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Effects of repeated fertilizer and cattle slurry applications over 38 years on N dynamics in a temperate grassland soil

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ABSTRACT

The effects of repeated synthetic fertilizer or cattle slurry applications at annual rates of 50, 100 or 200 m³ ha⁻¹ yr⁻¹ over a 38 year period were investigated with respect to herbage yield, N uptake and gross soil N dynamics at a permanent grassland site. While synthetic fertilizer had a sustained and constant effect on herbage yield and N uptake, increasing cattle slurry application rates increased the herbage yield and N uptake linearly over the entire observation period. Cattle slurry applications, two and four times the recommended rate (50 m³ ha⁻¹ yr⁻¹, 170 kg N ha⁻¹), increased N uptake by 46 and 78%, respectively after 38 years. To explain the long-term effect, a ¹⁵N tracing study was carried out to identify the potential change in N dynamics under the various treatments. The analysis model evaluated process-specific rates, such as mineralization, from two organic-N pools, as well as nitrification from NH₄⁺ and organic-N oxidation. Total mineralization was similar in all treatments. However, while in an unfertilized control treatment more than 90% of NH₄⁺ production was related to mineralization of recalcitrant organic-N, a shift occurred toward a predominance of mineralization from labile organic-N in the cattle slurry treatments and this proportion increased with the increase in slurry application rate. Furthermore, the oxidation of recalcitrant organic-N shifted from a predominant NH₄⁺ production in the control treatment, toward a predominant NO₃⁻ production (heterotrophic nitrification) in the cattle slurry treatments. The concomitant increase in heterotrophic nitrification and NH₄⁺ oxidation with increasing cattle slurry application rate was mainly responsible for the increase in net NO₃⁻ production rate. Thus the increase in N uptake and herbage yield on the cattle slurry treatments could be related to NO₃⁻ rather than NH₄⁺ production. The ¹⁵N tracing study was successful in revealing process-specific changes in the N cycle in relationship to long-term repeated amendments.

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1. Introduction

Repeated application of cattle slurry to grassland is a common farming practice in many areas of the world. Grasslands cover approximately 40% of the Earth's landmass (White et al., 2000). In Europe, approximately half of the agricultural area is grassland and slurry is regularly applied to a large proportion of it. Application of mineral N and/or organic-N in the form of manure has a positive effect on plant growth and N uptake (Shimizu et al., 2009). Organic-N has the advantage that it will increase the residual organic-N, thereby increasing the long-term fertilizer N value (Reijs et al., 2005).

Organic manures contain both a range of easily degradable organic C and N and mineral N compounds (Beauchamp et al., 1989). Studies have shown that repeated long-term applications of organic manures will enhance soil organic C and N contents, particularly the more labile soil organic matter, as well as soil microbial biomass and enzyme activity (Hao et al., 2003; Kirchmann et al., 2004; Mallory and Griffin, 2007; Zaman et al., 2004). At low rates of organic amendments it has been reported that the bacterial (and not the fungal) biomass is stimulated (Bittman et al., 2005; Marschner et al., 2003). The concentrations of soil organic C and N are often linearly related to the amount of manure applied, thus both the number of repeated amendments and the rate of application can affect the potentially available N in soil after slurry applications (Hao et al., 2003; Mallory and Griffin, 2007; Zaman et al., 2004). Furthermore, repeated slurry applications will have a long-term effect on soil characteristics such as the pore size distribution, water status or pH, which can indirectly

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influence soil C and N dynamics (Curtin and Wen, 1999; Thomsen et al., 1999). Since changes in organic C and N occur slowly (e.g. results from the Rothamsted Broadbalk study show that it may take a 100 years to reach a new equilibrium) (Kirchmann et al., 2004), observations from long-term amendment studies are extremely valuable.

Readily available C and N in organic manure increase the microbial biomass and its subsequent activity which in turn affects the mineralization rates (net release of NH_4^+ and NO_3^-) (Mallory and Griffin, 2007). A typical observation made after repeated long-term applications is that net NO_3^- production (net nitrification) is stimulated but not net NH_4^+ production (net ammonification) (Habteselassie et al., 2006). This leads to enhanced NO_3^- concentrations in the soil which can enhance NO_3^- leaching, potential denitrification activity and gaseous N emissions (Dambreville et al., 2006; Hao et al., 2003; Rochette et al., 2000; Zaman et al., 2004). One laboratory study showed that the rate of gross NO_3^- production after slurry application in a temperate grassland soil was enhanced more than 20-fold, possibly as a result of heterotrophic activity being stimulated (Müller et al., 2003). Another study showed that the availability of recently added N to soil was strongly affected by the N amendment history and whether organic or mineral N fertilizer had been applied (Mallory and Griffin, 2007). Organic manures tend to increase the more labile soil organic matter (SOM) which is characterized by an enhanced decomposability (Kirchmann et al., 2004; Sleutel et al., 2006). Thus, SOM mineralization is related to at least two conceptual SOM pools, decomposition of a passive/slow SOM pool and an active/labile SOM pool. Mallory and Griffin (2007) showed that such an approach was successful when predicting SOM mineralization (NO_3^- production) in soil after slurry application. They also showed that historic amendments reduced the availability of recently applied N, due to immobilization of N into the active SOM pool. High N immobilization is likely related to an increase in microbial biomass and may also explain the reduced leaching observed after slurry amendment in another grassland study (Bittman et al., 2005). Thus, there is evidence to suggest that N transformations after slurry application are affected by N amendment history.

Mallory and Griffin (2007) concluded that net rates are difficult to interpret because of the various production and consumption processes that occur simultaneously. While net N transformation studies will show how the availability of mineral N (NH_4^+ and NO_3^-) is affected, these studies fail to provide a mechanistic explanation of how the N amendment history affects specific N transformation pathways in soil. For instance, amendment with organic-N may enhance the gross NH_4^+ production in soil, but at the same time increasing organic C may also stimulate N immobilization into microbial biomass. Thus net NH_4^+ production rates may remain unaffected while the overall turnover of NH_4^+ may increase, leading to a more active and dynamic system.

We evaluated the dry-matter production and N uptake pattern from various field plots at a grassland site in Northern Ireland over the last 38 years. To explain changing biomass production and N uptake rates over this period, in particular under slurry application, it was hypothesized that slurry must have had an effect on the internal nitrogen Mineralization-Immobilization-Turnover (MIT) in the soil. Thus, a ^{15}N tracing study was carried out to identify potential impacts of antecedent slurry application on gross soil N transformations in the different treatments to help explain the observed long-term N uptake trends. To obtain a process-based understanding of the N dynamics under repeated slurry amendments, it is important to evaluate the individual gross N transformation rates (Mallory and Griffin, 2007). Therefore, in this study we used a ^{15}N tracing technique with a grassland soil to which one rate of synthetic fertilizer or slurry at three different rates had been

applied each year since 1970 (Christie, 1987). The ^{15}N tracing model considered pool size specific N transformation rates. Furthermore, in line with previous studies we used a model that considered two soil organic matter pools, an active (labile) and a slow (recalcitrant) soil organic matter pool (Mallory and Griffin, 2007; Thomsen and Olesen, 2000). The division into two conceptual SOM pools is supported by studies showing that the soil C:N ratio in long-term studies shifts toward labile SOM and higher enzyme activity with slurry applications (Kirchmann et al., 2004). Thus, the main aim of the current study was to show how repeated long-term applications of synthetic fertilizer and various rates of slurry applications influence the internal N dynamics, and in particular the individual gross N transformation rates, in a grassland soil. The potential N release (particularly in the form of NO_3^-) under various long-term slurry application rates has implications for N fertilizer recommendations and fertilizer and slurry management strategies.

2. Materials and methods

2.1. Site description and experimental setup

The field experiment from which the soil samples were taken was described in detail by Christie (1987). Briefly, the soil (a clay loam with 42% sand, 24% silt and 34% clay) has developed on glacial till overlying Silurian shales and greywackes, has an organic matter content of 4.25%, and its hydrogeological class number within HOST (Hydrology of Soil Types) is 18 *sensu* Boorman et al. (1995). The site is considered typical of a substantial area of grassland in Northern Ireland. Forty-eight rectangular plots, each 29.75 m² in area, were established in 1970 on a re-seeded perennial ryegrass sward at Hillsborough, Co. Down (O.S. Grid Reference J 244577) at an elevation of about 120 m above sea level. There are eight treatments: (1) unamended control (received fertilizer as in (2) for 3 years (1970–1972) and then no further nutrient applications); (2) fertilizer control receiving 200 kg N (as ammonium nitrate until June 1974 and subsequently as urea), 32 kg P and 160 kg K ha⁻¹ yr⁻¹ to maintain a balance of NPK inputs and outputs; (3)–(5) cow slurry applied at 50, 100 or 200 m³ ha⁻¹ yr⁻¹; (6)–(8) pig slurry applied at the same three rates. There were six replicates giving a total of 48 plots in a randomized block design with two replicates of each treatment fully randomized within each of three blocks. The sward was cut three times each year at the silage stage, approximately at the end of May, July and September. The fertilizer and slurries were applied by hand and by watering can respectively in three equal dressings, first in spring after annual soil sampling and again immediately after the first two herbage cuts.

