Measurement of Net Nitrogen and Phosphorus Mineralization in Wetland Soils Using a Modification of the Resin-Core Technique

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A modification of the resin-core method was developed and tested for measuring in situ soil N and P net mineralization rates in wetland soils where temporal variation in bidirectional vertical water movement and saturation can complicate measurement. The modified design includes three mixed-bed ion-exchange resin bags located above and three resin bags located below soil incubating inside a core tube. The two inner resin bags adjacent to the soil capture NH_4^+, NO_3^-, and soluble reactive phosphorus (SRP) transported out of the soil during incubation; the two outer resin bags remove inorganic nutrients transported into the modified resin core; and the two middle resin bags serve as quality-control checks on the function of the inner and outer resin bags. Modified resin cores were incubated monthly for a year along the hydrogeomorphic gradient through a floodplain wetland. Only small amounts of NH_4^+, NO_3^-, and SRP were found in the two middle resin bags, indicating that the modified resin-core design was effective. Soil moisture and pH inside the modified resin cores typically tracked changes in the surrounding soil abiotic environment. In contrast, use of the closed polyethylene bag method provided substantially different net P and N mineralization rates than modified resin cores and did not track changes in soil moisture or pH. Net ammonification, nitrification, N mineralization, and P mineralization rates measured using modified resin cores varied through space and time associated with hydrologic, geomorphic, and climatic gradients in the floodplain wetland. The modified resin-core technique successfully characterized spatiotemporal variation of net mineralization fluxes in situ and is a viable technique for assessing soil nutrient availability and developing ecosystem budgets.

Abbreviations: dw, dry weight; SRP, soluble reactive phosphorus; TC, total carbon; TN, total nitrogen.

Nutrient mineralization, the conversion of organic to inorganic forms, is a critical biogeochemical process in ecosystems (Binkley and Hart, 1989). The production of ammonia and orthophosphate from organic N and P, respectively, provides the preferred forms of nutrients for biotic uptake (Schimel and Bennett, 2004). Ammonification also is a rate-limiting step in coupled N transformations, from organic N to ammonia and then to nitrate via nitrification. In moist environments with low loading rates of nitrate, these coupled N cycling processes are essential to enhancing nitrate availability for denitrification that results in the permanent removal of N from the ecosystem (Reddy and Patrick, 1984).

Conversion of solid-phase organic to dissolved inorganic nutrients also has important implications for transport processes in ecosystems and landscapes. Particulate nutrients in low-energy environments, from upland soils to aquatic sediments, are typically less mobile than dissolved nutrients due to the faster settling rates of particles than solutes. As a result, mineralization with subsequent leaching from depositional wetland soils is likely more important than particulate nutrient erosion for regulating outputs from standing stocks of soil nutrients. Wetlands are widely known as important ecosystems for controlling water-quality processes in fluvial networks (Johnston, 1991). Floodplain wetlands in particular can have high

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sedimentation rates and depositional fluxes of N and P (Craft and Casey, 2000; Noe and Hupp, 2005; Olde Venterink et al., 2006; Kronvang et al., 2009) that can be a large percentage of the annual river load (Noe and Hupp, 2009). However, depositional fluxes are likely not permanent due in part to postdepositional mineralization of this sediment-associated N and P (Surridge et al., 2007; Kronvang et al., 2009). Therefore, identifying the controls and quantifying rates of nutrient mineralization in wetland ecosystems is critical to the understanding and prediction of nutrient transport and retention in landscapes.

Several methods have been developed to measure nutrient mineralization rates in soils (reviewed by Binkley and Hart, 1989). Two classes of methods measure either actual rates by in situ measurements or potential rates by laboratory incubation. Laboratory incubations are optimal for experimental manipulation of controlling factors and direct comparison of potential rates of mineralization in soils from multiple sources by maintaining homogenous experimental conditions. Field measurements generate more accurate rates of mineralization by having fewer disruptions of abiotic and biotic conditions in the source soils, and therefore are more desirable for estimating flux rates in relation to other ecosystem processes and budgets. Isotope labeling of the ammonium or orthophosphate pool in the field followed by measurement of the dilution of the labeled pool over time is considered to generate the least-biased data on gross N and P mineralization rates. However, isotope methods are the most expensive measurement, are logistically challenging, and can have methodological problems with rate estimation, microbial immobilization, and other violations of the assumptions of the method (Walbridge and Vitousek, 1987; Hart et al., 1994; Kellogg et al., 2006). Field incubation techniques span a range from relatively closed to open soil systems. The polyethylene bag (Eno, 1960) or bottle (Verhoeven and Arts, 1987) techniques fully enclose soil in a closed vessel with limited gas permeability and water movement for the duration of the incubation. Closed-top cores retain soil in a coring device with the top of the core tube closed to the atmosphere and precipitation (Raison et al., 1987). In contrast, the resin-core method permits exchange of water and gases with the enclosed soil and thus maintains a relatively "open" soil system that tracks conditions in the soil environment and minimizes feedback of inorganic nutrient accumulation on biogeochemical processes (DiStefano and Gholz, 1986; Binkley and Hart, 1989). However, all methods except isotopic dilution estimate net mineralization, disturb soils at least somewhat, and should only be considered as indexes of nutrient availability (Schimel and Bennett, 2004).

Wetlands are challenging ecosystems for making in situ mineralization measurements due to their highly dynamic hydrology and redox, both important controllers of mineralization rates. Past studies have frequently utilized laboratory incubations (e.g., Updegraff et al., 1995) or closed-vessel field incubations (e.g., Verhoeven and Arts, 1987) to measure mineralization rates in wetland soil. Both techniques are valuable for providing indexes of mineralization potential. By removing a soil sample from its temporally dynamic native conditions, both techniques introduce bias to estimation of actual in situ rates. Closed-vessel field incubations fix soil moisture to the initial condition over the duration of the incubation and greatly limit gas and water exchange with the surrounding system. Laboratory incubations can at best only approximate field conditions. The optimal method for measuring in situ rates of soil-nutrient mineralization in wetlands would be minimally biased by matching the conditions of the surrounding environment, inexpensive, and easy to interpret. I modified the DiStefano and Gholz (1986) design for resin cores in terrestrial soils for use in wetland soils, which can have bidirectional vertical exchange of surface and ground water that varies over time. Here I present the results from a study to test this new method, with the specific objectives of (i) evaluating the performance of the modified resin-core design; (ii) comparing the modified resin core with the polyethylene bag method; and (iii) demonstrating realistic spatiotemporal variation in net mineralization fluxes using the modified resin-core method.

MATERIALS AND METHODS

Modified Resin-Core Technique

The original resin-core design included two bags of mixed-bed ion exchange resin, one on top and one at the bottom of soil enclosed in a vertical core tube (DiStefano and Gholz, 1986). The upper resin bag traps external NH_4^+ and NO_3^- infiltrating the core from above, and the lower resin bag traps internal nutrients transported downward out of the core. Two cores are collected simultaneously, one initial core immediately returned to the laboratory and analyzed for extractable NH_4^+ and NO_3^-, and one resin core incubated in situ for a period of time before removal and laboratory extraction. Net ammonification and nitrification are calculated as the sum of the amount of NH_4^+ or NO_3^-, respectively, in the resin soil core and lower resin bag minus the amount in the initial soil core. Net nitrogen mineralization is the sum of ammonification and nitrification fluxes.

