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ORIGINAL ARTICLE

Biogas slurry use as N fertilizer for two-season *Zizania aquatica* Turcz. in China

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Abstract The development of ecological circular agriculture has been highly encouraged by the Chinese government to recycle agricultural wastes, reduce mineral fertilizer input, and protect the environment. Biogas slurry, a byproduct of biogas engineering developed in rural areas of China, could be used as N fertilizer for crop growth. The field experiments were conducted in 2014 and 2015 to study the plant growth responses and environmental impacts of applying biogas slurry to two-season *Zizania aquatica* Turcz. growth. The potential factors that restrict the rational use of biogas slurry were also clarified. Mineral N fertilizer can be completely or partly substituted by N fertilizer from biogas slurry to satisfy *Z. aquatica* plant growth. It was not at the cost of sacrificing yield, dry matter accumulation, N accumulation and physiological N use efficiency in the above-ground parts. However, the growth inhibition occurs when the N quantity in biogas slurry was 2 or 2.7 times higher than that of mineral N fertilizer. Vitamin C in non-shell swollen culms (as edible part) of *Z. aquatica* significantly increased after biogas slurry application. Biogas slurry application substantially increased the N concentrations, i.e., total N, NH_4^+–N, and NO_3^-–N in floodwater and delayed the time to reach national discharge standards. However, biogas slurry application did not affect the N concentrations in percolating water compared with the treatment with mineral N fertilizer only. Applying biogass slurry did not generate potential pollution risks by trace elements (Cu, Zn, Pb, Cr, Cd, As, and Hg) in the non-shell swollen culm and soil, and did not increase the nitrate content in non-shell swollen culm. We found the NH_4^+–N concentration in biogas slurry can account for 77–93% of total N and reflects the N level in biogas slurry to a great degree. Semi-quantitative color-based colorimetric methods possessing simple and fast characteristics should be developed to determine the NH_4^+–N concentration with the purpose of promoting reasonable use of biogas slurry in area of crop cultivation. Otherwise, excessive use of biogas slurry can adversely affect crops and increase environmental risks.

Keywords Ecological circular agriculture · Biogas slurry · Aquatic vegetable · Plant growth · Environmental effect

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Introduction

The Chinese government has decided to popularize ecological circular agriculture throughout the country to recycle agricultural wastes, reduce mineral fertilizer input, and protect the environment. Agriculture in China has fully developed in recent years. The total grain yield in China exceeded 621 million tons in 2015 and has consecutively increased in the past 12 years (National Bureau of Statistics of the People's Republic of China 2015). China is also a major producer of fruits and vegetables worldwide, with outputs reaching 260 and 700 million tons in 2014, respectively (Ministry of Agriculture of the People's Republic of China 2014). Mineral fertilizers, especially nitrogen (N) fertilizer, have mainly contributed to this output. As reported, a threefold increase in N fertilizer application has contributed to about 70% increase in grain production in China since 1980, which has consumed huge amounts of oil resources but polluted the environment (Zhu and Chen 2002).

By contrast, anaerobic digestion has become increasingly popular worldwide to satisfy the growing concerns on energy supply and, more importantly, reduce organic wastes (Abraham et al. 2007; Arthurson 2009; Yu et al. 2010). China is a major developer of biogas engineering by anaerobic digestion. Approximately 600 million m^3 of effluent could have been generated in rural areas in 2010 (Lu et al. 2012). However, the increasing popularity of anaerobic digestion has created another challenge that how to deal with large quantities of biogas slurry generated during this process. An 800 m^3-volume biogas system needs to discharge 15 tons of biogas slurry daily. Therefore, this condition will become an environmental issue if improperly managed.

Studies showed that biogas slurry is a high quality nutrient material for crop growth and can be used as nitrogenous fertilizer because it contains large amounts of plant nutrients, such as P, K, and particularly N (Dahiya and Vasudevan 1986; Lu et al. 2012; Sheets et al. 2015; Tan et al. 2016). Biogas slurry probably also contains some micronutrients, auximones, B vitamins, and humic acid, which are beneficial to plant growth, can improve soil fertility, and can enhance farm production (Liu et al. 2009; Islam et al. 2010). The Chinese government has invested much to push biogas slurry use in crop cultivations for the reuse of agricultural waste and reduction of mineral fertilizer input. However, biogas slurry holds some chemical characteristics involving liquidity, high pH, high ammonium (NH_4^+) content, and potentially high metal content (Hou et al. 2007; Lu et al. 2012; Svoboda et al. 2013), which differ with mineral fertilizers such as urea. Thus, studies must be performed to elucidate the effects of biogas slurry application on crop growth, yield, and environment and human health before its extensive use.

Researchers have studied biogas slurry utilization for crops, such as wheat (Garg et al. 2005), barley (Terhoeven-Urselmans et al. 2009), rice (Hou et al. 2007; Lu et al. 2012), leek (Elfstrand et al. 2007), maize (Svoboda et al. 2013), peanut (Zheng et al. 2016), and Chinese cabbage (Zhu et al. 2009). Most studies focused on the effects of biogas slurry on crop yield, soil properties, and fertility. Some researchers only selected environmental effects as their study point. However, the relatively integrated effects that involve crop growth, yield, nutrient use efficiency, agricultural product quality, and safety, as well as the potential environmental risks of applying biogas slurry to crop cultivation, are rarely investigated. Additionally, previous studies focused on cereal crops or conventional vegetables but not on aquatic vegetables, which account for a large proportion of the total vegetable planting area in southern China and require large amounts of nutrients during their entire growth.

Zizania aquatica Turcz. of Oryzeae has been cultivated for more than 2000 years and has historically been used mainly as an aquatic vegetable in China and some southeastern Asia such as Russia, Japan and Korea (Zhai et al. 2001; Guo et al. 2007). The smut fungus *Ustilago esculenta* causes enlargement of infected culms of *Z. latifolia.* The induced swollen culms are edible as a vegetable (Terrell and Batra 1982). There are two main types of cultivars cultivated in China at present. One is single-season *Z. aquatica* that can be harvested once each year, in the fall; the other is two-season *Z. aquatica* which can be harvested twice each year, in the fall and once the summer thereafter. The two-season cultivar is preferred by the Chinese farmers (Guo et al. 2007). By now, in China, *Z. aquatica* has been widely cultivated from northern Beijing to southern Guangdong province and Taiwan, and from eastern Sichuan province to Shanghai. The largest cultivated area in China is found in the region surrounding Tai Lake, Jiangsu and Zhejiang provinces (Chen 2002). It is estimated that more than 20,000 ha are under cultivation with *Z. aquatica* in Zhejiang province alone and that 500,000 tons of fresh product are harvested each year (Yu et al. 2003).

Improving the development of ecological circular agriculture in China will be more beneficial if biogas slurry, a byproduct of biogas engineering, can be used as mineral fertilizer (particularly N fertilizer) for *Z. aquatica* cultivation. Some farmers have replaced a partial amount of mineral fertilizer with biogas slurry during *Z. aquatica* growth in many places in China. However, a study should be conducted with the aim of assessing the plant growth responses and environmental impacts of applying biogas slurry in *Z. aquatica* cultivation. In addition, the feasibility of applying biogas slurry with irrigation should be studied. Field experiments were conducted in 2014 and 2015 in Jiaxing City, which is located in the Taihu Lake region of China. The study area is densely populated and has high potential for both cultivating *Z. aquatica* and developing biogas engineering because of large number of pig farms. In the present study, biogas slurry was applied to the plants at different rates and the yield, dry matter, and N use efficiency of *Z. aquatica*, N concentrations in floodwater and leachate, quality of agricultural product, and heavy metal contents in agricultural product and soil were measured. The results of this study would provide useful information that can be used to guide farmers in implementing best management practices in *Z. aquatica* cultivation.

Materials and methods

Experimental design

Field experiments were conducted in Jiaxing City, Zhejiang Province, China in 2014 and 2015. Jiaxing is located in the Hangjiahu Plain (30°50′N, 120°43′E) and is within the catchment of Taihu Lake. This area has a typical subtropical monsoon climate with an average annual temperature of 15–16 °C and an annual average rainfall of 1194 mm. Soil at the experimental site was identified as gleyed paddy soil [clay loam; mesic Mollic Endoaquepts (USDA/NRCS 1999)]. Prior to start of the field experiments, soil samples for site characterization were collected from the top 20 cm of the soil profile. The pH of the soil was 6.40 (in 1:2.5, soil to water) and organic C content, total N (TN), alkali-hydrolyzable N, Olsen P, NH_4-OAC-extractable K in the soil were 56.6, 3.53 g kg^{-1}, 215, 5.89 and 119 mg kg^{-1}, respectively.

Seven treatments based on the input N loading from N-fertilizer (NF) and/or biogas slurry (NB) were used in the experiments. The treatments were: no NF or NB (0N); N supplied completely by NF applied at traditional rate as practiced by farmers (1NF); from the N input equivalent to that received at 1NF half is supplied with NF and the other half with NB (0.5NFB); and N supplied only with NB at 1, 1.5, 2, and 2.7 times of N input equivalent to 1NF treatment (1NB, 1.5NB, 2NB and 2.7NB respectively). 1NF treatment received 450 kg N ha^{-1}, 165 kg P_2O_5 ha^{-1} and 230 kg K_2O ha^{-1} in first season (2014) and 485 kg N ha^{-1}, 180 kg P_2O_5 ha^{-1} and 250 kg K_2O ha^{-1} in second season (2015). Urea, single superphosphate and potassium chloride were used as the sources of N, P and K, respectively. From 0.5NFB to 2.7NB treatments the rates of P and K fertilizers were adjusted considering the P and K inputs from the biogas slurry. The fertilizers were applied in three splits per season (for N—35, 30, and 35%; for P and K—20, 30, and 50%) that correspond to three growth stages: seedling stage, early tillering stage, and early pregnant stage, respectively. Accordingly, fertilizer application was done on July 15, September 10, and October 9 in 2014 as for the first season, and on April 10, May 10, and June 4 in 2015 as for the second season. All mineral fertilizers were evenly dispersed by hand and biogas slurry was irrigated by 15 cm-diameter flexible pipes to each plot.

