Effect of a Urea and Urease/Nitrification Inhibitor Combination on Rice Straw Hydrolysis and Nutrient Turnover on Rice Growth

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Stabilized fertilizers that contain nitrification inhibitors and/or urease inhibitors are widely used in China. A pot experiment was conducted to analyze soil enzymatic characteristics related to carbon and nitrogen turnover and metabolism under the use of rice straw and stabilized fertilizer. Results showed that stabilized fertilizer exhibited the highest yield production, panicle numbers, and above-ground biomass. Compared with urea treatment with straw, adding inhibitors reduced soil organic carbon and the enzyme activity related to acquisition of carbon, but increased soil organic carbon accumulation, rice yield, and above-ground biomass. Stabilized fertilizer increased protease activity; however, it decreased N-acetyl-β-glucosaminide. Addition of straw significantly increased dissolved organic and microbial biomass carbon or nitrogen, as well as the enzyme activities of α-D-glucosidase, β-D-glucosidase, β-N-acetyl-glucosidase, and cellulase at the seedling and tillering stages. The principal components analysis showed that the synthesis of extracellular enzyme related to carbon and nitrogen acquiring act as a proxy for straw decomposing under nitrogen conditions. The combination delayed the release of ammonia, which affected the carbon and nitrogen coupling by microbial organisms. These results demonstrated a relationship between soil carbon and nitrogen dynamics and soil enzymes in different fertilization management.

Keywords: Enzyme mechanisms; C and N turnover; Stabilized fertilizer; Paddy soil; Rice straw

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INTRODUCTION

In China, agricultural residues are essentially a renewable biomass resource, and returning crop residues to field is a common agricultural management strategy (Lu *et al.* 2018). Straw biomass has been used as coating material for the controlled release of fertilizers into the soil, and possesses high density, good degradability, and superior controlled release properties (Lu *et al.* 2015; Chen *et al.* 2017). Research has shown that the addition of straw influences the nitrogen (N) uptake efficiency (Cucu *et al.* 2013). There is a great potential of straw to be combined with stabilized fertilizer; however there has been a need for research in this area. Stabilized fertilizers refers to fertilizers with urease inhibitors and/or nitrification inhibitors added during the production process (Wu *et al.* 2019).

Applying stabilized fertilizer has been demonstrated to be an effective agricultural management practice for N-fertilizer use, which reduces the loss of gas emission through prohibiting urea release and the nitrification process, increases the content of NH_4^+-N available for plant uptake, and then influences the nitrogen turnover of soil (Shi *et al.* 2015; Meng *et al.* 2020). In addition to the effect on N-transformation, research also shows that N-fertilization affects the rate of soil organic carbon (SOC) decomposition through regulating extracellular enzyme activities (Jian *et al.* 2016). Wang *et al.* (2019) considered that fertilization regimes had a significant influence on microbial communities and soil enzyme activities.

Soil enzymes play a key role in nutrient cycling, as well as the utilization of carbon (C) and nitrogen (N). They also contribute to the stock and export of soil dissolvable organic carbon (DOC) and dissolvable organic nitrogen (DON) pools (Zhang *et al.* 2015), and also correlate with soil microbial biomass (Wang *et al.* 2019). Cellulases are a group of hydrolytic enzymes that soil microbes produce to decompose polysaccharides. They include α-1,4-glucosidase (AG), β-D-glucosidase (BG), β-D-cellulobiosidase (CEL), and β-1,4-xylosidase (XYL). Glucosidase acts as a release agent for small molecular sugars, providing potential energy for microbes. The activity of α-glucosidase and β-glucosidase play an important role in the decomposition of cellulose of soil. Glucosidase is an important component of cellulolytic enzyme system (Fujita *et al.* 2018). The cellulase hydrolyzes cellulose into monosaccharides, which indicates the ability of soil to decompose plant residues. Kader *et al.* (2017) reported that glucosidase and cellulase are involved in the accumulation and conversion of organic C and that the hydrolysis of the terminal NH_2 group in amino acid is the limiting step of soil N-mineralization. In addition, the enzymes associated with microbial N-acquisition include β-1,4-N-acetyl-glucosaminidase (NA) and protease (PG) (Jian *et al.* 2016). Additionally, the N-acetyl-glucosidase degrades chitin into soluble subunits that can be absorbed and utilized by soil microorganisms (Yang *et al.* 2016).

Soil organic carbon (SOC) is the aggregate of humus, animal and plant residues, and microorganisms formed by microbial action. Soil dissolvable organic C (DOC) refers to the organic carbon that can be lost through a membrane with an aperture of 0.45 microns without evaporation during the analysis. Microbial biomass and soil enzymes work together as catalysts in the conversion of soil organic C to soluble organic C (Eagle *et al.* 2000; Bowles *et al.* 2013) and are also used as indicators of changes in soil properties induced by soil management (Kandeler *et al.* 1999), especially the C and N materials input. Straw added into paddy soil along with exogenous C mainly consists of protein and cellulose; and chitin is present in soils acting as fungal and macro faunal residue, thus increasing the reaction of soil substrate and the enzyme source. The enzyme correlating to chitin and cellulose is an important factor to characterize straw decomposition (Wang *et al.* 2016). As the available N source, especially the inorganic N source, and the living environment of soil microorganisms improves, it triggers increased soil enzyme activity (Geisseler and Scow 2014). The stabilized fertilizer coupled with straw N also affects the key extracellular enzyme activities and the turnover of C and N in paddy soil, and then affects the rice N adsorption and yield.

