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¹⁵N-NITRATE SIGNATURE IN LOW-ORDER STREAMS: EFFECTS OF LAND COVER AND AGRICULTURAL PRACTICES

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Abstract. Many studies have shown that intensive agricultural practices significantly increase the nitrogen concentration of stream surface waters, but it remains difficult to identify, quantify, and differentiate between terrestrial and in-stream sources or sinks of nitrogen, and rates of transformation. In this study we used the $\delta^{15}\text{N-NO}_3$ signature in a watershed dominated by agriculture as an integrating marker to trace (1) the effects of the land cover and agricultural practices on stream-water N concentration in the upstream area of the hydrographic network, (2) influence of the in-stream processes on the $\text{NO}_3\text{-N}$ loads at the reach scale (100 m and 1000 m long), and (3) changes in $\delta^{15}\text{N-NO}_3$ signature with increasing stream order (from first to third order). This study suggests that land cover and fertilization practices were the major determinants of $\delta^{15}\text{N-NO}_3$ signature in first-order streams. $\text{NO}_3\text{-N}$ loads and $\delta^{15}\text{N-NO}_3$ signature increased with fertilization intensity. Small changes in $\delta^{15}\text{N-NO}_3$ signature and minor inputs of groundwater were observed along both types of reaches, suggesting the $\text{NO}_3\text{-N}$ load was slightly influenced by in-stream processes. The variability of $\text{NO}_3\text{-N}$ concentrations and $\delta^{15}\text{N}$ signature decreased with increasing stream order, and the $\delta^{15}\text{N}$ signature was positively correlated with watershed areas devoted to crops, supporting a dominant effect of agriculture compared to the effect of in-stream N processing. Consequently, land cover and fertilization practices are integrated in the natural isotopic signal at the third-order stream scale. The GIS analysis of the land cover coupled with natural-abundance isotope signature ($\delta^{15}\text{N}$) represents a potential tool to evaluate the effects of agricultural practices in rural catchments and the consequences of future changes in management policies at the regional scale.

Key words: agriculture effects on stream-water N; catchment; denitrification; nitrification; nitrogen concentration in streams and rivers; rivers; stable-isotope analysis; stream order.

INTRODUCTION

The contribution of agriculture to nitrogen enrichment in large rivers (Meybeck 1982, Vitousek et al. 1997, Cole et al. 2004) and low-order streams (Clément et al. 2002, Groffman et al. 2004, King et al. 2005, Bernot et al. 2006) has been largely reported, but very few studies identified, quantified, and differentiated among terrestrial and in-stream sources and sinks of N. Recent works showed the importance of these low-order streams in N retention (Alexander et al. 2000, Peterson et al. 2001). Most of them were carried out with injections of isotopically enriched $^{15}\text{N-NH}_4$ (Mulholland et al. 2000, Tank et al. 2000, Peterson 2001), but little was done using the natural abundance of $^{15}\text{N-NO}_3$

(Kellman and Hillaire-Marcel 1998, Sebilo et al. 2003, Kaushal et al. 2006).

In agricultural regions, the nitrogen enrichment of aquatic systems is due to point or non-point sources of organic and mineral N fertilizers, which have specific isotopic compositions (Hübner 1986, Widory et al. 2004). In fact, mineral fertilizers reflect the isotopic composition of atmospheric N, from which they are synthesized, and have a $\delta^{15}\text{N}$ generally ranging between -4% and $+4\%$ (Shearer et al. 1974, Hübner 1986, Kendall 1998), while the isotopic signature of organic manures ranges between $+2\%$ and $+30\%$ (Heaton 1986, Hübner 1986, Macko and Orstom 1994). The $\delta^{15}\text{N}$ signature can thus be used to distinguish the origin (mineral or organic) of the $\text{NO}_3\text{-N}$ found in surface waters (Kohl et al. 1971, Kellman and Hillaire-Marcel 1998, Kendall 1998, Mayer et al. 2002).

However, the isotopic signature of these N forms fractionates as they move throughout the ecosystems, due to abiotic processes (volatilization of $\text{NH}_3\text{-N}$) and

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biotic processes (e.g., nitrification, denitrification, assimilation by the plants; Heaton 1986, Fogel and Cifuentes 1993, Macko and Orstom 1994, Robinson 2001). In small streams, these biotic processes occur during N advection through the water–sediment interface, affecting both nitrate concentrations (Brunke and Gonser 1997, Dahm et al. 1998, Pinay et al. 2002) and their isotopic signature (Alexander et al. 2000). Indeed, the nitrifying and denitrifying bacteria preferentially use lighter N forms ($^{14}\text{N-NH}_4$ and $^{14}\text{N-NO}_3$, respectively). These fractionation processes induce an enrichment with the ^{14}N isotope of the NO_3 during nitrification and an enrichment with the ^{15}N isotope of the residual NO_3 after denitrification (Mariotti et al. 1981, 1988, Kendall 1998, Dhondt et al. 2003, Fukada et al. 2004, Pardo et al. 2004, Groffman et al. 2006). As a result, $\delta^{15}\text{N-NO}_3$ signature can also be used as a marker of the nitrification and denitrification processes along river networks (Kellman and Hillaire-Marcel 1998). However this signature results from a complex mix between the nitrogen origins (Heaton 1986, Vitousek et al. 1997, Kendall 1998, Chang et al. 2002, Min et al. 2003, Elliott and Brush 2006, Voss et al. 2006) and the effects of biogeochemical processes (Mariotti et al. 1988, Högberg 1997, Kendall 1998, Clément et al. 2003, Sébilo et al. 2003, Vidon and Hill 2004).

Our present work focused on three objectives. First, we investigated whether the natural $\delta^{15}\text{N-NO}_3$ signature can be used as a marker of the human activities in the upstream part of the hydrographic network or, conversely, if the terrestrial origin of the $\text{NO}_3\text{-N}$ can be obscured by riparian and in-stream microbial processes (i.e., denitrification). We selected 11 first-order streams in four different types of sub-watersheds (i.e., dominated by forest, grassland, crop fields, and mixed crops–grasslands). In rural watersheds, riparian zones and stream sediments are frequently disturbed by stream regulation and may have poor efficiency in $\text{NO}_3\text{-N}$ load reduction. We thus hypothesized that human activities around the stream will be the major driver of stream $\text{NO}_3\text{-N}$ load and predicted that the $\delta^{15}\text{N-NO}_3$ signature will change with both agricultural land cover and fertilization practices (Mayer et al. 2002).

Second, we evaluated if $\delta^{15}\text{N-NO}_3$ signature can be used to highlight and identify the influence of in-stream microbial activities (nitrification or denitrification) at the reach scale (100 m and 1000 m long). We studied $\delta^{15}\text{N-NO}_3$ signature along a 100-m reach in each sub-watershed and along a 1000-m reach in a second-order stream. All studied streams had fine-sand bottom sediments. We hypothesized that $\text{NO}_3\text{-N}$ loads would be modified by microbial activities and predicted that $\delta^{15}\text{N-NO}_3$ signature should decrease in forested watersheds where nitrification is dominant (Pardo et al. 2004), while it should increase in reaches surrounded by crop fields where fine sediment, rich in organic matter, supports a significant denitrification activity (Lefebvre et al. 2004). Ground and interstitial waters were sampled

along the 1000-m reach to highlight potential groundwater inputs.

Our third objective was to differentiate the effects of in-stream processes and various hydrological sources of N on the $\delta^{15}\text{N-NO}_3$ signature with increasing watershed size (i.e., stream order). We measured the $\delta^{15}\text{N-NO}_3$ signature in 13 first-order, 5 second-order, and 2 third-order streams. The second-order and third-order stream watersheds were dominated by intensive agriculture. We thus hypothesized that $\delta^{15}\text{N-NO}_3$ signature will reflect mostly human activities in the upstream watershed (i.e., land cover and fertilization practices) and in-stream processes will be of secondary importance because the biotic capacity of the stream to remove N (by denitrification or algal assimilation) should be exceeded due to high $\text{NO}_3\text{-N}$ concentrations (Bernot and Dodds 2005, Gucker and Pusch 2006). Consequently, we predicted that $\delta^{15}\text{N-NO}_3$ signature will gradually attain (from first- to third-order streams) a stable value mostly explained by land cover in the upstream watershed.

