

Nitrogen dynamics in paddy soil applied with various ^{15}N -labelled green manures

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Abstract The effects of ^{15}N -labelled green manure (GM) application on rice yield and N uptake were investigated and compared with those of inorganic fertilizer (IF) and no fertilizer (NF) application. Nine GMs (white clover, Chinese milk vetch, hairy vetch, crimson clover, oats, rye, Italian ryegrass, white mustard, lacy phacelia) were either incorporated into or surface-applied on paddy soil. Among the nine GM treatments, the rice yield and N uptake tended to be higher in the white clover and hairy vetch treatments. The rice N uptake efficiency from the applied N source was highest in the incorporated hairy vetch (56%), followed by IF (52%), and incorporated white clover (44%), whereas for the other treatments there were no significant differences. The mineral fertilizer equivalent values of hairy vetch and white clover in case of the incorporation were 108% and 84%, respectively. Therefore, hairy vetch and white clover seem to be the most efficient organic N source as alternatives to inorganic N fertilizers. The N uptake efficiency of rice and residual N in soil derived from GM were higher in the incorporation treatments than that in the surface-applied treatments, indicating that N losses from the soil-plant system were lower with the incorporation.

Keywords Green manure · Hairy vetch · ^{15}N · N uptake · Rice · White clover

Introduction

Soil fertility is important for maintaining or improving plant growth and productivity. It can be effectively improved by applying organic matter to soil. Many studies have attempted to develop cultivation systems with organic materials (Ashraf et al. 2004; Azam 1990; Diekmann et al. 1993). Green manure (GM) is a type of organic material which can be used as nutrient source. The cultivation methods with GM offer several advantages that include decreased NO_3^- -N leaching and soil erosion and improved biological nitrogen fixation (Hartwig and Ammon 2002). Therefore, GM may be useful for increasing soil fertility and crop production.

With regard to fertilization, organic matter such as a GM can be potentially important sources of N for crop production. Soil N is often the most limiting element for plant growth and quality. Therefore, it is important to estimate the amount of N supply and the plant N uptake from the applied organic materials in order to optimize crop production. However, in comparison to inorganic fertilizers, it is difficult to appropriately estimate the amount of nutrient supply to crops from organic N source during cultivation, because it depends on the rate of decomposition and the mineralization processes of organic materials. Mineralization is a well-known complex process that

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is regulated by many environmental factors such as soil physicochemical properties, temperature, soil moisture, and biota. Therefore, the method using ^{15}N -labelled plant materials has been useful for direct estimation of plant N uptake from the GMs. Earlier studies have found that the N uptake by the subsequent crop was 6–25% of the input ^{15}N labeled GM, hairy vetch, ryegrass, phacelia, white clover, red clover, subterranean clover, field bean, and timothy in upland soils (Bergstrom and Kirchmann 2004; Harris et al. 1994; Jackson 2000; Muller and Sundman, 1988; Seo et al. 2006), and 19–50% of the ^{15}N labeled GMs sesbania and maize applied in paddy soils (Ashraf et al. 2004; Azam 1990; Diekmann et al. 1993). The rate of N uptake by the subsequent crop from the applied organic materials seems to be highly variable and depends on the nature of the organic materials and the soil system to which they are added (Wivstad 1999; Yaacob and Blair 1980). In order to evaluate the N-supplying potential of a GM, it is essential to investigate the N fate of various GMs simultaneously compared to the fate of inorganic fertilizers under same soil conditions. However, studies related to the fate of various GMs in the soil-paddy rice system by using the direct method with ^{15}N are limited (Fox et al. 1990). Moreover, a comparison of the effect of surface-application or soil incorporation on the N fates of various ^{15}N -labelled GMs has not been estimated.

In this study, nine types of GM were simultaneously examined in a paddy field with transplanted rice. The different GMs were incorporated in soil or surface-applied on soil. The objectives of this study were (1) to investigate the N fate of the ^{15}N -labelled GMs incorporated in soil or surface-applied on soil during the rice cultivation period in the Shikoku region of Japan and (2) to compare the N fate of the nine GMs with that of IF.

Materials and methods

Site and preparation of ^{15}N labeled green manure

A field experiment was conducted at the Ehime University Farm, Matsuyama, Japan (lat $33^{\circ}57'\text{N}$ $132^{\circ}47'\text{E}$). Figure 1 shows the monthly rainfall and temperature during the rice cultivation period.

White clover (*Trifolium repens* L.; WC), Chinese milk vetch (*Astragalus sinicus* L.; CMV), hairy vetch

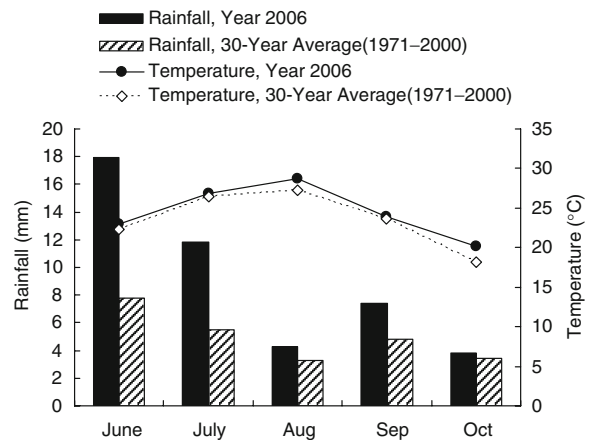


Fig. 1 Monthly rainfall and temperature at Matsuyama, Japan during the rice cultivation period

