Fate of applied urea ^{15}N in a soil-maize system as affected by urease inhibitor and nitrification inhibitor

L. Zhang[1,2], Z. Wu[1], Y. Jiang[1], L. Chen[1], Y. Song[1], L. Wang[3], J. Xie[3], X. Ma[4]

[1]*Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, P.R. China*
[2]*Graduate School of the Chinese Academy of Sciences, Beijing, P.R. China*
[3]*Agricultural Environment and Resources Research Center, Jilin Academy of Agricultural Sciences, Changchun, P.R. China*
[4]*Institute of Heilongjiang Academy of Agricultural Sciences, Harbin, P.R. China*

ABSTRACT

A micro-plot field experiment with reduced urea ^{15}N application was conducted to study the effects of urease inhibitor NBPT (N-(*n*-butyl) thiophosphoric triamide) and nitrification inhibitor DMPP(3,4-dimethyl-1H-pyrazolium dihydrogen) on the fate of applied urea ^{15}N; it aimed to find an efficient way to reduce the urea N application rate while improving the agronomic and environmental benefits. Five treatments were installed, i.e., 180 kg N/ha (N_1, conventional application rate), 126 kg N/ha (N_2, reduced to 70% conventional application rate), N_2 + NBPT, N_2 + DMPP, and N_2 + NBPT + DMPP. Compared with treatment N_1, all the other treatments had a significantly higher total ^{15}N recovery by both soil and plant ($P < 0.05$ 48.20, 41.39, 37.69, 38.85 and 34.83% soil recovery for N_2 + NBPT + DMPP, N_2 + DMPP, N_2 + NBPT, N_2 and N_1 treatment, respectively; and 42.68, 40.86, 40.25, 37.18 and 36.30% plant recovery for N_2 + NBPT + DMPP, N_2 + DMPP, N_2 + NBPT, N_2, and N_1 treatment, respectively). In the plant ^{15}N recovery, the ^{15}N absorbed in grain/stem was highest in treatment N_2 + NBPT + DMPP. The maize biomass and the maize yield had a slight increase in treatment N_2 + NBPT + DMPP, compared with those in treatment N_1. In sum, for the maize production in study area, N_2 + NBPT + DMPP application method would be a feasible way to ensure the normal maize yield while improving yield quality, saving urea fertilizer, and protecting the environment.

Keywords: nitrogen; fertilizer; N losses; N use efficiency

Agroecosystem relies on the N inputs from chemical and organic N fertilizers to sustain its productivity. However, N is a highly mobile element, which makes its efficient use and management a challenging task, especially for the over- N fertilized agricultural systems where significant N losses can occur via NO_3^- leaching, NH_4^+ runoff, and N gaseous emissions (Zaman et al. 2009). These N losses bring threat to the economical implications and environmental quality worldwide (Boyer et al. 2002, Ledgard et al. 2008, Vitousek et al. 2009, Zhang et al. 2009).

Various management strategies were adopted to improve the fertilizer N use efficiency and mitigate its losses from intensively cultivated lands, among which, moderately reducing fertilizer N application rate is a commonly adopted countermeasure (Chen et al. 2006, Zhao et al. 2006, Fan et al. 2007, Ju et al. 2007, 2009). Current researches usually combine less fertilizer application with multiple amendment or with better water management in order to avoid crop yield decrease (Fan et al. 2007, Ju et al. 2007, 2009). It must be indicated that these cooperative methods usually need extra labor. Amending with urease/nitrification inhibitors was also proved as an effective means to improve the fertilizer N use efficiency and mitigate its losses. (Chen et al. 1998, Xu et al. 2001, 2002, Giller et al. 2004, Yu et al. 2007, Ledgard et al. 2008, Yu et al. 2008, Zaman et al. 2008, 2009). Lots of studies

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focused on the agronomical, economical, and environmental effects under inhibitors amendment (Chen et al. 1998, Xu et al. 2001, 2002, Yu et al. 2007). However, few studies have been done on fate of N in the plant-soil system under reduced N application plus inhibitors amendment. Of the inhibitors nowadays used, N-(*n*-butyl) thiophosphoric triamide (NBPT) is an available urease inhibitor. Applied into soil, it can be quickly converted into its oxygen analogue N-(*n*-butyl) phosphoric triamide (NPBTO), which forms a tridentate ligand with soil urease (Manunza et al. 1999), retarding urea N hydrolysis. 3,4-dimethyl-1H-pyrazolium dihydrogen phosphate (DMPP) is an effective nitrification inhibitor, which can inhibit the activity of nitrifying bacteria responsible for the oxidation of NH_4^+ to NO_2^-, and thereby, reduce the NO_3^- leaching and N_2O emission (Abbasi and Adams 2000, Cameron et al. 2005, Di et al. 2007, Yu et al. 2008).

