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Nitrogen Fertilization Degrades Soil Aggregation by Increasing Ammonium Ions and Decreasing Biological Binding Agents on a Vertisol after 12 Years

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ABSTRACT

Nitrogen (N) fertilization degrading soil aggregation has been reported in many literatures; however, the mechanisms have not been clarified. A greater understanding of the impact of N fertilization on soil aggregation would be helpful for improving soil structure and sustaining high crop production. The objective of this study was to determine the impact of long-term N fertilization on soil aggregation and its associated with binding and dispersing agents. A 12-year (2008-2019) N fertilization field experiment on a Vertisol was selected because this trial covered a wide range of N application rates (0, 360, 450, 540, 630 and 720 kg N ha^{-1} yr^{-1}) and included straw management (straw return and straw removal) in a wheat (*Triticum aestivum* L.)-maize (*Zea mays* L.) cropping system. Soil samples of 0-20 cm depth were collected from 12 field treatments with 3 replications in 2019. Soil aggregate stability (MWD), soil organic carbon (SOC), glomalin-related soil proteins (GRSP), microbial biomass carbon (MBC), and N forms (NH_4^+ and NO_3^-) were determined. The long-term N fertilization under straw removal conditions reduced the MWD by 12-18% when the N rates increased from 0 to 720 kg ha^{-1} ($P < 0.05$). The MWD was positively associated to pH ($P < 0.05$) and MBC ($P < 0.05$) but negatively related to NH_4^+ ($P < 0.05$) and NO_3^- ($P < 0.05$). Compared with straw removal treatment, the straw incorporation treatment significantly improved the aggregating agents (e.g., SOC, GRSP and MBC) ($P < 0.001$) but did not affect the dispersing agent (e.g., NH_4^+) ($P > 0.05$) and consequently improved soil aggregation. Overall, our results indicate that long-term N fertilization may degrade soil aggregation because of the increases in monovalent ions (H^+, NH_4^+) and the decrease in MBC in soil acidification, especially when the applied dose exceeded 360 kg N ha^{-1} yr^{-1}, but straw incorporation can minimize the negative structural impacts in the Vertisol.

Key words: aggregating agents, aggregate stability, nitrogen fertilizer, soil structure, straw incorporation

INTRODUCTION

N fertilizers, as the essential inputs, are routinely applied into the soil to promote soil fertility and stimulate crop growth. As a result, N fertilization can increase the amount of residues through above- and below-ground biomass of crops as a C input into soils (Zhao *et al*., 2018). This can be expected that the increase in C input can result in an increase in SOC and further improve soil aggregation (Blanco-Canqui *et al*., 2014; Mustafa *et al*., 2020). However, the improvement in soil aggregate stability as SOC content increased was not observed on a Cambisol (Xie *et al*., 2015; Zhou *et al*., 2017) and a Fluvisol (Xin *et al*., 2016), a Plinthosol and Anthrosol in China (Yan *et al*., 2013). In the nine long-term (12 ~ 39 yr) fertilization experiments, Guo *et al*. (2019a) also found that the inorganic fertilization treatments (NP or NPK) resulted in similar or lower aggregate stability as control, although they contributed to the SOC content far higher than control. These negative structural impacts induced by NH_4^+-containing fertilization are often ignored, because they may be offset by visible costs such as increased SOC content. Soil aggregate stability has been extensively used for an important index of soil structural quality, because it influences many physical, chemical and biological processes of soil (Peng *et al*., 2015). Thus, it is important to understand in-depth how the use of N fertilizers influences on soil aggregation in the long-term if we wish to develop sustainable agriculture in the future.

A change in soil aggregation is likely associated with soil binding agents and/or dispersing agents (Guo *et al*., 2018, 2019a). Soil organic carbon (SOC), glomalin-related soil proteins (GRSP) and microbial biomass carbon (MBC), as three main biological stabilizers play an important role in soil aggregation. Generally, these binding agents positively correlate with aggregate stability in many studies (Zhang *et al*., 2014; Guo *et al*., 2019a). However, some long-term N fertilization experiments do not produce these positive correlations (Yan *et al*., 2013; Zhou *et al*., 2017). Applications of N fertilizers can accelerate particle organic matter decomposition (Neff *et al*., 2002), inhibit the production of GRSP (Jeske *et al*., 2018) and decrease MBC (Zang *et al*., 2016; Dai *et al*., 2018; Luo *et al*., 2020), which are not in favor of soil aggregation. The large accumulation of NH_4^+ ions after overuse of N fertilizers has raised questions about the impact of NH_4^+ on soil aggregate dispersion (Haynes and Naidu, 1998). For example, Yan *et al*. (2013), Xin *et al*. (2016) and Zhou *et al*. (2017) speculated that degraded soil aggregation possibly resulted from the accumulated NH_4^+ in soil associated with large applications of N fertilizers. Unfortunately, a correlation in statistical analysis between aggregate stability and NH_4^+ is not yet conducted. This is because many long-term fertilization experiments include only two rates, e.g., no N input as a control and N input. Thus, an overall assessment of the relationships between aggregate stability and binding agents and/or dispersing agents after long-term N fertilization is needed (Guo *et al*., 2018).

