Application of nitrogen loaded biochar in purifying agricultural wastewater and as a nitrogen releaser for rice production

Hongyang Chen[1], Yidi Sun[2], Yang Sun[1], Yanzhi Wang[1], Yanqi Li[1], Qi Wu[1*], Daocai Chi[1]
(1. *College of Water Conservancy, Shenyang Agricultural University, Shenyang 110866, China*;
2. *College of Hydraulic Science and Engineering, Yangzhou University, Yangzhou 225009, Jiangsu, China*)

Abstract: Herein a new approach to the application of agricultural eutrophic wastewater for rice plant cultivation is described. Biochar was used as a medium for the sorption of ammonia from simulated wastewater and subsequently as a nitrogen (N) releaser in the cultivation of rice plants. The main goals of this approach were to isolate ammonia from simulated wastewater and transfer it into rice cultivation, and or explore how exogenous N promoted the growth of rice. The results demonstrate that according to X-ray diffraction phase analysis, most of the properties of biochar were retained before and after loading NH_4^+-N. Compared with biochar, the crystal peak of AlOOH in N-loaded biochar (NLB) disappeared and the intensity of the crystal peak of $CuCaSe_2$ decreased, which was the important mechanism allowing it to adsorb 30.8% of the N present in simulated low N-concentration agricultural wastewater. The soil N content in NLB treatments was higher than in Non-NLB treatments during the critical tillering and reproductive growth stages. Moreover, the N adsorption-desorption process of NLB matched the N requirements of the rice plant, and thus greatly increased the tiller number by 11.9% and rice yield by 7.5%. These results indicated that the indirect use of ammonia derived from wastewater using biochar as a sorption and releasing medium for rice plant cultivation was promising. This is the first time that biochar was used for possibly indirect reuse of agricultural eutrophic wastewater and enhancement of rice plant growth.
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1 Introduction

As agricultural modernization accelerates, the stress of agricultural development in Northeast China on natural resources and the environment has intensified. In particular, the rapid growth of japonica rice planting areas has aggravated existing water shortages. Long-term excessive N application has impacted soil structure, resulting in low retention of soil N and high N load in nutrient water, which is a very serious soil-water problem in irrigated areas. The imbalanced N load phenomenon of "soil poor-water rich" is common in rice-irrigated areas, and the key to solving this problem is to regulate the distribution of N from space.

In recent years, high-performance adsorption materials such as biochar and clinoptilolite have emerged as important ways to remove water pollution barriers and are generally significant for the removal of N, phosphorus, and heavy metals from eutrophic wastewater. Biochar (straw biochar), which comes from farmland, is an environment-friendly adsorption material that is beneficial to the growth of crops after returning to the field[1-3]. The adsorption of biochar on NH_4^+-N is the result of ion exchange and the reaction of surface functional groups with external cations, which provide attachment sites for the adsorption of positive ions and make biochar an effective N fixator[4,5]. Biochar and clinoptilolite can adsorb 20%-35% of NH_4^+-N in eutrophic water (created by agricultural nutrient discharge), while the adsorption efficiency of these materials to NH_4^+-N in high-concentration urban domestic sewage was > 83.5%[6-8]. Xie et al.[9] reported that biochar not only loaded N from yellow water (urban domestic sewage) but also was applied as N releaser to farmland to release NH_4^+ through ion competition, thus improving the availability of soil N and promoting the emergence rate of legumes. By means of material characterization, Liu et al.[10] demonstrated that the biochar can be loaded with urea to produce a new type of fertilizer, and concluded that this new type of fertilizer had good prospects for application in green and sustainable agriculture. Therefore, biochar not only has the potential to reduce the degree of N enrichment in eutrophic water, but also to act as a nutrient carrier and participate in the spatial redistribution of N in rice irrigated areas. Although the N concentration of eutrophic water from agricultural discharge is lower than that of municipal and industrial wastewater, it can lead to algae outbreak, which seriously threatens the sustainable development of agriculture[11]. For agricultural production, eutrophic water is the most effective N source for biochar adsorption. However, there are few studies on the use of biochar as a nutrient carrier to remove N from eutrophic water or as a nutrient releaser to promote plant growth in agricultural production.

