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Long term-mineral nitrogen fertiliser recovery in cereals

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Abstract

Retention and remineralisation of soil nitrogen (N) originally applied as mineral fertiliser over multiple consecutive years may increase soil N supply to crops. Other than for organic manures, such cumulative effect has received limited attention for mineral fertilisers. The associated increment in crop N uptake, as compared to first-year uptake from fertiliser, can be expressed as fraction of annual N application rate. This here-called ΔRE is the difference between long-term (RE^{LT}) and first-year (RE^{1st}) recovery of mineral fertiliser N. This study aims to quantify ΔRE using data of nine long term experiments (LTEs) in Europe and North America.

RE^{1st} was assessed either by the ^{15}N isotope method, or by a zero-N subplot freshly superimposed on a long-term fertilised LTE treatment plot. RE^{LT} was calculated for all LTEs by comparing N uptake between long-term fertilised and control treatments. Using a mixed linear effect model, the effects of climate, crop type, experiment duration, average N rate, and soil clay content on ΔRE were evaluated. The effect of the type of method used in assessing RE^{1st} was evaluated too.

Across the nine LTEs, RE^{LT} of mineral fertiliser N was consistently higher than RE^{1st}. Mean ΔRE was 21.8% (± 8.75%, 95% CI) of annual N rate, with higher values for winter wheat than for maize. This shows that fertiliser-N retained in the soil and stubble may contribute substantially to crop N uptake in subsequent years. Our results suggest that an initial N recovery of 42% can increase to around 64% over time. Furthermore, ΔRE was not clearly related to long-term changes in topsoil total soil N stock, suggesting that ΔRE reflects a change in composition rather than size of the soil N pool. The long-term contribution of fertiliser-N to crop N supply should be considered in studies on N requirements for given yield targets, especially where future N rates deviate strongly from current rates.

Keywords: Cereal production; Nitrogen Use Efficiency; Long term experiment; Nitrogen recovery; 15N; Soil N retention

Highlights:

- **Nine long term cereal experiments in Europe and USA were analysed on N recovery of mineral fertiliser**

- **On average, N recovery increased from 42% in the first year to 64% in the long term.**

- **Delta recovery is larger for winter wheat than maize.**

- **Observed increase in soil N uptake are not proportionally explained by increases in soil N.**

1. Introduction

Long-term experiments (LTEs) such as the Broadbalk Wheat Experiment at Rothamsted Research (UK) show that external nitrogen (N) inputs can increase crop yields by two or three times (Rasmussen et al. 1998). Although ample N supply has led to increased food security in recent decades, it also causes environmental damage such as eutrophication of surface waters, loss of biodiversity, and global warming. On the other hand, in places with insufficient access to N inputs, severe food scarcity, low yields and soil depletion are apparent. Sustainable N management implies avoidance of excess application as well as avoidance of soil fertility depletion. This involves proper accounting for N requirements that meet crop needs for given target yields, both in short and long term. This is especially relevant in regions where drastic changes in fertiliser-N input are advocated or expected.

Research on inorganic N fertilisers has largely focused on N uptake in the year of application, and recommendation systems commonly account only for these first-year effects. Relatively few studies have aimed at quantifying the long-term effect of mineral fertiliser N on soil N and crop N uptake, although the need has been recognized (e.g. Thomsen et al. 2003). For organic manures, in contrast, long-term increments of total soil N, soil organic matter (SOM) and N uptake are well documented (e.g. Lund and Doss 1980; Schröder et al. 2005).

Yet inorganic fertiliser N inputs, too, may change the size or composition of the soil N pool in the long term - directly or via crop residues – and this could potentially sustain an increased annual soil N supply and associated crop N uptake. While multiple-year effects on soil N supply remain scarcely documented for mineral N fertilisers, several estimations have been made in the UK based on trials with ^{15}N-labelling. Sylvester-Bradley et al. (1987) calculated that 10% of mineral fertiliser applied was re-mineralised in the second year, 3% of the remainder in the third year and 1% in each of the following years. Similar values were found by others who followed the fate of fertiliser-derived ^{15}N for multiple years (e.g. Glendining et al. 2001; Macdonald et al. 2002).

LTE's have also been used to quantify long term apparent fertiliser N recovery by comparing annual crop N uptake in plots that did or did not receive fertiliser N during many years. Long term recovery thereby accounts for both the continuous soil N depletion in absence of fertiliser inputs, and the possible build-up of soil N under a regime of fertiliser input. Long-term N response curves, therefore, show steeper slopes of crop yield (at low and moderate N rates) than curves from the one-year trials typically used to inform fertiliser recommendation systems (Van Grinsven et al. 2021, in prep.).

Trends in long term N recovery, as seen in LTEs, may provide an upper estimate of the effect that sustained inorganic N inputs may have on soil N supply and crop yield. In the Bad Lauchstädt trial (Germany), a long-term increase in N recovery was observed between 1902 and 2016 (SI-Figure 5). However, such trends may also be caused by improvements in crop genotype, management, or climate. Bhogal et al. (1997) analysed an LTE at Ropsley (UK), and also found a positive trend in N recovery between 1978 and 1990. Their approach differed from Bad Lauchstädt in that multiple N application rates were used at Ropsley. Over time, N recovery differences between the respective N application rates appeared to increase. This suggests a positive effect of N rate on N retention and uptake, because higher N application rates showed larger increases in N recovery over time.

