scientific reports

Check for updates

OPEN

Evaluation of seven fermentation methods for enhancing the fertilizer potential of the liquid fraction of cow manure

Dong Zeng[1,2]✉, Xiaorui Ma[1,2], Yiwen Zhong[1,2], Wenxing Su[1,2]✉, Dechun He[1,2]✉ & Wangrong Liu[1,2]

Although the use of cow manure as a crop fertilizer has received widespread attention, the impact of different fermentation methods (FMs) on the potential of liquid fraction of cow manure as a crop fertilizer has not been thoroughly assessed. To address this gap, we investigated the effects of seven FMs over a 13-week period on the physicochemical characteristics of cow wastewater and evaluated its potential for reuse as a soil fertilizer. Our findings demonstrated that diverse FMs resulted in variations in average root length (ARL) and germination index (GI), whereas all FMs increased the germination percentage to over 80% after five weeks of fermentation. All FMs steadily reduced the levels of NO_3-N and total phosphorus. Furthermore, total nitrogen, NH_4-N, NO_3-N, and total phosphorus were significantly negatively correlated with the germination percentage, ARL, and GI, indicating that the fermented cow wastewater should be appropriately diluted or combined with other fertilizers. These results provide foundational data for assessing the impact of different FMs on cow liquid manure, which helps to select the optimal composting method according to crop-specific nutritional needs in the future.

Keywords Cow wastewater, Liquid fermentation, Fertilizer, Composting

The consumption of dietary animal protein has rapidly increased due to urbanization and rising disposable income in China. This trend is also observed globally, as socioeconomic development often leads to a shift from plant-based to animal-based food[1,2]. As a result, the global livestock and poultry industry has expanded, leading to the production of large amounts of excreta[1–3]. Wang et al.[4] estimated that the total weight of manure produced in China in 2017 is 1.99×10^{12} kg, which is close to the 1.90×10^{12} kg reported by the Ministry of Agriculture and Rural Affairs of the PRC in 2015. Although these wastes can serve as valuable materials for anaerobic fermentation to produce biogas or fertilizers[3,4]they can also pose serious environmental risks if not properly managed. Discharging them into the environment without appropriate treatment can result in soil and water pollution[3]. Therefore, timely and effective treatment is crucial to enable the reuse of organic matter in these wastes and prevent environmental contamination[5].

Livestock manure is often mixed with large volumes of urine and wash water, resulting in a highly diluted slurry. Therefore, solid-liquid separation is typically carried out before further processing of the manure excreta[6–8]. This makes the treatment of the liquid portion more practical and affordable[6]. Numerous studies have been conducted on the environmentally sound treatment and fertility of the solid and liquid fractions after separation[1,9–12]. For instance, constructed wetlands have been used to remove chemical and biological pollutants from swine wastewater, with the goal of reclaiming water for reuse[13]. Additionally, a self-sustaining synergetic microalgae-bacteria symbiosis system has been developed to achieve environmental sustainability[12]. The application of beetroot (*Beta vulgaris*) at a rate of 80 t/ha has also been shown to increase plant growth, marketable yield, and quality[14]. Furthermore, cow dung microorganisms have been found to naturally increase soil fertility through phosphate solubilization, and can also produce novel chemicals[15]. However, the direct use of raw livestock manure as fertilizer may pose a threat to crops due to the presence of insect eggs and

[1]South China Institute of Environmental Sciences, Ministry of Ecology and Environment, Guangzhou 510655, China. [2]Guangdong Engineering and Technology Research Center for System Control of Livestock and Poultry Breeding Pollution, Guangzhou 510655, China. ✉email: dongzeng_scies@163.com; suwenxing@scies.org; hedechun@scies.org

pathogenic bacteria. To mitigate these risks, livestock manure is typically fermented before being used as a crop fertilizer[4,16,17].

Fermenting the liquid fraction of livestock manure is a sustainable strategy for crop fertilization as it eliminates pathogens while retaining important nutrients such as nitrogen and phosphorus. Currently, various fermentation methods (FMs) have been studied[18]. One common practice in some European regions with intensive farming is anaerobic digestion, which produces a nutrient-rich digestate that can improve soil structure when applied in agriculture. This method is also efficient in reducing greenhouse gas emissions[18,19]. Additionally, membrane filtration is a technical solution that can effectively redistribute unbalanced nutrient concentrations in the liquid fraction[20–22]. However, due to economic and technical constraints, these technologies are not been widely used for treating the liquid fraction of livestock manure[23]. In China, traditional FMs are still the primary methods for managing the liquid fraction of livestock manure. However, their impact on fertilizing capacity has not been thoroughly evaluated. Although the germination percentage is an important indicator for assessing fertilizer availability[24–26]no prior studies have evaluated the fertility effect of fermented cow manure liquid fraction based on plant germination metrics. The purpose of this study is to assess the effects of different FMs on the potential of cow wastewater for soil fertilization, clarify the fertilizing characteristics of the fermented products, and provide reference for subsequent agricultural production and use. We compared the changes in pH, conductivity, total nitrogen (TN), nitrate, ammonia, water-soluble organic carbon (WSOC), total phosphorus (TP), and chemical oxygen demand (COD) of seven FMs, i.e., open (O), open with microbial agent (OMA), open with mixing (OM), shading ball (SB), G-membrane sealing (GS), H-membrane sealing (HS), and H-membrane sealing with microbial agent (HSMA), for 13 weeks. We also evaluated the potential of treated cow wastewater for fertilization by conducting germination experiments. These methods were selected because they are widely used in practice due to their low cost and operational simplicity, despite limited scientific evaluation of their effectiveness. This study provides fundamental data for assessing nutrient loss during fermentation of the liquid fraction of cow manure and supports the selection of optimal composting methods tailored to crop-specific nutritional needs.

