Role of Belowground Parts of Green Manure Legumes, *Crotalaria spectabilis* and *Sesbania rostrata*, in N Uptake by the Succeeding Tendergreen Mustard Plant

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**Abstract**: Using symbiotically N\(_2\)-fixing legumes as green manures is a way to supply N from the atmosphere to cropping ecosystems. Usually whole plants of the green manure are incorporated into soil; hence, the belowground parts as well as the aboveground parts would contribute to N transfer to succeeding crops. However, little is known about the contribution of the belowground parts alone. We assessed N transfer from belowground parts compared to whole plants of two legumes, *Crotalaria spectabilis* and *Sesbania rostrata*. Each of the legumes was grown approximately for 3 months in a 1/2000a Wagner pot filled with soil media, and then the roots alone (R) or shoot and root (S + R) were harvested and incorporated in the pots. Tendergreen mustard (*Brassica rapa*) as the succeeding crop was grown for 66 days in these pots without additional fertilizer. Although the amount of N in green manure in S + R pots was approximately 4-fold higher than that in R pots, differences in N uptake by tendergreen mustard between the S + R and R pots were smaller (1.7-fold for *C. spectabilis* and 2.3-fold for *S. rostrata*). This means that N recovery rate by tendergreen mustard was significantly higher in R than in S + R pots with either green manures. Differences in C/N ratio of the green manures could not likely explain the higher N recovery rate in R pots. Bioassay of the aqueous extracts from the green manure with lettuce seedlings suggested that growth inhibitory effects might be responsible for the lower recovery rate in S + R treatment.

**Key words**: Allelopathy, C-N ratio, Crop rotation, Nitrogen fixation, Nitrogen starvation.

Several tropical legumes such as *Crotalaria* and *Sesbania*, which play an important role in reducing mineral N fertilizer demand, are used as green manures in temperate regions such as eastern Asian countries, Japan, Korea and China. In order to improve soil fertility and maintain the sustainability, these N\(_2\)-fixing legumes should be more used in agricultural systems in these regions. The traits of dry matter production and N\(_2\) fixation in these legumes have been shown by many researchers (Martin et al., 1976; Ladha et al., 1996; Becker and Johnson, 1999; Fischler et al., 1999; Samba et al., 2002), and we also evaluated both positive and negative effects of the legumes on growth of the succeeding crops after incorporation of these legumes (Yano et al., 1994; Ohdan et al., 1995; Ohdan and Daimon, 1998a,b; Daimon and Kotoura, 2000; Uratani et al., 2004).

The flow of N from green manure to the succeeding crops mainly occurs through the great amount of N in the aboveground parts of these legumes, and much of the N would be provided after decomposition of the aboveground parts. As described previously, the chemical properties of the incorporating materials, such as the contents of N and lignin (L) and the ratios of C (Carbon)-N and L-N, affect the mineralization of the incorporated N (Yano et al., 1994; Ohdan and Daimon, 1998a,b; Uratani et al., 2004). In the materials with higher C/N and L/N ratios, the growth of the succeeding crops would severely compete with the microbial biomass for the organic N, and N immobilization immediately after incorporation will inhibit the growth of the succeeding crops.

After incorporation of the legumes as green manure, N is released not only from the aboveground parts, N releasing after incorporation of the green manure also occurs through the decomposition of the belowground parts of the legumes. Belowground N flow from legumes to the succeeding crops would occur in two ways. In addition to being mineralized by decomposition of roots and nodules, their debris is also the main source of N for the succeeding crops in the long-term N flow. On the other hand, release of nitrogenous compounds from the root system would be considerable in the short-term N flow to the succeeding crops. For example, belowground N transfer from legumes to the associated grasses has been examined in the mixed cropping systems, such as clover-grass associations (Chujo and Daimon, 1984; Høgh-Jensen and Schjoerring, 2001; Paynel et al., 2001; Paynel and Cliquet, 2003; Rasmussen et al., 2007). Several nitrogenous compounds such as ammonium, amino acids, and peptides have also been...
identified in exudates of the roots of soybean and alfalfa plants (Brophy and Heichel, 1989). The amounts of nitrogenous compounds released from legume root systems increase immediately after cutting the shoots, and the nitrogenous compounds would be then taken up by the associated grasses (Simpson, 1965; Frame, 1973; Chestnutt et al., 1980; Osman and Diek, 1982). N release via exudates from the root systems with active root nodules showing higher nitrogenase activity might also play important roles in short-term N flow even in the green-manure incorporation.

