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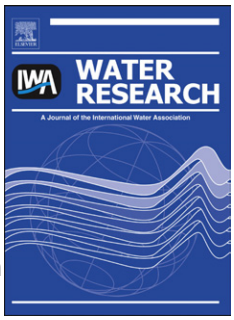

Evaluating the utility of 15n and 18o isotope abundance analyses to identify nitrate sources: A soil zone study

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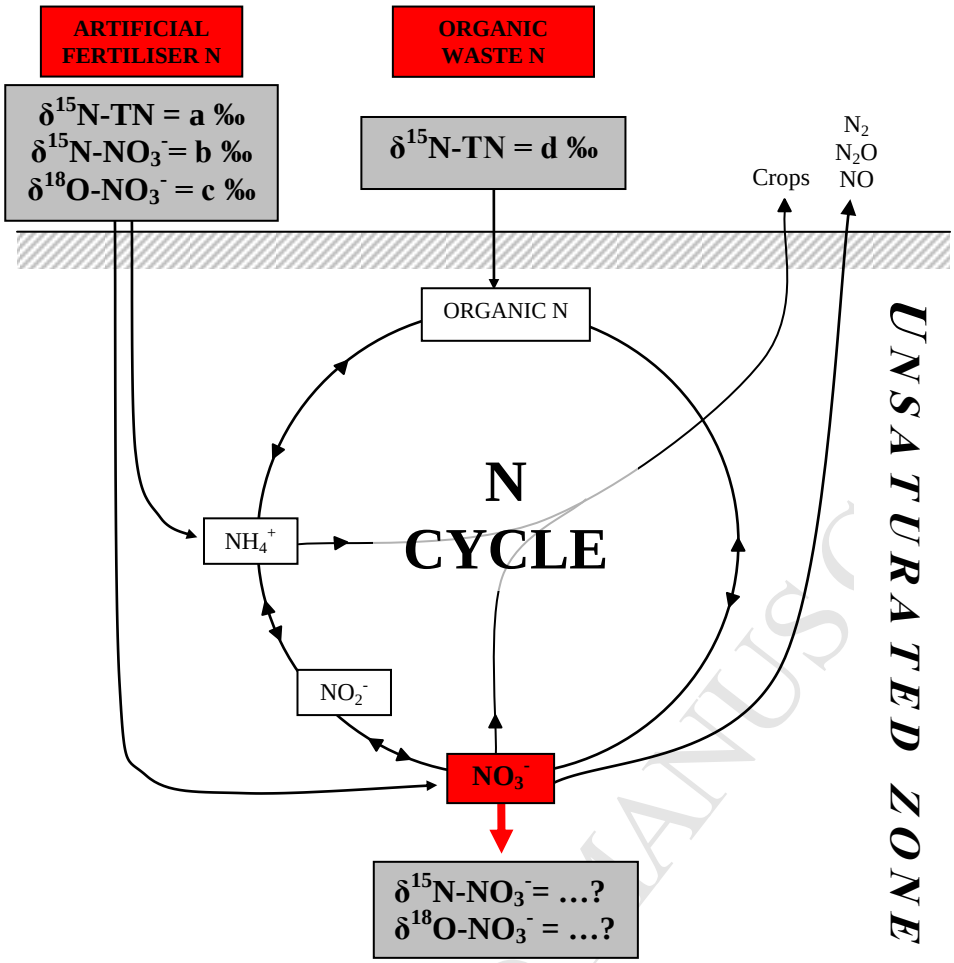
HIGHLIGHTS

- Using stable isotopes for NO_3^- source tracking in soils showed limitations.
- ^{15}N and ^{18}O contents in soil-water NO_3^- were confined to a narrow range of values.
- Tracking artificial fertiliser NO_3^- was possible in limited circumstances.
- Correlating $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ variables helped characterise nitrate sources.

Graphical Abstract



EVALUATING THE UTILITY OF ^{15}N AND ^{18}O ISOTOPE ABUNDANCE ANALYSES TO IDENTIFY NITRATE SOURCES: A SOIL ZONE STUDY

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ABSTRACT

^{15}N and ^{18}O isotope abundance analyses in nitrate (NO_3^-) (expressed as $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values respectively) have often been used in research to help identify NO_3^- sources in rural groundwater. However, questions have been raised over the limitations as overlaps in δ values may occur between N source types early in the leaching process. The aim of this study was to evaluate the utility of using stable isotopes for nitrate source tracking through the determination of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ in the unsaturated zone from varying N source types (artificial fertiliser, dairy wastewater and cow slurry) and rates with contrasting isotopic compositions. Despite NO_3^- concentrations being often elevated, soil-water nitrate poorly mirrored the ^{15}N content of applied N and therefore, $\delta^{15}N\text{-}NO_3^-$ values were of limited assistance in clearly associating nitrate leaching with N inputs. Results suggest that the mineralisation and the nitrification of soil organic N, stimulated by previous and current intensive management, masked the cause of leaching from the isotopic prospective. $\delta^{18}O\text{-}NO_3^-$ was of little use, as most values were close to or within the range expected for nitrification regardless of the treatment, which was attributed to the remineralisation of nitrate assimilated by bacteria (mineralisation-immobilisation turnover or MIT) or plants. Only in limited circumstances (low fertiliser application rate in tillage) could direct leaching of synthetic nitrate fertiliser be identified ($\delta^{15}N\text{-}NO_3^- < 0$ ‰ and $\delta^{18}O\text{-}NO_3^- > 15$ ‰). Nevertheless, some useful differences emerged between treatments. $\delta^{15}N\text{-}NO_3^-$ values were lower where artificial fertiliser was applied compared with the unfertilised controls and organic waste treatments. Importantly, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ variables were negatively correlated in the artificial fertiliser treatment ($0.001 \leq p \leq 0.05$, attributed to the varying proportion of fertiliser-derived and synthetic nitrate being leached) while positively correlated in the dairy wastewater plots ($p \leq 0.01$, attributed to limited denitrification). These results suggest that it may be possible to distinguish some nitrate sources if analysing correlations between δ variables from the unsaturated zone. In grassland, the above

correlations were related to N input rates, which partly controlled nitrate concentrations in the artificial fertiliser plots (high inputs translated into higher NO_3^- concentrations with an increasing proportion of fertiliser-derived and synthetic nitrate) and denitrification in the dairy wastewater plots (high inputs corresponded to more denitrification). As a consequence, nitrate source identification in grassland was more efficient at higher input rates due to differences in δ values widening between treatments.

KEY WORDS

Nitrate; soil-water; stable isotope; nitrate source; artificial fertiliser nitrogen; organic waste nitrogen

1. INTRODUCTION

The common occurrence of elevated nitrate (NO_3^-) levels in groundwater has long been a cause of concern for human/animal health (Stark and Richards, 2008) and the environment (discharge into surface-waters associated with eutrophic conditions (Howarth, 1988)). In response to these problems, environmental policies have been implemented in many countries. In the European Union for instance, legislation including the Nitrates Directive 91/676/EEC and the Groundwater Directive 2006/118/EC prohibits nitrate concentrations in aquifers to exceed the mandatory limit of 50 mg L^{-1} NO_3^- and requires that actions be taken in order to reverse or prevent any infringement (Stark and Richards, 2008). However, such a task remains challenging, partly because nitrate can come from multiple sources, which makes identifying and controlling the main contamination difficult.

To date, nitrate source identification has been a central topic to rural groundwater quality studies to help reduce nitrate occurrence. One of the commonly used methods are stable isotope analyses,

which investigate $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios in dissolved NO_3^- (referred to as $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values respectively). The interest in this technique came from the expectation that major N sources involved in the terrestrial N cycle (artificial fertilisers, human/animal organic wastes, soil nitrogen (N), atmospheric depositions) generate nitrate with characteristic and therefore recognizable δ values (Kendall and Aravena, 2000). Ideally, nitrate deposited or nitrified in soils would carry these characteristics unchanged while leaching to the water-table. However, complications can occur, especially if biochemical reactions that transform NO_3^- proceed in the unsaturated zone. Denitrification causes an elevation of both $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ (Chen and MacQuarrie, 2005), while mineralisation-immobilisation turnover (MIT) (rapid remineralisation of nitrate assimilated by bacteria) alters $\delta^{18}O\text{-}NO_3^-$ to within the range expected from nitrification (Mengis et al., 2001). Isotopic fractionation can also be caused by ammonia volatilisation, which shifts $\delta^{15}N$ in the remaining substrate towards higher values, resulting in higher $\delta^{15}N\text{-}NO_3^-$ for subsequently nitrified nitrate. Consequently, overlaps in nitrate δ values may occur between N source types early in the leaching process (Fogg et al., 1998), hence weakening nitrate source tracking in underlying groundwater.

The utility of using stable isotopes to identify nitrate sources in rural groundwater was evaluated through the determination of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values in soil-water from the unsaturated zone under varying N source types (artificial fertiliser, dairy wastewater and cow slurry) and rates with contrasting isotopic compositions. Three main aspects of stable isotope analyses were explored: i) efficiency in identifying NO_3^- sources, ii) ability to discriminate N treatments relative to one another and iii) influence of application rates on leaching and δ variables. Overall, an underlying purpose was to shed more light on nitrate dynamics in soils and improve our understanding of the leaching process.

2. MATERIALS AND METHODS

2.1 Sites description and N management

Experiments were conducted at Teagasc facilities (Irish Agriculture and Food Development Authority) on three Irish soils (Table 1) already set up for several Teagasc studies. Most soil-water sampling took place at Moorepark Research Centre (County Cork, 52º09'35" N - 8º16'28" W) where soils allow good drainage (Gibbons et al., 2006). Some samples were collected at Knockbeg near Oak Park Research Centre (County Carlow, 52º51'57" N - 6º54'45" W), where soils of medium to heavy texture are nonetheless well drained (Hooker, 2005). Additional sampling was also undertaken on coarse and excessively drained soils originating from Oak Park but transported to lysimeters at Johnstown Castle Research Centre (County Wexford, 52º17'35" N - 6º30'03" W) (Brennan et al., 2010). All three soils overlie free-draining sediments. The climate at these sites is temperate and oceanic. Temperatures remained mild during the sampling period, with the daily mean oscillating between -2.2 °C in winter (December/January) and 21.3 °C in summer (July/August) (Figure 1). Effective rainfall was nil in summer (July to September) and positive in-between with a peak around late autumn/early winter (Figure 1).