METHODS

Study site

The study was carried out in 11 sub-watersheds of $\sim 1 \text{ km}^2$ each, located at the French Long-Term Socio-Ecological Research (LTSER) site of “Pleine-Fougères” in Brittany, located in northwestern France. The study area is characterized by dairy farming and land cover is dominated by sown and permanent grasslands (36.4%), crop fields (36.7%), and beech forests (*Fagus sylvatica* L., 18.2%). A sample of sub-watersheds was selected by combining field observations (during February 2002) and land-cover map analyses (GIS data base since 2000). There were four types of land-cover pattern: (1) forests (called “forested,” F; considered as “control”; $n = 3$ sites), (2) sown grassland (called “grassland,” G; $n = 3$ sites), (3) mixed grassland and crops (called “mixed,” Mx; $n = 3$ sites), and (4) corn and cereal fields (called “crops,” C; $n = 2$ sites; Fig. 1, Table 1). An initial categorization of sub-watersheds was validated by the GIS analysis of the dominant land covers (Table 1): forested (100% wooded), sown grassland (more than 70%), mixed (50–70% of grassland and 20–50% of crops) and crops (>80% of crop fields, except for the C1 site, which presented only 44% of crops in June). All three forested sub-watersheds, the G2 site, and the Mx3 site were on granitic substratum, all other sub-watersheds were located on schist. The sub-watersheds also differed by their fertilization practices. No nutrient additions were applied to the forested sites, while cultivated sites were subjected to fertilization. The grassland received mostly mineral fertilizers ($101 \pm 52 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ [mean \pm SD] for permanent grassland), while crops were amended with either large amounts of mineral fertilizers (cereal fields) or both mineral forms and organic manures ($253 \pm 52 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ for maize fields, Herzog et al. 2006). Field and land-cover patterns were established by coupling geographical data (topograph-

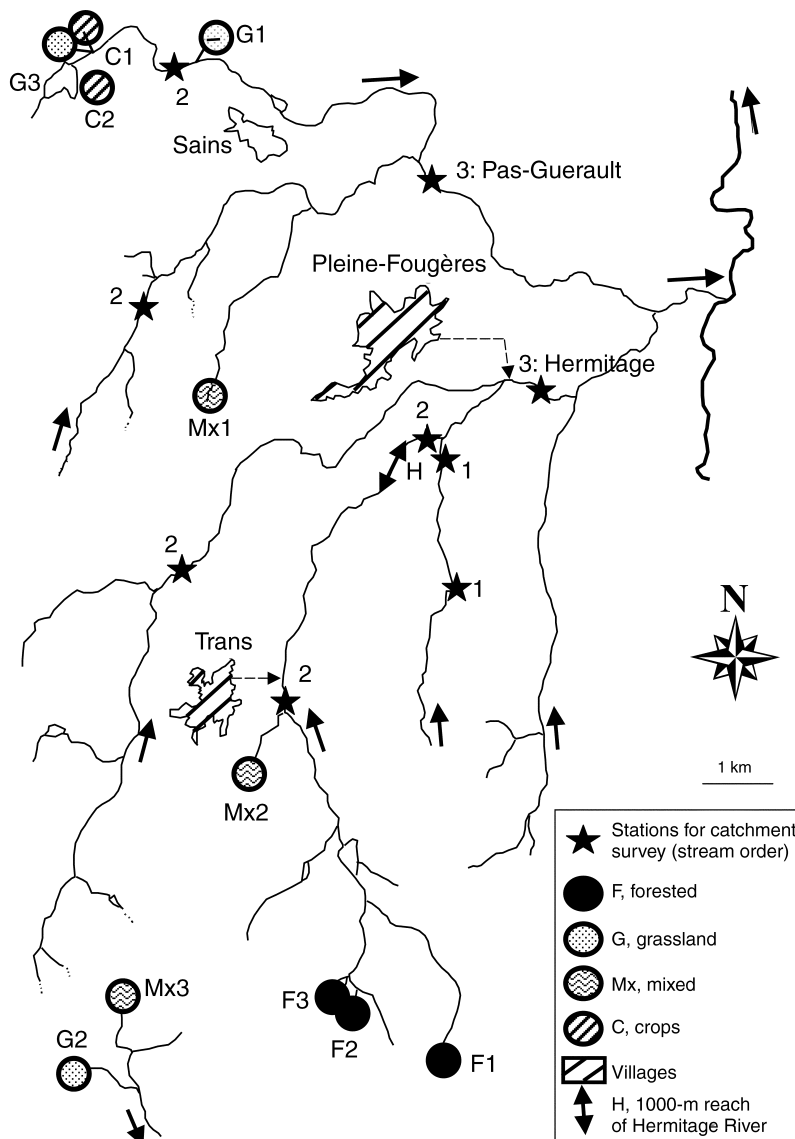


FIG. 1. Study area in northwestern France showing the 11 sub-watersheds (circles), the nine stations for the watershed survey (stars, with stream orders), the two villages (hatched), and the two wastewater discharges (thin dashed arrows). Single-headed arrows along each stream indicate direction of flow.

ical 1:25 000 maps) and field observations. The data bases were processed using a Geographical Information System (ArcGIS; ESRI 2007) to assess the surface area of each land-cover type upstream from each sampling stations.

In each sub-watershed we studied a 100-m reach on sandy substrate differing from the surrounding silt-clay soils. Stream widths ranged from 25 to 50 cm and the mean height of the stream banks was 20 cm. Discharge ranged from 0.5 to 5 L/s. Reaches were sampled at three stations located at the spring (groundwater upwelling) of the stream, and at sites located 50 m and 100 m downstream. The changes in NO₃-N concentrations and δ¹⁵N-NO₃ signature along the reaches were used to evaluate the impact of “in-stream” processes (i.e.,

nitrification in the forested reaches and denitrification in the reaches surrounded by crop fields), the changes in chloride concentrations and in the NO₃-to-chloride ratio were used to evaluate the influence of groundwater inputs along the 11 reaches. Samples were collected at two dates: during spring rising discharge (March 2002; temperature ranging between 9.3° and 12.9°C) and at the beginning of the low-water period (June 2002, between 12.1° and 15.1°C). In June, the sampling stations were moved slightly downstream because of drought (the reaches G3, Mx1 and Mx2 were dry in June). The resulting land-cover patterns of those sub-watersheds were slightly modified (Table 1), but differences between sub-watershed types remained similar.

TABLE 1. Percent cover of different land-cover types characteristic of the 11 sub-watersheds studied in the Long-Term Socio-Ecological Research (LTSER) site of Pleine-Fougères in Brittany, France.

Site	March 2002				June 2002			
	Forested	Grassland	Built area	Crops	Forested	Grassland	Built area	Crops
Forested								
F1	100				100			
F2	100				100			
F3	100				100			
Grasslands								
G1		80	20		30	70		
G2		94		6		95		5
G3		92		8		92	6	
Mixed grassland and crops								
Mx1		54	6	40		52		48
Mx2		70	3	27	38	61	1	
Mx3		66	1	33	6	70		24
Crops (corn and cereals)								
C1	5		2	81	24	32		44
C2			1	99		7		93

Notes: Land cover changed between March and June because water sampling stations were moved downstream following a drying out of the streams (see *Methods: Study site*). The land-cover type "built area" groups farms and villages. Blank data cells indicate 0%.