Vertisol, mainly situated in China's Huang-Huai-Hai Plain on an area of 4×10^6 ha, which is typically soil type producing a low and middle crop yield in this region (Guo *et al*., 2019b). According to our latest survey, farmers in this region have been using excessive N inputs (325 to 805 kg ha^{-1} yr^{-1}) to get high crop yields for wheat-maize cropping system (unpublished data). The use of N fertilizers in excess of crop demands may lead to a poor soil structure (Blanco-Canqui *et al*., 2014), which makes it difficult for soil tilth and crop production (Guo *et al*., 2018; Li *et al*., 2014). High crop yields are accompanied by a large amount of straw residue. straw incorporation has been shown to be a promising practice for reducing intense use of N fertilizer (Yin *et al*., 2018; Zhao *et al*., 2018) and improving soil structure (Guo *et al*., 2018). However, little information about the coupled incorporation of crop straw with different application rates of N fertilizers on soil aggregation is available. Thus, how the N fertilization rate and straw management in the Vertisol influence soil aggregation and associated binding or dispersing agents needs to be clarified urgently. We hypothesized that excessive N fertilization could degrade soil aggregation by decreasing the associated binding agents and increasing some dispersing agents in soil acidification. The optimal N fertilizer rate combined with straw incorporation could alleviate the negative structural impacts of excessive N fertilization due to increased biological binding agents. Thus, the purposes of this research were (1) to assess the impact of N application with straw management on soil aggregation and (2) to determine the relationships among aggregate stability and associated binding agents or dispersing agents.

MATERIALS AND METHODS

Site description and experimental design

A long-term field experiment (2008-2019) was conducted at the Agricultural Science and Technology Demonstration Center in Mengcheng county (33° 09′ N, 116° 33′ E), Anhui Academy of Agricultural Sciences, Province of Anhui, China. The soil is derived from fluvio-lacustrine sediments with a clay loam texture (31.5% sand, 38.0% silt and 30.5% clay), locally called as Shajiang black soil (Vertisol, Chinese Soil Taxonomy) (Li *et al*., 2011). Before the experiment was conducted in 2008, the soil (0-20 cm) contained 8.22 g kg^{-1} organic C, 0.99 g kg^{-1} total N, 0.67 g kg^{-1} total P, 57.8 mg kg^{-1} alkali-hydrolysable N, 21.6 mg kg^{-1} available P, and 197 mg kg^{-1} available K (Li *et al*., 2014).

This experiment was a randomized complete block design factorial of 2 × 6. Treatments included two straw management practices (straw return and straw removal) and six N fertilization rates (0, 360, 450, 540, 630 and 720 kg N ha^{-1} yr^{-1}). The initial aim of this experiment was to assess the optimal N amount in this region (Li *et al*., 2014). Moreover, such a wide range of N application doses could also help us clarify the relationship between N fertilization and soil aggregation. There were three replicates, and the area of each plot was 21.6 m^2 (5.4 m × 4 m). Phosphorous (P) and Potassium (K) used as primary fertilizers for the soil, 81 kg ha^{-1} P_2O_5 and 81 kg ha^{-1} K_2O for wheat season, 99 kg ha^{-1} P_2O_5 and 99 kg ha^{-1} K_2O for maize season. Approximately 55% (wheat) or 45% (maize) of N was applied before tillage as a basal fertilizer, and the remaining 45% (wheat) or 55% (maize) of N was used as a topdressing. During mid-October, wheat seeds were sown with a 12 rows wheat planter at a density of 225 plants m^{-2} and harvested in the first week of June. Maize seeds were sown with a 4 rows corn planter at a density of 6.75 plants m^{-2} during middle of June and harvested in the first week of October. The wheat variety was Jimai 22 and the maize hybrid was Zhengdan 958, respectively. Crop yields at harvest were measured based on a 14% moisture content from the whole plot (Zhou *et al*., 2017; Guo *et al*., 2019b). After the crop harvest every year, straw was chopped into ≤ 10 cm pieces by a chopping straw machine and then incorporated into the soil with a trailed rotary cultivator to ~ 15 cm depth in the straw return treatment, while in the straw removal treatment all straw was removed from the field.

Soil sampling

Five soil samples of each plot from the 0-20 cm layer were taken randomly by shovel and mixed by hand into a composite sample in the middle of March 2019 before N topdressing. Each soil sample was further divided into two fractions after removing stones and visible crop debris. For MBC and mineral N analysis, one subsample was immediately preserved at 4 °C and the other were dried in the air, sieved manually to < 5 mm and stored at room temperature (Guo *et al*., 2018). A part of the bulk soil samples was further ground to < 2 mm for determining the basic soil properties of the bulk soil. The rest of < 5 mm samples were used to select 3-5 mm aggregates for testing the water stability of aggregates. To determine the soil bulk density, three undistributed cores (100 cm^3) were taken from each plot in the 0-20 cm soil layer (Xin *et al*., 2016).