(*Vicia villosa* Roth.; HV), crimson clover (*Trifolium incarnatum* L.; CC), oats (*Avena sativa* L.; OT), rye (*Secale cereale* L.; RY), Italian ryegrass (*Lolium multiflorum* Lam.; IR), white mustard (*Sinapis alba* L.; WM), and lacy phacelia (*Phacelia tanacetifolia* Benth.; LP), all of which can be cultivated in the Shikoku region of Japan, were used as GM. The WC, CMV, HV, CC, OT, RY, IR, WM, and LP were cultivated in greenhouse pots (30×80×26 cm) supplied with $^{15}\text{NH}_4\text{Cl}$ (2.635 atom% excess) and harvested at the flowering stage. Air-dried tops and roots of the nine ^{15}N -labelled GMs were fragmented into small pieces (less than 5 cm in length).

Experiment 1: effects of GM on rice growth and N uptake

Twenty treatments comprising nine GM incorporations, nine GM surface-applications, and two controls (no fertilizer (NF) and inorganic fertilizer (IF)) were compared. A plastic pot (diameter: 20 cm, height: 30 cm, the bottom: opened) was installed in the paddy field (gray lowland paddy soil; 13.5% clay, 28.0% silt, 58.5% sand; total carbon: 1.46%; total nitrogen: 0.15%). The nine ^{15}N -labelled GMs (less than 5 cm in length) were incorporated in the soil or applied to the soil surface within the pot on 23 June 2006. The application rate and their chemical properties are shown in Table 1. The application rate of each GM was calculated on the basis of the plant weight ($\text{g}\cdot\text{m}^{-2}$) at harvest under practical farming conditions.

Table 1 Green manure application rate and chemical characteristics

	Application rate			Chemical characteristics				
	N			C	N	Lignin+cellulose	C/N	(Lignin+cellulose)/N
	Dry matter (g m ⁻²)	(g m ⁻²)	(g pot ⁻¹)					
White clover	448	13.3	0.44	376	31.7	346	11.9	10.9
Chinese milk vetch	199	4.7	0.15	393	24.8	473	15.8	19.1
Hairy vetch	449	16.2	0.53	427	39.7	376	10.8	9.5
Crimson clover	450	11.6	0.38	379	28.8	414	13.2	14.4
Oats	998	14.2	0.46	424	16.0	321	26.5	20.0
Rye	975	9.8	0.32	316	11.6	502	27.3	43.4
Italian ryegrass	996	10.4	0.34	249	11.5	624	21.7	54.5
White mustard	449	6.2	0.20	382	15.7	518	24.4	33.1
Lacy phacelia	446	8.6	0.28	420	21.0	324	20.0	15.4

For comparison, inorganic fertilizer was added at a rate of 8 g N m⁻² (0.26 g N pot⁻¹)

In the IF treatment, N was applied in the form of ¹⁵NH₄Cl (0.635 atom % excess) to the soil at the rate of 4 g m⁻² as basal fertilization (23 June 2006) and 2 g m⁻² each at the tillering (25 July 2006) and ripening stages (16 September 2006), respectively. On 23 June, P and K were applied to the soil at a rate of 6 g m⁻² in the form of P₂O₅ and KCl, respectively.

On 24 June 2006, the pots were flooded with tap water, and three seedlings per pot of rice *Oryza sativa* L. cv. Matsuyamamii were transplanted in each pot with four replicates per treatment.

Experiment 2: proportion of applied GM N remaining in soil

Air-dried tops and roots of each of the nine ¹⁵N-labelled GMs (less than 5 cm long) were enclosed in mesh bags (mesh size 1×1 mm, bag size 14×14 cm), and they were then placed in a layer at a depth of 10 cm or on the soil surface inside a soil filled plastic pot (similar to exp. 1) on 23 June 2006, with one replicate per treatment. On 24 June 2006, the pots were flooded with tap water, but no rice seedlings were transplanted to each pot.

Sampling and chemical analysis

Plant roots, leaves and stems, panicles and the residue in the mesh bag were separately collected at harvest, oven-dried at 70°C, and their dry weights measured. They were then ground to a powder. The total N concentration and abundance of ¹⁵N in the subsamples of the plants were determined using a mass spectrometer (Integra CN; SerCon Ltd, Cheshire, UK).

Calculations

The percentage of plant N uptake derived from applied N (%N_{dfan}), i.e. from the GM (%N_{dfgm}) or the IF (%N_{dfif}) can be calculated from Eq. (1).

$$\%N_{dfan} = \frac{\text{atom\% } ^{15}\text{N excess of rice plant in GM or IF}}{\text{atom\% } ^{15}\text{N excess of applied N in GM or IF}} \quad (1)$$

The N uptake efficiency (NUE) is the recovery rate of N by rice from the applied N source (NUE_{an}). The NUE rates of the GMs (NUE_{gm}) and IF (NUE_{if}) were calculated from Eq. (2).

$$\text{NUE}_{an}(\%) = \frac{\text{N uptake derived from the applied N (mass) in rice plant at harvest in GM or IF}}{\text{Amount of N applied in GM or IF}} \times 100 \quad (2)$$

We also computed the mineral fertilizer equivalent (MFE) value, i.e. the NUE_{gm} relative to the NUE_{if} , as described in Eq. (3).