Biogas slurry was collected from the Shuangqiao Black Pig Farm. Wastewater from the solid–liquid separation of pig farm waste (i.e., a mixture of pigs' dejecta and the wastewater from piggery cleaning) was anaerobically digested for 20–30 days or longer time under normal temperature digestion condition to produce biogas in a methane fermentation tank. The biogas slurry was then discarded and stored in a pond for experimental usage. Before irrigation, we collected two parallel samples of biogas slurry in plastic bottles after stirring biogas slurry body as evenly as possible. The chemical properties of biogas slurry for each irrigation treatment are shown in Table 1. The irrigation capacity of biogas slurry was defined as N rate supplied for each treatment divided by TN concentration in biogas slurry. The biogas slurry was transported by a collection vehicle with a storage tank to the test

Table 1 Chemical property of biogas slurry irrigated (mg L^{-1})

Season	Growth stage	TN	NH_4^+–N	NO_3^-–N	TP	TK	Cu	Zn	Pb	Cr	Cd	As	Hg
2014	Seedling	444	337	15.5	16.3	65.2	4.78	7.51	0.14	0.11	0.02	0.03	0.009
	Early tillering	798	694	20.3	19.1	76.4	9.55	5.23	0.15	0.09	0.03	0.05	0.006
	Early pregnant	1521	1141	26.5	26.2	115	6.63	10.5	0.13	0.06	0.02	0.07	0.011
	Standard deviation	549	403	5.51	5.10	26.1	2.40	2.64	0.01	0.03	0.01	0.02	0.003
2015	Seedling	395	307	12.3	10.3	33.6	11.1	9.42	0.17	0.07	0.04	0.04	0.008
	Early tillering	684	613	25.1	30.2	69.3	7.56	5.23	0.10	0.09	0.03	0.05	0.007
	Early pregnant	436	301	20.4	15.4	92.1	4.98	5.67	0.13	0.05	0.02	0.06	0.010
	Standard deviation	156	178	6.47	10.3	29.5	3.07	2.30	0.04	0.02	0.01	0.01	0.002

Data are presented as means of the parallel. Standard deviation in each growth season is for values of three growth stages

site for fertilization. A 15 cm-diameter flexible pipe was used to connect the storage tank and each plot. The treatments were applied in triplicates. Twenty-one plots each with an area of 84 m^2 (12 m × 7 m) were arranged in a randomized complete block design. The field levees of each plot were 35–40 cm high above the soil plane in the plot. The plastic film was buried in the ground at a vertical depth of 30 cm to avoid lateral seepage between two adjacent plots.

Two-season *Z. aquatica* variety Zhe911 was selected as the experimental material. It is widely cultivated in the Zhejiang and Jiangsu Provinces of China. The plants were transplanted on July 1, 2014. The distance between rows and hills was 70 cm × 80 cm. Floodwater level was maintained at a depth of 15–20 cm during the growth stage in each plot. However, floodwater level was reduced to 5–10 cm at the maximum tillering stage, which helped to inhibit excessive tillering that can't develop the field to a great degree. The swollen culms were harvested by hand from October 20 until November 10, 2014. Subsequently, the above-ground parts were removed when the weather was sufficiently cool to prevent crop regrowth. It was regrown in late March 2015 when the weather was sufficiently warm. It was harvested from June 16 to July 15, 2015.

Sampling and measurements

Eight representative hills of *Z. aquatica* plants in inner rows of each plot were selected to determine yield in 2014 and 2015 growing season. The fresh swollen culms were harvested and weighed for yield definitions. The above-ground parts (including leaf, stem and swollen culm) of four hills of plants chosen for yield in each plot were collected at harvest time. These above-ground parts were then oven-dried at 75 °C until a constant weight was reached for dry matter measurements. Subsequently, the samples were ground for N concentration measurements. The samples of fresh swollen culms removing shell (as edible part) were collected, some of which were oven-dried at 75 °C until a constant weight was reached, and some of which were used for quality analysis of agricultural products. The dried samples of non-shell swollen culms were ground for N concentration measurements. In 2015 season, some milled samples were sieved through a 0.149 mm mesh to use in the analyses of potentially toxic trace elements to confirm whether the edible part of *Z. aquatica* was polluted by heavy metal in biogas slurry after a relatively long irrigation period (in 2014 and 2015 growing seasons).

A composite soil sample mixed by five subsamples at depth of 0–20 cm was collected in each plot after harvest of *Z. aquatica* in 2015 with the same purpose as that mentioned above. Naturally air-dried and homogenized samples were ground with an agate mortar, passed through a 150 μm nylon sieve (100 mesh), and stored in closed polyethylene bags for analyses of potentially toxic trace elements.

The floodwater samples in plots were collected 1 day after each fertilization. Collection was repeated at 3 day intervals until similar NH_4^+–N contents were obtained among treatments. The water samples were filtered with general qualitative filter paper and subsequently frozen in −20 °C for TN, ammonium–

N (NH_4^+–N), and nitrate–N (NO_3^-–N) concentrations analyses.

PVC tubes with porous at one end were installed in each plot at a depth of 75 cm to collect percolating water since the groundwater depth is ~1 m in this region. The percolating water was pumped out several times with a hand pump before sample collection. Leachate samples were collected at 7 or 10 day intervals after fertilizer application during the growing season. The water samples were filtered with general qualitative filter paper and immediately stored in a freezer at −20 °C until the TN, NH_4^+–N, and NO_3^-–N concentrations analyses.

Chemical analysis of samples

Milled plant samples were digested with H_2SO_4–H_2O_2. Subsequently, the N concentration was determined by the Kjeldahl method. Vitamin C (Vc) in non-shell swollen culms was extracted with 2% oxalic acid and analyzed by the 2,6-dichlorophenolindophenol sodium salt titrimetric method (Liu et al. 2008). The total soluble sugar content was determined with a colorimetric method (Laurentin and Edwards 2003). Anthrone (0.15%) in ethanol was added to suitable aliquots of the extract or the standard sugar solution, mixed, and incubated for 15 min in a water bath at 90 °C. The absorbance was determined at 620 nm with a spectrophotometer (DR 5000 Spectrophotometer by HACH). The protein was analyzed based on a method in GB 5009.5-2010 (National Food Safety Protein in Foods). The milled samples of non-shell swollen culms were digested with $CuSO_4$–K_2SO_4–H_2SO_4. The N concentration was subsequently determined by the Kjeldahl method. The protein content was obtained by multiplying the N concentration by 6.25, which is a coefficient used to convert N content to protein. The nitrate (NO_3^-) content in non-shell swollen culms was determined according to the method as described by Miranda et al. (2001). The homogenate of the samples in hyperpure water was mixed with 5% salicylic acid in the sulfuric acid solution and incubated with 1.8% sodium hydroxide for 20 min at room temperature. The absorbance was read at 410 nm. The level of nitrate in all samples was calculated according to standard curves.

The concentrations of TN, NH_4^+–N, and NO_3^-–N in floodwater, percolating water, and biogas slurry were measured as the followings: The TN concentration was determined with a spectrophotometer at 220 and 275 nm (DR 5000 Spectrophotometer, HACH) after alkaline persulfate digestion; The NH_4^+–N content was measured by an indophenol blue colorimetric method; The NO_3^-–N concentration was analyzed with a spectrophotometer at 220 and 275 nm (DR 5000 Spectrophotometer, HACH). The total P content in biogas slurry was analyzed by the molybdenum blue method after persulfate digestion. The total K concentration in biogas slurry was determined by flame atomic absorption spectrophotometry (FP640; Shanghai Spectrum Instruments Co., Ltd, China). The pH of biogas slurry was determined by using a pH meter (FE 20; Mettler Toledo).

The potentially toxic trace elements (Cu, Zn, Pb, Cr, Cd, As, and Hg) in biogas slurry, soil, and non-shell swollen culms were measured by inductively coupled plasma mass spectrometry (Agilent Technologies, Inc., USA) after HNO_3 digestion (Lu et al. 2012; Duan et al. 2012).

Statistical analysis

Data were statistically analyzed with SPSS version 17.0. One-way ANOVA with the LSD post hoc test was performed to determine differences among different treatments. Significant differences at $P < 0.05$ are indicated by different letters. Figures were plotted with Sigma Plot version 10.0.

Results

Yield and dry matter accumulation of two-season *Z. aquatica*

The *Z. aquatica* yield was significantly reduced when no N fertilizer was supplied in both growing seasons. No significant differences were observed between 1NF versus the 0.5NFB, 1NB, 1.5NB, and 2NB treatments. However, the yield was significantly reduced by almost 10% in 2.7N compared with that in 1NF in 2014 (Fig. 1a). Further, the yield was reduced by 48.8% in 2.7NB and by 26.2% in 2NB compared with that in 1NF in 2015 (Fig. 1b).