The modified resin-core design for wetlands includes a total of six resin bags capping a soil core enclosed in a vertical core tube (Fig. 1). The two (upper and lower) inner resin bags capture internal NH_4^+, NO_3^-, as well as orthophosphate that are transported upward or downward out of the incubating soil; the two outer resin bags remove external inorganic nutrients transported downward or upward into the resin core; and the two middle bags serve as quality-control checks for the functioning of the two inner and two outer resin bags. Ideally, the two middle resin bags have no detectable inorganic nutrients at the end of each incubation.

Each resin bag consisted of a known mass of roughly 10-g dry weight (dw) equivalent mixed-bed ion-exchange resin beads (Rexyn I-300, Fisher Scientific, Pittsburgh, PA) and a 7.6-cm outer diameter nitrile rubber o-ring to maintain shape (Marco Rubber and Plastic Products, Inc., North Andover, MA) held in an undyed nylon stocking (Lajtha, 1988; Kayser-Roth, Inc., Greensboro, NC). Each bag was closed with two stainless-steel staples. Both the nylon and o-ring had substantial N and P contamination and thus were acid washed before use. The core tube is a 10-cm-long section of 7.8-cm-i.d. thin-wall PVC

pipe that was beveled and sharpened at the bottom to ease insertion into soil. Surficial 0- to 5-cm soil was sampled for both initial and resin core soils by inserting the core tube into the soil to a depth of 7.5 cm, removing the core with enclosed soil, and scraping 2.5 cm of soil out of the bottom of the core tube to make space for the lower resin bags. Three resin bags were placed both on top and below the enclosed soil in the core tube. The outside edge of each resin bag was sealed inside the core with silicone caulk (free of antimicrobial agents; Silicone I, GE, Fairfield, CT), to prevent flow bypass around the resin. Wire was then inserted through holes in the bottom and top lips of the core tube to keep the modified resin core intact and ease core extraction. Each modified resin core was replaced into its soil core hole, after excavating 2.5 cm deeper into the soil so that the top of the modified resin core was flush with the soil surface, and then nearby leaf litter was placed on top of the modified resin core. The modified resin cores were then left in place for roughly 1 mo to incubate before retrieval. In addition to estimating net ammonification, nitrification, and nitrogen mineralization by quantifying changes in extractable NH_4^+ and NO_3^-, net phosphorus mineralization was also estimated by measuring changes in extractable SRP.

Study Location

The modified resin core design was tested in the alluvial floodplain of Difficult Run, in Fairfax County, Virginia. Difficult Run is a fifth-order river in a crystalline Piedmont, urban watershed that is tributary to the Potomac River and the Chesapeake Bay. Mean annual discharge near the mouth (46 m above NGVD 29) of the 148 km^2 watershed is 1.76 $m^3\ s^{-1}$ (62.3 cfs; U.S. Geological Survey, 2007). Research was conducted at Tamarack Park (38°55′57″, 77°17′50″; Fairfax County Park Authority), located mid-watershed. The watershed upstream of this location has an area of 73.9 km^2, a slope of 0.0082, a total of 17% impervious surface, and land use is 32% developed, 43% forested, and 7% crop (Dianna Hogan, USGS, 2010, personal communication). This floodplain site is forested and receives occasional overbank flooding (discharge at mouth > 35 $m^3\ s^{-1}$) of short duration and more frequent backwater flooding. Soils are mapped as Codorus silt loam; fine-loamy, mixed, active, mesic Fluvaquentic Dystrudepts (NRCS, 2008). Dominant tree species included *Acer rubrum* L., *Fraxinus pennsylvanica* Marsh., *Quercus palustris* Münchh., *Platanus occidentalis* L., and *Betula nigra* L., with *Microstegium vimineum* (Trin.) A. Camus being the dominant herbaceous vegetation.

Sampling Design

Five sites were located along a floodplain flowpath, from a crevasse on the natural levee (Site 1), through the backswamp (Sites 2–4), and to a depression near a distributary channel that reconnects to the main channel (Site 5). These five sites include the range in elevation, hydrology, vegetation, soil texture, and organic content found in the floodplain. Corner markers of 1-m^2 square plots were installed at a random location at each site to fit a sampling quadrat that included 100 10- by 10-cm cells. Three modified resin cores were incubated and three initial soil cores were collected from randomly selected unused cells (not taken in pairs) in each of the five plots for each of 12 monthly deployments (for a total of 180 modified resin core incubations).

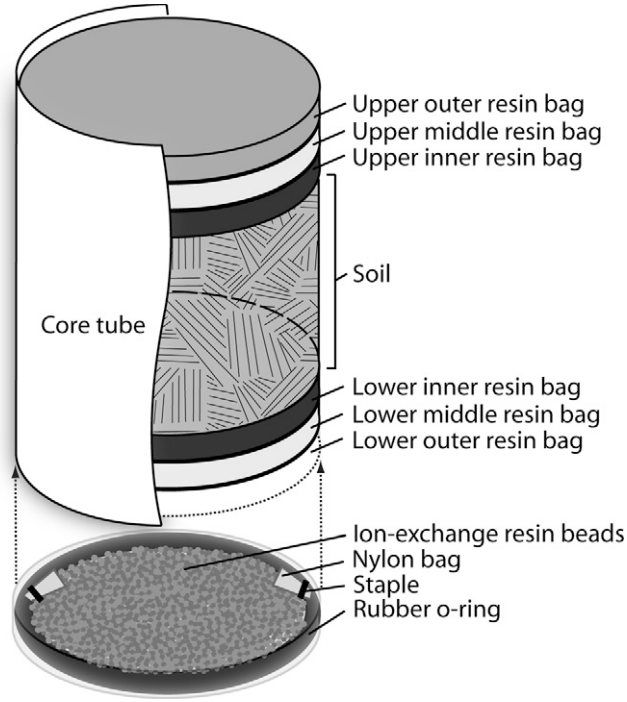

Fig. 1. Cutaway diagram of a modified resin core, in which surficial soil (0–5 cm) and six mixed-media ion-exchange resin bags are incubated inside a core tube. The two outer bags capture any NH_4^+, NO_3^-, or SRP entering the modified resin core from above or below, the two inner bags capture any inorganic nutrients transported out of the soil, and the two middle bags serve as a quality control check on the function of the two inner and two outer bags. A transparent example of an ion-exchange resin bag is shown with its rubber o-ring, ion-exchange beads, nylon bag, and staples.

Incubations began in August 2007, ended in August 2008, and lasted 29 d on average (23–34 d range).

Mineralization rates in the modified resin cores were simultaneously compared with the Eno (1960) buried polyethylene bag method during the June 2008 incubation. For this comparison, three replicate cores of surficial (0–5 cm) soil were collected from random cells in each plot, the soil was gently extruded from the core tube into a sealable 1.75-mil polyethylene bag, and small vertical slices of soil were collected from four locations on the edge of the core to quantify initial soil nutrient concentrations. The bag was sealed and placed in its core hole in the soil, incubated for 1 mo, and then collected and processed similar to the concurrent modified resin cores.