Soil chemical properties

Soil NH_4^+-N and NO_3^--N concentrations within the extracts were analyzed using a continuous flow analyzer (San++, SKALAR, Netherlands) from 10 g of moist soil with a 1:5 w/v 2 mol L^{-1} KCl. Total N (TN) was determined with fully automatic C/N elemental analyzer (Vario MAX CN, Elemental Co., Germany) after dry combustion (Sun *et al*., 2015). Soil pH was measured operating an electromagnetic instrument (Mettler Toledo Five Easy FE30, Switzerland), with 1:2.5 proportion of soil-to-water (Guo *et al*., 2018).

Soil aggregate stability

Measuring the stability of soil aggregates was adopted from the Le Bissonnais (1996) fast wetting method. The 3-5 mm sized aggregates were oven-dried at 40°C for 24 h to avoid contrasts in humidity, and then about 5 g aggregates (3-5 mm) were gently immersed in a glass beaker filled with 50 ml deionized water for 10 min. After pre-wetting treatment, the residual aggregates were transferred on a 0.053 mm sieve previously immersed in ethanol, which was gently hand moved in a helical movement with a 4 cm amplitude at a frequency of 25 min^{-1} oscillations for 2 min. After wet sieving, the aggregates acquired on the sieve were gathered and dried for 24 h at 40°C. Dry sieving was used to measure the different aggregate sizes using a set of six nested sieves with diameters of 2, 1, 0.5, 0.25, 0.10 and 0.053 mm. For each sample, stability of soil aggregate was determined with the mean weight diameter (MWD, mm):

$$MWD = \sum_{i=1}^{n} \bar{x}_i * w_i \qquad (1)$$

where $\bar{x}_i$ represents each aggregate size mean diameter, w_i is the aggregates mass ratio retained on every sieve, and *n* refers sieve's number.

Soil biological aggregating agents

Soil MBC was determined in 10 g fresh soil by the direct extraction method of chloroform fumigation (Vance *et al*., 1987). A TOC analyzer (Multi N/C3000, Analytik Jena, Germany) was used to calculate extractable C. The conversion factor of 0.45 was used to estimate MBC (Wu *et al*., 1990) as the difference in extractable C between the non-fumigated and fumigated samples.

The common method of potassium dichromate oxidation was used to determine soil organic carbon (SOC) (Lu, 2000). A 0.25 g sieved soil of < 0.149 mm was digested in $K_2Cr_2O_7$-H_2SO_4 solution using oil-bath heating at 220-230 °C. The determination of SOC content in the solution was titrated with the $FeSO_4$ solution.

Wright and Upadhyaya (1998) method was used to isolate and determine the glomalin-related soil proteins (GRSP). A 0.25 g sieved soil of < 2 mm was incubated at 121 °C for 90 min with 2 mL of 50 mmol L^{-1} citrate buffer (pH 8.0). After five extractions, the supernatant of each sample was gathered and centrifuged (SIGMA 3-18K, Germany) at 10000 g (10319 rpm) for 10 min. The GRSP content in the supernatant at 595 nm was determined using a spectrophotometer (UV-2450, Shimadzu, Japan). The standard used for the Bradford assay was bovine serum albumin.

Statistical analysis

Two-way Analysis of Variance (ANOVA) by using IBM SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used to test the main effects and interactions between the N application rate and straw management on crop yield, soil chemical properties, biological binding agents and structural stability. The least significant difference (LSD) of Fisher's test (LSD at $P < 0.05$) was used for multiple comparisons among means of them (n = 3). Linear regression function was used to describe the relationships between MWD and the SOC, GRSP, MBC and the pH, TN, NH_4^+, NO_3^- contents (Guo *et al*., 2019a). All regressions and figures were conducted using Origin 9.0 (Origin Lab, Northampton, MA, USA).

RESULTS

Crop yields

The annual yields of wheat and maize (2014-2018) are affected differently by the N application rate and straw management (Fig. 1). When the N rates increased from 360 to 630 kg ha^{-1}, the wheat and maize yields under straw removal conditions gradually increased by 9.7-22.7% and 1.63-2.9%, respectively. Relative to N fertilization at a rate of 630 kg ha^{-1} when straw was not added, the N rate of 720 kg ha^{-1} slightly reduced the wheat and maize yields by 2.13% and 1.9%, respectively. However, the straw return treatments significantly increased maize yield by 5-17% in comparison to the straw removal treatments ($P < 0.001$), but this effect was not observed for wheat yield ($P > 0.05$). Coupled incorporation of crop straw with a rate of 630 kg N ha^{-1} could achieve desired crop yields in this region (8710 kg ha^{-1} for wheat and 9928 kg ha^{-1} for maize). Clearly, N fertilizer is still essential input for increasing or maintaining crop yields of the intensively agricultural soils in this region.

Fig. 1

Fig. 1 Wheat yield (upper) and maize yield (bottom) under different nitrogen fertilizer rates and straw management on a Vertisol (2014-2018).

