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Rotational leys in arable cropping systems

Ley establishment, cropping system performance, and effects on soil fertility

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Abstract

Since the mid-20th century, European agriculture has become more specialised, resulting in increased labour productivity but also environmental challenges such as nutrient losses, high reliance on external inputs, and loss of soil organic matter, which contribute to reduced soil fertility. Reintroducing ley phases into arable crop rotations has been proposed as a strategy to improve soil fertility and reduce input dependency; however, their long-term effects in systems without manure inputs, as well as the potential of alternative establishment methods under changing climatic conditions, remain uncertain. This thesis evaluates the role of short-term leys in arable rotations using long-term experiments across Sweden, combined with field studies on clover autumn establishment of grass-clover leys in winter cereals and visual and image-based assessments of clover proportion. Rotational grass–legume ley increased cereal and oilseed brassica yields at low rates of nitrogen fertilisation. In winter wheat, less nitrogen fertiliser was required to achieve comparable grain nitrogen concentrations following grass–legume ley compared with without ley. Leys also enhanced topsoil carbon and nitrogen concentrations and potential nitrogen mineralisation, whilst bulk density was reduced at one site. Autumn establishment of clover in winter cereals was primarily influenced by management that affected light availability, with early cereal harvest increasing clover biomass, and red clover generally resulting in higher clover proportions and greater yields than white clover. Image-based methods for estimating clover proportion exhibited moderate accuracy, highlighting the need for additional calibration and further training before being useful in practice. Overall, short-term ley integration can contribute to a reduced reliance on nitrogen fertiliser and improved soil fertility, but the outcomes depend on management and site-specific factors.

Keywords: Autumn-sowing, clover, crop production, legumes, nitrogen mineralisation, soil fertility, soil organic matter

Vall i växtföljden i odlingssystem med ettåriga grödor

Valletablering, odlingssystemets produktivitet och effekter på markbördighet

Abstract

Sedan mitten av 1900-talet har det europeiska jordbruket blivit alltmer specialiserat, vilket har ökat arbetskraftens produktivitet men också bidragit till miljöproblem såsom näringsförluster, beroende av externa insatsmedel och minskad halt av organiskt material i jorden, vilket försämrar markens bördighet. Återinförande av vall i växtföljder kan vara en strategi för att förbättra markens bördighet och minska beroendet av insatsmedel; men långsiktiga effekter i system utan stallgödsel samt potentialen för alternativa etableringsmetoder under förändrade klimatförhållanden är otillräckligt studerade. Denna avhandling utvärderar effekten av kortvariga vallar i växtföljder utifrån långliggande fältförsök i Sverige, kombinerat med fältstudier av höstetablering av gräs-klövervall insådd i höstspannmål samt visuella och bildbaserade metoder för att uppskatta klöverandel. Avkastningen av spannmål och raps/rybs vid låga kvävegivor var högre i växtföljder med gräs–baljväxtvall, och i höstvete krävdes mindre kvävegödsel för liknande kvävehalter i kärnan än i växtföljder utan vall. Med vall i växtföljden var även kol- och kvävekoncentrationerna i matjorden och den potentiella kvävemineraliseringen högre än utan vall, medan jordens skrymdensitet var lägre på en av tre platser. Höstetablering av klöver i spannmål påverkades främst av odlingsåtgärder som styr ljusförhållanden; tidig spannmålsskörd ökade klöverbiomassan, och rödklöver gav högre klöverandelar och större avkastning än vitklöver. Bildbaserade metoder för att uppskatta klöverandel visade måttlig precision, vilket visar behovet av kalibrering och modellträning krävs innan metoden blir praktiskt användbar. Sammantaget kan kortvariga vallar i växtföljden bidra till minskat beroende av kvävegödsel och förbättrad markbördighet, men effekterna beror på skötsel och platsspecifika förhållanden.

Keywords: Höstetablering, klöver, växtproduktion, baljväxter, kvävemineralisering, markbördighet, markens organiska material

Dedication

To my family

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I. El Khosht, F. F., Bergkvist, G., Dahlin, A. S., Watson, C. A., Forkman, J., Nilsson, J. and Öborn, I. (2025). Rotational grass-legume leys increase arable crop yields, particularly at low N fertiliser rates. Field Crops Research, 32, 109835. https://doi.org/10.1016/j.fcr.2025.109835
- II. El Khosht, F. F., Wetterlind, J., Öborn, I., Bergkvist. G., Watson, C. A. and Dahlin, A. S. Short-term leys and mineral fertilisation increase potential N mineralisation rate and stable C and reduce bulk density (submitted)
- III. El Khosht, F. F., Bergkvist. G., Öborn, I., Nilsdotter-Linde, N., Hallin, O., Watson, C. A. and Dahlin, A. S. Successful autumn establishment of undersown grass-clover leys at high latitudes is supported by measures that reduce cereal-clover competition (submitted)
- IV. El Khosht, F. F., Parsons, D., Oliveira, J., Sashuang, S., Börjesson, T., Hallin, O., Nilsdotter-Linde, N., Bergkvist. G., Öborn, I., Watson, C.A., and Dahlin, A.S. Accuracy and predictive performance of image-based and visual estimates of clover proportion in young grass-clover leys (manuscript)

Paper I is published open access.

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Abbreviations

BC	Botanical composition
BCA	Between class analysis
CAP	Common agricultural policy
C	Carbon
DM	Dry matter
ha	Hectar
LTE	Long-term experiments
MIR	Mid-infrared
N	Nitrogen
P	Phosphorous
PCA	Principal component analysis
RGB	Red-green-blue
SOM	Soil organic matter
SOC	Soil organic carbon
t	Tonnes
UAV	Unmanned aerial vehicles

1. Introduction

Before the rise of agricultural specialisation in Europe during the mid-20th century, most farms operated as mixed systems in which livestock and crop production were integrated (de Roest et al., 2018; Robinson & Sutherland, 2002). This integration allowed farmers to recycle nutrients through manure and benefit from crop rotations, which in turn helped to reduce weed, pest, and disease pressure (Peyraud et al., 2014; Magdoff & van Es, 2021). However, during the mid-20th century, European agriculture experienced major transformations driven by the need to increase food production, rapid population growth, and technological developments. Advances such as the availability of relatively cheap inputs, including synthetic fertilisers, crop protection chemicals (pesticides), and high-yielding crop varieties enabled farmers to increase productivity and gradually separate livestock and crop production (Matson et al., 1997; Robinson & Sutherland, 2002; Peyraud et al., 2014). In addition to these technical and socio-economic drivers, agricultural policy played a key role in reinforcing this transition. Notably, the European Union's Common Agricultural Policy (CAP), introduced in 1962, promoted a model of agricultural modernisation based on specialisation, intensification, and structural changes towards larger farms and more regionally concentrated production systems. Similar structural changes towards agricultural specialisation also occurred outside the European Community, including Sweden, where agricultural policies and technological developments contributed to intensification and the restructuring of farming systems to improve efficiency (Robinson & Sutherland, 2002; Flygare & Isacson, 2003). Although this development improved labour productivity, it has been associated with several environmental challenges (Moraine et al., 2014). In specialised livestock systems, nutrient surpluses are common and may result in phosphorus (P) accumulation in soils and nitrogen (N) losses to surrounding water bodies and the atmosphere (Peyraud et al., 2014). In contrast, specialised arable farming systems are typically characterised by limited crop diversity where cereals dominate, resulting in a heavy reliance on inputs such as synthetic fertilisers and pesticides (Matson et al., 1997; Peyraud et al., 2014). These systems can impose negative effects on the environment through nutrient and pesticide leching, pesticide resistance, reduced biodiversity, and soil degradation, particularly through the loss of soil organic carbon (SOC) (e.g. Matson et al., 1997; Peyraud, et al., 2014). To address these challenges, attention is being increasingly directed towards management practices and cropping system designs that enhance soil health, reduce reliance on external inputs, and strengthen ecosystem services. In this context, reintroducing ley phases into arable crop rotations has been proposed as a potential strategy to improve the sustainability of modern cropping systems (Martin et al., 2020).

1.1 Swedish cropping systems: cereal dominance and ley distribution

Sweden encompasses a wide range of soil types and climatic conditions, from fertile clay-rich plains to lighter, more coarse-textured soils, and from milder southern regions to cooler northern areas, which together strongly influence agricultural production systems and may lead to site-specific responses to crop management. In Sweden, leys (i.e. forage crops of grasses and forage legumes) occupy approximately 38 % of the agricultural land (SJV, 2025), whilst an additional share of land is managed as permanent semi-natural pastures and meadows. Together, these cover roughly half of the agricultural area (SJV, 2025). However, these crops are largely confined to less productive areas (Reumaux et al., 2023; Chopin et al., 2026) and are predominantly used as feed for ruminant livestock.

The clay-rich and inherently fertile plains are instead dominated by annual crops, especially cereals (Reumaux et al., 2023). Cereals alone are grown on approximately 40 % of the total agricultural land (SJV, 2025). This has created a landscape where ley-based systems and annual cropping systems are spatially separated, reflected in the increasingly simplified crop rotations (Chopin et al., 2026). Reduced crop diversity in annual crop rotations has been linked to declines in ecosystem services provided by agricultural landscapes, including provisioning, regulating, cultural, and supporting functions, all of which are essential for sustaining agricultural production systems (Martin et al., 2020).

Over time, Swedish agriculture has become increasingly specialised, with crop and livestock production often separated between farms. Specialised arable farms, although fewer in number (approximately 27 % of all farms; SJV, 2025), typically manage a substantial share of the agricultural land. These farms are characterised by cropping systems dominated by annual crops, in which leys play a limited role, particularly in more productive arable regions on fertile soils (Chopin et al., 2026). Despite their potential to enhance soil functioning and long-term sustainability (Martin et al., 2020), leys continue to be underutilised within these systems.

1.2 Soil organic matter and soil fertility

Soil organic matter (SOM), comprised of approximately 58 % carbon (C) (Van Bemmelen factor), plays a key role in maintaining soil functions. It influences a range of chemical, biological, and physical processes, including soil aggregation, aeration, water-holding capacity, pH buffering, and nutrient retention and availability (Arshad & Martin, 2002). As such, SOM is a fundamental component of soil health, which can be defined as the soil's capacity to function as a living system that sustains plant productivity, maintains environmental quality, and supports ecosystem services (Bünemann et al., 2018). Thus, a decline in SOM may have a negative impact on soil health and its ability to support crop growth by reducing soil structural stability, which increases the risk of compaction, and decreasing biological activity, as well as water and nutrient availability to plants (Arshad & Martin, 2002).

Soil fertility, as part of the broader soil health framework, reflects the soil's capacity to sustain agricultural productivity (Bünemann et al., 2018) and is closely linked to the quantity and composition of SOM, because SOM regulates both the supply and retention of essential plant nutrients (Bashir et al., 2021). The formation and turnover of SOM are strongly influenced by the quality of organic inputs, as residue characteristics affect decomposition pathways and the stabilisation of organic matter in soil (Cotrufo et al., 2013; Córdova et al., 2018). Consequently, SOM consists of fractions with varying turnover rates, regulated by interacting biological, chemical, and physical processes, including microbial activity and the physical stabilisation of organic matter through associations with soil minerals (Baldock & Skjemstad, 2000; Williams et al., 2023). These processes play a central role in regulating soil N mineralisation and plant-available N supply (Korsaeth et al., 2002; Drinkwater & Snapp, 2007), as the decomposition of SOM constitutes a major source of N that is released through microbial mineralisation (Chen et al., 2014; Marzi et al., 2020). In addition, lower SOM levels can reduce the soil's cation exchange capacity, thereby decreasing its ability to retain nutrients and increasing the risk of nutrient losses (Oades, 1988; Bashir et al., 2021). Together, these changes may intensify the reliance on mineral fertilisers to sustain crop productivity.

1.3 Functional roles of ley in crop rotations

1.3.1 Effects of rotational leys on soil organic matter

Rotational leys influence several key soil properties that support soil fertility and crop production, particularly through their effects on SOM dynamics and nutrient cycling. The inclusion of ley periods in crop rotations has been shown to mitigate SOM losses typically associated with continuous annual cropping (e.g. Smith et al., 1997; Poeplau et al., 2011; Zani et al., 2021). This effect is generally attributed to longer periods of plant growth, greater root biomass, and increased rhizodeposition, which together enhance organic matter inputs (Bolinder et al., 2007; Börjesson et al., 2018). However, the extent to which SOM accumulates under rotational leys is strongly dependent on management and site-specific factors, such as the frequency of soil tillage, the use of organic fertilisers, the initial SOM content, and the proportion of leys within the rotation (Soussana et al., 2004; Bolinder et al., 2010; Keel et al., 2019). In many stockless arable farming systems, where both manure inputs and leys are often absent, the return of organic material to the soil may be limited compared to mixed or livestock-based systems, resulting in lower organic matter inputs (Magdoff & van Es, 2021).

Many long-term studies assessing the effects of rotational leys have included organic amendments, often manure, to simulate mixed crop–livestock systems (e.g. Bolinder et al., 2010; Albizua et al., 2015; Puerta et al., 2018; Jensen et al., 2022; Lang et al., 2025). However, this approach may introduce confounding effects, rendering it difficult to separate the specific effects of leys from those of additional organic inputs (Bergkvist & Öborn, 2011). This highlights the need for studies that isolate the effects of rotational leys on soil properties in systems without the use of other organic amendments.

1.3.2 Effects of rotational leys on nitrogen dynamics and pre-crop effects

Soil N availability depends on organic inputs, such as crop residues and manure, and mineral fertilisers (Chen et al., 2014), whilst the presence, quantity, and quality of these inputs determine changes in soil N pools. Rotational leys influence soil N dynamics through their capacity to enhance SOM, thereby increasing the pool of organic N available for mineralisation (Jarvis et al., 1996; Cotrufo et al., 2013). Notably, leys that include legumes can further increase soil N availability through biological N_2 fixation. Legume-derived residues are typically characterised by lower C/N ratios compared to those of non-N fixing crops (Talgre et al., 2012), which can promote more rapid mineralisation and N release (Kaleem Abbasi et al., 2015). As a result, rotational leys often generate positive pre-crop effects, such as increased grain yield and grain N concentration in subsequent crops, which are most evident under low levels of mineral N fertilisation (Nevens & Reheul, 2002; Poulton et al., 2023). However, the magnitude of these effects depends on the synchrony between N release and crop demand, as well as environmental conditions that influence mineralisation and N losses (Torstensson, 1998; Fontaine et al., 2024).