Lab Extraction and Analysis

Initial soil cores and modified resin cores were stored at 4°C overnight until processed the next day. Soils from both core types were removed from the core tubes, immediately weighed, and then homogenized with a spatula. The ion-exchange resin beads from each of the six bags in the modified resin core were removed and weighed. A 4-g dw equivalent mass subsample of field moist soil and 2-g wet weight subsample of resin beads from each of the six resin bags were each placed in a 50-mL centrifuge tube and then extracted with 40 mL of 2*M* KCl for 1 h on a 250-rpm shaking table. Soils and resin beads were subsampled to permit extractions in vessels of manageable size. The samples were then centrifuged at 3000 rpm (soils only) and filtered with a syringe through a 0.2-μm polyethersulfone filter (Pall Corporation,

Port Washington, NY). Extracts were stored at 4°C until analysis of inorganic nutrients on a segmented flow autoanalyzer (Astoria-Pacific, Astoria, OR) within 1 wk. The 2*M* KCl extracts were analyzed for SRP, NO_2^-, NO_3^-, and NH_4+ (Mulvaney, 1996; Reddy et al., 1998). Nitrite concentrations were below detection and we report NO_3^- as NO_3^- + NO_2^-. Every autoanalyzer run included an external reference standard that was diluted with 2*M* KCl and sample blanks (extracts from empty centrifuge tubes or new resin beads in empty centrifuge tubes).

A separate subsample of soil from each initial and modified resin core was measured for gravimetric moisture content (60°C until constant mass). Dried soils from the modified resin cores were then ground and passed through a 1-mm sieve; coarse organic matter was preground with a Wiley mill (Thomas Scientific, Swedesboro, NJ). Ground resin-core soils were analyzed for total carbon (TC) and total nitrogen (TN) (CHN analyzer; Thermo Electron, Milan, Italy) and loss-on-ignition by combusting at 400°C for 16 h (Nelson and Sommers, 1996), followed by microwave-assisted acid digestion, and measurement of total P (ICP-OES; PerkinElmer, Waltham, MA). The remaining soil from initial and modified resin cores was air dried and then analyzed for pH using a 1:2 soil to deionized water slurry (Robertson et al., 1999). Soil texture was measured for a single incubation (January 2008) using the hydrometer method (Gee and Bauder, 1986).

Mineralization Calculations

Mineralization rates were determined by comparing concentrations in the modified resin cores after field incubation to initial soil cores collected at the start of the incubation. Flux rates for modified resin cores were calculated as

(resin-core soil mol + upper inner bag mol + lower inner bag mol − initial soil mol)/duration of incubation

for SRP, NH_4^+, and NO_3^- to estimate net P mineralization, ammonification, and nitrification, respectively. Each of the three replicate modified resin cores in each plot were compared with the average of the three initial soil cores in that plot for each incubation. Fluxes measured using the buried bag technique were calculated as the difference between incubated soil and the initial soil sample within each bag. Net N mineralization was calculated as the sum of net ammonification and net nitrification. To estimate in situ fluxes for the development of ecosystem nutrient budgets and estimating potential inorganic nutrient losses from soil, incubations with net immobilization of N or P (negative mineralization) were not zeroed when calculating annual flux. Flux rates were expressed as mol m^{-2} d^{-1}, mol g^{-1} dw d^{-1}, and mol mol^{-1} d^{-1}.

Statistical Analyses

All variables were tested for homogeneity of variance and homoscedasticity and those variables failing the normality tests were transformed before analysis. An $\alpha = 0.05$ was used for each statistical test, unless indicated otherwise, using SAS 9.1 (SAS Institute, Cary, NJ). To test the efficacy of the modified resin-core design in this wetland, the cumulative amount of SRP, NH_4^+, and NO_3^- in the two middle resin bags was correlated with measurements of conditions in the cores (soil and other resin-bag parameters) using Pearson Product-Moment Correlation with a Bonferroni adjustment to evaluate significance ($P < 0.000794$) for the large number of comparisons. Differences between the modified resin-core and polyethylene-bag methods were tested using factorial two-way analyses of variance, with method and site as the main factors. The influence of spatial (five sites) and temporal variation (12 sequential incubations) in mineralization rates measured by the modified resin cores were tested using repeated-measures ANOVAs.

RESULTS

Site Description

The texture of surficial (0–5 cm) soils in the modified resin cores ranged from sandy loam at Site 1 on the natural levee, to silt loam at Site 2 past the levee, and silt at Sites 3, 4, and 5 in the backswamp (Table 1). Soil-particle size, D50, was smallest in the middle of the flowpath at Site 3. Soil pH generally decreased from Sites 1 to 5. Soil TC, TN, and gravimetric moisture content peaked at Sites 3 and 4, which also had the lowest soil bulk density. Soil TP was greatest at Sites 3 and 5, whereas extractable SRP concentrations in the initial soil cores were greatest at Sites 3 and 4. Extractable NH_4^+ was greatest at Sites 3 and 4, and extractable NO_3^- was greatest at Site 5. Site 1 had very low extractable inorganic nutrient concentrations.

Five overbank floods occurred during the year of modified resin-core incubations, on 20 April, 21 April, 9 May, 11–12 May, and 5 June 2008 (Fig. 2). These overbank events were of short duration, from 2.25 to 18.25 h, in this hydrologically flashy system. Changes in floodplain soil moisture coincided with different discharge regimes. Low soil gravimetric water content in the initial soil cores (bulk soil) occurred during periods of low base flow in late summer and early autumn (August–October 2007), followed by intermediate soil moisture when river discharge increased slightly during late autumn (starting November 2007). Backwater flooding occurred in late winter (February–March 2008), further increasing soil moisture. The overbank flooding occurred in spring (April–June 2008), coinciding with times of greatest soil moisture.

Evaluation of Modified Resin Cores

The two middle resin bags captured a small proportion of the cumulative total amount of resin-trapped nutrients in all six resin bags in each of the modified resin-core incubations. This indicates that the ion-exchange resin in the two inner and two outer bags was capable of trapping nearly all nutrients transported out of the incubated soil and transported into the resin core, respectively, over the duration of the incubations. The two middle resin bags together, on average, held 3.2% of NO_3^- (95% confidence interval: 2.5 to 4.0%, median: 1.4%) and 5.0% of NH_4^+ (3.5–6.6%, 0.9%) extracted from all six resin bags in each resin-core incubation. Slightly more SRP was captured by the middle resin bags, on average 8.7% (7.4–10.0%, 7.0%) of total resin-extractable SRP in each resin core.

The amount of SRP in the middle resin bags was associated with conditions inside the modified resin cores. The number of moles of

Table 1. Soil characteristics at the five Difficult Run floodplain sites. Data shown are mean ± one standard error in parentheses.