The enzyme activity helps to identify soil C and N conversions and kinetics after the addition of rice straw and the stabilized fertilizer in the northeast paddy soil. Therefore, it is essential to explore the relationship between the change of enzyme activity and C or N conversion. To date, most researchers have aimed at straw management and normal N fertilizer (Shi *et al.* 2015; Sihi *et al.* 2019), but few have focused on stabilized fertilizer with a new kind of inhibitors combination and rice straw. Hence, this study explored the impact of straw and stabilized fertilizer added on soil C and N turnover according to related enzymes and rice growth. Furthermore, this work considered the potential of rice straw and stabilized urea on straw hydrolysis and urea transformation. This provides theoretical data for straw and inhibitor combination to slow down nitrogen release and improve microbial activity, which accelerates the development of stabilized fertilizer of straw film coating and fertilization strategy in paddy fields.

EXPERIMENTAL

Experimental Site

An outdoor pot experiment was conducted at Shenyang National Agricultural Ecosystem Field Observation and Research Station (41°31 'N, 123°24' E) located in Shenyang, China. The tested paddy soil was taken from 0 to 20 cm soil layer of the experimental site (Shenyang Agricultural University Rice Research Institute). Soil was shielded at 5 mm to remove stones, crop stubble, and roots. The soil contained 20.43 g C per kg and 1.85 g N per kg soil, with a C/N of 11.04 and pH of 6.18. Rice straw comprised of 37.8 g C per kg and 5.96 g N per kg, with C/N ratio of 63.

Experimental Design

Four treatments were set in this experiment, *i.e.*, urea (N), urea plus straw (NS), stabilized urea (NI), and stabilized urea plus straw (NIS). Each treatment had three replicates; there were 36 pots in total and they were randomly arranged, and flood-cultivated for 120 days. Each pot was a plastic basin with a straight configuration. The diameter was 18 cm, and the height was 20 cm. Each pot was filled with 3 kg dry soil. Potassium chloride (KCl) and calcium superphosphate ($CaP_2H_4O_8$) were applied as basal fertilizer, with 100 mg P per kg soil and 150 mg K per kg soil. Stabilized urea was added in amounts of 60, 45, and 45 mg N per kg soil before culture, seedling stage, and tillering stage, respectively.

The new NI sample included urease plus inhibitor combination, which were 1% phenyl phosphorodiamidate (PPD) (Ourchem, Sinopharm Chemical Reagent Co. Ltd., Shanghai, China), 1% thiophosphoric triamide (NBPT) (Ourchem, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), and 2% 3,4-dimethylpyrazole phosphate (DMPP) (Maya Reagent Biotech, Jiaxing, China). The straw was added at 5 g/kg, which is equivalent to straw yield in a season. Meifeng 9 (*Oryza sativa* L.) was selected in this study, which is planted widely in northeast China. Crop management was performed consistently with local conventional farming practices.

The rice plant and soil were sampled after the seedling stage (June 4, 2018), tillering stage (June 25, 2018), and maturity stage (September 21, 2018). Seedling stage was about 7 days after rice plant transplanted and basal fertilizer application, which is the peak period that urea nitrogen hydrolysis. Tillering stage was the most vigorous in nitrogen metabolism in rice life and rice straw hydrolysis. It is important both in terms of nitrogen transformation and soil nutrient accumulation. To detect the effect of different nitrogen and straw on soil C and N accumulation, the soil and rice plant were evaluated after harvest.

Table 1. Experimental Design and the Basal Chemical Properties

Treatment	N Fertilizer (type)	Additive amount (mg kg^{-1} soil)	KCl (mg kg^{-1} soil)	$CaP_2H_4O_8$ (mg kg^{-1} soil)	Rice straw (g kg^{-1})	Soil C/N
N	Urea	150 (60,45,45)	105	150	0	13.95
NS	Urea	150 (60,45,45)	105	150	5	15.28
NI	Stabilized urea (urea+ PPD + NBPT + DMPP)	150 (60,45,45) +1%+1% +2%	105	150	0	14.23
NIS	Stabilized urea (urea+ PPD + NBPT + DMPP)	150 (60,45,45) +1%+1% +2%	105	150	5	14.38

