Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Fate and recovery of nitrogen applied as slow release brown coal-urea in field microcosms: ^{15}N tracer study

Biplob K. Saha[a], Michael T. Rose[b], Lukas Van Zwieten[b], Vanessa N.L. Wong[c], Terry J. Rose[d], Antonio F. Patti[a*]

[a]*School of Chemistry, Monash University, Clayton, Victoria, 3800, Australia*

[b]*NSW Department of Primary Industries, Wollongbar Primary Industries Institute, Wollongbar, NSW 2477, Australia*

[c]*School of Earth, Atmosphere & Environment, Monash University, Clayton, Victoria, 3800, Australia*

[d]*Southern Cross Plant Science, Southern Cross University, Lismore, NSW, 2480, Australia*

**Corresponding author. Email: bksaha@bau.edu.bd*

Supporting Information

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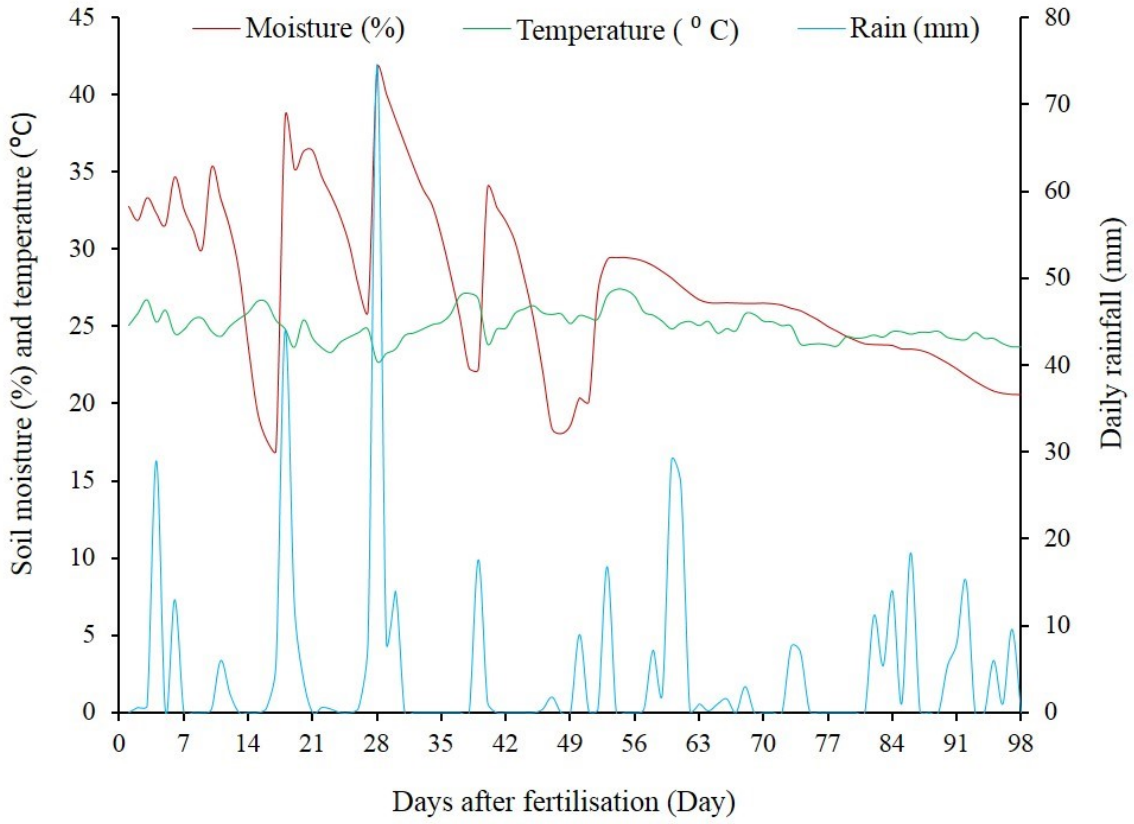

Fig. S1. Weather data at the experimental site during the growing period of sweet corn

