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Effects of atrazine application on soil aggregates, soil organic carbon and glomalin-related soil protein

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Abstract: Atrazine is still widely used in China. Atrazine residue (1.86–1 100 mg/kg) in the soil has exceeded the allowable limit (1.0 mg/kg), affecting soil structure and soil aggregate composition. To understand the long-term application of atrazine on soil aggregates and the binding agent, four treatments were established in cornfield planted since 1998, including without atrazine applied (AT_0), atrazine applied (28% atrazine, 1 200–1 350 mL/ha/year) once a year from 2012 to 2018 (AT_6, 167 mg/kg), from 2008 to 2018 (AT_{10}, 127.64 mg/kg) as well as from 2002 to 2018 (AT_{16}, 102 mg/kg) with three replications. Along with the increase of atrazine application time, the mass fraction of soil aggregates > 5 mm and 2–5 mm decreased significantly while the mass fraction of soil aggregates 0.5–2 mm and < 0.5 mm increased gradually, and the change of aggregate binding agents contents were the same as that of aggregates. The contents of soil organic carbon (SOC) and glomalin-related soil protein (GRSP) in the aggregates > 5 mm and 2–5 mm were significantly negatively correlated with the years of atrazine application. Our results show that although atrazine residue in the soil does not increase with the increased yearly application, its concentration is still markedly higher than the permitted limit value and seriously affected the content of SOC and GRSP of aggregates > 2 mm, which can lead to a decrease of soil aggregate stability and soil quality.

Keywords: herbicide; pollution; soil aggregation; glycoprotein; arbuscular mycorrhizal fungi

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a kind of synthetic triazine herbicide, which became used worldwide since 1959. Due to the high potential of pollution in soil, groundwater and mammal (Fan and Song 2014, Peréz-Iglesias et al. 2019, Fan et al. 2020), atrazine was removed from the list of approved products in the European Union and other countries after 2004 (Albuquerque et al. 2020). However, atrazine is still widely used in other countries, such as China, India and Brazil (Zhu et al. 2018). Atrazine detected in the agricultural soil was 1.86–1 100 mg/kg, which seriously exceeded the regulation permitted limit (1.0 mg/kg) in the soil (Zhang et al. 2019).

Although atrazine was designed to control target organisms (annual grass and broadleaf weeds), direct or indirect inverse effects on microbial community structures and activities were also found, which can adversely change the properties of agricultural soil quality in turn (Chen et al. 2014a). Chen et al. (2014b) reported that incubation with atrazine reduced soil microbial diversity, yet microbial diversity was characterised by suppression-recovery-stimulation with

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increased atrazine treated frequency (Fang et al. 2015). Meanwhile, the most abundant phyla Actinobacteria and Proteobacteria, especially atrazine-mineralising bacteria genus, showed positive correlations with atrazine concentrations in soils (Radivojević et al. 2006, Tu 2008, Fang et al. 2015, Gao et al. 2018). In the previous study of our research group, we found in the agriculture soil, atrazine increased the relative abundance of Actinobacteria significantly, yet inhibited the catalase activity gradually with atrazine application years increased (Liu et al. 2020). Few attempts have been made to assess the effect of long-term atrazine application on the physicochemical properties of agricultural soil in the field.

Soil aggregation is a dynamic process, and the microbial community is assumed to be an important factor in controlling this process. Atrazine reduced the relative abundance of most bacteria (Liu et al. 2020), and with the increase of atrazine content in farmland soil, arbuscular mycorrhizal fungi (AMF) spore density and species abundance decreased accordingly (Wang et al. 2015). The formation and stabilisation of soil aggregate require the action of soil organic carbon (SOC) and glomalin-related soil protein (GRSP) (Guo et al. 2018). As a glycoprotein, approximately 80% of GRSP is generated from hyphal and spores of arbuscular mycorrhizal fungi (Driver et al. 2005). AMF hyphal networks mesh soil particles together, and its metabolic products GRSP can also act as glue to bind soil particles together, which directly affects the stability of soil aggregates (Ji et al. 2019). GRSP is the part of the contribution to SOC and positively correlated with the content of SOC (Rillig et al. 2003, Singh et al. 2016). GRSP can indirectly increase the content of SOC, thus jointly improve the stability of aggregates and enhance resistance to soil erosion (Rillig et al. 2003, Zhu et al. 2019). Besides binding agents in the soil physicochemical process, SOC also acts food and energy source for the soil microbial community.

