Agr. Nat. Resour. 54 (2020) 245–254

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Release of plant nutrients and changes in the copies of N-cycling genes in response to soil amendment with rice straw and waste from a food seasoning industry

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Article Info

Article history:
Received 5 July 2018
Revised8 October 2018
Accepted 9 October 2018
Available online 30 June 2020

Keywords:
Decomposition,
Food seasoning waste,
N-cycling genes,
Quantitative polymerase chain reaction,
Rice straw

Abstract

Rice-derived organic matter is an important source of nitrogen for paddy soils, and this limiting nutrient is subsequently redistributed throughout the paddy and moved up the food chain. The effects were investigated of waste from a food seasoning industry (W_0,1,875 L/ha and 3, 125 L/ha) and urea fertilizer (100 kg/ha) on the decomposition of rice straw and the subsequent release of nitrogen, phosphorus, potassium and silicon by soil microbial communities. Soil amended with W_0 and urea fertilizer had a major impact on the decomposition of rice straw. However, biodegradation of rice straw did not result in large effects on the quantities of released organic matter, total nitrogen and available phosphorus, but it significantly ($p < 0.05$) affected the release of exchangeable potassium and available silicon. The abundance levels of 10 N-cycling genes (*amoA-B, nxrA, nxrB, narG, nirS, nirK, cnorB, nosZ3, hdh and hzo*) were also analyzed using quantitative polymerase chain reaction. In all treatments, the quantities of the *nxrB, nirS, nirK* and *nosZ3* genes were greater than for the other genes over the 84d rice straw decomposition period. Moreover, the quantities of the *nirS* and *nirK* genes were greater than those of *nosZ3* and *nxrB*. Nitrogen was estimated to be lost from the soil through nitrite reduction in the form of NO. The application of W_0 and urea fertilizer significantly ($p < 0.05$) increased the abundance of the *nirS* gene after the decomposition of rice straw over the 28d incubation period.

Introduction

Crop residues are substances left in the agriculture field after crops have been harvested and threshed. While these residues represent disposed waste material, they are also natural resource used to maintain soil health and productivity (Kibblewhite et al., 2008). For example, crop residues are known to maintain nutrients for plant growth, prevent erosion, and improve the physical and biological properties of soil (Kumar and Goh, 2000). Crop residues also help increase the organic carbon and nutrient contents of soils and thereby increase crop yields (Hooker et al., 1982). Crop residues add nitrogen

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https://doi.org/10.34044/j.anres.2020.54.3.03

content to soils and in particular, the paddy soil ecosystem plays an important role in the transport of nitrogen in soils (Galloway et al., 2008). The biological nitrogen cycle is one of the most important nutrient cycles in terrestrial ecosystem (Gregory et al., 2001). The benefit of nitrogen transformation is organic nitrogen immobilization to ammonium and nitrate, which supplies nutrition for plants (Haynes and Goh, 1978)

In addition to crop residues, other waste products are also used to amend soils to promote heath and improve productivity (Hossain et al., 2017). Waste products from food seasoning industry are increasing in most countries, including Thailand (Thongjoo et al., 2005). The recycling of waste from food seasoning could beneficially convert surplus farm waste into useful fertilizers to sustain the nutrient requirements of crops. For example, in the process of producing food seasonings, waste from yeast cultures (W_0) is produced and this material contains large amounts of plant nutrients (Chin-Ching et al., 2008). However, most wastes from the food seasoning industry are left unused and are causing environmental problems (Thongjoo et al., 2005).

Recent advances in molecular based approaches at the gene and genomic levels have allowed researchers to analyze the diversity of nitrogen metabolism genes in environmental samples (Taroncher-Oldenburg et al., 2003). Previous studies have examined the abundance of nitrogen metabolism genes from whole microbial communities present in genomic DNA extracted from soils (Kolb et al., 2003), and allowed researchers to target functional genes involved in nitrogen transformation processes including *nifH, amoA, nirS/K* and *nosZ*, which are involved in nitrogen fixation, ammonia oxidization, nitrate reduction, and nitrous oxide reduction, respectively (Francis et al., 2005).

While the addition of crop residues to soils has been shown to bolster the soil total nitrogen content, some of these materials are lost to the atmosphere via these nitrogen transformation reactions, primarily nitrification following denitrification (Lee et al., 2006). Nitrification is a two-step process consisting of the conversion of ammonia to nitrite ($NH_4^+ \rightarrow NO_2^-$) and nitrite to nitrate ($NO_2^- \rightarrow NO_3^-$), where the first step is carried out by ammonia-oxidizing bacteria (*amoA*-B) and ammonia-oxidizing archaea (*amoA*-A), while the second step is mediated by nitrite-oxidizing bacteria (*nxrA*) and nitrite-oxidizing archaea (*nxrB*) (Nicol et al., 2008). Ammonia-oxidizing bacteria and archaea also function in the rate-limiting step of nitrification and regulating the nitrogen dynamics in soil (De Boer and Kowalchuk, 2001). While nitrate in soil supplies nutrition for plants, it can also increase the mobility of nitrogen to groundwater through nitrate leaching (Singh and Sekhon, 1979). In contrast, denitrification is the reduction of nitrate to nitrogen gas (N_2), and this process is stimulated by high soil moisture conditions with limiting oxygen, and available nitrate and organic to drive activity of denitrifying microorganism (Luo et al., 1999).Complete denitrification is a four step process consisting of nitrate reduction ($NO_3^- \rightarrow NO_2^-$), nitrite reduction ($NO_2^- \rightarrow NO$), nitric oxide reduction ($NO \rightarrow N_2O$) and nitrous oxide reduction ($N_2O \rightarrow N_2$), which are carried out by microbes using the enzymes encoded by *narG*, *nirS/K*, *cnorB* and *nosZ*. The abundance of the *nosZ* gene has been used to estimate the denitrifier density (Ruiz-Rueda et al., 2009).

