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ORIGINAL PAPER

Annual and Long-Term Changes in N Pools and Fluxes in Field Lysimetric Investigation

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Abstract

Purpose Short- and long-term effects of a varying annual rate of nitrogen (N) inputs on plant N uptake and N balance at a constant mean level of fertilization are seldom studied. Applied N may or may not be utilized by crops. This leads to a positive, neutral or negative balance between inputs and outputs. Although the balance could not be exactly determined, it should subsequently change measurable pools or fluxes of N in soil during long-term field investigation.

Methods Influence of varying annual N fertilization rates on soil N pools (soil mineral nitrogen), N fluxes (outputs through plant N uptake and leaching) and balance between inputs and outputs, was studied in a 21-year field lysimetric experiment. We hypothesized that each N input into the soil or loss from the soil changes remaining pools and fluxes of N. We also hypothesized that a long-term deficit decreases, and a surplus increases, soil mineral nitrogen (N_{min}), plant N uptake and losses.

Results At given N input rate, the mean annual crop N uptake was higher than the inputs at 7 of 8 locations. An increasing rate of annual N input increased crop N uptake, with only a marginal effect on N_{min} concentration and leaching.

Conclusions Results show, that internal soil resources provided up to 100 kg N to annual N uptake and from 100 kg N ha^{-1} of annual N input, 40–50 kg N is harvested and the rest of added N is retained in soil and released later, independently on actual rate of input. Despite a negative N balance, long-term changes were limited. The results suggest that non-estimated sources of N help to maintain sustainability of the studied cropping systems.

Keywords Nitrogen fertilization · Plant nitrogen uptake · Lysimeter · Soil nitrogen

1 Introduction

Annual rates of N fertilization, defined as annual input of N into soil and expressed in kg N ha^{-1} year^{-1}, are usually quite variable even at a uniform level of N fertilization. They should reflect crop demand, content of soil mineral nitrogen (N_{min}, the sum of extractable NH_4^+ and NO_3^- concentration in soil) and anticipated yield. Nitrogen is usually applied with the aim of meeting plant demand, but the relation between N dosage and crop yield is not straightforward. Yield and plant N uptake should be related to input. However, annual input does not always correspond with plant N uptake (Fontaine et al. 2023). Above all, climatic conditions or pest attacks have a very strong effect on N uptake and its utilization. Therefore, applied N may or may not be utilized by crops. This leads to a positive, neutral or negative balance between inputs and outputs (Huang et al. 2017). Balance could not be exactly determined because it is difficult to quantify gaseous fluxes. However a loss or increase of N in soil should subsequently change measurable pools as soil N_{min} or fluxes of N in soil as plant uptake, leaching and denitrification. Nitrogen added in surplus may be stored in the biomass of living soil organisms or in soil organic matter (Daly et al. 2021). However, the amount of N that may be biologically utilized is limited. When N inputs exceed plant and microbial uptake, it increases losses from the system through leaching or gaseous losses (Körschens et al. 2013; Quemada et al., 2020). Losses from the soil can

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Table 1 Basic characteristics and climatic conditions of the studied field stations

Location	Abbreviation	Elevation (m a. s. l.)	Precipitation (mm)	Temperature (°C)
Lednice	(Led)	170	461	9.1
Uherský Ostroh	(Uho)	196	525	8.8
Věrovany	(Ver)	207	562	8.5
Žatec	(Zat)	285	451	8.3
Chrastava	(Chr)	345	798	7.1
Jaroměřice	(Jar)	425	481	8.0
Horažďovice	(Hor)	470	575	7.8
Vysoká	(Vys)	580	599	7.4

Table 2 Basic characteristics and classification of soils at field stations

Location	C_{ox} (%)	pH ($CaCl_2$)	Soil classification	Soil type
Led	1.35	6.6	Chernozem	Loam
Uho	1.55	6.4	Haplic luvisol	Loam
Ver	1.35	6.5	Chernozem	Loam
Zat	1.39	7.1	Chernozem	Loam
Chr	1.07	5.9	Haplic luvisol	Sandy loam
Jar	1.24	6.5	Haplic luvisol	Loam
Hor	1.26	6.2	Cambisol	Loamy sand
Vys	1.25	6.0	Albeluvisol	Loam

Table 3 Rates of fertilization

treatment	Rate of mineral fertilization (kg ha^{-1} year^{-1})			Rate of manure application (t ha^{-1}year^{-1})	Liming
	N	*P*	K		
Control	0	0	0	0	0
Manure	0	0	0	40	according to pH value
N1	60	13	33		
N2	90	26	66		
N3	120	52	132		

be as high as 100 kg N ha^{-1} (Ma et al. 2019). Quemada et al. (2020) report surplus of N in European farmlands up to 150 kg N ha^{-1} with potential for losses. It suggests that up to approximately half of N inputs into agricultural soil could be lost. In contrast, an insufficient rate of fertilization often leads to a negative N balance and decreasing soil fertility (Müller et al. 2011). However, the rate of plant uptake may also be maintained for a relatively long time even without fertilization (Clark et al. 2009) at the expense of internal soil resources. For example, Karimi et al. (2017) report a low, but stable N uptake at a rate of 30 kg N ha^{-1}, even with a significantly decreasing content of total N in the soil. They attributed this to the increasing yield of improved plant cultivars and better weed control. With a shortage of N, plant N uptake from soil reserves should decrease soil N availability and, over the long term, a negative N balance should also lead to depletion of soil organic reserves of N (Karimi et al. 2017).

The rate of nitrogen mineralization is related to the amount of organic matter in the soil (Lal 2020). Don et al. (2013) propose that the concentration of soil organic matter is a driving factor of decomposition in soil. A decreasing amount of organic matter should therefore reduce the release of plant-available N from soil organic reserves. Consequently, export in crop yield may be a strong means of N depletion.

The influence of different annual N rates on plant nitrogen uptake, nitrogen leaching and balance of measurable inputs and outputs were studied in a long-term lysimetric investigation. In calculation of N balance, inputs included N applied in fertilizers, N in rainwater and N_2 fixation. Outputs were plant N uptake and leaching. We hypothesized that the annual rate of N inputs or outputs induces an equivalent change in plant uptake, soil mineral N accumulation and N losses. We also hypothesized that a long-term deficit decreases, and a surplus increases, soil N_{min}, plant N uptake and losses over time. Data from nearby situated long-term experiment were used to provide the additional information on yield, plant N-uptake, total content of soil carbon and nitrogen at different N rates.