To evaluate the effect of in-stream processes over a longer distance, we studied a 1000-m reach of the Hermitage stream (noted "H" on Fig. 1) in a sector where the river is surrounded by wetlands covered by patches of forest (23%), grassland (40%), and crop fields (35%). The river width was 1.5 m and the discharge ranged between 75 and 100 L/s. Residence time of the water in the 1000-m reach was estimated a day before each sampling date, using NaCl injection. Samples were collected in three different seasons (June 2001, March and May 2002). The surface water was sampled three times at three locations upstream ($n = 3$ mean values) and downstream ($n = 3$ mean values) of the reach for electrical conductivity, $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations, and $\delta^{15}\text{N-NO}_3$ signature. We used electrical conductivity to estimate potential groundwater inputs along the reach, the electrical conductivity ranged from 500 to 800 $\mu\text{S/cm}$ in the groundwater, while it was generally close to 250 $\mu\text{S/cm}$ in the surface water of the reach. Groundwater and interstitial water were sampled at each date at three stations along the reach (with three piezometers at each station, $n = 3$ mean values). For groundwater, piezometers (1.5-m-long PVC tube, 2.5-cm internal diameter, screened on 10 cm from their bases) were installed 2 m from the bank at 1-m depth (below the soil surface). For interstitial water, mini-piezometers (1-m-long PVC tube, 1.7-cm internal diameter, screened on 5 cm from their bases) were inserted at each sampling date to 10-cm depth in the stream sediment at upwelling zones using a metallic bar inside the mini-piezometers that prevented contamination by surface water.

To study the effect of increasing stream order, 13 first-order, 5 second-order and 2 third-order streams within the Pas-Guerault and the Hermitage stream networks (Fig. 1) were sampled in June 2002 for $\text{NO}_3\text{-N}$, $\delta^{15}\text{N}$

signatures, and chloride (a conservative tracer mostly linked to manure amendment in this geological context). Percentages of land cover were calculated in the watershed located upstream of each station using a GIS data base. The wastewaters from the village of Trans (580 inhabitants) and Pleine-Fougères (1740 inhabitants) discharged into the Hermitage stream (dashed lines in Fig. 1). The total discharge of wastewater to the Hermitage stream was estimated at 10 L/s, but $\delta^{15}\text{N}$ signatures were not measured.

Water analyses

In each study site, water temperature, electrical conductivity (WTW LF92 thermo-conductimeter [Wissenschaftlich Technische Werkstätten, Weilheim, Germany]), and pH (IQ-Scientific Instrument, Carlsbad, California, USA) were measured in the field. Nutrient concentrations and $\delta^{15}\text{N}$ signatures were measured on 1-L water samples collected in the stream and stored in a cooler until analyses.

In the laboratory, water samples were filtered (Whatman GF/C) and analyzed by colorimetric methods: blue-indophenol for ammonium ($\text{NH}_4\text{-N}$; Rossum and Villaruz 1963) and diazotation for nitrite (N-NO_2 ; Barnes and Kollard 1951) using a Uvikon XS spectrophotometer (Bio-Tek Instruments, Milan, Italy). $\text{NO}_3\text{-N}$ was measured as nitrite following an automated cadmium-reduction method (APHA 1976). Silica (SiO_2) concentrations were determined following the Desire procedure (Desire 1957). Chloride concentrations were measured with a Sherwood chloride Analyzer 926 (Sherwood, Cambridge, UK) (Clarke 1950). Isotopic compositions of dissolved $\text{NO}_3\text{-N}$ were analyzed using diffusion methods (Sigman et al. 1997, Holmes et al. 1998). Samples of 500–1000 mL of water were filtered

(Whatman GF/C) and boiled to reduce the volume to 50 mL when needed (if NO₃⁻-N concentration <1 mg/L). A 50-mL subsample was placed into a diffusion vial with 3 g of NaCl to remove NH₄-N by alkaline diffusion (1 hour shaking); 0.5 g of DeVarda's alloy was then added to reduce nitrate to ammonium, and 1 g of MgO was added to buffer the samples (pH ~9.7), causing NH₄⁺ to convert to NH₃. Finally, an ammonia trap was added to fix the diffused NH₃ (filter pack: 1-cm-diameter GF/D Whatman filters, acidified with 25 μL of 2mol/L H₂SO₄ sandwiched between two 2.5-cm-diameter 10-μm pore size Teflon membranes [Millipore LCWP 02500; Millipore, Billerica, Massachusetts, USA]). The diffusion bottles were capped and shaken for 1 week at 40°C. The filter packs were then removed, dried in clean desiccators, and stored in clean scintillation vial until ¹⁵N analysis by a coupled elemental analyzer–isotope ratio mass spectrometer (Finnigan Delta S, Stable Isotope Laboratory, Marine Biological Laboratory, Woods Hole, Massachusetts, USA). Analytical deviation of ¹⁵N-NO₃ was assessed using standards (4 μmol/L and 1000 μmol/L NO₃) and sample replicates. As is conventional, N isotopic compositions was reported using delta notation, where δ¹⁵N = [(R_{SA}/R_{ST}) - 1] × 10³ and R = ¹⁵N/¹⁴N, expressed as deviation (in permils, ‰) of the sample (SA) from the standard (ST), which is N₂ in atmospheric air (δ¹⁵N_{air} = 0‰).

Statistical analyses

The statistical analyses of chemical characteristics were carried out with nonparametric tests. The Mann-Whitney *U* test was used to test the influence of the land-cover types (inter-type comparison), the differences between sub-watersheds of the same type (intra-type comparison), the seasonal effect, and the upstream–downstream differences for reach studies. Relationships between δ¹⁵N-NO₃ signature and NO₃-N concentrations or land-cover variables were explored with linear correlations (with log transformation when needed). Two principal component analyses (PCA) were performed on the physicochemical characteristics of the different sub-watersheds to estimate the effects of land cover and fertilization practices (inter-site variability) and their temporal changes (seasonal variability). All statistics were performed using Statistica software (StatSoft 2005).

A theoretical isotopic signature [δ¹⁵N]_{th} was calculated for the two third-order streams. Assuming the quantity of water released by each land cover is proportional to its surface area, we used the ratio of the different land-cover types in their watershed (RLC_f for forest, RLC_g for grassland, and RLC_c for crop fields) and the mean δ¹⁵N signature of the water flowing off each land-cover type (i.e., [δ¹⁵N]_f = -4.66‰ for forest, [δ¹⁵N]_g = +1.73‰ for grassland, and [δ¹⁵N]_c = +8.6‰ for crop fields in June 2002). The evapotranspiration by grassland and crop fields was assumed to be similar during the growing period, while RLC_f was reduced by

20% in each watershed to account for the higher evapotranspiration by trees (Robinson 1998, Calder et al. 2003, Robinson et al. 2003, Viaud et al. 2005). The theoretical isotopic signature [δ¹⁵N]_{th} was

$$[\delta^{15}\text{N}]_{\text{th}} = 0.8\text{RLC}_f \times [\delta^{15}\text{N}]_f + \text{RLC}_g \times [\delta^{15}\text{N}]_g + \text{RLC}_c \times [\delta^{15}\text{N}]_c. \quad (1)$$

This model is based on the assumption that the δ¹⁵N signature is only linked to the inputs of NO₃-N from the surrounding lands, without any effects from riparian wetlands and vegetation, in-stream microbial processes, or deep groundwater. Eq. 1 cannot be used to predict nitrate and chloride concentrations due to the high spatial and temporal variability of practices (quantities of amendment used within the same land-cover type), inducing therefore high variability in the quantities of solutes. The [δ¹⁵N]_{th} of the Hermitage stream was corrected for the input of wastewaters originating from Trans and Pleine-Fougères villages using a simple mixing model, where the downstream theoretical isotopic signature, [δ¹⁵N]_{dow}, was

$$[\delta^{15}\text{N}]_{\text{dow}} = \{Q_{\text{up}} \times [\delta^{15}\text{N}]_{\text{th}} + Q_{\text{ww}} \times [\delta^{15}\text{N}]_{\text{ww}}\} / Q_{\text{dow}}. \quad (2)$$

We used the mean upstream discharge of the stream (*Q*_{up} of 90 L/s), the mean wastewater discharge (*Q*_{ww} of 10 L/s for the two villages), the mean downstream discharge of the stream (*Q*_{dow} of 100 L/s), and a mean wastewater isotopic signature [δ¹⁵N]_{ww} of +15‰ (wastewater ranges from +10‰ to +20‰, Kendall 1998, Jordan et al. 1997). This value was consistent with the mean δ¹⁵N signature of +13‰ measured in wastewater produced by isolated farms in the studied area (range from +7.2‰ to +20.0‰, P. Marmonier, unpublished data).

