

EFFECTS OF BIOGAS SLURRY ON THE NUTRIENT CONTENT AND SOIL ENZYME ACTIVITY OF LIME CONCRETION BLACK SOIL

 Xihuan Zhang^{1,2,3,4},  Elina Zakharchenko^{4*},  Jiao Tang¹,
 Ngai Paing Tan²,  Roman Yaroshchuk⁴,  Natalia Kravchenko⁵,
 Inna Mozharivska⁵

¹Henan Institute of Science and Technology, Xinxiang, China

²Universiti Putra Malaysia, Serdang, Selangor, Malaysia

³Henan Engineering Technology Research Center for Major Invasive Alien Species Prevention and Control, Xinxiang, China

⁴Sumy National Agrarian University, Sumy, Ukraine

⁵Zhytomyr Polytechnic State University, Zhytomyr, Ukraine

Abstract. The objective of this study was to evaluate the effect of returning biogas slurry to the field on the fertility indicators of lime concretion black soil in Henan Province, China, with the aim of optimizing agricultural practices and promoting crop nutrient uptake and utilization. Four treatments were set up in the experiment, namely: CK (Control), CF (Chemical Fertilizer), BS50 (Chemical Fertilizer combined with Biogas Slurry Irrigation) and BS100 (Pure Biogas Slurry Irrigation). The application of biogas slurry significantly increased soil enzyme activity, which was particularly prominent in the 20-40 cm soil layer. This enhancement of enzyme activity was closely associated with the delayed senescence of winter wheat roots during the late growth stage, thereby facilitating crop nutrient uptake and utilization. Further analysis revealed a strong correlation between soil fertility status and enzyme activity, indicating that soil enzyme activity can serve as a reliable indicator for measuring the intensity of nutrient transformation. Elevated enzyme activity implies more active nutrient cycling and mineralization processes within the soil system. The decrease in phosphorus and nitrogen content under application of BS is attributed to the higher uptake of these nutrients by the winter wheat plants. Considering the dual requirements of efficient resource use (organic waste utilization), promotion of crop nutrient uptake, biogas slurry treatments were identified as the most suitable measures to improve the productivity and quality of lime concretion black soil under winter wheat cultivation systems.

Keywords: Soil fertility, enzyme activity, wheat, organic matter, fertilizer, biogas.

Corresponding Author: Elina Zakharchenko, Sumy National Agrarian University, Sumy, Ukraine, Tel.: +380989152603, e-mail: elionapolis@gmail.com

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1. Introduction

Lime concretion black soil (LCBS) is a crucial agricultural soil formed in the Huang-Huai-Hai Plain, China's major grain-producing region (Tang *et al.*, 2022a). LCBS covers millions of hectares across Henan and neighboring provinces and is associated with yield limitations. Understanding subsurface responses to BS directly supports region-specific strategies for nutrient management and soil health (Wang *et al.*, 2021). Characterised by a heavy texture and relatively low organic matter content, this soil type is typical of medium-and low-yield farmlands in China. Therefore, improving the fertility

of LCBS and increasing crop yields are of great significance for safeguarding global food security. Studies have shown that applying biogas slurry to the soil can enhance its physical, chemical and biological properties (Chernysh *et al.*, 2023). This practice not only enhances enzyme activity but also promotes crop growth and development, as well as boosts the crop's yield potential (Wentzel *et al.*, 2015). Currently, research on the relationship between soil enzyme activity and soil fertility is increasing. Notably, soil nutrient content is a direct reflection of soil fertility levels (Kharchenko *et al.*, 2019; Voitovyk *et al.*, 2023; Wu *et al.*, 2020; Chen *et al.*, 2024). A significant correlation exists between these two parameters (Dindar *et al.*, 2015; Wu *et al.*, 2021). Biogas slurry is a high-quality organic fertilizer derived from the mixed fermentation of diverse organic substrates, encompassing human and animal manure as well as crop straw (stems). It is abundant in essential macronutrients (e.g., nitrogen, phosphorus, potassium) and a broad spectrum of organic and inorganic compounds, including amino acids, proteins, nucleic acids, carbohydrates. These bioavailable substances can either be directly taken up by plants or undergo biotransformation in the soil, thereby exerting a positive influence on crop yield and improving key soil fertility indicators (Abdelsalam *et al.*, 2016; Czekala *et al.*, 2020). The application of biogas slurry enables the efficient treatment of large volumes of agricultural wastewater, mitigates pollution associated with chemical fertilizer use, contributes to environmental protection and reduces crop production costs (Datsko *et al.*, 2025; Han *et al.*, 2021). A consensus among agricultural experts and economists is that the utilization of plants and animal residues - with biogas slurry as a typical example - serves as an effective strategy for nutrient recycling in agricultural systems (Shevchuk & Hospodarenko, 2025). Notably, the efficient utilization of biogas slurry is not only economically viable but also plays a crucial role in sustaining closed-loop agriculture and maintaining ecological balance, thereby aligning with the goals of sustainable agriculture (Li *et al.*, 2025; Yang *et al.*, 2025; Wang *et al.*, 2021).

Primarily, researchers take soil samples from the topsoil and in this case, there is a missing subsurface response where BS actually accumulates or reacts. Specifically targeting the 20-40 cm depth addresses that gap (Chen *et al.*, 2020). This layer has different moisture dynamics, creating optimal conditions for unique enzyme patterns. Stratification causes a functioning 20-40 cm zone as a "reaction-retention" band for nitrogen and carbon from the soil. BS contains highly mobile NH_4^+ and dissolved organic carbon that move below 20 cm, where carbonate buffering and redox conditions differ from those in the topsoil. Recent studies indicate that BS strongly stimulates carbon-degrading enzymes and alters microbial activity - effects that are probably different at depths of 20-40 cm compared to 0-20 cm (Liang *et al.*, 2024). The 20-40 cm zone in the LCBS also contains higher levels of CaCO_3 and shows different pH and calcium (Ca^{2+}) levels compared to the top layer (Daunoras *et al.*, 2024). This study aims to analyze the changes in the nutrient content and enzyme activity induced by biogas slurry application, thereby evaluating its soil improvement effects across two soil depth intervals: 0-20 cm and 20-40 cm. Furthermore, this study investigates the relationship between nutrient content and enzyme activity to provide a scientific basis for the application of biogas slurry in lime-concretion black soil, ultimately facilitating the sustainable development of agriculture in this specific soil region.

2. Materials and methods

Research area. Soil samples were collected from a winter wheat field situated in Shangshui County, Zhoukou City (114°36' E, 33°45' N), southeastern Henan Province, China. This region features a temperate semi-humid monsoon climate, characterized by moderate annual temperatures and distinct seasonal variations. The mean annual precipitation in the area is 785.1 mm, with the majority of rainfall occurring from June to September, while the mean annual temperature is 14.5°C. The soil type of the experimental plots is LSBS.

The experimental design was implemented as follows:

Experimental design, sampling and materials. The experimental design was implemented as follows:

1. Control (CK) - without any fertilizers.
2. CF - application only chemical fertilizers $N_{180}P_{90}K_{75}$.
3. BS50 - application biogas slurry $N_{90}P_{10}K_{37.5}$ and chemical fertilizers $N_{90}P_{80}K_{37.5}$.
4. BS100 - application biogas slurry $N_{180}P_{20}K_{75}$ and chemical fertilizers $N_0P_{70}K_0$.

Nitrogen application at a rate of $180 \text{ kg} \cdot \text{ha}^{-1}$ (designated as N_{180}) was set as the core nutritional management practice for winter wheat. Seventy per cent of the biogas slurry was applied via basal irrigation five days prior to sowing. At the time of sowing, chemical fertilizers were incorporated into the soil, while the remaining thirty percent of the biogas slurry was topdressed through sprinkler irrigation during the elongation stage of winter wheat. When co-applying fertilizer, the soil water content from the sole biogas slurry irrigation treatment was used as the baseline and any additional water input from biogas slurry irrigation at different proportions was quantified and adjusted independently to ensure consistent soil moisture conditions across treatments. Post-sowing, supplementary irrigation was conducted to homogenize soil water content across all experimental plots.

The biogas slurry used in the experiment was sourced from Shangshui Muyuan Agriculture and Animal Husbandry Co., Ltd. Its basic physicochemical properties were determined as follows: total nitrogen (TN) = $1.31 \text{ kg} \cdot \text{m}^{-3}$, total phosphorus (TP) = $0.14 \text{ kg} \cdot \text{m}^{-3}$, total potassium (TK) = $0.52 \text{ kg} \cdot \text{m}^{-3}$, dissolved organic carbon (DOC) = $510.34 \text{ kg} \cdot \text{m}^{-3}$ and pH = 7.42. For chemical fertilization, triple superphosphate was used as the phosphorus source (P_2O_5), potassium sulfate as the potassium (K_2O) source and urea as the nitrogen (N) source. All phosphorus, all potassium and 70% of nitrogen were applied as basal fertilizers during sowing; the remaining 30% of nitrogen was topdressed in March, coinciding with the crop's elongation stage. Other field management practices (e.g., pesticide application) were performed in accordance with local conventional agricultural practices to ensure consistency with regional production conditions.

