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Higher than expected: Nitrogen flows, budgets, and use efficiencies over 35 years of organic and conventional cropping

A. Oberson [b,*,1], K.A. Jarosch [a,c,1], E. Frossard [b], A. Hammelehle [a,e], A. Fliessbach [d], P. Mäder [d], J. Mayer [a]

[a] Departement Agroecology and Environment, Agroecology and Environment, Agroscope, Reckenholzstrasse 191, Zurich 8046, Switzerland
[b] Institute of Agricultural Sciences, ETH Zurich, Eschikon 33, Lindau 8315, Switzerland
[c] Institute of Geography, University of Bern, Hallerstrasse 12, Bern 3012, Switzerland
[d] Department of Soil Sciences, Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, Frick 5070, Switzerland
[e] Landesbetrieb Landwirtschaft Hessen, Germany

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ABSTRACT

Organic and conventional cropping systems differ in type and amount of nitrogen (N) inputs. In organic cropping only organic fertilizers are permitted, while both organic and mineral fertilizers are used in conventional cropping. Fertilizer type and amount can affect N use efficiency of a cropping system, but contributions via symbiotic N fixation and changes in soil N stocks are rarely quantified based on field data when computing nutrient budgets. We calculated an N budget that accounts for these contributions based on annual data records for a period of 35 years at the Swiss DOK (bio-Dynamic, bio-Organic, Konventionell) field experiment. Here, different organic and conventional cropping systems have been maintained at two fertilization levels: typical for the respective system, and half these doses (low). Controls comprise a conventional treatment receiving solely mineral fertilizers and an unfertilized treatment. At the typical level, average fertilizer N inputs were 93 (bio-dynamic), 96 (bio-organic), and 171 (conventional system) kg N ha^{-1} yr^{-1}. Nitrogen output via harvested products regularly exceeded N input with fertilizers in all treatments. In each of the 7-year crop rotation periods, legumes (grass-clover ley, intercrops, soybean) were grown in three years. Their symbiotic N fixation was quantified based on ^{15}N studies and legume N yield data. It ranged from 75 to 122 kg N ha^{-1} per year of the DOK experiment, was slightly reduced under low fertilization and was the main N input for most treatments. Soil surface budgets (sum of N inputs from fertilization, symbiotic fixation, seeds, and deposition minus N outputs via crop harvests) yielded balances from −31 kg N ha^{-1} yr^{-1} (in non-fertilized control) to +46 kg N ha^{-1} yr^{-1} (conventional system with typical fertilization level). Nitrogen use efficiencies (NUE; N output with harvests as % of sum of N inputs) reached values >100 % in treatments with negative balances while NUE ranged from 85 % to 99 % in treatments with positive balances. Changes in topsoil (0–0.2 m) N stocks over time ranged from −26 to +9 kg N ha^{-1} yr^{-1} and declined in both unfertilized and mineral fertilized controls, and in systems receiving animal manure at low fertilization levels. Thus, positive soil surface N balances and animal manure are needed to maintain or increase topsoil N stocks. While NUE was generally high in all cropping systems there remains a trade-off between either soil N mining at higher NUE or potential N loss to the environment at lower NUE.

1. Introduction

Humans have greatly altered the global nitrogen (N) cycle over the past decades, with a dramatic increase in reactive N compounds in terrestrial and aquatic systems and the atmosphere (Gruber and Galloway, 2008; Steffen et al., 2015). Agricultural production plays a key role in the use and emission of reactive N compounds (Galloway et al., 2008). While the application of mineral fertilizer N has increased food production to sustain a growing world population (Jenkinson, 2001), recovery of applied mineral fertilizer N in crops and soil is often less than 50 %, suggesting that a large fraction of fertilizer N is lost to the environment (Crews and Peoples, 2005). This calls for more efficient N

* Corresponding author.
E-mail address: astrid.oberson@usys.ethz.ch (A. Oberson).
[1] These authors contributed equally to this work.

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use, i.e., an increase in the proportion of fertilizer N taken up by crop and/or retained in the soil to maintain soil quality and to be available to later crops, thereby reducing detrimental effects of excessive fertilizer use to the environment (Xia and Yan, 2023).

Replacement of synthetic mineral N fertilizers by organic (organic carbon (C) containing) N sources has been proposed as an approach to achieve more efficient N use in agriculture (Gardner and Drinkwater, 2009). The use of synthetic mineral N fertilizers is prohibited in organic cropping (IFOAM, 2019). Organic cropping relies on organic N sources such as animal manure and symbiotically fixed legume N. Soils that regularly receive animal manure have higher soil organic matter content than mineral fertilized soils (Edmeades, 2003; Koishi et al., 2020). However, animal manures are often subjected to high ammonia losses when applied to fields (Häni et al., 2016), and to N loss via nitrate leaching (Thomsen, 2005) or denitrification (Lesschen et al., 2011), similar to mineral N fertilizers (Frick et al., 2022b). Also, in the year of application, N recovery in crops from manure (slurries and solid) and legume residues is usually less than that from commercial mineral fertilizers (Webb et al., 2013). Even less information is available on the long-term use efficiency of organic N sources. An evaluation of N use in 80 European long-term field experiments that used bovine farmyard manure or bovine liquid slurry alone or combined with mineral fertilizers concluded that, in the long term, less animal manure N was recovered in crops than from mineral fertilizer (Zavattaro et al., 2017). However, the same study also demonstrated that long term application of animal manure increased soil N content, i.e., increased a potential future N source for crops, and these changes have not yet been considered in the efficiency evaluation.

Legumes integrated in crop rotations may add significant amounts of symbiotically fixed N to the soil-plant system (Peoples et al., 2009; Rasmussen et al., 2012). The cropping system affects the input of symbiotically fixed N in several ways. For instance, the proportion of fixed N in the legume decreases with increasing mineral N content in soil (Schipanski et al., 2010). The fixed N amount also depends on overall growth conditions of the legume, which affect its yield (Unkovich et al., 2010). Similar to organic N fertilizer, symbiotically fixed legume N may get incorporated into soil organic matter and become available to crops over years (Mayer et al., 2003). To determine the fertilizer and legume induced changes in soil N stocks with respect to large soil organic matter N stocks, long-term field studies are required.

A soil surface N budget of a soil-crop system compares N inputs entering the soil via the surface to N outputs via crop harvest over a defined period (Oenema et al., 2003). Inputs typically consider fertilization, symbiotic fixation, and N deposition while outputs include N withdrawal via harvested products. The difference between inputs and outputs denotes the balance; inputs exceeding outputs result in a positive balance, which indicates either N losses and/or a soil N stock increase, while a negative balance suggests soil N depletion. This can further be disentangled with a soil system budget that additionally considers observed changes in soil N stocks (Oenema et al., 2003). From budgets, indicators of N use efficiency (NUE) can be derived by dividing N outputs in harvested products by N inputs, either based on farm-gate budgets (EU Nitrogen Expert Panel, 2015; Quemada et al., 2020) or at the field level based on soil surface budgets (Chmelikova et al., 2021).

In the Swiss long-term field experiment DOK (bio-Dynamic, bio-Organic, Konventionell), organic and conventional cropping systems have been compared since 1978 (Mäder et al., 2002). Simple soil surface N budgets subtracted N withdrawal via harvested products from N inputs via manure and/or mineral fertilizers and resulted in negative balances, suggesting soil N depletion in all systems (Oberson et al., 2013). However, symbiotic N_2 fixation by legumes grown in the trial had not been included in those estimates. These inputs have now been measured (Oberson et al., 2007, 2013; Hammelehle et al., 2018) and can be integrated into a soil surface budget using plot specific records of legume N yields. Additionally, total N concentrations in the topsoil (0–0.2 m) have been recorded and can be used to calculate topsoil N stock changes. Combining these changes with the soil surface balance provides an estimate of total N losses from the topsoil-crop system.

The objective of this study was to quantify currently unaccounted for N inputs, namely symbiotic fixation and topsoil N changes, to complete N budgets for the period from 1985 until 2019; i.e., over 35 years of cropping, and to derive NUE. We aim to identify which cropping systems and related fertilization practices have higher NUE, and how this relates to sustainable N use in terms of minimizing N losses to the environment and maintaining soil N stocks.

2. Materials and methods

2.1. DOK long-term field experiment

The DOK long-term field experiment compares organic and conventional cropping systems since 1978 (Mäder et al., 2006; Krause et al., 2020). The experiment consists of crops grown in seven-year rotation periods. In this N budget study, we consider the period from the second to the sixth crop rotation period (1985–2019) because no significant treatment changes have taken place since 1985, as explained below.

The trial is located in Therwil near Basel, Switzerland (Mäder et al., 2006). The climate is mild, with a mean annual temperature of 10.5 °C and mean annual precipitation of 842 mm, resulting in a vegetation period of 210–215 days per year (Krause et al., 2020). The soil is a Haplic Luvisol developed on deposits of alluvial loess, of 0.9–1.3 m depth (Krause et al., 2020). The conception and experimental design of the DOK experiment can be found in recent presentations by Krause et al. (2020) and Krause et al. (2022). Three cropping systems, which differ mainly in fertilization and plant protection strategies, have been applied since the beginning (Table 1): two organic systems (bio-dynamic = BIODYN; bio-organic = BIOORG) that receive slurry and farmyard manure, and a conventional system (CONFYM) that receives slurry, farmyard manure and synthetic mineral fertilizers. Each of these systems is managed at two fertilizer input levels: typical (Level 2) and low (Level 1). Level 2 receives nutrient applications typical for the respective cropping system, while Level 1 receives half these amounts. For the organic systems, fertilization level 2 was determined by the annual manure production of 1.2 livestock units (LU) for the crop rotation periods 1, 2, and 3, and 1.4 LU from crop rotation period 4 onwards. The conventional system at typical fertilization level 2 is defined by the Swiss fertilization guidelines, which recommend moderate input levels compared to other Western European countries. Nutrient inputs from conventional treatments were somewhat higher during the early crop rotation periods because fertilizer input recommendations underwent revisions after the experiment began (Oehl et al., 2002). Additionally, a non-fertilized control (CTRLNON) and a control with exclusively mineral fertilizer inputs at typical fertilization level 2 (CTRLMIN) are maintained, resulting in a total of eight treatments. From 1978–1985 CTRLMIN was a non-fertilized control with conventional plant protection. Therefore, our investigation of long-term effects started with the second crop rotation period.