Previous studies on soil aggregates mainly focused on soil remediation, such as biochar (Liu et al. 2012), organic amendments (Zhang et al. 2014), straw return mode (Zhao et al. 2018) and so on. In this paper, long-term (up to 16 years) atrazine application in the maize field was conducted, and *in-situ* tests examined the effects of atrazine on soil aggregates and binding agents in farmland soils. The objectives of the present study were to determine (1) the effect of long-term atrazine application on soil aggregates and (2) the correlation between atrazine and soil aggregates binding agents, such as SOC and GRSP.

MATERIAL AND METHODS

Study sites. The study plot was located in Qiqihar of Heilongjiang province, northeastern China (47°31'N, 123°35'E). This region is in the middle temperate zone, continental monsoon climate, and is characterised by an annual mean temperature of 3.2 °C and annual mean precipitation of 415 mm. The main soil is Chernozem (78% sand, 10% clay and 12% silt) with the basic physicochemical characteristics as follows: pH 8.46, 14.3 mg/kg NH_4^+-N, 34.0 mg/kg NO_3^--N, 326 mg/kg total phosphorus, 4.27 mg/kg available phosphorus.

In this region, maize (*Zea mays* L.) was sown at the beginning of May and harvested at the beginning of October by traditional tillage since 1998. During the plantation, 28% atrazine was applied once a year in the middle of May with 1 200–1 350 mL/ha. The research plots were conducted with the different years of atrazine application, including 6 years from 2012 to 2018 (AT_6), 10 years from 2008 to 2018 (AT_{10}), and 16 years from 2002 to 2018 (AT_{16}) as well as without atrazine application (AT_0). There is a width of approximately 100 m buffer area established between each plot (about 30 m × 30 m). There are three plots in each treatment and twelve plots total. In addition, atrazine residue in the soil has been determined in our previous report (Liu et al. 2020) and was list as follows: 167 mg/kg in AT_6, 127.64 mg/kg in AT_{10} and 102 mg/kg AT_{16} treatment.

Soil sample collection. Sampling was conducted in August 2018. Soil samples (0–20 cm depth) were collected from each plot in each treatment by the five-point sampling method (Figure 1). These intact soil blocks were separately placed in rigid aluminum containers and kept at approximately 4 °C until the lab.

Soil aggregate fractions. In the lab, large soil blocks were broken into smaller pieces, and then small stones and plant residues were removed. The collected soil samples in each plot were homogenised and air-dried naturally at room temperature, subsequently saved for soil aggregate fractions.

500 g of air-dried soil was taken from each treatment, and the fraction of soil aggregates was determined dry sieving method. The soil aggregates were divided into four sizes: < 0.5, 0.5–2, 2–5, and > 5 mm (Kemper and Rosenau 1986). The portion of each aggregate's size was ground, mixed fully and sieved (< 2 mm) for SOC and GRSP determinations, respectively.

Determination of soil organic carbon. SOC in each aggregate size was measured with the high

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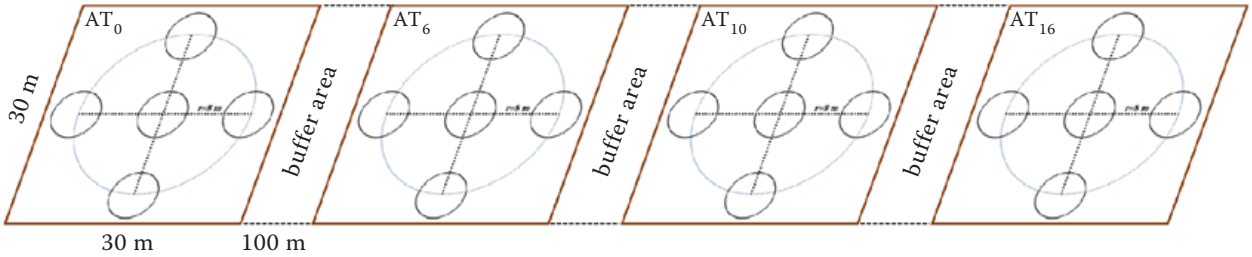

Figure 1. Five-point sampling method. "r" as radius. Take each soil sample center as the fixed point, first collect the soil about 1.0 kg at this point, then take this point as the center of the circle, 8 m as the radius of the circle circumference selected four sampling points, respectively collect 1.0 kg soil, the five points of the soil mixed evenly as a sample. AT_0 – without atrazine application; AT_6 – 6 years from 2012 to 2018; AT_{10} – 10 years from 2008 to 2018; AT_{16} – 16 years from 2002 to 2018

temperature external thermal potassium dichromate oxidation-volumetric method (Hogan et al. 2019). 0.2 g soil samples (through a 0.149 mm sieve) were shaken in a volumetric flask with silver sulfate, potassium dichromate solution and sulphuric acid. Then the soil samples were digested by an air condensation tube and titrated with an o-phenorphine indicator. Finally, the solution color changed from orange to green and then to brown-red.