2 Materials and Methods

2.1 Lysimetric Measurements

For the purpose of the current study, lysimetric measurements were performed at 8 locations of the Central Institute of Supervising and Testing in Agriculture in the Czech Republic (Tables 1 and 2). The results of 21 years (1996–2016) of measurements were evaluated. Long-term field experiment with different rate of NPK fertilization (Table 3) was established at all locations with mean annual N rates corresponding to 0, (N0), 60 (N1), 90 (N2) and 120 kg N ha^{-1} (N3). Lysimeters were set up at field sites with comparable management (rates of fertilization, crop sequence, soil cultivation) as used for treatments with middle rate of N addition (N2). Annual N input at these lysimetric sites was 70 kg N added in mineral fertilizer and the rest of N was provided as applications of manure that was performed in 4 year intervals. Crop rotation contained 25% legumes, 50% wheat and 25% row crops.

Nitrogen fertilizers were applied as ammonium sulphate during soil preparation before sowing. During vegetation, ammonium nitrate was applied as a side dressing. Phosphorus and potassium were applied as superphosphate and potassium chloride at the rates 26 kg P ha^{-1} and 66 kg K

ha^{-1} (Table 3). Organic fertilizers were applied to row crops as cattle manure at the rate of 40 t ha^{-1} (Table 3). The annual rate of fertilization was corrected according to the anticipated demands of crops. Liming was performed at pH lower than 6.5.

2.2 Sampling of Leachate and Rainfall Water

In each lysimeter, collectors of leachate were inserted into the wall of the working hole. The structure of the soil above the collector was not disturbed. Rainfall water percolated through the soil profile into the collector and was drained into a plastic bottle, where it accumulated. Bottles were situated in a pit, which was separated by a wooden board from the rest of the working hole. The working hole outside of the pit was filled with soil after the lysimeter construction was completed. The amount of water in the bottles was determined after each rainfall and samples of water were collected. Collectors were located 80 cm below the soil surface at the depth where water is hardly available for plant uptake.

At each location, collector of rainfall water was installed. Amount of rainfall water was determined after each rainfall and samples of rainfall water were collected. Samples of water were stored in plastic bottles at 4 °C until analysis.

2.3 Soil Sampling

Soil samples for determination of basic soil characteristics were collected from soil layer 0–30 cm in five terms during period 2013–2021 (2013, 2014, 2016, 2017 and 2021) at four locations (Hor, Chr, Jar, Uho) and before establishment of lysimeters at all lysimetric sites. Soil samples were sieved (2 mm) and air dried.

Soil samples for determination of N_{min} were periodically collected in two terms during the season: in early spring before fertilization (N_{spring}, Table 4), and after harvest ($N_{harvest}$) of the cultivated crops. Soil was sampled from two horizons: 0–40 cm and 40–60 cm. Samples were stored in plastic bags at -20 °C until analysis.

Table 4 Parameters calculated from results of lysimetric investigation

Parameter	Description
N_{spring}	Content of mineral N in soil layer 0–60 cm in spring before fertilization (kg N ha^{-1})
$N_{harvest}$	Content of mineral N in soil layer 0–60 cm after harvest (kg N ha^{-1})
N_{input}	Estimated annual rate of N input (kg N $ha^{-1}y^{-1}$)
N_{uptake}	N removed in harvested products (kg N ha^{-1} y^{-1})
N_{losses}	Leaching of NO_3^- below 80 cm (kg N ha^{-1} y^{-1})
$N_{balance}$	Nitrogen balance (kg N ha^{-1} y^{-1})
$N_{balance-cumulative}$	Cumulative nitrogen balance (kg N ha^{-1}) for whole period of measurements (21 years) calculated as a sum of N balances of the current year + N balance of all previous years

2.4 Soil and Leachate Analyses

Content of oxidizable soil carbon (C_{ox}) was determined using wet oxidation with acid dichromate, Content of total soil nitrogen (N_{tot}) was determined using Kjeldahl method (Zbíral et al. 2011). The pH value was measured in 1 *M* KCl extracts using a soil (fresh field-moist): solution ratio of 5 g: 25 ml (Zbíral et al. 2011).

The content of NH_4^+ and NO_3^- in soil and leachate were quantified colorimetrically using Berthelet reaction and absorbance at 660 nm for NH_4^+ and absorbance at 210 nm for NO_3^-according to ISO 14256-1 modified by Zbíral et al. (2011). Soil extraction was performed using 1 M KCl in ratio soil: solution 1:5.

2.5 Sampling of Harvestable Plant Products and Plant Analyses

Plant samples were taken after the harvest of the mature plants. Afterwards, the samples were homogenized in a laboratory knife mill and dried at 60 °C. Determination of total nitrogen in the plant biomass was carried out by the Kjeldahl method (Zbíral et al. 2014).

2.6 Calculations

Soil N_{min} concentration was calculated as a sum of NH_4^+ and NO_3^- concentration in dry soil. The content of N_{min} in soil layer 0–60 cm was calculated from the soil concentration of N_{min} in soil layers 0–40 and 40–60 and the bulk density of particular soil layers (Table 4). Plant N (N_{uptake}) uptake was estimated from the yield of plant products and content of N in the respective products. All harvested products were removed from the field. Atmospheric nitrogen input and losses, defined as leaching of N below 80 cm (N_{losses}, Table 4), were calculated from the volume of rainwater or leachate and the content of NH_4^+ and NO_3^- in water samples. Inputs (N_{input}) were calculated as the sum of N inputs in fertilizers and rainwater corrected for N_2 fixation by legumes. A rough estimation of the rate of N_2 fixation was based on the assumption that 80% of N in harvested legumes is provided by N_2 fixation and 70–90 kg N ha^{-1} remains in the soil after the harvest of alfalfa or clover, respectively (Klír et al. 2008). The annual N balance was calculated as the difference between inputs and outputs according to Eq. 1:

$$Equation1 : N_{balance} = N_{input} - (N_{harvest} + N_{losses}) \tag{1}$$

Table 5 Content of N_{min} in soil (kg N ha^{-1}) in spring (N_{spring}) and after harvest ($N_{harvest}$) during 21-year lysimetric investigation. Values are means ± standard deviation

