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EFFECTS OF LONG-TERM BIOGAS SLURRY APPLICATION ON SOIL PROPERTIES AND CROP YIELD IN THE NORTH CHINA PLAIN

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ABSTRACT

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Zhoukou is located in the southeast of Henan Province, China, which is the core area of grain production. Lime concretion black soil is one of the main low-yield soil types in this region, which has the characteristics of heavy clay, compact soil layer, poor aeration and low organic matter content, so it is urgent to carry out the task of improving the soil fertility. Biogas slurry contains a variety of nutrients, which can improve soil physical and chemical properties, increase soil enzyme activity, improve crop biological properties and promote crop growth.

However, the application of continuous large concentrations will have a negative impact on the growth of crops. Reasonable application of biogas slurry is particularly important in order to ensure the yield and quality of agricultural products, and does not pollute the soil and groundwater environment.

In this study, we systematically analyzed the effects of different proportions of combined application of biogas slurry and chemical fertilizer on the physicochemical properties and enzyme activities of different soil layers in the of lime concretion black soil of the Huang-Huai-Hai Plain, as well as the effects on the growth and development of winter wheat, and determined the appropriate amount of biogas slurry suitable for Zhoukou area, so as to provide a scientific basis for the improvement of the soil of the of lime concretion black soil, and provide a reference for the application of biogas slurry in Ukraine, the improvement of soil texture and the realization of sustainable agricultural development.

Soil bulk density comprehensively reflects the tightness and porosity of soil, and has a significant impact on water, fertilizer, gas, heat conditions in the soil and agricultural production. In this study, there were significant differences between different soil layers, and between each treatment and CK (control). After adding biogas slurry, the soil bulk density was significantly reduced, especially BS50 (50 % nitrogen from biogas slurry and 50 % nitrogen from fertilizer during winter wheat planting) soil bulk density was the smallest, and it was within the suitable bulk density range for winter wheat, which was conducive to the growth and development of winter wheat and the increase in yield.

In 0-10 cm soil layer, percentage of particle aggregates with size >2 mm was the highest for BS50 and for CF (only chemical fertilizers) was the lowest, which are 54.48 and 39.27 %, respectively. Percentage of 0.25-2 mm particle size aggregates was the highest for CF with a value of 53.61, which is significant different from others. Soil samples from BS50 had the lowest percentage of aggregates 0.053-0.25 mm with a value of 2.03, which is not significant different from BS100 (100% biogas slurry), but is significant different from other treatments. For aggregates of <0.25 mm particle size, CF had the smallest percentage - 1.94, but there is no significant difference between treatments. Long-term application of chemical fertilizer significantly decreased the number of large aggregates and increased the number of small aggregates, and application of biogas slurry was beneficial to increase the composition of large aggregates.

The stability of soil aggregates varies in different soil layers and under different treatment conditions. Biogas slurry treatment is beneficial to increase the MWD (mean weight diameter) of 0-10 cm, 10-20 cm, 20-40 cm force-stable aggregates and water-stable aggregates, with BS50 being the largest. The size order of MWD of force-stable aggregates and MWD of water-stable aggregates in the vertical direction is 0-10 cm>10-20 cm>20-40 cm, and both decrease with the increase of soil depth. The combined application of chemical fertilizers and biogas slurry made the MWD of force-stable and water-stable aggregates significantly higher than those of other treatments, increasing the stability and agglomeration of the soil. In the 0-10 cm soil layer, BS50 has the smallest percentage of aggregate destruction (PAD) value of 36.52, which is 45.0 % and 46.74 % lower than CK and CF respectively. The PAD of CF increased by 3.27 % compared to CK. In the 10-20 cm soil layer, the PAD value of BS50 is 35.70, which is 46.26 % and 47.71 % lower than CK and CF respectively. In the 20-40 cm soil layer, the PAD value of BS50 combined with biogas slurry and chemical fertilizers was the lowest (33.33), which was significantly different from each treatment. The PAD of CF was increased compared with CK. The single application of chemical fertilizer CF will make the stability of soil aggregates worse and destroy the aggregation of aggregates; while the combined application of organic fertilizer and chemical fertilizer will reduce the degree of damage to aggregates and facilitate the aggregation of aggregates. Reasonable fertilizer application, especially the BS50 treatment of biogas slurry and chemical fertilizers, has positive significance in maintaining the stability of soil aggregates and improving soil quality.

pH is an important chemical property of soil, which can affect the solubility of nutrient elements and ultimately affect the supply of nutrients in the soil. In this study, CF treatment can reduce pH, and application of biogas slurry can increase pH. Biogas slurry itself is alkaline and contains high cation content. The increase of base ions can effectively inhibit the soil acidification trend caused by long-term application of chemical fertilizer to a certain extent, and the acid-base environment of soil solution can be improved.

The application of biogas slurry in agricultural planting can significantly increase the nitrogen content of the soil, thereby satisfying the absorption of nutrients by crops. In 0-10 cm, 10-20 cm, and 20-40 cm, the total nitrogen content is highest by the BS50 treatment. The total nitrogen content is 0-10 cm>10-20>20-40 cm, the total nitrogen content decreased with the increase of soil depth.

Biogas slurry contains a large number of microorganisms, which can promote the propagation of soil microorganisms and enhance the activity of related enzymes. Application of biogas slurry has a certain impact on the soil available phosphorus content. Among all soil layers, the available phosphorus content of BS50 was the highest, which was 25.59 mg /kg, 22.50 mg/kg and 20.48 mg/kg, respectively, and there was a significant difference between BS50 and other treatments. In terms of soil depth, soil available phosphorus content decreased with the increase of soil depth, and the available phosphorus content was 0-10 cm>10-20 cm>20-40 cm, indicating that soil available phosphorus mainly concentrated in the surface layer. The content of soil available phosphorus was different in different soil layers and different treatment conditions, which indicated that the application of biogas slurry could improve the supply level of soil available phosphorus, but more is not always better, increasing the proportion of biogas suspension to BS75, B100 leads to a decrease in phosphorus content. In agricultural production, biogas slurry should be applied reasonably according to soil conditions and crop needs to increase soil available phosphorus content and promote crop growth.

Potassium has obvious effects on improving crop yield and agricultural product quality. Available potassium is an effective potassium that can be directly used by plants and plays an irreplaceable role in ensuring the normal growth and development of plants. Compared with CK, the content of available potassium in soil of each treatment increases to varying degrees. In different soil layers, the available potassium content of BS50 treatment combined with biogas slurry and chemical fertilizer was the highest, which was in 0-10 cm - 173.71 mg/kg, 10-20 cm - 156.63mg/kg and 20-40 cm - 144.06 mg/kg, respectively, and significantly increased compared with other treatments. Biogas slurry increased the content of available potassium in soil, which was beneficial to the absorption of potassium by winter wheat. Reasonable application of fertilizer and biogas slurry has positive significance to increase soil available potassium content, which is conducive to crop growth.

Application of biogas slurry had an effect on soil organic matter content. In 0-10 cm, 10-20 cm and 20-40 cm soil layers, the soil organic matter content was BS100>BS75>BS50>BS25>CF>CK, and the soil organic matter content increased with the increase of biogas slurry concentration. There were significant differences between all treatments and CK ($p<0.05$), and the soil organic matter content of BS100 was the highest (24.24 g/kg, 20.16 g/kg and 18.32 g/kg, respectively). Compared with CF, organic matter increased by 32.82 %, 24.44 % and 17.84 %, respectively, with the increase of soil layer, the organic matter content of different treatments decreased, and the organic matter content was 0-10 cm >10-20 cm > 20-40 cm.

We studied the effects of different proportions of biogas slurry and fertilizer on soil enzyme activity. The results showed that: the application of biogas slurry increased the activity of sucrase in soil, and with the increase of biogas slurry application, the activity of sucrase first increased until B75 and then decreased (BS100). The activity of sucrase with BS75 was the highest in 0-10 cm, 10-20 cm and 20-40 cm, which was 15.28 mg/g, 30.73 mg/g and 31.28 mg/g, respectively. Sucrase provides energy for organisms in soil, increases the intensity of organic carbon accumulation, decomposition and transformation, and increases soil fertility level.

Application of biogas slurry and chemical fertilizer can increase soil urease activity. The urease activity of combined application of biogas slurry and chemical fertilizer (BS25, BS50 and BS75) was higher than that of CF. With the increase of biogas slurry replacement ratio, the urease activity increased first BS50 and then decreased, indicating that the benefit of biogas slurry on soil was dose-dependent, and appropriate biogas slurry was beneficial to the improvement of soil urease activity, but excessive fertilization was counterproductive. In 0-10 cm, 10-20 cm, 20-40 cm, soil urease activity was the highest in BS50 treatment (235.33 ug/g, 337.79 ug/g and 351.45 ug/g, respectively), and was significantly different from that in other treatments. Combined application of biogas slurry and chemical fertilizer can enrich soil nitrogen pool, improve soil nitrogen transformation, increase the nitrogen source absorbed by winter wheat.

Both biogas slurry and chemical fertilizer increased soil acid phosphatase activity. With the increase of biogas slurry concentration, soil acid phosphatase increased with BS50 and then decreased on BS75-100. An appropriate amount of biogas slurry could catalyze the conversion of soil phosphorus into phosphorus easily absorbed by organisms, and improve its utilization rate. The activity of acid phosphatase with BS50 was the highest in different soil layers.

The order of catalase activities in 0-10 cm, 10-20 cm, and 20-40 cm soil was as follows: BS100 >BS75> BS50>BS25>CF >CK, and there was a significant difference between each treatment and CK ($P<0.05$), and there was no significant difference between CF and BS25 ($P > 0.05$), BS100 catalase activity was the highest, with 93.19 mmol/g, 95.11 mmol/g and 97.12 mmol/g, which were 12.35 %, 6.21 % and 5.86 % higher than CF, respectively. The activity of soil catalase reflects the process of soil organic oxidation, with the increase of the proportion of biogas slurry, the catalase activity increases. The biogas slurry has a significant promoting effect on soil catalase activity, which can accelerate the removal of hydrogen peroxide in organisms and soil to prevent damage, and the soil catalase activity of BS100 treatment is the largest, and the soil detoxification effect is the strongest. Long-term application of chemical fertilizers has a significant inhibitory effect on soil catalase activity, which can easily lead to the accumulation of root exudates, thereby aggravating the toxic effect of hydrogen peroxide on crops. Biogas slurry application can significantly increase soil catalase activity and accelerate the decomposition of toxic substances in soil.

Under the influence of soil fertility and productivity, the vertical distribution of soil enzymes has obvious regularity. Biogas slurry can improve the soil enzyme activity of winter wheat in 0-40 cm soil layer, especially in 20-40 cm soil layer, which is conducive to delaying the aging process of winter wheat root in the late growth period, promoting the transformation of soil nutrients, improving the ability of soil to supply fertilizer to winter wheat, and meeting the demand for nutrients during the growth and development of winter wheat, which is beneficial to the improvement of soil fertility and crop yield.

Finally, we measured the effects of different proportions of biogas slurry and chemical fertilizer on the growth and development of winter wheat. Both biogas slurry and chemical fertilizer application increased the yield of winter wheat, and there was a significant difference compared with CK ($p<0.05$). With the increase of biogas slurry proportion, the yield of winter wheat first increased and then decreased, and the yield of winter wheat with BS50 treatment was the highest, compared with CF, BS25, BS75 and BS100, the increases were 30.79 %,13.61 %, 4.31 % and 34.06 %, respectively, and there were significant differences between them ($p<0.05$). BS50 is the optimal combination of biogas slurry and chemical fertilizer, which can well match the nutrient demand of winter wheat at different growth and development stages, ensure the continuous supply of nutrients, promote winter wheat nutrient growth and nutrient accumulation, and achieve the best level of winter wheat yield.

The yield and yield composition of winter wheat are closely related. Chemical fertilizer and biogas slurry treatment can significantly increase the effective number of ear, grain number per ear and 1000-grain weight of winter wheat ($P<0.05$), so as to increase production. Yield was significantly correlated with the effective number of ear, grain number per ear and 1000-grain weight, and the correlation coefficients were 0.964, 0.974 and 0.870, respectively. The correlation between yield and grain number per ear is the highest. The high yield was achieved by increasing grain number per ear.

Different fertilization treatments had effects on the morphological indexes of winter wheat. Fertilization increased the plant height of winter wheat, and thus increased the ventilation and light transmittance of the bottom of the plant, which was conducive to the growth and development of winter wheat and the photosynthetic efficiency of leaves, so as to increase the yield of winter wheat. In the fertilization treatment, the plant height of BS treatment was higher than that of chemical fertilizer. The plant height treated with BS25, BS50, BS75 and BS100 was 77.47 cm, 79.52 cm, 78.08 cm and 78.02 cm, respectively. With the increase of biogas slurry proportion, the plant height first increased and then decreased, and plant height with the BS50 was the highest of 79.52 cm, but still belonged to the medium plant height. This can not only prevent lodging, but also enable the winter wheat nutrient body to obtain the appropriate spatial distribution and size, meet the nutrition required for wheat growth and growth, and is more conducive to the high yield of winter wheat.

The plant height is determined by several internode lengths, which extend at different stages of development, so they have different effects on yield. The first basal internode length is very important to plant lodging resistance wheat, the base of the first internode increases with fertilizer use, but the increase rate is different, compared with CK, CF, BS25, BS50, BS75, BS100 respectively increased by 1.81 %, 3.55 %, 0.68 %, 5.96 % and 6.19 %, BS100 has the greatest amplitude, increasing the risk of lodging. BS50 had the smallest increase, which was not only conducive to increasing plant height, but also had better lodging resistance.

The plant height component index of wheat (I) is the proportional relationship between the length between the upper and lower nodes of the stem, reflecting the information of the spatial arrangement of photosynthetic area and the operation and distribution of compounds. Fertilization can effectively improve the plant height component index of winter wheat. If the value of I is high, the lower node is shorter, the center of gravity is lower, the lower ear node is longer, the photosynthetic area above the blade node is increased, and the resistance to falling and photosynthesis are enhanced. The increase of plant height component index with BS50 made the ear under good light condition, which was conducive to increasing the yield of winter wheat. Plant height component index was significantly correlated with the effective number of ears, grain number per ear, 1000-grain weight, yield and plant height. The correlation coefficients between sub-panicle internode and 1000-grain weight, I1 and plant height were above 0.9, accounting for a higher proportion.

In the correlation analysis between yield and plant height component index, the correlation coefficient between yield and subear node was the highest (0.895), while the correlation coefficient between plant height and I1 and subear node was 0.971 and 0.954, respectively. Therefore, subear internode plays a key role in the formation of winter wheat yield. In practice, winter wheat with relatively high plant height and I1 should be selected within a moderate height range.

The increase of crop yield is based on the accumulation and distribution of dry matter. The combined application of biogas slurry and chemical fertilizer had a significant effect on straw biomass and aboveground biomass ($p<0.05$), and the highest values of straw biomass and aboveground biomass were BS75 and BS50, with the increase of biogas slurry proportion, even the biogas slurry completely replacing chemical fertilizer, both showed a downward trend.

Harvest index is the ratio of grain yield to aboveground total dry matter weight. It reflects the distribution ratio of crop assimilation products in grains and vegetative organs, and is an important index to evaluate crop yield level and cultivation effectiveness. Both fertilizer and combined biogas slurry increased the harvest index of winter wheat ($P<0.05$), and the harvest index of BS50 was the highest (0.48). Increasing biogas slurry within a certain range could improve both economic yield and harvest index. BS50 treatment can improve the growth and development of various organs in the later period of growth, coordinate the balanced distribution of dry matter, transport more photosynthetic products from source organs to reservoir organs, increase the flow and achieve the purpose of increasing production.

The factors affecting grain yield, biological yield and substance distribution were closely related to harvest index. Above ground biomass was significantly positively correlated with effective number of ear and grain number per ear, with correlation coefficients of 0.935 and 0.944, respectively, and the harvest index was also significantly positively correlated with effective number of ear and grain number per ear, with correlation coefficients greater than 0.9 (0.911 and 0.901, respectively). Therefore, it is possible to effectively combine high biological yield with high harvest index, and it is feasible to increase the yield by increasing effective number of ears and grain number per ear.

Key words: *biogas slurry, fertilizer, nutrients, soil fertility, yield, winter wheat, digestate, enzyme activity, soil structure, bulk density, nitrogen, phosphorus, potassium, organic matter, 1000 grain weight, growth and development.*

АНОТАЦІЯ

Чжан Сіхуан. Вплив довготривалого застосування дігестату на властивості ґрунту та врожайність сільськогосподарських культур в умовах Північно-китайської рівнини. – Кваліфікаційна праця на правах рукопису.

Дисертація на здобуття наукового ступеня доктора філософії за спеціальністю 201 – Агрономія. – Сумський національний аграрний університет. Суми, 2023 р.

Чжоукоу розташований на південному сході провінції Хенань в Китаї, який є основним районом виробництва зерна. Чорнозем щебенюватий на елювії вапняку є одним із основних малопродуктивних типів ґрунтів цього регіону, бо він є важкоглинистим, ущільненим, зі слабкою аерацією та низьким вмістом органічної речовини, тому вирішення завдань підвищення родючості цих ґрунтів є актуальним. Біогазова суспензія містить різноманітні поживні речовини, які можуть покращити фізичні та хімічні властивості ґрунту, підвищити активність ґрунтових ферментів, покращити ріст та розвиток рослини й сприяти отриманню приросту врожаю.

Однак, постійне застосування великих концентрацій біогазової суспензії матиме негативний вплив на ріст сільськогосподарських культур. Тому, її раціональне, науково обґрунтоване застосування є особливо важливим для отримання врожаїв та високої якості сільськогосподарської продукції без шкідливого впливу на ґрунтове середовище та підземні води.

У цьому дослідженні системно проаналізовано вплив комбінованого застосування біогазової суспензії та мінеральних добрив у різних співвідношеннях на фізико-хімічні властивості та активність ферментів різних шарів чорнозему щебенюватого на елювії вапняку рівнини Хуан-Хуай-Хай. Також визначено вплив удобрення на ріст і розвиток пшениці озимої, визначено відповідну кількість

біогазової суспензії, придатної для району Чжоукоу з метою створення наукової основи для покращення відповідного ґрунту й надання інформації та рекомендації для застосування біогазової суспензії, внесення якої покращує структурно-агрегатний склад грунту та реалізацію сталого розвитку сільського господарства.

Щільність ґрунту всебічно відображає складення і шпаруватість ґрунту, має значний вплив на водний, поживний, повітряний, тепловий режими ґрунту та сільськогосподарське виробництво. У цьому дослідженні було встановлено значні відмінності між різними шарами ґрунту, а також між варіантами із внесенням біогазової суспензії й мінеральних добрив та контролем (CK). Після додавання біогазової суспензії щільність ґрунту значно зменшилася, особливо на варіанті BS50 (50% азоту з біогазової суспензії та 50% азоту з мінеральних добрив), і знаходилася в межах прийнятного діапазону для озимої пшениці, що сприяло росту та розвитку рослини й збільшенню врожайності.

У шарі ґрунту 0-10 см, вміст агрегатів із розміром >2 мм на варіанті BS50 є найвищим, а CF (варіант із внесенням тільки мінеральних добрив) є найнижчим, що, відповідно, становить 54,48 і 39,27 %. Агрегатів розміром 0,25-2 мм на варіанті CF було найбільше - 53,6 %, що значно відрізняє його від інших варіантів. На варіанті BS50 отримано найменше значення - 2,03 % агрегатів розміром 0,053-0,25 мм, що незначно відрізняється від BS100 (внесення тільки біогазової суспензії), але суттєво відрізняється від інших варіантів. Агрегатів розміром менше 0,25 мм на варіанті CF є найменшим - 1,94 %, але істотної різниці між удобреними варіантами немає. Тривале застосування мінеральних добрив значно зменшує кількість великих агрегатів і збільшує кількість дрібних, а застосування біогазової суспензії сприяє збільшенню агрегатів більше 2 мм.

Стійкість ґрунтових агрегатів відрізняється в різних шарах ґрунту та за різного удобрення. Застосування біогазової суспензії є корисним для збільшення MWD (середньозважений діаметр) механічно стійких агрегатів й водостійких агрегатів в 0-10 см, 10-20 см, 20-40 см шарах ґрунту, причому на BS50 є найбільшим. Вертикальний розподіл MWD механічно стійких агрегатів і MWD водостійких агрегатів виглядає таким чином: 0-10 см >10-20 см >20-40 см, і обидва ці показники зменшуються з глибиною. Комбіноване внесення хімічних добрив і біогазової суспензії зробило MWD механічно стійких і водостійких агрегатів значно більшими, ніж інші варіанти удобрення, підвищивши стабільність і агломерацію ґрунту. У шарі ґрунту 0-10 см BS50 має найменше значення PAD (відсоток руйнування агрегатів) - 36,52, що на 45,0 % і 46,74 % нижче, ніж на варіантах CK і CF відповідно. PAD CF збільшився на 3,27 % порівняно з CK. У шарі ґрунту 10-20 см значення PAD BS50 становить 35,70, що на 46,26 % і 47,71 % нижче, ніж CK і CF відповідно. У шарі ґрунту 20-40 см значення PAD BS50 у поєднанні з біогазовою суспензією та хімічними добривами було найнижчим (33,33), що значно відрізнялося від інших варіантів. PAD CF був підвищений порівняно з CK. Одноразове внесення хімічних добрив CF погіршує стійкість ґрунтових агрегатів й запобігає агрегатуванню, тоді як сумісне внесення органічних і хімічних добрив зменшить ступінь пошкодження агрегатів і полегшить структуроутворення. Розумне внесення добрив, особливо застосування BS50 - біогазової суспензії та хімічних добрив, має позитивне значення для підтримки стабільності ґрунтових агрегатів та покращення якості ґрунту.

pH є важливою хімічним показником ґрунту, який може впливати на розчинність поживних елементів і, зрештою, впливати на надходження поживних речовин у ґрунт. Внесення тільки мінеральних добрив CF може знизити pH, а застосування біогазової суспензії може підвищити pH. Біогазова суміш сама по собі є лужною і містить високий вміст катіонів. Збільшення основ може певною мірою ефективно пригнічувати тенденцію підкислення ґрунту, спричинену тривалим застосуванням хімічних добрив, і кислотно-лужне середовище ґрунтового розчину може бути покращено.

Застосування біогазової суспензії на сільськогосподарських землях може значно підвищити вміст азоту в ґрунті. На глибинах ґрунту 0-10 см, 10-20 см і 20-40 см загальний вміст азоту є найвищим на варіанті BS50. Загальний вміст азоту має тенденцію з глибиною зменшуватися 0-10 см>10-20>20-40 см.

Біогазова суспензія містить велику кількість мікроорганізмів, які можуть сприяти розмноженню ґрунтових мікроорганізмів і посилювати активність відповідних ферментів. Застосування біогазової суспензії має певний вплив на вміст фосфору в ґрунті. Серед усіх шарів ґрунту доступний вміст фосфору для BS50 був найвищим і становив 25,59 мг/кг, 22,50 мг/кг і 20,48 мг/кг в шарах 0-10, 10-20 і 20-40 см відповідно і була встановлена значна різниця між BS50 та іншими варіантами. Вміст доступного фосфору в ґрунті зменшувався зі збільшенням глибини і мав таку тенденцію як 0-10 см>10-20 см>20-40 см, що вказує на те, що доступний фосфор в ґрунті в основному зосереджений у поверхневому шарі. Вміст доступного в ґрунті фосфору був різним у різних шарах ґрунту та різних умовах удобрення, що вказує на те, що застосування біогазової суспензії може покращити рівень забезпечення ґрунту доступним фосфором, але більше не завжди означає краще, підвищення частки біогазової суспензії до BS75, BS100 приводить до зниження вмісту фосфору. У сільськогосподарському виробництві біогазову суспензію слід застосовувати відповідно до типу ґрунту та потреб рослин, щоб збільшити вміст фосфору в ґрунті та сприяти росту врожаю.

Калій має очевидний вплив на підвищення врожайності та якості сільськогосподарської продукції. Доступний калій - це ефективний калій, який може безпосередньо використовуватися рослинами і відіграє незамінну роль у забезпеченні нормального росту та розвитку рослин. Порівняно з CK, вміст доступного калію в ґрунті на різних варіантах із застосуванням біогазової суспензії збільшується неоднаково. У різних шарах ґрунту вміст доступного калію у варіанті BS50 був найвищим, що становило у шарах 0-10 см - 173,71 мг/кг, 10-

20 см - 156,63 мгкг і 20-40 см - 144,06 мг/кг, і значно збільшився порівняно з іншими варіантами. Біогазова суспензія збільшила вміст доступного калію в ґрунті, що сприяло засвоєнню калію рослинами пшениці. Розумне застосування добрив і біогазової суспензії має позитивне значення для збільшення доступного вмісту калію в ґрунті, що сприяє росту культур.

Застосування біогазової суспензії вплинуло на вміст органічної речовини в ґрунті. У шарах ґрунту 0-10 см, 10-20 см і 20-40 см вміст органічної речовини в ґрунті мало таку тенденцію: BS100>BS75>BS50>BS25>CF>CK, а вміст органічної речовини в ґрунті збільшувався зі збільшенням концентрації суспензії біогазу. Існували значні відмінності між усіма варіантами удобрення та CK ($p<0,05$), а вміст органічної речовини в ґрунті BS100 був найвищим (24,24 г/кг, 20,16 г/кг і 18,32 г/кг відповідно). Порівняно з CF, органічна речовина зросла на 32,82 %, 24,44 % і 17,84 %, відповідно, зі з глибиною вміст органічної речовини при різному удобренні зменшився, і вміст органічної речовини мало таку тенденцію як 0-10>10-20 см>20-40 см.

Було вивчено вплив різних пропорцій біогазової суспензії та добрив на активність ґрунтових ферментів. Результати показали, що застосування біогазової суспензії підвищувало активність цукрози в ґрунті, а зі збільшенням у пропорції біогазової суспензії до BS75 активність цукрози підвищувалася, а потім при BS100 знизилася. Активність цукрози на варіанті BS75 була найвищою в 0-10 см, 10-20 см і 20-40 см, яка становила 15,28 мг/г, 30,73 мг/г і 31,28 мг/г відповідно. Цукроза забезпечує енергією організми в ґрунті, підвищує інтенсивність накопичення, розкладання і перетворення органічного вуглецю, підвищує рівень родючості ґрунту.

Застосування суспензії біогазу та хімічних добрив може підвищити активність уреази ґрунту. Уреазна активність комбінованого застосування біогазової суспензії та хімічних добрив (BS25, BS50 та BS75) була вищою, ніж CF.

Зі збільшенням коефіцієнту заміни біогазової суспензії активність уреази спочатку зросла (до BS50), а потім зі збільшенням частки знизилася, що вказує на те, що користь біогазової суспензії для ґрунту залежала від дози, і відповідна кількість була корисною для покращення активності уреази ґрунту, але надмірне внесення добрив було не продуктивним. У 0-10 см, 10-20 см, 20-40 см шарах грунту активність уреази ґрунту була найвищою на варіанті BS50 (235,33 мкг/г, 337,79 мкг/г і 351,45 мкг/г відповідно) і значно відрізнялася від інших варіантів. Комбіноване застосування біогазової суспензії та хімічних добрив може збагатити ґрунт на азот, покращити трансформацію азоту в ґрунті, збільшити поглинання рослинами пшениці озимої азоту.

Як біогазова суспензія, так і хімічне добриво, підвищили активність кислої фосфатази в ґрунті. Зі збільшенням концентрації біогазової суспензії активність кислої фосфатази підвищилася при застосуванні BS50, а потім, із збільшенням частки суспензії – знижувалася. Відповідна кількість біогазової суспензії може каталізувати перетворення фосфору ґрунту у фосфор, який легко поглинається організмами, і покращити швидкість його використання. Активність кислої фосфатази на варіанті BS50 була найвищою в різних шарах ґрунту.

Тенденція щодо активності каталази в грунту на глибинах 0-10 см, 10-20 см і 20-40 см була такою: BS100>BS75>BS50>BS25>CF>CK, й встановлено значну різницю між кожним варіантом удобрення та CK ($P<0,05$); відсутня істотна різниця між CF і BS25 ($P>0,05$), активність каталази BS100 була найвищою, з 93,19 ммоль/г, 95,11 ммоль/г і 97,12 ммоль/г, що було на 12,35 %, 6,21 % і 5,86 % вище, ніж CF, відповідно. Зі збільшенням частки біогазової суспензії активність каталази зростає, тобто суспензія має значний стимулюючий вплив на активність каталази ґрунту, що може прискорити видалення перекису водню в організмах і ґрунті, щоб запобігти пошкодженню. Активність ґрунтової каталази при застосуванні BS100 є найбільшою, а ефект детоксикації ґрунту є найсильнішим.

Тривале внесення хімічних добрив має значний пригнічуваний ефект на активність ґрунтової каталази, що може легко призвести до накопичення кореневих ексудатів, посилюючи тим самим токсичну дію пероксиду водню на посіви. Застосування біогазової суспензії може значно підвищити активність ґрунтової каталази та прискорити розкладання токсичних речовин у ґрунті.

Вертикальний розподіл ґрунтових ферментів має певні закономірності і залежить від родючості ґрунту. Біогазова суспензія може покращити активність ґрунтових ферментів пшениці озимої в шарі ґрунту 0-40 см, а особливо - в шарі ґрунту 20-40 см, що сприяє затримці процесу старіння коренів озимої пшениці в пізній період росту, сприяючи трансформації поживних речовин ґрунту, покращуючи здатність ґрунту постачати поживні елементи для рослини та задовольняючи потребу в поживних речовинах під час росту та розвитку рослин пшениці, що, в кінцевому рахунку, сприятиме покращенню родючості ґрунту та врожайності.

