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***Article***

# The Enhancement of Soil Fertility, Dry Matter Transport and Accumulation, Nitrogen Uptake and Yield in Rice via Green Manuring

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**Abstract:** Readily available chemical fertilizers have resulted in a decline in the use of organic manure (e.g., green manures), a traditionally sustainable source of nutrients. Based on this, we applied urea at the rate of 270 kg ha$^{-1}$ with and without green manure in order to assess nitrogen (N) productivity in a double rice cropping system in 2017. In particular, treatment combinations were as follows: winter fallow rice-rice (WF-R-R), milk vetch rice-rice (MV-R-R), oil-seed rape rice-rice (R-R-R) and potato crop rice-rice (P-R-R). Results revealed that green manure significantly ($p \leq 0.05$) improved the soil chemical properties and net soil organic carbon content increased by an average 117.47%, total nitrogen (N) by 28.41%, available N by 26.64%, total phosphorus (P) by 37.77%, available P by 20.48% and available potassium (K) by 33.10% than WF-R-R, however pH was reduced by 3.30% across the seasons. Similarly, net dry matter accumulation rate enhanced in green manure applied treatments and ranked in order: P-R-R > R-R-R > MV-R-R > WF-R-R. Furthermore, the total leaf dry matter transport (t ha$^{-1}$) for the P-R-R in both seasons was significantly higher by an average 11.2%, 7.2% and 36 % than MV-R-R, R-R-R, and WF-R-R, respectively. In addition, net total nitrogen accumulation (kg ha$^{-1}$) was found higher in green manure applied plots compared to the control. Yield and yield attributed traits were observed maximum in green manure applied plots, with treatments ranking as follows: P-R-R > R-R-R > MV-R-R > WF-R-R. Thus, results obtained highlight ability of green manure to sustainably improve soil quality and rice yield.

**Keywords:** Soil organic carbon; dry matter; nitrogen accumulation; milk vetch; rapeseed

## 1 Introduction

Rice is a staple food in China, and due to its rapidly increasing population, the demand for food is sharply rising. In order to meet rising demands, farmers generally apply large amounts of chemical fertilizers to maximize rice yields [1]. However, the application of chemical nitrogen (N) fertilizers to the paddy fields is associated with severe environmental issues [2,3]. This over-fertilization of nitrogen fertilizers has not only resulted in wastage of a valuable chemical input but has also led to deteriorated soil, water and atmospheric quality. The degraded soil quality due to increased soil acidification and structural damage, and decreased water table level, has started negative effects on rice yield [4]. Therefore, sustainably maximizing grain yield is a major challenge for rice production. The replacement of mineral fertilizers by organic substrates, such as green manure and straw, is considered a promising approach for the reduction of chemical fertilizer inputs [5,6].

The re-emergence of green manuring, a traditional method commonly applied in the past, is currently the focus of much attention in southern China as a sustainable nutrient supply for crop production and for its contribution to grain yield [7]. Crops, such as Chinese milk vetch (*Astragalus sinius*), Oilseed rape (*Brassica napus* L.), and potato (*Solanum tuberosum* L.) within double-rice rotations, are cultivated as wintergreen manure instead of a leaving field fallow, which is then incorporated into the soil either with rice straw or alone. In this triple cropping system, paddy rice is generally planted in the early spring (late February to mid-March ) and mid-summer (late July to early August), followed by leguminous crops in the winter (late October to early November). These crops are cultivated for various purposes, such as the maximum use of land [8]. However, due to the incorporation of crop residue [9], the enhanced uptake of nitrogen mobility and N fixation from the atmosphere [10], their cultivation generally alters soil fertility. For a sustainable, intensive cultivation based on a double cropping rice system, soil exhaustion must be avoided, while a balance must be determined in between the amount of N available following the early season rice harvest and the rice N requirements, thus avoiding N losses from leaching or denitrification [11].

Furthermore, the efficiency of rice N use under multiple green manure treatments remains to be fully understood. In particular, the involvement of such as chemical N, the subsequent crop responses and the N requirements of the plants are in need of a full assessment. Soil N mobility and rice N requirements rely on the internal flow of N within the soil. Moreover, the winter crops selected for the initial application of green manuring are key for the synchronization of soil N supply with rice N demand and for soil sustainability [12]. The use of green crops as manure is a rich source of N and has the ability to stimulate the use of early-season rice straw via microbial activity. Previous studies have demonstrated that the amendment of green manure and rice straw can alter microbial community composition and structures in paddy soil [13,14] or influence carbon sequestration and enhanced soil fertility [15]. Thus, green manure can reduce the strong dependence on synthetic fertilizers that is currently observed across the globe [16], consequently increase crop yields [17].

The long-term application of green manure has been observed to have the following effects on soil: reduction in bulk density, enhancement of porosity; increases in water-retention capacity; improvements in water-stable aggregate content and aggregate stability, increases in organic matter, total nitrogen, and available nitrogen contents [13]. In particular, nitrogen management can influence the dry matter accumulation and N uptake of crops [18]. The correlation between grain yield and dry matter (DM) production is frequently demonstrated, while DM is directly related to nutrient uptake and availability [19]. Meanwhile, dry matter accumulation and N uptake vary across the growth stages of rice [20]. Numerous studies have reported the occurrence of dry matter and nitrogen accumulation at the tillering and panicle initiation stages of rice [21,22]. However, due to a lack of knowledge, the majority of farmers in China apply more chemical fertilizers at earlier stages with the aim of maximizing DM [23]. This consequently leads to more non-productive tillers due to the higher tillering population. Non-productive tillers have a negative effect on rice growth as the act as competitors for assimilates and nutrients [24].

Thus, a sufficient amount of N must be available for plants in triple-cropping systems throughout the year in order to maintain a linear growth and soil sustainability. This is only possible through green manuring [25]. To date, much research has been conducted in monoculture and specific grain yield, with a focus placed on the influence of green manure on rice productivity, and variations in microbial and diazotrophic communities during the rice growing season [14]. However, the effects of the long-term application of different types of green manure on dry matter accumulation in paddy soil remains to be clarified. Thus, in order to overcome the limitations in the literature, the current study examined the influence of green manure crops on rice growth, yield, dry matter translocation and nitrogen use efficiency in a rice-rice-green manure rotation compared to the application of an inorganic N fertilizer. The aims of our study were as follows: (1) To investigate soil chemical properties in response to addition of green manures (2) To determine their effects on dry matter accumulation and nitrogen uptake and (3) To examine their influence on rice grain yield and yield attributes with minimum environmental and economic cost.

## 2 Materials and Methods

### *2.1 Experimental Site*

Experiments were performed during 2016–2017 in the experimental field at the Agriculture College of Guangxi University, China. The first winter crops were planted for green manuring in November 2016. In March (August) 2017, early (late) rice was transplanted, respectively. The soil at the test site is of the ultisol type, and the detail basic physiochemical properties (0–20 cm) in 2016 are reported in Tab. 1. We used the wintergreen manure variety of "Yujiang big leaf" for Green Vetch, "F13-2" for Rapeseed and "Dutch potato No. 15" for the potato variety. In addition, "Guiliangyou No.2" was the rice variety used in the early and late season. The climate is categorized as subtropical with a monsoon zone, and a mean annual precipitation of 1190 millimeters. Mean maximum and minimum temperatures ranged between 30.9–36.7°C and 23.8–27.3°C during the early season and 23.3–27.3°C and 11.5–18.1°C in the late season, respectively (Fig. 1).

