Urease activity and urea hydrolysis rate under coupling effects of moisture content, temperature, and nitrogen application rate

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Abstract: The traditional qualitative analysis of the individual factors on the kinetic parameters cannot sufficiently reveal the mechanism underlying urea hydrolysis in soil.   This study aimed at revealing the coupling effects of the three factors on urease activity (V_0), hydrolysis rate constant (K_u), and activation energy (E_a) and establishing the quantitative model for K_u under the coupling condition.   Laboratory culture experiments were conducted under different temperatures (T) (15°C, 20°C, 25°C, and 35°C), moisture contents (θ) (60%, 80%, and 100% of field capacities), and nitrogen application rates (F) (247 mg/kg, 309 mg/kg, 371 mg/kg, and 433 mg/kg).   The urea content was measured daily.   Results showed that the effects of moisture content, temperature, nitrogen application rate, and their interaction on V_0 and K_u were in the descending order: T, F, $T*F$, θ, $T*\theta$, $F*\theta$, $T*\theta*F$.   The effect of single factor and two-factor coupling on V_0 was extremely significant ($p<0.01$), whereas the effect of the three-factor coupling on V_0 was negligible.   The effects of three factors and their interaction on K_u were extremely significant ($p<0.01$).   The effects of moisture content, nitrogen application rate, and their interaction on E_a were in the descending order: F, θ, $F*\theta$.   The effects of two factors and their interaction on E_a were not significant.   The mean absolute percentage error (MAPE) values of the established K_{u-1} (θ, T, F) and $K_{u-2}(\theta, T, F)$ models were 3.14% and 4.60%, respectively.   The MAPE of the traditional Arrhenius model $K_{u-3}(T)$ was 6.75%.   The accuracy of the proposed three-factor interaction model was superior to that of the traditional single factor model.   The results supplemented the mechanism of urea hydrolysis and improved the prediction accuracy of K_u.

Keywords: urease activity, hydrolysis rate constant, Arrhenius model, activation energy, coupling effect

DOI: 10.25165/j.ijabe.20181102.3784

1   Introduction

Food security is a major agricultural topic and has been closely monitored by the Chinese government.   In recent years, the food supply maintains a growing trend but still takes the old way of "high amount of fertilizer but low yield".   Excessive use of chemical fertilizers exacerbates the environmental pollution[1-3].   This phenomenon has aroused great concern from the Chinese government, and the Ministry of Agriculture issued an official document for pollution control in 2015[4].   The document indicates that reducing the application rate of urea and improving the usage efficiency are effective to solve environmental pollution and promote grain production[4].   In China, urea is most widely used in agriculture, accounting for more than 75% of the total nitrogen use.   However, the use efficiency of urea is only approximately 32%[5].   Most nitrogen amounts in fertilizers in soil are removed via

ammonia volatilization.   Therefore, urea hydrolysis mechanism should be investigated to improve urea utilization efficiency, reduce gas emissions, and promote grain production.

Urease is a key catalytic enzyme involved in urea hydrolysis.   Urease activity is closely related to temperature, water content, and nitrogen application rate[6-8].   The effect of individual environmental factors on urease activity has been revealed, but the interaction effect of the three mentioned factors is still unclear.   Urea hydrolysis process is often described as a first-order kinetic model (Equation (1))[9,10].   The hydrolysis rate constant (K_u) is an important parameter in chemical reaction kinetics.   In agriculture, the different soil temperatures, moisture contents, and nitrogen application rates can exert different impact degrees on urea hydrolysis[11-13].   Temporal and spatial variabilities of environmental conditions are found in soils.   However, the value of K_u at a fixed moisture content, temperature, and nitrogen concentration is often used in the simulation studies on nitrogen transformation and transportation[14-16].   This method reduces the accuracy of numerical simulations.   The interaction effect of the three above-mentioned factors on K_u should be further studied.   Revealing the relationship between K_u and environmental factors and establishing the modified model of K_u can supplement the control mechanism of urea hydrolysis and improve the prediction accuracy of K_u.

$$\frac{\partial U}{\partial t} = -K_u * U \tag{1}$$

where, U is the urea content (mg/kg) at time t (d); K_u is the

hydrolysis rate constant, d^{-1}.