The applied fertilizers are typical for the respective cropping systems. Slurries and farmyard manure originate from farms managed according to the respective cropping system. Manure application is done according to good agricultural practice in terms of splitting of doses, strip application near crop rows, and incorporation of solid manure shortly after application. Because plots are relatively small (100 m^2) no big machinery has been used for fertilizer application and hand-held tools have been used for many years (e.g., watering cans for slurry application).

The DOK experiment has a split-split-plot design within a Latin square with four replicates and a plot size of 5 m × 20 m (Krause et al., 2020). The seven-year crop rotation is the same for all cropping systems and the same crop rotation is cropped with a time shift on three rotation units (subplots a, b, c) so that three of the seven crops are present each year for each cropping system. Crops of the most recent period

Table 1
Fertilization, plant protection, and crops in organic and conventional cropping systems and in unfertilized and mineral fertilized controls of the DOK long-term field experiment, from 1985 until 2019.

Characteristics	Cropping systems and control treatments							
	Unfertilized control (CTRLNON)	Bio-dynamic (BIODYN)		Bio-organic (BIOORG)		Conventional-manure (CONFYM)		Minerally fertilized control (CTRLMIN)[a]
Fertilization								
Fertilizer input level	zero	1=low	2=typical	1=low	2=typical	1=low	2=typical	2=typical
Type of fertilizer	Non-fertilized control	Aerobically composted farmyard manure (FYM) and slurry		Slightly aerobically rotted FYM and slurry		Stacked FYM and slurry and mineral fertilizer		Mineral fertilizers
Manure LU ha^{-1} yr^{-1}	0	0.7[b]	1.4[b]	0.7[b]	1.4[b]	0.7[b]	1.4[b]	0
Mineral fertilizer level	0	0	0	0	0	Completed to half	full norm[c]	Full norm[c]
Crops of rotation periods[d]								
2. Rotation (1985–1991)	Potato, GM[e]/Winter wheat, IC[f]/Beetroot/Winter wheat/Barley/Grass-clover/Grass-clover							
3. Rotation (1992–1998)	Potato/Winter wheat, IC[f] Beetroot/Winter wheat/Grass clover/Grass-clover/Grass-clover							
4. Rotation (1999–2005)	Potato/Winter wheat, GM[e]/Soybean, GM[e]/Silage maize/Winter wheat/Grass-clover/Grass-clover							
5. Rotation (2006–2012)	Silage maize/Winter wheat, GM[e]/Soybean, GM[e]/Potato/Winter wheat/Grass-clover/Grass-clover							
6. Rotation (2013–2019)	Silage maize, GM[e]/Soybean/Winter wheat, GM[e]/Potato/Winter wheat/Grass-clover/Grass-clover							
Plant protection[g]	Bio-dynamic	Bio-dynamic		Bio-organic		Integrated pest management		

[a] CTRLMIN was from 1978 to 1985 non-fertilized.
[b] Before 1992, 0.6 and 1.2 livestock units (LU) ha^{-1} yr^{-1}, respectively. The LU unit is defined by the average annual nutrient excretion by a cow with 600 kg live weight, which is 105 kg N, 15 kg P, 149 kg K, 12 kg Mg, and 37 kg Ca (Krause et al., 2020).
[c] Flisch et al. (2009); Richner and Sinaj (2017).
[d] The seven-year crop rotation is the same for all cropping systems.
[e] Followed by green manure (GM).
[f] Followed by intercropping (IC).
[g] For details see Krause et al. (2020).

(2012–2019) were silage maize (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], winter wheat I (*Triticum aestivum* L.), potatoes (*Solanum tuberosum* L.), winter wheat II, and two years of grass-clover (GC) ley. While the crop rotation period always lasted seven years, the type and order of crops have undergone slight modifications over time (Table 1). The crop rotation usually included two years of grass-clover ley, except from 1992 to 1998, when the ley lasted three years. Soybean was introduced in the fourth crop rotation period, starting in 1999. For this budget study all 96 plots were included, and N input and N output data were available for each plot unless stated otherwise.

Basic soil characteristics of the topsoil have been affected by treatments (Suppl. Table S1). Soils sampled in 2018 were slightly acidic, with organic C content corresponding to sufficient soil organic matter content (Richner and Sinaj, 2017). Available phosphorus (P) levels in the soils of treatments CONFYM2, CTRLMIN, and BIODYN2 were sufficient for the duration of the N budget calculations (1985–2019) while available P in all other treatments was low to very low. The available potassium (K) content was low to very low in soils of all treatments (Richner and Sinaj, 2017).

2.2. Nitrogen budget

Soil surface N budgets record the N that enters the soil via the surface and that leaves the soil via harvested crop N (Oenema et al., 2003). We calculated them on a yearly basis for the period from 1985–2019 (five crop rotation periods of seven years each, 35 years total) individually for each of the 96 plots and each of the 8 treatments (12 plots per treatment). The difference between annual N inputs and harvested N provided the soil surface N balance (SoilSurfBal) (Oenema et al., 2003):

$$\text{SoilSurfBal (kg N ha}^{-1}\text{ yr}^{-1}) = \text{Nfert} + \text{Nfix} + \text{Ndep} + \text{Nseed} - \text{Nharv} \quad (1)$$

where Nfert is the N input with animal manure and/or mineral fertilizer, Nfix is the amount of N symbiotically fixed by legumes, Ndep is atmospheric N deposition, Nseed accounts for N contained in seeds, and Nharv is the amount of N removed from the plot in the harvested plant products (all in kg N ha^{-1} yr^{-1}).

The budget was expanded to a soil system N budget, which as defined by Oenema et al. (2003) records all N inputs and N outputs including “gains or losses within and from the soil”. We considered the soil N stock change (ΔsoilN, N ha^{-1} yr^{-1}) in the 0–0.2 m topsoil, resulting in the following soil system balance (SoilSysBal):

$$\text{SoilSysBal (kg N ha}^{-1}\text{ yr}^{-1}) = \text{Nfert} + \text{Nfix} + \text{Ndep} + \text{Nseed} - \text{Nharv} - \Delta\text{soilN} \quad (2)$$

The ΔsoilN was calculated from changes in topsoil N concentrations over time (see below). A decrease in soil N (negative ΔsoilN) presents an additional source of N and thus an input in the budget, while an increase in soil N (positive ΔsoilN) is a sink, i.e., an N output in the budget. In contrast to Oenema et al. (2003) we had not measured specific N losses but estimated total N losses from the soil system balance. A positive balance provides an estimate of total N losses (comprising all possible N loss pathways and forms, including N_2) from the topsoil-crop system, while a deficit would suggest yet unaccounted sources.

2.3. Quantification of inputs and outputs

2.3.1. Nitrogen input with fertilizers

Nitrogen inputs with fertilizers (Nfert) were available from the data records and are based on the total N analyses of all organic fertilizers (slurries, farmyard manure) applied in the DOK experiment. Total N concentrations were multiplied by the mass (solid manure) or volume (slurries) of manure applied per plot. Total N analyses were carried out using Kjeldahl digestion (Agroscope, 2020). The N applied in mineral fertilizers was based on the N concentrations indicated by their supplier. Average N amounts applied from different N fertilizer types, including the mineral N contained in animal manure, and the distribution of N between slurries and farmyard manure, are shown in Table 2.

2.4. Symbiotic N_2 fixation in legumes

The amount of N symbiotically fixed (Nfix, in kg N ha^{-1} yr^{-1}) was calculated for grass-clover (GC) leys, for soybean (Sb), for legumes sown with intercrops (In), and green manure (GM):

Table 2
Nitrogen fertilization: average total N and mineral N inputs at typical fertilization level 2, 1985–2019 and by crop rotation period (CRP). Mineral N in animal manure is mostly ammonium. Animal manure was obtained from farms managed according to the respective cropping system and mainly produced from cow excreta (dairy or suckler cows). FYM=farmyard manure, largely composed of feces and bedding material; slurry is dominated by urine N. Synthetic mineral fertilizer was mostly calcium ammonium nitrate. Fertilization level 1 received half the input of level 2, in the same proportions of fertilizer types.

Period	— CRP2-6— 1985–2019		— CRP2— 1985–1991		— CRP3— 1992–1998		— CRP4— 1999–2005		— CRP5— 2006–2012		— CRP6— 2013–2019	
Treatment[a]	Total N	Mineral N	Total N	Mineral N	Total N	Mineral N	Total N	Mineral N	Total N	Mineral N	Total N	Mineral N
Fertilzer type	kg ha^{-1} yr^{-1}											
BIODYN												
Farmyard manure	51	1	55	1	48	1	63	0	54	1	35	1
Slurry	43	25	45	24	41	29	37	25	37	21	52	28
Total	93	26	100	26	89	30	100	25	91	22	87	30
BIOORG												
Farmyard manure	52	4	55	7	47	3	59	3	59	1	40	3
Slurry	44	27	38	23	33	21	68	44	34	19	45	26
Total	96	30	93	31	79	25	127	47	93	20	85	29
CONFYM												
Farmyard manure	51	9	49	8	47	6	51	8	56	10	51	14
Slurry	65	49	37	23	71	55	79	57	67	54	72	55
Synthetic mineral fertilizer	55	55	58	58	55	55	50	50	60	60	54	54
Total	171	113	144	89	173	116	180	115	184	124	176	123
CTRLMIN												
Synthetic mineral fertilizer	121	121	105	105	145	145	118	118	119	119	117	117

[a] For treatments see Table 1.

$$\text{Nfix (kg N ha}^{-1}\text{ yr}^{-1}) = \text{NfixGC} + \text{NfixSb} + \text{NfixIn} + \text{NfixGM} \quad (3)$$

Calculated Nfix included fixed N contained in aboveground and belowground biomass, and for the grass-clover ley the fixed N transferred to the associated grasses also (Table 3). For a given period, e.g., 35 years from 1985 to 2019, all Nfix was summed and then divided by 35 years in order to obtain the average annual Nfix input per year of the DOK experiment (as shown in Table 4).

