Hydrochemical and dual-isotope approach to the identification of denitrification in arable field drainage in the Wensum catchment, eastern England

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ABSTRACT

The global pool of reactive nitrogen has doubled in the last century in response to the need to increase food production with the consequent increase in fertiliser-derived reactive nitrogen detrimentally affecting aquatic ecosystems. This study investigates the spatial distribution and significance of denitrification in the lowland, agriculturally-impacted River Wensum catchment in eastern England as a natural attenuation process. To investigate the evidence for denitrification, the hydrochemical characteristics and dual stable isotope composition of nitrate (^{15}N and ^{18}O) were measured over a 15-month period, 2015–2017, in 63 samples of field drainage in predominantly clay loam and sandy clay loam soils under mainly arable cultivation. Microbially-mediated denitrification in field drainage was indicated by the gradient of the linear regression of ^{15}N$_{NO3}$ and ^{18}O$_{NO3}$ compositions with a value of 0.58. Dual fractionation of the nitrate isotopes yielded enrichment factors for δ^{15}N$_{NO3}$ (−4.52‰) and δ^{18}O$_{NO3}$, (−4.51‰) within the reported ranges for denitrification in aquatic studies. Soil type influenced denitrification, with a positive relationship between percentage clay and δ^{15}N$_{NO3}$ and δ^{18}O$_{NO3}$ values. The same relationship was observed for denitrification rates calculated via a simple mass balance approach, which ranged from 11.0 to 26.3 kg N ha^{-1} and accounted for 30–73% of the leached soil nitrogen. Higher denitrification rates were recorded in drainage areas with a greater soil clay content (>20% by weight). Comparing calculated dentification rates for individual drain areas with median δ^{15}N$_{NO3}$ values of drain samples demonstrated that an isotopic enrichment of +1‰ is associated with a denitrification rate of 2.6 kg N ha^{-1}. In conclusion, sustainable agricultural practices that maintain natural attenuation processes such as denitrification, for example by preserving and increasing the soil organic carbon content, are desirable to improve overall soil health to support ecosystem services that reduce nitrate pollution.

1. Introduction

The global cycling of nitrogen has doubled over the last century with an estimated 210 Tg N a^{-1} from all anthropogenic sources and 203 Tg N a^{-1} from combined natural sources (Galloway et al., 2003; Fowler et al., 2013), equating to 1.04 Tg N a^{-1} being produced synthetically for every 1 Tg N a^{-1} fixed naturally. This dramatic increase in global nitrogen production and use has vastly improved global food security but not without detrimental impacts on greenhouse gas emissions (Gao and Cabrera Serrenho, 2023), human health (Wolfe and Patz, 2002; Patel et al., 2022) and the environment, causing fluctuations in forest and grassland productivity and the acidification of lakes and streams (Aber et al., 1995; Kopáček et al., 2013; Webster et al., 2021; Yuan et al.,

2023). Furthermore, elevated levels of reactive nitrogen cause eutrophication, hypoxia events, loss of biodiversity and habitat degradation in freshwater and coastal ecosystems (Steffen et al., 2015; Wurtsbaugh et al., 2019; Sekar et al. 2022; Zhang et al., 2022). Therefore, the control of nitrate contamination in surface and subsurface water is vital for mitigating downstream water quality impairment (Cooper and Hiscock, 2023; Husic et al., 2023), for example through the adoption and implementation of the EU Nitrates Directive (Council of the European Communities, 1991) and EU Water Framework Directive (Council of the European Communities, 2000), and in reducing the substantial economic cost of diffuse pollution impacts (Sutton et al., 2011, 2021; Garcia-Hernandez et al., 2022).

Even though there has been a significant perturbation of the global

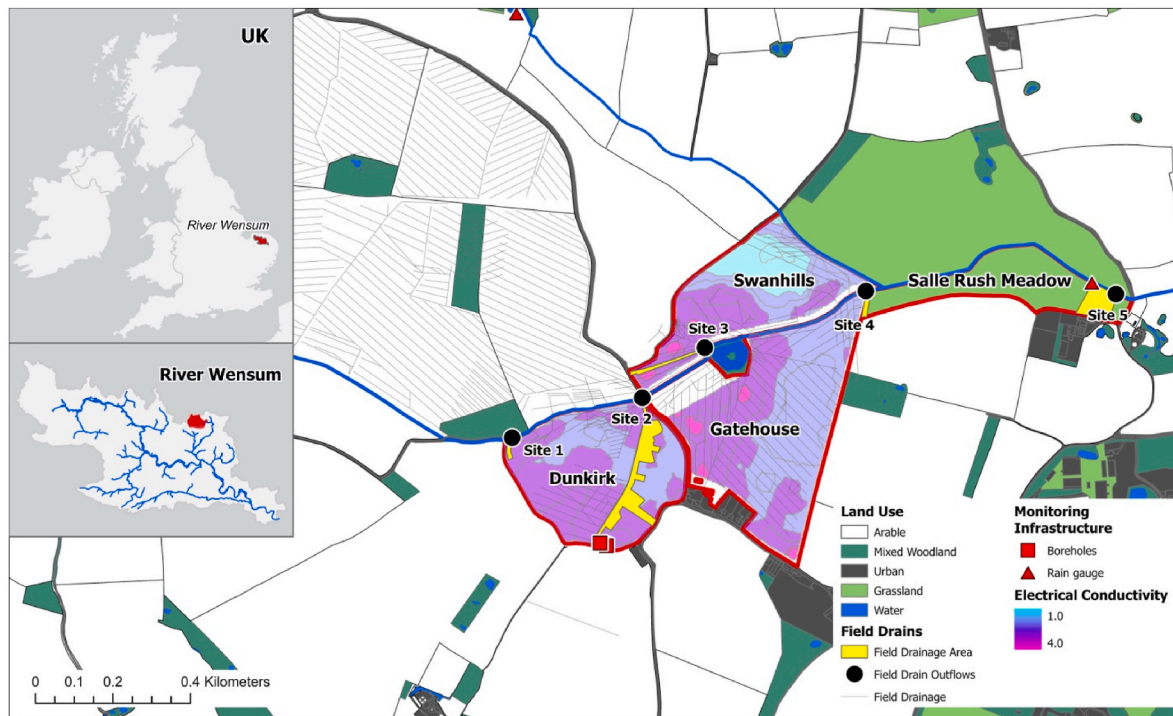**Fig. 1.** Location of the Blackwater sub-catchment study area in the River Wensum showing the position of field drain sampling sites. Sites 1 and 2 are the Dunkirk field drains (D3R and D1R, respectively), Site 3 the Swanhills field drain (D4L), Site 4 the Gatehouse field drain (D5R) and Site 5 the Salle Rush Meadow field drain. Also shown are the arable field drain network and the locations of a tipping-bucket rain gauge adjacent to Site 5 and a shallow observation borehole (MLBH4) adjacent to the southern boundary of Dunkirk field. Blue to pink background shading in the arable study fields shows the spatial distribution of relative soil electrical conductivity as a measure of increasing clay content in the 0–40 cm soil profile.

nitrogen cycle, Green et al. (2004) estimated that the mean global export of terrestrial nitrogen to rivers was just 18%, suggesting that river systems have the potential for high rates of nitrogen transformation, storage and removal through, for example, denitrification. The natural attenuation of nitrogen is a key stage of the nitrogen cycle in terrestrial and aquatic ecosystems through the reduction of nitrate (NO_3^-) to gaseous nitrogen species (NO, N_2O, N_2).

Denitrification represents an important loss process from many agricultural systems given that nitrogen is a major component in crop production (Tiedje, 1988; Gu et al., 2023). Nitrogen compounds in inorganic and organic fertilisers are oxidised in soils to nitrate, a highly soluble and therefore mobile form of nitrogen. Surface water nitrogen is then leached below the root zone and into groundwater (and eventually discharged into surface water bodies) or is incorporated into surface water directly by runoff. In areas where the water table is shallow and the underlying aquifer is unconfined, this infiltration of nitrogen to groundwater is of equal concern (Wang et al., 2016; Ascott et al., 2017; Burri et al., 2019).

Rates of soil nitrate production from fertiliser-derived ammonium and subsequent removal of nitrate by denitrification are influenced by the availability of an electron donor and acceptor (typically carbon and nitrate, respectively) (Boulton et al., 1998; Rivett et al., 2008; Wrage-Mönnig et al., 2018). Given that denitrification is an anaerobic process, oxygen availability is an important factor (Hofstra and Bouwman, 2005). The nitrogen cycle is intrinsically linked to climate and land management practices, which in turn govern soil conditions (Tiedje, 1988; McClain et al., 2003). Soil pH affects rates of denitrification, with more acidic conditions typically inhibiting denitrification and, as with many biological processes, an optimum temperature range exists (Šimek et al., 2000). Soil type is also an important factor in regulating rates of nitrogen cycling. For example, rates of nitrification are linked to the availability of ammonium, which is itself affected by the cation exchange capacity of soils. Also, crops such as legumes and clover are

capable of fixing nitrogen in the soil (Hofstra and Bouwman, 2005).

