### 1 Controlled-release urea combined with potassium chloride improved the soil

#### 2 fertility and growth of Italian ryegrass

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- 14 Abstract

15 A field experiment with a split-plot design was conducted to study the effect of nitrogen fertilizer type combined with different potassium fertilizer rates on the 16 soil fertility and growth of Italian ryegrass. The main plots were assigned to 17 controlled-release urea (CRU) and common urea, while low, moderate and high 18 19 potassium chloride (KCl) rates (150, 300 and 450 kg ha<sup>-1</sup>, respectively) were assigned to the subplots. The results showed compared with the common urea, 20 the CRU significantly increased the SPAD value, plant height, leaf area, and 21 22 photosynthetic index. Moreover, the dry and fresh yields of the CRU increased by 10.9-25.3% and 11.8-17.7%, respectively. At the same time, compared with 23 24 the KCl150 and KCl450 treatments, the KCl300 treatment resulted in better 25 plant growth. Overall, the CRU×KCl300 maximized the soil inorganic nitrogen 26 and different soil potassium forms. The root length, volume, surface area, 27 average diameter, tips and branches were also improved, and there was a 28 significant N×K interaction effect on the tips. Our analysis corroborated the CRU combined with 300 kg ha<sup>-1</sup> KCl fertilization enhances crop growth by 29

## improving leaf photosynthesis, soil fertility, and yield and should be recommended as the best fertilizer ratio for Italian ryegrass production.

32 Keywords: Controlled-release urea; growth characteristics; Italian ryegrass;

33 potassium chloride; soil fertility, yield

#### 34 Introduction

Grassland accounts for 41.7% of China's total land area (Liu et al., 2018). For a long 35 time, as the main means of production and material basis, grasslands have made 36 important contributions to the development of animal husbandry, and grasslands have 37 38 an important ecological function that plays a central role in water and soil conservation, air purification, climate regulation and biodiversity conservation 39 40 (Binder et al., 2018). Italian ryegrass (Lolium multiflorum L.) is a globally cultivated grass. This grass has many tillers at its roots, grows quickly and has good grazing 41 42 resistance. Italian ryegrass has a strong adaptability and high yield and nutrition, which play a role in improving soil (Hussain et al., 2018; Svatos and Abbott, 2019). 43 Italian ryegrass is widely used in parks, golf courses and other recreational sporting 44 45 areas as the first choice of lawn grass in foreign countries (Fan et al., 2018). Various 46 production technologies and late management measures have also become new 47 research topics. At present, research on ryegrass in lawns, playgrounds and other fields in China is immature. A large number of researchers have focused on 48 management technology after ryegrass planting (Alves dos Santos et al., 2018; He et 49 al., 2020). 50

51 Nitrogen is not only the basis of forage genetic material but also the composition 52 of many important organic compounds (Bolinder et al., 2010). It is very important for 53 life cycle activities and the yield and nutritional quality of forage. Italian ryegrass does not have the ability to fix nitrogen, so nitrogen in the soil is a key factor in grass 54 55 growth and development (Woods et al., 2018). The application of nitrogen fertilizer can significantly improve the yield of Italian ryegrass, and the plant can absorb both 56 57 ammonium and nitrate nitrogen (Cavalli et al., 2016). Moreover, Italian ryegrass is a fast-growing forage grass with a high N requirement and, therefore, strongly relies on 58 59 N soil content to maintain adequate forage yield (Masoni et al., 2015).

60 The fertilizer utilization rate in China is generally low, mainly due to the rapid 61 dissolution of instant fertilizer after it is applied to the soil (Wang et al., 2018). The 62 crop cannot absorb and utilize nitrogen in time, which results in the loss of most of 63 the nitrogen in gaseous or water-soluble forms and causes a series of environmental 64 problems, such as eutrophication of surface water, nitrate pollution of groundwater and agricultural products, and ammonia and nitrogen oxides emitted to the ozone 65 layer (Ata-Ul-Karim *et al.*, 2017). In addition, it is difficult to apply fertilizer to 66 Italian ryegrass after every cutting, so decreasing the fertilizer application frequency 67 and improving the yield and quality of Italian ryegrass are the key problems to 68 address in the planting process (Martin et al., 2017). An effective solution may be to 69 70 develop a new type of slow-release fertilizer to meet the needs of crop growth.

In recent years, controlled-release urea (CRU) has been used worldwide (Geng *et al.*, 2016; Li *et al.*, 2018; Liu *et al.*, 2019). CRU can release nitrogen slowly in the form of a resin polymer coating, continuously supply nutrients needed for ryegrass growth, decrease the number of topdressings and labour intensity in the later stage, simplify cultivation technology, save time and labour, and reduce environmental pollution (Gaylord *et al.*, 1975).

77 Potassium chloride (KCl) is commonly used in agricultural production. It has a low price and high nutrient content, and the application of appropriate amounts can 78 79 promote the growth of Italian ryegrass (McDonnell, et al., 2018). In addition, 80 potassium can improve the photosynthetic capacity and disease resistance of plants 81 and then extend the green period (Hasanuzzaman et al., 2018). In addition, a single 82 application of nitrogen and potassium or mixed application of different proportions 83 can increase the fresh yield of Italian ryegrass, but the effect of a mixed application is 84 better than that of a single application (Oliveira *et al.*, 2017). According to the supply 85 and demand curve of soil for nutrient elements, the most appropriate fertilization amount and fertilization type can be formulated. 86

Nitrogen can improve photosynthesis and thus dry matter production (Jarvis,
1987). Potassium is very important for root growth and disease resistance (Snyder and
Cisar, 2000). The possible interactions between the two nutrients are unknown, as

90 they have not been sufficiently studied in Italian ryegrass. In previous studies on the 91 effect of N $\times$ K fertilizer application on crop growth, the positive interaction of N and 92 K reduced the cost of fertilizer and contributed to food security (Yang et al., 2016a, 93 2016b). Nitrogen fertilizer application increases the plant potassium absorption 94 efficiency, and potassium fertilizer application resolves the problem of nitrogen pollution by inducing in crops a high nitrogen absorption efficiency (Dong et al., 95 96 2010). Eventually, the mutual promotion of nitrogen and potassium enhances the yield and quality of crops (Yang et al., 2016a). This is a feasible way to increase the 97 98 potassium fertilizer input and improve the nitrogen utilization efficiency. Understanding the mechanism underlying the N×K interaction is vital to guide the 99 100 best practice of nutrient management in agricultural production.

Furthermore, there are few reports on the interaction application effects of CRU combined with KCl on Italian ryegrass growth. Hence, the objective of this study was to investigate the effects of CRU in combination with KCl on (i) soil inorganic nitrogen ( $NO_3^-$ -N and  $NH_4^+$ -N), (ii) soil potassium forms (available potassium, water-soluble potassium, exchangeable potassium and nonexchangeable potassium), (iii) growth and photosynthesis characteristics, and (iv) Italian ryegrass yield and fertilizer use efficiency.

#### **108** Materials and methods

109 Experimental site and material

110 The field experiment was arranged at the experimental base of the College of 111 Agriculture and Forestry Science, Linyi University, Linyi city, Shandong Province 112 (35°06'N, 118°17'E) in 2019, and this site has a continental climate typical of temperate monsoon areas. The temperature and relative humidity were  $30 \pm 5$  °C and 113 114  $45 \pm 5\%$  mm, respectively. The experimental site has four distinct seasons and 115 substantial light. The tested soil is sandy loam, which is classified as Typic Hapludalf according to the USDA classification (Soil Survey Staff 1999). The basic soil 116 117 properties are listed in Table 1.