In general, Crotalaria and Sesbania plants are incorporated during their vigorous vegetative stage showing high activity of N₂ fixation. In this stage, both roots and root nodules have higher N contents, and harvesting of the aboveground part for green manuring might induce releasing the substantial amounts of N compounds from the root systems. However, little is known about contribution of belowground parts of these plant species to the growth and N uptake of the succeeding crops in the cropping systems applied green manures, especially when the succeeding crops are sown immediately after incorporation. The objective of the present study is to investigate N benefits from belowground parts of C. spectabilis and S. rostrata in the succeeding tendergreen mustard plants grown immediately after incorporation.

Materials and Methods

1. Cultivation of green manure plant

Crotalaria spectabilis and Sesbania rostrata were grown in 1/2000 a Wagner pot containing 1:2 mixture of vermiculite and “Akadamatchi”, that is subsoil of Andosol with a pH (H₂O) of 5.2, EC of 0.06 ds m⁻¹, TN of 0.11%, ammonium N of 10.4 mg kg⁻¹, nitrate N of 13.4 mg kg⁻¹, Truog-P of 0.6 mg kg⁻¹, CEC of 30.7 cmol kg⁻¹, exchangeable Ca²⁺ of 0.71 cmol kg⁻¹, exchangeable Mg²⁺ of 0.17 cmol kg⁻¹, and exchangeable K⁺ of 0.25 cmol kg⁻¹. Chemical fertilizer (N:P₂O₅:K₂O = 3:10:10) was applied at the rate of 5 g per pot. Seeds of the green manure legumes were obtained from Kaneko Seeds Co. Ltd., Japan. They were sown in the pot on 22 June 2005. Rhizobial suspensions (OD₆₀₀: 0.2) were prepared from cultures of Bradyrhizobium sp. strain USDA3024 for C. spectabilis and Azorhizobium caulinodans strain U0207SRR for S. rostrata in YM medium and lactose medium, respectively (Somasegaran and Hoben, 1985). They were applied to the pot on 23 June 2005 and also added once a week till mid-July. After 7 days of sowing, 5 seedlings in a pot were thinned to the one plant per pot. The experiment was conducted outdoors with 20 pots per species.

On 12 September 2005, shoots and roots were sampled and nitrogenase activities of the root and stem nodules were determined in a detached system using the acetylene reduction assay (Hardy et al., 1968). Shoots and roots sampled separately were oven-dried, weighed, and then ground for analyzing N and C concentrations by the dry-combustion method using a Model NC-80 analyzer (Sumitomo Chemical Inc., Japan).

2. Cultivation of the succeeding tendergreen mustard plant

Each green manure species was grown in 20 pots. Five pots were used for measurement of growth of each species, five pots for incorporation of only roots, and five pots for incorporation of both shoots and roots as described below. Five pots out of 20 pots prepared for each species were not used, because only uniformly grown plants were used.

Green manure crops were harvested on 12 September 2005. Fresh weight of shoots and roots were weighed, cut into 3–5 cm length and mixed together (S+R) into the soil in the pot for culture of tendergreen mustard plants (succeeding crop). For evaluation of the effect of belowground parts on the N contribution to the succeeding crop, only the roots (R) were returned into the soil.