2.2. Determination of herbage dry-matter and % N in herbage

Herbage was dried at 60 °C and subsequently ground with a ball mill (Retsch, Germany). Total N in the herbage was determined by Kjeldahl digestion in the years from 1970 to 1988 and thereafter by using a Carlo Erba NA1500 elemental analyzer (Carlo Erba, Milan, Italy).

2.3. Experimental treatments used in laboratory study

For determination of gross N dynamics (see Section 2.4), soil samples were collected from treatments (1)–(5) in February 2007 to compare three application rates of cow slurry with both types of control. The treatments are referred to as control (CT), fertilizer control (CF), slurry-low, 50 m³ ha⁻¹ yr⁻¹ (SL), slurry-medium, 100 m³ ha⁻¹ yr⁻¹ (SM) and slurry-high, 200 m³ ha⁻¹ yr⁻¹ (SH). Ten soil cores were randomly taken per plot to a depth of 10 cm, sieved (<6 mm) and stored at 4 °C for one week before use. Within each

treatment, soil sampled from the two replicates in each block was combined to give triplicate composite samples. Immediately before the start of the experiment the soil was air-dried at 30 °C and passed through a 2 mm sieve. Soil subsamples were dried at 105 °C for 24 h to determine the oven-dry soil weight.

The equivalent of 100 g of oven-dry soil was placed in acid washed 500 ml preserving jars (Kilner jars). An aliquot of differentially ^{15}N labelled ammonium nitrate (NH_4NO_3) was uniformly applied over the soil surface in each jar using a pipette, in which either the ammonium ($^{15}\text{NH}_4\text{NO}_3$), nitrate ($\text{NH}_4^{15}\text{NO}_3$), or both moieties ($^{15}\text{NH}_4^{15}\text{NO}_3$) were labelled with ^{15}N at 60 atom % excess. The NH_4NO_3 was applied at a rate of $100 \mu\text{g N g}^{-1}$ ($50 \mu\text{g g}^{-1}$ as $\text{NH}_4^+\text{-N}$ and $50 \mu\text{g g}^{-1}$ as $\text{NO}_3^-\text{-N}$) in sufficient volume of liquid to attain a target soil water-filled pore space of 65%. The jars (225 in total) were incubated at 20 °C, prior to soil extractions at 24, 48, 72, 96 and 120 h after NH_4NO_3 application.

2.4. Determination of mineral N concentration and ^{15}N enrichment

The soil in each jar was extracted with 2 M KCl (1:1 w/v proportion of soil to KCl). Portions of each suspension were centrifuged immediately at $2000\times g$ for 5 min and the supernatant filtered sequentially through GF/D and GF/F glass-fibre papers (Whatman International Ltd, Kent, UK). Filtrates were stored at 4 °C prior to analysis within 2 days for concentration and ^{15}N enrichment of NH_4^+ and NO_3^- .

Concentrations of NO_3^- and NH_4^+ in the KCl extracts were determined by segmented-flow analysis (Technicon Random Access Automated Chemistry System 800+, Bran and Luebbe, Norderstedt, Germany). Nitrate was determined by the sulphanilamide–naphthylethylenediamine method after Cd reduction to NO_2^- . Ammonium was determined by the indophenol blue method (Keeney and Nelson, 1982).

The ^{15}N contents of the NO_3^- and NH_4^+ were determined by methods based on the generation of N_2O for Ion Ratio Mass Spectrometry (IRMS). The production of N_2O from NO_2^- and NO_3^- was based on the reaction between NO_2^- and NH_2OH in acid conditions, the NO_3^- having been reduced to NO_2^- with Cd (Stevens and Laughlin, 1994). The production of N_2O from NH_4^+ consisted of a diffusion stage where NH_3 was absorbed into H_2SO_4 and an oxidation step where recovered $(\text{NH}_4)_2\text{SO}_4$ was oxidized to N_2 by alkaline NaOBr , during which N_2O was produced as a by-product (Laughlin et al., 1997).

2.5. Quantification of gross N transformation rates

Gross soil N dynamics were determined with a ^{15}N tracing approach according to Müller et al. (2007). The principle of the method relies on the simulation of the experimental data with a N cycle model (Fig. 1) in combination with a model parameter optimization procedure. The aim of the optimization procedure is to determine a set of model parameters that produce a model output similar to the experimental data. The key of the procedure lies in the optimization algorithm which needs to unambiguously determine the model parameters. Here we use a Markov Chain Monte Carlo (MCMC) method which has been shown to produce reliable results for a large number of parameters (Knorr and Kattge, 2005; Metropolis et al., 1953; Müller et al., 2007). The model (Fig. 1) considered ten gross N transformations: M_{Nrec} , mineralization of recalcitrant organic-N to NH_4^+ ; M_{Nlab} , mineralization of labile organic-N to NH_4^+ ; $I_{NH_4_{Nrec}}$, immobilization of NH_4^+ into recalcitrant organic-N; $I_{NH_4_{Nlab}}$, immobilization of NH_4^+ into labile organic-N; O_{NH_4} , oxidation of NH_4^+ to NO_3^- (autotrophic and heterotrophic nitrification); O_{Nrec} , oxidation of recalcitrant organic-N to NO_3^- (heterotrophic nitrification); I_{NO_3} , immobilization of NO_3^- to recalcitrant organic-N and

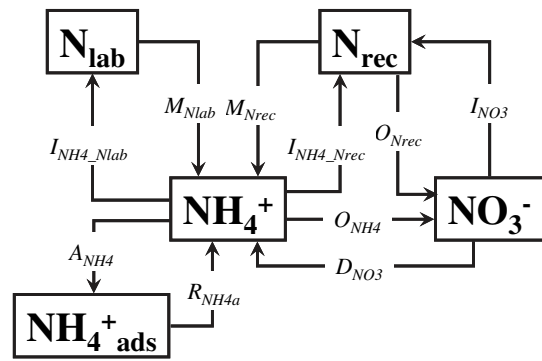


Fig. 1. ^{15}N tracing model for analysis of gross soil N transformation rates. Abbreviations are explained in the text (Section 2.5).

D_{NO_3} , dissimilatory NO_3^- reduction to NH_4^+ ; A_{NH_4} , adsorption of NH_4^+ on cation exchange sites; $R_{NH_4_{ads}}$, release of adsorbed NH_4^+ . The transformation rates were calculated either by zero- or first-order kinetics. The model calculated gross N transformation rates by simultaneously optimizing the kinetic parameters for the various N transformations by minimizing the misfit between modelled and observed (mean \pm standard deviations) NH_4^+ and NO_3^- concentrations and their respective ^{15}N enrichments (Müller et al., 2004). A unique parameter set was optimized for the entire duration of the study. Analyses using this parameter optimization concept in previous studies have shown that the mineralization of two conceptual organic-N pools produced realistic NH_4^+ dynamics (e.g. Cookson et al., 2006; Huygens et al., 2007; Müller et al., 2004). The advantage of ^{15}N tracing studies over the commonly used pool dilution technique (Kirkham and Bartholomew, 1954) which determines gross N production and consumption rates is, that process related N rates of the mineralization-immobilization-turnover (MIT) dynamics can be evaluated (Rütting and Müller, 2007). For instance, gross N mineralization rates from various SOM pools (labile, recalcitrant SOM pools; M_{Nlab} and M_{Nrec} in Fig. 1) can be quantified as well as process related nitrification rates (related to the oxidation of NH_4^+ and organic-N; O_{NH_4} and O_{Nrec} in Fig. 1).

Parameter optimization was carried out with a Markov Chain Monte Carlo Metropolis algorithm (MCMC-MA) which performs a random walk in model parameter space and is very robust against local minima (Müller et al., 2007). The misfit function between the simulation output and observations, (see eq. (3) in Müller et al., 2007) takes into account the variance of the individual observations. Thus, the uncertainties in the experimental data are taken into account for parameter quantification and can have an impact on the standard deviation of each parameter (Rütting and Müller, 2007). The optimization procedure samples the probability density function (PDF) of parameters, from which parameter averages and standard deviations are calculated. The MCMC-MA routine is programmed in the software MatLab (Version 7, The MathWorks Inc.), which calls models that are set up in Simulink (Version 6, The MathWorks Inc.). A description of the model and the optimization procedure can be found in Müller et al. (2007).

