

Nitrogen stable isotopes in streams: effects of agricultural sources and transformations

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Abstract. The nitrogen stable isotope ratio of biological tissue has been proposed as an indicator of anthropogenic N inputs to aquatic ecosystems, but overlap in the isotopic signatures of various N sources and transformations make definitive attribution of processes difficult. We collected primary consumer invertebrates from streams in agricultural settings in Wisconsin, USA, to evaluate the relative influence of animal manure, inorganic fertilizer, and denitrification on biotic $\delta^{15}\text{N}$. Variance in biotic $\delta^{15}\text{N}$ was explained by inorganic fertilizer inputs and the percentage of wetland land cover in the watershed, but not by animal manure inputs. These results suggest that denitrification of inorganic fertilizer is the primary driver of $\delta^{15}\text{N}$ variability among the study sites. Comparison with previously collected stream water $\text{NO}_3\text{-N}$ concentrations at the same sites supports the role of denitrification; for a given N application rate, streams with high biotic $\delta^{15}\text{N}$ had low $\text{NO}_3\text{-N}$ concentrations. The lack of a manure signal in biotic $\delta^{15}\text{N}$ may be due its high ammonia content, which can be dispersed outside the range of its application by volatilization. Based on our findings and on agricultural census data for the entire United States, inorganic fertilizer is more likely than manure to drive variability in biotic $\delta^{15}\text{N}$ and to cause excessive nitrogen concentrations in streams.

Key words: agriculture; denitrification; fertilizer; manure; nitrogen stable isotopes; primary consumer invertebrates; watershed; Wisconsin, USA.

INTRODUCTION

Agriculture contributes excessive amounts of nitrogen (N) to surface and ground waters, which has led to degradation of aquatic ecosystems (Vitousek et al. 1997, Carpenter et al. 1998). Mitigation of this degradation requires an understanding of the sources, transport, transformation, and fate of agricultural N. Nitrogen stable isotope ratios ($^{15}\text{N}/^{14}\text{N}$, expressed as $\delta^{15}\text{N}$), at natural abundances, are increasingly used to examine the N cycle because many N sources and transformations produce characteristic $\delta^{15}\text{N}$ values. Several authors have proposed the use of $\delta^{15}\text{N}$ as an indicator of anthropogenic N inputs (Kohl et al. 1971, Lake et al. 2001, McKinney et al. 2002, Cole et al. 2004, Vander Zanden et al. 2005, Anderson and Cabana 2006, LeFebvre et al. 2007), yet it is still unclear whether this is a correct interpretation in all circumstances. A given $\delta^{15}\text{N}$ value can be generated by multiple combinations of sources and transformations, making multi-source mixing models unreliable (Hauck et al. 1972) and definitive attribution of processes difficult (Heaton 1986, Högberg 1997, Robinson 2001, Bedard-Haughn et al. 2003). For example, the two primary sources of agricultural N, manure and inorganic fertilizer, have different initial $\delta^{15}\text{N}$ values ($\sim 5\%$ and $\sim 0\%$, respec-

tively) (Kendall 1998). The $\delta^{15}\text{N}$ of manure is often further elevated through ammonia volatilization during storage and application, and N from both sources is subject to further $\delta^{15}\text{N}$ increases through denitrification (Kendall 1998). Thus, the high $\delta^{15}\text{N}$ values that have been reported for aquatic ecosystems in agricultural settings (Harrington et al. 1998, Vander Zanden et al. 2005, Anderson and Cabana 2006) could be primarily an indicator of animal waste pollution or of N loss through denitrification.

The objective of this study was to evaluate the relative importance of N sources and transformations in determining biotic $\delta^{15}\text{N}$ in agriculture-influenced streams. Distinguishing these components is important because it will help determine whether $\delta^{15}\text{N}$ is more useful as an indicator of a degrading (animal waste) or a mitigating (denitrification) process. In mixed agricultural landscapes, a key management question is to determine whether row crop or animal agriculture is causing a greater proportion of N pollution. In addition, understanding landscape processes that drive variation in $\delta^{15}\text{N}$ in aquatic ecosystems will also facilitate the use of this metric in food web studies (e.g., Cabana and Rasmussen 1996). To address our objective, we measured $\delta^{15}\text{N}$ in primary consumer invertebrates from a set of streams with widely varying watershed characteristics. Our methods improve on several shortcomings of previous studies. First, we excluded streams with significant urban influence to avoid confounding human wastewater effects on $\delta^{15}\text{N}$ (McKinney et al. 2002, Cole

Manuscript received 19 February 2008; revised 23 September 2008; accepted 23 October 2008. Corresponding Editor: K. N. Eshleman.

