Use efficiency and residual effect of ^{15}N-labelled ryegrass green manure over a 9-year field micro-plot experiment

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Abstract

A 9-year field micro-plot experiment was conducted to investigate the use efficiency, residual effect, and fate of ^{15}N-labelled ryegrass green manure in soil-crop system, so as to offer useful information for reasonable application of green manures on meadow burozem. Results showed that high application rate (600g per plot) of ^{15}N-labelled ryegrass green manure resulted in reduction of percent recovery (PR)[15]N_{plant} and PR[15]N_{soil} by 25.0% and 12.8%, respectively, and elevation of percent loss (PL)[15]N by 31.2%, as compared with low application rate. The ^{15}N utilization and loss could be divided into three phases, i.e., high uptake and fast loss in the first and second growth seasons, low uptake and slow loss in the following four growth seasons, and little change in the last three growth seasons. This suggested that the green manure N had a long residual effect but its loss was faster at early phase, and thus, rational application of green manure was of considerable importance to reduce its N loss while improve its N use efficiency.

Keywords: ^{15}N-labelled, green manure, N use efficiency, percent recovery, percent loss, fate

1. Introduction

Green manuring is a good management practice in agricultural production, because it can improve soil fertility and quality (Kautz *et al.*, 2004; Astier *et al.*, 2006; Stark *et al.*, 2007; Zhang and Fang, 2007; Lee *et al*., 2010; Zavattaro *et al*., 2012), and also, supply N, a primary limiting nutrient for crops (Drinkwater *et al.*, 1998; Pypersa *et al*., 2005; Elfstrand *et al*., 2007; Xavier *et al*., 2013). This practice has received much attention (Toomsan *et al*., 2000; Elfstrand *et al*., 2007; Tejada *et al.*, 2008). It is a conventional agricultural practice in South China, but is less adopted in Northeast China. Many studies have evaluated the use efficiency, residual effect, and fate of chemical fertilizer N (Zhu and Chen, 2002; Abril *et al*., 2007; Ju *et al*., 2009; Zhang *et al*., 2011; Chuan *et al*., 2013), but the utilization and fate of green manure originated N has been very rarely studied. The ability of green manure to release N nutrient relies on its chemical composition (Chaves *et al*., 2006, 2008; Tejada and Gonzalez, 2006; Matos *et al*., 2008). Under field in situ conditions, the decomposition of green manures is complex, and is controlled by numerous factors such as availability of carbon and nitrogen, the biochemical nature, and soil and climatic factors, etc (Chaves *et al.*, 2004; Tejada *et al.*, 2008; Matos *et al.*, 2008, 2011).

In order to maximize the N use efficiency while minimize the N loss, it is important to understand the behavior and fate of green manure N under field condition. In this study, a 9-year (from May 1999 to October 2007) field micro-plot experiment with upland rice growth was conducted on a meadow burozem in Shenyang, Northeast China to investigate the effects of applying different amounts of ^{15}N-labelled ryegrass green manure once at the start of the experiment on the crop yield, ^{15}N recovery by crop and soil, and ^{15}N loss in the soil-crop system. The objective was to investigate the potential applicability of green manure on meadow burozem.

2. Materials and Methods

2.1 Study site

The experiment was conducted at the National Field Observation and Research Station of Shenyang Agro-ecosystems, a member of Chinese Ecosystem Research Network (CERN) established in 1987 (Figure 1). This station locates on the Lower Liao River Plain, with a humid and semi-humid continental monsoon climate of warm-temperate zone. The mean annual temperature is 7-8 °C, with the minimum and maximum mean monthly temperature in January (-13 °C) and July (24 °C), respectively. The mean active accumulated temperature ($\geq$10 °C) is 3300-3400 °C, total solar radiation is 5410-5600 kJ cm^{-2}, duration of frost-free season is 147-168 d, and mean annual precipitation is about 700 mm. The tested soil (Hapli-Udic Cambisols in Chinese soil taxonomy, corresponding to Inceptisols in US soil taxonomy) is meadow burozem, and soil texture is silty loam (Table 1).