Anaerobic ammonium oxidation (anammox), in which ammonium is converted directly to nitrogen gas with nitrite as the electron acceptor, is performed under anoxic conditions ($NH_4^+ \rightarrow N_2$) by anaerobic ammonium oxidizing bacteria (Mulder et al., 1995). The rate of this process can be estimated by the quantification of *hdh* and *hzo* genes.

It is important to determine the benefits and potential adverse effects of the addition of rice straw and food seasoning industry waste to soils before the practice is recommended to farmers for adoption. Therefore, the objectives of the present study were to determine the effects of waste from food seasoning industry on: 1) the decomposition of rice straw and the release of plant nutrients and 2) the abundances of N-cycling genes during the decomposition of rice straw.

Materials and Methods

Soil, rice straw and food seasoning waste

Soil samples were collected from Bang Sai, Ayutthaya, Thailand from the 0-15 cm depth, air-dried and sieved to 2 mm to remove coarse plant debris. After collection and thorough mixing, soil samples were dried, ground and analyzed for pH, electrical conductivity (EC), organic matter using the Walkley-Black procedure (Walkley and Black, 1934), total nitrogen using the Kjeldahl method (Jackson, 1965), available phosphorus using the Bray II method (Bray and Kurzt, 1945), exchangeable potassium using extraction in 1M NH_4OAc at pH 7.0 (Pratt, 1965) and available silicon using extraction in 0.01 M $CaCl_2$ (Thomas et al., 2011).

Rice (*Orysa sativa* L.) straw was sampled from plots after grain harvest, dried at 70°C, and cut into 5 cm lengths. This straw was obtained from the photoperiod-insensitive rice cultivar RD31 whose chemical properties included total nitrogen (1.10%), total phosphorus (0.07%), total potassium (1.17%) and total silicon (3.42%).

Food seasoning industry waste (W_0) was obtained from Thai Foods International Co., Ltd., Bangkok, Thailand. The waste (W_0) was produced during the production of mono sodium glutamate. The $W_0$was a yeast liquid supernatant (excluding yeast cells) from a separate glutamate process and contained 0.02% total nitrogen, 0.056% total phosphorus, 0.309% total potassium and 0.07% total silicon content at an initial pH of 5.49and EC of 41.5 dS/m.

Rice straw decomposition and nutrient release

The experiment was conducted in a greenhouse at Kasetsart University, Bangkhen Campus, Bangkok, Thailand from 31 July 2015 to 23October 2015. The decomposition of the rice straw and nutrient (N, P, K and Si) release were studied using nylon litterbags (Louiser and Parkinson, 1979). The experimental study pots were arranged in a complete randomized design (CRD), with three replicates. Five treatments were investigated: control pots (without rice straw), two rice straw decomposition pots (without W_0 [RS] and with W_0 1,875 L/ha (RSW [1,825]), and two rice straw decomposition pots with W_0 plus urea fertilizer (W_0 1,875 L/ha + urea 100 kg/ha

(RSW [1,825]+U) and W_0 3,125 L/ha+ urea 100 kg/ha (RSW [3,125]+U). A 30 kg aliquot of air-dried soil was added to each plastic basin (65 cm diameter, 22 cm height), and 22 g of rice straw was weighed into litter bags (15 × 20 cm with a mesh size of 2 mm to exclude macro fauna). Litterbags were placed on the soil surface and soil was strewn on the top of the litterbags to imitate farming practices for incorporating the rice straw into the soil. All pots were moistened with the same amount of water then added with W_0 and urea fertilizer. Finally, all pots were flooded to maintain the water level at 5 cm above the soil surface. The litterbags and soil were collected at days 7, 14, 28, 42 and 84 after decomposition. The weight loss of the decomposing rice straw was determined after washing and drying the remaining rice straw at 60°C. After collection and through mixing, each soil sample was dried, ground and analyzed for pH, EC, organic matter, total nitrogen, available phosphorus, exchangeable potassium and available silicon.

Measurement of the abundance of various N-cycling genes (amount of nitrifying bacteria, denitrifying bacteria and anaerobic ammonium oxidizing bacteria)

The soil under the litterbags was collected at days 14, 28, 42 and 84 after decomposition, followed by thorough mixing and stored at -80°C prior to DNA extraction.

DNA was extracted in duplicate from 0.4 g aliquots of soil from each sample using the DNeasy Power Soil kit (QIAGEN; Hilden, Germany) according to the manufacturer's instructions. DNA concentrations were measured using a BioPhotometer (Eppendorf; Enfield, CT, USA). The DNA samples were 10-fold diluted and stored at -20°C.

