



## Article

# Soil Aggregates and Aggregate-Associated Carbon and Nitrogen in Farmland in Relation to Long-Term Fertilization on the Loess Plateau, China

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**Abstract:** Soil aggregation plays a critical role in the maintenance of soil structure and crop productivity. Fertilization influences soil aggregation, especially by regulating soil organic carbon (SOC) and total nitrogen (TN) contents in aggregate fractions. Here, we conducted a fixed-site field experiment to quantify the effect of five N application rates: 0, 75, 150, 225, and 300 kg·N·ha<sup>-1</sup>, denoted as N0, N75, N150, N225, and N300, respectively, on soil aggregate stability, aggregate-associated SOC and TN sequestration and crop productivity. Soil aggregates were divided into >0.25 (>5, 5–2, 2–1, 1–0.5, 0.5–0.25) and <0.25 mm through wet and dry sieving methods. The results showed that long-term fertilization increased the proportion of macro-aggregates (>0.25 mm), decreased the proportion of micro-aggregates (<0.25 mm), and improved the aggregates stability. Compared with N0, the proportion of micro-aggregates in N225 was significantly decreased by 66.45% under wet sieving, while the proportion of >5 mm macro-aggregates in N225 was significantly increased by 19.24% under dry sieving ( $p < 0.05$ ). With the increase in N application rate, the bulk SOC and TN contents first increased and then decreased, and the SOC and TN of N225 were significantly increased by 17.75% and 72.33% compared with N0 ( $p < 0.05$ ). More specifically, fertilization promoted the distribution and enrichment of SOC and TN in macro-aggregates and reduced the C/N of the micro-aggregates and the contribution of SOC and TN in the micro-aggregates. Compared with N0, the contribution rate of macro-aggregates to SOC and TN of N225 under wet sieving was significantly increased by 84.13 and 17.18%, respectively, while the C/N of micro-aggregates of N225 under wet and dry sieving methods was significantly decreased by 45.95 and 31.74%, respectively ( $p < 0.05$ ). Moreover, fertilization improved the yield, and N225 significantly increased the total yield by 80.68% compared with N0 ( $p < 0.05$ ). In conclusion, N225 was the suitable N application for improving soil aggregate stability, carbon and nitrogen sequestration, and crop productivity on the Loess Plateau, China.

**Keywords:** fertilization; soil aggregate stability; soil organic carbon; total nitrogen; aggregate-associated carbon and nitrogen; yield



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## 1. Introduction

Soil aggregates are the basic unit of soil structure. The distribution and structure of soil aggregates play an important role in maintaining good physical properties, maintaining fertilizer, and reducing soil erosion [1]. According to their various formation processes, they are mainly separated into micro-aggregates (<0.25 mm) and macro-aggregates (>0.25 mm) [2]. Soil aggregates and SOC are closely correlated [1,3,4]. Since soil aggregates provide physical protection for soil organic matter (SOM), increase organic carbon stability, and protect internal organic carbon from microbial deterioration [5]. Therefore, the

structure and dynamics of soil aggregates could be crucial in regulating C and N sequestration. In addition, SOC is an essential cementitious material that can improve soil particle agglomeration and improve the soil aggregate structure [1,3]. As a result, characterizing the soil aggregates distribution and aggregate stability and the distribution of carbon and nitrogen can assist in determining if farmland soils are a source or sink of carbon in the global carbon cycle and how the OM cycle alters following fertilizer application.

The Loess Plateau has contributed considerably to the country's food supply in Northwest China [6]. However, soil problems caused by over-fertilization are endless and extensive. Under intensive agricultural cropping systems, farmers overuse mineral fertilizers for maximum yield, leading to heavy native SOC mineralization [7]. The poor soil caused by severe soil erosion and excessive SOC mineralization has become one of the factors limiting sustainable agricultural development in this region [8]. As a result, improving soil carbon sequestration through sensible agricultural management strategies holds great potential. Numerous innovations in N management have been suggested to assure food security and lessen environmental effects. For example, reducing fertilizer application promoted N utilization rate [9], combining the application of organic and inorganic fertilizers, and adding straw to fertilizers [10]. Previous research has demonstrated that these improved N fertilization methods have significantly increased carbon and nitrogen sequestration [11] while decreasing negative environmental effects [12]. There needs to be more information on the effects of improved N management on SOC and TN sequestration and aggregate-associated C and N in fields with multi-year straw returning in this region.

It is difficult to identify the influence of N input on soil aggregates due to the complexity of soil structure and the decomposition of each aggregate size fraction [13]. N addition may indirectly affect soil structure by simultaneously changing biological factors (mainly roots and fungal mycelium), but it may also affect soil structure by affecting abiotic soil properties [14]. Zhou et al. (2013) studied the influence of organic and inorganic fertilization on soil agglomeration using X-ray scanning aggregate technology. They pointed out that organic fertilizer could promote soil agglomeration, while inorganic fertilizer had no effect after 25 years [15]. According to Guo et al. (2022) 12-year fertilization experiment, N application can degrade soil agglomeration by increasing ammonium ions and reducing biological binding agents. Especially when N application exceeds a certain threshold, soil agglomeration decreases. However, straw returning can significantly increase the content of aggregate agents (SOC, GRSP, and MBC) and promote soil aggregation [16]. Mustafa et al. (2020) pointed out that long-term application of NPK fertilizer and combined application of organic and inorganic fertilizers can improve soil agglomeration, stability, and the associated OC and TN stocks in aggregates, as well as the associated OC mineralization, which is controlled by the aggregate size [17]. Yan et al. (2021) studied the effects of different N application rates on aggregate-related carbon and nitrogen, indicating that  $120 \text{ kg N ha}^{-1}$  could effectively increase SOC and TN under a wheat–soybean planting system and increase the content of water-stable macro-aggregates [18]. In addition, due to soil heterogeneity and different management measures, previous studies on the effects of fertilization on soil aggregate carbon and nitrogen sequestration were inconsistent. For example, Zou et al. (2018) reported that SOC and TN contents declined as particle size increased [19]. Gelaw A M. et al. (2015) also noted that soil clay particles were primarily responsible for higher SOC and TN contents [3]. On the contrary, Zheng et al. (2018) pointed out that SOC and TN are mainly distributed in the  $>1 \text{ mm}$  size fraction aggregate [20]. Studies also demonstrated that macro-aggregate dynamics influence SOM stabilization [21]. However, the effects of improved N management on soil aggregates stability, carbon and nitrogen sequestration, and SOC and TN concentration of aggregate fractions in this region still need to be clarified.

The aggregate size fraction distribution and the SOC and TN distributions of aggregates are affected by the choice of the aggregate sieving process [22]. Wet sieving aggregates analysis is more suitable for evaluating and predicting erosion due to rainfall [23]. Therefore, the wet sieving method is frequently used to examine the turnover and stability of

SOC, the C and N distributions of aggregate fractions, soil fertility status, water retention capacity, corrosion resistance, and other features [4,24]. Nevertheless, it is unavoidable that the usage of water, osmotic expansion pressures, and cementing chemicals will cause aggregates to disintegrate and lose their soluble and microbial carbon contents [23]. On the other hand, dry sieving aggregates analysis understandably relates to wind erosion effects, which profoundly influence soil fertility, resistance to degradative forces, and soil erosion [25]. However, numerous studies have evaluated aggregate stability and size distribution, emphasizing the wet sieving method or dry sieving only [23,24]. Only very few studies have focused on both wet and dry sieving methods simultaneously for aggregate distribution [25], and there are even fewer records of such studies in this study area. Considering the climatic conditions and the characteristics of farmland soil in the Loess Plateau, it is essential to explore further the fertilization management measures for soil resistance to wind erosion and water erosion.

However, SOC and TN accumulation and dynamics are lengthy, and long-term experiments can produce convincing results. The goals were to: (i) determine how long-term N fertilization would affect the aggregate size distribution pattern and aggregate stability using both wet and dry sieving methods; (ii) measure the SOC and TN distribution pattern; (iii) analyze the potential sequestration or depletion of SOC and TN in various aggregate size fractions and the impact of soil aggregation on the storage of SOC and TN; (iv) the impact of N fertilization on C/N ratio of aggregate fractions; (v) and the effect of N fertilization on crop yield. We hypothesized that appropriate N application would benefit soil aggregate stability, carbon and nitrogen sequestration, and crop productivity. In addition, we aim to provide farmers in the area with suitable fertilization management options for sustainable agricultural development on the Loess Plateau, China.

## 2. Materials and Methods

### 2.1. Site Description

The experiment was carried out at the Institute of Water-saving Agriculture in arid areas of China (IWSA) (108°04' E, 35°20' N). The experimental site is located 524 m above sea level. The annual average temperature is 13.5 °C, the average annual rainfall is about 550–660 mm, and the annual mean evaporation is 993.2 mm. The site belongs to the warm temperate semi-humid monsoon climate, and the precipitation is mainly concentrated from July to September each year. The soil was classified as Eum-orthic Anthrosol (Udic Haplustalf in the USDA system). Soil physical and chemical properties (0–60 cm) at the experiment's beginning are listed in Table 1.

**Table 1.** Soil physical and chemical properties of different soil layer depths before sowing.

| Soil Depth (cm)                                   | 0–10             | 10–20            | 20–40            | 40–60            |
|---------------------------------------------------|------------------|------------------|------------------|------------------|
| Bulk density ( $\text{g}\cdot\text{cm}^{-3}$ )    | $1.22 \pm 0.56$  | $1.25 \pm 0.48$  | $1.29 \pm 0.25$  | $1.31 \pm 0.17$  |
| SOC ( $\text{g}\cdot\text{kg}^{-1}$ )             | $9.12 \pm 0.32$  | $8.11 \pm 0.27$  | $4.21 \pm 0.18$  | $3.97 \pm 1.55$  |
| TN ( $\text{g}\cdot\text{kg}^{-1}$ )              | $0.94 \pm 0.03$  | $0.90 \pm 0.04$  | $0.51 \pm 0.05$  | $0.36 \pm 0.02$  |
| Available P ( $\text{mg}\cdot\text{kg}^{-1}$ )    | $12.51 \pm 0.59$ | $11.57 \pm 0.33$ | $6.05 \pm 0.27$  | $5.84 \pm 0.43$  |
| Available K ( $\text{mg}\cdot\text{kg}^{-1}$ )    | $93.74 \pm 3.08$ | $86.70 \pm 5.34$ | $74.26 \pm 4.85$ | $62.85 \pm 3.58$ |
| pH                                                | $7.89 \pm 0.47$  | $7.80 \pm 0.58$  | $7.21 \pm 0.38$  | $7.04 \pm 0.29$  |
| Sand (%)                                          | $5 \pm 0.17$     | $4 \pm 0.21$     | $2 \pm 0.08$     | $2 \pm 0.18$     |
| Silt (%)                                          | $68 \pm 1.58$    | $65 \pm 2.37$    | $58 \pm 3.01$    | $56 \pm 2.44$    |
| Clay (%)                                          | $27 \pm 0.88$    | $31 \pm 0.75$    | $42 \pm 0.98$    | $44 \pm 0.67$    |
| Textural class (USDA)                             | Silty clay loam  | Silty clay loam  | Silty clay       | Silty clay       |
| $\text{CaCO}_3$ ( $\text{g}\cdot\text{kg}^{-1}$ ) | $46 \pm 1.14$    | $48 \pm 1.20$    | $50 \pm 1.25$    | $53 \pm 1.35$    |

### 2.2. Experimental Design and Management

A fixed-site field experiment was carried out for consecutive years (2010–2020). The planting system is a wheat–maize cropping system. A single-factor randomized complete block design was used in the experiment with five N application rates with three replicates:

0, 75, 150, 225, 300 kg·N·ha<sup>-1</sup> (N0, N75, N150, N225, and N300). The planting area of the plot was 18 m<sup>2</sup> (3 m × 6 m). The winter wheat (Xinong-979) was sown in mid to late October, planted with a 20 cm inter-row spacing, sowing amount of 225 kg·ha<sup>-1</sup>, and harvested in early June next year. Summer maize (Zhengdan-958) was sown in mid of June, planted with a 60 cm inter-row spacing, sowing 83,333 plants·ha<sup>-1</sup>, and harvested in early October. All P and K fertilizers were uniformly spread as base fertilizers at 150 kg·ha<sup>-1</sup> (calcium phosphate) P<sub>2</sub>O<sub>5</sub> and 60 kg·ha<sup>-1</sup> K<sub>2</sub>O (potassium chloride). N fertilizer in the wheat season was used as base fertilizer, and N fertilizer in the maize season was applied according to a topdressing ratio of 4:6. Topdressing was carried out at the jointing stage. Before wheat sowing, the soil was prepared by straw powder, tilling, and rotary tillage. Maize is sown with hard stubble. The straw returning method was wheat straw with high stubble returning to the field and maize straw with total pulverized returning to the field, and the amount of returning to the field was wheat straw (4500 kg·ha<sup>-1</sup>) and maize straw (9000 kg·ha<sup>-1</sup>). The crop was grown as rainfed with no irrigation for all experimental years. In addition, pesticide and insecticide management was carried out according to local practices.

### 2.3. Soil Sampling

After the wheat harvest, samples were collected from four soil layers (0–10, 10–20, 20–40, and 40–60 cm) in June 2020, with three random sampling points in each plot. The soil samples from three sampling points in each soil layer of each plot were thoroughly mixed into one composite soil sample weighing about 2 kg. Sixty soil samples (15 plots × 4 depths) were packed in hard plastic cassettes and taken to the laboratory for further processing. Each sample was mixed thoroughly and gently, breaking the soil clods, and avoiding soil deformation from mechanical compression. Pebbles, plant residues, and creatures were removed, and the soil was air-dried at room temperature. After natural air drying, samples were divided into fractions of >5 mm, 5–2 mm, and <2 mm through dry sieving. Then, according to the ratio of the three soils to the undisturbed soil, three samples of 200 g mixed soil samples were collected for dry sieving analysis, and three samples of 100 g mixed soil were collected for wet sieving analysis.

