

1 **Effects of biogas slurry on crop yield, physicochemical properties and**  
2 **aggregation characteristics of lime concretion soil in wheat-maize rotation in the**  
3 **North China Plain**

4

5

6 **Jiao Tang<sup>1,2,3,4</sup>, Anthony J. Davy<sup>5</sup>, Wei Wang<sup>3</sup>, Xihuan Zhang<sup>3</sup>, Dafu**

7 **Wu<sup>3,4</sup> Lin Hu<sup>3</sup>, Jinzhong Yin<sup>3</sup>**

8

9 <sup>1</sup>Farmland Irrigation Research Institute, Chinese Academy of Agricultural Sciences, Xinxiang  
10 453000, China

11 <sup>2</sup>Key Laboratory of High-efficient and Safe Utilization of Agriculture Water Resources, Chinese  
12 Academy of Agricultural Sciences, Xinxiang 453000, China

13 <sup>3</sup>School of Resource and Environment, Henan Institute of Science and Technology, Xinxiang  
14 453003, China

15 <sup>4</sup>Post-doctoral research and development Base, Henan Agricultural University, Zhengzhou,  
16 450002, China

17 <sup>5</sup>School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich  
18 NR47TJ, UK

19 **Corresponding author**

20 Dafu Wu

21 E-mail address: wudafu1965@126.com

22

23 ORCID: Jiao Tang <https://orcid.org/0000-0002-9673-3713>

24 Anthony J. Davy <https://orcid.org/0000-0002-7658-7106>

25

26

27 **Abstract**

28 **Purpose:** Biogas slurry is a potential sustainable substitute for chemical fertilizers and  
29 a soil amendment to restore soil organic matter depletion and structural deterioration.  
30 The effects of substituting biogas slurry for chemical fertilizer on a lime concretion  
31 soil in the North China Plain were investigated.

32 **Methods:** A field experiment examined the consequences of applying different  
33 proportions (0, 25, 50, 75 and 100%) of biogas slurry, while maintaining the same  
34 total nitrogen supply, over a period of 5 years. We determined effects on crop yield  
35 (winter wheat/summer maize rotation), soil physiochemical properties and  
36 aggregation characteristics (using dry- and wet-sieving) in the last experimental year.

37 **Results:** All fertilizer treatments increased crop yield relative to a control. 50%  
38 substitution by biogas slurry gave the highest yield of wheat and maize. Increasing  
39 substitution progressively increased soil pH, water holding capacity, organic matter  
40 content, total nitrogen content, available phosphorus and potassium contents and C/N  
41 ratio; conversely, bulk density declined. Soil aggregate size distribution and stability  
42 were improved to varying degrees by biogas slurry substitution, in comparison with  
43 chemical fertilizer application alone and unamended control; optimal values for  
44 macro-aggregate mass proportion, fractal dimension and percentage of aggregate  
45 destruction were achieved with 50% substitution, coinciding with the highest crop  
46 yield.

47 **Conclusions:** Lime concretion black soil was readily amenable to improvement by  
48 biogas slurry application, while maintaining optimal crop yields. Its use should be a  
49 priority for environmentally coordinated crop production and animal husbandry in this  
50 region.

51

52 **Keywords:** crop yield, physiochemical properties, aggregation stability, biogas  
53 slurry, lime concretion black soil, North China Plain

54

55

56

## 57 **1 Introduction**

58 Long-term, continuous application of chemical fertilizers, as part of agricultural  
59 management for intensive cropping, can intensify land degradation. The resulting  
60 problems, including depletion of soil organic matter, deteriorating soil structure,  
61 nutrient imbalances and loss of soil biodiversity, can hinder the development of  
62 sustainable of agricultural ecosystems (Chen et al. 2019; Bansal et al. 2020; Fan et al.  
63 2020; Kamran et al. 2021). Such degradation is the case in the North China Plain  
64 (NCP), the major crop production area of China, which is mainly cultivated using an  
65 intensive annual crop rotation of wheat and maize (Wang et al. 2019; Zhu et al. 2021).

66  
67 The deep soils, gently sloping terrain and equable climate of the NCP are conducive  
68 to large-scale agricultural production and great significance is attached to improving  
69 the soil structure and chemical nutrient retention (Chen et al. 2019). There are nearly  
70 3.7 million ha of lime concretion black soil, which supports low to medium farm  
71 yields (Chen et al. 2019; Kan et al. 2020). This clay-rich, heavy soil is characterized  
72 by a poor structure, swelling-shrinkage with wetting/drying cycles and difficult  
73 workability (Wei et al. 2018). Low organic matter content and transient soil fertility  
74 may severely limit crop yields and the development of sustainable agriculture (Zheng  
75 et al. 2017). Chemical fertilizers have become the ubiquitous choice of growers, who  
76 lack sources of organic fertilizer and face constraints on labor costs (Dai et al. 2019).  
77 Therefore, it is desirable to seek a sustainable organic substitute for chemical  
78 fertilizer.

79  
80 In China, more than 400 million tons of nitrogen-rich manure are produced annually  
81 by livestock and poultry husbandry (Yang et al. 2018). In the absence of appropriate  
82 treatments to prevent the discharge of raw manure, disposal causes environmental  
83 problems, particularly from soil pollution and water eutrophication (Cavalcante et al.  
84 2019; Rahaman et al. 2021; Wang et al. 2021). Biogas engineering, using an anaerobic  
85 digestion process, provides both a suitable treatment and a valuable energy resource,  
86 and has been widely adopted around the world (Badagliacca et al. 2020). The residual  
87 liquid fraction from anaerobic digestion (biogas slurry) is rich in organic matter and  
88 plant nutrients, and represents a valuable resource for arable agriculture (Wang et al.  
89 2021). Apart from addressing environmental problems, such recycling alleviates an  
90 increasing disconnection between large-scale animal husbandry and arable production  
91 (Du et al. 2018; Badagliacca et al. 2020; Chen et al. 2020). Although benefits of using  
92 biogas slurry have been widely reported, few long-term quantitative field studies have  
93 investigated the effects of biogas slurry on crop yield and soil structure, particularly in  
94 lime concretion black soil (Zheng et al. 2016; Bosch-Serra et al. 2017; Zheng et al.

95 2017).

96

97 Soil structure depends on the aggregation of soil particles into larger units, with pore  
98 spaces between them that allow the passage and storage of water and air (Bosch-Serra  
99 et al. 2017). These aggregates constitute the basic unit of soil structure and regulate  
100 physicochemical properties and biological processes, thereby affecting soil slaking  
101 and erosion, aeration and infiltration, and nutrient release and accumulation, as well  
102 as biodiversity (Kemper and Rosenau 1986; Dai et al. 2019; Grant et al. 2020). Thus,  
103 aggregate formation and stability play a vital role in soil fertility and rehabilitation in  
104 agricultural ecosystem (Six et al. 2004; Abbott and Murphy 2007; Diacono and  
105 Montemurro 2010; Zhu et al. 2021). The addition of organic materials is an  
106 economically viable and effective way to promote aggregate formation and they are  
107 widely applied in traditional agricultural production (Badagliacca et al. 2020;  
108 Garcia-Franco et al. 2021; Kamran et al. 2021). However, previous work has focused  
109 on the effects of soil ameliorations with solid organic amendments such as livestock  
110 manure, compost or green manure, rather than liquid organic amendments  
111 (Bosch-Serra et al. 2017; Chen et al. 2019; Dai et al. 2019).

112

113 The aims of the research described here were to investigate the effects of the  
114 application of biogas slurry, as a replacement for chemical fertilizer, on the crop yield,  
115 soil physicochemical properties and aggregate characteristics of lime concretion black  
116 soil. Different combinations of biogas slurry and chemical fertilizer were applied in a  
117 field experiment over five consecutive years. We hypothesized that: (1) fertilizer  
118 substitution with biogas slurry could maintain or even increase the crop yield in this  
119 type of soil; (2) fertilizer substitution with biogas slurry would modify soil  
120 physicochemical properties and soil aggregation, improving its stability; (3) crop  
121 yield increases would be associated with improved nutrient and water retention and  
122 aggregate characteristics. An optimal proportion of biogas slurry substitution was  
123 sought to provide a theoretical and practical basis for coordinated arable farming,  
124 animal husbandry and sustainable land use in this region.

125

## 126 **2 Materials and methods**

### 127 **2.1 Study site**

128 A field experiment was established in the village of Shangji (Zhangming Town,  
129 Shangshui County) in the Henan province of China (33°63'N, 114°28'E, 33.72 m  
130 a.s.l). It is located in the southern part of the North China Plain in an area with a long  
131 cultivation history. The region experiences a warm-temperate continental climate,  
132 with a mean annual temperature of 14.5 °C and mean annual precipitation of 785 mm

133 (averages from 1975 to 2019). Most of the rainfall (>70%) is concentrated between  
134 June and September. Average annual sunshine duration is 2095 h, and the average  
135 annual frost-free period lasts for 223 days.