1. Measurement of N_2O emissions

One static has chamber was installed in each microcosm which was closed with an airtight lid having a rubber septum for the measurements of N_2O flux. The headspace concentration of N_2O was measured at three-time events (0, 30, and 60 min after closing the static gas chambers) during each measuring day. At each measurement, a 12 mL gas sample was withdrawn from the headspace of the gas chambers by an airtight syringe (SGE, 25MDR-LL-GT). The gas sample was then transferred into a 12 mL airtight glass vial which was pre-evacuated and flushed with argon and then re-evacuated. The gas sample was collected in the glass vial and N_2O concentration was measured within one week. Gas samples were analyzed for N_2O using an Agilent 7890A gas chromatograph (GC) fitted with a Gerstel MPS autosampler. The N_2O flux was determined with the help of a calibration curve prepared from the reference gas with a known concentration of 1 ppm. Linear interpolation of the gas concentrations were used to calculate the flux and cumulative N_2O emissions[1].

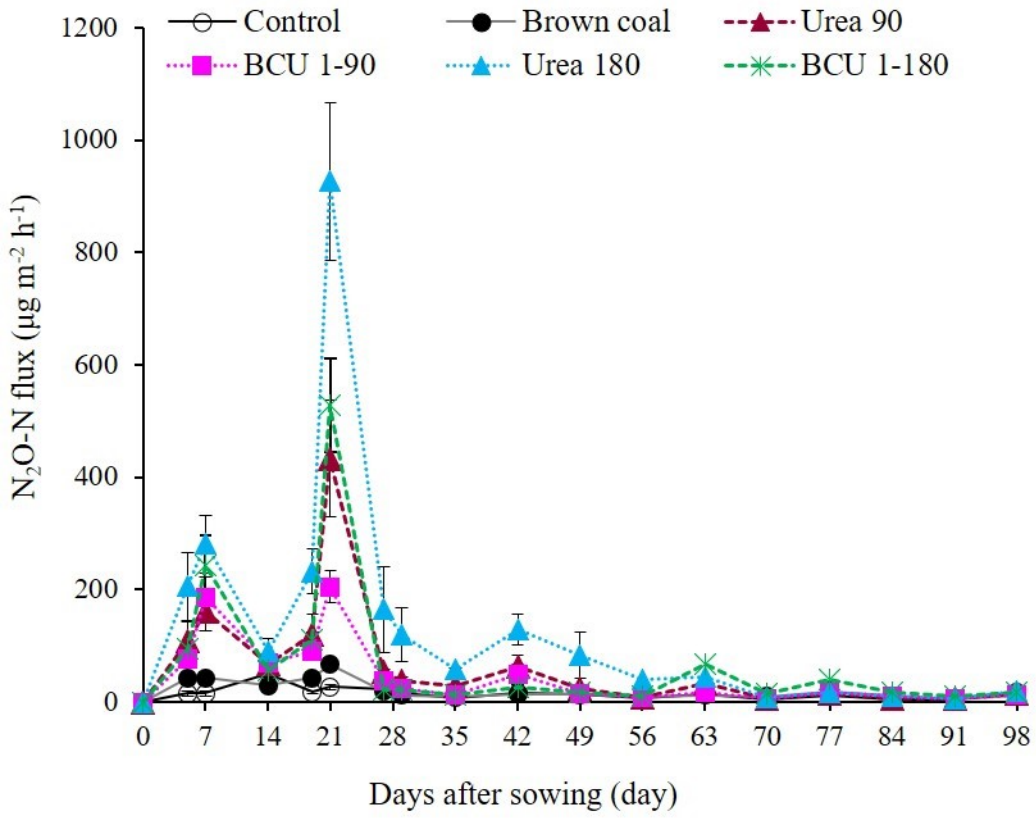

Fig. S2. Effect of BCU granules and urea on the daily N_2O emissions from soil. The error bars indicate the standard error among the replicates (n=4).