$$MFE(\%) = \frac{NUE_{gm}}{NUE_{if}} \times 100 \quad (3)$$

The Physiological N use efficiency (PNUE), i.e. the ratio of the rice grain yield to the N uptake derived from the applied N in rice, as described in Eq. (4).

$$PNUE(gg^{-1}) = \frac{\text{Rice grain yield (GM or IF)} - \text{rice grain yield (NF)}}{\text{N uptake derived from the applied N (mass) in rice plant at harvest in GM or IF}} \quad (4)$$

The agronomic efficiency (AE), i.e. the ratio of the rice grain yield to the amount of N applied, as described in Eq. (5).

$$AE(gg^{-1}) = NUE \times PNUE = \frac{\text{Rice grain yield (GM or IF)} - \text{rice grain yield (NF)}}{\text{Amount of N applied in GM or IF}} \quad (5)$$

The added N interaction (ANI) is defined as effects of the fertilizer N addition on the uptake of soil N by crop (Jenkinson et al. 1985), i.e. subtracting plant N uptake derived from soil N (N_{dfs}) in NF from N_{dfs} in GM or IF, as described in Eq. (6).

$$ANI(g \text{ pot}^{-1}) = N_{dfs}(\text{GM or IF}) - N_{dfs}(\text{NF}) \quad (6)$$

Statistical analysis

The differences among the means were analyzed by two-way ANOVA and Tukey-Kramer test, and the correlation coefficient was obtained using Pearson's correlation analyses with the software KyPlot (KyensLab Inc., Tokyo, Japan).

Results

Dry weight and yield of rice

At harvest, the dry weight, yield, and tiller number of rice in the GM-incorporated treatments (except for IR) and in the surface-applied treatments (except for IR and LP) were higher than those in the NF (Table 2). The dry weight, yield, and tiller number in the IF were the highest compared to all treatments. Rice dry

weights and yields in the incorporated HV and WC treatments were not significantly lower than those in the IF treatment. However, these values were significantly lower in the other GM treatments. The dry weight and yield tended to be higher in the WC and HV treatments and lower in the IR treatment. The root dry weight was lower with the incorporated IR and the surface-applied LP treatments than that with the other GM, IF and NF treatments. The rice root and total dry weight were significantly different between the nine GM treatments. Averages of dry weight, yield, and tiller number in the nine GM-incorporated treatments were significantly higher than those in the surface-applied treatments.

Rice N uptake derived from fertilizer (GM or IF) and soil

Table 3 shows N uptake by rice. N uptake derived from the fertilizer (GM or IF) was most significant with the incorporated HV treatment (0.30 g pot^{-1}) compared to all other GM treatments, followed by the incorporated WC (0.19 g pot^{-1}) and IF (0.14 g pot^{-1}) treatments. Further, N uptake from soil in the incorporated HV (1.25 g pot^{-1}) and WC (1.14 g pot^{-1}) treatments was lower than that in the IF treatment (1.36 g pot^{-1}). The total N content of rice was significantly higher in the incorporated HV

Table 2 Rice dry weight, grain yield and tiller number at harvest

	Dry weight (g pot ⁻¹)				Grain yield (g pot ⁻¹)	Tillers pot ⁻¹
	Root	Stems and leaves	Panicles	Total		
Incorporation						
White clover (WC)	26.9 ab	39.3 abc	39.7 ab	105.9 abc	35.6 a	18.5 bc
Chinese milk vetch (CMV)	26.9 ab	34.7 bcd	36.0 abc	97.6 abc	34.7 ab	16.0 bcd
Hairy vetch (HV)	33.0 a	45.5 ab	40.8 ab	119.2 ab	38.7 a	19.5 ab
Crimson clover (CC)	28.1 ab	34.4 bcd	33.6 abc	96.0 abc	30.8 ab	16.0 bcd
Oats (OT)	21.3 ab	32.4 bcd	32.1 bc	85.8 bcd	29.1 ab	15.0 bcd
Rye (RY)	17.7 ab	30.2 cd	32.3 abc	80.2 cd	28.9 ab	15.3 bcd
Italian ryegrass (IR)	11.0 b	23.0 d	22.8 c	56.8 d	20.2 b	12.5 d
White mustard (WM)	24.6 ab	34.8 bcd	34.6 abc	94.0 abcd	32.9 ab	15.3 bcd
Lacy phacelia (LP)	15.1 ab	33.8 bcd	34.8 abc	83.7 bcd	31.8 ab	15.3 bcd
Control						
No fertilizer (NF)	17.3 ab	30.3 cd	30.1 bc	77.6 cd	26.5 b	14.3 cd
Inorganic fertilizer (IF)	33.9 a	49.6 a	46.2 a	129.7 a	42.7 a	22.5 a
<i>F</i> value	*	*	*	*	*	*
Surface-application						
White clover (WC)	19.4 ab	32.7 b	32.5 ab	84.6 b	26.7 ab	15.8b
Chinese milk vetch (CMV)	16.3 b	32.3 b	31.3 ab	79.9 b	29.1 ab	15.5b
Hairy vetch (HV)	23.5 ab	36.1 ab	34.3 ab	93.8 ab	32.4 ab	15.5b
Crimson clover (CC)	21.4 ab	34.3 b	35.5 ab	91.2 ab	32.9 ab	16.3b
Oats (OT)	18.2 ab	33.7 b	33.0 ab	84.9 b	30.4 ab	16.5ab
Rye (RY)	17.3 b	32.1 b	31.1 ab	80.5 b	28.0 ab	15.0b
Italian ryegrass (IR)	15.9 b	29.7 b	28.7 b	74.3 b	23.5 b	15.5b
White mustard (WM)	17.3 b	34.3 b	30.9 ab	82.5 b	30.3 ab	15.3b
Lacy phacelia (LP)	11.8 b	26.2 b	26.7 b	64.7 b	23.6 b	11.5b
Control						
No fertilizer (NF)	17.3 b	30.3 b	30.1 ab	77.6 b	26.5 ab	14.3b
Inorganic fertilizer (IF)	33.9 a	49.6 a	46.2 a	129.7 a	42.7a	22.5a
<i>F</i> value	*	*	*	*	*	NS ^e
Green manure average						
(A) Incorporation	22.7	34.2	34.1	91.0	31.4	15.9
(B) Surface-application	17.9	32.4	31.5	81.8	28.5	15.2
<i>F</i> value						
1) Between green manure	*	NS	NS	*	NS	NS
2) Between (A) and (B)	*	*	*	*	*	*
1)×2)	NS	NS	NS	NS	NS	*