Up to four treatments were investigated (Table 2) - application of artificial N fertiliser (all three soils), dairy wastewater (Moorepark), cow slurry (Moorepark) and no application (Moorepark, Oak Park) - on two types of land use - permanent intensively managed grassland (perennial ryegrass *Lolium perenne L.*, Moorepark and Oak Park) and tillage (winter wheat *Triticum aestivum L.* and spring barley *Hordeum vulgare L.*, Knockbeg) - at different application rates (Moorepark and Knockbeg). Except for urea, synthetic fertiliser N applied to artificial fertiliser plots consisted of ammonium (NH_4^+) and nitrate, the latter fraction amounting to between 41 and 50 % of annual N inputs. Distinctly, dairy wastewater (washings from milking parlours, dairies, run-off from cattle

house, etc) and slurry (mixture of urine, faeces and water) hardly contained any nitrate, almost all N being organically bound or in ammonium form (Table 3) as expected from anaerobic storage. Soil nitrogen, 95 % of which is generally expected to be in the form of insoluble organic matter (Whitehead, 1995), was another potential source of N examined in the unfertilised controls. Atmospheric N deposition rates, estimated between 6 to 20 kg N/ha/year in Ireland (Jordan, 1997), were not deemed to contribute to a large extent to the total N inputs when compared with treatment N rates. Primary N fixers like clover were controlled in the swards to prevent any N fixation occurring. N inputs could therefore be all accounted for by treatment N applied.

The pre-existing soil-water sampling units were designed to avoid cross-contamination between these single N source treatments. In Moorepark, 8×8 m plots separated from each other by a 3 m buffer strip were instrumented with one to three ceramic suction cups that were installed at four 'shallow' (0.9 m, 1.0 m, 1.2 m and 1.5 m) and three 'deep' depths (2.0 m, 2.5 m and 3.0 m) (Gibbons et al., 2006). At Knockbeg, 12×30 m plots with a 3 m buffer strip were instrumented with six to eight ceramic suction cups at a single depth of 1.5 m (Hooker, 2005). Lysimeters of Oak Park soils consisted of three outdoor cylindrical undisturbed soil monoliths per treatment, 0.6 m diameter by 1 m depth (Brennan et al., 2010). All three soils had a previous history of intensive farming. However, Moorepark and Knockbeg treatment plots had been set up in early 2001, i.e. more than a year before the first sampling event, to allow residual nitrate from previous treatments to be flushed out of the soil profile (favoured by free drainage and high precipitation). Lysimeters of Oak Park soils had been isolated from their original environment since the early 1990's, and have been subject to lower N inputs with cut only regime and no animal dung/urine deposition since that time.

2.2 Sampling programme

Suction cups were sampled less than fourteen days after applying a negative initial pressure up to 50 mBar, while lysimeters were sampled at the outlet where leachates drained by gravity. Sampling time was dictated by recharge conditions and nitrate concentrations in the unsaturated zone, both being sufficiently high after the start of the recharge season in late autumn (Gibbons et al., 2006) to allow isotopic analyses. Variable drainage conditions within plots meant that a single suction cup rarely yielded enough nitrate for isotopic analyses, and therefore, samples often had to be aggregated (Table 3). In Moorepark, forty soil-water samples collected on four occasions (December 2002, June 2003, April 2004 and June 2004) were the result of combining the 'shallow' depths (labelled as depth 1.0 m) and the 'deep' depths (labelled as depth 2.5 m). Likewise, nine samples collected at Knockbeg (March 2004) were the result of aggregating replicated cups. Distinctly, all six lysimeters of Oak Park soil (November 2003) yielded enough water and nitrate for chemical and stable isotope analyses.

2.3 Chemical and isotope abundance analyses

Upon collection into polyethylene bottles, water samples were kept chilled into cool boxes for transport to the laboratory, where they were 0.45 μm nylon-filtered and stored at 4 °C. NO_3^- concentrations, reported in mg L^{-1} NO_3^- (Table 4), were measured within 24 hours of collection by cadmium reduction with a flow injection analyser (Bran & Luebbe Auto-Analyser AA3) (Minet, 2007).

Within 48 hours of collection, 6.2 mg NO_3^- (i.e. 100 μmol) were extracted according to a simplified 'ion-exchange resin method' best suited for freshwater samples with high nitrate (> 25 mg L^{-1} NO_3^-) and low dissolved organic carbon (DOC) levels (typically < 5 mg L^{-1} C) (Minet et al., 2011). In brief, water samples were i) treated with barium chloride to precipitate out major O-bearing

contaminants (mainly sulphate), ii) passed through an anion exchange resin highly selective to nitrate (barium cations and DOC had been left in solution); this was followed by iii) nitrate elution with a hydrochloric solution, iv) neutralisation with silver oxide to form silver nitrate ($AgNO_3$) and v) freeze-drying. % N analyses of $AgNO_3$ (Table 4) confirmed a consistent preparation, 87 % of samples (48/55) displaying values between 6.2 and 8.2. Sample preparation was also efficient since the median % N value was 7.6, which compared well with the value of 8.2 % N expected for pure $AgNO_3$. It should be noted that the extraction lost efficiency with low nitrate water, as witnessed in the Oak Park control and the Moorepark slurry plots from which a few $AgNO_3$ samples of low purity (% N between 1.7 and 5) were produced. The nature of that contamination was unknown but it was not thought to be nitrogenous. In fact, % C analysis results remained below detection limit to rule out the presence of DOC, i.e. a common source of unwanted N. $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values (Table 4), determined in duplicate by Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS), were expressed in permil ‰ (relative to AIR and VSMOW respectively) using the standard definition of the δ-value of the heavier isotope (h) of a given chemical element (E), $\delta^hE = \{(R_{sample} - R_{std}) / R_{std}\}\times 1000$, where R represents $^{15}N/^{14}N$ or $^{18}O/^{16}O$ ratios in samples (R_{sample}) and standards (R_{std}) (Kendall and Aravena, 2000). Analyses were carried out as reported in Minet et al. (2011). For quality control and normalisation purposes a laboratory standard L-alanine ($\delta^{15}N_{AIR}$ = -1.7 ‰) was run in blocks each before, during, and after actual samples. Similarly, reference material IAEA-NO-3 ($\delta^{18}O_{VSMOW}$ = 25.6 ‰) was run and the $\delta^{18}O$ consensus value ($\delta^{18}O_{VSMOW}$ = 25.6 ‰) used to quality control and normalise $\delta^{18}O$ sample values. Silver boats (4 × 6 mm) used to encapsulate samples and reference materials were also inserted empty at the beginning of each batch for blank correction. Quality of ^{18}O isotope abundance analysis by TC/EA-IRMS was later monitored by analysis of reference materials USGS34 and USGS35 ($\delta^{18}O_{VSMOW}$ = -27.9 ‰ and 57.5 ‰, respectively). Observed $\delta^{18}O$ values for USGS34 and USGS35 were -28.0 ±0.1 ‰ and 57.4 ±0.2 ‰, respectively. Standard deviations (SDs) for scale corrected $\delta^{15}N$ values of $AgNO_3$ soil-

water samples were in line with the analytical precision of the instrument (≤ 0.1 ‰, measured from L-alanine): SDs was always better than 0.3 and ≤ 0.1 ‰ in 96 % of the samples (53/55). For $\delta^{18}O$ values of $AgNO_3$ soil-water samples, SDs were ≤ 0.5 in 60 % of the analysed samples (32/53) but always better than 1.1 ‰ (analytical precision ≤ 1.1 ‰ in batches where $AgNO_3$ soil-water samples were analysed).

In addition to soil-water, applied N and soils (top 10 cm) were analysed. Fertilisers were finely ground before measuring $\delta^{15}N$-TN ($\delta^{15}N$ in total nitrogen), while the nitrate fraction of two nitrate fertilisers was extracted and converted into silver nitrate for $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- determination. Dairy wastewater and slurry, frozen after collection, were freeze-dried and finely ground for $\delta^{15}N$-TN measurements. Two representative samples of each soil were frozen after collection, dried at 60 ºC, 2 mm-sieved and finely ground. 0.5 g was then decalcified in duplicate with an acid washing (13 mL of 0.5 M HCl) (carbonates can generate carbon monoxide m/z 28 that interferes with ^{15}N analysis), rinsed twice with deionised water and dried before insoluble N was analysed for $\delta^{15}N$-TN in tin capsules (Midwood and Boutton, 1998). For information, $\delta^{15}N$-TN in acidified soil was very similar to that of untreated samples (results not shown), the difference between the two methods (acidified minus untreated) ranged from -0.4 to +0.2 ‰. Differences in soil C content before and after acidification were within the analytical precision of the instrument (results not shown), suggesting that soil contained little carbonate. All CF-IRMS measurements were duplicated

2.4 Data analysis

The efficiency of stable isotope analyses in identifying NO_3^- sources was estimated after comparing $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- data measured in soil-water nitrate with values expected from applied N

(all three soils). The ability to discriminate N application types relative to one another was gauged by comparing δ values between treatments (Moorepark, Oak Park). Further disparities were sought from Spearman's rank correlation coefficients r_s between δ variables and nitrate concentration (Moorepark, Knockbeg). The influence of application rates on δ values and nitrate leaching was examined within treatments (Moorepark, Knockbeg). Finally, δ values were compared at two depths representing different points in the leaching process (Moorepark).

