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RESEARCH ARTICLE

Effects of urea enhanced with different weathered coal-derived humic acid components on maize yield and fate of fertilizer nitrogen

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Abstract

Humic acid (HA) is a readily available and low-cost material that is used to enhance crop production and reduce nitrogen (N) loss. However, there is little consensus on the efficacy of different HA components. In the current study, a soil column experiment was conducted using the ^{15}N tracer technique in Dezhou City, Shandong Province, China, to compare the effects of urea with and without the addition of weathered coal-derived HA components on maize yield and the fate of fertilizer-derived N (fertilizer N). The HA components were incorporated into urea by blending different HA components into molten urea to obtain the three different types of HA-enhanced urea (HAU). At harvest, the aboveground dry biomass of plants grown with HAU was enhanced by 11.50–21.33% when compared to that of plants grown with U. More significantly, the grain yields under the HAU treatments were 5.58–18.67% higher than the yield under the urea treatment. These higher yields were due to an increase in the number of kernels per plant rather than the weight of individual kernels. The uptake of fertilizer N under the HAU treatments was also higher than that under the urea treatment by 11.49–29.46%, while the unaccounted N loss decreased by 12.37–30.05%. More fertilizer-derived N was retained in the 0–30 cm soil layer under the HAU treatments than that under the urea treatment, while less N was retained in the 30–90 cm soil layer. The total residual amount of fertilizer N in the soil column, however, did not differ significantly between the treatments. Of the three HAU treatments investigated, the one with an HA fraction derived from extraction with pH values ranging from 6 to 7, resulted in the best improvement in all assessment targets. This is likely due to the abundance of the COO/C–N=O group in this HA component.

Keywords: humic acid enhanced urea, maize, aboveground dry biomass, fertilizer N uptake, fertilizer N residue

1. Introduction

Nitrogen (N) is one of the most important and limiting nutrients in cereal production. The adequate supply of chemical N fertilizer is central to the high crop productivity achieved in modern agriculture. There has been a dramatic escalation in the consumption of chemical N fertilizer in China, which increased from 2.865 Tg in 1970 to 23.942 Tg in 2013, with the consumption in 2013 accounting for

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approximately 22.34% of the total consumption worldwide (107.080 Tg) (FAO 2014; NBSPRC 2014). However, this rapid increase does not always translate into a continuous increase in crop yield. In fact, the excessive and inappropriate use of chemical N has led to stagnant or even declining maize production in most provinces in China, inflicting unnecessarily high input costs and threatening both food security and environmental quality (Fan *et al.* 2007, 2012; Zhou *et al.* 2008; Vitousek *et al.* 2009; Shi *et al.* 2012; Shen *et al.* 2013). This has been attributed to the fact that 52% of applied N is lost through ammonia volatilization, nitrous oxide emission, leaching, runoff, and so on (Zhu and Chen 2002; Zaman *et al.* 2009). Given that urea costs 340 US dollars per Mg (data for 2013 from the China Nitrogen Fertilizer Industry Association), N loss in China leads to a financial loss of more than 4.15 billion US dollars per year. Therefore, there is an urgent need to develop strategies to mitigate fertilizer-derived N (fertilizer N) losses and/or improve N use efficiency. Towards this end, controlled release coated-urea, alginic acid-enhanced urea, humic acid-enhanced urea and other modified fertilizers have been developed to slow down urea hydrolysis and to reduce the loss of N fertilizer (Purakayaths and Katyal 1998; Zaman *et al.* 2009; Zhao *et al.* 2012).

Humic acid (HA) has plentiful acidic functional groups, a high specific surface area, high cation exchange capability, as well as strong absorption properties (Zheng 1991; Stevenson 1994). These characteristics are conducive to simulating plant growth and nutrient uptake, acidizing fertilizer microsites and preserving NH_4^+, which mitigates ammonia volatilization (Thorn and Mikita 1992; Mackowiak *et al.* 2001; Quaggiotti *et al.* 2004; Ahmed *et al.* 2008; Kasim *et al.* 2009; Reeza *et al.* 2009; Celik *et al.* 2010; El-Mekser *et al.* 2014; Tan 2014). Moreover, as commercial HA is commonly derived from low-rank coal, it can be produced at low cost, while at the same time providing a solution to the environmental problem of disposing of an industrial waste product. Therefore, HA enhanced urea (HAU) stands out as an important and promising product, both for enhancing the uptake of fertilizer N, and for being ecologically sustainable and socially acceptable (Zhao *et al.* 2012).

