Urea Release by Intermittently Saturated Sediments from a Coastal Agricultural Landscape

Mason D. King, Ray B. Bryant,* Louis S. Saporito, Anthony R. Buda, Arthur L. Allen, Lindsey A. Hughes, Fawzy M. Hashem, Peter J. A. Kleinman, and Eric B. May

Abstract

Urea-N is linked to harmful algal blooms in lakes and estuaries, and urea-N-based fertilizers have been implicated as a source. However, the export of urea-N-based fertilizers appears unlikely, as high concentrations of urea-N are most commonly found in surface waters outside periods of fertilization. To evaluate possible autochthonous production of urea-N, we monitored urea-N released from drainage ditch sediments using mesocosms. Sediments from a cleaned (recently dredged) drainage ditch, uncleaned ditch, forested ditch, riparian wetland, and an autoclaved sand control were isolated in mesocosms and flooded for 72 h to quantify urea-N, NH₄⁺-N, and NO₃⁻-N in the floodwater. Sediments were flooded with different N-amended solutions (distilled H₂O, 1.5 mg L⁻¹ NH₄⁺-N, 3.0 mg L⁻¹ NH₄⁺-N, 2.6 mg L⁻¹ NO₃⁻-N, or 5.1 mg L⁻¹ NO₃⁻-N) and incubated at three water temperatures (16, 21, and 27°C). Urea-N concentrations in mesocosms representing uncleaned and cleaned drainage ditches were significantly greater than nonagricultural sediments and controls. While flooding sediments with N-enriched solution had no clear effect on urea-N, warmer (27°C) temperatures resulted in significantly higher urea-N. Data collected from field ditches that were flooded by a summer storm showed increases in urea-N that mirrored the mesocosm experiment. We postulate that concentrations of urea-N in ditches that greatly exceed environmental thresholds are mediated by biological production in sediments and release to stagnant surface water. Storm-driven urea-N export from ditches could elevate the risk of harmful algal blooms downstream in receiving waters despite the dilution effect.

Core Ideas

- Elevated urea-N can increase the frequency and toxicity of algal blooms.
- Urea-N initially present in dry sediment does not explain surface water urea-N.
- Elevated urea-N in ditches is likely due to autochthonous sediment production.
- Urea-N in agricultural drainage ditch water is higher than in nonagricultural sites.
- Urea-N is higher at warmer temperatures.

Urea-N can enhance the growth rate or toxicity of certain harmful algal blooms (Thessen et al., 2009; Finlay et al., 2010; Solomon et al., 2010; Belisle et al., 2016). Urea-N concentrations above the relatively low value of 0.06 mg N L⁻¹ have been correlated with high P. fuciformis spp. abundance in Chesapeake Bay tributaries (Glibert et al., 2004). This phenomenon has drawn attention to sources of urea-N to surface water, and elevated urea-N concentrations issuing from agricultural watersheds have led some researchers to suggest a link to the land application of manures and urea-N synthetic fertilizers (Glibert et al., 2001, 2005, 2006; Lomas et al., 2002; Thorén et al., 2003). Indeed, global urea-N fertilizer consumption ballooned 100-fold from the 1960s to the 2000s (Glibert et al., 2006), part of a trend of dramatically increasing fertilizer N use since the mid-twentieth century. Due in part to restrictions on inorganic fertilizers because of security concerns, urea-N comprises perhaps 60% of global N fertilizer use on an annual basis, and this proportion is expected to grow (Glibert et al., 2014). However, although small losses of agriculturally applied urea-N could be biologically important in receiving waters (Glibert et al., 2006), the export of untransformed urea-N fertilizer is likely minor. The rapid hydrolysis of urea-N in soils generally precludes substantial export from fertilizer applications (Fisher et al., 2016). Losses via leaching and overland flow are brief and typically small even under heavy rainfall conditions (Han et al., 2015; Kibet et al., 2016). In a synoptic watershed study on the Delmarva Peninsula, Tzilkowski (2013) found no evidence that storms in the weeks after poultry manure application led to increased urea-N in streams. Additionally, the highest observed urea-N concentrations (up to 0.220 mg L⁻¹) in the watershed were typically measured in small drainage ditches during nonfertilization periods in summertime when flows were stagnant and ditches were hydrologically disconnected from regional groundwater. Over the sampling period of March 2010 to June 2011, urea-N concentration in ditch drainage water (0.067 mg L⁻¹) was significantly higher than in the shallow groundwater system below the ditch (0.026 mg L⁻¹), indicating the potential for autochthonous (i.e., in situ) urea-N generation associated with warm, nutrient-rich conditions within the ditch itself (Tzilkowski, 2013).

