Isotopic signals of summer denitrification in a northern hardwood forested catchment

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Despite decades of measurements, the nitrogen balance of temperate forest catchments remains poorly understood. Atmospheric nitrogen deposition often greatly exceeds streamwater nitrogen losses; the fate of the remaining nitrogen is highly uncertain. Gaseous losses of nitrogen to denitrification are especially poorly documented and are often ignored. Here, we provide isotopic evidence ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) from shallow groundwater at the Hubbard Brook Experimental Forest indicating extensive denitrification during midsummer, when transient, perched patches of saturation developed in hillslopes, with poor hydrological connectivity to the stream, while streamwater showed no isotopic evidence of denitrification. During small rain events, precipitation directly contributed up to 34% of streamwater nitrate, which was otherwise produced by nitrification. Together, these measurements reveal the importance of denitrification in hydrologically disconnected patches of shallow groundwater during midsummer as largely overlooked control points for nitrogen loss from temperate forest catchments.

nitrogen cycle | denitrification | stable isotopes | forested watershed | streamwater chemistry

Many forested catchments export far less nitrogen (N) in streamwater than they receive in atmospheric deposition (1, 2). The rest of the deposited N may accumulate in vegetation or soil organic matter, or be lost in gaseous form. Losses of N to denitrification, the microbial reduction of aqueous nitrate (NO_3^-) to nitrous oxide (N_2O, a greenhouse gas) and N_2 gas, are extremely difficult to measure due to the difficulty in directly measuring N_2 fluxes and due to the high degree of spatiotemporal variability in redox conditions and substrate sources (3). Many past studies using a range of measurements (streamwater nitrate isotopic composition, the acetylene block technique, N_2O emissions, and mass balance calculations) have concluded that denitrification in temperate forests is highly uncertain or generally unimportant (e.g., refs. 4–8).

Nitrogen budgets are particularly perplexing in the northern hardwood forests at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire, USA, where atmospheric deposition has supplied 6–8 kg N $ha^{-1}{\cdot}yr^{-1}$ for half a century, a rate ∼5–10 times preindustrial levels (7–10). Accumulation of N in plant biomass ceased in the early 1990s (10, 11), while streamwater inorganic N export from catchments across the HBEF and nearby streams decreased to <1 kg N $ha^{-1}{\cdot}yr^{-1}$, for reasons that remain elusive (9, 10, 12). These N flux measurements imply increasingly important roles for N gas loss or storage in soil organic matter. However, both processes are so difficult to quantify that the fate, drivers, and consequences of the "missing" N remain unknown, at the HBEF and elsewhere (8–10, 12).

Measurement of the dual isotopic composition of NO_3^- ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) provides a powerful tool to identify NO_3^- sources and to infer its loss to denitrification (13–16). Values of $\delta^{18}O_{NO3}$ differ greatly between NO_3^- in precipitation and NO_3^- produced by nitrification (refs. 13–16, Table S1), which is the microbial oxidation of NH_4^+ to NO_3^-. Measurements of $\delta^{18}O_{NO3}$ have enabled detection of direct contributions of precipitation NO_3^- to streamwater (Table S1), especially during snowmelt, when catchments often release large quantities of NO_3^- (7, 8). However, past $\delta^{18}O_{NO3}$ measurements show that nitrification—not precipitation—supplies the vast majority of NO_3^- in streamwater at the HBEF (10, 17, 18) and other forested catchments (refs. 14 and 19, Table S1).

Nitrate isotopic composition reflects not only NO_3^- sources but also fractionation from a range of processes (14, 20). During denitrification, heterotrophic microbes consume organic carbon using NO_3^- as an electron acceptor under low-oxygen conditions, in a 5:4 molar ratio of carbon:NO_3^-. If NO_3^- is not replenished or consumed by other processes, denitrification progressively enriches both ^{18}O and ^{15}N in the residual NO_3^-, with an O:N fractionation ratio of 0.4–0.7 in the field (14, 20, 21) and up to 1.0 in laboratory studies (22). The fractionation ratio is the slope of the relationship between $\delta^{18}O_{NO3}$ and $\delta^{15}N_{NO3}$. Dual isotopic enrichment and these enrichment ratios provide evidence of denitrification. Dual isotope analysis of NO_3^- has provided evidence for denitrification in large aquifers (e.g., ref. 20) and in drainage waters receiving heavy agricultural N loads (e.g., refs. 21 and 23), whereas recent catchment studies in a subtropical forest (15) and a warm Mediterranean grassland (24) have reported isotopic evidence of denitrification in soil or groundwater. However, dozens of past studies of stream $\delta^{18}O_{NO3}$ and $\delta^{15}N_{NO3}$ in naturally vegetated temperate and boreal catchments (refs. 14 and 19, Table S1), including the HBEF (10, 17), have revealed little if any isotopic evidence of denitrification.