Determination of glomalin-related soil protein. The amount of GRSP in each aggregate size was determined by Coomassie brilliant blue method (Wright et al. 1998). Extraction of total GRSP (T-GRSP): 1 g of soil sample was added to (8 mL, pH 8.0) 50 mmol sodium citrate extractant and extracted at 103 kPa, 121 °C for 60 min. After extraction, the soil sample was centrifuged rapidly at 111 xg for 6 min, and the supernatant was retained. The operation was repeated for continuous extraction until the supernatant did not appear the typical red-brown of GRSP. The mixture of the extract can be preserved for 3 days at 4 °C.

Extraction of easily extractable GRSP (EE-GRSP): the same method as described above, but only performed once. Colorimetry: 0.5 mL of extract and 5 mL Coomassie brilliant blue G-250 stain was added to the centrifugal tube. After 2 min of color reaction, the colorimetric analysis at 595 nm by Ultraviolet-Visible Spectrophotometer (TU-1810, Beijing, China) was performed. The standard curve of bovine serum albumin was drawn. The curve was fitted linearly with concentration as abscissa and absorbance as ordinate. The fitting equation was $y = 2.25x + 0.004$ and $R^2 = 0.9999$. The GRSP content can be obtained by introducing the absorbance value of the sample into the equation.

Data analysis. A one-way analysis of variance (ANOVA) was used to determine the difference of atrazine residues, the mass fraction of aggregates and the contents of SOC, T-GRSP and EE-GRSP among four treatments (Tukey, $n = 3$). Excel 2007 (Microsoft, USA) was used as the linear fitting equation to analyse the correlation between atrazine application years, soil aggregates and their binding agents with different particle sizes.

RESULTS

Distribution characteristics of soil aggregate mass fraction. After the analysis of soil aggregates fraction in the four treatments, the aggregates 0.5–2 mm had the highest mass fraction, ranging from 38.7% to 42.2%, followed by aggregates 2–5 mm, ranging from 18.9% to 29.8%, aggregates > 5 mm ranged from 11.8% to 21.0%, and mass fraction of aggregates < 5 mm was 10.5–27.2% (Table 1). With atrazine application year increased, the fraction of aggregates > 5 mm and 2–5 mm gradually decreased, especially aggregates > 5 mm in AT_{16} decreased significantly compared to AT_0, AT_{10} and AT_{16} ($P < 0.05$). Meanwhile, the fraction of aggregates < 0.5 mm increased significantly with the application year increased ($P < 0.05$). There was no significant difference of aggregates 0.5–2 mm between different atrazine application years (Table 1).

Total glomalin-related soil protein and easily extractable glomalin-related soil protein contents in soil aggregates. The content of T-GRSP in soil aggregates > 5 mm and 2–5 mm decreased gradually with atrazine application years increased (Table 1). The content of those T-GRSP in AT_{16} treatment was the lowest, which was 1.42 mg/g and 1.37 mg/g, re-

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Table 1. The fraction of each soil aggregates size and related total glomalin-related soil protein (T-GRSP), easily extractable GRSP (EE-GRSP) and soil organic carbon (SOC) content with different atrazine application year