The lack of N input significantly decreased the dry matter accumulation in above-ground parts of *Z. aquatica* by 24.0–40.3% in 2014 and 16.4–48.8% in

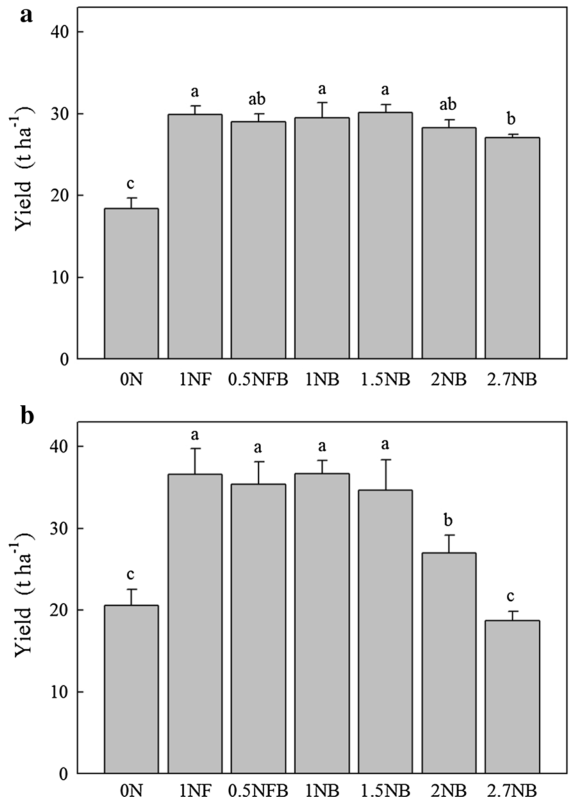


Fig. 1 Yield of *Z. aquatica* in the 2014 (**a**) and 2015 (**b**) growing seasons. *Error bars* represent the standard deviation; *different lowercase letters* indicate significant differences ($P < 0.05$) among treatments

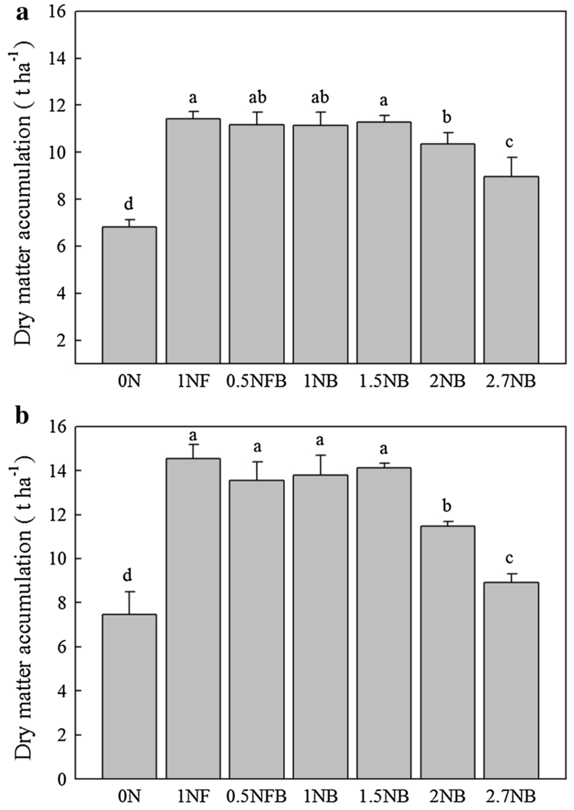


Fig. 2 Dry matter accumulation in above-ground parts of *Z. aquatica* plants in the 2014 (**a**) and 2015 (**b**) growing seasons. *Error bars* represent standard deviation; *different lowercase letters* indicate significant differences ($P < 0.05$) among treatments

2015. No obvious differences in dry matter development were observed between 1NF versus 0.5NFB, 1NB, and 1.5NB in both growing seasons. However, the dry matter was significantly reduced by 9.44 and 21.4% in 2NB and 2.7NB in 2014 and by 21.1 and 38.6% in 2015, respectively (Fig. 2a, b).

N accumulation and physiological N use efficiency (PNuE) of two-season *Z. aquatica*

The N accumulation in above-ground parts was significantly restrained by 16.6% for 2.7NB, whereas 0N was 103% lower than 1NF in 2014. However, a significant decrease was found in the 2NB and 2.7NB treatments in 2015 by 13.8 and 30.5%, respectively, except for 0N with a decrease of 59.2% compared with 1NF (Fig. 3a, b).

The PNuE of plants is defined as the biomass accumulation relative to the N accumulation (Shi et al. 2010). The absence of N application markedly increased the PNuEs of above-ground parts of *Z. aquatica* by 16.3–22.4% in 2014 and by 20.3–29.7% in 2015 compared with other treatments. PNuEs were not obviously affected in the 0.5NFB, 1NB, and 1.5NB treatments as compared with the 1NF treatment. However, the 2NB and 2.7NB treatments decreased the PNuE by 4.65 and 5.91% in 2014 and by 8.56 and 11.8% in 2015, respectively (Fig. 4a, b).

N concentration in floodwater

Immediately after the application of mineral N fertilizer or biogas slurry in both growing seasons, the TN and NH_4^+–N concentrations in floodwater (Fig. 5a–d) were higher than 30 and 25 mg L^{-1}, respectively, which are the critical levels set in the **National Integrated Wastewater Discharge**

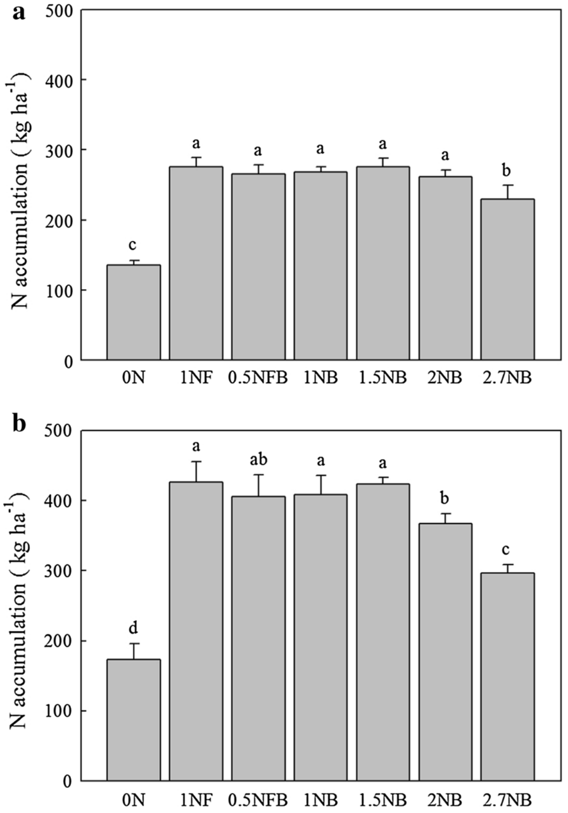


Fig. 3 N accumulation in above-ground parts of *Z. aquatica* plants in the 2014 (**a**) and 2015 (**b**) growing seasons. *Error bars* represent standard deviation; *different lowercase letters* indicate significant differences ($P < 0.05$) among treatments

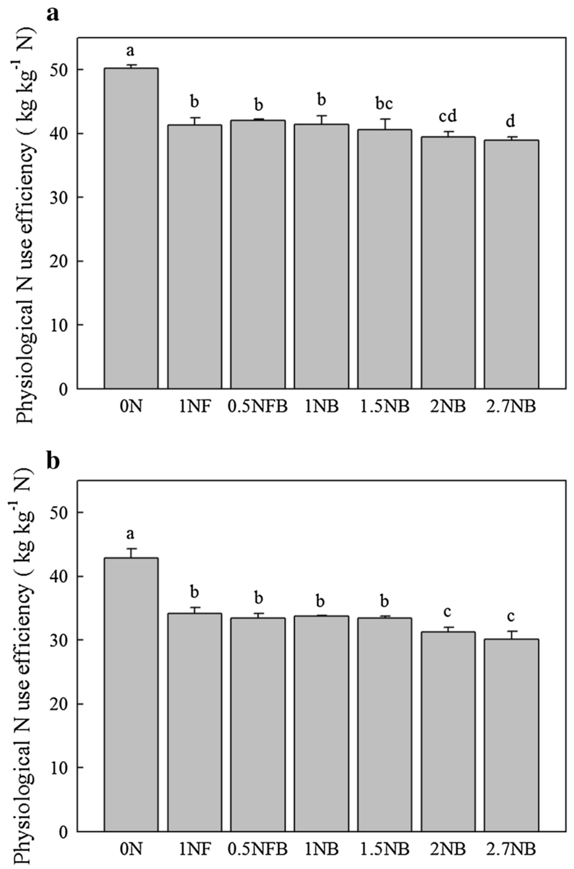


Fig. 4 Physiological N use efficiency in above-ground parts of *Z. aquatica* plants in the 2014 (**a**) and 2015 (**b**) growing seasons. Physiological N use efficiency in the above-ground parts of *Z. aquatica* plants: dry matter accumulation (kg ha^{-1}) divided by the N accumulation (kg ha^{-1}) in above-ground parts of *Z. aquatica* plants. *Error bars* represent standard deviation; *different lowercase letters* indicate significant differences ($P < 0.05$) among treatments

Standard of China (GB 8978-1996). However, the treatments involving biogas slurry application had obviously higher concentrations than the 1NF treatment in most cases, especially for 1NB, 1.5NB, 2NB and 2.7NB treatments. Moreover, TN and NH_4^+–N concentrations showed increased tendency with increasing of biogas slurry input. The TN (or NH_4^+–N) content decreased below critical level on the 7th, or 10th, or 13th day, which indicated that the high N concentrations in floodwater did not immediately reach critical levels. Although the application of more biogas slurry increased the NO_3^-–N content in floodwater, the maximum was only 10.6 mg L^{-1} in 2014 or 13.2 mg L^{-1} in 2015. No critical level of NO_3^-–N concentration is set in the National Integrated Wastewater Discharge Standard of China (GB 8978-1996). But, its maximum in floodwater was far below the national standards for discharge concentrations of TN and NH_4^+–N (Fig. 5e, f).

N concentration in percolation water

There were no obvious differences in N concentrations (TN, NH_4^+–N, and NO_3^-–N) in percolating water in most instances between treatments received biogas slurry and 1NF, which implied biogas slurry application showed the similar effects on the N contents in percolating water in comparison with mineral N fertilizer application. Interestingly, N applications by mineral N fertilizer or biogas slurry did not significantly increase the N content in the percolating water of this area (Fig. 6a–f).

As shown in Fig. 6a–f, the TN, NH_4^+–N, and NO_3^-–N concentrations were in the ranges of 1.16–4.59, 0.2–2.10, and 0.51–2.03 mg L^{-1}, respectively, in the 2014 and 2015 growing seasons for all treatments.

However, the NH_4^+–N contents exceeded 0.5 mg L^{-1} (a critical level according to the National Groundwater Quality Standard of China or GB/T 14848-1993) during most of the *Z. aquatica* growth stages. By contrast, the NO_3^-–N concentrations were much lower than 5.0 mg L^{-1} (a critical level according to the National Groundwater Quality Standard of China or GB/T 14848-1993). This critical level was not observed for the TN concentration in percolating water.