Significance

Denitrification is the most poorly understood process in the terrestrial N cycle. As a result, terrestrial N budgets are wildly unbalanced and our ability to address global nitrogen pollution is fundamentally constrained. Denitrification is controlled by multiple factors, often exhibiting extraordinary variation in time and space, especially in terrestrial environments. Temperate forests regularly receive much larger inputs of precipitation N than they export in streamwater. The fate of the rest has been elusive. We present stable isotope measurements revealing extensive evidence of denitrification from temperate-forest shallow groundwater in midsummer, even as concurrent measurements of streamwater show little sign of denitrification. These measurements support the importance of a disputed nitrogen removal process and its occurrence at a previously missed time and location.

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To investigate the role of denitrification at the HBEF, we measured NO_3^- isotopic composition throughout watershed 3 (WS3), a hydrologic reference catchment drained by Paradise Brook within the HBEF (Fig. 1), during the first two weeks of July 2011, close to the warmest part of the year (Fig. S1). Sampling encompassed nine shallow groundwater wells, a seep, and 19 stream sites along Paradise Brook and its tributaries. The shallow groundwater wells accessed water from saturated soil within the solum above the C horizon at depths between 30 and 115 cm. Three of the nine wells were close to (<2 m) or within the perennial stream channel; the other six were more distal (≥4 m) and upgradient from the perennial channel. Four rain events occurred during the sampling period (0.8–12.3 mm, 26.8 mm total); all contained NO_3^- and NH_4^+ concentrations that exceeded those in streamwater and groundwater by an order of magnitude (Table 1 and Fig. 2 *A* and *B*). Nitrogen export over the study period amounted to 0.006 ± 0.003 kg N ha^{-1}, consisting of 24% NO_3^-, 13% NH_4^+, and 63% dissolved organic nitrogen (DON). Streamflow over the sampling period (5.3 mm) exported less than 2% of rainfall N input (0.335 ± 0.087 kg N ha^{-1}). The remaining 98% was retained within the catchment or lost via denitrification. If this 98% were denitrified in soil or shallow groundwater, it gives a maximum denitrification rate of 5.0 (3.6–6.4) kg N ha^{-1} if extrapolated over the growing season. Although this figure represents the maximum loss of N to denitrification, recent extrapolations of N_2 and N_2O flux measurements from soil cores from the HBEF found denitrification rates during the growing season higher than previous estimates and equal to or higher than atmospheric deposition while follow-up measurements found rates ranging from 4–10 kg N $ha^{-1}{\cdot}y^{-1}$ at HBEF (25).

What Is the Source of Streamwater NO_3^-?

Most streamwater NO_3^- in WS3 appeared to have been produced by microbial nitrification (Fig. 3*A*). That is, stream $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values fell within the expected range for a nitrification source (see *Methods*), similar to past measurements in forested catchments (refs. 14 and 19, Table S1). Streamwater $\delta^{18}O_{NO3}$ values averaged −3.3‰ at baseflow (Table 1), slightly lighter than the theoretically expected value (14, 26, 27) of microbially produced NO_3^- at this site of 1.7 ± 0.1‰, as the combination of one oxygen atom from air ($\delta^{18}O_{O2}$ = 23.5‰) and two from local water ($\delta^{18}O_{H2O}$ = −9.2 ± 0.2‰). This difference is likely due to well-documented kinetic isotope effects during nitrite oxidation and incorporation of water by nitrite reductase, and exchange of oxygen between NO_2^- and water during nitrification (16, 28–30), resulting in consistently low $\delta^{18}O_{NO3}$ values in streamwater at baseflow (Fig. 3*A*). Nitrification occurs in aerobic soils and in the stream itself. Previous studies of stream additions of NH_4^+ below the weir at WS3 (31, 32) and $^{15}NH_4^+$ elsewhere at the HBEF (33) show rapid in-stream NH_4^+ uptake, with uptake lengths of <20–61 m during baseflow conditions. In-stream nitrification can account for 10–100% of NH_4^+ uptake in WS3, and in-stream NO_3^- uptake equals or exceeds in-stream nitrification (32). This past work demonstrates that in-stream recycling rapidly produces and consumes stream NO_3^-, processes that can alter stream NO_3^- isotopic composition to strongly reflect an in-stream nitrification source (19).

