Biological manure substitution's impact on grain yield, nitrogen recovery efficiency, and soil biochemical properties (#94811)

First submission

Guidance from your Editor

Please submit by **2 Mar 2024** for the benefit of the authors (and your token reward) .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files
Download and review all files from the materials page.

4 Figure file(s)
2 Table file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Biological manure substitution's impact on grain yield, nitrogen recovery efficiency, and soil biochemical properties

Zhili Sun [1] , **Chengshun Wang** [1] , **Jiabao Wang** [2] , **Gang Wu** [2] , **Manman Yuan** [2] , **Haiming Zou** [Corresp., 1] , **Yixiang Sun** [Corresp. 2]

[1] College Resource & Environment, Anhui Science & Technology University, Chuzhou, Anhui, China

[2] Institute of Soil and Fertilizer, Anhui Academy of Agricultural Sciences, Key Laboratory of Nutrient Cycling and Arable Land Conservation of An Hui Province, Hefei, Anhui, China

Corresponding Authors: Haiming Zou, Yixiang Sun
Email address: zouhm@ahstu.edu.cn, sunyixiang@aaas.org.cn

Fertilization plays a crucial role in ensuring global food security and ecological balance. In a comprehensive three-year experiment, we investigated the impact of substituting innovative biological manure for chemical fertilization on rice productivity and soil biochemical properties. Our findings highlight a significant improvement in both rice yield and straw weight. Specifically, applying 70% of total chemical nitrogen (N) fertilization with 2000 kg ha^{-1} of biological manure resulted in a substantial 13.6% increase in rice yield and a remarkable 34.2% boost in straw weight. In comparison to the conventional local farmer practice of applying 165 kg N ha^{-1}, adopting 70% of total N with biological manure demonstrated superior outcomes, particularly in enhancing yield components and spike morphology. Fertilization treatments led to elevated levels of soil microbial biomass carbon and N. However, a nuanced comparison with local practices indicated that applying biological manure alongside urea resulted in a slight reduction in N content in vegetative and economic organs, along with decreases of 10.4%, 11.2%, and 6.1% in N recovery efficiency (NRE), respectively. Prudent N management through the judicious application of partial biological manure fertilizer in rice systems could be imperative for sustaining productivity and soil fertility in southern China.

Biological manure substitution impacts on grain yield, nitrogen recovery efficiency, and soil biochemical properties

Zhili Sun [1], Chengshun Wang [1], Jiabao Wang [2], Gang Wu [2], Manman Yuan [2], Haiming Zou [1*], Yixiang Sun [2*]

[1] Anhui Science & Technology University, College Resource & Environment, Donghua Rd 9, Chuzhou 233100, China

[2] Key Laboratory of Nutrient Cycling and Arable Land Conservation of An Hui Province, Institute of Soil and Fertilizer, Anhui Academy of Agricultural Sciences, Hefei 230001, China

[*] Corresponding author: Yixiang Sun and Haiming Zou

Email: sunyixiang@aaas.org.cn and zouhm@ahstu.edu.cn

Abstract

Fertilization plays a crucial role in ensuring global food security and ecological balance. This study investigated the impact of substituting innovative biological manure for chemical fertilization on rice (*Oryza sativa* L) productivity and soil biochemical properties based on a three-year experiment. Our results suggested rice yield and straw weight were increased under manure addition treatment. Specifically, 70% of total amount of nitrogen (N) fertilizer substituted by biological manure resulted in a substantial 13.6% increase in rice yield and a remarkable 34.2% boost in straw weight. In comparison to the conventional local farmer practice of applying 165 kg N ha^{-1}, adopting 70% of total N with biological manure demonstrated superior outcomes, particularly in enhancing yield components and spike morphology. Fertilization treatments led to elevated levels of soil microbial biomass carbon and N. However, a nuanced comparison with local practices indicated that applying biological manure alongside urea resulted in a slight reduction in N content in vegetative and economic organs, along with decreases of 10.4%, 11.2%, and 6.1% in N recovery efficiency (NRE), respectively. Prudent N management through the judicious application of partial biological manure fertilizer in rice systems could be imperative for sustaining productivity and soil fertility in southern China.

Keywords: N recovery efficiency, soil biochemical property, rice production, biological manure fertilizer.

1 Introduction

Agricultural practices play a pivotal role in global food security and ecosystem health, with fertilizer application being a critical aspect. Traditionally, chemical nitrogen (N) fertilizers have been widely used to improve soil fertility and increase crop yields. However, the associated ecological and environmental consequences have raised concerns, urging researchers to explore alternative strategies (Smith et al., 2016; Doğan et al., 2023). Seeking innovative approaches that promote sustainable agriculture, considering the complex interplay between soil health, crop yield, and environmental sustainability could be required in shaping the future of agriculture towards more eco-friendly and resilient practices (Tilman et al., 2017; Singh et al., 2023).

Manure, a traditional and organic nutrient source, is gaining recognition as a promising alternative to chemical fertilizers (Ng et al., 2024). Beyond supplying essential nutrients for crop development, its utilization presents the added benefit of recycling organic matter into the soil. As the agricultural paradigm undergoes transformations, it becomes crucial to comprehensively evaluate the ramifications of partial and complete substitution with manure applications (Hou et al., 2023). Understanding the holistic impact of transitioning to manure-based practices is essential for informed and sustainable agricultural decision-making, ensuring a balanced approach to nutrient management and soil health.

As global concerns about the environmental footprint of agriculture intensify, there is a paramount need to explore sustainable practices that balance productivity with ecological stewardship. The investigation into the partial substitution by manure applications represents a crucial step in evaluating the feasibility and consequences of adopting organic alternatives on a large scale. Thus, this study aims to elucidate the impacts of a novel biological manure application on grain yield, soil nutrient and microbial biomass in rice system during 2020 – 2022 in southern China.

2 Method and materia

2.1 Site description and experimental design

A three-year experiment was conducted to assess the impact of various nitrogen fertilization strategies on rice productivity in He County, Anhui province, China (N31.76828, E118.20394, elevation 18 m) from 2020 to 2022. The experimental site, characterized by a subtropical climate, had an average annual temperature of approximately 15.8 ℃ and annual precipitation of 1067 mm. The predominant soil profile was hydragric paddy soil, equivalent to Inceptisols in the U.S. soil classification system. Initial soil properties at depths of 0–20 cm in 2020 were as follows: pH 6.04, soil organic matter 14.8 g kg-1, total N content 1.07 g kg^{-1}, available phosphate 36.6 mg kg^{-1} and available potassium 147 mg kg^{-1}.

