Responses of water-stable aggregates, their associated organic carbon and crop yield to the application of biogas slurry in a fluvo-aquic soil of the North China Plain

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Abstract

Crop productivity under intensive agriculture depends on the maintenance of fertility and soil structure. Chemical fertilizers are effective in supplying nutrients for crops but eventually lead to loss of the soil organic carbon that is important in supporting soil structure. Biogas slurry is an alternative to chemical fertilizers that potentially can both provide nutrients and promote organic carbon content. A field experiment was conducted to compare the effects of applying biogas slurry (BS), chemical fertilizer (CF), or a combination of both with 50% of nitrogen derived from each (BSCF) and no fertilization control (CK) to a fluvo-aquic soil of the North China Plain. All fertilization treatments had equal nutrient supply and were continuously applied over four years. Annual yields of wheat and maize were determined. Water-stable aggregates in size classes >5 mm, 2-5 mm, 0.25-2 mm, 0.053-0.25 mm and <0.053 mm in the topsoil of 20 cm were separated by wet sieving; mean weight diameter (*MWD*), geometric mean diameter (*GMD*), percentage of aggregates destruction (*PAD*) and fractal dimension (*D*) were derived. The concentrations of organic carbon associated with the aggregates were measured and the distribution of soil organic carbon (SOC) was quantified. Fertilization treatments enhanced total crop yield by 49.1%-75.0%. Fertilization also increased the mass proportions of water-stable macro-aggregates by 81.6%-164.4%, *MWD* by 100.0%-264.3%, *GMD* by 54.5%-227.3%, while decreasing *PAD* by 15.4%-47.8 and *D* by 1.0-3.8% (all relative to CK). The highest proportions of macro-aggregates, the greatest aggregate stability and the greatest crop yield all resulted from BSCF. All fertilization treatments substantially increased the SOC content of water-stable aggregates, with greater relative contribution in macro-aggregates. Increased SOC in macro-aggregates was mainly attributable to mass proportion changes rather than changes in their SOC concentrations. However, SOC concentration tended to account for a larger proportion of the total changes in micro-aggregates. BSCF also produced the highest total soil organic carbon stocks in aggregates (40.3% and 57.3% greater than CF and CK, respectively). Our work demonstrates that half substitution of chemical fertilizer with biogas slurry is a practical management to enhance soil aggregation and its stability that is associated with a higher crop yield than either treatment individually. It also promotes organic carbon accumulation in soil aggregates, which will support sustainable agricultural development in fluvo-aquic soil.

KEYWORDS: carbon sequestration, chemical fertilizer, nitrogen fertilization, soil aggregate stability, soil organic carbon

1 INTRODUCTION

Soil organic carbon (SOC) plays a crucial role in maintaining and improving soil quality in agro-ecosystems (Lal, 2004; Wang *et al.*, 2018; Kan *et al.*, 2020). Its amount and composition regulate varied physical, chemical and biological processes that underlie soil fertility (Six & Paustian, 2014; Pan *et al.*, 2021). Topsoil also represents a large organic carbon reservoir in terrestrial ecosystems that might help mitigate increasing atmospheric carbon dioxide concentrations and so influence future climate scenarios (Okolo *et al.*, 2020; Pan *et al.*, 2021). Soil aggregates, the small clumped or granular structures formed by soil particles, constitute a fundamental unit of soil structure (Six & Paustian, 2014; Garcia-Franco *et al.*, 2020). Their composition and stability can determine certain processes that are important to soil fertility and the assessment of its quality, particularly erosion, compaction and crusting (Cavalcante *et al.*, 2019; Zhong *et al.*, 2021). A stable aggregate structure may favor seedling emergence, root extension and ultimately crop yield by improving porosity, and thus gas exchange and drainage. It also may confer a significant capacity for organic carbon sequestration (Badagliacca *et al.*, 2020; Fan *et al.*, 2020 Okolo *et al*., 2020). Previous reports have demonstrated close relationships between soil aggregation and organic carbon (Barreto *et al*., 2009; Wang *et al*., 2018; Zhang *et al*., 2019). SOC can serve as both core and cement in the process of soil aggregation, thus promoting stability (Lal *et al.*, 2007; Kamran *et al.*, 2021). It has been estimated that more than 90% of the organic carbon is resident in topsoil aggregates and this carbon pool is sensitive to agricultural managements (Six & Paustian, 2014). Stable aggregates, in turn, can provide strong adsorption sites and spatial isolation between organic carbon and microbes, which could reduce SOC decomposition and promote its accumulation (Garcia-Franco *et al*., 2020). Significant differences in organic carbon sequestration have been found among aggregates of various sizes, and attributed to these protection mechanisms (Fan *et al*., 2020; Garcia-Franco *et al*., 2020; Okolo *et al*., 2020). In general, carbon associated with macro-aggregates is sequestered by physical protection, and has greater absolute concentrations than micro-aggregates (Six *et al*., 2002; Gulde *et al*., 2008; Pan *et al*., 2021). In contrast, the vast majority of SOC is in micro-aggregates and silt-clay particles, which are considered to have higher stability and slower turnover time than macro-aggregates (Hernandez-Soriano *et al*., 2016). Water-stable aggregates, separated by wet sieving, are often used to indicate resistance to water erosion in humid and semi-humid regional agro-ecosystems (Dai *et al*., 2019; Zhang *et al*., 2019). Previous research has focused predominantly on the overall organic carbon changes at bulk and aggregate scales. Studies of the quantities of organic carbon specifically associated with different sizes of water-stable aggregates should improve fundamental understanding of SOC accumulation.