### 2.4. Aggregate Separation

Classification of aggregates by the Savinov method. Mechanical stability aggregates were obtained by the dry sieving method, and water-stable aggregates were obtained by the sieving method. The wet sieving method uses an agglomerate analyzer (TPF-100). Firstly, four 100 g air-dried soil samples were placed into a 5 mm sieve and slowly immersed with 5 mL of deionized water for 5 min before sieving. Then, the samples were placed into a shaker of 5 sieves (5, 2, 1, 0.5, and 0.25 mm) in series and vertically shaken with a stroke length of 13 mm and a frequency of 45 strokes min<sup>-1</sup>. Precautionary measures were taken to ensure the soil aggregates on the topmost sieve were consistently below the water surface during each oscillation. After sieving, aggregates remaining in each sieve were flushed into separate glass evaporating dishes. Those remaining in the bucket were <0.25 mm size fractions aggregate, and a total of six aggregate size fractions were collected (>5, 5–2, 2–1, 1–0.5, 0.5–0.25, and <0.25 mm). The aggregate retained in the respective sieve was transferred to an evaporating glass dish, and the weight of each part was measured after drying at 40 °C for 2–3 days. Each aggregate sample was ground to pass through a 0.25 mm sieve and used to determine SOC and TN content. The dry sieving method uses a vibration mechanical sieving instrument (DM185, Shanghai Decode Information Technology Co., LTD., Shanghai, CN). Firstly, 200 g air-dried soil samples were placed into a shaker consisting of five sieves (5, 2, 1, 0.5, and 0.25 mm) in series and shaken 5 min with a rotation speed of 270 times min<sup>-1</sup>. After sieving, the remaining aggregates in each sieve were collected separately, and six aggregate size fractions were collected (>5, 5–2, 2–1, 1–0.5, 0.5–0.25, and <0.25 mm). Then, the aggregates were weighed and stored at room temperature. Each aggregate sample was ground to pass through a 0.25 mm sieve and used

to determine SOC and TN content. We did three repetitions for each sample for both dry and wet sieving. The sand correction was performed for each aggregate-size class in two sieving methods because sand was not considered part of the aggregates.

### 2.5. Determination of Soil Aggregate Stability

The mean weight diameter (MWD), the geometric mean diameter (GMD), the fractal dimension (D), the aggregate destruction rate (PAD), and unstable aggregate index ( $E_{LT}$ ) were calculated by the following formula [22]:

$$MWD(mm) = \sum_{i=1}^n x_i w_i \quad (1)$$

$$GMD(mm) = \text{Exp} \left[ \frac{\sum_{i=1}^n w_i \ln x_i}{\sum_{i=1}^n w_i} \right] \quad (2)$$

where  $x_i$  is the average diameter of aggregates in any size fractions;  $w_i$  is the proportion of aggregates corresponding to  $x_i$ .

$$\log \left[ \frac{M(r < \bar{x}_i)}{M_T} \right] = (3 - D) \log \left( \frac{\bar{x}_i}{x_{\max}} \right) \quad (3)$$

where  $\bar{x}_i$  is the average diameter of each aggregate size fractions (mm);  $M(r < \bar{x}_i)$  is the weight of the aggregates  $< \bar{x}_i$  (g);  $x_{\max}$  is the maximum diameter of the aggregates (mm);  $M_T$  is the total weight of aggregates.

$$PAD(\%) = \frac{DR_{0.25} - WR_{0.25}}{DR_{0.25}} \times 100\% \quad (4)$$

$$E_{LT}(\%) = \frac{(W_T - WR_{0.25})}{W_T} \times 100\% \quad (5)$$

where  $DR_{0.25}$  and  $WR_{0.25}$  are the proportion of  $>0.25$  mm aggregate fractions in wet sieving and dry sieving;  $M_T$  is the total weight of aggregates.

### 2.6. Determination of Soil Organic Carbon and Total Nitrogen in Each Size Fraction

SOC and TN were determined by  $K_2Cr_2O_7$ - $H_2SO_4$  oxidation method and the semi-micro Kjeldahl method [26]. The samples of bulk soil and different aggregate size fractions were analyzed.

The contribution rate of aggregate fractions to SOC and TN (PC-As and PN-As) was calculated as follows:

$$PC - As(\%) = \frac{C - A_S(g \cdot kg^{-1}) \times A_S(\%)}{SOC(g \cdot kg^{-1})} \times 100\% \quad (6)$$

$$PN - As(\%) = \frac{N - A_S(g \cdot kg^{-1}) \times A_S(\%)}{TN(g \cdot kg^{-1})} \times 100\% \quad (7)$$

where PC-As and PN-As are the contributing rates of aggregate fractions to SOC and TN; C-As and N-As are the SOC and TN contents in each aggregate size fraction.

### 2.7. Crop Yield

At maturity in each experimental year, three central rows were harvested from each plot. The harvested plants were threshed using a mini thresher to separate the grains. The grains were weighed to record the crop yield.

## 2.8. Statistical Analysis

Microsoft Excel 2010 software (Microsoft Corp., Redmond, WA, USA) was used for data processing and chart drawing. SPSS-19.0 software (IBM Corp., Armonk, NY, USA) was used for single-factor variance statistical analysis of the test data, and the least significant range method (LSD-t method) was used for multiple comparisons. The significance level was set as  $p < 0.05$ . Analysis of variance (ANOVA) was conducted following the general linear model (GLM) procedure to evaluate single-factor effects and interaction effects. Aggregate size, soil layer depth, fertilization, and interactions were treated as fixed effects. Values presented in the tables are mean values ( $n = 3$ ). Origin 2021 (OriginLab Corp., Northampton, MA, USA) was used for principal component analysis, correlation analysis, and graphical presentation. Spearman's correlation was used to establish a relationship among the studied parameters. The Smart PLS 3 (SmartPLS GmbH Corp., Oststeinbek, Schleswig-Holstein, GER) was used for the partial least squares path models (PLS-PM).

## 3. Results

Three-way ANOVA analyses showed that soil aggregates distribution and the contributing rate of aggregate fractions to SOC and TN were significantly influenced by aggregate size fractions (S), and the interaction between aggregate size fractions (S) and soil depth (D) and fertilization treatment (T), but not significantly affected by D and T. Three-way ANOVA analyses indicated that T, D, and S and their interactions significantly affected the SOC and TN contents in aggregate size fractions and C/N of aggregate size fractions (Table S1). Two-way ANOVA analyses showed that water-stable stability and mechanical stability, and SOC and TN were significantly influenced by T, D, and their interaction (Table S2).

### 3.1. Aggregate Size Distribution

The proportion of  $<0.25$  mm aggregate fractions in wet sieving and  $>0.25$  mm aggregate fractions in dry sieving accounted for the most significant proportion (74.88 and 95.53%), and the proportion of  $>5$  mm aggregate fractions in macro-aggregates in dry sieving was the largest (56.43%) (Table 2). The difference in aggregate size fractions was  $<0.25$ ,  $>5$ ,  $0.5-0.25$ ,  $1-0.5$ ,  $5-2$ ,  $2-1$  mm in descending order in wet sieving;  $>5$ ,  $5-2$ ,  $2-1$ ,  $1-0.5$ ,  $<0.25$ ,  $0.5-0.25$  mm in descending order in dry sieving. With the deepening of soil depth, the proportion of macro-aggregates ( $>5$ ,  $5-2$ ,  $2-1$ ,  $1-0.5$ , and  $0.5-0.25$  mm) decreased gradually, while the corresponding micro-aggregates ( $<0.25$  mm) showed an increasing trend under two sieving methods. With the increase in N application, the proportion of  $>5$ ,  $5-2$ ,  $2-1$ ,  $1-0.5$ , and  $0.5-0.25$  mm aggregates obtained by wet sieving showed a “Λ” shape and was the highest at N225, while the proportion of  $<0.25$  mm aggregates showed a “V” shape and was the lowest at N225. Compared with N0, the proportion of  $>5$ ,  $5-2$ ,  $2-1$ ,  $1-0.5$ , and  $0.5-0.25$  mm aggregates in N225 were significantly increased by 13.30, 3.55, 3.46, 5.80, and 7.45%, respectively, and the proportion of  $<0.25$  mm aggregates were significantly decreased by 66.45% ( $p < 0.05$ ). With the increase in N application rate, the proportion of  $>5$  mm aggregates obtained by dry sieving first increased and then decreased, which was higher in N150 and N225, and significantly increased by 26.77% and 19.24% compared with N0, respectively ( $p < 0.05$ ). The proportion of  $5-2$ ,  $2-1$ ,  $1-0.5$ , and  $0.5-0.25$  mm aggregates decreased first and then increased, and the values of N150 and N225 were lower than those of other treatments. Compared with N0, the proportion of  $<0.25$  mm aggregates in N75, N150, and N225 had no significant difference, while N300 significantly increased by 134.52% ( $p < 0.05$ ).

**Table 2.** Impact of nitrogen fertilization on the soil aggregates distribution in different soil layer depths under two sieving methods.

| Soil Depths (cm) | Fertilization Treatment | Soil Aggregates Distribution (%) |         |         |          |             |          |
|------------------|-------------------------|----------------------------------|---------|---------|----------|-------------|----------|
|                  |                         | >5 mm                            | 5–2 mm  | 2–1 mm  | 1–0.5 mm | 0.5–0.25 mm | <0.25 mm |
| Wet sieving      |                         |                                  |         |         |          |             |          |
| 0–10             | N0                      | 3.02d                            | 2.81d   | 2.92b   | 4.95c    | 7.34a       | 78.96a   |
|                  | N75                     | 12.29b                           | 4.54bc  | 3.84b   | 8.18ab   | 7.92a       | 63.23c   |
|                  | N150                    | 7.08c                            | 3.81c   | 3.49b   | 6.91b    | 9.22a       | 69.50b   |
|                  | N225                    | 16.92a                           | 4.84b   | 5.35a   | 8.59a    | 8.21a       | 56.10d   |
|                  | N300                    | 4.04cd                           | 6.08a   | 5.61a   | 8.31ab   | 7.67a       | 68.29b   |
| 10–20            | N0                      | 17.21b                           | 4.18ab  | 2.49b   | 3.50c    | 4.67b       | 67.95b   |
|                  | N75                     | 6.10c                            | 2.93b   | 2.64b   | 4.62b    | 6.93a       | 76.78a   |
|                  | N150                    | 17.25b                           | 3.42b   | 2.40b   | 4.02bc   | 6.09a       | 66.81bc  |
|                  | N225                    | 34.28a                           | 5.41a   | 4.28a   | 6.07a    | 6.74a       | 43.22d   |
|                  | N300                    | 19.26b                           | 4.68ab  | 3.84a   | 6.43a    | 6.30a       | 59.48c   |
| 20–40            | N0                      | 0.00d                            | 0.49c   | 1.36c   | 3.47b    | 6.22b       | 88.46b   |
|                  | N75                     | 0.00d                            | 0.22c   | 0.86d   | 1.97c    | 4.13c       | 92.82a   |
|                  | N150                    | 10.92a                           | 3.80a   | 2.65a   | 4.58a    | 5.82b       | 72.23d   |
|                  | N225                    | 1.99c                            | 3.47a   | 2.73a   | 4.38ab   | 7.98a       | 79.46c   |
|                  | N300                    | 5.05b                            | 2.24b   | 1.90b   | 3.98ab   | 5.40b       | 81.42c   |
| 40–60            | N0                      | 0.00b                            | 0.00c   | 0.75c   | 2.73b    | 5.23b       | 91.29a   |
|                  | N75                     | 0.00b                            | 0.77b   | 2.68a   | 4.92a    | 7.32a       | 84.31c   |
|                  | N150                    | 0.55a                            | 1.11a   | 1.28b   | 5.10a    | 6.08b       | 85.89bc  |
|                  | N225                    | 0.00b                            | 0.50b   | 1.47b   | 4.14a    | 6.86ab      | 87.04b   |
|                  | N300                    | 0.00b                            | 0.74b   | 1.71b   | 5.85a    | 7.37a       | 84.33c   |
| Dry sieving      |                         |                                  |         |         |          |             |          |
| 0–10             | N0                      | 22.95c                           | 31.85b  | 26.28a  | 11.16a   | 4.13a       | 3.62a    |
|                  | N75                     | 34.24b                           | 29.05b  | 21.06b  | 9.62a    | 3.12b       | 2.91b    |
|                  | N150                    | 45.09a                           | 28.55b  | 15.86c  | 6.19b    | 2.01c       | 2.31b    |
|                  | N225                    | 34.94b                           | 36.54a  | 18.77bc | 5.82b    | 1.41d       | 2.52b    |
|                  | N300                    | 29.05bc                          | 34.13ab | 21.54b  | 9.71a    | 2.92b       | 2.66b    |
| 10–20            | N0                      | 55.38c                           | 21.80a  | 12.20a  | 5.78a    | 2.47ab      | 2.37b    |
|                  | N75                     | 61.16c                           | 18.08ab | 9.86b   | 5.49a    | 2.79a       | 2.62b    |
|                  | N150                    | 67.02b                           | 13.51b  | 8.32c   | 5.32a    | 2.77a       | 3.06ab   |
|                  | N225                    | 68.63b                           | 15.28b  | 7.67c   | 3.97b    | 1.97b       | 2.48b    |
|                  | N300                    | 79.66a                           | 8.78c   | 4.00d   | 2.48c    | 1.68b       | 3.39a    |
| 20–40            | N0                      | 65.13b                           | 16.63a  | 8.48ab  | 4.89ab   | 2.21b       | 2.68b    |
|                  | N75                     | 66.05b                           | 13.92b  | 7.86b   | 5.26a    | 3.14a       | 3.77b    |
|                  | N150                    | 73.87a                           | 11.95bc | 5.28c   | 3.35b    | 1.98b       | 3.57b    |
|                  | N225                    | 66.35b                           | 15.00ab | 8.88a   | 4.60ab   | 1.86b       | 3.32b    |
|                  | N300                    | 65.46b                           | 10.84c  | 5.64c   | 4.10b    | 3.66a       | 10.31a   |
| 40–60            | N0                      | 54.72b                           | 19.73a  | 9.77a   | 6.17ab   | 3.65b       | 5.97b    |
|                  | N75                     | 59.73b                           | 17.97a  | 9.91a   | 5.63ab   | 2.94c       | 3.83c    |
|                  | N150                    | 65.25ab                          | 14.68b  | 7.01b   | 4.63b    | 2.99c       | 5.44b    |
|                  | N225                    | 66.38a                           | 13.13b  | 7.61b   | 5.26b    | 3.12bc      | 4.50c    |
|                  | N300                    | 47.51c                           | 12.86b  | 7.99b   | 6.79a    | 6.86a       | 17.99a   |

N0, N75, N150, N225, and N300 indicates treatments of 0, low-, medium-, high-and excessive dose N application ( $\text{kg N ha}^{-1}$ ), respectively. Different lowercase letters in the same column indicate significant differences among different N treatments at 0.05 levels.