Agricultural commodity quality and heavy metal risk in non-shell swollen culms and soil

As shown in Table 2, the Vc content in non-shell swollen culms of *Z. aquatica* increased by 12.6–17.8% in 2014 and 13.6–17.0% in 2015 after biogas slurry treatment compared with 1NF. However, increasing the biogas slurry input did not significantly increase Vc content. Obviously, the increased protein content was observed in 2NB and 2.7NB, particularly 2.7NB, which was probably caused by increasing N application rate. No significant differences in the total sugar content were found among treatments. Moreover, the biogas slurry application did not increase the nitrate content in non-shell swollen culms of *Z. aquatica*.

There were no significant differences in Cu, Zn, Pb, Cr, Cd, As, and Hg concentrations in non-shell swollen culm or soil between 0N and the treatments supplied with N fertilizer. Moreover, biogas slurry application did not increase the heavy metal contents, regardless of the application rates. Heavy metal contents in non-shell swollen culm were below the critical values stipulated in the National Maximum Levels of Contaminates in Foods of China (GB2762-2005), the **National Maximum Levels of Cu in Foods of China (GB13106-91)**, and the **National Maximum Levels of Zn in Foods of China (GB15199-94)**. The detected heavy metal contents in soil were below the critical values stipulated in the National Soil Environmental Quality Standard of China (GB15618-1995) (Tables 3, 4).

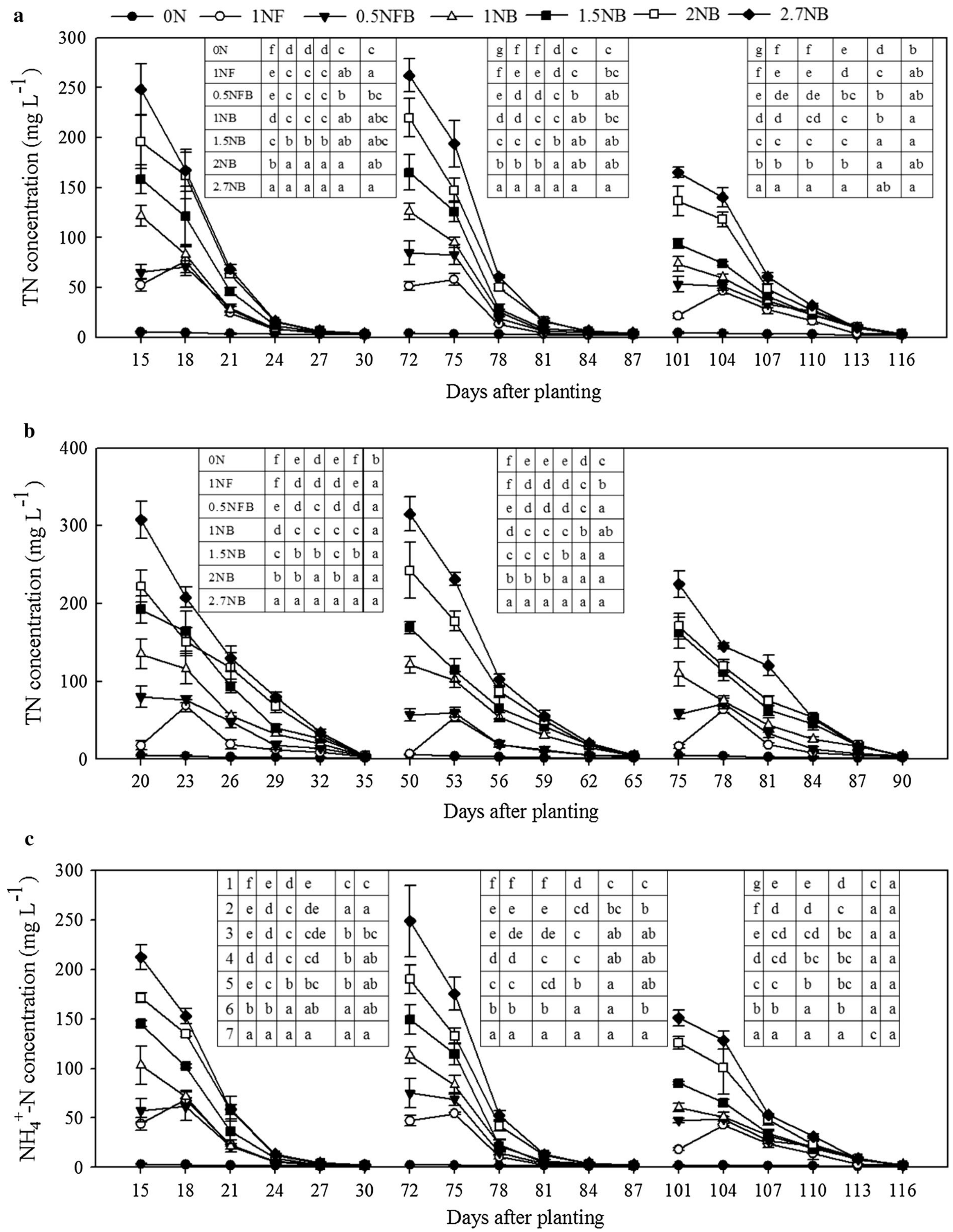
Discussion

Excessive biogas slurry application suppressed growth, yield formation, and PNuE

As a kind of N fertilizer, biogas slurry could completely or partially replace mineral N fertilizer for the growth of *Z. aquatic*. Similar findings were reported in other crops, such as rice, wheat, maize, and komatsuna (Hou et al. 2007; Lu et al. 2012; Svoboda et al. 2013). However, excessive use of biogas slurry adversely affects the *Z. aquatica* yield, dry matter, and N accumulation in above-ground parts. In addition, the growth suppression was more serious when continuously applied in the second growing season (Figs. 1a, b, 2a, b, 3a, b). Moreover, the plant height, leaf length, and stem length also decreased because of excessive application (unpublished). Similar results have been observed in other crops, such as bhendi and fodder maize, although these crops showed various responses to different rates of biogas slurry application (Geeta and Sreenivasa 2002; Islam et al. 2010).

Fig. 5 TN, NH_4^+–N, and NO_3^-–N concentrations in floodwater after biogas slurry application in the 2014 (**a**, **c**, **e**) and 2015 (**b**, **d**, **f**) growing seasons. *Columns from left to right in each table above the variation curve* correspond to the sampling date order. *Different lowercase letters in the same column* indicate significant differences ($P < 0.05$) among treatments. *Abbreviation ns at the top row of the table* indicates no significant differences ($P < 0.05$) among treatments ▶

NH_4^+ is an inorganic nitrogen source that can be deleterious to the growth of plant species when absorbed as the sole N source (Schortemeyer et al. 1997; Kotsiras et al. 2005; Dai et al. 2008). Rice is an NH_4^+-tolerant species that can also be negatively affected by high NH_4^+ concentrations in solution (Balkos et al. 2010). Chen et al. (2013b) found that different rice varieties had diverse tolerance levels to high NH_4^+ concentrations in solution. The levels of NH_4^+ in soils frequently reach the critical amount, which negatively affects plant growth. These negative effects are manifested as stunted root growth, yield suppression, and leaf chlorosis (Britto and Kronzucker 2002). We collected biogas slurry samples from eight pig farms in Jiaxing City and found NH_4^+–N is a dominant form of N that accounts for 77–93% of the TN in biogas slurry (data unpublished). These levels were similar with those obtained in other reports (65–90%) (Chen et al. 2013a; Svoboda et al. 2013). In the present study, as much as 300 mg L^{-1} NH_4^+–N was present in floodwater after biogas slurry application. Moreover, NH_4^+–N above 150 mg L^{-1} existed in floodwater for 6 days or more (Fig. 5c, d). This phenomenon may lead to high NH_4^+ concentrations around the *Z. aquatica* root. Additionally, Li et al.


a
0N
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2NB
2.7NB
TN concentration (mg L-1)
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0.5NFB e c c c b bc
1NB d c c c ab abc
1.5NB c b b b ab abc
2NB b a a a a ab
2.7NB a a a a a a
g f f d c c
f e e d c bc
e d d c b ab
d d c c ab bc
c c c b ab ab
b b b a ab ab
a a a a a a
g f f e d b
f e e d c ab
e de de bc b ab
d d cd c b a
c c c c a a
b b b b a ab
a a a a ab a
15 18 21 24 27 30 72 75 78 81 84 87 101 104 107 110 113 116
Days after planting
b
TN concentration (mg L-1)
400
300
200
100
0
0N f e d e f b
1NF f d d d e a
0.5NFB e d c d d a
1NB d c c c c a
1.5NB c b b c b a
2NB b b a b a a
2.7NB a a a a a a
f e e e d c
f d d d c b
e d d d c a
d c c c b ab
c c c b a a
b b b a a a
a a a a a a
20 23 26 29 32 35 50 53 56 59 62 65 75 78 81 84 87 90
Days after planting
c
NH4+-N concentration (mg L-1)
300
250
200
150
100
50
0
1 f e d e c c
2 e d c de a a
3 e d c cde b bc
4 d d c cd b ab
5 e c b bc b ab
6 b b a ab a ab
7 a a a a a a
f f f d c c
e e e cd bc b
e de de c ab ab
d d c c ab ab
c c cd b a ab
b b b a a b
a a a a a a
g e e d c a
f d d c a a
e cd cd bc a a
d cd bc bc a a
c c b bc a a
b b a b a a
a a a a c a
15 18 21 24 27 30 72 75 78 81 84 87 101 104 107 110 113 116
Days after planting