2.6. Calculation procedures, statistics and presentation of results

The total annual dry-matter production was calculated as the sum of the three harvests per year. The values for cut 3 in 1982 and cut 2 in 1998 were missing. They were estimated based on 5 year averages before and after the respective cut. Nitrogen uptake by plants was determined by multiplying the individual dry-matter production of each plot with the associated % N content. The total N uptake was calculated as the yearly sum. Both the dry-matter yield

and N uptake are presented as averages \pm standard deviations. Linear regressions of N uptake vs. time (years) were calculated for each treatment. The slope of this relationship showed how plant N uptake changed over time as a result of long-term applications of repeated amendments.

Statistical analysis was carried out with MatLab (Version 7., The MathWorks Inc.). Test of normality and equal variance tests were carried out with the MatLab procedures *lillietest* and *vartestn* respectively. The analysis of variance and pair-wise comparison were calculated via the MatLab procedures *anovan* and *multcompare* at a significance level of 0.05, using a Tukey's least significant difference procedure. The statistical analysis considered the factors Year, Cut (harvest), Block (repetitions) and Treatment (N treatment).

To obtain the most suitable parameter set for the ^{15}N tracing analysis that was able to simulate the observed data, variations of the model depicted in Fig. 1 were evaluated. These modifications included/excluded N pools (such as NH_4ads), N transformations (e.g. D_{NO_3} , O_{Nrec}) and variations of kinetic settings (e.g. trying out various combinations of zero- and first-order kinetics) for the different N transformations. The most suitable model was identified based on Akaike's Information Criterion (AIC) for each model version. Such an approach is in line with general parameter estimation procedures (Cox et al., 2006) and has been successfully used for parameter estimation with this ^{15}N tracing model (e.g. Müller et al., 2009; Rütting et al., 2011; Zhang et al., 2011). For N transformations following first-order kinetics, average gross N rates were calculated by integrating the gross N rates over the experimental period, divided by the total time (Rütting and Müller, 2007). A one-way ANOVA was calculated based on the averages and standard deviations of the individual N transformation rates to identify if the five treatments were significantly different. Pair-wise comparisons of the N transformation rates for all treatment combinations were calculated with the Holm–Sidak test. Based on the actual experimental repetitions, the least significant differences (LSD) were calculated for each N transformation rate (presented as an error bar in Figs. 4 and 5). This presents the most conservative way to calculate LSDs because the number of iterations for the determination of averages \pm standard deviations for each N transformation was much higher. However, the observed error in the observations is linked to the number of actual repetitions, and is reflected in the probability density function (PDF) of each parameter (Rütting and Müller, 2007). The ANOVA and pair-wise comparisons for each N transformation were calculated with SigmaPlot (Version 11.0, Systat Inc.) and the LSDs were calculated in Excel 2003 (Microsoft Corp.). The presentation of results focused on the main gross N transformation rates, mainly mineralization (from labile and recalcitrant organic-N) and nitrification (NH_4^+ and organic-N oxidation) which represented 68–92% of the observed N dynamics in all treatments. For the calculation of net NH_4^+ and net NO_3^- production (Fig. 5) the rates related to the consumption of NH_4^+ and NO_3^- (Table 1) were taken into account, i.e.

$$\text{net } \text{NH}_4^+ \text{ production} = M_{\text{Nrec}} + M_{\text{Nlab}} + R_{\text{NH}_4\text{a}} + D_{\text{NO}_3} - I_{\text{NH}_4\text{Nrec}} - I_{\text{NH}_4\text{Nlab}} - A_{\text{NH}_4} - O_{\text{NH}_4} \quad (1\text{a})$$

$$\text{net } \text{NO}_3^- \text{ production} = O_{\text{NH}_4} + O_{\text{Nrec}} - I_{\text{NO}_3} - D_{\text{NO}_3} \quad (1\text{b})$$

3. Results

3.1. Dry-matter production and N uptake between 1970 and 2009

Annual dry-matter production and N uptake between 1970 and 2009 are presented in Fig. 2 for five treatments (control, CT;

Table 1

N transformation rates (averages \pm stdev in brackets) after 38 years of repeated synthetic fertilizer and increasing slurry applications in permanent grassland. Rates are in $\mu\text{g N g}^{-1} \text{d}^{-1}$ (for explanation of the N rates see section 2.5) (kinetics: 0 = zero-order, 1 = first-order).

N rates	Kinetics	Control	Fert-Cont	Slurry-low	Slurry med	Slurry-high
M_{Nrec}	0	4.74(0.16)	4.45(0.51)	3.50(1.47)	2.42(0.47)	1.04(0.71)
$I_{\text{NH}_4\text{Nrec}}$	0	0.34(0.24)	1.01(0.47)	0.05(0.03)	3.84(0.44)	1.87(0.44)
M_{Nlab}	1	0.29(0.13)	0.66(0.45)	4.00(1.55)	3.82(0.40)	4.63(0.97)
$I_{\text{NH}_4\text{Nlab}}$	1	0.54(0.22)	0.33(0.19)	0.33(0.31)	0.65(0.49)	0.91(0.50)
O_{Nrec}	0	0.02(0.02)	0.05(0.02)	0.36(0.13)	1.87(0.27)	2.58(0.22)
I_{NO_3}	0	0.08(0.07)	0.04(0.03)	0.06(0.03)	0.14(0.12)	0.07(0.05)
O_{NH_4}	0	0.07(0.04)	1.43(0.07)	2.16(0.15)	5.73(0.15)	10.21(0.21)
D_{NO_3}	0	0.01(0.00)	0.05(0.00)	0.06(0.04)	0.10(0.01)	0.12(0.01)
A_{NH_4}	1	0.40(0.25)	0.30(0.22)	0.21(0.14)	0.22(0.04)	0.18(0.11)
$R_{\text{NH}_4\text{a}}$	1	0.04(0.03)	0.06(0.06)	0.15(0.08)	1.41(0.34)	0.39(0.18)

control + fertilizer, CF; slurry-low, SL; slurry-medium, SM; slurry-high, SH). In general, dry-matter yield and N uptake showed a similar trend: decreasing in the CT treatment, staying more or less constant in the CF and SL treatments and increasing over time in the SM and SH treatments. The statistical analysis revealed that there was only a statistically significant treatment effect but no effect with respect to time or cutting regime. The initial mean yields were similar in all treatments, ranging from 670 (SL) g m^{-2} to 770 (SH) g m^{-2} . In the years 1970–1972 the CT plots received fertilizer at the same rate as the CF plots (Fig. 2). In 1973, the first year without N application, the mean yield decreased in CT from 730 g m^{-2} to approx. 450 g m^{-2} and then further to 230 g m^{-2} in the last 3 years (2007–2009) of the observation period. In the CF and SL treatments the mean yields stayed more or less constant over the observation period at 770 g m^{-2} and 620 g m^{-2} respectively. The average yields of the SM and SH treatments were 930 g m^{-2} and 1050 g m^{-2} , respectively. These were not only higher than the yields in the other treatments ($p < 0.05$) but also showed an increase over time from 710 and 750 g m^{-2} , respectively in the first 3 years to 1090 and 1290 g m^{-2} , respectively in the last 3 years (2007–2009) of the observation period ($p < 0.05$).

The annual N uptake differed with treatment ($p < 0.05$). In the CT plots the mean annual N uptake decreased slightly from 6.8 g N m^{-2} (1973–1975) to 4.0 g N m^{-2} (2007–2009) (as indicated by the regression slope of -0.065). The fertilizer effect was similar in all treatments in the first 3 years of the study (ranging from 10.3 g N m^{-2} (SL) to 13.1 g N m^{-2} (SH)). In the CF and SL treatments the mean annual N uptake stayed more or less constant at 12.6 and 9.3 g N m^{-2} , respectively while it increased over time in the SM and SH treatments from 11.4 and 13.1 g N m^{-2} , respectively (1970–1972) to 16.6 and 23.3 g N m^{-2} , respectively (2007–2009). The slope of the relationship between N uptake and time was dependent on the slurry application rate and was highest in the SH treatment (i.e. slopes of the SL, SM and SH treatment were 0.018, 0.150 and 0.244 respectively, Fig. 2). Based on average N uptake rates in the years 1970–1972 and 2007–2009, the additional N uptake stimulated by slurry application over time corresponded to 2.4, 51.8 and 102.3 kg N ha^{-1} in the SL, SM and SH treatments, respectively.

