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RESEARCH ARTICLE

Nitrogen leaching losses following biogas slurry irrigation to purple soil of the Three Gorges Reservoir Area

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Abstract
Ammonium (NH_4^+) in biogas slurries is readily nitrified into very mobile soil nitrate (NO_3^-) to promote nitrogen (N) leaching concerning which a few studies, however, have been reported. These slurries are regularly applied through irrigation to purple soil in the Three Gorges Reservoir Area, and therefore we explored the consequent N leaching there with a plot experiment. Biogas slurry irrigation was carried out with nitrogen application rates of 0, 48, 144, 240, 336, and 480 kg N/ha. As a result, the last two rates have triggered N leaching being detrimental to groundwater safety. In addition, N leaching was negatively correlated with soil microbial biomass, diversity, and respiration, indicating a potential technique to prevent it with soil heterotrophs activated by increased ratios of carbon to nitrogen (C/N) in biogas slurries.

Keywords Purple soil · Biogas slurry · Nitrogen leaching · Nitrification

Introduction

Biogas slurries, a by-product of biogas projects, contain nutrients and thus are recycled as fertilizers to increase crop yields. However, significant gaps persist in our knowledge on their environmental impacts. To bridge these gaps, the scientific community has been making efforts, but to date researches are far from complete (Insam et al. 2015; Möller and Müller 2012; Nkoa 2014). A few studies have dealt with the nitrogen (N) leaching in soils following application of biogas slurries, although it may pose risks to groundwater. It mainly derives from very mobile nitrate (NO_3^-), into which the ammonium (NH_4^+) in biogas slurries, accounting for a large percentage (35 to 81%) of total nitrogen (Möller and

Highlights
• Applying biogas slurry at common nitrogen application rates triggered N leaching.
• The N leaching was due to soil nitrification promoted by the biogas slurry.
• Increasing slurry C/N might prevent the N leaching but needs further confirmation.

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Müller 2012; Nkoa 2014), is readily nitrified (Abubaker et al. 2013; Abubaker et al. 2012; Alburquerque et al. 2012; Goberna et al. 2011; Gomez-Brandon et al. 2016; Grigatti et al. 2011; Johansen et al. 2013; Sanger et al. 2014; Sawada and Toyota 2015; Senbayram et al. 2009).

Academic works have measured higher and lower rates of N leaching in the absence and presence of plants, respectively. After entering some bared soils, the biogas slurries resulted in three times as much NO_3^- leaching as the manure led to (Goberna et al. 2011), and in the N leaching accounting for 31% (Cheng et al. 2017) or 16% (Sänger et al. 2011) of the applied rate of nitrogen. However, if the maize or grasses were included, such ratios would be lower at 6 to 12% (Matsunaka et al. 2006), 8% (Svoboda et al. 2013a, b), or 1% (Svoboda et al. 2013a).

The two levels of N leaching may confuse questions as to which of them to refer to and then whether the other carries weight despite in fact the vital information provided by both. The less alarming level seems to outweigh because the usage of plants makes conditions more real. However, the other is also essential for our understanding of the processes that biogas slurry NH_4^+ go through, including volatilization, adsorption, immobilization, nitrogen uptake, and nitrification (Amlinger et al. 2003). In addition, it enables a simpler fate of NH_4^+ for exploration that getting rid of crops to separate the nitrogen uptake from other processes. Moreover, the clearer fate better fits experiments on the N leaching derived from biogas slurries per se, and on potential (the maximum likelihood) and risk of N leaching (USEPA 1998).

Here, we investigate the N leaching in purple soil following biogas slurry irrigation of the Three Gorges Reservoir Area. This area is critical for the water quality of the Yangtze River, the world's third longest river, because it covers a 660-km stretch (Gao 2017; Gleick 2009). It however is vulnerable to N leaching, because the purple soil accounts for 69% of arable lands (Guan et al. 2014), and is characterized by shallow depths of up to 50 cm. According to reports, recent years have witnessed increasing environmental concerns raised by agricultural nitrogen loads there (Gao 2017; Ma et al. 2016; Zhang et al. 2015).