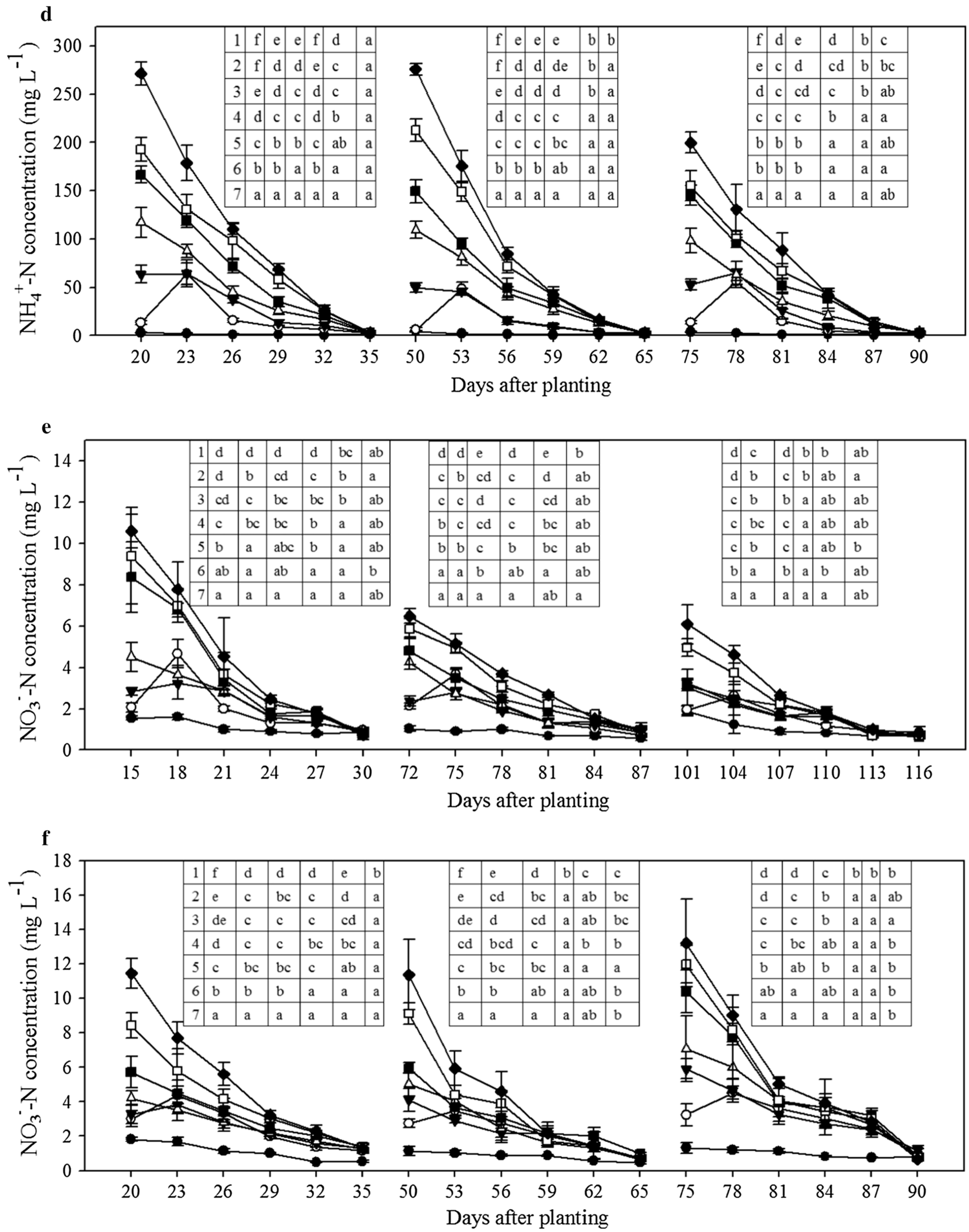


Fig. 5 continued

(2012a) confirmed that the growth and lateral root development of *Arabidopsis* was considerably influenced by NH_4^+ toxicity when leaves were exposed to high NH_4^+ levels as compared with the root. In the present study, partial stems of *Z. aquatica* were exposed to high NH_4^+ levels for several days, which might have contributed to growth depression. Chen et al. (2013b) suggested that the decreased N use efficiency in plants may be closely associated with the increased futile NH_4^+ cycling in roots and NH_4^+ toxicity, which might be the principal reason for decreased PNuE levels in above-ground parts of *Z. aquatica*.

Biogas slurry application has been widely recognized to improve the quality of crops or fruits (Liu et al. 2009; Xue et al. 2012). The preset study demonstrated that biogas slurry application significantly increased the Vc content in non-shell swollen culms of *Z. aquatica* but did not adversely affect the nitrate content (Table 2). In addition to N, P, and K, biogas slurry contains other important nutritional substances, such as carbohydrates, amino acids, microelements, hormones, and crude proteins, which might play an important role in improving product quality (Liu et al. 2009; Islam et al. 2010).

Environmental effects of biogas slurry applied on Z. *aquatica*

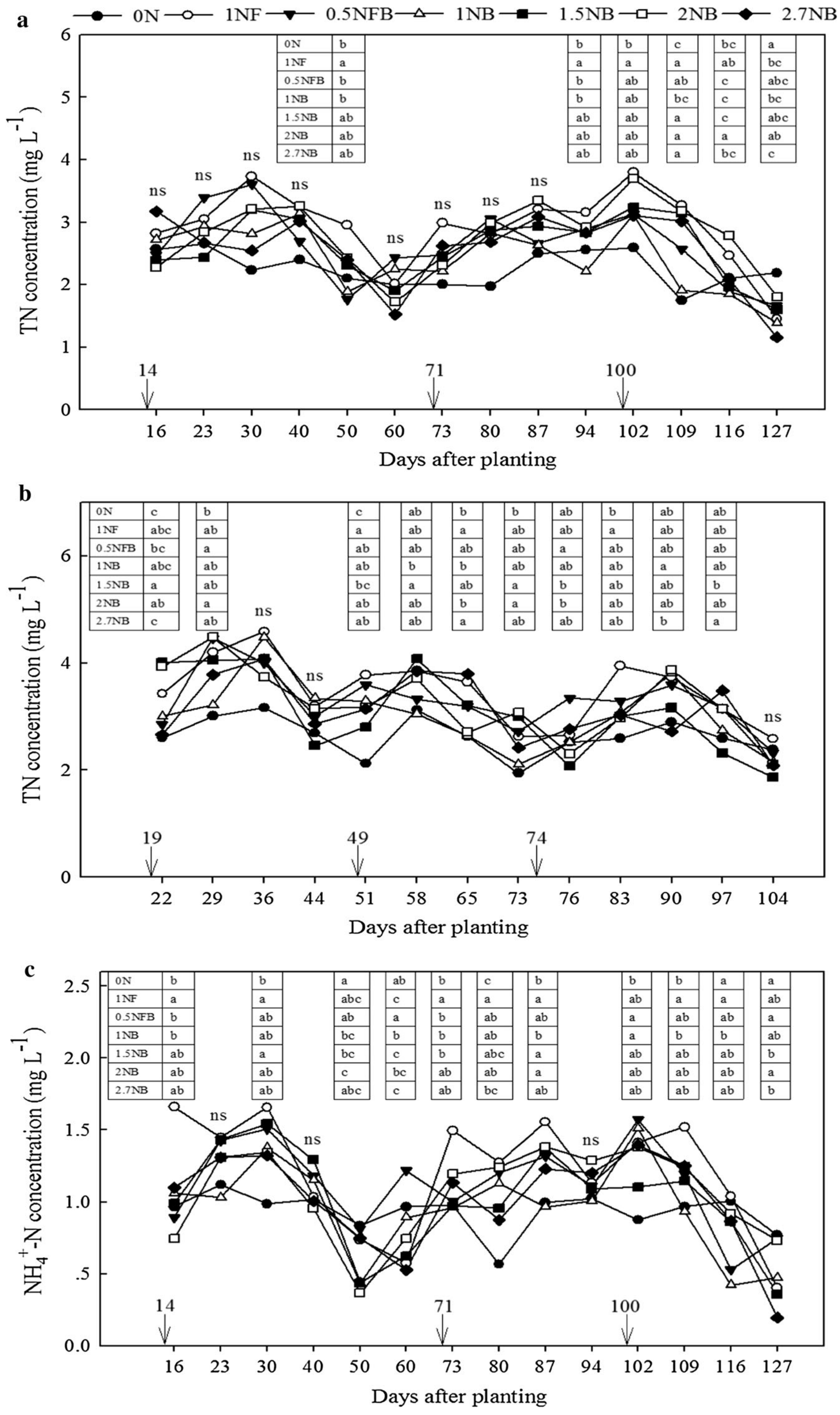
The environmental effects after application of biogas slurry have gained an increasing amount of attention. In this study, biogas slurry irrigation and mineral N fertilizer application showed the similar effects on the N contents in percolating water (Fig. 6a–f); these results are similar to the reports of Lu et al. (2012) and Chen et al. (2013a). The soil characteristics in test area of purple clay-based paddy soil may be closely related to our results because high soil density and poor water penetration capability were observed (Li et al. 1984).

High concentrations of NH_4^+–N in floodwater could be a potential risk to ambient water bodies when runoff events occur (Chen et al. 2016). In the present study, high TN and NH_4^+–N concentrations of up to ~ 300 mg L^{-1} were observed in floodwater after biogas slurry was irrigated; these concentrations increased with more input and reduced to the safe level after 10–13 days or longer time (Fig. 5a–d). Runoff events occurring during this period can introduce considerable risks to the water body. However, a high ridge of field is usually built to keep high water level in field for aquatic vegetable growth. For example, farmers usually build 35–40 cm or higher ridges for planting *Z. aquatica* in fields. Therefore, the runoff event does not normally occur only if 150–200 mm of precipitation occurrs within a short period of time. In our study, runoff events did not occur in the 2014 and 2015 growing seasons.

The rapidly decreasing NH_4^+–N content in floodwater could be mainly attributed to ammonium (NH_3) volatilization and nitrification–denitrification processes. Previous studies showed that the application of biogas slurry produced 2–4 times more NH_3 and nitrous oxide (N_2O) than the mineral fertilizers, which was probably aggravated by applying more biogas slurry (Hou et al. 2007; Jacobi et al. 2009; Chen et al. 2013a). Furthermore, N uptake by crops also can decrease the NH_4^+–N content in floodwater. The study suggests that NH_3 volatilization in paddy fields was considerably reduced when the mineral N fertilizer was applied at the elongation stage as compared with the early tillering stage; this trend is attributed to the strong ability of N uptake by rice plants at elongation stage (Cassman et al. 1998). In our study, the required time to reduce NH_4^+–N to safe levels was relatively long, with more biogas slurry input. The main reasons for this trend were (1) the large quantity of irrigation and the increased NH_4^+–N concentration in floodwater and (2) the decreased N uptake of *Z. aquatica* plants by excessive use of biogas slurry.