Although influenced by climate and vegetation cover, soil aggregation and its stability have been seen to be strongly regulated by organic and inorganic material inputs (Abiven *et al*., 2009; Garcia-Franco *et al*., 2020). Especially in agricultural production systems, organic matter inputs mainly derive from crop residues, roots and organic fertilizers (Liu *et al.*, 2014). Organic fertilizer application directly increases organic carbon input, but also indirectly facilitates accumulation of residual plant biomass (Cavalcante *et al*., 2019; Tang *et al.*, 2022a). The North China Plain (NCP) is a significant agricultural production region where soil degradation has occurred in recent decades, due to intensive annual crop rotation, high mineral fertilizer applications, and insufficient organic matter input (Du *et al.*, 2018; Kan *et al.*, 2020). The regionally typical fluvo-aquic soil is characterized by poor soil structure and low organic content, which limit its potential for crop yield (Du *et al*., 2018; Kan *et al*., 2020; Zhu *et al.*, 2021). In addition, animal husbandry in this region has been increasing rapidly and biogas engineering has been developed as an economical and environmental solution to achieve safe disposal of the manure generated (Tang *et al*., 2022). Apart from producing large amounts of renewable energy, anaerobic digestion yields enormous volumes of biogas slurry, a by-product, which is regarded as an alternative to chemical fertilizer in providing available nutrients, organic matter and bioactive substances for crop growth and soil fertility (Abubaker *et al*., 2012; Wang *et al.*, 2021; Tang *et al*., 2022a). Considerable effort has been devoted to investigating the effects of biogas slurry combined, with chemical fertilizer, on crop performance and yield, soil nutrients and microbial properties (Zheng *et al*., 2017; Zhang *et al*., 2021; Tang *et al*., 2022b), but little information is currently available on its consequences for soil water-stable aggregate characteristics and the accumulated organic carbon within them.

Against this background, we hypothesized that biogas slurry in combination with chemical fertilizer could promote water-stable aggregation and enhance the associated organic carbon sequestration in the topsoil, while increasing crop yield. Furthermore, such changes of soil organic carbon within water-stable aggregates would depend on aggregate size. Thus, the objectives of the research were：1) to examine the influence biogas slurry application on the composition and stability of water-stable aggregates and crop yield; 2) to investigate the changes in organic carbon within aggregate fractions. We sought to compare the effects of biogas slurry and chemical fertilizer applications, separately and in combination, in a field experiment. This aimed to identify the extent to which biogas slurry offers an alternative to chemical fertilizer, while providing support for the improvement of soil structure and the sequestration of organic carbon in fluvo-aquic soil, and promoting the rational utilization of regional livestock and poultry waste in the NCP.

2 MATERIALS AND METHODS

2.1 Study area

The study was conducted at the pig biogas slurry resource utilization demonstration base in Gubei village (33°94'N, 114°32'E, average altitude 55 m), Fugou County, Henan Province, China, which is located in the central region of the NCP. Terrace deep soils formed by sediment accumulation in this area are conducive to crop cultivation. The area represents a warm-temperate, continental monsoon climate, with an annual temperature of 14.4 °C and frost-free period of 215 days (30-year averages from 1990 to 2020). Annual precipitation is 611 mm (45-year averages from 1975 to 2020), of which more than 70% falls in summer (June to September). The soil is classified as fluvo-aquic according to the soil taxonomy system of China and as Fluventic Ustochrept in the soil taxonomy of the USDA. Basic agrochemical properties prior to the experiment were: pH of 7.32 (ratio of soil to water, 1:2.5 by mass), organic matter of 10.9 g kg^{-1}, total nitrogen content of 1.25g kg^{-1}, available phosphorus content of 26.8 mg kg^{-1}, available potassium content of 242.0 mg kg^{-1}. Coarse sand (>0.2 mm), fine sand (0.02-0.2mm), silt (0.002-0.02 mm) and clay（<0.002 mm) content were 75.2%, 12.8%, 6.0%, and 6.0%, respectively according to the international classification system.

2.2 Experimental design and soil sampling

A long-term field experiment involved biogas slurry application was established in September 2016. The treatment comprised a control, with no biogas slurry or chemical fertilizer application (CK) and three fertilization treatments with equal nitrogen input: chemical fertilizer application only (CF); biogas slurry application only (BS) and biogas slurry combined with chemical fertilizer, such that 50% of nitrogen was derived from each (BSCF). Nutrient inputs in the wheat and maize growing seasons were the same, including N 180 kg ha^{-1}, P_2O_5 90 kg ha^{-1}, and K_2O 90 kg ha^{-1}, which is the local recommendation. Phosphorus and potassium were supplemented as heavy superphosphate ($Ca(H_2PO_4)_2$•$CaHPO_4$) and potassium sulfate (K_2SO_4), respectively, to maintain consistent nutrient amounts input in all fertilization treatments. In each treatment, a plot of 67 m^2 (10 m ×6.67 m) in area was designed as a replicate. These four treatments arranged in a randomized complete block design with five replicates. Biogas slurry was obtained from the No.4 breeding farm of Fugou Muyuan Animal Husbandry. Pig manure, urine and part of the piggery washing water were collected and processed at temperatures of 30-40 °C with a retention time of 7-10 days in a biogas engineering fermentation cylinder. Anaerobic fermentation digestate was separated by solid-liquid filter separators, and the liquid residue, the biogas slurry, was transferred to a storage pond covered with black high-polyester material for secondary fermentation for at least 6 months before farmland application (Figure.1A). The physicochemical properties of the biogas slurry used in the experiment are detailed in Table 1.