Many experiments have been conducted to investigate the effects of HAs or compound fertilizers containing HA on the growth of cereal crops and on fertilizer N utilization efficiency. Van Vuuren and Claassens (2009) reported that maize yield under the treatment with a mixture of HA and urea was 20–46% higher than under normal urea, and that fertilizer application could be reduced by 10–20% and 30–50% on alkaline and acidic soils, respectively, by using HAU as a topdressing instead of urea. Thus, using HA can provide a huge economic benefit. Reeza *et al.* (2009) reported that applying a combination of humic/fulvic acids and urea suppressed ammonia volatilization by 12.92–20.12%, when compared with normal urea. Other studies demonstrated that applying a combination of HAs and urea inhibits ammonia volatilization at an early cultivation stage, thus ensuring a higher and more sustainable N supply in the soil for subsequent stages of plant growth (Chen *et al.* 2007; Yusuff *et al.* 2009; Yuan *et al.* 2014). Low-rank coal is used to manufacture the compounds containing HA and urea, and the effectiveness of these fertilizers has been investigated. Saha and Patti (2016) demonstrated that after the application of brown coal-urea (BCU) blends, there was a 27 and 23% increase in the biomass yield of silver beet in neutral and acid soils, respectively, as well as a 29 and 13% decrease in N_2O emissions, respectively. In addition, they investigated the levels of soil ammonium, nitrate, potentially mineralizable N and total N, and found that application of BCU blends maintained a higher amount of potentially mineralizable N in soil and altered N cycling and its availability to crops.

Most previous studies have concentrated on the effects of different ratios of urea and HA combinations on crop yield and apparent N efficiency (Liu *et al*. 2009; Yusuff *et al*. 2009; Saha and Patti 2016; Li *et al*. 2017). There has been very little investigation of the effects of urea enhanced with HAs with different characteristics and of the fate of fertilizer N. It is known that HA compounds with different structures differ in their ability to enhance plant growth, stimulate nutrient uptake and suppress ammonia volatilization (Nardi *et al.* 2000; Reeza *et al.* 2009; Jindo *et al.* 2012). In addition, the current practice is to either physically blend HA with chemical fertilizers or to apply the two separately. It has been proposed that adding HA into melted urea to generate a new fertilizer is likely to achieve better crop yields (Liu *et al.* 2009; Yuan *et al.* 2014; Li *et al.* 2017). In this paper, we report the results of an investigation into the effects of HAU, produced by adding HA into melted urea, on the growth and yield of maize grown in a column experiment in Dezhou City, Shandong Province, China. We evaluated the effects of urea enhanced with different HA components fractionated from weathered coal (Zhang *et al.* 2017a) on dry matter accumulation and maize yields and on the fate of applied fertilizer N. The aim of this investigation is to identify an efficient method to enhance maize yield and improve N use efficiency, and thereby, to improve the agronomic output and environmental sustainability of maize in northern China.

2. Materials and methods

2.1. Materials

HA fractions ($HA_{3–4}$, $HA_{6–7}$, and $HA_{9–10}$) obtained by pH-fractionation of weathered coal (Zhang *et al.* 2017a) were used as the raw material in this experiment, and the basic characteristics of these fractions are shown in Table 1. HAU fertilizers were produced by adding 0.10 g of an HA fraction into 19.90 g molten ^{15}N urea (^{15}N abundance 10.24%, produced by the Institute of Chemical Industry in Shanghai, China) at 130°C. Molten ^{15}N urea with no HA addition was prepared as the control sample. After thoroughly mixing and cooling to ambient temperature, the ensuing product was ground and passed through a 0.25-mm screen to ensure that the product was uniform in size. The products synthesized with $HA_{3–4}$, $HA_{6–7}$, and $HA_{9–10}$ were labelled as HAU1, HAU2, and HAU3, respectively, and ^{15}N urea with no HA addition was labelled U. The basic compositional properties of the experimental fertilizers were determined using the methods described in "urea containing humic acid" (HG/T 5045-2016) published by the Ministry of Industry and Information Technology of China (Table 2). In addition, monopotassium phosphate and potassium chloride were used as sources of phosphorus (P) and potassium (K).

2.2. Field trials

The soil column cultivation experiment was conducted at Dezhou Station of the Chinese Academy of Agricultural Sciences, Shandong, China (36°50´N, 116°34´E, altitude 21.2 m). The geographical region is classified as a warm, temperate, semi-humid and monsoon climate. The average annual rainfall is 569.6 mm, the average annual temperature is 13.4°C, and the mean annual water surface evaporation is 2 094.5 mm. This area is a typical winter wheat-summer maize rotation region in North China. Soil columns with open ended polyvinyl chloride pipes (25 cm in diameter and 100 cm in length) were installed as shown in Fig. 1-A. After a 90-cm hole was dug, 95 cm of the pipe was buried with the bottom 5 cm of the pipe pressed in undisturbed field soil, facilitating contact with the ground soil below. The top 5 cm of the pipe protruded above the ground level to prevent runoff and lateral contamination. Fluvo-aquic light loam that received no fertilizer input for three years was collected from a nearby field and was used to fill each test column as described below. The soil from the 0–20 and 20–90 cm layers was collected separately. Then, the soil was air-dried, ground, and passed through a 1-cm sieve. The basic physical and chemical properties of the collected soil are summarized in Table 3.