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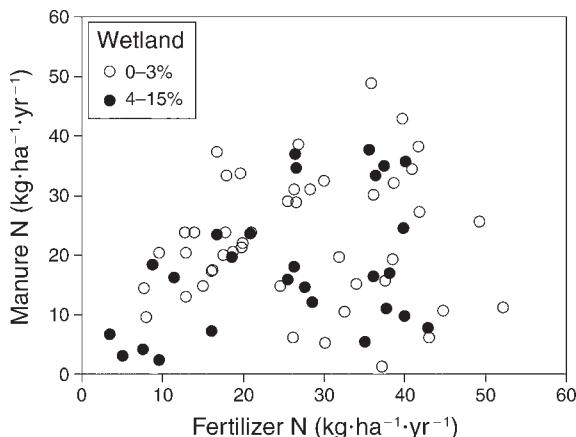


FIG. 1. Estimated N application rates as fertilizer (N_f) and manure (N_m) in watersheds of the 72 sampled sites. The symbols indicate the percentage of wetland land cover in the watersheds of the sampled sites. The 4% cutoff is used to distinguish sites whose watersheds have high amounts of wetland from those with low amounts of wetland. The value of 4% as a cutoff is arbitrary in relation to the statistical analyses, which use percentage of wetland as a continuous variable.

et al. 2004, Steffy and Kilham 2004). Second, we selected sample sites so that potential explanatory variables were uncorrelated, making independent estimation of their effects possible (Anderson and Cabana 2006). And third, we evaluate our findings for biotic $\delta^{15}\text{N}$ against stream water $\text{NO}_3\text{-N}$ concentrations at the same sites and against the predicted effect of denitrification on the remaining N pool. Our results support a complex picture of the drivers of aquatic $\delta^{15}\text{N}$, one that involves additive and interactive effects of N sources, transport, and transformation.

METHODS

Site selection

We conducted a synoptic survey of streams in Wisconsin, which has broad gradients of landscape factors with the potential to influence biotic $\delta^{15}\text{N}$. Seventy-two stream sites were selected from a larger set that was studied by the U.S. Geological Survey in 2001–2003 (Robertson et al. 2006). At each site, stream water nutrient concentrations were measured monthly between April and October (for details, see Robertson et al. 2006). Watersheds for these sites ranged from 5 to 1083 km^2 (median = 25 km^2). Sites were selected to orthogonally span gradients of three watershed variables that were expected to influence $\delta^{15}\text{N}$: (1) N application as inorganic fertilizer, (2) N application as manure, and (3) wetland land cover (a surrogate for denitrification potential; Fig. 1). This sampling design minimized intercorrelation of potential explanatory variables, which can limit interpretation of results (King et al. 2005). We also excluded sites with $>5\%$ watershed urban land cover to minimize the effects of human waste on $\delta^{15}\text{N}$ (Steffy and Kilham 2004).

Invertebrate collection and isotope analysis

We measured $\delta^{15}\text{N}$ in primary consumer invertebrates collected from each stream site during July and August of 2005. Primary consumers are considered good long-term indicators of system status because they integrate temporal isotopic variability of algal and inorganic nitrogen and because their low trophic position minimizes variability due to omnivory (Cabana and Rasmussen 1996). Primary consumer $\delta^{15}\text{N}$ is expected to reflect the average $\delta^{15}\text{N}$ of the biologically available inorganic N pool in the stream, plus the trophic fractionation factor, which is $\sim 3.4\%$ (Vander Zanden and Rasmussen 2001).

