Productivity and Profitability of Mechanized Deep Nitrogen Fertilization in Mechanical Pot-Seedling Transplanting Rice in South China

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Abstract

Background: The deep nitrogen (N) fertilization coupled with mechanical pot-seedling transplanting rice (DNF-MPT) is an effective alternative to traditional transplanting rice with broadcasting fertilizer, however, little is known about its effects on grain yield, nutrient accumulation, and economic profitability. In present study, a two-year field experiment was conducted in early seasons (March-July) of 2019 and 2020. All seedlings were transplanted by DNF-MPT, whereas four treatments were designed as: MD: mechanized deep placement of all fertilizers as basal fertilizer; MDB: mechanized deep placement of 70% fertilizers as basal fertilizer, 30% broadcasting fertilizers at panicle initiation stage; MB: manual broadcasting of 40%, 30% and 30% fertilizers at the transplanting, tillering and panicle initiation stages, respectively; and CK: no fertilizer was applied during entire growth stages.

Results: The results indicated that MD treatment substantially improved the grain yield by 33.48-36.35%, total nitrogen accumulation (TNA) by 26.38-44.15%, total phosphorus accumulation (TPA) by 28.72-30.23%, and total potassium accumulation (TKA) by 25.61-37.33%, respectively, compared with MB treatment. Deep placement of N fertilization treatments i.e., MD and MDB remarkably promoted root morphological indexes and total root dry weight. Furthermore, nitrate reductase (NR), glutamine synthetase (GS) activities and total chlorophyll content (total Chl) of leaves were also enhanced under deep placement of N fertilizer. Overall, the MD treatment had the highest benefit cost ratio (BCR) owing to high gross returns and low input costs.

Conclusions: Deep placement of N fertilizer coupled with mechanical pot-seedling transplanting rice could be better alternative to conventional rice production system with more economic benefits.

Background

Rice (*Oryza sativa* L.) is one of the major food crops in the world and consumed as staple food for over 50% of the world population (Abid et al., 2015). China is the largest rice producer, currently accounting for 35% of the world's total rice production (Kong et al., 2017). Therefore, rice production becomes very important to feed the growing population in China and other parts of the world (Chen et al., 2018). At present, transplanting is still the most important rice planting method in most parts of China, which is accounting for 50% of country's rice cultivation area (Peng et al., 2009). With the rapid economic development, the transfer of most labor to cities has caused the lack of labor in rural areas. Thus, the traditional transplanting method is not suitable for the current development of Chinese agriculture (Bhushan et al., 2007; Knight et al., 2011). Therefore, improving the mechanization in rice production systems could solve the problem of labor shortage with better economic efficiencies.

At present, the mechanical carpet-transplanting method is mainly used for rice transplanting in China (Hu et al., 2014). This approach has some limitations, including high sowing rates, poor seedlings, and severe mechanical damage to seedlings during transplanting (Zhi et al., 2017). However, there are many advantages in mechanical pot-seedling transplanting rice, including cultivating elder seedlings with soil bowl, almost no damage in mechanical transplanting precisely, designing basic seedlings accurately, forming an appropriate number of tillers and large productive panicle. Moreover, it can improve the aeration and light penetration, lodging resistance, and boost photosynthetic production capacity in the middle and late stages of the rice population, which is conducive to get high yield on sustained basis (Zhang and Gong., 2014). Therefore, it is imperative to develop alternative rice planting technology based on machine transplanting with mechanical deep fertilization so as to meet farmers’ requirements and national food security and sustainability.

Nitrogen (N) is one of the most important nutrients for rice productivity (Wang et al., 2018). Balanced use of N fertilizer could significantly enhance crop productivity without environmental and economic constraints (Yan et al., 2018). However, surface application of N fertilizer often results in loss of N fertilizer through ammonia volatilization and runoff, thus reducing the utilization efficiency of N fertilizer (Watanabe et al., 2009). Deep fertilization effectively reduced the volatilization and loss of fertilizer on the soil surface, improved the absorption of fertilizer by crops, and increased the fertilizer utilization rate from 30~50% (Mohanty et al., 1998). The appropriate fertilization depth could not only promote the growth and development of rice, but also improve nutrient accumulation and decrease the appearance of diseases and insect pests (Wu et al., 2017). In addition, Liu et al. (2015) observed that deep N fertilization could effectively reduce the greenhouse gas emissions including N₂O and NH₃, and decrease N loss under field conditions, thus improves N utilization and ultimately rice yield. Recently, mechanized transplanting coupled with deep placement of N fertilizer is an accurate and reliable technology, which is of great significance to improve rice productivity (Zhu et al., 2019). It further enhanced the root volume by 84.11% for soil depths > 15 cm compared to broadcasting fertilizer (Chen et al., 2018). Furthermore, deep fertilization e.g., to place the fertilizer near the rice root is beneficial for its rapid uptake via growing roots (Liu et al., 2017).

Although deep N fertilization coupled with mechanical pot-seedling transplanting rice is an effective rice production strategy, nevertheless, little is known about the its effects on grain yield, nutrient accumulation, root characteristics, and economic benefits in rice. Therefore, the present study was conducted to assess the superiority of mechanical pot-seedling transplanting over traditional production system with the hypothesis that DNF-MPT could improve grain yield, nutrient acquisition, root indexes, and economic benefits of rice.
Materials And Methods

Experimental site and materials

Deep nitrogen fertilization coupled with mechanical pot-seedling transplanting machine (DNF-MPT) was developed by Changzhou YaMeiKe mechanical Co., Ltd. (Changzhou, China) (Fig. 1). The field experiments were performed at Experimental Research Farm (23.13 °N, 113.81 °E), South China Agricultural University. The soil of experimental field was sandy loam with 1.03 g kg\(^{-1}\) total N, 1.08 g kg\(^{-1}\) total P, 20.23 g kg\(^{-1}\) total K, 73.32 mg kg\(^{-1}\) available P, 104.55 mg kg\(^{-1}\) available K, and 21.56 g kg\(^{-1}\) organic C.