Other studies which aim to assess the long-term effect of mineral N application on crop N uptake use the residual effect of historically applied N on current uptake after changing N application rates (e.g. Maaz and Pan 2017; Petersen et al. 2010; Thomsen et al. 2003). Petersen et al. (2010) studied several experiments in Scandinavia where a wide range of new N rates were superimposed on historical N rates. The effect of historical N rates was found to be small compared to the effect of the newly established N rates on crop N uptake.

In this study we present a new analysis that borrows elements from the above cited studies. We evaluate the difference between short- and long-term N recovery (ΔRE). Next, we apply this method to cereal-based LTEs found suitable for the purpose of quantifying the long term effect of inorganic fertiliser N application on crop N uptake from the soil N pool.

2. Materials and methods

First, the literature was searched for LTEs with suitable experimental set-ups (Sections 2.1 and 2.2). Subsequently, RE^{LT}, RE^{1st}, and ΔRE were calculated for a number of data sets within each LTE (Sections 2.3, 2.4 and 2.5). Finally, a meta-analysis was conducted to find the mean ΔRE and try to explain observed variation.

2.1 Data selection and criteria

Data were collected from journal articles that reported information about LTEs. The selection criteria to include a study in this analysis were 1) at least one long-term fertilised and one long-term unfertilised (control) plot should be present to quantify RE^{LT}; 2) there should be either a ^{15}N or subplot experiment to quantify RE^{1st}; 3) the experiment should have run for at least five years, preferably longer, so that long-term effects may have become more apparent over time. Using the search terms "Long-term" and "Cereal" and "^{15}N" and/or "subplot" in Google Scholar and Web of Science, five useful experiments were selected. Another experiment was obtained from the Catch-C database, which contains over 300 long-term experiments within Europe. Of these, only one matched the selection criteria. Via personal communication, three more useful experiments were found. In total, this resulted in nine useful long-term experiments, which contained data from 11 experimental sites. When data were not fully provided in an article, they were obtained either by personal communication or by analysing figures from the article using Webplotdigitizer (Rohatgi 2020).

2.2 Characteristics of LTEs included in the meta-analysis

The selected experiments suitable for the calculation of ΔRE were located in Europe and North America (Figure 1). Crop residues (excluding roots and stubble) were removed from the field in the experiments, except for the LTEs in Kiel and Iowa. An overview of other meta-data and slight deviations from the above methods to calculate ΔRE is provided in Table 1 for all experiments. Such deviations include irreversible modifications in the experimental setup (instead of using subplots) enabling the calculation of RE^{1st}. A detailed description of all experiments is provided in SI-Table 1.

Figure 1: Locations of selected long-term experiments in North America (A) and Europe (B).

Table 1: Descriptive information about the experiments that were used to calculate ΔRE.
Crop type: WW= winter wheat, SB= spring barley, M=maize

Name of experiment	**Location**	**Crop type**	**Duration experiment (yrs.)**	**Sampling year(s)**	**Soil clay content (%)**	***RE*^{1st} method**	**Source**
Broadbalk	Harpenden, UK	WW	138-141	1980-1983	28	^{15}N	(Powlson et al. 1986)
Ropsley	Ropsley, UK	WW	15 or 16	1992-1993	27	^{15}N and Subplot	(Bhogal et al. 1997)
Oklahoma-222 and 502	Oklahoma, USA	WW	21 and 20 resp.	1989	20	^{15}N. Because total N uptake was not measured in 1989, a value was calculated using crop yield as a reference from total N uptake in 1991.	(Raun et al. 1999) and personal communication with Jagman Dhillon and Bill Raun
Monmouth	Monmouth, USA	M	12	1994-1996	24	^{15}N	(Stevens et al. 2005)
Salisbury	Salisbury, USA	M	5	1977	15	^{15}N	(Meisinger et al. 1985)
Iowa-central and southern	Ames and Chariton, USA	M	16	2015	20 and 15 resp.	^{15}N and Subplot	(Poffenbarger et al. 2018) and personal communication with Hanna Poffenbarger
Kiel	Kiel, Germany	WW & SB	7 or 9	1997-1999	15	^{15}N	(Sieling and Beims 2007) and personal communication with Klaus Sieling
Hoosfield	Harpenden, UK	SB	119-121	1970-1972	20	Subplot method. However, instead of introducing a subplot an complete alteration of the experimental design was used to calculate *RE*^{1st} and *RE*^{LT} (SI-Equation 1).	eRA database http://www.era.rothamsted.ac.uk/ and personal communication with Margaret Glendining
Bad Lauchstädt	Bad Lauchstädt, Germany	Rotation	80	1979-1982	21	*ΔRE* was directly calculated instead of first calculating *RE*^{1st} and *RE*^{LT} (SI-Equation 1).	(Körschens et al. 2002) and personal communication with Ines Merbach and João Vasco Silva.