118 The tested forage is 'Bluesign' Italian ryegrass, which is produced by Suqian 119 Chengzhiyang Seed Industry Co., Ltd., China. The seeding rate of the Italian ryegrass

was 25 kg ha<sup>-1</sup>. The fertilizers used included CRU and ordinary fertilizers. The CRU 120 121 (containing N 43%, with a release period of almost 3 months in distilled water at 122 25 °C) (Fig. 1) fertilizer was produced by the State Key Laboratory of Nutrition 123 Resources Integrated Utilization, China. The CRU fertilizer was formulated as round 124 particles with a regular shape and smooth surface, and it was coated with an epoxy 125 resin (low curing shrinkage, strong adhesion and chemical resistance). The ordinary 126 fertilizers included urea (containing N 46%), calcium superphosphate (containing  $P_2O_5$  14%), and KCl (containing K<sub>2</sub>O 60%), which were provided by Jinzhengda 127 128 Ecological Engineering Group Co., Ltd., and Kingenta Ecological Engineering Group 129 Co., Ltd., China.

130 Experimental design

131 A split-plot design with three replications was used for the experiment. Specifically, 132 the nitrogen types (CRU and urea) were the main plots, KCl rates (150, 300 and 450 kg ha<sup>-1</sup>) were the subplots, and no N or K fertilization was the control. The main plot 133 covered an area of 45  $m^2$  (3 m wide and 15 m long), and the subplot covered an area 134 of 15  $m^2$  (3 m wide and 5 m long). Each plot received a basal application of 300 kg 135 ha<sup>-1</sup> N and 200 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> All fertilizers (except common urea) were applied once 136 137 with hands before sowing seeds. In particular, urea was applied twice in total, with 138 60% before sowing seeds and 40% after the second clipping stage. The Italian 139 ryegrass was sown on April 4, 2019. CRU (10 g) fertilizer was weighed and placed 140 into the mesh bag (8 cm width and 10 cm length), the bags were sealed, and this 141 process was repeated 18 times. Then, these bags were put into the ploughed soil layer 142 before the Italian ryegrass was sown to determine the release characteristics of the 143 CRU fertilizer buried in the soil.

144 *Sampling and measurement* 

The release of the CRU in the soil was determined by the buried bag method. Similarly, 10 g of CRU particles were put in a mesh bag (8 cm wide and 10 cm long) buried in a cement tank at a depth of 15-20 cm during fertilization. The bags were collected on the 10<sup>th</sup>, 20<sup>th</sup>, 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup>, and 120<sup>th</sup> days after burial. Three bags were collected each time, washed and dried to constant weight at 60 °C, and the nitrogen

release rate was calculated according to the weight of the remaining fertilizerparticles.

Soil and plant samples were collected on May 18, 2019 (first clipping), June 14, 2019 (second clipping), July 16, 2019 (third clipping), and August 10, 2019 (fourth clipping), and the physiological indexes of Italian ryegrass under different treatments were observed and measured.

156 The 0-20 cm soil samples were collected by a 5-point sampling method (2) sampling points in the fertilizer row, 3 sampling points in the plant row). The contents 157 of NO<sub>3</sub><sup>-</sup>N and NH<sub>4</sub><sup>+</sup>-N in fresh soil (0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> extraction) were determined 158 immediately using an AA3 continuous flow analyser (Bran-Luebbe, Norderstedt, 159 160 Germany). The remaining soil was dried by naturally existing air and ground through 161 2 mm and 0.25 mm sieves, and the organic matter (potassium dichromate external 162 heating method), soil total N (semi micro Kelvin method), available phosphorus (pH 8.5, 0.5 mol  $L^{-1}$  NaHCO<sub>3</sub> extraction, molybdenum blue colorimetry) and available 163 potassium (1 mol L<sup>-1</sup> NH<sub>4</sub>OAc extraction, flame photometer method) contents were 164 165 determined (Zheng et al., 2016).

166 The leaf area of Italian ryegrass was measured by a leaf area meter (Yaxin-1241, 167 Yaxinliyi, China). The SPAD value was measured by a hand-held chlorophyll meter 168 (SPAD-502, Minolta, Japan). Besides, the Li-6400 portable photosynthetic apparatus 169 (LI-COR, Lincoln, NE, USA) was also used for the determination. The leaf photosynthetic indicators were measured from 9:00-10:00 a.m. Under sunny and 170 171 cloudless weather, the net photosynthetic rate  $(P_n)$ , stomatal conductance  $(G_s)$ , 172 intercellular carbon dioxide concentration ( $C_i$ ) and transpiration rate ( $T_r$ ) were measured before the second clipping. 173

After measuring the physiological indexes of the plant, ten successive plants above the roots from each subplot were clipped with scissors, and the height of the stubble was the same as that of the ground. The fresh weight of the yield of each treatment was recorded. Then, the clipped grass was sealed in the file bag according to the treatment label, placed in the oven at 105 °C for 30 minutes, and dried at 65 °C for 72 hours, and then, the dry weight of the yield was recorded. Finally, the nitrogen and potassium contents of the plants were analysed. The plant total nitrogen contents were determined by digestion with  $H_2SO_4$ - $H_2O$  using the micro-Kjeldahl method. The potassium content of the plant was digested by  $H_2SO_4$ - $H_2O$  and determined by a flame photometer. The nitrogen and potassium uptake were calculated according to the nitrogen and potassium content and dry mass weight of each plot. The nitrogen use efficiency (NUE) and potassium use efficiency (KUE) were calculated (Rietra *et al.*, 2017).

187 The root samples were scanned with a flatbed image scanner (Epson 188 Expression/STD LC-4800 scanner). The images were analysed by WinRHIZO 189 commercial software (Regent Instruments, 2001) to determine the root volume, total 190 length, diameter, surface area, and numbers of tips and branches.

191 *Statistical analyses* 

192 Microsoft Excel 2010 was employed for data processing, and Sigma Plot software 193 version 10 (MMIV, Systat Software Inc., San Jose, CA, USA) was used to draw the 194 figures. Data were subjected to analysis of variance (ANOVA) and mean separation 195 tests as a split-plot factorial design with three replications. Concretely, the data were 196 analysed using Statistical Analysis System version 9.2 (SAS Institute Cary, NC, 2010) 197 with a two-way ANOVA at a significance level of 0.05, with nitrogen type and 198 potassium rate as the independent variables. Two-way ANOVAs were performed to 199 determine the effects of N, K and their interactions on the leaf area, leaf SPAD, leaf 200 photosynthesis chlorophyll parameters, root morphology, yield and nutrient uptake of 201 Italian ryegrass. One-way ANOVAs were performed to test for significant differences between treatments of NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and soil K informs. A Duncan multiple range 202 203 test was carried out to determine if significant (p<0.05) differences occurred between 204 individual treatments (Tang and Feng, 2002).