Experimental treatments and design

Two rice cultivars i.e., *Wufengyou615* (WFY615) and *Yuxiangyouzhan* (YXYZ), widely grown locally were grown in 96 m\(^2\) (6.0 m × 16.0 m) plots under randomized complete block design (RCBD) with three replicates. The YaraMila-Compound fertilizer (N: P\(_2\)O\(_5\): K\(_2\)O =15%: 15%: 15%), manufactured by YaraMila Fertilizer Company, was applied to both rice cultivars with 150 kg N ha\(^{-1}\). The application rate was for N fertilizer application treatments. Two fertilization methods were adopted i.e., mechanized 10 cm depth fertilization (M) and broadcasting fertilizer (B). All seedlings were transplanted by DNF-MPT. Four fertilizer management practices were designed as follow; MD: mechanized deep N fertilization as basal fertilizer; MDB: mechanized deep placement of 70% fertilizers as basal fertilizer, and 30% broadcast fertilizers at panicle initiation stage; MB: all fertilizers were broadcasted with 40%, 30% and 30% of total fertilizers at the transplanting, tillering and panicle initiation stage, respectively; and CK: no fertilizer was applied during entire growth stage. In both seasons, the strategies for water management were the same as adopted by local farmers. Some chemical reagents such as herbicide, imidacloprid, tricyclazole and carbendazim were adopted to prevent and control common weeds, insects and diseases, so as to avoid damaging the growth of rice.

Yield and yield-related traits

At maturity, the yield was recorded from a harvested-area of 6 m\(^2\) in each treatment. Twenty rice plants from each treatment were sampled randomly and the averaged values were calculated for number of productive panicles per hill. Six rice plants were sampled from each treatment to determine yield-related traits according to Pan et al. (2017).

Total above-ground biomass (TAB) and determination of nutrient accumulation

The TAB was determined according to Pan et al. (2017). After the TAB measurements, each part was ground into powder for estimation of total nutrient accumulation. Afterwards, each portion of the plant sample (0.2 g) was digested with a mixture of concentrated H\(_2\)SO\(_4\) and H\(_2\)O\(_2\) solution at 320°C for 2 h. The N concentrations in each part were determined according to Pan et al. (2017), the P concentrations in each part were determined according to Olsen (1954), and the K concentration was determined through a flame photometer.

Detection of total chlorophyll content (total Chl) and net photosynthetic rate (Pn)

The Chl a, Chl b and total Chl contents were estimated according to Du et al. (2018).

At the heading stage, the Pn of the flag leaves was measured from ten representative plants by LI-6400XT Portable Photosynthesis System (LICOR, Inc., USA). The photosynthetically active radiation (PAR) was set at 1200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) provided by a 6400-2B LED light source.

Determination of physiological activities

To determine physiological traits, about twenty-five leaves were collected from each treatment. The samples were stored at -80 °C for determination of physiological activities. The glutamine synthetase (GS) and nitrate reductase (NR) activities were determined according to the methods of Masclaux et al. (2000).

Roots sampling and measurements

Root sampling measurements were completed according to Pan et al. (2016). Eight rice seedlings in each plot were taken out with slurry after transplanting in one week, then eight PVC cylinders with the diameter of 20 cm (30 cm high) were inserted into the soil with a depth of 25 cm in situ, and the slurries in the cylinder were taken out. The mesh bag (20 cm diameter) was inserted into each PVC cylinder, then the uniform slurries
were returned into the mesh bag. Finally, the rice seedling of each hill was planted into the mesh bag. The mesh bag could ensure the free passage of water and nutrients, and prevent the root system from passing through of the mesh bag. Eventually, the buried cylinders were taken out of the soil. At heading stage, four rice plants were removed from the mesh bag, all roots were carefully rinsed with clean running water. The root morphology indexes were determined according to Pan et al. (2016).

**Economic profitability**

The economic profitability is determined by cost of rice production and the net income generated from the selling price of rice. The costs of rice production include labor costs, fertilizer costs, machinery use costs, and various input costs. Labor costs include farming, irrigation, pesticide and other different agricultural operations. The costs of fertilizer mainly include the expenditures of nitrogen, phosphorus and potassium fertilizer. Machinery costs include the rent of machinery for agricultural operations. Input costs include the cost of seeds, fertilizers and farm pesticides. All input costs were determined by local average price in two-year. Net income was determined by subtracting various costs and expenses from the calculated total return, the ratio of gross return to total production cost was estimated as the benefit-cost ratio (BCR).

**Data analysis**

The experimental data were analyzed using DPS3.11 (Data Processing System, Hangzhou, China). All figures were drawn with Origin 9.0. The differences amongst means of the experimental treatments were separated using the least significant difference (LSD) test at 0.05 probability level (ANOVA). Correlation analyses were analyzed by using Statistix8.1 (Analytical Software, Tallahassee, FL, USA).

**Results**

**Grain yield and yield-related traits**

Grain yield and yield-related traits varied to some extent under different N application treatments (Table 1). The grain yield of MD treatment was remained the highest in both years. Mean grain yield of both rice cultivars under MD were 8.03–8.35 t ha\(^{-1}\), which was 33.48–36.35% higher than the MB treatment. The MD treatment resulted the highest number of productive panicles ha\(^{-1}\) and spikelet per panicle with 257.16 × 10\(^4\) – 274.21 × 10\(^4\) ha\(^{-1}\) and 178.25–185.96, respectively, which was significantly higher than MB treatment. The MD and MDB treatment were not differed significantly (P > 0.05) in number of productive panicles ha\(^{-1}\) and spikelet per panicle. Substantial difference was found in the grain yield and yield-related traits between nitrogen treatments (p < 0.05). Furthermore, the N × Y factor also had prominent effect on spikelet per panicle and harvest index (HI).
Table 1
Effects of mechanized deep placement of nitrogen fertilization on yield and its components of rice during 2019 and 2020

