Sedimented Organic Nitrogen Isotopes in Freshwater Wetlands Record Long-Term Changes in Watershed Nitrogen Source and Land Use

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Although historic land use is widely recognized as an important determinant of watershed N cycling, efforts to examine land use legacy effects are limited by incomplete historical data. This research evaluates N isotopes of sedimented organic matter ($\delta^{15}N_{org}$), in a palynological context, as a long-term proxy of changes in N source to wetland biota. N and S isotope measurements of organic sediments, fossil plant fragments, and living plants are used to explore isotope stratigraphies of wetland sediment cores. Processes potentially contributing to isotope stratigraphies are investigated including the following: a change in N source, diagenesis, and denitrification. We document the $\delta^{15}N_{org}$ stratigraphy of a core from the Smithsonian Environmental Research Center, MD, U.S.A. spans approximately 350 years, during which time $\delta^{15}N_{org}$ increases from +2‰ to +7‰. Reconstructed population density and wastewater inputs to the watershed suggest that the increase in $\delta^{15}N$ reflects changing land use from forested conditions to increasing nutrient inputs from human waste. Our results illustrate the importance of hydrologic connectivity in delivering waste-derived N in a watershed characterized by relatively low human population density. These results also demonstrate how this approach can expand the temporal horizon over which we can assess human impacts to watershed N dynamics.

Introduction

Land use is an important determinant of nitrogen (N) biogeochemistry, whether in terms of N cycling (*1*, *2*), N fluxes (*3*, *4*), or N sources to watersheds (*5*, *6*). Isotopic studies comparing land uses in small watersheds have shown that forested ecosystems generally have $\delta^{15}N$ values <+3‰ in soils, groundwater, and surface water, whereas watersheds with significant waste contributions from pasturage, feedlots, or septic systems generally have $\delta^{15}N$ values >+8‰ (e.g., refs *7* and *8*). In particular, Mayer et al. report that large watersheds with >80% forest cover generally have low $\delta^{15}N_{NO3-}$ (+3.5 to +5.5‰), whereas watersheds with >15% agricultural or urban land use have higher $\delta^{15}N_{NO3-}$ (+6 to +9‰) (*5*). Such studies provide valuable insight into contemporary dynamics between land use, human activities, and N sources to ecosystems.

Given the importance of contemporary land use on nutrient sources to watersheds, it is also important to consider that land use has changed dramatically in most Eastern U.S. watersheds since European settlement. For example, during the peak period of agricultural land clearance in the mid- to late-1880s, it is estimated that only 20% of the mid-Atlantic area was forested (*9*). The historic N dynamics resulting from such changes are unknown; however, it has been shown that historic land use affects contemporary N cycling (*2*). Attempts to understand N dynamics in watersheds prior to major anthropogenic N additions are generally limited to modeling exercises or examination of "pristine" watersheds. These approaches are limited by the lack of historical data for validating modeled results and the accuracy of comparing "pristine" watersheds given ubiquitous atmospheric nitrate deposition, as well as the myriad factors that affect N cycling in individual watersheds (e.g., vegetation type, climate, etc.).

In addition to 400 years of land use change in the eastern U.S., N sources to watersheds have also changed as a result of human activities. In the absence of human additions to the N cycle, ecosystems obtain N via biological fixation and, to a lesser extent, from lightning and forest fires. Of N-fixing organisms, symbiotic plant-bacteria associations provide the largest amount of fixed N in upland and wetland ecosystems (*10*). $\delta^{15}N$ derived from biological fixation generally varies over a small range (0 to +2‰) due to its derivation from atmospheric N (isotopically defined as 0‰). However, since 1890, human activities (e.g., fossil fuel combustion, fertilizer production) have increased fixed N contributions worldwide 9-fold (*11*). As fixed N, these inputs are readily available for biotic uptake. In addition, these anthropogenic inputs have relatively distinct ranges of $\delta^{15}N$ values. For example, fertilizer $\delta^{15}N$ generally ranges from −2‰ to +4‰ (*12*), whereas wet atmospheric deposition of nitrate in the U.S. ranges from −11‰ to +3‰ (*13*) and nitrate derived from wastewater, septic effluent, or manure generally has $\delta^{15}N$ values ranging from +8‰ to +22‰ (*14*, *15*).

This research investigates the use of freshwater wetlands as archives of land use change, human disturbance, and the associated impacts to the N cycle over long temporal scales. In particular, we examine the use of $\delta^{15}N$ of sedimented organic N ($\delta^{15}N_{org}$) from freshwater wetlands as a means for reconstructing long-term changes in watershed N sources to wetland biota. As wetland biota assimilate inorganic N, the isotopic composition of the biomass acquires the $\delta^{15}N$ signature of the N source with minimal fractionation (<2‰) (*16*). Ultimately, this organic N pool returns to the wetland surface and is buried by sediment, recording changes in N source to biota over time.

Previous sediment core studies in lakes and coastal waters have demonstrated the utility of using isotopes to distinguish multiple N sources to aquatic biota, including atmospherically derived N (*17*), biologically fixed N (*18*), wastewater effluent N (*19*), and salmon-derived N (*20*). Other work has shown that sedimented organic N isotopes record eutrophication of lakes and coastal waters (*21*, *22*). However, water column N cycling and trophic interactions in lakes and coastal waters can complicate the transfer of $\delta^{15}N$ from primary producers into the sedimentary record. In comparison, as the main primary producer in wetlands, wetland plants provide a direct link to evaluating nutrient sources without the complications of trophic transfer. In addition, freshwater wetlands regulate water, nutrient, and sediment fluxes at the terrestrial–aquatic interface, much closer to potential nutrient sources.

