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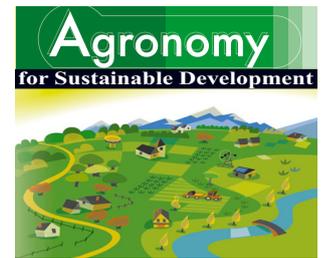
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Research article

Evidence of soil pollution by nitrates derived from pig effluent using ^{18}O and ^{15}N isotope analyses

Nicolas PAYET^{1,2*}, Eric NICOLINI¹, Karyne ROGERS³, Hervé SAINT MACARY², Michel VAUCLIN⁴

¹ Laboratoire Géosciences Réunion, 15 avenue René Cassin, BP 7151, 97715 Saint-Denis Messagerie Cedex 9, La Réunion, France

² CIRAD, UPR Recyclage et Risque, 97408 Saint-Denis, La Réunion, France

³ National Isotope Centre, GNS Science, PO Box 31-312, Lower Hutt, New Zealand

⁴ Laboratoire d'Étude des Transferts en Hydrologie et Environnement (LTHE), UMR 5564, CNRS, INPG, IRD, UJF, BP 53, 38041 Grenoble Cedex 9, France

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Abstract – In Réunion Island, expanding human populations, urbanization and agriculture during the last 50 years have all contributed to a steady increase in the level of nitrates in drinking water. Various nitrate point sources are responsible for the nitrate contamination around the island including chemical fertilizers, animal effluent applied to pasture and crops, and urban waste such as sewage and domestic waste water. In terms of agricultural fertilizers, pig effluent is the most widely used, but the cumulative effects of slurry applications on soil water and groundwater are unknown. Our objectives were (1) to characterize and follow in situ the fate of nitrogen through the subsurface after application of pig effluent onto a cultivated soil using stable nitrate isotopes, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$, and (2) to compare the isotopic signatures of Réunion Island's principal aquifers with results from the experimental site to infer potential contamination sources. The study was conducted on an experimental field site planted with maize in the western part of Réunion Island during the rainy season. A control site with no fertilizer application to the maize was compared with the investigation site which had pig effluent applied once a year. The site which had pig effluent applied over one year had an average maximum surface soil water $^{15}\text{N}\text{-NO}_3^-$ value of +9.0‰ at 0.45 m depth. This signature was significantly more enriched in ^{15}N than the corresponding subsurface soil water $^{15}\text{N}\text{-NO}_3^-$ value of +3.8‰ at 10 m depth. The control site average maximum surface soil water $^{15}\text{N}\text{-NO}_3^-$ value of +3.6‰ at 0.45 m is similar to the subsurface pig effluent application plot. This indicates that nitrates derived from pig effluent have not reached 10 m depth in the subsurface, even though over the last 18 months this site was subjected to two effluent applications, each around 200 kg N ha⁻¹, and more than 1900 mm of rain, more than half of which drains directly into the root zone. This slow migration shows that mobilization of nitrates through cultivated soil can take many tens of years before infiltrating and contaminating the saturated zone situated at several tens, and in places, hundreds of meters depth. On an island-wide scale, an isotopic assessment of nitrates from the experimental site's soil water and other drinking water wells highlights a nitrogenous contamination derived primarily from urban and/or agriculture via effluent application.

nitrates / nitrogen and oxygen isotopes / pig effluent / groundwater contamination / tropical island

1. INTRODUCTION

Nitrate levels found naturally in groundwater are usually less than 2 ppm (Davis and Suárez, 1974). However, nitrates are often associated with intensive applications of synthetic fertilizers, organic manures or urban waste water. Excessive levels in drinking water can cause serious health problems or drive eutrophication processes, damaging ecosystems (Spalding et al., 1993). Maximum allowable nitrate levels fixed by European regulations are capped at 50 mg L⁻¹. Unfortunately, the environmental impact of contaminants rich in nitrogen on local groundwater quality is poorly known in small tropical islands, relative to temperate zones (Cameron et al., 1995; Carey et al., 1997; Dauden and Quilez, 2004;

Mantovi et al., 2006, among others). Pollution effects from modern agriculture and urban growth on small islands infrastructures dating from the 1960s onwards are now causing serious concern, due to degradation of drinking water quality (Duwig et al., 1998; Muñoz-Carpena et al., 2002; Dong-Chan et al., 2005). Moreover, these contaminants are now appearing in coastal zones where, particularly in tropical regions, they affect coral reef ecosystems (D'Elia et al., 1981; Lewis, 1987; Lapointe et al., 1990; Smith et al., 1990).

In Réunion Island, 48% of all drinking water originates from groundwater and one-third of these sites have nitrate levels higher than 10 mg L⁻¹ (OLE, 2008). Over the last 30 years, the nitrate level of Réunion Island's drinking water has been increasing, concurrently with population growth, and expanding urbanization and agricultural activities. As agricultural