In China, the consumption of chemical fertilizer-N increased rapidly since the early 1970s from 2.865 Tg in 1970 to 24.8 Tg in 1998, which accounts for ca. 30% of the world total (China Agriculture Yearbook, 1980–1999). It was estimated that the apparent recovery for agricultural production in China might be 30–35% (Zhu and Chen 2002), which causes unnecessarily high economic input and severe environmental problem.

In northeast China, the farmers often apply up to 180 kg N/ha (according to a survey of 100 farmers) to keep a high maize yield on chernozem, one of the main agricultural soils (about 20.2% of agricultural land, Wang et al. 2009) in this region. Under such a high N application rate, the use efficiency of applied N is relatively low (approximately 35%). So, in this paper, a micro-plot field experiment was conducted in Gongzhuling City of Jilin Province, Northeast China to investigate the effects of reduced N application supplemented with NBPT and/or DMPP amendment on the fate of applied ^{15}N in a chernozem-maize system, aimed to approach an efficient way to reduce the urea N application rate while improving the agronomic and environmental benefits in Northeast China.

MATERIALS AND METHODS

Field site. The field site is located in Gongzhuling City, Jilin province (123°26'17''E, 41°44'51''N). The annual temperature is 4.5°C, and the annual precipitation is 450–650 mm. The soil is chernozem (USDA), with its basic characteristics listed in Table 1.

Field experiment and lab analysis. Chernozem (USDA) was selected as test soil (Table 1), and maize cultivar Zhengdan 958 was chosen as test crop. Five treatments were installed, i.e., conventional N application rate (180 kg N/ha, N_1), reduced N application rate (126 kg N/ha, N_2), reduced N application rate plus NBPT (N_2 + NBPT), reduced N application rate plus DMPP (N_2 + DMPP), and reduced N application rate plus NBPT + DMPP (N_2 + NBPT + DMPP). Each treatment had three replicates. The micro plots (1.26 × 0.80 m) were enclosed with iron panel, which was inserted into soil 60 cm deep and the upper edge was 5 cm above the soil surface. Before sowing (May 2, 2008), each plot was amended with 28 g CaH_2PO_4 (24 kg P_2O_5/ha) and 20 g KCl (34 kg K_2O/ha) as basal dressing. The labeled urea-^{15}N (10.12 atom % excess) supplied by the China Shanghai Chemical Institute was also applied as basal fertilizer. Both NBPT and DMPP supplied by the J & K CHEMICAL LIMITED were mixed with urea-^{15}N at a rate of 1% (w/w). Urea was mixed with inhibitor and then mixed with P and K fertilizer. Surface soil (0–15 cm) was dug up, then 10 kg surface soil was mixed with fertilizer and spread into micro plot uniformly. Then all the surface soil left was put back to the plot. After watering, seeds were sown on May 2, 2008.

Maize in the micro-plots was sampled at physiological yield maturity (September 28, 2008). Three samples were taken from each plot and then partitioned into the organs (root, stem and grain) to determine plant dry matter yield and total N uptake. Plant material was ground to < 0.25-mm sieve before analyses for total N (Kjeldahl method) and ^{15}N abundance (Mat-251 mass spectrometer, Finnigan, Germany).

Table 1. Main physical and chemical properties of test soil

Total N	Total P	SOC	Available N	Olsen-P	pH	CEC (cmol/kg)	Sand	Silt	Clay	Bulk density (g/cm^3)
(g/kg)			(mg/kg)				(g/kg)			
1.35	0.49	14.87	25.87	14.8	6.2	22.43	110	400	580	1.13

SOC – soil organic carbon; available N – sum of soil NH_4^+-N; (NO_3^- + NO_2^-)-N; pH, soil:water = 1:2.5; CEC – cation exchange capacity

Soil (0–60 cm) samples were taken from the micro-plots after each harvest using a steel corer of 20-mm diameter. From each micro-plot, two 60 cm deep soil cores were obtained and partitioned into 20-cm segments, with segments of the same depth being composited. Three samples were taken from each plot.