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Experimental Section

This research was conducted in a suburbanizing Coastal Plain watershed (1192 ha) at the Smithsonian Environmental Research Center, MD, U.S.A. (38°51 N, 76°32 W) (see Supporting Information). Two sediment cores were taken from Mill Swamp, a 59 ha, freshwater, nontidal, herbaceous wetland that flows into Muddy Creek, a tributary to Rhode River and the Chesapeake Bay. The two cores were taken approximately 100 m apart, with the SERC2 core upstream of the SERC 1 core.

The sediment cores were collected using a vibrocorer and refrigerated upon return to the lab. After extrusion, sediments were divided into 1-cm increments for all subsequent analyses. Organic N and C isotopes were determined on increments from 1 to 10 cm and every 8−10 cm downcore (n=24). Organic sulfur was extracted at 5 sediment intervals (1−2 cm, 40−41 cm, 69−70 cm, 78−79 cm, 93−94 cm) for isotopic analysis. Plant fragments were extracted from sediments at intervals adjacent to those analyzed for isotopes and pollen (n=8).

Pollen was extracted from sediments using methods documents by Faegri and Iverson (23), counted, and identified in increments from every 8 to 10 cm (n=19). Sedimentation rates were reconstructed following Brush (24), wherein changes in pollen assemblages are matched with historic records of land use change (see Supporting Information). Sedimentation rates were reconstructed for each 1-cm sediment interval between dated horizons by adjusting the average sedimentation rate in proportion to pollen concentrations (24). Because pollen counts were conducted every 8−10 cm, interpolation was used to calculate sedimentation rates in intervals where pollen counts were not available. The pollen biomarkers used for dating sediments include the ratio of Quercus to Ambrosia (initial land clearance) and the abundance of Ambrosia (peak land clearance) and Castanea (extinction of American chestnut) pollen. The U.S. Department of Agriculture, Agricultural Research Service Lab (Beltsville, MD) measured ^{137}Cs concentrations in sediments from 1 to 10 cm and identified the 1964 surface as the sediment interval where the peak ^{137}Cs content occurred. Modern plants near the core site were identified, freeze-dried, and ground. Fossil plant fragments were extracted from core sediments using density separation (25) and analyzed for δ^{15}N and δ^{34}S.

For organic N analyses, inorganic soluble NO_3^- and exchangeable NH_4^+ were extracted from bulk sediment subsamples with 2 M KCl. Residual organic N was dried and homogenized. Inorganic C was removed from separate bulk sediment subsamples using HCl vaporization. $\delta^{34}S_{org}$ was determined on separate bulk sediment subsamples after sequential extraction of acid volatile sulfur, chromium reducible sulfur, and elemental sulfur (26). N isotopes were analyzed at UC-Davis using a Europa Scientific Hydra 20/20 mass spectrometer. S isotopes were analyzed at the USGS in Menlo Park, CA using a Micromass Optima EA-IRMS. S isotopes of organic S and modern plants were analyzed in duplicate or triplicate. The limited plant fragment material recovered during density separation generally prohibited replication. The precision of isotopic measurements is ±0.13‰ for δ^{15}N, and ±0.5‰ for δ^{34}S. Isotope values are reported as parts per thousand (‰ or permil) relative to standards of known composition using eq 1

$$\delta \ [‰] = (R_x/R_s - 1)*1000 \quad (1)$$

where R_x and R_s are the ratios of the heavy to light isotope (e.g., ^{15}N/^{14}N, ^{34}S/^{32}S) in the sample and standard, respectively. N and S isotopes are reported relative to atmospheric N_2 (Air) and Cañon Diablo Troilite (CDT), respectively.

Local changes in human population densities were reconstructed from 1675 to 1993 using multiple sources. Local changes in housing density in the watershed were reconstructed using historic atlases (27), historic topographic maps (28, 29), and aerial photography (30). Population densities were estimated using census data for household size in Anne Arundel County from 1850 to 2000 (31). Prior to available census data, Earle's (32) reconstructed colonial land use history (1650−1783) of All Hallow's Parish, which encompasses watershed 121, is used to approximate human and animal population densities in watershed 121. Although All Hallow's Parish (20 719 ha) is larger than watershed 121 (1192 ha), these data are unusually detailed and allow comparisons of land use change and sediment core characteristics prior to 1850. To estimate general trends in animal populations after 1783, census data for Anne Arundel County (1880, 1900, 1940, and 1992) were used to calculate animal unit (AU) densities based on ref 33.

Results

Core Chronology and Sedimentation History. The 94 cm core (SERC2) taken from Mill Swamp spans approximately 350 years and, based on Munsell color, pollen counts, and texture, shows no indication of discontinuity. A total of 2116 pollen grains representing 36 plant types (genus, family, or species) were identified and counted. The pollen biomarkers used to date intervals and reconstruct sedimentation rates are shown in Figure 1 (see Supporting Information). Initial land clearance, indicated by the sharp decrease in the ratio of Quercus (oak) to Ambrosia (ragweed) pollen, is dated 1650 based on regional settlement history (32). Peak land clearance, indicated by the largest proportion of Ambrosia pollen, relative to other herbaceous and arboreal species, is dated as 1880 based on the highest percentage of improved farmland in Anne Arundel County reported by the U.S. Census Bureau. The decline of Castanea due to the American chestnut Blight is dated 1910 and is indicated by a sharp decrease in the concentration of Castanea pollen. The 1964 horizon is identified in the sediments at 7−8 cm where peak ^{137}Cs concentrations occur, corresponding with peak atmospheric fallout in the northern hemisphere. Below 11 cm, ^{137}Cs concentrations are below detection limits. The top of the core is dated as the year collected (2000).