Також було встановлено вплив різних пропорцій біогазової суспензії та хімічних добрив на ріст і розвиток рослин пшениці озимої. І суспензія, і застосування хімічних добрив підвищили врожайність; було виявлено значну різницю у варіантів з удобренням порівняно з контролем ($p<0,05$). Зі збільшенням частки біогазової суспензії врожайність пшениці зросла до BS50, а потім почала знижуватися із подальшим збільшенням частки суспензії. Урожайність пшениці на варіанті BS50 була найвищою порівняно з CF, BS25, BS75 і BS100, збільшення становило 30,79 %, 13,61 %, 4,31 % і 34,06 % відповідно, і між ними спостерігалися достовірні відмінності ($p<0,05$). BS50 - це оптимальна комбінація біогазової суспензії та хімічного добрива, яка може добре відповідати потребам пшениці озимої в поживних речовинах на різних стадіях росту та розвитку, забезпечувати безперервне їх надходження та акумуляцію, сприяти росту та досягати найкращого рівня врожайності пшениці озимої.

Урожайність і елементи продуктивності урожаю пшениці озимої тісно пов’язані між собою. Внесення хімічних добрив та біогазової суспензії може значно збільшити продуктивний стеблостій, кількість зерен у колосі та масу 1000 зерен ($P<0,05$). Урожайність суттєво корелювала з кількістю продуктивних стебел, кількістю зерен в колосі та масою 1000 зерен, а коефіцієнти кореляції становили 0,964, 0,974 та 0,870 відповідно. Найвищий зв’язок встановлено між урожайністю та кількістю зерен у колосі. Таким чином, високий урожай досягався за рахунок збільшення кількості зерен у колосі.

Різні способи підживлення впливали на морфологічні показники озимої пшениці. Внесення добрив збільшило висоту рослини, а отже, збільшило надходження повітря та світлопроникність до нижньої частини, що сприяло росту та розвитку озимої пшениці та фотосинтетичній ефективності, що вплинуло на підвищення врожайності. При застосуванні біогазової суспензії висота рослини була вищою, ніж при застосуванні мінеральних добрив. Висота рослини на варіантах BS25, BS50, BS75 та BS100 становила 77,47 см, 79,52 см, 78,08 см та 78,02 см відповідно. Зі збільшенням частки біогазової суспензії висота рослини збільшувалася до BS50, де вона була найвищою - 79,52 см, і далі зменшувалася, але все ще належала до рослин середньої висоти. Це може не тільки запобігти виляганню, але й дозволить рослинам пшениці озимої отримати відповідну площу живлення, надати необхідні для росту та росту поживні елементи, і більше сприятиме формуванню високого врожаю.

Висота рослини визначається міжвузлями, які подовжуються на різних стадіях розвитку, тому вони по-різному впливають на врожайність. Довжина першого основного міжвузля дуже важлива для пшениці, а саме для стійкості до вилягання. Основа першого міжвузля збільшується з використанням добрив, але швидкість збільшення відрізняється й порівняно з CK, на варіантах CF, BS25, BS50, BS75, BS100 відповідно, збільшилася на 1,81 %, 3,55 %, 0,68 %, 5,96 % і

6,19 %, BS100 має найбільшу амплітуду, що підвищує ризик вилягання. Тобто, цей показник на варіанті BS50 мав найменший приріст, що не тільки сприяло збільшенню висоти рослини, але й підвищувало стійкість до вилягання.

Індекс висоти рослин пшениці (I) — це пропорційне співвідношення між довжиною верхнього і нижнього вузлів стебла, що відображає інформацію про просторове розташування фотосинтетичної зони й роботу та розподіл сполук. Внесення добрив може ефективно покращити індекс висоти рослин озимої пшениці. Якщо значення I високе, нижній вузол коротший, центр ваги нижчий, нижній вушний вузол довший, фотосинтетична область над лопатевим вузлом збільшується, а стійкість до падіння та фотосинтез посилюються. Збільшення індексу висоти рослин за допомогою BS50 формувало продуктивні стебла за умов доброго освітлення, що сприяло підвищенню врожайності пшениці озимої. Індекс висоти рослин значно корелював з продуктивним стеблостоєм, кількістю зерен у колосі, масою 1000 зерен, урожайністю та висотою рослин. Коефіцієнти кореляції між міжвузлями та масою 1000 зерен, I1 та висотою рослини були вище 0,9, що покращує архітектоніку рослини та її стійкість до вилягання. У кореляційному аналізі між урожайністю та індексом висоти рослини, коефіцієнт кореляції між урожайністю та вузлом кущення був найвищим (0,895), тоді як коефіцієнт кореляції між висотою рослин та I1 та вузлом кущення становив 0,971 та 0,954 відповідно. Отже, вузол кущіння відіграє ключову роль у формуванні врожаю. На практиці пшеницю озиму з відносно високою висотою рослин і I1 слід вибирати в межах помірного діапазону висоти.

Підвищення врожайності ґрунтується на накопиченні і розподілі сухої речовини. Комбіноване внесення біогазової суспензії та мінеральних добрив мало значний вплив на масу соломи та в цілому надземну біомасу ($p<0,05$), а найвищі значення отримано на варіантах BS75 та BS50, однак, зі збільшенням частки біогазової суспензії, і навіть на варіанті BS100, який повністю замінює мінеральні добрива, обидва показники показали тенденцію до зниження.

Індекс збору врожаю – це відношення врожаю зерна до надземної загальної маси сухої речовини. Він відображає співвідношення розподілу продуктів асиміляції рослин у зерні та вегетативних органах і є важливим показником для оцінки рівня врожайності та ефективності вирощування культур. Як мінеральні добрива, так і комбінації їх з біогазовою суспензією, підвищували індекс урожаю пшениці озимої ($P<0,05$), а індекс урожаю на варіанті BS50 був найвищим (0,48). Збільшення частки біогазової суспензії в певному діапазоні може покращити як врожайність, так і індекс збору врожаю. Застосування варіанту удобрення BS50 може покращити ріст і розвиток різних органів у більш пізній період росту, скоординувати збалансований розподіл сухої речовини, транспортувати більше продуктів фотосинтезу від донорів до рецепторів, збільшити потік і досягти мети збільшення виробництва.

Фактори, що впливають на врожайність зерна, у цілому врожайність рослини і розподіл речовин, тісно пов'язані з індексом урожаю. Надземна біомаса значно позитивно корелювала з продуктивним стеблостоєм та кількістю зерен у колосі, з коефіцієнтами кореляції 0,935 та 0,944 відповідно, а індекс урожаю також значно позитивно корелював з продуктивним стеблостоєм та кількістю зерен у колосі, з більшими коефіцієнтами кореляції ніж 0,9 (0,911 і 0,901 відповідно). Таким чином, можна ефективно поєднувати високу біологічну врожайність з високим індексом врожаю, а також можливо підвищити врожайність шляхом збільшення кількості продуктивних стебел та кількості зерен у колосі.

Ключові слова: *біогазова суспензія, добриво, поживні речовини, родючість ґрунту, урожайність, пшениця озима, активність ферментів, структура ґрунту, щільність грунту, азот, фосфор, калій, органічні речовини, маса 1000 зерен, ріст і розвиток.*

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CONTENT

ABBREVIATIONS

Control check (CK)

Biogas slurry (BS)

Chemical fertilizer (CF)

Total nitrogen (TN)

Available phosphorus (AP)

Available potassium (AK)

Organic matter (OM)

Mean weight diameter (MWD)

Percentage of aggregate destruction (PAD)

The plant height component indexes (I)

The plant height (L)

Harvest index (HI)

Acid phosphatase (ACP)

Catalase (CAT)

INTRODUCTION

Actuality of theme. Wheat, known as "world grain", is cultivated and consumed in almost every country of the world's five continents. The area and yield of wheat in the world have been the first among cereals for a long time. The yield and quality of wheat are closely related to the national economy and people's livelihood. Many studies have shown that wheat quality is not only affected by genetic factors, but also by wheat growing environment and cultivation factors. Fertilizer has the greatest effect on wheat yield and quality among many cultivation factors.

In crop cultivation, the utilization rate of chemical fertilizer as fertilizer is low, the production cost is high, and the soil quality is easy to decline. Biogas slurry is regarded as a good fertilizer source in agricultural production, containing a variety of nutrients required for plant growth such as N, P, K, rich organic matter and various growth hormones, vitamins, etc. It has the advantages of high fertilizer efficiency and easy absorption by plants, and has positive effects on improving the yield of agricultural products, reducing production costs and improving soil. Promoting the application of biogas slurry in agricultural production can not only provide fertilizer for farmland, but also reduce the environmental pollution of large-scale aquaculture waste, but also realize agricultural water-saving irrigation, which can effectively promote the organic combination of agriculture and animal husbandry development, and promote the promotion and application of efficient circular and low-carbon agricultural technology.

At present, there have been many research reports on the application of biogas slurry in farmland in China and other countries, but due to the climate environment, soil conditions, physical and chemical properties of biogas slurry in different regions there are great differences, the use of biogas slurry is also very different, otherwise it is easy to cause the decline of fertilizer utilization rate, crop growth inhibition, yield quality decline and other adverse effects. Therefore, in agricultural production should be adapted to local conditions, reasonable and efficient use of biogas slurry. In this study, winter wheat in Zhoukou lime concretion black soil was used as the research object to study the effects of different concentrations of biogas slurry on nutrient soil physical and chemical properties, enzyme activities and winter wheat yield, providing scientific basis for scientific application of biogas slurry, improving soil texture, increasing wheat quality and high yield, and realizing sustainable agricultural development.

"Storing grain in the land and storing grain in technology" is a national strategic measure of China and a guarantee for world food security. To make scientific fertilization scheme, optimize the use of biogas slurry in agricultural production, improve agricultural production efficiency and promote the sustainable development of agriculture is the necessary way to achieve soil health, planting science and improve the quality and yield of winter wheat.

Connection of work with scientific programs, plans, themes. This work was supported by the science and technology key project of Henan Province (212102110388) and by the key scientific research project at Henan Provincial University (20B210004), China. The research also was carried out in accordance with the thematic plans of research works of the department of agrotechnologies and soil science of the Sumy National Agrarian University and within the framework of the topics “Biologization of the farming system through a rational combination of methods of soil cultivation and sideration” 0115u0010055. In 2021 our project “Impact of organic fertilizers from biogas plants on microbiological, physical and chemical properties of soil and crop growth” was supported by minigrant due to participating in the project “Interuniversity cooperation as a tool for enhancement of quality of selected universities in Ukraine” financed within the Development Cooperation of the Czech Republic (Ministry of Foreign Affairs).

The purpose and objectives of the study. The purpose of the research is to determine the effect and the best amount of biogas slurry to improve the yield and quality of winter wheat. Set the following 4 tasks according to the goal：

- To determine soil physicochemical indexes, enzyme activities, straw and grain data at winter wheat harvest period after fertilizer and biogas slurry application;
- To compare the effects of fertilizer and biogas slurry application on each index and the correlation;
- To evaluate the effect of fertilizer and biogas slurry application;
- To determine the fertilizer type and application amount suitable for Zhoukou lime concretion black soil.

Object of study – nutritional value of biogas slurry and increasing yield and improving quality of winter wheat.

Subject of study. The effects of biogas slurry on improving the quality and efficiency of winter wheat were analyzed from the changes of soil physical and chemical properties, enzyme activities, straw and grain of winter wheat.

Research methods. The research mainly used the following research methods：

Observational method: select the samples that meet the requirements of research through observation.

Experimental method: obtains scientific data by controlling conditions, such as physical and chemical properties, enzyme activity, etc.

Literature method: to understand the research progress of the topic, so as to determine the appropriate concentration gradient of biogas slurry and the application amount of chemical fertilizer, methods for determination of physical and chemical properties and enzyme activity etc., and ensure the accuracy of the experimental results by reading and collecting the literature.

Statistical method: the enzyme activity, thousand-grain weight, protein content were analyzed to understand the influence of biogas slurry on winter wheat.

Induction method: according to the measured data, statistical analysis, etc., comprehensive analysis and comparison of fertilizer and biogas slurry application effect.

The scientific novelty:

The practicability of the topic: the research topic closely follows the urgent problems in the current agricultural development, using the combination of agriculture and animal husbandry as the research entry point, the return of biogas slurry to the field not only solves the digestion and effective utilization of biogas slurry, but also increases the grain yield and quality, which can be said “to kill two birds with one stone”, and in line with the concept of sustainable development.

The comprehensiveness of the experimental design: the concentration of biogas slurry was comprehensive (0, 25 %, 50 %, 75 %, 100 %), the soil layer was comprehensive (0-10 cm,10-20 cm, 20-40 cm), the measurement index was comprehensive (soil and winter wheat), and the change of different concentrations of biogas slurry in the soil layer and its impact on winter wheat could be fully understood, ensuring the accuracy of the experimental results.

The benefit of the research results: the research results determined the optimal concentration of biogas slurry used in Zhoukou lime concretion black soil, and confirmed the effects of biogas slurry on agricultural production, such as increasing fertility, stress resistance and production, which can not only achieve the effect of increasing production and quality, but also reduce the environmental pollution of large-scale aquaculture waste, so that people realize the value of biogas slurry in economic benefits, social benefits and environmental protection. In the future, biogas slurry will be more and more widely used in promoting the organic combination of agriculture and animal husbandry to achieve low-carbon and efficient agriculture.

For the first time the effects of application of biogas slurry on soil and winter wheat in lime concretion black soil in Zhoukou were studied and evaluated comprehensively. The difference of biogas slurry with concentrations was determined, and the best biogas slurry application concentration BS50 was determined.

It was improved the application amount of biogas slurry in the combination of agriculture and animal husbandry, increased the yield and quality of wheat, and provided a basis for the scientific application of biogas slurry.

The practical significance of the results. Based on this research, the evaluation system of biogas slurry application effect was obtained, the relationship between soil and winter wheat measurement indexes was clarified, the soil fertility was improved, the growth and development of winter wheat was promoted, the yield and quality of winter wheat was increased. At the same time, the use of chemical fertilizer, the cost of agricultural production and environmental pollution were reduced, and agriculture and animal husbandry were closely combined. It is beneficial to the sustainable development of agriculture, and the research has been recognized by the Department of Education and the Department of Science and Technology of Henan Province (appendix A). Results of the experiment are implemented in the educational process of the Sumy National Academy of Sciences (appendix B).

The personal contribution of the applicant. This research is determined by the applicant and the scientific supervisor according to the applicant's situation and previous research work, the applicant has conducted the implementation of research, sampling, data analysis, review of the topic, and the writing of papers.

Approbation of dissertation results. The main items, research results and conclusions of the work during 2020 - 2023 were presented and discussed at the meetings of the Department of Agrotechnologies and Soil Science of Sumy National Agrarian University, Department of Education and the Department of Science and Technology of Henan Province, International scientific and practical conference dedicated to World Soil Day (Kyiv, NULES. 7.12.2021), International Scientific and Practical Conference «Honcharivski Chytannya» (Sumy National Agrarian University, 25.05.2021, 25.05.2022), International Scientific Conference «Global and national trends in life sciences» (Nizhyn, 6.04.2023).

Publications. Based on the results of the research, one article was published in a journal indexed in the Scopus database (Q1 quartile), 2 articles were published in professional journals, 1 article – in a journal of EU, 3 articles in scientific professional publications of China and in the proceedings of 4 conferences.

The structure and scope of the dissertation. The dissertation contains annotations, a list of abbreviations, introduction, five chapters, conclusions, a list of references, appendixes. The volume of dissertation is 151 pages of computer text, includes 13 tables and 32 figures.

CHAPTER 1
REVIEW OF EFFECTS OF BIOGAS SLURRY ON SOIL AND CROPS

1.1. Effects of biogas slurry on soil physicochemical properties

In recent years, with the development of agricultural production in China, the use of chemical fertilizer in agricultural production has been increasing, the multiple cropping index of cultivated land and the yield level of crops have been continuously improved, and the nutrient status of farmland soil has undergone significant changes. China's grain production is closely related to the input of nitrogen fertilizer, phosphate fertilizer and potassium fertilizer. However, improper application of fertilizer will lead to soil fertility degradation, which seriously threatens the sustainability of agricultural production. Long-term single use of inorganic chemical fertilizer leads to the deterioration of soil structural stability, the reduction of porosity, and the dispersion of micro-aggregates. As the number increases and the destruction rate of water stability structure increases, the cultivated layer of soil loses its original physical and chemical properties, which is not only detrimental to the development of plant roots, but also changes the water-gas-heat environment and affects the balance of fertilizer - soil - crop nutrient system (Zhang et al. 2010).

Biogas slurry is rich in N, P, K and other nutrients required by plants, and rich in humic acid, organic matter, trace elements and other nutrients. It is a kind of quick-acting and slow-acting organic compound fertilizer, they are found in the soil under the condition that nitrogen, phosphorus, potassium and other nutrients in the soil are equal, soil fertility can be significantly improved after application of biogas slurry, and the contents of nitrogen, phosphorus, potassium and trace elements in the soil can be increased (Gan et al. 2011), its physical properties are also improved (Wu et al. 2013). So far, biogas technology has been applied in more than 60 countries.

The results of long-term positioning experiments (Bronick et al. 2005; Kaiser et al.

2005) show that long-term single application of chemical fertilizer can increase the proportion of soil solid pores and soil bulk density, and at the same time reduce the field water capacity. Therefore, the application of chemical fertilizer combined with the application of organic fertilizer can significantly improve the physical and chemical properties of soil.

Moreover, many studies have found that the application of biogas slurry can improve soil pH to varying degrees and effectively prevent soil acidification caused by long-term application of chemical fertilizers. Zhu Yanli et al. (2012) studied the effects of biogas slurry application on soil physical and chemical properties and found that application of biogas slurry effectively improved soil pH and porosity, reduced soil bulk density, and increased soil total nitrogen, total phosphorus, total potassium and organic matter contents.

According to the research of Wang Zongshou (2007b), it can be found that the application of biogas slurry can increase soil pH by 0.23-1.03 units, with an increase rate of 5.37-24.07 %, soil organic matter increased by 0.14-3.11g/kg, with an increase rate of 0.44-9.80 %. Available phosphorus, available potassium and alkali-hydrolyzed nitrogen were all increased, and the increase rate was available phosphorus >Quick available potassium >Total phosphorus >Total nitrogen > Total potassium >Alkali hydrolyzed nitrogen. Wang Fuquan et al. (2015) took "Chuanmai 58" wheat as experimental material to study the effect of biogas slurry returning to wheat field on soil improvement. The results showed that biogas slurry could reduce soil bulk density, increase soil porosity and pH value, effectively prevent soil acidification, promote the formation of soil aggregate structure, increase soil water and fertilizer retention ability, and improve soil physical and chemical properties.

Zhang Li et al. (2015) conducted a 3-year field experiment and concluded that replacing fertilizer with biogas slurry could significantly improve the structure of newly reclaimed red soil and increase the contents of soil organic carbon, total nitrogen, available nitrogen, available phosphorus, available potassium, and available state content and micronutrients.

Studies have shown that biogas slurry irrigation can improve the biomass and metabolic activity of soil microorganisms, increase the activity of microorganisms, and enhance the ability of soil to fix organophosphorus. Then microorganisms can decompose and send some fixed phosphorus, which can be absorbed and utilized by plants, and finally reduce the pollution of leaching on groundwater (Oehl et al. 2004; Odlare et al. 2008).

Ni Liang et al. (2008b) and Kang Lingyun et al. (2011) also proved that surface soil nutrient content increased after application of biogas slurry. However, the experimental results of Kang Lingyun et al. also found that a large amount of phosphorus accumulated in surface soil and nitrogen leaching loss, a large amount of phosphorus accumulation will cause water eutrophication, and nitrogen loss will lead to groundwater pollution risk. It can be seen that the application of biogas slurry can improve the soil aggregate structure and soil quality, and at the same time may bring about secondary environmental problems such as water pollution, secondary salt collapse, soil acidification and compaction, which will become the focus of future research on the agricultural effect of biogas slurry, and carry out scientific and rational digestion of biogas slurry in farmland.

The pH of biogas slurry is mostly 7.5-8.0, which is alkaline, which can effectively improve soil acidification and balance soil pH. At the same time, rich elements such as nitrogen, phosphorus and potassium, organic matter and humic acid enter the soil system with the application of biogas slurry, changing the physical and chemical properties and distribution of soil.

· In the process of agricultural use of biogas slurry containing a large amount of organic matter, this organic matter is brought into the farmland. The organic matter acts as a nutrient for microorganisms, increasing the number of microorganisms and thus increasing the content of organic matter in the soil. Li Youqiang et al. (2014b) research results show that that replacing fertilizer with biogas slurry could significantly increase the contents of N, P, and K in wheat soil, with total nitrogen content increased by 15.3 %, available phosphorus content increased by 34.5 %, and slow-available potassium content increased by 6.2 %. After biogas slurry replaces fertilizer, cations in soil can be adsorbated by organic matter, thereby improving the soil's fertilizer holding capacity and buffering performance, providing a good environment for the growth of microorganisms in the soil, and keeping the soil loose.

Biogas slurry is an excellent organic liquid fertilizer, which can completely replace chemical fertilizer. A large number of experiments show that long-term application of biogas slurry can improve the physical and chemical properties of soil (Garg et al. 2005).

1.2. Effect of biogas slurry on soil enzyme activity

As one of the important indicators affecting soil biological activity and soil fertility, soil microbial enzyme activity is mainly derived from plant root exudates and soil microorganisms. It participates in the decomposition and transformation of soil organic matter and plays an important role as a catalyst in the biochemical reaction of soil. The strength of soil nutrient transformation can be reflected by the level of soil enzyme activity. Soil nutrients, microorganisms and other factors can also affect soil enzyme activity.

Tang Hua (2011) found in the process of applying biogas slurry to wheat fields that soil urease activity increased, but soil dehydrogenase activity decreased after agricultural application of biogas slurry. However, in the process of application of biogas slurry to paddy fields, it was found that the biogas slurry not only increased the soil urease activity, but also significantly increased the catalase and invertase activities (Ni et al. 2008b). Wang Gui fang et al (2009a) showed that the application of biogas slurry as top-based fertilization in apple orchards could increase the activities of urease and invertase in soil. Feng Wei et al. (2013) showed that the 1:3 complex application of biogas slurry with fertilizer and nitrogen fertilizer could increase the activities of soil protease and catalase. Wan Haiwen et al. (2017) showed that topdressing of wheat with biogas slurry application level of 22,500 kg/hm^2 could significantly increase the activities of soil catalase, alkaline phosphatase and urease. Enzyme activities can predict and influence soil fertility and improve soil conditions. Zhang Wudi et al. (2009) showed that application of biogas slurry can effectively improve soil enzyme activity and increase the number and diversity index of soil microorganisms.

Soil biological activity plays an important role in changing soil physical and chemical properties, and its biological activity is indirectly or directly affected by soil physical properties. Soil enzyme activity was affected by soil structure and mechanical composition, and the effect was obvious. Studies have confirmed that soil clay adsorbs soil enzymes or soil enzymes combine with humus molecules and exist in the soil through inorganic organic complex, which makes the enzyme activity of the same type of soil light soil lower than that of clay soil. Soil microaggregates and aggregates are an important index to represent the degree of "soil and fertilizer integration" and the level of soil fertility (Gao Ziqin et al. 1992). It was found that aggregates with the smallest particle size had the highest soil enzyme activity, which was consistent with the distribution of microorganisms (Mendes C et al al.1999), Feng Guiying (1999) tested the adsorption capacity of eight kinds of soil clay adsorbed by urease and found that soil clay could adsorb soil enzyme, but the adsorption capacity value was different. The interaction between soil clay and organic matter in urease adsorption can be shown by the relationship between the adsorption amount of soil clay and urease and their organic mass. Wang Jingkuan et al. (2000) tested the activities of multiple enzymes in typical brown soils with different fertility and their micro-aggregates in different areas of Liaoning Province and concluded that the activities of polyphenol oxidase, sucrase, neutral phosphatase and urease in low-fertility brown soils were significantly lower than those in high-fertility brown soils. Enzyme activity is also affected by soil porosity. Dick et al. (1988) believed that the diffusivity of gas and water in soil changed after soil compaction, which also affected soil biology and biological parameters, and concluded that soil porosity and water penetration had a great impact on nutrient cycling. Water, air and heat are affected by soil structure, which in turn affects the level of soil enzyme activity.

Soil enzyme activity is affected by soil chemical properties, mainly in the following three aspects: First, the activities of soil microorganisms and plant roots are affected by soil energy and nutrient status, and then affect enzyme activity; Second, the composition and characteristics of soil organic mineral complex and the content of soil organic matter determine the stability of enzymes. The activation and inhibition of soil enzymes are affected by certain chemicals. Soil total nitrogen content, soil organic carbon and soil phosphatase, urease, sucrase and protease are closely related (Zantua et al. 1977; Dick 1984); He Wenxiang (2001) concluded through research that soil samples in northern Shaanxi had a considerable relationship with pH value, cation substitution amount, alkali-soluble nitrogen, total nitrogen and organic matter, but it was negatively correlated with pH. Soil enzymes are directly related to the concentration of hydrogen ions in soil, the absorption capacity of soil and the total amount of adsorbable cations. The properties of adsorbed enzymes are determined by the properties of adsorbent cations. The accumulation of soil enzyme and the manifestation of enzyme activity are mainly through the clay soil with high base saturation and high humus content. The composition and physiological characteristics of the enzyme, the amount of production is determined by the pH value of the soil, but also determine the degree of preservation of enzyme activity and the amount of enzyme in the soil, at different pH values, the enzyme molecules will produce different dissociation, and only a certain form of dissociation of the enzyme has catalytic activity.

At present, with the continuous expansion of biogas projects at home and abroad, the annual output of biogas slurry is also increasing. It can be said that the application prospect of biogas slurry is very broad and the development potential is huge.

1.3. Effects of biogas slurry replacement of fertilizer on crop yield and quality

The resource utilization of biogas slurry plays an important role in the healthy and sustainable development of agriculture, which can not only improve soil fertility and pollution remediation, but also achieve the effect of increasing production and improving quality (Yu et al. 2010), which has the potential to replace or combine with fertilizers to produce pollution-free vegetables, thus achieving cost savings (Tiwari et al. 2000).

In farmland ecosystem, biogas slurry is mainly used as base fertilizer, topdressing fertilizer and foliar spraying to achieve high yield of crops. Wang Zhi et al. (2009b) significantly increased the corn yield by applying biogas slurry, with an increase rate of 7.64-10.34 %. Wu Huashan et al (2012a) found in the study on biogas slurry irrigation of corn fields that 50 % biogas slurry combined with chemical fertilizer could increase corn grain yield more than the replacement of full biogas slurry irrigation (Wu Huashan et al., 2012). Du Zhenjie (2014) also obtained the same result in the study of biogas slurry irrigating wheat fields. Huang Hongying et al. (2013a) concluded that the highest yield was achieved when the concentration of rice biogas slurry replaced fertilizer was 75 %, and the highest yield was achieved when the concentration of wheat biogas slurry replaced fertilizer was 50 %. Wang Yongcui et al. (2010b) concluded through research and comparison that the application of biogas slurry and nitrogen fertilizer in a certain proportion was conducive to the accumulation of dry matter of silage maize and affected the yield, and the recommended application amount was $90m^2/hm^2$.

Panicle number per unit area, grain number per panicle, 1000-grain weight and seed setting rate are the factors of yield. Shao Wenqi et al. (2017) studied the effects of biogas slurry on rice growth and yield, and the results showed that: $750t/hm^2$ is a watershed of biogas slurry application. When the application rate of biogas slurry is lower than this standard, the increase of biogas slurry replacement ratio can significantly improve the tillering ability and plant height of rice. Compared with traditional chemical fertilizer treatment, the total panicle number of rice can be increased by biogas slurry replacement.

He Shunmin et al. (2005) showed that biogas slurry was an organic fertilizer with relatively slow fertilizer efficiency. During the growth and development of rice, the plant height and tillering number of rice were higher than that of the control treatment when biogas slurry was applied in combination with chemical fertilizer, and the growth was stable in the early stage, while the color transformation was good in the later stage by applying different concentrations of biogas slurry. Li Youqiang et al. (2014b) took Yangmai16 as the experimental material and showed that the application of biogas slurry promoted the tillery ability of wheat and increased the head rate. Due to the application of biogas slurry, the SPAD of wheat increased, the photosynthetic capacity was strengthened, and the dry matter accumulation was significantly increased. Therefore, the wheat yield was significantly higher than that of the control treatment, and the optimal fertilizer replacement amount of biogas slurry was 60.000-75.000 kg/hm^2. Wang Wenbo et al. (2014) obtained the same result through different concentrations of biogas slurry irrigation: the rice yield of biogas slurry instead of fertilizer was greater than that of fertilizer treatment. Wan Haiwen et al (2017) found that due to the application of biogas slurry in the jointing stage of maize, the leaf green content of leaves increased, which promoted the progress of maize photosynthesis and the accumulation of dry matter in the plant, so it can be seen that the application of biogas slurry had a significant yield increase effect.

It can be seen that biogas slurry replacing fertilizer can improve the yield of rice and wheat mainly by increasing tillering number, plant height, head rate, thousand grain weight, etc. Meanwhile, biogas slurry replacing fertilizer can significantly or extremely significantly increase the yield of other crops, and the application amount of biogas slurry has a more suitable application range.

Biogas slurry has been widely used in agricultural production, but biogas fermentation broth has different effects on increasing production of different crops. In wheat after watering biogas fermentation liquid, yield 20 kg more than after water and urea, increase 10 % leaf spraying made wheat grow strong and resistant to lodging. The combined application of biogas fermentation liquid with nitrogen and phosphorus fertilizer was 20.5 % higher than that of control, 14.5 % higher than that of nitrogen fertilizer alone, and 5.6 % higher than that of phosphorus fertilizer (Gupta et al. 2002).