205 **Results** 

206 *Release characteristics of the CRU fertilizer* 

The release characteristics of the CRU fertilizer in the soil were released in the form of "S", reaching 80% in approximately 100 days (Fig. 1). The release period of the CRU in the soil was almost 120 days: the release rate was slow within 0~60 days, the

210 release rate increased within 60~90 days, and the nutrient decline period was within

- 211 90~120 days, which met the nitrogen demand of Italian ryegrass.
- 212 Soil inorganic nitrogen and potassium form

The contents of  $NO_3^{-}N$  and  $NH_4^{+}N$  in the soil in the control treatment were the 213 lowest among all the treatments in the whole plant growing season (Fig. 2). With the 214 215 increase in the KCl application rate, the contents of  $NO_3^--N$  and  $NH_4^+-N$  in the soil changed little, which was not related to the type of nitrogen application. However, at 216 the early stage of growth, the contents of  $NO_3^-N$  and  $NH_4^+-N$  in the urea treatment 217 218 were higher than those in the CRU treatment, but after the second clipping, the contents of  $NO_3$ -N and  $NH_4^+$ -N in the urea treatment decreased rapidly; in addition, 219 the contents of  $NO_3^-$ -N and  $NH_4^+$ -N in the urea treatment were lower than those in the 220 221 CRU treatment.

222 Overall, the contents of available K, water-soluble K, exchangeable K and 223 nonexchangeable K were significantly affected by the KCl application rate, and of the 224 treatments, the control treatment had the lowest values in different developmental 225 stages (Fig. 3). The contents of soil available K, water-soluble K and 226 nonexchangeable K decreased gradually, but that of exchangeable K showed a 227 fluctuating trend. Regardless of which type of nitrogen fertilizer was combined, the 228 contents of soil available potassium, water soluble potassium and exchangeable 229 potassium improved with the increase in the KCl application rate, and there was a 230 significant difference between the different potassium fertilizer treatments.

231 *Plant height and leaf area* 

232 In the whole growth period of Italian ryegrass, the plant heights of the different 233 fertilization treatments increased first and then decreased (Table 2). There was no significant difference between the different fertilization treatments at the first and 234 235 second clipping stages, but the advantage of the CRU treatment group was obvious; in addition, the average value was higher than that of the control treatment. During the 236 237 mowing period of the third and fourth clippings, Italian ryegrass growth was in the transition period from maturity to senescence, the plant demand for nutrients 238 239 decreased, and the ability to absorb fertilizer also decreased. Under the same amount

240 of applied nitrogen, the plants in the KCl150 and KCl300 treatments were taller, and 241 those in the KCl450 treatment were shorter. At the same time, the plant height of the 242 CRU treatment group was significantly greater than that of the urea treatment groups. 243 Similarly, the leaf area of Italian ryegrass in the whole growth period increased 244 first and then decreased with the growth of the plant (Table 3). At the second clipping 245 period, the apparent leaf area increased the fastest. The leaves of the plants were long 246 and thin, and there was no difference between the fertilization treatments at the first 247 and second clipping stages, but fertilization was more suitable for its growth; in 248 addition, the leaf area of the plants with fertilization was relatively large. During the 249 third and fourth clipping stages, in comparison with the control and common urea 250 treatments, the CRU treatments resulted in a significant difference, which indicated 251 that the CRU could delay plant senescence to some extent. In addition, the effect of 252 nitrogen fertilizer on ryegrass leaf area was greater than that of KCl fertilizer, and the 253 sustainability of combined application is more important. There was no significant 254 N×K interaction effect on the plant height and leaf area (except at the third clipping).

#### 255 *Leaf SPAD and photosynthetic index*

256 The effect of different fertilization treatments on the SPAD value of Italian ryegrass 257 was different (Table 4). At the first clipping stage, the SPAD value of the 258 CRU×KCl300 treatment was the highest, 17.1% higher than that of the control 259 treatment. During the whole growth period of Italian ryegrass, the SPAD value 260 increased first and then decreased, and the SPAD value of the CRU treatment was 261 higher than that of the common urea treatment. Under the three levels of KCl fertilizer, 262 the SPAD value of the KCl150 treatment in the whole growth period was low, which 263 indicates that the insufficient use of potassium had a certain impact on the SPAD 264 value. The SPAD value of the CRU×KCl300 treatment was the highest at the third 265 and fourth stages, while the effect of nitrogen on the SPAD value of ryegrass was 266 greater at the later stage. In comparison with common urea, the application of CRU 267 had a greater advantage in improving the SPAD value. There was no significant N×K 268 interaction effect on the SPAD value (except at the second clipping).

269 The nitrogen fertilizer types and KCl rates affected the photosynthesis indicators,

but there was no significant difference between their interaction effects (Table 5). In comparison to the urea treatments, the CRU treatment improved the  $P_n$ ,  $G_s$  and  $T_r$  and lowered the  $C_i$ . In addition, the photosynthesis indicators increased with increasing KCl rates. There was no significant N×K interaction effect on the photosynthesis indicators (except  $T_r$ ), and of the treatments, the CRU×KCl300 treatment performed the best in terms of Italian ryegrass leaf photosynthesis.

276 *Root morphology* 

The nitrogen fertilizer types and potassium fertilizer rates significantly affected the 277 278 root morphology (total length, surface area, average diameter, root volume, and 279 numbers of tips and branches) (Table 6). Concretely, the CRU treatments increased 280 these parameters compared with the urea treatments. In addition, the moderate KCl 281 rate treatments markedly improved the root morphology compared with the low- and 282 high-KCl-rate treatments despite any nitrogen fertilizer type. However, there was no 283 significant N×K interaction effect (except the tips), and of the treatments, the 284 CRU×KCl300 treatment performed the best in terms of Italian ryegrass root growth.

285 *Yield* 

286 The fresh and dry Italian ryegrass yields of the CRU treatment increased continuously 287 in four clipping periods (Tables 7 and 8), those of the control treatment and common 288 urea treatment decreased at the third clipping stage, and the difference was significant. 289 In the whole growth period, under the CRU treatment conditions, there was no 290 difference in the treatment with different amounts of KCl fertilizer, which indicated 291 that the factors affecting the fresh and dry yields of Italian ryegrass were due more to 292 nitrogen fertilizer than to potassium fertilizer. However, there was no significant N×K interaction effect on the yield. When clipping at the second stage, the highest dry 293 yields of the CRU×KCl300 and CRU×KCl450 treatments were 1.75 g plant<sup>-1</sup>, which 294 295 were 5.4% and 2.9% higher than those of the urea×KCl300 and urea×KCl450 296 treatments, respectively, at the same potassium rate. During the third and fourth stages, 297 the growth trend of the plants increased slowly, the demand for nutrients decreased, and the CRU slowly released nitrogen for plant growth. Thus, CRU was more 298 299 beneficial than common urea. In addition, potassium fertilizer had little influence on

300 the yield. There was no significant N $\times$ K interaction effect on the dry yield and fresh

- 301 yield (except at the fourth clipping).
- 302 *Nitrogen and potassium use efficiency*

The nitrogen fertilizer application significantly affected nitrogen uptake and NUE, and the potassium fertilizer application significantly affected potassium uptake and KUE (Table 9). The nitrogen uptake and NUE of the CRU treatments were significantly higher than those of the urea treatments. In addition, potassium uptake increased with increasing KCl rate, but the KUE had the opposite trend. In total, the CRU×KCl300 treatment resulted in the highest nutrient uptake and use efficiency.