RESULTS

Sub-watershed study

The physicochemical characteristics of the water in each sub-watershed were compared with two principal-components analyses (PCA; Fig. 2). The first axis of the PCA accounted for 72% in March (spring rising discharge) and 52% in June (low-water period) of the physicochemical parameters variance (Fig. 2A). It opposed silica (negative pole) to nitrogen concentrations, δ¹⁵N-NO₃ signatures, and electrical conductivities (positive pole). The second axis of the PCA accounted for 16% of the variance in March and 17% in June. It opposed the stations with high NH₄-N concentrations (negative pole) with those of high pH values (positive pole).

Within the two first axes plan of the PCA (Fig. 2B), the forested sub-watersheds (F1 to F3) were clearly separated from grassland (G1 to G3) and cultivated sub-watersheds (Mx and C stations), which presented the highest N concentrations and highest electrical conduc-

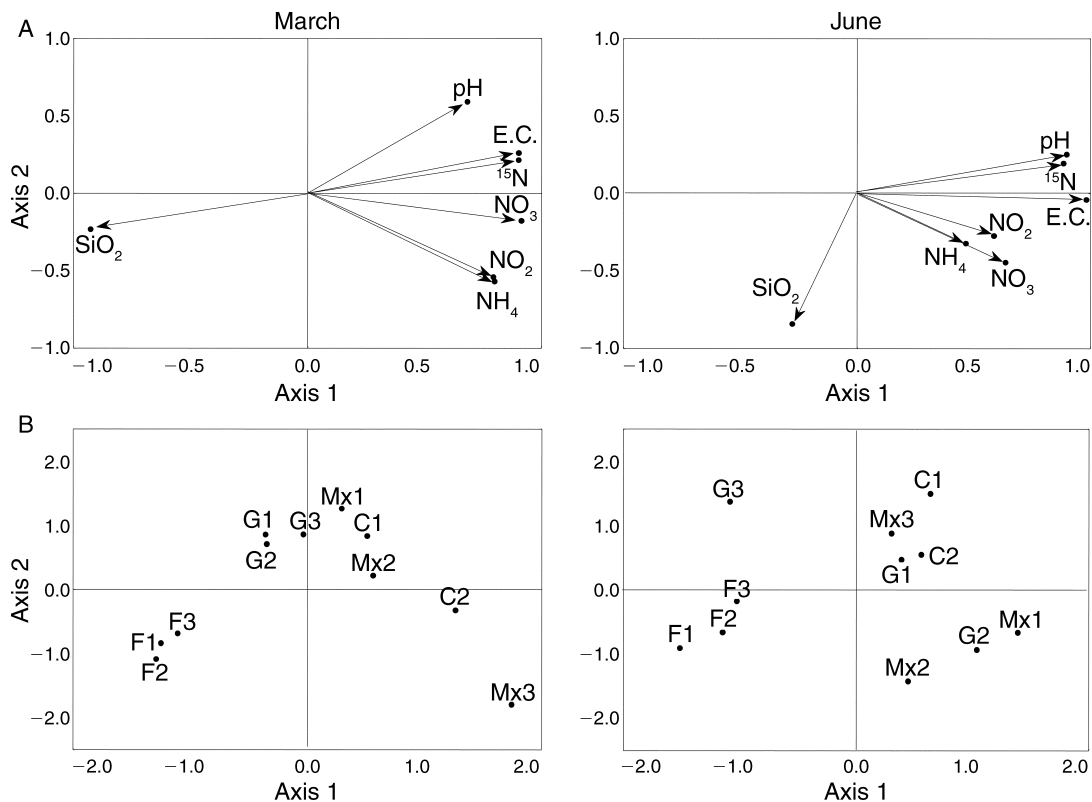


FIG. 2. Results of principal-components analyses (PCA) for March spring rising discharge (left-hand panels) and the June low-water period (right-hand panels) ($n = 33$ samples [11 reaches, 3 sites/reach] for each period) based on (A) physicochemical characteristics of stream waters and (B) on the same plane, location of the three sample sites from the 11 micro-catchments. Abbreviations: in (A), E.C. = electrical conductivity; NO_3 = nitrate; ^{15}N = nitrate isotopic signature; NO_2 = nitrite; NH_4 = ammonium; pH; SiO_2 = silica; in (B) F = forested, G = grassland, Mx = mixed, and C = crops.

tivities. This gradient was clear during spring rising discharge (March), but less obvious during the low-water period (June). A strong inter-site variability in water characteristics of the cultivated areas resulted in a poorly structured distribution of mixed (Mx) and dominant crop (C) sub-watersheds at both dates. The geological characteristics of the substratum (granite or schist) had minor effects on surface water chemistry compared to land-cover types: G2 and Mx3 sub-watersheds were clearly separated from the forested sub-watersheds.

During both hydrological periods $\text{NO}_3\text{-N}$ concentrations in forested sub-watersheds were significantly lower than in sites with a mixed grassland-crop or dominant crop pattern, while intermediate values were measured in the dominant-grassland sub-watersheds (Fig. 3). Highest variability in $\text{NO}_3\text{-N}$ concentrations was measured in reaches draining mixed sub-watersheds.

The $\delta^{15}\text{N}\text{-NO}_3$ signature of the water of the forested sub-watersheds was significantly lower than in the other sites (U tests, $P < 0.01$; Fig. 3), with average values ranging from $-3.3 \pm 1\%$ for spring rising discharge to $-4.7 \pm 0.7\%$ for low-water period. $\delta^{15}\text{N}\text{-NO}_3$ was intermediate in sown-grassland sub-watersheds where mean values were $2.1 \pm 1.1\%$ for spring rising discharge

and $1.7\% \pm 1.8\%$ for low-water period. Finally, $\delta^{15}\text{N}\text{-NO}_3$ in stream water of mixed grassland-crop (Mx) and dominant crop (C) sub-watersheds was always positive. In mixed sub-watersheds, $\delta^{15}\text{N}\text{-NO}_3$ averaged $9.1\% \pm 0.6\%$ during spring rising discharge and $5.1\% \pm 0.6\%$ during low-water period. In dominant-crop sub-watersheds, $\delta^{15}\text{N}\text{-NO}_3$ was $9.4\% \pm 0.7\%$ during spring rising discharge and $8.6\% \pm 1.1\%$ during low-water period. The increase of $\text{NO}_3\text{-N}$ concentrations and $\delta^{15}\text{N}\text{-NO}_3$ with increasing crop land cover in the sub-watersheds was consistent during the spring rising discharge period, but less clear during the low-water period due to high variability within watershed types.

Ammonium ($\text{NH}_4\text{-N}$) concentrations (Fig. 3) were significantly lower during low-water period (June) than during spring rising discharge (March, U test, $P < 0.1$), except for sown grassland site G2. In March the $\text{NH}_4\text{-N}$ concentrations were similar for forest and sown-grassland sub-watersheds, but significantly higher in mixed grassland-crop and dominant-crop sites (U test, $P < 0.05$; Fig. 3). In June, this pattern was not clear because of high $\text{NH}_4\text{-N}$ concentrations in a sub-watershed with sown grassland (G2) and two mixed sub-watersheds (Mx1 and Mx2).