1.3.3 Effects of rotational leys on soil biological activity and weed regulation

Leys can support a range of ecological processes that are important for crop production, including a reduced risk of soil erosion, improved nutrient cycling, pest regulation, and enhanced water retention (Albizua et al., 2015; Martin et al., 2020). These effects are primarily fuelled by extended vegetative soil cover and reduced soil disturbance, which together create favourable conditions for biological activity and soil functioning during the ley phase (Martin et al., 2020). Reduced soil disturbance in rotations that include perennial leys, compared with systems based solely on annual crops, often increases the abundance and activity of soil biota. This resultingly strengthens functions such as N mineralisation and contributes to improved soil structure through, for example, earthworm burrowing (van Eekeren et al., 2008; van Eekeren et al., 2009). Rotational leys can also contribute to weed regulation through several mechanisms. Extended plant cover can promote weed-seed predation (Meiss et al., 2010), whilst dense vegetation and repeated cutting suppress weed growth and reproduction (Liebman & Davis, 2000; Meiss et al., 2008). Furthermore, the absence of regular soil disturbance during the ley phase reduces opportunities for weed seed germination, which may further contribute to lower weed pressure (Döring et al., 2017).

1.4 Ley mixtures, competing and complementary traits

Grasses and forage legumes, such as white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), and lucerne (*Medicago sativa* L.), represent distinct functional groups that differ in seasonal growth dynamics and how they respond to cutting or grazing (Black et al., 2009). Whilst this section focuses on grass–clover interactions that are relevant for establishment, other forage legumes, such as lucerne, may exhibit different growth dynamics due to contrasting functional traits. The success of an individual species in mixed stands is largely determined by traits related to resource acquisition, such as canopy development for light interception, nutrient uptake efficiency, and the ability of legumes to fix atmospheric N_2 (Black et al., 2009). Grasses generally exhibit faster early growth than white and red clover, which can be advantageous during the early stages of crop development (Black et al., 2009). However, growth patterns also differ between clover species. White clover spreads through branched stems that develop into lateral stolons which grow along the soil surface. Through these stolons, white clover can adjust its growth in response to light availability by expanding laterally towards areas with greater light penetration (Marcuvitz & Turkington, 2000). Red clover, in contrast, grows more vertically, and its larger leaves enable it to compete more efficiently for light than white clover when grown in dense mixtures with grass (Huber et al., 1998; Black et al., 2009). Moreover, grasses are generally more tolerant to lower temperatures than white and red clover, whose growth and shoot elongation strongly decline under cool conditions (Black et al., 2009). This may provide a competitive advantage for grasses in terms of light capture and biomass production during periods with low temperatures, such as late autumn and early spring. Competition for light can be particularly important during autumn and winter, when light availability is limited (Haynes, 1980), and during the grass stem elongation phase in spring, when rapidly growing grasses may further reduce light availability for the clover canopy (Ergon & Bakken, 2022).

In addition to above-ground competition for light, grasses and legumes also differ in their below-ground resource acquisition (Haynes, 1980). Grasses typically develop dense, fibrous root systems that are concentrated in the upper soil layers. White clover forms a shallow root system that develops from its stolons, whilst red clover possesses a deeper taproot, allowing access to water and nutrients from deeper soil layers (Black et al., 2009). Lucerne also forms a deep taproot, often extending beyond that of red clover, which further enhances these benefits and additionally improves soil structure, for example by alleviating soil compaction and reducing soil bulk density (Annicchiarico et al., 2015). These differences in rooting patterns may promote complementary resource use in mixed leys, thereby improving resource efficiency and enhancing weed suppression (Frankow-Lindberg, 2012; Connolly et al., 2018; Helgadóttir et al., 2018). Additionally, legumes contribute biologically fixed N through symbiosis with rhizobia (Siqueira da Silva et al., 2012), whilst grasses are generally more efficient at taking up soil mineral N (Nyfeler et al., 2009). Thus, grass–legume mixtures often demonstrate greater N acquisition and biomass production (Nyfeler et al., 2011) and may achieve higher yields with lower fertiliser inputs than when each species is grown alone (Nyfeler et al., 2009).

1.5 Establishing leys under Nordic climatic conditions

In the Nordic region, rotational leys are commonly established as mixtures of grasses and legumes, usually grass–clover mixtures (Parsons et al., 2014). Relatively low spring temperatures can decelerate the early development of spring-sown ley crops (Black et al., 2009). As a result, undersowing grass-clover seeds into spring cereals has become the predominant establishment method, as the cereal crop can act as a protection crop, reducing weed pressure during establishment, whilst allowing a cereal harvest in the establishment year (Parsons et al., 2014). Although undersowing ley in spring cereals can provide advantages compared with sowing the ley alone, it reduces management flexibility because establishment must coincide with spring-sown crops.

In recent years, farmers' experiences and official statistics have highlighted increasing issues with spring and early summer drought, which may reduce the reliability of spring crop establishment in certain regions of Sweden (SJV, 2023; 2024). In response, interest has increased regarding establishment strategies that shift part of the establishment phase to autumn, when soil moisture conditions may be more favourable. One such strategy is undersowing leys into winter cereals.

In Sweden, winter cereals occupy a substantial share of the cereal area. Winter wheat is the dominant winter cereal, representing 90% of national wheat production (SJV, 2025). Winter wheat is often favoured over spring wheat because autumn-sown crops are already established at the onset of spring growth, enabling more efficient use of soil moisture from winter and early spring and leading to higher yields than spring-sown crops (Hay & Porter, 2006; Koppel et al., 2020). Autumn sowing may also distribute field operations more evenly throughout the year. These characteristics make winter cereals a potentially suitable protection crop for undersowing ley crops. However, establishing clover in autumn under Nordic climatic conditions can be challenging due to limited daylength and reduced light availability, low temperatures that slow down early growth and establishment, and the generally lower frost tolerance of clover compared with grasses (Laidlaw and McBride, 1992; Black et al., 2009; Guðleifsson, 2010). These factors may consequently complicate the establishment of mixed grass–clover leys.

Considering the projected lengthening of the vegetation period in Northern Europe due to climate change, including warmer autumn temperatures (Anav et al., 2019; SMHI, 2025), autumn establishment of ley crops may become an increasingly viable strategy. Nevertheless, successful autumn establishment of clover at northern latitudes may still be constrained by limited light availability and short daylengths, which restrict plant growth despite favourable temperature conditions (Black et al., 2009). When perennial forage crops are undersown in cereal crops, competition from the cereal canopy can further suppress the development of the ley crop (Gecaitė et al., 2021). Increasing light availability to the undersown ley, for example through early cereal harvest, may therefore improve clover establishment.

Field experiments from southern Sweden (approximately 55–59° N) indicate that autumn-sown clover can be successfully established under favourable conditions (Hallin, 2022). However, it is important to note that these experiments focused on pure stands of clover. Thus, it remains unclear whether similar establishment success can be achieved in mixed grass–clover leys undersown in cereal crops, where competition for light and other resources may further limit establishment. Moreover, it is unclear the extent to which practices that were successful in southern Sweden can be applied at higher latitudes, where shorter daylengths and cooler temperature regimes may impose greater limitations.

1.6 Botanical composition in grass-clover leys

The botanical composition (BC) of mixed grass-clover leys, defined as the relative proportion of different plant species, is a key determinant of forage quality and N dynamics (McDonagh et al., 2017). Botanical composition is influenced by several factors, including fertilisation, weather, soil conditions, and diseases (Annicchiarico et al., 2015). In addition, management strategies such as grazing or cutting affect species performance within the ley. Grasses generally possess a good tolerance to both cutting and grazing. White clover is more suitable for grazing than red clover due to its stoloniferous growth and low-positioned growing points, which are typically not damaged during grazing. In contrast, red clover has higher-positioned growing points and a solitary crown and is therefore more sensitive to grazing and trampling damage, making it better-suited to be cut for silage or hay (Black et al., 2009).

Including legumes such as clover in the ley can result in high yields whilst simultaneously requiring lower mineral N inputs than grass-only leys due to the legumes' ability to fix atmospheric N (Nyfeler et al., 2009; Nyfeler et al., 2011). Maintaining a target clover proportion and forage protein content requires careful management of N fertilisation, as high N inputs can favour grasses over clover (McDonagh et al., 2017). Thus, BC can serve as a key indicator for adjusting management practices, such as fertiliser application and cutting regimes, to steer the ley towards the desired levels of productivity and forage quality. Achieving this necessitates an accurate assessment of BC across the field. However, BC can vary substantially within and between fields and over time (Annicchiarico et al., 2015) making it difficult to both assess and manage using conventional methods. Therefore, there is a clear need for reliable and practical methods to estimate BC.

1.6.1 Methods for assessing botanical composition

Botanical composition can be determined through botanical analysis, a process in which field samples are collected and manually sorted into species or functional groups (e.g. grass, clover, and weeds), and their dry weight proportions are subsequently calculated (Peratoner & Pötsch, 2019). Although this method provides detailed results, it is highly time-consuming, which limits its practical applicability under field conditions. Biomass-based measurements may also be influenced by sampling variability, particularly when based on subsamples from heterogeneous field conditions. Therefore, a practical tool for determining clover proportions in the field would be valuable, provided that it is quick, simple, and accurate.

Consequently, simpler and more rapid approaches have been explored. One method for assessing the BC in mixed leys is visual estimation of the proportion of each species within a field. This approach is inexpensive, does not require specialised equipment, and is relatively fast. However, the accuracy of visual estimations has proved to be limited and influenced by factors such as species morphology, growth stage, and the individual who is carrying out the observation (Vittoz & Guisan, 2007; Peratoner & Pötsch, 2019).

Digital approaches using image analysis and remote sensing offer non-destructive and time-efficient alternatives for estimating BC (Wachendorf et al., 2017). Recent advances include machine-learning techniques that distinguish clover and grass in Red-Green-Blue (RGB) imagery from ground-based cameras (Grüner et al., 2020) or unmanned aerial vehicles (UAVs), as well as hyperspectral methods that can predict forage traits such as N concentration (Oliveira et al., 2024). Tools that analyse field images, such as CloverSense (Aarhus University, Denmark), apply deep learning to classify grass, clover, and weeds, and have shown promising potential for field-based estimation of BC. However, image-based estimates may be affected by segmentation errors and canopy occlusion, which can influence the accuracy of clover proportion estimates (Skovsen et al., 2017; Skovsen et al., 2021). In practical evaluations, CloverSense has been reported to underestimate clover content, especially at high clover proportions (Nötkreatursstiftelsen & Agroväst, 2024). Earlier research suggests that combining image data with crop-height information can improve accuracy (Sun et al., 2021). However, knowledge regarding the performance of image-based estimates under different field conditions, growth stages, and species mixtures remains limited. Thus, it is currently unclear how such factors influence the accuracy of these methods, which restricts their reliability for practical applications.

1.7 Knowledge gaps in the role, establishment, and assessment of rotational grass-legume leys

The reintegration of leys into crop rotations has gained increasing attention in recent years as a part of efforts to enhance the sustainability and resilience of agricultural systems (Martin et al., 2020; Cooledge et al., 2022). Leys can be integrated either through closer integration of crop and livestock systems or through alternative uses for ley biomass in arable farming, such as substrates for biogas production or as raw material for biorefinery processes (Tidåker et al., 2014; Micke et al., 2023). However, several knowledge gaps persist regarding the role and management of leys in arable systems. Many studies on ley effects in crop rotations have included organic amendments such as manure (e.g. Albizua et al., 2015; Puerta et al., 2018; Lang et al., 2025), which makes it difficult to disentangle the specific effects of ley cultivation itself on soil processes, nutrient dynamics, and subsequent crop performance. Furthermore, knowledge is limited regarding the establishment of mixed grass–clover leys during autumn under Nordic climatic conditions, particularly when undersown in winter cereals, where limited light availability may constrain establishment. In addition, although image-based approaches show promising results for estimating BC in grass-clover leys, the reliability of these methods across different conditions and species mixtures is not sufficiently understood. Addressing these knowledge gaps is important for improving the integration of leys into arable farming systems and for supporting their practical management.

1.8 Aims and Objectives

Using long- and short-term agricultural field experiments, the overall aim of this thesis was to assess the role of rotational leys in Nordic arable cropping systems, with a particular emphasis on annual crop performance, soil fertility, and ley establishment strategies. The following four specific objectives were addressed:

- Quantify the long-term effects of including grass or grass-legume leys in crop rotations under different mineral N fertilisation rates on crop yield, grain N concentration, seed oil concentration, and soil carbon and nitrogen concentrations (Paper I).
- Assess the effects of leys and mineral N fertilisation on soil fertility indicators, including potential N mineralisation rate, alkyl-C abundance, and bulk density (Paper II).
- Evaluate autumn establishment of mixed grass-clover leys undersown in winter cereals, with a focus on clover establishment and performance (Paper III).
- Evaluate image-based and visual methods for estimating clover proportion in grass-clover leys and assess their potential for predicting first harvest clover proportion using early-season estimates (Paper IV).

2. Material and methods

This thesis is based on long- and short-term agricultural field experiments conducted in Sweden which address different aspects of rotational leys, including their effects on annual crop performance and soil fertility, their establishment, and methods for estimating their BC. The experiments were carried out at several sites representing a range of climatic and soil conditions. Site characteristics are summarised in Table 1. This chapter summarises the materials and methods used in Papers I-IV (Figure 1), whilst detailed descriptions are provided in the respective papers.

Table 1. Location, coordinates, altitude, mean annual air temperature and precipitation, and soil characteristics of the experimental sites included in the thesis

Location	**Coordinates (º N, º E)**	**Altitude (m)**	**Temp. (°C)**	**Precip. (mm)**	**pH (H_2O)**	**Soil type**
Stenstugu	57.4º N, 18.3º E	39	7.7	564	7.1	Loam
Lanna	58.2º N, 13.1º E	74	7.3	584	6.3	Silty clay loam
Säby	59.5º N, 17.4º E	9	6.7	541	6.7	Silt loam
Färjestaden	56.7º N, 16.5º E	36	8.3	529	6.4	Sandy loam/Loamy sand
Eldsberga	56.6º N, 13.0º E	11	8.4	850	6.5	Silt loam
Länghem	57.6ºN, 13.2ºE	182	7.3	1095	6.1	Sandy loam
Ljung	58.0ºN, 13.1º E	161	7.3	822	6.0[1]	Sandy loam[2]
Uppsala	59.9ºN, 17.7ºE	9	6.7	541	5.9	Silt loam
Offer	63.1ºN, 17.8º E	150	3.8	606	6.0	Silty clay loam
Ås	63.3ºN, 14.6º E	384	3.5	558	6.4	Gravely loam
Röbäcksdalen	63.5ºN, 20.1º E	6	4.1	637	6.0	Clay silt loam
Öjebyn	65.2ºN, 21.2º E	7	3.1	538	6.1	Silt loam–Loamy sand

Average temperature, precipitation and altitude data were obtained from the nearest available meteorological station from Swedish Meteorological and Hydrological Institute (1991-2020 normals), and altitude data were estimated from maps using the reported coordinates. All values should be regarded as approximate site values. Unless otherwise stated, soil pH and soil type data are based general site sampling or published site descriptions.

