

1 **Controlled-release urea combined with potassium chloride improved the soil**
2 **fertility and growth of Italian ryegrass**

3 Jibiao Geng¹, Xiuyi Yang^{1*}, Xianqi Huo¹, Jianqiu Chen^{1,2}, Shutong Lei¹, Hui Li¹, Ying
4 Lang¹, Qianjin Liu^{1*}

5 ¹Shandong Provincial Key Laboratory of Water and Soil Conservation and Environme
6 ntal Protection, State Key Laboratory of Nutrition Resources Integrated Utilization,
7 College of Agriculture and Forestry Science/Resources and Environment,
8 Linyi University, Linyi, Shandong 276000, China

9 ²Kingenta Ecological Engineering Group Co., Ltd., Linshu, Shandong 276700, China

10 *these authors are corresponding authors

11 Corresponding authors:

12 Xiuyi Yang. Email: woshiyangxiuyi@163.com.

13 Qianjin Liu. Email: liuqianjin@163.com.

14 **Abstract**

15 **A field experiment with a split-plot design was conducted to study the effect of**
16 **nitrogen fertilizer type combined with different potassium fertilizer rates on the**
17 **soil fertility and growth of Italian ryegrass. The main plots were assigned to**
18 **controlled-release urea (CRU) and common urea, while low, moderate and high**
19 **potassium chloride (KCl) rates (150, 300 and 450 kg ha⁻¹, respectively) were**
20 **assigned to the subplots. The results showed compared with the common urea,**
21 **the CRU significantly increased the SPAD value, plant height, leaf area, and**
22 **photosynthetic index. Moreover, the dry and fresh yields of the CRU increased**
23 **by 10.9-25.3% and 11.8-17.7%, respectively. At the same time, compared with**
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**
29 **CRU combined with 300 kg ha⁻¹ KCl fertilization enhances crop growth by**

30 **improving leaf photosynthesis, soil fertility, and yield and should be**
31 **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**

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

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
54 does not have the ability to fix nitrogen, so nitrogen in the soil is a key factor in grass
55 growth and development (Woods *et al.*, 2018). The application of nitrogen fertilizer
56 can significantly improve the yield of Italian ryegrass, and the plant can absorb both
57 ammonium and nitrate nitrogen (Cavalli *et al.*, 2016). Moreover, Italian ryegrass is a
58 fast-growing forage grass with a high N requirement and, therefore, strongly relies on
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
65 and agricultural products, and ammonia and nitrogen oxides emitted to the ozone
66 layer (Ata-Ul-Karim *et al.*, 2017). In addition, it is difficult to apply fertilizer to
67 Italian ryegrass after every cutting, so decreasing the fertilizer application frequency
68 and improving the yield and quality of Italian ryegrass are the key problems to
69 address in the planting process (Martin *et al.*, 2017). An effective solution may be to
70 develop a new type of slow-release fertilizer to meet the needs of crop growth.

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

77 Potassium chloride (KCl) is commonly used in agricultural production. It has a
78 low price and high nutrient content, and the application of appropriate amounts can
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
86 amount and fertilization type can be formulated.

87 Nitrogen can improve photosynthesis and thus dry matter production (Jarvis,
88 1987). Potassium is very important for root growth and disease resistance (Snyder and
89 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×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
95 pollution by inducing in crops a high nitrogen absorption efficiency (Dong *et al.*,
96 2010). Eventually, the mutual promotion of nitrogen and potassium enhances the yield
97 and quality of crops (Yang *et al.*, 2016a). This is a feasible way to increase the
98 potassium fertilizer input and improve the nitrogen utilization efficiency.
99 Understanding the mechanism underlying the N×K interaction is vital to guide the
100 best practice of nutrient management in agricultural production.

101 Furthermore, there are few reports on the interaction application effects of CRU
102 combined with KCl on Italian ryegrass growth. Hence, the objective of this study was
103 to investigate the effects of CRU in combination with KCl on (i) soil inorganic
104 nitrogen (NO_3^- -N and NH_4^+ -N), (ii) soil potassium forms (available potassium,
105 water-soluble potassium, exchangeable potassium and nonexchangeable potassium),
106 (iii) growth and photosynthesis characteristics, and (iv) Italian ryegrass yield and
107 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 ($35^{\circ}06'N$, $118^{\circ}17'E$) in 2019, and this site has a continental climate typical of
113 temperate monsoon areas. The temperature and relative humidity were 30 ± 5 °C and
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
116 according to the USDA classification (Soil Survey Staff 1999). The basic soil
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

120 was 25 kg ha⁻¹. The fertilizers used included CRU and ordinary fertilizers. The CRU
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
127 P₂O₅ 14%), and KCl (containing K₂O 60%), which were provided by Jinzhengda
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
133 kg ha⁻¹) were the subplots, and no N or K fertilization was the control. The main plot
134 covered an area of 45 m² (3 m wide and 15 m long), and the subplot covered an area
135 of 15 m² (3 m wide and 5 m long). Each plot received a basal application of 300 kg
136 ha⁻¹ N and 200 kg ha⁻¹ P₂O₅. All fertilizers (except common urea) were applied once
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*

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

150 release rate was calculated according to the weight of the remaining fertilizer
151 particles.