3.2. ^{15}N tracing analysis

The observed and simulated concentrations and ^{15}N enrichments of NH_4^+ and NO_3^- agreed well for all treatments (Fig. 3). The changes in NH_4^+ and NO_3^- concentrations and the dilution of the $^{15}\text{NO}_3^-$ excess when NO_3^- was labelled indicated that NO_3^- production increased gradually from the CT treatment to the treatment with the highest slurry application (SH) (Fig. 3).

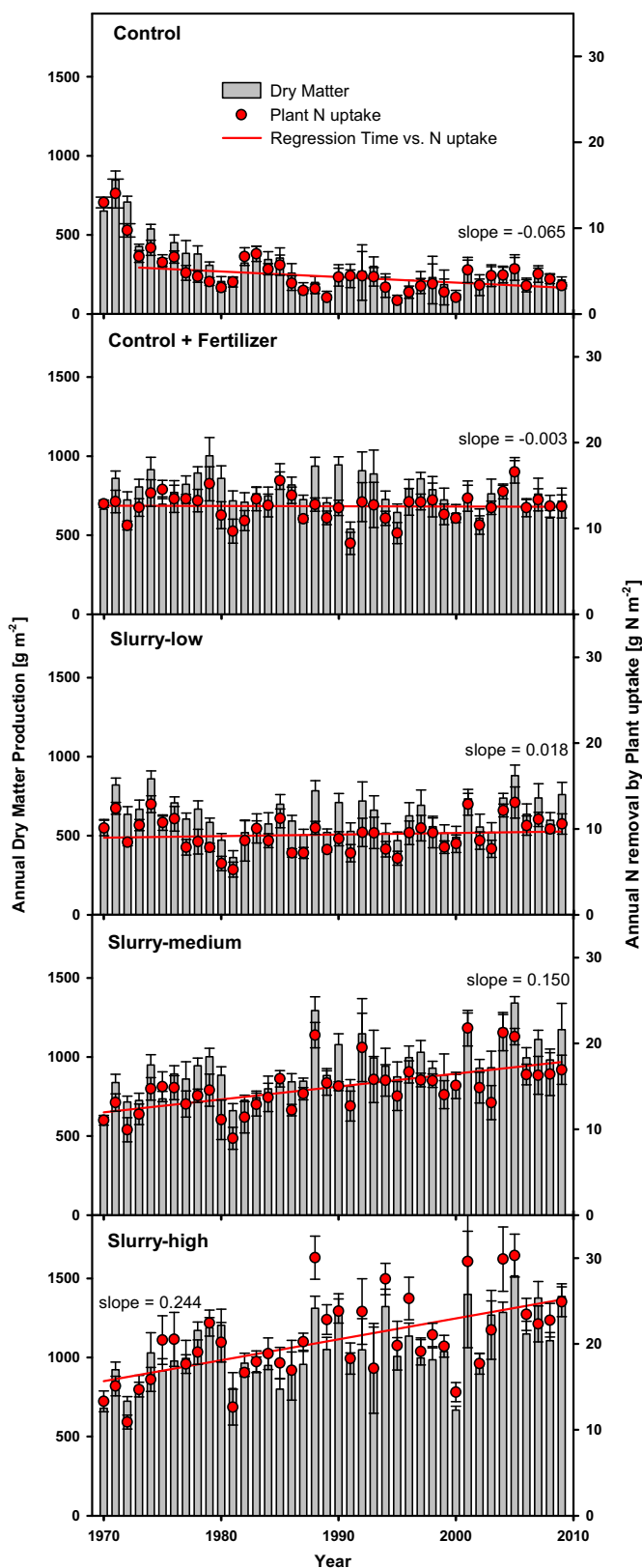


Fig. 2. Effect of fertilizer (Control + Fertilizer) and different slurry application rates $50 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$ (slurry-low), $100 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$ (slurry-medium) and $200 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$ (slurry-high) on herbage yield (g m^{-2}) and plant N uptake (g N m^{-2}) of a permanent grassland between 1970 and 2009.

3.3. NH_4^+ dynamics under long-term fertilizer and increasing slurry application rates

Ammonium production via mineralization ($M_{\text{tot}} = M_{\text{Nrec}} + M_{\text{Nlab}}$) of organic-N was not significantly different between treatments and ranged from $5 \mu\text{g N g}^{-1} \text{ d}^{-1}$ to $7.5 \mu\text{g N g}^{-1} \text{ d}^{-1}$ (Fig. 4a). The model estimated rates of mineralization from recalcitrant organic-N (M_{Nrec}) and from labile organic-N (M_{Nlab}). Values of M_{Nrec} decreased from $4.7 \mu\text{g N g}^{-1} \text{ d}^{-1}$ in the CT treatment to $1.0 \mu\text{g N g}^{-1} \text{ d}^{-1}$ in the SH treatment with significantly different rates between CT and the two highest slurry application rates (SM, SH) and between CF/SL and SH ($P < 0.05$) (Fig. 4a). The M_{Nlab} rates increased from $0.3 \mu\text{g N g}^{-1} \text{ d}^{-1}$ in the CT treatment to $4.6 \mu\text{g N g}^{-1} \text{ d}^{-1}$ in the SH treatment with significant differences between CT/CF and all slurry treatments (Fig. 4a). M_{Nlab} was not statistically different among the slurry treatments (for the dynamics of the N_{rec} pools see Section 3.6). The model calculated total NH_4^+ consumption as the sum of NH_4^+ immobilization into microbial biomass associated with the recalcitrant (M_{Nrec}) and labile (M_{Nlab}) organic-N pool, adsorption on exchange sites (A_{NH_4}) and oxidation of NH_4^+ to NO_3^- (O_{NH_4}) (Fig. 1). On average adsorption (A_{NH_4}) accounted for $<5\%$ of total mineralized NH_4^+ (M_{tot}) in all treatments. Immobilization into organic-N pools accounted for on average 34% of M_{tot} . Oxidation of NH_4^+ to NO_3^- (O_{NH_4}) was 1, 28, 29, 92 and 180% of M_{tot} for treatments CT, CF, SL, SM and SH, respectively. Moreover, O_{NH_4} accounted for 5 (CT)–78% (SH) of the total NH_4^+ consumption. The highest relative NH_4^+ consumption rates via O_{NH_4} (55–78%) were associated with the slurry treatments (Table 1).

3.4. NO_3^- dynamics under long-term fertilizer and increasing slurry application rates

The model analysis showed that NO_3^- production occurred in all treatments via NH_4^+ oxidation (O_{NH_4}) and organic-N oxidation (O_{Nrec}). Both O_{NH_4} and O_{Nrec} responded non-linearly to fertilizer and increasing slurry application rates (Fig. 4b). The total rate of NO_3^- production ($O_{\text{NH}_4} + O_{\text{Nrec}}$) ranged from $0.09 \mu\text{g N g}^{-1} \text{ d}^{-1}$ (CT) to $12.8 \mu\text{g N g}^{-1} \text{ d}^{-1}$ (SH) and rates were significantly different from each other ($p < 0.05$) (Fig. 4b). NH_4^+ oxidation (O_{NH_4}) in the control and slurry treatments accounted on average for 81% of total NO_3^- production ($O_{\text{NH}_4} / (O_{\text{NH}_4} + O_{\text{Nrec}})$), while in the fertilizer treatment it accounted for 97% of total NO_3^- production (Fig. 4b).

The consumption of NO_3^- was calculated in the model as the sum of NO_3^- immobilization (I_{NO_3}) and dissimilatory NO_3^- reduction to NH_4^+ (D_{NO_3}) (range: 0.09 – $0.23 \mu\text{g N g}^{-1} \text{ d}^{-1}$) and was found to be an order of magnitude smaller than NH_4^+ consumption (range: 1.4 – $13.2 \mu\text{g N g}^{-1} \text{ d}^{-1}$). While in the CT treatment more than 91% of NO_3^- consumption was related to immobilization, in the CF and slurry treatments this value ranged from 35% to 58%, indicating that DNRA became relatively more pronounced with N application. However, DNRA rates were relatively small compared to the other N transformation rates (Table 1).

