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Rotational grass-legume leys increase arable crop yields, particularly at low N fertiliser rates

Fatima F. El Khosht [a,*], Göran Bergkvist [a], A. Sigrun Dahlin [a], Christine A. Watson [a,c], Johannes Forkman [a], Johan Nilsson [b], Ingrid Öborn [a]

[a] Department of Crop Production Ecology, Swedish University of Agricultural Sciences (SLU), P.O. Box 7043, Uppsala SE-750 07, Sweden
[b] Department of Energy and Technology, Swedish University of Agricultural Sciences (SLU), Uppsala SE-750 07, Sweden
[c] Department of Rural Land Use, SRUC, Craibstone Estate, Aberdeen AB21 9YA, UK

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ABSTRACT

Including perennial leys in crop rotations can increase arable crop yield and soil organic carbon content. However, ley effects are often confounded by differences in manure addition, and it is unclear how the effects change over time or are impacted by ley species. Using 48 years of data from long term experiments at three locations in Sweden, this study examined the effects of including a two-year sole-grass or mixed grass-legume ley compared with only arable crops in six-year rotations, on crop production, and soil carbon and nitrogen under different nitrogen (N) fertiliser rates but without manure additions. Rotational leys resulted in greater oilseed and cereal grain yields at zero and low N fertilisation, particularly when legumes were included in the ley. The effect was evident for grain yields throughout the rotation and similar over crop rotation cycles. However, there were no yield differences between rotations at the highest N rate. With a grass-legume ley in the rotation, less N fertiliser was required to obtain similar grain N concentrations in winter wheat as in the rotation without ley. Concentrations of topsoil C and total-N, across cycles and N rates, were higher in rotations with ley. Topsoil C was maintained between the 2nd and 8th cycle in all treatments except at the low N fertiliser rate in the rotation without ley. Including short-term grass-legume leys in crop rotations with only arable crops may be a way to reduce the dependence on N fertiliser and still maintain topsoil C.

1. Introduction

The separation of agriculture into specialised arable and livestock-based farming systems has resulted in the use of simplified crop rotations and exclusion of leys (consisting of perennial legumes, grasses, forbs or a mixture of these) on many farms. In agricultural areas with fertile soils, arable crops that rely heavily on fossil fuel-derived inputs such as fertilisers and pesticides (Oomen et al., 1998; Peyraud et al., 2014) have replaced leys. For example, a recent study of crop sequence patterns and diversity in farming systems in Sweden showed that ley is far less frequently grown on the fertile plains (high productivity zones) than in regions with mixed landscapes (Reumaux et al., 2023). Specialisation and intensification of production have led to increased labour productivity but have also had negative impacts on the environment (Moraine et al., 2014). Specialised livestock systems commonly operate with a nutrient surplus, resulting in phosphorus (P) accumulation in soil and nitrogen (N) losses to water and air (Peyraud et al., 2014). Specialised arable farming systems may also impact negatively on the environment through nutrient and pesticide leaching (Franzluebbers et al., 2011), and pesticide resistance (Storkey et al., 2019), decreased biodiversity (Oomen et al., 1998) and soil degradation in terms of e.g. loss of soil organic carbon (SOC) (Goidts and van Wesemael, 2007; Peyraud et al., 2014).

Re-introducing perennial leys in arable crop rotations has been suggested as a step towards creating more sustainable systems by providing ecosystem services such as nutrient provision and weed and pest suppression (Albizua et al., 2015; Martin et al., 2020). For example, less disturbance in systems with perennial leys as compared to only arable cropping can increase the quantity of soil biota and thus improve soil functions such as N mineralisation (van Eekeren et al., 2009). In addition, the longer periods of vegetation cover during ley phases promote weed seed predation and decay (Meiss et al., 2010), and increase competition that in combination with regular cutting of the crop, prevents production of new weed seeds and exhaust many perennial weed

* Corresponding author.
E-mail address: fatima.el.khosht@slu.se (F.F. El Khosht).

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species (Meiss et al., 2008). Therefore, the abundance of weeds in the system is likely to be reduced. In turn, the need for inputs of fertiliser and pesticides decreases leading to both environmental and economic benefits (Tidåker et al., 2014, 2016). Furthermore, leys may reduce the loss of SOC associated with arable crop cultivation (Smith et al., 1997; Poeplau et al., 2011; Zani et al., 2021) through extended growth periods, larger amounts of exudates and increased root biomass resulting in larger soil organic matter (SOM) inputs (Bolinder et al., 2007; Börjesson et al., 2018). In addition, roots contribute more to soil C sequestration than shoot residues (Kätterer et al., 2011; Lange et al., 2015). In conversion from arable land to permanent grassland, leys have the potential to mitigate climate change (IPCC, 2022) and have been found to accumulate about 0.4 Mg C $year^{-1}$ (Kätterer et al., 2008) to 0.8 Mg C year (IPCC, 2000). In short-term leys, however, less carbon is potentially accumulated, and the amount is greatly affected by management practices such as ploughing frequency between the ley phases (Soussana et al., 2004), C inputs in manure or other bulky organic fertiliser and duration of ley in the rotation (Bolinder et al., 2010; Keel et al., 2019). Therefore, it is not clear whether accumulation of C can be sustained over time with low proportions of ley in the rotation.

Organic matter (e.g. crop residues, manure or compost) and N fertiliser are the main sources of organic and inorganic soil N (Chen et al., 2014). Thus, soil N concentrations and changes in these are related to the amounts and types of N inputs through mineral fertilisers and organic amendments such as manure and compost. Legume residues contain more N (particularly in roots and root nodules) and have a lower C/N ratio than non-N fixing crop residues as a result of biological N_2 fixation (Talgre et al., 2012). Hence, inclusion of forage legumes in rotational leys can give an additional increase in soil N and associated decrease in soil C/N ratio.

Positive pre-crop effects of rotational leys on succeeding crops such as greater grain yields and higher N concentration have been reported by others, particularly at no or low N fertiliser application rates (e.g. Nevens and Reheul, 2002; MacLaren et al., 2022; Poulton et al., 2023). The ley age may influence the pre-crop effect, where older grass leys can give an increased advantage, such as improved grain yield and N concentration in the subsequent crops, over younger grass leys (Christensen et al., 2009;). The effect of ley age in mixed grass-clover leys also interacts with the effect of proportion of legume in the ley, since a high proportion of legumes benefits improved yields (Kramberger et al., 2014) and the proportion of legumes generally decreases over time (Brophy et al., 2017), particularly if the legume is red clover (Annicchiarico et al., 2015). Oilseed rape generally yields well after N-rich pre-crops (Fordoński et al., 2016), however, oil concentrations has been found to decrease with increased N availability in favour of an increase in protein (Brennan, 2016; Tian et al., 2020). While positive pre-crop effects of ley are documented, studies rarely assess the effects beyond two years after ley incorporation, although some stretch to three years after ley (e.g. Nevens and Reheul, 2002).

In long-term studies on the effect of leys, organic amendments, such as manure, are often added to the ley to simulate mixed crop/livestock systems, resulting in confounding effects (Bergkvist and Öborn, 2011). However, leys can be introduced in the crop rotations for uses other than as ruminant feed, e.g. as substrate in biogas plants (Tidåker et al., 2014) or to produce various products through fractionation in a bio-refinery (Parajuli et al., 2015; Micke et al., 2023). Therefore, the association of leys with animal manure is no longer obvious and it is important to distinguish the effect of leys from the effect of manure.