The 16S rRNA and 10 N-cycling genes (*amoA*-B, *nxrA*, *nxrB*, *narG*, *nirS*, *nirK*, *cnorB*, *nosZ3*, *hdh* and *hzo*) were quantified using quantitative polymerase chain reaction (qPCR)as previously described (Tomasek et al., 2017) (Table 1). Nitrification gene abundances were determined for *amoA*-B (primers amoA-1F/amoA-2R; Rotthauwe et al., 1997), *nxrA* (primers F1norA/R2norA; Wertz et al., 2008) and *nxrB* (primers nxrB169f/ nxrb638r; Pester et al., 2014). Denitrification gene abundances were determined for *narG* (primers narG-1960m2fE/narG2050m2R; López-Gutiérrez et al., 2004), *nirS* (primers m-cd3AF/m-R3cd; Kandeler et al., 2006), *nirK* (primers nirK876F/1040R; Henry et al., 2004), *cnorB* (primers cnorB-BF/cnorB-BR; Dandie et al., 2007) and *nosZ3* (primers nosZ2F/nosZ2R; Henry et al., 2006). Anammox gene abundances were determined for *hzo* (primers HZOQPCR1F/ HZOQPCR1R; Long et al., 2013) and *hdh* (primers hdh-1-F/ hdh-2-R; Wang et al., 2016). For each gene, a standard curve was generated using gBlock Gene Fragment synthesized by Integrated DNA Technologies, Inc. (Coralville, IA, USA). Dilutions were determined based on the required copy numbers which contained 3,000,000, 300,000, 30,000, 3,000, 300, and 30 copies. Quantification was based on the fluorescence intensity of the SYBR Green Super mix, and reactions for each sample were carried out in the RocheLightCycle480II machine (Roche, Mannheim, Germany). Each assay was conducted in a Light Cycler 480 Multi well Plate 96 (Roche, Mannheim, Germany)

Table 1 Primers and quantitative polymerase chain reaction (qPCR) parameters

Gene name	Primer set	Primer sequence (5'→3')	qPCR specifications	qPCR protocol	Size
BAC515F	U515F	GTGCCAGCMGCCGCGGTAA	0.8 µL each primer	(95°C:30s, 50°C:30s, 72°C:30s)x 40	292
	U806R	GGACTACHVGGGTWTCTAAT	10^{-2} dilution		
amoA-B	amoA-1F	GGGGTTTCTACTGGTGGT	0.5 µL each primer+1.6 µL $MgCl_2$	(95°C:45s, 57°C:30s, 72°C:1m)x 45	491
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC	1 dilution		
nxrA	F1norA	CAGACCGACGTGTGCGAAAG	0.5 µL each primer	(95°C:30s, 55°C:45s, 72°C:40s)x 45	323
	R2norA	TCC ACA AGG AAC GGA AGG TC	10^{-1} dilution		
nxrB	nxrB169f	TACATGTGGTGGAACA	1.2 µL each primer+1.6 µL $MgCl_2$	(95°C:45s, 56°C:45s, 72°C:45s)x 45	485
	nxrB638r	CGGTTCTGGTCRATCA	10^{-1} dilution		
narG	narG_1960m2fE	TAYGTSGGGCAGGARAAACTG	0.5 µL each primer	(95°C:15s, 57°C:30s, 72°C: 15s)x 45	109
	narG_2050m2R	CGTAGAAGAAGCTGGTGCTGTT	10^{-1} dilution		
nirS	m-cdAF	AACGYSAAGGARACSGG	2 µL each primer	(50°C:2m, 95°C:30s, 63°C58°→C	407
	m-R3cd	GASTTCGGRTGSGTCTTSAYGAA	10^{-1} dilution	(-1°C/cycle):30s, 58°C:30s, 72°C:30s, 83°C:30s)x 55	
nirK	nirK876F	ATYGGCGGVCAYGGCGA	0-5 µL each primer	(95°C:30s, 63°C58°→C (-1°C/cycle): 30s, 58°C:30s,	164
	nirK1040R	GCCTCGATCAGRTRTGGTT	10^{-1} dilution	72°C:30s)x 51	
cnorB	cnorB-BF	AIGTGGTCGAGAAGTGGCTCTA	0.3 µL each primer+0.4 µL 20 mg/mL BSA	(95°C:15s, 60°C:30s, 72°C:15s)x 45	177
	cnorB-BR	TCTGIACGGTGAAGATCACC	10^{-1} dilution		
nosZ3	nosZ2F	CGCRACGGCAASAAGGTSMSSGT	1.2 µL each primer	(95°C:30s, 65°C60°→C (-1°C/cycle): 30s, 60°C:30s,	267
	nosZ2R	CAKRTGCAKSGCRTGGCAGAA	10^{-1} dilution	72°C:30s)x 51	
hzo	HZOQPCR1F	AAGACNTGYCAYTGGGGWAAA	1.0 µL each primer+1.6 µL $MgCl_2$	(95°C:45s, 53°C:45s, 72°C:35s, 75°C:35s)x 50	224
	HZOQPCR1R	GACATACCCATACTKGTRTANACNGT	1 dilution		
hdh	Hdh-1-F	GGTGGTTTGAGGGGTTCCAA	0.5 µL each primer	(95°C:30s, 54°C:30s, 72°C:30s)x 45	338
	Hdh-2-R	TATGGCGACCTCTGTGCATC	10^{-1} dilution		

with three replicates for each standard, negative control and DNA template. Amplification was performed in a total reaction volume of 20 µL containing 10 µL of 2 × iTaq™Universal SYBR® Green Super mix, 5 µL of DNA template or gBlock Gene Fragment and the forward and reverse primers. $MgCl_2$ and bovine serum albumin (BSA) concentrations were optimized (Table 1). Nuclease-free water was added to bring the reaction volume to 20 µL.

