or have no effect (Hai et al., 2010, Lou, Xu, Wang, Sun, & Liang, 2011) on SOC concentration. The application of N and P fertilizers significantly enhanced SOC and N concentrations by 15 and 27%, respectively, and also enhanced soil aggregate stability (Dou et al., 2016; Ling et al., 2014; Tian et al., 2017). Conversely, SOC concentration has decreased, partly as a result of long-term excessive application of mineral fertilizer (N, P, K), in the North China Plain region (Yang, Zhao, Huang, & Lv, 2015).

In addition, N and P fertilizer application rates have a significant impact on aggregate-associated OC and N (Fisk, Ratliff, Goswami, & Yanai, 2014; Wang et al., 2018). Microaggregates significantly increased at an N fertilization application rate of 200 kg ha^{-1} yr^{-1} relative to minimum N fertilization (0 kg ha^{-1} yr^{-1}) (Piazza, Pellegrino, Moscatelli, & Ercoli, 2020; Six & Paustian, 2014). Additionally, Piazza et al. (2020) and Stewart, Halvorson, and Delgado (2017) found that high N fertilization (200 kg ha^{-1} yr^{-1}) promoted soil aggregation and SOC accumulation in occluded microaggregates and reduced the activity of soil enzymes while shifting the soil microbial community structure toward taxa that are common under soil degradation. The mechanisms for these effects may be that high N addition decreases soil enzyme activity (Wang et al., 2015a), which leads to the redistribution of the microbial community within macroaggregates and microaggregates (Wang et al., 2018). Furthermore, a low P fertilization rate (<150 kg P_2O_5 ha^{-1} yr^{-1}) reduced soil N concentration (Zhang, Xie, Ni, & Zeng, 2019). Applying P fertilization rates below 10 to 30 kg ha^{-1} will limit the mineralization and enrichment of soil nutrients, resulting in unbalanced soil nutrient levels (Valkama, Uusitalo, Ylivainio, Virkajärvi, & Turtola, 2009). Many studies have measured the cycling of C or N with N + P fertilization in the field (Belanger et al., 2017; Dai et al., 2016). However, it is unclear how N and P fertilization affects aggregate formation and the distribution of soil aggregate-associated OC and N concentration (Wang et al., 2018), and most studies to date have ben short-term (no more than 10 yr) experiments (Li et al., 2020; Zhao et al., 2018).

Core Ideas

- 35-yr continuous N and P fertilization affected C and N distribution in aggregates.
- N + P fertilization did not significantly alter aggregate stability.
- N and P fertilization increased soil organic C content by 16 to 35% over the control.
- N + P fertilization decreased large macroaggregate-associated organic C.

Aggregate stability is a vital factor of soil quality that is influenced by soil fertilization management and land use (Castro, Lourenço, Guimarães, & Fonseca, 2002; Sithole, Magwaza, & Thibaud, 2019), which was indicated by the mean weight diameter (MWD) (Kemper & Rosenau, 1986). Aggregate size distribution can be interpreted from the MWD parameter (Karami, Homaee, Afzalinia, Ruhipour, & Basirat, 2012): when the MWD value is higher, the erosion resistance ability of the soil is stronger (i.e., the soil structure is more stable) (Chaplot & Cooper, 2015). Sithole et al. (2019) found that aggregate stability and soil aggregation increased at application rates of 0 to 100 kg N ha^{-1} yr^{-1} but may decrease at an application rate of 200 kg N ha^{-1} yr^{-1}. By using ^{13}C tracers, Jastrow, Miller, and Boutton (1996) found that large macroaggregates contained more OC than microaggregates. Macroaggregate-associated OC turns over more rapidly than microaggregate-associated OC (Rabbi, Wilson, Lockwood, Daniel, & Young, 2014; Six, Conant, Paul, & Paustian, 2002). Previous studies have shown that large macroaggregates (>0.25 mm) were affected first after fertilization; the number of macroaggregates was reduced and the macroaggregate-associated OC concentration decreased (Post & Kwon, 2010; Whalen, Quancai, & Liu, 2003). The OC concentration decreased in the 0.053- to 0.25-mmaggregates in soil, probably through the fast decay rates of old C in these fractions, compared with large macroaggregates (>2 mm) (Fröberg et al., 2013). In addition, changes in aggregate size and stability can indicate changes in soil

TABLE 1 Soil characteristics in 1983 before the field experiments

Soil layer	pH (H_2O)	SOC	TN	Alkaline N	Available P	Available K
cm		g kg^{-1}		mg kg^{-1}		
0–20	8.0	6.60	0.4	23.50	9.94	94.00

structure and quality (Du et al., 2017b). The effects of different sized aggregates on nutrient retention and supply in soil vary (Lipiec, Walczak, & Witkowska-Walczak, 2007), possibly due to different microbial decomposition rates in each size of aggregate. Nitrogen fertilization can increase plant productivity and accelerate SOC turnover (Dou et al., 2016; Li et al., 2019). Long-term N fertilization (6 yr) might stabilize SOC by increasing macroaggregates and microaggregates within large macroaggregates (Chen et al., 2017). Previous studies have shown that long-term (23 yr) mineral fertilization not only increased N concentration in bulk soil but also in macroaggregates (>2 and 0.25–2 mm) (Wang et al., 2018). However, the responses of aggregate-associated OC and N to long-term application of N or P fertilizers and various fertilizer application rates have not been well understood and soil aggregation may vary in surface soil and subsoil in response to long-term N and P fertilization with different application rates.

Therefore, the main objective of this study was to determine the effects of long-term application of different rates of N, P, or N + P fertilization in the wheat (*Triticum aestivum* L.)–maize (*Zea mays* L.) croplands of the North China Plain on (a) OC and N concentrations in bulk soil, and (b) soil aggregate stability and the distribution of OC and N associated with aggregates at different soil depths. The results are useful in understanding the mechanism of SOC and N stabilization and sequestration. This can aid in developing better N and P fertilization and land management strategies to maintain soil structure and quality in intensive agroecosystems such as in the North China Plain.