Characteristic†	Site 1	Site 2	Site 3	Site 4	Site 5
Bulk density, g dw cm^{-3}	0.98 (0.02)	1.02 (0.01)	0.76 (0.01)	0.78 (0.02)	0.87 (0.02)
D50, μm	108.5 (22.9)	30.3 (5.0)	11.2 (1.6)	13.4 (2.3)	16.7 (3.3)
Clay, %	1.4 (0.3)	4.7 (0.7)	12.0 (1.6)	10.5 (1.9)	8.5 (1.4)
Silt, %	27.6 (4.5)	63.4 (4.6)	84.6 (0.9)	81.9 (2.4)	78.9 (4.0)
Sand, %	71.0 (4.8)	31.9 (5.3)	3.4 (2.2)	7.6 (4.2)	12.5 (5.3)
pH	5.92 (0.05)	5.66 (0.06)	5.54 (0.05)	5.71 (0.06)	5.25 (0.05)
Gravimetric moisture, %	32.9 (1.2)	44.8 (0.7)	62.6 (1.0)	60.9 (1.7)	57.8 (0.9)
Organic content, %	2.8 (0.2)	5.2 (0.1)	9.7 (0.1)	9.5 (0.4)	7.4 (0.2)
Total phosphorus, μmol P g^{-1}	8.2 (0.2)	14.4 (0.2)	24.9 (0.4)	19.1 (0.3)	23.7 (0.6)
Total C, %	1.31 (0.10)	2.16 (0.07)	3.95 (0.14)	4.14 (0.22)	2.88 (0.11)
Total N, %	0.086 (0.009)	0.126 (0.005)	0.248 (0.007)	0.221 (0.008)	0.192 (0.007)
Extractable SRP in initial core, μmol P g^{-1} dw	0.0009 (0.0002)	0.0015 (0.0006)	0.0019 (0.0002)	0.0022 (0.0003)	0.0014 (0.0002)
Extractable NH_4^+ in initial core, μmol N g^{-1} dw	0.05 (0.01)	0.14 (0.04)	0.29 (0.05)	0.27 (0.04)	0.20 (0.04)
Extractable NO_3^- in initial core, μmol N g^{-1} dw	0.13 (0.02)	0.18 (0.04)	0.15(0.03)	0.20 (0.03)	0.33 (0.09)

† D50, median soil particle size; dw, dry weight; SRP, soluble reactive phosphorus.

P of SRP in the two middle resin bags was positively correlated with the moles of SRP in the two inner bags (Pearson Product-Moment Correlation, $n = 180$, all reported $P < 0.001$, $r = 0.607$), the moles of SRP in the two outer bags ($r = 0.472$), P mineralization rate ($r = 0.533$), the moles of NO_3^- in the two outer bags ($r = 0.286$), soil moisture ($r = 0.421$), soil organic content ($r = 0.295$), and soil TN concentration ($r = 0.260$), and was negatively correlated with nitrification rate ($r = -0.302$). In other words, the modified resin-core design performed relatively more poorly for P with greater P mineralization rates, wetter conditions, and with more transport of SRP into and out of the core. The negative association between the amount of SRP in middle bags and soil nitrification rate could be explained by less P being mineralized and transported when conditions were more oxidizing and conducive to the buildup of NO_3^- in these wetland soils.

The number of moles of NH_4^+ in the two middle bags was associated with more NH_4^+ in the two inner ($r = 0.350$) and two outer bags ($r = 0.321$). The number of moles of NO_3^- in the two middle bags was only correlated with the amount of NO_3^- in the two inner bags ($r = 0.349$). Thus, for measurements of N-flux rate, the modified resin-core design also performed relatively more poorly when more inorganic N was in transport into or out of the core.

The dual inner resin bags were crucial to measuring net mineralization fluxes in these wetland soils. The vast majority of net mineralized SRP and NO_3^- was captured on the inner resin bags and did not build up in the soil of the modified resin cores. The two inner resin bags cumulatively captured 1.40 μmol SRP, 7.60 μmol NH_4^+, and 67.64 μmol NO_3^- on average during incubations. In comparison, the average differences in soil extractable nutrients between the modified resin cores and the initial cores were −0.03 μmol SRP (immobilization), 19.20 μmol NH_4^+ (production), and −6.32 μmol NO_3^-. The upper inner bag on average captured 70.7% of SRP and 75.3% of NH_4^+ and the lower inner bag captured 71.8% of NO_3^- of the cumulative amount captured by the two inner resin bags. In other words, SRP and NH_4^+ transport through the soil was mostly upward and NO_3^- transport was mostly downward. The differences in the predominant direction of vertical transport may be due to more SRP and NH_4^+ production and transport upward through the soil during wetter periods with groundwater discharge, and more NO_3^- production and leaching during drier periods with groundwater recharge.

The outer resin bags provide a measure of external loading of nutrients to the surficial soil. The two outer bags cumulatively captured a total of 5.56 μmol SRP, 10.42 μmol NH_4^+, and 29.73 μmol NO_3^- entering the resin cores on average each incubation, or 40.8 μmol SRP m^{-2} d^{-1}, 76.5 μmol NH_4^+ m^{-2} d^{-1}, and 218.3 μmol NO_3^- m^{-2} d^{-1}. Of these combined fluxes to the two outer resin bags, 85.0% of SRP, 47.2% of NH_4^+, and 63.6% of

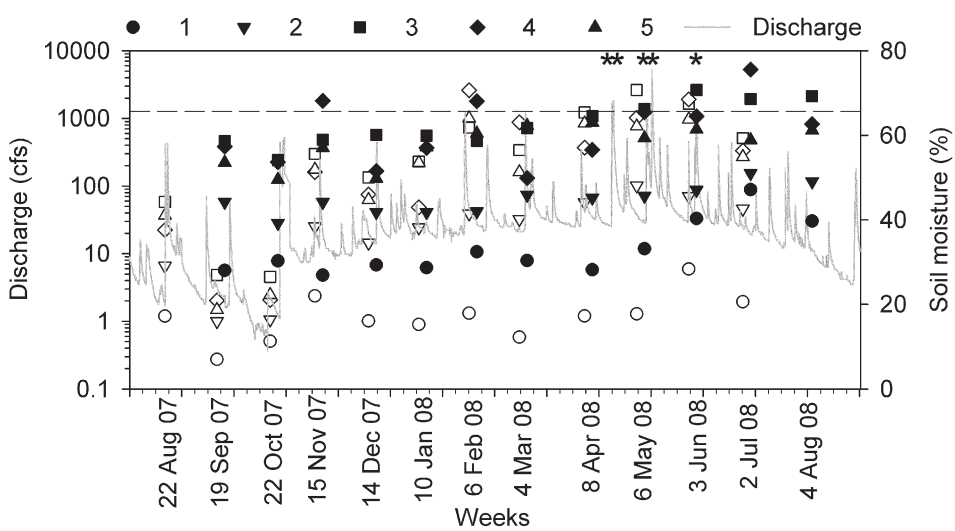

Fig. 2. River discharge, the threshold for overbank floodplain inundation (dashed line), and mean gravimetric soil moisture content in modified resin cores (closed symbols) and initial soil cores (open symbols) from each of the five floodplain sites (1–5, different symbol shapes) through time. Individual overbank flooding events are indicated by asterisks.

NO_3^- was trapped on the upper outer bag. In conclusion, both the inner and the outer resin bags were crucial to the accurate measurement of net mineralization inside the resin cores by capturing both internal and external inorganic nutrients transported out of or into the incubating soil.