Results

Changes in physicochemical parameters of liquid fraction of cow manure during fermentation

All seven FMs resulted in an increase in the pH of the liquid fraction of cow manure. However, the pH of the HS and HSMA methods stabilized earlier (by the fifth week after fermentation) (Fig. 1A). Conductivity exhibited clear variation among the methods. The HS method showed a steady increase over the first six weeks, followed by stabilization. In contrast, the HSMA method initially decreased during the first four weeks, returned to its

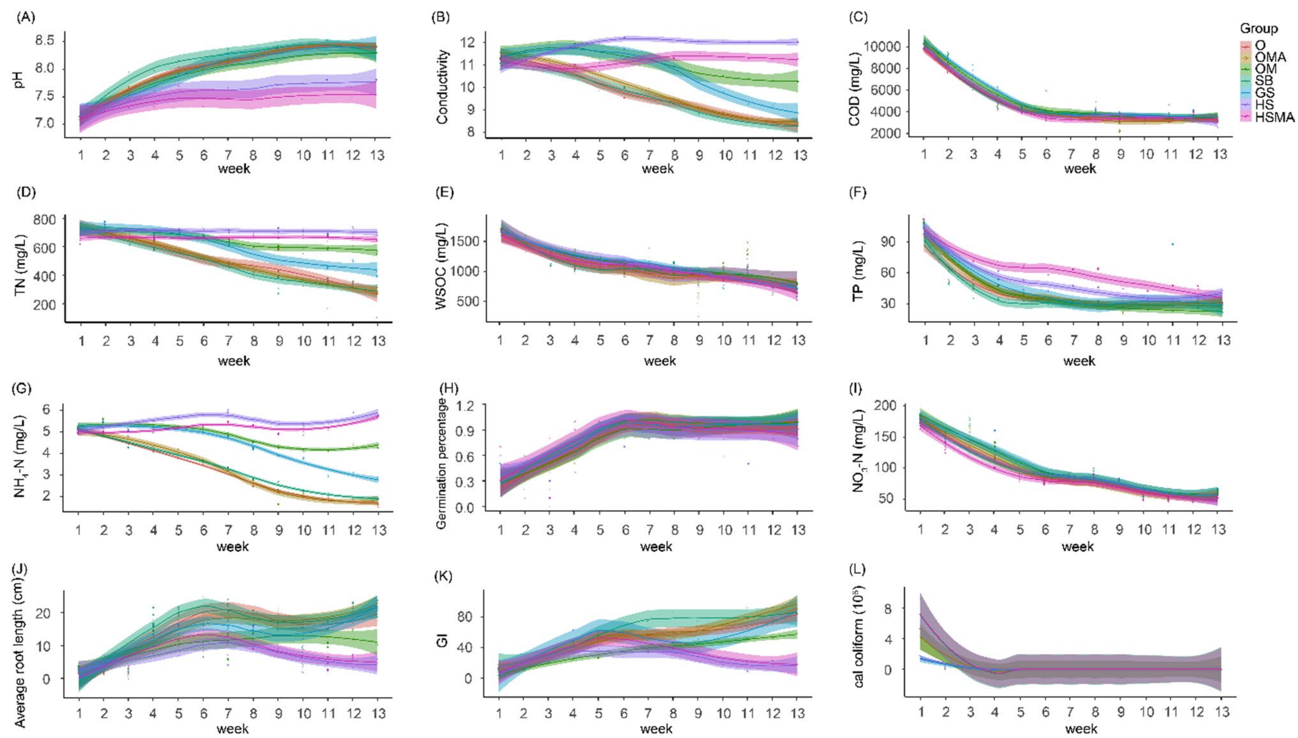

Fig. 1. Changes in physicochemical parameters during fermentation process. (**A**) pH; (**B**) conductivity; (**C**) chemical oxygen demand (COD); (**D**), total nitrogen (TN); (**E**) water-soluble organic carbon (WSOC); (**F**) total phosphorus (TP); (**G**) ammonia nitrogen; (**H**), germination percentage; (**I**) nitrate nitrogen; (**J**) average root length; (**K**) germination index (GI); (**L**) fecal coliform. Shaded areas are 95% confidence intervals. (**O**) Open; OMA, open with microbial agent; OM, open with mixing; SB, shading ball; GS, G-membrane sealing; HS, H-membrane sealing; HSMA, H-membrane sealing with microbial agent.

baseline level by week eight, and then stabilized. Conductivity under the OM and GS methods slightly increased during the first four weeks and subsequently declined, ending at a level lower than the baseline by week 13. The O, OMA, and SB methods showed a consistent decrease in conductivity throughout the fermentation (Fig. 1B). The COD levels across all methods declined rapidly in the first six weeks and then plateaued (Fig. 1C). N levels in the HS and HSMA methods remained relatively unchanged, while other methods showed a gradual decrease, with the greatest reduction observed in the O, OMA, and SB methods (Fig. 1D). The WSOC decreased steadily in all methods, with similar magnitudes of reduction (Fig. 1E). The TP concentrations in the HS and HSMA methods continued to decline, whereas TP in the remaining methods decreased rapidly in the first five weeks and then stabilized (Fig. 1F). The NH_4-N concentrations in the HS and HSMA methods remained almost unchanged, while the other methods decreased steadily (Fig. 1G). The NO_3-N levels decreased consistently across all treatments (Fig. 1I). Germination percentage increased rapidly in the first five weeks, exceeding 80% in all treatments, and then remained stable (Fig. 1H). Although some fluctuations were observed, the SB and GS methods showed the most stable germination percentages above 90% after week five (Fig. S1). The trends in average root length and GI mirrored each other. After fermentation, the HS and HSMA treatments showed the lowest root length and GI, followed by the OM, while the other methods yielded higher values (Fig. 1J, K). Fecal coliform levels dropped below 20 MPN/L in all treatments by the fifth week (Fig. 1L).

Weekly conversion rates of COD, WSOC, TN, NO_3-N, NH_4-N, and TP revealed significant differences across treatments and fermentation stages (Kruskal-Wallis rank sum test with Dunn post-hoc test, $P < 0.05$; Fig. S2). For instance, in week 1, the HSMA treatment caused the fastest reductions in COD, WSOC, NO_3-N, and NH_4-N, while TN increased. The SB treatment produced the fastest TP decline (> 50%). Similar weekly variations were observed across the 13 weeks, with no consistent pattern indicating that microbial addition uniformly influenced nutrient changes. Furthermore, mixing (as in OM) was expected to enhance NH_4-N oxidation by increasing DO levels, yet our results showed no significant reduction in NH_4-N in open liquid compost with mixing (Kruskal-Wallis rank sum test with Dunn post-hoc test, $P > 0.05$; Fig. 2 and S2). Although different FMs significantly influenced COD levels ($P < 0.05$), post-hoc comparisons revealed that significant differences only occurred during the first nine weeks, with no significant differences observed after week 10 (Dunn post-hoc test, $P > 0.05$; Table S1).