[1] Soil pH data from the Swedish University of Agricultural Sciences (SLU) Soil and Crop Inventory.

[2] Soil type data from the Swedish Digital Arable Land Map (DSMS), provided by the Swedish University of Agricultural Sciences (SLU) and the Geological Survey of Sweden (SGU).

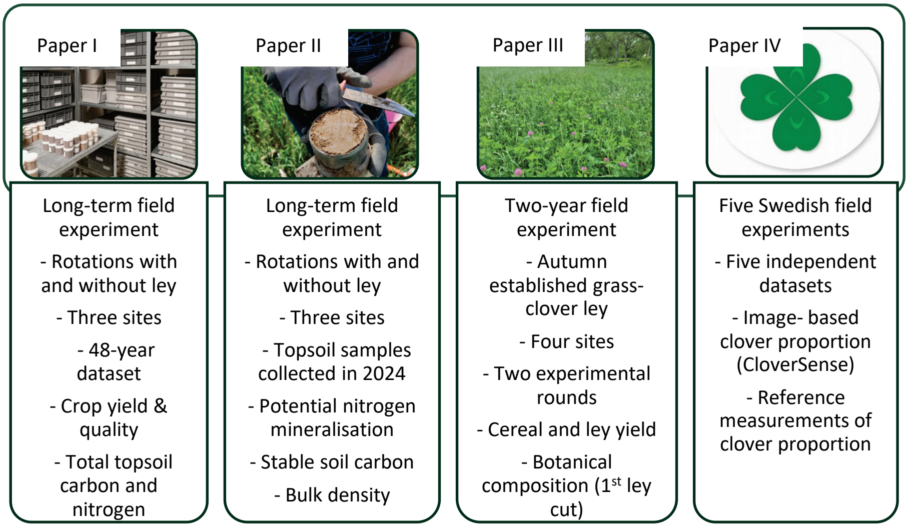


Figure 1. Overview of the field experiments, datasets, and study design underlying Papers I–IV.

2.1 Study sites

Papers I and II are based on a long-term experimental (LTE) series of the Swedish University of Agricultural Sciences, entitled “Rotations with and without ley” (R4-1103) which is located at three sites: Stenstugu, Lanna (Figure 2), and Säby (Table 1). It investigates crop rotations with and without grass or grass-legume leys, along with four N-fertilisation rates. The experimental series was established during the period 1965 to 1967 and is still ongoing.

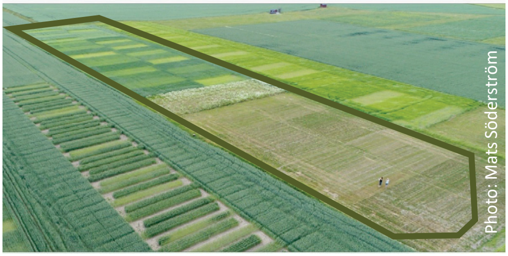


Figure 2. Drone image of the experimental field at Lanna in 2022. The experiment (R4-1103) is located in the centre of the image and is outlined in the figure. The experiment is characterised by a distinct gradient of green shades reflecting different N fertilisation rates, as well as a white-flowering fallow phase and recently harvested ley plots.

Paper III is based on a two-year field experiment series, “Autumn establishment of mixed leys” (R6-0365), which was conducted at four locations in southern and central Sweden: Färjestaden, Eldsberga, Länghem, and Uppsala (Table 1). The experiment evaluated ley establishment in winter cereals and establishment occurred in the autumn of 2021 and 2022 at the respective sites, with measurements performed over two consecutive growing seasons. Weather conditions during the study period were generally close to long-term averages, with some site-specific variation in precipitation (Paper III).

Paper IV is based on five datasets derived from separate field experiments and on-farm data collected across Sweden between 2017 and 2024. Dataset I was collected from experimental sites in northern Sweden: Offer, Ås, Röbäcksdalen, and Öjebyn. Datasets II and IV originated from field experiments at Länghem, whilst Dataset III originated from ley fields at Ljung (Table 1). Dataset V originated from the same experimental series (R6-0365) described for Paper III.

2.2 Experimental designs (Papers I-III)

The LTE series “Rotations with and without ley” was used to assess the long-term effects of including grass-only and grass-legume leys on soil and crop performance. The experiment was comprised of three six-year rotations: (i) a rotation with two years of grass-only ley, (ii) a rotation with two years of grass-legume ley, and (iii) a rotation with only annual crops. Four crop-specific mineral N fertiliser rates were included in the experiment (Table 2).

At Stenstugu, an unreplicated split-strip-plot design was used that consisted of six main plots representing each rotation year (1-6), with rotations (grass-legume ley, grass ley, no-ley) assigned to columns and N rates (N0-N3) to rows (Figure 3a). At Lanna and Säby, the unreplicated experiment also included rotation year on the main plots, but with rotations on subplots and N rates on sub-subplots (Paper I) (Figure 3b). The sub-subplot size was 7.3 m x 16 m at Stenstugu, 8 m x 15 m at Lanna, and 4.75 m x 18.65 m at Säby. Each phase of the rotation was present every year, meaning that all crops in the rotations were represented annually.

(a)

Grass-legume ley rotation (1)		Grass ley rotation (1)		No ley rotation (1)	
N1	N3	N0	N2	N1	N2
N2	N0	N1	N3	N0	N3

(b)

Grass-legume ley rotation (1)	Grass ley rotation (1)	No ley rotation (1)
N0	N0	N0
N1	N1	N1
N2	N2	N2
N3	N3	N3

Figure 3. Schematic illustration of one experimental block from the two field trial designs used in the long-term experiment R4-1103, showing one rotation year. (a) The experimental design at Lanna and Säby follows a split-split-plot design including rotation year as main plots, with rotations on subplots and nitrogen fertilisation rates on sub-subplots. (b) The experimental design at Stenstugu follows a split-strip-plot design with rotation year as main plots and rotations (grass–legume ley, grass ley, and no ley) randomised to columns and nitrogen fertilisation rates (N0–N3) to rows.

Table 2. Crop sequences within each rotation and N fertilisation rates per crop (kg ha-1; N0–N3) in the long-term experiment series R4-1103 at Stenstugu, Lanna, and Säby established in 1966, 1965, 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**
[1]Oilseed brassicas/ Winter wheat 1	0; 60; 120; 180/ 0; 45; 90; 135	[1]Oilseed brassicas/ Winter wheat 1	0; 60; 120; 180/ 0; 45; 90; 135	[1]Oilseed brassicas/ Winter wheat 1	0; 60; 120; 180/ 0; 45; 90; 135
Winter wheat 2	0; 45; 90; 135	Winter wheat 2	0; 45; 90;135	Winter wheat 2	0; 45; 90; 135
Spring oat	0; 40; 80; 120	Spring oats	0; 40; 80; 120	Spring oats	0; 40; 80; 120
Spring barley[2] (undersown)	0; 60; 60; 60	Spring barley[2] (undersown)	0; 60; 60; 60	Spring barley	0; 40 80 120
Grass-legume ley 1[3]	0; 0-80; 0-160; 0-240	Grass ley 1	0; 80; 160; 240	Spring wheat	0; 60; 120; 180
Grass-legume ley 2[3]	0; 0–45; 0–90; 0–135	Grass ley 2	0; 45; 90; 135	Black/chemical fallow	0

[1]Due to challenges during crop establishment and different types of yield losses (e.g. insect damage), oilseed brassica was replaced by winter wheat following the completion of a six-year rotation at Säby (2017), Lanna (2019), and Stenstugu (2022).

[2]Spring barley was undersown with either grass-legume or grass ley in rotations with ley.

[3]Nitrogen fertilisation of the grass-legume ley was adjusted according to visual assessment of legume proportion prior to applications (<25%: full dose; <50%: 70% dose; >50%: no fertiliser).

The experiment R6-0365 was established to evaluate autumn establishment and the subsequent performance of mixed grass–clover leys undersown in winter cereals under different cereal harvest systems and cereal species. A randomised complete block split-split plot design was used, consisting of two cereal harvest systems as main plots, three winter cereal species on subplots, and four ley mixtures as sub-subplots (Figure 4). The sub-subplot sizes were 1.75 m × 12 m at Färjestaden, 1.5 m × 12 m at Eldsberga, 1.6 m × 12 m at Länghem, and 1.5 m × 16 m at Uppsala. The management timeline is presented in Figure 5.

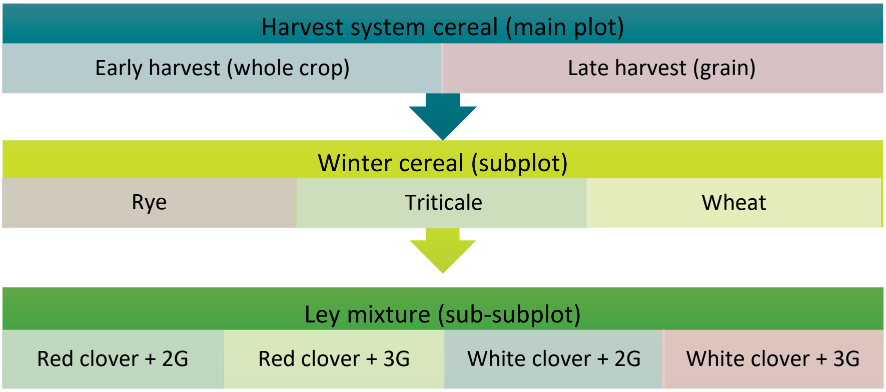


Figure 4. Schematic illustration of the three factors within the two-year experiment R6-0365 with harvest system of cereals on main plots, winter cereal species on subplot, and ley mixture on sub-subplot. Clover species: red clover (*Trifolium pratense* L.); white clover (*Trifolium repens* L.). Grass species mixture 2G = timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.); 3G = timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds), and perennial ryegrass (*Lolium perenne* L.).

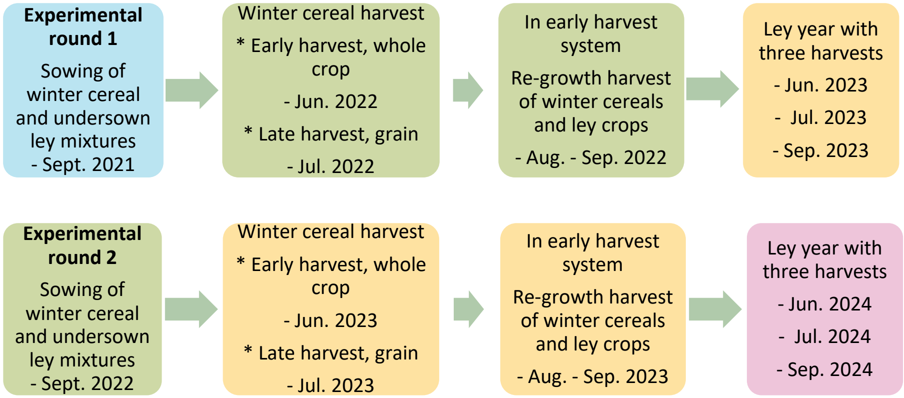


Figure 5. Management timeline of the experiment R6-0365 established in Sweden at Färjestaden, Eldsberga, Länghem, and Uppsala in 2021 and 2022. Colours indicate years: blue = 2021, green = 2022, yellow = 2023, and pink = 2024.

2.3 Data collection and analysis methods

2.3.1 Long-term experiment on rotations with and without ley (Papers I-II)

In Paper I, crop and soil data spanning a 48-year period from the LTE (R4-1103) were used, starting with the second six-year cycle (cycle 2) of the experiment. The data covered the period 1971–2020, with a slight variation in the starting years between sites (1971–1973). The crop data included yields from the first four annual crops in each rotation across all N fertiliser rates, as well as oil concentration in oilseed brassicas and grain N concentration in cereals. Ley yields and biomass N concentrations were also included for the low (N1) and high (N3) N fertilisation rates. The data were compiled from historical records and recently analysed archived samples. Soil data was comprised of topsoil C and N concentrations from historical measurements up to the 1990s, complemented by archived samples from 2011–2020 that were analysed in 2021. To ensure comparability across sites and years, only rotation cycles with consistent management and complete datasets were included in the analyses. The first cycle was excluded due to differences in nutrient management between rotations, and later cycles were selected based on available data for specific variables.

For Paper II, topsoil samples were collected during early spring and early summer in 2024. Early spring samples were collected in plots where winter wheat 1, winter wheat 2, and spring oats were grown in 2023. These samples were used to determine potential N mineralisation rate and alkyl-C abundance (based on the alkyl-C spectral region). Bulk density sampling was conducted in early summer in standing crops (Figure 6a). To minimise the influence of current vegetation, plots with the same crops across all rotations (winter wheat 1, winter wheat 2, and spring oats) were selected.

Figure 6. (a) Bulk density sampling in winter wheat at the Säby experimental site in June 2024 (Paper II), and (b) an autumn-established grass–clover ley prior to the first harvest at Länghem in June 2024 (Paper III).

2.3.2 Two-year experiment on autumn establishment of mixed leys (Paper III)

In Paper III, data on cereal yields was used, including both whole-crop and grain yields. In cases where cereals were harvested as a whole crop, a regrowth harvest was performed later in the season, and this was also used in the analysis. Additionally, yields from the different leys were included, with three harvests conducted during the ley year.

For clover proportion measurements at the first ley harvest (Figure 6b), subsamples were collected for botanical analysis, and species composition was based on dry weight proportions of clover, grass, and weeds. At the second and third harvests, species proportions were estimated through visual assessments.

2.3.3 Datasets for clover proportion assessment (Paper IV)

Five independent datasets from separate research projects were used to represent a range of agronomic and observational contexts which visual and image-based clover assessments are typically performed under. These included variations in seasonal timing (reflecting different crop developmental stages), species composition, weather conditions during data collection (including variation in light conditions, e.g. cloud cover and occasional precipitation), as well as crop height and soil cover. Examples of images across datasets are presented in Figures 7a–e. Across all datasets, clover proportion was estimated using the CloverSense web platform.

Reference measurements were obtained through botanical analysis based on harvested biomass, either on a frame basis (i.e. biomass harvested within the same area as the image that visual estimates were based on) or on a plot basis (i.e. subsamples collected across the plot). Information on sampling procedures and spatial scales is provided in Table 2.

Dataset I was obtained from four locations in northern Sweden (Offer, Ås, Röbäcksdalen, and Öjebyn) during 2017–2019. CloverSense estimates were compared with frame-wise reference measurements of BC, and crop height was recorded (Table 2).

