Student thesis series INES nr 636

Effective soil organic carbon monitoring in perennial agriculture systems

Sampling protocol development and evaluation

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2024
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Maja Holm (2024).
Effective soil organic carbon monitoring in perennial agriculture systems – Sampling protocol development and evaluation
Effektiv mätning av markkol i perenna jordbrukssystem – Utveckling och utvärdering av mätprotokoll
Master's degree thesis, 30 credits in Physical Geography and Ecosystem science
Department of Physical Geography and Ecosystem Science, Lund University

Level: Master of Science (MSc)

Course duration: *January* 2023 until *January* 2024

Disclaimer

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Master thesis, 30 credits, in Physical Geography and Ecosystem Science

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Abstract

Perennial agriculture systems are gaining ground as a more sustainable alternative to conventional annual agriculture, partly for their potential to increase the soil organic carbon (SOC) content. Carbon farming is another hot topic for SOC sequestration, as it creates economic incentives for farmers. The main purpose of this study was to measure the baseline SOC stock for a larger research project, where the SOC balance of a field with the perennial grain Kernza™ will be compared to conventional annual crops grown on a control field during five years. The SOC stock of the test and a control plot in Alnarp, south Sweden has been determined to 136.76 Mg SOC ha^{-1} for Kernza™ and 150.06 Mg SOC ha^{-1} for the control, through extensive field sampling and laboratory analysis. When evaluating different sampling protocols regarding stratification and sample size, it was found that in order to accurately detect relevant changes in SOC over a short time frame, a large number of samples was required. In this study, stratification was not effective to reduce the required number of samples. This study implies that there is a need for robust SOC sampling designs for research and the carbon farming market alike.

Keywords: physical geography, ecosystem science, soil organic carbon, soil sampling, perennial crops, carbon sequestration, carbon farming, Kernza™, SOC stock.

Acknowledgements

I would like to express my gratitude towards the following people and actors for their much-appreciated help along the way:

Jonas Ardö *Supervisor*
Karl Ljung *Lab master*
Ryan Davidson and Peter Kornacher *Soil sampling team*
Svensk Kolinlagring *Inspiration, field experience and stratification data*
Daniel Holm *Statistics support*

Abbreviations:

SOC – soil organic carbon
SIC – soil inorganic carbon
BD – bulk density
MDD – minimum detectable difference
IDW – intermediate distance weighted
SK – Svensk Kolinlagring *(Swedish Carbon Sequestration)*
GHG – greenhouse gas

1 Introduction

Soil organic carbon sequestration in agricultural soils is one of the key options for cost-effective climate change mitigation, which also leads to additional benefits like improved soil health and resilience as well as food security (Lal, 2011; Ledo et al., 2020; Nayak et al., 2019). Since agriculture compromises a third of all arable land, the potential for achieving large scale carbon sequestration is huge. There is also a large economic and environmental interest in instrumentalising carbon sequestration to offset GHG emission by selling carbon credits (*carbon farming*), and initiatives for selling carbon credits on the voluntary market are increasing in number (van der Voort et al., 2023).

One management method for improved carbon sequestration in agricultural soils is to grow perennial instead of annual crops. This is mainly due to the large root systems of perennials, the year round vegetation cover and reduced soil disturbance (Ledo et al., 2020). However, there is significant lack of scientific evidence on the carbon sequestration capacity of perennial crops (Ledo et al., 2020). A study of the recently developed perennial crop Kernza™ showed that it was a strong carbon sink during the 4.5 years of study (de Oliveira et al., 2018), but further studies of Kernza™ in different climatic contexts, soils and management systems are needed in order to determine its' potential in replacing annual crops and sequestering atmospheric carbon at a large scale.

Detecting changes in soil organic carbon (SOC) content over time spans shorter than 5-10 years is difficult (Arrouays et al., 2018; Vandenbygaart & Angers, 2006). This is due to several factors including the comparatively large background content of SOC, the slow and dynamic nature of SOC build-up and its high spatial variability (Vandenbygaart & Angers, 2006). Accurate estimations of SOC stocks in individual fields as well as on a landscape level is therefore both time and cost consuming. Despite the difficulties, there is an obvious need to detect changes in SOC content over shorter time spans (Arrouays et al., 2018). For climate change research and the carbon credit market alike, accurate, reliable and affordable methods for measuring carbon sequestration are required (Paul et al., 2023; van der Voort et al., 2023).

Svensk Kolinlagring (*Swedish Carbon Sequestration*) is currently the only carbon credit initiative on the Swedish market. As of now, their carbon credits are based on standard values of carbon sequestration (300 kg C ha^{-1} yr^{-1}) (Svensk Kolinlagring, 2022b). However, they are working towards using field data as well as modelling to verify the amount of carbon that has been sequestered on the fields (Svensk Kolinlagring, 2022b). Amongst other things, they are investigating how this can be done in a cost-efficient yet accurate manner, and have established their own sampling protocol, largely based on FAO (UN Food and Agriculture Organisation) recommendations.

Both perennial crops and carbon sequestration are hot topics. At Lund University, the recently started research project *Capturing Carbon in Perennial Systems* (*Perennial*) will investigate whether a transition from annual to perennial crops can effectively help Sweden reach net zero emissions in 2045, and net-negative emissions after that. As part of this, the perennial grain crop Kernza™ is grown on almost 10 ha in Alnarp, south Sweden, where it will be compared to conventional farming on a neighbouring control field. Fluxes of carbon, energy and water between the soil, the biosphere and atmosphere will be monitored in order to estimate the ecosystem responses to the shift to perennial crops as well as to environmental variations. As for any effective study on carbon sequestration, a rigorous baseline measurement of SOC is required in both a test field and a control field (Nayak et al., 2019).

1.1 Aim

The primary aim of this thesis is to determine the total SOC stock in the Kernza™ test and control fields in Alnarp prior to the sowing, through extensive field sampling and data analysis. In order to do this, a sampling protocol tailored to the site will be created. The result of the field sampling will serve as a baseline measurement for the *Capturing Carbon in Perennial Systems* research project. The results will also be used to assess the performance and applicability of different sampling designs for estimating SOC in different contexts: both scientifically and on the carbon credit market. Specifically, results derived from the sampling protocol developed in this study will be compared to results derived from a simulation of the Svensk Kolinlagring sampling protocol. It will also be investigated whether stratification can be used in order to reduce the required number of samples, by evaluating both the stratification scheme of Svensk Kolinlagring and a stratification scheme created from remote sensing data of seasonal productivity.

1.2 Research questions

1. What is the SOC content and stock of the Kernza™ test and control plots in Alnarp, estimated by the *Perennial* sampling protocol created for this thesis?
2. What is the SOC content and stock of the Kernza™ test and control plots in Alnarp when estimated by a simulation of the Svensk Kolinlagring sampling protocol?
3. Are the *Perennial* and Svensk Kolinlagring protocols able to measure the expected or desired rates of carbon sequestration?
4. In this context, could a stratified sampling design reduce the required number of samples?

2 Background

2.1 The carbon cycle in agricultural soils

As photosynthesizing plants take up CO_2 from the atmosphere and store it as organic compounds in biomass, atmospheric carbon is transformed into terrestrial carbon. Through aerobic decomposition by microbes, the carbon is eventually returned to the atmosphere as CO_2 (Weil & Brady, 2017). Much smaller fractions are returned as CH_4 through anaerobic decomposition. Hence, the carbon cycling in soils is an important regulator of the atmospheric greenhouse gas (GHG) levels (Weil & Brady, 2017). In fact, 17 % of global GHG emissions are derived from agriculture (Tubiello et al., 2013).

Inputs of carbon first occur in more labile forms in the topsoil, in forms of plant debris or root exudates – the active fractions – where it can be readily used as energy by soil microorganisms. Large labile carbon compounds eventually transforms into more stable fractions, where it can stay in the soil for decades or centuries, as the carbon compounds are protected from decay either physically or chemically, allowing SOC compounds to accumulate in the soil (Weil & Brady, 2017). Microorganisms have an essential role in soil C cycling. They contribute to both degradation and mineralization of SOC which decreases the soil C storage, but also increase the amount of recalcitrant metabolites, which increases the residence time of SOC (Chenu et al., 2019).

Depending on management, climate and soil type, the world's agroecosystems have lost 25-75 % of their pool of SOC (Haddaway et al., 2017; Horwath & Kuzyakov, 2018; Lal, 2011). This has illuminated the potential of soils to provide cost-effective climate mitigation by sequestering CO_2 from the atmosphere, while also improving soil health (Lal et al., 2021;

Stanton et al., 2018). The long-term balance between the losses and gains of carbon in the soil determines whether the soil acts as a carbon source or sink. If the input of organic compounds to a soil increases, its carbon stock will increase until a new equilibrium value between gains and losses is reached (Chenu et al., 2019). Soils also contain significant amounts of inorganic carbon, but while most soils contain varying amounts of SOC, inorganic carbon is primarily found in soils in arid and semiarid regions. In the context of carbon sequestration in Swedish agricultural lands, SOC is more influential (Lal, 2011). Still, when determining the C stock of an agricultural field, both soil organic carbon and soil inorganic carbon (SIC) need to be considered, as SIC can make up a non-negligible amount of the total carbon (TC).

Although all soils have a finite capacity to store carbon by protecting it from mineralization, the SOC pool in most agricultural ecosystems is well below this capacity (Chenu et al., 2019; Weil & Brady, 2017). Soils can continue sequestering carbon for decades until a new equilibrium is reached, where it can be stored for millennia (Weil & Brady, 2017). However, in the topsoil (0-30 cm), the timeline is likely decades (Demenois et al., 2021). A soil's carbon storage potential is defined as the maximum C gain in soil C stock that can be attained under a certain timeline and climate. The sequestration potential refers to the maximum gain in SOC that allows a net removal of atmospheric CO_2, under a certain timeline and climate (Chenu et al., 2019).

2.2 SOC sequestration in agricultural soils

Land managers can sequester carbon by changing the balance between SOC gains and losses (Weil & Brady, 2017), and it is estimated that agricultural lands have the potential to sequester up to 66% of historical C loss if managed properly (Ledo et al., 2020). While carbon outputs are mainly controlled by pedoclimatic conditions, the inputs of carbon are determined by farming practices (Kätterer & Bolinder, 2022). Conventional agricultural land management practices like tillage, monoculture, and fertilization have a great effect on the carbon cycle in the soil (Horwath & Kuzyakov, 2018; Kätterer & Bolinder, 2022). Soil disturbance like tillage leads to accelerated losses of carbon, both through increase decomposition rates and increased erosion. As soil aggregates are broken up during tillage, SOC previously protected from decay by being adsorbed on soil colloid surfaces or in soil aggregates, gets exposed to oxygen and can thus start decomposing more rapidly (Horwath & Kuzyakov, 2018). Whether increased inputs of C leads to SOC sequestration and an increase in the C stock, or only prevents C loss compared to the previous situation depends on both management and land use history. The input of C that is required for maintaining the C balance is proportional to the size of the C stock and it is therefore more relevant to focus on changes in C storage rather than changes in SOC sequestration (Kätterer & Bolinder, 2022).

Increasing the organic carbon content in agricultural land may come with several major benefits for the farmer: Improved soil biodiversity, increased fertility and productivity, improved water retention and greater crop resilience, while reducing erosion, soil compaction and nutrient runoff (Chenu et al., 2019; Lal, 2011; Moinet et al., 2023). Increasing SOC in soils poor in organic matter can trigger a positive feedback loop: increasing SOC leads to increased aggregation, which helps reduce soil erosion and carbon loss. Increasing SOC also increases fertility, which leads to higher plant productivity and thus higher input of organic matter from plants (Chenu et al., 2019; Kätterer & Bolinder, 2022).

Agriculture management strategies that are often promoted for their carbon sequestration potential include, but are not limited to:

- No tillage / conservative tillage

- Cover crops
- Organic amendments additions, e.g., biochar or compost
- Year-round vegetation cover
- Agroforestry / perennial crops
- Integrated grazing
- Increased crop rotation diversity

These strategies all contribute to altering the balance between carbon inputs in outputs in different ways (Chenu et al., 2019; Horwath & Kuzyakov, 2018; Kätterer & Bolinder, 2022; Stanton et al., 2018). However, they all come with economic cost for the farmers and without any guarantees for increased SOC content at the farm level, as most reported C sequestration values originate from research fields where the same treatment have been done year in and year out (Horwath & Kuzyakov, 2018; Stanley et al., 2023).

2.3 Perennial crops and Kernza™

Listed above as a management method for promoting carbon sequestration, perennial crops have recently gained significant attention as a promising alternative to annual crops. In contrast to annual cropping systems, which most often are greenhouse gas emitters, perennial systems may have negative or net zero emissions (Ledo et al., 2020). Perennial cropping systems have an array of advantages over annual varieties, including no-till, reduced use of fertilizers, less irrigation, higher water use efficiency and less machinery work (de Oliveira et al., 2018). They also generally have larger root systems.

Plants root have great influence on soil health and properties such as on SOC content and microbial communities, and SOC pools are primarily regulated by root residues, since the contribution of belowground inputs from roots to SOC is much higher than the aboveground input from residues (Chenu et al., 2019; Pugliese et al., 2019). The majority of SOC in agroecosystems is stored in the top 30 cm of soil, since the roots of most agricultural crops don't extend very deep into the soil. However, deep rooted plants like perennials can extend far deeper into the soils, where the carbon compounds are protected from decay, and by placing C inputs in deeper soil layers, the efficiency of C input is higher (Chenu et al., 2019). Perennial crops also promote SOC sequestration by increasing aggregation, which protects the SOC molecules from decay (Ledo et al., 2020; Sprunger et al., 2018). Therefore, a transition from annual deep-rooted plants like perennials are considered a promising method for C sequestration and climate mitigation (Ledo et al., 2020; Pugliese et al., 2019; Smith et al., 2020).

According to The Land Institute (2023), grains make up around 70 % of our caloric consumption. A switch from annual extractive agriculture systems to perennial systems could therefore pose a great possibility and opportunity for creating a sustainable and regenerative agriculture. The Land Institute is a non-profit agriculture research organisation located in Kansas, US, which has recently developed the perennial grain crop Kernza™ by domesticating intermediate wheat grass (*Thinopyrum intermedium*), which is a relative to annual wheat. It is now grown on small scales in the US and Europe by both farmers and researchers. The yield is currently far from that of annual wheat, but the development process is ongoing with new varieties and increasing yields with each breeding cycle (The Land Institute 2023).

One of the main characteristics of Kernza™ is its unusually deep roots which can extend up to 3 m into the ground. A study by Sprunger et al. (2018) found that after four years, perennial intermediate wheatgrass had 15 times more root mass than annual wheat and de Oliveira et al. (2018) found that Kernza™ was a strong carbon sink (-340 g C m^{-2} yr^{-1} or -1478 g C m^{-2} in total) during the 4.5 years of experiment. However, a later study of the same field found that

depending on how much biomass is harvested, it can also act as a carbon source or be carbon neutral. By changing the fertilizer load during the course of the experiment, they also found that lower fertilization led to higher ecosystem respiration, making it a weaker net sink of CO_2 (de Oliveira et al., 2020). The extensive root system of Kernza™ has also been shown to lead to a higher water use efficiency (de Oliveira et al., 2020).

2.4 Carbon farming

Apart from promoting soil health, there is a general consensus among scientists that increasing soil carbon levels is crucial to mitigate climate change, and thus contributing to achieving carbon neutrality (Horwath & Kuzyakov, 2018). By the look of current available technologies, it will not be possible to achieve carbon neutrality by 2050 without carbon dioxide removal. Increasing the SOC levels in agricultural soils is considered to have a high potential for this. Although, this is also associated with high economic costs for farmers, as mentioned earlier (Paul et al., 2023). For example, it has been shown that without economic incentive, French farmers could only sequester 0.66 Mt C yr^{-1} while the technical potential was as high as 8.43 Mt C yr^{-1} (Demenois et al., 2021). Thus, giving economic value to SOC sequestration in agricultural soils is an important step in achieving its potential for climate change mitigation and regulation.

On this basis, numerous studies and initiatives have investigated how to instrumentalise carbon stocks as an active way of offsetting carbon emissions – so called *carbon farming*. This includes – among many others – the 4p1000 Initiative launched by the French government in 2015 and FAO's Global Soil partnership established in 2012. The 4p1000 Initiative based on a thought experiment suggesting that if the C stock of all soils globally would increase with 0.4 % per year, a substantial amount of CO_2 would be removed from the atmosphere (Rumpel et al., 2020). Since then, numerous similar initiatives, frameworks, and voluntary markets have opened up worldwide, where farmers can register their fields and actions and be economically compensated for making changes in management to achieve carbon sequestration (Paul et al., 2023). FAO released their GSOC MRV protocol in 2020, providing a framework and guidance on how to measure, monitor, report and verify changes in SOC levels that result from changes in farming management (FAO, 2020).

Svensk Kolinlagring (*Swedish Carbon Sequestration*) is the only carbon farming framework in Sweden, and in late 2022 they launched the first version of their carbon credit program. They're a non-profit initiative and common platform operating on the voluntary market, connecting different actors to enable increased carbon sequestration in Swedish agricultural soils (Svensk Kolinlagring, 2022b). They have developed their own protocol for measurement, verification and reporting (MRV), based on FAO's recommendations in the GSOC MRV protocol as well as other scientific resources. However, they and virtually every other carbon farming scheme share issues regarding both the verification, additionality and permanence of sequestered carbon, which are all core concepts of carbon offsets (Demenois et al., 2021; Paul et al., 2023; Stanley et al., 2023). Additionality refers to whether the carbon sequestered by the farmer is additional to what would have been sequestered without the carbon farming incentive. The length of time that the carbon remains in the soil without being released into the atmosphere again is referred to as permanence, while verification refers to whether it can be reliably confirmed that a specified amount of carbon has in fact been offset through sequestration (FAO, 2020).

2.5 The main hurdle: Monitoring SOC

The primary obstacle for effective monitoring of C sequestration in agricultural soils, and thus in creating an MRV protocol that is reliable and widely applicable, is that changes in SOC are inherently difficult to monitor in a cost- and time efficient manner. Another limitation is a lack of understanding on how SOC content is influenced by climate, management and soil properties (Smith et al., 2020). This has led to large differences in soil sampling frameworks and protocols between different actors. Still, there is dire need for a consensus on accurate and reliable methods for monitoring and measuring SOC. This is true for both scientific research, national reporting and carbon farming (Paul et al., 2023; Smith et al., 2020; Stanley et al., 2023), as accurate mapping of the C stock in fields essential for capturing the temporal changes and ensuring both verification and permanence of carbon sequestration in the soil (van der Voort et al., 2023).

Efficient monitoring of SOC sequestration depends on the ability to measure SIC with sufficient accuracy to detect relevant changes (Arrouays et al., 2018). Several different factors contribute to the complication of detecting SOC changes: insufficient or unsuitable sampling designs, the spatial and temporal variability of SOC, variability in bulk density, unmet statistical assumptions as well as laboratory and soil processing methods variations (Paul et al., 2023; Stanley et al., 2023). Another major factor is that changes in SOC content are low compared to the total stock of SOC, and since the spatial variation of SOC content within fields is often comparatively high, they are hard to detect. This is likely the main driver of uncertainty (Arrouays et al., 2018; Smith et al., 2020; Stanley et al., 2023). Additional challenges in SOC monitoring include the reversibility of carbon sequestration due to the discontinuation of certain management practices, and climate change or climate variability (Smith et al., 2020).