Each column was filled with the soil derived from the field as shown in Fig. 1-A: the bottom 60 cm of the column was filled with the soil collected from the field at a depth of 20–90 cm, and the remaining 30 cm was filled with field soil collected from a depth of 0–20 cm and mixed with the prepared test fertilizers (see below). In total, each column was filled with 50 kg dry soil, with compaction and watering carried out for each 30 cm of the soil column filled to ensure that the soil moisture content was 60% of field capacity. Prior to sowing maize (*Zea mays* L.), the top 30 cm of soil in the column was mixed thoroughly with a base fertilizer applied at a rate of 0.1 g N kg^{-1}, 0.2 g P_2O_5 kg^{-1}, and 0.2 g K_2O kg^{-1} of soil dry weight. The treatments in this experiment were the four synthetic fertilizers described above: urea, HAU1, HAU2, and HAU3. Columns filled with soil without urea application were also prepared in a similar manner to establish a base level (i.e., to account for naturally existing ^{15}N) for the ^{15}N measurements. Five replicates of each treatment were prepared, and all columns were distributed in a randomized block arrangement in the field (schematic diagram shown in Fig. 1-B). Four maize seeds (Zhengdan 958) were sown by hand in each column on June 25, 2015 and thinned to one seedling at the trefoil stage to ensure near uniform growth over the five columns receiving the same fertilizer treatment (Fig. 1-C). Field management was done in accordance with practices of local farmers. The maize was harvested manually at maturity on September 30, 2015 after 107 days of growth. Precipitation, irrigation, daily mean temperature, and daily mean sunshine duration during the growing season were recorded and are shown in Fig. 2. The total amounts of precipitation and irrigation were 263.8 and 120 mm, respectively.

2.3. Sample preparation and analysis

At harvest, the aboveground portion of each plant was

Table 1 Elemental and structural composition of the humic acid (HA) fractions derived from Chinese weathered coal

Sample1)	Element composition (%)				Relative proportions of C-containing functional groups in HA fractions determined by ^{13}C NMR spectroscopy (%)							
	C	H	N	O	Alkyl C	O-alkyl		Aromatics		Aromatic C–O	COO/N–C=O	Ketones/Aldehydes
						OCH	OCq	C–H	C–C			
$HA_{3–4}$	59.73	5.00	1.28	33.99	1.60	1.76	0.20	49.47	27.66	9.31	10.02	0.00
$HA_{6–7}$	60.83	4.44	1.11	33.62	0.16	1.86	0.00	44.35	33.07	8.90	11.78	0.02
$HA_{9–10}$	55.89	3.97	0.94	39.20	0.46	3.44	0.12	39.04	38.17	9.20	9.58	0.08

1) $HA_{3–4}$, $HA_{6–7}$, and $HA_{9–10}$ were humic acid (HA) fractions obtained by pH-fractionation of weathered coal.
Data are from Zhang *et al.* (2017a).

Table 2 Basic properties of the experimental fertilizers

Fertilizer[1)]	Humic acid (HA) content (%)	N content (%)	^{15}N abundance (%)	Biuret content (%)
Urea	0.00	44.21	10.24	1.12
HAU1	0.51	44.01	10.23	1.14
HAU2	0.50	44.00	10.23	1.13
HAU3	0.51	44.00	10.24	1.15

[1)] HAU1, urea enhanced with $HA_{3–4}$; HAU2, urea enhanced with $HA_{6–7}$; HAU3, urea enhanced with $HA_{9–10}$.
Data are the average of three measurements.

cut at the soil-surface level. The spike traits (kernels per spike, spike diameter, spike length, row number and kernels per row) were measured. Each plant was separated into five parts, grains, leaves, stems, bracts, and cobs, fixed at 105°C for 30 min and oven-dried at 60°C to a constant weight. Next, the hundred-kernel weight was recorded. The individual oven-dried parts of each plant were weighed and ground to a fine powder in a high-speed mill and passed through a 0.149-mm mesh sieve. The soil samples from the 0–15, 15–30, 30–50, 50–70 and 70–90 cm layers of each column were collected using a soil auger (2.5 cm in diameter). The soil was air-dried, ground and passed through a 0.149-mm sieve. All soil and plant samples were analyzed for N concentration and ^{15}N abundance using elemental analyzer-isotope ratio mass spectrometry (Vario MAX CN Carlo Erba NA1500; Elementar Analysensysteme GmbH, Germany).

2.4. Calculations and statistical analysis

The fate of fertilizer N was calculated according to Yang *et al.* (2013) and Zhang *et al.* (2017b). The differences ($P<0.05$) between treatments were determined by least significant difference (LSD) multiple comparisons following one-way analysis of variance (ANOVA) using SAS 8.0 Software (SAS Institute Inc., USA). Redundancy analysis (RDA) (Canoco 5.0 software Microcomputer Power, USA) was applied to establish the relationship between the structural characteristics of HAs and aboveground dry matter allocation or the fate of fertilizer N.