2.4.1. Symbiotic fixation in grass-clover leys

Every crop rotation period of seven years contained two years of grass-clover ley, except the third rotation, with three years (Table 1). Thus, from 1985 to 2019 each plot was under grass-clover for 11 years. Because there are three crop rotation units, plot specific data on dry matter, N yield, and clover proportions from 33 cultivation years were available. The N fixed in grass-clover leys was determined as follows:

$$\text{NfixGC (kg N ha}^{-1}\text{ yr}^{-1}) = \text{NfixAgCl} + \text{NfixBgCl} + \text{NfixTransAgGr} + \text{NfixTransBgGr} \quad (4)$$

with NfixAgCl the amount of N fixed in aboveground clover biomass, NfixBgCl the amount of N fixed belowground in clover roots and rhizodeposition, NfixTransAgGr the amount of fixed N transferred to the associated grass aboveground biomass, and NfixTransBgGr to the grass roots (all in kg N ha^{-1} yr^{-1}). The amount of N fixed in aboveground biomass was determined using plot specific clover N yields (recorded for each year under grass-clover ley) and proportions of N in clover derived from the atmosphere (PNdfa, %) determined in all treatments of the DOK trial during a two-year study using the ^{15}N natural abundance method (Oberson et al., 2013). The treatment specific means of PNdfa were used, ranging from 85 % to 91 % for white clover, and from 83 % to 89 % for red clover (Oberson et al., 2013). Belowground input of fixed N was calculated by multiplying N fixed in aboveground clover biomass by a factor of 0.4, based on a two-year ^{15}N labeling study carried out in selected treatments of the DOK (Hammelehle et al., 2018). Fixed N transferred from clover to the associated grasses was calculated using treatment specific proportions of grass N derived from clover (PNdfc, %) obtained by the ^{15}N natural abundance method (Oberson et al., 2013) and plot specific grass N yields. The PNdfc were available for each treatment and were time dependent, with lower proportions for the first year (3–25 %) and higher proportions for the second year (43–55 %) (Oberson et al., 2013). Fixed N contained in grass roots was calculated by multiplying the fixed N contained in aboveground grass biomass by a factor of 0.2, which was also determined in a model grass-clover mixture grown over two seasons in the DOK experiment (Hammelehle, 2018). For more details see supplementary method description 1.

2.4.1.1. Symbiotic fixation in soybean.

Soybean was cropped during each of the seven-year crop rotation periods, beginning in the 4th rotation period (Table 1). Across all crop rotation units, data from nine soybean cultivation years was available for the periods from 1999 until 2019. Symbiotic N_2 fixation in soybean was calculated as the sum of aboveground (NfixAgSb) and belowground (NfixBgSb) fixation:

$$\text{NfixSb (kg ha}^{-1}\text{ yr}^{-1}) = \text{NfixAgSb} + \text{NfixBgSb} \quad (5)$$

NfixAgSb was calculated by:

$$\text{NfixagSb (kg ha}^{-1}\text{ yr}^{-1}) = \text{Nyield x PNdfaSb/100} \quad (6)$$

with the N yield obtained by multiplying soybean grain dry matter yield (kg ha^{-1}) with the N concentration analyzed in grains (both variables always determined on samples from each plot). The proportion of N derived from the atmosphere in soybean (PNdfaSb, %) was determined for soybean growing in 2004 in plots of all treatments except BIODYN1, BIOORG1 and CONFYM1 (Oberson et al., 2007) and in 2009 for soybean growing in all treatments except BIODYN1, BIODYN2 and CONFYM1 (Hammelehle et al., 2013). The average PNdfaSb from both studies was used. BIODYN1 was assumed to have the same fixation rate as BIODYN2, and CONFYM1 the same as CONFYM2. In both studies, the ^{15}N natural abundance method (Shearer and Kohl, 1986) was used to determine the PNdfaSb. Average PNdfaSb across all treatments was 44 % in 2004, and 71 % in 2009, resulting in an average of 57 %, with treatment specific averages ranging from 50 % (CTRLMIN) to 64 % (BIOORG1).

NfixBgSb was calculated by:

$$\text{NfixBgSb (kg N ha}^{-1}\text{ yr}^{-1}) = \text{NfixAgSb x 0.645} \quad (7)$$

where 0.645 was the ratio between belowground N and aboveground N determined for soybean (Hammelehle et al., 2013).

2.4.1.2. Symbiotic fixation in intercrops and green manure.

Intercrops were grown during crop rotation periods one and two, while green manure was grown from the first crop rotation period onwards (Table 1), but not all of them contained legumes. For the study period from 1985 until 2019, intercrops containing legumes were grown only once while green manure containing legumes was grown four times on each plot. Nitrogen fixation was estimated using literature values for the PNdfa of the legume species contained in the mixtures (Büchi et al., 2015), and the legume proportion in the mixture was assumed to be the number of legume species divided by the total number of species in the mixture. More details, including consideration of treatment specific yields and belowground input of fixed N are given in Supplementary method description 2.

2.4.2. Nitrogen deposition

N deposition data was obtained from hectare-based models of the division Air Pollution Control and Chemicals of the Swiss Federal Office for the Environment for the period from 1990 until 2010. Total annual atmospheric N deposition ranged from 19.0 and 21.2 kg N ha^{-1} yr^{-1} and we thus assumed an annual N deposition of 20 kg N ha^{-1} yr^{-1} for each plot.

2.4.3. Nitrogen in seed

The N contained in seed was estimated based on amounts of seeds applied per plot (kg ha^{-1}) and seed N concentrations by suppliers, where available. If no seed N concentrations were available, reference data (Flisch et al., 2009) or mean N seed concentrations from similar crops were used.

2.5. Nitrogen removed by harvested plant material

The N removed by harvested plant material (Nharv) was determined by

$$\text{Nharv (kg N ha}^{-1}\text{ yr}^{-1}) = \text{DMyield x Nconc} \quad (8)$$

with total dry matter yield of crops (DMyield in t ha^{-1}) and total N concentration measured in harvested plant material (Nconc in kg t^{-1}). These data had been measured for each of the 96 plots for all harvested material that was removed from the plots over 35 years.

2.6. Soil N stock changes

Soil N stock changes (0–0.2 m topsoil of 1 ha) between 1984 and 2018 were calculated based on measured soil N concentrations and bulk densities. Each year after harvest of the main crop, the topsoil (0–0.2 m) of each plot was sampled as a composite of 15–20 soil cores 3 cm in diameter (Krause et al., 2022). Samples were air dried and passed through a 2 mm sieve. Thereafter, finely ground sub-samples were analyzed for total C and N concentrations. Because laboratory personnel, instruments and analytical protocols changed over time, original data showed variation that could not be explained by the treatments. Therefore, in 2018 archived topsoil samples from all plots and from every second year between 1984 and 2018 were re-analyzed using an Elementar Vario Max Cube, as in Krause et al. (2022). After visual inspection for outliers, 42 of the total 1728 observations were excluded, two samples due to obvious measurement error and 40 samples because the sample order had most likely been confounded. The remaining 1686 observations on soil N concentrations were used for the calculation of N stock changes over time. The starting point in 1984 was chosen to allow for the time needed for plot equilibration after installation of the trial in 1978 and because from 1985 onwards the CTRLMIN treatment was in place.

Bulk density of the 0–0.2 m topsoil layer had been determined for each plot in three different years during crop rotation period 1 (Suppl. Table S1). These three values were averaged, resulting in an average individual soil bulk density value for each plot. Values varied from 1.20 to 1.46 t m^{-3}, with an overall average of 1.32 t m^{-3}. Mean soil bulk densities of crop rotation period 1 were not significantly affected by treatment (Suppl. Table S1). Soil N stocks in the 0–0.2 m topsoil layer were calculated as follows:

$$\text{N stock (kg N ha}^{-1}) = \text{Nconc} \times \text{d} \times \rho \quad (9)$$

with Nconc = soil N concentration (kg t^{-1}), d = soil depth (0.2 m), ρ = bulk density (t m^{-3}).

The annual rates of soil N stock changes (ΔsoilN, kg N ha^{-1} yr^{-1}) between 1984 and 2018 were derived from the slope of a linear regression between the N stock (kg ha^{-1}) in the topsoil (0–0.2 m) and years since 1984 (t), where b is the intercept (or the model-derived initial N stock in 1984) of this function.

$$\text{N stock} = \Delta\text{soilN} \times t + b \quad (10)$$

Negative ΔsoilN indicates a decline in topsoil N stock with time while positive values indicate an increase.

2.7. Nitrogen use efficiency indicators

The soil surface budget derived NUE describes the efficiency of use of the combined N inputs with fertilizers, symbiotic fixation, seeds, and atmospheric deposition (SoilSurfNUE) (EU Nitrogen Expert Panel, 2015):

$$\text{SoilSurfNUE (\%)} = \frac{Nharv}{Nfert + Nfix + Nseed + Ndep} x100 \quad (11)$$

with Nharv, Nfert, Nfix, Nseed and Ndep explained above.

Additionally, the following soil system budget derived NUE including the soil N stock change was computed:

$$\text{SoilSysNUE (\%)} = \frac{Nharv}{Nfert + Nfix + Nseed + Ndeposition - \Delta soilN} x100 \quad (12)$$

where the ΔsoilN is subtracted because declines (negative values) present a source while positive values present a sink. The NUE indicators were calculated for each crop rotation period and based thereon for the entire duration from 1985 until 2019. The averages of all inputs and of all outputs per crop rotation period were calculated for each of the 12 plots per treatment, and budgets and NUE indicators were derived for each plot. The exception is ΔsoilN, which was also computed for each plot, but over the period from 1984 to 2018 (see above).

2.8. Statistical data evaluation

Statistical analyses were carried out as in Oberson et al. (2013) using the Linear Mixed Models procedure in the statistical analysis package SYSTAT 13 (Systat Software Inc., Chicago, USA). Data were fitted to a one-factorial mixed effect model (treatment + error) if all eight treatments were included, or a two-factorial mixed effect model ([cropping system + fertilization level + cropping system x fertilization level] + error) using fertilizer levels 2 and 1 for BIODYN, BIOORG, and CONFYM. Testing of the treatment effect included all treatments, and the standard error of the mean (SEM) and the Least Significant Difference (LSD) were derived from this analysis. For analysis of variance, percentages were transformed using arcsine- transformation. Statistical evaluation of soil N stock changes (ΔsoilN) were based on factorial ANOVA using JMP® Pro 14.1.0, using the plots' clay content as co-variable, as described in Krause et al. (2022). Figures were made in R version 4.2.1 (R Core Team, 2022) using the ggplot2 package (Wickham, 2016) or in Microsoft Excel.

3. Results

3.1. Nitrogen inputs

The average annual total N input from 1985 until 2019 ranged from 96 (CTRLNON) to 310 (CONFYM) kg N ha^{-1} yr^{-1} (Table 4). CTRLMIN, BIOORG2 and BIODYN2 had similar total N inputs of around 240 kg N ha^{-1} yr^{-1}, while the low fertilization level 1 treatments received between 180 and 219 kg N ha^{-1} yr^{-1}.