Different letters in each column denote significant differences within treatments (Tukey-Kramer test, $P < 0.05$, $n = 4$)

^eNS, not significant at $P > 0.05$

(1.55 g pot⁻¹) and in the IF treatment (1.50 g pot⁻¹) than that in the incorporated IR (0.78 g pot⁻¹). The N uptake derived from the surface-applied HV was not significantly lower (0.10 g pot⁻¹) and that from the

other surface-applied GMs (0.02–0.08 g pot⁻¹) was significantly lower than that from the IF (0.14 g pot⁻¹). The N uptake from soil and total N content of rice in the all surface-applied GM treatments was lower

than those in the IF treatment. Total N uptake in the incorporated GM treatments, except for IR, and surface-applied GM treatments, except for IR and LP, were higher than that in the NF (0.99 g pot^{-1}). Added N interaction (ANI) was observed both in the GM and IF treatments. ANI in the IF treatment was significantly higher than that in the incorporated IR and the surface-applied LP treatments.

There were significant differences in N uptake derived from the GM as well as in the total N content in rice between the nine GM treatments. However, in the case of N uptake derived from soil, there were no significant differences (Table 3). The average of rice N uptake derived from the incorporated GM and that of rice N uptake derived from soil were significantly higher than those in the surface-applied GM treatment. Therefore, the average of total N content of rice in the incorporated treatment was significantly higher than that in the surface-applied treatment.

The percentage of N uptake derived from fertilizer (IF or GM) was much lower than that from soil: 8.2% in the case of IF, 3.2–19.1% in GM incorporated treatments, and 2.0–8.1% in GM surface-applied treatments (Fig. 2a). Distribution of N from IF or GM in the roots, stems and leaves, panicles differed widely between treatments (Fig. 2c), while distribution of N from soil did not differ (Fig. 2b).

Dynamics of rice N uptake and utilization

The NUE (the recovery rate of N by rice from the applied IF or GM) was highest in the incorporated HV (55.6%), followed by IF (51.6%), and incorporated WC (43.5%) whereas for the other treatments there were no significant differences (Table 3). In the surface-applied treatment, this rate was also high for HV (19.2%) and WC (18.7%). The recovery rates of N from the incorporated GMs, except for HV and WC, and all the surface-applied GMs were significantly lower than that from the IF. The MFE value (the N uptake efficiencies of GM relative to IF) of HV and WC was higher than that of the other GMs, 108%, 84.3% in case of the incorporation, 37.2%, 36.2% in case of the surface-application, respectively. While the PNUE (the ratio of the rice grain yield to the Ndfan in rice) and AE (the ratio of the rice grain yield to the amount of N applied) of HV, WC were lower than those of IF. The incorporated CMV has the highest PNUE and AE of all GMs.

The average of NUE of the incorporated GMs (29.5%) was significantly higher than that of the surface-applied GMs (13.3%). The means of PNUE and AE were not significantly higher with the incorporated GMs than those with the surface-applied GMs.

Residual N in soil derived from GM

At rice harvest, the residual N of GMs in soil was investigated in exp. two using litter bags (Fig. 3). The amount of residual N derived from the incorporated GM was 24.5–53.7%, and it was 7.8–23.8% from the surface-applied GM. N derived from soil in the residual GM was due to immobilized N. This value was computed by the ^{15}N tracer technique. The amount of residual N derived from soil was 0.0–12.9% in the case of incorporated treatments and 0.0–21.5% in the case of surface-applied treatments. The average residual N from incorporated GMs (36.6%) was higher than that from the surface-applied treatments (13.7%).

Relationships between N fate and the chemical characteristics of GM

Significant relationships were identified between the N fate and the chemical characteristics of GMs (Fig. 4). The N uptake efficiency of rice derived from GM was significantly related to the N (%) of GM in the incorporated treatments, but not significantly related in the surface-applied treatments (Fig. 4a). The proportion of applied GM N remaining in soil residual N was significantly related to the (lignin + cellulose) (%) of GMs in the incorporated treatments and with N (%), C/N, (lignin + cellulose)/N in the surface-applied treatments (Fig. 4b). The relationship between N fate and the chemical characteristics of GM in the incorporated treatments was different from that in the surface-applied treatments.