To address the perceived risk of loss of crop productivity and soil fertility in arable farming systems because of reduced use of perennial leys and manure, several long-term experiments (LTEs) were initiated in Sweden in the 1950s and 1960s (Bergkvist and Öborn, 2011). These experiments compared traditional crop rotations used in mixed (crop and livestock) farming systems with simpler crop rotations used in modern systems dominated by arable crops. These experiments continue to provide long-term data that are valuable in understanding how different cropping systems influence crop yields, and soil C and N in the long-term (Bolinder et al., 2010; Shi et al., 2022). One series of LTEs in Sweden, replicated at three sites, evaluates the effect of two-year leys in six-year rotations on arable crop yields in the rotation under four different rates of mineral N fertiliser (Wallgren and Rådberg, 1989). Previous studies on the effects of crop rotations have been performed based on data from this LTE series (Persson et al., 2008; Nilsson et al., 2023). Persson et al. (2008) evaluated the effect of rotation under different N rates on soil C and winter wheat grain yield from 1975 to 2006 and Nilsson et al. (2023) assessed the site-specific effect on environmental performance based on crop yield and soil C data during eight rotation cycles at two (high and low) N fertiliser rates. Persson et al. (2008) found that the effect of N fertiliser on winter wheat yields increased over time at two of the sites and in one of these sites the N effect was greater with leys in the rotation if it included legumes. In addition, the modelled carbon input to the soil with leys was about twice that without the leys. Nilsson et al. (2023) used a life cycle assessment approach to evaluate data from two of the N rates and found the smallest environmental impact with two-year mixed ley at the low N rate. They also established positive effects of using leys on soil carbon content and average yields of the arable crops, especially at the low N rate.

In the present study, we assessed how the influence of ley in the crop rotation interacts with N fertiliser rates on the arable crop yields and quality, topsoil C and total-N and how this changed over 48 years. We hypothesise that;

(H1) Rotational leys have a positive influence on arable crop yields and grain protein, with a greater positive effect of legume-based leys than grass-only leys.

(H2) Rotational leys have a positive effect on topsoil C compared to rotations with arable crops, and leys with legumes reduce soil C/N ratio.

(H3) Addition of N fertiliser masks the effects of rotational leys and legumes on arable crop yields, grain protein and topsoil C and total-N.

(H4) The residual effect of leys on arable crop yields and topsoil C and total-N increases over rotation cycles.

2. Materials and methods

2.1. Experimental sites and set-up

Crop and soil data from the Swedish University of Agricultural Sciences LTE series (code R4–1103) were obtained for its three locations in Sweden: Lanna (58.20 N 13.07E), Stenstugu (57.36 N 18.26E) and Säby (59.49 N 17.42E). The ongoing experiment started in 1965 at Lanna, 1966 at Stenstugu and 1967 at Säby. Mean initial topsoil C concentration measured at the establishment of the experiments was 1.4 % at Lanna, 1.5 % at Stenstugu and 1.7 % at Säby. Additional site and soil characteristics and climate conditions are presented in Table S1 in Supplementary Material (SM). The LTE consists of three six-year rotations (Table 1). Two of the rotations include ley, one a two-year grass-only ley and one a two-year mixed grass-legume ley, together with four arable crops. The third rotation comprises five years of arable crops and one year of fallow, where the spontaneous vegetation has been controlled with tillage, herbicides or mowing (Table S2) as needed to prevent an increase in the weed seed bank. Management practices are presented in Table S2 in SM and seed rates, species and varieties of ley crops in Table S3 in SM. The four N fertiliser rates differ depending on crop and are presented in Table 1. A change in N fertiliser composition was made in the late 1990 ́s to contain 50 % ammonium-N, compared with nitrate-N only in earlier years. Varieties of individual crops are the same in all rotations per site, and the same variety is kept in the rotations while it is available on the market. At Lanna and Säby, an unreplicated split-split-plot design is used (Fig. S1 in SM), with rotation phase (1−6) on main plots, rotation (grass-legume ley, grass ley, no-ley) on subplots and N rates (N0-N3) on sub-subplots. The unreplicated experiment at Stenstugu also comprises six main plots with different rotation phases, but within each main plot, rotations are randomised to columns and N

Table 1
Crop sequences in each of the crop rotations and N fertilisation treatments per crop (kg ha^{-1} (N0-N3)) in three long-term experiments conducted at Lanna, Stenstugu and Säby starting in 1965, 1966 and 1967, respectively.

Grass-legume ley rotation		Grass ley rotation		No-ley rotation	
Crop	N rate (kg ha^{-1}) N0, N1, N2, N3	Crop	N rate (kg ha^{-1}) N0, N1, N2, N3	Crop	N rate (kg ha^{-1}) N0, N1, N2, N3
Oilseed rape[a]	0, 60, 120, 180	Oilseed rape[a]	0, 60, 120, 180	Oilseed rape[a]	0, 60, 120; 180
Winter wheat	0, 45, 90, 135	Winter wheat	0, 45, 90,135	Winter wheat	0, 45, 90, 135
Spring oats	0, 40, 80, 120	Spring oats	0, 40, 80, 120	Spring oats	0, 40, 80, 120
Spring barley (undersown)	0, 60, 60, 60[c]	Spring barley (undersown)	0, 60, 60, 60[c]	Spring barley	0, 40, 80, 120
Grass-legume ley 1[b]	0, 0–80, 0–160, 0–240	Grass ley 1	0, 45 + 35, 90 + 70, 135 + 105	Spring wheat	0, 60, 120, 180
Grass-legume ley 2[b]	0, 0–45, 0–90, 0–135	Grass ley 2	0, 45, 90, 135	Fallow	0

Due to frequent problems with damage, mainly by insects, oilseed crops were replaced with winter wheat after completion of a six-year rotation starting at Säby in 2017, Lanna in 2019 and Stenstugu in 2022. In the ley rotations, spring barley was undersown with grass-legume or grass ley.

[a] All plots, also in the N0 treatment, were fertilised with 30 kg N ha^{-1} in autumn prior to sowing of oilseeds to enable a successful establishment in all N treatments. Additional amounts of N fertiliser in the N1-N3 treatments were given according to plan in spring. Oilseed crops were initially winter turnip rape at Säby and winter oilseed rape at the other sites. Due to poor winter survival the winter oilseed crops were occasionally replaced with spring rape or spring turnip rape.

[b] N fertilisation of the grass-legume ley was based on proportion of legumes determined by visual grading prior to fertilisation, i.e. at < 25 % legumes a full dose was applied; < 50 % legumes a 0.7 dose was applied; and > 50 % legumes no fertiliser was applied.

[c] The same N rate was applied to the undersown spring barley in order to support ley establishment.

rates to rows (Fig. S2 in SM). Thus, at each site, there are 72 sub-subplots. At Lanna, the sub-subplot size is 8 m x 15 m, at Säby 4.75 m x 18.65 m and at Stenstugu 7.3 m x 16 m. At each site, all crops (i.e. phases) of the rotations are present every year (space for time substitution).

2.2. Crop and soil sampling

Arable crops and ley biomass were harvested and yield determined from an area of at least 24 m^2. Leys were harvested two to three times in the first year and once in the second year, at a cutting height of 8–10 cm. Since 2009, visual estimates of percentage of grasses and legumes were made in the grass-legume ley prior to each harvest.

Topsoil samples were collected annually, except from 1993 to 2005. The topsoil samples were collected in autumn in the N1 and N3 spring oat plots. Each topsoil sample (0–20 cm) was collected using a soil corer (diameter 2.8 cm) and consisted of at least 20 subsamples distributed evenly across the plot excluding a border of 50 cm along plot edges.