Biogas slurry irrigation was carried out with nitrogen application rates of 0 to 480 kg N/ha to analyze the consequent leachate and soil for experimental results, which may lead to sensible precautions against unawareness undermining the safety of the environment.

Material and methods

Soil and biogas slurry

Both purple soil and biogas slurry were similar to those in a study, with respect to the sources and characteristics (Table 1) (Cheng et al. 2017). The silty clay loam was collected at the National Monitoring Station of Purple Soil Fertility and Fertilizer Efficiency, Chongqing, China (106° 25′ 45 ″ N, 29° 49′ 18″ E), being located in the Three Gorges Reservoir Area. The slurry was collected from a cattle farm in Banan, Chongqing, China. After sedimentation, slurry in the upper layer was passed through a 1-mm sieve and diluted with tap water.

Table 1 Characteristics of soil and biogas slurry

Soil	Clay	Silt	Sand	FC	pH	$CaCO_3$
	30%	42%	28%	38.3%	6.81	1.13%
	CEC (cmol (+)/kg)	TOC (g/kg)	TN (mg/kg)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	SMBN (mg/kg)
	18.29	4.15	501.3	10.73	7.36	2.3
Slurry	pH	TN (mg/L)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)	NO_2^--N (mg/L)	COD (mg/L)
	7.73	949.1	761.0	37.66	7.70	7888

Methods of analysis reported (Cheng et al. 2017)

FC field capacity; *CEC* cationic exchange capacity; *TOC* total organic carbon; *TN* total nitrogen; *SMBN* soil microbial biomass nitrogen; *COD* chemical oxygen demand

Experimental plot setup

Smaller plots (SPs) measuring 24 cm long × 18 cm wide × 20 cm deep and larger plots (LPs) measuring 60 cm long × 40 cm wide × 60 cm deep were employed (Fig. 1). In order to achieve a density of 1.25 g/cm^3 in all plots, they were homogeneously packed with the air-dried aggregates of diameter less than 5 mm rather than intact soil. They were run outside but shielded from rains and kept bare during this experiment.

Experimental design

Biogas slurry irrigation with a rate of 25 mm/week lasted for 12 weeks, equivalent to 0 (tap water), 144, and 336 kg N/ha for the SPs (r0 to r336) and 0, 48, 144, 240, 336, and 480 kg N/ha for the LPs (R0 to R480). Both the irrigation rate (Fessehazion 2012) and the nitrogen application rates (Amon et al. 2006; Bertora et al. 2008; de la Fuente et al. 2012; Gericke et al. 2012; Köster et al. 2011; Monaco et al. 2011; Quakernack et al. 2012; Svoboda et al. 2013a, b; Win et al. 2014; Wu et al. 2013) are commonly used. SPs had two replicates while the repetition of LPs was impossible to perform due to high consumption of air-dried soil (2.2 to 3.2 tons). This is consistent with some studies suffering from shortages of materials (e.g., soil, wastewater) (Castillo et al. 2001; Hedström and Amofah 2008; Jellali et al. 2010).

Sampling and analysis

Leachate was collected under plots by containers to determine pH (PB-10; Sartorius, Göttingen, Germany), total nitrogen (TN) (MEPC 1990), ammonium nitrogen (NH_4^+-N) (MEPC

