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Effect of short-term perennial leys on life cycle environmental performance of cropping systems: An assessment based on data from a long-term field experiment

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ABSTRACT

Modern agriculture's dependence on the intensive use of inputs, such as chemical fertiliser and pesticides, leads to high environmental impacts and, possibly, vulnerability in food security, since most of these inputs are imported from other countries. This calls for more sustainable and resilient agricultural practices. Diversification of crop rotations, e.g. by including perennial leys, enhances provision of ecosystem services, leading to healthier crops and increased yields. Perennial crops also increase soil organic carbon (SOC) stocks, which is interesting from a global warming mitigation perspective. In addition, legume-rich leys can utilise atmospheric nitrogen (N) through symbiotic association with N_2-fixing bacteria. However, few studies have evaluated the effects of short-term perennial leys in rotation on cropping system performance over long periods and under different conditions. In this study, we used data from three sites in a long-term experiment in Sweden (initiated in the 1960 s), in combination with Life Cycle Assessment methodology, to assess the environmental and yield effect of including ley in crop rotations. Two N fertiliser regimes (High, Low) in combination with three six-year crop rotations, consisting of either i) two-year mixed grass-legume ley, ii) two-year pure grass ley or iii) annual crops without ley, were compared. Environmental impacts (climate impact, energy resource depletion, eutrophication potential) of the different combinations were quantified per kg harvested crop (expressed in cereal units, CU) and per hectare. The lowest environmental impact, at all sites, was found for the crop rotation with two-year mixed ley under the Low N regime. On average, this combination resulted in 329 g lower GHG emissions per kg CU than the crop rotation without ley and Low N, primarily due to lower input of chemical N fertiliser, which reduced the impact from fertiliser production and soil N_2O emissions. Comparison of mean SOC change over the study period revealed reduced SOC stocks for all rotations and all sites, especially in the rotation without ley. Therefore, including short-term perennial leys, especially leys containing legume species, in crop rotations can be a useful tool in meeting policy targets on reducing the environmental impacts of agriculture, and in reducing the dependence on purchased agricultural commodities. However, despite the potential benefits of rotational leys, the market demand for the produced ley biomass may be insufficient. Hence, incentives to increase demand are necessary to promote large-scale adoption, for example, for use in bioenergy production and feed.

1. Introduction

Following the *Green Revolution*, chemical fertilisers and biocides have increased food production and helped sustain the growing global population (MacLaren et al., 2022). They have also allowed farmers to specialise in a few crops and abandon the diverse crop rotations that characterised European agriculture since the introduction of perennial grass-legume crops during the 19th century (Mudgal et al., 2010). However, intensive use of inputs in agriculture is known to be directly linked to environmental impacts such as global warming, eutrophication, biodiversity loss and extensive energy use (Campbell et al., 2017; Foley et al., 2011; Stoate et al., 2001; Tang et al., 2021). Reducing the dependence on purchased input commodities could increase cost-efficiency, reduce the environmental impact (Tidåker et al., 2014,

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2016) and enhance regional security of the supply of agricultural goods. The importance of the latter has recently been demonstrated with the invasion of Ukraine, which is causing deep geopolitical disruption in the European area (World Bank, 2022). The resulting high price fluctuations in agricultural input commodities have brought new challenges for farmers, who can no longer rely on business-as-usual. Therefore, new strategies for building resilience to current and future shocks and stresses must be developed, while still avoiding further aggravating upcoming challenges such as global warming.

One potential strategy for reducing the current dependence on agricultural inputs is to promote ecosystem services by re-introducing more diversified crop rotations (MacLaren et al., 2022; Nemecek et al., 2015; Tamburini et al., 2020). Research has shown that diversification of crop rotations can increase nutrient delivery, keep crops healthier, increase yields and reduce yield losses due to weather extremes (Bergkvist and Båth, 2015; Bowles et al., 2020; Gaudin et al., 2015). In particular, the inclusion of perennial crops, such as temporary leys in cropping systems with a high proportion of cereals, has been shown to reduce dedicated pests, with distance in time and space having a large influence on pest occurrence (Kirkegaard et al., 2008). If properly managed, it can also mitigate nitrogen (N) leaching, leading to reduced eutrophication (Larsson et al., 2005).

A key challenge for resilient agricultural systems is to find a sustainable and reliable supply of N. Grass-legume mixtures can provide substantial amounts of N, up to 500 kg N ha^{-1}, with low environmental burden via biological N-fixation through symbiotic association with N_2-fixing bacteria (Carlsson and Huss-Danell, 2003; Peoples et al., 2019). Inclusion of legumes in leys can, therefore, be used to reduce dependence on synthetic N fertiliser (Ledgard and Steele, 1992), a highly resource-intensive agricultural input that causes environmental impacts from its production and use (Galloway et al., 2003; IEA, 2021; Jensen et al., 2012; Tian et al., 2019). However, diversification measures to promote ecosystem services may have differing effects depending on where and how they are adopted, and they can reduce obtainable yield (Tamburini et al., 2020). In addition, economic forces tend to favour cost-efficient specialist cropping systems over the more long-term benefits of diversification (Reckling et al., 2016; Zander et al., 2016).

Since the beginning of agriculture, soils have been a source of atmospheric carbon dioxide (CO_2) through depletion of soil organic carbon (SOC) (Lal, 2010). The rate of depletion has been accelerated by the specialisation of arable agriculture, with systems dominated by annual crops. Temporary leys have the potential to sequester considerable amounts of C in agricultural soils by shifting the SOC equilibrium to a higher level (Börjesson et al., 2018; Englund et al., 2023; Poeplau et al., 2015). High plant diversity within the ley mixture itself may also be a driver of SOC sequestration, by promoting belowground SOC input and an increased contribution from microbial necromass (Bai and Cotrufo, 2022; Kagiya et al., 2019). This SOC sequestration potential, in combination with low associated costs, has generated interest in using agricultural soils as a negative emissions approach to remove CO_2 from the atmosphere with the aim of reducing global warming (Minx et al., 2018; Smith et al., 2016). Within the European Union (EU) alone, SOC sequestration potential is estimated to be between 9 (Frank et al., 2015) and 58 million ton CO_2 per year (Lugato et al., 2014). However, SOC sequestration rate is highly dependent on, for example, soil properties, climate, farming system and current soil C content (Bolinder et al., 2020; Kätterer et al., 2012). This makes it difficult to predict the soil C effect of various cropping systems. Studies investigating soil C effects within agricultural systems often rely on modelling because of the protracted nature of soil C changes and lack of available measured data (Goglio et al., 2015; Nilsson et al., 2020a, 2020b; Poeplau et al., 2015). However, the use of models entails significant uncertainty, and the underlying theory has been challenged (Dungait et al., 2012; Schmidt et al., 2011). This uncertainty reduces the overall effectiveness of models in accounting for soil C changes (Stockmann et al., 2013), leading Goglio et al. (2015) to conclude that field data should be used where possible.

Many of the reported benefits of ley cultivation are based on field studies involving livestock, where manure is used as fertiliser on cultivated leys (Bolinder et al., 2010; Jarvis et al., 2017; Poeplau et al., 2015). This set-up makes it difficult to assess the effect of the ley itself and does not distinguish the effect of N-fixing legumes. In fact, there are currently few long-term field experiments where the effects of ley and manure on crop yields and soil C can be separated (De Los Rios et al., 2022). However, a long-term field experiment is being conducted at three sites in Sweden where crop rotations with and without perennial leys are being compared and where only mineral fertilisers have been used since the early 1970s (Persson et al., 2008).

