Experiment 2 .

Effect of Vegetable Soybean Decomposition on Quantity of N-uptake in Kale

(*Brassica oleracea* **var.alboglabra**)

Objective

1. To monitor quantity of nitrogen release by vegetable soybean green manure decomposition
2. To find out the quantity of N-uptake of kale from mineralized vegetable soybean, using Isotope ^{15}N technique

Materials and Methods

Experimental design

The experiment was conducted on the area previously grown to the vegetable soybean. Kale was used as the following crop to determine the benefit of plant nutrient mineralization from vegetable soybean plant residues in the soil with the properties of 1.35% OM , 36 ppm extractable P, 80 ppm extractable K, 1223 ppm Ca, 155 ppm Mg and 8 ppm Mn. Before kale growing, unlabelled and ^{15}N-labelled vegetable soybean residues were incorporated into the soil at the rate of 0, 2.0, 2.3 and 2.4 t/rai of vegetable soybean biomass, along with 0, 2.23, 2.39 and 2.29 %N, then the seed beds were prepared for transplanting the kale. After three weeks of incorporation, the 21-day-old kale seedlings were planted at 15x15 cm spacing on 1.2x6 m plot size of each treatment. The treatments were carried out on the plot where vegetable soybean was previously planted so that kale was grown without addition of fertilizer. Thus, the treatments and experimental design of this experiment were the same as previous treatments of growth and nitrogen fixation efficiency of vegetable soybean by spraying wet azadirachtin for pest control and round up for weed control for every treatment.

Plant sampling and analysis

Ten plants of kale in ^{15}N unlabelled zone were randomly harvested at 21, 28, 35, 42, 49 and 56 days after planting (DAP) to determine weight accumulation in terms of fresh and dry weight. In ^{15}N labelled zone of 15x15 cm, the vegetable plants were harvested about 56 days for nitrogen accumulation analyses. All plant samples were dried in hot-air oven at 70^0C for 48 hrs and ground for total nitrogen and isotope nitrogen (^{15}N) analysis, using the methods which was previously described for soybean plant analysis.

Soil analysis for nitrogen mineralization

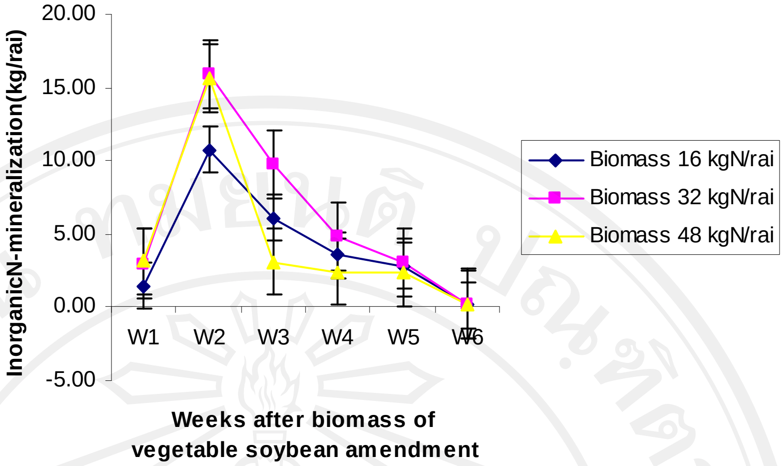
Soil was collected from the 0-15 cm depth of vegetable-grown plots at 7, 14, 21, 28, 35 and 42 days after planting. Nitrogen mineralization was determined in the form of inorganic nitrogen (NH_4^+-N and NO_3^-- N). Inorganic nitrogen extractions from soil samples were made by extracting 10 gm of oven-dry basis in 100 ml of 2 M KCl. The extractant was filtered and analysed for NH_4^+ and NO_3^- content, using Steam-Distillation Method (Mulvaney,1996).