The speed of urea hydrolysis rate is closely related to temperature[12]. The relationship between K_u and temperature can be described by the Arrhenius equation[7]. Arrhenius argued that the logarithmic value ln (K_u) of the hydrolysis rate constant (K_u) is linearly related to the reciprocal (1/T) of the thermodynamic temperature (T) (Equation (2))[7]. Equation (2) can also be simplified to another simple linear form (Equation (3))[17]. The value of ln (K_u) and 1/T do not show a good linear relationship[18-20]. The reason is attributable to interference from other factors in the soil. Therefore, the Arrhenius model needs to be improved reasonably to contain additional critical environmental factors. Accordingly, the quantitative relationship between the factors and the rate constant can be expressed accurately.

$$\ln(K_u) = \ln(A) - \frac{Ea}{R * T_r} \qquad (2)$$

$$\ln(K_u) = a + \frac{b}{T_r} \qquad (3)$$

where, A is the pre-exponential factor; E_a is the activation energy, kJ/mol; R is the gas constant (8.314 J/(mol·K)); a and b are constants; T_r is thermodynamic temperature, K.

The effects of moisture content and nitrogen application rate on urea hydrolysis rate cannot be ignored[11-21]. The rate of urea hydrolysis in soil can decrease with the increase in water content, that is, a significant negative correlation exists between the two factors[11]. A significant negative correlation also exists between the nitrogen application and hydrolysis rate[22]. This relationship is due to the non-competitive substrate inhibition of urease and the inactivation of urease[13,23]. However, traditional Arrhenius model and first-order kinetic model did not consider the effects of moisture content and nitrogen application rate on K_u. In conclusion, temperature, moisture content, and nitrogen application rate are the key influential factors of urea hydrolysis and should therefore be considered in establishing the hydrolysis parameter model.

Using the sample data of the laboratory culture experiments, this study aimed at revealing the coupling effect of temperature, moisture content, and nitrogen application rate on urease activity and hydrolysis parameters and establish a high-precision parametric model of K_u for the simulation studies of nitrogen transformation and transportation.

2 Materials and methods

2.1 Materials

Soil was sampled from the Pomology Institute of the Shanxi Academy of Agricultural Sciences, and the samples were air dried and crushed to pass a 10-mesh sieve. Soil type was sandy loam, and pH was 8.84. The moisture content and field capacity were 21.63 g/kg and 217.79 g/kg, respectively. The NO_3^--N and NH_4^+-N contents were 30.2 mg/kg and 25.7 mg/kg, respectively. All chemicals used in the experiments were of analytical reagent grade and obtained from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China).

2.2 Treatment

This study was mainly performed at the soil laboratory of Taiyuan University of Technology. The laboratory culture method was used to study the soil urea hydrolysis under different water contents, nitrogen application rates, and temperatures. Moisture content included three levels of θ_{60}, θ_{80}, and θ_{100}, which corresponded to 60%, 80%, and 100% of field capacities,

respectively. Nitrogen application rate was divided into four levels of F_{247}, F_{309}, F_{371}, and F_{433}, which corresponded to 247 mg/kg, 309 mg/kg, 371 mg/kg, and 433 mg/kg, respectively. Temperature was divided into four levels of T_{15}, T_{20}, T_{25}, and T_{35}, which referred to 15°C, 20°C, 25°C, and 35°C, respectively. This experiment included 48 treatments. All treatments were repeated three times. A total of 150 g (oven-dry basis) of soil was added to a series of 500 mL glass beakers. The beakers were treated with urea solution and deionized water to adjust the design level. The beakers were covered with a polyethylene film with several tiny holes. The beakers were incubated at different temperatures (15°C, 20°C, 25°C, and 35°C) for 9 days. The loss of soil water through evaporation was replenished daily with deionized water. The urea contents under different treatments were measured daily.

Soil urea hydrolysis rate is closely related to urease activity. Thus, a urease activity experiment should be conducted to reveal the mechanism of urea hydrolysis. In the urease activity experiment, the setting of various factors and levels was the same as those in the urea hydrolysis experiment. To prevent microbial proliferation, 30 mL of toluene was added to 150 g of soil sample and allowed to stand for 15 min. Then, urea solution and the citrate buffer solution with pH 6.7 were added to the soil to reach the design level of the soil urea concentration and water content. The beakers were incubated at different temperatures (15°C, 20°C, 25°C, and 35°C) for 24 h. In addition, a non-urea group was set as a blank control. Urease activity was expressed as milligrams of ammonium nitrogen released per kilogram of soil in 24 h.