It is important to apply a systems perspective when evaluating the environmental impacts of crop cultivation (Henryson et al., 2019). Life Cycle Assessment (LCA) is frequently used to assess the environmental impact of agricultural products and is accepted by policymakers in both public and private organisations (Brandão et al., 2022). In LCA, emissions and resources used during the whole (cradle-to-grave) or parts (e.g. cradle-to-gate) of the life cycle of a product or process are considered (ISO, 2006a, 2006b).

The overall aim of this study was to assess the effect on crop yield and environmental performance of including ley in crop rotations, focusing on the comparison between a pure grass ley and a legume-grass ley mixture at the same levels of N fertiliser application. The analysis was based on data from the long-term field experiment running at three sites in Sweden. Specific objectives were to:

- Quantify the effect of ley in crop rotations on annual crop yield in the rotation, total crop rotation yield, and SOC stock under different fertiliser regimes.
- Compare climate impact, energy resource depletion and eutrophication potential of including grass or grass/legume ley in crop rotations, using LCA methodology.

2. Methods and materials

2.1. Experimental sites and set-up

The study was based on data from the ongoing long-term field experiment at three sites in southern Sweden with different climates and soil properties: Säby (59°49 N; 17°42 E), Lanna (58°20 N; 13°07 E) and Stenstugu (57°36 N/; 8°26 E). The characteristics of these sites, which have been in operation since 1969, 1965 and 1968, respectively, are shown in Table S1. in Supplementary Material (SM). The aim of the experiment is to investigate the long-term effects of including ley in three crop rotations (*Mixed-Ley*, *Grass-Ley*, *No-Ley*) under four different N fertiliser regimes. The three crop rotations consist of six-year rotations with the first four crops in each rotation being identical and the last two being different (Table 1). Data for two of the four N fertiliser levels (the highest and second lowest, referred to here as *High N* and *Low N*) were used in this study, because SOC was only measured in these treatments. Thus in total, data from six treatment combinations (three crop rotations × two N fertiliser levels) at each site were included in the analysis.

At Säby and Lanna, the experiment follows a split-split-plot design with crop rotations and N-levels included as subplots and sub-subplots,

Table 1
The composition of the crop rotations in a long-term Swedish field experiment at three different sites in southern Sweden, which supplied data used in this study to evaluate the environmental effect of rotational leys.

Rotation	Mixed-Ley	Grass-Ley	No-Ley
I	Oilseed crop	Oilseed crop	Oilseed crop
II	Winter wheat	Winter wheat	Winter wheat
III	Oats	Oats	Oats
IV	Barley	Barley	Barley
V	Legume-Grass Ley I	Grass Ley I	Spring wheat
VI	Legume-Grass Ley II	Grass Ley II	Fallow

respectively. At Stenstugu, a split-strip-plot design is used, with crop rotations and N-levels arranged as rows and columns (Fig. S1 in SM). All six crops in each rotation are cultivated each year in neighbouring main plots. Thus, there are as many replicates as there are crops in the rotation. This design means that comparisons between the treatments can only be made over time, because each main plot is in a different position in the rotation. The Mixed-Ley treatment consists of red clover (at Säby and Stenstugu lucerne is also included in the seed mixture) and timothy, while the pure Grass-Ley is a mixture of the grass species meadow fescue and timothy. More information on the study sites and experimental set-up can be found in Persson et al. (2008).

Yield data for each treatment at Säby in the period 1969–2016, Lanna in the period 1965–2012 and Stenstugu in the period 1968–2015 were used to calculate mean yield for the entire crop rotation, and for each crop in the rotation. Mean yield was then used to compare land occupation, i.e. yield per m^2 agricultural land. Soil organic C was measured in the topsoil (0–20 cm) once per rotation (after the oat crop), except between the years 1993 and 2005. Subsoil samples (40–60 cm) were also collected and analysed. However, as no significant changes were observed during the assessed period for any of the treatments, the subsoil data was not incorporated into the LCA.

To estimate mean SOC stock change per rotation, a random intercept and slope model that takes into account the SOC change for each plot, and then calculates mean SOC change for each site and treatment (Zuur et al., 2009), was applied to the collected data (see regression plots in (see regression plots in Fig. S2, S3, and S4 in SM). The data used to assess the change in SOC were collected from the beginning of the field experiment until the most recent samples analysed in 2020. The C content (%) was converted to kg C per ha using the equation:

$$SOC\ \left(\frac{kg\quad C}{ha}\right) = \frac{SOC(\%)}{100} \bullet \rho \bullet V \qquad (1)$$

where SOC is soil organic carbon content, ρ is soil bulk density at each site and V is volume of 1 ha of topsoil (to 20 cm depth). Using the pedotransfer functions for Swedish agricultural soils developed by Kätterer et al. (2006), topsoil density was estimated to be 1.27, 1.31 and 1.52 g cm^{-3} at Säby, Lanna and Stenstugu, respectively.

2.2. Life Cycle Assessment

2.2.1. Goal and scope

LCA methodology was used to quantify and compare the environmental impact, in terms of greenhouse gas (GHG) emissions, energy resource depletion and eutrophication potential. The system boundaries were set from cradle to farm-gate, including the complete crop rotation for *Mixed-Ley*, *Grass-Ley* and *No-Ley* (Fig. 1). Life cycle inventory (LCI) was performed for the following processes:

- Production of fertiliser and pesticides
- Seed cultivation
- Field operations, including fuel production and consumption, and production and maintenance of machinery
- Crop drying,
- Emissions to water (N and phosphorus (P) leaching) and emissions to the atmosphere (nitrous oxide (N_2O), ammonia (NH_3) and nitrogen oxides (NOx))
- SOC changes

An important concept in LCA methodology is the functional unit, which is used as the basis for quantification, i.e. the environmental impact is quantified per functional unit. The functional unit should be chosen with respect to the goals of the study, but it is sometimes not obvious which is most suitable and several can be included in the assessment (Klöpffer and Grahl, 2014). Here, we applied two separate functional units: i) kg harvested cereal units (CU) and ii) ha of agricultural land. The CU concept, which was developed by the German authorities to make agricultural productivity more comparable, converts harvested mass to CU by determining the animal feeding value of each agricultural product and normalising it to the reference crop (barley) (Brankatschk and Finkbeiner, 2014). The animal feeding value is based on the protein, lipid, fibre and carbohydrate content of the crop and the proportion fed to specific animal species (cattle, pigs, poultry and horses) (Brankatschk and Finkbeiner, 2014). The CU can be used in LCA studies to allocate environmental burden between crops in a rotation (Brankatschk and Finkbeiner, 2015; Goglio et al., 2018) and as a functional unit for the entire crop rotation (Henryson et al., 2019; Prechsl et al., 2017).