Dataset II was collected in 2020 at Länghem in southern Sweden (Table 2). CloverSense and visually assessed estimates were compared with plot-wise reference measurements of BC based on subsamples.

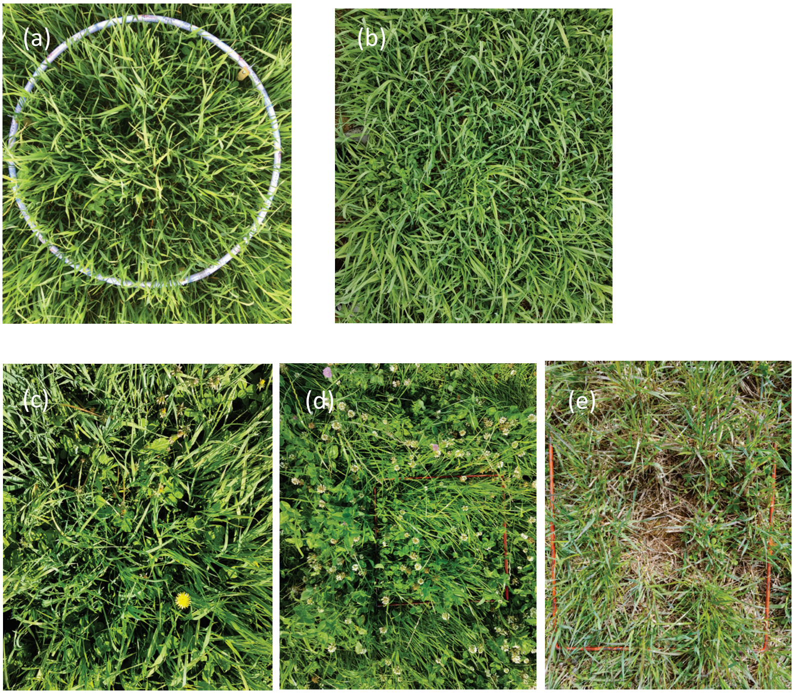


Figure 7. Example field images from the five datasets, illustrating the original images and for datasets I, IV, and V, the frame used to define the analysed area prior to clover proportion estimation. Images from (a) dataset I, (b) dataset II, (c) dataset III, (d) dataset IV, and (e) dataset V.

Table 3. Summary of dataset characteristics

Dataset	[1]Number of sites	Sampling period	[2]Species composition	Growth stages	Frame type	Weather	Reference measurements (spatial scale)
I	4	Jun.–Aug. Jun.–Jul.	**1 mixture:** T+RC	Vegetative–flowering	Round hoop Ø 0.8 m	Stable and clear	Frame-wise
II	1	May–Sep.	**3 mixtures:** T+RC+WC T+MF+PRG+RC+WC T+TF+RC+WC	Vegetative–flowering	NA	Variable	Plot-wise
III	1	28 May 27 Jul.	T+MF+PRG+RC+WC	Vegetative–flowering	NA	Variable	Plot-wise
IV	1	Late Jul.	**1 mixture:** T+MF+PRG+RC+WC	Vegetative–flowering	0.25m^2 quadrat	Variable	Frame-wise
V	4	Late Apr.–Early May	**4 mixtures**: T+MF ±PRG+RC/WC	Vegetative	0.25m^2 quadrat	Variable	Plot-wise at 1st harvest

[1] Sites included locations in southern to northern Sweden, spanning a latitude range of 56.6°N–65.2°N.

[2] Species abbreviations: T = timothy *(Phleum pratense* L.); MF = meadow fescue (*Festuca pratensis* Huds.); PRG = perennial ryegrass (*Lolium perenne* L.); TF = tall fescue (*Festuca arundinacea* Schreb.); RC = red clover (*Trifolium pratense* L.); WC = white clover (*Trifolium repens* L.).

Dataset III was collected in 2020 at Ljung in southern Sweden (Table 2). CloverSense and visually assessed estimates were compared with plot-wise reference measurements based on subsamples.

Dataset IV was derived from sampling conducted in 2020 at Länghem. CloverSense and visually assessed estimates were compared with frame-wise reference measurements based on biomass samples from the same area as the images (Table 2).

Dataset V originated from the field experiment described in Paper III (R6-0365) (Table 2). Clover proportion was estimated in spring, approximately one month prior to the first harvest, using CloverSense and visually assessed estimates within the same sampling frame. These estimates were compared with plot-wise reference measurements based on subsamples obtained at the first harvest.

2.4 Laboratory methods

2.4.1 Total nitrogen and carbon (Paper I)

The methods used to quantify crop N concentration evolved over time throughout the experimental period. Early analyses were based on the Kjeldahl method and were later replaced by combustion using elemental analysers, which have been shown to provide comparable results (Sader et al., 2004). More recently, grain protein and oil concentration have been determined by near-infrared transmittance analysis, with N concentration calculated using crop-specific conversion factors (Tkachuk, 1977). Analytical methods for soil C and N also evolved over time, shifting from wet combustion and loss on ignition approaches to dry combustion methods, which were consistently applied in the later part of the experimental period.

2.4.2 Potential net N mineralisation and alkyl-C in soil (Paper II)

Soil samples were analysed to determine potential net N mineralisation rates using an anaerobic incubation method, following established procedures described by Canali & Benedetti (2005), with minor modifications (Paper II). The rates were calculated from changes in ammonium-N concentration before and after incubation. Soil C composition was assessed based on alkyl-C signal intensity which was determined using diffuse reflectance infrared

Fourier transform spectroscopy (DRIFT) in the mid-infrared (MIR) region on prepared soil samples (Paper II). Differences in alkyl-C signal intensity between treatments were used to indicate variation in the contribution of more stable soil organic C fractions.

2.5 Statistical data analysis

Statistical analyses were primarily conducted using linear mixed-effects models. Fixed effects typically included experimental treatments such as crop rotation and N fertilisation, whilst random effects accounted for variation related to the experimental design and data structure. Model assumptions of normality and homogeneity of variance were evaluated and response variables were transformed when necessary. The specific models and additional analyses for each paper are described below.

2.5.1 Long-term field experimental data (Paper I)

Data were analysed using linear mixed-effects models. Crop rotation, N fertiliser rates, and rotation cycles were included as fixed effects, together with their interactions, whilst site, year, and plot (nested within sites) were included as random effects. Tukey's HSD test was used for pairwise comparisons when significant effects were detected. Analyses were performed in JMP Pro 16.

2.5.2 Potential N mineralisation, stable C, and bulk density (Paper II)

Potential net N mineralisation and bulk density data were analysed using linear mixed-effects models. The experimental design of the LTE differed slightly between sites; therefore, site-specific random structures were specified to account for variation associated with plots and differences among crops used as replicates within sites. Crop rotation, N fertiliser rate, and site were included as fixed effects, together with their interactions. Analyses were conducted using the mixed procedure in SAS, with degrees of freedom estimated using the Kenward–Roger method and the Tukey adjustment for pairwise comparisons. Additionally, site-specific analyses were performed to further examine patterns within individual sites using JMP Pro 17, using similar model structures as described above.

Multivariate analysis of spectral data was performed separately for each site to account for site-specific spectral variation related to differences in soil properties such as SOM composition, mineralogy, and texture, and to avoid confounding treatment effects with larger between-site variation, even within the selected alkyl-C spectral region. Principal component analysis (PCA) followed by between class analysis (BCA) was used to evaluate the effects of crop rotation and N fertilisation on spectral variation. Statistical significance of factor effects was assessed using permutation tests. Spectral preprocessing included baseline offset and linear baseline correction in The Unscrambler (v14.2, AspenTech) to reduce background variation in the MIR spectra, and multivariate analyses were performed in R.

2.5.3 Autumn establishment of grass-clover leys (Paper III)

Data were analysed using linear mixed-effects models in JMP Pro 17. Fixed effects included site and year, cereal species, harvest system of cereal, clover species, and grass mixture with their interactions, whilst block structure within fields was included as a random effect. Separate models were used for ley yield and BC, and for cereal yield and regrowth following early harvest; harvest system of cereal was not included in the model for cereal yield analysis, as whole crop and grain yields were analysed separately, and regrowth was only assessed after early cereal harvest. Tukey's HSD test was used for pairwise comparisons when significant effects were detected.

2.5.4 Estimations of clover proportions (Paper IV)

For Paper IV, agreement between clover proportion estimates obtained using image-based CloverSense, visual assessment, and reference measurements was evaluated separately for each dataset (Datasets I–IV) using descriptive and regression-based approaches. Differences between estimates and reference measurements were visualised using scatter plots to assess agreement patterns, and linear regression analyses were performed to quantify these relationships.

For the predictive analysis (Dataset V), linear regression models were fitted with clover proportion determined by botanical analysis at the first harvest as the response variable and early-season CloverSense or visual estimates as explanatory variables.

Analyses were carried out separately for each dataset to enable comparisons of method performance across various agronomic and observational contexts.

3. Results

3.1 Effects of rotational leys on crop performance and soil fertility

3.1.1 Long-term effects of rotational leys on crop performance (Paper I)

At zero N fertiliser (N0), yields of oils seed brassica, winter wheat, and spring oats were greatest in the grass-legume ley rotation, increasing by approximately 0.4, 0.9 and 0.6 t DM ha^{-1}, respectively, compared with the no-ley rotation (Figure 8a–c). Yields in the grass ley rotation at N0 were generally intermediate, with winter wheat and spring oats yielding approximately 0.3 t DM ha-1 more than in the no-ley rotation. The yield benefit of the ley declined with increasing N fertiliser rate. The fourth crop, spring barley, had lower seeding rates in the ley rotations than in the no-ley rotation, but still yielded on average 0.3 t DM ha-1 more in the grass-legume ley rotation than in the no-ley rotation at N0. At higher N rates (N1–N3), differences in yield also reflected differences in N fertilisation management between rotations (undersown vs. sole-sown barley; Table 2).

Although average yields varied between rotation cycles, the relative effect of ley inclusion remained consistent over time. However, both winter wheat and spring oats exhibited stronger yield responses to N fertilisation in the later compared to the earlier cycles.

Quality variables responded to both rotation and N fertiliser rate. Grain N concentration in cereals generally increased with increasing N fertiliser rates, although only for rates above N1. In winter wheat, grain N concentrations at N1 and N2 in the grass-legume ley rotation were similar to those at N2 and

N3, respectively, in the no-ley rotation (Figure 8b). In addition, winter wheat in later cycles showed higher grain N concentrations and a stronger response to N fertilisation. Grain N concentration in wheat was strongly correlated with gluten concentration (R^2=0.96, Paper II). In spring oats, no rotational effect on grain N concentration was detected. In spring barley, the highest grain N concentration was recorded in the no-ley rotation at N3, which also received a higher N fertiliser rate than the corresponding ley treatments (Table 2). Oil concentration in oilseed brassicas was about one percentage unit higher in the grass ley and no-ley rotations than in the grass-legume ley rotation, and 1–2 percentage units higher at N0–N1 than at N2–N3.

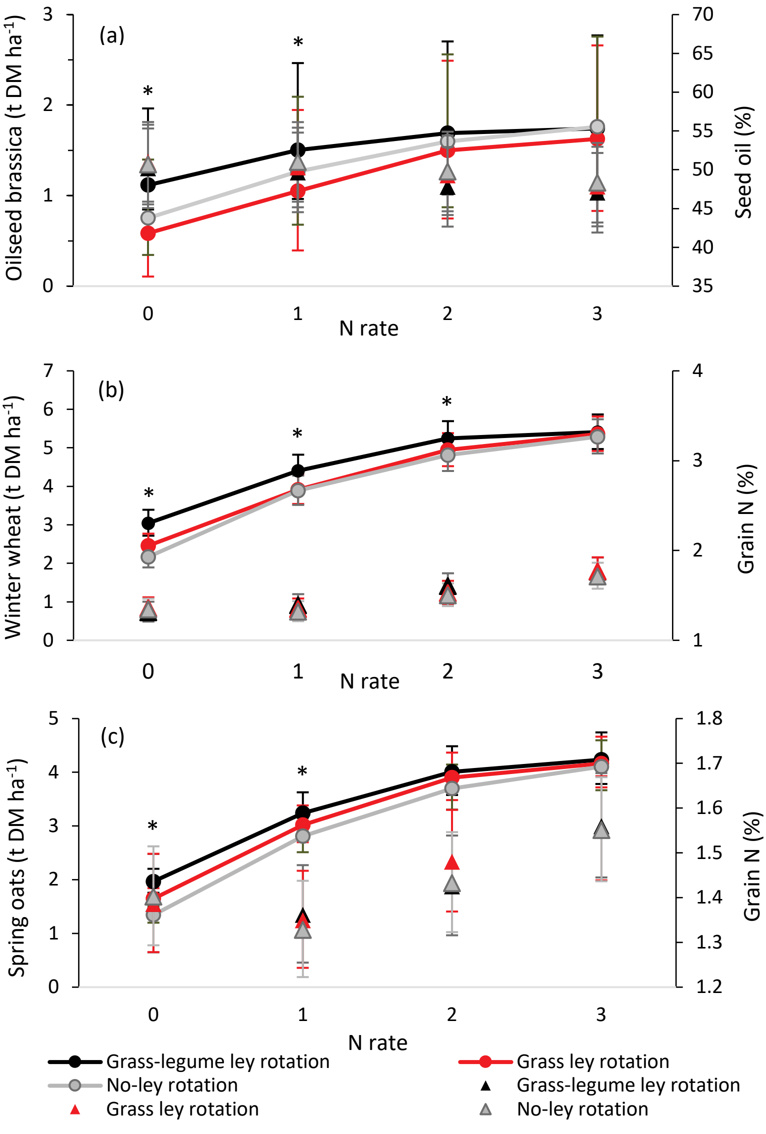


Figure 8. Seed and grain dry matter yields of the first three crops in the rotation, averaged across sites and eight rotation cycles, at four mineral N fertiliser rates: (a) oilseed brassicas, (b) winter wheat, and (c) spring oats, following either two years of grass or grass–legume ley, or spring wheat and fallow (no-ley rotation). Oil concentrations (oilseed brassicas) and grain N concentration (cereals) are shown on the right Y axis (triangles). Mineral N rates (N0–N3) were 0, 60, 120, and 180 kg N ha^{-1} for oilseed brassicas; 0, 45, 90, and 135 kg N ha^{-1} for winter wheat; and 0, 40, 80, and 120 kg N ha^{-1} for spring oats. Asterisks (*) indicate significant yield differences between rotations. Error bars represent 95% confidence intervals.

3.1.2 Soil fertility characteristics (Papers I and II)

Across cycles and N fertiliser rates, ley rotations had higher average topsoil total C and N concentrations than the no-ley rotation, by approximately 0.2 and 0.01 percentage units, respectively. In addition, the highest N fertiliser rate (N3) resulted in higher topsoil C and N concentrations than the lowest rate (N1), when averaged across rotations and cycles.