Invertebrates were collected from riffles with a D-frame net and by hand picking from rocks. Three to 30 individuals of each taxon were then placed in plastic centrifuge tubes and kept on ice until they could be frozen (maximum 72 h). Invertebrate guts were not removed, nor was a consistent gut clearance time provided, because these procedures do not significantly affect measured stable isotope ratios of primary consumer invertebrates (Jardine et al. 2005). Samples were dried at 60°C for 48 h, ground to a fine powder, and packed into tin capsules. Isotope analysis was conducted on a Europa Hydra 20/20 continuous flow isotope ratio mass spectrometer (Europa Scientific, Crewe, UK) by the University of California–Davis Stable Isotope Facility. Stable isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation of a sample (m) from a standard (s) material (atmospheric nitrogen, set at 0‰; $\delta^{15}\text{N} = ([R_m/R_s] - 1) \times 1000$, where $R = {}^{15}\text{N}/{}^{14}\text{N}$). Twenty percent of the samples were analyzed in duplicate; the mean standard error of replicates was 0.20‰.

At least one of two common primary consumer invertebrate taxa (baetid mayflies and hydropsychid caddisflies) was present at all sites. Hydropsychids can be omnivorous (Merritt and Cummins 1996), so guts from hydropsychids at a subset of study sites were examined. Guts contained mostly diatoms, with some detrital plant material and very little material of obvious animal origin. We tested for differences in $\delta^{15}\text{N}$ between these two taxa at a subset of sites and found no difference (paired t test; $n = 18$, mean difference = 0.12‰, $P = 0.85$). Reported $\delta^{15}\text{N}$ values for each site are either one of these taxa, or when both were collected, the mean of the two.

Calculation of nitrogen source and transformation variables

We analyzed publicly available data in a geographic information system to calculate variables that have the potential to influence $\delta^{15}\text{N}$ in aquatic ecosystems. All variables were calculated for the watersheds of sampled stream sites, which were delineated by Robertson and others (2006). Calculation of N source variables was based primarily on agricultural census data, which is mostly reported by county. Because counties are larger

than most of the study watersheds, we used the following methods to interpolate patterns at the smaller spatial grain of these watersheds.

Nitrogen application as inorganic fertilizer (N_f) (in kilograms per hectare per year) was estimated for all U.S. counties by Battaglin and Goolsby (1994) based on county reports of fertilizer sales in 1991 (the most recent data available). Despite the time gap between the fertilizer data and our biological sampling, agricultural census data indicate that the extent and geographical distribution of cropland in Wisconsin has been nearly constant during this period; $R^2 = 0.99$ for a regression of "cropland acres harvested" by county from the 1992 and 2002 agricultural censuses (USDA 2002). To generate N_f estimates for the watersheds in this study, cropland application rates for each county in Wisconsin were calculated by dividing the total N content of fertilizer sold by the area of cropland in the county (from Wisland; WDNR 1998). For each study watershed, N_f was calculated as the area-weighted mean N_f of county-specific cropland areas in each watershed multiplied by the proportion of the watershed that is cropland.

Nitrogen application as manure (N_m) (in kilograms per hectare per year) was estimated by a variation of the method of Burkart and James (1999), which used animal population data and estimates of the N content of manure from each type of animal. The U.S. Census of Agriculture (USDA 2002) reports animal populations by county and the number of farms with each type of animal by zip code. To increase the spatial resolution of manure application estimates, we combined data at these two scales through the following method. For each animal type (t), the number of animals was divided by the number of farms with that type of animal in each county (c), giving the mean number of animals per farm (D_{tc}). Polygons of area (A_{zc}) were then formed by intersecting zip codes (z) and counties (mean $A_{zc} = 67 \text{ km}^2$). The number of farms with a given animal type (F_{tzc}) was calculated for each polygon as the number of farms in the zip code multiplied by the proportion of the zip code represented by that polygon. Thus, for each polygon,

$$N_m = \frac{\sum_{t=1}^n (D_{tc} \times F_{tzc} \times E_t)}{A_{zc}}$$

where E_t is the mean annual N content of manure excreted by animal type t . For each study watershed, N_m was calculated by first converting the polygon layer to a raster grid, then calculating the mean grid cell value in each watershed. Values of E_t were taken from Burkart and James (1999), who report estimates of both gross (total excreted) and net (remaining N after losses to ammonia volatilization) E_t . Gross E_t values were used in the primary analysis of this study, but in later sections, the potential relevance of net E_t is discussed. In this study N_m only includes N excreted by cattle for two reasons. First, 93% of the total amount of N excreted by animals

in Wisconsin is from cattle (based on total animal numbers in 2002 census). And second, other animals (pigs and poultry, in particular) are raised in more concentrated populations, making the above methods of geographically allocating animal distributions less reliable. Manure from highly concentrated animal populations is also more likely to be transported elsewhere for land application.