	Fraction (%)				T-GRSP (mg/g)				EE-GRSP (mg/g)				SOC (mg/g)			
	> 5	2–5	0.5–2	< 0.5	> 5	2–5	0.5–2	< 0.5	> 5	2–5	0.5–2	< 0.5	> 5	2–5	0.5–2	< 0.5
	(mm)															
AT_0	20.96 ± 0.72^a	29.81 ± 1.55^a	38.73 ± 1.42^a	10.50 ± 0.45^d	1.56 ± 0.03^a	1.51 ± 0.01^a	1.17 ± 0.01^a	0.85 ± 0.02^a	1.27 ± 0.01^a	1.24 ± 0.01^a	0.88 ± 0.01^a	0.57 ± 0.01^a	21.64 ± 1.10^a	21.52 ± 0.99^a	13.42 ± 0.20^a	6.75 ± 1.49^a
AT_6	17.53 ± 1.54^b	25.70 ± 2.98^{ab}	40.05 ± 5.11^a	16.72 ± 3.00^c	1.51 ± 0.04^b	1.45 ± 0.02^b	1.18 ± 0.01^a	0.87 ± 0.03^a	1.23 ± 0.03^b	1.20 ± 0.01^b	0.88 ± 0.01^a	0.58 ± 0.01^a	18.56 ± 1.58^b	18.21 ± 0.99^b	13.65 ± 2.28^a	7.17 ± 0.91^a
AT_{10}	14.96 ± 0.52^b	22.79 ± 0.24^{bc}	40.68 ± 0.59^a	21.57 ± 0.31^b	1.49 ± 0.01^b	1.43 ± 0.02^b	1.19 ± 0.01^a	0.87 ± 0.01^a	1.21 ± 0.01^b	1.18 ± 0.04^b	0.89 ± 0.02^a	0.59 ± 0.01^a	17.98 ± 0.91^b	17.07 ± 0.20^b	13.76 ± 0.20^a	7.64 ± 0.86^a
AT_{16}	11.79 ± 1.28^c	18.91 ± 0.52^c	42.15 ± 1.51^a	27.15 ± 0.86^a	1.42 ± 0.01^c	1.37 ± 0.01^c	1.19 ± 0.02^a	0.89 ± 0.02^a	1.16 ± 0.01^c	1.09 ± 0.01^c	0.90 ± 0.02^a	0.60 ± 0.01^a	14.79 ± 0.71^c	14.22 ± 0.69^c	14.10 ± 0.20^a	8.16 ± 0.86^a

Data represent mean ± standard deviation (n = 3). Different letters indicate significant differences (P < 0.05) between different atrazine application years in each aggregate particle size. AT_0 – without atrazine application; AT_6 – 6 years from 2012 to 2018; AT_{10} – 10 years from 2008 to 2018; AT_{16} – 16 years from 2002 to 2018

spectively. Compared to treatment without atrazine application, the content of T-GRSP with atrazine application decreased significantly (P < 0.05), such as lower content in AT_{16} than in AT_6 and AT_{10} treatment (P < 0.05). The T-GRSP content of aggregates 0.5–2 mm and < 0.5 mm increased with atrazine application year increased, but not significantly.

A similar trend of EE-GRSP and T-GRSP content was observed in each aggregate size (Table 1). In aggregates > 5 mm and 2–5 mm, EE-GRSP content gradually decreased with atrazine application years increased. The lowest values of EE-GRSP in aggregates > 5 mm and 2–5 mm were in AT_{16} treatment, 1.16 mg/g and 1.09 mg/g, which was significantly lower than that in AT_6 and AT_{10}, respectively (P < 0.05). Compared with AT_0, the content of EE-GRSP in the other three treatments decreased significantly (P < 0.05). In addition, the content of T-GRSP and EE-GRSP in each aggregates size did not change significantly in AT_6 and AT_{10}, and there was no significant difference in T-GRSP and EE-GRSP of aggregates 0.5–2 mm and < 0.5 mm between different atrazine application year.

Distribution characteristics of soil organic carbon content in soil aggregates. In soil aggregates > 5 mm and 2–5 mm, the SOC content gradually decreased with the increase of atrazine application time, and the lowest content was 14.79 mg/g and 14.22 mg/g in AT_{16} treatment, respectively (Table 1). Compared with AT_0, the SOC content in the other three treatments decreased significantly (P < 0.05), and the content of AT_{16} was significantly lower than that in AT_6 and AT_{10} treatment (P < 0.05).

The correlation between atrazine application years, soil aggregates and their binding agents with different particle sizes. In Figure 2, aggregate mass fractions > 5 mm and 2–5 mm were negatively correlated with increased atrazine application time (R^2 = 0.93, P < 0.01; R^2 = 0.89, P < 0.01). The mass fraction of aggregates < 0.5 mm had a significant positive correlation with increased atrazine application time (R^2 = 0.96, P < 0.01). The content of T-GRSP in > 5 mm and 2–5 mm were negatively correlated with the application years of atrazine (R^2 = 0.80, P < 0.01; R^2 = 0.96, P < 0.01) (Figure 3). The content of EE-GRSP > 5 mm and 2–5 mm were negatively correlated with the application years of atrazine (R^2 = 0.87, P < 0.01; R^2 = 0.86, P < 0.01) (Figure 4). The content of SOC in > 5 mm and 2–5 mm were negatively correlated with the application years of atrazine (R^2 = 0.85, P < 0.01; R^2 = 0.93, P < 0.01) (Figure 5).