Over the 48-year period, topsoil C concentrations declined under low N input (N1) in the no-ley rotation, whereas they were maintained in the ley rotations at the same N rate (Figure 9a). At the highest N input rate (N3), C concentrations were maintained in all rotations. Topsoil total N concentrations declined over time regardless of rotation and N rate, decreasing by approximately 0.015 percentage units between the earlier cycles (2–3) and later cycles (8–9) (Paper I; see also Figure 10).

Across sites, the potential N mineralisation rate ranged from 2.5 to 3.0 g N kg^{-1} soil d^{-1} and was approximately 0.4 g N kg^{-1} soil d^{-1} higher in ley-based rotations than in the no-ley rotation. In the site-specific analysis, soils from the grass-legume ley rotation at Stenstugu demonstrated an additional increase in potential N mineralisation compared with the grass ley rotation. Mineral N fertilisation was also associated with higher potential N mineralisation compared with the unfertilised treatment (N0), whereas an increase in fertiliser rates did not lead to further significant increases.

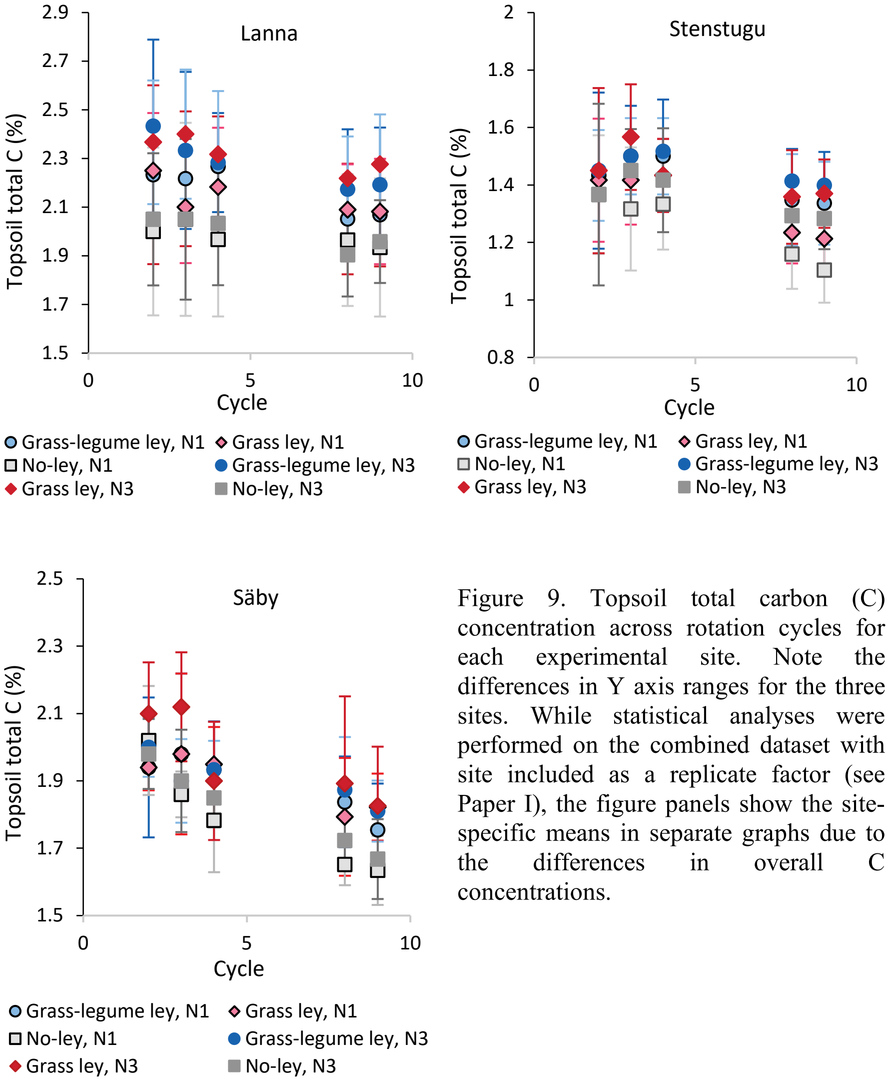
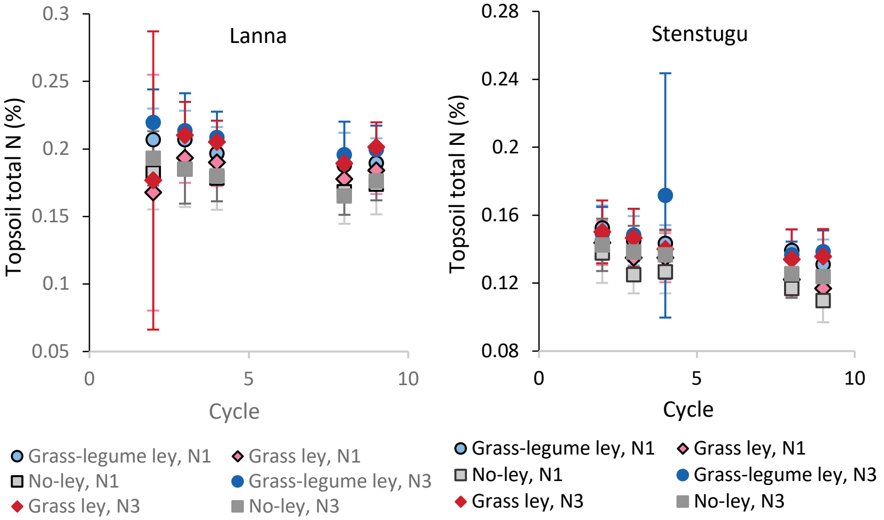


Figure 9. Topsoil total carbon (C) concentration across rotation cycles for each experimental site. Note the differences in Y axis ranges for the three sites. While statistical analyses were performed on the combined dataset with site included as a replicate factor (see Paper I), the figure panels show the site-specific means in separate graphs due to the differences in overall C concentrations.



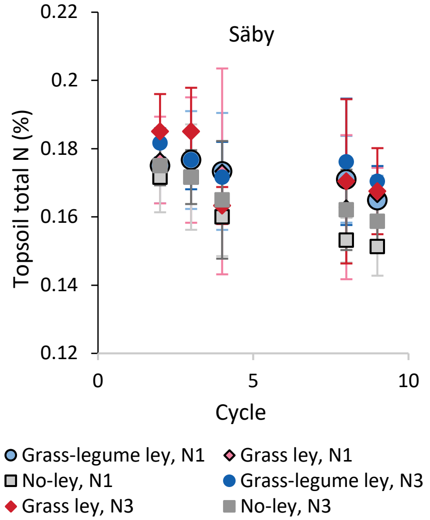


Figure 10. Topsoil total nitrogen (N) concentration across rotation cycles for each experimental site. Note the differences in Y axis ranges for the three sites. While statistical analyses were performed on the combined dataset with site included as a replicate factor (see Paper I), the figure panels show the site-specific means in separate graphs due to the differences in overall N concentrations.

The abundance of stable C, indicated by the strength of alkyl-C spectral signals, was higher in ley-based rotations than in the no-ley rotation across all sites. This pattern was supported by significant between-class differences in the BCA (randtest, $p < 0.05$) and is illustrated by the separation of rotations along the first axis (BCA axis 1) (Figure 11).

Moreover, the response to mineral N fertilisation differed between crop rotations and sites. At Stenstugu, N fertilisation increased stable C in all rotations, including the grass-legume ley rotation. In contrast, at Lanna and Säby, sites that are characterised by more fine-textured soils and higher total C concentrations (Table 1 in Paper II), soils under the grass-legume ley rotation exhibited weaker responses to mineral N fertilisation. Soils under the grass ley and the no-ley rotations generally demonstrated increases in stable C in response to N fertilisation across sites but varied in magnitude (Figure 12). The observed effect of treatments was supported by permutation tests (randtest; Paper II).

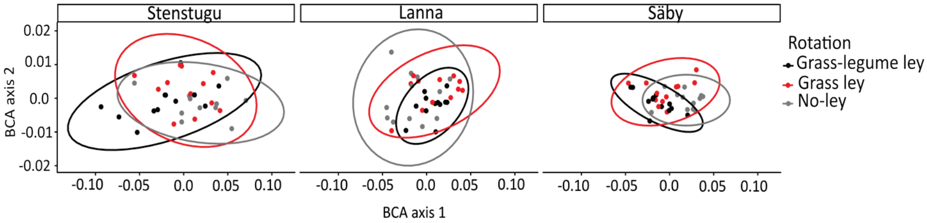


Figure 11. Between-class analysis (BCA) of alkyl-C related spectral signals showing the ordination of soil samples by crop rotation at three sites (Stenstugu, Lanna, Säby). Each point represents a soil sample, coloured by crop rotation. Ellipses represent the 95% confidence regions for each class (i.e. crop rotation), illustrating within-class variability. Separation of rotations along the first axis (BCA axis 1) reflects differences in alkyl-C-related spectral signals.

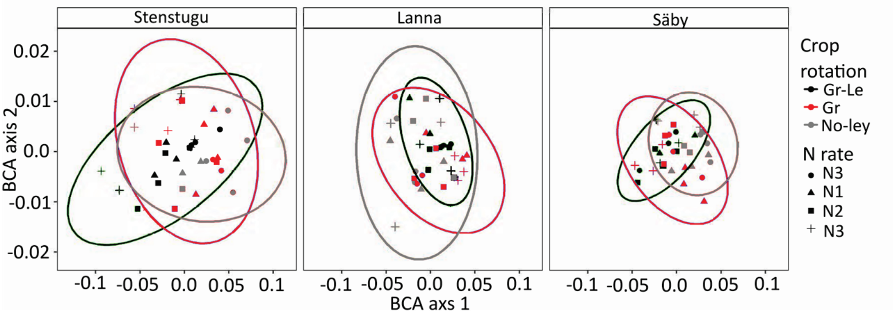


Figure 12. Between-class analysis (BCA) of alkyl-C related spectral signals showing the ordination of soil samples by crop rotation and mineral N fertilisation rate at three sites (Stenstugu, Lanna, Säby). Each point represents a soil sample, coloured by rotation and symbolised by N fertilisation rate. Ellipses represent the 95% confidence regions for each class (i.e. crop rotation × N rate), illustrating within-class variability. Separation of rotations along the first axis (BCA axis 1) reflects differences in alkyl-C-related spectral signals.

On average across sites and rotations, bulk density in the upper topsoil layer (0–14 cm) ranged from 1.36 to 1.41 g cm^{-3} across all N fertiliser rates (N0–N3). Bulk density was approximately 0.04 g cm^{-3} higher in unfertilised soils compared with N-fertilised soils, with no significant differences found among the fertiliser rates (N1–N3). In the site-specific analysis at Stenstugu, bulk density was lower under the grass-legume ley rotation than under the no-ley rotation (1.55 and 1.60 g cm^{-3}, respectively), whilst the grass ley showed intermediate values.

3.2 Establishment and assessment of clover in ley systems

3.2.1 Clover establishment and performance under autumn sowing (Paper III)

Following the system-level effects of ley inclusion on soil and crop performance (Section 3.1), this section focuses on the establishment and performance of clover within ley systems. Despite an autumn establishment, total ley yields (harvests 1–3) were within the typical range of productive forage systems in Sweden, yielding 7–14 t DM ha^{-1}. Averaged across treatments, yields were about 1.2 t DM ha^{-1} greater in mixtures of red clover than white clover (Figure 13a). Mixtures without perennial ryegrass produced 0.17 t DM ha^{-1} more than those that did include perennial ryegrass when averaged across site–year combinations, although no statistically significant differences were found within individual site–year combinations.

Clover proportion in the first ley harvest varied widely among site–year combinations, ranging from 0 to 45 % when averaged across treatments. Proportions were generally higher in mixtures of red clover than white clover (Figure 13b), with maximum values reaching 50 % and 20 %, respectively. Clover proportion was also higher following early cereal harvest (Figure 13c), averaged across site–year combinations and grass mixtures. In general, clover proportions were the highest in red clover mixtures following early cereal harvest, then red clover mixtures after late cereal harvest. These levels were often comparable to those in white clover mixtures following early harvest, although responses varied among site–year combinations. Under late cereal harvest, cereal species also affected clover proportion, with the lowest proportions following rye, which also produced the highest grain yields, whereas no differences in clover proportion were observed between triticale and wheat. Although patterns observed in the first ley harvest were also evident at the second and third harvests, the differences between treatments declined.

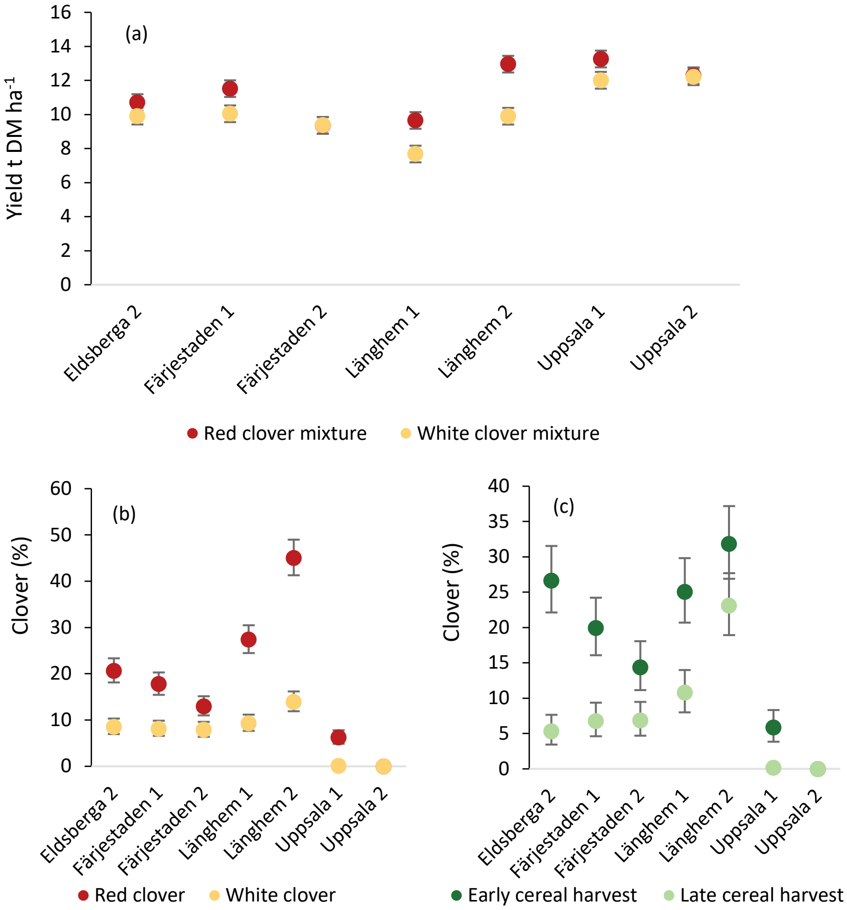


Figure 13. Total dry matter ley yields and clover proportions following autumn establishment across site–year combinations. (a) Total ley yield across three harvests for red clover and white clover mixtures. (b) Clover proportion in the first ley harvest for red clover and white clover mixtures. (c) Clover proportion in the first ley harvest under early and late cereal harvest. The full statistical analysis, including interactions among site–year combinations, cereal species, harvest system of cereal, clover species, and grass mixture, is presented in Paper III.

