Original Paper

Apparent Nitrogen Mineralization Rates of Several Green Manures Incorporated in Soil and the Application Effects on Growth of Komatsuna Plants

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土壤にすき込まれた数種緑肥窒素のみかけの無機化率と
コマツナに対する施用効果

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1. Introduction

Raising crop productivity and production are becoming increasingly important to meet growing food requirements imposed by the incessantly increasing the world population (Yildirim and Guvence 2005), which is expected to reach 8 billion by 2020. That higher population necessitates greater food production (Eilitta *et al.*, 2004). During the last century, agriculture worldwide has experienced changes in farm size, marketing strategies, and biological and economic diversity, dependence on external non-

renewable resources and vulnerability to urbanization, climate change, and volatile global markets (Cherr *et al.*, 2006). Decreased soil productivity resulting from excessive soil erosion, intensive cultivation, and plant nutrient losses and depletion is a well-documented constraint to crop production in many parts of the world (Denish *et al.*, 2001). Recently, producers, consumers, government agencies, and researchers have therefore expressed increasing interest in economically viable alternatives that harness ecological and biological processes, on-farm resources, and diversification (Cherr *et al.*, 2006). During the 1980s and 1990s, green manure/cover crop (GMCCs) systems became a popular agricultural technology in studies and developmental efforts, especially for tropical and subtropical smallholders or farmers, to increase food production.

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Organic amendments have received renewed attention as improvements of soil fertility for crop production (Khan *et al.*, 2008). In addition to manure application, crop residue management is a key to supplying organic matter to soil and is expected to establish sustainability in cooperation with conservation tillage systems (Quemada 2004). Use of GMCCs is an option for crop residue management. In fact, GMCCs supply high amounts of organic matter to soil and improve soil structure via promotion of aggregation and increased water infiltration capacity (Andraski *et al.*, 2003 ; Sainju *et al.*, 2005 ; Miguez and German 2005 ; Sogbedji *et al.*, 2006) and root growth of succeeding crops (Sainju *et al.*, 2005), and to control soil erosion (Snapp *et al.*, 2005 ; Boller *et al.*, 1993). Recently the role of carbon sequestration has received much attention (Coppens *et al.*, 2007).

Other roles of GMCCs include temporary catching of nutrients remaining in fields, prevention of nutrients from leaching and assurance of their supply to succeeding crops. From this perspective, winter GMCCs have been used increasingly to scavenge remaining nitrogen (N) in soils after crop harvesting in summer or autumn, to return organic matter to the soil (Kuo and Jellum 2000), and to increase the N supply for succeeding summer crops (Sainju *et al.*, 2007 ; Vaughan and Evanylo 1998), thereby reducing N leaching (Odhiambo and Bomak 2001 ; Kavdir and Smucker 2005 ; Torstensson *et al.*, 2006 ; Clark A. 2007 ; Kuo and Sainju 1998). Cover cropping can increase soil organic carbon and N concentration by as much as 4–12% compared with no cover cropping or no N-fertilizer application (Sainju *et al.*, 2003 ; Monneveux *et al.*, 2005). Particularly, leguminous GMCCs can fix large amounts of N. Tian *et al.* (2000) reported that most tropical leguminous GMCCs can fix nitrogen of 25–115 kg N ha⁻¹ during 17 weeks, which is about 70% of the accumulated N.

However, the full benefit of using GMCCs will depend on the synchrony between the tim-

ing of N supply via mineralization of residues incorporated into soil and N demand of subsequent crops (Vyn *et al.*, 2000). Restricted uptake of organic N by crops is attributed to the lack of synchrony between the N release from the residue and N demand by the crop. In general, the mineralized N supply becomes available too late for the crop demand, especially in the case of slowly decomposing residues. The mineralization rate of N from crop residues has been linked to their structural and chemical characteristics or “residue quality”, to the soils physical and chemical properties and biological activities, and to environmental factors such as temperature and moisture (Bajjukya *et al.*, 2006).