In this study, the dual stable isotopes of nitrate (^{15}N and ^{18}O), through the measurement of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$, provide a valuable tool in contributing understanding to agricultural nitrogen cycling. The application of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values to determine the source of nitrate within a system relies on the level of isotopic fractionation during its production and cycling, under the assumption that no subsequent biogeochemical cycling occurs that might alter the original isotopic composition of the source material. Including the measurement of $\delta^{18}O_{NO3}$ provides an additional level of investigation of biogeochemical processes that are not captured solely using nitrogen isotopes (Sigman et al., 2005). Some processes affect ^{15}N in the same way, and hence are associated with the same range or fractionation of ^{15}N, overlapping when the sources of nitrogen share a similar isotopic range. Hence, measurement of both $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values allows for the separation of such processes (Kellman and Hillaire-Marcel, 2003). While some processes may overlap in terms of their impact on $\delta^{15}N_{NO3}$ values, rendering them indistinguishable from one another without the measurement of $\delta^{18}O_{NO3}$ values, the same can also apply to sources of nitrate.

The aim of the research presented here is to use the dual stable isotopes of nitrate to identify the presence and significance of microbially-mediated denitrification within field drainage in a lowland arable catchment and to examine the influence of soil characteristics on the rates of denitrification. The quantification of field-scale denitrification in respect of occurrence and rates is challenging and is identified as an area for further study (Rivett et al., 2008).

Artificial drainage of soils using subsurface field drains (tiles) is a common practice on many agricultural fields and acts to discharge water rapidly to streams, bypassing the groundwater system (Spalding and Exner, 1993). High concentrations of nitrate are often released from these systems and so knowledge of the sources and processes controlling nitrogen losses in under-drained soils is necessary to inform farm

Table 1

Soil chemical characteristics at 0–30 cm and particle size distribution at 0−30, 0−60 and 0−90 cm depth for fields associated with sampled field drains. Soil major ion concentrations in mg kg^{-1}. Sand, silt and clay fractions as weight percentages from Hama-Aziz (2016).

Field	Sample	P	K	Mg	NO$_3$	NH$_4$	0−30 cm			30−60 cm			60−90 cm		
							Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
Dunkirk	D1	12.0	73.8	57.4	47.73	0.80	61	22	17	46	31	23	53	20	27
	D2	12.0	72.8	48.2	24.00	1.51	59	24	17	47	36	17	35	23	42
	D3	32.6	173.0	76.4	70.28	0.94	62	22	16	43	25	32	35	23	42
	D4	25.6	57.1	42.8	38.14	0.95	63	21	16	55	30	15	24	27	20
Swanhills	SW1	30.80	109.0	37.7	156.91	12.80	67	20	13	75	14	7	92	4	4
	SW2	21.20	92.3	55.5	171.50	11.80	66	20	14	55	29	16	45	33	22
	SW3	21.40	108.0	55.0	97.24	0.73	59	24	17	45	34	21	34	29	37
	SW4	12.40	94.0	58.0	127.30	1.17	70	20	10	54	32	14	72	16	12
Gatehouse	GH1	51.4	105.0	50.1	17.92	0.90	63	22	15	53	30	17	42	31	27
	GH2	27.0	86.9	58.1	108.29	1.61	61	23	16	43	33	24	38	25	37
	GH3	40.0	148.0	51.1	123.76	1.26	55	23	22	41	24	35	31	24	45
	GH4	20.80	129.0	44.4	160.88	3.67	65	23	12	66	25	9	75	17	8

management practices. The results of this study add to existing literature regarding natural nitrogen attenuation in an intensive arable setting, an important consideration of any catchment nitrogen budget.

2. Study area and experimental methods

2.1. Study area

This study focuses on the commercial Salle Park Estate located within the headwaters of the Blackwater sub-catchment of the lowland calcareous River Wensum, Norfolk, UK (52°47′09″N, 01°07′00″E) (Fig. 1). The area experiences a temperate maritime climate, with a mean annual temperature of 10.5 °C and a mean annual precipitation total of 684 mm (1991–2020) (Meteorological Office, 2023). The Estate is situated 40–50 m above Ordnance Datum (OD) (sea level) with gentle slopes (<1°) meaning that subsurface leaching rather than surface runoff is the dominant pollution pathway. Intensive arable cropping comprises 79% of the land use and is managed with a seven-year rotation of winter wheat, winter and spring barley, winter oilseed rape, spring beans and sugar beet. The Estate also includes 15% improved grassland, 5% mixed woodland and 1% rural settlements.

The bedrock geology is Cretaceous Chalk (>20 m) overlain by Mid-Pleistocene chalky, flint-rich, argillaceous glacial tills of the Sheringham Cliffs Formation (Bacton Green Till Member; 0.2–7 m depth) and Lowestoft Formation (Lowestoft Till Member; 8–16 m depth), with interdigitated bands of glaciofluvial and glaciolacustrine sands and gravels (Lewis, 2011; Cooper et al., 2018). Soils developed on the glacial deposits are predominantly clay loam to sandy clay loam (<0.5 m depth) of the argillic brown earths (Freckenham series) and stagnogley (Beccles series) groups which, together with the argillaceous tills, result in moderately impeded drainage conditions. Soil organic carbon (SOC) content within the study area is widely depleted due to long-term agricultural cultivation. SOC contents in the range of 1.06–2.25% by weight were recorded in the top 0–30 cm (Hama-Aziz, 2016), values at or below the minimum 2% by weight threshold associated with healthy soils (Loveland and Webb, 2003).

Surface water channels draining the sub-catchment have been extensively deepened and straightened to reduce water residence times resulting in the disconnection of water courses from the floodplain. The more clay-rich areas of the sub-catchment are extensively under-drained by a dense network of subsurface agricultural field drains installed at a depth of 100–160 cm (Fig. 1). Measured drain discharges are typically less than 0.2 L s^{-1}, although can be as high as 10 L s^{-1} during the winter and dry up entirely during the summer (June–September), with discharge also varying greatly depending upon depth, catchment area and antecedent moisture conditions (Hama-Aziz et al., 2017). Outram et al. (2016) reported that the highest daily discharge volumes and

solute fluxes occur when shallow groundwater levels are within 0.6 m of the ground surface when the catchment exhibits a strong response to precipitation events.

2.2. Experimental design

Within the study area, five sampling sites (1−5) were located along a 1.6 km stream reach (Fig. 1). At each sampling site, samples from a field drain were collected for major and minor ions and nitrogen stable isotope analysis to investigate evidence for microbially-mediated denitrification. The field drains are considered to represent soil processes in terms of transformations and attenuation of nitrogen in the soil matrix. The field drains sampled at Sites 1 and 2 under-drain the Dunkirk field, at Site 3 the Swanhills field and at Site 4 the Gatehouse field. The field drain at Site 5 under-drains Salle Rush Meadow that is not suitable for arable cropping and is managed as grazing grass.

The selection criteria for the Dunkirk, Gatehouse and Swanhills fields under arable cultivation were based on consideration of differences in soil type, tillage regime and fertiliser application history. The soil particle size distribution in these fields, from ground level to 90 cm depth, is heterogeneous in nature, typical for soils developed on glacial tills, and provided the opportunity to examine denitrification in soils with varying texture. Tillage regime also varied between the study fields. The Dunkirk and Gatehouse fields were under a reduced (non-inversion) tillage regime, while the Swanhills field was managed by a direct-drill (no-till) method. Selecting fields under different tillage regimes enabled a comparison of the effect of cultivation method on soil denitrification potential. In general, each of the arable fields received a comparable annual application of nitrogen fertiliser (~210–245 kg ha^{-1}) during the study period. Therefore, given the differences in soil type and tillage regime, nitrogen fertiliser applications were not considered to be a significant variable when comparing the arable fields in terms of soil denitrification rates.

2.3. Field methods

Soil chemical and physical characteristics were recorded as part of a wider study into the efficacy of in-field measures such as cover cropping and reduced tillage methods to limit nutrient losses from arable agriculture (Cooper et al., 2017, 2020). Data for the arable fields Dunkirk, Gatehouse and Swanhills are included in Table 1. In addition, the Salle Park Estate commissioned shallow (0–40 cm) and deep (0–120 cm) soil profile electrical conductivity surveys. Variations in electrical conductivity measured using a towed, trailer-mounted electromagnetic instrument are caused principally by soil type and water holding capacity but stone content, soil depth and compaction will also have an effect (SOYL, 2023). The results of the shallow profile survey are shown in Fig. 1.

At monthly intervals between November 2015 and January 2017, covering two winter recharge periods, a 1 L grab sample was collected from each of the five field drain outflows in 1 L polypropylene bottles, yielding 63 samples in total for analysis. Field measurements of water temperature, dissolved oxygen (DO), pH and electrical conductivity of field drain outflows were taken using a Hanna HI9025 pH meter, Hanna HI9146 DO meter and a Fisher Scientific Accumet AP75 electrical conductivity meter. Field drain discharge was also recorded using a graduated bucket and stopwatch.

In addition to field drain measurements, precipitation with a 15-min resolution was recorded with a Casella tipping-bucket rain gauge located adjacent to Site 5, and shallow groundwater levels were monitored at Merrison's Lane borehole 4 (MLBH4). Observation borehole 4 was drilled to a depth of 3.6 m in glacial till deposits and completed with 50-mm diameter casing, slotted from 1.0 to 3.6 m. The top elevation of the borehole is 42.98 m OD. The borehole was equipped with a pressure transducer (Mini-Diver, Schlumberger), which recorded pressure at 15-min resolution, barometrically compensated by linear interpolation using a barometer located in the borehole.