Denitrification potential was estimated as the percentage of wetland land cover in the watershed (from Wisland; WDNR 1998). Wetlands are good environments for denitrification because they often contain anoxic sediments and high levels of organic carbon (Myrold and Tiedje 1985). Their presence is indicative of flat topography, which also promotes denitrification by slowing the movement of water and dissolved materials, thus allowing denitrifying reactions to progress further than in better drained landscapes (Saunders and Kalff 2001).

Statistical analysis

The objective of our analysis was to evaluate the relative influence of fertilizer N, manure N, and wetland land cover on primary consumer $\delta^{15}\text{N}$ in the study streams. We also tested for an effect of watershed area on the relationships among N source/transformation variables and primary consumer $\delta^{15}\text{N}$. Because correlations among potential explanatory variables were minimal (highest Pearson correlation coefficient was 0.22, between N_f and N_m) and there were few variables, we conducted linear regressions (all subsets, including interactions). To meet assumptions of normality and constant variance for regressions, the two N source variables were square-root transformed and the wetland variable and watershed area were log transformed.

RESULTS AND DISCUSSION

Among the sampled sites, estimated N applications as fertilizer and manure were comparable (means, $N_f = 27 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$, $N_m = 21 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$). Wetland made up 0–15% of the watersheds of sampled sites (mean, 3.9%). Primary consumer $\delta^{15}\text{N}$ ranged from 5‰ to 18‰ and was significantly correlated with N_f ($P < 0.001$, $R^2 = 0.27$) and wetland cover ($P < 0.001$, $R^2 = 0.25$), but not with N_m ($P = 0.36$; Fig. 2). Nitrogen as manure was also not a significant variable in any of the multiple regression models. Nitrogen as fertilizer and wetland were both significant ($P < 0.001$) in all models that included them. The multiple regression model with the highest R^2 was $\delta^{15}\text{N} = 3.72 + 0.80(\text{sqrt}(N_f)) + 0.41(\log(\text{wetland}) \times \text{sqrt}(N_f))$ ($R^2 = 0.57$; Fig. 2C), which indicates that wetland cover most influences $\delta^{15}\text{N}$ through its interaction with fertilizer N. Watershed area was not a significant variable in any of the multiple regression models, which indicates that it did not modulate the mean effects of N_f and wetland on $\delta^{15}\text{N}$. Watershed area also did not affect the variability of the residuals from the best regression model (Fig. 2C)

