Sveriges lantbruksuniversitet
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Tea time for soils

– Decomposition experiments in Swedish long-term field trials

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– decomposition experiments in Swedish long-term field trials

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Abstract

Soils represent a large carbon pool, with almost twice the amount contained in living plant biomass and the atmosphere combined. Consequently, soil has a significant impact on the global C cycle and it is suggested that soil organic C (SOC) sequestration is one of the most cost-effective alternatives to counteract climate change.

The literature was reviewed regarding the influence of abiotic and biotic factors on SOC dynamics, and how SOC stocks vary with management practices and cropping systems. I also screened the literature for different methodologies to study carbon and litter decomposition. I decided to use the Tea bag index (TBI) approach. The TBI uses two types of commercially available tea bags, and characterizes decomposition dynamics in terms of two parameters, the decomposition rate (k) and stabilization factor (S).

I hypothesized that the TBI would be sensitive enough to quantify the impact of common agricultural practices on litter decomposition under a wide range of pedo-climatic conditions. I selected treatments from 13 long-term field trials that allowed me to compare: perennial forage versus annual crops ($N = 4$), fertilized versus unfertilized fields ($N = 6$) and different tillage practices ($N = 3$).

The results showed that the two TBI parameters were sensitive to both management practices and cropping systems. Fertilized plots showed higher stabilization (S) than unfertilized plots, but there were no differences on decomposition rates (k). The effect of different tillage practices on k and S were variable across sites and treatments, although ploughing tended to result in lower decomposition rates, compared to direct drilling or shallow cultivation treatments. The TBI was most sensitive when comparing perennial forage versus annual cropping systems, where the measurements for forage generally indicated higher S values and lower k values. The results also showed that climate, through precipitation and air temperature, had a large impact on stabilization ($R^2 = 0.33$), while its effect on decomposition rates was limited. The decomposition rates were found to be significantly affected by soil properties such as clay content ($R^2 = 0.13$) and soil C/N-ratios ($R^2 = 0.19$). It can be concluded that the TBI approach is a useful tool to characterize decomposition dynamics and to identify climate-smart agricultural management practicies under different pedo-climatic conditions.

Sammanfattning

Marken är ett stort, och viktigt, kollager då det innehåller nästan dubbelt så mycket kol som atmosfären och den levande biomassan tillsammans. Därmed spelar marken också en signifikant roll i den globala kolcykeln där det organiska kolets inbindning i marken (SOC) föreslås vara ett av de mest kostnadseffektiva alternativen för att bromsa klimatförändringarna. I denna uppsats använder vi oss av Tea bag index (TBI) -metoden för att mäta kolets stabilisering och nedbrytning i ett antal svenska långliggande fältförsök.

Litteraturgenomgången beskriver hur abiotiska och biotiska faktorer påverkar det organiska kolet i marken, och hur detta påverkas av olika brukningsmetoder och odlingssystem. Den behandlar även olika metoder för att studera nedbrytningen av kol och organiskt material.

Vår hypotes var att TBI skulle vara en tillräckligt känslig metod för att se skillnader i kolets dynamik i olika brukningssystem och en rad olika markförhållanden. Vi studerade behandlingar såsom odling av vall jämfört med annuella grödor ($N = 4$), gödslade och ogödslade fält ($N = 6$) och olika kultiveringsmetoder ($N = 3$) i totalt 13 olika långliggande svenska fältförsök.

Resultaten visade att de två parametrarna S och k, erhållna från TBI, var känsliga nog för att märka skillnader i både brukningsmetoder och odlingssystem. Trenden var att stabiliseringen (S) var högre i gödslade led jämfört med ogödslade. Även om några signifikanta skillnader i nedbrytningshastigheten (k) hos dessa inte kunde upptäckas. Effekterna av olika kultiveringsmetoder var varierande både mellan de olika platserna och mellan behandlingarna, men plöjning verkade resultera i lägre nedbrytningshastighet, jämfört med direktsådd och grundare kultivering (5-7 eller 10-12 cm djup). TBI påvisade dock starkast känslighet när etablerade vallar jämfördes med annuella grödor, där resultaten indikerade att vallen genererade högre S och lägre k än motsvarande annuella gröda. Resultaten visade även att klimatet, som en funktion av lufttemperatur och nederbörd, hade stor inverkan på stabiliseringen ($R^2 = 0.33$), medan dess effekt på nedbrytningshastigheten var begränsad. Nedbrytningshastigheten påverkades dock av jordegenskaper som lerhalt ($R^2 = 0.13$) och C/N kvot ($R^2 = 0.19$).