The field experiment employed a randomized complete block design with plots measuring 60 m^2 (6 m × 10 m) and three replications. Three treatments were applied: the control treatment (N0) with 0 kg N ha^{-1}; the conventional N treatment (N100) following local practices, applying 165 kg N ha^{-1}; the manure plus 70% of the total 165 kg N ha^{-1} application (N70BM). Each experimental plot received 60 kg P_2O_5 of calcium superphosphate (12%) and 75 kg K_2O ha^{-1} potassium sulfate (60%). The biological manure fertilizer, jointly developed by our experimental lab and Anhui Serte Fertilizer Co., Ltd, primarily utilized pig manure and straw as raw materials. It exhibited an effective viable count (Bacillus amyloliquefaciens SQR9) of ≥ 200 million g^{-1}, organic matter content of ≥ 50%, moisture content of ≤ 30%, and nutrient content of 15.6 g N kg^{-1}, 26.1 g P_2O_5 kg^{-1}, and 64.8 g K_2O kg^{-1} in granular powder form. The popular rice cultivar “Fuxiangzhan” was employed in this study. Field management practices, including tillage, weed, and pest control, were consistent with local farmer practices.

2.2 Field sampling and analysis

Grain yield was determined by harvesting the whole plot area and weighing all leaves and stems for recording straw. The plant samples were oven dried to constant weight at 60 ℃ and then was measured N content in grain and straw by an elemental analyzer (Vario Max CN, Elemeta Analysensysteme GmbH, Hanau, Germany). Accumulated N content was calculated as the product of N content and dry matter weight.

Soil samples were collected using the 5-point method at depths of 20 cm with three replications after the rice harvest for each season. The fine root, gravel and plant residues were removed in each soil sample. Then, the soil was divided into two parts: one served as air-drying soil through a 0.25 mm sieve for determining soil properties, and another one was stored at 4 ℃ for measuring the ammonium, nitrate, microbial biomass C (MBC) and N (MBN) and soil enzyme activity. Soil NH_4^+−N and NO_3^-−N was measured by the continuous flow analyzer (FIAstar 5000 Analyzer, Foss Tecator, Hillerød, Denmark). Soil MBC and MBN can be determined using the chloroform fumigation extraction method. After fumigation, microbial residues are extracted, and organic carbon and nitrogen are quantified (Vance et al., 1987).

Soil catalase activity was assessed following the potassium permanganate titration method (Guan et al., 1986; Wang et al., 2020). A soil sample (2 g) was mixed with 40 ml distilled water and 5 ml hydrogen peroxide (3%), shaken for 30 min, and filtered. A 25 ml filtrate was titrated to pink with 0.1 M potassium permanganate.

Urease activities were determined as Guan and Yin's method (Guan et al., 1986; Yin et al., 2014). Soil (2 g) was treated with 10 ml urea (10%), 20 ml citrate buffer (1 M, pH 6.7), and 1 ml methylbenzene. After incubation at 37°C for 24 hours, the solution was filtered. 1 ml filtrate was mixed with 20 ml distilled water, 4 ml sodium phenolate hydroxide, and 3 ml sodium hypochlorite.

NH_4^+−N was analyzed after 20 min using a spectrophotometer at 578 nm. Urease activity was expressed as milligrams of NH_4^+−N per gram of dry soil released in 24 hours.

The invertase activity was assessed following the protocols outlined by Guan et al. (1986). Using 3,5-dinitrosalicylic acid, invertase activity was measured with sucrose solution as the substrate. The results were expressed as the mass (mg) of glucose per gram of soil after a 24-hour incubation period.

Apparent N recovery efficiency (NRE) was calculated by N concentration and plant biomass as defined by a previous study (Conant et al., 2013).

$$\text{NRE} = \frac{\text{N uptake (fertilized)} - \text{N uptake (none} - \text{fertilized)}}{\text{amount of N applied}}$$

2.3 Statistical Analysis

To test the difference between different N management (i.e. N0, N100 and N70BM) for grain yield, biomass and soil properties, one-way analysis of variance (ANOVA) and the least significant differences (LSDs, $P < 0.05$) was conducted in SPSS 20.0 (SPSS, inc., 2011, Chicago IL, USA).

3 Result

3.1 Grain yield and straw weight

Grain yield and straw weight were significant differences under fertilizer treatments (Fig. 1). Compared with N0, 100% N application (N100) increased the average yield by 1221 kg ha^{-1} and straw weight by 1913 – 1963 kg ha^{-1}, respectively ($P < 0.05$,Table S6). Manure substitution with 30% of total N amount (N70BM) increased the average yield by 13.6% and straw weight by 34.2%, respectively ($P < 0.05$,Table S6). Both grain yield and straw weight under 30% of total N substituted by manure were slightly smaller than that under 100% of total N application.

3.2 N recovery efficiency and N uptake in plant

Fertilization regime increased N content in economic and vegetative organs (*P* < 0.05, Fig. 2a,Table S6) and N recovery efficiency (*P* > 0.05, Fig. 2b). In detail, N content in grain yield and straw at harvest was highest by 111 kg N ha^{-1} and 49.8 kg N ha^{-1} under N100 treatment, respectively. N content in plant under N70BM treatment was 10.4% and 11.2% slightly lower than that under N100 (*P* > 0.05), respectively (Fig. 2a). Similarly, N recovery efficiency under N100 treatment was slightly higher by 6.1% compared with N70BM treatment (*P* > 0.05, Fig. 2b).

3.3 Physiological morphology

Fertilization regime influenced yield components and N concentrations in grain and straw (Table 1). Detailly, there is no significant difference in spike length, kernel grain weight, grain number and N concentration in straw between with and without fertilization. The average effective spike and N concentration in grain among N management treatments were 20.6 – 21.6% and 2.3 – 14.5% higher than that under N0, respectively. In general, the practice for N70BM treatment was superior to slightly increase the spike length, kernel grain weight and grain number while the practice for N100 has potential to enhance effective spike and N concentration in plant compared with other treatments.

3.4 Soil biochemical property

3.4.1 Soil nutrient and microbial biomass

Soil nutrient and microbial biomass at harvest for different fertilization regime are shown in Fig. 3. The average soil ammonium and nitrate N contents varied from 0.06 – 0.1 g kg^{-1} and 3.0 – 5.2 g kg^{-1} under all treatments, respectively (Fig. 3 a and b). The lower ammonium N content was observed from N70BM while the lower nitrate N content was observed from N100, with the average value of 0.08 g kg^{-1} and 3.9 g kg^{-1}, respectively. These values showed no significant differences as compared to those with N0 treatment.