Each treatment was arranged with three biological replicates, following a randomized complete block design (RCBD): 4 treatments, 3 replications, three soil samples were taken for each plot $n=4 \times 3 \times 3=36$. Each replicate plot had an area of 66.7 m^2 . The winter wheat sowing rate (cultivar Fengde Cunmai 21) was 75 ths. seeds per hectare. Following the 2020-2022 wheat harvest, in early June, three quadrates were randomly chosen in each treatment area using the "S" shaped sampling method. Soil samples were taken using a soil drill at depths of 0-20 cm and 20-40 cm. Large soil aggregates, plant roots and crop residues were manually removed from the fresh soil samples immediately after collection. A subsample of the fresh soil was stored at 4°C for subsequent soil enzyme activity determination; the remaining soil was air-dried at room temperature and passed through a 2 mm mesh sieve to remove coarse fractions, which

were then used for the analysis of soil nutrient contents and soil organic matter (SOM) content.

Determination method. Soil enzyme activities were determined following the methods described by Guan (1986). Specifically: Sucrase activity was assayed via the 3,5-dinitrosalicylic acid (DNS) colorimetric method and expressed as mg glucose per gram of soil ($\text{mg glucose}\cdot\text{g}^{-1}\text{ soil}$) under incubation conditions of 37°C for 24 h. Urease activity was measured using the indophenol blue colorimetric method, with units expressed as $\text{mg NH}_3\text{-N}$ per gram of soil ($\text{mg NH}_3\text{-N}\cdot\text{g}^{-1}\text{ soil}$) after incubation at 37°C for 24 h. Catalase activity was determined by ultraviolet (UV) spectrophotometry and reported as $\text{mmol H}_2\text{O}_2$ per gram of soil ($\text{mmol H}_2\text{O}_2\cdot\text{g}^{-1}\text{ soil}$) following incubation at 25°C for 24 h. Acid phosphatase activity was quantified via the disodium phenyl phosphate colorimetric method, with activity expressed as mg phenol per gram of soil ($\text{mg phenol}\cdot\text{g}^{-1}\text{ soil}$) under incubation at 37°C for 24 h. The soil nutrient content was analyzed using established conventional methods (Bao, 2000). The organic matter (SOM) content was determined by the potassium dichromate redox titration method.

Data Analysis Methods. Statistical analyses were performed using SPSS 22.0 software (IBM Corporation, Armonk, NY, USA) and Microsoft Office Excel (Microsoft Corporation, Redmond, WA, USA). Differences in soil urease activity, sucrase activity, acid phosphatase activity, catalase activity, soil organic matter (SOM) content, total nitrogen (TN) content and available phosphorus (AP) content among different winter wheat treatments were evaluated via one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) for post-hoc comparisons. In the presentation of statistical results, different lowercase letters indicate significant differences at the $p < 0.05$ level.

3. Results and discussion

Effects of fertilization treatments on soil nutrient contents

Total nitrogen. Soil total nitrogen (TN) includes all organic and inorganic nitrogen fractions in the soil; it functions as both a direct nitrogen source for plants and a long-term nitrogen reservoir and thus represents a critical indicator of soil capacity to supply plant-available nitrogen. This characterization provides a scientific basis for evaluating soil fertility status and formulating optimized fertilization management strategies. Consistent with previous findings (Abubaker *et al.*, 2012; Bachmann *et al.*, 2011), numerous studies have confirmed that the application of biogas slurry in agricultural systems can significantly increase soil nitrogen content, which in turn helps meet the nitrogen demand of crops during their growth and development.

Figure 1 presents the soil total nitrogen (TN) content in the experimental plots (treatments: CK, CF, BS50, BS100) after winter wheat harvest. In the surface soil layer (0-20 cm), the soil TN content followed the order: $\text{CK} > \text{CF} > \text{BS100} > \text{BS50}$. Specifically, significant differences in TN content were observed between the biogas slurry treatments (BS50, BS100) and the control treatments (CK, CF) ($p < 0.05$). In the subsurface soil layer (20-40 cm), the TN content of BS50 and BS100 was lower than that of CK and CF. Among all treatments, BS50 exhibited the lowest TN content, with reductions of 11.5%, 27.9% and 37.9% compared to BS100, CF and CK, respectively and these differences were statistically significant ($p < 0.05$). Across both soil layers, the minimum TN content was recorded in the BS50 treatment: $1.19\text{ mg}\cdot\text{g}^{-1}$ in the 0-20 cm layer and $0.79\text{ mg}\cdot\text{g}^{-1}$ in the 20-40 cm layer. Additionally, the effect of fertilization treatments on soil TN content

weakened with increasing soil depth. Notably, the TN content in the 20-40 cm layer is significantly lower than that in the 0-20 cm layer across all treatments.

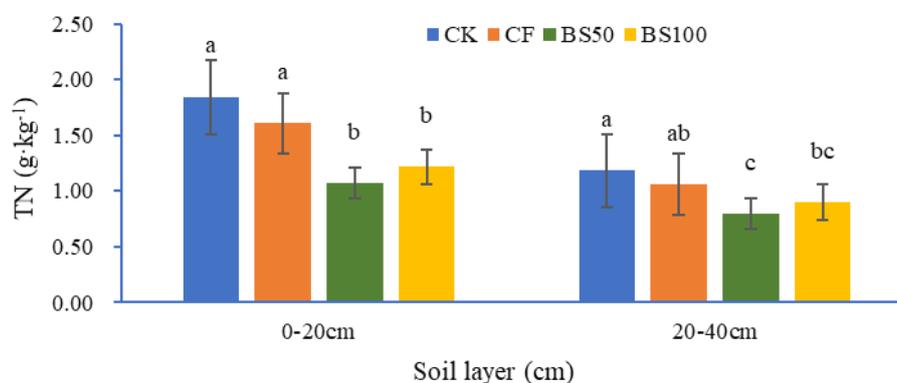


Figure 1. Total nitrogen content in the soil of the experimental plots after winter wheat harvest (The statistical analyses were determined using Duncan's multiple-range test, different letters above bars indicate significant differences between treatments ($P < 0.05$). CK, no fertilization; CF, conventional fertilization with chemical fertilizer; BS50, 50 % nitrogen from biogas slurry and 50 % nitrogen from fertilizer; BS100, only biogas slurry for winter wheat)

The organic nitrogen in biogas slurry exhibits a slow-release property and cannot be directly utilized by plants (Cheng, 2016). After application to the soil, the organic nitrogen pool in the soil is slowly released over time; it only becomes available to plants following mineralization. Chantigny et al. (2004) reported that 12%-15% of the nitrogen from biogas slurry was incorporated into the soil organic nitrogen pool within 6 hours of application and approximately 26%-34% of the nitrogen remained in the soil as organic matter after a full crop growth cycle. In contrast, the inorganic N fraction in biogas slurry can be rapidly taken up and utilized by crops. This combination of slow-release organic nitrogen (which provides a sustained low-level nitrogen supply over an extended period) and readily available inorganic nitrogen allows biogas slurry to better meet the continuous and efficient nitrogen demand of crops, thereby facilitating crop nutrient absorption and growth. Soil TN content dynamics are regulated by multiple biogeochemical processes, including biological nitrogen fixation, organic nitrogen mineralization, nitrogen immobilization, nitrification and denitrification (Kovalenko *et al.*, 2024; Kolisnyk *et al.*, 2025; Lopushniak *et al.*, 2022). Each of these processes plays a pivotal role in shaping the availability and temporal variation of nitrogen in the soil ecosystem. Notably, the response of soil TN content to biogas slurry application remains relatively variable: previous studies have shown that soil TN content may either increase or decrease following biogas slurry incorporation into the field (Wu *et al.*, 2020).

Organic matter. Soil organic matter (SOM) is the primary source of substrates for soil enzymes; it plays a vital role in soil formation processes and the development of soil fertility. Additionally, SOM serves as a key reservoir of carbon and nitrogen for soil microorganisms and thus maintains a close link with soil nutrient status (Zanella *et al.*, 2022; Zhang *et al.*, 2022). Figure 2 depicts the effects of different fertilization treatments (CK, CF, BS50, BS100) on soil SOM content. In the 0-20 cm surface layer, SOM content followed the order: CK>CF>BS50>BS100, with each treatment exhibiting a significant difference compared to CK ($p < 0.05$). In the 20-40 cm subsurface layer, the order of SOM content was identical to that in the 0-20 cm layer (CK>CF>BS50>BS100) and significant differences were observed among all treatments ($p < 0.05$). Notably, SOM

content in the biogas slurry treatments (BS50, BS100) was lower than that of CK and CF and BS50 was 31.2% and 8.1% lower than that of CK and CF, whereas BS100 was 39.6% and 19.4% lower than that of CK and CF, respectively. The soil organic matter content of BS100 was the lowest and decreased in all different treatments in the deeper layer.