* Corresponding author: nicolas.payet@univ-reunion.fr

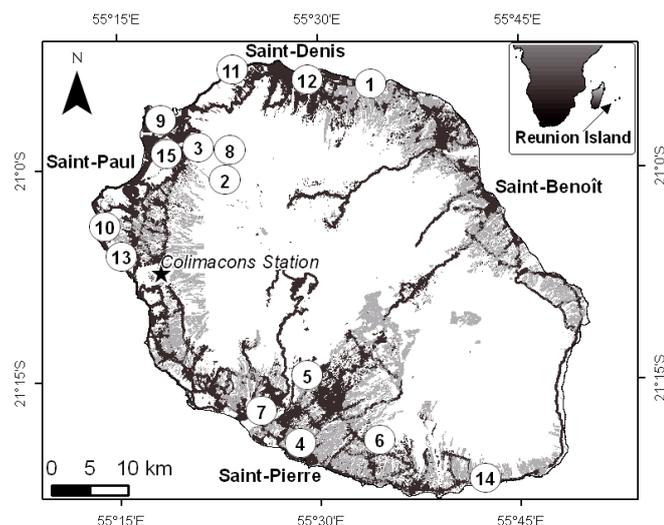


Figure 1. Map of Réunion Island's land use (gray: agricultural area; black: urban zone). Site location numbers are indicated in Table IV. Urban and agricultural areas are located primarily around the circumference of the island due to its relief.

development has evolved over the last two decades, there has been a concurrent increase in the use of chemical fertilizer and more recently, effluent derived from animal manure on pastures and crops. Réunion Island's main crop is sugar cane, which accounts for 50% of the crops planted on cultivated land around the island (Fig. 1). The distribution of effluent onto arable soils and crops as organic manure is one of the most common methods employed to recycle this excess waste.

Even though soil produces, and hence already contains nitrogen, the use of nitrogen isotopic tracers is usually the only way to follow the action and fate of introduced fertilizers (Smith et al., 1990). Sugar cane is usually fertilized with synthetically made nitrogen fertilizers, and occasionally animal effluent. Identification of the nitrogen's origin is possible due to the relative $\delta^{15}\text{N}$ difference between atmospherically-derived chemical fertilizers and soil nitrogen. Waste water and animal waste can also be distinguished (Fig. 2) from other sources of nitrate on the basis of their elevated $\delta^{15}\text{N}$ values, derived from fractionation and enrichment of organic nitrogen during the ammonification process, which precedes nitrification (Hubner, 1986).

Synthetic chemical fertilizers generally exhibit relatively low $\delta^{15}\text{N}$ values between -8 and $+5\text{‰}$ (Kendall, 1998) as they are synthesized directly from atmospheric nitrogen. Soil nitrate, formed by natural oxidation of organic material, generally shows $\delta^{15}\text{N}$ values ranging between $+3$ and $+6\text{‰}$ (Kendall, 1998) and lies between chemical fertilizers and waste water/animal manure. However, it can be difficult to differentiate between natural soil nitrate and human and animal waste (Fig. 2). The use of nitrogen isotopes is often insufficient to resolve this overlap, as clearly indicated by Kellman and Hillaire-Marcel (2003). Moreover, $\delta^{15}\text{N}$ values of various sources can be modified by denitrification processes which enrich the $\delta^{15}\text{N}$ values of the residual nitrates (Böttcher et al.,

1990). On the other hand, plant consumption of nutrients has little effect on soil nitrogen fractionation (Mariotti et al., 1981; Hubner, 1986). Hence, the dual use of nitrogen ($\delta^{15}\text{N-NO}_3^-$) and oxygen ($\delta^{18}\text{O-NO}_3^-$) isotopes to identify the origin of nitrates (Fig. 2) appears to be more effective than $\delta^{15}\text{N}$ alone (Kendall, 1998; Kendall and Aravena, 1999; Silva et al., 2002). In theory, for every three oxygen atoms present in a nitrate molecule formed via nitrification, two come from water and one from atmospheric oxygen (Böttcher et al., 1990). Nitrates therefore exhibit two distinct isotopic signatures: (i) that of nitrogen, and (ii) that of the in situ water present at the time of nitrate formation during nitrification. The initial isotopic ratio is subsequently changed by a specific isotope fractionation owing to denitrification. The enrichment of the heavier nitrogen isotope is about twice as high for oxygen (ratio of $\approx 1:2$) and agrees well with other studies (Böttcher et al., 1990).

The study objectives were twofold: (i) to characterize and follow in situ the fate of nitrogen through the subsurface after application of pig effluent onto a cultivated soil using stable isotopes ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) of the nitrates, and (ii) to compare the isotopic signatures of Réunion Island's principal aquifers with results from the experimental site to infer potential contamination sources.

2. MATERIAL AND METHODS

2.1. Site description

The study was conducted at Colimaçons experimental field station, located in the western part of Réunion Island ($21^\circ 7' \text{ S}$, $55^\circ 18' \text{ E}$, 780 m above sea level) (Fig. 1) during the 2004–2005 austral summer. Réunion Island's climate is tropical, with a hot and humid season during December and April, and a dry season between May and November. The soil horizon is more than 2 m thick, and is an andic cambisol according to Feder and Findeling (2007). Subsoil consists of lava flows (hawaïte), which has varying levels of compacted fractures and weathering (ancient scoria).