The GS method showed the highest TN concentrations in weeks 2 and 3, while the HS method maintained the highest TN from week 5 onward (Table S2). The NO_3-N concentrations in GS were significantly higher than those in HSMA between weeks 3 and 6, and higher than in HS at week 13 (Kruskal-Wallis rank sum test with Dunn post-hoc test, $P < 0.05$; Table S3). For NH_4-N, OM had significantly higher levels than OMA in week 1 and higher than O in week 2. HS had higher concentrations than SB in week 3 and higher than O and OMA in weeks 4–13 (Kruskal-Wallis rank sum test with Dunn post-hoc test, $P < 0.05$; Table S4). The TP concentrations varied over time. HSMA had the highest TP in most weeks, whereas SB had the lowest from weeks 2–8, OMA had the lowest in weeks 9–10, and OM had the lowest in weeks 11–13 (Table S5).

Effects of different FMs on the germination

Although FMs caused significant differences in germination percentage at week 13 (Kruskal-Wallis rank sum test, $P < 0.05$), Dunn's test did not show any significant differences throughout the process ($P > 0.05$; Table S6). However, SB consistently produced longer root lengths, while HS yielded the shortest (Table S7).

Correlations between physicochemical parameters and germination traits

Spearman correlation analysis indicated that the germination percentage, GI, and root length were positively correlated with pH ($r > 0.6$, $P < 0.05$), and negatively correlated with WSOC, NO_3-N, NH_4-N, TN, TP, COD, and conductivity ($r < -0.6$, $P < 0.05$). Regression analysis revealed that WSOC, NO_3-N, TP, TN, COD, and pH were significantly associated with germination percentage in multiple linear models, while NH_4-N and conductivity showed significant univariate associations ($P < 0.05$; Fig. 2). Similar trends were observed for GI and root length (Fig. S3). Fecal coliform counts showed positive correlations with WSOC, NO_3-N, NH_4-N, TN, TP, COD, and conductivity ($P < 0.05$), and negative correlations with pH ($P < 0.05$; Fig. 2).

Discussion

Cow manure is a cheap and readily available bioresource on our planet[15,27]. Currently, it is commonly used either for fermentation to produce renewable energy such as biogas[28,29]or as an agricultural organic fertilizer[19]. Organic manure derived from animal waste holds great promise, and has a clear advantage over chemical fertilizers in various aspects, including improving soil health and fertility, maintaining soil biodiversity, and enhancing crop productivity[30–33]. Gao et al.[34] reported that in a 38-year fertilization experiments in double-cropping rice systems, he combination of N, P, and K with cow manure significantly improved rice yield and soil fertility, although balanced fertilization was necessary. Cheng et al.[35] found cow manure compost increased the abundance of pollutant-degrading bacteria in black soil, but had inhibitory effects in saline-alkali soils. Moreover, while compost addition increased imazethapyr degradation by 12.6% in black soil, it decreased it by 7% in saline-alkali soils. Despite growing interest in manure as a fertilizer[5,36–38]the effect of different FMs on the fertilizing potential of the liquid fraction of cow manure has not been comprehensively evaluated. Most previous studies focus on reducing nutrient loads to avoid environmental pollution[8]with few addressing fertilizer effectiveness[39]. Our results indicated that different FMs significantly affected average root length and GI, although all methods raised germination percentage above 80% after six weeks. According to the Agricultural Industry Standard of China (NY/T 525–2021), a GI ≥ 70% is required. Only the O, OMA, SB, and GS methods met this standard after 13 weeks of fermentation. The lower GI in OM, HS, and HSMA methods may be due to their higher TN and NH_4-N concentrations (Fig. 1), which were negatively correlated with germination percentage, root length,