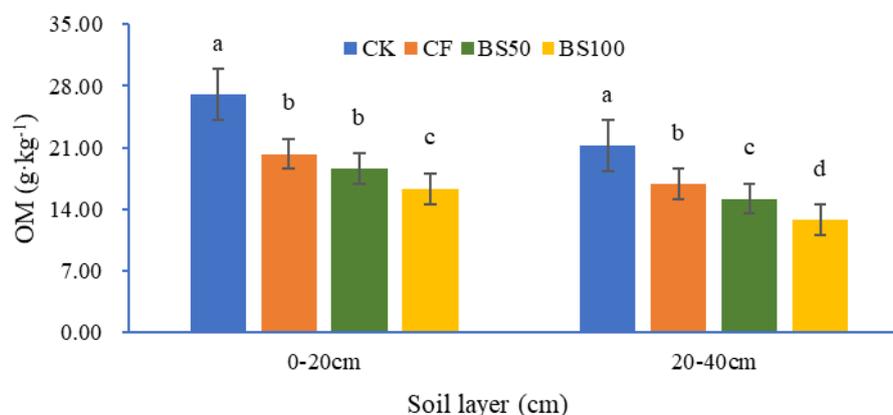


Figure 2. Organic matter content in the soil of the experimental plots after winter wheat harvest (The statistical analyses were determined using Duncan's multiple-range test, different letters above bars indicate significant differences between treatments ($P < 0.05$). CK, no fertilization; CF, conventional fertilization with chemical fertilizer; BS50, 50 % nitrogen from biogas slurry and 50 % nitrogen from fertilizer; BS100, only biogas slurry for winter wheat)

Biogas slurry is a liquid byproduct of the anaerobic fermentation of organic materials (e.g., crop straw, animal manure) and it is rich in organic matter, nitrogen (N), phosphorus (P), potassium (K) and other nutrients. Compared with chemical fertilizers, the nutrients in biogas slurry are more readily available for crop uptake; they also improve the soil environment around crop roots (Xu *et al.*, 2017), promote root development and thus function as a “quick-available nutrient bank” for plant roots. Additionally, organic compounds in biogas slurry (e.g., cellulose, lignin) are decomposed into soil organic matter by soil microorganisms, which not only increases SOM content but also enhances plant nutrient absorption efficiency - conditions that are highly favorable for crop growth (Koszel & Lorencowicz, 2015).

In the present study, SOM content in the BS100 was lower than that in BS50. This observation suggests that the combined application of biogas slurry and chemical fertilizer promotes the accumulation of soil SOM. In contrast, the exclusive use of biogas slurry (as in BS100) may be unfavorable for soil SOM accumulation, leading to a reduction in SOM content. For the CF treatment (chemical fertilizer alone), no organic matter was input alongside nitrogen application; this resulted in the consumption of native soil organic carbon, which in turn caused a decline in SOM content - explaining why SOM content in fertilized treatments (CF, BS50, BS100) was lower than that in CK. These findings align with previous research by Yu *et al.* (2012), who demonstrated that biogas slurry application significantly increases soil microbial biomass and enzyme activity. This process effectively accelerates the transformation and utilization of soil organic matter, which ultimately contributes to improved soil fertility.

Available phosphorus. Phosphorus (P) is an essential component of critical plant organic compounds, such as phospholipids and nucleoproteins (Garcia-Berumen *et al.*, 2025). It is also involved in a wide range of plant metabolic processes, exerting a significant influence on crop yields and the improvement of desirable plant traits. For

these reasons, soil available phosphorus (AP) content is widely used as a key indicator for assessing soil fertility (Zhu *et al.*, 2020).

As presented in Figure 3, in the 0-20 cm surface soil layer, soil AP content followed the order: CK > CF > BS100 > BS50. Specifically, AP-content in the biogas slurry treatment (BS50, BS100) was lower than that in CK and CF; compared to CF, AP content decreased by 22.7% in BS50 and by 7.8% in BS100, respectively. Results from one-way ANOVA showed no significant difference in AP content between CK and CF ($p > 0.05$), whereas significant differences were observed among the other treatment pairs ($p < 0.05$). In the 20-40 cm subsurface soil layer, the order of soil AP content differed from that in the surface layer, being CF > CK > BS100 > BS50. Here, both BS50 and BS100 had significantly lower AP content than CK ($p < 0.05$), while no significant difference was detected between CK and CF ($p > 0.05$). Across all soil layers, the biogas slurry treatments (BS50, BS100) consistently exhibited the lowest AP content. Furthermore, soil AP content showed a steady decline with increasing soil depth, with BS50 displaying the most pronounced reduction.

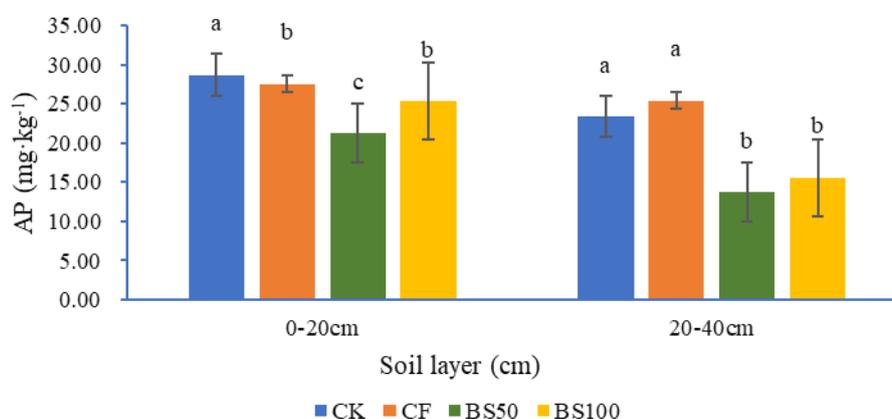


Figure 3. Available phosphorus in the soil of the experimental plots after winter wheat harvest (The statistical analyses were determined using Duncan's multiple-range test, different letters above bars indicate significant differences between treatments ($P < 0.05$). CK, no fertilization; CF, conventional fertilization with chemical fertilizer; BS50, 50 % nitrogen from biogas slurry and 50 % nitrogen from fertilizer; BS100, only biogas slurry for winter wheat)

The soil available phosphorus (P) content in the BS50 treatment was lower than that in the BS100 treatment. This phenomenon may be attributed to the following reasons: first, enhanced nitrogen (N) supply promotes vigorous crop growth, which in turn increases the demand for essential P (Wang *et al.*, 2013) and the subsequent absorption and utilization of P by winter wheat would consequently reduce the soil available P content. Second, biogas slurry inherently contains a certain amount of P, a portion of which is readily decomposable and releasable. Moreover, biogas slurry application can increase the soil organic matter content; during the decomposition of organic matter, substances such as organic acids are produced, which can reduce the fixation of inorganic P, facilitate the dissolution of organic P and ultimately elevate the soil available P content. This mechanism is consistent with the finding in the present study that the soil organic matter content in the BS100 treatment was lower than that in the BS50 treatment. Additionally, the effect of biogas slurry on the available P content is also modulated by the original soil properties (Wang *et al.*, 2017). Previous research on P fertilizers to soil can significantly increase the content of total P, organic P and inorganic P (Xie *et al.*,

2004). More importantly, this combined application can effectively drive the transformation of non-labile P to labile P and this effect can be sustained over an extended period. Furthermore, it exerts a positive impact on improving the crop uptake efficiency of N and P, thereby creating more favorable conditions for crop growth.

The nutrients in subsoil predominantly originate from the leaching and migration of soluble surface substances, driven by vertical water movement. In the present study, soil nutrient concentration in the 0-20 cm layer was consistently higher than that in the 20-40 cm layer. This observation was strongly associated with the inherent high clay content and compaction characteristics of the lime concretion black soils in the study area; such textural properties impede the downward migration of both surface soil nutrients and soil moisture.

Notably, biogas slurry exerts a more pronounced effect on topsoil nutrient dynamics. In the absence of crop nutrient uptake, soil available nutrients derived from biogas slurry accumulate preferentially in the topsoil (Xue *et al.*, 2014). With increasing soil depth, the content of available nutrients decreases gradually - this pattern confirms that soil exhibits a retention effect on available nutrients. This finding provides a critical theoretical basis for optimizing the utilization of biogas slurry-derived available nutrients by crops.