Fertilizers were the main N input in conventional treatments at typical fertilization level 2 where CTRLMIN received 121 and CONFYM2 171 kg N ha^{-1} yr^{-1}). In CONFYM2 about one third of it was applied in the form of synthetic mineral N (Table 2). The organic treatments at typical fertilization level 2 received less than 100 kg ha^{-1} yr^{-1} of fertilizer N (Table 4). Fertilizer N inputs changed somewhat over time (Fig. 1a).

Symbiotic fixed N was the dominant N source in most treatments (Table 4). The input of fixed N per year of the DOK trial exceeded 110 kg N ha^{-1} yr^{-1} in all treatments except for CTRLMIN and CTRLNON. Symbiotic fixed N was not significantly affected by the cropping system and only slightly lower at the low fertilization level 1 than in the typical fertilization level 2 (Table 4). Grass-clover leys were the main source of symbiotically fixed N (Table 3, Fig. 2). Nitrogen fixation per grass-clover cultivation year ranged from 185 to 301 kg N ha^{-1} yr^{-1}, which translated into 58–95 kg fixed N per year over the entire observation period of 35 years (Fig. 2). Most fixed N was contained in aboveground clover biomass. However, relevant amounts were also contained in belowground clover N (roots, rhizodeposition) and transferred to the associated grass (Table 3). The amount of fixed N in clover above and belowground was not affected by the cropping system or by fertilization level (Table 3), but fixed N transferred to the grass was. Soybean fixed above- and belowground between 109 and 200 kg N ha^{-1} per year of soybean cultivation, or 9–17 kg per year of DOK trial. Intercrops and green manure contributed between 7 and 14 kg ha^{-1} yr^{-1} over the entire period (Fig. 2). Symbiotic fixation inputs fluctuated over time but maintained the level (average values per crop rotation period, Fig. 1b) except for treatment CTRLNON, where it markedly decreased over time.

Nitrogen deposition and N input via seeds summed to 21 kg N ha^{-1} yr^{-1} (Table 4). Nitrogen deposition contributed 21 % of the input to CTRLNON though was of little importance for the treatments fertilized at typical fertilization level 2; N input with seeds having values around 1 kg N ha^{-1} yr^{-1} were negligible.

3.2. Nitrogen output with harvests

The average yearly N export via harvested products was highest in CONFYM2 (264 kg N ha^{-1} yr^{-1}) and lowest, by almost half, in CTRLNON (128 kg N ha^{-1} yr^{-1}) (Table 4).

Low fertilization level 1 treatments were on average 87 % of the harvested N export of typical fertilization level 2 treatments (Table 4). The average N export via harvested products remained nearly stable for all fertilized treatments (Fig. 1c). Harvest N exports were higher for CTRLNON during the second than during later crop rotation periods. Nitrogen exports were highest from grass-clover leys and lowest for potatoes in all treatments (Suppl. Fig. S1).

3.3. Soil N stock change

Soil N stocks were affected by treatment and decreased for most treatments with time, except for treatments that had received animal manure at typical fertilization level 2 (BIODYN2, BIOORG2, CONFYM2) (Fig. 3a, b). The changes in soil N stock with time translated into yearly changes from −26.2 kg N ha^{-1} yr^{-1} (CTRLNON) to +9.3 kg N ha^{-1} yr^{-1}

Table 3
Symbiotic fixation by legumes included in the crop rotation of the DOK experiment, in kg fixed N per legume cultivation year. Symbiotically fixed N (Nfix) was determined for aboveground (Ag) and belowground (Bg) plant parts; for grass-clover leys additionally fixed N transferred (NfixTransGr) to the grass was included. Integration of these values into the balance (Table 4) considered the number of cultivation years during which a specific legume, or legume containing intercrop, or green manure had been cropped on each plot during the 35 years. The number of cultivation years were as follows: 11 grass-clover, 3 soybean, 5 intercrop (1) or green manure (4). The number n indicates the total number of data points per treatment, obtained from number of years multiplied by 12 plots per treatment.

Treatment	Grass clover leys						Soybean		Intercrops & Green manure
	Nfix		NfixTransGr		Yield DM	Clover	Nfix	Nfix	Nfix
	AgClover	BgClover	AgGrass	BgGrass	Clover+Grass	Prop	Ag	Bg	AgBg
	kg N ha^{-1} yr^{-1}				dt ha^{-1}	% of DM	kg N ha^{-1} yr^{-1}		
CTRLNON	105	42	33	6.7	71	40	67	43	51
BIODYN1	159	64	43	8.8	105	41	108	70	90
BIOORG1	162	65	36	7.4	107	41	114	74	74
CONFYM1	165	66	36	7.2	123	39	103	67	82
BIODYN2	172	69	50	10.3	118	40	112	72	83
BIOORG2	170	68	42	8.6	120	39	121	78	82
CONFYM2	160	64	46	9.4	142	32	109	70	95
CTRLMIN	125	50	49	10.0	129	30	93	60	90
SEM	8.2	3.3	2.5	0.5	2.1	1.8	3.0	1.9	5.3
LSD	22.7	9.2	7.8	1.4	5.9	5.0	8.3	5.3	14.6
n	132	132	132	132	132	132	36	36	60
Anova source of variation with (DF)									
Treatments[a] (7)	***	***	***	***	***	***	***	***	***
CropSys (S)[b] (2)	n.s.	n.s.	**	*	***	*	***	***	***
FertLev (F)[b] (1)	n.s.	n.s.	***	***	***	*	*	*	*
S x F[b] (2)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*, **, *** significant at p<0.1, 0.05, 0.01 and 0.001 probability level, respectively; n.s. not significant; SEM standard error of the mean from Anova; LSD = Least significant difference; DF degrees of freedom.

[a] Treatment denotes analysis over all eight treatments (cropping systems BIODYN, BIOORG, CONFYM at both fertilizer levels and control treatments described in Table 1).

[b] Two-way Anova by cropping system (S), fertilization level (F) and their interaction (S x F) including systems BIODYN, BIOORG, and CONFYM at fertilizer input levels 1 and 2.

Table 4
Nitrogen budget for 35 years of the DOK experiment (1985–2019): Inputs with fertilizers, symbiotic fixation, N deposition, seed and N export via harvested products and resulting N balances. Balance 1 is the difference between total inputs (by fertilizers, symbiotic fixation, seeds, deposition) minus output via harvested products, balance 2 additionally includes the topsoil N stock change, all in kg^{-1} ha^{-1} yr^{-1}. Nitrogen use efficiency (NUE, %) describes N output via harvested products in percentage of input, with total inputs in NUE 1, and additionally including topsoil N stock change in NUE 2.

Treatment	CTRLNON	BIODYN1	BIOORG1	CONFYM1	BIODYN2	BIOORG2	CONFYM2	CTRLMIN	SEM	LSD	Anova source of variation			
											Tr[a]	S[b]	F[b]	SxF[b]
DF[c]											7	2	1	2
kg N ha^{-1} yr^{-1}														
Inputs Total	96	180	180	219	237	236	310	242	3.2	8.8	***	***	***	***
Fertilizers	0	47	48	85	93	96	171	121	1.4	3.9	***	***	***	***
SymbFix	75	112	111	112	122	119	117	99	2.9	8.0	***	n.s.	***	n.s.
Seeds	1.4	1.4	1.4	1.3	1.4	1.4	1.3	1.4						
Deposition	20	20	20	20	20	20	20	20						
Outputs														
Harvested products	128	189	190	223	214	213	264	240	3.2	8.9	***	***	***	**
Balance 1 Soil Surface	-31.1	-8.7	-9.6	-4.5	22.9	23.7	45.9	2.1	2.1	5.8	***	***	***	***
Topsoil N stock change	-26.2	-9.1	-10.0	-11.2	9.3	1.2	-0.7	-10.0	1.9	14.4	***	n.s.	***	n.s.
Balance 2 Soil system	-4.9	0.4	0.3	6.7	13.6	22.5	46.6	12.1	2.1	5.8	***	***	***	***
%														
NUE 1 Soil surface	133	105	106	102	91	90	85	99	1.0	2.8	***	***	***	***n.s.***
NUE 2 Soil system	104	100	100	97	94	91	85	95	0.9	2.5	***	***	***	**

All values are averages of n=60 values, which have been aggregated by the 5 crop rotation periods for each of the 12 replicate plots per treatment, except for soil N stock change (regression across data from 1984 to 2018, see material and methods); *, **, *** significant at p<0.1, 0.05, 0.01 and 0.001 probability level, respectively; n.s. not significant; SEM standard error of the mean from Anova; LSD = Least significant difference; DF degrees of freedom.

[a] Treatment (Tr) denotes analysis over all eight treatments (cropping systems BIODYN, BIOORG, CONFYM at both fertilizer levels and control treatments described in Table 1).

[b] Two-way Anova by cropping system (S), fertilization level (F) and their interaction (S x F) including systems BIODYN, BIOORG, and CONFYM at fertilizer input levels 1 and 2.

(BIODYN2) (Table 4, Fig. 3b). The greatest decrease in soil N occurred in the CTRLNON treatment. The decline in soil N stock under low fertilization level 1 differed significantly from typical fertilization level 2, under which stocks were maintained or increased. Soil N stock in treatments receiving animal manure at low fertilization level 1 decreased similarly to those under mineral fertilization in CTRLMIN.

3.4. *Nitrogen balance*

The soil surface balance, which subtracted N exports via harvests from inputs via fertilizers, symbiotic fixation, deposition, and seeds, ranged from −31 to +46 kg ha^{-1} yr^{-1} (Table 4). It was positive or in equilibrium for all typical fertilization level 2 treatments. Thus, the inputs of these treatments exceeded or balanced the N withdrawal from harvests. Low fertilization level 1 treatments had a moderate deficit of around −8 kg N ha^{-1} yr^{-1}. In contrast, in CTRLNON the N withdrawal exceeded the inputs to a greater degree, resulting in the N deficit of −31 kg N ha^{-1} yr^{-1}.

Inclusion of topsoil N stock changes in the soil system budgets resulted in equal or positive balances for all treatments except CTRLNON (Table 4). CTRLNON still had a deficit of −5 kg N ha^{-1} yr^{-1} while BIODYN1 and BIOORG1 were close to zero. The other treatments had average N surpluses from +7 (CONFYM1) to +47 (CONFYM2) kg N ha^{-1} yr^{-1}.

The balances changed over time, with changes following similar patterns for all treatments (Fig. 1d). Because soil stock changes were considered constant over time, the soil system balance had the same temporal evolution as the soil surface balance (Suppl. Fig. S2).

