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Source apportionment of nitrate pollution in surface water using stable isotopes of N and O in nitrate and B: a case study in Flanders (Belgium)

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ABSTRACT

Excess application to fields of animal manure or mineral N fertilizers is generally assumed to be the major source of nitrate pollution in surface and groundwater in agricultural areas in Flanders (Belgium). The Flemish Environment Agency (VMM) suspects, but could not prove, that discharges of untreated domestic sewage or discharges containing mineral N fertilizers from hydroculture in greenhouses are also contributing to the nitrate pollution observed in some of the agricultural areas. The objective of this study was to investigate, using stable isotope data of dissolved nitrate ($\delta^{15}N$ and $\delta^{18}O$) and boron ($\delta^{11}B$), if these suspected sources significantly contribute to the nitrate pollution observed in selected surface water monitoring points in Flanders. Based on our nitrate and boron isotope results, nitrate input from domestic sewage was estimated to range between 5 and 50% in the investigated sampling points. Our nitrate isotope results confirmed significant inputs from mineral nitrate fertilizers, potentially originating from greenhouse discharges, in areas with intensive greenhouse cropping. Nitrate isotope data also indicated the potential occurrence of natural nitrate attenuation through denitrification, which could partially explain lower nitrate concentrations generally observed in summer period.

1. Introduction

For the implementation of the Nitrates Directive (91/976/EEC)[1], different policy measures have been taken to reduce nitrate pollution originating from agriculture in surface and groundwater in Flanders. Excess application to fields of animal manure and mineral fertilizers is generally assumed to be the major source of nitrate pollution in agricultural areas. Three consecutive Manure Action Plans (MAP) have been implemented in Flanders since 1996, which mainly aimed at reducing and controlling inputs from those agricultural sources. In order to evaluate the effectiveness of the measures taken, an extensive MAP monitoring network has been established in agricultural areas since 1999 by the Flemish Environment Agency (VMM) for monitoring nitrate concentrations in surface and groundwater. A positive evolution of surface water quality in Flanders has been observed during 1999-2003, as the number of MAP points with nitrate concentrations exceeding the 50 mg/l limit decreased from 59% to 32%[2]. However, improvement in surface water quality is stagnating during the last five years and elevated nitrate concentrations (>50 mg NO3 l^{-1}) are currently still observed in 42% of the MAP surface water monitoring points[2,3].

VMM suspects, but could not prove, that discharges of untreated domestic sewage or discharges containing mineral fertilizers from hydroculture in greenhouses are also contributing to the nitrate pollution observed in some of the agricultural areas. Thus appropriate remedial measures (connecting houses to the public sewage system or implementing individual wastewater treatment, legislation controlling discharges from hydroculture) could not yet be implemented there.

Several studies[4,5,6] have demonstrated that nitrate nitrogen and oxygen isotope ratios ($^{15}N/^{14}N$ and $^{18}O/^{16}O$, expressed as $\delta^{15}N$ and $\delta^{18}O$ values) can be used for tracing contaminant sources, since nitrate originating from different sources is often characterized by distinct $\delta^{15}N$ and $\delta^{18}O$ values (Fig. 1a). Recent studies[7,8,9] have shown that the stable isotope ratio of boron ($^{11}B/^{10}B$, expressed as $\delta^{11}B$ value) can be used as an additional tracer, and more specifically to distinguish between nitrate originating from animal manure or domestic sewage (Fig. 2).

The objective of this study was to investigate, using stable isotope data ($\delta^{15}N$ and $\delta^{18}O$ of nitrate and $\delta^{11}B$), if other suspected sources than animal manure (discharges of untreated domestic sewage, discharges from hydroculture in greenhouses) significantly contribute to the nitrate pollution observed in a number of surface water MAP points selected by VMM.

2. Materials and methods

2.1. Study area and sampling

VMM selected nine MAP surface water sampling points located at small streams in agricultural areas for this study. The MAP identification number, location, sampling dates and suspected nitrate sources for the samples supplied by VMM are summarized in Table 1 (all chemical data monitored by VMM for the MAP-points including nitrate concentrations are available from the VMM website[3]). Six MAP points were located in the northern region of Flanders (Hoogstraten, Wuustwezel, Ravels, Rijkevorsel) and were sampled monthly during one year. Three MAP points were

located in a region in central Flanders (Sint Katelijne Waver) with intensive greenhouse cropping in hydroculture and were sampled only once or twice.
In MAP points 79670 and 79700, mainly manure input was suspected. In MAP points 71200 and 85400, VMM suspected a contribution from domestic sewage, since many houses are not yet connected to the public sewer system in that region. In the remaining MAP points, a contribution was suspected from discharges containing mineral fertilizers from hydroculture in greenhouses.
Water samples were taken by VMM and transported at 0°C to IRMM. Upon arrival at IRMM samples were stored at -20°C and defrosted prior to isotope analysis.

Samples were also collected from potential nitrate sources in the study area in order to characterize their nitrate and B isotopic composition. Potential sources included animal manure, mineral nitrate fertilizers, discharges from greenhouses, untreated domestic sewage, soil organic N and atmospheric deposition (Table 2).
Animal manure samples (two pig manure and cow manure samples) have been collected from manure pits at local farms in the study area. For characterization of the nitrate in greenhouse discharges, we collected samples from two greenhouse feeding solutions and two mineral nitrate fertilizers (KNO_3 and $Ca(NO_3)_2$) which are used in these feeding solutions. VMM supplied two sewage samples (influent to a water purification plant) mainly charged with domestic sewage and one domestic sewage sample was collected by IRMM (sewage 1,2 and 3, respectively in Table 2). For characterization of nitrate originating from the mineralization and nitrification of soil organic N, two agricultural soil samples were collected. A water sample was taken from a lake in a nature reserve in Wuustwezel to characterize surface water without anthropogenic nitrate inputs (non-contaminated surface water). Nitrate in atmospheric deposition was assessed by collecting rainwater at IRMM during a rainfall event in August 2007.

2.2. Nitrate isotope analysis

Sample preparation for dissolved nitrate isotope analysis in surface water and domestic wastewater was executed according to Silva et al. (2000)[10], with some modifications. This method consists of extraction of the NO_3^- on an anion exchange resin (2 mL Bio-Rad AG1-X8 in the Cl-form, 200-400 mesh size), elution of the NO_3^- with HCl and conversion to solid $AgNO_3$ for IRMS analysis. Modifications to the original method were that samples were passed through a cation exchange resin column (2 mL Bio-Rad AG50W-X8 in the H-form,100-200 mesh size) on top of the anion exchange resin column (to prevent trapping of dissolved organics on the anion column) using a vacuum manifold system. Water samples were filtered on 0.45 µm polycarbonate filters before loading onto the ion exchange columns and between 6 and 12 mg of NO_3^- were loaded. Because of the much lower nitrate concentrations in the rainwater sample (Table 2) and larger sample volumes needed, sample preparation for this sample was performed according to Chang et al. (1999)[11]. This sample preparation method is similar to the method of Silva et al. (2000)[10], but larger amounts of resin (5mL) and anion exchange resin with different mesh size (100-200 mesh size) are used. The nitrogen and oxygen isotopic composition of the $AgNO_3$ ($\delta^{15}N$ and $\delta^{18}O$ value) was analyzed using a thermal conversion carbon reduction elemental analyzer (TC-EA system from ThermoFinnigan) linked to a continuous flow isotope ratio mass spectrometer (IRMS MAT252 and Delta V Advantage from

ThermoFinnigan). Isotope reference materials (IAEA-N3, USGS32) were included in the measurement sequences to normalize the $\delta^{15}N$ and $\delta^{18}O$ results. All $\delta^{15}N$ values in this report were expressed in ‰ relative to the international standard AIR; all $\delta^{18}O$ values were expressed in ‰ relative to the international standard VSMOW.