2.2. Experimental site

From 2002 onwards, maize (a local open-pollinated variety) was grown on the experimental site, which had previously been uncultivated since 1983 with only natural rainwater irrigation. Two plots which had no fertilizer applied for the last 19 years were investigated at the site; a control plot of 570 m² which was not fertilized during the study, and another plot of 620 m² which had an annual application of pig effluent slurry (*Sus scrofa*) during the two-year study. The first effluent application occurred in October 2003, and a second in November 2004. Each application corresponded to around 200 kg N ha⁻¹ with the majority of nitrogen as ammonium (76% in 2003 and 89% in 2004) and occurred three weeks before the maize was sown at the rate of 64 m³ per hectare. The bulk nitrogen isotopic signature of the pig effluent was analyzed in 2004 ($\delta^{15}\text{N} = +11.3\text{‰}$) and corresponds to literature values

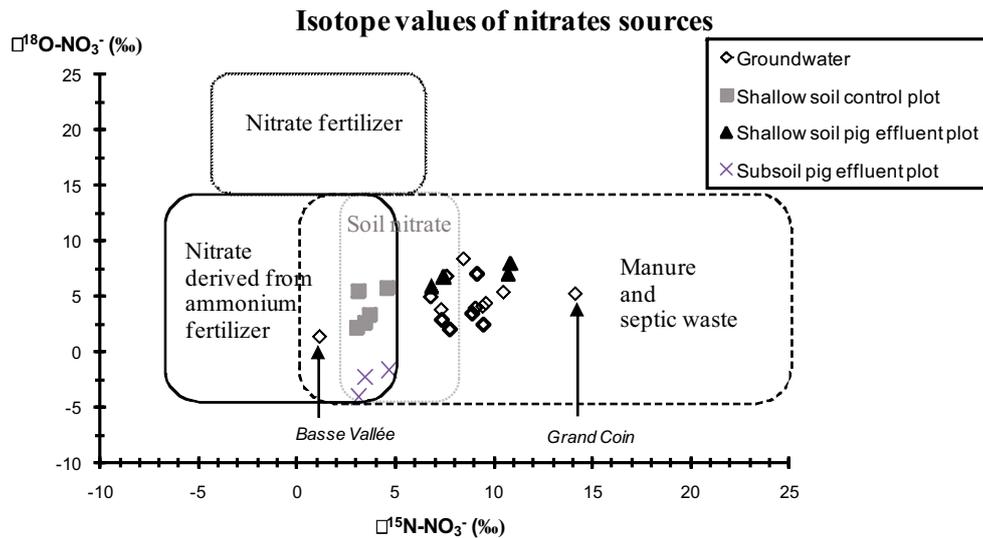


Figure 2. Typical range of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of nitrate from various sources (from Kendall, 1998). Nitrate isotopic signatures from the experimental site (soil and subsoil) and groundwater nitrates from Réunion Island. Soil and subsoil water from the control site appear depleted in ^{15}N relative to water from the fertilized plot and Réunion's groundwaters. These drinking waters have isotopic values which lie between two end members; Grand Coin and Basse Vallée. Grand Coin corresponds to groundwater which has infiltrated an intensive cattle farming region, whereas Basse Vallée represents a pristine forested zone.

(Kendall, 1998). The maize was harvested each year at the end of March. The dry weight yield was 8.4 T ha^{-1} and 13 T ha^{-1} for the control plot and pig effluent plot, respectively.

Two lysimeters were installed vertically into the soil surface at a depth of 0.45 m in the ground, each equipped with a porous suction cup. An experimental pit was dug downstream of each experimental plot to a depth of 10 m. Two lysimeters equipped with porous suction cups were inserted into the subsoil at 10 m depth horizontally (with a very slight incline) to a depth of 3 m into the altered bedrock wall of each pit under each site (Fig. 3). Lysimeters were subjected to a vacuum of -70 hPa for 24 h prior, and allowed monthly collection of soil and subsoil water when there was sufficient moisture. Sampling occurred from January to April for soil samples, and February to April for the subsoil samples (which accounts for the lower number of subsoil samples). Drinking waters were sampled directly from wells in June 2005, at the end of the rainy season. Rainwater was captured in a closed container with a layer of mineral oil in the rain collector to prevent evaporation. Analysis of the isotopic composition of rainwater ($\delta^{18}\text{O}-\text{H}_2\text{O}$ and $\delta^2\text{H}-\text{H}_2\text{O}$) was carried out on cumulative monthly rainfall samples.

The large time variability in $\delta^{18}\text{O}$ rainwater (Tab. I) requires weighted mean averages according to rainfall. An example of calculation of $\delta^{18}\text{O}_{\text{WM}}$ for the period 26/11/04 to 27/04/05 (Tab. I) is:

$$\delta^{18}\text{O}_{\text{WM}} = (-6.68) \times \left(\frac{250 + 36}{36 + 250 + 109} \right) + (-3.39) \times \left(\frac{109}{36 + 250 + 109} \right). \quad (1)$$

where WM: weighted mean.

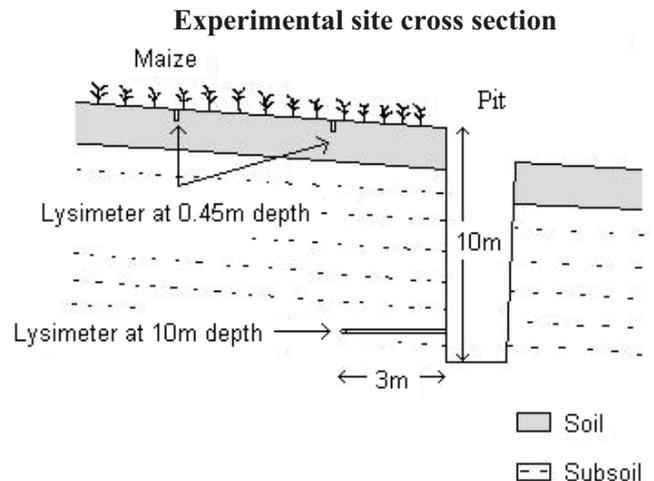


Figure 3. Experimental site cross-section. Lysimeters were installed vertically into the soil surface of a plot cultivated with maize, at a depth of 0.45 m in the ground and each equipped with a porous suction cup. An experimental pit was dug downstream from each experimental plot to a depth of 10 m. Two lysimeters equipped with porous suction cups were inserted into the subsoil at 10 m depth horizontally to a depth of 3 m into the altered bedrock wall of each pit under each site.