Fresh soil samples were sieved with 5 mm mesh. An aliquot of 30 g soil was extracted with 100 ml 2.0M KCl on a rotary shaker (175 r.p.m.) at 25°C for 30 min, filtered, and kept in freezer (4°C) before analysis. Soil NH_4^+-N and (NO_3^- + NO_2^-)-N concentrations were determined by AAIII Continuous Flow Analyzer. For the measurement of ^{15}N enrichment, the liberated NH_3 was absorbed in 10mM H_2SO_4, the $(NH_4)_2SO_4$ solution was concentrated up to 0.25 mg N/ml, and the $^{15}N/^{14}N$ ratio was measured with JAS CO-150 ^{15}N Analyzer (Axmann 1990).

Soil total N concentration was determined by Kjeldahl digestion (Keeney and Nelson 1982). 1 g soil (sieved with 0.25 mm mesh) was digested with K_2SO_4 + $CuSO_4$ + Se (10:1:0.1) and H_2SO_4 for about 8 h. An aliquot of the digest containing 1 mg N was distilled into 10mM H_2SO_4, and the $^{15}N/^{14}N$ was measured as described before.

Plant samples were oven-dried at 70°C, and their N concentration was determined by Kjeldahl digestion (Keeney and Nelson 1982). 0.3 g plant (ground < 0.25 mm) was digested with K_2SO_4 + $CuSO_4$ + Se (10:1:0.1) and H_2SO_4 for about 4 h. An aliquot of the digest containing 1 mg N was distilled into 10mM H_2SO_4, and the $^{15}N/^{14}N$ was measured as described before.

The double-distillation procedure of Pruden et al. (1985) was used to minimize ^{15}N memory effect, and each ^{15}N analysis was tri-replicated.

Soil organic C and total N were determined by elemental analyzer (Elementar Analysensysteme Vario EL III, Germany). Soil available N was analyzed colorimetrically using a continuous flow autoanalyzer (AutoAnalyzer 3, BRAN + LUEBBE, Germany). Soil pH was measured in a 1:2.5 soil: water suspension. Soil Olsen-P was extracted with 0.5M $NaHCO_3$ on a reciprocal shaker for 30 min, and the P concentration in the filtrate was determined colorimetrically (880 nm) by using the procedure of Olson and Sommers (1982). Soil total phosphorus was determined by the method of Keeney and Nelson (1982). Soil CEC was determined according to Sumner and Miller (1996). Soil bulk density was measured by using the volumetric ring method (Blake 1965). Soil particle size distribution was determined by pipette method.

Calculation and statistical analysis. All data were computed on oven-dried weight basis. The urea-^{15}N recovery by soil and plant was calculated based on the natural ^{15}N enrichment of relative samples from unfertilized N plot. The difference between soil total ^{15}N and mineral ^{15}N (NH_4^+-^{15}N and NO_3^--^{15}N) was considered as soil organic ^{15}N. Significance test was analyzed by the Duncan's multiple range test using SPSS 11.5 software for Windows, with a confidence interval of 95%.

RESULTS AND DISCUSSION

Soil ^{15}N recovery. Compared to the N_1 treatment, soil total ^{15}N recovery in the other four treatments was significantly higher (*P* < 0.05 48.20, 41.39, 37.69, 38.85 and 34.83% for N_2 + NBPT + DMPP, N_2 + DMPP, N_2 + NBPT, N_2 and N_1 treatments, respectively). In addition, soil total ^{15}N recovery in the N_2 + NBPT + DMPP treatment was greater than the other three N reduction treatments (*P* < 0.05). The N_2 + DMPP and N_2 + NBPT + DMPP treatments significantly enhanced NH_4^+-^{15}N recovery (*P* < 0.05), but N_2 and N_2 + NBPT did not affect it. The (NO_3^- + NO_2^-)-^{15}N recovery did not change under reduced N application and reduced N application with inhibitor(s) treatments. Organic ^{15}N recovery was not affected by the N_2 treatment. In contrast, organic ^{15}N recovery was by 54.3, 208.5, and 316.5% higher under the N_2 + NBPT, N_2 + DMPP, and N_2 + NBPT + DMPP treatments, respectively (Figure 1).