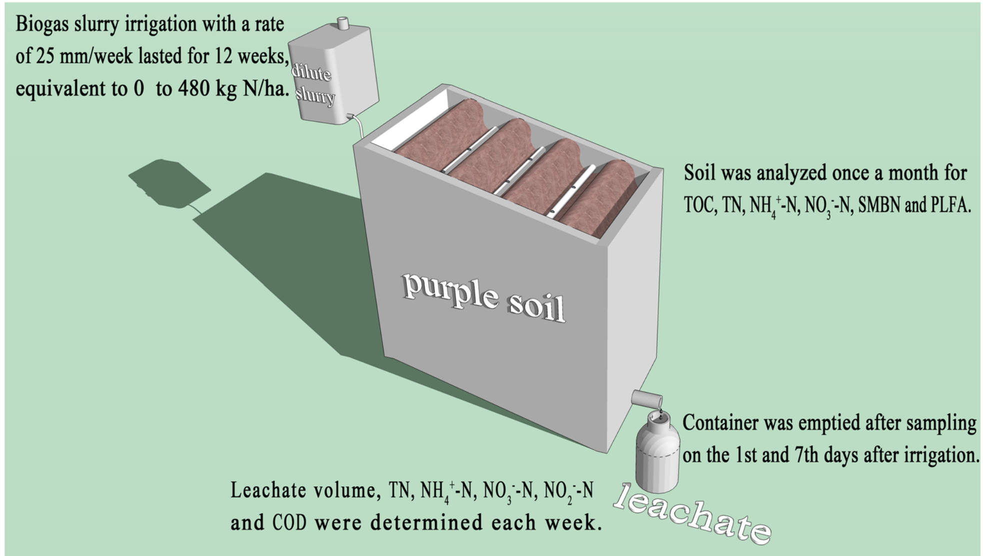

Fig. 1 Experimental plot, design, sampling, and analysis

2009), nitrite nitrogen (NO_2^--N) (MEPC 1987), nitrate nitrogen (NO_3^--N) (CNEMC 2007), and chemical oxygen demand (COD) (COD DRB 200 and COD DR1010; Hach, Loveland, USA). Each parameter was determined by triplicate measurements. Sampling took place on the first day and the last day each week after each irrigation. The first days could represent their weeks as regards the quality of leachate, since they contributed about 100% and 69% to the weekly volumes for LPs and SPs, respectively.

Each layer of soil (0 to 20 cm; 20 to 40 cm; and 40 to 60 cm) was analyzed once a month for $pH_{\text{-water}}$ (1:5) (Pansu and Gautheyrou 2003), total organic carbon (TOC) (Cao et al. 1983), total nitrogen (TN) (Xu 1992), ammonium nitrogen (NH_4^+-N) (MEPC 2012), nitrate nitrogen (NO_3^--N) (MEPC 2012), and soil microbial biomass nitrogen (SMBN) (Cabrera and Beare 1993). Phospholipid fatty acid (PLFA) in the surface soil (0 to 20 cm) sampled from LPs at the end of experiment was determined (Wang et al. 2016). The measurement of any soil indicator was performed in triplicate or quadruplicate.

Statistical analysis

The C respired, N nitrified, TN leached, NO_3^--N leached, and N denitrified were calculated to indicate soil respiration, nitrification, N leaching, NO_3^- leaching, and denitrification, respectively (Cheng et al. 2017). Then, ordination analysis (*Canoco* CCA) was applied, across the gradient of nitrogen application rates, to nitrogen leaching-related indices (e.g., N nitrified, TN leached, and NO_3^--N leached), soil microbial indices (e.g., SMBN, PLFA, and C respired), and experimental treatments (as r0 to r336 and R0 to R480) (Palmer 2015). One-way analysis of variance (ANOVA) followed by post hoc test was used to determine differences between treatments.

Results and discussion

N leaching raised environmental concern

None of total nitrogen loads, leachate nitrogen concentrations (as TN and NO_3^--N), temporal trends in the concentrations, and soil temperature conditions were suggestive of low risks of N leaching, when the nitrogen application rates of 336 and 480 kg N/ha had been employed (as r336, R336, and R480).

