Nitrogen Mineralization of Maturity-Stage Green Manure and its Application Effects on Rice Growth, Yield, and Nitrogen Use Efficiency

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Abstract

In recent years, modern agriculture relying on chemical fertilizers (CFs) has been facing concerns over sustainability, which involve fluctuating fertilizer prices and deteriorating soil fertility. Green manure (GM) is organic matter that can replace CFs and contribute to improving soil fertility. Currently, the use of GM in rice production is limited to the basal application at the flowering stage. However, the application of GM at the flowering stage can lower nitrogen use efficiency (NUE) because of rapid nitrogen (N) mineralization from the early growth stage of rice and possible long upland periods. Thus, this study focused on the use of maturity-stage GM, which might improve NUE because the GM mineralizes N until the late growth stage of rice. Moreover, the pattern of N mineralization from GMs, which changes with cultivars, growth stage, and incorporation timing, affects rice growth and yield. This requires that the characteristics of N mineralization should be clarified to promote appropriate use of GMs.

Therefore, the objectives of this study were (1) to clarify physicochemical properties and N mineralization of maturity-stage GM (crimson clover, hereafter CC) and its application effects on rice growth and NUE, through the comparison with flowering-stage GM, (2) to evaluate rice growth, NUE, yield, and quality when flowering- and maturity-stage CC were incorporated at the same timing, (3) to clarify the pattern of N mineralization from flowering- and maturity-stage hairy vetch (HV), CC, and white clover (WC) under flooded condition, which involved different upland periods, and (4) to evaluate denitrification loss during upland periods.

For the first objective, a cultivation experiment was conducted from October 2017 to September 2018 using 1/5,000a Wagner pots and six treatments of fertilizer and GM application: no fertilizer (NF), CF, GM incorporation at the flowering stage without (GMF) or with topdressing (GMF+T), and GM incorporation at the maturity stage without (GMM) or with topdressing (GMM+T). The total N application rates were 1.01–1.26 g pot^{-1} in GMF(+T) and 0.62–0.86 g pot^{-1} in GMM(+T) which were higher than that in CF (0.24 g pot^{-1}). The total

nitrogen (TN) contents of shoots and roots from flowering-stage GM were 2.3% and 1.5%, while those from maturity-stage GM were 1.9% and 1.3%, respectively. The C/N ratios of shoots and roots from flowering-stage GM were 16.8 and 16.9, respectively, while those from maturity-stage GM were 21.4 and 18.3, respectively. The dry matter weight (DMW), TN, P_2O_5, and K_2O of GM lowered, while the C/N ratio increased as GM matured. The N mineralized from GM at 30 °C under the flooded incubation for 13 weeks was 34% (97 mg kg^{-1}) of TN supplied from GM at the flowering stage, while it was 71% (126 mg kg^{-1}) of TN supplied from GM at the maturity stage. This difference was probably because GM incorporation at the flowering stage involved more denitrification loss due to a longer upland period. The large amount of mineral N available in GMM and GMM+T increased tiller number and TN content of rice aboveground part, which resulted in significantly larger dry matter weight at the panicle formation (PF) stage and N uptake at the active tillering (AT) and PF stages, compared with GMF and GMF+T. The NUE in GMM and GMM+T (14–21%) were significantly higher than those in GMF and GMF+T (4–6%).

For the second objective, a cultivation experiment was conducted from October 2018 to September 2019 followed by the cultivation experiment from October 2017 to September 2018 in the first year. The experiment in the second year included the same treatments as the first year. The total N application rates were 0.79–0.87 g pot^{-1} in GMF(+T) and 0.63 g pot^{-1} in GMM(+T) which were higher than that in CF (0.24 g pot^{-1}). The TN contents of shoots and roots from GM in GMF(+T) were 2.69–2.76% and 1.40–1.60%, respectively, while those in GMM(+T) were 2.68–2.80% and 0.92–1.01%, respectively. The C/N ratios of shoots and roots from GM in GMF(+T) were 13.9–14.5 and 15.4–20.1, respectively, while those in GMM(+T) were 14.2–15.0 and 25.4–28.0, respectively. Inhibited initial growth recovered less in GMM(+T) than in GMF(+T) because mineralized N supplied from maturity-stage GM before the AT stage was estimated to be 20%–30% lesser than that from the flowering-stage GM. The availability of N was likely to be high in GM treatments for later growth stages, which reduced unproductive tillers. The brown rice yield was higher in GMF(+T), followed by GMM(+T) and CF. The NUE

at the harvest stage in GMF(+T) (46%–52%) and GMM(+T) (38%–42%) was significantly lower than that in CF (67%) because of inhibited initial growth. The results indicated that using maturity-stage GM for rice production might require the application of basal fertilizer to promote initial growth.

For the third and fourth objectives, N mineralized from three species of GMs (HV, CC, and WC) collected both at the flowering- and maturity-stages was measured through incubation tests. The incubation tests included five upland periods (0, 1, 2, 3, 4 weeks) at 20 °C, and then each upland period involved eight flooded periods (0, 1, 2, 4, 6, 8, 12, 16 weeks) at 30 °C. Flowering-stage HV mineralized 52% of total N input at flooded week 0 under upland week 3 because of low C/N ratios (13.3 in shoots and 11.6 in roots). The percentage of N mineralized until flooded week 4 was about 50% and more for flowering-stage GMs under upland week 0, whereas maturity-stage GMs mineralized 40–58% after 4 weeks till 16 weeks. Denitrification loss under the upland condition increased markedly when the upland period became more than 2 weeks. The denitrification loss is likely attributed to easily mineralized fraction of N in GM, which is mineralized until flooded week 4. Thus, maturity-stage GMs, which have a higher C/N ratio than flowering-stage, are advantageous of lowering denitrification loss during the upland period.

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List of Abbreviations and Acronyms

AT: Active tillering stage

CC: Crimson clover

CF: Chemical fertilizer

CMV: Chinese milk vetch

DAT: Days after transplanting

DMW: Dry matter weight

GM: Green manure

GMF: Green manure incorporation at the flowering stage without topdressing

GMF+T: Green manure incorporation at the flowering stage with topdressing

GMM: Green manure incorporation at the maturity stage without topdressing

GMM+T: Green manure incorporation at the maturity stage with topdressing

HA: Harvest stage

HV: Hairy vetch

MN: Mineralized nitrogen

N: Nitrogen

NF: No fertilizer

NUE: Nitrogen use efficiency

PF: Panicle formation stage

TC: Total carbon

TK: Total potassium

TN: Total nitrogen

TP: Total phosphorus

WC: White clover

Chapter 1

Introduction

1.1 Background

In recent years, modern agricultural techniques that use chemical fertilizers (CFs) have been facing concerns that threaten its sustainability. The prices of CF have remained high for the last decade, with fluctuations caused by the rising costs of fossil fuels and concerns over the depletion of raw materials to produce CFs (Baligar and Bennett, 1986; World Bank, 2019). In addition, the long-term use of CFs in agricultural production has caused the deterioration of soil fertility, including reduced soil organic carbon (C) and degraded biological activity (Liu et al., 2010; Miao et al., 2011).

Green manure (GM) is organic matter that can replace CFs and contribute to improving soil fertility. Singh et al. (1994) mentioned that legume GM, such as sweet clover, red clover, and alfalfa, provided nitrogen (N) ranging from 45 to 173 kg ha^{-1} to succeeding crops. In rice cultivation, grain yield was equivalent to or higher than conventional cultivation with a CF, when the Chinese milk vetch (CMV) or hairy vetch (HV) was used as a basal application with topdressing(s) (Azuma et al., 2017; Kawase and Kitazima, 1994). Chen (1988) reported that the incorporation of CMV for four consecutive years improved soil organic matter in rice fields by 0.1–0.9%.

The use of GM in agricultural production has rapidly declined since the advent of CFs (Chen, 1988; Rosegrant and Roumasset, 1988; Yasue, 1991). On the other hand, the GM use in agriculture has been renewed as a sustainable practice in terms of soil fertility management and low input of energy and fertilizer resources.

1.2 Previous studies on the use of green manure in rice production and focus of this thesis

There have been many studies on the use of GM in rice production. Kawase and Kitazima (1994) reported that using CMV as a basal application with a topdressing resulted in grain yield equivalent to conventional cultivation with CF. Asai et al. (2013) showed that the basal application of CMV alone did not cause a large yield reduction in an early rice variety compared with cultivation using CF. Azuma et al. (2017) reported that grain yield was greater than cultivation with CF when HV was used as a basal application with two topdressings. Appropriate incorporation rates and timings have been reported for CMV and HV to maximize yield and avoid adverse effects on growth because of the decomposition of carbohydrates (Ishikawa, 1988; Azuma et al., 2017). Chen (1988) and Ishikawa (1988) reported that the consecutive incorporation of CMV improved soil organic matter in rice fields.

In Japan, the use of GM in rice production has been basically limited to basal application at the flowering stage, because GM at this stage provides more mineralized N from the early growth stage of rice than GM at other growth stages, due to its peak amount of N and low C/N ratio (Ishikawa, 1988). However, the nitrogen use efficiency (NUE) of rice plants is likely to be low when GM is incorporated in the soil at the flowering stage. Asagi and Ueno (2009) reported that the NUE of rice plants was 55.6% in HV followed by white clover (WC) (43.5%), crimson clover (33.3%), and CMV (25.7%) when air-dried flowering-stage GMs were incorporated 1 day before transplanting rice. This low NUE is because of the N supplied from GM incorporated at the flowering stage, which is rapidly mineralized after incorporation, can be excess to what the crop's mineral N requirement is (Kawase and Kitazima, 1994).

In addition, because the growth of GM varies depending on weather conditions, the N supply from GM can be high enough to cause lodging and disease (Japan Soil Association, 2012). In this case, the excessive N is reduced by adjusting an upland period between the incorporation of GM and the flooding before transplanting rice. Azuma et al. (2017) reported that the ratio of mineralized N to the total N supplied from GM decreased from about 60% to 20% when the upland period increased from 7 to 28 days. On the other hand, this decrease in the mineralized N indicates that N from GM was lost by denitrification and leaching (Buresh and De Datta, 1991; George et al., 1992). Thus, N from GM incorporated at the flowering stage can be used inefficiently in rice production.

Green manure incorporated at the maturity stage can improve the NUE of rice plants because maturity-stage GM, which has a higher C/N ratio than the flowering-stage GM, supplies mineralized N until the late growth stage of rice (Ishikawa, 1988). Becker et al. (1995) mentioned that organic materials, which slowly release N, have the potential to synchronize the N supply with the N demand of rice, which leads to reducing N loss. Meanwhile, there have been limited studies that focus on the use of maturity-stage GM in rice production. Nagumo et al. (2014) showed through incubation tests at 25 °C that N mineralized from maturity-stage CMV (C/N ratio: 17.1) in one month was 17.9% of the total N added, which was markedly lower than 53–57.8% (28-days incubation at 23 °C) reported for the flowering-stage CMV (Ishikawa, 1963). In addition, Nagumo et al. (2014) reported that N mineralized from maturity-stage CMV during the late growth stage was insufficient to replace the topdressing. Maturity-stage GM needs to be studied further in terms of its physicochemical properties and N mineralization as well as its application effects on rice growth, yield, and NUE, to consider the appropriate use of GM in rice production.

1.3 Research objectives and thesis outline

This thesis focused on the use of maturity-stage GM in rice production. The objectives of this thesis were (1) to clarify physicochemical properties and N mineralization of maturity-stage GM (crimson clover, hereafter CC) and its application effects on rice growth and NUE, through the comparison with flowering-stage GM, (2) to evaluate rice growth, NUE, yield, and quality when flowering- and maturity-stage CC were incorporated at the same timing, (3) to clarify the pattern of N mineralization from flowering- and maturity-stage HV, CC, and WC under flooded condition, which involved different upland periods, and (4) to evaluate denitrification loss during upland periods.

Chapter 2 describes the study on the first objective. To complete this objective, a cultivation experiment using 1/5,000a Wagner pots was conducted from October 2017 to September 2018 at Kyushu University farm in Fukuoka Prefecture, Japan. This experiment included six treatments of fertilizer and GM application: no fertilizer (NF), CF, GM incorporation at the flowering stage without (GMF) or with topdressing (GMF+T), and GM incorporation at the maturity stage without (GMM) or with topdressing (GMM+T). Each treatment was prepared for three growth stages of rice: active tillering (AT), panicle formation (PF), and harvest (HA). The incorporation dates were April 29 for flowering-stage GM and May 23 for maturity-stage GM. The upland period was 45 days for flowering-stage GM and 21 days for maturity-stage GM. The physicochemical properties of flowering- and maturity-stage GM were measured and mineralized N from GM was estimated through incubation tests. The growth parameters (plant length, number of the tillers, SPAD readings, dry matter weight) and nutrient contents (TN, P_2O_5, and TK) were measured for rice at each growth stage. The application effects of maturity-stage GM were investigated on growth and NUE of rice by comparing with the treatments with flowering-stage GM and CF.

Chapter 3 describes the study on the second objective. To complete this objective, a cultivation experiment using 1/5,000a Wagner pots was conducted from October 2018 to September 2019 followed by the cultivation experiment from October 2017 to September 2018 in the first year. The experiment in the second year included the same treatments as the first year. The incorporation timing of GM was the same both for flowering- and maturity-stage GM (10 days before transplanting). Estimating mineralized N from GM and measuring rice growth and

NUE were performed with the same procedures in the first year experiment. This chapter also included measurements of yield components, yield, and protein content in brown rice. The application effects of maturity-stage GM on growth, yield, and NUE of rice were compared with the flowering-stage GM under the same timing of the GM incorporation.

Chapter 4 describes the study on the third and fourth objectives. To complete this objectives, we cultivated HV, CC, and WC using planters from October 2018 to June 2019 at Kyushu University farm. Mineralized N from flowering- and maturity-stage GMs was estimated through incubation tests. The incubation tests included five upland periods (0, 1, 2, 3, 4 weeks) at 20 °C, and then each upland period involved eight flooded periods (0, 1, 2, 4, 6, 8, 12, 16 weeks) at 30 °C. N mineralization patterns and denitrification loss were evaluated under different upland periods using three cultivars of flowering- and maturity-stage GM.

Chapter 2

Physicochemical Properties and Nitrogen Mineralization of Maturity-Stage Green Manure and its Application Effects on Growth of Rice Cultivated in a Wagner Pot

2.1 Introduction

In recent years, modern agricultural techniques that use chemical fertilizers (CFs) have been facing concerns that threaten its sustainability. The prices of CF have remained high for the last decade, with fluctuations caused by the rising costs of fossil fuels and concerns over the depletion of raw materials to produce CFs (Baligar and Bennett, 1986; World Bank, 2019). In addition, the long-term use of CFs in agriculture production has caused the deterioration of soil fertility, including reduced soil organic carbon (C) and degraded biological activity (Liu et al., 2010; Miao et al., 2011).

Green manure (GM) is organic matter that can replace CFs and contribute to improving soil fertility. Singh et al. (1994) mentioned that legume GM, such as sweet clover, red clover, and alfalfa, provided N ranging from 45 to 173 kg ha^{-1} to succeeding crops. In rice cultivation, grain yield was equivalent to or higher than conventional cultivation with a CF, when the Chinese milk vetch (CMV) or hairy vetch (HV) was used as a basal application with topdressing(s) (Azuma et al., 2017; Kawase and Kitazima, 1994). Chen (1988) re-ported that the incorporation of CMV for four consecutive years improved soil organic matter in rice fields by 0.1–0.9%.

In Japan, the use of GM in rice production is basically limited to basal application at the flowering stage, because GM at this stage provides more mineralized N from the early growth stage of rice than GM at other growth stages, due to its peak amount of N and low C/N ratio (Ishikawa, 1988). However, the nitrogen use efficiency (NUE) of rice plants is likely to be low when GM is incorporated in the soil at the flowering stage. Asagi and Ueno (2009) reported that the NUE of rice plants were 43.5% (white clover), 25.7% (CMV), 55.6% (HV), and 33.3% (crimson clover, hereafter CC), when flowering-stage GMs were incorporated one day before transplanting rice. This low NUE is because of the N supplied from GM incorporated at the flowering stage, which is rapidly mineralized after incorporation, can be excess to what the crop's mineral N requirement is (Kawase and Kitazima, 1994). The total amount of N supplied from GM incorporated at the flowering stage can reach 200 kg ha^{-1} (Kawase and Kitazima, 1994; Schulz et al., 1999). Meanwhile, the total N uptake of IR-8 (improved variety) was 164 kg N ha^{-1} and Peta (traditional variety) was 143 kg N ha^{-1}, of which, the N uptake of the panicle accounts for 71 % and 48%, respectively (Yoshida, 1981).

In addition, because the growth of GM varies depending on weather conditions, the N supply from GM can be high enough to cause lodging and disease (Japan Soil Association, 2012). In this case, the excessive N is reduced by adjusting an upland period be-tween the incorporation of GM and the flooding before transplanting rice. Azuma et al. (2017) reported that the ratio of mineralized N to the total N supplied from GM decreased from about 60% to 20% when the upland period increased from 7 to 28 days. On the other hand, this decrease in the mineralized N indicates that N from GM was lost by denitrification and leaching (Buresh and De Datta, 1991; George et al., 1992). Thus, N from GM incorporated at the flowering stage can be used inefficiently in rice production.

Green manure incorporated at the maturity stage can improve the NUE of rice plants because maturity-stage GM, which has a higher C/N ratio than the flowering-stage GM, supplies mineralized N until the late growth stage of rice (Ishikawa, 1988). Becker et al. (1995) mentioned that organic materials, which slowly release N, have the potential to synchronize the N supply with the N demand of rice, which leads to reducing N loss. Meanwhile, there have been limited studies that focus on the use of maturity-stage GM in rice production. Nagumo et al. (2014)

showed through incubation tests at 25 °C that N mineralized from maturity-stage CMV (C/N ratio: 17.1) in one month was 17.9% of the total N added, which was markedly lower than 53–57.8% (28-days incubation at 23 °C) reported for the flowering-stage CMV (Ishikawa, 1963). In addition, Nagumo et al. (2014) reported that N mineralized from maturity-stage CMV during the late growth stage was insufficient to replace the topdressing. Maturity-stage GM needs to be studied further in terms of its N mineralization and NUE to consider the appropriate use of GM in rice production.

Among legume GMs, which involve biological N fixation, CMV has been conventionally used in rice production (Yasue, 1991), while recently the use of HV is expected (Azuma et al., 2017). On the other hand, characteristics of more GMs should be clarified to promote an appropriate use of GMs under different conditions of field drainage, local weather, and cropping system. Crimson clover has large plant height and relatively high C/N ratio among legume GMs (Asagi and Ueno, 2009; Japan Soil Association, 2012; Okumura and Koinuma, 2017), which might result in different nutrient supply, N mineralization, and the effects on rice growth. However, there have been limited investigations about the use of crimson clover in rice production. The objectives of this chapter were to clarify physicochemical properties and N mineralization of maturity-stage GM (CC) and the effects of the GM on the growth and NUE of rice cultivated in a Wagner pot, through the comparison with flowering-stage GM.

2.2 Materials and Methods

2.2.1 Pot experiment

A cultivation experiment using 1/5,000a Wagner pots was conducted from October 2017 to September 2018 at Kyushu University farm in Fukuoka Prefecture, Japan. The pots placed outside were managed under a similar condition of rainfall and sunshine with other paddy fields at the farm. The experiment included six treatments of fertilizer and GM application: no fertilizer

(NF), CF, GM incorporation at the flowering stage without (GMF) or with topdressing (GMF+T), and GM incorporation at the maturity stage without (GMM) or with topdressing (GMM+T). We included the treatments of GM with topdressings (GMF+T, GMM+T), because Nagumo et al. (2014) reported that N mineralized from maturity-stage CMV during the late growth stage was insufficient to replace the topdressing. The six treatments were laid out in randomized complete block designs with three replications. Each treatment was prepared for three growth stages of rice: active tillering (AT), panicle formation (PF), and harvest (HA).

The pots were filled with 3.5 kg paddy soil (dry weight basis) collected from Kyushu University Farm, which was passed through a 10-mm sieve. The soil type, pH, cation exchange capacity, total nitrogen (TN), total phosphorus (TP), and exchangeable K were clay loam, 5.8, 11.17 cmol kg^{-1}, 0.18%, 0.16% and 0.32 cmol kg^{-1}, respectively. Crimson clover (*Trifolium incarnatum* L.) was sown in pots involving GM application (GMF, GMF+T, GMM, and GMM+T) at a seed rate of 0.06 g pot^{-1} (3 g m^{-2}) on October 23, 2017. Eight plants of GM were maintained in each pot after germination. Liquid fertilizer (HYPONeX, HYPONeX Japan Corp., Ltd.) was applied on December 11 in all the pots of six treatments at a rate of 0.06 g N pot^{-1} (3 g N m^{-2}), 0.1 g P_2O_5 pot^{-1} (5 g P_2O_5 m^{-2}), and 0.05 g K_2O pot^{-1} (2.5 g K_2O m^{-2}), because the leaves of CC plants, which partly turned purple, showed a symptom of phosphate deficiency. The moisture condition of soil in all pots was adjusted between 30% and 60% of the maximum water holding capacity during cultivation. The drain outlet of a Wagner pot remained open.

In the treatments of GMF and GMF+T, GM was incorporated at the flowering stage on April 29, 2018. Shoots of CC plants were cut from the soil surface and measured for total fresh weight. The shoots were then cut into 1 cm pieces, from which about 5 g was used for measuring moisture content (oven-drying at 70 °C for 48 h). Roots were also cut into about 1 cm pieces. The pieces of roots, which were mixed well with the pieces of shoots, were incorporated in the soil. In

GMM and GMM+T, GM was incorporated at the maturity stage on May 23, following the same procedure as that of the flowering stage.

In the CF treatment, basal fertilizer was mixed well with soil on June 18 with the application rate of 0.16 g N pot^{-1} (8 g N m^{-2}), 0.16 g P_2O_5 pot^{-1} (8 g P_2O_5 m^{-2}), and 0.16 g K_2O pot^{-1} (8 g K_2O m^{-2}). For other treatments, soil in each pot was mixed well. Then, all the pots were flooded with tap water and the soil was mixed with water on the same day. Three 22-day-old seedlings of rice were transplanted into each pot on June 21. The rice cultivar used in the experiment was Genki Tsukushi (*Oryza sativa* L.). For CF, GMF+T, and GMM+T treatments, topdressing was applied two times on August 3 and August 10 each with the application rate of 0.04 g N pot^{-1} (2 g N m^{-2}) and 0.04 g K_2O pot^{-1} (2 g K_2O m^{-2}). The water was maintained at a level of about 3–5 cm above the soil surface during the rice growth period.