Nitrogen mineralization is a conversion of organic N into inorganic N, whereas immobilization is a converse process occurring via microorganisms ; both are important processes to develop models that estimate the N amount that becomes available to crops in arable land (Christensen 1999 ; Cabrera *et al.*, 2005 ; Schomberg *et al.*, 2006). The mineralization rate of crop residue in soil depends on the nitrogen form, carbon form, C/N ratio (Brandsaeter *et al.*, 2008), and tillage methods and cropping practices, as well as soil properties such as temperature, moisture, and aeration (Kuo *et al.*, 1996). It is also affected by physical, chemical, biochemical, and microbiological soil parameters (Vinther *et al.*, 2004). On a global scale, climate is the best predictor of decomposition kinetics (Johnson *et al.*, 2007).

Nitrogen content or C/N ratio of organic matter is a principal determinant of N availability, irrespective of the mode of management of residues (Sainju *et al.*, 2005 ; Kuo and Jellum 2002). The optimal C/N ratio for rapid decomposition of organic matter is 15 : 1–25 : 1 (Sullivan 2003). One advantage of legumes in cropping systems is that their biomass residue tends to decompose quickly in soil and supply a substantial amount of N to soils, although their effectiveness on crop growth is poorer

than that of mineral fertilizers.

For farmers who have introduced GMCCs in their husbandry, the N availability of the GMCCs residues to the succeeding crop should be considered to avoid increased N leaching resulting from excessive fertilization. A careful GMCC management program during the growth period might enable farmers to maximize the dry matter yield through the N supply for subsequent crops (Odhambo *et al.*, 2001). Stute and Posner (1995) examined the synchrony between legume nitrogen release and corn demand in the Upper Midwest and they showed that in addition to releasing N in synchrony with the uptake pattern of corn, legumes released N in adequate amounts for corn production in this region. Bergstrom and Kirchmann (2004) also measured the recovery by succeeding crop and the leaching of nitrogen derived from GMs and stated that benefits of legume-based GMs should be evaluated with regard to the timing of N release and demand for N by the plant. However, few reports in the literature in Japan describe the synchronization of nutrient release from GMCCs incorporated in soil and nutrient demand by succeeding crops.

Objectives of this study were to evaluate the N availability of green manures (GMs) incorporated into soil in relation to their chemical properties, and to improve the synchrony between the N release from residues and N demand by succeeding crops.

2. Materials and Methods

A pot experiment was conducted in a plastic greenhouse at the Field Science Center, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan. The experiment was started on 15 December 2007 and conducted for 90 days until 28 February 2008. As organic N sources, crop residues of four kinds were used : hairy vetch (*Vicia villosa* Roth., HV, C/N=13.2), sunflower (*Helianthus annuus* L., SF, C/N=13.4), crotalaria (*Crotalaria*

breviflora DC., CR, C/N=21.2), and upland rice (*Oryza sativa* L., UR, C/N=38.1). They were collected from an upland field of the Field Science Center and dried in an oven at 70°C and then cut to about 1 cm length. Each residue was mixed with 350 g volcanic ash soil, *Silic Andisol*, which was collected from the upland field and passed through a 2 mm sieve, was then packed into Neubauer pots. The mixing rate of residues was determined by the amount of nitrogen as 107.7 mg N pot⁻¹, which was equal to 200 kg N ha⁻¹. In addition to these treatments, two treatments were prepared : a chemical fertilizer plot (F) mixed with the same ratio of ammonium sulfate and a control plot (C) with no residue. Every treatment was triplicate and all pots were added beforehand, respectively, with 350 kg P₂O₅ ha⁻¹ as fused phosphate and 200 kg K₂O ha⁻¹ as potassium sulfate.

The pots were placed in the plastic greenhouse, where a heating mat accompanied with a plastic cover maintained the minimum soil temperature at 15°C. The maximum and minimum air temperatures under the cover were 32.8°C and 3.3°C, respectively, through the growing period. After two weeks from the residue mixing, on 15 December, 15 seeds of komatsuna (*Brassica rapa* L. var. *peruviridis*) were planted to all pots at 1 cm depth. Soil moisture contents were maintained at 360–400 g kg⁻¹ by adding deionized water two or three times a week.