2.3 Determination method

Determination of urea content: 5 g (oven-dry basis) of soil and 50 mL of ultrapure water were added into the flasks, placed inside a mechanical shaker for 0.5 h, and oscillated at a frequency of 50 Hz. The soils and solution were separated by filtration with a filter membrane (0.45 μm), and the urea content in the pellucid filtrate was determined through high-performance liquid chromatography (Thermo Fisher Scientific Corporation, America). The specific parameter setting was as follows: sample size of 30 μL, flow rate of 1 mL/min, mobile phase (95% ultrapure + 5% acetonitrile), and chromatographic column (C18, 4.6 mm×150 mm, 3 μm) with a chromatographic column temperature of 30°C and wavelength of 190 nm.

Determination of NH_4^+-N contents: 5 grams (oven-dry basis) of soil and 100 mL of KCl solution (1 mol/L) were added into flasks and then placed inside a mechanical shaker and oscillated at a frequency of 50 Hz for 1 h. The soils and solution were separated by filtration with a filter membrane (0.22 μm), and the NH_4^+-N contents in the pellucid filtrate were determined with a continuous flow analyzer (AA3, Bran Luebbe, Germany).

2.4 Data processing

A first-order model (Equation (1)) based on kinetic theory was used to describe the dynamic process of urea hydrolysis. E_a can be calculated by Equation (2). A regression analysis was conducted using the 1stopt 7.0 software to calculate the parameters of the models from the experimental data. The three-way ANOVA was performed with IBM SPSS Statistics 19 software, and the figures were plotted with Origin 9.1 software. The simulation effects of the models were evaluated using the mean absolute percentage error (MAPE) and the determination coefficient (R^2), which were calculated as follows:

$$MAPE = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{C_i - C_i'}{C_i}\right| \times 100\% \qquad (4)$$

$$R^2 = \sum_{i=1}^{n}(C_i' - \overline{C})^2 / \sum_{i=1}^{n}(C_i - \overline{C})^2 \qquad (5)$$

where, C_i, C_i', and $\overline{C}$ represent the measured, calculated, and average value of K_u (d^{-1}), respectively; n is the number of evaluated data points.

3   Results

3.1   Urease activity and hydrolysis rate constant under different treatments

Urease is a catalyst for urea hydrolysis, and its activity is indicative of the urea hydrolysis rate. Figure 1 shows the urease activity (V_0) under the coupling condition of moisture content, nitrogen application rate, and temperature. When temperature increased from T_{15} to T_{35}, and nitrogen application rate increased from F_{247} to F_{433}, the soil urease activity showed an increasing trend on power function. The soil urease activity increased by 0.15-0.62 times and 0.19-0.40 times in this process, indicating that the increased temperature and nitrogen application rate promoted urease activity. The urease activity treated with θ_{100} was 0.77-0.98 times of that of θ_{60}, which indicated that soil urease activity was inhibited when the soil water content increased. The three-way ANOVA of V_0 was performed to reveal the coupling effect of the three factors, and the results are shown in Table 1. The table shows that the effect of single factor and two-factor coupling on urease activity was extremely significant ($p<0.01$), whereas the effect of the three-factor coupling on urease activity was trivial. The effects of three factors and their interactions on urease activity were in the descending order: T, F, θ, $T*F$, $T*\theta$, $F*\theta$, $T*\theta*F$.
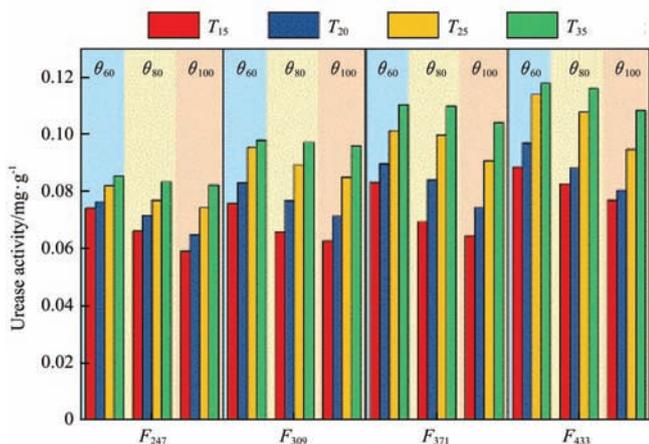
Figure 1   Urease activity under the coupled effect of water-nitrogen-temperature

Table 1   Three-way ANOVA of urease activity and urea hydrolysis rate constant under the coupled effect of water-temperature-nitrogen