2.3 Quantifying long-term N recovery

Long-term N recovery was calculated based on LTEs where fixed levels of mineral fertiliser N were maintained over many years (Figure 2; Equation 1). To calculate RE^{LT} from an LTE, at least one N application rate and a control plot must be present in the experimental set-up. The control plot should have received zero N, with P and K application at the same rate as the fertilised plot. In this manner, the additional crop N uptake (compared to the 0N treatment) is the long term N recovery from fertiliser and can be expressed per kg N applied. As this method takes a zero-N treatment as a reference, it should be referred to as 'long-term *apparent* recovery' (as opposed to labelled N recovery), but the term 'apparent' is omitted for brevity in the remainder of the text.

$$RE^{LT} = \frac{U^{+N} - U^{0N}}{\text{annual N applied (+ N)}} \quad (1)$$

With:

U^{+N}: annual N uptake from long-term fertilised plot (kg N/ha)

U^{0N}: annual N uptake from long-term non-fertilised (control) plot (kg N/ha)

Annual N applied is in kg N/ha.

Note that both the +N and 0N treatments here refer to the long-term treatments that are still being continued, undisturbed by recent interventions made to assess short-term recovery. Note also that first-year recovery is included in RE^{LT}.

2.4 Quantifying first-year N recovery

Within the long-term trial fields, two types of superimposed short-term experiments were considered suitable to calculate RE^{1st}: 1) introduction of 0N subplot (as illustrated by Figure 2, Left); 2) mineral fertiliser application with a ^{15}N isotope (Figure 2, Right). RE^{LT} and RE^{1st} were both calculated for the year in which such short-term experiments were run. For comparison with the long term situation, it is imperative that the long-term treatments are being continued unchanged.

Method 1: Introducing a subplot

Experiments with a newly introduced control subplot (only receiving PK application without mineral N fertiliser), enable calculation of RE^{1st} by subtracting measured N uptake in the control subplot from the N uptake in the main plot and dividing by the long-term N application rate (Equation 2).

$$RE^{1st} = \frac{U^{+N} - U_{sub}^{0N}}{\text{annual N applied (+ N)}} \quad (2)$$

With:

U^{+N}: N uptake from fertilised plot (kg/ha)

U_{sub}^{0N}: N uptake from non-fertilised (control) subplot (kg/ha), where the historic long-term N rate was discontinued in the year of observation.

Method 2: Using ^{15}N

Alternatively, most of the LTEs allowed to calculate RE^{1st} from observations on first-year 15N recovery from fertiliser labelled with the 15N isotope (Powlson et al. 1986). This approach assumes that the two isotopes undergo chemical and biological transformations in the same manner. The ^{15}N taken up by the crop was divided by the amount of 15N applied:

$$RE^{1st} = \frac{U^{15N}}{\text{15N applied}} \quad (3)$$

With:

U^{15N}: ^{15}N uptake (kg/ha/yr)

^{15}N application (kg N/ha/yr)

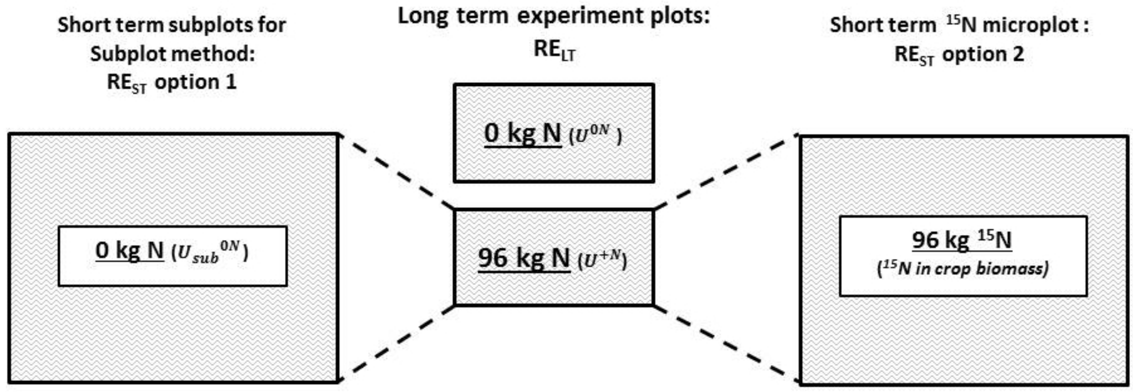

Figure 2: Examples of short-term experiments within a long-term trial which allow for the calculation of ΔRE. In the middle, two fields are shown that have continuously received either mineral N fertiliser or no fertiliser. Often such treatments are part of a larger setup with multiple N rate treatments. At the right, an example of a ^{15}N subplot is shown superimposed on the original treatment. The ^{15}N subplot receives the same N rate as the historic N rate, but now the fertiliser N is labelled. At the left, an example is shown where a new control subplot is introduced. The underlined numbers indicate the amount of applied mineral N fertiliser (these are examples only). Between brackets is indicated what is measured on these plots, corresponding to the notation in equations 1, 2, and 3.

2.5 Delta recovery (*ΔRE)*

The main response variable in this analysis, delta recovery (*ΔRE*), was introduced to express the cumulative effect of long-term mineral N application on crop N uptake. We define *ΔRE* as the difference between first-year N recovery (RE^{1st}) and long-term N recovery (RE^{LT}) in above-ground crop biomass, both measured in the same year.