The winter wheat cultivars 'Xinong 979' (2016-2018) and 'Zhoumai 18' (after 2018) and the maize variety Yuyu 30 were sown during the experimental period. Residue mulching is a typical field management practice in this region. After crop harvest, residues were chopped to 2-3 cm for maize straw and to 6-7 cm for wheat straw. For the wheat growing season, 70% of the urea, and all the heavy superphosphate and potassium sulfate were applied as basal fertilizers. They were broadcast evenly onto the soil surface before plowing for all fertilization treatments in each year. The maize residues and fertilizer were incorporated into the soil by rotary tillage with an offset disc harrow with depth of 15 cm. Wheat was directly sown using a grain drill at 3-5 cm depth. The remaining 30% of urea was broadcast as top-dressing onto the soil surface in the following March. For the maize growing season, no tillage was used and the wheat straw was left on the soil surface. Chemical fertilizer application including 70% of the urea, and all of the heavy superphosphate and potassium sulfate was concurrent with sowing by a direct drilling disk planter set to 3-6 cm depth. The remaining of urea was a supplementary top-dressing at the elongation stage in mid-July. Biogas slurry was applied by a modified micro-spraying hose connecting to the biogas slurry transmission network (Figure.1B). For wheat, 70% of the biogas slurry was applied before sowing, and the remaining 30% during the overwintering period. For maize, biogas slurry was applied on two occasions before tasseling, according to the weather conditions and degree of soil drought, but the interval between applications was not less than 7 days (Figure.1C). The chemical fertilizer application and control were irrigated with ground water at the same times, using the same type of modified micro-spraying hose, and the same volume as the biogas slurry application. Pumped ground water was supplied so as to maintain the same water input for all the experimental treatments. Conventional crop managements including herbicide and insecticide applications were consistently carried out during the whole experimental period.

Wheat yield was determined by randomly harvesting a quadrat with area of 1 m^2 and maize yield was measured by collecting 20 plants at random, from each plot at harvest time. Sampled wheat grain and maize kernels were dried at 130 °C for 19 hours and 103 °C for 72 hours in a convection oven. Data were scaled proportionally to give wheat and maize yields per hectare. After maize harvest, five randomly selected undisturbed soil blocks (10 cm ×10 cm × 20 cm) of topsoil (0-20 cm) were immediately taken by shovel in each plot and placed into rigid plastic boxes to prevent extrusion during transportation on 1 October 2020. These samples were air-dried in the laboratory and then gently manually fragmented along natural cracks in the clods. Visible stones, plant debris and soil animal corpse were removed with forceps, and soil was passed through a 10 mm sieve. All samples from the same plot were carefully mixed to form a composite sample. At the same time, core samples were collected at the same location in each plot using a custom stainless steel cylinder (200 mm height × 50 mm diameter) to determine bulk density (BD).

2.3 Soil aggregate separation and analysis

BD was measured using cutting ring method (Lu, 1999). Water-stable aggregates were separated by wet sieving method as described by Tang *et al*. (2022a). Firstly, 100 g of air-dry soil was distributed evenly on the top sieve stacked over a series of sieves with mesh sizes of 5, 2, 0.5, 0.25 and 0.1 mm, spaced about 5 cm apart. Mechanical-stable aggregate size fractions were separated and collected by shaking for 5 min at 30 vibrations min^{-1} on a Retsch AS200 Control (Retsch Technology, Düsseldorf, Germany). Dry mass proportions of different fractions were obtained by dividing the total weight. Secondly, 50 g samples, reconstituted according to their dry mass proportion, were placed on the top layer of the sieve set in a TF-100 water-stable aggregate analyzer (Shunlong Instrument, Shaoxing, China) with the soil in the topmost sieve just submerged with deionized water for 5 min. These sieves were programmed to oscillate up and down by 5 cm 30 times min^{-1} for 2 min. Fractions less than 0.1 mm in diameter were determined by a sequence of procedures involving sedimentation, decanting and drying. Soil retained on each sieve was collected and dried to a constant weight at a temperature of 40 °C for 48 hours. The recovery ratio of aggregates after wet-sieving reached 90%-99%, calculated as the total mass of separated water-stable aggregate fractions recovered from the soil sample weight (50g). Finally, the organic carbon within all water-stable aggregate fractions was determined with an external heating method using potassium dichromate (Bao, 2008).

2.4 Calculations and statistical analysis

Mean weight diameter (*MWD*) and geometric mean diameter (*GMD*) were calculated by the following formula (Kemper & Rosenau, 1986):

$$MWD = \sum_{i=1}^{n} w_i * x_i;$$

$$GMD = \exp\left(\sum_{i=1}^{n} w_i Inx_i \Big/ \sum_{i=1}^{n} w_i\right)$$

where w_i is the mass proportion (%) of aggregate fraction of the total sample weight, x_i is the average diameter (mm) of each aggregate fraction, n is the numbers of sieves.

Percentage of aggregates destruction (*PAD*) is the ratio of >0.25 mm aggregates remaining between dry sieving and wet sieving, which is estimated by the following equation:

$$PAD = \left(\frac{MR_{0.25} - WR_{0.25}}{MR_{0.25}}\right) * 100\%;$$

where $MR_{0.25}$ and $WR_{0.25}$ are the mass proportions of > 0.25 mm mechanical-stable and water-stable soil aggregates (%), respectively.

A mass-based model for fractal dimension (*D*) was estimated following Perfect and Blevins (1997):

$$(3\text{-}D)\ln\frac{x_i}{x_{max}} = \ln\frac{M(r<x_i)}{M_t};$$

where *M* is the sum of aggregate masses separated by sieves less than x_i; M_t is the total aggregate mass; x_{max} is the maximum value for the aggregate size (10 mm). Linear regressions were fitted to determine fractal dimension by the least-squares method.

