Nitrogen Cycling in an Ecological Farming System of Milk Vetch Culture—Pig-Raising—Biogas Fermentation—Rice Culture

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Abstract : The ^{15}N – labeled technique was used to study the stock, transformation, fate and utilization efficiency of N in the farming—pig husbandry—biogas ecosystem in rice areas. It was shown that the crude protein digestibility of the ensilaged milk vetch by pig was 53.76%, the recovery rates of ensilaged milk vetch N from the pig feces and urine were 39.36% and 24.71%, respectively. The recovery rate of biogas fermentation N of pig feces and urine was 97.9%. The quantity of alkali-hydrolysable N was 2.6 times as great as before the fermentation. The mixed application of milk vetch, biogas-tank sludge and chemical fertilizer could promote N partitioning to rice grain, therefore it was advantageous to yield improvement. The residual quantity in soil of the fertilizer N was equivalent to 2.0 – 2.5 times that of only chemical fertilizer and yet gaseous loss N of the latter was 2.6-8.2 times that of the former. The comprehensive economic effect of the whole ecosystem increased by times, compared with the only milk vetch-early rice-late rice pattern, and ecological effect and social effect were also very prominent.

Key words : Biogas fermentation, Ecosystem, Fate of nitrogen, ^{15}N–labeled milk vetch, Pig, Rice.

Milk vetch (*Astragalus sinicus* L.) is a kind of traditional green manure in the rice growing areas in Jiangxi Province, P. R. China. It is a main resource of organic fertilizer for rice, and has played an important role in producing grain and improving soil fertility. In recent years, owing to the increasing application of chemical fertilizer, especially owing to the lower direct economic effect of green manure, the sown area of milk vetch has declined remarkably, and so the yield of fresh milk vetch decreased relevantly. Perhaps this is obviously negative effect on the sustainable development of agriculture in the rice growing areas. Therefore, on the basis of the widespread farming system of milk vetch—rice—rice, in this study, milk vetch was used as both fertilizer and feed—fermenting pig feces and urine into biogas—returning biogas-tank sludge (a mixture of the dross and the liquid, some below) to fields. A complex ecological pattern of farming, animal husbandry and biogas generation was set up; and through food chains and the additional chain of biogas fermentation, the increased function and effects of green manure and the whole pattern was achieved. ^{15}N-labled tracer determination method was used here to study mainly the stock, transformation, fate and the utilization efficiency of N in the pattern, so as to provide scientific basis for improving economic effect of green manure and for setting up an agricultural ecological pattern fit for the rice growing areas.

Materials and Methods

1. ^{15}N–labeled green manure

Milk vetch was planted in a low fertility soil to which ammonium sulfate with a ^{15}N abundance of 50.5% was applied. Fresh milk vetch with a ^{15}N abundance of 11. 85% was cut at the blooming stage, which was used partly as the rice base fertilizer and partly as ensilaged feed.

2. Raising of pigs and biogas fermentation of pig feces and urine

The two pigs used in the experiment were Long-white Yorkshire-local tribasic hybrid castrated pigs of the same age, which were raised separately in Dracher metabolic cages. The experiment consisted of three periods : (1) the pre-experimental period of 6 days, during which the pigs were adapted to the experimental environment. The pigs were supplied with a diet composed of normal ensilaged milk vetch and proper amount of N-free starch; (2) the experimental period of 2 days, in which the pigs were fed 3 times a day. The experimental diet for the pigs was composed of 15N-labeled ensilaged feed of 750 g for each time. Based on the dried mass balance, it was composed of 46.7% ensilaged feed, 51.3% N-free starch and a little salt and sugar. The feed had a ^{15}N abundance of 11.58%; and (3) the post-experimental period of 4 days, during which the pigs were supplied with a diet composed of the same components as the pre-experimental diet. Throughout the raising process, the pig feces and urine were collected and tested at

scheduled intervals. Some parts of the pig feces and urine collected during the experimental periods were chosen and stored in a hermetically sealed chamber for biogas fermentation for 3 months, and the fertilizer of biogas-tank sludge with a ^{15}N abundance of 2.73% was then obtained. At the same time, the unlabeled N in pig feces and urine was stored in practical biogas pool for fermentation for 6 months.