2.3. Analytical method

The nitrogen content and isotopic composition of dried pig effluent were analyzed in triplicate at the Stable Isotope Laboratory (GNS Science) using a Europa Geo 20/20 (PDZ Europa Ltd., UK) isotope ratio mass spectrometer, interfaced to an ANCA-SL elemental analyzer in continuous flow mode

Table I. Values of isotopic composition of rainfall (‰) from the field station measured on several different dates. R is the cumulative rainfall between two dates.

Date	R (mm)	$\delta^{18}\text{O-H}_2\text{O}_{\text{rain}}$	$\delta^{18}\text{O}_{\text{WM}}$
26/11/2004	-	-	-
08/12/2004	36	-1.9	-1.9
05/01/2005	250	-7.4	-6.7
04/02/2005	109	-3.4	-5.8
23/02/2005	293	-6.8	-6.2
23/03/2005	335	-6.4	-6.3
27/04/2005	95	-10.6	-6.6
Total	1118		

(EA-IRMS). Standards and blanks were included during each run for calibration. The analytical precision of the measurements is $\pm 0.2\%$, and the reproducibility of the results is within $\pm 0.3\%$ for nitrogen (2σ).

The nitrate concentration of water recovered from both the shallow soil and deep subsoil lysimeters was determined using ion chromatography (Dionex DX 100). Oxygen isotopes from water ($\delta^{18}\text{O-H}_2\text{O}$) were determined by an automated water equilibration machine (WES), coupled to a dual inlet Geo 20–20 mass spectrometer (PDZ Europa Ltd. UK). 1 mL of water was equilibrated in 6.5 mL ‘Exetainers’ (Labco Ltd UK) with CO_2 for 8 h at 50 °C. Internal laboratory standards are calibrated to international standards, V-SMOW and V-SLAP, and are used in each run, before and after every 8 samples. Analytical precision, determined by using laboratory standards, is $\pm 0.15\%$ for $\delta^{18}\text{O}$ (2σ).

Oxygen and nitrogen isotopes of the nitrate ($\delta^{18}\text{O-NO}_3$ and $\delta^{15}\text{N-NO}_3$) derived from water were analyzed using the bacterial conversion method at Reston Stable Isotope Laboratory (USGS). Analytical error is 0.5‰ for $\delta^{15}\text{N}$ and 1.0‰ for $\delta^{18}\text{O}$ (2σ). All stable isotope ratios are expressed in the usual delta per mil (‰) notation relative to the respective international standards:

$$\delta_{\text{sample}}(\text{‰}) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (2)$$

where R is the $^{15}\text{N}/^{14}\text{N}$ or $^{18}\text{O}/^{16}\text{O}$ of the sample and standard, respectively. The internationally recognized standard for nitrogen is atmospheric N_2 gas, which, by definition, has a per mil value of 0, and for oxygen is Standard Mean Ocean Water (V-SMOW).

It is possible to calculate the theoretical $\delta^{18}\text{O}$ value of the initial water (Böttcher et al., 1990) from the $\delta^{18}\text{O-NO}_3$ value by using the equation:

$$\delta^{18}\text{O-NO}_3 = 2/3 \delta^{18}\text{O-H}_2\text{O}_{\text{theoreticalrainwater}} + 1/3 \delta^{18}\text{O}_{\text{air}} \quad (3)$$

where $\delta^{18}\text{O}_{\text{air}} = + 23.5 \text{‰}$ according to Kroopnick and Craig (1972).

3. RESULTS AND DISCUSSION

This study reports the in situ fate of nitrogen through the topsoil to the subsurface after application of pig effluent onto

a cultivated soil using stable isotopes and compares the results with nitrates found in Réunion Island’s principal aquifers.

3.1. Evolution of nitrate concentration in soil and subsoil

3.1.1. Control plot

During this investigation, we found that nitrate levels of soil water sampled at 0.45 m vary between 6.6 mg L^{-1} and 49.1 mg L^{-1} (Tab. II). These levels correspond to natural nitrate production via mineralization of organic soil material, which can dominate particularly in summer, when temperatures are between 21 and 30 °C, and soil humidity is up to 55 to 60% due to heavy rainfall (Payet et al., 2009). It was not possible to acquire subsoil water samples at 10 m depth due to the fractured nature of the rock, which did not retain water.

3.1.2. Pig effluent plot

In soil water sampled from 0.45 m, our results show elevated nitrate levels, ranging between 100.5 and 281.1 $\text{mg NO}_3^- \text{L}^{-1}$ (Tab. II), and are considerably higher than the control plot. These levels are most likely attributed to nitrification of effluent applied in October 2003 and November 2004. In contrast, corresponding subsoil nitrate levels (Tab. III) are significantly lower, 1.1 to 12.6 $\text{mg NO}_3^- \text{L}^{-1}$, than those recorded in the soil. Therefore, it is likely that nitrates derived from effluent nitrification have not penetrated through the subsurface to the subsoil lysimeter at 10 m depth after two consecutive fertilizer applications. Isotopic analyses of the in situ nitrates will enable confirmation of this hypothesis in Section 3.3.