Plant ^{15}N recovery. No significant differences in root ^{15}N recovery were observed among the treatments (*P* > 0.05) (Figure 2a). In comparison with N_1 treatment, ^{15}N recovery in stem did not change under treatments of N_2, N_2 + NBPT, N_2 + DMPP, but significantly decreased by 21.26% under the N_2 + NBPT + DMPP treatment. The N_2, N_2 + NBPT and N_2 + DMPP treatments did not affect ^{15}N recovery in grain and whole plant while the N_2 + NBPT + DMPP treatment significantly enhanced them by 31.64 and 17.57%, respectively. ^{15}N recovery in grain accounted for the majority of ^{15}N recovery in plants (Figure 2b). Either the N_2 or N_2 + DMPP affected the proportion of root ^{15}N recovery in the whole plants. However, the N_2 + NBPT and N_2 + NBPT + DMPP treatments caused significant reductions in the proportion of root ^{15}N recovery (Figure 2b). Compared to the N_1 treatment, all the other four N reduction treatments significantly reduced the proportion of stem ^{15}N recovery. Compared with N_1 treatment, the proportion of grain ^{15}N recovery in the other four treatments was significantly higher (P < 0.05). In addition, the proportion of stem ^{15}N recovery under the N_2 + NBPT + DMPP treatment was also greater than the other three N reduction treatments. Moreover, all the four N reduction treatments stimulated the ^{15}N allocation ratio of grain/stem (P < 0.05) (Figure 2b).

Urea ^{15}N use efficiency and maize yield. In comparison with the N_1 treatment, the urea ^{15}N use efficiency increased significantly under the N_2 + NBPT + DMPP treatment only (17.57%). Maize biomass was reduced by 5.26% under the N_2 treatment, and did not change under the N_2 + NBPT, N_2 + DMPP or N_2 + NBPT + DMPP treatment. The N_2 treatment caused a significant decrease (6.08%) in grain yield. No changes in grain yield were observed under the other three N reduction treatments (Figure 3).

An overall scene about the fate of applied urea ^{15}N in test chernozem-maize system (Figure 4) showed that 71–86% of applied ^{15}N was absorbed by plant and remained in soil, and 13–28% was lost via different ways. In comparison with the N_1 treatment, the proportions of ^{15}N recovery in plants and soil as well as total recovery were significantly greater (26.90, 21.43 and 22.14% for plant, soil and total recovery, respectively) under the N_2 + NBPT + DMPP treatment only. In contrast, the loss of applied ^{15}N was significantly