Bacterial 16S rRNA gene (primers U515F/U806R; Caporaso et al., 2012) and N-cycling genes fragments were amplified using the thermo cycling conditions described in Table 1. All reactions were carried out a melting curve at 90°C for 5s, 60°C for 3s and cooling at 40°C for 30 s.

The qPCR efficiency (E) was calculated according to the equation $E= [10^{(-1/slope)}]-1$. The PCR reaction runs had efficiencies of 96%, 88%, 92%, 96%, 87%, 90%, 80%, 88%, 82%, 87% and 86% for the 16S rRNA, *amoA-B, nxrA, nxrB, narG, nirS, nirK, cnorB, nosZ3, hdh* and *hzo*, respectively. The R^2values of all standard curves were greater than 0.99. The no-template controls resulted in undetectable values in all samples. Gene copy numbers were reported as per gram of dry soil.

Statistical analysis

Data were analyzed using the Statistical Tool for Agricultural Research (STAR) routine for a CRD (Clewer and Scarisbrick, 2001). Treatment effects were compared using F tests, while multiple comparisons of mean were assessed using Duncan's new multiple range test (Duncan, 1955). Significance differences between treatments were tested at $p < 0.05$. The effect of decomposition of rice straw on nutrient release and N-cycling genes was tested using linear regression analysis.

Results and Discussion

Soil properties

The chemical properties of the tested soil included total nitrogen (0.18%), soil organic matter (3.38%), available phosphorus (0.004%), exchangeable potassium (0.018%) and available silicon (0.016%) at an initial soil pH of 4.55and EC of 0.70 dS/m.

Rice straw decomposition

During the study period, a rapid decrease in the dry weight of the rice straw was observed in all treatments at day 14 (Fig. 1). However, no statistically significant differences were found in the decomposition rate in the treatment of rice straw supplemented with W_0 (3,125 L/ha) and urea fertilizer compared with the treatment of rice straw supplemented with W_0 (1,825 L/ha) and urea fertilizer. The dry weight of the rice straw in the litterbag supplemented with W_0 (3,125 L/ha) and urea fertilizer decreased more than for the other treatments in the period 28–84 d and was significantly different from the other treatments. This may have been due to the W_0 containing yeast and some nutrients (Chin-Ching et al., 2008) which increased the decomposition rate. The results showed that the decomposition rate in rice straw supplemented with W_0 (1,875 L/ha) and urea fertilizer was higher than that of rice straw with W_0 (1,875 L/ha) but not by a significant amount during study period.

Nevertheless, in the current study, the decomposition rate may not have replicated conventional farming practice for several seasons. Permeable bags with a mesh size of 2 mm were used in the study to exclude macro fauna and consequently, the decomposition rate might have been much lower than in a rice field and the abundance

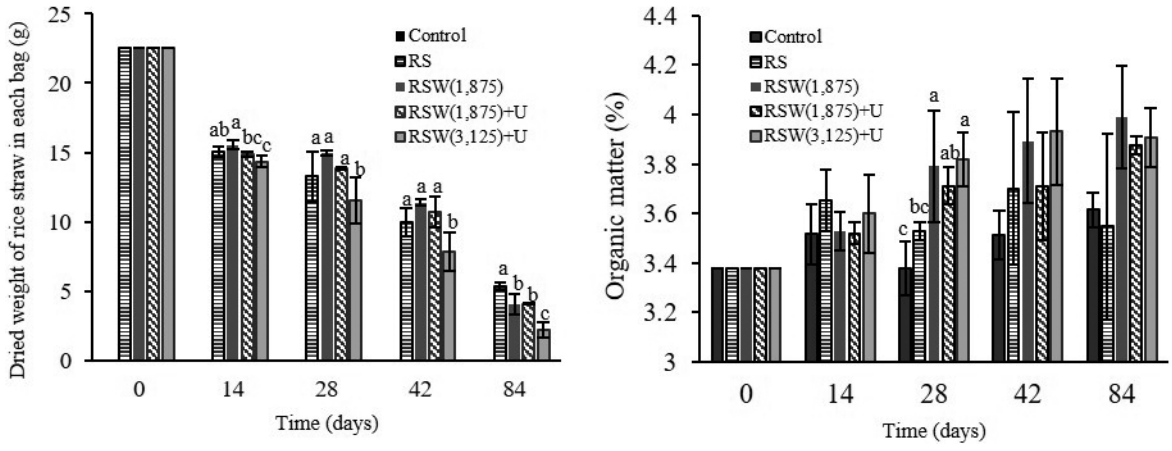

Fig. 1 Histograms showing mean of (A) remaining rice straw; (B) organic matter throughout the decomposition period using litterbags; error bars represent ± SD ($n = 3$); different lowercase letters above bars indicate significant differences ($p < 0.05$) at each time point; a set of histograms without superscription denote non-significant difference among means ($p > 0.05$); Control = without rice straw; RS= rice straw; RSW(1,875)= W_0 1,875 L/ha, RSW(1,875)+U= rice straw with W_0(1,875) plus urea fertilizer; RSW (3,125)+U= rice straw with W_0(3,125)+plus urea fertilizer

of invertebrates might be higher in rice fields with straw amendment. The impact of such factors would depend on the mode of residue application since scattering residues on the surface might have favored different groups of invertebrates from those more suited to the material being incorporated into the soil. The abundance of functional groups of invertebrates and their relative contribution to decomposition would vary over time with stronger effects of invertebrates and faster decomposition rates at the beginning of the season (Schmidt et al., 2015). The dynamics of nutrient cycling are probably different and correlated with decomposition rate.

