Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

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– an analysis of Swedish long-term experiments and SOC modelling

Lina Wu

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Lina Wu

Supervisor: Lorenzo Menichetti, SLU, Department of Ecology
Assistant supervisor: Thomas Kätterer, SLU, Department of Ecology
Examiner: Martin Bolinder, SLU, Department of Ecology

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Walk on Earth as if you
were kissing her with your feet

- *Thich Nhat Hanh*

This research included an internship at Greppa Näringen to get applied knowledge of the consultant tool Odlingsperspektiv, which was financially supported by KSLA.

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Abstract

C sequestration in soils is a potential way to mitigate climate change and increase the fertility of arable soils. Ley has long been known to deliver more C to its root systems compared to annual crops. Therefore, it has been suggested as a potential C sequestration strategy. My objective was to evaluate the SOC sequestration potential of ley in Nordic countries by studying three Swedish long-term experimental sites. From each site a ley rotation and barley monoculture rotation were selected, and mineral- was compared to combined fertilization. Two of the sites (in N. Sweden) were measured for SOC stocks, while corresponding data was available for the third site (in S. Sweden). Higher topsoil SOC stocks was found for ley rotation than barley rotation for all sites. The effect from SOC stocks on soil aggregate stability was determined on the soil samples by analysing readily dispersible clay (RDC) and water stable aggregates (WSA). Ley might have influenced RDC positively, while mineral fertilization and WSA was found to correlate positively.

As a second part of the study, I modelled SOC stock changes with ICBM, a SOC model used in National SOC Inventory reporting system and in the consultant tool OP. OP uses a simplified version of ICBM with default values. Samples from the soil archive from each of the northern experiments were included in the modelling. I used yield data records to estimate belowground C inputs from plant residues using shoot to root ratios. The predictions with OP matched in many cases better to measured SOC stocks than ICBM, suggesting that the default values in OP worked relatively well.

The models matched the measured SOC stocks in the southern site. However, the models did not always apply to the northern sites. One explanation lies in the high uncertainty when estimating initial SOC stocks and imprecision in estimations of belowground C. Although the SOC stocks decreased in the northern sites (all treatments), the SOC stocks remained higher in ley rotation than in barley rotation. Combined fertilization had a positive effect upon the SOC sequestering potential of ley at the northern sites. This could however not be evaluated for the southern site. Finally, the SOC stocks increased for ley rotation in the southern site and the SOC stocks in ley rotation in one of the northern sites did not start to decrease until after 30 years, suggesting a SOC preserving potential in addition to the observed SOC sequestering potential of ley.

Keywords: carbon sequestration, soil aggregate stability, readily dispersible clay, water stable aggregates, SOC stocks, ICBM, Odlingsperspektivet

Sammanfattning

Kolinlagring i åkermark är ett potentiellt sätt att mildra klimatförändringarna och öka markbördigheten. Man har länge känt till att vall levererar mer kol till rotsystemen än annuella grödor, varför vallodling föreslagits som en potentiell kolinlagringsmetod. Mitt syfte med den här studien var att utvärdera vallens kolinlagringspotential i nordiskt klimat genom att följa tre svenska långliggande försök. Från vart och ett av försöken valdes en vallväxtföljd och en korn monokultur växtföljd, och effekten från mineralgödsel jämfördes med en kombinerad gödselstrategi. Kolförrådet mättes genom jordprovtagning för två av försöken i norra Sverige. För det tredje försöket, i södra Sverige, fanns motsvarande data redan tillgänglig. Vall växtföljden lagrade in mer kol i matjordsskiktet än korn växtföljden. För att utvärdera kolförrådets effekt på aggregatstabilitet blev proverna från de norra försöken analyserade för lätt dispergerbara lerpartiklar (RDC) och vattenstabila aggregat (WSA). Det visade sig att vall kan ha en positiv påverkan på RDC, medan mineralgödselmedel och WSA korrelerade positivt.

Som en andra del av min studie modellerades de historiska kolförändringarna med ICBM, en kolbalansmodell som används i det svenska nationella systemet för rapportering av växthusgaser (NIR) och i rådgivningsverktyget Odlingsperspektiv (OP). OP använder en förenklad version av ICBM baserat på standardiserade värden. För modelleringen analyserades även jordprover från det historiska arkivet på kol innehåll, och skördedata användes för att uppskatta mängden tillfört underjordiskt kol från ovanjordiska växtrester, genom att använda skott-till-rot kvoter. Simuleringarna med OP överensstämde i många fall bättre till de uppmätta kolförråden än ICBM, vilket antyder på att de standardiserade värden i OP fungerade relativt väl.

De två modellerna (OP och ICBM) matchade de uppmätta kolförråden för det södra försöket. Dock var modellerna inte applicerbara på de norra försöken. En förklaring till detta var den historiska användningen av marken. Innan försökets start dränerades en av platserna, som orsakade en snabb förändring från anaerob till aerobt tillstånd, något som kolmodellen inte tar hänsyn till. Trots att resultaten visar på att kolförrådet minskade i de norra försöken, förblev kolförrådet större i vallväxtföljden än i kornväxtföljden. En kombinerad gödselstrategi medförde en positiv effekt på kolinlagringspotentialen hos vall i de norra försöken.

Slutligen ökade kolförrådet i vallväxtföljden i det södra försöket, och därutöver började inte kolförrådet minska förrän 30 år efter försöksstarten i ett av de norra försöken, vilket antyder att vall har utöver en kolinlagrande effekt även en kolpreserverande effekt.

Nyckelord: kolinlagring, aggregatstabilitet, readily dispersible clay, waterstable aggregates, kolförråd, ICBM, Odlingsperspektivet

Lägg tillbaka kolet där det hör hemma

EU satsar över 100 miljoner kronor på att hitta säkra lager i berggrunden för att pumpa ner koldioxid. En billigare metod skulle vara att lagra kolet i marken istället. Markbördigheten skulle öka, samtidigt som koldioxidhalterna i atmosfären skulle minska.

Ända sedan mänskligheten började bruka jorden har mark odlats bort, och stora mängder kol har därmed frigjorts till atmosfären. I odlade svenska mulljordar sjunker marknivån med upp till 1 meter på 50 år till följd av bortodlingen och kompaktering. Sjunkande kolhalter i mineraljordar försämrar markstrukturen eftersom organiska föreningar kittar ihop markpartiklar till aggregat. En god markstruktur är A och O för en bördig jord, då den får en bättre vattenhållandeförmåga och i många fall en bättre näringsstatus. Marken blir mer lättgenomtränglig för rötter och mer tålig mot yttre påverkan.

En potentiell metod för att lagra kolet i marken är genom vallodling, eftersom fleråriga grödor utvecklar ett större rotsystem som i sin tur utsöndrar kolrika organiska substanser i större utsträckning än ettåriga grödor. Eftersom kolhalterna i marken varierar i fält och förändras långsamt så är det viktigt med långliggande försök för att kvantifiera koldynamiken. I denna studie har jag därför provtagit och analyserat data från tre långliggande försök i Ås, Röbäcksdalen och Lanna i vall respektive korn växtföljd. Resultaten från 2018 visar att vall lagrar mer kol i matjordsskikten än korn. Dessutom visade det sig att vallodling ledde till att jordaggregaten stod emot regn bättre än aggregaten i kornväxtföljden.

För att undersöka den historiska effekten av vallodling på koldynamiken användes ICBM (en markkolberäkningsmodell). Med hjälp av ICBM förutsågs koldynamiken för de olika växtföljderna. Men det visade sig att ICBM endast matchade mätningarna i Lanna. Det visade sig att vallodlingen successivt lagrat mer kol i marken än spannmål genom åren (medan en långsam minskning av markkol observerades i kornväxtföljden) i Lanna försöket. En del av förklaringen till varför kolmodellen inte stämde med försöken i Norrland var att innan man anlade försöket i Röbäcksdalen dränerade den kolrika gräsmarken. I och med dräneringen började markkol att brytas ner i rasande takt, vilket håller i sig än idag i korn växtföljden. Kolförrådet i spannmålsväxtföljden sjönk som mest med cirka 60 ton kol per hektar mellan åren 1965 till 2016 i Röbäcksdalen.

I Ås upptäcktes även att kolhalterna behölls på en stadig nivå i vall växtföljden och inte förrän 30 år efter försökets början började sjunka, vilket antyder en kolpreserverande effekt hos vall. Kolhalterna för vallväxtföljderna som fått stallgödsel observerades även ha minskat mindre i snitt än vallväxtföljderna som fått enbart mineralgödselmedel för de norrländska försöken. Slutligen, kan vi dra slutsatsen att vallodling leder till högre kolförråd än spannmålsodling. Vallodlingen har därför en potential att bidra till minskade växthusgasutsläpp från jordbruket.

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Abbreviations

AG	Aboveground
BD	Bulk density
BG	Belowground
GHG	Greenhouse gas
ICBM	Introductory Carbon Balance Model
NVSW	No vacuum slow wetting technique
OP	Odlingsperspektivet
RDC	Readily dispersible clay
S:R	Shoot to root ratio
SOC	Soil organic carbon
SOM	Soil organic matter
WC	Water content
WSA	Water stable aggregates

1 INTRODUCTION

The soils of the world have lost 25-75% (10-30 t C/ha) of their soil organic carbon (SOC) stocks and have been a source of greenhouse gas (GHG) emissions since the dawn of agriculture (Lal, 2013). Over long-term time scales, the entity of SOC loss is proportional to the potential SOC sink capacity and is therefore higher in the tropics than in temperate climates (Lal, 2013). A decrease in SOC concentration is also linked with a decrease in soil quality and nutrient use efficiency (Lal, 2013), as SOC is positively related to the biological and physical soil characteristics (Hati et al., 2007). SOC lowers the bulk density, which improves the water-holding capacity and increases soil microbial activity (Yang et al., 2011), thus supporting an increased soil biodiversity and ecosystem function (Barrios, 2007). Moreover, SOC contributes to aggregate stability, and this enhances soil fertility in long-term perspective (Marschner et al., 2003; Blanchet et al., 2016). A critical threshold of approximately 1.1% SOC has therefore been set for the root zone (Lal, 2013). For some parts of the world this threshold is currently not met, and therefore improving the SOC concentrations are important for food security in many low-income countries (ibid).

Increasing SOC content provide with greater soil resilience towards extreme weather conditions (Haddaway et al., 2017) and mitigates climate change (Minasny et al., 2017). To integrate agriculture as a part of the climate change solution was “4/1000 Initiative” launched at COP21 by France, which aims to counteract the annual increase of CO_2 in the atmosphere by increasing SOC stocks by 0.4% per year (Rhodes, 2016; Jordbruksverket, 2018a; KSLA, 2018). Applied to the world’s total soil surface would result in about 30% annual reduction of GHG emissions, and thereby halt the annual increase of anthropogenically emitted CO_2 (Rhodes, 2016; Minasny et al., 2017). A zero net emission target has been proposed to be reached by year 2045 in Sweden and SOC sequestration is one of the measurements included in this proposal (Jordbruksverket, 2018a).

The SOC pool is the third largest C pool on Earth (Lal, 2013), about twice the size of the atmospheric C pool and almost three times larger than the C stored in aboveground biomass (Post et al., 1982; Eswaran et al., 1993). Even small changes in SOC could therefore cause a significant change to atmospheric concentrations of CO2 (Schimel D.S et al., 2012; Minasny et al., 2017). Arable soils contain approximately 12-18% of the global SOC pool (Schlesinger, 1991; FAO, 2014), but their C concentrations varies greatly between fields and regions (Minasny et al., 2017). Due to the high spatial variation but also because of the relatively slow mineralization rate of SOC stocks, changes in SOC are rarely observable within a time span of a few years (Franz et al., 2003). This makes it difficult to study changes in SOC stocks unless the time scale embraces multiple decades. Long-term field experiments and soil carbon monitoring are therefore essential to understand SOC dynamics (Kätterer et al., 2004). By investigating the SOC changes in relation to the history of land use and management, soil type and hydrology, along with other climatic conditions, we can have an idea of SOC stocks to expect in the future (Kätterer, Andrén & Persson, 2004).

2 BACKGROUND

2.1 The mechanism of soil carbon sequestration

When litter from crop- and animal residues falls to the ground, a decomposition process by the soil micro- and macrofaunal starts (Eriksson et al., 2011). The decomposition is mainly mediated by microbes, as soil animals accounts for only 10-15% of the decomposition (Wolters, 2000) and abiotic chemical oxidation another 5% (Lavelle et al., 1993). The microbial community occupies less than 1% of the soil volume and SOC degradation is therefore constrained to where the local SOC concentration is high (Ekschmitt et al., 2008). The first fraction of organic matter to be degraded are easily decomposable materials like sugars, starches and simple proteins (AgriInfo, 2015). This occurs during the first two years of the decomposition cycle of organic material (Lützow et al., 2006). The decomposition of this material, conventionally termed labile soil organic matter, is the first phase of soil organic matter (SOM) decomposition when approximately 67% of initial C is degraded (ibid). After this first phase a slower decomposition follows, which lasts for about 10-100 years, where an additional 23% of initial C is degraded, also referred to as the intermediate SOM (Lützow et al., 2006). The remaining C (refractory SOM) is eventually turned into more stable compounds that ultimately complete the decay process which can last up to thousands of years (ibid). Some part of the organic material that reaches the soil can therefore remain for many decades, and these SOM stabilization mechanisms are the reasons why soil can accumulate C.

What the main reason for the greater stability of the old SOM pool remain unknown. The main processes behind SOM persistence (considered proportional to a compound's mean residence time) are currently explained by a combination of selective preservation, spatial inaccessibility and chemical interactions (see list below for description, adapted from Sollins, Homann & Caldwell 1996). Chemical recalcitrance of the SOM seems to be important in the initial phase of decomposition, and later spatial inaccessibility and organo-mineral interactions increase in importance (ibid).

(1) Selective preservation due to the OM's own chemical composition of recalcitrant compounds such as lignin and polyphenols (Six et al., 2002),
(2) Spatial inaccessibility, soil aggregates can provide with physical protection by forming barriers between microbes and enzymes and their substrates, which consequently controls microbial turnover (Elliott & Coleman, 1988) and
(3) Chemical interactions between SOM, soil minerals (clay & silt particles) or other OM (Six et al., 2002).

More recently, a "soil-centered" approach has suggested that labile, high-quality root litter that is rapidly decomposed contribute to SOM formation (Cotrufo et al., 2013), as recalcitrant compounds, such as lignin and plant lipids have been observed to have a faster turn-over rate than the bulk of the organic matter. These labile compounds have shown to be able to persist for decades in soil as they associate to soil minerals (Knorr et al., 2005; Amelung et al., 2008; Grandy & Neff, 2008; Marschner et al., 2008).

The longevity (e.g. persistence) of the global SOC sink is still debated and much attention has been raised to the possibility of converting C input into passive SOM pools with longer (~infinite) residence time (Wheeler, 2014; He et al., 2016).

2.2 Climate affect the SOC stocks globally

Abiotic factors such as precipitation and temperature are the most important external factors that influence SOC stock changes. High temperature, long growing season and rainfall might lead to an enhanced plant growth, and thereby increased SOC stocks (Lu et al., 2013). Higher temperature further increases the availability of mineral nitrogen to plants which enhances plant growth (Melillo et al., 2002). Higher temperatures, on the other hand, are expected to speed up the decomposition of SOM and thus lowering the SOC stocks (Smith, 2005). It has however been observed that SOC sequestration is enhanced under warm conditions, which suggests that SOC balance is more driven by input than by its decomposition rate (Thornley & Cannell, 1997; Post & Kwon, 2000). Decomposition also depends on aeration and available moisture content as an adequate level is needed to allow decomposition (Schlesinger, 1986; Smith, 2005; Baritz et al., 2010). The balance between precipitation and evapotranspiration have therefore a strong impact on whether C stocks increase or decrease.

Very few soils in the world keep an uniform temperature in the upper layers, and the intensity of solar irradiation is critical for driving soil warming (Paul, 2015). From a study conducted on soils along a north-south transect in central United States by Jenny (1961) was OM content observed to decrease 2 to 3 times for each rise of 10° C mean annual air temperature. Globally greater SOC stocks are found at higher latitudes due to the low temperature regimes (Minasny et al., 2017), and in humid tropics because of high precipitation and high net primary production (Baccini et al., 2012) see fig. 1.