Enzyme activity

Soil enzyme activity is regulated by a multitude of factors and variations in environmental conditions, research objects and sampling timing often lead to substantial discrepancies in the observed vertical distribution patterns of enzyme activity across different studies. As a sensitive bioindicator of soil fertility, soil enzyme activity is primarily influenced by fertilization strategies, the source and chemical composition of fertilizers, tillage and cultivation practices, the application of pesticides and soil amendments, vegetation cover types and inherent soil properties (e.g., texture, pH) coupled with regional climatic conditions (Skubała *et al.*, 2025). Additionally, it is further modulated by the accumulation of pollutants and toxic substances in the soil matrix, as well as the incidence and intensity of soil erosion processes (Kovalzhy *et al.*, 2024; Sternik *et al.*, 2015; Strelbytska *et al.*, 2025). Soil phosphatase, urease and sucrase activities are widely recognized as key indicators for characterizing soil phosphorus/nitrogen supply potential and soil organic matter accumulation. In contrast, catalase activity is commonly used to assess the overall biological activity and humification degree of soil systems (Ma *et al.*, 2021). Previous studies have consistently demonstrated that biogas slurry application can effectively enhance soil enzyme activities (Hao *et al.*, 2011; Yang *et al.*, 2017). Specifically, Wan *et al.* (2017) reported that biogas slurry application significantly increased sucrase, alkaline phosphatase and urease activities in the topsoil of wheat fields. Additionally, under the condition of equal total nitrogen input, Zhang and Wang (2011) found that both sole biogas slurry application and its combination with chemical fertilizers promoted soil microbial biomass and activity, while concurrently increasing soil enzyme activities and reducing the microbial carbon-to-nitrogen (C:N) ratios. Among these treatments, the combined application of biogas slurry and chemical fertilizers exhibited the most pronounced positive effects.

The effects of chemical fertilizer application, biogas slurry application and their combined application on soil enzyme activities are presented in Tables 1-4. Within the same soil depth, values followed by different lowercase letters indicate a significant difference among treatments at the $p < 0.05$ level.

Table 1. Urease activity in the soil of the experimental plots after the winter wheat harvest

| Treatment | 0-20 cm | 20-40 cm |
|-----------|--------------------------|---------------------------|
| CK | 145.53±2.03 ^d | 249.45±1.17 ^c |
| CF | 204.29±4.97 ^b | 294±5.75 ^b |
| BS50 | 219.26±2.8 ^a | 353.31±11.01 ^a |
| BS100 | 163.25±5.17 ^c | 299.1±1.86 ^b |

Note: The statistical analyses were determined using Duncan's multiple-range test, different letters above bars indicate significant differences between treatments ($P < 0.05$). CK, no fertilization; CF, conventional fertilization with chemical fertilizer; BS50, 50 % nitrogen from biogas slurry and 50 % nitrogen from fertilizer; BS100, only biogas slurry for winter wheat

Table 2. Sucrase activity in the soil of the experimental plots after winter wheat harvest

| Treatment | 0-20cm | 20-40cm |
|-----------|------------------------|-------------------------|
| CK | 5.28±0.27 ^c | 6.43±0.2 ^d |
| CF | 7.54±0.3 ^b | 15.65±0.57 ^c |
| BS50 | 8.53±0.06 ^a | 26.78±0.52 ^a |
| BS100 | 7.82±0.04 ^b | 18.16±0.45 ^b |

Note: The statistical analyses were determined using Duncan's multiple-range test, different letters above bars indicate significant differences between treatments ($P < 0.05$). CK, no fertilization; CF, conventional fertilization with chemical fertilizer; BS50, 50 % nitrogen from biogas slurry and 50 % nitrogen from fertilizer; BS100, only biogas slurry for winter wheat

Table 3. Acid phosphatase activity in the soil of the experimental plots after winter wheat harvest

| Treatment | 0-20cm | 20-40cm |
|-----------|------------------------|------------------------|
| CK | 2.56±0.09 ^c | 0.73±0.04 ^c |
| CF | 3.04±0.07 ^b | 2.37±0.1 ^a |
| BS50 | 3.73±0.09 ^a | 2.54±0.1 ^a |
| BS100 | 3.64±0.13 ^a | 1.19±0.16 ^b |

Note: The statistical analyses were determined using Duncan's multiple-range test, different letters above bars indicate significant differences between treatments ($P < 0.05$). CK, no fertilization; CF, conventional fertilization with chemical fertilizer; BS50, 50 % nitrogen from biogas slurry and 50 % nitrogen from fertilizer; BS100, only biogas slurry for winter wheat

Table 4. Catalase activity in the soil of the experimental plots after winter wheat harvest

| Treatment | 0-20cm | 20-40cm |
|-----------|-------------------------|-------------------------|
| CK | 83.92±2.01 ^b | 85.61±0.87 ^c |
| CF | 86.12±1.11 ^b | 91.41±0.83 ^b |
| BS50 | 93.37±0.84 ^a | 95.76±1.49 ^a |
| BS100 | 95.31±0.41 ^a | 97.78±1.44 ^a |

Note: The statistical analyses were determined using Duncan's multiple-range test, different letters above bars indicate significant differences between treatments ($P < 0.05$). CK, no fertilization; CF, conventional fertilization with chemical fertilizer; BS50, 50 % nitrogen from biogas slurry and 50 % nitrogen from fertilizer; BS100, only biogas slurry for winter wheat

Research demonstrates that, relative to the control (CK), the combined application of biogas slurry and chemical fertilizers significantly enhances soil enzyme activity; notably, biogas slurry-based treatments outperform sole chemical fertilizer (CF) application. Among these, the treatments with biogas slurry at a 50% concentration (BS50)

exhibit a remarkable promoting effect on soil enzyme activity, with catalase activity being particularly elevated. The mechanisms by which biogas slurry effectively improves soil enzyme activity are summarized as follows:

1. *Direct enzyme supplementation and substrate provision.* Biogas slurry contains substantial intrinsic enzymes, which can directly augment soil enzyme reserves upon application. Moreover, its abundant, easily decomposable organic matter serves as a rich substrate for various soil enzymes, thereby boosting their catalytic activity. These intrinsic enzymes in biogas slurry act as “exogenous enzyme supplements” when introduced into the soil. Furthermore, the increased soil organic matter content provides protective sites for enzymes, facilitates microbial proliferation and thereby further stimulates the enhancement of soil enzyme activity (Abubaker *et al.*, 2012).

2. *Stimulation of microbial metabolism and residue decomposition.* Soil enzymes are primarily accumulated through the reproductive and metabolic activities of soil microorganisms, as well as the decomposition of animal and plant residues (Sun *et al.*, 2021; Zhernova *et al.*, 2023). Biogas slurry is rich in bioavailable nutrients and its application can improve soil stability (Tang *et al.*, 2022b), while increasing the contents of soil organic matter and essential nutrients - particularly readily available nitrogen, phosphorus and potassium (NPK). This provides sufficient nutrients and water for microbial growth and reproduction, consequently increasing the abundance of soil microorganisms and further enhancing soil enzyme activity (Zhang *et al.*, 2021a).

3. *Improvement of the soil ecological environment.* Biogas slurry functions as a high-quality organic fertilizer, characterized by a comprehensive nutrient composition, abundant available nutrients and both immediate and sustained effects on soil and plants. It optimizes the soil environment by effectively regulating soil water, nutrients, gas and heat conditions and promotes the sustainable cycling of the soil ecoenvironment. This improved soil ecological niche indirectly contributes to the enhancement of soil enzyme activity. However, it is important to note that excessive application of biogas slurry may lead to a reduction in soil enzyme activity, which is consistent with the findings reported by Feng *et al.* (2014). Soil enzyme activity is closely associated with the mineralization of soil nutrients and the decomposition of organic matter (Böhme *et al.*, 2005). Enhanced soil enzyme activity signifies vigorous substances and energy conversion processes in the soil, which in turn promote favorable plant growth conditions (Ullah *et al.*, 2019). Thus, the BS50 treatment exhibits particular benefits for the growth and development of winter wheat. Urease activity was affected by fertilizer application, with moderate effects observed in both the topsoil and subsoil layers. In the 0-20 cm layer, the highest urease activity was recorded under the BS50 treatment, with an average of 219,26 $\mu\text{g}\cdot\text{g}^{-1}$, compared to 145,53 $\mu\text{g}\cdot\text{g}^{-1}$ in the CK treatment. Notably, as the proportion of nitrogen derived from biogas slurry increased from 50 to 100%, urease activity decreased, falling below the level observed in the CF treatment. In the 20-40 cm soil layer, urease activity was significantly higher across all treatments, with an average increase of 61-71% relative to the 0-20 cm layer. However, at this depth, no statistically significant difference was found between the CF and BS100 treatments.