2 | MATERIALS AND METHODS

2.1 | Study sites

The long-term fertilization field experiment has been carried out since 1983 at the Quzhou Experimental Station of the China Agricultural University (36°51′N, 115°01′E, 36 m above sea level), located in Quzhou County, Hebei Province, China. In the study area, the mean annual precipitation is about 542.8 mm, with 65.7% falling from July to September. The mean annual temperature is 13.0 °C. This region is within the warm temperate semiarid monsoon climate zone, and the soil type is saline-alkali soil (USDA), the texture is silt loam (10% sand, 78% silt, and 12% clay) (Ludwig, Hu, Niu, & Liu, 2010). The basic chemical properties of the soil before the field experiment are shown in Table 1.

TABLE 2 Amount of nitrogen (N) and phosphorus (P) fertilization

Treatment	N levels	P levels
	kg ha^{-1} yr^{-1}	
Control	0	0
N_1	270	0
N_2	540	0
P_1	0	67.5
P_2	0	150
N_1P_1	270	67.5
N_1P_2	270	150
N_2P_1	540	67.5
N_2P_2	540	150

2.2 | Experimental design and soil sampling

The experiment was a randomized block design with N and P as the two factors at three levels compared with the control treatment without any fertilization (Table 2). The two factors were applied in three replicates as random arrangements to 27 plots (Table 2), each with an area of 46.2 m^2 (4.2 by 11 m) and included three N levels (0, 270, and 540 kg ha^{-1} yr^{-1}) and three P levels (0, 67.5, and 150 kg ha^{-1} yr^{-1}). The N fertilizer was urea (Shanxi Lanhua Technology Venture Co. Ltd) at 46% N and the P fertilizer was superphosphate (Yunnan Yuntianhua International Chemical Co. Ltd) with 43% P_2O_5. Nitrogen fertilizer was incorporated to 20 cm and applied twice during winter wheat and summer maize growth: 30 (N fertilizer) and 50% (P fertilizer) at sowing (1 October) and 20% (N fertilizer) at 176 d after sowing. In maize, 30% of the N fertilizer and 50% of the P fertilizer was applied at sowing (10 June) and 20% (N fertilizer) at 40 d after sowing (20 July). Phosphate fertilizer was applied only once as the base fertilizer before wheat planting. The winter wheat and summer maize were in a double-cropping system, the prevalent agricultural system in the region.

‘Han 6172’ winter wheat (early October to early June) and ‘Zheng dan 958’ summer maize (mid-June to later September) were planted. Depending on rainfall availability, irrigation (60–80 mm each time) was applied to the winter wheat (three times) and maize (two or three times) with a sprinkler system. In June 2017, soil samples from 0- to 20-cm and 20- to 40-cm were collected after winter wheat harvesting to reduce temporal variability (Marschner, Umar, & Baumann, 2011). Three soil cores per plot were randomly collected with a hand auger (4.1 cm in diameter) and pooled to make a composite sample. The soil samples were brought to the laboratory and air-dried at room temperature. Before sieving, large plant residues and stones were removed. After sieving through an 8-mm sieve, the different size aggregates were separated and determined. At the same time, a portion of the samples was sieved through a 2-mm sieve for determination of bulk soil OC, total N concentration, and soil pH.

Soil pH was measured with a glass electrode in a 1:2.5 soil/water suspension. The SOC was determined by a standard potassium dichromate digest method. Total N was measured via the Kjeldahl method. Alkaline N was determined by the alkaline-hydrolyzed diffusion method. To determine the available P, soil samples were first extracted with a $HClO_4$–H_2SO_4 solution and 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5). Subsequently, the Olsen-P method was used. Available K was extracted with an ammonium acetate solution (NH_4OAc, 1 mol L^{-1}) and then determined with a flame photometer.

2.3 | Determination of soil water-stable aggregates

The water-stable aggregates in the soil were separated by wet sieving following the procedure by Cambardella and Elliot (1993). Briefly, 50 g of air-dried bulk soil was spread on top of a 2-mm sieve submerged in deionized water. The soil samples were left immersed in the water for 5 min and then sieved by moving the sieve 3 cm vertically 30 times per minute. The materials remaining on the three sieves (2, 0.25, and 0.053 mm) were transferred to beakers. The large macroaggregates (>2 mm), macroaggregates (2–0.25 mm), microaggregates (0.25–0.053 mm), and silt + clay fractions (<0.053 mm) were dried at 60 ˚C for 48 h. The OC and N concentrations in each aggregate size fraction were determined. The recovery rates of the soil aggregate mass, SOC, and N were 94, 93, and 96%, respectively.

Organic C concentrations in bulk soil and each aggregate were determined via the dichromate oxidation method. For this method, external heating in an oil bath (170–180 ˚C for 5 min) was applied and a correction factor of 1.1 was used (Bao, 2000). The N concentration in bulk soil and each aggregate was analyzed via the Kjeldahl method (Nelson & Sommers, 1973). Briefly, each sample was subjected to high-temperature digestion with concentrated H_2SO_4 and catalysts to convert organic and inorganic forms of N to ammonium. Ammonium is then determined by acidimetric titration followed by alkaline distillation of ammonia. Soil pH was measured with a glass electrode in a 1:2.5 (v/v) soil/water suspension.

2.4 | Data analysis

The mean weight diameter (MWD) was calculated to assess the effects of long-term N and P fertilization at different application rates on soil structure, following the equations from Kemper and Rosenau (1986).

$$\mathrm{MWD} = \sum\nolimits_{i=1}^{n} d_i \times w_i, \tag{1}$$

where d_i is the mean diameter (mm) of each aggregate fraction, w_i is the weight proportion of each aggregate size in the total sample weight, and n is the number of sieves.

$$\mathrm{AOC}_i = \left(\frac{\mathrm{OC}_i \times M_i}{W}\right) \times 100\% \tag{2}$$

$$\mathrm{AN}_i = \left(\frac{N_i \times M_i}{W}\right) \times 100\%, \tag{3}$$

where AOC_i is the aggregate-associated SOC, OC_i is the OCconcentration per aggregate, AN_i is the aggregate-associated nitrogen in the soil, N_i is the N concentration per aggregate, M_i is the aggregate mass, and W is the weight of the bulk soil.