Biogas slurry is a decomposed water-soluble fertilizer. Biogas slurry has been widely used in crop research, especially in vegetables and food crops. Many studies have shown that the application of biogas slurry can improve the yield and quality of crops, reduce the use of fertilizers, and achieve the effect of low investment and high yield. Wang Fuquan et al. (2015) concluded through the study and analysis of wheat-rape rotation system that biogas slurry instead of chemical fertilizer treatment could improve wheat quality to a certain extent compared with whole chemical fertilizer treatment. Xu Weihong et al. (2005) also mentioned in their published articles that biogas slurry fertilization had a good yield increase effect on many crops, especially in the impact on crop quality.

Conclusions to Chapter 1

Therefore, it is necessary to systematically analyze the effects of different proportions of combined application of biogas slurry and chemical fertilizer on soil physical and chemical properties and enzyme activities in different soil layers of lime concretion black soil in Huang-Huai-Hai Plain, as well as the effects on the growth and development of winter wheat, and determine the appropriate dosage of biogas slurry in Zhoukou area, so as to provide a scientific basis for soil improvement of lime concretion black soil.

CHAPTER 2
CONDITIONS, MATERIALS AND METHODS OF RESEARCH

2.1. The materials

2.1.1 The site

The research area is located in Leizhuang Village(114°36′ E, 33°45′ N), Dawu Township, Shangshui County, Zhoukou City, Henan Province. The area is located in the southwest of Shangshui County. The overall terrain is relatively flat and low in the east, with an altitude of 48-54 m. Rich in hydraulic resources, the annual average precipitation 785.1 mm, mainly concentrated in June to September, the annual frost-free period of about 200 days, the soil type is lime concretion black soil, suitable for agricultural production.

"China's wheat in Henan Province, Henan wheat in Zhoukou City", Zhoukou city is an important wheat production base in Henan province, the annual total grain production of more than over 9 billion kilograms, ranking first in the province, can provide a large number of commercial grain to the country every year, to ensure national food security has made important contributions. And Shangshui County of Zhoukou city is the national super grain production county, wheat grain output is about 560,800 tons/year.

The specific geographical location is shown in Figure 2.1. This precipitation data is obtained from the Meteorological Bureau by my tutor in China.

According to long-term observations, the highest temperature in the region occurred in June, which is also the event of the winter wheat harvest, the highest temperature in 2020-2022 occurred in June 2022, with an average maximum temperature of 29°C, and the low temperature weather occurred in January and December every year, and the lowest average temperature in 2020-2022 was in

December and January 2022, and January 2020, which is suitable for the growth of winter wheat (Fig. 2.2, appendix C). Precipitation rate are showed in Appendix D.

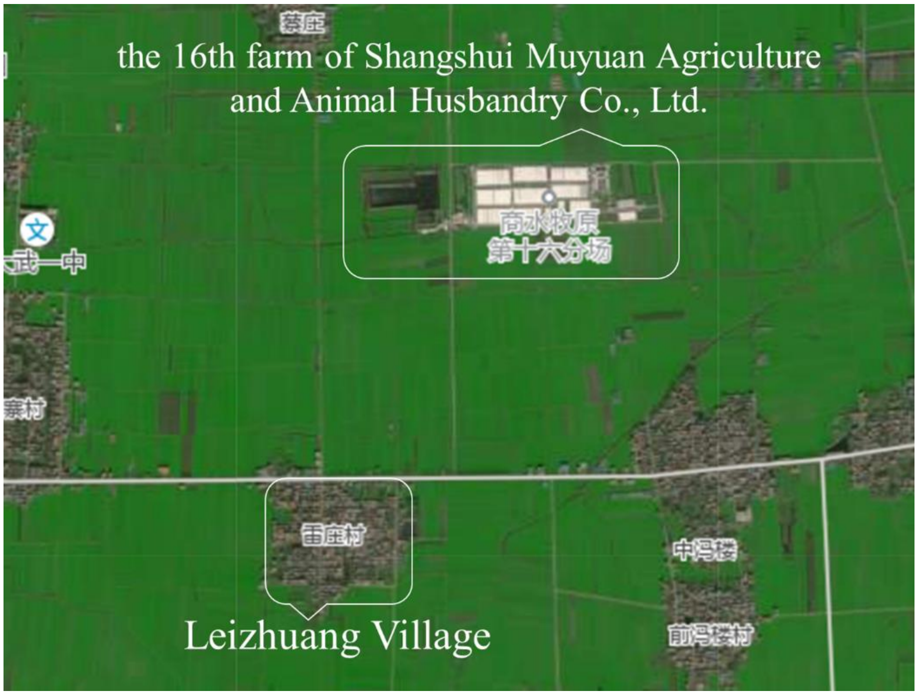

Fig. 2.1. Location of experimental plots

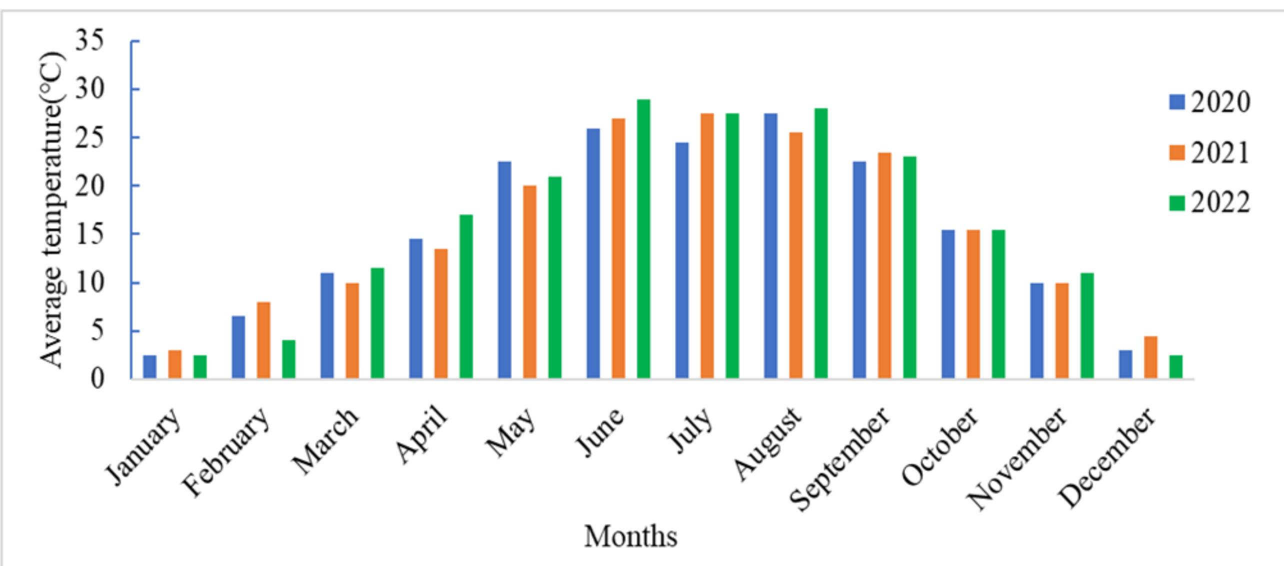

Fig. 2.2. Average temperature per month for 2020-2022

2.1.3. The biogas slurry

The biogas slurry comes from Shangshui Muyuan Agriculture and Animal Husbandry Co., Ltd., which is mainly engaged in pig breeding and is a national key leading enterprise in agricultural industrialization. It has realized automatic pig breeding, and the number of pig farms in 2022 has reached 61,201 million, ranking first in the world.

Shangshui Muyuan Agriculture and Animal Husbandry Co., Ltd. has 11 farms in Shangshui Country, and the biogas slurry used in the field is taken from the nearby biogas slurry storage tank of the 16th farm. The main raw material is the liquid mixture of pig manure and urine, and also includes the washing water of some pig pens. By using microbial anaerobic fermentation process, the fermentation temperature is controlled to 35-40 ℃, and the retention time is 7-10 days according to seasonal changes. Aquaculture water consumption and fecal waste discharge vary with the breeding season to a certain extent. Since the breeding scale and the quantity of feedings in the farms remain basically the same during the experimental period, and the feeding method and fermentation process also adopt a standardized process, the physical and chemical properties of the biogas slurry produced can be ensured to be relatively stable and uniform (Fig. 2.3).

Fig. 2.3. Biogas slurry fermentation process

The basic composition of the biogas slurry is shown in Table 2.1

Table 2.1

Basic properties of the selected biogas slurry

Index	Content
pH	7.48
Total nitrogen (kg/m^3)	1.36
Total phosphorus (kg/m^3)	0.15
Total potassium (kg/m^3)	0.57
Organic matter (kg/m^3)	18.64

2.2. Test methods

2.2.1 Sample Site Settings

In this experiment, based on the principle of equal nitrogen content (the total fertilizer application in wheat growing season was controlled at N 180 kg/ha), a total of 6 treatments were set up, and each treatment was set up with 3 replicates. The random block arrangement was adopted, and the area of each replicate area was about 66.7 m^2.

The treatments were as follows:

② Control (CK), that is, no application of biogas slurry and chemical fertilizer;

② Full fertilizer (CF), all chemical fertilizer nitrogen;

③ Fertilizer and biogas slurry irrigation (BS25), 25 % nitrogen from biogas slurry and 75 % nitrogen from fertilizer during winter wheat planting;

④ Fertilizer and biogas slurry irrigation (BS50), 50 % nitrogen from biogas slurry and 50 % nitrogen from fertilizer during winter wheat planting;

⑤ Fertilizer and biogas slurry irrigation (BS75), 75 % nitrogen from biogas slurry 25 % nitrogen from chemical fertilizer during winter wheat planting;

⑥ Biogas slurry irrigation (BS100), only biogas slurry irrigation during winter wheat planting.

Fertilization inputs in wheat season were N 180 kg/ha, P_2O_5 90 kg/ha and K_2O 90 kg/ha. In order to ensure equal phosphorus and potassium nutrients in each biogas slurry treatment except CK, heavy superphosphate ($Ca(H_2PO_4)_2 \cdot CaHPO_4$) and potassium sulfate (K_2SO_4) were used to supplement the specific fertilizer application amount, as shown in Table 2.2.

Table 2.2

Nutrient supply conditions of biogas slurry irrigation proportion in wheat cultivation

Treatments	Biogas Slurry(BS)			Chemical Fertilizer(CF)		
	N (kg/ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)	N (kg/ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)
CK	0	0	0	0	0	0
CF	0	0	0	180.00	90.00	75.00
BS25	45.00	5.00	18.75	135.00	85.00	56.25
BS50	90.00	10.00	37.50	90.00	80.00	37.50
BS75	135.00	15.00	56.25	45.00	75.00	18.75
BS100	180.00	20.00	75.00	0	70.00	0

70 % of the biogas slurry needs to be watered 5 days before sowing, fertilizer is applied at the time of sowing, and the remaining 30 % of the biogas slurry is applied into the soil by sprinkler irrigation during winter irrigation. When applying fertilizer at the same time, the water brought in by single biogas slurry irrigation treatment was taken as the standard, and the water brought in by different proportions of biogas slurry irrigation was deducted. After seeding, water irrigation was carried out to keep the water input of all plots equal.

The winter wheat variety used in the test was Fengde Cunmai 21, a wheat variety with high yield, high quality and multiple resistance, belonging to the semi-winter high-quality variety commonly planted in this area. Nitrogen fertilizer was applied according to different treatments, and other management was consistent with that of local wheat.

2.2.2 Sample collection and processing

2.2.2.1 Soil sample collection

After wheat harvest at the end of May and the beginning of June from 2020 to 2022, three quadrates were randomly selected in each treatment area according to the "S" shaped sampling method, and soil samples at the upper and lower levels of 0-10 cm and 10-20, 20-40 cm were respectively taken with soil drill. The soil bulk density was determined by a ring knife to collect the original soil samples of the corresponding soil layer (Fig. 2.4). At the same time, parallel samples were randomly taken from pairs treated without biogas slurry under similar terrain.

Fig. 2.4. Soil sampling for bulk density definition

The plant residues, roots and large stones were removed and brought back to the laboratory. Part of the fresh soil was stored in a refrigerator at 4°C for the determination of soil enzyme activity, and part of the fresh soil was air-dried through different soil screens to determine the soil physicochemical properties.

2.2.2.2 Winter wheat sample collection

After physiological maturity of winter wheat each year, 20 representative winter wheat plants in each plot were randomly selected, and their plant height, base internode length and subear internode length were measured, and the average value of each index was calculated. At the same time, a representative 1 m double-row area was randomly selected to calculate the number of ears per m^2, which was converted into the number of ears per hectare according to the sowing row spacing of wheat. In addition, 20 wheat ears of uniform size were randomly collected in the selected 1m double-row area to investigate the number of grains per ear. After the harvest of 1m double-row area wheat was threshed and dried, 1000 grains were randomly selected, the 1000-grain weight was measured, and the average value was obtained 5 times. Finally, an area of 1 m×1 m was randomly selected in each plot for actual yield measurement, and the dried grain weight and dry matter weight of straw in the ground were weighed to obtain the actual yield and dry matter accumulation in the ground, and the harvest index was calculated.

Theoretical yield of wheat = the number of ear ×grain number per ear×thousand grain weight, because the units of thousand grain weight is different from other, so the thousand grain weight is plotted separately.

2.2.3. Measurement indexes and methods

2.2.3.1 Determination of basic components of biogas slurry

The contents of total nitrogen, total phosphorus and total potassium in biogas slurry were determined by referring to the Monitoring and Analysis Method of Water and Wastewater [21], and the specific methods are shown in Table 2.3.

Table 2.3

The basic properties of biogas slurry

Index	*Method*
pH	Acidometer method
Total nitrogen	Ultraviolet spectrophotometry
Total phosphorus	Spectrophotometry
Total potassium	Flame photometry
Organic matter	Potassium dichromate REDOX titration method

2.2.3.2. Determination of soil physical and chemical properties

The analysis of soil physical and chemical properties was determined by referring to the methods in the textbook of soil agrochemical analysis. The specific methods are shown in Table 2.4.

Table 2.4

The determination method of soil physical and chemical properties

Index	*Method*
pH	Acidometer method
Bulk density	Ring knife series analysis
Aggregate	Wet screening
Total nitrogen	Semi-micro Kelvin method
Rapidly available phosphorus	Ammonium fluoride hydrochloric acid extraction - molybdenum antimony resistance colorimetric method
Quick available potassium	NH_4Oac extraction, flame spectrophotometry
Organic matter	Potassium dichromate REDOX titration

2.2.3.3 Calculation of experimental indexes

Soil aggregate

Soil aggregate failure rate (PAD), mean weight diameter of water-stable aggregates (MWD) were calculated using the following formula:

PAD= $(DR_{0.25} - WR_{0.25}) / DR_{0.25}$ *100%

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

Where $DR_{0.25}$ and $WR_{0.25}$ are the aggregate content obtained by dry screening and wet screening methods greater than 0.25mm, respectively. x_i is the average diameter of any adjacent aggregate size (mm), wi is the proportion of aggregate weight of a certain aggregate size.

Wheat plant height composition index

Wheat plant height composition index refers to a certain proportion between the length of the upper and lower internode of the stalk, and its value is the ratio of the length of any internode and the sum of the length of the internode plus the next internode (In) or the ratio of the sum of the length of the subspike internode and the top second stem to the plant height (I_L). The ratio determines the planting position and configuration of the leaves on the stem, and is therefore closely related to yield traits.

Set plant height as L, Ln as the NTH internode length, n as the top-down node, and I as the component index of plant height. I_L and In between nodes can be obtained according to the following formula:

$I_L=(L_1+L_2)/ L$; $In=L_n/ (L_n+L_{n+1})$

In the formula, L is the plant height, and L_1, L_2, L_n and L_{n+1} are the first, second, n and n+1 internode length from top to bottom.

The Harvest Index

The Harvest Index (HI), also known as the Coefficient of economy, is the ratio of Economic yield (grains, fruit, etc.) to the above-ground biomass at the time of harvest.

The formula is as follows: The Harvest Index = yield/biomass x100%.

2.2.3.4 Determination of soil enzyme activity and data analysis

The determination method of soil enzyme activity was referred to the "Soil Enzymes and Their Research Methods" edited by Guan Songyin and the specific methods were shown in Table 2.6.

Data analysis and were performed using Excel2019 and Spss25.0.

Table 2.6

The determination method of soil enzyme activity

Index	Method	Unit
Urease	Indophenol blue colorimetry	$ug \cdot g^{-1}$ (37℃, 24h)
Sucrase	3, 5-dinitrosalicylic acid colorimetric method	$mg \cdot g^{-1}$ (37℃, 24h)
Acid phosphatase	Colorimetric method of disodium phenyl phosphate	$mg \cdot g^{-1}$ (37℃, 24h)
Catalase	Ultraviolet spectrophotometry	$mmol \cdot g^{-1}$ (25℃,24 h)

2.2.3.5 Reagents and instruments

The main reagents and instruments used in the experiment are shown in the following table 2.7 and 2.8.

Table 2.7

The experimental reagents list

3-5 dinitrosalicylic acid	Toluene	Sucrose	Hydrogen peroxide
Aluminium sulfate	Sodium hydroxide	Sodium hypochlorite	Vitriol
Phenol	Sodium chloride	Boric acid	Ethanol

Table 2.8

Instruments and equipments used in the experiments

Device Name	Model	Manufacturer
Electronic analytical balance	FA2104	Changzhou lucky electronic equipment limited
Microcoder	SpectraMax Plus384	Molecular Devices，America
Full temperature oscillating incubator	HZQ-F160	Jintan Hualong experimental instrument factory
pH meter	Starter 3100	Ohaus
Spectrophotometer	724	Shanghai Youke Instrument Co., LTD
thermostatic water bath	HS-4	Jintan Hualong experimental instrument
Electric blast drying oven	101-2AB	Tianjin Test Instrument Co., LTD
Conductivity meter	Starter 3100c	Ohaus
Protein detector	HD-97-1	Shanghai Jiapeng Technology Co., LTD

Conclusions to Chapter 2

Zhoukou City is one of the main wheat producing areas in China, the flat terrain is very suitable for mechanized operations, and the wheat planting area is very wide. Zhoukou has a national key leading enterprise of agricultural industrialization whose scale of intensive pig raising ranks among the top in China, which provides favorable conditions for the necessity, feasibility and popularization of this study.

The experimental design, sampling quantity, measurement methods, instruments and equipment, data statistics and analysis methods of this study show that this study can obtain a sufficient number of field records and ensure the accuracy of the measurement results. The results obtained by comprehensive analysis will provide a scientific basis for the application of biogas slurry.

CHAPTER 3

EFFECTS OF COMBINED APPLICATION OF BIOGAS SLURRY AND CHEMICAL FERTILIZER ON SOIL PHYSICOCHEMICAL PROPERTIES

Soil is the carrier of plant growth and development, and soil physical and chemical properties are an important index of soil quality. The physical and chemical properties of soil can be divided into soil physical properties and soil chemical properties, including soil bulk density, aggregate and so on. Soil chemical properties include the content of various elements in the soil, of which the most important and most commonly used are the three major nutrients N, P and K in the soil.

3.1. Effect of combined application of biogas slurry and chemical fertilizer on physical properties in soil

3.1.1. Soil bulk density

Soil bulk density is a comprehensive reflection of soil porosity, texture, structure and other physical properties, and is an indicator to measure soil tightness. Soil tightness is one of the important physical properties of soil, which directly affects soil fertility and plant root development. Appropriate bulk density is conducive to the spread of crop roots and the accumulation of root mass, and can improve crop biological yield and economic yield (Garcia et al. 1988; Gao 2009).

Fig. 3.1 shows the bulk density of winter wheat with different proportion of biogas slurry in different soil layers. In 0-10 cm soil layer, there were significant differences between the treatments and CK ($p<0.05$), CF decreased by 3.15 % compared with CK, and there was significant difference between BS and CF ($p<0.05$), with the increase of biogas slurry, the bulk density first decreased BS 50 and then increased (to BS100). The bulk density of BS50 was the smallest with a value of 1.24, which is

10.17 % and 7.25 % lower than CK and CF, respectively. There were significant differences with all treatments (except BS75) ($p<0.05$).

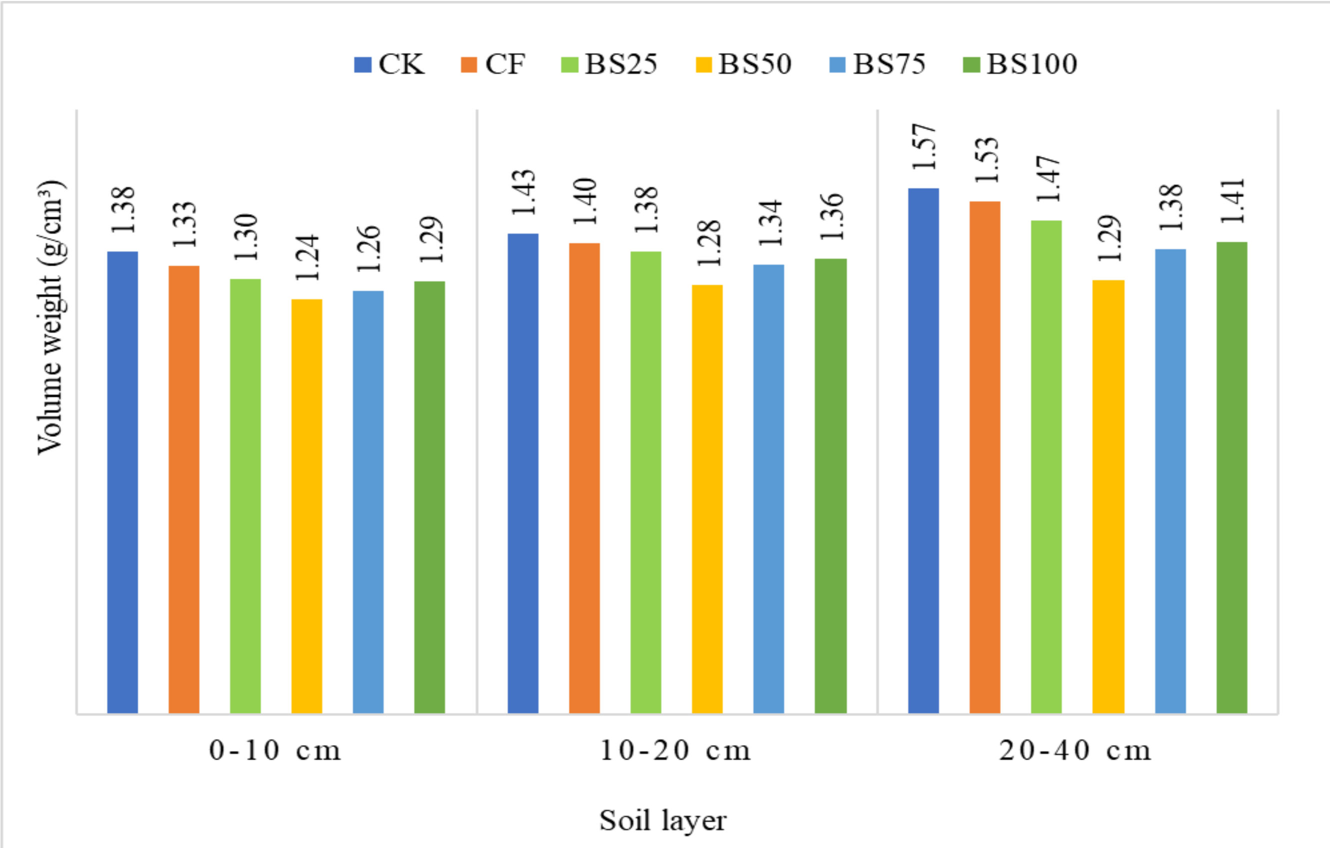

Fig. 3.1. The bulk density of winter wheat with different proportion of biogas slurry in different soil layers

In the 10-20 cm soil layer, the order of soil bulk density is BS50<BS75<BS100<BS25<CF<CK, there were significant differences between each treatment and CK ($p<0.05$), CF decreased by 1.86 % compared with CK, and there was significant difference between BS and CF ($p<0.05$), the bulk density of BS50 was the lowest (1.28), and compared with CK and CF were decreased by 10.72 % and 9.03 %, respectively, showing significant differences ($p<0.05$), BS75 and BS100 had no significant difference ($p>0.05$), there was no significant difference between BS100 and BS25 ($p>0.05$).

In the 20-40 cm soil layer, the order of soil bulk density was consistent with that in the 0-10 cm and 10-20 cm soil layers, and there were significant differences between each treatment and CK ($p<0.05$), CF decreased by 2.66 % compared with CK, and there was significant difference between BS and CF ($p<0.05$), the bulk density of BS50 was the lowest (1.29), compared with CK, CF decreased by 17.45 % and 15.19 %, respectively, and there were significant differences among all treatments ($p<0.05$).

In addition to sandy soil, generally speaking, the bulk density of the soil with the same texture can generally reflect the soil structure. The smaller the bulk density, the more loose the soil, the better the structure, on the contrary, indicating that the soil is compact and the structure is poor. The texture of lime concretion black soil is heavy, coupled with the lack of organic fertilizer input and agricultural machinery rolling for many years, the soil bulk density is high, which has become one of the important factors restricting crop growth. Some studies have reported that the appropriate growth bulk density of rice, wheat and cotton is 1.2-1.3 $g•cm^{-3}$ (Shen et al. 1996; Suuster 2011). In this study, the addition of biogas slurry significantly reduces the soil bulk density, especially the soil bulk density of BS50 is the lowest, and in the appropriate range of wheat bulk density, it is conducive to the growth and development of winter wheat and the improvement of yield. This is consistent with the research results of Zheng et al. (Li et al. 2010; Zheng et al. 2020) and Li et al. (Li et al. 2010; Zheng et al. 2020), biogas slurry can reduce soil bulk density.

3.1.2. Soil aggregares

3.1.2.1. Particle size distribution of soil aggregates

As a basic component of soil structure, particle size composition and quantity of soil aggregates are important indicators to measure soil physical quality (Hai et al. 2020). Soil aggregate is closely related to soil water permeability, air permeability, organic matter storage and mineral nutrient supply capacity, and directly or indirectly affects soil fertility. It not only plays an important role in crop growth and development, but also is closely related to soil erosion resistance (Bosch-Serra et al. 2017; Liu et al. 2018). The measurement of particle size composition and stability parameters of soil aggregates is an important part of soil health investigation and soil quality assessment (Lal et al. 2000). In general, according to the size of the soil aggregate, it can be divided into macroaggregates and microaggregates, of which >0.25 mm aggregates are called macroaggregates, and the <0.25 mm aggregate is called a microaggregate (Tisdall et al. 1982). Among them, soil macroaggregates play an important role in protecting soil active organic carbon from the influence of enzymes and microbial activities, and the more content of macroaggregates, the more stable soil structure (T. et al. 2016). At the same time, dry screening and wet screening methods are often used to determine the soil mechanical stability and water stability of aggregates. Force-stable aggregates are related to soil resistance to mechanical destructive force, while water-stable aggregates are closely related to soil water erosion and water disintegration resistance (Wei et al. 2013; Bosch-Serra et al. 2017).

Particle size distribution of mechanically stable aggregates

In the 0-10 cm soil layer, increasing with the proportion of biogas slurry, the mass composition ratio of soil aggregate with 2 mm particle size mechanically stable aggregates showed a trend of first increasing (to BS50) and then decreasing (to BS100) (Fig. 3.2). The composition of soil aggregates with 2 mm of BS50 was the highest (54.58), which was significantly difference with other treatments ($p<0.05$).

The soil aggregates with >2 mm have particle size from 39.27 CF to 50.69 BS75. For soil aggregates with particle size of 0.25-2 mm, the proportion of mass composition first increased (CF), then decreased (to BS75) and then increased with the increase of the proportion of biogas slurry (BS100).

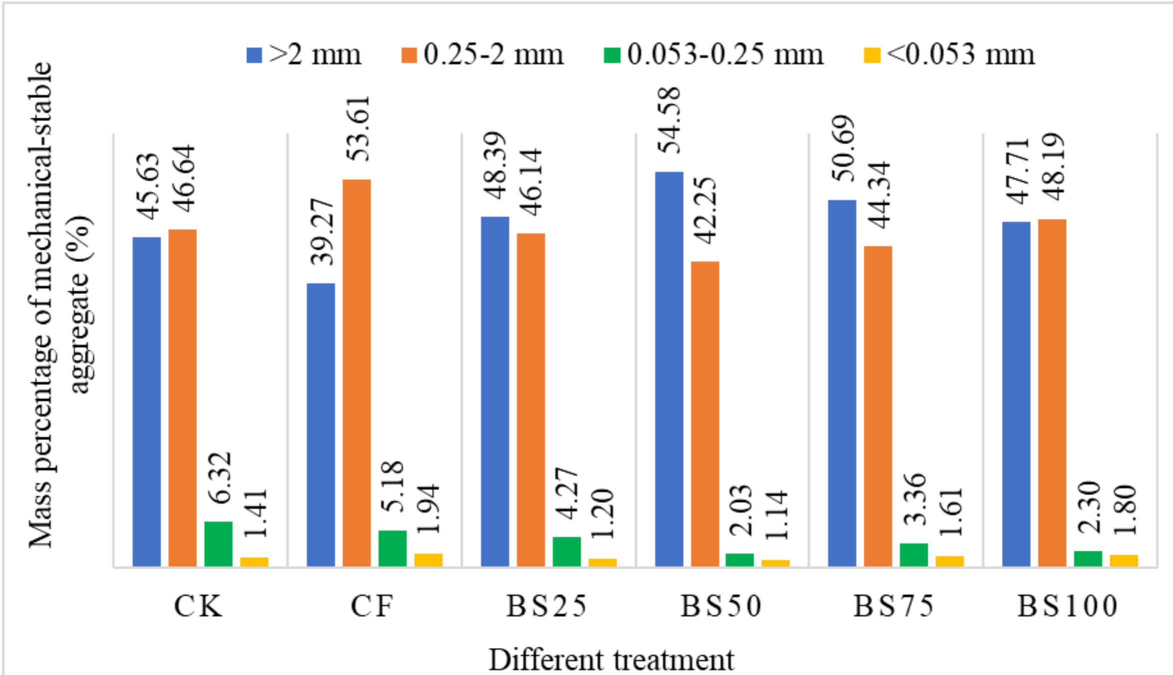

Fig. 3.2. Mass percentage of mechanical-stable soil aggregates (0-10 cm)

Under the condition of single application of CF, the proportion of mass composition of aggregate was the highest (53.61), and there was a significant difference with other treatments ($p<0.05$), increased by 14.94 % and 11.25 % compared with CK and BS100. For the soil aggregate of 0.053-0.25 mm particle size, CK has the largest value of 6.32, 22.01 % higher than CF, and there is a significant difference between CF and other treatments. CF treatment is larger than BS treatment, and BS treatment value is between 2.03-4.27, and BS50 and BS100 have no significant difference ($P>0.05$), there were significant differences among other treatments ($p<0.05$). For soil aggregates with particle size less than 0.053 mm, CF is the largest, which first decreased and then increased with the increase of biogas slurry proportion.