Slutsatsen är att TBI-metoden är ett användbart verktyg för att karaktärisera det organiska markkolets dynamik och för att identifiera klimatsmarta brukningssystem vid olika markförhållanden.

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Abbreviations

C	Carbon
GHGs	Greenhouse gases
ICBM	Introductory Carbon Balance Model
MRT	Mean residence time
SAS	Statistical Analysis Software
SOC	Soil organic carbon
SOM	Soil organic matter
TBI	Tea bag index

1 Introduction

Global warming is of great concern and highly ranked on the political agenda. It depends mainly on increased emissions of greenhouse gases (GHGs) such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) (Schaufler et al., 2010). At the climate meeting in Paris in December, 2015, world leaders agreed to halt the temperature increase due to global warming at a maximum of 2 degrees Celsius (Regeringskansliet, 2015). To reach this agreement, there is a need to significantly decrease GHG-emissions worldwide. Some engineers and researchers are currently even examining possibilities to capture what has been emitted to the atmosphere during the last century. In that regard, soil organic carbon (SOC) is an important pool and any changes therein can affect atmospheric CO_2 concentrations, contributing to mitigate GHG-emissions (Paul, 2015).

Indeed, soils contain more carbon than the living biomass and the atmosphere combined (Fig. 1) and have therefore a central role in the global carbon cycle (Lal, 2004). The SOC content of soils is determined by the balance between inputs and outputs. The inputs come from the annual return to soil of above- and belowground plant residues, as well as from recycled organic materials. The outputs are mainly from the annual decay of SOC as a result of biological activity. When the SOC balance is positive, soils can act as a sink of atmospheric CO_2 (Bolinder et al., 2007b; Kätterer et al., 2012). Increased SOC contents do not only contribute to offset CO_2, it is also primarily essential for the overall quality and productivity of soils (Halvorson et al., 2002; Lal, 2004; Liu et al., 2009).

Historically, most agro-ecosystems have been subject to significant losses (25 to 75%) of SOC since the 1850’s. Mainly because of increased mineralization and soil erosion; this is largely attributed to tillage associated with the conversion of e.g., grassland and forests into agricultural land (Lal, 2013). It should be possible to store more C in soils and it is considered that agro-ecosystems have a carbon sink potential to recapture 50 to 66% of its historical losses. With a global potential of 0.4 to 1.2 Pg of SOC annually, where 1 Pg equals 0.47 ppm in the atmosphere, this is equivalent to an offset by 5 to 15% of CO_2 from yearly global fossil-fuel emissions (Lal, 2004; 2013).

The change in the balance between SOC storage and release depends on several factors, such as soil type, moisture, temperature, farming system and soil management. Since the decomposition of SOC is slow compared to the high background level of SOC stocks, this balance is best studied in long-term experiments where treatment effects have accumulated over decades so that they become measurable (Kätterer et al., 2012). Proposed strategies to increase the soil carbon pool are numerous and include soil restauration, no-till farming, fertilization, cover crops, water management, agroforestry and growing on spare lands (Lal, 2004). In this study, we used the Tea Bag Index (TBI), which is an innovative approach to assess potential drivers of SOC release and litter stabilization (Keuskamp et al., 2013). This was done using several long-term field experiments located across a wide range of pedo-climatic conditions in Sweden representing different management practices.