2.2.2 Measurement of physicochemical properties of green manure

Green manure was cultivated in three investigation pots, each for the flowering and maturity stages to measure its physicochemical properties. GM in the investigation pots were cultivated during the same period and under the same management as the pot experiment. The physicochemical properties of GM were measured using shoots and roots, which were collected from the investigation pots when GM was incorporated at the flowering and maturity stages in the treatments of GMF, GMF+T, GMM, and GMM+T. Shoots were cut from the soil surface, while roots were collected by removing soil carefully; fresh weights of shoot and root parts were measured separately. Then, half of each part was used for measuring the moisture content, TN, phosphorus (P_2O_5), and potassium (K_2O). The shoot and root parts were oven -dried at 70 °C for 48 h and were ground to a fine powder using a sample mill (Cyclotec 1093, Foss, Hilleroed, Denmark). The TN and total carbon (TC) were measured using a CHN coder (MT-5, Yanaco) with three replications. For the measurement of P_2O_5 and K_2O, the ground shoot and root were separately digested by H_2SO_4-H_2O_2 Kjeldahl digestion method with three replications (Ohyama et al., 1991). P_2O_5 was measured by the ascorbic acid method, and K_2O was measured using an atomic absorption spectrophotometer (Z-5300, Hitachi High-Technology Co., Tokyo, Japan) (Murphy and Riley, 1962).

2.2.3 Estimation of nitrogen mineralized from green manure

The N mineralized from GM during the rice growth period was estimated through incubation tests. Moist soil and GM (shoots and roots), which were sampled at the flowering and maturity stages each from three investigation pots, were used for the incubation tests. In addition, moist soil sampled from the three pots with bare soil, which were managed in the same manner as the pot experiment during the cultivation period of GM, were used for the incubation tests. The shoots and roots of GM, which were cut into about 1 cm pieces, were mixed well with moist soil (approximately 120 g dry weight basis) and were transferred into a 500-mL glass bottle. The average dry weights of the shoot and root parts used for the incubation tests were 1.28 g and 0.31 g at the flowering stage, respectively, and 0.86 g and 0.16 g at the maturity stage, respectively. The weights of the shoot and root parts were determined by multiplying their total weights in the investigation pots with the ratio of the soil weight used in the incubation tests (approximately 120 g) to soil weight filled in a pot (3,500 g).

The moisture condition of the soil was adjusted to 60% of the maximum water holding capacity during the incubation under the upland condition. The temperature during the incubation was 20 °C, which was about the average of the daily mean temperature at the experimental site between the beginning of May and mid-June. The incubation periods were 45 days for GM collected at the flowering stage, which corresponded to the upland period after GM incorporation in the treatments of GMF and GMF+T. The incubation periods were 21 days for GM collected at the maturity stage, which corresponded to an upland period in the treatments of GMM and

GMM+T. Moist soil collected from the pots with bare soil at the flowering and maturity stages was also incubated under the same condition.

After the incubation under the upland condition, soil was divided for four periods of incubation tests under the flooded condition at 30 °C: 0, 4, 7, and 13 weeks. The periods corresponded to each growth stage of transplanting, AT, PF, and HA, respectively, based on an accumulation temperature at the experimental site. Approximately 30 g soil (dry weight basis) each for the 4, 7, and 13-week incubation was transferred from a glass bottle into a 30 ml polypropylene tube (62.543, Sarstedt, Nümbrecht, Germany) with distilled water. Soil filled in a tube was about 6 cm in depth, and the water above the soil surface was about 2.5 cm. After each of the incubation periods, soil and water in a tube were transferred into a 500-mL glass bottle with 150 ml of 10% KCl. For 0-week incubation, approximately 30 g soil was not transferred into a polypropylene tube and stored in a glass bottle, to which 150 ml of 10% KCl was added. Then, mineralized N was extracted by shaking a glass bottle for 30 min. The extract was filtered through a No. 5B filter paper (Advantec, Tokyo, Japan). Then, ammonium (NH_4–N) and nitrate (NO_3–N) in the extract were measured by the indophenol and Cataldo methods, respectively (Cataldo et al., 1974; Cataldo et al., 1975). Finally, N mineralized from GM incorporated at the flowering and maturity stages was estimated using the difference in the mineralized N between the soil mixed with GM and bare soil.

2.2.4 Measurement of rice growth

The plant length and the number of tillers per pot were measured weekly from 7 days after transplanting (DAT) to 84 DAT. SPAD readings were measured weekly from 21 DAT using a chlorophyll meter (SPAD-502, Konica Minolta Holdings, Inc., Tokyo, Japan). The SPAD readings were measured for the second fully expanded leaf blade in the longest tiller, while they were measured for the flag leaf after heading (63 to 84 DAT). Measurements were performed four times on both sides of the midrib of the leaf blade (two times on each side), midway between the leaf base and tip, and then the average value was calculated. The positions on a leaf for measuring SPAD readings were determined according to Chubachi et al. (1986).

2.2.5 Measurement of NPK content in rice aboveground part, dry matter weight, and NPK uptake of rice aboveground part

Rice aboveground part in each pot was cut from the soil surface at the AT stage on July 20 (28 DAT) and PF stage on August 2 (42 DAT). Rice aboveground part at the HA stage were not sampled because they were damaged by wild boars. The aboveground part was oven- dried at 70 °C for 48 h and they were then measured for dry matter weight (DMW). Next, the aboveground part was milled using the sample mill. The milled samples were digested by H_2SO_4-H_2O_2 Kjeldahl digestion method. The TN content in the rice plant was measured by the indophenol method. TP and TK were measured by the same methods used for GM.

2.2.6 Calculation of nitrogen use efficiency of rice aboveground part

The NUE of the rice aboveground part was calculated as

$$\text{NUE (\%)} = \frac{\text{N uptake in a GM or CF pot} - \text{N uptake in a no fertilized pot}}{\text{TN applied from GM and CF}} \times 100 \tag{2.1}$$

Here, GM pots include GMF, GMF+T, GMM, and GMM+T. In this study, the mineralized NUE of the rice aboveground part was also calculated using

$$\text{Mineralized NUE (\%)} = \frac{\text{N uptake in a GM or CF pot} - \text{N uptake in a no fertilized pot}}{\text{N mineralized from GM} + \text{TN applied from CF}} \times 100 \tag{2.2}$$

The values of N mineralized from GM in the denominator of Eq. (2.2) were calculated using mineralized N estimated through incubation tests.

2.2.7 Statistical analysis

The difference in the mean values between two treatments was tested by the t test at a 5% probability level using Microsoft Excel (Tokutsu, 2002). The difference in the mean values among more than two treatments was tested by the Tukey method at a 5% probability level using STATISTIX 8 (Analytical Software, Tallahassee, FL, USA).

2.3 Results

2.3.1 Physiochemical properties of green manure

Table 2.1 shows the physiochemical properties of GM collected at the flowering and maturity stages. For the shoot part, the moisture content was 68.3% at the flowering stage, which was about 20% higher than that at the maturity stage (47.0%). Dry matter weight was significantly higher at the flowering stage (37.3 g pot^{-1}) than at the maturity stage (28.6 g pot^{-1}). The TN, P_2O_5, and K_2O content at the flowering stage were 2.3%, 0.51%, and 1.32%, respectively, and they were significantly higher than those at the maturity stage (TN: 1.9%, P_2O_5: 0.39%, and K_2O: 0.80%). The TC content was around 40% at the both growth stages. The C/N ratio was higher at the maturity stage (21.4) than at the flowering stage (16.8), although their difference was not significant. For the root part, the moisture content was significantly higher at the maturity stage (57.3%) than that at the flowering stage (46.6%). Dry matter weight was significantly larger at the flowering stage (9.0 g pot^{-1}) than that at the maturity stage (5.3 g pot^{-1}). The TC, TN, P_2O_5, and K_2O were lower than those in the shoot. The C/N ratio was 16.9 and 18.3 at the flowering and maturity stages, respectively.

Table 2.1 Physicochemical properties of green manure

Plant parts	Growth stage	Moisture (%)	Dry matter weight (g pot^{-1})	Total %				C/N ratio
				TC	TN	P_2O_5	K_2O	
Shoot	Flowering	68.3 a	37.3 a	39.0 a	2.3 a	0.51 a	1.32 a	16.8 a
	Maturity	47.0 b	28.6 b	40.2 a	1.9 b	0.39 b	0.80 b	21.4 a
Root	Flowering	46.6 b	9.0 a	24.6 a	1.5 a	0.36 a	0.82 a	16.9 a
	Maturity	57.3 a	5.3 b	23.2 a	1.3 a	0.28 b	0.74 a	18.3 a

Average values in the table were measured for three investigation pots (*n*=3). Different letters indicate that mean values between flowering and maturity are significantly different ($p < 0.05$) according to the t test.

2.3.2 N, P_2O_5, and K_2O application rate of chemical fertilizer and green manure

Table 2.2 lists the N, P_2O_5, and K_2O application rates of the CF and GM. The application rate of N from the GM was higher in the GMF ranging from 1.01–1.24 g pot^{-1} (Average: 1.14 g pot^{-1}) than that in the GMM ranging from 0.62–0.81 g pot^{-1} (Average: 0.74 g pot^{-1}). The total N application rates in the GMF and the GMM were about five and three times larger than that in CF (0.24 g pot^{-1}), respectively. The total application rates of P_2O_5 (0.26 g pot^{-1}) and K_2O (0.64 g pot^{-1}) in GMF were about 1.6 and 2.7 times larger than those in CF (P_2O_5: 0.16 g pot^{-1}, K_2O: 0.24 g pot^{-1}), respectively. For GMM, the total application rates of P_2O_5 (0.15 g pot^{-1}) and K_2O (0.31 g pot^{-1}) in GMM were similar with those in CF.

2.3.3 Nitrogen mineralized from green manure

Figure 2.1 shows the N mineralized from GM at 30 °C for the incubation periods of 0, 4, 7, and 13 weeks under flooded condition. N mineralized from the GM incorporated at the flowering and maturity stages had similar levels at the 0 week. Then, mineralized N increased quickly in the fourth week of incubation. The rate of N mineralized was higher in the incorporation at maturity stage, and mineralized N reached 70.9 mg kg^{-1} at four weeks, which was about 20 mg kg^{-1} higher than that in the incorporation at flowering stage. The mineralized rate of N slowed down after four weeks. The mineralized N at 13 weeks in the incorporation at flowering and maturity stages were 97.1 and 126.1 mg kg^{-1}, respectively. Differences in the mean values at each incubation period were not significant between flowering and maturity stages.

Table 2.2 N, P_2O_5, and K_2O application rates of chemical fertilizer and green manure

Treatments	Green manure*	Chemical Fertilizer**			Total***
		Basal	1st Topdressing	2nd Topdressing	
N application rates (g pot^{-1})					
NF	-	-	-	-	0
CF	-	0.16	0.04	0.04	0.24
GMF	1.01-1.24(1.14)	-	-	-	1.01-1.24(1.14)
GMF+T	0.97-1.39(1.17)	-	0.04	0.04	1.17-1.26(1.22)
GMM	0.62-0.81(0.74)	-	-	-	0.62-0.76(0.71)
GMM+T	0.58-0.83(0.74)	-	0.04	0.04	0.79-0.86(0.83)
P_2O_5 application rates (g pot^{-1})					
NF	-	-	-	-	0
CF	-	0.16	-	-	0.16
GMF	0.23-0.28(0.26)	-	-	-	0.23-0.28(0.26)
GMF+T	0.22-0.31(0.26)	-	-	-	0.25-0.26(0.26)
GMM	0.13-0.17(0.15)	-	-	-	0.13-0.16(0.15)
GMM+T	0.12-0.17(0.15)	-	-	-	0.15-0.16(0.15)
K_2O application rates (g pot^{-1})					
NF	-	-	-	-	0
CF	-	0.16	0.04	0.04	0.24
GMF	0.57-0.70(0.64)	-	-	-	0.57-0.70(0.64)
GMF+T	0.55-0.78(0.66)	-	0.04	0.04	0.70-0.74(0.72)
GMM	0.27-0.35(0.32)	-	-	-	0.27-0.33(0.31)
GMM+T	0.25-0.36(0.32)	-	0.04	0.04	0.39-0.42(0.41)

N, P_2O_5, and K_2O application of GM (green manure) included N from shoots and roots, which was estimated by the product of dry matter weight (DMW) and TN, P_2O_5, and K_2O (%). The DMW of shoots was measured for each pot where GM was incorporated. The DMW of roots was estimated by multiplying the average DMW of roots in the investigation pots with the ratio of DMW of shoots in each pot to the average DMW of shoots in the investigation pots. Physicochemical properties of GM were measured using three investigation pots each for the flowering and maturity stages. Average values of TN, P_2O_5, and K_2O (%) each in shoots and roots in the investigation pots were used as TN, P_2O_5, and K_2O (%) in shoots and roots in the pot experiment.

* GM was incorporated at the flowering stage on April 29 and at the maturity stage on May 23. The ranges and the averages in parenthesis were obtained by measuring GM cultivated in nine pots (*n*=9) for evaluating rice growth at three growth stages: active tillering, panicle formation and harvest.

** Basal fertilizer was applied on June 18. Topdressing was applied on August 3 (1st) and August 10 (2nd).

*** The ranges and the averages in parenthesis were obtained by measuring GM cultivated in three pots (*n*=3) for evaluating rice growth at harvest stage.

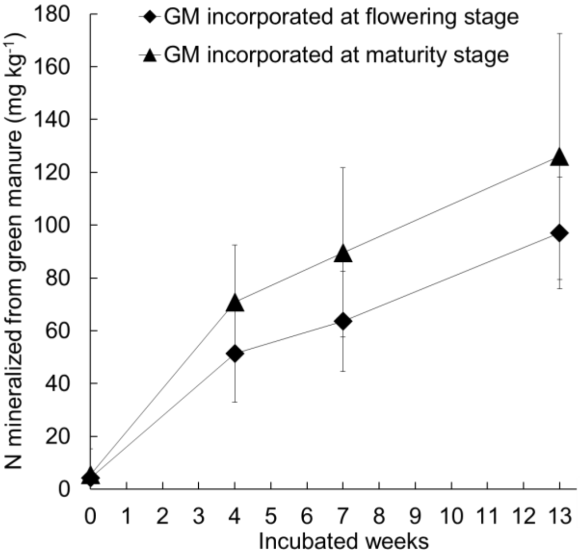

Figure 2.1 Nitrogen (N) mineralized from green manure estimated through incubation tests at 30 °C under flooded condition. Error bars represent SD (standard deviation). N mineralized from GM incorporated at the flowering and maturity stages was estimated using the difference in the mineralized N between the soil mixed with GM and bare soil. *n*=3

2.3.4 Rice growth

Plant length rapidly increased from 7 to 14 DAT and then gradually increased from 14 to 63 DAT under all treatments, which reached 78 cm in CF at 63 DAT followed by 72.9 cm in GMM+T, 71.6 cm in GMF+T, 69.9 cm in GMF, 68.1 cm in NF, and 67.2 cm in GMM (Fig. 2.2). The plant length before the AT stage (7, 21, and 28 DAT) and that after heading (63 to 84 DAT) was significantly higher in CF than that under treatments where GM was incorporated (GMF, GMF+T, GMM, and GMM+T).

Tiller number (tillers pot^{-1}) rapidly increased in CF before the AT stage (28 DAT) and reached about 30, while tiller numbers at 28 DAT were 25.7 in GMM, 25.6 in GMM+T, 22.6 in GMF, 20.4 in GMF+T, and 14.3 in NF, respectively (Fig. 2.3). After 28 DAT, tiller numbers in GMM and GMM+T increased over 30, whereas those in CF declined to 24 at 84 DAT, which are similar to those in GMF and GMF+T. Tiller numbers before the AT stage were significantly higher in CF than in all other treatments. On the other hand, tiller numbers after the AT stage were significantly higher in GMM and GMM+T than NF, GMF, and GMF+T, while the values tended to be higher than CF.

SPAD readings were high in GMM and GMM+T before the PF stage (49 DAT), which gradually declined from about 39 at 21 DAT to 34 at 42 DAT (Fig. 2.4). For other treatments, SPAD readings sharply declined from about 39 to 30 during the same period. SPAD readings after the PF stage till the beginning of the ripening period (70 DAT) tended to be higher in GMF+T, GMM, and GMM+T. SPAD readings were significantly higher in GMM and GMM+T between 28 and 49 DAT compared with other treatments (except for GMF+T at 49 DAT), while most of the values between 56 and 84 DAT did not show significant differences among all the treatments.

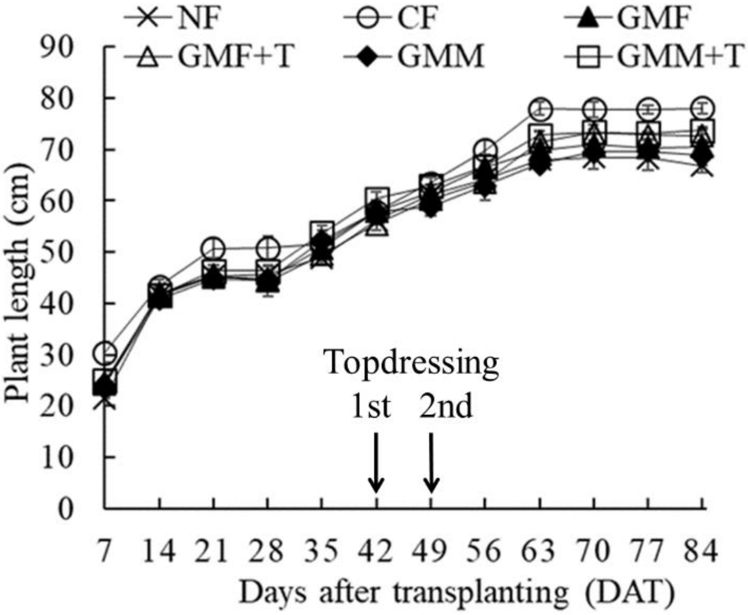

Figure 2.2 Plant length of rice. Error bars represent SD (standard deviation). *n*=9 from 7 to 28 DAT, *n*=6 from 35 to 42 DAT, *n*=3 from 49 to 84 DAT

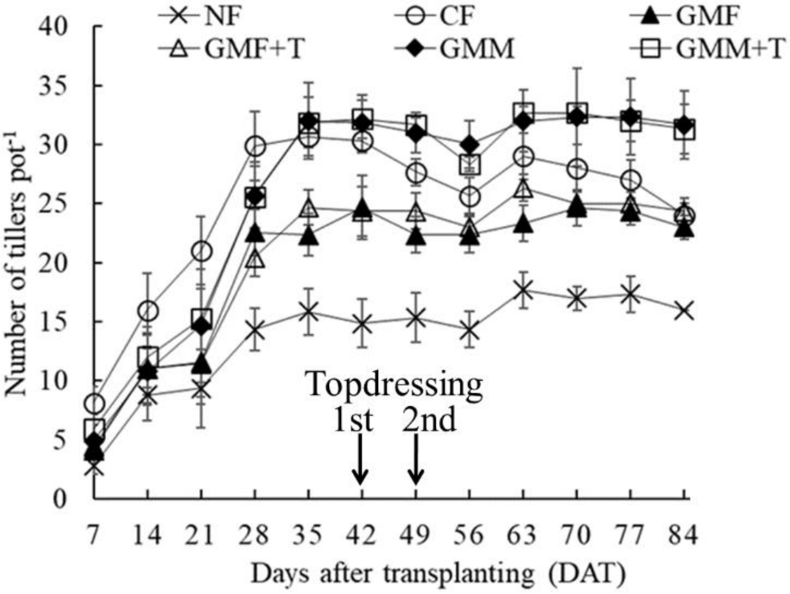

Figure 2.3 Number of tillers pot^{-1} of rice. Error bars represent SD (standard deviation). *n*=9 from 7 to 28 DAT, *n*=6 from 35 to 42 DAT, *n*=3 from 49 to 84 DAT

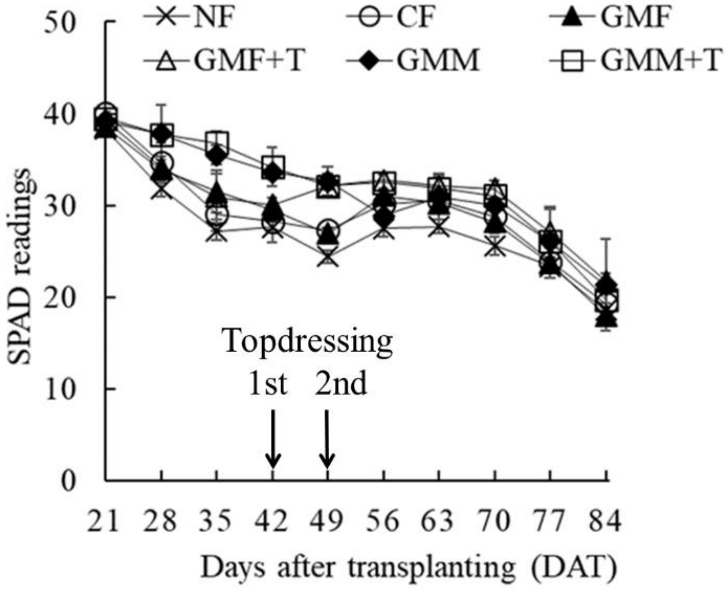

Figure 2.4 SPAD readings of rice. Error bars represent SD (standard deviation). *n*=9 from 21 to 28 DAT, *n*=6 from 35 to 42 DAT, *n*=3 from 49 to 84 DAT

2.3.5 Nutrient contents, dry matter weight, and nitrogen uptake

Table 2.3 lists the nutrient (TN, P_2O_5, and K_2O) contents in the rice aboveground part. The TN contents at the AT stage were significantly higher in GMM (2.18%) and GMM+T (2.12%) compared with those in NF, CF, and GMF. The TN contents at the PF stage ranged from 0.81–1.07%, which were lower than in the AT stage. They were significantly higher in GMM and GMM+T than that in CF. P_2O_5 contents at the AT and PF stages were not significantly different among CF and the other treatments involving GM incorporation (except for GMM+T at the PF stage). The K_2O contents at the AT stage were higher than 3.0% in NF, CF, GMF, and GMF+T, which were significantly higher than that in GMM (2.20%) and GMM+T (2.35%). The K_2O contents at the PF stage lowered in the range from 2.28–2.46% in NF, CF, GMF, and GMF+T, whereas their values were significantly higher than those in GMM (1.74%) and GMM+T (1.49%).

The dry matter weight of the rice aboveground part ranged from 4.8–11.3 g pot^{-1} at the AT stage and from 10.5–24.9 g pot^{-1} at the PF stage (Fig. 2.5). The DMW at the AT stage was significantly lower in the treatments with GM incorporation than that in CF. The DMW at the PF stage was significantly higher in CF, GMM, and GMM+T than that in GMF and GMF+T.

Figure 2.6 shows the N uptake of the rice aboveground part (g pot^{-1}) at the AT and PF stages. The N uptake ranged from 0.070–0.199 g pot^{-1} at the AT stage and from 0.088–0.244 g pot^{-1} at the PF stage. The N uptake at the AT stage was significantly higher in CF, GMM, and GMM+T than GMF and GMF+T, while it was significantly higher in GMM and GMM+T than CF, GMF, and GMF+T at the PF stage.