	N_{spring}	$N_{harvest}$
Hor	121.9 ± 75.4	90.8 ± 53.3
Chr	51.0 ± 31.0	59.2 ± 29.1
Jar	77.9 ± 47.9	93.6 ± 62.8
Led	87.7 ± 37.0	70.9 ± 36.9
Ver	76.3 ± 39.9	57.4 ± 21.4
Uho	68.8 ± 33.6	60.4 ± 41.9
Vys	84.7 ± 61.5	105.3 ± 77.8
Zat	109.4 ± 62.2	101.4 ± 135.5

The cumulative balance was calculated as the sum of the annual rates of N balance. The N balance of the current year was added to the N balance of the previous years.

2.7 Statistical Analyses

Statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, USA). Correlations between measured parameters were calculated using Pearson's correlation coefficient. Hypotheses were tested for whole data sets and in individual locations.

Long-term trends of measured parameters were evaluated using a nonparametric Sen's method for estimating the slope of a linear trend (Salmi et al. 2002). The significance of observed trends was tested using a nonparametric Mann-Kendall test for testing the presence of a monotonic increasing or decreasing trend. Yield, N-uptake and content of soil C_{ox} and N_{tot} in 50 year experiment were evaluated using three way ANOVA with year, location and treatment were factors. As their values had similar trends in all stations, they were evaluated together across all locations.

3 Results

3.1 Pools and Fluxes of N at Lysimetric Sites

Values of N_{min} were quite variable in both terms of sampling and were in the range 51–122 kg N ha^{-1} in spring and 59–105 kg N ha^{-1} after harvest, respectively (Table 5). When annual rates of inputs in fertilizers and rainfall water in the range 80–120 kg N ha^{-1} were corrected for N_2 fixation, their values were 97–175 kg N ha^{-1} (Table 6, table S1). Plant N uptake was the main way of N output, and the rate of leaching was lower. Uptake of N by plants was highest at the station Uho (194 kg N ha^{-1}, Table 6) and lowest at Chr (94 kg N ha^{-1}). At other stations, uptake of 120–150 kg N ha^{-1} was observed. Leaching of NO_3^- was low at stations Hor, Led and Ver, where it was up to 5 kg N $ha^{-1}y^{-1}$. Values of 8 and 13 kg N $ha^{-1}y^{-1}$ were observed at stations Jar and Chr, and values between 20 and 30 kg N $ha^{-1}y^{-1}$ were determined at stations Vys, Uho and Zat.

3.2 Balance Between Inputs and Outputs

The mean annual balance between inputs and outputs was negative at most studied locations (Table 6). The highest N deficit was observed at the station Uho. At this location, a high rate of plant N uptake and significant leaching of NO_3^- (N_{losses}) were observed. A negative N balance at the rate of -30 kg N ha^{-1} was observed at stations Zat and Led. At these stations, leaching was low, but export in harvested products exceeded N inputs.

At the stations Ver, Chr, Vys and Hor, the annual deficit was from −11 to -16 kg N ha^{-1}.

A rate of balance reaching a neutral value was determined at the station Jar.

Table 6 Mean rates of N inputs in fertilizers, rainwater, total input (N in fertilizer + N in rainwater corrected for N_2 fixation) (N_{input}), plant N uptake (N_{uptake}), losses of N through leaching (N_{losses}) and balance of N ($N_{balance}$) (kg N ha^{-1} y^{-1}) during 21-year lysimetric investigation. Values are means ± standard deviation

	Fertilizer *N*	Rainwater *N*	N_{input}*	N_{uptake}	N_{losses}	$N_{balance}$
Hor	86.0 ± 106.5	8.1 ± 2.2	142.6 ± 125.9	150.9 ± 84.7	4.8 ± 4.9	-13.1 ± 121.9
Chr	79.1 ± 47.7	10.6 ± 10.2	97.3 ± 39.1	94.5 ± 30.6	13.7 ± 16.4	-10.9 ± 50.2
Jar	100.3 ± 63.3	19.2 ± 6.5	167.9 ± 78.7	150.9 ± 57.1	8.2 ± 19.6	8.7 ± 70.4
Led	56.2 ± 41.8	22.7 ± 13.8	118.5 ± 96.9	151.2 ± 80.1	0.5 ± 1.6	-33.1 ± 80.5
Ver	77.7 ± 57.4	13.5 ± 7.9	106.2 ± 59.9	122.0 ± 36.9	0.2 ± 0.4	-16.0 ± 62.3
Uho	99.9 ± 52.7	18.6 ± 15.3	174.9 ± 95.8	194.4 ± 81.2	27.3 ± 63.6	-46.8 ± 103.8
Vys	81.3 ± 57.2	15.9 ± 5.5	156.2 ± 93.1	149.8 ± 81.7	20.7 ± 14.2	-14.3 ± 54.7
Zat	93.4 ± 66.7	15.0 ± 9.9	117.8 ± 62.3	122.1 ± 42.7	29.1 ± 107.7	-33.4 ± 110.5

*Corrected for N_2 fixation

Table 7 Pearson's correlation coefficients between content of N_{min} in soil in spring (N_{spring}) and after harvest ($N_{harvest}$), total N input (N in fertilizer + N in rainwater corrected for N_2 fixation), (N_{input}), plant N uptake (N_{uptake}), losses of N through leaching (N_{losses}), balance of N ($N_{balance}$) and cumulative N balance ($N_{balance-cumulative}$) for data from individual stations in the 21-year field study. Pairs of variables without any correlation are not shown. The level of significance is given in parentheses, significant coefficients ($p<0.05$) are highlighted in bold, n = number of correlated pairs