In terms of agricultural application, the influence of biochar on plant growth is still uncertain. Biochar contains a variety of

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Biographies: Hongyang Chen, PhD, research interest: green and efficient crop water use and environment, Email: chenhygs@163.com; **Yidi Sun**, PhD, Lecturer, research interest: efficient utilization of agricultural water and soil resources, Email: yidisun0626@outlook.com; **Yang Sun**, PhD, research interest: green and efficient crop water use and environment, Email: sy18102447288@163.com; **Yanzhi Wang**, PhD, research interest: theory and technology of water saving irrigation, Email: 13304058353@163.com; **Yanqi Li**, PhD, research interest: theory and technology of water saving irrigation, Email: lyq111999@outlook.com; **Daocai Chi**, PhD, Professor, research interest: green and efficient crop water use and environment, Email: chidaocai@syau.edu.cn.
***Corresponding author: Qi Wu**, PhD, Associate Professor, research interest: nanomaterials and optimized utilization of agricultural resources, Mailing address: No.120, Dongling Road, Shenyang City, 110866, China, Tel: +86-15909825713, Email: qiwu0701@syau.edu.cn.

nutrients which can be added to the soil to slow-release nutrients and benefit plant growth[12]. However, El-Naggar et al.[13] reported that soil nutrient supply and plant growth are inhibited after the addition of biochar in farmland. Scholars have attributed this inconsistency to external production environmental factors while ignoring differences in the material's internal structural characteristics (decisive structural parameters affecting ion adsorption). Using material characterization techniques such as X-ray diffraction, Hagab et al.[14] concluded that the condensation reaction between functional groups on the surface of nano zeolites and external ions was important in promoting N adsorption and peanut growth. In fact, many papers showed that biochar with similar cation exchange capacity, specific surface area, and carbon-oxygen content exhibited significantly different effects following on-farm applications[1,15,16]. Therefore, examining differences in the internal structures of biochar is an important precursor to qualitative evaluation of biochar as a tool to improve soil and water environment. In addition, in agricultural production, when the N released by biochar matches the growth requirements of the plant, it can enhance plant growth. However, how does biochar compensate for plant growth after adsorbing N from eutrophic water? What is the relationship between the water purification by biochar and the promotion of plant growth? To date, there are no studies addressing these questions.

Therefore, in this study X-ray diffraction phase analysis and SEM (Scanning Electron Microscope, SEM) sample morphology analysis were used to compare microstructure before and after biochar loading with N. NLB was applied to farmland and the growth of rice plants was monitored. The aims of this study were to 1) propose a new approach to utilizing low-concentration eutrophic agricultural wastewater based on biochar, 2) explore the microscopic adsorption rules of N recovered from eutrophic water by biochar, and N release from biochar in farmland environments, and 3) verify the effect of NLB on rice plant growth and its potential to solve the N "soil poor-water rich" problem in irrigated areas.

2 Materials and methods

2.1 Biochar and soil

Biochar used in this study was pure straw biochar with a particle size of about 0.25 mm. It was purchased from Shenyang Longtai Biological Engineering Co., Ltd., Liaoning Province, China. The specific surface area was 6.16 m^2/g. The cation exchange capacity was 668-757 mmol/kg and the porosity of biochar was 11%. Soil samples originated from the Donggang experimental irrigation station (39°52′48″N, 123°34′48″E and 8.1 m above mean sea level), Liaoning Province, in North-Coast China. Soils, sieved for 60 mesh after natural air drying, were sampled with an "S" shape at 0-30 cm depth. The main physical and chemical properties of the experimental soil were 11.4% sand, 66.7% silt, 21.9% clay; the bulk density, 1.50 g/cm^3; pH, 6.61; available phosphorus, 32.33 mg/kg; available potassium, 56.56 mg/kg; total N, 677 mg/kg; organic matter, 9.02 g/kg.