Soil chemical properties

Under straw removal conditions, there was a rapid decline in soil pH by 0.51-1.67 units over 12 years period when the N rates increased from 0 to 720 kg ha^{-1} ($P < 0.001$), and straw return did not alleviate the acidification process ($P > 0.05$) (Table 1). It was not surprising that N fertilization increased the TN, NH_4^+-N, and NO_3^--N content significantly ($P < 0.001$). The straw return treatment substantially increased TN by 20.5-46.2%, and NO_3^--N by 53.8-126% compared with the straw removal procedure, but decreased NH_4^+-N by 6.84-31.3% ($P < 0.001$). Our results indicate that long-term increased N fertilization strongly influence soil pH and N forms (NH_4^+ and NO_3^-) in intensively farmland soils.

TABLE I

The effects of nitrogen fertilizer rates and straw management on some basic soil properties of a Vertisol after 12 years.

Straw management	N rate	pH	TN[b]	NH_4^+-N	NO_3^--N
	(kg ha^{-1})		g kg^{-1}	mg kg^{-1}	
Removal	0	7.10 Aa	0.87 Bc[a]	4.53 Ac	2.26 Bb
	360	6.59 Ab	0.93 Bc	4.78 Abc	3.25 Ba
	450	6.34 Ab	0.97 Bbc	5.97 Aabc	3.40 Ba
	540	5.53 Ac	0.99 Bbc	6.31 Aab	3.25 Ba
	630	5.57 Ac	1.17 Ba	6.45 Aab	4.02 Ba

	720	5.43 Ac	1.08 Bab	7.69 Aa	3.57 Ba
Return	0	7.14 Aa	1.08 Ab	4.22 Ab	3.64 Ac
	360	6.45 Ab	1.36 Aa	4.14 Bb	5.00 Ab
	450	6.28 Abc	1.29 Aa	4.10 Bb	5.57 Ab
	540	5.87 Acd	1.35 Aa	4.66 Bb	7.35 Aa
	630	5.62 Ade	1.41 Aa	4.53 Bb	7.17 Aa
	720	5.55 Ae	1.35 Aa	6.58 Aa	5.39 Ab
N rate effect		***	***	***	***
Straw effect		ns	***	***	***
N rate × Straw effect		ns	ns	ns	**

[a)]Distinct lowercase characters within the same column represent the significant difference among different nitrogen fertilization treatments; Distinct uppercase characters represent the significant difference between different treatments for straw management.
*, * * and * * * represent significant difference at $P < 0.05$, $P < 0.01$, and $P < 0.001$ level; ns: not significant ($P > 0.05$).
[b)]TN: total nitrogen.

Biological binding agents

The impacts of straw management and N fertilizer rates on three biological aggregating agents shown in Table 2. N fertilization under straw removal conditions substantially increased the SOC content by 11.1-23.7% and decreased the MBC content ($P < 0.001$) but did not alter the content of GRSP ($P > 0.05$). In comparison to the straw removal treatment, the straw return treatment significantly improved the SOC content by 28-36%, GRSP content by 13.1-22.7%, and MBC content by 86.9-224% ($P < 0.001$). Our results demonstrate that the negative effects of N fertilization on GRSP and MBC should not be ignored; however, the benefits of straw incorporation on soil binding agents should be further highlighted.

TABLE II

The effects of nitrogen fertilizer rates and straw management on the SOC, GRSP, and MBC content of a Vertisol after 12 years

Straw management	N rate (kg ha^{-1})	SOC[b] g kg^{-1}	GRSP[c]	MBC[d]
Removal	0	9.25 Bb[a]	1.37 Ba	0.15 Ba
	360	10.3 Bab	1.47 Ba	0.14 Ba
	450	10.3 Bab	1.47 Ba	0.15 Ba
	540	11.2 Ba	1.40 Ba	0.06 Bb
	630	11.4 Ba	1.40 Ba	0.08 Bb
	720	11.3 Ba	1.43 Ba	0.05 Bb
Return	0	12.0 Ab	1.61 Aa	0.28 Aa
	360	14.0 Aa	1.67 Aa	0.29 Aa
	450	14.0 Aa	1.67 Aa	0.32 Aa
	540	14.4 Aa	1.72 Aa	0.26 Aa
	630	14.3 Aa	1.71 Aa	0.25 Aab
	720	15.1 Aa	1.67 Aa	0.17 Ab
N rate effect		***	ns	***

Straw effect	***	***	***
N rate × Straw effect	ns	ns	ns

[a]Distinct lowercase characters within the same column represent the significant difference among different nitrogen fertilization treatments; Distinct uppercase characters represent the significant difference between different treatments for straw management.
*, * * and * * * represent significant difference at $P < 0.05$, $P < 0.01$, and $P < 0.001$ level; ns: not significant ($P > 0.05$).
[b]SOC: soil organic carbon;
[c]GRSP: glomalin-related soil proteins; [d]MBC: microbial biomass carbon.