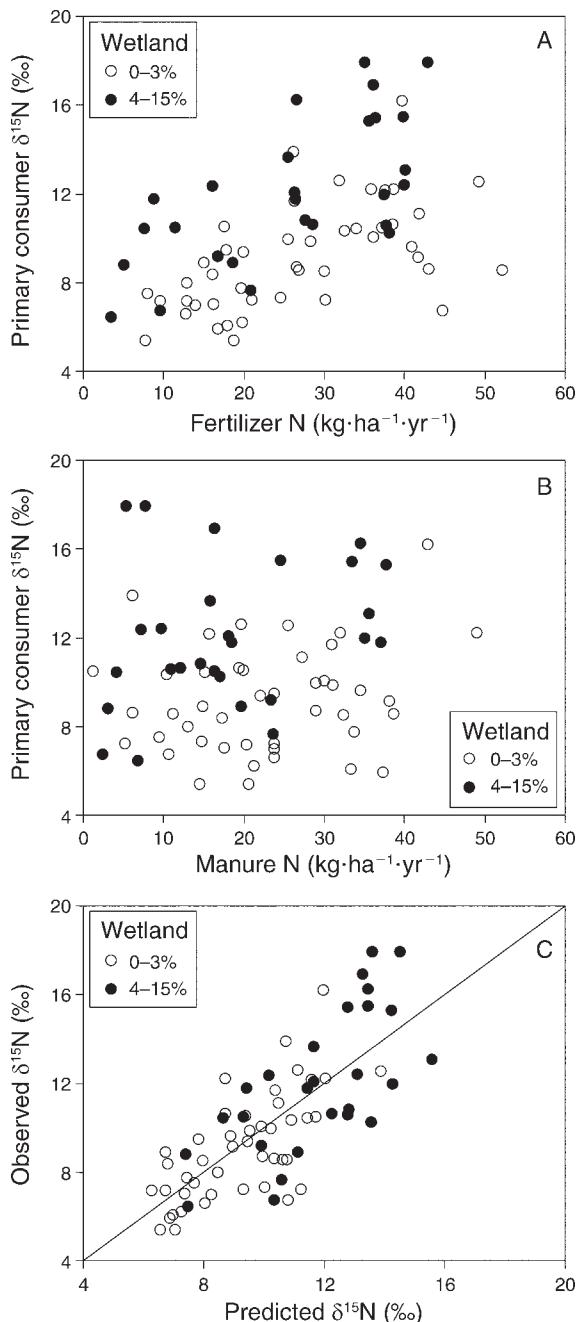


FIG. 2. Relationships between primary consumer $\delta^{15}\text{N}$ and (A) fertilizer N (N_f) application rate in the watershed ($P < 0.001$, $R^2 = 0.27$) and (B) manure N (N_m) application rate ($P = 0.36$). (C) Predicted vs. observed $\delta^{15}\text{N}$ based on the model $\delta^{15}\text{N} = 3.72 + 0.80[\text{sqrt}(N_f)] + 0.41[\log(\text{wetland}) \times \text{sqrt}(N_f)]$ ($R^2 = 0.57$).

(Bartlett's test, $P = 0.60$ for three watershed size categories, 5–30, 31–150, and 151–1083 km^2).

These results indicate that application rate of fertilizer N and its subsequent denitrification explain the observed patterns in $\delta^{15}\text{N}$ of primary consumers from Wisconsin agricultural streams. To further evaluate these findings, we examined how growing-season mean stream water

nitrate concentrations ($\text{NO}_3\text{-N}$ in milligrams per liter; from Robertson et al. 2006) were related to the total N application rate ($N_t = 0.3(N_m) + 0.9(N_f)$) in each watershed, adjusted for estimated losses due to ammonia volatilization (70% loss for manure, 10% loss for inorganic fertilizer; from Burkart and James 1999). While there is a significant positive correlation between N_t and stream $\text{NO}_3\text{-N}$ concentrations (Fig. 3), $\text{NO}_3\text{-N}$ concentrations are highly variable at high N application rates. Biotic $\delta^{15}\text{N}$ explains a significant amount of this residual variation ($P = 0.02$, additional $R^2 = 0.03$) in the model $\log(\text{NO}_3\text{-N} + 1) = 0.21 + 0.044(N_t) - 0.043(\delta^{15}\text{N})$. For a given N application rate, streams with high $\delta^{15}\text{N}$ had low nitrate concentrations (Fig. 3), a pattern that is consistent with N loss by denitrification.

The dominant influence of inorganic fertilizer N (compared to manure N) on biotic $\delta^{15}\text{N}$ is surprising because on average our study sites had comparable watershed inputs of these two N sources (Fig. 1) and because other studies have found strong correlations between livestock distributions and $\delta^{15}\text{N}$ in streams (Vander Zanden et al. 2005, Anderson and Cabana 2006). However, there are several factors that help explain our results. First, it is possible that our estimates of manure amounts were less accurate than our estimates of fertilizer amounts. The spatial grain at which livestock numbers are reported in agricultural census reports make it difficult to map their distributions. In contrast, our method for mapping fertilizer application is probably more precise because cropland is mapped at a much higher spatial resolution. Furthermore, manure may be transported away from where it is produced before it is applied to land. And finally, although manure from animals other than cattle contains only 7% of all manure N in Wisconsin, in some locations this may amount to a significant quantity. While these potential sources of error may weaken the precision of our estimate of the true

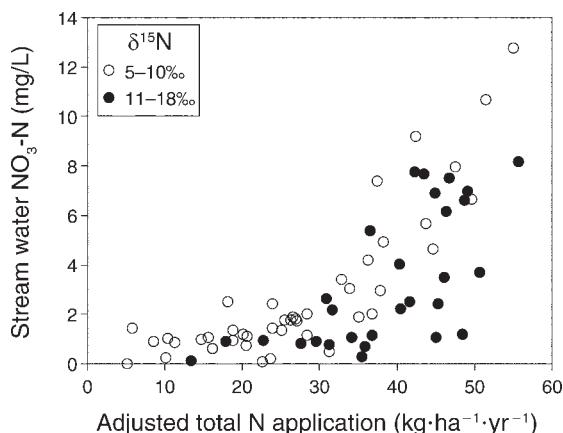


FIG. 3. Relationship between stream water nitrate concentration and adjusted total N application rate, N_t (manure and fertilizer), in the watershed. Biotic $\delta^{15}\text{N}$ explains a significant amount ($P = 0.02$) of the residual variation in this relationship.

relationship between manure application and biotic $\delta^{15}\text{N}$, they are unlikely to completely mask it.