Copyright © American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. 5585 Guilford Rd., Madison, WI 53711 USA. All rights reserved.

doi:10.2134/jeq2016.08.0304
This is an open access article distributed under the terms of the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
Received 22 Aug. 2016.
Accepted 5 Feb. 2017.
*Corresponding author (ray.bryant@ars.usda.gov).

Abbreviations: HDPE, high density polyethylene.
Nearly half of the land on the Delmarva Peninsula is used for agricultural production (Denver et al., 2004), and the southern portion of the peninsula is low-relief coastal plain that relies on vast networks of drainage ditches to make fields arable (Needelman et al., 2007a; Vadas et al., 2007; Maryland Department of Agriculture, 2015). Ditches are hydrologically dynamic, transitioning from lotic to lentic conditions when the water table drops, and often drying out mid-summer through autumn (Needelman et al., 2007a; Strock et al., 2007; Vadas et al., 2007). Sedimentation of eroded soil and organic matter requires periodic dredging of ditches to maintain hydrologic function (Smith and Pappas, 2007). Ditch cleaning results in removal of organic matter, plants, and microbial communities that facilitate biogeochemical cycling (Smith and Pappas, 2007; Strock et al., 2007; Taylor et al., 2015). Agricultural drainage ditches in this area are cleaned as needed to remove vegetation and sediment and reestablish the original depth. On average, cleaning occurs every 10 yr. Ditches have received growing attention for their dual role as conduits for N export (Schmidt et al., 2007) and biogeochemical hotspots (McClain et al., 2003; Needelman et al., 2007b), but urea-N production and transformation in ditches has not been thoroughly characterized.

Growing evidence suggests that biogeochemical N cycling may exert a major control on urea-N concentrations in the environment, including autochthonous generation (Lomas et al., 2002; Siuda and Chróst, 2006; Bogard et al., 2012; Tzilkowski, 2013). An important source of urea-N in marine environments is efflux from sediment (Lomstein et al., 1989; Thomsen and Jähmlich, 1998). Tzilkowski (2013) hypothesized that urea-N released from sediments could explain the high urea-N concentrations found in ditches and wetlands characteristic of agricultural coastal plain headwater basins, but this phenomenon remains untested. Consequently, our objective was to assess the potential for intermittent sediments from agricultural drainage ditches and wetlands to release urea-N to overlying surface waters. Working on the lower Delmarva Peninsula, we used mesocosms to monitor urea-N accumulation in stagnant surface waters overlying sediments collected from representative ditch and wetland environments. We hypothesized that sediments from agricultural drainage ditches and wetlands incubated under warm, N-enriched conditions would yield higher surface water urea-N concentrations than sediments from forested ditches and control sediments consisting of autoclaved sand. To corroborate mesocosm experimental results, we conducted field sampling in selected drainage ditches under analogous conditions.

Materials and Methods

Laboratory Mesocosm Experiments

Site Description and Mesocosm Design

A suite of laboratory mesocosms containing sediments from intermittent ditches in Somerset County, MD, was collected in October 2014 from four different locations. Sites included an uncleaned ditch, a cleaned ditch (i.e., recently dredged), a ditch draining a conifer forest, and a riparian wetland (Fig. 1). Loose, unconsolidated sediments were loamy, consisting primarily of a mixture of soil particles from ditch bank sloughing of silt loam surface horizons and sandy subsoil horizons exposed by dredging. Autochthonous organic matter settled from the water column comprised a minor portion of sediments.

Fig. 1. Sampling sites from which sediment was collected for the mesocosm experiment (green circles) and from which water samples were drawn after a rainstorm filled drainage ditches: uncleaned ditches (red circles) and cleaned ditches (yellow circles). These sites are located on a farm (top right, bottom right panels) and nearby wetland (bottom left panel) in Somerset County, MD, on the Delmarva Peninsula (top left panel).
By combustion, organic carbon contents in the cleaned ditch, uncleaned ditch, forest ditch, and wetland were 13, 25, 48, and 65 g kg⁻¹, respectively. Porosity and density varied considerably across the spectrum of sediment sources, reflecting the difference in organic matter content.