Soil structural stability

The use of N fertilization under straw removal conditions did not alter soil bulk density, while the straw return treatment reduced the soil bulk density by 6-12% ($P < 0.001$) as compared to the straw removal treatment (Fig. 2). It was noted that 720 kg N ha^{-1} under straw return conditions slightly increased soil bulk density by 6.26% relative to the 630 kg N ha^{-1} level. The MWD under straw removal conditions presented a sharp decrease from 1.26 mm to 1.03 mm when the N input levels increased from 0 to 720 kg ha^{-1} ($P < 0.05$) (Fig. 3). However, the straw return treatment increased the MWD by 26-48% ($P < 0.001$) compared to the straw removal treatment.

Fig. 2

Fig. 2 The effects of nitrogen fertilizer rates and straw management on soil bulk density of a Vertisol after 12 years.

Fig. 3

Fig. 3 The effects of nitrogen fertilizer rates and straw management on soil aggregate stability (MWD) of a Vertisol after 12 years.

The associations among aggregate stability and SOC, GRSP, and MBC under different N fertilization rates combined with straw management are shown in Fig. 4. Under the straw removal conditions, MWD had a significant association with MBC ($R^2 = 0.61$, $P < 0.05$) and a negative association with SOC ($R^2 = 0.93$, $P < 0.001$) but no interaction with GRSP ($R^2 = 0.08$, $P > 0.05$). These relationships between MWD and binding agents became weak when straw return was involved. Interestingly, MWD also demonstrated a highly positive association with pH ($R^2 = 0.87$, $P < 0.01$) and negatively associated with NH_4^+ ($R^2 = 0.68$, $P < 0.05$) and NO_3^- ($R^2 = 0.83$, $P < 0.05$) under straw removal conditions (Fig. 5a, c and d). Thus, optimized N input and straw incorporation could minimize risks of soil structural deterioration in the study region.

Fig. 4

Fig. 4 Relationships among soil aggregate stability (MWD) and soil organic carbon (SOC), glomalin-related soil proteins (GRSP), and microbial biomass carbon (MBC) in the Vertisol after 12 years.

Fig. 5

Fig. 5 Relationships among soil aggregate stability (MWD) and pH, total nitrogen (TN), NH_4^+, and NO_3^- in the Vertisol after 12 years.

DISCUSSION

N fertilization under straw removal conditions substantially lowered soil aggregate stability ($P < 0.05$) compared with the non-N fertilized treatment (Fig. 3), although the SOC content also considerably increased ($P < 0.001$) (Table 2), which is inconsistent with many reports in the literature that N fertilization not only increased SOC content but also improved soil aggregation and MWD (Guo *et al*., 2018, 2019a; Mustafa *et al*., 2020). This discrepancy may be related to the changes in some aggregating agents (e.g., GRSP and MBC). In this study, we found that N fertilization did not alter the GRSP content (Table 2), which is in agreement with previous results from a Cambisol (Guo *et al*., 2019a) and Fluvisol (Dai *et al*., 2013), but differs from other data obtained in a Vertisol (Guo *et al*., 2018) and Cambisol (Xie *et al*., 2015) that the N application from inorganic sources increased the GRSP content. The effect of N fertilization on GRSP content produced by arbuscular mycorrhizal fungi (AMF) is largely related to N application rate (Sun *et al*., 2018). Here, the N application level in our present study and the first two studies mentioned above is higher than 300 kg N ha^{-1}. This high-level N input could decrease the amount of AMF biomass (Jeske et al., 2018) and thus inhibit GRSP production (Dai *et al*., 2013; Sun *et al*., 2018; Guo *et al*., 2019). We also found that the content of MBC has decreased substantially with an increased amount of N inputs ($P < 0.001$) (Table 2) and a positive correlation of MBC with MWD (Fig. 4c). Some previous studies have demonstrated that the leading cause of this reduced MBC appears to be a rapid decline in soil pH induced by long-term elevated N fertilization (Zang *et al*., 2016; Dai *et al*., 2018). These findings confirm the part of our hypothesis that soil acidification from high-level N input can inhibit GRSP and reduce MBC and consequently decrease soil aggregation.