152 Soil and plant samples were collected on May 18, 2019 (first clipping), June 14,
153 2019 (second clipping), July 16, 2019 (third clipping), and August 10, 2019 (fourth
154 clipping), and the physiological indexes of Italian ryegrass under different treatments
155 were observed and measured.

156 The 0-20 cm soil samples were collected by a 5-point sampling method (2
157 sampling points in the fertilizer row, 3 sampling points in the plant row). The contents
158 of NO_3^- -N and NH_4^+ -N in fresh soil ($0.01 \text{ mol L}^{-1} \text{ CaCl}_2$ extraction) were determined
159 immediately using an AA3 continuous flow analyser (Bran-Luebbe, Norderstedt,
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
163 8.5, $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ extraction, molybdenum blue colorimetry) and available
164 potassium ($1 \text{ mol L}^{-1} \text{ NH}_4\text{OAc}$ extraction, flame photometer method) contents were
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
170 photosynthetic indicators were measured from 9:00-10:00 a.m. Under sunny and
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
173 measured before the second clipping.

174 After measuring the physiological indexes of the plant, ten successive plants
175 above the roots from each subplot were clipped with scissors, and the height of the
176 stubble was the same as that of the ground. The fresh weight of the yield of each
177 treatment was recorded. Then, the clipped grass was sealed in the file bag according
178 to the treatment label, placed in the oven at $105 \text{ }^\circ\text{C}$ for 30 minutes, and dried at $65 \text{ }^\circ\text{C}$
179 for 72 hours, and then, the dry weight of the yield was recorded. Finally, the nitrogen

180 and potassium contents of the plants were analysed. The plant total nitrogen contents
181 were determined by digestion with H₂SO₄-H₂O using the micro-Kjeldahl method. The
182 potassium content of the plant was digested by H₂SO₄-H₂O and determined by a
183 flame photometer. The nitrogen and potassium uptake were calculated according to
184 the nitrogen and potassium content and dry mass weight of each plot. The nitrogen
185 use efficiency (NUE) and potassium use efficiency (KUE) were calculated (Rietra *et*
186 *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
202 between treatments of NH₄⁺-N, NO₃⁻-N, and soil K informs. A Duncan multiple range
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*

207 The release characteristics of the CRU fertilizer in the soil were released in the form
208 of “S”, reaching 80% in approximately 100 days (Fig. 1). The release period of the
209 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*

213 The contents of NO_3^- -N and NH_4^+ -N in the soil in the control treatment were the
214 lowest among all the treatments in the whole plant growing season (Fig. 2). With the
215 increase in the KCl application rate, the contents of NO_3^- -N and NH_4^+ -N in the soil
216 changed little, which was not related to the type of nitrogen application. However, at
217 the early stage of growth, the contents of NO_3^- -N and NH_4^+ -N in the urea treatment
218 were higher than those in the CRU treatment, but after the second clipping, the
219 contents of NO_3^- -N and NH_4^+ -N in the urea treatment decreased rapidly; in addition,
220 the contents of NO_3^- -N and NH_4^+ -N in the urea treatment were lower than those in the
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
234 significant difference between the different fertilization treatments at the first and
235 second clipping stages, but the advantage of the CRU treatment group was obvious; in
236 addition, the average value was higher than that of the control treatment. During the
237 mowing period of the third and fourth clippings, Italian ryegrass growth was in the
238 transition period from maturity to senescence, the plant demand for nutrients
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,

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

276 *Root morphology*

277 The nitrogen fertilizer types and potassium fertilizer rates significantly affected the
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
293 interaction effect on the yield. When clipping at the second stage, the highest dry
294 yields of the CRU×KCl300 and CRU×KCl450 treatments were 1.75 g plant⁻¹, which
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,
298 and the CRU slowly released nitrogen for plant growth. Thus, CRU was more
299 beneficial than common urea. In addition, potassium fertilizer had little influence on

300 the yield. There was no significant N×K interaction effect on the dry yield and fresh
301 yield (except at the fourth clipping).

302 *Nitrogen and potassium use efficiency*

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

309 **Discussion**

310 *Soil inorganic nitrogen and potassium form*

311 The contents of soil inorganic nitrogen (NO_3^- -N and NH_4^+ -N) in the 0-20 cm soil
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
321 NO_3^- -N and NH_4^+ -N increased significantly from the second clipping stage to the
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
326 obvious during the whole growth stage of Italian ryegrass.