3.2.2 Comparison of methods for estimating clover proportion and early-season prediction of clover in the first harvest (Paper IV)

Overall, image-based CloverSense estimates tended to overestimate clover at low clover proportions (approximately 0–15 %) and underestimate clover at higher proportions (≥20 %), with underestimation increasing as the proportion of clover increased. This pattern was consistently observed across datasets and is illustrated in the pooled data (Figure 14a). Linear regressions between CloverSense estimates (Y) and clover proportions from reference measurements (X) across the four datasets showed variable correlations (R^2 = 0.30–0.81), with slope coefficients of 0.35–0.69 and intercepts of 1.7–9.1.

No clear relationship was found between the difference in grass and clover height and CloverSense estimation errors (Dataset I). Agreement between estimated and reference clover proportions varied across sampling occasions during the growing season, although the overall pattern of overestimation at low clover proportions and underestimation at higher proportions remained consistent (Paper IV).

Visually assessed clover proportion showed similar patterns to CloverSense estimates, which are also illustrated in the pooled data (Figure 14b).

Early-season estimates, conducted approximately one month prior to the first harvest, from both CloverSense and visual assessment explained approximately one-third of the variation in clover proportion from reference measurements at the first harvest. Prediction errors for CloverSense and visual assessments were 12–13 percentage units, indicating similar predictive performance between the two methods.

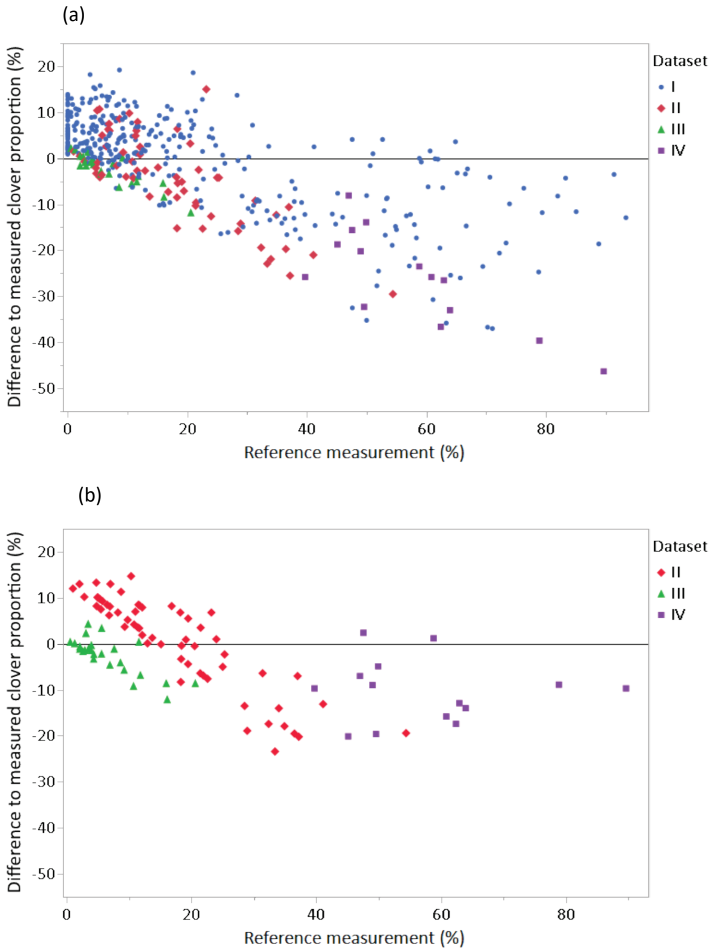


Figure 14. Difference between clover proportion estimates and clover proportions obtained from reference measurements. The horizontal line indicates zero difference, where negative values indicate underestimation and positive values indicate overestimation relative to the reference measurements. (a) CloverSense estimates; (b) visually assessed estimates. Different symbols and colours indicate different datasets I–IV. Data from all datasets were pooled to illustrate overall patterns.

4. Discussion

Maintaining crop productivity whilst preserving soil fertility in specialised arable farming systems with a limited use of temporary leys in crop rotations and little or no manure inputs can be a challenging process (Matson et al., 1997; Shi et al., 2022). Long-term experiments initiated to address concerns regarding declining soil fertility in such systems provide valuable opportunities to evaluate the role of rotational leys in sustaining soil function and crop performance (Bergkvist & Öborn, 2011). The results of this thesis demonstrate that the inclusion of leys in arable crop rotations without manure application can contribute to improved soil fertility and support crop productivity, particularly under low mineral N inputs (Papers I and II). Alongside these system-level effects, the studies included in the thesis also highlight practical aspects of integrating grass-clover leys into arable rotations, including methods for autumn establishment of mixed leys under Nordic conditions and approaches for assessing clover proportions in the field (Papers III and IV). Together, these findings contribute to a broader understanding of the agronomic benefits of ley inclusion into arable rotations, whilst also underscoring opportunities to integrate grass-clover ley by undersowing it in winter cereals and the conditions that influence successful clover establishment.

4.1 Crop productivity and soil fertility in ley-based rotations

4.1.1 Crop responses to rotational leys under different mineral N rates

Yield responses to rotational leys varied depending on the rate of mineral N fertiliser input. Although leys, particularly grass-legume leys, increased crop yields under no or low fertiliser rates, yield differences between rotations were small at higher N rates. This suggests that the relative contribution of soil-derived N compared with applied fertiliser becomes less important at high mineral input rates, consistent with previous findings (Drinkwater & Snapp, 2007). This may explain the similar yields and grain N concentrations measured at the higher N fertiliser rates regardless of rotation, despite the increased potential N mineralisation rates observed in the ley rotations. Grass leys generally displayed intermediate responses, often not significantly differing from either the grass-legume ley or no-ley rotations, indicating more limited effects than systems including legumes. Consequently, greater amounts of residual soil N may remain following harvest in the grass-legume ley rotation at higher N fertiliser rates, thereby increasing the risk of N losses to the environment. Such patterns have been reported in earlier studies which show that high N inputs from ley residues can result in substantial amounts of mineral N remaining in the soil, increasing the risk of losses through leaching or gaseous emissions when not fully utilised by crops (Torstensson, 1998; Saarijärvi et al., 2007).

The greater yield response of following crops in the grass-legume ley rotation at low N inputs is likely linked to the contribution of biologically fixed N and the relatively low C/N ratio of legume-derived residues, which may promote rapid decomposition and N mineralisation (Peoples et al., 2009; Chen et al., 2014). Increased yields persisted throughout the annual crop phase of the grass-legume ley rotation compared to no ley under both no and low mineral N fertiliser inputs. Thus, these results indicate that the ley effects extend beyond the immediate pre-crop effect and may persist for up to four years.

Winter wheat and spring oats showed stronger responses to mineral N fertilisation in later cycles than in earlier. This may primarily reflect the decline in topsoil total N concentrations over time, which likely reduced the indigenous soil N supply and increased crop dependence on fertiliser N, consistent with relationships reported by Poulton et al. (2023). Changes in fertiliser composition during the experimental period, from nitrate-based products to formulations containing ammonium, may also have contributed, particularly to grain N responses. Gradual replacement of cultivars over time may likewise have influenced responsiveness to applied N. On the other hand, the magnitude of the yield response did not increase over time, which may be related to the unchanged total topsoil C concentrations and the decline in total topsoil N over time. This suggests that the observed effects were primarily driven by the continued mineralisation of ley residues, as opposed to long-term changes in SOM.

In addition to yield effects, rotational grass-legume leys were particularly effective at improving the grain quality of winter wheat. Higher grain N and gluten concentrations at lower N fertiliser rates suggest that less N fertiliser is required to achieve a grain quality comparable to a rotation without grass-legume ley, likely due to increased availability and uptake of N from ley-derived residues. The consistently higher gluten concentration in the grass-legume ley rotation may further indicate improved N availability during the later crop development stages, which is known to favour gluten formation (Xue et al., 2016).

Oil concentration in oilseed brassicas was slightly lower following grass-legume ley, which may reflect higher soil N availability in line with previous findings of the negative relationship between N supply and oil concentration (Brennan et al., 2016; Tian et al., 2020). When considering oil yield across N fertilisation rates, the lower seed oil concentration in the grass-legume ley rotation appeared to be balanced by greater seed yields at low N rates. Taken together, these findings suggest that an important benefit of grass-legume leys is their capacity to supply readily available N following ley termination, which may reduce the need for mineral N inputs in subsequent crops. This could lower production costs and decrease the risk of N losses to the environment. Although improved N supply was likely important, other mechanisms may also have contributed to the observed yield responses.

However, for ley cultivation to be relevant in specialised arable systems, the produced biomass must serve a purpose beyond just livestock feed, and practical constraints such as the need for specialised machinery may limit its adoption of leys.

4.1.2 Soil fertility processes in ley rotations

The yield benefits observed in ley-based rotations at no or low N inputs were associated with improvements in soil N supply, reflecting the higher mean concentrations of total topsoil C and N observed in the ley rotations, as well as potential improvements in soil physical properties that may enhance root development and nutrient uptake efficiency. However, despite these higher mean concentrations in the ley-based rotations, total topsoil N declined over time across all rotations and N fertiliser rates, with lower values observed in the later rotation cycles compared with the earlier ones. Similar long-term trends have been reported in other arable systems, where soil N appear more sensitive than soil C to continued crop removal and N losses (Poulton et al., 2023). This likely reflects the greater susceptibility of N to export in harvested crops and losses through leaching or gaseous emissions, whereas soil C can be retained in more stable organic matter pools (Drinkwater & Snapp, 2007). Thus, whilst the ley rotations maintained higher N levels relative to the no-ley rotations, they did not prevent a long-term decline in total soil N concentrations. The higher mean C and N concentrations were likely the result of greater SOM inputs through the longer vegetation periods and greater root biomass of ley crops (Bolinder et al., 2007; Börjesson et al., 2018), as well as less topsoil C mineralisation because of the absence of tillage during ley phases (Meurer, et al., 2018). The interpretation of improved soil N supply associated with higher total C and N levels is supported by the higher potential N mineralisation rates measured in the ley rotations. This is consistent with both the stronger yield responses at low N fertiliser rates and the well-established relationship between SOM and the supply of mineralisable N, where higher organic C levels provide a larger pool of organically bound N that can potentially become available to plants through mineralisation (Jarvis et al. 1996).

The relatively low C/N ratio of legume-derived residues, compared with non-N_2-fixing crops, may promote faster decomposition and earlier net N mineralisation, thereby increasing N supply to the subsequent crops (Kaleem Abbasi et al., 2015). This effect of legume inclusion on potential N mineralisation rate was only pronounced at the Stenstugu site, which is characterised by a coarser soil texture and lower total C concentration, indicating a more responsive system to N-driven increases in organic C inputs (Bolinder et al., 2020; Gocke et al., 2023). This site-specific responsiveness is supported by Nilsson et al. (2023), who, based on an analysis of the same long-term experiment, reported that soil organic carbon (SOC) depletion was reduced under high N fertiliser inputs, with particularly pronounced effects at the Stenstugu site.

Although mean concentrations of C and N were higher in the ley rotations when averaged across time (cycles) and N fertiliser rates, this did not translate into an increase in topsoil total C over time, contrasting to previous studies (e.g. Jensen et al., 2022; Lang et al., 2025). This suggests that the system was close to a steady state throughout the study period, where C inputs were similar to decomposition losses. One likely explanation is the relatively high initial topsoil C concentrations at the experimental sites, which are known to limit further increase in soil C due to the higher inputs required to reach a new equilibrium (Bolinder et al., 2010). Additionally, the absence of manure application likely restricted C inputs compared with studies where manure was applied to the ley systems (e.g. Jensen et al., 2022; Lang et al., 2025). Furthermore, the relatively short duration of the ley phase (two out of six years) limited the period during which increased C inputs and reduced soil disturbance could promote soil C accumulation. In contrast, in the no-ley rotation, total C concentrations decreased at low mineral N fertiliser rates but remained unchanged at the high N rate. This suggests that the inclusion of leys helped to maintain soil C concentrations under low N fertiliser inputs.

The ley rotations were also associated with a greater abundance of more stable organic C fractions (alkyl-C). This is consistent with the higher mean total C concentrations observed in the ley rotations and with previous findings from Swedish agricultural soils, where alkyl-C has been shown to be positively correlated with SOC (Wetterlind et al., 2022). However, the difference in soil C between rotations may have also been influenced by the one-year fallow in the no-ley rotation, which likely resulted in lower biomass inputs than during the ley phases. This reduction in C inputs could have possibly led to a net loss of SOM due to decomposition not being compensated by sufficient organic C inputs (Karbozova–Saljnikov et al., 2004), thereby contributing to lower total C compared with the ley rotations.

The increased abundance of alkyl-C is consistent with a greater presence of stable SOM fractions. This does not necessarily imply a shift in SOM composition but may instead reflect an overall increase in total C, resulting in greater amounts of both stable and labile SOM fractions. In line with this, the higher potential N mineralisation rate observed in the ley rotations suggests that these systems may also contain greater amounts of labile and N-rich SOM fractions. However, this could not be directly assessed with the methods applied in this study. The weaker increase in alkyl-C abundance in response to mineral N fertilisation in the grass-legume ley rotation, compared with the other rotations, may indicate that systems which are partly reliant on biological N fixation are less affected by external N inputs. This may be due to a greater reliance on internally regulated N cycling (Fontaine et al., 2024), as legume-based ley systems have been shown to increase the availability of soil N and thus reduce the need for external N inputs (Poulton et al., 2023). As a result, SOM dynamics and composition in these systems may already resemble those of more highly fertilised systems.