Isotope analysis of mineral nitrate fertilizers was executed on bulk samples by TC-EA-IRMS. Isotope analysis of nitrate in the manure samples could not be performed using anion exchange resins because of the high concentration of dissolved organics and suspended solids. Therefore, total N $\delta^{15}N$ analysis was executed on freezedried and homogenized manure samples using a flash combustion elemental analyzer (EA NC 2500 system from Interscience) linked to a continuous flow IRMS (Delta Plus from ThermoFinnigan). For $\delta^{15}N$ analysis of the soil organic N, the agricultural soil samples were extracted with Milli-Q water to remove any nitrate (extraction of 10 g dry soil in 30 ml water on an orbital shaker at 200 rpm during 30 min) and dried overnight at 60°C. $\delta^{15}N$ analysis was executed on homogenized bulk samples using EA-IRMS.

The expanded uncertainty (k=2) on the reported $\delta^{15}N$ and $\delta^{18}O$ results is approximately 0.2‰.

2.3. Boron isotope analysis

Prior to ion exchange separation, water samples were filtered on a 0.45 µm polycarbonate filter. Ion exchange separation was applied using a column containing 100 µL Amberlite IRA 743 resin (125-250 mesh fraction). The resin bed was successively cleaned with 0.1 M HCl, deionised water and 0.5 M NaOH and conditioned with deionised water. Water samples (pH 8-9) containing 2 µg B were loaded onto the column by gravity dripping. After loading, the column was successively washed with deionised water, 0.6 M NaCl and deionised water. Boron was eluted and collected in a 2.5 mL aliquot of 0.1 M HCl which was evaporated at a temperature of 54-58°C to a final volume of +/- 15 µL. The boron was separated from residual organic material by sublimation in an inverted conical vial at a temperature of 75-80°C. After cooling, the droplet collected in the vial base was first evaporated to almost-dryness with D-mannitol solution and was then loaded onto a Ta filament with a graphite-ethanol mix and a caesium carbonate solution. Boron isotope measurements were made by single filament technique on a magnetic sector thermal ionisation mass spectrometer equipped with a single Faraday collector. Signal intensities were measured for $Cs_2{}^{10}BO_2$ and $Cs_2{}^{11}BO_2$ at mass 308 and 309. $^{11}B/^{10}B$ isotope ratios are corrected for ^{17}O contribution at mass 309 ($^{11}B/^{10}B_{corrected}$ = $^{11}B/^{10}B_{measured}$ - 0.00078). $\delta^{11}B$ values are expressed in ‰ relative to the measured NIST standard SRM 951.

Boron isotope analysis has been executed on five surface water samples (from MAP-points), one non-contaminated surface water sample, one sewage sample and one greenhouse feeding solution.

2.4. Chemical analysis

Nitrate and ammonium concentration analysis was performed by spectrophotometry (cuvette tests and spectrophotometer from Hach-Lange). Boron concentration analysis was performed by spectrophotometry and by ICP-OES (analyses performed by VITO).

2.5. Estimation of nitrate source contributions

Contributions of nitrate sources to a mixture (nitrate in surface water samples) were quantified using an isotope mixing model based on mass balance equantions. Using N and O isotopes of nitrate, contributions from up to three different nitrate sources could be estimated based on following mass balance equations (3 source mixing model, f stands for fraction of a source in the nitrate mixture):

$$\delta^{15}N_{mixture} = \sum_{i=1}^{3} f_{source,i} * \delta^{15}N_{source,i}$$

$$\delta^{18}O_{mixture} = \sum_{i=1}^{3} f_{source,i} * \delta^{18}O_{source,i}$$

$$1 = \sum_{i=1}^{3} f_{source,i}$$

For samples where B isotope data were available, contributions from up to four different nitrate sources could be estimated based on following mass balance equations (4 source mixing model):

$$\delta^{15}N_{mixture} = \sum_{i=1}^{4} f_{source,i} * \delta^{15}N_{source,i}$$

$$\delta^{18}O_{mixture} = \sum_{i=1}^{4} f_{source,i} * \delta^{18}O_{source,i}$$

$$\delta^{11}B_{mixture} = \sum_{i=1}^{4} f_{source,i} * \delta^{11}B_{source,i}$$

$$1 = \sum_{i=1}^{4} f_{source,i}$$

3. Results and discussion

3.1. Nitrate source samples

3.1.1. Literature values

Typical ranges of δ^{15}N and δ^{18}O values reported in literature[12] for nitrate derived or nitrified from the following sources are shown in Fig. 1a: manure, domestic sewage, NO_3-fertilizers (e.g. KNO_3, $Ca(NO_3)_2$), reduced N-fertilizers (e.g. urea, $(NH_4)_2SO_4$), soil N and atmospheric deposition. δ^{15}N values of NO_3-fertilizers and reduced N-fertilizers typically range around 0‰, since they are produced from atmospheric N_2 (international standard for expressing δ^{15}N values). The N in reduced N-fertilizers, animal manure, sewage and soil organic matter is transformed into NH_4 (ammonification) and further into NO_3 (nitrification) through microbial activity in soils. Due to volatilization of ^{15}N-depleted NH_4, NO_3 originating from animal manure and sewage generally shows relatively higher and overlapping δ^{15}N values, compared to other sources. During nitrification, two oxygen atoms are incorporated from soil H_2O (δ^{18}O values of water normally range from -25 to +4‰) and one from ambient O_2 (δ^{18}O values of atmospheric O_2 is approximately +23.5‰)[12]. Hence the δ^{18}O value for nitrate formed through nitrification is expected to range between -10 and +10‰ (Fig. 1a). Mineral NO_3-fertilizers, where the O is mainly derived from atmospehric

O_2, show elevated $\delta^{18}O$ values around +23.5‰ ($\delta^{18}O$ value of atmospehric O_2). For nitrate in atmospheric deposition, reported $\delta^{15}N$ values range between -10 to +15‰, while $\delta^{18}O$ values range from +25 to 75‰[12]. Microbial denitrification (reduction of nitrate to N_2O and N_2 under anaerobic conditions) may affect the original nitrate isotopic signature according to a relatively predictable pattern, causing $\delta^{15}N$ and $\delta^{18}O$ values to increase in a ratio of roughly 2:1. This corresponds with a slopes of 0.5 for the denitrification trend in Fig. 1a and b.

$\delta^{11}B$ values reported in literature for nitrate sources[7,8,9,13-17] were plotted versus the $\delta^{15}N$ values in Fig. 2. While $\delta^{15}N$ values overlap for sewage and manure, sewage is generally characterized by lower $\delta^{11}B$ values compared to manure. This is due to the elevated presence of synthetic sodium perborates (bleaching agent added to cleaning products) with a typical $\delta^{11}B$ signature between -5 and 10‰[14,17]. Mineral fertilizers and sewage typically show overlapping $\delta^{11}B$ values.

3.1.2. Experimental values

Results from the chemical and isotopic characterization of the nitrate source samples are summarized in Table 2. The nitrate and B isotope results were also plotted in Fig. 1 and 2 for comparison with literature values.