Correlated parameters		Location								
		All n = 21	Hor n = 21	Chr n = 21	Jar n = 21	Led n = 21	UHO n = 20	Ver n = 19	Vys n = 21	Zat n = 19
N_{input}	N_{uptake}	**0.580** (0.000)	0.375 (0.09)	0.201 (0.383)	**0.645** (0.002)	**0.602** (0.004)	**0.542** (0.014)	0.242 (0.317)	**0.867** (0.001)	**0.507** (0.027)
N_{uptake}	$N_{harvest}$	-0.152 (0.054)	-0.150 (0.517)	0.090 (0.698)	-0.372 (0.097)	-0.060 (0.797)	-0.350 (0.131)	**0.492** (0.032)	**-0.460** (0.048)	-0.225 (0.355)
N_{spring}	N_{uptake}	0.073 (0.357)	**0.489** (0.024)	0.320 (0.157)	-0.324 (0.150)	-0.233 (0.309)	0.199 (0.400)	0.266 (0.270)	-0.246 (0.310)	-0.146 (0.551)
$N_{balance}$	$N_{harvest}$	0.147 (0.063)	0.275 (0.228)	**-0.445** (0.043)	**0.506** (0.019)	-0.268 (0.240)	0.126 (0.595)	-0.272 (0.259)	-0.052 (0.832)	0.172 (0.480)
$N_{balance-cumulative}$	$N_{harvest}$	0.095 (0.232)	0.066 (0.774)	-0.097 (0.675)	**0.513** (0.017)	-0.010 (0.964)	**0.541** (0.014)	-0.001 (0.996)	**-0.573** (0.010)	0.120 (0.623)
$N_{harvest}$	N_{losses}	-0.05 (0.523)	0.089 (0.701)	**0.457** (0.037)	-0.248 (0.279)	-0.252 (0.270)	-0.055 (0.816)	-0.039 (0.873)	-0.129 (0.600)	0.101 (0.681)

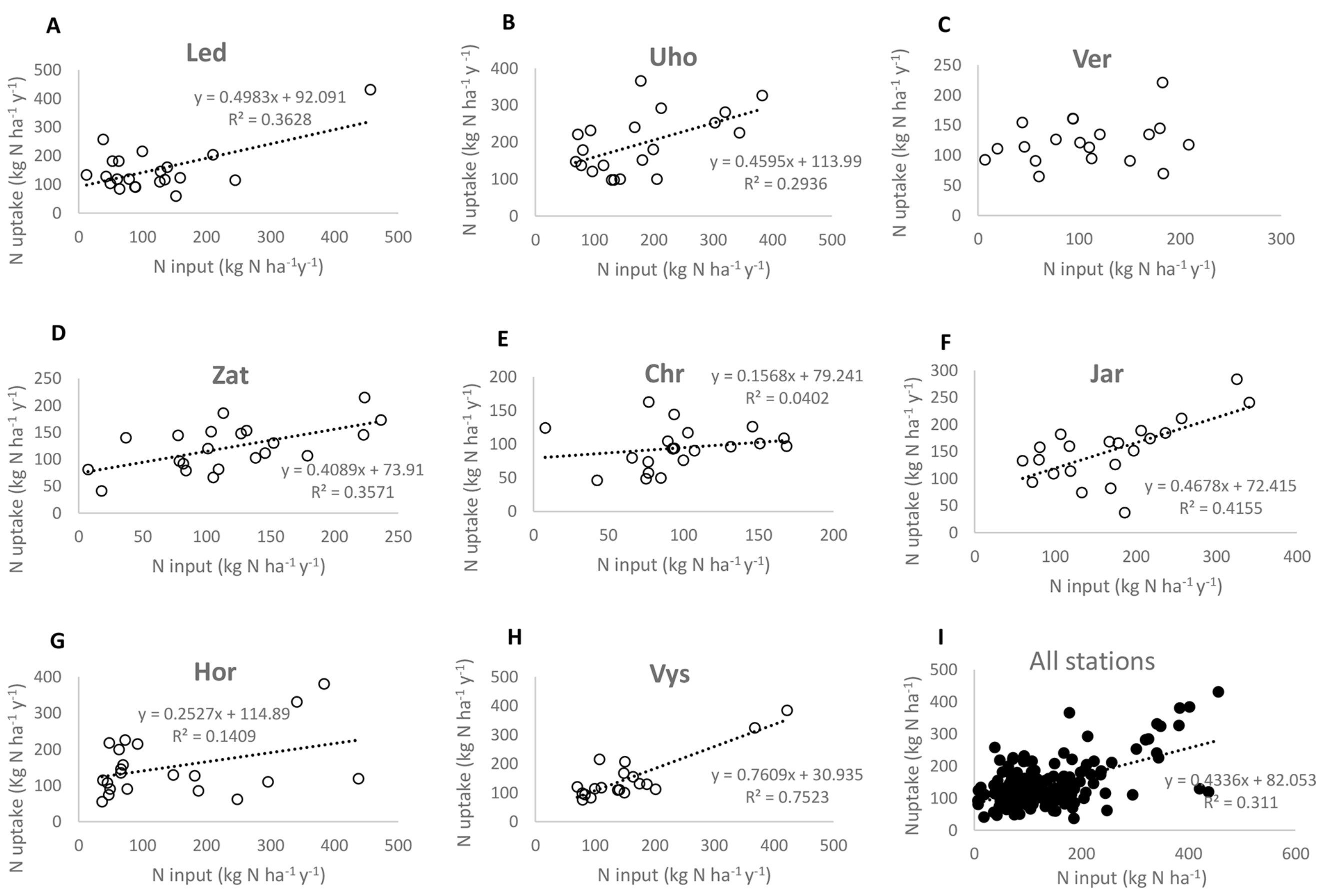

Fig. 1 Correlation between annual rate of N input (N_{input}) and N uptake (N_{uptake}) (kg N ha^{-1} y^{-1}) at lysimetric field sites Led (**A**), Uho (**B**), Ver (**C**), Zat (**D**), Chr (**E**), Jar (**F**), Hor (**G**), Vys (**H**) and at all stations (**I**)

3.3 Correlations Between Pools and Fluxes at Field Sites

In the general data set and at stations Jar, Led, Uho, Vys and Zat, plant N_{uptake} was correlated with N_{input} (Table 7; Fig. 1). No correlation between inputs and N uptake was determined at locations Ver, Hor and Chr. N_{uptake} was related to N_{spring} at the station Hor. At Ver, N uptake was correlated with $N_{harvest}$.