Results and Discussion

Nitrogen mineralization

Potential mineralization of the incorporated detached pods of vegetable soybean plants into soil revealed that mineralizable inorganic nitrogen was continuously released during incubation period of six weeks. Two forms of inorganic-N, NH_4^+ and NO_3^{--} , were determined because they are the major compounds directly mineralized from organic nitrogen and usable by plants. Naturally, NH_4^+ is released from decomposition of organic nitrogen existing in plant tissue through ammonification process, mediated by microorganisms, and then a part of it is transfromed into NO_3^- by nitrification process (Alexander *et al* .,1977).

Apparent net nitrogen mineralization under field condition (native soil-N not included) after incorporation of soybean plant showed gradual release with time and reached a maximum at 2 weeks after amendment. The treatment of 16 kgN/rai (biomass 2.0 t/rai), showed lower mineral nitrogen compared to the other treatments (Appendix Table 1). In amended soil after two week of incubation, mineralizable N reached its maximum level and sharply decreased at the 6th week.

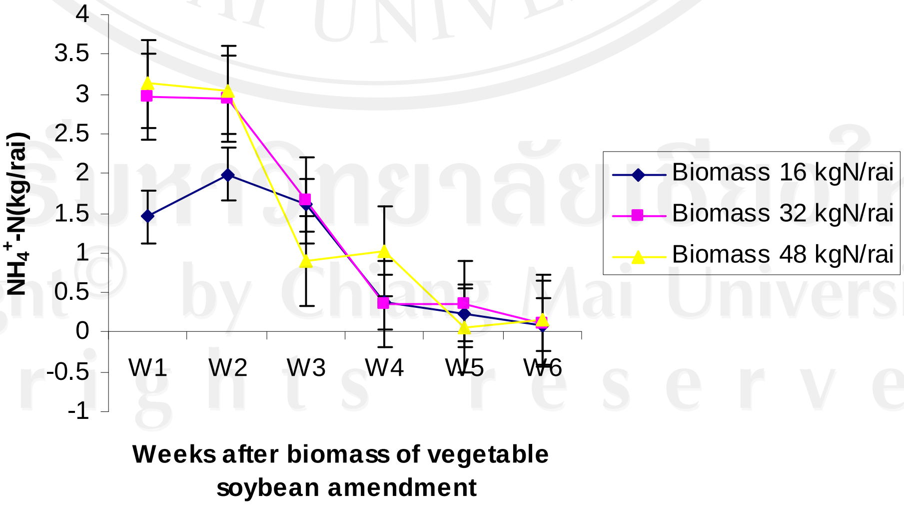


Fiqure1 Quantity of inorganic- N mineralization during 1-6 weeks after amendment of vegetable soybean residues T2 = soybean residue incorporation at 2.0 t/rai (16kgN/rai); T3 = soybean residue incorporation at 2.3 t/rai (32kgN/rai) ; T4 = soybean residue incorporation at 2.4 t/rai (48kgN/rai).

Higher Inorganic-N mineralized nitrogen in the soil treated with higher N fertilizer might be because of higher N content in soybean biomass. However, there were not significant differences among the rates of N fertilizer applied to soybean (Appendix Table 1) where the inorganic-N mineralization was in the average of 10.75-15.95 kg/rai, gradually declined after the second week. At the third week, remarkable rise of inorganic-N mineral was higher than 1st week but less than 2nd week in the amended soil which might be due to the mineralization of immobilized N in microbial biomass (Fig1). Nevertheless, the average inorganic-N mineralized in amended soil in the treatment 32 kgN/rai was the highest when compared with all treatments after the second week but not significantly different. Inorganic-N mineralization continuously declined to be a minimal amount at the final period of experiment (6th week), with the average of 0.18 kgN/rai (Appendix Table 1).

NH_4^+-N mineralization

Generally, mineralization of organic N in amended soil is firstly converted to be NH_4^+-N, rapidly generated in high quantity in the first two weeks (Figure 2), there after declined until less than 1 kg/rai at the 4th week. There was NH_4^+-N in treated and untreated soil at the same amount at the fifth and sixth week. This result indicated that organic N in plant residue was exhausted from amended soil. The 48 kgN/rai fertilized soybean mineralized the highest NH_4^+-N at 3.13 and 3.05 kgN/rai at the first and second week (Appendix Table 2), then rapidly decreased to 0.9 , 1.02, 0.05 and 0.16 kg/rai at the 3 , 4 , 5 and 6 week, respectively.