N leaching was relatively undesirable, given total nitrogen loads accounting for $13 \pm 3\%$ and $20 \pm 11\%$ of the nitrogen applied to SPs and LPs, respectively. These proportions, along with 16 to 30% for the bare soils (Cheng et al. 2017; Sänger et al. 2011), varied in excess of 1 to 12% for the soils with herbs (Matsunaka et al. 2006; Svoboda et al. 2013a, b). This is because immediate infiltration of irrigation water left little time for denitrification of soil NO_3^- and no chance of NO_3^- uptake was provided in this study. However, if restored, the nitrogen uptake might blind one to the environmental impacts and risks of biogas slurries per se.

N leaching raised environmental concerns as the nitrogen application rate achieved 336 kg N/ha (r336, R336, and R480). Meanwhile, the leachate TN became significantly higher than the counterparts in the other treatments (Sig.$<$ 0.05, Fig. 2(a and b)), together with the leachate NO_3^--N of 23.07 ± 18.12 mg/L and 45.47 ± 22.08 mg/L for SPs and LPs, respectively (Fig. 2(e and f)). The NO_3^--N concentrations were far short of the reported around 100 mg/L (Goberna et al. 2011). However, substantial proportions of them were beyond the local threshold for groundwater (30 mg/L) (MEPC 1994), 30% and 73% for SPs and LPs, respectively; and the nitrate can have an easy access to groundwater due to the limited depths of purple soil. In addition, N leaching (leachate TN and NO_3^--N) was positively correlated to nitrogen application rates (N applied) and in close proximity to the higher ones (r336, R336, and R480) as demonstrated in ordination analysis (Fig. 4). In fact, nitrogen application rates of over 336 kg N/ha are highly likely to occur in the Three Gorges Reservoir Area employing an average rate of 287 kg N/ha, and particularly in the upstream regions where flooded arable lands (or paddy fields) receiving little biogas slurry take, however, larger proportions than their counterparts in the downstream regions; accordingly, groundwater quality in the upstream area is worth investigating in further studies. The average rate was estimated according to that 20% of about 350,000 t of the manure TN load produced by livestock and poultry is processed in anaerobic digesters and then applied to a total area of 243,940 ha of available and arable lands (Chen et al. 2008; Huang et al. 1999, 2017; Wei et al. 2013; Yang et al. 2012).

N leaching increased over time once the higher rates of nitrogen were applied (r336, R336, and R480), as indicated by very high correlations between leachate TN and time ($R^2 > 0.91$, Fig. 2(a and b)). Leachate TN increased by 4.07, 2.57, and 7.97 mg/(L × week) for r336, R336, and R480, respectively. Such high correlations were interesting but seldom reported.

The N leaching could be even greater due to increased soil temperatures and intensified application of biogas slurries. Soil temperatures were recorded three times per day as 9.8 ± 2.6 °C and 10.0 ± 2.8 °C for SPs and LPs, respectively. They were much colder than 25 to 35 °C, the optimum range for nitrification promoting N leaching (Harmsen and Kolenbrander 1965). Therefore, the observed N leaching was conservative compared with its potential. In addition, it could be enhanced during autumn plowing in the Three Gorges Reservoir Area, provided that this monsoon season has witnessed air temperatures averaging out to 32 °C (TQW 2018) and a vast majority of annual output of biogas slurries being applied.