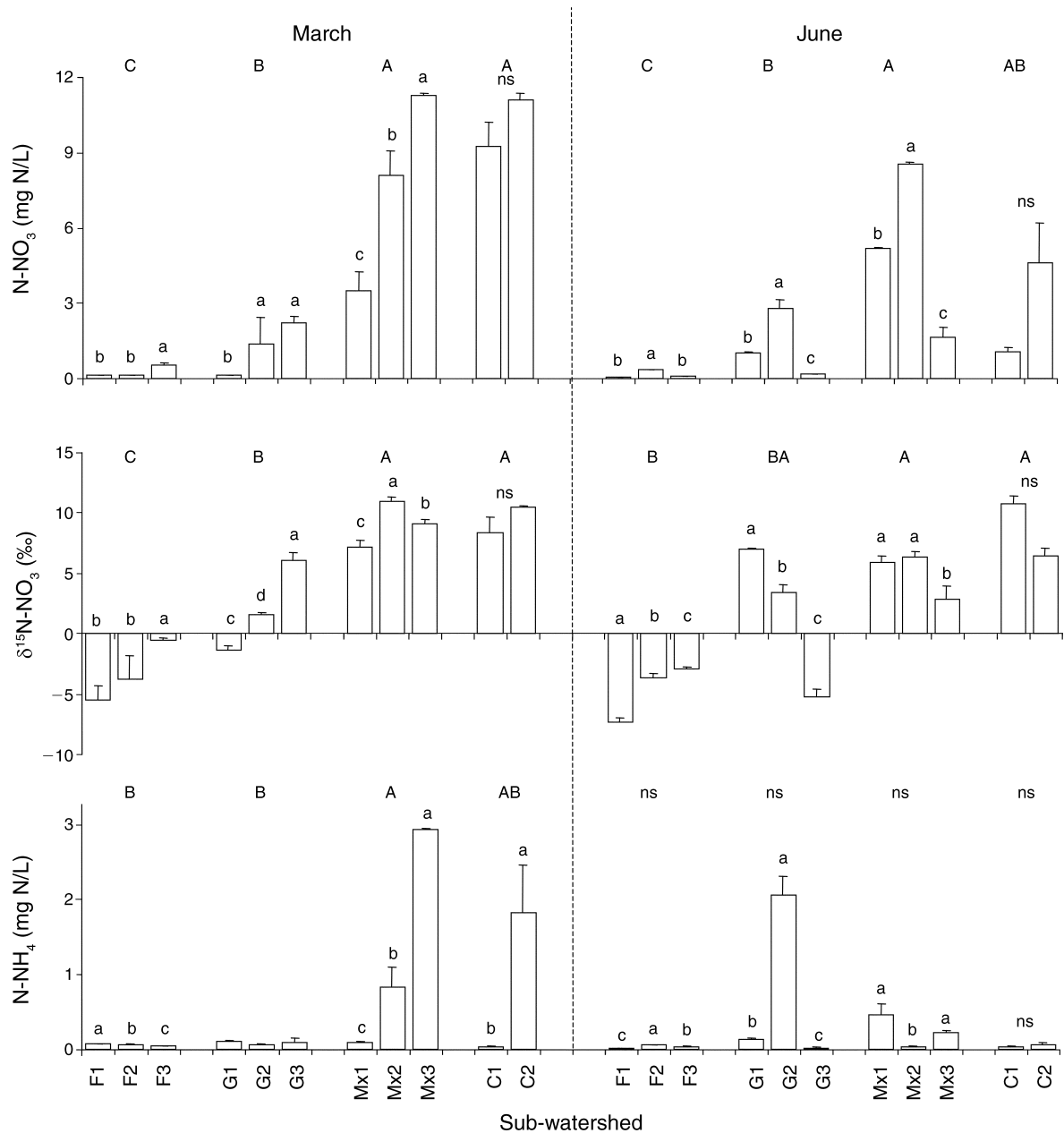


FIG. 3. Nitrate (N-NO₃) concentrations, δ¹⁵N nitrate signature, and ammonium (N-NH₄) concentrations during March spring rising discharge and June low-water period in the 11 sub-watersheds (F = forested, G = grassland, Mx = mixed, C = crops). Values are mean + SE for n = 3 samples at each date. Significant differences are noted by different letters: capital letters indicate inter-type U test; lowercase letters indicate intra-type U test; ns = nonsignificant.

Regardless of the season, the NO₃-N concentrations were significantly correlated with δ¹⁵N-NO₃ (Fig. 4). Yet the correlation coefficient was weaker in June than in March when sub-watersheds C1 and G1 were distinctly above the curve with high δ¹⁵N-NO₃ signature.

In-stream nitrogen processes along the reaches

There were no significant differences in δ¹⁵N-NO₃ signatures along the 100-m reaches, whatever the

sub-watershed land use. In forested watershed, the δ¹⁵N-NO₃ signature remained similar along the reaches or even slightly increased (in F2 in March) and no changes were observed for NO₃-N and chloride concentrations, or for the NO₃-to-chloride ratio. In the mixed grassland-crop (Mx) and dominant-crop (C) sub-watersheds, the δ¹⁵N-NO₃ signature remained similar along the reaches or decreased slightly (in C1 in June). In the same way, the NO₃-N and chloride concentrations as

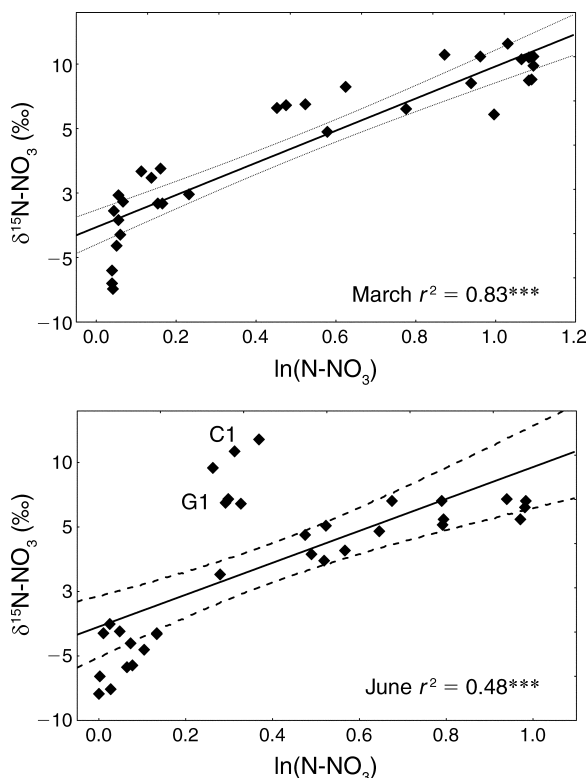


FIG. 4. Correlations between $\delta^{15}\text{N}$ nitrate signature and the logarithm of nitrate concentrations (N-NO_3) for March spring rising discharge (upper panel) and June low-water period (lower panel) in the 11 sub-watersheds. The solid line is the regression curve; dotted lines and dashed lines denote 95% confidence limits. Sub-watersheds C1 and G1 are labeled in June because of their position above the regression curves.

well as the NO_3 -to-chloride ratio remained mostly similar, even for the C1 reach. The only noticeable change occurred for the C2 reach in June, where the NO_3 -N concentrations decreased from upstream to downstream while chloride concentrations increased, but the $\delta^{15}\text{N-NO}_3$ signature remained stable.

Along the 1000-m reach, we measured negligible changes in electrical conductivity from upstream to downstream (Table 2), indicating minor groundwater inputs along the reach. Small but significant changes were observed for NO_3 -N concentrations in June 2001 (slight decrease) and May 2002 (slight increase), while NH_4 -N concentrations slightly decreased in March and May 2002. At all the three dates, the $\delta^{15}\text{N-NO}_3$ signatures were not significantly different between upstream and downstream parts of the reach. Characteristics of interstitial and ground waters were rather similar (Table 3), with very low NO_3 -N, but high electrical conductivity and NH_4 -N concentrations during all sampling periods. The $\delta^{15}\text{N-NO}_3$ signatures were generally positive (except for groundwater in May 2002), but highly variable (see standard deviations in Table 3).