**Table 1:** Soil physical and chemical properties prior to the green manure application (2016)

| Properties | Soil | Properties | Soil |
|---|---|---|---|
| Porosity (%) | 40.12 | Total N (g $kg^{-1}$) | 1.14 |
| Moisture (%) | 11.23 | Total P (g $kg^{-1}$) | 0.62 |
| Bulk density (g $cm^{-3}$) | 1.38 | Total K (g $kg^{-1}$) | 11.23 |
| pH (water) | 5.6 | Available N (mg $kg^{-1}$) | 123.24 |
| SOC (g $kg^{-1}$) | 16.12 | Available P (mg $kg^{-1}$) | 23.13 |
| SOM (g $kg^{-1}$) | 27.72 | Available K (mg $kg^{-1}$) | 79.42 |
| C: N ratio | 14.14 | | |

### *2.2 Crop Management and Experimental Design*

Experiments were performed on a randomized block design under three replications with four treatments: Winter Fallow-Rice-Rice (WF-R-R) (control); Milk Vetch-Rice-Rice (MV-R-R); Rapeseed-Rice-Rice (R-R-R); and Potato-Rice-Rice (P-R-R). The crops were harvested, finely chopped, and mechanically mixed (0–20 cm) with soil and applied to the respected plots following early and late planting. The milk vetch and rapeseed were turned back to the field during the flowering period, while the potato tubers were harvested and the stalk and straw were turned back to the field. Following the ploughing of the field, the embedded straw was made to rot by applying water for slightly longer than one month. The early rice (planted on March 10) was then transplanted on April 10, while the late rice (planted on July 16) was transplanted on August 7. The transplanted plants were 10 cm apart, with a row spacing of 30 cm and one seedling per each hill. Urea (46% N) fertilizer was applied uniformly at a rate of 270 kg N $ha^{-1}$ in three splits (5:3:2) at basal, tillering and panicle initiation stage, respectively.

Calcium superphosphate was applied as a basal dose uniformly to all treatments at a rate of 135 kgPha$^{-1}$. Potassium chloride was applied at a rate of 180 kg K ha$^{-1}$ in two splits (5:5) basal and tillering stage.

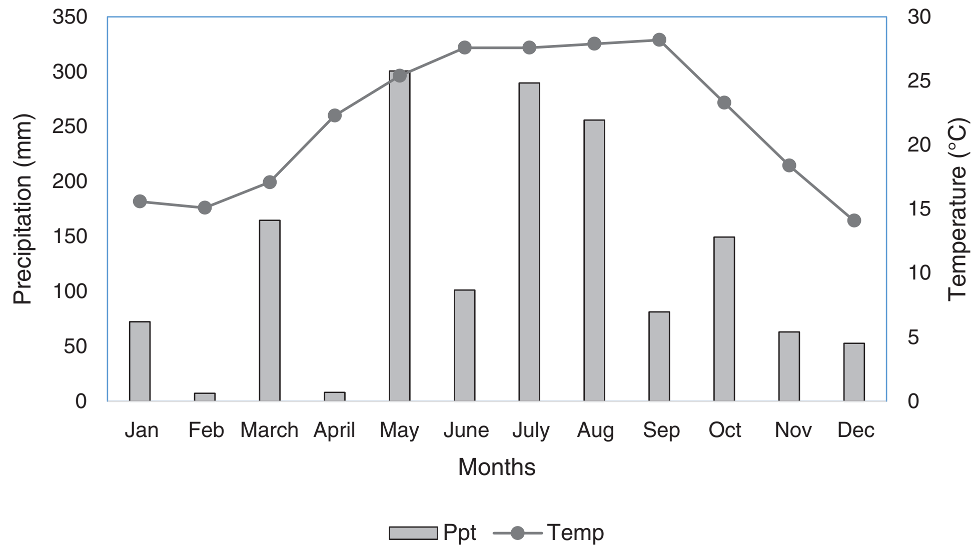


**Figure 1:** Mean average monthly temperature (°C), precipitation (mm) in 2017 during both seasons

### *2.3 Soil Sampling for Measurements*

Soil samples were taken before cultivation of green manures in winter 2016 and prior to early and late seasons of 2017. Soil from each treatment was randomly sampled in five locations (3 replicates) via a soil auger at a depth of 0–20 cm across the seasons. The soil samples were air-dried and passed through a 2 mm sieve in order to determine their chemical composition. Moreover, samples were again passed through a 0.25 mm sieve to determine soil organic carbon (SOC) via the method [26]. Total organic matter was calculated by multiplying SOC with 1.72. Total N (TN) was examined by digesting 2 g soil samples using the salicylic acid–sulfuric acid–hydrogen peroxide method [27]. TN levels were then analyzed according to the micro-Kjeldahl procedure of Jackson [28]. Furthermore, total phosphorus (TP) was tested using the ascorbic acid method [29], while total potassium (TK) was calculated using standard stock solution by dissolving potassium chloride in distal water. Potassium was determined using an atomic absorption spectrophotometer at 7665 R (Z-5300; Hitachi, Tokyo, Japan) after samples were digested. Available N (AN) was determined via the extraction from the soil samples [30], while available P (AP) was extracted based on Olsen's method with 0.5 m NaHCO3 solution adjusted to a pH of 8.5 [31]. In order to determine available K (AK), soil samples were air-dried, passed through a 2 mm sieve, transferred to a 100 ml polyethylene bottle together with 50 ml of ammonium acetate/acetic acid solution, where AP was extracted using the method of Leaf & Neuberger [32].

### *2.4 The Data were Taken on the Following Parameters across the Seasons*

#### *2.4.1 Dry Matter Accumulation and Transportation*

To determine the dry matter and its translocation at the grain filling stage, samples were taken of each treatment from all three replications at heading and maturity. Rice plant samples were divided into leaves, stems + sheets, and panicles. Samples were put in the oven and dried till to gain a steady weight. To record dry matter at the tillering, heading and maturity stages of rice, 15 hills were investigated in each plot randomly. Samples were divided in to leaves, stem, and panicles according to the growth stage. The samples were kept in an oven at 75°C for 48 hours and dried to a constant weight. Dry matter weight at each growing stage tillering, heading, and maturity was investigated. The following parameters were taken accordingly,

Dry matter during growth period = difference between dry matter in the two growing periods

Dry matter accumulation rate = dry matter/growth period days

Dry matter transport of stems and leaves (t $ha^{-1}$) = dry weight of stems and leaves at heading stage—the dry weight of stems and leaves at maturity

Stem and leaf dry matter transport rate efficiency = stem and leaf material transport capacity/dry weight of stems at heading × 100

Dry matter conversion rate of stems and leaves = dry matter transport capacity of stems and leaves/dry weight of spikes × 100

Contribution rate of dry matter to panicles dry weight after heading = (dry weight at maturity—dry weight at heading stage)/dry weight at maturity × 100

#### *2.4.2 Nitrogen Accumulation, Transport, and Efficiency*

Nitrogen accumulation in each part: The separated, dried stems, leaves, and spikes were taken to measure nitrogen accumulation in each growth stage across the seasons.

Total nitrogen accumulation in each period: The sum of nitrogen accumulation in stem, leaf, and ear of rice plants per unit area at each growth period.

Nitrogen accumulation proportion and rate during growth periods: The difference in nitrogen accumulation between the two growth stages of early and late-season rice.

Nitrogen dry matter production efficiency: The ratio of the dry matter accumulation per unit area to the total nitrogen accumulation per unit area.

Nitrogen use efficiency: The ratio of rice grain yield per unit area to total nitrogen accumulation per unit area.

Nitrogen harvest index: The ratio of nitrogen accumulation in panicles of mature rice to total nitrogen accumulation in plants.

Nitrogen transport: The difference between nitrogen accumulation in stems and leaves of rice at the heading stage and stem and leaf accumulation at maturity.

Nitrogen transport efficiency: The ratio of nitrogen transport capacity to nitrogen accumulation in stem and leaf of rice at the heading stage.

#### *2.4.3 Rice Yield and Yield Attributes*

Actual yield: The undisturbed five central rows from each plot were harvested for grain yield during the maturity of rice, dried and weighed, and the actual yield was calculated.