2.3. Chemical analyses of seeds, grain, ley biomass and soil

Methods and equipment for determining N concentration (%) in grains have changed during the study period. It was initially determined by the Kjeldahl method, followed by dry combustion (LECO, USA) which has been shown to be comparable (Sader et al., 2004). Currently near-infrared transmittance analyser (Infratec NOVA/Infratec 1241) is used providing concentrations of grain protein and seed oil. Protein percentage is then converted to N% by dividing by a crop-specific factor, i.e. 5.7 for wheat and 6.25 for oats and barley (Tkachuk, 1977). Ley biomass N concentration is analysed using dry combustion on an auto-analyser (LECO).

Soil samples collected annually were air-dried (25̊C) and sieved prior to analyses. Samples collected annually between 1972 and 1992 and again between 2005 and 2010 were analysed in the year of collection, whereas samples from 2011 to 2020 were archived prior to analysis. During the period 1972–2010, methods and equipment used for C and N analysis changed. Initially, soil C was determined by loss on ignition with correction for soil clay content (Wiklander, 1976) or by wet combustion, i.e. the Walkley-Black method, while dry combustion methods with Ströhlein instruments and various LECO instruments have been used since the early 1990s. The reliability of the method of analysing carbon concentrations in the beginning of the experimental period was tested by reanalysing archived topsoil samples from the years 1972 and 1973 and comparing the new results with the older. The C concentration in recent and older analysed samples showed high correlation ($R^2 = 0.91$), leading us to conclude that the older analysis results could be used. To determine soil total-N, the Kjeldahl method was used before the LECO instrument was available.

The archived soil samples from 2011 to 2020 were analysed in autumn 2021. Total-C and total-N were determined by dry combustion at 1350̊C according to the Dumas method on an auto-analyser (LECO CNS 2000, USA). At the Lanna and Säby sites, topsoil pH was ≤ 6.7 (S1) and the carbonate test with 10 % HCl droplets gave negative results; total-C was thus assumed to be equal to SOC and used in the statistical analysis. At Stenstugu, since topsoil pH was > 6.8, a sub-set of soil samples from 2011 to 2020 and 1972–1973 were further analysed for carbonate C (LECO TruMac CN). For this analysis, the samples were first combusted at 550°C and analysed for total-C, potential remaining carbonate-C was then combusted at 1350°C where the carbonate-C concentration corresponded to the difference in analysis results between the two combustion temperatures. Carbonate C concentration was below the report limit (0.02 %) in 92 % of the samples. Based on this, the carbonate-corrected values were used in the statistical analysis of samples from 1972 to 1973 and 2011–2020. Since the carbonate concentration in the analysed samples were too low to significantly influence the results, the original data without any correction factor were used for the remaining samples. Stocks of C and N could not be calculated, due to lack of information on soil bulk density. Therefore, the concentrations of SOC and total-N were used in statistical analysis.

2.4. Data availability and statistical data analyses

Data used in the present analysis (Table S4) were from the second six-year cycle (cycle 2) onwards at each experimental site, starting in 1972 at Lanna, 1973 at Stenstugu and 1974 at Säby. The first cycle was excluded due to differences in nutrient management between treatments. For soil data, crop rotation cycles 5–7 were excluded because samples were not taken in all years. For crop quality data, cycles 2–7 were not included in the oilseed rape oil concentration analysis nor the winter wheat gluten analysis since this data collection started in later cycles. Rotational cycles were only included when data was available from all sites. This was the case for winter wheat and spring barley grain N concentration in cycle 5 and ley N concentration in cycles 6–7 (Table S4).

The data were analysed using linear mixed-effects models fitted in JMP Pro 16 to address following research questions: i) long-term effects of crop rotation on soil C and N concentrations and ii) on arable crop yields and quality; iii) influence of N fertiliser rate on these effects; and iv) changes in the effects over time. The model used to assess the effects on soil and crop data included fixed effects of rotation, N fertiliser rates and cycles (i.e. a six-year full rotation cycle), and all interactions between rotation, N fertiliser rate and cycle. In addition, random effects of sites (i.e. used as replicate, see Nilsson et al., 2023 for site-specific results and Fig. S3 for site-specific topsoil C concentrations), (calendar-) years and plots (nested within sites) were included in the model. Random effects of two-way interaction of sites and N fertiliser rates, and sites and rotations, and three-way interaction of sites, N fertiliser rates and rotation were also included in the model when this improved the Akaike information criterion (AIC). This was the case for the response variables arable crops and ley yields, winter wheat and spring oat grain N and ley biomass N, for which more data were available than for soil (Table S4). When analysis of variance identified a statistically significant difference, the Tukey HSD test was used to identify treatments that differed significantly. To meet the assumptions of normality and homogeneity in the analysis, the response variables oilseed rape yield, winter wheat grain yield and grain N, spring oat grain yield and grain N, spring barley grain yield and grain N, and ley biomass yield and N concentration were either square root- or log-transformed before analysis. In addition, ley production was analysed as total yield combining all harvests per year and both ley years regarding biomass yield and N concentration.

3. Results

3.1. Arable crop seed and grain yields

Yields of all crops except spring barley were, on average, significantly greater with the grass-legume ley in the rotation than without ley (Table 2). The difference in yields between rotations decreased with increasing N rate, causing a significant interaction for all crops (Fig. 1). At the higher N rates, the yield differences between rotations were generally not significant, except for spring barley where rates differed between rotations (Fig. 1). The average yields changed between cycles, but the effect of rotation was similar during the whole study period (Table 2). Winter wheat and spring oats responded more to N application in later than earlier cycles causing a significant interaction (Table 2; Table S5).

The crop rotation with grass-legume ley had the greatest mean yields of oilseed rape, winter wheat and spring oats, across N rates and cycles. With no (N0) and low (N1) addition of N fertiliser, the positive influence on arable crop yields of having grass-legume ley in the rotation persisted through the whole crop rotation (except N1 for oilseed rape), however, the effect decreased with increasing N rates and generally with years after termination of the ley (Fig. 1; Table S6).

Across cycles, mean oilseed rape yield at N0 was 0.53 Mg DM ha^{-1} and 0.36 Mg DM ha^{-1} greater with grass-legume ley in the rotation than with grass ley and without ley, respectively. Addition of N fertiliser reduced the yield-increasing effect and at N2 and N3 yields were similar in all rotations (Fig. 1a). Winter wheat grain yields (second crop succeeding ley in the ley rotations) had greater mean yields across cycles at N0-N2 in the rotation with grass-legume ley than in the other rotations (Fig. 1b). The greatest yield difference between rotations was recorded at N0, where the grain yield was about 0.88 Mg DM ha^{-1} and 0.59 Mg DM ha^{-1} greater with grass-legume ley in the rotation than without ley or with grass ley, respectively (Fig. 1b). In the grass ley rotation at N0, winter wheat showed a yield increase of about 0.30 Mg ha^{-1} compared with the no-ley rotation. For mean grain yield of spring oat (third crop after ley in the ley rotations), the differences in yields across cycles between rotations was smaller than for previous crops (Fig. 1c). Still, at N0, mean spring oat grain yield across cycles in the grass-legume ley rotation was greater than yields in the no-ley and grass ley rotations. In the grass ley rotation, recorded mean spring oat yield at N0 (across cycles) was also greater than in the no-ley rotation. Mean spring barley grain yield across cycles was greater at N0 and N1 in the rotation with grass-legume ley than in the rotation without ley (Fig. 1d). The N rates (N1-N3) applied to spring barley differed between rotations with and without ley (see Table 1) and were always 60 kg N ha^{-1} with undersown ley to equalize conditions for ley establishment. Despite 20 kg N ha^{-1} lower rate at N2 to undersown spring barley, yields in the ley rotations were as great as for sole-grown spring barley in the no-ley rotation. However, at N3, when the N rate was 60 kg N ha^{-1} lower to the undersown spring barley, yields were greater in the no-ley rotation (Fig. 1d).