3.5. *Nitrogen use efficiency indicators*

Soil surface budget based NUE indicators were generally high, usually greater than 80%, with sometimes unrealistic values higher than 100 % (Table 4). Fertilization level had a stronger influence on them than fertilizer type applied to the different cropping systems, with higher NUE at low fertilization level 1 than at typical fertilization level 2. The soil system budget based NUE included the soil N stock change in the topsoil. For all treatments except BIODYN2, BIOORG2 and CONFYM2, it was lower than the soil surface budget derived NUE, because the net N decline in their topsoils presented an additional N input. In contrast, the augmentation of soil N stock, as in BIODYN2, increased this NUE as compared to soil surface NUE.

4. Discussion

The N budget of the DOK field experiment was established based on 35 years of detailed data records, from 1985 until 2019. Essential data for the setup of the N budget were recorded every year and for each of the 96 plots, creating a uniquely complete database. Biological N_2 fixation by legumes contained in the crop rotation, including belowground N and fixed N transferred to grass in grass-clover leys has been quantified in several ^{15}N isotope studies carried out in the DOK experiment (Oberson et al., 2007, 2013; Hammelehle et al., 2018). Before 1985 all treatments except CTRLMIN had already been in place for seven years; soils had thereby adjusted to the cropping systems (Maire et al., 1990). In the following we discuss the roles and accuracy of specific inputs and outputs, and the resulting balances and NUE indicators. Whilst N from the various sources was used efficiently, soil N stocks declined in some treatments, indicating a soil quality vs N use efficiency trade-off.

4.1. *Nitrogen inputs with fertilizers*

The amount of N applied with fertilizers (mineral and/or manure) at typical fertilization level 2 corresponded to the typical levels applied in organic and conventional mixed crop-livestock farms in Switzerland (Krause et al., 2020). In comparison to CONFYM, both organic systems

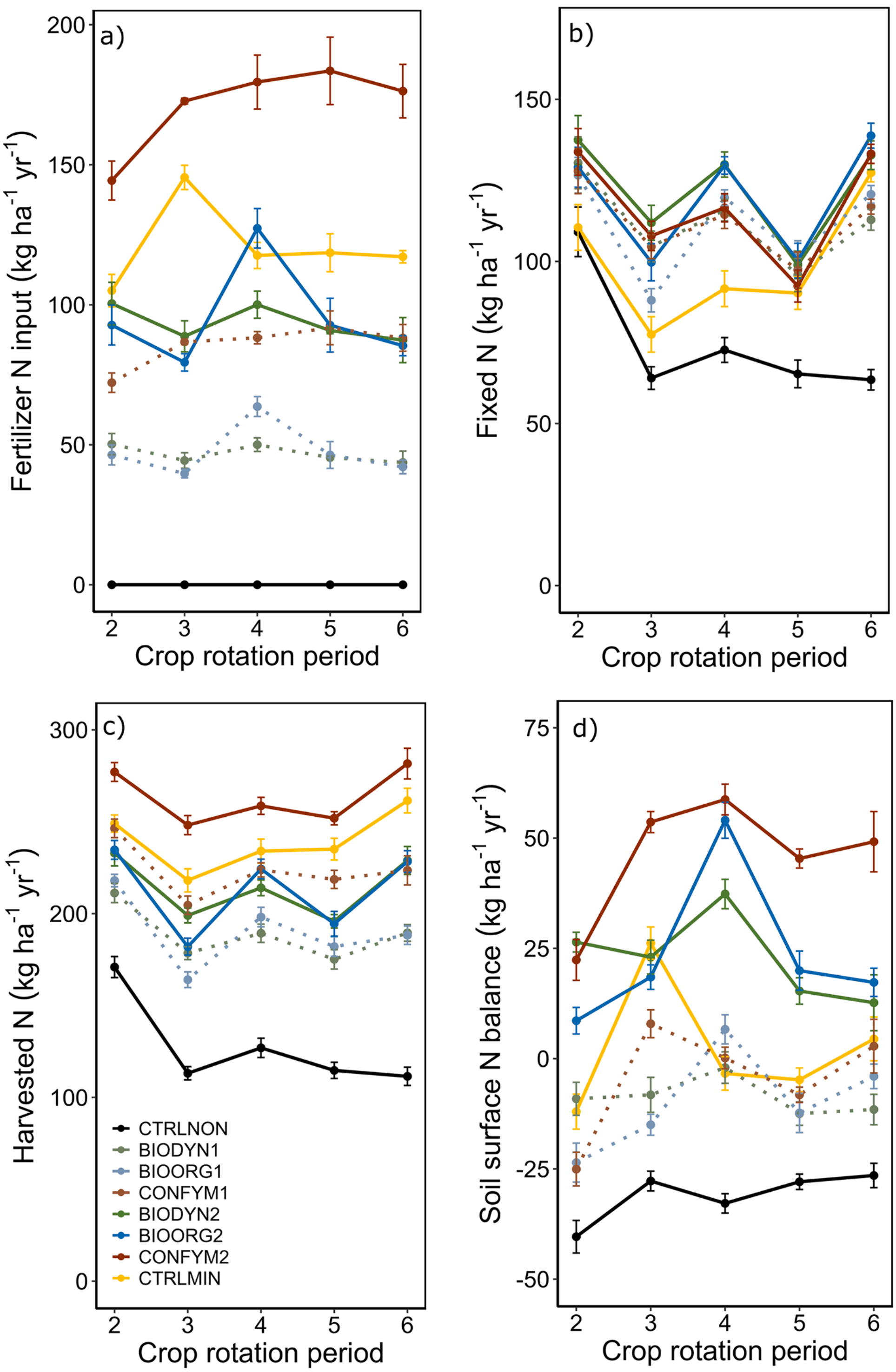

Fig. 1. Development of N inputs, outputs and balances over time. Panel a) is fertilizer N input, panel b) symbiotic N fixation, panel c) N output via harvested products, and panel d) soil surface balance per crop rotation period. Each data point shows the mean and standard error of n = 84 values (7 years with 12 plots) per treatment.

at typical fertilization level 2 received only 55 % of the total N input, while CTRLMIN received around 70 % (Table 2, Table 4). Organic systems received some mineral N input via slurries. This input was around 25 % of the mineral N input in CONFYM, because CONFYM received synthetic mineral N in addition to animal manure (Table 2). Still, CTRLMIN was the treatment with the greatest mineral N input. Thus, treatments differed both in total and in readily plant available mineral N input by fertilizers.

Total N fertilization levels changed somewhat over the course of the DOK field experiment (Fig. 1a). Explanations lay in the history of fertilization in the experiment, e.g., an increase of animal manure from 1.2 to 1.4 LU at the typical fertilization level, changes in farmers providing the animal manure, and/or revisions in fertilization recommendations (for details see Supplement 4).

Average amounts of fertilizer N applied at the typical fertilization level 2 of the conventional treatments (kg N ha^{-1} yr^{-1} 171 for

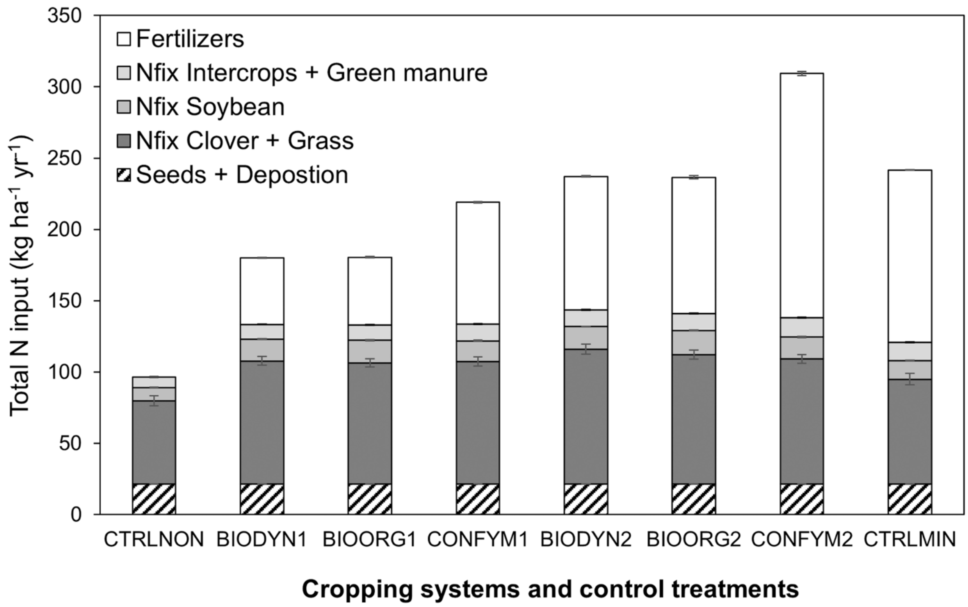

Fig. 2. Contribution of specific N inputs to total N inputs. Stacked bars indicate mean inputs, error bars the standard error of n = 420 values (35 years with 12 plots per treatment) per input type.

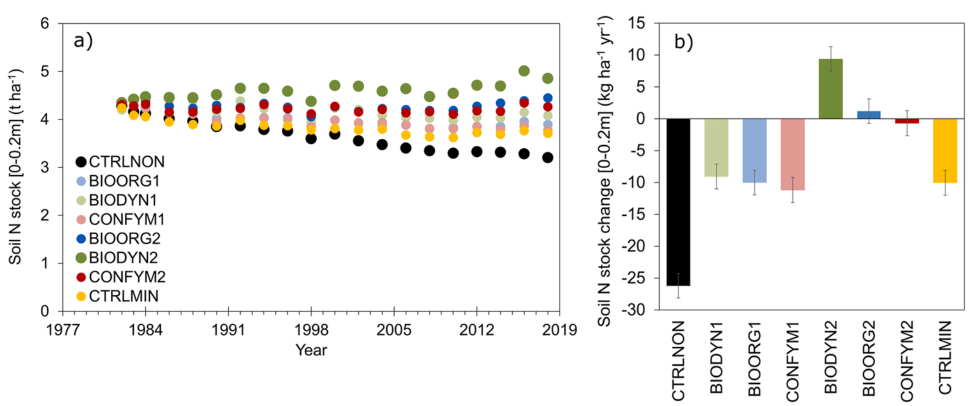

Fig. 3. Soil N stocks under different treatments. Panel a) shows soil N stocks over time, where each data point is the mean of n = 8–12 values per treatment and year. Panel b) shows the resulting mean annual N stock changes based on total N concentrations determined between 1984 and 2018 in 0–0.2 m soil depth and the soil bulk density measurements in crop rotation one. Error bars in panel b) indicate standard error derived from analysis of variance.