2.2 Experiment design

2.2.1 Adsorption experiments

To determine the NH_4^+-N adsorption efficiency of biochar, deionized water was used to configure NH_4^+ concentration of 0.8, 1.6, 2.4 mg/L NH_4Cl (AR, Sinopharm Chemical Reagents Co., Ltd.) solution, and placed in three groups of 100 mL centrifuge tubes. Each group of centrifuge tubes was added with a mass of 0.3, 0.9, 1.5, 3, 6 g of biochar, respectively. The centrifuge tube was placed in a thermostatic oscillator (200 r/min, 25°C) for the timing of oscillation, and a small amount of solution was filtrated at 0, 5, 10, 30, 60, 90 min after the beginning of oscillation, and the concentration of NH_4^+-N was measured by CFA (Seal Analytical, AutoAnalyzer 3, Germany). Sample measurements were repeated three times. The adsorption efficiency of biochar to NH_4^+-N was calculated according to Equation (1)[17]. Biochar after adsorption equilibrium (NLB) was prepared in large quantities for the field experiment by mixing biochar with simulated low-concentration eutrophic agricultural wastewater (NH_4Cl solution) in a 500 L container, mechanically stirring for 30 min, and then extracting for natural air drying after 2 h. The preparation process is stopped until enough NLB are prepared.

$$W = \frac{C_0 - C_1}{C_0} \tag{1}$$

where, W is the N adsorption efficiency of biochar; C_0 is the initial concentration of NH_4^+ in solution, mg/L; C_1 is the equilibrium concentration of NH_4^+ in solution, mg/L.

2.2.2 Lysimeter experiments

The lysimeter experiment was a single factor design with three treatments and three replicates. The three treatments were: 1) no NLB treatment (NLB_0, blank control), 2) 10 t/hm^2 NLB treatment (NLB_{10}), and 3) 20 t/hm^2 NLB (NLB_{20}). NLB was applied to the surface soil as a base fertilizer and was then mixed with the 0-15 cm soil layer by ploughing. The rice variety "Dongyan 18", a late-maturing variety with a fertility period of about 150 d, was chosen for testing. Based on the traditional fertilization method in the custom, N (172.5 kg/hm^2) as urea was applied in three parts: 50% basal before transplanting, 30% 10 d after transplanting (DAT) and 20% 15 d after the jointing-booting stage, respectively. K (60 kg/hm^2) was applied in the form of potassium sulfate in two fractions: 50% basal and 50% 15 d after the jointing-booting stage, respectively. P (P_2O_5, 75 kg/hm^2) was applied as the basal fertilizer. The irrigation pattern was the usual local irrigation regimes, alternate wet-dry (AWD) irrigation, as shown in Table 1. Other agronomic measures were carried out with reference to local traditional management techniques.

Table 1 Traditional irrigation regimes

Irrigation regimes	Parameters	Early tillering stage	Middle tillering stage	Late tillering stage	Jointing-booting stage	Heading-flowering stage	Grain-filling stage
AWD	Water layer/cm	3-5	0-3	0	0-5	0-5	0-3
	Lower limit of soil water potential/kPa	0	0 to –5	–25 to –35	0 to –5	0 to –5	–10 to –20

2.2.3 Sample measurements

For tillering dynamics, five plants of each plot were marked for observing tiller number. The observation was made at the 3-5 d intervals before the joint-booting stage, followed by 10-15 d intervals until grain ripening. During rice plants growth period, fresh soil samples were collected with an auger from depths of 15-30 cm to prevent any interferences of N absorption by NLB. The collected samples were immediately transferred to insulated plastic containers, and visible roots were removed. At the end of growing stage, grain yield was calculated based on 14% moisture content. The NH_4^+-N and NO_3^--N were extracted from fresh soil samples with 2M KCl, the suspension was filtered by hydrophilic filter membrane and the filtrate was frozen (–20°C) for later analysis. Inorganic N concentrations in the KCl extracts were measured by the CFA (Seal Analytical, Autoanalyzer 3, Germany)[18].

2.3 Statistical analysis of data

Origin 2021 scientific mapping and data analysis software was used for mapping and analysis of variance (ANOVA). The separation of the means was performed using least significant difference (LSD). An X-ray diffractometer (XRD, Bruker, D8 Advance) was used to analyze the changes in mineral composition of biochar before and after N loading. Jade 6.5 was used for phase analysis of XRD data. Crystal compounds in the samples were identified by comparing diffraction data against a database compiled by the Joint Committee on Powder Diffraction and Standards. Morphology characteristics of biochar before and after N loading were tested by a scanning electron microscope (SEM, Zeiss, Supar55).