The Swedish agricultural soils are in comparison to many other agricultural soils in the world quite rich in SOC because of low temperatures, high precipitation and the common cropping system of perennial grasses (Ericson & Mattsson, 2000). The short and intense growing season causes the soil biological activity to decrease (Bolinder et al., 2007a). These factors, together with the frequent input of manure, increases the SOC stocks (ibid). The average SOC content of mineral soils in Sweden is 2.4% in the 0-20 cm layer, while at 20-40 cm depth the C content generally decreases to 1.4% C and even more at 40-60 cm of 0.6% C (Eriksson et al., 2010).

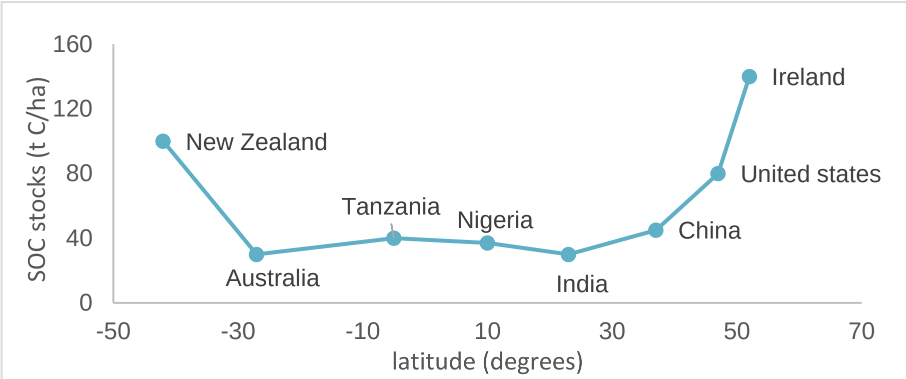

Figure 1. *Topsoil SOC stocks as a function of latitude (adapted from Minasny et al., 2017).*

To identify the realizable C sequestration potential in soils is it necessary to work out SOC steady state levels, e.g. equilibrium (Minasny et al., 2017). SOC models based on first-order kinetics predict an increasing or decreasing trend, that levels off at an equilibrium point that is usually far in time and has been observed in many long-term experiments (Paul et al., 1996; Huggins et al., 1998; Kong et al., 2005). The SOC stocks of a certain ecosystem are, under native conditions, in steady state (Stewart et al., 2007; Eriksson et al., 2011) determined by the C inputs of that ecosystem and soil type (Johnston et al., 2009). However, agricultural and pastoral ecosystems have demonstrated the possibility to exceed native SOC stocks, through increased C inputs and therefore increasing the steady state of the system to an equilibrium with higher SOC stocks (Russell, 1960; Barrow, 1969; Ridley et al., 1990; Ismail et al., 1994). The physiochemical processes in soils determines the total surface area, which leads to a saturation phenomenon of SOC (Kemper & Koch, 1966; Hassink, 1997). Thus, every steady state level is specific not only to a certain farming system but also to soil types (Johnston et al., 2009), implying that, while a grassland soil should have a higher steady state level than a cropland, a clay soil should have a higher steady state level than a sandy soil.

Climate, by influencing the SOC decomposition kinetics, contributes to the determination of the steady state level. Temperate climates have been estimated to require up to 100 years to reach steady state after a land-use change, whilst soils in boreal regions can require several centuries, and the tropics only 10 years (Smith, 2005). The latest update of the Swedish National Inventory report confirmed that the carbon content in many of the Swedish mineral soils are at steady state (Swedish Environmental Protection Agency, 2017; Jordbruksverket, 2018a).

2.3 Possible strategies to improve SOC sequestration in agricultural soils

Suitable farm management options can sequester SOC to the great benefit of increased SOM content (Lal, 2016). Even if the SOC level have reached steady state for an ecosystem it is important to maintain the C-sequestering management, as the accumulated C otherwise would be lost, usually faster than it was accumulated (Smith et al., 1996). In a review including temperate grasslands 36±5% of the accumulated SOC was lost after 17 years after conversion to cropland, SOC that had required centuries to accumulate (Poeplau et al., 2011). Also, by introducing new SOC sequestering methods the C sequestration rate in Sweden could be enhanced, and the potential is high on especially mineral soils with annual cropping systems in comparison to organic soils, pasture and long-term ley (Jordbruksverket, 2018a). Currently mineral soils are sequestering approximately 0,4 Mton/ha and year, half of it sequestered by living biomass (ibid).

An increase in SOC stocks in agricultural soils can be obtained by increasing the C input and/or decreasing the output (Freibauer et al., 2004). Crop residues, manure and other organic amendments provide to C input, as well as irrigation and fertilization indirectly (Stewart et al., 2007). The C sequestration potential based on the long term trials of SLU, decreases in following order: ley > salix > manure and cover crop > straw residue (Naturvårdsverket, 2017). Globally the following measures has been reported to have the highest C sequestering potential: afforestation (~0.6 t C/ha year), conversion to pasture and organic amendments (~0.5 t C/ha year respectively), residue incorporation (~0.35 t C/ha year), no or reduced tillage (~0.3 t C/ha year) and crop rotation (~0.2 t C/ha year) (Minasny et al., 2017)[1] .

The amount of SOM, the quality (C/N ratio) and decomposition rate influence soil aggregation (Hadas et al., 1994; Sun et al., 1995). Adding organic residues

[1] A list on SOC sequestering measures and their potential can be found in table 2 in Freibauer et al. (2004).

with high C/N ratio have showed an immediate but transient response on soil stability, whereas larger quantities of moderate quality has been shown to stabilize aggregates for longer periods (Hadas et al., 1994). Avnimelech & Cohen (1989) concluded that organic amendments with C/N ratios between 15 and 40 resulted in the greatest soil structural improvement. Besides, SOM quality influences soil water content and N availability of organic residues' decomposition rate and therefore its effect upon soil stability (Hadas et al., 1994).

2.3.1 Ley as a SOC sequestering farm management method

The focus of this study is on the SOC sequestration potential of leys, studied for three Swedish long-term trials. Management practices such as conversion of cropland to grasslands, avoided conversion of grasslands and improved grassland management have all been investigated as SOC sequestrating options for agriculture (IPCC, 2000; Lal, 2004; Smith, 2004), as well as SOC sequestering effect from short rotational leys (Persson et al., 2008). Ley has the capacity to allocate more C to the roots due to a longer growth period than annual crops, which leads to higher C input over time (Paustian et al., 1996; Bolinder et al., 2010). Moreover, the root-derived C has a longer turn-over time than above-ground C, which makes it particularly important to allocate C to roots (Kätterer et al., 2011; Zhou et al., 2018). Ley also evaporates more, causing a lower soil water content and temperature, which in turn slows down the decomposition rate and more SOM is thus conserved (Kätterer & Andrén, 2009). The reduced tillage in ley systems also leads to less decomposition and higher SOM than high-intensity tillage systems (Zhou et al. 2018). Frequent tillage was observed to increase SOC decomposition rate by 20% in a Swedish long-term experiment (LTE) (Bolinder et al., 2012). Furthermore, the legume component in leys have been shown to play an important role to SOC accumulation because of the symbiotic associations between roots and N2 fixing bacteria (Kaye et al., 2000; Binkley, 2005; Fornara & Tilman, 2008). The explanation seem to lie in the stimulation of microbial growth during the decomposition of the N-rich legume litter, as the Rhizobium species are able to produce extracellular polysaccharides that are adsorbed onto fine silt and clay particles (Marshall, 1969; Fehrmann & Weaver, 1978). The leguminous plants have moreover a deeper root system that deposit root-C that can associate with organominerals at deeper depths than perennial grasses, which may contribute significantly to a greater C storage in subsoil (Hansson & Andrén, 1987).

Studies have shown that it is possible to increase annual crops yields by incorporating rotational leys to the crop rotation, especially in combination with mineral fertilizer (Uhlen & Kolnes, 1994; St-Martin, 2017). One of the explanations to the increased yields can be that of the added N by the legume component in leys (Chalk, 1998), but also due to the disease break from the provided perennial crop

(Berzsenyi 2000). However, many studies that have demonstrated a positive effect from leys on soil C are associated with the continuous addition of manure (Paustian et al., 1996; Conant et al., 2001) and since manure itself has a positive impact on SOM (Persson & Kirchmann, 1994), it is hard to distinguish the origins of the positive effects on SOC (Persson et al., 2008). A LTE study indicate that a two-year ley can have a positive effect on soil carbon, although no manure was added (but mineral fertilizers) (ibid). At the same time was the lack of manure application partly the explanation to why a ley rotation caused slow decrease of soil carbon to one of the sites (ibid).

2.3.2 The impact of manure on SOC stocks

The relationship between manure and increased crop productivity was known already in the golden age of the Greeks (Sartron 1959 as cited in Bolinder 2012). Manure application have been shown to increase the content of SOM, especially in combination with mineral fertilizers (Masto et al., 2006; Liu et al., 2010). An increased nutrient status of the soil is thereby increased, as well as manure contributes to hydraulic conductivity, aggregate stability, soil microbial biomass and a reduced bulk density that all enhances soil fertility in long-term perspective (Marschner et al., 2003; Blanchet et al., 2016).

Today manure is often considered more effective in increasing SOC levels than mineral fertilizers, but this is not always apparent on yield outcome (Hijbeek et al., 2017). Mineral fertilization increases plant growth, which in turn results in a higher return of OM to soil e.g. litter, roots and crop residues. Mineral fertilization might eventually increase the SOC stock this way (Haynes & Naidu, 1998a; Smith, 2005). The quality of manure is of importance as the ratio between organic C and N, that is added to soil determines whether N is to be released or fixed in SOM as it decomposes (Johnston et al., 2009). In an experiment at Rothamsted (started 1942) where four types of organic manures were compared, all but the biosolids (sewage sludge) were observed to have released mineral N (ibid). The biosolids had instead fixed mineral N (ibid).

2.4 The influence from plants on soil structure

Soil structure plays a crucial role in determining SOC persistence (Six et al., 2000; Blanco-Canqui & Lal, 2004), and therefore specific steady state levels of SOC concentrations (Six & Paustian, 2014). Soil structure has a major influence upon biological activity as it is within the pore space of aggregates where a continuous gas exchange take place, which controls biotic activity and thus SOM turnover time (Ball, 2013).

Soil structure is defined by the heterogeneous arrangement of soil particles and void space at a given time (Kay et al., 1988). Soil aggregates are formed when soil particles bind together by chemical interactions and substances derived from root exudates and microbial activity (Amézketa, 2015; Paul, 2015). Soils consist of different sized aggregates and pores between them (Tisdall & Oades, 1982). The microaggregates are formed by the flocculation of fine silt, clay, amorphous minerals and humic substances (Paul, 2015). Flocculation occurs under the physical forces of freezing and thawing, the compressive and drying effect of roots and the mechanical alimentary action by soil fauna (Dexter, 1988). Macroaggregates are formed when a network of plant roots, fungal hyphae and fibrous organic matter bind micro-aggregates together, long enough to allow chemical linkage (Paul, 2015). Plant roots also have a drying effect in soils which pull organic compounds closer together which increase the possibility of chemical linkage (Tisdall & Oades, 1982). The roots further supply the soil with decomposable organic residues, polyvalent cations and a large microbial population, and residues that are often physically protected because of the ability of roots to grow into very small pores (Morel *et* al., 1990; Pojasok & Kay 1990).

Soil structure is defined by its form but also by its stability (Kay et al., 1988). Soil aggregate stability is the capability of soil aggregates to remain intact under various stresses (Angers & Carter, 1995) such as the impact from water, wind and management (Amézketa, 2015). Aggregate stability influences many physical and biogeochemical processes in agricultural soils, such as soil erosion, water storage capacity and biological activity, and is therefore linked to soil quality and crop production (Amézketa, 2015). Microaggregates are stabilized by both organic and inorganic agents. Inorganic stabilizing agents consists of clay particles, polyvalent metal cations, oxides, hydroxides (especially Fe and Al), carbonates and gypsum (Amézketa, 2015). Chemical additions that contain divalent cations (especially Ca) increase the cation exchange capacity in soil solution, and consequently enhances clay flocculation and aggregate stability (Weill *et* al., 1989). Clay particles are particularly strongly bound together by inorganic and organic cements and electrostatic bonds (Amézketa, 2015). The organic stabilizing agents consists of polysaccharides, the more persistent effect from polymers and aromatic compounds on micro-aggregates and the temporarily stabilizing effect from roots and hyphae on macro-aggregates (Tisdall & Oades, 1982). These stabilizing agents contribute to the physical protection of humic material within clay and silt-sized aggregates (Skjemstad et al., 1993).

About half of the net fixed C in plants is transferred belowground due to root turnover and exudation (Nguyen, 2009) which contributes to the soil structure formation (Jastrow et al., 1996). The plant-root C influences for example water repellency, which improves the soil stability towards disruption by wetting, bypass flow and surface runoff (Hallett et al., 2001). The same authors found that pasture soils repel water better than no-tilled soils and even better than ploughed soils (ibid). Other studies show that pasture and forage crops increase the soil resistance towards slaking in comparison to annual cropping systems (Haynes & Swift, 1990; Perfect et al., 1990b; Pojasok & Kay, 1990b; Angers et al., 1992; Haynes & Francis, 1993). Therefore, wet aggregate stability (Ball et al., 2005) and the overall soil structure can be improved by ley cropping systems (Ball & Douglas, 2003), especially on soils with poor structure (Watson et al., 2002; Ball et al., 2005).

The symbiotic interaction in leguminous plants can promote micro- and macroaggregation through the binding of rhizobial polysaccharides (Clapp et al., 1962; Tisdall & Oades, 1982; Haynes & Beare, 1997; Alami et al., 2000). Leguminous plants can further stabilize SOC as they increase inorganic N in topsoil (Kaye et al., 2000; Sainju et al., 2003) which could prevent the formation of lignin-degrading enzymes and increase the formation of recalcitrant compounds (Berg, 2000; Binkley, 2005; Du et al., 2014). Perennial grasses have been observed to contribute to more dense and strong stable macroaggregates, (that are enriched with SOC) because of their high root length density (Materechera et al., 1992). Manure have also demonstrated positive effect on root growth and the associated root exudation, which can increase macroaggregation (Weill, Mckyes & Kimpe 1989).

2.5 Destabilization of soil aggregates

The destabilization of soil aggregates and consequent degradation of soil structure leads to long-term effects such as reduced water infiltration, poor crop emergence, and increased risks of soil erosion and run-off (Dexter & Czyz, 2000).

Climate strongly affects the degree of soil aggregation. Particularly water content changes (wetting-drying) and air temperature (freezing-thawing) over the year influence soil structure in a dynamic way (Amézketa, 2015). Water influences the main destabilizing mechanisms of agricultural soils which are clay dispersion, clay swelling (ibid) and slaking (Barzegar et al., 1996) see fig. 2. The first step in the loss of soil structure has been suggested to be the disintegration of macroaggregates into microaggregates (i.e. slaking), as clay dispersion is a time-dependent chemical process and therefore assumed to be the second step in the break-down process of soil aggregates (Abu-Sharar et al., 1987; Bissonnais, 1990; Quirk & Murray, 1991; Shainberg et al., 1992; So & Aylmore, 1993). Although clay particles are strongly bound together (Amézketa, 2015), soils with high clay content have been found to have a lower aggregate stability (Ternan et al., 1996), as the clay traits depend on the mineralogy (Amézketa, 2015). Clays with large specific surface area (e.g. Smectite) contribute to the interaction capacity and therefore aggregate stability, whereas clays with low specific surface area (e.g. Illites) are most sensitive towards clay dispersion (El-Swaify, 1976; Olphen, 1977; Arora & Coleman, 1979; Oster et al., 1980; Shainberg & Letey, 1984). The effect of clay minerals is however hard to assess as soils usually contain a mixture of clay minerals (Amézketa, 2015).

2.5.1 Slaking – the breakdown of macroaggregates into microaggregates

Slaking is the complete disintegration of aggregates during wetting (Barzegar et al., 1996). Slaking occur especially on dry aggregates when they are rapidly wetted as the clay swells and the air entrapped inside aggregates burst out (Angulo-

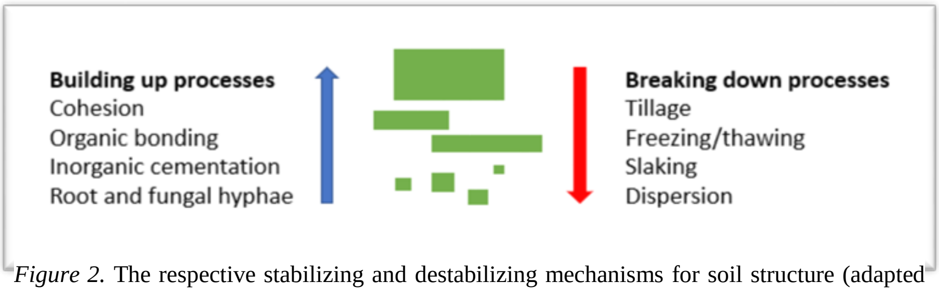

Figure 2. The respective stabilizing and destabilizing mechanisms for soil structure (adapted from Díaz-Zorita, Perfect & Grove (2002).