The sources of uncertainty listed above are often of the same magnitude as the change in SOC obtained through management interventions during the time frame often used by carbon farming schemes (Stanley et al., 2023), meaning that the monitoring protocol needs to be able to in a statistically reliable way detect changes in SOC content that are larger than the variation of SOC content within the studied area. This is often referred to as the minimum detectable difference (MDD) (Stanley et al., 2023). Several studies have shown that in order to detect significant change in SOC content, the timespan needs be around 10 years or more (Smith et al., 2020). However, there is an obvious need for monitoring protocols to be able to detect changes in shorter timespans, as carbon farming programs generally work with timespans of 3-5 years. Since the timeline for the Perennial research project is 5 years, this also applies to this study.

How much carbon can be sequestered in a field after five years? Knowing this is important for setting an appropriate MDD, but it's a complex question. Ledo et al. (2020) found that SOC increased 20 % (±10 %) during a 20-year period following a transition from annual to perennial crops, which means an average of 5% SOC increase per every 5 years. However, the increase was not linear: the SOC content increased sharply in the beginning, then dropped into steady state. A Danish research project found that the SOC content increased 4% during the 5 years following a transition from annuals to perennials, whereas continuous annual crop had no effect on SOC content (Chen et al., 2022).

To reduce potential error and reduce the MDD, a large number of samples is often necessary (Smith et al., 2020; Stanley et al., 2023). Determining this number should require power analysis based on the spatial heterogeneity on the field level, in order to get accurate estimations of the total C stock (Stanley et al., 2023), rather than an arbitrary number. The latter is currently

the case for most existing carbon farming protocols, both FAO and Svensk Kolinlagring included.

2.6 Sampling design

2.6.1 Stratification

One option for reducing the number of samples required is to stratify the field into more homogenous sub-areas, or strata, based of factors influences carbon stocks (Aynekulu, 2011; Potash et al., 2022; The Earth Partners, 2012b). A stratified sampling design can lead to a more efficient estimation of mean SOC with a reduced number of samples (Potash et al., 2022). Several scientific resources as well as existing carbon farming protocols recommend this, including FAO (UN Food and Agriculture Organisation), VCS (Verified Carbon Standard), Gold Standard and Svensk Kolinlagring.

As Donovan (2013) effectively puts it, a stratified sampling design is most effective when the following three conditions are met:

- Variability within strata is minimized
- Variability between strata is maximized
- The stratification covariates (e.g., slope, vegetation, management system) are strongly correlated with soil carbon change.

Several different stratified sampling designs exist. On this matter, FAO provides relevant and applicable recommendations, suggesting either *directed stratified random* or *stratified simple random* sampling, depending on if there is sufficient auxiliary data on SOC variability across the area (FAO, 2020).

The directed stratified random approach can be used when there is previous data explaining the variability of SOC within the study plot. For this method, the area of interest is divided into a minimum of three subplots – strata – based on similar characteristics that are correlated with soil carbon change. Within each stratum, a minimum of three sampling locations are randomly selected, and composite samples are collected at or near this location. FAO (2020) recommends using a minimum of three sampling locations.

For the stratified simple random approach, the area of interest is systematically divided into subplots or strata of equal size. Within each stratum, sampling locations are randomly selected. At each sampling location, a number of composite samples are collected. The FAO (2020) protocol recommends using a minimum of five strata. Regardless of sampling design, FAO recommends using 5-15 subsamples.

Common stratification covariates are long term average NDVI (Normalized Difference Vegetation Index), altitude maps, yield maps, modelled SOC content, soil type or soil minerals, to mention a few (Bettigole et al., 2023; FAO, 2020). However, most frameworks and protocols including FAO lack detailed guidance on how to perform the stratification and based on which covariates (Potash et al., 2022; Stanley et al., 2023). There is a free tool available for stratification called Stratifi, launched by quickcarbon.org. However, there is limited data available for non-US countries. While FAO gives guidance on how to address the number of samples needed post stratification, they do not give guidance on how to perform the stratification. One exception is VCS, who present a methodology for creating strata within the area of interest (The Earth Partners, 2012a) that is comparatively user friendly.

Stratification procedures can thus become somewhat arbitrary due to subjectivity in the choice of covariates (Arrouays et al., 2018; Potash et al., 2022). While covariates like NDVI, yield maps or soil type can have a correlation with SOC content, a comparison with the preliminary study of the area could be useful for exploring this correlation. Potash et al. (2022) found that a stratification based on Sentinel-2 SOC index offered substantial improvement over simple random, but that the magnitude of the improvement was uncertain. In a study by Bettigole et al. (2023), simple random, grid and cLHS (conditioned latin hypercube sampling) were more efficient than stratified sampling at farm scale. Grid sampling was very efficient on small study sites, but less so at larger sites (Bettigole et al., 2023).

2.6.2 Composite samples

Composite samples are a common recurrence in the soil monitoring field. They consist of two or more different second order samples, or subsamples, that are pooled into one homogenised first order sample. If the composite sample is sufficiently homogenised, the analysis should provide a measure on SOC content that is equal to the mean value of the subsamples, if they had been analysed individually (FAO, 2019). Compositing samples is a way of reducing both the cost and time of SOC analysis. Compared to individual analysis of each subsample, compositing reduces power by decreasing the effective sample size (Stanley et al., 2023). The compositing of samples can be done to varying extent, from pooling 3-5 subsamples from a small sampling area to full compositing, where all samples are pooled together.

If finances are unlimited, the best option would be to analyse each subsample individually, which minimises error, this is rarely the case. Because of this, compositing samples is recommended in most cases, except if the spatial scale is really small – such as a few meters (FAO, 2019). While FAO (2020) recommends 5-15 subsamples, it has been found that the benefit of increasing the subsample number beyond five is negligible (Arrouays et al., 2018).

2.6.3 Sampling depth

Another important factor for accurate soil C monitoring is the sampling depth. In common agriculture practice and in the case of many scientific studies, only the top 30 cm of the soil is sampled (Zhang & Hartemink, 2017), since this in accordance with IPCC (2006) Kyoto protocol recommendations for soil C inventory (Nayak et al., 2019). However, the sampling depth required by Jordbruksverket (Swedish Board of Agriculture) is just 20 cm (Gustafsson, 2010). This fails to reflect changes in deeper soil layers, which is problematic for several reasons.

Deep soil layers are important in carbon sequestration. While most of the measurable changes in SOC happens in the top 30 cm, these gains or losses are often temporary while changes in SOC content in deep layers happens very slowly (Chenu et al., 2019). In deeper soil levels, SOC is protected from decay and can stay in the soil for decades or millennia (Weil & Brady, 2017). Other factors are that gains in SOC in the top soil layers may be offset by losses in deeper levels (Stanley et al., 2023), and that the SOC variation differs between layers (Nayak et al., 2019).

Further, SOC effects from management interventions can be detected down to around 1 m or more (FAO, 2019), and to be able to accurately catch changes in the total C stock, the entire root zone should be included (Nayak et al., 2019). This is especially important for perennial systems like Kernza™ fields, where the root zone tends to be deeper than that of annual varieties.

In the case of perennial crops, the fields are never ploughed or tilled, or only when the grain needs replanting, depending on variety – or if the field is discontinued and a new grain is established. However, in annual fields, it is important to include the entire plough depth, due to stratification of SOC (Vandenbygaart & Angers, 2006). This is also true when looking at the effect of no till, which is not measurable unless the entire plough depth is included (Smith et al., 2020).

Thus, deep sampling is required in order to make reliable conclusions about carbon sequestration and climate change mitigation (Arrouays et al., 2018; Stanley et al., 2023). This is especially true for certain management changes or interventions where the true effect isn't seen unless sampling at deeper levels (Smith et al., 2020). However, sampling changes in deeper soil layers (> 30 cm) require specific equipment or machinery and are costly. Further, they often don't appear until after several years (> 10 years), so depending on the purpose and timespan of the study, a more shallow sampling design might be more suitable (FAO, 2019).

FAO's GSOC MRV protocol still only requires sampling to 30 cm depth (FAO, 2020). The MRV protocol of Svensk Kolinlagring involves sampling to 60 cm in default cases, and to 90 cm at chosen sites (Svensk Kolinlagring, 2022a). When sampling deeper than 30 cm it is common practice to divide the soil depth into fixed depth layers, e.g., 0-10, 10-30, 30-50 and 50-100 cm or 0-30, 30-60 and 60-90cm, which are analysed individually (FAO, 2020). Compared to sampling by soil horizons, fixed depth intervals are preferrable for SOC stock assessments (Arrouays et al., 2018; Zhang & Hartemink, 2017).

2.7 Measurements

To be able to accurately estimate the SOC stock of a field, the two most important measurements are SOC content, which essentially is the concentration of carbon in the soil, and the bulk density of the soil (BD). It is also sometimes recommended that soil inorganic carbon (SIC) is analysed, especially in soils rich in mineral C compounds. Even though majority of C in humid region soils is stored as SOC (Lal, 2011), SIC can still constitute a significant amount of the total C stock depending on soil type, and should thus be included in the analysis in cases where deemed necessary (Nayak et al., 2019).

The most common method for bulk density measurements is the intact core method. This is done by collecting a known undisturbed volume of soil, using a cylinder of a known volume (often metal) that is driven horizontally into a vertical soil profile. The soil sample inside the cylinder is dried and weighed (FAO, 2020). BD measurements at depth are notoriously time- and cost-consuming to collect, but nonetheless important. Even if the total C stock is relatively homogenous across a field, variability in BD could prevent reliable detection of changes in the C stock. Thus, failing to factor in the variability of BD, e.g. treating it as a fixed value, can lead to inaccurate assumptions about the total C stock since this greatly underestimates the uncertainty (Stanley et al., 2023).

When estimating C stock by using standard bulk density measurements, the C stock is estimated down to a fixed depth (e.g., 30, 60 or 100 cm). However, SOC sequestration changes the density of the soil, which can create some bias and potential errors (Rovira et al., 2022). The density of organic matter is lower than the density of minerals, and so the BD of the soil is negatively related to SOC content, meaning that the BD decreases with SOC sequestration (Rovira et al., 2022). This is especially relevant for systems where there has been a change in tilling regime, as in the transition from annual to perennial grains, since tilling can alter the soil density and compaction (Haddaway et al., 2017; Wendt & Hauser, 2013). Conventional tilling may increase

compaction below the plough depth – which increases BD – and decrease compaction and BD above the plough depth. On the contrary, no-till may increase compaction above the plough depth (Haddaway et al., 2017; Wendt & Hauser, 2013).

Thus, the source of the error is that C stocks are being compared at fixed depths that contain different soil masses (Wendt & Hauser, 2013), which means that fixed depth BD can lead to both over- and underestimation of the total C stock (Rovira et al., 2022). An alternative measurement to fixed depth BD that compensates for the change in soil compaction is equivalent soil mass (ESM). Here, fixed mass layers are compared instead of fixed depth layers, which compensates for the management driven changes in soil compaction (Rovira et al., 2022). However, for worldwide C stock comparisons across different soil types, climates and vegetation types, the fixed depth BD approach is still preferable, due to difficulties in establishing a standard value of ESM (Rovira et al., 2022).

Lastly, the described approach for estimating field SOC stock also involves quantification of the fine (<2 mm) and coarse (>2 mm) soil. The coarse fractions (>2 mm) have very limited capacity of storing SOC and are removed, in order to be able to estimate the SOC stock accurately. SOC analysis is thus only performed on the fine fractions. While it is not necessary for estimating the current C stock of a field, both N content and grain size fractions further than the <2 / >2 mm division can give information about the carbon sequestration potential of the field (Chenu et al., 2019; Kirkby et al., 2014). SOC and N are directly linked, and SOC sequestration cannot occur in the absence of N (Horwath & Kuzyakov, 2018). Optimum carbon sequestration often requires additional N input, especially the formation of fine fraction soil organic matter (SOM) particles, which are considered a more resilient form than larger more labile SOM particles (Chenu et al., 2019; Kirkby et al., 2014; Nayak et al., 2019). In a study comparing the C and N dynamics in annual and perennial cropping systems, it was found that both crop type and management influence the C and N dynamics and content of soils, especially the topsoil (Means et al., 2022). Therefore, both N content and soil grain fractions of the Alnarp field are interesting from of a carbon sequestration potential view, even if they're not strictly related to the estimation of the total C stock.

2.8 Laboratory analysis

While the variability of lab analysis results is low, there can be significant difference between different lab methods and instruments, making it important to use the same method and instrument for all samples within a study. This can also make it difficult to compare studies and/or fields that have been analysed with different instruments or methods (Stanley et al., 2023). However, when compared to spatial heterogeneity, the variability of lab assays contributes little to overall uncertainty, given that the same method and instrument is used (Stanley et al., 2023).

The most widely used laboratory methods for analysing the C content in soil are wet oxidation and dry combustion. While wet oxidation is easy, cheap, and requires minimal equipment it has drawbacks in form of high variability and requiring site-specific correctional factors (Nayak et al., 2019). Dry combustion can be done in two ways: Loss on ignition (LOI) or automatic elemental analysis (EA). LOI is good and cheap, but can have slightly ambiguous results compared to EA, partly due to the fact that SOC and SIC can't be separated using this method (Nayak et al., 2019). EA is the most correct method for analysing C content in soil and is thus recommended by several sources (FAO, 2020; Nayak et al., 2019; Stanley et al., 2023) It requires very small samples and takes only a short time per sample, but costs of initial purchase and maintenance are high.

By analysing both total carbon (TC) content and total organic carbon (TOC) content, the inorganic carbon content can be calculated as the difference between TC and TOC. When analysing TOC, the inorganic content is removed by treatment with hydrochloric acid (HCl). However, this is most efficient when the SIC content is high (Nayak et al., 2019). Nitrogen (N) content can be analysed at the same time as C, and thus doesn't require any additional analysis. The EA operation is automatic and turns solid phase C or N in the samples into gas (CO_2 or N_2) through a combustion reaction in a furnace. The gasses then pass through a gas chromatography column where they are separated, before they are quantified using a thermal conductivity detector (Brodie et al., 2011). In this way, the exact C and N content in the samples is obtained.

A more novel method for quantifying the C content of soils is spectroscopic analysis (Smith et al., 2020). Although an interesting and promising alternative as it is cost- and time-efficient method not requiring any wet lab assays (Nayak et al., 2019), it is outside the scope of this thesis.

3 Method

3.1 Research site and context

The four-year research project Capturing Carbon in Perennial Cropping Systems (*Perennial*), led by Jonas Ardö at the Department of Physical Geography at Lund University, aims to investigate whether a transition from annual to perennial grain crops could become an effective way to help Sweden reach its climate goals. Part of this project is to cultivate Kernza™ on a 9.6 ha field in Alnarp, situated between Malmö and Lund, where the carbon balance will be measured with flux towers. This is included in the study area for this thesis, which – as mentioned – serves as a baseline study of SOC and other soil parameters. This carbon balance will be compared to that of a conventional cropping field of annual wheat on a control field neighbouring the Kernza™ field. The control plot does not have fixed boundaries since the annual wheat is grown on a larger area, but the EC area reached by the EC tower is equivalent in size to the area of the Kernza™ field and will also be part of the baseline study, since any gains in SOC under Kernza™ will be confirmed against the control. The combined area of the two fields studied in this thesis – Kernza™ and control – is 18.9 ha and is also included in this thesis, although mainly for comparative purposes. Apart from the CO_2 flux, standard meteorological variables as well as important soil parameters will be monitored during later stages of research.

Table 1. Areas of the research plots; Used for field sampling, calculations and analysis.

	Kernza™	**Control**	**Total**
Area (m^2)	96401	92168	188570
Percentage of total area	51.1 %	48.9 %	100 %

3.2 Data

The data used for this MSc thesis include:

- Soil data from the Alnarp research site and its surroundings from a field sampling campaign 2022, including soil organic matter (SOM), micro- and macronutrients and other parameters. Source: Swedish Agricultural University / The Alnarps Egendom farm
- Ortophotos from 1998, 2004, and 2018 (raster). Resolution: 0.25x0.25 m. Source: Lantmäteriet

- Elevation map (GSD-Elevation Grid 2+). Resolution: 2x2 m. Source: Lantmäteriet
- Soil type map (Jordartskartan). Resolution: 25x25m. Source: Lantmäteriet.
- Kernza™ area extent and EC tower positions (vector shapefile). Source: Lund University
- Stratification of the Alnarp research site based on the Svensk Kolinlagring protocol (raster). Resolution: 10x10 m. Source: Svensk Kolinlagring.
- Seasonal Productivity (SPROD) data (raster) from 2017—2022. Resolution: 10x10 m. Source: Copernicus
- Moisture map (SLU Markfuktighetskartan) of the research site. Resolution: 2x2 m. Source: Lantmäteriet
- Register map 1935—1978 (Ekonomiska kartan) 1:10 000. Source: Lantmäteriet.

3.3 Sampling protocol and field sampling

3.3.1 Creating a sampling protocol

The creation of a suitable sampling protocol for the *Perennial* research project was an essential part of this MSc study, as well as performing the actual field sampling campaign. The creation of the sampling protocol, the field sampling campaign as well as the laboratory analysis described in 3.4 were all done within the scope and time frame of this MSc and was primarily conducted by the thesis author. The sampling protocol – described in section 3.3.1 to 3.3.4 – was a result of thorough literature research (partly summarised in the background of this thesis) and consideration of the circumstances regarding the research site, economic funds, available equipment and time.

The literature research encompassed the extensive SOC monitoring protocol provided by FAO and primarily aimed to be used for carbon farming schemes, as well as a great number of research articles on SOC monitoring, carbon farming and perennial crops. VCS (Verified Carbon Standard), the world's most widely used GHG credit program, also provided advice and instructions on SOC sampling design and monitoring. All relevant information gathered from this material was categorised and saved in a "literature database" for future review and trace back. In order to determine the sampling protocol for the Kernza™ field in Alnarp that would suit the larger Perennial research project as well as this MSc, this methodology is a compromise of choosing the best option using the resources available. Or as Arrouays et al. (2018) puts it (p. 635):

"The choice of a method for monitoring SOC changes should always be a trade-off between sampling effort, minimizing soil disturbance, and statistical power."

One main constraint was the timing and length of the soil sampling period, which was limited to when the fields needed to be harvested, sown or fertilized, as well as when relevant staff and equipment was available. Other limiting factors were the lack of concrete scientific guidance in the literature on how to perform a stratification prior to the study as well as the time-consuming nature of laboratory work.

3.3.2 Sampling design

Several sources suggested doing a pre-sampling to get an idea of the SOC variability of the area (FAO, 2020; Smith et al., 2020; Stanley et al., 2023). Within the time frame and scope of this study it was not possible to perform a proper pre-sampling, but an existing soil survey from 2022 included data on SOM content across a larger area around the research site. From this survey, 26 points with SOM data were selected from the agriculture field where the research site would be. These 26 points were treated as pre-sampling data. The area containing these 26

points was larger than the test and control plots, but at this stage the exact location of the test and control plots were not fully known. This larger area however appeared to be representative of the test and control plots in terms of elevation, soil type and management history. The SOM (g SOM g soil^{-1}, or %) data was converted into SOC (g SOC g soil^{-1}, or %) based on the conventional assumption that SOC on average constitutes 58% of SOM (Pribyl, 2010). Following this, the standard deviation (STD) was calculated and used as a measure of heterogeneity. The 2022 soil survey only sampled the top 30 cm, but since topsoil heterogeneity generally should guide decisions around sample size (Stanley et al., 2022), it was not regarded as a cause of concern. To avoid confusion regarding the use of percentages (%), the unit g SOC g soil^{-1} is in this thesis used when referring to the measured or calculated absolute values or changes of SOC in the soil, and the percentage (%) is used when referring to relative values or relative changes.