136

137 The terrain is relatively flat, favoring agricultural production. Summer maize and  
138 winter wheat have been cultivated in long-term rotation. Maize is generally sown in  
139 early June and harvested in late September, whereas wheat is seeded in the middle of  
140 October and reaped in the following June. The soil is classified as a Lime Concretion  
141 Black Soil according to the soil taxonomy system of China and as a Vertisol in the  
142 Soil Taxonomy of the USDA. Increasing use of chemical fertilizers since privatization  
143 in 1990 had led to soil degradation and surface crusting. The topsoil (surface 15 cm)  
144 contained 17.8 g kg<sup>-1</sup> organic matter, 0.9 g kg<sup>-1</sup> total Kjeldahl nitrogen, 24.7 mg kg<sup>-1</sup>  
145 available phosphorus and 226.0 mg kg<sup>-1</sup> available potassium, with a pH of 5.8, before  
146 the field experiment.

147

148 Near to the experimental area, a medium-sized fattening pig farm was established in  
149 March 2015, with an annual production capacity of about 3.0×10<sup>5</sup> pigs. Appropriate  
150 biogas plant and storage facilities were installed at the start of production. The main  
151 raw materials for the biogas plant reactor were pig excreta, as well as pigsty flushing  
152 water. Microbial anaerobic digestion was carried out at temperature of 30-40 degrees  
153 centigrade with a retention time of 7-10 days according to season. After passing  
154 through solid-liquid filter separators, biogas slurry from digestate was transferred to a  
155 storage pool covered with black high-polyester film. Standardized production  
156 schedules and processing guaranteed relative consistent component of slurry over the  
157 experimental period. The main properties of biogas slurry are: dry matter is 1.5-2.4%,  
158 pH is 7.6-7.7 with total nitrogen contents (TN), ammonia-N, total phosphorus (TP)  
159 and total potassium (AK) contents of 1150.0-1250.0 mg L<sup>-1</sup>, 810.0-880.0 mg L<sup>-1</sup>,  
160 275.1-320.5 mg L<sup>-1</sup>, 350.0-610.0 mg L<sup>-1</sup>, respectively.

161

## 162 **2.2 Experimental design**

163 A series of six biogas slurry application treatments with five replicates were randomly  
164 established on 5 June 2015. These fertilization treatments comprised different  
165 substitution proportions of biogas slurry, while maintaining the same total nitrogen  
166 supply, including: complete chemical fertilizer with no biogas slurry (CF); 25%  
167 chemical fertilizer substituted by biogas slurry (BS25); 50% chemical fertilizer  
168 substituted by biogas slurry (BS50); 75% chemical fertilizer substituted by biogas  
169 slurry (BS75) and 100% biogas slurry applied with no chemical fertilizer (BS100,). In  
170 addition, an unamended control was designed without biogas slurry or chemical  
171 fertilizer application. Each replicate plot had an area of 134 m<sup>2</sup> (20 m long × 6.7 m

172 wide).

173

174 Zhoumai 18 and Xuke 328 were the wheat and maize varieties chosen. The seed  
175 sowing rates were 225 kg ha<sup>-1</sup> and 75000 plants ha<sup>-1</sup>, respectively according to local  
176 recommendation. Crop seeds were sown in rows oriented in an east-west direction at a  
177 depth of 40 mm using a mechanical drill. The CF treatment was designed to provide  
178 225 kg ha<sup>-1</sup> of nitrogen as urea, with 90 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 90 kg ha<sup>-1</sup> K<sub>2</sub>O applied using  
179 superphosphate and potassium sulfate, respectively, for each of the two crops. Seventy  
180 percent of the urea was applied at planting (for maize in early June and for wheat in  
181 early October), and the remainder as top-dressing applied at the elongation stage (for  
182 maize in mid-July and for wheat in following March, respectively). Both  
183 superphosphate and potassium sulfate were applied concurrently with wheat and  
184 maize seed planting. For each treatment, all the amounts of total nitrogen, phosphorus  
185 and potassium provided by biogas slurry and chemical fertilizer are shown in Table 1.

186

187 Residue mulching was carried out before biogas slurry and chemical fertilizer  
188 application. The remaining maize stalks were incorporated by rotary tillage with an  
189 offset disc harrow with depth of 15 cm after maize harvesting at the beginning of  
190 October. All wheat straw was smashed with a straw crusher and spread on the soil  
191 surface. No tillage was applied before maize seed planting at the beginning of June  
192 (Pu et al. 2019). Because of no tillage, corn seeds were planted in rows exactly  
193 between the previously harvested wheat rows. A modified micro-spraying hose called  
194 "Small White Dragon" was connected to biogas slurry transmission network and used  
195 to apply biogas slurry. Biogas slurry was thus directly applied to the soil surface  
196 quantitatively, using an electromagnetic flowmeter after crop harvest. Two hoses  
197 connected to the same biogas slurry outlet were arranged in parallel to cover an area  
198 of 66 m<sup>2</sup> (20 m long and 3.3 m wide) with the help of a booster pump. Sprayed biogas  
199 slurry was infiltrated into soil via gravity. In order to maintain consistent water supply  
200 for all of treatments, pumped ground water supplied at the same time was adjusted to  
201 complement the volume of water in the biogas slurry, using the same modified  
202 micro-spraying equipment. Other field management measures including herbicides  
203 and insecticides application were carried out according to local agricultural practices.

204

### 205 **2.3 Sampling and analysis**

206 For crop yield, wheat was harvested and measured by selecting a 1 m<sup>2</sup> area at maturity  
207 at the beginning of June, 2020. Maize yield was measured by random collecting 20  
208 corn plants in each of the replicate plots before soil sampling. Finally, the yield per  
209 hectare is calculated based on the grain moisture content of 13%. Following 5 years of  
210 consecutive wheat-maize rotation, the main cropping system in this area, soil samples

211 were collected after the maize harvest in early October 2020. From each plot, five  
212 randomly selected soil samples were taken from the upper 15 cm, using a cylindrical  
213 corer (50 mm height × 50 mm diameter) and combined. These samples were air-dried,  
214 mixed and passed through a 2-mm sieve to remove debris and litter for determining  
215 soil chemical properties. Additional, undisturbed, bulk soil samples (150 mm × 80  
216 mm × 50 mm) from a similar depth were obtained by spade at the same sample points.  
217 Visible roots and crop residues were removed before breaking up these soil clods  
218 along their natural cracks into pieces <10 mm in diameter (Kan et al. 2020). Then  
219 these samples were air-dried in the shade for determining aggregation characteristics.

220

221 Soil bulk density (BD) and soil water-holding capacity (WHC) were obtained using  
222 the cutting ring method for the undisturbed soil from the upper 15 cm in the field (Lu  
223 1999). Soil pH was measured in a soil-water aqueous extract (1:2.5 by mass) after 30  
224 min shaking at low speed (Orion Star 310p, Thermo, USA). Soil organic matter  
225 content (SOM) was determined by the potassium dichromate oxidation method and  
226 conversion coefficient (1.724) (Bao, 2008). The total N was estimated by titration of  
227 distillations after Kjeldahl digestion. Soil available phosphorus (AP) and potassium  
228 (AK) were measured using the Olsen and Dean method and flame atomic absorption  
229 spectrophotometry, respectively (Bao 2008).

230

231 Soil aggregate size fractions were estimated using the dry and wet sieving techniques,  
232 to obtain mechanical-stable and water-stable aggregates, respectively (Kemper and  
233 Rosenau 1986). 200 g air-dried bulk soil were distributed evenly on the top of a 5 mm  
234 sieve stacked over a nest of 30-cm diameter sieves with successive mesh sizes of 2, 1,  
235 0.5, 0.25 and 0.053 mm, and spaced about 5 cm apart. Different aggregate size  
236 fractions were weighed immediately after sieving for 2 min at one oscillation per  
237 second. For wet sieving, 100 g air-dry soil from each dry sieving fraction was placed  
238 on the top of the same set sieves with the soil in the topmost sieve just submerged  
239 with deionized water for 5 min. The sieves were programmed move up and down by 3  
240 cm at one oscillation per second for 2 min. Soil retained on each sieve was collected  
241 and weighed after drying at 40 °C for 48 h. Size fractions smaller than 0.053 mm in  
242 diameter were determined by a sequence of procedures including sedimentation,  
243 decanting and drying.

244

#### 245 **2.4 Calculation of aggregation characteristic parameters**

246 Mean weight diameter (MWD) and geometric mean diameter (GMD) were calculated  
247 based on the percentage weight of soil in each range to the total soil sample, which  
248 could be used as an index reflecting the aggregate size distribution (Kemper and  
249 Rosenau 1986). MWD and GMD were estimated:

250

$$251 \quad \text{MWD} = \sum_{i=1}^n w_i * x_i;$$

252

$$253 \quad \text{GMD} = \exp\left(\frac{\sum_{i=1}^n w_i \ln x_i}{\sum_{i=1}^n w_i}\right)$$

254

255 where  $w_i$  is the mass proportion of corresponding size fraction to the total dry sample  
256 weight,  $x_i$  is the mean diameter of any adjacent aggregates particle size range  
257 separated by sieving.