3.2. Arable crop seed and grain quality

The inclusion of a grass-legume ley in the rotation affected arable crop quality parameters, i.e. concentrations (%) of oil in oilseed rape, grain N in cereals and gluten in winter wheat, up to two years after ley termination (Table 3, Table S7-S8). The N rate had a clear impact throughout the rotation, but in winter wheat and spring barley the effect depended on rotation (Table 3). Oil concentration was smaller when oilseed crops followed grass-legume ley compared to grass leys or fallow and after higher N rates (Table 3). In rotations with grass-legume ley, grain N concentrations in winter wheat at N1 and N2, across cycles, were similar to grain N concentrations at N2 and N3 respectively in the no-ley rotation (Fig. 2a; Table S9). In addition, mean gluten concentration in winter wheat across cycles (cycle 7 and 8) and N rates was one percentage unit greater in the rotation with grass-legume than in the no-ley rotation; unaffected by N rate (Table 3). The gluten concentration also increased with N rate (Table 3) and was correlated with grain N concentration (R^2=0.96). The greatest concentration of grain N in spring barley was recorded in the no-ley rotation at N3. At other N rates, grain N in spring barley was similar in all rotations (Fig. 2b).

The effect of N rate on oilseed rape oil concentration and cereal grain N concentrations differed between cycles (Table 3; Table S10). The difference in oil concentration between N rates was larger in cycle 9 than in cycle 8 (Fig. 3a).Winter wheat grain N concentration was generally higher and responded more to higher N rates in later cycles than in the three first cycles (Fig. 3b). For spring barley, a significant effect of N rate on grain N concentration across rotations was only recorded in early cycles (Fig. 3d; Table S10).

3.3. Soil carbon and nitrogen

In topsoil (0–20 cm), mean soil C concentration across N rates and cycles was around 0.15 % units higher with ley in the rotation than

Table 2
Results of fixed effect tests in oilseed rape, winter wheat, spring oats and spring barley grain yield (JMP pro16) across three long-term experiments, each with eight six-year cycles of data, in terms of nominator and denominator (DEN) degrees of freedom (DF) and P-value per response variable in experiments according to Table 1. Some cycles contain missing yield data and differences in DFDen between variables are results of missing values.

	Oilseed rape			Winter wheat			Spring oats			Spring barley		
Treatment	DF	DFDen	P-value	DF	DFDen	P-value	DF	DFDen	P-value	DF	DFDen	P-value
Rotation	2	4	0.015	2	4	0.001	2	4	< 0.001	2	4	0.192
N rate	3	6	< 0.001	3	6	< 0.001	3	6	< 0.001	3	6	< 0.001
Rotation x N rate	6	11	< 0.001	6	13	< 0.001	6	12	< 0.001	6	12	< 0.001
Cycle	7	392	< 0.001	7	510	< 0.001	7	354	< 0.001	7	478	< 0.001
Rotation x Cycle	14	1210	0.401	14	1325	0.717	14	1345	0.988	14	1314	0.672
N-rate x Cycle	21	1209	0.729	21	1324	< 0.001	21	1344	< 0.001	21	1313	0.021
Rotation x N rate x Cycle	42	1202	1	42	1328	1	42	1345	1	42	1314	0.999

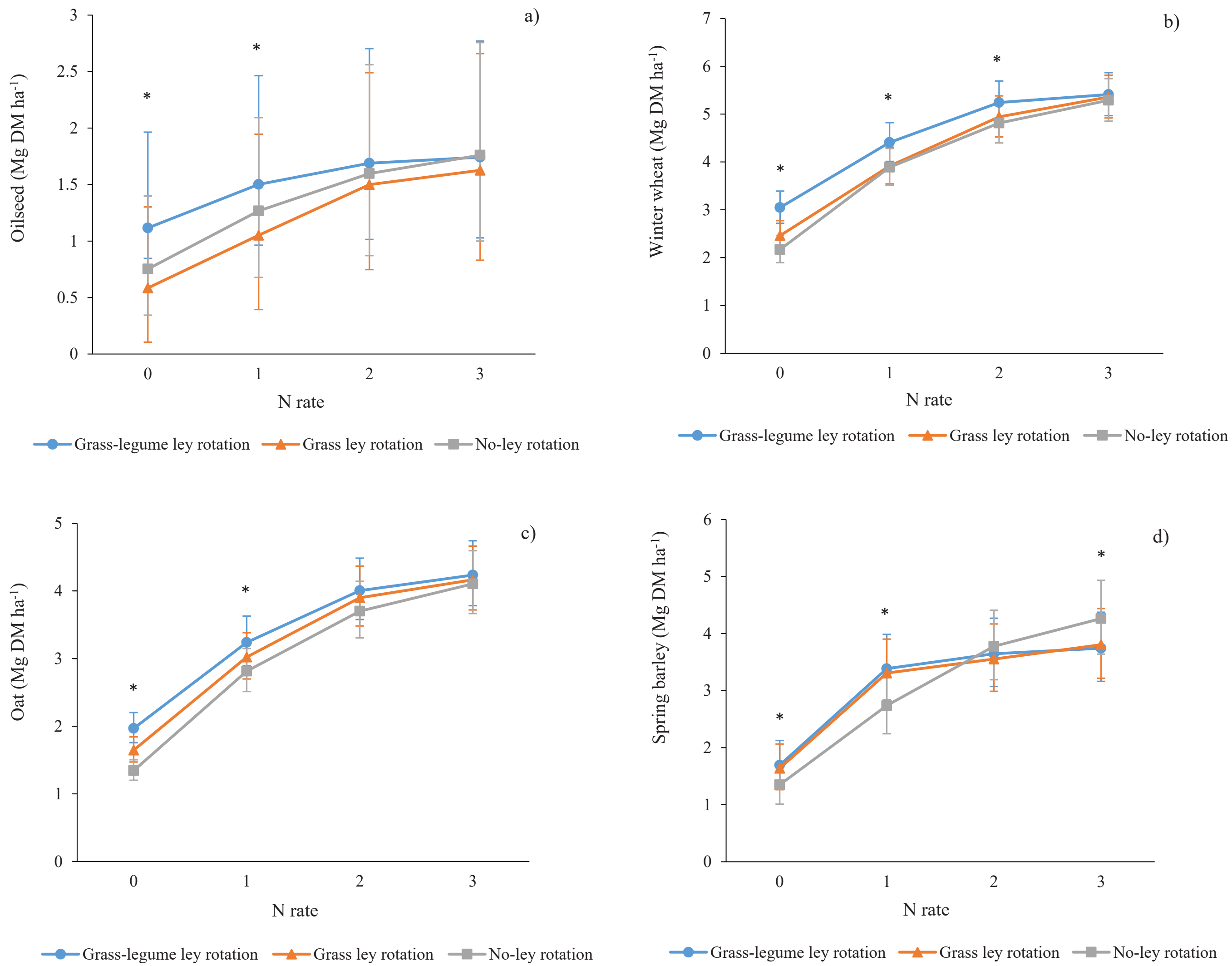

Fig. 1. Arable crop seed and grain dry matter yields, averaged across sites (see Table 1), 8 cycles of six-year crop rotations, in different rotations per N fertilisation rate (Table 1) of (a) oilseed rape grown as the first crop, (b) winter wheat as the second crop, (c) spring oat as the third crop and (d) spring barley as the fourth crop after two years of grass ley or mixed grass-legume ley, or spring wheat and fallow. The spring barley was under-sown in the ley rotations and thus N rates were adapted such that N1-N3 were all fertilized with 60 kg N ha^{-1}, while the N-rate in the no-ley rotation increased by 40 kg N ha^{-1} over the treatment sequence. Stars (*) indicate where yields differed significantly between rotations at specific N rate. Error bars indicate confidence intervals.