NO_3 -N concentrations and $\delta^{15}\text{N}$ as a function of stream order

With increasing stream order, the variability of land-cover patterns decreased (Fig. 5) to reach intermediate values in the third-order streams (Pas-Guerault and Hermitage streams), close to the values measured for the entire Long-Term Socio-Ecological Research (LTSER) area (36.4% grassland, 36.7% crop, and 18.2% forest). Similarly, the variability of NO_3 -N concentrations, $\delta^{15}\text{N}$ signatures, and chloride decreased with increasing stream order (Fig. 6). In first-order streams, NO_3 -N concentrations ranged from 0.1 to 12 mgN- NO_3 /L, from -5% to $+11.8\%$ for $\delta^{15}\text{N-NO}_3$, and from 14.7 to 55.7 mg/L for chloride. In contrast, the third-order streams had very similar NO_3 -N concentrations (5.9 and 5.7 mg NO_3 -N/L for Pas-Guerault and Hermitage streams, respectively), $\delta^{15}\text{N-NO}_3$ signatures ($+4.3\%$ and $+4.5\%$, respectively), and chloride concentrations (42 and 38 mg/L, respectively). These values were similar to those measured in the mixed grassland-crop sub-watersheds for the same period (5.1 ± 0.3 mgN/L, $+5.06\% \pm 1.92\%$, and 34.7 ± 1.8 mg/L [mean \pm SD], respectively). The variability of the NO_3 -to-chloride ratio decreased

TABLE 2. Changes in electrical conductivity (Elec. cond.), NO_3 -N and NH_4 -N concentrations, and $\delta^{15}\text{N-NO}_3$ signature upstream and downstream of the 1000-m reach of the Hermitage stream (northwestern France) on the three sampling dates.

Station	Elec. cond. ($\mu\text{S}/\text{cm}$)	NO_3 -N (mgN/L)	$\delta^{15}\text{N-NO}_3$ (‰)	NH_4 -N (mgN/L)
June 2001				
Upstream	230.7 ± 2.1	4.88 ± 0.05	7.87 ± 0.12	0.025 ± 0.003
Downstream	232.0 ± 1.0	4.55 ± 0.03	8.00 ± 0.10	0.030 ± 0.006
M-W	ns	*	ns	ns
March 2002				
Upstream	235.0 ± 0.1	3.20 ± 0.02	7.20 ± 0.10	0.306 ± 0.003
Downstream	235.3 ± 0.2	3.16 ± 0.02	7.35 ± 0.05	0.294 ± 0.006
M-W	ns	ns	ns	*
May 2002				
Upstream	239.7 ± 0.6	4.58 ± 0.12	5.63 ± 0.38	0.047 ± 0.016
Downstream	240 ± 0.5	4.78 ± 0.02	6.10 ± 0.40	0.032 ± 0.002
M-W	ns	*	ns	*

Notes: Data are means \pm SD; $n = 3$ measures per site. M-W: Mann-Whitney U test between upstream and downstream stations (ns = nonsignificant; * $P < 0.05$).

TABLE 3. Electrical conductivity (Elec. cond.), $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations, and $\delta^{15}\text{N}\text{-NO}_3$ signature in the interstitial and ground waters along the 1000-m reach of the Hermitage stream on the three sampling dates.

Station	Elec. cond. ($\mu\text{S}/\text{cm}$)	$\text{NO}_3\text{-N}$ (mgN/L)	$\delta^{15}\text{N}\text{-NO}_3$ (‰)	$\text{NH}_4\text{-N}$ (mgN/L)
June 2001				
Interstitial water	318 ± 51	0.13 ± 0.16	3.63 ± 4.63	0.610 ± 0.094
Groundwater	516 ± 152	0.34 ± 0.43	3.97 ± 4.09	0.153 ± 0.201
March 2002				
Interstitial water	497 ± 263	0.13 ± 0.02	8.00 ± 7.43	1.011 ± 0.393
Groundwater	649 ± 238	1.61 ± 1.53	7.70 ± 2.57	0.802 ± 0.548
May 2002				
Interstitial water	299 ± 43	0.24 ± 0.21	9.97 ± 10.6	0.727 ± 0.237
Groundwater	499 ± 186	0.09 ± 0.08	-1.5 ± 4.91	1.362 ± 0.952

Note: Data are means \pm SD; $n = 3$ measures per site.

with increasing stream order: it ranged from 0.001 to 0.235 in the first-order stream and reached very similar values in the two third-order streams (0.140 and 0.150 for Pas-Guerault and Hermitage streams, respectively). Similar mean $\text{NO}_3\text{-N}$ -to-chloride ratio was measured in the mixed grassland-crop sub-watersheds for the same period (0.145 ± 0.09). When all samples were considered together (Fig. 7), the $\delta^{15}\text{N}\text{-NO}_3$ signature was positively correlated to the percentage of crop and negatively correlated to the percentage of forest in the watershed area. No significant relationship was found with the percentage of grassland.

Theoretical $\delta^{15}\text{N}$ signatures calculated for both third-order streams using their watershed land covers were consistent with the measured value in the Pas-Guerault stream ($+4.35\text{‰}$ calculated cf. $+4.30\text{‰}$ measured; Fig. 6), but consistently different in the Hermitage stream ($+2.11\text{‰}$ calculated cf. $+4.50\text{‰}$ measured). When the wastewaters of Trans and Pleine-Fougères villages were included in the calculation of the theoretical $\delta^{15}\text{N}$ signatures of the Hermitage stream, the value reached $+3.40\text{‰}$ (a value still less than the measured value).

DISCUSSION

Relationships between land cover and water chemistry in headwater streams

The chemical characteristics of headwater streams highlighted the dominant influence of land-cover pattern and, to a lesser extent, of bedrock geology (Valett et al. 1996, Lefebvre et al. 2005). High silica concentrations and low pH in forested sites highlighted their location on granite bedrock, but the G2 and Mx3 sub-watersheds (also located on granite) were grouped together with other agricultural sub-watersheds located on schist, demonstrating the critical influence of the land cover on surface water chemistry. As predicted, land cover in the watershed (and the associated fertilization practices) also significantly influence the nitrate concentration of the draining streams. This relationship between agricultural activities and stream water nitrogen loads has been well established (Vitousek et al. 1997, Alexander 2000, Naiman et al. 2002). In our study the forested sites under limited anthropogenic influence presented the lowest

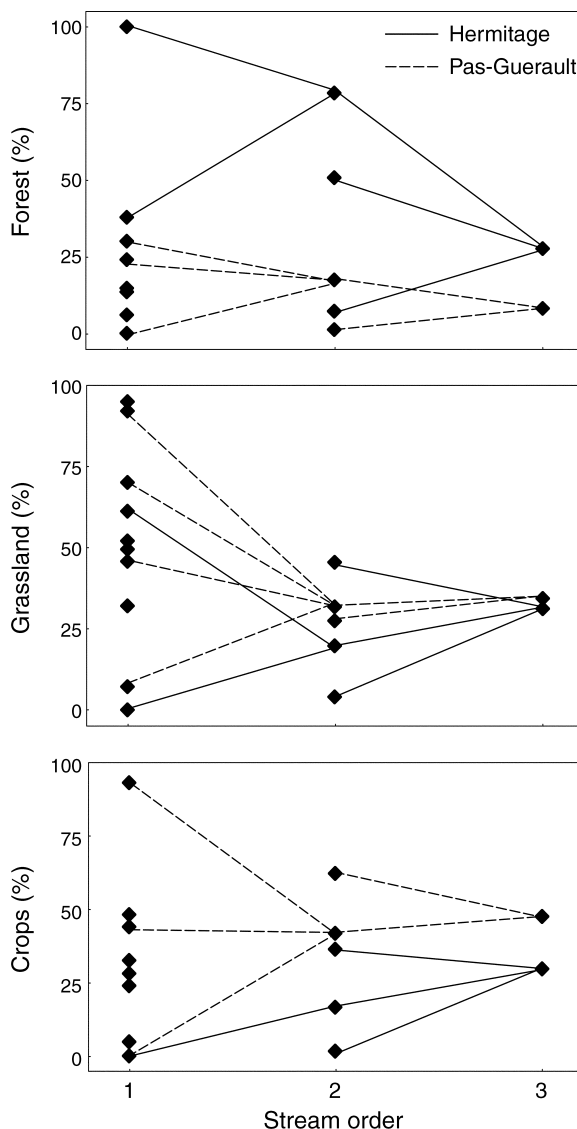


FIG. 5. Changes in land use (percent cover of different land-cover types) with stream orders. Solid lines denote the Hermitage stream network, and dashed lines denote the Pas-Guerault stream network. The first-order stream stations are not all related to second-order stream study sites (see Fig. 1).