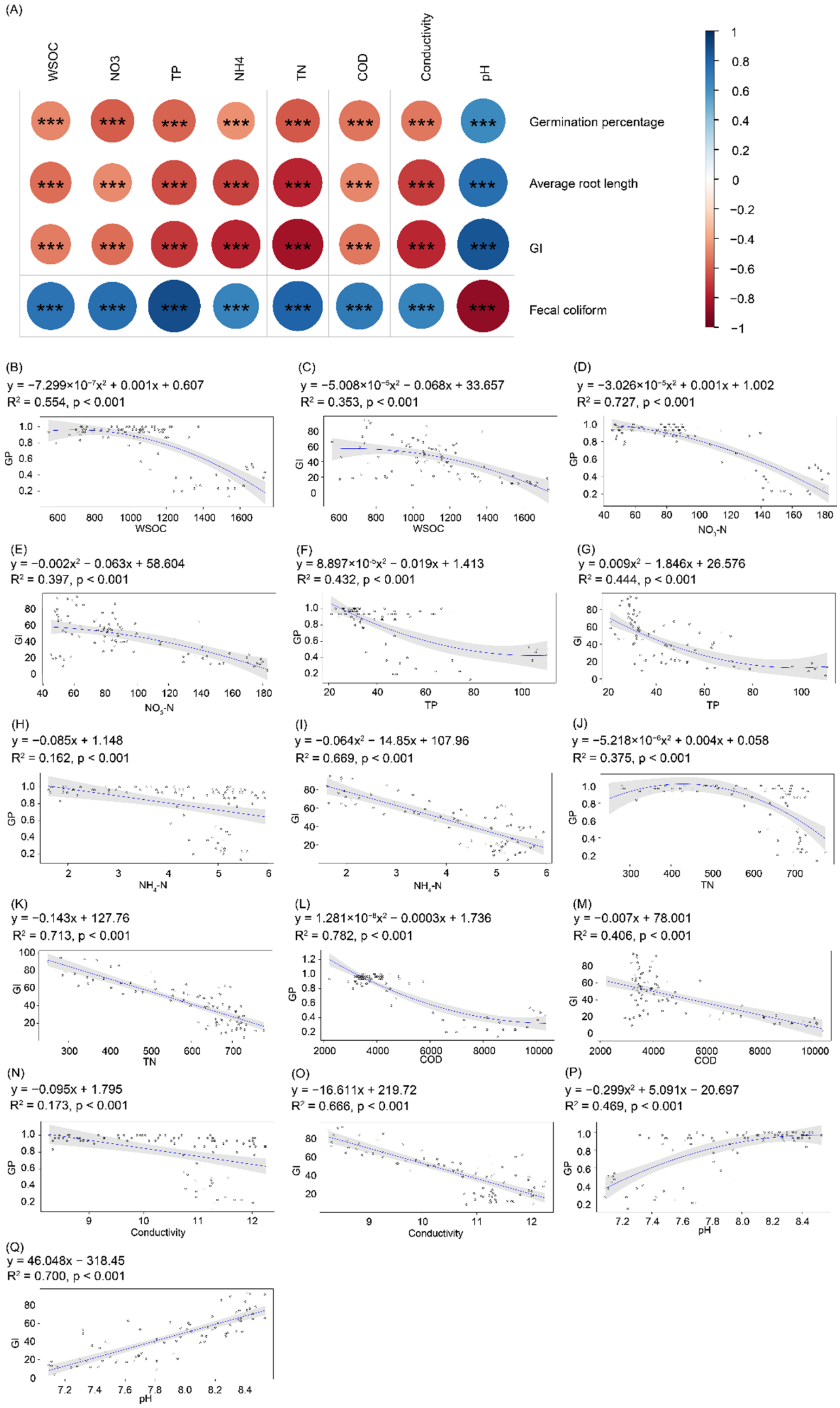

Fig. 2. Correlation between physicochemical parameters of liquid fraction of cow manure and germination. (**A**) Spearman correlation between physicochemical parameters of liquid fraction of cow manure and germination and fecal coliform; (**B**) correlation between water-soluble organic carbon (WSOC) and germination percentage (GP); (**C**) correlation between water-soluble organic carbon (WSOC) and germination index (GI); (**D**) correlation between NO_3-N and GP; (**E**) correlation between NO_3-N and GI; (**F**) correlation between TP and GP; (**G**) correlation between TP and GI; (**H**) correlation between NH_4-N and GP; (**I**) correlation between NH_4-N and GI; (**J**) correlation between TN and GP; (**K**) correlation between TN and GI; (**L**) correlation between COD and GP; (**M**) correlation between COD and GI; (**N**) correlation between conductivity and GP; (**O**) correlation between conductivity and GI; (**P**) correlation between pH and GP; (**Q**) correlation between pH and GI. The gray areas indicate the 95% confidence interval. *** $p < 0.001$.

and GI (Fig. 2A). Therefore, diluting these fermented liquids or combining them with other fertilizers may help achieve acceptable GI values[40].

A previous study reported that the fermentative process of mixed livestock manure reduced the pH, electric conductivity, TN, NH_4-N, and TP, while increasing NO_3-N[41]. In contrast, our results showed increased pH and decreased steadily in NO_3-N, COD, WSOC, and TP during fermentation. This discrepancy likely arises from differences in FMs and conditions. Closed fermentation systems (HS and HSMA) retained high TN and NH_4-N, whereas open systems (O, OMA, and SB) more effectively reduced these nutrients - except for OM. The H-membrane may restrict oxygen entry, limiting aerobic denitrification and reducing nitrogen volatilization. In the OM method, mixing likely released organic nitrogen into suspension but did not allow sufficient oxidation, resulting in high TN and NH_4-N concentrations. Additionally, the addition of microbial agents had no substantial impact on fermentation outcomes. Nitrogen and phosphorus are crucial for fertilizer value[42–44]. Compared to chemical fertilizers, organic fertilizers release nitrogen more slowly, leading to higher nitrogen use efficiency, crop yield, and grain quality[45,46]. Previous studies showed that cattle and sheep manure composts had higher pH, NO_3-N, total carbon, and C/N ratios, while pig and poultry composts had higher EC, NH_4-N, TN, and TP[47]. Our results showed that all methods, except HS and HSMA, significantly reduced TN and NH_4-N. In all treatments, NO_3-N and TP declined steadily. Moreover, NH_4-N levels in open fermentation were consistently lower than in closed systems during mid and late fermentation, indicating that aerobic conditions promoted ammonia oxidation. However, mixing had no substantial effect on NH_4-N levels. Importantly, TN, NH_4-N, NO_3-N, and TP were negatively correlated with germination percentage, root length, and GI, supporting the recommendation to dilute fermented liquid manure or blend it with other fertilizers to reduce phytotoxicity.

Electrical conductivity (EC) reflects soluble salt content, which may negatively impact plant growth if too high[40]. Patel and Lakdawala[48] noted that EC > 2.5 dS/m is toxic to most crops. All fermented liquids in our study exceeded this threshold, indicating that dilution or blending is necessary before application.

According to Patel and Lakdawala[48]optimal pH for liquid fertilizers lies between 6.5 and 7.8. Only HS and HSMA methods produced liquid with pH values within this range; the others exceeded 7.8. Although higher pH was positively correlated with germination percentage, further research is required to understand its effect on different crop species.

The fertilizer value of fermented manure depends on both crop species and application rate[49]. Valentinuzzi et al.[49] reported that the liquid digestate releases nutrients faster than solid manure pellets. Our study evaluated seven FMs using cucumber seeds under laboratory conditions. Future work should assess the effects on other plant species and in field conditions.

Conclusions

Different FMs of the liquid fraction of cow manure caused varying effects on average root length, and GI. However, all FMs increased the germination percentage to over 80% after six weeks of fermentation. The HS and HSMA methods maintained TN concentration and conductivity throughout fermentation and kept the pH within an appropriate range, while the other FMs steadily reduced the TN and NH_4-N content in the liquid fraction. All FMs steadily reduced NO_3-N and TP. The TN, NH_4-N, NO_3-N, and TP were negatively correlated with germination percentage, average root length, and GI, indicating that the fermented liquid fraction should be appropriately diluted or used in combination with other fertilizers to mitigate the adverse effects of nitrogen and phosphorus on seed germination. These findings provide fundamental data for evaluating the impact of different FMs on nutrient loss in cow manure liquid fraction, which may assist in selecting the optimal composting methods based on crop-specific nutritional requirements. Moreover, this study is the first to assess the influence of various FMs on the humification of cow liquid manure and to propose seed GI as a potential indicator for evaluating humification. However, due to the limitations in experimental conditions, microbial composition and biological parameters such as biomass, enzyme activity, and substrate conversion rates were not assessed. Since the microbial community plays a key role in fermentation, future studies should include these biological indicators to clarify the mechanisms underlying the physicochemical changes observed across different FMs.