Soil microbial biomass C (MBC) and N (MBN) were generally higher in applied N treatments than in the zero N treatment. The largest MBC and MBN occurred in N70BM, while the lowest MBC and MBN occurred in N0 treatment. N fertilization significantly affected MBC and MBN, which showed an increase by 24.3 – 37.3% and 35.5 – 93.3% for the MBC and MBN in response to N fertilization ($P < 0.05$, Fig. 3c and d,Table S6).

3.4.2 Soil enzyme activity

The soil enzyme activities differed among fertilization regimes (Fig. 4). High N addition had the largest soil sucrase and urease enzyme activities involved in C and N cycling, which are 17.8 – 47.6 % and 31.9 – 48.1% higher than those from other treatments, respectively ($P < 0.05$, Fig. 4a, b,Table S6). The lowest catalase enzyme activity was observed under N100 treatment ($P < 0.05$, Fig. 4c,Table S6). Similarly, the average highest C-acquisition enzyme activities occurred under N100 treatment ($P > 0.05$, Fig. 4d).

4 Discussion

4.1 Fertilizer regime impacts on grain yield, physiological morphology and N uptake

Previous studies have shown that suitable N management can increase plant growth, grain yield and its components (Makino et al., 2022). Grain yield, a key indicator of agricultural productivity, relies on the intricate interplay between yield components and N remobilization to economic organs in plant. Unraveling these connections is crucial for maximizing grain yield while reducing N losses to environment (Long et al., 2006; Liu et al., 2020). Our study showed that properly managing N application by urea or biological manure could significantly increase grain yield and N uptake in economic organs in plant, with the magnitude ranging from 7640 kg ha^{-1} to 9305 kg ha^{-1} and from 99 kg ha^{-1} to 131 kg ha^{-1}, respectively (Fig. 1 and Fig. 2). This is in agreement with previous studies that reported fertilization significantly contributed to increased biomass production through enhancing photosynthesis efficiency and mobilizing N to leaves and stems in plant (Yoon et al., 2020; Makino et al., 2022). However, compared to N100 treatment, biological manure fertilizer substitution slightly reduced grain yield, its components and accumulated N in plant. This could be because of rich nutrients from chemical N and biological manure fertilizer, which effectively supply sufficient N in leaves and stems over the vegetative period, promoting the remobilization of N to economic organs over the reproductive period (Iqbal et al., 2021). Nutrients were slowly released from biological manure fertilizer, which improves plant growth and meets N requirements, thus minimizing mineral N losses (Qaswar et al., 2020). Compared to the single N fertilization treatment, a small increase in spike length, kernel grain weight and grain number per spike was found under N70BM (Table 1). This could be because a slower nutrient release rate of biological manure enhanced the synchronization of N supply and accelerated the remobilized N in vegetative organs to economic organs at the later growth period. Additionally, this could be associated with the difference in assimilation distribution between tiller and main shoot due to the response of tiller inhibition gene to different N type (Duggan et al., 2005; Moeller and Rebetzke, 2017).

4.2 Fertilization regime effects on soil nutrient, microbial biomass and enzymes

Of the soil nutrients involved in this study, soil ammonium and nitrate consistently exhibited the most uniform responses across different treatments (Fig. 3a and b). The ANOVA showed that soil nutrient content under fertilization was slightly smaller compared to that in plots with zero nitrogen application. The increase in fertilizer application might have led to a more efficient uptake of nutrients by crops, resulting in enhanced crop yield and nutrient absorption despite the slightly reduced soil nutrient content (Li et al., 2019). This agrees with that the additional nutrients supplied through fertilization compensated for the marginal decrease in soil nutrient levels, contributing to improved crop performance and nutrient utilization (Govindasamy et al., 2023).

Organic manure increased soil microbial biomass in comparison with single chemical N addition (Fig. 4), which could be ascribed to the increased organic C and nutrient availability (Zhang et al., 2020). High amount of manure application in soil serves as substrates and energy for increasing microbial growth and activity (Ling et al., 2022). Our results suggest that the MBC and MBN were increased under N70BM compared with the single chemical N treatment (Fig. 3c and d). This is in agreement with previous study reported that abundant organic materials mixed with soil can provide rich nutrients for microbial metabolism, which in turn accelerates microbial growth and enzyme secretion, especially in the catalase (Zheng et al., 2019; Wang et al., 2022; Fig. 4c).

Past studies in paddy soil found that soil enzyme activity was enhanced by N amendments (Xiao et al., 2023 [urease and phosphatase]; Li et al., 2022 [β-1,4-N-acetylglucosaminidase]; Sharma et al., 2021 [urease and L-asparaginase]; Hu et al., 2023 [β-1,4-glucosidase, β-N-acetyl-glucosaminidase and phenol oxidase]). This is in accordance with our results showing that soil urease and sucrase activity was increased under N100 and N70BM treatments. An elevation in urease activity facilitates the conversion of soil organic nitrogen into ammonium nitrogen through mineralization. This ammonium nitrogen can subsequently adsorb onto soil particles, making it readily available for plant uptake (Liang et al., 2003). However, soil catalase activity could be repressed by fertilization. This could be attributed to the alteration in soil microbial communities and their metabolic activities when applying fertilization to soil (Zheng et al., 2019). Our results indicated that urease and sucrase activity in paddy soil were decreased while catalase activity occurred an increase when using biological manure compared to chemical N addition (Fig. 4). The observed phenomenon may be attributed to the release of substances by organic manure, leading to an increase in soil C/N ratio (Li et al., 2018; Yang et al., 2023). This alteration in C/N ratio could prompt shifts in soil microbial communities, given the presence of two nitrogen forms—organic and inorganic compounds (Luo et al., 2016; Wu et al., 2020). Consequently, these changes may influence urease and sucrase enzyme activities. It is important to note that catalase activity might exhibit distinct responses in the context of these alterations. To elucidate the effect of biological manure on soil C acquisition enzyme activity, more work should be made in the future.