The minimum number of samples (n) required to meet a satisfactory MDD, based on assumptions made from reading previous research, was calculated using the following equation (FAO, 2020):

$$n \geq \left(STD * \frac{t_\alpha + t_\beta}{MDD}\right)^2$$

Equation 1. Calculation of the minimum required number of samples (n), where t_α is the two-sided critical value of the t-distribution at a given significance level (α) (frequently taken as 5 to 10%; 0.05-0.1) and t_β is the one-sided quartile of the t-distribution corresponding to a probability of type II error β (being 1 − β the statistical power; frequently 80 to 90% (FAO, 2020).

The standard deviation of SOC in the 2022 soil survey was 0.43 g SOC g soil^{-1}, which was used as STD in equation 1. Several different options and combinations with varying MDD (3-10 g SOC g soil^{-1}) and statistical power t_β (80-90 %) were calculated in order explore how *n* changed depending on these values. Ideally, the MDD expressed in relative terms would be 3 % or lower, as demonstrated by Chen et al. (2022), and the statistical power (t_β) 90 % with a p-value (t_α) of 5 %. A 3 % difference corresponds to an absolute difference of 0.056 g SOC g soil^{-1}, based on the mean SOC content of the previous soil survey (1.869 g SOC g soil^{-1}). This results in a minimum of 53 samples.

The next decision regarding the study design was how the samples should be distributed across the study area, and whether stratification would be a suitable option to reduce the number of samples needed. The auxiliary data potentially suitable to use as covariates for stratification available at this stage were soil type and elevation from Lantmäteriet. The resolution of the soil type raster was 25 m and the number of soil types within the test and control fields were between three and five. Soil type could theoretically have served as strata, but since it was not clear whether this had an actual correlation with SOC at the research site, this was not done. As for elevation, the maximum difference in elevation was less than 5 m across the entire field, which did not meet the conditions formulated by Donovan (2013) listed in the background. Thus, due to the lack of suitable auxiliary data at this stage, a clear methodology on how to perform the stratification as well as time limitations the decision was made to not stratify the field. Instead, a systematic grid sampling approach was used, as it has been shown to be efficient at field scale (Bettigole et al., 2023). It would also serve as a good basis for further analysis of simulations of other sampling options.

In the end, 72 sampling locations were systematically spread across the total area in a grid pattern (test and control plots combined), a seen in fig. 1 below. The number of 72 was derived from the minimum of 52 samples, with added margin to allow for sample loss or other unforeseen circumstances. The size of the grid cells was roughly 58x50 m, as this allowed 72 samples to be placed systematically across the study area, roughly corresponding to the standard 50x50 m cells often used in soil and ecological studies. In a few cases where the sampling point interfered with the location of the EC tower cable, the sampling point was moved a few meters. An additional location was added later near one of the EC towers due to practical circumstances, and the total amount of sampling points was thus 73.

This corresponds to an MDD of 0.048 g SOC g soil^{-1} with the given standard deviation of 0.43 g SOC g soil^{-1}. If the mean SOC content of the previous survey is maintained both spatially and temporally, the relative MDD of SOC content would be 2.57 %.

Figure 1. Map of all 73 sampling locations, both SOC and BD, at the Alnarp research site. EC towers and deep samples are also included.

3.3.3 SOC content

Following the suggestions of Arrouays et al. (2018) and FAO (2020), it was concluded that composite samples consisting of 5 subsamples would be suitable for this study to reduce the cost and time of both analysis and field sampling, while maintaining accuracy. As for depth, three equally distributed depth intervals were deemed as a good trade-off, as having more than three intervals would significantly increase the number of samples to analyse and having less would reduce the accuracy of the result. The expected deep roots of Kernza™ as well as other factors called for sampling down to 1 m. Thus, all locations were sampled at three depth intervals: 0-30 cm (A), 30-60 cm (B) and 60-100cm (C) at five subsample locations.

The samples were taken with a soil drill (Wintex MCL3) attached to a small tractor, collecting five cores or subsamples. Each core/subsample was then split into the three different depth intervals and put into corresponding paper bags, to form three composite samples (i.e., 1A, 1B, 1C) per main sampling location. The subsamples were around 100 g each (per depth), and the composite sample weight was thus around 500 g. The subsamples were placed roughly 1 m apart on a straight line (horizontal in the map above), with the middle subsample at the original sample location, as this reduced both time and effort of the field sampling.

3.3.4 Bulk density

Bulk density samples were only collected at 15 of the 73 sampling points (fig. 1). Ideally, all sampling points would have been sampled for bulk density, but this would have been both incredibly time- and cost-consuming as well as cause significant soil disturbance. The choice of sampling points for bulk density were initially chosen in a systematic manner, resembling a larger grid, but as deep pits were added later, the final placement sampling locations were not entirely systematically placed, as seen in fig. 1. An excavator was used to dig 1 m deep pits. Bulk density was then sampled at roughly 15, 45 and 80 cm depth – the midpoints of the three depth intervals –, using the intact core method, as recommended by several sources including FAO (2020).

The material used was a 50x50 mm bulk density cylinders with a handle, large rubber hammer, knife and bucket. The cylinder was hammered horizontally into the vertical walls of the soil pit and then carefully removed to prevent sample loss. This was repeated until an undisturbed sample could be collected. The sample was then emptied into the bucket and transferred to a labelled paper bag (e.g., 1A, 1B, 1C). At all locations where bulk density was sampled, additional samples for fractioning and texture analysis were collected for the possibility of later research by other actors.

While it was considered to also calculate the equivalent soil mass, this was disregarded due to time limitations as well as limitations in available data. Calculating the equivalent soil mass requires knowing the total weight (both <2 mm and >2 mm fractions) of all samples. Unfortunately, these values were not accurate enough due to variable sample loss while collection the soil cores. However, since the full soil depth of 1 m is sampled in this survey, this will likely be able to capture the majority of SOC change.

3.3.5 Deep samples

Seven out of the 73 locations were also sampled at 100-130 cm (D), 130-160 cm (E) and 160-200 cm (F), as seen in fig. 1 above. An excavator dug deep pits (200 cm) in conjunction with the BD sampling described in the paragraph below. The purpose of these deep samples was primarily to get an initial perception of the deep soil characteristics in the field.

To collect samples of SOC content, soil was collected from each depth interval (D, E, F) by scraping a vertical layer from the walls, using a small spade and a bucket. These samples thus only contained one subsample. Even though the heterogeneity of SOC distribution is significantly less at 1-2 m depth, seven samples will probably not be enough to determine any statistically reliable change in SOC over the course of five years.

Bulk density was collected in an identical manner as described in 3.3.4, at the vertical midpoint of the D, E and F depths in the seven deep pits.

3.4 Laboratory methods

All soil samples regardless of further analysis were dried at 40° for a week, within two days of sampling. All composite samples intended for SOC, SIC and N analysis were then homogenised into a fine powder, grinding them by hand using a mortar and pestle, until all soil aggregates could pass through a 2 mm sieve. Any mineral material larger than 2 mm were put aside after sieving, and both partitions (<2 mm and >2 mm) were labelled and weighed. The main reason for this method was that no other equipment was available at the time. All bulk density samples were weighed in their bags and the mean bag weight was subtracted.

Since there was previous knowledge from other research in the nearby research fields at Lönnstorp that the soil at the Alnarp site was potentially rich in inorganic C, an additional step for removing inorganics and measuring only the organic content was included in the laboratory analysis protocol. Otherwise, the SOC stock of the soil would most likely be overestimated. Laboratory analysis of N content was also included.

Total organic carbon (TOC) and total carbon (TC) content were analysed using elemental analysis (EA), as this is now considered the most accurate method. From each homogenised composite sample, two 10-20 mg subsamples were extracted and put in a closed tin (Tn) capsule for analysis of total carbon (TC), and an open silver (Ag) capsule for analysis of total organic carbon (TOC). The capsules where then put in labelled plastic well plates, holding 8x12 capsules each. The individual weight of all samples was noted as well as their position in the plate. The Tn capsules were carefully folded with pincers.

To remove any inorganic components, the samples intended for TOC analysis (open Ag capsules) were acidified with hydrochloric acid (HCl) using the capsule method described by Brodie et al. (2011). The capsules were transferred from the plastic plate to a glass plate on a 50°C hotplate in a fume cupboard and 10 µl of deionized water was added using an automatic pipette to wet the samples prior to acidification. After this, 1M HCl was added in the following proportions: 10 µl, 50µl, 50 µl and 100 µl, without letting the sample dry out between the additions. Thus, a total of 210 µl of HCl was added. The reaction was monitored by visual inspection. While Brodie et al. (2011) used slightly different proportions, these ones used in this study proved more suitable to the size and character of the samples as well as the size of the capsules. Then, the acidified samples were left on the hotplate overnight to dry out.

Once dry, the acidified samples in the Ag capsules were carefully folded as to not leak any sample material, and put into a second capsule, this time Tn, which was also carefully folded. In case of visible sample loss due to overflow during the acid reaction, the whole process of weighing and acidification was repeated for the sample. This also applied to any other mistakes while handling or analysing the samples.

All capsules, both TOC and TC, where run through a Costech ECS 4010 elemental analyser (EA) with a 1020°C furnace, connected to an online software system. The instrument carousel held 50 samples, out of which every tenth sample (a total of four per round) were calibration samples containing known amounts of acetanilide. This way, it was possible to keep track of when the instrument needed calibration or removal of ashes from the GC column, as well as making sure the measurements were accurate. The instrument needed calibration roughly every 100-150 sample and for each calibration run, four acetanilide standards (a series of 0.2 mg, 0.5 mg, 1 mg and 2 mg) and two samples with known C content were run on their own. N content was obtained from both the TOC and TC samples, since this is always included in EA analysis.

To account for sample heterogeneity – since only 10-20 mg out of the full 300-800 g samples were analysed – 20 replicate samples were run on one randomly selected sample. The variability of these replicate samples was used as a measurement of the combined uncertainty of incomplete homogenisation, inherent variability within the sample and variability of the acidification process.

3.5 Data analysis

3.5.1 Bulk density

The bulk density was calculated using the volume of the cylinder and the weight of the sample. This value was then corrected based on the fine earth fraction (ff) (<2 mm) vs the coarse mineral (>2 mm) fraction, since the C stock of a field is calculated based on the SOC content of the fine earth fraction. It was calculated as follows:

$$ff = \frac{Net\ weight\ of\ < 2\ mm\ partition}{(Net\ weight\ of\ < 2\ mm\ partition) + (Net\ weight\ of\ > 2\ mm\ partition)}$$

Equation 2. Calculation of fine earth fraction.

The next step was to interpolate BD over the entire field based on the field data of the BD of fine earth (<2 mm). Inverse Distance Weighted (IDW) was chosen as interpolation technique since it is widely used for spatial interpolation of soil data, including BD (Chun-Chih et al., 2016; Sajid et al., 2013).

The Inverse Distance Weighted method uses a weighed combination of sample values, which gives more weight to nearby samples and less to samples further away. It's based on the assumptions that nearby samples is more related to the interpolated location than distant samples (Sajid et al., 2013). An alternative method which is also commonly used is Ordinary Kriging (OK). Both Chun-Chih et al. (2016) and Sajid et al. (2013) have compared these two methods in the context and found that while neither of them manage to reflect the true variation of BD, both options are suitable for spatial analysis of BD and that the difference between them is marginal. One known disadvantage of the IDW method is that the quality of the interpolation can be decreased if the samples are unevenly distributed and that it is sensitive to outliers (Chun-Chih et al., 2016).

The combined area of the Kernza™ and control plots was used as extent for the interpolation with 6x11 raster cells, since this reflected the distribution of the 73 sampling points fairly well. This corresponded to a spatial resolution of 62*58 m. The distance coefficient (*P*) was set to 2.0, as this was the default setting in QGIS 3.28.2-Firenze. No other settings were adjustable in this version of QGIS. Then, cell values at each of the 73 sampling points were extracted. In this way, each sampling point was assigned an individual unique BD value. The performance of the interpolation was evaluated by calculating the RMSE of the interpolated data and the sampled data.

To estimate the uncertainty of the field sampled BD measurements an error of up to 3 g per field BD sample was assumed, due to the difficulty of collection a perfect undisturbed sample, corresponding to an uncertainty of ± 0.122 g cm^{-3}. Other factors such as scale precision were disregarded, as it was assumed that this would be negligible in comparison to the field sampling error. The RMSE of the IDW interpolation was used as a measurement of uncertainty for this step. The total uncertainty of BD was calculated using both the sampling error and the

interpolation error. The fractional uncertainty of BD was calculated by dividing the total absolute uncertainty with the mean value of BD for each sampling depth.

3.5.2 SOC content

The first step was to check whether the SOC data followed a standard distribution, by producing histograms and performing a Jarque-Bera test (Jarque, 2011) on the values obtained by analysing the TOC samples. This was mainly an informative step with the purpose of guiding further analysis, since using parametric tests for datasets that do not follow a normal distribution can be an issue when comparing two datasets to each other, e.g., comparing the C stock of a field at two different points in time to evaluate the difference (Stanley et al., 2023). If the dataset fails to meet the necessary requirements of common parametrical tests such as the Student t-test or ANOVA, nonparametric alternatives should be used instead (Stanley et al., 2023).

The mean SOC content as well as its median, standard deviation and range for each of the three depth layers were calculated and compared. The absolute MDD was calculated using Equation 1 presented in an earlier section of this methodology. Relative MDD was calculated by dividing the absolute MDD with the mean SOC content.

To estimate the uncertainty of the SOC measurements, the standard variation of the 20 replicas was used as a combined measurement of uncertainty due to incomplete homogenisation, inherent variability within the sample or variability of the acidification process. Any uncertainties derived from the EA analysis itself were regarded as negligible in comparison. The fractional uncertainty was calculated by dividing the absolute uncertainty with the mean value of SOC for each depth.

Lastly, SOC maps of each depth across the total area were created by interpolating the SOC content across the field, again using IDW as interpolation technique. The distance coefficient was 2.0 (*P*), which was default settings in QGIS 3.28.2-Firenze. The combined area of the two research plots was used as interpolation extent of 5719*3413 pixels, which was suggested by QGIS. This corresponds to a resolution of 0.1 m. These maps were only created for visual representation and the pixel values were not used for any calculations.

3.5.3 SOC stock

The SOC stocks of the different plots and depths of the research site, expressed both as Mg OC ha^{-1} (metric tonnes per hectare) and kg OC m^{-3}, were calculated with an adapted version of an equation given by Tadiello et al. (2022). The reason for the adaptation was that most equations given in scientific resources did not take more than one value of OC and BD into consideration, while this study includes many individual data points. However, the same results are yielded when using the mean SOC content of the plot if all decimals are preserved. The equation given by FAO (2020) was not used due to being unsuitable for the sampling design used in this MSc, as it relies on equivalent soil mass data.

The SOC stock was calculated for the total area as well as for both the Kernza™ and control plots separately. For each depth layer (A, B and C), the total SOC stock was calculated with equation 3:

$$SOC\ stock\ \left[\frac{MgSOC}{ha}\right] = 0.1 * LT * A * ff * (OC_x{*}BD_x + \ldots + OC_u{*}BD_u)$$

Equation 3. SOC stock calculation.

Where LT [m] is layer thickness, A [m^2] is the total area of the research plot divided by the number of sampling points (e.g., 73 for the total area), OC_x is the soil organic carbon fraction at sampling point x and BD_x [kg m^{-3}] is the interpolated BD at sampling point x. Sampling points x and u refer to the ID of the first and last point within the area. The factor of 0.1 converts it to Mg SOC ha^{-1} from kg m^{-3}. The factor ff refers to fine earth fraction, which is the weight fraction of the <2 mm soil particles compared to the full sample, calculated in an earlier step.

To obtain the total SOC stock of the depth layers for the Kernza™ and control plots individually, only the sampling points located within the plots were used, as well as the respective areas of the plots. Since the layer thickness was not constant – 0.3 m for A and B, but 0.4 m for C – the unit of kg m^{-3} was used for comparing the SOC stocks of the layers to each other. When referencing the SOC stock using the unit Mg SOC ha^{-1}, it is required to specify the depth of the measured soil layer. Just as for SOC content, the absolute MDD of the SOC stock was calculated with Equation 1. By dividing this with the mean SOC stock, the relative MDD was obtained.

The fractional uncertainty of the SOC stock was calculated as the sum of the fractional uncertainties of BD and SOC content.

3.5.4 Workflow chart

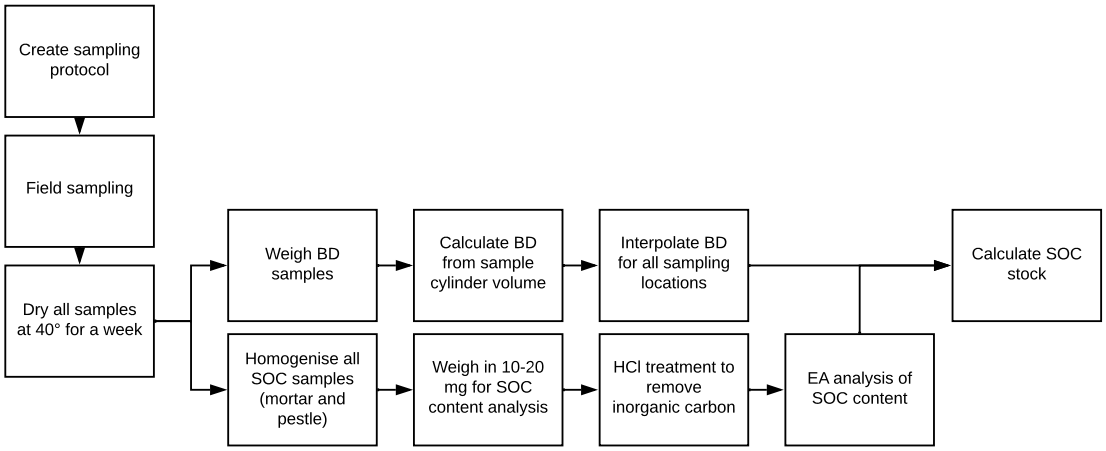

Figure 2. Workflow chart of the soil organic carbon (SOC) stock analysis.

Fig. 2 shows an overview of the workflow of lab and field work, from creating the sampling protocol to calculating the SOC stock (Equation 3).

3.6 Svensk Kolinlagring methodology: Simulation and analysis

The Svensk Kolinlagring MRV protocol was applied on the Alnarp research site. In this case, the protocol proposed a total of 12 samples, six on each field, since the Kernza™ and control plots would have been regarded as separate fields (Svensk Kolinlagring, 2022a). This sampling design entailed using stratified simple random instead of grid sampling, with three strata per plot and two samples per stratum, i.e., a total number of 12 samples. SK uses biomass index and elevation data as stratification covariates. They randomly place the required number of sampling points – depending on field size – within each stratum, with a 15 m buffer at the field edges. The stratification data was obtained from SK. Two of the sample points collected for the

Perennial baseline measurement were randomly selected within each stratum, and these were used for all further calculations and analysis.

