Time-dependent regulation of soil aggregates on fertilizer N retention and the influence of straw mulching

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Abstract:

Fertilizer nitrogen (N) turnover is highly controlled by soil aggregation. However, the functions of the various aggregates that regulate long-term fertilizer N retention under conservation management remain unexplored. In this study, ^{15}N-labeled fertilizer was initially applied *in situ* to investigate the effects of maize straw mulching on fertilizer N allocation in soil aggregates at a decadal scale. The topsoil was fractionated into macroaggregate, microaggregate, and silt-clay (SC) fractions. Macroaggregate was further divided into particulate organic matter (POM) and mineral-associated organic matter (MAOM). A higher enrichment factor of fertilizer N than of soil total N in macroaggregate indicated that the fertilizer N was more apt to incorporation into macroaggregate. The fertilizer N in the bulk soil declined gradually to 84.0% by the 13th year. Temporally, the reduction proportion of fertilizer N in the SC fraction was the largest before 5th years, whereas macroaggregate was the main reactive spot for fertilizer N transformation from 9 to 13 years. Therefore, the function of aggregates was time-dependent in controlling fertilizer N retention and turnover via the release of previously entrapped fertilizer N, but encapsulated the subsequently applied N (i.e., unlabeled fertilizer), whereas mineral adsorption contributed to the long-term stabilization of fertilizer N. Compared with fertilization alone, straw mulching improved aggregates stability, favored the initial fertilizer N retention in macroaggregate by enriching fertilizer N in POM, and reduced the proportion of N loss in MAOM after 9 years. These finding indicate that the improvement in fertilizer N stability related to straw decomposition was sequentially attributed to the enhancement of aggregate encapsulation and persistent interaction with soil minerals. Therefore, this study provides new insights into the functional heterogeneity of soil aggregates at different time stages and the intricate interplay between carbon availability-controlled fertilizer N retention and the

improvement in soil aggregation.

Keywords: ^{15}N-labeled fertilizer; allocation dynamics; soil aggregates; maize straw mulching

Introduction

As a fundamental element in guaranteeing agricultural productivity, improving the soil N use efficiency is crucial for food security and environmental safety (Gentile et al., 2008; Gao et al., 2020). Long-term soil N retention and supply are closely related to the mineralization-immobilization turnover (Martens et al., 2006; Farzadfar et al., 2021), and such a transformation process is critically controlled by soil aggregation and adherence to mineral surfaces (Six et al., 2004; Lehmann and Kleber, 2015) in addition to the close linkage between carbon (C) and N availability. The critical soil aggregation process is an encapsulation process with an internal turnover of components, including particulate organic matter (POM), which is consistently dominant in plant-derived structural compounds and mineral-associated organic matter (MAOM) (Six et al., 2000; Cotrufo et al., 2015). Recent studies have found that nitrogenous components, either bound to clay minerals or enriched in POM, can be encapsulated in soil aggregates for stabilization (O'Brien and Jastrow 2013; Zhu et al., 2018). However, in the reverse process, N-containing components in macro and microaggregate can be released along with aggregate fragmentation (Six et al., 2000). Therefore, the functions of various aggregates and the mechanisms of long-term soil N stability and turnover remain poorly understood.

Synthetic N fertilizer is available for crop uptake in the current growing season, and the residual fertilizer N contributes to soil N pool build up and reutilization by the crop and soil biota (Yan et al., 2020). The dynamics of residual fertilizer N involved in soil N turnover are highly aggregate fraction-specific, as indicated by the ^{15}N labeling technique (Canfield et al., 2010; Dorodnikov et al., 2011; Battye et al., 2017). In an 8-year field experiment with ^{15}N-labeled NH_4NO_3, van Groenigen et al. (2002) found a faster turnover of fertilizer N in macroaggregate than in microaggregate. This difference was ascribed to the loose bonding of fungal hyphae and plant-root binding agents in the macroaggregate compared to the chemically persistent organic components and crystalline oxides in the microaggregate. In contrast, based on the cultivation of ryegrass on separated soil aggregate fractions for 42 days, Duan et al. (2021) found a higher use efficiency of fertilizer N retained in the silt-clay (SC) fraction than in macroaggregate, implying the stabilization of fertilizer N via aggregate formation. The functional variation in differently sized aggregate fractions can be shaped by environmental conditions, for example, soil type and management practices (Balabane, 1996; Bimüller et al., 2014; Murphy et al., 2016; Bhattacharyya et al., 2019); however, the possible time-dependent fertilizer N translocation along with soil aggregate fragmentation-formation processes has been neglected in such snapshot-based studies. Therefore, tracing the dynamic allocation of ^{15}N-labeled fertilizer in soil aggregates is essential for exploring the regulatory mechanism of the soil microstructure on residual fertilizer N retention and turnover.

Conservation tillage with crop straw return to the field, as an effective practice for agricultural sustainability, can enhance fertilizer N accumulation in soil (Moran et al., 2005; Gentile et al., 2009; Yuan et al., 2021). Critically, the decomposition of crop straw gives rise to reactive hotspots and fosters the microbial assimilation of inorganic fertilizer N into organic forms (Liu et al., 2016; Zhou et al., 2023). The production and accumulation of adhesive microbial residues are conducive to soil aggregation and enhance the physical protection of soil N (Rahman et al., 2019). Crop straw return enhances the 1.5-year retention of fertilizer N in soil aggregates (Gentile et al., 2013), especially by extending the mean residence time in macroaggregate (Kong et al., 2009; Bhattacharyya et al., 2019). Moreover, enhanced POM formation after plant residue input favors an increase in the capacity of macroaggregate as reservoirs for fertilizer N retention (Chivenge et al., 2011). However, the effects of crop straw return on fertilizer N turnover over time, which is essentially regulated by the divergent functions of different aggregates, remain unclear in field experiments.

To better understand how soil aggregation is associated with the retention and release of fertilizer N in conservation tillage management, we conducted a ^{15}N-labeled fertilization experiment with and without maize straw mulching. ^{15}N-labeled fertilizer was applied initially, and the dynamic allocation of residual fertilizer N in the soil aggregate fractions was determined over a 13-year period. We hypothesized that (1) the function of different soil aggregate fractions in controlling fertilizer N turnover is time-dependent, considering the vulnerability of physical protection; (2) macroaggregate dominates the long-term fertilizer N turnover process owing to the dispersal of the previously enveloped portion, but clay minerals contribute to the persistent stability of fertilizer N; and (3) maize straw mulching favors the retention of fertilizer N in macroaggregate by strengthening soil aggregation and enhancing the immobilization of fertilizer N in the POM fraction.

2. Materials and methods

2.1 Site description

A ^{15}N-labeling fertilization field experiment with conservation tillage was conducted in 2007 at the National Field Observation and Research Station of Shenyang Agroecosystem (41°31′N, 123°24′E), located in Shenyang City, Liaoning Province, China. The region has a typical temperate continental monsoon climate, with a mean annual temperature of 8.2 °C and a mean annual precipitation of 700 mm. The soil type is classified as Alfisols (Soil Survey Staff, 2003), with 13.0% sand, 66.1% silt, and 20.7% clay. Maize (*Zea mays L.*) has been cultivated for more than 20 years, and both above- and belowground biomass was removed before the start of our experiment.

2.2 Experimental design

Micro-plots with an area of 2.08 m^2 (1.6 m × 1.3 m) were established with a completely randomized design. Each plot was surrounded by polyvinyl chloride boards (with the cuboids extending 15 cm above the ground and penetrating 35 cm underground) to prevent the lateral migration of ^{15}N-labeled fertilizer. Moreover, a 2.5 m protective row between the micro-plots was designed to reduce the mutual interference between neighboring plots. Within all micro-plots and protective rows, no-tillage cultivation of maize was implemented at a mean density of 57700 plants ha^{-1} (12 plants per micro-plot), and fertilizer was applied annually at a local rate of 200 kg N ha^{-1}, 30 kg P ha^{-1} (KH_2PO_4), and 58 kg K ha^{-1} (summed by KH_2PO_4 and K_2SO_4). ^{15}N-labeled fertilizer, $(^{15}NH_4)_2SO_4$ (^{15}N, 99 atom%), was applied at three stages: maize sowing, jointing, and silking, at a ratio of 1:2:1. Two treatments with three replicates were selected for this study: (1) only ^{15}N-labeled fertilizer was applied in the 1st year (2007), followed by the annual application of unlabeled $(NH_4)_2SO_4$ with aboveground biomass removal (^{15}NF); (2) combined with annual maize straw mulching, ^{15}N-labeled fertilizer was applied in 2007, and unlabeled fertilizer was applied in subsequent years (^{15}NFS). Two corresponding control treatments were established in 2007: annual application of unlabeled N fertilizer (^{14}NF) and annual application of unlabeled N fertilizer combined with maize straw mulching (^{14}NFS). In the maize straw mulching treatment, the amount of unlabeled maize straw applied was approximately equivalent to half of the annual straw yield (ca. 5.8×10^3 kg ha^{-1}) and was used to cover the soil surface in each micro-plot after being cut into small pieces (10 cm in length). This corresponds to an annual input of 2500 kg C ha^{-1} and 48 kg N ha^{-1} (Hu et al., 2015).