The effects of N fertilization rates, P fertilization rates on SOC and total N, aggregate-associated OC and N, and MWD at each soil depth were analyzed separately via two-way ANOVA. The results of the ANOVA for each indicator were compared among different N and P fertilizers in the same soil layer. However, the difference between the two soil depths was not statistically significant. Mean values were separated (i.e., the difference between the two averages) via the LSD method at the 5% level of significance in Data Processing Station software version 7.05 (Zhejiang University).

3 | RESULTS

3.1 | Effects of N and P fertilization on aggregate mass distribution

Long-term N and P fertilization at different application rates affected the mass distribution of water-stable aggregates (Figure 1). Across all treatments, the silt + clay fraction (<0.053 mm) was dominant, accounting for 36 to 54% of the bulk soil at the 0- to 20-cm depth (Figure 1a). Large macroaggregates constituted the smallest fraction, accounting for 3 to 13% of the bulk soil. Long-term application of N fertilization or P fertilization increased the silt + clay fraction under the 540 kg N ha^{-1} yr^{-1} (N_2) and 150 kg P ha^{-1} yr^{-1}

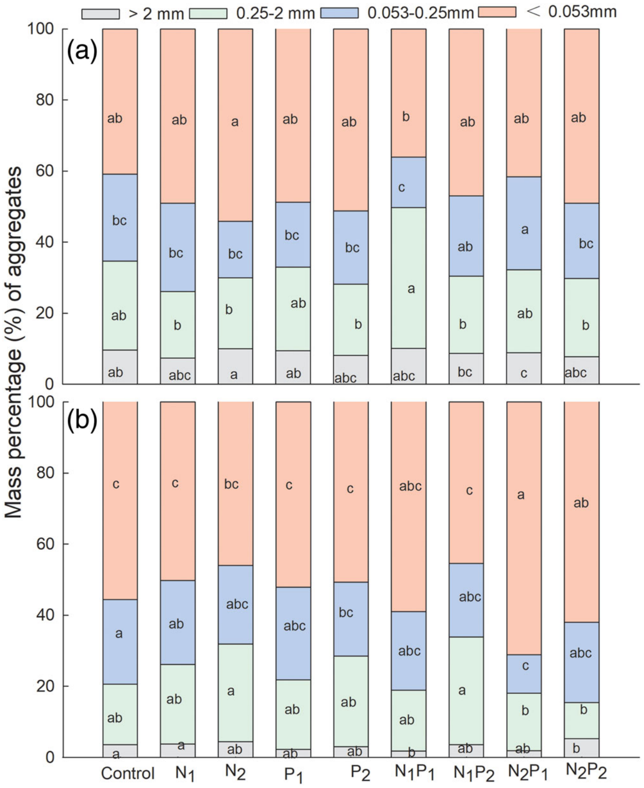

FIGURE 1 Mass distribution of four different size aggregates (>2 mm, 0.25–2 mm, 0.053–0.25 mm, and <0.053 mm) under P and N fertilization at 0 to 20 cm (a) and 20 to 40 cm (b). Bars followed by a similar letter within a soil layer are not significantly different among treatments according to the LSD means separation test. N_1, 270 kg N ha^{-1} yr^{-1}; N_2, 540 kg N ha^{-1} yr^{-1}; P_1, treatment with 67.5 kg P ha^{-1} yr^{-1}; P_2, treatment with 150 kg P ha^{-1} yr^{-1}; N_1P_1, 270 kg N ha^{-1} yr^{-1} + 67.5 kg P ha^{-1} yr^{-1}; N_1P_2, 270 kg N ha^{-1} yr^{-1} + 150 kg P ha^{-1} yr^{-1}; N_2P_1, 540 kg N ha^{-1} yr^{-1} + 67.5 kg P ha^{-1} yr^{-1}; N_2P_2, 540 kg N ha^{-1} yr^{-1} + 150 kg P ha^{-1} yr^{-1}. Similar notation is used in all other figures

(P_2), whereas N + P fertilization increased the proportion by 30% of macroaggregate mass ($P = .06$). In addition, different amounts of N + P fertilization influenced the mass distribution of the macroaggregate, microaggregate, and silt + clay fractions. There was a slight increase in the macroaggregate and microaggregate fractions under N + P fertilization, but the silt + clay fraction decreased compared with other N or P fertilizer treatments, except for the microaggregate fraction in the of N_1P_1 treatment (270 kg N ha^{-1} yr^{-1} + 67.5 kg P ha^{-1} yr^{-1}).

Similar to the 0- to 20-cm depth, the silt + clay fraction accounted for 45 to 71% (23.3 ± 2.4–34.6 ± 2.2 g, $P = .03$) of the mass of the total aggregates at 20- to 40-cm depth. The mass of large macroaggregates was the lowest of the four size aggregates at 20- to 40-cm depth (Figure 1). Compared with the control, long-term application of N fertilizer alone increased the amount of large macroaggregates and macroaggregates, whereas the silt + clay fractions and microaggregates decreased. The combined application of N and P fertilizers increased the silt + clay fraction by 46 and 43% (both $P < .05$), respectively, for N_2P_1 (540 kg N ha^{-1} yr^{-1} + 67.5 kg P ha^{-1} yr^{-1})and N_2P_2 (540 kg N ha^{-1} yr^{-1} + 150 kg P ha^{-1} yr^{-1}).

3.2 | Soil and water-stable aggregate-associated OC and N

Across all treatments, SOC was greatest in the silt + clay fraction, ranging from 34 to 48% at 0- to 20-cm depth (Figure 2). Long-term application of N, P, and N + P fertilization increased OC by 2 to 35% and the N concentration by 3 to 20% in bulk soil at the 0- to 20-cm depth (Figure 2, Figure 3). The OC and N were higher in macroaggregates than in microaggregates, especially under the N_1P_1 treatment. However, N + P fertilization had a much stronger effect on the increase in the OC and N concentrations in bulk soil but decreased the OC (50%) and N (33%) concentrations in the large macroaggregates, compared with N or P fertilization alone. In all treatments, the concentrations of soil N in the silt + clay fraction were the highest (Figure 3). Fertilization rates significantly ($P = .03$) affected the SOC concentration. Higher rates of N fertilizer increased the OC concentration, especially in the silt + clay fraction, whereas P fertilization alone significantly ($P < .05$) affected the N concentration but did not significantly ($P > .05$) affect the SOC concentration.

