Introduction

Soil structure exerts important influences on the conditions and workability of soil. Optimal soil structure can decrease erodibility [1], support plants by regulating water conditions, and optimize aeration levels and the release of available nutrients [2-3].

As an important agricultural practice, fertilization can change soil structure [4-6]. The effects of fertilizers on soil structure are complex and depend on the types of fertilizers, environmental conditions, fertilization regime, and initial soil C contents [7-8]. Some studies have reported that long-term applications of chemical fertilizers increased water-stable aggregation, porosity, and hydraulic...
conductivity, but decreased bulk density; these changes were attributed to the increased crop yields and returns of organic matter [9]. Other studies, however, have reported that the application of chemical fertilizers exacerbated clay dispersal, adversely affecting soil aggregation [3, 10]. Soil structure could thus be degraded with a reduction in aggregate stability. Simansky et al. [11] even reported that chemical fertilization (NPK) did not have statistically significant influence on increasing the soil structure stability of orthic luvisol. In addition, organic matter was a major contributor to aggregate stability because it provided important biological binding agents, which decreased the breakdown of aggregates by slaking, swelling, or even osmoting stress [7, 12-14]. Some studies have reported that aggregate stability was higher in soil fertilized with manure than in soil fertilized with chemical fertilizers [15-16]. However, other studies have reported that the rate of organic input or soil characteristics (essentially C and clay contents) had no evident effect on aggregate stability [17-18].

Suitable measures for characterizing soil structure are necessary for evaluating the impact of fertilizer application on soil quality [3]. Three broad categories of soil structure are generally recognized: single-grained, massive, and aggregated [19]. An aggregated structure is the most desirable condition for plant growth and the most sensitive index of soil structure [1, 20]. Water-stable aggregates (WSAs), mean weight diameter (MWD), and geometric mean diameter (GMD) have been widely used to analyze aggregate stability [21-23]. Moreover, aggregate state, aggregate degree, and dispersion rate determine the ability of soil to resist disturbance and serve as indicators of soil structure [24]. Fractal dimension is also a powerful tool used to characterize aggregate-sized distributions for monitoring soil structure [25-27]. Using the fractal method to estimate soil structure changes under practices in conventional tillage/no tillage rotation, Wang et al. [28] found that MWD and GMD were increased while fractal dimension decreased.

The Loess Plateau of China is characterized by concentrated precipitation, dry climate, intensive evaporation, and frequent natural disasters [29]. The loessial soil (Entisols in the USDA classification system) widely distributed in the region is characterized by silty loams of low fertility (soil organic matter content 6.15±2.4 g kg⁻¹) and poor structural stability for resisting disturbances such as tillage and erosion [23]. To date, few studies have quantified the changes of soil aggregate structure caused by long-term fertilization on the hilly Loess Plateau. Unfavorable soil properties combined with inappropriate fertilization management, such as excessive chemical fertilization, a preference for nitrogen (N) fertilizers, and no manure application can lead to a significant degradation of soil structure and fertility [2, 10, 30], which could further become serious obstacles to local agricultural development.

The long-term application of fertilizer provides a good opportunity formulating rational strategies for maintaining soil health [3, 31-32]. Based on the fundamental role of soil organic matter on the formation of aggregates, the study of the association between fertilization and soil structure could provide scientific and practical reference for the development of sustainable fertilization practices. The main objectives of this study were thus: 1) to determine the effects of different fertilization practices on soil aggregate stability and structure and 2) to offer an appropriate nutrient-supply strategy for local farmers based on a 19-year long-term fertilization system.

Materials and Methods

Study Site

The long-term fertilization experiment began in 1995 at the Ansai Field Experimental Station (109°19′E, 36°51′N) in a typically hilly region of the Loess Plateau. The area has a semiarid climate with a mean annual temperature of 8.8°C and an average of 203 frost-free days. The mean annual precipitation in the area is 505 mm, which falls mostly from July to September. The experimental plots are located on tableland along a river. The soil is typical cultivated loessial soil, which originated from wind deposits and is characterized by a yellow color, an absence of bedding, a silty texture, looseness, macroporosity, and wetness-induced collapsibility [33].