3. Pot-culture experiment

(1) The soil used in the experiment was a paddled paddy soil derived from red clay at pH 6.2, organic matter 27.9 g kg^{-1}, total N 1.42 g kg^{-1}, available P 13 mg kg^{-1}, available K 145 mg kg^{-1}, and alkali - hydrolysable N 127 mg kg^{-1}. The experiment was done by the simulation method, in galvanized iron tubes (33 cm in height and 28 cm in inside diameter). In the lower part of the tube, a plowpan with a depth of 9 cm was packed to have bulk density of 1.5 g cm^{-3} (accounting for 8.3 kg dried soil), above which soil was filled in for use as the cultivated layer (17 cm in height, accounting for 11.5 kg dried soil). The tube wall above the surface of the soil, 7 cm in height, was left to keep the water-remaining layer. The water seeped through the plowpan was taken as seepage water, which was continually collected at scheduled intervals throughout the rice growing period. The seepage water was led into a plastic basin filled with pebbles and then was piped into 0.5 mol L^{-1} sulfuric acid solution with a concentration (Fig 1).

(2) The experimental rice variety was Chao Feng Zao. Phosphate and potash fertilizers were used as the basal fertilizers. Three hills were transplanted in each pot with 5 rice seedlings for each hill. Four treatments as follows were set : 1. No nitrogen fertilizer (CK); 2. ^{15}N ammonium sulfate; 3. Combined application of green manure and ammonium sulfate [(a) 1/2 ^{15}N green manure N + 1/2 ^{14}N ammonium sulfate, and (b) 1/2 ^{15}N ammonium sulfate + 1/2 ^{14}N green manure N] ; 4. Combined application of biogas-tank sludge and ammonium sulfate [(a) 1/2 ^{15}N biogas-tank sludge N + 1/2 ^{14}N ammonium sulfate, and (b) 1/2 ^{15}N ammonium sulfate + 1/2 ^{14}N biogas-tank sludge N]. Each treatment or sub-treatment was repeated three times. Nutrient elements were applied to the cultivated layer with the amount of 945 mg N, 473 mg P$_2$O$_5$, and 945 mg K$_2$O per pot, the same amount of P.K was applied in the treatment with N-free fertilizer. Fertilizers were applied at one time just prior to transplanting rice seedlings, except that the green manure was applied 10 days earlier. The abundance (%) of the 15N-labeled fertilizers were : 12.51% for ammonium sulfate, 11.85% for green manure (milk vetch), and 2.73% for biogas-tank sludge. The phosphate and potash fertilizers were added in the form of calcium magnesium phosphate and potassium chloride to organic manure. At the ripening stage, samples of rice grains, rice straws, rice roots, soils in the cultivated layer and plowpan were collected.
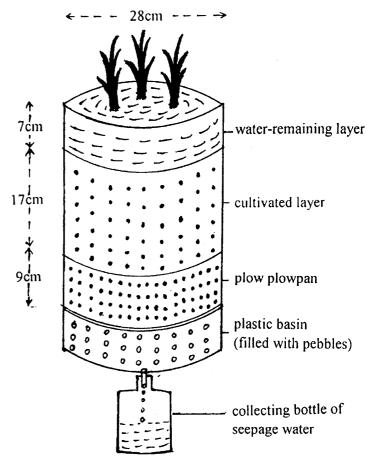

Fig. 1. A schematic diagram of pot culture.

4. Analytical methods

Total nitrogen was determined by the improved Kieldahl method. ^{15}N abundance was determined by means of the Bremmer method (Bremmer 1965) with МИ 1305 mass spectrometer. Soil pH was determined with a glass electrode pH meter (1 : 5 w/v of soil : H$_2$O). Organic matter was determined by the K$_2$Cr$_2$O$_7$ oxidizing method. Soil-available P and K were extracted by the NaHCO$_3$ and NH$_4$OAc methods, respectively.

5. Calculation of ^{15}N recovery and seepage losses

The fraction of nitrogen derived from ^{15}N-labeled fertilizer in the plant and soil and the percent ^{15}N recovered by the plant and that remaining in soil and entering into seepage water were calculated by the following equation.

Percent N in plant (soil, water) derived from fertilizer (% Ndff).

$$= \frac{^{15}\text{N atom \% excess in plant (soil, water)}}{^{15}\text{N atom \% excess in fertilizer}} \times 100$$

Fertilizer N in plant (soil, water) (mg pot^{-1} or total seepage water^{-1})

$$= \frac{\text{\% Ndff} \times \text{Total N in plant (soil, water)}}{100}$$

Fertilizer N recovery in plant (soil) and seepage losses (%)

$$= \frac{\text{Fertilizer N in plant (soil, water)}}{\text{Fertilizer N applied}} \times 100$$

Table 1.   Crude protein digestibility of the ensilaged milk vetch by pigs.