The rate of $N_{balance}$ was negatively correlated with $N_{harvest}$ at station Chr, while a positive correlation was found at Jar. Cumulative N balance was correlated with $N_{harvest}$ at stations Jar and Uho, and negatively correlated at the station Vys.

$N_{harvest}$ was found to have a significant effect on N_{losses} at station Chr. In the general data set, N_{min} was not related to N_{losses}.

3.4 Long-term Trends at Field Sites

When trends for all stations were estimated, only the cumulative balance showed significant changes (Table 8; Fig. 2). Other trends were insignificant. No significant long-term changes in the estimated parameters were observed at Jar. At other stations, significant changes in different parameters were found.

Cumulative N balance significantly decreased at all stations except station Jar. The highest rate of decrease was observed at the station Uho (49 kg N ha^{-1} y^{-1}).

At stations Chr and Ver, N_{spring} decreased over time at the rate 1.7 and 2.1 kg N $ha^{-1}y^{-1}$, respectively. The content of N_{spring} and $N_{harvest}$ significantly decreased at Uho, the decrease being 2.6 kg N ha^{-1} y^{-1} for N_{spring} and 2.6 kg N ha^{-1} y^{-1} for $N_{harvest}$, respectively.

Increasing content of soil mineral N was observed at Vys. N_{spring} and $N_{harvest}$ increased significantly at the rate 2.6 and 6.0 kg N ha^{-1} y^{-1}, respectively. Plant N uptake and annual N balance decreased only insignificantly over time.

3.5 Yield and N Uptake in 50 Year of Field Trial

Yield of all crops increased with increasing rate of fertilization up to the level of N1 (Fig. 3). Higher rates than N1 were without additional effect on yield. N uptake increased up to the rate of fertilization N3 (Fig. 4).

3.6 Soil Organic C and N in Soil

The content of C_{ox} and N_{tot} were higher in treatments wit mineral fertilization than in control and manure treatments, differences between fertilized treatments being insignificant (Fig. 5).

4 Discussion

At the outset we expected that increasing the annual rate of N inputs would increase N_{min} in soil, N uptake and losses. We also supposed that a long-term deficit decreases, and a surplus increases, soil N_{min} and plant N uptake. These assumptions were only partially supported by the obtained data. When general trends across all stations were evaluated, the most visible effect was a correlation between inputs and plant N uptake. This was observed at most of the studied locations. At the rest of the locations (Hor, Chr and Ver), plant N uptake was related to N_{min} in spring at Hor, and N input + N_{min} in spring at Chr. Taken together, N added as fertilizer or released during winter significantly supports plant uptake. When effect of fertilizers was evaluated only for nonlegumes with exclusion of N_2 fixation, effect of fertilization was insignificant at most of stations (Fig. S1). Significant effect was only observed at the station Zat. Legumes have high biomass yield and nitrogen content in biomass. Most of their N uptake is from N_2 fixation and it is immediately used for their production. Therefore, they strongly contribute to correlation between inputs and outputs.

The effect of inputs on uptake was insignificant at Ver. Although the rate of input also slightly increased N_{uptake} at this station, the effect was too weak to be significant. It can be explained by the increased ability of the respective soil to store and release plant available N, independently of the actual rates of N inputs (Mulvaney et al. 2001; Osterholz et

Table 8 Sen slope estimate of changes in content of N_{min} in soil in spring (N_{spring}) and after harvest ($N_{harvest}$), total N input (N in fertilizer + N in rainwater corrected for N_2 fixation), (N_{input}), plant N uptake (N_{uptake}), losses of N through leaching (N_{losses}), balance of N ($N_{balance}$) and cumulative N balance ($N_{balance-cumulative}$) for data from individual stations in the 21-year field study. Significance of changes was estimated using the Mann-Kendall test. Asterisks mark significance at the level 0.05 (*), 0.01 (**) and 0.001 (***)

	All	Hor	Chr	Jar	Led	Uho	Ver	Vys	Zat
N_{spring}	-0.305	-1.397	-1.732 *	-0.759	0.130	-2.658 *	-2.120 *	2.604 *	2.355
N_{uptake}	-0.435	1.313	-1.116	2.371	2.161	0.846	-0.320	-2.250	0.380
N_{losses}	0.874	0.147	0.204	0.000	0.000	0.000	0.000	-0.002	0.443
$N_{harvest}$	0.173	-1.260	-1.254	-2.092	1.258	-2.045 *	0.000	6.592 **	0.865
N_{inputs}	-0.808	-0.446	-0.560	1.962	-2.578	0.004	1.620	-4.178	3.959
$N_{balance}$	-1.263	-3.151	-0.844	0.386	-7.034	-0.583	3.363	-2.000	1.132
$N_{balance-cumulative}$	-23.605 **	-12.458 **	-10.187 ***	2.356	-45.294 ***	-49.461 ***	-14.217 ***	-30.775 ***	-34.179 ***
$N_{balance-cumulative}$	-56.4 ***	-60.5 ***	-18.4 ***	-41.2 ***	-87.1 ***	-103.7 ***	-28.6 ***	-67.5 ***	-44.1 ***