Soil organic matter content

The results showed no difference in the soil organic matter (SOM) content in all treatments after day 14 (Fig. 1). The SOM content in the treatment of rice straw supplemented with W_0 (3,125 L/ha) and urea fertilizer was significantly higher than the control, rice straw without $W_0$and rice straw with W_0 (1,875 L/ha) after 28 days. However, the SOM content in the treatment of rice straw supplemented with W_0 (3,125 L/ha) and urea fertilizer was not significantly different from that supplemented with W_0 (1,875 L/ha) and urea fertilizer. The results of the SOM content analysis associated with the rice straw decomposition experiment indicated there was a higher rate of decomposition of rice straw in the treatment supplemented with W_0 (3,125 L/ha) and urea fertilizer. These experimental results were similar to those reported by Chin-Ching et al. (2008) who indicated that the soluble organic carbon fraction increased in soil amended with green manure containing wastewater from the food seasoning industry.

Soil pH and electrical conductivity

The soil pH of all treatments decreased during days 0–14 (Fig. 2), but these differences in soil pH were not significant. It was possible that the decrease in soil pH was due to the production of organic acids from plant residues (Gotoh and Onikura, 1971). During days 28–84, the results showed that the soil pH increased in all rice-straw pot treatments [RS, RSW (1,825), RSW (1,825) + U, RSW (3,125) + U]. These pH levels were significantly higher than those of the control. The subsequent increase in soil pH was likely due to decarboxylation

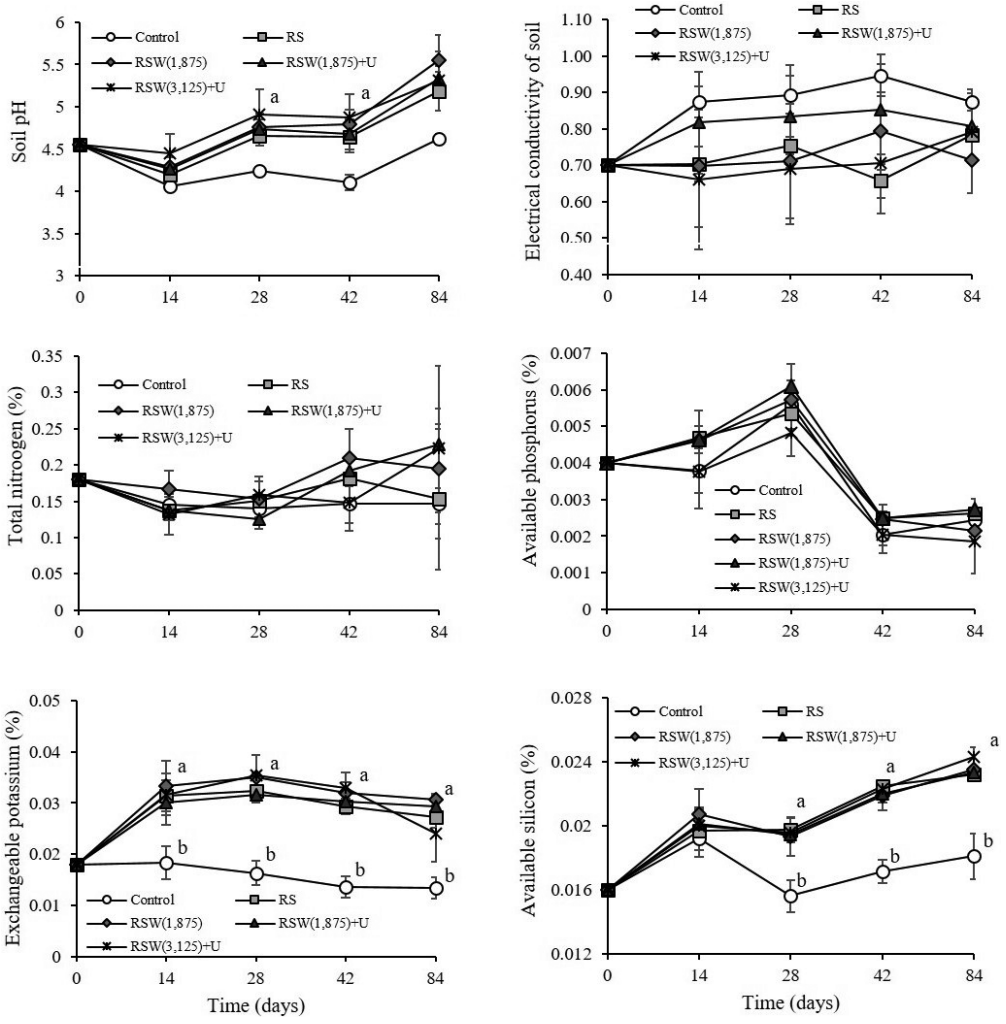

Fig. 2 Mean of (A) pH; (B) electrical conductivity; (C) total nitrogen; (D) available phosphorus; (E) exchangeable potassium; (F) available silicon throughout the straw decomposition period over time; error bars represent ± SD (n = 3); different lowercase letters indicate significant differences ($p < 0.05$) at a time point

of organic anions by microorganisms in the water logged soil(Raison, 1979) and proton consumption by ammonification of nitrogen in the plant residue (Wong and Swift, 2003).During days 0–14, the quantities of nitrogen-cycling genes decreased and then slightly increased after day 14 (Fig. 4). Most microorganisms grow best around neutral pH values of 6.5–7.0, as the soil pH influences the microbial community and biomass composition with a pH less than 4.5inhibitingmicrobial activity (Johannes et al., 2009). Linear regression analysis revealed that the amount of organic matter significantly affected the soil pH (Fig. 3). The EC values in all treatments were not significantly different *throughout* the *study* (Fig. 2).