2.2. Preparation of ^{15}N-labelled ryegrass green manure

Ryegrass was grown in four 1m×1m micro-plots, with 5 rows and 20 cm row spacing in each micro-plot. Labelled (^{15}NH$_4$)$_2$SO$_4$ (Shanghai Institute of Chemical Industry) with 6.72 atom% ^{15}N was top-dressed at a rate of 100 kg N ha^{-1} at elongation stage, and concentrated superphosphate (60 kg P ha^{-1}) and potassium sulfate (90 kg K ha^{-1}) were applied as basal fertilizers. The ryegrass was harvested at earing-flowering stage in 1998, and dried at 65 °C to constant weight. All ryegrass plants were cut into < 0.5 cm pieces and stored as ^{15}N-labelled green manure with 2.70 atom% ^{15}N. The basic physical and chemical properties of ryegrass are present in Table 1.

2.3. Experimental design

The field micro-plot experiment was conducted from May 1999 to October 2007. Each plot was inserted with a 30 cm × 50 cm PVC cylinder. The cylinders were 1.5 m in interval to avoid the cross-contamination, and their top was 10 cm above ground level. Three treatments were set up, i.e., no N fertilization (CK), low application rate of ^{15}N-labelled ryegrass green manure (L-RGM, 200 g ryegrass each micro-plot, corresponding to 59.6 g N m^{-2}), and high application rate of ^{15}N-labelled ryegrass green manure (H-RGM, 600 g ryegrass each micro-plot, corresponding to 178.8 g N m^{-2}). Each treatment had three replicates.

The ^{15}N-labelled ryegrass green manure was applied once to 0-20 cm soil layer in October 1998 (before soil freezing), and the concentrated super-phosphate (60 kg P ha^{-1}) and potassium sulfate (90 kg K ha^{-1}) were applied as basal fertilizers each year. Upland rice was sowed on 1 May 1999-2001, 3 May 2002, 1 May 2003-2005, and 2 May 2006-2007. Plant and soil samples were collected on 1 October 1999, 2 October 2000, 1 October 2001-2004, 30 September 2005, 1 October 2006, and 2 October 2007, respectively.
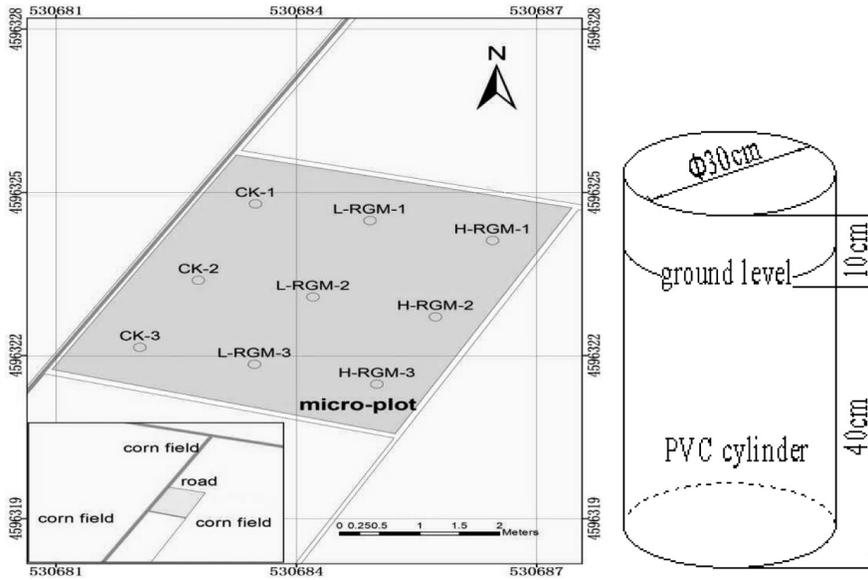
Figure 1. Sketch diagram of study site and PVC cylinder

Table 1. Physical and chemical characters of tested soil and ryegrass green manure

		Total C	Total N	Total P	Total K	Olsen-P	Available K	C/N	pH	Sand	Silt	Clay
		-----------------g kg⁻¹-----------------				-------mg kg⁻¹-------				-------%-------		
Soil	0-20cm	11.51	1.05	0.35	21.35	9.23	127.0	11.0	6.3	14.7	60.4	24.9
	20-40cm	8.44	0.81	0.37	23.40	7.60	123.7	10.4	7.2	13.0	60.3	26.7
Ryegrass green manure (RGM)		411.50	21.15					19.4				

2.4. Sampling and analytical methods

Plant samples collected from each micro-plot were separated into grain and straw, directly dried at 65 °C to constant weight, and ground and sieved for total N and atom% ^{15}N analysis. Soils at 0-20 and 20-40 cm depth from each micro-plot were collected with a 2cm-diameter soil auger using five-point sampling method (Lu, 2000), and were air-dried and sieved for the measurement of total N and atom% ^{15}N.