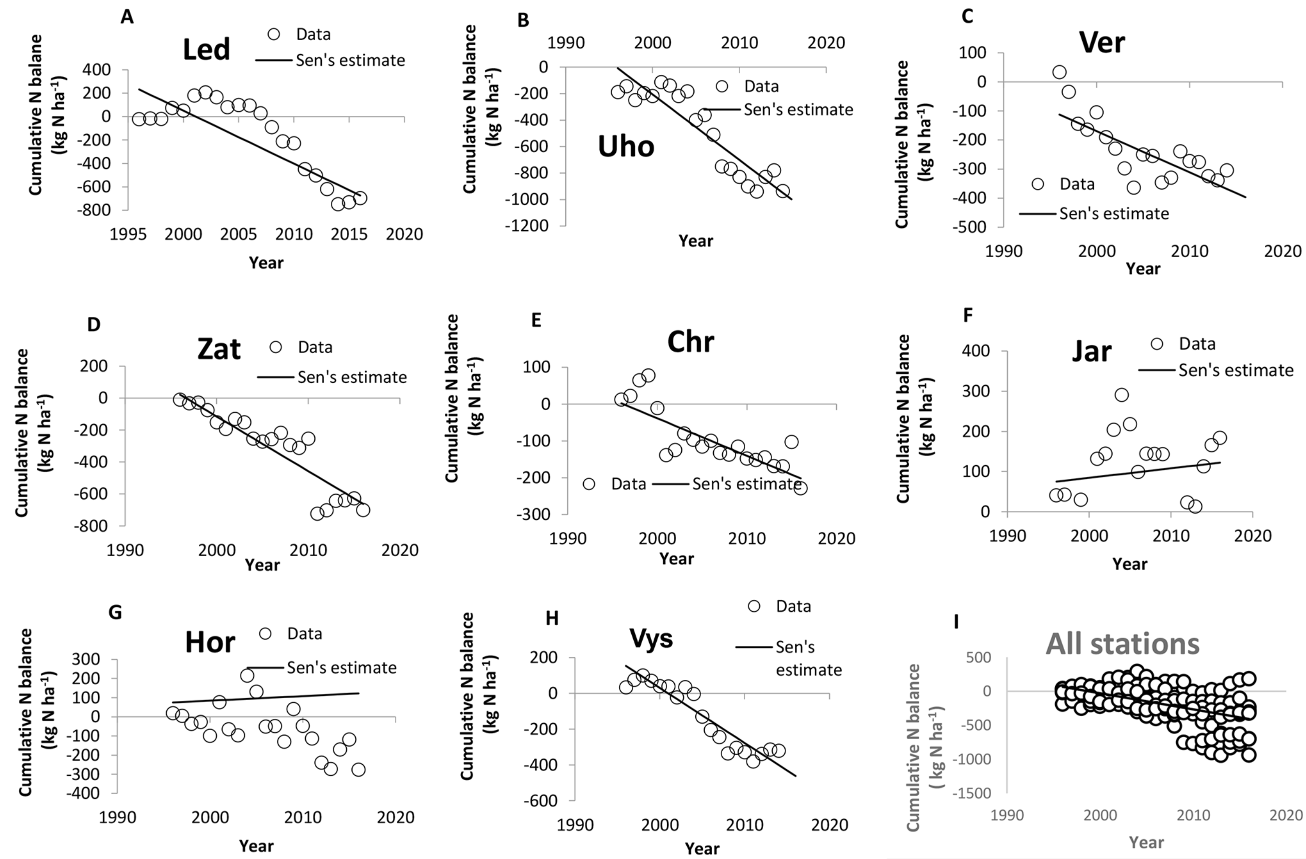

Fig. 2 Trends of cumulative N balance (kg N ha^{-1}) at lysimetric field sites Led (**A**), Uho (**B**), Ver (**C**), Zat (**D**), Chr (**E**), Jar (**F**), Hor (**G**), Vys (**H**) and at all stations (**I**) over 21 years

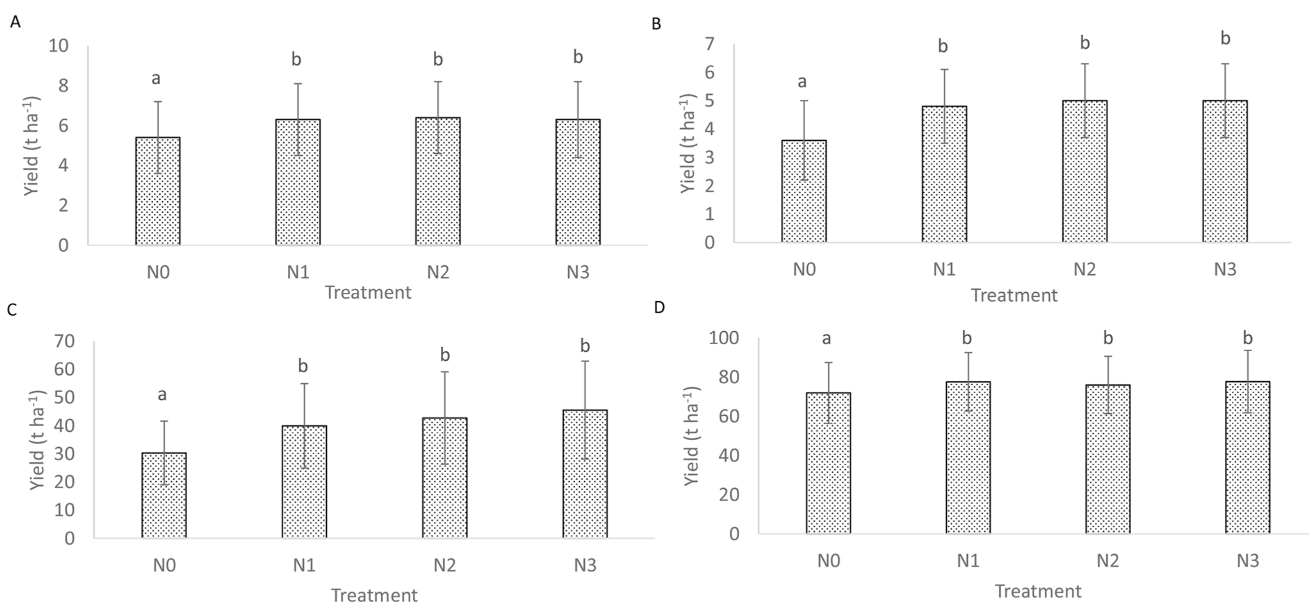

Fig. 3 Yield (t ha^{-1}) of wheat (**A**), barley (**B**), potatoes (**C**) and sugar-beet (**D**) in long-term field experiment. The treatments N0, N1, N2, N3 correspond to the NPK fertilization, with N added at a mean annual rate of 0, (N0), 60 (N1), 90 (N2) and 120 kg N ha^{-1} (N3). Values represent mean and standard deviation