3.2. Water isotopes

Isotopic composition of $\delta^{18}\text{O-H}_2\text{O}$ versus $\delta^2\text{H-H}_2\text{O}$, derived from rainfall between December 2004 and April 2005, as well as water samples recovered from lysimeters in the soil at 0.45 m and subsoil at 10 m at both sites correlate with the global meteoric water line ($R^2 = 0.95$). This correlation between soil and subsoil waters which lie on the meteoric water line suggests that water percolating through soil and subsoil does not undergo any significant evaporative processes (Craig, 1961). These results agree well with potential evapotranspiration climate data taken at the field station calculated according to Monteith (1981) and are relatively weak, around 2 to 3 mm each day, reported by the French Meteorological Office of Réunion Island.

3.3. Source assessment using nitrate $\delta^{18}\text{O-NO}_3^-$ and $\delta^{15}\text{N-NO}_3^-$ isotopic signatures

Nitrate isotopic values ($\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$) of soil and subsoil water, and groundwaters are presented in Figure 2.

Table II. Nitrate concentration (mg L^{-1}) and isotopic composition (‰) of nitrate and water measured in soil at 0.45 m below the control plot (CP) and pig effluent plot (PEP). N.B.: trw is theoretical $\delta^{18}\text{O}$ rainwater expressed in ‰ .

Date	NO_3		$\delta^{15}\text{N-NO}_3$		$\delta^{18}\text{O-NO}_3$		$\delta^{18}\text{O-H}_2\text{O soil}$		$\delta^{18}\text{O-H}_2\text{O trw}$	
	CP	PEP	CP	PEP	CP	PEP	CP	PEP	CP	PEP
12/01/2005	12.8	202.0	+3.0	+6.9	+2.2	+5.8	-2.8	-3.6	-8.5	-3.0
17/02/2005	49.1	176.4	+3.2	+7.5	+5.4	+6.7	-4.9	-4.5	-3.7	-1.7
09/03/2005	23.4	281.1	+4.6	+10.8	+5.7	+7.0	-5.7	-4.8	-3.2	-1.3
30/03/2005	6.6	200.2	+3.7	+10.8	+3.3	+8.0	-5.3	-5.2	-6.8	+0.2
27/04/2005	11.2	100.5	+3.5	-	+2.6	-	-5.8	-5.5	-7.9	-
Mean	20.6	192.0	+3.6	+9.0	+3.8	+6.8	-4.9	-4.7	-6.0	-1.5

Table III. Nitrate concentration (mg L^{-1}) and isotopic composition (‰) of nitrate and water measured in the subsoil at 10 m below the pig effluent plot (PEP).

Date	NO_3	$\delta^{15}\text{N-NO}_3$	$\delta^{18}\text{O-NO}_3$	$\delta^{18}\text{O-H}_2\text{O}$	$\delta^{18}\text{O-H}_2\text{O trw}$
	PEP	PEP	PEP	PEP	PEP
19/02/2005	1.1	*	*	+5.5	*
09/03/2005	4.0	+4.7	-1.7	+5.7	-14.3
01/04/2005	12.6	+3.5	-2.3	+5.7	-15.2
27/04/2005	3.0	+3.2	-4.1	+5.7	-18.0
Mean	5.2	+3.8	-2.7	+5.7	-15.8

NB: "trw" is the theoretical $\delta^{18}\text{O}$ rainwater expressed in ‰ . * No isotope analysis.

Our results show soil nitrate $\delta^{15}\text{N}$ values from the control plot at 0.45 m depth are significantly less than those of the pig effluent plot. Soil nitrate $\delta^{15}\text{N}$ values from the control plot range between +3.0 and +4.6 ‰ , with an average of +3.6 ‰ , compared with +6.9 to +10.8 ‰ , with an average of +9.0 ‰ from the pig effluent plot (Tab. II). The control plot results confirm the absence of applied fertilizer and that these nitrates are derived from a natural soil mineralization process (Heaton, 1986; Aravena et al., 1993), as is shown in Figure 2 (Kendall, 1998). The nitrate isotopic composition of soil water from the pig effluent plot has an isotopic signature approaching that of the applied pig effluent, i.e. +11.3 ‰ , consistent with the effluent applications carried out on this plot.

Nitrates extracted from soil water sampled during March and April show more positive $\delta^{15}\text{N}$ values than those sampled in January and February (Fig. 4). Considering that soil water samples are taken at 0.45 m, this increase is consistent with surface leaching of nitrate from pig effluent comprising higher $\delta^{15}\text{N}$ values, i.e. +11.3 ‰ . The vertical leaching rate can be estimated to be approximately 0.45 m for every 1 m of rain falling during the study period (Payet et al., 2009). Increasing $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ isotopic values from February onwards (Tab. II) can potentially be attributed to denitrification processes due to warmer, humid weather in these months. This process preferentially consumes the lighter ^{14}N isotope, leaving the residual nitrate enriched in ^{15}N . A linear regression obtained using the four points from the nitrate isotopic analyses confirms a hypothesis of denitrification (Böttcher et al., 1990). In effect, the direct coefficient of this regression from $^{18}\text{O}/^{15}\text{N}$ is 0.35, close to 1/2. Measurement of total soil nitrogen shows a large N loss during February to March 2005 of

around 150 kg N ha^{-1} which is associated with higher rainfall during this summer period. This reduction is not associated with nitrate lexiviation or nitrogen uptake by the maize (Payet et al., 2009), and strongly suggests denitrification processes induced by microbial activity, which prefers very humid soil conditions, higher temperatures and soil rich in organic matter.

