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Effects of slow-release urea fertilizers on urease activity, microbial biomass, and nematode communities in an aquic brown soil

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Abstract A field experiment was carried out at the Shenyang Experimental Station of Ecology (CAS) in order to study the effects of slow-release urea fertilizers high polymer-coated urea (SRU1), SRU1 mixed with dicyandiamide DCD (SRU2), and SRU1 mixed with calcium carbide CaC_2 (SRU3) on urease activity, microbial biomass C and N, and nematode communities in an aquic brown soil during the maize growth period. The results demonstrated that the application of slow-release urea fertilizers inhibits soil urease activity and increases the soil NH_4^+-N content. Soil available N increment could promote its immobilization by microorganisms. Determination of soil microbial biomass N indicated that a combined application of coated urea and nitrification inhibitors increased the soil active N pool. The population of predators/omnivores indicated that treatment with SRU2 could provide enough soil NH_4^+-N to promote maize growth and increased the food resource for the soil fauna compared with the other treatments.

Keywords: slow-release urea, urease activity, microbial biomass, nematode community, aquic brown soil.

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Developing slow-release urea fertilizers is an important method for increasing urea nitrogen (N) use efficiency and for achieving agro-product safety, since these fertilizers may release their N in synchrony with plant growth. Furthermore, it may be possible to provide sufficient N in a single application to satisfy plant requirements, while maintaining a very low concentration of mineral N in the soil throughout the growth season[1].

Many slow-release urea forms, such as coated, chemically and bio-chemically modified, and granulated forms, have been developed and applied to a number of different plant species growing under a range of diverse environments[2—5]. All of these modifications changed the release rate of urea N from slow-release forms, but could not influence its subsequent nitrification and denitrification.

Nitrification inhibitors were used to retard the nitrification of hydrolyzed NH_4^+-N from urea in order to further promote the efficiency of slow-release urea N and to decrease leaching out of the transformed nitrate[1, 6—9]. Although the effects of slow-release urea

forms on soil mineral N content and the recovery of fertilizer N have been assessed widely in field trials [10], information on the influence of their application on soil biological activities and soil fauna is not available. Studies on the effects of varying substrate (urea) concentrations in assaying soil urease activity have shown that urea hydrolysis increases with increasing urea concentration until the amount of urea added is enough to saturate the urease with substrate [11]. Thus, soil urease activity varies with the N release rate of conventional and slow-release urea fertilizers.

Soil microbial biomass is a source and sink of soil nutrients [12], which may be influenced by the N transformation in soil systems. Xu et al. [13] have shown that the regulation of urea transformation by inhibitors can increase the soil microbial biomass N. The total free-living soil nematode population partly controls the soil microbial biomass and plays an important role in nutrient cycling[14—17]. The variation in soil microbial biomass is followed by a change in the soil nematode population. Dicyandiamide (DCD) and coated calcium carbide (CCC) were effective in inhibiting the activity of *nitrosomonas,* and thus, the nitrification of ammonium[18, 19]. Its effects on soil urease activity, microbial biomass, and nematode population should be investigated.

The field experiment described in this paper deals with the effects of slow-release urea fertilizers high polymer coated urea and its combination with the nitrification inhibitors dicyandiamide (DCD) or calcium carbide (CaC_2) on soil urease activity, microbial biomass C and N, and nematode communities in an aquic brown soil.

1 Experimental

1.1 Site

The field work was conducted at the Shenyang Experimental Station of Ecology (41°31′N, 123°22′E), Chinese Academy of Sciences, a Chinese Ecosystem Research Network (CERN) site established in 1990 in the Sujiatun District, Liaoning Province, Northeast China. The station is located in the continental temperate monsoon zone, with a dry-cold winter and a warm-wet summer. The annual mean temperature is 7.0—8.0℃. Annual precipitation averages 650—700 mm, and the annual non-frost period is 147—164 days. The soil at the study site is classified as aquic brown soil, which is suitable for growing maize, soybean, and rice.