The soil OC and N concentrations were significantly ($P < .05$) lower at the 20- to 40-cm depth than at the 0- to 20-cm depth (Figure 2B, Figure 3b). Similar to the results at the 0- to 20-cm depth, the long-term application of N-only or P-only fertilizers increased SOC and N concentrations in the bulk soil at 20 to 40 cm in all the treatments compared with the control (Figure 2b, Figure 3b). The increase in the OC and N concentrations in soil was mainly from the macroaggregate and silt + clay fraction, and secondarily from the microaggregate fraction. The SOC concentration of the 540 kg N ha^{-1} yr^{-1} application was higher than that under 270 kg N ha^{-1} yr^{-1}, whereas N concentration in the bulk soil was higher for the application of 270 kg N ha^{-1} yr^{-1}. Nitrogen-only fertilization increased the OC concentration in the silt + clay fraction, whereas P-only fertilization increased the OC concentration in the large macroaggregates and macroaggregates.

3.3 | Organic carbon and N concentrations in aggregate fractions

Long-term fertilization with N and P significantly ($P = .03$) affected the OC and N concentrations in the different size aggregates (Figure 4, Figure 5). In all treatments, the

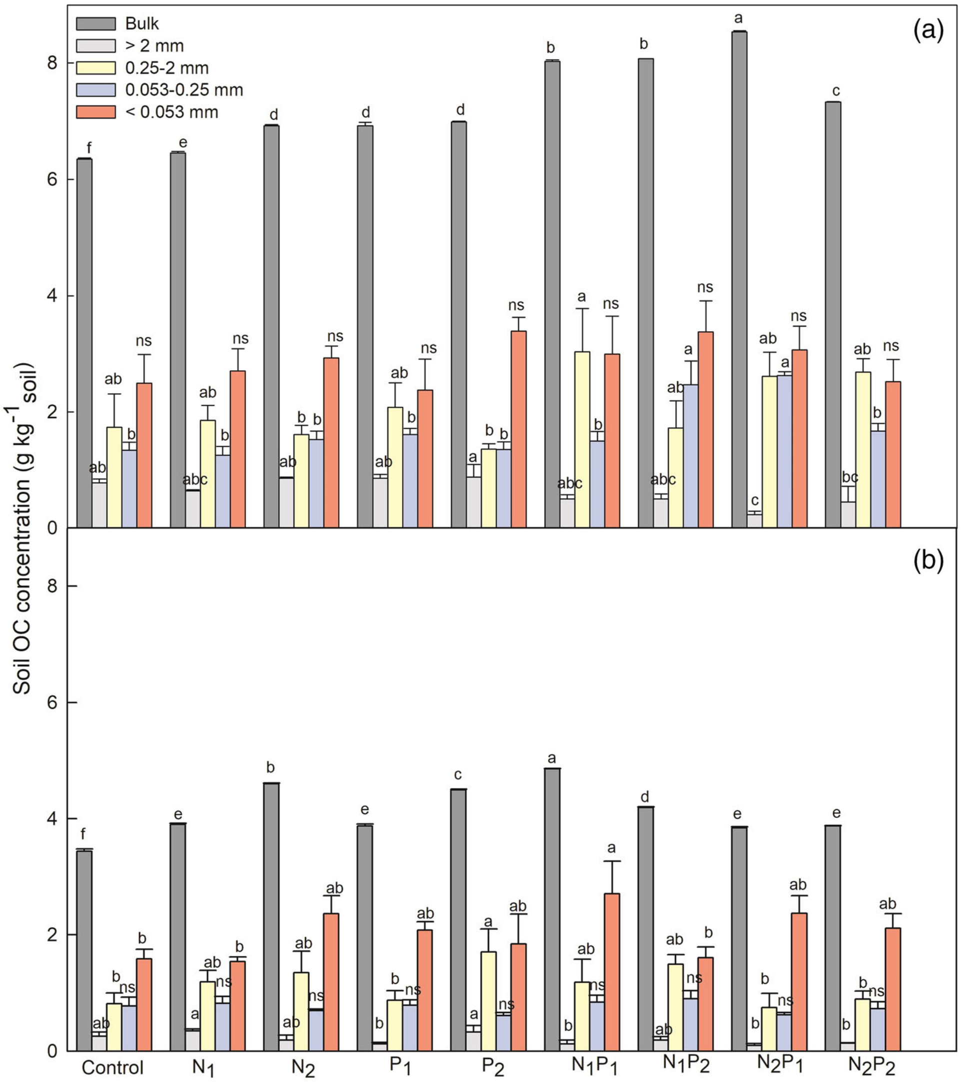

FIGURE 2 Contribution of organic carbon (OC) in aggregates (>2 mm, 0.25–2 mm, 0.053–0.25 mm, and <0.053 mm) to soil OC (g kg^{-1} soil) at 0 to 20 cm (a) and 20 to 40 cm (b). Bars followed by a similar letter within a soil layer are not significantly different among treatments according to the LSD means separation test. ns, not significant

silt + clay fraction had the lowest OC and N concentrations (5.70 g C kg^{-1} aggregate, 0.66 g N kg^{-1} aggregate) per aggregate weight compared with the other fractions at 0- to 20-cm depth (Figure 4a, Figure 5a). Except for in the silt + clay fractions, N + P fertilization increased the N concentration in aggregates relative to other treatments, particularly for the large macroaggegates and macroaggregates (Figure 5a). Nitrogen-only fertilization increased the OC concentration in the macroaggregates, whereas P-only fertilization reduced the OC concentration in the microaggregates. The N_2P_2 treatment had the largest OC concentration in the macroaggregates (Figure 4a) and N_2P_2 had the largest N concentration in the large macroaggregate and macroaggregate fractions (Figure 5a).