2.3 Soil sampling

After harvesting in late September, soil samples were collected in the 1st (2007), 5th (2011), 9th (2015), and 13th (2019) years. In each plot, three separated topsoil samples (0–10 cm) were collected using a cylindrical soil aggregate sampler with a diameter of 10 cm. To obtain representative soil samples, each soil sample was broken into pieces of approximately 1 cm^3 according to the natural cracks, the three separate samples were remixed, and a quarter of the composite sample was collected as a replicate for soil aggregates fractionation.

2.4 Soil aggregates fractionation

Soil aggregate fractionation was performed based on the wet-sieving method described by Six et al. (1998). Accordingly, bulk soil was separated into macroaggregate (2000–250 μm), microaggregate (250–53 μm), and silt-clay (SC) (<53 μm) fractions.

The fraction >2000 μm was almost negligible and thus not included in our analyses. Briefly, a 50 g air-dried sample was placed in a 2000 μm sieve and immersed in deionized water for 5 min. The separation of soil aggregates was achieved by manually moving the sieve up and down by 3 cm, with 50 repetitions over 2 min. Soil particles that passed through a 2000 μm sieve when retained in a 250 μm sieve were collected as macroaggregate, whereas those in the 250–53 μm sieve were collected as microaggregate. Subsequently, soil particles passed through a 53 μm sieve were centrifuged for 30 min at 4000 rpm and separated as the SC fraction. Floating organic matter, mainly straw residue, was discarded according to the definition of soil organic matter (Six et al., 1998). The recovery rate of the soil during aggregate fractionation was over 98%, indicating the accuracy and reliability of the method employed in this study.

As a hotspot site with active metabolism, the macroaggregate fraction was further fractionated into POM and MAOM fractions using a density- and particle size-based method modified from Six et al. (1998) and van Groenigen et al. (2002). Briefly, 10 g of macroaggregate was placed in a 100 mL centrifugation tube containing 50 mL NaI solution with a density of 1.80 g cm^{-3}. The tube was gently inverted five times and then the suspension was filtered through a 0.45 μm filter membrane to obtain the free particulate organic matter (fPOM). The remaining soil was shaken for 12 h with 10 glass beads and 50 mL NaI (1.80 g cm^{-3}) to break down soil aggregates. After shaking, the tube was centrifuged at 4000 rpm for 60 min, and the supernatant was filtered with a 0.45 μm filter membrane to obtain the occluded particulate organic matter (oPOM). The pellet was dispersed and separated using a 53 μm sieve, with the >53 μm fraction defined as >53 μm particulate organic matter ($POM_{(>53)}$) and the <53 μm fraction defined as mineral-associated organic matter (MAOM). In this study, the fPOM and oPOM fractions were combined into one fraction owing to their minimal mass and named as $POM_{(f+o)}$ fraction. The stability index of the soil aggregates, known as the mean weight diameter (MWD) (van Bavel, 1950), was calculated as follows:

$$\mathrm{MWD} = \sum_{i=1}^{n}(\overline{\mathrm{X}}_i \, \mathrm{W}_i) \quad (1)$$

where $\overline{\mathrm{X}}_i$ is the average diameter of the soil aggregates (mm) and W_i is the mass proportion of each soil aggregate fraction (%).

2.5 Fertilizer N analyses

The concentration of soil total N (TN) was measured using an elemental analyzer (Model CN; Vario Macro Elemental Analyzer System, GmbH, Germany). The ^{15}N abundance of the physical fractions was analyzed using an isotope ratio mass spectrometer (EA-IRMS, Flash EA1112, Delta plus XP; Thermo Fisher Scientific Inc., Waltham, MA, USA). The fertilizer N concentration was calculated as follows:

$$\text{Fertilizer N (mg kg}^{-1}) = (\mathrm{AT}_e - \mathrm{AT}_c)/\mathrm{AT}_f \times \text{TN (mg kg}^{-1}) \quad (2)$$

where Fertilizer N (mg kg^{-1}) is the concentration of fertilizer N in the bulk soil and physical fractions, AT_e is the ^{15}N abundance of the soil sample in ^{15}NF and ^{15}NFS treatments, AT_c is the natural ^{15}N abundance in the ^{14}NF and ^{14}NFS treatments, and AT_f is the ^{15}N abundance of the applied fertilizer (99 atom%).

The enrichment factor (E*f*) is the relative ratio of the TN or fertilizer N concentration in the physical fraction to that in the bulk soil (E*f* > 1 indicates that TN or fertilizer

N is relatively enriched in this fraction; E*f* < 1 indicates a relative depletion in this fraction), which was calculated as follows:

$$\mathrm{E}f = \mathrm{E}f_i / \mathrm{E}f_{bs} \tag{3}$$

where $\mathrm{E}f_i$ is the concentration of TN (mg kg^{-1}) or fertilizer N (mg kg^{-1}) in the individual fractions and $\mathrm{E}f_{bs}$ is the concentration of TN or fertilizer N in the bulk soil.

The stock (S_f, mg kg^{-1}) of TN or fertilizer N in the individual fractions was calculated as follows:

$$\mathrm{S}_f\ (\mathrm{mg\ kg^{-1}}) = \mathrm{C}_f\ (\mathrm{mg\ kg^{-1}}) \times \mathrm{M}_f\ (\%) \tag{4}$$

where C_f (mg kg^{-1}) is the concentration of TN (mg kg^{-1}) or fertilizer N (mg kg^{-1}) in this fraction and M_f (%) is the relative mass of the corresponding fraction.

The relative stock distribution (P_f, %), calculated as the proportion of TN or fertilizer N stock in individual fractions relative to the bulk soil, was assessed as follows:

$$\mathrm{P}_f(\%) = \mathrm{S}_f\ (\mathrm{mg\ kg^{-1}}) / \mathrm{S}_T\ (\mathrm{mg\ kg^{-1}}) \times 100 \tag{5}$$

where S_T (mg kg^{-1}) is the sum of the S_f of TN (mg kg^{-1}) or fertilizer N (mg kg^{-1}) in bulk soil.

The reduction proportion of fertilizer N in the soil aggregates, including the cumulative and phased reduction proportions, was calculated as follows:

$$\text{Cumulative reduction proportion } (\%) = [\mathrm{FN}_i(\mathrm{mg\ kg^{-1}}) - \mathrm{FN}_1(\mathrm{mg\ kg^{-1}})] / \mathrm{FN}_1(\mathrm{mg\ kg^{-1}}) \times 100 \tag{6}$$

$$\text{Phased reduction proportion } (\%) = [\mathrm{FN}_{j+4}(\mathrm{mg\ kg^{-1}}) - \mathrm{FN}_j(\mathrm{mg\ kg^{-1}})] / \mathrm{FN}_j(\mathrm{mg\ kg^{-1}}) \times 100 \tag{7}$$

where FN_1 (mg kg^{-1}) is the concentration of residual fertilizer N after the first growing season, FN_i and FN_j (mg kg^{-1}) are the concentrations of residual fertilizer N at specific sampling points, i = 5, 9, 13 and j = 1, 5, 9, respectively.