The seeds of tendergreen mustard (Brassica rapa cv. Rakuten) were sown in the pots on 29 September 2005. No fertilizer was applied. Ten days after sowing, nine seedlings in a pot were thinned to 3 plants per pot. From 20 days after sowing, length and width of the largest leaf in each plant were measured every 10 days, and the SPAD values (SPAD-502, Minolta, Co. Ltd., Japan) at the center of the leaf blade beside the main vein were also measured to determine the N status of the plant. Soil solution was collected with a fiber-type soil moisture sampler (DIK-301B, Daiki Rika, Co. Ltd., Japan) once a week. Inorganic nitrogen concentration of the sample was determined by a microdiffusion method (Mulvaney et al., 1997). On December 5 2005, tendergreen mustard plants were sampled, and oven-dried, weighed, and ground for analyzing N concentrations. The data were analyzed by the T-test for determining significant differences in the mean of each parameter between S+R and R incorporation.

3. Evaluation of inhibitory effect of aqueous extracts of green manure plant

For evaluation of inhibitory effect of shoot and root of green manure legumes on growth of the succeeding tendergreen mustard plant, a bioassay using a testing plant, lettuce (Lactuca sativa cv. Great Lakes 366), for germination and seedling growth affected by application of aqueous extracts of different plant parts was conducted. Shoots and roots were sampled from 5 pots on 12 September, and shoots were also separated into leaves and stems, and then oven-dried at 70°C for 2 days, and ground into a fine powder. A 1.25 g of dried powder was placed in a 300 ml Erlenmeyer flask and then 100 ml of distilled water was added. After shaking for 60 min at 120 rev min⁻¹ in the dark,
Table 1. Growth, N content and acetylene reduction activity (ARA) of *Crotalaria spectabilis* and *Sesbania rostrata* grown as green manure crops at 81 days after sowing.

| Plant species | Plant parts | Fresh weight (g plant⁻¹) | Dry weight (g plant⁻¹) | N content (mg plant⁻¹) | C/N ratio | ARA (C₂H₄ μmol hr⁻¹ plant⁻¹) |
|---------------|-------------|---------------------------|------------------------|------------------------|-----------|-------------------------------|
| *C. spectabilis* | Shoot       | 162                       | 32.0                   | 646                    | 20.9      | –                             |
|                | Root        | 97                        | 13.4                   | 178                    | 27.2      | 12.0                          |
|                | Total       | 258                       | 45.4                   | 824                    | 22.2      | 12.0                          |
| *S. rostrata*  | Shoot       | 219                       | 65.9                   | 1151                   | 24.8      | 6.9                           |
|                | Root        | 117                       | 30.0                   | 287                    | 26.0      | 5.0                           |
|                | Total       | 336                       | 95.9                   | 1438                   | 25.1      | 11.9                          |

Values are means of 5 pots sampled out of 20 pots in each species.

Fig. 1. Changes in length, width, and SPAD value of the largest leaf in tendergreen mustard plant grown on the soil after incorporation of different parts of *C. spectabilis* and *S. rostrata* plants. Values are mean of five pots with three plants per pot. Vertical bars indicate the standard error of the mean.
the mixtures were filtered through No. 2 filter paper (Advantec Toyo Kaisha, Ltd., Japan) and then through a membrane filter with 0.45 μM pore size (Nihon Milipore Ltd., Japan). The effect of the aqueous extracts at different concentrations 0.05, 0.25 and 1.25% (V/V) were examined in this experiment. Seeds of lettuce were surface-sterilized in sodium hypochlorite solution (2% active chlorine) with 0.01% Tween 20 for 15 min. The aqueous extracts were added into a Petri dish with No. 2 filter paper, and twenty seeds were placed on filter paper. They were incubated at 26°C in the dark. Distilled water was used as a control.

Germination of the seeds was measured every day and the lengths of hypocotyl and radicle were measured on all seedlings after 4 days of culture. The experiment was conducted with 3 dishes for each treatment. The data in each treatment were analyzed by a two-way ANOVA (plant parts x concentrations) and Tukey’s LSD was calculated to determine differences in the means among these treatments, when F values were significant.