Source	Urease activity (V_0)			Hydrolysis rate constant (K_u)		
	Sum of squares	F-value	P-Value	Sum of squares	F-value	P-Value
T	0.017	378.082	<0.001	74769.514	6697.022	<0.001
F	0.010	222.873	<0.001	30354.741	1284.150	<0.001
θ	0.003	85.860	<0.001	7394.418	112.203	<0.001
$T*F$	1.313E-3	9.483	<0.001	1480.021	30.944	<0.001
$T*\theta$	6.547E-4	7.095	<0.001	255.124	8.001	<0.001
$F*\theta$	6.487E-4	7.112	<0.001	112.198	4.692	<0.001
$T*F*\theta$	4.075E-5	1.472	0.117	101.005	4.547	<0.001

Figure 2 shows the hydrolysis rate constant (K_u) under the coupling effect of moisture content, nitrogen application rate, and temperature. When the soil temperature increased from T_{15} to T_{35}, the value of K_u increased by 1.96-2.66 times, indicating that temperature had a significant effect on K_u. At the same level of nitrogen application rate and moisture content, the increase in temperature increased the urease activity (Figure 1), which in turn promoted the urea hydrolysis rate ($\partial U/\partial t$) and decreased the residual substrate concentration (U). K_u must increase with the increase in temperature according to Equation (1).
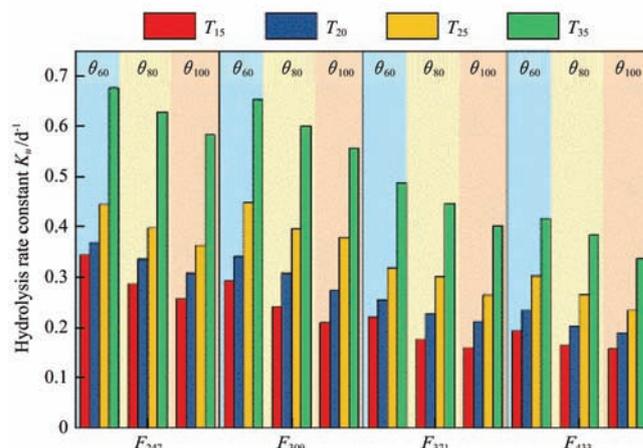
Figure 2   Urea hydrolysis rate constant (K_u) under the coupled effect of water-nitrogen-temperature

When soil moisture content increased from θ_{60} to θ_{100}, K_u decreased by 13.9%-28.7%. At the same level of nitrogen application rate and temperature, the increase in moisture content inhibited the urease activity (Figure 1), which in turn decreased the urea hydrolysis rate ($\partial U/\partial t$) and increased the residual substrate concentration (U). K_u must decrease with the increase in moisture content according to Equation (1).

In analyzing the response of nitrogen application rate to K_u, the increase in nitrogen application rate and V_0 could not account for the relative size of the residual substrate concentration because of the different initial nitrogen application rates. At this point, the dynamic process of the substrate concentration should be analyzed. During the entire hydrolysis process, when the nitrogen application rate increased from F_{247} to F_{433}, the residual substrate concentration (U) increased by 1.75-16.83 times (Figure 1) whereas K_u decreased by 0.56-0.68 times (Figure 2); the product of the two must be greater than 1. Therefore, high nitrogen application rate means high urease activity (V_0), high concentration (U), and low constant (K_u). The three-way ANOVA of K_u was performed to reveal the coupling effect of the three factors, and the results are shown in Table 1. Table 1 shows that the effects of three factors and their interaction on K_u were extremely significant ($p<0.01$). The effects of three factors and their interaction on K_u were in the descending order: T, F, θ, $T*F$, $T*\theta$, $F*\theta$, $T*\theta*F$. Therefore, the effects of individual factors and multi-factor interaction on K_u should be considered in establishing a quantitative model.

3.2   Activation energy (E_a) of urea hydrolysis under different treatments

E_a is an important thermodynamic indicator that characterizes the difficulty in chemical reactions. This indicator reflects the energy barriers that need to be overcome when urea hydrolysis occurs. Figure 3 shows E_a calculated by the Arrhenius equation under different treatments. E_a increased exponentially with the