| Treatment | Productive panicle (10^4 ha^{-1}) | Spikelet per panicle (%) | Grain filling weight (g) | 1000-grain- index (%) | Harvest yield (t ha^{-1}) |
|-----------|-----------------------------------|--------------------------|--------------------------|-----------------------|--------------------------|
| **2019**  |                                   |                          |                          |                       |                          |
| WFY615    | CK                                | 130.95 ± 5.92b           | 139.57 ± 6.65b           | 76.13 ± 0.08a         | 21.62 ± 0.31b            | 0.66 ± 0.03a             | 4.23 ± 0.33d             |
|           | MDS                               | 253.97 ± 3.53a           | 158.62 ± 3.07ab          | 80.22 ± 0.04a         | 23.34 ± 0.11a            | 0.66 ± 0.02a             | 7.17 ± 0.45b             |
|           | MBS                               | 277.78 ± 4.33a           | 180.19 ± 4.93a           | 83.15 ± 0.03a         | 23.74 ± 0.28a            | 0.60 ± 0.05a             | 8.09 ± 0.33a             |
|           | MD                                | 170.63 ± 7.92b           | 147.30 ± 6.92b           | 81.30 ± 0.04a         | 22.14 ± 0.61b            | 0.62 ± 0.06a             | 5.45 ± 0.49c             |
|           | mean                              | 208.33                    | 156.42                   | 80.20                 | 22.71                    | 0.64                     | 6.24                     |
|           | YXYZ                              | 130.95 ± 4.54c           | 149.14 ± 5.77b           | 83.28 ± 0.02a         | 19.21 ± 0.12c            | 0.64 ± 0.02a             | 3.37 ± 0.33c             |
|           | MDS                               | 234.12 ± 7.92ab          | 160.17 ± 4.24ab          | 86.55 ± 0.02a         | 19.68 ± 0.43a            | 0.6 ± 0.05ab             | 7.09 ± 0.57a             |
|           | MBS                               | 250.24 ± 3.81a           | 176.41 ± 1.78a           | 86.42 ± 0.05a         | 21.71 ± 0.47a            | 0.50 ± 0.06c             | 7.67 ± 0.33a             |
|           | MD                                | 186.51 ± 6.01b           | 157.90 ± 2.99ab          | 82.11 ± 0.05a         | 20.75 ± 0.25b            | 0.55 ± 0.01b             | 5.80 ± 0.37b             |
|           | mean                              | 200.40                    | 160.91                   | 84.59                 | 20.34                    | 0.57                     | 5.98                     |
| **2020**  |                                   |                          |                          |                       |                          |                          |                          |
| WFY615    | CK                                | 184.11 ± 4.57c           | 149.14 ± 5.77b           | 83.28 ± 0.02a         | 19.21 ± 0.12c            | 0.64 ± 0.02a             | 3.37 ± 0.33c             |
|           | MDS                               | 264.43 ± 2.98ab          | 173.32 ± 2.97ab          | 81.51 ± 0.41a         | 23.16 ± 0.23a            | 0.57 ± 0.01a             | 7.57 ± 0.74b             |
|           | MBS                               | 270.67 ± 3.86a           | 191.79 ± 3.77a           | 82.11 ± 0.05a         | 22.14 ± 0.61b            | 0.55 ± 0.01b             | 7.67 ± 0.33a             |
|           | MD                                | 220.37 ± 2.98bc          | 160.84 ± 5.81ab          | 80.12 ± 0.86ab        | 22.37 ± 0.64ab           | 0.57 ± 0.01a             | 6.92 ± 0.26b             |
|           | mean                              | 234.76                    | 166.74                   | 80.42                 | 22.65                    | 0.57                     | 7.18                     |
|           | YXYZ                              | 150.09 ± 4.21c           | 131.05 ± 1.31c           | 77.83 ± 1.06c         | 19.75 ± 0.56a            | 0.48 ± 0.02c             | 4.76 ± 0.68c             |
|           | MDS                               | 244.25 ± 3.86a           | 176.59 ± 6.31ab          | 79.67 ± 0.13b         | 19.87 ± 0.19a            | 0.52 ± 0.03a             | 7.67 ± 0.07a             |
|           | MBS                               | 264.07 ± 5.60a           | 180.18 ± 2.55a           | 80.77 ± 0.28a         | 21.61 ± 0.92a            | 0.49 ± 0.01bc            | 8.38 ± 0.94a             |
|           | MD                                | 208.39 ± 6.97b           | 149.74 ± 3.34bc          | 80.15 ± 0.76ab        | 20.01 ± 1.65a            | 0.50 ± 0.01b             | 6.22 ± 0.95b             |
|           | mean                              | 216.50                    | 159.39                   | 79.61                 | 20.31                    | 0.50                     | 6.76                     |
| **Anova** | Year(Y)                           | ns                       | ns                       | ns                    | *                       | ns                       | ns                       |
|           | Cultivar(C)                       | ns                       | ns                       | ns                    | **                      | ns                       | ns                       |
|           | Nitrogen(N)                       | **                      | **                      | **                   | **                     | **                     | **                     |
|           | Y × C                             | ns                       | ns                       | ns                    | ns                     | ns                       | ns                       |
|           | Y × N                             | ns                       | *                       | ns                    | ns                     | ns                       | ns                       |
|           | N × C                             | ns                       | ns                       | ns                    | ns                     | ns                       | ns                       |
|           | Y × N × C                         | ns                       | *                       | ns                    | ns                     | ns                       | ns                       |