Sediment history was reconstructed using methods documented by Brush (24) (see Supporting Information). Error associated with sedimentation rate reconstructions was estimated using first-order propagation of error (34). These calculations indicate that error associated with sedimentation rate reconstructions are <0.04 cm yr^{-1}. Large variations in sedimentation rates are apparent over the timespan of this core and range from 0.05 to 1.16 cm yr^{-1}. Sedimentation rates increased nearly 15-fold from the mid-1600s to the mid-1800s due to deforestation, agricultural development, and subsequent erosion. Since the mid-1800s, sedimentation rates decreased but are still 7-fold higher than the rates prior to settlement. These trends in sedimentation rates are similar to those reported by Brush (35) for cores taken throughout the Chesapeake Bay estuary.

Isotope and Concentration Stratigraphies. Figure 2 illustrates the isotope and elemental concentration stratigraphies in the SERC2 core. In 24 measurements of $\delta^{15}N_{org}$, values ranged from +1.7‰ to +6.9‰. $\delta^{15}N_{org}$ values generally increase from the bottom of the sediment profile to the top. Plant fragments extracted from core sediments exhibit a similar trend as $\delta^{15}N_{org}$. The δ^{15}N of plant fragments are on average 1.3‰ lower than $\delta^{15}N_{org}$. δ^{34}S values of organic S and plant fragments are generally <0‰ and generally decrease from the bottom of the core to the surface. The average isotopic composition of 5 species of living plants (*Sagittaria latifolia, Cinna arundinacea, Cornus amomum, Salix nigra,*
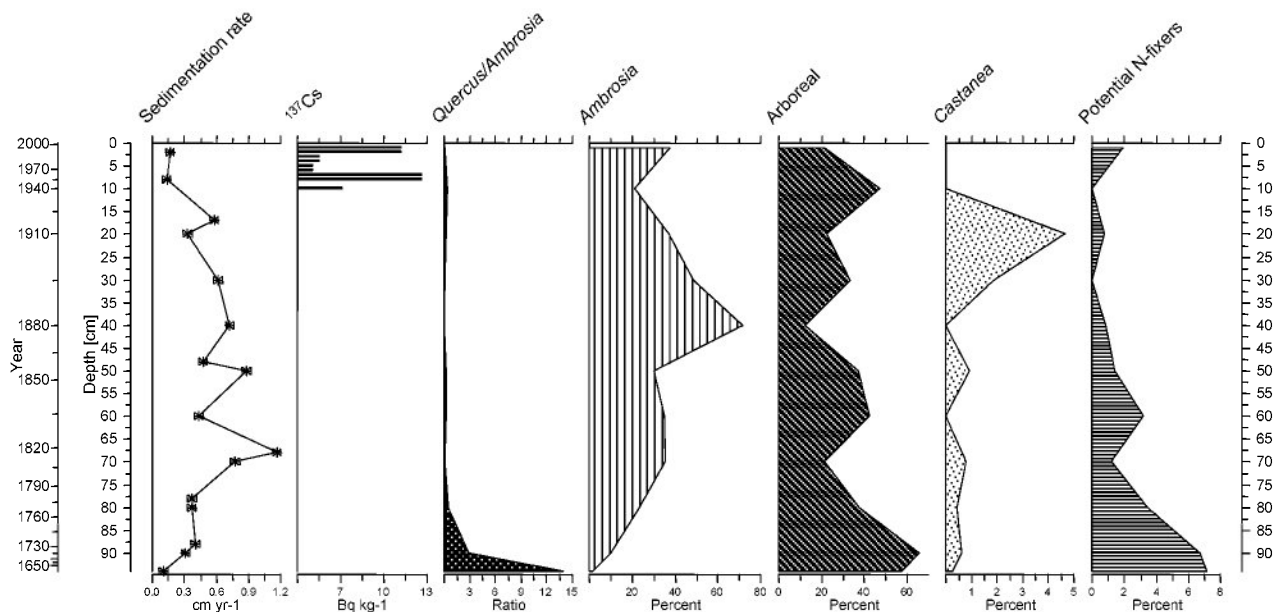
FIGURE 1. Reconstructed sedimentation rates, ^{137}Cs concentration, profiles of pollen biomarkers used for dating sediments, and relative abundance of pollen from potential N-fixing plants. Depth from the surface [cm] is shown on the left axis with corresponding chronology.
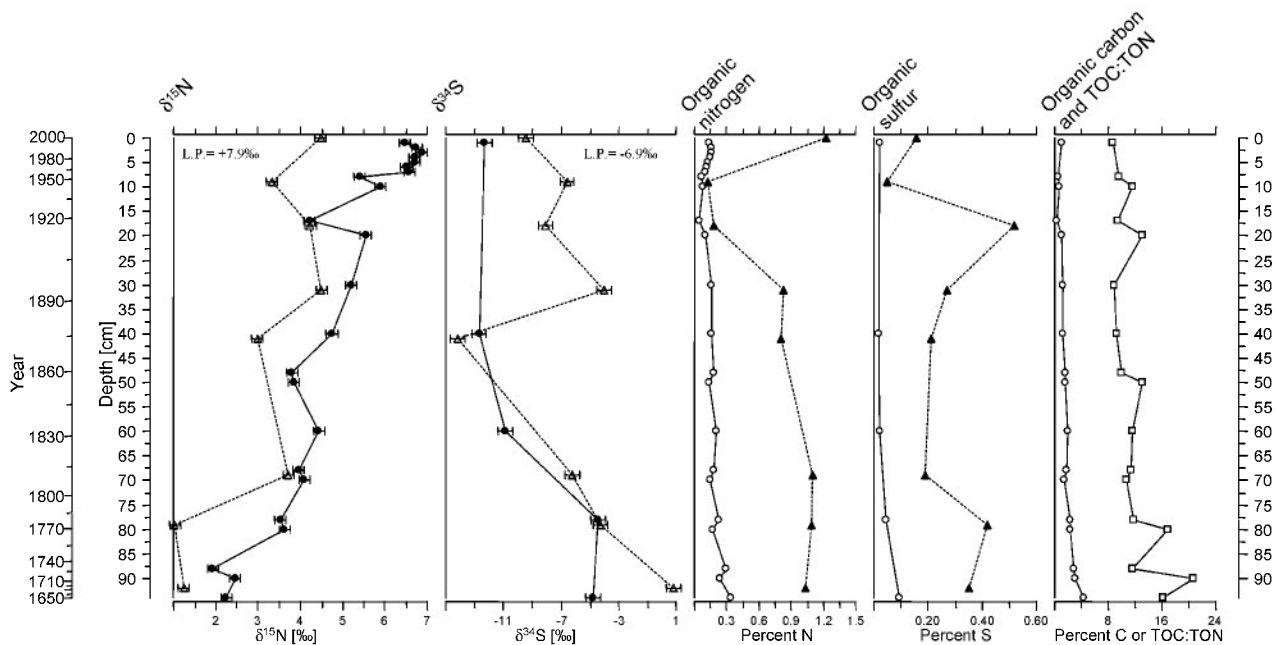
FIGURE 2. Isotope and elemental stratigraphies in the SERC2 core. Depth from the surface [cm] is shown on the left axis with corresponding dates. Organic sediment fractions are shown as solid lines with solid circles for isotopic values ($\delta^{15}N_{org}$, $\delta^{34}S_{org}$) and hollow circles for concentration (percent organic N, organic S, organic C). Plant fragments extracted from core sediments are shown as dashed lines with a hollow triangle for isotopic values and a solid triangle for elemental concentrations. The mean isotopic value of living plants collected near the core site is indicated for $\delta^{15}N$ and $\delta^{34}S$ ($n=5$). The molar ratio of TOC:TON is shown with hollow squares.