Table 3
Mean concentration (%) of oil in oilseed rape, N in winter wheat grains, gluten in winter wheat grains, N in spring oat and spring barley grains by rotation and N rate across sites and cycles (see Table 1). P-values show the effect of rotation, N rate, rotation x N rate and N rate x cycle, and SE is the model-based standard error per response variable. Oilseed rape oil concentration and winter wheat gluten analysis were not collected in cycles 2–7. Cycle 5 is excluded from the winter wheat and spring barley grain N analysis due to missing data from one site.

	Oilseed rape (n = 138)		Winter wheat (n = 361)		Winter wheat (n = 234)		Spring oats (n = 309)		Spring barley (n = 336)	
Treatment	Oil	P-value	Grain N	P-value	Gluten	P-value	Grain N	P-value	Grain N	P-value
Rotation		0.016		0.018		0.017		0.557		0.117
Grass-legume ley	48.7^{A}		1.51^{A}		21.7^{A}		1.43		1.38	
Grass ley	49.6^{B}		1.49^{AB}		21.1^{AB}		1.44		1.37	
No-ley	49.9^{B}		1.46^{B}		20.6^{B}		1.43		1.41	
SE	1.770		0.030		1.886		0.018		0.033	
N rate		0.006		< 0.001		< 0.001		< 0.001		< 0.001
N0	50.6^{A}		1.34^{A}		17.6^{A}		1.39^{A}		NA	
N1	50.3^{A}		1.35^{A}		18.3^{A}		1.35^{A}		NA	
N2	49.0^{B}		1.54^{B}		21.9^{B}		1.45^{B}		NA	
N3	47.7^{C}		1.75^{C}		26.7^{C}		1.55^{C}		NA	
SE	1.771		0.033		1.892		0.018			
Rotation x N rate		0.157		0.020		0.232		0.461		0.002
Cycle		1.103		< 0.001		0.471		< 0.001		< 0.001
Rotation x Cycle		0.829		0.923		0.709		0.715		0.880
N rate x Cycle		0.004		< 0.001		0.693		< 0.001		< 0.001
Rotation x N rate x Cycle		0.511		1.000		0.885		1.000		1.000

Values within columns with different superscript letters are significantly different (P < 0.05, Tukey HSD). NA = not applicable since N rates were different in the spring barley (Table 1).

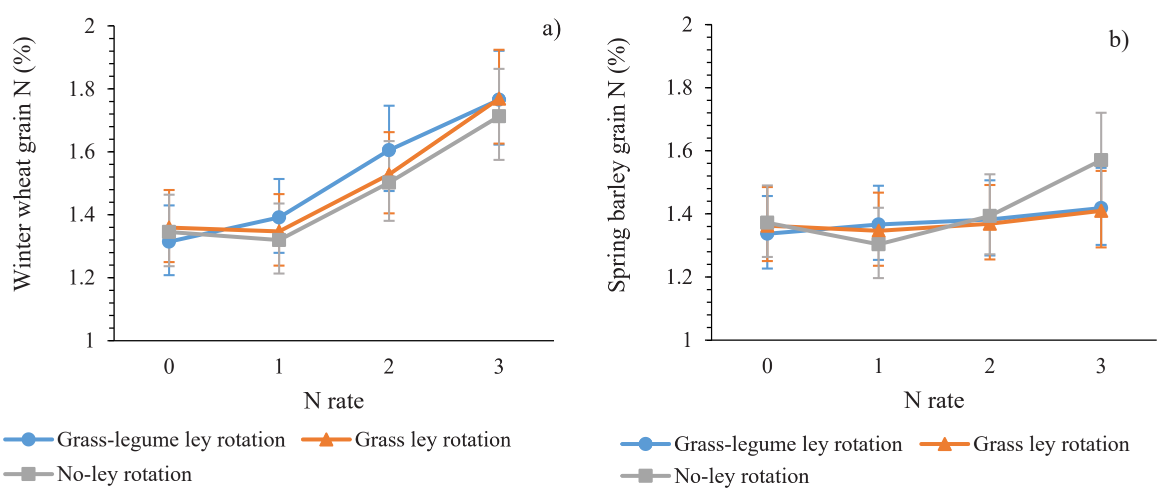

Fig. 2. Grain N concentration in (a) winter wheat grown as the second crop after two years of ley (in the mixed grass-legume ley and grass ley rotations) or spring wheat and fallow (in the no-ley rotation) and (b) grain N concentration in spring barley grown in the fourth year after ley (in the ley rotations) and after spring wheat (in the no-ley rotation), per rotation and N rate average across cycles of full six-year crop rotations. Cycle 4 is excluded due to missing data. Error bars indicate confidence intervals.

without ley (Table 4). The higher N rate (N3) also led to topsoil C concentrations that were about 0.07 % units higher than in N1 (across rotations and cycles) (Table 4). The topsoil C concentration changed differently between cycles depending on rotation and N rate, causing a three-way interaction (Table 4; Table S11). With ley in the rotation, topsoil C did not decrease significantly at any of the N rates (Fig. 4; Table S12). In the no-ley rotation at N1, the topsoil C concentrations in cycle 8 and 9 were about 0.2 % lower than in cycle 2 (Fig. 4), whereas at N3, the topsoil C concentration remained stable. The large variation in topsoil C concentration is mainly due to the large differences in average concentration between sites (being used as replicates) and are presented in Fig. S3.

The effect of leys and N rates on topsoil N concentration were similar to those on topsoil C. With grass-legume or grass ley in the rotation, mean topsoil N across N rate and cycles was about 0.014 % units higher than without ley (Table 4). Higher topsoil N was also recorded at N3, where average N concentration, across rotations and cycles, was 0.007 % units higher than at N1 (Table 4). Topsoil N decreased by 0.015 % units across rotations and N rates between cycle 2–3 and cycle 8–9 ((P < 0.001; Table S16). Mean C/N ratio, across cycles, was higher in the rotations with grass ley than in the rotations with grass-legume ley or no ley, irrespective of N rate and cycle (Table 4).

3.4. Ley yield and N concentration

The yield of the grass-legume ley was on average 6.06 Mg DM ha^{-1} and similar at all N rates (Fig. 5a). The grass ley yield increased with N applied (Table S14, Fig. 5a). At N0, the grass-legume ley yielded 3.30 Mg DM ha^{-1} more than the grass ley (Fig. 5b), this was also the N rate where the grass-legume ley resulted in the greatest increase of arable crop grain yields (Fig. 2). Yields of both ley types were similar in the first and last cycle and remained stable throughout, except for a peak in cycle 7 reaching approximately 9.00 Mg DM ha^{-1} and 7.00 Mg DM ha^{-1} in the grass-legume and grass ley, respectively (Fig. 5b). The yield trends of the two ley types were similar in most years.