Note: WFY615, Wufemgyou615, YXYZ, Yuxiangyouzhan. Within a column, means followed by the same letter are not significantly different at the 0.05 probability level according to least significant different test (LSD0.05). The same as below.
The root morphological indexes were different to some extent under different N application treatments at the HS stage (Table 2). The root morphological indexes for MD treatment were substantially improved for both rice cultivars. Mean total root volume (TRV) of both rice cultivars under MD were 17.81–21.59 cm$^3$ per hill, and the total root length (TRL) was 69.79–77.46 m per hill, root superficial area (RSA) was 12.94–13.29 cm$^2$ per hill, respectively. Moreover, during the two early seasons, difference in TRV, TRL, and RSA between the MD and MB treatments was significant, however, no significant difference was found between deep fertilizer treatment. The highest root dry weight (RDW) was recorded in MD treatment, which was significantly higher 17.21–24.11% than MB treatment. Significant differences were found in the root morphological traits and RDW between N treatments (P < 0.05), moreover, the Y × C factor also had obvious impact on TRV and RSA. The Y × N × C factor had also prominent impact on RDW and ARD.
Table 2
Effects of mechanized deep placement of nitrogen fertilization on root morphological indexes at heading stage during 2019 and 2020

| Treatment | TRV (cm³/hill) | TRL (m/hill) | RDW (g/hill) | RSA (cm²/hill) | ARD (mm/hill) |
|-----------|----------------|--------------|--------------|----------------|---------------|
| **2019**  |                |              |              |                |               |
| WFY615    |                |              |              |                |               |
| CK        | 11.33 ± 0.87c  | 56.63 ± 1.77c| 6.22 ± 0.88b | 9.38 ± 0.44c   | 9.55 ± 0.45b  |
| MDB       | 17.04 ± 4.11b  | 68.22 ± 8.34b| 10.11 ± 0.80a| 12.27 ± 2.08ab | 10.40 ± 0.03a |
| MD        | 22.47 ± 1.83a  | 79.25 ± 2.83a| 10.35 ± 0.71a| 13.02 ± 0.71a  | 10.61 ± 0.31a |
| MB        | 13.37 ± 1.39bc | 60.79 ± 3.71bc| 6.88 ± 1.02b | 10.42 ± 0.92bc | 8.95 ± 0.23b  |
| mean      | 16.05          | 66.22        | 8.39         | 11.27          | 9.88          |
| **YXYZ**  |                |              |              |                |               |
| CK        | 11.69 ± 1.24c  | 56.36 ± 4.01c| 5.97 ± 0.51c | 9.57 ± 1.12b   | 9.62 ± 0.23ab |
| MDB       | 15.29 ± 1.36b  | 63.67 ± 2.51b| 9.89 ± 0.95b | 11.39 ± 0.62a  | 9.39 ± 0.47b  |
| MD        | 19.39 ± 1.98a  | 72.99 ± 1.36a| 11.36 ± 0.54a| 11.46 ± 0.34a  | 10.36 ± 0.52a |
| MB        | 12.24 ± 0.67bc | 56.48 ± 2.76c| 9.38 ± 1.22b | 10.15 ± 0.69ab | 9.43 ± 0.47b  |
| mean      | 14.65          | 62.38        | 9.15         | 10.64          | 9.70          |
| **2020**  |                |              |              |                |               |
| WFY615    |                |              |              |                |               |
| CK        | 14.68 ± 1.09c  | 63.43 ± 2.21c| 5.45 ± 1.04b | 11.08 ± 0.55c  | 9.71 ± 0.15c  |
| MDB       | 21.15 ± 1.96a  | 73.57 ± 1.68ab| 9.33 ± 0.99a | 14.35 ± 0.42a  | 12.57 ± 0.36a |
| MD        | 20.70 ± 1.70a  | 75.66 ± 3.98a| 9.91 ± 1.70a | 15.12 ± 0.99a  | 9.45 ± 0.26c  |
| MB        | 17.81 ± 0.83b  | 69.77 ± 3.46b| 8.17 ± 0.93ab| 12.65 ± 0.86b  | 10.77 ± 0.03b |
| mean      | 18.58          | 70.61        | 9.22         | 13.3           | 10.63         |
| **YXYZ**  |                |              |              |                |               |
| CK        | 10.48 ± 0.55c  | 54.91 ± 1.12b| 6.12 ± 1.48b | 8.96 ± 0.28b   | 9.57 ± 0.21b  |
| MDB       | 14.53 ± 2.96ab | 66.13 ± 2.52a| 9.13 ± 1.16a | 12.00 ± 0.63a  | 10.50 ± 0.24a |
| MD        | 16.23 ± 1.38a  | 66.58 ± 6.02a| 9.07 ± 0.27a | 12.86 ± 1.5a   | 9.56 ± 0.33b  |
| MB        | 11.72 ± 1.24bc | 57.42 ± 2.81b| 7.08 ± 1.58ab| 9.58 ± 0.70b   | 9.39 ± 0.31b  |
| mean      | 13.24          | 61.26        | 7.85         | 10.85          | 9.76          |
| **Anova** |                |              |              |                |               |
| Year(Y)   | ns             | ns           | ns           | ns             | ns            |
| Cultivar(C)| ns           | ns           | ns           | ns             | ns            |
| Nitrogen(N)| **          | **           | **           | **             | **            |
| Y × C     | **            | ns           | ns           | **             | ns            |
| N × C     | ns            | ns           | ns           | ns             | **            |
| Y × N     | ns            | **           | ns           | ns             | ns            |
| Y × N × C | ns            | ns           | *            | ns             | **            |

Note: TRV: total root volume, TRL: total root length, RDW: root dry weight, RSA: root superficial area, ARD: average root diameter.