Pilea pumila) from the core site vicinity is +7.9‰ and −6.9‰ for $\delta^{15}N_{org}$ and $\delta^{34}S_{org}$, respectively.

Intrawetland Comparison. The effect of wetland spatial heterogeneity on the $\delta^{15}N_{org}$ stratigraphy was assessed using a second sediment core taken approximately 100 yards downstream of the SERC2 core. Sediment history in this downstream core was reconstructed using the same techniques as in SERC2 (^{137}Cs and pollen biomarkers) to allow for comparisons of $\delta^{15}N_{org}$ in sediments of similar ages between the two cores. The $\delta^{15}N_{org}$ values in sediments of similar age are not significantly different before about 1950 (paired t-test, $p=0.10$). After about 1950, $\delta^{15}N$ values of the downstream core are significantly different (paired t-test, $p<0.05$) and are an average of 0.55‰ higher than $\delta^{15}N$ values

for equivalent dates in the upstream core. However, the small differences between the isotope stratigraphies in the two cores suggest that the stratigraphy of $\delta^{15}N_{org}$ in wetland sediments is recording a systematic change from low $\delta^{15}N_{org}$ values around the time of European settlement to higher $\delta^{15}N_{org}$ values in more recent sediments in both cores.

Population Density Reconstruction. Local changes in human population densities were reconstructed for watershed 121 from 1675 to 1993 and are shown in Table 1. Over this time period, population density in watershed 121 increased nearly 16-fold from 0.03 to 0.47 person ha^{-1}. From 1675 to 1783, human population density steadily increased by approximately 6-fold. During the 1800s, a village was established upstream of the present wetland and resulted in

TABLE 1. Reconstructed Housing Density, Population Density, and Wastewater Inputs to Watershed 121

year	housing density (houses ha^{-1})	persons per household[a]	population density [person ha^{-1}][b]	watershed 121 population	total wastewater N input to watershed 121 [kg N yr^{-1}][c]	wastewater N load [kg N/ha]
1675			0.03	41	349	0.29
1685			0.05	57	488	0.41
1695			0.06	75	642	0.54
1705			0.07	86	740	0.62
1715			0.08	92	790	0.66
1725			0.09	108	931	0.78
1735			0.10	121	1038	0.87
1745			0.12	141	1212	1.02
1755			0.13	151	1301	1.09
1765			0.15	174	1496	1.26
1776			0.16	196	1686	1.41
1783			0.17	201	1726	1.45
1876	0.04	8.12	0.32	382	3285	2.76
1904	0.05	5.02	0.26	306	2632	2.21
1957	0.09	4.23	0.37	440	2464	2.07
1972	0.11	4.23	0.47	560	3136	2.63
1991	0.15	3.16	0.46	546	3058	2.57
1993	0.15	3.16	0.47	565	3164	2.65

[a] Derived from Anne Arundel County, MD Census data from 1860, 1910, 1950, and 1990 U.S. Censuses. [b] Population density from 1675 to 1783 reconstructed using Earle, 1975. [c] Assumes 8.6 kg N person^{-1} from 1675 to 1950 and 5.6 kg N person^{-1} from 1950 to 1993.

the population density increase evident in 1876. From 1876 to 1993, the housing density in watershed 121 increased nearly 4-fold, while the housing occupancy rate decreased. The net result was a 1.5-fold increase in population density during 1876—1993. From these estimates, we estimate that the total watershed population increased from approximately 41 in 1675 to about 565 people in 1993.