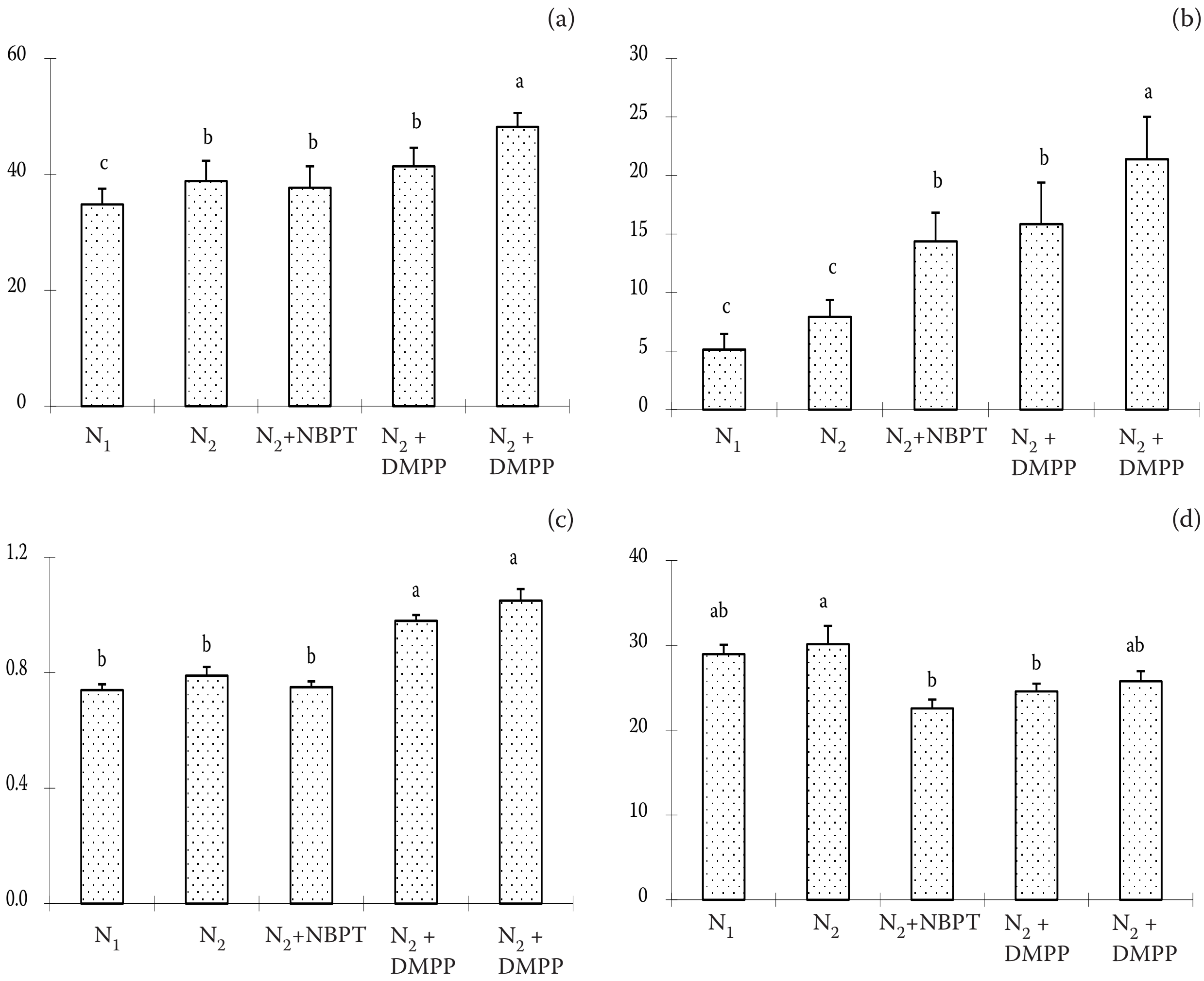


Figure 1. (a) Total 15N recovery (% of applied 15N); (b) organic 15N recovery (% of applied 15N); (c) NH_4^+-15N recovery (% of applied 15N); (d) NO_3^- + NO_2^+-15N recovery (% of applied 15N) Mean value ± SD (n = 3). Different letters among treatments indicate statistically different values (P < 0.05). Organic 15N, the difference between soil total 15N and mineral 15N (NH_4^+-15N and NO_3^--15N + NO_2^--15N). N_1, 180kg N/ha; N_2, 126 kg N/ha; N_2 + NBPT, 126 kg N/ha + urease inhibitor(N-(n-butyl) thiophosphoric triamide); N_2 + DMPP, 126 kg N/ha + nitrification inhibition(3,4-dimethyl-1H-pyrazolium dihydrogen phosphate); N_2 + NBPT + DMPP, 126 kg N/ha + urease inhibitor (N-(n-butyl) thiophosphoric triamide) + nitrification inhibition (3,4-dimethyl-1H-pyrazolium dihydrogen phosphate). Vertical bars denote standard deviation of the mean (average of 3 15N field experiments)