Results

1. Growth and N accumulation of green manure plant

The seedlings of the two green manure plant species 4–5 days after sowing exhibited vigorous growth under the pot experiment conditions. The growth phase of the plants harvested on 12 September 2005 (81 days after sowing) differed between the two species. *C. spectabilis* was under a vegetative stage and *S. rostrata* was under mid anthesis.

In *C. spectabilis*, shoot dry weight was 2.4 times heavier than root dry weight, and higher value in ratio of shoot to root in N content was also found (Table 1). The C/N ratio was higher in roots than in shoots, but less than 30 even in the roots not showing a higher value considered for being slowly decomposed.

Dry weight and N content of shoot and root harvested on 12 September were greater in *S. rostrata* than in *C. spectabilis* (Table 1). They were approximately 2 times as high as those in *C. spectabilis*. However, the ratio of shoot to root in dry weight and N content did not differ between the two plant species. The C/N ratio in shoot was slightly higher in *S. rostrata* than in *C. spectabilis*, but was also less than 30.

The value of acetylene reduction activity (ARA), which was evaluated as a sum of the values in root nodules and stem nodules in *S. rostrata*, did not differ between the two plant species.

2. Growth and N uptake of the succeeding tendergreen mustard plant

Seedlings of tendergreen mustard emerged at 5 days after sowing, and they grew well exhibiting vigorous leaf expansion. From 20 days after sowing, length and width of the leaf (largest in each plant) of tendergreen mustard plant grown in R pots became greater than those in S + R pots until 40–50 days in both pots with *C. spectabilis* and *S. rostrata* (Fig. 1). The SPAD value of the largest leaf did not differ between the plants grown after R and S + R incorporation until 40–50 days after sowing. However, at 60 days after sowing, the value was higher in S + R pots than in R pots with both legumes (Fig. 1).

There was a definite difference in inorganic N concentration between the soil solutions collected from the pots incorporated with R and S + R (Fig. 2). The N concentrations became markedly higher in the soil of S + R pots than in R pots 4 and 2 weeks after incorporation of *C. spectabilis* and *S. rostrata* (Fig. 1). The SPAD value of the largest leaf did not differ between the plants grown after R and S + R incorporation until 40–50 days after sowing. However, at 60 days after sowing, the value was higher in S + R pots than in R pots with both legumes (Fig. 1).
especially in those incorporated with *S. rostrata*.

Table 2 shows the growth and total N content of tendergreen mustard plants grown after incorporation of different parts of *C. spectabilis* and *S. rostrata* at 66 days after sowing.

| Plant species | Plant parts | Fresh weight of incorporated materials (g pot⁻¹) | N content of incorporated materials (mg pot⁻¹) | Fresh weight (g pot⁻¹) | Dry weight (g pot⁻¹) | N content (mg pot⁻¹) | N recovery rate (%) |
|---------------|-------------|-----------------------------------------------|-----------------------------------------------|------------------------|----------------------|---------------------|---------------------|
|               |             | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| *C. spectabilis* | R           | 89    | 164  | 253   | 9.3   | 9.5  | 18.8  | 1.8   | 1.4  | 3.2   | 24    | 21   | 45    | 27.4  |
|                | S+R         | 233   | 742  | 975   | 14.0  | 9.5  | 23.5  | 2.3   | 1.2  | 3.6   | 53    | 25   | 78    | 10.5  |
| T-test         |             | **    | n.s  | **    | **    | **   | **    | **    | **   | **    | **    | **   | **    | **    |
| *S. rostrata*  | R           | 135   | 330  | 465   | 12.0  | 10.3 | 22.3  | 2.2   | 1.5  | 3.7   | 35    | 22   | 57    | 17.3  |
|                | S+R         | 339   | 1339 | 1678  | 16.8  | 9.3  | 26.2  | 2.7   | 1.4  | 4.0   | 93    | 36   | 129   | 9.7   |
| T-test         |             | *     | *    | n.s   | n.s   | **   | **    | **    | **   | **    | **    | **   | **    | **    |

*Values are means of 5 pots with three plants per pot. R: only roots were incorporated. S+R: both shoots and roots were incorporated.*

*, **Significantly different between R and S+R treatments in each plant species at the 0.05 and 0.01 probability levels, respectively. n.s: not significant.