The measured total N $\delta^{15}N$ values in the animal manure and soil samples fell within the ranges reported in literature for nitrate nitrified from the respective sources (Table 2 and Fig. 1a). However, the actual $\delta^{15}N$ values of nitrate originating from these sources may be different from the measured $\delta^{15}N$ values, due to isotope fractionation associated with nitrification and ammonia volatilization. $\delta^{18}O$ in H_2O has been analyzed for one surface water sample by TC-EA-IRMS, and the resulting $\delta^{18}O$ value (-7.4‰) fell within the range of -6 to -8‰ reported for local rainwater[18]. This corresponds with a theoretical $\delta^{18}O$ value of +3‰ for nitrate nitrified from the animal manure and soil organic N samples, which was plotted in Fig. 1a for these sources. The $\delta^{18}O_{NO3}$ value measured in sewage (+9.4‰) was higher than this theoretical $\delta^{18}O$ value, but fell within the literature range. The $\delta^{15}N$ and $\delta^{18}O$ values of mineral fertilizer samples and atmospheric deposition also plotted within literature value ranges. The experimental $\delta^{15}N$ and $\delta^{18}O$ values for atmospheric deposition were comparable to the values reported by Deutsch et al. (2006)[19] for multiple rainwater samples collected during one year in northern Germany ($\delta^{15}N$=0.1‰; $\delta^{18}O$=51.7‰).

Boron concentrations in the pig manure samples were relatively low compared to the values reported by Komor (1997)[13] (1430-8190 μgl^{-1}), no other reported values were found for cow manure. The $\delta^{11}B$ value of the pig manure sample was in the lower range of other reported values. Boron concentrations in the sewage samples were also relatively low compared to values reported in other studies (40-1100 μgl^{-1})[7-9,14,15,17]. The $\delta^{11}B$ value of the greenhouse feeding solution plotted in the range reported in literature for mineral fertilizers (Fig. 2). The B concentration in saturated solutions of mineral fertilizer samples was too low (below 10 μgL^{-1}) to enable B isotope analysis.

3.2. Surface water samples

Chemical and isotope analysis results for the surface water samples (from the nine MAP points and one non-contaminated surface water) are summarized in Table 3. For MAP points 69000, 82870, 71200, 85400, 79670 and 79700, samples taken between

February and June 2006 were selected for nitrate isotope analysis in order to study possible variations of the isotopic signature in function of time or nitrate concentration. In general, nitrate concentrations were most elevated and higher than the 50 mg l^{-1} norm set by the Nitrates Directive in winter and spring period (February to April) and relatively lower in summer period (May-June). MAP point 79670 showed elevated nitrate concentrations during the entire sampling period.

Different plots were made for interpretation of the isotope data: $\delta^{18}O_{NO3}$ versus $\delta^{15}N_{NO3}$ values (Fig. 1a and b), $\delta^{11}B$ versus $\delta^{15}N_{NO3}$ values (Fig. 2), $\delta^{18}O_{NO3}$ values versus the inverse NO3 concentrations (Fig. 3) and $\delta^{11}B$ values versus the inverse B concentrations (Fig. 4).

In Fig. 1a, mixing lines were drawn between the isotopic signatures of potential nitrate sources or mixing end members in the study area: reduced N fertilizers, NO3 fertilizers (including greenhouse discharges) and sewage or manure. A limited number of the isotope data points for the surface water samples (samples 1,15,16,18) plotted within or on the border of the region outlined by these mixing lines. This indicates that the isotopic signature in those data points could be explained by mixing between the nitrate sources taken into account. However, most of the data points plotted outside the outlined region, which indicates a contribution from an additional nitrate source (atmospheric deposition) or that the nitrate isotopic signature was affected by isotope fractionation (most likely denitrification). Fig. 3 and Fig. 4 relate the nitrate and B isotopic signatures in the nitrate sources and water samples to the corresponding concentrations. Isotope data were plotted versus inverse concentrations, since mixing between two sources with different nitrate concentrations is then represented by straight mixing lines.

A first group of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ data points (samples 1,2,5,6,8,9,10,11,12,15-19) plotted in the region of nitrate originating from animal manure or sewage (Fig. 1a). These samples were taken from MAP points 69000, 82870 and 85400 in March and April and from MAP points 79670, 71200 and 79700 between March and June. In Fig. 3 these data points also plotted close to the experimental data for animal manure and sewage samples, which indicates that nitrate pollution in those points could be largely attributed to animal manure or sewage input.

For samples 1,15,16 and 18 (which plotted within or on the border of the mixing region) we estimated contributions from manure or sewage, NO3 fertilizers and reduced N fertilizers using the measured $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ sample values (3 source mixing model). For the N fertilizer end-member in the mixing model, a $\delta^{15}N_{NO3}$ value of 0‰ (in the middle of the literature range) and a $\delta^{18}O_{NO3}$ value of +3‰ was taken. For the animal manure or sewage end-member, the largest experimental $\delta^{15}N$ value for manure (+13.1‰) and a $\delta^{18}O_{NO3}$ value of +3‰ were taken. Experimental values for the KNO_3 sample were taken for the NO3 fertilizer end-member. The calculated source contributions are shown in Table 4, indicating major contributions from manure or sewage (between 78 and 91%), and minor contributions from NO3 or reduced N fertilizers (between 0 and 13%).

The $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values of samples 2,5,6,8,9,10,11,12,17 and 19 (plotting outside the mixing region) indicate that nitrate in these samples had been subject to denitrification. The potential original nitrate isotopic compositions before denitrification then fall on a line following the denitrification trend (Fig. 1b). The approximate changes in the nitrate isotopic signature corresponding with 25% or 50%

denitrification of an original nitrate mixture (5‰ or 12‰ increase of the $\delta^{15}N_{NO3}$ value, respectively) are also indicated in Fig 1b. Contributions of the nitrate sources were estimated for the intersections of this denitrification trend with mixing lines between (1) mineral NO3 fertilizers and manure or sewage (implicating 0% contribution from reduced N fertilizers) and (2) reduced N fertilizers and manure or sewage (implicating 0% contribution from NO3 fertilizers) (Fig. 1b and Table 4).

In order to distinguish between animal manure or sewage as nitrate source, additional B isotope analysis has been executed on one sample from each of these MAP points (samples 2, 6, 9, 12, 15 and 18). Fig. 2 shows that the $\delta^{11}B$ values of these samples were considerably higher than the experimental value and literature values for sewage, and that their $\delta^{11}B$ versus $\delta^{15}N_{NO3}$ data points plotted within the range reported for animal manure sources. Since only one experimental B isotope value was available for animal manure, a hypothetical $\delta^{11}B$ value of +25‰ (in the middle of the range reported in literature) was assigned to the animal manure mixing end-member in Fig. 4. Mixing lines between animal manure, sewage and non-contaminated surface water were plotted to study mixing processes between these nitrate sources. Samples 2, 6, 12, 15 and 18 showed similar $\delta^{11}B$ values and B concentrations (Table 3) and their data points plotted relatively close to the hypothetical mixing line between animal manure and non-contaminated surface water (Fig. 4). Sample 9 (MAP point 71200) was characterized by a lower $\delta^{11}B$ value, a considerably higher B concentration (Table 3) and plotted closer to the hypothetical mixing line between animal manure and sewage (Fig. 4). This could indicate some input from nitrate originating from sewage. For these six samples, we estimated contributions from four potential nitrate sources (manure, sewage, NO3 fertilizers and atmospheric deposition) using the $\delta^{11}B$, $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ sample values (4 source mixing model). For samples 15 and 18 the measured $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values were used for the calculations. For the other samples, which plotted outside the $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ mixing diagram, values at the intersection of the denitrification trend with the mixing line between mineral NO3 fertilizers and manure were used (Fig 1b). Literature $\delta^{11}B$ values were taken for animal manure (25‰) and atmospheric deposition (37‰) sources. The estimated contributions and uncertainty on these estimates are shown in Table 4. Estimated contributions from manure ranged from 42% (sample 9) to 85% (sample 6). Contributions from sewage were estimated to range from 5% (sample 6) up to 52% (sample 9) but we remark that the uncertainties on these estimates were relatively large. Discriminant analysis also indicated that sewage was the main contribution in sample 9, whereas manure was the main contribution in the other samples. As already indicated by estimates using the three source mixing model, contributions from NO3 fertilizers were small. The estimated contribution from atmospheric deposition was similar in all samples and ranged between 4 and 7%.