Table 2.3 Nutrient content in the aboveground parts of rice

Treatments	TN (%)		P_2O_5 (%)		K_2O (%)	
	AT*	PF*	AT	PF	AT	PF
NF	1.46 b	0.84 bc	0.88 a	0.72 ab	3.23 a	2.46 a
CF	1.42 b	0.81 c	0.70 b	0.67 b	3.13 a	2.33 a
GMF	1.66 b	0.90 bc	0.83 ab	0.69 ab	3.13 a	2.28 a
GMF+T	1.79 ab	0.96 ab	0.84 ab	0.69 ab	3.13 a	2.29 a
GMM	2.18 a	1.07 a	0.82 ab	0.80 ab	2.20 b	1.74 b
GMM+T	2.12 a	0.97 ab	0.81 ab	0.82 a	2.35 b	1.49 b

Rice aboveground part was collected at the AT stage on July 20 (28 DAT) and PF stage on August 2 (42 DAT). Different letters indicate that mean values among treatments are significantly different ($p < 0.05$) according to the Tukey method. n=3

* AT and PF represent active tillering and panicle formation, respectively.

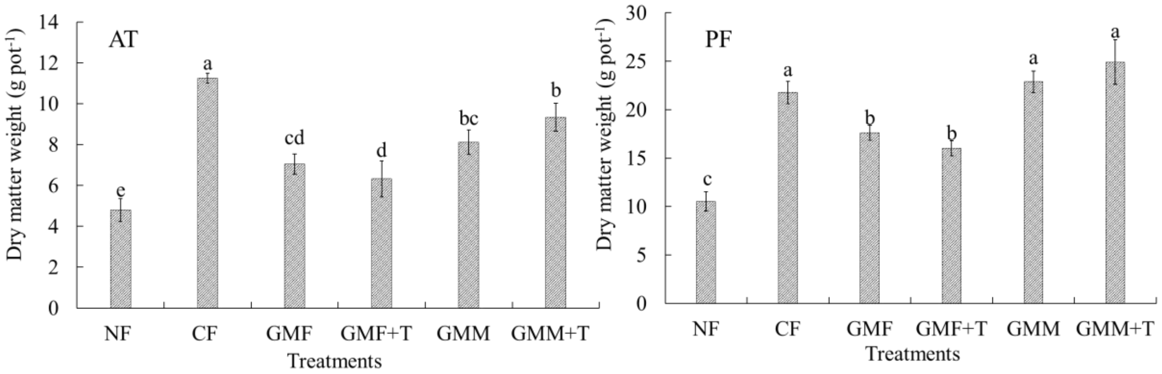

Figure 2.5 Dry matter weight of rice aboveground part at the active tillering stage (AT) and panicle formation stage (PF). Error bars represent SD (standard deviation). Different letters indicate that mean values among treatments are significantly different ($p < 0.05$) according to the Tukey method. (n=3)

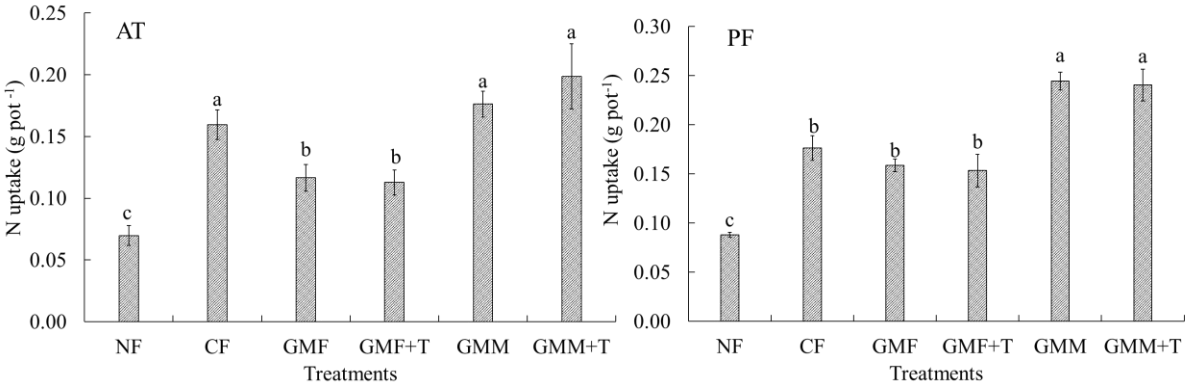

Figure 2.6 Nitrogen (N) uptake (g pot^{-1}) of rice aboveground part at the active tillering stage (AT) and panicle formation stage (PF). Error Bars represent SD (standard deviation). Different letters indicate that mean values among treatments are significantly different ($p < 0.05$) according to the Tukey method. (n=3)

2.3.6 Nitrogen use efficiency of rice aboveground part

Table 2.4 lists the NUE and mineralized NUE of the rice aboveground part. The NUE was only 4–6% in GMF and GMF+T, which was markedly lower than that in CF: 56% at the AT and 55% at the PF. The NUE in GMM and GMM+T, which ranged from 14–21%, were significantly higher than those in GMF and GMF+T. The mineralized NUE in GMM and GMM+T ranged from 28–36%, which was higher than that in GMF and GMF+T, where it ranged from 21–25%. The mineralized NUE in the treatments where GM was incorporated was still markedly lower than that in CF.

Table 2.4 Nitrogen use efficiency (NUE) and mineralized NUE of rice aboveground part

Treatments	N from basal application* (g pot^{-1})	N uptake (g pot^{-1})		NUE%		Mineralized N from CF/GM*** (g pot^{-1})		Mineralized NUE%	
		AT**	PF**	AT	PF	AT	PF	AT	PF
NF	–	0.07 c	0.09 c	–	–	–	–	–	–
CF	0.16	0.16 a	0.18 b	56 a	55 a	0.16	0.16	56 a	55 a
GMF	1.14	0.12 b	0.16 b	4 c	6 c	0.20	0.28	23 c	25 bc
GMF+T	1.17	0.11 b	0.15 b	4 c	5 c	0.21	0.30	21 c	22 c
GMM	0.74	0.18 a	0.24 a	14 b	21 b	0.38	0.47	28 bc	33 b
GMM+T	0.74	0.20 a	0.24 a	18 b	20 b	0.36	0.48	36 b	32 b

Rice aboveground part was collected at the AT stage on July 20 (28 DAT) and PF stage on August 2 (42 DAT). Different letters indicate that mean values among treatments are significantly different ($p < 0.05$) according to the Tukey method. n=3

*Topdressings were applied after collecting rice aboveground part at the PF stage. Thus, only basal application was included in the calculation of NUE at the AT and PF stages.

** AT and PF represent active tillering and panicle formation, respectively.

*** Mineralized N from GM was calculated using mineralized N at four weeks (AT) and seven weeks (PF) estimated through incubation tests (Fig. 2.1).

2.4 Discussion

2.4.1 Physiochemical properties of green manure

Ishikawa (1988) reported that the moisture content in the shoot of CMV decreased at the pod formation stage, resulting in the decrease of fresh weight. Our result similarly showed a decrease in the moisture content in the shoot at the maturity stage, whereas the moisture content in the root was higher at the maturity stage (Table 2.1). The DMW of shoot was about 4–5 times at the flowering stage and 3–4 times at the maturity stage as large as the previous report (Asagi and Ueno, 2009; Sullivan, 2003). Ishikawa (1988) showed that the shoot dry weight of the CMV was the largest at the latest growth stages: maturity and pod formation. In this study, however, the DMW of the shoot and the root at the maturity stage were smaller than those at the flowering stage. The reason for these results was not clear.

The TN in the shoot at the flowering stage (2.3%, Table 2.1) was lower than that in the CMV: 3.73% and 3.32% (Ishikawa, 1988; Kawase and Kitazima, 1994). The lower TN observed in the CC cultivated in this study is probably because of the low nutrient supply from the soil. The TN in the shoot of the CC was higher (2.88%) than that in the shoot of CMV (2.48%) when both GMs were cultivated in the same soil (Asagi and Ueno, 2009).

The TN in the shoot lowered at the maturity stage (later growth stage), which followed the reports about the CMV (Ishikawa, 1988). As the CMV matures, its crude protein decreases, whereas the soluble carbohydrates and crude cellulose increase (Ishikawa, 1988). This results in a higher C/N ratio at a later growth stage: 15.4 at pod formation stage (Ishikawa, 1988). This study also observed a higher C/N ratio at the maturity stage (21.4) than that at the flowering stage (16.8) (not significantly different). The P_2O_5 and K_2O contents in the shoot at the flowering stage were 0.51% and 1.32%, respectively, which were markedly lower than 0.94% for P_2O_5, and 3.49% for K_2O in the CMV (Kawase and Kitazima, 1994). The P_2O_5 and K_2O in the shoot at the maturity stage lowered as observed in TN.

For the root parts, TN (1.5%), P_2O_5 (0.36%), and K_2O (0.82%) at the flowering stage were markedly lower than 3.00% for TN, 1.00% for P_2O_5, and 2.34% for K_2O in the CMV (Kawase and Kitazima, 1994). The C/N ratio of 16.9 was higher than 14.0 in the CMV (Kawase and Kitazima, 1994). The TN, P_2O_5, and K_2O in the root tended to decrease at the maturity stage, as observed in the shoot.

As mentioned above, the DMW and TN, P_2O_5, and K_2O in the shoot and the root tended to be larger at the flowering stage. This resulted in larger N, P_2O_5, and K_2O application rates from GM in the treatments of GMF and GMF+T (Table 2.2).

2.4.2 Mineralized nitrogen from the incorporation of green manure

The N mineralized from GM at 13 weeks was 97 mg kg^{-1} at the flowering stage (Fig. 2.1), which accounted for 34% of TN from GM added in the soil (286 mg kg^{-1}). It was 126 mg kg^{-1} at the maturity stage (Fig. 2.1), which accounted for 71% of TN from GM (178 mg kg^{-1}). This difference in the ratios of mineralized N to TN was probably because N mineralized from GM incorporated at the flowering stage was lost due to denitrification. Aulakh et al. (2000) reported that the denitrification loss from GM was 3.7 mg kg^{-1} for 16 days under the upland condition at 35 °C, when the N application rate of *Sesbania* (C/N ratio: 14) was 100 mg kg^{-1}. Moreover, Aulakh et al. (2000) revealed that the rate of denitrification increased instantly under flooded condition; the rate showed a peak value in two days after flooded incubation started, which was about ten times as the peak rate under upland condition in the GM incorporation.

Azuma et al. (2017) reported through the incubation tests that the ratios of the mineralized N to TN from HV (approximate C/N ratio: 16 for stem, 8 for leaf) under the flooded condition for four weeks at 30 °C decreased from 64% to less than 30%, when the upland periods before the flooded incubation increased from one to four weeks. This result indicates that denitrification loss increases as the upland period becomes longer.

In this study, the upland period at the flowering stage was 45 days, which was longer than that at the maturity stage (21 days). In addition, mineralizable fraction of GM is likely to be high at the flowering stage because of its lower C/N ratio (Table 2.1). Thus, GM incorporated at the flowering stage, which was mineralized more than that at the maturity stage, probably involved more denitrification loss. Moreover, a small amount of N mineralized from GM at 0 week at the flowering stage indicates that denitrification loss mostly occurred during the upland period (Fig. 2.1).

Green manure incorporated at the maturity stage (C/N ratio: 21.4 for shoot, 18.3 for root) also might have involved denitrification loss, considering the experimental result that timothy grass, which has similar C/N ratio of 20.6, increased mineralized N rapidly in four days after the upland incubation started (Hirose, 1973). On the other hand, maturity-stage GM, which increases hardly decomposable carbohydrate, might have reduced N mineralized during the flooded period (Ishikawa, 1988). Nagumo et al. (2014) reported that N mineralized from maturity-stage Chinese milk vetch (C/N ratio: 17.1) in 168 days was 36% of the total N added under the flooded incubation at 25 °C.

2.4.3 Rice growth

The plant length before the AT stage (7, 14, and 28 DAT) was significantly higher in CF than in the treatments with GM incorporation (Fig. 2.2). The basal application rate of N in CF was 0.16 g pot^{-1} (Table 2.2), while N mineralized from GM at four weeks was 51.4 mg kg^{-1} (0.18 g pot^{-1}) at the flowering stage and 70.9 mg kg^{-1} (0.25 g pot^{-1}) at the maturity stage (Fig. 2.1). A higher amount of mineralized N was expected to be available in the treatments with GM incorporation. Thus, smaller plant length in the treatments indicates that GM incorporated in the soil produced organic acid under the flooded condition, which retarded rice growth (Ishikawa, 1988). Ishikawa (1963) reported that incorporation of the CMV (N application rate: 1 g pot^{-1}), which involved upland periods of 5 and 10 days, lowered the plant length at the AT stage. Then, the plant length became similar with the treatment of ammonium sulfate application after the AT stage (Ishikawa, 1963). This study also showed that plant length after the AT stage was not significantly different between CF and treatments involving GM incorporation. The plant length was significantly higher in CF after heading (63 to 84 DAT). The possible causes of the higher plant length in CF were topdressings applied at 43 DAT and 50 DAT, and a smaller tiller number compared with that in GMM and GMM+T, which promotes the elongation of tillers.

Tiller numbers before the AT stage were significantly higher in CF than that in the treatments with GM incorporation (Fig. 2.3). This result indicates that tillering was inhibited by GM incorporation as reported by Ishikawa (1963), considering a larger amount of N mineralized from GM at four weeks. Tiller numbers in GMM and GMM+T were generally over 30 after 35 DAT until maturity stage, because a large amount of mineral N was available during this period. The N mineralized from GM incorporated at the maturity stage reached 126.1 mg kg^{-1} (0.44 g pot^{-1}) at 13 weeks (Fig. 2.1), which was about two times the TN application in CF (0.24 g pot^{-1}). On the other hand, the amount of N mineralized from GM incorporated at the flowering stage was 97.1 mg kg^{-1} (0.34 g pot^{-1}) at 13 weeks (Fig. 2.1), which seemed to be insufficient to recover the tiller number inhibited in GMF and GMF+T. The decline in the tiller number in CF after 42 DAT indicates that the mineral N supply during the late growth stage did not meet the amount required to maintain the tiller number around 30 pot^{-1}.

The SPAD readings were significantly higher in GMM and GMM+T between 28 and 49 DAT than that in other treatments (except for GMF+T at 49 DAT). This was because N mineralized from GM incorporated at the maturity stage was higher than that from GM incorporated at the flowering stage and the mineral N supply from the basal application in CF (Fig. 2.1). A larger mineral N supply until the late growth stage probably resulted in higher SPAD readings before the beginning of ripening (70 DAT) in GMM and GMM+T (Fig. 2.1).

2.4.4 Nutrient contents, dry matter weight, and nitrogen uptake

The TN contents in the rice aboveground part tended to be higher in GMM and GMM+T than that in other treatments at both AT and PF stages (Table 2.3). This was because a large amount of mineral N was available in GMM and GMM+T (Fig. 2.1). The P_2O_5 contents in the treatments with GM incorporation (except for GMM+T at PF stage) were not significantly different from that in CF. The basal application rate of P_2O_5 in CF was 0.16 g pot^{-1}, while P_2O_5 applied from GM was 0.26 g pot^{-1} at the flowering stage and 0.15 g pot^{-1} at the maturity stage (Table 2.2). Thus, the P_2O_5 available for the rice in the treatments with GM incorporation was probably comparable to that available for the CF treatment. Although P_2O_5 uptake at the PF stage was significantly larger in GMM and GMM+T than CF, GMF, and GMF+T (Table 2.5), this result was due to difference in DMW (Fig. 2.5). The K_2O contents in GMM and GMM+T were lower than that in CF. This resulted in smaller K_2O uptakes for the rice aboveground part, which were 0.18–0.22 g pot^{-1} at the AT stage and 0.37–0.40 g pot^{-1} at the PF stage, compared with CF: 0.35 g pot^{-1} at AT and 0.51 g pot^{-1} at PF stage (Table 2.5). The amount of K_2O supply from GM was 0.64–0.66 g pot^{-1} at the flowering stage and 0.32 g pot^{-1} at the maturity stage, whereas that in CF was 0.16 g pot^{-1} from the basal application. Although K_2O supply from GM seems to be larger than that in CF, the supply only depends on the soil without fertilizer application. The K_2O content in CF was supplied from the basal application as well as the soil. Thus, the K_2O uptake can be inhibited in GMM and GMM+T due to smaller amount of K_2O supply compared with that in CF.

The DMW at the AT stage was significantly lower in the treatments with GM incorporation than CF (Fig. 2.5). This result reflected a smaller plant length and reduced the tiller number at 28 DAT in GMF, GMF+T, GMM, and GMM+T (Fig. 2.2, Fig. 2.3). The DMW at the AT stage were significantly correlated with plant length (r=0.694, p<0.01), tiller number (r=0.880, p<0.01), and the product of plant length and tiller number (r=0.924, p<0.01) at 28 DAT. Here, the product of plant length and tiller number is an indicator conventionally used to estimate the DMW of rice (Kumagai et al., 1991). The DMW at the PF stage was significantly larger in GMM and GMM+T than that in GMF and GMF+T, because the tiller numbers at 42 DAT in GMM and GMM+T were significantly larger than those in GMF and GMF+T. The DMW at the PF stage were significantly correlated with tiller number (r=0.943, p<0.01), and the product of plant length and tiller number (r=0.959, p<0.01) at 42 DAT. The DMW at the HA stage was not measured because samples were damaged by wild boars. On the other hand, the product of plant length (cm) and tiller number (tillers pot^{-1}) at 84 DAT was significantly lager (p<0.01) in GMM+T (2312 cm· tillers pot^{-1}) than that in GMF (1623 cm· tillers pot^{-1}) and GMF+T (1769 cm· tillers pot^{-1}). This result indicates that the DMW excluding head part in GMM+T are expected to be larger than that in GMF and GMF+T.

The N uptake of the rice aboveground part was significantly larger in GMM and GMM+T than that in GMF and GMF+T at the AT and PF stages (Fig. 2.6). This is because TN contents and DMW in GMM and GMM+T tended to be high or are significantly higher than those in GMF and GMF+T (Table 2.3, Fig. 2.5). Because the N uptake at the HA stage was not measured, we compared the products of plant length, tiller number, and SPAD readings, which can be an indicator of N uptake excluding head part (Araki et al., 2006). Although the products in GMM (47446 g·cm) and GMM+T (45631 g·cm) showed larger values compared with GMF (29224 g·cm) and GMF+T (38457 g·cm) at 84 DAT, their differences were not significant (p<0.05).

2.4.5 Nitrogen use efficiency of rice aboveground part

Higher values of NUE in GMM and GMM+T (14–21%) were because more N mineralized under smaller amount of N applied from GM resulted in larger N uptake compared with the case of GMF and GMF+T (4–6%) (Table 2.2, Figs. 2.1 and 2.6). Meanwhile, NUE in the treatments with GM incorporation were markedly lower than those in CF (Table 2.4). This was probably because large amount of N applied from GM was not efficiently used for rice growth. From the results of the incubation tests, 66% and 29% of TN applied from GM at the flowering and maturity stages, respectively, were assumed to be not available during the rice growth, because N mineralized from GM was lost due to denitrification or N was not mineralized. Asagi and Ueno (2009) reported 33.3% of NUE, when the air-dried CC was incorporated one day before transplanting rice. It is difficult to compare the NUE in this study with the result by Asagi and Ueno (2009) due to different growth stages. However, the NUE in GMF (4–6%) and GMF+T (4–5%) at the AT and PF stages might be markedly lowered by denitrification loss under the long upland period (45 days).

Mineralized NUE was calculated based on N mineralized during the rice growth, which didn't include unavailable fraction of N mentioned above. However, mineralized NUE were 21–25% in GMF and GMF+T and 28–36% in GMM and GMM+T, which were still lower than those in CF. The estimates of mineralized N from GM at the flowering stage were 0.18 g pot^{-1} and 0.22 g pot^{-1} at the AT and PF stages, respectively. For GM at the maturity stage, the estimates of mineralized N were 0.25 g pot^{-1} and 0.31 g pot^{-1} at the AT and PF stages, respectively. These amounts of mineralized N, which were larger than basal N application from CF (0.16 g pot^{-1}), was not efficiently absorbed by rice plant. This can be partly because rice growth was retarded at the early growth stage due to organic acid produced by GM incorporation as suggested from plant length and tiller number (Figs. 2.2 and 2.3). Moreover, mineralized N might be overestimated because incubation tests don't involve leaching loss as the upland condition in pot experiment.

2.5 Conclusions

The objectives of this chapter were to clarify physicochemical properties and N mineralization of maturity-stage CC and the effects of the GM on the growth and NUE of rice cultivated in a Wagner pot, through the comparison with flowering-stage GM. The following conclusions were drawn:

1. The dry matter weight, TN, P_2O_5, and K_2O of GM lowered, while the C/N ratio increased as GM matured.
2. The N mineralized from GM at 13 weeks' incubation under flooded condition was 34% (97 mg kg^{-1}) of TN supplied from GM at the flowering stage, while it was 71% (126 mg kg^{-1}) of TN supplied from GM at the maturity stage.
3. Large amounts of mineral N available in GMM and GMM+T resulted in larger values of tiller number after the AT stage, DMW at the PF stage, TN in rice aboveground part at the AT stage, and N uptake of rice at the AT and PF stages compared with GMF and GMF+T.
4. Nitrogen use efficiency at the AT and PF stages were higher in GMM and GMM+T (14–21%) than GMF and GMF+T (4–6%), while the values were markedly lower than those in CF (55–56%).

Chapter 3

Evaluation of Rice Growth, Nitrogen Use Efficiency, and Yield under the Incorporation of Flowering- and Maturity-Stage Green Manure at the Same Time

3.1 Introduction

Legume green manure (GM), which involves biological N fixation, is organic matter that can replace chemical fertilizers (CFs) and improve soil fertility. In rice production, GM cultivation used for basal application has rapidly declined because CFs are widely used in agricultural production (Yasue, 1991). However, the reliance of agriculture on CFs has recently suffered from the high cost of CFs and the deterioration of soil fertility (Gao et al., 2006; Liu et al., 2010; Miao et al., 2011; World Bank, 2019). In addition, agricultural practices with low input of energy and fertilizer resources are becoming important considering the limited resources and effects on the environment worldwide. In this context, the use of GM has been renewed as a sustainable practice in agriculture.

There have been many studies on the use of GM in rice production. Kawase and Kitazima (1994) reported that using Chinese milk vetch (CMV) as a basal application with a topdressing resulted in grain yield equivalent to conventional cultivation with CF. Asai et al. (2013) showed that the basal application of CMV alone did not cause a large yield reduction in an early rice variety compared with cultivation using CF. Azuma et al. (2017) reported that grain yield was greater than cultivation with CF when hairy vetch (HV) was used as a basal application with two topdressings. Appropriate incorporation rates and timings have been reported for CMV and HV to maximize yield and avoid adverse effects on growth because of the decomposition of carbohydrates (Ishikawa, 1988; Azuma et al., 2017). Chen (1988) and Ishikawa (1988) reported that the consecutive incorporation of CMV improved soil organic matter in rice fields.

