



Winter nitrification contributes to excess NO_3^- in groundwater of an agricultural region: A dual-isotope study

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[1] Conventional thinking is that bacterial nitrification leading to labile nitrate in fertilized agricultural soils of northern regions greatly diminishes during winter. We have carried out seasonal water sampling over 2 years to understand the fate of nitrate present in a rapidly responding groundwater/surface water system. Nitrate results show no seasonal $\delta^{15}\text{N}$ trend. Significant $\delta^{18}\text{O}$ downward shifts were observed between the spring-summer and autumn-winter periods of 2003–2004 (10.0‰) and 2004–2005 (1.3‰). Using mass-balance mixing calculations of soil leachate with groundwater and assuming seasonal nitrification, we reproduce the observed water and nitrate oxygen-isotope trends. These calculations suggest that nitrification takes place throughout all seasons. We also use a hydroclimatic index to establish a relationship between $\delta^{18}\text{O}$ values in nitrate and recharge weighted by temperature. Our findings imply that nitrifying activities occur all year long and that winter nitrate production is high. This conclusion has important implications for modeling the nitrogen cycle of regions where seasonal changes in soil water mark the oxygen isotopes of nitrate.

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1. Introduction

[2] Production and loss of NO_3^- from soil during winter have long been recognized [e.g., Heaney and Nyborg, 1988]; however, the processes involved have not been examined using groundwater (GW) characteristics in regions of intense agricultural activities, and the significance of this loss has not been fully considered. Soil processes affecting the N-bearing components during cold periods are only partly known under field conditions. Soil bacterial denitrification occurring at 0°C has been measured during winter and at snowmelt in snow-covered agricultural fields [van Bochove et al., 2000]. Considering the diversity of soil–N cycle microbiological processes, other N transformations are also likely active in cold soils. The literature on N- NO_3^- concentrations measured in winter soil-water samples is sparse with most papers primarily addressing total quantity of soil nitrate. Winter nitrification has been examined for various soil types and, in general, a decrease in nitrate production accompanies a decrease in temperature [Campbell et al., 1970]. A few studies have investigated the effects of winter temperatures typical of temperate regions on the release of N from plant residues in laboratory experiments. They propose that nitrifying bacteria are still active at temperatures as low as 0°C , that the processes

favoring nitrification can occur during the December–March period, and that the production of nitrate in winter can be important [Chantigny et al., 2002; Cookson et al., 2002; Mengis et al., 2001; Heaney and Nyborg, 1988].

[3] Catchment modeling of nitrate-N export from temperate forests to surface water (SW) suggests that the concentration of nitrate-N is unchanged throughout the year, but the nitrate-N flux to surface water is the highest during the late winter-spring snowmelt period [Creed et al., 1996]. This observation is explained by bacterial activity occurring under the snowpack which provides NO_3^- to the soil that is leached over short periods during snowmelts as there is no uptake by plants [Brooks et al., 1998; Creed et al., 1996]. Groundwater denitrification in riparian zones of agricultural regions during cold period has been documented using $\delta^{15}\text{N}$ values, but the period of nitrification has not been determined [e.g., Dhondt et al., 2002]. Overall, in agricultural regions, nitrate dynamics during winter are not completely understood, and losses of soil N- NO_3^- could be due either to denitrification or to leaching [Heaney and Nyborg, 1988]. Results linking winter nitrification to nitrate loss by leaching to aquifers have not been reported, and $\delta^{18}\text{O}$ values of nitrate dissolved in GW have not been used to demonstrate such a link.

[4] Many areas of the Maritime Provinces of Canada are dedicated to intense agriculture. In areas of potato cultivation, concentrations of N- NO_3^- in soil leachates can reach as much as 65 mg L^{-1} , and may have an average of 38 mg L^{-1} over a 2-year period ($28\text{--}48 \text{ mg L}^{-1}$ [Milburn et al., 1990, 1997]). These concentrations have been reported to diminish during winter [Zebarth et al., 2003]. The yearly concentration averages are high and represent a threat to drinking water quality. A related crucial issue is that GW

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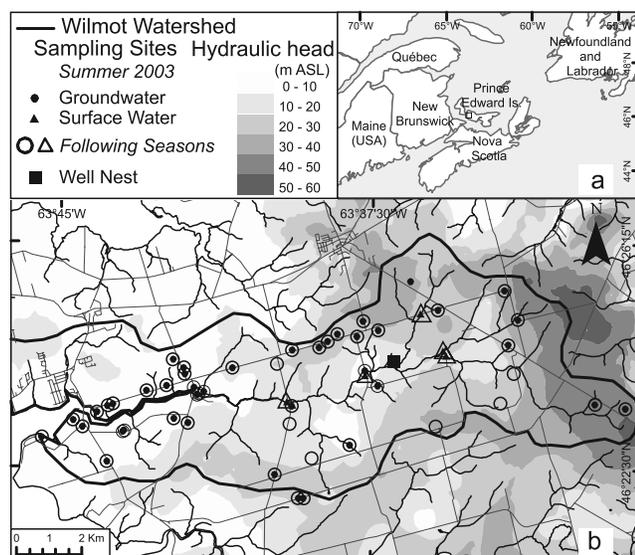


Figure 1. (a) Map showing the location of the Wilmot watershed. (b) Water table map showing the delineation of the watershed and the distribution of sampling sites, including the observation well nest.

constitutes the only source of potable water on Prince Edward Island (PEI). Nitrate concentrations in GW have generally been increasing over time. In places, concentrations are now in excess of the recommended Canadian maximum concentration limit of $10 \text{ mg L}^{-1} \text{ N-NO}_3^-$ [Health Canada, 2003]. An understanding of the export of nitrate from soils to GW is therefore required to aid in the development of strategies to reduce the detrimental effects of nitrate leaching from agricultural soils, to estimate the environmental risk related to autumn spreading of manures, and to provide adequate information for modeling the N cycle in agricultural watersheds. A quantitative assessment of the nitrate contribution from various sources in the study area is presented elsewhere [Savard et al., 2007]. Here we combine the analyses of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $[\text{NO}_3^-]$ of nitrate dissolved in GW and SW to evaluate, under real agricultural conditions, the period of nitrification and of transfer of soil nitrate to the aquifer of the Wilmot watershed on PEI. Our specific objectives are to (1) document the seasonal extent of nitrification in these agricultural soils; (2) assess seasonal export of nitrate from the agricultural soils to the regional aquifer; and (3) help characterize the local nitrogen cycle.