In the pig effluent plot, subsoil at 10 m depth has an average $\delta^{15}\text{N-NO}_3$ value of +3.8 ‰ . This contrasts significantly with the pig effluent plot $\delta^{15}\text{N-NO}_3$ value of +9.0 ‰ from the soil at 0.45 m, but is close to the $\delta^{15}\text{N-NO}_3$ value of +3.6 ‰ obtained for the control plot soil at 0.45 m. It is likely that effluent-derived nitrates have not penetrated down to the lysimeter sampling depth at 10 m in the subsoil, even though there were two effluent applications, and more than 1900 mm of rain, more than half of which drained directly into the root zone during the 18 months of the study. This can also be explained by the anion retention of andesitic soils which slow the nitrate flux relative to the water flux (Shoji et al., 1993; Reynold-Vargas et al., 1994; and Katou et al., 1996).

Although this study aims to understand the migration of nitrates in the subsoil, it appears the nitrates are found in very low concentrations at 10 m depth, and do not display an isotopic signature similar to the pig effluent applied 18 months earlier. This demonstrates that the vertical migration of nitrates is extremely slow in the soils and subsoils of Réunion Island.

3.4. Origin of $\delta^{18}\text{O}$ in nitrates

Our study shows soil water $\delta^{18}\text{O-NO}_3$ values of the control plot are different from those of the pig effluent plot: $\delta^{18}\text{O-NO}_3$

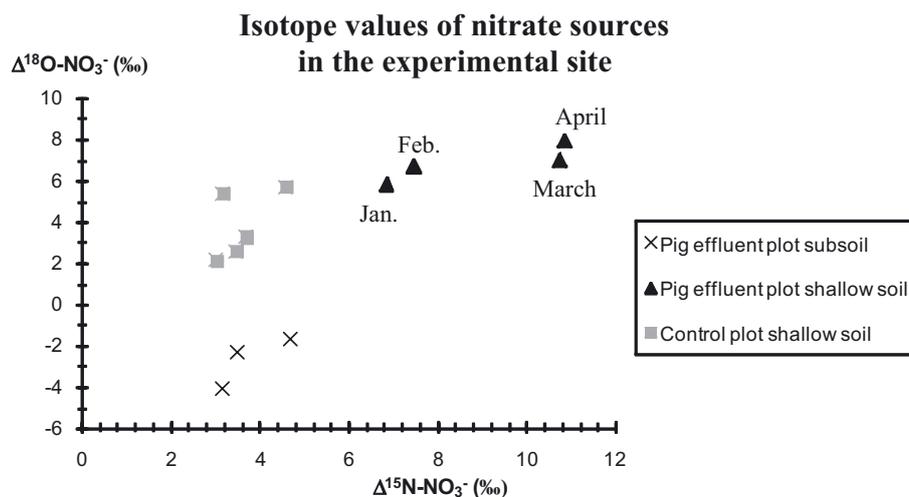


Figure 4. Nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values (‰) derived from soil and subsoil water. Nitrates derived from the pig effluent-fertilized subsoil exhibit similar ^{15}N values to natural soil mineralization of the control plot. However, nitrates found in the surface soil of the pig effluent-fertilized plot are enriched in ^{15}N , as they are derived from the nitrification of the pig effluent spread over this plot. Isotopic variations between samples taken in January/February and those taken in March/April are attributed to denitrification. Subsoil nitrates from the pig effluent-fertilized plot are depleted in ^{18}O relative to surface soils, as they were most likely formed several years earlier, and are derived from ^{18}O -depleted rainwater, such as that produced by cyclones.

values range between +5.8 and +8.0‰ with an average of +6.9‰ for the pig effluent plot, while the control plot has lower $\delta^{18}\text{O}\text{-NO}_3^-$ values ranging between +2.2 and +5.7‰, with an average of +3.8‰ (Tab. II). The two plots received the same amount of rainfall, characterized by a $\delta^{18}\text{O}$ isotopic mean weighted value of around -6.6‰ over the period of the study from December 2004 to April 2005 (Tab. I). Soil water $\delta^{18}\text{O}\text{-H}_2\text{O}$ values of the control plot and pig effluent plot at 0.45 m are very similar: -4.9‰ and -4.7‰, respectively (Tab. II), and are slightly enriched relative to precipitation during this study which has $\delta^{18}\text{O}\text{-H}_2\text{O}_{\text{WM}}$ of -6.6‰ (Tab. I). The enrichment of soil water is most likely due to mixing of recent depleted rainwater with older enriched rainwater from previous rainfall events.