2. Measurement of NH_3 emissions

The NH_3 volatilisation was measured using polyurethane foam absorbers. The absorbers were placed into plastic petri dishes and soaked with 20 mL of 0.05 M sulfuric acid solution. The NH_3 emissions were measured for 12 h during each sampling event. To prevent contamination with environmental NH_3, the absorbers were maintained in plastic bags until installation on the PVC tubes. one absorber was installed at the top of each PVC tube. The petri dishes on the upper side of the absorbers prevented capture of NH_3 from atmosphere. After this, the absorbers were removed and deep-frozen in plastic bags immediately. At the end of the experimental period, the absorbers were disassembled, and components were washed with deionised water. For each absorber, the petri dish was washed with approximately 30 mL of water with a wash bottle, above the foam placed in a Buckner funnel attached to a Kitassato and a vacuum pump. For complete removal of the acidic solution, the absorber was washed a second time with deionised water. The solution collected in the Kitassato was then transferred to a volumetric flask and the final volume was made up to 100 mL by adding deionised water. The NH_4^+-N concentration was determined colorimetrically by reacting with salicylate and hyphochlorite in a buffered alkaline solution contain sodium nitroprusside as a reductant [2]. The NH_3 flux (mg N m^{-2} h^{-1}) was then calculated using the equation of [3].

$$Ammonia\ flux = \frac{C \times V}{a \times D} \qquad (1)$$

Where, C is NH_3 concentration in the acid trap (mg dm^{-3}); V is the volume of acid (dm^3); a is total cross-section area (m^2) of soil column and D is duration (h) of each sampling.

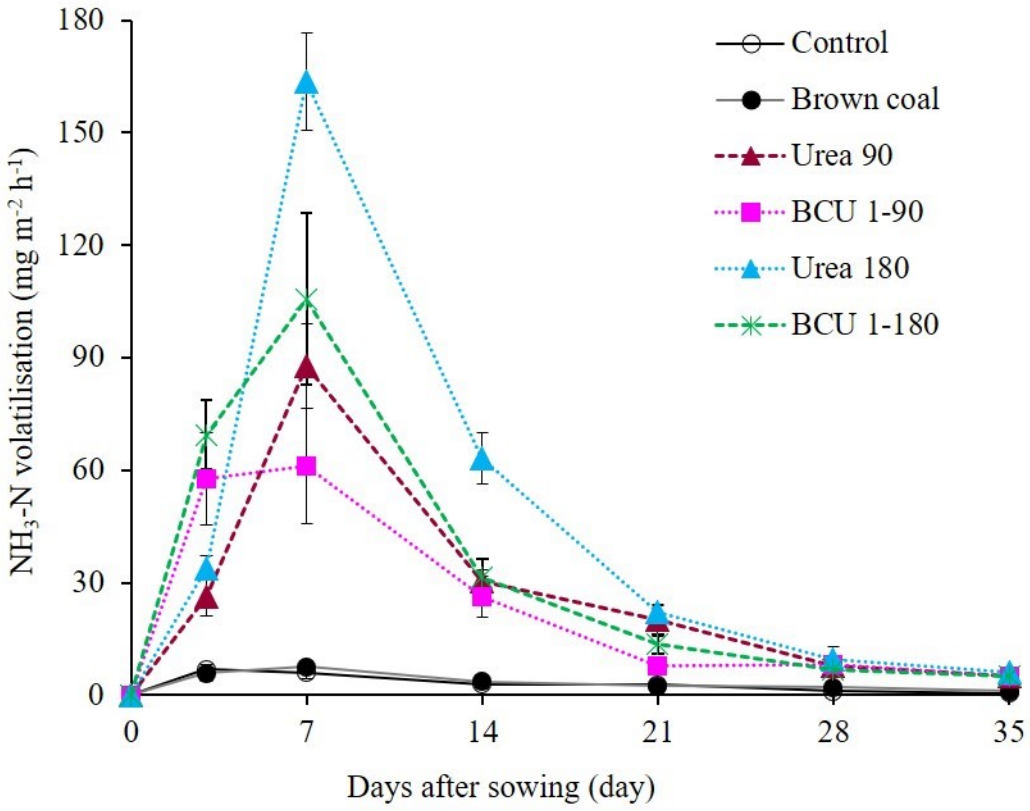

Fig. S3. Effect of BCU granules and urea on the daily NH_3 emissions from soil during the early growth stage of sweet corn. The error bars indicate the standard error among the replicates (n=4).