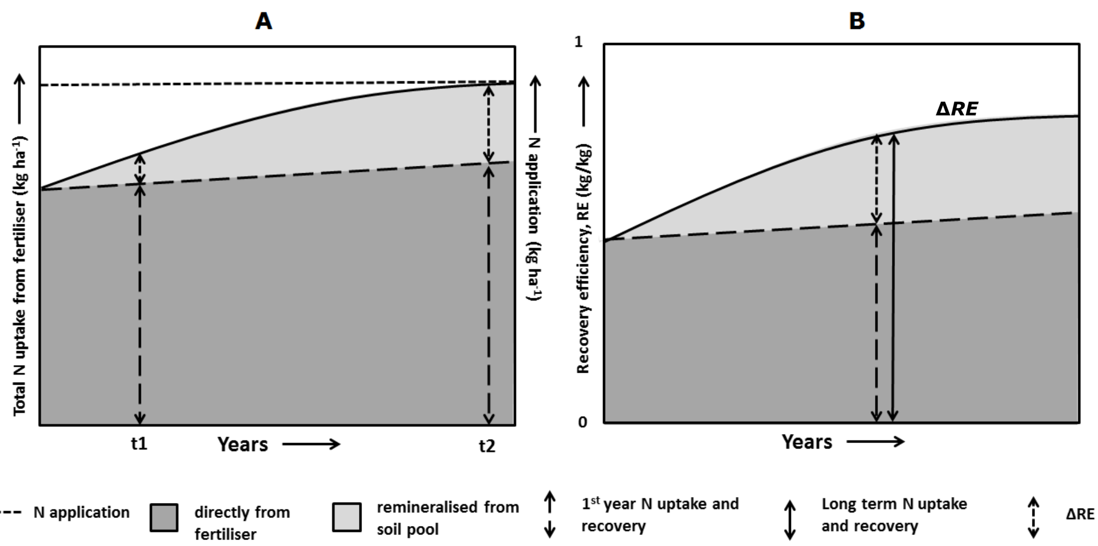

Figure 3: Hypothetical development of total N uptake and fertiliser N recovery, with continuous mineral N application over time. A) A certain fraction of applied N is directly taken up by the plant (short-term: i.e. in year of application), as indicated by the dashed arrows. The short-term recovery fraction increases over time in this graph, which can be caused by improvements in e.g. cultivars or management. It would also be possible that this line decreases when for example less efficient cultivars are introduced over time. The uptake of remineralised fertiliser N from an increasing organic N pool size (long-term) is indicated by the smaller dotted arrows. B) Recovery shows the same pattern as uptake, now divided by N application rate: the large dashed arrows indicate the fraction of annually applied N that is taken up in the season of application (short-term recovery), the full line arrow indicates the long-term N recovery. The small dotted two-sided arrow indicates Delta recovery (ΔRE): the difference between long- and short-term recovery. Uptake from the native soil N stock (that existed prior to start of LTE, and is dwindling over time) is not shown here.

As explained, first-year N recovery (RE^{1st}) refers to the fraction of N taken up from fertiliser in the year of application (Figure 2B, large dotted arrow). Long-term N recovery (RE^{LT}), in contrast, also includes uptake of N applied in earlier years (Figure 2B, solid black arrow). The difference between RE^{1st} and RE^{LT} *(ΔRE,* Equation 4) results from uptake of fertiliser-N that was retained in the soil and released beyond the year of application. Therefore, *ΔRE* could be thought of as 'delayed N recovery' and is expressed as percentage of annual N application rate (%):

$$\Delta RE \equiv RE^{LT} - RE^{1st} \tag{4}$$

Total aboveground biomass was used to determine crop N uptake as a basis for calculating all recovery values (RE^{1st}, RE^{LT}, *ΔRE*) , with the exception of two LTEs (Oklahoma 222 and 502) where only harvested grain was used. Cereal crops (wheat (*Triticum aestivum*), spring barley (*Hordeum vulgare*) and maize (*Zea mays*)) were the main focus of this study because these are the crops most commonly grown in long-term studies.

2.6 Meta-analysis

Relevant data from the nine LTEs were compiled in a database. Based on these, 66 sets of data were constructed which allowed for calculating RE^{1st}, RE^{LT} and *ΔRE*. Sixty-six observations were found as data from multiple years, crop types, and N application rates was available for some experiments.

Every observation included information about experimental location, year, N application rate, RE^{1st} calculation method and N uptake. Most studies that were included in this analysis did not provide a measure of variance for N uptake. Besides, for some studies every data point included only a single observation. Therefore, the number of replicates was used as a weighting factor for the data points, including the number of years and the number of true field replicates. Experiment location and sampling year were included as random effects. For both RE^{1st}, RE^{LT} and *ΔRE*, the normality of distribution was checked using density plots (SI-Figure 2).

2.6.1 RE_{1st} calculation method comparison

First, the extent to which the method used to calculate RE^{1st} (^{15}N or Subplot method) affected *ΔRE* was tested. This comparison was performed both on the whole dataset and on a subset of the experiments in which both methods were used. Using only LTEs where both ^{15}N and subplot method were available gives a straightforward comparison. However, as this selection reduced the sample size and the other analyses were performed using all data points, the type of method was also added as an explanatory variable for *ΔRE* using a mixed-effects model from the nlme package in R (Pinheiro et al. 2020) on the whole dataset.