### 3.2. Soil Aggregate Stability

The indexes of soil aggregate stability are shown in Table 3. With the increase in the N application rate, the MWD and GMD were first increased and then decreased, while the D, PAD, and  $E_{LT}$  were first decreased and then increased under the two sieving methods, with the N225 as the critical value. Under wet sieving, compared with N0, MWD and GMD of N225 were significantly increased by 99.97 and 10.72%, while the D value was significantly decreased by 3.83% ( $p < 0.05$ ). Under dry sieving, compared with N0, MWD and GMD of N225 were significantly increased by 72.94 and 15.30%, while the D value was significantly decreased by 12.37% ( $p < 0.05$ ). Compared with N0, the PAD and  $E_{LT}$  of

N225 were significantly decreased by 19.21 and 18.63% ( $p < 0.05$ ), respectively. With the deepening of soil depth, the MWD and GMD were significantly decreased, while D, PAD, and  $E_{LT}$  were significantly increased in two sieving methods ( $p < 0.05$ ).

**Table 3.** Impact of nitrogen fertilization on the soil aggregate stability indices including MWD, GMD, D, PAD, and  $E_{LT}$  under two sieving methods.

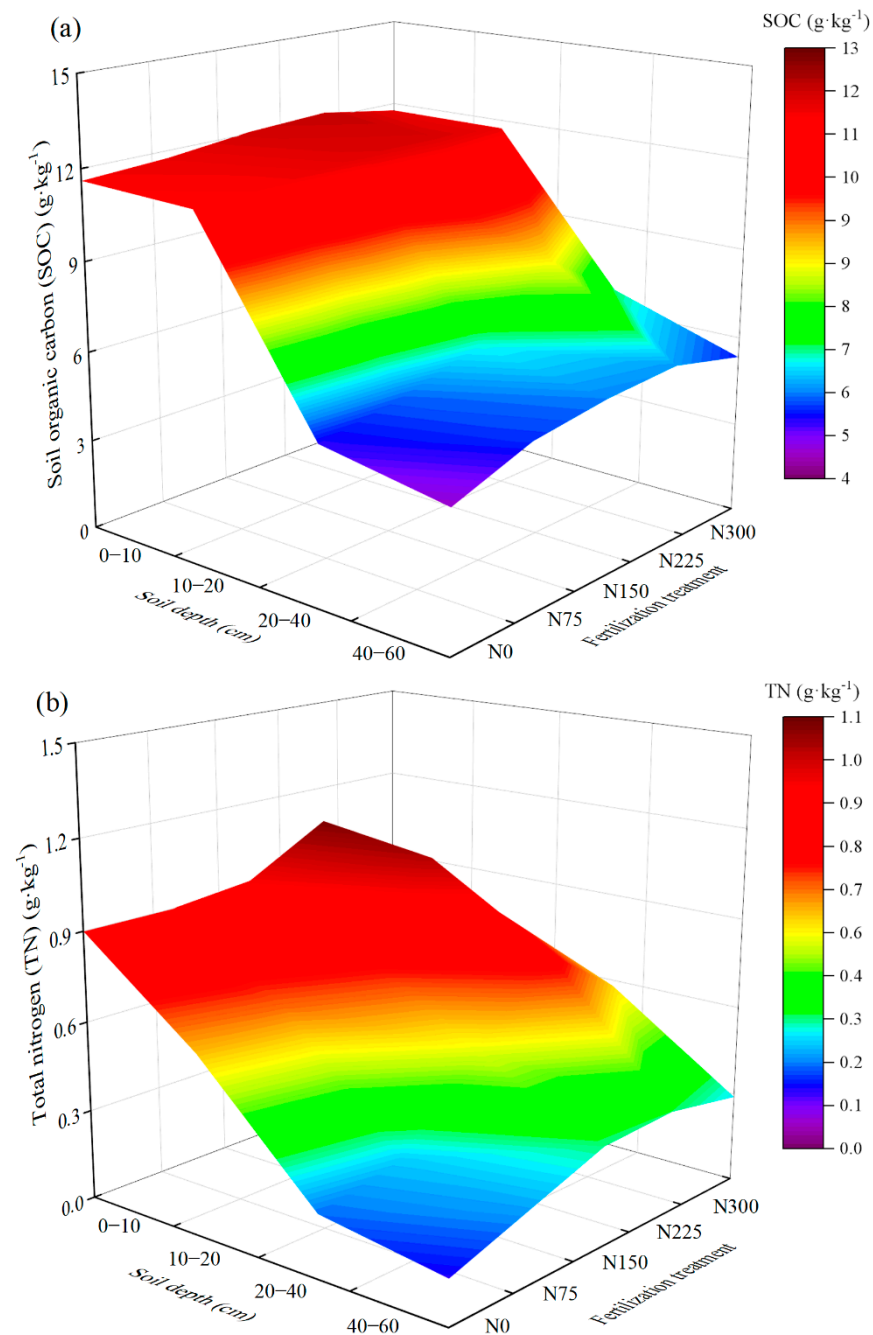
| Index               | Soil Depth (cm) | Fertilization Treatment |        |         |        |        |
|---------------------|-----------------|-------------------------|--------|---------|--------|--------|
|                     |                 | N0                      | N75    | N150    | N225   | N300   |
| Wet sieving         |                 |                         |        |         |        |        |
| MWD (mm)            | 0–10            | 0.54d                   | 1.21b  | 0.85c   | 1.54a  | 0.77cd |
|                     | 10–20           | 1.46b                   | 0.73c  | 1.45b   | 2.63a  | 1.65b  |
|                     | 20–40           | 0.24d                   | 0.21d  | 1.06a   | 0.49c  | 0.63b  |
|                     | 40–60           | 0.21d                   | 0.28b  | 0.30a   | 0.25c  | 0.27b  |
|                     | AVG             | 0.61c                   | 0.61c  | 0.92b   | 1.23a  | 0.83b  |
| GMD (mm)            | 0–10            | 0.26d                   | 0.41b  | 0.32c   | 0.50a  | 0.33c  |
|                     | 10–20           | 0.43bc                  | 0.28c  | 0.42bc  | 0.90a  | 0.51b  |
|                     | 20–40           | 0.20c                   | 0.19c  | 0.34a   | 0.25b  | 0.26b  |
|                     | 40–60           | 0.19c                   | 0.21a  | 0.21a   | 0.20b  | 0.21a  |
|                     | AVG             | 0.27c                   | 0.27c  | 0.33b   | 0.46a  | 0.33b  |
| D                   | 0–10            | 2.95a                   | 2.84c  | 2.90b   | 2.78d  | 2.91b  |
|                     | 10–20           | 2.79b                   | 2.92a  | 2.79b   | 2.55c  | 2.76b  |
|                     | 20–40           | 3.00a                   | 3.00a  | 2.86d   | 2.95b  | 2.93c  |
|                     | 40–60           | 3.00a                   | 2.99ab | 2.99b   | 3.00ab | 2.99b  |
|                     | AVG             | 2.93a                   | 2.94a  | 2.88b   | 2.82c  | 2.90b  |
| Dry sieving         |                 |                         |        |         |        |        |
| MWD (mm)            | 0–10            | 3.11c                   | 3.65b  | 4.23a   | 3.88ab | 3.49bc |
|                     | 10–20           | 4.60c                   | 4.81c  | 5.01bc  | 5.15b  | 5.58a  |
|                     | 20–40           | 4.99bc                  | 4.96bc | 5.34a   | 5.02b  | 4.78c  |
|                     | 40–60           | 4.46b                   | 4.72a  | 4.91a   | 4.95a  | 3.76c  |
|                     | AVG             | 4.29c                   | 4.53b  | 4.87a   | 4.75a  | 4.40bc |
| GMD (mm)            | 0–10            | 2.22c                   | 2.66b  | 3.29a   | 3.05a  | 2.60b  |
|                     | 10–20           | 3.58c                   | 3.75c  | 3.90bc  | 4.19b  | 4.64a  |
|                     | 20–40           | 3.97b                   | 3.78b  | 4.31a   | 3.96b  | 3.18c  |
|                     | 40–60           | 3.16b                   | 3.56a  | 3.65a   | 3.72a  | 1.97c  |
|                     | AVG             | 3.23c                   | 3.44b  | 3.79a   | 3.73a  | 3.10c  |
| D                   | 0–10            | 2.30a                   | 2.11b  | 1.81c   | 1.89c  | 2.11b  |
|                     | 10–20           | 1.69a                   | 1.61ab | 1.55b   | 1.38c  | 1.09d  |
|                     | 20–40           | 1.49b                   | 1.58b  | 1.27c   | 1.51b  | 1.72a  |
|                     | 40–60           | 1.79b                   | 1.67bc | 1.58c   | 1.59c  | 2.18a  |
|                     | AVG             | 1.82a                   | 1.74a  | 1.55b   | 1.59b  | 1.78a  |
| PAD (%)             | 0–10            | 78.16a                  | 62.13c | 68.78b  | 54.97d | 67.43b |
|                     | 10–20           | 67.17b                  | 76.16a | 65.75bc | 41.77d | 58.05c |
|                     | 20–40           | 88.14b                  | 92.54a | 71.20d  | 78.75c | 79.30c |
|                     | 40–60           | 90.73a                  | 83.69c | 85.08bc | 86.43b | 80.89d |
|                     | AVG             | 81.05a                  | 78.63a | 72.70b  | 65.48c | 71.42b |
| E <sub>LT</sub> (%) | 0–10            | 78.96a                  | 63.23c | 69.50b  | 56.10d | 68.29b |
|                     | 10–20           | 67.95b                  | 76.78a | 66.81bc | 43.22d | 59.48c |
|                     | 20–40           | 88.46b                  | 92.82a | 72.23d  | 79.46c | 81.42c |
|                     | 40–60           | 91.29a                  | 84.31c | 85.89bc | 87.04b | 84.33c |
|                     | AVG             | 81.66a                  | 79.29a | 73.61b  | 66.45c | 73.38b |

N0, N75, N150, N225, and N300 represent 0, low-, medium-, high-, and excessive N application rate ( $\text{kg N ha}^{-1}$ ), respectively. MWD, mean weight diameter; GMD, geometric mean diameter; D, the fractal dimension; PAD, aggregate destruction rate;  $E_{LT}$ , unstable aggregate index. Different lowercase letters in the same row indicate significant differences among different N treatments at 0.05 levels.

### 3.3. SOC and TN

With the increase in the N application rate, the SOC and TN first increased and then decreased in all soil layer depths, reaching the highest value at N225 (Figure 1). Compared with N0, the SOC in 0–10, 10–20, 20–40, and 40–60 cm soil layer depths of N225 were

significantly increased by 4.99, 4.60, 63.45, and 30.95%, respectively ( $p < 0.05$ ), the TN in 0–10, 10–20, 20–40, and 40–60 cm soil layer depths of N225 were significantly increased by 20.90, 68.82, 259.39, and 125.78%, respectively ( $p < 0.05$ ). Compared with the average SOC and TN of N0, the average SOC of N75, N150, N225, and N300 was significantly increased by 5.03, 10.28, 17.75, and 8.20%, respectively, and the average TN was significantly increased by 11.92, 37.38, 72.33, and 34.58% ( $p < 0.05$ ). The SOC and TN decreased significantly with the deepening of soil layer depths ( $p < 0.05$ ).



**Figure 1.** Impact of nitrogen fertilization on the SOC and TN contents in different soil depths ((a), SOC; (b), TN). N0, N75, N150, N225, and N300 indicates treatments of 0, low-, medium-, high-, and excessive dose N application (kg N ha<sup>-1</sup>), respectively.

### 3.4. Aggregate Associated SOC, TN, and C/N

#### 3.4.1. Aggregate Associated SOC and TN

The SOC and TN contents in each aggregate size fraction obtained by the wet sieving method differ significantly, which increased firstly and then decreased with decreasing aggregate size and presenting an “Λ” shape (Figure 2a,c). The difference in the SOC content in aggregate size fractions (C-WSAs) was 2–1, 5–2, 1–0.5, 0.5–0.25, >5, and <0.25 mm in descending order, and the difference in the TN contents in aggregate size fractions (N-WSAs) was 1–0.5, 2–1, 0.5–0.25, 5–2, >5, and <0.25 mm in descending order ( $p < 0.05$ ). The macro-aggregates showed an advantage in carbon and nitrogen enrichment, especially the 2–1 mm aggregates with higher SOC and TN contents (10.00 and 0.71 g·kg<sup>−1</sup>). The micro-aggregates had the lowest values (6.69 and 0.53 g·kg<sup>−1</sup>). The SOC and TN contents in each aggregate size fraction under wet sieving were significantly decreased with the deepening of soil layer depths ( $p < 0.05$ ). With the increase in the N application rate, the SOC content of >0.25 mm aggregates first increased and then decreased and was significantly increased by 12.46, 40.10, 26.77, and 24.65% compared with N0, respectively, and the SOC content of 2–1 mm aggregates was significantly increased by 9.30, 20.37, 18.42, and 9.86%, respectively. The SOC content of <0.25 mm aggregates was increased gradually, significantly increasing by 9.23, 12.12, 12.95, and 18.60%, respectively ( $p < 0.05$ ). With the increase in N application rate, the TN content of >0.25 mm aggregates increased by 1.09, 32.27, 19.51, and 27.32% compared with N0, respectively. In contrast, the TN content of <0.25 mm aggregates first increased and then decreased and significantly increased by 10.20, 36.73, 67.35, and 34.69% compared with N0, respectively ( $p < 0.05$ ). The average SOC and TN contents (average aggregate size and soil layer depths) first increased and then decreased with the increase in N application. Compared with the average SOC and TN of N0, the average SOC content of N150 and N225 was significantly increased by 36.13% and 24.81%, and TN content was significantly increased by 32.84% and 25.59%, respectively ( $p < 0.05$ ).