Volatilized NH_3 can return to land and water by the dry deposition of NH_3 or the dry–wet deposition of NH_4^+; these phenomena can contribute to acidification and eutrophication of water bodies, thereby causing undesirable changes in natural ecosystems (Bouwman et al. 2002). N_2O is a major greenhouse gas that is 250 times more effective as an absorber of infrared radiation than CO_2 for a 100-year time frame (Ruser and Schulz 2015). Therefore, simple and effective technologies should be developed to limit the NH_3 and N_2O emissions induced by irrigating biogas slurry.

The heavy metal pollution in agricultural commodities and soil has become a growing concern. In this study, the heavy metal content in the non-shell swollen culms and soil did not increase after applying the biogas slurry (Tables 3, 4). Similar findings were reported by Lu et al. (2012) and Montemurro et al. (2008). The first possible reason for this trend is that



a

0N	b	b	b	c	bc	a
1NF	a	a	a	a	ab	bc
0.5NFB	b	b	ab	ab	c	abc
1NB	b	b	ab	bc	c	bc
1.5NB	ab	ab	ab	a	c	abc
2NB	ab	ab	ab	a	a	ab
2.7NB	ab	ab	ab	a	bc	c

b

0N	c	b	c	ab	b	b	ab	b	ab	ab
1NF	abc	ab	a	ab	a	ab	ab	a	ab	ab
0.5NFB	bc	a	ab	ab	ab	ab	a	ab	ab	ab
1NB	abc	ab	ab	b	b	ab	ab	ab	a	ab
1.5NB	a	ab	bc	a	ab	a	b	ab	ab	b
2NB	ab	a	ab	ab	b	a	b	ab	ab	ab
2.7NB	c	ab	ab	ab	a	ab	ab	ab	b	a

c

0N	b	b	a	ab	b	c	b	b	b	a	a
1NF	a	a	abc	c	a	a	a	ab	a	a	ab
0.5NFB	b	ab	ab	a	b	ab	ab	a	ab	ab	a
1NB	b	ab	bc	b	b	ab	b	a	b	b	ab
1.5NB	ab	a	bc	c	b	abc	a	ab	ab	ab	b
2NB	ab	ab	c	bc	ab	ab	a	ab	ab	ab	a
2.7NB	ab	ab	abc	c	ab	bc	ab	ab	ab	ab	b

Fig. 6 TN, NH_4^+–N, and NO_3^-–N concentrations in percolation water during different growth stages in the 2014 (**a**, **c**, **e**) and 2015 (**b**, **d**, **f**) growing seasons. *Different lowercase letters in the same column above sampling date* indicate significant differences ($P < 0.05$) among treatments. *Abbreviation ns at the top row of table* indicate no significant differences ($P < 0.05$) among treatments. *Arrow with day on the top* indicated the day of fertilization event after planting

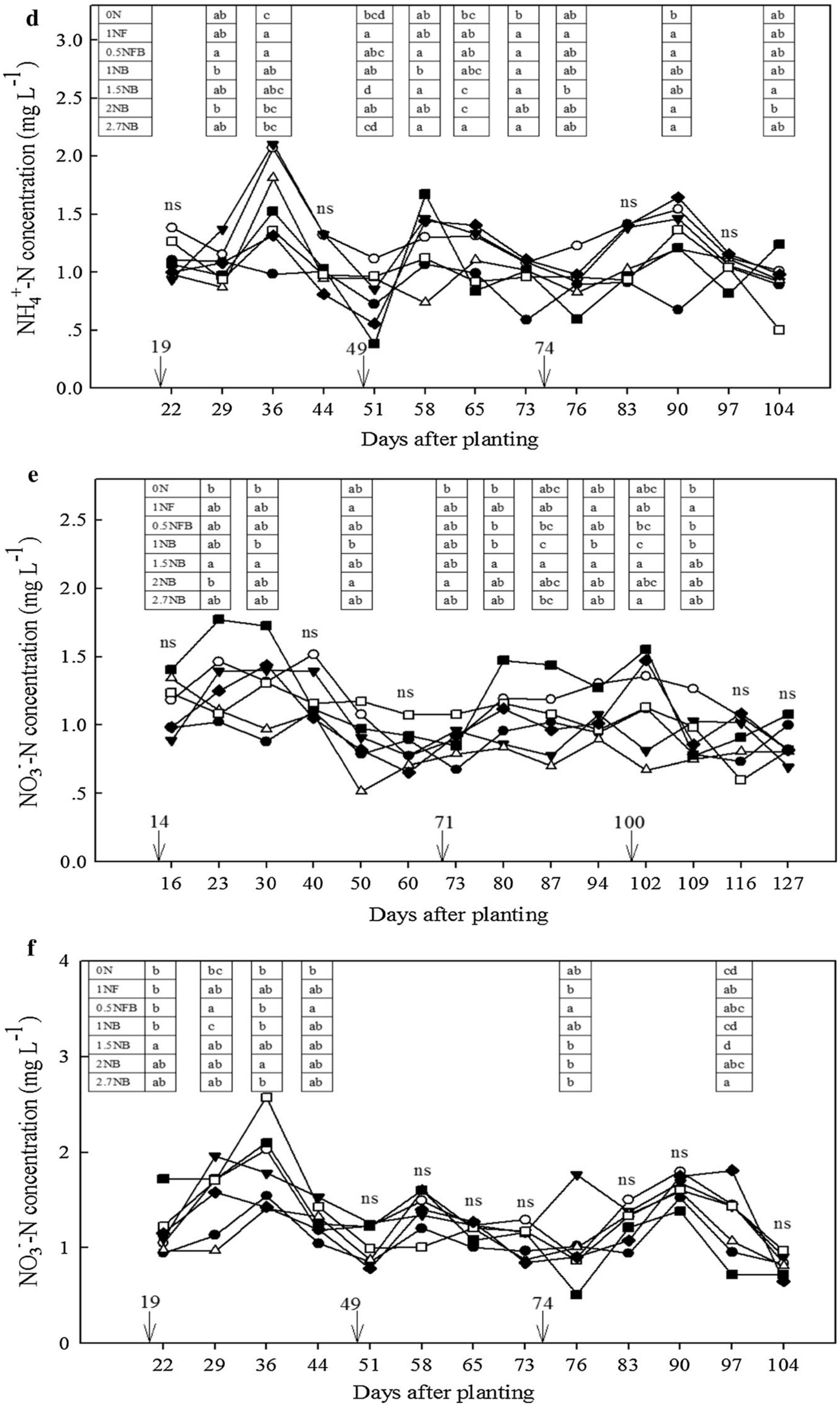


Fig. 6 continued

Table 2 Quality of non-shell swollen culm in 2014 and 2015 growing season

Growing season	Treatments	Vitamin C (mg kg^{-1})	Total sugar (%)	Protein (%)	Nitrate (mg kg^{-1})
2014	0N	59.6 ± 3.9c	1.76 ± 0.18ns	18.9 ± 0.73d	36.0 ± 4.9b
	1NF	60.8 ± 5.9bc	1.72 ± 0.24	20.5 ± 0.58bc	52.2 ± 6.8a
	0.5NFB	68.4 ± 9.6ab	1.70 ± 0.06	20.2 ± 0.38c	53.6 ± 10.9a
	1NB	71.2 ± 5.2a	1.77 ± 0.16	20.6 ± 0.52bc	49.3 ± 7.0a
	1.5NB	71.6 ± 5.1a	1.69 ± 0.19	20.9 ± 0.36bc	48.0 ± 10.8a
	2NB	69.2 ± 3.0a	1.78 ± 0.18	21.2 ± 0.12ab	49.1 ± 4.9a
	2.7NB	68.7 ± 4.9ab	1.82 ± 0.29	21.7 ± 0.07a	48.7 ± 6.1a
2015	0N	62.0 ± 2.1b	1.89 ± 0.22ns	22.4 ± 0.76d	29.7 ± 3.0b
	1NF	61.6 ± 2.6b	1.82 ± 0.36	24.9 ± 0.94c	43.7 ± 4.5a
	0.5NFB	72.1 ± 7.2a	1.81 ± 0.33	25.3 ± 0.62c	40.4 ± 4.6a
	1NB	70.0 ± 5.4a	1.94 ± 0.47	25.4 ± 0.53c	38.5 ± 4.3a
	1.5NB	72.1 ± 3.3a	2.00 ± 0.32	25.6 ± 0.52bc	39.7 ± 4.2a
	2NB	70.8 ± 4.0a	1.87 ± 0.24	26.8 ± 0.17b	38.2 ± 5.0a
	2.7NB	70.4 ± 1.2a	1.84 ± 0.22	28.6 ± 1.36a	38.6 ± 3.7a

Data are presented as mean ± SD of three replicates. The different lowercase letters (s) in the same column indicate significant differences ($P < 0.05$) among treatments of each growing season. Values with ns at the top of each column season indicate no significant differences ($P < 0.05$) among treatments of each growing season

the heavy metal concentrations are low in biogas slurry, which was also reported by Arthurson (2009) and Lu et al. (2012). Furthermore, Marcato et al. (2009) reported the lower bioavailability of Cu and Zn in anaerobically digested pig slurry than in the raw slurry. Second, the heavy metal concentrations of biogas slurry were significantly lower than the background levels in the soil. Finally, high concentrations of organic matter in biogas slurry can form complexes with heavy metals, thereby decreasing the available amount for plant uptake (Lo et al. 1992; Marcato et al. 2009). However, the cumulative long-term effect of heavy metals in agricultural commodities and soil must be further monitored to ensure environment and human safety.