Discussion

Historic Evidence for Source Change. Prior to major human N inputs, ecosystems generally obtained N via biological N fixation; this N source is isotopically similar to atmospheric N$_2$ (~ 0—2‰). Hu et al. (18) document that δ^{15}N and pollen stratigraphies can record the presence of historic biological N-fixation in sediments. Here, we examined the presence of plant taxa known to have symbiotic relations with N-fixing bacteria in the palynological record, including *Alnus*, *Leguminosae*, *Myricaceae*, *Cornus*, and *Rosaceae* (10, 36). Potential N-fixing plant taxa were more abundant in older sediments (Figure 1), suggesting that historically more N may have been derived from this source. The percent facultative N-fixers and δ^{15}N$_{org}$ are inversely related ($R^2 = 0.67$, $p < 0.01$). This suggests that lower δ^{15}N$_{org}$ values in older sediments reflect the contribution of this fixed N or the derivation of N from this source after recycling through soil organic matter.

Reconstructed population density in watershed 121 suggests that the trend of increasing δ^{15}N$_{org}$ reflects a change in the N source associated with historic land use change, as forested conditions gave way to initial land clearance (1650), peak land clearance (1880), increased population densities, and increasing N inputs derived from human waste. As shown in Table 1, we estimate that the net population in watershed 121 has increased by an order of magnitude from 1675 to 1993. This population increase would have contributed an increasing load of high δ^{15}N$_{NO3-}$ to the system over time from wastewater effluent. However, the relative amount of waste-derived N contributed by human populations likely varied with time according to sanitation practices. For example, during the mid-1800s, privy vault-cesspool systems were commonly used for waste disposal (37). Privy vault-cesspool systems are generally comprised of unlined holes in the ground from which waste flowed directly into the subsoil. Although septic tanks were introduced in the late 1800s (37),

discharge of effluent into subsurface drain fields did not become common practice until the mid-1900s (38). It is estimated that the direction of septic tank effluent into subsurface drains and leaching fields reduces N losses by 35% (39).

Given changes in sewage disposal practices and population, we reconstructed waste-derived N loads to watershed 121 from 1675 to 2000. For this analysis, wastewater N loads from 1950 to 1993 are assumed to be derived from septic tanks and contribute 5.6 kg N person^{-1} yr^{-1}. This loading rate was reported by Castro et al. (40) and was determined using wastewater treatment plant releases of N and associated populations served by sewers. The loading rate reported by Castro et al. falls within the range of directly measured septic tank effluent (3.6—7.8 kg N per capita annually) reported by the U.S. EPA (38). Prior to 1950, it is assumed that sanitation practices consisted of privy-vault cesspool systems with average N loadings that are 65% higher than those of septic tanks with drain fields (8.6 kg N person^{-1} yr^{-1}). Using these assumptions, we estimate that based on population growth in the watershed, wastewater N loads to watershed 121 increased by an order of magnitude between 1675 and 1993 from 0.29 to 2.65 kg N ha^{-1} yr^{-1}.

The temporal trends in reconstructed population density and wastewater N inputs are generally reflected in δ^{15}N$_{org}$. As shown in Figure 3A,B, population density and corresponding waste-derived N in the watershed increased steadily from 1650 to 1783. δ^{15}N$_{org}$ values are $\sim +2$‰ from 1650 to 1730 and begin to increase after about 1730 when population density exceeds 0.09 person ha^{-1}. Although accurate population densities are not available from the late 1700s to the mid-1800s, a small village was established by 1876. The high population density in 1876, coupled with the higher occupancy rate during this period (Table 1), results in the highest estimated loads of wastewater-derived N to watershed 121 as shown in Figure 3B. This period from 1730 to 1876 was accompanied by a steady increase in δ^{15}N$_{org}$ from $\sim +2$ to $\sim +5$‰. The introduction of septic tanks (~ 1950) and a decrease in household size reduced estimated net inputs of wastewater N from 1910 to 1950. Post World War 2 suburbanization results in nearly a 30% increase in population density after 1957. After 1972, watershed population growth levels out. During the period from 1876 to 2000, δ^{15}N$_{org}$ values continue the generally increasing trend.
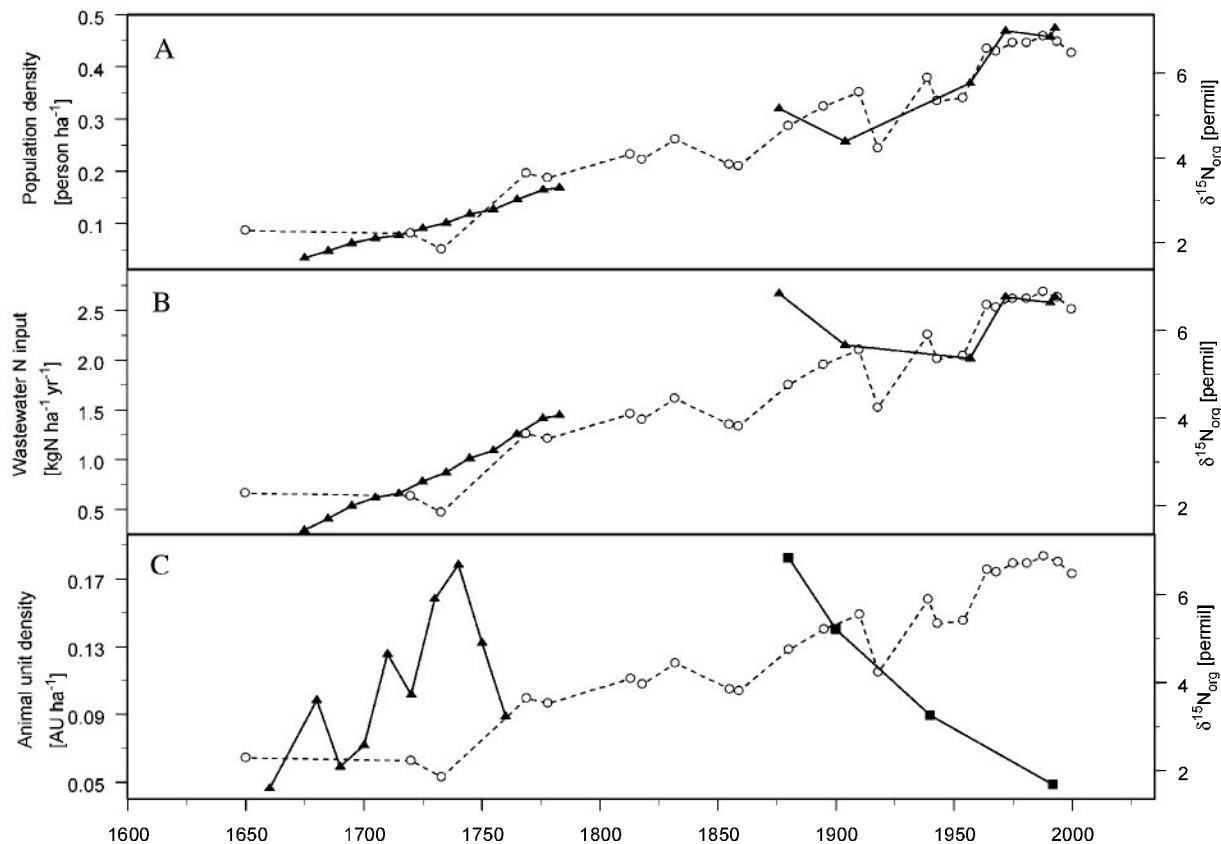
FIGURE 3. A comparison of temporal changes in reconstructed population density, waste effluent N, approximate animal unit density, and $\delta^{15}N_{org}$ in watershed 121 from 1650 to 2000. (A) Human population density (solid triangles) and $\delta^{15}N_{org}$ (hollow circles). (B) Waste effluent N inputs (solid triangles) and $\delta^{15}N_{org}$ (hollow circles). (C) Animal unit densities for watershed 121 (solid triangles) and Anne Arundel County (solid squares).