The sampling scheme for the Kernza™ and control plots can thus be seen below (fig. 3); three strata with two samples per stratum for both plots. The SK protocol doesn't include any pre-sampling to determine the number of required samples but rely on fixed numbers of required samples determined by the total area (minimum 3 samples per hectare) (Svensk Kolinlagring, 2022a).

Based on the SK sampling protocol described above, the following analysis was conducted as part of this thesis:

1. Calculating SOC content and stock according to the SK protocol and compare this to the SOC stock calculated with the *Perennial* methodology described above
2. Calculating SOC content and stock mean, median, STD and MDD of the SK protocol and compare to *Perennial* data
3. Evaluating the performance and applicability of the stratification

The analysis and comparison of other methodological procedures, like BD sampling techniques and/or interpolation as well as sampling depth have been regarded as outside the scope of this thesis.

Figure 3. Svensk Kolinlagring sampling map for both research plots in Alnarp, including strata and selected sampling points (6 per plot). Stratification covariates were biomass index and elevation data. Data source: Svensk Kolinlagring.

The SOC stocks of the Kernza™ and control plots based on the SK protocol were calculated separately, identical to the Perennial protocol, but due to the 30 m buffer zone between the two plots, the SOC stock of the total area was not calculated this time. The SOC stock was calculated first per strata, using the strata areas (m^2) and the respective mean SOC stock (kg SOC m^{-3}) of

the selected sampling points within them. To get the total SOC stock of the Kernza™ and control plots, the strata sub stocks of each plot were summarised. The SOC stock was calculated both in kg SOC m^{-3} and Mg SOC ha^{-1}. Due to the buffer zones, the sampled areas are smaller than the Perennial areas: 78 300 m^2 (Kernza™) and 74 800 m^2 (control).

STD and MDD of each plot were calculated in the same manner as earlier. Using this information, it was also investigated whether it would be possible to determine if SOC sequestration of 300 kg SOC yr^{-1} could be detected at the end of the 5-year Perennial research program based on the SK protocol, since this is the SOC sequestration standard value that Svensk Kolinlagring uses.

To evaluate the performance and applicability of the stratification, a Kruskal-Wallis H test was done to check if there were statistically significant differences in SOC % between the different strata. Without significant differences between strata, choosing stratified random over simple random or grid sampling to reduce the required number of samples would be unmotivated as it requires extra labour. The Kruskal-Wallis H test is the non-parametric equivalent of the more frequently used one-way ANOVA (MacFarland & Yates, 2016). The main motivation for choosing the Kruskal-Wallis H test was that it doesn't assume that the data follow a normal distribution, as well as being more suitable than ANOVA when there are large differences in group sizes, which was the case. However, it has slightly lower statistical power (MacFarland & Yates, 2016; Stanley et al., 2023).

First, the following hypotheses were formulated:

Null hypothesis: The SOC content is equal in all strata, i.e., there is no significant difference in SOC content between the strata.
Hypothesis: There is a significant difference in SOC content between at least two strata.

Next, the Kruskal-Wallis H values were calculated for each depth. Since 0.05 was chosen as p-value, the H value would have to be smaller than the Chi Square value of 5.991 in order to prove the null hypothesis, and greater than 5.991 in order to prove the hypothesis. To further check whether the stratification was successful, the SOC variability within the strata was evaluated by calculating the STD and comparing it to the STD of all samples.

3.7 Stratification with seasonal productivity data

This section describes the procedure of investigating whether Sentinel-2 Seasonal Productivity Data (SPROD, source) can be used as a stratification variable. SPROD data is derived from time-series of Plant Phenology Index (PPI) data, as the sum of all daily PPI values from the start to the end of the growing season. PPI is a vegetation index developed for monitoring growth, which is better suited for Sweden than NDVI, partly because it takes snow cover into account (Jin & Eklundh, 2014). The SPROD data had a resolution of 10x10 m and was supplied by Lund University.

In QGIS, the mean SPROD value of six consecutive years (2017-2022) of raster data were calculated across the entire area, to see if there was any correlation between SOC content and seasonal productivity when expressed as SPROD values. This was then clipped to match the extents of the Kernza™ and control plots. The Kernza™ and control plots were treated separately in this section, allowing for better comparison with the SK methodology. Both rasters were stratified into three strata: low (1), mid (2) and high (3), as seen in table 2. This was done by reclassifying the mean SPROD raster according to the table below. The maximum and

minimum values were determined by the total range of SPROD values within the raster, and the range width was divided by three to determine the width of each category. The strata can be seen in fig. 4 below.

Table 2. Maximum and minimum values of the resampled categories of the mean SPROD raster.

Strata	Kernza™	Control
Total range	111.6—183.8	117.5—190.5
1 (Low)	111.6—135.6	117.5—141.8
2 (Mid)	135.6—159.7	141.8—166.1
3 (High)	159.7—183.8	166.1—190.5

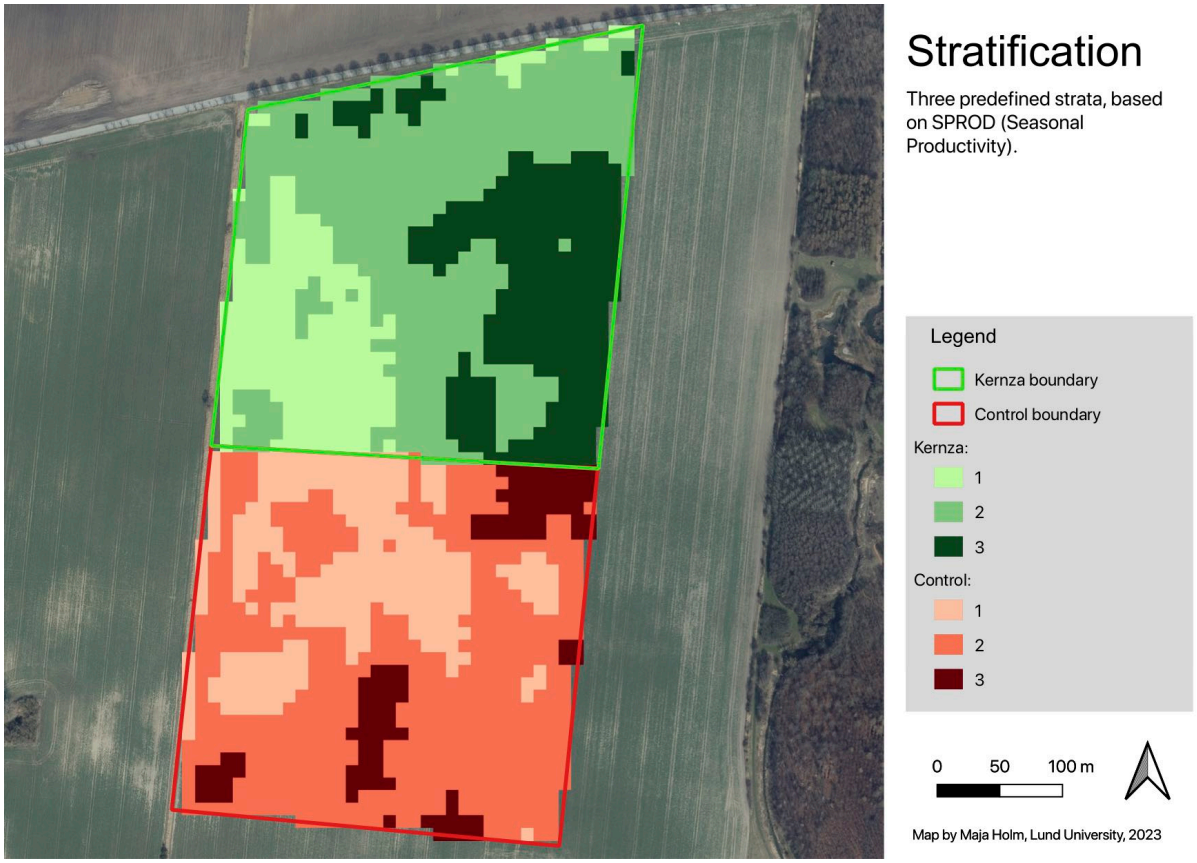

Figure 4. Seasonal productivity (SPROD) stratification map of both Kernza™ and control in Alnarp, three predefined strata per plot based on the mean SPROD values 2017—2022.

Next, all SOC sampling locations were assigned a value of their respective strata (mid, low or high), by sampling the mean SPROD raster values. As for the SK simulation methodology, the Kruskal-Wallis H test was used to assess whether there were significant differences in SOC content between the different strata, i.e., if the stratification was successful. The same null hypothesis, hypothesis and critical cut-off value (5.991) were used.

4 Results

4.1 Bulk density

4.1.1 Field sampling

In total, BD was sampled at 15 locations at three different depths (A, B, C) across the total area. These are presented in fig. 5 above. Out of these, 8 were in the control plot and 7 in the Kernza™ plot. By visual inspection of the scatter plot above, it appeared that while there was a variation of BD, there were no obvious outliers. The mean BD and the standard deviation (STD) are presented in table 3. "Fine earth" refers to the <2 mm fraction, while "full sample" refers to both the <2 mm and >2 mm fractions. Since SOC stock is calculated based on the BD of the fine earth fraction (FAO, 2020), the BD of the full sample is only presented here as additional information. The mean and STD are both similar for the full sample and the <2 mm sample.

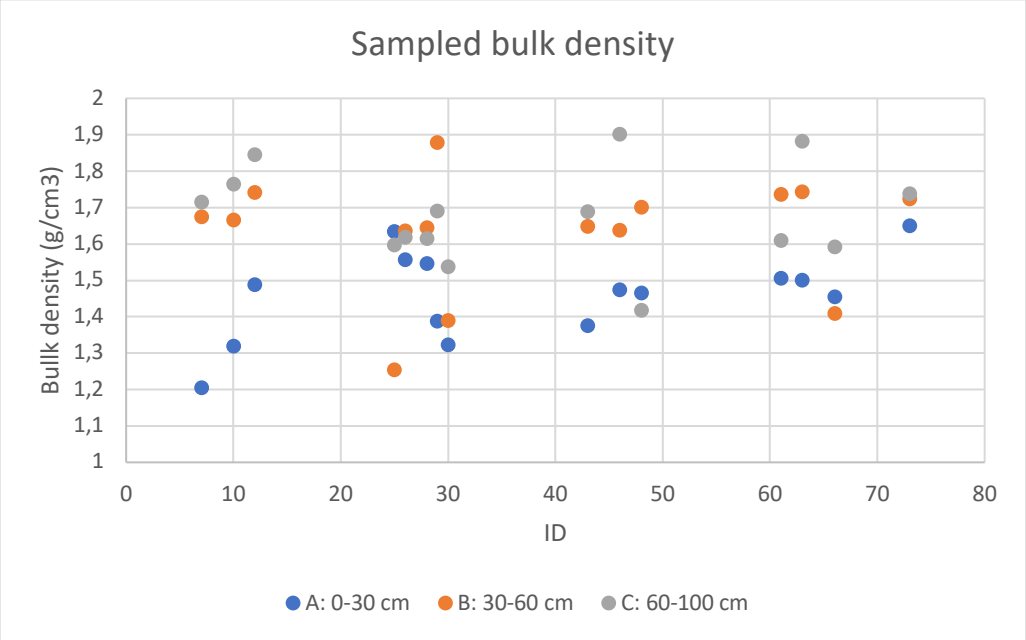

Figure 5. Scatter plot of all 15 field sampled bulk density (BD) data points, collected from the research site in Alnarp 2023. Sampling points with ID 7, 10, 12, 25, 26, 28, 29 and 30 represent the control plot, while points 43, 46, 48, 61, 63, 66 and 73 represent the Kernza™ plot.

Table 3. Bulk density statistics. The range, mean and STD are expressed in the unit *g* cm^{-3}.

Kernza™	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**
Number of samples	15	15	15
Range	1.38—1.65	1.41—1.73	1.42—1.90
Mean (fine earth)	1.49	1.66	1.69
STD (fine earth)	0.08	0.12	0.17
Mean (full sample)	1.50	1.67	1.72
STD (full sample)	0.08	0.12	0.15
Control	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**
Number of samples	15	15	15
Range	1.21—1.63	1.25—1.88	1.53—1.85
Mean (fine earth)	1.43	1.61	1.67
STD (fine earth)	0.15	0.20	0.10
Mean (full sample)	1.47	1.66	1.73
STD (full sample)	0.14	0.18	0.12

Table 3 continued.

Total area	0-30 cm (A)	30-60 cm (B)	60-100 cm (C)
Number of samples	15	15	15
Range	1.21—1.65	1.25—1.88	1.42—1.90
Mean (fine earth)	1.46	1.63	1.68
STD (fine earth)	0.12	0.16	0.13
Mean (full sample)	1.49	1.67	1.72
STD (full sample)	0.11	0.15	0.13

The BD of fine earth got progressively higher in the deeper soil layers, ranging from a mean of 1.46 to 1.68 g cm^{-3} for the total area (table 3). The range also followed the same pattern. This tendency is also vaguely apparent in the scatter plot above. The standard deviation was quite low for all depth layers and areas, from 8 % of the mean for depth A, 10 % for depth B and 8 % for depth C. The mean BD values of the Kernza™ and control plot were similar, but the Kernza™ values were slightly higher.

4.1.2 Fine earth fraction

There was little difference between the mean and STD of the A and B depth layers, while the C layer had a slightly lower percentage of fine earth and a higher STD (table 4). This tendency supports what was observed by visual inspection while field sampling, which was that the texture – and thus the fine earth fraction – of the B and C depth layers varied frequently across the field, from more clay to more sand.

Table 4. Mean and STD of the fine earth fraction (<2 mm) [%] of the total area.

% <2mm	A: 0–30 cm	B: 30–60 cm	C: 60–100 cm
Mean [%]	97.9	98.0	96.9
Median [%]	98.1	98.2	97.8
STD [%]	1.5	1.9	3.4

4.1.3 Uncertainty

The error due to irregularities in field sample collection was estimated to be maximum 3 g per sample, and with the BD cylinder volume of 25.544 cm^3, this equaled to an estimated error of ± 0.122 g cm^{-3}. The fractional uncertainty (based on the mean BD of the full dataset) for each depth is expressed in the table below.

Table 5. Fractional uncertainty of field sampled BD.

Fractional uncertainty	0-30 cm (A)	30-60 cm (B)	60-100 cm (C)
Total area	0.098	0.139	0.089

4.1.4 Deep pits

The sampled bulk density data from the deep pits (100-200 cm) include seven data points for depth layers 100-130 cm (D) and 130-160 cm (E), and four data points for the depth layer 160-200 cm (F). The smaller number of data points for the deepest layer F is due to that in three of the deep pits, the water table was higher than 200 cm, and these points could thus not be sampled. Both fine earth (<2 mm) and full samples BD is presented, although the SOC is stored only in the fine earth fraction. See table 6 below.

Table 6. Mean BD values of all deep pits and their standard deviation as well as relative standard deviation.

Deep pits (total area)	**100–130 cm (D)**	**130–160 cm (E)**	**160–200 cm (F)**
Number of samples	7	7	4
Range	1.45—1.77	1.50—1.87	1.54—1.80
Mean (<2mm) [g cm^{-3}]	1.68	1.69	1.64
STD (<2mm) [g cm^{-3}]	0.11	0.11	0.11
Mean (full sample) [g cm^{-3}]	1.72	1.74	1.70
STD (full sample) [g cm^{-3}]	0.14	0.16	0.16

While the small number of samples prevent any statistically reliable conclusions, it appears that both mean BD and the STD were similar across all three layers D, E and F.

4.2 Interpolation of bulk density

For each depth interval, a BD raster of the entire field was interpolated from the fine earth BD data at the 15 sampling points, using Inverse Distance Weighted (IDW) as interpolation method.

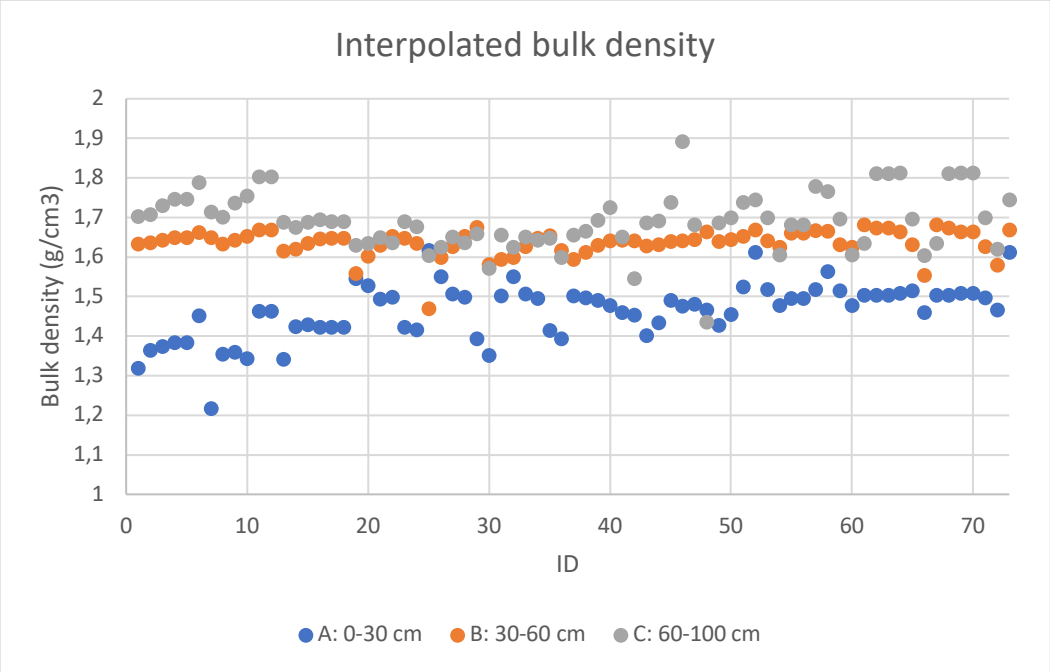

Figure 6. Scatter plot of interpolated bulk density (BD). Points with ID 1-36 represent the control plot and 37-73 represent the Kernza™ plot. Based on field samples collected in Alnarp 2023.

Figure 6 shows the interpolated BD data for all 73 sampling points. This dataset showed a more visible tendency of BD increasing with depth. Since the values are sampled for a raster and the cell locations don't match the sampling locations perfectly, some points are assigned the same value due to being located in the same raster cell.

4.2.1 Statistics

The range, mean and the STD of BD were calculated from extracted values from the interpolated BD raster at all 73 sampling locations, as seen in the table 7.

Table 7. Statistics of the fine earth (<2 mm) fraction.

Kernza™	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**
Range [g cm^{-3}]	1.41—1.61	1.55—1.68	1.44—1.89
Mean (<2 mm) [g cm^{-3}]	1.49	1.65	1.70
STD (<2 mm) [g cm^{-3}]	0.041	0.028	0.087
Control			
Range [g cm^{-3}]	1.22—1.62	1.47—1.67	1.57—1.80
Mean (<2 mm) [g cm^{-3}]	1.43	1.63	1.68
STD (<2 mm) [g cm^{-3}]	0.081	0.037	0.056
Total area			
Range [g cm^{-3}]	1.22—1.62	1.47—1.68	1.44—1.89
Mean (<2 mm) [g cm^{-3}]	1.46	1.64	1.69
STD (<2 mm) [g cm^{-3}]	0.070	0.033	0.074

The values were again slightly higher for the Kernza™ plot, but the difference between these and the control plot values was small (0.02—0.06). Compared to the sampled BD data, the variation (STD) was lower, which is a known disadvantage of IDW interpolation (Sajid et al., 2013). Comparing the STD of the field samples and the interpolated data, the STD of the field samples is roughly the double for the A and C depths, while it's triple for the B depth.