Yield component factors: Rice yield and yield attributes were calculated in plants harvested for grain yield. Grain yield was expressed in t $ha^{-1}$ at 14 % moisture content.

### ***2.5 Statistical Analysis***

Data were analyzed of following analysis of variance (Statistix 8, Analytical Software, Tallahassee, FL, USA), Microsoft Excel for figures and means of cultivars were compared based on the least significant difference test (LSD) at the ($p \leq 0.05$) probability level.

## 3 Results

### 3.1 Soil Chemical Properties

Results compiled show a significant effect ($p \leq 0.05$) of green manuring (GM) on soil organic carbon (SOC), total N (TN), available N (AN), total phosphorus (TP), available P (AP) and available K (AK) (Tab. 2). An average maximum SOC 30.92 g kg$^{-1}$, TN 1.43 g kg$^{-1}$ and AN 149.2 mg kg$^{-1}$ was noted in milkvetch-rice-rice (MV-R-R) treated plots, across the seasons. Higher TP 0.87 g kg$^{-1}$ and AP 28.12 mg kg$^{-1}$ was recorded in potato-rice-rice (P-R-R), while maximum AK 128.11 in rapeseed-rice-rice (R-R-R). Compared with winter-fallow-rice-rice (WF-R-R), treatment MV-R-R, R-R-R and P-R-R significantly increased SOC by 138.94%, 107.26% and 106.18%, TN by 32.4%, 23.14% and 30.55%, AN by 33.57%, 24.31% and 22.05%, TP by 33.33%, 33.33% and 45.12%, AP by 4.03%, 13.63% and 26.95%, similarly AK by 16.11%, 42.13% and 34.77% across the seasons, respectively. However a decrease in pH was recorded in green manure treated plots and ranked in order WF-R-R > P-R-R > R-R-R > MV-R-R in early season while WF-R-R > P-R-R > MV-R-R > R-R-R for late season.

**Table 2:** Effect of green manuring on Soil chemical properties before transplantation, early and late seasons (2017)

| SS | TT | pH | SOC | TN | AN | TP | AP | AK |
|---|---|---|---|---|---|---|---|---|
| | WF-R-R | 5.64 a | 13.24 d | 1.03 d | 115.16 d | 0.61 c | 22.13 d | 93.13 d |
| ER | MV-R-R | 5.25 b | 29.42 a | 1.34 b | 155.78 a | 0.79 b | 27.53 b | 107.83 c |
| | R-R-R | 5.32 c | 25.36 c | 1.28 c | 141.41 b | 0.84 a | 25.67 c | 127.61 a |
| | P-R-R | 5.45 d | 27.24 b | 1.41 a | 138.54 c | 0.89 a | 29.12 a | 120.6 b |
| | WF-R-R | 5.62 a | 12.64 d | 1.14 d | 108.24 d | 0.59 c | 22.17 d | 87.13 d |
| LR | MV-R-R | 5.56 c | 32.42 a | 1.53 a | 142.62 a | 0.82 a | 26.53 b | 112.83 c |
| | R-R-R | 5.54 c | 28.29 b | 1.38 c | 136.32 b | 0.77 b | 24.67 c | 128.62 a |
| | P-R-R | 5.58 b | 26.12 c | 1.42 b | 134.12 c | 0.85 a | 27.12 a | 122.34 b |

Note: SS: season, TT: treatment ER: Early rice LR: late rice, Soil organic carbon (SOC) g kg$^{-1}$, Total Nitrogen (TN) g kg$^{-1}$, Available Nitrogen (AN) mg kg$^{-1}$, Total Phosphorus (TP) g kg$^{-1}$, Available Phosphorus (AP) mg kg$^{-1}$, Available Potassium (AK) mg kg$^{-1}$. Means followed by different letters within column are significantly different from each other at ($p \leq 0.05$).

### 3.2 Dry Matter Accumulation

Dry matter, a function of metabolic activity, is an important indicator of economic yield. The effect of dry matter on dry matter accumulation was varied across seasons (Figs. 2A,2B). Results indicate that in the early rice, the dry matter accumulation determined from the green manure plots during the seedling to tillering period was found smaller than that of the control treatment. However, the accumulation of dry matter was tended to increase linearly from heading to maturity, in the green manure plots than control treatment and ranked in order (P-R-R > R-R-R > MV-R-R > WF-R-R). For the early season rice, the growth rate was inhibited during tillering, yet from tillering to maturity, the growth was promoted, which consequently increased the accumulation of final dry matter. In the late season, the dry matter accumulation during tillering, heading and maturity in the green manure plots was significantly ($p \leq 0.05$) greater than that of the control. Moreover, dry matter accumulation was greater for the late-season rice compared to the early rice. Our results imply that green manuring was able to sustainably increase the growth and accumulation of final dry matter in rice.

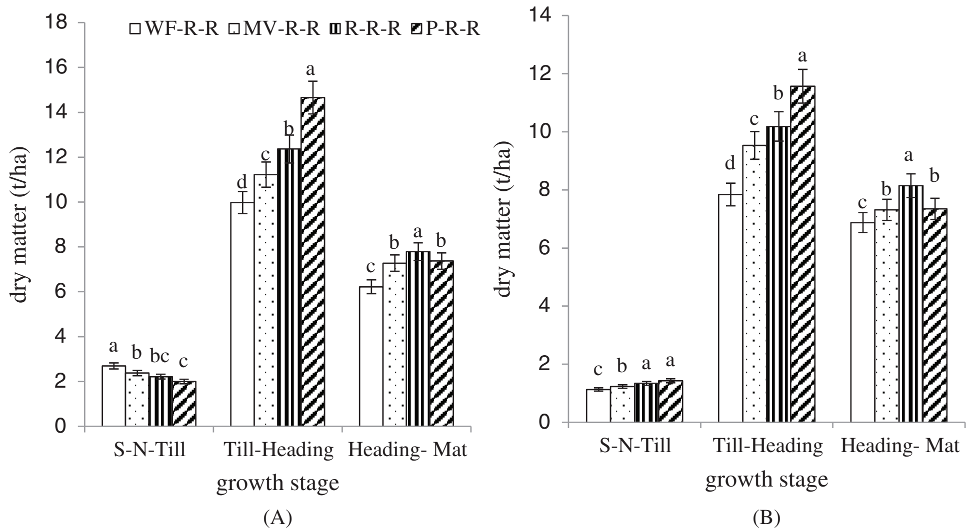


**Figure 2:** Effect of green manuring on dry matter of each growth period of rice early (A) and rice late (B)
Note: S-N-Till (seedling to tillering), Till-Heading (Tillering-Heading), Heading-Mat (Heading-Maturity). Vertical bars represent the standard error of mean. Different letters above the columns represent statistical differences from each other at ($p \leq 0.05$)

### *3.3 Dry Matter Accumulation Rate*

In the early rice, the dry matter accumulation rate for the number of days under green manuring was observed to be significantly lower than that of the control treatment at the tillering stage (Tab. 3). However, the accumulation rate was significantly higher ($p \leq 0.05$) from tillering to heading at 3.80%, 13.40%, 35.33%, and from heading to maturity at 18.68%, 17.93% and 16.87% in MV-R-R, R-R-R and P-R-R, than WF-R-R, respectively. Furthermore, the dry matter accumulation rate in the green manuring plots was found maximum than that in the control treatment over the whole growing period. For the late rice, the dry matter accumulation rate was observed to be significantly higher at all growing periods from tillering to maturity in green manure plots compared to the control treatment. In general, the dry matter accumulation rate in the green manure applied plots ranked in the order of P-R-R > R-R-R > MV-R-R relative to WF-R-R. The results revealed that for the late rice, green manuring enhanced soil fertility and sustainable growth in rice. The net growth production during all growth periods across the seasons was 4.58%, 10.87%, and 21.67% higher for the P-R-R treatment compared to those of R-R-R, MV-R-R, and WF-R-R, respectively.