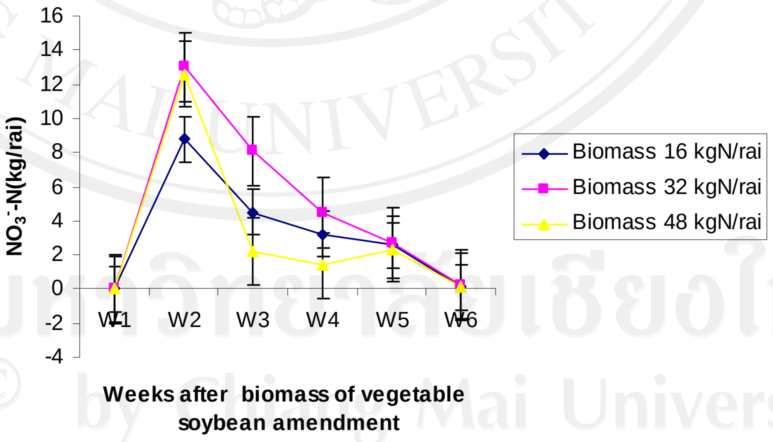
Fiqure 2 Mineralization quantity of NH_4^+-N during 1-6 weeks after amendment of vegetable soybean residues T2 = soybean residue incorporation at 2.0 t/rai (16kgN/rai) ; T3 = soybean residue incorporation at 2.3 t/rai (32kgN/rai) ; T4 = soybean residue incorporation at 2.4 t/rai (48kgN/rai).

Similarly, NH_4^+-N mineralization was also found in the treatment of 32 kgN/rai at 2.96 and 2.94 kgN/rai at the first and second week (Appendix Table2), then rapidly decreased to 1.66 , 0.35 , 0.35 and near zero at the 3 , 4 , 5 and 6 week, respectively. Fertilized soybean in this treatment was not significantly different from the previous treatment.. Though NH_4^+-N in the 1st-6thweek fertilized soybean was not significantly (Appendix 2.1, 2.2, 2.3, 2.4, 2.5 and 2.6) lower than treatment 4th and 3rdat the first two weeks of incorpolation but decrease rates were similar after 3rd week. However, NH_4^+-N was also detected in the treatment of 16 kgN/rai at the amount of 1.46 kgN/rai to 0.22 kg/rai and near zero at the end of experiment.. These results were similar to those reported by Carmen *et al.* (2000) who found that the application of soybean green manure biomass at 110 and 140 kg/ha gave 30 kg NH_4^+-N/ha in the first week then declined rapidly within 3 weeks and Kumar *et al* .(2006) found that NH_4^+-N was mineralized in the amount of 159.36-167.5 kg/ha at the 3rd week of green manure biomass of *Sesbania aculata* incorporating and gradually declined .

NO_3^--N mineralization in soil

The amount of NO_3^--N mineralized from fertilized soybean plant residues were not significantly different after a period of two weeks of incorporation (Appendix 3).

The greatest amount of NO_3^--N was released from the 32 and 48 kgN/rai treatment which amounted to the average of 13 and 12.6 kgN/rai, respectively (Appendix Table 3). The subsequent treatment was that of 16 kgN/rai which was mineralized at 8.76 kgN/rai. At the third and fourth weeks, NO_3^--N mineralization was relatively decreased in all treatments of fertilized plants in a little unusual manner. Decreasing of NO_3^--N in the highest of N fertilizer application was at the lowest when compared with that of 16 and 32 kgN/rai. However, these NO_3^--N quantities were not significantly different ($P \leq 0.05$) (Appendix Table 3). Although mineralization of 16 and 48 kgN/rai fertilization seemed to be stable in the fifth week but the 32 kgN/rai fertilization sharply declined (Figure3). However, mineralizable N in the form of NO_3^--N almost disappeared at the sixth week.



Fiqure 3 Mineralization quantity of NO_3^--N during 1-6 weeks after amendment of vegetable soybean residues. T2 = soybean residue incorporation at 2.0 t/rai (16kgN/rai); T3 = soybean residue incorporation at 2.3 t/rai (32kgN/rai) ; T4 = soybean residue incorporation at 2.4 t/rai (48kgN/rai).