One potential mechanism behind our results is the likelihood that much of the N in manure (up to 70%) can be lost during storage and application through ammonia volatilization (Lander et al. 1998). Variability in the chemical composition of different animal manures and in manure management practices can influence the proportion of ammonia that is volatilized (Powell et al. 2004). Typical manure management practices in Wisconsin, daily hauling to fields and broadcast-spreading, facilitate ammonia volatilization (Powell et al. 2007). Some of this ammonia can be deposited locally, thereby remaining available for loss to aquatic systems in the same area in which it was produced (Fowler et al. 1998). However, ammonia can also be transported long distances (hundreds of kilometers) before it is deposited (Ferm 1998), thereby “evening out” the ecosystem effects of fine-scale spatial heterogeneity in its production. In contrast, very little fertilizer N (<10%) is lost to the atmosphere (Meisinger and Randall 1991), so in general, its ecosystem effects are felt where or downstream from where it is applied. This difference in the spatial fidelity of agricultural N sources may partly explain our results and should be considered in any agricultural N budget.

Other biogeochemical transformations of N in agricultural soils fractionate ^{15}N , notably nitrification. However, because both the substrate (ammonium) and the product (nitrate) of nitrification remain available for hydrologic transport and biological uptake, the net effect of nitrification on the $\delta^{15}\text{N}$ of aquatic biota should be negligible. Denitrification and volatilization are fundamentally different from other processes because they transport N from the soil–water complex to the atmosphere and, in the case of denitrification, make it biologically nonreactive (Bedard-Haughn et al. 2003).

Studies that have compiled N budgets for large regions of the United States indicate that manure makes up a smaller proportion of total agricultural N than inorganic fertilizer (Howarth et al. 1996, Burkart and James 1999). Additionally, the spatial distribution of the largest manure and fertilizer N inputs are largely nonoverlapping (USDA 2002). In Wisconsin, despite similar amounts of manure and fertilizer N, our analysis found a much stronger signal from fertilizer N in the $\delta^{15}\text{N}$ of aquatic biota. Furthermore, the relationship in Fig. 3 suggests a threshold value of watershed N inputs ($\sim 35 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$), above which stream water $\text{NO}_3\text{-N}$ can reach very high concentrations. To assess the broader implications of these patterns, we extended our methods for calculating N sources to all U.S. counties (Fig. 4). We used the threshold estimated above ($35 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ total agricultural N input) to identify counties where the sum of agricultural N inputs is most likely to result in high N concentrations in streams. Thirty-two percent of U.S. counties have agricultural N inputs above the threshold. Of these “high-risk”

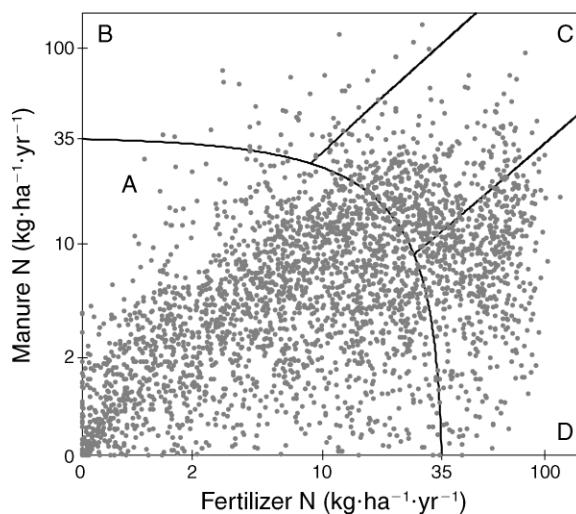


FIG. 4. Estimated manure and fertilizer N application rate for U.S. counties ($n = 3111$). Sixty-eight percent of counties (section A) have $<35 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ of total N application, which is the threshold in the relationship shown in Fig. 3. Of the counties that are above this threshold, 54% (D) are dominated (>75%) by synthetic fertilizer N, 5% (B) are dominated by manure N, and 41% (C) have mixed N sources.