The field site was planted with maize (*Zea mays* L.) from 1990 to 2002, and the chemical properties of the 0—20 cm soil layer before trial were pH 6.7, 20.2 g/kg organic matter, 0.69 g/kg total nitrogen, 11.2 mg P/kg, and 90.1 mg K/kg. 12 experimental plots, 162 m^2 each, were planted with a monoculture of maize in 2002. The treatments were: (i) conventional urea (CK), (ii) urea coated with high polymer (SRU1), (iii) SRU1 mixed with DCD (SUR2), and (iv) SRU1 mixed with CaC_2 (SRU3), which were arranged in random and with three replicates[17].

1.2 Soil sampling

0—20 cm soil samples were collected from three plots of each treatment on 12 May (seedling stage), 19 June (jointing stage), 8 July (male tetrad stage), 28 August (filling stage), and 26 September (ripening stage) 2002, respectively. Each soil sample comprised of 5 cores (5 cm diameter). Soils collected for chemical and biological assays were sieved through a 2-mm mesh screen, and half of them were dried at room temperature for those analyses requiring air-dried soil [20]. Both moist and dry subsamples were kept at 4℃ before the analysis.

1.3 Chemical measurements

Soil inorganic NO_3^- and NH_4^+ were determined by extraction with 2 mol/L KCl, steam distillation, and titration[21]. Total N was determined by Kjeldahl digestion, followed by NaOH distillation, and measured by titration with 25 mmol/L H_2SO_4 in boric acid indicator[22]. Total C was analyzed by dry combustion using a TOC 5000 (Shimadzu, Kyoto) analyzer. pH was determined using a glass electrode on a pH meter with a soil:water ratio of 1:2[20].

1.4 Biological measurements

Soil microbial biomass C (MBC) and N (MBN)

were measured using the fumigation-extraction method[23,24]. Fumigated and non-fumigated soils were extracted with 100 mL 0.5 mol/L K_2SO_4 for 30 min, and the extracts were analyzed for microbial biomass C and N by a TOC 5050 (Shimadzu, Kyoto) analyzer. The C obtained from fumigated samples minus that from non-fumigated samples was taken to represent the microbial-C flush, and converted to microbial biomass C using the relationship: microbial C=1/0.38 C flush. Microbial biomass N was estimated by the relationship: microbial N=1/0.45 N flush[25]. Soil urease activity was determined using urea as substrate, incubating for 5 h at 37℃ and measuring the remaining urea using a colorimetric method[26]. Nematodes were extracted from 100 g soil (fresh weight) of each sample by using sugar flotation and centrifugation[16], and the nematode populations were expressed per 100 g dry weight soil[15]. All extracted nematodes in each sample were counted and identified, to genus level if possible, using an inverted compound microscope. The classification of trophic groups was assigned to: (i) bacterivores (BF); (ii) fungivores (FF); (iii) plant-parasites (PP); and (iv) omnivore-predators (OP), based on the known feeding habitats or stoma and esophageal morphology[15, 27].

1.5 Statistical analysis

All the data across the 5 sampling dates were subjected to the statistical analysis of variance (ANOVA) using the SAS statistical package. Differences with $p<0.05$ were considered significant.

2 Soil urease activity

Fig. 1 shows that the soil urease activity in all treatments of slow-release urea fertilizers was lower at the seedling stage compared with CK treatment, but demonstrated an obvious increase during the jointing stage. Thereafter, no significant differences were found between treatments. Significant positive correlation was found between soil urease activity and soil NH_4^+-N content (table 1).

3 Soil microbial biomass C and N

The soils following SRU1 and SRU3 treatments had a somewhat higher microbial biomass C content than following the CK treatment, and the SRU1 treatment resulted in the highest value, whereas the SRU2 treatment resulted in a lower soil microbial biomass C content (fig. 2).

The soil microbial biomass N content following treatments with slow-release urea fertilizers was lower during the seedling stage than following the CK treatment, but was higher thereafter. The SRU3 treatment resulted in the highest value of soil microbial biomass N content (fig. 3).