Assessments were performed for eight full six-year rotations, i.e. in total 48 years. Field operations included for the different crops were based on the average treatment for each site and crop in the study period (Table 2). According to the field experiment design, Legume-Grass Ley I in *Mixed-Ley* was only fertilised once a year, while Grass Ley I in *Grass-Ley* was fertilised twice, before and after the first cut. The second-year leys (Legume-Grass Ley II, Grass Ley II) only had one cut, to allow for oilseed crop seeding time. Application of N fertiliser in *Mixed-Ley* was decided depending on the legume proportion, with a higher percentage of legumes resulting in a lower amount of N fertiliser (or no N fertiliser if

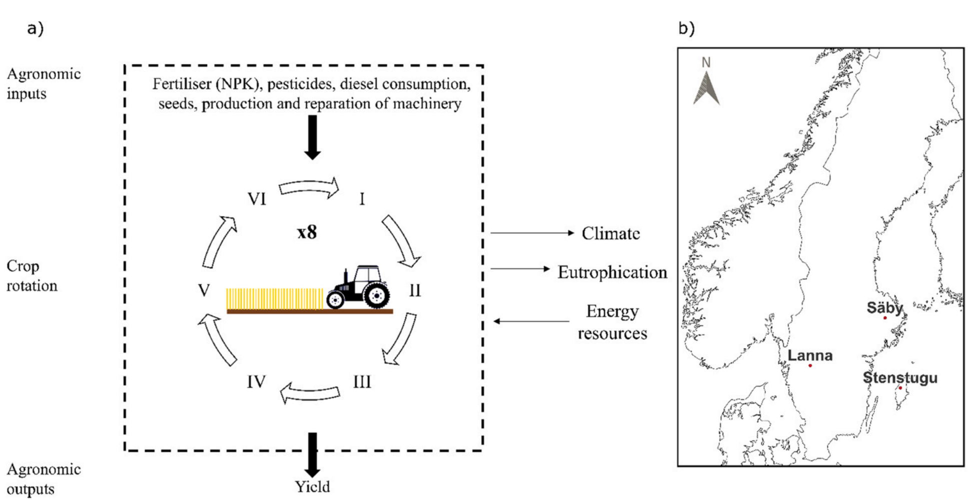

Fig. 1. a) Schematic overview of the system analysed, with assessments performed for eight full six-year rotations, and b) location of the study sites Säby (59°49´N/17°42´E), Lanna (58°20´N/13°07´E) and Stenstugu (57°36´N/18°26´E) in southern Sweden; the background map was generated using the free and open-source software QGIS.

Table 2
Field operations performed in each crop in the Mixed-Ley, Grass-Ley and No-Ley rotations in the long-term field experiment at three sites in southern Sweden.

Operation	Oilseed	Winter wheat	Oats	Barley	Mixed ley I[a]	Mixed ley II[a]	Grass ley I[b]	Grass ley II[b]	Spring wheat	Fallow
Harrowing	3	3	3	3	0	0	0	0	3	0
Fertilisation	1	2	1	1	1	1	2	1	1	0
Sowing	1	1	1	1	1	0	1	0	1	0
Application of pesticides	1	2	1	1	0	0	0	0	1	0
Harvesting	1	1	1	1	0	0	0	0	1	0
Stubble cultivation	1	1	1	1	0	1	0	1	1	0
Mowing	0	0	0	0	2	1	2	1	0	0
Ploughing	1	1	1	1	0	1	0	1	1	1

a) Only in the *Mixed-Ley* rotation. b) Only in the *Grass-Ley* rotation. c) Only in the *No-Ley* rotation.

the legume content was above 50%), based on the assumption that a high biomass would be produced with less N fertiliser if the proportion of legume was high. The mean N application over the evaluated period was utilised in LCA (Table 3). The Fallow in *No-Ley* was left untreated in the autumn after spring wheat harvesting.

2.2.2. Life cycle inventory

Data on yields, SOC content, fertiliser rates and field operations used in LCI were taken from the database for the long-term field experiment. The CU for each crop rotation and site was calculated as:

$$CU = \sum_{i=1}^{n=6} \bar{y}_i \bullet x_i \tag{2}$$

where CU (kg) is total cereal units of the crop rotation at a specific site, $\bar{y}_i$ (kg) is mean yield of crop *i* in all years assessed and x_i is the CU conversion factor for crop *i* (taken from Supplementary Material to Brankatschk and Finkbeiner, 2014) (see Table S2 in SM).

No environmental burden was allocated to the straw, since it was left on the field and was not considered an output from the system assessed. Land occupation was determined by calculating the area required to produce 1 kg CU in each of the treatment combinations.

Data on application rates of N, P and potassium (K) were taken from the field experiment guidelines (Table 3). Since the N application rate in *Mixed-Ley* varied depending on the legume content, the mean N application rate for each site and fertiliser scheme was calculated separately for Legume-Grass Ley I and II.

Emissions and energy use from production of fertiliser and pesticides, and production, maintenance and use of machinery were calculated using data from Ecoinvent (www.ecoinvent.org) (Table 4). Inputs

Table 3
Fertiliser application rate (kg nitrogen/phosphorus/potassium ha^{-1}) in the Low N and High N treatments in the long-term field experiment at three sites in southern Sweden. The values are based on experiment instructions.

Crop	Low N	High N
Oilseed	90/75/143	210/75/143
Winter wheat	45/0/0	135/0/0
Oats	40/0/0	120/0/0
Barley	*/75/293	*/75/293
Legume-Grass Ley I	* */0/0	* */0/0
Legume-Grass Ley II	* */0/0	* */0/0
Grass Ley I	80/0/0	240/0/0
Grass Ley II	45/0/0	135/0/0
Spring wheat	45/0/0	135/0/0
Fallow	0/0/0	0/0/0

[*] Barley was fertilised with 60 kg N ha^{-1} in both *Low N* and *High N* in the rotations with ley (where the ley was undersown in the barley), while in the *No-Ley* rotation the barley received 40 kg N ha^{-1} and 120 kg N ha^{-1} in the *Low N* and *High N* scenario, respectively. * *Amount of N fertiliser in Legume-Grass Ley was based on the legume content. In the *Low N* regime at Säby, Lanna and Stenstugu, mean N application rate was 32, 34 and 29 kg ha^{-1} in Legume-Grass Ley I and 32, 36 and 37 in Legume-Grass Ley II, respectively. In the *High N* regime, it was 89, 101 and 87 in Legume-Grass Ley I and 89, 107 and 112 in Legume-Grass Ley II at Säby, Lanna and Stenstugu, respectively.

Table 4
Data used in Life Cycle Inventory. Ecoinvent data are based on v.3.9 cut-off by classification methodology. Abbreviations denote the geographical resolution of the dataset, where RER = Europe, CH = Switzerland, SE = Sweden and GLO = Global.

Input	LCI dataset
N fertiliser*	Ammonium nitrate production, RER
P fertiliser* *	Triple superphosphate production, RER
K fertiliser* * *	Potassium chloride production, RER,
Pesticides	Pesticide production, unspecified, RER
Ploughing	Tillage, ploughing, CH
Harrowing	Tillage, harrowing, by spring tine harrow, CH
Fertilisation	Fertilising, by broadcaster, CH
Sowing	Sowing, CH
Pesticide application	Application of plant protection product, by field sprayer, CH
Harvesting	Combine harvesting, CH
Stubble cultivation	Tillage, cultivating, chiselling, CH
Mowing	Mowing, by rotary mower, CH
Heavy fuel oil	Heavy fuel oil, burned in refinery furnace, Europe without Switzerland
Electricity	Market for electricity, high voltage, SE
Diesel production	Market for diesel, Europe without Switzerland
Diesel combustion	Diesel, burned in agricultural machinery, GLO

* 33.5% N. * *20% P. * * * 47% K.

of pesticides (herbicides, fungicides and insecticides) were based on national statistics for the specific region and for specific crops for each field (SCB, 2011). The cereals in the crop rotations were assumed to be harvested at 20% dry matter (DM) and then dried to 14%, and the oilseed crop was assumed to be harvested at 15% DM and dried to 9%, based on data from Edström et al. (2005). The demand for heating oil was set to 5.4 MJ per kg of evaporated water and electricity use in the process to 17 kWh per kg grain (Edström et al., 2005). No further processing of the ley biomass was included. To account for seed cultivation, we subtracted the seed rate (6 kg seed per ha for oilseed, 210 kg for winter wheat, 205 kg for oats, 170 kg for barley and 230 kg for spring wheat) from the yield (Ahlgren et al., 2011). The seed rate for the ley crops was set at 24 kg per ha in both *Mixed-Ley* and *Grass-Ley*. Diesel use for producing the ley seeds was assumed to be 19.4 MJ per kg (Prade et al., 2015) and emissions from sowing were based on the Ecoinvent dataset (Table 4).