In addition to human waste inputs of N, livestock animals also likely contributed waste-derived N during the post-European settlement of watershed 121. As shown in Figure 3C, we estimate that during 1650−1783, animal unit (AU) densities in watershed 121 increased nearly 4-fold between 1660 and 1745 to 0.18 AU ha^{-1}. U.S. Agricultural Census (1880−1992) data for Anne Arundel County show a similarly high AU density in 1880. After 1880, AU density in Anne Arundel County decreases to 0.04 AU ha^{-1}, which is similar to 1660 levels in watershed 121. While the large increase in AU density would have been accompanied by a corresponding increase in waste inputs, we find it interesting that $\delta^{15}N_{org}$ does not reflect this increase in AU density. The relatively diffuse distribution of animal waste inputs to the landscape (e.g., pasture or applied as fertilizer), coupled with above ground delivery, may account for the relative insensitivity of the $\delta^{15}N_{org}$ signal to AU density. This lack of correlation between $\delta^{15}N_{org}$ and AU density supports our argument that hydrologic connectivity is important in transporting human-derived waste effluent to downstream ecosystems.
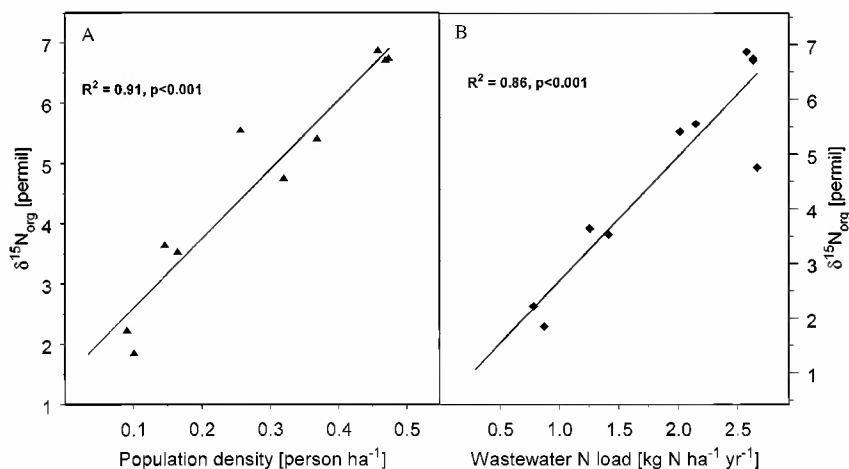
To compare population density and wastewater loads directly with $\delta^{15}N_{org}$, we used a subset of $\delta^{15}N_{org}$ values that fell within 6 years of population density and wastewater estimates (n=10, mean difference = 3 years). In this subset of data, $\delta^{15}N_{org}$ values are strongly correlated with population density (R^2=0.91, p<0.001) and wastewater N inputs (R^2=0.86, p<0.001) for equivalent periods (Figure 4).

These land use history reconstructions suggest that increasing contributions from waste sources are responsible for the increasing $\delta^{15}N$ values in the core. Other potential N sources to the system, including industrial fertilizer and atmospheric deposition are ruled out based on isotopic values for these sources, which would generally not result in increasing $\delta^{15}N$ values with increasing inputs. Other con-

temporary studies have shown that $\delta^{15}N$ values in biota are correlated with wastewater N inputs (e.g., refs 6 and 41). In particular, Mayer et al. reported higher $\delta^{15}N$ values (>+6.5‰) in riverine nitrate from 16 large watersheds in the Eastern U.S. with wastewater contributions >1.5 kg ha^{-1} (5). In watershed 121, this threshold (1.5 kg ha^{-1}) of wastewater contributions was crossed between 1783 and 1876, although $\delta^{15}N_{org}$ values began increasing before 1783. In this same study, Mayer et al. also report correlations between population density and $\delta^{15}N_{NO3-}$ where population densities ranged from 0.08 to 5.6 person ha^{-1} (5). In watershed 121, we observe this same relationship, with stronger correlations, at a much lower range of population densities (Figure 4). This suggests that examination of long-term changes in a single watershed document the contributions of wastewater N at lower population densities than possible when comparing a cross-section of watersheds.