Jaramillo et al., 2016). Therefore, slaking has been reported to be less intensive in coarse aggregates due to less entrapped air compression (Bresson, 1995).

Macroaggregate stability has been found to decrease by a continuous water content change (Soulides & Allison, 1961; Tisdall et al., 1978; Lehrsch et al., 1991; Mulla et al., 1992), and by an increase in water content (Gerard, 1987; Coote et al., 1988; Perfect et al., 1990a; b; Gollany et al., 1991; Caron et al., 1992; Rasiah et al., 1992; Chan et al., 1994). But changes in water content have also been observed to increase water-stable macro aggregation, e.g. formation of aggregates tolerant to water (Utomo & Dexter, 1982; Dexter, 1988; Barzegar et al., 1995). Aggregate stability depends on external disruptive forces and internal bond strength, which both are inversely related to the antecedent soil water content (Gollany et al., 1991). A high antecedent water content result in more slaking resistant soil aggregates (ibid). A high clay content generally implies less risk for slaking, but the aggregate strength is also increased by a higher clay content, which can in turn increase the slaking forces (Angulo-Jaramillo et al., 2016).

2.5.2 Clay dispersion

Clay dispersion is the separation of soil aggregates into single particles and is influenced by many factors such as soil texture, clay mineral type, SOM, soil salinity and exchangeable cations (Almajmaie et al., 2017). Moreover, clay dispersion is dependent on soil water content as a critical minimum has been found (Emerson, 1967; Kay & Dexter, 1990; Watts et al., 1996), which has been suggested to be close to the plastic limit (Emerson, 1967; Watts et al., 1996). Drying of the soil counteract clay dispersibility as small suspended mineral particles are pulled together and the possibility for bonding increase (Kemper & Rosenau, 1984, 1986; Kemper et al., 1987; Bullock et al., 1988; Dexter, 1988; Mulla et al., 1992; Lehrsch & Brown, 1995), and thereby aggregation (Dexter, 1988; Oades, 1993).

The impact of rainfall can cause clay dispersion. The force from rain drops causes fine soil particles to detach and subsequently, drawn into soil pores which clog the pores and form a crust on the soil surface (Legout et al., 2005; Bu et al., 2013). The crust cement further when the soil dries up why a higher energy input is required to fragmentize again, which makes the soil more difficult to work (Kay & Dexter, 1992). In other words, large amounts of dispersible clay causes the tensile strength[2] of dry aggregates to increase (Kay & Dexter, 1992; Watts & Dexter, 1997; Elmholt et al., 2008). Especially intensive tillage on soil with low SOC content under wet conditions makes the soil vulnerable to clay dispersion (Watts & Dexter, 1997; Schjønning et al., 2012). Clay dispersibility, like slaking (author's note) is reduced with increasing soil aggregate size (Abu-Sharar, 1993). An increased aggregate size therefore decreases the tensile strength in soils and lowers the risk for cementation (Braunack et al., 1979; Munkholm et al., 2006).

SOC plays a crucial role for soil aggregation (Tisdall & Oades, 1982) by acting over the particle charge (Goldberg et al., 1990). However the effect on aggregate stability from SOM is still controversial as the addition of organic anions have shown to increase clay dispersion (Frenkel & Shainberg, 1980; Shanmuganathan & Oades, 1983; Durgin & Chaney, 1984; Goldberg & Forster, 1990; Kosmas & Moustakas, 1990; Heil & Sposito, 1993, 1995; Kretzschmar et al., 1993; Tarchitzky et al., 1993; Piccolo & Mbagwu, 1994; Itami & Kyuma, 1995). SOM has also been demonstrated to affect upon the proportion of clay which influences clay dispersibility and the vertical migration of clay colloids (Schjønning et al., 2012). Clay dispersibility has therefore been determined by the degree to which fine mineral particles are saturated with clay/SOC ratios of 10 (Hassink, 1997; Dexter et al., 2008) and clay+silt/SOC of 20 (Schjønning et al., 2012).

2.6 Soil aggregate stability test

Organic agents contribute to soil aggregate stabilization, and the physical protection of SOM (Tisdall & Oades, 1982). Soil aggregate stability is therefore also an indicator of SOM content (ibid).

[2] The tensile strength is defined as the strength of bonds between the components of any hierarchical soil level e.g. between each aggregate (Dexter, 1988).

Two tests have been proposed to be used to determine soil aggregate stability, as water stable aggregates reflect macroaggregate stability and dispersed clay reflect microaggregate stability (Amézketa, 2015). The wet-sieve technique used to determine macro-aggregate stability simulates the action of wetting and measures the slaking forces (Truman et al., 1990). Whereas the dispersed clay colloid test simulates the mechanical energy from rain droplets and its impact upon the breakdown of clay- and silt aggregates (Miller & Baharuddin, 1986) by exposing the aggregates to varying levels of disruptive energy through shaking (Williams et al., 1966; Dong et al., 1983; Rengasamy et al., 1984; Pojasok & Kay, 1989; Kay & Dexter, 1990; Bartoli et al., 1992). The clay which disperse when no energy is applied is called "spontaneously dispersible clay" whereas the clay which disperse due to a small amount of energy is called "readily dispersible clay"(Dexter & Czyz, 2000).The clay behaviour (e.g. flocculation/dispersion) analysed in the dispersed colloid test is important (Pojasok & Kay, 1989; Carter & Mele, 1992) as flocculation is the basis for soil aggregation (Dexter, 1988).

2.7 Summarizing the factors governing SOC balance through modelling

All factors controlling SOC accumulation or reduction can be represented in a SOM model. Many models are available, but they are in general all based on a first order kinetic (e.g. decay proportional to SOM mass) consequently leading to a faster decomposition with higher OM input, and multiple pools representing different organic compound classes (Menichetti, personal communication). The structure of SOC models can be generalized as Equation 1:

$$\frac{dC}{dt} = I(t) + R(t) \cdot H \cdot K \cdot C$$

Equation 1. The derivative of the generalized SOC model structure. I represents the C inputs, H the humification transfer between pools, K the decay of SOC, C the mass of pools and R the edaphic influences added together.

ICBM, the *Introductory Carbon Balance Model* is used for the Swedish national reporting system for GHGs inventories and in the farmers' decision tool Odlingsperspektivet (OP). ICBM is in its simplest form a two-component model and comprises two SOC pools: Young (recently added OM) and Old (stabilized SOC stocks), two decay constants and three parameters for litter input, a humification coefficient and a factor for external influences (fig. 3) (Andrén & Kätterer, 1997). The model have in its simplest form default values for S:R for different crops and climatic factor for each of the 8 Swedish agricultural production regions.

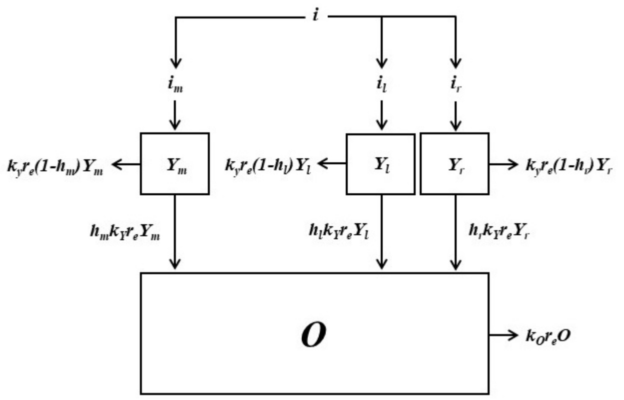

Figure 3. ICBM flow chart. i = C inputs divided into: manure (i_m), aboveground (i_l) and belowground (i_r). k_y and k_o = 1st-order decomposition rate constants for the young (Y) and old pools (O). h= humification coefficient, r_e= climate parameter.

The mean annual inputs of C (denoted by *i*, fig. 3) is usually estimated from shoot and root residues of crops and weed plants including rhizodeposition, and/or the addition of manure (Kätterer et al., 2004). The C inputs from roots are derived by plant C allocation coefficients based on reviews on shoot to root ratios for respective crop (paragraph 8.2.1) (Andrén & Kätterer, 1997).

The outflows follow linear kinetics with specific decomposition rates (k_Y and k_O), and therefore decomposition speeds up with higher OM input. In the original model formulation by Andrén & Kätterer (1997) the decomposition parameters are held constant, with values k_Y = 0,8 and k_O = 0,007. The decomposition rates are affected by soil, climate, and management practices. These are represented in ICBM by the climate factor r_e (Bolinder et al., 2012). The calibration of the climate factor, r_e from annual mean was normalized to 1 in Central Sweden (Ultuna frame trial), (Andrén et al., 2007). The value of re allows immediate comparisons between sites, even regions in terms of decomposition (ibid). It was for example estimated that a Swedish soil in steady state would lose 41% of its SOC content after 30 years if moved to Kenya (ibid).

The climate factor r_e is composed by three components: soil water content (r_w), soil temperature (r_T) and tillage intensity (r_c) (Bolinder et al., 2012). All three components control the climatic condition for soil biological activity in a multiplicative manner (Andrén et al., 2007). The soil water content generally increases with C content in soils, but also depend on soil texture (Rawls et al., 2003). The driving factors for r_w are daily precipitation and potential evapotranspiration data inherent to Green Area Index (GAI) (Kätterer et al., 2008; Bolinder et al., 2012), plus two soil parameters: water content at wilting point[3] and at field capacity[4] (Bolinder et al., 2012). The difference between the two parameters defines the capacity to store plant available water. The r_w and r_T components are estimated with a soil climate module connected to ICBM driven by meteorological data and commonly used assumptions on relationships between temperature, soil water content and biological activity (Andrén et al., 2004, 2007; Bolinder et al., 2007a; b). The parameter for tillage intensity r_c is estimated from expert opinon, with values usually ranging from 10% to 30% for perennial forage crop and annual crops, respectively (Andrén et al., 2004; Kätterer et al., 2008).

The humification coefficient (*h*) is the fraction of annual outflux from Y pool that enters O pool. The humification is not affected by external influences but depends on variation in litter quality (Kätterer et al., 2004). The coefficient *h* can be approximated as the fraction remaining after 5-10 years from litter bags and ^{14}C experiments (Andrén & Kätterer, 1997). Humification coefficients of 0.125 and 0.250 for above- and belowground crop residues, and 0.310 for manure is usually used in the ICBM model (Andrén & Kätterer., 1997; Bolinder et al., 2012).

ICBM can be used as an instrument for predicting soil carbon balances in Swedish agricultural soils and answer questions like: is a system losing or sequestering soil C, how will climate change influence SOC dynamics and how much the SOC pool will increase by changing litter amount (ibid). For calculating the steady state for a certain cropping system and its management the following (Equation 1) can be used for three different inputs:

Equation 2. Steady state formula for three different inputs: manure, aboveground and belowground input (Kätterer & Andrén, 2001).

$$Yss = \frac{i_{root}}{k_y r_e} + \frac{i_{manure}}{k_y r_e} + \frac{i_{shoot}}{k_y r_e}$$

$$Oss = h_{root}\frac{i_{root}}{k_o r_e} + h_{manure}\frac{i_{manure}}{k_o r_e} + h_{shoot}\frac{i_{shoot}}{k_o r_e}$$

2.7.1 Estimating C inputs by S:R ratios

To drive a SOC model we need, besides the climatic data, the input of C to the soil. The soil C inputs from crop residues can be divided into two major sources:

[3] The permanent wilting point is defined as the minimal water content required by the plant in order not to wilt, from where it is not possible to recover overnight, usually at 1500kPa tension (Kätterer *et al.*, 2006).

[4] Field capacity is defined as the water content after 3 days of drainage (when the downward movement has decreased) after a thoroughly rain, usually less physically stringent (Kätterer *et al.*, 2006).

aboveground (AG) i.e. straw, stubble and surface debris and belowground (BG) i.e. root biomass in soil at harvest, root turnover[5] and exudates (Bolinder et al., 2002). Kätterer et al. (2011) found that root-derived C contributes about twice more stable soil C than the same amount of aboveground crop residues. Perennial forage crops, with its larger root biomass are considered to have a higher relative C allocation to belowground than annual crops (Bolinder et al., 2007b). However, it is more difficult to obtain accurate estimates of annual C inputs to soils from belowground residues than those from aboveground (Steen 1982 as cited in Bolinder 2002) why it often has been neglected in studies on crop residue C inputs (Paustian et al., 1996). The estimates on BG plant biomass have often been based on fixed allometric relationship between AG and BG parts (Hu et al., 2018), in so called shoot to root (S:R) ratios, although these ratios are subject to large variation (Bolinder et al., 2002).

There is great variability in the reported S:R ratios between crop species (Paustian et al., 1990; Zagal, 1994). A S:R of 7.4±3.6 (n=59) for barley was reported in a review including studies from Canada and the US (Bolinder et al., 2007b). This is quite close to the S:R ratio of 7.1 derived from a Finnish study (Pietola & Alakukku, 2005). An older study from Uppsala suggested a S:R of 6.25 suggested for barley (Hansson et al., 1986). There is a significant difference between perennial forage crops and annual crops regarding S:R ratios as forage crops change over time (Weaver & Zink 1946; Troughton 1957; Hansson, A. C. & Andrén 1987). Hansson & Andrén (1987) found that the root biomass of red clover increased by 15% and timothy 192% the following year. An equilibrium between net root growth and root turnover in forage crops has been estimated to require 2 to 4 years (Troughton, 1957). The S:R ratio of timothy has been estimated to 2.1 the first year and 0.60 the following years in Canada (Bolinder et al., 2002), which is close to the Finnish study which found a S:R ratio of 1.9 for barley (Ilola et al., 1988). The S:R ratio for red clover was estimated to 1.82 the first year and 1,38 the second year (Bolinder et al., 2002).

[5] e.g. individual roots dying and decomposition

3 AIM OF THE STUDY

The aim of this study was to obtain a better quantitative and qualitative understanding of the effect of ley rotations on SOC stocks and soil aggregation. Therefore, soil samples from Röbäcksdalen and Ås (R8-74) long-term experiments in northern Sweden with different crop rotations (ley vs. barley) and two fertilization regime (mineral fertilization vs combined mineral fertilization and manures) were collected and analysed. The lab analysis composed of elementar analysis (C&N) and micro- and macro-aggregate stability tests to evaluate the response from ley upon soil aggregation. The soil samples were analysed at eight different depths for their:

- Soil organic matter content (C_{tot} and N_{tot})
- Soil stability indicators (RDC and WSA)
- pH and bulk density

The experiments are associated to a soil bank with archived soil samples since the beginning of the experiment, and these samples were also analysed for C_{tot} and N_{tot}. These time series were utilized to understand the trends over time of the different treatments. The time series of SOC contents were then described by adjusting the ICBM model, to deepen the understanding for SOC dynamics. The model was also used to calculate the SOC steady state levels. A long-term experiment in Lanna (R3-0021 and R3-0020) was included for the modelling.

3.1 Research questions

I. Is there a significant effect of ley rotation in comparison to barley monoculture rotation on SOC stocks? And, do fertilization treatment have an impact on C stocks?

II. Are the observed field data comparable to the simulation results obtained with the ICBM and OP model?

III. Can SOC contribute to a greater soil aggregate stability so that aggregates remain intact under the impact of rainfall (e.g. clay dispersion) and rapid wetting (e.g. slaking)? And if so, does ley rotation influence aggregates?

3.2 Hypothesis

I. It was hypothesized that ley rotation contributes significantly to SOC stocks in comparison to barley monoculture rotations, and that the effect is increased with manure addition.

II. It was hypothesized that the ICBM parameters relating to site-specific soil properties, initial conditions and climate can explain the relative differences in SOC stocks for contrasting cropping systems in different regions.

III. It was hypothesized that SOC impact soil aggregate stability positively, and that ley rotation would increase aggregate stability (connected with Hypothesis I).