Relative contribution of aggregate-associated organic carbon was obtained as follows (Sun *et al.*, 2005):

$$\text{Relative contribution}_{\text{organic carbon}} = \text{SOC}_{\text{amount-fraction}} / \text{SOC}_{\text{amount-total}}$$

$$\text{SOC}_{\text{amount-fraction}} = \text{SOC}_{\text{concentration-fraction}} * \text{M}_{\text{fraction}}$$

where $\text{SOC}_{\text{amount-total}}$ is the sum of organic carbon amount within measured aggregates, $\text{SOC}_{\text{amount-fraction}}$ is the organic carbon amount in specific soil aggregate fractions (g C kg^{-1} aggregates), $\text{SOC}_{\text{concentration-fraction}}$ is the organic carbon concentration of aggregates (g C kg^{-1} aggregates), and $\text{M}_{\text{fraction}}$ is the mass proportion of aggregates for total measured soil samples (%).

Assuming that changes in OC accumulation relative to the control within any particular treatment, aggregate fraction were caused by changes in OC concentration in the fraction (F1) and by changes in the mass of the fraction (F2), the contributions of these two components were calculated by the method of Qiu *et al* (2012):

$$F_1 = M * \Delta C$$

$$F_2 = \Delta M * C$$

where ΔM is the mass change of a fraction (g), M is the mass of the aggregate fraction in the control (g), C is the treatment OC concentration of the aggregate fraction (g kg^{-1}) and ΔC is the change in OC concentration in this aggregate fraction (g kg^{-1}). The contribution rates of concentration or mass changes were calculated as percentages of F_1 or F_2 of the total changes.

SOC stock reflects changes in carbon accumulation within water-stable aggregates. Because there was no significant difference in soil bulk density among treatments，it was directly calculated using the following depth-based method (Emde *et al.*, 2021):

$$\text{SOC stock (Mg C ha}^{-1}\text{ aggregates)} = \text{SOC}_{\text{concentration}} \times BD \times H \times 0.1$$

where SOC concentration is the weighted organic carbon content of water-stable aggregates (g C kg^{-1} aggregates), *BD* is the bulk density (g cm^{-3}), *H* is the depth of soil layer (cm) and 0.1 is the conversion factor for Mg ha^{-1}.

All data were tested for normality and homogeneity of variance prior to analysis. Crop yield and parameters for soil aggregates and aggregate-associated organic carbon were compared for significant differences by one-way ANOVA followed by the least significant range method (LSD) at $P<0.05$. All statistical analyses were carried out in Microsoft Excel 2013 and SPSS 26 (SPSS Inc., Chicago, USA). All graphs were plotted using Origin 2021(Origin Lab Corp, Northampton, USA).

3 RESULTS

3.1 Crop yield

Annual yields of wheat and maize, as well as the total yields during the whole experimental period, are shown in Table 2. Compared with the control (CK), all fertilization treatments significantly increased the yields of both crops consistently over the experiment. Biogas slurry in combination with chemical fertilizer (BSCF) produced higher yields of wheat than other treatments, except for in the first year, where there was no significant difference between chemical fertilizer (CF) and BSCF. Repeated application of the BSCF treatment progressively improved wheat yield, with the largest yield of 7088 kg ha^{-1} obtained in the fourth year. Similarly, maize increased in annual yield to 12,110 kg ha^{-1} in BSCF. Total crop yield, in comparison with CK, was increased by 49.1% for BS, 63.7% for CF and 75.0% for BSCF.

3.2 Mass proportions of water-stable soil aggregates

Most of the water-stable aggregates were dominated by smaller size fractions of less than 0.1 mm across the treatments, accounting for 39.1%-65.0%. Aggregates in the range of 0.1-0.25 mm and 0.25-2 mm were the next most abundant (Table 3). All fertilization treatments significantly increased the mass proportions of macro-aggregates, by 81.6%-164.4%, relative to CK. Especially, chemical fertilizer (CF) or biogas slurry (BS) application alone resulted in significantly greater proportions of large macro-aggregates, particularly in the >5 mm and 2–5 mm fractions. The BSCF treatment was extremely effective in maintaining the highest mass proportions of every macro-aggregate fraction larger than 0.25 mm, and the lowest proportions of micro-aggregates less than 0.1 mm. In the 0.1-0.25 mm aggregate fraction, the mass proportions resulting from BSCF and CF treatments were similar, near 15.0%, and significantly lower than CK. The BS treatment produced a further significant decrease, down to 12.2%.

3.3 Water-stable soil aggregate stability indices

The various trends in aggregate size were more clearly summarized by the four indices of stability (Fig. 2). The mean weight diameter (*MWD*) was increased by 157% and 100% in the BS and CF treatments, respectively, in comparison with CK. However, BSCF had the highest values of *MWD*, reaching 1.5 mm. The geometric mean diameter (*GMD*) followed the same trend (i.e. BSCF>BS>CF>CK), with the greatest value of 0.36 mm, also obtained in BSCF treatment. However, the percentage aggregates destruction (*PAD*) and the fractal dimension (*D*) showed similar relationships but with an inverse trend, i.e. with the highest value in CK being 76.8% and 2.9, respectively. Differences were all significant among the treatments for values of *MWD*, *GMD* and *PAD*, although there was no significant difference between BS and CF for *D*.