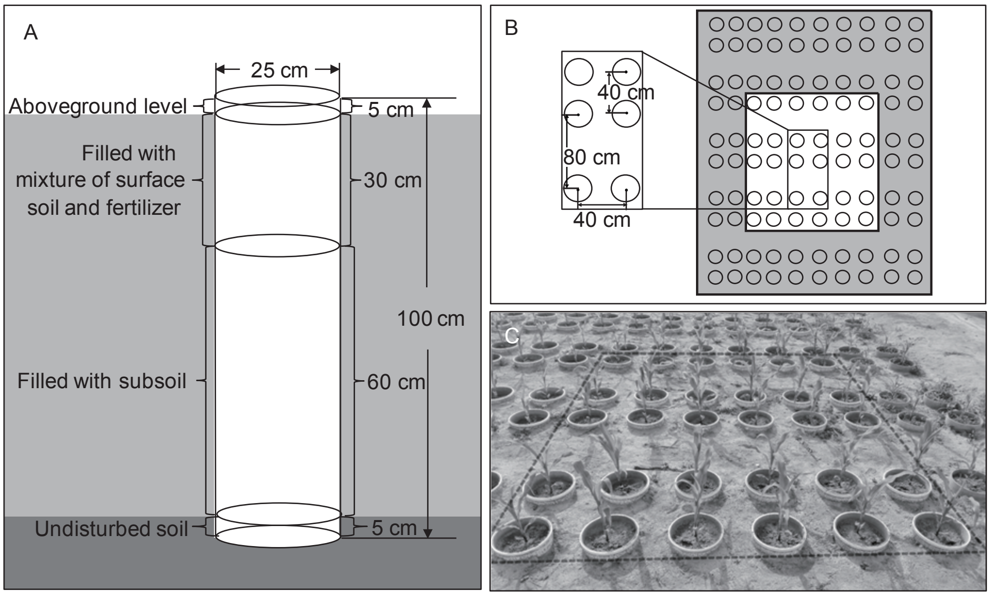

Fig. 1 Schematic diagrams of the soil columns used for maize cultivation and the experimental plot. A, a diagram showing how columns were filled with soil. B, an aerial schematic diagram of the soil column arrangement. The small circles indicate the positions of the soil columns and are divided into two classes. Specifically, the treatments were arranged in the central area (white background) and were surrounded by guarding rows (gray background). C, image showing maize cultivation in the field. The columns were arranged in a square. The black rectangle outlines the location of the treatment samples, which are surrounded by the guarding rows.

Table 3 Basic physicochemical properties of the experimental soil

Origin	pH	Organic matter (g kg^{-1})	Total N (g kg^{-1})	Alkaline hydrolyzed N (mg kg^{-1})	Olsen P (mg kg^{-1})	Exchangeable K (mg kg^{-1})
Topsoil (0–20 cm)	8.49	11.29	0.78	53.68	17.79	131
Subsoil (20–90 cm)	8.54	10.70	0.69	58.15	8.80	115

3. Results

3.1. Aboveground dry biomass and grain yields

HAU application significantly enhanced the aboveground dry biomass of the maize compared with the urea treatment (Table 4). The total dry biomass of the shoot and of individual organs was the highest under the HAU2 treatment. The total dry biomass under HAU2 was 393.96 g pot^{-1}, which significantly exceeded that under urea by 21.33%. The grain dry biomass under HAU2 was 211.98 g pot^{-1}, which was 18.67, 5.58 and 7.55% higher than that under urea, HAU1 and HAU3 treatments, respectively. HAU2 had a significantly higher (11.96%) leaf dry biomass than HAU1, while the difference between HAU2 and HAU3 was not significant. RDA was conducted to analyze the relationship between aboveground dry biomass (response variables) and HA functional groups (explanatory variables) (Fig. 3). The aboveground dry biomass was positively correlated with COO/N–C=O content, negatively correlated with aromatic C–O content, and not significantly associated with aromatic C–C and aromatic C–H. When the effects on individual parts of the shoot were analyzed, the dry biomass of grains and cobs were found to be tightly correlated with COO/N–C=O content, while the dry biomass of leaves and bracts were closely correlated with aromatic C–O content. To further investigate the increase in grain yield, the grain yield components and ear traits were measured. Similar to grain yield, kernel number per plant and row number per ear were the highest under HAU2 (Table 5). However, hundred-kernel weight and other ear traits showed slight differences.

3.2. Aboveground N uptake and its distribution

Total N uptake and fertilizer N uptake are shown in Table 6. At maturity, the total N uptake under HAU2 was 4.321 g

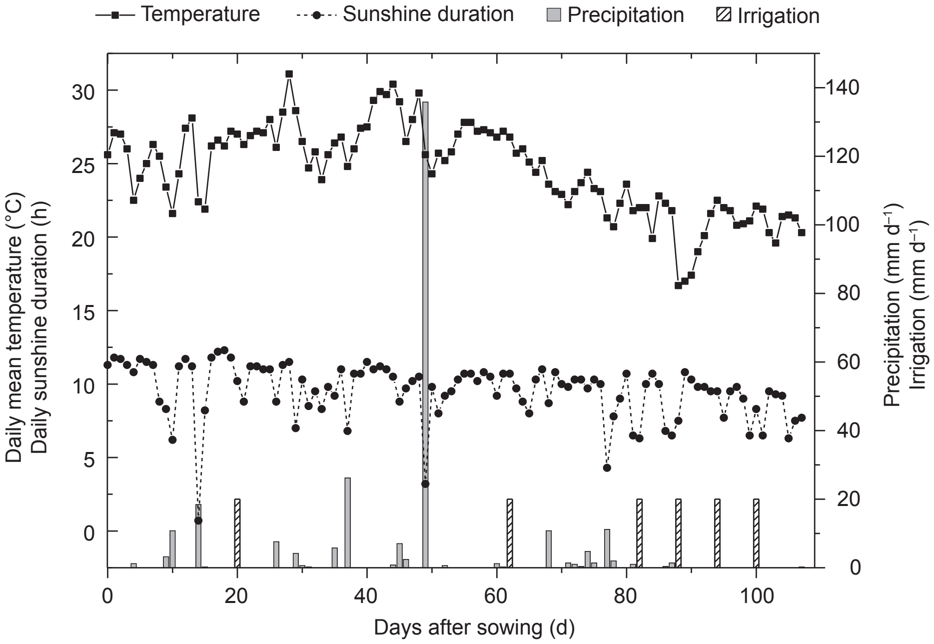

Fig. 2 Daily mean temperature, daily mean sunlight duration, precipitation and irrigation during the maize growing season in this experiment.