![Fig. 3](image-url)  
Changes in germination rate of lettuce plant treated with aqueous extracts of different parts of *C. spectabilis* and *S. rostrata* harvested at 81 days after sowing at different concentrations (%). Values are means of 3 Petri dishes with 20 seeds per a dish. Vertical bars indicate the standard error of the mean.
calculated from these values, N recovery percentage in R pots was significantly higher than that in S+R pots with both legumes (i.e. 27.4% in R and 10.5% in S+R pots, with C. spectabilis).

The dry weight and total N contents tended to be higher in the pots incorporated with S. rostrata, than with C. spectabilis, while the percentage of N recovery by the succeeding tendergreen mustard plant was higher in C. spectabilis than in S. rostrata.

3. Inhibitory effect of incorporated materials

Germination and seedling growth of lettuce differed among treatments with the aqueous extracts from each plant part of the two legumes at 0.05, 0.25, and 1.25%. After 1 to 3 days of culture, germination of lettuce was definitely inhibited by the extract of all parts of C. spectabilis at the concentration of 1.25% (Fig. 3). The leaf extract of S. rostrata at 1.25% inhibited the germination after 1 or 2 days of culture, while the extract of the stem and root had no inhibitory effect at any concentration.

Similar trends of inhibitory effects on seedling growth, which was evaluated as lengths of hypocotyl and radicle after 4 days of culture, were observed (Table 3). Leaf extracts at the concentrations of 0.25 and 1.25% in C. spectabilis severely reduced length of radicle, and the inhibition was also found in stem extracts. On the other hand, leaf extracts of S. rostrata at 1.25% reduced hypocotyl length by 85% compared with extracts of stem and root, while radicle length was drastically inhibited by the leaf extracts at all concentrations, and it was also inhibited by the extracts of stem and root at both 0.25 and 1.25%.

Discussion

Previous studies on the effect of green manure legumes, such as Crotalaria and Sesbania species, on N contribution to the growth and N uptake of the succeeding crops such as wheat and spinach plants, showed an apparent positive N benefit from the legume to the succeeding crop (Yano et al., 1994; Ohdan and Daimon, 1998a,b; Daimon and Kotoura, 2000; Uratani et al., 2004). As reviewed by Daimon (2006), prerequisites for efficient application of green manure are selection of an appropriate plant species and the identification of the appropriate time of incorporation into soil for the succeeding crops. Of several prerequisites, potentials of dry matter production and N$_2$ fixation and changes in chemical properties of incorporated materials such as C/N ratio, lignin (L) content, and L/N ratio, have previously been identified in C. spectabilis and S. rostrata, showing that a large biomass with lower ratios of C/N and L/N based on a higher N content could provide a greater benefit to the succeeding crops grown soon after incorporation (Ohdan and Daimon, 1998b; Daimon and Kotoura, 2000). This was in agreement with the present experiments; N uptake by the succeeding tendergreen mustard plant depended on the total amounts of N input as incorporated materials, as shown by the difference in N content of the succeeding crop between the S+R pots and the R pots (Table 2).

In this experiment, we further investigated the N flow especially through roots, which should also be considered as an important prerequisite for efficient application of each green manure legume. Relative to total N content of whole plant, N in the root harvested on 12 September amounted to 21.6% (178 mg/824 mg) and 19.9% (287 mg/1438 mg) in C. spectabilis and S. rostrata, respectively (Table 1). On the other hand, N recovery rate by the succeeding tendergreen mustard plant was significantly higher in the R pots than in the S+R pots in both legumes (27.4% in the R pots compared with 10.5% in the S+R pots for C. spectabilis, 17.3% in the R pots and 9.7% in the S+R pots for S. rostrata, shown in Table 2). Moreover, higher values in the length and width of the maximum leaf of the tendergreen mustard plant examined soon after sowing were also found in the R pots, and also SPAD value did not vary between the R pots and S+R pots (Fig. 1), indicating that the succeeding crop might utilize considerable amounts of N accumulated in roots.