Nitrogen concentration in the grass-legume ley was about 2.80 %

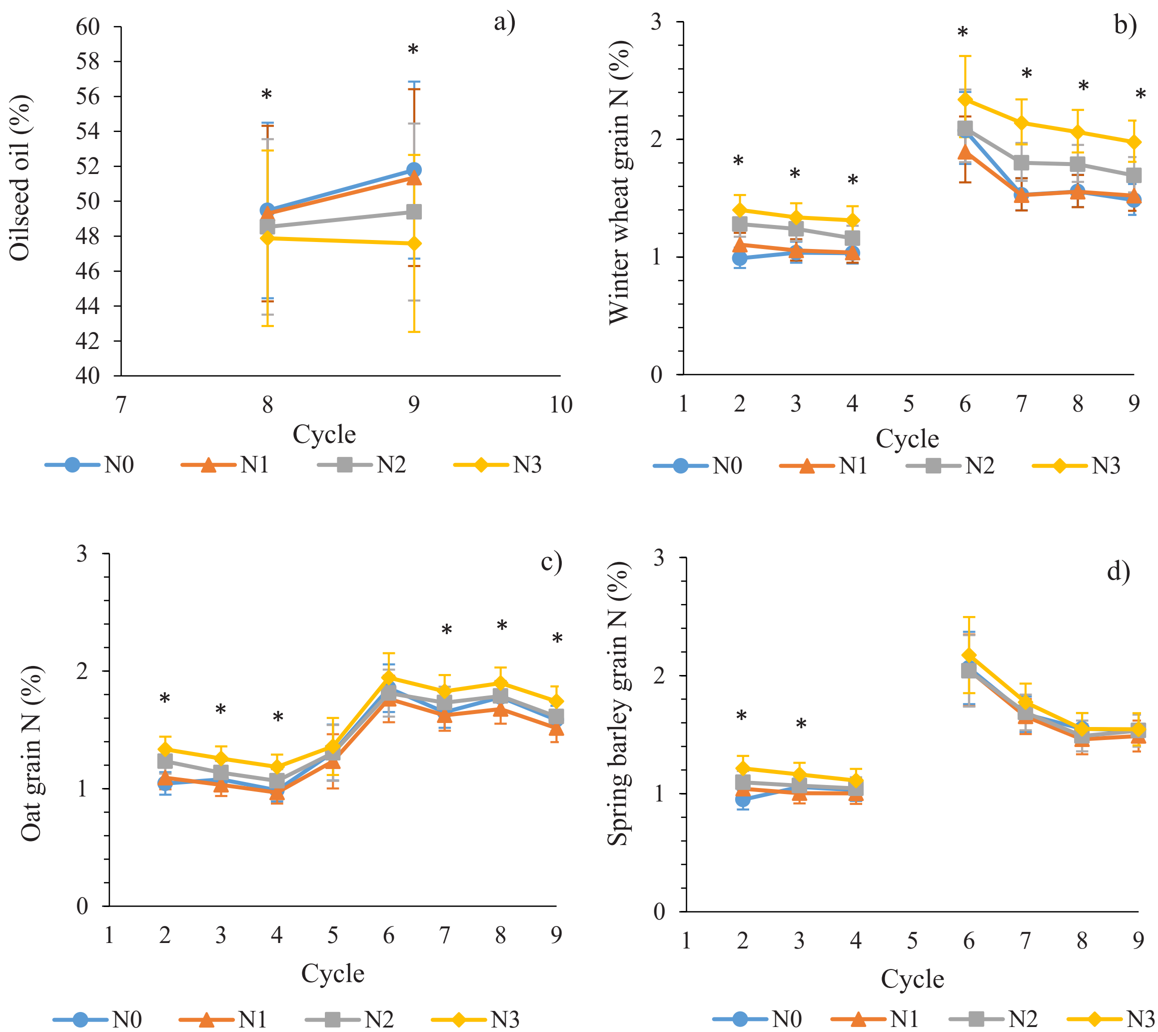

Fig. 3. Trends in oilseed rape oil and grain N concentration averaged over rotations per N rate. (a) Oil in oilseed rape grown as the first crop, (b) grain N in winter wheat as the second crop, (c) grain N in spring oats as the third crop and (d) grain N in spring barley grown the fourth year after two years of grass- or mixed grass-legume ley or spring wheat and fallow. Cycles 1–6 are excluded from the analysis on oil concentration in oilseed rape, cycle 5 from the winter wheat grain N analysis and the spring barley grain N analysis, due to missing data. Stars (*) indicate where yields differed significantly between N rates in specific crop rotation cycles. Error bars indicate confidence intervals.

regardless of N rate, while in the grass ley it increased by about 0.50 % units from N2 to N3, approaching the N concentration of the grass-legume ley (Fig. 5b, Table S15). The N concentration of the ley yield also differed between cycles (Table S14), increasing over time in both rotations from 1.81 % to 1.86 % in cycle 2–5–2.99–3.21 % in cycle 8–9 (Table S16).

4. Discussion

4.1. Arable crop yields and seed and grain quality

The general increasing yields and associated grain N effect of rotational leys on arable crop yields, with greater positive effect when legumes were included, support hypothesis H1. The effect of legumes decreased with increased N fertiliser rates. At the highest N rate, the effect was no longer significant (Fig. 1) thus H3 stating that N fertilisers reduce the effect of leys and legumes on following crop yield was supported. A greater effect of legumes at low N rates (Fig. 5) was expected due to inputs of N to the system through biological N_2 fixation by the legumes (Peoples et al., 2009) and ability to produce biomass at low N rates. Other potential benefits that may be obtained with rotational leys, such as improved soil physical conditions (Berdeni et al., 2021; Puerta et al., 2018) and reduced dependence on pesticides (Lechenet et al., 2016), were not investigated in this study. However, since the positive effect of leys on arable crops yields was not visible at higher N rates, the N rich legume residues with low C/N ratio and potentially high mineralisation rates (Chen et al., 2014) were likely to be the main driver of greater yields at low N rates. The positive effect of leguminous ley on arable crop yields was greatest in the first two years after ley (Fig. 1). In addition, later in the rotation, yields were more similar in both ley rotations at low N rates, but still greater compared to the no-ley rotation potentially due to greater inputs of organic matter (Bolinder et al., 2012) and higher biological activity (Albizua et al., 2015). Contrary to hypothesis H4, the effect of ley did not increase over rotation cycles, reflecting the small changes in soil C and N concentrations over time, and thus H4 was rejected. This further indicates that it was mainly short-term effect of the leys, such as greater production of biomass with low C/N ratio and its mineralisation, that increased the arable yields in the rotation. In the grass-legume ley rotation at N0 and N1, increased yields persisted throughout the arable crop phase of the rotation, although the effect declined with years after ley incorporation. Most commonly only the immediate pre-crop effects of leys are studied although Nevens and Reheul (2002) looked at the three-year effect of three years of grass ley, grazed after cutting, on subsequent silage maize

Table 4
Mean topsoil carbon (C) concentration (%) (across nitrogen (N) rates and cycles) per rotation and mean topsoil C (%) (across rotations and cycles) per N rate; mean topsoil N concentrations (%) (across N rates and cycles) per rotation and mean topsoil N (%) (across rotations and cycles) per N rate; mean carbon/nitrogen (C/N) ratio (across N rates and cycles) per rotation and mean C/N ratio (across rotations and cycles) per N rate, P-values for each response variable on the effect of rotation, N rate and rotation x N rate, rotation x N rate x cycle and model-based standard error (SE).

