Nitrogen Uptake by Faba Bean from $^{15}$N-Labelled Oilseed-Rape Residue and Chicken Manure with Ryegrass as a Reference Crop

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Abstract: The effects of soil amendment with oilseed-rape residue (OSRR) and chicken manure (CM) on the growth and nitrogen (N) uptake of faba bean (Vicia faba L.) were assessed in a pot experiments with Italian ryegrass (Lolium multiflorum Lam.) as a reference crop. A $^{15}$N isotope dilution method was used to estimate the amount of N derived from the residue (OSRR and CM) and from atmosphere through N$_2$ fixation in the plants. Dry weights (DW) of shoots and whole plants were heaviest in the plants grown on the soil amended with CM (CM plants) followed by the plants grown on the soil amended with OSRR (OSRR plants) and control plants in this order. There were significant differences (p<0.05) in dry weight between CM, OSRR and control plants. DW of roots was also increased with amendment with either CM or OSRR in faba bean, but it was decreased in ryegrass. The amount of total N in both roots and shoots were increased by application of either CM or OSRR in both faba bean and ryegrass. The amount of N$_2$ fixed by faba bean cultured on 1.2 kg soil amended with 10g residue (CM or OSRR) was 85.9 mg pot$^{-1}$ but total N in faba bean derived from OSRR and CM was 192 and 374 mg pot$^{-1}$, respectively. The percentage of N derived from atmosphere to total N in faba bean plants ranged from 15.9 to 26.5%. The amount of N taken up by faba bean and ryegrass plants from CM were larger than those from OSRR by 81.0 and 54.3%, respectively. Soil N balance was calculated as the difference between the amount of N applied (including fixed) and taken up by the plants. The N balance of soil amended with OSRR after cultivation of faba bean was 72.2% higher than that of soil amended with CM, and that after cultivation of ryegrass was 89.9% higher.

Key words: Chicken manure, Faba bean, N uptake, Nutrition, Oilseed-rape residue, Ryegrass.

Nitrogen (N) is the most limiting factor for crop production (Prasad et al., 1990). The supply of N from manures and others organic substrates contributes to satisfy the N demand of growing crops. The final N status of a crop is determined by the N availability throughout the growing season and the crop N demand. Some studies evaluating the fate of $^{15}$N in oilseed-rape residue and poultry manure showed that N recovery is 28 and 51% for oilseed-rape leaves with C/N= 9 (Dejoux et al, 2000) and poultry manure with C/N= 2.4 (Rees and Castle, 2002), respectively. Faba bean (Vicia faba L.) is an economically important legume crop with widespread commercial production and utilization. It is well known that legume crops are important to maintain soil fertility with their high N$_2$-fixing ability (Zapata et al., 1987; Amanuel et al., 2000; van Kessel and Hartley, 2000). Hitherto, there is little information as to what extent organic manure in soil interferes with N$_2$ fixation of legumes. Roper (1983) reported that nitrogenase activity of free-living bacteria positively correlated with straw decomposition in soil, but did not provide quantitative data on biological N$_2$ fixation. Although there is evidence that organic amendment to soil improves crop yields and soil fertility (Ghosh and Sharma, 1999; Kumar et al., 1999), the organic material may have various effects on N$_2$ fixation of legumes. The effect of faba bean on soil N balance depends on the difference between the inputs and outputs of N in the system. A positive soil N balance after faba bean cultivation has been reported (Zapata et al., 1987; Amanuel et al., 2000).

The $^{15}$N isotope dilution method (McAuliffe et al., 1958) using $^{15}$N-labelled organic materials has been used in estimating crop N uptake from organic N inputs (Azam et al., 1995; Jensen, 1994; Vanlauwe et al., 1996). Moreover, the $^{15}$N-isotope dilution technique is widely used to estimate N$_2$ fixation in legumes (Jensen, 1986; Amanuel et al., 2000). Further, Witty (1983) reported that Italian ryegrass was a suitable reference crop for faba bean.

The present study was conducted to (1) assess the...
effect of $^{15}$N-labelled oilseed-rape residue and chicken manure on faba bean growth, (2) measure the amount of atmospheric N$_2$ fixed by faba bean and the amount of N derived from $^{15}$N-labelled oilseed-rape residue and chicken manure by faba bean and ryegrass plants, (3) quantify N uptake by faba bean from $^{15}$N-labelled oilseed-rape residue and chicken manure and (4) estimate the N balance of the soil amended with residue or manure after planting of faba bean.