5 Conclusion

Proper N management significantly influences grain yield, components, and nitrogen remobilization in plants, crucial for agricultural productivity. Substitution of biological manure for chemical fertilization significantly enhances rice yield and straw weight. Application of 70% total chemical N plus 2000 kg ha^{-1} biological manure proves superior to local practices, especially in maintaining rice yield and an increase in yield components and soil microbial biomass C and N. Fertilization influenced soil ammonium and nitrate content, with slightly smaller nutrient content in fertilized plots, compensated by efficient nutrient uptake. Prudent N management, particularly the judicious use of partial biological manure, is crucial for sustaining productivity and soil fertility in southern China.

Acknowledgements

This research was supported by Key Research and Development Program of Anhui Province (2023n06020019) and the Young Backbone Talents Project of Anhui Academy of Agricultural Sciences (QNYC-202210, QNYC-202209and XXBS-202214).

Declaration of competing interest

The authors declared that there is no conflict of interest either financially or otherwise.

References

Conant, Richard T., Berdanier, Aaron B. and Grace, Peter R., 2013. Patterns and trends in nitrogen use and nitrogen recovery efficiency in world agriculture. *Global Biogeochemical Cycles* **27**(2): 558-566. https://doi.org/10.1002/gbc.20053.

Doğan, Buhari, Shahbaz, Muhammad, Bashir, Muhammad Farhan, Abbas, Shujaat and Ghosh, Sudeshna, 2023. Formulating energy security strategies for a sustainable environment: Evidence from the newly industrialized economies. *Renewable and Sustainable Energy Reviews* **184**: 113551. https://doi.org/10.1016/j.rser.2023.113551.

Duggan, B. L., Richards, R. A., van Herwaarden, A. F. and Fettell, N. A., 2005. Agronomic evaluation of a tiller inhibition gene (tin) in wheat. I. Effect on yield, yield components, and grain protein. *Australian Journal of Agricultural Research* **56**(2): 169-178. https://doi.org/10.1071/AR04153.

Govindasamy, Prabhu, Muthusamy, Senthilkumar K., Bagavathiannan, Muthukumar, Mowrer, Jake, Jagannadham, Prasanth Tej Kumar, Maity, Aniruddha, Halli, Hanamant M., G. K., Sujayananad, Vadivel, Rajagopal, T. K., Das, Raj, Rishi, Pooniya, Vijay, Babu, Subhash, Rathore, Sanjay Singh, L., Muralikrishnan and Tiwari, Gopal, 2023. Nitrogen use efficiency—a key to enhance crop productivity under a changing climate. *Frontiers in Plant Science* **14**. https://doi.org/10.3389/fpls.2023.1121073.

Guan, SY, Zhang, Desheng and Zhang, Zhiming, 1986. Soil enzyme and its research methods. *Agricultural, Beijing* **1986**: 274-297.

Hou, Qiong, Ni, Yuemin, Huang, Shan, Zuo, Ting, Wang, Jian and Ni, Wuzhong, 2023. Effects of substituting chemical fertilizers with manure on rice yield and soil labile nitrogen in paddy fields of China: A meta-analysis. *Pedosphere* **33**(1): 172-184. https://doi.org/10.1016/j.pedsph.2022.09.003.

Hu, Quanyi, Liu, Tianqi, Ding, Huina, Li, Chengfang, Tan, Wenfeng, Yu, Ming, Liu, Juan and Cao, Cougui, 2023. Effects of nitrogen fertilizer on soil microbial residues and their contribution to soil organic carbon and total nitrogen in a rice-wheat system. *Applied Soil Ecology* **181**: 104648. https://doi.org/10.1016/j.apsoil.2022.104648.

Iqbal, Anas, He, Liang, Ali, Izhar, Ullah, Saif, Khan, Aziz, Akhtar, Kashif, Wei, Shangqin, Fahad, Shah, Khan, Rayyan and Jiang, Ligeng, 2021. Co-incorporation of manure and inorganic fertilizer improves leaf physiological traits, rice production and soil functionality in a paddy field. *Scientific Reports* **11**(1): 10048. https://doi.org/10.1038/s41598-021-89246-9.

Li, Weitao, Kuzyakov, Yakov, Zheng, Yulong, Liu, Ming, Wu, Meng, Dong, Yuanhua and Li, Zhongpei, 2022. Effect of long-term fertilisation on enzyme activities and microbial community composition in the rice rhizosphere. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science* **72**(1): 454-462. https://doi.org/10.1080/09064710.2021.2011394.

Li, Weitao, Wu, Meng, Liu, Ming, Jiang, Chunyu, Chen, Xiaofen, Kuzyakov, Yakov, Rinklebe, Jörg and Li, Zhongpei, 2018. Responses of Soil Enzyme Activities and Microbial

Community Composition to Moisture Regimes in Paddy Soils Under Long-Term Fertilization Practices. *Pedosphere* **28**(2): 323-331. https://doi.org/10.1016/S1002-0160(18)60010-4.

Li, Zhiguo, Zhang, Runhua, Xia, Shujie, Wang, Li, Liu, Chuang, Zhang, Runqin, Fan, Zhanhui, Chen, Fang and Liu, Yi, 2019. Interactions between N, P and K fertilizers affect the environment and the yield and quality of satsumas. *Global Ecology and Conservation* **19**: e00663. https://doi.org/10.1016/j.gecco.2019.e00663.

Liang, Wei, Wu, Zhen-bin, Cheng, Shui-ping, Zhou, Qiao-hong and Hu, Hong-ying, 2003. Roles of substrate microorganisms and urease activities in wastewater purification in a constructed wetland system. *Ecological Engineering* **21**(2): 191-195. https://doi.org/10.1016/j.ecoleng.2003.11.002.

Ling, Jun, Zhou, Jie, Wu, Gong, Zhao, De-Qiang, Wang, Zhi-Tong, Wen, Yuan and Zhou, Shun-Li, 2022. Deep-injected straw incorporation enhances subsoil quality and wheat productivity. *Plant and Soil*. https://doi.org/10.1007/s11104-022-05660-6.

Liu, Chuang, Wang, Li, Cocq, Kate Le, Chang, Changlong, Li, Zhiguo, Chen, Fang, Liu, Yi and Wu, Lianhai, 2020. Climate change and environmental impacts on and adaptation strategies for production in wheat-rice rotations in southern China. *Agricultural and Forest Meteorology* **292-293**: 108136. https://doi.org/10.1016/j.agrformet.2020.108136.

Long, Stephen P., Zhu, Xin-Guang, Naidu, Shawna L. and Ort, Donald R., 2006. Can improvement in photosynthesis increase crop yields? *Plant, Cell & Environment* **29**(3): 315-330. https://doi.org/10.1111/j.1365-3040.2005.01493.x.