At each site, shallow sediment cores (23.5 cm diam. × 10 cm depth, approximately 4000 cm³) were excavated with shovels and placed intact into 2-gallon high density polyethylene (HDPE) buckets (23.5 cm diam. × 21.5 cm height). Herbaceous plants, if present, were trimmed to approximately 30 cm in height. Control mesocosms were created by autoclaving 4000 cm³ of washed, screened sand (Quikrete play sand, Atlanta, GA), which served as a substitute for sediment. Buckets containing sediments or autoclaved sand (controls) were flooded, thereby serving as open-top, noncirculating mesocosms.

Testing the Effects of Temperature and Nitrogen Enrichment

To evaluate the effect of temperature on urea-N release by sediments, mesocosms were randomly assigned to different water-bath temperature treatments of 16, 21, or 27°C, representing a range of ambient water temperatures during spring, summer, and autumn in the study area. Water baths consisted of modified aquaculture tanks that were partially filled, heated to 21 or 27°C using aquarium baron heaters with electronic temperature controllers or chilled to 16°C using in-line aquarium chillers, and monitored over the course of the experiment.

In addition to temperature treatments, we also sought to test whether flooding sediments with inorganic N–enriched water would affect urea-N release from sediments to the floodwater. Mesocosms were randomly assigned to be flooded with saturating solutions composed of distilled water, 1.5 mg L⁻¹ NH₄⁺-N, 3.0 mg L⁻¹ NH₄⁺-N, 2.6 mg L⁻¹ NO₃⁻-N, and 5.1 mg L⁻¹ NO₃⁻-N solution prepared from NH₄Cl or NaNO₃. The lower concentrations represent values commonly observed in ditch monitoring data, and the higher concentrations are double those values. The factorial design of this experiment is shown in Table 1.

Mesocosm Sediment and N Analysis

Mesocosms were placed in constant-temperature water baths, immediately flooded with 3.0 L of saturating solution, and maintained under continuous, dim, indoor light to minimize primary production in the floodwater. Two 20-mL HDPE scintillation vials were filled with surface water from each mesocosm at 1, 2, 4, 8, 16, 24, 48, and 72 h and stored at 5°C until processing and analysis. The contents of one vial were filtered with an AP40 glass fiber filter (0.7 μm pore size) for the analysis of urea-N; the contents of the second vial were filtered through a cellulose membrane (0.45 μm) for analysis of NH₄⁺-N and NO₃⁻-N. Concentrations of urea-N, NH₄⁺-N, and NO₃⁻-N were determined colorimetrically with a QuikChem 8500 Series 2 Flow Injection Analyzer System (Lachat Instruments) using QuikChem methods 10-206-00-1-B (diacetyl monoxime method) for urea-N, 10-107-06-2-A (salicylate-hypochlorite method) for NH₄⁺-N, and 10-107-04-1-A (Cd reduction method) for NO₃⁻-N (Lachat Instruments, 2003, 2008a, 2014). Ammonium-N was measured as $\sum(NH_4^+-N, NH_3-N)$, and NO₃⁻-N was measured as $\sum(NO_3^--N, NO_2^-N)$.

To assess changes in sediment N content, cores were taken from each mesocosm before experimental flooding and again after floodwater was removed. Cores were collected using a clean, open-faced soil probe (1.27 cm diam.), immediately air dried to ambient laboratory humidity for 72 h, homogenized, and sieved (2-mm mesh). Soluble N was extracted from the sediment with 2 M KCl (Francis and Haynes, 1991; Mulvaney, 1996). The extract was filtered and analyzed for N as described previously, but with an updated NO₃⁻-N method, QuikChem 10-107-04-1-R (Lachat Instruments, 2008b). Urea-N, NH₄⁺-N, and NO₃⁻-N concentrations in extract were used to calculate air-dry sediment concentrations on a mass basis.