The influence of maize straw mulching on the reduction in fertilizer N (ISR, %) was assessed as follows:

$$\text{ISR (\%)} = [\text{RR}_{FS}(\%) - \text{RR}_{F}\ (\%)]/\text{RR}_{F}(\%) \times 100 \qquad (8)$$

where RR_{FS} (%) and RR_F (%) represent the reduction proportions of fertilizer N in the ^{15}NFS and ^{15}NF treatments, respectively.

2.6 Statistical analyses

Normality of the data was tested before analysis using the Kolmogorov–Smirnov test. Homogeneity of variance was assessed using Levene's test. All statistical analyses were performed using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA). To test the main effects of straw mulching, temporal effect, aggregate-size class, and their interaction effects, a linear mixed-effect model analysis was conducted with straw mulching, temporal effect, and aggregate-size class as fixed factors, and blocks as random factors. Statistically significant differences in soil parameters (e.g., mass distribution, concentration, enrichment factor, relative stock, and reduction proportion) within the individual fractions and treatment but in different years were assessed using one-way ANOVA, followed by the Bonferroni test at a 95 % confidence level (P < 0.05). Paired t-tests were performed to determine differences in soil parameters between the two treatments in the same year and aggregate fraction. Figures were plotted using OriginPro 2015 (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1 Mass distribution of soil aggregates during 13 years of conservation tillage

In the N fertilizer alone treatment (^{15}NF), the relative mass proportions of macroaggregate, microaggregate and SC fractions accounted for 18.8%, 42.4%, and 38.8%, respectively, in the 1st year, and the distribution remained unchanged throughout the experimental period (Table 1). In contrast, in the treatment with consecutive maize straw mulching at half of the annual straw yield (^{15}NFS), the relative proportion of macroaggregate increased from 18.4% to 24.4% after 13 years, accompanied by a decrease in the SC fraction from 37.7% to 33.6%. The mean weight diameter of the soil aggregates increased from 0.21 mm to 0.26 mm over time under maize straw mulching (Table 1). In the macroaggregate subfractions, the relative proportions of $POM_{(f+o)}$, $POM_{(>53)}$, and MAOM were 3.9%, 19.2%, and 76.9%, respectively, in the ^{15}NF treatment (Table 1). Maize straw mulching increased the proportion of the $POM_{(f+o)}$ fraction from 3.8% to 6.7% by the 13th year ($P < 0.05$), and accordingly reduced that of the MAOM fraction from 76.4% to 67.9% ($P < 0.05$).

3.2 Dynamic distribution of soil TN in soil aggregates

The concentration of soil TN increased with time, and the aggregate size increased ($P < 0.05$, Tables 2 and 3); thus, the enrichment factor (E*f*) of TN remained larger than 1 in macroaggregate, whereas it was lower than 1 in the SC fraction (Table 2). Until the 13th year, the TN concentration in bulk soil (0–10 cm) increased from 1.0 g kg^{-1} (1st year) to 1.1 g kg^{-1} in the ^{15}NF treatment. The maximum increase was observed in macroaggregate (22.7%), followed by microaggregate (9.0%) and SC fractions (6.7%)

compared with the 1^{st} year. In the ^{15}NFS treatment, 13-year maize straw mulching increased soil TN concentration in bulk soil to 1.2 mg kg^{-1}, and the concentrations in the individual fractions were 1.7 g kg^{-1} (macroaggregate), 1.3 g kg^{-1} (microaggregate), and 1.0 g kg^{-1} (SC fraction) (Table 2). In the 1^{st} year, the relative stocks of TN in the macroaggregate, microaggregate, and SC fractions ranged between 22.8–23.4%, 44.1–45.6% and 31.5–32.5%, respectively, with no significant differences between the two treatments (Table 2). By the 13^{th} year, the relative TN stock in the macroaggregate of the ^{15}NFS treatment (32.5%) was higher than that in the ^{15}NF treatment (23.7%). However, the change in the relative TN stock in SC fraction showed an opposite trend, being 25.5% in the ^{15}NFS treatment and 31.3% in the ^{15}NF treatment.

The TN concentration within the macroaggregate subfractions was significantly influenced by the physical fraction and sampling time ($P < 0.05$, Tables 3 and 4). The TN concentration was the highest in $POM_{(f+o)}$, followed by $POM_{(>53)}$, and was the lowest in the MAOM fraction; thus, the E*f* of TN was larger than 1 in the $POM_{(f+o)}$ fraction, whereas it was lower than 1 in the $POM_{(>53)}$ and MAOM fractions (Table 4). In the 1^{st} year, there was no significant difference in the TN concentrations between the two treatments. Till the 13^{th} year, TN concentration in the ^{15}NF treatment increased to 12.9 g kg^{-1} in $POM_{(f+o)}$, 1.6 g kg^{-1} in $POM_{(>53)}$, and 1.1 g kg^{-1} in MAOM fraction; whereas in the ^{15}NFS treatment, straw mulching significantly increased TN concentration to 14.2 g kg^{-1} in $POM_{(f+o)}$, 1.7 g kg^{-1} in $POM_{(>53)}$ and 1.1 mg kg^{-1} in MAOM fraction, respectively (Tables 3 and 4). In the ^{15}NF treatment, the relative TN stocks remained constant over time, at 28.9% in $POM_{(f+o)}$, 18.9% in $POM_{(>53)}$, and 52.2% in MAOM (Table 4). Comparatively, maize straw mulching gradually changed the distribution pattern by increasing the relative TN stock to 44.4% in $POM_{(f+o)}$, whereas decreasing to 35.2% in the MAOM fraction by the 13^{th} year.

3.3 Temporal distribution of the initially applied fertilizer N in soil aggregates

The concentration of the initially applied fertilizer N in the soil was highly specific to aggregate size (Fig. 2; Table 3). It remained the highest in macroaggregate across all sampling periods, followed by microaggregate, and was the lowest in the SC fraction. The E*f* of fertilizer N in the macroaggregate was larger than that of TN, whereas the opposite trend was observed for the microaggregate and SC fraction (Fig. 3; Table 2). As a result, the relative stock of fertilizer N in the macroaggregate remained larger than that of TN, whereas it was lower in the SC fraction (Fig. 4; Table 2).

The concentration of fertilizer N in individual aggregate fractions was significantly influenced by the treatment and duration of the experiment ($P < 0.05$, Fig. 2; Table 3). In the ^{15}NF treatment, the residual fertilizer N in the bulk soil (0–10 cm) was 45.2 mg kg^{-1} after 1 year of fertilization, accounting for 27.0% of the applied amount. In the 13^{th} year, the residual fertilizer N decreased to 7.6 mg kg^{-1}, with a loss of 84.0% (Fig. 2). During the initial 5 years, the reduction proportion of fertilizer N in the SC fraction was higher than that in the macroaggregate (Fig. 5); thus, the E*f* of fertilizer N in the macroaggregate increased with time (Fig. 3). From 5 to 9 years, the reduction proportion of fertilizer N did not differ significantly across the aggregate fractions. From 9 to 13 years, fertilizer N declined at a higher rate in the macroaggregate than in the microaggregate and SC fractions (Fig. 5).

In the ^{15}NFS treatment, the fertilizer N concentration in bulk soil was 47.1 mg kg^{-1} in the 1st year, but declined to 8.8 mg kg^{-1} by the 13th year and was significantly higher than that in the ^{15}NF treatment ($P < 0.05$). Compared with N fertilizer application alone, maize straw mulching significantly increased the fertilizer N concentration in macroaggregate by 5.5% ($P < 0.05$) in the 1st year (Fig. 2b) and tended to slow the reduction in macroaggregate over the initial 5 years (Fig. 6a). Maize straw mulching slowed down the reduction proportion in the SC fraction after 9 years (Fig. 6a), and the remaining fertilizer N concentration was 21.5% ($P < 0.05$) higher in the 13th year than in the ^{15}NF treatment (Fig. 2b). In the 1st year, the relative stock of fertilizer N in the two treatments was similar: approximately 27%, 40%, and 33% in the macroaggregate, microaggregate, and SC fractions, respectively. In the ^{15}NF treatment, the relative stock of fertilizer N among aggregates remained constant over time, whereas maize straw mulching increased the relative stock of fertilizer N to 35.6% in macroaggregate, 41.0% in microaggregate, and 23.4% in SC fractions until the 13th year.