3 Results and discussion

3.1 Determination of NLB adsorption efficiency

The relationship among NH_4^+-N adsorption efficiency, biochar dosages, and initial N concentration was modeled based on the adsorption test data as shown in Figure 1. The adsorption efficiency of NH_4^+ increased with increasing biochar dosage and initial NH_4^+ concentration in the low concentration NH_4^+-N solution environment. The fitted equation for NH_4^+-N adsorption by biochar was as follows:

$$Z = 0.8763 \times \frac{1}{1+\left(\dfrac{X}{3.4314}\right)^{-0.5539}} \cdot \frac{1}{1+\left(\dfrac{Y}{1.1999}\right)^{-2.5627}} + 0.0158 \quad (2)$$

where, X represents biochar dosage, g; Y represents the initial N concentration in solution, mg/L; and Z represents the adsorption efficiency of biochar.

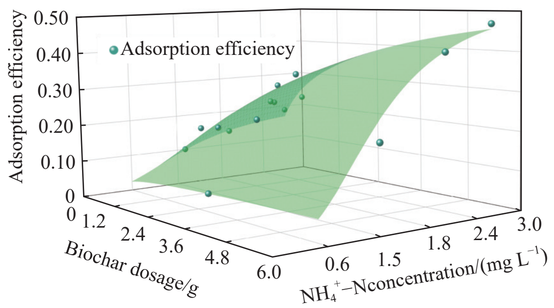


Figure 1 Absorption efficiency of biochar to NH_4^+ under simulated low-concentration eutrophic agricultural wastewater conditions

This experiment determined that the adsorption efficiency of biochar for NH_4^+-N in simulated low-concentration eutrophic agricultural wastewater was 30.8%.

3.2 Characterization and analysis

The XRD diffraction pattern of the biochar before and after loading N is shown in Figure 2. The X-ray diffraction of the biochar before loading N showed 10 crystal peaks at 2θ = 20.99, 22.19, 26.70, 28.08, 36.66, 39.52, 42.54, 50.20, 60.11, and 67.89, while the X-ray diffraction of NLB showed 7 crystal peaks at 2θ = 21.01, 26.71, 28.05, 42.60, 50.30, 60.20, and 68.40. XRD analysis showed that most properties, e.g. the structural integrity of the material, were preserved after biochar was loaded with N[14,19]. First, the silicon compound in the structure of the biochar surface have become silicon ions, providing adsorption sites for NH_4^+ to attach to the biochar surface and pore channels (note that the SiO_2 crystal peak of B in Figure 2 is much higher than that of NLB). Second, some metal cations on the surface or in the pore channels of biochar were exchanged with NH_4^+, allowing the biochar to achieve N loading[20,21]. This is evident in comparing diffraction data between B and NLB (Figure 2); the crystal peak of AlOOH in NLB disappeared and the intensity of the crystal peak of $CuCaSe_2$ decreased. Yang et al. (2020) showed that the adsorption pathways of biochar for NH_4^+ are electrostatic adsorption, ion exchange, and surface precipitation[22]. Through material characterization techniques and adsorption experiments, Kizito et al.[23] demonstrated that rice husk biochar adsorbed NH_4^+ via ion exchange. From the XRD analysis here, it is clear that biochar adsorbed NH_4^+ easily in its ion exchange sites and void channels through ion exchange and physical attachment. In addition, The surface morphology of the biochar before and after N loading was characterized by a scanning electron microscope. It can be seen from Figure 3 that the surface of biochar was relatively smooth. In contrast, NLB had small crystals with N attached to its surface, which looked rougher, and this helped to increase the total specific surface area. Therefore, NLB had a higher adsorption capacity[24]. In addition, the biochar had sharp edges and corners, while NLB surface had many debris-like particles. This indicated that the surface of the biochar may be wrapped with ammonium. From the above, the altered microstructure of biochar is an important mechanism allowing it to adsorb 30.8% of the N present in simulated low-concentration eutrophic agricultural wastewater.