Discussion

Effects of GM on rice growth and N uptake

At harvest, the rice dry weight, yield, and total N uptake in the GM-incorporated treatments (except for IR) and in the surface-applied treatments (except for

Table 3 Uptake of N derived from green manure or inorganic fertilizer, N derived from soil, N use efficiency by rice, the relative efficiency, and productive efficiency

	N uptake (g pot ⁻¹)				NUE ^j (%)	MFE ^k (%)	PNUE ^l (g g ⁻¹)	AE ^m (g g ⁻¹)
	Ndfan ^g	Ndfs ^h	Total	ANI ⁱ				
Incorporation								
White clover (WC)	0.19 b	1.14 ab	1.33 ab	0.15 ab	43.5 abc	84 abc	48 ab	21 abc
Chinese milk vetch (CMV)	0.04 d	1.20 ab	1.24 ab	0.21 ab	25.7 cde	50 cde	209 a	54 ab
Hairy vetch (HV)	0.30 a	1.25 ab	1.55 a	0.26 ab	55.6 a	108 a	41 ab	23 ab
Crimson clover (CC)	0.13 bcd	1.08 ab	1.21 ab	0.09 ab	33.3 cde	64 cde	34 ab	11 bc
Oats (OT)	0.07 cd	1.22 ab	1.29 ab	0.23 ab	15.9 cde	31 cde	35 ab	6 bc
Rye (RY)	0.11 bcd	0.99 ab	1.10 ab	-0.01 ab	34.5 bcd	67 bcd	22 ab	7 bc
Italian ryegrass (IR)	0.05 d	0.73 b	0.78 b	-0.26 b	15.4 e	30 e	-122 b	-19 c
White mustard (WM)	0.05 d	1.05 ab	1.11 ab	0.06 ab	26.7 cde	52 cde	117 ab	32 ab
Lacy phacelia (LP)	0.04 d	1.09 ab	1.14 ab	0.10 ab	15.3 de	30 de	122 ab	19 abc
Control								
No fertilizer (NF)	–	0.99 ab	0.99 ab	–	–	–	–	–
Inorganic fertilizer (IF)	0.14 bc	1.36 a	1.50 a	0.37 a	51.6 ab	100 ab	120 a	62 a
<i>F</i> value	*	NS ⁿ	*	*	*	*	*	*
Surface-application								
White clover (WC)	0.08 bc	1.10 ab	1.18 ab	0.11 ab	18.7 b	36 b	2 a	0.3 ab
Chinese milk vetch (CMV)	0.02 e	1.00 ab	1.02 ab	0.01 ab	12.2 bc	24 bc	141 a	17 ab
Hairy vetch (HV)	0.10 ab	1.13 ab	1.23 ab	0.14 ab	19.2 b	37 b	58 a	11 ab
Crimson clover (CC)	0.05 cd	1.13 ab	1.18 ab	0.13 ab	14.3 bc	28 bc	118 a	17 ab
Oats (OT)	0.03 de	1.13 ab	1.17 ab	0.14 ab	7.4 c	14 c	113 a	8 ab
Rye (RY)	0.05 de	1.03 ab	1.08 ab	0.04 ab	14.5 bc	28 bc	32 a	5 ab
Italian ryegrass (IR)	0.05 de	0.91 ab	0.95 ab	-0.09 ab	13.8 bc	27 bc	-65 a	-9 b
White mustard (WM)	0.03 de	0.97 ab	0.99 ab	-0.03 ab	12.2 bc	24 bc	151 a	18 ab
Lacy phacelia (LP)	0.02 de	0.76 b	0.78 b	-0.23 b	7.3 c	14 c	-145 a	-11 b
Control								
No fertilizer (NF)	–	0.99 ab	0.99 ab	–	–	–	–	–
Inorganic fertilizer (IF)	0.14 a	1.36 a	1.50 a	0.37 a	51.6 a	100 a	120 a	62 a
<i>F</i> value	*	NS	*	*	*	*	NS	NS
Green manure average								
(A) Incorporation	0.11	1.08	1.19	0.09	29.5	57	56	17
(B) Surface-application	0.05	1.02	1.07	0.03	13.3	26	45	6
<i>F</i> value								
1) Between green manure	*	NS	*	*	*	*	NS	NS
2) Between (A) and (B)	*	*	*	NS	*	*	NS	NS
1)×2)	*	NS	NS	NS	*	*	NS	NS

Different letters in each column denote significant differences within treatments (Tukey-Kramer test, $P < 0.05$, $n = 4$)

^g *Ndfan* N derived from applied N (green manure or inorganic fertilizer)

^h *Ndfs* N derived from soil

ⁱ *ANI* Added nitrogen interaction, the value calculated by subtracting plant N uptake derived from soil N in the no fertilizer treatment from N uptake derived from soil in the green manure or inorganic fertilizer treatment

^j *NUE* The N uptake efficiency, the recovery rate of N by rice from the applied N (green manure or inorganic fertilizer)

^k *MFE* The mineral fertilizer equivalent value, the N uptake efficiencies of green manure relative to inorganic fertilizer

^l *PNUE* The Physiological N use efficiency, the ratio of the rice grain yield to the N uptake derived from the applied N (green manure or inorganic fertilizer) in rice

^m *AE* The agronomic efficiency, the ratio of the rice grain yield to the applied N (green manure or inorganic fertilizer)