258

259 Percentage of aggregates destruction (PAD) was also determined as a structure  
260 stability index after wet sieving estimated (Zhang and Horn 2001):

261

$$262 \quad \text{PAD} = \left(\frac{\text{MR}_{0.25} - \text{WR}_{0.25}}{\text{MR}_{0.25}}\right) * 100\%;$$

263

264 where  $\text{MR}_{0.25}$  and  $\text{WR}_{0.25}$  are equal to the mass proportion of  $> 0.25\text{mm}$   
265 mechanical-stable aggregate and water-stable soil aggregate (%), respectively.

266

267 A mass-based model for fractal dimension (D) was represented quantitatively using  
268 the structure characteristics of soil aggregates and reflected the uniformity of soil  
269 texture (Tyler and Wheatcraft 1992).

270

$$271 \quad (3\text{-D}) \ln \frac{x_i}{x_{max}} = \ln \frac{M(r < x_i)}{M_t};$$

272

273 where M is the sum aggregate mass from sieves less than  $x_i$ ;  $M_t$  is the total mass of  
274 aggregates;  $x_{max}$  is maximum value for the aggregate size. Linear regression was  
275 used to calculate fractal dimension by the least-squares method.

276

## 277 **2.5 Statistical analysis**

278 All data were tested for normality and homoscedasticity prior to analysis. Crop yield,  
279 soil physiochemical properties and aggregation indexes were compared for significant  
280 differences by one-way ANOVA followed by a Fisher's least significant difference  
281 (LSD) post hoc test at  $p < 0.05$ . Mantel tests were adopted to test the linear correlation  
282 between pairs of proximity matrices. These tests have been widely applied to explore  
283 the relationship between a group of environmental factors and microbial composition.  
284 In order to apply them to soil aggregate indexes, soil physiochemical properties and  
285 crop yield, the package Vegan in the statistical software R 3.6.1 was used. Other



286 statistical analyses were performed with SPSS 22.0 (SPSS Inc., Chicago, USA) and  
287 graphs were plotted with Origin Pro 2019b (Origin Lab Corp, USA).

288

## 289 **3 Results**

### 290 **3.1 Crop production**

291 There were significant differences in wheat and corn yield, as well as the total yield,  
292 after 5 years of experimental treatment (Table 2). Compared with unamended control  
293 (CK), fertilization treatments all dramatically increased crop yield. For wheat, the  
294 yield changed with increasing substitution proportion. The largest yield was obtained  
295 with 50% substitution by biogas slurry (BS50), reaching 6775 kg ha<sup>-1</sup>, which was  
296 slightly larger than 25% substitution (BS25) but much higher than other fertilization  
297 treatments. For maize, there was a similar trend with the greatest yield (12690 kg ha<sup>-1</sup>)  
298 also in BS50. No significant difference was found between BS25 and chemical  
299 fertilizer alone (CF) or BS75. Total crop yield, in comparison with CK, was increased  
300 by 55% for CF, 63% for BS25, 76% for BS50, 65% for BS75 and 48% for BS100.

301

### 302 **3.2 Soil physicochemical properties**

303 Substantial differences in mean soil pH among treatments were seen (Fig. 1A).  
304 Continued application of chemical fertilizer alone (CF) significantly increased soil  
305 acidity, relative to the CK. All biogas slurry treatments significantly increased pH in  
306 comparison to the CK and CF. The highest pH was recorded in BS50 treatment,  
307 although that was not significantly higher than BS25; higher rates of substitution with  
308 slurry (BS75 and BS100) resulted in slightly lower pH.

309

310 Soil bulk density was highest in CK and not significantly different between CK and  
311 CF (Fig. 1B). Increasing substitution proportions of slurry progressively lowered bulk  
312 density, although it was not until BS50 that effect became significant. Soil  
313 water-holding capacity (Fig. 1C) and organic matter content (Fig. 2A) both showed  
314 the opposite trend – being lowest in the CK and CF, and then significantly higher with  
315 increasing slurry substitution, to a maximum in BS100. Water-holding capacity in  
316 BS100 was 41% higher than the CK and 63% higher than the CF.

317

318 Soil total nitrogen content, available phosphorus and available potassium all showed  
319 similar trends (Fig. 2B-D). These were significantly lower in the control than in all the  
320 biogas slurry substitution treatments. Few significant differences were found among  
321 substitution treatments: none for available P (Fig. 2C), only one for total N (BS50 was  
322 anomalously higher than other treatments; Fig. 2B) and there was considerable  
323 variation in available K (Fig. 2D). The soil C/N ratio was significantly enhanced by

324 substitution by slurry only once its substitution proportion exceeded 50% (Fig.2E).

325

### 326 **3.3 Soil aggregate mass proportions**

327 The distributions of mechanically stable aggregates were dominated by the fractions  
328 of 2-5 mm and 0.5-1.0 mm (24.3-47.4% and 27.3-39.2%, respectively, across  
329 treatments) with micro-aggregates (<0.25 mm) representing less than 6.5% (Table 3).  
330 Differences in aggregate mass proportion were observed among the treatments. The  
331 total proportion of macro-aggregate particles (>0.25 mm) was similar in the CK and  
332 CF but significantly greater in all treatments that had received biogas slurry  
333 substitution. Chemical fertilizer application alone significantly decreased the  
334 proportion of larger aggregates (>5 mm and 2-5 mm) and those in the 0.053-0.25 mm  
335 size range, but increased aggregate particles in the range of 0.25-0.5 mm, when  
336 compared with unamended control. Application of biogas slurry noticeably reduced  
337 the largest aggregate fraction (>5mm), as well as the 0.053-0.25 mm and <0.053 mm  
338 fractions, relative to the unamended control. Conversely, it significantly increased  
339 representation in the 0.5-1 mm aggregate fraction (Table 3).

340

341 In contrast to the mechanically stable aggregates, the water-stable aggregates were  
342 dominated by smaller size fractions (0.25-0.5, 0.053-0.25 and <0.053 mm) and  
343 micro-aggregates (<0.25 mm) represented 42-68% of the totals across treatments  
344 (Table 4). Chemical fertilizer application alone resulted in significantly lower  
345 representation of larger aggregates (particularly the >5 mm and 1-2 mm fractions) and  
346 concomitantly greater representation of some smaller aggregates (0.5-1 mm and  
347 0.25-0.5 mm), relative to the unamended control. Moderate applications of biogas  
348 slurry (BS25 and BS50) were extremely effective in maintaining higher fractions of  
349 macro-aggregates (>0.25 mm) relative to both unamended control and chemical  
350 fertilizer alone, but this effect was not seen at the higher application rates (BS75 and  
351 BS100).

352

### 353 **3.4 Soil aggregation stability characteristics**

354 Mean weight diameter (MWD) and geometric weight diameter (GMD) of aggregates  
355 showed very similar patterns among treatments, although the water-stable values were  
356 consistently much lower than the mechanical-stable values (Fig. 3A, B). The MWD of  
357 mechanical-stable aggregates was largest in unamended control and lowest with  
358 chemical fertilizer application alone. However, increasing application of biogas slurry  
359 had a significant positive effect on the GMD of mechanical-stable and water-stable  
360 aggregate, up to maximum at 50% substitution with slurry, with diminishing returns  
361 thereafter. The mechanical-stable and water-stable values of GMD were smallest with  
362 chemical fertilizer application alone, and significantly lower than other treatments.

363 Increasing substitutions with biogas slurry increased values to a maximum at 50%  
364 substitution. Beyond that, these values declined again, such that 100% substitution  
365 was not significantly different from the unamended control for water-stable values of  
366 GMD.

367

368 Determinations of fractal dimension (D) reflected the smaller aggregate sizes after  
369 wet-sieving, with water-stable fractal dimensions being consistently higher than their  
370 mechanical counterparts. The differences between treatments were generally small,  
371 although 50% substitution with slurry yielded significantly the lowest values (Fig. 3C).  
372 This treatment also resulted in clearly the lowest percentage aggregate destruction  
373 (PAD) values observed the experiment (Fig. 3D). Unamended control, chemical  
374 fertilizer application alone and 100% slurry substitution produced the highest values  
375 of PAD, although these were not significantly different.

376

### 377 **3.5 Correlations among crop yield and soil properties**

378 Mantel test correlations among soil aggregation characteristics, physicochemical  
379 properties and crop yield confirmed that the distance of soil aggregate indexes was  
380 extremely significant positively correlated with soil physiochemical properties (Fig.  
381 4A). Crop yield distance was similarly significant positively correlated with  
382 physiochemical properties (Figure 4B) and soil aggregate indexes (Fig. 4C).