Reduced bulk density in ley-based rotations was only observed at the Stenstugu site, but this nevertheless suggests the potential for structural improvements following ley phases, which is in accordance with previous findings (Jarvis et al., 2017). Such effects are often more pronounced in coarse-textured soils with low initial SOM levels, which tend to respond more strongly to increased N inputs (Oades, 1993; Ling et al., 2025). As a result, increased SOM can contribute to improved soil structure and reduced bulk density over time (Arvidsson, 1998; Bolinder et al., 2010). In contrast, clay-rich soils frequently exhibit smaller structural responses to management because clay–mineral interactions and natural swell–shrink processes already promote aggregate formation (Oades, 1993). This could explain the lack of ley-induced effects on bulk density at the other sites, which had either a higher clay content (Lanna) or lower sand concentrations (Säby). Yet, the application of N fertiliser reduced bulk densities at all sites, compared to without fertilisation, suggesting that increased biomass production and related SOM inputs can influence soil structure even in clay-rich and/or less coarse textured soils. However, such effects are likely smaller and more difficult to detect. These results suggest that rotational leys may contribute to structural improvement in coarse-textured soils where soil structure is more dependent on SOM content (Oades et al., 1993).

4.2 Autumn establishment of grass-clover leys and clover assessments

4.2.1 Autumn establishment of grass-clover leys

Higher clover proportions and ley yields were generally observed in red clover mixtures compared with white clover mixtures, suggesting that clover species was the primary determinant of clover abundance in the ley. This is consistent with the greater competitive ability of red clover for light during establishment, due to its more upright growth habit compared with white clover (Black et al. 2009). Early cereal harvest also increased clover proportions at most sites, although this effect was not consistent across site–year combinations, indicating that reducing light competition earlier in the season may promote clover performance.

The importance of early growth conditions was reflected in the site-specific responses observed at the experimental sites. At the southern sites, white clover mixtures reached adequate clover proportions, particularly when cereals were harvested early. This indicates that favourable conditions, such as higher temperatures and a longer growth period before frost, can partly compensate for the lower light competitiveness of white clover (Black et al. 2009).

At the northernmost site (Uppsala), red clover achieved a more successful establishment than white clover, and early cereal harvest was crucial to reach adequate clover proportions, indicating that species choice and management practices that reduce light competition are particularly important when establishing grass-clover leys in autumn at these latitudes. The complete absence of clover in Uppsala at the second experimental round further illustrates how autumn establishment can be constrained by seasonal weather conditions, particularly in environments with a narrow sowing window. Overall, these results indicate that successful autumn establishment of grass–clover leys in northern environments is possible but strongly depends on clover species and establishment conditions, particularly the ability to tolerate light competition and achieve sufficient establishment before winter.

4.2.2 Clover estimation methods and early-season clover prediction

Maintaining a target clover proportion and forage protein content requires management of N fertilisation, as high N inputs can favour grasses over clover. Botanical composition can thus serve an indicator for adjusting, for example, fertiliser application rates to achieve desired levels of clover proportions and forage quality. The agreement between image-based CloverSense estimates and biomass-derived reference measurements was moderate, with overestimation at low clover proportions and underestimation at high proportions. One limitation is that canopy cover images represent visible clover proportions, whereas botanical analysis reflects biomass-based proportions, rendering it difficult to reach a complete agreement between the two approaches (Skovsen et al., 2017). Although the image-based method is designed and trained to estimate biomass fractions from canopy images, the relationship between visible cover and biomass is not 'fixed' and may vary depending on factors such as species structure, occlusion, and growth stage. For example, differences in growth habit between clover species, as well as changes in canopy structure over time due to, for instance, flowering, can alter the relationship between the two-dimensional canopy cover and actual biomass. These factors may have contributed to the observed differences between the methods. Additional sources of disagreement may include camera setup, image scale, and lighting conditions, which influence pixel-based classification (Skovsen et al., 2017; Skovsen et al., 2021) and are difficult to control under field conditions. Low-growing species such as white clover (Black et al., 2009) may be partially covered by taller vegetation, reducing the correspondence between canopy images and biomass-based clover proportions, although this effect appeared to be limited in this study. The presence of senescent plant material and flowers may alter the canopy colour and texture, thereby affecting pixel-based species classification (Skovsen et al., 2021) and contributing to the reduced agreement between CloverSense and reference measurements observed in this study. The bias found with CloverSense was relatively systematic, suggesting that it may be predictable and could be potentially reduced through calibration approaches. Such approaches could account for non-linear relationships between canopy cover and biomass and may be additionally improved through training data that represents a wider range of field conditions, growth stages, and image characteristics.

Visually assessed clover proportions exhibited similar deviation patterns to CloverSense estimates. These deviations may reflect known limitations that are inherent to visual methods for estimating species compositions, including observer bias and difficulty in separating species in dense canopies, where small differences are challenging to detect (Vittoz & Guisan, 2007; Peratoner & Pötsch, 2019).

Differences in sampling scale between estimates and biomass-based reference measurements may have also contributed to the disagreement between methods. In Datasets II and III for example, CloverSense estimates were derived from a limited number of images that represent smaller areas than the biomass-based reference measurements and visual assessments, which may not capture spatial variability within heterogeneous fields. Moreover, reference measurements based on subsamples may not fully represent the true clover proportions. Together, differences in spatial scale and sampling approach likely contributed to the observed disagreement between estimated and measured values. However, whilst this probably contributed to random variation in the data it likely does not explain the systematic pattern of overestimation at low clover proportion and underestimation at high proportions observed across datasets.

Accurate assessment of clover proportions is important for fertiliser management, as higher clover abundance can reduce the need for N-fertiliser inputs and influence forage quality in mixed grass-clover leys (Nyfeler et al., 2009; McDonagh et al., 2017). Highly accurate estimates, however, may not be necessary for management decisions such as N fertilisation, because approximate estimates of clover proportion are often sufficient in practice. This assumes that estimates are sufficiently reliable and preferably supported by calibrated relationships between canopy cover and biomass. In this study, deviations of around 20 percentage units, as observed with CloverSense at higher clover proportions, may affect fertilisation decisions, whilst overestimation at very low clover levels may result in reduced N application when clover contribution is minimal. The reported increase in deviations with higher clover abundance indicates that clover proportion was a primary factor influencing the agreement, whereas effects of sampling time during the growing season appeared to be less consistent.

Early-season image-based estimates using CloverSense and visual assessments demonstrated limited accuracy in predicting clover proportions at the first harvest, with low R^2 values and relatively large prediction errors. This indicates a limited potential for accurate quantitative prediction under the conditions of this study. The limited accuracy was not unexpected, as both grass and clover continue to grow between image acquisition and the first harvest. The similar performance between the two methods suggests that the advantage of CloverSense over visual assessments is limited within this context. Whilst CloverSense may reduce observer-related variability and provide more consistent estimates, visual assessments by trained observers may achieve comparable accuracy under field conditions (Peratoner & Pötsch, 2019). Certain limitations were also observed in the image classification, as senescent plant material and bare soil were classified as green cover, highlighting the challenges in distinguishing between living vegetation and background material.

Early-season estimates may provide indicative information of clover proportion, allowing areas with relatively high or low clover abundance prior to first harvest to be identified.

Although this study was conducted at small plot scale, image-based approaches could be applied at larger scales, for example through the use of drones, which would enable a rapid assessment of clover distribution across fields. However, the observed systematic bias and variability under field conditions suggest that further calibration and training under a wider range of conditions are needed before the method can be reliably applied in practice. With improved accuracy, CloverSense has the potential to serve as a practical tool that supports management decisions, particularly when approximate estimates of clover proportion are sufficient.

4.3 The role of leys in arable cropping systems – concluding discussion

Using long- and short-term agricultural field experiments, the overall aim of this thesis was to assess the role of rotational leys in Nordic arable cropping systems, with a particular emphasis on annual crop performance, soil fertility, and ley establishment strategies. Previous research has shown positive effects of leys on SOM and soil fertility, but these effects are often studied in systems where leys are combined with manure inputs (e.g. Albizua et al., 2015; Puerta et al., 2018; Jensen et al., 2022; Lang et al., 2025), making it difficult to separate their individual contributions. The results of this thesis demonstrate that rotational leys, even without manure application and when included for only two years in a six-year rotation, can improve soil fertility and support crop performance under reduced mineral N fertiliser inputs. This highlights their potential role in reducing external inputs in stock-less arable farming systems. These results also show that site-specific factors, such as soil texture and initial C levels, may influence the magnitude of these effects. In addition, the findings demonstrate practical opportunities for integrating grass-clover leys into arable systems through autumn-sowing with winter cereals, and for supporting management decisions through field-based monitoring of clover abundance. Whilst the image-based method exhibited a moderate agreement with reference measurements, its potential for practical use depends on further calibration and validation.

Despite these benefits, the wider adoption of leys in specialised arable systems will depend on their practical and economic feasibility. In particular, the need for different machinery and management compared with annual cropping systems represents a potential barrier. Addressing such constraints, along with developing viable pathways for biomass utilisation, will be important for enabling the integration of leys into stock-less arable systems.

5. Conclusions

- Grass-legume leys increased yields of subsequent annual crops at low or no mineral N input and reduced the N fertiliser required to achieve target grain N and gluten concentrations in winter wheat, with similar grain N levels achieved at lower N rates than in no-ley rotations. Rotational leys also helped to maintain topsoil C levels under low N inputs.
- Ley rotations increased potential N mineralisation rates and stable C abundance compared with no-ley rotations. Mineral N fertilisation also increased N mineralisation and reduced bulk density across all sites, whereas ley effects on bulk density were only observed at the more coarse-textured site with a relatively low initial C.
- Successful autumn establishment of undersown grass-clover ley in winter cereals depend on early sowing and reduced crop competition. Red clover mixtures performed better overall, and early cereal harvest increased clover proportions.
- Visual and image-based (CloverSense) methods showed moderate agreement with reference measurements on clover proportion. Whilst visual estimation relies on observer experience, the CloverSense estimates exhibited systematic bias, indicating that further calibration is required before the method can be reliably used in decision support.
- Overall, rotational leys, especially those with legumes, can enhance soil fertility and reduce the reliance on mineral N fertiliser in Nordic arable systems, although the effects depend on soil conditions and management, and can be supported by practical establishment strategies such as autumn sowing in winter cereals.

6. Future research

While the results of this thesis provide insights into the role of rotational leys without manure application in arable cropping systems, several aspects need further investigation. As fallow periods are less common in modern cropping systems and may have influenced soil fertility indicators, further studies should compare no-ley rotation without fallow to ley-based systems to better isolate ley effects. Further studies on of the effects of varying the number of ley years (without manure) within crop rotations would be valuable, as this study only assed a two-year ley period within a six-year rotation, limiting conclusions about the optimal duration of ley phases. Moreover, this study did not address all components of the SOM, and further research is needed to assess changes SOM composition. The accuracy of the image-based approach for estimating clover proportion could be improved. This includes evaluating calibration approaches, as well as addressing classification challenges such as distinguishing between living vegetation and background material (e.g. bare soil). In addition, for leys to be relevant in specialised arable systems, further research is needed on practical and economically viable strategies for managing and utilising ley biomass. This includes addressing logistical constraints, such as limited access to harvesting equipment, and exploring practical and economically viable solutions for biomass management and utilisation.

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Popular science summary

Before the mid-20th century, most European farms were mixed systems in which livestock and crop production were closely linked. Nutrients were recycled through manure, and crop rotations included leys—perennial forage crops made up of grasses and/or legumes. These leys provided fodder for livestock, helped to control weeds, pests, and diseases, and aided in maintaining soil fertility. From the 1950s onwards, agriculture became increasingly specialised and intensified in response to factors such as the growing food demand, availability of cheap synthetic fertilisers, crop protection chemicals (pesticides), and agricultural policies promoting such developments. These changes increased productivity but also contributed to a gradual separation of livestock and crop production. Whilst this development improved labour efficiency, it also created environmental challenges. In specialised livestock systems, nutrient surpluses lead to nitrogen losses to water and air, and to phosphorus accumulation in soils. Over time, this accumulation increases the risk of phosphorus losses to water, contributing to eutrophication. In specialised arable systems, crop diversity is often low and cereals dominate, resulting in a greater reliance on fertilisers and pesticides. This can have negative effects on biodiversity and the surrounding environment. Such arable systems can also contribute to soil degradation, particularly through declines in soil organic matter, which is essential for many soil functions.

Previous research suggests that reintroducing perennial leys into crop rotations can improve soil fertility and reduce environmental impacts in modern agriculture. This is due to several benefitable effects of leys, including their ability to supress weeds and diseases, as well as their potential to increase soil organic matter, which is essential for many soil functions to sustain crop productivity. However, most previous studies have focused on systems where leys are combined with manure inputs, which are known to enhance overall soil fertility, and less attention has been given to the role of leys in arable systems without manure.

This thesis examines the role of grass and grass-legume leys in arable crop rotations in Sweden. The thesis also evaluates the autumn establishment of grass–clover leys sown together with winter cereals, with a focus on clover performance. It also includes an evaluation of methods for estimating and predicting clover proportions in the field, which are important for informing ley management.

Results from Papers I and II show that rotations including two years of grass or grass-clover ley in a six-year rotation improved soil fertility. Soil carbon concentrations were maintained in the rotations with ley, unlike the rotation without ley, where soil carbon declined at low nitrogen fertiliser inputs. Ley rotations had a greater capacity to mineralise nitrogen. Together with higher carbon and nitrogen concentrations, this suggests improved soil fertility. Rotations including grass–legume leys also led to higher crop yields when little or no mineral fertiliser was used. In addition, winter wheat grain quality could be maintained with lower fertiliser inputs compared with the system without ley.

Building on the demonstrated benefits of grass-legume leys, Paper III explored an additional strategy to integrate grass-clover leys into crop rotations by sowing them together with winter cereals in autumn, rather than establishing them in spring together with spring-sown cereals, which is the current common practice. The results show that clover can successfully establish under current climatic conditions, but this success strongly depends on species choice and management. In particular, clover establishment and performance were improved when more light-competitive clover species (red clover) were used, and when the cereal crop was harvested early, allowing more light to reach the ley.

In Paper IV, CloverSense, a photo-based method for estimating the proportion of clover in grass-clover leys, was evaluated alongside visual estimates by comparing both to reference measurements. Both showed moderate agreement with the reference and performed similarly. CloverSense showed systematic biases, but these could potentially be reduced through further calibration and training. With improved accuracy, the method could help to identify fields with a relatively high or low clover content, allowing nitrogen fertilisation to be adjusted accordingly.

For leys to be relevant in arable farms without livestock, the ley biomass must be used as something other than animal feed, for example, in systems such as biogas production where the residues can be returned to the soil as fertiliser. With such uses, leys could help to improve soil fertility and support crop production with a reduced reliance on external inputs.