Other Factors that May Contribute to Stratigraphic Changes in $\delta^{15}N_{org}$. The strong correlations we observe between $\delta^{15}N_{org}$, human population density, and wastewater N inputs to watershed 121 suggest the stratigraphic trends we observe result from increasing input of waste-derived $\delta^{15}N$ characterized by high $\delta^{15}N$ values. However, several additional factors must also be considered.

As in any paleoecological study, it is important to recognize the potential for postdepositional or diagenetic alterations of sediments. Sediment and soil profiles affected by diagenetic processes generally have increasing $\delta^{15}N$ values with depth and decreasing N concentrations and C:N ratios with depth (42, 43). In this study, we observed decreasing $\delta^{15}N_{org}$ and slight increases in the percent organic N, organic C, organic S, and TOC:TON with depth. Although we cannot rule out the influence of diagenetic alterations, the stratigraphic trends we observed in sediment cores for this study are generally

FIGURE 4. Correlations between $\delta^{15}N_{org}$ and (A) reconstructed population density and (B) reconstructed wastewater inputs to watershed 121 for equivalent timeperiods.

opposite those documented in diagenetically altered sediments. Further, despite large differences in N concentration between bulk organic N and plant fragments, the average difference in $\delta^{15}N$ between these pools is only 1.3‰. The low N concentrations in bulk sediment relative to plant fragments and living plants suggests that labile nitrogenous compounds (e.g., amino acids) have decomposed, leaving a residual refractory organic N pool very similar isotopically to both living plants and extracted plant fragments. These results are supported by Fogel et al. (25) in their study of diagenesis in wetland organic matter where they concluded that $\delta^{15}N$ of bulk sediment remained stable, despite variations in $\delta^{15}N$ of specific macromolecules.

Denitrification in wetlands is an important process for mediating inorganic N inputs from the landscape. As such, it is important to acknowledge the potential for isotopic fractionations associated with denitrification and how these fractionations could impact the $\delta^{15}N_{org}$ in the sedimentary record. While it is impossible to reconstruct historic denitrification rates, we use an additional isotope, $\delta^{34}S_{org}$, to consider how long-term changes in redox status may have affected $\delta^{15}N_{org}$ stratigraphy.

In principle, sulfate reduction only occurs after all available nitrate is reduced. However, in heterogeneous natural systems, nitrate and sulfate reduction likely occur together in response to dynamic redox conditions, especially in wetland sediment—water interfaces. The fractionations associated with nitrate and sulfate reduction are large, with reported enrichment factors of 1.005 to 1.04 (44) and 1.02 to 1.03 (45), respectively. These fractionations can result in residual nitrate and sulfate pools available for plant uptake with high $\delta^{15}N$ and $\delta^{34}S$ values and can thus lead to high $\delta^{34}S$ and $\delta^{15}N$ in sedimented organic matter.

If increased denitrification rates were responsible for the observed long-term increase in $\delta^{15}N_{org}$, it is expected that $\delta^{34}S_{org}$ values would show a corresponding increase. As shown in Figure 2, $\delta^{15}N_{org}$ values increase upcore, whereas $\delta^{34}S_{org}$ values decrease upcore. Similarly, we found that the N and S isotopic composition of plant fragments extracted from core sediments are inversely related. While we cannot rule out absolutely the influence of denitrification rates on long-term $\delta^{15}N_{org}$ values, the fact that $\delta^{15}N$ and $\delta^{34}S$ values in organic sediment fractions and plant fragments are not positively correlated suggests that reduction is not the primary mechanism responsible for the observed increase in $\delta^{15}N_{org}$. It should be noted that we are not suggesting that denitrification does not occur in this wetland, but rather that it does not seem to be a controlling factor in the $\delta^{15}N_{org}$ and $\delta^{34}S_{org}$ stratigraphies. For example, it may be the case that

denitrification occurs in areas of the wetland that are not hydrologically connected to our core sites, in anaerobic microsites, or well below the rooting zone of wetland plants.

Ecological Significance. Based on the land use classification put forth by Theobald (46), the human population density in watershed 121 has ranged from "exurban" in the late 1600s to "suburban" beginning in the 1950s. Other studies report correlations between $\delta^{15}N$ in biota and the proportion waste effluent N in watersheds with much higher population densities (e.g., refs 6 and 41). Nonetheless, we find that human waste contributions are the most likely factor contributing to increasing $\delta^{15}N_{org}$ values in the sedimentary record. These results suggest that nonpoint source N from low-density, suburban watersheds may be more important than previously thought in terms of N inputs to coastal waters.

These results also demonstrate the importance of considering hydrologic connectivity when estimating N sources to watersheds. Historically, wastewater was commonly disposed into belowground cesspools or privy pits. More recently, septic tanks deliver wastewater into leaching fields. Both of these disposal techniques deliver waste-derived N directly into the soil subsurface where it can move into ground and surface water. For example, Valiela et al. (39) report lower watershed loss rates of septic-derived N than for atmospheric deposition and fertilizer (65%, 91%, 84%, respectively) due to the direct connectivity of septic waste N to subsurface waters. This suggests that hydrologic connectivity and landscape position may cause waste effluent from low density, suburban watersheds to be a more ecologically important N source than much higher density urban areas served by sewer and wastewater treatment plants.