2.6.2 Calculation of mean *ΔRE*

To calculate a weighted average of *ΔRE* across all studies, a mixed effect model was used from the nlme package in R (Pinheiro et al. 2020). In cases where both methods to calculate RE^{1st} were available, one value for *ΔRE* was included for each of the two methods.

2.6.3 Mixed-effects model estimation and selection

Besides quantifying *ΔRE*, this study also aimed to quantify the potential effects of several co-variables on *ΔRE* (Equation 5) such as crop type, experiment duration, average N application, soil type and climate. Co-variables were standardized to the same unit to enable comparison between studies. The effect of co-variables was tested in several combinations using mixed-effects models. To find the combination of co-variables that best fitted the data, a model selection was performed with the "dredge" function from the Mumin package (Barton 2019), based on the corrected Akaike information criterion (AICc). Models were considered to be different when $\Delta AICc > 2$. Fixed effects which were tested are provided in Equation 5. Co-variable values were mostly obtained from the published articles. In addition, the climate zone for each LTE was characterised by the Global Yield Gap Atlas approach, defining three main features: growing degree days, aridity index, and temperature seasonality (Van Wart et al. 2013). The variable named "crop residue retention" indicated whether crop residues were removed or kept on the field after the harvest. (the latter was the case in two LTEs)

$$\Delta RE \sim \text{Growing degree days} + \text{Temperature seasonality} + \text{Aridity index} + \text{Crop type} + \text{experiment duration} + \text{average N application} + \text{soil clay content} + \text{method} + \text{crop residue retention} + \varepsilon \quad (5)$$

2.6.4 Total Soil N

In addition to the co-variables that are shown in Equation 5, the effect of total soil N on soil N recovery was examined. The total soil N difference between the control plot and long-term fertilised plot could help to explain variation in *ΔRE.* Including total soil N as a co-variable in equation 5 was not optimal, because soil N data were only available for three experiments. Therefore, the relative increase in total soil N (i.e., in fertilised plot versus unfertilised plot) was compared with the relative increase in soil N uptake (again in fertilised plot versus unfertilised plot):

$$\text{relative soil N uptake increase} = \frac{\Delta RE * \text{N rate}}{U^{0N}} * 100\% \quad (6)$$

3. Results

3.1 Observed *ΔRE* across nine experiments

In 61 out of 66 cases, RE^{LT} was larger than RE^{1st} (Figure 4). When including all data-points, mean RE^{1st} and RE^{LT} were 42% (±13%, 95% confidence interval [CI]) and 62% (±14%, 95% CI) of annual N application rate, respectively. In Experiments 222 and 502 in Oklahoma, relatively low N recoveries were observed, probably because they refer to N recovery in grain only. When removing these (N=7) observations, mean RE^{1st} and RE^{LT} were respectively 51% (±9%, 95% CI) and 67% (±14%, 95% CI) of the annual application rate. For three observations, RE^{LT} exceeded 100%. For these, the increment in N uptake (compared to the control treatment) was larger than the amount of N applied. All of these points were retained in the overall analysis.

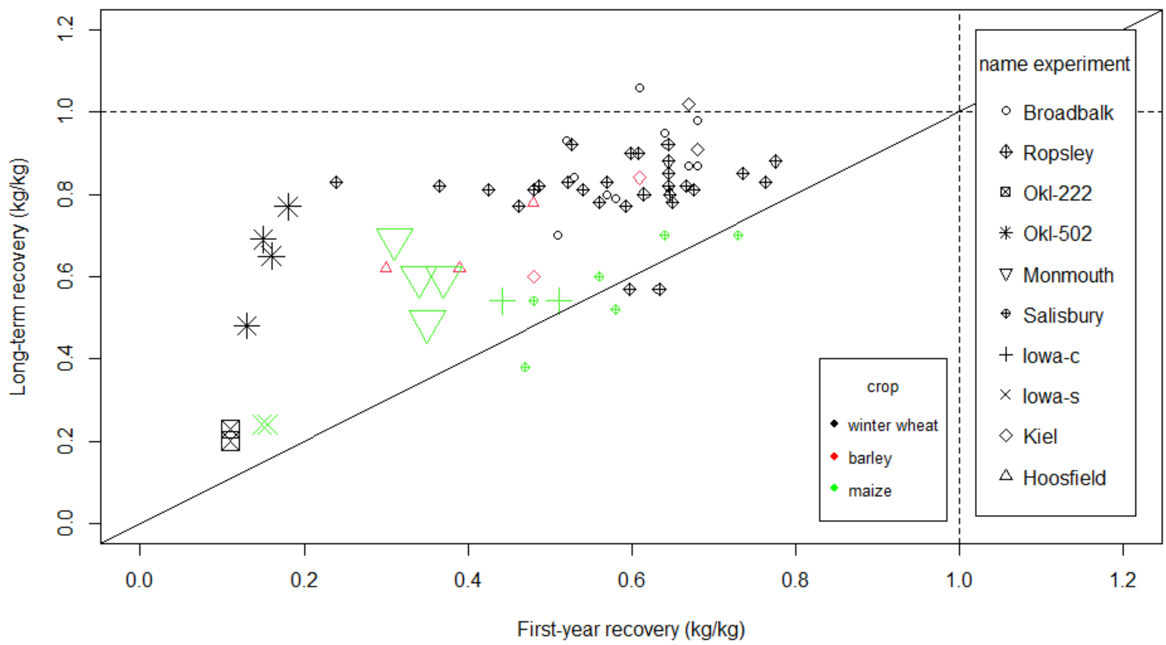

Figure 4: Short- and Long-term recovery for winter wheat, spring barley and maize (N=66). The diagonal solid black line indicates $RE^{LT} = RE^{1st}$. Point-size indicates the weight based on sample size. Note that data from the LTE Bad Lauchstädt is excluded from this graph as there were no calculated values of long and first-year recovery; only direct calculation of ΔRE was possible (see Table 1; explanation in SI, see SI Equation 2).