Soil total carbon was measured by using a TOC-5000A automatic analyzer (Shimadzu Corporation, Japan); total P and K were measured with sodium carbonate fusion and molybdenum antimony-ascorbic acid colorimetric method. Soil available P and K were determined by extraction with sodium bicarbonate and ammonium acetate, respectively, and soil pH was determined in soil slurry with a soil (g) to water (ml) ratio of 1:2.5 using a glass electrode pH meter.

Soil and plant total N was determined by using a TruSpec CN Elemental Analyzer (Leco Corporation, USA), and the atom% ^{15}N was measured by Finnigan DELTA plus XP Stable Isotope-Ratio Mass Spectrometers (USA), with the operation procedures carried out from lower to higher atom% ^{15}N to avoid cross-contamination.

2.5. Methods of calculation

Plant or soil total ^{15}N ($C_{p,s}$, kg N ha^{-1}), percent recovery of ^{15}N by plant or soil (PR^{15}N$_{plant}$ or PR^{15}N$_{soil}$), and percent loss of ^{15}N (PL^{15}N) were calculated by the formulae:

$$C_{p,s} = C * \frac{(b-c)}{(a-c)} \qquad (1)$$

$$PR^{15}N_{plant} \text{ or } PR^{15}N_{soil}, \% = \frac{C*(b-c)}{C_{ryegrass}*(a-c)}*100 \qquad (2)$$

$$PL^{15}N, \% = 100\% - \text{total } PR^{15}N_{plant} - PR^{15}N_{soil} \qquad (3)$$

Where C is the amount (kg N ha^{-1}) of plant or soil total N, $C_{ryegrass}$ is the amount of applied ^{15}N-labelled ryegrass green manure (kg N ha^{-1}), a is the atom% ^{15}N of ^{15}N-labelled ryegrass green manure, b is the atom% ^{15}N of treated plant or soil total N, and c is the atom% ^{15}N of background plant or soil total N.

2.6. Data processing

One-way and two-way Analysis of Variance (ANOVA) with SPSS 13.0 statistical package was used to examine the effects of ^{15}N-labelled ryegrass green manure application rate, sampling time, and their interactions on the grain yield, ^{15}N uptake of upland rice and fate of ^{15}N-labelled green manure. Differences with a probability level of $p < 0.05$ were considered significant.

3. Results

3.1. Grain yield and ^{15}N uptake of upland rice

The grain yield of upland rice significantly declined with increase of consecutive growth seasons ($p < 0.001$, Table 2, Figure 2). Application rate of ryegrass green manure significantly affected the grain yield of upland rice ($p < 0.001$, Table 2, Figure 2). A significant interactive effect was observed in the grain yield of upland rice between application rate and time ($p < 0.001$, Table 2, Figure 2). As compared with low application rate, high application rate significantly increased the grain yield of upland rice by 35.4% across nine consecutive growth seasons, and this increasing trend strengthened in 1999-2002, and weakened in 2003-2007 ($p < 0.001$, Table 2, Figure 2).
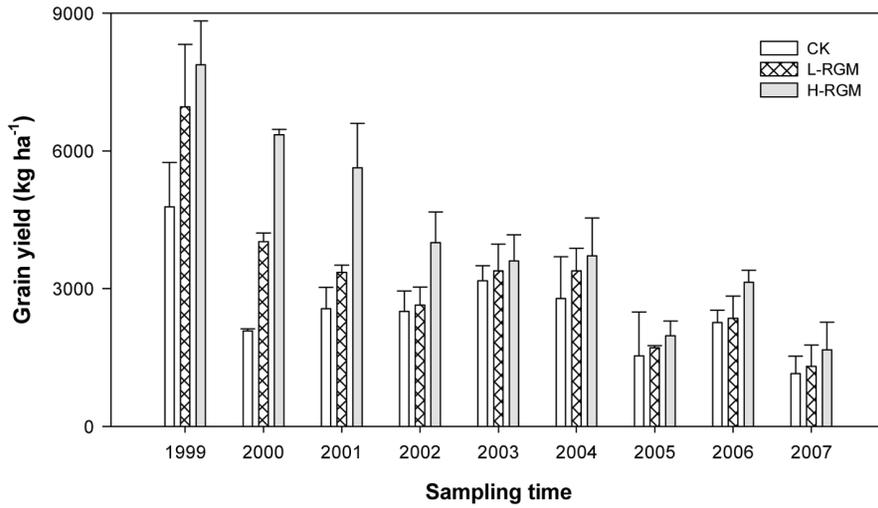
Figure 2. Grain yield of upland rice across nine consecutive growth seasons (Bars indicate standard errors)