The calculated mean SOC change (kg ha^{-1}) was used in the LCA model to estimate the average change in SOC per rotation and was converted to CO_2 based on the atomic weight ratio of C to CO_2. Direct soil N_2O emissions were estimated using the IPCC Tier I approach (IPCC, 2019), with the emissions factor for temperate wet climates (0.016 kg N_2O-N kg N^{-1}) and mean change in SOC content per rotation. Indirect N_2O emissions were calculated using the IPCC Tier I approach, where N_2O from volatilised N and N from leaching are both included. Nitrogen leaching was estimated using the farm management tool VERA, described in Aronsson and Torstensson (2004). Phosphorus leaching was estimated using data from Johnsson et al. (2016), who calculated mean leaching rates for 22 regions in Sweden using the ICECREAMDB model. The data used represented leaching and runoff rates for specific crops and soil textures (Johnsson et al., 2016). Emissions factor for N volatilisation at field level was set to 0.033 kg NH_3 and 0.04 kg NO_x per kg applied N fertiliser (EMEP/EEA, 2016).

2.2.3. Life cycle impact assessment

The environmental aspects considered were climate impact, energy resource depletion and eutrophication potential. The climate impact was assessed using GWP_{100}, applying the characterisation factors in Forster et al. (2021). Resource use in terms of energy resources was calculated using the abiotic depletion potential method for energy carriers developed by Van Oers et al. (2002) and updated in Van Oers and Guinée (2016). This method is included in the set of indicators used in the EU Environmental Footprint version 3.0 (Crenna et al., 2019). Eutrophication potential was assessed using the CML method (Guinée, 2002), a simple approach for assessing potential eutrophication which assumes that all N and P discharged to the environment can cause eutrophication by placing the indicator at the point of emission, and thereby not including the fate of the emissions (Henryson et al., 2019).

3. Results

3.1. Yields

The yield effect of ley on the first four crops in the rotation was assessed by comparing the difference in yield for each individual crop between the ley rotations and *No-Ley* (Figs. 2a-2c). Under the *Low N* regime, inclusion of ley in the rotations at all sites gave higher mean yield of the first four crops except for oilseed, with higher yields observed in *No-Ley* than in *Grass-Ley*. The largest positive yield effect was observed for *Mixed-Ley*, which was most evident when comparing the yield expressed in CU. Under the *High N* regime, the difference between the ley rotations and the *No-Ley* rotation was small, but barley yields were considerably lower in the ley rotations at all sites, most likely because less N was applied to the barley in the ley rotations in order to ensure good establishment of the undersown ley crop (Table 3). Across all study sites, the mean aggregated effect of ley on yield of the first four crops under the *Low N* fertiliser regime was 1.69 and 0.51 Mg CU ha^{-1} for *Mixed-Ley* and *Grass-Ley*, respectively. Under the *High N* regime, the yield response was − 0.29 and − 0.45 for *Mixed-Ley* and *Grass-Ley*, respectively. Mean yield of each crop in the different crop rotation and sites is shown in Table S3 in SM.

When expressed in CU, total yield of all crops in the rotation was clearly higher in the ley rotations than in *No-Ley* (Fig. 3a). Moreover, under the *Low N* regime, total yield was higher in *Mixed-Ley* than in the *Grass-Ley* rotation, particularly at Lanna and Stenstugu. Under the *High N* regime, the opposite effect was found in Säby and Lanna, i.e. higher total yield in *Grass-Ley* compared with *Mixed-Ley*, although the difference was small. Total crop rotation yield was higher under *High N* than *Low N*. Higher total yield means lower land occupation in terms of area required to produce 1 kg CU. Consequently, the land occupation was lowest for *Mixed-Ley* under the *Low N* regime and *Grass-ley* under *High N*, respectively. In general, the *High N* regime resulted in a lower land occupation than *Low N*. Across all study sites, the mean ley yield effect on the total crop rotation in *Mixed-Ley* and *Grass-Ley* was, respectively, 6.58 and 4.23 Mg CU ha^{-1} for *Low N* and 3.72 and 4.51 Mg CU ha^{-1} for *High N*.

The contribution of each crop to total CU of the rotation was similar for the two ley rotations, where Ley I (i.e. the first year of ley) and Ley II (i.e. the second year of ley) contributed between 35% and 38% of total CU, and Ley I made a larger contribution than Ley II in both *Mixed-Ley* and *Grass-Ley* (Fig. 3b). In the *No-Ley* rotation, the largest contribution to the CU was made by the winter wheat crop, which alone accounted for 29% and 27% of total CU in the *Low N* and *High N* fertiliser regime, respectively.

3.2. Soil organic carbon

Estimated mean SOC change, which was used in the LCA, indicated that all treatments resulted in depletion of SOC, leading to atmospheric CO_2 emissions. However, there was large variation between replicate plots, as indicated by the error bars in Fig. 4. Changes in SOC in all plots are shown in Fig. S2. Under the *Low N* regime at Säby and Stenstugu, the greatest depletion of SOC stock occurred in the *No-Ley* rotation (153 and 199 kg C ha^{-1} $year^{-1}$, respectively) and the least depletion in the ley rotations (70 and 133 kg C ha^{-1} $year^{-1}$ in *Mixed-Ley* and 76 and 169 kg C ha^{-1} $year^{-1}$ in *Grass-Ley* at Säby and Stenstugu, respectively). The *High N* regime resulted in lower SOC stock depletion at Säby, and particularly at Stenstugu. However, at Lanna, there was almost no difference in SOC change between the rotations under the *Low N* regime, whereas under *High N* the greatest SOC stock depletion was found in the *Mixed-Ley* rotation (Fig. 4).

The mean difference in SOC between the ley rotations and *No-Ley* after eight rotations, i.e. 48 years, over all sites was 2.54 and 2.49 Mg ha^{-1} for *Mixed-Ley* and *Grass-Ley,* respectively, under the *Low N* regime. Under the *High N* regime, the difference was 1.43 and 2.71 for *Mixed-Ley* and *Grass-Ley*, respectively.