Confirming NH_4^+ content in biogas slurry for its improved reasonable use in crop cultivation

The Chinese government has encouraged the recycling of biogas slurry within agriculture system to develop ecological circular agriculture and reduce mineral fertilizer application rates (Li et al. 2012b; Qin 2015). However, the problem is that it is difficult for farmers to determine the N content in biogas slurry applying for crops or other plants growth. High NH_4^+ content in biogas slurry and its excessive application can damage the plants. Although, previous studies have recommended a total range of application rates or one certain rate on selected crops (Wu et al. 2011; Lu et al. 2012; Zheng et al. 2016). The application rate of biogas slurry is still difficult to confirm because of large variations in the N concentration. In the present study, 395–1521 mg L^{-1} TN and 301–1141 mg L^{-1} NH_4^+–N were detected in biogas slurry (Table 1). Additionally, biogas slurry samples were collected from eight pig farms in the Jiaxing area; the observed TN content ranged from 260 to 1358 mg L^{-1}, with 236–1196 mg L^{-1} of NH_4^+–N accounting for 77–93% of the TN (data unpublished). Lu et al. (2012) reported TN and NH_4^+–N concentrations of 531–1117 and 492–733 mg L^{-1}, respectively. NH_4^+–N is the primary N form in biogas slurry because methane is extracted, whereas N in the substrate is conserved in biogas residues, thereby increasing the proportion of N in NH_4^+–N and reducing the C/N ratio (Gutser et al. 2005). The strong redox potential and alkaline conditions in biogas slurry are also important contributors (Lu et al. 2012). Therefore, the optimal N concentrations should first be understood before

Table 3 Heavy metal content in non-shell swollen culm in 2015 growing season (mg kg^{-1})

Treatments	Cu	Zn	Pb	Cr	Cd	As	Hg
0N	0.34 ± 0.05ns	3.13 ± 0.61ns	0.038 ± 0.006ab	0.071 ± 0.009ns	0.017 ± 0.003ns	0.020 ± 0.004ns	0.006 ± 0.001ns
1NF	0.36 ± 0.05	3.19 ± 0.72	0.041 ± 0.008ab	0.077 ± 0.005	0.020 ± 0.005	0.023 ± 0.005	0.006 ± 0.001
0.5NFB	0.34 ± 0.01	2.66 ± 0.74	0.044 ± 0.009a	0.074 ± 0.011	0.016 ± 0.001	0.020 ± 0.004	0.006 ± 0.001
1NB	0.35 ± 0.06	3.25 ± 0.80	0.036 ± 0.007ab	0.076 ± 0.015	0.016 ± 0.002	0.018 ± 0.004	0.006 ± 0.001
1.5NB	0.35 ± 0.06	3.20 ± 0.26	0.031 ± 0.003b	0.068 ± 0.014	0.018 ± 0.001	0.024 ± 0.003	0.006 ± 0.000
2NB	0.38 ± 0.03	3.01 ± 0.50	0.039 ± 0.008ab	0.066 ± 0.003	0.017 ± 0.001	0.025 ± 0.006	0.007 ± 0.001
2.7NB	0.38 ± 0.04	3.26 ± 0.41	0.037 ± 0.003ab	0.072 ± 0.014	0.017 ± 0.001	0.021 ± 0.003	0.006 ± 0.001
Standard	10	20	0.1	0.5	0.05	0.05	0.01

Data are presented as the mean ± SD of three replicates. The different lowercase letters in the same column indicate significant differences ($P < 0.05$) among treatments. Values with ns at the top of each column indicate no significant differences ($P < 0.05$) among treatments. The standard values indicate the critical level for all treatments according to the National Maximum Levels of Contaminates in Foods of China (GB2762-2005), the National Maximum Levels of Cu in Foods of China (GB13106-91), and the National Maximum Levels of Zn in Foods of China (GB15199-94)

Table 4 Heavy metal content in soil in 2015 growing season (mg kg^{-1})

Treatments	Cu	Zn	Pb	Cr	Cd	As	Hg
0N	29.4 ± 2.6ab	80.6 ± 6.6ns	46.1 ± 9.1ns	68.6 ± 5.7ns	0.14 ± 0.01ns	9.61 ± 0.89ns	0.14 ± 0.01ns
1NF	30.9 ± 2.3ab	74.4 ± 8.5	52.8 ± 9.5	73.6 ± 5.5	0.15 ± 0.02	9.87 ± 0.73	0.13 ± 0.02
0.5NFB	32.5 ± 1.3a	81.2 ± 9.2	49.9 ± 9.2	67.8 ± 8.9	0.15 ± 0.01	8.80 ± 1.17	0.14 ± 0.03
1NB	31.1 ± 2.0ab	86.0 ± 8.1	51.1 ± 14.3	68.4 ± 4.1	0.15 ± 0.01	9.59 ± 1.09	0.11 ± 0.02
1.5NB	29.6 ± 2.7ab	70.9 ± 15.4	53.4 ± 13.0	66.8 ± 4.1	0.14 ± 0.01	10.1 ± 1.5	0.13 ± 0.02
2NB	27.4 ± 1.7b	69.8 ± 7.6	49.7 ± 11.0	69.7 ± 12.1	0.15 ± 0.01	10.1 ± 1.1	0.11 ± 0.01
2.7NB	30.3 ± 3.4ab	72.1 ± 13.3	45.2 ± 13.4	67.3 ± 10.0	0.14 ± 0.02	9.88 ± 1.2	0.13 ± 0.03
Standard	50	200	250	250	0.30	30	0.3

Data are presented as mean ± SD of three replicates. The different lowercase letters in the same column indicate significant differences ($P < 0.05$) among treatments. Values with ns at the top of each column indicate no significant differences ($P < 0.05$) among treatments. The standard values indicate the critical levels according to the National Soil Environmental Quality Standard of China (GB15618-1995) biogas slurry can be reasonably and safely used for crop cultivation.

TN or NH_4^+–N concentrations in water are generally measured by spectrophotometry in the laboratory. The determination of TN content is complicated because of the high-temperature and high-pressure digestion processes and the spectrophotometry at UV–light. However, NH_4^+–N is measured via an indophenol blue colorimetric method by spectrophotometry at the visible light range and does not need to undergo digestion (Peng et al. 2011). Moreover, NH_4^+–N is the most prevalent form of N in biogas slurry, and a large amount of N levels is present. Therefore, the development of simple and quick methods to determine the NH_4^+–N content in biogas slurry should be feasible and beneficial to quantify biogas slurry irrigation rates. At present, semi-quantitative methods are used for NH_4^+–N determination in related commercial products. Nevertheless, the low-concentration of NH_4^+–N (0–100 mg L^{-1}) in water was analyzed but was found inconvenient for high NH_4^+–N measurement in biogas slurry. To promote the reasonable use of biogas slurry for crop cultivation, semi-quantitative or precise methods should be developed to determine the NH_4^+–N concentration in biogas slurry.

Conclusion

Biogas slurry was used as N fertilizer to partially or completely substitute mineral N fertilizer and satisfy two-season *Z. aquatica* (an aquatic vegetable) growth, including the yield formation, dry matter development, N uptake, and N utilization. Nevertheless, excessive application (2 or 2.7 times higher N in the biogas slurry relative to the mineral N fertilizer in our study) suppressed normal growth, which was probably caused by NH_4^+ toxicity. The application of biogas slurry improved the quality of non-shell swollen culm but maintained safe concentrations of nitrate and heavy metal content. Furthermore, biogas slurry application did not cause heavy metal pollution in the soil or N pollution in leakage water. However, the N concentrations in floodwater are a major threat to ambient water bodies after the application of biogas slurry as compared with mineral N fertilizer. High NH_4^+–N and the large variation of N content in biogas slurry impedes its reasonable use as nitrogenous fertilizer for crop cultivation. Thus, semi-quantitative or more precise methods are necessary, especially to determine the NH_4^+–N concentration in biogas slurry, given its large proportion of TN content and relatively simple measurement.

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References

Abraham ER, Ramachandran S, Ramalingam V (2007) Biogas: can it be an important source of energy? Environ Sci Pollut Res Int 14:67–71. doi:10.1065/espr2006.12.370

Arthurson V (2009) Closing the global energy and nutrient cycles through application of biogas residue to agricultural land-potential benefits and drawbacks. Energies 2:226–242. doi:10.3390/en20200226

Balkos KD, Britto DT, Kronzucker HJ (2010) Optimization of ammonium acquisition and metabolism by potassium in rice (*Oryza sativa* L. cv. IR-72). Plant Cell Environ 33:23–34. doi:10.1111/j.1365-3040.2009.02046.x

Bouwman AF, Van Vuuren DP, Derwent RG, Posch M (2002) A global analysis of acidification and eutrophication of terrestrial ecosystems. Water Air Soil Pollut 141:349–382. doi:10.1023/A:1021398008726

Britto DT, Kronzucker HJ (2002) NH_4^+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584. doi:10.1078/0176-1617-0774

Cassman KG, Peng S, Olk DC, Ladha JK, Reichardt W, Dobermann A, Singh U (1998) Opportunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. Field Crops Res 56:7–39. doi:10.1016/S0378-4290(97)00140-8

Chen ZL (2002) The research on introduction and cultivation of Chinese wild rice shoots in the Washington region of America. J Wuhan Bot Res 20:43–47

Chen DJ, Jiang LN, Huang H, Toyota KK, Dahlgren RA, Lu J (2013a) Nitrogen dynamics of anaerobically digested slurry used to fertilize paddy fields. Biol Fertil Soils 49:647–659. doi:10.1007/s00374-012-0752-8

Chen G, Guo SW, Kronzucker HJ (2013b) Nitrogen use efficiency (NUE) in rice links to NH_4^+ toxicity and futile NH_4^+ cycling in roots. Plant Soil 369:351–363. doi:10.1007/s11104-012-1575-y

Chen MP, Sun F, Shindo JK (2016) China's agricultural nitrogen flows in 2011: environmental assessment and management scenarios. Resour Conserv Recycl 111:10–27. doi:10.1016/j.resconrec.2016.03.026

Dahiya AK, Vasudevan P (1986) Biogas plant slurry as an alternative to chemical fertilizers. Biomass 9:67–74. doi:10.1016/0144-4565(86)90013-2

Dai GZ, Deblois CP, Liu SW, Juneau P, Qiu BS (2008) Differential sensitivity of five cyanobacterial strains to ammonium toxicity and its inhibitory mechanism on the photosynthesis of rice-field cyanobacterium Ge-Xian-Mi

(*Nostoc*). Aquat Toxicol 89:113–121. doi:10.1016/j.aquatox.2008.06.007

Duan GL, Zhang HM, Liu YX, Jia Y, Hu Y, Cheng WD (2012) Long-term fertilization with pig-biogas residues results in heavy metal accumulation in paddy field and rice grains in Jiaxing of China. Soil Sci Plant Nutr 58:637–646. doi:10.1080/00380768.2012.726597