2. The Wilmot Watershed and Aquifer

[5] We selected the Wilmot watershed-aquifer system because it is ideally suited to the study of nitrate transfer from soils to aquifer due to the intensity of agricultural production, availability of wells for sampling, and accessibility of records for GW and SW quantity and quality. The Wilmot River basin is located in west central PEI, and is approximately 5 km wide and 17 km long (Figure 1). Topographic elevations range from sea level to the west to 90 m above sea level (asl) in the eastern area of the watershed. From headwater tributaries, the river flows

southwest and drains an area of about 87 km^2 . Residential land use represents 9% of the watershed area, whereas forests account for another 15%. Agriculture dominates land use in the watershed, occupying the remaining 76% of the total land area [Atlantic AgriTech, 2007]. Practices in the Wilmot watershed are characterized by seeding and fertilizer application in the range of 75 kg ha^{-1} during late spring. Row-crop potato farming accounts for approximately 80% of agricultural land use. Plant residues are left in the field after harvesting [Atlantic AgriTech, 2007].

[6] The climate of PEI is humid-continental, with long, fairly cold winters and warm summers (Figure 2a). The meteorological conditions recorded during the 2 years of investigation were typical of yearly conditions in the Wilmot region (Figure 2a). Mean annual precipitation recorded for the period of 1961–2005 was 1153 mm. Most of the precipitation falls as rain (73%, or 841 mm), and the remainder falls as snow (27%, or 311 mm). The mean annual temperature is 5.3°C , and mean monthly temperatures range from -7.9°C in January to $+18.5^\circ\text{C}$ in July.

[7] The substrate of the entire province consists primarily of fine- to medium-grained sandstone (80–85%), mudstone (siltstone and claystone), and conglomerate of the Upper Carboniferous to Lower Permian “Prince Edward Island Redbeds” [Van de Poll, 1983] (from CSPG Atlantic Region Lexicon). The sandstone is highly fractured. Vertical to

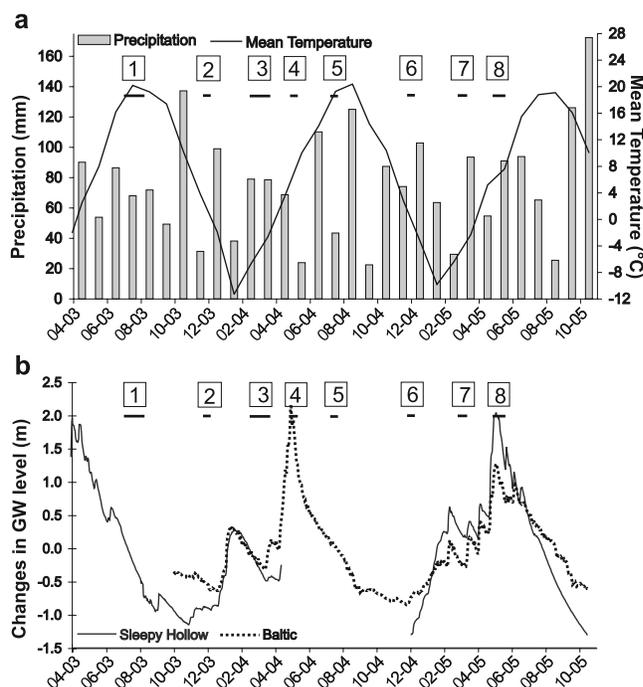


Figure 2. (a) Cumulated precipitation and mean monthly temperature for the Wilmot region from April 2003 to October 2005. (b) Hydrographs of groundwater level relative to mean levels of the Sleepy Hollow and Baltic wells outside the Wilmot watershed, but typical of the region and completely covering the periods of sampling (numbers in squares refer to seasons specified in Table 1). Data from the Prince Edward Island Department of Fisheries, Environment and Energy.

Table 1. Sampling Periods Over the Two Yearly Cycles

Season	Sampling Period		n			Season of Sampling
	Beginning	End	Precipitation	Surface Water	Groundwater	
Summer	8 Jul 2003	4 Aug 2003	3	6	41	1
Autumn	28 Sep 2003	2 Dec 2003	3	4	20	2
Winter	2 Mar 2004	10 Mar 2004	0	3	20	3
Spring	31 Mar 2004	3 Jun 2004	7	4	20	4
Summer	6 Jul 2004	16 Sep 2004	4	6	49	5
Autumn	29 Sep 2004	19 Dec 2004	1	4	25	6
Winter	13 Jan 2005	10 Mar 2005	4	4	25	7
Spring	5 Apr 2005	5 May 2005	2	8	27	8

subvertical fractures occur in addition to fractures parallel to bedding planes [Francis, 1989]. Most fractures are found in the uppermost 20 m of the rock aquifer. Surface geology for most of the Wilmot Basin is composed of a ground moraine characterized as a permeable sand-phase till, with local areas of clay-sand phase till. The thickness of these surface deposits ranges from less than a meter to more than 15 m, averaging about 3.6 m.

[8] The Wilmot aquifer has been characterized as a fractured porous media, with fractures representing the main GW flow paths, and matrix pores determining its storage characteristics. Permeability and flow are significantly reduced below the aquifer depth of 30m [Paradis *et al.*, 2006]. At the regional scale, the conceptual model of the aquifer is represented by a permeable unit of mixed-porosity sedimentary rocks, covered by a permeable till of variable thickness. The recharge is in the range of 25% of annual precipitation, which represents on average 410 mm yr⁻¹. Groundwater is typically of a Ca-Mg-HCO₃⁻ type, slightly alkaline with moderately high hardness and total dissolved solids [Somers *et al.*, 1999].