Materials and methods

Experimental site conditions and pretreatment of cow excreta

The liquid fraction of cow manure excreta was obtained from the Qinglong Base of Guangzhou Huamei Milk Co., Ltd. (113.391 E, 23.689 N; Guangzhou, Guangdong, China). The base had a stock of approximately 3,500 dairy cows, with a total of 8 breeding pens, was collected using scrapers and transferred to a cesspool. Well-mixed raw manure was then pumped from the homogenization cesspool to a solid-liquid separator. After dewatering, the liquid portion entered a dedicated excrement pool for treatment and subsequent discharge (Fig. 3). The wastewater used in this experiment was the liquid fraction obtained from this excrement pool.

Experimental design and sample collection

The study was conducted at the outdoor experimental area of the College of Natural Resources and Environment, South China Agricultural University between October 8, 2021, and January 3, 2022. The average temperatures for each month during the experiment were 26, 22, 16, and 15 °C. The study consisted of seven common FMs, i.e., O, OMA, OM, SB, GS, HS, and HSMA, respectively (Table 1). Each method included three replicate treatment units, all of which injected 350 L liquid fraction for experiment into a 400 L white polyethylene drum with 75 cm of the bottom diameter, 93 cm of the top diameter, and 77 cm of the height (Longfei, Chnagzhou, Jiangsu, China; Fig. S4). G-membrane was a customized waterproof and ammonia permeable membrane. H-film was a HDPE anti-seepage film purchased from the market. The microbial agent used was Wangnongbao manure fermentation starter (Zhengzhou, Henan, China), with 360 mL of the mixed starter added to each treatment

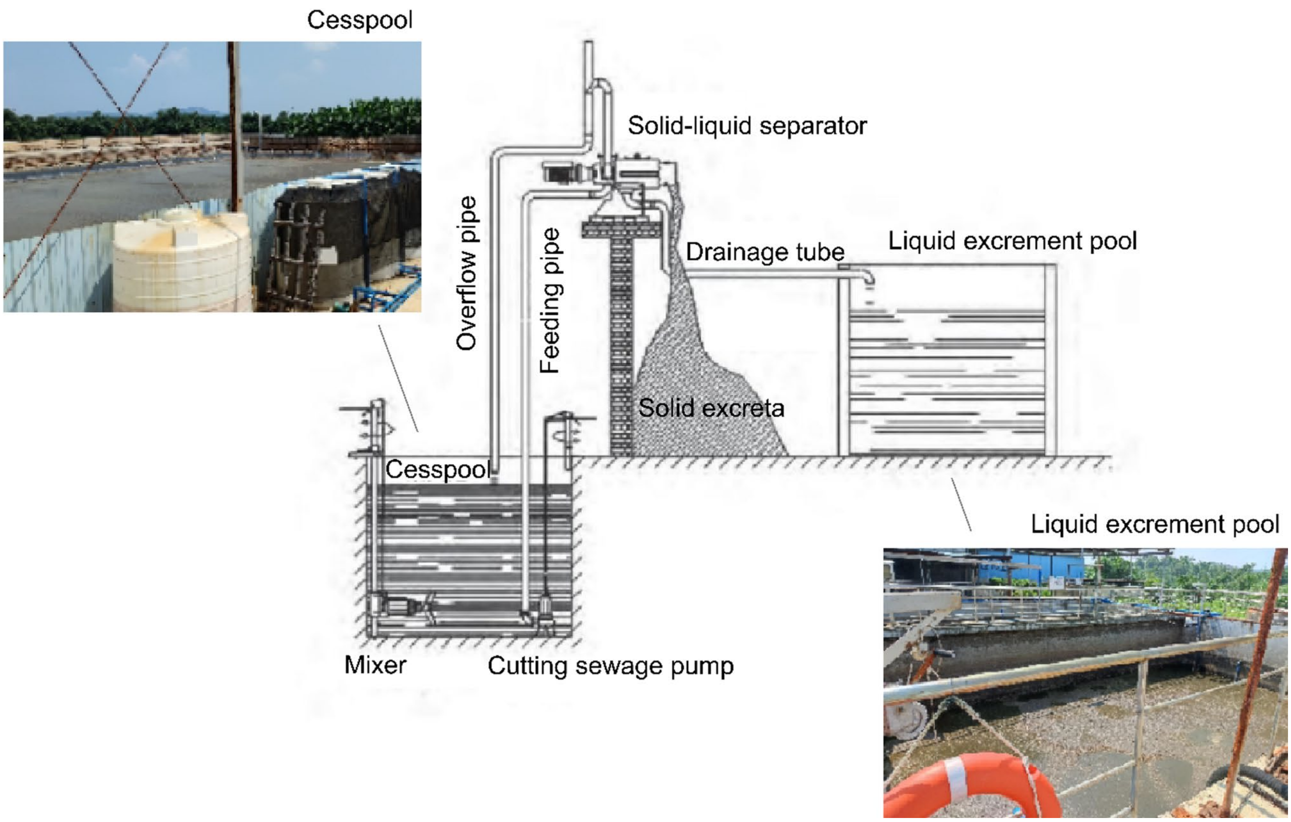

Fig. 3. Diagram of solid-liquid separation and on-farm photos.

Method	Overlay				Microbial agent	Mixing
	Open	H-membrane sealing	G-membrane sealing	Shading ball		
O	√					
OMA	√				√	
OM	√					√
SB				√		
GS			√			
HS		√				
HSMA		√			√	

Table 1. Seven fermentation methods used in this study. G-membrane is a customized waterproof and ammonia permeable membrane. H-film is a HDPE anti-seepage film purchased from the market. The microbial agent is the fermentation starter of Wangnongbao manure (Zhengzhou, Henan, China) purchased in the market. Each unit that was added microbial agent was added 360-mL of mixed fermentation starter. The fermentation liquid in the OM method was continuously mixed using an electric mixer with 50 rpm.

that included a microbial agent. In the OM method, the fermentation liquid was steadily stirred with an electric mixer at 50 rpm.