After germination, extra komatsuna plants except five plants per pot were thinned on December 30 for the first sampling, and the thinned plants were dried in an oven at 80°C for two days and milled for analysis after measuring their weight. For the second to fourth sampling, three pots that each pot had five plants were taken from each treatment every two weeks, on 14 and 28 of January, and 11 and 25 of February. The plants and soils were separated carefully and the plants were treated similarly to those of the first sample. The soils were air-dried at room temperature. Nitrogen contents of plants were analyzed in

every pot using an NC analyzer (NC-80 AUTO ; Sumika Chemical Analysis Service, Ltd.). Inorganic N in soil was extracted using an 18mM L⁻¹ calcium chloride solution and determined using a total carbon analyzer with a nitrogen unit (TOC-VCSH+TNM ; Shimadzu Corp.).

Mineralized N derived from GM residues was calculated by subtracting absorbed N of control from that of each treatment. The recovery rate of N by komatsuna plants from the amendments and the N recovery rate were calculated, respectively, using the following equations.

$$\text{Recovery rate (\%)} = \frac{(\text{N uptake in treatments}) - (\text{N uptake in Control})}{\text{N added}} \times 100$$

where the unit of each N amount is mg N pot⁻¹.

$$\text{Nitrogen mineralization rate (\%)} = \frac{(\text{N uptake by plants}) + \{(\text{SIN in treatments}) - (\text{SIN in Control})\}}{\text{N added (mg pot}^{-1})} \times 100$$

where SIN means soil inorganic nitrogen and the unit of each N amount is mg N pot⁻¹. Denitrification from the residues was not measured in this study.

3. Results and Discussion

1) Plant Growth

Growth response of komatsuna plants to the tested GM residues showed great variation. Until 30 days after planting (DAP) on 14 January 2008, the dry weight of komatsuna plants was unaffected by treatments except for those of plants from the UR plot (Fig. 1). However, at 45 DAP, the dry weights were separable into three groups: the group with the highest weight, which included F, SF and HV; the middle group, including CR and C; and the lowest, UR. At 60 DAP, the dry weight in F plot became distinctly the highest out of the first group. This tendency persisted until harvesting time at 75 DAP. In fact, the HV and SF plots showed the same growth pattern. Their growth followed that of the F plot. The growth in the CR plot was very low and then improved after 45 DAP, but no significant differ-

ence from the C plot was found. For the UR plot, growth has always been lower than C. These results might be explainable by the difference of N supply from the residues because sufficient nutrients aside from N were added to all plots beforehand. Differences of the N supply from the residues indicate a difference of mineralization patterns of residual N.

2) Nitrogen Uptake and the Recovery Rate

Table 1 presents the absorbed nitrogen by komatsuna derived from the residues. The values were calculated by subtracting the N uptake in C plot, to which residues and fertilizers

Fig. 1 Changes of dry weight of komatsuna plants applied with fertilizer and green manure residues
Abbreviation is the same as Table 1.
Different letters mean significant differences at $P < 0.05$ (Fischer's PLSD).

Table 1 Nitrogen amount absorbed by komatsuna, derived from green manure residues, by subtracting nitrogen of control plot

Treatments	Days after planting			
	30	45	60	75
	(mg N pot ⁻¹)			
F	19.2 ^a	38.0 ^a	42.0 ^a	54.0 ^a
HV	10.6 ^b	12.8 ^c	14.0 ^b	17.8 ^b
SF	11.1 ^b	17.1 ^b	15.7 ^b	21.0 ^b
CR	-2.8 ^c	3.0 ^d	4.7 ^c	4.1 ^c
UR	-5.9 ^c	-5.5 ^e	-11.8 ^d	-9.3 ^d

* Different letters mean significant differences at $P < 0.05$ (Fischer's PLSD).

* F : Fertilizer, HV : Hairy vetch, SF : Sunflower, CR : Crotalaria, UR : Upland rice.