The IDW interpolation was able to predict BD with good accuracy when compared to the field data, especially at 0-30 cm (A) and 60-100 cm (C). The root mean square error (RMSE) of the interpolated BD compared to the sampled data is presented in table 8.

Table 8. The RMSE of the interpolated BD raster.

Total area	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**
RMSE [g cm^{-3}]	0.021	0.105	0.027

Combined with the estimated sampling error of ±0.122 g cm^{-3}, the RMSE presented in the table 8 contributes to the estimated combined fractional uncertainties presented in table 9. This is calculated for the full dataset only, due to the small dataset of field samples.

Table 9. Combined fractional uncertainty of bulk density.

Fractional uncertainty	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**
Total area	0.099	0.140	0.089

Thus, the interpolation of BD combined with the field sampling contributed to 9.9 % uncertainty for the A depth, 14.0 % for the B depth and 8.9 % for C.

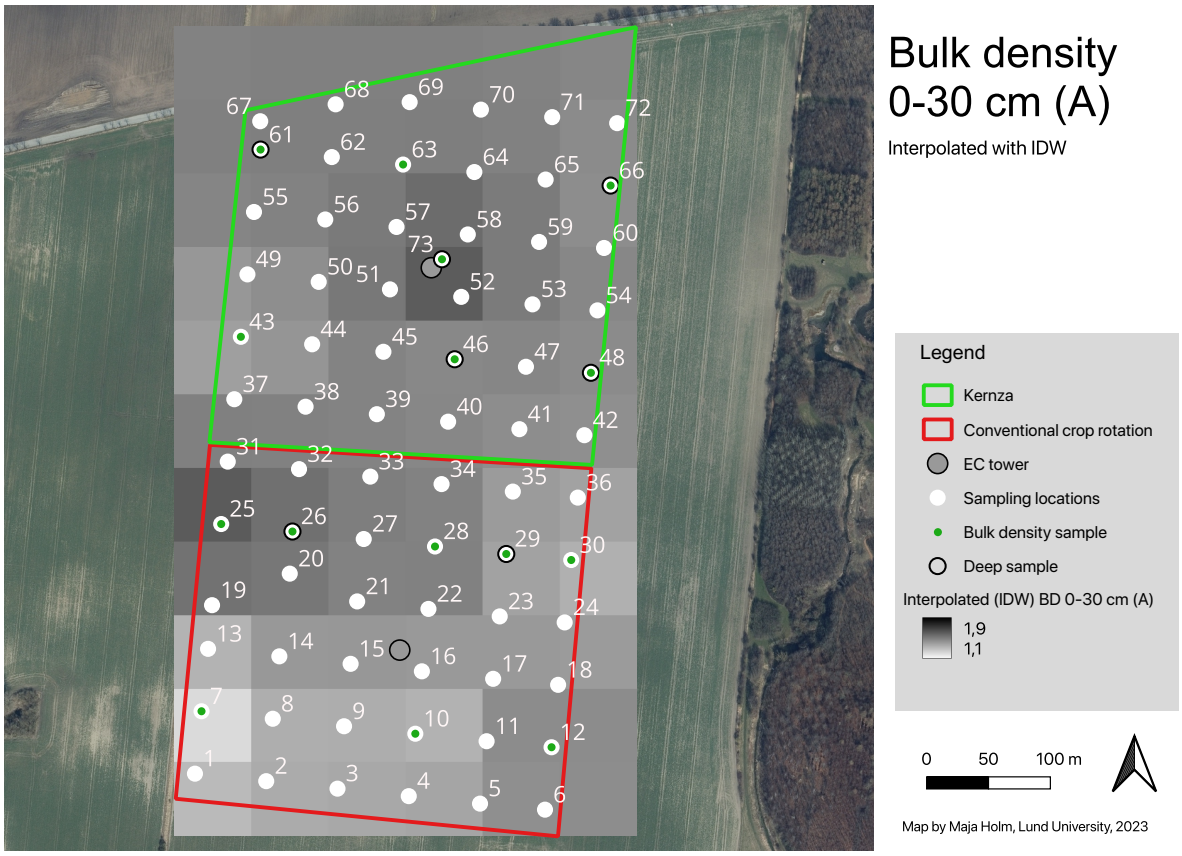

*Figure 7. Interpolated BD raster at 0—30 cm and all 73 sampling locations. Conventional crop rotation refers to the control plot. Based on 15 points of field data collected at the research site in Alnarp 2023. The spatial resolution was 62*58 m.*

*Figure 8. Interpolated BD raster at 30—60 cm and all 73 sampling locations. Conventional crop rotation refers to the control plot. Based on 15 points of field data collected at the research site in Alnarp 2023. The spatial resolution was 62*58 m.*

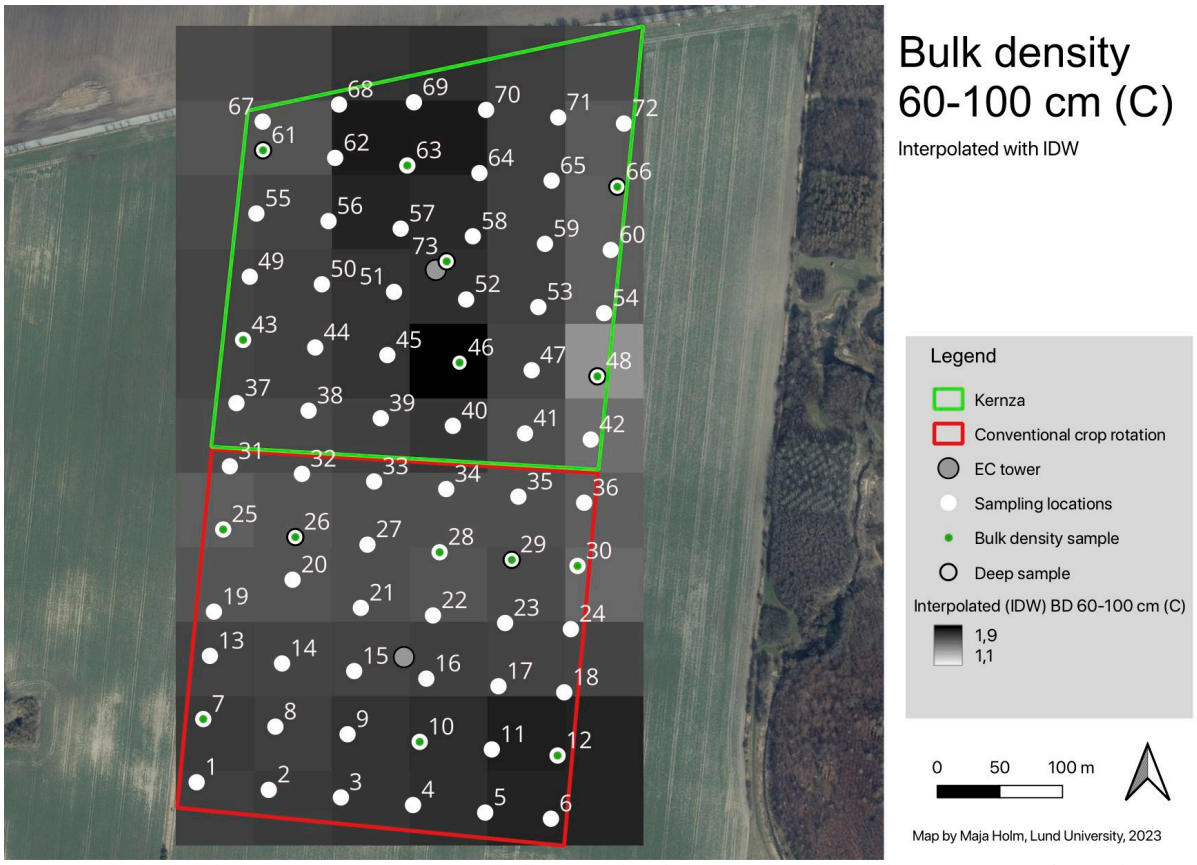

*Figure 9. Interpolated BD raster at 60—100 cm and all 73 sampling locations. Conventional crop rotation refers to the control plot. Based on 15 points of field data collected at the research site in Alnarp 2023. The spatial resolution was 62*58 m.*

The maps (fig. 7-9) above are visual representations of the interpolated BD values at the A, B and C depths. The overall darker shades of the B and C depths compared to the A depth show the positive relationship between BD and depth.

4.3 SOC content

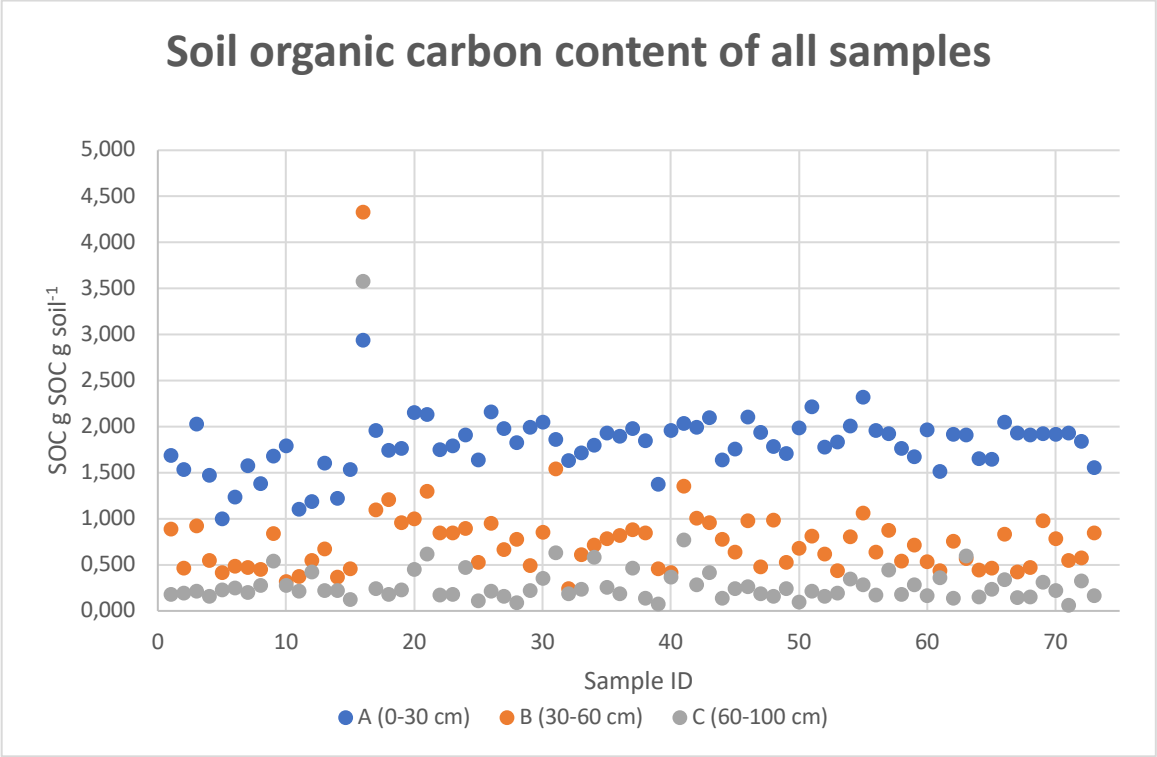

Figure 10. Scatter plot of the soil organic carbon (SOC) content (g SOC g soil^{-1}) of all 73 analysed field samples. Collected from the research site in Alnarp 2023.

The scatter plot above (fig. 10) shows the SOC content (g SOC g soil^{-1}) of all analysed samples. The SOC content were highest for depth A and lowest for depth C, while depth B had medium high levels and was more similar to depth C than to depth A. All three data points showing extraordinarily high SOC content belong to sampling point 16, with the SOC content being especially high for depth B (4.335) and C (3.582), but also very high for depth A at 2.942. However, the three samples (16A, 16B and 16C) were analysed individually and independent of each other. Thus, the conclusion was made that these outlier values where not due to measurement error, but to some intrinsic characteristic of sampling location 16. Therefore, they were included in all further data analysis and had equal influence on the results as the other samples, despite being obvious outliers.

4.3.1 Data distribution

The Jarque Bera values of the full dataset (total area) were roughly 97 (A), 15 969 (B) and 33 706 (C), with p-values of 7.50*10^{-22}, 0 and 0 respectively. Since the p-value in this case needs to be above 0.05 to prove a normal distribution (Jarque, 2011), none of the three datasets followed a standard distribution. The only dataset showing a normal distribution (p value: 0.149) was the A depth of the Kernza™ plot.

When excluding sampling location 16, the full dataset (total area) of depth B had a p-value of 0.182 which proved a normal distribution, as well as depth B of the control plot with a p-value of 0.437. The Kernza™ depth A dataset still showed a normal distribution.

4.3.2 Statistics

Table 10. Descriptive statistics of the Kernza™ and control plots as well as the total (combined) area. To avoid confusion with relative MDD, the unit g SOC g soil^{-1} is used instead of *%* for the SOC content.

Kernza™	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**
Number of samples	37	37	37
Range [g SOC g soil^{-1}]	1.380 – 2.321	0.422 – 1.356	0.068 – 0.772
Mean [g SOC g soil^{-1}]	1.877	0.710	0.260
Median [g SOC g soil^{-1}]	1.920	0.685	0.224
STD [g SOC g soil^{-1}]	0.192	0.227	0.145
Absolute MDD [g SOC g soil^{-1}]	0.030	0.035	0.023
Relative MDD [%]	1.60 %	4.99 %	8.73 %
Control	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**
Number of samples	36	36	36
Range [g SOC g soil^{-1}]	1.001 – 2.942	0.247– 4.335	0.089 – 3.582
Mean [g SOC g soil^{-1}]	1.745	0.826	0.372
Median [g SOC g soil^{-1}]	1.648	0.616	0.227
STD [g SOC g soil^{-1}]	0.361	0.668	0.569
Absolute MDD [g SOC g soil^{-1}]	0.057	0.106	0.090
Relative MDD [%]	3.27 %	12.80 %	24.26 %
Total area	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**
Number of samples	73	73	73
Range [g SOC g soil^{-1}]	1.001 – 2.942	0.247 – 4.335	0.068 – 3.582
Mean [g SOC g soil^{-1}]	1.812	0.768	0.312
Median [g SOC g soil^{-1}]	1.843	0.717	0.225
STD [g SOC g soil^{-1}]	0.293	0.496	0.414
Absolute MDD [g SOC g soil^{-1}]	0.033	0.055	0.046
Relative MDD [%]	1.80 %	7.18 %	14.72 %

4.3.3 Mean, median, STD and depth

As seen in table 10, the SOC content of the Kernza™ and control plots were overall similar, with slightly higher mean and median SOC content in the A depth of Kernza™ and slightly higher mean SOC content in the B and C depths of the control plot. The medians on the other hand were close to identical in both plots for the B and C depths. The mean SOC content decreased with depth in all cases, as most SOC is stored at shallow depths. The range of SOC content for the three depth layers doesn't appear to have tendency with depth, even though the mean SOC content does. In the Kernza™ plot, the STD also decreases with depth. For the total area, the STD was more consistent while it increased with depth in the control plot. There doesn't appear to be a relationship between STD and depth.

The outlying point 16 is the main driver of statistical differences between the total area and Kernza and control plots, as it belongs to the control plot. In the scatter plot in fig. 10, this point is clearly identifiable. When 16A, 16B and 16C were excluded from the control plot, the STD decreased from 0.361 to 0.301 in A, from 0.688 to 0.296 in B and from 0.569 to 0.145 in C. This is a noteworthy difference, making the control field more similar to the Kernza™ field. The respective STD values of the total area (point 16 excluded) were 0.263 (A), 0.261 (B) and 0.144 (C). In this case, there was a slight decrease in STD with depth.

The large difference between mean and median values between the Kernza™ and control plots can also be explained by point 16, which increases the mean but not the median. When point

16 was excluded, the mean SOC content (g SOC g soil^{-1}) for the control plot was 1.710 (A), 0.723 (B) and 0.277 (C). Without point 16, the mean SOC content for the total area was 1.796 (A), 0.719 (B) and 0.267 (C).

4.3.4 Absolute and relative minimum detectable difference (MDD)

The absolute MDD of SOC content in the Kernza™ plot A depth was 0.057 g SOC g soil^{-1}. This corresponds to a relative MDD of 3.27 %, which matched and exceeded the desired relative MDD of 3 %. The B depth layer of Kernza™ had a slightly relatively higher relative MDD than absolute MDD due to the lower mean SOC content and does not match the desired MDD of 3 %. Still, it could prove low enough to detect the expected rate of changes in SOC content over the course of the research experiment in the A and B depth layers. As for the C layer, the mean SOC content was so low that even if the absolute MDD was 0.023 g SOC g soil^{-1}, this amounted to a relative MDD of 8.73 %.

In the control plot, the absolute MDD of the A depth was 0.057 g SOC g soil^{-1}, corresponding to a relative MDD of 3.27 %. This matches the desired relative MDD of 3 % but is almost double the MDD of the Kernza™ plot. The absolute MDD of both the B and C depths of the control plot is much higher than for the Kernza™ plot. Their relative MDDs are 13.52 % (B) and 25.18 % (C). This is due to the higher STD in the control plot, i.e., the presence of point 16. When calculated without point 16, the relative MDD is 2.67 % (A), 6.56 % (B) and 8.26 % (C).

Regarding the total area, the relative MDD is very low for the A depth: 1.80 %. For the B and C depths, the absolute and relative MDD sits in between that of the control and Kernza™ plot.

4.3.5 Uncertainty

The combined variability due to acidification irregularities, incomplete homogenisation and/or inherent variability within the samples was estimated to ± 0.066 g SOC g soil^{-1}. The uncertainty due to measurement errors during the EA process was deemed negligible. The fractional uncertainties are presented below in table 11. This was calculated based on the mean SOC of the total area for each depth. Again, the low mean SOC content of the deeper soil levels increased the uncertainty.

Table 11. Fractional uncertainty of SOC content.