Not all stream $\delta^{18}O_{NO3}$ fell within the expected range for a nitrification source, particularly during or immediately after storm events. On 3 July 2011, all streamwater samples had substantially elevated $\delta^{18}O_{NO3}$ values (17.3 ± 3.3‰), reflecting a small (3.7 mm) rainfall event that rapidly contributed NO_3^- to the stream (Figs. 2 *C* and *E* and 3*A*). A two end-member mixing calculation using $\delta^{18}O_{NO3}$ (see *SI Text*) indicated that rainfall contributed 29–34% of stream NO_3^- at the WS3 weir on 3 July, and 5–16% of stream NO_3^- from an event (9.1 mm) on 9 July. Precipitation-derived NO_3^- in streamwater in headwater catchments is typically detected only during snowmelt and is rarely observed during the growing season (Table S1). These results confirm that streams can export pulses of atmospheric nitrate unaltered by microbial cycling even during summer. At WS3, streamwater export of atmospherically derived NO_3^- amounted to just 0.3% of the estimated NO_3^- flux that rained onto the catchment during the 3 July event and 0.1–0.3% of the 9 July event (see *SI Text*). The entire WS3 channel network, including dry channels, spans 1.8% of the catchment area (34), such that the estimated flux of NO_3^- that rained onto this channel area more than sufficed to supply the rainfall-derived NO_3^- observed in stream export at the weir, even if >80% of this channel area were dry during mid-July (see *SI Text*).

Groundwater Denitrification Is Consuming Soil NO_3^-

The isotopic composition of NO_3^- in the six groundwater wells upgradient and away (≥4 m) from perennial streamflow showed substantial enrichment in both ^{15}N and ^{18}O, falling far outside the expected ranges of NO_3^- sources from deposition or nitrification (Table 1 and Figs. 2 *D* and *F* and 3*B*). Samples from these wells showed a strong positive relationship between $\delta^{18}O_{NO3}$

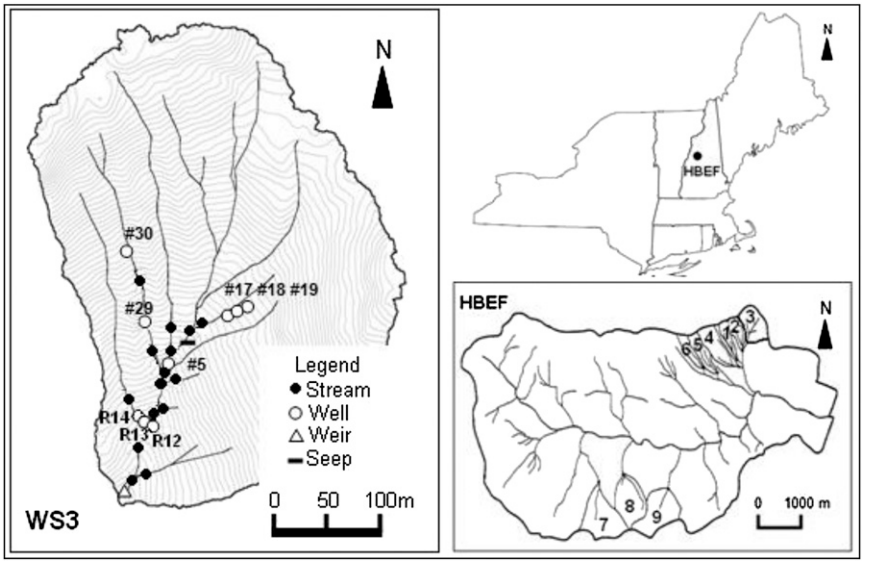

Fig. 1. Location of HBEF in the northeast United States (*Top Right*), showing HBEF watersheds 1–9 (*Bottom Right*) and watershed 3 (WS3; *Left*). WS3 shows drainage network comprising Paradise Brook and tributary channels, with sampling locations from the weir (triangle), streams (filled circles), wells (empty circles; including wells ≥4 m from surface streamflow in July 2011 (JD05, JD17, JD18, JD19, JD29, JD30) and those <2 m (R12, east bank; R13 in-stream; R14, west bank) and a seep (dash). Contour interval is 3 m; elevation range is 537–732 m.