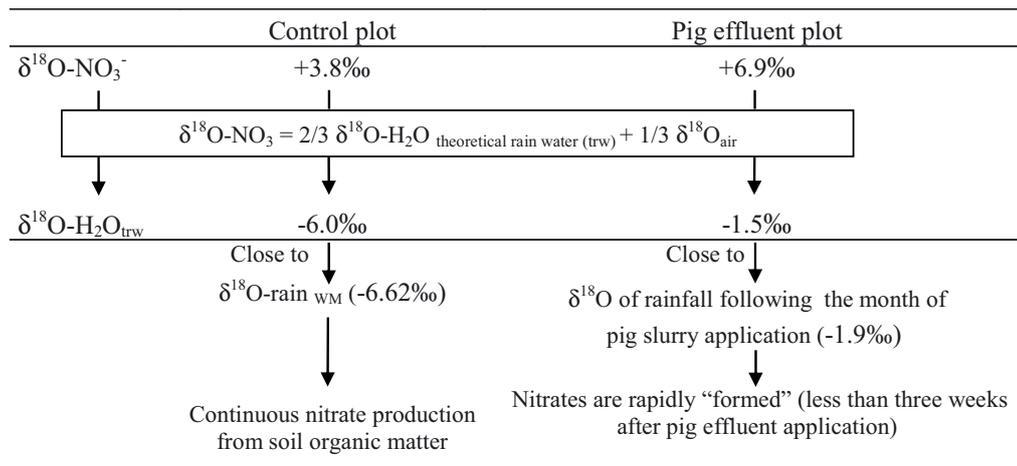
Nitrates derived from the mineralization of reduced nitrogen products such as NH_4 and NO_2 in the unsaturated zone have two isotopically distinct sources of oxygen (Aleem et al., 1965; Anderson and Hooper, 1983; Yoshinari and Wahlen, 1985): one-third of nitrate oxygen is usually derived from air (+23.5‰) and two-thirds from rainfall. Therefore, the variability of the nitrate oxygen isotope derived from nitrification of ammonium-rich effluent depends on the isotopic composition of meteoric water. Taking into account differences between the pig effluent site which has a $\delta^{18}\text{O}\text{-NO}_3^-$ value of +6.9‰, and the control site which has a $\delta^{18}\text{O}\text{-NO}_3^-$ value of +3.8‰, it appears in the present case that the nitrates are formed from different rainfall events. In Réunion Island, the variability of the isotopic composition of rainfall can change significantly, even in the same location, notably during a cyclone event (Nicolini et al., 1989). The isotopic composition of soil water which could have formed the nitrate $\delta^{18}\text{O}$ according to the process outlined above can be calculated (Tab. II) from equation (3).

We calculate soil from the control plot (Tab. II) has an average theoretical rainfall $\delta^{18}\text{O}$ value (Eq. (2)) of -6.0‰. It is close to the weighted mean $\delta^{18}\text{O}$ value of -6.6‰, which is the rain which fell during the period of the experiment (Tab. I). The oxygen isotopic signature of nitrate from the control plot soil at 0.45 m depth therefore reflects the isotopic signature of its precipitation. These nitrates are generated continuously in the shallow soil, along with other soil mineralization processes of the organic matter.

In contrast, the pig effluent plot has an average theoretical rainfall $\delta^{18}\text{O}$ value (Eq. (2)) of -1.5‰ (Tab. II), which is considerably more enriched in comparison with the recent rainfall $\delta^{18}\text{O}$ value of -6.6‰ (Tab. I). We suggest that nitrates were rapidly “formed”, in a matter of several weeks after effluent application (three weeks according to Morvan et al., 1997), as there was a period of isotopically light rainfall with $\delta^{18}\text{O}_{\text{rain}}$ values of -1.9‰ at the beginning of December 2004 (Tab. I) which fell immediately after effluent application (Fig. 5).

Moreover, we notice a strong contrast between $\delta^{18}\text{O}\text{-NO}_3^-$ from pig effluent plot soil ranging from +5.8‰ to +8.0‰, and pig effluent plot subsoil ranging from -4.1‰ to -1.7‰ (Fig. 4, Tabs. II and III). Two possibilities of interpretation are considered.

- If we consider that the ratio of oxygen incorporation from H_2O and O_2 is 2:1 (Eq. (2)), then the $\delta^{18}\text{O}\text{-NO}_3^-_{\text{subsoil}}$ values of the pig effluent plot could be explained by two possible interpretations: (i) the $\delta^{18}\text{O}\text{-NO}_3^-_{\text{subsoil}}$ values would correspond to a $\delta^{18}\text{O}\text{-H}_2\text{O}_{\text{theoreticalrainwater}}$ value of -14.3 to -18.0‰ (Tab. III). This suggests very depleted ^{18}O rainfall derived from cyclonic events of previous years, i.e. Dyna cyclone, January 2002; (ii) there is significant isotope fractionation during the incorporation of oxygen from H_2O and O_2 into the newly formed nitrate.



where $\delta^{18}\text{O}_{\text{air}} = +23.5\text{‰}$; WM: weighted mean

Figure 5. Formation of oxygen isotopes in nitrates by rainwater. Using ^{18}O values of soil nitrates and monthly rainwater, it is possible to determine when nitrates are formed in the soil.

Or,

- The ratio is not 2:1 (Aravena et al., 1993; Kendall et al., 1995; Wassenaar, 1995; Böhlke et al., 1997; Kendall, 1998; Mayer et al., 2001). If we assume a $\delta^{18}\text{O}-\text{HO}$ value of -5‰ in precipitation corresponding to 800 m asl (Nicolini et al., 1998), the ratio explaining the isotopic values of $\delta^{18}\text{O}-\text{NO}_3^-$ subsoil water would be 5:1 and not 2:1.