Elfstrand S, Bath B, Martensson A (2007) Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. Appl Soil Ecol 36:70–82. doi:10.1016/j.apsoil.2006.11.001

Garg RN, Pathak H, Das DK, Tomar RK (2005) Use of flyash and biogas slurry for improving wheat yield and physical properties of soil. Environ Monit Assess 107:1–9. doi:10.1007/s10661-005-2021-x

Geeta GS, Sreenivasa MN (2002) Influence of biogas spent slurry on growth and yield of bhendi. J Maharashtra Agric Univ 27:64–65

Guo HB, Li SM, Peng J, Ke WD (2007) *Zizania latifolia* Turcz. cultivated in China. Genet Resour Crop Evol 54:1211–1217. doi:10.1007/s10722-006-9102-8

Gutser R, Ebertseder T, Weber A, Schraml M, Schmidhalter U (2005) Short-term and residual availability of nitrogen after long-term application of organic fertilizers on arable land. J Plant Nutr Soil Sci 168:439–446. doi:10.1002/jpln.200520510

Hou H, Zhou S, Hosomi M, Toyota K, Yosimura K, Mutou Y, Nisimura T, Takayanagi M, Motobayashi T (2007) Ammonia emissions from anaerobically digested slurry and chemical fertilizer applied to flooded forage rice. Water Air Soil Pollut 183:37–48. doi:10.1007/s11270-007-9353-9

Islam MDR, Rahman SME, Rahman MDM (2010) The effects of biogas slurry on the production and quality of maize fodder. Turk J Agric For 34:91–99. doi:10.3906/tar-0902-44

Jacobi HF, Moschner CR, Hartung E (2009) Use of near infrared spectroscopy in monitoring of volatile fatty acids in anaerobic digestion. Water Sci Technol 60:339–346. doi:10.2166/wst.2009.345

Kotsiras A, Olympios CM, Passam HC (2005) Effects of nitrogen form and concentration on yield and quality of cucumbers grown on rockwool during spring and winter n southern Greece. J Plant Nutr 28:2027–2035. doi:10.1080/01904160500311102

Laurentin A, Edwards CA (2003) A microtiter modification of the anthrone-sulfuric acid colorimetric assay for glucose-based carbohydrates. Anal Biochem 315:143–145. doi:10.1016/S0003-2697(02)00704-2

Li RA, Cao XF, Yu ZY (1984) Genetic characteristics and classification of the paddy soils developed on the bluish purple clay and the quaternary red clay in Zhejiang province. Acta Agric Univ Zhejiangensis 3:315–323 **(in Chinese with English abstract)**

Li BH, Li Q, Xiong LM, Kronzucker HJ, Krämer U, Shi WM (2012a) Arabidopsis plastid AMOS1/EGY1 integrates abscisic acid signaling to regulate global gene expression response to ammonium stress. Plant Physiol 160:2040–2051. doi:10.1104/pp.112.206508

Li FJ, Dong SC, Li F (2012b) A system dynamics model for analyzing the eco-agriculture system with policy recommendations. Ecol Model 227:34–45. doi:10.1016/j.ecolmodel.2011.12.005

Liu ZH, Jiang LH, Li XL, Hardter R, Zhang WJ, Zhang YL, Zheng DF (2008) Effect of N and K fertilizers on yield and quality of greenhouse vegetable crops. Pedosphere 18:496–502. doi:10.1016/S1002-0160(08)60040-5

Liu WK, Yang QC, Du LF (2009) Soilless cultivation for high-quality vegetables with biogas manure in China: feasibility and benefit analysis. Renew Agric Food Syst 24:300–307. doi:10.1017/S1742170509990081

Lo KSL, Yang WF, Lin YC (1992) Effects of organic matter on the specific adsorption of heavy metals by soil. Toxicol Environ Chem 34:139–153. doi:10.1080/02772249209357787

Lu J, Jiang LN, Chen DJ, Toyota K, Strong PJ, Wang HL (2012) Decontamination of anaerobically digested slurry in a paddy field ecosystem in Jiaxing region of China. Agric Ecosyst Environ 146:13–22. doi:10.1016/j.agee.2011.10.011

Marcato CE, Pinelli E, Cecchi M, Winterton P, Guiresse M (2009) Bioavailability of Cu and Zn in raw and anaerobically digested pig slurry. Ecotoxicol Environ Safe 72:1538–1544. doi:10.1016/j.ecoenv.2008.12.010

Ministry of Agriculture of the People's Republic of China (2014) http://www.zzys.moa.gov.cn/

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5:62–71. doi:10.1006/niox.2000.0319

Montemurro F, Canali S, Convertini G (2008) Anaerobic digestates application on fodder crops: effects on plant and soil. Agrochemica 52:297–312

National Bureau of Statistics of the People's Republic of China (2015) http://www.stats.gov.cn/tjsj/zxfb/201602/t20160229_1323991.html

National Groundwater Quality Standard of China (1993) GB/T 14848-1993. Standards Press of China, Beijing

National Integrated Wastewater Discharge Standard of China (1999) GB 8978-1996. Standards Press of China, Beijing

National Maximum Levels of Contaminates in Foods of China (2005) GB2762-2005. Standards Press of China, Beijing

National Maximum Levels of Cu in Foods of China (1991) GB13106-91. Standards Press of China, Beijing

National Maximum Levels of Zn in Foods of China (1994) GB15199-94. Standards Press of China, Beijing

National Soil Environmental Quality Standard of China (1995) GB15618-1995. Standards Press of China, Beijing

Peng SZ, Yang SH, Xu JZ, Luo YF, Hou HJ (2011) Nitrogen and phosphorus leaching losses from paddy fields with different water and nitrogen managements. Paddy Water Environ 9:333–342. doi:10.1016/j.agwat.2014.04.008

Qin BT (2015) Sustainable agricultural development in rural China: the way of addressing agricultural pollution. Sustainable development in rural China. Chapter 2, pp 13–22. doi:10.1007/978-3-662-46476-2_2

Ruser R, Schulz R (2015) The effect of nitrification inhibitors on the nitrous oxide (N_2O) release from agricultural soils. J Plant Nutr Soil Sci 178:4–12. doi:10.1002/jpln.201400251

Schortemeyer M, Stamp P, Feil B (1997) Ammonium tolerance and carbohydrate status in maize cultures. Ann Bot 79:25–30. doi:10.1006/anbo.1996.0298

Sheets JP, Yang LC, Ge XM, Wang ZW, Li YB (2015) Beyond land application: emerging technologies for the treatment and reuse of anaerobically digested agricultural and food waste. Waste Manag 44:94–115. doi:10.1016/j.wasman.2015.07.037

Shi WM, Xu WF, Li SM, Zhao XQ, Dong GQ (2010) Responses of two rice cultivars differing in seedling-stage nitrogen use efficiency to growth under low-nitrogen condition. Plant Soil 326:291–302. doi:10.1007/s11104-009-0007-0

Svoboda N, Taube F, Wienforth B, Klup C, Kage H, Herrmann A (2013) Nitrogen leaching losses after biogas residue application to maize. Soil Tillage Res 130:69–80. doi:10.1016/j.still.2013.02.006

Tan F, Wang Z, Zhouyang SY, Li HL, Xie YP, Wang YP, Zheng YM, Li QB (2016) Nitrogen and phosphorus removal coupled with carbohydrate production by five microalgae cultures cultivated in biogas slurry. Bioresour Technol 221:385–393. doi:10.1016/j.biortech.2016.09.030

Terhoeven-Urselmans T, Scheller E, Raubuch M, Ludwig B, Joergensen RG (2009) CO_2 evolution and N mineralization after biogas slurry application in the field and its yield effects on spring barley. Appl Soil Ecol 42:297–302. doi:10.1016/j.apsoil.2009.05.012

Terrell EE, Batra LR (1982) *Zizania latifolia* and *Ustilago esculenta*, a grass-fungus association. Econ Bot 36:274–285. doi:10.1007/BF02858549

USDA/NRCS (1999) Soil taxonomy. A basic system of soil classification for making and interpreting soil surveys, 2nd edn. U. S. Government Printing Office, Washington

Wu FG, Wang L, Wang J, Yuan JF, Wang HM, Li G, Wang BX (2011) Effects of biogas slurry content on development and yield and quality of flue-cured tobacco leaf. Hubei Agric Sci 8:1606–1610 **(in Chinese with English abstract)**

Xue YF, Shi ZQ, Chen J, Yan SH, Zheng JC (2012) Promotion of the growth and quality of Chinese cabbage by application of biogas slurry of water hyacinth. Appl Mech Mater 195–196:1200–1206. doi:10.4028/www.scientific.net/AMM.195-196.1200

Yu XP, Li JR, Shi JM, Deng SQ, Deng CR, Wu C, Li BF (2003) The aquatic vegetable, Jiaobai (*Zizania caduciflora* L.) and its safe production in Zhejiang Province. Acta Agric Zhejiang 15(3):109–117 (**in Chinese with English abstract**)

Yu FB, Luo XP, Song CF, Zhang MX (2010) Concentrated biogas slurry enhanced soil fertility and tomato quality. Acta Agric 60:262–268. doi:10.1080/09064710902893385

Zhai CK, Lu CM, Zhang XQ, Sun GJ, Lorenz KJ (2001) Comparative study on nutritional value of Chinese and North American wild rice. J Food Compos Anal 14:371–382. doi:10.1006/jfca.2000.0979

Zheng XB, Fan JB, Cui J, Wang Y, Zhou J, Ye M, Sun MM (2016) Effects of biogas slurry application on peanut yield, soil nutrients, carbon storage, and microbial activity in Ultisol soil in southern China. J Soils Sediments 16:449–460. doi:10.1007/s11368-015-1254-8

Zhu ZL, Chen DL (2002) Nitrogen fertilizer use in China—contributions to food production impacts on the environment and best management strategies. Nutr Cycl Agroecosyst 63:117–127. doi:10.1023/A:1021107026067

Zhu K, Choi HL, Yao HQ, Suresh A, Oh DI (2009) Effects of anaerobically digested pig slurry application on runoff and leachate. Chem Ecol 25:359–369. doi:10.1080/02757540903193114