A second group of samples (samples 4, 14, 20, 21, 22) showed higher $\delta^{18}O_{NO3}$ values (above 10‰) and equal or lower $\delta^{15}N_{NO3}$ values (below 12‰) compared to the first group of samples (Fig. 1a), which all plotted outside the mixing region between NO3 fertilizers, reduced N fertilizers and manure or sewage. All of these samples were taken between February and April and showed elevated nitrate concentrations. Most of these samples originated from MAP points where input from greenhouse discharges was expected next to animal manure input (MAP points 82870, 263100, 376220 and 37525). One of these samples originated from MAP point 79670, where only manure input was expected.

The higher $\delta^{18}O_{NO3}$ values in these samples indicate inputs from mineral nitrate fertilizers, atmospheric deposition or a combination of both sources, since these sources are characterized by higher $\delta^{18}O_{NO3}$ values and lower $\delta^{15}N_{NO3}$ values compared to manure and sewage. The fact that their isotopic signatures plot outside the mixing region NO3 fertilizers/reduced N fertilizers/manure or sewage in these sample could thus be explained by (1) denitrification of an original mixture between NO3 fertilizers, reduced N fertilizers and manure or sewage or (2) input from atmospheric deposition to such mixture. Contributions of the nitrate sources were estimated for the intersections of the denitrification trend with mixing lines between (1) mineral NO3 fertilizers and manure or sewage (implicating 0% contribution from reduced N fertilizers) and (2) mineral NO3 fertilizers and reduced N fertilizers (implicating 0% contribution from manure or sewage) (Fig. 1b and Table 4).

For samples 4,20,21 and 22 significant contributions from NO3 fertilizers, potentially originating from greenhouse discharges, were estimated, ranging between 13% (sample 22, MAP point 375250) and 76% (sample 20, MAP point 263100).

Sample 14 (MAP point 79670, sampled in February 2006) showed a similar elevated nitrate concentration compared to samples 15 and 16 (sampled in March and April) but a considerably higher $\delta^{18}O_{NO3}$ value. The estimated contribution from NO3 fertilizers in this sample ranged between 11 and 30%, which indicates a potential input from greenhouse discharges.

A third group of samples (3, 7, 13) was taken during summer period (May or June) and showed relatively low nitrate concentrations compared to other sampling dates (Table 3). Samples 7 and 13 were characterized by higher $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values compared to samples taken in March or April, while sample 3 only showed a higher $\delta^{18}O_{NO3}$ value.

The higher nitrate isotopic signatures combined with lower nitrate concentrations in samples 7 and 13 may indicate that the nitrate in these three MAP points had been subject to denitrification, which is more likely to occur in summer period than in winter period due to the higher ambient temperatures.

Contributions of the nitrate sources in potential mixtures before denitrification were estimated for the intersections of the denitrification trend with mixing lines between (1) mineral NO3 fertilizers and manure or sewage (implicating 0% contribution from reduced N fertilizers) and (2) mineral NO3 fertilizers and reduced N fertilizers (implicating 0% contribution from manure or sewage) (Fig. 1b and Table 4). The estimated nitrate contributions from mineral NO3 fertilizers then ranged between 23% (77% N fertilizers) and 40% (60% manure or sewage) for sample 7 and between 10% (90% N fertilizers) and 26% (74% manure or sewage) for sample 13.

The $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values in sample 3 could be interpreted in two ways. They could either indicate (1) denitrification of an original mixture of NO3 fertilizers with reduced N fertilizers, manure or sewage or a combination of these sources (Fig. 1b), or (2) indicate a mixture between mineral NO3 fertilizers and manure or sewage, diluted by rainwater with low nitrate concentration (Fig. 3). However, the elevated B concentration in this sample (Table 3) suggests that the second hypothesis is less likely, since the generally observed low B concentration in rainwater (reported values range from 1.5 to 7 $\mu g\ l^{-1}$) would also have resulted in a dilution of the B concentration. This hypothesis will be verified through additional $\delta^{11}B$ analysis on this samples, since B in rainwater is characterized by relatively high $\delta^{11}B$ values (Fig. 2 and 4). According to the denitrification trend, estimated nitrate contributions from mineral NO3 fertilizers ranged between 33% (67% reduced N fertilizers) and 50% (50% manure or sewage) in this sample (Fig. 1b and Table 4).

4. Conclusions

- $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values indicated that the elevated nitrate concentrations observed in MAP points 69000, 82870, 71200 and 85400 during winter/spring period (March and April) and in MAP points 79670 and 79700 during winter/spring (March and April) and summer period (May or June) could be attributed to excess animal manure application or domestic sewage discharges. Additional $\delta^{11}B$ analysis results combined with nitrate isotope data enabled to estimate relative contributions from manure and sewage using a 4 source isotope mixing model. Contributions from manure were estimated to range from 42% (MAP point 71200) up to 85% (MAP point 82870); contributions from sewage were estimated to range from 5% (MAP point 8287) up to 52% (MAP point 71200).
- $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values indicated that there were significant inputs from mineral NO3 fertilizers, potentially originating from greenhouse discharges, in MAP points 263100, 376220, 375250, 82870 (February and June), 69000 (May) and 79670 (February). The estimated contributions from NO3 fertilizers or greenhouse discharges ranged between 13% and 76%.
- $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values of samples taken in summer period in MAP points 69000, 82870, 71200 and 85400, which showed significantly lower NO3 concentrations than in winter period, indicated that the nitrate probably had been subject to denitrification. This suggests that the lower NO3 concentrations observed in summer period in these MAP points could partially be explained by natural nitrate attenuation through denitrification, next to reduced inputs from the assumed nitrate sources.
- For estimating the NO3 contributions originating from manure, reduced N fertilizers and soil N using the isotope mixing model, $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values were taken based on literature values. Determination of experimental values for nitrate originating from these sources would enable more reliable estimates of the source contributions.
- Large ranges of $\delta^{11}B$ values are reported in literature for mineral fertilizers, manure and domestic sewage. Determination of experimental $\delta^{11}B$ values for these sources in the study area is needed for more accurate estimation of nitrate source contributions using isotope mixing models.

5. Perspectives

- $\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$ and $\delta^{11}B$ analysis of samples taken during an entire monitoring year (e.g. bimonthly analysis) would enable to detect changes in function of time of nitrate source contributions and to estimate yearly averages of source contributions for individual MAP points. Upscaling of this isotope monitoring to a larger number of MAP points could enable to estimate nitrate source contributions on an annual basis in river sub-catchments or catchments. This new information, obtained through additional isotope analyses, could potentially be used to validate nutrient emission models (e.g. the SENTWA model used by VMM) which are used to simulate environmental effects of policy interventions to reduce nitrate pollution at different scales.