3. Soil sampling, preparation and analysis

After harvest, 3 soil samples were collected from each microcosm to measure the mineral N, mineralisable N, total C and N in the soil. The soil samples were collected with a hand auger to a depth of 0-50 cm. The depth of the different soil profile layers was: top (0-15 cm), middle (16-30 cm) and bottom (31-50 cm). Soil was removed from each section were mixed thoroughly and foreign materials (roots, gravels, stones) were removed. The soil was then air dried and sieved through a 2 mm sieve. A representative soil sample was obtained by reducing the bulk volume by following the quartering process. Soil pH was determined at a soil-to-water ratio of 1:5 (WP 80 Reference pH Meter, Anpros Phy LTD., Victoria, Australia). Mineral N was extracted from soils with 2 M KCl using a 1:2.5 soil: extractant ratio. The soil extracts were filtered through Advantech filter paper 42 prior to analysis for mineral N species. The mineral N concentrations of soil were also measured following the colorimetric method. Total C and N in soil was measured using a high-frequency induction furnace CHN analyser (Vario Micro Cube). The soils were finely ground using a mortar and pestle prior to C and N analysis. Phosphorous, exchangeable Ca, Mg, K, extractable S, Al, and DTPA Fe were measured according to the standard methods[4].

4. Leachate analysis

To each microcosm, one ceramic suction lysimeter was installed to a depth of 50 cm for the collection of leachates. Leachate collection was conducted at 42 and 98 days after fertilisation (DAF). The two leachates were combined before analysis. Leachates were filtered through Advantech filter paper 42 prior to analysis. The total N in leachate was analysed at the Water Studies Centre, School of Chemistry, Monash University using Lachat Flow Injection (Quikchem 8500 Series 2) analysis coupled with microwave digestion. For the ^{15}N isotopic analysis the leachates were freeze dried in a freeze drier. The ^{15}N isotopic composition were measured using an elemental analyser (Carlo Erba/Fisons/Interscience) coupled via a ConFlo II interface to an isotope ratio mass spectrometer (EA-IRMS, Finnigan Delta S).

Table S1

Volume of leachate collected during two leaching events from soil (values are mean ± standard error, n = 4).

Treatments	Volume of leachate (mL)		
	Leachate 1	Leachate 2	Average
Control	17.4±0.84	22.2±1.84	39.6±2.68
Brown coal	13.0±0.51	12.6±1.12	25.6±1.63
Urea 90	16.3±0.42	19.8±0.92	36.0±1.32
BCU 90	15.6±0.48	18.4±1.11	34.0±1.51
Urea 180	17.6±0.35	22.8±0.76	40.4±1.11
BCU 180	15.4±0.45	17.8±0.99	33.2±1.44

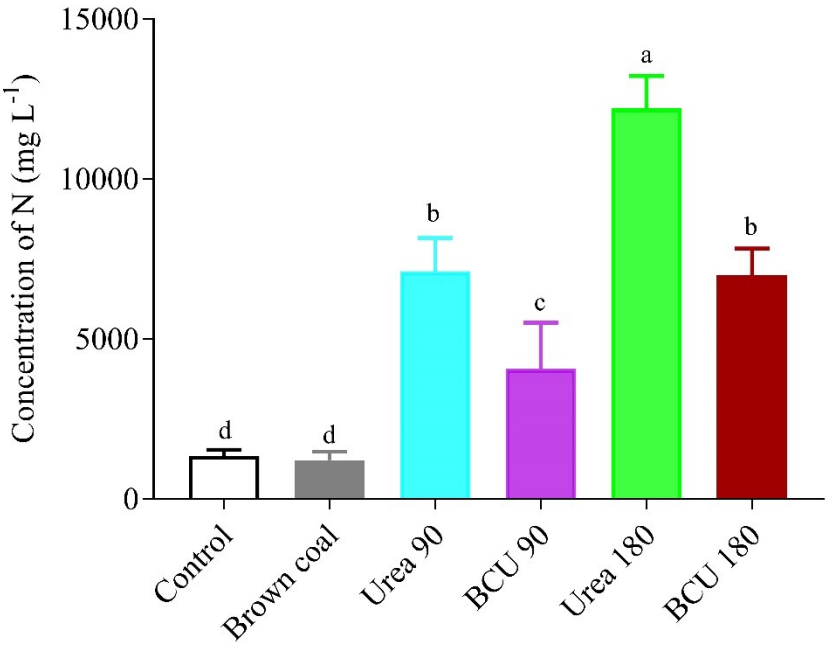

Fig. S4. Concentration of N in leachate. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4).