CONFYM2, 121 for CTRLMIN, Table 4) were less than the average annual N input of 225 kg N ha^{-1} in 18 conventional mixed crop-livestock pilot farms in Germany. In the same network, Chmelikova et al. (2021) reported that the organic mixed livestock farms had an average fertilizer N input of 91 kg N ha^{-1} yr^{-1} (Chmelikova et al., 2021), while Lin et al. (2016) reported an average N input with farmyard manure of 79 kg N ha^{-1} yr^{-1} for a crop rotation on an experimental organic farm in Germany. These values are similar to or lower than the 95 kg N ha^{-1} yr^{-1} applied with fertilizers to the organic treatments at typical fertilization level 2 of the DOK experiment (Table 2). The higher N input in organic systems of the DOK experiment is due to the underlying manure production level of 1.4 LU ha^{-1}, as compared to 0.87 LU ha^{-1} on the 18 organic mixed crop-dairy farms studied by Chmelikova et al. (2021). Livestock density underlying the manure application in the DOK experiment is also higher than the 1.06 LU ha^{-1} on 14 organic mixed crop-dairy farms in Denmark (Halberg et al., 1995). Thus, differences in fertilizer N input between conventional and organic systems of the DOK experiment were somewhat lower than between conventional and organic farms in other European countries.

4.2. *Input with symbiotic N_2 fixation*

Symbiotic N_2 fixation by legumes was a major N source in most treatments, most with an average N input of more than 100 kg N per year of the DOK experiment (Table 4). This shows the critical importance of the quantification of symbiotic fixation to quantify N budgets and NUE correctly. Treatment specific values ranging from 75 to 122 kg N ha^{-1} yr^{-1} (Table 4) were higher than all earlier estimates of symbiotically fixed N inputs into the DOK trial, either because below-ground N and/or N transfer to the associated grasses of grass-clover mixtures had not been accounted for (Bosshard, 2007), or because not all legume yield records had been included (Frossard et al., 2016). The values of the current study are also higher than values modeled for the DOK trial by Autret et al. (2020) based on the calculation method of Anglade et al. (2015), for four of the eight DOK treatments. Their values ranged from 65 (CTRLNON) to around 75 kg N ha^{-1} yr^{-1} for CTRLMIN, CONFYM2 and BIOORG2. Our values were also higher than fixed N inputs estimated for organic (75 kg N ha^{-1} yr^{-1}) and conventional (21 kg N ha^{-1} yr^{-1}) mixed crop-livestock (dairy) farms by Chmelikova et al. (2021). This underscores the value of plot- and year-specific data records in this experiment.

4.2.1. Critical evaluation of N fixed in legumes

Clover planted in mixtures provided the major input of symbiotically fixed N, contributing on average 75 % of the fixed N (Fig. 2). Nitrogen fixation with clover was also highest in absolute terms per cultivation year (Table 3) and per year of the DOK trial, because clover contained in grass-clover leys was the most often cropped legume (on each plot during 11 of the 35 study years) (Table 3, Fig. 2). Therefore, uncertainties related to estimates of N fixed with clover would have the greatest impact. For the PNdfa in clover biomass, we used treatment specific means ranging from 83 % to 91 %, based on Oberson et al. (2013). Such high proportions have regularly been reported for grass-clover mixtures receiving similar fertilizer N doses, and with similar clover proportions in the sward (Nyfeler et al., 2011; Lüscher et al., 2014). In grasslands, symbiotically fixed N contained in legume aboveground biomass can range from 100 to 380 kg of N ha^{-1} $year^{-1}$ (Lüscher et al., 2014). Thus, together with recorded clover proportions and ley yields, we consider the estimates of N fixed in aboveground clover biomass ranging from 105 to 172 kg ha^{-1} per cultivation year of grass-clover appropriate (Table 3).

Fewer studies have been done on belowground N inputs and transfer of clover N to associated grasses. We calculated belowground N by multiplying N fixed in aboveground clover biomass by a factor of 0.4 (Hammelehle et al., 2018). This factor corresponds to a shoot-to-belowground N ratio of 2.5, which is similar to a shoot to root N ratio of 2.46 proposed by Unkovich et al. (2010) for annual pasture legumes.

For the N transfer from clover to grass we used a proportion of grass N derived from clover of 3–25 % in the first year, and a higher proportion for the following years (43–55 %) based on Oberson et al. (2013). More recent values obtained in the DOK experiment using ^{15}N natural abundance and enriched ^{15}N techniques confirmed the proportions for the second year, but found a higher average proportion of 44 % for the first year (Hammelehle, 2018). In contrast, Pirhofer-Walzl et al. (2012) found a proportion of 13% of grass N derived from legumes during the first year of a grass-legume-herb mixture. Amounts of grass N derived from clover (Table 3) are similar to those of 30 and 45 kg N ha^{-1} yr^{-1} for years one and two, respectively, reported by Nyfeler et al. (2011) for grass-clover mixtures receiving 150 kg N ha^{-1} with mineral fertilization. Thus, our results on belowground fixed legume N input into the soil and fixed legume N transferred to the grass are within values reported for leys. At the same time, these inputs are highly dependent on sward composition and environmental conditions.

The N fixed in soybean contributed on average around 13 % of the fixed N input into the DOK treatments (Fig. 2). It was obtained using an average PNdfa of 57 % (Oberson et al., 2007; Hammelehle et al., 2013). The PNdfa of soybean can vary greatly. Unkovich et al. (2010) reported an average PNdfa of 48 %, ranging from 0 % to 90 % across soybean cropped in Australia, and Peoples et al. (2009) reported an average of 68 % for the PNdfa determined in soybean cropped in different regions of the world, with a range of 13–95 %. The PNdfa used for our calculations fell into this broad range. Still, the PNdfa in soybean is affected by the availability of mineral N in soils (Schipanski et al., 2010), which in turn is affected by the climatic conditions of the year (Gill et al., 1995; Jabloun et al., 2015). Also, because soybean is a sole crop, greater availability of mineral N in one year than in another would not be compensated for by greater N uptake of the non-fixing plant, as in grass-clover leys (Nyfeler et al., 2011). Therefore, the PNdfa may have varied across different cropping years. For some treatments the difference in PNdfa obtained by Oberson et al. (2007) and Hammelehle et al. (2013) was as much as 20%. Belowground N input by soybean was calculated using the ratio of 0.645, determined in the DOK experiment by Hammelehle et al. (2013), which is similar to the root to shoot ratio of 0.61 proposed for soybean by Unkovich et al. (2010).

Legumes contained in intercrops and green manure fixed 50–95 kg ha^{-1} yr^{-1} (Table 3). This range is comparable to 78–128 kg N fixed by cover crops sown in a field experiment between two barley crops in Denmark (Li et al., 2015), and to 38–67 kg fixed N reported by Amossé et al. (2013) for cover crops on six organic farms in France. The somewhat higher values from Li et al. (2015) include N fixed in root biomass (like our estimates), while Amossé et al. (2013) include amounts of N fixed only in aboveground biomass. The fixed N inputs by the intercrops contributed on average 10% of fixed N (Fig. 2). Hence, under- or overestimations of that value would have less impact than N fixed by clover or soybean.

Symbiotic fixation was an important N input in all treatments (Table 4, Fig. 2). It was lowest in CTRLNON, most probably due to limitations in nutrients such as K and P in the soils of that treatment (Suppl. Table S1) (Oberson et al., 2013; Hammelehle et al., 2018). Limited P and K supply can limit N_2 fixation, as shown for white clover grown hydroponically, where limited P and K supply restrict N_2 fixation through changes in the relative growth of roots, nodules, and shoots (Hogh-Jensen et al., 2002; Hogh-Jensen, 2003). Nutrient limitation of symbiotic N_2 fixation can further be concluded from the decreasing amounts of N fixed in CTRLNON with time (Fig. 1b), because P and K availability in CTRLNON decreased with time (Oehl et al., 2002; Gunst et al., 2013). Interestingly, symbiotic N_2 fixation was only slightly, though significantly, lower under low fertilization level 1 than typical fertilization level 2 (Table 3, Table 4), despite lower P and K availability in level 1 than level 2 soils (Suppl. Table S1). However, the yield of the associated grasses was significantly reduced compared to level 2, and lower K and P concentrations in clover shoots under fertilization level 1 than 2 suggest that these elements may as well become limiting for legume yield and symbiotic fixation (Oberson et al., 2013). Thus, if fixation is to be maintained, then alternative K and P fertilizers such as nutrients recycled from urban wastes will be needed (Möller et al., 2018).

Another key determining factor that may have counteracted N fixation is the higher availability of mineral N under typical fertilization level 2 than under low fertilization level 1 (Table 2), with level 1 treatments receiving half the dose of level 2. This effect was demonstrated by lower fixed N values in CTRLMIN than in other treatments (Table 4, Fig. 2). The down-regulation on symbiotic fixation by mineral N availability has repeatedly been shown both for soybean (Herridge and Brockwell, 1988) and for clover growing in mixtures with grasses, where high availability of mineral N from synthetic fertilizers reduced symbiotic N_2 fixation activity of clover and clover proportion in the sward (Hebeisen et al., 1997; Nyfeler et al., 2011). Hence, for optimizing the input of symbiotically fixed N, organic or organo-mineral fertilization is of advantage compared to sole mineral fertilization (Tables 3, 4).

4.2.2. Impact of fixed N on N budget and NUE

Nitrogen fixation was a major input to all treatments, and errors related to this calculation would hence affect the balance and NUE. Assuming that N fixation was underestimated, such that this input would have been 110 % of the values shown in Table 4, the soil surface balance would increase accordingly. Because total N fixation was similar in all fertilized treatments, it would be plus 10–12 kg N input ha^{-1} $year^{-1}$. The soil surface budget NUE would decrease about 5 % (Suppl. Table S2). In contrast, assuming that N fixation had been overestimated, the balance would, with only 90 % of the fixation inputs shown in Table 4, decrease accordingly and the NUE would increase on average by about 5 %. This would have resulted in unrealistically high soil surface NUE > 100 % for even more treatments. Thus, we may rather have under- than overestimated N fixation.