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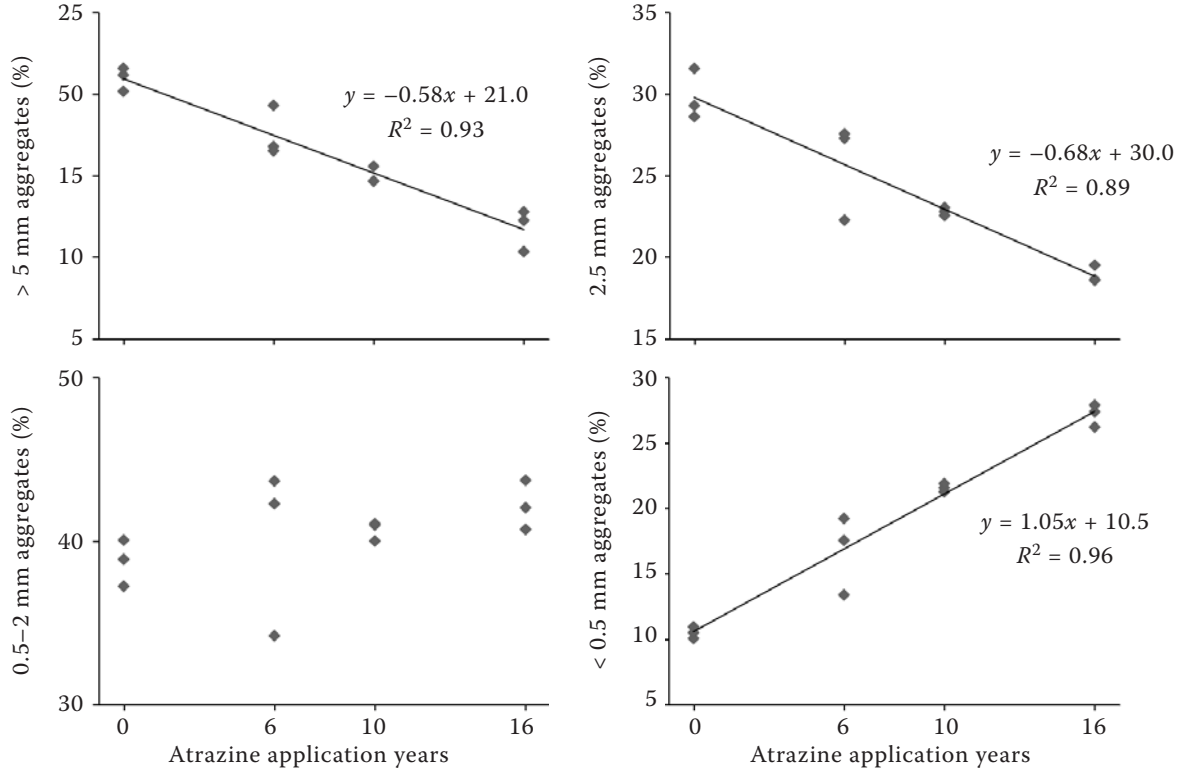

Figure 2. The correlation between atrazine application year and the fraction of soil aggregates with different particle sizes