These results were similar to those reported by Carmen *et al.*(2000) who found that the application of soybean green manure biomass at 110 to 140 kg/ha nitrogen release in soil peaked at 80-120 kg NO_3^--N ha^{-1}. This peak of nitrogen release occurred 2 to 6 weeks after green manure application and Kumar *et al* .(2002) reported that the peak NO_3^--N content was observed at 5th week after incorporation of 118.4-123.4 kg/ha. In summation, organic nitrogen in the plant tissue which was transformed to be NH_4^+-N and NO_3^--N by mineralization were useful for growth of kale when incorporated vegetable soybean in the soil. From the study on accumulated fresh weight of kale (Table5), Carmen *et al* .(2000) reported that the N content of 60 to 74 day soybean green manure varied between 110-140 kgN/ha, soybean decomposed rapidly, losing 30 to 70% of their biomass within 5 weeks after application. Soil nitrate contents increased corresponding to green manure with a maximum increase of 80 to 100 kg NO_3^--N /ha with incorporated soybean. The peak N release occurred 2 to 3 wk. (Alva, 2006). In addition to soil amendments such as manure to enhance the biological qualities of agricultural soil, which undergo transformation with the release of inorganic N forms, ammonium(NH_4^+-N) and nitrate (NO_3^--N) forms, the NH_4^+-N rapidly converts to NO_3^--N under favorable soil conditions. The concentration of NH_4^+-N varied from 50 to 280 mg/kg, decreased while that of NO_3^--N increased rapidly and concentration generally stabilized about 40 days.

Table 5 Fresh weight of kale at the different growth stages respond to amendment of green manure plant growth and nitrogen translocation (kg/rai)

Week Treatment	1st week 4 DAP	2nd week 21 DAP	3rd week 28 DAP	4th week 35 DAP	5th week 42 DAP	6th week 48 DAP	% increase
Control	28.68 b	91.87 c	242.64 b	741.18	946.16 b	995.10 b	0
Biomass2.0 t/rai	33.25 ab	127.71 ab	327.21 a	783.67	1079.31a	194.87ab	20.08
Biomass2.3 t/rai	34.02 ab	100.20 bc	304.66ab	885.47	1029.69ab	262.11ab	26.83
Biomass2.4 t/rai	41.92 a	139.04 a	296.74ab	876.04	1073.93 a	1345.79 a	35.24
LSD$_{0.05}$	10.10	34.05	82.04	ns	119.25	302.52	
CV(%)	14.7	14.9	14.09	16.28	5.8	12.6	

Means with the same letter are not significantly different at alpha level =0.05

The growth variation of fresh weight of kale

The growth variation of fresh weight of kale seemed to be dependent on the amount of fertilizer applied to vegetable soybean. Almost of fresh biomass at each plant age was significantly different, except at the fourth week after transplanting, where biomass 2.4 t/rai soybean gave the highest vegetable fresh weight, followed by the treatment of 2.3 t/rai. However, the fresh weight of nitrogen fertilized soybean treatments were significantly higher than that of the control. Overall fresh weight accumulation of vegetable tended to respond to higher nitrogen fertilizer applied to soybean plants. The vegetable biomass increasing was inconsistent with nitrogen mineralization from fertilized soybean residues. More mineralized nitrogen was obtained in nitrogen treated soybean and led to increased vegetable weight. This result indicated that vegetable growth did not depend on nitrogen applied to soybean but depended on the biomass of soybean residues. Biomass of soybean residues were 1,594.67, 1,996.67, 2,274 and 2,447 kg/rai in the applied nitrogen treatments of 0, 16, 32 and 48 kgN/rai , respectively. Indeed , yield of kale was not significantly different between the applied biomass treatments of 2,274 and 2,447 kg/rai due to low difference in soybean residues in these two treatments. Incorporation of green manure applied biomass with 1,996.67 and 2,447 kg/rai were not significantly different. In 3rd and 4th week, growth responses of kale in the treatment incorporation of green manure applied biomass with 2,274 kg/rai(T_3) was highest at 304.66 kg/rai and 885.47 kg/rai, however, 4th week was not significantly different at $p \leq 0.05$ (Appendix 4.4.), After that the accumulation of fresh weight of kale in 5th and 6th week after transplantation were significantly different at $p \leq 0.05$. Though most of treatments were significantly different at $p \leq 0.05$, and percent increase of fresh weight when compared with treatment 1 (Control) after harvest were found that treatment incorporation of green manure applied with 1,996.67, 2,274 and 2,447 kg/rai increased fresh weight 20.08, 26.83 and 35.24%, respectively (Table5). On the other hand, when compared the accumulation of fresh weight of kale by analysis of variance for each week by fixed treatment, it was found that all treatments had weight from accumulated 1 week-6 weeks significant at 0.05 probability levels (Appendix 5.1-5.4). These results were similar to those reported by Choe Kangrak *et al.*(2006) who found that no mulching and mulching with rice straw on growth and yield of kale, no significant effects were observed, and the report by AVRDC (1998) that when used N source (ammonium sulfate 21-0-0) and N rate 60-240 kg/ha for Chinese cabbage, the marketable yields were between 14.9-18.9 t/ha and non significant at $P \leq 0.05$.