Data Analysis and Statistics

The mesocosm experiment was replicated three times for statistical comparisons among treatments, yielding five types of sediment (four sites and a control), three temperature treatments, and five types of saturating solution (75 unique conditions, Table 1). Data were analyzed using SAS 9.3 (SAS Institute, 2011). The normality of data was assessed visually and with the Shapiro–Wilk test. Data were transformed by $\log_{10}(x + 1)$ to normalize main effects in the data set prior to testing for differences among main effects (i.e., site, temperature, and saturating solution). All concentration data reported herein were back-transformed. Differences in mesocosm surface water N concentration were evaluated for each time point using a repeated measures procedure with PROC MIXED and a least squares mean separation procedure (SAS 9.3, SAS Institute, 2011). Differences in initial sediment N concentration among sites were evaluated by analysis of variance using PROC GLM and Tukey’s Studentized Range mean separation procedure. Despite unequal variance among sites (Levene’s Test), sufficient sample size ($n = 45$), balanced groups, and a near normal distribution allowed for robust parametric analysis (Ramsey, 1980). Changes in mesocosm sediment N concentration before and after the experiment were assessed with paired $t$ tests. Linear regressions were used to evaluate the importance of N diffusion from mesocosm sediment to the surface water according to the following model:

$$[\text{surface water } N]_t = m[\text{sediment } N]_0 + b$$

that is, surface water N concentration at time $t$ as a linear function of the N present in sediment prior to the experiment (i.e., 2 M KCl extractable). This regression model was run using surface water concentration data for urea-N, NH₄⁺-N, and NO₃⁻-N, where $t = 8$ h and $t = 72$ h, time points representative of when
mesocosms were judged to be well equilibrated after flooding and when the highest urea-N concentrations were generally found. For all statistics evaluated, results were considered significant at $\alpha = 0.05$.

**Corroborating Mesocosm Results by Field Sampling Stormwater-Filled Ditches**

For comparison with laboratory mesocosm results, five lentic farm ditches (Fig. 1) were sampled following two consecutive summer rainstorms that filled the drainage ditches. Ditches 1, 2, and 3 were uncleaned ditches, and Ditches 4 and 5 were cleaned by dredging in 2014. Automatic sampler hose intakes on Avalanche Transportable Samplers (Teledyne Isco) were housed in perforated 5-gallon HDPE buckets buried in the sediment up to the neck several weeks before deployment to ensure intake submergence and minimal interference from fine sediment and macrofauna. Samplers were programmed to draw 250-mL water samples at time intervals identical to the mesocosm experiment until the ditch dried. Rainfall was recorded over the period of water sampling every 5 min using a Texas Electronics TE525 tipping bucket rain gauge and a CR1000 datalogger (Campbell Scientific, Inc.).

The first of the two storm events started at 11:45 PM EDT on 10 Aug. 2015, depositing 3.8 cm of rain over 9.5 h and rapidly submerging the previously dry, exposed sediments under several centimeters of water. These conditions matched those simulated in the mesocosm experiment, so automatic samplers were deployed in the ditches. The samplers were activated on 11 Aug. 2015, 18 h after the start of the storm. Soon after activation, a second rainstorm occurred, delivering an additional 1.6 cm of rainfall in 4.5 h. No discharge was observed, and the lentic pools were judged to reflect the stagnant conditions simulated in the mesocosm experiment. A YSI EXO2 Multiparameter Sonde was deployed in Ditch 2 for the duration of water sampling to measure water temperature (YSI, Inc.). Water samples were retrieved within 6 d for N analysis as described previously (Lachat Instruments, 2008a, 2008b, 2014).