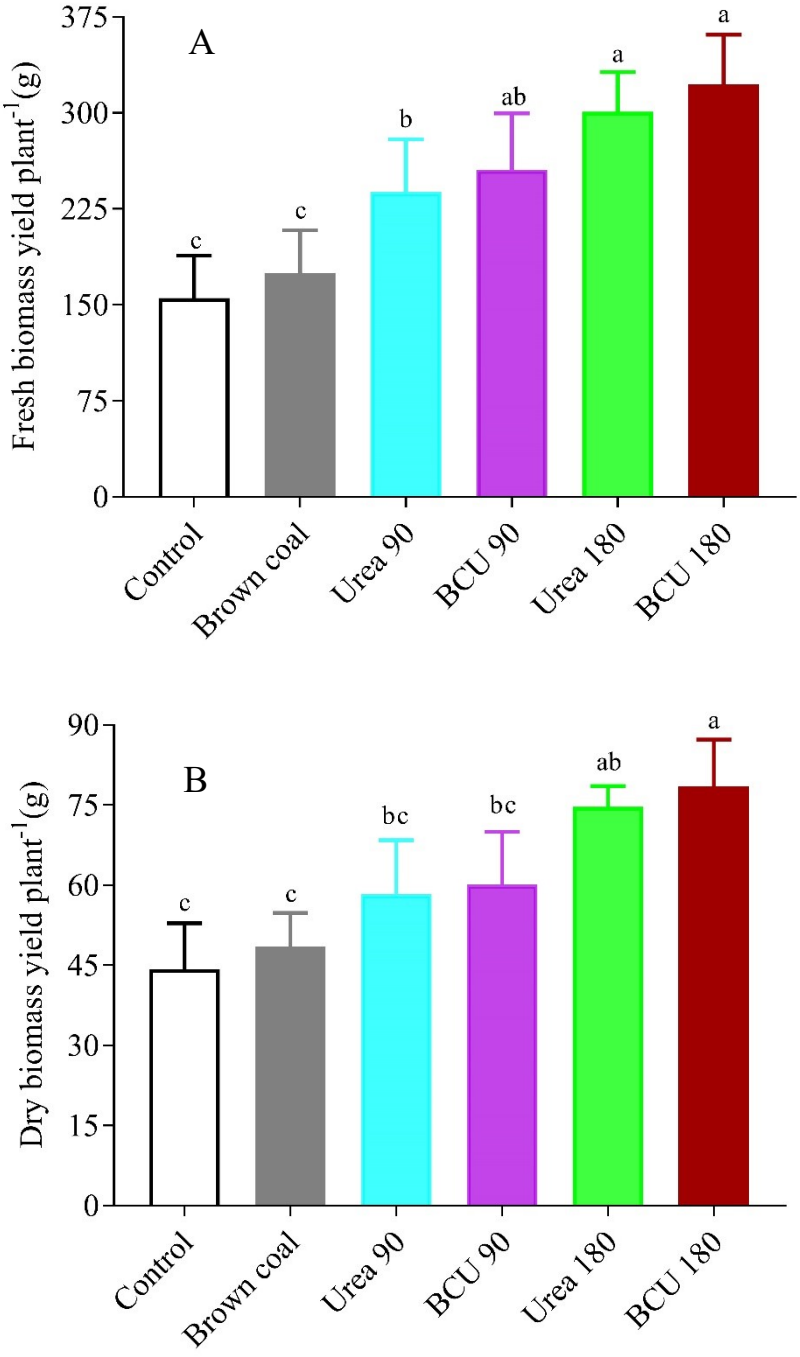

Fig. S5. Effect of BCU granules and urea on the fresh (A) and dry (B) biomass yield of sweet corn. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4).