Sucrase activity in the 0-20 cm layer ranged from 5.28 to 7.82 $\text{mg}\cdot\text{g}^{-1}$ across the various treatments, with no significant difference ($p > 0.05$) between the CF and BS100 treatments. Similar to urease, sucrase activity increased in the subsoil, especially in plots receiving fertilization. In the 20-40 cm layer, significant differences were observed among all treatments, with the highest sucrase activity detected under the BS50 treatment. Catalase activity in the 0-20 cm layer ranged from 83.92 to 95.32 $\text{mmol}\cdot\text{g}^{-1}$, while in the

20-40 cm layer, it varied from 85.61 to 97.78 mmol•g⁻¹. In the topsoil (0-20 cm), no significant differences in catalase activity were observed among CK, CF, BS50 and BS100 treatments. These results suggest that the application of organic matter in the form of biogas slurry affects catalase activity; however, increasing the nitrogen rate from BS50 to BS100 does not enhance this enzymatic activity. In the 20-40 cm layer, the control treatment exhibited the lowest catalase activity, while, consistent with the topsoil layer, no significant difference was detected between BS50 and BS100 treatments. Given that catalase functions as a key enzyme, it is important to emphasize that variations in its activity can reflect the potential impacts of external factors on the development of the soil microbiome. Notably, uncultivated soils show higher catalase activity in the upper 0-20 layer. Based on long-term research, Samuel et al. (2018) demonstrated that the combined application of organic and mineral fertilizers enhances both catalase and phosphatase activities. The most significant positive effect was observed with the incorporation of green manure (a mixture of lupine, rapeseed and oats) and farmyard manure, whether used alone or in combination with mineral fertilizers. Long-term exclusive use of chemical fertilizers led to a decline in phosphatase activity, whereas manure application promoted it. This indicates that the incorporation of organic amendments and plant residues plays a vital role in nutrient transformation processes, enhancing both phosphatase and catalase activities. Zhang et al. (2022) highlighted a current challenge in modern agriculture: excessive reliance on chemical fertilizers and the predominance of nitrogen over phosphorus and potassium. Their findings confirmed that the activities of urease, catalase and sucrase are strongly correlated with organic carbon content. Increasing the proportion of organic fertilizers relative to mineral ones from 20% to 80% significantly elevated the activity of these enzymes.

Acid phosphatase activity showed a decreasing trend with soil depth across the 0-40 cm profile, with significantly higher enzyme activity in the upper 0-20 cm layer compared to the 20-40 cm layer. These observations are consistent with the findings of Li et al. (2007). Two key factors likely account for this pattern. First, the application of biogas slurry enriched the topsoil with essential nutrients conducive to microbial growth, resulting in increased concentrations of organic matter, phosphorus and microbial biomass. Given that the fibrous roots of winter wheat are predominantly concentrated in the top layer, the improved aeration at this depth facilitates more vigorous microbial respiration and metabolism, thereby supporting higher phosphatase activity. Second, the lower root density in the deeper layer leads to less favorable moisture and temperature conditions, coupled with reduced soil aeration. These constraints negatively affect soil structure and porosity, consequently limiting microbial activity. Additionally, the decrease in organic matter content at this depth diminishes the capacity of soil microbes to synthesize enzymes (Wang *et al.*, 2016). Collectively, these factors contribute to the observed vertical variation in acid phosphatase activity within the upper 40 cm of soil. A distinct pattern characterizes the vertical distribution of soil enzymes, which is closely linked to the productivity and fertility of different soil layers. Liu (2021) demonstrated that within the 0-60 cm soil profile the activities of urease, alkaline phosphatase and sucrase exhibit a decreasing trend from the surface layer to deeper layers. In contrast, catalase activity reaches its peak in the subsurface layer between 20 and 40 cm (20-40 cm). Similarly, urease activity increased with soil depth from 0 to 40 cm. In contrast, invertase and catalase activities decreased, with a significant concentration in the upper 0-20 cm layer, as reported by Guo et al. (2012). Additionally, their study indicated that catalase activity is higher in deeper soil layers, while urease, invertase and alkaline

phosphatase activities decline with increasing depth, showing marked accumulation in the upper 0-20 cm layer. In this study, all three soil enzymes, except for acid phosphatase, exhibited higher activity levels in the 20-40 cm layer than in the 0-20 cm layer. Two possible explanations for this pattern are the progressive increase in root secretions during the later stages of winter wheat growth and the heightened activity of deep roots (Xiong *et al.*, 2003). Research has indicated that in the later developmental stages of winter wheat, root activity in deeper soil layers tends to be higher than that in the surface layer. Roots located at depths of 20-40 cm display greater metabolic activity, leading to an increased release of root exudates into the soil. This influx simulates soil enzyme activity. Furthermore, these exudates act as a crucial carbon source for soil microorganisms, facilitating their growth and reproduction, which in turn further enhances enzymatic function in the soil.

Our results revealed that the activities of four enzymes are highly sensitive to both chemical fertilization and biogas slurry application. The incorporation of biogas slurry into the soil significantly enhances enzyme activity in the 0-40 cm soil layer, with a particularly pronounced effect observed in the 20-40 cm layer. This enhancement contributed to delaying senescence in the winter wheat root system during the later growth stages and mitigating the decline in activity of the upper root zone. Thereby enabling the entire root system to maintain higher physiological activity (Wang, 2017). Such conditions promote increased soil biological activity, facilitate soil nutrient transformation processes and enhance the soil's nutrient supply capacity, which in turn fulfils the nutritional demands of crops throughout their growth cycle. Ultimately, these effects exert a positive impact on soil fertility and winter wheat yield.

Relationships between soil characteristics. In recent years, investigating the associations between soil enzyme activity and soil nutrient factors has become a prominent research focus in soil science (Yao *et al.*, 2021; Zhang *et al.*, 2021b). Most researchers contend that soil enzymes are derived from the reproductive and metabolic activities of soil microorganisms, as well as the decomposition of plant and animal residues. These enzymes show a strong correlation with soil fertility and its overall status and thus serve as valuable indicators of soil fertility (Gil-Sotres *et al.*, 2003). Our study revealed no significant correlation between acid phosphatase, sucrase and catalase across the four different treatments (Table 5).

Table 5. Pearson correlation analysis of soil enzyme activity and nutrient

| | Urease | Total nitrogen | Sucrase | Organic matter | Acid phosphatase | Available phosphorus | Catalase |
|----------------------|--------|----------------|---------|----------------|------------------|----------------------|----------|
| Urease | 1 | -0.769 | 0.892 | -0.661 | -0.434 | -0.809 | 0.508 |
| Total nitrogen | | 1 | -0.712 | 0.802 | 0.199 | 0.862 | -0.756 |
| Sucrase | | | 1 | -0.685 | -0.175 | -0.759 | 0.654 |
| Organic matter | | | | 1 | 0.058 | 0.841 | -0.904 |
| Acid phosphatase | | | | | 1 | 0.226 | 0.134 |
| Available phosphorus | | | | | | 1 | -0.762 |
| Catalase | | | | | | | 1 |

Our study revealed no significant correlation between acid phosphatase, sucrase and catalase across the four different treatments. However, notable correlations were observed among other enzymes: sucrase showed positive correlations with urease and catalase, with correlation coefficients of 0.892 and 0.654, respectively; urease was also

significantly correlated with acid phosphatase and catalase, with correlation coefficients of 0.434 and 0.508, respectively. This results indicate that soil enzymes not only play specific roles in driving soil nutrient conversion and cycling but also participate in the cycling and transformation of other soil nutrient factors. Moreover, nutrient cycling and transformation involve not just individual enzymes, but multiple enzymes that are closely associated and interact with one another. Thus, soil nutrient characteristics are collectively regulated by a suite of enzymes (Ye *et al.*, 2013). During crop growth, plants continuously absorb nutrients from the soil, while root metabolism enhances soil organic matter content and enzyme activity - thereby improving soil fertility. This reciprocal relationship facilitates the renewal and dynamic change of soil fertility. Studies have confirmed a strong correlation between soil fertility level and enzyme activity (Li *et al.*, 2021), with soil enzyme activities being significantly influenced by soil organic carbon, nitrogen and phosphorus contents (Jian *et al.*, 2016). In contrast, based on ecological-economic optimal allocation model, microorganisms reduce enzyme production when resources are non-limiting, leading to a common nutrient content and enzyme activity (Čapek *et al.*, 2019; He *et al.*, 2020). Our data showed that, except for acid phosphatase, urease, sucrase and catalase were significantly negatively correlated with the content of organic matter, total nitrogen and available phosphorus. Relevant studies have also indicated that increased nutrient availability reduces the difficulty for microorganisms to acquire these substances, thereby decreasing the activity of related catalytic enzymes (Burns *et al.*, 2013). In other words, when nutrients are insufficient to meet the growth demands of winter wheat, microorganisms may enhance enzyme secretion to promote nutrient release from the soil, facilitating nutrient uptake and utilization by wheat. However, the relationship between soil enzyme activities and soil biochemical properties during the nutrient cycling process is complex. For instance, some studies have confirmed a significant positive correlation between soil nutrient content and enzyme activities (Sun *et al.*, 2021). In the present study, no significant correlation was observed between acid phosphatase activity and soil nutrients, which is consistent with the findings of Zheng *et al.* (2018).