2.4. Laboratory methods

The laboratory methods employed in the Science Analytical Facility at the University of East Anglia for major and minor ions, dissolved organic carbon (DOC) and stable isotopes of nitrate, together with the limits of detection and precision used to report water analyses, are described in the accompanying Supplementary Information. In brief, concentrations of anions (NO_3^-, NO_2^-, Cl^-, SO_4^{2-}) were measured by liquid chromatography using a Dionex ICS 2000. Cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) were measured by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) using a Varian Vista Pro axial ICP-OES. Sample volumes prevented direct measurement of HCO_3^- and so concentrations were calculated by ionic balance using the hydrochemical modelling software PHREEQC (Parkhurst and Appelo, 2013).

Ammonium concentrations were measured by liquid chromatography using a Dionex ICS 5000. DOC concentrations were measured by high temperature combustion using a Skalar Formacs HT TOC/TN analyser equipped with a LAS-160 sampler and non-dispersive infrared (NDIR) detector.

The measurement of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values followed the well-established denitrifier method (Sigman et al., 2001; Casciotti et al., 2002). The delta notation (δ) is used to express the ratio of heavy to light isotopes relative to the international standards (AIR for nitrogen and Vienna Standard Mean Ocean Water (V-SMOW) for oxygen), calculated as:

$$\delta(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \qquad \text{Equation 1}$$

where R_{sample} and $R_{standard}$ represent the ratio of heavy to light isotopes (for example, $^{15}N/^{14}N$) in the sample and standard, respectively (Kendall, 1998).

2.5. Data analysis

The sources of nitrate in surface water and groundwater bodies typically have a $\delta^{15}N$ composition of −10 to ~ +30‰, with denitrification capable of causing further enrichment (Kendall et al., 2007). There are comparatively fewer studies that report $\delta^{18}O_{NO3}$ values of nitrate sources and so the ranges are less constrained. Fig. S1 in the Supplementary Information shows the typical sources of nitrate for an agricultural system demonstrating that, in many cases, a single isotopic value (for example, just ^{15}N) is insufficient to separate sources.

To analyse stable isotope fractionation following a Rayleigh-type process, the Rayleigh equation relates the isotopic enrichment with the nitrate concentration value by an enrichment factor, as follows:

$$\delta_s = \delta_{so} + \varepsilon \ln(C/C_o) \qquad \text{Equation 2}$$

where δ_s is the measured $\delta^{15}N_{NO3}$ or $\delta^{18}O_{NO3}$ value (‰), δ_{so} is the $\delta^{15}N_{NO3}$ or $\delta^{18}O_{NO3}$ value before denitrification (‰), ε is the isotopic enrichment factor (‰), C is the nitrate concentration of the denitrified water (mg L^{-1}) and C_o is the nitrate concentration of the original water (mg L^{-1}). Equation (2) can be rearranged as:

$$\delta_s = \varepsilon \ln C + (\delta_{so} - \varepsilon \ln C_o) \qquad \text{Equation 3}$$

A plot of δ_s versus ln$C$ yields a straight regression line, the slope of which has a value equal to the enrichment factor, ε.

To estimate the amount of denitrification occurring in the soil zone, a simple mass balance was employed based on the difference between the mass of nitrogen applied to a field drainage area and the mass of dissolved nitrogen exported in the field drain. From available farm data for the year 2015/16 when the study fields were under winter barley feed, 200 kg ha^{-1} of ammonium nitrate fertiliser (equivalent to 70 kg N ha^{-1}) were applied to each of the study fields on three occasions in February, March and April 2016, plus a further application of 100 kg ha^{-1} (equivalent to 35 kg N ha^{-1}) to the Swanhills field in May 2016. No further applications were made during the remainder of the year. The crop-specific amount of nitrogen leached equates to the amount of nitrogen fertiliser applied (210 or 245 kg N ha^{-1}) multiplied by a soil leaching coefficient (0.20) derived from published studies in areas of England with similar physical conditions such as soil type, topography and nutrient management practice (Johnes, 1996; Zhang and Hiscock,
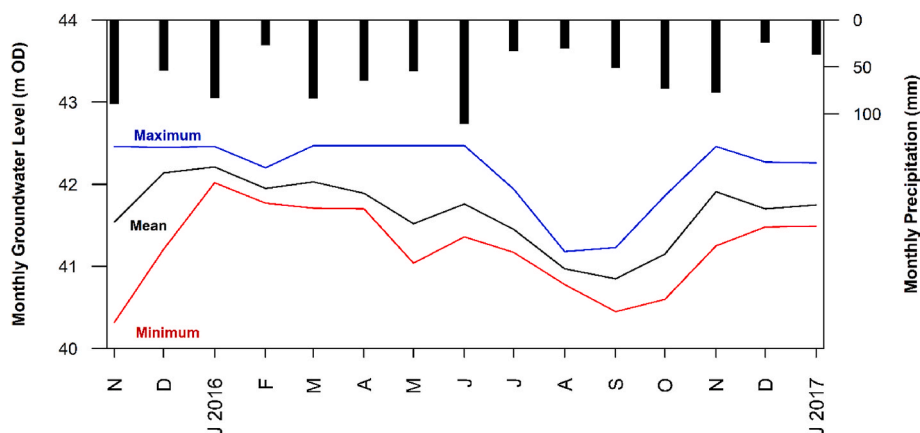
Fig. 2. Monthly precipitation (mm) recorded adjacent to Site 5 and monthly mean, minimum and maximum groundwater levels (m OD) measured at MLBH4 (see Fig. 1 for locations) for the period November 2015–January 2017.
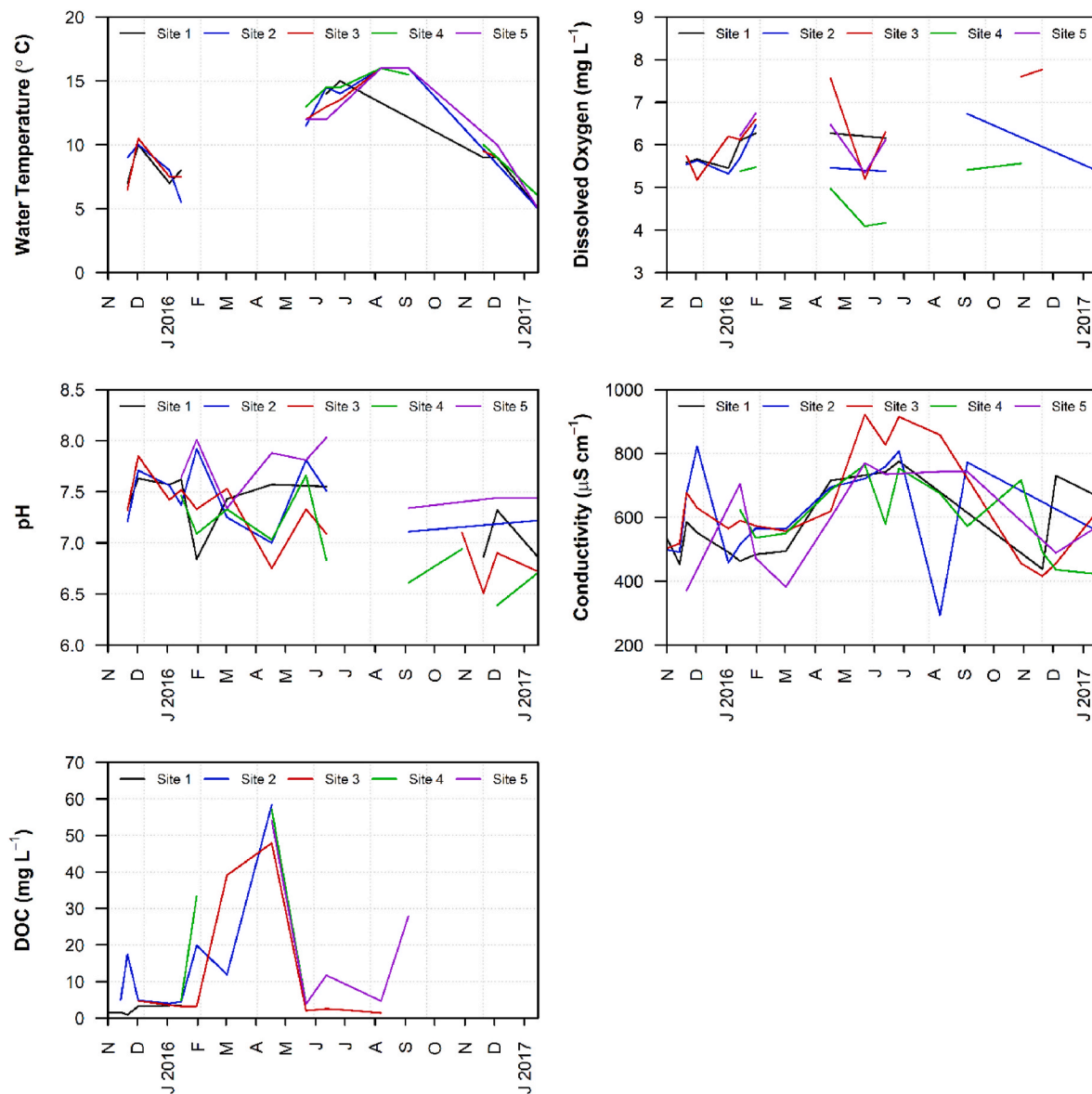
Fig. 3. Time series plots of field measurements and dissolved organic carbon (DOC) for field drain samples at Sites 1–5 for the period November 2015–January 2017.