Materials and Methods

1. Trial description

The study was undertaken in a polyvinyl house at Gifu University from Nov. 10, 2002 to Feb. 18, 2003. Outside the polyvinyl house, the daily highest temperature ranged from 1.3 to 18.7 °C with an average of 10.4 ± 3.5 °C and the daily lowest temperature from −4.5 to 10.5 °C with an average of 2.6 ± 3.1 °C. Plants were raised in 1/10000a Wagner’s pots filled with soil 1.2 kg in dry weight. The soil used in this experiment was a Brown lowland sandy loam soil collected from near the Nagara River in Gifu. The soil was sieved to remove stones and other dust materials and air dried before use. It consisted of 40.7% sand, 32.6% silt, and 26.8% clay and the main physicochemical characteristics were pH (H$_2$O) 5.3; EC 0.025 dS m$^{-1}$; bulk density of 1.41 g cm$^{-3}$; specific gravity of 2.54 g cm$^{-3}$. It contained 0.58 mg g$^{-1}$ total N; 5.85 mg g$^{-1}$ total C; 90.5 mg kg$^{-1}$ P; 2.97 mol, kg$^{-1}$ CEC; 30.5, 28.6, 8.9, and 40.6 mg kg$^{-1}$ of Na$^+$, Ca$^{2+}$, Mg$^2+$, and K$^+$, respectively; and 57.2, 18, and 33.5 mg kg$^{-1}$ of HCO$_3^-$, Cl$^-$, and SO$_4^{2-}$, respectively; and 2.35 % organic matter. Two types of raw $^{15}$N-labelled organic residues were obtained from Shoukou Tsushou Co.Ltd., Shoukou Tsushou Co.Ltd., Sugito Institute, Shoukou Tsushou Co.Ltd., Sugito Institute, Japan. Faba bean (Vicia faba L. var. major) cv. Ryousai-issun and Italian ryegrass (Lolium multiflorum Lam. cv. Mammoth B) were grown in the above-described soil with three treatments, in a 2 (species) x 3 (treatments) factorial experiment laid out in a randomized complete block design with four replicates. The three treatments were: Amendment with $^{15}$N-labelled (OSRR), and $^{15}$N-labelled (CM) and control without amendment (CONT). No chemical fertilizers were supplied to the pots. Three faba bean seeds and 12 ryegrass seeds were sown per pot. Three weeks later, seedlings were thinned to one per pot for faba bean, and six per pot for ryegrass. The pots were kept weed-free and watered as necessary throughout the experimental period.

| Property | Oilseed rape residue | Chicken manure |
|----------|---------------------|---------------|
| pH$^a$   | 5.6                 | 6.2           |
| EC$^a$   | 1.3                 | 15.2          |
| Total N (%) | 5.53              | 5.93         |
| $^{15}$N atom excess (%) | 4.7037        | 0.4697       |
| Total C (%)     | 50.3         | 16.6         |
| C/N             | 9.1           | 2.8          |
| P (g kg$^{-1}$)  | 23.9         | 3.7          |
| K (g kg$^{-1}$)  | 3.1          | 1.8          |

$^a$The pH and EC were measured in the extracts of OSRR and CM in distilled water [1:20 (w/v) on a dry weight basis].

2. Plant harvest and analysis

The plants were harvested 100 days after sowing (DAS) at the late flowering stage of faba bean. Each plant was cut at ground level to separate into the top (leaves, stems and reproductive organs) and the underground portion (roots). The underground portion was washed carefully under running tap water to remove soil particles and plant debris. Shoots and roots were oven-dried at 70 °C for 72 hrs and their dry weights were measured. The oven dried shoot and roots were ground to pass a 0.5 mm sieve to estimate N content. Total N was determined by a CN analyzer, Shimadzu Corporation, Japan. The $^{15}$N/$^{14}$N-isotope ratio was determined by a Mass Spectrometry (Thermo Electron EA1110-DELTA plus advantage ConFlo III System, Shoukou Tsushou Co.Ltd., Sugito Institute, Japan).

3. Calculations

The atom % $^{15}$N excess was calculated from the difference of the atom % $^{15}$N in the plant material (root and shoot in faba bean and ryegrass) and organic residue (oilseed-rape residue and chicken manure) and that of the natural abundance in the atmosphere (0.3663%). The amount of N derived from the residue (Ndfr), the atmosphere (Ndfa), and the soil (Ndfs) in the plants, and that of N taken up from the residue were calculated using equations 1-8 according to IAEA (2001):

\[
\text{N yield (mg pot}^{-1}\text{) of each plant part} = \frac{\text{Dry matter of each plant part (g/pot)} \times \text{N conc. (mg/g)}}{100}
\]  