counties (Fig. 4, regions B–D), manure inputs dominate (>75%) in only 5% (B), whereas inorganic fertilizer dominates in 54% (D). The relative proportions in these categories were very similar when alternative (20 and 50 $\text{kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$) thresholds were used to identify high-risk counties. This pattern suggests that nutrient management policies that focus on inorganic fertilizer will be more effective at reducing the incidence of N contamination in surface waters than policies that focus exclusively on manure. It also suggests that, nationally, denitrification of inorganic fertilizer N has a greater potential to drive variability in aquatic $\delta^{15}\text{N}$ than does manure.

Our results provide support for separate but interacting effects of N supply and denitrification on biotic $\delta^{15}\text{N}$ in streams. The amount of ^{15}N enrichment caused by denitrification depends on the fractionation factor and the extent to which the denitrification reaction progresses (Mariotti et al. 1981). The fractionation factor is an increasing asymptotic function of the initial concentration of the substrate ($\text{NO}_3\text{-N}$) (Bryan et al. 1983). The extent of reaction progress is dependent upon the availability of suitable conditions, which include anoxia, a supply of organic carbon, and sufficient time. Assuming a linear relationship between N concentration and denitrification rate (Pina-Ochoa and Alvarez-Cobelas 2006), higher initial N concentrations and better conditions for denitrification will result in a larger difference between the $\delta^{15}\text{N}$ of the initial N pool and the residual N pool following denitrification. Using an experimentally derived relationship (Bryan et al. 1983), we plotted the change in $\delta^{15}\text{N}$ as a function of initial $\text{NO}_3\text{-N}$ concentration and the extent to which denitri-

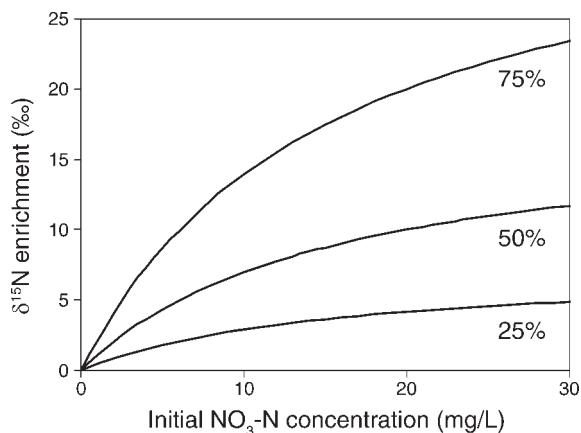


FIG. 5. Theoretical relationships between initial $\text{NO}_3\text{-N}$ concentration and $\delta^{15}\text{N}$ enrichment of the remaining $\text{NO}_3\text{-N}$ pool when denitrification reactions proceed to different extents (25%, 50%, and 75% of $\text{NO}_3\text{-N}$ converted to N_2 ; based on Bryan et al. 1983).

fication depletes the N pool (Fig. 5). We were unable to use this relationship to estimate the extent of denitrification in the watersheds of our study streams because the initial concentrations of $\text{NO}_3\text{-N}$ entering denitrifying environments were unknown. However, the qualitative similarity between the theoretical curves (Fig. 5) and our observed data (Fig. 2A) provides further support for the role of denitrification in determining $\delta^{15}\text{N}$.

The influence of wetlands on biotic $\delta^{15}\text{N}$ in streams appears to reflect their role in promoting denitrification. Among our study sites, variation in wetland land cover that was relatively independent of agricultural intensity allowed this pattern to be identified. Our data do not allow us to distinguish whether denitrification is actually occurring in wetlands or the presence of wetlands is simply an indicator of other landscape conditions that facilitate this process. Other studies have measured denitrification in poorly drained agricultural fields (e.g., Pennock et al. 1992), riparian zones (e.g., Ostrom et al. 2002), and stream channels (Kellman and Hillaire-Marcel 1998). The lack of influence of watershed size on biotic $\delta^{15}\text{N}$ supports the notion that most N removal occurs either prior to its entry into stream channels or in low-order channels (Alexander et al. 2000). In contrast with a study by LeFebvre et al. (2007), we did not observe an influence of watershed size on variation in biotic $\delta^{15}\text{N}$ after accounting for N source/transformation variables. Regardless of where exactly denitrification occurs, landscapes that contain wetlands can mitigate the effects of N loss from agricultural lands (Mitsch et al. 2001, Hey 2002, Trepel and Palmeri 2002, Zedler 2003). Conversely, well-drained landscapes appear to be less capable of buffering the loss of excess N, suggesting that agricultural nutrient management is more important in these areas (Jordan et al. 1997).