**Table 3:** Effect of green manuring on dry matter accumulation rate (kg $ha^{-1}$ $d^{-1}$) during the growth period of rice

| SS | TT | SN-Till | Till-Head | Head-Mat | AGP |
|---|---|---|---|---|---|
| ER | WF-R-R | 50.85 a | 277.16 d | 188.57 c | 142.07 c |
| | MV-R-R | 44.04 b | 287.90 c | 227.43 b | 151.34 b |
| | R-R-R | 40.32 b | 316.98 b | 236.09 a | 157.54 b |
| | P-R-R | 34.56 c | 396.13 a | 223.33 b | 168.05 a |
| LR | WF-R-R | 26.84 c | 313.75 c | 196.41 c | 134.28 c |
| | MV-R-R | 29.32 b | 366.59 a | 208.93 b | 151.89 b |
| | R-R-R | 31.13 ab | 339.56 b | 232.75 a | 163.93 a |
| | P-R-R | 33.27 a | 373.22 a | 209.94 b | 168.17 a |

Note: AGP: All growth Period, SN-Till-Seedling to tillering, Till-Head-Tillering to heading, Head-Mat Heading to maturity.

### 3.4 Stem (Leaf) Dry Matter

Stem reserves from pre-anthesis assimilation are an important source of grain filling and yield. Our results indicate that green manuring was able to prolong the vegetative stage of the rice plants (Tab. 4). In particular, in the early rice, stem material transport levels under green manuring were determined as negative. This indicates that the stem at heading to maturity was still growing. Furthermore, no significant differences were observed in the leaf dry matter transport rate for MV-R-R, R-R-R, and WF-R-R, while the transport rate for P-R-R was significantly greater than WF-R-R by 13.14%. Moreover, the stem and leaf dry matter transport rate under the green manure treatments was significantly ($p \leq 0.05$) lower than that of the control treatment. Although the dry matter conversion rate of the stem and leaf to spike at heading stage was lower for the green manure plots compared to that of WF-R-R, however the difference was not significant ($p \leq 0.05$) for all treatments except R-R-R which was 6.44% minimum than the control treatment. Furthermore, the contribution rate of the dry matter to spike dry weight following heading in the green manure treatments was greater than that of the control treatment (R-R-R > MV-R-R > P-R-R > WF-R-R). The stem transport capacity of each treatment for the late rice was determined as negative, again indicating that the stem for each green manure treatment at heading was still growing. The stem transport rate was found to be higher for the P-R-R treatment, with the remaining treatments exhibiting rates in the order of MV-R-R > WF-R-R > R-R-R. Similarly, the leaf dry matter transport rate was observed to be 71.26%, 10.37%, and 4.19% higher in P-R-R compared to WF-R-R, MV-R-R, and R-R-R, respectively. In addition, the green manuring treatments exhibited a leaf dry matter transport capacity that was 43.24%, 48.69%, and 52.54% higher in P-R-R, R-R-R, MV-R-R than WF-R-R, respectively. Moreover, compared to the control, the green manure treatments were associated with maximum stem and leaf dry matter transfer rates at heading and stem (leaf) dry matter conversion rate to panicle dry weight, and ranked as follows: MV-R-R > R-R-R > P-R-R > WF-R-R. The difference between the green manure and control dry matter contribution rate to spike dry weight following heading was smaller in the late rice compared to the early rice, with minimum levels associated with the P-R-R treatment.

**Table 4:** Effect of green manuring on Stem (Leaf) dry matter transport (SLDMT) t ha$^{-1}$, Leaf dry matter transfer rate (LDMTR) %, Leaf dry matter conversion rate (LDMC) % and Contribution rate of dry matter to panicle dry weight after heading (CRODMTPDWAH)% of rice

| SS | TT | SDMT | LDMT | LDMTR | LDMC | CRODMTPDWAH |
|---|---|---|---|---|---|---|
| ER | WF-R-R | 0.74 a | 1.35 b | 37.41a | 12.22 a | 56.46 b |
| | MV-R-R | −0.18 b | 1.33 b | 34.06 b | 11.81 ab | 64.45 a |
| | R-R-R | −0.36 c | 1.36 b | 31.96 c | 11.48 b | 65.75 a |
| | P-R-R | −0.16 b | 1.54 a | 32.84 bc | 12.14 a | 58.14 b |
| LR | WF-R-R | −1.07 b | 0.87 d | 40.33 b | 10.21 b | 80.61 a |
| | MV-R-R | −1.01 b | 1.35 c | 49.57 a | 13.63 a | 73.65 b |
| | R-R-R | −1.43 c | 1.43 b | 49.43 a | 13.34 a | 75.93 b |
| | P-R-R | −0.32 a | 1.49 a | 48.44 a | 13.34 a | 65.76 c |

### 3.5 Nitrogen Accumulation in Stem, Leaf and Spike

The results showed that nitrogen accumulation was significantly ($p \leq 0.05$) affected by green manures in early season at tillering stage as compared to winter fallow, depicted in Fig. 3A. The maximum nitrogen in stem 21.44 g kg$^{-1}$ and 28.61 g kg$^{-1}$ in leaf was recorded in P-R-R treatment. Moreover, at heading stage the concentration of nitrogen varied in stem, leaf and spike. In stem N content was significantly higher in green manuring treatments as compared to WF-R-R, while the concentration was found non-significant between green manuring and winter fallow treatment in leaf and spikes (Fig. 3B). At maturity, the concentration of N was found higher in stem and leaf of green manuring treatments, while the effect was found non-significant in spikes. GM enhanced the vegetative growth, delayed the maturity therefore slowdown the transportation of N from leaf to spike in early season. The concentration of nitrogen in spike at maturity was ranked in order R-R-R > P-R-R > WF-R-R > MV-R-R (Fig. 3C). Moreover, in late season the nitrogen concentration was found non-significant between GM and WF-R-R at tillering stage in both stem and leaf (Fig. 3D). However, at heading stage the concentration of N was higher in WF-R-R in stem (10.25 g $kg^{-1}$) and leaf (25.22 g $kg^{-1}$), while the results was found non-significant between GM and WF-R-R treatments in spike (Fig. 3E). At maturity the concentration of N in stem was maximum in WF-R-R (5.48 g $kg^{-1}$) minimum in R-R-R (4.16 g $kg^{-1}$), while the effect was found non-significant in leaf N concentration. Moreover, the N concentration in spike was found higher in R-R-R (12.42 g $kg^{-1}$) which was statistically at par ($p \leq 0.05$) with P-R-R (Fig. 3F).

### *3.6 Total Nitrogen Accumulation*

Fig. 4A demonstrates that nitrogen uptake was lower at tillering for the green manure treatments compared to the control, with the maximum uptake observed for WF-R-R. However, nitrogen usage was maximized at heading and maturity, while the uptake of the green manure plots was significantly higher than that of the control (MV-R-R > R-R-R > P-R-R > WF-R-R). As compared to early rice, maximum nitrogen uptake was observed in green manure applied plots at the tillering stage in late season. In addition, the heading stage also exhibited higher uptake percentages, with the order of P-R-R > R-R-R > MV-R-R > WF-R-R observed at both tillering and heading (Fig. 4B). Interestingly, this trend was not observed at maturity, with greater nitrogen uptake levels recorded in the control compared to the green manure treatments (WF-R-R > R-R-R > P-R-R > MV-R-R). The results indicate that in the late rice, the nitrogen uptake at tillering and heading under the green manure plots was greater than the control, with inhibit N uptake in later stages. However, total nitrogen uptake was found greater in the green manure plots compared to the control.