**Results and Discussion**

**Laboratory Mesocosms**

**Sediment and Surface Water Urea-N Differences by Site**

Mesocosm sediments showed differences in N concentrations before the experiment ($n = 45$) (Fig. 2). Urea-N concentrations in cleaned and uncleared ditch sediments (0.377 and 0.436 mg kg$^{-1}$, respectively) were significantly lower than in forest and wetland sediment (0.906 and 0.794 mg kg$^{-1}$, respectively), and the cleaned ditch was no different than the control (0.234 mg kg$^{-1}$). Average sediment urea-N concentrations (0.234–0.906 mg kg$^{-1}$) were an order of magnitude greater than those observed by Nielsen et al. (1998) in agricultural soils (0.020–0.035 mg kg$^{-1}$). The composition of inorganic N at our sites reflects known patterns in sediments and soils. For instance, N-enriched ditch sediments have low C:N ratios typical of agricultural coastal plain soils (Fisher et al., 2016). Low C:N conditions likely drive higher net N mineralization and nitrification rates, resulting in the N pool being dominated by NO$_3^-$-N (Aber et al., 1995; Chen and Stark, 2000; Hall and Matson, 2003; Schimel and Bennett, 2004). Cleaned ditches have lower organic matter and N (Smith and Pappas, 2007; Shigaki et al., 2009), which explains why the cleaned ditch in our study had the lowest inorganic N concentrations of any site.

Once flooded, mesocosms with sediments from cleaned and uncleaned ditches had significantly higher surface water urea-N concentrations than the other two sites and the control when data were pooled across treatments of temperature and saturating solution (Fig. 3a). There was no consistently significant difference between surface water urea-N concentrations in uncleaned and cleaned ditches. Concentrations of NH$_4^+$-N showed less distinct patterns than urea-N over the course of the experiment (Fig. 3b). Concentrations of NO$_3^-$-N (Fig. 3c) in surface water were significantly higher in cleaned and uncleared ditch mesocosms than other sites, climbing rapidly and peaking at 8 h (11.04 and 13.57 mg L$^{-1}$, respectively), before declining substantially by 72 h (3.96 and 4.41 mg L$^{-1}$, respectively).

Surface water urea-N concentrations were not proportional to initial sediment concentrations (compare Fig. 2, 3). Initial sediment urea-N concentrations were used to estimate urea-N mass in the mesocosms and thereby calculate the maximum potential concentration in the 3.0-L liquid phase should initial urea-N diffuse throughout. Urea-N mass observed in mesocosms surface water over the course of 72 h, expressed as a percentage of the aforementioned estimated maximum potential concentrations, were 20% for the cleaned ditch, 17% for the uncleared ditch, 5% for wetland, 5% for forest, and 7% for the control. This shows that urea-N found in surface water represents a small percentage of that which exists in the sediment. Furthermore, if the mechanism controlling urea-N concentrations in the surface water was simple diffusion from a static sediment urea-N pool, one would expect these percentages to be similar among sites. Linear regression demonstrated that initial sediment urea-N concentrations could not predict urea-N concentrations in surface water at 8 h ($p = 0.014$, $r^2 = 0.03$) or at 72 h when urea-N was highest ($p = 0.417$, $r^2 = 0.00$). Urea-N in surface water did not reflect sediment concentrations to the same extent as mobile NO$_3^-$-N when it peaked at 8 h ($p < 0.0001$, $r^2 = 0.47$), or immobile NH$_4^+$-N when it peaked at 72 h ($p < 0.0001$, $r^2 = 0.17$). Taken together, these data indicate that simple diffusion of a static urea-N pool from sediments to overlying water does not explain surface water urea-N concentrations.

Comparison of urea-N from mesocosms representing different sites generally supports our hypotheses that urea-N can accumulate in surface water overlying flooded sediments that...
denitrification was the mostly likely explanation for the universal decrease in NO$_3^-$-N after 8 h (Qiu and McComb, 1996; Taylor et al., 2015). Denitrification can be inhibited by ditch cleaning (Smith and Pappas, 2007); however, the lack of significant differences in NO$_3^-$-N between the cleaned and uncleaned ditches shows that the denitrification potential of cleaned ditch sediments had recovered in the year since it was dredged.

Effects of Nitrogen Source on Urea-N

Saturating solutions of different N types were used to assess the effect of external N sources on urea-N dynamics in surface water. When results were pooled by temperature treatment and site, we found that saturating N type and concentration had no clear effect on surface water urea-N concentrations in mesocosms, although low concentration NH$_4^+$-N (1.5 mg L$^{-1}$) solution did lead to significantly greater urea-N at 48 and 72 h. Urea-N concentrations were not considerably different whether mesocosms were saturated with distilled water or N solution, and the hypothesis that urea-N accumulation in surface water would be enhanced by enriched NO$_3^-$-N or NH$_4^+$-N was not supported by these data. At the concentrations studied herein, external inputs of dissolved inorganic N to ditches from surface runoff or subsurface flow may not directly affect processes resulting in high urea-N concentrations.