3. RESULTS

3.1 Nitrate concentrations

Unfertilised controls and slurry treatments generated little to moderate nitrate leaching, with NO_3^- concentrations in soil-water as low as 2.6 mg L^{-1} and no higher than 34 mg L^{-1} (Figure 2). By contrast, the other N treatments clearly increased N losses. In the artificial fertiliser plots/lysimeters, NO_3^- levels in soil-water ranged from 13.2 to 152.2 mg L^{-1}. The second largest range of values was met in the dairy wastewater treatment where NO_3^- concentrations were between 20 and 108.8 mg L^{-1}.

3.2 Comparisons between measured and expected δ values

- Isotopic composition of N sources and expected δ values for soil-water nitrate

Noticeable differences were observed between all N sources in terms of ^{15}N content (Table 5). Artificial fertilisers had by far the lowest $\delta^{15}N$ values ($\delta^{15}N$-TN and $\delta^{15}N$-NO_3^- between -1.5 and 0.4 ‰), followed by dairy wastewater ($\delta^{15}N$-TN of 3.8 ‰) and then slurry (greatest ^{15}N-enrichement with $\delta^{15}N$-TN of 10.2 ‰). Soil N from unfertilised controls displayed intermediate values between

these last two N sources, with δ^{15}N-TN ranging between 3.8 ‰ (see Oak Park soil) and 5.4 ‰ (see Moorepark soil) (Knockbeg fertilised soil noticeably showed δ^{15}N-TN very similar to Moorepark soil values). Since mineralisation causes little isotopic fractionation and δ^{15}N-NO_3^- shifts towards δ^{15}N-TN of the source as nitrification goes on in non N-limited systems (Kendall and Aravena, 2000), it was expected that in the hypothetical absence of any interference, nitrate derived from these N sources would display δ^{15}N-NO_3^- in soil-water within the same ranges.

Three main theoretical ranges were expected for δ^{18}O-NO_3^- in soil-water. Firstly, leached synthetic nitrate should display values slightly above 18 ‰, as measured in fertiliser nitrate (Table 5). Secondly, other forms of N that underwent nitrification should have much lower δ^{18}O-NO_3^-. If assuming that the oxidation of ammonia incorporates two oxygen atoms (O) from water (H_2O) and one from air (O_2) (Andersson and Hopper, 1983; Hollocher, 1984), a theoretical range of δ^{18}O-NO_3^- values can be calculated for biologically formed nitrate (Equation 1).

$$\delta^{18}\text{O-NO}_3^- = 2/3\ \delta^{18}\text{O-H}_2\text{O} + 1/3\ \delta^{18}\text{O-O}_2 \qquad \text{(Equation 1)}$$

Based on δ^{18}O-H_2O measured between -8.9 and -5.9 ‰ in local groundwater (Minet, 2007) and δ^{18}O-O_2 of 23.5 ‰ reported for atmospheric O_2 (Kroopnick and Craig, 1972), δ^{18}O-NO_3^- nitrification values could then range between 1.9 and 3.9 ‰. However, Equation 1 makes the few (debatable) assumptions that i) O_2 and H_2O contributions to O incorporation observed under laboratory conditions with chemolithoautotrophic organisms are similar in undisturbed natural soils, ii) O incorporation occurs without isotopic fractionation, iii) δ^{18}O-O_2 of incorporated O is identical to that of atmospheric O_2 and iv) δ^{18}O-H_2O of incorporated O is identical to that measured in precipitation, soil-water or groundwater bulk samples (Kendall and Aravena, 2000).

Between synthetic and nitrified NO_3^-, a great variety of other values were also expected from mixed fertiliser applications. Based on % N applied as artificial NO_3-N (Table 2), it was estimated that urea plus calcium ammonium nitrate (CAN) (Moorepark) could give rise to $\delta^{18}O\text{-}NO_3^-$ between 8.6 and 9.9 ‰, SuperNet and compound 18-6-12 to values between 9.0 and 10.2 ‰ (Knockbeg, high rate spring barley), and other applications to values between 10 and 11.2 ‰. Overall, artificial fertiliser N could then generate a third set of $\delta^{18}O\text{-}NO_3^-$ values between 8.6 and 11.2 ‰.

- Comparison of measured and expected δ values for soil-water nitrate

Measured and expected $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values are plotted in Figure 3. Except for two low rate spring barley samples from Knockbeg (Lsb treatment), which yielded values of -0.4 ‰, $\delta^{15}N\text{-}NO_3^-$ in soil-water from artificial fertiliser treatments (between 0.4 and 6.6 ‰) was found to be consistently ^{15}N-enriched in comparison with the range of -1.5 to 0.4 ‰ expected from the analysis of artificial fertilisers. In the dairy wastewater treatment, soil-water $\delta^{15}N\text{-}NO_3^-$ (between 4.3 and 7.6 ‰) was likewise slightly higher than the value of 3.8 ‰ expected from the wastewater analysis. By contrast, the slurry treatment plots leached nitrate with $\delta^{15}N\text{-}NO_3^-$ between 5.1 and 9.2 ‰, i.e. lower than the value of 10.2 ‰ expected from the slurry analysis. In the Moorepark unfertilised control plot however, $\delta^{15}N\text{-}NO_3^-$ in the suction cups was measured between 5.1 and 7.2 ‰, which encompassed the 5.2 to 5.4 ‰ range of soil $\delta^{15}N$-TN (from the analyses of 0-10 cm depth soil samples). In the Oak Park control lysimeters, measured $\delta^{15}N\text{-}NO_3^-$ in the drainage water (between 2.3 and 2.9 ‰) was slightly lower than the 3.8 to 3.9 ‰ range of Oak Park soil $\delta^{15}N$-TN.

High $\delta^{18}O\text{-}NO_3^-$ above 15 ‰ was recorded in Lsb samples. However, much lower $\delta^{18}O\text{-}NO_3^-$ were observed in other artificial fertiliser plots and lysimeters (between -1.4 and 7.4 ‰). These values were well below the range expected from the direct leaching of artificial pre-formed nitrate (about

18 ‰) or from the leaching of a mixture of artificial nitrate and nitrified artificial N (between 8.6 and 11.2 ‰). In fact, $\delta^{18}O\text{-}NO_3^-$ widely encompassed the range expected for nitrification (calculated between 1.9 and 3.9 ‰). On the other hand, soil-water samples collected from dairy wastewater, slurry and unfertilised treatments showed a narrow range of $\delta^{18}O\text{-}NO_3^-$ values between -1.2 and 2.8 ‰, overlapping with the lower end of the range expected for nitrification.

3.3 Comparisons of measured δ values between N treatments

- $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values

Despite an overlap with the lower end of the dairy wastewater range between 4 and 5 ‰, most $\delta^{15}N\text{-}NO_3^-$ values measured in Moorepark artificial fertiliser plots were lower than in other Moorepark treatments (Figure 4A). On the other hand, $\delta^{15}N\text{-}NO_3^-$ in Moorepark slurry treatments and the control were very similar, and their ranges were encompassed by that of the dairy wastewater treatments (except for one outlier). $\delta^{18}O\text{-}NO_3^-$ values followed a different pattern (Figure 4B), all values being within the range displayed by soil-water nitrate from the fertiliser plots (between -1.4 and 2.9 ‰).

Some contrasts were also observed between treatments on Oak Park soils. Artificially fertilised lysimeters displayed $\delta^{15}N\text{-}NO_3^-$ values which were 1.2 ‰ lower than the unfertilised controls (Figure 5A). Conversely, $\delta^{18}O\text{-}NO_3^-$ was higher by 3.4 ‰ where artificial fertiliser was applied (Figure 5B).

- Correlation coefficients

Strong relationships with high Spearman's rank correlation coefficients ($0.691 \leq r_s$ absolute value ≤ 0.946) and high significance levels ($p \leq 0.05$ at the very least) were observed in artificial fertiliser treatments at Moorepark (Table 6A) and Knockbeg (Table 6B). Nitrate concentration (y coordinate) was negatively correlated with $\delta^{15}N\text{-}NO_3^-$ (x coordinate) (slopes of -38 and -15.6 in Moorepark and Knockbeg respectively) while positively correlated with $\delta^{18}O\text{-}NO_3^-$ (x coordinate) (slopes of 21.7 and 6 in Moorepark and Knockbeg respectively). Consequently, $\delta^{18}O\text{-}NO_3^-$ (y coordinate) was negatively correlated with $\delta^{15}N\text{-}NO_3^-$ (x coordinate) (slopes of -1.1 and -2.1 in Moorepark and Knockbeg respectively).

By contrast, organic wastes applied to Moorepark soils were not associated with strong relationships. No significant correlation was detected in the slurry treatment (Table 6D), while only one significant correlation ($p \leq 0.01$) occurred in the dairy wastewater plots (Table 6C): $\delta^{18}O\text{-}NO_3^-$ (y coordinate) was correlated with $\delta^{15}N\text{-}NO_3^-$ (x coordinate) (slope of 0.63). Not enough data were available in the controls (Moorepark, Oak Park) to explore relationships.

3.4 Comparisons of nitrate concentrations and δ values between application rates

- Artificial fertiliser treatments

Nitrate concentrations in Moorepark soil-water markedly increased under higher application rates of artificial fertiliser (Figure 6A): means of 35.6, 73.2 and 130.1 mg L^{-1} NO_3^- at low, medium and high rate, respectively. Larger fertiliser inputs were associated with lower $\delta^{15}N\text{-}NO_3^-$ (means of 4.6, 3.7 and 2.6 ‰ at low, medium and high rate, respectively) and higher $\delta^{18}O\text{-}NO_3^-$ values (means of -0.2, 0.4 and 2.4 ‰ at low, medium and high rate, respectively) in soil-water (Figure 6B).