Acknowledgments

Supporting Information Available

Site location, sediment chronology, and methods for reconstructing sediment history. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supplementary Information

Sedimented Organic Nitrogen Isotopes in Freshwater Wetlands Record Long-term Changes in Watershed Nitrogen Source and Land Use

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4 pages total including coversheet. 1 figure, 1 table.

Figure S1: Location of two sediment cores from Watershed 121 at the Smithsonian Environmental Research Center in Edgewater, Maryland, USA.
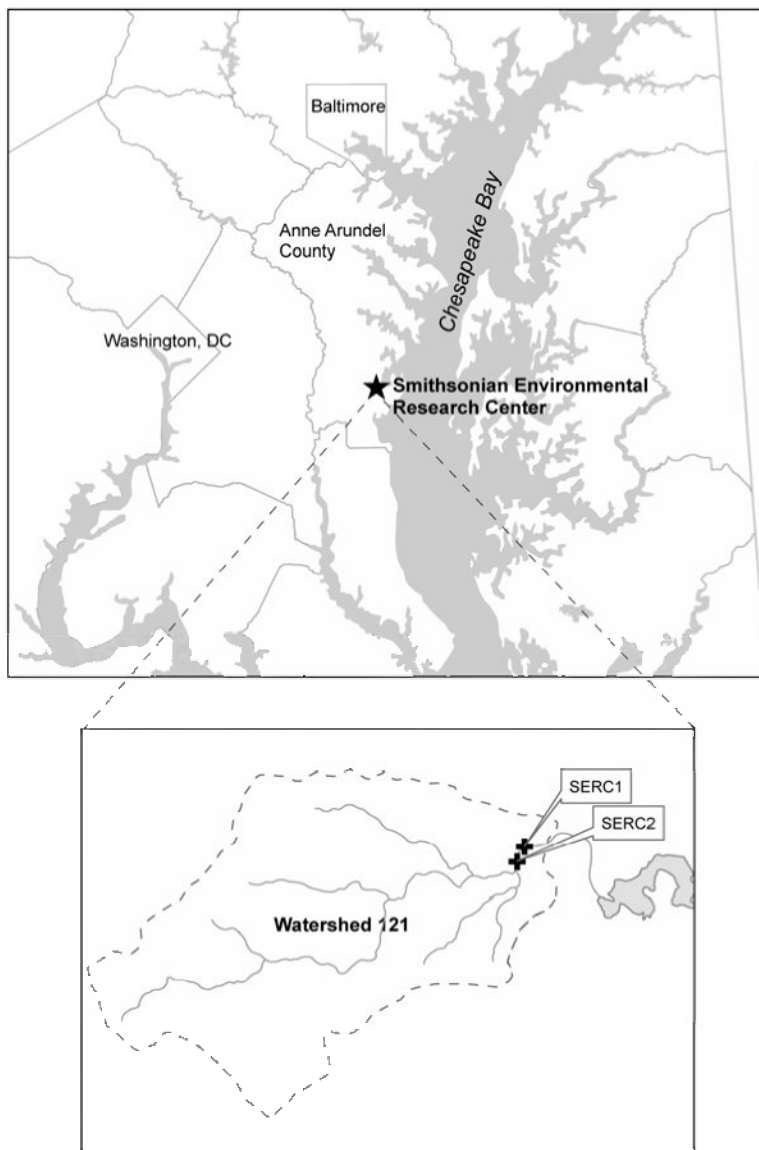
Table S1. Sediment chronology reconstruction.

Depth [cm]	Date of horizon	Source of Date	Pollen grain count	Sedimentation rate [cm/yr]	Sedimentation rate error [cm/yr]	Estimated Chronology
1-2	2000	Date of collection.	64	0.17		2000-1994
7-8	1964 (1954-1968)	^{137}Cs	-	0.13	± 0.018	1964-1954
8-9			126	0.13		1954-1943
10-11			28	0.58		1939-1937
17-18			49	0.33		1918-1914
20-21	1910 (1910-1930)	Decrease in *Castanea*.	274	0.62	± 0.044	1910-1908
30-31			233	0.73		1895-1894
40-41	1880 (1870-1890)	*Ambrosia >10%, Quercus/Ambrosia <1.*	129	0.47	± 0.024	1880-1877
48-49			111	0.88		1859-1857
50-51			225	0.43		1855-1851
60-61			84	1.16		1832-1830
68-69			125	0.78		1819-1816
70-71			260	0.37		1814-1809
78-79			264	0.37		1779-1774
80-81			243	0.40		1770-1766
88-89			311	0.31		1734-1729
90-91	1720 (1710-1740)	*Ambrosia <10%, Quercus/Ambrosia >1.*	384	0.06	± 0.002	1720-1699
91-92			331	0.09		1699-1686
93-94			477	0.06		1670-1650
94-95	1680 (1620-1680)	*Ambrosia < 1%, Quercus/Ambrosia >10.*	438	0.08	± 0.003	1650-1634

Calculation of sedimentation rates:

$$R_{i-j} = \frac{\bar{n}}{n_{i-j}} \times \bar{R} \qquad (1)$$

where R_{i-j} is the sedimentation rate for interval i to j;

$\bar{n}$ is the average number of pollen grains per unit area in the interval;

n_{i-j} is the number of pollen grains per unit area in the interval i to j;

$\bar{R}$ is the average sedimentation rate ($\frac{d}{t}$) where d is the depth of the dated horizon and t is the time in years. Between horizons where pollen was counted, sedimentation rates were linearly interpolated. Between dated horizons, offsets in chronology from linear interpolation of sedimentation rates were normalized by the ratio of years between dated horizons and estimated years in between dated horizons reconstructed from sedimentation rates. Error associated with sedimentation rate calculations are estimated using first order propagation of error from Benjamin and Cornell (1970) (see reference #34 in main text). This calculation considers the ranges of dates for each horizon as indicated in the table.