On the other hand, nitrogen use efficiency (NUE) of rice plants is assumed to be low in rice production with GM application. Asagi and Ueno (2009) reported that the NUE of rice plants was 43.5% (white clover), 25.7% (CMV), 55.6% (HV), and 33.3% (crimson clover, hereafter CC) when GMs collected at the flowering stage were incorporated 1 day before transplanting rice. The NUE can be lowered further when GM incorporation requires the reduction of excess N input, which can cause lodging and disease, by increasing the upland period before transplanting rice. Mineralized N from GM decreases when the upland period after incorporation of GM increases (Kawase and Kitazima, 1994; Azuma et al., 2017). Ko et al. (2020a) reported that NUE of rice plants was 4% at the active tillering (AT) stage and 6% at the panicle formation (PF) stage when the treatment with CC incorporation involved a 45-days period of the upland condition. The low NUE indicated that N supplied from GM is not efficiently used in rice production and can cause environmental impacts through leaching.

Currently, the use of GM in rice production is limited to the basal application at the flowering stage. The flowering-stage GM rapidly mineralizes N from the early growth stage of rice (Kawase and Kitazima, 1994), which might not be synchronized with the N demand of rice. In addition, the N supply from GM can be excessive because GM growth varies depending on weather conditions (Japan Soil Association, 2012). This can lead to low NUE in rice production with GM application. Meanwhile, supplying N mineralized from GM during the late growth stage of rice, instead of being excessively supplied at the early growth stage, can improve NUE and substitute for topdressings.

Maturity-stage GM can supply mineralized N until the late growth stage of rice because of its higher C/N ratio compared with that of the flowering-stage GM (Ishikawa, 1988; Yasue, 1991; Nagumo et al., 2014). On the other hand, few studies have focused on the use of maturity-stage

GM in rice production. Nagumo et al. (2014) found that maturity-stage CMV reduced the mineralized N supply at the early growth stage of rice, resulting in a reduction in panicle number. Moreover, Nagumo et al. (2014) suggested that mineralized N supplied from maturity-stage CMV did not replace topdressing, although continuously mineralized N supply was estimated to occur until the late growth stage of rice. Ko et al. (2020a) reported that NUE of rice plants cultivated with maturity-stage GM (CC) was higher than that with flowering-stage GM. However, the result was mainly affected by different upland periods before transplanting rice rather than differences in physicochemical properties between flowering- and maturity-stage GM. Thus, the objective of this chapter was to evaluate rice growth, NUE, yield, and quality when flowering- and maturity-stage GM were incorporated at the same time. This study examined the feasibility of applying maturity-stage GM to rice production.

3.2 Materials and Methods

3.2.1 Cultivation of green manure and rice

A cultivation experiment was conducted using 1/5,000a Wagner pots from October 2018 to September 2019 at the Kyushu University farm in Fukuoka Prefecture, Japan. This experiment was conducted following a cultivation experiment in the first year explained in chapter 2. The experiment in the second year included six treatments of fertilizer and GM application: NF, CF, incorporation of flowering-stage GM without (GMF) or with topdressing (GMF+T), and incorporation of maturity-stage GM without (GMM) or with topdressing (GMM+T). We included the treatments of GM with topdressings (GMF+T, GMM+T) because only the basal application of GM was expected to suffer a shortage of N supply during the late growth stage (Nagumo et al., 2014). The six treatments were laid out in a randomized complete block design with three replications. Each treatment was prepared for three growth stages of rice: AT, PF, and harvest (HA).

In the first year, pots were filled with 3.5 kg of paddy soil (dry weight basis) collected from the Kyushu University Farm, which was passed through a 10 mm sieve. Before the cultivation experiment, the soil type, pH, CEC, TN, TP, and exchangeable potassium (K) were clay loam, 5.8, 11.17 cmol kg^{-1}, 0.18%, 0.16%, and 0.32 cmol kg^{-1}, respectively. After the first year of the experiment, the average values of pH, TN, and TP were 6.1, 0.15%, and 0.10%, respectively. Crimson clover (*Trifolium incarnatum* L.) was sown in pots involving a GM application (GMF, GMF+T, GMM, and GMM+T) at a seed rate of 0.06 g pot^{-1} (3 g m^{-2}) on November 13, 2018. Eight GM plants were maintained in each pot after complete germination. Liquid fertilizer (HYPONeX, HYPONeX Japan Corp., Ltd.) was applied on February 4, 2019, in all pots of the six treatments because the leaves of CC plants showed a symptom of phosphate deficiency. The application rates of N, P_2O_5, and K_2O were 0.06 g pot^{-1} (3 g m^{-2}), 0.1 g pot^{-1} (5 g m^{-2}), and 0.05 g pot^{-1} (2.5 g m^{-2}), respectively. The moisture condition of the soil in all pots was adjusted between 30% and 60% of the maximum water-holding capacity during cultivation, leaving the drain outlet of the Wagner pots open.

In the first year, GM was incorporated at the flowering stage on April 29, 2018, for treatments GMF and GMF+T and at the maturity stage on May 23 for treatments GMM and GMM+T. The different incorporation dates, which resulted in different upland periods before flooding between GM incorporation at the flowering and maturity stages, mainly affected the growth and NUE of rice (Ko et al., 2020a). Thus, in the second year, we incorporated flowering- and maturity-stage GM on June 8, 2019 (10 days before flooding) to evaluate the differences in their effects on rice production. The flowering- and maturity-stage GM in each cultivation pot were collected on April 19 and June 1, respectively. Shoots of CC plants were cut at the soil surface, and roots were collected by carefully removing soil. The soil from the same treatment, including NF and CF treatments, was mixed well and divided into equal weights to fill each of the three replications on June 1.

The shoots and roots were then air-dried and kept at room temperature. Among shoots and roots collected from each pot, 10% of their total weight, which was mixed in the same treatment, was used for incubation tests and measurement of chemical properties of GM. The rest of the shoots or roots (90% of total weight) were separately mixed in the same treatment and cut into approximately 1 cm pieces. Each of the mixed shoots and roots was divided into three equal weights. Then, each equal division of shoots and roots was mixed with soil in each of the three pots on June 8. For other treatments, soil in each pot was mixed well.

In the CF treatment, basal fertilizer was mixed well with soil on June 18. The application rates of N, P_2O_5, and K_2O were 0.16 g pot^{-1} (8 g m^{-2}), 0.16 g pot^{-1} (8 g m^{-2}), and 0.16 g pot^{-1} (8 g m^{-2}), respectively. For other treatments, soil in each pot was mixed well. Then, all the pots were flooded with tap water and the soil was mixed with water on June 18. Three 22-day-old seedlings of the rice Genki Tsukushi (*Oryza sativa* L.) were transplanted into each pot on June 21. For CF, GMF+T, and GMM+T treatments, topdressing was applied twice on August 3 and August 14, and each had N and K_2O application rates of 0.04 g pot^{-1} (2 g m^{-2}). Water was maintained at a level of approximately 3–5 cm above the soil surface during the rice growth period. An insecticide for rice stem borers was applied on July 31 and an insecticide for planthoppers was applied on August 16 and September 3. In addition, a fungicide for rice blast was applied on August 30.

3.2.2 Measurement of the physicochemical properties of green manure

The physiochemical properties of GM were measured for shoots and roots collected separately from the same treatment. The shoots and roots parts oven-dried at 70 °C for 48 h and ground to a fine powder using a sample mill (Cyclotec 1093, Foss, Hilleroed, Denmark). The total carbon (TC) was measured using a CHN coder (MT-5, Yanaco, Tokyo, Japan) with two replications. For the measurement of TN, phosphorus (P_2O_5), and potassium (K_2O), the ground shoots and roots were separately digested by the H_2SO_4-H_2O_2 Kjeldahl digestion method with three replications (Ohyama et al., 1991). TN and P_2O_5 were measured using the indophenol method (Cataldo et al., 1974) and the ascorbic acid method (Murphy and Riley 1962), respectively. K_2O was measured using an atomic absorption spectrophotometer (Z-5300, Hitachi High-Technology Co., Tokyo, Japan) (Murphy and Riley, 1962). The fraction of N, which is easily mineralized in the shoots, was extracted from 0.5 g of ground powder with 5 mL of 0.1 M phosphate buffer saline (pH 6.8) plus 0.5 g of insoluble polyvinylpyrrolidone with three replications (Ohtake et al., 1994). The mixture was shaken with a rotary shaker for 30 min, followed by centrifugation at 2330 × g for 5 min. Then, 1 mL of the supernatant filtered through a 0.45 μm filter was digested by the H_2SO_4-H_2O_2 Kjeldahl digestion method. Finally, the extracted fraction of N was measured using the indophenol method. We defined the fraction as extractable N.

3.2.3 Estimation of nitrogen mineralization from green manure

The N mineralized from GM during the rice growth period was estimated through incubation tests. Moist soil (approximately 120 g dry weight basis) was sampled from each pot on June 1. The soil samples from the NF, GMF, and GMM treatments were used for the incubation tests. The shoots and roots of GM, which were cut into approximately 1 cm pieces, were mixed well with moist soil (approximately 90 g dry weight basis) and transferred into a 500-mL glass bottle. Three bottles each were prepared for each of the three growth stages in the same treatment. The application rate of TN from GM was determined to be approximately the same as that in a pot where the treatment and growth stages were the same. The application rate of TN from the flowering-stage GM was 193 mg kg^{-1} (dry soil weight basis) at the AT stage, 227 mg kg^{-1} at the PF stage, and 226 mg kg^{-1} at the HA stage. The application rate of TN from maturity-stage GM was 231 mg kg^{-1} at the AT stage, 228 mg kg^{-1} at the PF stage, and 179 mg kg^{-1} at the HA stage.

The moisture condition of the soil was adjusted to 60% of the maximum water-holding capacity during incubation under upland conditions. The temperature during the incubation was 20 °C, which was approximately the average daily mean temperature at the experimental site during the upland period in this experiment (June 8 to 17). The incubation period was 10 days for both the flowering- and maturity-stage GM.

After incubation under the upland condition, the soil in each bottle was divided for three periods of incubation tests under flooded conditions at 30 °C: 1, 2, and 4 week(s) for the AT stage; 0, 5, and 7 weeks for the PF stage; and 9, 11, and 13 weeks for the HA stage. Approximately 30 g of soil (dry weight basis) was transferred from a glass bottle into a 30 mL polypropylene tube (62.543, Sarstedt, Nümbrecht, Germany) with distilled water. Then, the incubation test was conducted under flooded conditions and mineralized N was measured at each incubation weeks following the procedures described in section 2.4.2. Finally, N mineralized from the flowering- and maturity-stage GM was estimated using the difference in the mineralized N between the soil mixed with GM and the soil from the NF treatment.

3.2.4 Measurement of rice growth

Plant length and the number of tillers per pot were measured weekly from 7 DAT to 98 DAT. SPAD readings were measured weekly from 21 DAT using a chlorophyll meter (SPAD-502, Konica Minolta Holdings, Inc., Tokyo, Japan), following the procedures described in section 2.4.3.

3.2.5 Measurement of dry matter weight, NPK content, and nitrogen uptake of the aboveground parts of rice

The aboveground parts of rice in each pot were cut at the soil surface at the AT stage on July 19 (28 DAT), PF stage on August 2 (42 DAT), and HA stage on September 27 (98 DAT). Dry matter weight (DMW), NPK content, and N uptake were measured following the procedures described in section 2.4.4.

3.2.6 Measurement of yield components, yield, and protein content

Yield components, yield, and protein content of brown rice were measured using the samples collected at the HA stage. The samples were air-dried and the numbers of panicles and rough rice per pot were counted. The rough rice was then husked by an impeller husker (FC2K, Otake Seisakusyo Co., Ltd., Aichi, Japan), and brown rice was obtained. Next, the brown rice was sorted using a 1.85-mm sieve, and the number of sorted gains over the sieve was counted. A seed counter (WAVER IC-0, AIDEX Co., Ltd., Aichi, Japan) was used to count the number of rough rice and sorted grains. The sorted grains were then weighed and the moisture content was measured using a grain moisture tester (Riceter, Kett Electric Laboratory, Tokyo, Japan). From these measurement results, yield components including the number of rough rice per panicles, percentage of ripened grains, and 1000-grain weight were calculated. To measure the protein content, we oven-dried brown rice at 70 °C for 48 h, and the sample was then milled using a Cyclone Sample Mill. Each of the milled samples was digested by the H_2SO_4-H_2O_2 Kjeldahl digestion method. The TN content in brown rice was then measured using the indophenol method. The protein content was calculated by multiplying the TN content of brown rice by the conversion coefficient of protein (5.95) (Jones, 1931). Each value for 1000-grain weight, brown rice yield, and protein content was standardized to 15.0% moisture content.

3.2.7 Calculation of nitrogen use efficiency of the aboveground parts of rice

The NUE and mineralized NUE of the aboveground parts of rice were calculated using the following equations:

$$\text{NUE (\%)} = \frac{\text{N uptake in a GM or CF pot} - \text{N uptake in a non-fertilized pot}}{\text{TN applied from GM and CF}} \times 100 \qquad (3.1)$$

$$\text{Mineralized NUE (\%)} = \frac{\text{N uptake in a GM or CF pot} - \text{N uptake in a non-fertilized pot}}{\text{N mineralized from GM} + \text{TN applied from CF}} \times 100 \qquad (3.2)$$

Here, GM pots include GMF, GMF+T, GMM, and GMM+T. The values of N mineralized from GM in the denominator of Eq. (3.2) were calculated using mineralized N estimated through incubation tests.

3.2.8 Statistical analysis

The difference in the mean values was tested by the Tukey method at a 5% probability level using STATISTIX 8 (Analytical Software, Tallahassee, FL, USA). For the comparison of mean values among treatments that involved a missing value (GMM at PF), an analysis of variance was conducted with an estimated missing value by reducing the degrees of freedom of the total and residual variances by 1 (Okuno and Haga, 1969). Then, the difference in the mean values among treatments was tested using the Tukey method at a 5% probability level (Yamauchi, 2008). The analysis was performed using macros written in Microsoft Excel.

3.3 Results

3.3.1 Physiochemical properties of green manure

Table 3.1 shows the physiochemical properties of the flowering- (GMF and GMF+T, hereafter GMF(+T)) and maturity-stage GM (GMM and GMM+T, hereafter GMM(+T)) incorporated for cultivating rice at the AT, PF, and HA stages. The average values of DMW for shoots, which ranged from 24.9 to 29.0 g pot^{-1}, were not significantly different among treatments. The average values of DMW for the roots tended to be higher in the flowering-stage GM (GMF(+T)) than in the maturity-stage GM (GMM(+T)).

Some values of TC in shoots were greater than 40% in the maturity-stage GM and higher than the values in the flowering-stage GM, which ranged from 37.95% to 39.07%. However, the average values were not significantly different among treatments. The average values of TC in roots ranged from 24.64% to 27.98% and were not significantly different among treatments. The TN content in shoots ranged from 2.55% to 2.84% in flowering-stage GM, whereas the value involved larger variation in the maturity-stage GM (2.30% to 3.14%). The average values were not significantly different. The average values of TN in the roots tended to be higher in the flowering-stage GM than in the maturity-stage GM.

The extractable N in shoot was around 2%. The average values of P_2O_5 content in shoot ranged 0.46–0.54%, while the values in root were in lower range of 0.20–0.35%. The average values of K_2O content in shoot tended to be higher in flowering-stage GM (1.33% in GMF and 1.20% in GMF+T) than those in maturity-stage GM (0.60% in GMM and 0.82% in GMM+T). The average values of K_2O content in root were around 0.7%. The average values of C/N ratio in shoot, which ranged from 13.9–15.0, were not significantly different. The average values in root tended to be higher in maturity-stage GM than those in flowering-stage GM.

Table 3.1 Physicochemical properties of green manure

Plant parts	Treatment*	Growth stage	Dry matter weight (g pot^{-1})	TC (%)	TN (%)	Extractable N (%)	P_2O_5 (%)	K_2O (%)	C/N ratio
Shoot	GMF	AT	23.2	38.99	2.55	2.08	0.59	1.54	15.3
		PF	25.9	39.07	2.74	2.07	0.52	1.36	14.3
		HA	25.7	38.10	2.77	2.03	0.43	1.08	13.9
		Average	24.9a	38.72a	2.69a	2.06b	0.51ab	1.33a	14.5a
	GMF+T	AT	34.1	38.99	2.84	2.03	0.55	1.31	13.7
		PF	27.4	38.33	2.70	2.02	0.47	1.15	14.2
		HA	25.6	37.95	2.75	1.97	0.40	1.13	13.8
		Average	29.0a	38.42a	2.76a	2.01c	0.47ab	1.20ab	13.9a
	GMM	AT	25.5	41.45	3.03	2.05	0.56	0.98	13.7
		PF	27.7	41.10	2.72	2.01	0.45	0.42	15.1
		HA	26.3	37.18	2.30	2.01	0.36	0.41	16.1
		Average	26.5a	39.91a	2.68a	2.02bc	0.46b	0.60b	15.0a
	GMM+T	AT	29.8	40.63	2.78	2.14	0.58	0.64	14.6
		PF	32.6	38.72	3.14	2.13	0.54	0.91	12.3
		HA	21.5	39.39	2.49	2.06	0.50	0.90	15.8
		Average	28.0a	39.58a	2.80a	2.11a	0.54a	0.82ab	14.2a
Root	GMF	AT	6.1	29.88	1.35	–	0.33	0.77	22.1
		PF	6.4	25.95	1.33	–	0.35	0.64	19.5
		HA	5.8	28.12	1.51	–	0.33	0.73	18.6
		Average	6.1ab	27.98a	1.40ab	–	0.34a	0.71a	20.1ab
	GMF+T	AT	8.1	23.53	1.56	–	0.36	0.95	15.1
		PF	6.4	27.60	1.63	–	0.34	0.56	17.0
		HA	5.6	22.80	1.60	–	0.35	0.51	14.2
		Average	6.7a	24.64a	1.60a	–	0.35a	0.67a	15.4b
	GMM	AT	4.2	26.36	0.90	–	0.18	0.83	29.3
		PF	4.5	23.64	0.95	–	0.21	0.46	24.8
		HA	2.1	27.42	0.91	–	0.21	0.77	30.0
		Average	3.6b	25.81a	0.92c	–	0.20b	0.69a	28.0a
	GMM+T	AT	4.7	22.95	0.96	–	0.24	0.67	23.9
		PF	5.4	26.88	1.29	–	0.27	0.88	20.8
		HA	1.4	24.84	0.79	–	0.21	0.49	31.5
		Average	3.8b	24.89a	1.01bc	–	0.24b	0.68a	25.4a

AT, PF, and HA represent active tillering, panicle formation, and harvest stages, respectively. Different letters indicate that mean values among treatments are significantly different ($p < 0.05$) according to the Tukey method. n=3

*GM incorporated in GMF and GMF+T and in GMM and GMM+T was collected on April 19 and on June 1, respectively. Shoots or roots in the same treatment were mixed to analyze physicochemical properties at each growth stage.

3.3.2 N, P_2O_5, and K_2O application rates of chemical fertilizer and green manure

Basal N application rates from GM were mostly around 0.8 g pot^{-1}, which was five times as large as that in CF, although the values varied from 0.55 to 1.10 g pot^{-1} (Table 3.2). The total N application rates in GMF (0.79 g pot^{-1}) and GMF+T (0.87 g pot^{-1}) were 3.3 and 3.7 times larger than that in CF (0.24 g pot^{-1}), respectively. The rates in GMM(+T) (0.63 g pot^{-1}) were 2.5 times larger than that in CF. Basal P_2O_5 application rates from GM ranged from 0.10 to 0.22 g pot^{-1} and were similar to that in CF (0.16 g pot^{-1}). The total application rates of K_2O were larger in GMF, GMF+T, and GMM+T (0.28–0.40 g pot^{-1}) than that in CF (0.24 g pot^{-1}), whereas the rate in GMM (0.12 g pot^{-1}) was half of that in CF.

3.3.3 Nitrogen mineralized from green manure

Figure 3.1 shows the N mineralized from GM at 30 °C for incubation periods from 0 to 13 weeks. The N mineralization pattern was slightly different between the flowering- and maturity-stage GM during the period of 0–4 weeks. For the flowering-stage GM, mineralized N rapidly increased from a relatively large value of 17.6 mg kg^{-1} at 0 week to 63.0 mg kg^{-1} at 1 week, which was 33% of TN application from GM. The mineralized N continued to increase from 1 week to 7 weeks and slowly reached the maximum value of 156.6 mg kg^{-1} (69% of TN application) at 11 weeks. For the maturity-stage GM, the N mineralized at 0 week was only 4.4 mg kg^{-1}. The value rapidly increased from 0 to 1 week (43.1 mg kg^{-1}, 19% of TN application) and continued to increase from 1 to 4 weeks. The mineralized N rapidly increased from 4 to 5 weeks and slowly reached the maximum value of 158.2 mg kg^{-1} (88% of TN application) at 13 weeks.

Table 3.2 N, P_2O_5, and K_2O application rates of chemical fertilizer and green manure

Treatments	Green manure*			Chemical fertilizer**					Total***
				Basal			1st topdressing	2nd topdressing	
	AT	PF	HA	AT	PF	HA	HA	HA	
N application rates (g pot^{-1})									
NF	–	–	–	–	–	–	–	–	0
CF	–	–	–	0.16	0.16	0.16	0.04	0.04	0.24
GMF	0.67	0.79	0.79	–	–	–	–	–	0.79
GMF+T	1.10	0.84	0.79	–	–	–	0.04	0.04	0.87
GMM	0.81	0.80	0.63	–	–	–	–	–	0.63
GMM+T	0.87	1.09	0.55	–	–	–	0.04	0.04	0.63
P_2O_5 application rates (g pot^{-1})									
NF	–	–	–	–	–	–	–	–	0
CF	–	–	–	0.16	0.16	0.16	–	–	0.16
GMF	0.16	0.16	0.13	–	–	–	–	–	0.13
GMF+T	0.22	0.15	0.12	–	–	–	–	–	0.12
GMM	0.15	0.13	0.10	–	–	–	–	–	0.10
GMM+T	0.18	0.19	0.11	–	–	–	–	–	0.11
K_2O application rates (g pot^{-1})									
NF	–	–	–	–	–	–	–	–	0
CF	–	–	–	0.16	0.16	0.16	0.04	0.04	0.24
GMF	0.40	0.39	0.32	–	–	–	–	–	0.32
GMF+T	0.52	0.35	0.32	–	–	–	0.04	0.04	0.40
GMM	0.28	0.14	0.12	–	–	–	–	–	0.12
GMM+T	0.22	0.35	0.20	–	–	–	0.04	0.04	0.28

N, P_2O_5, and K_2O application rates of GM (green manure) included those from shoots and roots, which were calculated by the product of dry matter weight (DMW) and TN, P_2O_5, and K_2O (%) shown in Table 1. AT, PF, and HA represent active tillering, panicle formation, and harvest stages, respectively.

* GM was incorporated on June 8 (10 days before flooding).