3.4 Distribution and accumulation of organic carbon within aggregate fractions

The concentrations of soil organic carbon (SOC) in different aggregate fractions varied from 5.8 to 16.8 g kg^{-1} aggregate (Table 4). The effects of the fertilization treatments were most marked in the largest macro-aggregate (>5 mm), where the addition of BS, either alone or in combination, increased the concentration of SOC by 44.9%-121.5%, relative to CK. The combined treatment (BSCF) also significantly increased SOC concentration in the 2-5 mm fraction and the 0.1-0.25 mm fraction. In the

<0.1 mm fraction, except for the fact that CF significantly increased the concentration of SOC relative to CK (9.0 g C kg^{-1} aggregate vs 6.3 g C kg^{-1} aggregate), there was no significant difference among CK, BS and BSCF.

Fertilization treatments had notable effects on the relative distribution of SOC among aggregate fractions (Fig. 3). A considerable part of SOC tended to accumulate in the <0.1 mm fraction, accounting for 54.4% in the CK. However, this contribution was remarkably reduced in all the fertilizer treatments, particularly for BSCF. Conversely, biogas slurry application (both in BS and BSCF) greatly increased the SOC relative contribution in the >5 mm and 2-5 mm fractions. Values for BS and BSCF were 6.3- and 7.5-fold greater, respectively, than in CK. There were no obvious contribution trends among the 0.1-0.25 mm and 0.25-2 mm fractions.

Changes in SOC accumulation within water-aggregate fractions (relative to the control) caused by fertilization treatments could be partitioned into those attributable to the SOC concentration within a fraction and those attributable to the mass of that fraction (Table 5). In the larger macro-aggregates (>2 mm), the changes brought about by the fertilization treatments were positive and predominantly due to the mass proportion changes. Effects on smaller macro-aggregates (0.25-2 mm) were also positive but aggregate SOC concentration was of similar importance to mass proportion. Changes among the aggregates <0.25 mm were generally larger and sometimes negative; the SOC concentration component was nearly as important as, or more important than, the mass proportion component. The greatest effects were in the micro-aggregates in the range of 0.1-0.25 mm for the BS treatment, where a negative change in concentration was offset by a positive change in the mass proportion component.

Total soil organic carbon stocks within water-stable aggregates were increased by fertilization treatments (Table 6), although no significant difference was seen between CK and BF (20.6 Mg C ha^{-1}aggregates vs 23.7 Mg C ha^{-1} aggregates). BSCF increased total carbon stock by 40.3% and 57.3%, relative to CF and the control, respectively. However, corresponding changes in bulk density were negligibly small.

4 DISCUSSION

4.1 Crop production and water-stable aggregates

Our crop yield data has confirmed that fertilization increased wheat and maize yield on a fluvo-aquic soil, and that biogas slurry as an alternative to chemical fertilizer could maintain or even increase productivity (Table 2). Similar results were reported by Xu *et al* (2019) showing that appropriate biogas slurry application effectively improved crop yield and soil properties in the rice-rape rotation system. Furthermore, repeated biogas slurry combined with chemical fertilizer application has proved of progressive benefit for crop production, with a gradual rise in yields for both crops evident over time under the current nutrient-demanding annual wheat-maize rotation system. This is consistent with a report by Hernández *et al* (2013) that annual application of pig slurry (PS) stimulated barley yield in low-fertility Mediterranean soils; residual effects were evident for the second and third experimental years, showing the value of PS as resource capable of supplying organic matter and plant nutrients, ultimately to improve crop growth. Therefore, repeated biogas slurry application, particularly in combination with chemical fertilizer, appears to have great potential to boost yield and advance agricultural sustainability in the NCP region.

Biogas slurry application significantly promoted the formation of macro-aggregates from small soil particles, and increased mass proportions of water-stable aggregates more than 0.25 mm in size (Table 3). In the NCP, clay minerals would be expected to be the dominant primary particles of fluvo-aquic soil, with soil organic matter serving as a key cementing agent (Kamran *et al*., 2021). Compared with conventional solid organic material (animal manure, compost and straw), liquid biogas slurry can be more easily distributed and spread on the soil surface and would directly provide organic core and cement for the process of soil particle aggregation (Yu *et al*., 2012; Meng *et al*., 2014; Bosch-Serra *et al*., 2017; Zhu *et al*., 2021). Furthermore, the macromolecules with high specific surface areas and the functional groups of hydrophilic colloids contained in biogas slurry were likely to have been conducive to soil aggregation (Du *et al*., 2018). Compared with the control, chemical fertilizer application also had advantageous effects on macro-aggregate formation, by producing more biomass residues, but its efficacy was less than biogas slurry application alone and significantly less than the combined application. However, it was not entirely consistent with previous research, which had suggested that long-term application of chemical fertilizer did not promote the formation of soil aggregates (Li *et al.*, 2019). Such a disparity could have arisen from differences in soil texture, environmental and nutrient conditions, crop species or agricultural managements (Barreto *et al*., 2009; Garcia-Franco *et al*., 2020; Poblete-Grant *et al.*, 2020). Overall, incorporation of biogas slurry into the fertilization regime appears to be particularly effective in promoting soil aggregation and improving soil structure in a carbon-depleted fluvo-aquic soil.