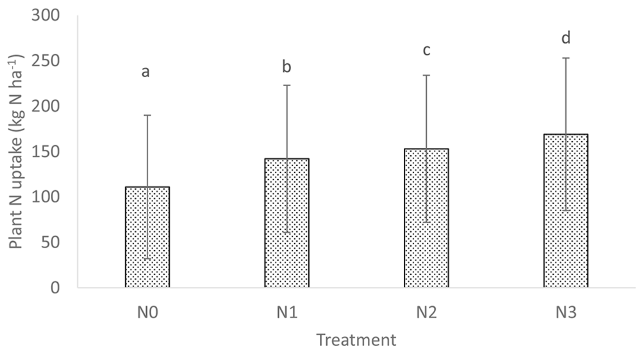

Fig. 4 Mean plant N uptake (kg N ha^{-1} y^{-1}) in long-term field experiment. The treatments N0, N1, N2, N3 correspond to the NPK fertilization, with N added at a mean annual rate of 0, (N0), 60 (N1), 90 (N2) and 120 kg N ha^{-1} (N3). Values represent mean and standard deviation

al. 2017), especially the part of the N inputs that enter the soil from the roots of leguminous plants, N from organic fertilizers and N immobilized in the rhizosphere. This portion of the applied N can be utilized and stored by microbial populations in the soil, and it needs some time to appear in plant-available form (Yan et al. 2020; Han et al. 2024). Plant-available N from organic sources may be released in the years following N input. When a sufficient amount of potentially available N accumulates in soil, this soil may become non-responsive to fertilization (Mulvaney et al. 2001). All these factors can weaken the direct effect of inputs on plant uptake at all stations, but this is most evident at the station Ver. The suggested dependence on soil-derived N at the station Ver is also supported by the correlation between N_{uptake} and $N_{harvest}$. It implies that processes in the rhizosphere soil release N (Fontaine et al. 2023; Holz et al. 2023) that is used by plants, and its residue increases the N_{min} pool after harvest. Fontaine et al. (2023) describe storage of N at higher and release at lower rate of actual N availability in rhizosphere. N in soil accumulates as microbial residues bond at mineral surfaces or in decomposing particulate organic matter. At low N availability it could serve as a source of available N. These processes could explain discrepancy of N inputs and outputs. However, possible range of soil accumulation or release was not estimated in field conditions and its contribution to observed results in current study is unclear.

The content of N_{min} in the soil was within the lower range of values reported for agricultural soils (Giacometti et al. 2013; Hartl and Erhart 2005; Kelner et al. 1997). At the same time, inputs were still in the range that does not support soil N_{min} accumulation and losses – the N_{min} concentrations in the soil profile were in the order of units of mg N kg^{-1} up to 10 mg N kg^{-1}. In context of previously published models of increasing N availability (Ma et al. 2019), it is the range that does not support extensive losses of N. Increasing N rate increases at first the yield, then the N uptake and later the mineral N accumulates together with increased losses. It limits the potential for undetermined N losses.

The main way of N output was plant N uptake, at a grain yield level of 6 t ha^{-1}. N losses by leaching were lower than plant uptake. The rates of leaching are in agreement with the rate of losses reported by Sestak et al. (2014).

Export of N in harvested crop products and NO_3^- leaching resulted in a negative N balance at most locations despite correction of inputs for N_2 fixation. Balance incorected for N_2 fixation was even more negative. It could be interpreted that rates of fertilization used in this study were insufficient to satisfy plant uptake. However, the N balance calculated in this study is only the balance between measurable inputs and measurable outputs. Undetermined inputs and outputs may (and probably they did) significantly affect the real availability of N for plants.

Difference between values of cumulative N balance corrected and uncorrected for N_2 fixation suggests strong contributions of N_2 fixation as N source in studied soils.

The anticipated correlation between N balance and $N_{harvest}$ was observed only at the station with the highest (Jar) or lowest (Uho) rate of N balance.

This supports the hypothesis that a long-term deficit decreases (Uho), and a surplus increases (Jar), soil N_{min}. At

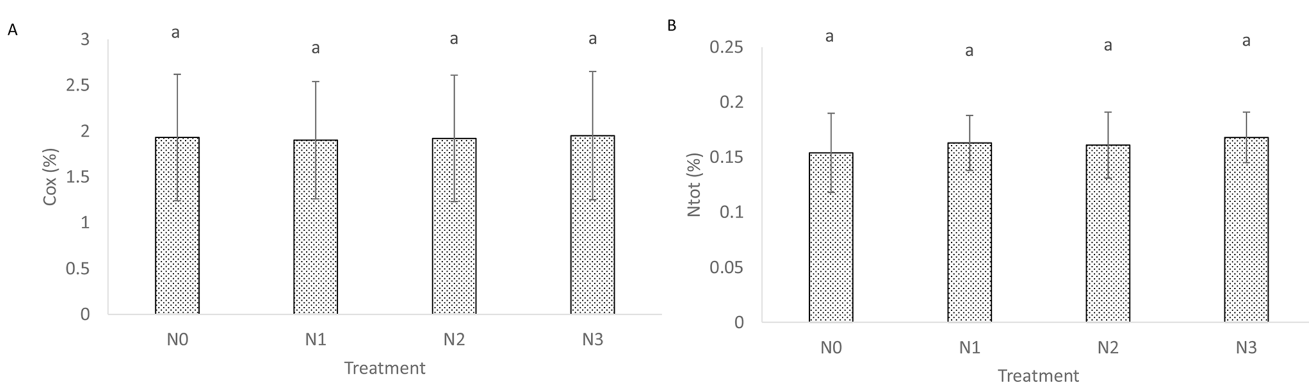

Fig. 5 Mean content of C_{ox} (**A**) and N_{tot} (**B**) (%) in soil in long-term field experiment. The treatments N0, N1, N2, N3 correspond to the NPK fertilization, with N added at a mean annual rate of 0, (N0), 60 (N1), 90 (N2) and 120 kg N ha^{-1} (N3). Values represent mean and standard deviation

the station Jar, soil N_{min} increased with an increasing rate of N balance and cumulative N balance. This suggests that unused N accumulates in soil at a rate proportional to the excess N, in agreement with our hypothesis. It also agrees with the rate of N balance, which was positive only at this station. At Uho, a decreasing cumulative N balance significantly decreased $N_{harvest}$. This suggests that a long-term annual N deficit decreases N availability in soil, and plant productivity is maintained at the expense of soil N reserves (Clark et al. 2009; Karimi et al. 2017). However, no significant long-term decrease in plant N uptake was observed, and it can be neither proved nor disproved from available data whether the plants really exhausted soil N. At other stations, no significant effect of N balance on the measured parameters was observed. This may be explained by unexpected N inputs, which weaken the anticipated effects and also help to maintain an almost neutral N balance. A surplus or deficit of N could therefore significantly change the measured parameters only at the station with the highest (Jar) or lowest (Uho) rate of N balance.