Luo, Xuesong, Fu, Xiaoqian, Yang, Yun, Cai, Peng, Peng, Shaobing, Chen, Wenli and Huang, Qiaoyun, 2016. Microbial communities play important roles in modulating paddy soil fertility. *Scientific Reports* **6**(1): 20326. https://doi.org/10.1038/srep20326.

Makino, Amane, Suzuki, Yuji and Ishiyama, Keiki, 2022. Enhancing photosynthesis and yield in rice with improved N use efficiency. *Plant Science* **325**: 111475. https://doi.org/10.1016/j.plantsci.2022.111475.

Moeller, Carina and Rebetzke, Greg, 2017. Performance of spring wheat lines near-isogenic for the reduced-tillering 'tin' trait across a wide range of water-stress environment-types. *Field Crops Research* **200**: 98-113. https://doi.org/10.1016/j.fcr.2016.10.010.

Ng, Zheng Yang, Ajeng, Aaronn Avit, Cheah, Wai Yan, Ng, Eng-Poh, Abdullah, Rosazlin and Ling, Tau Chuan, 2024. Towards circular economy: Potential of microalgae – bacterial-based biofertilizer on plants. *Journal of Environmental Management* **349**: 119445. https://doi.org/10.1016/j.jenvman.2023.119445.

Qaswar, Muhammad, Jing, Huang, Ahmed, Waqas, Dongchu, Li, Shujun, Liu, Lu, Zhang, Cai, Andong, Lisheng, Liu, Yongmei, Xu, Jusheng, Gao and Huimin, Zhang, 2020. Yield sustainability, soil organic carbon sequestration and nutrients balance under long-term combined application of manure and inorganic fertilizers in acidic paddy soil. *Soil and Tillage Research* **198**: 104569. https://doi.org/10.1016/j.still.2019.104569.

Sharma, Sandeep, Singh, Pritpal, Choudhary, O. P. and Neemisha, 2021. Nitrogen and rice straw incorporation impact nitrogen use efficiency, soil nitrogen pools and enzyme activity in rice-wheat system in north-western India. *Field Crops Research* **266**: 108131. https://doi.org/10.1016/j.fcr.2021.108131.

Singh, Kshitij, Guleria, Vanshika, Kaushal, Shilpa and Shubham, 2023. Utilization of Biofertilizers and Plant Growth Promoters in Hydroponic Production System. *Current*

Journal of Applied Science and Technology **42**(37): 13-23. https://doi.org/10.9734/cjast/2023/v42i374243.
Smith, Pete, Davis, Steven J., Creutzig, Felix, Fuss, Sabine, Minx, Jan, Gabrielle, Benoit, Kato, Etsushi, Jackson, Robert B., Cowie, Annette, Kriegler, Elmar, van Vuuren, Detlef P., Rogelj, Joeri, Ciais, Philippe, Milne, Jennifer, Canadell, Josep G., McCollum, David, Peters, Glen, Andrew, Robbie, Krey, Volker, Shrestha, Gyami, Friedlingstein, Pierre, Gasser, Thomas, Grübler, Arnulf, Heidug, Wolfgang K., Jonas, Matthias, Jones, Chris D., Kraxner, Florian, Littleton, Emma, Lowe, Jason, Moreira, José Roberto, Nakicenovic, Nebojsa, Obersteiner, Michael, Patwardhan, Anand, Rogner, Mathis, Rubin, Ed, Sharifi, Ayyoob, Torvanger, Asbjørn, Yamagata, Yoshiki, Edmonds, Jae and Yongsung, Cho, 2016. Biophysical and economic limits to negative CO_2 emissions. *Nature Climate Change* **6**(1): 42-50. https://doi.org/10.1038/nclimate2870.
Tilman, David, Clark, Michael, Williams, David R., Kimmel, Kaitlin, Polasky, Stephen and Packer, Craig, 2017. Future threats to biodiversity and pathways to their prevention. *Nature* **546**(7656): 73-81. https://doi.org/10.1038/nature22900.
Vance, E. D., Brookes, P. C. and Jenkinson, D. S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* **19**(6): 703-707. https://doi.org/10.1016/0038-0717(87)90052-6.
Wang, Gang, Ren, Ying, Bai, Xuanjiao, Su, Yuying and Han, Jianping, 2022. Contributions of Beneficial Microorganisms in Soil Remediation and Quality Improvement of Medicinal Plants, Plants.
Wang, Haiyan, Wu, Jiangqi, Li, Guang and Yan, Lijuan, 2020. Changes in soil carbon fractions and enzyme activities under different vegetation types of the northern Loess Plateau. *Ecology and Evolution* **10**(21): 12211-12223. https://doi.org/10.1002/ece3.6852.
Wu, Lipeng, Ma, Hui, Zhao, Qinglei, Zhang, Shirong, Wei, Wenliang and Ding, Xiaodong, 2020. Changes in soil bacterial community and enzyme activity under five years straw returning in paddy soil. *European Journal of Soil Biology* **100**: 103215. https://doi.org/10.1016/j.ejsobi.2020.103215.
Xiao, Qingqing, He, Boping and Wang, Su, 2023. Effect of the Different Fertilization Treatments Application on Paddy Soil Enzyme Activities and Bacterial Community Composition, Agronomy.
Yang, Yining, Chen, Yao, Li, Zhe, Zhang, Yuanyuan and Lu, Lunhui, 2023. Microbial community and soil enzyme activities driving microbial metabolic efficiency patterns in riparian soils of the Three Gorges Reservoir. *Frontiers in Microbiology* **14**. https://doi.org/10.3389/fmicb.2023.1108025.
Yin, Rui, Deng, Huan, Wang, Hui-li and Zhang, Bin, 2014. Vegetation type affects soil enzyme activities and microbial functional diversity following re-vegetation of a severely eroded red soil in sub-tropical China. *CATENA* **115**: 96-103. https://doi.org/10.1016/j.catena.2013.11.015.
Yoon, Dong-Kyung, Ishiyama, Keiki, Suganami, Mao, Tazoe, Youshi, Watanabe, Mari, Imaruoka, Serina, Ogura, Maki, Ishida, Hiroyuki, Suzuki, Yuji, Obara, Mitsuhiro, Mae, Tadahiko and Makino, Amane, 2020. Transgenic rice overproducing Rubisco exhibits increased yields with improved nitrogen-use efficiency in an experimental paddy field. *Nature Food* **1**(2): 134-139. https://doi.org/10.1038/s43016-020-0033-x.
Zhang, Xu, Qu, Jisong, Li, Hong, La, Shikai, Tian, Yongqiang and Gao, Lihong, 2020. Biochar addition combined with daily fertigation improves overall soil quality and enhances water-

fertilizer productivity of cucumber in alkaline soils of a semi-arid region. *Geoderma* **363**: 114170. https://doi.org/10.1016/j.geoderma.2019.114170.