Table 4 Effect of different fertilizer treatments on grain, leaf, stem, bract, and cob dry biomass at harvest

Treatment[1)]	Aboveground dry biomass (g pot^{-1})					
	Grain	Leaf	Stem	Bract	Cob	Total
Urea	178.63 b	57.96 b	47.13 b	19.96 a	21.01 b	324.69 b
HAU1	200.78 a	59.53 b	57.62 ab	18.57 a	25.53 ab	362.03 ab
HAU2	211.98 a	66.65 a	65.42 a	22.03 a	27.87 a	393.96 a
HAU3	197.12 a	62.79 ab	64.75 a	19.58 a	25.29 ab	369.54 a

[1)] HAU1, urea enhanced with HA_{3-4}; HAU2, urea enhanced with HA_{6-7}; HAU3, urea enhanced with HA_{9-10}. HA, humic acid.
Data are mean values (n=5), and different letters in the same column indicate significant differences (P<0.05) between fertilizer treatments.

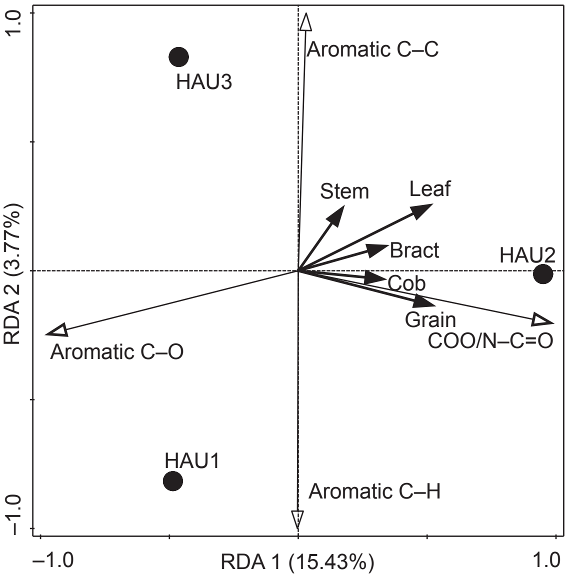

Fig. 3 Redundancy analysis (RDA) of maize aboveground dry biomass constrained by humic acid (HA) functional groups under different treatments. HAU1, urea enhanced with $HA_{3–4}$; HAU2, urea enhanced with $HA_{6–7}$; HAU3, urea enhanced with $HA_{9–10}$.

pot$^{–1}$, which was 42.94% higher than that under urea, and 16.53 and 12.12% higher than that under HAU1 and HAU3, respectively (Table 6). The total N uptake by the leaf, stem, bract, and grain was also the highest under HAU2. The total amount of fertilizer N taken up by all aboveground parts ranged from 0.611 to 0.791 g pot$^{–1}$, with the highest uptake observed under HAU2 (Table 7), similar to the trend observed for total aboveground dry biomass (Table 4) and total N uptake (Table 6). Compared with urea, HAU increased fertilizer N uptake in the aboveground parts of the plant (Table 6). Most N accumulated in grain, and the total N uptake by grain under the HAU treatments, which ranged from 0.415 to 0.456 g pot$^{–1}$, was significantly higher than that (0.358 g pot$^{–1}$) under U. As for the different HAU treatments, the fertilizer N uptake by grain under the HAU2 treatment was higher than that under the HAU1 and HAU3 treatments by 14.36 and 5.56%, respectively. Similarly, plants grown under the HAU1 and HAU3 treatments showed significantly lower fertilizer N uptake by the leaf than those grown under HAU2. However, fertilizer N uptake by the cob was the highest (0.082 g pot$^{–1}$) under the HAU3 treatment. No significant difference in fertilizer N uptake by the stem and bract was observed.

3.3. Fertilizer N residue and its distribution in the soil column

After maize was harvested, a fair amount (19.00–20.67%) of fertilizer N remained in the 0–90 cm soil profile (Fig. 4). There was no significant difference in the amount of total residual fertilizer N in the column between the urea and HAU treatments (Table 7), but a slightly higher value (5.53% higher than the mean) was observed for the HAU3 treatment. The distribution of residual fertilizer N in the 0–15, 50–70, and 70–90 cm soil layers was also not significantly different (Fig. 4). However, under HAU2, the 15–30 cm layer

Table 5 Maize yield components and ear traits under different treatments

Treatment[1)]	Kernel number (no. plant$^{–1}$)	Hundred-kernel weight (g)	Ear diameter (mm)	Ear length (cm)	Row number (no. ear$^{–1}$)	Kernel number (no. row$^{–1}$)
Urea	576 b	31.30 a	51.41 a	17.78 a	15.6 ab	40.2 a
HAU1	617 ab	32.84 a	52.96 a	18.75 a	14.4 b	39.6 a
HAU2	655 a	32.67 a	52.95 a	18.65 a	16.0 ab	40.0 a
HAU3	638 a	31.38 a	51.54 a	18.72 a	16.4 a	40.8 a

[1)] HAU1, urea enhanced with $HA_{3–4}$; HAU2, urea enhanced with $HA_{6–7}$; HAU3, urea enhanced with $HA_{9–10}$. HA, humic acid.
Data are mean values (*n*=5), and different letters in the same column indicate significant differences ($P<0.05$) between fertilizer treatments.