3.5. Net mineral N production

Net NH_4^+ and NO_3^- production rates in the treatments were calculated with eqs. (1a) and (1b), respectively. Net NH_4^+ production rates ranged from -7 (SH) to 5 (SL) $\mu\text{g N g}^{-1} \text{ d}^{-1}$ showing that in the two highest slurry treatments a net NH_4^+ consumption was observed (Fig. 5a). The net NH_4^+ production was significantly different between the SH treatment and treatments CT, CF and SL and between the SM and SL treatment. All other combinations were not significantly different. Calculation of net NH_4^+ production without the consideration of NH_4^+ oxidation (O_{NH_4}) resulted in net NH_4^+ production rates between 3 and $7.1 \mu\text{g N g}^{-1} \text{ d}^{-1}$ which were not significantly different among all treatments (data not shown). Thus, differences in NH_4^+

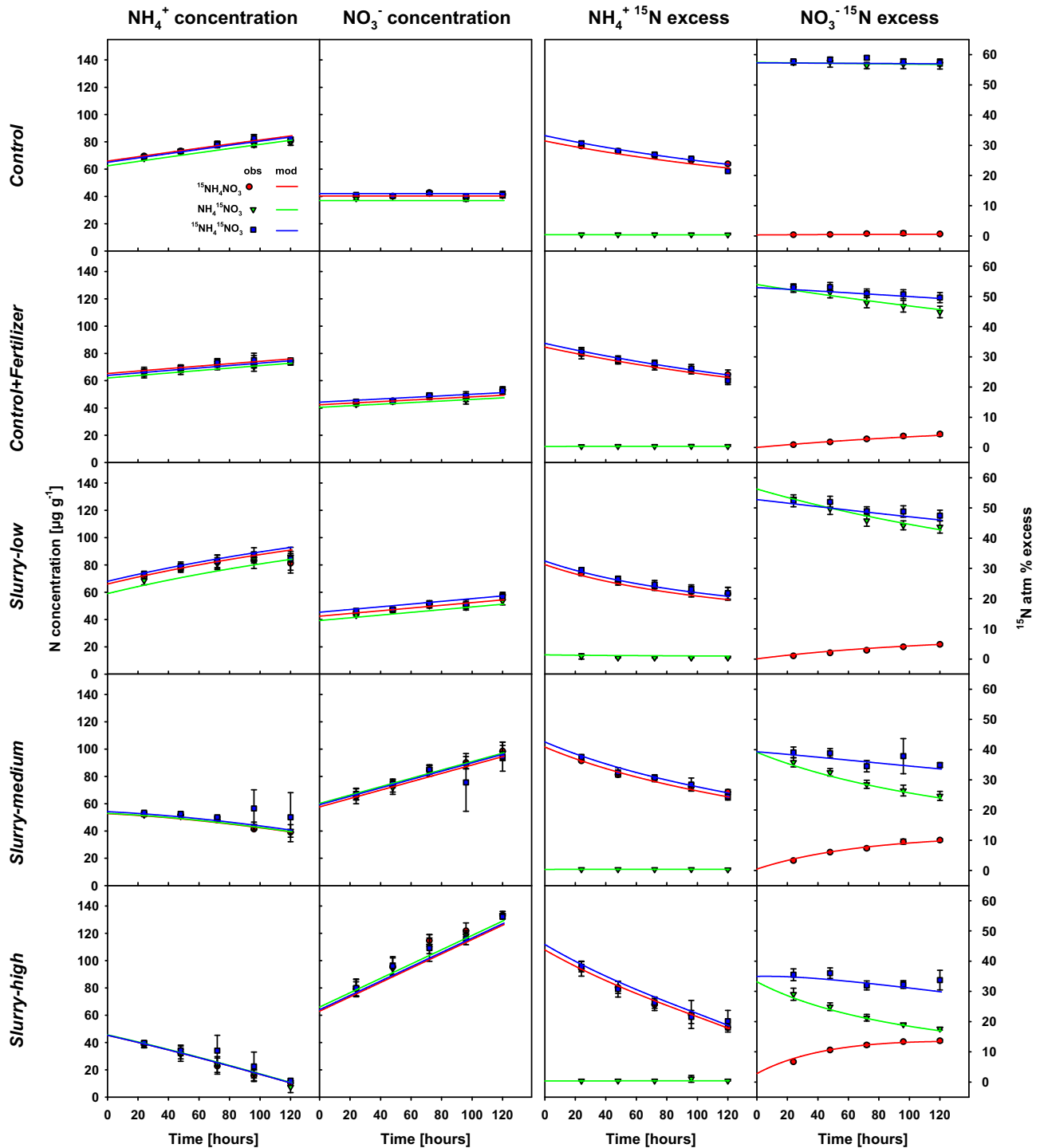


Fig. 3. Observed (avg \pm sd) and modelled concentrations and ^{15}N enrichments of ammonium and nitrate after application of differentially labelled NH_4NO_3 to a grassland soil that had previously received repeated applications of synthetic fertilizer or different rates of cattle slurry.

oxidation (O_{NH_4}) rate are mainly responsible for the observed effect on net NH_4^+ production in the various treatments. Total NO_3^- production in the CT treatment matched the total NO_3^- consumption (NO_3^- production/ NO_3^- consumption = 1). However, NO_3^- production was 16, 20, 32 and 68 times higher than NO_3^- consumption in the CF, SL, SM and SH treatments, respectively. The imbalance between production and consumption of NO_3^- is reflected in the net NO_3^- production rates which increased significantly between all treatments ranging from $0 \mu\text{g N g}^{-1} \text{d}^{-1}$ (CT) to $12.6 \mu\text{g N g}^{-1} \text{d}^{-1}$ (SH) (Fig. 5a).

3.6. Dynamics of the recalcitrant organic-N (N_{rec}) for the production of mineral N

The model calculated rates of NH_4^+ ($M_{N_{\text{rec}}}$) and NO_3^- production ($O_{N_{\text{rec}}}$) originating from the recalcitrant organic-N (N_{rec}) pool in the soil. The production of mineral N via these two pathways relative to total mineral N production accounted for 94, 86, 48, 45 and 42% in the CT, CF, SL, SM and SH treatments, respectively. The total production of mineral N ($\text{NH}_4^+ + \text{NO}_3^-$) from N_{rec} , ranged

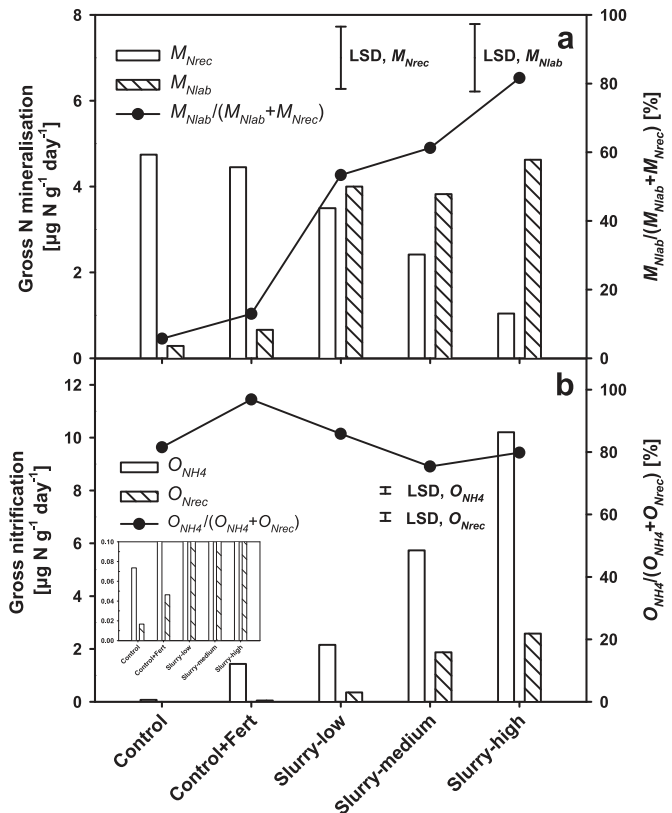


Fig. 4. (a) Gross N mineralization rates from recalcitrant (M_{Nrec}) and labile (M_{Nlab}) organic matter and the relative proportion of M_{Nlab} to total mineralization ($M_{Nrec} + M_{Nlab}$) and (b) gross NH_4^+ oxidation rates (O_{NH_4}) and organic-N oxidation rates (O_{Nrec}) and the relative proportion of O_{NH_4} to total nitrification ($O_{NH_4} + O_{Nrec}$) in a permanent grassland soil that had previously received repeated applications of synthetic fertilizer or different rates of cattle slurry. LSD, least significant difference when comparing any two means for $P = 0.05$.