Effects of Water Temperature on Urea-N

Unlike saturating N solutions, water temperatures did appear to play an important role in urea-N accumulation in surface waters. Warmer water temperatures accentuated N dynamics when data were pooled by site and saturating solution ($n = 75$) (Fig. 4). The higher temperatures enhanced urea-N and NH$_4^+$-N accumulation in surface water and accelerated nitrification and denitrification (Ryden, 1986; Qiu and McComb, 1996; Dobie and Smith, 2001). Temperature appears to have affected the rates of biological urea-N production and transformation (Hasan, 2000; Siuda and Chróst, 2006). Baseflow surveys of nearby watersheds showed that urea-N concentrations in shallow waters tend to be higher during warm summer months (Tzilkowski, 2013). During the warm season, biomass and organic N are high, discharge is low (Dukes and Evans, 2006), and sediment production of urea-N is likely to be enhanced (Therkildsen and Lomstein, 1994). The hydrolysis of large portions of newly produced urea-N would explain the accumulation of NH$_4^+$-N at 27°C (Fig. 4b) (Lomstein et al., 1989; Therkildsen and Lomstein, 1994). The data shown in Fig. 4 support the hypothesis that high temperatures lead to significantly higher urea-N concentrations in the intermittent surface water overlying sediments.

Because site and temperature affected urea-N concentration, but saturating solution had little effect, we compared the effect of site at 27°C when data were pooled across saturating solutions. At this smaller sample size ($n = 15$), only the cleaned ditch showed significantly higher urea-N than other sites (data not shown).

The biological origin of urea-N and its rapid transformation in the environment make environmental concentrations notably variable (Berman et al., 1999). Urea-N concentrations measured in mesocosm surface water, ranging from <0.015 to 0.477 mg L$^{-1}$, were similar to values reported for freshwater wetlands, ponds, streams, and ditches (Park et al., 1997;
When urea-N concentration data were pooled across water temperature and saturating solution treatments to show the effect of site (Fig. 3) and across site and saturating solution to show the effect of water temperature (Fig. 4), resultant mean values are relatively low, barely exceeding the 0.06 mg L$^{-1}$ associated with an abundance of *Pfiesteria* spp. (Glibert et al., 2004). However, individual observations of urea-N concentrations in mesocosm water representing ditch sites and high temperatures frequently exceeded 0.12 mg L$^{-1}$, twice the concentration associated with harmful algae blooms.

### Sediment N Changes after Flooding

A comparison of sediment N concentrations before and after experimental saturation for 72 h revealed significant changes in urea-N and NO$_3^-$-N, but not NH$_4^+$-N (Fig. 5), indicating that urea-N and NO$_3^-$-N were actively cycled in the sediments. Sediment urea-N decreased in the uncleaned ditch and increased in the wetland, highlighting urea-N as a dynamic participant in sediment N cycling. The decrease in NO$_3^-$-N at all four sites reflects denitrification in the sediments, with the exception of the biologically inactive control that showed a small increase after being saturated with NO$_3^-$-N–containing solutions. An analogous change in NH$_4^+$-N for control sediments was not detected.