383

## 384 **4 Discussion**

385 Fertilization is a key element of modern agricultural production (Abbott and Murphy  
386 2007, Chen et al. 2020). Five years of continuous treatment in our experiment have  
387 shown that substituting biogas slurry for chemical fertilizer can maintain productivity  
388 under intensive crop production in a wheat-maize annual rotation. Indeed, a  
389 combination of slurry and chemical fertilizer gave the highest yields of both crops,  
390 suggesting a synergistic effect beyond simple replacement of nutrient supply.  
391 Consequently, the results of the present study supported the first hypothesis that  
392 appropriate biogas slurry utilization could maintain or even increase the crop yield in  
393 a lime concretion black soil. Much of previous work on biogas slurry application has  
394 implicated the roles of soil texture, underlying fertility, tillage management and crop  
395 species as factors in increased yield, in crops such as rice, wheat, peanut and maize  
396 (Galvez et al. 2012; Zheng et al. 2016; Zheng et al. 2017; Rahaman et al. 2021). It is  
397 important therefore to examine its effects on the soil itself.

398

399 Soil fertility depends on a range of physiochemical conditions that are related to its  
400 organic matter content (Abbott and Murphy 2007; Diacono and Montemurro 2010;

401 Badagliacca et al. 2020). A common approach to fertility maintenance involves  
402 amendment with allochthonous organic matter, such as livestock manure, green  
403 manure, composts and biochar (Du et al. 2018; Greenberg et al. 2019; Li et al. 2020).  
404 Our experiment confirmed that biogas slurry application was also effective in this  
405 respect when applied to a lime concretion black soil. The findings supported our  
406 second hypothesis that biogas slurry applicant would have physicochemical effects  
407 relative to both conventional chemical fertilizer treatment and unamended control.  
408 Soil pH is an important determinant of nutrient availability to plants and is used as an  
409 indicator of fertility (Yang et al. 2018; Shi et al. 2019). Because of its carbonate  
410 content, lime concretion black soil has a high buffering capacity (Luo et al. 2015;  
411 Bosch-Serra et al. 2017). Nevertheless, untreated control and chemical fertilizer  
412 treatments had become distinctly acidic, whereas substitution with biogas slurry  
413 significantly increased pH to near neutral (6.4-7.2) and the greatest buffering was  
414 obtained with moderate biogas slurry applications (25% and 50% substitution). These  
415 results are consistent with findings that biogas slurry application improved buffering  
416 capacity on a fluvio-aquic soil of the NCP due to the abundance of polysaccharides,  
417 humic acids and basic cations in digestate (Du et al. 2018). The less effective  
418 mitigation of acidification at higher substitution fractions might be explained by acid  
419 inputs exceeding the capacity of soil aggregation processes and microbial  
420 consumption to deal with them (Shi et al. 2019). However, whether this acidification  
421 continues to intensify or mitigate after prolonged biogas slurry application time (>5  
422 years) still requires investigation.

423

424 Biogas slurry application clearly reduced soil bulk density and increased  
425 water-holding capacity in proportion to the amount of slurry added. Previous studies  
426 have also shown a decrease in bulk density with increasing slurry substitution  
427 application in the Indo-Gangetic alluvial tract and Southern China (Garg et al. 2005;  
428 Zheng et al. 2017). The driver of these effects would have been soil organic matter,  
429 which also increased in proportion to the amounts of biogas slurry added (Diacono  
430 and Montemurro 2010; Fan et al. 2020; Li et al. 2020). The increase could be directly  
431 from the biogas slurry and indirectly by enhanced decomposition of crop residues and  
432 stubbles (Galvez et al. 2012; Grant et al. 2020). The highest soil organic matter  
433 content recorded in the experiment (27.4 g kg<sup>-1</sup>) is toward the upper end of the range  
434 for intensive agricultural production in this area (30-year average, 12.5-17.5 g kg<sup>-1</sup>,  
435 unpublished data).

436

437 Organic matter is also a source of nutritional elements for plants; it provides the  
438 energy source for microbial metabolism and its associated mineralization yields  
439 available elements (Abbott and Murphy 2007; Mondini and Sequi 2008). Our results

440 demonstrated that biogas slurry was a more than adequate substitute to maintain soil  
441 total nitrogen, available phosphorus and available potassium concentrations. Similar  
442 results were reported by Xu et al (2019) in Southwest China. Lime concretion black  
443 soils are generally characterized by nitrogen deficiency but have adequate phosphorus  
444 and potassium. Therefore, biogas slurry application would have contributed to  
445 balancing this nutritional maladjustment and to facilitating nitrogen transformations.  
446 Only the 50% substitution with biogas slurry gave slightly higher residual nitrogen  
447 content than the chemical fertilizer alone. However, there could have been losses  
448 ascribable to volatilization of ammonium-nitrogen, as about 70% of the nitrogen in  
449 biogas slurry presents in the form of  $\text{NH}_4^+$  in our research (Wang et al. 2021), or  
450 leaching of poorly adsorbed nitrate-nitrogen. Both could ultimately have negative  
451 environmental impacts by contributing to climate warming and water eutrophication  
452 (Gericke et al. 2012). Although greater than in the control, available phosphorus was  
453 not significantly different in any of the other treatments, even though previous work  
454 suggested that phosphorus availability was enhanced by labile phosphorus from  
455 biogas slurry, as well as by stimulation of native soil phosphorus release (Grant et al.  
456 2020; Niyungeko et al. 2020). Some of the available phosphorus could have been  
457 fixed by  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in lime concretion black soils. Available potassium in the  
458 slurry substitution treatments was similar to that in the chemical fertilizer, although  
459 interpretation is complicated by potentially large amounts mineralized from straw  
460 mulches and their reserves (Luo et al. 2020). Biogas slurry application would promote  
461 mineralization by stimulating decomposer activity (Chen et al. 2020). The observed  
462 changes of soil C/N ratio would also be likely to affect the soil microbial biomass and  
463 nutrient fixation or release (Jiang et al. 2017). In comparison with complete chemical  
464 fertilizer application, biogas slurry substitution increased the soil C/N ratio  
465 proportionately, suggesting that its influence on the concentration of soil organic  
466 matter was greater than that on total nitrogen.

467

468 Organic matter also affects soil aggregate structure, the physical foundation of soil  
469 fertility (Kemper and Rosenau 1986; Abiven et al. 2009). Aggregates and the pore  
470 spaces between them determine soil aeration, drainage and permeability, influencing  
471 the activities of soil biota (Ceotto and Spallacci 2006; Du et al. 2016). After 5 years,  
472 biogas slurry application had substantial effects in promoting soil aggregation and  
473 stabilization, in comparison with exclusive use of chemical fertilizers. In general,  
474 macro-aggregates with a diameter of 0.25-10 mm are important indicators of soil  
475 physical, chemical and biological properties, and have been called ideal aggregates  
476 (Shi et al., 2019), although micro-aggregates with of diameter  $< 0.25$  mm also would  
477 affect soil aeration and microbial activities (Pan et al. 2021). All biogas slurry  
478 substitution proportions contributed to macro-aggregate formation and aggregate

479 stability, whether mechanical- or water-stable aggregates. Although organic matter has  
480 an important role in improving soil aggregate structure, the effects we observed were  
481 not linear (Diacono and Montemurro 2010; Shahbaz et al. 2017). Macro-aggregate  
482 mass proportions ( $MR_{0.25}$  and  $WR_{0.25}$ ), widely accepted as another important indicator  
483 of good structure (Liu et al., 2020), peaked at 50% substitution and then declined  
484 again, such that values at 100% were similar to those in the untreated controls  
485 although still significantly higher than for chemical fertilizer.

486

487 Differences between mechanical- and water-stable parameters, particularly expressed  
488 as percentage aggregates destruction (PAD) reinforced the finding that slurry  
489 substitution increased stability, with an optimum stability at 50% substitution.  
490 Aggregate distribution, rather than being lognormal as previously assumed, has fractal  
491 characteristics and so fractal dimension (D) represents a theoretically improved index  
492 of soil structural distribution (Tyler and Wheatcraft 1992, Six et al. 2004). The lower  
493 value of D at 50% substitution again indicated greater aggregate stability and  
494 macro-aggregate composition (Tyler and Wheatcraft 1992). Similar results were  
495 obtained by Zheng et al (2016) from biogas slurry application to upland red soil in  
496 southern China. As a Vertisol, lime concretion black soil is characterized by high clay  
497 content, dominated by smectite, which favors macro-aggregation formation and  
498 swelling-shrinkage (Okolo et al. 2020). Increasing application with biogas slurry  
499 might mask the effects of smectite, and slightly aggravate aggregate slaking because  
500 of exchangeable cations (Bosch-Serra et al. 2017).

