Urea losses in flooded soils with established oxidized and reduced soil layers*

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Summary. Laboratory batch incubation experiments were conducted to determine fate of urea-15N applied to floodwater of four rice soils with established oxidized and reduced soil layers. Diffusion-dependent urea hydrolysis was rapid in all soils, with rates ranging from 0.0107 to 0.0159 h⁻¹ and a mean rate of 0.0131 h⁻¹. Rapid loss of 53%–65% applied urea-15N occurred during the first 8 days after application, primarily by NH₃ volatilization. At the end of 70 days, an additional 20%–30% of applied urea-15N was lost, primarily through nitrification—denitrification processes. The soil types showed significant differences in total applied urea-15N recovery. Conversion of urea-15N to N₂-15N provided direct evidence of urea hydrolysis followed by nitrification-denitrification in flooded soils.

Key words: Nitrogen transformations — Flooded soil — Nitrogen loss — Nitrification — Denitrification — Urea — NH₃ volatilization

Urea is a major source of N fertilizer for rice. Urea is highly unstable when added to flooded rice soils, because it is rapidly hydrolyzed to NH₄⁺—N and subsequently removed through (1) plant uptake, (2) ammonia volatilization, (3) nitrification—denitrification, and (4) leaching. In many previous studies (DeLaune and Patrick 1970; Sahrawat 1980; Vlek and Carter 1983), urea transformations were conducted using air-dried oxidized soils which were treated with urea and subsequently flooded, with losses of urea measured in the soils. It is well established that flooded rice soils are characterized by an oxidized surface layer (thickness is inversely related to the organic matter content of the soil) and an underlying anaerobic soil layer (Mitsui 1954; Reddy and Patrick 1984). Both oxidized and reduced soil layers can significantly influence urea hydrolysis and subsequent N loss from soils (Savant et al. 1985; Lindau et al. 1988). To date very little information has been made available on the effect of these two layers on the fate of urea applied to flooded rice soils.

The purpose of the present study was to determine the fate of labelled urea-15N applied to the floodwater in four soils with established aerobic and anaerobic soil layers.

Materials and methods

Soils used in the study include two from the United States (Crowley silt loam from Louisiana and Yolo silt loam from California) and two from India [Cuttagar sandy clay loam, a rice soil from Central India (Orissa state), and Coimbatore clay loam, a rice soil from southern India (Tamil Nadu state)]. All soils were obtained from the plow layer (0–15 cm). Selected properties of the soils are given in Table 1. In experiment 1, 25 g soil (oven-dry basis) was added to 50-mI test-tubes (25×150 mm³; 24 tubes per soil type) containing 25 ml deionized distilled water (Fig. 1a). The soils were incubated in the dark at 28°C for 60 days to establish the oxidized and reduced soil zones. During the pre-incubation period, floodwater levels were maintained at 5 cm depth by adding distilled water periodically. At the end of the pre-incubation, 5 ml of the floodwater was removed and replaced with 5 ml urea solution enriched with 10 atom % ¹⁵N (equivalent to 50 mg urea—N kg⁻¹ soil). At the end of 0, 1, 2, 4, 8, 15, 30, and 70 days after the addition of urea, the contents of three tubes were analyzed for various ¹⁵N fractions. Floodwater was removed and analyzed for urea-N and NH₄⁺—N, and the remaining soil was extracted with 2 M KCl. The extracted solutions were analyzed for urea-N and NH₄⁺—N. Residual soil was analyzed for organic-N. Urea-N, NH₄⁺—N, and organic-N in the samples were analyzed according to the procedures described by Bremner (1982), Keeney and Nelson (1982), and Bremner and Mulvaney (1982), respectively. All samples were subsequently analyzed for their labelled ¹⁵N content (Hauck 1982). A separate incubation with flooded Crowley, Cuttagar, and Coimbatore soils was conducted to determine the effects of urea hydrolysis on floodwater pH. The method was identical to that described above, except that Yolo soil was not included. Floodwater pH was measured by a combination glass electrode and pH meter, over a period of 40 days.

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Table 1. Selected properties of the soils used in the study

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH (H₂O)</th>
<th>CEC (meq/100 g dry weight soil)</th>
<th>Organic C (g kg⁻¹)</th>
<th>Organic N (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crowley silt loam</td>
<td>5.8</td>
<td>9.4</td>
<td>7.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Yolo silt loam</td>
<td>5.6</td>
<td>18.1</td>
<td>8.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Cuttack sandy clay loam</td>
<td>6.1</td>
<td>15.1</td>
<td>4.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Coimbatore clay loam</td>
<td>8.1</td>
<td>31.6</td>
<td>10.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