2 Background

2.1 Soil organic carbon stocks in agro-ecosystems

Agricultural soils in Europe contain typically 2 to 5% soil organic matter (SOM), and this SOM has a carbon (C) content of about 50% (Pribyl, 2010; Merante et al., 2017). The amount of soil organic C (SOC) depends on historical soil forming factors (e.g., type of vegetation, topography) and evolves with abiotic (climate and specific soil properties) and biotic (e.g., microorganisms and earthworms) factors, and is also influenced by current land use and management practices (Lal, 1998; Paul, 2015).

The total amount of SOC in agro-ecosystems is considered the best indicator for soil quality since it influences a large number of physical, biological and chemical soil properties. It is also important for crop productivity because it is a source of major plant nutrients (Carter et al., 1997; Lal, 2013). Furthermore, the total pool of SOC is also significant from a large-scale perspective. There is approximately 2300 Pg of C down to a 2 m depth where 1500 Pg are found down to 1 m depth with as much as 700 Pg in the arable soil layer (<30 cm) (Fig. 1) (Kätterer et al., 2012; Paustian et al., 2016).

The total amount of SOC in the arable layer of agricultural soils is usually divided into at least three different pools, having more or less stable carbon compounds depending both on chemical and physical characteristics. A labile pool with a mean residence time (MRT) less than a few years, a more stable pool with an intermediate MRT and a passive pool with a MRT ranging from centuries to millennia (Stockmann et al., 2013). Generally, 1 to 10% of the total SOC is in the labile pool, consisting of microbial biomass and organic material in early stages of decomposition, most of which is intimately associated with clays (Paul, 2015).

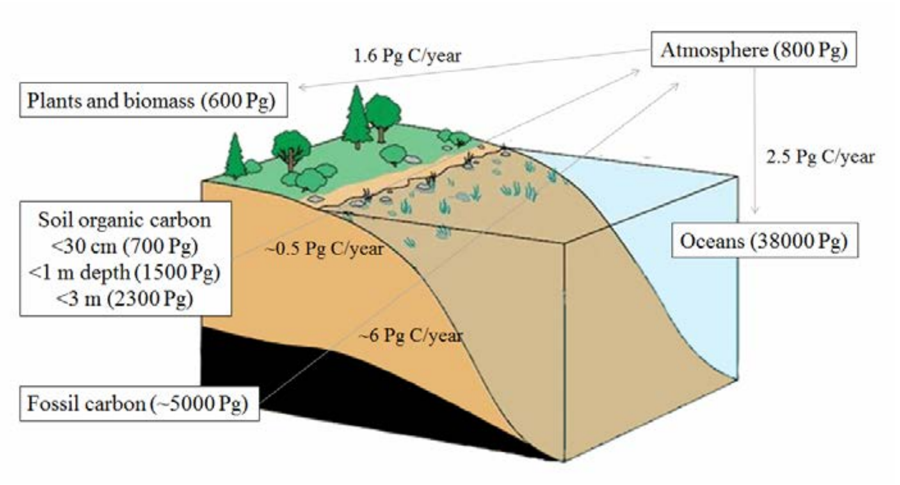

Figure 1. Global carbon stocks and net annual flows ($P=10^{15}$).

The SOC balance in agro-ecosystems is dynamic and determined by carbon inputs and outputs. The inputs come from above-ground post-harvest crop residues (e.g., straw, stubble) and below-ground rhizodeposits derived from roots (Kätterer et al., 2014). Additional sources of inputs come from recycled organic materials such as manures, including composts, sewage sludge and other industrial co-products. A fraction of these annual C inputs enters the SOC pools during the decomposition process; the rest is lost as CO_2. A large proportion of the annual carbon outputs are attributed to the decomposition of SOC stocks already present in soil, equally important are the CO_2 outputs from the decomposition of fresh organic material. Losses through soil erosion can also be important and some leaching of soluble SOC compounds may also occur. The decomposition process is a result of soil biological activity and is influenced by soil climatic conditions and management practices (Fig. 2). Carbon is being sequestered when the organic inputs are greater than the outputs, and through decomposition the former is gradually transformed into more or less stable organic compounds, subsequently stored in the SOC pools with longer MRT (Kätterer et al., 2012; Stockmann et al., 2013). It is considered that SOC sequestration is one of the most cost-effective alternatives to counteract climate change because of its importance for soil productivity and other ecosystem services (Freibauer et al., 2004).