### *3.7 Nitrogen Accumulation Proportion and Rate*

It is revealed from the results that the effect of green manuring on the proportion and rate of N accumulation varied across stages and seasons (Tab. 5). Accumulation proportions and rates from seedling to tillering were significantly lower ($p \leq 0.05$) in the green manure plots compared to the control treatment. However, the opposite was true from tillering to heading, with proportions and rates recorded as 14.54 and 27.14%, 41.09 and 21.17%, and 45.04 and 73.42% significantly higher in MV-R-R, R-R-R, P-R-R than WF-R-R, respectively. At maturity, nitrogen accumulation proportions in R-R-R and P-R-R were significantly lower than that of the control. However, that of MV-R-R was 4.50% (non-significantly) higher than that of the control. In the late rice, the nitrogen proportion of green manure treatments was found to be statistically at par ($p \leq 0.05$) to WF-R-R treatment, with nitrogen accumulation rates 10.63%, 11.70%, and 22.34% greater in the MV-R-R, R-R-R, and P-R-R treatments than WF-R-R, respectively. The N proportions and accumulation rates subsequently increased from tillering to heading for MV-R-R, R-R-R, and P-R-R compared to WF-R-R by 6.88 and 11.52%, 10.70 and 10.08%, and 9.64 and 10.08%, correspondingly. Following this, similar to the early rice, from heading to maturity, the nitrogen proportion and accumulation rates of the green manure plots were significantly lower than those of the control.

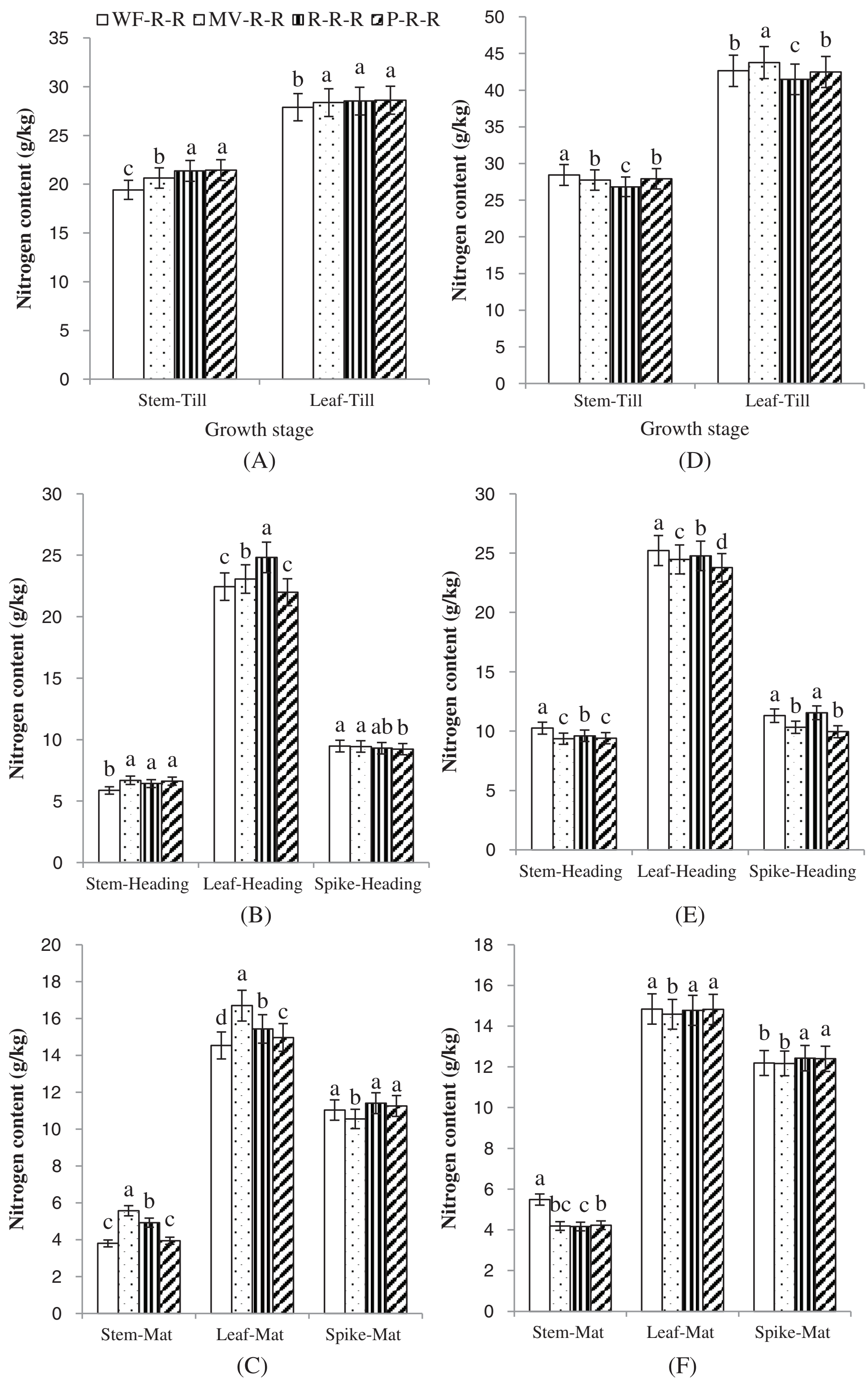


**Figure 3:** Effect of green manuring on nitrogen accumulation at each part of rice early (A-C) and rice late (D-F). Note: Till (N content at tillering stage in stem and leaf), Heading (N content at heading stage in stem, leaf and spike), Mat (N content at maturity stage in stem, leaf and spike)

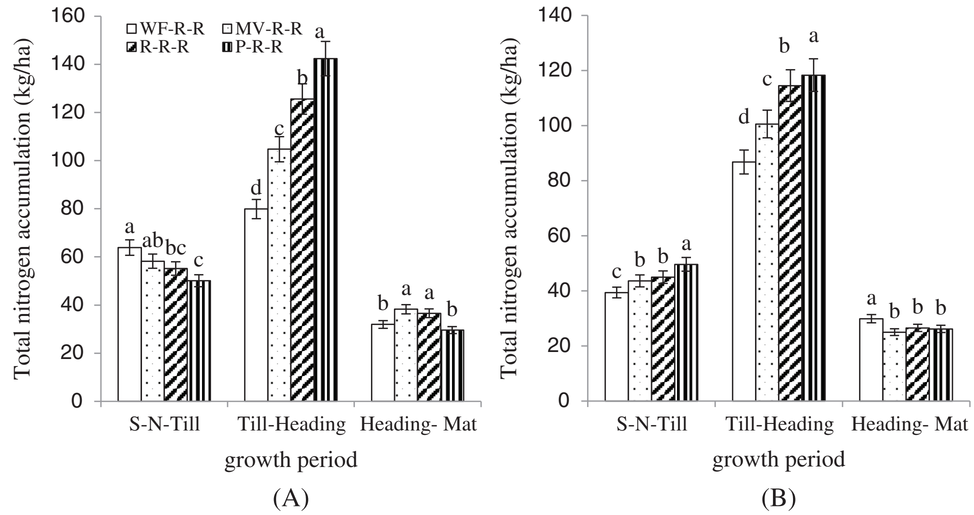


**Figure 4:** Effect of green manuring on total nitrogen accumulation at each growth stage of rice early (A) and rice late (B)