Fractional uncertainty	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**
Total area	0.0365	0.0862	0.2118

4.4 Interpolation of SOC content

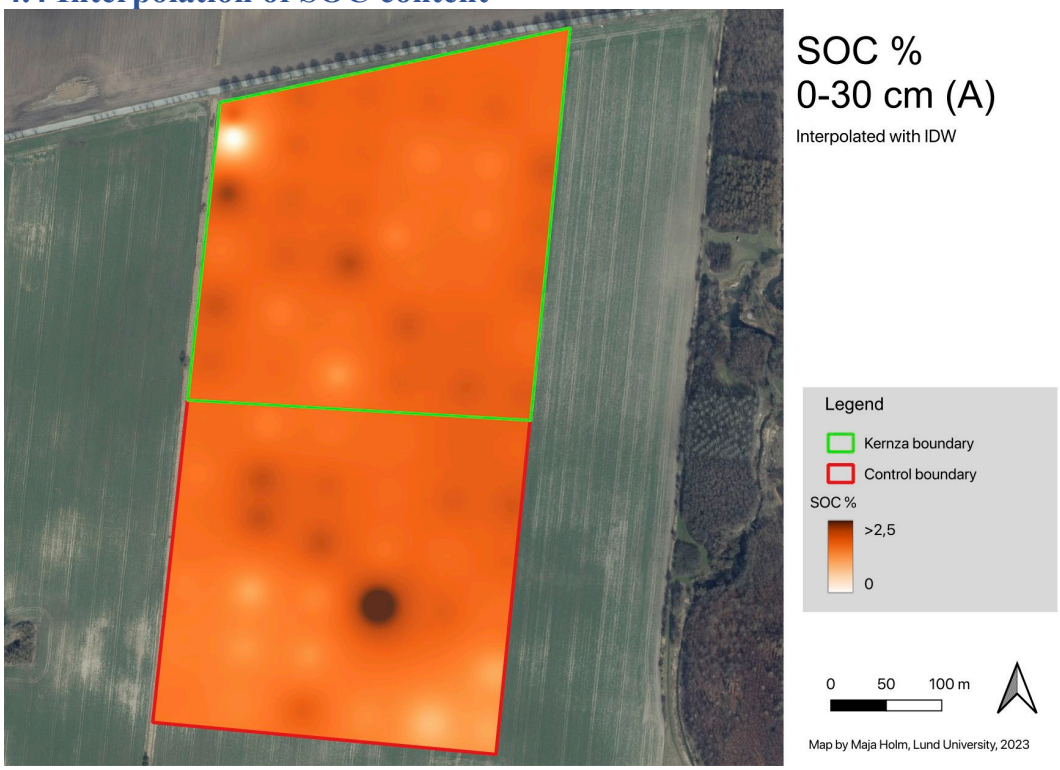

Figure 11. Soil organic carbon (SOC) content of the Kerna™ and control plots at 0—30 cm, interpolated across the total area. Based on 73 field samples collected at the research site in 2023. The resolution was 0.1 m.

Figure 12. Soil organic carbon (SOC) content of the Kerna™ and control plots at 30—60 cm, interpolated across the total area. Based on 73 field samples collected at the research site in 2023. The resolution was 0.1 m.

Figure 13. Soil organic carbon (SOC) content of the Kerna™ and control plots at 60—100 cm, interpolated across the total area. Based on 73 field samples collected at the research site in 2023. The resolution was 0.1 m.

The maps shown in fig. 11-13 are visual representations of the SOC content of the total area, both Kernza™ and control plots. The pixel values were interpolated through IDW from the data points and are thus only approximate, as the original data values were not preserved when interpolated through IDW. The outliers 16A, 16B and 16C are clearly identifiable. In fig. 11 (depth A), a white dot in the upper left corner represents an outlying value that was corrected after the creation of the maps (61A).

4.5 SOC stock

4.5.1 Statistics

The following values for SOC stock have been calculated based on the SOC data presented above as well as the interpolated BD values for each point. Worth noting is that the layer thickness of A and B is 0.3 m, while it is 0.4 m for C, which makes the comparison in SOC stock between layers more suitable when measured in kg SOC m^{-3} than in Mg SOC ha^{-1} (metric tonnes per hectare).

Table 12. SOC stock of layers A, B, C and combined. *Abs MDD* refers to absolute MDD and *Rel MDD* refers to relative MDD.

Kernza™	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**	**0–100 cm**
Mean [kg SOC m^{-3}]	28.03	11.66	4.42	13.68
STD [kg SOC m^{-3}]	2.76	3.72	2.47	2.26
Tot stock [kg SOC]	810 700	337 300	170 400	1 318 300
Tot stock [Mg SOC ha^{-1}]	84.10	34.99	17.67	136.76
Abs MDD [Mg SOC ha^{-1}]	1.29	1.74	1.55	3.53
Rel MDD	1.54 %	4.98 %	8.74 %	2.58 %

Table 12 continued.

Control	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**	**0–100 cm**
Mean [kg SOC m^{-3}]	25.04	14.75	7.68	15.01
STD [kg SOC m^{-3}]	5.53	11.32	11.14	8.57
Tot stock [kg SOC]	692 300	407 800	283 000	1 383 000
Tot stock [Mg SOC ha^{-1}]	75.12	44.24	30.70	150.06
Abs MDD [Mg SOC ha^{-1}]	2.63	5.38	7.05	13.57
Rel MDD	3.50 %	12.16 %	22.97 %	9.04 %
Total area	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**	**0–100 cm**
Mean [kg SOC m^{-3}]	26.56	13.18	6.02	14.33
STD [kg SOC m^{-3}]	4.58	8.46	8.13	6.22
Tot stock [kg SOC]	1 502 300	745 800	454 400	2 701 400
Tot stock [Mg SOC ha^{-1}]	79.67	39.55	24.10	143.26
Abs MDD [Mg SOC ha^{-1}]	1.53	2.82	3.61	6.90
Rel MDD	1.92 %	7.14 %	15.00 %	4.83 %

The total as well as the mean SOC stock (kg SOC m^{-3}) decreased with depth in all cases. In the Kernza™ plot, 61 % of the SOC stock was in the A depth layer, 26 % in B and 13 % in C. The equivalent for the control plot were 50 % (A), 29 % (B) and 20 % (C), and for the total area 56 % (A), 28 % (B) and 17 % (C). The mean SOC stock of the A depth layer was higher in Kernza™ plot than in the control plot, but the mean SOC stock of the B and C depths were higher in the control plot. The STD of SOC stock in the B and C depths were several times larger in the control plot than in the Kernza™ plot. Again, the differences in mean and total SOC stock between the Kernza™ and control were mostly due to the inclusion of the outlying point 16, which had more extreme values in the B and C depths than in A.

The relative MDD of the SOC stock was similar to that of SOC content in all cases. Just as for SOC content, the MDD of the A depth matched or exceeded the desired MDD (3 %) in both plots, while it is much larger in the deeper soil levels. Especially the B and C depths of the control plot had very high MDD, due to the high STD. The relative MDD of the total sampling depth was 2.58 % for Kernza™ – which matches and exceeds the desired 3 %. On the other hand, it was 9.04 % for the control plot. Again, point 16 explained the main difference between the two plots. Without it, the relative MDD of the full sampling depth was 5.09 % for the control, and the absolute MDD 6.82 Mg SOC ha^{-1}.

With a preserved number of samples (73), the absolute MDD of the SOC stock for the entire sampling depth (0-100cm) was 3.53 Mg SOC ha^{-1} for Kernza™ and 13.57 Mg SOC ha^{-1} for the control plot. For the total area, it was 6.90 Mg SOC ha^{-1}. Assuming a SOC sequestration of 0.3 Mg SOC ha^{-1} yr^{-1} (the Svensk Kolinlagring standard value) throughout the full sampling depth of 1 meter, statistically reliable changes in SOC would be detectable in around 12 years for the Kernza™ plot and in 45 years for the control plot. For the total area, changes would be detectable in 23 years. However, if it is assumed that all the A depth on its own could sequester 0.3 Mg SOC ha^{-1} yr^{-1}, this change would be detectable in 4.5 years in the Kernza™ plot. The number of samples required to obtain a 3 % relative MDD of the full sampling depth based on the known mean and STD presented in table 12 is 28 samples for Kernza™ and 237 samples for the control plot.

4.5.2 Uncertainty

The combined fractional uncertainty of sampling error of BD, interpolation of BD and SOC content variability is presented in the table below.

Table 13. Total fractional uncertainty.

Fractional uncertainty	0-30 cm (A)	30-60 cm (B)	60-100 cm (C)
Total area	0.135	0.225	0.300

The full SOC stock of the Kernza™ plot was thus 136.76 ± 24.57 Mg SOC ha^{-1}, or a total field stock of 1 318 300 ± 236 500 kg SOC. The full SOC stock of the control plot was 150.06 ± 29.31 Mg SOC ha^{-1}, or a total field stock of 1 383 000 ± 270 100 kg SOC.

4.6 Deep pits

The SOC content and stock of the 100—130 cm (D), 130—160 cm (D) and 160—200 cm (D) depths are presented in table 14.

Table 14. SOC content and stock of the D, E and F depths of the total area.

Total area	100–130 cm (D)	130–160 cm (E)	160–200 cm (F)
Number of samples	7	7	4
Mean [g SOC g $soil^{-1}$]	0.177	0.183	0.174
Median [g SOC g $soil^{-1}$]	0.200	0.176	0.178
STD [g SOC g $soil^{-1}$]	0.052	0.096	0.058
Mean SOC stock [kg SOC m^{-3}]	2.97	3.04	2.83
Total SOC stock [kg SOC]	167 900	172 000	213 200
Total SOC stock [Mg SOC ha^{-1}]	8.90	9.12	11.31

The number of samples was too low to be able to predict anything with certainty, but the values in table 14 give an idea of the SOC content and stock of the deeper soil. The mean SOC content and stock appeared to remain similar across the different depths. The total stock was higher for the F depth, but since this was calculated with a depth of 0.4 m instead of the 0.3 m used for D and E, comparing the mean SOC stock is more accurate.

4.7 Svensk Kolinlagring protocol simulation

Table 15. Kernza™ and control SOC statistics of the SK simulation. Values within brackets refer to the full dataset, presented earlier. Letters *A* and *B* in the two last rows refer to two different ways of calculating the MDD. The first, *A*, is calculated with the STD and sample size of the SK dataset, while *B* is calculated with the sample size of the SK dataset, but the STD of the full *Perennial* dataset (table 10). *Abs MDD* refers to absolute MDD and *Rel MDD* refers to relative MDD. The unit of absolute MDD is Mg SOC ha^{-1}. For mean, STD and MDD of SOC content the unit is g SOC g $soil^{-1}$.

Kernza™	0-30 cm (A)	30-60 cm (B)	60-100 cm (C)	0–100 cm
Sample size	6	6	6	
Content mean	1.746 *(1.877)*	0.562 *(0.710)*	0.188 *(0.260)*	
Content STD	0.141 *(0.192)*	0.134 *(0.227)*	0.069 *(0.145)*	
Content MDD	0.055 *(0.030)*	0.052 *(0.035)*	0.027 *(0.023)*	
Stock [kg SOC m^{-3}]	26.78 *(28.03)*	9.91 *(11.66)*	3.69 *(4.42)*	12.47 *(13.67)*
Stock [Mg SOC ha^{-1}]	80.34 *(84.09)*	29.72 *(35.00)*	14.22 *(17.67)*	124.80 *(136.76)*
Abs MDD A	2.58 *(1.29)*	2.14 *(4.98)*	2.3 *(1.45)*	6.69 *(3.53)*
Rel MDD A	3.2% *(1.5%)*	7.2% *(1.5%)*	15.6% *(8.7%)*	5.4% *(2.6%)*
Abs MDD B	3.36 *(1.29)*	4.53 *(1.74)*	4.02 *(1.45)*	9.18 *(3.53)*
Rel MDD B	4.0% *(1.5%)*	15.1% *(5.0%)*	26.0% *(8.7%)*	7.0% *(2.6%)*

Table 15 continued.

Control	**0-30 cm (A)**	**30-60 cm (B)**	**60-100 cm (C)**	**0–100 cm**
Sample size	6	6	6	
Content mean	1.710 *(1.745)*	0.631 *(0.826)*	0.215 *(0.372)*	
Content STD	0.144 *(0.361)*	0.211 *(0.688)*	0.032 *(0.569)*	
Content MDD	0.060 *(0.057)*	0.082 *(0.106)*	0.012 *(0.090)*	
Stock [kg SOC m^{-3}]	24.30 *(25.04)*	10.57 *(14.75)*	3.75 *(7.67)*	11.96 *(15.01)*
Stock [Mg SOC ha^{-1}]	72.91 *(75.11)*	31.71 *(44.24)*	15.01 *(30.70)*	119.63 *(150.06)*
Abs MDD A	1.06 *(2.63)*	3.49 *(5.38)*	1.40 *(7.05)*	5.73 *(13.57)*
Rel MDD A	4.3% *(3.5%)*	11.0% *(12.2%)*	5.5% *(23.0%)*	4.8% *(9.0%)*
Abs MDD B	6.44 *(2.63)*	13.17 *(5.38)*	17.28 *(7.05)*	33.80 *(13.57)*
Rel MDD B	8.83% *(3.5%)*	41.54% *(12.2%)*	115% *(23.0%)*	28.3% *(9.0%)*

Table 15 shows the statistics of the Kernza™ and control plots, when calculated with the stratified sampling design of the SK protocol. For easier comparison, the equivalent values of the unstratified *Perennial* dataset (also presented in table 10 and 12) are put side by side in parenthesis.

The SOC statistics calculated with the SK protocol generally followed similar tendencies as the full dataset, with decreasing SOC content and stock with depth. The mean SOC content as well as the total SOC stock were lower in all cases when calculated with the stratified sampling design of the SK protocol. Quite notably, the STD was lower than for the Perennial dataset in all cases, likely due to the smaller sample size. The exclusion of the outliers 16A, 16B and 16C also contributed to the lower estimation of the total SOC stock in the control plot compared to the *Perennial* calculation, as well as the much lower STD of the control plot.

When the MDDs of both SOC (g SOC g $soil^{-1}$) and stock (Mg SOC ha^{-1}) (**A**) were calculated with the sample size and STD of the smaller stratified dataset of 6 samples per plot, the relative MDD was generally larger than the *Perennial* MDD for Kernza™ and lower for control plot. The lower STD of SOC of the SK dataset was thus not enough to compensate for the smaller sample size (6 compared to 36-37). When calculated with the same sample size but with the more accurate SOC (g SOC g $soil^{-1}$) STD of the full dataset – described with the letter **B** in table 15 – the MDD of the SOC stock was higher in all cases. Overall, the smaller stratified sample size captures the general tendency of SOC variability and stock but does not manage to accurately estimate the overall stock or the MDD.

Based on the MDD calculated with the more accurate *Perennial* STD, it would take 112 years to verify a total increase in SOC of 300 kg SOC ha^{-1} in the control plot and 31 years in the Kernza™ plot.

4.8 Stratification

4.8.1 Svensk Kolinlagring

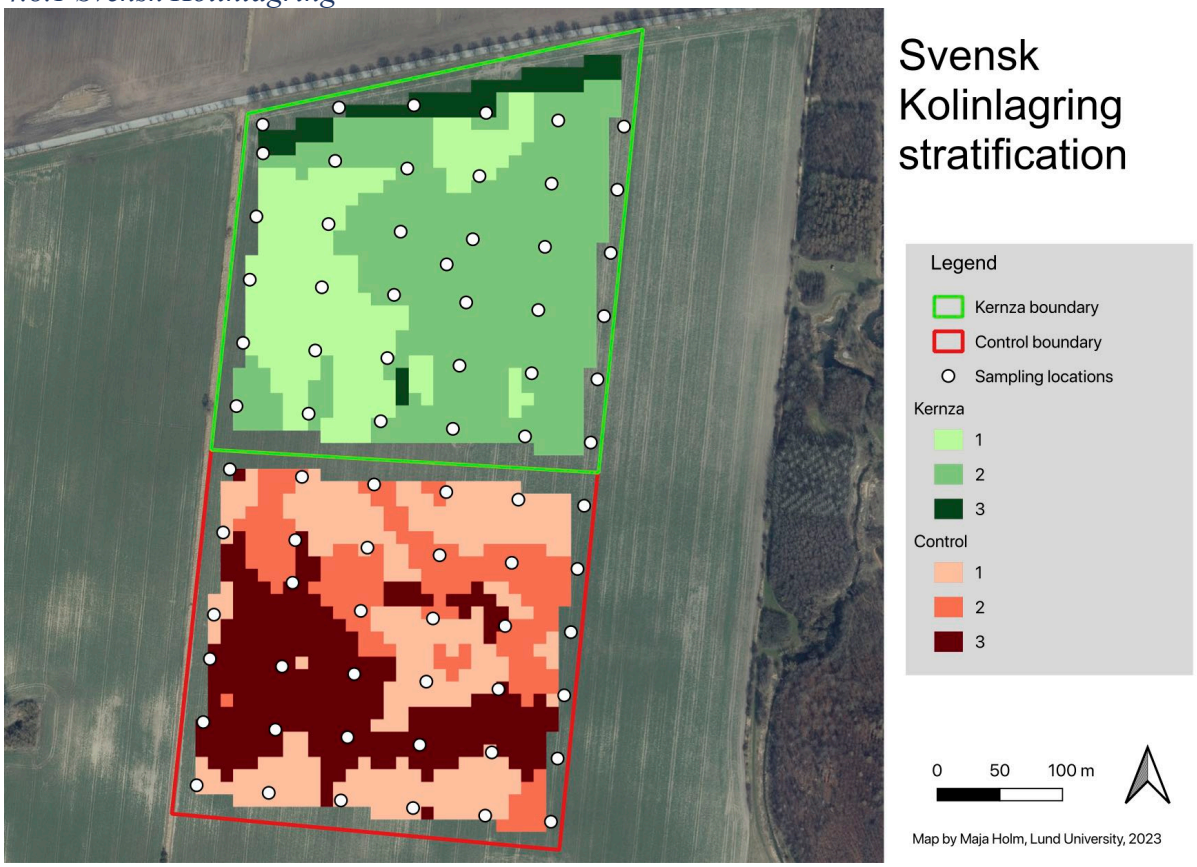

Figure 14. Svensk Kolinlagring strata for both Kernza™ and control plots with all 73 sampling locations at the Alnarp research site, including those that fell within the 15 buffer zones at the plot edges. Source of stratification data: Svensk Kolinlagring.

Table 16. Basic SOC statistics for the different strata in both research plots. These results were generated prior to a correction of the SOC content data. Thus, points 10A and 11A are missing from the dataset used for this analysis due to time limitations. For point 61A, an incorrectly small value was used.

Kernza™	**Stratum 1**	**Stratum 2**	**Stratum 3**	**Total**
Sample size	5	19	3	27
Mean [g SOC g soil^{-1}]	1.791	1.854	1.325	1.841
Median [g SOC g soil^{-1}]	1.714	1.910	1.920	1.930
STD [g SOC g soil^{-1}]	0.170	0.190	1.040	0.341
Control	**Stratum 1**	**Stratum 2**	**Stratum 3**	**Total**
Sample size	11	7	9	27
Mean [g SOC g soil^{-1}]	1.797	1.891	1.631	1.751
Median [g SOC g soil^{-1}]	1.764	1.831	1.581	1.758
STD [g SOC g soil^{-1}]	0.471	0.208	0.295	0.356

The Svensk Kolinlagring stratification and all sampling locations are seen in fig. 14. The smaller sample size (27 compared to 36 or 37) is explained by that several sampling points fell in the excluded buffer zone (as seen in fig.14) as well as the missing samples 10A and 11A. By comparing the mean, median and STD of the strata presented in the table above, no clearly discernible patterns in SOC g SOC g soil^{-1} between the different strata in either of the research plots appeared, which indicates that this stratification was ineffective.

When comparing the mean SOC content between the Kernza™ strata, it first appeared as if they strata were different from each other – however, when looking at the medians, it became evident that it was not the case. The STD of strata 1 and 2 were lower than the total, but the STD of stratum 3 was triple that of the total. For the control plot, the mean and median of the strata were more similar both within and between strata, and the points were more evenly distributed across the strata. The STD was also more similar than for Kernza™.

According to the Kruskal Wallis H test, there were no significant differences in SOC content of the A depth between the different strata in either of the two research plots. The H value was 0.798 for the Kernza™ plot and 3.737 for the control plot, with a critical cut-off value of 5.991. Since both 0.798 and 3.737 were smaller than the critical cut-off value, this did not reject the null hypothesis, which was that the SOC content of the strata would be equal. This showed that in this case and with this methodology, stratifying the field prior to field sampling was not an effective way of reducing the required number of samples.