Unlike Moorepark soils, nitrate leaching and artificial fertiliser inputs on the Knockbeg tillage plots did not increase together (Figure 7A). In the spring barley plots, high NO_3^- levels were observed at the lower fertiliser application rate (mean of 149 mg L^{-1} NO_3^-), whereas lower concentrations were measured at high rate (mean of 85.8 mg L^{-1} NO_3^-). In the winter wheat plots, NO_3^- concentrations were slightly above 50 mg L^{-1} NO_3^- regardless of the fertiliser application rate. Stable isotope ratios in Knockbeg tillage plots (Figure 7B) also followed a different pattern from that witnessed at Moorepark artificial fertiliser plots. The lowest $\delta^{15}N\text{-}NO_3^-$ (-0.4 ‰) was measured in low rate spring barley plots (Lsb), along with the highest $\delta^{18}O\text{-}NO_3^-$ values (15.1 and 17 ‰). $\delta^{15}N\text{-}NO_3^-$ in other Knockbeg artificially fertilised plots were higher and $\delta^{18}O\text{-}NO_3^-$ much lower.

- Dairy wastewater treatments

Higher applications rates of dairy wastewater were associated with a moderate increase in soil-water nitrate concentrations (Figure 8A): means of 43.7 and 62.7 mg L^{-1} NO_3^- at medium and high rate, respectively. Higher inputs were accompanied with a slight increase in both $\delta^{15}N\text{-}NO_3^-$ (means of 4.9 and 5.8 ‰ at medium and high rate, respectively) and $\delta^{18}O\text{-}NO_3^-$ (means of 0.9 and 1.2 ‰ at medium and high rate, respectively) (Figure 8B).

- Slurry treatments

Minor differences in soil-water nitrate concentrations or isotopic values were observed between low, medium and high application rates of slurry. Except for one outlier, nitrate levels remained consistently low (< 23 mg L^{-1} NO_3^-). The only sample from the low rate plot showed the highest $\delta^{15}N\text{-}NO_3^-$ value (9.2 ‰), whereas medium and high rate plots showed similar ranges (between 5.1 and 6.2 ‰). As for $\delta^{18}O\text{-}NO_3^-$, it ranged between -1 and 2.3 ‰.

3.5 Influence of depth on nitrate concentrations and δ values

Comparing nitrate concentrations between the 2.5 m and 1.0 m depths of the Moorepark plots (Table 4) showed high variations within treatments and sampling events. The difference ranged from -25.8 to +54.4 mg/L NO_3^- in the artificial fertiliser plots (eight comparisons), from -52.4 to +39.9 mg/L NO_3^- in the dairy wastewater plots (six comparisons), from +11.9 to +14 mg/L NO_3^- in the in slurry plots (two comparisons) and from -6.3 to -4.4 mg/L NO_3^- in the control plot (two comparisons). However, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values fell within similar ranges at both depths. In the artificial fertiliser plots, the difference in δ values between 2.5 m and 1.0 m depths ranged from -0.5 to +2.3 ‰ ($\delta^{15}N\text{-}NO_3^-$) and from -1.7 to 0.4 ‰ ($\delta^{18}O\text{-}NO_3^-$). In the dairy wastewater plots, this difference ranged from -1.5 to +2.8 ‰ ($\delta^{15}N\text{-}NO_3^-$) and from -1.4 to +1.7 ‰ ($\delta^{18}O\text{-}NO_3^-$), whereas in the slurry and the control plots, it ranged from -0.8 to +0.2 ‰ ($\delta^{15}N\text{-}NO_3^-$) and from -3.2 to +0.1 ‰ ($\delta^{18}O\text{-}NO_3^-$).

4. DISCUSSION

4.1 Identifying nitrate sources

In a context favourable to NO_3^- source tracking (soils very responsive to varying N inputs with nitrate concentrations often elevated, good drainage assumed to limit denitrification and the associated isotopic fractionation), it was hypothesised that leached nitrate would display the isotopic signature expected from treatment N (section 3.2). However, the ^{15}N content of soil-water nitrate poorly reflected that of applied N (Figure 3), highlighting the limitations of using ^{15}N to clearly associate nitrate leaching to N inputs. Despite concentrations up to three times the limit set in the Nitrates Directive and Groundwater Directive (Figure 2), nitrate in artificial fertiliser and

dairy wastewater plots was consistently ^{15}N-enriched in comparison with treatment N, except for two low rate spring barley soil-water samples (Lsb) ('FER_Kno' samples in Figure 3). By contrast, the little nitrate leached from the slurry plots displayed δ^{15}N-NO_3^- lower than expected. These findings suggested that nitrate leaching originated dominantly from a source with an intermediate ^{15}N content higher than in artificial fertiliser and dairy wastewater but lower than in slurry. This description matched that of soil insoluble organic N (micro-organisms, plant and root residues, organic molecules, decomposed organic matter) whose δ^{15}N-TN was measured across all three soils between 3.8 and 5.6 ‰ (in agreement with values reported by Heaton (1986)). Experiments in Moorepark (δ^{15}N-TN in soil N between 5.2 and 5.4 ‰) supported such an assumption as the unfertilised control plot, whose main N source is soil organic N, yielded nitrate with intermediate δ^{15}N-NO_3^- (between 5.1 and 7.2 ‰). In the Oak Park controls, δ^{15}N-NO_3^- was a bit lower than 3.8 ‰, but values were still higher than δ^{15}N-TN of artificial fertiliser applied to fertilised lysimeters. NO_3^- levels recorded close to 25 mg L^{-1} in the Moorepark control (Table 4) confirmed that soils in previously intensively managed grassland can release large quantities of N through mineralisation of organic matter (Gill et al., 1995). Oak Park controls leached comparatively far less nitrate, but these lysimeters had a lower N input history for more than a decade. In the case of slurry applications, lower quantities of applied N than in other treatments (Table 2), a larger fraction of organically bound N (i.e. N not readily available) and favouring of bacterial immobilisation (due to higher C/N ratio) possibly meant slower nitrate release from soils (Hoekstra et al., 2010). Studies have also shown that when applied as readily available nitrogen, almost all added N ends up incorporated in the biomass while very little is left unused (Cookson et al., 2002). At the same time, such addition can induce a positive priming effect, i.e. the mineralisation and nitrification of soil organic N is stimulated (Kuzyakov et al., 2000). Artificial fertiliser and dairy wastewater treatments, whose N consisted largely of NO_3^- and NH_4^+ (Table 3), may then have promoted nitrate

production from soil organic N, herein further masking the cause of leaching from the isotopic prospective.

Except for both Lsb samples (low rate spring barley), $\delta^{18}O\text{-}NO_3^-$ in all artificial fertiliser treatments ranged far below ranges expected for synthetic fertiliser ('FER' samples in Figure 3), which questioned the utility of ^{18}O to identify artificial fertiliser nitrate. Values were in fact much closer to the expected range of nitrification. A combination of two factors may have contributed to explain the absence of higher $\delta^{18}O\text{-}NO_3^-$ values in the soil-water from the artificial fertiliser experiments. Mengis et al. (2001) linked this to the occurrence of MIT processes: large gross rates of NO_3^- immobilisation by bacteria followed by rapid remineralisation would mask the original high $\delta^{18}O\text{-}NO_3^-$ values (similarly, the mineralisation and nitrification of fertiliser nitrate taken up by plants will have the same effect). An alternative hypothesis by Roadcap et al. (2002) is that fertiliser pre-formed nitrate is rapidly taken up by plants, leaving only the non-nitrate fraction (i.e. NH_4^+) available for nitrification and subsequent leaching. However, some plants (especially grass) tend to utilise NH_4^+ more readily than NO_3^- (Whitehead, 1995), the latter possibly being too mobile an anion to be entirely taken up. Therefore, fertiliser NH_4^+ seemed unlikely to be a primary source of nitrate leaching, giving more credence to the MIT hypothesis. Interestingly, the data from the two Lsb plots, i.e. occurrence of high $\delta^{18}O\text{-}NO_3^-$ (> 15 ‰) in conjunction with low $\delta^{15}N\text{-}NO_3^-$ (< 0 ‰) and high nitrate concentrations (> 120 mg L^{-1} NO_3^-), proved that direct leaching of fertiliser nitrate can be identified using dual stable isotope analyses in the soil zone in some limited circumstances (see section 4.3).

$\delta^{18}O\text{-}NO_3^-$ in controls, dairy wastewater and slurry plots should match values expected for biologically formed nitrate. Instead, measured values narrowly overlapped with the lower end of the 1.9 to 3.9 ‰ range calculated for nitrification ('CTL', 'SLR' and 'DW' samples in Figure 3). These results highlighted the difficulty to predict nitrification $\delta^{18}O\text{-}NO_3^-$ values from Equation 1 (section

3.2), which does not take into account potential nitrite-water oxygen exchange or isotopic fractionations (kinetic fractionation during O incorporation from O_2 and H_2O, equilibrium fractionation for the nitrite-water equilibrium) (Snider et al., 2010). Laboratory experiments with marine nitrifiers suggest that the overall isotopic enrichment by nitrification is negative (Snider et al., 2010). If these studies are valid in natural soil conditions, microbial nitrate should be depleted in ^{18}O relative to O_2 and H_2O, and calculated $\delta^{18}O\text{-}NO_3^-$ value should then be lower than that given by Equation 1, as suggested by our results. However, other parameters can complicate this picture (e.g. evaporation and respiration in top soil), and most studies have in fact reported $\delta^{18}O\text{-}NO_3^-$ values higher than that given by Equation 1 (Snider et al., 2010). Using groundwater $\delta^{18}O\text{-}H_2O$ rather than soil-water values in Equation 1 probably added some bias to the calculation. Using precipitation $\delta^{18}O\text{-}H_2O$ values (-8.1 to -2.7 ‰) from the closest International Atomic Energy Agency station at Valentia Island (IAEA/WMO, 2006) would have only widened the gap between calculated and observed $\delta^{18}O\text{-}NO_3^-$ values.

Depth at Moorepark had no noticeable effect on $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$, which remained relatively unchanged within treatment and sampling event at 1.0 m and 2.5 m depth (section 3.5). This suggests that soil-water nitrate underwent very little transformation below the 1.0 m zone, and therefore, the isotopic signature was acquired earlier during the leaching process.