501

502 Correlations among crop yield, soil aggregation indexes and physiochemical  
503 properties across different treatments provide insights into coordinated change  
504 involving crop production and soil fertility parameters, and thus support the third  
505 hypothesis. It is likely that these correlations devolve from a complex of factors  
506 associated with organic matter and arising from biogas slurry application. Organic  
507 matter, especially humus, is one of the main cementing materials in aggregate  
508 formation (Plaza-Bonilla et al. 2010). Compared with solid materials, biogas slurry  
509 had high flow characteristics, thus increasing contact area between soil particle and  
510 organic material (Bosch-Serra et al. 2017). Polysaccharides could also act as  
511 transitory binding agents to initiate aggregate formation (Tisdall and Oades 1982;  
512 Ceotto and Spallacci 2006; Abiven et al. 2009). Exogenous organic materials could  
513 bind to soil particles to increase intra-particle cohesion within aggregates and  
514 inter-aggregate hydrophobicity (Okolo et al. 2020). On the other hand, salinity could  
515 induce soil aggregate slaking and dispersion, as  $Na^+$  could replace  $Ca^{2+}$  in clay  
516 mineral particles and  $Mg^{2+}$  adsorbed on the surface of soil (Abiven et al. 2009; Meng  
517 et al. 2014). Organically enriched soils might also have more exchange sites as was

518 found by Yang et al. (2019) and Badagliacca et al. (2020).

519

520 Overall, substitution of biogas slurry for chemical fertilizers on lime concretion black  
521 soil of NCP had beneficial effects on its physicochemical properties and aggregation  
522 characteristics that were correlated with crop yield, with optimal values occurred at 50%  
523 substitution. Clearly, this approach deserves wider application in this region.  
524 Nevertheless, local conditions and resources need to be taken into account  
525 (Garcia-Franco et al. 2020). In particular, the economic effectiveness of a piping  
526 layout for biogas slurry transportation and application depends on proximity to animal  
527 breeding enterprises and farmers might need to adjust the timing of application  
528 according to the degree of drought and local crop nutritional requirements (Wang et al.  
529 2021). Furthermore, the potential disadvantages of biogas slurry application should  
530 not be ignored: secondary salinization of the soil derived from its considerable salt  
531 content, heavy metal accumulation originating from feed additives (Cavalcante et al.  
532 2019), and groundwater pollution (e.g. excessive nitrate content and antibiotic  
533 contamination) and ammonia volatilization (Rahaman et al. 2020). Further, long-term,  
534 work on the improvement of lime concretion black soil should encompass ecological  
535 safety and economic feasibility, as well as the relationships between soil aggregates,  
536 nutrients and crop yield.

537

## 538 **5 Conclusions**

539 Substitution of biogas slurry for fertilizer application on the lime concretion black soil  
540 in North China Plain represents a promising approach to sustaining crop yield and  
541 improving soil characteristics. A field experiment demonstrated the utility of biogas  
542 slurry application in reducing soil bulk density, while enhancing its water-holding  
543 capacity, organic matter, available phosphorus, available potassium and C/N ratio. All  
544 biogas slurry substitution proportions contributed to macro-aggregate formation and  
545 stability, relative to chemical fertilizer alone. Furthermore, the 50% substitution with  
546 biogas slurry gave optimal crop yield, soil aggregate structure and stability, and pH  
547 and total nitrogen content. This proportion of substitution with biogas slurry is  
548 recommended for more sustainable land and fertilizer use on lime concretion black  
549 soils in this region, where crop production can be integrated with intensive animal  
550 husbandry.

551

## 552 **Acknowledgements**

553 This research was supported by the Key Laboratory of High-efficient and Safe  
554 Utilization of Agriculture Water Resources of CAAS (2019AA02), Applied Research  
555 Program of Key Scientific Research Projects in Henan Province (20B210004),

556 Postdoctoral Research Grant in Henan Province, China (201903042) and Plant  
557 Protection of Key Discipline Project of Henan Province. Special thanks are due here  
558 to Fei Wang and Yongpeng Xu from Muyuan Husbandry Company and all members  
559 from 513 scientific group for their valuable assistance during the 5-year experimental  
560 period. Finally, this manuscript was dedicated to my newborn baby named Yuxin in  
561 2021.

562

## 563 **Declarations**

564 The authors declare that they have no conflict of interest.

565

## 566 **References**

- 567 Abbott LK, Murphy DV (2007) What is soil biological fertility. Springer, Dordrecht
- 568 Badagliacca G, Petrovicova B, Pathan SI et al (2020) Use of solid anaerobic digestate and no-tillage  
569 practice for restoring the fertility status of two Mediterranean orchard soils with contrasting  
570 properties. *Agr Ecosyst Environ* 300:107010. <https://doi.org/10.1016/j.agee.2020.107010>
- 571 Bansal S, Yin XH, Savoy HJ et al (2020) Long-term influence of phosphorus fertilization on organic  
572 carbon and nitrogen in soil aggregates under no-till corn-wheat-soybean rotations. *Agron J*  
573 112:2519-2534. <https://doi.org/10.1002/agj2.20200>
- 574 Bao SD (2008) Soil Agrochemical Analysis. China Agriculture Press, Beijing.
- 575 Bosch-Serra àD, Yagüe MR, Poch RM et al (2017) Aggregate strength in calcareous soil fertilized with  
576 pig slurries. *Eur J Soil Sci* 68:449-461. <https://doi.org/10.1111/ejss.12438>
- 577 Cavalcante JS, Favaretto N, Dieckow J et al (2019) Long-term surface application of dairy liquid  
578 manure to soil under no-till improves carbon and nitrogen stocks. *Eur J Soil Sci* 1-12.  
579 <https://doi.org/10.1111/ejss.12920>
- 580 Chao F, Zhang SR, Li JL et al (2021) Partial substitution of rice husk for manure in greenhouse  
581 vegetable fields: Insight from soil carbon stock and aggregate stability. *Land Degrad Dev* 1-11.  
582 <https://doi.org/10.1002/ldr.4021>
- 583 Chen L, Li F, Li W et al (2019) Organic amendment mitigates the negative impacts of mineral  
584 fertilization on bacterial communities in Shajiang black soil. *Appl Soil Ecol* 150:103457.  
585 <https://doi.org/10.1016/j.apsoil.2019.103457>
- 586 Chen ZM, Wang Q, Ma JW et al (2020) Soil microbial activity and community composition as  
587 influenced by application of pig biogas slurry in paddy field in southeast China. *Paddy Water*  
588 *Environ* 18, 15-25. <https://doi.org/10.1007/s10333-019-00761-y>
- 589 Dai HC, Zang HD, Zhao YX et al (2019) Linking bacterial community to aggregate fractions with  
590 organic amendments in a sandy soil. *Land Degrad Dev* 30:1828–1839.  
591 <https://doi.org/10.1002/ldr.3383>
- 592 Diacono M, Montemurro F. (2010) Long-term effects of organic amendments on soil fertility. A review.  
593 *Agron Sustain Dev* 30:401-422. [https://doi.org/10.1007/978-94-007-0394-0\\_34](https://doi.org/10.1007/978-94-007-0394-0_34)
- 594 Du ZJ, Xiao YT, Qi XB et al (2018) Peanut-shell biochar and biogas slurry improve soil properties in  
595 the North China Plain: a four-year field study. *Sci Rep* 8:13724.  
596 <https://doi.org/10.1038/s41598-018-31942-0>
- 597 Fan RQ, Du JJ, Liang AZ et al (2020) Carbon sequestration in aggregates from native and cultivated  
598 soils as affected by soil stoichiometry. *Biol Fert Soils* 56:1109-1120.



599 <https://doi.org/10.1007/s00374-020-01489-2>

600 Galvez A, Sinicco T, Cayuela ML et al (2012) Short term effects of bioenergy by-products on soil C  
601 and N dynamics, nutrient availability and biochemical properties. *Agr Ecosyst Environ* 160:3-14.  
602 <https://doi.org/10.1016/j.agee.2011.06.015>

603 Garcia-Franco N, Walter R, Wiesmeier M et al (2020) Biotic and abiotic controls on carbon storage in  
604 aggregates in calcareous alpine and prealpine grassland soils. *Biol Fert Soils* 57:203–218.  
605 <https://doi.org/10.1007/s00374-020-01518-0>

606 Garg RN, Pathak H, Das DK et al (2005) Use of flyash and biogas slurry for improving wheat yield  
607 and physical properties of soil. *Environ Monit Assess* 107:1-9.  
608 <https://doi.org/10.1007/s10661-005-2021-x>

609 Gericke D, Bornemann L, Kage H, et al (2012) Modelling ammonia losses after field application of  
610 biogas slurry in energy crop rotations. *Water Air Soil Poll* 223:29-47.  
611 <https://doi.org/10.1007/s11270-011-0835-4>

612 Grant P, Suazo-Hernández J, Condrón L et al (2020) Soil available P, soil organic carbon and  
613 aggregation as affected by long-term poultry manure application to Andisols under pastures in  
614 Southern Chile. *Geoderma Regional* 21: e00271. <https://doi.org/10.1016/j.geodrs.2020.e00271>

615 Greenberg I, Kaiser M, Polifka S et al (2019) The effect of biochar with biogas digestate or mineral  
616 fertilizer on fertility, aggregation and organic carbon content of a sandy soil: Results of a  
617 temperate field experiment. *J Soil Sci Plant Nut* 182:824–835. [https://doi.org/](https://doi.org/10.1002/jpln.201800496)  
618 [10.1002/jpln.201800496](https://doi.org/10.1002/jpln.201800496)

619 Jiang YF, Guo K, Sun L et al (2017) Spatial Variability of C-to-N Ratio of Farmland Soil in Jiangxi  
620 Province. *Environ Sci China* 38: 3840-3850. <https://doi.org/10.13227/j.hjcx.201702193>

621 Kamran M, Huang L, Nie J et al (2021) Effect of reduced mineral fertilization (NPK) combined with  
622 green manure on aggregate stability and soil organic carbon fractions in a fluvo-aquic paddy soil.  
623 *Soil Till Res* 211:105005. <https://doi.org/10.1016/j.still.2021.105005>

624 Kan ZR., Ma ST, Liu QY et al (2020) Carbon sequestration and mineralization in soil aggregates under  
625 long-term conservation tillage in the North China Plain. *CATENA* 188:104428.  
626 <https://doi.org/10.1016/j.catena.2019.104428>

627 Kemper WD, Rosenau RC (1986) Aggregate stability and size distribution. American Society of  
628 Agronomy-Soil Science Society of America, Madison.