In the 10-20 cm soil layer and the 20-40 cm soil layer, the mass composition ratio of the mechanically stable soil aggregate is basically the same as that of the 0-10 cm soil layer (Fig. 3.3, 3.4).

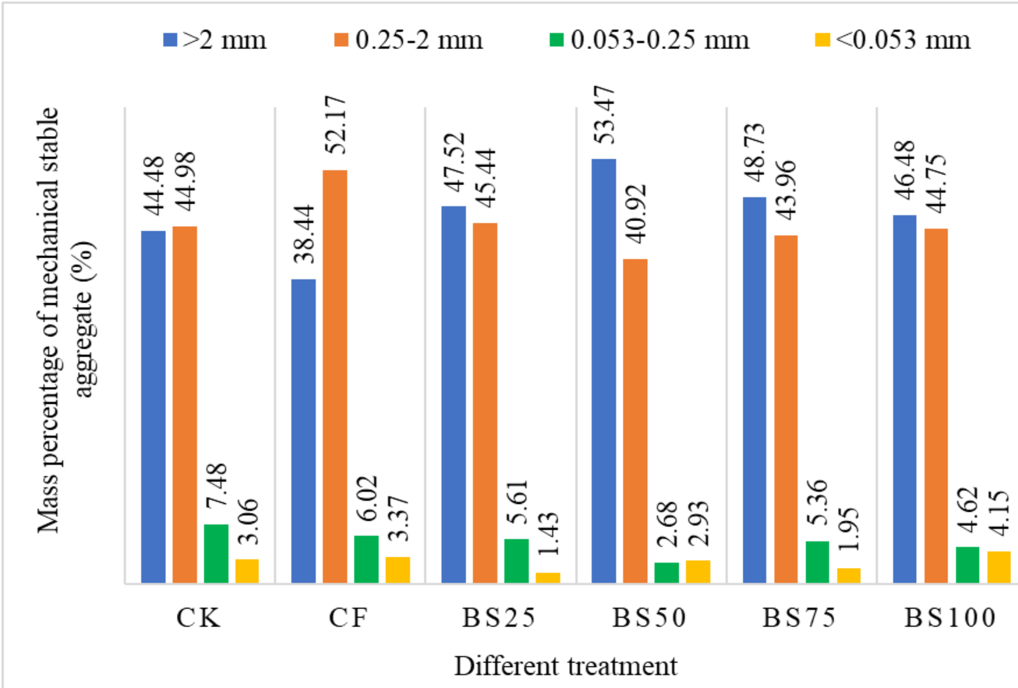

Fig. 3.3. Mass percentage of mechanical-stable soil aggregates (10-20 cm)

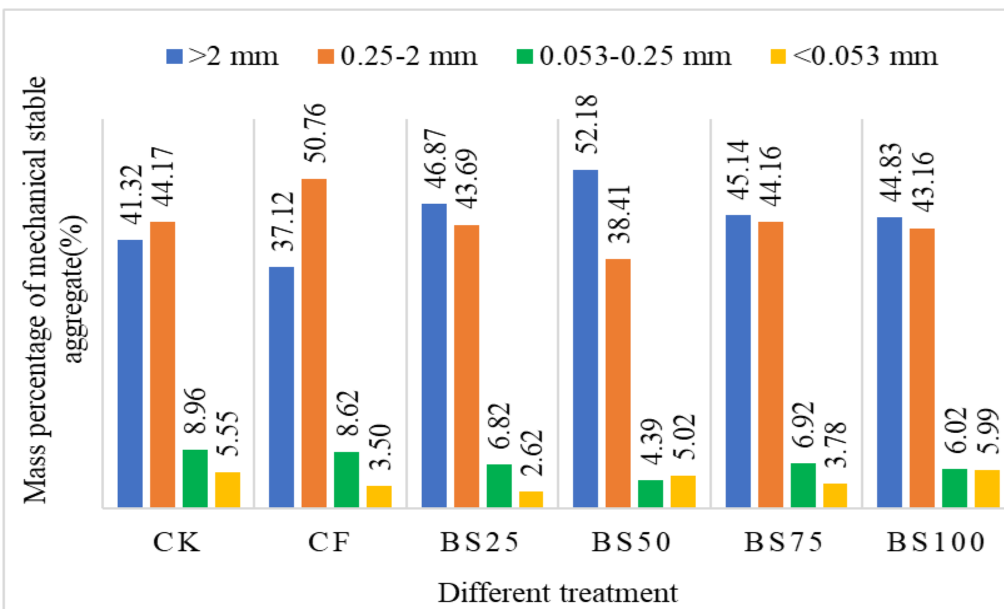

Fig. 3.4. Mass percentage of mechanical-stable soil aggregates (20-40 cm)

Particle size distribution of water-stable aggregates

In 0-10 cm soil layer, the mass composition ratio of water-stable soil aggregates larger than 2 mm showed that the biogas slurry treatment was larger than CK and CF (Fig. 3.5).

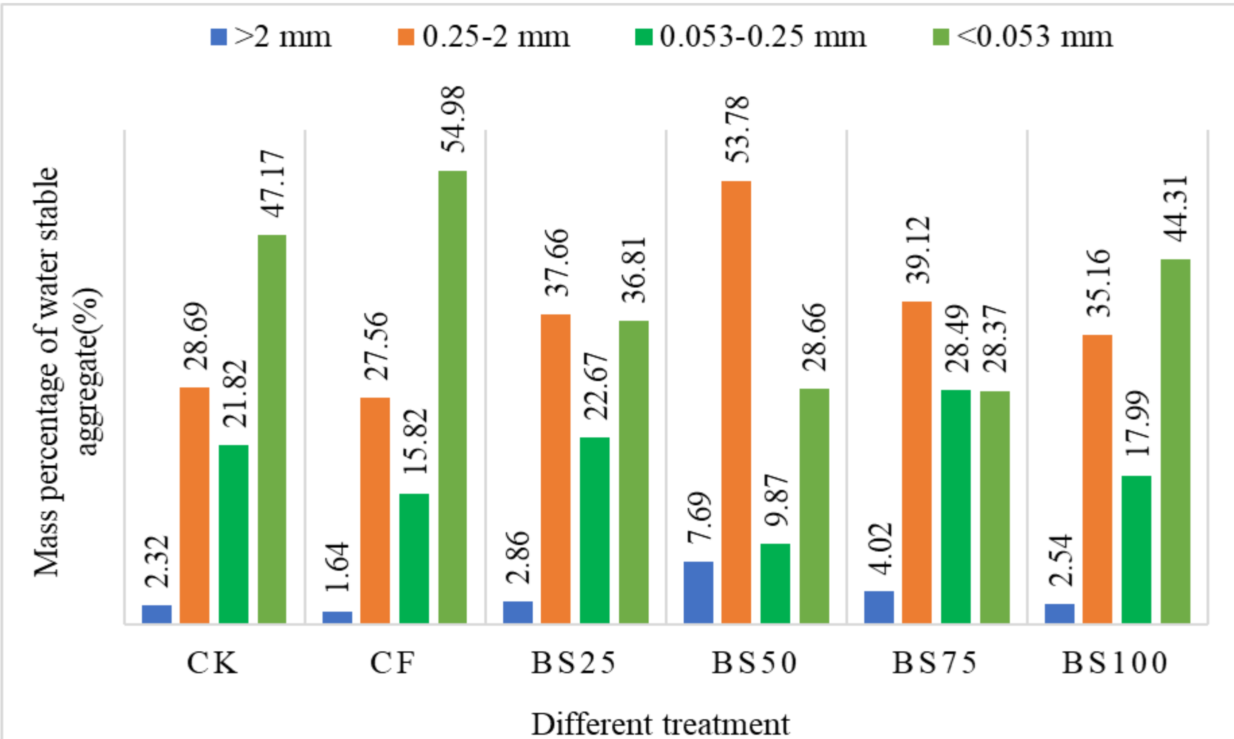

Fig. 3.5 Mass percentage of water-stable soil aggregates (0-10 cm)

The mass composition of water-stable soil with 2 mm particle size by BS50 was the highest (7.69), and there were significant differences between the others ($p<0.05$), compared with CK, CF decreased the mass composition of soil aggregates with 2 mm particle size and is 29.31 % lower than that of CK. For the water-stable soil aggregates of 0.25-2 mm particle size, the mass composition ratio of water-stable soil aggregates first increased (to BS50) and then decreased with the increase of biogas slurry proportion, and the BS50 value was the highest, which was 53.78, and there was a significant difference between the two treatments ($p< 0.05$), CF decreased 0.25-2 mm water-stable soil aggregate mass composition, and there was no significant difference between BS100 and BS25 ($p<0.05$), BS75 and BS25 had no significant difference ($p>0.05$).

For the water-stable soil aggregates of 0.053-0.25 mm diameter, there was no obvious regularity in each treatment, and the mass composition ratio of BS50 soil aggregates was the smallest (9.87), which decreased by 37.61 % compared with CF, and there was a significant difference between with others($p<0.05$). BS75 was the biggest, its value was 28.49, compared with CK and CF increased by 30.57 % and 80.09 %, respectively, and there were significant differences between them ($p<0.05$).

The water-stable soil aggregates of 0.053 mm particle on the biogas slurry BS75 was the smallest, but there was no significant difference between BS50 and BS75 ($p>0.05$), CF was the largest, and there were significant differences between treatments (<0.05).

The change trend of 10-20 cm and 20-40 cm is basically the same as that of 0-10 (Fig. 3.6, 3.7).

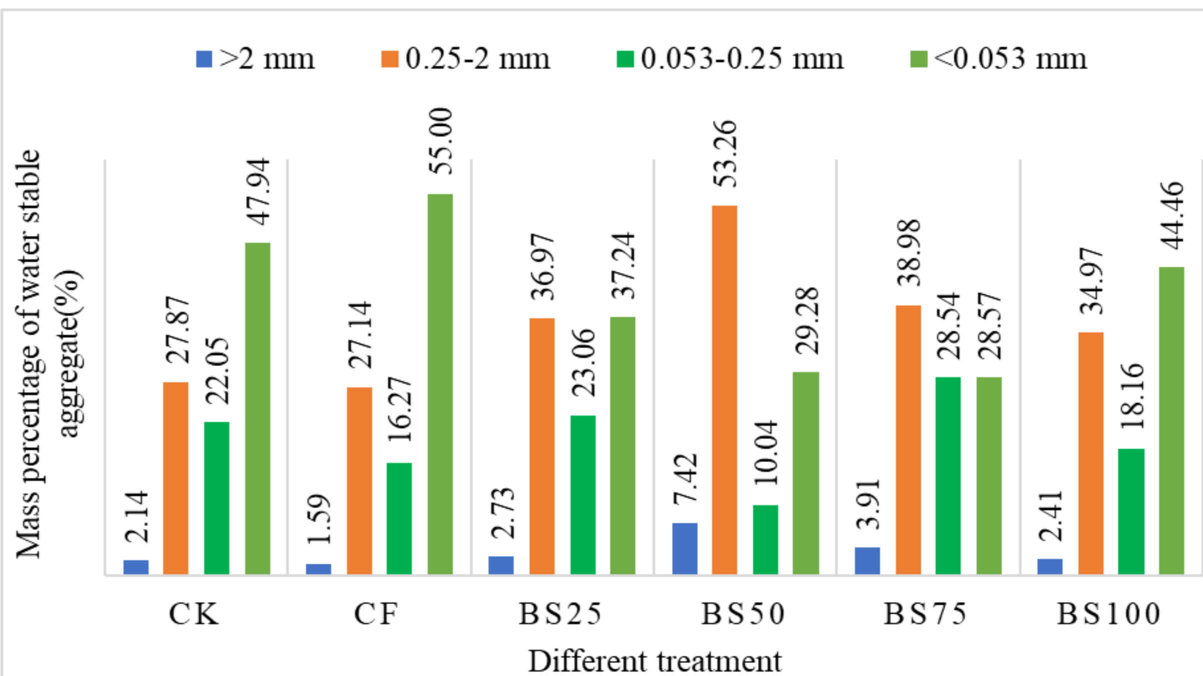

Fig. 3.6. Mass percentage of water-stable soil aggregates (10-20 cm)

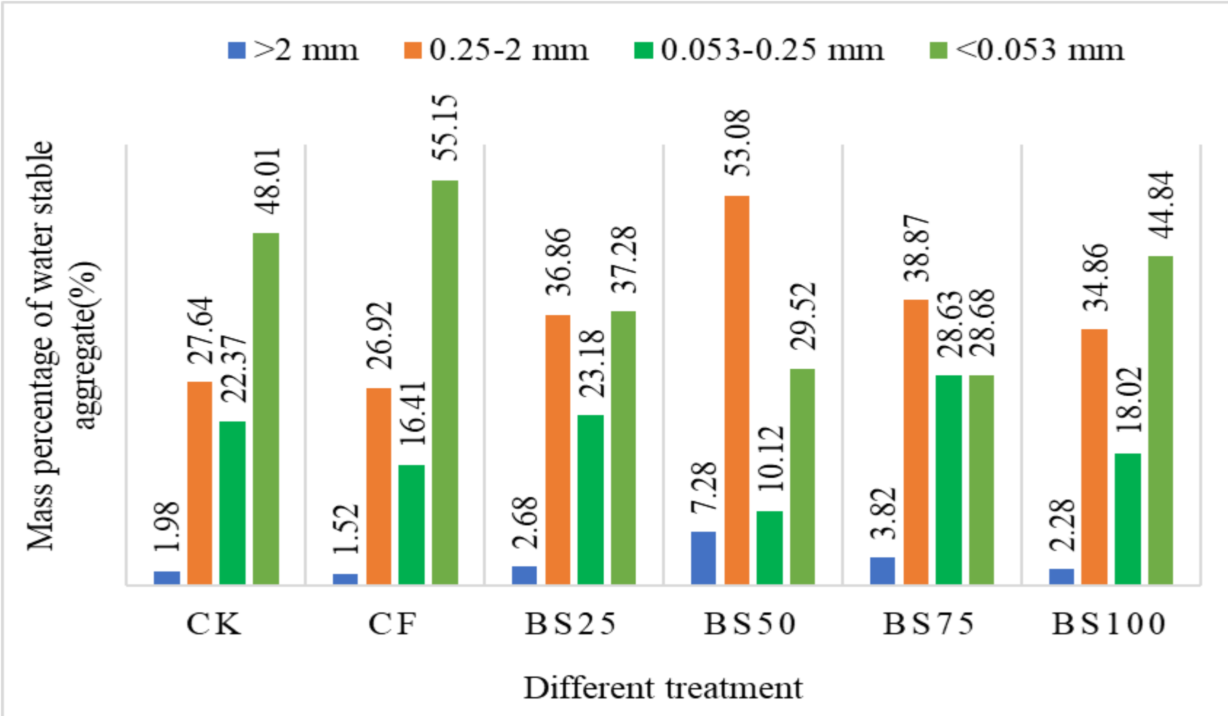

Fig.3.7. Particle size distribution of water-stable soil aggregates (20-40 cm)

In this study, it was found that different fertilization treatments could significantly change the particle size distribution and adjust the mass composition of aggregates with different particle sizes.

Long-term application of chemical fertilizer significantly reduced the number of large aggregates and increased the number of small aggregates. The reason for this phenomenon may be that long-term application of chemical fertilizer affects the soil environment, thus reducing the adhesion of organic matter in the soil. At this time, the adhesion of soil organic matter is not enough to maintain the existence of soil large aggregates, so that the small soil aggregates fall off from the original large aggregates. The application of biogas slurry significantly increased the number of soil macroaggregates, because the application of biogas slurry reduced the adverse impact of chemical fertilizer on the soil environment, and the biogas slurry contained organic matter, which could reduce the cohesion of soil and inhibit the formation of bad structures. At the same time, small particle size aggregates promote the formation of large aggregates through the gumming action of organic matter and microorganisms, forming soil benign water-stable aggregates.

3.1.2.2. Influence on the stability of soil aggregates

The stability of soil aggregates, like its particle size distribution, is an important index reflecting soil quality, and is directly related to the adaptability and coordination of soil to different environments. Soil structural water stability refers to the ability of soil particles to resist hydraulic dispersion and maintain their original state under the action of water flow, which is closely related to soil aggregates, especially water-stable aggregates. Common soil aggregate stability parameters include mean weight diameter (MWD), aggregate failure rate (PAD), etc.

Mean weight diameter (MWD)

MWD (Mean weight diameter) is a common index reflecting the size distribution of soil aggregates, and the lower the value, the lower the stability of aggregates.

Mechanically stable aggregate (MWD)

In 0-10 cm, 10-20 cm, 20-40 cm soil layers, the order of MWD of mechanically stable aggregates was CF<CK<BS100<BS25<BS75<BS50, CF were the smallest, 2.97, 2.90 and 2.81 respectively, which were decreased by 9.31 %, 8.90 % and 5.98 % compared with corresponding CK, and there were significant differences ($p<0.05$), the BS50 increased the MWD of the mechanically stable aggregate, and the BS50 was the largest, and the soil layers were 3.76, 3.67 and 3.57, respectively, which increased by 26.48 %, 26.52 % and 26.98 % compared with the corresponding CF, and there were significant differences ($p<0.05$) (Fig. 3.8).

The vertical order of MWD of the mechanically stable aggregate is 0-10 cm>10-20 cm>20-40 cm; all of them decreased with the increase of soil depth.

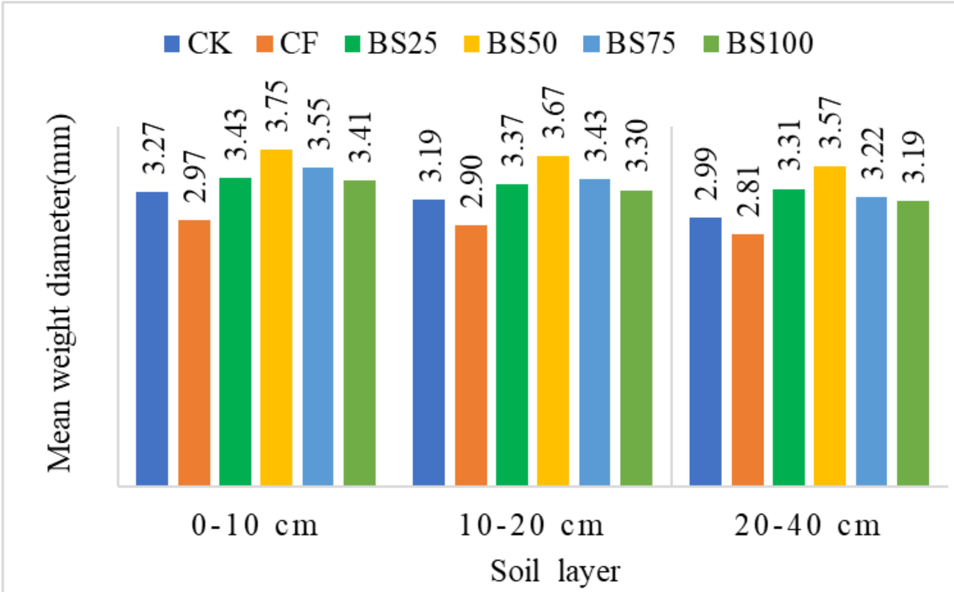

Fig. 3.8. Mean weight diameter of mechanical-stable soil aggregates

Water stable aggregate MWD

The MWD water-stable aggregates in 0-10 cm, 10-20 cm and 20-40 cm was significantly affected by each fertilization treatment ($P<0.05$) (Fig. 3.9). The MWD of soil water stability aggregates treated with BS50 was the highest, with the values of 1.09, 1.07 and 1.06 in each soil layer, respectively, and there were significant differences between BS50 and others ($P<0.05$). Compared with CK, CF decreased the MWD of water-stable aggregates by 11.93 %, 9.86 % and 8.99 % in each soil layer, respectively, and there were significant differences among all treatments (<0.05).

In terms of soil profile, MWD of water-stable aggregates decreased with the increase of soil depth under different treatments, ranging from 0-10 cm>10-20 cm>20-40 cm.

The results showed that MWD of dynamic stability and water stability of aggregates were significantly higher than those of other treatments. MWD can comprehensively reflect the size distribution and composition ratio of soil aggregates.

Generally, the larger the MWD, the better the stability and the higher the agglomeration degree (Kemper et al. 2018; Liu et al. 2020).

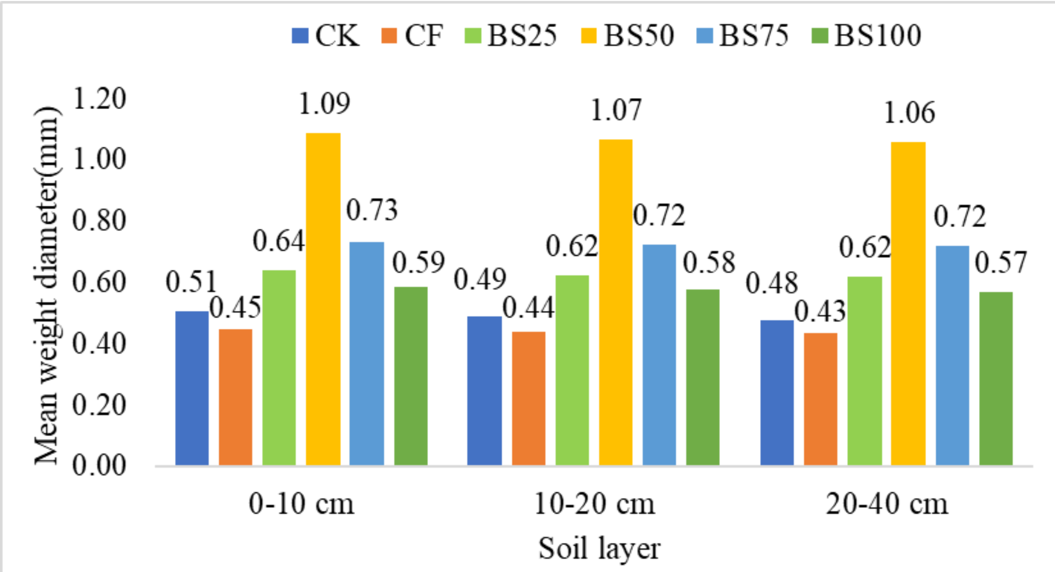

Fig. 3.9. Mean weight diameter of Water-stable soil aggregates

Percentage of aggregate destruction (PAD)

Percentage of aggregate destruction (PAD0.25) represents the particle size >0.25 mm after wet and dry screening. The ratio of soil aggregates of 0.25 mm can directly demonstrate the stability of soil aggregates and explain the degree of dispersion of soil aggregates caused by hydraulic damage. The greater the ratio, the more drastic the disintegration of aggregates, the more unstable the soil structure, and the increased degree of soil degradation.

In the 0-10 cm soil layer, PAD was significantly different among different treatments. CF increased PAD by 3.27 % compared with CK; BS decreased PAD by 3.27 % compared with CK; BS50 had the smallest PAD (36.52), and CF decreased by 45.00 % and 46.74 % compared with CK (Fig. 3.10). In the 10-20 cm soil layer, the trend was the same as that in the 0-10 cm soil layer. With the increase of biogas slurry proportion, PAD was increased (BS75-BS100). The PAD value of BS50 was 35.70, and compared with CK and CF, it was decreased by 46.26 % and 47.71 %, respectively, and had significant differences with other treatments (P< 0.05). The PAD of CF increased by 2.77 % compared to CK, but there was no significant difference between the two (P>0.05).

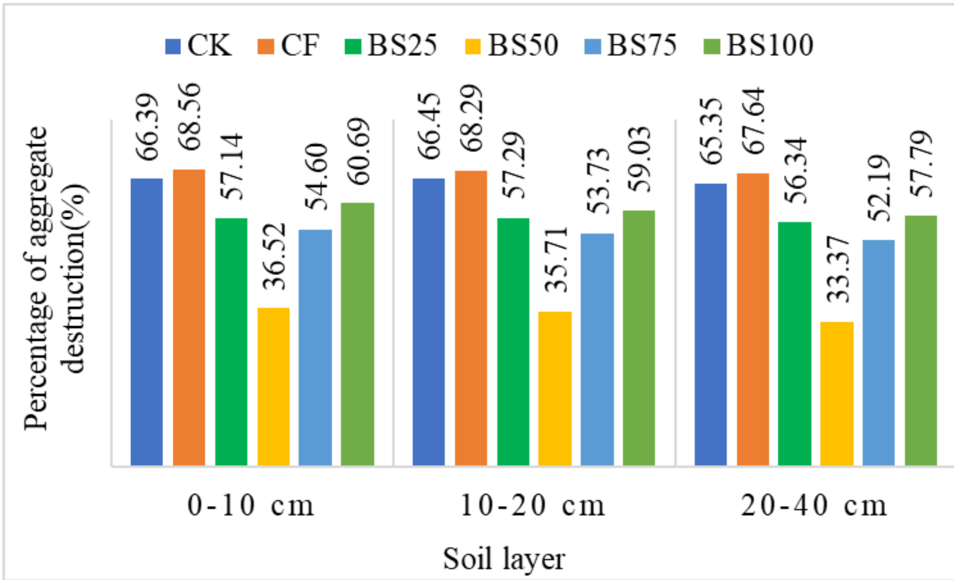

Fig. 3.10. Percentage of aggregate destruction (PAD)

In the 20-40 cm soil layer, PAD changes ranged from 33.33 to 67.64, compared with CK, CF increased PAD, and BS decreased PAD. Th value of PAD with BS50 applied with biogas slurry and chemical fertilizer was the smallest, with a value of 33.33, which was significantly different from other treatments (P>0.05). Single application of chemical fertilizer makes the stability of soil aggregates worse, thus destroying aggregates. Combined application of organic fertilizer and chemical fertilizer can reduce the damage degree of aggregates and is conducive to create aggregates.

During the wet screening process, the non-water-stable aggregates continue to crack due to the physical dispersion caused by the charged colloid biionic layer structure, the dissolution of adhesive substances during the agglomeration process, the difference in expansion of clay minerals from different sources, and the pressure difference between the internal and external water environment of the aggregates [Bronik & Lal 2005; Fan et al., 2011]. The results of this study showed that compared with CK, the PAD value of co-application of fertilizer and biogas slurry was the smallest, indicating that chemical fertilizer combined with biogas slurry was conducive to improving the stability of large aggregates. This is similar to the research results of Bosch-Serra et al. (2017) on the effects of porcine biogas slurry irrigation on soil aggregates.

3.2. Effect of combined application of biogas slurry and chemical fertilizer on chemical properties in soil

3.2.1. Soil pH

Soil pH value is an important chemical property of soil, which determines the pH of soil (Tang et al. 2013), can affect the solubility of nutrient elements in soil, and ultimately affect the supply of nutrients in soil. The application of chemical fertilizer can improve crop yield, but excessive application of chemical fertilizer can lead to or aggravate the disconnection of H^+ cycle, which leads to the decrease of soil pH and soil acidification (Barak et al. 1997; Xu et al. 2002). According to the research (Xu et al. 2016), in the field of lime concretion black soil, because of the large amount application of chemical fertilizer, the lime concretion black soil has developed from the original alkaline to the present acidic. In this study, the pH value of soil under different treatments after wheat harvest is shown in Fig. 3.11. It can be seen from the figure that the pH value is BS50>BS75>BS100>BS25>CK>CF in 0-10 cm. The pH of CF (5.76) is the lowest, and there is a significant difference between CF and CK ($p<0.05$). Compared with CF and CK, BS significantly increased soil pH, and the pH (6.46) of BS50 was the highest, and there were significant differences between BS50 and other treatments ($p<0.05$). In the 10-20 cm, the pH was still BS50>BS75>BS100>BS25>CK>CF, the pH (5.84) of CF is the lowest, and there is a significant difference between CF and CK ($p<0.05$), the pH (6.59) of BS50 was the highest, and the pH of BS50 was 10.58 % and 12.72 % higher than that of CK and CF, respectively, and there were significant differences between BS50 and other treatments ($p<0.05$). In 20 to 40 cm, the pH value was in the same order as 0-10 and 10-20. The pH value of CF was the lowest (5.92), and there was a significant difference between CF and CK ($p<0.05$), the pH of BS50 was the highest (6.69), and the pH of BS50 was 8.32 % and 13.01 % higher than that of CK and CF, respectively, and there were significant differences between BS50 and other treatments ($p<0.05$).