Methods

Rice plants were harvested at three sampling times. In order to inhibit the respiration of plants, avoid the loss of biomass, and reduce the measurement error of biomass, the plants were green retarded at 85 °C for 2 h, then oven-dried until constant weight at 65 °C, and ground to powder to detect the total nitrogen content (Elementar, Thermo, Heraeus, Germany). The ammonia (NH_4^+-N) and nitrate (NO_3^--N) contents were analyzed using 2 mol/L KCl (100 mL), which was digested with 20 g fresh soil, and determined with a continuous flow analyzer at the wavelengths of 660 nm and 540 nm (AA3; Bran Luebbe, Heidelberg, Germany) (Yang *et al.* 2016). Microbial biomass C and N were determined by chloroform fumigation method (Joergensen 1996; Joergensen and Mueller 1996). The ratio of soil to extract liquid used was 1:4. Microbial biomass nitrogen (MBN) was measured using a Vario TOC Select Analyzer (Elementar, Heraeus, Germany). The fumigation coefficient N was 0.54, and C was 0.45. Determination of soil dissolved organic C and N: the extracted solution was filtered through 0.45-um filter membrane and determined by an elemental analyzer (TOC). Protease activity was determined by the Ladd and Butler (1972) method for control and substrate samples, the control was added after the soil sample incubation and prior to analysis. The extracellular enzyme activities of α-D-glycosidase enzymes (AG), β-D-glycosidase enzymes (BG), N-acetyl-β-glucosaminide enzymes (NA), β-D-xyloside (XYL), and β-D-cellobioside enzyme (CEL) were determined as described with colorimetric determination methods described by Marx *et al.* (2001). Briefly, 1.0 g fresh soil was homogenized with 100 mL 50 mM acetate buffer (pH = 5.0), 10 μM 4-methyl umbrella ketone as a reference and 200 μM substrates were dispensed into the black 96-well microplate, then covered and incubated at 2 °C for 4 h in the dark. The reaction was stopped by adding 50 μL 0.5M NaOH. The fluorescence was determined immediately in a fluorometer (BMG Labtech, Offenburg, Germany) at 360 nm excitation and 460 nm emission.

Statistical Analysis

All statistical analysis were conducted by Excel (Microsoft, Office 2010, Washington, USA) and SPSS 16.0 software (SPSS Inc., SPSS16.0, Chicago, USA), one-way analysis of variance (ANOVA) with Duncan's method was performed for nutrient contents and soil enzyme activities at different management practice ($p < 0.05$). Principal component analysis (PCA) analysis was conducted by Canoco 5 (Micro-computer Power, Ithaca, New York, USA) with data represented by mean value ($n = 3$). Origin 8.0 (OriginLab Inc, Origin 8.0, Guangzhou, China) was used for drawing.

RESULTS AND DISCUSSION

Nitrogen Uptake and Utilization by Rice Plants

According to the results of rice biomass and nitrogen adsorption, as shown in Table 2, no significant effect was observed at the seedling stage ($P > 0.05$), while N and NI samples indicated significantly higher biomass at the tillering stage ($P < 0.05$). However, at the maturation stage, NS harvest provided the worst yield, biomass, N adsorption, and the number of panicles. The rice biomass and yields of N, NI, and NIS samples increased 23.0%, 27.8%, 22.8%, and 16.4%, 31.2%, 25.2%, respectively, compared with NS. The worst effect of urea with organic material might be because of the amount of carbon source addition that made the competition of plant and microbial more intense (Inselsbacher *et al.* 2010). It also increased NH_3 volatilization and thus reduced the available N for rice growth (San *et al.* 2011). The NI and NIS had the highest yield, panicle numbers, and above-ground biomass. Silva *et al.* (2017) showed that NBPT increased crop yield 5.3%; the yield was significantly higher with the inhibitor combinations in this experiment ($P < 0.05$). In addition, the stabilized urea alleviated the yield reduction compared with urea plus straw. The NIS had the highest N adsorption content; the results were constant with Ma (2015) in the upland soil. When both the agronomic and economic effects were considered, the stabilized urea and coupled with rice straw were recommended.

Table 2. Yield, Panicle Number, Above-ground Biomass, and the Nitrogen Adsorption of Rice

	Seedling Biomass	Tillering Biomass	Maturation Biomass	Maturation Yield	Panicle Number	N Adsorption
Unit	g pot^{-1}	g pot^{-1}	g pot^{-1}	g pot^{-1}	Ears	g N kg^{-1}
N	0.70 ± 0.06a	6.16 ± 0.84a	67.24 ± 2.17a	20.54 ± 2ab	11 ± 0.43a	51.17 ± 5.38ab
NS	0.74 ± 0.03a	3.47 ± 0.18b	54.68 ± 9.92b	17.64 ± 1b	6 ± 0.99b	39.72 ± 5.05b
NI	0.73 ± 0.16a	6.45 ± 2.27a	69.90 ± 4.13a	23.15 ± 0.58a	12.33 ± 1.6a	47.46 ± 6.80ab
NIS	0.81 ± 0.06a	3.98 ± 0.20b	67.16 ± 3.24a	22.09 ± 0.58a	10.33 ± 1.71a	55.32 ± 13.51a

The values in the table represent the mean of three replicates. Small letters indicate the significant difference among treatments at the level of 0.05

Effects of Stabilized Fertilizer and Straw on Soil NH_4^+-N and NO_3^--N Contents