The study lasted for 13 weeks, at weekly sampling on Monday. Considering the evaporation of the units, the units were filled with water to approximately 350 L and stirred manually before each sampling, and then 2-L samples were collected by the multi-point sampling method and transported to the laboratory at low temperature in an insulated box with ice bags for subsequent analysis.

Determination of physicochemical parameters

Conductivity and pH were measured using the HQ40d portable digital multi-parameter analyzer (Hach, USA) during sampling. The sample and surrounding temperatures were detected using a wireless automatic temperature recorder (Huahanwei, Shenzhen, China). TN was determined using ultraviolet spectrophotometry with alkaline potassium persulfate digestion[50]. Nitrate and ammonium nitrogen were determined using flow injection analyzer spectrophotometry as previously described[51,52]. WSOC was determined using the purple red complex spectrophotometry as previously described[53]. Total phosphorus was detected using molybdenum blue method[54,55]. COD was detected using spectrophotometry with COD digester (DRB200, Hach, USA)[56]. GI was detected using Baimeng Jinyan No. 4 cucumber seeds (PiShu Agricultural Technology Co., Ltd, Xuzhou, Jiangsu, China) according to a previous description[57]. Briefly, the number of germinated seeds was counted from the first to seventh days during the seed germination testing. Seeds were germinated when the radicle had emerged by more than 2 mm. The GI was calculated by:

$$\mathrm{GI} = \sum \frac{\mathrm{G_t}}{\mathrm{D_t}}$$

where $\mathrm{G_t}$ is the number of germinated seeds on the *t*th day and $\mathrm{D_t}$ is the number of corresponding germination days. The average of three replicates was used for the data analysis.

Weekly conversion rates (CR) of COD, WSOC, TN, NO_3-N, NH_4-N, and TP were calculated according to the formula:

$$CR_i = \frac{C_{i+1} - C_i}{C_i}$$

where CR_i is the *i*th conversion rate. C_i and C_{i+1} is the concentrations of physicochemical parameter in the *i* and *i + 1* weeks, respectively.

Data analysis

Data were enunciated as the mean ± standard deviation. Kruskal-Wallis rank sum test with Dunn post-hoc test was conducted using R FSA version 0.9.6 package (https://cran.r-project.org/web/packages/FSA/index.html) to test the differences in data between FMs. Spearman analyses were conducted using R psych version 2.5.3 (https://cran.r-project.org/web/packages/psych/index.html), reshape2 version 1.4.4 (https://cran.r-project.org/web/packages/reshape2/index.html), and corrplot version 0.95 (https://cran.r-project.org/web/packages/corrplot/index.html) packages. Linear correlation analysis was conducted using R basicTrendline (https://github.com/PhDMeiwp/basicTrendline) package.

Data availability

All relevant data are available from the authors upon request and the corresponding author will be responsible for replying to the request.

Received: 30 November 2024; Accepted: 28 August 2025
Published online: 21 October 2025

References

1. Hu, Y., Cheng, H. & Tao, S. Environmental and human health challenges of industrial livestock and poultry farming in China and their mitigation. *Environ. Int.* **107**, 111–130. https://doi.org/10.1016/j.envint.2017.07.003 (2017).
2. Hu, H., Li, X., Wu, S. & Yang, C. Sustainable livestock wastewater treatment via phytoremediation: current status and future perspectives. *Bioresour Technol.* **315**, 123809. https://doi.org/10.1016/j.biortech.2020.123809 (2020).
3. Li, F., Cheng, S., Yu, H. & Yang, D. Waste from livestock and poultry breeding and its potential assessment of biogas energy in rural China. *J. Clean. Prod.* **126**, 451–460. https://doi.org/10.1016/j.jclepro.2016.02.104 (2016).
4. Wang, Y. et al. Biogas energy generated from livestock manure in china: current situation and future trends. *J. Environ. Manage.* **297**, 113324. https://doi.org/10.1016/j.jenvman.2021.113324 (2021).
5. Mengqi, Z., Shi, A., Ajmal, M., Ye, L. & Awais, M. Comprehensive review on agricultural waste utilization and high-temperature fermentation and composting. *Biomass Convers. Biorefinery*. **13**, 5445–5468 (2021).
6. Riaño, B. & García-González, M. C. On-farm treatment of swine manure based on solid–liquid separation and biological nitrification–denitrification of the liquid fraction. *J. Environ. Manage.* **132**, 87–93. https://doi.org/10.1016/j.jenvman.2013.10.014 (2014).
7. Cocolo, G., Hjorth, M., Zarebska, A. & Provolo, G. Effect of acidification on solid–liquid separation of pig slurry. *Biosyst Eng.* **143**, 20–27 (2016).
8. Wang, M., Kong, W., Yan, S., Ai, P. & Zhang, Y. Effect of solid-liquid separation on removal of total solid and pollutants from pig manure wastewater before anaerobic digestion. *Trans. Chin. Soc. Agric. Eng.* **34**, 235–240. https://doi.org/10.11975/j.issn.1002-6819.2018.17.031 (2018).
9. Dennehy, C. et al. Greenhouse gas emissions from different pig manure management techniques: a critical analysis. *Front. Environ. Sci. Eng.* **11** (11). https://doi.org/10.1007/s11783-017-0942-6 (2017).
10. Adekiya, A. O. et al. Different organic manure sources and NPK fertilizer on soil chemical properties, growth, yield and quality of Okra. *Sci. Rep.* **10** https://doi.org/10.1038/s41598-020-73291-x (2020).
11. Ribeiro, J. V. S., Semensato, L. R. & Vendruscolo, E. P. Increasing doses of cattle manure for organic Chili pepper production. *Rev. Agric. Neotrop.* **7**, 109–112 (2020).
12. Qu, W., Zhang, C., Chen, X. & Ho, S. H. New concept in swine wastewater treatment: development of a self-sustaining synergetic microalgae-bacteria symbiosis (ABS) system to achieve environmental sustainability. *J. Hazard. Mater.* **418**, 126264. https://doi.org/10.1016/j.jhazmat.2021.126264 (2021).
13. Bôto, M. L., Dias, S. M., Crespo, R. D., Mucha, A. P. & Almeida, C. M. R. Removing chemical and biological pollutants from swine wastewater through constructed wetlands aiming reclaimed water reuse. *J. Environ. Manage.* **326**, 116642. https://doi.org/10.1016/j.jenvman.2022.116642 (2023).
14. Dlamini, V. C. et al. Effects of cattle manure on the growth, yield, quality and shelf life of beetroot (*Beta vulgaris L. cv.* Detroit Dark Red). *J. Exp. Agric. Int.* **42**, 93–104. https://doi.org/10.9734/jeai/2020/v42i130455 (2020).
15. Gupta, K. K., Aneja, K. R. & Rana, D. Current status of cow Dung as a bioresource for sustainable development. *Bioresour Bioprocess.* **3**, 28. https://doi.org/10.1186/s40643-016-0105-9 (2016).
16. Shi, F. et al. Static composting of cow manure and corn stalk covered with a membrane in cold regions. *Front. Bioeng. Biotechnol.* **10**, 969137. https://doi.org/10.3389/fbioe.2022.969137 (2022).