Treatment	Topsoil C	P-value	Topsoil N	P-value	Topsoil C/N ratio	P-value
Rotation		< 0.001		< 0.001		< 0.001
Grass-legume ley	1.85 [A]		0.173 [A]		10.6[B]	
Grass ley	1.84 [A]		0.168 [A]		10.9 [A]	
No ley	1.69[B]		0.157[B]		10.7[B]	
SE	0.229		0.0166		0.033	
N rate		0.013		0.003		0.348
N1	1.76 [A]		0.16 [A]		10.8	
N3	1.83[B]		0.17[B]		10.7	
SE	0.23		0.017		0.330	
Rotation x N rate		0.619		0.801		0.755
Rotation x N rate x Cycle		0.035		0.363		0.341

Values within columns with different superscript letters are significantly different ($P < 0.05$, Tukey HSD).

and fodder beat and likewise found a positive yield effect that decreased with years after ley.

Rotational ley, although more markedly when it included legumes, had a positive impact on grain N and gluten concentrations in winter wheat (Table 3). Less N fertiliser was required to obtain similar grain N concentrations, probably due to more available N from legume residues, than in the no-ley rotation. Regarding gluten, the concentration was higher in the grass-legume ley rotation regardless of N rate, whereas winter wheat grain N and gluten concentration in the grass ley rotation were intermediate and not significantly different from either rotation (Table 3). The effect of grass-legume ley persisted at all N rates, especially for the gluten concentration and thus H3 was rejected. Higher gluten concentration with legumes in the rotation suggests N availability and plant uptake at later maturity stages of winter wheat, which favour gluten formation (Xue et al., 2016). Increased availability of N and its timing seemed to be the main driver for the improved winter wheat grain quality in the grass-legume ley rotation. Poulton et al. (2023) detected increased grain protein concentrations in spring wheat after grass-legume ley as compared to grass only ley while Eriksen et al. (2006) found similar benefits on gluten concentration of both grass- and grass-clover ley with an increased effect with longer leys. No increasing effect of ley on arable grain quality was seen over time, contradicting hypothesis H4. This further supports the idea that the greater amount of fresh organic matter caused both yield and quality benefits of ley, which may be attained rapidly.

The lower oil concentration in rapeseed grown after the grass-legume ley may be explained by the higher topsoil N concentration in this rotation (Table 4) and supported previous evidence of the negative impact of N fertiliser on oil concentration (Tian et al., 2020) (Table 3). The greater spring barley grain yield and N concentration at N3 in the no-ley rotation can be considered as an N fertiliser effect (Table 1). Generally, seed and grain yields did not increase during the 48-year period studied, despite use of new varieties with greater yield potentials in later years (e.g. Peltonen-Sainio et al., 2009).

Cereal protein concentrations were higher in later cycles (cycle 6–9) than in earlier cycles (Fig. 3). Modern varieties have been shown to use N more efficiently (Fernando and Sparkes, 2020), but since old varieties generally produce a higher protein concentration than modern varieties under low N conditions (Baresel et al., 2005), more efficient N use of new varieties is not a likely explanation of the higher protein concentrations in later cycles. Instead, the change in N fertiliser composition to contain 50 % ammonium N, compared with nitrate N only in earlier cycles is a more likely explanation of at least some of the increase in protein. The application time in relation to crop development was maintained. As N is released more slowly from ammonium than nitrate and therefore less likely to be lost, N would have been available at later crop development stages resulting in increased grain protein content (Fuertes-Mendizábal et al., 2012). The later release of N from ammonium fertiliser could also help to explain the lack of yield increase in later cycles despite modern cultivars, since N availability early in the season has more impact on yields (e.g. Efretuei et al., 2016; Zebarth et al., 2007). There was no increasing effect of ley in the rotation on seed oil concentration, so hypothesis H4 is not supported. However, less data was available for oilseeds and thus a comparison was only possible between the last two cycles.

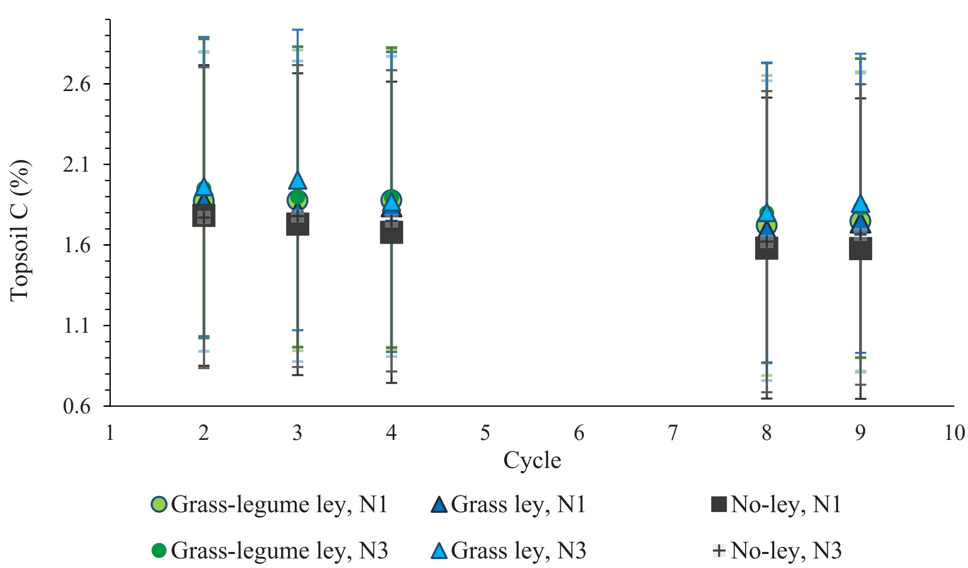

Fig. 4. Mean topsoil carbon (C) concentrations (%) per rotation and nitrogen (N) rate over cycles. Error bars indicate confidence intervals. Number of samples per cycle was 102 or 108. Cycles 5–7 are excluded because samples were not taken in all years during these cycles. Topsoil C concentration in the no-ley rotation at N rate N1was significantly lower in cycle 8 and 9 compared to cycle 2 ($P < 0.05$, Tukey HSD).

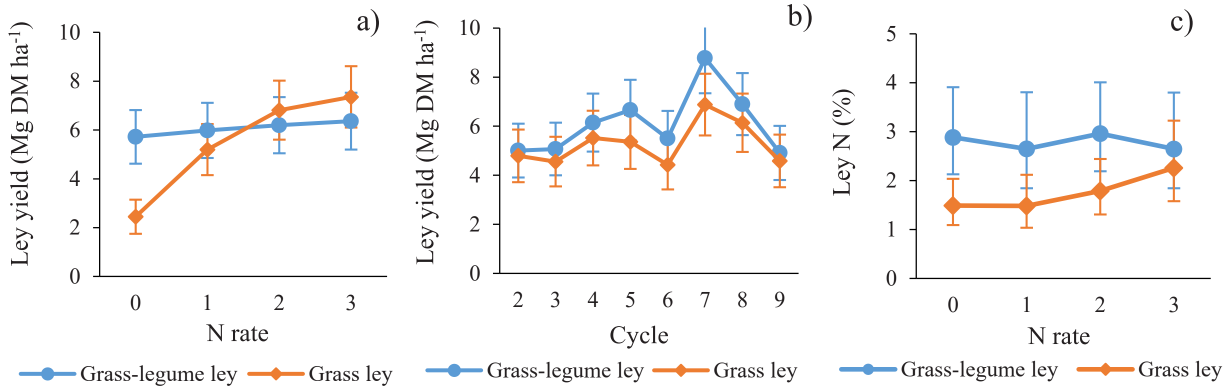

Fig. 5. Ley total dry matter yield and nitrogen (N) concentration in the grass-legume ley and grass ley. (a) Ley biomass yield per N rate (N0-N3) across cycles, (b) ley biomass yield per cycle of full six-year crop rotations and (c) ley N concentration per N rate and ley type across cycles. For ley N concentration, cycles 6–7 are excluded from the analysis due to missing data. Error bars indicate confidence intervals.