The increase in some dispersive ions (e.g., NH_4^+) could be another reason for the adverse effect on soil aggregation (Haynes and Naidu, 1998). In our research, a negative association between aggregate stability and NH_4^+ ($R^2 = 0.68$, $P < 0.05$) (Fig. 5c) was observed. This indicate that the accumulation of NH_4^+ may also be a primary explanation for decreased aggregate stability after long-term increased N fertilization. In several soils of China (Yan *et al*., 2013; Xin *et al*., 2015; Zhou *et al*., 2017; Guo *et al*., 2019a) and other regions in the world (Blanco-Canqui *et al*., 2014), soil structural deterioration has been reported as a consequence of a high level of NH_4^+-forming fertilizer. They speculated that this could happen because soil colloids were dispersed by the accumulated NH_4^+ from the synthetic N fertilizers in soil. However, due to limited data points (only with or without N addition treatments) or a narrow range of N application rates (from 0 to 200 kg N ha^{-1} yr^{-1}), an evidence of the relationship between aggregate stability and NH_4^+ has not yet definitive. In reality, the high crop yields in this area is depend largely on the excessive use of synthetic N fertilizers (from 325 to 805 kg N ha^{-1} yr^{-1}) (unpublished data). The annual N application rate in the present study ranged from 0 to 720 kg ha^{-1}, providing an opportunity to evaluate the adverse structural effects of NH_4^+ fertilization. Shen et al. (2010) reported that a 2-year overuse of N fertilizer (456 and 570 kg N ha^{-1}) on vegetable soil in China significantly reduced the NH_4^+ nitrify ability of soil organisms in low pH conditions and hence the NH_4^+ content increased sharply by 71-83%. Sun *et al*. (2015) also observed that the content of NH_4^+ in the acidified soil (Vertisol) increased massively by 60% in the wheat harvest over a period of 30 years, owing to its lower abundance and diversity of ammonia oxidizing archaea (AOA). Furthermore, the 4-year nitrification experiment verified that nitrification during the fall-through-spring period is highly inhibited by low temperatures (Kyveryga *et al*. 2004). Due to its long persistence, this slow nitrification accelerated NH_4^+ accumulation in the surface soil until late spring, causing the dispersion of soil particles. In our study, higher rates of N fertilization at low temperature during the wheat season (averaged 10 °C) and relatively acidic conditions (pH < 6) may have led to the accumulation of NH_4^+. Clearly, N fertilization, particularly when overly applied, may add a significant amount of NH_4^+ in some undesirable circumstances, by limiting ammonia oxidizers for nitrification and degrading soil aggregation.

Straw incorporation has great potential to reduce the dependency on higher N fertilizers, and sequester more carbon in soil. It is estimated that a return of 100% crop straw to the soil could counterbalance 29% of the total N fertilizer consumption (Yin *et al*., 2018) and contribute approximately 40% of the total SOC storage (Zhao *et al*., 2018). Furthermore, several incubation experiments (Rahman *et al*., 2017, 2018; Yu *et al*., 2019) and field trials (Zhang et al., 2014; Zhang *et al*., 2017; Guo *et al*., 2018) have revealed the beneficial effects of incorporating straw on soil aggregation. Reasons may be provided for the greatest increase in SOC, GRSP and MBC content serving as binding agents in the aggregation process. In this study, we observed that integration straw with N fertilization did not display a declining trend in MWD, in comparison with N fertilization alone treatments; however, aggregate stability improved by 2-6% relative to 360 kg ha^{-1} of N fertilization (Fig. 3). This is because the positive effects of straw incorporation on soil binding agents (SOC, GRSP and MBC) may offset the negative effects of N fertilization on soil aggregation. On the other hand, we also found that integrating straw incorporation with N fertilization (ranged from 0 to 630 kg N ha^{-1}) accelerated the conversion of NH_4^+ into NO_3^- (Table 1), which may be due to the increase in abundance and diversity of ammonia-oxidizing bacteria (AOB) (Zhang *et al*., 2019). Our findings highlight that a consistently high N inputs may not be feasible for the intensive wheat-maize rotation in this region, coupled incorporation of crop straw with optimized N input as a sustainable management strategy could alleviate some adverse structural effects of excessive N fertilization.

CONCLUSIONS

Our study provides a direct evidence that long-term N fertilization degrades soil aggregation because it creates more monovalent ions (H^+, NH_4^+) in soil acidification despite increasing SOC, especially when the applied N rate exceed 360 kg ha^{-1} in a wheat-maize cropping system. In contrast with sole N fertilization, straw incorporation increased remarkably the aggregating agents but did not affect the dispersing agents and thus improved soil aggregation. Thus, the optimized N input and straw incorporation should be considered while sustaining agricultural system in the future.

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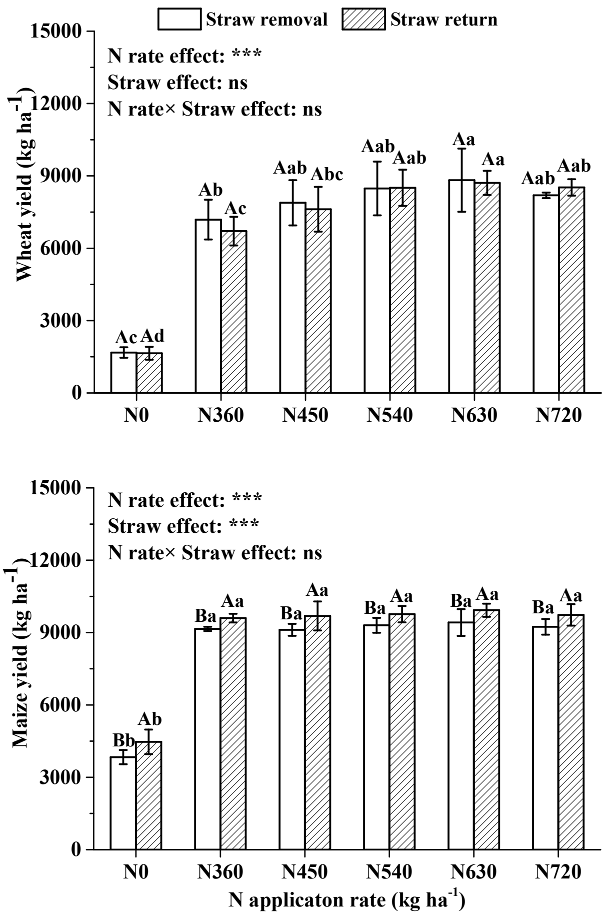