17. Song, Y. et al. Co-composting of cattle manure and wheat straw covered with a semipermeable membrane: organic matter humification and bacterial community succession. *Environ. Sci. Pollut Res.* **30**, 32776–32789. https://doi.org/10.1007/s11356-022-24544-x (2022).
18. Camilleri-Rumbau, M. S. et al. Treatment of manure and digestate liquid fractions using membranes: opportunities and challenges. *Int. J. Environ. Res. Public. Health.* **18**, 3107. https://doi.org/10.3390/ijerph18063107 (2021).
19. Song, Y. et al. Microbiological and technological insights on anaerobic digestion of animal manure: A review. *Fermentation* **9**, 436. https://doi.org/10.3390/fermentation9050436 (2023).
20. Camilleri-Rumbau, M. S. et al. Ultrafiltration of separated digestate by tubular membranes: influence of feed pretreatment on hydraulic performance and heavy metal removal. *J. Environ. Manag.* **250**, 109404. https://doi.org/10.1016/j.jenvman.2019.109404 (2019).
21. Camilleri-Rumbau, M. S. et al. Application of aquaporin-based forward osmosis membranes for processing of digestate liquid fractions. *Chem. Eng.* **371**, 583–592. https://doi.org/10.1016/j.cej.2019.02.029 (2019).
22. Shi, L. et al. Nutrient recovery from pig manure digestate using electrodialysis reversal: membrane fouling and feasibility of long-term operation. *J. Membr. Sci.* **573**, 560–569. https://doi.org/10.1016/j.memsci.2018.12.037 (2019).
23. Zhang, Z. et al. Membrane processes for resource recovery from anaerobically digested livestock manure effluent: opportunities and challenges. *Curr. Pollut Rep.* **6**, 123–136. https://doi.org/10.1007/s40726-020-00143-7 (2020).
24. Hafez, M., Popov, A. I. & Rashad, M. Integrated use of bio-organic fertilizers for enhancing soil fertility-plant nutrition, germination status and initial growth of corn (Zea Mays L). *Environ. Tech. Innov.* **21**, 101329. https://doi.org/10.1016/j.eti.2020.101329 (2021).
25. Yang, Y. et al. Selection of sensitive seeds for evaluation of compost maturity with the seed germination index. *Waste Manag.* **136**, 238–243. https://doi.org/10.1016/j.wasman.2021.09.037 (2021).
26. Kaewtaphan, P., Maniin, P., Nilkong, P., Aninbon, C. & Teamkao, P. Effect of organic fertilizer rates on grain yield and seed qualities of rice. **20**, 1067–1074 (2024).
27. Yadav, A., Gupta, R. & Garg, V. Organic manure production from cow dung and biogas plant slurry by vermicomposting under field conditions. *Int. J. Recycl. Org. Waste Agric.* **2**, 21. https://doi.org/10.1186/2251-7715-2-21 (2013).
28. Liu, M. et al. Improvement of cow manure anaerobic digestion performance by three different crop straw biochars. *Environ. Tech. Innov.* **31**, 103233. https://doi.org/10.1016/j.eti.2023.103233 (2023).
29. Wang, Y. et al. Biogas energy generated from livestock manure in china: current situation and future trends. *J. Environ. Manag.* **297**, 113324. https://doi.org/10.1016/j.jenvman.2021.113324 (2021).
30. Yue, X., Zhang, J., Shi, A., Yao, S. & Zhang, B. Manure substitution of mineral fertilizers increased functional stability through changing structure and physiology of microbial communities. *Eur. J. Soil. Biol.* **77**, 34–43. https://doi.org/10.1016/j.ejsobi.2016.10.002 (2016).
31. Guo, Z., Zhang, Z., Zhou, H., Wang, D. & Peng, X. The effect of 34-year continuous fertilization on the SOC physical fractions and its chemical composition in a vertisol. *Sci. Rep.* **9**, 2505. https://doi.org/10.1038/s41598-019-38952-6 (2019).
32. Iqbal, A. et al. Organic manure coupled with inorganic fertilizer: an approach for the sustainable production of rice by improving soil properties and nitrogen use efficiency. *Agronomy* **9**, 651. https://doi.org/10.3390/agronomy9100651 (2019).
33. Ali, I. et al. Biochar addition coupled with nitrogen fertilization impacts on soil quality, crop productivity, and nitrogen uptake under double-cropping system. *Food Energy Secur.* **9** fes3.208 (2020).
34. Gao, P. et al. Improvement of soil fertility and rice yield after long-term application of cow manure combined with inorganic fertilizers. *J. Integr. Agric.* **22**, 2221–2232. https://doi.org/10.1016/j.jia.2023.02.037 (2023).
35. Cheng, L. et al. The various effect of cow manure compost on the degradation of Imazethapyr in different soil types. *Chemosphere* **337**, 139325. https://doi.org/10.1016/j.chemosphere.2023.139325 (2023).
36. Kamilaris, A., Engelbrecht, A., Pitsillides, A. & Prenafeta-Boldú, F. X. Transfer of manure as fertilizer from livestock farms to crop fields: the case of Catalonia. *Comput. Electron. Agric.* **175**, 105550. https://doi.org/10.1016/j.compag.2020.105550 (2020).
37. Iqbal, A. et al. Combined application of manure and chemical fertilizers alters soil environmental variables and improves soil fungal community composition and rice grain yield. *Front. Microbiol.* **13**, 856355. https://doi.org/10.3389/fmicb.2022.856355 (2022).
38. Zhang, Q. et al. Comprehensive assessment of the utilization of manure in china's croplands based on National farmer survey data. *Sci. Data*. **10**, 223. https://doi.org/10.1038/s41597-023-02154-7 (2023).
39. Sigurnjak, I. et al. Fertilizer performance of liquid fraction of digestate as synthetic nitrogen substitute in silage maize cultivation for three consecutive years. *Sci. Total Environ.* **599–600**, 1885–1894. https://doi.org/10.1016/j.scitotenv.2017.05.120 (2017).
40. Haji, K., Zekeria, Y. & Misrak, K. Production of bioorganic liquid fertilizer from cow manure and banana peels. *Adv. Res. J. Biotechnol.* **4**, 98–102 (2020).
41. Kim, S. R., Lee, J., Lee, M. G., Sung, H. G. & Hwang, S. G. Analysis of microbial communities in solid and liquid pig manure during the fertilization process. *Sci. Rep.* **14**, 72. https://doi.org/10.1038/s41598-023-50649-5 (2024).
42. Hua, W. et al. Manure application increased crop yields by promoting nitrogen use efficiency in the soils of 40-year soybean-maize rotation. *Sci. Rep.* **10** (14882). https://doi.org/10.1038/s41598-020-71932-9 (2020).
43. Lu, X. et al. Long-term application of fertilizer and manures affect P fractions in Mollisol. *Sci. Rep.* **10** https://doi.org/10.1038/s41598-020-71448-2 (2020).
44. Reuland, G., Sigurnjak, I., Dekker, H., Michels, E. & Meers, E. The potential of digestate and the liquid fraction of digestate as chemical fertiliser substitutes under the RENURE criteria. *Agronomy* **11**, 1374. https://doi.org/10.3390/agronomy11071374 (2021).
45. Kumar, K. A., Swain, D. K. & Bhadoria, P. B. S. Split application of organic nutrient improved productivity, nutritional quality and economics of rice-chickpea cropping system in lateritic soil. *Field Crop Res.* **223**, 125–136. https://doi.org/10.1016/j.fcr.2018.04.007 (2018).
46. Zhang, M. et al. Increasing yield and N use efficiency with organic fertilizer in Chinese intensive rice cropping systems. *Field Crop Res.* **227**, 102–109. https://doi.org/10.1016/j.fcr.2018.08.010 (2018).
47. Wan, J. et al. Livestock manure type affects microbial community composition and assembly during composting. *Front. Microbiol.* **12**, 621126. https://doi.org/10.3389/fmicb.2021.621126 (2021).
48. Patel, D. H. & Lakdawala, M. M. Study of soil's nature by pH and soluble salts through EC of Kalol-Godhra taluka territory. *Der Chem. Sin.* **5**, 1–7 (2014).
49. Valentinuzzi, F. et al. The fertilizing potential of manure-based biogas fermentation residues: pelleted vs. liquid digestate. *Heliyon* **6**, e03325. https://doi.org/10.1016/j.heliyon.2020.e03325 (2020).
50. Koistinen, J., Sjöblom, M. & Spilling, K. Total nitrogen determination by a spectrophotometric method. *Methods Mol. Biol.*. **1980**, 81–86. https://doi.org/10.1007/7651_2019_206 (2019).
51. Liang, Y., Yan, C., Guo, Q., Xu, J. & Hu, H. Spectrophotometric determination of ammonia nitrogen in water by flow injection analysis based on NH_3- o-phthalaldehyde -Na_2SO_3 reaction. *Anal. Chem. Res.* **10**, 1–8. https://doi.org/10.1016/j.ancr.2016.10.001 (2016).
52. Hu, J. et al. A method of the determination nitrate nitrogen (NO_3^--N) in high-salinity water samples. *Chem. Pap.* **76**, 1739–1744. https://doi.org/10.1007/s11696-021-02007-w (2022).
53. Zhou, Y. et al. Measurement report: molecular composition, optical properties, and radiative effects of water-soluble organic carbon in snowpack samples from Northern xinjiang, China. *Atmos. Chem. Phys.* **21**, 8531–8555. https://doi.org/10.5194/acp-21-8531-2021 (2021).