ⁿ *NS* not significant at $P > 0.05$

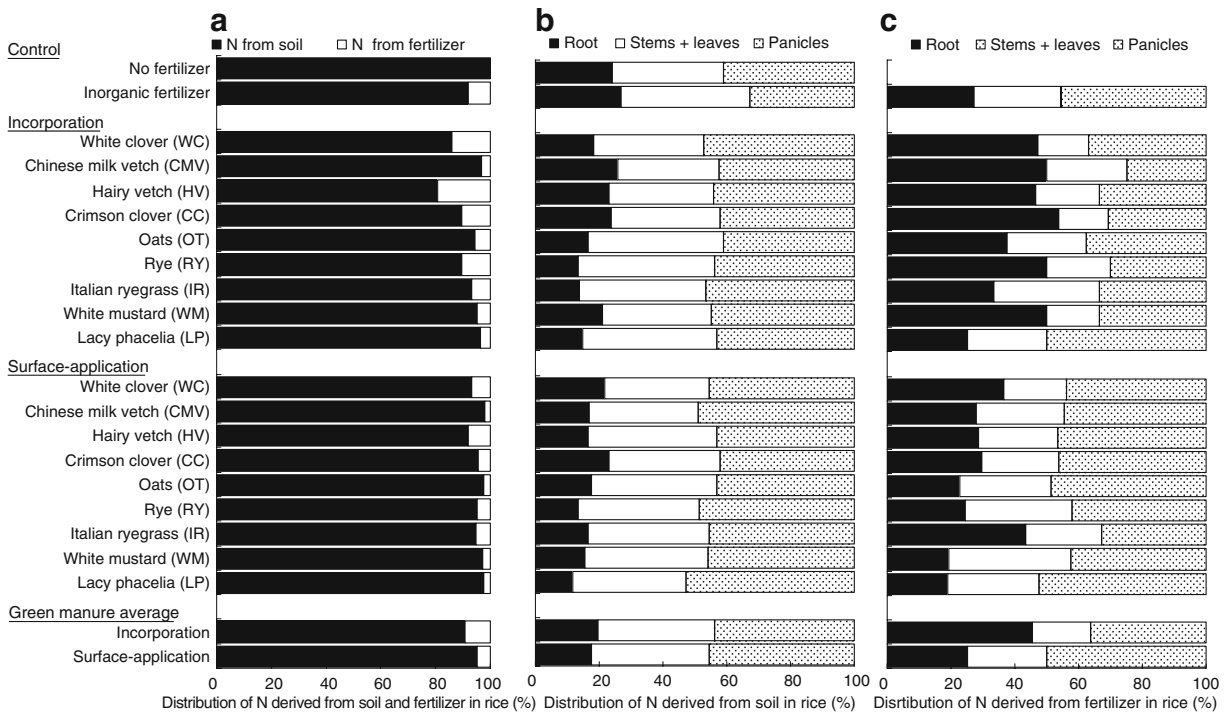


Fig. 2 Distribution of N derived from applied materials (inorganic fertilizer or green manure), and soil in rice (a) and distribution of soil derived (b) and derived from applied (c) into roots, stems & leaves and panicles of rice ($n=4$)

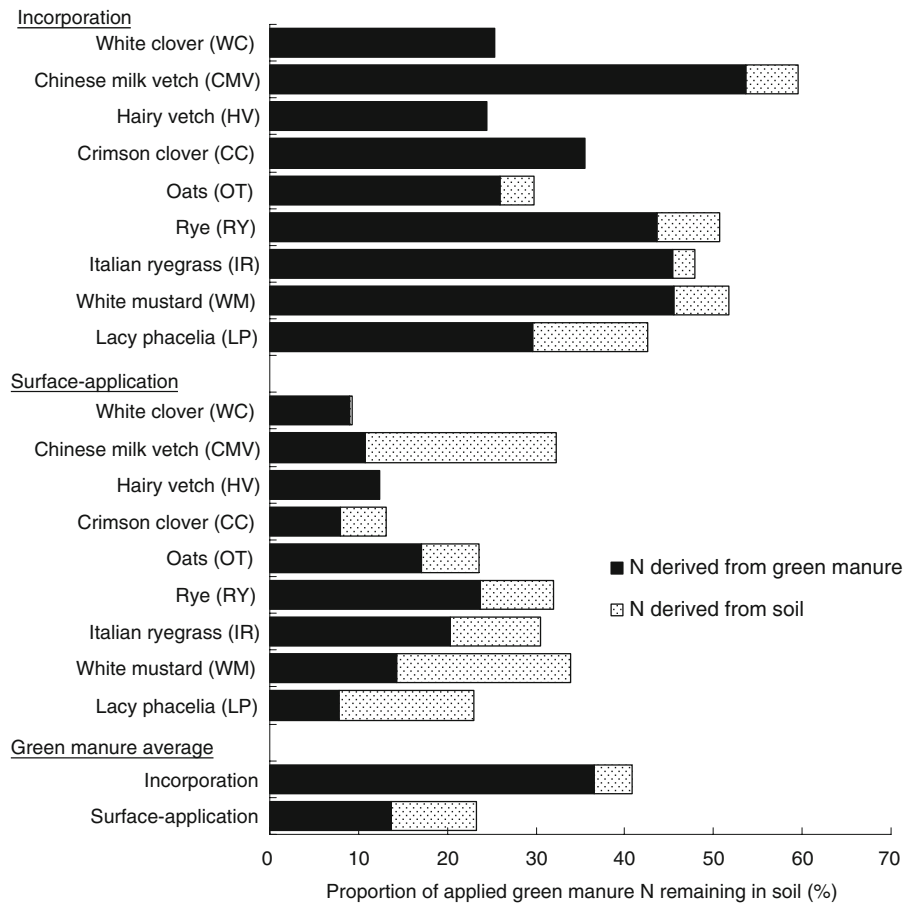
IR and LP) were higher than those in the NF (Tables 2, 3). These values tended to be higher in the WC and HV treatments. The results indicate the positive effects of GM application, excluding the GMs IR and LP, on rice yield and N uptake. Moreover averages of dry weight, yield, and N uptake in the nine GM-incorporated treatments were significantly higher than those in the surface-applied GM treatments. These results suggested that differences in both the type of GM and application methods (incorporation or surface application) influenced rice growth and N uptake. The positive effects on rice growth, yield, and N uptake were higher in the GM incorporation treatments than that in GM surface-application treatments.