\[
\% \text{Ndfr} = \frac{\text{atom} \% ^{15}\text{N excess of sample}}{\text{atom} \% ^{15}\text{N excess of residue}} \times 100
\]
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\[ N \text{ residue yield (mg pot}^{-1}) = \frac{N \text{ yield (mg/pot)} \times \%\text{Ndfr}}{100} \] \hspace{1cm} (3)

\[ \%\text{Ndfr (weighted average)} = \frac{\text{Total N residue yield}}{\text{Total N yield}} \times 100 \] \hspace{1cm} (4)

\[ \%\text{Ndfa} = \left(1 - \frac{\%\text{Ndfr}_F}{\%\text{Ndfr}_NF}\right) \times 100 \] \hspace{1cm} (5)

\[ \text{N}_2 \text{ fixed (mg pot}^{-1}) = \frac{\%\text{Ndfa} \times \text{total N in fava bean}}{100} \] \hspace{1cm} (6)

\[ \%\text{Ndfs} = 100 - \%\text{Ndfr} - \%\text{Ndfa} \] \hspace{1cm} (7)

\[ \% \text{ N recovery from residue} = \frac{\text{Amount of N in the plant derived from the residue}}{\text{Amount of N applied as residue}} \times 100 \] \hspace{1cm} (8)

where: N conc., N concentration. The index F denotes the fixing plant (faba bean) and the index NF denotes the non-fixing plant (ryegrass). Sample is fava bean or ryegrass. Residue is OSRR or CM.

The soil N balance after fava bean and ryegrass cultivation was obtained by subtracting N output from N input according to Amanuel et al. (2000):

\[ \text{Soil N balance} = (N_f + \text{Ndfa}) \text{ - Ng} \]

where: N\text{f}, the applied N; N\text{dfa}, the total fixed N; and Ng, the N in fava bean shoot or ryegrass shoot.

4. Statistical analysis

All data collected for various studies were subjected to the analysis of variance appropriate to the design. Significance of the difference between treatments was tested by the F-test. The significant differences between the treatments were compared by the Duncan’s test (p<0.05).

Results

Dry weight (DW) of whole plant and DW of shoots were heaviest in the plants grown on the soil amended with OSRR (CM plants) followed by those grown on the soil amended with OSRR (OSRR plants) and control plants in this order (Table 2). DW of root was also significantly (p<0.05) increased by amendment with CM or OSRR in fava bean but was significantly (p<0.05) reduced by the same amendment in ryegrass.

The N concentration in the plants varied with the
In faba bean, the N concentration tended to be the highest in leaves followed by shoots and roots in this order, irrespective of the soil amendment. Also in ryegrass the N concentration was higher in shoots than in roots.

The total N in roots and shoots was significantly (p<0.05) increased by amendment with either CM or OSRR. The amount of total N in shoots and the whole plant was larger in CM plants than in OSRR plants in both species, but that in roots was larger in CM plants than in OSRR plants only in faba bean and not in ryegrass.

Amendment with OSRR and CM increased DW of faba bean plants by 1.7- and 3.3-fold, respectively, and total N of faba bean by 3.0- and 5.3-fold, respectively, compared with the control.

Table 4 shows the effect of soil amendment on the amount of N used for growth of faba bean and ryegrass plants (available N) and the percentage of N derived from soil (%Ndfs), residue (%Ndfr) and atmosphere (%Ndfa) in the plants. Neither faba bean nor ryegrass plants showed any significant difference in %Ndfr and %Ndfs between the CM and OSRR plants. The amount of N derived from residue (Ndfr) was larger in CM plants than in OSRR plants in both faba bean and ryegrass (Fig. 1). Even though there was no significant difference in %Ndfs between OSRR and CM plants in both species (Table 4), the amount of N derived from soil (Ndfs) in CM plants was 139.8 and 101.3% higher than that in OSRR plants in faba bean and ryegrass, respectively (Fig. 1). No significant difference in %Ndfa or Ndfa was observed between OSRR and CM plants of faba bean (Table 4 and Fig. 1).

The percentage of N taken up from residue (manure) to total N in CM plants was 81.0 and 54.3% higher than that in OSRR plants in faba bean and ryegrass, respectively (Table 4). After harvest of faba bean and ryegrass, the soil N content was significantly higher in the soil amended with OSRR than that amended with CM. Faba bean plants tended to take up less amount of soil N than ryegrass. The N balance of the soil amended with OSRR after cultivation of faba bean and ryegrass was 72.2 and 89.9%, respectively, higher than that of the soil amended with CM.