Table 2. Results of ANOVA on the grain yield, ^{15}N uptake by plant and fate of ^{15}N-labelled green manure

	Grain yield	^{15}N uptake	PR^{15}N$_{plant}$	PR^{15}N$_{soil}$	PL^{15}N
Time (T)	24.7***	19.9***	15.7***	24.3***	61.1***
Ryegrass green manure (RGM)	40.6***	128.3***	55.3***	18.5***	309.1***
T * RGM	2.73*	11.3***	0.02	0.07	0.37

PR^{15}N$_{plant}$ and PR^{15}N$_{soil}$ represent the percent recovery of ^{15}N by plant and soil, respectively. PL^{15}N was the percent loss of ^{15}N. *,**, and *** denotes statistically significant at $p< 0.05$, 0.01, and 0.001, respectively.

The ¹⁵N uptake by upland rice also significantly declined with increasing consecutive growth season, and there was a significant difference in the ¹⁵N uptake between the treatments with low and high N application rates of ryegrass green manure ($p < 0.001$, Table 2, Figure 3). A significant interactive effect was observed in the ¹⁵N uptake by upland rice between green manure application rate and sampling time ($p < 0.001$, Table 2, Figure 3). As Compared with low application rate of ryegrass green manure, high application rate remarkably elevated the ¹⁵N uptake by 134.4%, and this increasing trend also strengthened with time from 1999 to 2002, but weakened from 2003 to 2007 ($p < 0.001$, Table 2, Figure 3).
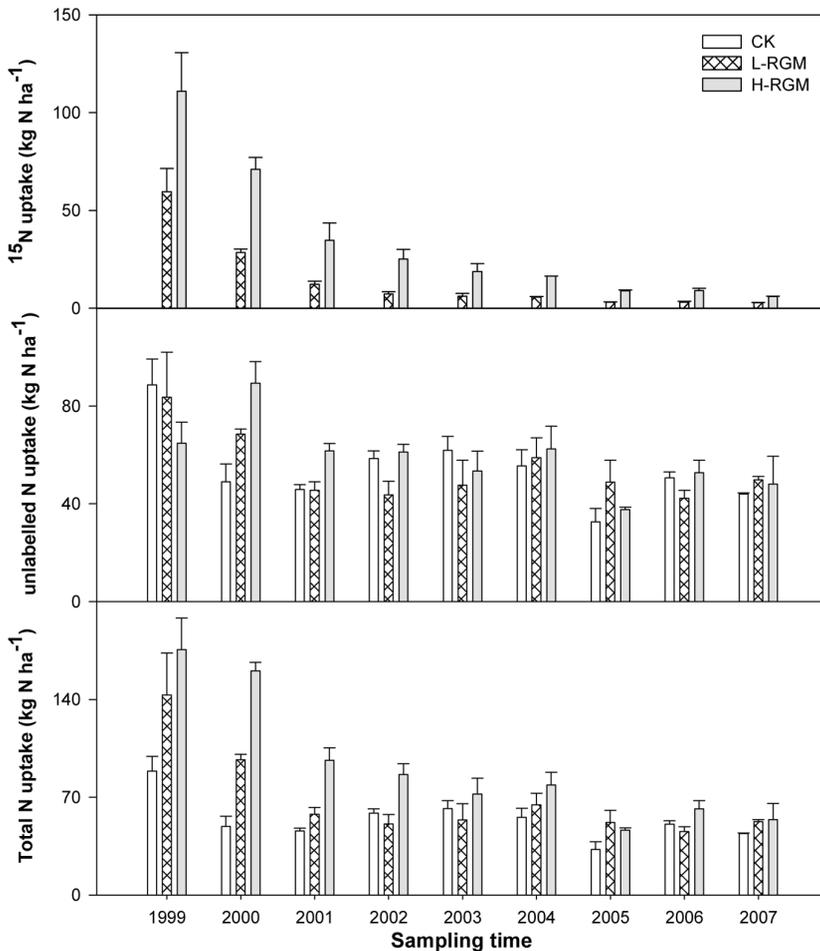