CEC, cation exchange capacity

Fig. 1a, b. Schematic representation of the floodwater–soil systems used in experiment 1 (1250 µg urea-N which is equivalent to 254.6 µg N cm⁻²) and b experiment 2 (46.2 mg urea-N which is equivalent to 1200 µg N cm⁻²)

A second experiment (experiment 2) was conducted to obtain direct evidence of nitrification and denitrification following urea-15N hydrolysis, by measuring the gaseous end product N₂-15N. The experimental setup was similar to that described by Patrick and Reddy (1976) and Lindau et al. (1988). The laboratory reactors consisted of a gas sampling unit attached to the flask, and a side arm containing 1 N H₂SO₄ to trap volatilized NH₃ (Fig. 1b). Two hundred grams of air-dried soil (0.5 mm mesh sieve) were placed in the reactors containing 200 ml distilled water (two reactors per soil type) and incubated at 25 °C for a period of 70 days in the dark, with the mouth of the flask open to the air. During the pre-incubation period, O₂ diffusion through the floodwater established an aerobic soil layer at the soil-water interface. After 70 days, labelled urea (29 atom % 15N) was applied to the floodwater at a rate of 1200 µg N cm⁻² (120 kg N ha⁻¹). On a soil mass basis, the concentration of urea-N was 230 mg N kg⁻¹ of soil. The flask tops were then sealed and incubated for 6, 13, 20, 27, 34, and 40 days. At the end of each incubation period, a 10 ml gas sample was obtained and stored in a pre-vacuumed vacutainer. The reactors were then opened for 2 h, allowing equilibration with the laboratory atmosphere, and sealed until the next sampling period. The rubber septa of the vacutainers were sealed with silicon glue. The gas samples were analyzed on a Finigan MAT Delta E isotope mass spectrometer equipped with a dual inlet and a collector assembly for simultaneous analysis of masses 28, 29, and 30. At the end of each incubation, only the headspace gas was analyzed. At the end of 40 days, after the headspace gas was sampled, acid traps were removed and the soil in the reactors was vigorously shaken for 1 h, to release soil-trapped gases into the headspace. The gases were analyzed for N₂-15N as described above. The acid in the traps was analyzed for NH₄⁺-N according to the procedure described by Kenney and Nelson (1982), and was subsequently analyzed for labelled 15N content (Hauck 1982).

Results and discussion

The urea-15N disappearance from floodwater and from soil is presented in Fig. 2. The urea loss from floodwater was due to (1) urea hydrolysis in the floodwater, and (2) urea diffusion from floodwater to the underlying oxidized and reduced soil layers. In experiment 1, significantly less of the urea hydrolysis product NH₄⁺-15N accumulated in the floodwater than in the underlying soil layers, suggesting that the active zone for urea hydrolysis was in the soil layers. These results correlate with the results obtained by Vlek et al. (1980) and Savant et al. (1985), who reported very little urease activity in floodwater, with urea largely hydrolyzed to NH₄⁺ at the soil–floodwater interface. Floodwater and soil NH₄⁺-15N from urea hydrolysis at the soil-water interface diffused both into the water column and the underlying soil column. In experiments by Vlek and Craswell (1979), NH₃ volatilization proceeded rapidly following urea hydrolysis, and was directly related to increased NH₄⁺ in the floodwater 5 days after the urea addition. Ammonia volatilization proceeded rapidly because of the increased floodwater pH caused by urea hydrolysis, as shown in Fig. 3. Within the first 4 days after the addition of urea-15N to the floodwater, NH₄⁺-15N concentrations peaked in the floodwater, with up to 23%, 11%, 11%, and 3% of the urea-15N applied measured as NH₄⁺-15N in the floodwater of Yolo, Crowley, Cuttack, and Coimbatore soils, respectively. Virtually all urea-15N and NH₄⁺-15N disappeared from the floodwater between 8 and 15 days after the urea addition in all soils tested.

Urea hydrolysis rates followed first-order kinetics, with k (rate constant, h⁻¹) values of 0.0119, 0.0107, 0.0159, and 0.0140 h⁻¹ for Crowley, Yolo, Coimbatore, and Cuttack soils, respectively. No significant differences were observed in the urea hydrolysis rates, and the mean rate constant was 0.0131 h⁻¹. In studies conducted by Lindau et al. (1989), urea added directly to oxidized and reduced soil suspensions (without established floodwater zones, or lengthy pre-incubation periods) gave much higher hydrolysis rates, with k values of 0.028 – 0.403 h⁻¹ for reduced soils and 0.054 to 0.369 h⁻¹ for oxidized soils. Similarly, the urea hydrolysis rates of Crowley silt loam soil adjusted to 20% water content (weight: weight) were 0.023 h⁻¹ at 15 °C and 0.101 h⁻¹ at 35 °C, respectively (Vlek and Carter 1983). In our experi-
ments, the rates of urea hydrolysis were influenced by urea diffusion from the floodwater to the underlying soils, the zone of active urea hydrolysis. In addition, the low soil urease activity may have been due to decreased oxygen partial pressures in soils flooded for long periods of time (Savant et al. 1985). It was likely that a significant amount of urea was hydrolyzed in the oxidized layer. The thickness of the oxidized layer in soils flooded for 130 days (as measured by the thickness of the reddish brown layer formed by the oxidation of ferrous iron) was between 7.8 and 9.4 mm for the four soils tested.