increase in nitrogen application rate and linearly with moisture content. E_a had a stronger response to nitrogen application rate than to moisture content. When the moisture content and nitrogen application rate increased, the energy barrier (E_a) that must be overcame also increased. Consequently, K_u decreased. The effects of moisture content, nitrogen application rate, and their interaction on E_a were in the descending order: F, θ, $F*\theta$. The effects of two factors and their interaction on E_a were not significant.
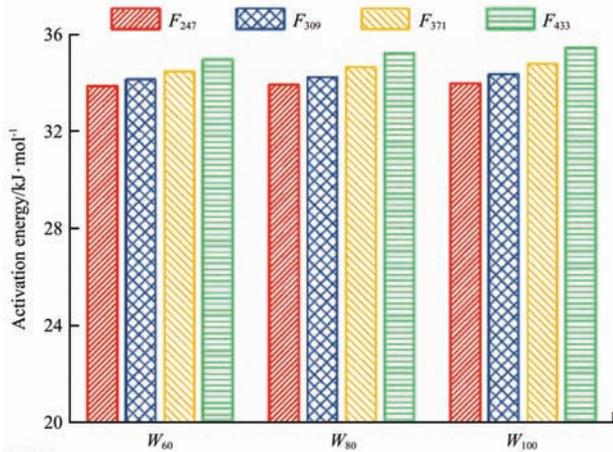
Figure 3   Activation energy (E_a) under the coupled effect of water-nitrogen

3.3   Establishment of the coupling model of K_u

From the experiment results, the relationship between K_u and moisture content, nitrogen application rate, and temperature can be described by Equations (6)-(8), respectively. The average values of determination coefficient of the three models were 0.977, 0.963, and 0.994, respectively; this finding showed a satisfactory fitting accuracy. Considering the existence of the interaction between the three factors, Equations (6)-(8) were multiplied to obtain a quantitative model $K_{u-1}(\theta, T, F)$ of K_u under the coupling condition of temperature, moisture content, and nitrogen application rate. The $K_{u-1}(\theta, T, F)$ model was shown as Equation (9).

$$K_u(\theta) = K_{u\theta} * \exp(a * |b - \theta|) \tag{6}$$

$$K_u(T) = K_{uT} * \exp(a * |b - T|) \tag{7}$$

$$K_u(F) = K_{uF} * \exp(a * |b - F|) \tag{8}$$

$$K_{u-1}(\theta, F, T) = K_{u\theta FT} * \exp(a * |b - \theta| + c * |d - F| + e * |f - T|) \tag{9}$$

where, $K_{u\theta}$, K_{uT}, and K_{uF} are the values of K_u under standard moisture content (θ_{100}), standard temperature (T_{15}), and standard nitrogen application rate (F_{433}), respectively; θ, T, and F are the moisture content (g/kg), temperature (°C), and nitrogen application rate (mg/kg), respectively; $K_{u\theta TF}$ is the value of K_u under the standard coupling condition of the three factors (θ_{100}, T_{15}, F_{433}); a, b, c, d, e, and f are constants.

Another three-factor coupling model $K_{u-2}(\theta, T, F)$ of K_u was established in this study. According to Arrhenius' theory, the relationship between ln (K_u) and $1/T$ can be described by Equation (3)[17]. The determination coefficient (R^2) of the linear model between ln (K_u) and $1/T$ was 0.93-0.98. Therefore, the Arrhenius equation $K_{u-3}(T)$ can be applied to soil urea hydrolysis. After a reasonable improvement, the accuracy of $K_{u-3}(T)$ model may be further improved. The classical Arrhenius model $K_{u-3}(T)$ only represents the relationship between temperature and K_u. However, the effects of moisture content and nitrogen application rate on K_u

were very significant ($p<0.01$). Therefore, the influence of moisture content and nitrogen application rate should be considered in improving the $K_{u-3}(T)$ model. Given the significant interaction between the three factors, Equations (3), (6) and (8) were multiplied to obtain another coupling model $K_{u-2}(\theta, T, F)$ of K_u based on Arrhenius' model. The $K_{u-2}(\theta, T, F)$ model was shown as Equation (10).

$$K_{u-2}(\theta, T, F) = a * \exp(b * |c - \theta| + d * |e - F| + f / T) \tag{10}$$