Soil inorganic nitrogen is the preferred form that the microorganisms and rice would be absorbing. According to Fig. 1, all treatments showed a negative trend of NH_4^+-N content at seedling stage. The application of straw immobilized more NH_4^+ ions and NI showed an inhibitory function of nitrification, which slowed down the process of urea hydrolysis and nitrification, reduced the release of NH_4^+ions. Although the trend was obvious in the tillering stage, NIS had the highest NH_4^+-N content. The combination of rice straw and stabilized fertilizer delayed the peak of NH_4^+ release (Fig. 1). Thereafter, for rice cultivation, the NH_4^+ release character of NIS meets the rice demand, which is lower in seedling but higher in tillering stage (Chauhan *et al.* 2017). The delayed release of NH_4^+-N and rice high uptake in tillering coincide. This combination reduces the loss caused by large release and little demand, which means that there is a timing effect that is benefit for fertilizer utility and rice growth. While for NO_3^--N content, the straw addition significantly reduced the NO_3^--N content in the tillering stage and increased it in the maturation stage ($P < 0.05$), indicating the persistent release of NO_3^--N with straw addition, which may increase the risk of gaseous release (N_2O and NO) through de-nitrification (Xia *et al.* 2016). According to Ye and Horwath (2016), who concerned that water saturated conditions and high soil organic C (SOC) may lead to higher risk of N_2O emission. Further, the lower NO_3^--N in tillering stage occurred because of the immobilization of NH_4^+ by microbial biomass (Fig. 2). However, there were no significant influences of NO_3^--N on stabilized fertilizer and normal urea.

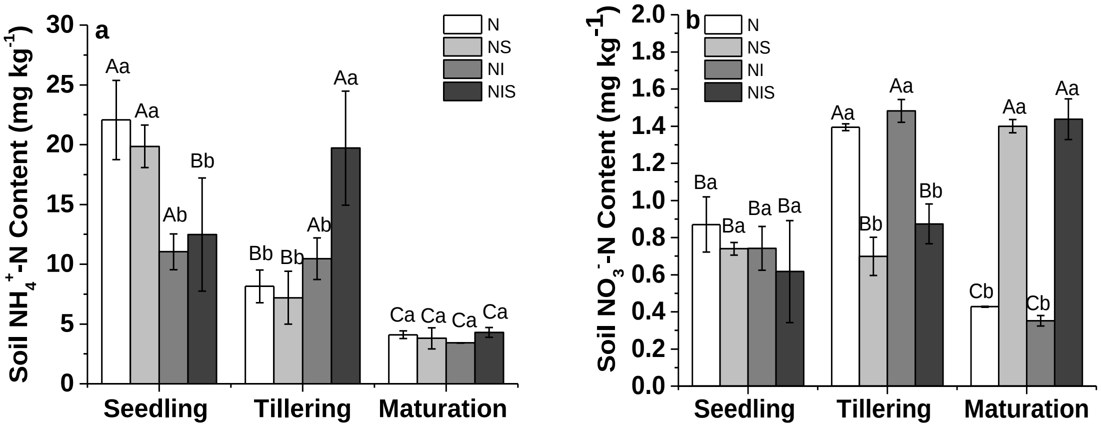

Fig. 1. Soil NH_4^+-N and NO_3^--N contents with straw and fertilizer applications. a: NH_4^+-N content; b: NO_3^--N content. Values are the means of three; lowercase letter represents the significant difference between treatments during the same sampling period (Duncan, $P < 0.05$).

Effects of Stabilized Fertilizer and Straw on Soil Microbial Biomass C and N, and Dissolved Organic C and N

Soil dissolvable organic carbon (DOC) and microbial biomass carbon (MBC) were both effective soil carbon pools, which are variable components in soil and can be easily used by microorganisms in the process of transformation and mineralization. The addition of straw was expected to promote the microbial assimilations of C and N (Fig. 2), the soil contents of DOC, dissolvable organic nitrogen (DON), MBC, and microbial biomass nitrogen (MBN), which significantly increased at seedling and tillering stages ($P < 0.05$). Research shows that flooding conditions had a tendency to reduce C mineralization with a lower net residue-C mineralization. The mineralized C mainly comes from the recalcitrant pools, such as cellulose and lignin (Olivier and Horwath 2000). However, a higher SOC content effectively improved the crop nutrient supply and soil properties, increased the biodiversity, and enhanced the microbial activity (Yang *et al.* 2015). Similarly, straw addition stimulated soil respiration rate and total phospholipid fatty acids (Pan *et al.* 2016; Zhou *et al.* 2020). It also led to microbial assimilation of soil organic nitrogen, such as amino acids, to meet their need for C, N, and energy (Geisseler and Horwath 2014), and reduced the loss of urea and straw nitrogen. Meanwhile, the dead metabolism of microorganisms can release nitrogen for absorption and the subsequent utilization by crops in the later growth stage (Geisseler *et al.* 2011).

The inhibitor played an important part in the rice seedling and tillering stages. No significant effect was found during N and NI treatments. Compared with NS, stabilized urea with straw (NIS) reduced soil MBN and increased the DON content (Fig. 2). That discrepancy may cause the mineralized N in a later growth stage to meet the demand of rice, which may explain the highest N adsorption of UIS as shown in Table 2. A study by Geisseler *et al.* (2017) showed that fertilizer application could increase soil MBC and SOC contents. Except for delaying the hydrolysis of urea, the addition of inhibitors also increased the content of organic carbon (Fig. 2c), which was constant as reported by Wang *et al.* (1991). The combination of rice straw and inhibitor significantly influenced soil DOC and MBC ($P < 0.05$). Geisseler (2010) reported that the SOC positively correlated with the amount of cellulose added, which also showed a better N supply pattern. The higher SOC and improved soil nutrient status mainly attributed higher rice yield (Bi *et al.* 2009). Hence, the combination enhanced the turnover capacity of straw and the fixation effect of microorganisms on fertilizer nitrogen. The C-N coupling effect was found for straw decomposition and N acquisition.