Fig. 1 Wheat yield (upper) and maize yield (bottom) under different nitrogen fertilizer rates and straw management on a Vertisol (2014-2018). Bars are standard deviations on columns (Number = 3). Distinct lowercase characters represent the significant difference among different nitrogen fertilization treatments; Distinct uppercase characters represent the significant difference between different treatments for straw management.

*, * * and * * * represent significant difference at $P < 0.05$, $P < 0.01$, and $P < 0.001$ level; ns: not significant ($P > 0.05$).

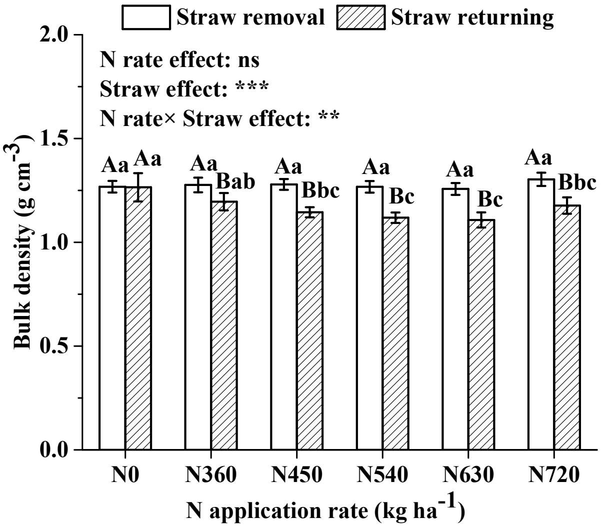

Fig. 2 The effects of nitrogen fertilizer rates and straw management on soil bulk density of a Vertisol after 12 years.

Bars are standard deviations on columns (Number = 3). Distinct lowercase characters represent the significant difference among different nitrogen fertilization treatments; Distinct uppercase characters represent the significant difference between different treatments for straw management.

*, * * and * * * represent significant difference at $P < 0.05$, $P < 0.01$, and $P < 0.001$ level; ns: not significant ($P > 0.05$).

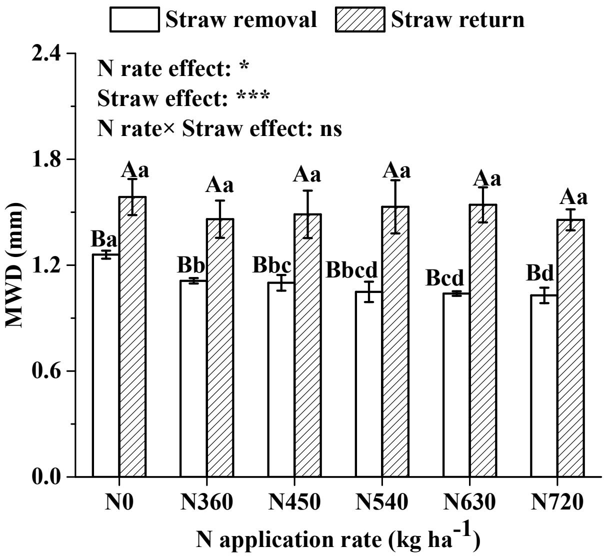

Fig. 3 The effects of nitrogen fertilizer rates and straw management on soil aggregate stability (MWD) of a Vertisol after 12 years. Bars are standard deviations on columns (Number = 3). Distinct lowercase characters represent the significant difference among different nitrogen fertilization treatments; Distinct uppercase characters represent the significant difference between different treatments for straw management.

*, * * and * * * represent significant difference at $P < 0.05$, $P < 0.01$, and $P < 0.001$ level; ns: not significant ($P > 0.05$).