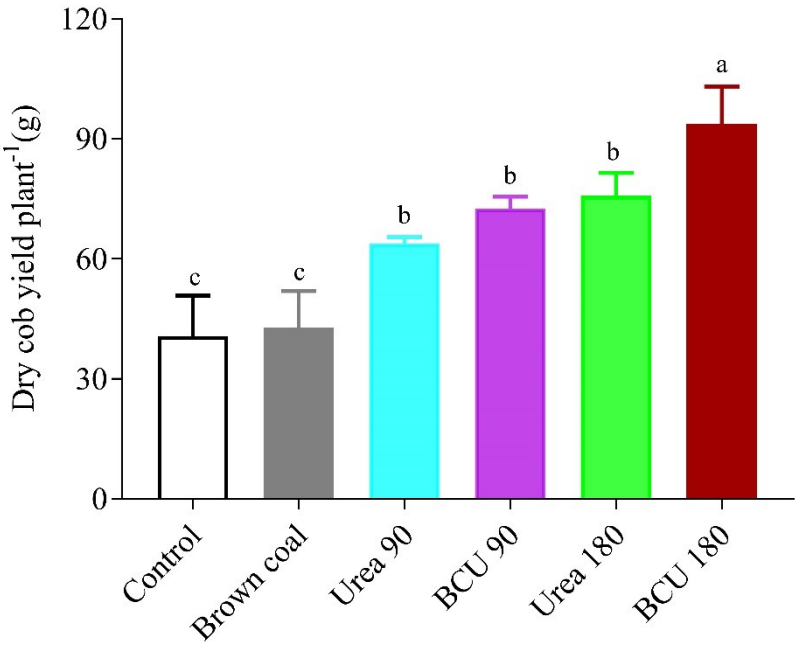

Fig. S6. Effect of BCU granules and urea on the dry cob yield of sweet corn. Bars with different letters differ significantly according to Tukey-test at $p<0.05$ and the error bars indicate the standard error among the replicates (n=4).

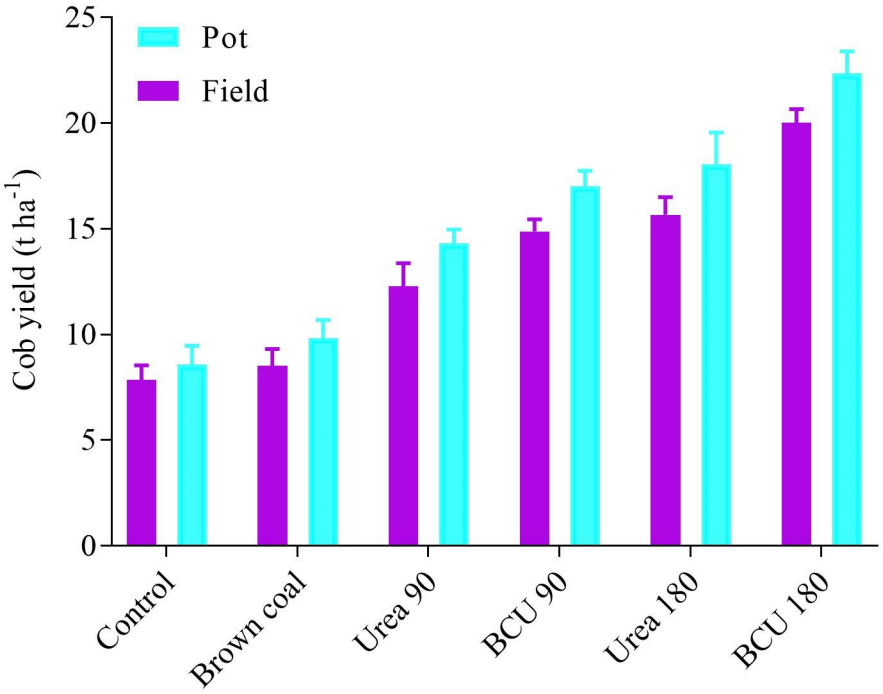

Fig. S7. Yield comparison of main plot and microcosm. The error bars indicate the standard error among the replicates (n=4).