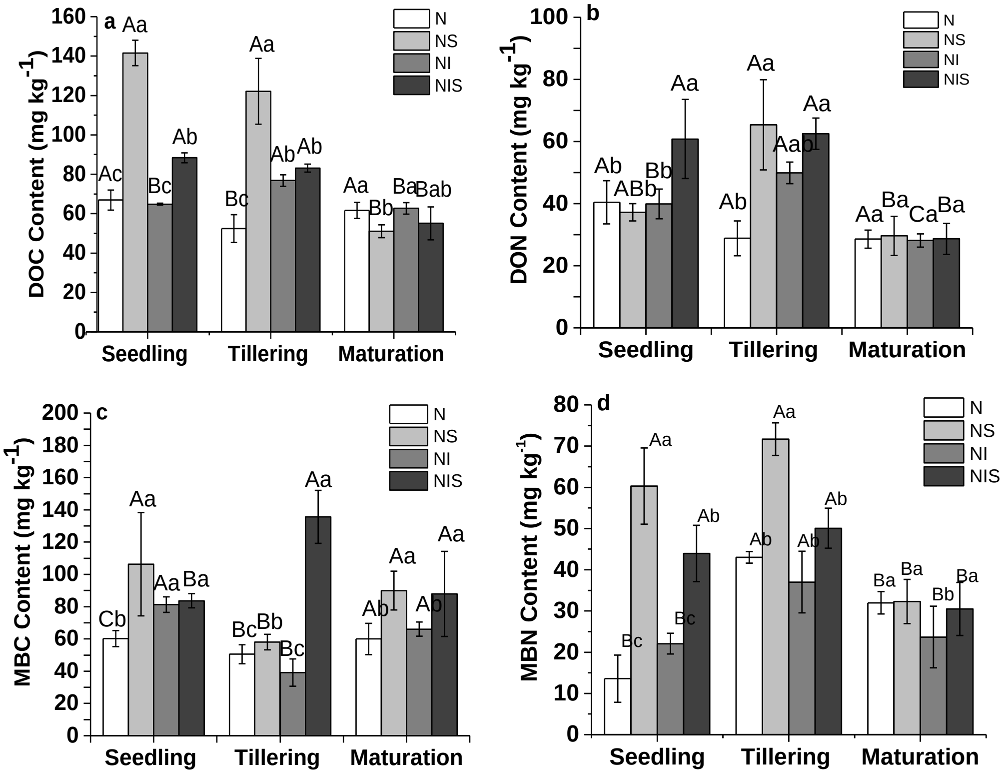

Fig. 2. The content of dissolvable organic C (DOC) and N (DON) and soil microbial biomass C (MBC) and N (MBN) contents; a: DOC; b: DON; c: MBC; d: MBN. Uppercase letters represent the significant difference of treatments at different times of the same treatment, and lowercase letters represent the significance of different treatments at the same time (Duncan, $p < 0.05$).

Response of Stabilized Fertilizer and Straw on Soil Enzyme Activity

Activity of enzymes related to carbon conversion

Soil enzyme activities varied significantly with different managements of straw and stabilized fertilizer ($P < 0.05$), which also changed in various rice growth stages (Figs. 2 and 3). Straw addition significantly improved the activity of α-glucosidase, β-glucosidase, β-xylosidase, and β-cellulose compared to single urea application ($P < 0.05$, Fig. 3). However, the stabilized fertilizer inhibited the activities of α-glucosidase, β-xylosidase, and β-cellulose activities. Compared with NI, the AG enzyme activity of NS and NIS increased 32.4% and 56.4% in the seedling stage, 44.5% and 6.1% in the tillering stage, and 155.4% and 69.7% in the maturation stage, respectively (Fig. 3A). The BG enzyme was involved in the accumulation and conversion of organic carbon and played an important role in the decomposition of straw until maturation stage. The BG was dominated in NS and NI treatments at the seedling stage, while NS and NIS treatments dominated at the tillering stage (Fig. 3B). The variation of β-xylosidase (XYL) activity was consistent with α-glucosidase activity (Fig. 3A, Fig. 3C).