The SOC and TN contents in each aggregate size fraction obtained by the dry sieving method differ significantly, which gradually increases with the decrease in aggregate size (Figure 2b,d). The difference in the SOC content in aggregate size fractions (C-MSAs) was 0.5–0.25, <0.25, 1–0.5, >5, 5–2, and 2–1 mm in descending order, and the difference in the TN content in aggregate size fractions (N-MSAs) was <0.25, 0.5–0.25, 1–0.5, 5–2, 2–1, and >5 mm in descending order ( $p < 0.05$ ). The 0.5–0.25 and <0.25 mm aggregates had higher SOC and TN content (9.05 and 8.52 g·kg<sup>−1</sup>; 0.67 and 0.67 g·kg<sup>−1</sup>). The SOC and TN content in each aggregate size fraction under dry sieving was significantly decreased with the deepening of soil layer depths ( $p < 0.05$ ). With the increase in N application rate, the SOC content of >0.25 mm aggregates first increased and then decreased and was significantly increased by 12.77, 19.90, 30.00, and 18.66% compared with N0, respectively, and the SOC content of 0.5–0.25 mm aggregates was significantly increased by 53.52, 55.15, 66.53, and 60.57%, respectively. The SOC content of <0.25 mm aggregates was significantly increased by 46.50, 42.30, 58.96, and 68.21% compared with N0, respectively ( $p < 0.05$ ). With the increase in the N application rate, the TN content of >0.25 mm aggregates significantly increased by 6.99, 39.93, 69.38, and 17.19% compared with N0, respectively, and the SOC content of 0.5–0.25 mm aggregates significantly increased by 8.43, 41.21, 71.11, and 18.16%, respectively. The TN content of <0.25 mm aggregates was increased by 0.89, 34.52, 62.03, and 13.12% compared with N0, respectively ( $p < 0.05$ ). The average SOC and TN contents (average aggregate size and soil layer depths) first increased and then decreased with the increase in N application. Compared with N0, the average SOC and TN content of N225 significantly increased by 30.00 and 69.38% ( $p < 0.05$ ), respectively.

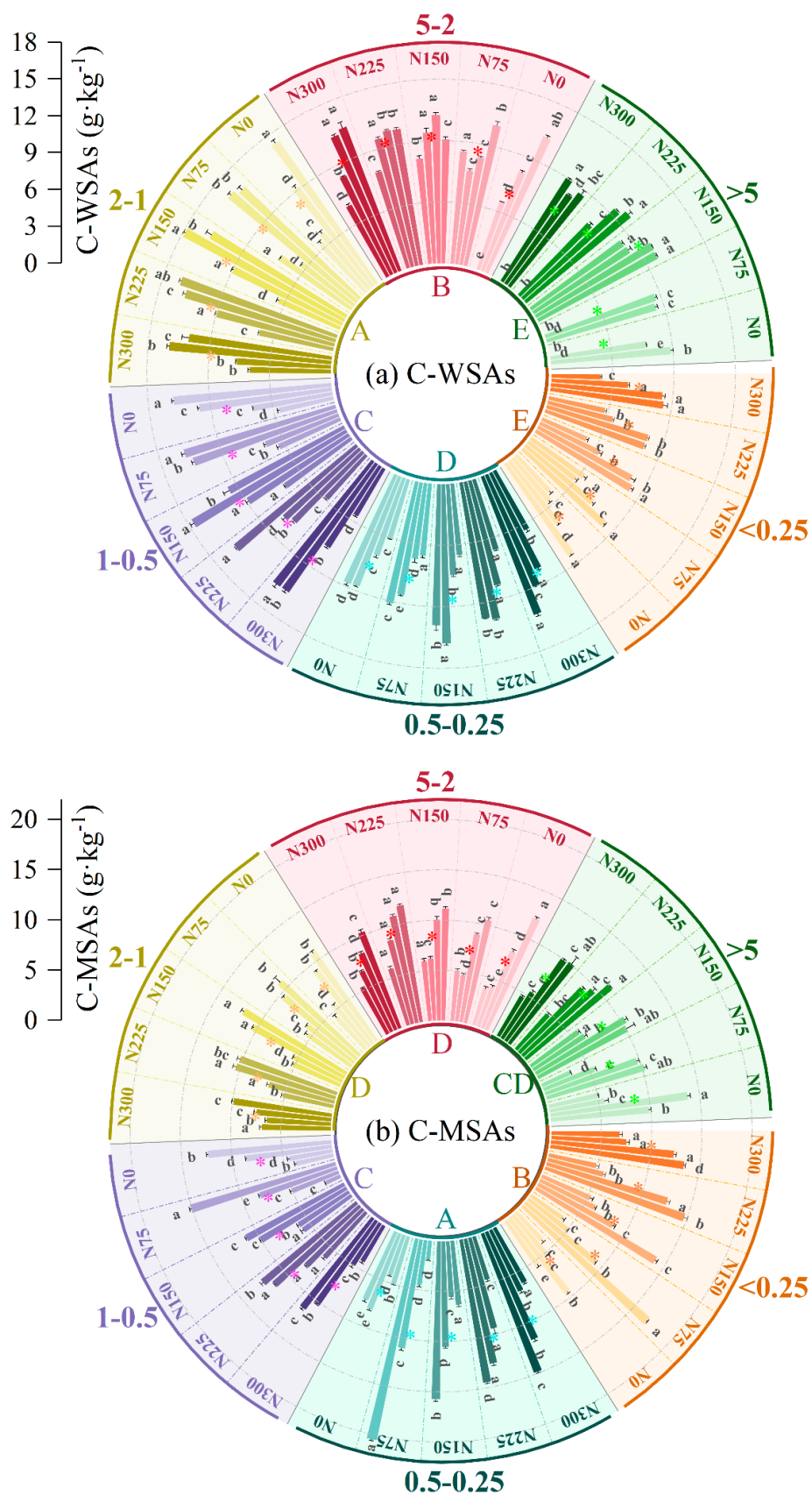
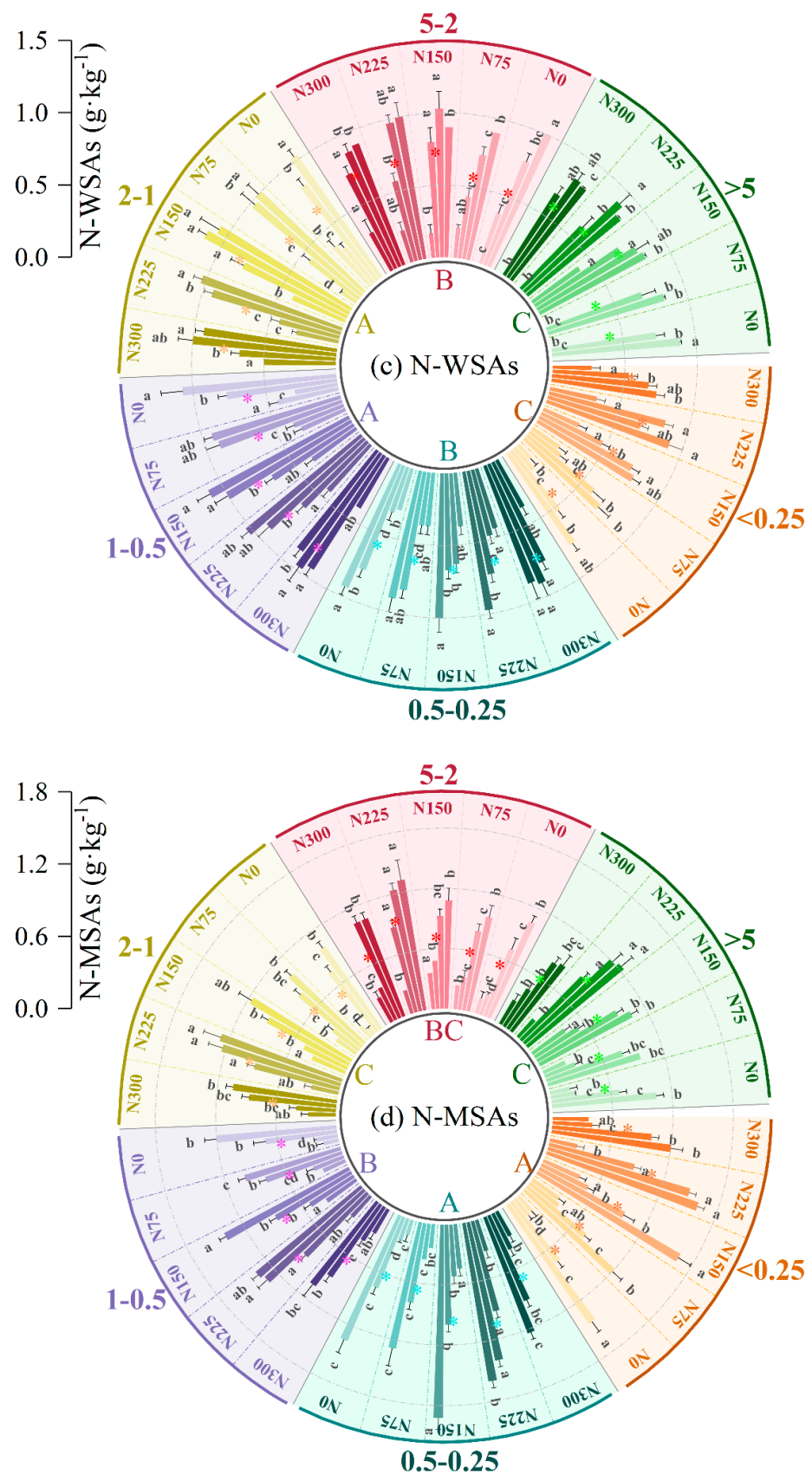


Figure 2. Cont.



**Figure 2.** Impact of nitrogen fertilization on the SOC and TN content in each aggregate size fraction in different soil layer depths under two sieving methods. (a), C-WSAs, the SOC content in aggregate

fractions under wet sieving; **(b)**, C-MSAs, the SOC content in aggregate fractions under dry sieving; **(c)**, N-WSAs, the TN content in aggregate fractions under wet sieving, **(d)**, N-MSAs, the TN content in aggregate fractions under dry sieving). Four adjacent columns arranged counterclockwise under each fertilization treatment represent different soil depths of 0–10, 10–20, 20–40, and 40–60 cm, respectively. N0, N75, N150, N225 and N300 indicates treatments of 0, low-, medium-, high-, and excessive dose N application ( $\text{kg N ha}^{-1}$ ), respectively. Different lowercase letters indicate significant differences among five N treatments at 0.05 levels at the same aggregate size and soil depth. The asterisk indicates the corresponding value of each aggregate size and each fertilization treatment at the average soil depth. The >5, 5–2, 2–1, 1–0.5, 0.5–0.25 and <0.25 represent the different aggregate size fractions (mm). Different capital letters indicate significant differences among six aggregate size fractions at the level of 0.05.

### 3.4.2. Contributing Rate of Aggregates in SOC and TN

Under the wet sieving method, the contributing rate of aggregate fractions to SOC and TN was first decreased and then increased with the aggregate size decreased, in which <0.25 mm aggregates accounted for the most significant proportion (58.36 and 67.04%) (Figure 3a,c). The difference in the contributing rate of aggregate fractions to SOC (PC-WSAs) was <0.25, >5, 0.5–0.25, 1–0.5, 2–1, and 5–2 mm in descending order, and the difference in the contributing rate of aggregate fractions to TN (PN-WSAs) was <0.25, >5, 0.5–0.25, 1–0.5, 2–1, and 5–2 mm in descending order ( $p < 0.05$ ). With the deepening of soil layer depths, the contributing rate of >0.25 mm aggregates to SOC and TN gradually decreased. In contrast, the contributing rate of <0.25 mm aggregates to SOC and TN gradually increased. With the increase in the N application rate, the contributing rate of >0.25 mm aggregates to SOC first increased and then decreased, and the corresponding contributing rate of <0.25 mm aggregates to SOC first decreased and then increased. Compared with N0, the contributing rate of >0.25 mm aggregates to SOC significantly increased by 16.86, 70.83, 84.13, and 56.86%, respectively, and the contributing rate of <0.25 mm aggregates to SOC decreased significantly by 0.26, 14.95, 26.82, and 7.62%, respectively ( $p < 0.05$ ). With the increase in the N application rate, the contributing rate of >0.25 mm aggregates to TN gradually increased, and the corresponding contributing rate of <0.25 mm aggregates to TN gradually decreased. Compared with N0, the contributing rate of >0.25 mm aggregates to TN significantly increased by 5.79, 20.66, 17.18, and 24.14%, respectively, and the contributing rate of <0.25 mm aggregates to TN significantly decreased by 0.74, 12.55, 15.57, and 9.50%, respectively ( $p < 0.05$ ).

Under the dry sieving method, the contributing rate of aggregate fractions to SOC and TN was first decreased and then increased with the aggregate size decreased, in which >5 mm aggregates accounted for the most significant proportion (50.96 and 50.58%) (Figure 3b,d). The difference in the contributing rate of aggregate fractions to SOC and TN (PC-MSAs and PN-MSAs) was >5, 5–2, 2–1, 1–0.5, <0.25, and 0.5–0.25 mm in descending order ( $p < 0.05$ ). With the deepening of soil layer depths, the contributing rate of >0.25 mm aggregates to SOC and TN gradually decreased. In contrast, the contributing rate of <0.25 mm aggregates to SOC and TN gradually increased. Among the five N treatments, there was no significant difference in the contributing rate of aggregate fractions to SOC and TN of N0, N75, N150, and N225 ( $p > 0.05$ ). However, the contributing rate of >0.25 mm aggregates to SOC and TN of N300 significantly decreased by 16.47% and 18.93, respectively, and the contributing rate of <0.25 mm aggregates to SOC and TN of N300 significantly increased by 262.64 and 94.52%, respectively ( $p < 0.05$ ).