Added N interaction in rice has been reported by Ashraf et al. (2004), Azam et al. (1991) and Diekmann et al. (1993). In the present study, the positive interaction was also observed in the GM (except for the incorporated RY, IR and the surface-applied IR, WM, LP) and IF treatments (Table 3). The application of GM and IF may increase the volume of soil explored by roots, and pool substitution or isotope displacement is expected to occur as suggested by

Jenkinson et al. (1985). While there was the negative added N interaction in the incorporated IR and the surface-applied LP treatments, which may be due to the lower growth of root in these treatments (Table 2). The reason for the lower root growth is not clear, but it is much more likely that because the IR and LP actually cause N immobilization (in spite of not too high C/N), the N supply for the rice is as poor for these as it is in the NF treatment.

The percentage of rice N derived from the IF or GM was 8.2% in the case of IF, 2.0–19.1% in GM treatments (Fig. 2a). These values were similar to that of a recent study where the reported rates of N uptake by rice in paddy soil were determined by the ^{15}N direct method, i.e. 16–18% from sesbania and 20–22% from maize (Ashraf et al. 2004). Distribution of N from IF or GM in rice differed widely between treatments, while distribution from soil did not differ (Fig. 2b, c). Distribution of N derived from IF or GM was affected by the differences in the applied N sources and application methods (incorporation or surface-application), but the distribution derived from soil was not affected. The distribution may be

Fig. 3 Proportion of applied green manure N and soil derived N remaining in soil at rice harvest in litter bags on soil surface or incorporated into the soil ($n=1$)



influenced by the variable rates of N mineralization from GMs and the same mineralization rates from soil among the different treatments.

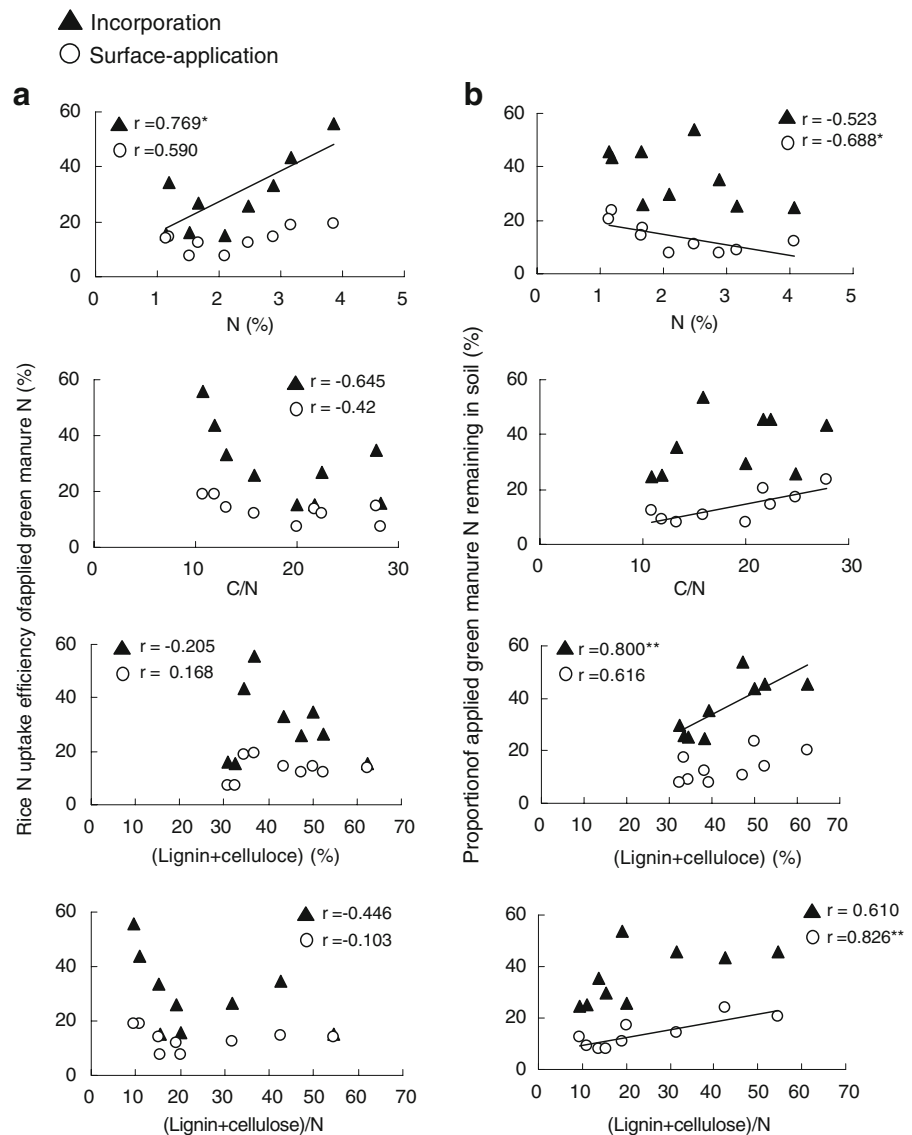
Dynamics of rice N uptake and utilization