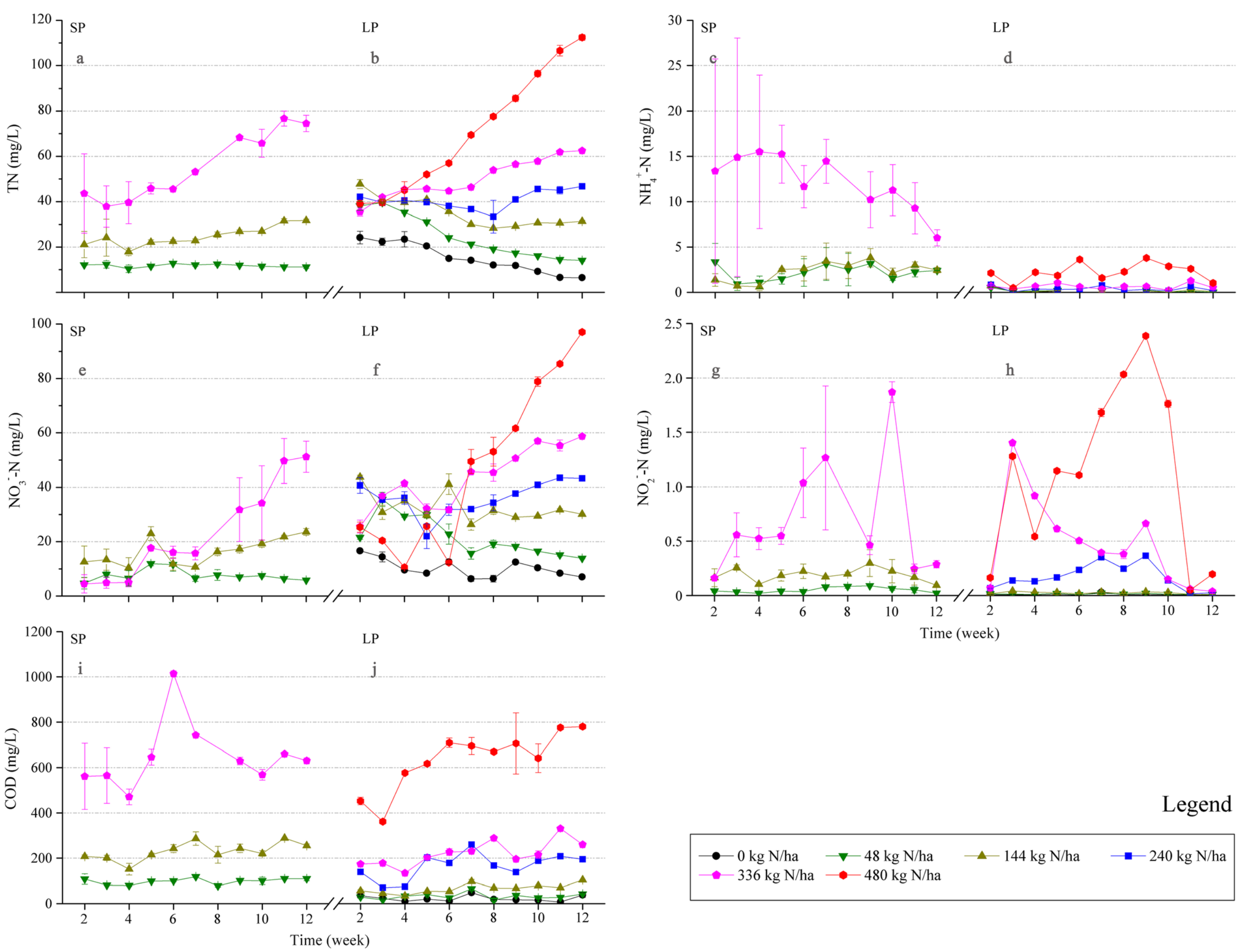

Fig. 2 Nitrogen content (as total nitrogen, ammonium nitrogen, nitrate nitrogen and nitrite nitrogen, a to h) and chemical oxygen demand (COD, i and j) of leachate from smaller plots (SP) and larger plots (LP) by nitrogen application rates. Leachate was sampled in the first day after each irrigation; and sampling did not take place in the first week; and analysis was not performed for the treatment r336 in the eighth week