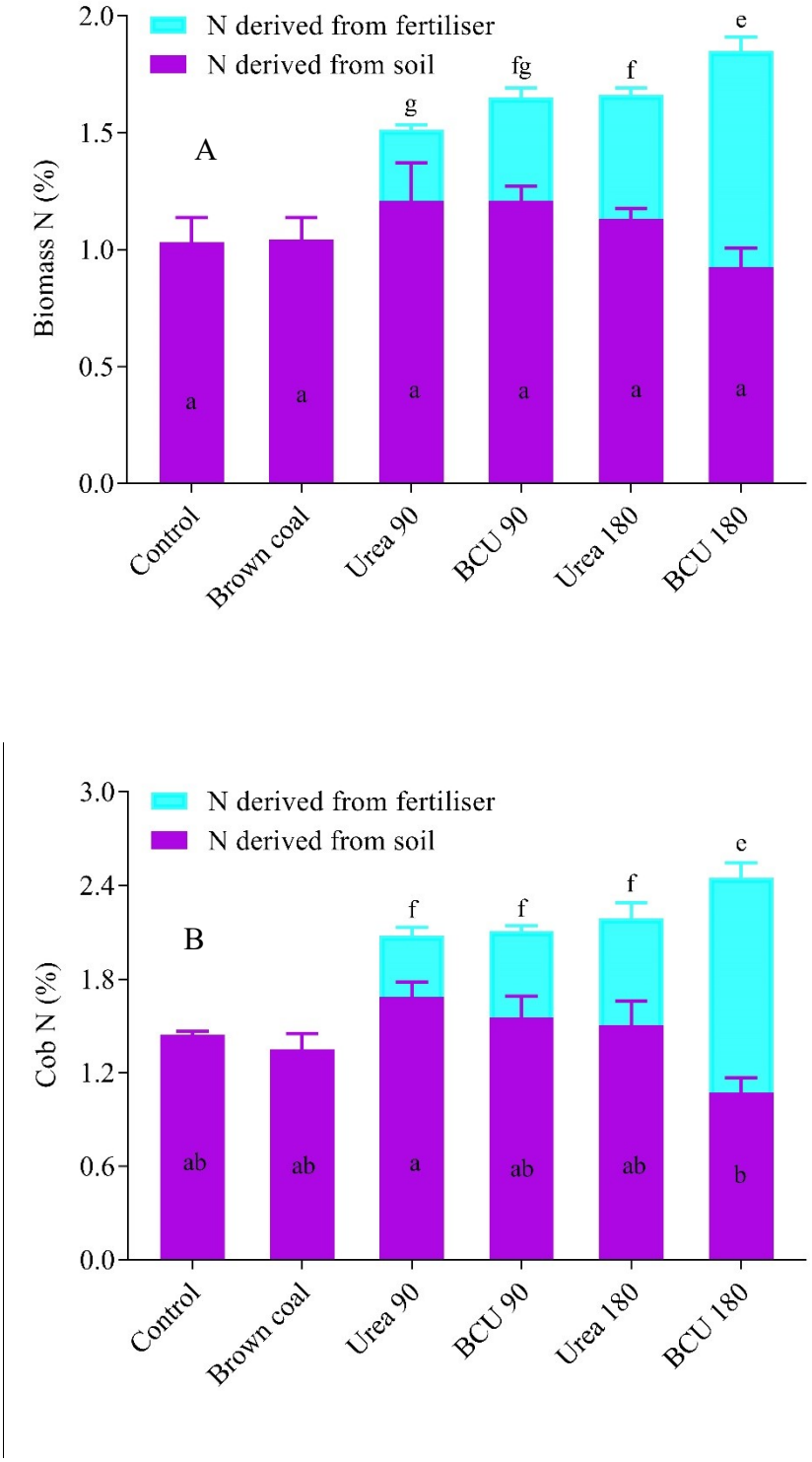

Fig. S8. Effect of BCU granules and urea on the biomass (A) and cob (B) N concentration of sweet corn. Bars with different letters differ significantly according to Tukey-test at $P<0.05$ and the error bars indicate the standard error among the replicates (n=4).

Table S2

Soil total C content at various depth of soil profile (values are mean ± standard error, n = 4)

Treatments	Soil C (%)		
	Top (0-15 cm)	Middle (16-30 cm)	Bottom (31-45 cm)
Control	4.59±0.16	3.51±0.13	2.80±0.08
Brown coal	4.62±0.02	3.58±0.12	2.81±0.05
Urea 90	4.67±0.11	3.75±0.10	2.81±0.11
BCU 90	4.75±0.08	3.78±0.12	2.83±0.16
Urea 180	4.69±0.07	3.74±0.26	2.87±0.18
BCU 180	4.78±0.06	3.83±0.21	2.84±0.26

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