4.2 Discriminating between N treatments

Differences useful for differentiating some N source treatments from one another emerged in section 3.3. Essentially, artificial fertiliser plots/lysimeters leached nitrate with $\delta^{15}N\text{-}NO_3^-$ clearly lower than in unfertilised controls or where dairy wastewater and slurry were applied (Figures 4A and 5A). Similar differences have often been reported in the literature between artificial fertiliser

and organic waste, but more rarely between artificial fertiliser and soil N (Fogg et al., 1998; Kendall and Aravena, 2000). Two concomitant factors may explain this outcome. Firstly, applied N influences the ^{15}N content of soil organic N and the subsequent product of nitrification (Choi et al., 2002). In the case of artificial fertiliser applications, the incorporation of N with low $\delta^{15}N$ in the tissues of plants, micro- and macro-living organisms should generate NO_3^- slightly ^{15}N-depleted compared with other treatments. Secondly, the small proportion of artificial fertiliser N not taken up by the biomass mixes with soil-derived nitrate and lowers soil-water $\delta^{15}N\text{-}NO_3^-$ values. No such difference was revealed between the dairy wastewater and control treatments, as N source $\delta^{15}N$-TN values of dairy wastewater and soil organic N were much closer to one another. More elevated $\delta^{15}N\text{-}NO_3^-$ values could have been expected from slurry plots owing to ammonia volatilisation and denitrification that typically trigger large isotopic fractionation (Kendall and Aravena, 2000). However, slurry was used as a diffuse source that provided a limited N and C supply less likely to shift $\delta^{15}N\text{-}NO_3^-$ towards very high values. Besides, rainy conditions often met in Ireland (Figure 1) could have curtailed ammonia volatilisation, not only from applied slurry but also from applied dairy wastewater and artificial fertiliser (which both contain large quantities of ammonium). Less contrasting results were observed with $\delta^{18}O\text{-}NO_3^-$. All values in the Moorepark treatments fell within the range of the artificial fertiliser plots, close to that expected for nitrification (Figure 4B). $\delta^{18}O\text{-}NO_3^-$ was higher in the Oak Park fertilised lysimeters than in the controls (Figure 5B), but linked with low $\delta^{15}N\text{-}NO_3^-$, this suggests that some synthetic nitrate was leached (the more favourable drainage conditions might give less time to bacteria for MIT to fully operate).

Matrices of correlation coefficients (Table 6) further highlighted differences between treatments. (While these coefficients cannot be taken as a definite guide to relationships owing to correlations forced by outliers or the possibility of non-linearity often met with stable isotopes, they provide indications of relationships worthy of further exploration.) Under artificial fertiliser applications,

497 higher nitrate concentration was related with lower δ^{15}N-NO_3^- ($p \leq 0.0001$) and higher δ^{18}O-NO_3^- (p
498 ≤ 0.01), which is consistent with the leaching of an increasing proportion of synthetic NO_3^- and
499 fertiliser-derived nitrate (i.e. nitrified artificial NH_4^+). Importantly, δ^{15}N-NO_3^- was negatively
500 correlated with δ^{18}O-NO_3^- ($0.001 \leq p \leq 0.05$ depending on the soil), which contrasted with the
501 positive correlation observed with dairy wastewater application ($p \leq 0.01$). With a slope of 0.63, the
502 latter correlation suggested the occurrence of denitrification affecting the entire nitrate pool, in line
503 with other studies where slopes close to 0.5 have been reported (Chen and MacQuarrie, 2005).
504
505 ***4.3 Influence of application rates on δ values***
506
507 The apparent linear response of grassland soil leaching (Moorepark) to applications of artificial
508 fertiliser was related to input rates in a systematic pattern (section 3.4) similarly witnessed by
509 Barraclough et al. (1984): increasing CAN application rate resulted in much higher soil-water
510 nitrate concentrations (Figure 6A) and proportionally higher losses of synthetic NO_3^- and fertiliser-
511 derived nitrate (Figure 6B), widening the gap in δ^{15}N-NO_3^- values with the control and organic
512 wastes treatment plots. These results also highlighted that highly managed grassland can be at risk
513 of high nitrate leaching, even though application rates in Moorepark fertiliser plots were high but in
514 line with Teagasc recommendations based on the relationship between soil analysis and nutrient
515 requirements (Coulter et al., 2002).
516 No such systematic pattern was observed in tillage soils (Knockbeg). Higher application rates for
517 spring barley and winter wheat matched Teagasc recommendations, but in the lower rate Lsb,
518 fertiliser inputs were below agronomic crop requirements (Figure 7A). The highest nitrate levels
519 were recorded in the Lsb plots, as previously observed by Hooker (2005), where δ^{15}N-NO_3^- and
520 δ^{18}O-NO_3^- values were close to that of synthetic nitrate (Figure 7A). Reasons for this unexpected
521 outcome were unclear.

Increase in the application rates of organic wastes led to far less contrasting results than from the artificial fertiliser applications. $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values from the dairy wastewater plots increased slightly (along with nitrate levels) (Figures 7A and 7B), suggesting limited denitrification of the whole NO_3^- pool stimulated by larger carbon availability and higher soil moisture content (Whitehead, 1995). As for the slurry treatment, nitrate levels were very low and δ values remained within similar ranges irrespective of the input rates.

5. CONCLUSIONS

- ^{15}N isotope abundance analyses in nitrate were of limited assistance in clearly associating the origin of NO_3^- in the unsaturated zone with N inputs, because soil-water nitrate poorly mirrored the ^{15}N content of applied N in spite of NO_3^- concentrations often elevated. Results suggested that leaching originated dominantly from soil organic N, whose mineralisation and nitrification may have been stimulated by previous intensive managements and current N treatments (priming effect), herein masking the cause of leaching. Direct leaching of synthetic nitrate was unexpectedly identified in tillage under the lower fertiliser rate in the spring barley plots. Despite this, variations which may be useful to differentiate some N source treatments from one another emerged: $\delta^{15}N\text{-}NO_3^-$ values were consistently lower where artificial fertiliser was applied than in unfertilised controls and organic waste treatments (dairy wastewater, slurry).
- ^{18}O isotope abundance analyses in soil-water nitrate were of little use to identify nitrate sources as most $\delta^{18}O\text{-}NO_3^-$ values were close to the range expected for nitrification, regardless of the N treatment. This was attributed to the occurrence of mineralisation-immobilisation turnover processes.

- Relationships between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ differed drastically in soil-water from the artificial fertiliser and the dairy wastewater treatments: δ variables were negatively correlated in the former ($0.001 \leq p \leq 0.05$, attributed to the varying proportion of fertiliser-derived and synthetic nitrate being leached) and positively correlated in the latter ($p \leq 0.01$, attributed to limited denitrification). These results suggest that it may be possible to distinguish artificial fertiliser and organic wastes contaminations if analysing correlations between δ variables in the unsaturated zone.
- In grassland, higher artificial fertiliser input rates were associated with a sharp rise in nitrate concentrations and a growing proportion of fertiliser-derived and synthetic nitrate leached. As a consequence, the differences in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values between the artificial fertiliser treatment and other treatments became more noticeable.

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647
648 **TABLE TITLES**
649
650 Table 1: Soil classification (FAO-UNESCO, 1988), particle size analysis and organic matter content
651 (% OM) in topsoil ([1] Kramers et al., 2009; [2] Teagasc, unpublished data; [3] Brennan et al., 2010).
652 Table 2: Description of N treatments (unfertilised control as 'CTL', artificial fertiliser as 'FER',
653 dairy wastewater as 'DW', cow slurry as 'SLR'), application rates (high as 'H', medium as 'M',
654 low as 'L'), land use (grassland as 'g', spring barley as 'sb', winter wheat as 'ww'), application
655 times and N inputs characteristics over Moorepark, Knockbeg and Oak Park soils.
656 Table 3: Mean concentrations of total nitrogen (TN), ammonium ($NH4^+$-N) and nitrate nitrogen
657 (NO_3^--N) in cow slurry (6.6 ±2.7 ‰ dry matter content) and dairy wastewater at Moorepark
658 Research Centre between 2002 and 2004 (Ryan et al., 2006)
659 Table 4: Nitrate concentrations, stable isotope composition of soil-water nitrate and % N content of
660 silver nitrate (AgNO3) under different N treatments (unfertilised control as 'CTL', artificial
661 fertiliser as 'FER', dairy wastewater as 'DW', slurry as 'SLR'), application rates (high as 'H',
662 medium as 'M', low as 'L'), land use (grassland as 'g', spring barley as 'sb', winter wheat as
663 'ww') and depths (with detail of aggregated depths for Moorepark soil experiment) on
664 Moorepark, Knockbeg and Oak Park soils.
665 Table 5: Stable isotope composition of total nitrogen (TN) and nitrate fractions (artificial fertilisers
666 only) in N sources at Moorepark (Moo), Knockbeg (Kno) and Oak Park (Oak) (adapted from
667 Minet (2007)).

Table 6: Correlation matrices of Spearman's rank r_s values between nitrate concentration, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ in **A**) Moorepark fertiliser plots (FER_Moo, $15 \leq n \leq 16$), **B**) Knockbeg fertiliser plots (FER_Kno, n = 9), **C**) Moorepark dairy wastewater plots (DW_Moo, n = 12) and **D**) Moorepark slurry plots (SLR_Moo, n = 7) (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

FIGURE TITLES

Figure 1: Monthly precipitation, calculated monthly effective rainfall (precipitation minus evapotranspiration minus surface runoff) and daily air temperature at the weather station in Oak Park Research Centre between February 2002 and June 2004 (Minet, 2007).

Figure 2: Ranges of soil-water nitrate concentration in the unfertilised controls (CTL, n = 8), cow slurry (SLR, n = 7), dairy wastewater (DW, n = 12) and artificial fertiliser treatments (FER, n = 27) across all soils.

Figure 3: Scatterplot of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values measured in soil-water nitrate collected from Moorepark (Moo), Knockbeg (Kno) and Oak Park soils (Oak) subject to applications of artificial fertiliser (FER), dairy wastewater (DW), slurry (SLR) or no application (CTL), and comparison with δ values expected from the analysis of each treatment N source (delineated by boxes).