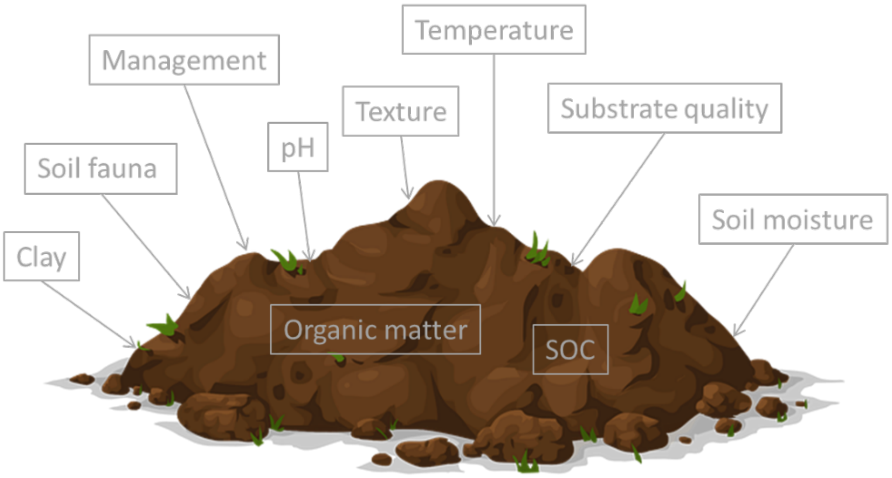

Figure 2. Schematic figure showing factors affecting organic matter and SOC dynamics.

2.2 Abiotic factors and soil organic carbon dynamics

Abiotic factors influence the activity in soils in many ways. Soil biological activity is critically affected by soil properties and climatic driven factors such as soil moisture and temperature (Brockett et al., 2012; Paul, 2015). Fluctuations in temperature and moisture levels influence various processes in the soil that becomes more or less important during different environmental conditions (Paul, 2015). For example, moisture affects pH, which in turn affects enzyme activity and microbial community structure. Knowledge about abiotic factors is therefore crucial for understanding the complex and dynamic decomposition processes in the soil.

2.2.1 Moisture

Soil moisture affects the aeration status, solves materials, influences osmotic pressure and the pH in the soil (Paul, 2015). Water is an agent of transport and acts as a solvent and reactant in important biochemical reactions. It is considered that soil moisture, next to temperature, determines respiration and is therefore one of the most important environmental factors affecting soil biological activity, carbon cycling and productivity in terrestrial ecosystems (Moyano et al., 2012).

The relationship between soil moisture and respiration in ecosystems is variable and influenced by factors including soil type, total pore space and bulk density (Moyano et al., 2012). However, soil respiration is usually well correlated with different soil properties. With a stable temperature, soil moisture has been found to be the dominant environmental factor controlling respiration (Flanagan & Johnson, 2005). Water potential is the best predictor of respiration rates in soil, but measurements of water potential often leads to large errors (Moyano et al., 2012). Orchard & Cook (1983) found a log-linear relationship between microbial activity and water potential, where a decrease in water potential by -0.01 MPa caused a 10% decrease of microbial activity. In a sandy African soil, the water content could explain as much as 75% of the total variation in decomposition (Nhantumbo et al., 2009).

The optimal soil moisture levels for respiration is usually around field capacity (approximately 60% water filled pore space (Schaufler et al., 2010)) and at lower levels there is a decline in respiration (Davidson et al., 2000). In a silt loam, at moisture levels exceeding 0.15 m^3/m^3, corresponding to 42% of field capacity Xu et al. (2004) found indications that moisture was not a limiting factor for respiration. Under this value, respiration steadily decreased while soil moisture decreased depending on osmotic stress. High soil moisture can decrease respiration due to lower aeration (Davidson et al., 2000; Schaufler et al., 2010). Different communities of microorganisms and microfauna are active at different water potentials (Paul, 2015). For example, at water potentials near field capacity (water films ≥5 μm) protozoa are active. Microorganisms can be active at even lower water potentials.