Fig. 2 Recovery rates of nitrogen derived from the green manure residues during 75 days, in comparison with fertilizer (F). Different letters mean significant differences at $P < 0.05$ (Fischer's PLSD).

had not been applied. The N supply from every residue was quite low in comparison with the nitrogen fertilizer, as reported by Baijukya *et al.* (2006). Komatsuna in HV and SF plots absorbed greater amounts of N than in CR and UP, but much more in the F plot. For the CR plot, N uptake was lower than the control at 30 DAP, which indicates "nitrogen starvation", in which soil microbes used even soil mineral N to propagate themselves using residue carbon when residues with a high C/N ratio were incorporated. In the UR plot, nitrogen starvation was generated from the beginning and continued throughout the period.

The recovery rate of N in amendments for 90 days became maximum for the F plot: it was about 50% (Fig. 2). Among the GMs, SF and HV showed comparatively high rates of 15–18%, but only 5% in the CR plot. For UR, it became about –8% from the calculation.

3) Inorganic Nitrogen Remaining in Soil

Characteristics of GM residues affected the inorganic N level in soil during the growth period. The F plot retained the highest level, although it decreased rapidly with the growth of komatsuna (Fig. 3). In the HV and SF plots, 70–80 mg N kg⁻¹ of nitrogen, the difference between these plots and control, was increased by the addition of the residue at 30 DAP; it decreased gradually with growth thereafter.

Fig. 3 Changes of inorganic nitrogen in soils applied with fertilizer and green manure residues. Abbreviation is the same as Table 1. Different letters mean significant differences at $P < 0.05$ (Fischer's PLSD).

On the other hand, 10–20 mg N kg⁻¹ of nitrogen was supplied in the CR plot and there seemed less N supply from the residue in UR plot or in the control. Apparently, the residues of HV and SF had a high share of easily decomposable fraction of organic N in comparison with CR and UR.

4) Apparent Nitrogen Mineralization Rates of GMs

It has been documented that nitrogen mineralization of soil organic matter and incorporated crop residues is an important N source for crop production, along with N fertilizers (Christensen, 1999). When GMs are used as a N source, rapid mineralization is expected because an efficient amount of N should be supplied to crops during the growth period. The N mineralization rate is a useful index of the availability of organic amendments. In this study, apparent N mineralization rates of GMs were calculated by the subtraction method, instead of the gross N mineralization rates.

Apparent N mineralization for the residue of HV and SF is promoted at the initial stage. The rates had already reached 36% at 45 days after incorporation (DAI) (Fig. 4). Apparently, these residues contained large amounts of the readily decomposable N fraction. Then the mineralization continued moderately; the rate became

Fig. 4 Patterns of apparent nitrogen mineralization of incorporated green manures during 90 days

Different letters mean significant differences at $P < 0.05$ (Fischer's PLSD).

greater than 40% at 90 DAI. For CR, the apparent N mineralization was much slower than the two residues described above; the rate became about 20% at 75 DAI. In UR, the mineralization was not generated up to 75 DAI; the rate ended up at about 5%, even at 90 DAI.

It is also documented that the mineralization rates of incorporated residues depend on their characteristics, form, and content of the organic nitrogen contained and their C/N ratios (Kuo *et al.*, 1996; Horimoto *et al.*, 2002). In this experiment, a strong negative correlation was found between the apparent N mineralization rates and the C/N ratios (Fig. 5). Consequently, the difference in the N availability of the tested GMs is explainable by the C/N ratios. GMs with the lower C/N ratio will be decomposed more quickly. For SF, the C/N ratio of the whole plant is usually higher than 30 in the stage the seeds fully ripen, but it is still low with around 15 before ripening. The SF residue used in this experiment was a younger one, for which the C/N ratio was 13.4. The apparent N mineralization rates of HV and SF, for which C/N ratios were both lower than 15 were about 40% during 90 days; this value is apparently equal to that of manure or compost. For CR, Wang and McSorly (2003) and Valenzuela and Smith (2002) reported that the C/N ratio of this GM was within a range of 13.5–24 and that CR had high N availability. However, that range, 13.5–24, is

Fig. 5 Relationship between C/N ratio and apparent nitrogen mineralization rate of green manures incorporated for 90 days

apparently too wide to estimate the N availability; the availability will be variable. The low rate of apparent N mineralization and the availability (Figs. 1, 2) for CR in the current experiment, for which the C/N ratio was 21.2, might be reasonable. However, if CR were cut and incorporated at a younger stage, higher nitrogen availability could be expected because of the lower C/N ratio.