Zheng, Qing, Hu, Yuntao, Zhang, Shasha, Noll, Lisa, Böckle, Theresa, Dietrich, Marlies, Herbold, Craig W., Eichorst, Stephanie A., Woebken, Dagmar, Richter, Andreas and Wanek, Wolfgang, 2019. Soil multifunctionality is affected by the soil environment and by microbial community composition and diversity. *Soil Biology and Biochemistry* **136**: 107521. https://doi.org/10.1016/j.soilbio.2019.107521.

Figure legend

Fig. 1 Boxplots summarizing variation in rice grain yield (a) and straw weight (b) under different fertilizer treatments between 2020 and 2022. For each boxplot, the central mark is the median, the red square indicates the mean value, the edges of the box are the 25th and 75th percentiles, and the whiskers extend to the extreme data points not considered to be outliers. Different lowercase letters represent significant differences ($P < 0.05$) between the different N treatments.

Fig. 2 Variations of N uptake between vegetative and economic organs (a) and NRE at harvest among different N treatments during 2020 – 2022. The error bar represents the standard deviation of each index in each treatment. Different lowercase letters represent significant differences ($P < 0.05$) between the different N treatments.

Fig. 3 Average soil ammonium, nitrate (a, b) and microbial biomass C/N (c, d) in each treatment from 2020 to 2022. The error bar represents the standard deviation of each index in each treatment. Different lowercase letters represent significant differences ($P < 0.05$) between the different N treatments.

Fig. 4 Boxplots summarizing variation in soil sucrase, urease and catalase under different fertilizer treatments between 2020 and 2022. For each boxplot, the central mark is the median, the red point indicates the mean value, the edges of the box are the 25th and 75th percentiles, and the whiskers extend to the extreme data points not considered to be outliers. The error bar represents the standard deviation of each index in each treatment. Different lowercase letters represent significant differences ($P < 0.05$) between the different N treatments.

Table 1 Changes of yield components and N concentrations in grain and straw at harvest during 2020 – 2022.

Figure 1

Grain yield and straw weight in rice

Fig. 1 Boxplots summarizing variation in rice grain yield (a) and straw weight (b) under different fertilizer treatments between 2020 and 2022. For each boxplot, the central mark is the median, the red square indicates the mean value, the edges of the box are the 25th and 75th percentiles, and the whiskers extend to the extreme data points not considered to be outliers. Different lowercase letters represent significant differences (*P* < 0.05) between the different N treatments.

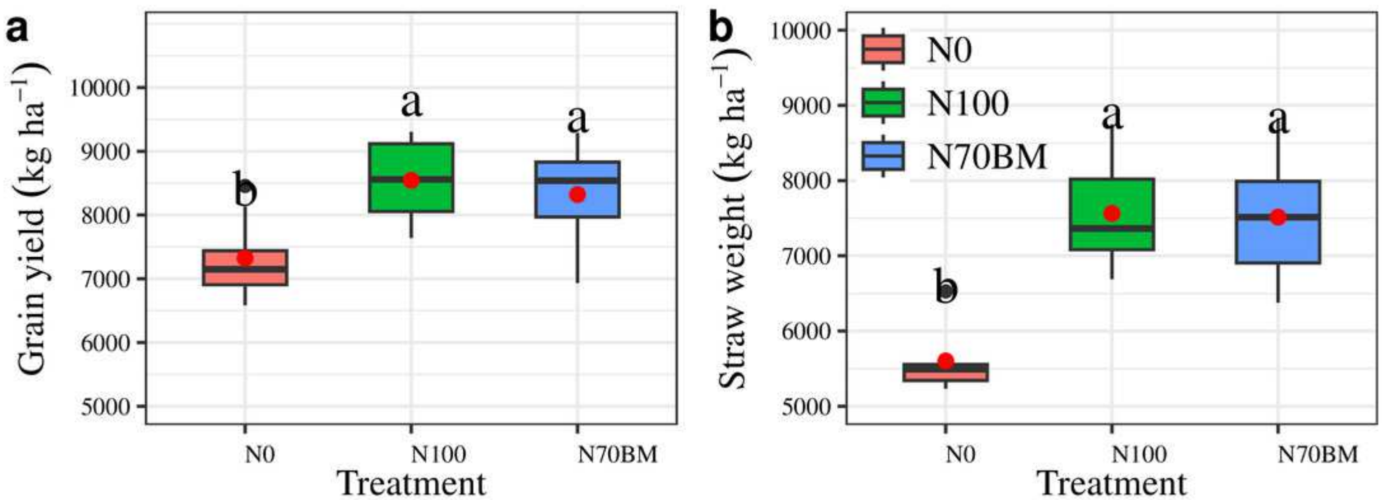

Figure 2

N uptake and NUE in rice

Fig. 2 Variations of N uptake between vegetative and economic organs (a) and NRE at harvest among different N treatments during 2020 – 2022. The error bar represents the standard deviation of each index in each treatment. Different lowercase letters represent significant differences (*P* < 0.05) between the different N treatments.