Table 1. Concentrations of NO_3^-, NH_4^+, DON, and DOC (μM), and $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ of water samples from watershed 3, Hubbard Brook Experimental Forest, New Hampshire July 2011

		Concentration						Isotopic composition, ‰	
Sample type	N	NO_3^-, μM	NH_4^+, μM	DON, μM	DOC, μM	DOC:DON, molar ratio	DOC:NO_3^-, molar ratio	$\delta^{15}N_{NO3}$	$\delta^{18}O_{NO3}$
Rainfall	4	27.3 ± 12.4	54.7 ± 12.0	2.4 ± 0.8	N/A	N/A	N/A	−5.1 ± 3.0	59.2 ± 9.7
Seep	6	1.6 ± 0.6	0.8 ± 0.5	11.3 ± 3.0	303 ± 56	26 ± 6	218 ± 103	−1.9 ± 1.0	−2.4 ± 3.3
Wells ≥4 m from stream	21	2.3 ± 2.4	2.3 ± 1.7	8.7 ± 1.8	364 ± 105	41 ± 11	353 ± 271	10.9 ± 5.4	16.7 ± 4.6
Wells <2 m from stream	28	3.4 ± 2.2	1.5 ± 1.0	7.1 ± 2.5	210 ± 59	32 ± 11	92 ± 79	3.3 ± 4.0	2.3 ± 8.4
Streams, 3 July	7	2.4 ± 1.3	0.8 ± 0.2	8.1 ± 3.4	247 ± 43	28 ± 9	129 ± 69	−3.3 ± 3.0	15.9 ± 3.6
Streams, 4–14 July	48	1.8 ± 1.2	1.2 ± 0.8	5.9 ± 2.7	236 ± 96	42 ± 15	197 ± 162	−0.9 ± 2.1	−1.6 ± 2.7
Weir, 4–14 July	8	2.0 ± 1.1	1.0 ± 0.6	5.3 ± 2.4	219 ± 88	43 ± 16	137 ± 83	−1.4 ± 1.8	0.4 ± 3.7

Mean ±1 SD are shown. Values are combined by sample type across sites and dates, except as noted for 3 July.

and $\delta^{15}N_{NO3}$ ($r^2 = 0.68$) with a slope of 0.76. This slope represents the fractionation ratio of O to N (Fig. 3*B*) and serves as a diagnostic of denitrification (14, 20–22). This regression of $\delta^{18}O_{NO3}$ versus $\delta^{15}N_{NO3}$ excluded one sample (Well JD05, 12 July) that had a lighter isotopic composition and higher NO_3^- concentration than the others (Fig. 2 *B* and *D*), although downward extrapolation of the $\delta^{18}O_{NO3}/\delta^{15}N_{NO3}$ relationship directly intersected this sample point (Fig. 3*B*), which could represent a high-concentration NO_3^- end-member before isotopic fractionation by denitrification. The isotopic composition of this sample fell in the expected range for microbial nitrification and may reflect newly nitrified NO_3^-.