**Table 5:** Effect of green manuring on nitrogen accumulation proportion (PP) (%) and rate (AR) ($kgha^{-1}d^{-1}$) during growth period of rice early and rice late

| SS | TT | S-N-Till | | Till-Head | | Head-Mat | |
|---|---|---|---|---|---|---|---|
| | | PP | AR | PP | AR | PP | AR |
| ER | WF-R-R | 36.34 a | 1.21 a | 45.46 d | 2.22 d | 18.19 a | 0.97 b |
| | MV-R-R | 28.93 b | 1.08 ab | 52.07 c | 2.69 c | 19.01 a | 1.20 a |
| | R-R-R | 25.38 c | 1.00 b | 57.80 b | 3.22 b | 16.83 b | 1.11 a |
| | P-R-R | 22.55 d | 0.86 c | 64.14 a | 3.85 a | 13.31 c | 0.90 b |
| LR | WF-R-R | 25.25 a | 0.94 c | 55.59 c | 3.47 b | 19.15 a | 0.85 a |
| | MV-R-R | 25.78 a | 1.04 b | 59.42 b | 3.87 a | 14.80 b | 0.72 b |
| | R-R-R | 24.19 a | 1.05 b | 61.54 a | 3.82 a | 14.27 bc | 0.76 b |
| | P-R-R | 25.56 a | 1.15 a | 60.95 a | 3.82 a | 13.49 c | 0.75 b |

### *3.8 Nitrogen Dry Matter, Grain Production Efficiency and Harvest Index*

Nitrogen dry matter production efficiency (NDMPE) in the early rice was observed to be lower in MV-R-R and R-R-R compared to WF-R-R, while that of P-R-R was statistically at par ($p \leq 0.05$) with WF-R-R (Tab. 6). Moreover, nitrogen grain production efficiency (NGPE) was significantly higher by 13.23%, 17.30%, and 17.98% in WF-R-R compared to MV-R-R, R-R-R, and P-R-R, respectively. Similarly, the nitrogen harvest index (NHI) was 16.92%, 11.35%, and 7.80% higher in WF-R-R than MV-R-R, R-R-R, and P-R-R, respectively. For the late rice, NDMPE was higher by 5.07%, 4.06% and 3.20% in MV-R-R, R-R-R and P-R-R than WF-R-R, respectively. However, there were no significant differences in NGPE between WF-R-R and the green manure treatments. Furthermore, the green manure treatments exhibited greater NHI compared to the control and ranked in order, R-R-R > MV-R-R > P-R-R > WF-R-R.

**Table 6:** Effect of green manuring on nitrogen dry matter production efficiency (NDMPE) (kg $kg^{-1}$) nitrogen grain production efficiency (NGPE) (kg $kg^{-1}$) and nitrogen harvest index (NHI) (%) of rice

| SS | TT | NDMPE | NGPE | NHI |
|---|---|---|---|---|
| ER | WF-R-R | 107.54 a | 46.37 a | 69.23 a |
| | MV-R-R | 103.81 b | 40.95 b | 59.21 d |
| | R-R-R | 102.93 b | 39.53 b | 62.17 c |
| | P-R-R | 108.25 a | 39.30 b | 64.22 b |
| LR | WF-R-R | 101.49 b | 41.68 a | 66.58 b |
| | MV-R-R | 106.77 a | 41.10 a | 71.38 a |
| | R-R-R | 105.70 a | 39.34 a | 71.65 a |
| | P-R-R | 104.80 a | 38.63 a | 71.35 a |

### *3.9 Stem (Leaf) Nitrogen Transportation Rate and Efficiency*

Tab. 7 indicates that in the early rice, the nitrogen transport rate of stems in the P-R-R treatment was 26.39% greater than that of the control treatment, yet the MV-R-R and R-R-R treatments exhibited significantly lower values compared to the control (WF-R-R > R-R-R > MV-R-R). Similarly, nitrogen transportation efficiency was also determined to be lower in the green manure plots compared to the control (WF-R-R > P-R-R > R-R-R > MV-R-R). In addition, the leaf nitrogen transport rates of R-R-R and P-R-R were significantly greater (26.78% and 16.39%) than those of WF-R-R. The opposite was true for the MV-R-R treatment, yet the difference was not significant. Leaf nitrogen transport efficiencies of MV-R-R, R-R-R, and P-R-R were lower than WF-R-R by 12.88%, 3.03%, and 9.10%, respectively. This indicates that for the early rice, the leaf nitrogen transport efficiency of stems and leaves treated by green manuring was generally low compared to the control. In the late rice, the nitrogen transport capacity and efficiency in both the stems and leafs were significantly greater in the green manure plots than the control treatment. In particular, the stem and leaf N transportation rates were greater in the MV-R-R, R-R-R, and P-R-R treatments by 35.96 and 27.85%, 40.76 and 34.50%, and 68.34 and 33.74% compared to the WF-R-R treatment. Moreover, stem nitrogen efficiency was significantly higher by 30.19%, 27.87%, and 41% in MV-R-R, R-R-R, and P-R-R than the control. Similarly, leaf nitrogen transport efficiency was significantly greater ($p \leq 0.05$) in the green manure plots (MV-R-R > R-R-R > P-R-R > WF-R-R).

**Table 7:** Effect of green manuring on Stem (Leaf) nitrogen transportation, SLNT (kg $ha^{-1}$) and Stem (Leaf) nitrogen transportation efficiency, SLNTE (%) of rice

| SS | TT | SNT | SNTE | LNT | LNTE |
|---|---|---|---|---|---|
| ER | WF-R-R | 15.98 b | 42.83 a | 48.05 c | 59.47 a |
| | MV-R-R | 6.64 d | 14.54 d | 47.17 c | 52.27 c |
| | R-R-R | 9.17 c | 19.64 c | 60.92 a | 57.69 a |
| | P-R-R | 20.84 a | 39.14 b | 55.93 b | 54.29 b |
| LR | WF-R-R | 17.81 d | 35.01 c | 35.32 c | 64.90 b |
| | MV-R-R | 25.62 c | 47.46 b | 46.75 b | 69.95 a |
| | R-R-R | 26.93 b | 46.37 b | 50.05 a | 69.82 a |
| | P-R-R | 36.30 a | 53.07 a | 49.66 a | 67.86 a |

### *3.10 Yield and Yield Attributes*

Tab. 8 shows that the effect of green manure on rice grain yield and yield attributes varied across seasons. More specifically, in the early (late) rice, the yield was 5.82% (15.38%), 5.39 (7.75%), and 1.1% (2.45%) higher in P-R-R compared to WF-R-R, MV-R-R, and R-R-R. Furthermore, Maximum number of effective panicles and panicle$^{-1}$ spikelets were recorded for the green manure plots compared to the control in both early and late rice, yet the difference was non-significant for panicle$^{-1}$ spikelets in late rice. The early rice exhibited a greater grain filling percentage in the control (WF-R-R > MV-R-R > P-R-R > R-R-R). In contrast, however the P-R-R, R-R-R and MV-R-R treatments were associated with greater grain filling percentages compared to the control for the late rice (8.86%, 7.81% and 1.12%, respectively). There were no significant differences ($p \leq 0.05$) in thousand grain weight among treatments in the early season, yet for the late season, the treatments were ranked as follows: P-R-R > WF-R-R > R-R-R > MV-R-R.