** Basal fertilizer was applied on June 18. Topdressing was applied on August 3 (1st) and August 14 (2nd).

*** Total values were the average on three pots (*n*=3) at the HA stage.

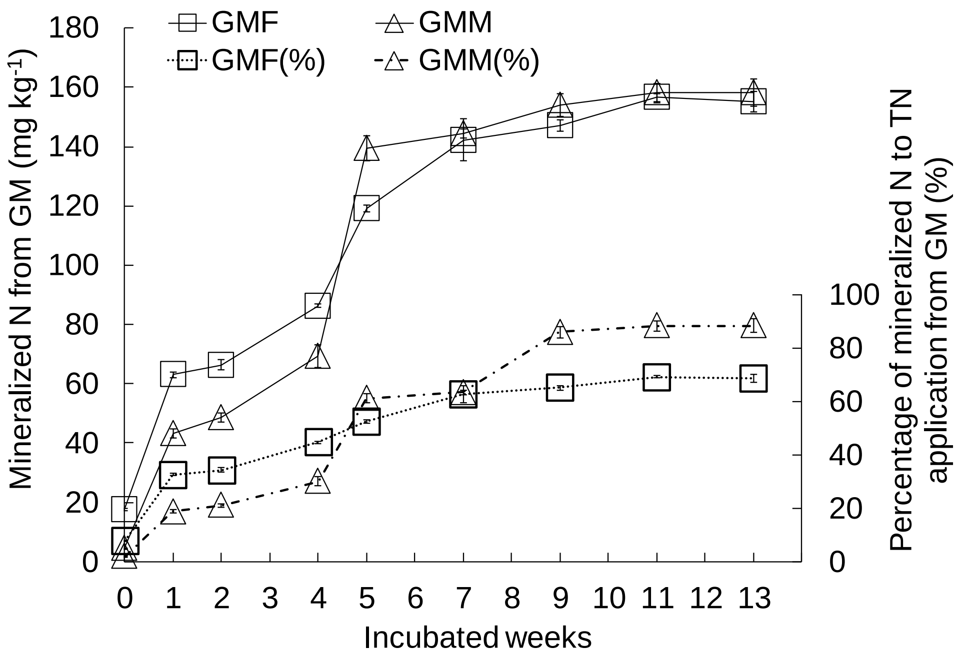

Figure 3.1 Nitrogen (N) mineralized from green manure (GM) estimated through incubation tests at 30 °C under flooded condition. The upland period before flooding was 10 days. N mineralized from flowering- and maturity-stage GM was estimated using the difference in the mineralized N between the soil mixed with GM and soil from the NF treatment. Error bars represent standard deviation. *n*=3

3.3.4 Rice growth

Plant length was significantly higher in CF from 7 to 21 DAT than in other treatments (Fig. 3.2). In the GM treatments, plant length during the period was not significantly different or significantly lower than that in NF, whereas it was significantly higher in GMF(+T) than in CF from 35 to 49 DAT (Fig. 3.2). Finally, plant length in GMF+T showed the largest value among the treatments and was significantly higher than that in GMM from 70 to 98 DAT.

Tiller number (tillers pot^{-1}) was significantly higher in CF than in other treatments before the AT stage (28 DAT) (Fig. 3.3). The tiller number reached peak values of 56 at 35 DAT in CF, approximately 55 at 42 DAT in GMF(+T), and greater than 40 at 42 DAT in GMM(+T). Then, the value sharply declined, and finally was approximately 30 in GMF(+T), 20 in CF, and approximately 18 in GMM(+T) at 98 DAT. Tiller numbers from 70 to 98 DAT were significantly larger in GMF(+T) than in CF and GMM(+T).

Differences in SPAD readings, except for NF, were mostly not significant among the treatments (Fig. 3.4). On the other hand, SPAD readings at 35 and 42 DAT were significantly higher in the treatments with GM than in CF, when SPAD readings in CF sharply declined from 41.5 at 28 DAT to 26.2 at 42 DAT.

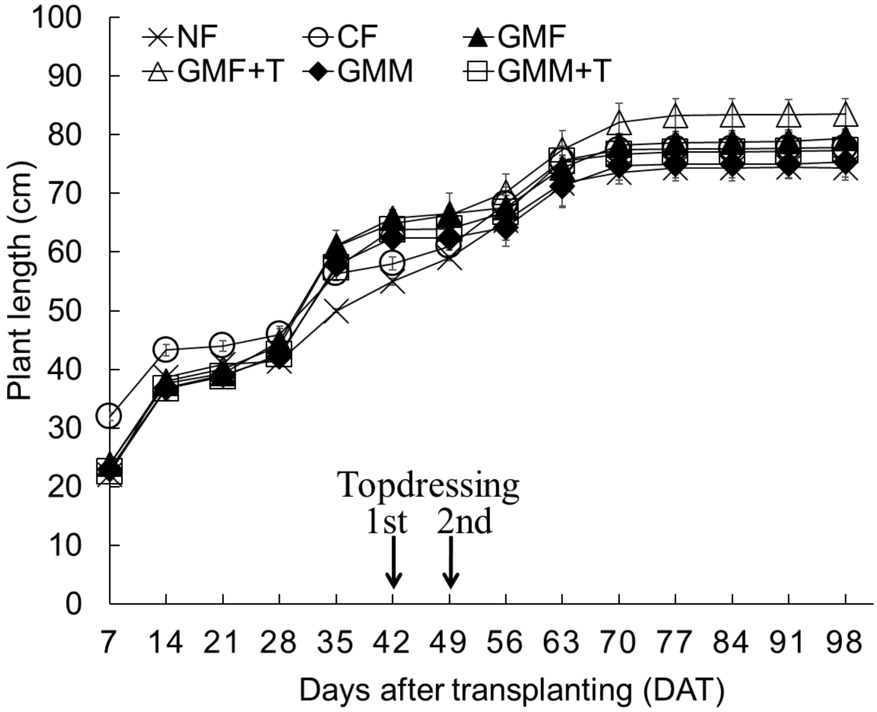

Figure 3.2 Plant length of rice. Error bars represent standard deviation. *n*=9 from 7 to 28 DAT, *n*=6 from 35 to 42 DAT except for GMM (*n*=5) where one replication was damaged by rice stem borers, *n*=3 from 49 to 98 DAT.

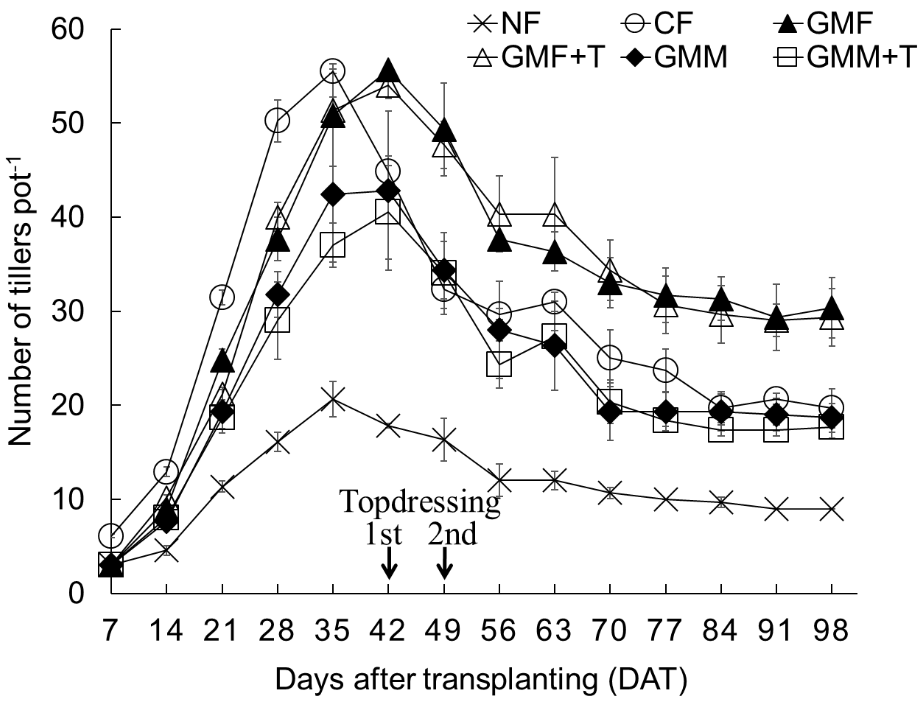

Figure 3.3 Number of tillers pot^{-1} of rice. Error bars represent standard deviation. *n*=9 from 7 to 28 DAT, *n*=6 from 35 to 42 DAT except for GMM (*n*=5) where one replication was damaged by rice stem borers, *n*=3 from 49 to 98 DAT.

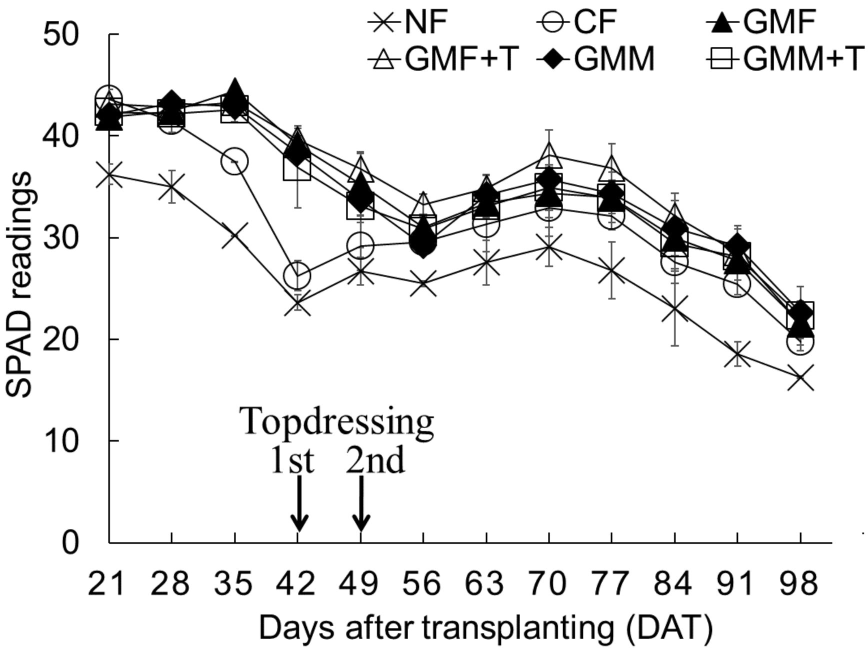

Figure 3.4 SPAD readings of rice. Error bars represent standard deviation. *n*=9 from 21 to 28 DAT, *n*=6 from 35 to 42 DAT except for GMM (*n*=5) where one replication was damaged by rice stem borers, *n*=3 from 49 to 98 DAT.

3.3.5 Nutrient content, dry matter weight, and nitrogen uptake

Table 3.3 lists the nutrient (TN, P_2O_5, and K_2O) contents in the aboveground parts of rice. The TN contents at the AT stage tended to be higher in GMF (2.99%) and GMF+T (3.14%) than those in GMM (2.88%) and GMM+T (2.80%), which were significantly higher than that in CF (1.83%). The TN content in the shoots declined toward the later growth stage; the ranges were from 0.89% to 1.63% at the PF stage and from 0.39% to 0.53% at the HA stage. The TN content in the shoots at the HA stage was significantly higher in the treatments with GM incorporation than in the CF. The P_2O_5 and K_2O contents in the shoots declined toward the later growth stage, as shown by the TN contents. The P_2O_5 content was significantly higher in the treatments with GM incorporation than in the CF at the AT and HA stages. The K_2O contents were significantly higher or tended to be higher in CF than in the treatments with GM incorporation. The TN and K_2O contents in panicle were not significantly different among the treatments, whereas the P_2O_5 content in the panicles tended to be higher in GMF(+T).

The DMW of the aboveground rice parts ranged 1.8–6.3 g pot^{-1} at the AT stage, 5.6–24.3 g pot^{-1} at the PF stage, and 19.2–82.7 g pot^{-1} at the HA stage (Fig. 3.5). The DMW at the AT stage was significantly greater in CF than in other treatments, except for GMF, whereas the value at PF was significantly greater in GMF(+T) than in CF. The DMW at the HA stage was significantly larger in GMF(+T) than that in CF and GMM(+T). The N uptake ranged 0.03–0.14 g pot^{-1} at the AT stage, 0.05–0.40 g pot^{-1} at the PF stage, and 0.11–0.55 g pot^{-1} at the HA stage (Table 3.4). The N uptake at the AT stage was significantly greater in GMF(+T) than in GMM(+T). The N uptake at the PF and HA stages was greater in GMF(+T), followed by GMM(+T) and CF.

Table 3.3 Nutrient content in the aboveground parts of rice

Treatments	TN (%)				P_2O_5 (%)				K_2O (%)			
	AT	PF	HA-shoot	HA-panicle	AT	PF	HA-shoot	HA-panicle	AT	PF	HA-shoot	HA-panicle
NF	1.75c	0.91d	0.39c	0.84a	1.05b	0.81a	0.35b	0.55c	3.41a	2.61a	1.17a	0.31a
CF	1.83c	0.89d	0.41c	0.84a	0.93c	0.74b	0.37b	0.61bc	3.28ab	2.32b	1.18a	0.30a
GMF	2.99ab	1.48b	0.48b	0.85a	1.09b	0.72bcd	0.43a	0.66ab	3.03b	1.50c	0.60b	0.27a
GMF+T	3.14a	1.63a	0.50b	0.87a	1.15a	0.71d	0.43a	0.68a	2.99b	1.47c	0.75b	0.32a
GMM	2.88b	1.32c	0.53a	0.86a	1.07b	0.71cd	0.46a	0.62b	2.36c	1.26d	0.67b	0.33a
GMM+T	2.80b	1.36bc	0.50b	0.86a	1.04b	0.74bc	0.45a	0.62ab	2.30c	1.12d	0.65b	0.30a

The aboveground parts of rice were collected at the AT stage on July 19 (28 DAT), PF stage on August 2 (42 DAT), and HA stage on September 27 (98 DAT). AT, PF, and HA represent active tillering, panicle formation, and harvest stages, respectively. Different letters indicate that mean values among treatments are significantly different ($p < 0.05$) according to the Tukey method. n=3 except for GMM at PF (n=2) where one replication was damaged by rice stem borers.

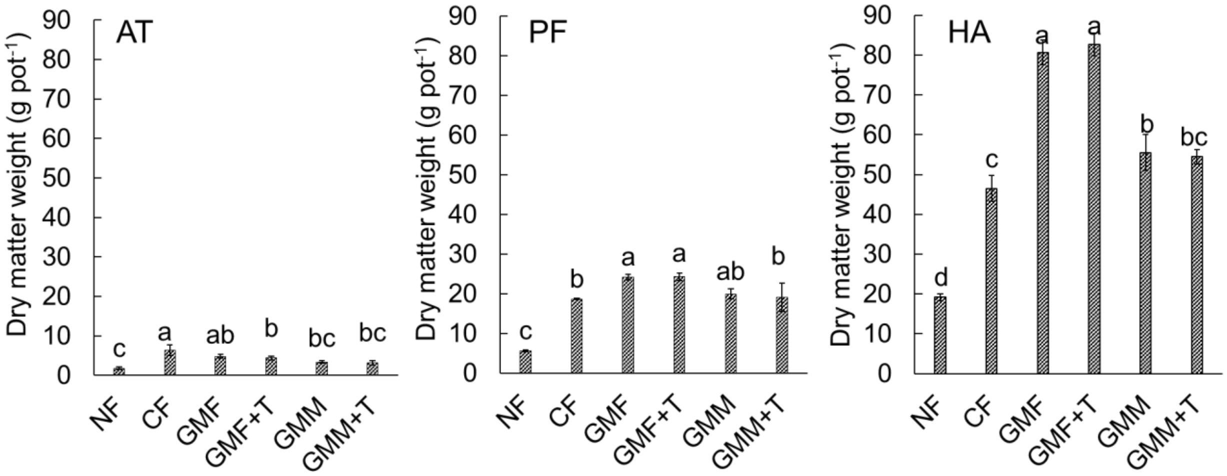

Figure 3.5 Dry matter weight of the aboveground parts of rice at the active tillering stage (AT), panicle formation stage (PF), and harvest stage (HA). Error bars represent standard deviation. Different letters indicate that mean values among treatments are significantly different ($p < 0.05$) according to the Tukey method. $n=3$ except for GMM at PF ($n=2$) where one replication was damaged by rice stem borers.

3.3.6 Nitrogen use efficiency of the aboveground parts of rice

Table 3.4 lists the NUE and mineralized NUE of the aboveground parts of rice. The NUE at AT was 52% in CF, whereas the value was markedly smaller in the GM treatments: GMF (17%), GMF+T (10%), GMM (8%), and GMM+T (7%). The NUE in the GM treatments gradually increased to approximately 50% in GMF(+T) and approximately 40% in GMM(+T) at the HA stage, but those values were significantly lower than 67% in CF. Compared with NUE, the mineralized NUE increased by more than 20% at the HA stage in GMF (76%) and GMF+T (72%), which was not significantly different from that in CF (67%). The mineralized NUE in GMM and GMM+T at the HA increased by 5% and 12%, respectively, compared with the NUE; however, these values were significantly smaller than those in CF and GMF(+T).

3.3.7 Yield components, yield, and protein content

Table 3.5 shows the yield components, yield, and protein content of the brown rice. The number of panicles was 29 in GMF and 28 in GMF+T, which were significantly higher than in GMM(+T) (18) and CF (16). The number of rough rice was 1645 in GMF+T and 1618 in GMF, which were significantly higher than in GMM (1029), GMM+T (1016), and CF (742). The number of rough rice per panicle ranged from 42–58, which tended to be higher in the GM treatments than that in the CF. The percentage of ripened grains was not significantly different among treatments. The 1000-grain weight was significantly greater in CF than in GMF. The brown rice yield was higher in GMF(+T), followed by GMM(+T) and CF. The protein content was significantly higher in the GM treatments (7.5–7.7%) than in CF (7.0%).

Table 3.4 Nitrogen use efficiency (NUE) of the aboveground parts of rice

Treatments	N application (g pot^{-1})*			N uptake (g pot^{-1})			NUE%			Mineralized N from CF/GM (g pot^{-1})**			Mineralized NUE%		
	AT	PF	HA	AT	PF	HA	AT	PF	HA	AT	PF	HA	AT*	PF*	HA
NF	–	–	–	0.03c	0.05d	0.11d	–	–	–	–	–	–	–	–	–
CF	0.16	0.16	0.24	0.11ab	0.16c	0.27c	52a	72a	67a	0.16	0.16	0.24	52a	72a	67a
GMF	0.67	0.79	0.79	0.14a	0.35a	0.52a	17b	38b	52b	0.30	0.50	0.54	37ab	61a	76a
GMF+T	1.10	0.84	0.87	0.14a	0.40a	0.55a	10b	41b	46bc	0.49	0.52	0.62	23bc	66a	72a
GMM	0.81	0.80	0.63	0.10b	0.27b	0.37b	8b	26c	43bc	0.24	0.51	0.55	27bc	42b	48b
GMM+T	0.87	1.09	0.63	0.09b	0.26b	0.36b	7b	19c	38c	0.29	0.61	0.50	21c	34b	50b

The aboveground parts of rice were collected at the AT stage on July 19 (28 DAT), PF stage on August 2 (42 DAT), and HA stage on September 27 (98 DAT). AT, PF, and HA represent active tillering, panicle formation, and harvest stages, respectively. Different letters indicate that mean values among treatments are significantly different ($p < 0.05$) according to the Tukey method. $n=3$ except for GMM at PF ($n=2$) where one replication was damaged by rice stem borers.

*Topdressings were applied after collecting the aboveground parts of rice at the PF stage. Thus, only basal application was included in the calculation of NUE at the AT and PF stages.

** Mineralized N from GM was calculated using mineralized N at four weeks (AT), seven weeks (PF), and thirteen weeks (HA) estimated through the incubation tests (Fig. 3.1).

Table 3.5 Yield, yield components and protein content

Treatments	No. of panicles pot^{-1}	No. of rough rice pot^{-1}	No. of rough rice panicle^{-1}	Percentage of ripened grains (%)	1000-grain weight (g)*	brown rice yield (g pot^{-1})*	protein content*
NF	8 c	334 d	42 b	79 a	20.6 ab	5.4 d	7.4 ab
CF	16 b	742 c	47 ab	85 a	21.4 a	13.5 c	7.0 b
GMF	29 a	1618 a	55 ab	80 a	20.0 b	25.7 a	7.5 a
GMF+T	28 a	1645 a	58 a	79 a	20.5 ab	26.7 a	7.5 a
GMM	18 b	1029 b	58 a	78 a	20.8 ab	16.8 bc	7.6 a
GMM+T	18 b	1016 b	58 a	85 a	21.1 ab	18.2 b	7.7 a

Different letters indicate that mean values among treatments are significantly different ($p < 0.05$) according to the Tukey method. $n=3$

*Each value for 1000-grain weight, brown rice yield, and protein content was standardized to 15% moisture content.

3.4 Discussion

3.4.1 Rice growth, nutrition, yield, and protein content

Plant length and tiller number before the AT stage in the GM treatments were smaller than those in the CF treatments (Figs. 3.2 and 3.3). Our pot experiment in the first year showed a similar trend of lower initial growth in the GM treatments (Ko et al., 2020a). More mineralized N was supplied from the flowering-stage GM than from the maturity-stage GM for the period of 0–4 weeks (Fig. 3.1), but tiller numbers in the GM treatments were similar before 14 DAT (Fig. 3.3). In addition, plant length in the GM treatments before 21 DAT was not significantly different or significantly lower than that in NF (Fig. 3.2). These results suggest that initial growth was inhibited mainly by organic acid produced by GM incorporation, as shown in previous studies (Ishikawa, 1963). Watanabe (1984) mentioned that incorporating an excessive amount of GM causes anaerobic fermentation of GM after flooding, which inhibits the initial growth of rice. The ferrous iron (Fe^{2+}), which is increased under reduction condition of the soil caused by GM application, is also a possible factor of the inhibited initial growth. On the other hand, Ishikawa (1963) indicated that the adverse effect of increased Fe^{2+} on rice growth was small compared with that of organic acid.

The tiller number in GMM(+T) was larger than that in GMF(+T) in the first year experiment (Ko et al., 2020a), whereas the tiller number in GMF(+T) was larger than that in GMM(+T) in the second year experiment (Fig. 3.3). This was because mineralized N was expected to be greater before the AT stage in GMF(+T) than in GMM(+T), considering the incubation results from 0 to 4 weeks (Fig. 3.1). In the second year experiment, the incorporation timing of GM was the same among the GM treatments (10 days before transplanting). Thus, less N was likely lost because of denitrification and leaching during the upland period and denitrification after flooding (Aulakh et al., 2000), compared with GMF(+T) in the first year involving the long upland period (45 days). The loss of mineralized N increases because of denitrification and leaching as the upland period increases longer (Buresh and De Datta, 1991; Azuma et al., 2017). On the other hand, the differences in mineralized N from 0 to 4 weeks were not explained by the C/N ratio and extractable N, which were not significantly different between flowering and maturity-stage GM (Table 3.1, Fig. 3.1). Possibly increased cellulose, which was expected to increase as GM matured (Ishikawa, 1988; Yasue, 1991), might have lowered N mineralization of the maturity-stage GM from 0 to 4 weeks. The percentage of mineralized N to TN application from maturity-stage GM was 5.8–14.7% lower from 0 to 4 weeks than that from flowering-stage GM (Fig.3.1).