3.4 Temporal distribution of the initially applied fertilizer N in macroaggregate subfractions

In the macroaggregate subfractions, fertilizer N concentration was significantly influenced by the physical fraction size ($P < 0.05$, Table 3). The E*f* of fertilizer N in $POM_{(f+o)}$ remained the largest (>1) among the fractions, followed by $POM_{(>53)}$ (>1) and was the lowest in MAOM (<1). The E*f* of the fertilizer N was larger than that of TN in $POM_{(f+o)}$ whereas it was lower in MAOM (Fig. 3; Table 4). In the ^{15}NF treatment, the residual fertilizer N in the 1^{st} year was 499.4 mg kg^{-1}, 57.2 mg kg^{-1}, and 20.7 mg kg^{-1}, in $POM_{(f+o)}$, $POM_{(>53)}$, and MAOM, respectively (Fig. 2c). By the 13^{th} year, the residual fertilizer N had decreased by 83.0%, 76.0%, and 64.4% in the $POM_{(f+o)}$, $POM_{(>53)}$, and MAOM fractions, respectively. The E*f* of fertilizer N in $POM_{(f+o)}$ increased from 7.7 to 9.8 in years 1 to 5 and then declined to 7.3 after 13 years, whereas those in the $POM_{(>53)}$ and MAOM fractions increased with time throughout the experiment (Fig. 3c, 3d).

The concentration of fertilizer N in the macroaggregate subfraction was significantly influenced by straw mulching and experimental duration ($P < 0.05$; Fig. 2; Table 3). After maize residue mulching for 1 year, the concentration of fertilizer N in the three subfractions was not significantly different from that in the N fertilizer application alone. Subsequently, the residual fertilizer N concentration in $POM_{(f+o)}$ was significantly higher in the ^{15}NFS treatment than in the ^{15}NF treatment after 5 years; the same pattern was observed for MAOM after 9 years (Fig. 2). Until the 13^{th} year, maize straw mulching slowed the reduction of fertilizer N by 7.7% and 4.6% in the MAOM and $POM_{(f+o)}$ fractions. In the 1^{st} year, the relative stock of fertilizer N in $POM_{(f+o)}$, $POM_{(>53)}$, and MAOM was 41.6–42.9%, 24.6–25.0% and 32.5–33.4%, respectively, with no significant difference between the two treatments (Fig. 4). In the ^{15}NF treatment, the relative stock of fertilizer N in the $POM_{(f+o)}$ decreased to 27.4% and increased to 50.6% in the MAOM fraction until the 13^{th} year. Maize straw mulching increased the stock distribution of fertilizer N in $POM_{(f+o)}$ to 49.3 % in the 5^{th} year, and decreased it to 42.8% in the 13^{th} year; whereas the relative stock of fertilizer N in MAOM decreased to 28.1% in the 5^{th} year and increased to 32.2% in the 13^{th} year.

4. Discussion

4.1 Influence of maize straw mulching on soil aggregation and fertilizer N distribution

The concentration of soil TN increased with increasing aggregate size, and the increase remained the highest in macroaggregate over time (Tables 2 and 3), highlighting the improved protective effect of aggregation on soil N storage under no-tillage and crop residue return management (Paul, 2016; Liu et al., 2023). In addition, the notably higher E*f* of fertilizer N than that of TN in macroaggregate (Fig. 3; Table 2) indicated the preferential retention of the newly applied fertilizer N in macroaggregate during soil aggregation. Such protection could not be solely attributed to the physical encapsulation of fertilizer N in aggregates; it could also be associated with the function of the macroaggregate serving as a reactive hotspot to harbor soil microorganisms immobilizing fertilizer N (Duan et al., 2021). Residual fertilizer N was abundant in POM, especially in $POM_{(f+o)}$ over the MAOM fraction within macroaggregate (Fig. 2), further indicating that the input and decomposition of plant debris could provide available C to improve microbial activity and further assimilation of fertilizer N (Jilling et al., 2020). Additionally, the belowground biomass remained in the field after the maize harvest in this study; thus, the fertilizer N-containing root debris could be consecutively decomposed into macroaggregate and possibly contribute to the retention of fertilizer N in the POM fraction (Cotrufo et al., 2015). Nevertheless, the MAOM fraction, which is dominant in the mass fraction within macroaggregate (Table 1), is considered the structural foundation for aggregate formation and is the major reservoir of fertilizer N in macroaggregate via mineral adsorption and chemical/hydrogen bonds to form mineral-organic complexes in addition to physical encapsulation (Sollins et al., 2006; Dorodnikov et al., 2011).

Soil aggregation processes and fertilizer N distribution among the aggregates are intensively controlled by agricultural management. Compared with the time-independent aggregate composition in the treatment with inorganic N fertilizer alone, maize straw mulching favored soil aggregation and improved aggregate stability (Table 1), which is consistent with the results of previous studies by Zhang et al. (2014) and Cao et al. (2020). This promotion effect was enhanced over time, suggesting that consecutive crop residue returns dynamically reshaped the soil microstructure. Maize straw mulching increased the relative mass proportion of $POM_{(f+o)}$ in the macroaggregate (Table 1), implying that the enhanced formation of macroaggregate was mainly attributed to the increased input and maintenance of fresh crop residues in the cultivated soil layer (Tisdall and Oades, 1982; Six et al., 2000; Wang et al., 2022).

In addition to strengthening soil aggregation, maize straw mulching tended to enhance the retention of the initially applied fertilizer N in the soil (Fig. 3; Table 3), which was partially attributed to the improved microbial immobilization induced by increased C availability, as previously reported (Liu et al., 2016; Hu et al., 2019; Zhou et al., 2023). Essentially, the increase in fertilizer N concentration in macroaggregate was higher than that in the microaggregate and SC fractions, especially the enrichment of fertilizer N in the POM fraction (Fig. 3), indicating that the improvement effect of maize straw mulching on fertilizer N retention mainly resulted from the enhanced immobilization of fertilizer N in macroaggregate, especially in the POM fraction as a reactive hotspot. Compared with root debris-derived POM, the higher C/N ratio of maize straw-derived POM increased the microbial demand for inorganic N, that is, it facilitated microbial assimilation of applied fertilizer N, thus improving the fertilizer N retention efficiency in macroaggregate (Fig. 4). Therefore, the enhanced protection of fertilizer N in macroaggregate after maize straw return may be attributed to the joint effect of improving the retention efficiency of POM and increasing the storage capacity with enhanced soil aggregation. In the non-aggregated SC fraction, the maintenance of fertilizer N is mainly controlled by the physical/chemical adsorption and microbial immobilization (Sollins et al., 2006), with the latter being highly dependent on C availability. The migration of low-molecular-weight components from maize straw to the SC fraction could stimulate the microbial demand for fertilizer N and produce adhesive extracellular polymeric substances and necromass to improve the retention of fertilizer N on the mineral surface (Fonte et al., 2007; Kong et al., 2007). Nevertheless, the greater increase in the *Ef* of residual fertilizer N in macroaggregate than in the SC fraction under maize straw mulching (Fig. 2) implied that the improved residual fertilizer N retention could be attributed to the increased reactive hotspots with enhanced soil aggregation rather than mineral-associated stabilization.