Across all data-points, mean *ΔRE* was 21.8% (±8.75%, 95% CI) of the annual N application rate. When excluding three high *ΔRE* observations from Oklahoma Experiment 502, mean *ΔRE* was slightly lower, namely 19.9% (±8.96%, 95% CI) of annual N application rate.

3.2 Influence of co-variables on ΔRE

No severe collinearity was observed between the co-variables included in the full model (i.e. including all co-variables; SI Figure 1). The two models with the lowest AICc values include crop type, method, and crop residue retention, without or with clay content (Table 2, Figure 5). Winter wheat showed a significantly higher *ΔRE* than maize (p=0.017). *ΔRE* did not significantly differ between the other crop types. Soil clay percentage was included as a predictive variable for *ΔRE* in the second-best model. However, the estimated slope of 0.87% of annual N application rate per percent clay content was not significant (p=0.33) when included as a sole variable. The effect of clay content on RE^{LT} seemed more evident, but was not significant (p=0.20, SI-Figure 4). The type of method to assess RE^{1st} was included as an explanatory variable in the best model when using data from all experiments (Figure 5C). In the experiments that allowed for both methods to calculate RE^{1st} (Ropsley, Iowa-central and Iowa-southern, Figure 5D), *ΔRE* was not significantly different between both calculation methods (P=0.18). However, when removing one outlier, the ^{15}N method showed a significantly higher *ΔRE* than the Subplot method (P=0.008). The difference between both methods amounted to 7.4% recovery of annual N application, caused by lower RE^{1st} values from ^{15}N experiments compared to the Subplot experiments. Lastly, crop residue retention was included in all selected models. However, when including crop residue retention as a sole variable, no significant difference (P=0.17) was observed between retention and removal of crop residues. Crop residues were retained at two of the nine LTEs.

Table 2: Model results of a model without co-variables, with all co-variables and the four best models based on AICc model selection. ΔRE estimates are only given for the model without co-variables because there are multiple estimates for the other models.

Co-variable	**Only Δ*RE***	**Full model**	**Model1**	**Model 2**	**Model 3**	**Model 4**
Growing degree days	-	**+**	-	-	-	-
Temperature seasonality	-	**+**	-	-	-	-
Aridity index	-	**+**	-	-	-	-
Crop type	-	**+**	**+**	**+**	-	-
Duration long-term	-	**+**	-	-	-	**-**
Average N application	-	**+**	-	-	-	-
Soil clay content	-	**+**	-	**+**	**+**	-
Method	-	**+**	**+**	**+**	**+**	**+**
Residue retention	-	**+**	**+**	**+**	**+**	**+**
AICc - value	551.7	580.8	540.8	540.9	543.3	543.9
ΔRE estimate	**21.8 (±8.75%, 95% CI)**					