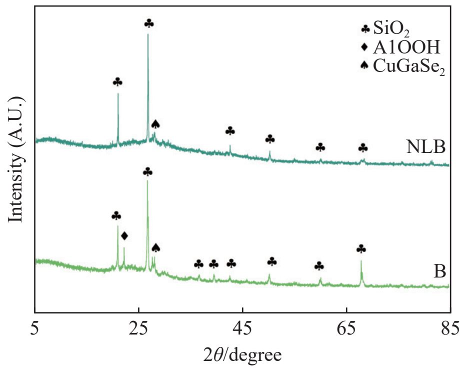


Figure 2 XRD patterns of NLB and B

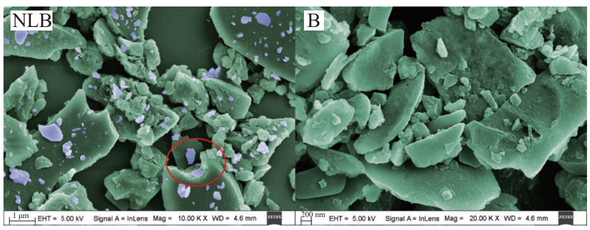
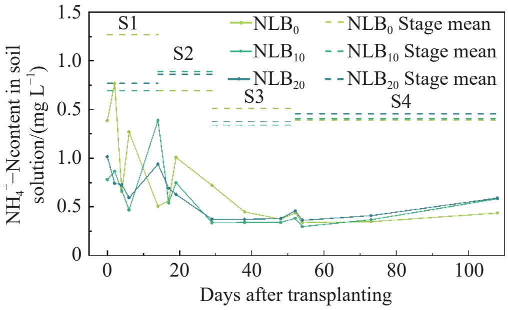


Figure 3 SEM images of NLB and B

3.3 Changes in soil NH_4^+-N

Soil NH_4^+-N changed obviously in all treatments (Figure 4). Soil NH_4^+-N content was higher in the early stage and lower in the later stage, as expected given rice plants' demand for N[25]. Based on the N demand and growth of rice plants, the growth period was divided into four stages; the non-critical growth stage (S1), the critical tillering growth stage (S2), the non-critical growth stage (S3), and the critical reproductive growth stage (S4)[26]. The peak and mean values of NH_4^+-N concentration in the NLB treatment were lower than those in the NLB_0 treatment (Figure 4). Plant N utilization was lower during S1, but NLB continued to adsorb soil NH_4^+-N, resulting in lower NH_4^+-N levels in the soil which was consistent with results in Haefele et al.[27] During S2, the average value of soil NH_4^+-N in the NLB treatment was much higher than in the NLB_0 treatment. S2 is a time of accelerated tiller differentiation in rice, and plant N requirement increases in order to ensure sufficient tillers. Meanwhile, the continued desorption of ammonia by NLB results in higher ammonia levels in the soil in the NLB_{10} and NLB_{20} treatments. During S3, according to traditional practice, farmers tend to lower N supply in order to improve the effective tillering rate. During this stage the average soil NH_4^+-N content in the NLB treatment was lower than that in the NLB_0 treatment (Figure 4), perhaps due to the low N demand of rice and the continuous absorption of N from tillering fertilizer by NLB. Under appropriate external environmental conditions, biochar can desorb or adsorb NH_4^+-N to regulate soil nutrients according to the following equation[28]:

$$NLB-X+nNH_4^+ \rightleftharpoons NLB-NH_4^+ + X^{n+} \quad (3)$$



Note: NLB_0 stage mean, NLB_{10} stage mean and NLB_{20} stage mean represent the mean of NH_4^+-N concentration in soil solution at every stage, respectively.

Figure 4 Dynamics and mean values of soil NH_4^+-N content in different treatments

When abundant tillering fertilizer was applied during S3, the equation shifted to the right and soil NH_4^+-N content significantly decreased[29]. X in the above equation may be Cu or other metal ions (Figure 2). During S4, rice plants need a large amount of N to ensure adequate supply of reproductive nutrients. However, the soil N content in NLB treatments was higher than in NLB_0 treatments. This was because increased rice N demand during S4 lead to the left shift of Equation (3). Overall, N adsorption and desorption rules match the N requirement for plant growth, which is beneficial to the plant. This study also demonstrated that biochar could adsorb 30.8% of the NH_4^+ present in simulated eutrophic wastewater, and the NH_4^+-N release process of NLB was well-matched with the N requirement of rice plants.