Detection of chlorophyll contents and net photosynthetic rate (Pn)
The total Chl at whole growth stage and Pn at HS stage were differed to some extent under different N application treatments (Table 3). For example, the MD treatment resulted in the highest total Chl for both rice cultivars, and remained significantly higher than other treatments at MT stage. Total Chl content was remained lower at PI stage than MT stage, with marginal difference among treatments whereas total Chl was noted the lowest at the HS stage for each treatment. Mean total Chl at HS stage of both rice cultivars under MD were 2.47–3.06 mg g$^{-1}$, which was 25.15–55.12% higher than MB treatment, respectively. The highest Pn was obtained for MD treatment for both rice cultivars, and mean Pn under MD were 20.27–22.17 µmol m$^{-2}$ s$^{-1}$, which was 24.63–25.57% higher than MB treatment. Furthermore, the similar trend in Pn was observed i.e., MD > MDB > MB > CK.
Table 3
Effects of mechanized deep placement of nitrogen fertilization on chlorophyll contents and net photosynthetic rate during 2019 and 2020

| Treatment | Total Chl (mg·g⁻¹) | Net photosynthetic rate (µmol m⁻²s⁻¹) |
|-----------|-------------------|--------------------------------------|
|           | MT | PI | HS |                    |                      |
| 2019      |    |    |    |                    |                      |
| WFW615    |    |    |    |            |                      |
| CK        | 1.38 ± 0.07c       | 1.90 ± 0.05b                      | 0.91 ± 0.07c         | 13.60 ± 0.76c        |
| MDB       | 3.09 ± 0.16b       | 3.28 ± 0.06a                      | 3.06 ± 0.29a         | 18.89 ± 0.19b        |
| MD        | 3.94 ± 0.50a       | 3.36 ± 0.19a                      | 3.00 ± 0.03a         | 22.45 ± 0.32a        |
| MB        | 3.11 ± 0.18b       | 2.03 ± 0.07b                      | 2.49 ± 0.27b         | 17.81 ± 1.65b        |
| mean      | 2.88            | 2.64            | 2.37            | 18.19               |
| YXYZ      |    |    |    |            |                      |
| CK        | 2.27 ± 0.12c       | 1.81 ± 0.09c                      | 1.27 ± 0.05b         | 12.18 ± 0.96c        |
| MDB       | 2.77 ± 0.07b       | 3.57 ± 0.14a                      | 1.04 ± 0.05b         | 19.14 ± 0.18a        |
| MD        | 3.36 ± 0.16a       | 3.84 ± 0.18a                      | 2.27 ± 0.54a         | 20.29 ± 0.99a        |
| MB        | 2.27 ± 0.02c       | 3.12 ± 0.08b                      | 1.50 ± 0.14b         | 16.02 ± 0.41b        |
| mean      | 2.67            | 3.09            | 1.52            | 16.91               |
| 2020      |    |    |    |            |                      |
| WFW615    |    |    |    |            |                      |
| CK        | 1.45 ± 0.25c       | 2.05 ± 0.09c                      | 1.01 ± 0.06c         | 15.58 ± 1.36c        |
| MDB       | 3.18 ± 0.11b       | 3.11 ± 0.03b                      | 2.98 ± 0.11a         | 21.48 ± 1.72ab       |
| MD        | 3.7 ± 0.07a        | 3.88 ± 0.06a                      | 3.12 ± 0.15a         | 23.89 ± 1.92ab       |
| MB        | 2.9 ± 0.15b        | 2.97 ± 0.1b                       | 2.4 ± 0.07b          | 19.50 ± 0.25b        |
| mean      | 2.81            | 3.01            | 2.38            | 18.11               |
| YXYZ      |    |    |    |            |                      |
| CK        | 2.20 ± 0.18d       | 1.98 ± 0.31b                      | 0.96 ± 0.11c         | 11.18 ± 0.8d         |
| MDB       | 3.13 ± 0.06b       | 3.27 ± 0.18a                      | 1.81 ± 0.2b          | 19.11 ± 0.8b         |
| MD        | 3.35 ± 0.1a        | 3.42 ± 0.48a                      | 2.66 ± 0.04a         | 20.24 ± 0.4a         |
| MB        | 2.4 ± 0.08c        | 3.23 ± 0.06a                      | 1.67 ± 0.14b         | 16.51 ± 0.34c        |
| mean      | 2.77            | 2.98            | 1.78            | 16.76               |

Anova:
- Year(Y): ns
- Cultivar(C): ns
- Nitrogen(N): **
- Y × C: ns
- Y × N: **
- N × C: ns
- Y × N × C: ns

Note: MT: mid-tillering stage; PI: panicle initiation stage; HS: heading stage; MS: maturity stage; The same as below.

Total above-ground biomass
Significant differences were noted in the total above-ground biomass at whole growth stage under different N application treatments (Fig. 2). For example, the TAB for deep fertilizer treatments (MD and MDB) were significantly higher than MB and CK treatment at PI and HS stage. However, no significant difference was found between MD and MDB. Mean TAB at MS stage of both rice cultivars under MD were 14.11–14.66 t ha$^{-1}$, which was 21.17–31.99% higher than MB treatment, respectively. Moreover, the MD and MDB treatment was remained statistically similar (P > 0.05) at MS stage. In the whole growth period, the similar trend for TAB of both rice cultivars was observed. It was also found that TAB was remained the highest at MS stage for each treatment than at other stages.

**Nutrient accumulation**

Significant differences were also observed in the total N accumulation (TNA) at whole growth stage under different N application treatments (Fig. 3). For instance, there were no significant differences in MD, MDB and MB treatments at MT stage, however, all treatments were substantially higher than CK treatment. The MD treatment had the highest TNA at PI stage, as compared to other treatments. Moreover, the MD treatment significantly increased the TNA in 2019, however, no significant difference was found between MD and MDB treatment in 2020. Mean TNA at MS stage of both rice cultivars under MD were 186.01-190.04 kg ha$^{-1}$, which was 26.38–44.15% higher than MB treatment, respectively. On the other hand, the MD and MDB treatments statistically similar (P > 0.05) at MS stage. In the whole growth period, similar trends were observed for TNA of both rice cultivars.