Nitrogen accumulation and nitrogen translocation

Nitrogen accumulated in kale was derived from mineralization of incorporated soybean manures and significant difference in % N observed in this study was found only between control treatment and fertilized soybean manures. Among the fertilized soybean manure treatments, there were no significant differences in percentage of N content at the value of 2.23, 2.39 and 2.29 in the treatments of soybean biomass 2.0 , 2.3 and 2.4 t/rai, respectively, while in the control was 1.81% (Appendix 6.1)

Table 6 Nitrogen content in terms of %N, atom%^{15}N abundance and atom%^{15}N excess in kale plant grown on soil incorporated with different nitrogen fertilized soybean manures.

Sample No.	% N	atom%^{15}N abundance	atom%^{15}N excess
Biomass2.0 t/rai	2.23	0.67	0.27
Biomass2.3 t/rai	2.39	0.80	0.43
Biomass2.4 t/rai	2.29	0.69	0.48
$LSD_{0.05}$	ns	ns	ns
CV%	9.48	14.60	7.65

Means with the same letter are not significantly different at alpha level =0.05

Total percentage of N content in enriched plant tissue consisted of ^{14}N and ^{15}N. A part of ^{15}N from N-mineralized in kale tissue exhibited in isotopic nitrogen, atom%

^{15}N which was expressed in atom%^{15}N abundance. Trend of atom%^{15}N abundance content was similar to that of total percentage N. The atom% ^{15}N abundances in manured kale were significantly higher than control treatment. Nevertheless, there was no statistical difference in ^{15}N abundance among soybean biomass manure treatments. The average amount of ^{15}N abundances shown in Table6 were 0.37, 0.67, 0.80 and 0.69 in control, 2.0, 2.3 and 2.4 t/rai soybean biomass manure treatments, respectively (Appendix 6.2). The absolute value of atom % ^{15}N in kale derived from mineralization of soybean manure was a subtraction of atom %^{15}N abundance by atom%^{15}N natural abundance. A substractive value was expressed in atom %^{15}N atom excess. The atom % ^{15}N excess in kale was not dependent on the quantity of N fertilizer applied to soybean plants. The appearance of atom % ^{15}N excess in kale tissue was significantly different at 0.01, 0.27 , 0.43 and 0.33 in the treatment of control, 2.0 , 2.3 and 2.4 t/rai of soybean biomass, respectively (Appendix 6.3) where the highest was in the treatment of soybean biomass 2.3 t/rai. The ^{15}N excess existed in control treatment indicated that isotopic nitrogen was derived from natural atom% ^{15}N occurred in the soil. The net percentage of nitrogen derived from soybean was calculated by following equation

$$\text{fNdfF} = \frac{\text{atom\% }^{15}\text{N excess in kale}}{\text{atom\% }^{15}\text{N excess in fertilizer}}$$

$$\text{atom \% }^{15}\text{N excess In kale} = \text{atom\%}^{15}\text{N in kale} - \text{Natural abundance}$$