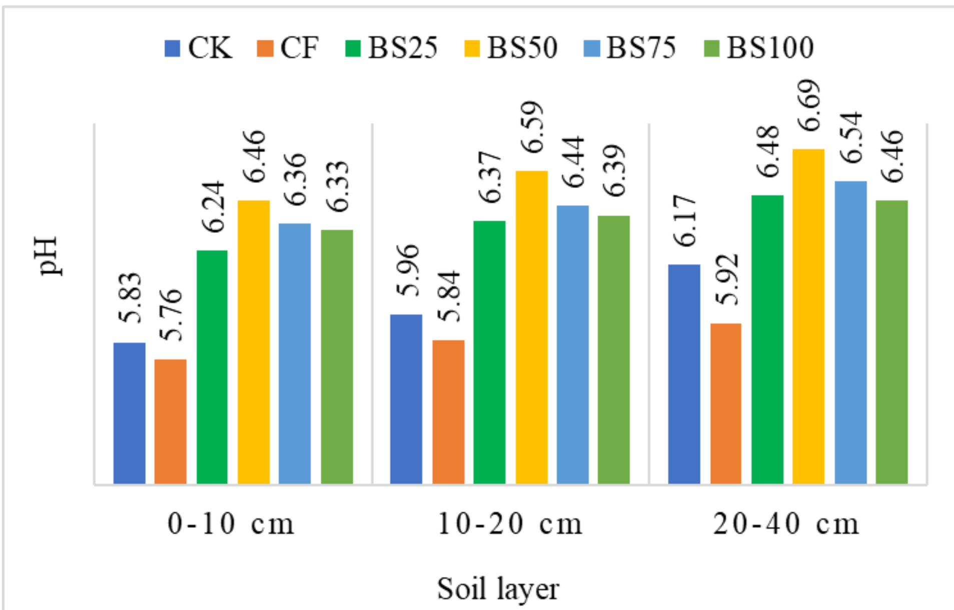

Fig. 3.11. Soil pH with different proportion of biogas slurry in different soil layers

In general, this study confirmed the view that CF reduces the value of pH. Long-term excessive application of inorganic fertilizer causes a large part of nitrogen to enter the soil, and the subsequent nitrification of ammonium ions and leaching of nitrate ions can reduce the value of pH. Application of biogas slurry can increase the value of pH. Biogas slurry itself is alkaline and contains high cation content. The increase of base ions can effectively inhibit the soil acidification trend caused by long-term application of chemical fertilizer to a certain extent, and the acid-base environment of soil solution can be improved. This is consistent with the results of previous studies (Wang 2007a; Qin 2009; Wei et al. 2017; Yu et al. 2017; Huang et al. 2021). However, previous studies have also shown that biogas slurry reduces pH value (Lin et al. 2019; Hu et al. 2020) or did not change much (Cai et al. 2014).

Different research results are inconsistent, which may be due to the differences in soil type, physical and chemical properties, basic nutrient characteristics of biogas slurry and plant species, resulting in different properties of soil colloid, soil colloid will adsorb potassium, calcium, magnesium plasma, these ions and the ions in the soil solution in a state of dynamic equilibrium, when the substitution between them, will affect the pH of the soil.

pH is a common index for evaluating soil quality or soil fertility, and it is also an indispensable index for characterizing soil quality. Under the current agricultural development, soil acidification has become a common process with the long-term application of chemical fertilizers. In general, the application of biogas slurry or combined application of biogas slurry and chemical fertilizer is more effective than the application of chemical fertilizer alone. Their pH values are relatively high, indicating that they can reduce the effect of chemical fertilizer on soil acidification process. For different soil improvement purposes, specific types and amounts of biogas slurry should be allocated.

3.2.2. Total nitrogen in soil

Nitrogen is an essential nutrient element for plants and one of the important material bases of soil fertility. Soil total nitrogen, including all forms of organic and inorganic nitrogen, is the source and reservoir of soil total nitrogen and available nitrogen supply to plants, and comprehensively reflects the nitrogen status of soil (Zhang et al. 2008b). Nitrogen is one of the most important components of various proteins, not only for winter wheat, but for all living things.

In this study, under different treatment conditions (CK, CF, BS25, BS50, BS75, BS100), the soil total nitrogen content after winter wheat harvest was shown in Fig. 3.12. As can be seen from Fig. 3.12, in the 0-10 cm soil layer, the order of soil total nitrogen content is BS50>BS75>BS25>BS100>CF>CK, there were significant differences between each treatment and CK ($p<0.05$), fertilizer treatment increased total nitrogen content, CF increased by 6.20 % compared with CK, and the total nitrogen content first increased (to BS50) and then decreased (BS75-100) with the increase of biogas slurry proportion.

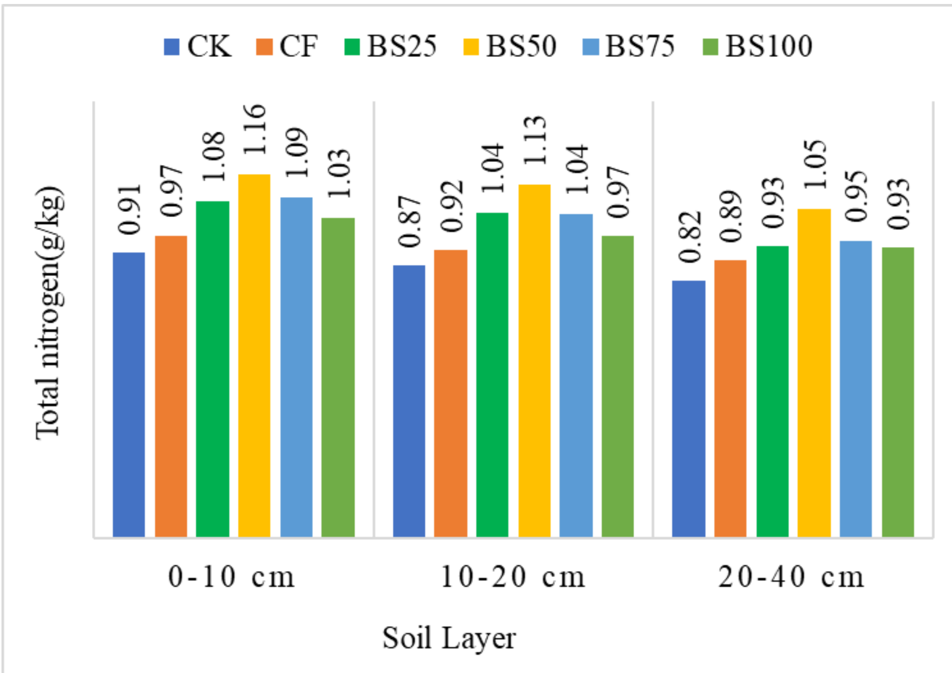

Fig. 3.12. Soil total nitrogen content with different proportion

The total nitrogen content of BS50 was the highest, and its value was 1.16 $g \cdot kg^{-1}$, which increased by 27.37 % and 19.93 % compared with CK and CF, respectively. In the 10-20 cm soil layer, the order of total nitrogen content was BS50>BS25>BS75>BS100>CF>CK, there were significant differences between each treatment and CK ($p<0.05$), CF increased by 5.73 % compared with CK, and there was significant difference between BS and CF ($p<0.05$). The total nitrogen content of BS50 was the highest (1.13 g•kg^{-1}), which was increased by 22.38 % compared with CF, and there were significant differences between treatments ($p< 0.05$), no significant difference was found between BS25 and BS75 ($p>0.05$). Less nitrogen was notices in BS75-100. The sequence of soil total nitrogen content in 20-40 cm soil layer was BS50>BS75>BS25>BS100>CF>CK, there were significant differences between each treatment and CK ($p<0.05$), CF increased by 8.10 % compared with CK. With the increase of biogas slurry proportion, total nitrogen first increased and then decreased, but no significant difference was found among BS25, BS75 and BS100 ($p>0.05$), BS50 had the highest total nitrogen content (1.05 g•kg^{-1}), which was significantly different from that of other treatments ($p<0.05$).

Nitrogen is a nutrient element with high absorption by plants. The total nitrogen content of soil can be used to measure the total nitrogen storage and nitrogen supply potential of soil. The nutrients in biogas slurry are mainly available nutrients, which are easy to be absorbed and utilized by crops after application in soil, and easy to be decomposed by microorganisms in soil, so as to improve the content of total nitrogen and available nutrients in soil, so as to improve the physical and chemical properties of soil to a certain extent.

Numerous studies (Bachmann et al. 2011; Abubaker et al. 2012) showed that the application of biogas slurry in agricultural planting could significantly increase the content of soil nitrogen, thus satisfying the nutrient absorption of crops.

In the same soil layer, with the increase of biogas slurry, the soil total nitrogen content first increased BS25-50 and then decreased BS75-100, and there was a significant difference with CK, indicating that biogas slurry can improve the growing environment of winter wheat, increase the soil total nitrogen content, increase the soil nitrogen supply capacity and fertility level, and the effect of combined application of biogas slurry and chemical fertilizer is better than that of single application of chemical fertilizer. This is consistent with the research results of Li et al. (2014a) and Wang et al. (2011)

The soil total nitrogen content of BS50 in 0-10 cm, 10-20 cm and 20-40 cm was the highest, and the total nitrogen content in soil was 0-10 cm>10-20> 20-40 cm, soil total nitrogen content decreased with the increase of soil depth, which was consistent with the research results of Cai et al. (2014). Crops absorb nutrients through roots, and the distribution of roots in soil is usually an important factor affecting their nutrient absorption. With the advancement of wheat growth process, root activity tends to move down to deep soil (Wang et al. 2001). In the late growth period of winter wheat, the root activity in the 20-40 cm soil layer was higher than that in the 0-20 soil layer, and more root secretions entered the soil, which increased soil urease activity and decreased soil total nitrogen content.

3.2.3. Content of available phosphorus in soil

Phosphorus is not only an important component of many important organic compounds in plants, such as phospholipids and nucleoproteins, but also participates in various metabolic processes in plants in various ways, playing an obvious role in crop yield and maintaining the good characteristics of varieties (Zeng et al. 2010) . Therefore, the level of phosphorus content is often used as an evaluation index of soil fertility. Soil available P is an indicator of the level of phosphorus nutrient supply in soil. The level of phosphorus content in soil reflects the storage and supply capacity of phosphorus in soil to a certain extent (Lu 1998).

As can be seen from Fig. 3.13, appendix E, the order of available phosphorus content in 0-10 cm soil layer was BS50>BS25>BS75>CF>BS100>CK, there were significant differences between all treatments ($p<0.05$), CF increased by 15.56 % compared with CK, and there were significant differences between BS and CK ($p<0.05$), BS50 had the highest available phosphorus content (25.59 mg•kg^{-1}), which was increased by 46.56 % and 26.83 % compared with CK and CF, respectively, and had significant differences with other treatments ($p<0.05$).

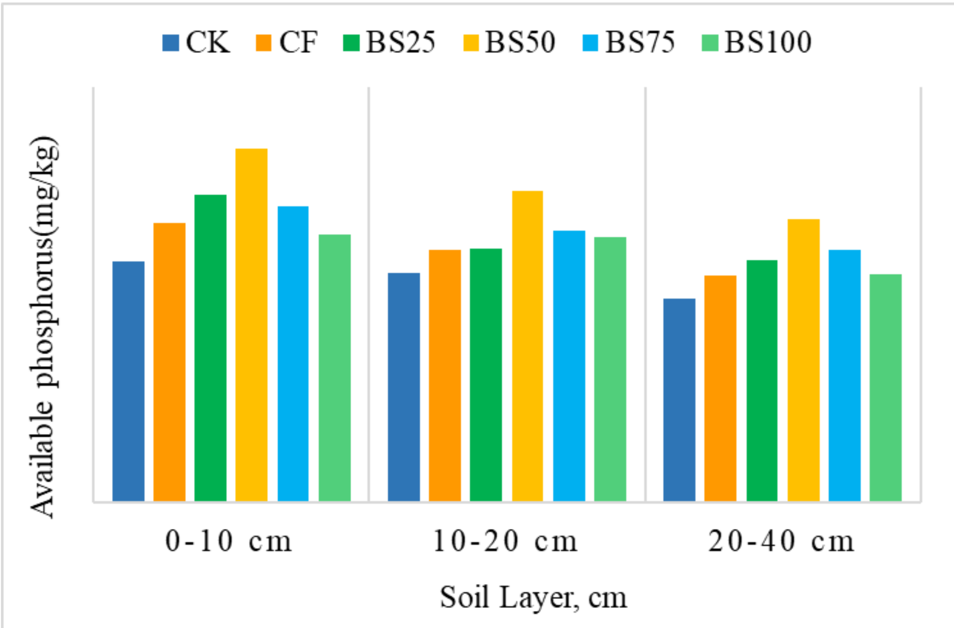

Fig. 3.13. Soil available phosphorus content with different proportion of biogas slurry in different soil layers

The soil available phosphorus content in 10-20 cm soil layer was BS50>BS75>BS100>BS25>CF>CK, there were significant differences between each treatment and CK ($p<0.05$), CF increased by 10.16 % compared with CK, and there was no significant difference between CF and BS25 ($p>0.05$), as the proportion of biogas slurry increased, the available phosphorus content first increased (BS25-50) and then decreased (BS75-100), and the available phosphorus content of BS50 was the highest (22.50 mg•kg^{-1}), which was significantly different from other biogas slurry treatments ($p<0.05$), and increased by 35.60 % and 23.09 % compared with CK and CF, respectively.

The content of available phosphorus in 20-40 cm soil layer was BS50>BS75>BS25>BS100>CF>CK, there were significant differences between each treatment and CK ($p<0.05$). Compared with CK, CF increased by 11.08 %, and BS50 soil available phosphorus content was the highest (20.48 $mg•kg^{-1}$), which was significantly different from other biogas slurry treatments ($p<0.05$), and increased by 38.96 % and 25.10 % compared with CK and CF, respectively.

Biogas slurry contains a large number of microorganisms, which can promote the propagation of soil microorganisms and enhance the activity of related enzymes to a certain extent (Liu 2007). Application of biogas slurry is conducive to the activation of soil phosphorus and increase the availability of soil phosphorus (Wang et al. 2017). In this study, after the harvest of winter wheat, the soil available phosphorus content first decreased (BS25-BS50) and then increased (BS75-100) with the increase of the content of biogas slurry, indicating that the application of biogas slurry improved the soil available phosphorus supply level, but more is not always better.

Among all soil layers, the content of available P in BS50 was the highest, and the content of available P decreased with the increase of soil layer, and the content of available P in soil was 0-10 cm>10-20>20-40 cm, which is consistent with the research results of Hui (2017), in the vertical direction, the soil available phosphorus content is mainly concentrated in the soil surface, which is significantly higher than the content in the lower layer of the same sampling point.

3.2.4. Content of available potassium in soil

Potassium is one of the three essential fertilizer elements for plant growth and development, many plants require a large amount of potassium, and its content in plants is second only to nitrogen (Horst 1995). Potassium has an obvious effect on improving crop yield and improving the quality of agricultural products, and it can also improve the ability of plants to adapt to the external adverse environment, so it is known as quality element and resistance element.

Available potassium is the available potassium that can be directly utilized by plants, which plays an irreplaceable role in ensuring the normal growth and development of plants. Compared with CK, the content of available potassium in soil with each treatment increased to varying degrees, as shown in Fig. 3.14, appendix F. As can be seen from the figure, in 0-10 cm soil layer, fertilization increased the available potassium content, and CF increased by 6.53 % compared with CK ($P<0.05$), the effective potassium content of BS50 was the highest, which was increased by 25.73 %, 18.02 %, 10.39%, 7.14 % and 12.31 % compared with CK, CF, BS25, BS75and BS100, respectively, and there were significant differences between them($P<0.05$), BS25 and BS100 had no significant difference ($P>0.05$).

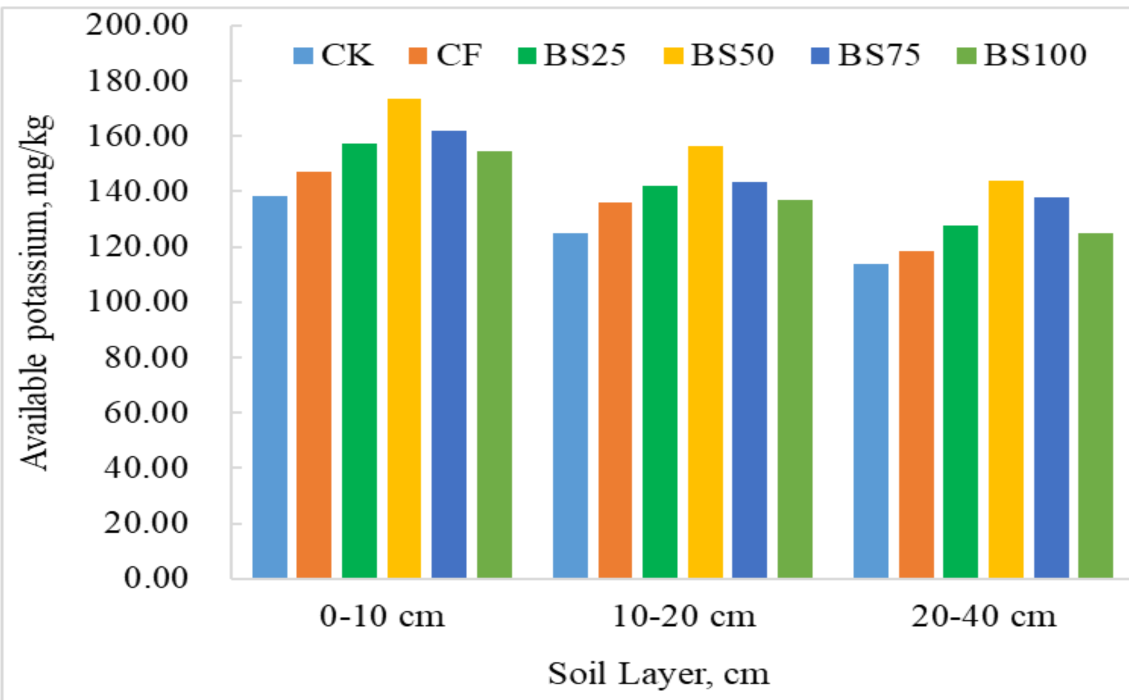

Fig. 3.14. Soil available potassium content with different proportion of biogas slurry in different soil layers

In the 10-20 cm soil layer, the available potassium content first increased and then decreased with the increase of biogas slurry proportion, and the available potassium content of BS50 was the highest, with a value of 156.63 mg•kg^{-1}, which was increased by 15.30 % compared with CF, and there was a significant difference between the treatments ($P<0.05$), there was no significant difference between CF and BS100 ($P>0.05$), no significant difference was found between BS25 and BS75 ($P>0.05$).

In 20-40 cm soil layer, the order of available potassium content was BS50>BS75>BS25>BS100>CF>CK, CF increased by 4.13 % compared with CK($P<0.05$), there was no significant difference between BS100 and BS25 ($P>0.05$), and the available potassium content of BS50 was the highest, which was increased by 26.54 %, 21.52 %, 12.64 %, 4.62 % and 15.20 %, respectively, compared with CK, CF, BS25, BS75 and BS100, showing significant differences ($P<0.05$).

Biogas slurry increased the content of available potassium in soil, which was conducive to the absorption of potassium by winter wheat, which was consistent with previous research results (Dong et al. 2021; Xu et al. 2021).

Biogas slurry contains potassium (Baróg et al. 2020). The application of biogas slurry may change the symbiotic relationship between plants and microorganisms, and improve the turnover rate of soil potassium through the transformation and activation of microorganisms, thus increasing the content of available potassium.

With the increase of soil layer from 0 to 40 cm, the available K content decreased, and the order of available K content was 0-10 cm>10-20 cm>20-40 cm, consistent with the results of Wu et al. (Wu et al. 2014).

3.2.5. Organic matter in soil

Soil organic matter content is an important index of soil fertility and one of the main sources of plant nutrition. Soil organic matter not only improves the physical properties of soil by maintaining soil fertility and buffering, but also plays an important role in soil formation and fertility evolution as the main source of enzymatic substrates in soil. At the same time, soil organic matter is also an important C source and N source for the growth and metabolism of soil microorganisms, which is closely related to soil nutrient content (Zhang et al. 2008a).

The effects of different treatments (CK, CF, BS25, BS50, BS75, BS100) on soil organic matter content are shown in Fig. 3.15. As can be seen from the figure, the order of soil organic matter content in 0-10 cm, 10-20 cm and 20-40 cm soil layers is BS100>BS75>BS50>BS25>CF>CK, soil organic matter content increased with the increase of biogas slurry proportion after winter wheat harvest, which was consistent with the research results of Guo et al. (Guo et al. 2022).

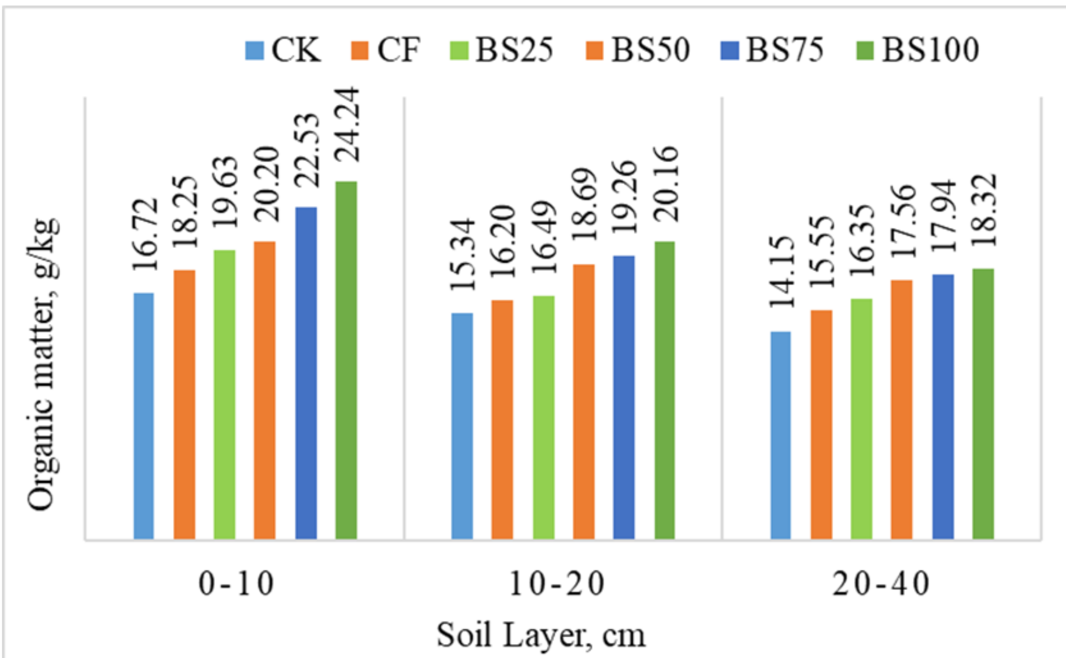

Figure 3.15. Soil organic matter content with different proportion of biogas slurry in different soil layers

There were significant differences between all treatments and CK ($p< 0.05$), the soil organic matter content of BS100 was the highest, which was 24.24g•kg^{-1}, 20.16g•kg^{-1} and 18.32 g•kg^{-1}, respectively, and increased by 32.82 %, 24.44 % and

17.84 % compared with CF, respectively. Fan et al. studied the effect of application of biogas slurry on soil nutrients and believed that application of biogas slurry could increase the content of organic matter in soil, and application of biogas slurry could increase the content of organic matter in soil more than application of chemical fertilizer (Fan et al. 2011), this may be mainly because in the process of biogas fermentation, most of the easily degradable substances are hydrolyzed, acidified and degraded under the action of microorganisms, but they are not completely degraded. Secondly, the biogas slurry contains a large number of organic substances such as cellulose, hemicellulose and lignin that are difficult to degrade. After being applied to the soil, it slowly decomposes under the action of multiple microorganisms over a certain period of time. Finally, it will be transformed into soil organic matter, thus greatly increasing the content of soil organic matter treated by biogas slurry. Relevant studies have shown that the application of organic matter to soil can not only promote the decomposition of organic matter, but also increase the content of soil organic matter, improve the quality of humus, and thus improve the fertilizer supply capacity of soil (Huang 2000; Li et al. 2009).

With the increase of soil layer, the content of organic matter in different treatments decreased, and the content of organic matter was 0-10 cm> 10-20 cm>20-40 cm, which is consistent with the results of Wang et al. (2019).

Conclusions to Chapter 3

In this study, there were significant differences between different soil layers, and between each treatment and CK. After adding biogas slurry, the soil bulk density was significantly reduced, especially BS50 soil bulk density was the lowest, and it was within the suitable bulk density range for winter wheat, which was conducive to the growth and development of winter wheat and the increase in yield. It was found that different fertilization treatments could significantly change the particle size distribution and adjust the mass composition of aggregates with different particle sizes.

The stability of soil aggregates varies in different soil layers and under different treatment conditions. Biogas slurry treatment is beneficial to increase the MWD of 0-10 cm, 10-20 cm, 20-40 cm force-stable aggregates and water-stable aggregates, with BS50 being the largest.

CF treatment can reduce pH, and application of biogas slurry can increase pH.

The application of biogas slurry in agricultural planting can significantly increase the nitrogen content of the soil, thereby satisfying the absorption of nutrients by crops. In 0-10 cm, 10-20 cm, and 20-40 cm, the total nitrogen content is highest by the BS50 treatment.

The content of soil available phosphorus was different in different soil layers and different treatment conditions, which indicated that the application of biogas slurry could improve the supply level of soil available phosphorus, but more is not always better.

Biogas slurry increased the content of available potassium in soil, which was beneficial to the absorption of potassium by winter wheat.

The soil organic matter content increased with the increase of biogas slurry concentration. The soil organic matter content of BS100 was the highest.

CHAPTER 4
EFFECT OF COMBINED APPLICATION OF BIOGAS SLURRY AND CHEMICAL FERTILIZER ON SOIL ENZYME ACTIVITY

4.1. Effect of different proportions of biogas slurry on enzyme activity in the same soil layer

4.1.1. Sucrase

Sucrase in soil, also known as invertase, is involved in the conversion of carbohydrates to hydrolyze sucrose into glucose and fructose, which can be converted into nutrients that can be utilized by plants and microorganisms. The activity of sucrase can reflect the degree of soil maturation and fertility (Wang et al. 2006). The activity of sucrase in soil is affected by many factors, such as the content of organic matter, the number of microorganisms and the intensity of soil respiration (Ni et al. 2008a).

The effects of different treatments (CK, C, BS25, BS50, BS75, BS100) on soil sucrase activity are shown in Fig. 4.1. As can be seen from the figure, the sucrase activity of different treatments was higher than that of CK（$p<0.05$. The order of sucrase activity in 0-10 cm was BS75>BS100>CF> BS50> BS25>CK. The sucrase activity of BS75 and BS100 was higher than CF, and the sucrase activity of BS75 was the highest (15.28 mg/(g*d)), and there were significant differences between BS75 and other treatments ($p<0.05$). The order of sucrase activity in 10-20 cm was BS75>BS50>CF>BS100>BS25>CK, and the sucrase activity of BS75 was the highest (30.73mg/(g*d)), and there were significant differences between BS75 and other treatments ($p<0.05$). The order of sucrase activity in 20-40 cm was BS75>BS50>BS25>BS100>CF>CK, and there were significant differences among different treatments ($p<0.05$). The highest sucrase activity of BS75 was 31.28 mg/(g*d).

In this study, the soil sucrase activity applied by biogas slurry was greater than CK, indicating that biogas slurry increased soil sucrase activity, which provided energy for organisms in soil, increased the accumulation of soil organic carbon and the intensity of decomposition and transformation, and increased soil fertility. Soil sucrase activity in BS75 was the highest and soil biological activity was the strongest. With increasing of biogas slurry to BS100 soil sucrase activity decreased.

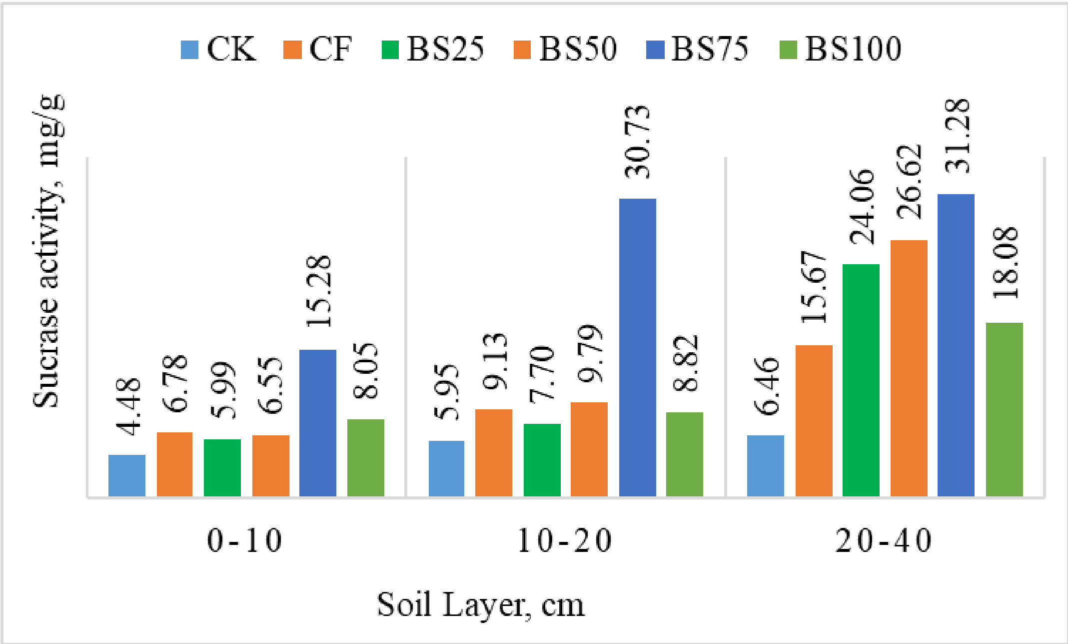

Figure 4.1. Soil Sucrase activity and distribution in different soil depth with different fertilization

Biogas slurry can increase the activity of sucrase, possibly because the conversion ability of soil carbon is closely related to the content of soil organic matter. Biogas slurry increases the content of organic carbon, provides more enzymatic substrate for sucrase, and improves the activity of sucrase to the greatest extent, which is similar to the research results of Hu et al. (2021).