TABLES

Table 1. MAP identification number, location, sampling dates and suspected nitrate sources in the investigated MAP points

MAP number	Location	Sampling dates	Expected nitrate sources
79670	Hoogstraten	monthly from 02/2006 until 03/2007	manure
79700	Hoogstraten	monthly from 03/2006 until 03/2007	manure
71200	Wuustwezel	monthly from 03/2006 until 03/2007	manure, domestic sewage
85400	Ravels	monthly from 03/2006 until 03/2007	manure, domestic sewage
69000	Wuustwezel	monthly from 03/2006 until 03/2007	manure, greenhouse discharges
82870	Rijkevorsel	monthly from 02/2006 until 03/2007	manure, greenhouse discharges
375250	Sint Katelijne Waver	02/03/2007	manure, greenhouse discharges
263100	Sint Katelijne Waver	01/03/2007 and 03/04/2007	manure, greenhouse discharges
376250	Sint Katelijne Waver	05/03/2007	manure, greenhouse discharges

Table 2. Chemical and isotopic characterization of the nitrate source samples

Sample	Chemical composition			Isotopic composition			
	NO_3 (mg l^{-1})	NH_4 (mg l^{-1})	B (µg l^{-1})	$\delta^{15}N_{Total}$ (‰)	$\delta^{15}N_{NO3}$ (‰)	$\delta^{18}O_{NO3}$ (‰)	$\delta^{11}B$ (‰)
Pig manure 1	71	1430	1926	13.1	n.d.	n.d.	15.5
Pig manure 2	63	1330	663	12.7	n.d.	n.d.	n.d.
Cow manure 1	67	300	465	11.7	n.d.	n.d.	n.d.
Cow manure 2	26	423	870	13.1	n.d.	n.d.	n.d.
Greenhouse 1	1907	n.d.	1870	n.d.	2.9	22.1	0.7
Greenhouse 2	974	3.7	n.d.	n.d.	n.d.	n.d.	n.d.
KNO_3 fertilizer	n.d.	n.d.	<10[b]	n.d.	0.5	25.2	n.d.
$Ca(NO_3)_2$ fertilizer	n.d.	n.d.	<10[b]	n.d.	1.4	24.4	n.d.
Sewage 1	48.2	9.4	126	n.d.	11.3	9.4	7.2
Sewage 2	4.4	14.1	120	n.d.	n.d.	n.d.	n.d.
Sewage 3	11.3	1.3	180	n.d.	n.d.	n.d.	-1.5
Agricultural soil 1	n.d.	n.d.	n.d.	6.2	n.d.	n.d.	n.d.
Agricultural soil 2	2.1[a]	n.d.	n.d.	3.9	n.d.	n.d.	n.d.
Atmospheric deposition	2.1	n.d.	< d.l.[c]	n.d.	-4.0	52.1	n.d.

[a] nitrate concentration in a soil extract (extraction ratio 30 ml Milli-Q water/10 g dry soil)

[b] boron concentration in a saturated mineral fertilizer solution (250 and 1000 g l^{-1} Milli-Q water for KNO_3 and $Ca(NO_3)_2$, respectively)

[c] below detection limit

Table 3. Chemical and isotopic characterization of the surface water samples

Sample number	MAP number	Sampling date	Chemical composition		Isotopic composition		
			NO_3 (mg l^{-1})	B (µg l^{-1})	$\delta^{15}N_{NO3}$ (‰)	$\delta^{18}O_{NO3}$ (‰)	$\delta^{11}B$ (‰)
1	69000	02/03/2006	75	64	12.0	5.5	n.d.
2	69000	04/04/2006	58	69	12.2	6.4	16.4
3	69000	03/05/2006	18	105	10.1	15.2	n.d.
4	82870	06/02/2006	50	n.d.	8.3	22.1	n.d.
5	82870	06/03/2006	103	65	12.4	6.5	n.d.
6	82870	05/04/2006	100	85	12.5	6.3	21.3
7	82870	07/06/2006	23	93	18.7	17.5	n.d.
8	71200	02/03/06	71	75	14.1	7.7	n.d.
9	71200	04/04/06	57	117	14.5	7.4	14.5
10	71200	03/05/06	37	n.d.	17.5	9.2	n.d.
11	85400	08/03/2006	59	54	11.6	6.8	n.d.
12	85400	10/04/2006	45	60	13.5	7.7	19.7
13	85400	10/05/2006	12	63	16.5	12.2	19.2
14	79670	06/02/2006	75	n.d.	9.8	10.3	n.d.
15	79670	06/03/2006	99	67	10.2	5.8	19.7
16	79670	05/04/2006	77	n.d.	10.5	4.6	n.d.
17	79670	07/06/2006	79	n.d.	14.6	7.5	n.d.
18	79700	06/03/2006	101	69	11.0	4.7	17.3
19	79700	08/05/2006	49	n.d.	13.1	5.0	n.d.
20	263100	03/04/2007	161	420	9.6	22.9	4.9
21	376220	02/03/2007	80	n.d.	6.9	17.6	n.d.
22	375250	02/03/2007	44	n.d.	12.1	12.2	n.d.
23	Non-contaminated	15/06/2006	1.7	18	n.d.	n.d.	19.8

Table 4. Estimation of the contribution (% of total nitrate) from nitrate pollution sources in surface water samples based on nitrate N and O isotope data using a three source mixing model, and based on B and nitrate N and O isotope data using a four source mixing model (uncertainties on the estimates using the four source mixing model are also indicated).

Sample number	MAP number	Sampling date	3 source mixing model			4 source mixing model							
			manure or sewage	NO3 fertilizers	Reduced N fertilizers	manure		sewage		NO3 fertilizers		atmospheric deposition	
			Contr. (%)			Contr. (%)	*Unc. (%)*	Contr. (%)	*Unc. (%)*	Contr. (%)	*Unc. (%)*	Contr. (%)	*Unc. (%)*
1	69000	02/03/2006	91	9	0								
2	69000	04/04/2006	42-87	0-13	0-58	56	*16*	38	*26*	1	*15*	5.7	*3.8*
3	69000	03/05/2006	0-50	33-50	0-67								
4	82870	06/02/2006	0-26	67-74	0-33								
5	82870	06/03/2006	46-88	0-12	0-54								
6	82870	05/04/2006	46-88	0-12	0-54	85	*22*	5	*34*	4	*19*	5.6	*5.2*
7	82870	07/06/2006	0-60	23-40	0-77								
8	71200	02/03/06	42-86	0-14	0-58								
9	71200	04/04/06	44-84	0-16	0-58	42	*22*	52	*56*	0	*39*	5.5	*6*
10	71200	03/05/06	42-86	0-14	0-58								
11	85400	08/03/2006	43-85	0-15	0-58								
12	85400	10/04/2006	43-85	0-15	0-58	72	*22*	17	*47*	3	*31*	7	*5.1*
13	85400	10/05/2006	0-74	10-26	0-90								
14	79670	06/02/2006	0-70	11-30	0-89								
15	79670	06/03/2006	78	13	9	72	*18*	19	*26*	3	*13*	6.6	*3.9*
16	79670	05/04/2006	80	7	12								
17	79670	07/06/2006	43-72	0-28	0-58								
18	79700	06/03/2006	84	8	8	59	*16*	37	*23*	0	*11*	4.1	*3.5*
19	79700	08/05/2006	73-93	0-7	0-27								
20	263100	03/04/2007	0-24	68-76	0-32								
21	376220	02/03/2007	0-37	52-63	0-48								
22	375250	02/03/2007	0-66	13-34	0-87								