3.3. Life Cycle Assessment

3.3.1. Greenhouse gas emissions

The lowest GHG emissions per kg CU were found in the *Mixed-Ley* rotation under the *Low N* fertiliser regime (Figs. 5a-5c). This was most evident at Lanna and Stenstugu, where GHG emissions from *Mixed-Ley* under the *Low N* regime corresponded to 81% and 77% of those from *Grass-Ley*. At Säby, the same treatment corresponded to 90% of those from *Grass-Ley*. The highest estimated emissions per CU were found for the *No-Ley* rotation at all sites. Under the *High N* regime, the difference was smaller between the two ley rotations. At Lanna, GHG emissions per CU were lower in *Grass-Ley* than in *Mixed-Ley*. The *High N* application regime resulted in greater emissions from production of N fertiliser and

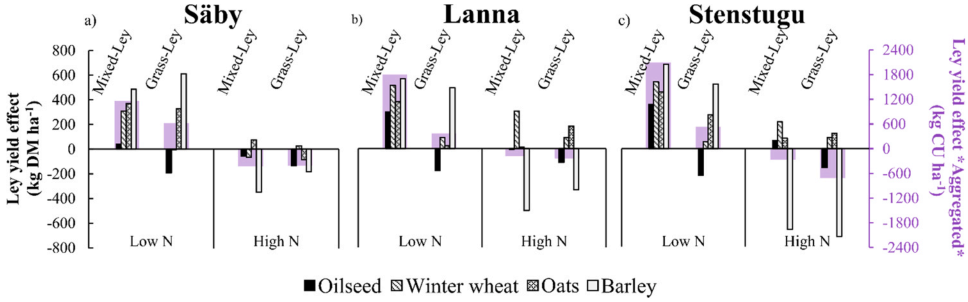

Fig. 2. Effects of ley inclusion on yield of the first four crops (oilseed, winter wheat, oats, barley) in the Mixed-Ley and Grass-Ley compared to the No-Ley rotation under the different N fertiliser regimes assessed at (a) Säby, (b) Lanna and (c) Stenstugu. Purple bars indicate difference in aggregated cereal units (CU) of the first four crops.

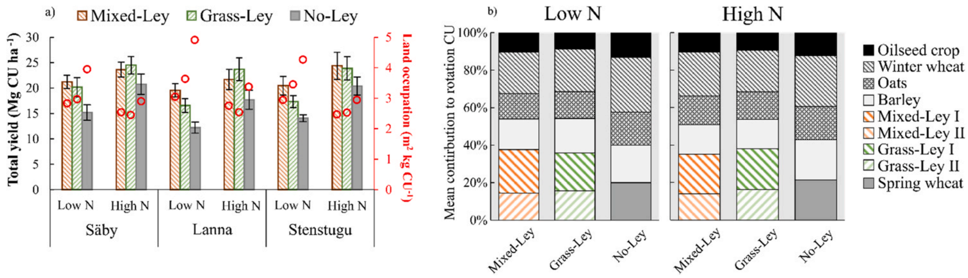

Fig. 3. (a) Mean total yield of the full rotation (cereal units (CU) ha^{-1}, error bars represent 95% confidence interval) at each site and for each crop rotation and fertiliser regime, where red circles represent land occupation (m^2 needed to produce 1 kg CU). (b) Mean contribution of each crop to total CU for each treatment.

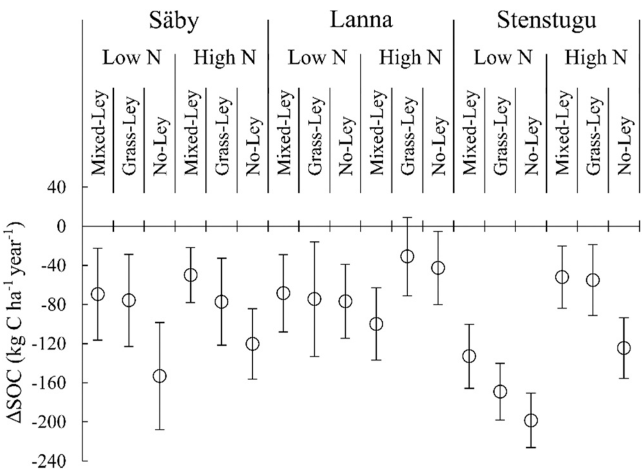

Fig. 4. Change in calculated soil organic carbon (SOC) stock in the topsoil (20 cm depth) in each treatment at the Säby, Lanna and Stenstugu sites. The mean value for each treatment is marked with a circle, error bars represent 95% confidence interval.

also greater soil N_2O emissions per ha and per kg CU. Soil organic C depletion in all treatment combinations and at all sites resulted in additional CO_2 emissions from the systems. Compared with the *No-Ley* rotation, the average GHG emissions from *Mixed-Ley* were 329 and 188 g CO_2-eq lower per kg CU for *Low N* and *High N*, respectively, whereas *Grass-Ley* resulted in 200 and 147 g CO_2-eq lower GHG emissions per kg CU for *Low N* and *High N*, respectively (Fig. 5d).

3.3.2. Energy resource depletion and eutrophication potential

Similarly to the findings for climate impact, energy resource depletion and potential eutrophication were lowest per CU in the *Mixed-Ley* rotation under the *Low N* regime (Fig. 6). In the *Low N* regime, the majority of total energy depletion originated from field operations, while in *High N* a higher proportion of energy depletion came from the agricultural inputs. This was because of the higher N fertiliser rate, which caused greater total depletion both per ha and per CU compared with *Low N*. The greatest energy resource depletion per CU was in the *No-Ley* rotation, while the greatest energy depletion per ha was in the *Grass-Ley* rotation, due to the larger total N input in that rotation.

Nitrogen emissions, predominantly in terms of leaching, contributed most to eutrophication potential at each site. Phosphorus leaching also had a considerable impact, particularly at Lanna (Fig. 6). Simulated impacts were lower for the other sources of eutrophication included in

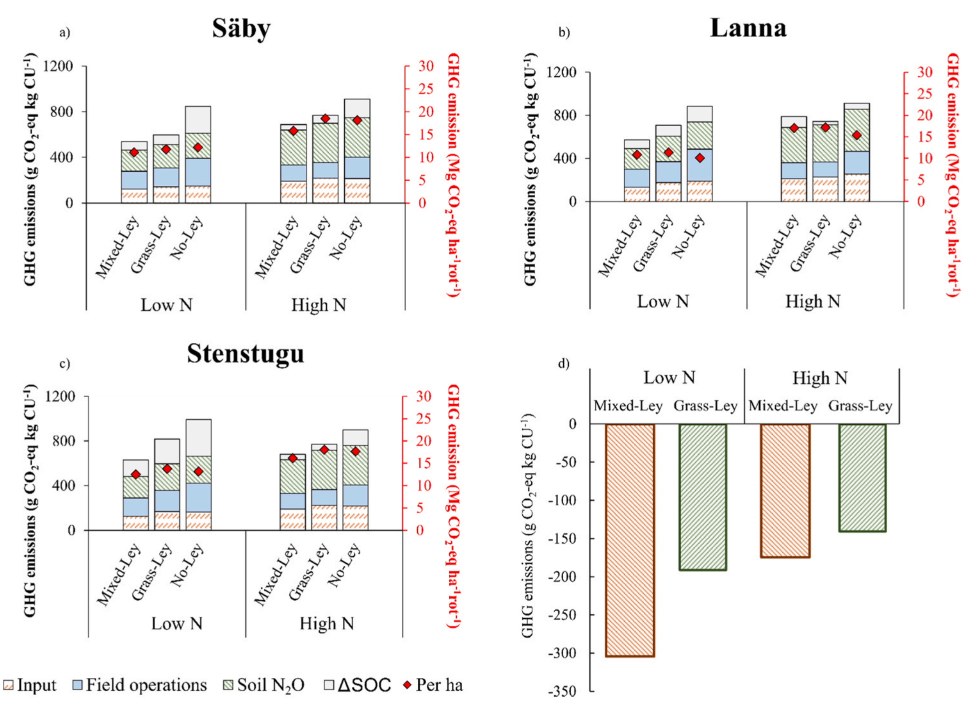

Fig. 5. Greenhouse gas (GHG) emissions per kg cereal units (CU) (bars, left axis) and per hectare agricultural land (red diamonds, right axis) at (a) Säby, (b) Lanna and (c) Stenstugu and (d) mean difference in emissions across all sites between the ley rotations (Mixed-Ley, Grass-Ley) and the No-Ley rotation (values on bars indicate total GHG emissions in g CO_2.eq per kg CU).