The number of unproductive tillers was the largest in CF (Fig. 3.3). This can be attributed to the low TN content (0.89%) in rice at the PF stage in CF, which was also indicated by a sharp decline in SPAD readings before applying topdressing at 42 DAT (Table 3.3, Fig. 3.4). Tanaka and Garcia (1965) reported that weak tillers started to die when N in the rice culms was approximately 0.8% during the ear-primordium development stage. Thus, higher TN in rice probably reduced unproductive tillers in the GM treatments. As a result, the panicle number was significantly higher in GMF(+T) than that in CF (Table 3.5). Although the tiller number in GMM(+T) was inhibited before the AT stage, the panicle number was not significantly different from that in CF. Higher TN content in rice at PF, which could increase the amount of nitrogen per panicle, might have increased the number of rough rice per panicle in the GM treatments (Wada, 1969). This increased the number of rough rice per pot in the GM treatments, resulting in higher brown rice yield for GMF(+T), followed by GMM(+T) and CF. Thus, the yield was not reduced in the GM treatments, despite the tiller number being inhibited before the AT stage. This result indicated that available N accumulated in the GM treatments for the later growth stage because the uptake of mineralized N by rice was inhibited before the AT stage. This increased N availability during the late growth stage could be the reason for the results wherein growth, yield components, and yield were not significantly different between the treatments of GM alone and GM with topdressings. On the other hand, high SPAD readings during the ripening period resulted in increased protein content in the GM treatments as shown by Mori et al. (2010) (Tables

3.6, Fig. 3.4). This result indicated that GM application, which can increase available N during the late growth stage, is likely to keep N in rice high during the ripening period and negatively affect rice palatability.

In the first year, K_2O in rice shoots was significantly lower in GMM(+T) than in CF (Ko et al., 2020a). In the second year, K_2O in rice was significantly lower in GMF(+T) (except for the AT stage) and GMM(+T) than in CF (Table 3.3). This was because K_2O supplied from GM only depended on the soil without fertilizer application. A deficiency in K_2O can reduce yield and increase disease severity (Williams and Smith, 2001). Thus, long-term rice production with GM incorporation alone will decrease potassium in the soil, which can increase the risk of K_2O deficiency in rice.

3.4.2 Nitrogen use efficiency of rice

The values of NUE at the AT stage were significantly lower in the GM treatments (GMF:17%, GMF+T:10%, GMM:8%, GMM+T:7%) than in CF (52%) (Table 3.4). This low NUE followed the results in the first year (Ko et al., 2020a). The NUE in CF was similar to the value in the first year (56%). Meanwhile, the NUE in GMF(+T) increased compared with the value in the first year (4%). This was because more mineralized N was supplied in the second year because of the shorter upland period (10 days), which increased N uptake at the AT stage (0.14 g pot^{-1}, Table 3.4) compared with 0.11–0.12 g pot^{-1} in the first year (upland period: 45 days). In flowering-stage GM, mineralized N at 4 weeks was 86.2 mg kg^{-1} (Fig. 3.1), which was greater than 51.4 mg kg^{-1} in the first year. In addition, N application rates from GM (0.67–1.10 g pot^{-1}, Table 3.2) were smaller than those in the first year (1.14–1.17 g pot^{-1}), contributing to increased NUE at the AT stage. In contrast, the NUE in GMM(+T) was lower than that in the first year (14%–18%). Considering mineralized N at 4 weeks (69.2 mg kg^{-1}) was similar to the 70.9 mg kg^{-1} in the first year, initial growth was likely more inhibited in the second year. The N uptake at the AT stage was 0.09–0.10 g pot^{-1} (Table 3.4), which was lower than that in the first year (0.18–0.20 g pot^{-1}). In addition, N application rates from GM (0.81–0.87 g pot^{-1}, Table 3.2) were greater than that in the first year (0.74 g pot^{-1}), contributing to the reduced NUE at the AT stage.

NUE at the PF stage markedly increased in GMF(+T) (38%–41%) compared with that in the first year (5%–6%). This occurred because the greater mineralized N supply increased N uptake (0.35–0.40 g pot^{-1}, Table 3.4) compared with 0.15–0.16 g pot^{-1} in the first year. Mineralized N at 7 weeks was 142.2 mg kg^{-1} (Fig. 3.1) compared with 63.5 mg kg^{-1} in the first year. NUE at the HA stage in the GM treatments, which was 46%–52% in GMF(+T) and 38%–42% in GMM(+T), was significantly lower than that in CF (67%) (Table 3.4). However, the NUE in GM treatments was greater than the 33.3% reported by Asagi and Ueno (2009). This probably occurred because Asagi and Ueno (2009) incorporated GM 1 day before flooding, which likely inhibited initial growth to a greater extent.

Mineralized NUE at the AT stage was significantly lower in GM treatments (except for GMF) than that in CF (Table 3.4), which showed that available N was not effectively used for rice growth, indicating that the initial growth of rice was inhibited by GM incorporation. Mineralized NUE at the PF and HA stages in GMF(+T) was not significantly different from CF, whereas mineralized NUE in GMM(+T) was significantly lower than that in CF. A possible reason was that the greater mineralized N supply before the AT stage, which was expected from mineralized N from 0 to 4 weeks of incubation, probably recovered the inhibited initial growth in GMF(+T) (Fig. 3.1). On the other hand, mineralized N supplied from maturity-stage GM before the AT stage might not be sufficient to recover inhibited initial growth of rice, resulting in significantly lower mineralized NUE at the PF and HA stages. Mineralized NUE at the PF in GMF(+T) (61%–66%) was markedly larger than that in the first year (22%–25%). This was probably because the abundant N that mineralized from the flowering-stage GM before the PF stage, which was more than double that in the first year, recovered the inhibited initial growth and promoted vegetative growth.

The differences in NUE and mineralized NUE between flowering- and maturity-stage GM might be attributed to their different patterns of N mineralization. The percentage of mineralized N from flowering-stage GM was larger (8–45%) from 0 to 4 weeks than that from maturity-stage GM (2–30%). This pattern of earlier mineralization promoted initial growth to increase NUE of rice cultivated with flowering-stage GM. Meanwhile, the percentage of mineralized N from maturity-stage GM was larger (89%) at 13 weeks than that from flowering-stage GM (69%). This difference indicated that maturity-stage GM involved less denitrification loss during the upland period. However, N mineralized more during the late growth stage was not efficiently used for rice growth and resulted in lower NUE.

3.4.3 Application of maturity-stage green manure in rice production

In the first year of our pot experiment, upland periods before flooding were 45 days and 21 days when GM was incorporated at the flowering and maturity stages, respectively (Ko et al., 2020a). The experiment showed that a shorter upland period because of GM incorporation at the maturity stage reduced denitrification and leaching loss, resulting in increased rice growth and NUE. The results indicated that incorporating GM at the maturity stage is advantageous when transplanting dates are separated from the flowering stage of GM.

In the second year of our experiment, GM was collected at the flowering and maturity stages and then air-dried. The upland period before flooding was 10 days when air-dried flowering- and maturity-stage GM were incorporated at the same time. The amount of mineralized N from maturity-stage GM was 20%–30% less before 4 weeks of incubation compared with that from the flowering-stage, whereas the amount after 4 weeks was similar between the flowering- and maturity-stage GM. The reduced mineralized N from maturity-stage GM recovered inhibited tillering less than the treatments with the incorporation of flowering-stage GM, which reduced panicle number resulting in decreased yield and lowered NUE. Nagumo et al. (2014) also reported that the incorporation of maturity-stage CMV tended to reduce the panicle number because of decreased mineralized N supply at the beginning of the growth stage. In this study, the C/N ratio was similar between flowering- and maturity-stage GM, which resulted in a relatively close pattern of N mineralization. However, the pattern of N mineralization can change depending on the extent of the maturity of GM. For CMV, N mineralized in 1 month at 25 °C was 17.9% of the total N added at the maturity stage, whereas N mineralized in 28 days at 23 °C was 53–57.8% at the flowering stage (Ishikawa, 1963; Nagumo et al., 2014). This large difference in mineralization implies that maturity-stage GM can inhibit initial growth more significantly than the extent observed in this study. Thus, the use of maturity-stage GM might require the application of basal fertilizer to promote initial growth, as mentioned by Nagumo et al. (2014).

The initial growth of rice with GM treatments was inhibited in both years of the experiment. Yasue (1991) concluded that damage to rice growth because of organic acid and other harmful substances is avoided by incorporating GM from 7 to 14 days before transplanting. However, rice growth was inhibited in the second year, although the upland period before flooding was 10 days. This was probably because the application amount of GM (approximately 40 g N m^{-2}) was at least 2–2.5 times larger than that reported in previous studies (Ishikawa, 1988; Nagumo et al., 2014; Azuma et al., 2017). The large amount of GM application implied that a large amount of organic acid was generated. Furthermore, our pot experiment was in an undrained condition that tended to suffer root damage (Ishikawa, 1988). In actual paddy fields, the concentration of organic acids can be diluted by paddling or temporary drainage and irrigation before transplanting (Azuma et al., 2017). Thus, inhibition of initial growth by GM incorporation is expected to be reduced in paddy fields compared with pot cultivation.

3.5 Conclusions

The objective of this chapter was to evaluate rice growth, NUE, yield, and quality when flowering- and maturity-stage GM were incorporated at the same time. The following conclusions were drawn:

1. Initial rice growth in the GM treatments was inhibited before the active tillering (AT) stage, probably because of the organic acid produced during flooded conditions.
2. The availability of N was likely to be high in GM treatments for later growth stages, which reduced unproductive tillers. As a result, the panicle number was significantly higher in GMF(+T) and was similar in GMM(+T) when compared with that in CF.
3. Brown rice yield was higher in GMF(+T), followed by GMM(+T) and CF.
4. Protein content in brown rice was significantly higher in GM treatments than that in CF.
5. The NUE at the harvest stage in GMF(+T) (46%–52%) and GMM(+T) (38%–43%) was significantly lower than that in CF (67%) because of inhibited initial growth.

Chapter 4

Nitrogen Mineralization from Flowering- and Maturity-Stage Green Manures under Flooded Condition after Involving Different Upland Periods

4.1 Introduction

Leguminous green manure (GM) is organic matter that has been conventionally used as a nitrogen (N) source for crops. The use of GMs in agricultural production has rapidly declined since the advent of chemical fertilizers (CFs; Chen, 1988; Rosegrant and Roumasset, 1988; Yasue, 1991). On the other hand, GM use has been renewed in agriculture as a sustainable practice with a low required input of energy and fertilizer resources. This is partly because farmers have recently experienced fluctuating prices in CFs caused by the rising cost of fossil fuels and concerns over the depletion of the necessary raw materials for CFs (Baligar and Bennett, 1986; Rosegrant and Roumasset, 1988; World Bank, 2019).

Previous studies regarding GM use in Japan have mainly focused on basal application in rice production, traditionally using Chinese milk vetch (*Astragalus sinicus* L.; CMV) and using hairy vetch (*Vicia villosa* Roth; HV) in recent years (Watanabe, 1984; Ishikawa, 1988; Azuma et al., 2017). The appropriate incorporation rate and timing have been studied to maximize yield and avoid the growth inhabitation caused by the decomposition of carbohydrates (Ishikawa, 1988; Azuma et al., 2017). In these studies, using GMs as a basal application with topdressing(s) has resulted in the production of an equivalent or greater yield compared with conventional cultivation using CF (Kawase and Kitazima, 1994; Azuma et al., 2017).

On the other hand, the current use of GMs in rice production, which is limited to basal application at the flowering stage, can lower the nitrogen use efficiency (NUE) of rice plants. This is because the N supply from flowering-stage GMs may not be synchronized with the N demand of rice, which changes in relation to the growth stage. Flowering-stage GMs rapidly mineralize N during the early growth stage of rice (Kawase and Kitazima, 1994), while a report on the improved variety of IR-8 demonstrated a case in which the N uptake of the panicle accounted for 71 % of the total uptake (Yoshida, 1981). Asagi and Ueno (2009) reported that a case in which the NUE of rice plants was 55.6% in hairy vetch, followed by white clover (*Trifolium repens* L.; WC) at 43.5%, crimson clover (*Trifolium incarnatum* L.; CC) at 33.3%, and CMV at 25.7%. In this case, air-dried flowering-stage GMs were incorporated 1 day before transplanting the rice. Ko et al. (2020b) have estimated that the NUE of rice was 52%, which is 15% lower compared with cultivation using CF, when air-dried flowering-stage CC were incorporated 10 days before flooding. In addition, GMs can supply excessive N that causes lodging and disease because their growth depends on weather conditions (Japan Soil Association, 2012). The excessive N thus needs to be reduced through denitrification during an upland period before transplanting the rice, which will further lower the NUE. Ko et al. (2020a) have reported that the NUE of rice plants at the panicle formation stage decreased from 21% to 6% when the upland period was increased from 21 to 45 days, after incorporating crimson clover. The low NUE indicates that N from the GM was lost due to denitrification and leaching (Buresh and De Datta, 1991; George et al., 1992), a process which could have negative environmental impacts.

Meanwhile, supplying N mineralized from GM during the late growth stage of rice can improve its NUE by avoiding an excessive N supply in its early growth stage. Maturity-stage GM, which has a higher C/N ratio than flowering-stage GM, can slowly mineralize N until the late growth stage of rice (Ishikawa, 1988; Yasue, 1991; Nagumo et al., 2014). Through incubation tests under flooded conditions at 25 °C, Nagumo et al. (2014) have demonstrated a case in which 17.9% of the total N added was mineralized from maturity-stage CMV (C/N ratio: 17.1) in one month. This pattern of N mineralization was slower than the 53%–57.8% for flowering-stage CMV incubated at 23 °C for 28 days revealed by Ishikawa (1963). This difference in the pattern of N mineralization affects the rice growth, yield, and NUE. Nagumo et al. (2014) and Ko et al.

(2020b) have both reported that applying maturity-stage GM in rice production can inhibit initial growth, thus reducing yield. In one study, the NUE of rice was found to be 43% under the application of maturity-stage crimson clover, lower than the 52% under the application of flowering-stage GM (Ko et al., 2020b).

Currently, no noticeable effects caused by the incorporation of maturity-stage GMs on yield and NUE have been reported in rice production. However, the pattern and the amount of N mineralization can change due to a variety of factors, such as the species of the GMs, the biomass amount of the GMs, and the length of the upland period after incorporation (Frankenberger and Abdelmagid, 1985; Oglesby and Fownes, 1992; Azuma et al., 2017; Ko et al., 2020a). Moreover, these factors depend on various conditions, such as the local weather, cropping system, field drainage, irrigation, and the farmers’ work schedule. This indicates that applying maturity-stage GMs will be a favorable option under certain conditions. For example, Ko et al. (2020b) suggest that incorporating GMs at the maturity stage is advantageous for reducing the denitrification loss when the dates for transplanting rice are kept separate from the flowering stage of the GMs. Thus, the characteristics of N mineralization should be better clarified to optimize the use of GMs under different conditions in rice production. The objectives of this study were to clarify the patterns of N mineralization from flowering- and maturity-stage GMs under flooded conditions following different upland periods and to evaluate the denitrification loss occurring during the upland periods.

4.2 Materials and Methods

4.2.1 Cultivation of green manures

HV, CC, and WC were cultivated from October 2018 to June 2019 at Kyushu University farm in Fukuoka Prefecture, Japan. We prepared 12 planters in total, four for cultivating each of the three species. The planters were 60 cm in length, 17 cm in width, and 16.5 cm in depth. First, light stones were placed at the bottoms of the planters. Then, they were filled with 7.6 kg of Futsukaichi soil (dry weight basis), which had been collected from Kamigoka, Chikushino city, Fukuoka prefecture, Japan. The Futsukaichi soil was passed through a 2 mm sieve after soybeans had been cultivating. The soil type, pH, cation exchange capacity, total nitrogen (TN), total phosphorus (TP), and exchangeable K were clay loam, 7.1, 8.35 cmol kg^{-1}, 0.09%, 0.14%, and 0.23 cmol kg^{-1}, respectively. The date of seeding was October 7. The seed rates were 0.5 g $planter^{-1}$ for HV, 0.3 g $planter^{-1}$ for CC, and 0.3 g $planter^{-1}$ for WC. After germination in a greenhouse, 6 planters were placed outside. The remaining 6 planters were placed in a phytotron at 15 °C considering possible growth inhibition in the GM cultivated outside during winter. The GMs cultivated in the phytotron were moved outside on March 1, as the GMs grew similarly both outside and in the phytotron. The moisture conditions of the soil in all planters were adjusted to between 30% and 60% of the maximum water holding capacity during cultivation. Finally, two of the four planters of each species were used to collect the flowering- and maturity-stage GMs, one having been placed in the phytotron for the first section of the GM cultivation and the other having been cultivated entirely outside.

4.2.2 Measurement of physiochemical properties of green manures and soil physical properties

Flowering- and maturity-stage GMs were collected on March 25 and May 27 for the HV, on April 1 and June 12 for the CC, and on April 18 and June 24 for the WC. The GM shoots were cut at the soil surface, and the roots were collected by carefully removing the soil. The fresh weights of the shoots and roots were measured separately. The GMs used for incubation tests were preserved in Ziploc® bags and stored in a refrigerator at 4 °C, while 5 g of the shoots and 1–5 g of the roots were used to measure the moisture content (using oven-drying at 70 °C for 48 h), the total carbon (TC), and the TN. The oven-dried shoots and roots were ground to a fine powder using a sample mill (Cyclotec 1093, Foss, Hilleroed, Denmark). The TC was then measured using a CHN coder (MT-5, Yanaco, Tokyo, Japan) with two replications. For TN measurement, the ground shoots and roots were separately digested by H_2SO_4-H_2O_2 Kjeldahl digestion method with three replications (Ohyama et al., 1991). The TN was then measured using the indophenol method (Cataldo et al., 1974).

To measure the soil properties, the soil in two planters each of the flowering- and maturity-stage GM was mixed well and passed through a 2 mm sieve. Then, moist soil (approximately 100 g on a dry weight basis) was sampled to measure moisture content (oven-drying at 105 °C for 24 h) and maximum water holding capacity (Kawaguchi and Ojima, 1965). The rest of the soil was preserved in a plastic bag for use in incubation tests.

4.2.3 Measurement of nitrogen mineralized from green manures

The N mineralized from the GMs was measured through incubation tests using both flowering- and maturity-stages GMs. The tests included five upland periods (0, 1, 2, 3, and 4 weeks) at 20 °C, and each upland period involved eight flooded periods (0, 1, 2, 4, 6, 8, 12, and 16 weeks) at 30 °C (Fig. 4.1).

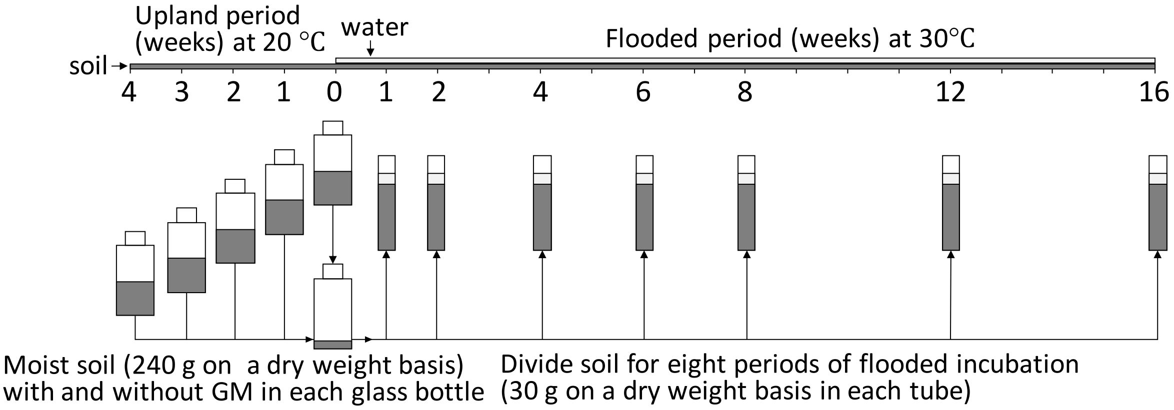

Figure 4.1 Procedures of incubation tests including eight flooded periods (0, 1, 2, 4, 6, 8, 12, and 16 weeks) at 30 °C following five upland periods (0, 1, 2, 3, and 4 weeks) at 20 °C.

The shoots and roots of the GMs, which were cut into approximately 1 cm pieces, were mixed well with moist soil (approximately 240 g on a dry weight basis) and were transferred into a 500-mL glass bottle. The N application rate was adjusted to 100 mg kg^{-1} dry soil. The amount of shoots and roots added was determined using the ratio between them on a dry weight basis, which was calculated based on the total fresh weight and moisture contents of the shoots and roots measured when the GMs were collected. Nevertheless, moisture content of the GMs, especially for the shoots at the flowering stage with high moisture content, decreased during the one-week period of preservation before the incubation tests. Thus, actual N application rates were finally calculated based on moisture contents measured for the shoots and roots when the incubation tests were conducted. The TN was also measured for pieces of the shoots and roots used in the incubation tests to calculate the N application rates. Three bottles each with and without GM were prepared for each of the five upland periods. The moisture conditions of the soil were adjusted to 60% of the maximum water holding capacity during upland incubation.

After upland incubation, the soil in each bottle was divided for eight periods of incubation tests under flooded conditions (0, 1, 2, 4, 6, 8, 12, and 16 weeks). Approximately 30 g of soil (dry weight basis) was transferred from a glass bottle into a 30 mL polypropylene tube (62.543, Sarstedt, Nümbrecht, Germany) with distilled water. The soil in each tube was approximately 6 cm in depth, and the water above the soil surface had a depth of approximately 2.5 cm. After each of the incubation periods, the soil and water in the tubes were transferred into a 500 mL glass bottle with 150 ml of 10% KCl. For the flooded 0-week sample, approximately 30 g soil was not transferred into a polypropylene tube and stored in a glass bottle, to which 150 ml of 10% KCl was added. Then, the mineralized N (MN) was extracted by shaking the bottle for 30 min. The extract was filtered through a No. 5B filter paper (Advantec, Tokyo, Japan). Then, the ammonium (NH_4–N) and nitrate (NO_3–N) in the extract were measured using the indophenol and Cataldo methods, respectively (Cataldo et al., 1975). The nitrate (NO_3–N) from the flowering- and maturity-stage GMs was only measured at 0 and 1 weeks and at 0 weeks of flooded incubation, respectively. Finally, the N mineralized from the flowering- and maturity-stage GMs was estimated using the difference in the MN content between the soil mixed with GM and that without GM.