Soil microbial biomass and respiration was found to be highly correlated with mean annual precipitation (Liu et al., 2009; Brockett et al., 2012). Generally, wetter climates show higher decomposition rates than dryer ecosystems (Portillo-Estrada et al., 2016). Liu et al., (2009) observed that increases in respiration due to increased soil moisture could derive from stimulated crop growth, and thereby higher allocation of plant-derived carbon to the soil through root-exudates and above-ground plant litter.

In dryer climates, the biological activity is more sensitive for rainfall pulses, and sudden increases in respiration and decomposition after rainfall can be observed. This is suggested to depend on peaks in biological activity (within an hour after rainfall) following improved living conditions for the microbes (Xu et al., 2004).

2.2.2 Temperature

Temperature influences biological, chemical and physical soil properties and is seen as the most important environmental factor for processes in soil (Paul, 2015). Temperature is, besides the physical and chemical properties of the carbon substrate, the determinant of the activation energy needed in enzymatic reactions in the soil (Davidson & Janssens, 2006). Generally, cooler climates lead to decreased soil biologic activity and decreased decomposition compared to warmer climates (Bolinder et al., 2007a).

However, there is still no scientific consensus about how exactly the decomposition of organic matter depends on temperature, and the lack of consensus seems to derive from differences in experimental conditions, as well as the influence of other confounding factors (Kirschbaum, 2006). Giardina & Ryan (2000) found that SOC decomposition rates in forest soils over a global mean annual temperature gradient where "remarkably constant", suggesting that microbial activity and decomposition was not controlled by temperature and that increased temperatures alone could not stimulate increased decomposition. Nevertheless, Schaufler et al. (2010) observed an exponential increase in CO_2 emissions with increased temperatures up to 40 degrees, and Kirschbaum (1995) explained that an increase by 10 ˚C often doubled the microbial activity. Liu et al. (2009) found that warmer climates with no limiting moisture content increased the decomposition rates, but at low moisture levels and elevated temperatures respiration would not be stimulated due to water stress and decreased plant growth.

Lefèvre et al. (2014) found a relation between SOC stability and temperature sensitivity and correlated it with biological activation energy, where more energy is needed to break down more stable C-structures. Earlier conclusions by Reichstein et al. (2005) declared that it was "premature to conclude that stable carbon is more sensitive to temperature than labile organic matter", based on the results from empirical studies. Haddix et al. (2011) describes that some researchers have found labile SOC to be more temperature sensitive, while others have found that stable SOC is the most temperature sensitive, and some studies have indicated that the sensitivity of different SOC fractions to temperature are the same, regardless of their stability. Davidson & Janssens, (2006) argued that dividing SOM into pools based on temperature sensitivity was too simplistic, pointing out the complexity and the many other factors affecting the decomposition of SOC.

Soil temperature is significantly positively correlated with air temperature during spring and summer, but not in autumn and winter (Qian et al., 2011). Between 1958 and 2008 a warming trend of 0.26-0.30 ˚C/decade could be seen at soil depths from 5 to 150 cm, associated to the warming trend in air temperatures during the same period. Whether decomposition of SOC will increase or decrease because of global warming has been a key question in climate change research for a long time, and most studies indicates that SOC would decrease seriously with global warming (Kirschbaum, 1995).

2.2.3 pH

Soil pH is derived by the proton supply from atmospheric and organic sources and their reactions with bases in the soil (Paul, 2015). High concentrations of protons lower the pH (acidic) and low concentrations increase the pH (basic).

The pH affects both solubility and ionization of elements in the soil solution and has an influence on other factors affecting microbial activity in soil (Paul, 2015). The acidity of a soil indicates the availability of nutrients and toxicity which can be estimated due to its correlations with pH (Thomas, 1996). Soil pH also correlates with soil microbial community structure (Brockett et al., 2012). Decomposition of organic material has some influence on pH since decarboxylation (removal of C-atoms from C-chains) of organic anions such as amino acids and carbohydrates leads to increased pH (Yan et al., 1996).