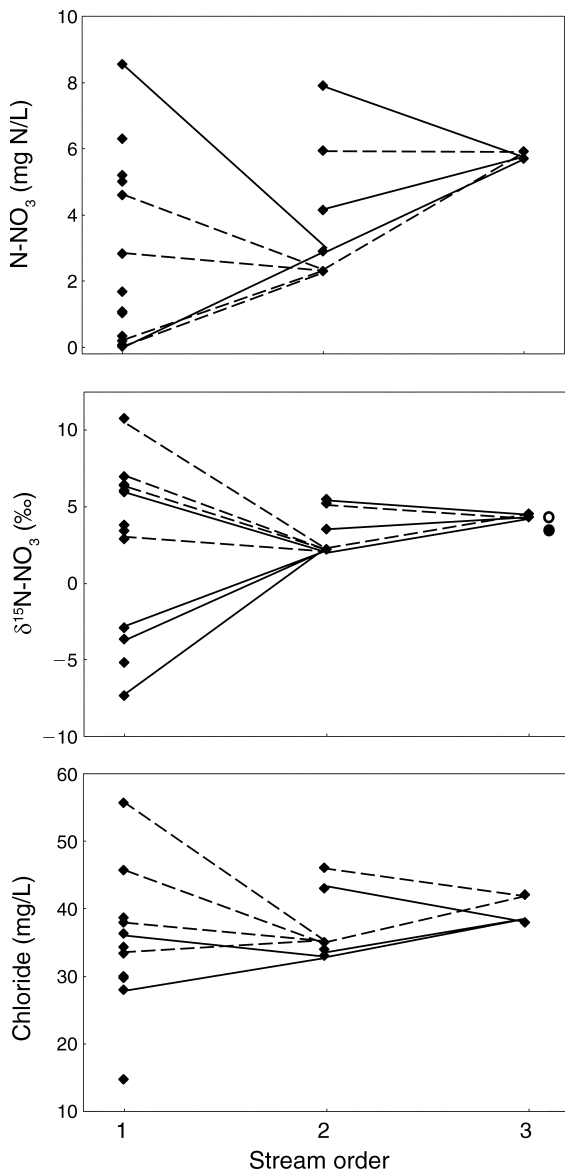


FIG. 6. Changes in nitrate concentrations, $\delta^{15}\text{N-NO}_3$ signatures, and chloride concentrations with stream orders, formatted as in Fig. 5. Theoretical $\delta^{15}\text{N}$ signatures calculated for Hermitage stream (solid circle) and Pas-Guerault stream (open circle) are shown.

inorganic-N concentrations and similar water characteristics. Among agricultural sub-watersheds, there was a clear gradient from moderate inorganic-N concentrations (from 0.5 to 3 mg $\text{NO}_3\text{-N/L}$) in streams draining sown grasslands to high concentrations (>3 mg $\text{NO}_3\text{-N/L}$) in streams draining crop fields.

High nitrogen concentrations measured in mixed grassland-crop and dominant-crop sub-watersheds reflected the intensive fertilization. Differences in the type and amount of fertilizers applied for the different land-cover types (see above) can partly explain the differences in N loads between sub-watersheds. Sown grasslands

were fertilized with mineral compounds; they were mown and/or grazed and tilled only every 4–8 years. The semipermanent vegetation cover of these sown grasslands contributed to retain nitrogen more effectively than the exposed soils of cultivated fields during winter (Cheverry 1998, Neveu et al. 2001). In the studied area, no winter covers were implemented after small-grain cereal and corn were harvested, and the arable lands used for corn production were fertilized with mineral compounds and, for some of them, with additional organic manures. These streams located at the head of watersheds represent the first interface

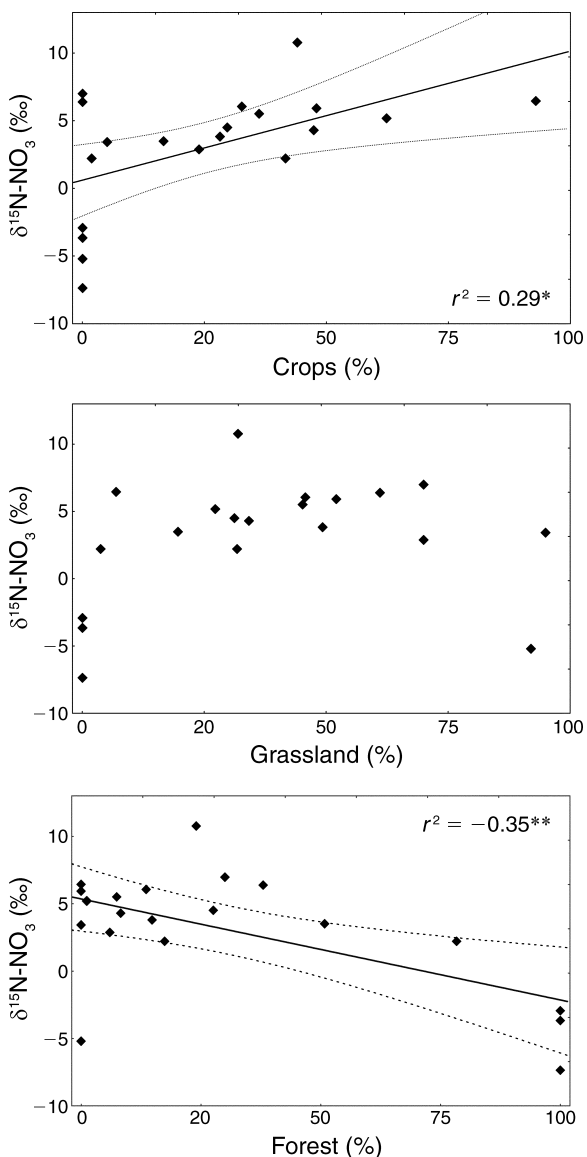


FIG. 7. Correlations between $\delta^{15}\text{N-NO}_3$ signatures and percent cover of land-cover types in the upstream catchments for the June low-water period in the 13 stations considered together (the solid line is the regression curve; dotted lines show 95% confidence limits).

between terrestrial and aquatic environments (Ward 1989, Stanford and Ward 1993) and they are therefore highly sensitive to human activities occurring on uplands (Cheverry 1998, Quinn and Stroud 2002). This short time lag between agricultural practices and water quality response in first-order streams can explain the strong inter-site variability in N loads during both spring rising discharge and low-water periods.

Agricultural practices and isotopic signatures

The non-fertilized forested sub-watersheds had stream water with the lowest $\text{NO}_3\text{-N}$ concentrations and consistently negative $\delta^{15}\text{N-NO}_3$ values. Similar results were obtained in forested sites, where the degradation of the organic matter followed by nitrification led to production of $\text{NO}_3\text{-N}$ depleted in ^{15}N (Mariotti et al. 1988, Pardo et al. 2004). The isotopic differences recorded among the three other types of sub-watersheds (G, Mx, and C) were certainly related to fertilization. In the studied area, the average $\delta^{15}\text{N-NO}_3$ in streams surrounded by sown grasslands ($+2.1\text{‰} \pm 1.1\text{‰}$) was lower than that recorded in mixed grassland-crop ($+9.1\text{‰} \pm 0.6\text{‰}$) and crop-dominated ($+9.4\text{‰} \pm 0.7\text{‰}$) sub-watersheds. The grasslands were generally amended with mineral fertilizers (with low $\delta^{15}\text{N-NO}_3$; Shearer et al. 1974, Hübner 1986, Kendall 1998), while corn fields were fertilized with mineral compounds and additional organic manures (Herzog et al. 2006) with high $\delta^{15}\text{N-NO}_3$ (Heaton 1986, Hübner 1986, Macko and Orstom 1994). This combination of different agricultural practices in the different land-cover types explained the positive exponential correlations observed between the $\text{NO}_3\text{-N}$ concentrations and their $\delta^{15}\text{N}$ during both hydrological periods (Fig. 4). These correlations resulted from a concomitant increase in both $\text{NO}_3\text{-N}$ concentrations and $\delta^{15}\text{N}$ enrichment with increasing fertilization in the sub-watersheds (as observed by Harrington et al. [1998]). The high $^{15}\text{N-NO}_3$ concentrations measured in C1 and G1 in June (Fig. 4) may be the result of wide local use of organic manures.