Experimental Pig. No.	Take forage(g)	Tot. N absorbed(g)	Dry wt. of feces(g)	Tot. N of feces(g)	Digestibility of CP (%)
1	1050	13.62	189.6	6.378	53.17
2	1050	13.62	190.7	6.219	54.34
Average	1050	13.62	190.2	6.299	53.76

Table 2.   The amount of pig feces and urine and the recovery rates of the ensilaged milk vetch ^{15}N from the pig feces and urine.

Experimental Pigs. No.	Feces			Urine		
	Feces excreted (g)	^{15}N recovery mg	%	Urine excreted(g)	^{15}N recovery mg	%
1	3261.1	506.1	39.53	15031.1	300.7	23.49
2	3777.8	500.0	39.06	16758.2	331.9	25.93
Average	3523.5	503.1	39.30	15894.6	316.3	24.71

The amount of the 15N-labeled ensilaged milk vetch taken-in by each pig is 1050 g, of which the enrichment amount of ^{15}N is 1280 mg.

Results

1. The digestibility of crude protein in the ensilaged milk vetch by the pigs

After a two-day feeding of ^{15}N labeled ensilaged milk vetch, on the basis of the excretory status of ^{15}N in the pig feces, the analytical data from the ^{15}N excretory peak period were collected, and the formula of feed digestibility used in the animal breeding science was used to calculate the crude protein(crude protein $= N \times 6.25$) digestibility of the ensilaged milk vetch by the pigs. The formula is

$$D\% = (A - B) / A \times 100$$

where D is the digestibility of a certain nutritional component in feed; A the intake of a certain nutritional component; B a certain nutritional component in feces.

It can be seen from the crude protein digestibility (Table 1) that the crude protein digestibility of the ensilaged milk vetch by the two experimental pigs was quite similar, averaged 53.75%, which reached the normal standard. This showed that the experimental pigs could well digest and assimilate the crude protein in the ensilaged milk vetch.

The N components which were not digested and assimilated by pig bodies were excreted in the form of pig feces and urine. The amount of excrement and ^{15}N recovery are shown in Table 2. The rates of ^{15}N recovery from the ensilaged milk vetch in the two experimental pig feces were 39.53% and 39.06%, (average 39.30%), while in urine were 23.49% and 25.93%, (average 24.71%). From Table 2, it can be seen that the amount of N in the pig feces and urine was still significantly high, which amounted to 64% that of N in the ensilaged milk vetch.

2. N changes in the pig feces and urine after the fermentation

After hermetical fermentation of the pig feces and urine in a small container for three months (biogas was not used), the recovery rate of N in pig feces and urine from the biogas-tank sludge was up to 97.9%, with little loss. This value is similar to that obtained by the researchers from the Soil and Fertilizer Institute of Shanghai Academy of Agricultural Sciences (Liu and Jin 1992). After 6 months of fermentation in biogas pool, during which biogas-tank sludge was collected regularly, the recovery rate of N in pig feces and urine from biogas -tank sludge was about 76%. The reason for the loss of N could be the release of NH_3 with the biogas throughout the utilization process of biogas, or as a result of the decrease of the ratio of C to N in the biogas pool owing to the long fermentation period. As for this, further study is needed. It can be seen in Table 3 that the alkali -hydrolysable N content in the biogas-tank sludge amounted from 45.7% to 52.3% of total N after the fermentation of the pig feces and urine, which was equivalent to 2.2-2.6 times of that before the fermentation. Therefore, fermentation was advantageous to the improvement of fertilizer utilization efficiency.

3. Effect of green manure and biogas-tank sludge on rice yield and N partition

(1) Effect of green manure and biogas-tank sludge on rice yield and N accumulation

It can be seen from Table 4 that the rice grain yield in each treatment with application of different fertilizers was higher than that of CK. The order of yield increase was treatment 4 > treatment 3 > treatment 2. This showed that under the condition of equivalent N, if organic fertilizer was applied in combination with chemical fertilizer, the yield of the rice grain was higher than that with only chemical fertilizer. Moreover, the combined application of biogas-tank sludge and chemical fertilizer showed a better result. Correlative analysis was carried out between the N accumulation and dry weights

Table 3. Effect of biogas fermentation on change of N content in pig feces and urine.

Fertilizer	Fermentation in small container (for 3 months)			Fermentation in biogas pool (for 6 months)		
	Total N (g)	Recovery of N (%)	Alk hydr- N of tot. N (%)	Total N (kg)	Recove ry of N (%)	Alk hydr- N of tot. N (%)
Pig feces and urine (before fermentation)	28.5	—	20.1	5.64	—	21.2
Biogas-tank sludge	27.9	97.9	52.3	4.29	76.06	45.7

Table 4. Effect of different fertilizers applied on rice yield and N accumulation (g pot^{-1}).