2011). The predicted soil leachate concentration for each field drain during the 12-month period November 2015–October 2016 was then derived as follows:

up by plants and, of the nitrogen that was not taken up by plants, 32–37% was incorporated into the soil organic matter pool, representing approximately 11–14% of total applied fertiliser-derived nitrogen.

$$\text{Soil leachate concentration} \left(\text{mg N L}^{-1}\right) = \frac{0.2 \times \text{fertiliser application rate} \left(\text{kg N ha}^{-1}\right) \times 10^6 \times \text{drain area (ha)}}{\text{volume of field drainage (L)}} \qquad \text{Equation 4}$$

The volume of field drainage was derived from the product of the field drain area and the 12-month precipitation total (755.8 mm) multiplied by an annual runoff coefficient (0.273; Outram et al., 2016), yielding an infiltration of 206.3 mm.

Sebilo et al. (2013) showed, using ^{15}N-labelled fertiliser-derived nitrate to identify the long-term fate of nitrogen applied to agricultural soils over 30 years, that 61−65% of applied fertiliser nitrogen was taken

Hence, a rate of incorporation of fertiliser-derived nitrogen into the soil organic matter pool of 14% was applied when estimating denitrification rates for the study fields.

In addition to the arable fields, for Salle Rush Meadow (Site 5) under grazing grass, the soil leachate nitrogen concentration is calculated based on an organic fertiliser (manure) application rate for an area of grazing grass of 6.16 ha with 30 head of cattle with an associated excretion rate of 56.3 kg N a^{-1} per animal (ADAS, 2021). For a soil leaching coefficient of 0.162 (Johnes, 1996), this equates to an annual
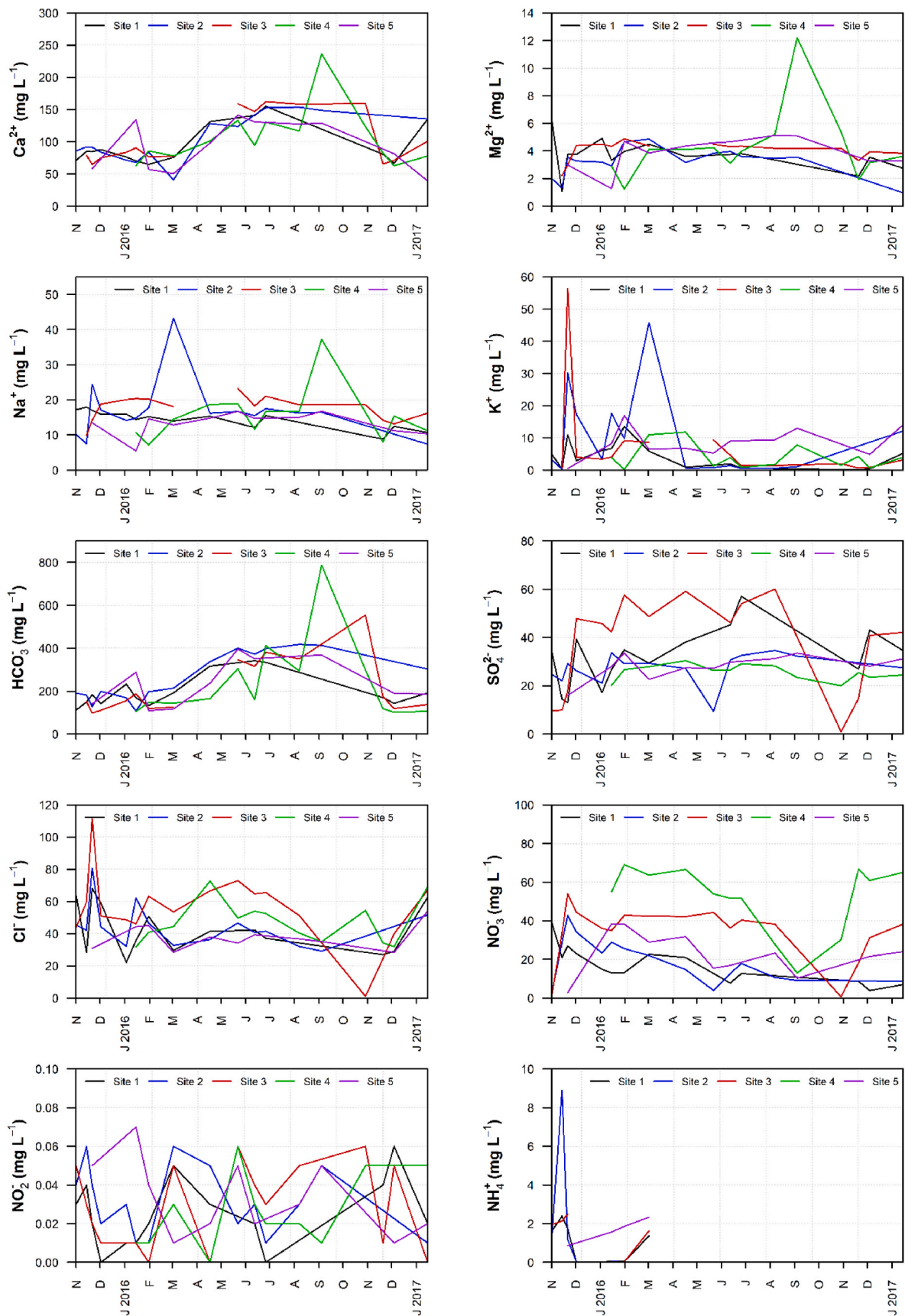
Fig. 4. Time series plots of major and minor ions for field drain samples at Sites 1–5 for the period November 2015–January 2017.
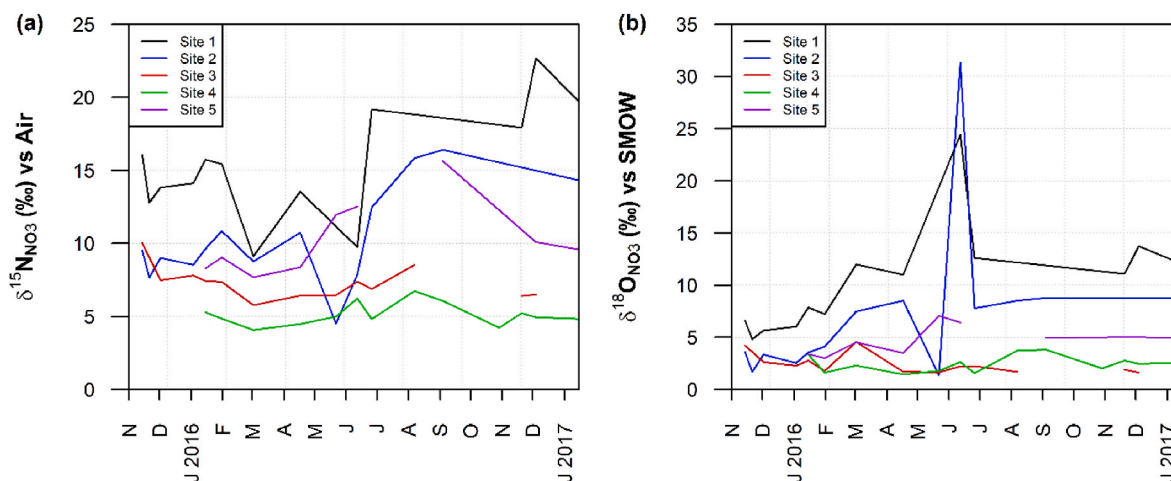
Fig. 5. Time series plots of (a) $\delta^{15}N_{NO3}$ and (b) $\delta^{18}O_{NO3}$ composition for field drain samples at Sites 1–5 for the period November 2015–January 2017.

input of 44.4 kg N ha^{-1} applied to a drain area of 0.62 ha.

3. Results

3.1. Field measurements and dissolved organic carbon

During the field drain sampling period, November 2015–January 2017, precipitation totalled 894.0 mm. Monthly precipitation (Fig. 2) ranged from 24.0 mm in December 2016 to 110.6 mm in June 2016. The mean monthly groundwater level ranged from a maximum of 42.21 m OD in January 2016 before declining towards a minimum level of 40.85 m OD in September 2016 (Fig. 2). The overall lowest groundwater level of 40.32 m OD was recorded in November 2015 and several months (March–June and November 2016) experienced the overall maximum recorded level of 42.47 m OD. With reference to the shallow groundwater level at MLBH4, all months (except for July–October 2016) experienced maximum groundwater levels above 42.2 m OD, the level at which the water table is within 0.6 m of the ground surface and the highest daily discharge volumes might be expected (Outram et al., 2016). The monthly mean groundwater level only exceeded 42.2 m OD in January 2016.