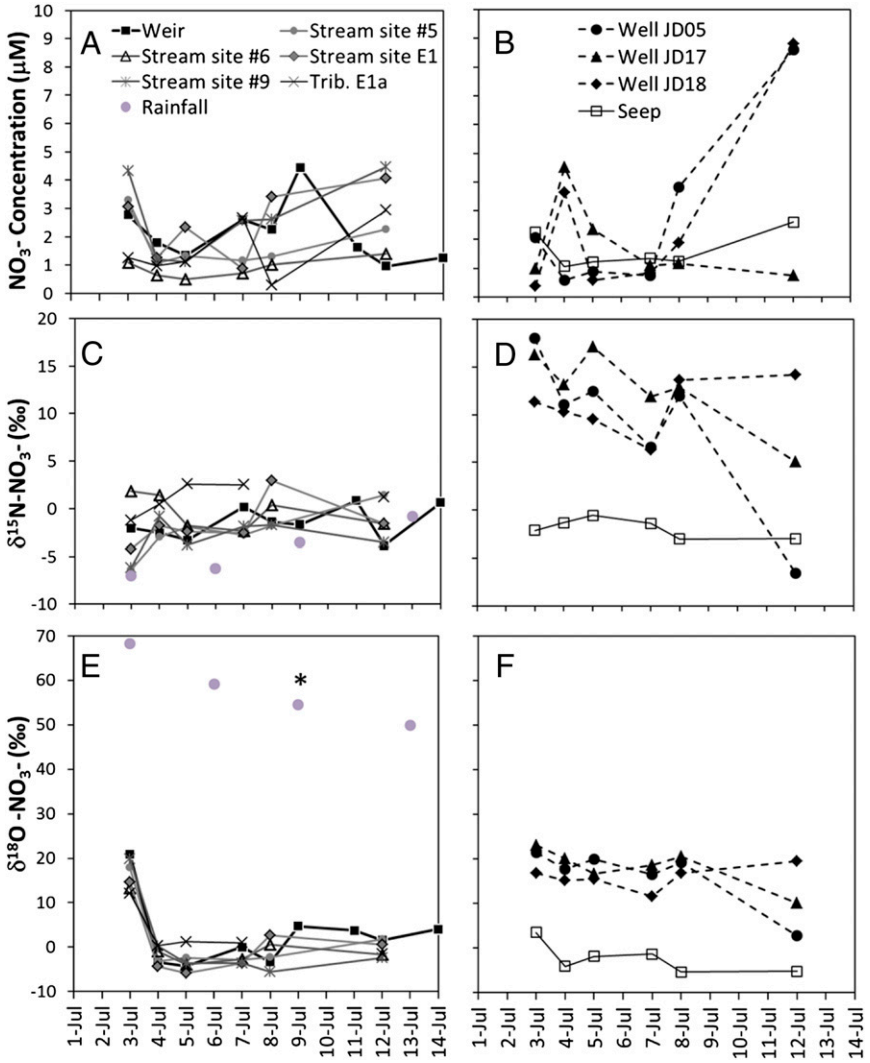

Fig. 2. Temporal pattern of nitrate concentration (*A* and *B*), $\delta^{15}N_{NO3}$ (*C* and *D*), and $\delta^{18}O_{NO3}$ (*E* and *F*) for rainfall and streams (*A*, *C*, and *E*) and for wells (≥4 m from surface flow) and the seep (*B*, *D*, and *F*). Symbols are denoted in *A* and *B* for each site type. The asterisk in *E* denotes an estimated rainfall $\delta^{18}O_{NO3}$ value, as the average of adjacent dates. Isotopic values are expressed per mil (‰) relative to established standards, Vienna Standard Mean Ocean Water (VSMOW) for $\delta^{18}O$ and air for $\delta^{15}N$.

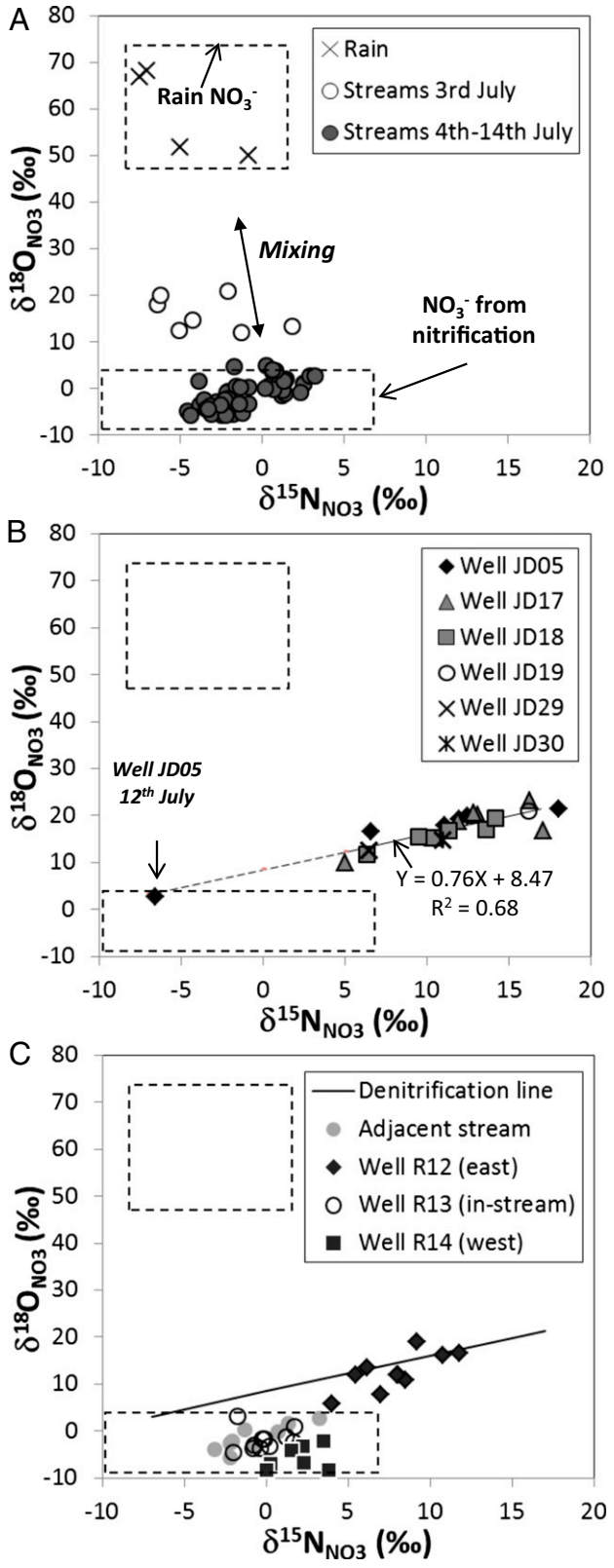

Fig. 3. Isotopic composition of nitrate ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) samples collected during July 2011 in watershed 3, Hubbard Brook, New Hampshire, USA. Measurements are shown from (*A*) rain and streamwater separated by date between 3 July (open circles) and 4–14 July (closed circles); (*B*) wells ≥4 m from surface streamflow, displayed by well, with regression line determined from samples excluding well JD05 on 12 July (solid line) extrapolated to this point (dashed); (*C*) wells <2 m (black) displayed by well, adjacent stream samples (gray), and the denitrification line determined in *B*. Dashed boxes indicate the ranges of isotopic composition of two nitrate sources, rain and microbial nitrification (see *Methods* for explanation). Isotopic values are expressed per mil (‰) relative to established standards, VSMOW for $\delta^{18}O$ and air for $\delta^{15}N$.