4.1.2. Urease

Urease is an obligate amidase that can catalyze the hydrolysis of peptide bonds in organic molecules, catalyze the hydrolysis of urea into CO_2 and NH_3, and provide nitrogen source for the growth of plants and microorganisms (Li et al. 2008b). As an important participant in biochemical reactions, urease plays a key role in the energy flow and material circulation of ecosystems (Cao et al. 2016). So soil quality can be assessed by urease activity (Dindar et al. 2015).

Studies have found that co-application of biogas slurry with other fertilizers can help improve soil environment and enzyme activity, promote nutrient conversion and crop utilization (Wei et al. 2011), in addition, the application proportion affects the physical, chemical and biological properties of soil (Chai et al. 2023; Wei et al. 2023).

Under different treatment conditions (CK, CF, BS25, BS50, BS75, BS100), the content of soil urease activity is shown in Fig. 4.2.

As can be seen from Fig. 4.2, in the 0-10 cm soil layer, the sequence of soil urease activity is as follows: BS50>BS75>BS25>CF>BS100>CK, there were significant differences between each treatment and CK ($P<0.05$). The urease activity of BS50 was the highest (235.33ug/g), which was 16.37 %,10.47 %, 9.95 % and 43.56 % higher than that of CF, BS25, BS75 and BS100, respectively. There were significant differences between BS75 and CF and BS100 ($P<0.05$), no significant difference was found between BS25 and BS75($P>0.05$).

In the 10-20 cm soil layer, the sequence of soil urease activity was BS50>BS75>BS25>CF>BS100>CK, there were significant differences between each treatment and CK($P<0.05$). The urease activity of BS50 was the highest (337.79 ug/g), which was significantly different from that of other treatments ($P<0.05$), compared with CF, BS100 increased. In the 20-40 cm soil layer, the sequence of soil urease activity was BS50>BS25>BS75>BS100>CF>CK, there were significant differences between each treatment and CK ($P<0.05$), sucrase activity CF increased by 17.38 % compared with CK, the sucrase activity of BS treatment was higher than CF, and there was no significant difference between BS100 and CF ($P>0.05$), BS50, BS25, BS75 were significantly different from CF ($P<0.05$). The urease activity of BS50 was the highest (351.45 ug/g), which was significantly different from that of other treatments ($P<0.05$).

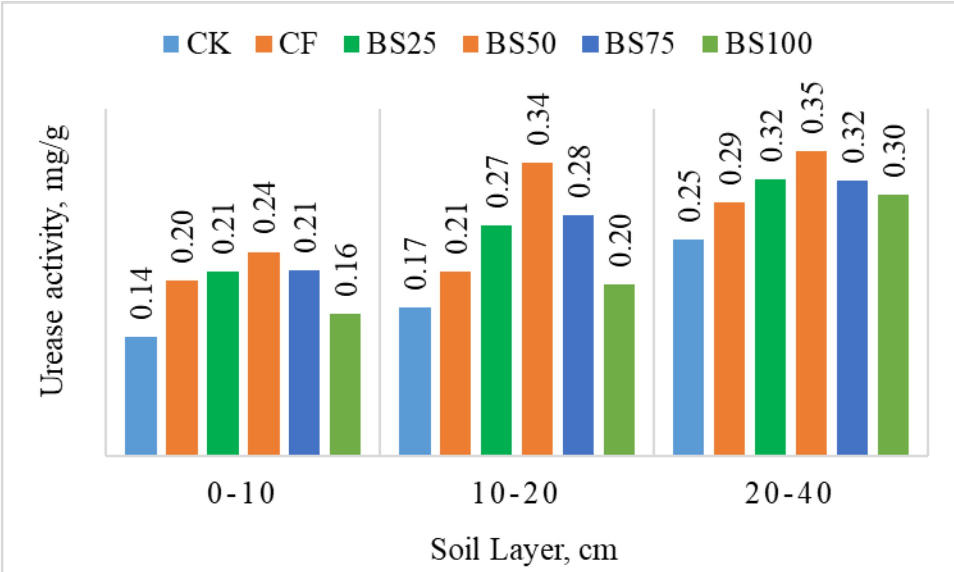

Fig. 4.2. Soil urease activity and distribution
in different soil depth with different fertilization

In summary, the urease activity of BS and CF was higher than that of CK, indicating that biogas slurry or chemical fertilizer could improve the urease activity of soil. The urease activity of biogas slurry (BS25, BS50, BS75) was higher than that of CF, and the combined application of biogas slurry and chemical fertilizer had a significant activation effect on the urease activity. It is beneficial to increase soil urease activity and soil nitrogen pool, improve soil nitrogen conversion status, increase plant nitrogen sources, and increase soil nitrogen conversion rate.

With the increase of biogas slurry proportion, the urease activity increased first and then decreased, indicating that the benefit of biogas slurry on soil was dose-dependent. This is consistent with previous studies (Huang et al. 2016). Appropriate co-application of biogas slurry with chemical fertilizer can improve soil urease activity, but excessive fertilization is counterproductive.

In the 0-10 cm,10-20 cm and 20-40 cm, the urease activity of BS50 was the highest and the utilization rate of biogas slurry was the highest. Co-application of biogas slurry with chemical fertilizer can improve soil urease activity, on the one hand, because biogas slurry is rich in easily decomposed nitrogen compounds and biological organisms, which can promote urease activity. At the same time, biogas slurry is a good substrate for soil enzymes, which can introduce urease into the soil (Zafar-Ul-Hye et al. 2022). On the other hand, co-application of biogas slurry with chemical fertilizer can effectively improve soil structure, significantly increase soil fertility, promote soil microbial reproduction, and thus fix and release more soil urease (Liu et al. 2022).

4.1.3 Phosphatase

Phosphatase can catalyze the hydrolysis of organophosphorus compounds to produce phosphoric acid and other substances, and its activity can characterize soil fertility, especially soil phosphorus content (Trasar-Cepeda et al. 2008).

Phosphatase is an adaptive enzyme, and the activity of phosphatase is enhanced when the content of soil available phosphorus is low.

Figure 4.3, appendix G show that the activity sequence of soil acid phosphatase in 0-10 cm soil layer is as follows: BS50>BS75>BS100>BS25>CF>CK and the acid phosphatase activity of BS50 was the highest (4.10 nmol/g*d), which was significantly different from that of other treatments ($P<0.05$), increased by 33.80 %, 21.04 % and 14.94 % compared with CK, CF and BS100, respectively.

The activity sequence of acid phosphatase in 10-20 cm soil layer was BS50>BS25>CF>BS75>BS100>CK, and there were significant differences between each treatment and CK ($P<0.05$), the acid phosphatase activity of BS50 was the highest (3.21 nmol/g*d), which was 13.55 % higher than CF.

The activity sequence of acid phosphatase in 20-40 cm soil layer was BS50>BS25>CF>BS75>BS100>CK, there were significant differences between each treatment and CK ($P<0.05$), the acid phosphatase activity of BS50 was the highest (2.60 nmol/g*d), which was increased by 12.57 % compared with CF.

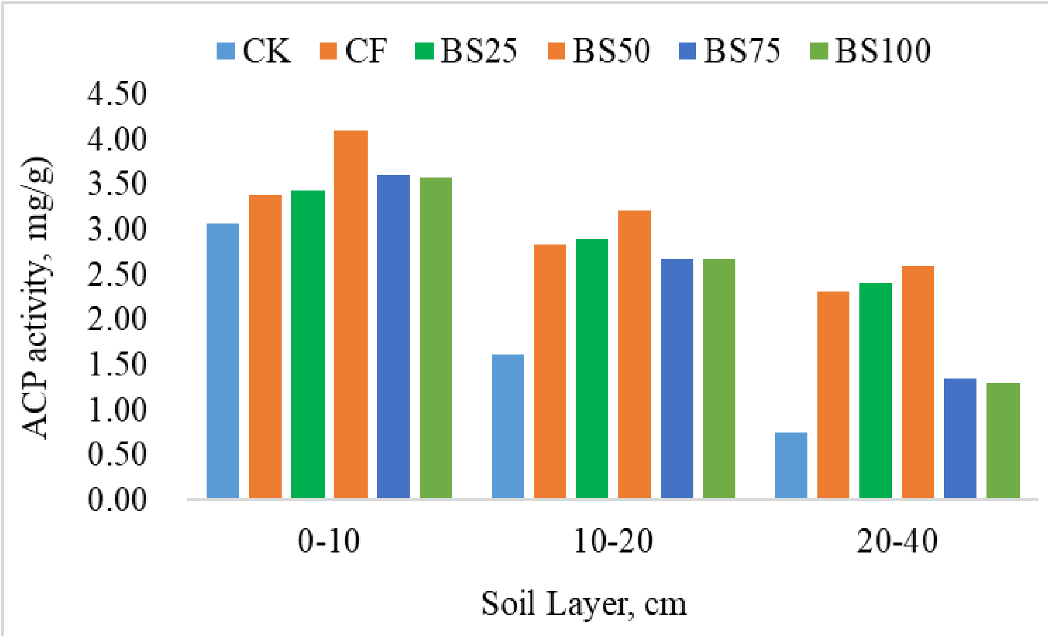

Fig. 4.3. Soil acid phosphatase activity and distribution
in different soil depth with different fertilization

As described above, the acid phosphatase activity of BS and CF was greater than that of CK, and both biogas slurry and chemical fertilizer increased soil acid phosphatase activity. As the proportion of biogas slurry increased, acid phosphatase in soil first increased and then decreased, indicating that an appropriate amount of biogas slurry can catalyze the conversion of phosphorus in soil into phosphorus easily absorbed by organisms, and improve its utilization rate. Biogas slurry can promote enzyme activity by stimulating microbial activity. Hao Xianjun et al. (2011) showed that the application of biogas slurry significantly improved the phosphatase activity of cabbage continuous cropping soil.

4.1.4 Catalase

Catalase belongs to the class of REDOX reductases, which can catalyze the decomposition of hydrogen peroxide into water and oxygen ($H_2O_2 \rightarrow H_2O+O_2$) to prevent the accumulation of hydrogen peroxide from causing harm to organisms (Wan et al. 2017). Its activity is related to soil respiration intensity and microbial activity, which reflects the intensity of soil microbial life activity to a certain extent, and can be used as an indicator of the activity intensity of soil organic matter (Zhou et al. 2022).

The effects of different treatments (CK, CF, BS2, BS50, BS75, BS100) on soil catalase activity are shown in Fig. 4.4. As can be seen from Fig. 4.4, the order of catalase activity in 0-10 cm, 10-20 cm, and 20-40 cm is as follows: BS100 >BS75>BS50>BS25>CF>CK, there were significant differences between each treatment and CK ($P<0.05$), there was no significant difference between CF and BS25 ($P>0.05$), BS100 had the highest catalase activity, which was 93.19 mmol•g^{-1}, 95.11 mmol•g^{-1}, 97.12 mmol•g^{-1}, and increased by 12.35 %, 6.21 % and 5.86 % compared with CF, respectively.

The activity of catalase reflects the process of organic oxidation in soil. In this study, with the increase of the ratio of biogas slurry, the activity of catalase increases, indicating that the application of biogas slurry has a significant promotion effect on catalase activity, which can accelerate the removal of hydrogen peroxide from organisms and soil, so as to protect it from damage. The activity of catalase is the highest when BS100 is applied, which shows the strongest detoxification effect. The activity of catalase with CF was lower than that of BS, indicating that long-term application of chemical fertilizer had a significant inhibitory effect on soil catalase activity, which was easy to lead to the accumulation of root secretions, thus aggravating the toxic effect of hydrogen peroxide on crops.

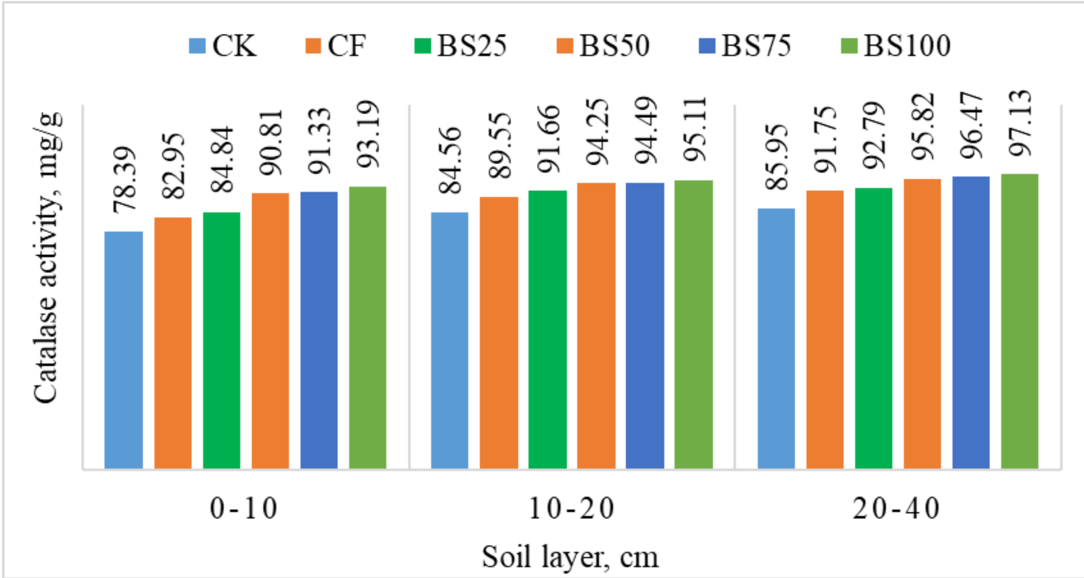

Fig. 4.4. Soil catalase activity and distribution
in different soil depth with different fertilization

Among different biogas slurry replacement ratios, BS50, BS75 and BS100 showed significant differences compared with CK, CF and BS25($P<0.05$), but there was no significant difference between BS50, BS75 and BS100, indicating that biogas slurry can improve catalase activity, but with the increase proportion of biogas slurry, the improvement of catalase activity was limited. The BS50 could improve the oxidation resistance of winter wheat and reduce the formation and release of peroxide in winter wheat. The accumulation of peroxides in living cells in soil was reduced, and the relatively stable REDOX balance of soil was maintained. Application of biogas slurry can significantly increase the activity of catalase in soil, which may be due to the rich ions and active substances in biogas slurry, which stimulate the activity of catalase and accelerate the decomposition of toxic substances in soil.

4.2. Vertical changes of enzyme activity in different soil layers with the same concentration

Soil enzyme system is an important part of the physiological and biochemical characteristics of soil. It actively participates in the material cycle and energy transformation in the ecosystem, and is one of the important components of soil. Affected by the soil fertility and productivity level in each soil layer, the vertical distribution of soil enzymes has obvious regularity.

Liu's study showed that the activities of sucrase, alkaline phosphatase and urease decreased from top to bottom at 0-60 cm, while the activity of catalase was the highest in the subsoil layer of 20-40 cm (Liu 2021).Guo et al. (2012) measured soil enzyme activities on the meadow grassland and found that urease activities increased with the increase of soil layer at 0-40 cm, invertase and catalase activities decreased with the increase of soil layer, and there was an obvious surface accumulation in 0-20 cm. Zhao and Wang (1995) showed that the catalase activity increased with the deepening of soil layer, and the activities of urease, invertase and alkaline phosphatase in the vertical distribution showed that the upper layer was higher than the lower layer. According to Yang's study, soil urease activity decreased in 0-30 cm, while catalase activity did not change regularly (Yang et al. 2012).

Soil enzyme activity is affected by many factors, due to the different conditions of the study area, the research object and the research time, the same soil enzyme activity shows different changes in the vertical distribution (Zhao et al. 1995; Li et al. 2012; Yang et al. 2012).

After winter wheat harvest, the effects of the same fertilization treatment on enzyme activity in different soil layers as summary were shown in Figure 4.5. As can be seen from Figure, under the same fertilization condition, the sequence of enzyme activity was 20-40 cm>10-20 cm>0-10 cm (except acid phosphatase), all of them increased with the increase of soil layer, which was consistent with the research results of Guo et al. (Guo et al. 2012).

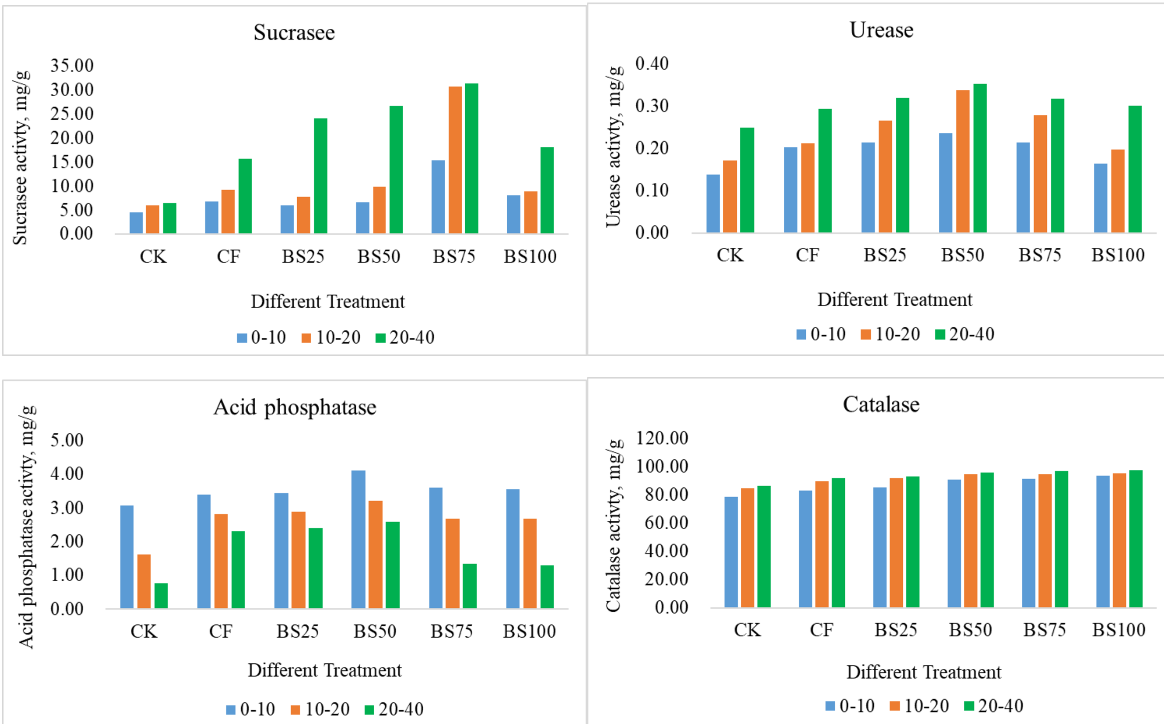

Fig. 4.5. The vertical variation of enzyme activity

in different treatments

This may be related to factors such as enhanced deep root activity and gradual increase of root secretions in late stage of winter wheat (Xiong Mingbiao et al., 2003). Soil enzymes are mainly derived from the secretory activities of soil microorganisms and crop roots (Tadano et al. 1993). The research of Wang et al. (2001) showed that with the advancement of wheat growth process, root activity tended to move down to deep soil. In the late growth period of winter wheat, the root activity in 20-40 cm soil layer was higher than that in 0-20 soil layer, and more root secretions entered the soil, which increased enzyme activity. At the same time, root exudates also provide sufficient carbon source for the activity and growth of microorganisms, improve the activity and reproduction rate of soil microorganisms, and increase soil enzyme activity.

In addition, with the deepening of the soil layer, the soil compactness increases and the permeability is poor, which promotes the catalase activity to increase, decomposes the root-harmful hydrogen peroxide in the soil into water and oxygen, and reduces and directly eliminates the harm caused by hydrogen peroxide in the soil environment. The release of catalase can protect the root system.

Moreover, the surface root system is greatly affected by air temperature, dry and wet soil and other factors, and the living environment conditions are unstable, which also accelerates the senescence of the upper root and reduces the enzyme activity of the soil (Wang et al. 2001).

The vertical distribution of urease activity in winter wheat soil was beneficial to increase the yield of winter wheat.

Acid phosphatase activity decreased from top to bottom in 0-40 cm, that is, phosphatase activity in 0-20 cm was higher than that in 20-40 cm, which was consistent with the research results of Li et al. (2007).This may be caused by two reasons, on the one hand, the application of biogas slurry prepared a rich source of nutrients for soil microorganisms, high content of organic matter, phosphorus and microbial content is also high, and winter wheat roots belong to the beard root system, mainly concentrated in the 0-20 cm, where ventilation condition is better, soil microbial respiration intensity increases and metabolism is active, therefore, the phosphatase activity in 0-20 cm was higher. On the other hand, in the 20-40 cm, winter wheat roots are less, soil hydrothermal conditions and air permeability become worse, and soil porosity becomes smaller, which restricts the normal metabolic activities of soil, while the content of organic matter decreases, and the enzyme production ability of soil microorganisms is restricted (Wang et al. 2016). Therefore, the combination of these factors led to the hierarchical distribution of soil acid phosphatase at the depth of 0-40 cm.

The four soil enzyme activities measured in this study were sensitive to the application of fertilizer and biogas slurry. Biogas slurry returning to field could improve the enzyme activities of soil in the 0-40 cm, especially in the 20-40 cm, which was beneficial to delay the aging process of winter wheat root system in the later growth period and slow down the decline of the activity value of the upper root system. In addition, the overall root system can maintain a larger root activity value (Wang 2017), which is conducive to maintaining a higher biological activity of soil, promoting the transformation of soil nutrients, improving the fertilizer supply capacity of soil to crops, and meeting the nutrient demand during the growth and development of crops. This will play a favorable role in improving soil fertility and increasing the yield of winter wheat.

As a biocatalyst for the intensity of some processes in soil, soil enzymes are the metabolic power of soil that plays an executing role in some cycles of soil, which is closely related to fertilization, tillage, soil structure and type and other agricultural management measures. Generally, the performance of soil enzyme activities in soil reflects the state of soil at that time. Moreover, soil enzymes are sensitive to the changes of external environmental factors to a certain extent, and when soil ecological environment deteriorates, changes in their activities can be used to provide early warning (Badiane et al. 2001). Soil enzyme activity is a comprehensive method to reflect soil quality changes (soil fertility and evolution process, etc.), and it is of great significance to explore the changes of soil structure and function and the sustainable use of land resources.

Conclusions to Chapter 4

The application of biogas slurry increased the activity of sucrase in soil, and with the increase of biogas slurry application, the activity of sucrase first increased and then decreased. The activity of sucrase with BS75 was the highest in 0-10 cm, 10-20 cm and 20-40 cm, which was 15.28mg•g^{-1}, 30.73 mg•g-1 and 31.28 mg•g^{-1}, respectively. Sucrase provides energy for organisms in soil, increases the intensity of organic carbon accumulation, decomposition and transformation, and increases soil fertility level.

Application of biogas slurry and chemical fertilizer can increase soil urease activity. The urease activity of combined application of biogas slurry and chemical fertilizer (BS25, BS50 and BS75) was higher than that of CF, and the combined application of biogas slurry and chemical fertilizer significantly activated the urease activity in soil, which was beneficial to the improvement of soil urease activity. With the increase of biogas slurry replacement ratio, the urease activity increased first and then decreased, indicating that the benefit of biogas slurry on soil was dose-dependent, and appropriate biogas slurry was beneficial to the improvement of soil urease activity, but excessive fertilization was counterproductive. In 0-10 cm, 10-20 cm, 20-40 cm, soil urease activity was the highest in BS50 treatment (235.33ug g^{-1}, 337.79 ug g^{-1} and 351.45 ug g^{-1}, respectively), and was significantly different from that in other treatments. Combined application of biogas slurry and chemical fertilizer can enrich soil nitrogen pool, improve soil nitrogen transformation, increase the nitrogen source absorbed by winter wheat, and increase the rate of soil nitrogen transformation.

Both biogas slurry and chemical fertilizer increased soil acid phosphatase activity. With the increase of biogas slurry concentration, soil acid phosphatase first increased and then decreased. An appropriate amount of biogas slurry could catalyze the conversion of soil phosphorus into phosphorus easily absorbed by organisms, and improve its utilization rate. The activity of acid phosphatase with BS50 was the highest in different soil layers.

The order of catalase activities in 0-10 cm, 10-20 cm, and 20-40 cm soil was as follows: BS100>BS75>BS50>BS25>CF>CK, and there was a significant difference between each treatment and CK ($P<0.05$), and there was no significant difference between CF and BS25 ($P>0.05$), BS100 catalase activity was the highest, with 93.19 mmol•g^{-1}, 95.11 mmol•g^{-1} and 97.12 mmol•g^{-1}, which were 12.35 %, 6.21 % and 5.86 % higher than CF, respectively. The activity of soil catalase reflects the process of soil organic oxidation, with the increase of the proportion of biogas slurry, the catalase activity increases, and the biogas slurry has a significant promoting effect on soil catalase activity, which can accelerate the removal of hydrogen peroxide in organisms and soil to prevent damage, and the soil catalase activity of BS100 treatment is the largest, and the soil detoxification effect is the strongest. Long-term application of chemical fertilizers has a significant inhibitory effect on soil catalase activity, which can easily lead to the accumulation of root exudates, thereby aggravating the toxic effect of hydrogen peroxide on crops. Biogas slurry application can significantly increase soil catalase activity and accelerate the decomposition of toxic substances in soil.

Under the influence of soil fertility and productivity, the vertical distribution of soil enzymes has obvious regularity. Biogas slurry can improve the soil enzyme activity of winter wheat in 0-40 cm soil layer, especially in 20-40 cm soil layer, which is conducive to delaying the aging process of winter wheat root in the late growth period, promoting the transformation of soil nutrients, improving the ability of soil to supply fertilizer to winter wheat, and meeting the demand for nutrients during the growth and development of winter wheat, which is beneficial to the improvement of soil fertility and crop yields of winter wheat.

CHAPTER 5
THE EFFECT OF CO-APPLICATION OF BIOGAS AND CHEMICAL FERTILIZER ON THE GROWTH AND DEVELOPMENT OF WINTER WHEAT

5.1. Winter wheat yield

Crops are the mainstay of agricultural production, and crop yield is an important indicator of agricultural productivity. Winter wheat is one of the most important food crops globally, and its increased yield has a significant impact on global food and nutritional security. Biogas contains a large amount of fast-acting nutrients, especially ammonium nitrogen which accounts for more than 70 % of the total nitrogen (Jin et al. 2011). At the same time, its high content of water-soluble organic matter can be utilized by soil microorganisms as an energy source to facilitate their decomposition of organic nitrogen in the substrate (Feng et al. 2011a). Therefore, both fast and slow nutrient supply can be achieved. Biogas fertilizer also contains amino acids, vitamins, sugars, antibiotics, physiologically active substances, etc., as fertilizer application can effectively promote crop growth (Matsi et al. 2003; Garg et al. 2005; Chen et al. 2011a; Gericke et al. 2012; Sun et al. 2012).

Li Youqiang (2014c) studied the effect of biogas application on wheat yield, and the results showed that the application of biogas treatments were all extremely significant yield increase compared with the control, and the highest wheat yield of 6525.00 kg•hm^2 was achieved in the treatment of basal fertilizer 60,000 kg•hm^{-2} of biogas + 150 kg•hm^2 of urea. Evens and Smitht (1977) used biogas slurry and biogas residue produced by fermentation of pig and cow dung as raw materials to grow corn, and the results showed that biogas slurry could not only promote the growth of corn but also increase the yield of corn.

In this study, the effects of different treatments on the yield of winter wheat were shown in Fig. 5.1. Both biogas slurry and chemical fertilizer application increased the yield of winter wheat, with significant differences compared with CK ($p<0.05$), CF increased by 7.52 t/ha (27.30 %) compared with CK. There were significant differences between biogas slurry treatment and CF ($p<0.05$) with the increase of biogas slurry, winter wheat yield first increased until 9.83 t/ha BS50 and then decreased to BS100, which was consistent with previous research results (Shangguan et al. 2000; Matsi et al. 2003; Garg et al. 2005; Liu et al. 2009; Olesen et al. 2009) .

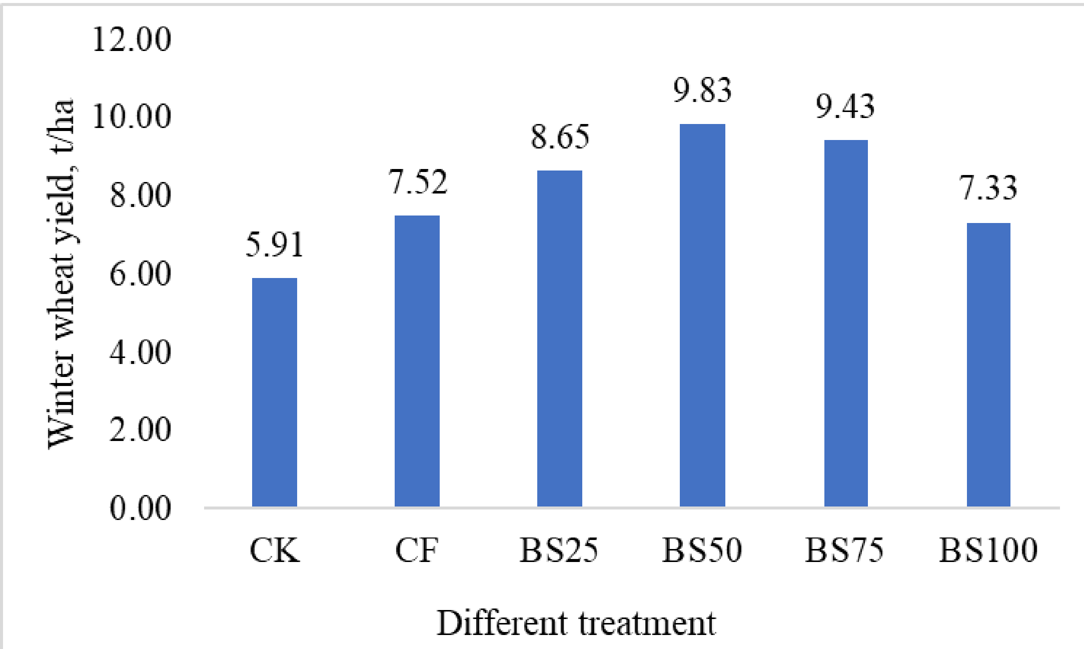

Fig. 5.1. Effects of different treatment on winter wheat yield

Compared with CF, BS25, BS75 and BS100, the increase BS50 was 30.79 %, 13.61 %, 4.31 % and 34.06 %, respectively, and there were significant differences between BS50 and other treatments($p<0.05$).