Within 4 days after the urea addition, a maximum of 5%–10% of the urea-15N was recovered as urea-15N in the oxidized and reduced soil layers. The urea accumulation in the soils indicated that the rate of urea diffusion into the soil exceeded the rate of urea hydrolysis. Between 4 and 8 days after the urea addition, a maximum of 30%–40% of urea-15N was recovered in the soil as NH4+–15N. An additional 5%–10% of the urea-15N added was recovered in the organic-15N fraction. Virtually complete urea hydrolysis had occurred in the soils between 8 and 15 days after the urea addition to the floodwater. By 8 days after the urea addition, 55%–65% of the urea-N added had been lost by NH3 volatilization, and nitrification–denitrification reactions.

Seventy days after the urea addition (Table 2), some of the added urea-15N remained as organic-15N (8.8%–14.1%), soil NH4+–15N (1.1%–6.6%), and soil urea-15N (0.1%–1.3%). A total of 81%–89% of the urea-15N added had been lost by NH3 volatilization and nitrification–denitrification reactions.

In experiment 2, gaseous N2-15N fluxes were measured in all four soils after labelled urea-15N was applied to the floodwater (Fig. 4a). Gaseous N2-15N loss-

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**Fig. 2a–d.** Transformations of labelled urea-15N applied to floodwater of a Crowley silt loam, b Coimbatore clay loam, c Yolo silt loam, and d Cuttack sandy clay loam. Soils were pre-incubated for 60 days. Each value is mean of three replicates ■ Total 15N recovered, ● floodwater urea-15N, ▲ soil urea-15N, * soil organic-15N, ○ floodwater NH4+–15N, △ soil NH4+–15N.

**Fig. 3.** Effect of urea hydrolysis on the floodwater pH of three soils with established oxidized and reduced zones. Soils were pre-incubated for 60 days. Each value is mean of two replicates.
Table 2. Mass balance of urea-\textsuperscript{15}N, 70 days after application to flooded soil. Urea was applied (1.25 mg N per tube or 50 mg N kg\textsuperscript{-1} soil urea-N enriched with 10 atom \% \textsuperscript{15}N) to floodwater after 60 days of flooding.

<table>
<thead>
<tr>
<th>\textsuperscript{15}N fraction\textsuperscript{a}</th>
<th>Crowley</th>
<th>Yolo</th>
<th>Coimbatore</th>
<th>Cuttack</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH\textsubscript{4}\textsuperscript{+}-N\textsuperscript{2}</td>
<td>6.7a</td>
<td>2.4c</td>
<td>5.4b</td>
<td>5.1b</td>
</tr>
<tr>
<td>Organic N</td>
<td>10.9b</td>
<td>8.8c</td>
<td>14.1a</td>
<td>9.5c</td>
</tr>
<tr>
<td>Total N recovered</td>
<td>17.6b</td>
<td>11.2d</td>
<td>19.5a</td>
<td>14.6c</td>
</tr>
<tr>
<td>N unaccounted for</td>
<td>82.4</td>
<td>88.8</td>
<td>80.5</td>
<td>85.4</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values are means of three replications and represent the percentage of added \textsuperscript{15}N.

\textsuperscript{b} For each N fraction, values with the same letter are not significantly different at P≤0.05.

\textsuperscript{c} Includes urea-\textsuperscript{15}N.

![Graph](https://via.placeholder.com/150)

**Figure 4a.** Gaseous N\textsubscript{2}–\textsuperscript{15}N production in four rice soils amended with labelled urea-N. Application rate was 1200 µg N cm\textsuperscript{-2} or 230 mg N kg\textsuperscript{-1} of soil applied as urea-N enriched with 29 atom \% \textsuperscript{15}N. a Gaseous N\textsubscript{2}–\textsuperscript{15}N in the headspace above floodwater and trapped in the soil, 40 days after application of urea to the floodwater.

![Graph](https://via.placeholder.com/150)

**Figure 4b.** Gaseous N\textsubscript{2}–\textsuperscript{15}N in headspace and gaseous N\textsubscript{2}–\textsuperscript{15}N trapped in soil.