629 Li JY, Yuan XL, Ge L et al (2020) Rhizosphere effects promote soil aggregate stability and associated  
630 organic carbon sequestration in rocky areas of desertification. *Agr Ecosyst Environ* 304: 107126.  
631 <https://doi.org/10.1016/j.agee.2020.107126>

632 Liu HM, Li RY, Gao JJ et al (2020): Research progress on the effects of conservation tillage on soil  
633 aggregates and microbiological characteristics. *Eco Environ Sci* 29: 1277-1284.  
634 <https://doi.org/10.16258/j.cnki.1674-5906.2020.06.025>

635 Lu RK (1999) Soil and Agro-Chemical Analysis Methods. China Agricultural Science and Technology  
636 Press, Beijing.

637 Luo SS, Gao G, Wang SJ et al (2020) Long-term fertilization and residue return affect soil  
638 stoichiometry characteristics and labile soil organic matter fractions. *Pedosphere* 30: 133-143.  
639 [https://doi.org/10.1016/S1002-0160\(20\)60031-5](https://doi.org/10.1016/S1002-0160(20)60031-5)

640 Meng QF, Sun YT, Zhao J et al (2014) Distribution of carbon and nitrogen in water-stable aggregates  
641 and soil stability under long-term manure application in solonchic soils of the Songnen plain,  
642 northeast China. *J Soils Sediment* 14:1041-1049. <https://doi.org/10.1007/s11368-014-0859-7>

643 Niyungeko C, Liang X, Liu C et al (2020) Effect of biogas slurry application on soil nutrients,  
644 phosphomonoesterase activities, and phosphorus species distribution. *J Soils Sediment* 20:  
645 900-910. <https://doi.org/10.1007/s11368-019-02435-y>

646 Okolo CC, Gebresamuel G, Zenebe A et al (2020) Accumulation of organic carbon in various soil  
647 aggregate sizes under different land use systems in a semi-arid environment. *Agr Ecosyst Environ*  
648 297:106924. <https://doi.org/10.1016/j.agee.2020.106924>

649 Pan JX, Wang JS, Zhang RY et al (2021) Microaggregates regulated by edaphic properties determine  
650 the soil carbon stock in Tibetan alpine grasslands. *Catena* 206:105570.  
651 <https://doi.org/10.1016/j.catena.2021.105570>

652 Plaza-Bonilla D, Cantero-Martínez C, Lvaro-Fuentes J (2010) Tillage effects on soil aggregation and  
653 soil organic carbon profile distribution under Mediterranean semi-arid conditions. *Soil Use*  
654 *Manage* 26:465-474. <https://doi.org/10.1111/j.1475-2743.2010.00298.x>

655 Pu C, Kan ZR, Liu P et al (2019) Residue management induced changes in soil organic carbon and  
656 total nitrogen under different tillage practices in the North China Plain. *J Integr Agr* 18: 1337-1347.  
657 [https://doi.org/10.1016/S2095-3119\(18\)62079-9](https://doi.org/10.1016/S2095-3119(18)62079-9)

658 Rahaman MA, Zhan XY, Zhang QW et al (2020) Ammonia volatilization reduced by combined  
659 application of biogas slurry and chemical fertilizer in maize–wheat rotation system in North China  
660 Plain. *Sustainability* 12:4400. <https://doi.org/10.3390/su12114400>

661 Rahaman MA, Zhang QW, Shi Y et al (2021) Biogas slurry application could potentially reduce N<sub>2</sub>O  
662 emissions and increase crop yield. *Sci Total Environ* 778: 146269. <https://doi.org/10.1016/j.scitotenv.2021.146269>

664 Shahbaz M, Kuzyakov Y, Heitkamp F (2017) Decrease of soil organic matter stabilization with  
665 increasing inputs: Mechanisms and controls. *Geoderma* 304:76-82. <https://doi.org/10.1016/j.geoderma.2016.05.019a>

667 Shi RY, Liu ZD, Li Y et al (2019) Mechanisms for increasing soil resistance to acidification by  
668 long-term manure application. *Soil Till Res* 185:77-84. <https://doi.org/10.1016/j.still.2018.09.004>

669 Six J, Bossuyt H, Degryze S, et al (2004) A history of research on the link between (micro)aggregates,  
670 soil biota, and soil organic matter dynamics. *Soil Till Res* 79:7-31.  
671 <https://doi.org/10.1016/j.still.2004.03.008>

672 Tyler SW, Wheatcraft SW (1992) Fractal scaling of soil particle-size distributions: analysis and  
673 limitations. *Soil Sci Soc Am J* 56:362-369. <https://doi.org/10.2136/sssaj1992.03615995005600020005x>

675 Wang WG, Zhang YH, Liu Y et al (2021) Managing liquid digestate to support the sustainable biogas  
676 industry in China: Maximizing biogas linked agroecosystem balance. *GCB Bioenergy* 13: 880-892.  
677 <https://doi.org/10.1111/gcbb.12823>

678 Wang X, Qi JY, Zhang XZ et al (2019) Effects of tillage and residue management on soil aggregates  
679 and associated carbon storage in a double paddy cropping system. *Soil Till Res* 194:104339.  
680 <https://doi.org/10.1016/j.still.2019.104339>

681 Wei CL, Gao WD, Whalley WR et al (2018) Shrinkage characteristics of lime concretion black soil as  
682 affected by biochar amendment. *Pedosphere* 28:713–725. [https://doi.org/10.1016/S1002-0160\(18\)60041-4](https://doi.org/10.1016/S1002-0160(18)60041-4)

684 Xu M, Xian Y, Wu JF et al (2019) Effect of biogas slurry addition on soil properties, yields, and  
685 bacterial composition in the rice-rape rotation ecosystem over 3 years. *J Soil sediment*  
686 19:2534-2542. <https://doi.org/10.1007/s11368-019-02258-x>

687 Yang XD, Ni K, Shi YZ et al (2018) Effects of long-term nitrogen application on soil acidification and  
688 solution chemistry of a tea plantation in China. *Agr Ecosyst Environ* 252:74-82. [https://doi.org/](https://doi.org/10.1016/j.agee.2017.10.004)  
689 [10.1016/j.agee.2017.10.004](https://doi.org/10.1016/j.agee.2017.10.004)

690 Zhang B, Horn R (2001) Mechanisms of aggregate stabilization in Ultisols from subtropical China.  
691 *Geoderma* 99:123-145. [https://doi.org/ 10.1016/S0016-7061\(00\)00069-0](https://doi.org/10.1016/S0016-7061(00)00069-0)

692 Zheng XB, Fan JB, Cui Y et al (2016) Effects of biogas slurry application on peanut yield, soil  
693 nutrients, carbon storage, and microbial activity in an Ultisol soil in southern China. *J Soil*  
694 *Sediment* 16:449-460. [https://doi.org/ 10.1007/s11368-015-1254-8](https://doi.org/10.1007/s11368-015-1254-8)

695 Zheng XB, Fan JB, Xu L et al (2017) Effects of combined application of biogas slurry and chemical  
696 fertilizer on soil aggregation and C/N distribution in an Ultisol. *Plos One* 12:e0170491.  
697 [https://doi.org/ 10.1371/journal.pone.0170491](https://doi.org/10.1371/journal.pone.0170491)

698 Zhu LY, Zhang FL, Li LL et al (2021) Soil C and aggregate stability were promoted by bio-fertilizer on  
699 the North China Plain. *J Soil Sci Plant Nut.* 21: 2355–2363.  
700 <https://doi.org/10.1007/s42729-021-00527-8>

701  
702  
703  
704  
705

706 Table 1 Amounts of total nitrogen, phosphorus and potassium provided by different biogas slurry and  
 707 chemical fertilizer applications for each crop  
 708  
 709

Treatment	Application Amount m <sup>3</sup> ha <sup>-1</sup>	Biogas Slurry			Chemical Fertilizer		
		N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
		kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>
CK	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CF	0.0	0.0	0.0	0.0	225.0	90.0	90.0
BS25	46.9	56.3	14.1	22.5	168.7	75.9	67.5
BS50	93.8	112.6	28.2	45.0	112.4	61.8	45.0
BS75	140.7	168.9	42.3	67.5	56.1	47.7	22.5
BS100	187.6	225.0	56.4	90.0	0.0	33.6	0.0

710 Treatments: CK, unamended control; CF, chemical fertilizer alone; BS25, 25% biogas slurry  
 711 substitution; BS50, 50% biogas slurry substitution; BS75, 75% biogas slurry substitution; BS100, 100%  
 712 biogas slurry with no chemical fertilizer.