3.4   Calibration and verification of the coupling model of K_u

The parameters of $K_{u-1}(\theta, T, F)$ and $K_{u-2}(\theta, T, F)$ were determined by 80% of the total samples. Table 2 shows the specific parameter, and Figure 4 shows the linear consistency and relative error between the observed and calculated values of the calibration samples. The linear consistency of $K_{u-1}(\theta, T, F)$ model was the best, that of the $K_{u-2}(\theta, T, F)$ model followed, and that of the $K_{u-3}(T)$ model was the worst (Figure 4a). The MAPE values of the $K_{u-1}(\theta, T, F)$ model, $K_{u-2}(\theta, T, F)$, and $K_{u-3}(T)$ were 2.89%, 3.67%, and 5.36%, respectively. In conclusion, the calibration effects of the three models were in the descending order: $K_{u-1}(\theta, T, F)$, $K_{u-2}(\theta, T, F)$, $K_{u-3}(T)$. K_u was predicted using the three models which had been calibrated and the verification samples (20% of the total samples). The prediction results in Figure 5 show that the consistency between the observed and predicted values calculated by the $K_{u-1}(\theta, T, F)$ model was better than that of the two other models. For the verification samples, the MAPE values of $K_{u-1}(\theta, T, F)$, $K_{u-2}(\theta, T, F)$, and $K_{u-3}(T)$ models were 3.14%, 4.60%, and 6.75%, respectively. The $K_{u-1}(\theta, T, F)$ model described the relationship between K_u and the three factors better than the two other models did. Thus, the $K_{u-1}(\theta, T, F)$ model was recommended for the quantitative studies.

Table 2   Fitting parameters of $K_{u-1}(\theta, T, F)$ model and $K_{u-2}(\theta, F, T)$ model

Model	$K_{u\theta TF}$	a	b	c	d	e	f
$K_{u-1}(\theta, T, F)$	0.157	2.251E-3	834.033	2.885E-3	422.949	−0.041	51.016
$K_{u-2}(\theta, F, T)$		0.659	2.348E-3	192.898	2.864E-3	423.073	−24.507
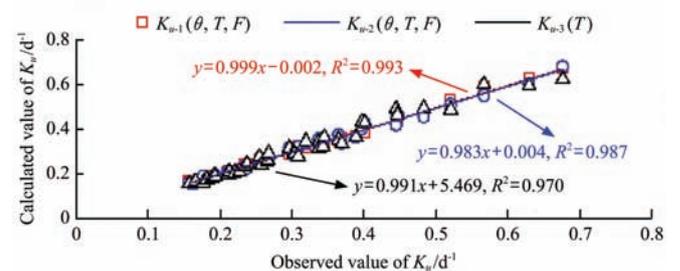
a. Observed and calculated values of K_u
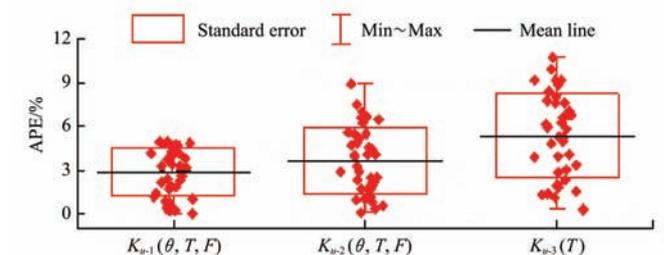
b. APE

Figure 4   Linear relationship and absolute percentage error (APE) between observed and calculated value obtained from the three models
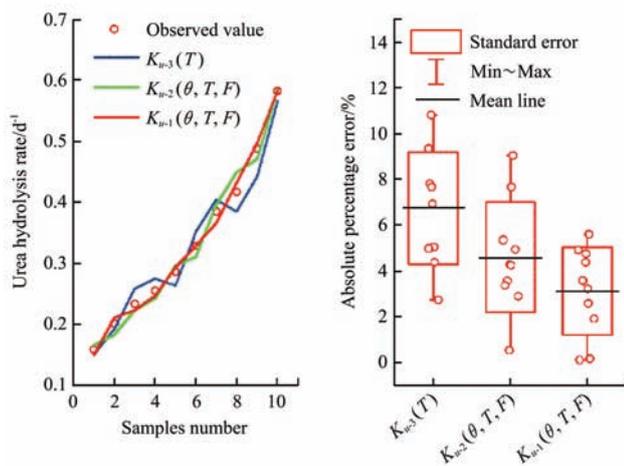
Figure 5   Comparison between observed and calculated value
obtained from the three models

4   Discussion

4.1   Determination method of urea content

In many studies on urea hydrolysis characteristics, the urea content is often determined by the diacetyl monooxime method[24-26]. This method is complicated and time consuming. The soil extraction and the various chemical reagents need to be mixed, and the reaction must be completed first prior to the determination. The sensitivity of the method is 0.04 mg/L[26]. The method of high performance liquid chromatography, which belongs to the physical separation method, was used in this study. This method can directly determine the urea content of soil extraction and avoid the systematic error caused by the chemical treatment process unlike previous reports. The sensitivity of this method is 0.006 mg/L. Compared with those of traditional methods, the results of this study would be more accurate and reliable.