Figure 4: Distribution of **A**) $\delta^{15}N\text{-}NO_3^-$ and **B**) $\delta^{18}O\text{-}NO_3^-$ measured in soil-water nitrate from the following Moorepark treatment plots: control (CTL), slurry (SLR), dairy wastewater (DW) and artificial fertiliser (FER).

Figure 5: Distribution of **A**) $\delta^{15}N\text{-}NO_3^-$ and **B**) $\delta^{18}O\text{-}NO_3^-$ measured in soil-water nitrate from the following Oak Park treatment lysimeters: control (CTL) and artificial fertiliser (FER).

692 Figure 6: Variations of **A**) mean soil-water NO_3^- concentration (± standard deviation) with
693 application rate and **B**) $\delta^{15}N\text{-}NO_3^-$ with $\delta^{18}O\text{-}NO_3^-$ (with linear regression line) in the Moorepark
694 artificial fertiliser treatment (high rate H marked with hollow rounds, medium rate M with
695 diamonds, low rate L with crosses; grassland (g) land use).
696 Figure 7: Variations of **A**) mean soil-water NO_3^- concentration (± standard deviation) with
697 application rate and **B**) $\delta^{15}N\text{-}NO_3^-$ with $\delta^{18}O\text{-}NO_3^-$ (with linear regression line) in the Knockbeg
698 artificial fertiliser treatment (high rate H marked with rounds and low rate L with crosses; spring
699 barley (sb) land use designated by shaded marks and winter wheat (ww) by unshaded marks).
700 Figure 8: Variations of **A**) mean soil-water NO_3^- concentration (± standard deviation) with
701 application rate and **B**) $\delta^{15}N\text{-}NO_3^-$ with $\delta^{18}O\text{-}NO_3^-$ (with linear regression line) in the Moorepark
702 dairy wastewater treatment (high rate H is marked with hollow rounds and medium rate M with
703 diamonds; grassland (g) land use).
704

Table 1: Soil classification (FAO-UNESCO, 1988), particle size analysis and organic matter content (% OM) in topsoil ([1] Kramers et al., 2009; [2] Teagasc, unpublished data; [3] Brennan et al., 2010).

Soil	FAO soil classification	Depth (m)			% sand	% silt	% clay	% OM
Moorepark [1]	haplic cambisol	0.00	–	0.15	53	31	16	8.5
		0.15	–	0.55	55	37	8	3.7
Knockbeg [2]	haplic luvisol	0.00	–	0.30	44	34	22	nd
		0.30	–	0.70	37	33	20	nd
Oak Park [3]	haplic cambisol	0.00	–	0.20	67	23	11	4.9
		0.20	–	0.45	68	20	12	3.4

Nd not determined

Table 2: Description of N treatments (unfertilised control as 'CTL', artificial fertiliser as 'FER', dairy wastewater as 'DW', cow slurry as 'SLR'), application rates (high as 'H', medium as 'M', low as 'L'), land use (grassland as 'g', spring barley as 'sb', winter wheat as 'ww'), application times and N inputs characteristics over Moorepark, Knockbeg and Oak Park soils.

Soil & *treatment*	Application rate (kg N/ha/yr)	Land use	Reps	Type of N applied (% of annual input)	Application time	% N applied as artificial NO_3-N
Moorepark						
CTL	–	g	1	–	–	–
FER	H (387)	g	1	urea (18), CAN (82)	January to September [3]	41 [4]
	M (286)	g	1	urea (18), CAN (82)	January to September [3]	41 [4]
	L (174)	g	1	urea (18), CAN (82)	January to September [3]	41 [4]
DW	H (343)[1]	g	1	dairy wastewater (100)	May & November	0
	M (171)[1]	g	1	dairy wastewater (100)	May & November	0
SLR	H (160)[2]	g	1	slurry (100)	March	0
	M (105)[2]	g	1	slurry (100)	March	0
	L (53)[2]	g	1	slurry (100)	March	0
Knockbeg						
FER	H (137.5)	sb	2	SuperNet (56), 18-6-12 (44)	March to April	43.4 [4]
	L (105)	sb	2	SuperNet (43), 20-0-15 (57)	March to April	50 [4]
	H (225)	ww	3	CAN (100)	March to April	50 [4]
	L (187.5)	ww	2	CAN (100)	March to April	50 [4]
Oak Park						
CTL	–	g	3	–	–	–
FER	H (390)	g	3	urea (18), CAN (82)	January to September [3]	41 [4]

1 estimated from total nitrogen concentrations (257 and 430 mg L^{-1} N in May 2003 and November 2001 respectively (Gibbons et al., 2006)) and volumes applied (high rate: 500 m^3, medium: 250 m^3) in May and November

2 estimated from total nitrogen concentration (3548 mg L^{-1} N in March 2002 (Gibbons et al., 2006)) and volumes applied (high rate: 45 m^3, medium: 30 m^3, low: 15 m^3)

3 urea was applied in late January (single application), CAN was applied quasi-monthly between April and September in seven equal applications

4 calculated from N applied (% annual input) and % NO_3-N provided by manufacturers for urea (0 % NO_3-N), calcium ammonium nitrate (CAN) (50 % NO_3-N), SuperNet (50 % NO_3-N), NPK compounds 18-6-12 (35 % NO_3-N) and 20-0-15 (50 % NO_3-N)

Table 3: Mean concentrations of total nitrogen (TN), ammonium ($NH4^+$-N) and nitrate nitrogen (NO_3^--N) in cow slurry (6.6 ±2.7 ‰ dry matter content) and dairy wastewater at Moorepark Research Centre between 2002 and 2004 (Ryan et al., 2006)

Parameter	**cow slurry (mg/kg, n = 18)**	**dairy wastewater (mg/L, n = 28)**
Total nitrogen (TN)	3392 ±687	289 ±194
Ammonium (NH_4^+-N)	≈ 1696 [1)]	149 ±109
Nitrate (NO_3^--N)	≈ 0 [2)]	≤ 0.2

1) Estimated from TN concentration and an assumed NH_4^+-N/TN ratio of 0.5 (Whitehead, 1995)

2) Assumed null due to high ammonia levels (nitrification inhibited), high dissolved organic carbon and anaerobic conditions (denitrification promoted) (Whitehead, 1995)

Table 4

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Table 4. Nitrate concentrations, stable isotope composition of soil-water nitrate and % N content of silver nitrate (AgNO3) under different N treatments (unfertilised control as 'CTL', artificial fertiliser as 'FER', dairy wastewater as 'DW', slurry as 'SLR'), application rates (high as 'H', medium as 'M', low as 'L'), land use (grassland as 'g', spring barley as 'sb', winter wheat as 'ww') and depths (with detail of aggregated depths for Moorepark soil experiment) on Moorepark, Knockbeg and Oak Park soils.