Holik *et al.* (2019) in their study on Cambisols subjected to organic fertilization, mineral fertilization and their combination, found that these treatments had only a marginal effect on soil enzyme activities. The primary response to fertilization was observed in soil respiration. However, the authors emphasized that the combined application of organic and mineral fertilizers enhanced soil microbiological activity. In summary, soil enzymes respond to changes induced by environmental or management practices and play a crucial role in mediating soil biogeochemical processes. The activity level of soil enzymes can serve as an indicator of efficiency of soil nutrient transformation.

4. Conclusion

1. Both chemical fertilizers and biogas slurry affect the content of soil organic matter, total nitrogen and available phosphorus. Compared with the sole application of chemical fertilizers, the BS50 and BS100 biogas slurry treatments promote the decomposition, transformation and mobilization of soil nutrients. The decrease in phosphorus and nitrogen content when applying biogas slurry is explained by the higher uptake of these elements by the winter wheat crop, which requires more detailed analysis.

2. Biogas slurry application significantly increased soil enzyme activity, with a particularly pronounced effect in the 20-40 cm layer. This enhancement played a crucial role in delaying root senescence during the late growth stages of winter wheat, which contributed to improved soil fertility and increased crop yield.

3. A strong positive correlation was observed between soil fertility level and soil enzyme activity. Higher enzyme activity was associated with increased nutrient mineralization rates and more efficient nutrient cycling in the soil ecosystem. Consequently, soil enzyme activity serves as an effective indicator of the intensity of soil nutrient transformation processes.

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References

- Abdelsalam, E., Samer, M., Attia, Y., Abdel-Hadi, M., Hassan, H. & Badr, Y. (2016). Comparison of nanoparticles' effects on biogas and methane production from anaerobic digestion of cattle dung slurry. *Renewable Energy*, 87, 592-598. <https://doi.org/10.1016/j.renene.2015.10.053>
- Abubaker, J., Risberg, K. & Pell, M. (2012). Biogas residues as fertilisers - effects on wheat growth and soil microbial activities. *Applied Energy*, 99, 126-134. <https://doi.org/10.1016/j.apenergy.2012.04.050>
- Bachmann, S., Wentzel, S. & Eichler-Löbermann, B. (2011). Codigested dairy slurry as a phosphorus and nitrogen source for *Zea mays* L. and *Amaranthus cruentus* L. *Journal of Plant Nutrition and Soil Science*, 174, 908-915. <https://doi.org/10.1002/jpln.201000383>
- Bao, S.D., (2000). *Agrochemical Analysis of Soil*, 3rd edition. Beijing: China Agriculture Press. (In Chinese).
- Böhme, L., Langer, U. & Böhme, F. (2005). Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. *Agriculture, Ecosystems & Environment*, 109, 141-152. <https://doi.org/10.1016/j.agee.2005.01.017>
- Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., ... & Zoppini, A. (2013). Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology and Biochemistry*, 58, 216-234. <https://doi.org/10.1016/j.soilbio.2012.11.009>
- Čapek, P., Starke, R., Hofmockel, K.S., Bond-Lamberty, B. & Hess, N. (2019). Apparent temperature sensitivity of soil respiration can result from temperature driven changes in microbial biomass. *Soil Biology and Biochemistry*, 135, 286-293. <https://doi.org/10.1016/j.soilbio.2019.05.016>
- Chantigny, M.H., Angers, D.A., Morvan, T. & Pomar, C. (2004). Dynamics of pig slurry nitrogen in soil and plant as determined with ¹⁵N. *Soil Science Society of America Journal*, 68, 637-643. <https://doi.org/10.2136/sssaj2004.6370>
- Chen, L., Li, F., Li, W., Ning, Q., Li, J., Zhang, J., ... & Zhang, C. (2020). Organic amendment mitigates the negative impacts of mineral fertilization on bacterial communities in Shajiang black soil. *Applied Soil Ecology*, 150, 103457. <https://doi.org/10.1016/j.apsoil.2019.103457>

- Chen, Q., Song, Y., An, Y., Lu, Y. & Zhong, G. (2024). Soil microorganisms: Their role in enhancing crop nutrition and health. *Diversity*, 16(12), 734. <https://doi.org/10.3390/d16120734>
- Cheng, J.B. (2016). Control of soil nitrogen leaching during biogas slurry irrigation by slurry C/N. Dissertation, Southwest University, Chongqing. (In Chinese).
- Chernysh, Y., Roy, I., Chubur, V., Shulipa, Y. & Roubik, H. (2023). Co-digestion of poultry litter with cellulose-containing substrates collected in the urban ecosystem. *Biomass Conversion and Biorefinery*, 13(6), 4803-4815. <https://doi.org/10.1007/s13399-021-01582-y>
- Czekała, W., Lewicki, A., Pochwatka, P., Czekała, A., Wojcieszak, D., Józwiakowski, K. & Waliszewska, H. (2020). Digestate management in polish farms as an element of the nutrient cycle. *Journal of Cleaner Production*, 242, 118454. <http://dx.doi.org/10.1016/j.jclepro.2019.118454>
- Datsko, O., Melnyk, O., Kovalenko, I., Butenko, A., Zakharchenko, E., Ilchenko, V., ... & Solokha, M. (2025). Estimation of the content of trace metals in Ukrainian military-affected soils. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 53(1), 14328. <https://doi.org/10.15835/nbha53114328>
- Datsko, O., Zakharchenko, E., Butenko, Y., Rozhko, V., Karpenko, O., Kravchenko, N., ... & Khtystenko, A. (2024). Environmental aspects of sustainable corn production and its impact on grain quality. *Ecological Engineering & Environmental Technology*, 25(11), 163-167. <https://doi.org/10.12912/27197050/192537>
- Daunoras, J., Kačergius, A. & Gudiukaitė, R. (2024). Role of Soil Microbiota Enzymes in soil health and activity changes depending on climate change and the type of soil ecosystem. *Biology (Basel)*, 13(2), 85. <https://doi.org/10.3390/biology13020085>
- Dindar, E., Şağban, F. & Başkaya, H.S. (2015). Evaluation of soil enzyme activities as soil quality indicators in sludge-amended soils. *Journal of Environmental Biology*, 36, 919-926.
- Feng, D.N., Wu, J., Yang, G., Zhang, L.W., Zhang, Y.H. & Wang, J.W. (2014). Influence of long-term applications of biogas slurry on microbial community composition and enzymatic activities in surface soil under rice-rape rotation. *Journal of Agro-Environment Science*, 33, 1644-1651. <https://doi.org/10.3390/microorganisms12040716>
- Fu, J.Z., Zhou, S.M., Qian, H.Y., Guo, F.F., Teng, Z.K., Yang, J., ... & He, D.X. (2023). Relationships between spatiotemporal distribution of root main morphological and quantitative traits and contents of soil available nutrients in wheat (*Triticum aestivum* L.). *Journal of Nuclear Agricultural Sciences*, 37, 626-637. (In Chinese).
- Garcia-Berumen, J.A., de la Torre, J.A.F., de los Santos-Villalobos, S., Espinoza-Canales, A., Echavarría-Cháirez, F.G. & Gutiérrez-Bañuelos, H. (2025). Phosphorus dynamics and sustainable agriculture: The role of microbial solubilization and innovations in nutrient management. *Current Research in Microbial Sciences*, 8, 100326. <https://doi.org/10.1016/j.crmicr.2024.100326>
- Gil-Sotres, F., de la Peña, M.L. & Trasar-Cepeda, C. (2003). A comment on the article by de la Paz-Jiménez et al., Soil quality: A new index based on microbiological and biochemical parameters. *Biology and Fertility of Soils*, 37, 260. <https://doi.org/10.1007/s00374-002-0529-6>
- Guan, S.Y. (1986). *Soil Enzymes and Research Methods*. Beijing: China Agriculture Press, 274-340. (In Chinese).
- Guo, M.Y., Chao, K.T., Yu, J.C., Xu, L.J., Wang, L.J., Jia, S.J. & Xin, X.P. (2012). Soil microbial characteristic and soil respiration in grassland under different use patterns. *Acta Agrestia Sinica*, 20, 42-48. (In Chinese).
- Han, J., Fan, D.W., Guo, Y.H., Shen, J.H. & Han, J.G. (2021). Effect of pig slurry on the availability of heavy metals in soils with different pH. *Research of Agricultural Modernization*, 42(2), 321-329. <http://dx.doi.org/10.13872/j.1000-0275.2021.0028> (In Chinese).
- Hao, X.J., Hong, J.P. & Qiao, Z.W. (2011). Effect of biogas slurry on biological properties of cabbage continuous cropping soil. *Chinese Journal of Applied and Environmental Biology*,