Nutrient release

The total nitrogen content of the soil decreased during the initial 28 d and increased afterward, but was not significant different (Fig. 2). A decrease in total nitrogen likely occurred because the addition of plant residues with a high C:N ratio may have immobilized nitrogen (Kumar and Goh, 2000). Bacon (1987) reported that incorporation of rice straw into the soil after harvest led to slower decomposition and immobilization of nitrate.

The addition to soil of waste from the food seasoning industry did not significantly affect the available phosphorus content in the soil (Fig. 2). During days 0–28, available phosphorus increased but afterward, the available phosphorus substantially decreased because iron and aluminum were released after flooding the acid soil (Patrick Jr. and Mahapatra, 1968). *Phosphate* precipitated with *iron and aluminum* in the soil creates iron and aluminum phosphate minerals (Patrick Jr. and Mahapatra, 1968). The current experimental results were similar to those reported by McLaughlin et al. (1988) who indicated that crop residue may not significantly enhance phosphorus in soils over the short-term.

During days 14–84, all rice straw decomposition pots (RS, RSW [1,875], RSW [1,875]+U, RSW [3,125]+U) had significantly higher potassium release than those of the control treatment (without decomposition of rice straw) as shown in Fig. 2, since rice straw contains high levels of potassium (Witt et al., 1999). These experimental results were consistent with Verma and Bhagat (1992) who reported that the incorporation of rice straw in wheat led to a slight increase in the availability of phosphorus, manganese and zinc and an observed increase in the availability of potassium. Yadvinder-Singh et al. (2004) also reported that incorporation of residual crops caused an increase in the exchangeable potassium contents in soil, but these results differed from Tian et al. (1992), who reported that most of the potassium in the rice residue was released in less than 41 d.

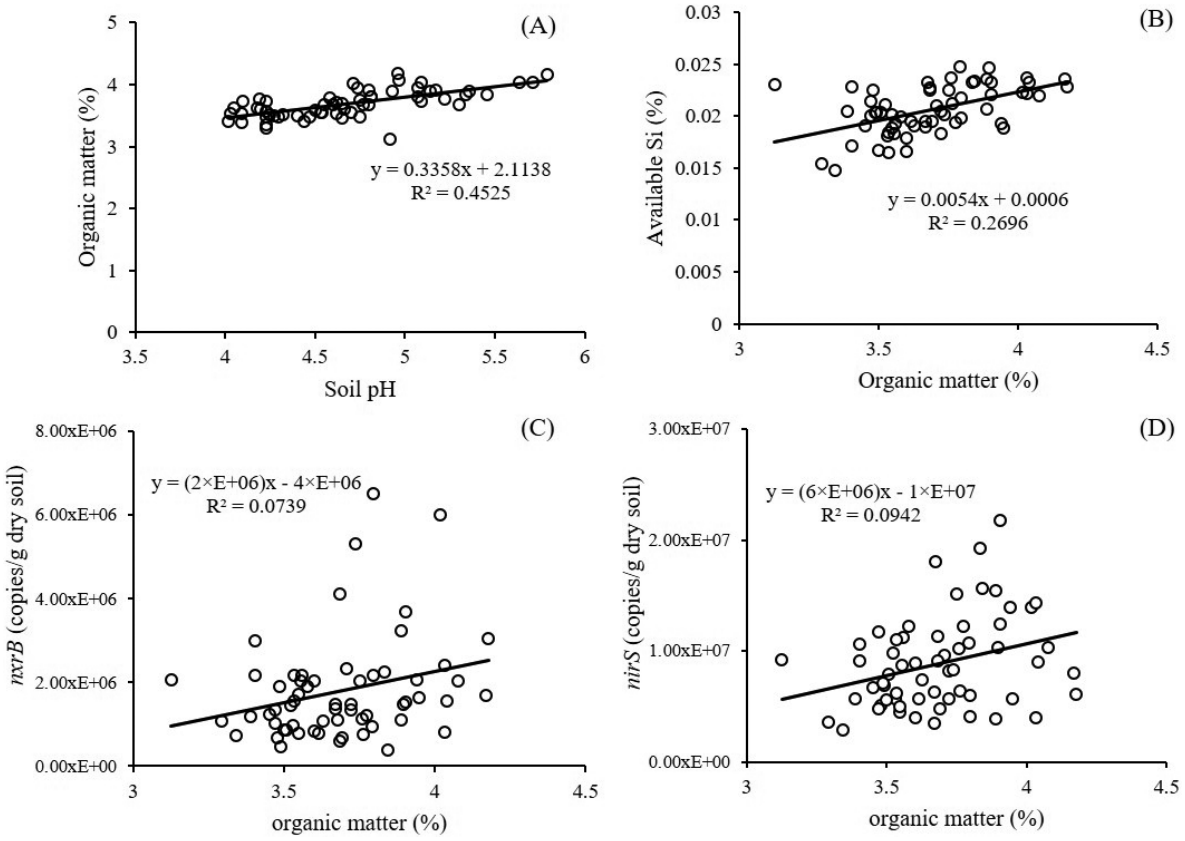

Fig. 3 Regression of (A) soil pH on organic matter; (B) organic matter on silicon release; (C) organic matter on quantity of *nxrB* gene; (D) organic matter on quantity of *nirS* gene, where all explanatory variables are significant in models at $p < 0.05$ and R^2 is the coefficient of determination