If denitrification consumes NO_3^- in a quasi-closed system, either over time (e.g., in a pocket of water in the soil or groundwater) or through space, (e.g., in a body of water flowing along a flow path), and is the only process altering $\delta^{15}N_{NO3}$ values, the progressive consumption of a finite NO_3^- pool yields an increase in the $\delta^{15}N$ of the residual NO_3^- in a quantitative relationship that defines an isotope enrichment factor between product and substrate ($\varepsilon_{p\text{-}s}$ $^{15}N_{NO3}$ ‰), as approximated by a modified Rayleigh closed-system model (20). Measurements from the WS3 wells did not show a clear relationship between $\delta^{15}N_{NO3}$ and NO_3^- concentration, likely due to spatiotemporal heterogeneity in nitrification rates and substrate $\delta^{15}N$ values. If denitrification was occurring in transient patches of saturated soil in the soil profile above the groundwater, sporadic pulses of partially and variably denitrified soil water may have reached each well independently. However, if the 12 July sample from well JD05 (Figs. 2 *B* and *D* and 3*B*) represents the NO_3^- concentration and $\delta^{15}N_{NO3}$ of groundwater before denitrification and the mean NO_3^- concentration and $\delta^{15}N_{NO3}$ of the rest of these samples represent partially denitrified NO_3^-, denitrification produced an isotope enrichment factor of −13‰ (−5‰ to −17‰). This value falls within the literature range for field measurements of denitrification, which average −16‰ and typically range from −6‰ to −23‰ (14, 21, 35). Together, these data indicate the pervasive occurrence of denitrification in soil or shallow groundwater of NO_3^- that had been produced by microbial nitrification, yielding groundwater with very low NO_3^- concentrations (<3 μM).

The three wells in or near (<2 m) the perennial stream showed varied isotopic signals (Fig. 3*C*). The east well (R12) showed isotopic evidence of denitrification similar to the ≥4-m wells. That is, most samples from this well showed evidence of dual isotope fractionation with high $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values that fell near the same denitrification line as was observed in the >4-m wells (Fig. 3 *B* and *C*). However, a few samples fell below this line. Their isotopic composition may be explained most simply as a two end-member mix of partially denitrified NO_3^- in groundwater and the NO_3^- in streamwater. Mixing these two NO_3^- sources would lower both $\delta^{15}N_{NO3}$ and, especially, $\delta^{18}O_{NO3}$ values in these east well samples (Fig. 3*C*). Mixing may be the result of a hyporheic flow path bringing water from the stream to the shallow groundwater in the riparian zone. Nitrate in the west well (R14) did not show isotopic evidence of denitrification, and nitrate in the in-stream well (R13) isotopically resembled nearby streamwater. These measurements exemplify the spatial variability of denitrification and the importance of sampling different water sources to ascertain the occurrence and magnitude of denitrification within catchments.

There have been few direct measurements of denitrification at the HBEF (4–8), and the process has been considered unlikely to affect the unexplained long-term decrease in NO_3^- export (10). These and many other past efforts to detect denitrification in forested catchments have focused on surface soils or streams, but recent measurements of soil gases (O_2, CO_2, CH_4, N_2O) (36) and denitrification enzyme activity (37) at WS3 indicate that deeper soil horizons and shallow groundwater could potentially support significant amounts of denitrification, particularly in summer. The results here show that hotspots (38) for denitrification were identified during the sampling period in shallow groundwater or saturated soil, rather than streamwater, although the isotopic signal of denitrification may have been masked if coupled nitrification/denitrification were occurring in-stream. Denitrification hotspots