- 17, 384-387. <http://dx.doi.org/10.3724/SP.J.1145.2011.00384> (In Chinese).
- He, Q.Q., Wu, Y.H., Bing, H.J., Zhou, J. & Wang, J.P. (2020). Vegetation type rather than climate modulates the variation in soil enzyme activities and stoichiometry in subalpine forests in the eastern Tibetan Plateau. *Geoderma*, 374, 114424. <http://dx.doi.org/10.1016/j.geoderma.2020.114424>
- Holík, L., Hlisnikovský, L., Honzík, R., Trögl, J., Burdová, H. & Popelka, J. (2019). Soil microbial communities and enzyme activities after long-term application of inorganic and organic fertilizers at different depths of the soil profile. *Sustainability*, 11(12), 3251. <https://doi.org/10.3390/su11123251>
- Jian, S.Y., Li, J.W., Chen, J., Wang, G.S., Mayes, M.A., Dzantor, K.E., ... & Luo, Y.Q. (2016). Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. *Soil Biology and Biochemistry*, 101, 32-43. <https://doi.org/10.1016/j.soilbio.2016.07.003>
- Kharchenko, O., Zakharchenko, E., Kovalenko, I., Prasol, V., Pshychenko, O. & Mishchenko, Y. (2019). On problem of establishing the intensity level of crop variety and its yield value subject to the environmental conditions and constraints. *AgroLife Scientific Journal, University of Agronomic Sciences and Veterinary Medicine of Bucharest*, 8(1), 113-120.
- Kolisnyk, O., Bahorka, M., Yurchenko, N., Barylko, M., Zakharchenko, E., Prokopenko, R., ... & Kryvozub, V. (2025). Evaluation of effectiveness indicators of the application of foliar fertilizers in the sustainable economic development of wheat winter productivity. *International Journal of Ecosystems and Ecology Science*, 15(4), 37-44. <https://doi.org/10.31407/ijees15.406>
- Koszel, M., Lorencowicz, E. (2015). Agricultural use of biogas digestate as a replacement fertilizers. *Agriculture and Agricultural Science Procedia*, 7, 119-124. <https://doi.org/10.1016/j.aaspro.2015.12.004>
- Kovalenko, V., Kovalenko, N., Gamayunova, V., Butenko, A., Kabanets, V., Salatenko, I., ... & Vandyk, M. (2024). Ecological and technological evaluation of the nutrition of perennial legumes and their effectiveness for animals. *Journal of Ecological Engineering*, 25(4), 294-304. <https://doi.org/10.12911/22998993/185219>
- Kovalzhy, N., Rieznik, S., Butenko, A., Havva, D., Degtyarjov, V., Hotvianska, A., ... & Nozdrina, N. (2024). Activity of cellulose-degrading microorganisms in typical chernozem under different fertilization systems of strawberries (*Fragaria*). *Agriculture and Forestry*, 70(3), 105-113. <https://doi.org/10.17707/AgricultForest.70.3.07>
- Li, C.X., Chen, F., Wang, J.Z. & Li, Y.J. (2007). Effect of different tillage practices on soil enzyme activity. *Chinese Journal of Soil Science*, 38, 601-603. (In Chinese).
- Li, M.D., Li, X.D., Niu, H.B., Yang, X.W., Jin, H.Y. & He, D.X. (2017). Relationship between drought resistances, deep rooting characteristics and vigor of roots within deep-soil-layer of different wheat cultivars. *Journal of Triticeae Crops*, 37, 666-672. (In Chinese).
- Li, P., Jiang, D., Wang, S., Zhang, X., Zhang, Y., Wang, Y., ... & Jian, N. (2025). Effect of biogas slurry on the nutrient cycling and micro-organisms community in two types of soil, *BioResources* 20(1), 1755-1770. <https://doi.org/10.15376/biores.20.1.1755-1770>
- Li, S.C., Wei, W.Y., Jun, W.C., Ju, L.Q., Hong, W.Z., Sheng, Y.D. & Li, Z.J. (2021). Effects of land use conversion on soil extracellular enzyme activity and its stoichiometric characteristics in karst mountainous areas. *Acta Ecologica Sinica*, 41, 4140-4149. <https://doi.org/10.5846/stxb202007161864> (In Chinese).
- Liang, X., Wang, H., Wang, C., Wang, H., Yao, Z., Qui, X., ... & Wang J. (2024). Unraveling the relationship between soil carbon-degrading enzyme activity and carbon fraction under biogas slurry topdressing. *Journal of Environmental Management*, 356, 120641. <https://doi.org/10.1016/j.jenvman.2024.120641>.
- Liu, M.Y., (2021). Effects of Returning of Winter Green Manure and Straw on Soil Physical and Chemical Properties and Enzyme Activity: Master Thesis, Hebei University of Engineering, Han Dan. (In Chinese).
- Lopushniak, V., Hrytsuliak, H., Gamayunova, V., Kozan, N., Zakharchenko, E., Voloshin, Y., ...

- & Kotsyubynska, Y. (2022). A dynamics of macro elements content in eutric podzoluvisols for separation of wastewater under Jerusalem artichokes. *Journal of Ecological Engineering*, 23(4), 33-42. <https://doi.org/10.12911/22998993/14626>
- Ma, N., Chen, Z.W. & Zhang, Q.W. (2021). Effects of different types of straw returning on soil organic carbon contents and enzyme activity: A review. *Jiangsu Agricultural Sciences*, 49, 53-57. (In Chinese).
- Samuel, A.D., Bungau, S., Tit, D.M., Melinte, C.E., Purza, L. & Badea, G.E. (2018). Effects of long term application of organic and mineral fertilizers on soil enzymes. *Revista de Chimie*, 69(10), 2608-2612. <https://doi.org/10.37358/RC.18.10.6590>
- Shevchuk, O.V., Hospodarenko, H.M. (2025). Comparative characteristics of digestate and other organic fertilizers. *Bulletin of Uman National University of Horticulture*, 1, 42-48. <http://dx.doi.org/10.32782/2310-0478-2025-1-42-48>
- Skubała, K., Styburski, J. & Chowaniec, K. (2025). Assessment of the suitability of dehydrogenase activity as a biomarker in the plant rhizosphere soil and lichen thalli for trace element pollution around a zinc smelter - a preliminary research. *Rocznik Ochrona Środowiska*, 27, 361-372. <https://doi.org/10.54740/ros.2025.029>
- Sternik, V., Martsinovsky, V. & Melnik, V. (2015). The content of the enzyme catalase in the soil on the territory of the gas station Rivne. *Lesya Ukrainka Eastern European National University Scientific Bulletin: Series: Biological Sciences*, 2(302), 113-118. (In Ukrainian).
- Strelbytska, T., Sakhno, A., Novitska, L., Romanenko, S. & Nitsenko, V. (2025). Fertilizer Application as a factor of economic security in achieving effective land use in the conditions of environmental development. *Rocznik Ochrona Środowiska*, 27, 253-268. <https://doi.org/10.54740/ros.2025.021>
- Sun, X., Ye, Y., Ma, Q., Guan, Q. & Jones, D.L. (2021). Variation in enzyme activities involved in carbon and nitrogen cycling in rhizosphere and bulk soil after organic mulching. *Rhizosphere*, 19, 100376. <https://doi.org/10.1016/j.rhisph.2021.100376>
- Tang, J., Davy, A.J., Wang, W., Zhang, X., Wu, D., Hu, L. & Yin, J. (2022a). 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. <http://dx.doi.org/10.1007/s42729-022-00817-9>
- Tang, J., Wang, W., Pan, F.F., Yin, J.Z., Zhang, X.H., Wu, D.F., ... & Du, Y.M. (2022b). The effects of biogas slurry irrigation on aggregation and stability of fluvoaquic soil in Huang-Huai-Hai Plain. *Journal of Irrigation and Drainage*, 41, 10-17. (In Chinese).
- Ullah, S., Ai, C., Huang, S., Zhang, J., Jia, L., Ma, J., ... & He, P. (2019). The responses of extracellular enzyme activities and microbial community composition under nitrogen addition in an upland soil. *PLoS One*, 14, e0223026. <https://doi.org/10.1371/journal.pone.0223026>
- Voitovyk, M., Butenko, A., Prymak, I., Mishchenko, Yu., Tkachenko, M., Tsiuk, O., ... & Havryliuk, O. (2023). Influence of fertilizing and tillage systems on humus content of typical chernozem. *Agraarteadus*, 34(1), 44-50. <https://doi.org/10.15159/jas.23.03>
- Wan, H.W., Jia, L.L., Zhao, J.Q., Feng, Y.Z., Yang, G.H. & Ren, G.X. (2017). Effects of topdressing biogas slurry on photosynthesis characteristics of wheat and soil enzyme activities and nutrients. *Journal of Northwest A&F University (Nat.Sci.Ed.)*, 45, 35-44. (In Chinese).
- Wang, J., Wang, Z., Gu, F., Liu, H., Kang, G., Feng, W., ... & Guo, T. (2021). Tillage and irrigation increase wheat root systems at deep soil layer and grain yields in lime concretion black soil. *Scientific Reports*, 11, 6394. <https://doi.org/10.1038/s41598-021-85588-6>
- Wang, L.D., Wang, F.L., Guo, C.X., Han, F.G., Wei, L.Y. & Li, F.M. (2016). Review: Progress of soil enzymology. *Soils*, 48, 12-21. (In Chinese).
- Wang, M.F., Chen, S., Zhu, J., Liu, S.L., Chen, Q., Li, J.J. & Xu, J.X. (2017). Effects of application of biogas slurry on the form and mobility of soil phosphorus in vegetable greenhouse field. *Journal of Agricultural Resources and Environment*, 34, 368-375. (In Chinese).