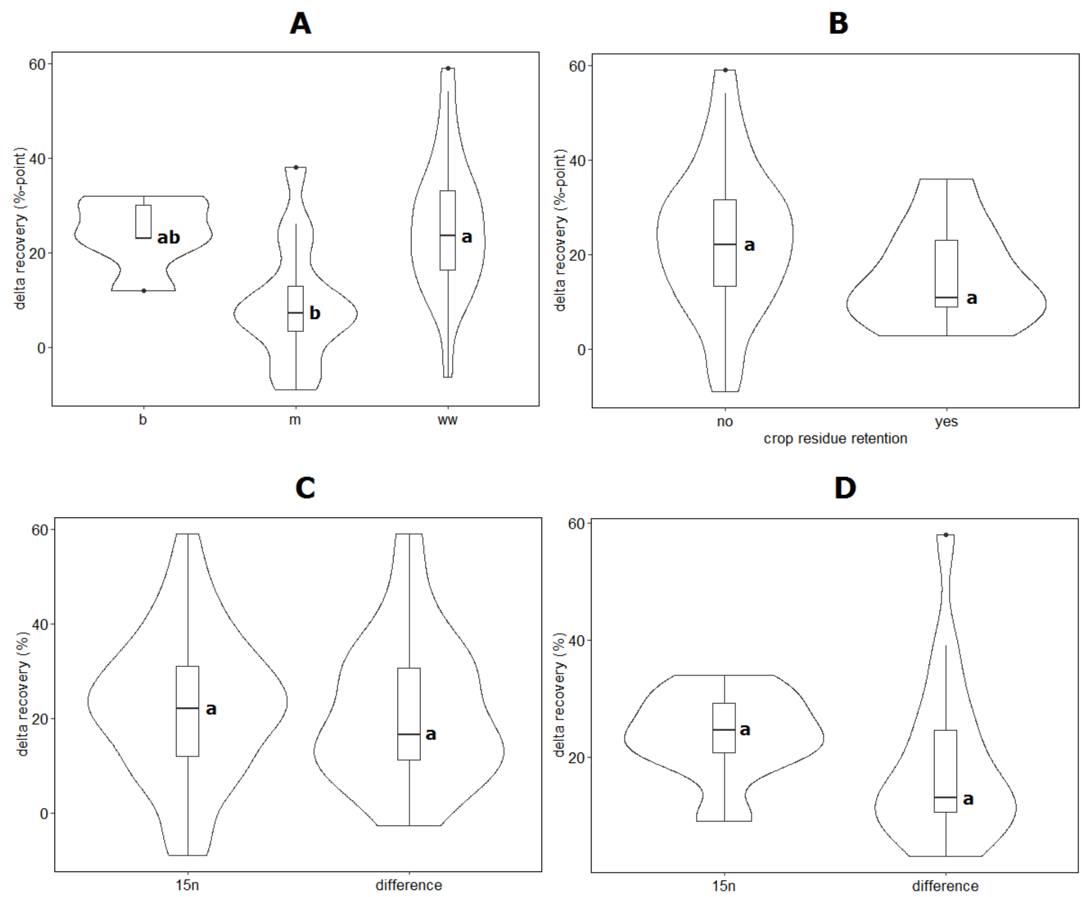

Figure 5: Violin and scatter plots of the distribution of ΔRE, separated for crop types (A) and clay percentage (B), and method (C&D) Within each violin plot, a boxplot indicates the median, lower (1th) and upper (3rd) quantile of the data. In plot B, ΔRE is plotted against clay content. Plot C illustrates the distribution of all data points, Plot D contains only data from experiment which allowed for both ^{15}N and Subplot method. If letters near the median are identical then there is no significant difference. b = barley; m = maize; ww = winter wheat.

In three out of nine experiments, total soil N data were available. Topsoil total soil N was, on average, 10% higher on fertilised plot compared to the control plot. N uptake from the soil, by contrast, increased by 86% on average, relative to unfertilsied control (SI-Figure 6). There was however no significant correlation between the relative increments of N uptake from the soil and total soil N (*P*=0.41), respectively.

4. Discussion and Conclusion

4.1 Long-term recovery is consistently higher than short-term recovery

Our results show a consistent positive *ΔRE*, which indicates that N originating from earlier mineral fertiliser applications contributes to crop N uptake in years after application. This corresponds well with ^{15}N studies which followed the fate of a single applied mineral ^{15}N over multiple years (e.g. Dourado-Neto et al. 2010; Glendining et al. 2001; Macdonald et al. 2002). Nitrogen retention was assessed in those studies by measuring the fraction of applied ^{15}N that ends up in the soil pool or crop. Additionally, when following ^{15}N in the soil for multiple years, the return to the soil of fertilizer-derived N via crop residues was also measured in those studies. While this method allows to follow a ^{15}N 'spike' (once applied) over several years, it does not allow quantification of the cumulative effect over many years with the same annual mineral N addition. Jenkinson et al. (2004) followed such a single ^{15}N pulse for nearly 20 years in old grassland where grass was harvested every year. In the first year after application, about 47% of applied ^{15}N was recovered. Cumulated over the following years of the experiment, another 17% of the initially applied ^{15}N was recovered in aboveground biomass, which is quite similar to the mean value of an additional 21.8% recovery for cereals found in this study.

Glendining et al. (1996) also found evidence for a positive *ΔRE*, as they describe the same method that is here called "Subplot method" in an experiment where N application was withheld for one season. The experiment was performed on Broadbalk, from which data were used in this study as well, but from other years. In the year that N fertiliser was not provided to the long-term plots, N uptake was higher on the "withheld" plots which had previously received fertiliser N, compared to a long-term control plot which never received N fertilizer. The maximum additional N uptake compared to the long-term N_0 treatment was found to be 29 kg N ha^{-1} on a plot which previously received 192 kg N ha^{-1}. The corresponding *ΔRE* value would be 15.1% of N applied. This method gives the same indication of the long-term effects from continuous mineral N application as is described in this study. However, the interpretation of their results was somewhat difficult due to weed growth. Nonetheless, their reported value corresponds well to the value of *ΔRE* that was found in this study.

As a single indicator of interest, RE^{1st} has been reported multiple times before (a.o. Dobermann 2005; Ladha et al. 2016). Based on mineral ^{15}N experiments, RE_{1st} is typically found to be around 50% (e.g. Petersen 2001; Powlson et al. 1986). In this study, we found an average RE^{1st} of 42%, which is somewhat lower. However, this slightly lower RE^{1st} is caused by the observations from Oklahoma, where only wheat grain was used to determine N recovery instead of total aboveground biomass. When the results from both Oklahoma experiments were excluded, an average RE^{1st} of 51% was found, while the average *ΔRE* only changed by 2.5%-point (to a *ΔRE* of 19.3%).

As reported values for N uptake were sometimes based on a number of replicates, weighting was used to give observations composed of multiple replicates a higher importance. However, weighing can also result in a bias towards those agro-ecological conditions for which a higher number of replicates happened to be available. Despite this, the estimated mean *ΔRE* only increased by 0.5%-point when excluding weights in the mixed-effects model. Furthermore, the model selection results did not change when weights were excluded.