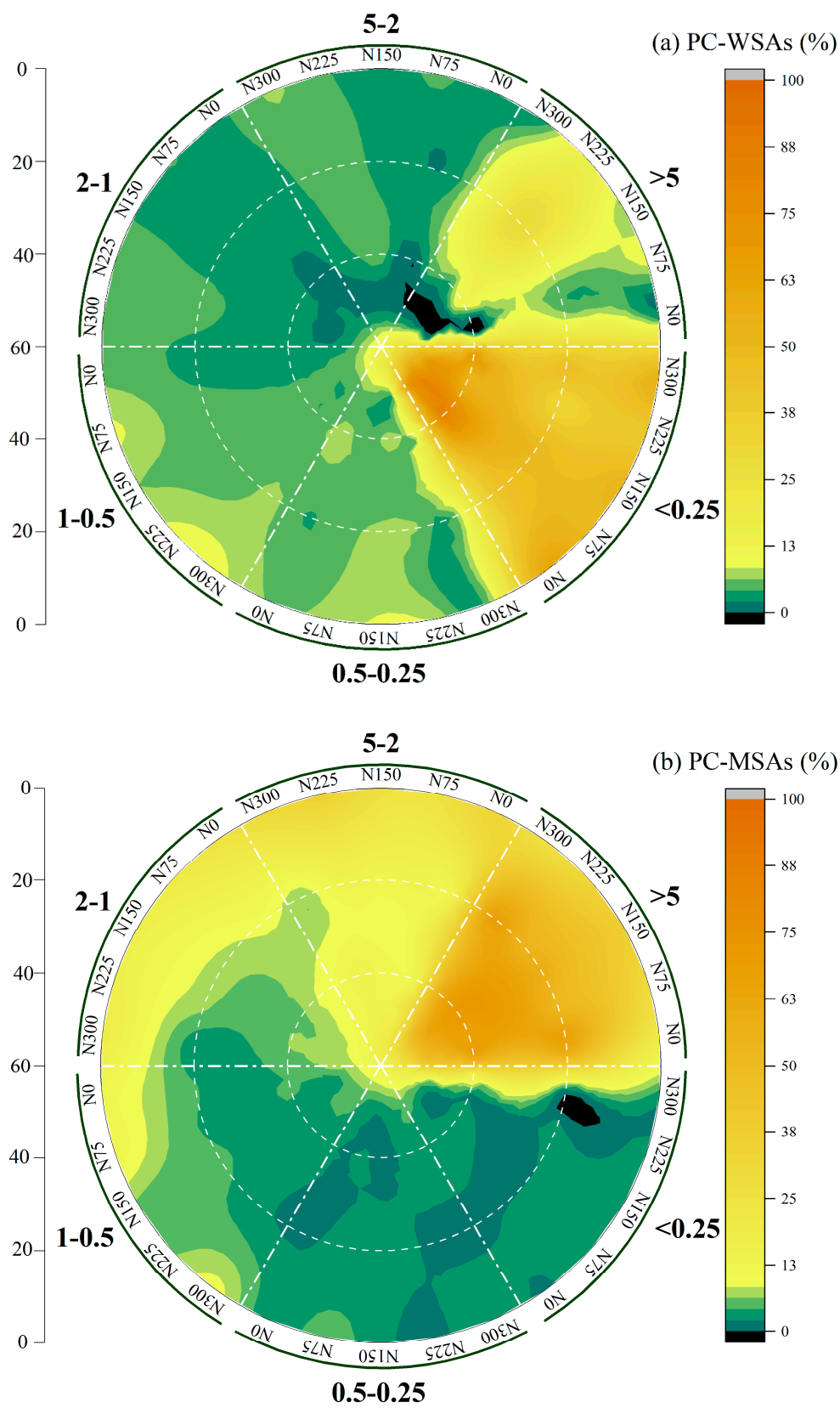
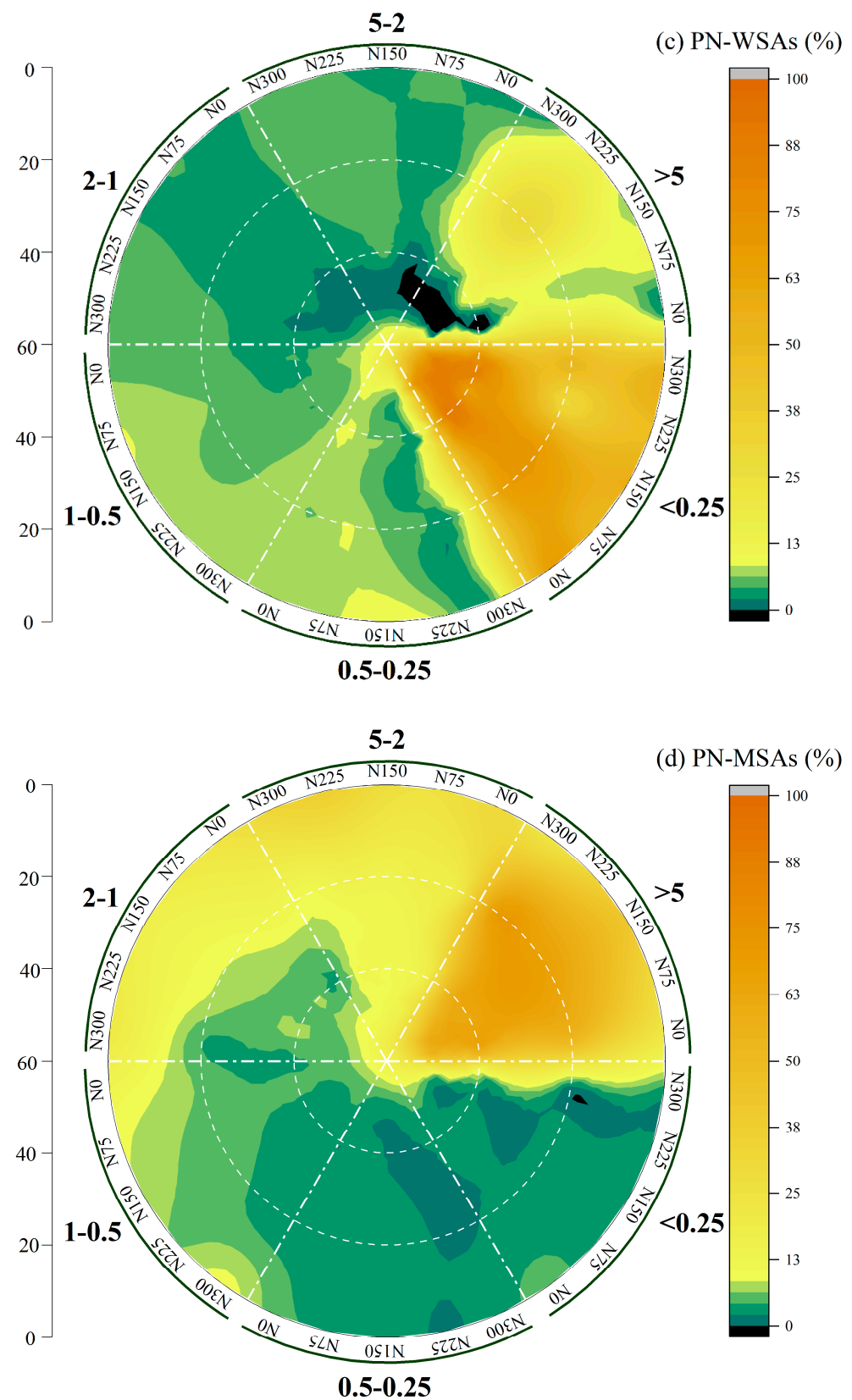


Figure 3.
 Cont.



**Figure 3.** Impact of nitrogen fertilization on the contributing rate of aggregate size fractions to SOC and TN in different soil layer depths under two sieving methods. (a), PC-WSAs, the contributing rate of aggregate size fractions to SOC under wet sieving; (b), PC-MSAs, the contributing rate of aggregate size fractions to SOC under dry sieving; (c), PN-WSAs, the contributing rate of aggregate

size fractions to TN under wet sieving, (d), PN-MSAs, the contributing rate of aggregate size fractions to TN under dry sieving). The scales on the figure left represent different soil layer depths (cm). N0, N75, N150, N225 and N300 indicates treatments of 0, low-, medium-, high-, and excessive dose N application ( $\text{kg N ha}^{-1}$ ), respectively. The >5, 5–2, 2–1, 1–0.5, 0.5–0.25, and <0.25 represent the different aggregate size fractions (mm).

### 3.4.3. C/N of Aggregate Size Fractions

The C/N of aggregate size fractions was first increased and then decreased with the decrease in aggregate size under the two sieving methods (C/N-WSAs and C/N-MSAs) (Figure 4). The C/N ratio of 5–2 mm aggregates was the highest (18.11), followed by 2–1 mm aggregates (16.63), and the minimum value was >5 mm aggregates (9.31) under the wet sieving method. The C/N ratio of 2–1 mm aggregate was the largest (20.00), and that of <0.25 mm aggregate was the largest (15.62) under the dry sieving method. The difference in the C/N of aggregate size fractions under the wet sieving (C/N-WSAs) was 5–2, 2–1, <0.25, 0.5–0.25, 1–0.5, and >5 mm in descending order, and the difference in the C/N of aggregate size fractions under the dry sieving (C/N-MSAs) was 2–1, 5–2, >5, 0.5–0.25, 1–0.5, and <0.25 mm in descending order ( $p < 0.05$ ). With the deepening of soil layer depths, the C/N of aggregate size fractions gradually increased under the two sieving methods.

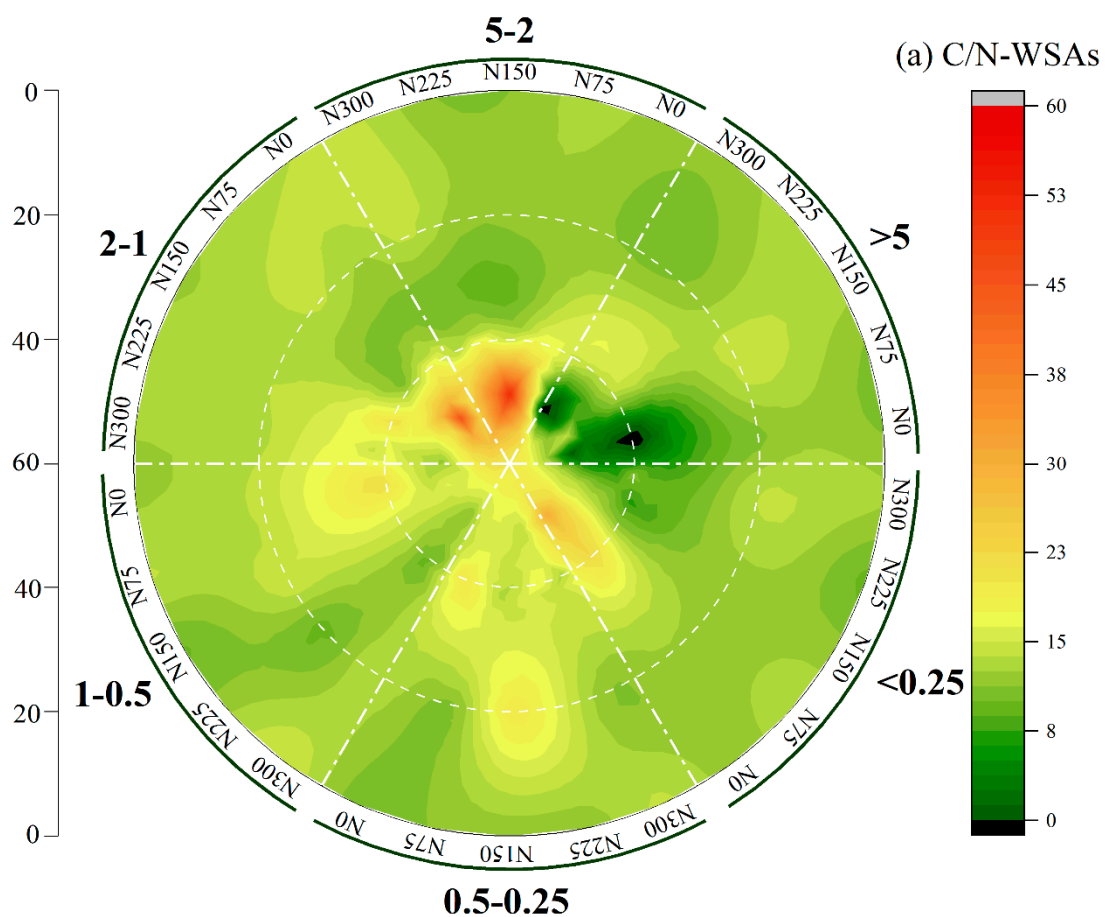
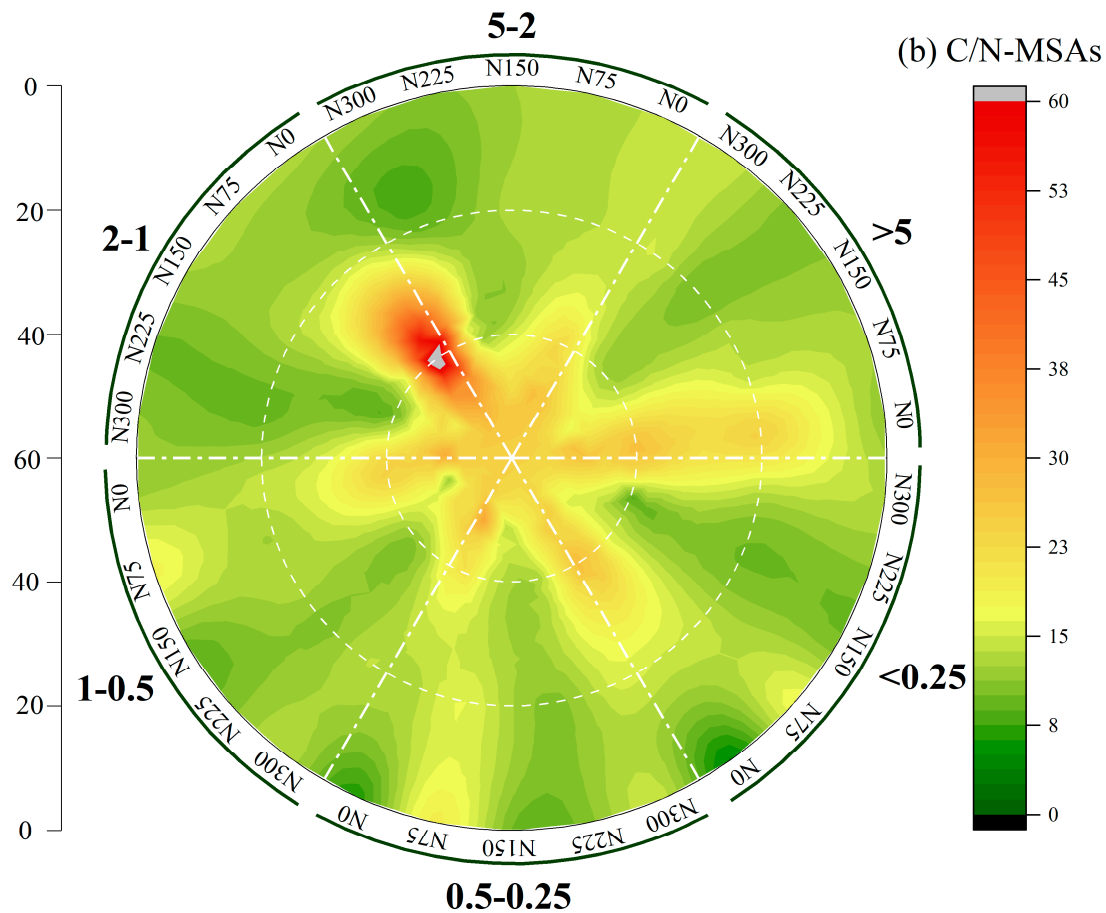


Figure 4. Cont.