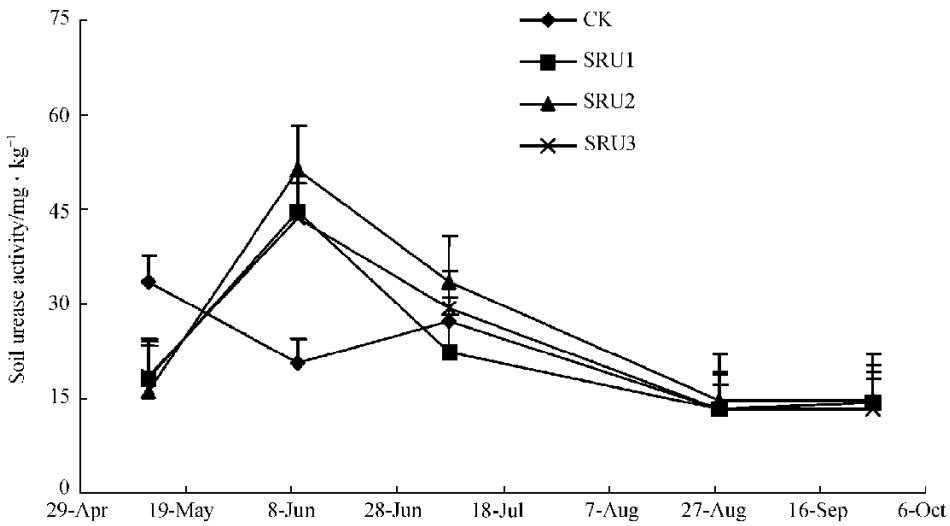

Fig. 1. The effect of slow-release urea fertilizers on the soil urease activity.

Table 1 Correlations between soil chemical and biological properties

	A	B	C	D	E	F	G	H	I	J	K	L	M
Total C (A)	1.000												
Total N (B)	**0.386***	1.000											
C/N (C)	**–0.829**	0.144	1.000										
NO_3^- (D)	**0.255**	–0.166	**–0.407**	1.000									
NH_4^+ (E)	0.155	**–0.267**	**–0.341**	**0.810**	1.000								
Urease (F)	0.185	–0.249	**–0.383**	**0.945**	**0.884**	1.000							
MBC (G)	**0.274**	–0.192	**–0.337**	**0.441**	**0.443**	**0.405**	1.000						
MBN (H)	0.195	**–0.258**	**–0.395**	**0.776**	**0.638**	**0.744**	**0.509**	1.000					
TNEM** (I)	0.026	**0.370**	0.195	**–0.290**	**–0.273**	**–0.403**	–0.206	**–0.389**	1.000				
BF (J)	0.097	**0.291**	0.079	–0.230	–0.241	**–0.328**	0.045	**–0.372**	**0.656**	1.000			
FF (K)	0.001	0.030	–0.009	**0.274**	**0.327**	0.197	0.023	0.171	**0.348**	**0.273**	1.000		
PP (L)	–0.008	**0.335**	0.214	**–0.314**	**–0.298**	**–0.398**	**–0.272**	**–0.360**	**0.924**	**0.352**	0.142	1.000	
OP (M)	–0.005	0.029	–0.011	–0.007	-0.004	–0.056	–0.230	0.015	–0.133	–0.091	0.067	–0.195	1.000

* Bold data indicate significant correlations between the two items (p<0.05). ** TNEM, total nematode.