4.2.4 Estimation of denitrification loss during upland periods

The destination of the TN input from the GM after 16 weeks of flooded incubation consists of the MN from the GM after 16 weeks, the denitrification loss during the upland period, the denitrification loss during the flooded period, and other factors, as shown in Equation (4.1). Assuming that the term of “others” included the remaining N in the soil and measurement/estimation errors. Any measurement errors can be attributed to differences in the moisture contents and TN of shoots and roots between the samples used for the measurement and incubation tests as well as estimation errors for denitrification loss.

$$\mathrm{DTN}_{\mathrm{f16}}(w) = \mathrm{MN}_{\mathrm{f16}}(w) + \mathrm{DNL}_{\mathrm{u}}(w) + \mathrm{DNL}_{\mathrm{f}}(w) + \mathrm{Others} \tag{4.1}$$

Here, the w in parentheses represents the upland weeks before the flooded incubation. $\mathrm{DTN}_{\mathrm{f16}}(w)$ is the destination of the TN input from the GM after 16 weeks of flooded incubation, $\mathrm{MN}_{\mathrm{f16}}(w)$ is the MN from the GM after 16 weeks of flooded incubation, $\mathrm{DNL}_{\mathrm{u}}(w)$ is the denitrification loss during the upland period, and $\mathrm{DNL}_{\mathrm{f}}(w)$ is the denitrification loss during the flooded period. The $\mathrm{DNL}_{\mathrm{f}}(w)$ was estimated using the nitrate detected after 0 and 1 weeks of flooded incubation, which was finally lost during the flooded period. The value of $\mathrm{DNL}_{\mathrm{u}}(w)$ was estimated using the difference in MN content during the flooded period between upland week 0 and an arbitrary upland week, as shown in Equation (4.2).

$$\mathrm{DNL}_{\mathrm{u}}(w)= \mathrm{MN}_{\mathrm{f16}}(0) + \mathrm{DNL}_{\mathrm{f}}(0) - \mathrm{MN}_{\mathrm{f16}}(w) - \mathrm{DNL}_{\mathrm{f}}(w) \tag{4.2}$$

Assuming that the MN during the flooded period was equal to the MN after 16 weeks along with the denitrification loss during the flooded period.

4.3 Results

4.3.1 Physiochemical properties of green manures and nitrogen application rates

Table 4.1 shows the physiochemical properties of the GMs and the N application rates. For the shoot portions, the moisture contents at the flowering stage were greater than 70%, a value markedly higher than that found at the maturity stage. The maturity-stage CC was markedly dried, with a moisture content of only 14%. The moisture contents in the root portions were similar between the flowering and maturity stages. The ratio of shoot/root to total application weight was around 0.5 for the flowering-stage HV and WC. On the other hand, the ratios of shoot to total application weight were larger for the maturity-stage HV (0.81) and for the CC (flowering: 0.72, maturity: 0.92).

The TC contents in the shoots from the HV and CC were higher at the maturity stage than at the flowering stage. The TC contents of WC were similar between the flowering and maturity stages. The TN contents in the shoots were similar between the flowering and maturity stages for CC (1.90% and 1.87%) and WC (3.24% and 3.08%), whereas the TN content for HV was markedly higher at the flowering stage (3.13%) than at the maturity stage (1.96%). Thus, the C/N ratios of shoots in the HV had a large difference between the flowering stage (13.3) and the maturity stage (22.2). The C/N ratios of the shoots were 21.6 and 23.1 for CC and 13.0 and 13.6 for WC at the flowering and maturity stage, respectively. The TC and TN contents of the roots were lower than those of shoots. The TC contents in roots of the HV and CC were higher at the maturity stage, resulting in larger C/N ratios of 15.7 for HV and 33.4 for CC at the maturity stage, compared with 11.6 for HV and 19.5 for CC at the flowering stage.

The total N application rate from the GMs was around 100 mg kg^{-1} dry soil (equal to our target rate) for HV at the maturity stage and for WC at both stages. Meanwhile, the rates were higher than the target rate for flowering-stage HV (133 mg kg^{-1}), flowering-stage CC (193 mg kg^{-1}), and maturity-stage CC (134 mg kg^{-1}).

Table 4.1 Physicochemical properties of green manures and nitrogen (N) application rates

Green manure*	Growth stage	Plant portion	MC%	Ratio to total application weight (dry weight basis)	Total %		C/N ratio	N application rate (mg kg^{-1})	Total N application rate (mg kg^{-1})
					TC	TN			
HV	Flowering	Shoot	74.6	0.55	41.55	3.13	13.3	96	133
		Root	44.9	0.45	17.46	1.51	11.6	37	
	Maturity	Shoot	32.6	0.81	43.52	1.96	22.2	85	104
		Root	40.1	0.19	28.43	1.81	15.7	19	
CC	Flowering	Shoot	78.9	0.72	41.03	1.90	21.6	166	193
		Root	58.3	0.28	15.92	0.82	19.5	27	
	Maturity	Shoot	14.0	0.92	43.19	1.87	23.1	130	134
		Root	47.8	0.08	23.39	0.70	33.4	4	
WC	Flowering	Shoot	82.4	0.41	42.18	3.24	13.0	62	109
		Root	65.8	0.59	24.66	1.67	14.8	47	
	Maturity	Shoot	57.6	0.48	41.86	3.08	13.6	60	89*
		Root	64.0	0.52	20.71	1.40	14.8	30	

HV: hairy vetch, CC: crimson clover, WC: white clover, MC: Moisture content, TC: Total carbon, TN: Total nitrogen.

Flowering- and maturity-stage GMs were collected on March 25 and May 27 for HV, on April 1 and June 12 for CC, and on April 18 and June 24 for WC, respectively.

*The N application rate (mg kg^{-1}) is on a dry soil weight basis.

**The value is not equal to the summation of each plant portion because the value was rounded to integer.

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4.3.2 Nitrogen mineralization from green manure

For the flowering-stage HV (Fig. 4.2(a)), the MN following upland week 0 rapidly increased from 14.5 mg kg^{-1} at flooded week 0 to 99.8 mg kg^{-1} at flooded week 2. The MN at flooded week 0 increased in relation to increases in the upland period except for following upland week 4. The MN contents were 44.1, 62.4, and 69.6 mg kg^{-1} following upland week 1, 2, and 3, respectively. Then, the MN gradually increased and reached its maximum value at flooded week 4. The maximum MN content was 94.7 mg kg^{-1} after upland week 1, followed by 93.0 mg kg^{-1} after upland week 2, and 83 mg kg^{-1} after upland week 3. The MN reached a maximum of only 34.1 mg kg^{-1} following upland week 4. For maturity-stage HV (Fig. 4.2(b)), the MN continued increasing until flooded week 16 under all the upland periods, at slower rates compared with flowering-stage HV. The MN at flooded week 16 decreased in relation to increasing the upland period. The MN contents were 99.5 mg kg^{-1} following upland week 0, 94.1 mg kg^{-1} following upland week 1, 87.8 mg kg^{-1} following upland week 2, 80.2 mg kg^{-1} following upland week 3, and 58.2 mg kg^{-1} following upland week 4.

For flowering-stage CC (Fig. 4.3(a)), the MN at flooded week 0 was 7.3 mg kg^{-1} following upland week 0, while the values were greater than 20 mg kg^{-1} in the cases of upland weeks 1–4. The rates of N mineralization were slower than those observed for flowering-stage HV. The MN gradually increased and approached a maximum value at flooded week 4 following upland week 0 (94.7 mg kg^{-1}), and at flooded week 8 following upland week 1 (99.0 mg kg^{-1}), upland week 2 (90.4 mg kg^{-1}), and upland week 3 (81.0 mg kg^{-1}). The MN following upland week 4 reached an approximately maximum value of 43 mg kg^{-1} at flooded week 4. For maturity-stage CC (Fig. 4.3(b)), the MN kept increasing until flooded week 16 under all the upland periods, as observed for HV. The MN reached 123.1, 121.6, and 115.7 mg kg^{-1} at flooded week 16 following upland weeks 0, 1, and 2, respectively. The MN at flooded week 16 decreased following upland week 3 and 4 to 92.4 and 88.4 mg kg^{-1}, respectively.

For flowering-stage WC (Fig. 4.4(a)), the MN content at flooded week 0 was relatively high, through only in the case of a 1-week upland period (20.9 mg kg^{-1}). The N mineralization for flowering-stage WC was slower than that observed for flowering-stage HV. The MN gradually increased and approached its maximum value at flooded week 6 following upland week 0 (94.3 mg kg^{-1}), at flooded week 8 following upland week 1 (93.8 mg kg^{-1}), and at flooded week 16 following upland week 2 (80.8 mg kg^{-1}). The MN contents following upland weeks 3 and 4 reached approximately maximums at flooded week 8 of 46.9 and 39.8 mg kg^{-1}, respectively. For maturity-stage WC (Fig. 4.4(b)), the MN at flooded week 0 had small negative values for upland weeks 1, 2, and 4. The MN gradually increased and approached its maximum value (94.3 mg kg^{-1}) at flooded week 8 following upland week 0. The MN continued increasing until flooded week 16 following upland weeks 1–4, as observed for HV and CC. The MN reached 99.9 and 91.7 mg kg^{-1} at flooded week 16 following 0 and upland week 1, respectively. The MN at flooded week 16 decreased following upland weeks 2, 3, and 4 to 72.3, 70.6, and 62.2 mg kg^{-1}, respectively.

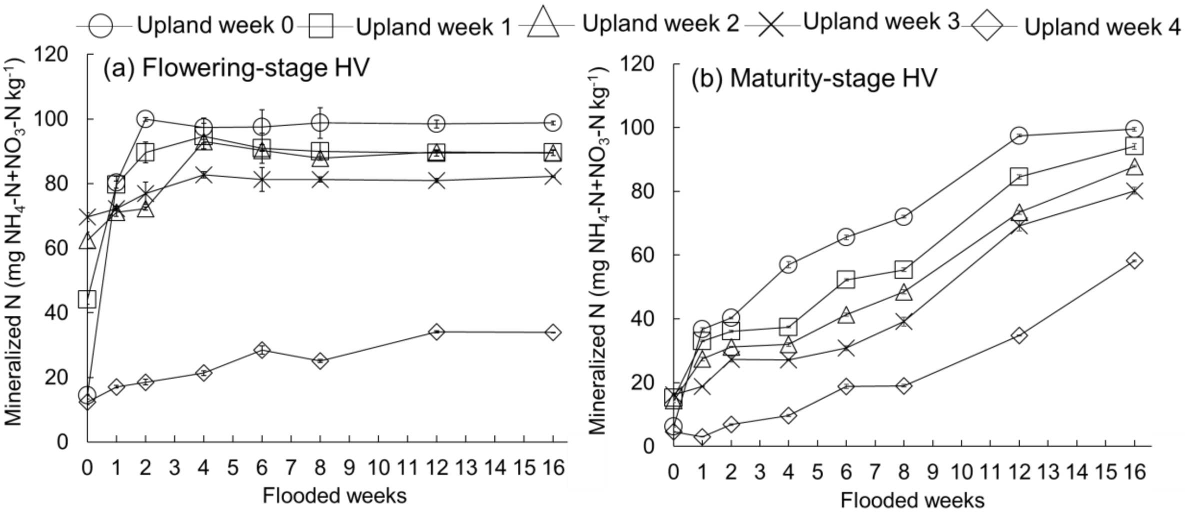

Figure 4.2 Nitrogen (N) mineralized from (a) flowering-stage hairy vetch (HV) and (b) maturity-stage HV estimated through incubation tests at 30 °C under flooded condition. The incubation tests under flooded conditions involved either of five upland periods (0, 1, 2, 3, and 4 weeks) at 20 °C. The N mineralized from HV was estimated using difference in mineralized N between the soil mixed with HV and soil without HV. Error bars represent standard deviation. *n*=3

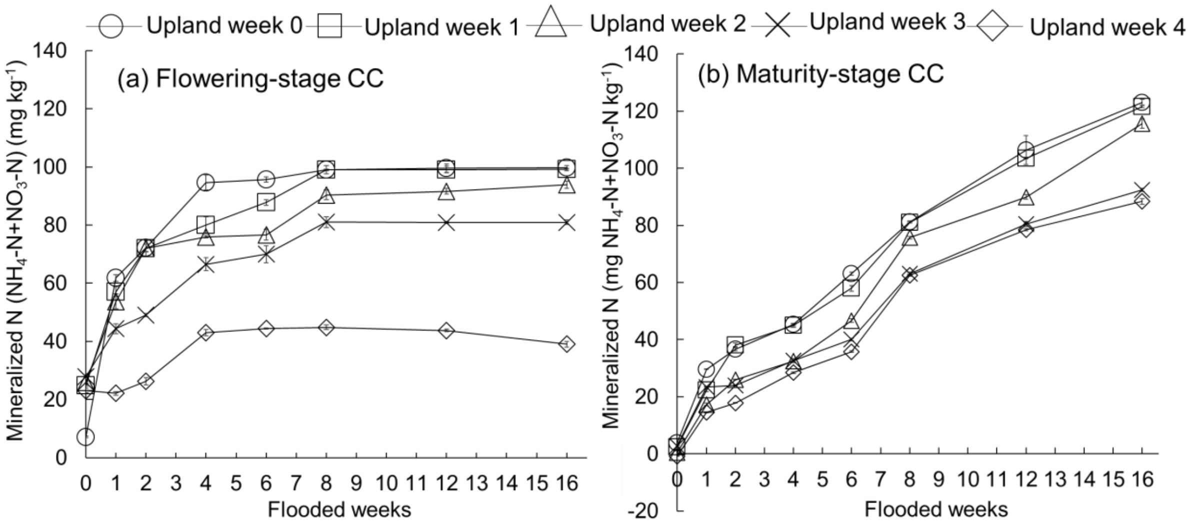

Figure 4.3 Nitrogen (N) mineralized from (a) flowering-stage crimson clover (CC) and (b) maturity-stage CC estimated through incubation tests at 30 °C under flooded condition. The incubation tests under flooded conditions involved either of five upland periods (0, 1, 2, 3, and 4 weeks) at 20 °C. The N mineralized from CC was estimated using the difference in mineralized N between the soil mixed with CC and soil without CC. Error bars represent standard deviation. *n*=3

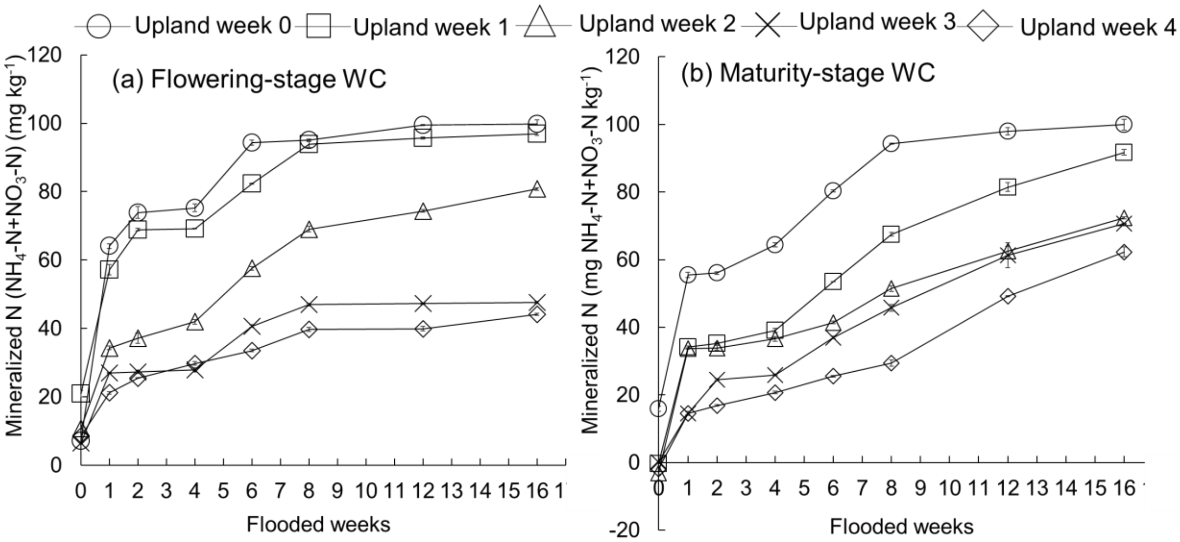

Figure 4.4 Nitrogen (N) mineralized from (a) flowering-stage white clover (WC) and (b) maturity-stage WC estimated through incubation tests at 30 °C under flooded condition. The incubation tests under flooded conditions involved either of five upland periods (0, 1, 2, 3, and 4 weeks) at 20 °C. The N mineralized from WC was estimated using the differences in mineralized N between the soil mixed with WC and soil without WC. Error bars represent standard deviation. *n*=3

4.3.3 Destination of total nitrogen input from green manure after 16 weeks of flooded incubation

For flowering-stage HV (Fig. 4.5(a)), the MN_{f16} ranged from 82.2 (62% of TN input from HV) to 89.6 mg kg$^{–1}$ (67%) following upland weeks 1–3, and the values decreased from 98.8 mg kg$^{–1}$ (74%) following upland week 0. Meanwhile, the MN_{f16}(4) sharply declined to 33.9 mg kg$^{–1}$ (25%), and the amount of DNL_u(4) showed a high value of 53.6 mg kg$^{–1}$ (40%). The amount of DNL_f increased as the upland period increased, while overall the amount was small, ranging from 5–11.2 mg kg$^{–1}$ (4%–8%). The “others”, which were assumed to include the remaining N in the soil and measurement errors, accounted for 26%. For maturity-stage HV (Fig. 4.5(b)), the MN_{f16} decreased as the upland period increased. The MN_{f16} was 96% of the TN input from HV after 0 weeks, 91% after 1 week, 85% after 2 weeks, 77% after 3 weeks, and 56% after 4 weeks. In contrast, DNL_u increased as the upland period increased from 1–4 weeks. After weeks 1–4, the DNL_u was 6 (6%), 12.2 (12%), 19.8 (19%), and 41.8 mg kg$^{–1}$ (40%), respectively.

For flowering-stage CC (Fig. 4.6(a)), MN_{f16} was found to decrease in relation to increasing upland period, similar to the trend observed for flowering-stage HV. The MN_{f16}(4) sharply declined to 39 mg kg$^{–1}$ (20%). The amount of DNL_u increased from 1.2 (1%) to 52.4 mg kg$^{–1}$ (27%) as the upland period increased from 1 to 4 weeks. Small amounts of DNL_f were detected following upland week 0 (0.6 mg kg$^{–1}$, 0.3%) and upland week 4 (9 mg kg$^{–1}$, 5%). “Others” accounted for 48%. For maturity-stage CC (Fig. 4.6(b)), MN_{f16}(3) and MN_{f16}(4) declined to 92.4 (69%) and 88.4 mg kg$^{–1}$ (66%), respectively, compared with the 123.1 mg kg$^{–1}$ (92%) for MN_{f16}(0). The DNL_u(3) and DNL_u(4) were 31.3 (23%) and 35.3 mg kg$^{–1}$ (26%), respectively, which are relatively high levels.

For flowering-stage WC (Fig. 4.7(a)), MN_{f16}(2) (80.8 mg kg$^{–1}$, 74%) decreased, while MN_{f16}(3) (47.5 mg kg$^{–1}$, 44%) and MN_{f16}(4) (44.2 mg kg$^{–1}$, 41%) markedly decreased compared with MN_{f16}(0) (99.7 mg kg$^{–1}$, 91%). The DNL_u (3) and DNL_u (4) were high: 52 mg kg$^{–1}$ (48%) and 55.3 mg kg$^{–1}$ (51%), respectively. For maturity-stage WC (Fig. 4.7(b)), the MN_{f16}(2),

MN_{f16}(3), and MN_{f16}(4) sharply declined to 72.3 (81%), 70.6 (79%), and 62.2 mg kg^{-1} (70%), respectively. The DNL_u (2), DNL_u (3), and DNL_u (4) were high: 27.6 mg kg^{-1} (31%), 29.3 mg kg^{-1} (33%), and 37.7 mg kg^{-1} (42%), respectively. “Others” accounted for −12%.

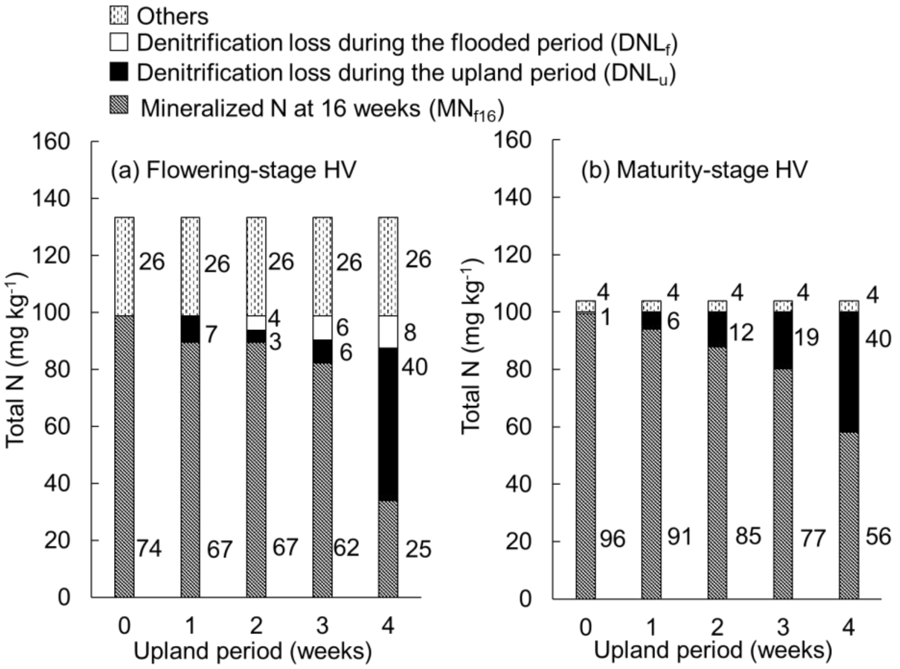

Figure 4.5 Destination of total nitrogen (TN) input from (a) flowering-stage hairy vetch (HV) and (b) maturity-stage HV after 16 weeks of flooded incubation. “Others” include the remaining N in the soil and measurement/estimation errors. Numbers in the graphs represent percentage of each component relative to TN input. The summation of percentage is not always 100 because the percentage of each component is rounded to integer.

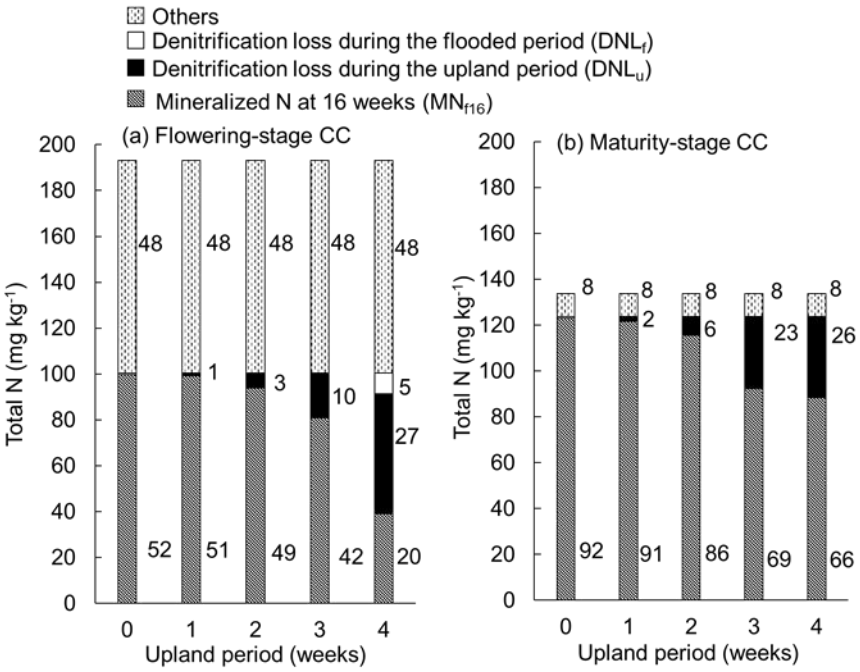

Figure 4.6 Destination of total nitrogen (TN) input from (a) flowering-stage crimson clover (CC) and (b) maturity-stage CC after 16 weeks of flooded incubation. "Others" include the remaining N in the soil and measurement/estimation errors. Numbers in the graphs represent percentage of each component relative to TN input. The summation of percentage is not always 100 because the percentage of each component is rounded to integer.