#### 309 **Discussion**

#### 310 Soil inorganic nitrogen and potassium form

The contents of soil inorganic nitrogen (NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N) in the 0-20 cm soil 311 312 layer were greatly affected by fertilization and were related to the type of fertilizer, 313 the frequency of fertilization and the amount of fertilization (Zheng et al., 2016). In 314 the present study, the contents of nitrate nitrogen and ammonium nitrogen at depths of 315 0-20 cm were significantly affected by nitrogen fertilization, but no significant 316 difference was found between the potassium fertilization applications. In the 317 beginning, urea rapidly dissolved and released substantial nitrogen, while the CRU 318 initially released less nitrogen; thus, the soil inorganic nitrogen content of the urea 319 treatment was higher than that of the CRU treatment at the first clipping stage. 320 However, due to the continuous release of nitrogen from the CRU, the contents of  $NO_3$ -N and  $NH_4^+$ -N increased significantly from the second clipping stage to the 321 322 fourth clipping stage compared with that in the urea treatment. Zheng et al. (2016) 323 also found that the application of CRU mixed with urea significantly increased the 324 soil inorganic nitrogen compared with the application of normal urea, especially in the 325 later growth stage. The effect of the KCl rate on soil inorganic nitrogen was not obvious during the whole growth stage of Italian ryegrass. 326

The application of potassium fertilizer significantly affects the content of soil potassium and the form of soil potassium (Yang *et al.*, 2017; Li *et al.*, 2017). In the present study, potassium application significantly increased the contents of soil

330 available K, water-soluble K, exchangeable K and non-exchangeable K, similar to other research results (Kurbah and Dixit, 2019). However, quick-acting fertilizers 331 332 such as KCl are easily converted into non-exchangeable K, which leads to a decrease in soil potassium availability (Chen et al., 2020). In total, the contents of soil available 333 334 K, water-soluble K, exchangeable K and non-exchangeable K increased with 335 increasing KCl rate. The contents of soil available K and water-soluble K had a 336 significant positive correlation, where a similar presence was also found in exchangeable K and non-exchangeable K (Table 10). The water-soluble K contributed 337 338 the most to  $NO_3^-N$ , and the available K had similar effects on  $NH_4^+-N$ . In addition, the contents of soil available potassium in the KCl300 and KCl450 treatments were 339 340 similar. Thus, a moderate amount of potassium fertilizer could maintain a high 341 potassium content in the soil, which would supply enough potassium nutrition for 342 Italian ryegrass (Jiménez-Calderón et al., 2018). In addition, we found that the 343 interaction effect of N×K on soil available potassium or inorganic nitrogen was not 344 obvious during the whole growth stage of Italian ryegrass. Yang et al. (2016b) also 345 found that the interaction effect of nitrogen and potassium fertilization on soil 346 potassium and nitrogen was not obvious in a cotton field.

347 *Growth index* 

348 To understand the growth of crops over time, it is necessary to determine their 349 nutritional status. The traditional determination of leaf colour is to detect the 350 chlorophyll content, which has high accuracy, but it takes considerable work and time 351 (Croft et al., 2017). Plant height, chlorophyll, leaf area and photosynthetic index are 352 important parameters to characterize crop photosynthetic production capacity, crop 353 growth and nutritional status (Lin et al., 2019). The results showed that in comparison 354 to the CRU treatment, the urea treatment significantly reduced the plant height, SPAD, leaf area and photosynthetic index, which might have led to plant senescence. 355

In addition, the application of KCl significantly increased the chlorophyll content in Italian ryegrass. Within a certain range of KCl applications, the chlorophyll content increased with increasing KCl applications, but beyond this threshold, the effect of KCl was weakened, thus affecting the ornamental value of Italian ryegrass. This

outcome may have been due to the decrease in assimilative capacity, enzyme content
and enzyme activity caused by the lack or excess of potassium, further leading to the
decrease in leaf area and photosynthesis and thus affecting photosynthesis (Lu *et al.*,
2017). Generally, the CRU×KCl300 treatment improved the leaf photosynthesis of
Italian ryegrass.

365 Roots play an important role in crop growth and yield formation (Bandeoğlu et 366 al., 2004). In the present study, the application of the CRU and moderate KCl rate 367 significantly increased root length, surface areas and the numbers of tips and branches 368 compared with other treatments, and there was a significant N $\times$ K interaction effect on 369 the numbers of tips. These results suggested that suitable nitrogen and potassium 370 fertilizer application can enhance root growth and thereby increase the uptake of 371 nutrients (Enriquez-Hidalgo et al., 2018). In total, there was no significant N×K 372 interaction effect on the growth index. Dong et al. (2010) also found a positive 373 correlation between the duration of reproductive growth and the appropriate amount 374 of N or K application, but the interaction effect of N×K was not obvious.

375 Yield and fertilizer use efficiency

Nitrogen and potassium are the key factors affecting the yield of Italian ryegrass. Under sufficient nitrogen, the leaves of Italian ryegrass are thick green and have strong growth and a high yield of fresh grass, and under nitrogen deficiency, the leaves of Italian ryegrass are yellow and have poor growth (Vleugels *et al.*, 2017).

380 The results showed that in comparison to those of the urea treatment and the 381 control treatment, the fresh and dry yields of the CRU treatment were highest and 382 increased by 20.5-53.2%. Studies have also shown that CRU effectively promotes the 383 ability of photosynthesis to produce organic matter and then increase plant yield (Van 384 Eerd et al., 2017; Miyatake et al., 2019). In this study, the second and third clipping 385 stages were the period of high yield of Italian ryegrass and the period of high yield of 386 CRU. This highlighted that the N uptake of Italian ryegrass in the first growing stages 387 is lower, which can be functional to the presented pattern of N release in soil (Masoni 388 et al., 2015). In the present study, the yield of the CRU treatment was higher than that 389 of the common urea treatment in the four clipping periods, especially in the middle

390 and later stages of Italian ryegrass growth, which occurred because the CRU slowly 391 released nitrogen and met the growth demand of Italian ryegrass. In general, the 392 nitrogen fertilizer type markedly affected the fresh and dry yield, but the application 393 amount of potassium fertilizer had little effect on the yield. Moreover, no significant 394  $N \times K$  interaction effect was found in the present study, which was different from Yang 395 et al. (2016a). Hence, it is necessary and important to carry out further field 396 experiments on the accurate rate and timing of N and K. Through a comparison, the yield of the CRU×KCl450 treatment was found to be the highest in the early stage, 397 398 but considering the later stage yield, fertilizer utilization rate, economic benefits and 399 other factors, the whole growth period of the CRU×KCl300 treatment was the most 400 suitable treatment combination of all treatments, which improved the yield of Italian 401 ryegrass.

402 There are many parameters to describe the efficiency of fertilizer utilization. The 403 key to improving the efficiency of fertilizer utilization is nutrient absorption (Xue et 404 al., 2017). In this study, NUE and KUE were used. Regardless of the amount of KCl 405 applied, the NUE of the CRU treatment was significantly higher than that of the urea 406 treatment, which might have been due to high nitrogen absorption, and Gaylord et al. 407 (1975) also found similar results. In addition, the KCl rate had a significant effect on 408 potassium absorption and KUE. The KUE values of the KCl300 treatment were 409 higher than those of the KCl150 and KCl450 treatments. Therefore, the CRU×KCl300 410 treatment could improve the NUE and KUE of Italian ryegrass. In the present study, at 411 the same N rate, the inorganic nitrogen concentration in the soil of the CRU was 412 higher than that in the urea treatments, which indicated that more N remained in the 413 soil, thus reducing the N leaching loss. Masoni et al. 2015 also found that the increase 414 in nutrient use efficiency could also minimize unfavourable effects on the 415 environment, mainly leaching.

#### 416 Conclusion

The nitrogen fertilizer type and potassium fertilizer rate had significant effects on Italian ryegrass growth, yield and soil fertility, but there was no significant N×K interaction effect. The CRU released nitrogen slowly, which was consistent with the

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420 nitrogen demand of Italian ryegrass during the whole growth and development period, simplifying the cultivation technology. This study found that the amount of potassium 421 422 fertilizer had no significant effect on the growth of Italian ryegrass in the early stage, 423 but in the middle and late stages of ryegrass growth, the CRU×KCl300 treatment improved plant SPAD and root morphology, delayed senescence of Italian ryegrass, 424 425 and significantly increased the yield and fertilizer use efficiency. Hence, the 426 CRU×KCl300 treatment is recommended as the best fertilization ratio for Italian 427 ryegrass, and this recommendation can serve technical support for Italian ryegrass 428 production and fertilization.