There was also evidence that aggregate stability was increased by fertilization treatments. In our experiment, the highest values of *MWD* and *GMD* and the lowest values of *PAD* and *D* were found after the application of biogas slurry combined with chemical fertilizer; the next highest and lowest measures, respectively, resulted from biogas slurry application alone (Fig. 2). It is reasonable to conclude that biogas slurry was beneficial to aggregate stability. Two possible explanations are likely responsible for this observation. One is that organic matter can promote the inter-particle cohesion within aggregates to inhibit their breakdown (Noellemeyer *et al.*, 2008; Abiven *et al*., 2009). The other is the hydrophobicity of aggregates is enhanced by organic matter input, thus resisting their breakdown by slaking (Abiven *et al*., 2009). Both scenarios allow maintenance of large water-stable aggregates and improve aggregate stability. Other studies have suggested that the polysaccharides present in biogas slurry might bind through hydrogen bonds with oxygen atoms on the surface of clay mineral crystals to enhance their stability (Clarholm *et al.*, 2015). We found apparent facilitation effects from combining biogas slurry with chemical fertilizer. The most likely reason might involve large quantities of exchangeable base cations remaining in the biogas slurry, which might potentially begin to inhibit soil aggregation after excessive biogas slurry applications (Meng *et al*., 2014; Bosch-Serra *et al*., 2017). Soluble salts would have been ingested with animal feed, and then excreted and retained though the anaerobic digestion process (Cavalcante *et al*., 2019). Especially, the monovalent Na^+ ions could reduce mutual attraction between soil colloids, causing dispersion and slaking of aggregates that were not water-stable (Bosch-Serra *et al*., 2017). The lower quantity of biogas slurry application in combination treatment might therefore have been enough to improve soil structure without invoking the potential salinization problems associated with biogas slurry application alone. An electrical conductivity in the soil greater than 2 ds cm^{-1} could result in serious consequences for crop growth (Deinlein *et al*., 2014). Although half substitution of chemical fertilizer with biogas slurry appears to be a promising measure to increase aggregate stability, the desalination of biogas slurry may be necessary before its long-term use, and monitoring of soil cations should be considered (Wang *et al*., 2021; Tang *et al*., 2022a).

4.2 Organic carbon changes associated with water-stable aggregates

In our experiment, small macro-aggregates (0.25-2 mm) generally possessed the highest SOC concentrations (Table 4), which was consistent with the findings of similar research in the NCP and Loess Plateau of China (Wang *et al*., 2018; Wang *et al*., 2022). In addition, all fertilization treatments produced augmented organic carbon concentrations associated with most aggregate fractions, and biogas slurry combined with chemical fertilizer proved to be particularly effective. Similar increases in the SOC associated with water-stable aggregates have been reported after application of bio-fertilization to this same soil type of the NCP (Zhu *et al*., 2021), and after manure application in the solonetzic soils of Songnen plain, northeastern China (Meng *et al*., 2014). Although much of the increased SOC was due to the greater mass fractions (Table 5), we found that SOC concentrations were also enriched in small macro-aggregates and micro-aggregates within 0.1-0.25 mm (Table 4).

Mass variations and organic carbon concentrations within the aggregate fractions were both responsible for the distribution of organic carbon within aggregates of different sizes. Micro-aggregates less than 0.25 mm accumulated the most organic carbon in the absence of fertilization inputs, reaching 73.5%. For macro-aggregates in the fertilization treatments, considerable increases in mass proportions (89.2%-98.8%) rather than increases in SOC concentration (1.2%-13.3%) were responsible for the changes in SOC accumulation (Table 5). Previous field experiments have suggested that organic fertilizer application tended to enhance organic carbon deposition in larger aggregates by promoting soil aggregation itself and increasing their associated carbon concentrations (Yu *et al*., 2012; Li *et al*., 2019). Biogas slurry or chemical fertilizer application individually reduced the relative contribution of micro-aggregates, whereas their combined effect was synergistic in reducing it further (Fig. 3). The combined treatment also produced the highest total organic carbon accumulation within water-stable aggregates (Table 6). Paradoxically biogas slurry application reduced total soil organic carbon accumulation in comparison with chemical fertilizer. This might be on account of limiting organic carbon content and high nitrogen content in biogas slurry, which could have promoted microbial metabolism and accelerated organic carbon decomposition and mineralization after its application to the topsoil (Xu *et al.*, 2019; Poblete-Grant *et al*., 2020). Chemical fertilization can also provide additional carbon to soil by increasing crop residues, which could offset mineralization of native SOC (Fan *et al*., 2020). During our field experiment, all of the residual crushed cornstalk and wheat straw in each treatment was incorporated into the soil, and so the increase of soil organic carbon after fertilization would have come from a combination of the organic amendment itself and crop residues (Fig 1C). Plentiful accessible nutrients and labile carbon in biogas slurry would serve as a resource and an energy source for soil microorganisms, which in turn influence decomposition processes (Bosch-Serra *et al*., 2017). Humus derived from crop residues is regarded as binding agent for clay particles that promotes cohesion of soil particles to form micro-aggregates (Zhang *et al*., 2019). The polysaccharides synthesized by microorganisms also promote the transformation of ready-formed micro-aggregates into larger sized aggregates. Additionally, root exudates and fungal hyphae derived from soil microorganisms can bind fine soil particles into larger aggregates (Pan *et al*., 2021). Future studies are needed to investigate the stability and origin of the organic carbon fractions associated in different sized aggregates in order to determine their turnover paths and the persistence of enhanced organic carbon in soil.

In the North China Plain (NCP), one of the most intensive crop-producing areas of China, inappropriate cultivation practices and fertilizer management are causing deterioration of soil structure and depletion of organic carbon, with implications for national food security and regional carbon sequestration (Kan *et al*., 2020; Zhong *et al*., 2021). Biogas slurry as an alternative to chemical fertilizer, clearly has considerable potential to address soil degradation problems and increase crop yield (Poblete-Grant *et al*., 2020; Wang *et al*., 2021). In particular, half substitution of chemical fertilizer with biogas slurry not only resulted in greater representation and stability of macro-aggregates, but also enhanced their associated organic carbon concentrations, both of which facilitated carbon accumulation in macro-aggregate fractions (Table 6). However, the easily decomposable materials in biogas slurry may have a transient effect on aggregate stability, so continuous biogas slurry application might need to be adopted as best agricultural practice (Abiven *et al.*, 2009). It should be acknowledged that there are several potential drawbacks from biogas slurry application, such as soil salinization, ammonia volatilization and groundwater contamination. Given these issues, local optimal practices should be developed specifically to minimize adverse environmental effects and maximize carbon sequestration, while seeking to ensure food supply security.