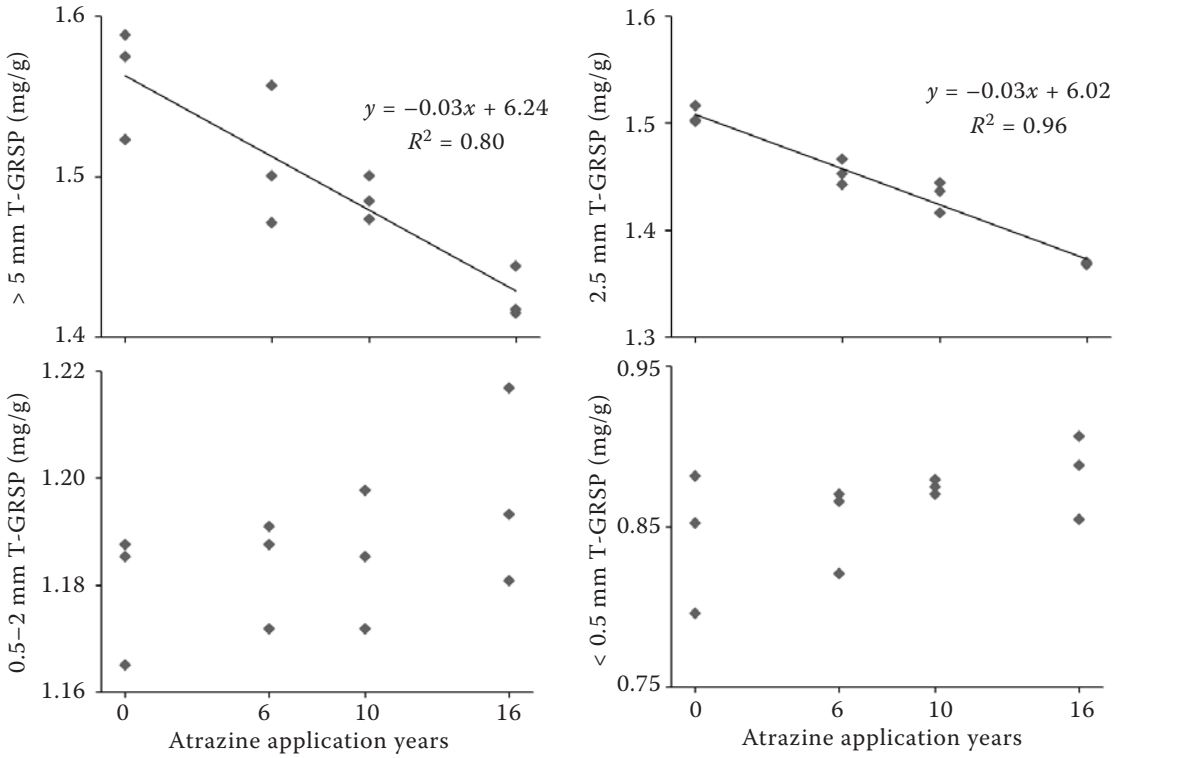

Figure 3. The correlation between atrazine application year and the content of total glomalin-related soil protein (T-GRSP) with different particle sizes

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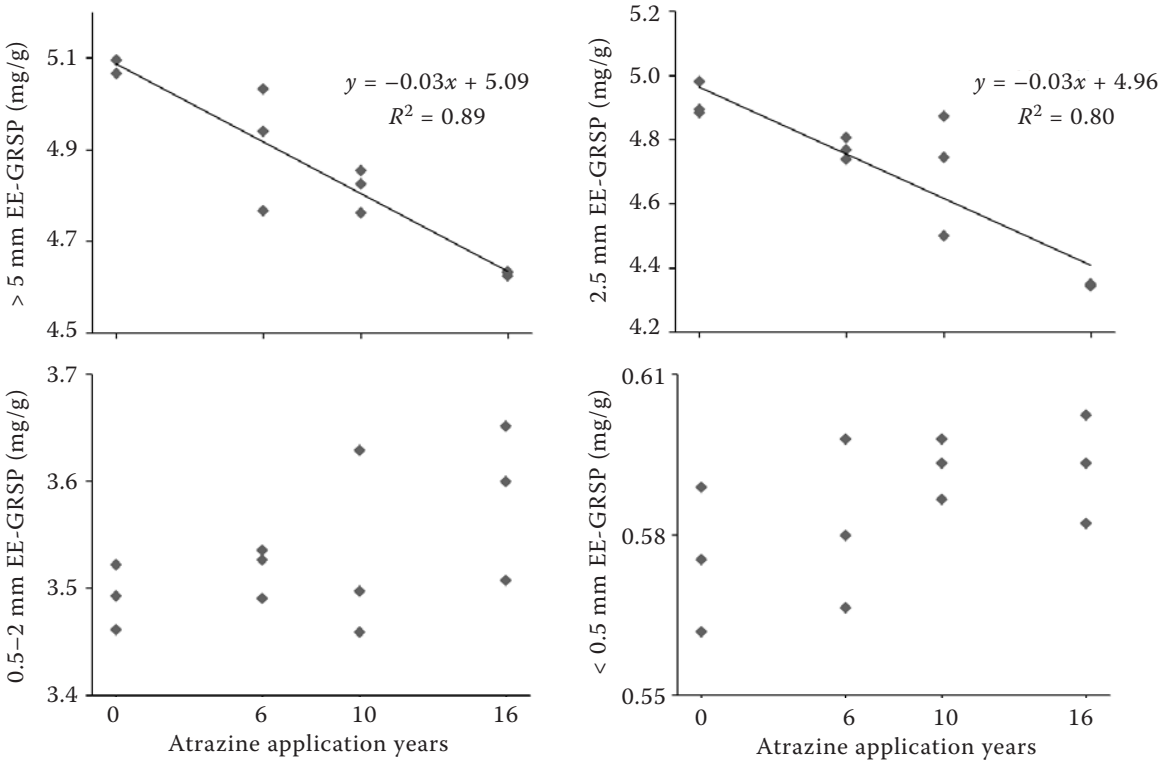