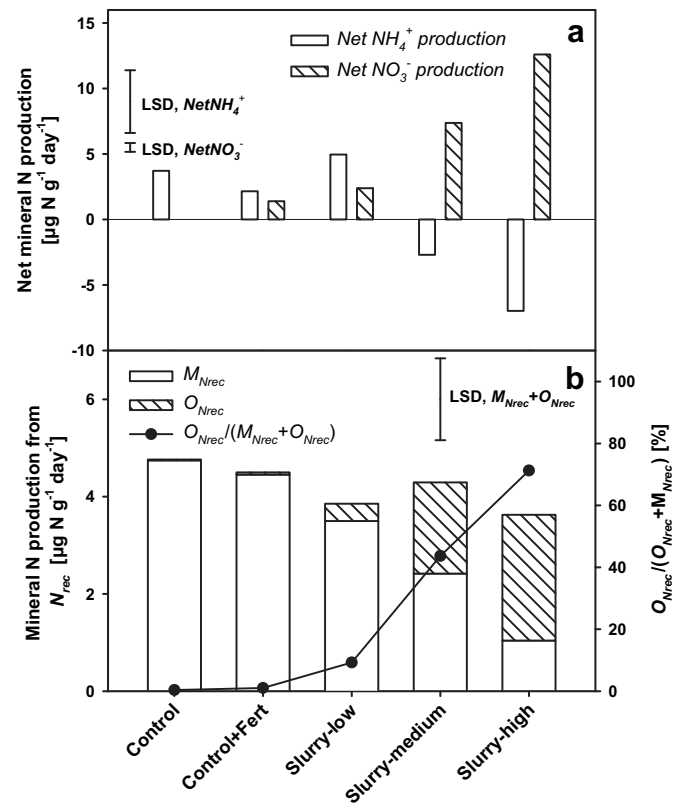


Fig. 5. (a) Net NH_4^+ and NO_3^- production rates and (b) rates of mineral N (NH_4^+ and NO_3^-) production from recalcitrant organic-N (N_{rec}) and the relative proportion of NO_3^- production (O_{Nrec}) to total mineral N production from N_{rec} in a permanent grassland soil that had previously received repeated applications of synthetic fertilizer or different rates of cattle slurry. LSD, least significant difference when comparing any two means for $P = 0.05$.

from $3.6 \mu\text{g N g}^{-1} \text{d}^{-1}$ to $4.7 \mu\text{g N g}^{-1} \text{d}^{-1}$ (Fig. 5b) and was not statistically different among treatments. However, the proportion of NO_3^- production (O_{Nrec}) from total N_{rec} oxidation increased significantly from 0% (CT) to 71% (SH) (Fig. 5b).

4. Discussion

4.1. Permanent grassland and slurry applications

Organic manures such as cattle slurry have a high fertilizer value and can therefore potentially replace or supplement synthetic fertilizers. Taking into account the energy-demanding process for the production of synthetic fertilizer it may be possible to considerably reduce the CO_2 emissions via a shift toward slurry based or organic systems (Scialabba and Müller-Lindenlauf, 2010). However, repeated slurry applications may alter the soil nutrient dynamics and N availability in the long term (Kirchmann et al., 2004) and thus affect grassland productivity as well as the production of greenhouse gas emissions. This current study of repeated applications of fertilizer and slurry applications over a 38 year period is particularly valuable because it provides important information on the long-term effect these amendments have on herbage production and soil N dynamics. Grasslands comprise approximately 93% of the total area farmed in Northern Ireland of which 85% is regarded as permanent grassland (>5 years old) (Census, 2009). The total volume of slurry produced by housed animals in Northern Ireland is approx. 14 million $\text{m}^3 \text{yr}^{-1}$ at 6% dry-matter (Frost et al.,

2005), which if evenly spread over the total grassland area would be equivalent to approx. $15 \text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$. The Nitrates Directive Action Programme has a farm limit of $170 \text{kg N ha}^{-1} \text{yr}^{-1}$ from livestock manures. Using an average N content of 3kg N m^{-3} for dairy slurry at 6% DM (MAFF, 2000), this organic-N loading would be reached with $57 \text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$ (close to the SL treatment). In the current study, the $50 \text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$ treatment (SL) would be representative of the application rate on many farms in Northern Ireland, but some fields may receive up to $100 \text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$. The highest rate of slurry application in this study ($200 \text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$) is higher than allowed under the Nitrates Directive. The experiment was setup in 1970 before the Nitrates Directive was in place and is still continuing. Results from this study are therefore an ideal resource to evaluate not only the effect of different slurry application rates but also the effect that repeated applications have on N dynamics.

4.2. Effect of fertilizer and slurry application on dry-matter production and N uptake

Dry-matter production was positively affected in response to N supplied via fertilizer and slurry applications. While in the CT treatment a small continuous decline in N uptake occurred, the other treatments showed either a constant response over the 38 years (CF and SL treatments) or an increasing response over time (SM and SH) (Fig. 2). The average dry-matter production of $7748 \text{kg N ha}^{-1} \text{yr}^{-1}$ from the CF treatment where $200 \text{kg N ha}^{-1} \text{yr}^{-1}$ was applied is within the range of dry-matter yield reported for lowland UK grasslands (between 7000 and $8000 \text{kg N ha}^{-1} \text{yr}^{-1}$, Whitehead,

2000). The main difference between fertilizer and slurry treatments is the additional supply of organic material contained in the slurry which is known to affect the size and the turnover of soil organic matter (SOM) (Sleutel et al., 2006). Both, fertilizer and slurry had an immediate effect on herbage yield and N uptake as indicated by the fertilizer response in the first 3 years of the study. Based on the observed N uptake in the CF treatment ($200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) in the first 3 years (1970–1972) and assuming a direct relationship between N uptake and mineral N supply, the apparent N fertilizer effect of the slurry would be 170, 183 and $216 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for the SL, SM and SH treatments, respectively. However, the N supply (based on a N content in the slurry of 3 kg N m^{-3}) would be 150, 300 and 600 kg N ha^{-1} respectively. This highlights the inefficiency of N use at high slurry application rates (Fig. 6). In contrast to the fertilizer treatment, slurry stimulated additional N supply to the plants over the years which resulted in a linear increase in N uptake over time. The increase was dependent on the slurry application rate (Fig. 2). The effect on N supply to plants is also reflected by the dose-response curves between N supply and N uptake. This relationship is clearly non-linear indicating a reduced efficiency of N uptake with increasing slurry application rates. Furthermore the dose-response curves are clearly different between the beginning and the end of the observation period (Fig. 6). The dose-response curves (assuming 3 kg N m^{-3} in the slurry) show three important aspects: (1) not all N supplied via slurry is available for plant uptake (2) N is used very inefficiently, especially in the high slurry treatments and (3) after decades of slurry application the N supply can increase substantially (almost 80% in the SH treatment) thus internal N supply mechanisms are stimulated by slurry or rather the organic fraction contained therein. However the increasing inefficiency with application rates shows the increased risk of N losses at higher application rates which is of environmental concern due the threat of NO_3^- leaching and/or gaseous N emissions.

Thus changes in the dose-response curve are most likely a reflection of the adaptation of the internal N cycle (Mallory and Griffin, 2007). The additional annual increase in N supply to the sward over the 38 years amounted to 0.18, 1.5 and $2.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (slope in Fig. 2 multiplied by 10 to convert g m^{-2} to kg N ha^{-1}) in the SL, SM and SH treatments, respectively. Thus, any tangible benefit in yield and N uptake is only seen after decades of slurry applications.

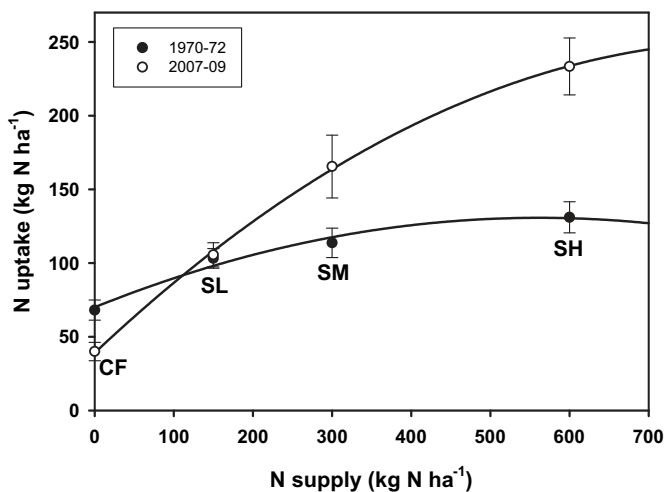


Fig. 6. Dose-response curves (plant N uptake vs. N supply, averages \pm stdev) due to increasing slurry application rates in a permanent grassland at the beginning (1970–1972) and at the end of the observation period (2007–2009). The parameters of the quadratic regression equations ($y = a + bx + cx^2$) are for 1970–1972: $a = 70$; $b = 0.22$, $c = -1.9e-4$; $R^2 = 0.98$ and for 2007–2009: $a = 39$; $b = 0.51$, $c = -3.0e-4$; $R^2 = 1$. (Treatments are indicated in the graph with the respective abbreviations).