The mechanism of N loss from the urea-N applied to the floodwater in experiment 1 was as follows. Urea was diffused from the floodwater to the underlying soil, where it was hydrolyzed into NH\textsubscript{4}\textsuperscript{+}–N. The NH\textsubscript{4}\textsuperscript{+}–N for Crowley, Cuttack, Yolo, and Coimbatore soils, respectively (Fig. 4b). These results suggest that measuring headspace gases to determine gaseous fluxes can underestimate the overall gaseous N\textsubscript{2} production by denitrification (Lindau et al. 1988). Including both soil-entrapped and headspace gases, N\textsubscript{2}–\textsuperscript{15}N rate losses were in the range 0.66–1.64 µg N\textsubscript{2}–\textsuperscript{15}N cm\textsuperscript{-2} day\textsuperscript{-1} after an initial 7-day lag phase for the four soils. In experiment 2, the total N\textsubscript{2}–\textsuperscript{15}N produced during 40 days of incubation was in the range 26.4 – 65.6 µg N cm\textsuperscript{-2}. Recovery of \textsuperscript{15}N in the acid traps represented only 0.4%–0.9% of the applied urea–\textsuperscript{15}N (data not shown), suggesting that in the second incubation study, NH\textsubscript{4} volatilization was not a major mechanism of N loss. In experiment 1, 75%–80% of the applied urea–\textsuperscript{15}N was lost in the first 40 days, which is equivalent to an N loss of 191 to 204 µg N cm\textsuperscript{-2}. Using the N\textsubscript{2}–\textsuperscript{15}N loss results from experiment 2, it was estimated that 13%–34% of the total urea-N loss within 40 days after the urea addition in experiment 1 was due to denitrification, with the rest due to NH\textsubscript{4} volatilization.

In experiment 1, NH\textsubscript{4} volatilization probably played a significant role in the N loss, as indicated by the rapid increase in the floodwater pH, rapid increase in NH\textsubscript{4}–\textsuperscript{15}N in the floodwater, and rapid decrease in overall \textsuperscript{15}N recovery during the first 8 days after the urea addition. A further indication of the importance of NH\textsubscript{4} volatilization is the 7-day lag phase in N\textsubscript{2}–\textsuperscript{15}N production (denitrification) after the urea addition in experiment 2. In studies by Vlek and Craswell (1979), using closed forced-draft systems, NH\textsubscript{4} volatilization led to losses of 50% of the urea within 2–3 weeks. Several studies (Mikkelsen et al. 1978; Vlek and Craswell 1981) have identified NH\textsubscript{4} volatilization as a key mechanism regulating losses of urea applied to floodwater enriched with algae. Between 40 and 70 days after the urea addition in experiment 1, gradual decreases in soil NH\textsubscript{4}–\textsuperscript{15}N, and negligible amounts of floodwater NH\textsubscript{4}–\textsuperscript{15}N, indicated that the remaining \textsuperscript{15}N loss (3%–7%) was due to nitrification–denitrification reactions. Lindau et al. (1988) found that nitrification–denitrification was a much slower process than NH\textsubscript{4} volatilization, with only 5% of the urea–\textsuperscript{15}N applied to the flood. Crowley soil transformed into gaseous N\textsubscript{2}–\textsuperscript{15}N in 10 days, while 44% of the urea–\textsuperscript{15}N applied was transformed to gaseous N\textsubscript{2}–\textsuperscript{15}N in 33 days. In another study, Buresh and Austin (1988) measured the (N\textsubscript{2}+N\textsubscript{2}O)–\textsuperscript{15}N flux in the headspace of flooded, fallow rice soil, 17 days following urea addition to the floodwater. Their results indicated that the (N\textsubscript{2}+N\textsubscript{2}O)–\textsuperscript{15}N flux accounted for less than 1.1% of the applied urea–\textsuperscript{15}N, while the total gaseous loss (as measured by \textsuperscript{15}N balance) estimated from the N unaccounted for was 40% of the applied urea–\textsuperscript{15}N. In the present study, soil-entrapped N\textsubscript{2}–\textsuperscript{15}N accounted for up to 65% of the total N\textsubscript{2}–\textsuperscript{15}N produced by denitrification, which may explain the lower denitrification rates found in the literature.
was diffused both into the water column and the underlying soil column. During the 1st week after application, depending on the physico-chemical environment at the floodwater—soil interface, about 50% of the urea-N was transformed into NH₃ and probably lost through volatilization. Between 8 and 70 days, the remaining NH₄⁺ –N was probably nitrified in the oxidized surface zone and subsequently denitrified in the reduced zone into gaseous end products.

In this study, the soil types showed statistically significant differences in overall N recovery from the applied urea. The applied urea-¹⁵N recovery was in the range 11.2%–19.5%, in the order Coimbatore > Crowley > Cuttack > Yolo soils, probably due to differences in soil organic C content and cation exchange capacity. Detection of N₂-¹⁵N in the headspace provided direct evidence of urea conversion to N₂ as a result of urea hydrolysis, nitrification, and denitrification.

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