Figure 4. The correlation between atrazine application year and the content of easily extractable glomalin-related soil protein (EE-GRSP) with different particle sizes

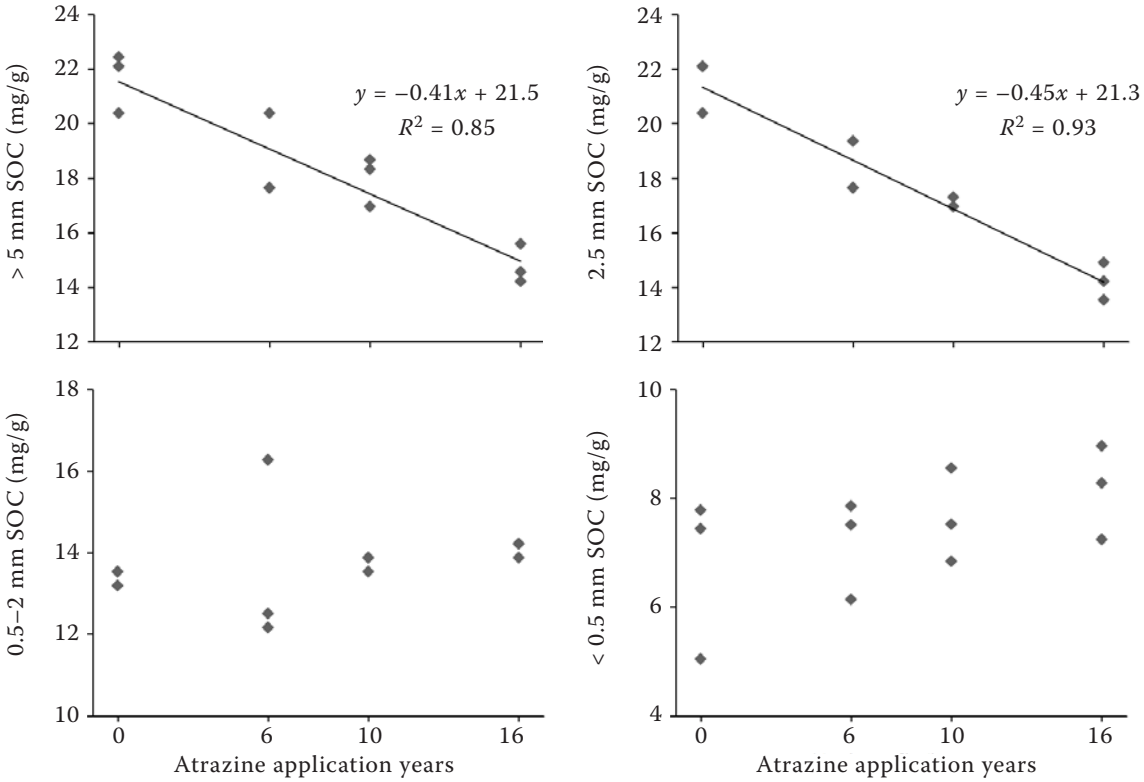

Figure 5. The correlation between atrazine application year and the content of soil organic carbon (SOC) with different particle sizes

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DISCUSSION

Effects of atrazine application on soil aggregates. As atrazine application time increased in this study, mass fraction of > 5 mm and 2–5 mm soil aggregate gradually decreased and transformed into small aggregates 0.5–2 mm and < 0.5 mm (Table 1). Photosynthetic microorganisms such as eukaryotic algae and prokaryotic cyanobacteria are commonly found in topsoil (Knapen et al. 2007) that can form microbial crusts to stabilise aggregates. Herbicide affected photosynthetic microbial crusts and consequently impaired soil aggregation (Crouzet et al. 2019), as aggregate particle sizes and composition ratio affects soil quality. "Stable" soils were characterised by a larger percentage of macroaggregates and a lower percentage of microaggregates, and conversely for the "unstable" soils (Shi et al. 2020). Therefore, the results show that atrazine application in the field affects the stability of soil structure in farmland.