Results of field drain measurements are shown in Fig. 3. Temperature values reflected seasonal differences, ranging between 5 and 10 °C in the autumn/winter and 11–16 °C in spring/summer. At all sites, field drain DO concentrations ranged between 4 and 8 mg L^{-1} and, therefore, exceeded the assumed threshold for the onset of anaerobic denitrification (<2 mg L^{-1}; Zumft, 1997; Rivett et al., 2008). The reliability of the field DO measurements is uncertain given the turbulent conditions and aeration of the bulk water discharging from the drain outflows. Therefore, it is unlikely the DO measurements represent the anaerobic conditions developed within micro-sites in the soil profile (Hamersley and Howes, 2002; Rivett et al., 2008). All sites were circum-neutral in terms of pH, although the autumn/winter samples in 2016/17 were slightly more acidic, reflecting the onset of rainfall recharge. Values of electrical conductivity ranged between 300 and 900 μS cm^{-1}, with most of the lower values again associated with autumn/winter precipitation events.

DOC concentrations in field drain samples are also presented in Fig. 3 and display high variability within and between sites. Sites 2–5 recorded values of >40 mg L^{-1} in spring 2016 but lower values, typically <20 mg L^{-1}, were recorded for all sites in other months.

3.2. Major and minor ions

Results of major and minor ion concentrations of drain samples are shown in Figs. 4 and 6a. In general, the sites demonstrated little seasonal variability but larger intra-site variability. The range of nitrate

concentrations tended to be greatest (up to 69 mg L^{-1} at Site 4 in February 2016) during the autumn/winter months and lowest (<20 mg L^{-1}) during the late summer/early autumn months (a concentration of 0.73 mg L^{-1} was recorded at Site 3 in early November 2016), with the wide range of nitrate concentrations reflecting its high mobility within the field drainage system. Nitrite concentrations were negligible for all samples (n = 69) with a mean concentration of 0.03 mg L^{-1} (1σ = 0.02 mg L^{-1}). The smaller number of ammonium results (n = 27) demonstrated a wide range of values (0.01–8.88 mg L^{-1}) with a mean value of 1.31 mg L^{-1} (1σ = 1.77 mg L^{-1}). The peak ammonium concentration in November 2015 coincided with a period of rainfall (8.6 mm) two days prior to and on the day of sampling.

Chloride and sulphate concentrations ranged largely between 25–70 and 20–70 mg L^{-1}, respectively, throughout the sampling period. Peaks in chloride concentrations (up to 111 mg L^{-1} at Site 3 in November 2015) reflected the post-harvest flushing of solutes from the soil profile, although this was not observed for sulphate. Concentrations of bicarbonate showed that most samples recorded between 100 and 400 mg L^{-1}, with a small number of peak values (up to 787 mg L^{-1} at Site 4) in autumn 2016. Calcium concentrations across all sites were predominantly in the range 50–150 mg L^{-1} with, as for bicarbonate, a small number of higher values (up to 236 mg L^{-1} at Site 4) observed in autumn 2016. Sodium, potassium and magnesium concentrations showed less variation with values largely in the ranges 10–20, 0.1–10 and 2–5 mg L^{-1}, respectively. Occasional peaks in concentrations were observed, for example potassium recorded 56 mg L^{-1} at Site 3 in November 2015, corresponding to the peak observed in chloride concentration.

3.3. Stable isotopic composition of nitrate

$\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values for all field drain samples are presented in Fig. 5 and, respectively, Fig. 6b and c. The temporal variation in stable isotope composition (Fig. 5) differentiates lower isotope values for Sites 3 and 4 compared to Sites 1, 2 and 5. The highest $\delta^{15}N_{NO3}$ values ($>+15‰$) are observed for Site 1 during the autumn-winter sampling periods in contrast to the occurrence of highest $\delta^{18}O_{NO3}$ values ($>+20‰$) observed for Sites 1 and 2 in summer 2016. Median $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values (Fig. 6) were significantly (p < 0.01) highest at Site 1 and lowest at Site 4. The $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values measured at Site 5 under grazing grass were significantly higher (median values of $+9.6‰$ and $+4.9‰$, respectively) than those at Sites 3 and 4 under arable cropping (p < 0.01) and reflect a manure source of nitrogen input (see Fig. S1). The greatest variation in spring/summer $\delta^{15}N_{NO3}$ values was measured at Site 2 whilst Site 4 was the least variable. Similarly, spring/ summer $\delta^{18}O_{NO3}$ values were most variable at Site 1 and least variable at Site 3. The autumn/winter samples followed the same pattern in terms
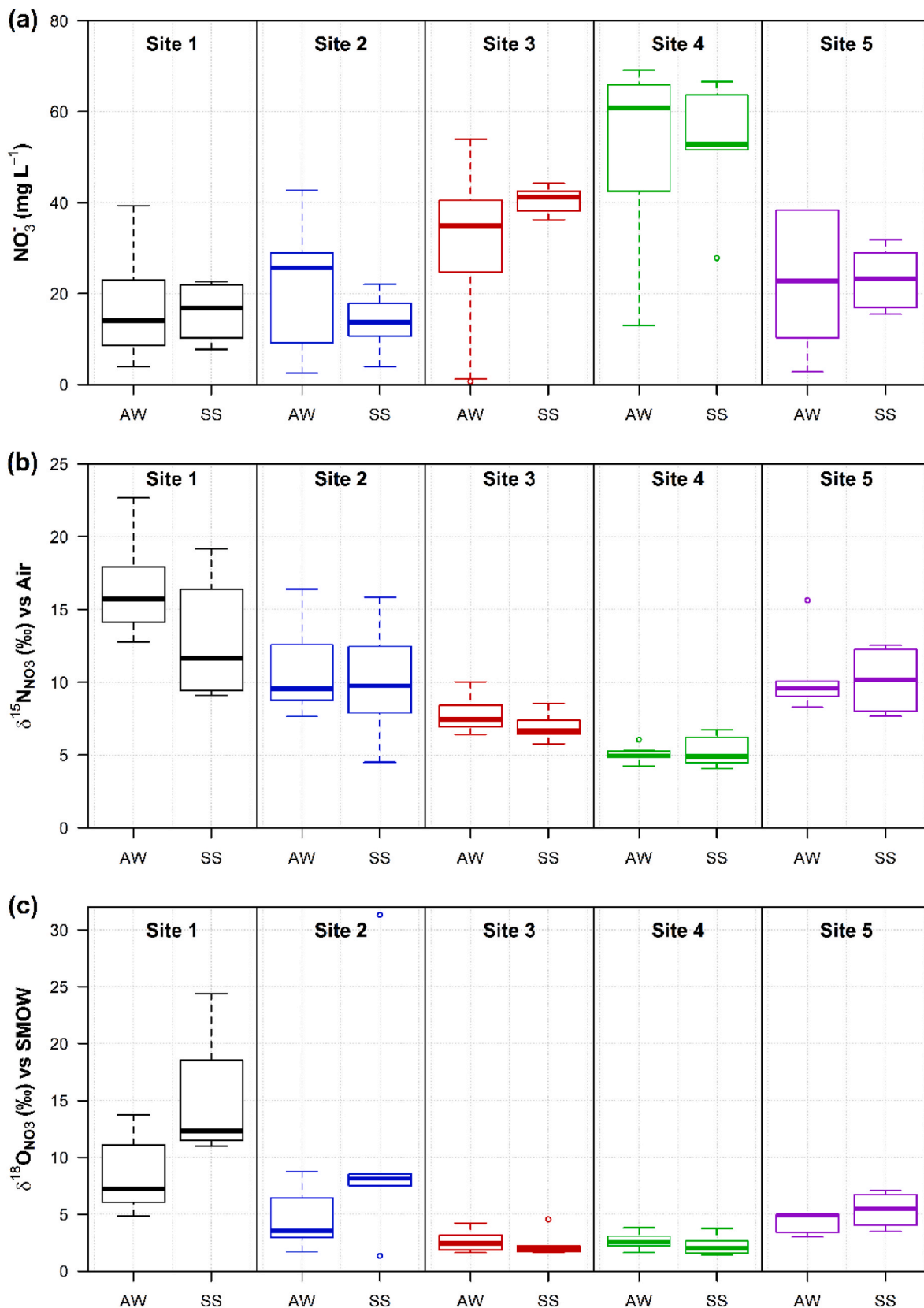
Fig. 6. Box and whisker plots showing intra-site comparison of (a) field drain nitrate concentration, (b) $\delta^{15}N_{NO3}$ composition and (c) $\delta^{18}O_{NO3}$ composition between autumn/winter (AW) (November 2015–February 2016 and September 2016–January 2017) and spring/summer (SS) (March–August 2016) samples at each sampling site. The solid central line is the median, the boxes the interquartile range, the whiskers 1.5x the interquartile range and the open circles are the outliers.
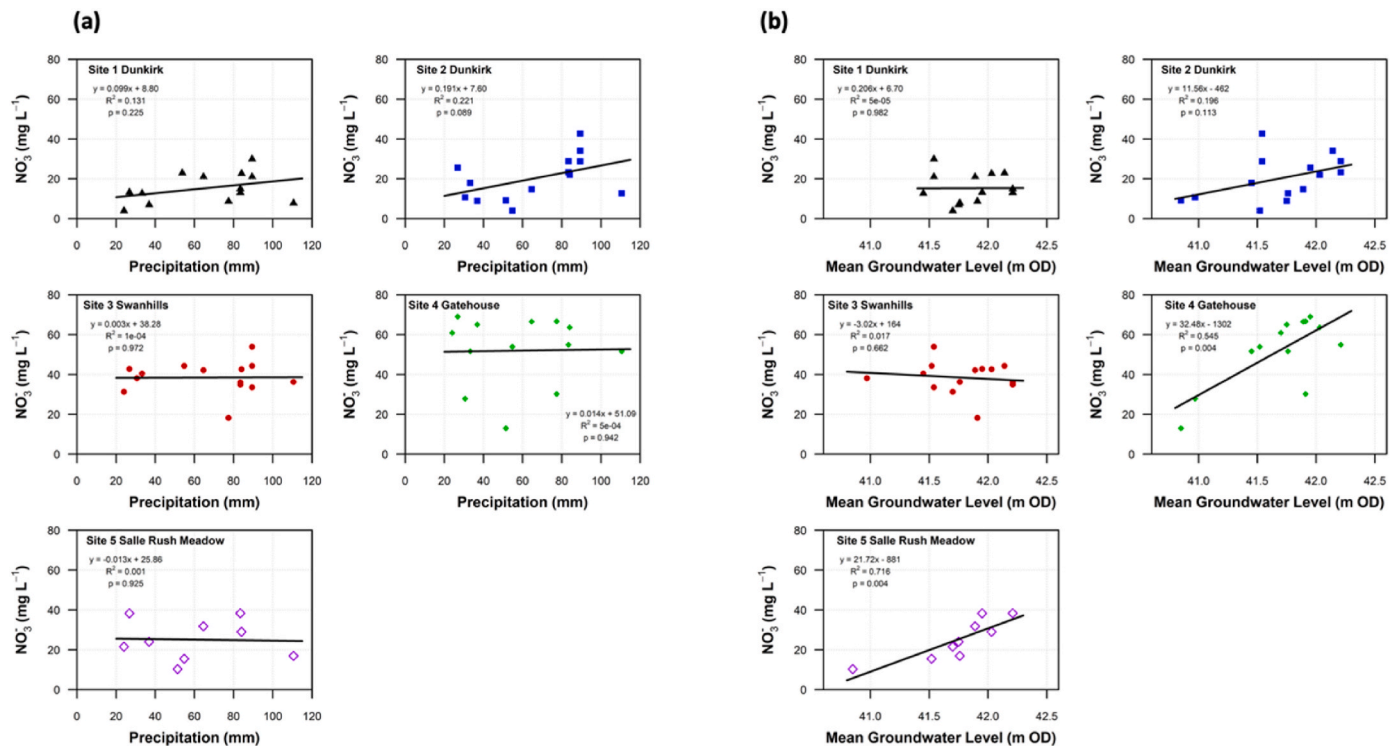
Fig. 7. Relationships between field drain nitrate concentration (mg L^{-1}) and (a) monthly precipitation (mm) and (b) monthly mean groundwater level (m OD) for Sites 1–5 for the period November 2015–January 2017.