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

330 available K, water-soluble K, exchangeable K and non-exchangeable K, similar to
331 other research results (Kurbah and Dixit, 2019). However, quick-acting fertilizers
332 such as KCl are easily converted into non-exchangeable K, which leads to a decrease
333 in soil potassium availability (Chen *et al.*, 2020). In total, the contents of soil available
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
337 exchangeable K and non-exchangeable K (Table 10). The water-soluble K contributed
338 the most to NO_3^- -N, and the available K had similar effects on NH_4^+ -N. In addition,
339 the contents of soil available potassium in the KCl300 and KCl450 treatments were
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,
355 leaf area and photosynthetic index, which might have led to plant senescence.

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

360 outcome may have been due to the decrease in assimilative capacity, enzyme content
361 and enzyme activity caused by the lack or excess of potassium, further leading to the
362 decrease in leaf area and photosynthesis and thus affecting photosynthesis (Lu *et al.*,
363 2017). Generally, the CRU×KCl300 treatment improved the leaf photosynthesis of
364 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×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*

376 Nitrogen and potassium are the key factors affecting the yield of Italian ryegrass.
377 Under sufficient nitrogen, the leaves of Italian ryegrass are thick green and have
378 strong growth and a high yield of fresh grass, and under nitrogen deficiency, the
379 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×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
397 yield of the CRU×KCl450 treatment was found to be the highest in the early stage,
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 al.*
404 *et 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**

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

420 nitrogen demand of Italian ryegrass during the whole growth and development period,
421 simplifying the cultivation technology. This study found that the amount of potassium
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
424 improved plant SPAD and root morphology, delayed senescence of Italian ryegrass,
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**

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

434 **Acknowledgment**

435 The authors appreciate American Journal Experts (AJE) for English language editing.

436 **Author contributions**

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

441 **Funding**

442 The present study was supported by the Shandong Provincial Natural Science
443 Foundation, China (ZR2018PD001), China Postdoctoral Science Foundation
444 (2019M652428), Project of Introducing and Cultivating Young Talent in the
445 Universities of Shandong Province (Soil Erosion Process and Ecological Regulation),
446 and Natural Science Foundation of China (31500371/31700553).

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Figure Legends

Fig. 1 Release of nitrogen from CRU.

Fig. 2 Change of the soil NO₃⁻-N and NH₄⁺-N contents.

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

Table 1 Part properties of tested soil before Italian ryegrass planting in 2019.

pH value (2.5:1)	Organic matter (g kg ⁻¹)	Total N (g kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)
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.

Treatment	First clipping (cm)	Second clipping (cm)	Third clipping (cm)	Fourth clipping (cm)
Nitrogen fertilizer 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 fertilizer rate (kg ha⁻¹)				
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 c	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	8.32 b
Urea×KCl300	16.20 a	24.43 abc	20.00 b	9.16 b
Urea×KCl450	16.07 a	25.03 ab	19.33 b	7.03 b
Source of variance				
N	0.1619	0.1735	0.0023	<0.0001
K	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.

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

Treatment	First clipping (mm²)	Second clipping (mm²)	Third clipping (mm²)	Fourth clipping (mm²)
Nitrogen fertilizer 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 fertilizer rate (kg ha⁻¹)				
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	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 variance				
N	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
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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.

Table 4 The leaf SPAD value of Italian ryegrass under different treatments.

Treatment	First clipping	Second clipping	Third clipping	Fourth clipping
Nitrogen fertilizer type				
CRU	49.76 a	54.70 a	27.49 a	17.44 a
Urea	44.37 b	57.66 b	20.92 b	10.39 b
Potassium fertilizer rate (kg ha⁻¹)				
150	45.72 b	54.83 a	24.17 a	14.33 a
300	46.47 ab	57.73 a	25.97 a	15.02 a
450	49.00 a	55.97 a	22.48 a	12.40 a
N×K interaction				
Control	45.67 c	50.93 c	25.97 ab	13.66 b
CRU×KCl150	50.00 ab	51.66 c	27.56 ab	14.86 ab
CRU×KCl300	47.66 bc	59.62 a	29.70 a	22.43 a
CRU×KCl450	51.60 a	52.74 bc	25.20 ab	15.03 ab
Urea×KCl150	41.43 d	58.02 ab	20.76 ab	13.80 b
Urea×KCl300	45.27 c	55.80 abc	22.23 ab	7.60 b
Urea×KCl450	46.40 bc	59.16 a	19.76 b	9.77 b
Source of variance				
N	0.0008	0.0956	0.002	0.0047
K	0.0746	0.3603	0.2125	0.507
N×K	0.1102	0.0436	0.8487	0.039

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.