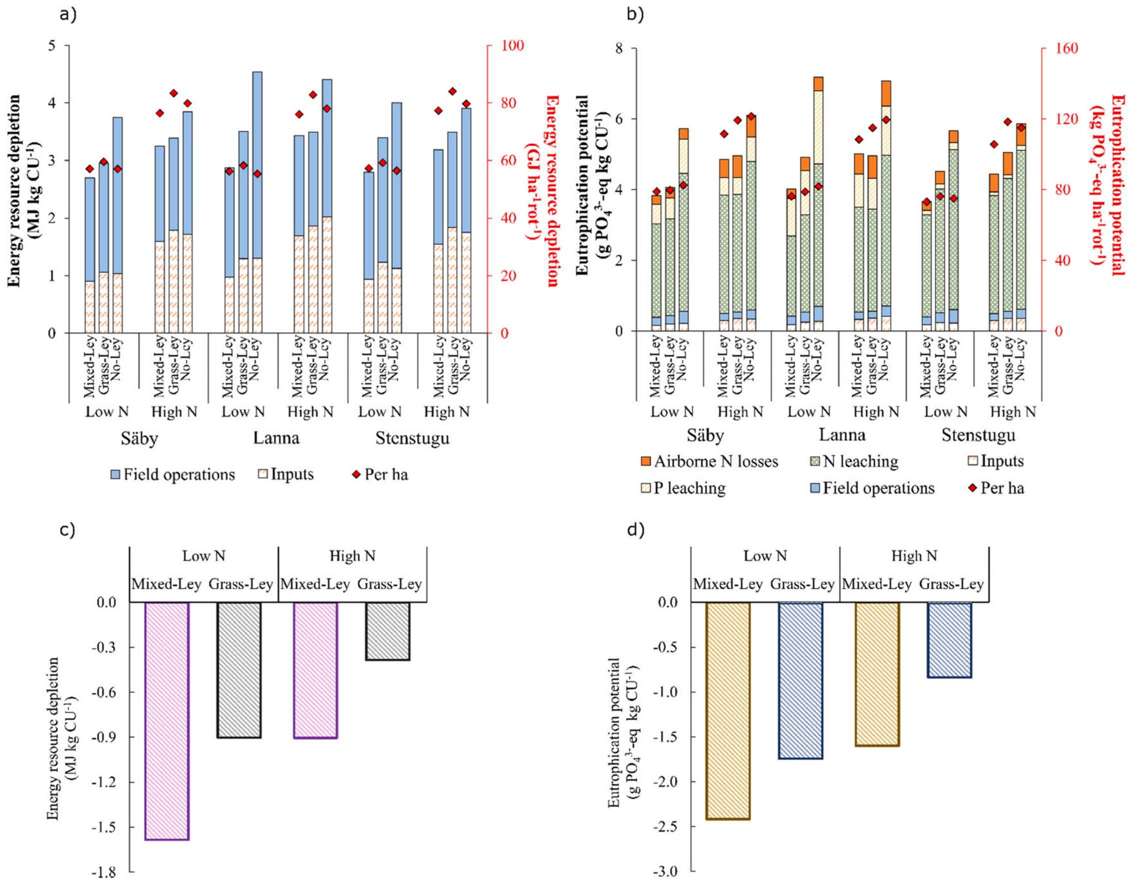

Fig. 6. (a) Fossil energy resource depletion and (b) eutrophication potential in the different treatments at the Säby, Lanna and Stenstugu sites, per kg cereal units (CU). Red diamonds indicate total impact per hectare of agricultural land for a whole rotation, and mean difference across all sites between ley rotations and the No-Ley rotation in (c) energy resource depletion and (d) eutrophication potential (values on bars indicate total impact per kg CU).

the assessment. Eutrophication potential was generally higher under the *High N* fertiliser regime, per CU and per ha agricultural land, than under the *Low N* fertiliser regime.

4. Discussion

4.1. Effects on biomass yield and soil organic carbon

Inclusion of two-year leys had a positive effect on yield of the first four crops in the rotation under the *Low N* fertiliser regime (Figs. 2a-2c). This effect was especially evident in the *Mixed-Ley* rotation (legume-grass ley), where the first four crops at all sites showed higher yields than those in the rotation without ley (*No-Ley*). A similar positive influence on yield following diversification of cropping systems has been reported in previous studies (e.g. Nunes et al., 2018; Ponisio et al., 2015). Marini et al. (2020) found specifically that diversification by including two- to three-year grass-legume leys in six- to seven-year crop rotations increased mean yield of winter and spring cereals by 0.86 and 0.39 Mg per ha and year, respectively. In the present study, aggregated mean yield of the four first crops increased by 1.69 and 0.51 Mg CU per ha for *Mixed-Ley* and *Grass-Ley*, respectively, under the *Low-N* regime. This yield effect of ley could be attributable to enhancement of ecosystem services leading to e.g. improved soil health and pest control (Tamburini et al., 2020), nutrient conservation by leys and symbiotic atmospheric N fixation in the *Mixed-Ley* rotation (MacLaren et al., 2022). Angus et al. (2015) found that the more unrelated the preceding crop, the greater the yield effect on wheat crops, which supports the theory that diversification has a positive effect on yields. In contrast, Garland et al. (2021) suggest that it is not diversification itself that is most important, but rather the proportion of the year with crop cover. A similar positive yield effect of ley inclusion was not observed for the *High N* fertiliser regime, which indicates that some of the positive effects on biomass provisioning gained through diversification were lost by increased N fertiliser application. Similarly, MacLaren et al. (2022) observed that diversification with legumes increased yields under low N fertilisation, but had little to no effect under high N fertiliser rates. In line with our results, they also found that pure grass leys had positive yield effects, indicating that perennial leys provide different provisioning functions than annual crops (MacLaren et al., 2022). Thus, diversifying crop rotations by including leys, especially mixed legume-grass leys, can be a strategy to maintain yields under a lower N application regime because of environmental concerns, high prices or ambitions to be self-sufficient.

The mean yield of the entire six-year crop rotation expressed in CU was higher in the ley rotations than in the *No-Ley* rotations (Fig. 3a), resulting in lower land occupation in the ley rotations. Land occupation is an important aspect when assessing the environmental impact of agricultural systems, as clearing new agricultural land is one of the major drivers of the negative climate and biodiversity impacts of agriculture (Foley et al., 2011). In general, crop rotation yield was higher and land occupation was lower for *Mixed-Ley* compared with *Grass-Ley* under the *Low N* regime, and vice versa under the *High N* regime. The lower total yield in the *No-Ley* rotation was partly attributable to the one-year fallow with no harvested biomass. While CU increased with the ley rotations, production of annual crops decreased. Without opportunities to harness the ley biomass, the net effect could be greater land occupation, instead of a reduction. However, there are several options for increasing ley biomass utilisation. These include expanded utilisation as feed for ruminants and monogastric animals such as pigs (Zira et al., 2023), production of protein concentrates through biorefineries for high-quality feed or food applications (Santamaría-Fernández and Lübeck, 2020; Jørgensen et al., 2022), and use as a feedstock for bioenergy (Englund et al., 2023).