Lowland rice is reported to take up 19–50% of applied GM N with ^{15}N tracer technique (Ashraf et al. 2004; Azam 1990; Diekmann et al. 1993). In the present study, the N uptake efficiency ranged between 15–56% of applied N in the incorporated GM treatments, 7–19% in the surface-applied GM treatments, and 52% in IF treatment, so these efficiencies were within the reported range. However there were differences in NUE among treatments. This may be influenced by the differences of rate of N mineralization from GM and the timing of N mineralization from GM and N demand of rice.

The MFE (mineral fertilizer equivalent) value of applied organic nutrient sources has been generally

regarded as the most useful index of organic material N efficiency, evaluating the N-supplying potential of organic matter compared to that of inorganic fertilizer. The MFE values of HV and WC in case of the incorporation were higher than that of the other GMs, 108% and 84%, respectively. In the case of surface-application, this value was also highest in HV, followed by WC. The efficiencies of GM were 29–108% in case of the incorporated GMs and 14–37% in the case of surface-applied GMs. Therefore, HV and WC seem to be the most efficient N source as alternative for inorganic N fertilizers. However the PNUE (physiological N use efficiency) and AE (agronomic efficiency) of HV, WC were much lower than those of IF. In order to optimize rice grain yield, it is important to increase the PNUE and AE of HV, WC. The incorporated CMV has lower MFE value than that of HV and WC, but it has the highest PNUE and AE of all GMs. Therefore high application rate of CMV seem to increase rice yield.

Fig. 4 Relationship between the chemical characteristics of green manure and rice N uptake efficiency (a) and proportion of applied GM N remaining in litter bags in/on soil (b). r denotes Pearson correlation coefficients, * Significant at the 0.05 level, ** Significant at the 0.01 level



Fate of fertilizer N

The average of N uptake efficiencies of the incorporated GMs (29.5%) was significantly higher than that of the surface-applied GMs (13.3%, Table 3). The average residual N from incorporated GM (36.6%) was higher than that from the surface-applied treatments (13.7%, Fig. 3). The rates of N uptake by rice and residual N in soil derived from incorporated GMs were higher than those from the surface-applied GMs. Detailed studies remains to clarify the precise N dynamics of the green manures in the paddy soil. However these results indicated that N losses from the

soil-plant system were the lower in the case of the incorporation treatments. NH_3 volatilization is an important pathway of N loss (Mikkelsen 1987). Daily NH_3 volatilization losses correlated with the NH_4^+ concentration of the flooded water (Vlek and Craswell 1979). In case of the incorporation, the GM was in deeper layer of soil than that in case of the surface-application, NH_4^+ generated from GM in deeper layer was relatively immobile. Therefore the concentration of NH_4^+ in flooded water may be lower with the GM incorporated treatment than that with the GM surface-application. These may induce a lower N loss in incorporated GM treatments than in surface-applied

GM treatments, resulted in higher N uptake, growth and yield in the GM incorporated treatments than those in GM surface-applications.

Relationships between N fate and the chemical characteristics of GM

The N mineralization rates of legume residues have been related to their (polyphenol + lignin)/N, polyphenol/N, lignin/N, lignin, and polyphenol (Baijukya et al. 2006; Fox et al. 1990; Handayanto et al. 1997; Palm and Sanchez 1991). In the present study, the N uptake efficiency by rice from GM was related to the N (%) of the incorporated GMs, but was not related with the surface-applied GMs (Fig 4). There was a difference in this relationship between the GM incorporated and surface-applied treatments, suggesting that the effect of N (%) of GMs on rice N uptake in the incorporated treatment was different from that in the surface-application treatment. This may be induced by different mineralization processes between GM incorporation in soil and surface-application on soil.

Conclusions

Among the nine GM treatments, the rice yield and N uptake tended to be higher in the HV and WC treatments. The rice N uptake efficiency from GM was also higher with HV and WC than that with the other GMs both in the incorporated and surface-applied treatments. Moreover the mineral fertilizer equivalent values of HV and WC in case of the incorporation were 108% and 84%, respectively. Therefore, HV and WC seem to be the most efficient N source as alternatives to inorganic N fertilizer in the Shikoku region, Japan. The rice N uptake efficiency and residual N in soil derived from GM were higher in the incorporation treatments than those in the surface-applied treatments, indicating lower N losses from the soil-plant system in case of the incorporation. Inorganic fertilizer is the most efficient N source for crop production, but fertilizer production consumes large quantities of fossil fuel that may susceptible to scarce availability and increasing prices in the current world market (Becker et al. 1995). Therefore incorporation of a GM with high mineral fertilizer equivalent such as HV and WC may result in saving an application of inorganic fertilizers, leading to

an improvement of the sustainability of these crop production systems.

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