The N uptake of the sward in the SL, SM and SH treatments increased between the first 3 years (1970–1972) and last 3 years (2007–2009) of the observation period by 2, 52 and 102 kg N ha^{-1} , respectively corresponding to a relative increase, based on the initial fertilizer response (1970–1972), of 2, 46 and 78% in the SL, SM and SH treatment, respectively. While it is known that repeated long-term slurry applications have an effect on SOM content and turnover, particularly on the labile SOM pool, the detailed mechanisms affecting soil N dynamics are still largely unknown (Kirchmann et al., 2004; Mallory and Griffin, 2007).

4.3. Application of a ^{15}N tracing analysis

The effect of slurry on gross N mineralization and nitrification rates has been studied before (Habteselassie et al., 2006; Zaman et al., 1999, 2004) but not after such a long-term amendment period as in the current study. Moreover in previous studies the pool dilution technique has been applied and this only quantifies the gross NH_4^+ and NO_3^- production and consumption rates and does not further differentiate specific gross N rates. To identify, for instance, gross N mineralization rates from labile and recalcitrant SOM, as suggested by Mallory and Griffin (2007), a ^{15}N tracing approach is required (Müller et al., 2007). Since the aim of this study was to identify potential N dynamics and obtain a mechanistic understanding of the simultaneously operating gross N transformations after repeated long-term fertilizer and slurry applications, a laboratory study was carried out. As shown previously, the rates obtained in such a study may not necessarily reflect field rates, which will vary in response to environmental conditions, but rather reflect the potential gross N dynamics after long-term N applications under conditions of the experiment (Arnold et al., 2008). However, the rates of mineralization and nitrification obtained in the current study are within the range reported in other grassland studies (Booth et al., 2005).

4.4. Long-term effect of fertilizer and slurry applications on N mineralization in grassland soils

Previous studies have shown that repeated slurry applications affect the size of the labile organic-N pool (Habteselassie et al., 2006). Christie and Beattie (1989) showed an increase in the soil microbial biomass with increasing slurry application rates after 17 years at the current study site. Here we showed that a shift in mineralization toward labile organic-N occurred which was stimulated by increasing slurry additions (Fig. 4a). Interestingly the overall gross NH_4^+ production (Fig. 4a) was not statistically different among treatments which is in line with other long-term studies (Habteselassie et al., 2006; Hao et al., 2003). Furthermore, instead of a net NH_4^+ production, as observed in the treatments CT, CF and SL, a net NH_4^+ consumption was observed in the two highest slurry treatments (Fig. 5a). Calculations with and without considering O_{NH_4} showed that the NH_4^+ turnover under the slurry treatments was governed by the rate of NH_4^+ oxidation which was affected by the different slurry application rates. The total gross N mineralization rates ($5\text{--}7.5 \mu\text{g g}^{-1} \text{ d}^{-1}$) were comparable with those of other long-term studies where sewage sludge was applied (Zaman et al., 2004) but were only half the gross rates determined in treatments where liquid dairy waste had been applied over 6 years (Habteselassie et al., 2006). In the present study we have shown that total mineralization was only marginally affected by N application (Fig. 4a) and that the declining mineralization toward NH_4^+ (M_{Nrec}) was compensated by oxidation toward NO_3^- (O_{Nrec}) (heterotrophic nitrification) (Fig. 5b), which may be a mechanism driven by a shift in the microbial community structure feeding on recalcitrant organic-N (N_{rec}) (Laughlin et al., 2009; Marschner et al., 2003). The NH_4^+

consumption was mainly driven by oxidation of NH_4^+ to NO_3^- (O_{NH_4}) (Fig. 4b). Fast NH_4^+ consumption and rapid NO_3^- production have also been observed in other long-term studies (Mallory and Griffin, 2007). Here we showed that a shift toward labile organic-N and the increase in heterotrophic nitrification are most likely crucial mechanisms leading to NO_3^- build-up and are related to the supply of organic constituents in slurry providing substrates for microbial growth (Laughlin et al., 2008; Müller et al., 2003).

4.5. Long-term effect of fertilizer and slurry applications on nitrification in grassland soils

Slurry application increased the oxidation of both NH_4^+ and organic-N so that the ratio of the two processes stayed more or less the same for all slurry treatments. In the CF treatment NO_3^- was almost exclusively produced by NH_4^+ oxidation (Fig. 4b). The increase in nitrification due to mineral and organic amendments is a general phenomenon (Schimel and Bennett, 2004). In addition to autotrophic nitrification, which was the only NO_3^- production process considered by Schimel and Bennett (2004), it is the activity and related NO_3^- production of heterotrophic nitrifiers, as stimulated by the organic amendments, that can explain the N dynamics under long-term slurry amendments. In the current study, the supply of organic material with the slurry stimulated heterotrophic processes that produced NO_3^- as well as NH_4^+ .

5. Conclusions

The gradual increase in herbage yield and N uptake during long-term repeated slurry applications showed that N dynamics changed gradually in response to organic-N amendments, stimulating additional N uptake and plant growth. Results also show that an application of $200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (e.g. supplied via mineral fertilizer) was suboptimal (Fig. 6). Highest plant growth was achieved at the highest slurry application rate but at this rate N was used very inefficiently, promoting potentially high N losses. Processes related to the turnover of organic-N are essential for the understanding of long-term changes and productivity of grassland ecosystems. There was a large impact on soil NH_4^+ and NO_3^- dynamics in soil that had received repeated slurry applications over 38 years. Increasing slurry application rates promoted a number of changes in the soil, including a shift from N mineralization of recalcitrant organic-N to labile organic-N, a shift from NH_4^+ production toward NO_3^- production of the Nrec pool and a stimulation of autotrophic and heterotrophic nitrification. The NO_3^- increase is of concern due to its ecological significance, increasing the potential for NO_3^- losses via leaching and/or gaseous N emissions. This is supported by a 9 year study adjacent to the current site which showed that the loss of NO_3^- to drainage water was linearly related to N input (Watson et al., 2000). At recommended levels of slurry application ($50 \text{ m}^3 \text{ ha}^{-1}$) no additional N was produced during long-term repeated slurry applications. This study provides a process-based explanation of how repeated slurry applications affect the internal mineralization-immobilization-turnover in permanent grassland soils. To link the observed results with biological function in the various treatments, future work should focus to identify the microbial community patterns and the related activity that have developed under long-term repeated N amendments.