4.2   Urease activity and hydrolysis rate constant under different treatments

When moisture content increased from 50% to 100% of field capacities, the urease activity increased from 0.22 mg/g to 0.28 mg/g and the percentage of increase was 26.2%[6]. This previous finding is contrary to the results of this study. When moisture content increased from 60% to 100% of field capacities, the urease activity decreased by 2.1% to 22.5%. This difference might be due to different soil particle gradations. The previous proportions of soil sand, granule, and clay are 66.5%, 17.5%, and 16.0%. By contrast, the present results were 34.6%, 51.5% and 13.9%. Thus, the texture of the two soil samples differed. Given the strong viscosity of soil, the effects of increased moisture content on soil air permeability was larger than that in the previous report. In the current study, the increase in moisture content indicated worse air permeability, low urease activity, and slow hydrolysis rate.

This study showed a positive correlation between temperature and urease activity, which is similar to that of MOYO et al.[7] However, the response of temperature to urease activity in the present study differed from that in the previous study. MOYO[7] showed that the urease activity of Kahola and Smoltm soils increases two and three times, respectively, when temperature increases from 15°C to 35°C. However, the current results were between 0.15 and 0.62 times. The difference of the results was due to the different nitrogen application rates in the two studies.

In this study, the nitrogen application rate was from 247 mg/kg to 433 mg/kg, and the urease activity did not reach saturation. On the contrary, in MOYO's study, the nitrogen application rate is 1000 mg/kg, and the urease activity is saturated[7]. The response intensity of the urease activity to temperature might differ under different nitrogen fertilizer concentrations. Urease activity depends mainly on the soil temperature and moisture content but not on the soil nitrogen concentration[8]. In this study, not only the temperature and moisture content had a significant effect on urease activity but also the nitrogen application rate. This finding slightly differed from those of previous research. Sardans's research was carried out in the field, and the results may be disturbed by other factors[8]. Meanwhile, this study was carried out under a factor-controllable laboratory experiment, and the results could be reliable. When urea concentration increased, the urea molecules adsorbed in the urease activity site and the interface between the two increased. Therefore, the hydrolysis rate was promoted.

The effect of single factor on urease activity is currently being explored[6-8, 12], and the studies reveal the effect of single factors on urease activity but ignore the effect interaction between the factors. In this research work, a supplementary study was conducted to reveal the interaction effect of temperature, moisture content, and nitrogen application rate on urea hydrolysis. The effects of the three factors and their interaction on V_u were in the descending order: T, F, θ, $T*F$, $T*\theta$, $F*\theta$, $T*\theta*F$.

4.3   Kinetics of urea hydrolysis under different treatments

Hydrolysis rate ($\partial U / \partial t$) increases first and then decreases with the increase in urea concentration[23], which is different from this study's result. This study showed that hydrolysis rate ($\partial U / \partial t$) enhanced with the increase in urea concentration. In Singh's report[23], the moisture content was 50% and nitrogen application rate was less than 200 mg/kg. However, the moisture content was between 19.2% (60% of field capacities) and 32% (100% of field capacities) and nitrogen application rate was between 247 mg/kg and 433 mg/kg in this study. The result difference between the two studies might be due to the different urease activity to nitrogen application rate under different moisture contents and nitrogen application rates. In Singh's study[23], the soil water content is relatively high, soil permeability is poor, urease is strongly inhibited, and its activity level is low. At this point, the increase in nitrogen application rate may have toxic effects on urease activity, thereby reducing the hydrolysis rate. In the current study, the moisture content was acceptable, and the increase in nitrogen application rate might increase the urease activity (V_0), thereby promoting the hydrolysis rate ($\partial U / \partial t$).

The current soil temperature was positively correlated with hydrolysis rate ($\partial U / \partial t$), which was similar to the previous study[12,27,28]. This relationship was mainly caused by three reasons. First, when the temperature increased from 15°C to 35°C, the urease activity increased by 0.15-0.62 times (Figure 1), thereby accelerating urea hydrolysis. Second, when the temperature increased from 15°C to 35°C, the diffusion amount of ammonium nitrogen from the liquid phase to the gas phase increased by 3.2 to 17.9 times (Figure 2), thereby resulting in the right moving of the chemical reaction balance (Equation (11)) and the increase in hydrolysis rate[29]. Third, the increase in temperature increased the percentage of activated molecules and the probability of effective collision between molecules; these conditions promoted the hydrolysis reaction[30].

$$CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2 \qquad (11)$$

The effects of single factors on kinetics of urea hydrolysis are currently being examined, thereby revealing the relationship between the factors and kinetic parameters[12,23,27,28]. However, the interaction effect of the three factors on the kinetics of urea hydrolysis should be further explored. In this study, the effects of three factors and their interaction on K_u were in the descending order: T, F, θ, $T*F$, $T*\theta$, $F*\theta$, $T*\theta*F$. Moreover, the effect of three individual factors and their interaction on K_u was extremely significant ($p<0.01$). These findings supplemented the mechanism of urea hydrolysis.