4.2. Soil carbon and nitrogen

Mean concentrations of topsoil C and total N (across N rates and cycles) were higher in the rotations with ley than without ley (Table 4), supporting hypothesis H2 that there would be a positive impact of ley on topsoil C. This is in line with the site-specific results on life cycle environmental performance of the three crop rotations (Nilsson et al., 2023). Current results also concur with previous findings from the same LTE by Persson et al. (2008) reported twice as much C inputs in the rotations with ley using a modelling approach, as well with results from other LTEs (e.g. Jarvis et al., 2017; Jensen et al., 2022). Higher mean C and N levels in the ley rotations may reflect longer periods of active growth and greater root biomass production by ley crops compared with arable crops, resulting in more SOM inputs in rotations with ley (Bolinder et al., 2007; Börjesson et al., 2018). Smaller topsoil C mineralisation due to absence of soil tillage (Meurer et al., 2018) during the ley phase may also have contributed to the higher soil C concentration in the ley rotations. The one-year fallow in the no-ley rotation may also affect the comparison of the three rotations regarding topsoil C concentration due to a smaller biomass of the spontaneous vegetation than that of the ley crops. Moreover, fallow is not as common in farming systems today as at the time of the LTE establishment. More efficient herbicides mean that fallow is not required to control weeds and earlier maturation of cereals means winter oilseed rape can be established after a cereal crop rather than waiting until the following year. Therefore, this crop rotation could be redesigned to maintain its contemporary relevance. This may include replacing the fallow with a grain legume crop or other spring-sown crop that is suitable to grow after spring wheat and before a winter crop.

During the 48-year study period, topsoil C in the ley rotations did not change significantly at either N rate (N1, N3). This implies that the current system was similar, in terms of C input, to the cropping system preceding the experiment and that the differences in C concentrations between rotations was achieved during the first experimental cycle. However, in the grass ley rotation, results were less clear with indications that the topsoil C started to decrease over time in N1 (Table S12). Without ley in the rotation, the concentration of topsoil C decreased over time at N1, being lower in cycles 8 and 9 than in cycle 2 (Table S12). However, at N3, topsoil C did not differ significantly between cycles. These findings are in line with Persson et al. (2008) who showed a larger impact of N rate on C inputs in the grass-ley and no-ley rotation. It suggests that high N application, at an environmental cost (Nilsson et al., 2023), is a way to maintain soil C with only arable crops in the rotation, as shown previously in long-term studies (e.g. Melero et al., 2011; Kätterer et al., 2014; Ghafoor et al., 2017). Availability of plant nutrients (N, P and sulphur (S)) has been shown to improve the transformation of C-rich residues such as cereal straw into stable forms of SOM by increasing the net humification efficiency (Kirkby et al., 2013). The observed maintenance of topsoil C concentrations at the higher N rate (N3) may thus be a result of more nutrients being available for this transformation at N3, besides increased yields and subsequent increased organic matter inputs.

One reason for the maintenance, rather than the hypothesised increase, of topsoil C in the ley rotations (H4), is possibly that the previous land use with mixed farming included both ley and animal manure, as was common practice before specialisation started in the 1950s and 1960s. The rotation with ley in the experiment may thus not constitute an increase in ley proportion and C inputs compared with the previous land use. Moreover, when the LTE was established, drainage was improved and the intensity and frequency of tillage increased. In other LTEs, the initial soil C concentration has been found to have a profound impact on whether it is possible to maintain soil C levels, with higher initial concentrations being more difficult to maintain (Bolinder et al., 2010). In our study, the relatively large proportion of arable crops in the rotations probably also prevented a build-up of organic matter explaining why no increasing effect of leys on soil C and N over rotation cycles was observed, contradicting hypothesis H4. To obtain an increase in soil C, the literature suggests that a larger proportion of perennial ley in the rotation is needed to compensate for loss of soil C during the arable crop years (Bolinder et al., 2010). In experiments in northern Sweden with initial SOC concentration of 2.8–4.8 %, even four years of ley in a six-year rotation and addition of manure were insufficient to maintain or increase SOC (Bolinder et al., 2010). However, on a sandy soil in Denmark with initial SOC concentration of 1.6 %, including two years of ley in a six-year rotation gave an increase in SOC over a 30-year period but in that case, manure was also applied (Jensen et al., 2022). Small changes in soil C have been recorded with ley duration exceeding three years (Hu et al., 2024). The soils in our study had similar topsoil C concentrations (1.4 %-1.7 %) and proportion of ley as in Jensen et al. (2022), the difference was that their trials included manure and these did not. We found that topsoil C was maintained over 48 years across the three study sites while Jensen et al. (2022) recorded an increase of 5.0 Mg C ha^{-1} reached 20 years after inclusion of ley.

Topsoil total-N showed a similar decrease across cycles in all rotations and N rates (Table S5), showing greater depletion of N than soil C, although their changes often are coupled (Zinn et al., 2018). The trend towards higher topsoil N concentration in the grass-legume rotation (Table 4) probably explains the smaller mean C/N in the rotation with grass-legume ley than in the grass ley rotation, as hypothesised in H2. Greater N content in grass-legume ley residues compared with grass ley residues has been well documented (e.g. Chen et al., 2014; Nyfeler et al., 2024). No effect of higher N rates on C/N ratio was observed in this study, in line with previous studies (e.g. Persson and Kirchmann, 1994), perhaps due to the coupled increase in topsoil C.

5. Conclusions

The effects of rotational leys without manure application on arable crop yield quantity and quality and on soil C and N were investigated over 48 years using LTE data from three experimental sites. Results showed that at low N rates, legume-based leys contributed to greater arable crop yields up to four years after ley incorporation yet with a decline in effect with years after ley. Legumes in the ley also reduced the amount of N fertiliser needed to obtain given grain N and gluten concentrations in winter wheat when compared to the no-ley rotation. Rotational leys (grass and grass-legume ley) complemented low rates of N fertiliser in maintaining topsoil C, although inclusion of legumes in the ley increased this effect and additionally reduced the topsoil C/N ratio. At high rates of N fertiliser (N3) the positive impact of ley was masked and similar yields and topsoil C were maintained regardless of rotation type. Thus, the positive impact of ley appeared to result from the effects of increased biomass inputs and high mineralisation rates due to low C/N ratio, which was achieved in the short term after ley incorporation. Adding short-term grass-legume leys to crop rotations has the potential to maintain crop productivity and topsoil C at low N fertiliser rates and without manure application on stockless farms. Rotational leys could thus be used as a measure towards sustainable agriculture systems with less dependency on fossil fuel-derived inputs.

CRediT authorship contribution statement

Fatima F. El Khosht: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Göran Bergkvist:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **A. Sigrun Dahlin:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Christine A. Watson:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Johannes Forkman:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Johan Nilsson:** Writing – review & editing. **Ingrid Öborn:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

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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.fcr.2025.109835.

Data Availability

Data will be made available on request.

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