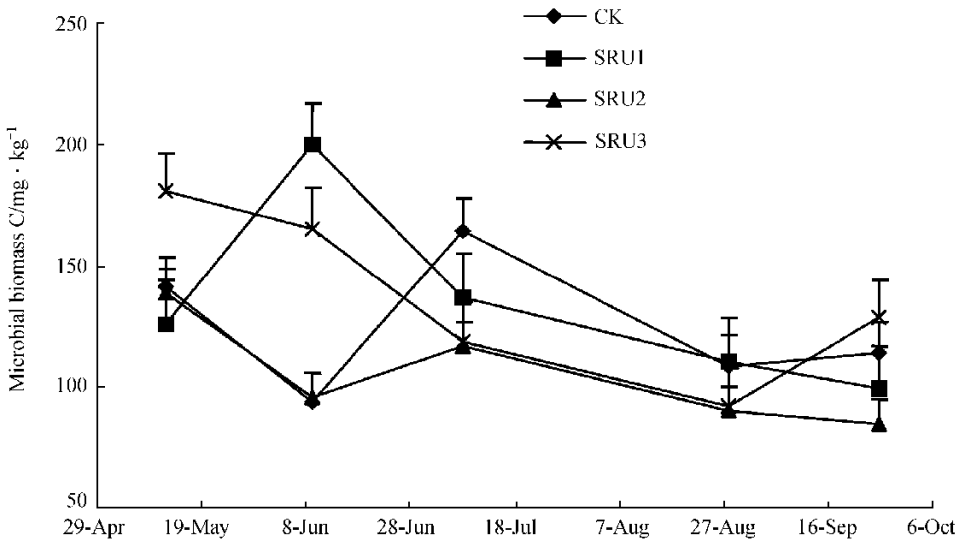

Fig. 2. The effect of slow-release urea fertilizers on soil microbial biomass C.

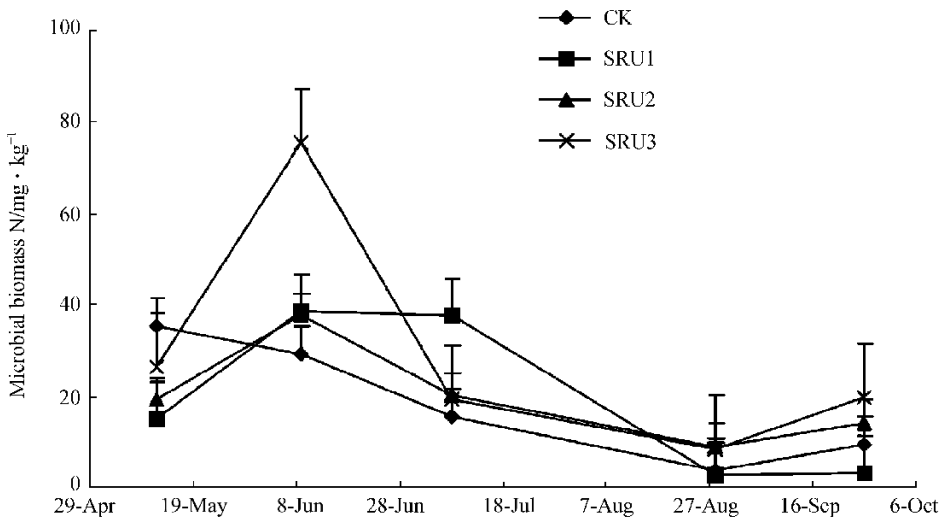

Fig. 3. The effect of slow-release urea fertilizers on soil microbial biomass N.

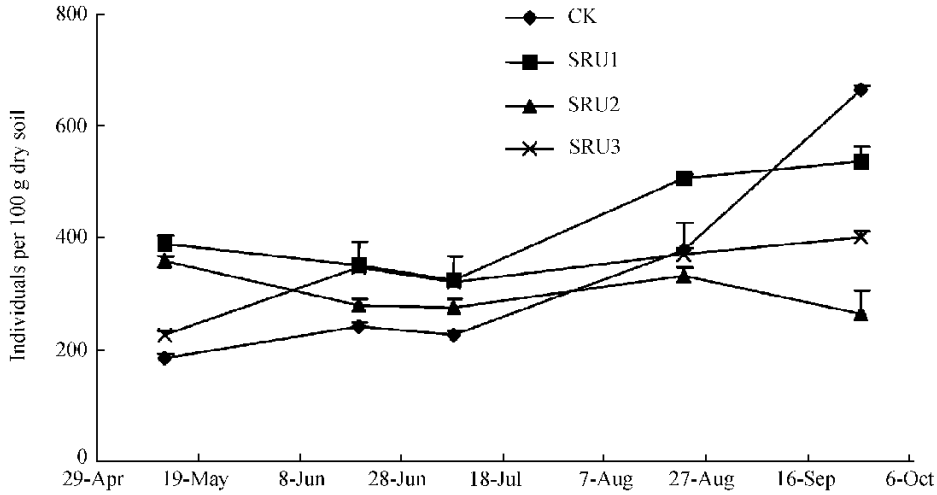

Fig. 4. The effect of slow-release urea fertilizers on individuals of total nematodes.