[9] A map of the water table of the unconfined Wilmot aquifer provides a general picture of GW flow towards the Wilmot River and its estuary (Figure 1b). The fluctuations of the water level in monitoring wells throughout the province demonstrate the seasonal response to regional climate. The response includes a major spring recharge event followed by a summer decline of the water table. Then a moderate recharge event occurs at the end of the autumn, and last, a decline in the water table through the winter is punctuated by periodic recharge events prior to the next spring recharge (Figure 2b). The winter recharge occurs as a result of winter rain and snowmelt. Depending on the frost conditions in the soil, this recharge can be very significant. For example, the hydrograph separation for the Baltic and Hollow wells in 2004 shows that about 31% of the recharge has occurred during winter, 51% during spring, 9% during summer, and 9% during autumn. The recharge was calculated following the approach of Healy and Cook [2002]. The relatively high hydraulic conductivity of the overburden and the rock formations in the Wilmot basin results in a very rapid response of the water table. The lag time between precipitation events and the rise of the groundwater level is approximately 5 days [Paradis *et al.*, 2006]. Moreover, GW discharge (base flow) accounts for approximately 65% of the Wilmot

River flow based on long-term annual records, and may account for nearly 100% during summer.

3. Sampling and Analytical Methods

3.1. Fieldwork and Sampling

[10] Water level measurements were obtained from accessible domestic wells, which reach an average depth of 18 m in the aquifer, are of open hole construction, and are generally cased down to the overburden-rock contact. The water level is generally below the casing, in the rock formations. Groundwater samples were obtained from a three-level monitoring-well nest located near the center of the basin and from domestic wells using outdoor taps devoid of treatment systems (Figure 1b). The three-level monitoring-well nest (depths of 1, -31, and -74 m relative to sea level) was installed for GW sampling of discrete depth intervals. A 1.5-m-long PVC screen surrounded by a sand pack was installed in the deepest section of each well; this section was isolated from the rest of the aquifer using bentonite. GW samples from the three discrete sections were collected over three sampling periods from September 2004 to May 2005 (data not included in Table 1).

[11] Prior to sampling, the wells were purged of 2–3 well volumes until temperature, pH, and conductivity values stabilized. Dissolved oxygen levels were measured from filled bottles at the surface. Unfiltered samples obtained for analyses of water oxygen isotopes ($\delta^{18}\text{O}$) and N concentrations ([N-NO₃⁻]) were refrigerated during transport and storage. Samples obtained for analyses of nitrate isotopes ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) were filtered using a 0.45- μm filter to remove particulate matter. After filtering, a spectrophotometer was used to estimate the concentrations of NO₃⁻ and other ions to determine how much GW would be needed for the ion-exchange resin extraction. Using this sampling protocol, we collected water from 16 domestic wells during eight field sampling periods, i.e., once every season during the period of June 2003 to May 2005 (Table 1). Summer collection involved the largest number of sampling sites (Table 1; seasons 1 and 5). Winter sampling periods (3 and 7) were carried out after or during recharge events (Figure 2b).

[12] Stream samples were obtained from the edge of the Wilmot River at a depth of at least 10 cm, where water flow was swift. The initial stream sampling during summer 2003 was completed when the river water can be assumed to represent base flow. Precipitation samples were obtained

Table 2. Summary of Sample Types and Analytical Methods Used in This Study

	Instrument	Tracer	n
<i>Precipitation</i>			
H ₂ O	Equilibration-IRMS	δ ¹⁸ O	18
<i>Surface Water</i>			
H ₂ O	FIA	[NO ₃]	39
	Equilibration-IRMS	δ ¹⁸ O	27
NO ₃	EA-CF-IRMS	δ ¹⁵ N	35
	TC/EA-IRMS	δ ¹⁸ O	37
<i>Groundwater</i>			
H ₂ O	FIA	[NO ₃]	315
	Equilibration-IRMS	δ ¹⁸ O	240
NO ₃	EA-CF-IRMS	δ ¹⁵ N	272
	TC/EA-IRMS	δ ¹⁸ O	274

from three 30.5-cm-diameter collectors. To avoid evaporation, a minimum 2.5-cm layer of oil was added to the bottom of the collectors. They were fitted with funnels that matched their diameter at the top and narrowed to a couple of centimeters at the bottom.

3.2. Analytical Methods

[13] Our hydrogeochemical study uses [N-NO₃⁻], δ¹⁵N, and δ¹⁸O and water δ¹⁸O values obtained for precipitation, SW, and GW samples. All water analyses of [N-NO₃⁻] were performed at the water quality laboratory of Agriculture and Agri-Food Canada (Québec). Nitrate concentrations were determined by flow injection analysis (FIA) colorimetric method (Lachat) for which the detection limit was 0.04 mg L⁻¹ N-NO₃⁻ and the precision was 0.09 mg L⁻¹.

[14] All isotope analyses were performed at the Delta-Lab of the Geological Survey of Canada (Québec). Aliquots of 0.5 mL were analyzed for their water δ¹⁸O ratios using an online water-gas equilibration in continuous flow with an isotope ratio mass spectrometer (IRMS; Table 2) (Gas Bench-Delta Plus^{XL}). Average precision on duplicates (n = 74) of samples for the δ¹⁸O ratios were 0.06‰. The cation- and anion-exchange resin protocols proposed by the U.S. Geological Survey (USGS) [Chang *et al.*, 1999; Silva *et al.*, 2000] were modified to include HCl decarbonization and removal of SO₄²⁻ prior to water filtration using the anion columns. Extraction and neutralization of nitrate collected on the columns were performed following recommendations of the USGS. The resulting silver nitrate was analyzed for δ¹⁵N and δ¹⁸O using online combustion- and pyrolysis-IRMS systems, respectively. In the same order, average precision obtained on sample duplicates (n = 86, 161) were of 0.1 and 0.2‰.