713  
 714  
 715  
 716  
 717  
 718  
 719  
 720  
 721  
 722  
 723  
 724  
 725  
 726  
 727  
 728  
 729  
 730  
 731  
 732  
 733  
 734  
 735  
 736  
 737  
 738  
 739  
 740  
 741  
 742  
 743  
 744  
 745  
 746  
 747  
 748  
 749  
 750

751  
752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800

Table 2 Wheat yield, maize yield and total yield of the last crops in response to biogas slurry and chemical fertilizer application to lime concretion black soil

Treatment	Wheat yield kg ha <sup>-1</sup>	Maize yield kg ha <sup>-1</sup>	Total yield kg ha <sup>-1</sup>
CK	4361 ±268e	6708 ±262e	11069 ±304e
CF	6045 ±471cd	11100 ±765c	17145 ±692c
BS25	6461 ±289ab	11545 ±352bc	18006 ±228b
BS50	6775 ±231a	12690 ±305a	19465 ±328a
BS75	6351 ±201bc	11935 ±369b	18286 ±440b
BS100	5903 ±214d	10510 ±297d	16413 ±422d

Treatments: CK, unamended control; CF, chemical fertilizer alone; BS25, 25% biogas slurry substitution; BS50, 50% biogas slurry substitution; BS75, 75% biogas slurry substitution; BS100, 100% biogas slurry with no chemical fertilizer. Values sharing same lowercase letters in the same column are not significantly different at  $p < 0.05$ .

801  
802  
803  
804  
805

806 **Table 3** Mass proportions of mechanical-stable aggregates in response to biogas slurry and chemical  
807 fertilizer application to lime concretion black soil

808  
809

Aggregate size class mm	Treatment					
	CK %	CF %	BS25 %	BS50 %	BS75 %	BS100 %
>5	12.1 ±1.0a	5.3 ±0.7d	8.8 ±0.8b	6.5 ±1.0c	4.1 ±0.6e	9.3 ±0.8b
2-5	39.4 ±3.5b	31.9 ±2.3c	38.7 ±3.2b	47.4 ±3.7a	41.7 ±3.6b	24.3 ±2.8d
1-2	7.3 ±1.1b	8.1 ±1.0b	6.7 ±1.0b	6.7 ±0.8b	7.3 ±0.7b	19.0 ±2.5a
0.5-1	27.3 ±1.6c	32.9 ±2.6b	35.9 ±2.2ab	32.4 ±3.3b	37.7 ±3.0a	39.2 ±3.8a
0.25-0.5	7.4 ±1.1b	16.1 ±1.5a	6.8 ±0.9b	5.0 ±0.6c	6.4 ±0.6b	5.1 ±0.8c
0.053-0.25	4.7 ±0.6a	3.9 ±1.0b	2.0 ±0.5c	1.5 ±0.1c	2.0 ±0.5c	2.2 ±0.4c
<0.053	1.7 ±0.2a	1.9 ±0.5a	1.0 ±0.1b	0.5 ±0.2c	0.9 ±0.2b	1.1 ±0.3b
MR <sub>0.25</sub>	93.5 ±6.8d	94.2 ±1.4d	97.0 ±5.8b	99.0 ±2.6a	95.6 ±8.6c	96.7 ±1.5b

810 Treatments: CK, unamended control; CF, chemical fertilizer alone; BS25, 25% biogas slurry  
811 substitution; BS50, 50% biogas slurry substitution; BS75, 75% biogas slurry substitution; BS100, 100%  
812 biogas slurry with no chemical fertilizer. Values sharing same lowercase letters in the same column are  
813 not significantly different at  $p < 0.05$ .

814  
815  
816  
817  
818  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
830

831 Table 4 Mass proportions of water-stable aggregates in response to biogas slurry and chemical

832 fertilizer application to lime concretion black soil

833

834

Aggregate size class mm	Treatment					
	CK	CF	BS25	BS50	BS75	BS100
	%	%	%	%	%	%
>5	0.7 ±0.1c	0.2 ±0.0d	2.3 ±0.3a	1.1 ±0.0b	1.1 ±0.1b	0.8 ±0.1c
2-5	1.8 ±0.2c	1.5 ±0.2c	0.7 ±0.2d	5.6 ±0.9a	2.5 ±0.3b	1.6 ±0.3c
1-2	3.2 ±0.4c	1.6 ±0.2d	9.2 ±1.5b	10.8 ±1.0a	1.6 ±0.2d	2.4 ±0.2cd
0.5-1	9.7 ±1.4c	10.8 ±2.5c	14.5 ±2.5b	27.0 ±2.2a	11.5 ±1.5c	10.3 ±1.8c
0.25-0.5	17.9 ±1.7bc	18.3 ±1.8bc	15.5 ±1.3cd	13.8 ±2.5d	25.2 ±2.3a	20.4 ±2.3b
0.053-0.25	20.3 ±3.5b	13.6 ±2.1c	21.7 ±2.3b	10.9 ±1.9c	29.0 ±2.5a	20.3 ±3.0b
<0.053	46.4 ±2.6b	54.1 ±3.0a	36.2 ±4.9c	30.8 ±1.7d	29.1 ±2.8d	44.3 ±2.3b
WR <sub>0.25</sub>	33.3 ±2.4cd	32.3 ±1.7d	42.2 ±3.7b	58.2 ±1.5a	41.9 ±1.6b	35.4 ±1.5c

835 Treatments: CK, unamended control; CF, chemical fertilizer alone; BS25, 25% biogas slurry  
836 substitution; BS50, 50% biogas slurry substitution; BS75, 75% biogas slurry substitution; BS100, 100%  
837 biogas slurry with no chemical fertilizer. Values sharing same lowercase letters in the same column are  
838 not significantly different at  $p < 0.05$ .

839

840

841

842

843

844

845

846

847

848

849

850

851

852

853

854

855

856

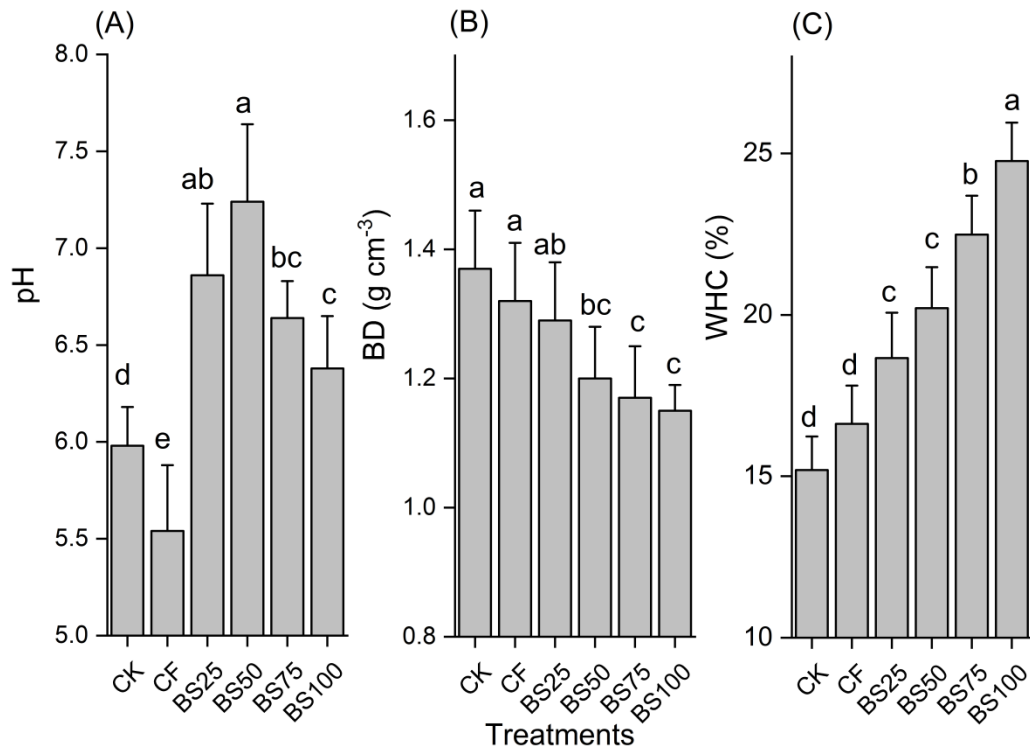
857

858

859

860

861



862

863 Figure. 1 Effects of combinations of chemical fertilizer and biogas slurry application on properties of  
 864 lime concretion black soil: (A) pH; (B) Bulk density (BD); (C) Water-holding capacity (WHC).