4 MATERIALS AND METHODS

4.1 Long-term field experiments

Long term field experiments (LTEs) are often defined as older than 20 years (Smith et al., 1997; Rasmussen et al., 1998). In Sweden many LTEs were initiated after World War II, as the separation between predominantly livestock-oriented and cropping-oriented farm management caused a great concern on how the new crop rotations and cropping systems would affect soil fertility and yields (Bergkvist & Öborn, 2011). The different treatments within the LTEs have a substantial impact on soil traits as they are more than 40 years old now (ibid). The design of the LTEs regarding crop rotation and cropping systems present all crops in a rotation yearly which makes them suitable for studies of annual variation. However, they are often constrained to only one or two true replicates, due to size or initial design limitations (ibid). LTEs are nevertheless crucial for studies of SOC dynamics in the field, since the average time scale for SOC decomposition last decades to centuries (Trumbore, 2000).

4.2 Experimental sites

The study sites were located to Ås and Röbäcksdalen in northern Sweden and Lanna in southern Sweden (fig. 4). Both the sites in northern Sweden belong to the long-term field experiment series R8-74 – Monocultures in northern Sweden initiated in 1965. The two experiments R3-0021 and R3-0020 in Lanna started 1981 with the objective to evaluate the effect of humusbalance in long-term ley cropping system and the effect of straw incorporation and nitrogen fertilization in cereal monoculture (Börjesson et al., 2018).

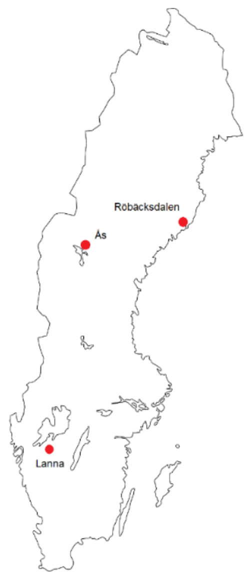

Figure 4. The experimental sites.

Röbäcksdalen was previously a poorly drained grassland, which explains its high SOC concentrations (4,84 %) at the start of the experiment (Ericson 1994 cited in Bolinder et al. 2010). The soil type is silty clay loam with clay content of 9 % (Andersson & Wiklert, 1977). No literature was found regarding the historical cropping use in Ås. The staff at the experiment station in Ås however, estimated it to have been 6-10 year long ley cropping system (Lars Ericson, personal communication). The soil type in Ås is a sandy loam, with clay content of 21% (Andersson & Wiklert, 1977) and SOC concentration of 3.85% (Ericson 1994 cited in Bolinder et al. 2010). In general, the soil structure is relatively weak in both Ås and Röbäckdalen and prone to slaking and crust formation (Bolinder et al., 2010). The SOC content prior to the start of Lanna experiment was assumed to be 2% for all treatments and BD 1,335 g/cm3 in ley and BD 1,415 g/cm3 for cereal monocultures (Börjesson et al., 2018). Data for each site is included in Table 1.

Table 1. Soil type and clay/silt content for the northern sites (St-Martin, 2017) and the southern site (Börjesson et al., 2018). Climate data adapted from weatherbase.com and Köppen climate classification was used (Peel et al., 2007). The coordinates was updated June 2018.

Site	*Position*	*Soil type*	*Clay (%)*	*Silt (%)*	*Climate classification*	*Average T °C (yearly)*	*Average P mm (yearly)*
Röbäcksdalen	63°48'29.2" N, 20°14'16.4" E	Silty clay loam	9	69	Dfc	2.8	530.9
Ås	63°14'57.4" N, 14°33'43.7" E	Sandy loam	21	38	Dfc	2.8	495.3
Lanna	58.34°N, 13.10°E		43			6.1	558

The two northern experiments include 6 year long crop rotations. The crop rotations included in this thesis were: undersown barley followed by five years of ley (B) and a monoculture of barley (F). At the beginning of the experiments in northern Sweden three fertilization treatments were included: 1) mineral NPK fertilization corresponding to plant uptake, 2) double amount of mineral NPK fertilization and 3) mineral NPK fertilization as in (1) plus additional 10 t/ha solid manure per year (10kg N/ha). In 2010 the first mineral fertilization treatment (1) was removed so that the fertilization treatments now consist of 2) mineral NPK fertilization corresponding to plant uptake (new update), and 3) mineral NPK fertilization as in (2) plus additional 20 t/ha liquid manure (30kg N/ha), for details see Table 2.

The change in 2010 caused the removal of the first row of plots in Röbäcksdalen (fig. 5). For this study the change of fertilization treatment (from 1 to 2) caused a more drastic change to plots 54 and 63 in Röbäcksdalen and plots 1, 13, 54 and 63 in Ås. The whole treatment 2 was removed in Ås and therefore is the change not consistent between the two sites. For this study is treatment 2 abbreviated (-) whereas treatment 3 is abbreviated (+).

The Lanna experiment is similar to the northern experimental sites, but only 4 year long, e.g. undersown barley followed by 3 years of ley, and barley monoculture. The fertilization treatment in the Lanna experiment consist of 0, 50, 100 and 150kg N/ha in ley rotation, the barley rotation received 40, 80 and 120 kg N/ha.

Table 2. Fertilization treatments for the two different crop rotations. Fertilization change in 2010 in parenthesis. All amendments were applied in autumn. Source: PM for R8-74 - Monocultures in northern Sweden.

Crop rotation	*1: low NPK*	*2: high NPK*	*3: low NPK+manure*
Barley monoculture (F)	40	80 (80)	30 (50)
Ley (B)	75	150 (130)	75 (100)

Seeding takes place in spring. The barley monoculture treatment is ploughed in the autumn and straw is incorporated into the soil. The ley consists of a mix of approximately 80% timothy (*Phleum pratense*), 11% meadow fescue (*Festuca pratensis*) and 9% red clover (*Trifolium pratense*) at all sites. The ley is harvested twice per year, except for the last ley year (V) when ley is not harvested but terminated with a glyphosate application. The straw from the barley is removed at every start of the rotation cycle. The soil sampling was thereby carried out on one of the ley (V) as 2018 closes the 9th crop rotation cycle.

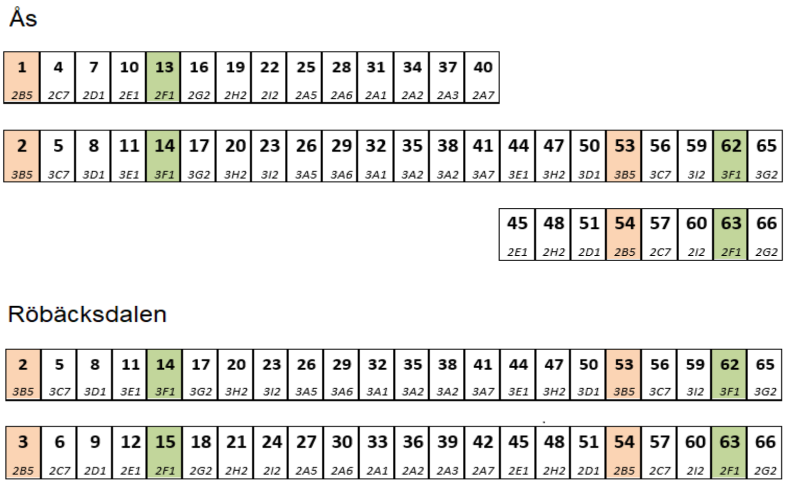

Figure 5. *The soil sampling position in Ås and Röbäcksdalen. Orange colouring denotes crop rotation B (ley) and green crop rotation F (barley monoculture).*

4.3 Soil sampling

The soil sampling included soil corer samples and bulk density samples in Ås and Röbäcksdalen. The samples were taken in mid-May 2018 (before sowing).
Three bulk density soil samples were taken in each plot using a stainless-steel cylinder (ø7,2 cm, V= 407.15 cm3) from the top of the soil. The turf of ley was removed. After collection, the samples were transported to the laboratory, weighed and left to dehydrate in the oven at 105°C for 72 hours. The dry soil weight was measured to determine the water content. Bulk density (BD), was calculated by dividing the dry soil weight <2 mm by the cylinder volume (Paul, 2015).

In addition to the bulk density sampling were five soil corer samples taken per plot. The subsoil was included in the sample, and therefore the soil samples were divided in 8 depth intervals: 0-20, 20-22.5, 22.5-25, 25-27.5, 27.5-30, 30-35, 35-40 and 40-50 cm. Different depth intervals were used in a few plots in Ås to account for the compression of the soil. All soil corer samples from each depth layer were pooled together and stored in the fridge at 5 °C.

Approximately 5 g topsoil and subsoil from the soil corer sampling was dried in 40 °C for 72 hours before sieving (<2 mm). The subsample was homogenised by pouring the soil sample over the edge of two containers until a suitable amount was derived (Magnus Simonsson, personal communication). A ball-mill was used to ground the subsamples into finer particles. Subsamples of 100 mg were sent to lab for C and N analysis according to the Dumas method performed by Elementar Vario Max analyser. When the C and N content were derived the numbers were used to calculate the amount of organic C per depth level multiplied with the bulk density. Additionally, historical soil samples from both Ås and Röbäcksdalen were obtained and prepared in a similar way for C and N analysis. The historical soil sample procedure was the following: a minimum of 10 subsamples were sampled within a circle of 1m from the center of the plot, pooled together and a subsample is stored (PM from R8-74).

Another fraction of the soil corer samples (approx. 50 g) were used for determining readily dispersible clay and another for macro-aggregate stability (see the paragraphs below).

4.4 Readily dispersible clay test (turbidimetry)

The test was performed on both field moist soil, according to (Pojasok & Kay, 1989; Getahun et al., 2016) and on air-dry soil as suggested by (Le Bissonnais, 1996). The topsoil for each plot was carefully crumbled through 8 mm sieve (the air-dry soil was then left to air dry for 72 hours). 10±3 g of soil (corrected to water content) were then put in 125 ml plastic shaking bottle (ø = 4 cm and height = 11.2 cm) and 80 ml of distilled water were added. The bottles were tilted to reduce the energy input before attaching them on the end-over-end shaking device ø = 21,3 cm (fig. 6). The device was set to a rotation speed of ~ 33 rpm. Time interval of 2 min shaking was chosen according to (Schjønning et al., 2012) and after that were the bottles were removed and left to sedimentate for 230 minutes.

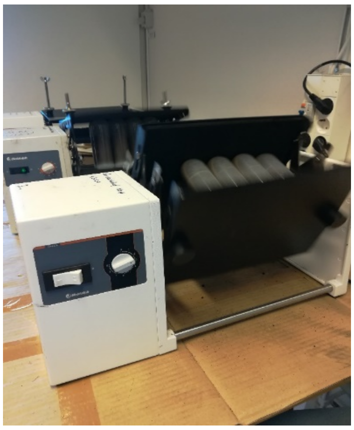

Figure 6. The end-over-end shaking device.

The top of the suspension (60 ml) was then extracted with a pipette, containing the dispersed clay, and transferred to a 100 ml glass beaker. 10 ml of the suspension was extracted with a pipette while the suspension was constantly stirred with a magnetic stirrer, transferred to a pre-weighed glass beaker and dried at 110 °C. The mass of readily dispersed clay (RDC) was determined on a 4 decimal precision scale.

4.5 pH determination

The method for pH determination ((Alef et al., 1995) was adjusted to half the amount of soil and distilled water of the original method description. 5 g of air dried and sieved (< 2 mm) soil were put into plastic scintillation vials and 12,5 ml of distilled water was added into each vial. The solution was shortly stirred, (approx. 1 min), left 1 hour standing, and subsequently stirred a second time before measuring pH using pH meter PHM 93.

4.6 Wet sieving

Before determining macro-aggregate stability, 2±0,15 g soil (air dried and sieved: 1-2 mm) for each replicate were pre-wetted. A modified no vacuum (atmospheric) slow wetting technique (NVSW, Dickinson et al., 1991) was used to pre-wet the aggregates.

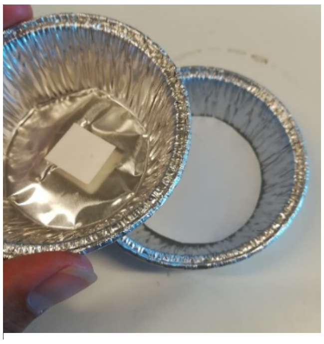

Figure 7. Picture on the wetting vessels.

Wetting vessels were constructed from aluminium baking tins (ø =5 cm, fig. 7). Two tins were needed per sample. A 3 cm straight line was cut in the middle of the top tin and a wick (6 x 2,5 cm) was cut from a Munktell 00R filter and introduced into the cut[6]. Two sheets of filter (cut to adjust to the smaller size) were placed at the bottom tin, and another one was placed at the top tin. 3 ml of distilled water to the bottom tin were added before the sampled soil added. The soil in the wetting vessels were left to slowly wet for approximately 1 hour.

Figure 8. Eijkelkamp wet sieving apparatus.

The wet sieving procedure was performed according to Kemper & Rosenau (1986). The pre-wetted soil was placed in the sieve holders of the wet sieving apparatus (fig. 8). The cans below the sieve holders were filled with 75 ml distilled water, covering the aggregates when at the lowest level of the cycle but not at the highest level. After 3 min of raising and lowering action from the apparatus the cans containing the water unstable aggregates were emptied into 100 ml beakers and refilled with the dispersing solution of 2 g/l NaOH (for all soil samples pH < 7). The apparatus was set on continuous oscillation, and after about 5 min the aggregates were carefully rubbed with a spoon to disintegrate all water stable aggregates left in the sieve holders. The cans containing the stable aggregates, were then emptied in beakers, leaving only visible sand particles and root fragments in the sieve holders. The remaining fraction in the sieve holders (minus eventual visible roots which were discarded) was also put into beakers for obtaining an exact total weight of each sample. All beakers were then placed in the oven at 110 °C to dry before mass determination on a 4-decimal scale.

The fraction of water stable aggregates (WSA) were calculated using the formula (Equation 3) adopted from (Angulo-Jaramillo et al., 2016):

Equation 3. Water stable aggregate equation. SA = mass stable aggregates, SM = mass sand.

$$WSA\ (\%) = \frac{SA - SM}{\text{Original soil mass} - \text{SM}} * 100$$

[6] Instead of Whatman 4 filter paper were Munktell 00R used. Retention <10 µm (Whatman 12-15 µm). Filtrationspeed 120 Hz sec/100ml (Whatman 40 Hz sec/100ml).

4.7 Data treatment

As part of the thesis, historical data were gathered from the archive of the experimental series R8-74 regarding ley and barley crop rotations in Röbäcksdalen and Ås. The data was collected from several sources and homogenized into a consistent form. Historical soil samples were further analysed with the same methodology as our own sampling method for a consistent evaluation.

Yield data from all the years since the start of the field trials were extracted and used in ICBM and OP to predict SOC stock changes. Yield data from an equivalent LTE (R3-0021 and R3-0020) in Lanna (southern Sweden) was collected to compare the sites and to evaluate the potential to use the models for different climatic regions. The yield data was harmonized to recover missing data and the C concentration of the crops was assumed to be 0.45 (Ma et al., 2018). The climate factor re_{clim} was composed by a temperature and moisture factor, re_{temp} and re_{wat} respectively. Re_{temp} was calculated from soil temperature reduction function according to Andrén et al. (2004). Soil temperature was calculated from air temperature (ibid). The moisture factor was calculated according to the function in Moyano et al. (2012) which utilizes relative water content. Relative water content was calculated from a soil water balance function that consider precipitation and evapotranspiration, the latter calculated from wind speed, radiation and crop coverage according to FAO guidelines as described in Andrén et al. (2004). The amount of C from manure was derived using the generally recommended NH_{4+}-N relationship and C/N content in manure (Jordbruksverket, 2018b).