Table 6 Total nitrogen and fertilizer nitrogen uptake by aboveground maize organs under different treatments

Treatment[1)]	N uptake by different organs of maize (g pot$^{–1}$)									
	Grain		Leaf		Stem		Bract		Cob	
	Total N	Fertilizer N	Total N	Fertilizer N	Total N	Fertilizer N	Total N	Fertilizer N	Total N	Fertilizer N
Urea	2.081 b	0.358 c	0.614 b	0.177 b	0.187 b	0.044 b	0.075 ab	0.017 a	0.066 b	0.015 b
HAU1	2.608 a	0.415 b	0.680 b	0.186 b	0.277 a	0.059 a	0.065 b	0.014 a	0.079 b	0.016 b
HAU2	2.965 a	0.456 a	0.887 a	0.242 a	0.291 a	0.059 a	0.096 a	0.018 a	0.082 b	0.016 b
HAU3	2.796 a	0.421 b	0.652 b	0.175 b	0.233 ab	0.050 ab	0.071 ab	0.016 a	0.102 a	0.020 a

[1)] HAU1, urea enhanced with $HA_{3–4}$; HAU2, urea enhanced with $HA_{6–7}$; HAU3, urea enhanced with $HA_{9–10}$. HA, humic acid.
Data are mean values (*n*=5), and different letters in the same column indicate significant differences ($P<0.05$) between fertilizer treatments.

Table 7 Fate of fertilizer nitrogen (N) at maturity under different treatments

Treatment[1)]	Fertilizer N uptake		Residue in 0–90 cm soil layer		Loss	
	Uptake (g pot^{-1})	Recovery ratio (%)	Residue (g pot^{-1})	Residue ratio (%)	Loss (g pot^{-1})	Loss ratio (%)
Urea	0.611 c	40.73	0.290 ab	19.33	0.599 a	39.94
HAU1	0.690 b	46.00	0.285 b	19.00	0.524 b	35.00
HAU2	0.791 a	52.73	0.290 ab	19.33	0.419 c	27.94
HAU3	0.681 b	45.40	0.310 a	20.67	0.509 b	33.93

[1)] HAU1, urea enhanced with HA$_{3-4}$; HAU2, urea enhanced with HA$_{6-7}$; HAU3, urea enhanced with HA$_{9-10}$. HA, humic acid.
Data are mean values (*n*=5), and different letters in the same column indicate significant differences (*P*<0.05) between fertilizer treatments.

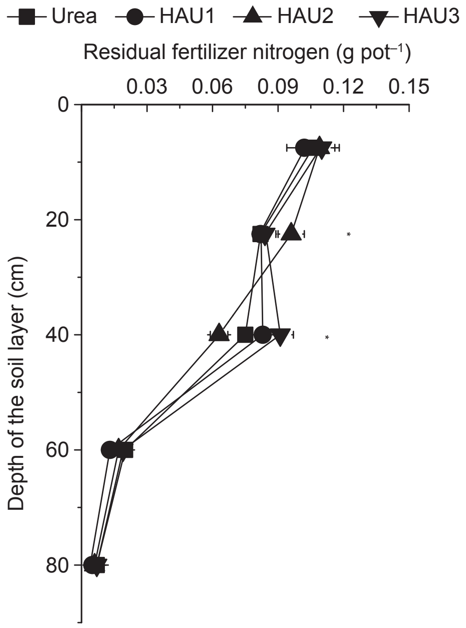

Fig. 4 Residual fertilizer nitrogen in different soil layers under different treatments. HAU1, urea enhanced with HA$_{3-4}$; HAU2, urea enhanced with HA$_{6-7}$; HAU3, urea enhanced with HA$_{9-10}$. HA, humic acid. Horizontal error bars represent the standard errors (*n*=5). * indicates significant difference among the treatments at *P*<0.05.

contained the highest residual fertilizer N (0.096 g pot^{-1}), which was 0.16 times higher than that in the corresponding soil layer of U. The HAU treatments also significantly decreased the amount residual fertilizer N in the 30–50 cm soil layer compared with the urea treatment. Most residual N remained in the 0–50 cm layer, accounting for 87.67–95.67% of total residual fertilizer N. The residual fertilizer N in the 0–30 cm soil layer under the HAU2 and HAU3 treatments (0.204 and 0.193 g pot^{-1}, respectively) was higher than that under the urea treatment.