4. Results

[15] Nitrate characteristics in GW and SW generally show similar tendencies during the 2 years of sampling, but the ranges of values obtained for SW are much narrower than those for GW (Figures 3a–3c). Groundwater NO₃-N concentrations exhibit a broad range from site to site but remain relatively constant at each site throughout all the sampling periods (data listed by Savard *et al.* [2007]). There is no apparent correlation between GW nitrate concentration and

well depth, nor is there any obvious spatial trend when results are examined over the extent of the watershed. Seasonal GW averages for NO₃-N ranged between 7.2 and 5.5 mg L⁻¹ throughout 2003–2004, and between 8.1 and 6.9 mg L⁻¹ for 2004–2005 (Figure 3a; Table 3). The δ¹⁵N values of nitrate dissolved in GW vary only moderately regionally and at specific sites (data given by Savard *et al.* [2007]) throughout the eight periods of sampling (Figure 3b). Thus GW and SW nitrate contents and δ¹⁵N values exhibit relatively flat patterns. In contrast, the ranges observed for the δ¹⁸O values in nitrate vary significantly between the summer-autumn and winter-spring periods; the GW average downward shift was 10.0‰ for 2003–2004, and 1.3‰ for 2004–2005 (Figure 3c; Table 3). Similarly, the 2003–2004 and 2004–2005 SW seasonal samples show average downward shifts of 10.2 and 2.3‰, respectively (Figure 3c; Table 3). The average H₂O-δ¹⁸O values measured in GW and SW display a narrow range (–11.2 and –10.9‰; Figure 3d) and vary little for the eight sampling periods relative to the values obtained for nitrate in GW. In contrast, the δ¹⁸O_{H₂O} results for precipitation samples show large changes from season to season, winter and spring having the lowest average values (–16.7 ‰) and summer and autumn having the highest ones (–6.5 ‰; Figure 3d).

[16] A seasonal trend in individual wells is observed consistently for δ¹⁸O values of GW nitrate from all domestic wells. Individual downward shifts between the summer-autumn and winter-spring mean values range between +4.5 and 13.5‰, and 0.4–6.0‰ for 2003–2004 and 2004–2005, respectively. The highest values therefore appear during the 2003–2004 summer-autumn period (Figure 3c). The processes responsible for the seasonal shift will be addressed first for 2003–2004.

[17] The GW δ¹⁵N values as a function of N-NO₃⁻ concentration for 2003–2004 show no trend, i.e., a broad range of nitrate concentrations over a moderate range of δ¹⁵N ratios (not illustrated). The N-NO₃⁻ range observed in SW is much narrower but show similar δ¹⁵N-values as in GW. In the δ¹⁸O-δ¹⁵N space, the summer-autumn and winter-spring GW results cluster into two groups with similar δ¹⁵N ranges but distinct δ¹⁸O ratios separating the two populations (Figure 4). The GW and SW clusters broadly overlap, but GW samples show a wider spread of values.

[18] If seasonal NO₃⁻-δ¹⁸O patterns exist for the watershed GW and SW samples, similar patterns should be most clearly observed in discrete top portions of the rapidly responding aquifer. Seasonal patterns should disappear with increasing depth in the aquifer due to mixing and dispersion during longer transport in the deep portion of the aquifer. Sampling from the observation well nest during the 2004–2005 autumn to spring periods was conducted to verify this assumption. Seasonally averaged N-NO₃⁻ concentration diminished with depth (Figure 5a). The shallowest well shows the highest GW δ¹⁵N values during spring and winter, whereas the two deeper wells show an average of +3.3‰ for all three seasons (Figure 5b). The highest δ¹⁸O values are observed for GW from the shallowest well. Data from the two deeper wells show an average value of –4.2‰ (Figure 5c). Last, an autumn to winter decrease of δ¹⁸O values is observed in the shallowest well (from +1.3 to