Is it difficult to predict how long the soil can support plant uptake at decreasing N availability. Probably yield would decrease at some level and steady state will establish between inputs and outputs, that may last for long time (Müller et al. 2011; Yan et al. 2020).

The hypothesis that increasing $N_{harvest}$ increases N_{losses} was confirmed only at the station Chr. At this station, nitrogen balance was negatively correlated with $N_{harvest}$. This suggests that N_{min} indirectly decreases N balance through increased N losses. At the rest of the locations, the anticipated correlation between N input, N balance, N_{min} and leaching was not found. Added N is probably stored in and released from soil reserves independently of the actual rate of N inputs (Mulvaney et al. 2001).

The observed rates of negative N balance should significantly decrease the total content of N in the soil at some stations. At soil C content between 1 and 2%, total N content in soil layer 0–30 cm is between 3000 and 6000 kg N ha^{-1}. When the calculated values of cumulative loss at field sites are 200–900 kg N ha^{-1}, soil N reserves must substantially decrease. Although the data on soil organic matter were not available for lysimetric plots, they were determined in nearby situated field experiment where increasing N rates were applied. If negative balance decreases total N in soil at the N rate N2, content of N_{tot} would increase if the level of fertilization increases to N3. However, changes in N_{tot} and C_{ox} were not observed and their content was not increased by higher fertilization rate than N1 level of field experiment. It is also reasonable to expect that this lower N_{tot} content at negative N balance should lead to a decreased concentration of plant-available N in soil and plant N uptake. The effect of total N concentration on the mineralizable N pool was reported by Mattila et al. (2023) and Chiriac et al. (2025), among others. Decreasing soil fertility at an insufficient rate of fertilization in a 38-year field experiment was reported by Müller et al. (2011). They found a substantial reduction of plant N uptake in unfertilized soil, from approximately 150 kg N ha^{-1} to 50 kg N ha^{-1} in permanent grassland over 10 years, and a constantly decreased level of N uptake afterwards. A lower rate of N transformations at a lower total N content in soil was reported by Laungani et al. (2012).

The anticipated decline of available N was observed at the stations Chr, Uho and Ver. At other stations, the change of soil N content during a long-term investigation was probably not substantial enough to change the N_{min} pool significantly, and no effect on plant N uptake was found. The rate of plant uptake can also be maintained for a relatively long time even without fertilization (Clark et al. 2009). More probably, the weak effect of long-term N balance may be explained by additional inputs of N from undetermined sources. Possible sources of missing N could be stronger-than-expected symbiotic N_2 fixation, rhizosphere associative N_2 fixation and undetermined dry N deposition. The estimated rate of airborne N input was in the range of 10–20 kg N ha^{-1} at the studied locations. However, it is uncertain whether this value includes all airborne N. Weigel et al. (2000) report input of airborne N as high as 50 kg N ha^{-1} in similar climatic conditions in Germany. Underestimation of atmospheric N input could explain the discrepancy between the calculated loss of N at a negative N balance and the observed stability of N pools and fluxes in our field investigation. The other possible source of nutrients is nutrient uptake from subsoil layers (below 80 cm), which are considered to be lost N, although they may be partially available for cultivated crops (Kautz et al. 2013).

The results suggest the capacity of soils to maintain pools of N despite a significant surplus or deficit of N.

Besides undetermined inputs, undetermined outputs are other missing information in N balance. There are numerous gaseous fluxes, that release especially N_2 and N_2O. However, they are usually smaller than leaching (in order of units kg N ha^{-1}) and in conditions of lysimetric investigations, they are probably limited by N availability. Really high rates of losses through denitrification and leaching usually occurs at higher rates than used in the experiment (Sestak et al. 2014; Huang et al. 2017). At given rate of inputs, most of added N is consumed by plants. Van Groeningen et al. (2010) and others report that increased losses are possible at N rates higher than 120–150 kg N ha^{-1}. At studied locations, mean rates of N applied in fertilizers were lower and the rates of N uptake still increased with the rates of input (Fig. 4). At this range of input rates, plant uptake strongly competes on available N with gas producing processes.

Changes of N rate have not only direct effect on nitrogen availability, but it could change the rates of nitrogen transforming processes. Increasing rate of N fertilization could impair N_2 fixation by legumes. However, it is not possible to distinguish these effects in conditions of the experiment.

On the basis of results, general model of soil N retention and mobilization was proposed. The slope of regression between input and uptake is about 0.4–0.5. It means, that from 100 kg of annual input, 40–50 kg is recovered in crop yield and the rest of N is retained in soil. It is in agreement with results of Yan et al. (2020). N retained in soil is then released from soil reserves into plant available form and uptake by plants independently on actual rate of inputs. Significant portion of N input is derived from N_2 fixation. In this way, soil provides up to 100 kg N ha^{-1}. Soil is therefore important player in nitrogen fluxes.

5 Conclusions

An increasing annual rate of N input increased crop N uptake. From 100 kg of N input, 40–50 kg was recovered in crop yield, with significant role of legumes, but the rate of N input had a limited effect on NO_3^- leaching and soil N_{min} concentration. Export of N by plant uptake was the primary way of N output and, together with the significant but lower NO_3^- leaching, was responsible for the negative N balance. Despite a negative N balance and decreasing N_{min} availability, soil sustains crop productivity at an almost constant level, and long-term changes in plant N uptake were limited. Strong control of soil transformations over N availability for crops mask effects of inputs on visible fluxes. In addition, unestimated sources of N help to maintain the sustainability of the studied cropping systems. The results suggest that soil is important reservoir of N which stores added N and contributes by up to 100 kg N ha^{-1} of plant available N. Data on simple values of yield and inputs could therefore provide useful information about soil N transformations.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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