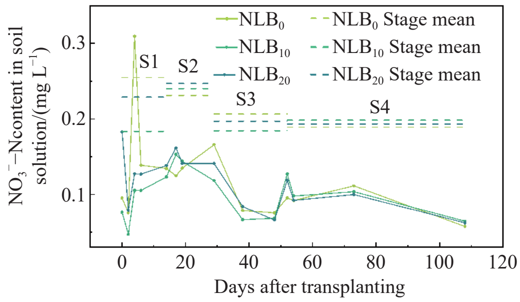
3.4 Changes in soil NO_3^--N

Changes in soil NO_3^--N content are shown in Figure 5. NLB_{10} and NLB_{20} treatments had similar effects on NO_3^--N content, and considerably differed from the effect of NLB_0 treatment. The soil NO_3^--N concentration curve of NLB_{10} and NLB_{20} treatment was gentler than that of the NLB_0 treatment. Overall, NO_3^--N content showed three peaks, and was generally consistent with the results of previous studies[30]. During S1, NO_3^--N content in each treatment was higher than during the subsequent stages (Figure 5). Because the nitrification reaction source formed by urea hydrolysis was sufficient, NH_4^+-N was accelerated into NO_3^--N according to the following equations[31].

$$CO(NH_2)_2 + H_2O = CO_2\uparrow + 2NH_3\uparrow \quad (4)$$

$$2NH_3 + 3O_2 = 2HNO_2 + 2H_2O \quad (5)$$

$$2HNO_2 + O_2 = 2HNO_3 \quad (6)$$



Note: NLB_0 stage mean, NLB_{10} stage mean and NLB_{20} stage mean represent the mean of NO_3^--N concentration in soil solution at every stage, respectively.

Figure 5 Dynamics and mean values of soil NO_3^--N content in different treatments

During S2, according to Equation (4-6), a large amount of desorbed NH_4^+-N increased the soil NO_3^--N content under NLB treatment, but NLB_0 treatment did not provide sufficient nutrients during the critical stage of rice vegetative growth. During S3, drought promoted the conversion of NH_4^+-N to NO_3^--N, resulting in high NO_3^--N content under NLB_0 treatment. In addition, NLB adsorbed NH_4^+-N into the internal structure of biochar and delayed the above transformation process, and this was conducive to desorption during S4 (the key stage of N demand) and thus beneficial to growth. There was no obvious difference in soil NO_3^--N content between treatments during S4, but the N content in both the NLB_{10} and NLB_{20} treatments was higher than in the NLB_0 treatment, therefore NLB treatment can provide an efficient supply of N for the reproductive growth of rice. The regulatory effect of all NLB treatments on NO_3^--N was better than that of the NLB_0 treatment across the entire rice growth period. It matched the N requirement of rice, and thus may promote the growth of rice plants.

3.5 Dynamic change of rice tillers

The curve of rice tillering number showed unimodal change (Figure 6). The curve increased dramatically 20 DAT and began to decline 40 DAT. At 60 DAT, the curve of the rice tiller number was gentle and remained stable. Compared with the NLB_0 treatment, the NLB_{10} treatment had the highest tiller number, and increased the effective tiller number up to 11.9%. NLB not only released N to promote tillers during the critical vegetative growth period of rice, but also provided a N source to produce effective tillers during the critical reproductive vegetative period. The important factors for increasing rice yield are to ensure a sufficient “source” and to form a large “sink”; that is, to properly increase the number of effective tillers and promote the formation of panicles[26]. This study showed that rice had a low tiller capacity and low N demand during S1. During S2, rice tiller growth accelerated and the NLB began to desorb the N fixed in the earlier stage to meet the needs of rice tiller growth. These stages were the “source” formation stages, during which the NLB adsorbs N to increase rice tillers. During the “sink” formation period, soil N concentration and rice tiller number under NLB treatment were greater than under NLB_0 treatment. The results indicated that during the reproductive growth stage, the N supply from NLB was beneficial to the effective tillers of rice. Perrin et al.[32] used clinoptilolite as an adsorbent to load NH_4^+-N and applied it to a farmland system with sandy soil. They found that the nitrogen-loaded clinoptilolite not only reduced N leaching from sandy soil but also supported higher plant productivity. Liu et al.[10] used biochar to load urea and mix it with bentonite to make a carbon-based fertilizer composite material. They found that urea-loaded biochar effectively regulated nutrient release, and had broad potential uses in sustainable agriculture. The present study concluded that biochar reused N resources from simulated wastewater and increased rice tillers by 11.9%.