Figure 3. ¹⁵N and unlabelled N uptake by upland rice across nine consecutive growth seasons (Bars indicate standard errors)

Table 3. Percent recovery of ^{15}N by plant and soil (PR^{15}Nplant and PR^{15}Nsoil) and percent loss of ^{15}N (PL^{15}N) across nine consecutive growth seasons

Sampling year	Treatments	PR^{15}N$_{soil}$	PR^{15}N$_{plant}$	PL^{15}N
1999	L-RGM	68.05±5.13	9.99±1.99	21.96±1.99
	H-RGM	61.03±4.17	6.21±1.10	32.76±1.10
2000	L-RGM	58.26±3.15	14.77±2.24	26.97±2.24
	H-RGM	53.39±2.38	10.18±1.44	36.43±1.44
2001	L-RGM	54.17±4.60	16.82±2.43	29.01±2.43
	H-RGM	47.48±5.76	12.12±1.89	40.40±1.89
2002	L-RGM	51.43±14.64	18.05±2.57	30.52±2.57
	H-RGM	43.23±3.76	13.53±2.06	43.25±2.06
2003	L-RGM	46.68±5.74	19.07±2.62	34.25±2.62
	H-RGM	40.23±1.93	14.58±2.16	45.20±2.16
2004	L-RGM	39.54±1.78	20.02±2.55	40.44±2.55
	H-RGM	35.12±2.56	15.49±2.17	49.39±2.17
2005	L-RGM	38.64±5.42	20.53±2.56	40.83±2.56
	H-RGM	33.17±4.05	15.98±2.14	50.84±2.14
2006	L-RGM	37.93±2.27	21.05±2.57	41.04±2.57
	H-RGM	31.88±4.85	16.49±2.09	51.63±2.09
2007	L-RGM	37.44±0.45	21.53±2.57	41.03±2.57
	H-RGM	31.50±5.53	16.82±2.10	51.68±2.10

The ^{15}N uptake by upland rice also significantly declined with increasing consecutive growth season, and there was a significant difference in the ^{15}N uptake between the treatments with low and high N application rates of ryegrass green manure ($p < 0.001$, Table 2, Figure 3). A significant interactive effect was observed in the ^{15}N uptake by upland rice between green manure application rate and sampling time ($p < 0.001$, Table 2, Figure 3). As Compared with low application rate of ryegrass green manure, high application rate remarkably elevated the ^{15}N uptake by 134.4%, and this increasing trend also strengthened with time from 1999 to 2002, but weakened from 2003 to 2007 ($p < 0.001$, Table 2, Figure 3).

3.2. Fate of ^{15}N-labelled ryegrass green manure in soil-crop system

The percent recovery of ^{15}N-labelled ryegrass green manure by plant (PR^{15}N$_{plant}$) significantly increased with increasing consecutive growth season ($p < 0.001$, Table 2, 3). As Compared with low application rate of ryegrass green manure, high application rate resulted in remarkable reduction of PR^{15}N$_{plant}$ by 25.0% ($p < 0.001$, Table 2, 3). The highest PR^{15}N$_{plant}$ was in the first growth season, accounting for an average of 41.7% of the total plant recovery in the two fertilization treatments. The corresponding proportions in the second, third, fourth, fifth, and sixth growth season were 22.9%,

10.6%, 7.0%, 5.5%, and 4.9%, respectively. After then, the PR^{15}N$_{plant}$ was less than 2.7%. These results showed that the utilization of ^{15}N by upland rice had three phases, i.e., high uptake in the first two growth seasons, low uptake in the following four growth seasons, and little uptake after then (Table 3).