4.3. Nitrogen removal with harvests

Nitrogen removal by harvested products was significantly affected by the treatments (Table 4, Fig. 1c) as expected from yield differences between these treatments (Knapp et al., 2023). Harvested N was closely related to the total N input (Fig. 4). The N output via harvested products fluctuated somewhat over time, with e.g., a decrease during the 3rd crop

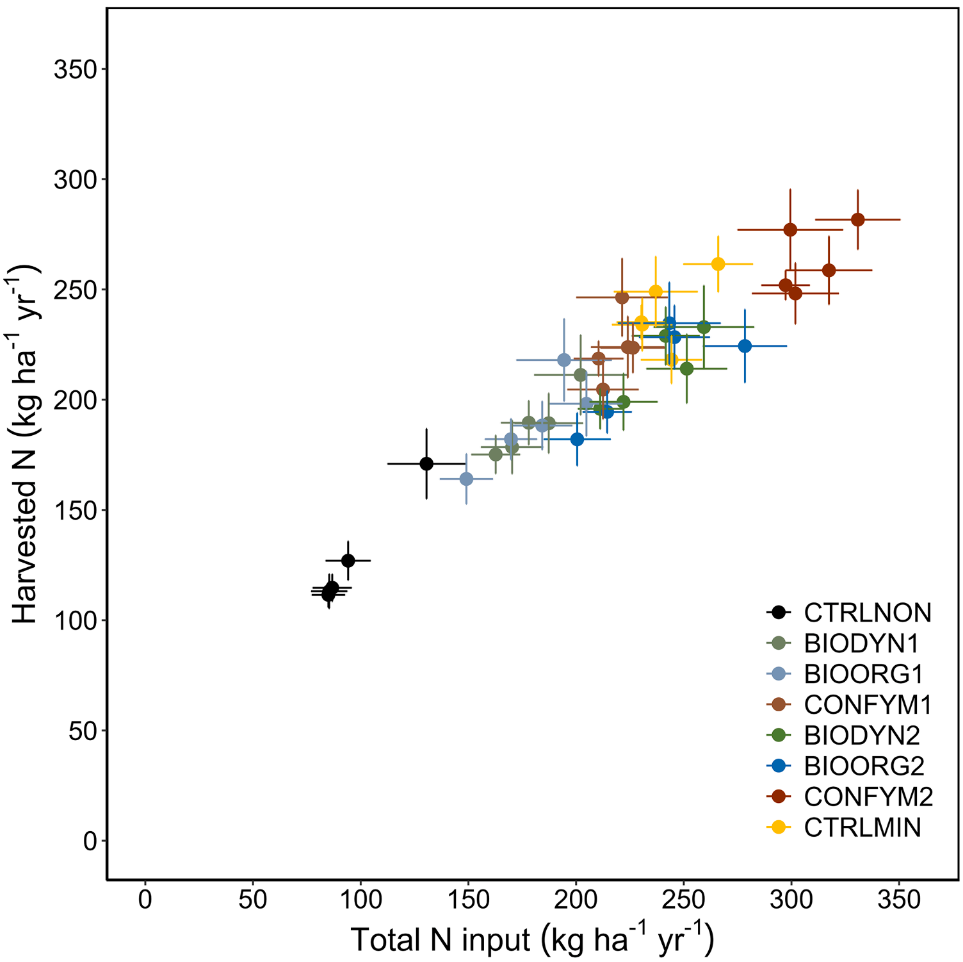

Fig. 4. Harvested N as a function of total N input. Each data point shows the mean and standard error of n = 84 values (7 years with 12 plots) per crop rotation period and treatment.

rotation (Fig. 1c). Decreases in N removal by harvested products were mainly due to a decrease in N output by the grass-clover leys (Suppl. Fig. S1). Overall, N removal via harvested products from a given treatment was stable for crop rotation periods 4–6. This agrees with Knapp et al. (2023) who reported stable yields at both fertilization levels of conventional and organic treatments, and of CTRLMIN of the DOK experiment between 1985 and 2019.

Average N removal with harvests of 264 kg N ha^{-1} yr^{-1} in CONFYM2 was greater than the 222 kg N ha^{-1} yr^{-1} average harvest N output from fields of 18 conventional mixed crop-dairy farms in Germany, but within their N output, ranging from 173 to 310 kg N ha^{-1} yr^{-1} (Chmelikova et al., 2021). This was possibly due to a lower proportion of grass-clover in their crop rotation (11 % of crop land) than in the DOK experiment (31 % from 1985 to 2019, Table 1), as grass-clover leys result in high N removal from plots (Suppl. Fig. S1). Average harvest N outputs were also high in organic systems of the DOK experiment fertilized at typical fertilization level 2 (around 213 kg N ha^{-1} yr^{-1}, Table 4) compared to the harvest N outputs from organically cropped fields of the 18 mixed crop-dairy farms studied by Chmelikova et al. (2021). They reported an average N removal by harvested products of 166 kg N ha^{-1} yr^{-1}, with a range of 110–236 kg N ha^{-1} yr^{-1}, although the organic farms had average grass-clover ley proportions of 40 % of crop land. From fields of an experimental farm in Southern Germany representing an organic mixed crop-livestock system with 1.4 LU per ha^{-1}, Lin et al. (2016) reported N output from the fields of 140 kg N ha^{-1} yr^{-1}. Thus, for conventional and organic treatments of the DOK experiment, N output via harvested products is somewhat above average, likely due to high N removal by grass-clover leys, and also to the favorable pedo-climatic conditions at the experimental site (very fertile Luvisol developed on Loess, deep soil profile, high field capacity, and sufficient precipitation).

4.4. Soil N stock changes

Negative to nearly balanced soil surface N values (Table 4) in the unfertilized CTRLNON, in treatments with low fertilization level 1, and in the CTRLMIN treatments, led to decreasing N stocks in their topsoils (0–0.2 m) (Fig. 3). This makes sense, because the soil surface budget did not consider N loss from fertilizers occurring at and after application (Webb et al., 2013; Häni et al., 2016). Soil N stocks declined in all treatments except CONFYM2 and BIOORG2 (stable) and BIODYN2 (increase) (Fig. 3). The same soil samples were analysed by Krause et al. (2022) who found the same pattern for soil organic C, as the average mass C/N ratio of 9.2 was not affected by treatments. Thus, farmyard manure input at typical fertilization level 2, but not at low fertilization level 1, maintained or increased soil organic C and N content. The beneficial effect of regular farmyard manure application on soil C and N concentrations in cropped soils has also been shown in other long-term field experiments (Johnston et al., 2009; Kong et al., 2009; Zavattaro et al., 2017). The greatest increase in soil organic C and N in BIODYN2 can be explained by the application of animal manure compost. Composting results in more stable organic matter compounds than those contained in fresh or fermented animal manure (Helgason et al., 2005). At the same time, during composting significant amounts of N can get lost via ammonia volatilization (Eghball et al., 1997), denitrification (He et al., 2001), or N leaching (Confesor et al., 2009). Thirteen years of manure compost application as compared to slurry application also increased soil organic C in a long-term tillage experiment established on a clayey soil in Switzerland (Krauss et al., 2017). In contrast, decreases in organic matter content in soils that have for decades been under synthetic mineral fertilization only, as with CTRLMIN, have been found in a range of cropped soils (Mulvaney et al., 2009). Sole N input of organic C-free synthetic mineral N fertilizer enhances microbial decomposition of soil organic matter (Drinkwater et al., 1998; Mulvaney et al., 2009). Manure application at low fertilization level 1, however, could not maintain soil organic C (Krause et al., 2022) and N stocks, i.e., it was not enough to sustain soil N turnover by replenishing soil N pools from fertilizers (Bosshard et al., 2008; Frick et al., 2022a).

In the soil system budget, we considered N decline in the topsoil as an additional N input, i.e., a source of N for crops. If the process underlying soil N decline was net soil N mineralization, then some of this mineralized soil N could also have gotten lost. For instance, mineralized soil N was the major source of leached nitrate under cropped soils studied on farm in Switzerland (Frick et al., 2022b). Soil N losses may occur when net mineralization exceeds plant N uptake, e.g., during winter (Frick et al., 2022b) or after soil tillage (Thomsen and Sorensen, 2006). Still, with a soil profile depth at the DOK experimental site of 0.95–1.3 m (Krause et al., 2020), some of this N may have been retained in deeper layers.

Subsoil N stock changes have not been considered in the budget and NUE calculations, as little data exists on layers below 0.2 m. Nitrogen concentrations have recently been measured in the 0.2–0.5 m depth on soil samples taken in 2019 and 2020 (Suppl. Fig. S3) and related N stocks been calculated using a bulk density of 1.5 t m^{-3}. Assuming that subsoil N stocks have not changed since the start of the DOK experiment in treatments BIODYN2, BIOORG2, and CONFYM2, then the lower N concentration measured in CTRLNON subsoils would translate into a decline (subsoil N mining) of 21 kg N ha^{-1} yr^{-1}. Likewise, lower N concentrations in subsoils under low fertilization level 1 treatments and CTRLMIN would indicate subsoil mining by around 7 kg N ha^{-1} yr^{-1}. If mined subsoil N is considered an additional N input to the soil system budget shown in Table 4, this would result in a positive balance of CTRLNON of around 16 kg N ha^{-1} yr^{-1}, which is in the range of 10 kg ha^{-1} yr^{-1} of N loss proposed for the CTRLNON treatment by Frossard et al. (2016) and Autret et al. (2020) (assuming that the surplus indicates potential loss, see below). The soil system balances of low fertilization level 1 treatments, and CTRLMIN would also increase and result in 7–19 kg N ha^{-1} yr^{-1}. Subsoil N would affect only the soil system budget derived NUE 2 (Table 4), with a minor reduction of about 3% for low fertilization level 1 treatments (Suppl. Table S2). As we assumed stable subsoil N concentrations for typical fertilization level 2 treatments, their budgets and NUE would remain unaffected. While topsoil N stocks reacted significantly to the soil surface budget (Fig. 5), more information on N stock changes in deeper layers, including N transfers between top- and subsoil, is needed to improve the understanding of N use.