3.5. Application to some Réunion Island’s aquifers

Nitrate isotopic compositions of 15 fresh water wells were sampled in June 2005 (Rogers et al., 2009) from around Réunion Island and are presented in Figure 2 and Table IV. The wells encompass a variety of different geological and climatic zones, and are exposed to varying land use (Fig. 1 and Tab. IV). Values obtained from the experimental control plot and pig effluent plot are also reported in Figure 2. Rogers et al. (2009) show that drinking water extracted from wells has nitrate levels ranging from 0.7 to 85.3 mg $\text{NO}_3^- \text{L}^{-1}$ (Tab. IV). According to Kendall (1998, Fig. 2), the majority of these wells have an isotopic composition which lies within the manure and septic waste zone, and soil nitrate zone, with two end members; Grand Coin, reflecting severe contamination from organic manure or animal effluent, and a high quality drinking water from “Basse Vallée”. The Basse Vallée groundwater lies in an overlapping region (Fig. 1), between natural soil nitrate and nitrate derived from chemical fertilizers, but it could be considered as a natural end member because of lack of surrounding agricultural activity. The Grand Coin well (located 1100 m above sea level) exploits an underground perched aquifer at a depth of around 100 m below the surface. In the region, pig and poultry farming and market gardening are well developed, and effluents derived from farming are

used extensively as fertilizers. The results from our study show that nitrates of Réunion Island’s fresh water wells have similar isotopic values to soil waters from the pig effluent plot from the experimental site (Fig. 2). This suggests that the majority of drinking waters from Réunion Island are affected by at least some level of nitrate contamination derived from human or animal waste. Therefore, chemical fertilizers are not considered to have a significant contribution to the nitrate signatures of Réunion Island’s drinking water.

4. CONCLUSION

Our results show nitrates produced naturally in soil by mineralization of soil organic matter were isotopically depleted (mean $\delta^{15}\text{N}-\text{NO}_3^- = +3.6\text{‰}$, mean $\delta^{18}\text{O}-\text{NO}_3^- = +3.8\text{‰}$) compared with nitrates derived from the nitrification of pig effluent (mean $\delta^{15}\text{N}-\text{NO}_3^- = +9.0\text{‰}$, $\delta^{18}\text{O}-\text{NO}_3^- = +6.8\text{‰}$). We show that the experimental site control plot has $\delta^{18}\text{O}-\text{NO}_3^-$ close to the $\delta^{18}\text{O}$ of precipitation, as nitrates are continuously produced from soil organic material. Concurrently, the experimental site pig effluent plot has $\delta^{18}\text{O}-\text{NO}_3^-$ close to the $\delta^{18}\text{O}$ of rainfall in the month following application, as they form from rapid nitrification of ammonia from pig effluent three weeks after application.

The site which had pig effluent applied attained an average maximum surface (0.45 m) soil water $^{15}\text{N}-\text{NO}_3^-$ value of $+9.0\text{‰}$. This signature was significantly more enriched in ^{15}N than the corresponding subsurface (10 m depth) soil water $^{15}\text{N}-\text{NO}_3^-$ value of $+3.8\text{‰}$. The control site surface (0.45 m) soil water $^{15}\text{N}-\text{NO}_3^-$ value of $+3.6\text{‰}$ is similar to the subsurface pig effluent application plot. This indicates nitrates derived from pig effluent have not reached the subsurface (10 m depth), even though over the last 18 months this site was subjected to two effluent applications, each around

Table IV. Altitude, nitrate concentration (mg L^{-1}) and isotopic composition (‰) of nitrates measured at several pumping wells and springs of Réunion Island (June 2005) from Rogers et al. (2009). Land use: A: agriculture; U: urban; M: mixed.

Name (number)	Origin	Altitude (m)	NO_3	$\delta^{15}\text{N-NO}_3$	$\delta^{18}\text{O-NO}_3$	Land use
Les Cafés (1)	Drilled well	73	13.6	+7.7	+6.7	M
Blanche (2)	Spring	250	4.6	+7.4	+2.8	A
Denise (3)	Spring	210	5.5	+8.9	+3.4	A
Salette (4)	Drilled well	81	20.7	+6.8	+4.9	A
Fargeau (5)	Drilled well	530	27.9	+10.5	+5.3	U
Leveneur (6)	Spring	774	11.7	+8.5	+8.3	A
Coco CGE (7)	Drilled well	69	10.7	+9.5	+4.0	M
Grand Coin (8)	Drilled well	1066	85.3	+14.2	+5.2	A
P11 (9)	Drilled well	37	16.2	+7.8	+2.0	M
Hermitage (10)	Drilled well	51	27.2	+7.4	+3.7	A
Pointe du Gouffre (11)	Spring	25	12.6	+9.2	+7.0	U
Forage Chaudron (12)	Drilled well	26	23.8	+9.6	+4.3	U
Trou d'Eau (13)	Spring	5	23.0	+9.1	+3.9	A
Basse Vallée (14)	Drilled well	150	0.7	+1.2	+1.3	A
La Plaine (15)	Drilled well	74	19.3	+9.5	+2.3	U

200 kg N ha^{-1} , and more than 1900 mm of rain. This emphasizes the significantly slow vertical nitrate migration through Réunion Island's soils and subsurface structures. Nonetheless, the vertical nitrate flux through the unsaturated zone is only delayed, and eventually these nitrates will pollute the unsaturated zone and, at some later time, the saturated zone and its associated aquifer. In conclusion, we determine that the nitrate contamination found in Réunion Island's groundwaters exhibits signatures consistent with those derived primarily from urban and/or agriculture via effluent application. Due to the slow nitrate migration through the soil into the unsaturated subsurface zone, and high level of urbanization over the last 50 years around the study zone, increasing nitrate levels in saturated zones from the western and northern regions would correspond mostly to nitrates produced by urban development.

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