of $\delta^{15}N_{NO3}$ values as the spring/summer samples, while the $\delta^{18}O_{NO3}$ values were significantly lower than the spring/summer samples at Sites 1 and 2 (p < 0.05).

4. Discussion

4.1. Field drainage hydrochemical characteristics

The major ion composition of the field drainage is influenced principally by the mineralogy of the chalky, argillaceous glacial tills on which the soils are developed. The field drain samples are dominated by calcium and bicarbonate with median concentrations for Sites 1–5 in the range 70–158 mg L^{-1} and 118–385 mg L^{-1}, respectively, with the lower values recorded during the autumn/winter sampling periods in response to dilution by precipitation. Hiscock (1993) showed that recharge entering the carbonate-rich soils developed on glacial tills in the adjacent Bure catchment achieved calcite saturation for high values of carbon dioxide partial pressure (PCO_2) in the range $10^{-2.1}$–$10^{-2.0}$. In addition, pyrite oxidation and sulphate reduction associated with the glacial tills in headwater regions evolve further bicarbonate and, to maintain calcite equilibrium, increase the dissolved PCO_2 of groundwater.

Sodium and chloride in field drain samples with median concentrations for Sites 1–5 in the range 11.2–18.6 mg L^{-1} and 35.0–65.0 mg L^{-1}, respectively, are controlled principally by precipitation inputs concentrated by evaporation in the soil zone leading to higher concentrations recorded in the spring/summer sampling period. Occasional peaks in concentration are observed in response to flushing events in both spring and autumn, as is also the case for magnesium and potassium.

Anthropogenic inputs, mainly agricultural fertiliser applications, lead to elevated nitrate concentrations (median values at Sites 1–5 in the range of 13.7–60.9 mg L^{-1}), with lower concentrations observed post-harvest prior to the onset of autumn/winter flushing of residual nitrogen from the soil zone. Although the overall nitrogen mobilisation and transport is greater during the winter (Outram et al., 2016), the actual

nitrate concentrations are not necessarily higher in the field drainage. For example, median nitrate concentrations are higher at Sites 1 and 3 during spring/summer (Fig. 6a) due to less dilution by precipitation, but also potentially by less available water limiting primary productivity and the uptake of nitrogen by the arable crop. In contrast, similar median nitrate concentrations are observed in autumn/winter and spring/summer at Site 5 that drains an area of grazing grass.

During months when the maximum groundwater level at MLBH4 exceeded 42.2 m OD with the potential to initiate high field drain discharge, nitrate concentrations of greater than 50 mg L^{-1} were consistently observed at Site 4 (Gatehouse) (except for November–December 2015) and at Site 3 (Swanhills) in November 2015. In general, the correlation of nitrate concentration with monthly precipitation (Fig. 7a) was weak for all sites (r^2 values < 0.22, p values > 0.09), but stronger for the correlation of nitrate concentration with shallow groundwater level at Sites 4 (r^2 = 0.55, p < 0.01) and 5 (r^2 = 0.72, p < 0.01) (Fig. 7b), as might be expected in that groundwater is responding to antecedent catchment conditions and the flushing of nitrate in the unsaturated zone.

There is little evidence from the stable isotope data for direct export of nitrate derived-fertiliser in field drainage, an observation also made by Outram et al. (2016) working in the same intensive arable catchment, in which legacy stores of nutrients buffer against changes in contemporary nutrient inputs and so exhibiting a state of biogeochemical stationarity (Green et al., 2014). In another study, Sebilo et al. (2013) showed that labelled ^{15}N incorporated into agricultural soils can persist as soil organic matter for up to 30 years following application. Hence, it is likely that the dissolved nitrate measured in the field drains is derived from mineralisation of soil organic matter, suggesting that a portion of the applied fertiliser-derived nitrate is immobilised in soil organic matter and later mineralised and lost as subsurface drainage.

Dissolved nitrate in field drain samples recorded $\delta^{18}O_{NO3}$ values across all sites of mostly between +1.4 and +13.7‰ (Fig. 5b), within the expected range of −5 to +15‰ indicative of soil nitrogen, and below the range reported for nitrate fertiliser (+15‰ to +25‰; Fig. S1) (Kendall,
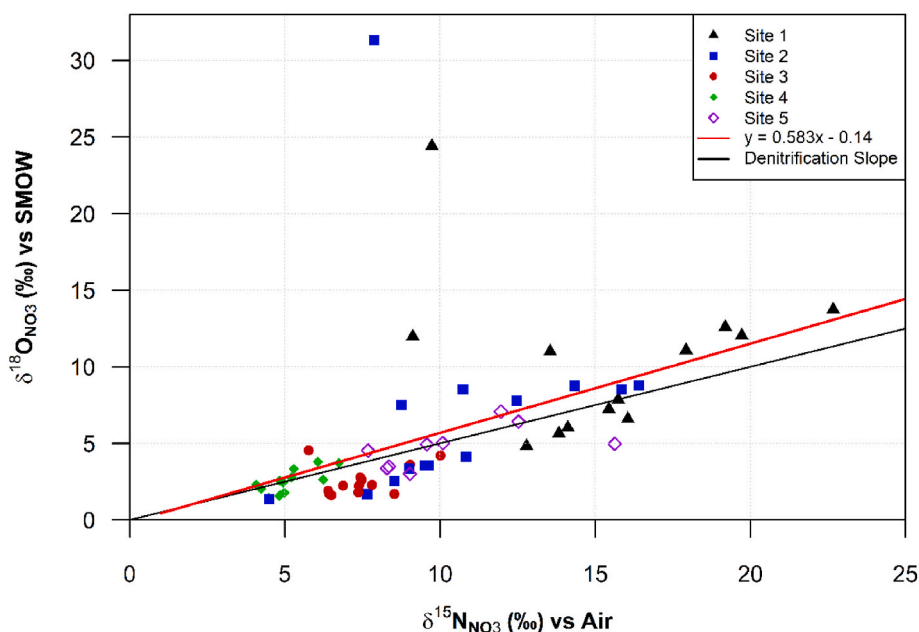
Fig. 8. Cross-plot of $\delta^{18}O_{NO3}$ versus $\delta^{15}N_{NO3}$ values of field drain samples from Sites 1−5 collected between November 2015–January 2017. The red line represents the best fit line to the data with a slope of 0.58, while the black line shows the theoretical slope of 0.5 associated with dual fractionation of $^{15}N_{NO3}$ and $^{18}O_{NO3}$ resulting from microbially-mediated denitrification.
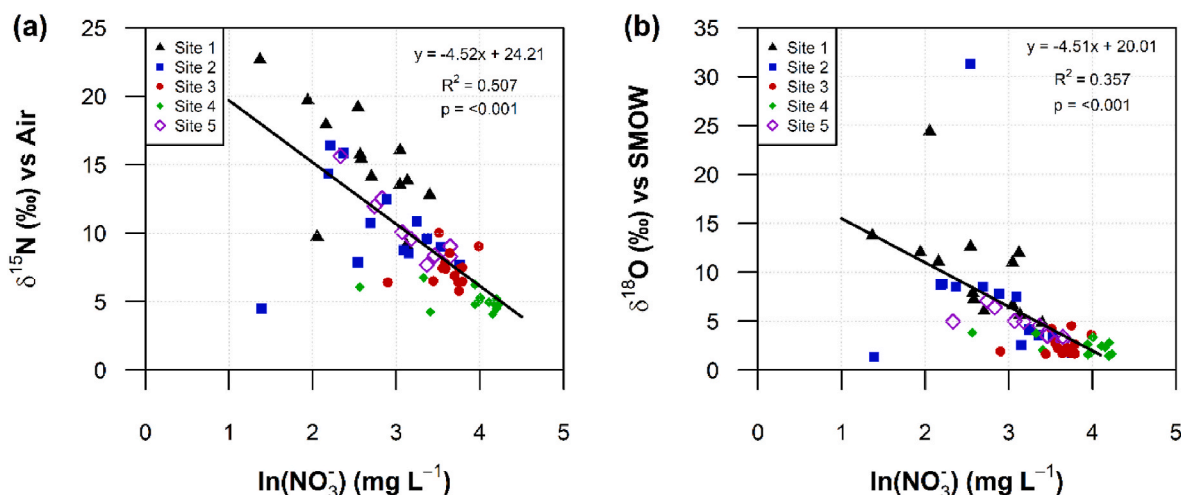
Fig. 9. Values of (a) $\delta^{15}N_{NO3}$ and (b) $\delta^{18}O_{NO3}$ composition plotted against $\ln(NO_3^-)$ concentration for all field drain samples collected between November 2015–January 2017.