For estimating the belowground input from ley and barley crop rotation from the aboveground data, the calculation was performed based on the different S:R ratios (see more in 2.8.1). S:R ratio of 7.4 ±3.6 for barley was used (Bolinder et al., 2007b). The ley yield was corrected according to the botanical analysis report on grass- and clover content. The grass content was treated as to contain only timothy as no significant difference between different grass species (timothy and fescue among the reported species) has been observed (Bolinder et al., 2002). For timothy a S:R ratio of 2.0 was used for the first year, averaged from Ilola et al. (1988) and Bolinder et al. (2002), and 0.60 for the following years in the crop rotation. A S:R ratio of 1.82 (first year) and 1.38 (following years) was used for red clover (Bolinder et al., 2002). The annual BG contribution from ley and barley consist of root exudation (65% of S:R biomass) plus root biomass when soil incorporation take place for respective

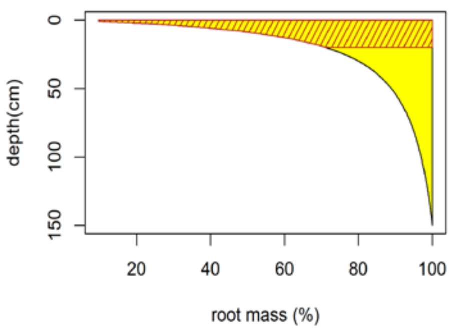

Figure 9. The root biomass for the crops were corrected for 20 cm depth with Michaelis-Menten-type function.

crop (Bolinder et al., 2007b). The root biomass was thereafter adjusted with a factor of 0.71 according to *Michaelis-Menten-type* function (fig. 9) to adapt the root biomass to 20 cm depth (to correlate to the SOC stocks calculations in ICBM) (Kätterer et al., 2011).

The annual AG input for ley consist of 25% litterfall and regrowth after last cutting (Martin Bolinder, personal communication). In the first year of ley, root exudates from barley was also added. At the end of the ley cycle, the input was increased to include an additional 100% of BG, stubble 25% and root biomass incorporation. In the long-term trial R8-74 approximately 8-10 cm of the hay stubble left, and another 3-4 cm stubble from ley (Boel Sandström, personal communication) why stubble is yet another factor to consider. It has been suggested from the Swedish GHG inventory to add another 25 % extra on the calculated ratios for stubble from cereals (Adolfsson, 2005). The AG input for barley consist of straw incorporation and an additional 25% for stubble. The accuracy of OP was compared to the ICBM modelling. OP uses the default S:R ratios of 10 for barley (3.1 for insown barley) and 2 for ley, based on literature review from N. America and Europe (personal communication, Bolinder). The corresponding default climate factors in OP is 0.69 for Ås, 0.67 for Röbäcksdalen and 1.04 for Lanna (Andrén et al., 2008). To evaluate the accuracy of the model predictions I calculated a forecast accuracy as indicator the root mean square error (RMSE) from the predicted SOC changes and the measured values. To calculate steady state level of each treatment Equation 2 was used.

To calculate the historical bulk density a linear regression test was performed based on the C and BD values (grouped by site) from soil samples taken in 2018. The linear regression was used to calculate the historical BD from the averaged C content in 1965 for the two sites. The historical BD was used in combination with the historical C content per treatment to evaluate SOC stock change from the beginning of the experiment to 2018. The SOC stocks were calculated as follows (Equation 4):

Equation 4. Formula for calculating SOC stock from bulk density where n is the number of soil layers (Don et al., 2011).

$$\begin{aligned}\text{SOC stock}\,(\text{Mg ha}^{-1}) = \sum_{i=1}^{n} &\text{SOC concentration}\,(\text{Mg Mg}^{-1})\\ &\times \text{bulk density}\,(\text{Mg m}^{-3})\\ &\times \text{soil volume}\,(\text{m}^{3}\,\text{ha}^{-1}),\end{aligned}$$

4.7.1 Statistical analysis

The statistical programme R and R studio was used to perform all statistical analysis (R Core Team, 2018). Regression analysis was used to determine the correlation between variables with Pearson's correlation coefficient r (ranging from -1 to 1 for negative and positive correlation respectively) between the different variables RDC or WSA with either water content, SOC, pH or BD, and between RDC and WSA. Another regression analysis was used to derive the coefficient of determination (r^2), which summarizes what percentage of total variation of the predicted variable was described by the variation in the predictor variable. ANOVA was used to examine the average effects of the selected management practices (crop rotation, fertilization and site) on the different variables. A two-way ANOVA was used to assess the main effect of each independent variable but also the interaction between them (if any).

Significance level was set to $p < 0.05$ for all tests unless otherwise specified.

5 RESULTS

5.1 SOC stock profile 2018

The SOC stock profile for 2018 showed that highest SOC content is found in the topsoil for both sites (fig. 11). The topsoil C stocks ranged from 49-97 t C/ha in Röbäcksdalen and 41-68 t C/ha in Ås. The topsoil samples were analysed with a two-way ANOVA with SOC stocks as predicted variable with crop rotation or fertilization and site as predictor treatments (*n*=4). The effect of crop rotation was significant on topsoil C stocks only (fig. 10), no significant effect of fertilization nor site was observed. The topsoil in ley rotation had on average 75.14 t C/ha (equivalent to 3.1% C) and barley rotation 51.77 t C/ha (2.2 % C) in Röbäcksdalen. In Ås the average topsoil C stocks was 63.27 t C/ha in ley rotation (2.45% C) and 54.0 t C/ha in barley rotation (1.96% C). The subsoil C (20-50 cm) decreased with depth at both sites and ranged from 2.7-9.9 t C/ha in Röbäcksdalen and 2.9-9.9 t C/ha in Ås. No significant effect from treatment or rotation was observed for subsoil C (fig. 11). The N content decreased with depth at both sites (fig. 11). ANOVA showed significance for C/N ratio with depth and site (fig. 12).

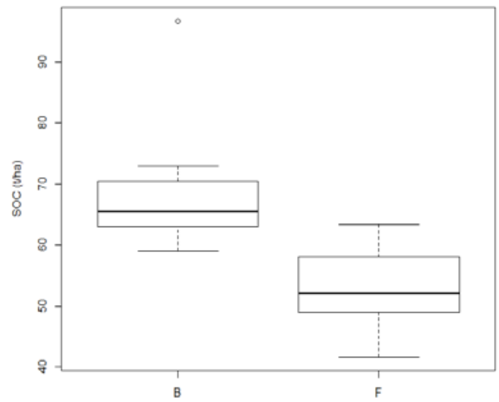

Figure 10. The effect from rotation on topsoil SOC stocks (2018).

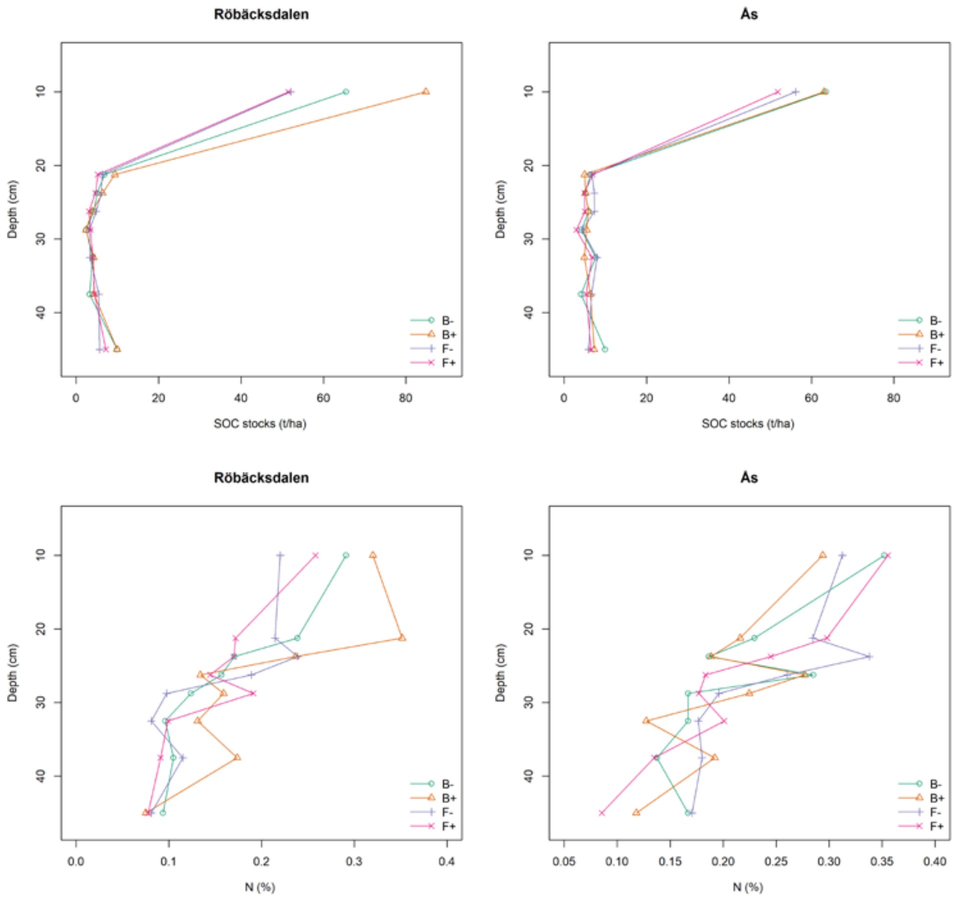

Figure 11. The SOC stock profile for Röbäcksdalen and Ås at top, and N profile below.

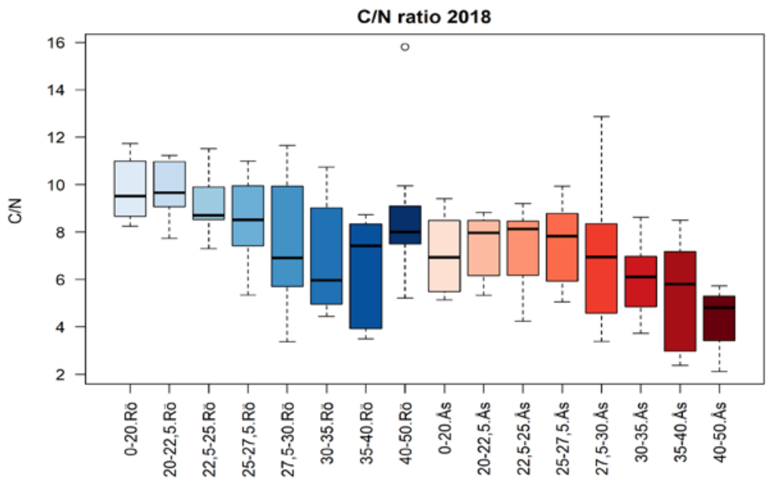

Figure 12. C/N ratio as a function of soil depth.

5.2 General soil properties

5.2.1 pH

Röbäcksdalen presented significantly lower pH than Ås, ranging from 5.2-5.9 whereas Ås presented pH levels of 5.5-6.8. Grouping the data for pH by crop rotation and site (*n*=4) in a two-way ANOVA test showed that ley rotation significantly lowers the pH in comparison to barley rotation (fig. 13). The regression test found a significant negative correlation between SOC stock and pH (r^2= 0.40). Thus, the results indicate that ley increases SOC stocks, and reduces pH (fig. 14). No significant result was found for fertilization treatments on pH.

5.2.2 Bulk density and water content

Bulk density (BD) differed significantly between the two sites. Röbäcksdalen had lower BD of 1.14-1.29 g/cm^3 whereas Ås had 1.25-1.49 g/cm^3. No significant effect of rotation or fertilization treatment (data not shown) on BD was observed. No significant linear relationship between SOC stocks and BD was found. However, there is an indication of a significantly lower BD for ley rotation than barley rotation in Ås (not observed in Röbäcksdalen).

Water content (WC) at the time of sampling was further negatively correlated (r = -0,87) with BD (r^2=0.75). Röbäcksdalen had in general a significant higher WC than Ås (21-25% WC in comparison to Ås of 12-17%, fig. 15). The slope of the regression between WC and BD are given in fig. 16.

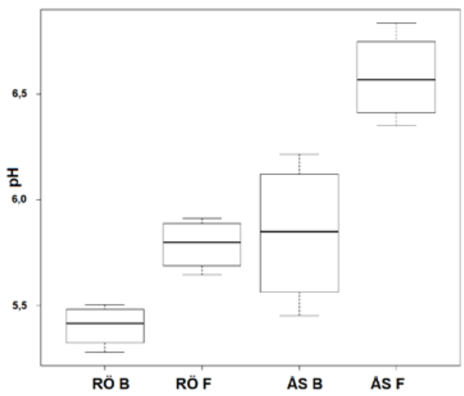

Figure 1. pH for the different crop rotations at the northern sites.

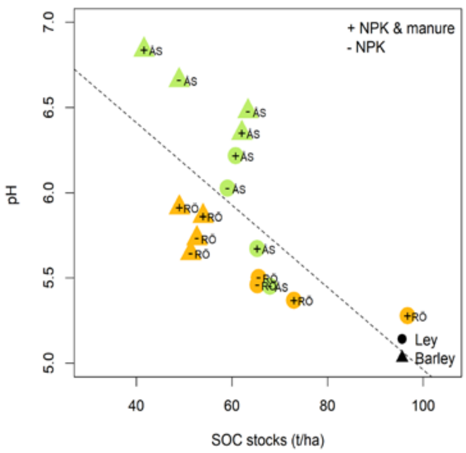

Figure 14. SOC stocks and pH.

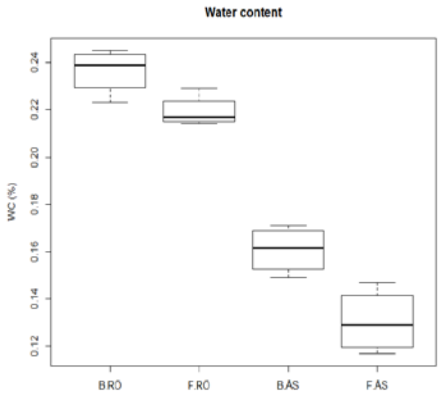

Figure 2. Water content observed for Röbäcksdalen and Ås and respective crop rotation.

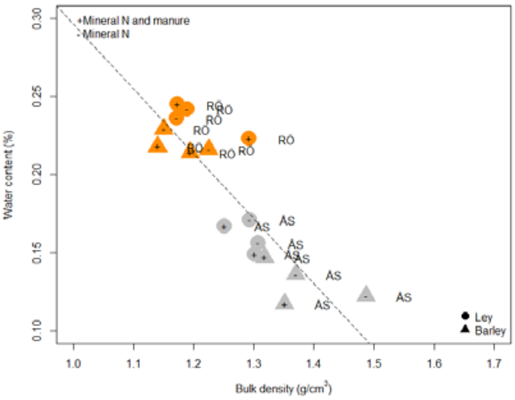

Figure 3. Water content and bulk density.

5.3 Soil aggregate stability

5.3.1 Readily dispersed clay (RDC)

The dispersibility test was performed on both dry and moist aggregates and calculations made on the mean values (by treatment) of aggregates recovered. The correlation between dry and moist aggregates was weak (fig. 17). The moist aggregate stability test presented a range of about 0-2 mg dispersed clay/g of soil, whereas dry aggregate stability had less variation, only up to 0.5 dispersed clay mg/g of soil. Most of the significant results regarding RDC were found for dry aggregates, why RDC is henceforth referred to the dry aggregates (when nothing else is stated).

A one-way ANOVA showed significantly (p=0.058) more RDC in Ås than in Röbäcksdalen (fig. 18), suggesting a structurally weaker soil in Ås. The results from a two-way ANOVA pointed out a small effect of site and rotation on RDC, which was significant only at a 90% confidence interval (p<0.1) so it should be considered with caution (fig. 19). No significant result was found for RDC and fertilization in the ANOVA one-way test.

Over all the sites there was a significant positive correlation between pH and RDC (r^2=0.42, r = 0.65, fig. 20).

According to the clay-saturation concept developed by Dexter et al. (2008) soils are unsaturated with SOC if the clay/SOC ratio is higher than 10. However, Schjønning et al. (2012) included the silt fraction, thus changing the clay+silt/SOC ratio to 20. In our study were silt+clay/OC ratio of 23-28 and 24 derived for ley rotation for Röbäcksdalen and Ås respectively. For barley rotation were higher ratios

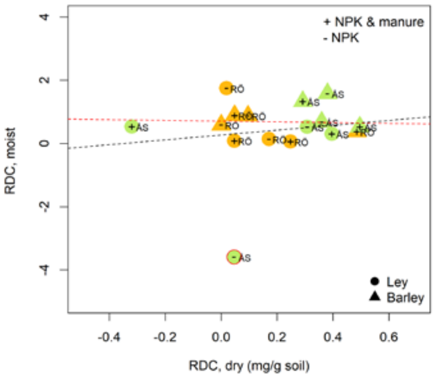

Figure 17. Correlation between dry and moist aggregates from the RDC test.