#### 429 Conflict of Interest Statement

Author Jianqiu Chen was employed by the company Kingenta Ecological Engineering
Group Co., Ltd. The remaining authors declare that the research was conducted in the
absence of any commercial or financial relationships that could be construed as a
potential conflict of interest.

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#### 436 Author contributions

J.Q.C. and X.Y.Y. conceived and designed the experiments; Q.J.L. and X.Q.H.
analyzed the data; J.B.G. and X.Y.Y. wrote the manuscript; S.T.L. and H.L. were
involved in the related discussion; Y.L. helps to improve the quality of the manuscript.
All authors reviewed the manuscript.

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#### **Figure Legends**

Fig. 1 Release of nitrogen from CRU.

Fig. 2 Change of the soil NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N contents.

Fig. 3 Change of soil available K, water soluble K, exchangeable K and

non-exchangeable K contents.

Table 1 Part p	roperties of teste	d soil before Italian	ryegrass j	planting in 2019.

pН	Organic					
value	matter	Total N	$NO_3$ -N	$\mathbf{NH_4}^+$ -N	Available P	Available K
(2.5:1)	(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	( <b>mg kg</b> <sup>-1</sup> )
6.52	6.88	0.73	46.09	31.22	39.82	182.08

Table 2 The plant height of Italian ryegrass under different treatments.

	First clipping	Second clipping	Third clipping	Fourth clipping
Treatment	( <b>cm</b> )	( <b>cm</b> )	( <b>cm</b> )	( <b>cm</b> )
Nitrogen fertiliz	zer type			
CRU	17.64 a	25.22 a	24.47 a	15.50 a
Urea	16.67 a	23.94 a	19.83 b	8.06 b
Potassium ferti	lizer rate (kg ha <sup>-1</sup>	·)		
150	17.78 a	23.47 a	22.70 a	11.17 b

300	16.48 a	25.17 a	21.62 a	13.08 a
450	17.20 a	25.12 a	20.63 a	11.08 b
N×K interaction				
Control	16.27 a	21.43 с	20.30 b	8.32 b
CRU×KCl150	17.83 a	24.57 abc	25.23 a	14.33 a
CRU×KCl300	16.77 a	25.90 a	23.23 ab	17.02 a
CRU×KCl450	18.33 a	25.20 ab	21.93 ab	15.16 a
Urea×KCl150	17.73 a	22.36 bc	20.17 b	<b>8.32</b> b
Urea×KCl300	16.20 a	24.43 abc	<b>20.00 b</b>	9.16 b
Urea×KCl450	16.07 a	25.03 ab	19.33 b	7.03 b
Source of variance	e			
Ν	0.1619	0.1735	0.0023	<0.0001
Κ	0.3002	0.2415	0.1858	0.0632
N×K	0.3855	0.6337	0.4809	0.5067

Note: Means followed by different lowercase letters in the same column were significantly different based on analyses with ANOVAs followed by Duncan tests (P < 0.05).

Control: no nitrogen and potassium fertilizer, CRU: polymer-coated urea, KCl: potassium chloride.

Treatment	First clipping (mm <sup>2</sup> )	Second clipping (mm <sup>2</sup> )	Third clipping (mm <sup>2</sup> )	Fourth clipping (mm <sup>2</sup> )	
Nitrogen fertiliz	er type				
CRU	295.72 a	542.99 a	475.30 a	171.64 a	
Urea	282.67 a	614.73 a	389.57 b	57.16 b	
Potassium fertili	izer rate (kg ha <sup>-1</sup>	<sup>1</sup> )			
150	287.10 a	522.52 a	396.50 a	111.93 a	
300	286.22 a	593.90 a	418.97 a	135.10 a	
450	294.27 a	620.17 a	<b>481.83</b> a	96.17 b	
N×K interaction	1				
Control	328.73 a	491.52 a	330.53 b	108.32 b	
CRU×KCl150	289.40 a	417.61 a	381.93 b	167.86 a	
CRU×KCl300	305.87 a	589.44 a	455.47 ab	184.50 a	
CRU×KCl450	291.92 a	622.02 a	588.53 a	162.63 a	
Urea×KCl150	284.85 a	627.56 a	411.12 b	56.07 cd	
Urea×KCl300	266.57 a	598.41 a	382.47 b	85.73 bc	
Urea×KCl450	296.63 a	618.39 a	375.13 b	29.76 d	
Source of varian	ice				
Ν	0.6001	0.3835	0.0319	<0.0001	
K	0.9588	0.5909	0.1536	0.0621	

Table 3 The leaf area of Italian ryegrass under different treatments.

N×K	0.7394	0.4862	0.0484	0.4916
	0.7574	0.4002	0.0404	0.4910

Note: Means followed by different lowercase letters in the same column were significantly different based on analyses with ANOVAs followed by Duncan tests (P < 0.05).

Control: no nitrogen and potassium fertilizer, CRU: polymer-coated urea, KCI: potassium chloride.

FIRST CHIMMINO		'l'hind olimping	Founth alimning
First clipping	Second clipping	i mra cupping	Fourth clipping
r type			
49.76 a	54.70 a	27.49 a	<b>17.44</b> a
44.37 b	57.66 b	20.92 b	10.39 b
er rate (kg ha <sup>-1</sup>	)		
45.72 b	54.83 a	<b>24.17</b> a	14.33 a
46.47 ab	57.73 a	25.97 a	15.02 a
<b>49.00</b> a	55.97 a	22.48 a	<b>12.40</b> a
45.67 c	50.93 с	25.97 ab	13.66 b
50.00 ab	51.66 c	27.56 ab	14.86 ab
47.66 bc	<b>59.62</b> a	<b>29.70</b> a	22.43 a
51.60 a	52.74 bc	25.20 ab	15.03 ab
41.43 d	58.02 ab	20.76 ab	13.80 b
45.27 с	55.80 abc	22.23 ab	7.60 b
46.40 bc	59.16 a	19.76 b	9.77 b
e			
0.0008	0.0956	0.002	0.0047
0.0746	0.3603	0.2125	0.507
0.1102	0.0436	0.8487	0.039
	44.37 b er rate (kg ha <sup>-1</sup> 45.72 b 46.47 ab 49.00 a 45.67 c 50.00 ab 47.66 bc 51.60 a 41.43 d 45.27 c 46.40 bc e 0.0008 0.0746	49.76 a       54.70 a         44.37 b       57.66 b         er rate (kg ha <sup>-1</sup> )         45.72 b       54.83 a         46.47 ab       57.73 a         49.00 a       55.97 a         45.67 c       50.93 c         50.00 ab       51.66 c         47.66 bc       59.62 a         51.60 a       52.74 bc         41.43 d       58.02 ab         45.27 c       55.80 abc         46.40 bc       59.16 a         e       0.0008       0.0956         0.0746       0.3603	49.76 a       54.70 a       27.49 a         44.37 b       57.66 b       20.92 b         er rate (kg ha <sup>-1</sup> )       45.72 b       54.83 a       24.17 a         46.47 ab       57.73 a       25.97 a         49.00 a       55.97 a       22.48 a         45.67 c       50.93 c       25.97 ab         50.00 ab       51.66 c       27.56 ab         47.66 bc       59.62 a       29.70 a         51.60 a       52.74 bc       25.20 ab         41.43 d       58.02 ab       20.76 ab         45.27 c       55.80 abc       22.23 ab         46.40 bc       59.16 a       19.76 b         e       0.0008       0.0956       0.002         0.0746       0.3603       0.2125

Note: Means followed by different lowercase letters in the same column were significantly different based on analyses with ANOVAs followed by Duncan tests (P < 0.05).