4.5. *Nitrogen budgets to unravel N losses from topsoil-plant system*

The soil system budget calculation assumes that all inputs and outputs shown in Eq. 2 affected only the 0–0.2 m topsoil layer. Indeed, some fertilizer N may have been translocated, or symbiotically fixed N been deposited into deeper layers. This would lead to an overestimation of our soil system balance, and thus to an underestimation of the NUE (Table 4), unless this downward transport would have been counterbalanced by crop N uptake from the subsoil (as discussed above for subsoil N and below for fertilizer N). In a previous study at the DOK experiment, Bosshard et al. (2009) applied ^{15}N-labeled fertilizers (ruminant slurry; synthetic mineral N) to winter wheat growing in microplots. At crop maturity between 20 % and 25 % of the labeled fertilizer N was recovered in the 0–0.18 m topsoil layer, but only 2–3 % in the deeper 0.18–0.28 m layer, suggesting that only minor quantities of fertilizer N reaches subsoil. However, the fertilizer N recovery in that deeper layer was one year later more than doubled (7–8 %), suggesting that downward movement continued to progress. The major proportion of the symbiotically fixed N input was contained in plant aboveground plant parts (Table 3). Belowground N was composed of roots and rhizodeposition. After 19 months of cultivation all root N, and more than 90 % of clover N rhizodeposition was recovered in the 0–0.25 m layer, and on average only 8 % in the subsoil (0.25–0.6 m) (Hammelehle et al., 2018). Moreover, the influence of the cropping systems on soil N concentrations decreases with depth (Fig. S3). All this indicates that the

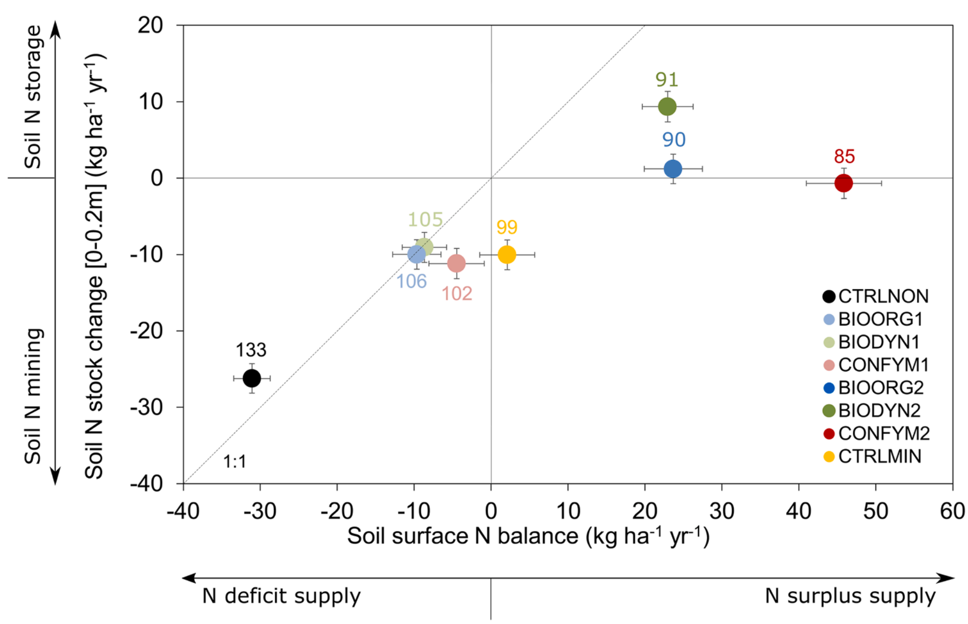

Fig. 5. Change in soil N stocks (Fig. 3b) as a function of soil surface N balance (Table 4). Each data point shows the mean and standard error of n = 210–211 measurements per treatment for soil N stock changes and n = 420 values (35 years with 12 plots) per treatment for soil surface balances. Values in the same color as data points are the soil surface budget derived nutrient use efficiency (NUE 1; Table 4).

subsoil may act as sink and source of N, yet that the main assumptions of the calculated soil system budget still hold.

The positive values of the soil system balance provide an estimate of N losses from the crop-topsoil system. For treatments fertilized at typical level 2, the soil system balance ranged from 12 to 47 kg N ha^{-1} yr^{-1}. These indicated losses were in the range of N losses via nitrate leaching, volatilization, and denitrification (N_2 and N_2O) modeled by Autret et al. (2020) for selected treatments of the DOK experiment. In contrast, they were less than the fertilizer N losses concluded from a study using ^{15}N labeled sheep manure and mineral fertilizer in microplots installed in the DOK experiment (Bosshard et al., 2009). In that study, 29 % of the mineral fertilizer, and 46 % of the urine-feces slurry N had not been recovered in the topsoil layer or in crops three years after application. These percentages of non-recovered fertilizer N would result in N fertilizer losses ranging from 20 to around 70 kg ha^{-1} yr^{-1} (Frossard et al., 2016). Fertilizer N losses determined by Bosshard et al. (2009) could have been overestimated because fertilizer N that has been transferred into soil layers deeper than 0.2 m can still be recovered by crops in following years. For instance, Hirte et al. (2018) found significant root biomass of wheat and maize cropped in the DOK experiment in the soil layer from 0.25 to 0.5 m soil depth, and even as deep as 0.75 m. Real N losses may thus fall between the values suggested by the soil system balance and the N losses shown in Frossard et al. (2016) based on the ^{15}N study of Bosshard et al. (2009). Overall, we consider the N losses in all treatments moderate, as compared to the average of 90 kg N lost annually per ha of agricultural land (including denitrified N_2 as main pathway) modeled by Velthof et al. (2009) across 27 EU member states.

4.6. *Efficient N use comes at a soil quality trade-off*

The soil surface budget based NUE 1 (Table 4) indicated that over the 35 years of the DOK trial, crops have converted N inputs at high efficiency (>85 %) into harvested N (Table 4). Both budget derived NUEs were significantly higher at low fertilization level 1 (97–106 %) than at typical fertilization level 2 (85–94 %). The soil system budget derived NUE was similarly high for the minerally fertilized CTRLMIN (95 %) and the manured BIODYN2 (94 %) treatment, suggesting that legume N combined with either animal manure or mineral fertilizer N can be used at high NUE. The CONFYM1 treatment at low fertilization level 1 combined high NUE (97–102 %) with high harvested N values (Table 4, Fig. 4). Legume N was a major input in most treatments (Table 4), and in our method approach, aboveground fixed N in clover and soybean was fully converted into harvested N. Still, calculations that separated fertilizer N use from that of other inputs (Supplement method 3) suggest that, at typical fertilization level 2, more than 70% of fertilizer N was converted into harvested N, with highest values (>80%) obtained for manures applied in BIODYN2 and mineral fertilizer in CTRLMIN (Suppl. Table S3).

NUE is usually greater in fields managed by researchers than in fields managed by farmers, as shown for mineral fertilizer N recovery in crops (Cassman et al., 2002; Ladha et al., 2005). Reasons for this are favorable pedo-climatic and topographic conditions at experimental fields of research stations, careful management by knowledgeable technical teams (e.g., absence of heavy machinery, manual labor for weeding or manure application in small plots, best management practices), and access to high quality genetic resources and other production means. From an on farm study, Chmelikova et al. (2021) reported soil surface budget derived NUE of 83–121 % in organic, and 61–114 % in conventional cropping systems, both receiving manure.

Similar NUE for treatments with mineral fertilizers and animal manure contrast with the higher short-term efficiency of mineral fertilizers than animal manure reported from both difference method approaches and ^{15}N studies (Webb et al., 2013; Chalk et al., 2020). However, studies using ^{15}N labeled manure have also consistently found greater recoveries in soil with animal feces and slurry compared with synthetic fertilizers (Bosshard et al., 2009; Frick et al., 2022a) which may, over the long term, translate into a greater residual fertilizer value of animal manure. Model calculations of Schröder et al. (2005) revealed that already 6–8 years after application, cattle slurry N reached about 80 % of the N fertilizer value of mineral fertilizer. With time, this value might further increase, though slowly, approaching similar NUE as those obtained with mineral fertilized systems.

The very high NUE in low fertilization level 1 treatments and CTRLMIN came at the expense of decreasing soil N stocks (Fig. 5) while the somewhat lower (but still high) NUE of CONFYM2, BIOORG2, and BIODYN2 was most probably related to higher N losses (indicated by the positive balances, Table 4). Our findings are in agreement with the EU Nitrogen Expert Panel (2015), which concluded that cropping systems with a soil surface budget based NUE of >100 % indicated actual soil N mining, while an NUE of 90–100% indicated a risk of soil N mining. Although animal manure application at typical fertilization level 2 maintained or increased soil N stocks, topsoil N stocks decreased under all low fertilization level 1 treatments (Fig. 3, Table 4). Since soil N decline paralleled an overall soil organic matter decline (Krause et al., 2022), this indicates a soil quality reduction and non-sustainable soil use (Bünemann et al., 2018). Thus, neither organic nor conventional cropping was run sustainably with a stocking density of 0.7 LU ha^{-1}. However, this threshold cannot be generalized, because the crop rotation of the DOK was designed in agreement with forage needs of 1.4 LU ha^{-1} (Krause et al., 2020). On farms, cropping systems with 0.7 LU ha^{-1} would probably be organized differently (e.g., less forage N removal, likely more green manure legume N input). Such systems would need alternative organic matter and nutrient inputs, e.g., from urban wastes (Möller et al., 2018; Xia and Yan, 2023).

5. Conclusions

Based on a data set of 35 years, different types of N budgets and NUEs were calculated for the different cropping systems of the DOK trial. Quantification of symbiotically fixed N inputs, based on ^{15}N studies and regular clover yield determinations, revealed that this input was underestimated in earlier studies. Nitrogen fixation by clover sown in mixtures was identified as a major N input. Its proper quantification requires accounting for belowground N and fixed N transferred to associated grass. Soil surface N balances were positive for all treatments receiving typical fertilization level 2, and nearly balanced under sole mineral fertilization. The derived NUE ranged from 85 % to 99 %. Thus, animal manure and mineral fertilizer-based treatments both had high NUE, probably due to favorable pedo-climatic and topographic conditions of the experimental site, where mineralized soil and residual fertilizer N may have been recovered by plant roots from deeper soil layers. Absence of fertilization and all treatments with low fertilization level 1 resulted in negative soil surface balances. The regular application of animal manure from 1.4 LU ha^{-1} maintained or increased topsoil N stocks, while topsoil N stocks declined under low fertilization level 1 in all cropping systems and with sole mineral fertilization. Thus, positive N balances are needed to maintain or increase topsoil N stocks in manured treatments, though the surpluses of 23–46 kg N ha^{-1} yr^{-1} are likely related to N losses to the environment. Our results indicate an efficiency-sustainability trade-off between efficient N use and mining of soil N reserves. Measures that further improve synchrony between plant N uptake and the presence of available N, and that increase N retention in soil during phases of reduced crop demand, may further increase NUE while maintaining soil N stocks. Treatments with reduced animal manure input require alternative organic matter and nutrient inputs.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2023.108802.

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