The percent recovery of ^{15}N-labelled ryegrass green manure by soil (PR^{15}N$_{soil}$) significantly decreased with increasing consecutive growth season ($p < 0.001$, Table 2, 3). Significant fertilization effect was observed in the PR^{15}N$_{soil}$ ($p < 0.001$, Table 2, 3). High application rate of green manure obviously decreased the PR^{15}N$_{soil}$ by 12.8%, as compared with low application rate (p < 0.001, Table 2, 3).

Similar to PR^{15}N$_{plant}$, the percent loss of ^{15}N-labelled ryegrass green manure (PL^{15}N) remarkably increased with increasing consecutive growth seasons ($p < 0.001$, Table 2, 3). As Compared with low application rate of ryegrass green manure, high application rate obviously enhanced the PL^{15}N by 31.2% ($p < 0.001$, Table 2, 3). The N loss of ryegrass green manure primarily occurred in the first growth season, accounting for an average of 58.5% of the total loss in two fertilization treatments. The corresponding proportions in the following five growth seasons were 9.7%, 6.3%, 4.6%, 6.4%, and 11.6%. After then, the PL^{15}N was 1.0%. These results showed that the N loss of ryegrass green manure can also be partitioned into three phases, i.e., fast loss in the first growth season, slow loss in the following five growth seasons, and little change in the last three growth seasons.

4. Discussions

Tejada *et al.* (2008) found that the incorporation of green manure had positive effects on soil physical, chemical, and biological properties, plant nutrition, and crop yield. Some results also indicated that green manuring increased plant leaf and root dry weight, grain yield, and crop N and P uptake (Astier *et al.*, 2006; Bilalis *et al.*,2009; Blaise, 2011; Rahman *et al.*, 2011). The present results showed that application of ryegrass

green manure significantly increased the upland rice dry weight and grain yield in the first growth season by an average of 62.9% and 55.1%, respectively. As compared with the control, the contribution of ryegrass green manure N was 85.2 kg N ha^{-1}, which was significantly higher than the results of Singh *et al.* (2010) that the incorporation of cowpea green manure significantly increased the mint fresh weight and oil yield by an average of 23.4% and 25.2% over the control, respectively, and the contribution of green manure N was equivalent to 30 kg N ha^{-1} when no fertilizer nitrogen was applied. This is possibly due to the differences in green manure, soil types, and climate conditions, etc.

Thonnissen *et al.* (2000) recorded the significant residual effect of soybean green manure on the dry matter accumulation of tomato and the N removal by succeeding maize, and was similar to that of 120 kg N ha$^{-1.}$ Yadav (2004) reported the small residual effect of S. aculeata green manure applied to rice on the agronomic efficiency, N recovery, and grain yield of succeeding wheat. Singh *et al.* (2010) also reported the significant residual effect of cowpea green manure on the fresh weight and oil yield of succeeding palmarosa. In our study, a longer residual effect of ryegrass green manure was observed on the grain yield and N recovery of upland rice in its succeeding eight growth seasons, and there were significant differences in the residual effect between the treatments with low and high application rates of ryegrass green manure. The average residual effect of ryegrass green manure N on the following upland rice in two treatments was equivalent to 129.2 kg N ha^{-1} (112.8 kg N ha^{-1} from 2000-2004, and was 16.4 kg N ha^{-1} from 2005-2007).

In present study, the percent recovery of green manure N by succeeding upland rice was higher in 2000-2004 (exceeding 85% of the total residual benefit of manure N), and lower in 2005-2007 (averagely accounting for 12.8% of the total residual benefit of manure N). This indicated a long-term sustained release of ryegrass green manure N, and this slow N release pattern depended on the residue quality and

environmental conditions (Quemada and Cabrera, 1997; Chaves *et al.*, 2006, 2008; Tejada and Gonzalez, 2006; Matos *et al*., 2008, 2011). After a period of nine growth seasons, the total percent recovery of green manure N by plant in the treatments with low and high application rate of ryegrass green manure was 21.5% and 16.8%, respectively. The total ^{15}N uptake amount accounted for 16.1% and 29.3% of total N uptake in the treatments with low and high application rate of ryegrass green manure among nine growth seasons, respectively (Figure 3). These results indicated that the use efficiency of ryegrass green manure N was lower. Some studies suggested that organic N from crop residue or manure showed lower N use efficiency than mineral N fertilizers (Harris *et al.*, 1994; Glendining *et al*., 1997, 2001; Kramer *et al.*, 2002).