**Table 8:** Effect of green manuring on yield and yield components of rice

| SS | TT | YI (t ha$^{-1}$) | EP ($10^4$/ha) | SPP | FGP (%) | 1000-GW (g) |
|---|---|---|---|---|---|---|
| | WF-R-R | 8.15 c | 279.78 b | 201 c | 64.66 a | 24.57 a |
| ER | MV-R-R | 8.24 bc | 298.45 ab | 203 bc | 60.93 ab | 24.44 a |
| | R- R-R | 8.59 ab | 326.89 a | 208 ab | 57.56 b | 24.16 a |
| | P- R-R | 8.72 a | 331.56 a | 213 a | 57.61 b | 24.09 a |
| | WF-R-R | 6.50 b | 240.45 b | 196 a | 67.65 b | 23.04 b |
| LR | MV-R-R | 6.96 ab | 252.22 b | 197 a | 68.41 b | 23.12 b |
| | R- R-R | 7.32 a | 256.00 a | 201 a | 72.94 a | 23.24 ab |
| | P- R-R | 7.50 a | 261.33 a | 199 a | 73.55 a | 23.73 a |

Note: EP: effective panicles, SPP: spikelets per panicle; FGP: filled grain percent; 1000-GW:1000-grain weight

## 4 Discussion

### *4.1 Green Manuring Ameliorated Soil Fertility, Dry Matter Production and Translocation*

Inorganic fertilizer is commonly applied to encourage the rapid growth of crops. Furthermore, it is immediately absorbed by plants following application, resulting in great nitrogen losses via leaching and ammonia volatilization [33]. In current study we found that green manuring is a substantial way to enhanced soil fertility as evident from results that soil chemical properties across the seasons were significantly ($p \leq 0.05$) improved by GM treatments than winter fallow (Tab. 2). This result is consistent with previous study concluding that soil fertility and biomass increase due to additional organic fertilizer application was significantly maximum than obtained from sole N fertilizer application under long-term fixed paddy field experiments conducted for more than ten years [24].Similar results were also reported by Pramanik et al. [34] that inclusion of winter crops as a green manure for subsequent crops are more effective for increasing the soil N supply than winter fallow. Dry matter content (the ratio of stem, leaf, dry panicle mass to fresh mass) is often used as an indicator of the particular resource strategies used by plants, namely, the trade-off extent between rapid assimilation and growth at one extreme, and efficient conservation of resources within well-protected tissues at the other [35]. In the current study, dry matter production performed positively to green manure during the period of heading to maturity for early rice. Furthermore, for the late rice, growth and dry matter accumulation was maximized in green manure plots across all stages. In particular, nitrogen uptake was observed to rank in the following order across both seasons: MV-R-R > R-R-R > P-R-R > WF-R-R. However, for the early rice, from the seedling to the tillering stage, green manure was observed to inhibit the growth of rice (Figs. 2A and 2B). This may be attributed to the slow and steady degradation of green manure, as well as the release of organic acid in the soil via microorganisms involved in the decomposition of the green manure, and reduction in pH (Tab. 2). In contrast, mineral nitrogen is a quick-release fertilizer that was observed to rapidly enhance growth. A similar trend was reported by DOU et al. [36], whereby that the harmful substances (mainly organic acids) released by straw were returned to the field, significantly reducing soil pH and the soil oxidation-reduction potential (EH). Such a phenomenon is not conducive to the growth of rice root systems, resulting in the significant inhibition of rice tillering.

In the late season, green manure was observed to have a significant effect on dry matter across all growing stages, resulting in greater levels of biomass accumulation compared to the winter fallow plots (Fig. 2B). Fen et al. [21] demonstrated that green manure in the form of straw was able to increase the number and activity of microorganisms, thus simultaneously enhancing the transformation and fixation of inorganic phosphorus in the soil. In addition, Wang et al. [37] expanding on the initial inhibitory effect of green manuring, whereby soil microorganisms in the soil greatly increased following the return of crop straw to the field. However, the crop straw C/N and C/P ratios are large, thus microorganisms need to absorb available nitrogen and phosphorus from the soil. This reduces the available nitrogen and phosphorus in the soil when the straw is initially returned, and subsequently inhibit the early stage growth of rice. However, significant increase occurs during the later growth stages due to maximum soil organic carbon and nitrogen availability. P-R-R was identified as the most promising amongst treatments in terms of growth and dry matter production. This may be a result of the substantial amount of nitrogen available via green potato manuring, as well as increases in soil fertility (Tab. 2). Ullah et al. [15] identified an increase in the number and activity of microorganisms in the soil due to the returning of plant straw into soil, simultaneously enhancing the transformation and fixation of organic nitrogen in the soil. Inorganic nitrogen is the main form of nitrogen uptake by plants [38]. Therefore, reductions in the available nitrogen and phosphorus in the soil results in insufficient amounts of these two elements for crop use, which consequently slows down the growth of rice. In the current study, green manuring enhanced the total growth of rice and dry matter accumulation across seasons compared to the control (Tab. 3). This may be attributed to the greater availability of nutrients in the soil throughout the seasons. Our results are in line with Huang et al. [39], who determined that growing winter fallow-rice-rice destroyed the soil nutrient status as well as the physical properties of reddish paddy soil. In addition, Xie et al. [40] reported that applying mineral fertilizer can result in low levels of soil organic matter and can also degrade crop productivity. Green manure cultivation has several advantages, including improved biological nitrogen fixation, and decreased $NO_3^-$-N leaching loss and soil erosion [8]. Similarly, Van Noordwijk et al. [41] indicate that the green manuring of crops not only transfers nutrients to the soil but can also lead to a deep root system for nutrient uptake. Thus, roots can absorb less available nutrients in deeper soils, thereby increasing the concentration of plant nutrients in the soil surface, reducing the use of fertilizer, and producing relatively large amounts of organic matter. This essentially leads to maximum dry matter production [42].

Our experiments demonstrate the significant effects of the treatments on dry matter translocation from the stem and leaf to spike dry weight at heading. In particular, the maximum and fasted translocation of assimilates from the stem to the spike was observed in the WF-R-R treatment. In contrast, the green manure plots demonstrated low translocation rates from the stem to the spike due to stem growth, which prolonged the vegetative stage of the green manure plants. However, the leaf translocation rate of green manures plots, was observed to higher across the seasons (Tab. 7). Our results indicated that due to the longer green period of the stems, a high photosynthetic ratio contributed maximum dry matter translocation at post-anthesis, while enhancing the sink capacity and the photosynthetic potential to grain ratio. The large amount of dry matter may be a result of the application of appropriate amounts of nitrogen fertilizer, which can promote the propagation of microorganisms, accelerating the decomposition of returning crop straw by microorganisms, and thus directly providing sufficient nitrogen amounts for crop growth. Source-sink relationships is the key way for the translocation of stimulants and this allocation determines the crop yield of plants. Similar to our findings, Wang et al. [25] reported that the photosynthetic products stored in the culm and leaves contribute to 20–30% of rice grain yield and can be transported to the spikelets and grains of rice after heading. Therefore, the apparent transportation ratio and the transformation ratio of dry matter play vital roles in grain yield formation. In the current study, the contribution rate of dry matter to spike dry weight after heading in green manure treatments was greater than that of the control in the early season, while the opposite was observed in the late season (Tab. 4). The difference in the conversion rate to panicle dry weight following heading between early and late seasons may be due to fact that the leaf dry matter transport rate and conversion ratio after heading were lower (greater) than the control during the early (late) season. Sangakkara et al. [43] documented significant increases in root dry weight and the root weight per unit length in maize following the application of green manure. They also noted that green manure is able to stimulate the partitioning of dry matter to roots, resulted maximum dry matter production and grain yield, thus playing an essential role in nutrient acquisition.