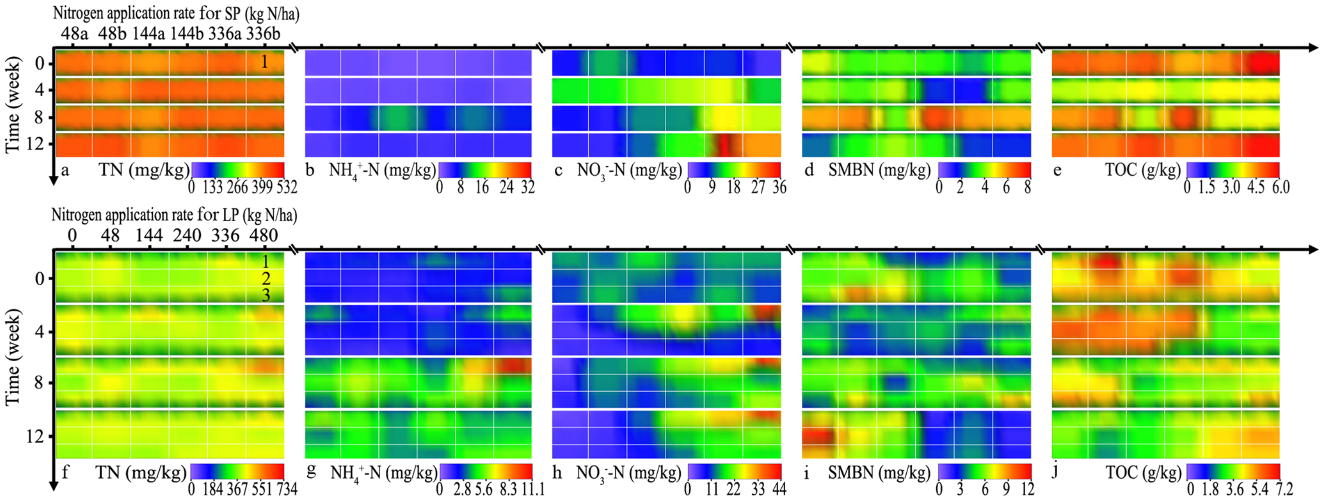

Fig. 3 Nitrogen content (as total nitrogen, ammonium nitrogen, nitrate nitrogen and soil microbial biomass nitrogen) and total organic carbon (TOC) of soil within smaller plots (SP, a to e) and larger plots (LP, f to j) by nitrogen application rates. Numbers *1*, *2*, and *3* indicate layers of 0 to 20 cm, 20 to 40 cm, and 40 to 60 cm, respectively (a and f); and letters *a* and *b* following a nitrogen application rate indicate its replicates (a to e)

N leaching was due to soil nitrification

The escalated N leaching accompanied by the higher nitrogen application rates was because of the high potential of digestate NH_4^+ to be nitrified into the very mobile NO_3^- in soils (Abubaker et al. 2012, 2013; Alburquerque et al. 2012; Goberna et al. 2011; Gomez-Brandon et al. 2016; Grigatti et al. 2011; Johansen et al. 2013; Sanger et al. 2014; Sawada and Toyota 2015; Senbayram et al. 2009).

It indicated soil nitrification that the predominant nitrogen changed from NH_4^+ in biogas slurry into NO_3^- in leachate. The principal form was NH_4^+ accounting for $80 \pm 13\%$ of TN in the biogas slurries, which fell into 35 to 81% in the reviews (Möller and Müller 2012; Nkoa 2014). But after irrigation, it was transformed into NO_3^- in the leachate with ratios of NO_3^--N to TN being $56 \pm 23\%$ and $90 \pm 20\%$ for SPs and LPs (Fig. 2(a–f)), respectively, in line with several studies (Matsunaka et al. 2006; Sänger et al. 2011; Svoboda et al. 2013a, b). Some percentages exceeded 100% due to the incomplete recovery of the method for determining TN (Cabrera and Beare 1993; Hagedorn and Schleppi 2000).

Another evidence of the nitrification was accumulations of NO_3^- in soil. Decreases in soil NO_3^- continued through the experiment when nitrogen was applied at the rates below 240 kg N/ha. However, as soon as that rate was achieved, they turned into increases accounting for 16% and 22% of the nitrogen inputs to SPs and LPs, respectively (Fig. 4(c and i)).