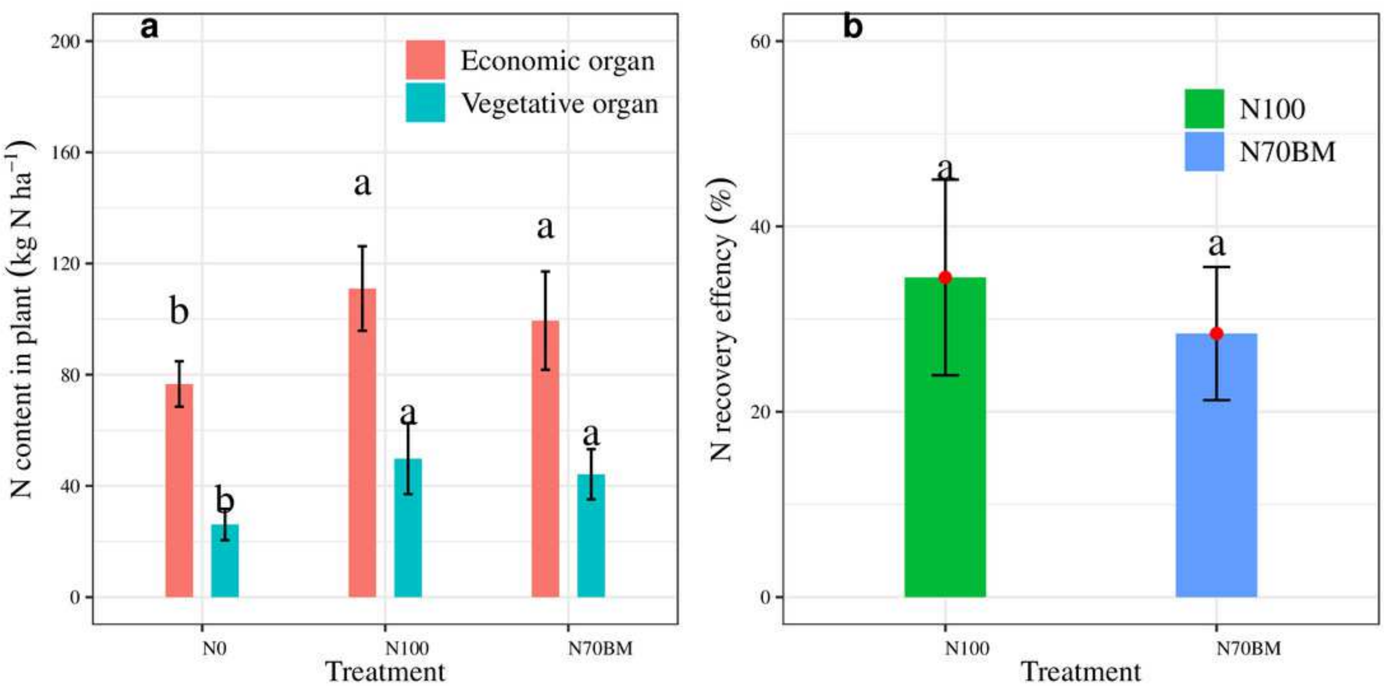

Figure 3

Soil nutrient and mirobial biomass in rice

Fig. 3 Average soil ammonium, nitrate (a, b) and microbial biomass C/N (c, d) in each treatment from 2020 to 2022. The error bar represents the standard deviation of each index in each treatment. Different lowercase letters represent significant differences (P < 0.05) between the different N treatments.

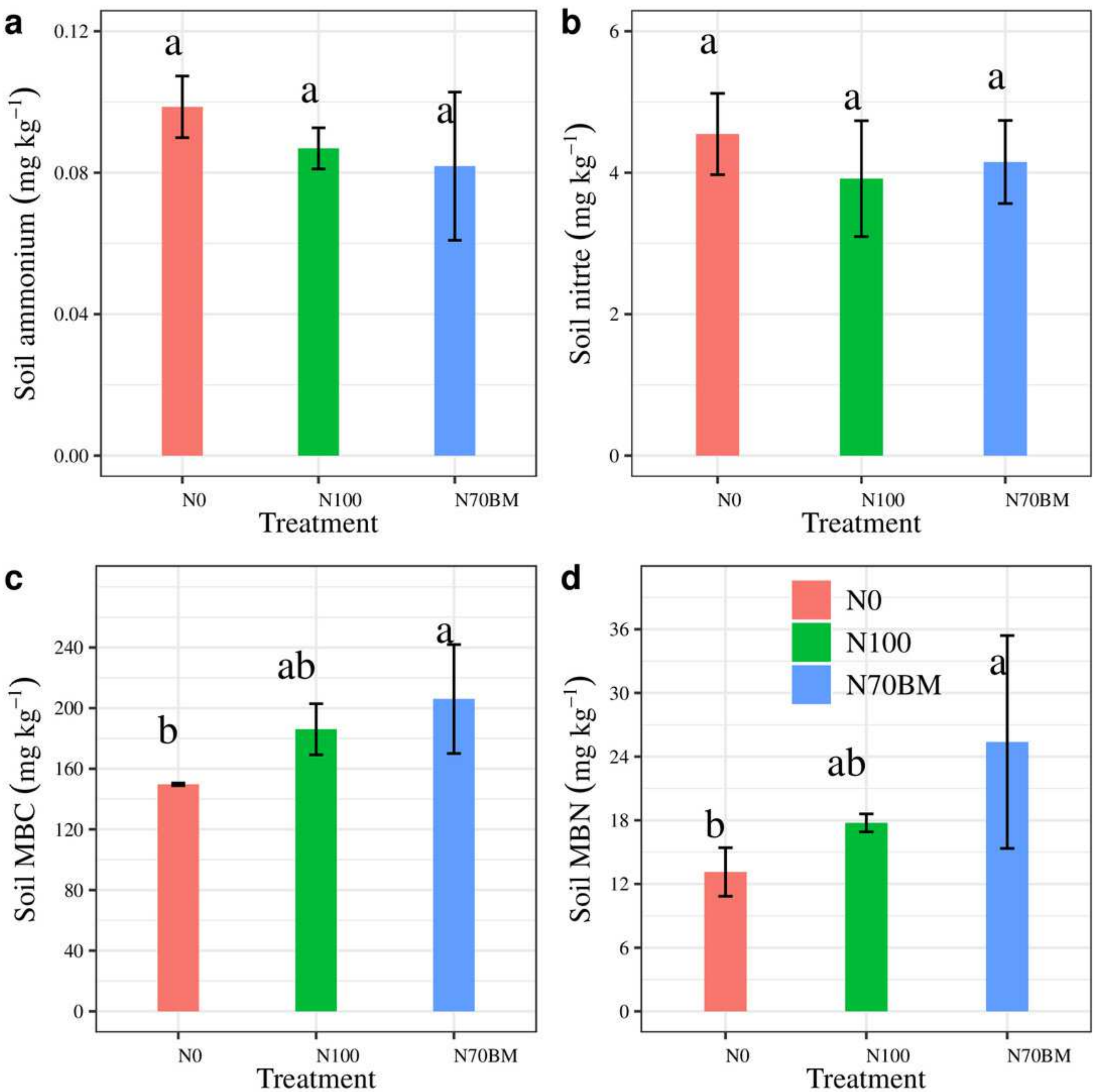
a
b
c
d
Soil ammonium (mg kg^{-1})
Soil nitrte (mg kg^{-1})
Soil MBC (mg kg^{-1})
Soil MBN (mg kg^{-1})
Treatment
N0
N100
N70BM
0.00
0.04
0.08
0.12
0
2
4
6
0
40
80
120
160
200
240
0
6
12
18
24
30
36
a
ab
b

Figure 4

Soil enzyme in rice

Fig. 4 Boxplots summarizing variation in soil sucrase, urease and catalase under different fertilizer treatments between 2020 and 2022. For each boxplot, the central mark is the median, the red point indicates the mean value, the edges of the box are the 25th and 75th percentiles, and the whiskers extend to the extreme data points not considered to be outliers. The error bar represents the standard deviation of each index in each treatment. Different lowercase letters represent significant differences ($P < 0.05$) between the different N treatments.