4.8.2 Seasonal productivity (SPROD)

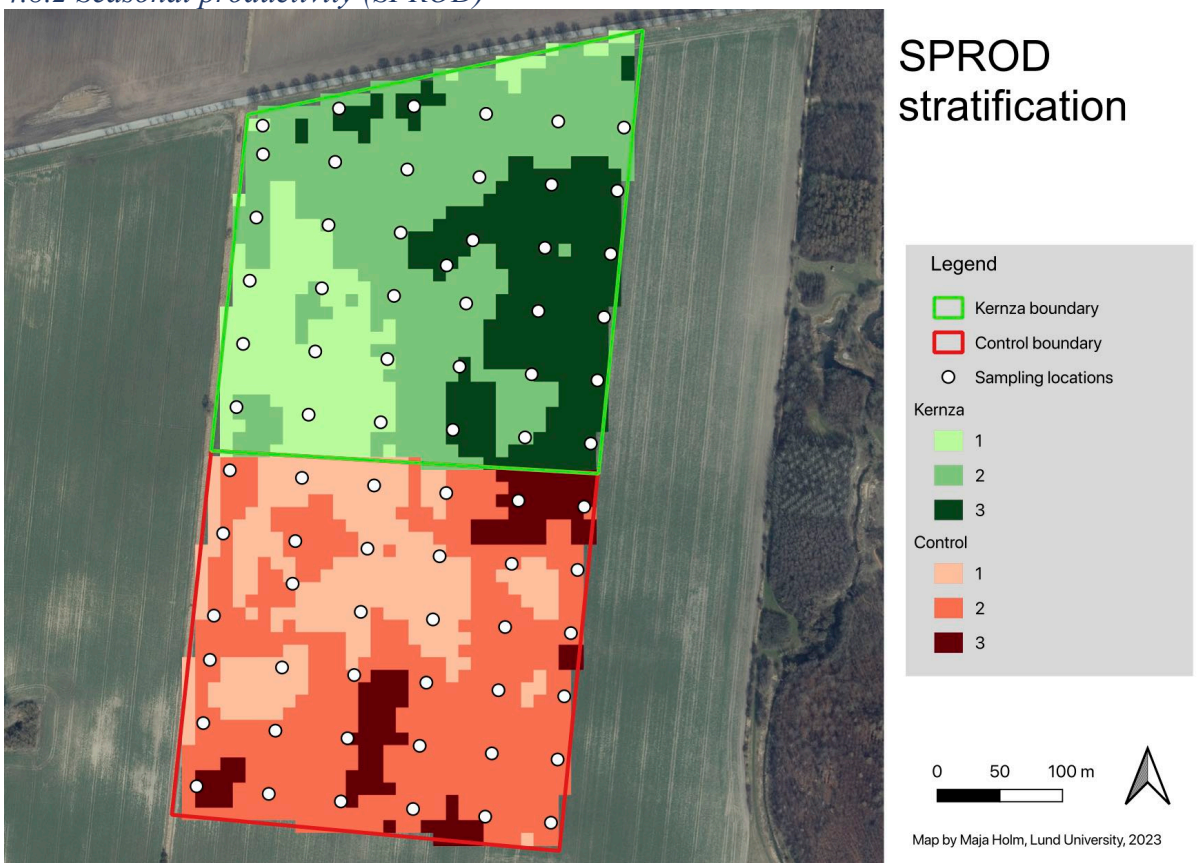

Figure 15. Strata of the Kernza™ and control plots at the research site in Alnarp, based on mean seasonal productivity (SPROD) values from 2017-2022. All 73 sampling locations.

Fig. 15 shows the result of the stratification with SPROD remote sensing data as well as all sampling locations. The Kernza™ and control field were treated separately, so the strata of the Kernza™ plot are not identical to the strata of the control plot.

Table 16. Basic SOC statistics of the different strata in both research plots.

Kernza™	**Stratum 1**	**Stratum 2**	**Stratum 3**	**Total**
Sample size	8	15	14	37
Mean [g SOC g soil^{-1}]	1.797	1.829	1.878	1.841
Median [g SOC g soil^{-1}]	1.806	1.936	1.945	1.930
STD [g SOC g soil^{-1}]	0.226	0.499	0.155	0.341
Control	**Stratum 1**	**Stratum 2**	**Stratum 3**	**Total**
Sample size	7	23	4	34
Mean [g SOC g soil^{-1}]	1.755	1.752	1.738	1.751
Median [g SOC g soil^{-1}]	1.751	1.764	1.795	1.758
STD [g SOC g soil^{-1}]	0.289	0.399	0.235	0.356

As for the SK strata, the total number of samples for the control plot is 34 instead of 36 due to two missing samples (10A and 11A). As for point 61A, an incorrectly small value was used. While there were some small differences in mean and median between the different strata in both plots (table 16), such as stratum 1 in the Kernza™ plot having slightly lower mean and median SOC content, it is not clear if there are any patterns by simple comparison.

However, the Kruskal Wallis H values were 0.069 for the control plot and 1.155 for the Kernza™ plot. In order to prove a significant difference in SOC content between the strata, the H values need to be greater than 5.991. This means that in this case, the null hypothesis was not rejected, i.e., there was no significant difference in SOC content between the strata defined by SPROD. Stratifying the fields based on SPROD in order to reduce the required number of samples is therefore most likely ineffective in this case.

5 Discussion

5.1 Summary of findings

The main purpose of this thesis has been fulfilled: Determining the SOC content and stock of the Kernza™ and control plots in Alnarp, serving as a baseline study for the *Perennial* research project. The SOC stock of the full sampling depth (0-100 cm) of the Kernza™ field was 136.76 ± 24.57 Mg SOC ha^{-1}, with a field total of 1318.30 ± 236.50 Mg SOC (table 12 and 11). The equivalent of the control plot was 150.06 ± 29.31 Mg SOC ha^{-1} with a field total of 1383.00 ± 270.10 Mg SOC. The mean SOC stock of the Kernza™ plot was 13.68 kg SOC m^{-3}, and 15.01 kg SOC m^{-3} of the control plot. This information is essential in determining the effect on SOC content of Kernza™ compared to the conventional cropping rotation in the control plot, at the end as well as during the *Perennial* research project.

The 0–30 cm (A) soil layer held most SOC – 61 % of the Kernza™ plot SOC stock, and 50 % of the control. While SOC content was by far highest in the topsoil, BD increased with depth in both plots. The mean SOC content was generally low compared to the median of Swedish soils (Eriksson, 2021), indicating that the field is nowhere near its maximum SOC storage capacity.

The aim of the sampling and analysis protocol designed for this study (sometimes referred to as the *Perennial* protocol) was to be able to determine the SOC content and stock with sufficient accuracy to detect relevant changes in SOC over the course of the 5-year *Perennial* research project. For the Kernza™ plot this was fulfilled, as the MDD of the full sampling depth of the Kernza™ plot was 2.58 % or 3.53 Mg SOC ha^{-1}. The MDD was 9.04 % or 13.57 Mg SOC ha^{-1} for the control plot (table 12). Without point 16, the MDD was 5.09 % or 6.82 Mg SOC ha^{-1}.

Thus, the target MDD of 3 % was technically not achieved regardless for the control plot, but 5.09% is much closer. While little to no changes in SOC content of the control plot are expected, any change in SOC in the Kernza™ plot will in this study be confirmed against the control, and it is therefore important that the MDD of the control plot is low enough. However, the absence of a control is not uncommon in previous research (Nayak et al., 2019).

When comparing the SOC content and stock of the *Perennial* sampling protocol to that of the Svensk Kolinlagring simulation protocol, it became apparent that the smaller sample size and stratified sampling design were not able to predict relevant changes over time. Further, the protocol was not able to verify the standard unit of SOC sequestration used by SK (300 kg ha^{-1} yr^{-1}) during a realistic timeline, as it would take 31 years to be able to detect this change in the Kernza™ plot, and 112 years in the control plot.

No correlation was found between strata and SOC content for either the SK stratification or the SPROD stratification. In this case, a stratified sampling design was therefore deemed ineffective in terms of reducing the required number of samples. Each subtopic is discussed individually in the following text.

5.2 Sampling design

The sampling protocol designed for this study was based on an extensive literature review as well as a previous soil survey and a calculation of the minimum number of samples required to obtain a certain minimum detectable difference (MDD), which was 52. The STD of the previous soil survey was 0.43 g SOC g $soil^{-1}$, calculated from the top 0—30 cm of 26 points collected from a larger area around the research site. The STD of SOC content at 0—30 cm calculated from all 73 sampling points in this study was 0.345 g SOC g $soil^{-1}$ (table 10). If the number of samples remains constant, MDD decreases with STD. Since the STD was in fact lower than that of the previous soil survey, the added margin in form of a total of 73 samples worked in favour of precision, allowing for some leeway in the form of an increased number of samples and consequently a lower MDD. Another factor that could have affected the outcome of the calculation of required number of samples is that the two plots ideally would have been treated as two separate plots from the start: While the MDD of SOC content for the total area is low, it is higher for the individual research plots since the number of samples is halved. When determining the SOC sequestration in the plots individually – which is required, since the *Perennial* research project will be a comparative study between Kernza™ and conventional management – the number of samples was smaller (37 and 36, respectively).

The decision to use composite samples consisting of five subsamples perhaps led to unnecessarily large samples, requiring more time and effort to homogenise than if the samples had been smaller. However, the use of composite samples is generally recommended (FAO, 2020), as the cost of analysing each subsample individually would be very high. One option could be to reduce the number of subsamples, but it's recommended to use the same sampling design for all sampling campaigns within the same research project (Stanley et al., 2023). The larger sample size also allows for future research.

The field sampling and lab methodology were mostly successful. When considering time-cost efficiency, the homogenisation process of crushing the soil samples by hand (using a mortar and pestle) was the least efficient part of the laboratory work, as it took a substantial amount of time. Another method, such as an electrically powered soil grinder, would have been preferable. In this case, there would be no need to reduce the number of subsamples. As for the field sampling, the planned methods worked out well. Collecting BD samples was the most difficult

process during field sampling, as collecting undisturbed samples requires careful handling. This is likely the main reason for the large uncertainty of BD, although its proportion compared with variability has not been quantified.

BD was only sampled at 15 locations, compared to the total 73 SOC samples, potentially leading to inaccurate estimations of SOC stock. Since every BD sample required an excavator to dig a 1 m pit, it was not practically doable to sample every location, but an increased number of samples would lead to higher accuracy. Another factor was that the 15 locations were not evenly distributed across the research area, due to insufficient planning. This has implications for the accuracy of the interpolation, as the interpolated value is more accurate at some locations than others.

5.3 Bulk density

While there was some spatial variation of BD, it was quite low compared to the SOC content. The relative STD of the A depth was 8 %, while it was 10 % for B and 8 % for C (based on the 15 field samples). When considering the mean BD, there appeared to be a tendency of increasing BD with depth, although though no statistical tests were done to confirm this. The mean sampled BD of Kernza™ was slightly higher than for the control field. The mean BD of the B and C depths were very similar, 1.63 g cm^{-3} (B) and 1.68 g cm^{-3} (C) for all 15 samples, while the mean BD of the A depth was 1.46 g cm^{-3} (table 2). However, the sampling error was estimated to ± 0.122 g cm^{-3}, which prevents robust conclusions about the difference between plots or depths, since the error was of similar magnitude as the differences. The lower BD of the topsoil could be explained by tilling, which temporarily decreases BD (Taylor et al., 2023).

The high BD of the lower soil layers could restrict root growth, potentially limiting the SOC sequestration effect of the long root system of Kernza™. While field sampling, variations in grain size were observed in the deeper soil layers, with very high clay content in some areas. This could restrict the root growth of Kernza™, as well as explain the high BD. In order to confirm this, a full grain size analysis is needed.

The STD of the interpolated BD was lower than the STD of the sampled BD. This is a known problem of IDW interpolation; it acts as a smoothing filter and often doesn't reflect the true variability (Sajid et al., 2013). Since IDW preserves the maximum and minimum sampled values, the STD can't be greater than that of the sampled data. This is also visible in the BD scatter plot (fig. 5), where the interpolated BD is visibly more homogenous. While some variation of BD was lost in the interpolation, the mean interpolated BD was very similar to the mean sampled BD. While using interpolated values of BD has its drawbacks, there are few alternatives. Taking one BD sample per SOC sampling location is very cost- and labour intensive and causes significant soil disturbance. Sometimes BD is treated as a fixed value, but failing to factor in the variability is not recommended as it can prevent reliable detection of SOC change (Stanley et al., 2023). Another commonly used option to BD is equivalent soil mass (ESM) (Rovira et al., 2022), which unfortunately was not suitable for this study due to irregular an inaccurate weights of the SOC composite samples.

The error derived from BD field sampling could well be either smaller or larger than ± 0.122 g cm^{-3}, as this is a post sampling estimation based on a maximum error of 3 g of soil per BD sample. This error is almost entirely due to the technical difficulty and time-consuming nature of collecting undisturbed samples. Other measurement errors were deemed negligible in comparison to the sampling error. The uncertainty derived from the BD interpolation was low compared to that derived from the field sampling. Thus, improving the field sampling accuracy

should be priority in order to reduce the uncertainty of BD. An increased and evenly dispersed number of samples could not only yield more accurate sampled BD data but also more accurately interpolated BD. Worth noting is that the RMSE of the BD interpolation – which is interpreted as uncertainty – is calculated by comparing the interpolated values and the sampled values at the sampling locations. I.e., the same data is used both as training data and validation data. A alternative, perhaps more suitable, validation of the IDW interpolation would have been e.g. cross validation (Caloiero et al., 2021).

5.4 SOC content

The mean SOC content of the 0-10 cm depth (A) was 1.887 g SOC g soil^{-1} for the Kernza™ plot and 1.745 g SOC g soil^{-1} for the control (table 10). Compared to the SOC content of other agricultural soils in the area, this is not unusual, although it is at the low end. The median SOC content of Swedish agricultural soils is 2.61 % (Eriksson, 2021). Thus, the soil is most likely far from its maximum capacity in terms of SOC storage. Since there were samples exhibiting as much as 4.335 g SOC g soil^{-1}, it is not unreasonable to assume that the SOC storage capacity is around this level. Although, these unusually high values could be due to some land history factor such as a drained bog, making it less representative of the surroundings (see discussion around point 16 further on). Also, maintaining this level of SOC content – at least in the topsoil – would require constant input of organic material, as an equilibrium between mineralization and sequestration of SOC is eventually reached.

There is a clear decrease in SOC content with soil depth, which is an expected behaviour of agricultural soil. The tilling depth is often around 30 cm, and conventional crops often have the majority of their root system above this depth (Fan et al., 2016). However, no full statistical analysis of the relationship between SOC content and depth was conducted. The variability (STD) of SOC content did not seem to have a clear relationship with depth. This was also observed for conventionally managed systems by Stanley et al. (2023).

The STD of SOC in the control plot was higher than in Kernza™ for all depths. When point 16 was excluded, the STD in the control plot decreased and was similar to that of the Kernza™ plot (table 10). When considering the full SOC dataset (Kernza™ and control combined), none of the depths showed a normal distribution, except for the 0—30 cm (A) of the Kernza™ plot. However, when point 16 was removed, the full dataset of depth B followed a normal distribution as well. The fact that the data generally isn't normally distributed is not abnormal per se, but this shows that a single outlier can have large influence on the result. In fact, the same tendency was observed for several factors – when excluding point 16, the two plots were a lot more identical. Point 16 was by far the most important driver of differences between the two research plots.

This leads to the elephant in the room: What happened at point 16? What is the cause of the abnormally high SOC content at this location, especially at depth? 16A was the least extreme of the three samples with 1.7 times higher SOC content than the mean – perhaps due to tilling which mixes the soil on a regular basis. The B and C samples were around 5 and 10 times higher than the respective mean. When looking at the scatter plot of SOC (fig. 9), point 16 stood out clearly, and the SOC measurements appear to have higher variation at points 1—20, all within the control plot. Since each sample is a composite of 5 subsamples, the high SOC content of point 16 could reflect some unusual conditions of a small area containing all subsamples, or an extremely high SOC content of one or several subsamples influencing the mean SOC content of the composite sample.

The two plots are geographical neighbours and appeared to be similar in terms of topography and recent land management. However, a brief study of older orthophotos (1998 and 2004), topographical maps, the Lantmäteriet Register map 1935—1975 (Ekonomiska kartan) and the Lantmäteriet soil moisture map (Markfuktighetskartan) suggested that point 16 is located near a well that is part of a drainage system. The area also looks both more wet and slightly lower in topography than the surroundings and appeared to be an accumulation zone for runo. This could be a possible explanation for the extreme values at point 16, leading to more anaerobic conditions and thus lower decomposition of SOC (i.e., higher SOC content). A more focused study on the local hydrology and topography is recommended in order to make an informed decision on whether point 16 could be excluded from the analysis, as well as if there are other similar areas in either of the research plots.

Part of the purpose of this thesis was to create a sampling design that could detect changes in SOC content with a target MDD of 3 % or lower. Since the calculation of this was based on the STD of SOC at 0-30 cm in previous soil survey, this target MDD is mainly applicable to the A depth (0-30 cm). For the A depth of the Kernza™ plot, this was achieved, as the MDD of the Kernza™ plot was almost half of that – 1.60 %. The control plot misses the bar ever so slightly with an A depth MDD of 3.27 %, which can be regarded as sufficient. For the total area, it was 1.80 %. This is despite the facts that the plots not being treated individually when considering the required number of samples, resulting in a smaller number of samples per plot than intended. The main factors contributing to this was the incorrect calculation of samples leading to more samples than intended, as well as the lower actual STD of the research plot than that of the 26 samples from the previous soil survey.

If the required number of samples is calculated in retrospect – using the known STD and mean of the SOC content and a relative MDD of 3 % (g SOC g soil^{-1}) – this resulted in 28 samples for Kernza™ and 237 for the control plot. This again highlights the great influence of spatial heterogeneity on SOC sampling uncertainty, as the main difference between the two plots was the STD of SOC content which in turn was mainly caused by point 16.

While it was increasingly difficult to detect relative changes in SOC content (relative MDD) at depth for both Kernza™ and control (greatly exceeding 3 %), absolute changes could still be detected at depth in the Kernza™ plot. The increasing relative MDD with depth is not due to increasing STD, but to the decreasing mean SOC content. In the control plot, the higher STD in the B and C depths (mainly due to point 16) caused higher absolute MDDs in the B and C depths than in A. While the higher MDD of the deeper soil layers is inevitable, it is unfortunate. Kernza™ is promoted for its extensive root systems and for its potential to sequester carbon at deeper soil layers, where it is protected from decay. The ability to detect changes in SOC in the B and C layers is therefore highly important.

Worth noting is that even if the relative MDD of the C depth is 8.73 % for Kernza™ and 24.26 % for control (8.26 % for control if point 16 is excluded), this corresponds to 0.023 g SOC g soil^{-1} for Kernza™ and 0.090 g SOC g soil^{-1} for control. Whether this is low enough is difficult to say, since there is limited previous research on the de facto SOC sequestration ability of Kernza™. The difference between the two plots is probably more of a concern, since any gain in SOC content under Kernza™ needs to be confirmed against the control, where the MDD is much higher.

Regarding uncertainty of SOC content due to sampling and/or analysis, it was 3.68 % for A, 8.62 % for B and 21.07 % for C. Comparing this to the relative MDD, this is cause of concern

as the uncertainty and relative MDD are of similar magnitude. However, this uncertainty or inherent variability will likely be similar in 5 years' time, given that the sampling method is the same.