1998). Interestingly, the two outliers recorded for Sites 1 and 2 in June 2016 (+24.4‰ and +31.3‰, respectively) corresponded to the month with an unusually high rainfall total (110.6 mm compared with a long-term (1991–2020) June average of 63.2 mm). Values of $\delta^{18}O_{NO3}$ >+18.0‰ suggest a contribution of nitrate-N from atmospheric precipitation (Amberger and Schmidt, 1987; Jung et al., 2020) in the field drainage during a month when there were no applications of fertiliser-N to the established crop and may also account for the higher median $\delta^{18}O_{NO3}$ values at Sites 1 and 2 in the spring/summer compared with the autumn/winter (Fig. 6).

4.2. Stable isotope evidence for denitrification

A clear distinction between the location of sampling sites in terms of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values is evident (Fig. 6b and c). When examined as a cross-plot (Fig. 8), the nitrate isotope data show a trend indicative of

denitrification with enrichment of both ^{15}N and ^{18}O in the residual nitrate. The slope of the linear regression in Fig. 8 is 0.58, within the range reported in the literature associated with denitrification (0.35–0.76) (Böttcher et al., 1990; Aravena and Robertson, 1998; Cey et al., 1999; Mengis et al., 1999; Fukada et al., 2003; Panno et al., 2006; Wexler et al., 2014) suggesting that microbially-mediated denitrification is occurring within the soil zone. Hence, those samples with $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values within the range associated with manure and septic waste, including Site 5 under grazing grass, are likely demonstrating enrichment of both N and O isotopic species of nitrified and subsequently denitrified soil ammonium.

Further evidence of denitrification is shown in Fig. 9 in which decreasing nitrate concentration is associated with increasing $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values as nitrate is consumed during denitrification, which in turn enriches the remaining pool of nitrate in ^{15}N and ^{18}O. The samples with the highest nitrate concentration (Site 4) are associated

Table 2

Soil water denitrification rates for the period November 2015–October 2016 under winter barley feed (Sites 1–4) and grazing grass (Site 5) based on the difference between calculated soil nitrate leaching and measured field drain nitrate concentrations. Also included are the interpolated soil clay, sand and silt contents for the top 0–30 cm in each of the drain areas (Sites 1–4) based on data presented in Table 1.

Site number and field location	Drain i.d.	Clay content (wt. %)	Sand content (wt. %)	Silt content (wt. %)	Drain area (ha)	Drainage volume (L)	Fertiliser/manure applied (kg N ha^{-1})	N leached (kg)	Soil leachate N (mg L^{-1})	Field drain N (mg L^{-1}) Min	Median	Max	Soil leachate N–median field drain N (mg L^{-1})	Denitrification rate (kg N ha^{-1}) Min	Median	Max	Median mass N denitrified (kg)
1 Dunkirk	D3R	24.00	42.16	33.00	1.293	2.667 × 10^6	210	46.7	17.51	0.90	4.74	9.66	12.76	16.20	26.33	34.26	34.0
2 Dunkirk	D1R	20.22	45.12	34.81	0.145	0.299 × 10^6	245	6.11	20.42	7.57	9.32	12.18	11.10	17.02	22.90	26.52	3.32
3 Swanhills	D4L	20.19	47.77	31.72													
4 Gatehouse	D5R	11.65	44.94	39.00	0.078	0.160 × 10^6	210	2.80	17.51	2.93	12.18	15.59	5.33	3.95	11.00	30.07	0.852
5 Salle Rush Meadow	Site 5	–	–	–	0.622	1.284 × 10^6	44.4	23.8	18.51	2.33	6.55	8.66	11.96	20.32	24.68	33.38	15.4
Total								79.4									53.6

with the lowest level of fractionation (that is, lowest $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values) and *vice versa* (Site 1). An increase in $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values with decreasing nitrate concentrations is characteristic of kinetic isotope fractionation induced by the breakage of N–O bonds during denitrification (Hiscock et al., 2011).

Calculated enrichment factors (Equation (2); Fig. 9) are −4.52‰ for $\delta^{15}N_{NO3}$ and -4.51‰ for $\delta^{18}O_{NO3}$, within the range for nitrogen isotopes reported for denitrification in various aquatic studies ($\varepsilon = -40$ to -5‰; Kendall, 1998), and similar to the reported range for oxygen isotopes ($\varepsilon = -8.0$ to -18.3‰; Fukada et al., 2004), with smaller values indicating a faster rate of denitrification. Wexler et al. (2012) obtained values of $\varepsilon = -6.2$‰ for $\delta^{15}N_{NO3}$ and -3.4‰ for $\delta^{18}O_{NO3}$ for a single, summer low-flow drainage sample in glacial till, also in the Wensum catchment. Comparable values of $\varepsilon = -5.9$‰ for $\delta^{15}N_{NO3}$ and -2.0‰ for $\delta^{18}O_{NO3}$ are reported by Deutsch et al. (2005) for tile drainage in sandy loam and loamy sand soils developed from moraine substrates in the Warnow catchment, north-east Germany. Also, based on data presented by Kellman and Hillaire-Marcel (2003), an enrichment factor for $\delta^{15}N_{NO3}$ of −3.2‰ is obtained for tile drainage in sandy clay loam soils developed on glacial sediments and post-glacial marine clays in the Prescott Drainage Basin, St Lawrence lowlands, Canada.

4.3. Denitrification rates and processes

Estimated rates of denitrification in the arable fields varied from 11.0 kg N ha^{-1} at Site 4 (Gatehouse) to more than twice this rate (26.3 kg N ha^{-1}) at Sites 1 and 2 (Dunkirk) based on median values of field drainage nitrate concentrations (Table 2). As observed with the pattern of measured $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values for field drain samples, the higher denitrification rates correspond to the more clay-rich soils in drainage areas discharging at Sites 1–3.

Electron donors are most likely contributed by bioavailable organic carbon (heterotrophic denitrification) and, where carbon is limiting, reduced sulphur species (autotrophic denitrification) given the presence of disseminated pyrite in the glacial tills of eastern England (Hiscock and Tabatabai Najafi, 2011). It is possible that denitrification involving a multiple electron donor system develops, for example where organic carbon, sulphide and iron minerals are all available (Rivett et al., 2008), as identified by Postma et al. (1991) for an unconfined sandy aquifer of Quaternary age in Jutland, Denmark. Anaerobic ammonium oxidation (anammox) using nitrate and nitrite as electron acceptors (Robertson et al., 2012) may also occur under water-saturated (high groundwater level) conditions when ammonium and nitrite availability is not limiting (Wang et al., 2020). In addition, the mutual coupling of respiratory ammonification and ammonium oxidation processes in the soil system is likely to promote the distribution of anammox (Wang et al., 2020).

For all Sites 1–5 in the headwaters of the Blackwater sub-catchment, the total mass of soil nitrogen removed by dentification (53.6 kg) represents 68% of the soil nitrogen leached from the soil zone (79.4 kg). The range of percentage nitrogen removal in headwater field drainage at the study sites (30–73%) is higher than the range of 15–30% for the wider Wensum catchment presented by Wexler et al. (2012), where denitrification at the catchment scale was identified as occurring increasingly downstream within extensive sand and gravel hyporheic zone deposits through which Chalk groundwater discharges to the main river channel.