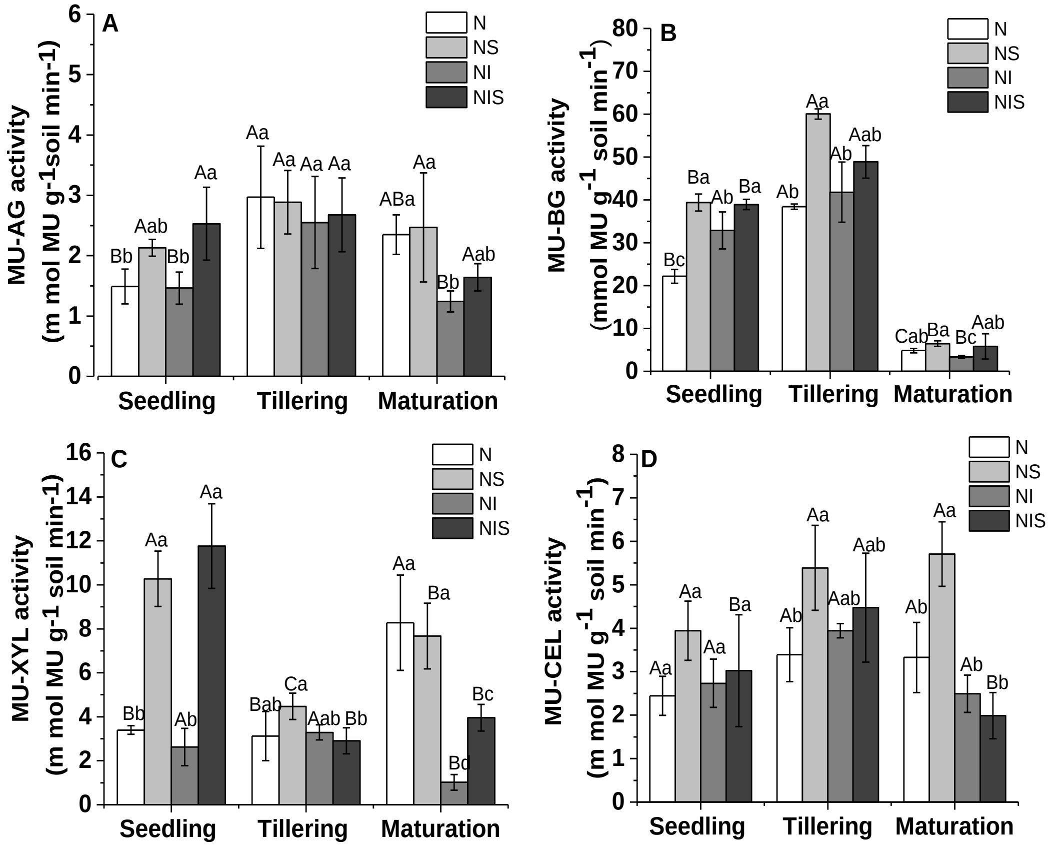

Fig. 3. Activity of α-D-glycosidase enzymes (MU-AG), β-D-glycosidase enzymes (MU-BG), β-D-xylosidase enzymes (MU-XYL), and β-D-cellulose (MU-CEL) enzymes related to the turnover of carbon in the soil. A: MU-AG; B: MU-BG; C: MU-XYL; D: MU-CEL

Compared with stabilized fertilizer, the NS and NIS treatments increased XYL activity by 291.6% and 348.5% in the seedling stage and 650.7% and 287.9% in the maturation stage, respectively, due to straw addition (Fig. 3C). According to Fig. 3D, NS had the highest β-cellulose (CEL) enzyme activity in the whole growth period; NS, NI, and NIS treatments increased the activity by 61.4%, 11.9%, and 23.9% at the seedling stage, and 58.8%, 16.3%, and 31.9% at the tillering stage, respectively, compared with urea alone. Qiu *et al.* (2011) reported that straw applied increased the organic matter, which is easily decomposed by small molecules, approximately 80% of cellulose of which is difficult to be decomposed. However, the products of cellulose degradation are glucose, cellobiose, and higher molecular weight oligosaccharides (Deng and Tabatabai 1994). Glucosidase and cellulase were the main participants in the hydrolysis and utilization of cellulose in the soil; the increase of the C-acquiring enzyme makes more C available to soil microorganisms (An *et al.* 2015). This explained why the decomposition of straw addition significantly improved the activity of hydrolysis enzymes compared to single urea application ($P < 0.05$). Moreover, with rice growth, the root system grows rapidly, the exudates and exfoliates of the root system increased, and the rich matrix promotes the increase of soil microorganisms and soil enzyme activity (Kader *et al.* 2017). However, there was no significant effect found in urea and stabilized fertilizer on soil carbon related hydrolytic enzymes under no straw addition.

Activity of enzymes related to nitrogen conversion

The index of soil N availability and the activity of N-acquiring enzyme such as N-acetyl-β-glucosaminidase (NA) and protease (PG) were measured, which was regulated by N availability. As shown in Fig. 4, the NA activity represented a trend of tillering stage > seedling stage > maturation stage. There was no significant effect on soil C-related hydrolytic enzymes of stabilized fertilizer ($P > 0.05$), but it showed an inhibitory effect on NA activity among the entire growing stage with or without straw. Meanwhile, PG showed adverse phenomena compared with NA enzyme.