Table 3. Effect of aqueous extracts of different parts of C. spectabilis and S. rostrata plants harvested at 81 days after sowing on lengths of hypocotyl and radicle of lettuce plant grown in a Petri dish.

| Aqueous extracts | Parameter measured | Control | Leaf | Stem | Root |
|------------------|--------------------|---------|------|------|------|
| C. spectabilis | Hypocotyl length (mm) | 15.5 a | 14.6 a | 15.3 a | 11.4 b | 15.5 a | 16.3 a | 15.7 a | 15.0 a | 16.3 a | 15.0 a |
| | Radicle length (mm) | 15.3 a | 13.1 bc | 10.0 d | 6.5 e | 14.8 a | 12.0 c | 7.6 e | 15.0 a | 15.5 a | 14.9 ab |
| S. rostrata | Hypocotyl length (mm) | 15.5 c | 16.0 abc | 15.8 abc | 13.7 d | 15.9 abc | 16.2 abc | 16.3 a | 15.7 bc | 15.9 abc | 16.2 ab |
| | Radicle length (mm) | 15.3 a | 12.9 b | 9.7d | 7.7 e | 14.7 a | 13.1 b | 10.7 cd | 14.7 a | 12.7 b | 11.1 c |

Values are means of 3 Petri dishes with 20 seedlings per a dish. Values in a row followed by the same letter are not significantly different at 0.01 probability level.

*: Concentrations of aqueous extracts.
Little has been known about N supply from belowground parts of the preceding legumes in crop rotation systems, especially in the system including green manure. However, short-term N transfer pathway from legume to associated crop in mixed cropping system has been reported by several authors. In mixed swards of legumes and grasses, for instance, associated legumes enable an increase in grass N uptake, because N fixed by the legume could be transferred to the grass via either death and decomposition of legume debris and/or exudation of inorganic nitrogenous compounds from the legume (Chestnutt et al., 1980; Høgh-Jensen and Schjoerring, 2001; Rasmussen et al., 2007).

Release of N from the legume roots through decay of roots and nodules or direct exudation from the root system have also been reported (Chujo and Daimon, 1984; Paynel et al., 2001; Paynel and Cliquet, 2003), and it has been known that the N release was drastically increased through photoassimilates deprivation due to shoot removal such as defoliation, cutting and shading (Osman and Diek, 1982; Daimon and Chujo, 1986). The sudden deprivation of photoassimilates that have been translocated to the root system including root nodules with higher N2-fixing activity, would also occur when shoots of the green manure legumes were harvested for being incorporated. We suggested that higher value of N recovery and earlier benefit of N in leaf growth in the R pots would be due to the pathway of this N flow from root system. However, the N flow from roots of these green manure legumes was not directly measured in the present experiment. Both N compounds released into the rhizosphere and their fate after incorporation of belowground parts of the legumes should be clearly defined in order to evaluate the role of N accumulated in belowground parts in the cropping systems including these green manure legumes.

On the other hand, the lower N recovery rate and earlier inhibition in leaf growth in the S + R pots compared with the R pots suggest that either more N was lost through microbial decomposition of the incorporated shoots and roots or that inhibitory substances released from shoots after decomposition severely control the N uptake of the succeeding crop as described below. Because C/N ratios of the incorporated materials were nearly the same between shoots and roots, mineralization rate after incorporation would not be so much different between the S + R and R pots, especially in earlier stage of decomposition. As described above, it is suspected that inorganic N released from active root nodules after shoot removal assists the reduction of competition between microbial uptake of the N and N absorption by the succeeding crop.

The N absorption by the succeeding crop would be expected to be higher when green manure legumes with much higher activity of N$_2$ fixation are incorporated. On the contrary, it would be lower when green manure legumes with lower N$_2$ fixation activity are incorporated. In the root system at flowering to maturity stage, the C/N ratio would be increased and a competition of the succeeding crop with the microbial biomass for N uptake would be increased. In the present experiment, shoots and roots of the legumes harvested on 12 September were examined to determine the effect of S + R and R applied as green manure. Further investigation would be required for more effective utilization of N accumulated in roots from the viewpoints of N$_2$ fixing abilities of these legumes.