Control: no nitrogen and potassium fertilizer, CRU: polymer-coated urea, KCI: potassium chloride.

Table 5 The leaf photosynthesis chlorophyll parameters of Italian ryegrass under different treatments at the second clipping stage.

	P <sub>n</sub>	$G_{ m s}$	T <sub>r</sub>	Ci
Treatment	(umol m <sup>-2</sup> s <sup>-1</sup> )	(umol mol <sup>-1</sup> )	(umol m <sup>-2</sup> s <sup>-1</sup> )	(umol m <sup>-2</sup> s <sup>-1</sup> )
Nitrogen fertiliz	zer type			

CRU	295.72 a	542.99 a	475.30 a	171.64 a
Urea	282.67 a	614.73 a	389.57 b	57.16 b
Potassium fertiliz	er rate (kg ha <sup>-1</sup> )			
150	287.10 a	522.52 a	396.50 a	111.93 a
300	286.22 a	593.90 a	418.97 a	135.10 a
450	294.27 a	620.17 a	481.83 a	96.17 b
N×K interaction				
Control	328.73 a	491.52 a	330.53 b	108.32 b
CRU×KCl150	289.40 a	417.61 a	381.93 b	167.86 a
CRU×KCl300	305.87 a	589.44 a	455.47 ab	184.50 a
CRU×KCl450	<b>291.92</b> a	622.02 a	588.53 a	162.63 a
Urea×KCl150	284.85 a	627.56 a	411.12 b	56.07 cd
Urea×KCl300	266.57 a	598.41 a	382.47 b	85.73 bc
Urea×KCl450	296.63 a	618.39 a	375.13 b	29.76 d
Source of variance	e			
Ν	0.6001	0.3835	0.0319	<0.0001
K	0.9588	0.5909	0.1536	0.0621
N×K	0.7394	0.4862	0.0484	0.4916

Note: Means followed by different lowercase letters in the same column were significantly different based on analyses with ANOVAs followed by Duncan tests (P < 0.05).

Control: no nitrogen and potassium fertilizer, CRU: polymer-coated urea, KCI: potassium chloride,  $P_n$ : photosynthetic parameters including net photosynthetic rate,  $G_s$ : stomatal conductance,  $C_i$ : intercellular carbon dioxide concentration,  $T_r$ : transpiration rate.

Table 6 The root morphology of Italian ryegrass under different treatments at the fourth clipping stage.

Treatments	Total length (cm)	Surface area (cm <sup>2</sup> )	Average diameter (mm)	Root volume (cm <sup>3</sup> )	Tips	Branches
Nitrogen fertilizer type	(cm)	(em )	(11111)	(em)		
CRU	1400.00 -	100.02 -	0.35 a	1.61 a	14956 12 a	16076.37 a
CRU	1480.89 a	180.23 a	0.35 a	1.01 a	14950 12 a	100/0.3/ a
Urea	1377.67 b	159.56 b	0.32 b	<b>1.49 b</b>	13947 .33 b	14872.35 b
Potassium fertilizer rate	e (kg ha <sup>-1</sup> )					
150	1393.00 с	164.50 b	0.32 b	1.51 c	14113.50 с	14989.31 c
300	1479.67 a	176.17 a	0.35 a	<b>1.60</b> a	14754.17 a	15905 04 a
450	1415.16 b	168.67 b	0.33 b	1.55 b	14487.33 b	15528.78 b
N×K interaction						
Control	1229.67 e	75.33 e	0.23 c	0.90 d	8142.02 e	10214.56 e
CRU×KCl150	1434.09 bc	173.66 b	0.33 b	1.56 bc	14521.72 bc	15490.34 с
CRU×KCl300	1545.33 a	188.45 a	0.36 a	<b>1.67</b> a	15411.23 a	16699.76 a

CRU×KCl450	1463.73 b	178.76 b	0.34 ab	1.61 ab	14935.33 ab	16039.72 b
Urea×KCl150	1352.01 d	155.33 d	0.31 b	<b>1.46</b> c	13705.49 d	14488.36 d
Urea×KCl300	1414.21 с	164.98 c	0.34 ab	1.52 bc	14097.34 cd	15111.23 с
Urea×KCl450	1367.03 d	159.35 cd	0.32 b	<b>1.49</b> c	14039.76 cd	15017.74 с
Source of variance						
Ν	<0.0001	<0.0001	0.0111	<0.0001	<0.0001	<0.0001
Κ	<0.0001	0.0013	0.0197	0.0003	0.0002	0.0004
N×K	0.063	0.3063	0.9734	0.1898	0.0321	0.0937

Note: Means followed by different lowercase letters in the same column were significantly different based on analyses with ANOVAs followed by Duncan tests (P < 0.05).

Table 7 The fresh yield of Italian ryegrass under different treatments.

Treatment	First clipping (g plant <sup>-1</sup> )	Second clipping (g plant <sup>-1</sup> )	Third clipping (g plant <sup>-1</sup> )	Fourth clipping (g plant <sup>-1</sup> )	Sum (g plant <sup>-1</sup> )
Nitrogen fertilize	r type				
CRU	3.35 a	7.01 a	8.18 a	<b>3.64</b> a	22.48 a
Urea	<b>2.98</b> b	6.48 a	7.54 b	2.56 b	19.56 b
Potassium fertiliz	er rate (kg ha <sup>-1</sup>	)			
150	3.29 a	6.42 a	8.29 a	<b>3.19</b> a	21.19 a
300	<b>3.10</b> a	6.95 a	<b>7.46</b> b	3.35 a	20.86 a
450	<b>3.11</b> a	6.86 a	<b>7.84</b> ab	<b>2.76 b</b>	21.01 a
N×K interaction					
Control	3.08 bc	5.49 c	5.51 d	2.44 b	16.52 c
CRU×KCl150	<b>3.36</b> a	6.84 ab	8.58 a	<b>3.66</b> a	22.45 a
CRU×KCl300	<b>3.32</b> ab	7.18 a	7.56 bc	<b>4.21</b> a	22.28 a
CRU×KCl450	<b>3.38</b> a	6.99 ab	8.42 ab	<b>3.06</b> b	22.72 a
Urea×KCl150	<b>3.22 ab</b>	5.99 bc	8.00 abc	2.72 b	19.93 b
Urea×KCl300	<b>2.88</b> c	6.71 ab	7.36 c	2.49 b	19.44 b
Urea×KCl450	<b>2.84</b> c	6.73 ab	7.27 c	<b>2.46</b> b	19.30 b
Source of variance	e				
Ν	0.0009	0.0663	0.0326	<0.0001	0.0003
К	0.1024	0.2379	0.1622	0.0215	0.8638
N×K	0.1235	0.6313	0.1577	0.0307	0.7555

Note: Means followed by different lowercase letters in the same column were significantly different based on analyses with ANOVAs followed by Duncan tests (P < 0.05).

Control: no nitrogen and potassium fertilizer, CRU: polymer-coated urea, KCI: potassium chloride.

Table 8 The dry yield of Italian ryegrass under different treatments.