For similarly sized aggregates, OC and N concentrations were lower at 20 to 40 cm than at 0 to 20 cm (Figure 4b, Figure 5b). At the depth of 20 to 40 cm, the OC concentration of the N_2P_2 and N_2P_1 treatments was significantly (P = .04) higher in large macroaggregates than under other treatments. The N concentrations under P_1 (67.5 kg P ha^{-1} yr^{-1}), N_1P_1, and N_2P_1 were significantly higher than under other treatments in the large macroaggregates and macroaggregates (Figure 5b). The combined application of N and P fertilizers increased the OC and N concentrations in the large macroaggregates, macroaggregates, and microaggregates at the 20- to 40-cm depth.

3.4 | Effects of N and P fertilization on aggregate stability

Long-term fertilization in separate N and P fertilizers did not significantly affect soil aggregate stability, as indicated by the

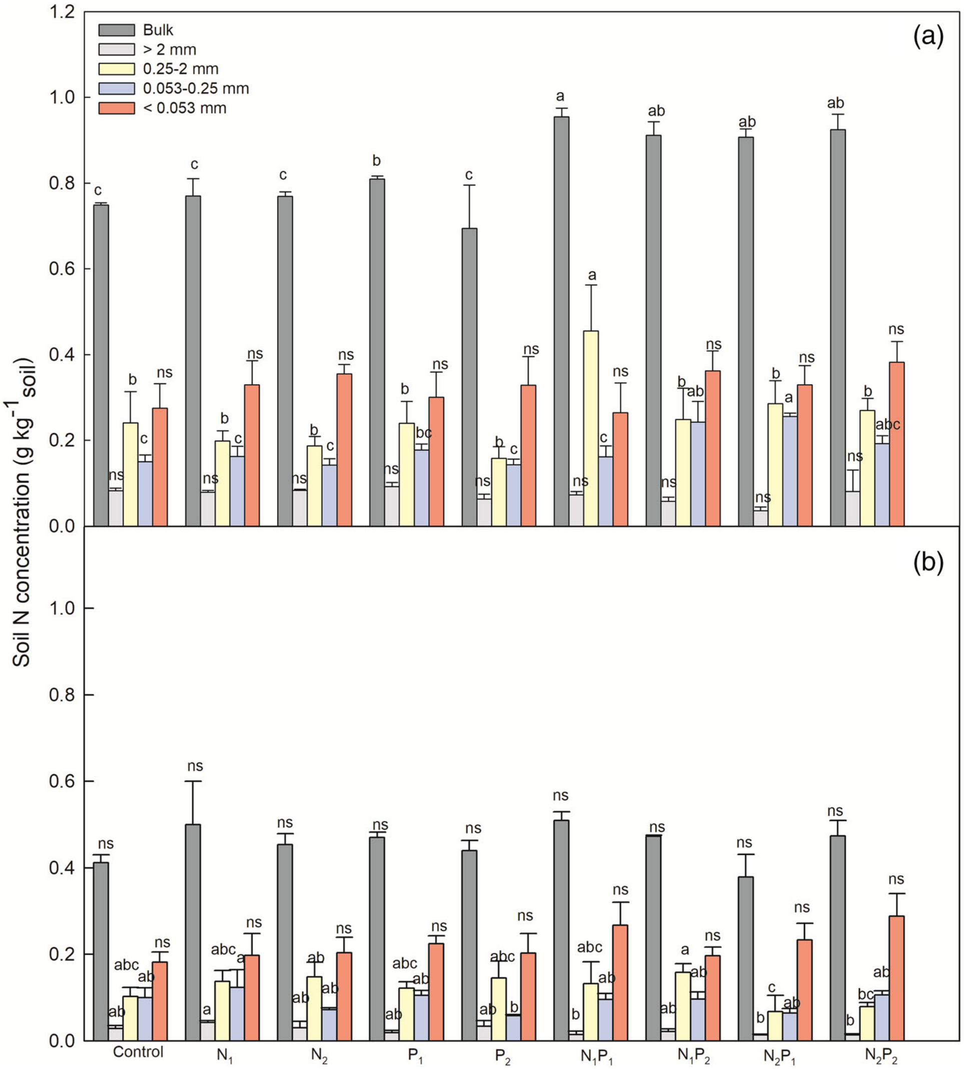

FIGURE 3 Contribution of N in aggregates (>2 mm, 0.25–2 mm, 0.053–0.25 mm, and <0.053 mm) (g kg^{-1} soil) from 0 to 20 cm (a) and 20 to 40 cm (b) to soil N under long-term N and P fertilization. Bars followed by a similar letter within a soil layer are not significantly different among treatments according to the LSD means separation test. ns, not significant

similar MWD at 0 to 20 and 20 to 40 cm. The MWD tended to decrease with depth compared with the control (Figure 6). At 0 to 20 cm, N_2P_1 had significantly ($P < .05$) lower aggregate stability than other treatments. At 20 to 40 cm, the aggregate stability in the N_2P_2 treatment was the lowest among all the treatments.

3.5 | Carbon/nitrogen ratios in bulk soil and aggregates

The C/N ratios in bulk soil were unaffected by the different treatments (Table 3). However, the C/N ratios in large macroaggregates significantly ($P < .05$) decreased under N + P fertilization. The N_2P_2 treatment significantly ($P < .05$) increased the C/N ratios of the 0.25- to 2-mm aggregates, whereas the N_1P_1 and 270 kg N ha^{-1} yr^{-1} + 150 kg P ha^{-1} yr^{-1} treatments reduced the C/N ratios in the 0.25- to 2-mm aggregates. The N_2 treatment significantly ($P < .05$) increased the C/N ratios of the microaggregate and silt + clay fractions. Under the P_1 and P_2 treatments, the C/N ratios in the large aggregate and silt + clay fractions increased significantly ($P < .05$) relative to other treatments (Table 3).