Treatments	Rice grain		Rice straw		Rice root		Whole plant	
	Dry weight	N accumulated	Dry weight	N accumulated	Dry weight	N accumulated	Dry weight	N accumulated
(1) CK (no N)	14.9	0.234	15.2	0.154	3.00	0.032	33.1	0.420
(2) A·S N	36.5	0.725	36.1	0.372	7.16	0.081	79.8	1.178
(3) 1/2 G·M N+ 1/2 A·S N	36.9	0.748	36.5	0.358	7.19	0.081	80.6	1.187
(4) 1/2 B·S N+ 1/2 A·S N	37.6	0.762	37.0	0.360	7.22	0.084	81.8	1.206
LSD0.05	1.51	0.0284	1.06	0.0115	0.44	0.0081	3.13	0.0345
LSD0.01	2.29	0.0430	1.60	0.0174	0.67	0.0122	4.74	0.0522

Data in treatment (3) and (4) are the average values of their two sub-treatments (same below);

A · S. refers to ammonium sulfate; G · M refers to green manure; B · S refers to biogas-tank sludge (same below).

of rice grains, rice straws, rice roots and the whole rice plants in each treatment. The relevancy coefficients were 0.9997**, 0.9967**, 0.9789**, and 0.9999**, respectively. This means that both the economical and biological outputs were closely correlated with N supply in the soil and fertilizers. The proportion value of N accumulation in rice grain to that in rice straw followed the order of treatment 4 (2.12) >treatment 3 (2.09) >treatment 2 (1.95). This result showed that the application of organic fertilizer in combination with chemical fertilizer could promote the partition of N to rice grain. Therefore, it was advantageous to the increase of rice grain yield. This finding was in agreement with the report by Wu and Ni (1990).

(2) The amounts and distribution of soil N and fertilizer N taken up by rice

Though different fertilization had a certain effect on the total amounts of soil N and fertilizer N taken up by rice, the distribution of soil N and fertilizer N taken up by rice was nearly identical, which were about 64-65% and 35-36%, respectively (shown in Table 5). This result was quite similar to that reported by Huang

(Huang. et al 1981). As for the two treatments to which organic manure was applied in combination with chemical fertilizer, the green manure N taken up by rice was 47.1% of the total N input. Biogas-tank sludge N taken up by rice was 48.7% of the total N input, which was slightly higher than the former. This result could be attributed to the more available N in the biogas-tank sludge. From the distribution of soil N and fertilizer N in each part of rice plant, a common tendency among all the fertilization treatment was observed. The rate of soil N accumulation was in the order of rice root (77-84%) >rice grain (64-65%) >rice straw(61-62%). However, the rate of fertilizer N accumulation was in the reverse order.

4. Fate of N in the ecosystem of farming-pig husbandry-biogas

(1) Fate of fertilizer N in the sub-ecosystem of paddy soil-rice

There are 4 kinds of N losses after fertilizer is applied to the soil. Assimilation by plant, soil residue and seepage loss was determined by 15N-labeled tracing method. The last one, gaseous loss was calculated by the differ-

Table 5. The amounts and distribution of fertilizer N and soil N taken up by rice.

Treatments		Rice grain		Rice straw		Rice root		Whole plant	
		mg pot^{-1}	%	mg pot^{-1}	%	mg pot^{-1}	%	mg pot^{-1}	%
(2) A·S N	A. S	249.2	35.36	137.3	38.11	12.9	16.13	399.4	34.88
	Soil	455.2	64.64	223.0	61.89	67.1	83.87	745.7	65.12
(3)1/2G·M	G. M	122.4	16.85	55.0	15.81	12.2	15.31	189.6	16.43
N+1/2	A. S	130.6	17.98	76.6	22.02	5.8	7.28	213.0	18.46
A·S N	Soil	473.4	65.17	216.3	62.17	61.7	77.41	751.4	65.11
(4)1/2B·S	B. S	132.4	17.88	60.0	17.14	12.4	15.23	204.8	17.47
N+1/2	A. S	134.2	18.12	75.8	21.66	6.2	7.62	216.2	18.45
A·S N	Soil	474.0	64.00	214.2	61.20	62.8	77.15	751.0	64.08

33.47 mg N brought by the rice seedlings is deducted from the amounts of N uptake by rice.