The total P accumulation (TPA) at whole growth stage were varied to some extent under different N applications treatments (Fig. 4). For example, no significant differences were noted in MD, MDB and MB treatments at MT stage for both rice cultivars, however, all treatments were remained significantly higher than CK. The MD treatment had the highest TPA at PI stage, as compared with other treatments. Moreover, the TPA as increased in MD treatment for $WFY615$, however, no significant differences was found between MD and MDB treatment in $YXYZ$ regarding TPA. Furthermore, deep fertilizer treatments i.e., MDB and MD were remained statistically similar (P > 0.05) at HS and MS stage. Mean TPA at MS stage of both rice cultivars under MD were 32.82–35.5 kg ha$^{-1}$, which was remarkable higher 28.72–30.23% than MB treatment, respectively. In the whole growth period, the similar trend for TPA of both rice cultivars was observed.

The total K accumulation (TKA) at whole growth stage were differed to some extent under different N applications treatments (Fig. 5). For example, there were no significant differences in MD, MDB and MB treatments at MT stage for both rice cultivars, however, all treatments were remained higher than CK treatment. The MD treatment had the highest TKA at PI stage, as compared to other treatments, whereas the TKA in $WFY615$ were increased under MD treatment in 2019, however, no remarkable difference was found between MD and MDB treatment in $YXYZ$. The MD treatment led to the highest TKA at MS stage, Furthermore, mean TKA at MS stage of both rice cultivars under MD were 173.76-182.63 kg ha$^{-1}$, which was 25.61–37.33% higher than MB treatment, respectively. Similar trend for TKA of both rice cultivars was observed during whole growth period.

**Glutamine synthetase (GS) and nitrate reductase (NR) activities**

The nitrate reductase (NR) activity at all growth stages were remained variable to some extent under different N applications treatments (Fig. 6). For $WFY615$, the NR activity of MD treatment was remained the highest among all treatments at MT stage during both years, however, MB and MDB treatment was not differed significantly. For both rice cultivars, as compared to other treatments, the MD treatment led to the highest NR activity at HS stage, furthermore, mean NR activity at HS stage of both rice cultivars under MD were remained 111.59-118.24% higher than MB treatment.

The glutamine synthetase (GS) activity at all growth stages differed under different N applications treatments (Fig. 7). For both rice cultivars, the GS activity of MD treatment was the highest among all treatment at MT stage, while significant difference was found among MD and MDB treatment. However, MD and MDB treatment was not differed significantly (P > 0.05). Compared with other treatments, the MD treatment led to the highest GS activity at HS stage. Furthermore, mean GS activity at HS stage of both rice cultivars under MD were remained 16.97–23.36% higher than MB treatment. Moreover, significant differences in GS activity were found in all treatments of $YXYZ$ in 2020.

**Economic profitability**

The total input, total return, net income, and BCR were differed under different N applications treatments (Table 5). Significant differences in total input were found among all treatments, while the highest total inputs in both seasons were observed in MB. However, MD generated maximum net income and total return, next to MDB and MB, while the lowest net income was observed in CK. The BCR was the highest in MD than all other treatments because of the highest total return and lower total input. Thus, present results demonstrated that mechanized deep N fertilization was a suitable and an economical fertilization technology for mechanically transplanted rice.
Correlation among root morphological indexes, nutrients accumulation, total above-ground biomass, and yield

The correlation coefficients amongst the root morphological indexes and nutrient accumulation i.e., TNA, TPA, and TKA, as well as TAB and yield (Table 4). The TNA had significant and positive correlations with root morphological indexes such as TRL, RSA, TRV, and RDW for both rice cultivars, correlations between TNA and ARD were remained non-significant. Moreover, the TPA had significant and positive correlations with TRV and RDW whereas the TKA has also significant associations with root morphological indexes except ARD. Moreover, significant and positive correlation among root morphological indexes, TAB and yield at was also noted at P<0.01.

Table 4
Correlation analysis between root morphological indexes and nutrients accumulation, total above-ground biomass, and yield

|       | TRV (cm³/hill) | RSA (cm²/hill) | RDW (g/hill) | TRL (m/hill) | ARD (mm/hill) |
|-------|----------------|----------------|--------------|--------------|--------------|
| TNA (kg ha⁻¹) | 0.4877* | 0.5071* | 0.4177* | 0.5136** | 0.2812 |
| TPA (kg ha⁻¹) | 0.4497* | 0.2113 | 0.6232** | 0.3496 | 0.1532 |
| TKA (kg ha⁻¹) | 0.6157** | 0.5874** | 0.6473** | 0.6299** | 0.1783 |
| TAB (t ha⁻¹) | 0.7476** | 0.7375** | 0.6359** | 0.7426** | 0.4305* |
| Yield (t ha⁻¹) | 0.7343** | 0.7639** | 0.6497** | 0.7607** | 0.5571** |

Note: TRV: total root volume, TRL: total root length, RDW: root dry weight, RSA: root superficial area, ARD: average root diameter, TNA: total nitrogen accumulation, TPA: total phosphorus accumulation, TKA: total potassium accumulation, TAB: total above-ground biomass.

Table 5
Production cost, gross return, and beneficial cost ratio (BCR) in 2019 and 2020