3.6 | Soil pH

After 35 yr, N fertilization or P fertilization did not significantly ($P > .05$) affected soil pH. However, N + P fertilization tended to decrease soil pH compared with the control at the 0- to 20-cm depth (Table 4). At 20 to 40 cm,

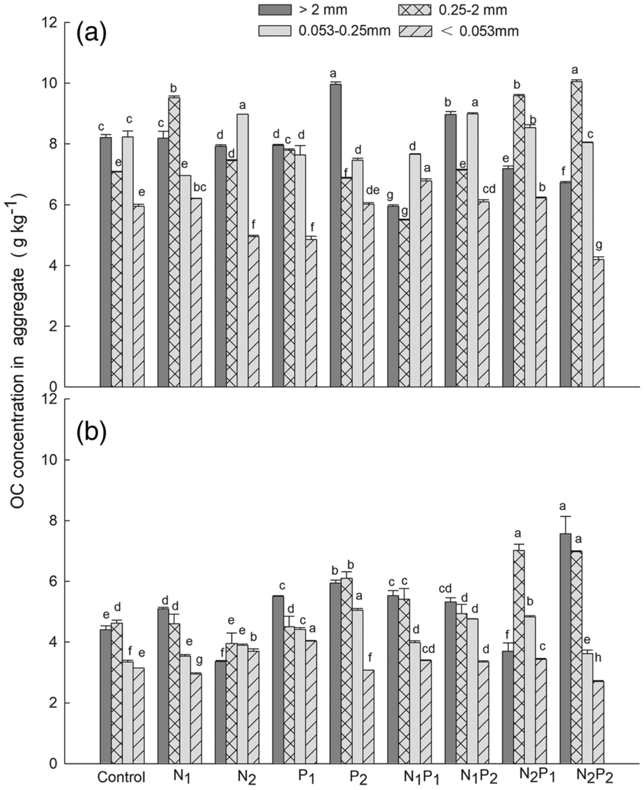

FIGURE 4 Organic carbon (OC) concentrations in four different sizes of soil aggregates at 0 to 20 cm (a) and 20 to 40 cm (b). Bars followed by a similar letter within a soil layer are not significantly different among treatments according to the LSD means separation test

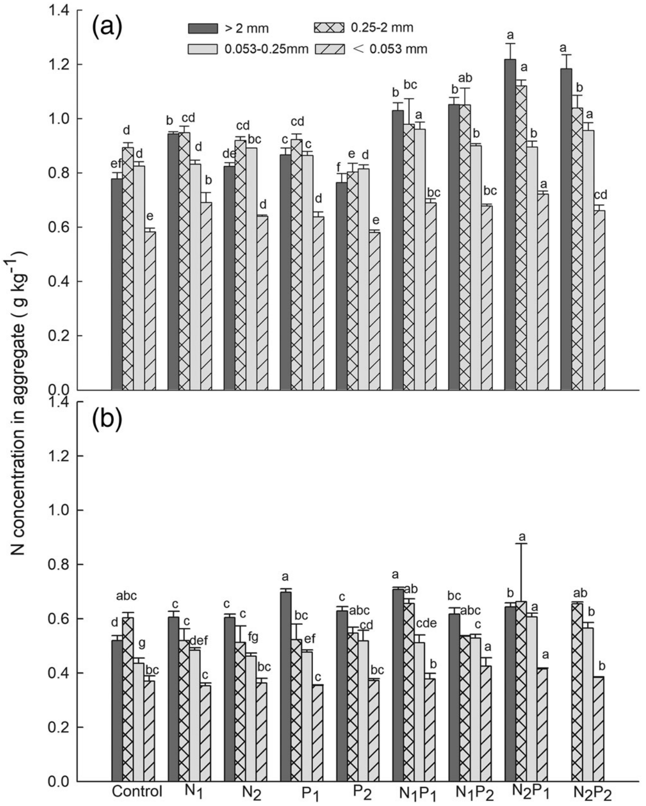

FIGURE 5 Nitrogen concentrations in different soil aggregate fractions at 0 to 20 cm (a) and 20 to 40 cm (b). Bars followed by a similar letter within a soil layer are not significantly different among treatments according to the LSD means separation test

chemical fertilization decreased soil pH when compared with the control.

4 | DISCUSSION

4.1 | Effects of long-term N and P fertilization on bulk soil OC and aggregate-associated OC concentrations

Long-term fertilization with N, P, or N + P not only enhanced SOC concentrations but also altered the OC distribution among aggregates of different sizes. With increasing rates of N fertilization, such as the application rate of 540 kg N ha^{-1} yr^{-1} compared with 270 kg N ha^{-1} yr^{-1}, the OC concentration in the large macroaggregates decreased but the macroaggregate-associated OC and the bulk soil OC concentration increased (Figure 2). Our results were similar to those of Janssens, Dieleman, Luyssaert, and Subke (2010), who found that adding N increased SOC sequestration through the reduction in soil respiration. The mechanism of this effect of N addition on increased OC stabilization could be through increased microaggregation within the macroaggregates (Chen et al., 2017). In addition, the increased soil acidity induced by N and P fertilization would decrease calcium carbonate (Fan et al., 2014), because the carbonates can increase large macroaggregates but decrease microaggregates (Boix-Fayos, Calvo-Cases, Imeson, & Soriano-Soto, 2001). Another possible mechanism is that unbalanced fertilization, particularly excess N without appropriate straw return or organic amendments, reduces SOC sequestration. Six, Elliott, and Paustian (1999) pointed out that microaggregates form around the OC particles inside large macroaggregates, so when OC is decomposed, large aggregates are broken and directly form the small aggregates. Across all treatments, SOC was mostly in the silt + clay fraction, ranging from 34 to 48% of SOC, whether at the 0- to 20-cm depth or the 20- to 40-cm depth (Figure 2). This result agreed with previous studies that used the same method, which found that in agricultural soils, specifically those of silty-loam soil texture, most of the OC is stored in the silt + clay fraction (Denef, Zotarelli, Boddey, & Six, 2007; Six, Elliott, & Paustian, 2000a).