The yield of winter wheat combined with biogas slurry and chemical fertilizer is higher than that of chemical fertilizer, because the filling period is the last period of wheat growth and development, and it is also the most critical period for the formation of wheat yield. If nutrients can be absorbed more fully in this period, the volume and weight of grains can also increase rapidly, thereby improving the yield and quality of wheat. Combined application of biogas slurry and chemical fertilizer can give full play to their respective advantages, and inorganic nitrogen in chemical fertilizer is conducive to the reasonable construction of plant type and optimization of wheat canopy structure during the vegetative growth period (Li et al. 2008a). Biogas slurry is rich in nutrients, vitamins, auxin and other bioactive substances (Liu et al. 2009; Li et al. 2010; Li 2011; Shang et al. 2011), which can improve the growth environment of wheat roots, enable the root growth point to develop rapidly, enhance root vitality (Kong et al. 2008), and help maintain a high level of nitrogen supply in soil during the reproductive growth period of wheat (Zhang et al. 2011), improve the photosynthetic characteristics of wheat leaves and increase wheat yield (Xie et al. 2010; Feng et al. 2011b).

At present, many studies have focused on the effects of different ratios of biogas slurry and inorganic fertilizer on crop yield. Most researchers believe that more biogas slurry is not always better, the best ratio may exist (Feng et al. 2011a). In this study, the highest yield of winter wheat was found in BS50 treatment, with biogas slurry and fertilizer N accounting for 50 % each, and too much or too little biogas slurry was not conducive to crop yield increase, and this result was also found in other crops (Xu et al. 2005; Liu et al. 2007).

BS50 is the optimal combination of biogas slurry and chemical fertilizer, which can well match the nutrient demand of winter wheat at different growth and development stages, ensure the continuous supply of nutrients, promote wheat nutrient growth and nutrient accumulation, and achieve the best level of wheat yield increase. Winter wheat yield reached the maximum in BS50, and decreased with the increase of biogas slurry proportion, which may be due to the large application amount of biogas slurry, strong nutritional growth, and late wheat ripening, and the yield decreased.

The yield of BS100 was lower than that of CF, and there was significant difference between them ($p<0.05$), indicating that BS100 will reduce the yield, which is consistent with the experimental results of Wu Huashan et al. (2012b) in corn, but there are studies on Chinese cabbage, cabbage, rice, corn and other crops that show that the BS100 will not reduce crop yield (Sun 2006; Tang et al. 2010; Cela et al. 2011). This may be related to climatic environment, crop species, soil fertility, biogas concentration, application times and periods, etc.

5.2. Composition of wheat yield

In the early 20th century, Engledow and Wadham (1923), in order to reveal the mechanism of controlling yield formation, decomposed the yield of cereal crops into panicle number, grain number per panicle and grain weight, and put forward the yield composition theory of quantitative yield analysis. Through this theory, we can not only intuitively understand the composition factors of yield, but also understand the formation law of yield from the changes of these factors, and play an important guiding role in the research and application of wheat and other crops high yield (China 2008), genetic breeding, cultivation physiology, demonstration and popularization. The three yield components of wheat have an ideal balance in each ecological environment, which depends on the local sunshine length and light and heat resources, and is affected by water and fertilizer supply (Hussain et al. 1998; Xu et al. 2008; Anderson 2010). The balance of yield factors is ecologically stable.

5.2.1. The number of ear, grain number per ear

The effective number of ear and grain number per ear of different treatments were BS50>BS75>BS25>CF>BS100>CK (Fig. 5.2). Biogas slurry and fertilizer application increased the number of ear and grain number per ear of winter wheat, and there were significant differences with CK ($P<0.05$). The number of ear and grains per ear applied with biogas slurry and chemical fertilizer were higher than those of CF, and there were significant differences between them ($P<0.05$), with the increase of biogas slurry, the number of ear and grains per ear increased first to BS50 and then decreased to BS100, and the number of ears and grains per ears of BS50 were the highest, which were $634*10^4$ pcs /ha and 36.2 grains per ear, respectively, which were 8.01 % and 17.03 % higher than CF, respectively.

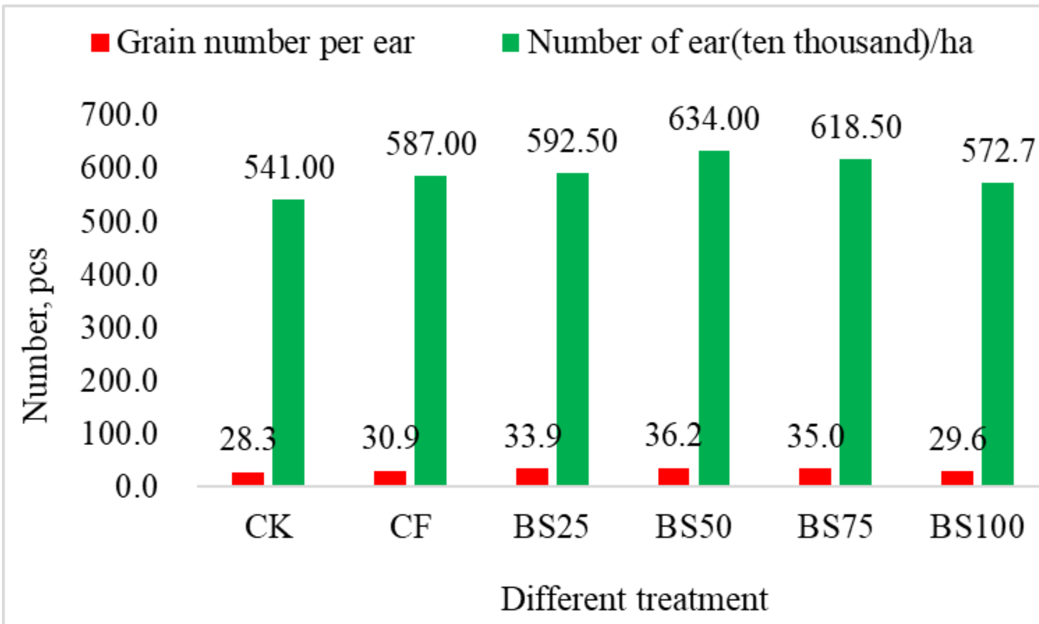

Fig. 5.2. The number of ear, grain number per ear

In the early stage, fertilizer supply of CF is sufficient, and higher fertilizer is used in the vegetative growth of winter wheat, resulting in too many ineffective tillers that are difficult to control. In the maturity stage, the photosynthetic capacity of leaves is poor due to insufficient nutrients, which affects the nutrient supply to seeds in the later stage, resulting in lower seed setting rate and ultimately lower yield. The nutrient release of pure biogas slurry was slow and the fertilizer utilization rate was relatively low, which caused nitrogen deficiency in the early stage of winter wheat, affected tillering and panicle differentiation, and led to the decrease of effective panicle number and grain number per ear. The combined application of biogas slurry and chemical fertilizer can give full play to their respective advantages, promote wheat growth, improve the early tillering ability and tillering rate of wheat, and increase the effective number of ear and grains number per unit area.

Biogas slurry is a kind of fertilizer with comprehensive nutrient content and rapid efficiency. Nitrogen nutrients, especially available nitrogen nutrients, are higher, and the application of biogas slurry on wheat shows rapid greening and more tillering, which can significantly improve the growth of wheat (Kong et al. 2008; Feng et al. 2010).

5.2.2. Grain weight

The long-term accumulation of wheat breeding knowledge shows that in order to increase yield, it is necessary to improve not only the "source" (the number of ear and grains per ear, etc.), but also the "reservoir" (Grain weight, etc.). 1000-grain weight of winter wheat is shown in Figure 5.3.

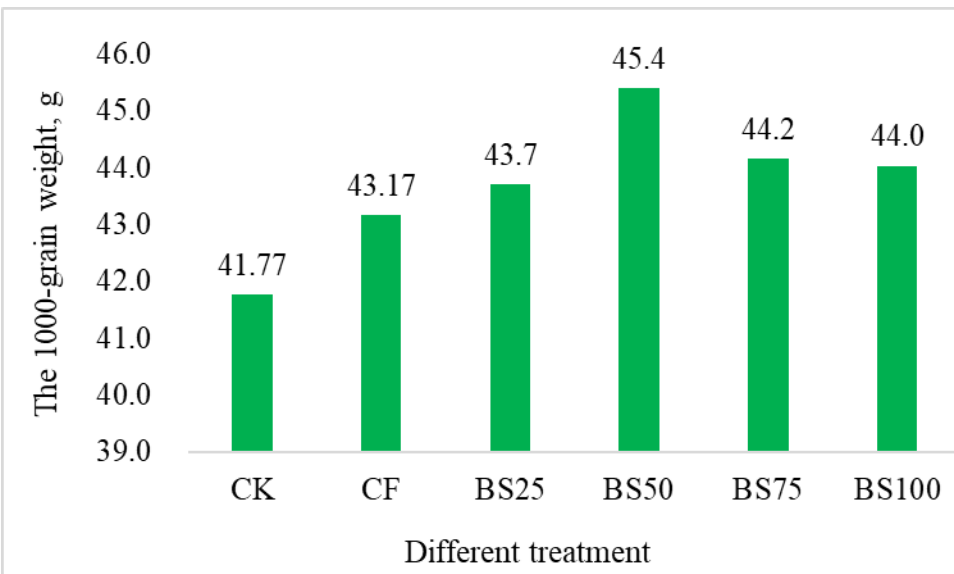

Fig. 5.3 The 1000-grain weight

Both fertilizer and biogas slurry increased the 1000-grain weight of winter wheat, and there were significant differences between them and CK ($P<0.05$), compared with

CF, biogas slurry increased the 1000-particle weight, and there was significant difference between the BS and CF ($P<0.05$), the 1000-grain weight of BS50 was the highest (45.4 g), which was increased by 5.17 %, 3.89 %, 2.79 % and 3.10 % compared with CF, BS25, BS75 and BS100, respectively.

Wheat filling period is a period that requires more nutrients, in this period, the more nutrients transported by the plant to the grain, the heavier the 1000-grain weight of wheat, the yield and quality of wheat are related to the number of photosynthetic products produced by wheat leaves in this period, protect the wheat leaves in this period, make the wheat leaves as long as possible and maintain a good function of manufacturing photosynthetic capacity. Thereby increasing the 1000-grain weight of wheat. Combined application of biogas slurry and chemical fertilizer can improve leaf photosynthetic performance, coordinate vegetative growth and reproductive growth, delay leaf senescence and increase 1000-grain weight.

Compared with CK and CF, the 1000-grain weight of BS100 was also significantly increased ($P < 0.05$), increasing by 5.43 % and 2.01 %, respectively. The reason may be that in the grain formation stage, the effective panicle number of BS100 was less, which made the population light transmission wind effect better, and was conducive to the photosynthesis and dry matter accumulation of wheat. At the same time, the number of grains as the bank organ was relatively small. It is also beneficial to increase grain weight.

Liu Fengling et al. (2009) sprayed biogas slurry at the regreening stage, jointing stage, heading stage and filling stage of winter wheat respectively, and compared with the control group sprayed with water, the 1000-grain weight of wheat increased by 2 g. Yang et al. (2005) also showed in their study on the effect of biogas slurry application in wheat fields that the 1000-grain weight of biogas slurry application at jointing stage and filling stage increased by 1.7 g compared with urea treatment, which was similar to the results in this study.

5.3. Correlation analysis of yield and yield composition

The yield and yield composition of different treatments were analyzed. The results showed that compared with CK, chemical fertilizer or biogas slurry could significantly increase the panicle number, grains per panicle and 1000-grain weight ($P<0.05$), thus having the effect of increasing production. Winter wheat yield from high to low order was BS50>BS75>BS25>CF>BS100>CK. The correlation analysis (Tab. 5.1) showed that the yield was significantly correlated with the number of ears and grains per ear and 1000-grain weight, and the correlation coefficients were 0.964, 0.974 and 0.870, respectively, among which the correlation between yield and grains per ear was the highest.

The yield, the effective number of ear and grain number of winter wheat treated with BS100 were lower than those of the combined application of biogas slurry and chemical fertilizer, mainly because the biogas slurry led to the vigorous vegetative growth of crops, and the late maturity of green and late maturity in the later stage seriously affected the number of ears and grains, and the yield was significantly correlated with the number of effective number of ears and grains per ears, resulting in a decrease in yield.

Table 5.1

Correlation analysis of yield and yield composition

Correlation				
	Number of ear ($\times10^4$ hm^2)	Grains per ear	1000-grain weight (g)	Grain yield (10^3 kg•hm^3)
Number of ears ($\times10^4$ hm^2)	1	0.928**	0.873**	0.964**
Grains per ear	0.928**	1	0.782**	0.974**
1000-grain weight（g）	0.873**	0.782**	1	0.870**
Grain yield (10^3 kg•hm^3)	0.964**	0.974**	0.870**	1

Compared with CK, the percentage increase of yield and yield composition is shown in Table 5.2. As can be seen from the table, among the three factors of yield composition, the increase of number of ear is the largest in general, which is 9.31 %, 19.79 %, 27.92 %, 23.56 % and 4.48 %, respectively, compared with CK, CF, BS25, BS50, BS75 and BS100. The main reason that fertilization can significantly increase yield is that the number of grains per ear is significantly increased, and the high yield can be achieved by increasing the number of grains per ear, which is consistent with the research results of Huang Hongying et al. (Huang et al. 2013b).

It can be seen that the high yield of winter wheat is not only determined by one factor, but is the result of the common influence and control of all factors. Understanding the relationship between yield and yield components is of great significance for improving the composition factors of winter wheat yield and increasing the yield of winter wheat. Therefore, we should take management and control measures such as nutrients, water and fertilizer, pay particular attention to increasing the accumulation of grain number per ear, take into account the effective number of ear and thousand grain weight, coordinate the relationship between yield components, and give full play to the potential of plant yield increase. The formation process of winter wheat yield is the process of dry matter production, distribution and transport.

Table 5.2

Percentage increase in yield and yield composition compared to CK

	Number of ear ($\times 10^4$ hm^2)	Grains per ear	1000-grain weight (g)	Grain yield (10^3 kg•hm^3)
CK	0	0	0	0
CF	8.50%	9.31%	3.35%	27.30%
BS25	9.52%	19.79%	4.63%	46.55%
BS50	17.19%	27.92%	8.70%	66.50%
BS75	14.33%	23.56%	5.75%	59.63%
BS100	5.85%	4.48%	5.43%	24.20%

5.4. Morphological indices of winter wheat

5.4.1 Plant height of winter wheat

It was concluded that plant height was an important factor affecting the lodging resistance of wheat. The higher the plant height, the weaker the lodging resistance, and shorter plant height significantly enhanced the lodging resistance of plants (Pinthus 1974; Berry et al. 2003)

Plant height is closely related to plant lodging, and plant height affects winter wheat yield indirectly. The plant height of combined application of biogas slurry and chemical fertilizer is shown in Figure 5.4. It can be seen from the figure that the average plant height of winter wheat under different treatments is 75-80 cm, which belongs to the medium plant height, which can not only prevent lodging, but also provide enough biological yield for high yield.

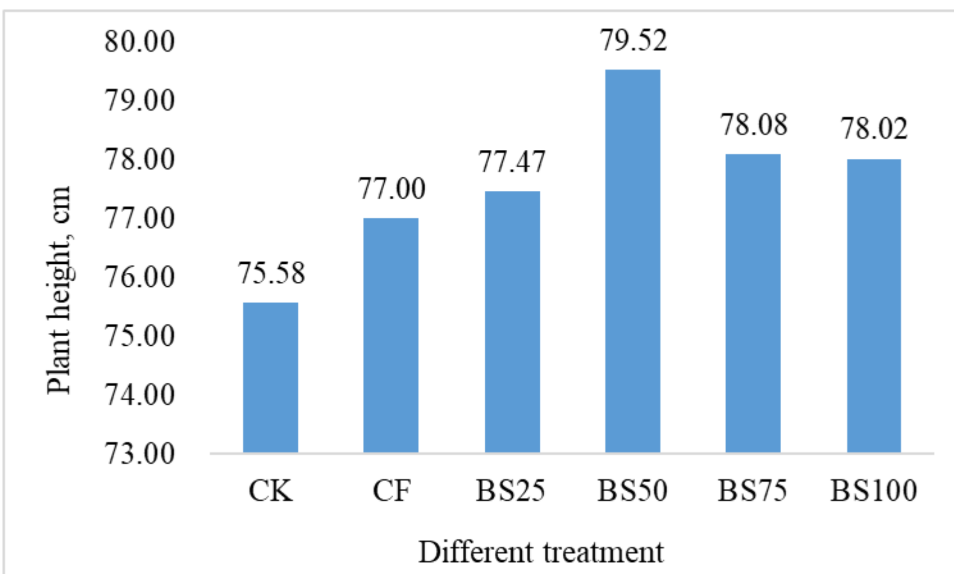

Fig. 5.4. Effect of Co-application of Biogas Slurry with Chemical Fertilizer on winter wheat height

Compared with CK, the plant height of CF, BS25, BS50, BS75 and BS100 increased by 1.88 %, 2.41 %, 5.22 %, 3.31 % and 3.22 %, respectively. Fertilization increased the plant height of winter wheat, increased the ventilation and light transmittance of plant bottom, and increased the growth and development of wheat and the photosynthetic efficiency of leaves. Thereby increasing the yield of winter wheat. The plant height of BS25, BS50, BS75 and BS100 was 77.47 cm, 79.52 cm, 78.08 cm and 78.02 cm, respectively. With the increase of biogas slurry proportion, the plant height increased first and then decreased, and the plant height of BS50 was the highest. Variety dwarfing reduces the risk of wheat lodging, but the high yield of wheat requires plant height to be kept within a certain range (about 70-100 cm) (Richards 1992; Flintham et al. 1997; Miralles et al. 2010).

Reasonable fertilization can promote the vegetative growth of winter wheat, coordinate the relationship between vegetative growth and reproductive growth of winter wheat, and suitable plant height is the premise of obtaining high yield of winter wheat. BS50 treatment can obtain suitable spatial distribution and size of winter wheat nutrients, meet the needs of wheat growth and growth, and is more conducive to the high yield of winter wheat.

5.4.2. Effects of combined application of biogas slurry and chemical fertilizer on first internode traits of wheat base

Plant height is determined by the length of several internodes, which extend at different stages of development, so they have different effects on yield traits. Wheat stem has the functions of supporting plants, transporting nutrients, producing and storing nutrients. However, due to variety, fertility, weather, pests and other reasons will lead to plant lodging. Lodging, the deviation of stems from natural vertical growth due to internal and external factors (Pinthus 1974) is an important limiting factor affecting the high and stable yield of wheat (Fischer et al. 1987; Berry et al. 2003).

Stalk lodging is mainly used in wheat production (Neenan et al. 1975), so stalk lodging is mainly used in the study of wheat resistance to lodging. Stalk lodging is a common occurrence in wheat growing areas in China, especially in Huang-Huai-hai growing areas, so it is an important research to improve stalk lodging resistance (Tian et al. 2005). In production, wheat lodging often occurs at the base of the stalk, so the quality of the stalk internode at the base of the stalk and the bending resistance determine the strength of wheat lodging resistance (Tian et al. 2005; Chen et al. 2011b) .

The length of the first basal node of wheat is very important for lodging resistance of wheat plants. The results of the first basal node characteristics under different treatments are shown in Fig. 5.5.

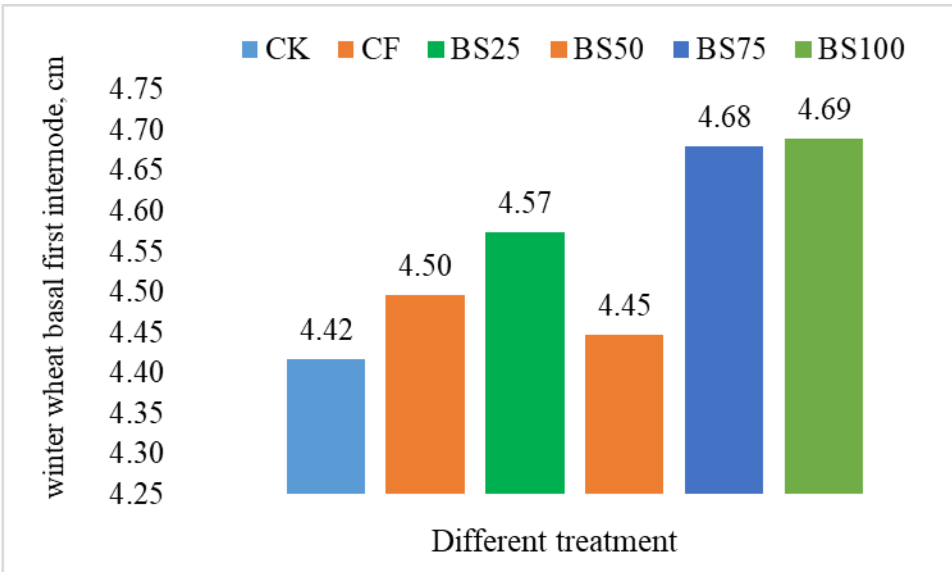

Fig. 5.5. Effect of Co-application of Biogas Slurry with Chemical Fertilizer on winter wheat basal first internode characteristic

As can be seen from the figure, the first internode of the base increased with fertilizer application, but the increase rate was different. Compared with CK, CF, BS25, BS50, BS75 and BS100, the increase rate was 1.81 %, 3.55 %, 0.68 %, 5.96 % and 6.19 %, respectively. BS100 had the largest range and increased lodging risk. BS50 has the lowest increase, which is beneficial to increase plant height, but has better lodging resistance.

Lodging is an important factor limiting grain yield, and studies have shown that lodging can result in 10-80 % grain yield reduction (Pendleton 1954; Wang et al. 1990; Jedel et al. 1991; Easson et al. 1993; Wei et al. 2008). There were significant differences between the first node of the base of different fertilization treatments, indicating that plant height and the traits of the first node of the base can be changed by adjusting the ratio of biogas slurry application, so as to improve the stress resistance of winter wheat and reduce the occurrence of lodging.

5.4.3. Winter wheat plant height composition index

The stem is the pillar of the ground part of the plant, and the structure of the stem directly affects the lodging resistance. The stem is also an important vegetative organ, and the node on the stem is the part of the vegetative leaves, so the morphological structure of the stem is closely related to the yield traits. Winter wheat plant height composition index is the proportional relationship between the length of the upper and lower nodes of the stem, reflecting the spatial arrangement of photosynthetic area and the information of the distribution of assimilates (Liu et al. 2003). In recent years, the research on plant height composition index mainly focused on wheat.

Previous studies (Duan et al. 2006; Zhu et al. 2009; Lu et al. 2014) pointed out that plant height, internode length, stalk thickness and fullness are important plant type indicators of wheat high yield and good quality, which determine dry matter storage capacity and lodging resistance of winter wheat plants, and thus affect wheat grain quality. Under normal conditions, the morphological indexes of different types of populations were different, among which the first internode length at the base was superior population>superior high-yielding population and superior high-yielding population, while the subspike internode and plant height were superior high-yielding population> superior high-yielding population and superior high-yielding population. The first basal internode was shortened, while the ratio of subspike internode to basal internode was increased, which could partly reflect the improvement of lodging resistance of wheat (Yao et al. 2013).

The effects of different treatments on internode and plant height composition index of winter wheat are shown in Figure 5.6 and Table 5.3. Variance analysis shows that there are significant differences between different treatments and CK ($p<0.05$), the longest subspike internode of BS50 was 30.81cm, and there were significant differences between BS50 and others ($p< 0.05$). There was no significant difference between CF and CK ($p>0.05$), there were significant differences between biogas slurry and CF, CK ($p<0.05$), in which BS50 had the longest internode (18.36 cm).

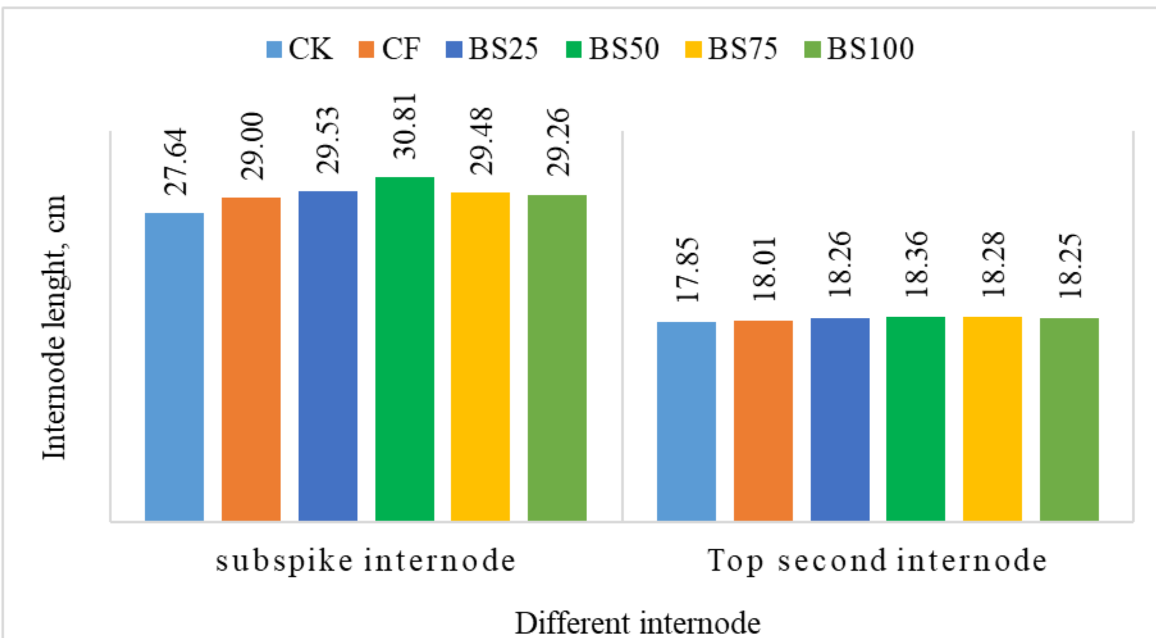

Fig. 5.6. Effect of Co-application of Biogas Slurry with Chemical Fertilizer on winter wheat subspike internode and top second stem

Compared with CK, the IL CF, BS25, BS50, BS75 and BS100 increased by 1.43 %, 2.51 %, 2.73 %, 1.64 % and 1.19 %, respectively. The IL value of BS50 was 0.618, which was consistent with the golden ratio. There were significant differences between I1 and CK in different treatments of winter wheat ($p<0.05$), the I1 value of

BS50 was the largest (0.627), and there were significant differences between BS50 and others ($p<0.05$). The higher the I value, the more beneficial it is to plant resistance and rational utilization of light source (Zhu et al. 2006).

Table 5.3

Effect of Co-application of Biogas Slurry with Chemical Fertilizer on winter wheat plant height composition index

	I_L	I_1
CK	0.602	0.608
CF	0.610	0.617
BS25	0.617	0.618
BS50	0.618	0.627
BS75	0.612	0.617
BS100	0.609	0.616

The plant height component index of winter wheat could be effectively increased by fertilization. When I value was high, the lower internode was shorter, the center of gravity was lower, the subear internode was longer, the photosynthetic area above the sword leaf node was increased, and the resistance to falling and photosynthesis were enhanced. The increase of plant height composition index of BS50 makes spike under good light condition, which is beneficial to increase yield.

5.5. Correlation between yield traits and plant height composition index

The I value not only reflects the spatial location of the photocompound area, but also reflects the information of the operation and distribution of the compounds, and comprehensively expresses the relationship between plant height, internode length and internode, so it is closely related to the population yield.

Wei Xiezhong et al. (1983) and Chen Weiwei et al. (1986) pointed out that wheat plant height composition index had a very significant positive correlation with yield.

The research results of Liu Zhaoye et al. (2003) show that each I value has a positive correlation with population yield, and the positive correlation between I1 and yield reaches a very significant level, which can be used as an important screening index for high-yield breeding. Xie Lingqin et al. (1996) studied the correlation between plant height component index and yield traits under different density conditions, and found that I value had little relationship with plant height, but was closely related to grain weight per ear, grain number per ear, ear length and 1000-grain weight, and pointed out that the combination with higher I value was conducive to selecting high-yield types.

Lei Zhensheng et al. (1996) pointed out that in the breeding of ultra-high yield wheat, the type with plant height of 75-85cm and plant height component index of IL > 0.62 should be selected under the condition of sparse sowing. Zhu Xinkai et al. (2009) concluded that IL and I 1 were significantly positively correlated with the number of grains per panicle and panicle weight.

In this study, the plant height component index of winter wheat was significantly correlated with number of ear, grain number per ear, 1000-grain weight, yield and plant height (Table 5.4). Plant height was significantly correlated with panicle number, grain number per spike, 1000-grain weight, yield, IL, I1, subspike internode and top second stem, with correlation coefficients of 0.842, 0.734, 0.911, 0.826, 0.710, 0.887, 0.954 and 0.905, respectively. The results showed that the effect of subspike internode and top second stem on plant height was relatively large, and the effect of the first basal internode on plant height was relatively small. Increasing the length of subspike internode and top second stem was conducive to increasing plant height, making ear in good light condition, increasing the number of ear, grain number per ear, 1000-grain weight of winter wheat, thus increasing the yield.