It should also be noted that N input through incorporation of shoots and roots has different effect on the growth of the succeeding tendergreen mustard plant. In the present experiment, the aqueous extracts from leaves of the two legumes strongly inhibited the seedling growth of the lettuce plants (Table 3), but those from the roots did not (Table 3). This is in agreement with our previous reports for some species in the genus *Crotalaria*, in which growth inhibition was confirmed by the experiment using a root-box and a growth pouch culture method (Ohdan et al., 1995, Daimon and Kotoura, 2000). *C. spectabilis* has also been reported to produce a substance toxic to livestock and nematodes, the pyroloziding alkaloid ‘monocrotaline’ (Martin et al., 1976, Lafranconi and Huxtable, 1984).

In a pot experiment, soil volume per plant might affect the root density, and the ratio of shoot/root would become higher due to the smaller volume of the soil compared with that under field conditions (Mayer et al., 2003). Especially in summer green manure legumes showing vigorous growth, the ratio would severely be influenced. However, approximately 20%, under 1/2000a Wagner pot conditions, would not be necessarily low, and it is possible that the fate of N accumulated in roots found in this pot experiment is similar to that under field conditions.

In conclusion, the present study indicated that belowground parts of *C. spectabilis* and *S. rostrata* harvested at vigorously growing phase could have an important role in N uptake of the succeeding tendergreen mustard plant sown immediately after incorporation. N uptake of the succeeding crop might be regulated by 1) the total N amounts of incorporated materials, 2) available N released from root systems immediately after incorporation, and 3) inhibitory substances produced from incorporated materials, especially through decomposition of shoots. For understanding the contribution of fixed-N by the green manure legumes in various cropping systems,
functions of the N accumulated in the belowground parts of the legumes need to be examined.

References

Becker, M. and Johnson, D.E. 1999. The role of legume fallows in intensified upland rice-based systems of West Africa. Nutr. Cycl. Agroecosyst. 53 : 71-81.

Brophy, L.S. and Heichel, G.H. 1989. Nitrogen release from roots of alfalfa and soybean grown in sand culture. Plant Soil 116 : 77-84.

Chestnutt, D.M.B., Bartholomew, P.W. and Binnie, R.C. 1980. The interaction of perennial ryegrass and timothy in mixtures and their reaction to clover and nitrogen in cut swards. Grass Forage Sci. 35 : 281-286.

Daimon, H. and Chujo, H. 1986. Plant growth and fate of nitrogen in mixed cropping, intercropping and crop rotation. 1. Growth acceleration of some temperate grasses in early stage of mixed cropping with red clover. Jpn. J. Crop Sci. 55 : 218-221*.

Daimon, H. and Chujo, H. 1986. Plant growth and fate of nitrogen in mixed cropping, intercropping and crop rotation. 2. Nitrogen content of wheat in association with pea or broad bean. Jpn. J. Crop Sci. 55 : 162-170*.

Daimon, H. and Kotoura, S. 2000. Incorporation of Crotalaria spectabilis grown at a high seeding rate inhibits the growth of the succeeding wheat crop. J. Agron. Crop Sci. 185 : 137-144.

Daimon, H. 2006. Traits of the genus Crotalaria used as a green manure legume on sustainable cropping systems. JARQ. 40 : 299-305.

Fischler, M., Wortmann, C.S. and Feil, B. 1999. Crotalaria (C. ochroleuca G. Don.) as a green manure in maize-bean cropping systems in Uganda. Field Crops Res. 63 : 137-143.

Frame, J. 1973. The yield response of a tall fescue/white clover sward to nitrogen rate and harvesting frequency. Grass Forage Sci. 28 : 139-148.