Experimental Design

The experimental field covered an area of 500 m² and was divided into 24 plots. The plots received three replicates of eight treatments: unfertilized control (CK), N fertilizer (N), phosphorus fertilizer (P), N + P fertilizer (NP), manure (M), manure + N fertilizer (MN), manure + P fertilizer (MP), and manure + N + P fertilizer (MNP) (Table 1). The plots were 2.3 × 6 m and were separated by 1-m protection rows in a randomized complete-block design.

Two crops, soybean (Glycine max L.) and corn (Zea Mays), were grown in annual rotation. The crops were sown in April at densities of 10 plants -m² for corn and 50 plants -m² for soybean. Following the local convention, the organic (manure), N (pure N), and P (P₂O₅) fertilizers were applied at rates of 500, 100, and 50 kg ha⁻¹.

<table>
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<th>Table 1. Fertilizer inputs to the treatments.</th>
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<td>Fertilizer</td>
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Note: CK, unfertilized but cropped control; N, nitrogen fertilizer; P, phosphorus fertilizer; NP, N+P fertilizer; M, manure; MN, M+N fertilizer; MP, M+P fertilizer; MNP, M+N+P fertilizer. + indicates the input of fertilizer.
respectively. The organic and P fertilizers were applied once at sowing. The N fertilizer was applied twice – 36% as a basal fertilizer and the remainder as a top-dressing at the jointing stage. The soil was plowed by donkeys before sowing each year and was manually heded once during the growing season. Residual plant material above the ground was removed from the plots after harvest at the beginning of October.

Soil Sampling

Soil samples were collected from the 0-20 cm layer of all plots after the crop harvest in October 2014. Undisturbed soil samples with dimensions of 20 x 10 x 5 cm were obtained using a spade from five random points in each plot for macroaggregates. Composite soil samples were collected from the same plots. After air-drying and removing visible pieces of crop residues and roots. Part of these samples were ground to pass through a 2-mm sieve for microbial biomass carbon (MBC), microaggregate- and particle size distribution measurements, and the remainder was ground to pass through a 0.25-mm sieve for soil organic carbon (SOC) and total nitrogen (TN) analysis.

Soil Analysis

Soil macroaggregates were measured using wet-sieving method [34]. Five size classes (>5, 2-5, 1-2, 0.5-1, 0.25-0.5 mm) were obtained with sieves of 5, 2, 1, 0.5, and 0.25 mm. Water-stable aggregates (WSAs) >0.25 mm were considered as macroaggregates. Soil microaggregates and particles were analyzed by a Mastersizer 2000 laser diffraction device [35]. For microaggregate determination, the samples were presoaked with distilled water for 24 h. For particle determination, samples were pretreated with 6% H2O2 to remove organic matter and we added 10% HCL for removing carbonates and oxides, and then soaked them with distilled water for 24 h. After removing the distilled water, the samples were chemically dispersed with 0.4% Calgon and mechanically dispersed in an ultrasonic bath for 30 seconds [24]. SOC was determined using the Walkley-Black method [36]. TN was determined by the Kjeldahl method [37]. MBC was measured by the fumigation-extraction method [38].

Calculation of Soil Aggregate Structure Index

MWD (mm) and GMD (mm), indices of aggregate stability were calculated as [15]:

\[ MWD = \sum_{i=1}^{n} \bar{R}_i w_i \]  
\[ GMD = \exp \left[ \sum_{i=1}^{n} w_i \ln \bar{R}_i \right] / \sum_{i=1}^{n} w_i \]  

…where \( \bar{R}_i \) is the average diameter of the openings of two consecutive sieves, and \( w_i \) is the weight ratio of the aggregates retained by sieve \( i \). The ratio of the weight of the aggregates retained by each sieve (>5, 2-5, 1-2, 0.5-1, and 0.25-0.5 mm) to the total weight of the aggregates was calculated for determining aggregate distribution.

Aggregate state (AS), aggregate degree (AD), and dispersion rate (DR) were proposed to evaluate soil aggregate structure via the following equations [24]:

\[ AS = n_1 - n_2 \]  
\[ AD = AS/n_2 \times 100\% \]  
\[ DR = n_3/n_4 \times 100\% \]

…where \( n_1 \) is the content of microaggregates >0.05 mm, \( n_2 \) is the content of particles >0.05 mm, \( n_3 \) is the content of microaggregates <0.05 mm, and \( n_4 \) is the content of particles <0.05 mm.