This study of first-order streams was limited to a single year and was restricted to the spring period. Spring was chosen for technical reasons (most of these streams generally are dry later in the year), and because it represents the best season for the buffering effect of riparian zones to be considered. Indeed, during spring both microbial denitrification and N plant uptake in the surrounding wetlands are high due, respectively, to frequent soil saturation and significant net primary productivity (Clément et al. 2002, Hefting et al. 2005). Nevertheless, the strong relationships between land cover and $\delta^{15}\text{N}$ signature must be carefully considered. Other combinations of processes may happen during other seasons (e.g., strong assimilation by stream algae, inputs of denitrified water from deep aquifer) that may hide the effect of human activities in the watersheds. Although more expensive than our single-isotope approach, the combined measurements of ^{15}N and ^{18}O

isotopes might help to better elucidate sources and transformations of nitrate in these first-order streams (Deutsch et al. 2005, 2006, Voss et al. 2006).

In-stream processes

The isotopic signature of manures has been shown to be modified by abiotic processes (e.g., volatilization of ammonia) and by microbiological fractionation (e.g., ammonification, nitrification, and denitrification) during their transit within soils, aquifers, and rivers (Mariotti et al. 1988, McMahon and Böhlke 1996, Högberg 1997, Fukada et al. 2004). The influence of microbial processes on the $\delta^{15}\text{N-NO}_3$ signature, such as denitrification, has been observed during the summer low-water period by several authors (Knowles 1982, Knowles and Blackburn 1993, Seitzinger et al. 1993, Clement et al. 2003, Sébilo et al. 2003). These studies suggest that the $\delta^{15}\text{N-NO}_3$ signature may also be used to highlight in-stream biophysical processes.

At the reach scale, for both 100 m and 1000 m long, nitrate concentrations and their $\delta^{15}\text{N}$ variations did not present any significant trend. At both scales it was not possible to distinguish the predicted influence either of nitrification in the forested reaches or of denitrification in agricultural reaches. This does not mean that $\text{NO}_3\text{-N}$ denitrification at the water-sediment interface did not occur in these rural streams. In parallel studies on the same streams, this process was measured in the laboratory with stream sediment sampled during both high- and low-water periods and a strong significant decrease in $\text{NO}_3\text{-N}$ concentrations was observed in the interstitial water of these streams (Lefebvre et al. 2004, 2005). The very low $\text{NO}_3\text{-N}$ contents measured in the interstitial water of the 1000-m reach (Table 3) support the occurrence of intense denitrification inside stream sediment, but with poor $\delta^{15}\text{N}$ signature. Denitrifying bacteria first use $^{14}\text{N-NO}_3$ for their metabolic need, but as it disappears they progressively use $^{15}\text{N-NO}_3$, making $\delta^{15}\text{N}$ signature less consistent (Clément et al. 2003).

The lack of $\delta^{15}\text{N}$ signature for denitrification along the studied reaches could result from (1) the large soil water inputs from annual crop fields and from the underlying groundwater, that potentially masked the benthic processes in these first-order streams, and (2) the short residence time of the water inside these 100-m reaches (~15–30 minutes) and the resulting short time of contact between water and sediments. These two factors would limit our ability to detect the influence of microbial processes (Kellman and Hillaire-Marcel 1998).

Along the 1000-m reach, changes in the $\delta^{15}\text{N}$ signature of surface water linked to denitrification could be hidden by coupled denitrification of surface water $\text{NO}_3\text{-N}$ (that increased $\delta^{15}\text{N-NO}_3$ signature) with nitrification of interstitial $\text{NH}_4\text{-N}$ (that reduced $\delta^{15}\text{N-NO}_3$ signature). These combined processes are difficult to highlight with $\delta^{15}\text{N}$ signature alone and $\delta^{18}\text{O}$ might be required for this. A large input of interstitial water and groundwater totally denitrified (i.e., with a low

$\delta^{15}\text{N}$) could also hide the in-stream processes, but our results do not support a massive outflow of groundwater to the river reach; little or no change in electrical conductivity of surface water along the reach (Table 2) suggests very limited, if any, interstitial and groundwater inputs.

¹⁵N-NO₃ signature and stream order

Our study confirmed that the isotopic composition of stream nitrate provides information about N origin on a whole-watershed basis (Spoelstra et al. 2001), and suggested that this isotopic signal changes with the stream order within the same watershed, because it integrates the upstream land covers and agricultural activities. NO₃-N concentrations and $\delta^{15}\text{N}$ variability strongly decreased in larger watersheds (i.e., second- and third-order streams), and presented intermediate values, similar to those measured in the mixed (grassland and crops) sub-watersheds. Similar decreases in variability with scale have been reported in other studies (Mérot and Durand 1995), but with lesser amplitude (Chang et al. 2002, Sébilo et al. 2003).

The progressive decrease in variability may be explained by a simple mixing of water originating from different land covers (Sébilo et al. 2003). This idea is supported by (1) the correlations, predicted in our hypotheses, between $\delta^{15}\text{N-NO}_3$ signatures and the surfaces devoted to crops (positive) or to forests (negative correlation) and (2) the similar decrease in the variability of chloride concentrations (mostly linked to manure amendment in the studied watersheds) and the NO₃-to-chloride ratio. However, the observed decrease in variability of $\delta^{15}\text{N-NO}_3$ signature along the stream network could result from different causes. On the one hand, the potential of both microbial NO₃-N denitrification and NO₃-N uptake by algae could be exceeded due to high NO₃-N concentrations in the studied streams (already observed by Bernot and Dodds [2005], Gucker and Pusch [2006]). In this case, the stream-water $\delta^{15}\text{N-NO}_3$ signature remains stable due to a saturation of biological activities. On the other hand, large input of denitrified groundwater (i.e., with low and constant $\delta^{15}\text{N-NO}_3$ signature) might dilute any extra microbiological NO₃-N fractionation by in-stream denitrification. As a result of this large-scale hydrological process, stream-water $\delta^{15}\text{N-NO}_3$ signature remains stable.

Finally, the calculation of a theoretical $\delta^{15}\text{N}$ signature using upstream land cover was successful for only one of the third-order streams (i.e., the Pas-Guerault stream), and remained below the observed value in the other one (i.e., the Hermitage stream). This deviation between measured and theoretical values of $\delta^{15}\text{N}$ could reflect differences in hydrologic yield of the sub-watersheds not properly accounted for by Eqs. 1 and 2. This deviation might also reflect uncertainty in the estimates of $[\delta^{15}\text{N}]_r$, $[\delta^{15}\text{N}]_g$, and $[\delta^{15}\text{N}]_c$ that were based on very small ($n = 3$) sample sizes. Alternatively, the high $\delta^{15}\text{N}$ signature measured in the Hermitage stream may be linked to

localized hot-spots of denitrification (in-stream, Lefebvre et al. 2006; or in the riparian wetlands, Clément et al. 2002) or to other point-source pollution not included in the calculation. The use of combined measurements of nitrogen and oxygen isotopes would be a pertinent method to further elucidate the origin of NO₃-N load at the river network scale (Voss et al. 2006).

In conclusion, the use of the $\delta^{15}\text{N-NO}_3$ signature as an integrator of upstream land cover linked to human activities may be of interest in rural watersheds, even if all biological and hydrological processes were not highlighted by this method. The combination of several elemental isotopic measurements (i.e., nitrogen and oxygen) may help to differentiate between the different sources of NO₃-N and the rate of its transformation. This is a crucial issue for water management. Nevertheless, when the stream order increased in our relatively small watersheds, we found a compelling parallel decrease in the variability of land patterns, stream water N-NO₃ concentrations, and $\delta^{15}\text{N-NO}_3$ values. At this scale (5–10 km² in the studied area), subtle changes in land cover and agricultural practices due to agricultural policies are predictable in the near future. Therefore, monitoring the variation of nitrate isotopic signature in these low-order streams could help to determine the time lag of response of river water quality to these landscape management changes.

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