- Wang, W., Zhang, Y., Liu, Y., Jiang, N., Zhao, Q. & Deng, L. (2021). Managing liquid digestate to support the sustainable biogas industry in China: Maximizing biogas-linked agro-ecosystem balance. *Global Change Biology Bioenergy*, 13(6), 880-892. <https://doi.org/10.1111/gcbb.12823>
- Wang, Y.Z., Chen, X. & Shi, Y. (2013). Phosphorus availability in cropland soils of China and related affecting factors. *Chinese Journal of Applied Ecology*, 24(1), 260-268. (In Chinese).
- Wentzel, S., Schmidt, R., Piepho, H.P., Semmler-Busch, U. & Joergensen, R.G. (2015). Response of soil fertility indices to long-term application of biogas and raw slurry under organic farming. *Applied Soil Ecology*, 96, 99-107. <http://dx.doi.org/10.1016/j.apsoil.2015.06.015>
- Wu, J., Pan, X., Yang, Y., Gao, C., Wang, Y. & Li M. (2020). Effects of biogas slurry utilization in long-term on soil nutrients and enzyme activities. *Current Investigations in Agriculture and Current Research*, 8(3), 1083-1090. <https://doi.org/10.32474/CIACR.2020.08.000286>
- Wu, J.C., Pan, X.Y., Yang, Y.H., Gao, C.M. & Wang, Y. (2021). Effects of long-term application of biogas slurry on soil nutrient content and enzyme activity. *Journal of Henan Agricultural Sciences*, 50, 76-86. <https://doi.org/10.15933/j.cnki.1004-3268.2021.07.010> (In Chinese).
- Xie, L., Lü, J., Zhang, Y. & Liu, L. (2004). Influence of long-term fertilization on phosphorus fertility of calcareous soil II. Inorganic and organic phosphorus. *The Journal of Applied Ecology*, 15(5), 790-794. (In Chinese).
- Xiong, M.B., Tian, Y.B., Lei, X.Z., Song, G.Y. & Cao, S.Y. (2003). Dynamics of soil nutrition and soil enzyme activity and their relationships during wheat growth. *Journal of Soil Water Conservation*, 17, 27-30. (In Chinese).
- Xu, Y.L., Tang, H.M., Cheng, A.W., Li, Y.F., Li, Y., He, W., ... & Wang, Y.P. (2017). Effects of different long-term fertilizer managements on soil nutrients and rice yield in double cropping paddy field. *Acta Agriculturae Boreali-Sinica*, 32, 192-197. <https://doi.org/10.7668/hbxb.2017.06.028> (In Chinese).
- Xue, L.H., Xie, X.Q., Duan, L.N. & Zhang, J.X. (2014). Effect of drip irrigation on growth, temporal and spatial distribution of root of winter wheat. *Agricultural Research in the Arid Areas*, 32, 1-9. (In Chinese).
- Yang, W., Cheng, Y., Wu, X., Zhou, J. & Liu, X. (2025). Effects of combined application of biogas slurry and chemical fertilizers on silage corn, soil nutrients and microorganisms. *Microorganisms*, 13(1), 2. <https://doi.org/10.3390/microorganisms13010002>
- Yang, Z.F., Chen, W.Q., Wang, W., Li, W.Y., He, X.B. & Yang, X.T. (2017). Effects of biogas slurry on soil microbial characteristics of broccoli. *Chinese Agricultural Science Bulletin*, 33(29), 112-115. <https://doi.org/10.11924/j.issn.1000-6850.casb17030139> (In Chinese).
- Yao, Y., Ma, Q., Zheng, G.L., Li, C.Y., Ding, J.F., Zhu, M., ... & Zhu, X.K. (2021). Effect of different straw returning methods on wheat yield, soil nutrients and physiological activities. *Journal of Triticeae Crops*, 41, 1-10. (In Chinese).
- Ye, X.F., Yang, C., Li, Z. & Jing, H.X. (2013). Effects of green manure in corporation on soil enzyme activities and fertility in tobacco-planting soils. *Plant Nutrition and Fertilizer Science*, 19, 445-454. <https://dx.doi.org/10.11674/zwyf.2013.0222> (In Chinese).
- Yu, W.W., Zhang, Z., Luo, S.R., Bi, S.L. & Naresh, S. (2012). Characteristics of purple vegetable soil under biogas slurry irrigation. *Transactions of the Chinese Society of Agricultural Engineering*, 28(16), 178-184. (In Chinese).
- Zanella, A., Ponge, J.F., Jabiol, B., Van Delft, B., De Waal, R., Katzensteiner, K., ... & Hager, H. (2022). A standardized morpho-functional classification of the planet's humipedons. *Soil Systems*, 6(3), 59. <https://doi.org/10.3390/soilsystems6030059>
- Zhang, C., Zhao, Z., Li, F. & Zhang, J. (2022). Effects of organic and inorganic fertilization on soil organic carbon and enzymatic activities. *Agronomy*, 12(12), 3125. <https://doi.org/10.3390/agronomy12123125>
- Zhang, K., Yan, Z., Li, M., Kang, E., Li, Y., Yan, L., ... & Kang, X. (2021a). Divergent responses of CO₂ and CH₄ fluxes to changes in the precipitation regime on the Tibetan Plateau: Evidence from soil enzyme activities and microbial communities. *Science of the Total Environment*, 801, 149604. <https://doi.org/10.1016/j.scitotenv.2021.149604>

- Zhang, W.G., Zhao, C.Z., Liu, J., Sun, S.Q., Zhao, Y.J. & Wei, J. (2021b). Effects of exogenous GR24 on biogas upgrading and nutrient removal by co-culturing microalgae with fungi under mixed LED light wavelengths. *Chemosphere*, 281, 130791. <https://doi.org/10.1016/j.chemosphere.2021.130791>
- Zheng, W., Gong, Q.L., Zhao, Z.Y., Liu, J., Zhai, B.N., Wang, Z.H. & Li, Z.Y. (2018). Changes in the soil bacterial community structure and enzyme activities after intercrop mulch with cover crop for eight years in an orchard. *European Journal of Soil Biology*, 86, 34-41. <https://doi.org/10.1016/j.ejsobi.2018.01.009>
- Zhernova, O., Hrosheva, O., Degtiarov, V. & Pachev, I. (2023). Biogenicity of typical chernozem by different fertilisation systems in the Kharkiv region of Ukraine. *Journal of Mountain Agriculture on the Balkans*, 26(5), 465-492. (In Russian).
- Zhu, C.W., Long, Q., Dong, S.G., Shi, K., Jiang, G.Y., Li, X.L., ... & Liu, S.L. (2020). Effects of rotary and deep tillage modes on soil microbial biomass carbon and nitrogen and enzyme activities in fluvo-aquic soil under wheat-maize rotation system. *Plant Nutrition and Fertilizer Science*, 26, 51-63. <https://dx.doi.org/10.11674/zwyf.19358>
- Zhang, H., Wang, G.L. (2011). Effects of biogas slurry combined with nitrogen fertilizer on soil microbial biomass and enzyme activities. *Journal of Anhui Agricultural Sciences*, 39, 16601-16603, 16723. (In Chinese)