4.2 Influence of crop type, soil clay content, and RE^{1st} calculation method

Crop type, soil clay content, crop residue retention, and RE^{1st} calculation method were the most important factors governing *ΔRE*, based on the model selection results. Winter wheat showed a significantly larger *ΔRE* than maize (Figure 5A), possibly because of its longer growing season, finer root system, and ability to root deeper, which would enable it to use mineralised N from soil organic matter more effectively (Thorup-Kristensen et al. 2009). Similarly, using a cover crop during the winter can potentially increase *ΔRE* for spring-sown crops. Soil type may also affect the ability to store, and re-mineralise fertiliser N. Soils with a higher clay content show a larger N immobilisation capacity compared to sandy soils (Cheshire et al. 1999). Crop residue retention was found to play an important role, based on the model selection results. In two experiments, crop residues were not removed from the field. It can be expected that *ΔRE* was higher in these experiments. However, no significant effect was found in our experiments. More experiments which retained crop residues at the field would have helped to get a better view on the effect of residues on *ΔRE.* Finally, the method used to estimate RE^{1st} (either ^{15}N or Subplot method) was found to affect *ΔRE*. However, this was only the case for the subset of experiments where both ^{15}N and the Subplot method could be used to calculate RE^{1st} and where the removal of one outlier resulted in a significant difference in *ΔRE* between methods.

^{15}N experiments are known to underestimate RE^{1st} (Jenkinson et al. 1985), which leads to higher values of *ΔRE* as the difference between RE^{1st} and RE^{LT} becomes larger. This is mainly caused by the phenomena collectively referred to as "added nitrogen interactions" (ANI). This includes pool substitution: replacement of unlabelled soil pool N by applied ^{15}N causes release of unlabelled N which the plant may take up, and so causes overestimation of the contribution by soil-N to crop N uptake (Jenkinson et al. 1985). Stepwise N rate experiments avoid these difficulties but are potentially afflicted with the "priming" issue (i.e. mineral N application increasing soil N mineralisation). Relatively, more ^{15}N experiments than 'subplot' experiments were found in the literature, possibly because their disturbing effect on the main LTE treatment plots is smaller.

4.3 Limitations of this study

4.3.1 Low variation in co-variable values

Climate, which was included in the regression model by using growing degree days, temperature seasonality, and an aridity index, did not significantly contribute to the explanation of observed variation in *ΔRE*, even when accounting for the influence of other co-variables. However, this could be due to the small variation in climate among the experimental locations. Most experimental sites were located around the same latitude, some with a more continental and others with a more maritime climate. For other climates, results may differ. However, no suitable long-term experiments were found beyond temperate climates.

The duration of the experiments did not affect *ΔRE*, in contrast to what was expected beforehand. A possible explanation is the lack of data-points in this study with an experimental duration between around 20 and 100 years (SI-Figure 3). When data points were clustered in two groups, the group above 80 years (14 out of 67 observations) showed an almost significantly higher *ΔRE* (by 8.7 % of N rate) than the group of experiments between 5 and 21 years (p=0.057). A second explanation could be that the soil N pool reaches a steady state (input=output) within a few decades, after which *ΔRE* would remain constant.

4.3.2 Total soil N

If a cumulative effect of mineral fertiliser N on soil N supply exists beyond the years of application, one would expect to find evidence by monitoring soil N stocks in LTEs. Previous studies reported clear, but relatively small increments in total soil N with increasing N application (Glendining and Powlson 1995; Macdonald et al. 1989; Petersen et al. 2010). Glendining and Powlson (1995) indicated that total soil N increased under higher mineral N application, but mineralisable N increased proportionally more. This suggests that soil pool quality changes, rather than the total N stock, are governing delRE. A similar conclusion was drawn in another study by Glendining et al. (1996). Bhogal et al. (1997) reported a 'break point' at around 150-160 kg N application, above which N recovery increased more proportional to total soil N compared to lower N rates. These findings correspond well to data from the experiments in this study, where increase in total soil N after long term mineral N application is much smaller than the increase in uptake from soil N. This suggests that *ΔRE* reflects a change in composition rather than size of the soil N pool. To our knowledge, there is still no conclusive explanation about the cause of the proportionally larger soil N uptake increase compared to total soil N increase.

4.4. Future implications of this study

Despite the potential difficulties with interpreting the specific value of *ΔRE*, a consistent positive value is certain. Additionally, it seems that crop type is the most important factor governing *ΔRE* (Table 2). More long-term experiments, with larger variation in all co-variables (e.g. assessing *ΔRE* in other situations, such as a tropical climate), can help to further develop understanding about sustainable N cycling. The outcomes of this study suggest that a current total N recovery of 42% can become, on average, 62% over time due to N retention in the soil. This is different from the simplified 50% that is commonly implemented at the moment, which does not consider soil N retention.

Due to the long term effect of fertiliser N retention on crop N uptake (expressed here as *ΔRE*), N yield response curves based on long term trials show steeper slopes than those based on short term trials. This shift should be taken into account in studies that seek to strike a balance between farm profit, food security and the environment. This is especially relevant in regions where N input rates are drastically changed. For example when grain output must steeply rise to feed a growing population such as in sub-Saharan Africa, or when N inputs are reduced to mitigate water pollution or greenhouse gas emissions such as in parts of Europe today.

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