may develop with the convergence of NO_3^- and dissolved organic carbon (DOC) produced by surface soils draining to low-oxygen zones in saturated soil and shallow groundwater. Mean growing-season concentrations of both NO_3^- and DOC draining from the forest floor (>20 μM NO_3^-; >1200 μM DOC) and upper mineral soils (Bh horizons; 19–26 cm depth; 8–25 μM NO_3^- and 700–900 μM DOC) at the HBEF (39) are relatively high compared with the groundwater measured here (<3 μM NO_3^-; <400 μM DOC; Table 1), and could supply both NO_3^- and DOC for denitrification in deeper soils or groundwater. Ratios of DOC to NO_3^- in all sample types in this study exceeded 5:4 by more than an order of magnitude (Table 1), indicating that carbon supply sufficed to support denitrification, even if a large fraction of the carbon was refractory. Recent measurements in WS3 show that O_2 concentrations in soil dip in summer coinciding with increases in soil respiration rate, and decrease sharply as water-filled pore space increases above ∼90% (36). This combination of substrates in low-O_2 soil or groundwater provides ideal conditions for denitrification to occur. This study provides evidence of the widespread but fragmented nature of denitrification in saturated soil and shallow groundwater in a temperate forested watershed. The existence of transient, perched patches of saturation in the soil that are poorly connected to streams (40, 41), where N then recycles rapidly (31–33), may explain why previous dual isotope studies based on streamwater samples alone—at HBEF and elsewhere—have shown little to no denitrification signal (Table S1). That is, even as denitrification occurs in saturated patches, these hotspots are connected poorly to surface streamwater (40, 41), which contains NO_3^- produced by local nitrification (Fig. 3*A*). Hydrological disconnection between perched saturated zones and stream channels and denitrification rates may both broadly covary seasonally with the warm, drying conditions of summer (42), creating fragmented patches of saturation (40) at the time when denitrification in these zones may be particularly active (36). Although it is possible that this NO_3^--depleted water is later exported to the stream (15), these data show concentrations of NO_3^- in groundwater equal to or higher than those of streamwater, suggesting that this was not the case at the time of sampling. If these episodes of fragmented denitrification have increased over time, for example, with the increases in summer temperature and precipitation that have been observed at this site (10, 43), then denitrification may have significantly contributed both to the amount of "missing N" in the ecosystem N balance and to the observed changes in this balance over time.

Methods

Site Description. This study focused on watershed 3 (WS3), a 41.2-ha hydrologic reference watershed at the HBEF (43°56′N, 71°45′W). Temperatures average −9 °C in January and 18 °C in July, and annual precipitation averages 1,400 mm, ∼70% as rain (44). WS3 is steep and south facing, with an elevation range of 537–732 m. The HBEF is covered by second-growth northern hardwoods naturally regenerated after harvesting between 1910 and 1917. WS3 is underlain by mica schist bedrock of the Silurian Rangeley Formation, and covered by Wisconsinan glacial tills. Spodosols of sandy loam to loamy sand texture comprise ∼80% of catchment soils, and Inceptisols and Histosols make up the rest (45). The C horizon occurs at ∼70 cm depth and has variable although generally lower hydraulic conductivity compared with the overlying B horizon (40). A shallow groundwater system with a transient saturated zone develops within the solum throughout the catchment (45). A more consistent saturated zone is present in the near-stream region that is typically hydrologically connected to surface water in perennial stream reaches and is at or near the surface beneath ephemeral and intermittent stream reaches (40). WS3 is drained by Paradise Brook, a second-order perennial stream fed by several ephemeral and intermittent tributaries, which together comprise 79% of the stream length within the catchment (45). The quickflow response of Paradise Brook to storm inputs is highly nonlinear, with a large and rapid runoff response observed when thresholds in soil moisture content and storm event size are exceeded (34).

Sample Collection and Analysis. Sampling encompassed 11 locations along Paradise Brook and 8 locations on tributaries, along with 1 seep and 9 shallow groundwater wells selected from >30 wells installed previously in WS3 (34, 40, 41), and chosen for their tendency to provide water during midsummer (40) and ease of repeated sampling. The wells included three wells in or within 2 m of the perennial channel of Paradise Brook and forming a transect across it (R12 on the east, R13 in-stream, and R14 on the west). The other six wells were ≥4 m away and upgradient from surface streamflow during July 2011, and included two wells located within a seasonally dry side-stream channel (JD29, JD30), one well ∼4 m from the perennial stream and generally upgradient from it (JD05), and three wells that form an upslope transect ∼3–29 m from a seasonally dry tributary (JD17, JD18, JD19). Well depth ranged from 30 cm (JD30) to 115 cm (JD05), with most wells accessing water from the lower mineral horizon. Three wells (JD05, JD17 and JD18), the seep, and seven sites on Paradise Brook and its tributaries were sampled on six dates on a near-daily basis (3, 4, 5, 7, 8, and 12 July 2011). The three near-stream wells were sampled up to nine times at 20–40 min intervals on 11 July 2011, along with concurrent sampling from Paradise Brook 0.5 m upstream of the wells. A mix of precipitation and throughfall was collected from a small clearing at the southern WS3 boundary using a rinsed 10-L plastic collector. Stream samples were collected with a cleaned high-density polyethylene collector. Well samples were collected using a peristaltic pump, after purging. Streamflow, rain gauge, and air temperature data were provided by the US Forest Service (44). Streamflow was measured using a sharp-crested V-notch weir at the watershed outlet, and rain was gauged by standard and weighing-recording.