The total percent N loss of green manure was 41.0% and 51.7% in the treatments with low and high application rate of ryegrass green manure, respectively. High application rate of ryegrass green manure declined N use efficiency and increased N loss, as compared with low application rate. This could be because that the mineralization and release of green manure N did not synchronize with the peak of crop N demand, and surplus mineral N occurred loss via ammonium volatilization, nitrification-denitrification and leaching processes. There was no correlation between the amount and percent of ^{15}N loss and uptake from green manure in each rice growth seasons. After 1 year of ryegrass application, the transfer amounts of ^{15}N labeled-ryegrass from topsoil (0-20cm) to subsoil (20-40cm) were 37.8 and 84.1 kg N ha^{-1} at the low and high application rate of ryegrass green manure, respectively and accounted for 6.3% and 4.7% of ryegrass green manure N applied. After a period of nine growth seasons, the corresponding amounts were 69.7 and 130.8 kg N ha^{-1}, and added up to 11.7% and 7.3% of ryegrass green manure N applied (Figure 4). These results indicated the obvious transfer of ryegrass green manure N from topsoil to deep soil and potential risk of N leaching loss.
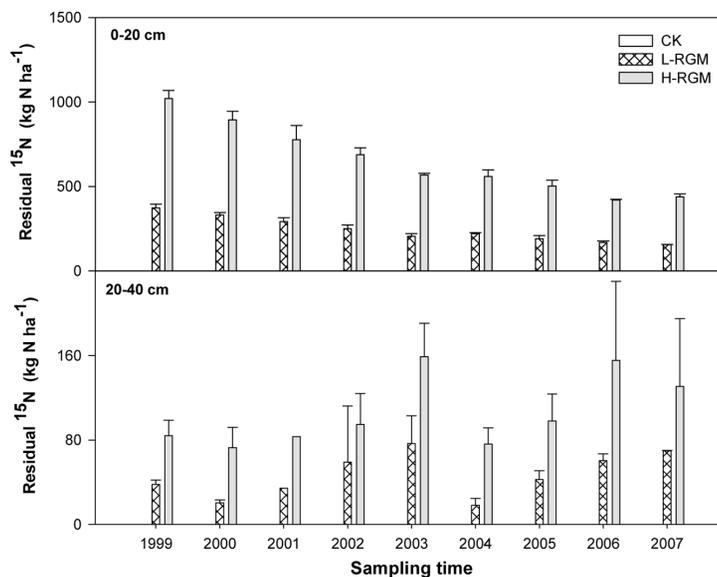

Figure 4. Residual amount of ^{15}N-labelled ryegrass green manure in soil across nine consecutive growth seasons (Bars indicate standard errors)

5. Conclusions

Application of ryegrass green manure on meadow burozem had significant effects on the grain yield and N uptake of upland rice in its current and consecutive growth seasons. Under high application rate of the green manure, the grain yield and N uptake of upland rice across nine growth seasons increased significantly, as compared with those under low application rate. The percent recovery of ^{15}N-labelled ryegrass green manure by plant and its percent loss in soil-crop system increased with increasing consecutive growth seasons, while the percent recovery of ^{15}N-labelled ryegrass green manure by soil was reverse, with the residual effect of ^{15}N-labelled ryegrass green manure being higher in the first five growth seasons and declined significantly in the following three growth seasons. High application rate of the ryegrass green manure remarkably declined the percent recovery of the manure N by plant and soil in soil-crop system, and elevated the percent loss of the manure N, as compared with low application rate. The ^{15}N utilization and loss could be partitioned into three phases, i.e., high uptake and fast loss in the first and second growth seasons, low uptake and slow loss in the following four growth seasons, and little change in the last three growth seasons. The green manure N had a longer residual effect but its loss was faster at early stages, and thus, reasonable application of green manure was of considerable importance to reduce its N loss while improve its N use efficiency.

Acknowledgements

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