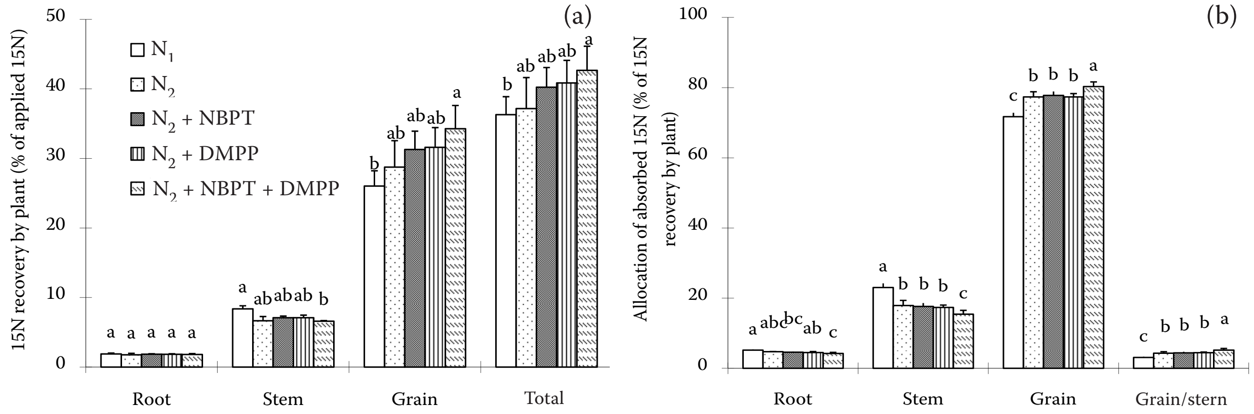
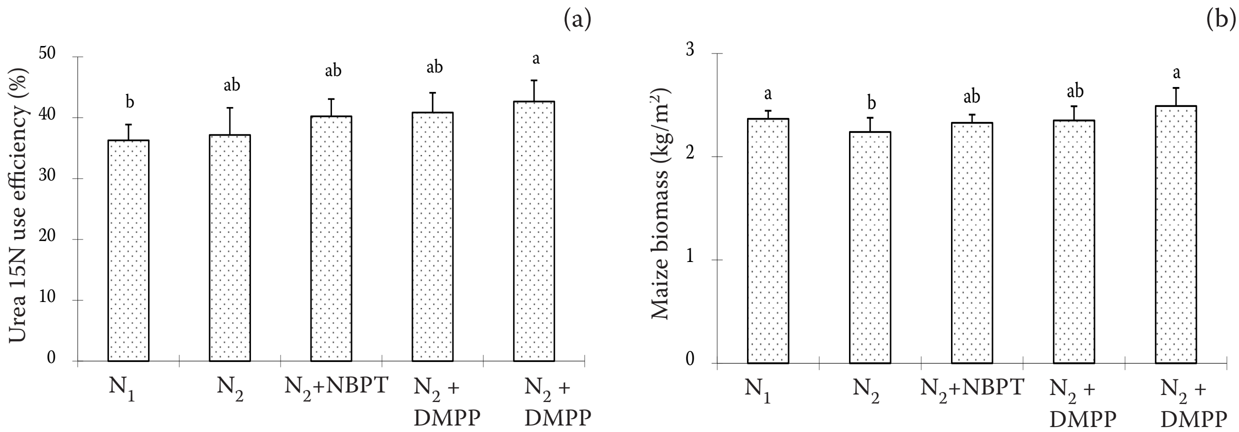


Figure 2. (a) 15N recovery by plant (% of applied 15N); (b) Allocation of absorbed 15N (% of 15N recovery by plant). Mean ± SD (n = 3). Different letters within treatments indicate significant differences (P < 0.05). For abbreviations see Figure 1

decreased (54.55%) by the N_2 + NBPT + DMPP treatment only.

In this study, the highest soil NH_4^+-^{15}N recovery observed in treatments with DMPP applied and the highest soil organic ^{15}N recovery in the treatment with combined application of DMPP and NBPT suggested the mitigation effect of DMPP on urea-^{15}N losses via nitrification-denitrification and the synergistic effect of DMPP and NBPT on the immobilization of remained soil ^{15}N. Similar results were obtained in the related researches with other soil types. Application of dicyandiamide (DCD) increased soil NH_4^+-N (Vilsmeier 1991, Zaman et al. 2009). Hydroquinone (HQ) enhanced the N immobilization of fertilizer ^{15}N by 5–30% in an alkaline soil (Wang et al. 1991). The combination of HQ and DCD increased the organic N in a brown soil. Figure 1 showed lower soil NO_3-^{15}N recovery by soil in the treatments with single and combined inhibitors applied. DMPP slowed down nitrification and therefore exhibited comparatively lower amounts of NO_3-^{15}N than N_1



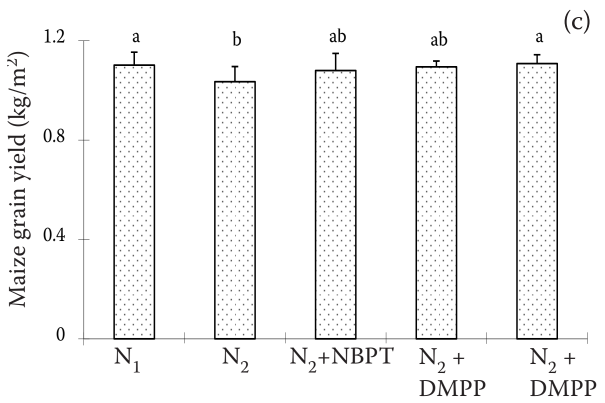


Figure 3. Percentage of plant recovery, soil recovery, total recovery and loss of applied 15N. Mean ± SD (n = 3). Different letters within treatments indicate significant differences (P < 0.05). For abbreviations see Figure 1