Where

fNdfF = Fraction of Nitrogen in kale derived from fertilizer

$$\text{atom } \%\ {}^{15}\text{N excess} \quad = \quad \text{atom\% excess Nitrogen 15}$$

$$\%\ \text{FUE}(\%\ \text{Fertilizer use efficiency}) = \frac{\text{N fert.yield in plant}}{\text{Fertilizer applied}} x100 \qquad (\text{IAEA,1983})$$

$$\%\ \text{FUE} \quad = \frac{\text{mg } {}^{15}\text{N in kale from vegetable soybean residues}}{\text{mg } {}^{15}\text{N in soil and vegetable soybean residues}} \text{ X } 100$$

FUE = Fertilizer use efficiency or Fertilizer utilization efficiency

Table 7. Calculate percentage and quantity of N-uptake and % FUE of kale from vegetable soybean residue decomposition.

Biomass quantities (t/rai)	%N-Uptake and %FUE in kale from vegetable soybean residue decomposition		
	%N-uptake	kgN/rai	%FUE
Biomass2.0(16kgN/rai)	29.59	10.70	32.73
Biomass2.3(32kgN/rai)	30.48	12.39	35.60
Biomass2.4(48kgN/rai)	31.01	12.53	37.37
Average	30.36	11.87	36.24
$\text{LSD}_{0.05}$	ns	ns	ns
CV%	10.93	20.35	5.04

Means with the same letter are not significantly different at alpha level =0.05

By calculation in this way, the result shows in Table 7, that percentage of mineralized N from soybean manure uptake by kale at 29.59, 30.48 and 31.01% in the treatment of soybean biomass 2.0, 2.3 and 2.4 t/rai respectively, and not significant (Appendix7.1). These amounts of uptake nitrogen were equivalent to 10.70 , 12.39 and 12.53 kgN/rai but, there were no significant differences among N-fertilized treatments (Appendix7.2). These results were similar to those reported by Ladd and Amato (1986) according to the comparision of ^{15}N uptake in wheat plants fertilized and applied ^{15}N-labelled soybean residues to plot cropped and labelled medic by labelled chemical fertilizer. The report showed that only 17% of legume N was taken up by the plant as compared to 46% of the fertilizer N. Conversely, 62% of the legume N remained in organic form in the soil as compared to only 29% of fertilizerN. Similarly, Ladd (1981) also found that only 10.9 -17.3% of the ^{15}N in medic material was taken up by subsequent wheat crops. A 1983 study by the same researchers found slightly higher ^{15}N uptake at 20.2 - 27.8% of the legume^{15}N added. This higher level of N uptake only provided 6-10% of the N needed by the crop. Futhermore, according to the study of Norman *et al.* (1990) in %N-uptake from soybean green manure to rice by applied ^{15}N-labelled soybean residues to plot cropped with rice, they found that 52% of residue N was mineralized from soybean and 11% of residue N was taken up by the rice. However, Yathaphutanon *et al.* (1995) found in the study that the residual effect of soybean stover was 36.72% of nitrogen in rice which was equal to 50.62 kgN/ha, and N came from the returned soybean (stover plus root nodule under the ground and soil where the soybean stover was returned). However, the percentage of FUE from soybean manure uptake by kale at 32.73, 35.60 and 37.37% in the treatment of soybean biomass 2.0, 2.3 and 2.4 t/rai, respectively, were not significant These results were similar to those reported by Hardy and Havelka (1975) that the world cereal grain nitrogen use efficiency would therefore be estimated at 33%.Furthermore, Dibb *et al*. (2003) had the same report that in the last 25 years, the agronomic efficiency of fertilizer N use on corn in the U.S. has increased 39%.However, research on farm fields in the U.S. and Asia shows that apparent single-year recovery efficiency for fertilizer N is usually below 50% and frequently below 40% and finally the research by Ardell *et al*.(2002) found that when 112 kgNha^{-1} was applied to onion on 18 May and 25 June, a total fertilizer N recovered was 39%.