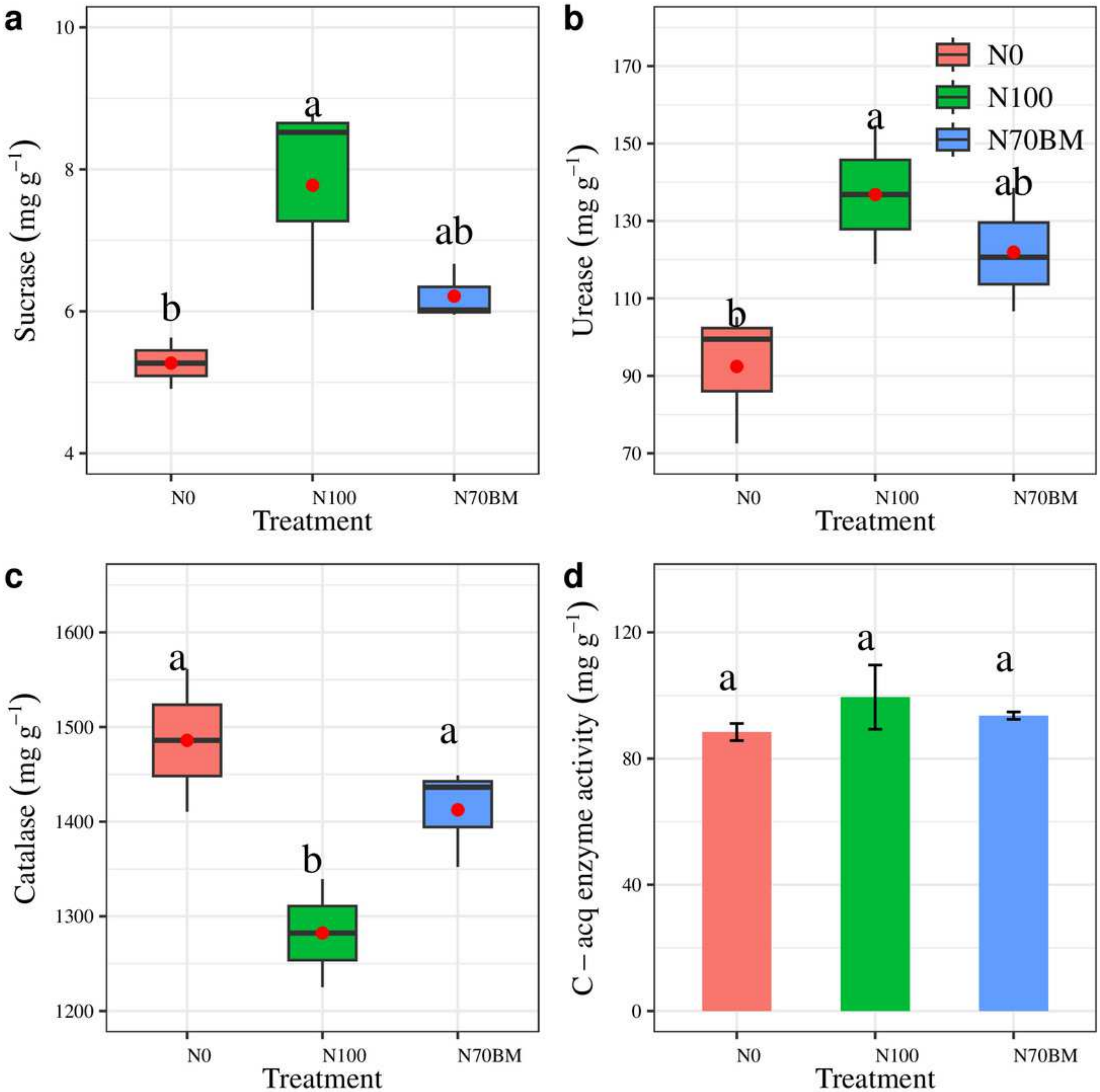
a
Sucrase (mg g^{-1})
10
8
6
4
a
ab
b
N0
N100
N70BM
Treatment
b
Urease (mg g^{-1})
170
150
130
110
90
70
N0
N100
N70BM
a
ab
b
N0
N100
N70BM
Treatment
c
Catalase (mg g^{-1})
1600
1500
1400
1300
1200
a
a
b
N0
N100
N70BM
Treatment
d
C − acq enzyme activity (mg g^{-1})
120
80
40
0
a
a
a
N0
N100
N70BM
Treatment

Table 1 (on next page)

Changes of gain yields, yield component and straw weight in rice under different N management practices.

Table 1 Changes of yield components and N concentrations in grain and straw at harvest during 2020 – 2022.

Table 1 Changes of yield components and N concentrations in grain and straw at harvest during 2020 – 2022.

Treatment	Spike length (cm)	Effective spike ($\times10^5$ ha^{-1})	Kernel grain weight (g^{-1})	Grain number (spike^{-1})	Straw N content (g kg^{-1})	Grain N content (g kg^{-1})
N0	26.1 ± 3.7a	16 ± 3.1b	22.4 ± 0.8a	164.3 ± 15.8a	5.2 ± 1.3a	11.0 ± 1.0b
N100	26.5 ± 3.6a	19.6 ± 2.5a	22.4 ± 1.1a	167.2 ± 28.6a	6.0 ± 1.6a	12.6 ± 1.2a
N70BM	27.6 ± 1.6a	19.3 ± 4.9a	22.5 ± 0.6a	169.9 ± 15.2a	5.8 ± 0.6a	11.3 ± 1.3ab