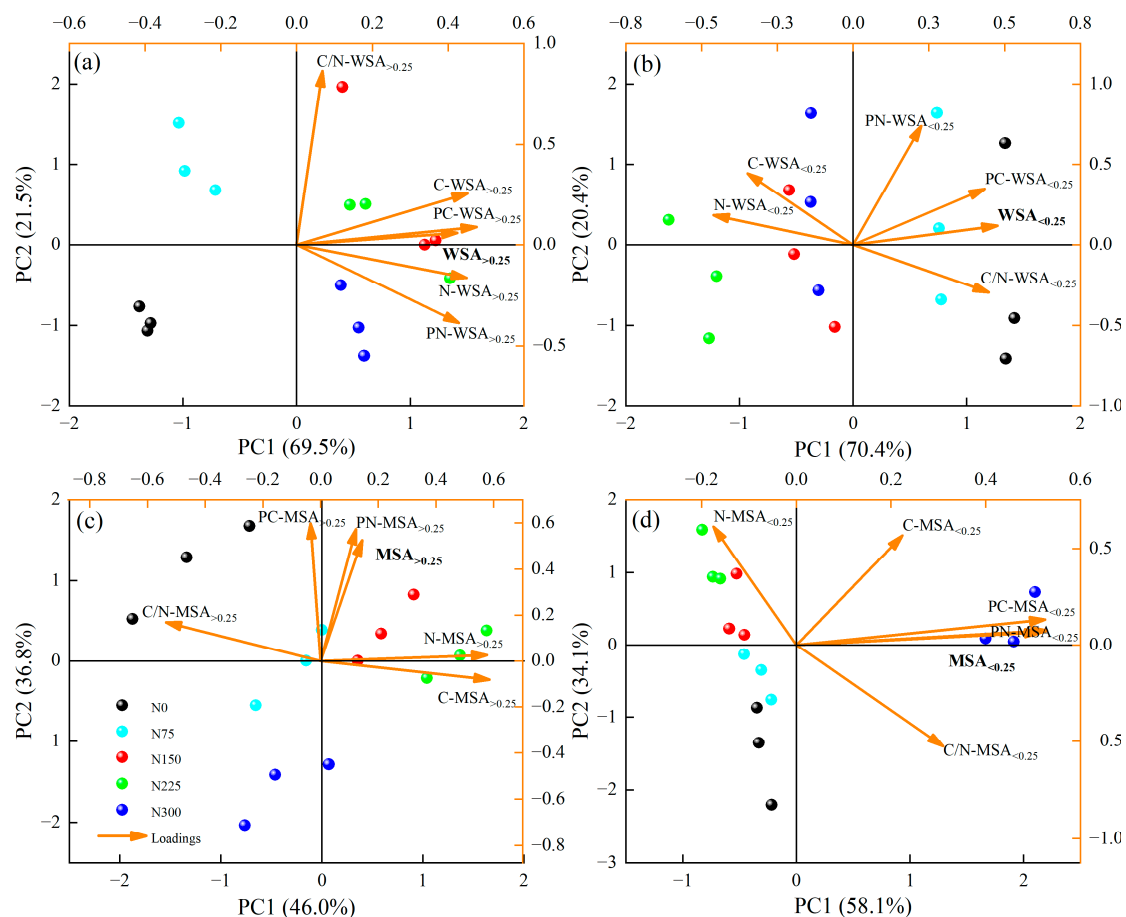
**Figure 4.** Impact of nitrogen fertilization on the C/N ratio of aggregate size fractions in different soil layer depths under two sieving methods (a), C/N-WSAs, the C/N ratio of aggregate size fractions under wet sieving; (b), C/N-MSAs, the C/N ratio of aggregate size fractions under dry sieving). The scales on the figure left represent different soil layer depths (cm). N0, N75, N150, N225, and N300 indicates treatments of 0, low-, medium-, high-, and excessive dose N application ( $\text{kg N ha}^{-1}$ ), respectively. The >5, 5–2, 2–1, 1–0.5, 0.5–0.25, and <0.25 represent the different aggregate size fractions (mm).

With the increase in the N application rate, the C/N of >0.25 mm aggregates first increased and then decreased, and the corresponding C/N of <0.25 mm aggregates first decreased and then increased under the wet sieving. Compared with N0, the C/N of >0.25 mm aggregates significantly increased by 36.79, 35.16, 22.15 and 4.56%, respectively, and the C/N of <0.25 mm aggregates significantly decreased by 15.47, 36.61, 45.95, and 32.99% compared with N0, respectively. Moreover, with the increase in the N application rate, the C/N of aggregate size fractions obtained by the dry sieving method first decreased and then increased and reached the minimum value in the N225 treatment. Compared with N0, the C/N of aggregate size fractions of N225 treatment were significantly decreased by 41.57, 24.76, 63.62, 28.79, 37.32, and 31.74%, respectively ( $p < 0.05$ ), with the decrease in aggregate size.

#### 3.4.4. Principal Component

The principal component (Figure 5) explains 91.0, 90.8, 82.8, and 92.2% of the variance in the original data, respectively. Five treatments were identified and separated. The results indicate that fertilization strongly influenced the proportion of macro-aggregates and micro-aggregates under two sieving methods. In Figure 5a, under wet sieving, N150, N225, and N300 are positive in PC1, and are positively affected by the proportion of macro-aggregates, the SOC and TN contents in macro-aggregate, and the contribution rate

of macro-aggregates to SOC and TN. N150 and N225 are positive in PC2 and positively affected by the C/N of macro-aggregates. In Figure 5b, under wet sieving, N0 and N75 are positive values in PC1, and are positively affected by the proportion of micro-aggregates, the C/N of micro-aggregates, and the contribution rate of micro-aggregates to SOC and TN. N150, N225, and N300 are negative values in PC1 and positively affected by the SOC and TN contents in micro-aggregate. In Figure 5c, under dry sieving, N0, N75, and N300 are negative values in PC1, and are positively affected by the C/N of macro-aggregates. N150 and N225 are positive values in PC1 and positively affected by the proportion of macro-aggregates, the SOC and TN contents in macro-aggregate, and the contribution rate of macro-aggregates to SOC and TN. In Figure 5d, under dry sieving, N0, N75, N150, and N225 are negative values in PC1, while N300 is positive values in PC1. N150 and N225 were positively affected by the TN content in the micro-aggregate, and N300 was positively affected by the proportion of micro-aggregates, the SOC content in the micro-aggregate, the contribution rate of micro-aggregates to SOC and TN, and the C/N of micro-aggregates.



**Figure 5.** Principal component analysis of macro-aggregates and micro-aggregates distribution was affected by aggregate associated SOC, TN, and C/N ratio under two sieving methods. (a),  $WSA_{>0.25}$ , the proportion of  $>0.25$  mm aggregate fractions in wet sieving; (b),  $WSA_{<0.25}$ , the proportion of  $<0.25$  mm aggregate fractions in wet sieving; (c),  $MSA_{>0.25}$ , the proportion of  $>0.25$  mm aggregate fractions in dry sieving; (d),  $MSA_{<0.25}$ , the proportion of  $<0.25$  mm aggregate fractions in dry sieving. C-WSAs, N-WSAs, C-MSAs, and N-MSAs, the SOC and TN content in aggregate fractions size under wet and dry sieving; PC-WSAs, PN-WSAs, PC-MSAs, and PN-MSAs, the contributing rate of aggregate size fractions to SOC and TN under wet and dry sieving; C/N-WSAs and C/N-MSAs, the C/N of aggregate size fractions under wet and dry sieving. N0, N75, N150, N225 and N300 indicates treatments of 0, low-, medium-, high-, and excessive dose N application ( $\text{kg N ha}^{-1}$ ), respectively.

### 3.5. Crop Yield

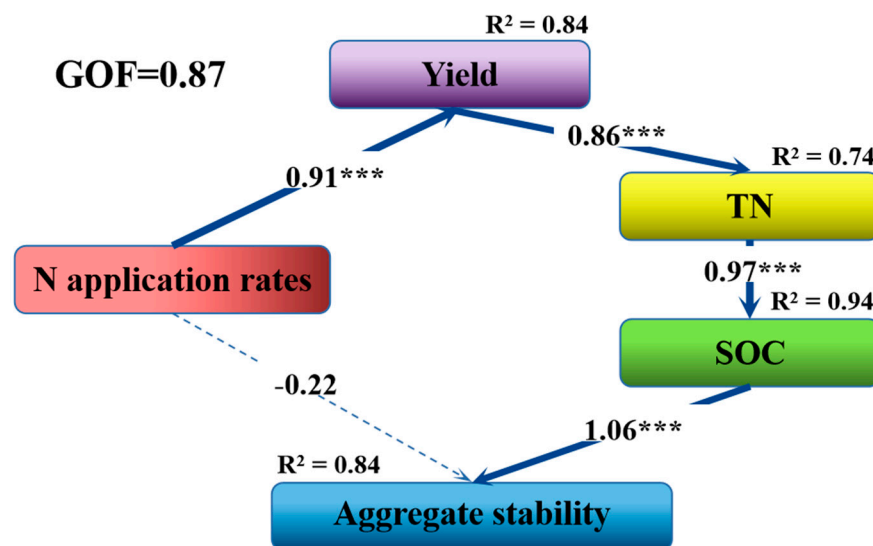
N application rates significantly affected crop yield (Table 4). The maximum crop yield was recorded from the N225. For winter wheat and summer maize yields, compared with N0, the crop yields of N75 increased by 17.47 and 21.54%, and those of N150, N225, and N300 significantly increased by 51.17 and 96.31, 62.43 and 95.68, 52.90 and 95.04%, respectively ( $p < 0.05$ ). For total yields, compared with N0, the crop yields of N75 increased by 19.70%, N150, N225, and N300 significantly increased by 75.95, 80.68 and 76.04% ( $p < 0.05$ ), respectively, among which N225 had the largest increase. There was no significant difference between N150, N225, and N300 ( $p > 0.05$ ).

**Table 4.** Impact of nitrogen fertilization on crop yield.

| T            | N0       | N75        | N150       | N225       | N300       |
|--------------|----------|------------|------------|------------|------------|
| Winter wheat | 4198.03b | 4931.47b   | 6346.03a   | 6818.86a   | 6418.93a   |
| Summer maize | 5111.18b | 6211.90b   | 10,033.77a | 10,001.48a | 9968.68a   |
| Total        | 9309.21b | 11,143.37b | 16,379.80a | 16,820.34a | 16,387.61a |

N0, N75, N150, N225, and N300 indicates treatments of 0, low-, medium-, high-, and excessive dose N application (kg N ha<sup>-1</sup>), respectively. Different small letters in the same row indicate significant differences among five N treatments at 0.05 levels.

The partial least squares path models (PLS-PM) were shown in (Figure 6). The N application rates had positive effects on yield, the yield had positive effects on SOC and TN, and then had a positive effect on water-stable stability and mechanical stability.



**Figure 6.** The partial least squares path models (PLS-PM) illustrate the effects of different N application rates on yield, TN and SOC, and soil aggregate stability. Soil aggregate stability are latent variables, which are indicated by the proportion of >0.25 mm aggregate fractions, MWD, and GMD. MWD, mean weight diameter; GMD, geometric mean diameter. The red and blue arrows indicate negative and positive flows of causality, respectively. Numbers on the arrowed lines and thickness of arrows indicate normalized path coefficient. The dotted grey arrows represent non-significant path relationships. R<sup>2</sup> beside the latent variables are the coefficients of determination. The GOF represents the goodness of fit. Asterisks represent significant effects (\*\*\*)  $p < 0.001$ .

## 4. Discussion

### 4.1. Aggregate Size Distribution

The mechanical stability aggregates obtained by dry sieving were mainly >0.25 mm, while the water-stable aggregates obtained by wet sieving were mainly <0.25 mm (Table 1). The different energy delivered to the soil under the two methods may result in different

aggregate distributions. In addition, it can be seen that the tested soil's mechanical resistance is strong, but the resistance to water erosion is poor. Nahidan and Nourbakhsh (2018) also support that aggregates tend to be micro-aggregates in wet sieving, while aggregates tend to be macro-aggregates in dry sieving [27]. The higher micro-aggregates under the wet sieving may be a function of ion hydration, that is, the rupture of weak aggregates and instability macro-aggregates due to the sudden release of additional stress and the dissolution of the cementing agent in water [28]. Macro-aggregates dominate the surface soil, while the deep soil is dominated by micro-aggregates (Table 1), which may be because the topsoil contains more returning OM [29].

Long-term N fertilization practices tended to improve the >0.25 mm aggregates but decreased the <0.25 mm aggregates (Table 1). Similar experiments have demonstrated that the application of N helps soil aggregate development [30,31]. Fertilization improved the soil aggregate stability by increasing crops' biomass and the amount of straw reentering the field and root secretions [32]. We also confirmed this result through PLS-PM (Figure 6). However, Wang et al. (2015) pointed out that N application had no discernible impact on aggregate distribution [13], which was different from our results mainly because the object of this study was temperate grassland, which was very different from the amount of straw returned was thrown back into the field in our study. Moreover, we found that macro-aggregates decreased, and micro-aggregates increased under N300 (Table 1). This also confirmed that the N300 was excessive. Excessive N application had a certain destructive effect on soil structure. Excessive N application rate leads to high OM mineralization degree in the aggregate, and the cohesion of SOM decreases, which makes it difficult to maintain soil macro-aggregates, eventually leading to the decomposition of >0.25 mm aggregates into <0.25 mm aggregates [33].

#### 4.2. Soil Aggregate Stability

The MWD, GMD, D, PAD, and  $E_{LT}$  are common indicators reflecting soil aggregate stability. The larger the value of MWD and GMD, the smaller the value of D, PAD, and  $E_{LT}$ , the more stable the soil structure and the lower the degradation degree [22,34]. In this study, the MWD and GMD of N150 and N225 were the highest, while the D value, PAD, and  $E_{LT}$  were the lowest (Table 2), indicating that an appropriate N application rate could increase the stability of the soil structure, prevent soil degradation, and diminish the risk of soil erosion. The reason may be that the C/N ratio of straw-returning soil can be adjusted under the condition of appropriate N application to enhance soil microbial activity and promote the growth of microbial mycelium. A large amount of mycelium can enhance soil cementing, which is conducive to increasing the number of macro-aggregates, improving MWD and GMD, and improving soil aggregate stability [32,35]. The PLS-PM analysis (Figure 6) also showed a strong correlation between fertilization, yield, carbon and nitrogen, and soil aggregate stability ( $p < 0.001$ ). However, fertilizer has a threshold effect on aggregates. Excessive N application may reduce cementing material and SOC mineralization, leading to macro-aggregate decomposition and soil aggregate stability reduction [36]. In addition, with the deepening of soil depth, the MWD and GMD gradually decreased. At the same time, D, PAD, and  $E_{LT}$  gradually increased, indicating that soil nutrient status and physical properties presented an upward and positive development.