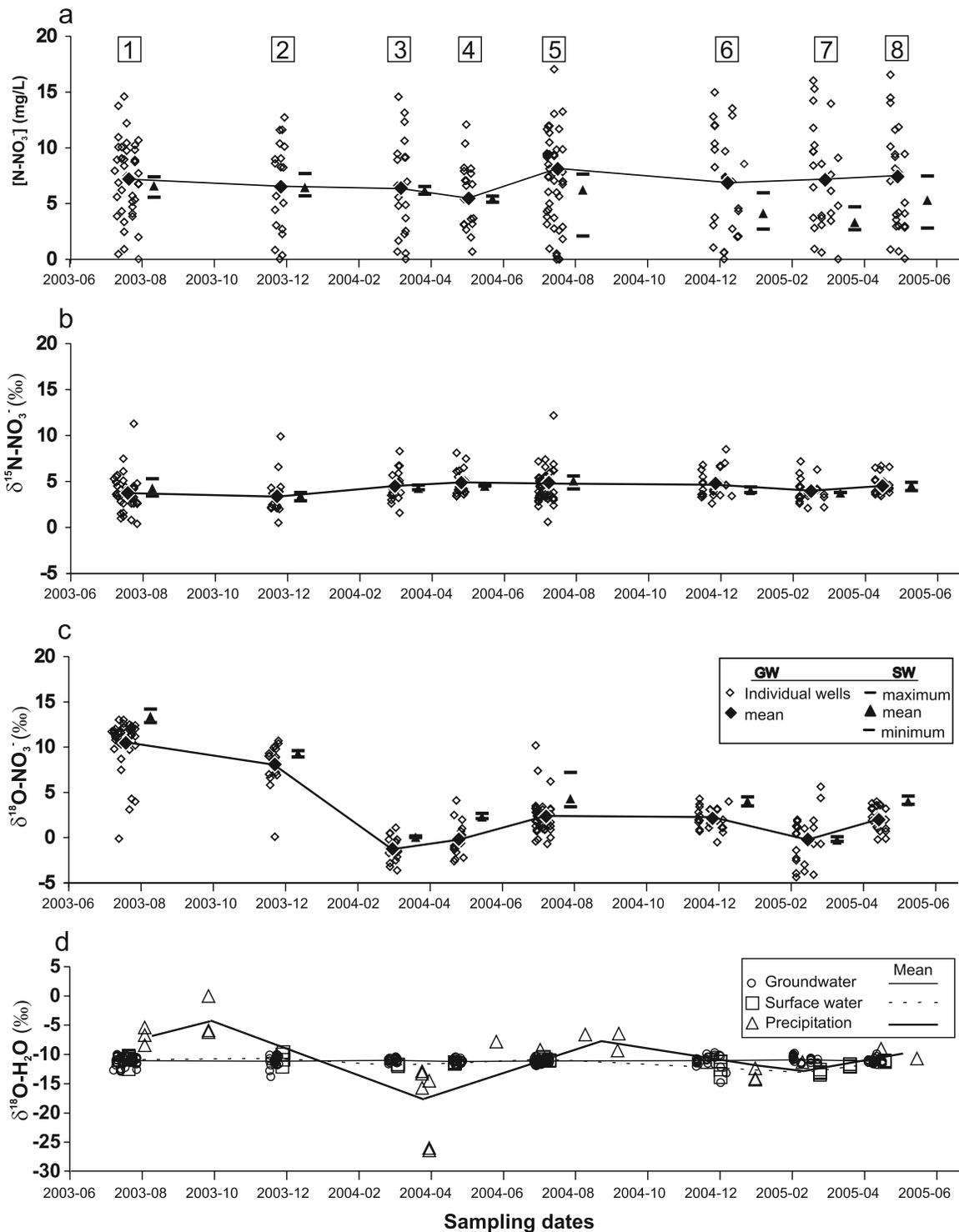


Figure 3. Results in groundwater (GW) and surface water (SW) relative to sampling dates. (a) Concentrations of N-NO_3^- . Periods of sampling are numbered 1 to 8 (see also Table 1). (b) Nitrogen isotope ratios. (c) Oxygen isotope ratios. The SW ranges and averages shown on the right of each GW seasonal population stand for the same period of sampling as for GW. Seasonal distribution of mean values over the 2003–2004 and 2004–2005 yearly cycles are shown by the solid curve. (d) Oxygen isotopes in H_2O .

–1.5‰). This seasonal decrease correlates with the seasonal pattern found regionally for domestic wells.

[19] A minimum daily nitrate load was calculated for each of the eight sampling seasons (Figure 6). For this purpose, we

used the estimated daily average volume of water recharging the aquifer, multiplied by the average concentrations measured in GW (Figure 3a). The estimated daily N load transferred to the aquifer ranges between 0.17 and 2.46 t d^{-1}

Table 3. Summary of Results Obtained for Each Sampling Period

Sampling Period	Number	N-NO ₃ , mg L ⁻¹				δ ¹⁵ N-NO ₃ , ‰				δ ¹⁸ O-NO ₃ , ‰				δ ¹⁸ O-H ₂ O, ‰			
		GW		SW		GW		SW		GW		SW		GW		SW	
		Avg ^a	σ ^b	Avg	σ	Avg	σ	Avg	σ	Avg	σ	Avg	σ	Avg	σ	Avg	σ
Summer	1	7.2	3.5	6.6	0.7	3.7	2.0	4.3	0.8	10.5	2.9	13.5	0.6	-11.1	0.8	-10.9	0.8
Autumn	2	6.5	4.0	6.5	0.8	3.4	2.1	3.5	0.4	8.1	2.4	9.4	0.3	-11.2	0.9	-10.7	1.1
Winter	3	6.4	4.4	6.2	0.3	4.5	1.6	4.3	0.3	-1.2	1.3	0.1	0.1	-11.0	0.3	-11.8	0.2
Spring	4	5.5	3.2	5.5	0.2	4.9	1.4	4.5	0.1	-0.2	1.7	2.4	0.3	-11.2	0.4	-11.5	0.1
Summer	5	8.1	7.0	6.3	2.1	4.9	3.4	5.1	0.5	2.3	1.9	4.4	1.4	-11.1	0.4	-10.8	0.3
Autumn	6	6.9	4.7	4.2	1.4	4.8	1.5	4.1	0.3	2.1	1.2	4.1	0.4	-11.1	1.1	-12.2	1.3
Winter	7	7.1	4.7	3.4	0.9	4.0	1.3	3.8	...	-0.2	2.7	-0.2	0.3	-10.9	0.5	-13.1	0.4
Spring	8	7.4	5.3	5.4	2.0	4.5	1.0	4.6	0.3	2.0	1.9	4.1	0.3	-11.2	0.4	-11.5	0.4

^aAverage values.^bStandard deviation.

for the entire watershed (Figure 6), corresponding to fluxes of 0.0007 to 0.0103 kg m⁻² yr⁻¹, respectively.

5. Interpretation and Discussion

[20] The consistency throughout the year in GW nitrate concentrations in domestic wells suggests that local sources of N are continually available. Given that site-specific nitrate fluxes and hydrogeological conditions may vary from well to well, it is not surprising to find the broad range of nitrate concentrations described above (Figure 3a). The lack of spatial trend in GW nitrate concentration underscores the local heterogeneity of the hydrogeological conditions, and suggests that the sources of nitrate present in GW are diffuse but unevenly distributed.

[21] Similarities between the nitrate trends for GW and SW suggest that the main source of nitrate present in SW is GW discharge to streams (Figure 3). This interpretation is consistent with the fact that base flow composes the dominant portion of total flow in the Wilmot River [Paradis *et al.*, 2006]. In addition, permeable soil and till units may favor vertical migration of nitrate to the water table rather than episodic overland transport by runoff.