The higher N and lower P fertilization (N_2P_1) treatment had the highest SOC concentration in bulk soil, whereas the N_2P_2 treatment significantly reduced the SOC concentration (Figure 2). The reduced SOC concentration was partially

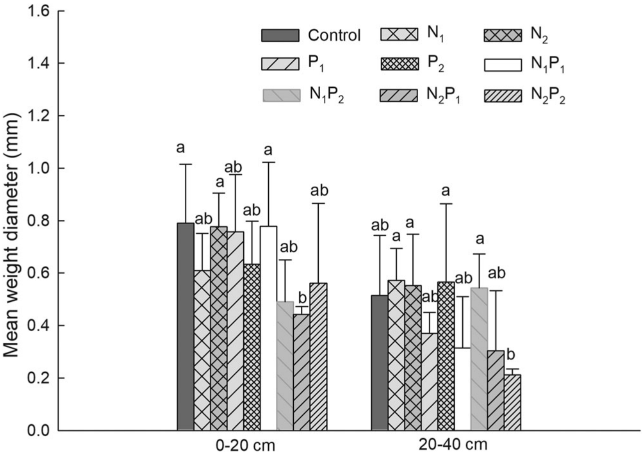

FIGURE 6 Mean weight diameter (MWD) of different treatments. Bars followed by a similar letter within a soil layer are not significantly different among treatments according to the LSD means separation test

because the increased P fertilization stimulated microbial activity during the decomposition of organic matter in soil (Neff, Townsend, Gleixner, & Lehman, 2002). The reduction caused by N + P fertilization in the macroaggregate-associated OC concentration resulted from (a) the decreased mass proportion of large macroaggregates in the soil (Figure 1), and (b) the reduced OC concentration in large macroaggregates (Figure 4). The results are similar to those of previous studies using the same method under similar crop rotations and soil types, which showed that N + P fertilization may have reduced C/N or C/P ratios in soils that would have otherwise been resistant to microbial degradation, and may also have stimulated soil microbial growth and promoted the decomposition of labile organic matter fractions, thereby decreasing the formation and stabilization of macroaggregates (Du, Ren, Hu, & Zhang, 2015; Six, Paustian, & Elliott, 2000b), as well as decreasing the OC concentration in the macroaggregates (Feng, Simpson, & Schlesinger, 2010; Kuzyakov, Friedel, & Stahr, 2000).

4.2 | Effect of long-term N and P fertilization on bulk soil N and aggregate-associated N concentrations

Nitrogen or P fertilizer had no significant effect on soil N and aggregate-associated N concentrations (Figure 3, Figure 5), which was in line with previous studies with different soil textures (Ahmed, Anjum, & Zhang, 2017; Chen et al., 2017; Li et al., 2010). The N concentration was lower at the 20- to 40-cm depth than at the to 0- to 20-cm depth (Figure 3, Figure 5). The N concentration in bulk soil under P_2 treatment was lower than under the other treatments, possibly caused by the increased crop yields in the P_2 treatment relative to P_1 (75 kg ha^{-1} yr^{-1}), resulting in greater N removal over time and decreasing the N concentration in the soil (Zhang et al., 2019). These results are in line with those of previous studies using the same method (Dou et al., 2016; Tian et al., 2015). Soil N mineralization ability and root growth can be improved by N fertilization (Dai et al., 2015; Shi et al., 2012), thereby increasing organic matter input into the soil. However, in the present study, increased N or P fertilization rates decreased N concentration in the 20- to 40-cm soil layer, mostly in the silt + clay fraction, and there was no significant difference among treatments (Figure 5b). In addition, with the increased N application rate, N concentrations tended to decrease (Figure 3), as seen previously by Sodhi, Beri, and Benbi (2009) and Zhu, Lei, and Shangguan (2018), which may have been caused by the alterations in the microbial community structure, diversity, subsequent function, and

TABLE 3 The C/N ratios in bulk soil and different sized aggregates (mean ± SE, $n = 3$)

Treatment	Bulk soil	Aggregates			
		>2 mm	0.25–2 mm	0.053–0.25 mm	<0.053 mm
Control (no N, no P)	8.91 ± 0.03a	10.59 ± 0.25b	8.47 ± 0.24b	10.38 ± 0.24abc	10.73 ± 0.08ab
N_1 (270 kg N ha^{-1} yr^{-1}, no P)	8.71 ± 0.43a	8.75 ± 0.02d	9.76 ± 0.17a	9.16 ± 0.15ef	9.63 ± 0.25c
N_2 (540 kg N ha^{-1} yr^{-1}, no P)	9.23 ± 0.18a	10.04 ± 0.07bc	8.37 ± 0.12b	10.67 ± 0.01a	8.29 ± 0.05e
P_1 (no N, 67.5 kg P ha^{-1} yr^{-1})	8.79 ± 0.11a	9.31 ± 0.17 cd	9.24 ± 0.19ab	9.14 ± 0.24de	7.70 ± 0.19e
P_2 (no N, 150 kg P ha^{-1} yr^{-1})	11.05 ± 1.67a	13.63 ± 0.39a	9.36 ± 0.12ab	9.28 ± 0.21 cd	11.54 ± 0.06a
N_1P_1 (270 kg N ha^{-1} yr^{-1}, 67.5 kg P ha^{-1} yr^{-1})	9.96 ± 1.37a	5.96 ± 0.06e	6.05 ± 0.34d	8.50 ± 0.05f	10.29 ± 0.13b
N_1P_2 (270 kg N ha^{-1} yr^{-1}, 150 kg P ha^{-1} yr^{-1})	9.25 ± 0.23a	8.73 ± 0.12d	6.82 ± 0.26 cd	10.56 ± 0.01ab	9.16 ± 0.11 cd
N_2P_1 (540 kg N ha^{-1} yr^{-1}, 67.5 kg P ha^{-1} yr^{-1})	10.32 ± 0.66a	6.11 ± 0.12e	9.08 ± 0.12ab	9.95 ± 0.35bcd	9.07 ± 0.09d
N_2P_2 (540 kg N ha^{-1} yr^{-1}, 150 kg P ha^{-1} yr^{-1})	8.93 ± 0.92a	5.86 ± 0.09e	9.60 ± 0.47a	7.97 ± 0.19ef	6.49 ± 0.33f