N leaching would be deterred by enlarged ratio of C/N in biogas slurry

Inactivated heterotrophs were responsible for the soil nitrification and consequent N leaching; therefore, activating them through enlarging the ratios of carbon to nitrogen (C/N) in biogas slurries may deter the N leaching.

Soil heterotrophs were influential in nitrogen dynamics of this study. They are able to break down organic matters to obtain carbon sources and release NH_4^+ during mineralization, thus influencing TOC in arable soils (Waksman and Starkey 1931). They were formidable compared with the carbon inputs through biogas slurries of this study, since interplot standard deviations of carbon applied to LPs accounted for only 4% of that of soil ΔTOC, consistent with a reported finding (Gomez-Brandon et al. 2016). Moreover, the increase of 26 kg N/ha in SMBN coincided with the TOC consumption of 12 t C/ha and the NH_4^+ accumulation of 16.5 kg N/ha in the treatment R0, although no biogas slurry was applied at all.

Soil heterotrophs were inactivated to free N leaching in treatments with the higher nitrogen application rates (r336, R336, and R480), as indicated by the microbial biomass (SMBN and PLFA contents), diversity (Shannon Index of PLFA), and respiration (C respired). These indicators each were negatively correlated to nitrogen application rates (Applied N) and distanced from the higher ones (r336, R336, and R480, Fig. 4), probably due to negative effects on heterotrophs of both a lack of carbon sources and an oversupply of biogas slurry NH_4^+ (Jansson et al. 1955; Kangmeznarich and Broderick 1980; Recous et al. 1990; Ricke and Schaefer 1991). As a result, redundant biogas slurry NH_4^+ for the inactive heterotrophs had promoted autotrophic nitrifiers, nitrification, NO_3^- accumulation, and the consequent N leaching.

The soil heterotrophs would be reactivated to deter N leaching through increasing biogas slurry C/N, because carbon sources assist heterotrophs to compete with autotrophic nitrifiers for NH_4^+ (Kaye and Hart 1997) and to denitrify NO_3^- (Broadbent and Clark 1965). The soil respiration (C