| Treatments | Production cost (US$ha⁻¹) | Gross return (US$ha⁻¹) | Net income (US$ha⁻¹) | BCR |
|------------|--------------------------|------------------------|----------------------|-----|
|            | Fertilizer application costs | Machinery costs | Labor costs | Input costs | Total input | Price (US$ t⁻¹) | GY (t ha⁻¹) | Total return | |
| 2019       |                          |                        |                      |                  |              |                  |            |              |     |
| CK         | 0                        | 0                      | 314.93               | 354.16           | 177.08       | 846.17d          | 390.12     | 1489.80d     | 643.63d | 0.76b |
| MDB        | 165.27                   | 47.03                  | 314.93               | 354.16           | 177.08       | 893.20b          | 390.12     | 2886.00b     | 1992.80b | 2.23a |
| MD         | 165.27                   | 34.58                  | 314.93               | 354.16           | 177.08       | 880.75c          | 390.12     | 3073.20a     | 2192.45a | 2.49a |
| MB         | 165.27                   | 76.09                  | 314.93               | 354.16           | 177.08       | 922.26a          | 39012      | 2193.75c     | 1271.49c | 1.38b |
| 2020       |                          |                        |                      |                  |              |                  |            |              |     |
| CK         | 0                        | 0                      | 314.93               | 354.16           | 177.08       | 846.17d          | 390.12     | 1700.92d     | 854.75d | 1.01d |
| MDB        | 165.27                   | 47.03                  | 314.93               | 354.16           | 177.08       | 893.20b          | 390.12     | 2972.71b     | 2079.51b | 2.33b |
| MD         | 165.27                   | 34.58                  | 314.93               | 354.16           | 177.08       | 880.75c          | 390.12     | 3312.12a     | 2431.37a | 2.76a |
| MB         | 165.27                   | 76.09                  | 314.93               | 354.16           | 177.08       | 922.26a          | 390.12     | 2563.08c     | 1640.82c | 1.78d |

Note: The grain yields were represented the average of two rice cultivars

Discussion

Effects of deep N fertilization on yield and yield-related traits
Previously, deep N fertilization was thought to improve rice yield by improving N uptake and overall N use efficiency with minimum N loss (Dan et al., 2019; Peng et al., 2019). Significant improvements in plant growth and crop yield were observed in plants applied with deep placement of N fertilizer compared with the broadcasting fertilizer (Bandaogo et al., 2015; Huda et al., 2016). Wu et al. (2017) reported that deep N fertilization could increase the tillering and panicle numbers in rice which on the other hand improve the rice yield compared with broadcasting fertilizer. Moreover, Kapoor et al. (2008) observed that deep N fertilization resulted in similar rice yields with reduced nitrogen loss and improved nitrogen efficiency as obtained from surface broadcasting with decrease in total urea application rate i.e., 40 kg ha$^{-1}$. In present study, the MD treatment increased rice yield owing to increase in number of productive panicles ha$^{-1}$ and spikelet per panicle compared to broadcasting fertilizer treatment. Deep N fertilization could possibly promote the N uptake at early growth stage of rice which may enhance tillering ability, as well as availability of N in the rhizosphere for a longer period of time required at later growth stage. Moreover, deep fertilization increases the release time of N fertilizer by keeping more NH$_4^+$ in the soil as non-exchangeable NH$_4^+$ (Ke et al., 2018).

The total above-ground biomass (TAB) is closely related to the growth and development of the crop, whereas the amount of dry matter accumulated in crops is the physical basis of ultimate yield (Hu et al., 2020). Our study showed that TAB from PI to MS stage under MD treatment was significantly higher than MB treatment (Fig. 2). Moreover, the TAB was significantly correlated with rice yield (Table 4) which could be recognized as an important high-yielding trait for rice plants, these results were in accordance with Zhi et al. (2017). Moreover, deep N fertilization could significantly improve the Pn and the Chl content of rice at heading stage compared with the broadcasting fertilizer (Table 3). Previously, Jia et al. (2008) found that the total Chl is directly proportional to photosynthesis within a certain range. Furthermore, deep fertilization treatments could maintain higher total Chl and Pn at HS stage, which was conducive to ensure the transportation of photosynthates towards panicle, leading to more spikelet per panicle in rice (Table 1). Significant positive correlation was noticed between TAB and root morphology indexes at HS stage (Table 4). Plants with deep fertilization treatment could absorb sufficient nutrients to ensure the dry matter accumulation of rice before PI stage (Fig. 3–5). Therefore, a strong root system could ensure to fulfill the nutrient demand of the above-ground plants parts, which was essential for increasing the rice yield.

In the present study, MD significantly improved the NR and GS activities compared to MB. Previous researches have shown that NR and GS were the main enzymes of N metabolism in higher plants (Martin et al., 2006; Zhong et al., 2018). The NR was the rate-limiting enzyme of nitrogen assimilation, which directly regulated the reduction of nitrate and thus regulated nitrogen metabolism (Masclaux et al., 2000). Hou et al. (2019) argued that the GS/GOGAT cycle as the first step in the conversion of inorganic nitrogen to organic nitrogen compounds is likely to be the main checkpoint for regulating plant nitrogen assimilation. We speculated that the production of nitrogen compounds in GS/GAGOT cycle was related to chlorophyll synthesis, which needs further investigations. Over-expressed GS increases the plant metabolism as well as increases the content of amino acids and total nitrogen, and thus affects crop yield (Cai et al., 2009). Moreover, MD treatment significantly increased the activity of NR and GS at all growth stages, especially in HS stage, which was one of the reasons for the higher TNA and rice yield.

**Effects of deep N fertilization on nutrient accumulation**

The nutrient application forms the basis for yield formation and are significantly affected by the fertilization mode. Some studies have found that deep fertilization could refer to rice nitrogen use efficiency by increasing the total N content of rice (Zhu et al., 2019). Moreover, the application of fertilizers enhances N, P, K uptake in rice as previously reported (Qiao et al., 2011). Guo et al. (2016) showed that deep nitrogen fertilization could also improve the utilization efficiency of P and K in rice. In the present study, deep fertilization treatment significantly increased the TNA, TPA, and TKA content of rice during MS stage, compared with broadcasting fertilizer. On the one hand, deep N fertilization could increase the growth and development of rice because of its effect for longer time period (Yao et al., 2018). On the other hand, deep N fertilization could promote the growth and development of rice root system (Table 2), The TNA, TPA, TKA and root morphological indexes were significantly and positively correlated among each other (Table 5). Stronger root system could enhance the uptake and utilization of nutrients by plants, promote the accumulation and transport of dry matter and nutrients in above-ground organs, and thus contribute to the increase of plant NKP nutrient accumulation (Tang et al., 2019). Some studies have shown that N, P, and K accumulation at young panicle differentiation stage is significantly positively correlated with rice yield, hence increasing nutrients at this stage was helpful to improve rice yield (Xue et al., 2011; Pan et al., 2016). However, it was observed that the deep fertilization treatment significantly enhanced the NPK accumulation at all growth stages (Fig 3-5), whereas the nutrients accumulation at PI stage was beneficial to the growth and development of rice, which had a great impact on the HS stage and even the whole growth period of rice. In addition, if the nutrient accumulation is the highest at the PI stage, it might cause excessive rice growth (HI of MD treatment was lower than MDB treatment) (Table 1).