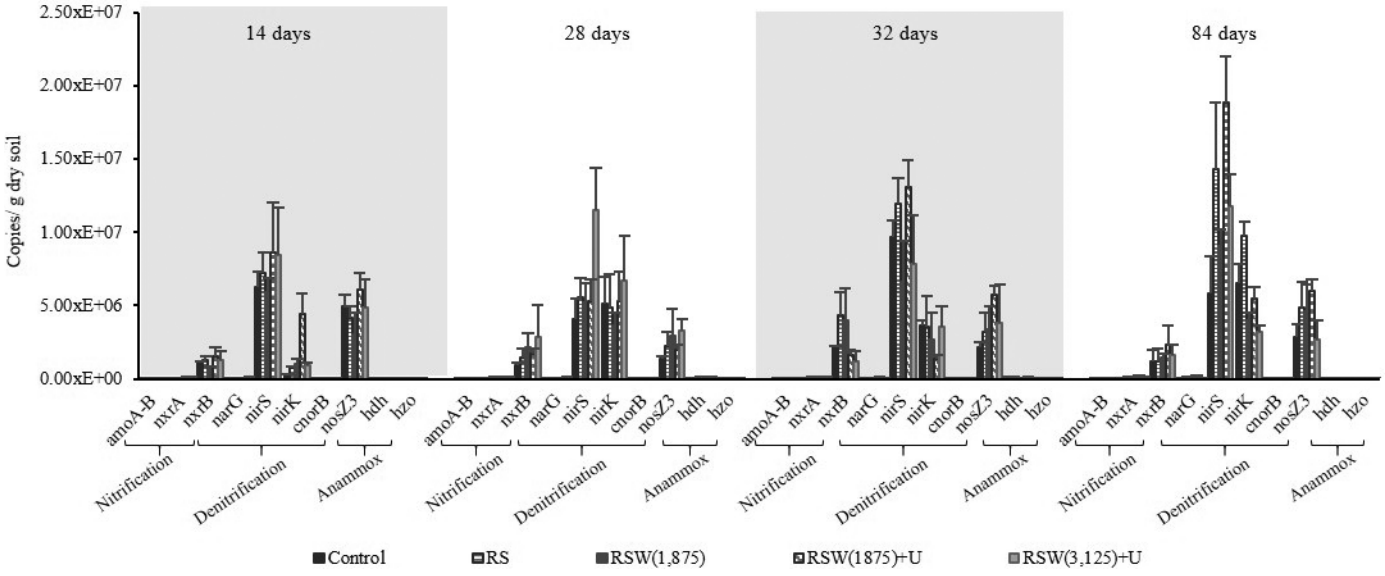

Fig. 4 Histograms showing mean of abundance of *amoA-B, nxrA, nxrB, narG, nirS, nirK, cnorB, nosZ3, hdh* and *hzo* genes in each treatment across 84 d decomposition of rice straw; error bars represent ± SD (n = 3)

The available silicon content of the soil was significantly greater in all rice straw decomposition pots (RS, RSW [1,875], RSW [1,875] + U, RSW [3,125] + U) compared to the control (Fig. 2). Linear regression analysis revealed that the amount of organic matter under the litterbags significantly affected silicon release (Fig. 3).

Quantification of the abundance of each N-cycling gene in response to different rice straw management

The quantities of 10 N-cycling genes fluctuated over the 84 d study period in all treatments (Fig. 4). The quantities of the *nxrB,nirS, nirK* and *nosZ3*genes were significantly greater than for the other genes in all treatments after 84 d (Fig. 4). Linear regression analysis revealed that the amount of organic matter in litterbags significantly increased the quantity of the *nxrB* and *nirS* genes (Fig. 3), but it did not affect the quantity of the *nirK* gene. During 0–84 d, the quantities of *nxrB* genes were significantly greater than for the *amoA–B* and *nxrA* genes. Per et al. (2012) reported that archaea were more abundant in acid soil than bacteria and archaea were an important group of microorganisms that controlled ammonium oxidation.

After incubation for 14 d, the quantity of the *nirS* gene was significantly greater than for the *nosZ3, nirK* and *nxrB* genes, respectively, but these differences were not significant (Fig. 5).

After 28 d, the quantities of the *nirK* and *nxrB* genes had increased from 14 d, but the quantities of the *nirS* and *nosZ3* genes had decreased (Fig. 5). The quantities of the *nxrB, nirK* and *nosZ3*genes in the different treatments were not significantly different. However, rice straw with W_0 (3,125 L/ha) and urea fertilizer had significantly greater quantities of *nirS* than the other treatments. The quantities of the *nxrB*, *nirS* and *nosZ3*genes in all treatments increased after 42 d, but the quantity of the *nirK* gene decreased (Fig. 5). Rice straw without W_0 and with W_0 (1,875 L/ha) had significantly greater amounts of the *nxrB* gene than the other treatments. Rice straw with W_0 (1,875 L/ha) and urea fertilizer had significantly greater amounts of the *nirS* gene than rice straw with W_0 (1,875 L/ha) and with W_0 (3,125 L/ha) and urea fertilizer. The quantities of the *nirK* and *nosZ3*genes in the different treatments were not significantly different.