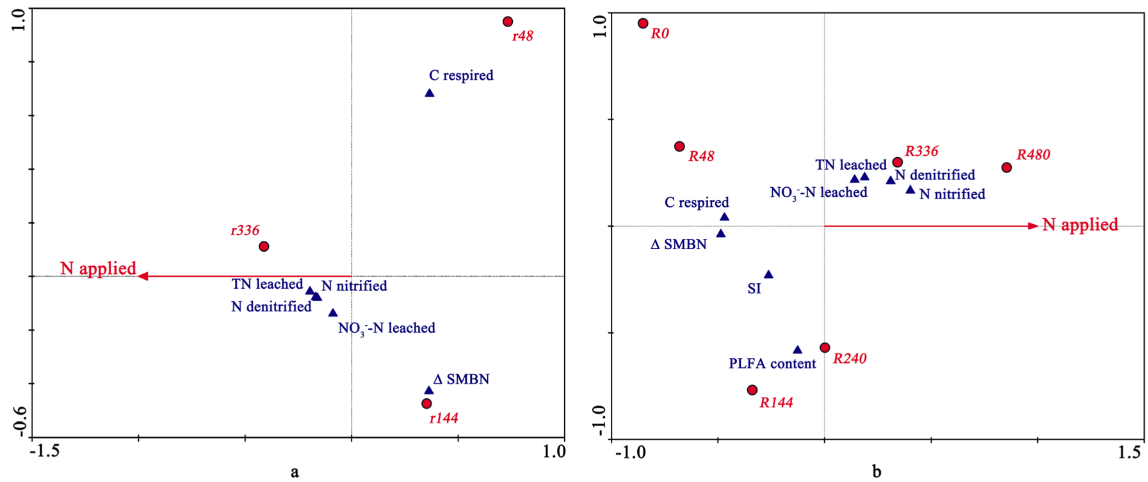

Fig. 4 Ordination analysis (*Canoco* CCA) of nitrogen leaching-related index (as nitrogen nitrified, total nitrogen leached, nitrate nitrogen leached, and nitrogen denitrified), soil microbial index (as Δ SMBN, PLFA content, SI and carbon respired) and experimental treatment (as r0 to r336 and R0 to R480) across the nitrogen application rate gradient for smaller plots (a) and larger plots (b). Note: ΔSMBN indicates the change in soil microbial biomass nitrogen in the surface layer (0 to 20 cm); and SI indicates Shannon Diversity Index of phospholipid fatty acid (PLFA) (b)

respired) was negatively correlated with the N leaching-related indices (as N nitrified, TN leached, and NO_3^--N leached, Fig. 4). In addition, negative correlations also persisted between the ratios of dissolved organic carbon (DOC) to TN in biogas slurries and the soil NO_3^- of an incubation experiment (Alburquerque et al. 2012).

In fact, it has been proved effective in a further test to prevent the N leaching with enlarged biogas slurry C/N ratios (Cheng et al. 2017). However, further confirmations are still needed.

Uncertainties in results

Measures, concerning negative impacts of the repacked soil and the lack of repetition for LPs, were taken to alleviate or recognize uncertainties in the results.

Not until 4 months after packing the plots did the experiment begin. During that time, the plots were exposed to rains. Therefore, soil processes such as mineralization, nitrification, and denitrification could be restored prior to the experiment to avoid unfavorable effects of the air-dried soil on the results (e.g., the priming effect derived from saturation (Kuzyakov et al. 2000)).

Comparability between LPs was monitored during the 4 months, so as to reasonably cancel the usage of LPs once any significant deterioration of it had been detected. The comparability was reflected by interplot standard deviations (SDs) of leachate quality. The leachate was collected after four rains varying from 7 to 32 mm, and analyzed for pH, TN, NH_4^+-N, and NO_3^--N, each of the four SDs averaged out to as low as 0.07, 1.91 mg/L, 0.07 mg/L, and 2.16 mg/L, respectively. As a result of the small SDs, LPs were considered suitable and subsequently put into operation.

SPs were regarded as a practical expedient for repeating LPs or, at least, a means to prove the reliability of the findings from LPs, due to two reasons. SPs were a scaled-down version of LPs. Besides, they repeated some nitrogen application rates of LPs. After experiment, the results from LPs were considered reliable. This is because they bear resemblance to those from SPs in respect of their temporal trends and the influences of nitrogen application rates on them (Figs. 2, 3, 4), and to their counterparts in two further studies including one published (Cheng et al. 2017) with reference to the significant N leaching and the preventive measure that they indicated.

Conclusions

Concern over N leaching in purple soil was raised by the biogas slurry irrigation employing nitrogen application rates of 336 and 480 kg N/ha; and one option is to increase biogas slurry C/N to prevent N leaching, but needs further confirmations.

Considerable percentages, 30 and 73% for SPs and LPs, respectively, of leachate samples were beyond the local threshold for NO_3^- in groundwater. Besides, the observed N leaching might be conservative compared with its potential, considering the soil temperatures remaining around 10 °C which was well below 25 to 35 °C, the optimum range for soil nitrification to produce very mobile NO_3^- and then facilitate N leaching. The upstream regions of the Three Gorges Reservoir Area are of interest to further studies investigating groundwater quality because possibly high nitrogen application rates occur there.

The N leaching-related indices (as N nitrified, TN leached, and NO_3^--N leached) were negatively correlated with the soil microbial biomass (SMBN and PLFA contents), diversity (Shannon Index of PLFA), and respiration (C respired), probably due to the situations for NH_4^+ either feeding autotrophic nitrifiers or heterotrophs and for NO_3^- either being leached or feeding heterotrophic denitrifiers.

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