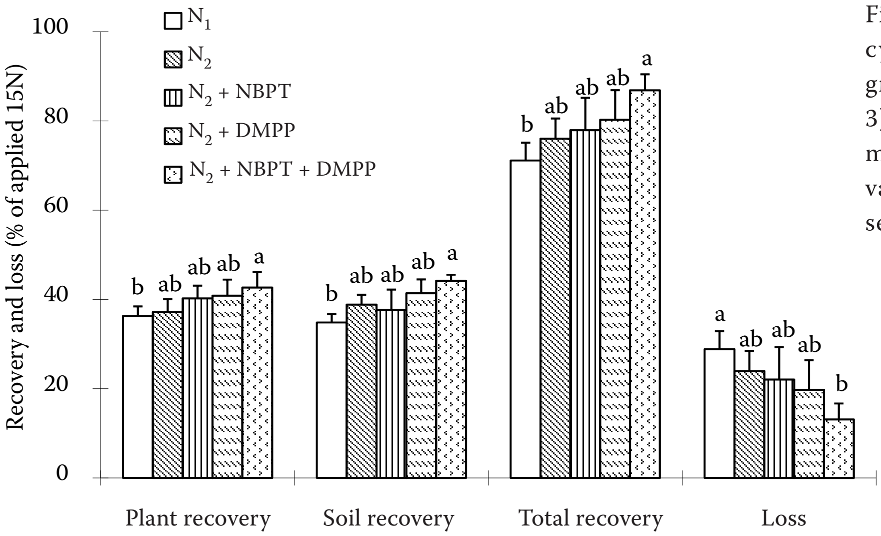


Figure 4. (a) Urea 15N use efficiency; (b) maize biomass; (c) maize grain yield. Mean value ± SD (n = 3). Different letters among treatments indicate statistically different values ($P < 0.05$). For abbreviations see Figure 1

and N_2. Such reduction in nitrification is related to the partial inhibition of the activity of nitrifying bacteria by DMPP (López et al. 2003). Di et al. (2007) also observed slow nitrification after application of urine with DCD to pasture soil. Slow nitrification in the N_2 + NBPT treatment is attributed to the delayed urea hydrolysis by urease inhibitor. A similar pattern of NO_3^- production from urea fertilizer coated with NBPT was also observed by other research work (Zaman et al. 2008). The lower NO_3-^{15}N in soil meant less risk of loss from leaching.

The NBPT, DMPP, and especially NBPT + DMPP increased the maize ^{15}N recovery and promoted the translocation of absorbed ^{15}N from stem to grain (Figure 2), suggesting their positive effects in improving grain quality. Recous et al. (1988a, b), Kiran and Patra (2003) and Giller et al. (2004) also reported the similar results in their studies with winter wheat, spring wheat and mint. They indicated that soil treated with inhibitors retained more available N for crop uptake by regulating the urea hydrolysis and nitrification-denitrification.

It was shown from our Figure 3 that reduced N application and its combination with amendment of NBPT or DMPP increased the urea-^{15}N use efficiency but decreased the maize biomass and grain yield, which implied that the reduced N application, even amended with single NBPT or DMPP, could not satisfy the N requirement of maize growth and development. The fact that the reduced N application plus NBPT and DMPP amendment increased the maize biomass and obtained the similar grain yield as the control illustrated the synergistic effect of NBPT and DMPP on eliminating the impact of deficient fertilizer N supply. A lot of research works were consistent with our work that the nitrogen use efficiency and crop yield significantly increased when urease and nitrification inhibitors were applied with fertilizer (Xu et al. 2001, 2002, Kiran and Patra 2003, Zaman et al. 2009).

It could be concluded that in the maize production on chernozem in the study area, to decrease the conventional urea N application rate by 30% and amend with both NBPT and DMPP would be a feasible way to ensure the normal maize yield while improving yield quality and soil N fertility, saving urea fertilizer, and protecting the environment.

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Corresponding author:

Prof. Zhijie WU, Institute of Applied Ecology, Chinese Academy of Sciences, P.O. Box 417, Shenyang 110016, P.R. China
phone: + 86 24 839 703 57, fax: + 86 24 839 703 00, e-mail: wuzhijiesy@yahoo.com.cn