3.4. Fate of fertilizer N

The main fates of fertilizer N include uptake by the plant, retention in the soil and loss *via* various mechanisms. The fates of fertilizer N in this study are shown in Table 7. The ratio of fertilizer N recovery by maize ranged from 45.40 to 52.73% for HAU, which was higher than that observed for urea (Table 7), but the corresponding residue ratios varied little. On the other hand, the percentage of fertilizer N lost varied from 27.94 to 39.94%, with treatments ranking as follows: U>HAU1 and HAU3>HAU2 (Table 7). This indicates that HAU significantly minimized the loss of fertilizer N. HAU2 led to a significantly higher ratio of fertilizer N uptake by the plant and a lower ratio of N loss than HAU1. Under HAU3, 20.67% of applied N remained in the soil, which was slightly higher than the ratio retained under other treatments. The lowest fertilizer N residual ratio (19.00%) was observed for HAU1.

RDA was performed to track the contributions of different HA functional groups with respect to the fate of fertilizer N. Fig. 5 shows the relationship between the fates of fertilizer N (response variables) under different HAU treatments and the structural characteristics of the corresponding HAs (explanatory variables). The first two axes (RDA 1 and RDA 2) explained 61.39 and 2.01% of the variation, respectively, and accounted for 63.4% of the total variation (*P*<0.01). Along RDA 1, the HAU2 treatment occupied the positive value, while HAU1 and HAU3 were negative. Residual fertilizer N was clearly separated from RDA 1. The uptake and loss of fertilizer N had opposite orientations and were more closely related to COO/N–C=O and aromatic C-O, respectively. Subsequent interactive-forward-selection revealed that COO/N–C=O explained 58.9% (*P*<0.01) of the total variation, and other explanatory variables were far less important.

4. Discussion

4.1. Effects of HAU fertilizers on maize biomass and grain yield

In this study, the biomass of maize plants subjected to HAU treatments was higher than those receiving the urea treatment by 11.50–21.33% (Table 4). This is in agreement with previous results suggesting that urea blended with HA could enhance the growth and yield of maize (Li *et al.* 2005;

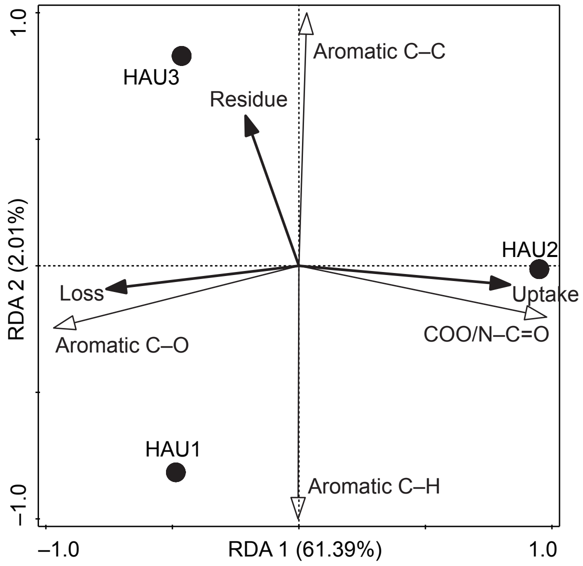

Fig. 5 Redundancy analysis (RDA) of the fate of fertilizer nitrogen constrained by the functional groups of humic acid (HA) under different treatments. HAU1, urea enhanced with HA$_{3–4}$; HAU2, urea enhanced with HA$_{6–7}$; HAU3, urea enhanced with HA$_{9–10}$.

Van Vuuren and Claassens 2009; Liu *et al.* 2016). However, the increases reported by these previous studies differ. This can be attributed to various factors, including differences in the use of fertilizer (species, amount, method) and study site (climate conditions, maize cultivar, soil fertility level, etc.). In this study, different types of HAU also had different effects on maize biomass. We have shown that this is likely due to the structural characteristics of HAs, which is in agreement with what has been reported in literature (Mato *et al.* 1972; Malcom and Vaughan 1979; Pflug and Ziechmann 1981; Muscolo and Sidari 2009).

Mato *et al.* (1972), Malcom and Vaughan (1979), and Pflug and Ziechmann (1981) suggested that carboxylic and hydroxylic functional groups play an important role in determining the activity of humic substances. Muscolo and Sidari (2009) reported that the carboxyl fraction of humic substances enhanced the fresh biomass of *Pinus nigra* calluses, while the phenolic fraction had an inhibitory effect on growth. Consistent with these findings, RDA revealed that maize biomass was positively related to COO/N–C=O content while negatively related to aromatic C–O content (Fig. 3). Considering there was lower N content in HA (Table 1), the structure of COO/N–C=O was more likely to be the carboxyl group (COO) instead of acylamino (N–C=O). Therefore, the enhancement of maize biomass was closely related to the HA with more carboxyl groups. Liang *et al.* (1999) suggested that the carboxyl groups in HAs could react with the acylamino group of urea to generate the highly stable humic acid-urea complex. The incorporation of urea into HA protects it from rapid hydrolysis, which allows the controlled release of urea and provides a continuous supply of nitrogen (Dong and Yuan 2009). Thus, the presence of more carboxyl functional groups ensures a more continuous nitrogen supply, and this potentially increases the accumulation of maize biomass (Li *et al.* 2005).