Soil & *treatment*	Application rate (kg N/ha/yr)	Land use	Aggregated depths (m)	Replicate	Time	mg L^{-1} NO_3^-	$\delta^{15}N$-NO_3^- (‰ AIR)	$\delta^{18}O$-NO_3^- (‰ V-SMOW)	% N (in $AgNO_3$)
Moorepark									
CTL	–	g	1.0 (1.2+1.5)	1	Jun-03	23.1	7.2 ± 0.1	0.7 ± 0.6	6.3 ± 0.1
		g	1.0 (0.9+1.2)	1	Apr-04	34.0	5.9 ± 0.1	1.6 ± 0.1	6.7 ± 0.1
		g	1.0 (0.9+1.2)	1	May-04	31.4	5.9 ± 0.1	0.6 ± 0.7	7.1 ± 0.1
		g	2.5 (2.5+3.0)	1	Jun-03	23.5	6.8 ± 0.1	1.6 ± 0.2	6.9 ± 0.1
		g	2.5 (2.0)	1	May-04	27.0	5.1 ± 0.1	0.0 ± 0.2	7.7 ± 0.1
FER	H (387)	g	1.0 (0.9+1.0)	1	Jun-03	101.9	2.9 ± 0.1	1.2 ± 0.1	7.8 ± 0.1
		g	1.0	1	Apr-04	136.3	2.7 ± 0.1	2.3 ± 0.5	7.8 ± 0.1
		g	1.0	1	May-04	150.0	2.4 ± 0.1	2.9 ± 0.2	7.7 ± 0.1
		g	2.5 (2.5+3.0)	1	Jun-03	112.2	2.5 ± 0.1		8.2 ± 0.1
		g	2.5 (2.5+3.0)	1	Apr-04	136.3	2.6 ± 0.1	2.7 ± 0.1	7.6 ± 0.1
		g	2.5	1	May-04	143.7	2.7 ± 0.1	2.9 ± 0.5	7.7
	M (286)	g	1.0	1	Dec-02	35.4	4.3 ± 0.1	0.3 ± 0.8	7.9 ± 0.1
		g	1.0 (1.0+1.2+1.5)	1	Jun-03	51.9	3.4 ± 0.1	1.6 ± 0.1	7.9 ± 0.1
		g	1.0	1	Apr-04	115.0	1.8 ± 0.1	1.5 ± 0.5	7.7 ± 0.1
		g	1.0 (0.9+1.0)	1	May-04	44.9	4.2 ± 0.1	0.6 ± 0.4	7.7 ± 0.1
		g	2.5	1	Dec-02	89.9	4.1 ± 0.1	-1.4 ± 0.2	7.9 ± 0.1
		g	2.5	1	Jun-03	69.6	3.9 ± 0.1	-0.1 ± 0.1	8.0 ± 0.1
		g	2.5	1	Apr-04	89.2	4.1 ± 0.1	0.8 ± 0.8	7.8 ± 0.1
		g	2.5	1	May-04	89.6	3.9 ± 0.1	0.0 ± 0.7	7.7 ± 0.1
	L (174)	g	1.0 (1.2+1.5)	1	Jun-03	27.9	4.9 ± 0.1	0.5 ± 0.2	8.0 ± 0.1
		g	2.5 (2.0+2.5)	1	Jun-03	43.4	4.4 ± 0.1	-1.0 ± 0.8	8.1 ± 0.1
DW	H (343)	g	1.0	1	Jun-03	24.2	6.1 ± 0.1	0.2 ± 0.2	7.4 ± 0.1
		g	1.0	1	Apr-04	108.8	4.9 ± 0.1	1.1 ± 0.5	7.8 ± 0.1
		g	1.0	1	May-04	76.2	4.5 ± 0.1	0.9 ± 0.3	7.8 ± 0.1
		g	2.5 (2.0+2.5)	1	Jun-03	49.9	4.9 ± 0.1	0.4 ± 0.2	8.1 ± 0.1
		g	2.5	1	Apr-04	56.4	7.6 ± 0.1	2.8 ± 0.8	7.7 ± 0.1
		g	2.5	1	May-04	60.5	6.5 ± 0.1	1.8 ± 0.2	7.7 ± 0.1
	M (171)	g	1.0	1	Dec-02	21.0	4.3 ± 0.1	-0.6 ± 0.1	7.3 ± 0.1
		g	1.0	1	Apr-04	48.4	4.9 ± 0.1	1.4 ± 1.0	7.6 ± 0.1
		g	1.0 (0.9+1.0)	1	May-04	20.0	6.1 ± 0.1	1.8 ± 0.6	6.4 ± 0.1
		g	2.5	1	Dec-02	49.7	5.1 ± 0.1	1.3	7.9 ± 0.1
		g	2.5	1	Apr-04	63.4	4.5 ± 0.1	1.0 ± 0.7	7.7 ± 0.1
		g	2.5	1	May-04	59.9	4.6 ± 0.2	0.4 ± 0.4	7.7 ± 0.1
SLR	H (160)	g	1.0 (0.9+1.0)	1	Apr-04	7.4	6.1 ± 0.1	0.6 ± 1.0	3.9 ± 0.2
		g	1.0 (0.9+1.2)	1	May-04	5.2	5.1 ± 0.1	1.0 ± 0.2	3.2 ± 0.2
		g	2.5 (3.0)	1	Jun-03	22.9	5.9 ± 0.1	0.0 ± 0.1	7.1 ± 0.1
		g	2.5 (2.0+3.0)	1	Apr-04	19.3	6.0 ± 0.1	0.7 ± 0.3	5.0 ± 0.1
	M (105)	g	1.0 (1.2+1.5)	1	Jun-03	16.0	5.7 ± 0.1	2.3 ± 0.3	6.8 ± 0.1
		g	2.5 (2.5+3.0)	1	Jun-03	30.0	6.2 ± 0.1	-1.0 ± 0.1	7.8 ± 0.1
	L (53)	g	2.5 (2.5+3.0)	1	Jun-03	5.7	9.2 ± 0.1	2.0 ± 0.1	2.7 ± 0.1
Knockbeg									
FER	H (137.5)	sb	1.5	1	Mar-04	87.0	2.0 ± 0.1	7.4 ± 0.2	7.6 ± 0.1
		sb	1.5	2	Mar-04	84.6	2.6 ± 0.1	5.5 ± 0.3	7.6 ± 0.1
	L (105)	sb	1.5	1	Mar-04	152.2	-0.4 ± 0.1	17.0 ± 1.1	7.4 ± 0.4
		sb	1.5	2	Mar-04	129.5	-0.4 ± 0.3	15.1 ± 0.1	7.7 ± 0.1
	H (225)	ww	1.5	1	Mar-04	68.6	3.7 ± 0.1	1.5 ± 0.7	7.6 ± 0.1
		ww	1.5	2	Mar-04	71.0	3.5 ± 0.1	1.0 ± 0.2	7.5 ± 0.1
		ww	1.5	3	Mar-04	13.2	5.6 ± 0.1	3.1 ± 1.1	6.2 ± 0.1
	L (187.5)	ww	1.5	1	Mar-04	48.9	4.2 ± 0.1	0.7 ± 0.3	7.5 ± 0.1
		ww	1.5	2	Mar-04	61.4	6.6 ± 0.1	3.8 ± 0.1	7.4 ± 0.1
Oak Park									
CTL	–	g	1.0	1	Nov-03	2.6	2.9 ± 0.1	1.5 ± 0.3	1.7 ± 0.1
		g	1.0	2	Nov-03	11.4	2.7 ± 0.1	-1.0 ± 0.6	4.9 ± 0.1
		g	1.0	3	Nov-03	6.5	2.3 ± 0.1	-1.2 ± 0.1	4.1 ± 0.1
FER	H (350)	g	1.0	1	Nov-03	29.7	1.1 ± 0.1	4.9 ± 0.4	6.9 ± 0.1
		g	1.0	2	Nov-03	35.6	0.4 ± 0.1	5.4 ± 0.2	6.9 ± 0.1
		g	1.0	3	Nov-03	60.5	0.4 ± 0.1		7.7 ± 0.1

Table 5: Stable isotope composition of total nitrogen (TN) and nitrate fractions (artificial fertilisers only) in N sources at Moorepark (Moo), Knockbeg (Kno) and Oak Park (Oak) (adapted from Minet (2007)).

N source (N fraction)	Soil	$\delta^{15}N$ (‰ AIR)	$\delta^{18}O$ (‰ V-SMOW)
ARTIFICIAL FERTILISERS *			
urea (TN)	Moo, Oak	-0.4 ± 0.1	n/a
CAN (TN)	Moo, Kno, Oak	-1.5 ± 0.1	n/a
CAN (NO_3^-)	Moo, Kno, Oak	0.4 ± 0.2	18.5 ± 0.3
SuperNet (TN)	Kno	0.1 ± 0.1	n/a
SuperNet (NO_3^-)	Kno	0.3 ± 0.2	18.2 ± 0.7
18-6-12 compound (TN)	Kno	0.2 ± 0.5	n/a
18-6-12 compound (NO_3^-)	Kno	nd	nd
ORGANIC WASTES			
Dairy wastewater (TN)	Moo	3.8 ± 0.2	n/a
Slurry (TN)	Moo	10 ± 0.2	n/a
SOIL INSOLUBLE NITROGEN **			
Moorepark soil (TN)	Moo	5.2 ± 0.2	n/a
	Moo	5.4 ± 0.1	n/a
Knockbeg soil (TN)	Kno	5.6 ± 0.1	n/a
	Kno	5.5	n/a
Oak Park soil (TN)	Oak	3.8 ± 0.1	n/a
	Oak	3.9 ± 0.1	n/a

nd not determined
n/a not applicable
* See fertiliser N description in Table 2 (footnote 3)
** Moorepark and Oak Park soils unfertilised, Knockbeg soil subject to application of artificial fertiliser

Table 6: Correlation matrices of Spearman's rank r_s values between nitrate concentration, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ in **A**) Moorepark fertiliser plots (FER_Moo, $15 \leq n \leq 16$), **B**) Knockbeg fertiliser plots (FER_Kno, n = 9), **C**) Moorepark dairy wastewater plots (DW_Moo, n = 12) and **D**) Moorepark slurry plots (SLR_Moo, n = 7) (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

A. FER_Moo	**$[NO_3^-]$**	**$\delta^{15}N\text{-}NO_3^-$**	**$\delta^{18}O\text{-}NO_3^-$**
$[NO_3^-]$	1		
$\delta^{15}N\text{-}NO_3^-$	-0.886****	1	
$\delta^{18}O\text{-}NO_3^-$	0.691**	-0.755***	1

B. FER_Kno	**$[NO_3^-]$**	**$\delta^{15}N\text{-}NO_3^-$**	**$\delta^{18}O\text{-}NO_3^-$**
$[NO_3^-]$	1		
$\delta^{15}N\text{-}NO_3^-$	-0.946****	1	
$\delta^{18}O\text{-}NO_3^-$	0.8**	-0.711*	1

C. DW_Moo	**$[NO_3^-]$**	**$\delta^{15}N\text{-}NO_3^-$**	**$\delta^{18}O\text{-}NO_3^-$**
$[NO_3^-]$	1		
$\delta^{15}N\text{-}NO_3^-$	-0.194	1	
$\delta^{18}O\text{-}NO_3^-$	0.098	0.672**	1

D. SLR_Moo	**$[NO_3^-]$**	**$\delta^{15}N\text{-}NO_3^-$**	**$\delta^{18}O\text{-}NO_3^-$**
$[NO_3^-]$	1		
$\delta^{15}N\text{-}NO_3^-$	0.214	1	
$\delta^{18}O\text{-}NO_3^-$	-0.679	-0.286	1

Figure 1

Figure 1: Monthly precipitation, calculated monthly effective rainfall (precipitation minus evapotranspiration minus surface runoff) and daily air temperature at the weather station in Oak Park Research Centre between February 2002 and June 2004 (Minet, 2007).

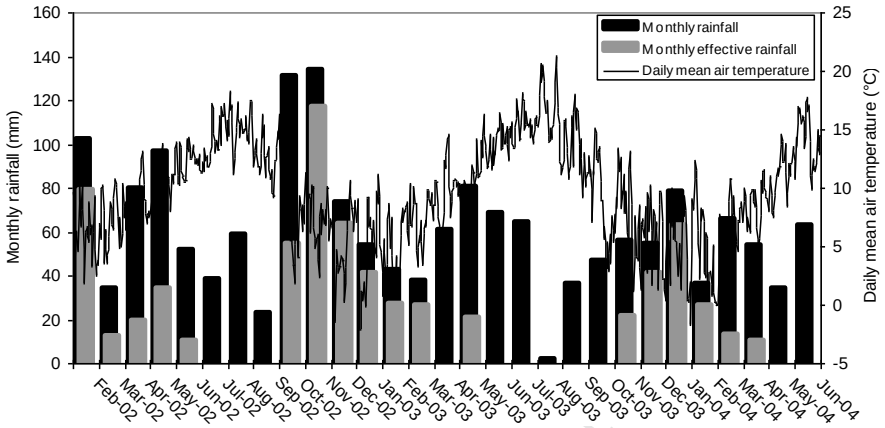


Figure 2

Figure 2: Ranges of soil-water nitrate concentration in the unfertilised controls (CTL, n = 8), cow slurry (SLR, n = 7), dairy wastewater (DW, n = 12) and artificial fertiliser treatments (FER, n = 27) across all soils.