4.2 Functional heterogeneity of soil aggregates in controlling N turnover and long-term maintenance of fertilizer N

Based on tracing the temporal changes in the initially applied ^{15}N-labeled fertilizer, residual fertilizer N was found to be dynamically involved in subsequent soil N turnover (Hu et al., 2015). The reduction in residual fertilizer N across aggregate fractions was time-dependent on a decadal scale (Fig. 5; Table 3), implying the functional heterogeneity of soil aggregates in controlling the long-term maintenance and availability of fertilizer N. Specifically, the release of fertilizer N from macroaggregate and microaggregate was lower than that from the SC fraction in the first 5 years (Fig. 5), confirming the positive effect of soil aggregation on the stabilization of residual fertilizer N (Bosshard et al. 2008). In contrast, fertilizer N in the non-aggregated mineral fraction is vulnerable to microbial attack; thus, it is mainly responsible for the short-term turnover of fertilizer N (Duan et al., 2021). As the experiment proceeded until the 13^{th} year, macroaggregate was gradually converted as the primary site for fertilizer N release (Fig. 5), whereas the increased TN in the soil was preferentially allocated to macroaggregate over microaggregate and SC fractions (Table 2). These findings imply that distinct encapsulation-release pathways co-regulate the net accumulation of soil N in macroaggregate (Six et al., 2000). The initially occluded fertilizer N in aggregates could be liberated upon the fragmentation of aggregates and possibly dispersed in soil matrices over time, whereas mobile N from multiple sources, for example, the subsequently applied fertilizer N, decomposed crop residue, and the inherent N in soil, could be encapsulated with the reformation of aggregates. Therefore, although the protection of aggregates on soil N was robust, the protected components changed temporally with the release of old N (from the initially applied fertilizer) and the encapsulation of new N (from the subsequent input). This indicates the temporal effect of aggregate protection and the mutable role of soil aggregation in controlling long-term N retention. Thus, our concept couples a high protective capability with a notable turnover of soil N in aggregates, providing a credible solution for explaining the dual function of soil aggregation. Contrary to the accelerated release of residual fertilizer N from macroaggregate over time, the reduction proportion of fertilizer N in the SC fraction slowed at intervals of 9 to 13 years (Fig. 5), whereas the retention ability of TN in the SC fraction was significantly lower than that in the macroaggregate. These findings indicate that mineral protection via sorption or chemical bonding was more prone to preserving the initially retained fertilizer N than physical encapsulation and thus contributed to a larger extent to the long-term intact stabilization of fertilizer N in soil.

The time-dependent control of fertilizer N retention and turnover by macroaggregate was closely associated with the distinct functions of the POM and MAOM fractions (Fig. 5; Table 3). The faster initial immobilization of fertilizer N in POM and its higher release than that from MAOM indicate the divergent roles of immobilization-based plant debris decomposition and adsorption-based mineral protection in fertilizer N retention and turnover (Fig. 5). The formation of complexes derived from fertilizer N with clay minerals could decrease the dispersal of fertilizer N to a greater extent than the free POM during the fragmentation of aggregates, and thus, primarily contributed to long-term fertilizer N stabilization (Yang et al., 2022). Comparatively, POM had a high potential to function as a temporary reservoir for fertilizer N retention and transformation by soil microorganisms owing to its larger C/N ratio, and then the immobilized fertilizer N, mostly as microbial-derived products, could be translocated into the mineral-associated fraction (Wang et al., 2020). In a laboratory incubation with ^{13}C-labeled maize residue addition, Li et al. (2016) found the “transfer” of residue-derived C from free organic C and coarse particulate organic C into MAOM fraction within aggregates, further supporting our findings from the perspective of C cycling. More importantly, the reduction proportion of fertilizer N in the MAOM fraction was lower than that in the SC fraction (Fig. 5), indicating that the stability of mineral-associated residual fertilizer N could be enhanced in macroaggregate owing to encapsulation by hierarchical aggregation, thus reducing the microbial accessibility of residual fertilizer N by double protection from mineral adsorption and occlusion in aggregates (Conen et al., 2008; De Clercq et al., 2015).

The turnover of fertilizer N in soil aggregates changed significantly under conservation tillage management. During the entire experimental period, maize straw mulching alleviated the release of fertilizer N in all physically separated fractions, especially in macroaggregate, indicating that the enhanced aggregation dominantly contributed to the long-term stabilization of fertilizer N in the soil. Nevertheless, improved protection was highly aggregate-specific and time-dependent. Maize straw mulching predominantly slowed down the decrease in fertilizer N in macroaggregate, especially in the subfraction of $POM_{(f+o)}$, during the initial 5 years, while shifting toward reducing the loss of fertilizer N in the SC and MAOM fractions after 9 years (Fig. 5), suggesting that maize straw return engendered functional divergence among the aggregate fractions in manipulating fertilizer N stabilization because of the changing competition between mineral adsorption and biotic assimilation of fertilizer N after exogenous organic C input. Maize straw return improves C availability and supplies reaction sites to enhance the microbial transformation of fertilizer N to organic forms (Liu et al., 2016; Zhou et al., 2023), thereby reducing the dispersal of fertilizer-derived POM from macroaggregate in the initial periods and extending the maintenance time of fertilizer N in aggregates. However, the subsequent input of unlabeled N had a stronger reactivity for plant residue decomposition in the macroaggregate and POM fractions than the aged organic N, leading to the translocation of the residual fertilizer N into mineral-associated fractions, thus contributing to the prolonged retention and stabilization of the residual fertilizer N.

Conclusions

Using the ^{15}N tracing technique, the divergent functions of various soil aggregate fractions that regulate the retention dynamics of fertilizer N were explored in a 13-year conservation tillage experiment. The initially applied fertilizer N was stabilized in the macroaggregate, but such protection was accompanied by soil N turnover owing to the "release old but store new" effect during the fragmentation-reformation of aggregates. The subsequent enhanced retention of residual fertilizer N in the SC fraction could be predominantly attributed to its strong interactions with clay minerals, and in turn, responsible for the long-term stabilization of fertilizer N in the soil. Maize straw mulching increased soil N storage while engendering functional divergence among aggregate fractions for fertilizer N stabilization. The improvement in fertilizer N retention could be jointly attributed to enhanced encapsulation with increasing soil aggregation at the initial stage and improved long-term stability of fertilizer N by promoting soil mineral protection.

Acknowledgments

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Figure captions

Fig. 1. Physical fractionation scheme employed for the separation of soil aggregates during the 1st, 5th,9th, and 13th years. NaI: sodium iodide (1.80 g cm^{-3}); fPOM: free particulate organic matter; oPOM: occluded particulate organic matter; $POM_{(>53)}$: >53 µm particulate organic matter; MAOM: mineral-associated organic matter. In this study, fPOM and oPOM fractions were combined into $POM_{(f+o)}$ fraction due to their minimal mass.

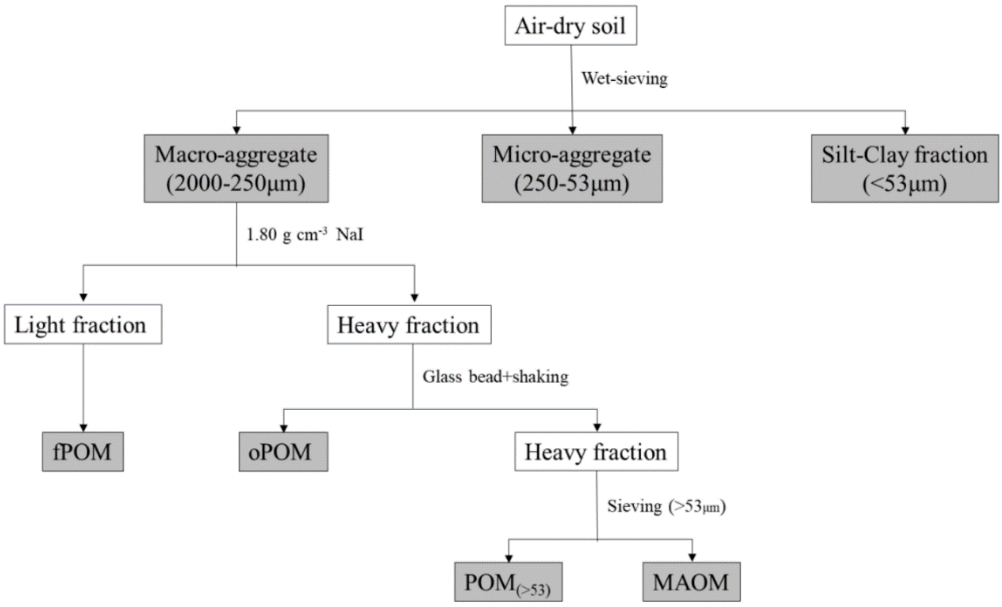

Fig. 2. Spatial and temporal dynamics of fertilizer N concentration in soil aggregate-size class in ^{15}NF (a) and ^{15}NFS (b) treatment and subfractions in macroaggregate in ^{15}NF (c) and ^{15}NFS (d) treatment during the 1st (1Y), 5th (5Y), 9th (9Y), and 13th (13Y) years (n = 3, error bars = SE). Different capital letters indicate the difference between treatments in an individual physical fractions and year ($P < 0.05$). Different small letters indicate the difference between years in an individual physical fractions and treatment ($P < 0.05$). ^{15}NF: only ^{15}N labeled fertilizer application; ^{15}NFS: ^{15}N labeled fertilizer application with maize straw mulching. Macro: macroaggregate fraction; Micro: microaggregate fraction; SC: silt-clay fraction. $POM_{(f+o)}$: free POM plus occluded POM fraction; $POM_{(>53)}$: POM over 53 μm size; MAOM: mineral associated organic matter.