Table 5.4

Correlation analysis between yield traits and plant height composition index

	IL	I1	Plant Height	First Internode Of Base	Subspike Internode	Top Second Stem
Number of ear	0.826**	0.898**	First internode of base	0.178	0.910**	0.738**
Grain Number Per Ear	0.832**	0.799**	0.734**	0.102	0.832**	0.707**
1000-Grain Weight	0.763**	0.908**	0.911**	0.253	0.928**	0.786**
Yield	0.850**	0.851**	0.826**	0.246	0.895**	0.793**
Il	1	0.866**	0.710**	0.103	0.877**	0.724**
I1		1	0.887**	0.052	0.971**	0.713**
Plant Height			1	0.375	0.954**	0.905**
First Internode of Base				1	0.223	0.592**
Subspike Internode					1	0.858**
Top Second Stem						1

** At level 0.01 (two-tailed), the correlation was significant

The correlation coefficients between subspike internode and number of ear, grain number per ear, 1000-grain weight, yield, IL, and I1 were 0.910, 0.832, 0.928, 0.895, 0.877, and 0.971, respectively. The correlation coefficients between IL and effective number of ear, grain number per ear, 1000-grain weight, yield and I1 were 0.826, 0.832, 0.763, 0.850 and 0.866, respectively. I1 was positively correlated with effective number per ear, grain number per ear, 1000-grain weight and yield, and the correlation coefficients were 0.898, 0.799, 0.908 and 0.851, respectively. There was no significant correlation between the first internode and number per ear, grain number per ear, 1000-grain weight, yield, IL and I1.

Comprehensive analysis showed that in the correlation analysis between plant height component index and yield traits, the correlation coefficients between subspike internode and number of ear, 1000-grain weight, I1 and plant height were all above 0.9, accounting for a relatively high proportion; while in the correlation analysis between yield and plant height component index, the correlation coefficient between yield and subspike internode was the highest, which was 0.895. The plant height index with high correlation coefficient with subspike internode was plant height and I1, and the correlation coefficient was 0.971 and 0.954, respectively. Therefore, subspike internode played a key role in the formation of winter wheat yield. In practical work, winter wheat with relatively high plant height and I1 should be selected within a moderate height range.

5.6. Above-ground dry matter accumulation of winter wheat

The increase of crop yield is based on the accumulation and distribution of dry matter. Winter wheat straw is a part of plant photosynthesis products, the quantity and quality of straw reflects the growth and development of plants, and is closely related to the yield of winter wheat. The biological yield of winter wheat is the weight of all dry matter harvested per unit area of land, including the dry weight of winter wheat stalks and grains. Biological yield is the result of continuous transportation, storage and accumulation of photosynthetic products of stems and leaves, which reflects the total productivity of winter wheat under certain cultivation conditions, is one of the important parameters reflecting the growth of winter wheat, and is also the key factor affecting the yield and income of winter wheat. Therefore, dry matter, as a photosynthetic product, is closely related to the yield, and it is helpful to understand the accumulation and transport rule of dry matter to implement reasonable control measures (Song et al. 2003).

The effects of different fertilization treatments on above-ground dry matter accumulation of winter wheat were shown in Fig. 5.7.

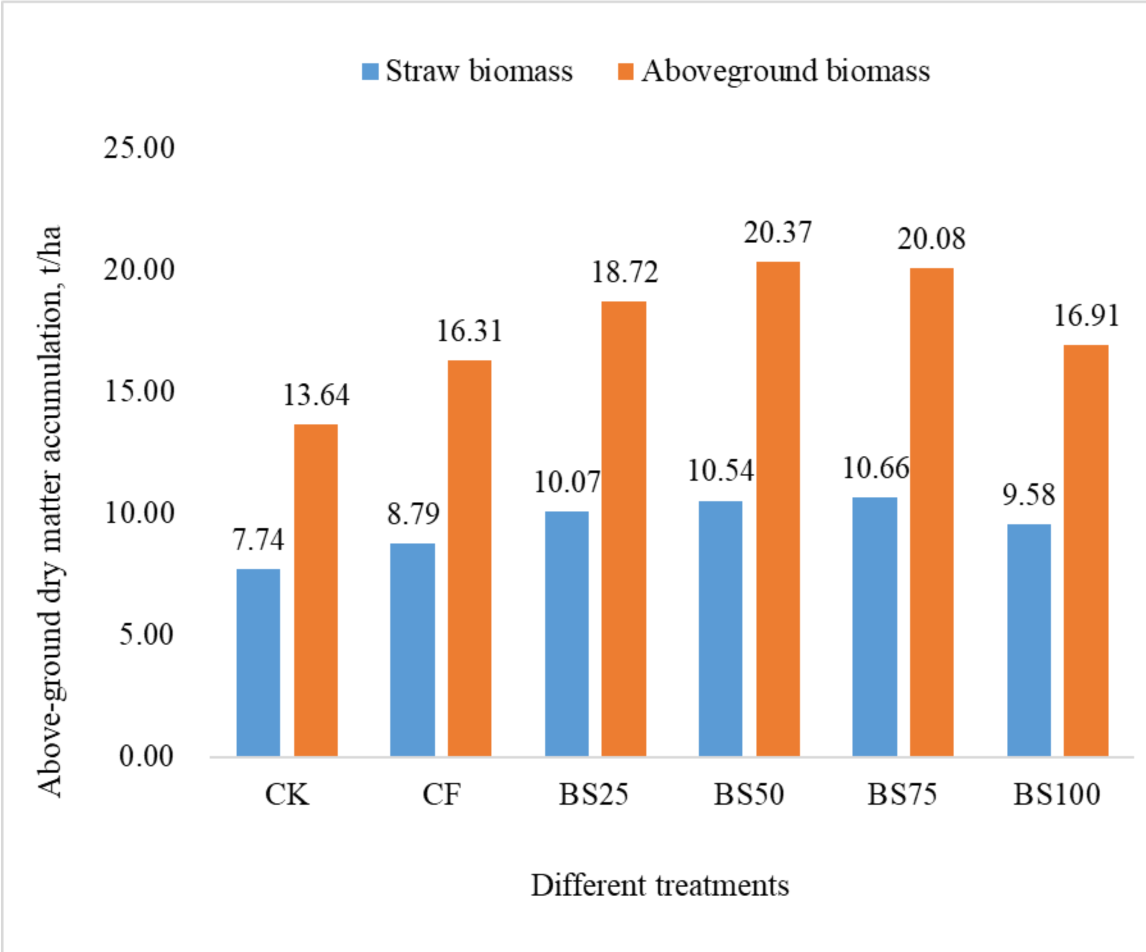

Figure 5.7. Accumulation of above-ground dry matter under different treatments

With different biogas slurry, the biomass of winter wheat straw was BS75＞BS50>BS25>BS100>CF>CK, the aboveground biomass was BS50>BS75>BS25>BS100>CF>CK, there were significant differences between fertilizer treatment and CK ($p<0.05$), compared with CF, combined application of biogas slurry and fertilizer had a significant effect on straw biomass and aboveground biomass ($p<0.05$), the straw biomass and aboveground biomass were the largest in BS75 and BS50, and their values were 10.66 t•hm^{-2} and 20.37 t•hm^{-2}, respectively, but both showed a decreasing trend with the continuous increase of the proportion of biogas slurry replacing chemical fertilizer, even after the biogas slurry completely replacing chemical fertilizer. Wang Yongcui et al. (2010a) found that the dry matter accumulation of corn silage was significantly increased after applying biogas slurry to the corn field, which was consistent with the results of this study. The straw biomass and above-ground biomass of BS100 were significantly lower than that of CF ($P < 0.05$), indicating that total application of biogas slurry was not beneficial to above-ground dry matter accumulation of winter wheat compared with combined application of biogas slurry fertilizer.

Harvest index is the ratio of grain yield to aboveground total dry matter weight, and it is a parameter to measure the coordination relationship between economic yield and biological yield. It reflects the distribution ratio of crop assimilation products in grains and vegetative organs, and is an important index to evaluate crop yield level and cultivation effectiveness. Studies have shown that biogas slurry treatment can increase the transport and distribution of dry matter from stem to grain (Zhang et al. 2017).

Both CF and fertilizer combined with biogas slurry increased the harvest index of winter wheat ($P < 0.05$), and the harvest index of BS50 was the highest (0.48), indicating that increasing biogas slurry in a certain range could improve both economic yield and harvest index. BS50 can improve the growth and development of various organs in the later period of growth, coordinate the balanced distribution of dry matter, transport more photosynthetic products from source organs to reservoir organs, increase the flow and achieve the purpose of increasing production. There was no significant difference between BS100 and CF ($P>0.05$), which may be due to the slow aging and high accumulation of dry matter in the late stage of BS100, but the poor transport of nutrient organs to seeds led to a decrease in harvest index.

In general, combined application of biogas slurry and chemical fertilizer can improve the harvest index of winter wheat and increase the yield of winter wheat, especially BS50 can further achieve high quality and high yield of winter wheat.

5.7. Correlation coefficient between harvest index and growth and development traits of winter wheat

Different traits have different effects on the yield index. As can be seen from Table 5.5, the yield index is positively correlated with grain yield, with a comparison coefficient of 0.887, and is also related to material production, distribution and organ development and construction.

Table 5.5

Correlation coefficient between harvest index and growth and development traits of winter wheat

Index	Straw biomass	Aboveground biomass	Harvest index
Yield	0.939**	0.988**	0.887**
Straw biomass	1.0	0.980**	0.685**
Aboveground biomass	0.980**	1	0.811**
Plant height	0.814**	0.833**	0.675**
The number of ear	0.864**	0.935**	0.911**
Grain number per ear	0.873**	0.944**	0.901**
1000-seed weight	0.871**	0.883**	0.705**
First base internode	0.456	0.342	0.006
Subear internode	0.847**	0.887**	0.774**
Top second stem	0.842**	0.826**	0.589*
IL	0.790**	0.837**	0.769**
I1	0.766**	0.826**	0.786**

By improving the harvest index, grain yield can be further improved. The high yield of winter wheat by increasing the harvest index should be based on a certain biological yield, and only when the biological yield reaches above medium, the high harvest index can achieve a higher yield. In this study, the harvest index was significantly positively correlated with effective number per ear, number of grain per ear and 1000-grain weight. Therefore, reducing stem dry matter allocation, increasing grain dry matter allocation, improving the transport ability of stalk integrates to grain, improving grain fruiting ability and increasing "reservoir" capacity during development were all beneficial to improving the harvest index of winter wheat.

The harvest index was positively correlated with plant height, IL, I1, subspike internode and top second stem, and the correlation coefficients were 0.675, 0.769, 0.786, 0.774 and 0.589, respectively, indicating that it was feasible to increase the yield index by increasing the plant height component index. The lower stem segment was shortened, the growing part of the leaves was reduced, the center of gravity was lowered, and the lodging resistance was enhanced. At the same time, increasing the length of subspike internode and top second stem can make the spacing of leaves in the upper canopy relatively sparse, which is conducive to the entry of light into the canopy and the interception of leakage light, so as to strengthen the apex advantage, improve the utilization rate of light energy, increase the plant height composition index, and then increase the harvest index and population yield.

Through the above analysis, it was found that the factors affecting grain yield, biological yield and substance distribution were closely related to the harvest index. The results of this study showed that above-ground biomass was positively correlated with effective panicle number and panicle number, and the correlation coefficients were 0.935 and 0.944, respectively, and the harvest index was also positively correlated with effective number of ear ear and grains per ear, and the correlation coefficients were also greater than 0.9, which were 0.911 and 0.901, respectively. Therefore, the effective combination of high biological yield and high harvest index was possible. It is feasible to increase the yield by increasing the effective number of ear and grain number per ear. This is consistent with the research results of Mcewan and Cross et al.(Henne et al. 1999; Courtois et al. 2003), that is, the selection of high-yield varieties takes both harvest index and biomass as selection indicators.

Crop yield is jointly determined by total dry matter quality and harvest index. Theoretically, improving total dry matter quality or harvest index can improve yield, while more studies believe that it is more important to further improve yield by improving total dry matter quality (Ying et al. 1998). High yield breeding must improve biological yield, and the focus of wheat breeding should be on the breakthrough of biological yield (Entcheva et al. 2001).

Conclusions to Chapter 5

Both biogas slurry and chemical fertilizer application increased the yield of winter wheat, and there was a significant difference compared with CK ($p<0.05$). BS50 is the optimal combination of biogas slurry and chemical fertilizer.

Chemical fertilizer and biogas slurry treatment can significantly increase the effective number of ear, grain number per ear and 1000-grain weight of winter wheat ($P<0.05$), so as to increase production.

In the fertilization treatment, the plant height of BS treatment was higher than that of chemical fertilizer. The plant height with the BS50 was the highest of 79.52.

The base of the first internode increases with fertilizer use, but the increase rate is different, compared with CK, CF, BS25, BS50, BS75, BS100 respectively increased by 1.81 %, 3.55 %, 0.68 %, 5.96 % and 6.19 %, BS100 has the greatest amplitude.

The increase of plant height component index with BS50 made the ear under good light condition, which was conducive to increasing the yield of winter wheat.

The combined application of biogas slurry and chemical fertilizer had a significant effect on straw biomass and aboveground biomass ($p<0.05$), and the highest values of straw biomass and aboveground biomass were BS75 and BS50. With the increase of biogas slurry proportion, even the biogas slurry completely replacing chemical fertilizer, both showed a downward trend.

Both fertilizer and combined biogas slurry increased the harvest index of winter wheat ($P<0.05$), and the harvest index of BS50 was the highest (0.48). BS50 treatment can improve the growth and development of various organs in the later period of growth.

Above ground biomass was significantly positively correlated with effective number of ears and grain number per ear, and the harvest index was also significantly positively correlated with effective panicle number and grain number per panicle, with correlation coefficients greater than 0.9.

CONCLUSIONS

1. There were significant differences between different soil layers in soil bulk density between each treatment and CK. After adding biogas slurry, the soil bulk density was significantly reduced, especially after applying BS50, that is very good for winter wheat growth.

2. Different fertilization treatments could significantly change the particle size distribution and adjust the mass composition of aggregates with different particle sizes.

3. Long-term application of chemical fertilizer significantly decreased the number of large aggregates and increased the number of small aggregates, and application of biogas slurry was beneficial to increase the composition of large aggregates.

4. The stability of soil aggregates varies in different soil layers and under different treatment conditions. Biogas slurry treatment is beneficial to increase the MWD of 0-10 cm, 10-20 cm, 20-40 cm force-stable aggregates and water-stable aggregates, with BS50 being the largest. The combined application of chemical fertilizers and biogas slurry made the MWD of force-stable and water-stable aggregates significantly higher than those of other treatments, increasing the stability and agglomeration of the soil. Reasonable fertilizer application, especially the BS50 treatment of biogas slurry and chemical fertilizers, has positive significance in maintaining the stability of soil aggregates and improving soil quality.

5. Mineral fertilizers can reduce pH, and application of biogas slurry can increase pH. Biogas slurry itself is alkaline and contains high cation content, the increase of base ions can effectively inhibit the soil acidification trend caused by long-term application of chemical fertilizer to a certain extent, and the acid-base environment of soil solution can be improved.

6. The application of biogas slurry in agricultural planting can significantly increase the nitrogen content of the soil, thereby satisfying the absorption of nutrients by crops. In 0-10 cm, 10-20 cm, and 20-40 cm， the total nitrogen content is highest by the BS50 treatment.

7. Application of biogas slurry has a certain impact on the soil available phosphorus content. Among all soil layers, the available phosphorus content of BS50 was the highest. The application of biogas slurry could improve the supply level of soil available phosphorus, but more is not always better.

8. The content of available potassium in soil of each treatment increases to varying degrees compared with control. In different soil layers, the available potassium content of BS50 treatment combined with biogas slurry and chemical fertilizer was the highest, which was 173.71 $mg•kg^{-1}$, 156.63$mg•kg^{-1}$ and 144.06 $mg•kg^{-1}$, in 0-10, 10-20, 20-40 cm respectively.

9. Application of biogas slurry had an effect on soil organic matter content. In 0-10 cm, 10-20 cm and 20-40 cm soil layers, the soil organic matter content was BS100>BS75>BS50>BS25>CF>CK, and the soil organic matter content increased with the increase of biogas slurry concentration.

10. The application of biogas slurry increased the activity of sucrase in soil to BS75 and decreased B100. The activity of sucrase with BS75 was the highest in 0-10 cm, 10-20 cm and 20-40 cm, which was 15.28$mg•g^{-1}$, 30.73 $mg•g^{-1}$ and 31.28 $mg•g^{-1}$, respectively.

11. The urease activity of combined application of biogas slurry and chemical fertilizer (BS25, BS50 and BS75) was higher than only mineral fertilizer application. The combined application of biogas slurry and chemical fertilizer significantly activated the urease activity in soil, which was beneficial to the improvement of soil urease activity. In 0-10 cm, 10-20 cm, 20-40 cm, soil urease activity was the highest in BS50

treatment (235.33 ug•g^{-1}, 337.79ug•g^{-1} and 351.45ug•g^{-1}, respectively), and was significantly different from that in other treatments.

12. Both biogas slurry and chemical fertilizer increased soil acid phosphatase activity, but the activity of acid phosphatase with BS50 was the highest in different soil layers.

13. The order of catalase activities in 0-10 cm, 10-20 cm, and 20-40 cm soil was as follows: BS100 >BS75> BS50>BS25>CF>CK, and there was a significant difference between each treatment and CK. BS100 catalase activity was the highest.

14. With the increase of the proportion of biogas slurry, the catalase activity increases. The soil catalase activity of BS100 treatment is the largest, and the soil detoxification effect is the strongest. Long-term application of chemical fertilizers has a significant inhibitory effect on soil catalase activity, which can easily lead to the accumulation of root exudates, thereby aggravating the toxic effect of hydrogen peroxide on crops. Biogas slurry application can significantly increase soil catalase activity and accelerate the decomposition of toxic substances in soil.

15. Biogas slurry can improve the soil enzyme activity of winter wheat in 0-40 cm soil layer, especially in 20-40 cm.

16. Both biogas slurry and chemical fertilizer application increased the yield of winter wheat. With the increase of biogas slurry proportion, the yield of winter wheat increased B25-B50 and then decreased B75-B100, and the yield of winter wheat with BS50 treatment was the highest (9,8 t/ha), compared with CF, BS25, BS75 and BS100, the increases were 30.79%,13.61%, 4.31% and 34.06%.

17. Chemical fertilizer and biogas slurry treatment can significantly increase the effective number of ears, grain number per ear and 1000-grain weight of winter wheat ($P<0.05$), so as to increase production. Yield was significantly correlated with the effective number of ear, grain number per ear and 1000-grain weight, and the correlation coefficients were 0.964,0.974 and 0.870, respectively.

18. Different fertilization treatments had effects on the morphological indexes of winter wheat. In the fertilization treatment, the plant height of BS treatment was higher than that of chemical fertilizer, The plant height treated with BS25, BS50, BS75 and BS100 was 77.47 cm, 79.52 cm, 78.08cm and 78.02cm, respectively.

19. The base of the first internode increases with fertilizer use, but the increase rate is different, compared with CK, CF, BS25, BS50, BS75, BS100 respectively increased by 1.81 %, 3.55 %, 0.68 %, 5.96 % and 6.19 %, BS100 has the greatest amplitude, increasing the risk of lodging. BS50 had the lowest increase, which was not only conducive to increasing plant height, but also had better lodging resistance.

20. Fertilization can effectively improve the plant height component index of winter wheat. The increase of plant height component index with BS50 made the ear under good light condition, which was conducive to increasing the yield of winter wheat. Plant height component index was significantly correlated with the effective number of ear, grain number per ear,1000-grain weight, yield and plant height.

21. The combined application of biogas slurry and chemical fertilizer had a significant effect on straw biomass and aboveground biomass ($p<0.05$), and the highest values of straw biomass and aboveground biomass were BS75 and BS50, however, with the increase of biogas slurry proportion, even the biogas slurry completely replacing chemical fertilizer, both showed a downward trend.

22. Both fertilizer and combined biogas slurry increased the harvest index of winter wheat ($P<0.05$), and the harvest index of BS50 was the highest (0.48). Increasing biogas slurry within a certain range could improve both economic yield and harvest index. BS50 treatment can improve the growth and development of various organs in the later period of growth.

23. The factors affecting grain yield, biological yield and substance distribution were closely related to harvest index. Above ground biomass was significantly positively correlated with effective ear number and grain number per ear, with

correlation coefficients of 0.935 and 0.944, respectively, and the harvest index was also significantly positively correlated with effective panicle number and grain number per panicle, with correlation coefficients greater than 0.9 (0.911 and 0.901, respectively).

PROPOSALS

Fertilization had effects on soil physicochemical properties, enzyme activities and growth of winter wheat, the effect of combined application of biogas slurry and chemical fertilizer is greater than that of single application of chemical fertilizer or biogas slurry. According to the situation of Zhoukou area and environmental protection, BS50 treatment of biogas slurry and chemical fertilizer is recommended, which is conducive to improving lime concretion black soil, improving soil fertility and increasing winter wheat yield.

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APPENDIXES

Appendix A

Certificate completion

Project Title: Research and application of biogas slurry fertigation combined with straw returning in aggregate formation and organic carbon accumulation of Lime concretion black soils

河南省科技攻关计划项目
结项证书
Completion Certificate of Henan Province Scientific and technological project

该项目提交的研究资料完整，总结报告系统详实，经审查符合结项要求，准予结项。

This certificate is awarded in recognition of satisfactory completion of research project.

河南省科学技术厅
科技计划专用章

2022 年 12 月 30 日

Signature：Department of Science and Technology of Henan Province

December 23, 2022

项目名称：沼液灌施配合秸秆还田模式在砂姜黑土团聚体形成及有机碳积累中的研究与应用

立项年度：2021 年

Approved Year: 2021

项目编号：212102110388

Project No: 212102110388

承担单位：河南科技学院

Organization: Henan University of Science and Technology

项目负责人：唐蛟

Chief Researcher: Tang Jiao

项目参加人（共 6 名）：

Researchers Information(6 in all):

吴大付、张喜焕、陈庆、崔世慧、王威、殷金忠

Wu Dafu, Zhang Xihuan, Chen Qing, Cui Shihui, Wang Wei, Yin Jinzhong

附件：
Attachments

2023 Xinxiang City Natural Science Academic Achievement Award first prize project list

2023 年新乡市自然科学学术成果奖一等奖项目名单

30	沼液施用对华北平原砂姜黑土分布区麦玉轮作体系作物产量和土壤理化性质及团聚体特征的影响	农学	论文奖	唐蛟	Anthony J Davy	王威	张喜焕	吴大付
30	Effects of Biogas Slurry on Crop Yield, Physicochemical Properties and Aggregation Characteristics of Lime Concretion Soil in Wheat–Maize Rotation in the North China	Agronomy	Paper Award	Tang Jiao	Anthony J Davy	Wang Wei	Zhang Xihuan	Wu Dafu

Continie App. E

Completion certificate of Henan Province key scientific research project

河南省高等学校重点科研项目结项证书

Science and Technology [2022] No.0465, Department of Education, Henan Province

豫教科技【2022】0465号

该项目提交的研究资料完整，结项报告系统详实，经审查符合结项要求，准予结项。

The research materials submitted by the project are complete, the content of the report is systematic and detailed, the certificate is awarded in recognition of satisfactory completion of research project.

Department of Education of Henan Province

June 20, 2022

河南省教育厅

2022年06月20日

Project Title: Construction and demonstration of large-scale intensive pig farming biogas slurry returning technology system in Henan Province

项目名称：河南省规模集约化生猪养殖沼液还田技术体系构建与示范

立项时间：2019年07月30日 Approved Date: July 30, 2019

项目编号：20B210004 Project No: 20B210004

承担单位：河南科技学院 Organization: Henan Institute of Science and Technology

项目负责人：吴大付 Chief Researcher: Wu Dafu

项目参加者（共6名）： Researchers Information(6 in all):

排序	姓名	性别	单位
2	唐蛟	男	河南科技学院资环学院
3	张喜焕	女	河南科技学院资环学院
4	任秀娟	女	河南科技学院资环学院
5	胡林	男	河南科技学院资环学院
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Sort	Name	Sex	Organization
2	Tang Jiao	Male	Henan University of Science and Technology
3	Zhang Xihuan	Female	Henan University of Science and Technology
4	Ren Xiujuan	Female	Henan University of Science and Technology
5	Hu Lin	Male	Henan University of Science and Technology
6	Zheng Bowen	Male	Henan University of Science and Technology

结项等级：合格 Grade: Qualified

Appendix B

З А Т В Е Р Д Ж У Ю
Проректор з науково-педагогічної
та навчальної роботи
д. б. н., професор

………………….. Ігор КОВАЛЕНКО

Д О В І Д К А

про впровадження результатів наукових досліджень у навчальному процесі

Видана **Чжан Сіхуан** у тому, що матеріали дисертаційної роботи «Вплив довготривалого застосування дігестату на властивості ґрунту та врожайність сільськогосподарських культур в умовах Північно-китайської рівнини», які опубліковані у статтях:

1.Tang J., Davy A. J., Wang W., **Zhang X**., Wu D., Hu L., Yin J. (2022). Effects of Biogas Slurry on Crop Yield, Physicochemical Properties and Aggregation Characteristics of Lime Concretion Soil in Wheat–Maize Rotation in the North China Plain. Journal of Soil Science and Plant Nutrition, 22, 2406–2417. https://doi.org/10.1007/s42729-022-00817-9

2.Zhang X., Zakharchenko E., Wu D., Tang J. (2022). Effects of Biogas Slurry Application on Wheat Yield and Quality. Sciences of Europe, 94, 3-5. https://doi.org/10.5281/zenodo.6616367

включено до навчальної програми (силабусу) дисципліни «Проблеми екологічного землеробства» та використовуються в навчальному процесі з підготовки фахівців ОС «Магістр» спеціальності 201 «Агрономія».

Довідка видана для подання до спеціалізованої вченої ради

Завідувач кафедри
агротехнологій та ґрунтознавства
д. с. – г. наук, професор

…………………….. Володимир ТРОЦЕНКО

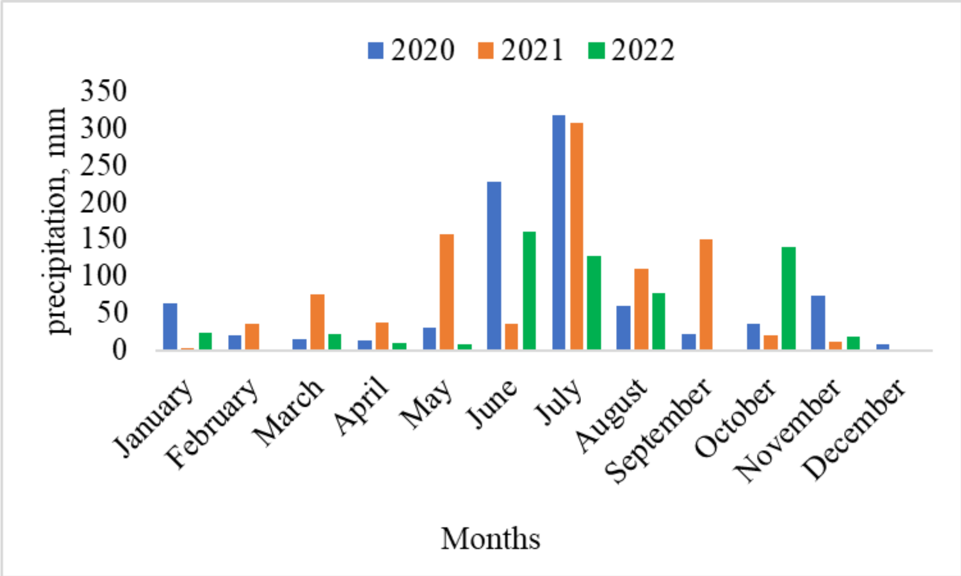
Apendix C

Average temperature per month for 2020-2022, ^{0}C

	2020	2021	2022
January	2.5	3	2.5
February	6.5	8	4
March	11	10	11.5
April	14.5	13.5	17
May	22.5	20	21
June	26	27	29
July	24.5	27.5	27.5
August	27.5	25.5	28
September	22.5	23.5	23
October	15.5	15.5	15.5
November	10	10	11
December	3	4.5	2.5

Appendix D

Average precipitation per month for 2020-2022



Month	2020	2021	2022
January	63.8	2.5	23.1
February	20.1	36.2	1.8
March	14.4	74.6	21.6
April	12.5	36.5	9.9
May	30.1	156.7	7.9
June	227.4	35.3	159.9
July	317.1	307.8	127.8
August	59.7	109.2	77.6
September	21.3	149.6	0
October	35.5	20.4	139.6
November	73.1	11.9	18.5
December	8.4	0.8	0.3

Appendix E

Soil available phosphorus content with different proportion of biogas slurry in different soil layers

Soil depth	CK	CF	BS25	BS50	BS75	BS100
0-10 cm	17.46	20.18	22.26	25.59	21.43	19.36
10-20 cm	16.59	18.28	18.40	22.50	19.64	19.15
20-40 cm	14.74	16.37	17.47	20.48	18.28	16.53

Appendix F

Soil available potassium content with different proportion of biogas slurry in different soil layers

Soil depth	CK	CF	BS25	BS50	BS75	BS100
0-10 cm	138.16	147.18	157.35	173.71	162.12	154.67
10-20 cm	124.75	135.84	141.97	156.63	143.33	136.89
20-40 cm	113.85	118.55	127.89	144.06	137.70	125.06

Appendix G

Soil acid phosphatase activity and distribution

in different soil depth with different fertilization

Soil depth	CK	CF	BS25	BS50	BS75	BS100
0-10 cm	3.07	3.39	3.43	4.10	3.61	3.57
10-20 cm	1.62	2.83	2.90	3.21	2.68	2.67
20-40 cm	0.76	2.31	2.41	2.60	1.34	1.31