**Effects of deep N fertilization on root morphological indexes**

The roots are the primary organ of water and nutrient uptake in rice, whereas root morphology and vigor were likely to be influenced by different N application methods and application dose (Pan et al., 2016; Turner, 2010). Yang et al. (2004) revealed that root morphology and vigor have significant correlation with rice yield. In present study, it was found that the root morphological indexes (total root volume, total root length, and
root superficial area) and root dry weight were significantly affected by deep fertilization (Table 2). Rice root system is relatively small at the early growth stages, thus its ability to uptake and/or absorb nutrients remains relatively weak whereas broadcasting fertilizer caused high concentrations of NH$_4^+$ under flood irrigation, which results in more ammonia volatilization with a significant loss of nutrients (Adviento-Borbe and Linquist., 2016; Yao et al., 2018). Moreover, correlation analysis also showed significant and positive associations among RSA, ARD, TRV and TAB, NPK nutrient accumulation, and yield (Table 5). These results showed that maintaining stronger root morphology is beneficial to absorb nutrients through extensive root system, which directly or indirectly beneficial for the growth of above-ground parts and the distribution of nutrients to get higher yields.

**Effects of deep N fertilization on economic profitability**

The MD treatment significantly reduced production costs compared to other treatments (Table 5). Mechanized deep placement of all fertilizers at once solves the problem of rural labor shortage to some extent, especially in some regions or seasons, however, the excessive demand for labor may affect timely planting of crops (Fernández and Schaefer, 2012; Paman et al., 2014; Emran and Shilpi, 2018). In present study, the highest yield was obtained by MD among all treatments, which was significantly higher than other treatments, indicating that fertilization method had a significant impact on rice yield, which was consistent with Pan et al., (2017) who reported that deep fertilization significantly increased the grain yield in direct-seeded rice irrespective of the types of N fertilizer applied. It was observed that the highest net income and BCR were generated by MD, which was mainly due to the combination of high yield and low production costs. Some studies on the application of deep N fertilization to increase the yield of other crops have also been reported (Tewari et al., 2007; Chen et al., 2020). Therefore, MD treatment could not only solve the problem of labor shortage but could also improve the rice yield. Deep placement of fertilizers in mechanized rice production has a broad application prospect in the current and future rice production system on sustainable basis.

**Conclusions**

Mechanized deep placement of all fertilizers as base fertilizer (MD) can improve the rice growth owing to substantial improvements in total above-ground biomass, total chl content, and net photosynthetic rate. The MD treatment also increased the grain yield, nutrient acquisition, physiological attributes, as well as also maintained a strong root system, and higher root dry weight at heading stage. Therefore, our results suggest that mechanized deep placement all fertilizers as base fertilizer may be an effective way to improve the rice productivity and sustainability.

**Abbreviations**

DNF-MPT: deep nitrogen fertilization coupled with mechanical pot-seedling transplanting rice; N: nitrogen; TNA: total nitrogen accumulation; TPA: total phosphorus accumulation; TKA: total potassium accumulation; NR: nitrate reductase; GS: glutamine synthetase; total Chl: total chlorophyll content; TRV: total root volume; TRL: total root length; RSA: root superficial area; RDW: root dry weight; ARD: average root diameter. Pn: net photosynthetic rate.

**Declarations**

**Ethics approval and consent to participate**

Not applicable

**Consent to Publish**

Not applicable

**Availability of data and materials**

Not applicable

**Competing interests**

The authors declare that they have no competing interests.

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Authors' contributions

SG and XR initiated and designed the research, LL, ZZ, HT, UA, MY, ZM and TY performed the experiments, LL and ZZ analyzed the data and wrote the manuscript, SG revised and edited the manuscript and also provided advice on the experiments. All authors read and approved the final manuscript.

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**Figures**

![Figure 1](image)

**Figure 1**

Pictorial view of deep nitrogen fertilization coupled with mechanical pot-seedling transplanting rice at the farm of South China Agricultural University, Guangzhou city, China. Note: The left picture is transplanting by mechanical pot-seedling transplanting rice. The right picture is 20 days after transplanting.
Figure 2

Effects of mechanized deep nitrogen fertilization on total above-ground biomass (TAB) in 2019 and 2020. (a, b) 2019; (c, d) 2020. Note: MT: Mid-tillering stage; PI: panicle initiation stage; HS: Heading stage; MS: maturity stage. Errors bars represent the mean standard error, and the data are mean values of three replications, the same as below.
Figure 3

Effects of mechanized deep placement of nitrogen fertilization on total nitrogen accumulation (TNA) in 2019 and 2020. (a, b) 2019; (c, d) 2020.
Figure 4
Effects of mechanized deep placement of nitrogen fertilization on total phosphorus accumulation (TPA) in 2019 and 2020. (a, b) 2019; (c, d) 2020.
Figure 5

Effects of mechanized deep placement of nitrogen fertilization on total potassium accumulation (TKA) in 2019 and 2020. (a, b) 2019; (c, d) 2020.
Figure 6

Effects of mechanized deep placement of nitrogen fertilization on nitrate reductase (NR) activities in 2019 and 2020. (a, b) 2019; (c, d) 2020.
Figure 7

Effects of mechanized deep placement of nitrogen fertilization on glutamine synthetase (GS) activity in 2019 and 2020. (a, b) 2019; (c, d) 2020.