#### 4.3. SOC and TN

Fertilization increased the SOC and TN contents (Figure 1). However, Huang et al. (2010) stated that the inorganic fertilizer application could maintain the SOC content in subtropical red soils [4]. In contrast, Studies demonstrated that the long-term addition of mineral fertilizer resulted in the de-aggregation effect [33]. Specific soil features, climate conditions, and cropping strategies can account for the inconsistent effects of N addition and variations in SOC and TN [37]. On the one hand, N fertilization increased the aboveground biomass, and straw returning increased soil carbon sources [33]. On the other hand, N fertilization promoted the decomposition and transformation of SOC, which put the SOC

budget in a dynamic state [38]. In our study, fertilization was beneficial to the increase in SOC and TN sequestration in the field of straw returning, which proved that C input was higher than C loss. The PLS-PM analysis (Figure 6) also showed a strong correlation between fertilization, yield, SOC, and TN ( $p < 0.001$ ). Excessive N application rate reduced the SOC and TN contents (Figure 1), possibly due to the higher carbon mineralization than carbon sequestration. Fertilization had a favorable impact on TN (Figure 1). With the increase in the N application rate, N availability and microbial effectiveness improved, thus increasing N retention [39]. In particular, with the help of straw returning, fertilization synchronizes soil available N supply with crop N demand, minimizes N loss, and stabilizes SOM [40].

TN and SOC had similar variation trends, demonstrating a close dynamic link between these components. It has been reported that C and N contents were linearly correlated with the structural components of C and N-type OM with similar changes and SOC changes in tandem with TN [41]. The SOC and TN content decreased gradually with the deepening of the soil depth (Figure 1). Bhattacharyya et al. (2010) demonstrated that SOC and TN were affected by soil depths and were mainly enriched in topsoil [31]. The reason may be that the topsoil receives more dead leaves, root exudates, and dead roots of plants. After decomposition and transformation by microorganisms, this OM enters the soil, and the residual organic carbon change into SOC [42].

#### 4.4. Aggregate Associated C and N

The SOC and TN contents in each aggregate size fraction obtained by two sieving methods differ significantly, where maximum values were recorded in  $>0.25$  mm aggregates (especially 2–1 mm aggregates) under wet sieving, while  $<0.25$  mm aggregates under dry sieving (Figure 2). The different distribution patterns of SOC and TN under different sieving methods are due to the different forms and intensity of energy input into the soil during the sieving process. The wet sieving method selected macro-aggregates with higher SOC and TN content, especially the 2–1 mm aggregates, as the dominant size fractions in terms of carbon and nitrogen retention. The results support the Nahidan and Nourbakhsh (2018) finding [27], indicating that macro-aggregates obtained by wet sieving had higher SOC and TN content than micro-aggregates. The dry sieving method selected the  $<0.25$  mm aggregates with high SOC and TN content, which may be due to mechanical forces. The micro-aggregates are likely to come from the decomposition of the unstable larger macro-aggregates. Particularly, micro-aggregates that cover the pore walls of large macro-aggregates are richer in solute mobility due to preferential deposition and stable biological exudates. They will quickly wear off during dry sieving, resulting in  $<0.25$  mm aggregates with a high SOC and TN content [43]. Due to the different soil physical and chemical properties, the stability and biochemical nature of key organic binding agents and aggregates of different sizes also differ in their role for carbon sequestration and stabilizations [44]. Hao et al. (2018) studied the response of aggregates to fertilization regime in Northeast China and indicated that aggregates with higher in aggregates with particle size  $> 0.053$  mm, especially 0.25–0.053 mm [45]. This difference from our study may be due to the high viscosity of the soil texture in this test position. Soil clay content is essential to organic carbon sequestration due to its large specific surface area and adsorption capacity [46]. Huang et al. (2010) pointed out that there was no difference in carbon concentration among large macro-aggregates, small macro-aggregates, and micro-aggregates, which may be attributed to the limited role that organic matter may play in the formation of red soil aggregates. Organic matter is not the main binder for accumulation in kaolinite-dominated red soils [10]. Lu et al. (2021) analyzed that aggregates with particle size  $> 2$  mm are dominant in terms of carbon sequestration for paddy soil [47], which is slightly different from our  $>0.25$  mm range. It is generally believed that anaerobic soil has higher carbon sequestration potential than well-aerated soil. Frequent dry–wet alternations of paddy soil lead to increased organic carbon adsorption on the surface of iron oxide in anaerobic soil, thus promoting the transition from micro-aggregates to macro-aggregates

under high organic consolidation [48]. In our work, the TN showed distributions similar to SOC as the aggregate size changed, consistent with [49]. In addition, the SOC and TN contents in all aggregates in the surface soil were significantly higher than that in the underlying soil (Figure 3), consistent with the results of most studies [50].

The contribution rate of the macro-aggregate to SOC and TN was higher than that of the micro-aggregate in the dry sieving method, and the contribution rate of the micro-aggregate to SOC and TN was higher than that of the macro-aggregate in the wet sieving method (Figure 3). It can be seen that the contribution rates of each aggregate size fraction to SOC and TN were strongly correlated with aggregate size fractions distribution. The macro-aggregates play a critical role in soil C and N sequestration in the dry sieving method. Organic materials, such as plant roots, root exudates, and fungal hyphae, are used as vectors by these micro-aggregates to create macro-aggregates gradually, effectively fixing these organic materials during the aggregate development and turnover process [51]. In addition, the contribution rate of micro-aggregate to SOC and TN was higher than that of macro-aggregate in the wet sieving method, indicating that there is higher decomposable SOM associated with macro-aggregates leads to the low retention of macro-aggregates rich in carbon.

N fertilization is beneficial to increase the SOC and TN content in aggregate size fractions (Figure 2), it can be seen that each aggregate size fraction did not reach the state of carbon saturation. The SOC and TN content of each aggregate were low under non-N (N0) and low N application (N75), which may be owing to microorganisms are involved in the transformation and synthesis of organic cementing materials required for the formation of aggregates. The straw itself has a high C/N, and microorganisms will also absorb specific N and other nutrient during the storage of OC. Appropriate fertilization benefits the distribution and sequestration of C and N in each aggregate, increases the contribution rate of macro-aggregates to SOC and TN, and decreases the contribution rate of micro-aggregate to SOC and TN under the wet sieving (Figure 3). Appropriate fertilization benefited the degree of agglomeration in macro-aggregates and promoted the sequestration of SOC in macro-aggregates due to the influence of coarse organic residues [52]. Excessive N rate reduced the SOC and TN contents in each size fraction, especially the contribution rate of macro-aggregates to SOC and TN under the dry sieving, which may be related to the fact that it aggravated the lack of other essential nutrients in plants, leading to a more significant excitation effect of crop root exudates on the mineralization of SOM, thereby reducing SOC.

#### 4.5. Aggregate Associated C/N Ratio

Soil aggregates regulate the C/N ratio and can reflect the soil's ability to store and recycle nutrients [53]. In our study, the macro-aggregates (especially 2–1 mm) had high SOC and TN content and high C/N ratio in wet sieving. The micro-aggregates (<0.25 mm) had high SOC and TN content and a low C/N ratio in dry sieving (Figure 4). We speculate that SOC in water-stable macro-aggregates (especially 2–1 mm) is relatively aged. The lowest C/N of the mechanical stability micro-aggregates (<0.25 mm) further confirms that, under the action of mechanical forces, these high-carbon and nitrogen micro-aggregates are likely to come from the decomposition of unstable macro-aggregates, especially those covering the pore walls of macro-aggregates. These micro-aggregates are conducive to microbial decomposition due to preferential deposition and relatively fresh OM. The soil C mineralization rate is higher than the N mineralization rate, leading to a low C/N [49]. Ying et al. (2010) also pointed out that the C/N ratio reduced to some extent with decreasing aggregate size [33]. The presence of the primary unstable C component and the higher conversion rate is indicated by higher organic carbon content, lower C/N ratio, and lower enrichment rate [35]. The C/N of aggregates in each size fraction gradually increased as the soil depth grew depth (Figure 4). This is consistent with the results of [41], which lower SOC mineralization rates and less disturbance in the lower soil depth could explain.

In our study, with the increase in the N application rate, the C/N of >0.25 mm aggregates first increased and then decreased, and the C/N of <0.25 mm aggregates first decreased and then increased, reaching the minimum value at N225 under the wet sieving method. The C/N of aggregate size fractions first decreased and then increased, reaching the lowest value at N225 under the dry sieving method. It can be seen that appropriate N application is beneficial to improve the C/N ratio of macro-aggregates, improve the C and N stability of macro-aggregates under the wet sieving method, reduce the C/N ratio of micro-aggregates under each aggregate size fraction under the dry sieving, and increase the decomposition rate of OM (Figure 4). On the one hand, the suitable N application rate is beneficial to adjust the returning straw with a high C/N ratio, improve the OM decomposition rate, and reduce the soil C/N ratio. On the other hand, appropriate N application rates contained higher macro-aggregates with higher quality residues (lower C/N ratios) and more unstable undecomposed SOM located on the surface of the macro-aggregates [41,54]. There is a higher C/N ratio for low N and excessive N treatment. In a high C/N ratio, according to basic stoichiometry theory, nitrogen is limited, which will increase microbial nitrogen mineralization [55]. However, Bhattacharyya et al. (2010) found no difference in C/N ratios between the mineral fertilized treatments [31], which may be the difference between the region and soil texture.

#### 4.6. Crop Yield

Fertilization can effectively promote the productivity of crops. In this study, the N application rate of 225 kg·ha<sup>-1</sup> is conducive to improving the crop yield. N fertilization reaches 300 kg·ha<sup>-1</sup>, and the crop yield decreases (Table 4). Fertilization, as a most common management practice, not only provides necessary elements for crop growth but also improves soil physical properties, changes aggregate distribution, affects soil carbon and nitrogen sequestration, regulates soil C/N ratio, and absorbs water and nutrients from the soil, and has a positive impact on crop yield [56]. Moreover, returning straw to the field after harvest is conducive to promoting the formation of organic carbon in the macro-aggregates, which is conducive to the formation of macro-aggregates [30], eventually leading to higher crop yields [57]. The PLS-PM analysis (Figure 6) also showed fertilization is conducive to higher crop yield, especially straw production, which will cause more straw to return to the soil, thereby increasing carbon and nitrogen sequestration and improving soil water-stable stability and mechanical stability. In addition, N fertilizer increasing the macro-aggregate has increased soil stability and C and N sequestration and decreased the C/N ratio, which is significantly related to yield, consistent with the conclusion of [58].

#### 5. Conclusions

Anthrosol has primarily mechanical stability macro-aggregates and water-stable micro-aggregates due to differences in mechanical force and water erosion resistance. Suitable N application rate (N225) improved soil aggregate stability by increasing macro-aggregates and decreasing micro-aggregates. Fertilization promoted the distribution and enrichment of carbon and nitrogen in macro-aggregates, reduced the C/N of micro-aggregates, and the contribution of micro-aggregates to SOC and TN, thus reducing the micro-aggregates. Non-N, less N, and excess N are not conducive to carbon and nitrogen sequestration in macro-aggregates, leading to the depolymerization effect of macro-aggregates. The N225 treatment had the highest yield among the different N fertilization treatments. In conclusion, long-term fertilization promoted the accumulation and storage of SOC and TN on the Loess Plateau. We recommend N225 as the appropriate N application rate, which is conducive to improving soil aggregate stability, carbon and nitrogen sequestration, and crop productivity on the Loess Plateau.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13051312/s1>, Table S1: Three-way ANOVA for the effect of aggregate size (S), soil depth (D) and fertilization treatment (T) on the soil aggregates distribution, the contributing rate of aggregate size fractions to SOC and TN, the SOC and TN content in each aggregate fractions size and C/N of aggregate size fractions under two sieving methods; Table S2: Two-way ANOVA for the effect of fertilization treatment and soil depth on the indicators related to soil aggregates stability, including MWD, GMD, D, PAD and ELT under two sieving methods.

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## References

1. Six, J.; Paustian, K.; Elliott, E.T.; Combrink, C. Soil Structure and Organic Matter I. Distribution of Aggregate-Size Classes and Aggregate-Associated Carbon. *Soil Sci. Soc. Am. J.* **2000**, *64*, 681–689. [\[CrossRef\]](#)
2. Wang, J.G.; Wei, Y.; Yu, B.; Li, Z.X.; Ma, R.M. Estimating the influence of related soil properties on macro- and micro-aggregate stability in ultisols of south-central China. *Catena* **2016**, *137*, 545–553. [\[CrossRef\]](#)
3. Gelaw, A.M.; Singh, B.R.; Lal, R. Organic Carbon and Nitrogen Associated with Soil Aggregates and Particle Sizes Under Different Land Uses in Tigray, Northern Ethiopia. *Land Degrad. Dev.* **2015**, *26*, 690–700. [\[CrossRef\]](#)
4. Huang, S.; Peng, X.X.; Huang, Q.R.; Zhang, W.J. Soil aggregation and organic carbon fractions affected by long-term fertilization in a red soil of subtropical China. *Geoderma* **2010**, *154*, 364–369. [\[CrossRef\]](#)
5. Six, J.; Frey, S.D.; Thiet, R.K.; Batten, K.M. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. *Soil Ecol. Soc. Am. J.* **2006**, *70*, 555–569. [\[CrossRef\]](#)
6. Zhang, Y.; Li, C.; Wang, Y.; Hu, Y.; Li, X. Maize yield and soil fertility with combined use of compost and inorganic fertilizers on a calcareous soil on the North China Plain. *Soil Tillage Res.* **2016**, *155*, 85–94. [\[CrossRef\]](#)
7. Qiu, Q.; Wu, L.F.; Ouyang, Z.; Li, B.; Xu, Y.; Wu, S.; Gregorich, E. Priming effect of maize residue and urea N on soil organic matter changes with time. *Appl. Soil Ecol.* **2016**, *100*, 65–74. [\[CrossRef\]](#)
8. Wooller, P.L. The impact of cultivation on carbon fluxes in woody savannas of Southern Africa. *Water Air Soil Pollut.* **1993**, *70*, 403–412. [\[CrossRef\]](#)
9. Zhao, R.F.; Chen, X.P.; Zhang, F.S.; Zhang, H.; Schroder, J.; Römhild, V. Fertilization and Nitrogen Balance in a Wheat–Maize Rotation System in North China. *Agron. J.* **2006**, *98*, 938–945. [\[CrossRef\]](#)
10. Huang, S.; Rui, W.; Peng, X.; Huang, Q.; Zhang, W. Organic carbon fractions affected by long-term fertilization in a subtropical paddy soil. *Nutr. Cycl. Agroecosyst.* **2010**, *86*, 153–160. [\[CrossRef\]](#)
11. Zhang, P.; Wei, T.; Li, Y.; Wang, K.; Jia, Z.K.; Han, Q.F.; Ren, X.L. Effects of straw incorporation on the stratification of the soil organic C, total N and C:N ratio in a semiarid region of China. *Soil Tillage Res.* **2015**, *153*, 28–35. [\[CrossRef\]](#)
12. Huang, T.; Ju, X.; Yang, H. Nitrate leaching in a winter wheat–summer maize rotation on a calcareous soil as affected by nitrogen and straw management. *Sci. Rep.* **2017**, *7*, 42247. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Wang, R.Z.; Dungait, J.A.J.; Creamer, C.A.; Cai, J.P.; Li, B.; Xu, Z.W.; Zhang, Y.G.; Ma, Y.N.; Jiang, Y. Carbon and Nitrogen Dynamics in Soil Aggregates under Long-Term Nitrogen and Water Addition in a Temperate Steppe. *Soil Sci. Soc. Am. J.* **2015**, *79*, 527. [\[CrossRef\]](#)
14. Tessa, C.; Papathanasiou, H.J.; Antje, F.; Karla, D.; Dietrich, H.; Homeier, J.; Yvonne, O.; Olsson, P.; Suárez, J.P.; Rillig, M.C. Increases in Soil Aggregation Following Phosphorus Additions in a Tropical Premontane Forest are Not Driven by Root and Arbuscular Mycorrhizal Fungal Abundances. *Front. Earth Sci.* **2016**, *3*, 89.