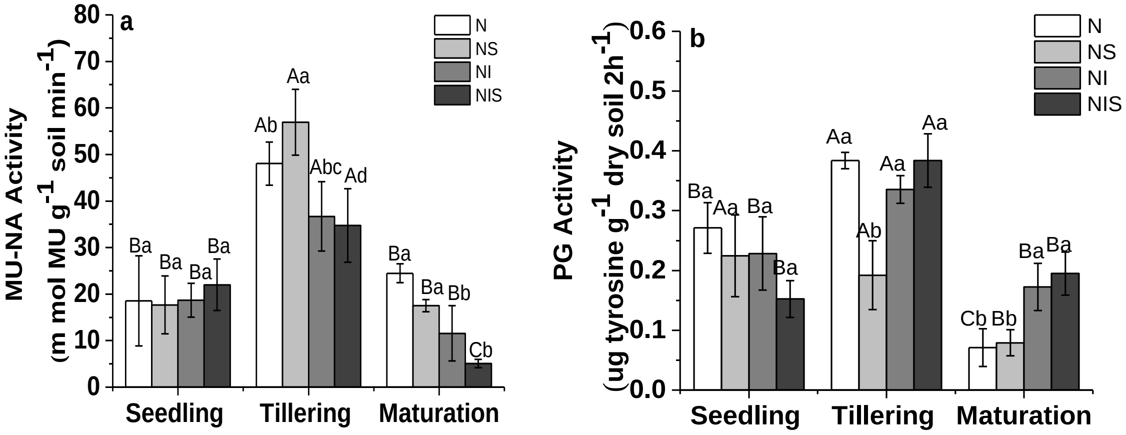

Fig. 4. Activity of N-acetyl-β-glucosaminidase (NA) and protease (PG) enzymes related to N turnover in soil. a: MU-NA; b: PG.

The activity of PG reflects soil N conditions, protease hydrolysis produced polypeptide, and then hydrolyzed to amino acid. The hydrolysis product is an important source of N for plants and microbes, after macromolecular aggregation under the catalysis of PG absorbed by microorganisms (Yang *et al.* 2017). Results showed that straw addition (NS, NIS) significantly increased soil protease activity at the tillering and maturation stages. Stabilized fertilizer showed a noticeably higher protease activity, which means that the inhibitor promoted the PG metabolisms and the microorganisms still had higher activity, and N turnover capacity, mainly due to the availability of nitrogen substrates by microorganism's absorption and utilization still be high compared with urea alone. Research showed that the nitrification inhibitor NBPT impeded the growth of ammonia-oxidizing archaea (AOA), and DMPP significantly reduced the ammonia oxidizing bacterial abundance, which significantly inhibited urea hydrolysis indirectly affecting nitrification (Dong *et al*. 2013; Meng *et al*. 2020). Geisseler *et al.* (2010) showed that the N turnover was determined by the amount and species of N source. This study showed that the domination of metabolism can be changed with added rice straw, which altered the C/N ratio of soil in the whole incubation period (Table 1). Yu *et al*. (2020) reported that straw and inhibitor influenced the fertilizer nitrogen transformation into soil organic pools. Rice straw significantly increased soil proteinase activity also ($P<0.05$, Fig 4). The present research found that stabilized fertilizer combined with straw may change the metabolic pathway of N, which increased protease activity but decreased NA activity, the specific bacteria needs further validation.

Principal Component Analysis Among Rice Yield, Soil Properties, and Soil Enzyme Activities

The principal component analysis (PCA) of enzyme activity with soil C and N is shown in Fig. 5.

The PCA produced two principal components. The first and the second principal components explained 76.60% of the total variability of the results. Results showed that the addition of straw significantly affected soil DOC, DON, MBC, and MBN ($P < 0.05$); the enzymes related to carbon conversion were closely related to the content of organic nitrogen in soil. Wang (2019) verified that the type of soil management employed affected microbial biomass and soil enzyme activities. Enzyme activities strongly depended on microbial activity and biomass, as microorganisms produce enzymes depending on resource supply and demand (Mooshammer *et al.* 2014). Soil DOC provides direct organic substrates for microbial growth and reproduction, and the increase of substrate carbon source improved microbial activity and caused the increase of MBN content (Qiu *et al.* 2011; Liang *et al.* 2019). Sampling times influenced the enzyme activities and soil carbon transformation, especially in the tillering stage, which had a strong effect on it. And there are similar separations among different treatments based on the enzyme activities in seedling stage and maturation stage (Fig.5). The results were also similar with Zhou *et al.* (2020).

To better understand the effect of straw and stabilized urea on soil properties and enzyme activities, Spearman’s correlation analysis was performed. According to Table 3, soil BG, NA, and PG enzymes and DOC, DON, NH_4^+-N, and NO_3^--N contents played an important role on rice yield. There was a negative correlation between the inhibitor and C-acquiring enzymes (AG, BG, XYL, and CEL), but the inhibitor could significantly increase the NA activity ($P < 0.05$, Table 3).

Geisseler (2014) reported that nitrogen reduced C-acquiring enzymes. The same effect was found of stabilized fertilizer in this experiment. Straw addition was positively related with CEL and XYL activities and soil DOC and MBN contents. Zhang (2014) also considered that carbon material addition was closely related to microbial decomposition characteristics under fertilization conditions. Especially under the condition of flooding and hypoxia, the activity of hydrolytic enzyme is inhibited, leading to a large amount of accumulation of organic matter (Freeman *et al.* 2001; Dunn and Freeman 2018). Moreover, stabilized fertilizer combined with straw may change the metabolic pathway of N. This may be explained by PG that had a significant positive correlation with NH_4^+-N, while NA was positively related to NH_4^+-N, NO_3^--N and negatively related to the inhibitor addition according to Table 3. Thus, there may be a difference between the metabolic processes used by the microorganisms.