How much of the uncertainty of SOC content analysis is due to insufficient homogenisation or to variations in acidification is unknown. The acidification process is a known source of uncertainty, especially when the SIC content is high (Brodie et al., 2011; Nayak et al., 2019). Whether the SIC content in the Alnarp soil is high or not is not fully known, although it has been speculated to be rather high, based on earlier soil analysis in nearby areas. Since the amount of total carbon (TC) was analysed in the lab as well as the total organic carbon (TOC), the SIC content can be calculated as the difference between TC and TOC. However, the results from the TC analysis were not available before the end of this study and have thus not been included or analysed.

Since the homogenisation was done by hand and interrupted at the point where all material could pass through a 2 mm sieve, it is also very possible that the samples were not fully homogenised. It is recommended to investigate this in order to better understand and potentially decrease the uncertainty.

5.5 SOC stock

When comparing the SOC stock of the two plots, they were almost equal when expressed as kg SOC. However, when expressed as Mg SOC ha^{-1}, it becomes apparent that the mean SOC stock of the control plot is higher, at 150.06 Mg SOC ha^{-1} compared to 136.76 Mg SOC ha^{-1}. The plots also differ in the distribution of SOC between the layers, as 61 % of the SOC stock in Kernza™ was in the 0—30 cm (A) depth, while only 50 % in the control. These differences are again explained by point 16, as it increased the mean SOC content of the 30—60 cm (B) and 60—100 cm (C) depths in the control.

The patterns of SOC stock are mainly a reflection of the those in SOC content. While there was spatial variability of BD it was reduced during the interpolation, as well being less than the SOC variability to begin with. Therefore, most of the discussion around mean, STD and MDD of SOC content also applies to the SOC stock. One issue regarding the loss of variability of BD due to the interpolation is that it could potentially lead to an underestimation of the MDD, since it's based on STD.

The main difference of the SOC stock results compared to SOC content is that the total SOC stock has been calculated as a sum of the depth layers. For the Kernza™ plot, the relative MDD of the total depth was more similar to the MDD of the A depth, at 2.58 % (total) compared to 1.54 % (A). For the control plot, the total depth MDD was 9.04 %, compared to 3.50 % (A). This reflects the distribution of the SOC between the layers, since Kernza™ has comparatively more SOC in the A layer than control. Notably, the total depth MDD for Kernza™ is more similar to the A depth than the total depth MDD for control, which is explained by the fact that 61 % of the Kernza™ SOC stock is in the A depth, whereas the equivalent is 50 % for the control plot.

Important to note is that the depth layers do not need to each gain 3 % SOC. It is enough if the sampling protocol is exact enough to detect a 3 % gain in total SOC stock. Most likely, the layers won't each gain 3 % of SOC during the upcoming five years of study. Gains happens first and foremost in the upper soil layers, and whether the Kernza™ roots are able to penetrate further into the soil and sequester SOC at depth remains to be seen. Thus, whether the C depth

MDD of 8.74 % (Kernza™) or 22.97 % (control) (table 12) are low enough to detect these changes depends on the de facto SOC sequestration in this layer during the research period. This is equivalent to 1.55 Mg SOC ha^{-1} (Kernza™) and 7.05 Mg SOC ha^{-1} (control), but whether the root system of Kernza™ manages to penetrate the hard clay and sequester these amounts of SOC in the deeper layers remains to be seen.

In the previously referenced study of Kernza™ by de Oliveira et al. (2018), the total sink over the 4.5 years of study was 1478 g C m^{-2}, corresponding to 147.8 Mg SOC ha^{-1}. If the Kernza™ plot in Alnarp is an as strong sink, the MDD of this study is by far enough to quantify the amount of sequestered SOC. The MDD of the total sampling depth (0—100 cm) was 3.53 Mg SOC ha^{-1} for Kernza™ and 13.75 Mg SOC ha^{-1} for the control (table 12).

Since the 147.8 Mg SOC ha^{-1} of the de Oliveira et al. (2018) study is around 42 times the 3.53 Mg SOC ha^{-1} (Kernza™) and 11 times the 13.75 Mg SOC ha^{-1} (control) changes would be detectable despite the high MDD of the control plot due to point 16, although with lower accuracy. In fact, the mean annual sink strength of the de Oliveira et al. (2018) study was 37.0 Mg C ha^{-1}, meaning that even the absolute MDD of the control plot would be detectable in less than 6 months. However, the de Oliveira et al. (2018) study was purely based on EC fluxes and not physical soil sampling and also took place in a different climatic context (Kansas, US), which make comparisons of this kind less relevant.

Another study also comparing fields of annual crops with Kernza™ (planted 5—17 years ago) found that the SOC stock was on average 4 ± 2 Mg SOC ha^{-1} higher under Kernza™ than under annual crops, with an average SOC gain of 0.4 ± 0.2 Mg SOC ha^{-1} yr^{-1} (van der Pol et al., 2022). This suggests that the absolute MDD of Kernza™ of 3.53 Mg SOC ha^{-1} could be low enough to detect changes in around 10 years' time, but not in 5. On the other hand, a recent study comparing Kernza™ to annual wheat found that while SOC content at 0—30 cm was slightly higher under Kernza™ after three years, it was actually higher under annual wheat *below* 30 cm (Taylor et al., 2023). Only SOC content was measured and not BD. The effect of tilling vs no-till also likely played a role here, as tilling redistributes SOC such that the SOC content can increase at depth (Taylor et al., 2023). Since the wheat was tilled and Kernza™ was not, this could explain the higher SOC under wheat below 30 cm.

Just as for SOC content, the low mean SOC stock of the B and C soil layers increases the uncertainty. The combined fractional uncertainty of the C depth (30%) SOC stock is more than double that of A (13.5%), due to the lower mean SOC content. While this increases the fractional error/uncertainty it doesn't increase the absolute error, which was the same for all depths. The fractional uncertainty was calculated for the total area only which potentially skews the results, but to similar degrees for both research plots. However, the main issue is that the combined uncertainty due to sampling error and interpolation BD and the SOC content variability is of the same magnitude as or higher than the MDD. This poses serious doubt about the ability to detect any relevant changes in the SOC stock over time.

The main driver of the combined uncertainty of the SOC stock estimations depended on the sampling depth. For the A and B depths, BD contributed most to the uncertainty (around 70%), while it was the inverse for the C depth, where the SOC analysis contributed around 70% to the total uncertainty. Further reducing the MDD would not only create an unpractical amount of labour, especially seeing that this study was already labour-intensive, but also be comparatively fruitless in comparison to the uncertainty. Therefore, the effort should be put into reducing the uncertainty of BD as well as SOC.

5.6 SOC content and stock: Svensk Kolinlagring protocol simulation

Both SOC content and stock were slightly underestimated when calculated with the SK dataset, but the general tendencies were the same as for the *Perennial* dataset with point 16 excluded. The six samples per hectare proposed by the SK protocol were not enough to verify a 3 % change of SOC content or stock for either of the two research plots. The relative SOC stock MDD of the full sampling depth was 5.4 % for Kernza™ and 4.8 % for the control when based on the STD of the presented dataset, which misses the 3 % target of the *Perennial* study but could still be low enough, depending on the de facto change in SOC under Kernza™.

However, these are somewhat incorrect assumptions, since the MDD is calculated with inaccurate values of STD. Having conducted the same analysis on the full *Perennial* dataset, a more accurate STD is known, and when the MDD was calculated with these STD values, it resulted in a relative MDD of 7.0 % in Kernza™ and 28.3 % in the control. The high value for the control is once again explained by point 16, which was by chance not included in the SK dataset but increased the *Perennial* STD value. While a 7.0 % increase in SOC stock is not impossible under Kernza™ (as shown by (de Oliveira et al., 2020)), a 28.3 % increase is unlikely. The large difference between the plots once again prevents reliable comparative conclusions.

The large difference in relative MDD depending on which STD is used is in agreement with a statement made by Stanley et al. (2023): *"If the MRV protocol does not require determining the number of samples necessary to detect a reasonable level of SOC sequestration, it could fail to reward legitimate sequestration or have a large chance of erroneously rewarding non-existent sequestration."* In this scenario, the SK protocol is at risk of doing this.

This leads to another question: How correct are the SK estimations of SOC stock and MDD? The uncertainty of the SK approach is increased by the probability element, while the underlying uncertainty due to sampling or analytical error is the same as for the *Perennial* approach. The outcome of the SK SOC stock calculation is highly dependent on which sampling points are randomly selected, which highlights one of the main issues of SOC measurements: Spatial heterogeneity. When a field sampling effort only includes a handful of datapoints that are randomly selected, it is impossible to know whether the values are representative of the field.

While the mean SOC content and SOC stock is underestimated with the SK approach in this study, the selection of sampling points was random and only one scenario was analysed. While the SK approach in this case resulted in an underestimation of the mean SOC content and stock, as well as and underestimation of SOC content STD and consequently also the MDD, this only reflects one out of many possible selection scenarios. In order to quantify the uncertainty as well as further evaluate the performance of the SK sampling protocol, a deeper analysis of probability and other possible scenarios should be made.

In this study, the number of samples per hectare proposed by the SK protocol is not enough to verify their standard rate of SOC sequestration of 0.3 Mg SOC ha^{-1} yr^{-1}. Assuming this standard rate, it would take 31 years to verify a single years' worth of SOC sequestration in the Kernza™ plot and 112 years in the control plot, based on the more accurate *Perennial* STD. If the result of the control plot is disregarded due to point 16, the 31 years for Kernza™ is still 30 years longer than required. Even with the low *Perennial* MDD of the Kernza™ field based on 37 samples, it would take 11 years to detect a SOC stock increase of 0.3 Mg SOC ha^{-1} yr^{-1}. It can

be concluded that while it is a conservative value – which is a good thing, as it lowers the risk of inaccurate claims of SOC having been sequestered –, it is not realistically measurable during the standard 5 years of contract that SK offers. Increasing the required sample size in the protocol to better capture the true mean and STD of SOC content is therefore recommended, even though it comes with additional costs.

However, the Svensk Kolinlagring soil sampling protocol is not used to verify SOC sequestration, nor is it claimed to be able to. The purpose of the physical soil sampling is rather to gain understanding of the SOC stocks and collect data, while the GHG offsets are generated from standardised values and modelling. Still, their protocol does not meet the requirements stated by many carbon farming critics, including Paul et al. (2023), Stanley et al. (2023) and Demenois et al. (2021). Carbon farming schemes must have a rigorous protocol for verification of SOC sequestration in order to fulfil their purpose of GHG offsets and climate change mitigation. If carbon credits are sold off unverified SOC sequestration, there is a serious risk of undermining the credibility of carbon credits as well as over- or underestimation of GHG offsetting. The protocol does however follow or exceed the guidelines proposed by FAO (2020).

While there is dire need for climate mitigation as well as improved soil health, there is substantial criticism on carbon farming, both existing protocols and as a concept, for not being able to meet the required criteria in terms of verification, permanence and additionality (Moinet et al., 2023; Paul et al., 2023; Stanley et al., 2023). Some issues regarding measurement and verification have been shown also in this study. Moinet et al. (2023) promote a soil-smart agriculture, rather than climate-smart, based on the inconsistent effects of carbon farming on SOC in soils, viewing the climate benefits of SOC sequestration as co-benefits to improved soil health. However, without economic incentives for farmers, it is unsure that sufficient action for soil health will be taken. This was shown by e.g. Demenois et al. (2021), as French farmers were able to sequester 12 times more carbon with economic incentives such as carbon farming (Demenois et al., 2021).

Worth mentioning is that this simulation of the SK protocol only accounted for sampling design (i.e., the number and distribution of samples). The comparison of BD and SOC analysis methods were not compared in this study. The simulation is rather an investigation of whether the stratified random approach can reduce the required number of samples and whether the SK protocol would be able to verify their standard value of SOC sequestration on the Alnarp research site. In the end this was not possible, which was also shown by the stratification analysis discussed in the next paragraph.

5.7 Stratification

Neither the strata generated by SK or from SPROD data showed any correlations between strata and SOC content in the 0-30 cm depth, as the Kruskal Wallis H value was below the critical cut-off value of 5.991 in all cases. For the SK stratification, the value was higher for the control plot, while it was the opposite for the SPROD stratification. It is possible that the SPROD strata could have had higher correlation with SOC content, if another classification method (e.g., some machine learning approach) had been used than the predefined ranges used in this study.

It has been shown that stratification is a useful tool for reducing the number of samples – if the process is straight forward and if the covariates have a strong correlation with SOC content (Donovan, 2013; Stanley et al., 2023). In this study, while the process of creating strata from SPROD data was straight forward and easy to repeat, there was no correlation between strata

and SOC content. In other words, none of the conditions in the background (formulated by Donovan (2013)) were met. It is possible that the SPROD values are better explained by the N content, which could readably be investigated since there is N data available for all sampling points.

The research site in Alnarp is a rather homogenous field with little elevation difference (<5 m), and similar recent land management history across the field. While there are a few different soil types within the field and obvious variations in grain size were observed while field sampling, the field is still very homogenous in a broader context. It is likely that stratified random sampling is more useful in more large-scale studies, or on more heterogenous fields – or based on different covariates. At the spatial extent of single fields – based on the results of this study – the stratified random sampling design used by Svensk Kolinlagring is less effective than the *Perennial* systematic grid sampling. It does however come with a significantly lower cost, as extensive soil sampling is notoriously cost- and time-consuming (Paul et al., 2023). While the *Perennial* protocol yielded more accurate results, the number of samples was sixfold.

When the required number of samples was recalculated from the known mean and STD of the full sampling depth and a target MDD of 3 %, 237 samples were needed for the control plot (point 16 included) while only 28 samples were needed for Kernza™. Thus, a much more efficient strategy for reducing the number of samples than stratification would be to make an informed decision regarding the inclusion of point 16. Further, since two sampling points were missing from the dataset at the time of analysis (10A and 11A) and an incorrect value (61A) was used, it is recommended to recalculate these results. The difference is however expected to be rather small, as the corrected values of all three sampling locations were similar to the mean SOC content.

When studying historical orthophotos and maps of the area, it appeared there was a potential relationship between SOC content and hydrology as well as topography, as previously mentioned regarding point 16. On soil moisture maps as well as elevation maps, there appeared to be similar tendencies as for the SOC content. Therefore, this could be an interesting option for further study on stratification covariates.

5.8 Limitations and future research

Several limitations of this study have already been presented. To conclude, the main issues of this study include but are not limited to: The similar magnitude of MDD and uncertainty (due to sampling and lab analysis), the unknown source of the SOC content uncertainty, the large difference in MDD between the Kernza™ and control plot and the fact that only one SK protocol simulation scenario was analysed. While sampling point 16 causes difficulties regarding the comparability of the two research plots, it also efficiently highlights one of the main hurdles of SOC monitoring, which is spatial heterogeneity.

Apart from the further research already suggested, it would also be interesting to look at the relationship between SOC and nitrogen content as well as tendencies regarding the hydrology and topography. Data on SIC and N are available for all sampling locations.

Future interesting research most of all involve the continuation of the *Perennial* research project. Gaining understanding of SOC dynamics under perennial crops like Kernza™ through comparison of conventional annual cropping systems is highly relevant, leading the way for a more sustainable future of agriculture as well as climate change mitigation (Crews et al., 2018).

6 Conclusions

The main purpose of this MSc thesis has been fulfilled: Determining the SOC stock of the Alnarp research plots, serving as a baseline measurement for the *Capturing Carbon in Perennial Systems* research project. The SOC stock of the Alnarp research sites was determined to 136.76 Mg SOC ha^{-1} for Kernza™ and 150.06 Mg SOC ha^{-1} for the control, with a mean SOC stock of 13.68 kg SOC m^{-3} (Kernza™) and 15.01 SOC m^{-3} (control) (table 12). The mean SOC content decreased with depth in both plots, with 1.877 (A), 0.710 (B) and 0.260 (C) g SOC g $soil^{-1}$ for Kernza™ and 1.745 (A), 0.826 (B) and 0.372 (C) g SOC g $soil^{-1}$ for the control (table 10). Bulk density also decreased with depth.

The relative MDD of the SOC stock was 2.58 % for Kernza™ and 9.04 % for control, regarding the full 1 m sampling depth (table 10). When the outlying point 16 was excluded, the relative MDD for the control plot was lower, at 5.09 %. The absolute MDD was 3.53 Mg SOC ha^{-1} for Kernza™ and 13.57 Mg SOC ha^{-1} for the control (6.82 Mg SOC ha^{-1} without point 16). Whether this is sufficient depends on the de facto change in SOC stock over the course of the experiment, as previous research is both limited and in disagreement. The target relative MDD of 3 % was reached for Kernza™, but not for the control plot.

Spatial heterogeneity is often named as the main culprit in detecting SOC stock change. This became apparent in this study, as one single outlier (point 16) caused substantial differences in SOC content and stock as well as MDD between the two research plots. This could prove problematic, since any SOC change under Kernza™ will be verified against the control during the *Perennial* research project. However, by further investigating the role of topography and hydrology as well as previous land management, point 16 could possibly be disregarded in future studies.

In this study, variability and uncertainty of lab assays proved to be as much of an issue as spatial heterogeneity for reliable and accurate detection of SOC change, as the uncertainty due to sampling error and variability was of similar magnitude as the MDD. While the BD uncertainty could primarily be reduced through more careful field sampling and more samples, it was unclear how much of the uncertainty of SOC content could be attributed to insufficient homogenisation and to variability of the removal of SIC through acidification.

The relative MDD of the SOC stock when calculated with the Svensk Kolinlagring protocol and the more accurate STD of SOC of the *Perennial* protocol was 7.0 % for Kernza™ and 28.3 % for control. While an increase in SOC stock of 7.0 % is not impossible, 28.3 % is unlikely – although this value can be reduced if point 16 can be excluded. However, the uncertainty of the SK protocol is higher compared to the *Perennial* protocol, due to the probability element of the random sampling point selection. This has not been quantified.

Neither the Svensk Kolinlagring stratification nor the SPROD (Seasonal Productivity) stratification proved effective in terms of reducing the required number of samples, as there were no significant differences in SOC content between the different strata in either case. In order to increase accuracy and decrease the risk of incorrectly assumed carbon offsets, a larger number of required samples is recommended for the Svensk Kolinlagring sampling protocol, preferably based on the STD of SOC within the field. While there is a high risk of both under- and overestimation of SOC sequestration with the use of currently available carbon farming sampling protocols, including that of Svensk Kolinlagring, there is an urgent need for increased soil carbon sequestration regardless of incentive.

This study was mainly in agreement with previous research – in order to reliable detect SOC stock change in short time frames, a large number of samples is indeed required. There are many hurdles involved in accurate measurement of SOC stock change, including spatial heterogeneity and variability of lab assays. While stratification can reduce this number in some scenarios, it is ineffective in others. Current carbon farming protocols, including Svensk Kolinlagring, should increase the required number of samples to increase the statistical certainty. If the sampling protocol is not adapted to fit the research site, there is risk of incorrect assumptions regarding the sequestration of SOC and false GHG offsets.

The outcome of the *Perennial* research project is highly interesting for the future of agriculture and the carbon farming market alike. More studies are needed in order to gain understanding of the effect on SOC dynamics of Kernza™. Perennial crops like Kernza™, tick several boxes of carbon sequestering farming management practice, but whether this will prove true in the Alnarp context remains to be seen.

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