15. Zhou, H.; Peng, X.; Perfect, E.; Xiao, T.; Peng, G. Effects of organic and inorganic fertilization on soil aggregation in an Ultisol as characterized by synchrotron based X-ray micro-computed tomography. *Geoderma* **2013**, *195*–196, 23–30. [\[CrossRef\]](#)
16. Guo, Z.; Wei, L.I.; Islam, M.U.; Wang, Y.; Zhang, Z.; Peng, X. Nitrogen fertilization degrades soil aggregation by increasing ammonium ions and decreasing biological binding agents on a Vertisol after 12 years. *Pedosphere* **2022**, *32*, 629–636. [\[CrossRef\]](#)
17. Mustafa, A.; Xu, M.; Atizaz, S.; Abrar, M.M.; Núñez-Delgado, A. Soil aggregation and soil aggregate stability regulate organic carbon and nitrogen storage in a red soil of southern China. *J. Environ. Manag.* **2020**, *270*, 110894. [\[CrossRef\]](#)
18. Yan, Z.J.; Zhou, J.; Nie, J.W.; Yang, Y.D.; Zhao, J.; Zeng, Z.H.; Marshall, M.R.; Peixoto, L.; Zang, H.D. Do cropping system and fertilization rate change water-stable aggregates associated carbon and nitrogen storage? *Environ. Sci. Pollut. Res.* **2021**, *28*, 65862–65871. [\[CrossRef\]](#)
19. Zou, C.; Li, Y.; Huang, W.; Zhao, G.; Pu, G.; Su, J.; Coyne, M.S.; Chen, Y.; Wang, L.; Hu, X. Rotation and manure amendment increase soil macro-aggregates and associated carbon and nitrogen stocks in flue-cured tobacco production. *Geofis. Int.* **2018**, *325*, 49–58. [\[CrossRef\]](#)
20. Zheng, H.; Liu, W.; Zheng, J.; Luo, Y.; Li, R.; Hao, W.; Qi, H.; Jian, L. Effect of long-term tillage on soil aggregates and aggregate-associated carbon in black soil of Northeast China. *PLoS ONE* **2018**, *13*, e0199523. [\[CrossRef\]](#)
21. Egan, G.; Crawley, M.J.; Fornara, D.A. Effects of long-term grassland management on the carbon and nitrogen pools of different soil aggregate fractions. *Sci. Total Environ.* **2018**, *613*, 810–819. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Okolo, C.C.; Gebresamuel, G.; Zenebe, A.; Haile, M.; Eze, P.N. Accumulation of organic carbon in various soil aggregate sizes under different land use systems in a semi-arid environment. *Agric. Ecosyst. Environ.* **2020**, *297*, 106924. [\[CrossRef\]](#)
23. Udom, B.E.; Ogunwale, J.O. Soil organic carbon, nitrogen, and phosphorus distribution in stable aggregates of an Ultisol under contrasting land use and management history. *J. Plant Nutr. Soil Ence.* **2015**, *178*, 460–467. [\[CrossRef\]](#)
24. Gelaw, A.M.; Singh, B.R.; Lal, R. Soil organic carbon and total nitrogen stocks under different land uses in a semi-arid watershed in Tigray, Northern Ethiopia. *Agric. Ecosyst. Environ.* **2014**, *188*, 256–263. [\[CrossRef\]](#)
25. Iticha, B.; Debele, T. Distribution and Stability of Soil Structural Aggregates in Relation to Organic Carbon under Changing Land Uses. *Am.-Eurasian J. Agric. Environ. Sci* **2017**, *17*, 119–127.
26. Lu, R.K. *Analytical Methods of Soil and Agricultural Chemistry China*; Agricultural Science and Technology Press: Beijing, China, 2000.
27. Nahidan, S.; Nourbakhsh, F. Distribution pattern of amidohydrolase activities among soil aggregates: Effect of soil aggregates isolation methods. *Appl. Soil Ecol.* **2018**, *125*, 250–256. [\[CrossRef\]](#)
28. Gale, W.J.; Cambardella, C.A.; Bailey, T.B. Root-Derived Carbon and the Formation and Stabilization of Aggregates. *Soil Sci. Soc. Am. J.* **2000**, *64*, 201–207. [\[CrossRef\]](#)
29. Kurmi, B.; Nath, A.J.; Lal, R.; Das, A.K. Water stable aggregates and the associated active and recalcitrant carbon in soil under rubber plantation. *Sci. Total Environ.* **2020**, *703*, 10. [\[CrossRef\]](#)
30. Zhang, X.F.; Xin, X.L.; Zhu, A.N.; Zhang, J.B.; Yang, W.L. Effects of tillage and residue managements on organic C accumulation and soil aggregation in a sandy loam soil of the North China Plain. *Catena* **2017**, *156*, 176–183. [\[CrossRef\]](#)
31. Bhattacharyya, R.; Prakash, V.; Kundu, S.; Srivastva, A.K.; Gupta, H.S.; Mitra, S. Long term effects of fertilization on carbon and nitrogen sequestration and aggregate associated carbon and nitrogen in the Indian sub-Himalayas. *Nutr. Cycl. Agroecosyst.* **2010**, *86*, 1–16. [\[CrossRef\]](#)
32. Banger, K.; Kukal, S.S.; Toor, G.; Sudhir, K.; Hanumanthraju, T.H. Impact of long-term additions of chemical fertilizers and farm yard manure on carbon and nitrogen sequestration under rice-cowpea cropping system in semi-arid tropics. *Plant Soil* **2009**, *318*, 27–35. [\[CrossRef\]](#)
33. Chen, Y.; Zhang, X.D.; He, H.B.; Xie, H.T.; Yan, Y.; Zhu, P.; Ren, J.; Wang, L.C. Carbon and nitrogen pools in different aggregates of a Chinese Mollisol as influenced by long-term fertilization. *J. Soils Sediments* **2010**, *10*, 1018–1026. [\[CrossRef\]](#)
34. Zhou, M.; Liu, C.; Wang, J.; Meng, Q.; Du, W. Soil aggregates stability and storage of soil organic carbon respond to cropping systems on Black Soils of Northeast China. *Sci. Rep.* **2020**, *10*, 265. [\[CrossRef\]](#) [\[PubMed\]](#)
35. He, Y.T.; Zhang, W.J.; Xu, M.G.; Tong, X.G.; Sun, F.X.; Wang, J.Z.; Huang, S.M.; Zhu, P.; He, X.H. 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.* **2015**, *532*, 635–644. [\[CrossRef\]](#)
36. Tisdall, J.M.; Oades, J.M. Organic matter and water-stable aggregates in soils. *Eur. J. Soil Sci.* **1982**, *33*, 141–163. [\[CrossRef\]](#)
37. Fan, J.; Ding, W.; Xiang, J.; Qin, S.; Zhang, J.; Ziadi, N. Carbon sequestration in an intensively cultivated sandy loam soil in the North China Plain as affected by compost and inorganic fertilizer application. *Geoderma* **2014**, *230*–231, 22–28. [\[CrossRef\]](#)
38. Xu, X.; Zhang, W.; Xu, M.; Li, S.; An, T.; Pei, J.; Jing, X.; Xie, H.; Wang, J. Characteristics of differently stabilised soil organic carbon fractions in relation to long-term fertilisation in Brown Earth of Northeast China. *Sci. Total Environ.* **2016**, *572*, 1101–1110. [\[CrossRef\]](#)
39. Gentile, R.M.; Vanlauwe, B.; Six, J. Integrated Soil Fertility Management: Aggregate carbon and nitrogen stabilization in differently textured tropical soils. *Soil Biol. Biochem.* **2013**, *67*, 124–132. [\[CrossRef\]](#)
40. Tian, G.; Ishida, F.; Keatinge, D.; Vanlauwe, B.; Diels, J. Combined Application of Organic Matter and Fertilizer. *Sustain. Soil Fertil. West Afr.* **2001**, *58*, 247–279.
41. Zhang, Y.; Li, P.; Liu, X.; Xiao, L.; Zhao, B. Effects of farmland conversion on the stoichiometry of carbon, nitrogen, and phosphorus in soil aggregates on the Loess Plateau of China. *Geoderma* **2019**, *351*, 188–196. [\[CrossRef\]](#)

42. Schmidt, M.; Torn, M.S.; Abiven, S. Persistence of soil organic matter as an ecosystem property. *Nature* **2011**, *478*, 49–56. [[CrossRef](#)] [[PubMed](#)]
43. Sainju, U. Carbon and Nitrogen Pools in Soil Aggregates Separated by Dry and Wet Sieving Methods. *Soil Sci.* **2006**, *171*, 937–949. [[CrossRef](#)]
44. Arai, M.; Miura, T.; Tsuzura, H.; Minamiya, Y.; Kaneko, N. Two-year responses of earthworm abundance, soil aggregates, and soil carbon to no-tillage and fertilization. *Geoderma* **2017**, *332*, 135–141. [[CrossRef](#)]
45. Hao, L.; Yu, C.; Zhang, Q.Y.; Zuo, B.B.; Du, W.L. Contrasting responses of bacterial and fungal communities to aggregate-size fractions and long-term fertilizations in soils of northeastern China. *Sci. Total Environ.* **2018**, *635*, 784–792. [[CrossRef](#)]
46. Ma, P.; Nan, S.; Yang, X.; Qin, Y.; Ma, T.; Li, X.; Yu, Y.; Bodner, G. Macroaggregation is promoted more effectively by organic than inorganic fertilizers in farmland ecosystems of China—A meta-analysis. *Soil Tillage Res.* **2022**, *221*, 105394. [[CrossRef](#)]
47. Lu, X.; Hou, E.; Guo, J.; Gilliam, F.S.; Li, J.; Tang, S.; Kuang, Y. Nitrogen addition stimulates soil aggregation and enhances carbon storage in terrestrial ecosystems of China: A meta-analysis. *Glob. Chang. Biol.* **2021**, *27*, 2780–2792. [[CrossRef](#)] [[PubMed](#)]
48. Yan, X.; Zhou, H.; Zhu, Q.H.; Wang, X.F.; Zhang, Y.Z.; Yu, X.C.; Peng, X. Carbon sequestration efficiency in paddy soil and upland soil under long-term fertilization in southern China. *Soil Tillage Res.* **2013**, *130*, 42–51. [[CrossRef](#)]
49. Wang, W.; Xie, X.L.; Chen, A.L.; Yin, C.M.; Chen, W.C. Effects of long-term fertilization on soil carbon, nitrogen, phosphorus and rice yield. *J. Plant Nutr. Fertil.* **2013**, *36*, 551–561. [[CrossRef](#)]
50. Chen, H.H.; Li, X.C.; Hu, F.; Shi, W. Soil nitrous oxide emissions following crop residue addition: A meta-analysis. *Glob. Chang. Biol.* **2013**, *19*, 2956–2964. [[CrossRef](#)]
51. Six, J.; Paustian, K. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. *Soil Biol. Biochem.* **2014**, *68*, A4–A9. [[CrossRef](#)]
52. Li, C.H.; Li, Y.; Tang, L.S. The effects of long-term fertilization on the accumulation of organic carbon in the deep soil profile of an oasis farmland. *Plant Soil* **2013**, *369*, 645–656. [[CrossRef](#)]
53. Ostrowska, A.; Porebska, G. Assessment of the C/N ratio as an indicator of the decomposability of organic matter in forest soils. *Ecol. Indic. Integr. Monit. Assess. Manag.* **2015**, *49*, 104–109. [[CrossRef](#)]
54. Wright, A.L.; Hons, F.M. Soil Aggregation and Carbon and Nitrogen Storage under Soybean Cropping Sequences. *Soil Sci. Soc. Am. J.* **2004**, *68*, 507. [[CrossRef](#)]
55. Qi, L.; Zhang, Y.; Liu, B.; Amonette, J.E.; Xie, Z. How does biochar influence soil N cycle? A meta-analysis. *Plant Soil* **2018**, *426*, 211–2255. [[CrossRef](#)]
56. Bhattacharyya, R.; Tuti, M.D.; Bisht, J.K.; Bhatt, J.C.; Gupta, H.S. Conservation Tillage and Fertilization Impact on Soil Aggregation and Carbon Pools in the Indian Himalayas Under an Irrigated Rice-Wheat Rotation. *Soil Sci.* **2012**, *177*, 218–228. [[CrossRef](#)]
57. Mikha, M.M.; Rice, C.W. Tillage and Manure Effects on Soil and Aggregate-Associated Carbon and Nitrogen. *Soil Sci. Soc. Am. J.* **2004**, *68*, 809–816. [[CrossRef](#)]
58. Xie, J.Y.; Hou, M.M.; Zhou, Y.T.; Wang, R.J.; Zhang, S.L. Carbon sequestration and mineralization of aggregate-associated carbon in an intensively cultivated Anthrosol in north China as affected by long term fertilization. *Geoderma* **2017**, *296*, 1–9. [[CrossRef](#)]

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