FIGURES

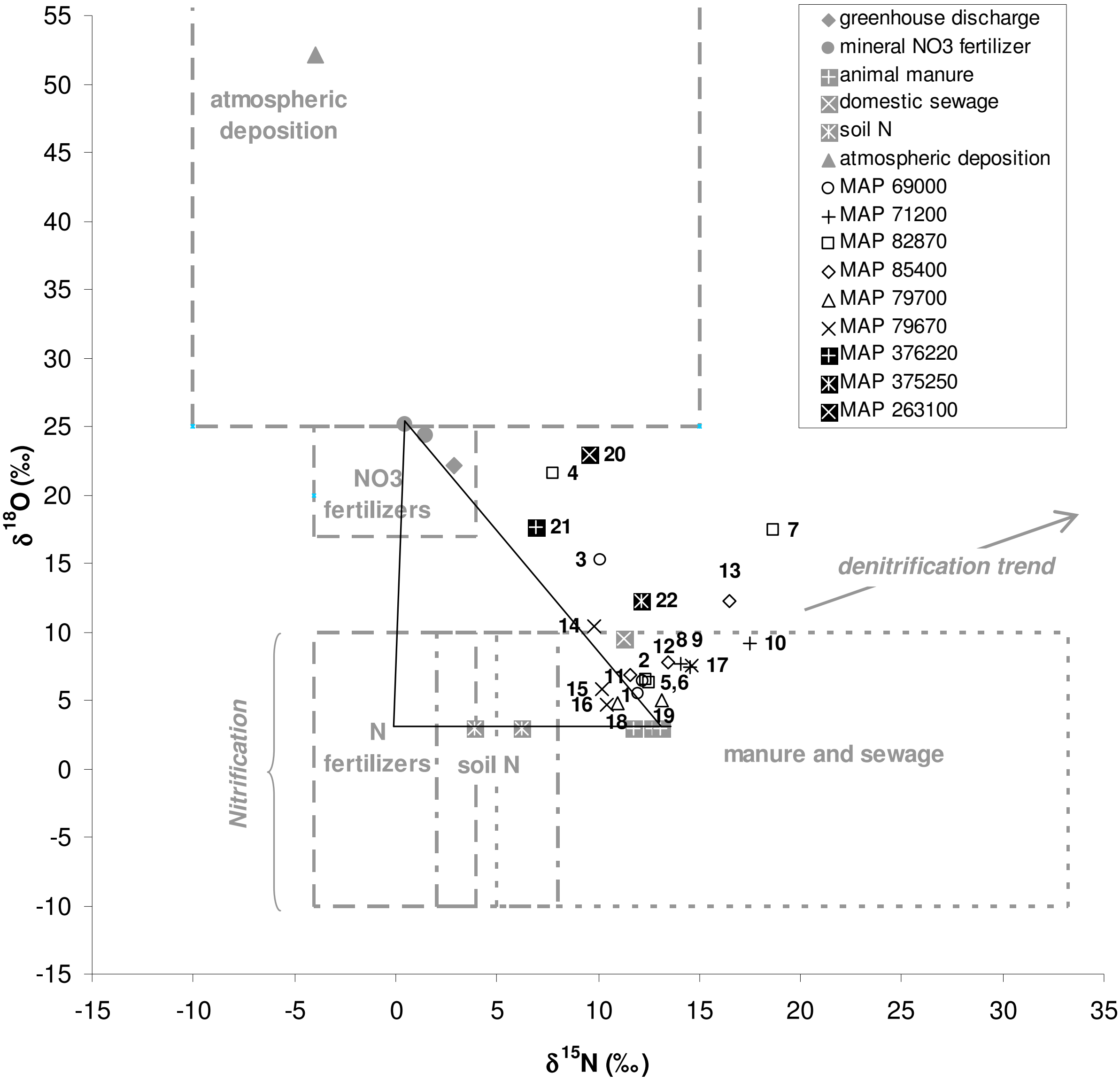

Figure 1a. $\delta^{18}O_{NO3}$ **versus** $\delta^{15}N_{NO3}$ **values of nitrate originating from different nitrate sources and in surface water samples from MAP points. The range of values reported in literature for nitrate sources is indicated by grey rectangles, experimental values for nitrate sources and surface water samples are indicated by grey and black symbols, respectively. Numbers indicate the surface water sample numbers as defined in Table 3. Black lines indicate mixing between NO3 fertilizer, reduced N fertilizer and manure or sewage end members.**