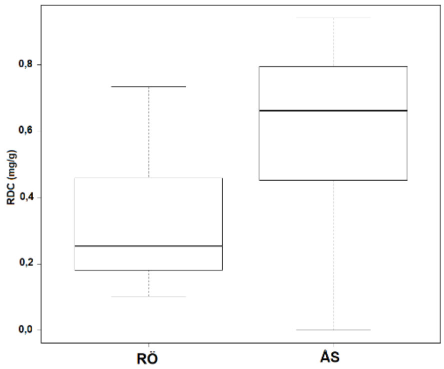

Figure 5. Amount of RDC between the sites.

of 35-36 and 30 derived for Röbäcksdalen and Ås respectively.

5.3.2 Water stable aggregation (WSA)

A two-way ANOVA with WSA as predicted variable and site and fertilization treatment as predictor treatments (n=4) showed significant correlation for fertilization (fig. 21). The fertilization treatments showed a wide span of water stability aggregation, with

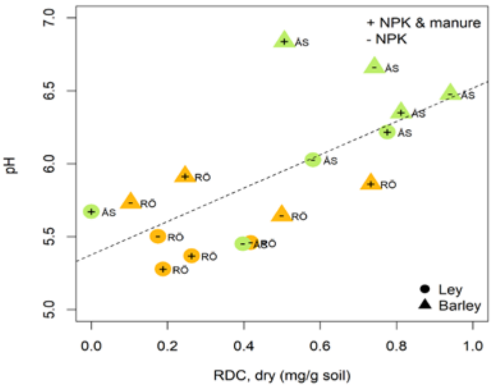

Figure 19. The effect from rotation on RDC.

Figure 204. Correlation between pH and RDC.

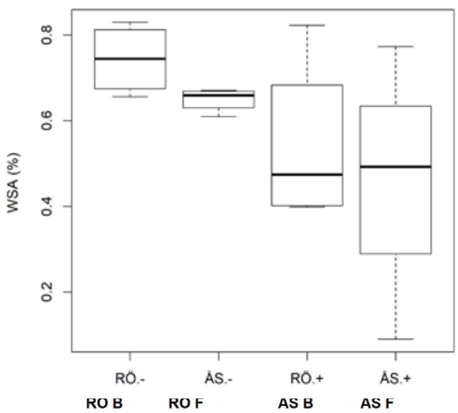

Figure 71. The effect from fertilization treatment on WSA.

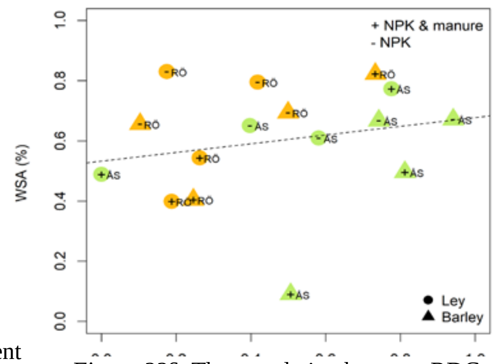

Figure 226. The correlation between RDC and WSA.

manure presenting a median value of approximately 45% WSA over the total soil mass, whereas mineral fertilization resulted in a higher water stability aggregation, about 70% (median value). No significant relationship was found between WSA and crop rotation or SOC content. No significant linear relationship was found between RDC and WSA (fig. 22). Nor was a linear relationship found between water stable aggregates or RDC and water content.

5.4 SOC stocks over time

The C analysis from 2018 showed that the original topsoil SOC content in Ås ranged from 2.3 to 3.2%, equivalent to 56 respectively 81 t C/ha, over all treatments. The original SOC content at Röbäcksdalen was significantly higher than at Ås, and ranged from 4.3 to 6.2 %, equivalent to 94 and 137 t C/ha, respectively (24). A two-way ANOVA with SOC stocks as predicted variable and rotation or fertilization and site as predictor elements showed that differences in SOC stocks was significant for site (p=3.93*10^{-6}) and fertilization (p=0.003) from the start of the LTE (n=4, fig. 23). In 2018 the same ANOVA test show that the SOC content differences have been evened out as the SOC content decreased in Röbäcksdalen, and no significant differences are seen between site or rotation (fig. 23).

The SOC stocks decreased for all treatments from 1965 to year 2016 in Ås. The SOC analysis results show that topsoil C in ley rotation (B) seem to have remained stable until 1993, before it started to decrease in Ås (fig. 24). The SOC in barley rotation (F) started to slowly decrease already from the start of the experiment. Based on the linear regression model the barley rotations lost the most SOC, ranging between 35% and 29% for F- and F+ respectively. The SOC stocks in ley rotations decreased by 21% and 17% for B- and B+, respectively. In summary the SOC losses in Ås were: 24.03 t C/ha for F-, 19.83 t C/ha for F+, 15.13 t C/ha for B- and 11.91 t C/ha for B+. The topsoil SOC content at Röbäcksdalen decreased from the beginning of the experiment in both rotations (fig. 25). The linear regression model based on the measured SOC values showed that the SOC stocks decreased by 53% in F-, 48% in B-, 43% in F+ and 19% in B+. The absolute SOC losses over the whole experiment duration was 59.65 t C/ha for B-, 56.05 t C/ha for F-, 40.14 t C/ha for F+ and 17.18 t C/ha for B+. Hence, the smallest SOC losses were observed for B+ in both Ås and Röbäcksdalen. Comparing fertilization treatment for Ås reveal the impact of the combined fertilization, the SOC losses were lowered by 0.06 t C/ha in ley rotation and 0.8 t C/ha in barley rotation. Similarly, the combined fertilization reduced the SOC losses in Röbäcksdalen by 0,83 t C/ha (ley rotation) and 0,31 t C/ha (barley rotation).

There are observable upwards trends in Ås for both rotations and B+ in Röbäcksdalen after 2010. The historical data available for subsoil SOC in both sites were few, and therefore no significant relationship due to rotation or treatment could be observed.

For Lanna, the increase was 15.05 t C/ha from 1981 to 2016, or 0.43 t C/ha per year in ley rotation receiving annual applications of 50kg N/ha and year, while the increase was lower for the rotations with 100 and 150kg N/ha (7.46 and 8.56 t C/ha, or 0.21 and 0.24 t C/ha year, respectively). The barley rotation in Lanna lost SOC, the highest losses were 7.5 t C/ha and were observed for the treatments receiving 40 and 80 kg N/ha, whereas a smaller loss of 4 t C/ha was observed for the treatment receiving 120kg N/ha (Fig. 26).

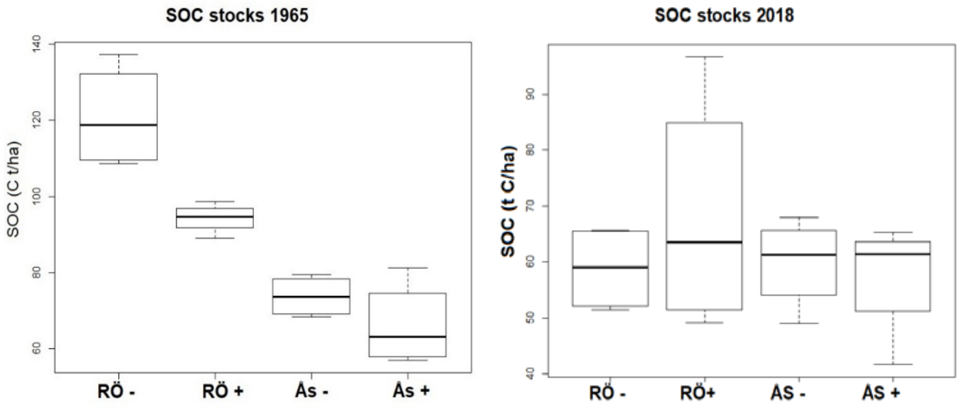

Figure 8. SOC stocks 1965 and 2018. NPK fertilization is denoted (-) and combined fertilization (+)

5.5 Model predictions and model fitness

The minimum and maximum S:R ratios for respective crop resulted in small differences in terms of SOC stock changes in ICBM. Due to the low differences are only the averaged values used for the calculations in the model prediction.

The historical SOC data revealed a negative SOC trend for all treatments in Ås. However, the ICBM model, predicted a small positive C accumulation in F+, B+ and B- for Ås, with a root mean square error (RMSE) of 19, 22 and 12, respectively. OP with default values showed smaller RMSE (difference of 1.6-2.5) than ICBM for all treatments except F+ in Ås. The difference between OP and ICBM predictions was particularly large in Ås for B-, where OP better described the observed negative trend. The average climate factor for Ås was 0.88 for the ley rotation and 0.79 for the barley rotation. This is slightly higher than the default value of 0.69 used in OP. The model predictions (ICBM and OP) for the treatments in Röbäcksdalen diverged more than in Ås. The RMSE values ranged between 42-48.5 (ICBM) and 28.8-39.6 (OP) over all treatments in Röbäcksdalen. Concerning just the direction of the change, the OP model predicted a decreased SOC stock for F-, F+ and B- as observed, whereas ICBM predicted a decrease only in F-. Hence, the default values used in OP gave more correct indications on SOC stock trends (e.g. increasing or decreasing) for Röbäcksdalen. In Röbäcksdalen, the average calculated climate factor in ICBM (0.37 and 0.45 for the barley and ley rotation, respectively) was substantially lower than the default climate factor of 0,67 in OP.

No model managed to replicate the drop in SOC stocks observed for the ley rotation in Ås after 1993, nor the gradual SOC decrease in barley rotation in Ås, and all treatments in Röbäcksdalen since the start of the experiment. In Lanna, the ICBM predictions matched better the observed SOC stocks in both rotations, particularly for the barley rotation. In Lanna, the RMSE for barley rotation was lower than in the northern sites and ranged between 2.4 and 4.1 (ICBM) and 2.6 and 3.44 (OP). Also, the ICBM prediction regarding ley rotation in Lanna matched the observed values quite well and RMSE ranged between 6.0-23 (ICBM) and 3.77-6.69 (OP). The largest RMSE was observed for ley rotation considering all treatments in the three sites. ICBM was overpredicting the SOC stocks for the ley rotation in Lanna, especially for the treatment receiving 150kg N/ha. For this site, the climate factor was higher in ICBM (1.3) than the default value used in OP (1.04).

The C input for all years was analysed with a three-way ANOVA with AG or BG C inputs as predicted variable and rotation, fertilization and site as predictor elements (*n*=36 for Lanna, *n*=52 for Ås and Röbäcksdalen, fig. 27). The effect of fertilization and site was significant regarding AG inputs, and all predictor elements showed significance for BG inputs. However, performing a two-way ANOVA test on yield data for only Ås and Röbäcksdalen, with rotation and site as predictor elements to AG C input showed significance for rotation. Barley rotation produced higher AG C input (0.94-1.13 t C/ha and year) than ley rotation (0.68-0.95 t C/ha and year) in Ås and Röbäcksdalen. A two-way ANOVA on BG C inputs with rotation and site as predictor elements showed however significantly higher BG C inputs for ley rotation (1.19-1.93 t C/ha and year) than barley rotation (0.23-0.29 t C/ha and year) in the northern sites.

The steady state levels of each treatment are described in Table 3. The steady state levels reflect the sequestration potential of each site and current management. Applying the steady state equations (Equation 2) to Ås predicted a final increase of 22 t C/ha for F-, whereas the other treatments are predicted to still lose 11 t C/ha for B-, 32 t C/ha for F+ and 65 t C/ha for B+ from 2018's observed C value. Ås would thus have a steady state level of 34.4 t C/ha for F-, 74.6 t C/ha for B-, 83.6 t C/ha for F+ and 128.9 t C/ha and year for B+. In Röbäcksdalen, all treatments are estimated to decrease, from 44.9 t C/ha (F-) up to 228.9 t C/ha (B+). Steady state level in Röbäcksdalen is around 97.0 t C/ha for F-, 195.5-220.8 t C/ha for B- and F+ and 313.7 t C/ha for B+. In Lanna barley rotations are predicted to increase by 12.15 up to 27.81 t C/ha to end at around 22-36.6 t C/ha. The ley rotations are estimated to decrease 61.6-189.1 t C/ha to end at a steady state level at around 123.5-143.8 t C/ha for the lower fertilization amounts and 249.7 t C/ha for the higher fertilization treatment.

Table 3. The steady state levels of each site, crop rotation and fertilization treatment. B denotes ley rotation and F barley rotation. Mineral fertilization is referred as (-) and combined fertilization as (+).

Site	*Treatment*	*Steady state (t C/ha)*	*Site*	*Treatment*	*Steady state (t C/ha)*
Röbäcksdalen	B+	313,72	*Lanna*	F 120 kg N/ha	36,61
	B-	220,78		F 80 kg N/ha	29,48
	F+	195,52		F 40 kg N/ha	22,10
	F-	96,95			
Ås	B+	128,86		B 150 kg N/ha	249,73
	F+	83,56		B 100 kg N/ha	143,70
	B-	74,61		B 50 kg N/ha	123,45
	F-	34,39			

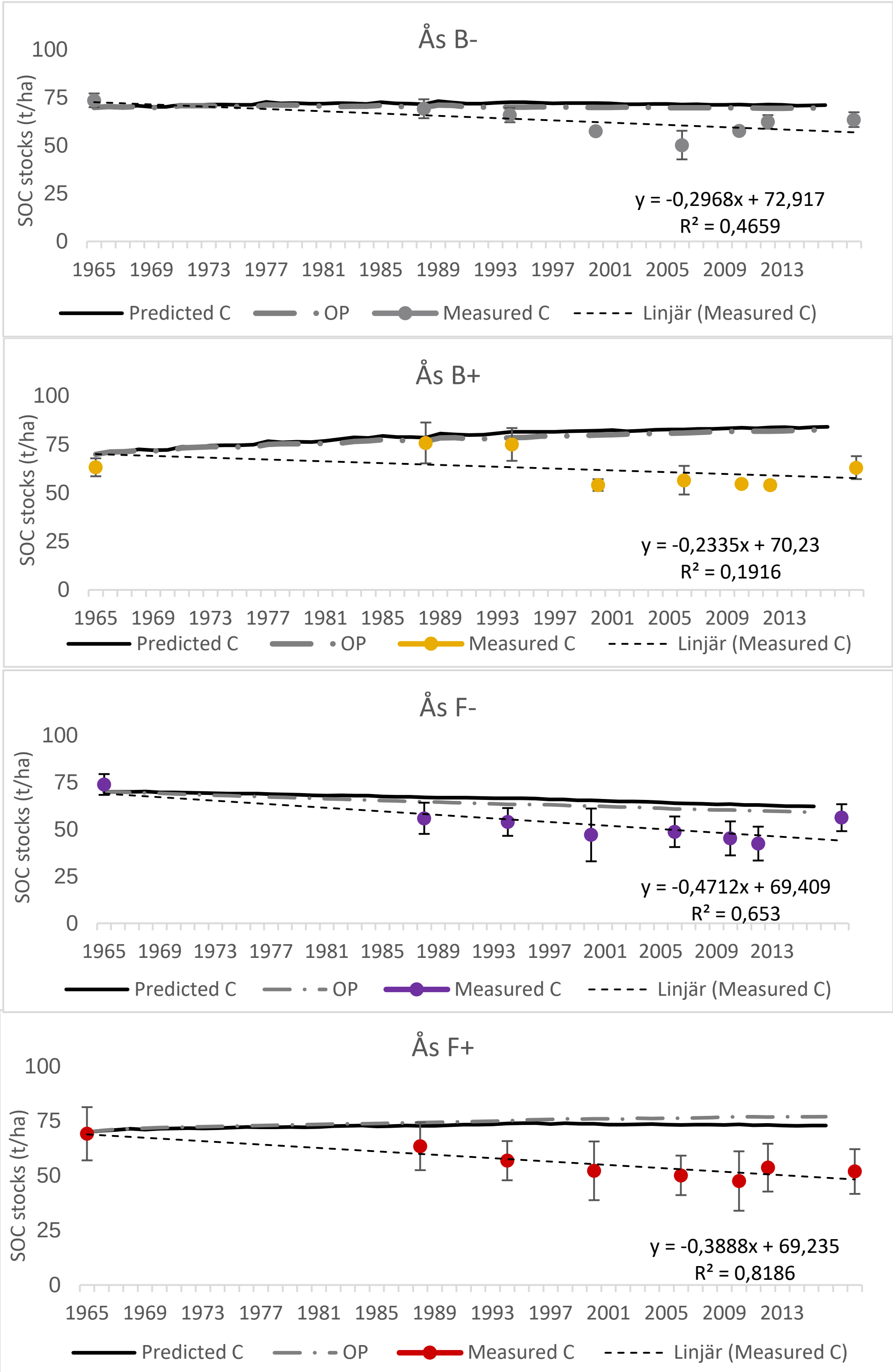

Figure 9. The SOC stock changes from 1965-2018 in Ås. Ley rotation is denoted by (B) and barley (F), fertilization treatment by + (combined fertilization) and - (mineral fertilization). Predicted C correspond to ICBM modelling. OP correspond to the modelling with default values.