54. Ganesh, S., Khan, F., Ahmed, M. K., Velavendan, P. & Pandey, N. K. Kamachi mudali, U. Spectrophotometric determination of trace amounts of phosphate in water and soil. *Water Sci. Technol.* **66**, 2653–2658. https://doi.org/10.2166/wst.2012.468 (2012).
55. Pradhan, S. & Pokhrel, M. R. Spectrophotometric determination of phosphate in sugarcane juice, fertilizer, detergent and water samples by molybdenum blue method. *Sci. World.* **11**, 58–62. https://doi.org/10.3126/sw.v11i11.9139 (2013).
56. Li, J. et al. A spectrophotometric method for determination of chemical oxygen demand using home-made reagents. *Desalination* **239**, 139–145. https://doi.org/10.1016/j.desal.2008.03.014 (2009).
57. Tan, M. et al. Genome-wide association analysis of seed germination percentage and germination index in *Brassica Napus* L. under salt and drought stresses. *Euphytica* **213**, 40. https://doi.org/10.1007/s10681-016-1832-x (2017).

Acknowledgements
The authors would like to thank Guangdong Meilikang Bio-Science Ltd. (Foshan, China) for assistance with data analysis.

Author contributions
D.Z., W.S., and D.H. conceived and designed the research. D.Z., X.M., Y.Z., and W.S. performed the research and acquired the data. D.Z., W.S., D.H., and W.L. analyzed and interpreted data. D.Z., W.S., and D.H. wrote the manuscript text. All authors reviewed the manuscript.

Funding
This study was supported by the Science and Technology Revitalization Mongolia Project (2020-Science and Technology Revitalization Mongolia - National Innovation Center – 14).

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-17921-2.

Correspondence and requests for materials should be addressed to D.Z., W.S. or D.H.