The calculated mean SOC stock decreased in all treatments, with or without inclusion of ley (Fig. 4). However, the values showed large variation between replicated plots, adding uncertainty to these results. At two of the sites, Säby and Stenstugu, SOC depletion was lower in rotations with ley for both fertiliser regimes. In contrast, SOC depletion at Lanna was greatest for the *Mixed-Ley* rotation under the *High N* regime. Inputs of organic matter to soils, for example, in the form of crop residues, have been shown to, under certain conditions, accelerate microbial activity and degradation of C already present in the soil (Blagodatskaya et al., 2011). These so-called priming effects are often used as an explanation when increased C inputs lead to elevated SOC decomposition (Poeplau et al., 2015). However, the mechanisms underlying this phenomenon and their interconnections are still not fully understood (Liu et al., 2020), and we have no further evidence to indicate that this mechanism was responsible for the higher SOC depletion in the *Mixed-Ley* under the *High N* regime at Lanna.

A study assessing the average SOC content over the entire duration of the Swedish long-term experiment across all sites has found significantly lower SOC stock in the crop rotation without ley (El Khosht et al., in prep). The lack of SOC sequestration seen in the present analysis, despite ley inclusion, may be due to the ley proportion being insufficient. Jarvis et al. (2017) compared the effect on soil properties of introducing different proportions of ley (1, 2, 3 and 5 years) in six-year rotations and found that the rotations with a higher proportion of ley resulted in larger C stock in the topsoil. However, the rotations with a higher ley proportion also received manure (Jarvis et al., 2017), which has been shown to have a significant effect on long-term SOC sequestration (Bolinder et al., 2020). Similarly, Zani et al. (2021) concluded that a larger proportion of temporary leys in a rotation had a positive linear correlation with SOC concentration when the ley proportion reached 30–40% of the full crop rotation. Moreover, a study by Henryson et al. (2022) investigating SOC content using national monitoring data in Sweden found higher SOC levels on beef and dairy farms than on arable and pig farms, which they attributed to differing proportions of ley and amount of applied manure in the different farming systems.

Another reason for SOC depletion in all rotations in the present study might be the initial SOC content in the soil (Kätterer et al., 2012). We do not have information about former land use, but it is plausible that the current management scheme includes e.g. fewer perennial crops than before the long-term field experiment was initiated. The straw from the annual crops was left in the field in the present study, which could have counteracted further SOC losses, although below-ground biomass is more recalcitrant and gives higher potential for SOC sequestration than above-ground residues (Kätterer et al., 2011; Menichetti et al., 2015; Rasse et al., 2005). One strategy to reduce SOC depletion may be to return part of the biomass to the soil, e.g. in the form of manure, biogas digestate or sewage sludge, which show high recalcitrance to degradation in the soil environment and may, therefore, be important in SOC stock build-up (Kätterer et al., 2011). Our results also indicated lower SOC depletion in *High N* compared with the *Low N* regime. This is in line with Kätterer et al. (2012), who concluded that there is a positive correlation between SOC storage and mineral N applied under Swedish conditions, due to increased biomass production at higher N application rates, which in turn increases the supply of organic matter to the soil. Moreover, Kirkby et al. (2014) showed that adequate availability of soil N is essential for the formation of stable soil organic matter.

4.2. Life Cycle Assessment

At all sites, the lowest cradle-to-farm-gate GHG emissions per CU were for the *Mixed-Ley* rotation under the *Low N* regime (Fig. 5). This mainly was due to higher yields and lower use of N fertiliser, which resulted in lower GHG emissions from both upstream fertiliser production and soil N_2O emissions. Energy resource depletion and eutrophication potential followed the same trend as seen for GHG emissions, with a lower impact per CU for *Mixed-Ley*, especially under the *Low N* regime (Fig. 6). Thus the *Low N* regime gave rotations with lower environmental burden per CU produced, especially in *Mixed-Ley*, mostly because of lower inputs and maintained high yields. However, lower overall biomass production in the *Low N* regime meant that more land was needed to produce the same amount of CU (Fig. 3a). This higher demand for agricultural land could lead to clearing of new land in the worst case scenario, resulting in a considerable additional environmental burden. Nevertheless, this is unlikely in Sweden because of the rather large amount of under-utilised land. According to Olofsson and Börjesson (2016) there are 88,000 ha of abandoned arable land in Sweden, while official statistics show that the area of arable land in use in Sweden decreased by 168,000 ha between 2000 and 2022 (Swedish Board of Agriculture, 2022). There is also potential for more efficient use of cultivated biomass, e.g. by people converting to a more plant-based diet in Western societies, thereby reducing pressure on existing agricultural land (Mottet et al., 2017). Reducing meat consumption has been suggested as a measure to combat global warming (Smith et al., 2019) and alleviate other environmental and human health issues (Martin and Brandão, 2017; Röös et al., 2020). Limiting the animal husbandry sector to using agricultural residues, such as ley biomass and crop residues, and grazing pastures with biodiversity value, as suggested by Karlsson (2022), would ease the pressure on agricultural land. It would also enable more extensive agricultural practices with more diversified cropping systems, which according to our results would entail lower GHG emissions per unit harvested yield. Furthermore, the expanding bio-economy, involving the replacement of fossil products with bio-based alternatives, is expected to increase demand for biomass (Popp et al., 2014). It is imperative to ensure that this increased demand is met without causing new environmental impacts.

Increasing the N fertiliser rate (from *Low N to High N*) generally increased GHG emissions per kg CU and use of N fertiliser had the strongest climate impact in the form of soil N_2O emissions, which is in line with previous findings (Goglio et al., 2015; Henryson et al., 2019). This implies that a technology transition to reduce GHG emissions from chemical N fertiliser production would have only a moderate effect on the total life-cycle GHG emissions. Therefore, to reduce the environmental impact of agriculture, conventional farmers must end their overuse of N fertiliser and learn from systems that are less reliant on chemical fertilisers (Foley et al., 2011). This will not be an easy task as it may result in lower yield per ha, with associated loss of income for farmers. It may, therefore, be argued that the need to incentivise measures that work towards closing the N cycle and low-fertiliser input systems that provide environmental benefits should make strong cases for the establishment of financial compensation schemes (Billen et al., 2021).

Many studies have reported SOC sequestration potential from including perennial crops in crop rotations (Bolinder et al., 2010; Börjesson et al., 2018; Kätterer et al., 2012). However, we observed SOC depletion for all rotations and at all sites when including two years of ley within six-year rotations. Changes in management practices that result in less SOC depletion than in a business-as-usual scenario are often considered to contribute to mitigation of global warming (Kätterer et al., 2012). In the present study, the ley rotations generally lost less C than the rotation without ley, which means that diversification through including ley crops in pure annual crop rotations had a net mitigating effect on CO_2 emissions from the soil. Such diversification will not remove current CO_2 from the atmosphere, but will reduce the future CO_2 concentration compared with business-as-usual (in our case the *No-Ley* rotation). Furthermore, SOC depletion was generally lower under the *High N* fertiliser regime, which may indicate that increased N fertilisation would be beneficial from a climate impact perspective. However, increasing N application to enable SOC sequestration would be a perilous strategy, since global warming mitigation from SOC sequestration will only continue until a new SOC equilibrium has been reached and N_2O emissions will continue to be elevated after that point, which may turn the system from a GHG sink into a GHG source (Lugato et al., 2018).