Hardy, R.W.F., Holsten, R.D., Jackson, E.K. and Burns, R.C. 1968. The acetylene-ethylene assay for N₂ fixation : Laboratory and field evaluation. Plant Physiol. 43 : 1185-1207.

Høgh-Jensen, H. and Schjoerring, J.K. 2001. Rhizodeposition of nitrogen by red clover, white clover and ryegrass leys. Soil Biol. Biochem. 33 : 439-448.

Lafranconi, W.M. and Huxtable, R.J. 1984. Hepatic metabolism and pulmonary toxicity of monocrotaline using isolated perfused liver and lung. Biochem. Pharmacol. 33 : 2479-2484.

Martin, J.H., Leonard, W.H. and Stamp, D.L. 1976. Legumes. In J. H. Martin, W. H. Leonard and D. L. Stamp eds., Principles of Field Crop Production. Macmillan Publishing, New York. 621-788.

Mayer, J., Buegger, F., Jensen, E.S., Schloter, M. and Heß, J. 2003. Residual nitrogen contribution from grain legumes to succeeding wheat and rape and related microbial process. Plant Soil 255 : 541-554.

Mulvaney, R.L., Khan, S.A. and Mulvaney, C.S. 1997. Nitrogen fertilizers promote denitrification. Biol. Fertil. Soils 24 : 211-220.

Ohdan, H., Daimon, H. and Mimoto, H. 1995. Evaluation of allelopathy in Crotalaria by using a seed pack growth pouch. Jpn. J. Crop Sci. 64 : 644-649.

Ohdan, H. and Daimon, H. 1998a. Evaluation of amount of nitrogen fixed in Crotalaria spp. and nitrogen turnover to the succeeding wheat. Jpn. J. Crop Sci. 67 : 193-199**.

Ohdan, H. and Daimon, H. 1998b. Growth of Crotalaria juncea and Sesbania cannabina under different underground water levels and their nitrogen contribution to the succeeding spinach plant. Jpn. J. Crop Sci. 67 : 467-472**.

Osman, A.E. and Dick, A.A.A. 1982. Effect of defoliation on yield and forage quality of some tropical grasses, legumes and their mixtures. Exp. Agric. 18 : 157-166.

Paynel, F., Murray, P.J. and Cliquet, J.B. 2001. Root exudates : a pathway for short-term N transfer from clover to ryegrass. Plant Soil 229 : 235-243.

Paynel, F. and Cliquet, J.B. 2003. N transfer from white clover to perennial ryegrass, via exudation of nitrogenous compounds. Agronimie 25 : 503-510.

Rasmussen, J., Eriksen, J., Jensen, E.S., Ebensen, K.H. and Høgh-Jensen, H. 2007. In situ carbon and nitrogen dynamics in ryegrass–clover mixtures : Transfers, deposition and leaching. Soil Biol. Biochem. 39 : 804-815.

Samba, R.T., Sylla, S.N., Neyra, M., Gueye, M., Dreyfus, B. and Ndoye, I. 2002. Biological nitrogen fixation in Crotalaria species estimated using the 15N isotope dilution method. Afri. J. Biotech. 1 : 28-44.

Simpson, J.R. 1965. The transference of nitrogen from pasture legumes to an associated grass under several systems of management in pot culture. Aust. J. Agric. Res. 16 : 915-926.

Somasegaran, P. and Hoben, H. J. 1985. Methods in Legume-Rhizobium Technology. University of Hawaii NifTAL project and MIRCEN, Hawaii. 1-367.

Urutani, A., Daimon, H., Ohie, M., Harada, J., Nakayama, Y. and Ohdan, H. 2004. Ecophysiological traits of field-grown Crotalaria mavena and C. pallida as green manure. Plant Prod. Sci. 7 : 449-455.

Yano, K., Daimon, H. and Mimoto, H. 1994. Effect of sunn hemp and peanut incorporated as green manures on growth and nitrogen uptake of the succeeding wheat. Jpn. J. Crop Sci. 63 : 137-143.

* In Japanese with English summary.
** In Japanese with English abstract.