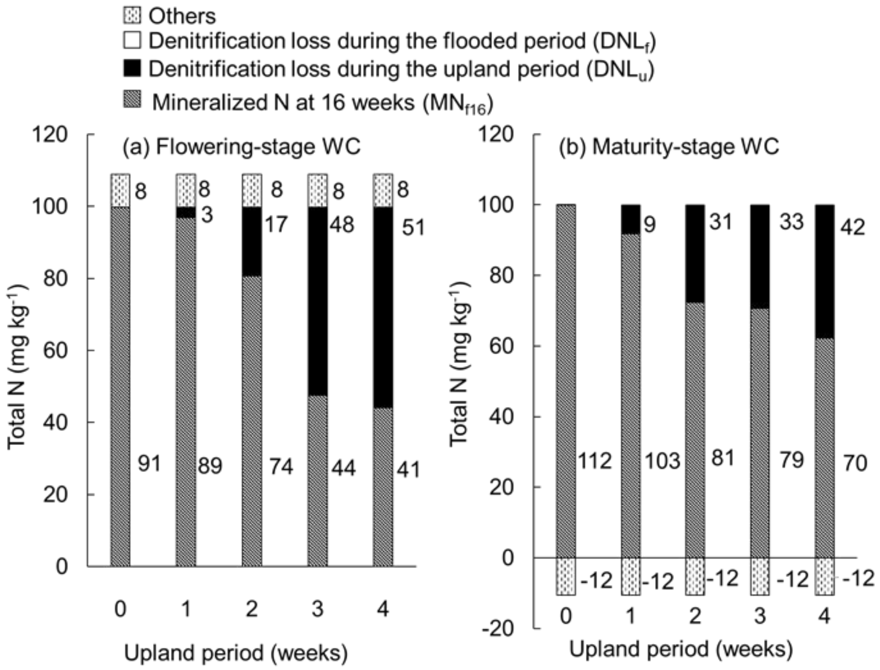

Figure 4.7 Destination of total nitrogen (TN) input from (a) flowering-stage white clover (WC) and (b) maturity-stage WC after 16 weeks of flooded incubation. “Others” include the remaining N in the soil and measurement/estimation errors. Numbers in the graphs represent percentage of each component relative to TN input. The summation of percentage is not always 100 because the percentage of each component is rounded to integer.

4.4 Discussion

4.4.1 Mineralized nitrogen at flooded week 0

The MN at flooded week 0 is discussed here as an indicator that can affect the initial growth of rice (Ando et al., 1988). A large amount of MN at flooded week 0, which increased from after upland weeks 0–3, was observed only in flowering-stage HV (Fig. 4.2). This result indicates that low C/N ratios of shoots (13.3) and roots (11.6) in flowering-stage HV promoted N mineralization during the upland period. The C/N ratios in flowering-stage HV were lower than those in maturity-stage HV and other GMs except for in the shoots of flowering-stage WC (Table 4.1). In the case of flowering-stage HV, the MN after upland weeks 1–3 increased because the amount of N mineralization was greater than the denitrification loss during the upland period. Meanwhile, there was a low level of MN in the flowering-stage HV following upland week 4. This result means that denitrification loss during the upland period markedly increased during the period from after 3 to 4 weeks for the flowering-stage HV (Fig. 4.5(a)). The MN at flooded week 0 showed relatively low levels for the GMs other than flowering-stage HV (Figs. 4.2–4.4). One of the reasons for this is that the amount of N mineralization from maturity-stage HV and CC was probably small during the upland periods for higher C/N ratios (Table 4.1). Another reason is that the denitrification loss increased following upland week 2 or 3, as shown in WC (Fig. 4.7), which is a shorter period than that for flowering-stage HV. The small negative values at flooded week 0 observed for maturity-stage WC (Fig. 4.4(b)) were probably caused by measurement errors rather than the immobilization of MN considering the relatively low C/N ratio measured compared with the other GMs (Table 4.1).

4.4.2 Pattern of nitrogen mineralization from flowering- and maturity-stage green manures

When GMs were used in rice production, the supply of MN needs to be discussed by separating that used for vegetative growth and that used for reproductive growth. Thus, the percentage of MN relative to TN input from the GMs was compared between the flooded period until 4 weeks and that after 4 weeks until 16 weeks (Figs. 4.8–4.10). For the flowering-stage GMs, the percentage until 4 weeks of flooding was 73% for HV (C/N ratio: 13.3 in shoot, 11.6 in root), 49% for CC (C/N ratio: 21.6 in shoot, 19.5 in root), and 69% for WC (C/N ratio: 13 in shoot, 14.8 in root) following upland week 0 (20 °C; Figs. 4.8(a), 4.99(a), and 4.10(a)). In contrast, the percentage after being flooded for 4 weeks until 16 weeks was 1% for HV, 3% for CC, and 22% for WC. This pattern of rapid N mineralization is in agreement with results obtained in former studies. For example, Ishikawa (1963) reported that the MN from flowering-stage CMV (dried powder, C/N ratio: 9) was 53%–57.8% in 28 days under flooded conditions at 23 °C. In another study, the MN from flowering-stage CMV (air-dried small pieces, C/N ratio: 17.3) was approximately 60% in one month under flooded conditions at a mean temperature of 28.5 °C (Saeki and Azuma, 1956). In a further study, the MN from flowering-stage HV (small fresh pieces less than 1 cm) was approximately 60% in 4 weeks under flooded conditions at 30 °C, following an upland period of 1 week at 14 °C (Azuma et al., 2017). In the present study, the percentage of MN in relation to TN input tended to increase when the C/N ratio was low. Meanwhile, the percentage for flowering-stage CC (49%) might have involved some measurement errors considering that the percentage of “others” was as large as 48%, which was larger than that in maturity-stage CC (8%) with higher C/N ratios (Table 4.1; Fig. 4.6).

For maturity-stage GMs, N mineralization until 4 weeks of flooding was lower in the HV and CC compared with the flowering-stage plants. The percentage of MN until 4 weeks of flooding was 55% for HV (C/N ratio: 22.2 in shoot, 15.7 in root) and 34% for CC (C/N ratio: 23.1 in shoot, 33.4 in root) following upland week 0 (Figs. 4.8(b), 4.9(b)), whereas the percentage after 4 weeks until 16 weeks increased more significantly compared with the flowering-stage plants (41% for HV and 58% for CC). These slower rates of N mineralization in HV and CC were caused by the higher C/N ratios in the maturity-stage compared with the flowering-stage. Nagumo et al. (2014) have also reported a case in which maturity-stage CMV (C/N ratio: 17.1) had a slow N mineralization of 17.9% of the total N input in one month under flooded conditions at 25 °C. This smaller percentage compared with our results is partly due to the fact that Nagumo et al. (2014) conducted their incubation tests at a lower temperature. For WC, the percentage of MN until 4 weeks of flooding was similar between the flowering- (69%) and maturity-stage WC (72%; Fig. 4.10). This is probably because their C/N ratios were almost identical (Table 4.1). The increase in the percentage of MN after 4 weeks until 16 weeks for the maturity-stage WC might have been due to overestimation considering that the percentage following upland week 0 was greater than 100%. The dates of sample collection between the flowering- and maturity-stage GMs were separated by approximately 2 months. Meanwhile, the changes in C/N ratios were different among the species. The C/N ratio of the shoots from HV increased to 22.2 at maturity-stage because of a decrease in the TN (1.96%) of the shoots from 3.13% at the flowering stage (Table 4.1). This large change in C/N ratio changed the N mineralization pattern markedly in the HV. The N mineralization of the maturity-stage CC also changed markedly compared with the flowering-stage CC (Fig. 4.9), although the increase in the C/N ratio was not as large as that observed in the HV. It is possible that increased cellulose levels, which were expected to increase as the GM matured (Ishikawa, 1988; Yasue, 1991), might have lowered the N mineralized from maturity-stage CC until four weeks, resulting in an increased percentage of MN after four weeks until 16 weeks.

4.4.3 Denitrification loss during the upland period

The amount of DNL_u increased as the upland period increased (Figs. 4.5–4.7). The decreases in the percentage of MN that occurred in relation to increases in the upland period were larger until 4 weeks of flooding than in the period after 4 weeks until 16 weeks (Figs. 4.8–4.10). Thus, the DNL_u values can likely be attributed to the easily mineralizable fraction of N in the GM. This result indicates that maturity-stage GMs are advantageous for lowering DNL_u for species that have a higher C/N ratio in the maturity stage than in flowering stage, such as HV and CC.

The upland periods during which denitrification loss sharply increased were generally longer than two weeks except for in the case of maturity-stage WC (Figs. 4.5–4.7). The results indicate that denitrification loss under upland conditions, in which the moisture condition of the soil was 60% of its maximum water holding capacity, increased markedly when the upland period extended longer than 2 weeks. However, this period might vary considering the result for maturity-stage WC, for which denitrification loss sharply increased after upland week 2 (Fig. 4.7).

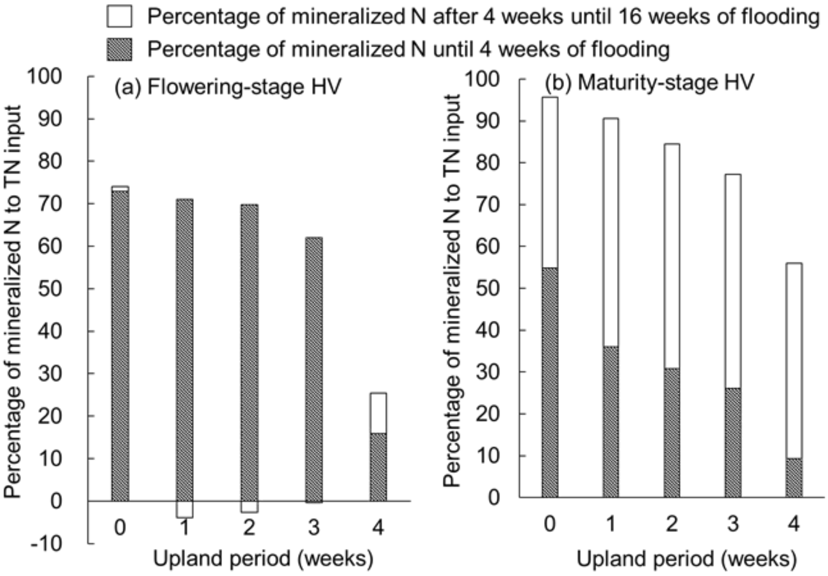

Figure 4.8 Comparison of the percentage of mineralized nitrogen (N) relative to total N from (a) flowering-stage hairy vetch (HV) and (b) maturity-stage HV between the flooded periods until 4 weeks and that after 4 weeks until 16 weeks.

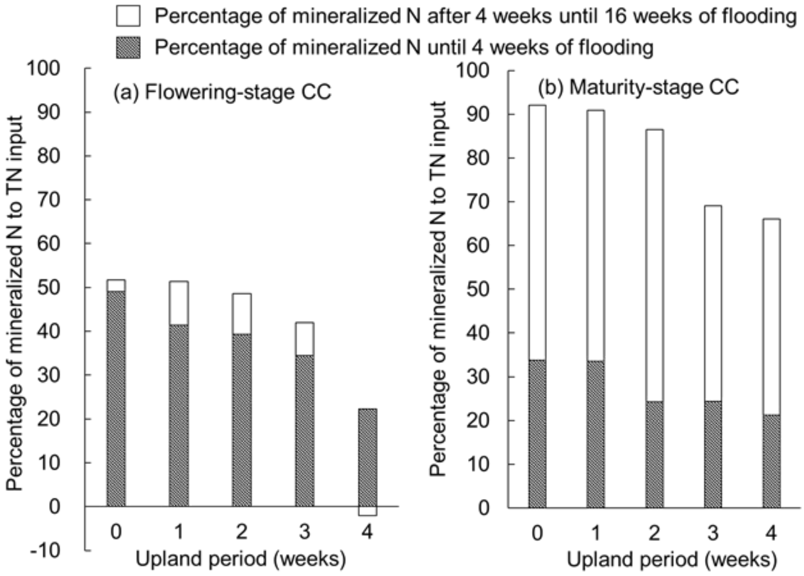

Figure 4.9 Comparison of the percentage of mineralized nitrogen (N) relative to total N from (a) flowering-stage crimson clover (CC) and (b) maturity-stage CC between the flooded periods until 4 weeks and that after 4 weeks until 16 weeks.

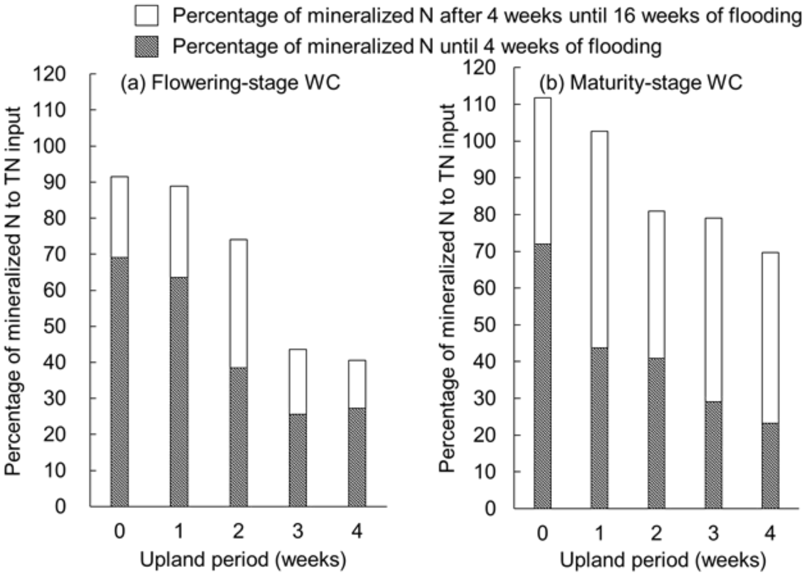

Figure 4.10 Comparison of the percentage of mineralized nitrogen (N) relative to total N from (a) flowering-stage white clover (WC) and (b) maturity-stage WC between the flooded periods until 4 weeks and that after 4 weeks until 16 weeks.

4.5 Conclusions

The objectives of this chapter were to clarify the patterns of N mineralization from flowering- and maturity-stage GMs under flooded conditions following different upland periods and to evaluate the denitrification loss occurring during the upland periods. The following conclusions were drawn:

1. A large amount of MN (52% of TN input) at flooded week 0 was observed in flowering-stage HV with a low C/N ratio following upland week 3.
2. The percentage of MN to TN input after being flooded for 4 weeks until after 16 weeks was 1% for flowering-stage HV and 3% for flowering-stage CC, whereas the percentage increased to 41% for maturity-stage HV and to 58% for maturity-stage CC. The results indicate that maturity-stage HV and CC slowly mineralize N until the late growth stage of rice.

3 The C/N ratios in maturity-stage HV (22.2 in shoots and 15.7 in roots) and CC (23.1 in shoots and 33.4 in roots) were larger than those in flowering-stage HV (13.3 in shoots and 11.6 in roots) and CC (21.6 in shoots and 19.5 in roots). The C/N ratios between the flowering and maturity stages were almost same in WC (approximately 13 in shoots and 14.8 in the roots).

4. Denitrification loss under upland conditions increased markedly when the upland period extended longer than 2 weeks.

Chapter 5

Summary and Conclusions

This thesis investigated the possibility of using maturity-stage green manure (GM) in rice production through two years' pot experiment and incubation tests. The first year experiment (chapter 2) aimed to clarify physicochemical properties and nitrogen (N) mineralization of maturity-stage GM (Crimson Clover, hereafter CC) and its application effects on rice growth and nitrogen use efficiency (NUE), through the comparison with flowering-stage GM. The second year experiment (chapter 3) aimed to evaluate rice growth, NUE, yield, and quality when flowering- and maturity-stage CC were incorporated at the same timing. The incubation tests (chapter 4) aimed to clarify the pattern of N mineralization from flowering- and maturity-stage hairy vetch (HV), CC, and white clover (CC) under flooded condition, which involved different upland periods, and to evaluate denitrification loss during upland periods.

In Chapter 2, a cultivation experiment using 1/5,000a Wagner pots was conducted from October 2017 to September 2018 at Kyushu University farm in Fukuoka Prefecture, Japan. This experiment included six treatments of fertilizer and GM application: no fertilizer (NF), chemical fertilizer (CF), GM incorporation at the flowering stage without (GMF) or with topdressing (GMF+T), and GM incorporation at the maturity stage without (GMM) or with topdressing (GMM+T). Each treatment was prepared for three growth stages of rice: active tillering (AT), panicle formation (PF), and harvest (HA), although treatments at the HA were not investigated because of damage by wild boars. The incorporation dates were April 29 for flowering-stage GM and May 23 for maturity-stage GM. The upland period was 45 days for flowering-stage GM and 21 days for maturity-stage GM. The dry matter weight, total nitrogen (TN), P_2O_5, and K_2O of GM lowered, while the C/N ratio increased as GM matured. The N mineralized from GM at 13 weeks' incubation under flooded condition was 34% (97 mg kg^{-1}) of TN supplied from GM at the flowering stage, while it was 71% (126 mg kg^{-1}) of TN supplied from GM at the maturity stage. This difference was probably because GM incorporation at the flowering stage involved more denitrification loss due to a longer upland period. The large amount of mineral N available in GMM and GMM+T increased tiller number and TN content of rice aboveground part, which resulted in significantly larger dry matter weight at the PF stage and N uptake at the AT and PF stages, compared with GMF and GMF+T. The NUE at the AT and PF stages were higher in GMM and GMM+T (14–21%) than GMF and GMF+T (4–6%), while the values were markedly lower than those in CF (55–56%).

In Chapter 3, a cultivation experiment using 1/5,000a Wagner pots was conducted from October 2018 to September 2019 followed by the cultivation experiment from October 2017 to September 2018 in the first year. The experiment in the second year included the same treatments as the first year. The upland period before flooding was 10 days when air-dried flowering- and maturity-stage GM were incorporated at the same time. Initial rice growth in the GM treatments was inhibited before the AT stage, probably because of the organic acid produced during flooded conditions. Inhibited initial growth recovered less in GMM(+T) than in GMF(+T) because mineralized N supplied from maturity-stage GM before the AT stage was less than that from the flowering-stage GM. The percentage of mineralized N to TN application from maturity-stage GM was 5.8–14.7% lower from 0 to 4 weeks than that from flowering-stage GM. This was probably because the cellulose content increased as GM matured. The availability of N was likely to be high in GM treatments for later growth stages, which reduced unproductive tillers. As the result, the panicle number was significantly higher in GMF(+T) and was similar in GMM(+T) when compared with that in CF. In addition, the rough rice number per panicle tended to be higher in the GM treatments. Thus, brown rice yield was higher in GMF(+T), followed by GMM(+T) and CF. However, protein content in brown rice was significantly higher in GM treatments than that in CF. The values of NUE at the AT stage were significantly lower in the GM treatments (GMF:17%, GMF+T:10%, GMM:8%, GMM+T:7%) than in CF (52%). This low NUE, which followed the results in the first year, was probably because of inhibited initial growth. Meanwhile, the NUE in GMF(+T) increased compared with the value in the first year (4%). This was because more mineralized N was supplied in the second year because of the shorter upland period (10 days). The NUE at the harvest stage in GMF(+T) (46%–52%) and GMM(+T) (38%–43%) was significantly lower than that in CF (67%) probably because of inhibited initial growth. This result also indicates that N mineralized more during the late growth stage was not efficiently used for rice growth resulting in lower NUE in rice cultivated with maturity-stage GM. The use of maturity-stage GM for rice production might require the application of basal fertilizer to promote initial growth.

In Chapter 4, N mineralized from three species of GMs (HV, CC, and WC) collected both at the flowering- and maturity-stages was measured through incubation tests. The incubation tests included five upland periods (0, 1, 2, 3, 4 weeks) at 20 °C, and then each upland period involved eight flooded periods (0, 1, 2, 4, 6, 8, 12, 16 weeks) at 30 °C. The C/N ratios in maturity-stage HV (22.2 in shoots and 15.7 in roots) and CC (23.1 in shoots and 33.4 in roots) were larger than those in flowering-stage HV (13.3 in shoots and 11.6 in roots) and CC (21.6 in shoots and 19.5 in roots). The C/N ratios between flowering- and maturity-stages were almost same in WC (approximately 13 in shoots and 14.8 in roots). Flowering-stage HV mineralized 52% of total N input at flooded week 0 under upland week 3 because of low C/N ratios (13.3 in shoots and 11.6 in roots). The percentage of N mineralized until flooded week 4 was about 50% and more for flowering-stage GMs under upland week 0, whereas maturity-stage GMs mineralized 40–58% after 4 weeks till 16 weeks. Denitrification loss under the upland condition increased markedly when the upland period became more than 2 weeks. The denitrification loss is likely attributed to easily mineralized fraction of N in GM, which is mineralized until flooded week 4. Thus, maturity-stage GMs, which have a higher C/N ratio than flowering-stage, are advantageous of lowering denitrification loss during the upland period.

Overall, this thesis revealed that maturity-stage GM, which mineralize N more during the late growth stage, can retard initial growth, resulting in reduced NUE and yield compared with flowering-stage GM. This indicates that NUE lowers when initial growth is inhibited for reduced N supply during the early growth stage. The use of maturity-stage GM for rice production might require the application of basal fertilizer to promote initial growth. On the other hand, a shorter upland period after GM incorporation reduced denitrification loss, resulting in increased rice growth and NUE. Thus, incorporating GM at the maturity stage is advantageous when transplanting dates are separated from the flowering stage of GM. In the incubation tests, maturity-stage WC slightly changed TN content and the pattern of N mineralization from flowering-stage unlike HV. Although this result can change depending on cultivation environment, this kind of species might be more advantageous for using at the maturity stage when transplanting dates were away from the flowering stage.

The initial growth of rice with GM treatments was inhibited in both years of the experiment, although the upland period before flooding was more than 10 days. This was probably because large amount of GM application (2–2.5 times larger than that reported in previous studies) produced a large amount of organic acid. Furthermore, pot experiment was conducted in an undrained condition that tended to suffer root damage. In actual paddy fields, the concentration of organic acids can be diluted by paddling or temporary drainage and irrigation before transplanting. Thus, inhibition of initial growth by GM incorporation is expected to be reduced in paddy fields compared with pot cultivation.

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