Populärvetenskaplig sammanfattning

Fram till mitten av 1900-talet dominerades europeiskt jordbruk av blandjordbruk, med både djurhållning och växtodling på samma gård. Näringsämnen återfördes till åkermarken genom stallgödsel, och växtföljder innehöll ofta vallar – fleråriga fodervaxter bestående av gräs och/eller baljväxter. Vallarna gav foder till djuren, men bidrog också till att hålla nere ogräs, skadegörare och sjukdomar samt till att bevara markens bördighet. Från 1950-talet och framåt blev jordbruket alltmer specialiserat och intensivt. Bakom utvecklingen låg bland annat en växande efterfrågan på livsmedel, tillgång på billiga mineralgödselmedel och kemiska bekämpningsmedel, samt jordbrukspolitik som gynnade denna omställning. Även om denna utveckling förbättrade arbetseffektiviteten skapade den också miljöproblem. I specialiserade djurhållningssystem kan överskott av näringsämnen leda till kväveförluster till vatten och luft samt till ansamling av fosfor i marken. Med tiden ökar risken att fosfor läcker ut till vattendrag och bidrar till övergödning. I specialiserade växtodlingssystem odlas ofta få grödor, där spannmål dominerar. Det ökar beroendet av gödselmedel och bekämpningsmedel och kan påverka både biologisk mångfald och omgivande miljö negativt. Sådana system kan också försämra markbördighet, bland annat genom minskade halter av organiskt material i jorden, vilket är viktigt för många av markens funktioner.

Tidigare forskning visar att fleråriga vallar i växtföljden kan förbättra markbördighet och minska jordbrukets miljöpåverkan. Det beror bland annat på att vallar kan hämma ogräs och sjukdomar samt öka mängden organiskt material i marken, vilket bidrar till funktioner som är viktiga för grödornas produktivitet. De flesta tidigare studier har dock undersökt system där vall kombinerats med stallgödsel, som i sig är känd för att stärka markbördighet. Vallarnas betydelse i växtodlingssystem utan stallgödsel har däremot fått mindre uppmärksamhet.

Denna avhandling undersöker rollen hos gräs- och gräs-baljväxtvallar i växtföljder med ettåriga grödor i Sverige. Avhandlingen utvärderar också höstetablering av gräs-klövervallar sådda tillsammans med höstspannmål, med fokus på klöverns etablering och tillväxt. Vidare utvärderas metoder för att uppskatta och förutsäga klöverandelar i fält, vilket kan ge underlag för planering och anpassning av vallskötseln.

Resultaten från artikel I och II visar att växtföljder som inkluderade två år med gräs- eller gräs-klövervall i en sexårig växtföljd förbättrade markbördigheten. Halterna av markkol bibehölls i växtföljder med vall, till skillnad från växtföljden utan vall, där markkolet minskade vid låga kvävegödselgivor. Växtföljder med vall uppvisade också högre potentiell kvävemineralisering. Tillsammans med högre halter av kol och kväve tyder detta på bördigare jordar. Växtföljder med gräs-baljväxtvallar gav också högre skördar när lite eller ingen mineralgödsel användes. Dessutom kunde kvaliteten på höstvete upprätthållas med lägre gödselgivor jämfört med systemet utan vall.

Med utgångspunkt i de positiva effekterna av gräs-baljväxtvallar undersökte artikel III en annan strategi för att integrera gräs-klövervallar i växtföljder. Vallarna såddes då tillsammans med höstspannmål på hösten, i stället för att etableras på våren tillsammans med vårsådda spannmål, vilket är vanligast i dag. Resultaten visar att klöver kan etablera sig väl under dagens klimatförhållanden, men att utfallet i hög grad beror på artval och skötsel. Klöverns etablering och tillväxt förbättrades särskilt när mer ljuskonkurrenskraftiga arter, såsom rödklöver, användes samt när spannmålsgrödan skördades tidigt så att mer ljus nådde vallen.

I artikel IV utvärderades CloverSense, en fotobaserad metod för att uppskatta andelen klöver i gräs-klövervallar, tillsammans med visuella bedömningar genom jämförelse med referensmätningar. Båda metoderna visade måttlig överensstämmelse med referensmätningarna och gav likvärdiga resultat. CloverSense uppvisade systematiska fel, men dessa skulle potentiellt kunna minska genom ytterligare kalibrering och träning. Med förbättrad noggrannhet skulle metoden kunna hjälpa till att identifiera fält med relativt hög eller låg klöverhalt, vilket skulle möjliggöra en anpassad kvävegödsling.

För att vallar ska vara relevanta på gårdar utan djurhållning behöver vallbiomassan användas till annat än djurfoder, till exempel som substrat i biogasproduktion där restprodukterna kan återföras till marken som gödsel. Med sådana användningsområden kan vallar bidra till förbättrad markbördighet och en växtodling med mindre beroende av externa insatsmedel.

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

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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

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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-sub-plots. 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 (25°C) 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 1350°C 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

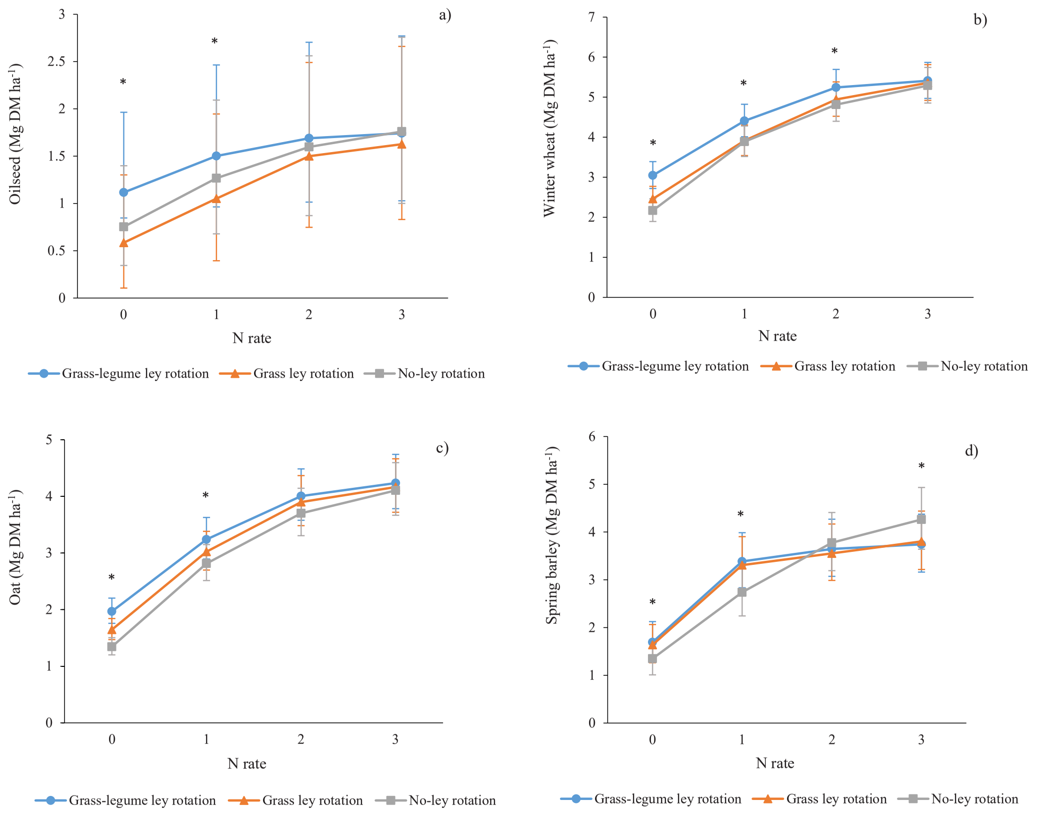

Fig. 1. Arable crop seed and grain dry matter yields, averaged across sites (see Table 1), 8 cycles of six-year crop rotations, in different rotations per N fertilisation rate (Table 1) of (a) oilseed rape grown as the first crop, (b) winter wheat as the second crop, (c) spring oat as the third crop 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 undersown 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).

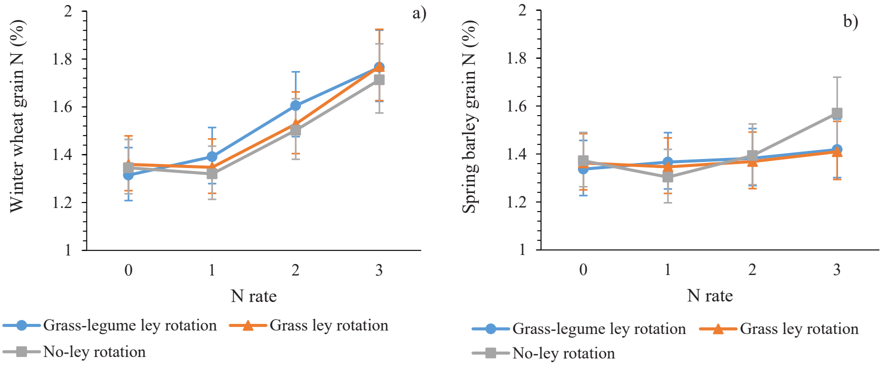

Fig. 2. Grain N concentration in (a) winter wheat grown as the second crop after two years of ley (in the mixed grass-legume ley and grass ley rotations) or spring wheat and fallow (in the no-ley rotation) and (b) grain N concentration in spring barley grown in the fourth year after ley (in the ley rotations) 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 %

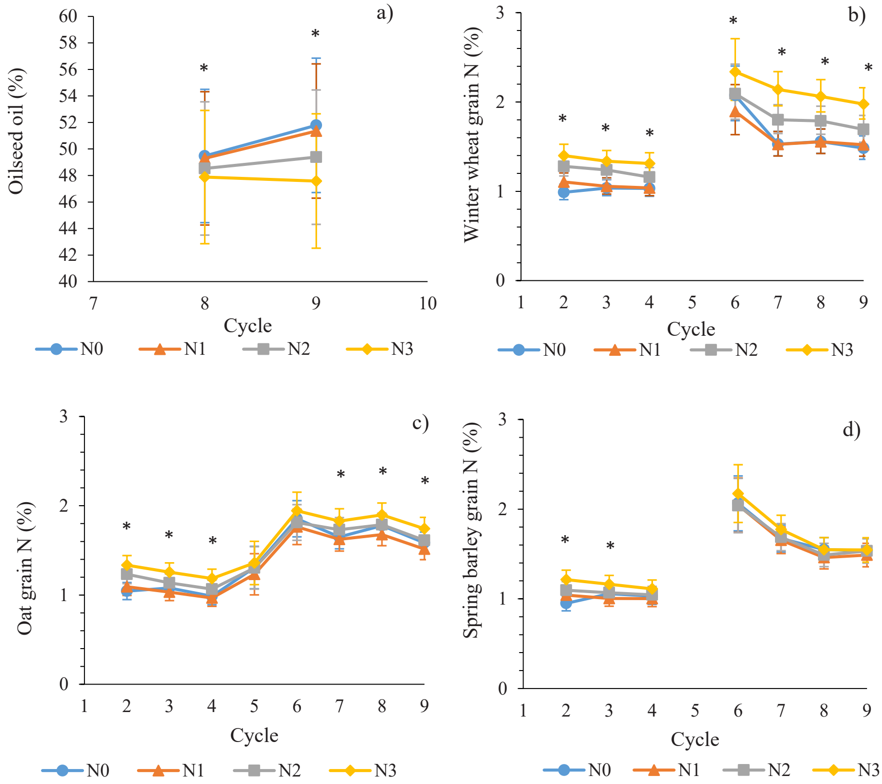

Fig. 3. Trends in oilseed rape oil and grain N concentration averaged over rotations per N rate. (a) Oil in oilseed rape grown as the first crop, (b) grain N in winter wheat as the second crop, (c) grain N in spring oats as the third crop and (d) grain N in spring barley grown the fourth year after two 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).

	Topsoil		Topsoil		Topsoil	
Treatment	C	P-value	N	P-value	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.

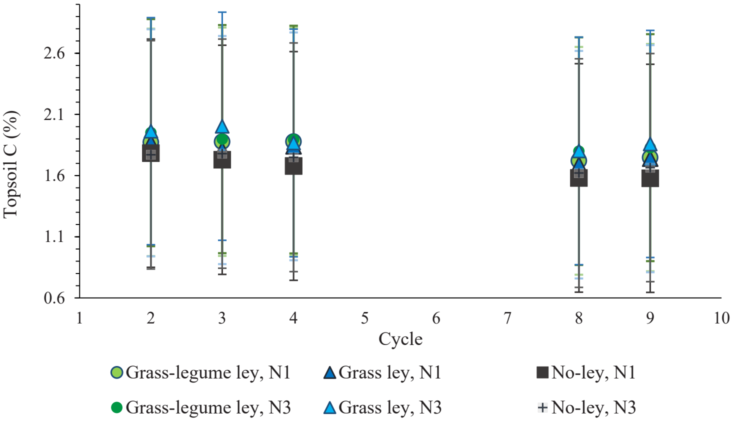

Fig. 4. Mean topsoil carbon (C) concentrations (%) per rotation and nitrogen (N) rate over cycles. Error bars indicate confidence intervals. Number of samples per cycle was 102 or 108. Cycles 5–7 are excluded because samples were not taken in all years during these cycles. Topsoil C concentration in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

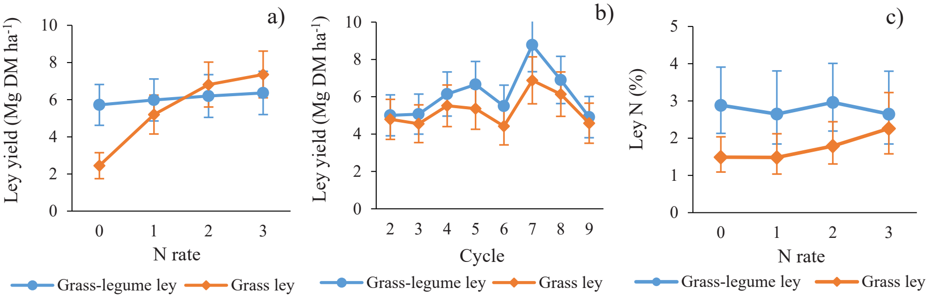

Fig. 5. Ley total dry matter yield and nitrogen (N) concentration in the grass-legume ley and grass ley. (a) Ley biomass yield per N rate (N0-N3) across cycles, (b) ley biomass yield per cycle of full six-year crop rotations and (c) ley N concentration per N rate and ley type across cycles. For ley N 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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Reintroducing leys into arable crop rotations may improve soil fertility and reduce input dependency. However, long-term effects of leys in systems without manure, and alternative establishment methods for changing climates remain uncertain. This thesis evaluates leys in cropping systems using Swedish field experiments. Results show that leys can reduce reliance on nitrogen fertiliser and improve soil fertility, and that clover in mixed leys can establish when undersown in autumn, with the magnitude of effects depending on management and site conditions.

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