The fractal dimension (D) of the soil aggregates was determined by Tuo et al. [39]:

\[ M(r < R_i)/M_T = (R_i/R_{\text{max}})^{-D} \]

…where \( R_i \) is the mean aggregate diameter (mm) of size class \( i \), \( M(r < R) \) is the cumulative mass of aggregates of size \( r \) less than \( R \), \( M_T \) is the total mass, \( R_{\text{max}} \) is the mean diameter of the largest aggregate, and \( D \) is the aggregate fractal dimension. The mean aggregate diameter was taken as the arithmetic mean of the upper and lower sieve sizes, and the mean diameter of the aggregates <0.002 mm was taken as 0.001 mm.

Statistical Analysis

The data were expressed as means ± standard deviations. One-way analysis of variance was performed by SPSS 18.0. Differences between means (at \( P<0.05 \)) were tested by multiple comparisons using the least significant difference test. Regression models and graphs were prepared using SigmaPlot 10.0 (Systat Software, Inc., Chicago, USA).

Results

Soil Properties and Crop Yield under Different Fertilizer Treatments

Soil properties showed significant differences between the treatments containing manure (M, MN, MP, and MNP) and only with chemical fertilizers (N, P, and NP) (\( P<0.05 \); Table 2). The contents of SOC, TN, and MBC were higher...
in treatments containing manure than in the chemical-fertilizer treatments. Compared with CK, SOC, TN, and MBC tended to increase in soils treated with manure. No remarkable differences in SOC, TN, MBC, and soil particles were found among the treatments with N, P, NP, and CK ($P>0.05$).

Fig. 1 shows the variation in crop yields among the eight treatments. Compared with CK, the mean yield was 6.1-87.6% higher in the chemical-fertilizer treatments (N, P, and NP), and 90.9-151.5% higher in the treatments containing manure (M, MN, MP, and MNP). Compared with CK, a significant increment in crop yield was observed among all fertilizer treatments except N and P ($P<0.05$). The highest crop yield was obtained in MN treatment, which was 151.5% higher than CK ($P<0.05$).

Soil Aggregate Stability under Different Fertilizer Treatments

WSAs accounted for 37.8-57.7% of the dry soil weight in all treatments (Fig. 2). Compared with CK, the proportions of WSAs were 6.2-17.5% lower in the N, P, and NP treatments, but were 12.6-25.9% higher in the M, MN, MP, and MNP treatments. Of the treatments with both manure and chemical fertilizer, MNP had the lowest proportion of WSAs. In contrast, the treatment with manure alone had the highest proportion of WSAs.

As shown in Fig. 3, the chemical-fertilizer treatments caused 15.9-36.4% decreases in MWD and 13.3-32.2% decreases in GMD relative to CK. MWD and GMD were lowest in P treatment. In contrast, MWD and GMD were 22.8-43.3% and 4.3-20.0% higher, respectively, in the treatments containing manure relative to CK. For these treatments, MWD and GMD were highest in M treatment, although not statistically significant.

Soil Aggregate Structure under Different Fertilizer Treatments

Aggregate state (AS), aggregate degree (AD), and dispersion rate (DR) varied to some degree among the eight treatments (Fig. 4). Compared with CK, AS was 1.4-
19.7% higher in M, MN, MP, and MNP treatments and 4.4-21.5% lower in N, P, and NP treatments. No significant difference was found among the fertilized treatments except P (P>0.05; Fig. 4a). AD was closely correlated with AS, and the lowest values both were obtained in P treatment among all fertilized treatments and CK (Fig. 4b). The changes in DR for all fertilized treatments, however, were opposite those of AS. The average DR was 6.1% lower for the treatments containing manure (M, MN, MP, and MNP) than for the chemical-fertilizer treatments (N, P, and NP; Fig. 4c).

Fig. 5 shows the variation in aggregate fractal dimension (D) among the eight treatments. The values of D were higher by 1.5-4.2% for the chemical-fertilizer treatments and lower by 0.4-2.9% for the treatments containing manure than for CK. Moreover, D was significantly negatively correlated with SOC, WSAs, and aggregate state, but positively correlated with dispersion rate (P<0.01 or P<0.05; Fig. 6).