Soil moisture in modified resin cores often tracked changes in the surrounding wetland soil. As previously mentioned, soil moisture was dynamic in space and time in response to geomorphic position, seasonal drought, and overbank inundation. The gravimetric moisture content within the modified resin cores typically increased during incubations when soil moisture content in the initial soil cores increased (e.g., incubation ending 15 Nov. 2007), and decreased when bulk soil moisture content decreased (e.g., incubation ending 4 Aug. 2008; Fig. 2). Thus, water could flow into and out of the modified resin cores. Ideally the soil moisture content in the modified resin cores also would be identical to the surrounding bulk soil. This was evaluated by comparing the gravimetric soil moisture content in the modified resin cores at the end of each incubation to the moisture content of initial soil cores collected at the same time for the subsequent incubation. Soil moisture content in modified resin cores was similar to bulk soil when soil moisture was moderate to high or increasing (Fig. 2 and 3). However, soil moisture inside the modified resin cores was greater than the surrounding bulk soil at locations and times with soil gravimetric moisture content < 30% (Fig. 3). In summary, water dynamics in the modified resin cores were similar to the bulk soil under the more typical wetter conditions in the floodplain wetland.

Methods Comparison: Modified Resin Core vs. Polyethylene Bag

The modified resin-core method was compared with the Eno (1960) polyethylene bag incubation method during concurrent incubations from June to July 2008. An overbank flood inundated the floodplain for 7 h on 5 June 2008 during this comparison. The modified resin-core method generated very different P mineralization flux estimates and N mineralization flux estimates than the polyethylene bag incubations (Table 2). Phosphorus mineralization rates were significantly greater, 18 times greater, using modified resin core compared with polyethylene bag incubations. Although there was no interaction between method and location, the backswamp sites in particular had larger mean differences in P mineralization rates between methods due to higher modified resin-core flux estimates. Site mean P mineralization rates estimated using the two techniques were negatively correlated (Pearson Product-Moment Correlation, $n = 5$, $r = -0.883$, $P = 0.047$).

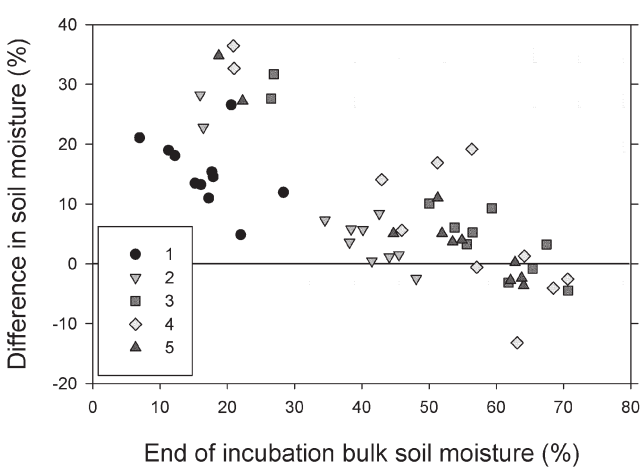

Fig. 3. The difference in mean soil gravimetric moisture content between modified resin cores and initial soil cores collected at the end of each incubation (modified resin core − initial core) relative to moisture content in the initial soil cores at the end of each incubation at each of the five floodplain sites (1−5).

Ammonification rates also were much greater in modified resin core than polyethylene bag incubations but the differences between methods were generally not statistically significant, except at certain locations as indicated by the nearly significant method by location interaction term. The near-levee location (Site 2) and backswamp (Site 4) had much higher ammonification rates in modified resin cores than in polyethylene bags, whereas ammonification in polyethylene bags exceeded that in modified resin cores at the backswamp depression (Site 5). Nitrification rates were similar between incubation methods. Finally, N mineralization rates in modified resin cores were more than twice the rate measured in polyethylene bag incubations but were not statistically different. Nitrogen mineralization rates estimated using the two techniques were not correlated across the wetland ($r = 0.205$, $P = 0.741$). In summary, modified resin-core incubations generated higher flux rates for most inorganic nutrients compared with polyethylene bag incubations, with a significant difference for P and reversal between net NH_4^+ production versus immobilization. The modified resin-core method generally was less spatially variable than the polyethylene bag method as indicated by the variability in N production rates, in particular, among replicates within each site.

The two methods had different responses to changing soil moisture in the wetland. In bulk soil, gravimetric moisture content decreased from the initial soil cores collected at the beginning of the incubations (t_0, mean = 54.7%) to initial cores at the end of the incubations (t_1, 46.8%) that were collected for the subsequent monthly modified resin-core incubations (Fig. 4). It is likely that the overbank flood temporarily increased soil moisture during the concurrent modified resin core and polyethylene bag incubations. Soil moisture in modified resin cores at the end of the incubations (t_1) increased slightly or not at all from values in the initial t_0 cores. In contrast, soil moisture in polyethylene bag incubations increased meaningfully at all sites from the initial t_0 cores to the end of the incubations (t1; Fig. 4). Final soil moisture was greater in the polyethylene bag incubations (67.1%) compared with the concurrent modified resin cores (60.2%; method: $P = 0.081$, site: $P < 0.001$, method × site: $P = 0.520$). Thus, the modified resin cores better tracked temporally varying soil moisture than polyethylene bag incubations.

Soil pH in modified resin cores tracked the increase in pH from t_0 (mean = 5.65) to t_1 (5.75) in initial cores, while

soil pH in polyethylene bags remained static over the incubation at t_0 initial core levels or decreased (Fig. 4). At the end of the concurrent incubations, soil pH in polyethylene bags (5.62) was more acidic than in modified resin cores (5.82; method: $P = 0.018$, site: $P = 0.190$, method × site: $P = 0.775$). Thus, soil pH in the modified resin cores tracked the temporal changes in soil observed in the initial cores but polyethylene bags did not.

Table 2. Comparison of areal net mineralization rates between the modified resin-core and polyethylene-bag methods during the concurrent deployment. Mineralization fluxes are shown as mean ± one standard error in parentheses. *P*-values are presented for factorial two-way ANOVAs with method and site as the main factors.

	Mineralization fluxes		ANOVA		
	Modified resin core	Polyethylene bag	Method	Site	Method × Site
	μmol P m^{-2} d^{-1}	μmol N m^{-2} d^{-1}	*P*		
P mineralization	18.0 (2.6)	1.0 (0.5)	<0.001	0.754	0.200
Ammonification	419 (187)	−12 (243)	0.140	0.489	0.077
Nitrification	205 (131)	259 (269)	0.391	0.065	0.950
N mineralization	624 (212)	247 (357)	0.351	0.213	0.376

Spatial and Temporal Variation in Rates

The rate of net areal P mineralization, prorated per day of the study, averaged 10.05 μmol P m^{-2} d^{-1} (1.14 kg P ha^{-1} yr^{-1}; or 10.09 μmol P m^{-2} d^{-1} when incubations with immobilization were defined as zero flux) over the year of incubations, but varied both among sites and time (Table 3). Mineralization of soil P was greatest at the backswamp depression Site 5, intermediate in the middle of the floodplain flowpath, and least at the levee Site 1 (Table 3). Phosphorus mineralization rates were more than three times greater at Site 5 than Site 1. Spatial variation was generally high within the 1-m^2 plot at most sites, as shown by the scatter in rates among the three replicate modified resin cores that were incubated each deployment (Fig. 5). Phosphorus mineralization rates fluctuated widely through the year, and the sites varied in their temporal dynamics. However, P mineralization was generally greatest from May to July and was slightly elevated in November when soil moisture increased following the dry summer. Low rates of net P immobilization occasionally occurred at several sites. Similar spatiotemporal patterns were found when net P mineralization was calculated on a dry-weight basis (prorated mean = 0.00024 μmol P g^{-1} dw d^{-1}; Table 3). However, P-specific rates of P mineralization, the amount of P mineralized per amount of soil TP, did not change along the floodplain flowpath (prorated mean = 0.00037 mol-P mol-P^{-1} d^{-1}) but were temporally variable.