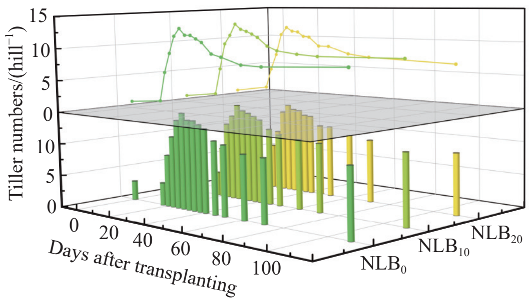
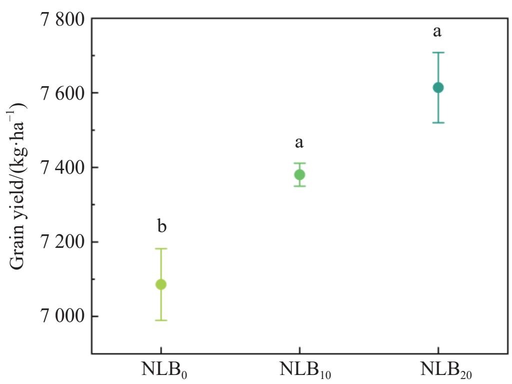


Figure 6 Rice tillering dynamics under different treatments

3.6 Grain yield

Figure7 shows the effect of adding NLB on rice yields. Compared with the treatment without NLB, either10 t/hm^2 or 20 t/hm^2 of NLB both facilitated a significant increase in rice grain yield, while there was no significant difference between NLB_{10} and NLB_{20}. According to the previous contents, NLB adsorbed N from low-concentration eutrophic wastewater and released it with N demand of rice plant, which were the main factors to promote rice grain yield. First, the NLB treatments brought the exogenous N in paddy soil to fully replenish the soil N pool. Second, NLB regulated the adsorption and release of soil N to match the N demand of rice plants, especially in the S2 and S4 stages. In addition, a higher rice tillers number in the NLB-added plots was also a indispensable reason for the increase in rice yield. As a result, NLB_{10} and NLB_{20} significantly increased rice grain yield by 4.2% and 7.5%, respectively. Clough et al.[33] reported that biochar effectively adsorbed NH_4^+-N and NO_3^--N and reduced N losses, which can be used as an agricultural fertilizer. Our experimental results demonstrated that NLB compensated soil N storage and thus increased rice yield. A vital study in this area showed that urea-loaded biochar was capable of controlled release of N into the soil for plants utilizing, and the N release rate reached 54.6%[10]. From the above results, NLB not only improved rice yield, but also provided powerful technique support to solve agricultural wastewater problems.



Note: Different letters above the point are significant at 0.05 probability level.

Figure 7 Grain yield under different treatments

4 Conclusions

In this study, an advanced approach to resource utilization of agricultural eutrophic wastewater based on biochar, an high-performance adsorbent material was proposed for the first time. Under weak acidic environment, the exchange of metal cations on the biochar structural surface with NH_4^+ and increase of specific surface area were the main reasons for biochar to load NH_4^+-N. NLB not only reduced N concentration in low concentration simulated wastewater by 30.8%, but also acted as a nutrient releaser for releasing N to promote plant growth during critical rice growth period. More importantly, the NLB in the rice field effectively regulated soil N to fit the plant's N demand. Eventually, compared to that without NLB treatment, the maximum number of rice tillers increased by 11.9%, and rice yield increased by 7.5%. Based on the advanced approach, the imbalanced N load phenomenon of "soil poor-water rich" in irrigation areas can be effectively solved.

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