865 Treatments: CK, unamended control; CF, chemical fertilizer alone; BS25, 25% biogas slurry  
 866 substitution; BS50, 50% biogas slurry substitution; BS75, 75% biogas slurry substitution; BS100, 100%  
 867 biogas slurry with no chemical fertilizer. Bars sharing lower-case letters are not significantly different  
 868 at  $p < 0.05$ .

869

870

871

872

873

874

875

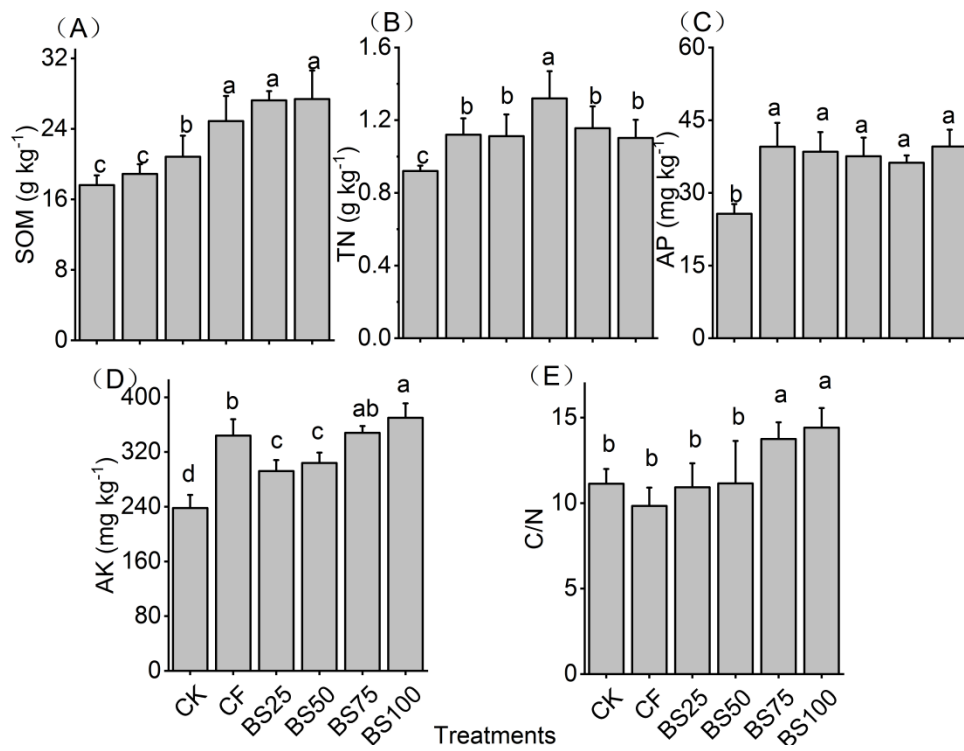
876

877

878

879





880

881 Figure. 2 Effects of combinations of chemical fertilizer and biogas slurry application on soil nutrient  
 882 concentrations and C/N in lime concretion black soil: (A) Soil organic matter content (SOM); (B) Total  
 883 nitrogen, (TN); (C) Available phosphorus (AP); (D) Available potassium (AK); (E) Carbon/nitrogen  
 884 ratio (C/N). Treatments: CK, unamended control; CF, chemical fertilizer alone; BS25, 25% biogas  
 885 slurry substitution; BS50, 50% biogas slurry substitution; BS75, 75% biogas slurry substitution; BS100,  
 886 100% biogas slurry with no chemical fertilizer. Bars sharing lower case letters are not significantly  
 887 different at  $p < 0.05$ .

888

889

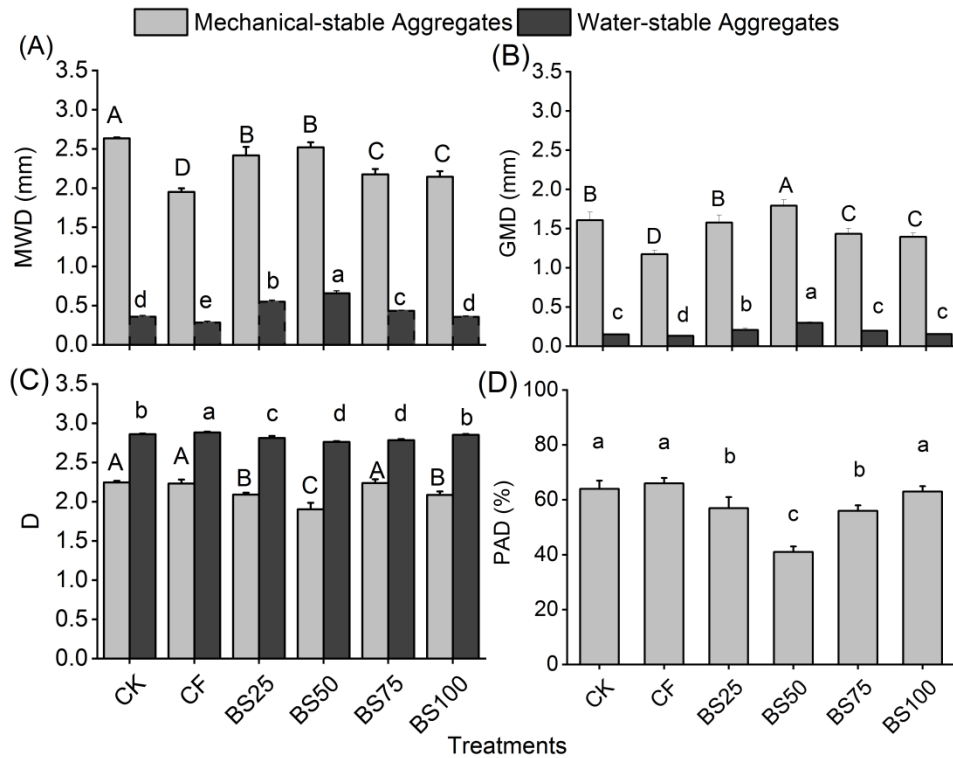
890

891

892

893

894



895

896 Figure. 3 Mechanical-stable and water-stable aggregation stability characteristics of the lime  
 897 concretion black soil in response to biogas slurry application treatments: (A) Mean Weight Diameter  
 898 (MWD); (B) Geometric Mean Diameter (GMD); (C) Fractal Dimension (D); (D) Percentage of  
 899 Aggregates Destruction (PAD). Treatments: CK, unamended control; CF, chemical fertilizer alone;  
 900 BS25, 25% biogas slurry substitution; BS50, 50% biogas slurry substitution; BS75, 75% biogas slurry  
 901 substitution; BS100, 100% biogas slurry with no chemical fertilizer. Bars sharing same uppercase or  
 902 lowercase letters were not significantly different at  $p < 0.05$  for mechanical-stable and water-stable  
 903 aggregates, respectively.

904

905

906

907

908

909

910

911

912

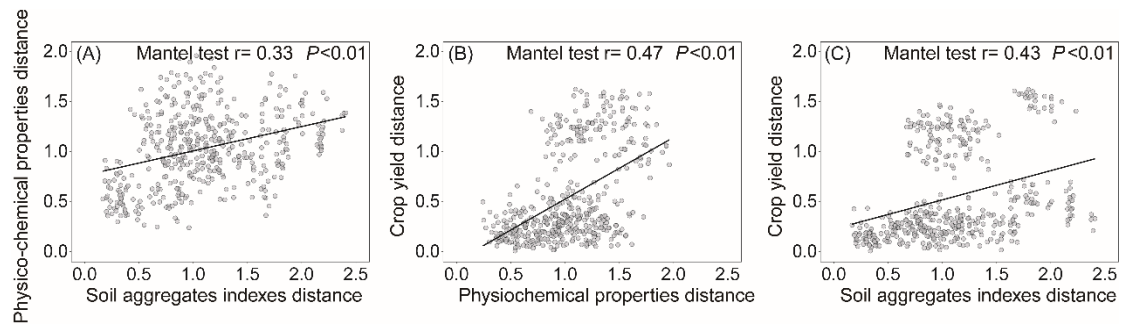
913

914

915

916

917



918

919 Figure. 4 Linear correlations between pairs of proximity matrices, using the Mantel test among soil  
 920 aggregate indexes, soil physicochemical properties and crop yield: (A) Physicochemical properties v.  
 921 Soil aggregates indexes; (B) Crop yield v. Physicochemical properties; (C) Crop yield v. Soil  
 922 aggregates indexes.

923

924

925