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References

- Arnold, J., Corre, M.D., Veldkamp, E., 2008. Cold storage and laboratory incubation of intact soil cores do not reflect in-situ nitrogen cycling rates of tropical forest soils. *Soil Biology & Biochemistry* 40, 2480–2483.
- Beauchamp, E.G., Trevors, J.T., Paul, J.W., 1989. Carbon sources for bacterial denitrification. *Advances in Soil Science* 10, 113–142.
- Bittman, S., Forge, T.A., Kowalenko, C.G., 2005. Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. *Soil Biology & Biochemistry* 37, 613–623.
- Boorman, D.B., Hollis, J.M., Lilly, A., 1995. Hydrology of Soil Types: A Hydrologically-based Classification of the Soils of the United Kingdom Report No. 126. Institute of Hydrology, Wallingford.
- Booth, M.S., Stark, J.M., Rastetter, E.B., 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecological Monographs* 75, 139–157.
- Census, A., 2009. The Agricultural Census in Northern Ireland. Department of Agriculture and Rural Development.
- Christie, P., 1987. Some long-term effects of slurry on grassland. *Journal of Agricultural Science, Cambridge* 108, 529–541.
- Christie, P., Beattie, A.J.M., 1989. Grassland soil microbial biomass and accumulation of potentially toxic metals from long-term slurry application. *Journal of Applied Ecology* 26, 597–612.
- Cookson, W.R., Müller, C., O'Brien, P.A., Murphy, D.V., Grierson, P.F., 2006. Nitrogen dynamics in an Australian semi-arid grassland soil. *Ecology* 87, 2047–2057.
- Cox, G.M., Gibbons, J.M., Wood, A.T.A., Craigan, J., Ramsden, S.J., Crout, N.M.J., 2006. Towards the systematic simplification of mechanistic models. *Ecological Modelling* 198, 240–246.
- Curtin, D., Wen, G., 1999. Organic matter fractions contributing to soil nitrogen mineralization potential. *Soil Science Society of America Journal* 63, 410–415.
- Dambreville, C., Hénault, C., Bizouard, F., Morvan, T., Chaussod, R., Germon, J.-C., 2006. Compared effects of long-term pig slurry applications and mineral fertilization on soil denitrification and its end products (N_2O , N_2). *Plant and Soil* 42, 490–500.
- Frost, J.P., Bailey, J.D., Stevens, R.J., 2005. Making Best On-farm Use of Plant Nutrients in Livestock manures. Agricultural Research Institute of Northern Ireland, Belfast.
- Habteselassie, M.Y., Stark, J.M., Miller, B.E., Thacker, S.G., Norton, J.M., 2006. Gross nitrogen transformations in an agricultural soil after repeated dairy-waste application. *Soil Science Society of America Journal* 70, 1338–1348.
- Hao, X., Chang, C., Travis, G.R., Zhang, F., 2003. Soil carbon and nitrogen response to 25 annual cattle manure applications. *Zeitschrift für Pflanzenernährung und Bodenkunde* 166, 239–245.
- Huygens, D., Rütting, T., Boeckx, P., Van Cleemput, O., Godoy, R., Müller, C., 2007. Soil nitrogen conservation mechanisms in a pristine south Chilean *Nothofagus* forest ecosystem. *Soil Biology & Biochemistry* 39, 2448–2458.
- Keeney, D.R., Nelson, D.W., 1982. Nitrogen – Inorganic Forms Part 2 Chemical and Microbiological Properties. vol. Monograph No. 9, second ed. ASA-SSSA, Madison, USA.
- Kirchmann, H., Haberhauer, G., Kandeler, E., Sessitsch, A., Gerzabek, M.H., 2004. Effects of level and quality of organic matter input on carbon storage and biological activity in soil: synthesis of a long-term experiment. *Global Biogeochemical Cycles* 18, GB4011. doi:10.1029/2003GB002204.
- Kirkham, D., Bartholomew, W.V., 1954. Equations for following nutrient transformations in soil, utilizing tracer data. *Soil Science Society of America Proceedings* 18, 33–34.
- Knorr, W., Kattge, J., 2005. Inversion of terrestrial ecosystem model parameter values against eddy covariance measurements by Monte Carlo sampling. *Global Change Biology* 11, 1333–1351.
- Laughlin, R.J., Stevens, R.J., Zhuo, S., 1997. Determining nitrogen-15 in ammonium by producing nitrous oxide. *Soil Science Society of America Journal* 61, 462–465.
- Laughlin, R.J., Stevens, R.J., Müller, C., Watson, C.J., 2008. Evidence that fungi can oxidize NH_4^+ to NO_3^- in a grassland soil. *European Journal of Soil Science* 59, 285–291.
- Laughlin, R.J., Rütting, T., Müller, C., Watson, C.J., Stevens, R.J., 2009. Effect of acetate on soil respiration, N_2O emissions and gross N transformations related to fungi and bacteria in a grassland soil. *Applied Soil Ecology* 42, 25–30.
- MAFF, 2000. Fertiliser Recommendations for Agricultural and Horticultural Crops. Ministry of Agriculture, Fisheries and Food London.
- Mallory, E.B., Griffin, T.S., 2007. Impacts of soil amendment history on nitrogen availability from manure and fertilizer. *Soil Science Society of America Journal* 71, 964–973.
- Marschner, P., Kandeler, E., Marschner, B., 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology & Biochemistry* 35, 453–461.
- Metropolis, N., Rosenbluth, A.W., Rosenbluth, M.N., Teller, A.H., 1953. Equation of state calculations by fast computing machines. *The Journal of Chemical Physics* 21, 1087–1092.
- Müller, C., Stevens, R.J., Laughlin, R.J., 2003. Evidence of carbon stimulated N transformations in grassland soil after slurry application. *Soil Biology & Biochemistry* 35, 285–293.

- Müller, C., Stevens, R.J., Laughlin, R.J., 2004. A ^{15}N tracing model to analyse N transformations in old grassland soil. *Soil Biology & Biochemistry* 36, 619–632.
- Müller, C., Rütting, T., Kattge, J., Laughlin, R.J., Stevens, R.J., 2007. Estimation of parameters in complex ^{15}N tracing models by Monte Carlo sampling. *Soil Biology & Biochemistry* 39, 715–726.
- Müller, C., Rütting, T., Abbasi, M.K., Laughlin, R.J., Kammann, C., Clough, T.J., Sherlock, R.R., Kattge, J., Jäger, H.-J., Watson, C.J., Stevens, R.J., 2009. Effect of elevated CO_2 on soil N dynamics in a temperate grassland soil. *Soil Biology & Biochemistry* 41, 1996–2001.
- Reijs, J.W., Sonneveld, M.P.W., Sørensen, P., Schils, R.L.M., Groot, J.C.J., Lantinga, E.A., 2005. Utilization of nitrogen from cattle slurry applied to grassland as affected by diet composition. *Agriculture Ecosystems and Environment* 118, 65–78.
- Rochette, P., van Bochove, E., Prévost, D., Angers, D.A., Côté, D., Bertrand, N., 2000. Soil carbon and nitrogen dynamics following application of pig slurry for the 19th consecutive year: II. Nitrous oxide and mineral nitrogen. *Soil Science Society of America Journal* 64, 1396–1403.
- Rütting, T., Müller, C., 2007. ^{15}N tracing models with a Monte Carlo optimization procedure provide new insights on gross N transformations in soils. *Soil Biology & Biochemistry* 39, 2351–2361.
- Rütting, T., Huygens, D., Straelens, J., Müller, C., Boeckx, P., 2011. Advances in ^{15}N -tracing experiments: new labelling and data analysis approaches. *Biochemical Society Transactions* 39, 279–283.
- Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591–602.
- Scialabba, N.E.-H., Müller-Lindenlauf, M., 2010. Organic agriculture and climate change. *Renewable Agriculture and Food Systems* 25, 158–169.
- Shimizu, M., Marutani, S., Desyatkin, A.R., Jin, T., Hata, H., Hatano, R., 2009. The effect of manure application on carbon dynamics and budgets in a managed grassland of Southern Hokkaido, Japan. *Agriculture Ecosystems and Environment* 130, 31–40.
- Sleutel, S., De Neve, S., Németh, T., Tóth, T., Hofman, G., 2006. Effect of manure and fertilizer application on the distribution of organic carbon in different soil fractions in long-term field experiments. *European Journal of Agronomy* 25, 280–288.
- Stevens, R.J., Laughlin, R.J., 1994. Determining nitrogen-15 nitrite or nitrate by producing nitrous oxide. *Soil Science Society of America Journal* 58, 1108–1116.
- Thomsen, I.K., Olesen, J.E., 2000. C and N mineralization of composted and anaerobically stored ruminant manure in differently textured soils. *Journal of Agricultural Science, Cambridge* 135, 151–159.
- Thomsen, I.K., Schjøning, P., Jensen, B., Kristensen, K., Christensen, B.T., 1999. Turnover of organic matter in differently textured soils II. Microbial activity as influenced by soil water regimes. *Geoderma* 89, 199–218.
- Watson, C.J., Jordan, S.D., Lennox, R.V., Smith, R.V., Steen, R.W.J., 2000. Inorganic nitrogen in drainage water from grazed grassland in Northern Ireland. *Journal of Environmental Quality* 29, 225–232.
- White, R.P., Murray, S., Rohweder, M., 2000. *Pilot Analysis of Global Ecosystems: Grassland Ecosystems*. World Resources Institute, Washington, DC.
- Whitehead, D., 2000. *Nutrient Elements in Grassland: Soil–Plant–Animal Relationships*. CAB International, Wallingford.
- Zaman, M., Di, H.J., Cameron, K.C., Frampton, C.M., 1999. Gross nitrogen mineralization and nitrification rates and their relationships to enzyme activities and the soil microbial biomass in soils treated with dairy shed effluent and ammonium fertilizer at different water potentials. *Biology and Fertility of Soils* 29, 178–186.
- Zaman, M., Matsushima, M., Chang, S.X., Inubushi, K., Nguyen, L.M., Goto, S., Kaneko, F., Yoneyama, T., 2004. Nitrogen mineralization, N_2O production and soil microbiological properties as affected by long-term applications of sewage sludge composts. *Biology and Fertility of Soils* 40, 101–109.
- Zhang, J., Zhu, T., Cai, Z., Müller, C., 2011. Nitrogen cycling in forest soils across climate gradients in Eastern China. *Plant and Soil*. doi:10.1007/s11104-010-0706-6.