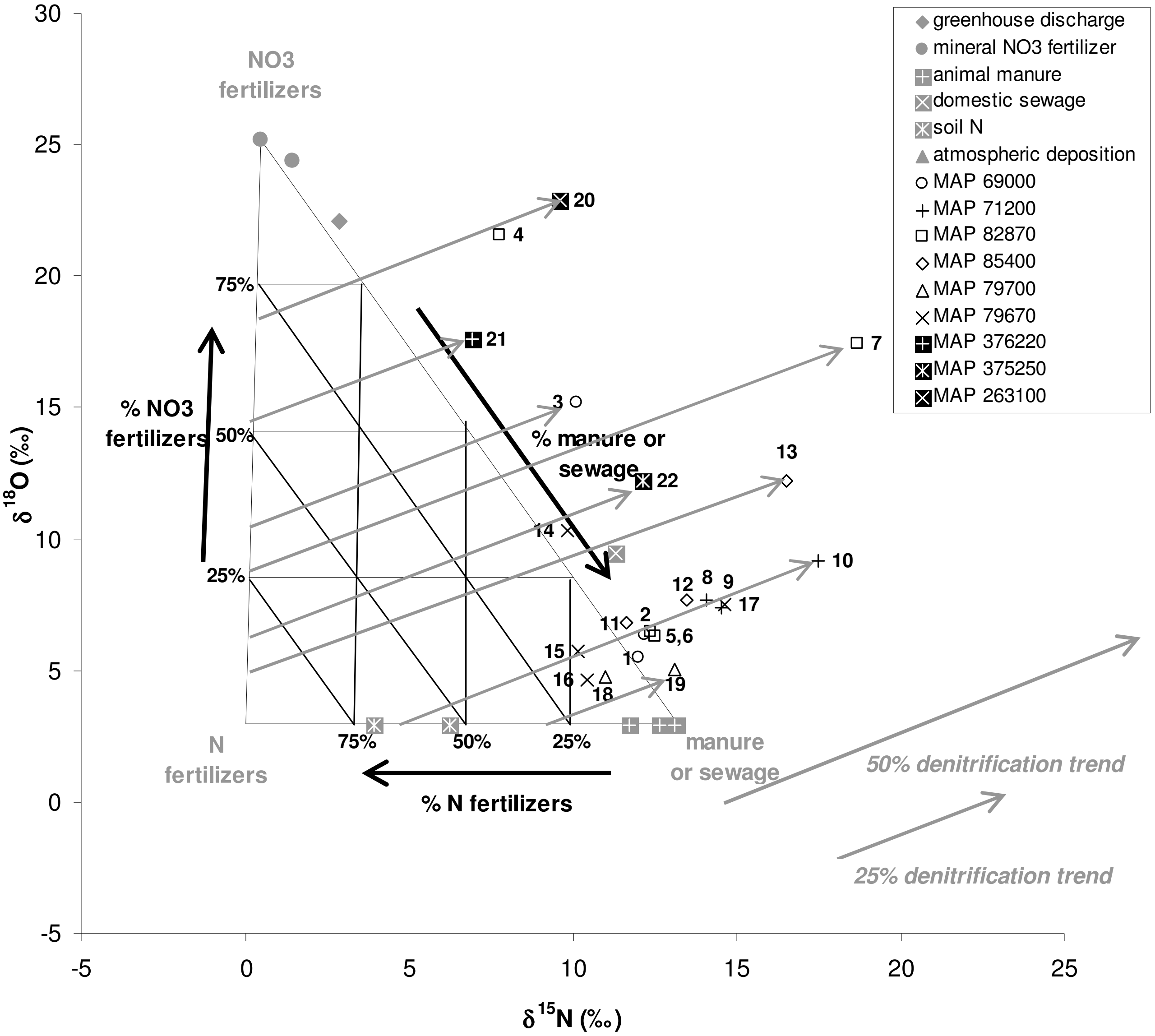

Figure 1b. $\delta^{18}O_{NO3}$ versus $\delta^{15}N_{NO3}$ values of nitrate originating from different nitrate sources and in surface water samples from MAP points. Black lines indicate the mixing diagram between NO3 fertilizer, reduced N fertilizer and manure or sewage end members. Grey arrows indicate denitrification trends and potential isotopic compositions before denitrification for the surface water samples.

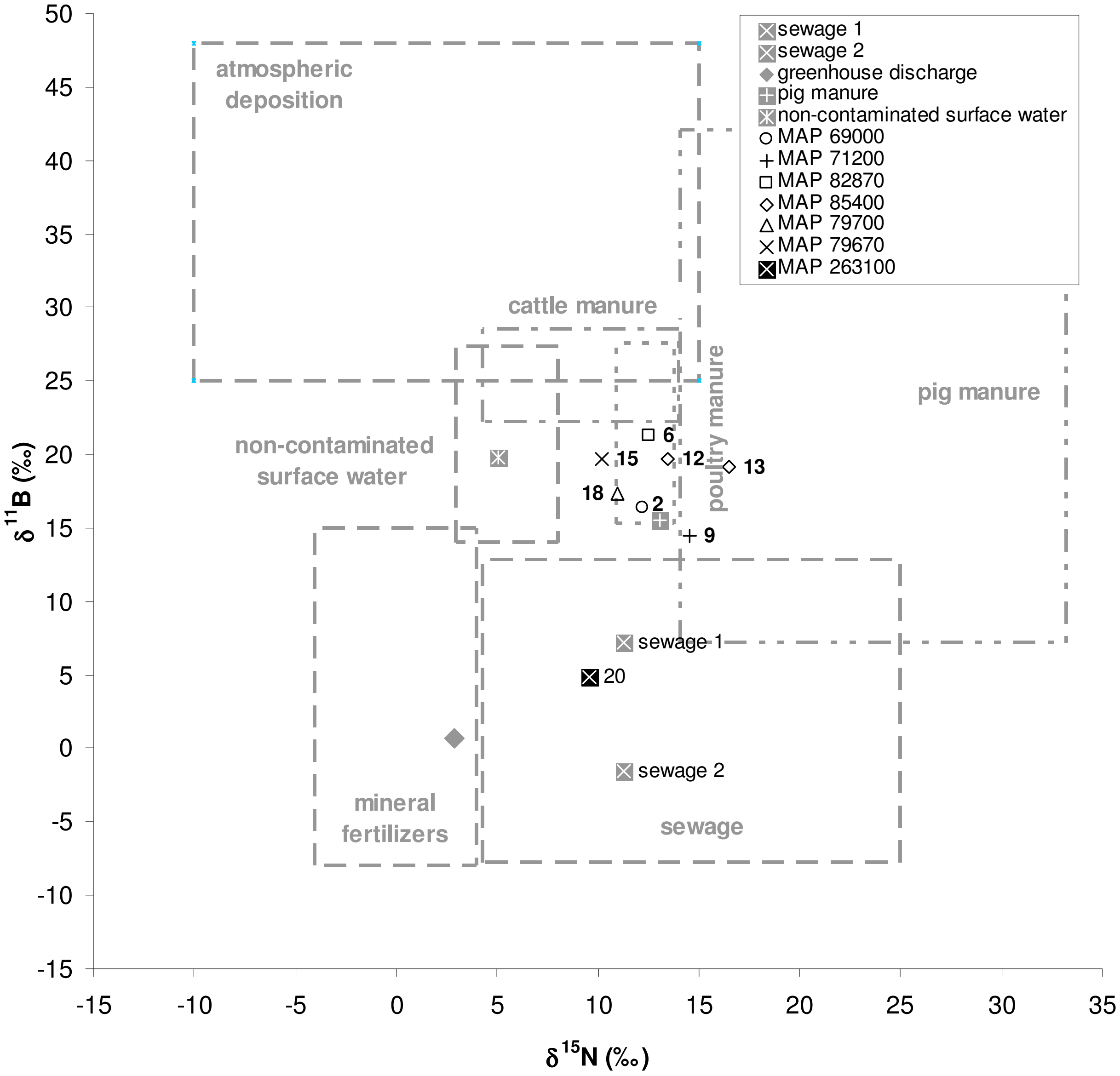

Figure 2. δ^{11}B versus $\delta^{15}N_{NO3}$ values of nitrate sources and selected surface water samples from MAP points. The range of values reported in literature for nitrate sources is indicated by grey rectangles, experimental values for nitrate sources and surface water samples are indicated by grey and black symbols, respectively.