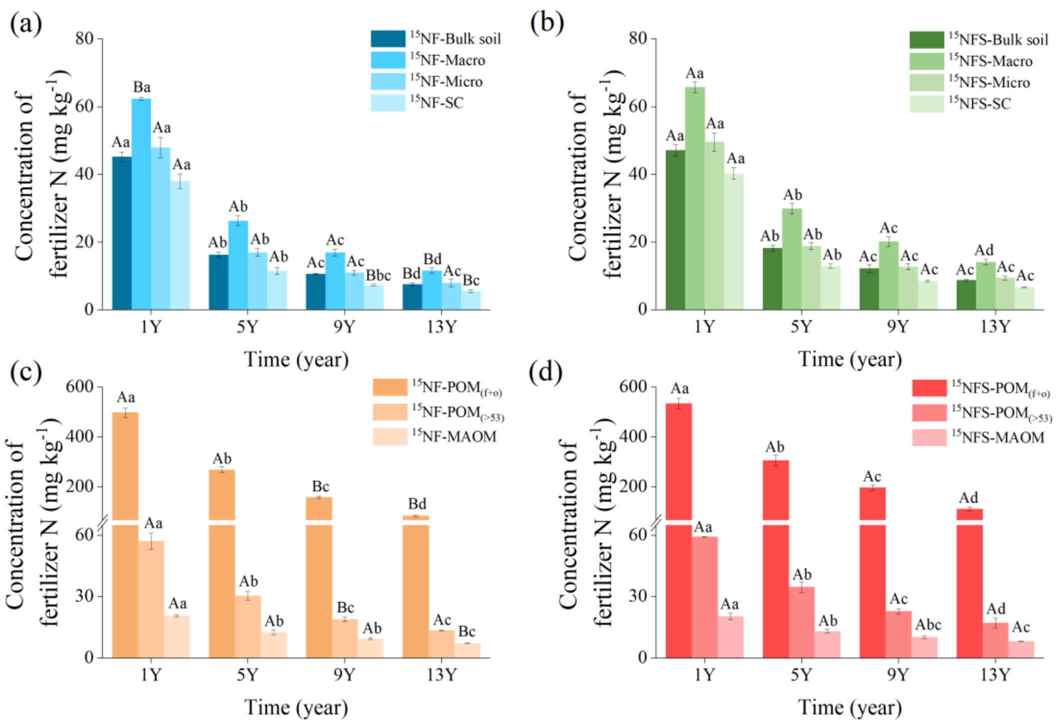

Fig. 3. Enrichment factor of fertilizer N in soil aggregate-size class in ^{15}NF (a) and ^{15}NFS (b) treatment and subfractions in macroaggregate in ^{15}NF (c) and ^{15}NFS (d) treatment during the 1st (1Y), 5th (5Y), 9th (9Y), and 13th (13Y) years (n = 3, error bars = SE). Different small letters indicate the difference between years in an individual physical fractions and treatment ($P < 0.05$). No letters indicate no significance.

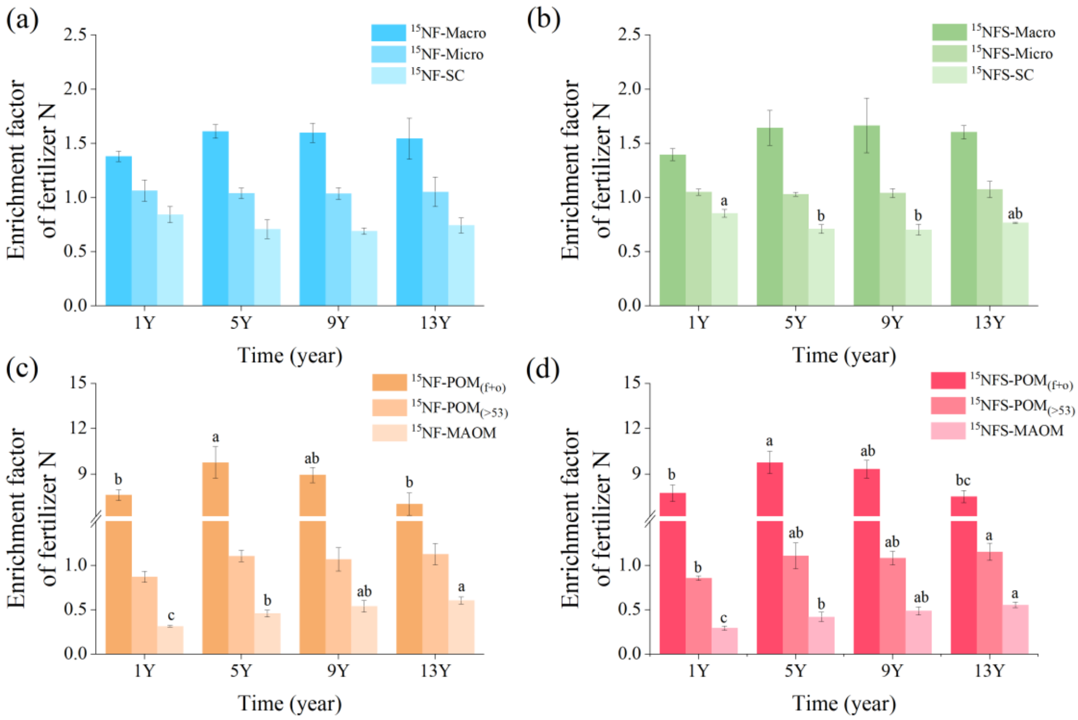

Fig. 4. Relative stock distribution of fertilizer N in soil aggregate-size class (a) and subfractions in macroaggregate (b) during the 1st (1Y), 5th (5Y), 9th (9Y), and 13th (13Y) years (n = 3, error bars = SE). Different capital letters indicate the difference between treatments in an individual physical fractions and year ($P < 0.05$); Different small letters indicate the difference between years in an individual physical fractions and treatment ($P < 0.05$); No letters indicate no significance.

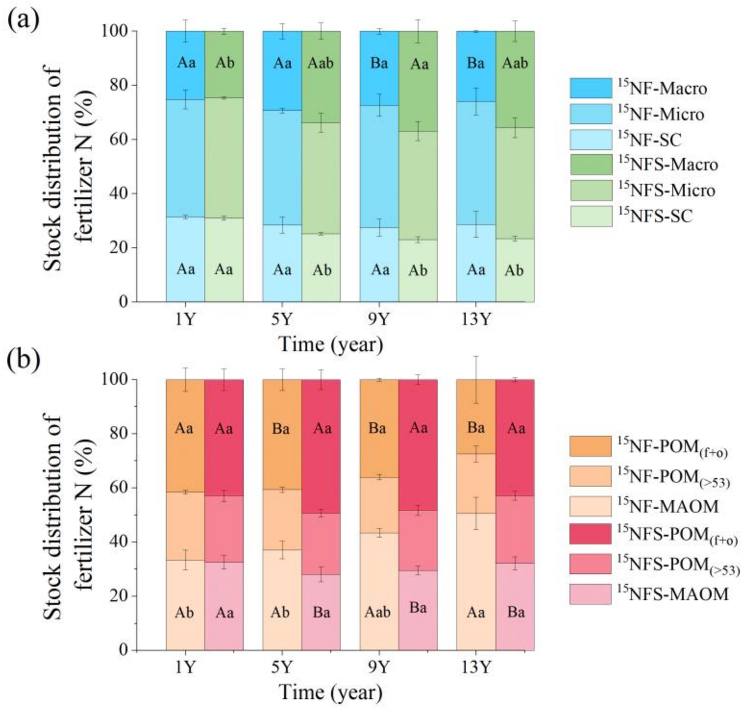

Fig. 5. Reduction proportion of fertilizer N in soil aggregate-size class in ^{15}NF (a) and ^{15}NFS (b) treatment and subfractions in macroaggregate in ^{15}NF (c) and ^{15}NFS (d) treatment (n = 3, error bars = SE). 1-5: years 1 to 5; 1-9: years 1 to 9; 1-13: years 1 to 13; 5-9: years 5 to 9; 9-13: years 9 to 13.