Net ammonification rates averaged 196.77 μmol-N m^{-2} d^{-1} but also varied widely among sites and time (Table 3). Area-specific rates of net NH_4^+ production were greatest in the middle of the flowpath, Site 3, and 12 times lower at the levee, Site 1. Within-site variation was particularly great during periods of greater ammonification rates (Fig. 5). Net NH_4^+ production peaked in late spring and early summer after overbank flooding events. Ammonia immobilization was common during several incubations. Mass-specific rates of ammonification (prorated mean = 0.0046 μmol N g^{-1} dw d^{-1}) had similar spatiotemporal variation as area-specific rates (Table 3). Nitrogen-specific rates of ammonification (prorated mean = 0.0011 μmol N μmol^{-1} N d^{-1}), the amount of NH_4^+ mineralized per amount of soil TN, were greatest at Site 2, lowest at Site 1, and also varied over time.

In contrast, area-specific rates of nitrification (prorated mean = 450.33 μmol N m^{-2} d^{-1}) varied twofold along the flowpath and were the lowest in the middle of the flowpath and greatest at levee and backswamp ends of the floodplain (Table 3). Mean net nitrification rates exceeded net ammonification rates at all sites except Site 3. Nitrification rates varied through time, but to a

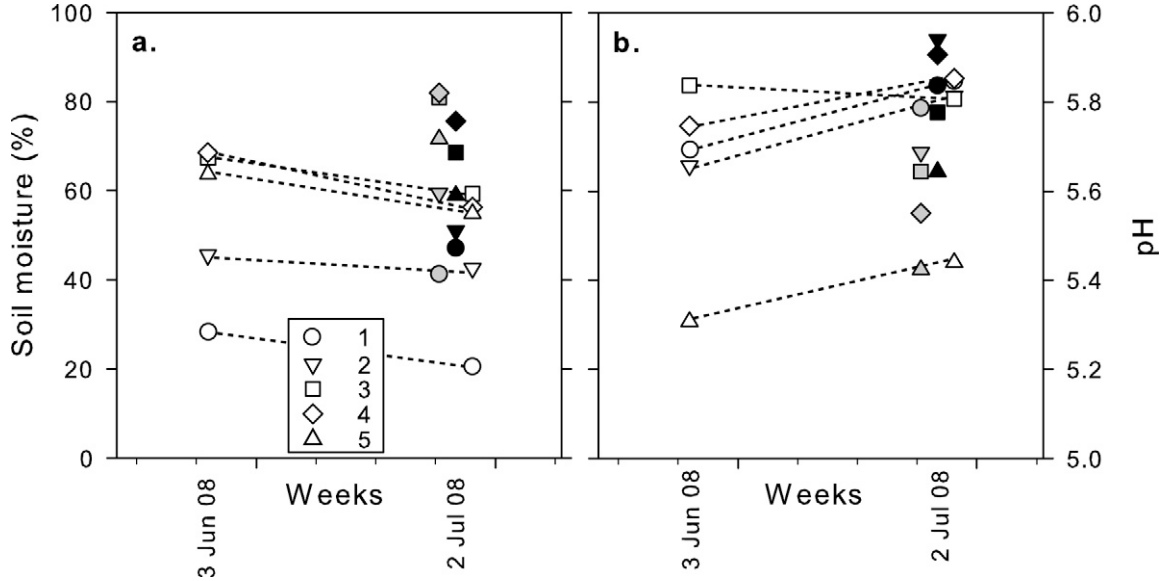

Fig. 4. Comparison of mean (a) soil gravimetric moisture content and (b) soil pH in initial soil cores (open symbols) and concurrent incubations of modified resin cores (closed symbols) and polyethylene bag (gray symbols) at the five floodplain sites (1–5). Dashed lines show the trajectory of soil conditions in bulk soil at each site during the incubations.

Table 3. Spatial variation (Site; five wetland sites) and temporal variation (Time; 12 sequential incubations) in net mineralization rates measured using the modified resin-core method. Mineralization rates are presented in areal-, mass-, and nutrient-specific units. *P* values are presented for repeated measures ANOVAs.

Mineralization	*P*-values			Daily mean†	Incubation mean all sites	Site incubation means				
	Site	Time	Site × Time			Site 1	Site 2	Site 3	Site 4	Site 5
P mineralization, μmol P m^{-2} d^{-1}	<0.001	<0.001	0.006	10.05	10.33	4.42a‡	10.39b	11.47bc	9.83ab	15.54c
Ammonification, μmol N m^{-2} d^{-1}	0.017	<0.001	<0.001	196.8	192. 5	22.3a	217.1ab	264.0b	227.6ab	231.3ab
Nitrification, μmol N m^{-2} d^{-1}	0.023	<0.001	<0.001	450.3	436.8	489.2ab	352.1ab	241.7a	566.0b	535.1ab
N mineralization, μmol N m^{-2} d^{-1}	0.112	<0.001	0.054	647.1	629.3	511.4	569.1	505. 7	793.6	766.4
P mineralization, μmol P g^{-1} dw d^{-1}	<0.001	<0.001	0.007	0.00024	0.00025	0.000096a	0.00021b	0.00030bc	0.00027bc	0.00036c
Ammonification, μmol N g^{-1} dw d^{-1}	0.007	<0.001	<0.001	0.0046	0.0045	0.00051a	0.0043ab	0.0069b	0.0057ab	0.0052ab
Nitrification, μmol N g^{-1} dw d^{-1}	0.008	<0.001	<0.001	0.010	0.010	0.010ab	0.0068a	0.0062a	0.014b	0.013ab
N mineralization, μmol-N g^{-1} dw d^{-1}	0.024	<0.001	0.037	0.015	0.014	0.011a	0.011a	0.013a	0.020a	0.018a
P specific mineralization, mol P mol^{-1} P	0.473	<0.001	0.179	0.00037	0.00038	0.00032	0.00041	0.00034	0.00038	0.00043
N specific mineralization, mol N mol^{-1} N	0.118	<0.001	0.127	0.0042	0.0041	0.0063	0.0041	0.0022	0.0041	0.0037

† Daily mean rates are prorated to take into account the varying duration of incubations.

‡ Means within the same row followed by a different letter are significantly different ($P < 0.05$) based on a Tukey's post-hoc test.

lesser degree than for ammonification, peaking in late summer and early autumn (Fig. 5). Net NO_3^- immobilization occurred occasionally, most often at Site 2. Mass-specific rates of nitrification (prorated mean = 0.010 μmol N g^{-1} dw d^{-1}) and N-specific nitrification rates (prorated mean = 0.0032 μmol N μmol N^{-1} d^{-1}) had similar patterns as area-specific rates (Table 3).