Table 3. Spearman's Rank Correlations Among the Soil Chemical Properties and Soil Hydrolase Activities (n = 9)

	AG	BG	CEL	XYL	NA	PG	DOC	DON	MBC	MBN	NH_4^+-N	NO_3^--N	Yield	Inhibitor	Straw
AG	1														
BG	0.360*	1													
CEL	0.573**	0.453**	1												
XYL	0.325*	-0.164	0.204	1											
NA	0.543**	0.679**	0.545**	0.074	1										
PG	0.285*	0.634**	0.075	-0.369*	0.497**	1									
DOC	0.132	0.425**	0.272	0.402**	0.180	0.007	1								
DON	0.372*	0.534**	0.317*	0.151	0.602**	0.319*	0.544**	1							
MBC	0.094	-0.101	0.024	0.312*	-0.112	-0.174	-0.125	-0.182	1						
MBN	0.500**	0.506**	0.479**	0.397**	0.471**	0.125	0.562**	0.326	0.239	1					
NH_4^+-N	-0.025	0.360*	-0.008	0.102	0.197	0.477**	0.420**	0.402**	0.018	-0.015	1				
NO_3^--N	0.516**	0.779**	0.325*	-0.276	0.731**	0.792**	0.147	0.528**	-0.178	0.364*	0.357*	1			
Yield	-0.265	-0.689**	-0.108	-0.065	-0.525**	-0.616**	-0.404**	-0.581**	0.243	-0.200	-0.700**	-0.664**	1		
Inhibitor	-0.253	-0.09	-0.270	-0.283*	-0.310*	0.155	-0.094	0.210	-0.168	-0.318*	-0.043	-0.052	0.045	1	
Straw	0.263	0.134	0.398**	0.471**	0.064	-0.158	0.501**	0.360*	0.104	0.471**	0.112	-0.038	-0.057	0.000	1

Note: Duncan method was used for correlation analysis, with * P < 0.05 and ** P < 0.01

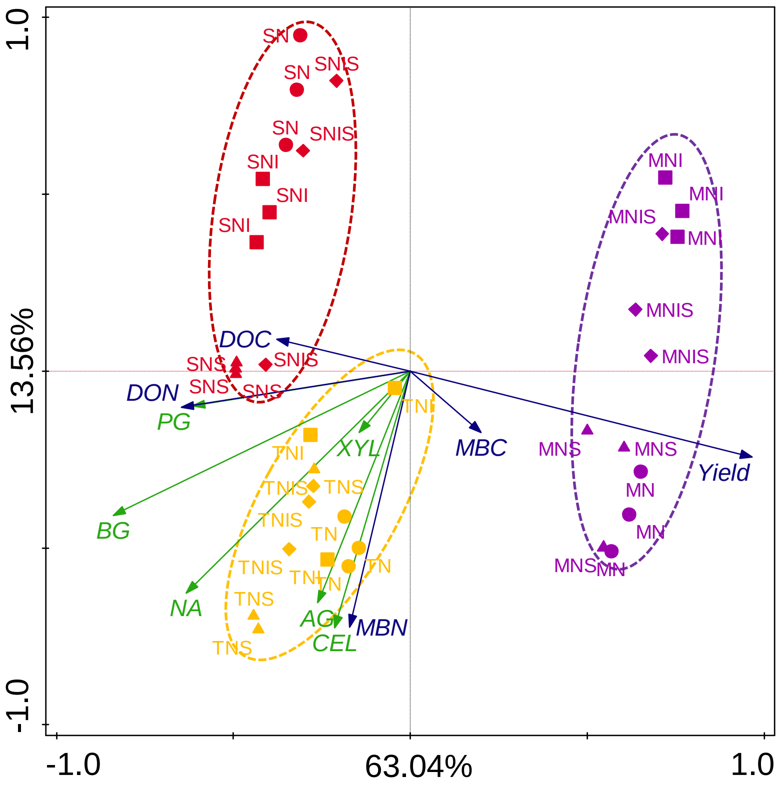

Fig. 5. PCA analysis on enzyme activity (α-D-glycosidase enzymes, AG; β-D-glycosidase enzymes, MU-BG); N-acetyl-β-glucosaminide enzymes, MU-NA; β-D-xyloside, MU-XYL; β-D-cellobioside enzyme, MU-CEL; Protease enzyme, PG) related to soil carbon nitrogen (DOC, DON, MBC, and MBN) in paddy soil (n = 9). The letter before the treatment abbreviations: S represents seedling stage, T represents tillering stage, and M represents maturation stage.

CONCLUSIONS

1. Rice straw significantly increased the dissolved carbon/nitrogen and microbial biomass carbon/nitrogen at the seedling and tillering stages, and it increased enzyme activity related to carbon turnover.
2. Stabilized fertilizer made of urea with urease inhibitor 1%PPD + 1%NBPT and nitrification inhibitor 2% DMPP enhanced N use efficiency, changed the metabolic pathway of N, mainly due to PG activity closely associated with NH_4^+-N compared with NA enzyme, and increased rice productivity.
3. There was a win-win effect of straw and stabilized fertilizer on soil organic carbon accumulation and crop productivity through increasing N efficiency by enhancing microbial activity. Rice straw coupled with the new type of stabilized fertilizer is a promising agricultural management strategy among paddy soils in north China.

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