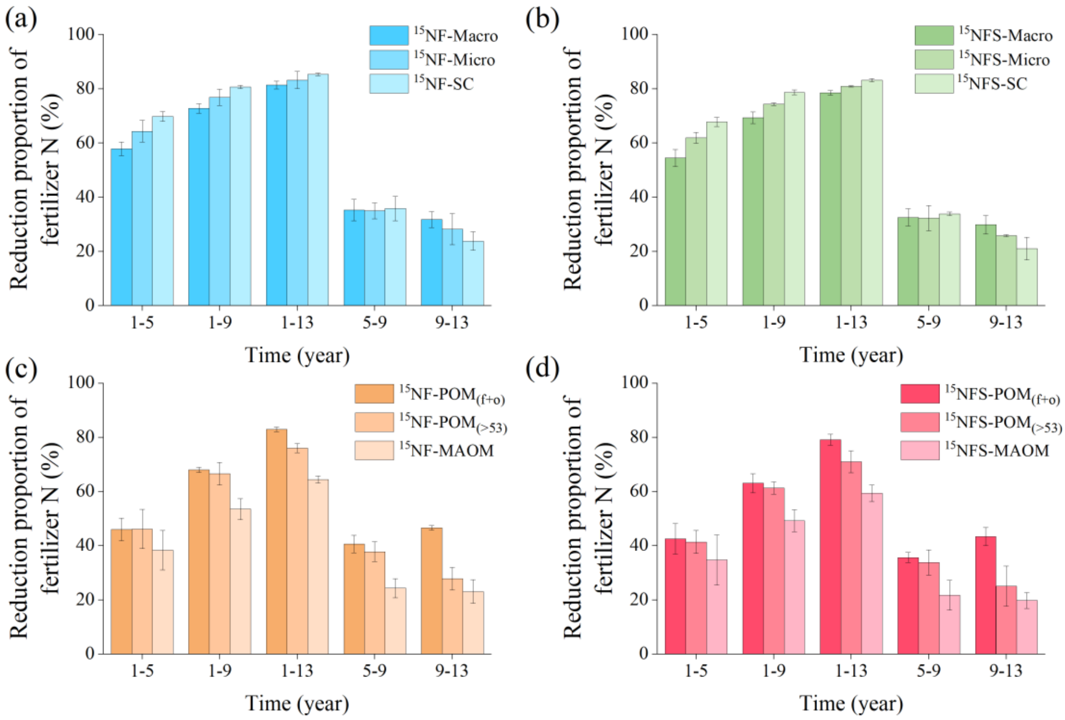

Fig. 6. The influence of maize straw mulching on the reduction proportion of fertilizer N in soil aggregate-size class (a) and subfractions in macroaggregate (b) (n = 3, error bars = SE).

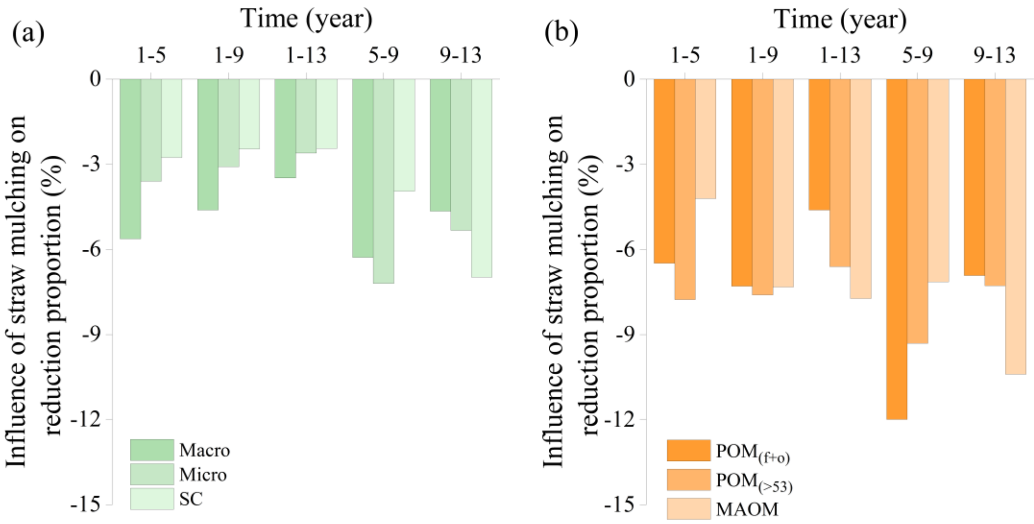

Table 1 Relative mass proportion and index of stability for soil aggregates.

Treatment	Year	Soil aggregate fraction (%)			Subfractions in macroaggregate (%)			MWD
		Macro	Micro	SC	$POM_{(f+o)}$	$POM_{(>53)}$	MAOM	
^{15}NF	1	18.80±2.85Aa	42.39±2.96Aa	38.82±0.11Aa	3.94±0.74Aa	20.61±1.87Aa	75.45±2.61Aa	0.22±0.02Aa
	5	18.32±1.72Aa	41.02±1.47Aa	40.66±0.87Aa	4.00±0.65Ba	19.18±0.67Aa	76.82±0.83Aa	0.21±0.01Aa
	9	17.08±1.09Ba	43.33±2.81Aa	39.60±2.95Aa	3.88±0.23Ba	18.67±1.09Ba	77.45±0.99Aa	0.20±0.01Ba
	13	17.19±0.92Ba	43.95±1.65Aa	38.86±2.20Aa	3.77±1.39Ba	18.40±0.99Ba	77.84±0.55Aa	0.20±0.01Ba
^{15}NFS	1	18.35±1.42Ab	43.94±1.34Aa	37.71±0.33Ba	3.83±0.39Ac	19.78±0.56Ab	76.39±0.17Aa	0.21±0.01Ab
	5	21.50±2.10Aab	41.40±1.81Aa	37.10±1.80Bab	5.47±0.26Ab	22.21±1.91Aab	72.32±2.12Bab	0.24±0.02Aab
	9	23.81±1.80Aab	41.08±1.51Aa	35.11±0.93Aab	5.99±0.24Aab	23.76±2.14Aab	70.25±2.06Bbc	0.26±0.01Aa
	13	24.43±2.47Aa	41.98±2.29Aa	33.59±1.58Bb	6.70±0.29Aa	25.43±1.10Aa	67.87±1.37Bc	0.26±0.02Aa

Note: Different capital letters indicate the difference between treatments in an individual physical fractions and year ($P < 0.05$). Different small letters indicate the difference between years in an individual physical fractions and treatment ($P < 0.05$). ^{15}NF: only ^{15}N labeled fertilizer application; ^{15}NFS: ^{15}N labeled fertilizer application with maize straw mulching. Macro: macroaggregate fraction; Micro: microaggregate fraction; SC: silt-clay fraction; $POM_{(f+o)}$: free POM plus occluded POM fraction; $POM_{(>53)}$: POM over 53 μm size; MAOM: mineral associated organic matter. MWD: Mean wight diameter. Values are means of three replicates (± SE).

Table 2 Concentration, enrichment factor, and relative stock distribution of soil total nitrogen (TN) in soil aggregates.