5 CONCLUSIONS

Fertilizer treatments had substantial effects on soil water-stable aggregate formation and their associated organic carbon, as well as increasing crop yield in an intensively cultivated fluvo-aquic soil. Increased macro-aggregate mass proportion and stability parameters were obtained from treatment with either biogas slurry or chemical fertilizer, but the greatest benefits were derived from a combination of both substances over four years in a field experiment. These treatments also increased organic carbon concentration, particularly in macro-aggregate fractions. However, changes of SOC within different water-stable aggregate fractions depended on the relative contribution of mass proportion and its SOC concentrations. Biogas slurry combined with chemical fertilizer achieved the greatest crop yield and produced the highest overall soil organic carbon stocks. These findings indicate that partial substitution of biogas slurry for chemical fertilizer is a promising management strategy for soil structure, crop yield improvement and organic carbon sequestration in North China Plain.

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DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table 1 Basic physicochemical properties of the experimental biogas slurry

Characteristics	
pH	7.4±0.3
Electrical conductivity (μs cm^{-1})	7830.0±550.0
Total salt content (g L-1)	2.5±0.2
Total organic carbon (g L^{-1})	4.3±0.4
Total nitrogen (g L^{-1})	1.5±0.2
Ammonia-N (g L^{-1})	1.1±0.1
Total phosphorus (mg L^{-1})	130.0±20.0
Total potassium (mg L^{-1})	650.0±90.0

Table 2 Annual wheat yield, maize yield and the total yield (kg ha^{-1}) within experimental period in response to biogas slurry and chemical fertilizer treatments

Crop Type	Year	Treatments			
		CK	BS	CF	BSCF
Wheat	2016~2017	4346±46c	5537±78b	6458±51a	6585±50a
	2017~2018	4150±43d	5659±44c	6368±60b	6790±81a
	2018~2019	3863±65d	5690±70c	6250±66b	6939±47a
	2019~2020	3792±99d	5709±61c	6028±62b	7088±61a
Maize	2016~2017	7138±79c	10545±213b	10987±23a	11356±98a
	2017~2018	6338±66d	10242±143c	11043±230b	11773±93a
	2018~2019	6582±57d	10040±67c	11216±224b	11975±63a
	2019~2020	6429±61d	10158±85c	11457±98b	12110±41a
Total	2016~2020	42639±129d	63580±335c	69806±296b	74615±347a

CK: control; BS: biogas slurry addition; CF: chemical fertilizer addition and BSCF: biogas slurry and chemical fertilizer addition. Values sharing same lowercase letters in a row with same year were not significantly different at $P< 0.05$.

Table 3 Mass proportion of water-stable aggregate fractions of fluvo-aquic soil in response to biogas slurry and chemical fertilizer treatments

Aggregate	**Treatments**			
Size Range (mm)	**CK（%）**	**BS（%）**	**CF（%）**	**BSCF（%）**
>5	1.6±0.1c	6.5±0.8b	5.4±0.8b	8.7±0.4a
2-5	2.4±0.3d	9.7±0.2b	5.1±0.5c	15.6±0.7a
0.25-2	13.4±0.7c	15.4±0.7b	16.8±1.2b	21.7±0.7a
0.1-0.25	17.6±0.5a	12.2±0.7c	15.4±0.9b	15.0±0.7b
<0.1	65.0±1.0a	56.2±1.7b	57.4±2.2b	39.1±1.0c
$WR_{0.25}$	17.4±0.9a	31.6±1.2b	27.2±1.8b	46.0±0.8c

$WR_{0.25}$ represents the mass proportions of water-stable aggregates >0.25mm.

Values sharing same lowercase letters in a row were not significantly different at $P< 0.05$.

Table 4 Concentration of associated organic carbon (g C kg^{-1} aggregates) in each water-stable aggregate fraction of fluvo-aquic soil in response to biogas slurry and chemical fertilizer treatments

Aggregate Size Range (mm)	**Treatment**			
	CK	**BS**	**CF**	**BSCF**
>5	6.5±0.4c	11.8±0.6b	6.7±0.5c	14.4±1.1a
2-5	10.2±1.3b	10.8±0.9b	12.3±0.3b	16.2±1.0a
0.25-2	12.5±1.3b	16.1±0.4a	16.8±0.4a	15.5±0.2a
0.1-0.25	8.1±0.2d	10.2±0.9c	14.0±0.7b	16.5±0.7a
<0.1	6.3±0.4bc	5.8±0.6c	9.0±0.3a	7.1±0.6b

Values sharing same lowercase letters in a row were not significantly different at $P < 0.05$.

Table 5 Percentage changes in SOC accumulation, relative to corresponding control value, in each water-stable aggregate fractions of fluvo-aquic soil in response to biogas slurry and chemical fertilizer treatments

	Treatment	>5 mm	2-5 mm	0.25-2 mm	0.1-0.25 mm	<0.1 mm
		%	%	%	%	%
F1	BS	12.5	1.7	60.2	-194.6	41.4
	CF	1.2	13.3	50.6	143.7	164.5
	BSCF	10.8	6.3	23.9	141.6	-37.9
F2	BS	87.5	98.3	39.8	294.6	58.7
	CF	98.8	86.7	49.4	-43.7	-64.5
	BSCF	89.2	93.7	76.1	-41.6	137.9

F1 represents component attributable to change in SOC concentration; F2 reflects component attributable to change in mass fraction.