TABLE 4 Soil pH under long-term N and P fertilization (mean ± SE, *n* = 3)

	pH	
Treatments	**0–20 cm**	**20–40 cm**
Control (no N, no P)	7.80 ± 0.00 abc	7.86 ± 0.02 a
N_1 (270 kg N ha^{-1} yr^{-1}, no P)	7.83 ± 0.01 ab	7.80 ± 0.02 ab
N_2 (540 kg N ha^{-1} yr^{-1}, no P)	7.87 ± 0.03 a	7.81 ± 0.01 ab
P_1 (no N, 67.5 kg P ha^{-1} yr^{-1})	7.69 ± 0.07 cdc	7.73 ± 0.01 c
P_2 (no N, 150 kg P ha^{-1} yr^{-1})	7.80 ± 0.02 abc	7.80 ± 0.00 ab
N_1P_1 (270 kg N ha^{-1} yr^{-1}, 67.5 kg P ha^{-1} yr^{-1}	7.74 ± 0.05 bcd	7.76 ± 0.04 bc
N_1P_2 (270 kg N ha^{-1} yr^{-1}, 150 kg P ha^{-1} yr^{-1})	7.73 ± 0.02 bcd	7.80 ± 0.01 ab
N_2P_1 (540 kg N ha^{-1} yr^{-1}, 67.5 kg P ha^{-1} yr^{-1})	7.65 ± 0.06 d	7.78 ± 0.03 bc
N_2P_2 (540 kg N ha^{-1} yr^{-1}, 150 kg P ha^{-1} yr^{-1})	7.70 ± 0.03 cd	7.73 ± 0.02 c

biological soil crusts that occur with increased N (Shen et al., 2019; Wang et al., 2015b). In the studied soil, the proportion of OC and N in the large macroaggregates decreased significantly, whereas the proportions of OC and N in the silt + clay fractions increased. This result could be related to the destruction of the large macroaggregates by traditional tillage methods and the reduction of particulate organic matter in large macroaggregates (Zhang et al., 2020).

4.3 | Effects of N and P fertilization on aggregate stability

Separate N and P fertilization did not significantly alter aggregate stability, compared with the N + P fertilizer application (Figure 6). Our results were similar to those of Xin, Zhang, Zhu, and Zhang (2016), who reported that aggregate stability did not differ between N or P fertilization and the control treatment under similar N and P application levels and soil depths to those on our study. The N_2P_1 and N_2P_2 treatments had significantly lower MWD at 0 to 20 and 20 to 40 cm than all other treatments, respectively (Figure 6). Generally, MWD was higher at 0 to 20 cm than at 20 to 40 cm for all the treatments. The results might be attributed to the higher amount of root residues in the surface soil than in deep soil layers (Cambardella & Elliott, 1993; Six et al., 2000a) and the higher physicochemical protection of C provided by soil aggregates in surface soil than at the subsurface levels (Chen et al., 2017).

The MWD increased with increasing N fertilization and decreased with P fertilization (Figure 6). These results were comparable with those of a previous study in which N fertilization decreased MWD and the proportion of macroaggregates, although that study had a different soil texture and the studied soil depth (Simansky, Tobiašová, & Chlpík, 2008). The relatively lower aggregate stability under N + P fertilization (N_1P_1, N_2P_1, and N_2P_2) might be attributed to the lower ratio of large macroaggregate mass to bulk soil mass (Figure 1) and lower OC concentrations in the large macroaggregates (Figure 2). Blanco-Canqui and Schlegel (2013) found that the mass of macroaggregates reduced with excessive application rates of P or N fertilizer. Previous studies have suggested that MWD is positively correlated with large macroaggregate-associated C; however, large macroaggregate-associated OC was minimal (Ghosh et al., 2018). Our results showed that the OC concentration in the large macroaggregates was lower than that in the other fractions (Figure 2), which was primarily because of the small mass of large macroaggregates. Therefore, long-term N and P fertilization affected the stability and size distribution of soil aggregates (Meng et al., 2014; Wei et al., 2011). However, the MWD of the control was higher than that of the other fertilizer treatments in this study (Figure 6), which may be because of large soil particles (>5 mm) in the control samples.

Overall, one of the major ways that long-term fertilization affected C cycling was by regulating soil aggregation. Our results revealed that mineral fertilization promoted changes in both the masses of different sized aggregates, as well as OC and N in bulk soil, which, in turn, affected the OC and N concentrations in aggregates. Further research is needed to determine how long-term N or P fertilizer application affects the soil structure and aggregate stability by influencing soil microbial biomass and community composition.

5 | CONCLUSIONS

Our results showed that long-term (35-yr) fertilization of N, P, and N + P at different application rates increased SOC and N concentrations by 16 to 35 and 14 to 22%, respectively, compared with the no-fertilizer treatment, in the wheat–maize cropland of the North China Plain. Long-term N + P fertilization had a much stronger effect on enhancing the SOC concentration than N fertilization or P fertilization alone.

Long-term application of N, P, and N + P fertilization altered the distribution of OC and N in different sized aggregates, although aggregate stability was not significantly altered, except under the N_2P_1 treatment. Among all aggregate sizes, SOC concentration was primarily distributed in the silt + clay fractions, with 34 to 48% of SOC. The N + P fertilization significantly decreased the large macroaggregate-associated OC, with this effect being driven by both the lower OC concentration in large macroaggregates and the lower mass proportions of large macroaggregates in the soil under fertilization. Additionally, different fertilization rates of N + P seemed to affect SOC concentrations, N concentrations, and the distribution of OC and N among the different sized aggregates. Therefore, our results suggest that optimized application rates of N + P fertilization are needed to maximize soil fertility and maintain soil structure. This would allow one to better uncover the role of soil stability in the control of SOC dynamics in the wheat–maize croplands of the North China Plain.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Huayan Zhang: data curation, software, writing – original draft. Ling'an Niu: data curation, methodology, project administration, resources, supervision, validation. Kelin Hu: investigation, supervision, validation. Jinmin Hao: funding acquisition, project administration, resources. Fan Li: data curation, investigation. Xiang Wang: project administration, software, writing – review and editing. Hong Chen: data curation, investigation.

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