The established $K_{u-1}(\theta, T, F)$ and $K_{u-2}(\theta, T, F)$ models showed more general applicability and better prediction accuracy than the traditional $K_{u-3}(T)$ model. First, when the traditional $K_{u-3}(T)$ model was adopted to quantify the relationship between K_u and the three factors (temperature, moisture content, and nitrogen application rate), 48 kinds of models had to be established to satisfy the different treatments. When the $K_{u-1}(\theta, T, F)$ and $K_{u-2}(\theta, T, F)$ models were adopted, one model could quantify the relationship between K_u and the three factors with all treatments. Second, the accuracies of the three models were in the descending order: $K_{u-1}(\theta, T, F)$, $K_{u-2}(\theta, T, F)$, $K_{u-3}(T)$. The results improved the prediction accuracy of K_u. Among them, the $K_{u-1}(\theta, T, F)$ model was advised to be used in the quantitative study on urea because of its low MAPE value. On the one hand, the $K_{u-1}(\theta, T, F)$ model brought into Equation (1) could achieve the dynamic prediction of urea hydrolysis under the coupling effect of moisture content, temperature, and nitrogen application rate, thus improving the kinetic mechanism of urea hydrolysis. On the other hand, the $K_{u-1}(\theta, T, F)$ model could be used as a source and sink term of the quantitative model of nitrogen transformation and transportation. Given that the $K_{u-1}(\theta, T, F)$ model considered the effect of environmental factors on hydrolysis, the accuracy of quantitative model of nitrogen transformation and transportation could be improved.

The difference in E_a between different reports is obvious. The value of E_a is 96.2 kJ/mol in Rachinskii's report[31] and 41.0 kJ/mol in Gould's report[32], respectively. In this study, the average value of E_a was 34.5 kJ/mol. This result was much smaller than the value reported by Rachinskii[31]. The observed variations could be due to differences in the techniques used in the two studies. The urea content is determined by the diacetyl monooxime method in Rachinskii's report[31]. By contrast, the method of high performance liquid chromatography was used in this study. Various assay techniques can induce conditions that influence differently the energy requirements for the formation of enzyme-substrate complex and thus the activation energy. The present results slightly differed from the findings reported by Gould[32]. However, the current urea hydrolysis was consistent with the first-order kinetics in Gould's study[32]. The control mechanism of the two studies was same. On the other hand, the experimental conditions differed between the two studies. The previous moisture content and nitrogen application are fixed at 24% and 200 mg/kg, respectively, unlike those in the present study. Previous studies only consider the temperature effect. By contrast, the current study not only considered the effects of moisture content, temperature, and nitrogen application rate but also the coupling effect among the three factors. The present results might be more accurate and reliable than the findings of the previous studies.

5 Conclusions

(1) The effects of moisture content, temperature, nitrogen application rate, and their interaction on V_0 and K_u were in the descending order: T, F, $T*F$, θ, $T*\theta$, $F*\theta$, $T*\theta*F$. The effects of single factor and two-factor coupling on V_0 were extremely significant ($p<0.01$), whereas the effect of the three-factor coupling on V_0 was insignificant. The effects of three factors and their interaction on K_u were extremely significant ($p<0.01$). The results represent an important stepping stone toward good understanding of the key mechanism of urea hydrolysis.

(2) The effects of moisture content, nitrogen application rate, and their interaction on E_a were in the descending order: F, θ, $F*\theta$. The effects of two factors and their interaction on E_a were not significant. These findings supplemented the mechanism of urea hydrolysis.

(3) The MAPE values of the established $K_{u-1}(\theta, T, F)$ and $K_{u-2}(\theta, T, F)$ models were 3.14% and 4.60%, respectively; the MAPE of the traditional Arrhenius model $K_{u-3}(T)$ was 6.75%. The accuracy of the proposed three-factor interaction model was superior to that of the traditional single factor model. The development of these two models represents an important stepping stone toward good understanding of the relationship between K_u and environmental factors. Therefore, this study provides a valuable way to predict urea hydrolysis rate.

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