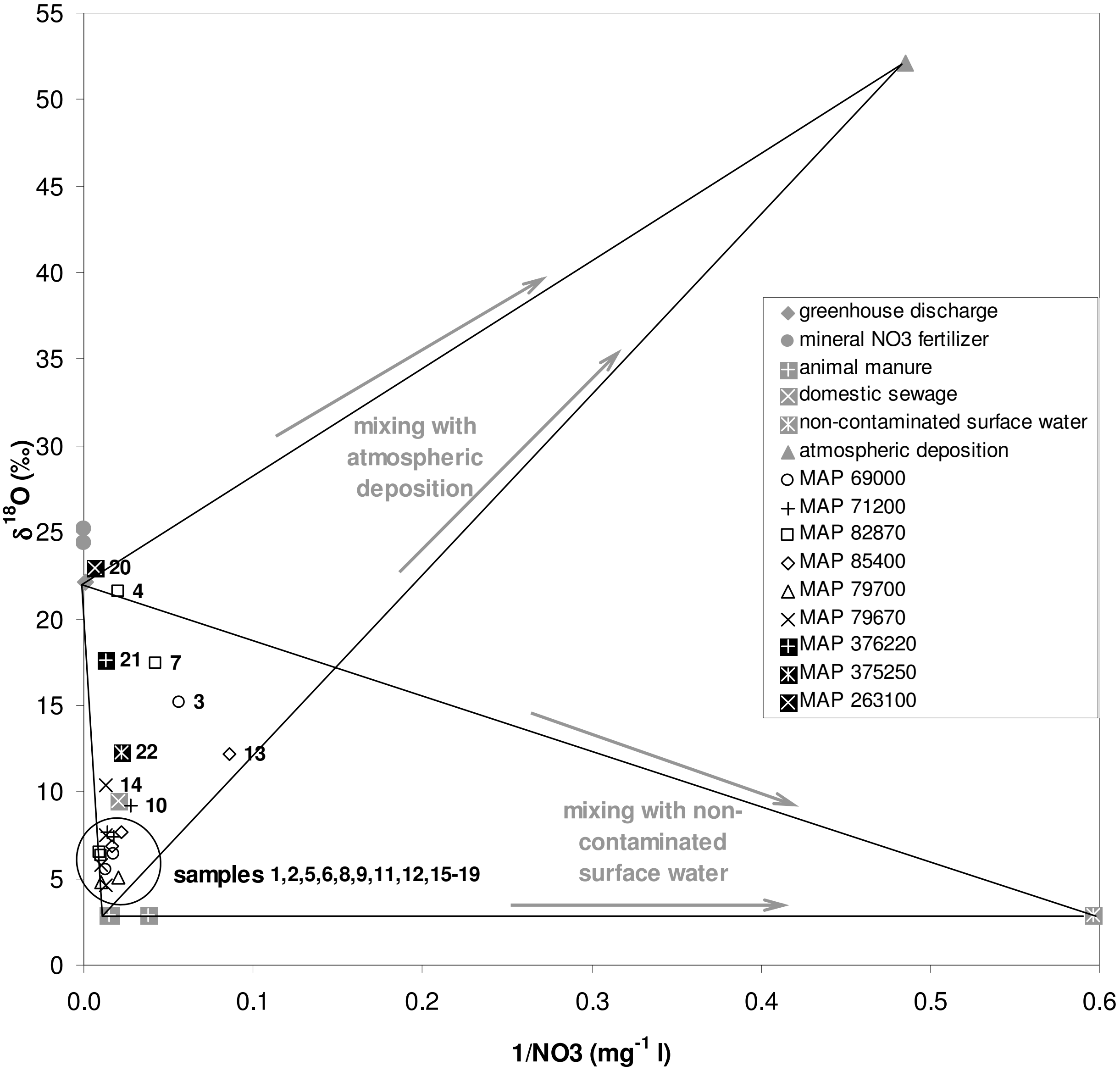

Figure 3. $\delta^{18}O$ **values versus the inverse nitrate concentrations in nitrate source samples (grey symbols) and in surface water samples (black symbols). Black lines indicate mixing between greenhouse discharge and animal manure end members with either atmospheric deposition or non-contaminated surface water end members.**

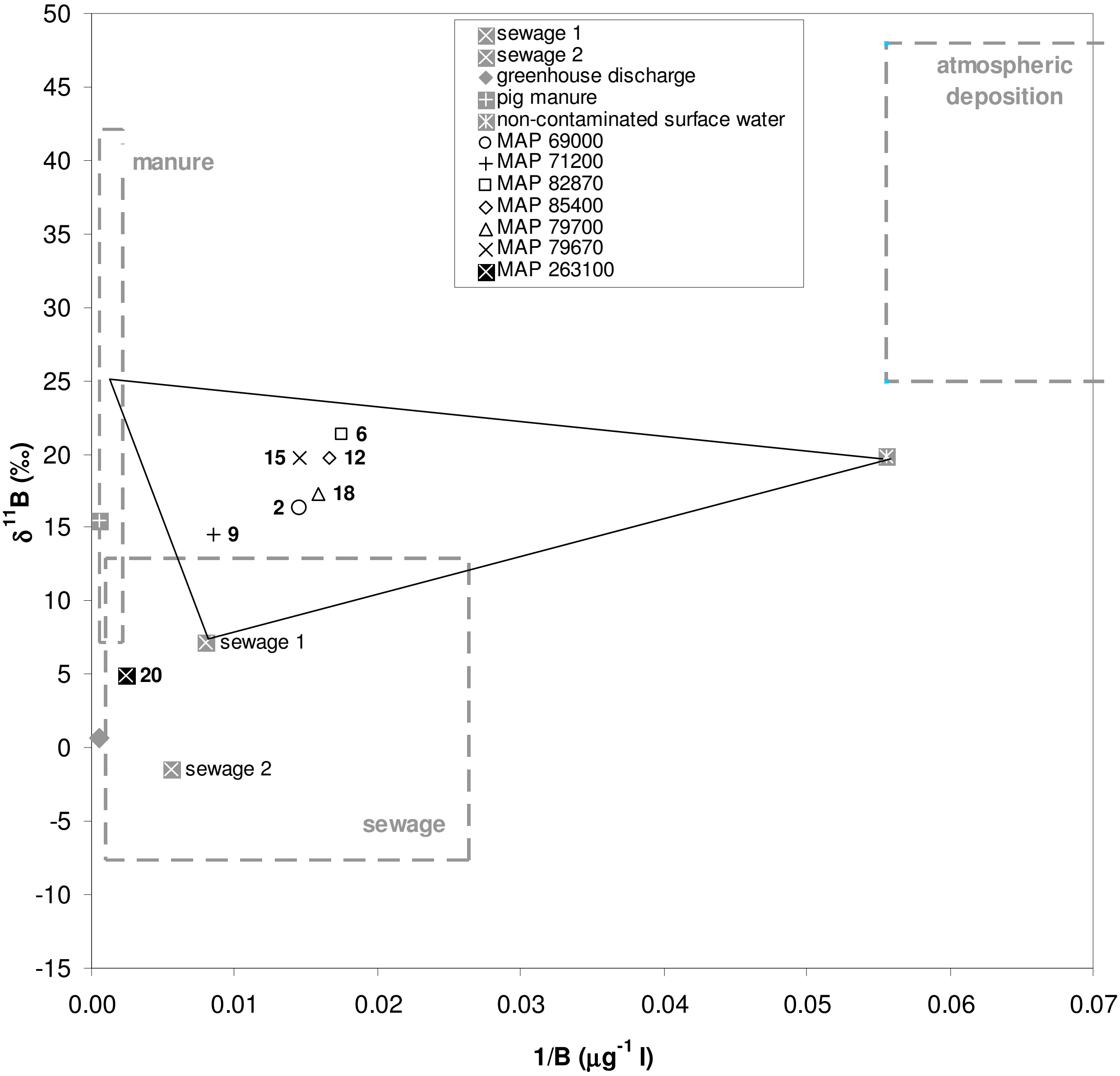

Figure 4. δ11B values versus the inverse boron concentrations in nitrate source samples (grey symbols) and in selected surface water samples (black symbols). The range of values reported in literature for nitrate sources is indicated by grey rectangles. Black lines indicate mixing between domestic sewage and non-contaminated surface water with a hypothetical animal manure end member.

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Abstract

Excess application to fields of animal manure or mineral fertilizers is generally assumed to be the major source of nitrate pollution in surface and groundwater in agricultural areas in Flanders (Belgium). The Flemish Environment Agency (VMM) suspects, but could not prove, that discharges of untreated domestic sewage or discharges containing mineral fertilizers from hydroculture in greenhouses are also contributing to the nitrate pollution observed in some of the agricultural areas. The objective of this study was to investigate, using stable isotope data of dissolved nitrate (δ^{15}N and δ^{18}O) and boron (δ^{11}B), if these suspected sources significantly contribute to the nitrate pollution observed in selected surface water monitoring points in Flanders. Based on our nitrate and boron isotope results, nitrate input from domestic sewage was estimated to range between 5 and 50% in the investigated sampling points. Our nitrate isotope results confirmed significant inputs from mineral nitrate fertilizers, potentially originating from greenhouse discharges, in areas with intensive greenhouse cropping. Nitrate isotope data also indicated the potential occurrence of natural nitrate attenuation through denitrification, which could partially explain lower nitrate concentrations generally observed in summer period.

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