Effects of atrazine application on soil organic carbon and glomalin-related soil protein. With the increase of atrazine application time, the SOC content of aggregates > 5 mm and 2–5 mm decreased significantly, while the SOC content of soil aggregates 0.5–2 mm and < 0.5 mm increased slightly. Crouzet et al. (2019) found that herbicide use impaired soil aggregation. Subtle variations in SOC content can lead to substantial changes in soil aggregate stability. Due to cementing microaggregates and primary particles to form macroaggregates by SOC, an increase in SOC content can result in an increase in macroaggregate fraction and a decrease in microaggregate fraction (Shi et al. 2020). However, the reduction in SOC content was accompanied by continuous soil aggregates degradation (Shi et al. 2020). Therefore, in this study, the SOC content in large aggregates decreased when transforming from large aggregates to small aggregates.

In addition, the T-GRSP and EE-GRSP content in aggregates > 5 mm and 2–5 mm decreased significantly with the increase of atrazine application time, while those content in aggregates 0.5–2 mm and < 0.5 mm increased slightly. Research recently discovered that herbicide use destroys AMF mycelia and spores (Ramos-Zappata et al. 2012, Druille et al. 2015). Additionally, our previous research showed that when atrazine content in farmland soil was higher than 0.28 mg/kg (atrazine content in soils in this study ranged from 102 mg/kg to 167 mg/kg), AMF spore density and species abundance decreased accordingly (Wang et al. 2015) (Figure 6). Moreover, pesticide application seriously threatens AMF diversity (Bedini et al. 2007). GRSP is AMF origin, and AMF status effectively mediated the metabolism of GRSP (Yang et al. 2017). Therefore, when AMF is affected, the GRSP content secreted by AMF decreases accordingly, explaining why GRSP content of large aggregates decreases with increasing atrazine application. This is another possible reason why aggregate stability decreased with increasing years of herbicide use.

The correlation analysis showed that soil aggregate SOC and GRSP contents > 5 mm and 2–5 mm were negatively correlated with the application life of atrazine. Alternatively, soil aggregate SOC and GRSP contents 0.5–2 mm and < 0.5 mm were positively correlated with the application life of atrazine, but the correlation was not significant (Figures 3–5). As the binding agents of aggregates, SOC and GRSP are closely related to the formation of aggregates (Bronick and Lal 2005, Chen et al. 2012). Herbicide use impaired soil aggregation (Crouzet et al. 2019), and the decline in SOC content was accompanied by continuous degradation in soil aggregates (Shi et al. 2020). The stability of soil aggregates is positively correlated with GRSP content (Wright et al. 1998). GRSP is a part of SOC, and the content of GRSP is positively correlated with SOC (Rillig et al. 2003, Singh et al. 2016). GRSP is generated from hyphal and spores of AMF and acts as a glue with AMF hyphae to bind soil particles together. Atrazine can affect AMF spore density and species abundance (Wang et al. 2015). Therefore, during long-term atrazine

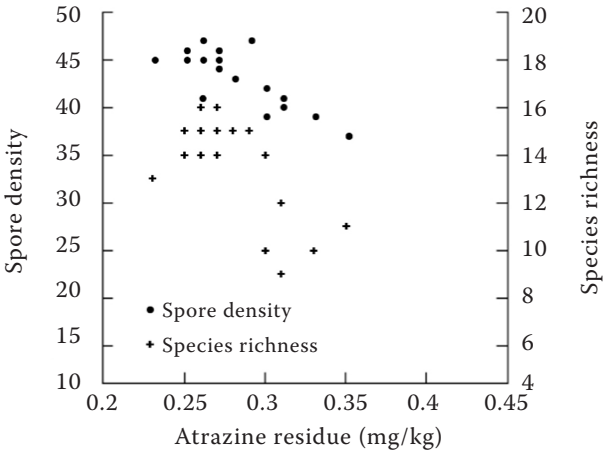

Figure 6. The change of arbuscular mycorrhizal fungi spore density and species richness with different atrazine residues

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application, atrazine will not only reduce the mass fraction of large-size aggregates but will also seriously impact the binding agents of corresponding particle size. Long-term atrazine application also affects aggregate binding agent content and weakens their cementing effect, causing larger-sized aggregates to degrade into small-size aggregates. Therefore, the development of environmentally friendly herbicides instead of atrazine application was recommended for future crop cultivation, which will be beneficial to protect the soil quality.

In conclusion, although atrazine residue did not increase with application year, its concentration was still nearly 100 times higher than the permitted limit, altering the proportion of aggregates with different particle sizes and negatively impacting the contents of SOC, T-GRSP and EE-GRSP of aggregates > 5 mm and 2–5 mm, thus reducing the stability of aggregates and harming farmland soil.

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