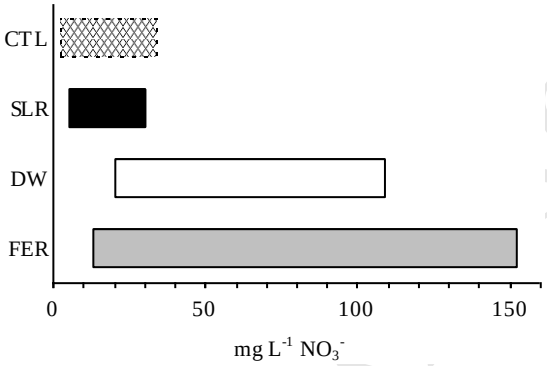


Figure 3

Figure 3: Scatterplot of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values measured in soil-water nitrate collected from Moorepark (Moo), Knockbeg (Kno) and Oak Park soils (Oak) subject to applications of artificial fertiliser (FER), dairy wastewater (DW), slurry (SLR) or no application (CTL), and comparison with δ values expected from the analysis of each treatment N source (delineated by boxes).

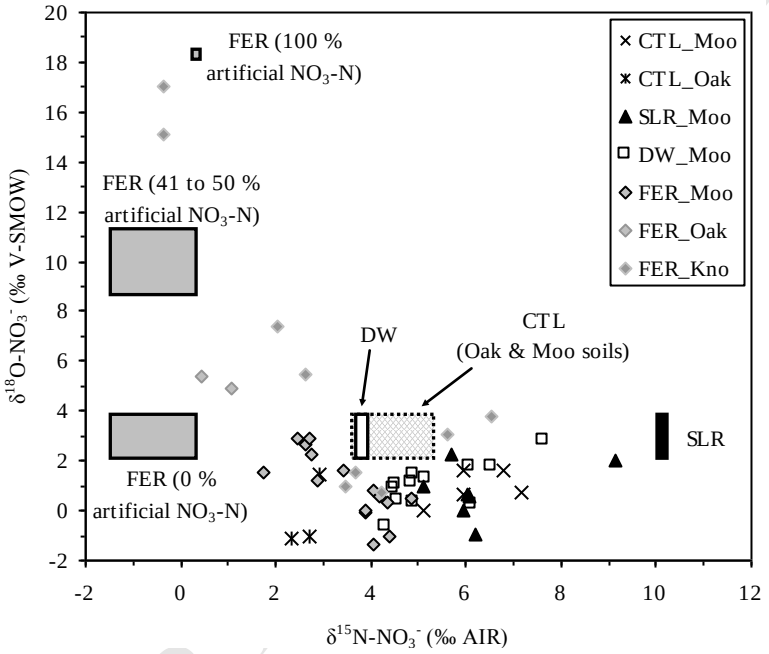
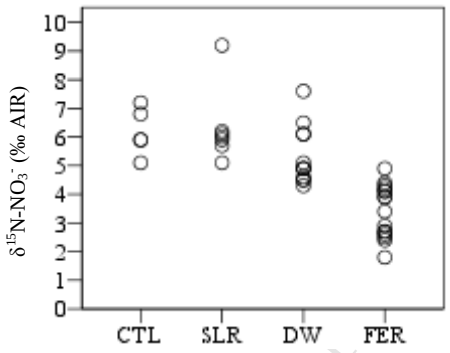


Figure 4: Distribution of **A**) $\delta^{15}N\text{-}NO_3^-$ and **B**) $\delta^{18}O\text{-}NO_3^-$ measured in soil-water nitrate from the following Moorepark treatment plots: control (CTL), slurry (SLR), dairy wastewater (DW) and artificial fertiliser (FER).

A.



B.

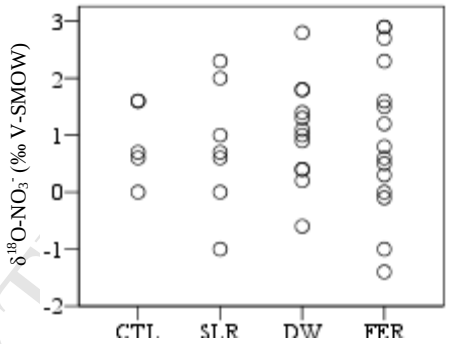
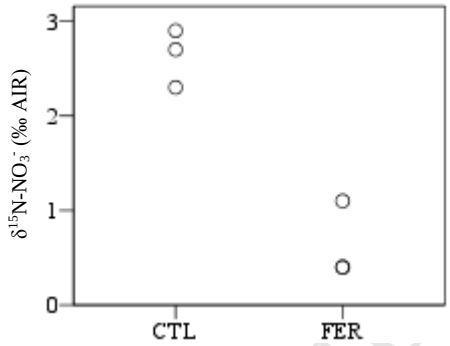


Figure 5

Figure 5: Distribution of **A**) $\delta^{15}N\text{-}NO_3^-$ and **B**) $\delta^{18}O\text{-}NO_3^-$ measured in soil-water nitrate from the following Oak Park treatment lysimeters: control (CTL) and artificial fertiliser (FER).

A.



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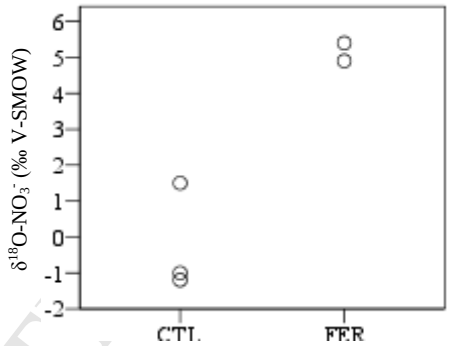
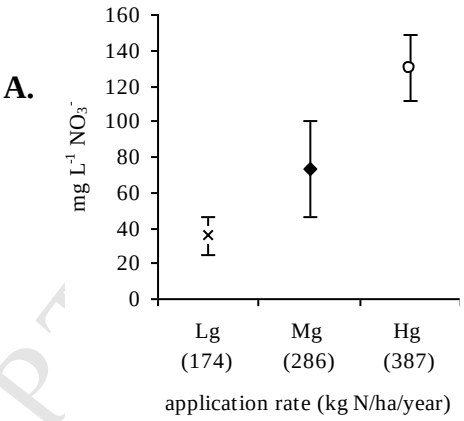


Figure 6: Variations of **A**) mean soil-water NO_3^- concentration (± standard deviation) with application rate and **B**) $\delta^{15}N\text{-}NO_3^-$ with $\delta^{18}O\text{-}NO_3^-$ (with linear regression line) in the Moorepark artificial fertiliser treatment (high rate H marked with hollow rounds, medium rate M with diamonds, low rate L with crosses; grassland (g) land use).



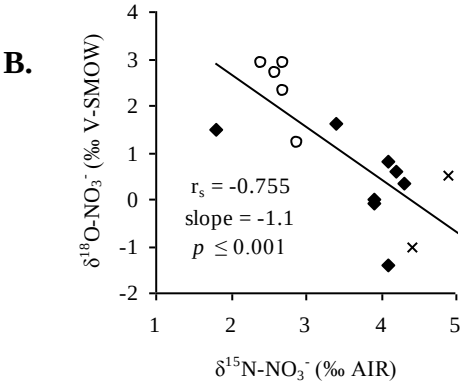
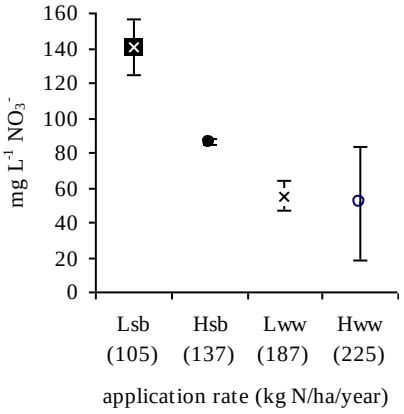


Figure 7: Variations of **A**) mean soil-water NO_3^- concentration (± standard deviation) with application rate and **B**) $\delta^{15}N\text{-}NO_3^-$ with $\delta^{18}O\text{-}NO_3^-$ (with linear regression line) in the Knockbeg artificial fertiliser treatment (high rate H marked with rounds and low rate L with crosses; spring barley (sb) land use designated by shaded marks and winter wheat (ww) by unshaded marks).

A.



B.

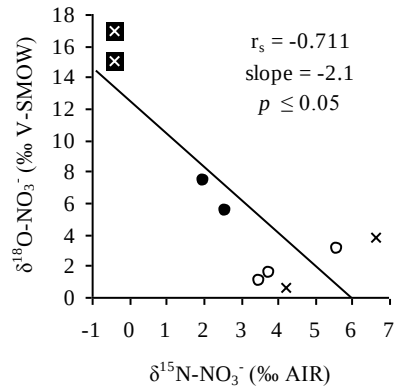
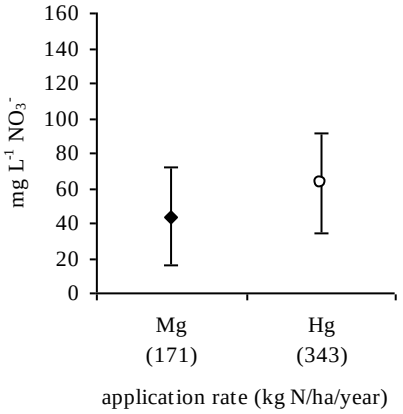


Figure 8

Figure 8: Variations of **A)** mean soil-water NO_3^- concentration (± standard deviation) with application rate and **B**) $\delta^{15}N\text{-}NO_3^-$ with $\delta^{18}O\text{-}NO_3^-$ (with linear regression line) in the Moorepark dairy wastewater treatment (high rate H is marked with hollow rounds and medium rate M with diamonds; grassland (g) land use).

A.



B.