A comparison of denitrification rate with nitrate stable isotope composition (Fig. 10) shows no significant relationship for $\delta^{18}O_{NO3}$ ($r^2 = 0.45$, $p = 0.33$) but a stronger relationship for $\delta^{15}N_{NO3}$ ($r^2 = 0.85$, $p = 0.08$) irrespective of land use (arable cropping and grazing grass). Based on the linear regression line (Fig. 10a), an isotopic enrichment of +1‰ for $\delta^{15}N_{NO3}$ is associated with a denitrification rate of 2.6 kg N ha^{-1}. When applied to Dunkirk field (Sites 1 and 2), this predictive relationship gives an enrichment of +14.0‰ for the complete denitrification of leached soil nitrogen (36.1 kg N ha^{-1}) under winter barley feed, comparable to the measured ranges in $\delta^{15}N_{NO3}$ values in field drainage for Sites 1 and 2 of +13.6‰ and +11.9‰, respectively.

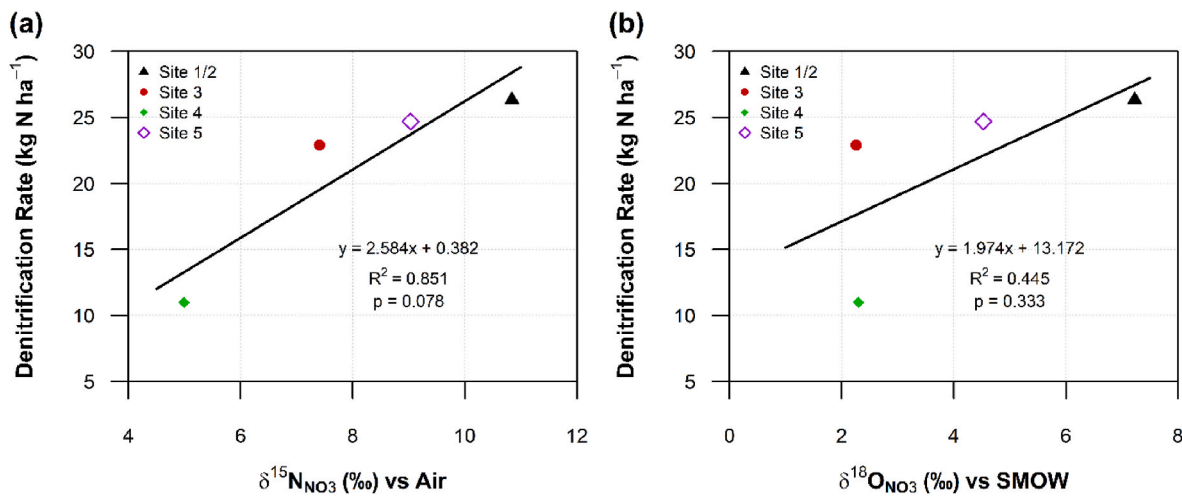**Fig. 10.** Relationship between median denitrification rate and (a) $\delta^{15}N_{NO3}$ and (b) $\delta^{18}O_{NO3}$ composition for field drain areas.

4.4. Influence of soil physical characteristics

The pore connectivity and water-filled pore space of a soil matrix are determined by its texture and structure, which in turn have a significant impact on the cycling of nutrients within and between microsites (Wier et al., 1992; Hofstra and Bouwman 2005; Włodarczyk et al., 2005; Castellano et al., 2013; Palta et al., 2014). For soil denitrification to occur, the soil texture must be within a narrow window of intergranular structure, where the pore connectivity is such that diffusion of substrates between oxic and sub-oxic zones can occur, but still maintain anaerobic conditions. It is well established that soil texture is a significant contributor to microbial nitrogen cycling owing to its influence on oxygen availability due to water retention, with nitrification commonly associated with moderate to dry conditions, and denitrification favoured by waterlogged, low oxygen soil conditions (De Klein and van Logtestijn, 1994; Hofstra and Bouwman 2005; Menyailo and Hungate, 2006; Palta et al. 2016). Also, soils with a higher clay content tend to have lower decomposition rates due to the strong bonds that exist between clay surface particles and organic matter. A high clay content increases the potential for aggregation within the soil matrix, resulting in organic matter molecules being physically protected from mineralisation (Bot and Benites, 2005).

The nitrate concentration and stable isotope data of arable field drainage (Fig. 6) in this study suggest that the soils at Sites 1 and 2 lie within the narrow range of conditions favourable to denitrification, while soil conditions at Sites 3 and 4 are more variable, dependent on differences in soil condition, primarily clay content and how this interacts with oxygen availability. Arable field drains recording the highest $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values (samples from Sites 1–3) were collected from drains in the most clay-rich areas (Fig. 1), supporting the importance of soil texture in soil denitrification, namely through its influence on soil moisture and, hence, oxygen availability. Site 1 has a greater fraction of clay content than the other arable field sites (24.0% by weight; Table 2) and exhibited a higher median $\delta^{15}N_{NO3}$ in the autumn/winter months (+15.7‰) compared with the spring/summer months (+11.7‰), possibly as a result of a high saturated water content enhancing denitrification in the clay-rich soils at this site during the autumn/winter period. In contrast, Site 4 has a lower fraction of clay than the other sites (11.7% by weight) and has correspondingly less enriched ^{15}N and ^{18}O, again demonstrating the influence of soil texture in promoting denitrification.

At the time of sampling, Site 4 was established under a reduced tillage method, with cultivation to a depth of 10 cm in which crop residues were incorporated into the soil following harvest and the void spaces generated through root growth were maintained. Therefore,

infiltration rates are likely to be enhanced under this cultivation method, with a greater hydrological connectivity and increased oxygen availability. Thus, in addition to other soil physical conditions (i.e., temperature) and the degree to which plant material is incorporated into soils (i.e., source material and decomposition affecting the $\delta^{15}N$ value of soil organic matter; Kendall et al., 2007), this study demonstrates that while soil texture is a dominant influence on the degree of fractionation of nitrate in field drainage, agricultural management regimes should also be considered.

Further evidence for denitrification in field drainage using a stable isotope approach is provided by Kellman and Hillaire-Marcel (2003) and Deutsch et al. (2005), also in catchments with sandy clay loam soils developed on glacial sediments. Kellman and Hillaire-Marcel (2003) found elevated $\delta^{15}N_{NO3}$ values and nitrate concentrations consistent with a pattern of denitrification with values repeatedly exceeding +15‰ in tile drainage during extended periods with little precipitation. Deutsch et al. (2005) observed a decrease in nitrate concentration and isotope enrichment in tile drainage from late July to the beginning of December suggesting that denitrification took place in this period, although it remained unclear whether this occurred in soils or in two small ponds connected to the drainage system.

In contrast, research by Smith and Kellman (2011) on drainage systems in sandy loam soils in Onslow, Nova Scotia showed that stable nitrogen isotope values for drainage water were consistent with the range of organic sources (manure and soil organic matter) through nitrification, with no evidence of denitrification causing a notable fractionation of the nitrate-N pool. In a study of tile drainage in silt loam soils in the semi-arid Palouse Basin of eastern Washington and northern Idaho, Kelley et al. (2013) also showed no stable isotope evidence for denitrification. Kelley et al. (2013) concluded that if denitrification were occurring in microsites where dissolved oxygen is controlled by diffusion in otherwise well-aerated unsaturated and saturated zones, it is not significant in governing nitrogen leaching dynamics at the field scale, which is dominated by soil nitrogen cycling by nitrification.

5. Conclusions

In this study, hydrochemical and stable isotopic data from arable field drainage in a region with clay loam and sandy clay loam soils developed on glacial till deposits have been interpreted together with soil physical characteristics. The field drainage represents major soil zone processes, namely denitrification, controlling the fate of nitrogen coupled with the oxidation of available organic carbon and reduced sulphur as electron donors. Denitrification is present within the soil zone, with greater fractionation of $^{15}N_{NO3}$ and $^{18}O_{NO3}$ isotopes occurring

where the soil texture is more clay rich. Clay-rich soils tend to contain more organic carbon owing to the charge on the particle surfaces and are also more water retentive due to their lower permeability, which together provide the opportunity for denitrification. At one site (Site 4), there is evidence showing the influence of cultivation regime on denitrification, with reduced tillage and the presence of crop residues in the subsurface resulting in higher infiltration rates (shorter residence times) and more oxygenated soil conditions that limit the isotopic enrichment of the soil water nitrate through denitrification.

Given that intensive agricultural practices have led to a decline in soil quality, with a third of all soils globally now considered moderately to highly degraded (FAO and ITPS, 2015), region-specific sustainable practices that enhance denitrification, for example by preserving and increasing the soil organic carbon content, should be encouraged to provide ecosystem services such as biodiversity and water quality benefits (Bünemann et al. 2018; Lehmann et al. 2020). When combined with mitigation measures such as improved fertiliser management and reduced tillage practices (Zhang et al., 2015), denitrification can aid the reduction of nitrate pollution, an important consideration in the sustainable transformation of global croplands (Gu et al., 2023).

Author contribution statement

Nicholas Gerrard: **Writing- original draft, Conceptualization, Formal analysis, Methodology, Investigation.** Kevin Hiscock: **Writing- original draft, Conceptualization, Formal analysis, Methodology, Funding acquisition.** Richard Cooper: **Writing-review and editing, Investigation, Data curation.** Alina Marca: **Writing-review and editing, Methodology, Investigation.** Gilla Sünnenberg: **Data curation, Formal analysis.**

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apgeochem.2023.105803.

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