**Discussion**

Growth and N uptake of faba bean cultivated on the soil amended with 15N-labelled oilseed-rape residue...
(OSRR) and chicken manure (CM) were examined using ryegrass as a reference crop. Faba bean and ryegrass responded differently to the soil amendments with OSRR and CM. The N balance of soil amended with OSRR and CM after faba bean cultivation was 36.3 and 50.2% higher than that after ryegrass cultivation, respectively. However, Senaratne and Hardarson (1988) reported that faba bean grown in sandy loam soil could cover as much as 80-90% of its N requirement through N₂ fixation. It seems likely that application of OSRR and CM to soil in the present experiment reduced the percentage of N derived from the atmosphere in the plants (%Ndfa). The %Ndfa ranged from 15.6 - 27.3% and 15.8 - 26.4% with a corresponding total amount of N₂ fixation ranging from 52.9 - 66.4 and 84.8 - 90.3 mg N pot⁻¹ in the shoot and whole plant, respectively. However, %Ndfa is known to vary from 19 to 97% according to Duc et al. (1988) and Schwenke et al. (1998). In the present experiment, N₂ fixation of faba bean might be depressed by the soil amendment with OSRR and CM (Table 4 & Fig.1), suggesting the well-known adverse effect of large amounts of available N to N₂ fixation. Hence, the low value of N₂ fixation of faba bean in this experiment does not support the claim by Roughley et al. (1983) and Hardarson et al. (1991) that N₂ fixation by faba bean is tolerant to higher N levels. They reported that when ammonium sulphate was applied at the rates of 20, 100, 200 and 400 kg N ha⁻¹, 85, 75, 60 and 43% of the N in faba bean, respectively, were derived from the atmosphere (%Ndfa). However, in our study we examined N₂ fixation in the plants grown on the soil containing approximately 500 mg organic N per pot (1.2 kg soil) (500 kg organic N ha⁻¹). Therefore, a further detailed research on N₂ fixation is required (i.e. starting from 20 mg N per pot).

The present result showed that dry weight and N yields of both faba bean and ryegrass were significantly increased by soil amendment with OSRR or CM indicating that they had a great demand for a readily available N.

The rate of N mineralization from manures is controlled, by the activity of decomposer, the physical environment (temperature, moisture etc.) and the chemical composition of the manures (Swift et al., 1979). Beauachamp (1986) and Chadwick et al. (2000) reported that N in manures with C/N of ≥ 15 is initially immobilize N, but that in manures with C/N of <15 is mineralized, and that the lower the C/N the greater the mineralization rate. Nitrogen recovery was calculated as the amount of ¹⁵N taken up by faba bean or ryegrass plant in relation to the amount of nitrogen added to the soil as residue. The rate of recovery (uptake by plants) from CM was higher than that from OSRR in both faba bean and ryegrass probably because C/N of CM (2.8) was lower than that of OSRR (9.1), in agreement with Chadwick et al. (2000). Moreover, the increase in dry weight and that in N uptake caused by application of OSRR and CM in faba bean and ryegrass may be attributed to the increase in available N released from them. The nutrient elements involved in OSRR and CM, C, N, P and K (Table 1), may also promote plant growth, as suggested by Abdelhamid et al. (2003).

The increase in the amount of soil N after cultivation of faba bean and ryegrass may be ascribed to the organic N added to the soil as residue. The soil N balance after cultivation of either faba bean or ryegrass was lower in the soil amended with CM than in the soil amended with OSRR, because a large amount of N was taken up to increase the dry matter by CM plants (plants grown on the soil amended with CM) than by OSRR plants. Zapata et al. (1987) also reported a positive soil N balance after cultivation and pod harvest of pods of faba bean. The N balance of soil amended with OSRR and CM after cultivation of faba bean was 36.3 and 50.2% higher, respectively, than those after cultivation of ryegrass. This is because faba bean has N₂ fixation ability but ryegrass does not.

In conclusion, this study demonstrated that OSRR and CM applied to soil increased the fertility of soil, like inorganic N fertilizer, and improved the growth of faba bean and ryegrass accelerating N uptake even in faba bean. The amount of N₅ fixed by faba bean grown on 1.2 kg soil amended with 10 g OSRR and CM was 85 and 90 mg per pot (1.2 kg soil), respectively, and that of N taken up by the plants from OSRR and CM was 192 and 374 mg per pot, respectively, in faba bean, and 226 and 374 mg per pot, respectively, in ryegrass. Faba bean took up about 35 and 53% of ¹⁵N-labelled OSRR and CM, respectively, during cultivation. The N balance of soil amended with OSRR and CM after cultivation of faba bean was 444 and 258 mg N per pot, respectively, and that after cultivation of ryegrass was 326 and 172 mg N per pot, respectively.

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