Solution concentrations of NO_3^-, NO_2^-, and NH_4^+ were measured using ion chromatography (Dionex ICS-2000; Dionex Corp.); all NO_2^- concentrations were below the detection limit (0.1 μM). Total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) were measured by combustion with a Shimadzu TOC-V_{CPN} and TNM-1 chemiluminescent detector (Kyoto, Japan). DON concentration was calculated by difference (DON = TDN − NO_3^- − NH_4^+). Samples for isotope analysis were frozen at −20 °C and later prepared at Cornell University using the denitrifier method (46, 47) to produce N_2O gas, which was sent to the Stable Isotope Facility, UC Davis, CA, for analysis of $\delta^{18}O$ and $\delta^{15}N$, with a precision of ±0.3‰ for both isotopes. Three rainfall, eight stream, and nine groundwater samples spanning a range of sites and dates were selected for measurement of $\delta^{18}O_{H2O}$ at the Stable Isotope Laboratory, School of Environmental Sciences, University of East Anglia, on a Picarro L1102-i with a precision of ±0.2‰.

Isotopic End-Member Determination. Expected isotopic values for NO_3^- source end-members were determined through a combination of field- and literature-based estimates. Rainfall NO_3^- was highly enriched in ^{18}O and slightly depleted in ^{15}N (Table 1 and Fig. 3), resembling previous measurements at the HBEF (10, 17). Nitrate produced by microbial nitrification reflects the $\delta^{15}N$ of the NH_4^+ that these autotrophic bacteria consume, as well as any fractionation that occurs during this process. Ammonium can be supplied both by atmospheric deposition and by the much larger flux of mineralization of organic N by heterotrophic microbes (7, 8). We measured $\delta^{15}N$ of soil samples collected from nine soil pits in WS3 (37) using a Finnigan MAT DeltaPlus isotope ratio mass spectrometer (Thermo Finnigan) at the Cornell Stable Isotope Lab. These $\delta^{15}N$ values averaged 1.2 ± 0.4‰ in the surface organic horizons (Oi/Oe) and increased to 5.2 ± 1.1‰ in the Oa horizon and 6.0 ± 1.1‰ in Bh and Bhs horizons at 10–65 cm depth, similar to prior measurements in WS3 (48). Mineralization causes negligible ^{15}N fractionation (14, 49) and so $\delta^{15}N_{NH4}$ from mineralization of this soil organic N should also have this range of ^{15}N values (0.5–7.5‰). Isotopic fractionation during nitrification should be negligible when NH_4^+ supply rate limits the rate of nitrification, but fractionation can occur when nitrification is slow or incomplete relative to the NH_4^+ supply, producing $\delta^{15}N_{NO3}$ values ∼2–20‰ lighter than those of the NH_4^+ substrate (14, 20, 49). At WS3, net nitrification consumes a highly variable fraction of mineralized N, but this fraction generally increases with soil depth (37). Together, these measurements indicate that microbially produced $\delta^{15}N_{NO3}$ could approach 7.5‰ in mineral soils or could be considerably lighter, particularly in surface soils, assumed here to range to −10‰ (14).

The $\delta^{18}O_{NO3}$ value for NO_3^- produced by microbial nitrification with no kinetic isotope effects or oxygen exchange should reflect nitrifier acquisition of two oxygen atoms from water and one from atmospheric O_2 ($\delta^{18}O_{NO3\text{-}nitrification}$ = 2/3 $\delta^{18}O_{H2O}$ + 1/3 $\delta^{18}O_{O2}$) (14, 26, 27). The $\delta^{18}O_{H2O}$ composition of streamwater and shallow groundwater during the sampling period was −9.2 ± 0.2‰ (n = 17) and the established value for $\delta^{18}O_{O2}$ of air is 23.5‰, yielding a value of $\delta^{18}O_{NO3}$ of 1.7‰ ± 0.1‰. However, kinetic isotope effects controlled by parameters including pH and temperature can occur during nitrite oxidation and during incorporation water, affecting the final

oxygen isotope composition of the nitrite (16, 28–30). This process is also affected by the residence time of nitrite, which is highly susceptible to microbially mediated oxygen exchange with water yielding markedly lighter $\delta^{18}O_{NO3}$ than expected otherwise (28–30).

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