Tollenaar *et al.* (1992) attributed higher grain yields to the stimulative accumulation of dry matter resulting from an increase in the size of the source or sink. Grain setting (kernel number) and grain filling (kernel weight), which are important processes in grain development, are also affected by the size of the source and sink (Tsimba *et al.* 2013). In maize individuals, kernel number and weight are the most crucial factors governing overall grain yield. In the present study, HAU treatments, especially HAU2, increased both the kernel number per ear and kernel weight (determined based on hundred-kernel weight in this study; Table 5), and there was more significant variation in kernel number than in kernel weight between treatments. This indicates that kernel number plays a more important role than kernel weight in the increase in grain yield we observed, especially under the HAU2 treatment, thus supporting the view that kernel number per plant is more susceptible to environmental conditions (Westgate *et al.* 1997; Borrás *et al.* 2009; Tsimba *et al.* 2013). In addition, in the current experiment, bare top was hardly observed, indicating that kernel abortion rarely occurred and that the kernels developed well after formation under all treatments. There is a large amount of literature arguing that kernel number per plant likely reflects the plant growth rate during the critical two-week period around silking (Uhart and Andrade 1995; Gambín *et al.* 2006; Amelong *et al.* 2015). Stimulation during the pre-silking period would explain the increase in observed kernel number per plant under the HAU treatments. Paponov *et al.* (2005) reported that increased kernel number per spike due to N fertilization is not associated with an increase in plant biomass, but rather is related to an increase in dry matter allocation to ears during the critical silking-to-anthesis period. Lee and Tollenaar (2007) suggested that the higher the allocation of dry matter to developing ears during the critical period of kernel establishment, the more the kernels produced per ear. Therefore, it is more likely that compared with urea treatment, HAU treatments accelerated maize growth during in the early stages of development (Mahler *et al.* 1994), leading to a large source, which ensured the establishment of a large sink.

4.2. Effects of HAU treatments on the fate of fertilizer N

In this study, the recovery of fertilizer N by the aboveground parts of the maize plants for all treatments ranged from 40.73 to 52.73%, which is higher than that reported for cereal plants worldwide (30–40%) and in China (30–35%) (Zhu and Chen 2002; Ladha *et al.* 2005). These differences could be attributed to the combined effects of climatic factors, soil fertility, and crop management (Wiesler 1998; Richter and Roelcke 2000). We found that there was a higher uptake of fertilizer N and lower unaccounted fertilizer N loss under the HAU treatments than under the urea treatment during the maize growing season (Table 7), indicating that HAU is a better fertilizer leading to higher N uptake and lower loss. This result is similar to the findings by Celik *et al.* (2010) and El-Mekser *et al.* (2014) that HAU could enhance N uptake while decreasing its loss.

We found that 87.67–95.67% of the residual fertilizer N remained in 0–50 cm soil layer (Table 7). More residual amount of fertilizer N in the 0–50 cm soil layer may be attributed to low precipitation during the crop season (Fang *et al.* 2006; Yang *et al.* 2011). In our study, there was variation in the amount of residual N in the different soil layers between the four treatments; there was more residual fertilizer N in the 15–30 cm soil layer under the HAU2 treatment than that under urea treatment, while the opposite was observed in the 30–50 cm soil layer. This is consistent with the results of previous studies (Yuan *et al.* 2014; Li *et al.* 2017) and suggests that HAU applications reduce the risk of fertilizer N leaching.

The uptake of fertilizer N by maize under HAU2 was significantly higher than that under HAU1 and HAU3 (Table 7), while the amount of residual fertilizer N under the HAU3 treatment was higher than that with HAU1 and HAU2 application (Table 7). RDA revealed that different HAs affected the fate of fertilizer N mainly by promoting fertilizer N uptake and that the functional group COO/N–C=O was positively correlated with the amount of fertilizer N uptake (Fig. 5). The COO/N–C=O functional group is likely the main factor responsible for the enhancement of urea use efficiency by HA. This is in agreement with the view of Liang *et al.* (1999) that the carboxyl, phenolic hydroxyl and other acid functional groups in HA react with urea, which is a weak base, generating stable humic acid-urea complexes that are resistant to hydrolysis. The *P*-value for RDA of fertilizer N fate (Fig. 5) was <0.01, which is lower than that observed for RDA of biomass (Fig. 3; *P*=0.274). This indicates that HA structural characteristics, especially COO/N–C=O content, have a greater effect on the fate of fertilizer N than on the dry biomass of individual maize organs. However, the underlying mechanism needs to be verified by future research on the effect of HA on the inhibition of urea hydrolysis.

5. Conclusion

We found that application of urea enhanced with HA improved maize biomass and grain yield by 11.50–21.33% compared with the application of urea alone. Further analysis indicated that kernel number per plant was a more significant factor than kernel weight in the improvement of maize grain yield. At the same time, HAU application also optimized fertilizer N fate. Specifically, HAU application significantly increased the recovery of fertilizer N by maize, while decreasing potential fertilizer N loss under the cropping conditions tested. Moreover, more residue N was found in 0–30 cm soil layer under the HAU treatments than that under urea treatment, while the opposite was observed in the 30–90 cm soil layer, suggesting that HAU, through some as yet unknown mechanism, reduced the leaching of fertilizer N. Of the three HAU treatments tested, HAU2 was optimal for enhancing maize growth and the use of fertilizer N, and we attribute this to the relatively high COO/N–C=O content of HA in HAU2.

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