[22] The calculated load of nitrate daily transferred to the aquifer is considered a minimum. Indeed domestic wells yield composite GW samples carrying average nitrate concentrations (6.9 mg L⁻¹) lower than nitrate average levels in soil leachates (19.1 mg L⁻¹) obtained for an experimental farm outside the Wilmot watershed (PEI) [Savard *et al.*, 2007]. Permission could not be obtained to sample soil

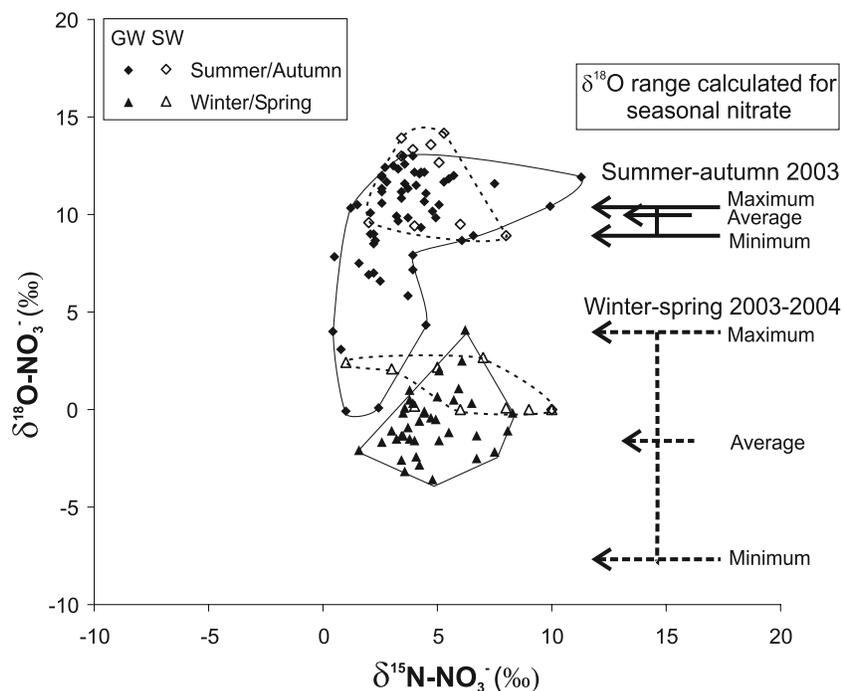


Figure 4. Graph of δ¹⁸O as a function of δ¹⁵N values for year 2003–2004. Solid curves delineate the fields of results for GW summer-autumn (up) and winter-spring (down), whereas the dotted curves delineate results for SW.

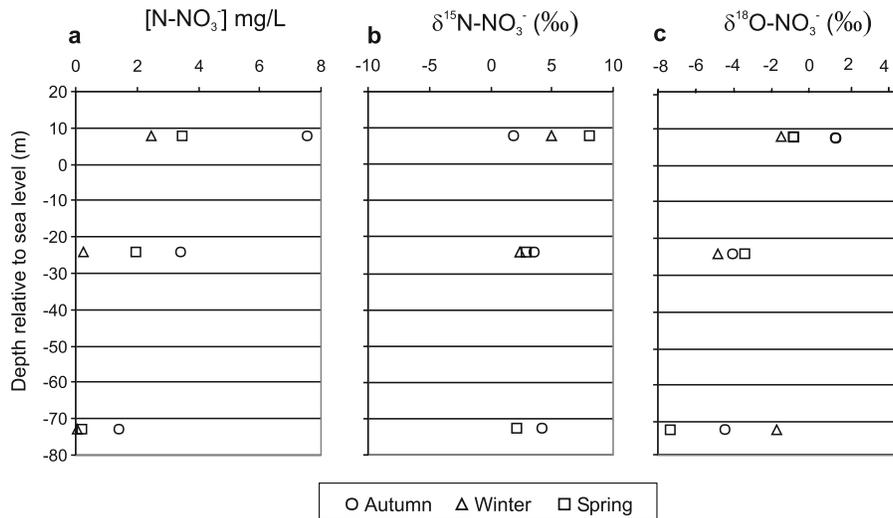


Figure 5. Results for seasonal groundwater (GW) sampling of the observation well nest during the 2004–2005 autumn to spring period. (a) N-nitrate concentration. (b) Nitrogen isotope ratios. (c) Oxygen isotope ratios.

water in the study area. However, the present exercise is aimed at comparing relative nitrate loads, not absolute values. A minimal estimate is therefore adequate. The calculations show that the cold periods of the two yearly cycles in the Wilmot watershed (seasons 3, 4, 7, and 8) release high N loads ($0.90\text{--}2.46\text{ t d}^{-1}$, Figure 6, or $0.0038\text{--}0.0103\text{ kg m}^{-2}\text{ yr}^{-1}$).

[23] The relationship between $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values clearly indicates that denitrification does not affect the GW nitrate (Figure 4). In addition, the nitrate concentration and $\delta^{15}\text{N}$ flat pattern suggests that the mixing of NO_3^- -rich water with NO_3^- -poor GW in domestic wells generates varying degrees of dilution of nitrate in GW.

[24] The differences of $\delta^{18}\text{O}_{\text{NO}_3^-}$ values between the summer-autumn and winter-spring periods (Figure 4) are likely determined by the points in time when nitrate is produced and subsequently exported to the Wilmot aquifer, and by the quantity of nitrate reaching the aquifer at these times. Nitrate produced during soil nitrification is reported to contain two oxygens from soil water and one oxygen from free O_2 [Andersson and Hooper, 1983; Kumar *et al.*, 1983; Hollocher, 1984; Mayer *et al.*, 2001]. Atmospheric O_2 has a known $\delta^{18}\text{O}$ value of $+23.5\text{‰}$. Soil water originates from local precipitation for which we have analyzed the seasonal $\delta^{18}\text{O}$ values (Figure 3d). Using these values, the $\delta^{18}\text{O}$ ratios of nitrate expected to have resulted from microbial activities during the two periods of interest can be calculated and used to interpret the observed $\delta^{18}\text{O}$ trends.