	First clipping	Second clipping	Third clipping	Fourth clipping	Sum
Treatment	(g plant <sup>-1</sup> )	(g plant <sup>-1</sup> )	(g plant <sup>-1</sup> )	(g plant <sup>-1</sup> )	(g plant <sup>-1</sup> )
Nitrogen fertilize	er type				
CRU	1.03 a	<b>1.74</b> a	<b>1.91</b> a	1.01 a	<b>5.68</b> a
Urea	0.94 b	<b>1.57</b> a	1.61 b	0.72 b	<b>4.85</b> b
Potassium fertiliz	zer rate (kg ha <sup>-1</sup>	)			
150	1.03 a	<b>1.74</b> a	<b>1.91</b> a	1.01 a	5.68 a
300	<b>0.94</b> b	<b>1.57</b> a	1.61 b	0.72 b	<b>4.85</b> b
450	<b>1.03</b> a	<b>1.74</b> a	<b>1.91</b> a	1.01 a	<b>5.68</b> a
N×K interaction					
Control	0.98 ab	1.44 ab	1.34 c	0.62 c	<b>4.38</b> d
CRU×KCl150	<b>1.02</b> a	<b>1.71</b> a	<b>1.95</b> a	0.92 b	5.61 ab
CRU×KCl300	<b>1.02</b> a	<b>1.75</b> a	1.79 ab	1.19 a	5.75 a
CRU×KCl450	<b>1.03</b> a	1.75 a	<b>1.98</b> a	0.92 b	5.68 ab
Urea×KCl150	0.95 b	<b>1.34 b</b>	1.71 abc	0.60 c	4.59 cd
Urea×KCl300	0.93 b	1.66 ab	1.67 abc	0.79 bc	5.06 bc
Urea×KCl450	0.96 ab	<b>1.70</b> a	1.45 bc	0.78 bc	4.88 cd
Source of variand	ce				
Ν	0.0018	0.0868	0.0171	0.001	0.0013
К	0.8091	0.1773	0.6016	0.0313	0.3924
N×K	0.9421	0.2891	0.263	0.2286	0.7365

Note: Means followed by different lowercase letters in the same column were significantly different based on analyses with ANOVAs followed by Duncan tests (P < 0.05).

Control: no nitrogen and potassium fertilizer, CRU: polymer-coated urea, KCI: potassium chloride.

Table 9 The nitrogen uptake, potassium uptake, nitrogen use efficiency (NUE) and potassium use efficiency (KUE) of Italian ryegrass under different treatments.

	N uptake	K uptake	NUE	KUE	
Treatment	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(%)	(%)	
Nitrogen fertilize	er type				
CRU	313.56 a	298.78 a	38.37 a	31.77 a 30.46 a	
Urea	298.78 b	297.22 a	33.45 b		
Potassium fertili	zer rate (kg ha <sup>-1</sup> )	)			
150	305.50 a	270.67 с	35.82 a	<b>33.58</b> a	
300	308.01 a	<b>304.00 b</b>	36.05 a	<b>34.79</b> a	
450	305.32 a	319.33 a	35.86 a	<b>24.98</b> b	
N×K interaction					
Control	202.33 с	212.61 d	-	-	

CRU×KCl150	Cl150 312.00 a 270.04 c		38.15 b	<b>33.69</b> a
CRU×KCl300	315.67 a	306.33 ab	<b>38.59</b> a	36.59 a
CRU×KCl450	313.22 b	320.09 a	38.36 ab	25.03 b
Urea×KCl150	299.12 b	271.33 с	33.49 с	<b>33.46</b> a
Urea×KCl300	300.36 b	301.67 b	33.50 с	32.99 a
Urea×KCl450	297.11 b	<b>318.64</b> a	33.35 c	24.93 b
Source of variance	e			
Ν	<0.0001	0.6245	<0.0001	0.2911
Κ	0.2975	<0.0001	0.1275	0.0002
N×K	0.7215	0.7333	0.1572	0.417

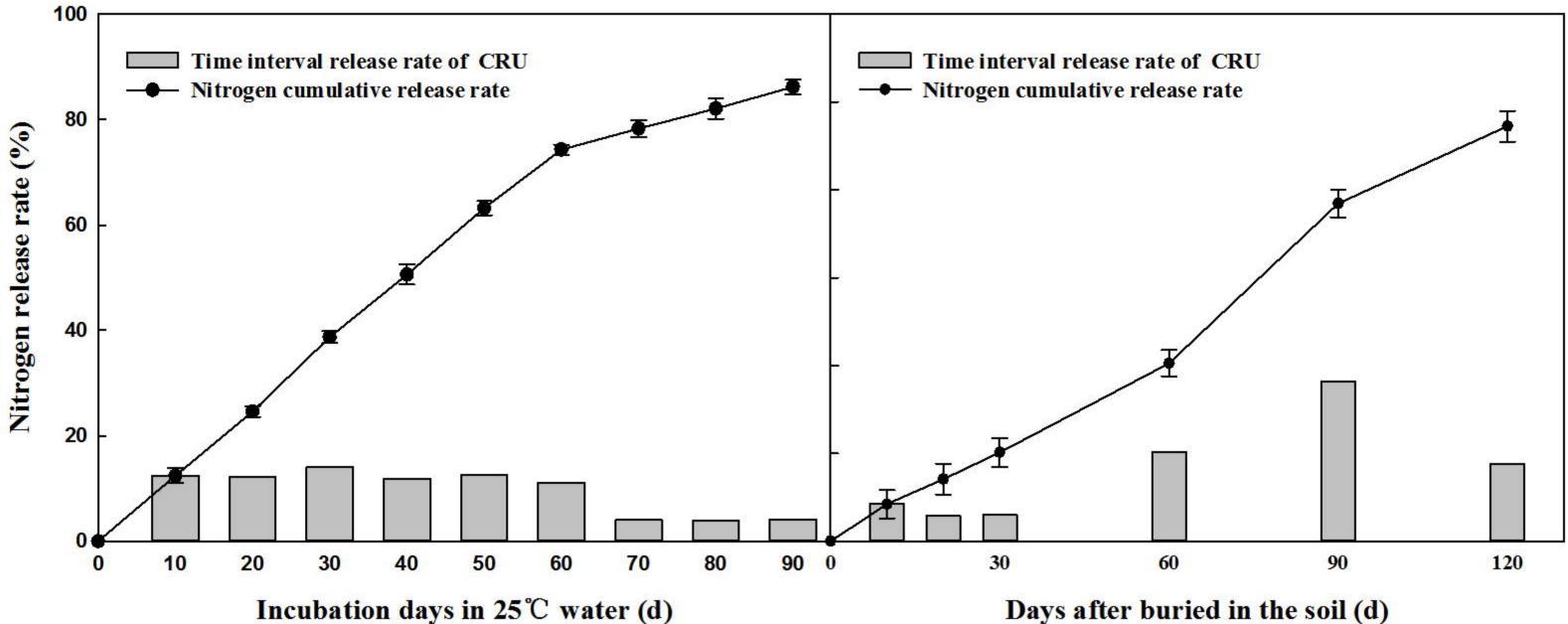
Note: Means followed by different lowercase letters in the same column were significantly different based on analyses with ANOVAs followed by Duncan tests (P < 0.05).