Area-specific rates of net N mineralization, the sum of ammonification and nitrification, did not vary spatially along the flowpath (prorated mean = 647.09 μmol N m^{-2} d^{-1} or 33.1 kg N ha^{-1} yr^{-1}, Table 3; or 679.40 μmol N m^{-2} d^{-1} when incubations with immobilization were defined as zero flux) due to high within-site variation. Nitrogen mineralization did vary through time with the lowest rates in winter and infrequent net immobilization occurring throughout the study (Fig. 5). However, the differing bulk densities among sites led to differences in mass-specific N mineralization rates among sites (prorated mean = 0.015 μmol N g^{-1} dw d^{-1}), with generally more N produced per unit mass near the backswamp than the levee (Table 3). However, N-specific rates of N mineralization did not vary along the flowpath but did vary through time (prorated mean = 0.0042 mol N mol^{-1} N d^{-1}).

DISCUSSION

Method Evaluation

The modified resin core method for use in wetland soils includes six ion-exchange resin bags, of which the two middle bags are used to assess the performance of the resin core. Ideally the two middle resin bags would have captured no ammonium, nitrate, or orthophosphate during an incubation because the two outer bags are designed to remove external nutrients transported into the modified resin core and the two inner bags are intended to trap internal nutrients transported out of the incubated soil. In this method development study, the amount and proportion of inorganic nutrients trapped on the middle resin bags were low compared with the inner and outer resin bags. Thus, both the outer and inner resin bags functioned effectively during the incubations of the modified resin cores, which is essential to the accurate estimation of mineralization rates. The low loading to the middle bags may suggest that they were not necessary to the function of measuring mineralization rate. However, continued use of middle bags is encouraged for assessing resin core performance. This is particularly true for deployments of modified resin cores under circumstances more likely to stress the design.

Relatively greater bypass of nutrients to the middle resin bags was correlated with greater nutrient availability during incubation, suggesting potential saturation of the ion-exchange resin in the inner or outer resin bags. Each of the three inorganic nutrients had more bypass to the middle resin bags when each had greater amounts trapped by the two inner resin bags. Thus, the modified resin-core design may be somewhat less effective with greater mineralization rates and/or more vertical water advection through the core. However, the overall low proportions of nutrients captured by the middle resin bags, on average 3 to 9%, indicate that resin saturation was not frequent or excessive. Because times and locations with relatively poorer performance of the modified resin cores were associated with greater mineralization rates, there is likely some degree of downward bias in estimation of the highest rates. This is also true for net N mineralization due to unmeasured denitrification losses of produced NO_3^-. The mass of resin in the inner and outer resin bags can be increased, or the duration of incubation decreased, for deployment in nutrient- or ion-enriched locations with stronger vertical transport of water through the cores. The bypass problem was minimized by increasing the mass of ion-exchange resin by 50% in both inner bags for incubations in permanently inundated created wetlands (K. Wolf and Noe, USGS, 2010, unpublished data) and by increasing resin mass by 50% in both outer bags for incubations in freshwater and oligohaline wetlands receiving diurnal tidal inundation (Noe, USGS, 2010, unpublished data). Brye et al. (2002) also modified the DiStefano and Gholz (1986) resin-core method to limit upward diffusion of $NO3^-$ in agricultural soils to the lower resin bag. The six-bag modification to the DiStefano and Gholz (1986) resin-core design presented here appears to be robust in conditions where there could be high external nutrient loading, high internal mineralization, and multidirectional water movement through the resin core (conditions that can also occur in terrestrial soils) while permitting quality-control checks of resin-core performance.

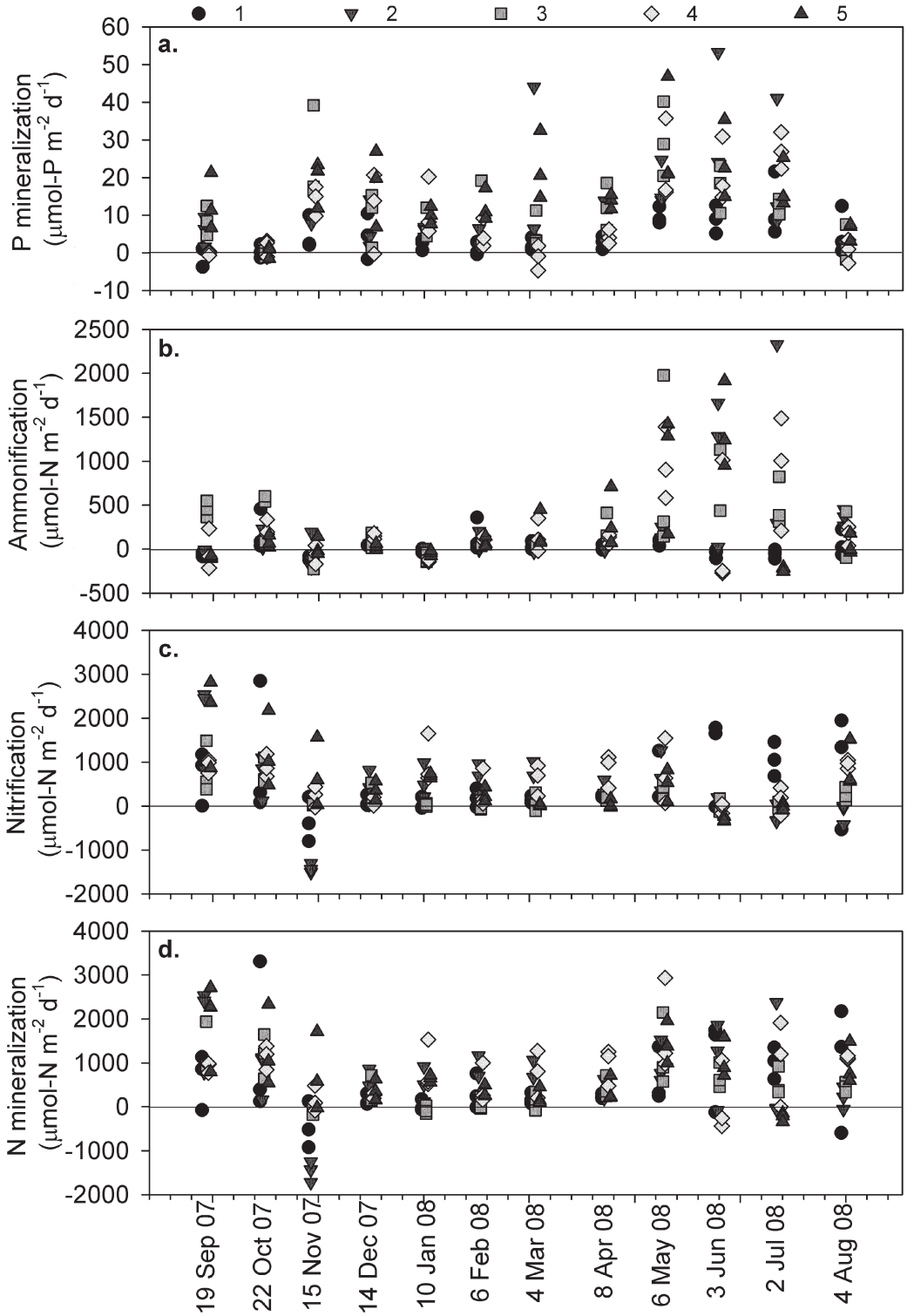

Fig. 5. Net nutrient mineralization rates in replicate modified resin cores at each of the five floodplain sites (1–5) through time: (a) net phosphorus mineralization; (b) net ammonification; (c) net nitrification; and (d) net nitrogen mineralization. Site symbols are slightly offset from their common monthly sampling dates.