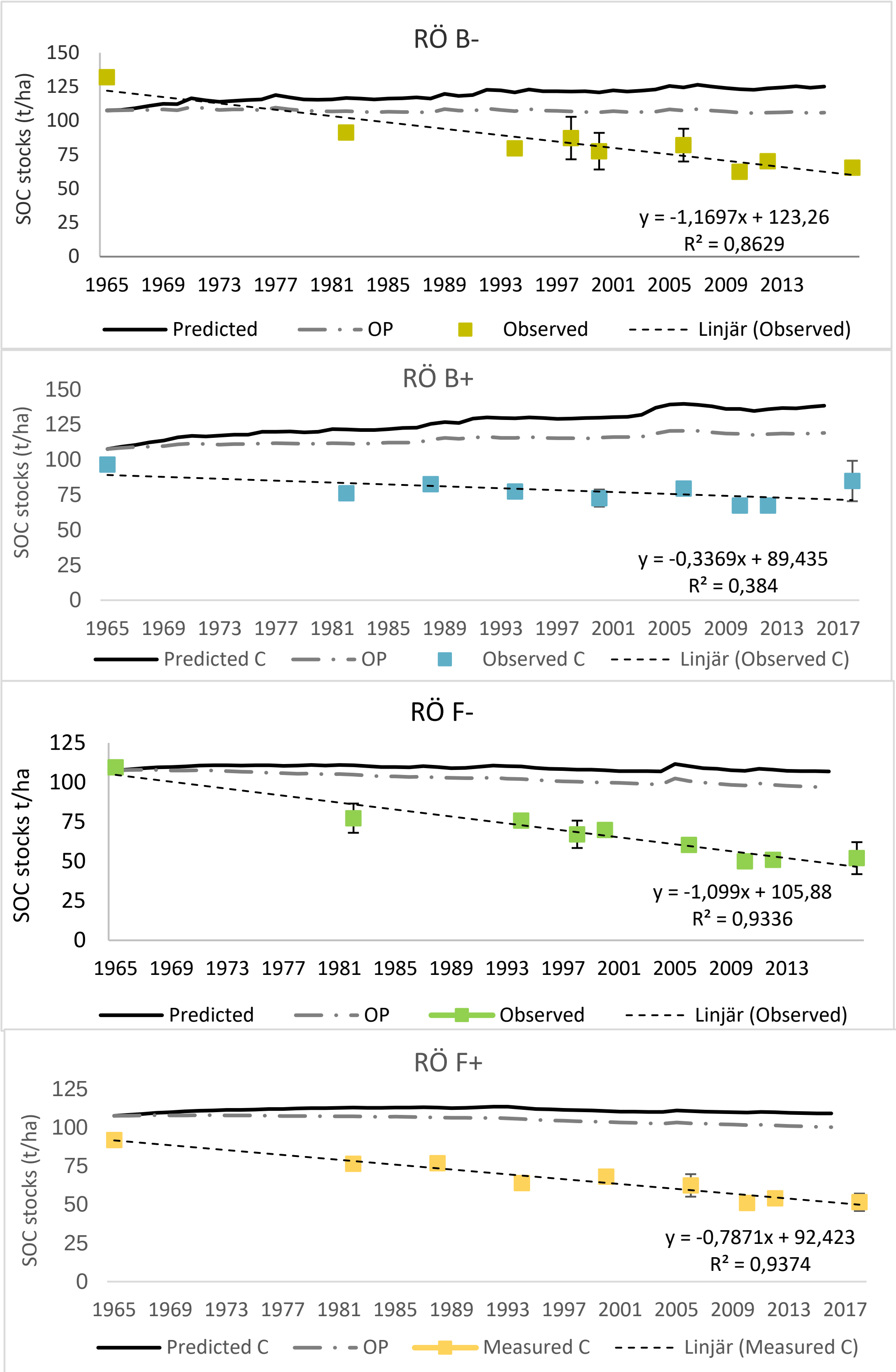

Figure 10. The SOC stock changes from 1965-2018 in Röbäcksdalen. Ley rotation is denoted by (B) and barley (F), fertilization treatment by + (combined fertilization) and - (mineral fertilization). Predicted C correspond to ICBM modelling. OP correspond to the modelling with default values.

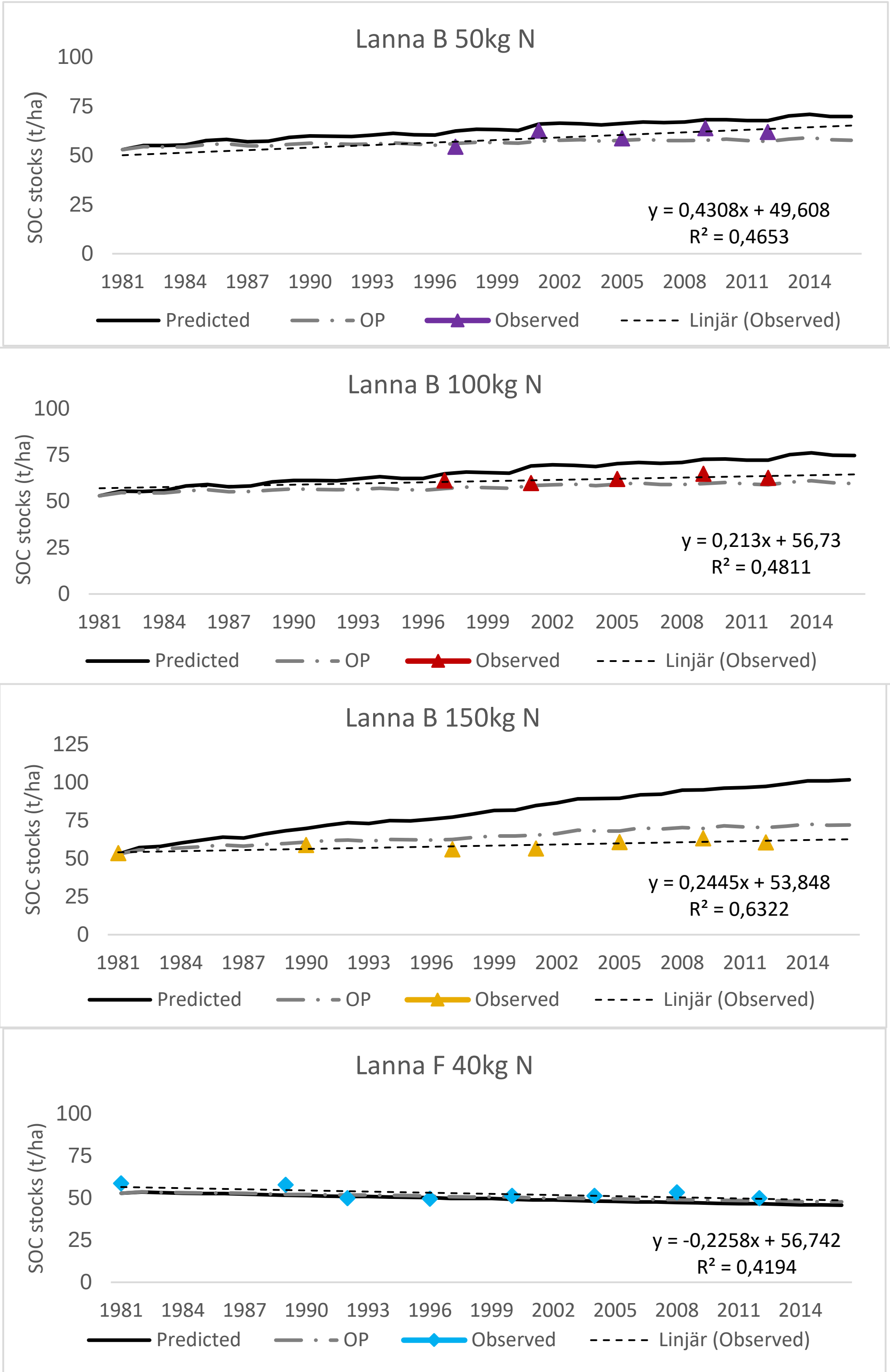

Figure 2612. The SOC stock changes from 1981-2016 in Lanna. Ley rotation is denoted by (B) and barley (F), fertilization treatment by NPK fertilization rates. Predicted C correspond to ICBM modelling. OP correspond to the model with default values.

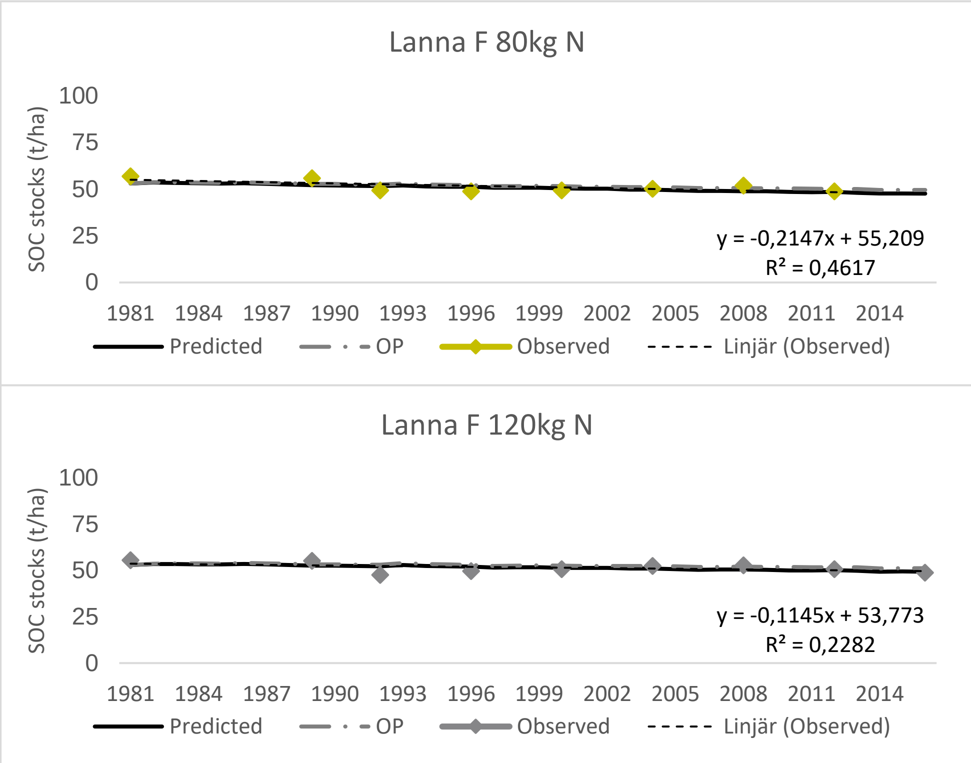

Figure 126. The SOC stock changes from 1981-2016 in Lanna. Ley rotation is denoted by (B) and barley (F), fertilization treatment by NPK fertilization rates. Predicted C correspond to ICBM modelling. OP correspond to the model with default values.

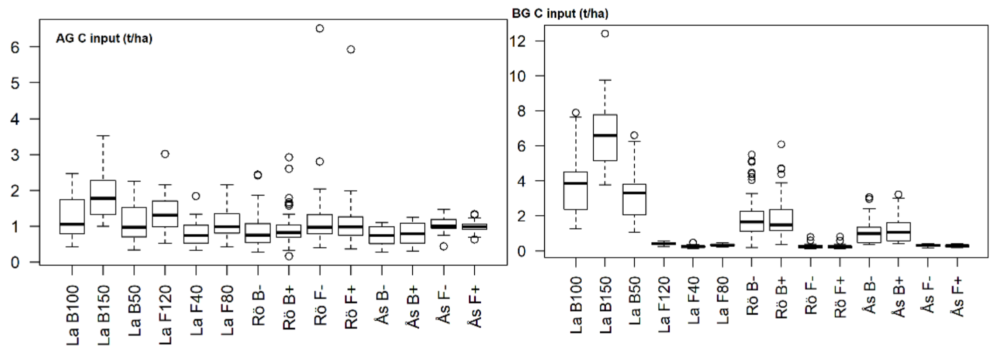

Figure 117. AG (aboveground) and BG (belowground) C inputs for all sites and treatments.

6 DISCUSSION

6.1 The influence of ley on SOC stocks

My results show that continuous ley have the capacity to sequester more C and preserve it for a longer period compared to barley monoculture. In Lanna, about 0,43 t C/ha and year was accumulated in ley rotation receiving 50kg N/ha, whereas for the ley rotations receiving higher amounts of N increased by 0.21-0.24 t C/ha and year. In comparison to barley rotation, the ley rotation sequestered 0.32-0.65 t C/ha and year over all fertilization rates in Lanna. Several studies have reported similar results to the higher range observed for Lanna, which indicate a good sequestration potential for ley (Paustian et al., 1996; Bolinder et al., 2010). For Nordic conditions, an estimated mean difference of 0.52 t C/ha and year between ley-arable systems and annual cropping systems have been reported (Kätterer et al., 2013). An European meta study suggest that perennial grasses and crops have a slightly higher potential soil C sequestration rate of 0.6 t C/ha and year (Freibauer et al., 2004). Even higher numbers of 1,1 t C/ha and year has been estimated if grass and clover is mulched (Soussana et al., 2006) and 1.20 -1.70 t C per ha and year has been estimated for Europe for the conversion of cropland to grassland (Smith et al., 2000; Smith, 2004).

The SOC balance of any crop and management seem, however, to depend also on the former cropping history of the site. Our results from Röbäcksdalen show how fast a site with originally high SOC levels can lose SOC due to drainage and cropping, even for a ley crop rotation with manure applications, as observed earlier by Ericson & Mattsson (2000). The results from Ås revealed that ley held SOC contents stable for a longer period than barley rotation, as it was observed not to decrease until 30 years after the start of the experiment, whereas the SOC in the barley rotation started to decrease from the beginning at all sites. Earlier studies demonstrated that SOM derived from ley is less decomposable in comparison to SOM from annual crops (Conant et al., 2001; VandenBygaart et al., 2003; Freibauer et al., 2004; Kätterer et al., 2012). A long-term study from Rothamsted (UK) found that steady state was reached after 125 years when an annual cropping system is converted to grassland (Freibauer et al., 2004; Johnston et al., 2009). Despite the SOC preservation capacity in ley, the SOC stocks still decreased over time and for all treatments in Ås and Röbäcksdalen. However, B+ lost the least SOC over time in comparison to the other treatments, particularly in Röbäcksdalen (17,2 t C/ha compared to 40-60 t C/ha lost in the other treatments in Röbäcksdalen). Due to the lower C losses and the C sequestering capacity of ley, the topsoil C data for 2018 was significantly higher for ley in comparison to barley rotation in Ås and Röbäcksdalen.

Barley rotation produced higher AG C input than ley rotation, but vice versa for BG C input. Our data suggest that SOC changes were driven mainly by BG inputs, which is consistent to a study showing that AG C input did not correlate significantly to SOC stock changes (Börjesson et al., 2018). It has also been shown that the fraction of BG residues that is humified is at least twice as high as that from AG residues (Kätterer et al., 2011). This is probably why perennial crops have a great C sequestering potential as they have been shown to allocate more C to the roots than annual crops (Paustian et al., 1996; Bolinder et al., 2010). Subsoil C has a promising capacity to preserve SOC as the turnover time and chemical recalcitrance of SOM increases with depth (Lorenz & Lal, 2005; Jenkinson & Coleman, 2008). However, my results from ley rotation showed no significant effect upon subsoil C, although ley contributed significantly more root C than barley. The C/N ratio decreased with soil depth, suggesting relatively more accumulation of organic material from microbial origin in the subsoil. This would be consistent with the older age observed in subsoil C and with the hypothesis (recently verified by Kallenbach et al., (2016)) that humified organic matter has been cycled several times through the microbial biomass (Mathieu et al., 2015). The longer the time scales of SOC and the less inputs from above, the more C/N ratio should eventually converge towards the C/N of microbial biomass (ibid). Poor site drainage and cold climatic conditions could be the reasons to the non-significant effect of treatments in subsoil C as previously observed in northern Sweden (Jarvis et al., 2017).