Table 6 Soil bulk density and organic carbon accumulation within water-stable aggregates of fluvo-aquic soil in response to biogas slurry and chemical fertilizer treatments

Treatments	**Bulk density**	**SOC accumulation in aggregates**
	g cm^{-3}	Mg ha^{-1}
CK	1.4±0.1a	20.6±1.1c
BS	1.4±0.1a	23.7±1.0c
CF	1.3±0.1a	28.9±0.8b
BSCF	1.3±0.1a	32.4±1.3a

Values sharing lowercase letters in a column are not significantly different at $P < 0.05$.

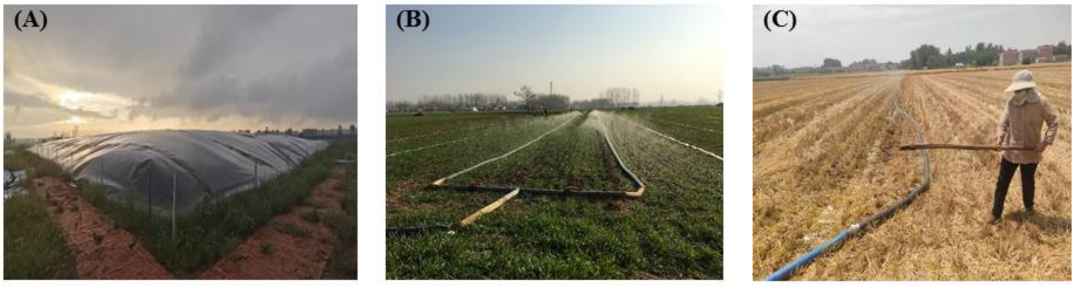

Figure 1 A container covered by black high-polyester material housing the secondary fermentation process (A) ,biogas slurry application using a modified micro-spraying hose (B) and residue mulching and biogas slurry application after crop harvest (C)

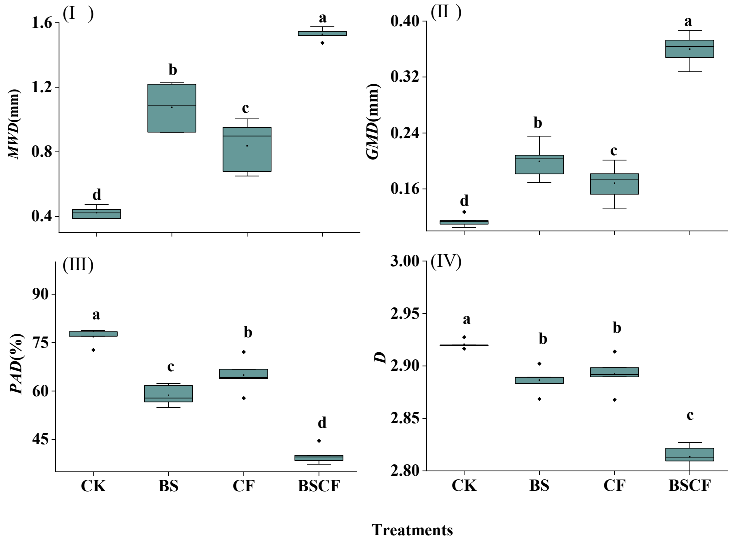

Figure 2 Box plots of water-stable aggregate stability indexes of fluvo-aquic soil in response to biogas slurry biogas slurry and chemical fertilizer managements: (Ⅰ) represents mean weight diameter (*MWD*); (Ⅱ) reflects geometric mean diameter (*GMD*); (Ⅲ) represents percentage aggregates destruction (*PAD*); (Ⅳ) reflects fractal dimension (*D*). Treatments include control (CK); Biogas slurry addition (BS); Chemical fertilizer addition (CF); Biogas slurry and chemical fertilizer addition (BSCF). Boxes sharing same lowercase letters were not significantly different at $P < 0.05$.

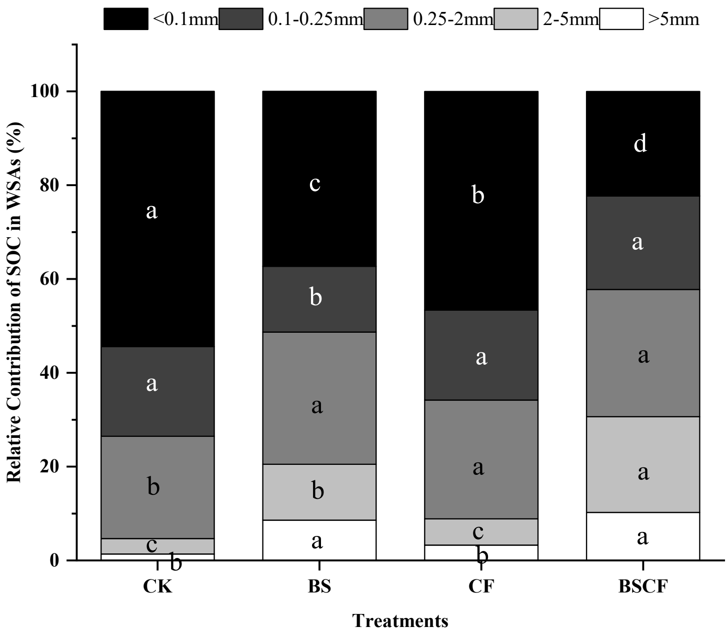

Figure 3 Relative contribution rates of SOC within different water-stable aggregate fractions of fluvo-aquic soil in response to biogas slurry and chemical fertilizer managements.

Bars sharing the same lowercase letters in the same aggregate fractions are not significantly different at $P < 0.05$.