4 Soil nematode communities

13 families and 22 genera were observed in the nematode suspensions. *Hellicotylenchus* was found to be the dominant genus. The number of total nematode individuals was higher following the SRUs treatment than following the CK treatments during the earlier growth stages, but exhibited an inverse trend during the ripening stage (fig. 4). Following the SRU2 treatment, the total number of nematodes, bacterivores and fungivores was higher than in the control during the early growth stage, while that of omnivore-predators was higher during the entire period. Following the SRU3 treatment, the number of fungivores and plant parasites was higher than in the control during the middle growth stage (table 2).

Significant differences (p<0.01) were observed in the number of bacterivores, fungivores and plant para sites between SRU treatments and the control during the study period, and the number of omnivore-predators was significantly higher following the SRU2 treatment than following the CK treatment (p<0.01). Significant positive correlations were observed between total nematodes, bacterivores, plant parasites and total nitrogen content (table 1).

Table 2 Nematode community structure in the 0—20 cm soil layer during the maize growth season

Trophic group	CKU	SRU1	SRU2	SRU3
Seedling				
BF	30±7	130±14	130±18	79±8
FF	19±3	37±1	45±0	19±5
PP	135±12	220±18	174±12	128±11
OP	0±0	0±0	11±0	0±0
Jointing				
BF	55±16	119±16	79±5	51±4
FF	37±12	64±15	53±7	36±16
PP	148±32	167±16	138±15	261±27
OP	0±0	0±0	9±1	0±0
Male tetrad				
BF	101±27	117±14	39±6	129±24
FF	0±0	25±5	21±3	32±10
PP	126±21	173±50	205±17	162±41
OP	0±0	11±2	9±1	0±0
Filling				
BF	121±24	101±29	77±14	77±18
FF	24±4	50±16	25±8	33±7
PP	233±28	354±38	218±18	261±15
OP	0±0	0±0	11±1	0±0
Ripening				
BF	192±8	105±29	80±6	97±9
FF	44±2	18±1	28±12	40±2
PP	429±10	411±35	147±45	251±3
OP	0±0	0±0	9±1	13±1

5 Discussion

The application of slow-release urea fertilizers in the field may inhibit soil urease activity during the earlier growth stages of maize by regulating the rate of urea release from its coated form[11], which results in a

higher soil NH_4^+-N content. This was confirmed by the significant positive relationship between soil urease activity and soil NH_4^+-N content (table 1). Bremner and Mulvaney (1978) indicated that nitrification inhibitors had little effect on soil urease activity[11]. The DCD and CaC_2 used in this study showed the same result. Soil available N increment could promote its immobilization by microorganisms[28]. CaC_2 has been used less often as a nitrification inhibitor [29]. However, coated CaC_2 was effective when applied in upland and flooded soil[1, 19]. In the present study, the CaC_2 in SRU3 was mixed with high polymer coated urea, and its effectiveness in inhibiting nitrification was higher than the effectiveness of DCD, for the lower nitrification rate during the entire growth period of maize.

In the present study, the SRU2 and SRU3 treatments had a higher amount of soil microbial biomass N than the SRU1 treatment, with the SRU3 treatment exhibiting the greatest effect, indicating that a combined application of coated urea and nitrification inhibitors can increase the soil active N pool. The reason that the SRU3 treatment resulted in the highest amount of soil microbial biomass N may be that the soil pH value during this treatment was near the optimal pH for microorganism growth. The optimum pH for microorganism growth has been shown to be around 7[30], while the pH of the test soil is 6.7. The applied CaC_2 may react with soil water [6, 19] and lead to an increase in the pH to a value near 7, thus influencing the amount of soil microbial biomass N.

The total free-living soil nematode population, which partly controls the microbial biomass through grazing on it[31], plays an important role in nutrient cycling by promoting N mineralization[14]. A positive correlation (r>0.291, table 1) was found between the number of bacterivores and the total nitrogen content. Bacterivores can release appreciable N by grazing on bacteria, and thus provide more available N for plant utilization and growth[33]. Since N is a dominant factor that limits plant growth, the SRU2 treatment can provide enough soil NH_4^+-N to promote the growth of maize, and provide enough food resource for soil biota. Because the omnivore-predators are the resource limitation[33], they exhibited an increasing trend and limited the numbers of plant-parasites. Nutrient turnover may be accelerated through the faunal-microbial interactions, which will favor the growth of maize.

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