6.2 The effect of manure on SOC stocks

The results revealed that a combined fertilization had an impact on lowering the SOC losses in Röbäcksdalen and Ås. In Ås, a combined fertilization resulted in lowering the SOC losses by 3-4 t C/ha and year in average for respective crop rotation, e.g. in F+ compared to F- and B+ compared to B-. In Röbäcksdalen was significantly more C lost than in Ås, lost B- and F- approximately the same amounts of C (56.0-59.7 t C/ha). A combined fertilization led to a reduction of SOC losses by 15.9 t C/ha for barley rotation, and about 42.5 t C/ha for ley rotation. Manure in Röbäcksdalen had thus a larger impact on mitigating the SOC decline in ley rotation. The literature reports other cases where manure applications increased the SOM content, particularly in combination with mineral fertilizers (Masto et al., 2006; Liu et al., 2010), as manure contains humified C (Kätterer et al., 2011). In my case where humified C was added to the treatments in Ås and Röbäcksdalen the C losses were reduced.

There was no significant impact of fertilization treatment on yields, since the combined fertilization produced similar yields to mineral fertilization in both Ås and Röbäcksdalen.

6.3 Model prediction and model fitness

ICBM and OP did not describe well the observed SOC stocks decreases in Ås and Röbäcksdalen, with some exception to B- and F- in Ås. The discrepancy between model predictions and observed SOC stocks could be in general be ascribed to the C inputs calculations, as the model kinetic parameters are relatively well tested and verified (Andrén et al., 2004).

The SOC stock drop in Röbäcksdalen is probably explained by the drainage of a former wetland rich in C. ICBM was not designed such a rapid change in management (i.e. from anaerobic to aerobic conditions). However, the SOC drop in Ås was also not well represented by the model. In this case, the C drop could be due to a historical cropping system richer in C than assumed. Another possibility is the interference from a change to more heavy machinery and deeper plowing depth, which could have “diluted” the SOC stocks in a bigger soil mass in year 2000. However, this was only observed for in ley rotation. The ICBM and OP applied better to Lanna and matched well the observed SOC trends, particularly in barley rotation. One possible explanation to the higher model fitness in Lanna could be that the soil in Lanna has a higher clay content than Ås and Röbäcksdalen. As ICBM is calibrated to Ultuna frame trial (Andrén et al., 2007) and soil in Ultuna has clay content similar to Lanna, the two soils in Lanna and Ultuna might behave more similarly than the sandy-loamy soils in Ås and Röbäcksdalen. Fine textured soils have a higher proportion of mineral and aggregate bound SOC that makes it more resistant to decomposition (Scott et al., 1999; Vesterdal et al., 2002), due to improved chemical interactions (Six et al., 2002) and, or the chemical sorption to mineral surfaces (Kaiser & Guggenberger, 2000). This explains the lower decomposition rate that has been observed in fine-textured soils than in coarse-textured soils (Verberne et al., 1990). According to this hypothesis the SOC in sandy-loamy soils would hence be lost faster, which could explain why barley rotation in Ås lost SOC faster than the model predicted with changed cropping system (from ley cropping system to annual cropping system). To calibrate to a more coarse-textured soil with lower clay content the humification coefficient could eventually be lowered to match the SOC stock changes better in Ås and Röbäcksdalen, which has been assumed before for coarse-textured fields (Kätterer et al., 2004).

The OP model simulated the SOC changes better for ley rotation over all sites (lower RMSE than ICBM). ICBM overpredicted the SOC stocks particularly for ley rotation in Lanna that received 150kg N/ha, which correspond to a higher AG and BG input for that particular treatment. The overprediction might indicate that a S:R ratio closer to 2 for ley is more probable than S:R ratios of 0.60 for timothy and 1.38 for red clover, as the higher S:R ratios of 2.0 and 1.82 for timothy and red clover respectively was applied only every 6th year. The ley composition indicated a legume proportion averaging only 30% (1st harvest) and 35% (2nd harvest) for Lanna, and 9% and 21% for Ås and Röbäcksdalen respectively. Therefore the S:R ratio of timothy (0.60) had a higher impact on the calculated BG inputs.

The observable upwards peaks in Ås for both rotations (B and F) and B+ in Röbäcksdalen after 2010 might indicate a new steady-state levels for respective treatment (Johnston et al., 2009). From the calculated steady states however, the SOC stocks for all treatments except for F- in Ås, and B+ in Röbäcksdalen, are expected to be further reduced, which might indicate that these recorded upward peaks are due to climate oscillations or differences between the soil sampling protocol in 2018 and the former sampling protocol. For Lanna we can conclude that the sequestration potential for barley, e.g. straw incorporation is about 22-36.6 t C/ha, whereas ley rotation can still sequester 123.5-143.8 t C/ha (the higher numbers represent the higher fertilization rates). Lanna might have thereby the potential to double the amount of SOC from the values observed in 2012. Summarizing the results from Lanna, F- in Ås and B+ in Röbäcksdalen would indicate steady state levels of 22.10-36.6 t C/ha for barley rotation and 123.45-313.72 t C/ha for ley rotation.

6.4 How SOC stocks relate to other soil parameters

A negative significant correlation between SOC stocks and pH was found from my results. The ANOVA test also showed that ley rotation significantly lowered the pH in comparison to barley rotation, in line with field results reported by Paul, K. I., Black & Conyers (2001). My results support the hypothesis of acidification by H+ excretion by plant roots (Mclaughlin et al., 1990; Dolling & Porter, 1994; Helyar & Porter, 2012) when organic acids, amino acids and other humic substances are produced when organic matter is decomposed (Paul, 2015).

My results showed that bulk density did not correlate to SOC content, in opposition to what has been previously affirmed by Eriksson et al., (2010) that for every C % increase, bulk density decrease until it reaches an end of 0.1 kg/dm^3, which correspond to a C content of 50% (e.g. organic soil). Also, Kätterer et al. (2006) found a similar relationship between SOC and bulk density, as it was observed to decrease exponentially (r^2=0.87).

The results show however that water content was negatively correlated with BD, which supports the hypothesis of an improved water-holding capacity in soils with lower BD (Yang et al., 2011).

6.5 Soil aggregate stability

My results indicate that SOM contribute to soil aggregate stabilization against clay dispersion, as Ås with its lower SOC content released more RDC than Röbäcksdalen, which is consistent with the study of Dexter & Czyz (2000). Similarly, the positive correlation between pH and RDC show that SOC might reduce RDC as pH and SOC stocks were negatively correlated (fig. 14). The results derived from ANOVA on RDC revealed however, significance for crop rotation at only 0.1 significance level, but there might be reasons to assume that ley decreased RDC as ley had a significant positive effect upon pH. The saturation concept developed by Schjønning et al. (2012) would indicate in all the sites analyzed here that the clay/silt fraction is not saturated with C, and hence still have potential to sequester more C. The observed trends and the steady state values calculated according to Equation 2 does not support this hypothesis.

No significant correlation was found between ley and WSA in our study. This is contrary to previous studies, as ley was shown to slow down the wetting rate because SOM acted as a binding agent between aggregates (Almajmaie et al., 2017). Other studies have found positive correlations between SOM and WSA, which in turn increases macroaggregate stability against slaking and disaggregation (Chaney & Swift, 1984; Fortun et al., 1989; Mbagwu & Piccolo, 1989). But organic bonds can also be broken and act as a deflocculant (Emerson, 1983; Goldberg & Forster, 1990; Itami & Kyuma, 1995), therefore is the effect from SOM on WSA not obvious. There seems to be an upper limit to which organic C can support this kind of aggregation, and only part of the SOM seem to be responsible for water stable aggregation (Tisdall & Oades, 1982). There are also indications that the spatial disposition of SOM seems to be more important than the type or amount, why SOM does not appear to be the major binding agent (ibid).

No significant effect of either mineral fertilization or combined fertilization was found by a one-way ANOVA test for RDC, although manure has been reported to increase RDC from accumulated K^+, Na^+ and NH^{4+} (Haynes & Naidu, 1998b). A study based on the Rothamsted LTE concluded that large amounts of manure (>35t/ha/year) over 100 years was required in order to observe any effect on soil aggregate stability (Edmeades, 2003). Therefore, the time scale in our experimental sites might be too small to observe significant results on RDC. Still my results indicate that the application of mineral fertilizers alone resulted in a higher water stable aggregation at both Ås and Röbäcksdalen. Results from other LTE indicates that fertilizer applications can improve water stable aggregation and BD along with other soil physical characteristics, especially when applied together with manures (Haynes & Naidu, 1998b). This as manures favoured the forming of slaking-resistant aggregates (Aoyama et al., 1999). There is however conflicting evidence in the literature regarding the effect of mineral fertilizers on soil aggregation (Yu et al., 2012), as some authors have reported an improved macroaggregation from the application of mineral fertilizers (Rasool et al., 2008; Lugato et al., 2010), as C was observed to increase microaggregation (Lugato et al., 2010).

No significant relationship between RDC and WSA was found for this study, contrary to many previous studies (Pojasok & Kay, 1989; Perfect et al., 1990a; Caron et al., 1992; Rasiah et al., 1992; Rasiah & Kay, 1994). These studies found that macro-aggregate stability generally decreased with increased amount of dispersible clay. This could be explained further by an increased surface area is exposed to clay dispersion when macro-aggregates breakdown (ibid). The lack of correlation between RDC and WSA was however reported by other authors, and these inconsistencies suggest that each test provides information about different aspects of soil stability (Bartoli et al., 1991; Mbagwu et al., 1993).

No significant relationship was found between RDC or WSA and water content in this study. As clay dispersion seem to be dependent on soil water content (Emerson, 1967; Kay & Dexter, 1990; Watts et al., 1996), and macroaggregate stability seem to lessen due to an increased water content (Gerard, 1987; Coote et al., 1988; Perfect et al., 1990a; b; Gollany et al., 1991; Caron et al., 1992; Rasiah et al., 1992; Chan et al., 1994), can my results be explained by the limitation the snapshot of the state of aggregate stability the time of sampling imply.

The field moist aggregates released more RDC than the dry aggregates which is in accordance with other studies (Pojasok & Kay, 1990a; Kjaergaard et al., 2004). In previous studies even field-moist samples that have been rewetted and thereafter drained showed considerable greater dispersion than dry aggregates (Schjønning et al., 2012). This however, might be due to the fact that rewetted soil did not immediately return the soil to original levels of dispersibility (Schjønning et al., 2012).

6.6 Evaluation of methods

6.6.1 Field soil sampling

The soil got compressed in three of the soil samples in Ås, which I accounted for by correcting the sampling depth intervals (assuming an equally distributed compression over the whole soil core). Sampling with instruments as shovels have been observed to cause less compression than augers in soil samples, and thereby influence less aggregate stability (Kemper & Rosenau, 1986; Jastrow & Miller, 1991). However, for this study soil augers were used which might have caused the decompression of some of the field samples. It is the small diameter of the soil augers as well as the hammering that causes the compaction (McIntyre, 1974a; McIntyre, 1974b; Campbell and Henshall, 1991; Freitag, 1971, all as cited in Díaz-Zorita et al., 2002). Compression is more likely to occur when the soil conditions are wet (Amézketa, 2015). At the time of soil sampling (before sowing) was the water content quite high, 21-25% in Röbäcksdalen and 12-17% in Ås. The compression could further have influenced the bulk density measurements in this study and might explain the non-existent correlation between bulk density and SOC stocks.

6.6.2 Data treatment

There were many missing values in the archived data concerning yield data, especially for ley in Ås to which I received data for only 1966-1991. The same is true for barley data in Lanna where I got data for only 15 out of 36 years. To fill in the missing values the yield data were averaged by treatment and site for all years, which might reflect the SOC stock changes quite well for barley treatment in Lanna. Because of missing ley data from Ås after 1991 an eventual subsequent yield change might not have been considered. As mentioned earlier neither ICBM nor OP could describe the SOC drop observed in ley rotation in Ås, where an eventual yield decrease could be a possible explanation. Moreover, the effect from the change in fertilization treatment in 2010 could not be evaluated in this study as the yield data were too few to note any major change in yield.

The S:R ratios set in OP could describe better the observed data in many treatments (see paragraph Model predictions and model fitness). There are difficulties with estimating the BG C input from S:R ratios, as they are subject to many sources of variation (Bolinder et al., 2002). Root biomass is much affected by both environmental and management factors (Hu et al., 2018). For example the S:R ratio might decrease under dry soil conditions because of the expansion of root systems in order to acquire more water from deeper levels (Turner & Begg 1978 as cited in Bolinder 2002). The S:R ratios are also commonly estimated at crop maturity, to account for the root biomass at harvest (Bolinder et al., 1997), however the root mass is at its maximum at about anthesis in spring cereals (Hansson &

Andrén, 1987; Pietola, 1995). Because of the difficulties to estimate S:R ratios, the use of fixed root biomass which considers the most influential factors of farming system (organic vs conventional) and crop species has been proposed as a reliable method to estimate root biomass without the need to consider AG biomass (Hu et al., 2018).

6.6.3 Soil aggregate stability

The slow wetting technique (NVSW) was chosen to pre-wet the air-dried soil before performing the wet-sieving test. The NVSW has been shown to be able to obtain water aggregate stability values that represent field conditions in temperate climates (Dickson et al., 1991) as dried aggregates for determination of water aggregate stability have shown to result in lower stability due to gravitational and capillary flow (Almajmaie et al., 2017).

Among the many different liquid solutions which have been tested in several studies from ethanol, benzene to ordinary tap water, distilled water is still the most commonly used liquid for wet-sieving techniques even if it has been demonstrated to produce clay dispersion (Amézketa, 2015). We chose however to use distilled water as it was available, although the risk of influencing the dispersibility test increased. Hence, the wet sieving method quantifies the loss of macro-aggregate stability in three different actions: slow wetting, shaking and chemical without discriminating the relative importance of each of them (Amézketa, 2015).

Because of the reported variation between the two approaches, we decided to perform the RDC test on both air dried and field-moist soil. And as we found most significant correlations with the data from air-dry aggregates in our test, we choose to refer most of our results to the air-dry aggregates. However soils seldom reaches the same water content as air dried soils in field condition if soil is not left bare (Kemper & Rosenau, 1984; Caron et al., 1992), and air-drying of soil samples may cause irreversible changes in chemical or physical characteristics which can alter the aggregate stability from its natural state (Alderfer, 1946; Reid & Goss, 1981; Churchman & Tate, 1987). Samples at field moisture might however aggravate the comparison of samples in different conditions or from different sites (Amézketa, 2015), and this is why it is preferable to either air dry or saturate under controlled conditions the samples prior to trying to standardize initial conditions (Le Bissonnais, 1996; Amézketa, 2015). However, other studies show that results from air-drying are still related to the antecedent moisture content and cropping history (Kemper & Rosenau, 1984; Caron et al., 1992).

All aggregate stability tests have in common that they try to predict long term behaviour of soils from the field from short term observations (Matkin & Smart, 1987). The statistical analysis was further limited by only 2 replicates from the experimental design of the LTEs.

7 CONCLUSION

Ley rotation significantly increased the SOC stock (up to 0.65 t C/ha and year) in comparison to barley rotation in Lanna. However, a combined fertilization can potentially increase the SOC sequestration potential for ley even more – as observed for the northern sites. Both cropping systems were observed to lose SOC for the two northern sites. However, relative to the barley rotation, the SOC losses were lower for ley rotations, particularly with combined fertilization. The SOC stocks in ley rotation in Ås decreased not until 30 years after the initialization of the experiment, why SOC derived from ley might have a SOC preserving potential as well as a SOC sequestering potential. My data indicate that ley lessened the amount of dispersible clay, which suggest that SOC increases soil resistance against rainfall.

The simulated SOC stocks with the models did not describe the SOC decrease in Röbäcksdalen and Ås (with exception for the mineral fertilization treatment). This, either because of the high uncertainty when estimating the initial SOC stocks or because of some imprecision in the estimation of the belowground inputs. The decreased SOC stocks in Röbäcksdalen was most likely connected to the drainage of a SOC rich grassland, an event that is not accounted for in the models. The models were shown to correspond well to the observed SOC stocks in Lanna, which is consistent with other studies using the same model on ley systems. OP matched in several cases better to the measured SOC stocks, which indicate that the default values in OP describe the SOC dynamics well. In general, although ICBM and OP represented the trends in some cases, the models should be applied with caution when considering the initial SOC stock states.

Overall, my study suggests that ley rotation both sequester and preserve SOC stocks in comparison to annual crops. Ley rotations is therefore a suitable strategy to mitigate the GHG emissions from the agricultural sector and at the same time increase the soil structure. However, these recommendations must also consider a sustainable usage of arable soils, so that ley production would not compete with food production.

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