**Discussion**

The MWD and GMD are crucial indicators of aggregate stability [14, 40]. The MWD reflects the proportion of macro aggregates [15], while the GMD estimates the size of the most frequent aggregate size class [41]. In our study, the result showed that the proportions of GMD and
MWD were lower in the chemical-fertilizer treatments (N, P, and NP) relative to CK (no fertilizer applied), consistent with the findings of Haynes and Naidu [10], who reported that chemical fertilizers have a negative influence on soil structure. In contrast, the MWD and GMD were significantly higher in soils treated with manure than in soils without manure [2].

Aggregate state (AS), aggregate degree (AD), and dispersion rate (DR) in our study were used to assess soil aggregate structure. The soils fertilized with both manure and chemical fertilizer showed a higher soil structure than soils treated with chemical fertilizer only (Fig. 4). These results were consistent with those of previous studies. Naveed et al. [3], using X-ray computed tomography, reported an obvious improvement in soil structure (wider distribution of pore sizes, higher pore connectivity, and higher biological activity) and related soil functions with increasing animal manure. Yang et al. [7] found that the application of manure increased the penetration resistance and shear strength of bulk soil.

The application of manure generally increases SOC content more than other fertilizer treatments (Table 2) supported by other reports [6, 42-43]. However, organic fertilizers affect soil aggregate stability and structure mainly through their effects on organic matter [7, 13-14]. Our results demonstrated that MWD was positively correlated with SOC content ($P<0.01$; Fig. 7). Organic fertilizers could lead to an increase in the proportion of WSAs, which was attributed to the input of additional organic residues and available C compared with that by the application of chemical fertilizers [2, 30, 44]. Furthermore, microbial biomass plays a fundamental role in soil organic matter by facilitating key functions and services such as soil aggregation, decomposition, and organic carbon mineralization [45-46]. Higher levels of SOC increase the availability of C substrates that stimulate microbial activity [46-48]. Microbial biomass contributes to C sequestration by producing polysaccharides and fungal hyphae that improve soil aggregation [49-51]. In contrast, the long-term application of chemical fertilizers may suppress microbial biomass, resulting in a substantial reduction in MBC and SOC accumulation [52]. Khan et al. [53] reported that N fertilizers increased SOC mineralization. In our study, the application of chemical fertilizers tend to increase crop yields (Fig. 1), but do not guarantee an increase in MBC and SOC contents, thus the aggregate stability of chemical-fertilizer treatments were slightly lower than that for CK. As a result, aggregate stability was lower in soil fertilized with chemical fertilizers than in soil fertilized with manure [15-16].

The heterogeneity of soil structure due to the distribution of aggregate sizes has recently been described by the fragmentation of fractal dimension [54]. Many factors that influence the fractal dimension include vegetation and erosion [19, 55-57]. Our results showed
that a combination of organic and inorganic fertilizers could decrease the aggregate fractal dimension (Fig. 5). The decrease may be attributed to the increase in the proportion of aggregates >5 mm with the input of an additional C source. Furthermore, fractal dimension was significantly correlated with SOC, WSAs, aggregate sate, and dispersion rate ($P<0.01$ or $P<0.05$; Fig. 6). Aggregate fractal dimension can thus indicate changes in soil structure associated with the long-term application of fertilizer in loessial soil [19].

**Conclusion**

The effects of fertilizers on soil aggregate stability and structure depend on the fertilizer type. Continuous application of manure (M), either alone or in combination with N and/or P, can increase water-stable aggregates (WSAs), mean weight diameter (MWD), geometric mean diameter (GMD), aggregate state (AS), and aggregate degree (AD). In contrast, the long-term application of NP, or N and P individually has lower values of these indexes than CK. The differences may be attributed to C source and microbial biomass, which cause differences in fungal hyphae in the loessial soil. Of the treatments in all fertilizers and CK, M had the highest proportion of WSAs, MWD, GMD, AS, and AD. Chemical fertilizers could increase crop yield, but did not improve soil structure. The present study indicated that the application of manure was a preferred strategy for improving soil fertility and for sustainable soil management in the Loess Plateau of China.

**Acknowledgements**

This research was financially supported by the key deployment project of the Chinese Academy of Sciences (KJZD-EW-TZ-G10), the Special Program for Basic Research of the Ministry of Science and Technology in China (2014FY210100), and the National Natural Science Foundation of China (41171422).

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