Table 6. Fate of fertilizer N.

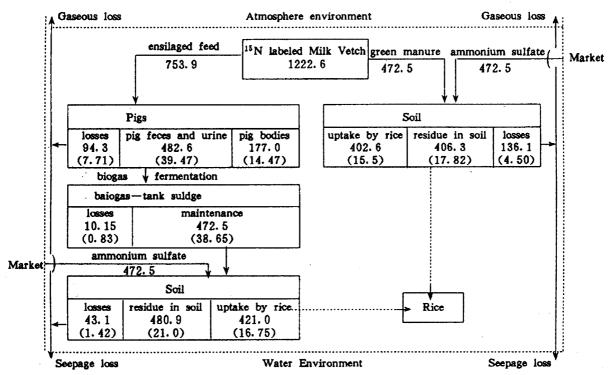| Treatment	Uptake of N by rice		Residue of N in soil		Seepage losses		Gaseous losses	
	mg	%	mg	%	mg	%	mg	%
(2)A·S N	399.4	42.26	206.2	21.82	1.04	0.11	338.4	35.81
(3)1/2G·M N+1/2 A·S N	402.6	42.60	406.3	43.00	3.85	0.41	132.25	13.99
(4)1/2B·S N+1/2 A·S N	421.0	44.55	480.9	50.89	1.65	0.17	41.45	4.39

Fig. 2. N cycling in the farming-pig husbandry-biogas ecosystem.
    (Values are expressed by mg. The data in the brackets are the percentages of milk vetch N.)

ence method. As Table 6 shows, under the experimental condition, the order of the rates of N uptake by rice plant from the fertilizer was treatment 4 >treatment 3 > treatment 2, which was identical with the effect on rice yield and the amount of N accumulation, mentioned above. The order of the N residue in the soil (including the cultivated layer and plowpan) in different treatments also was treatment 4 >treatment 3 >treatment 2. In treatment 2 to which only chemical fertilizer was applied, the N residue in the soil was half that in the former two treatments or less. Other reports (Zhu and Wen 1992) proved that more N residue in soil was advantageous to the maintenance and improvement of soil N content, and the effectiveness of the N residue in soil was remarkably higher than that of soil N. The order of the gaseous loss rates of total N input was, treatment 2 > treatment 3 > treatment 4, i. e. 35.81%, 13.99%, 4.39%, respectively, just contrary to that of the N residue rates in soil. The former was 2.6-8.2 times that of the latter two, making very remarkable difference. As for the seepage loss of total N input, the difference was very small. As a result, it could be concluded that the application of organic manure in combination with chemical fertilizer could not only improve the fertilizer utilization ratio and the rice yield, but also be very effective for soil improvement.

(2) Cycling pattern of N in the ecosystem of farming—pig husbandry—biogas

According to the data mentioned above, a schematic chart of the ecological cycle in the combination of farming-pig husbandry-biogas is drawn (Fig 2). The stock, transformation and fate of N in the ecosystem were expressed by absolute numbers in mg, and so were the output from the system into the environment (air, water and human society) and the input (chemical fertilizer) into the system from the environment (market). Figure 2 describes as follows. N in the system that contributed to biological rice products accounted for 35.25%, that to pig bodies 14.47% (from the data of He et al, 1994), and the residue in the soil 38.82%; the total N recovery rate was 85.54%; the N losses in the processes of rice planting, pig raising and biogas fermentation accounted for 14.46%, which was about half that (35.92%) of planting rice with application of only chemical fertilizer.

Discussion

The above experimental results show that in compari-

son with He Dian-yuan's pattern, this pattern substitutes ensilaged milk vetch for air drying milk vetch to feed pigs; adds a chain of biogas-fermenting pig feces and urine and fertilizing rice with biogas-tank sludge as a major fertilizer (about 1/2), hence its structure is more integrated and its function is improved. In other words : (1) This pattern not only exploited resources, especially milk vetch, but also increased their utilization ratio. (2) The recycling of nutrients was fully conducted, thus changing the one-shot utilization of recourses into repeated utilization. For the resources, half of the green manure was used for 3 times, and the pig feces and urine for twice. Nearly half amount of chemical fertilizer was saved, and more products were produced, not only rice grain but also pigs and biogas. (3) The biological mechanism of nitrogen cycling was used to exploit resources of fertilizer, feed and energy. This not only strengthened the effect of organic fertilizer on rice growth and soil improvement, but also played an important role in saving feed grain and improving the environment, hence making higher productivity and multiple ecological and social benefits of the system.

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