[25] The range of $\delta^{18}\text{O}$ values of 2003–2004 summer-autumn and winter-spring precipitation vary from -8.4 to -5.4‰ (average -6.5) and from -26.4 to -7.8‰ (average -16.7‰), respectively (Figure 3d). According to the Atlantic Fertilizers Institute [Jacques Whitford Environment, Ltd., 2001], the proportions of nitrate fertilizers and other N-bearing commercial products (ammonium nitrate, urea, etc.) generally used during spring in PEI are 24 and 76%, respectively. Note that the nonnitrate N species have to be bacterially mediated before they are transformed into nitrate. If nitrifying bacteria utilize soil waters with sum-

mer-autumn characteristics, assuming the above noted proportions of fertilizers and taking into account the high $\delta^{18}\text{O}$ values for nitrate fertilizers we have measured ($+30.0\text{‰}$), regional estimates for the summer-autumn nitrate mixture would be between $+8.9$ and $+10.4\text{‰}$ (average $+9.9\text{‰}$). These values fall in the middle of the summer-autumn cluster obtained for GW of domestic wells (Figure 4). If nitrifying bacteria utilize soil waters with winter-spring characteristics, assuming there is 5% of fertilizer nitrate ($+30$) in the mixture, the estimated $\delta^{18}\text{O}$ range for winter-spring nitrate is between -7.7 and $+4.0\text{‰}$ (average -1.6‰), covering the measured values of samples for that period (Figure 4). Therefore the $\delta^{18}\text{O}_{\text{nitrate}}$ values for the summer-autumn and winter-spring periods suggest active nitrification during all four seasons and rapid transfer of nitrate first to the aquifer and then to SW. The seasonal $\delta^{18}\text{O}$ trends in GW imply that precipitation is largely controlling the oxygen isotopic ranges in the soil water and, in turn, the values of bacterially mediated nitrate.

[26] On a site-specific basis, other processes such as mixing of shallow seasonal waters with older GW from greater depths in the aquifer likely influence seasonally observed values. Waters with distinct $\delta^{18}\text{O}_{\text{NO}_3^-}$ ratios exist along individual wells owing to seasonal replenishment of

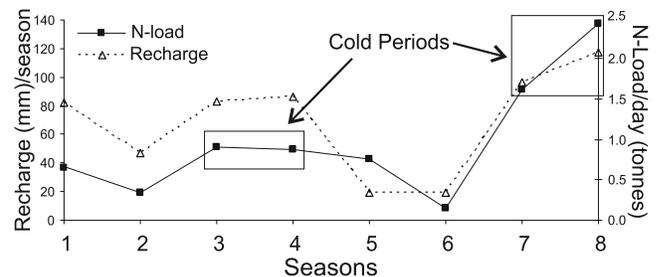


Figure 6. Daily average of seasonal N-NO_3^- transfer from soils over the entire watershed (87 km^2) to the aquifer, compared with the cumulated recharge for each season.

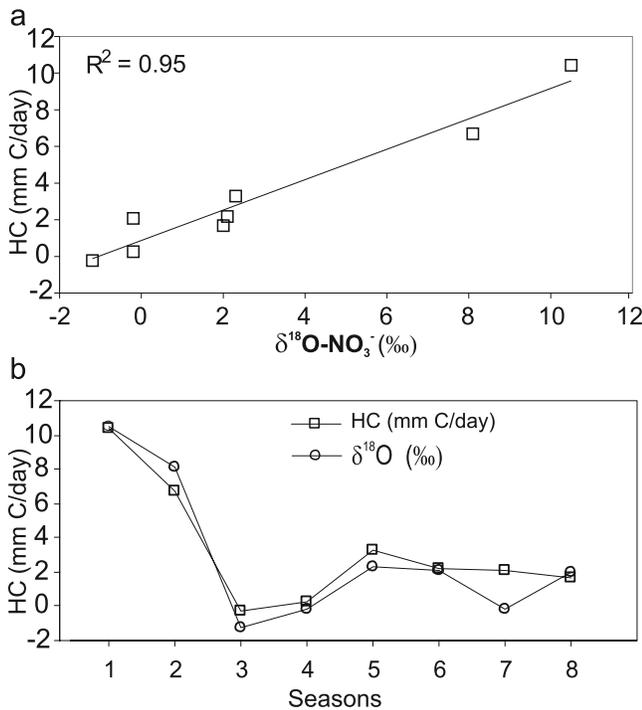


Figure 7. Empirical relationships between the hydroclimatic index (HC) and $\delta^{18}\text{O}$ values of nitrate in groundwater. (a) Linear relationship based on results obtained for the 2 years of sampling. (b) Covariation through time.

water by recharge of the upper part of the aquifer (Figure 5c). As noted earlier, the seasonal trends for $\delta^{18}\text{O}_{\text{NO}_3^-}$ and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ in GW of the domestic wells are not consistent; with $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ remaining relatively constant through the seasons (-11.2 to -10.5%) and $\delta^{18}\text{O}_{\text{NO}_3^-}$ values showing a marked shift between summer-autumn and winter-spring sampling periods (Figures 3c and 3d and Figure 5c). To explain this observation, calculations of isotopic ratios to be expected from various proportions of water mixtures were made. The classical equation of fluid mixing [Faure, 1986] was used to calculate the $\delta^{18}\text{O}$ values of mixtures of leachates (soil water and GW from the very top of the aquifer) with GW from deeper parts of wells (deep GW). The calculations indicate that $\delta^{18}\text{O}_{\text{nitrate}}$ and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ results obtained on either composite GW from domestic wells or discrete samples from the observation well nest can be all reproduced by mixing low proportions of nitrate-rich soil leachates (less than 25%) with high proportions of nitrate-poor deep GW. If GW from intermediate depths in the aquifer were considered as part of the mixture, the calculated maximum proportion of leachates would simply be lower than 25%.

[27] Under field conditions, the exact proportions of leachates and their $\delta^{18}\text{O}$ characteristics in mixtures will be largely controlled by climatic conditions. Indeed, the amount of precipitation translates into recharge, and air temperature controls the oxygen isotope fractionation during cloud distillation and local precipitation. These climatic conditions can vary significantly through time. It is therefore expected to observe significant $\delta^{18}\text{O}$ variations from year to year during specific periods of sampling. Differences of average nitrate $\delta^{18}\text{O}$ values for summer-autumn or winter-spring found in the present study certainly reflect

such changing hydroclimatic conditions (Figures 3c and 4). To illustrate this point, we have defined a hydroclimatic index (HC) which takes into account variations in temperature and recharge conditions for the various precipitation events between the sampling periods.

$$\text{HC} = \left(\sum_{i=1-D} R_i T_i \right) D^{-1}, \quad (1)$$

where R_i and T_i are the daily average recharge and air temperature, respectively, and D is the number of days between sampling sessions. The correlation between HC and average nitrate- $\delta^{18}\text{O}$ values found in seasonal GW explains 95% of the $\delta^{18}\text{O}$ variation (Figure 7). This empirical relationship expresses a solid covariation through the eight seasonal sampling periods, supporting our assumption that the hydroclimatic conditions largely control the average $\delta^{18}\text{O}$ values of nitrate produced by biological processes and transferred to the aquifer on a regional scale. The sessions of sample collection were performed every 95 days on average (average D). The correlation obtained suggests that the resetting of nitrate attributes and loads is significant after such a period of time in the upper part of the Wilmot aquifer. The minimal daily N load exported to the aquifer constitutes an estimate reflecting the importance of seasonal nitrate fluxes (Figure 6). This evaluation combined with $\delta^{18}\text{O}_{\text{NO}_3^-}$ characteristics indicates that winter and spring provide high rates of transfer during the two yearly cycles covered for the Wilmot watershed. Recharge is a crucial factor controlling the seasonal rate of transfer (Figure 6).