In a European Union context, lowering the environmental impact of agriculture is currently being promoted through several regulations and incentives, such as the European Green Deal (EU Commission, 2019), the Biodiversity Strategy for 2030 (EU Commission, 2020a), and the Farm to Fork Strategy (EU Commission, 2020b). Our results show that including perennial leys in crop rotations, especially leys containing legume species, can help achieve these targets by decreasing environmental impacts, with more prominent benefits under a Low N regime. Recommended fertiliser application rates fluctuate over time depending on the prices of fertilisers, cereals and other cash crops. The war in Ukraine and the subsequent heavily reduced availability of Russian natural gas on the European market led to historically high prices of N fertiliser in 2022 (World Bank, 2022). This type of market shock may increase interest in alternative sources of N fertiliser, e.g. N-fixing legume species. Provided that a market can be found for the ley biomass, inclusion of mixed legume-grass ley in crop rotations may increase the profitability of the cropping system, while also reducing the environmental impacts.

4.3. Limits, uncertainties

The study was based on empirical data from the long-term field experiment established in Sweden in the 1960 s, so the results were less affected by the inherent uncertainties often associated with modelling of agricultural systems. However, using empirical data adds other uncertainties, e.g. due to crop failure from pest attacks and extreme weather events. Some uncertainties are also associated with measuring methods, which may have caused e.g. the large variation in SOC change, showing considerable overlap of confidence intervals for the treatments assessed (Fig. 5). Moreover, due to lack of data on soil bulk density required to convert the measured SOC content (%) to SOC stock (kg C ha^{-1}), we used the pedotransfer functions developed by Kätterer et al. (2006). This approach has been used in several other studies (e.g. Börjesson and Kätterer, 2018; Hammar et al., 2017; Henryson et al., 2022), but is highly uncertain (Kätterer et al., 2006). Earlier findings have shown that fields predominately cultivated with ley crops, such as pastures, tend to have lower bulk density than fields that are annually ploughed (Tyson et al., 1990). To minimise this potential divergence between treatments, soil cores for the SOC assessment were sampled after the oats, three years after the incorporation of the ley crops. Furthermore, management practices in Swedish agriculture have changed since the beginning of the long-term field experiment, in particular for N fertiliser rates, where the *High N* regime corresponds to normal application rates today. Moreover, black fallow was common in Swedish cropping when the long-term field experiment was started, but is now less common as efficient herbicides have become more available (Kudsk and Streibig, 2003). However, increased herbicide resistance in tandem with tougher regulations may require new modes of weed control in future (Heap, 2014), which may lead to the return of fallow. In addition, winter oilseed rape should be established in early August at northern European latitudes to be sufficiently vigorous to survive winter and produce high yields. However, few crops were harvested before early August during the early years of the long-term experiment and ley crops that could be harvested after the first harvest were the best option as a preceding crop. With climate change and the development of efficient machinery, Swedish winter crops are starting to grow earlier in spring and spring sowing is earlier. In addition, earlier-maturing varieties have become available. Together, this has provided more options for preceding crops for winter oilseed rape. Thus, the differences between crop rotations may change over time. The lack of biomass harvesting in the *No-Ley* rotation means that this may not have given a fair comparison to the ley rotations, but on the other hand that rotation included one extra year of an annual cereal crop (spring wheat) with a relatively high CU conversion factor (Table S2). Adding another crop in *No-Ley* would likely have improved the results for land occupation and presumably also for life cycle environmental impact of this rotation. In addition, the emission savings from the less frequent use of field operations in the ley rotations compared to the *No-ley* could be reduced if a transition is made from fossil fuels, to power the agricultural machinery, to renewable energy.

The results of LCA studies depend on methodological choices, e.g. of functional unit and system boundary. These choices are particularly important for agricultural systems, because they generally deliver multiple functions and outputs. In agricultural LCAs, the most common functional units are dry or fresh matter mass of harvested crop, together with area of land used (Notarnicola et al., 2017). However, it has been argued that mass is a misleading functional unit because its function often varies between crops (Henryson et al., 2019). With the approach used here, the entire crop rotation was included within the system boundary, which means that no allocation between different crops in crop rotation was needed. The CU metric has been used in earlier studies, e.g. by Henryson et al. (2019) and Prechsl et al. (2017), and is used in agricultural statistics to capture the most important nutritional functions of crops (Brankatschk and Finkbeiner, 2014). One drawback of CU is that it is based on the feeding value of the agronomic outputs, although not all outputs may be used as feed. However, since the most common use of ley is as forage (Cederberg and Henriksson, 2020) and cereals in Sweden are used more for animal feed than for human consumption (Eklöf, 2014), we believe that this was a reasonable approximation. Moreover, the livestock species in Germany and Sweden are similar (FAO, 2016), justifying use of the same CU conversion factor. The CU conversion value for ley biomass was lower than for other crops in the rotation (Table S2). However, a wider utilisation area of ley biomass, e.g. enabled by processing in biorefineries, may suggest that ley biomass is potentially undervalued in the present study. Furthermore, the largest contributor to GHG emissions from ley was soil N_2O emissions, which are highly site-specific and can vary over time and under different management schemes (Butterbach-Bahl et al., 2013). Measurements of soil N_2O emissions are scarce, often resulting in LCA practitioners using the crude IPCC Tier I model, which was also the case in this study.

5. Conclusions

This study investigated the effects of including ley in crop rotations in terms of yield response, changes in SOC stock and environmental impact (climate impact, energy resource depletion and eutrophication potential). The results showed that inclusion of leys resulted in higher yields of annual crops in the same six-year rotation under a *Low N* regime, particularly for the rotation including a grass-legume ley. A weaker effect of ley inclusion on the yield of annual crops was observed under a *High N* regime. Total yield, i.e. of all crops in the rotation, was also larger for the ley rotations than for the rotation without ley, mainly due to the one-year fallow in the *No-ley* rotation.

Comparison of mean SOC changes indicated SOC stock depletion for all rotations and both fertiliser regimes at all three study sites, possibly due to high initial SOC content and/or insufficient proportion of ley in the rotation (two years of six). There were large variations in SOC changes between replicate plots, but mean SOC depletion was greater, across all sites, in the rotation without ley than in those with ley. The *High N* regime generally resulted in less SOC depletion.

The mixed ley rotation under the *Low N* regime gave the lowest climate impact, energy resource depletion, and potential eutrophication per kg CU, due to relatively high biomass yield per ha and lower input of purchased agricultural commodities (mainly N fertiliser). The latter reduced the upstream impacts from fertiliser production, and also soil N_2O emissions. Thus, inclusion of ley decreased the dependence on purchased agricultural inputs and lowered GHG emissions from the cropping system, and can therefore be used to help meet targets on reducing the environmental impact of agricultural systems. However, successful implementation will depend on market demand for the ley biomass produced, which can be generated by strengthening incentives for its use in e.g. bioenergy production and animal feed.

CRediT authorship contribution statement

Johan Nilsson: Conceptualization, Methodology, Writing – original draft **Fatima F El Khosht:** Data curation, Investigation **Göran Bergkvist:** Conceptualization, Writing – review & editing **Ingrid Öborn:** Conceptualization, Writing – review & editing **Pernilla Tidåker:** Supervision, Project administration.

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.eja.2023.126888.

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