4. Conclusion

The N supply to a succeeding crop, which is derived from incorporated GMs, is dependent on the mineralization rate. In GMs of four kinds tested in this experiment, the apparent N mineralization rate during 90 days after incorporation was highest for hairy vetch and sunflower. The growth of the succeeding crop, komatsuna, was good in their plots.

The apparent N mineralization rates of GMs showed a negative correlation with the C/N ratios; it was estimated that plant residues with a C/N ratio of less than 15 were desirable in view of the N supply to subsequent crops. Regarding the sunflower plant, it is important that the plant should be incorporated during a premature stage because its C/N ratio surpasses 20 when the seeds ripen.

Results show that cultivation and incorporation of hairy vetch is desirable for N supply to summer crops and immature sunflower to winter crops, from the standpoint of N recycling in fields.

Summary

Apparent nitrogen mineralization rates and the availability of released nitrogen to komatsuna plants succeeding green manures (GMs) incorporated to soil were investigated using a pot experiment to develop a reasonable crop rotation system that decreases environmental loads by ensuring nutrient cycling. Four GM residues—hairy vetch (HV), sunflower (SF), crotalaria (CR), and upland rice (UR)—were mixed with soil, respectively, to produce 200 kg N ha⁻¹. Furthermore, a fertilizer (F) plot with the same N amount of chemical fertilizer applied and a control (C) plot with no amendment were set. Komatsuna plants were grown for 75 days under the treatments in a plastic green house. Plants were sampled five times to determine their dry weights and nitrogen contents. Dry weight yields of komatsuna were highest in F plot, with those of HV and SF plots following, although that of UR plot was less than that of C plot. The N content of komatsuna showed similar results. Absorbed N, as derived from the amendments, was greatest in F plot, followed by HV and SF plots, although the UR plot had less than control. Regarding apparent nitrogen mineralization rates which were calculated by subtraction method, significant differences were found between HV and CR, and HV and UR plots, and between SF and CR, and SF and UR plots, but not between HV and SF. Inorganic nitrogen in soil was higher in HV and SF plots than CR and UR plots. The residues of HV and SF demonstrated preferred characteristics as GMs for a nitrogen supplier to succeeding crops.

Key Words

green manure, hairy vetch, sunflower, crotalaria, upland rice, apparent nitrogen mineralization rate, Synchronization, recovery rate, C/N ratio

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要 旨

養分循環の推進による環境負荷の少ない輪作体系の確立を目的として、数種緑肥作物を土壤にすき込んだ場合のみかけの窒素無機化率と後作物に対する可給性について、ポット試験により検討した。

4種類の緑肥作物（ヘアリーベッチ：HV、ヒマワリ：SF、クロタリリア：CR、陸稲：UP）の細断物をそれぞれ窒素量が一定（200 kg N ha⁻¹）になるように土壤に混合してポットに詰めた。SFは子実成熟前に収穫されたものであった。これら4試験区に加え、同窒素量の化学肥料区（F）および窒素無添加区（対照区、C）を設けた。ビニールハウス内で、資材混合2週間後にコマツナを播種し、75日間栽培した。途中で間引きを含め5回サンプリングを行い、乾物重と窒素含有率を測定した。

コマツナの乾物重および窒素含有率はF区>HV区≒SF区>C区>UR区となり、UR区は対照区より劣った。混合資材由来窒素の吸収量はF区で最大となり、HV区とSF区がこれに続き、UR区は計算上マイナスになった。差引法によって求めた残渣の窒素無機化率はHV区とSF区でCR区、UR区よりも有意に高かった。土壌中の無機態窒素もHV区とSF区が明らかにCR区、UR区より高かった。

以上の結果から、後作物への窒素供給すなわち後作物の窒素要求との同期性という点においては、供試したカバークロップのなかではヘアリーベッチと子実成熟前のヒマワリが最も望ましい性質を持つと考えられた。

キーワード

カバークロップ, ヘアリーベッチ, ヒマワリ, ク

ロタラリア, 陸稲, 窒素の無機化, 同期化, 残渣
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