### A Possible Role of Microbes in Urea-N Production and Release from Ditch Sediments

Our objective was to test whether sediment processes control surface water urea-N, but because the urea-N concentrations measured in this experiment reflect a balance between ongoing production and transformation in the sediment–water system, other sources of urea-N to surface water must first be ruled out. Limited light, the short duration of the experiment, and the use of distilled water to flood sediments eliminated potential urea-N sources in the surface water of mesocosms from photodegradation of dissolved organic N (Cornell et al., 1998), plankton excretion (Mitamura and Saijo, 1986; Cho et al., 1996), and macrofauna (Schmid, 1968; Regnault, 1987; Boucher and Boucher-Rodoni, 1988; Lomstein et al., 1989). By using mesocosms, we ruled out nonsediment sources of urea-N to surface water, so the most likely explanation for elevated urea-N concentrations is release from sediment. Sediments turn over urea-N rapidly (Lomstein et al., 1989; Therkildsen and Lomstein, 1994), and urea-N lost from the sediment matrix by diffusion can accumulate in surface water, where transformation rates are low (Delaune and Patrick, 1970; Thomsen and Jähmlich, 1998; Lomas et al., 2002; Thorén, 2007). Macrinovertrebrates and microbes can produce urea-N in marine and coastal sediments (Lomstein et al., 1989; Pedersen et al., 1993). We did not quantify the urea-N production rates of these respective groups, but we conjecture that microbes dominated the biomass of the sediments studied and correspondingly...
drew the dynamic changes in urea-N observed in surface water. The result that warm (27°C) temperatures were associated with significantly higher surface water urea-N supports this line of reasoning since microbial metabolism generally increases with temperature at the range of temperatures evaluated in our experiment. Nitrate dynamics showed a strong temperature-dependent response, and we speculate that temperature could similarly affect microbial metabolic pathways yielding urea-N. This explanation would account for the high concentrations of urea-N observed, especially since the ditch environment is characterized by shallow surface water in contact with a large area of biologically active sediment (Needelman et al., 2007b), and solutes in surface water could be further concentrated as water is lost to evaporation. These conditions are conducive to the accumulation of high urea–N concentrations from sediment release.

**Corroborating Mesocosm Results by Field Sampling**

Stormwater-Filled Ditches

Considerable increases in urea-N concentrations were observed in several farm drainage ditches hours to days after two consecutive storms filled the previously dry ditches (Fig. 6a). Out of the five ditches sampled, Ditches 1, 3, and 5 showed sustained increases in urea-N (100–291%) from 42 h until drying up completely. The highest sustained urea-N concentration (0.481 mg L⁻¹) was in a cleaned ditch (Ditch 5). Indeed, cleaned ditch sediment released the highest average urea-N in the mesocosm experiment, but this difference was only significant at 27°C. In situ maximum daily water temperatures following the storm were approximately 27°C, and mean water temperature was 23.5°C, approximating the 27°C condition in the mesocosm. The wide range in urea-N concentrations in field measurements was congruent with the range in measurements observed in the mesocosms.

The uncleaned ditches agree with typically low NH₄⁺-N under baseflow conditions (Fig. 6b) (Dukes and Evans, 2006; Strock et al., 2007; Tzilkowski, 2013), with the exception of the anomalously high NH₄⁺-N concentrations in Ditch 1 and Ditch 2 at 19 and 18 h, respectively. Higher NH₄⁺-N in cleaned ditches may reflect sediment release processes (Shigaki et al., 2009). Besides sediment release, N mineralization, including hydrolysis of urea-N, could have contributed to elevated NH₄⁺-N in Ditch 5. Notably, both the mesocosm experiment and field sampling showed relatively high urea-N positively associated with higher NH₄⁺-N.

Nitrate–N concentrations tended to peak during or shortly after the second rainfall event and decrease until ditches were dry (Fig. 6c). Storms can deliver pulses of NO₃⁻-N to ditches from the fields they drain (Schmidt et al., 2007; Vadas et al., 2007), adding substantially to nitrification in aerobic ditch water (Qiu and McComb, 1996), followed by rapid denitrification (Peterson et al., 2001; Tzilkowski, 2013; Taylor et al., 2015). Ditch 5, the site with higher urea-N and NH₄⁺-N, showed a lower NO₃⁻-N concentration than other sites and no increase from the second rain event. It is difficult to draw robust conclusions from this single observation, but it is notably consistent with mesocosm results that showed increasing urea-N levels coinciding with declining NO₃⁻-N due to denitrification.