Biotic $\delta^{15}\text{N}$ has been proposed as an indicator of anthropogenic N inputs (Lake et al. 2001, McKinney et

al. 2002, Cole et al. 2004, Steffy and Kilham 2004, Anderson and Cabana 2006, Bannon and Roman 2008). Several studies have found it to be a reliable indicator of urban wastewater inputs, even when these inputs do not greatly elevate background N concentrations (Cole et al. 2004, 2005, Steffy and Kilham 2004, Kroeger et al. 2006). However, in some settings $\delta^{15}\text{N}$ values may be more closely tied to the effects of biogeochemical transformations than to the original values of the N source(s). In general, we would expect source signature control where potential N sources differ greatly in their typical $\delta^{15}\text{N}$ values and the potential for biogeochemical transformations is low or relatively constant. For example, the signature of human or livestock waste should be detectable where large quantities (relative to ambient N loads) are discharged directly to surface waters, thus bypassing opportunities for biogeochemical transformation.

Our results provide further support for the use of aquatic primary consumer invertebrate isotopic composition as an indicator of landscape-scale N cycling. Despite differences in their feeding methods (Merritt and Cummins 1996), hydrosychid caddisflies and baetid mayflies had statistically equivalent $\delta^{15}\text{N}$ values in a given stream. This pattern either suggests a common N source profile for most components of primary production that enter stream food webs or that subtle trophic differences within a stream are overwhelmed by differences in N isotope profiles among streams. Other studies have found variability in the $\delta^{15}\text{N}$ of aquatic biota at a site over time (Finlay et al. 2002, Herwig et al. 2007). Most agricultural operations involve seasonally variable application of N to land, which should translate into temporal variability of $\delta^{15}\text{N}$ in streams. Our mid-summer sampling schedule was intended to capture a relatively stable period, but one in which the lagged isotopic effects of spring nutrient applications and temperature-accelerated biogeochemical cycling would be most evident in stream biota. Further research should explicitly assess temporal patterns of biotic $\delta^{15}\text{N}$ to better assess time lags associated with agricultural N transport, transformation, and incorporation into food webs.

An alternative to biotic $\delta^{15}\text{N}$ as an indicator of N cycling processes is the use of both N and O stable isotopes of $\text{NO}_3\text{-N}$. This dual isotope approach can more definitively distinguish denitrification from ^{15}N -enriched sources because denitrification enriches the ^{18}O content of the remaining $\text{NO}_3\text{-N}$ pool (Chang et al. 2002, Panno et al. 2006). However, isotope ratios of inorganic N species can be highly variable over short time periods (Kellman and Hillaire-Marcel 2003), which may limit their utility in synoptic surveys aimed at assessing landscape patterns.

In summary, the results of this study provide new insight into the relative influence of N sources and transformations on $\delta^{15}\text{N}$ of aquatic biota in streams. In Wisconsin, several lines of evidence point to denitrifica-

tion of inorganic fertilizer N as the dominant driver of $\delta^{15}\text{N}$ variability. Despite making up a comparable fraction of total agricultural N inputs, manure production patterns are not reflected in biotic $\delta^{15}\text{N}$, possibly because spatial variability in manure N production is muted by atmospheric transport of volatilized ammonia. These findings do not mean that manure N does not contribute to environmental problems associated with N enrichment of surface waters. Rather, they suggest that the spatial scope of influence of manure production is broader than would be inferred under the assumption that hydrologic processes (e.g., runoff, leaching) dominate its transport. A variety of approaches should be used to further test this hypothesis in diverse agricultural landscapes.

ACKNOWLEDGMENTS

Support for this research was provided by a grant from the USDA Natural Resources Conservation Service and by the Wisconsin Department of Natural Resources through the Wisconsin Buffer Initiative. The authors appreciate assistance by Lauren Taplinger, Lucas Joppa, Jeff Maxted, Dale Robertson, and Emily Stanley.

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