Fraction	Year	Concentration (g kg^{-1})		Enrichment factor		Relative stock (%)	
		^{15}NF	^{15}NFS	^{15}NF	^{15}NFS	^{15}NF	^{15}NFS
Macro	1	1.26±0.03Ac	1.27±0.03Ad	1.23±0.02Ab	1.23±0.04Ab	23.36±2.48Aa	22.85±1.50Ab
	5	1.40±0.01Bb	1.49±0.01Ac	1.34±0.03Aab	1.35±0.01Aa	24.26±1.65Aa	28.26±1.90Aab
	9	1.51±0.04Ba	1.62±0.01Ab	1.38±0.03Aa	1.40±0.02Aa	23.37±0.66Ba	31.73±2.13Aa
	13	1.55±0.02Ba	1.70±0.03Aa	1.40±0.06Aa	1.45±0.04Aa	23.68±0.92Ba	32.52±2.29Aa
Micro	1	1.05±0.01Ab	1.06±0.01Ac	1.03±0.03Aa	1.02±0.04Aa	44.15±2.77Aa	45.64±1.16Aa
	5	1.08±0.02Bab	1.15±0.02Ab	1.03±0.02Aa	1.04±0.01Aa	41.82±1.89Aa	41.93±2.02Aa
	9	1.14±0.02Ba	1.21±0.03Aab	1.04±0.01Aa	1.05±0.04Aa	44.80±2.61Aa	41.02±2.11Aa
	13	1.15±0.03Ba	1.27±0.03Aa	1.04±0.07Aa	1.08±0.04Aa	44.98±2.10Aa	41.98±1.86Aa
SC	1	0.85±0.00Ab	0.85±0.01Ac	0.83±0.03Aa	0.82±0.04Aa	32.49±0.32Aa	31.51±0.34Ba
	5	0.88±0.01Aab	0.91±0.02Ab	0.84±0.04Aa	0.82±0.02Aa	33.92±0.61Aa	29.81±1.31Bab
	9	0.88±0.02Bab	0.94±0.00Aab	0.81±0.02Aa	0.82±0.01Aa	31.83±2.39Aa	27.25±0.85Abc
	13	0.90±0.01Ba	0.97±0.02Aa	0.82±0.02Aa	0.82±0.03Aa	31.34±2.27Aa	25.50±0.85Bc

Note: Different capital letters indicate the difference between treatments in an individual physical fractions and year ($P < 0.05$). Different small letters indicate the difference between years in an individual physical fractions and treatment ($P < 0.05$). ^{15}NF: only ^{15}N labeled fertilizer application; ^{15}NFS: ^{15}N labeled fertilizer application with maize straw mulching. Macro: macroaggregate fraction; Micro: microaggregate fraction; SC: silt-clay fraction. Values are means of three replicates (± SE).

Table 3 Results of linear mixed-effect model testing (F values, with *P* values in parentheses) for the effects of treatment (S), temporal effect (T), aggregate-size class (A), and their interactions on concentration of fertilizer N. Values in bold indicate statistical significance at $P \leq 0.05$.

	S	A	T	S*A	S*T	A*T	S*A*T
Soil aggregate class	**30.8 (<0.001)**	**483.3 (<0.001)**	**2409.3 (<0.001)**	1.9 (0.162)	1.7 (0.916)	**35.5 (<0.001)**	0.6 (0.999)
Within macroaggregate	**41.7 (<0.001)**	**6925.3 (<0.001)**	**1193.8 (<0.001)**	**30.3 (<0.001)**	0.2 (0.889)	**810.1 (<0.001)**	0.2 (0.969)

Table 4 Concentration, enrichment factor, and relative stock distribution of soil TN in macroaggregate subfractions.

Treatment	Year	Concentration ($g\ kg^{-1}$)			Enrichment factor			Relative stock (%)		
		$POM_{(f+o)}$	$POM_{(>53)}$	MAOM	$POM_{(f+o)}$	$POM_{(>53)}$	MAOM	$POM_{(f+o)}$	$POM_{(>53)}$	MAOM
^{15}NF	1	9.71±0.14Ad	1.21±0.01Ad	0.91±0.01Ab	7.71±0.35Aa	0.96±0.02Aa	0.72±0.02Aa	28.90±4.39Aa	18.92±0.86Aa	52.18±5.15Aa
	5	11.29±0.16Bc	1.37±0.02Bc	0.94±0.02Ab	8.05±0.20Aa	0.98±0.01Aa	0.67±0.02Aa	31.25±3.48Ba	18.34±1.71Aa	50.41±2.25Ba
	9	12.19±0.13Bb	1.48±0.03Bb	1.01±0.02Aa	8.09±0.36Aa	0.99±0.05Aa	0.67±0.02Aa	30.74±1.58Ba	18.07±1.60Aa	51.19±1.60Ba
	13	12.92±0.14Ba	1.58±0.04Ba	1.06±0.02Aa	8.36±0.18Aa	1.02±0.01Aa	0.69±0.03Aa	29.64±8.26Ba	18.25±2.71Aa	52.11±5.90Ba
^{15}NFS	1	9.99±0.18Ad	1.24±0.02Ad	0.92±0.01Ab	7.89±0.20Ab	0.98±0.04Aa	0.73±0.02Aa	28.72±2.41Ac	18.32±0.68Aa	52.96±1.78Aa
	5	12.32±0.15Ac	1.47±0.01Ac	0.97±0.01Ab	8.27±0.10Aab	0.98±0.01Aa	0.65±0.01Ab	39.57±0.60Ab	19.13±1.61Aa	41.30±2.04Ab
	9	13.37±0.15Ab	1.60±0.02Ab	1.06±0.02Aa	8.25±0.10Aab	0.99±0.02Aa	0.65±0.02Ab	41.59±0.76Aab	19.76±2.05Aa	38.65±1.32Abc
	13	14.24±0.22Aa	1.71±0.04Aa	1.11±0.03Aa	8.39±0.20Aa	1.01±0.01Aa	0.66±0.03Ab	44.45±0.87Aa	20.30±0.28Aa	35.25±0.97Ac

Note: Different capital letters indicate the difference between treatments in an individual physical fractions and year ($P < 0.05$). Different small letters indicate the difference between years in an individual physical fractions and treatment ($P < 0.05$). ^{15}NF: only ^{15}N labeled fertilizer application; ^{15}NFS: ^{15}N labeled fertilizer application with maize straw mulching. $POM_{(f+o)}$: free POM plus occluded POM fraction; $POM_{(>53)}$: POM over 53 μm size; MAOM: mineral associated organic matter. Values are means of three replicates (± SE).

Supplementary Information

Table S1 Asymptotic significance (bilateral) (*P* < 0.001) of Kolmogorov-Smirnov test in soil aggregates.

Parameters	^{15}NF			^{15}NFS		
	Macro	Micro	SC	Macro	Micro	SC
Relative mass proportion	0.834	0.991	0.879	1.000	0.874	0.994
Concentration of TN	0.965	0.919	0.993	0.703	0.990	0.379
Enrichment factor of TN	0.997	0.760	0.998	0.967	0.922	0.985
Relative stock of TN	0.964	0.979	0.823	0.949	0.800	0.886
Concentration of fertilizer N	0.295	0.169	0.125	0.408	0.213	0.116
Enrichment factor of fertilizer N	0.964	0.786	0.971	0.873	0.937	0.827
Relative stock of fertilizer N	0.933	0.999	0.767	0.970	0.497	0.434
Reduction proportion of fertilizer N	0.786	0.667	0.436	0.617	0.616	0.389

Note: Macro: macroaggregate; Micro: microaggregate; SC: silt and clay fraction; ^{15}NF: only ^{15}N labeled fertilizer application; ^{15}NFS: ^{15}N labeled fertilizer application with maize straw mulching.

Table S2 Asymptotic significance (bilateral) ($P < 0.001$) of Kolmogorov-Smirnov test in subfractions of macroaggregate.

Parameters	^{15}NF			^{15}NFS		
	MAOM	$POM_{(>53)}$	$POM_{(f+o)}$	MAOM	$POM_{(>53)}$	$POM_{(f+o)}$
Relative mass proportion	0.813	0.972	0.870	0.834	0.895	0.880
Concentration of TN	0.949	0.969	0.897	0.881	0.920	0.908
Enrichment factor of TN	0.991	0.995	0.678	0.595	0.799	0.789
Relative stock of TN	0.830	0.874	0.619	0.570	0.693	0.266
Concentration of fertilizer N	0.541	0.808	0.570	0.526	0.627	0.856
Enrichment factor of fertilizer N	0.938	0.972	1.000	0.948	0.765	0.945
Relative stock of fertilizer N	0.963	0.923	0.290	0.924	1.000	0.989
Reduction proportion of fertilizer N	0.574	0.759	0.340	0.779	0.798	0.497

Note: ^{15}NF: only ^{15}N labeled fertilizer application; ^{15}NFS: ^{15}N labeled fertilizer application with maize straw mulching. $POM_{(f+o)}$: free POM plus occluded POM fraction; $POM_{(>53)}$: POM over 53 μm size; MAOM: mineral associated organic matter.