### *4.2 Green Manuring Improved Nitrogen Uptake, Transformation, and Nitrogen use Efficiency*

Green manure is a plant material that can be embedded into the soil. It is proved as an important alternative source of mineral fertilizers [44]. Moreover, N is the most important limiting nutrient in irrigated rice systems [45]. In the current study, the green manure treatments of MV-R-R, R-R-R, and P-R-R showed minimum total N accumulation than WF-R-R (control) in the early rice at the tillering stage (Fig. 4A). This may be a result of the insufficient nutrients for plant growth from green manure decomposition at the early stage, or reductions in pH due to substantial organic acid accumulation and biomass degradation. Our findings are in agreement with Deng et al. [46] who demonstrate that the harmful substances (mainly organic acids) released by straw returning to the field significantly reduce soil pH and soil oxidation-reduction potential (EH). These effects are not conducive to the growth of the rice root system, resulting in the significant inhibition of rice tillering and reductions in SPAD values. In the current study, total nitrogen accumulation from tillering to maturity stage was observed to be significantly higher in the green manure plots across seasons compared to the control, with P-R-R ranked as first amongst all treatments. This may be linked to the strong effect of the potato manure fertilization and the large amounts of available nitrogen and phosphorus. Fageria et al. [47] found that returning wheat straw to the field increased the amount of nitrogen, particularly at the tillering phase, thus improving the root activity and promoting dry matter accumulation and the absorption of mineral nutrients. Zou et al. [48] demonstrated significant improvements in the early growth of rice following the application of basal nitrogen fertilizer combined with returning straw to the field. Increases in the basal nitrogen fertilizer level enhanced the growth level and physiological activity of the upper and lower parts of the plant, including SPAD values, photosynthetic rate, root length, root diameter and root oxidation power, and also promoted the accumulation of assimilates.

In rice, both inorganic fertilizers and green manures are known to increase the plant availability of soil N via the added nitrogen interaction [49,50]. Rice yield is closely associated with sink-source circulation, where the sink size and source strength are important for the growth rate and yield formation [51]. In the current study, green manuring was observed to inhibit the nitrogen uptake and accumulation rate per day in early rice from seedling to tillering. This may be linked to decease in pH level and a high bulk density, with significant enhancements in both from tillering to heading (14.54 and 27.14%), (41.09 and 21.17%) (45.04 and 73.42%) in the MV-R-R, R-R-R and P-R-R treatments compared to WF-R-R, respectively. However, from heading to maturity, the N proportion and accumulation rate fell below those of the control, although this change was not significant. This may be attributed to greater post-anthesis biomass production and delayed physiological maturity in the green manure plots compared to the control. Our results are in agreement with [14], who reported that the greater biomass accumulation observed in the green manuring is also indicative of high growth rates in the rice plants. This is a result of a reduction in the soil bulk density and an increase in soil organic matter content and nutrients other than N. During the later ice seedling to heading stage, the nitrogen accumulation rate and proportion were consistently higher, with accumulation rates 10.63%, 11.70%, and 22.34% greater in MV-R-R, R-R-R, and P-R-R compared to WF-R-R. A similar trend was recorded from tillering to heading, while the accumulation rate reduced from heading to maturity in the green manuring plots (Tab. 5). The higher nitrogen ratio, a result of the substantial nutrient supply, led to a greater biomass production compared to the early season from seedling to heading (Tab. 2). Similar results are reported by Van Der Heijden et al. [52] and may be attributed to the additional effects of green manure on soil productivity.

The effects of green manuring on crop growth and nutrient use are linked to improvements in soil physiochemical properties, including bulk density, water conductivity, and carbon and N levels [53]. Increasing N use efficiency in crop production highly depends on the synchrony between crop N demand and the supply from various sources throughout the growing season [54]. In the current study, green manuring was found to be a sustainable source of energy for crop production. For the early rice, the nitrogen transport rate and efficiency in both the stem and leaf were observed to be lower in the green manuring plots than the control at heading. However, the opposite was true in the late-season (Tab. 7). Previous studies support our findings; green manure plants not only contain the primary macro-nutrients (N, P, and K), but also several medium- and micro-nutrients (e.g., Ca, Mg, and Si), which ensures a balanced, sustainable nutrient supply in paddy soil [55]. In addition, combining manures with chemical fertilizer can improve the ability of the soil to supply nitrogen by promoting the mineralization potential and rate of soil organic nitrogen, which subsequently promotes nitrogen absorption and accumulation in plants [56]. In addition, using milk vetch with chemical fertilizer is able to reduce soil bulk density and the SOC consumption rate in paddy fields, as well as increasing soil porosity and the proportion of macro aggregates [18]. Furthermore, it can increase soil enzyme activity and enhance soil microorganism resistance to environmental stresses. Islam et al. [57] reported that the substitution of chemical fertilizer by green manure improved the root growth environment for rice, and also increased the root density, biomass, activity, and nutrient absorption. In the current study, the application of green manure increased the total nitrogen transport rate and accumulation via the decomposition of organic components, which provided more available nitrogen and phosphorus than the control. This suggest that, despite reductions in the chemical fertilizer, the application of green manure to the soil may improve the quality and quantity of SOC over several years and improve the ability of soil to supply nitrogen [58]. Thus, the application of green manure can enhance the sustainability of rice production.

### *4.3 Green Manuring Increased Rice Yield by Increasing Yield-Related Traits*

Chemical fertilizers are commonly used to improve crop productivity as crops are able to immediately absorb them following their application [59]. However, combining chemical fertilizer with green manure reduces N losses, stimulates plant growth, and increasing NUE [33]. YANG et al. [60] reported that using green vetch as manure with urea as a basal application significantly increased N availability in soil and improved rice yields by 13.6–26.5%. Our results indicate that the application of green manure was able to significantly enhance rice grain yield in both the early and late seasons (Tab. 8). This may be attributed to the maximum levels of nutrients availability throughout the season, as well as a higher nitrogen accumulation, and post-anthesis biomass translocation. This is consistent with previous research. For example, Durán et al. [61]; Raheem et al. [62] demonstrated an increase in dry matter accumulation, the number of panicles and grains per panicle, and a significant increase in rice yield from the combination of emerald palm and chemical fertilizer. Moreover, Yao et al. [63] showed that returning rice straw into the field was able to increase the number of effective panicles and the yield of rice.

In the current study, the application of green manure in the winter crops was observed to promote rice yield via an increase in the number of spikelets across seasons and late season thousand-grain weight. Similar results were reported by Chen et al. [64,65], that green manure promoted the tillering period of rice, with the effective tiller increasing by 27.2%, however the seed setting rate and 1000 grain weight were observed to decrease significantly. Wang et al. [66] demonstrated that the application of green manure and the return of straw to the field reduced fertilizer amounts, improved effective panicles percentage resulted an increase in rice grain yield. In addition, Xing et al. [67] reported the significant promotion of the effective panicle number and increases in rice yield following two consecutive years of rice straw mulching. In a study by Sheehy et al. [68], rice yield was able to increase by 1.1–4.2% via the return of 1/4 of rapeseed to the whole field. The increasing yield observed in the current study is associated with the increase of grain number per panicle and the 1000 grain weight. Similarly, Wang et al. [69] documented an increase in the number of grains per ear and the 1000 grain weight from the return of rice straw to the field.

Ideally, from an agricultural productivity point of view, green manuring has a positive effects on the subsequent crops. Compared with pure-synthetic fertilization, the combination of winter crops with chemical fertilizer results in higher amounts of biomass, effective soil mineralization, and significantly increases the productivity of the subsequent late rice. Furthermore, the addition of other nutrients (e.g., potassium and phosphorus) and minerals via the application of winter crop biomass may directly increase rice yields.

## 5 Conclusion

Our results demonstrate that the combined application of winter crops (milk vetch, oilseed rape, and potato crop) with inorganic fertilizer significantly increased soil fertility, total biomass and nutrient productivity. Biomass yield increased linearly throughout the growth stages in both the early and late seasons. Increased nutrient inputs via the green manure stimulated the growth of the rice plants and significantly increased rice yields, soil N supply and dry matter accumulation. Average rice yields in plots that included winter crops as green manure were greater than those that were just applied with urea. Furthermore, combined application of GM and synthetic N fertilizer enhanced nitrogen availability in flooded rice soil, thus minimizing the environmental risks related to N leaching. Therefore, the application of winter crops as green manure for early and late rice may be able to maintain rice yield with lower amounts of N input. In summary, we highlight the potential of the potato crop as green manure combined with urea as an effective agronomic practice to increase biomass accumulation and nutrient productivity in a double rice cropping system.

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