After 84 d, the amount of the *nirS* gene was significantly greater than for the other genes (Fig. 5). Rice straw with W_0 (1,875 L/ha) and urea fertilizer had a significantly greater amount of the *nirS* gene than rice straw with W_0 (1,875 L/ha), rice straw with W_0 (3,125 L/ha) and urea fertilizer and the control. The abundance of the *nxrB* gene decreased, but the amount of the *nirK* gene were increased from day 14. However, the abundance of the *nirS* gene remained stable for all treatments. The amounts of the *nxrB, nirK* and *nosZ3*genes in the different treatments were not significantly different.

The abundance of the *nirS* gene was greater than for the other genes after 84 d (Fig. 5). Denitrification was always greater than nitrification and anammox after 84 d. Beauchamp et al. (1989) reported that carbon availability was one of the most important factors controlling the denitrification rate. Carbon availability fuels the majority of microbial processes and was an important source of electron donors in denitrification (Orr et al., 2007). Moreover, the litterbags were placed on the soil surface to prevent oxidation by oxygen. This layer induces more nitrification and denitrification than an ammox. Strous et al. (1997) reported that the anammox process was completely inhibited by the ammonium and nitrite concentration and oxygen. The abundance of nitrite reduction genes (*nirS* and *nirK*) was greater than the nitrous oxide reduction genes (*nosZ3*). During decomposition of the rice straw, the nitrogen in the soil was changed to nitric oxide (NO) by the nitrite reduction genes (*nirS* and *nirK*); however, this differed from Gillam et al. (2008), who reported that

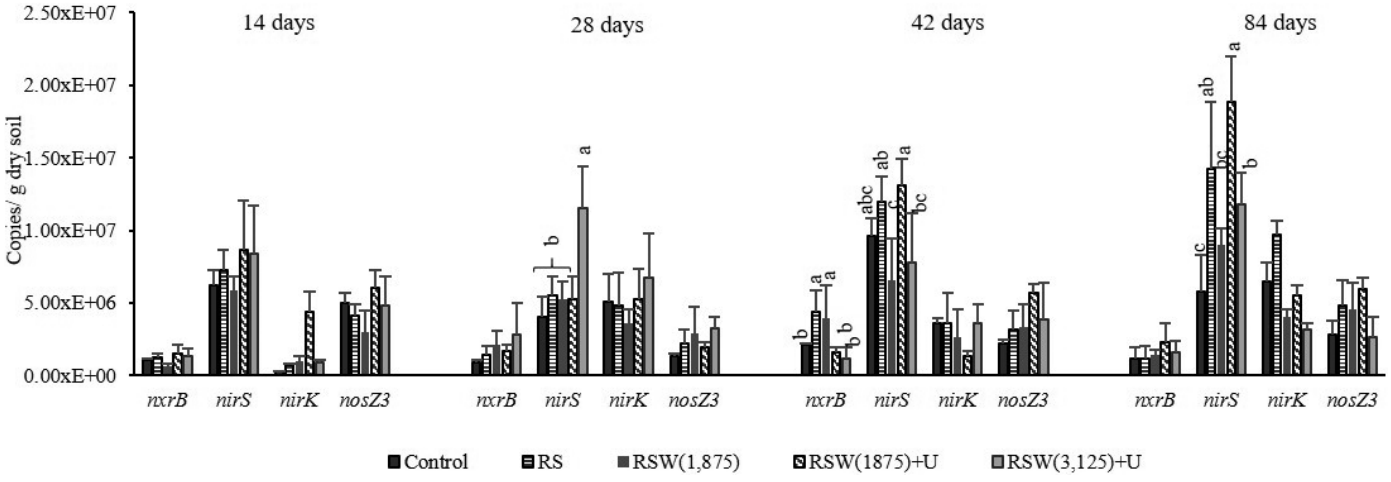

Fig. 5 Histograms showing mean abundance of *nirS, nirK, nosZ3 and nxrB* genes in each treatment across 84 d decomposition of rice straw; error bars represent ± SD ($n = 3$); different lowercase letters above bars indicate significant differences ($p < 0.05$) among mean within a gene; a set of histograms without superscripts denoted non significant difference among means ($p > 0.05$); treatment abbreviation was as described in Fig. 1

carbon availability increased the amount of N_2O. In addition, the different levels of W_0 (3,125 L/ha) and W_0 (1,875 L/ha) affected *nirS* gene abundance. The abundance of the *nirS* gene decreased after 28 d with W_0 (3,125 L/ha) and urea fertilizer, but the application of W_0 (1,875 L/ha) and urea fertilizer significantly increased the quantity of the *nirS* gene after decomposition for 28 d. All treatments with urea fertilizer added had high ammonium contents. The first reaction was the oxidation of ammonium to nitrite (NO_2^-) and then the nitrite was reduced to nitric oxide (NO), since the current experiment did not include any plants to uptake nitrite. For this experiment, nitrate was used as an electron acceptor in denitrification to reduce nitrate to nitrogen gases. The denitrification pathway to nitrogen gases has been established as via nitric oxide (NO) and nitrous oxide (N_2O) which are actually emitted to the atmosphere as greenhouse gases (Ravishankara et al., 2009).

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported, in part, by a grant from the Graduate School, Kasetsart University, Bangkok, Thailand and by the Center for Advanced Studies in Agriculture and Food, Kasetsart University, the Capacity Building of Kasetsart University Students on Internationalization Program; and the Minnesota Agricultural Experiment Station.

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