The results of field sampling show that the accumulation of urea-N in situ is accurately reflected in isolated mesocosms. Mesocosm results were consistent with the changes in inorganic N concentrations over time as well. This was most clear in Ditch 5, suggesting a positive association between urea-N and NH₄⁺-N and a negative association between urea-N and NO₃⁻-N. The processes controlling these three forms of N are not necessarily coupled, but their respective abundance does characterize biogeochemical dynamics in drainage ditches when high urea-N concentrations are found. Similarities between the mesocosm experiment and field sampled conditions include (i) exposure, dry ditch sediment that was flooded over a short time, (ii) hydrologic disconnection of ditches from deep groundwater, and (iii) relatively warm summer temperatures. Assuming that the amount of urea-N delivered in rainwater is inconsequential, the only possible remaining sources to the ditches studied are either transport from crop fields or autochthonous production in sediments. Two lines of evidence weigh in favor of the latter. First, given the rapid hydrolysis of urea-N in soil (Cartes et al., 2009; Fisher et al., 2016, Kibet et al., 2016), all of the urea-N that would have been applied to crops in the spring would have
been transformed in the two or more months elapsed since application. Second, the most striking increases in urea-N concentration occurred from 42 to 66 h and beyond (Fig. 6) instead of before or shortly after the last rain fell at 23 h. Although limited, these field data further support our conclusion from mesocosm data that urea-N in ditch surface water is the result of in-sediment production and release.

Environmental Implications

Whereas the lower limit of urea-N concentrations associated with abundance of harmful organisms (>0.06 mg L⁻¹) is relatively low (Gilbert et al., 2004), urea-N in precipitation can represent a substantial proportion of the organic N load delivered to coastal waters (e.g., Cornell et al., 2001; Jickells et al., 2013). Measured rainwater urea-N concentrations in the study area ranged from 0.03 to 0.04 mg L⁻¹ (Tzilkowski, 2013). Therefore, only small additions of urea-N from terrestrial sources are needed to exceed urea-N concentrations controlling noxious microplankton such as Pfiesteria spp. At high temperature conditions, we observed maximum urea-N concentrations up to 0.48 mg L⁻¹ in an individual mesocosm, as well as during field monitoring. If such high levels are widespread in a given watershed, then the export of this urea-N load could raise Chesapeake Bay tributary concentrations to the range at which Pfiesteria spp. outbreaks can be expected (>0.06 mg L⁻¹). Tzilkowski (2013) demonstrated that watershed urea-N loads are elevated during stormflow and suggested that storms flush urea-N downstream from the lentic ditches and wetlands that dominate coastal plain headwaters. In her study, the highest recorded peak urea-N concentration in stream flow (0.216 mg L⁻¹) occurred in a second-order, non tidal stream following Hurricane Irene in August 2011. Additionally, in a nearby Chesapeake Bay tributary (Chicamacomico River), high concentrations of urea-N were reported following storm events in June 1999 and in August of 1999 and 2001 (Gilbert et al., 2006). Agricultural sources of urea-N, such as poultry litter and urea fertilizer, are applied in March and April in this area of the Delmarva Peninsula (C.L. Fykes, J.O. Miller, and J.W. Lewis Jr., personal communication, 2016), and the complete hydrolysis of urea-N ranges from less than 24 h up to several days, well before the aforementioned summer rain events (Cartes et al., 2009; Fisher et al., 2016; Kibet et al., 2016). Thus, autochthonous urea-N production in drainage ditches under warm summer temperatures can explain elevated watershed urea-N concentrations better than the transport of untransformed amendments to crop fields.

The majority of urea-N production and transformation in coastal sediments is due to bacterial metabolism (Vogels and Van der Drift, 1976; Lomstein et al., 1989; Pedersen et al., 1993). We propose that bacterial metabolism in drainage ditch sediments is similar and that urea-N release from sediment explains the high concentrations in drainage ditches. The association of high urea-N concentrations with high temperatures also suggests a microbial source. Additional ditch monitoring and experimental studies are needed to quantify urea-N production and transformation in intermittent headwater sediments, to assess the prevalence of elevated urea-N concentrations in the landscape, to gauge the risk to water quality downstream, and to develop ditch management strategies to reduce urea export from agricultural ditches.

Acknowledgments

Special thanks are extended to the USDA-NIFA Capacity Building Grant Program, which funded this research, and to our USDA-ARS collaborators. We also thank the following UMES staff and students who assisted with data collection: Janice Donohoe, Peter Sang, Amon Kiptrotich, Tracie Bishop, Don Mahan, Earle Canter, Nancy Chepkeuter, Corrie Cotton, Sabrina Klick, Solomon Kirongo, Tedra Barker, Marvin Webb, Wilmelie Cruz-Marrero, Nelson Kimutai, Derrick Cheruiyot, Jennifer Ossai, Caitlin LaComb, Peter Kim, and Wahed Abdullah.

References