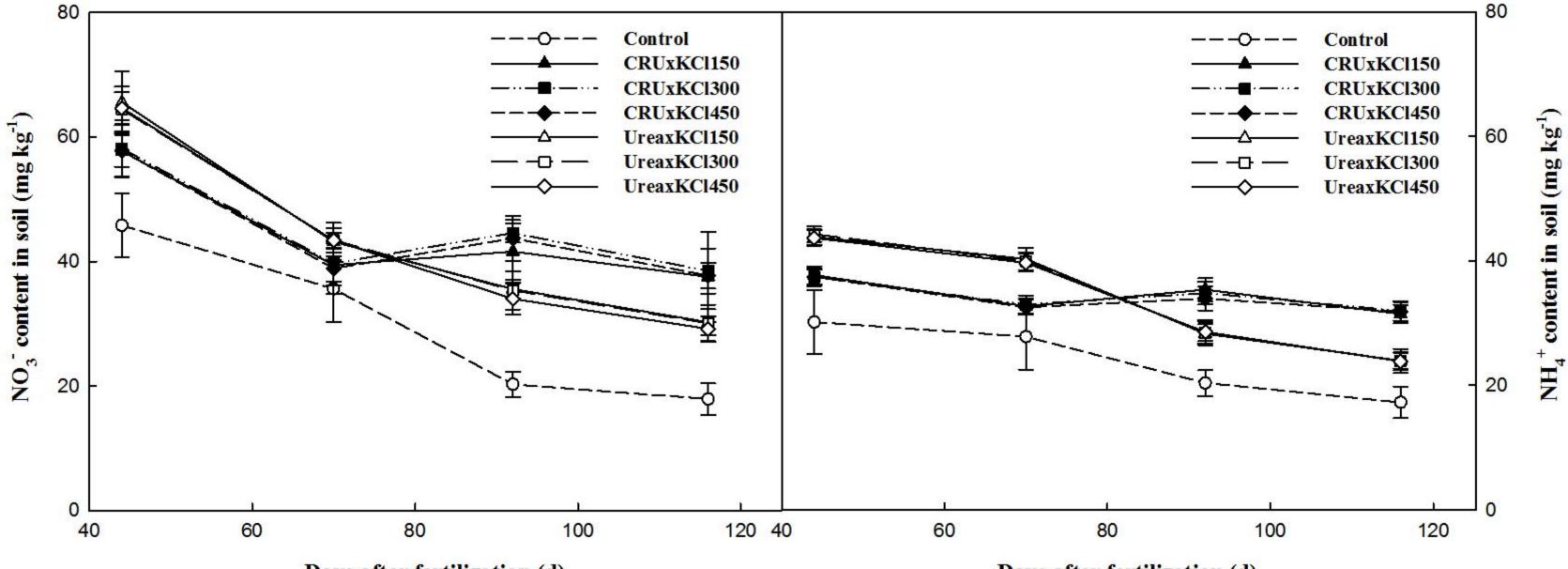
Control: no nitrogen and potassium fertilizer, CRU: polymer-coated urea, KCI: potassium chloride.

Table 10. Correlations between different forms of soil potassium and nitrogen

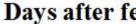
Correlation	Available K	Water	Exchangea	Non-exchan	NO <sub>3</sub> -N	NH4 <sup>+</sup> -N
coefficient		soluble K	ble K	geable K		
Available K	1					
Water soluble K	0.8733**	1				
Exchangeable K	0.6775**	0.2333*	1			
Non-exchangeable K	0.8455**	0.8248**	0.4423**	1		
NO <sub>3</sub> <sup>-</sup> N	0.7592**	0.8370**	0.2516*	0.5092**	1	
$NH_4^+-N$	0.7919**	0.7695**	0.4189**	0.5579**	0.8917**	1

Note: \* *P*<0.05; \*\* *P*<0.01.

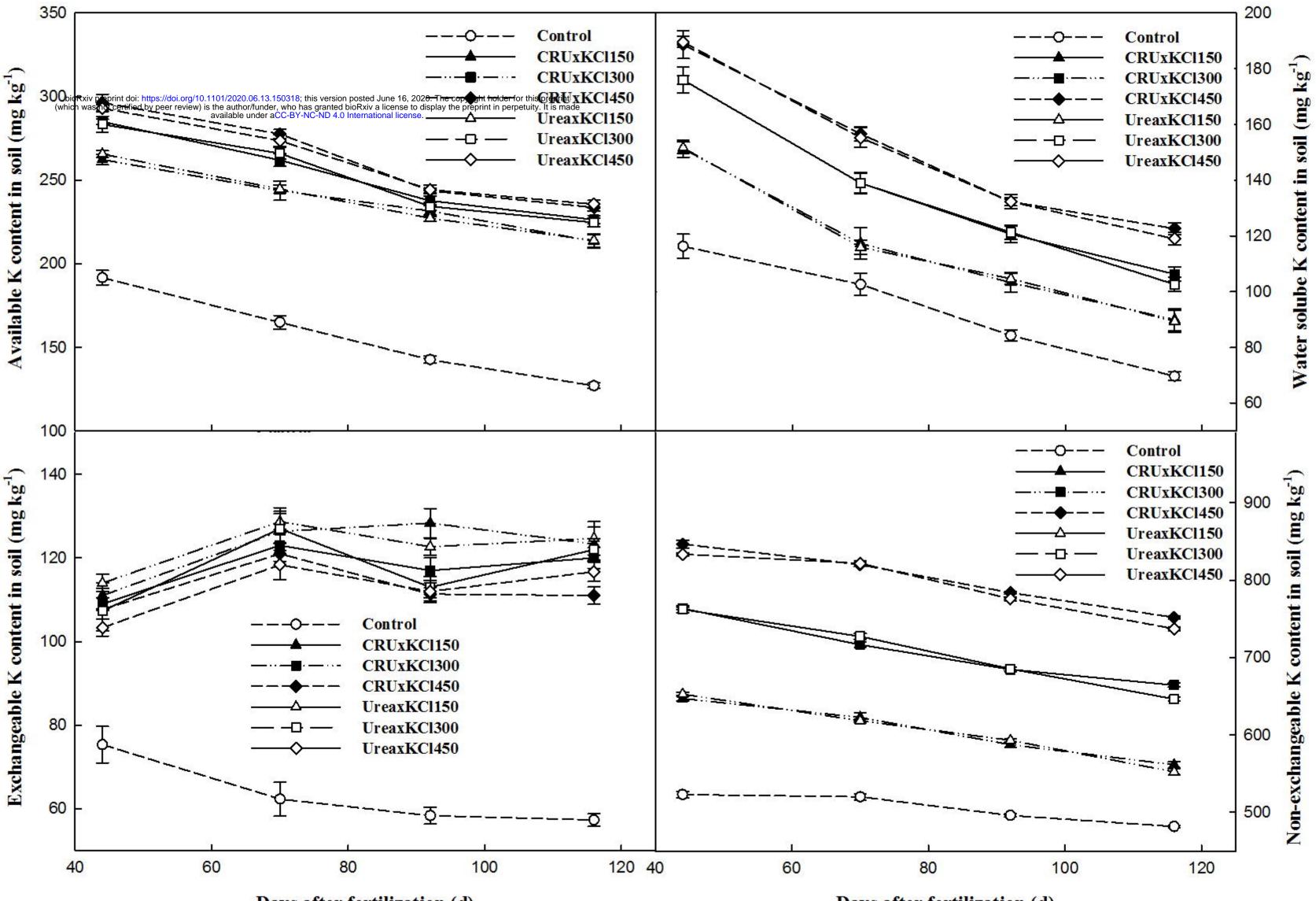




Days after fertilization (d)



Days after fertilization (d)



Days after fertilization (d)

Days after fertilization (d)