[28] Overall, we infer that the GW $\delta^{18}\text{O}_{\text{nitrate}}$ cycles in the Wilmot watershed result from ongoing nitrification in soils during the four Canadian seasons. This interpretation implies that soil microbial activity produces nitrate all year long, including winter, and that nitrate export from soils to GW takes place whenever recharge is occurring. Even though the rate of nitrification may diminish during cold periods, this is offset by the fact that the assimilation of N by plants is practically nonexistent during these periods. Fluctuations of air temperature and precipitation during the summer-autumn and winter-spring sequences control the $\delta^{18}\text{O}$ ratios of soil nitrate. The charge of new soil nitrate exported to the aquifer modulate the amplitude of the isotopic seasonal shifts at its top, relative to ^{18}O -depleted older nitrate present in deep GW. In other words, the isotopic ratios and amount of nitrate exported to the aquifer through leaching follows the dynamics driven by GW recharge.

[29] Seasonal $\delta^{18}\text{O}_{\text{NO}_3^-}$ patterns of nitrate in SW or GW of an agricultural watershed/aquifer system are reported here for the first time. The concept of discrimination of seasonal GW nitrate using oxygen isotopic ratios has been previously recognized [Wassenaar, 1995] and used to separate atmosphere and soil nitrate in the context of forested lands [Spoelstra et al., 2001]. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ ratios for potential sources of nitrate in GW were compiled from studies that took place all around the world [Kendall and Aravena, 2000]. This compilation also illustrates the process of bacterial denitrification occurring under reducing conditions which can diminish the nitrate load and modify

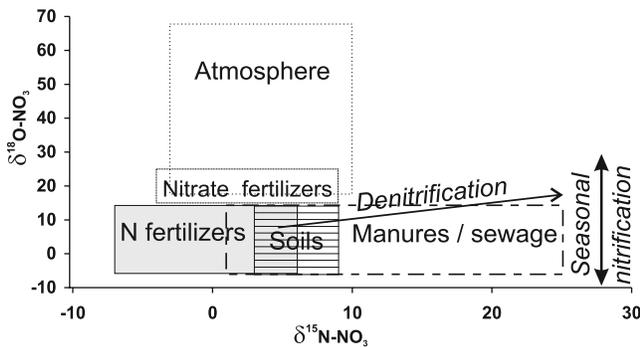


Figure 8. Graph of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of fields characteristic of potential sources of nitrate and main processes controlling changes of nitrate isotope ratios modified from Kendall and Aravena [2000] to include the seasonal effect on nitrification proposed in this article.

the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of residual nitrate (Figure 8). The compiled source domains show broad $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ ranges as they are compiled from a wide variety of contexts. In the light of the results of the present study, we suggest that changes in soil-water $\delta^{18}\text{O}$ values prior to seasonal mineralization and nitrification might have partly caused the broad vertical ranges compiled. This suggestion particularly applies to regions of temperate climate, but various rates of soil-water evaporation may also cause positive seasonal shifts under warm climate. We propose that it may be useful to illustrate seasonal nitrate production as a process affecting the $\delta^{18}\text{O}$ values of nitrate loads, i.e., to indicate that seasonal nitrification moves clusters of results vertically in the $\delta^{18}\text{O}$ - $\delta^{15}\text{N}$ space when compiling results for a given region (Figure 8). Finally, seasonal nitrification should be taken into consideration for understanding the N cycle, particularly for rapidly responding systems in regions where significant seasonal changes in climatic conditions mark the oxygen isotope ratios of precipitation.

[30] The selected study area of the Wilmot River presented conditions favorable to detecting seasonal soil nitrification and leaching to the aquifer. The Wilmot watershed is small and is dominated by intensive agricultural activities producing large quantities of nitrate. Its soils are highly permeable, favoring infiltration over runoff. Climatic conditions are such that a thick snow cover often protects soils from freezing fully and favors continuous activity of nitrifying bacteria, and that frequent thaws generate winter recharge. The Wilmot aquifer is characterized by a preferential fast flow in its upper part which presents excellent hydraulic links with the river as reflected by GW dominating its flow (base flow). Both the river and aquifer respond rapidly to recharge. Whereas most recharge takes place during spring and autumn, recharge during summer and winter can be significant as well. All these conditions combined with the seasonal data allow us to draw the three main conclusions of the present study.

6. Conclusions

[31] 1. Nitrification in agricultural soils dominated by potato cropping in the Wilmot watershed occurs all year long, and owing to an adequate snow cover, winter-

produced nitrate makes a significant contribution to the overall GW nitrate load.

[32] 2. The export of the bacterially produced nitrate from the agricultural soils to the regional aquifer takes place whenever recharge occurs, even during winter. Nitrate export from soil to the aquifer in the Wilmot region is rapid, as reflected by the preservation of seasonally distinct nitrate oxygen isotope characteristics in GW.

[33] 3. Determining the period of nitrification using $\delta^{18}\text{O}$ values provides a means to estimate the rate of transfer of nitrate from croplands to aquifers. Considering seasonal export of nitrate to the water system will help better quantify N budgets. Finally, the approach described here should be applicable in any region where climatic conditions (temperature during precipitation, evaporation) imprint $\delta^{18}\text{O}$ values of soil water seasonally.

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