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

Treatment	P_n ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	G_s ($\mu\text{mol mol}^{-1}$)	T_r ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	C_i ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
Nitrogen fertilizer 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 fertilizer rate (kg ha⁻¹)				
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	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 variance				
N	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, KCl: 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 ²)	Average diameter (mm)	Root volume (cm ³)	Tips	Branches
Nitrogen fertilizer type						
CRU	1480.89 a	180.23 a	0.35 a	1.61 a	14956.12 a	16076.37 a
Urea	1377.67 b	159.56 b	0.32 b	1.49 b	13947.33 b	14872.35 b
Potassium fertilizer rate (kg ha⁻¹)						
150	1393.00 c	164.50 b	0.32 b	1.51 c	14113.50 c	14989.31 c
300	1479.67 a	176.17 a	0.35 a	1.60 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 c
CRU×KCl300	1545.33 a	188.45 a	0.36 a	1.67 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	1.46 c	13705.49 d	14488.36 d
Urea×KCl300	1414.21 c	164.98 c	0.34 ab	1.52 bc	14097.34 cd	15111.23 c
Urea×KCl450	1367.03 d	159.35 cd	0.32 b	1.49 c	14039.76 cd	15017.74 c
Source of variance						
N	<0.0001	<0.0001	0.0111	<0.0001	<0.0001	<0.0001
K	<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 ⁻¹)	Second clipping (g plant ⁻¹)	Third clipping (g plant ⁻¹)	Fourth clipping (g plant ⁻¹)	Sum (g plant ⁻¹)
Nitrogen fertilizer type					
CRU	3.35 a	7.01 a	8.18 a	3.64 a	22.48 a
Urea	2.98 b	6.48 a	7.54 b	2.56 b	19.56 b
Potassium fertilizer rate (kg ha⁻¹)					
150	3.29 a	6.42 a	8.29 a	3.19 a	21.19 a
300	3.10 a	6.95 a	7.46 b	3.35 a	20.86 a
450	3.11 a	6.86 a	7.84 ab	2.76 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	3.36 a	6.84 ab	8.58 a	3.66 a	22.45 a
CRU×KCl300	3.32 ab	7.18 a	7.56 bc	4.21 a	22.28 a
CRU×KCl450	3.38 a	6.99 ab	8.42 ab	3.06 b	22.72 a
Urea×KCl150	3.22 ab	5.99 bc	8.00 abc	2.72 b	19.93 b
Urea×KCl300	2.88 c	6.71 ab	7.36 c	2.49 b	19.44 b
Urea×KCl450	2.84 c	6.73 ab	7.27 c	2.46 b	19.30 b
Source of variance					
N	0.0009	0.0663	0.0326	<0.0001	0.0003
K	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, KCl: potassium chloride.

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

Treatment	First clipping (g plant ⁻¹)	Second clipping (g plant ⁻¹)	Third clipping (g plant ⁻¹)	Fourth clipping (g plant ⁻¹)	Sum (g plant ⁻¹)
Nitrogen fertilizer type					
CRU	1.03 a	1.74 a	1.91 a	1.01 a	5.68 a
Urea	0.94 b	1.57 a	1.61 b	0.72 b	4.85 b
Potassium fertilizer rate (kg ha⁻¹)					
150	1.03 a	1.74 a	1.91 a	1.01 a	5.68 a
300	0.94 b	1.57 a	1.61 b	0.72 b	4.85 b
450	1.03 a	1.74 a	1.91 a	1.01 a	5.68 a
N×K interaction					
Control	0.98 ab	1.44 ab	1.34 c	0.62 c	4.38 d
CRU×KCl150	1.02 a	1.71 a	1.95 a	0.92 b	5.61 ab
CRU×KCl300	1.02 a	1.75 a	1.79 ab	1.19 a	5.75 a
CRU×KCl450	1.03 a	1.75 a	1.98 a	0.92 b	5.68 ab
Urea×KCl150	0.95 b	1.34 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	1.70 a	1.45 bc	0.78 bc	4.88 cd
Source of variance					
N	0.0018	0.0868	0.0171	0.001	0.0013
K	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, KCl: potassium chloride.

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

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

CRU×KCl150	312.00 a	270.04 c	38.15 b	33.69 a
CRU×KCl300	315.67 a	306.33 ab	38.59 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 c	33.49 c	33.46 a
Urea×KCl300	300.36 b	301.67 b	33.50 c	32.99 a
Urea×KCl450	297.11 b	318.64 a	33.35 c	24.93 b
Source of variance				
N	<0.0001	0.6245	<0.0001	0.2911
K	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, KCl: potassium chloride.

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

Correlation coefficient	Available K	Water soluble K	Exchangeable K	Non-exchangeable K	NO₃⁻-N	NH₄⁺-N
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		
NO₃⁻-N	0.7592**	0.8370**	0.2516*	0.5092**	1	
NH₄⁺-N	0.7919**	0.7695**	0.4189**	0.5579**	0.8917**	1

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