The more open design of resin cores allows them to better track temporal trends in soil abiotic conditions than closed incubations (Hart and Firestone, 1989). Soil-moisture content was highly variable over the duration of this study as indicated by the initial soil cores. Throughout the study, modified resin cores mostly succeeded in tracking soil moisture dynamics (Fig. 2). Soil moisture in the modified resin cores increased during wetting periods and decreased during drying periods, suggesting water exchange with surface water, atmosphere, and soil. Soil moisture content at the end of modified resin-core incubations typically was similar to the concurrently high bulk soil moisture found most places throughout the year in the floodplain wetland (Fig. 3). However, soils inside modified resin cores were wetter than surrounding soil if soil gravimetric

moisture content was <30%. Such low soil moisture was found on the natural levee and during seasonal drought. Enhanced moisture inside resin cores may be due to either the lack of plant uptake from severed roots inside the soil core, capture of water by resin bags over the incubation that was released into the resin-core soil, or textural differences between soil and resin beads that created a discontinuity in hydraulic conductivity and limited soil drainage (Hart and Firestone, 1989). During the concurrent incubations of modified resin cores and closed polyethylene bags, the modified resin cores better tracked the decrease in soil moisture and increase in soil pH in undisturbed soils (Fig. 4). Hanselman et al. (2004) also observed greater soil-moisture content in polyethylene bag incubations and to a lesser extent in the standard resin-core design in upland agricultural soils. Similarly, Hart & Firestone (1989) observed dynamic but elevated soil moisture in resin cores and static soil moisture in polyethylene bags that differed from bulk soil during times of changing soil moisture. Given the importance influence of moisture content on soil nutrient mineralization rates (De Neve and Hofman, 2002), the modified resin core design presented here likely provides less biased estimates of in situ net N and P mineralization than closed vessel incubations in wetland soils.

The use of ion-exchange resin bags was crucial to the measurement of P mineralization in our study. Most of the net P production in the modified resin cores was trapped by the two inner resin bags. In fact, on average, a small amount of SRP was immobilized in the soil over the incubations. The closed polyethylene-bag method, which only measures changes in extractable soil nutrients over the incubation, drastically underestimated net P mineralization compared with concurrent deployments of modified resin cores. This underestimation of P mineralization in closed polyethylene bags occurred despite the elevated soil moisture in the bags, which was associated with increased net P mineralization rates (2010, unpublished data). These patterns suggest that any mineralized or desorbed orthophosphate is quickly resorbed to the soil or immobilized by microbes (Froelich, 1988; Walbridge and Lockaby, 1994) unless it is first transported to the ion-exchange resin bags, where it is retained until sampled. Olde Venterink et al. (2002) also concluded that P mineralization could not be reliably assessed using the closed-top core incubation technique, which lacks ion-exchange resin.

Although N fluxes did not statistically differ between modified resin-core and polyethylene-bag incubations, large differences were apparent in mean immobilization vs. production and magnitudes of fluxes. Mean net ammonification rates were negative, or immobilizing, in polyethylene bags, but strongly positive in modified resin cores. Net nitrification rates were similar between the two methods. Mean net N mineralization in modified resin cores were more than twice that of polyethylene bags, but again were not significantly different. Similar to these findings, Hart and Firestone (1989) found two times greater N mineralization rates using resin cores compared with polyethylene-bag incubations in upland mature forest; this difference was attributed to differing moisture regimes and the capability of resin cores to allow natural N leaching compared with enhanced immobilization in closed-bag incubations. Fellman and D'Amore (2007) also observed greater N and P mineralization rates in aerobic lab incubations than with field polyethylene-bag incubations, possibly due to limited gas exchange with polyethylene-bag incubations in the field when inundated. Even with standing water, the more open modified resin cores would allow more natural rates of gas exchange than closed polyethylene bags would.

Comparison to Rates in Other Studies

The modified resin-core technique provided mineralization flux estimates that varied through space and time associated with hydrologic, geomorphic, and climatic gradients in the floodplain wetland (Fig. 5). In other words, the method was responsive to the expected ecosystem controls of mineralization rates. Measured rates of net N mineralization using the modified resin cores over 1 yr in the Difficult Run floodplain also were generally in the range measured by other studies. Area-specific net N mineralization rates varied from 506 to 794 μmol N m^{-2} d^{-1} (0.012 to 0.020 μmol N g^{-1} dw d^{-1}) along the floodplain flowpath in this study. In comparison, N mineralization ranged from 500 to 3200 μmol N m^{-2} d^{-1} (Pinay et al., 1995; incubations over 1 yr), 100 to 2600 μmol N m^{-2} d^{-1} (Wassen and Olde Venterink, 2006; incubations over 1 yr), 1800 to 10000 μmol N m^{-2} d^{-1} (Verhoeven et al., 2001; summer incubation), and 0.03 to 0.12 μmol N g^{-1} dw d^{-1} (Fellman and D'Amore, 2007; summer incubation) in other wetlands using other closed or semiclosed in situ techniques. Bowden (1984) demonstrated that gross N mineralization fluxes were twice as large as net fluxes in wetland sediments using the isotope dilution technique in laboratory incubations; net N mineralization flux for the surficial 5 cm of unmixed sediments was 1560 μmol N m^{-2} d^{-1}.

The modified resin cores produced similar net annual P mineralization rates (4.42–15.54 μmol P m^{-2} d^{-1}, 0.00010–0.00035 μmol P g^{-1} dw d^{-1}) compared with summer incubations by Fellman and D'Amore (2007; 0.00006–0.00059 μmol P g^{-1} dw d^{-1}) but a smaller range than summer incubations by Verhoeven et al. (2001; −1,291 to 12,785 μmol P m^{-2} d^{-1}). Thus, the modified resin cores produced mineralization rates within the range of other in situ studies also conducted over a year. In contrast, the in situ modified resin core incubations generated order of magnitude lower net P mineralization fluxes than 15-d laboratory incubations of wet soils using radioisotope dilutions to estimate gross P mineralization (0.00519 and 0.0116 μmol P g^{-1} dw d^{-1}, Kellogg et al., 2006).

CONCLUSIONS

Like the resin-core method in general (Binkley and Hart, 1989), the modified resin-core method is not inexpensive or effortless. Mixed media ion-exchange resin is expensive and labor requirements are high to build, process, and analyze the

modified resin cores. Nonetheless, the method is arguably less expensive and logistically and analytically simpler than isotopic dilution methods. Isotopic techniques also generally estimate gross mineralization fluxes, whereas the modified resin cores estimate net mineralization fluxes. Gross mineralization fluxes represent microbial activity and exceed net fluxes due to immobilization of inorganic nutrients during incubation (Bowden, 1984). The net mineralization fluxes measured by modified resin cores represent the amount of inorganic N and P produced and persisting in the soil or captured by ion-exchange resin during incubations. Laboratory incubations offer more control to test ex situ factors that influence mineralization rates or to assess variation in mineralization potential among sites. For in situ methods, modified resin cores represent a feasible middle ground for permitting sufficient replication of deployments that accurately characterize spatiotemporal variation of net N and P mineralization fluxes as an index of nutrient availability and potential effluxes in wetland soils.

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