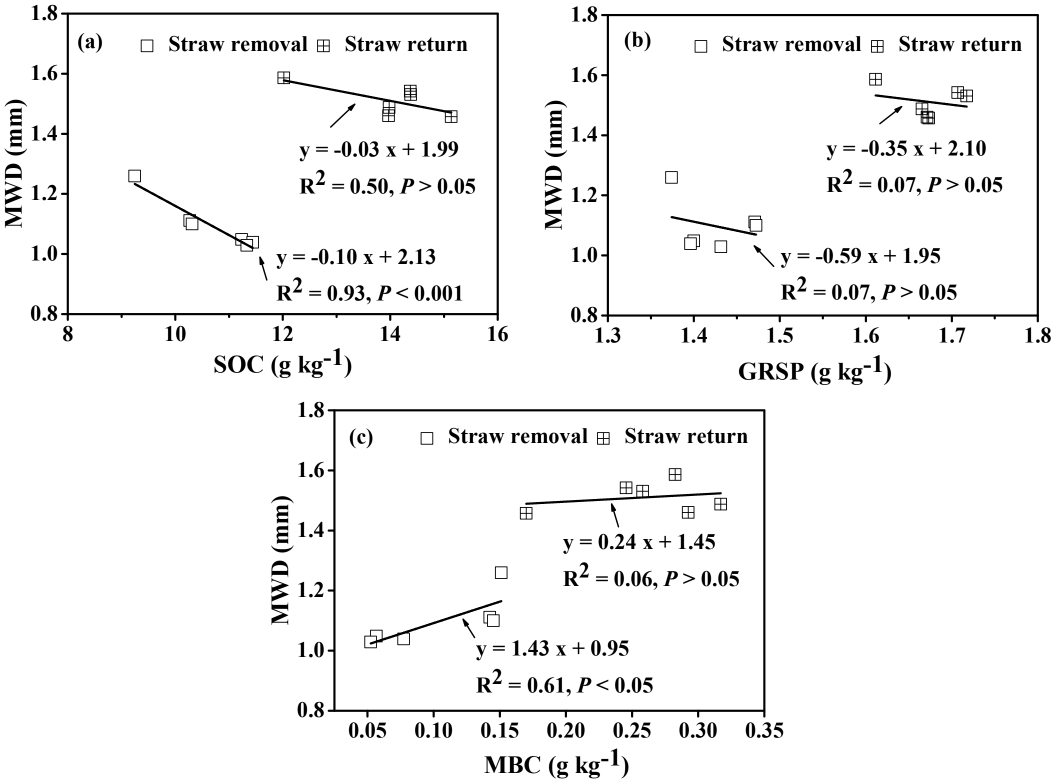

Fig. 4 Relationships among soil aggregate stability (MWD) and soil organic carbon (SOC), glomalin-related soil proteins (GRSP), and microbial biomass carbon (MBC) in the Vertisol after 12 years.

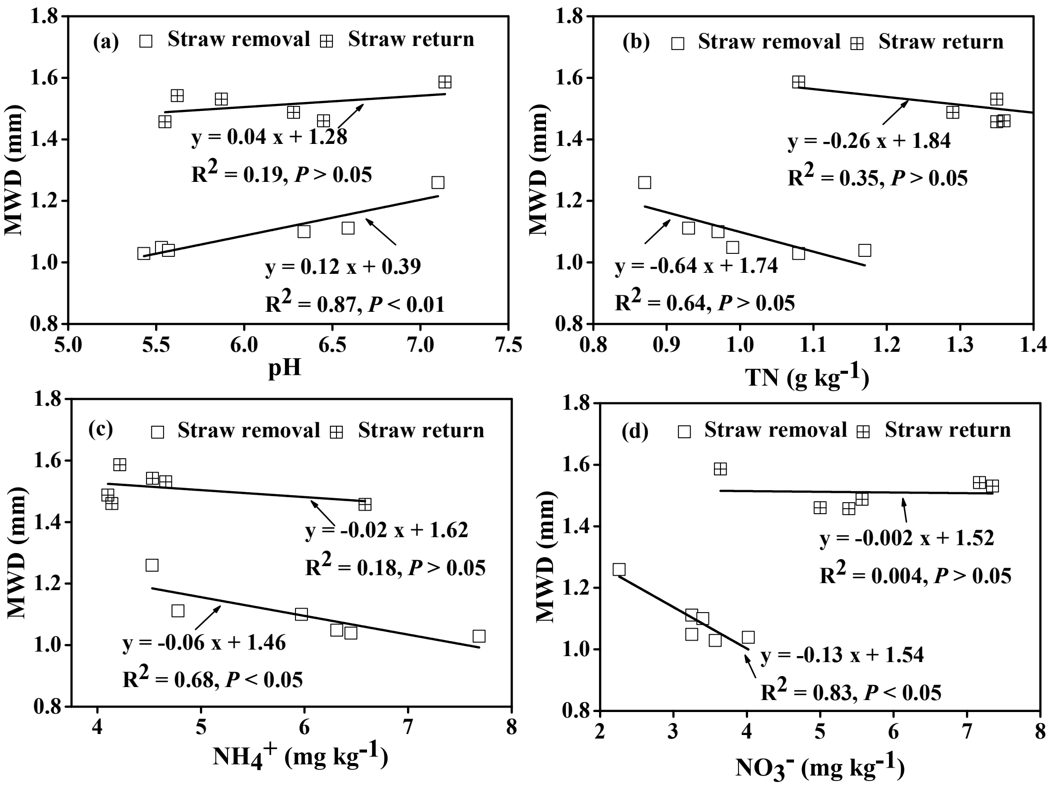

Fig. 5 Relationships among soil aggregate stability (MWD) and pH, total nitrogen (TN), NH_4^+, and NO_3^- in the Vertisol after 12 years.