Decomposition and biological activity in the soil in general, and thereby the decomposition, tends to decline with a decrease in pH. This makes pH a strong predictor of soil carbon content (Poeplau et al., 2015a). Rousk et al. (2010) examined soil pH's influence on phospholipid fatty acids (PLFA), which is an indirect measure of microbial biomass, over a decreasing pH gradient (pH 8.3 to 4.5) but could not find any significant effects on total concentration of PLFAs. However, the composition of the microbial community was clearly affected. Nevertheless, when estimating microbial soil biomass through substrate induced respiration, the biomass decreased by approximately 25% over a declining pH gradient.

2.2.4 Texture and soils

The soil environments are the most complex habitats in the world. Soil properties such as texture, aeration, structure, nutrients and carbon content influence all living organisms in different ways, making soil habitats very variable. The soil texture is a fundamental soil property affecting formation of soil structure, water characteristics and nutrient retention. Soil structure has a large influence on several processes that occurs in the soil, such as root development, water retention, cycling of nutrients and dispersal of chemicals (Paul, 2015). In general, the decomposition rate of SOC in agricultural soils is in the order of magnitude between 0.5 to 2.0%, annually, with the lower rates in fine-textured soils.

The effect of texture on the water availability influence both production and decomposition (Schimel et al., 1994). In particular, clay has a large impact on moisture-respiration relationships (Moyano et al., 2012). Soil carbon content is also generally linearly correlated to soil texture, increasing as clay content increase (Schimel et al., 1994). This is supposed to be caused by the larger specific surface area of clay, affecting water retention, availability (Moyano et al., 2012), stabilization (Jindaluang et al., 2013) and physical protection through aggregates (Schimel et al., 1994). Silt and sand has a lower impact on respiration and soil carbon levels (Moyano et al., 2012).

Furthermore, soil texture influences the ability of soil fauna to graze on the microbes (Hassink, 1994) and this indirectly affects the microbial species composition and function (Brockett et al., 2012).

2.2.5 Substrate quality

The decomposition of carbon also depends on substrate quality and its accessibility (Karberg et al., 2008). Substrate quality addresses properties such as nutrient concentration, physical structure, chemical properties and toxicity. Substrates containing labile compounds (e.g., sugars, amino acids) decompose faster than stable compounds. Stable (recalcitrant) compounds (e.g., lignin, chitin) are more difficult for the microorganisms to eat and digest, leading to slower decomposition (Karberg et al., 2008).

The ratio between carbon and nitrogen, C/N-ratio, in the organic material are one of the most important predictors for microbial activity in the early stages of decomposition. When the carbon content is high, relative to nitrogen, fungi are driving the decomposition and immobilizes the nitrogen. On the contrary, when the C/N-ratio is low, bacteria are favored and a fast-paced mineralization occurs (Paul, 2015).

2.3 Biotic factors and soil organic carbon dynamics

Soil biota plays a central role in soil processes, influencing nutrient cycling, availability, and decomposition (Brockett et al., 2012). Soil biota refers to the complete living community in a soil system. It consists of e.g. microorganisms, nematodes, microarthrophods, enchyatreids and earthworms (Paul, 2015). Numerous species of macrofauna can be found in the litter layer and at the soil surface, e.g. arthropods, diplopods, chilipods and snails. All organic material, sooner or later, passes through the microbial biomass (Hassink, 1994).

Soil microbial biomass consists of prokaryotes (bacteria, archea) and fungi. Prokaryotes hold as much C as plants, and 10 times more N, and play important roles in different metabolic pathways in the soil. This makes prokaryotes essential in global biogeochemical cycles and ecosystem functions (Paul, 2015). Fungi degrade organic material, forms mycorrhiza with plants which enables higher growth rates and increased production and redistributes nutrients and water in the soil (Paul, 2015). Their growth and cell wall formation leads to aggregation and stabilization, and fungal biomass turnover plays an important role in C-cycling and sequestration.

Soil fauna, the animals in the soil, facilitate bacterial and fungal activity and are involved in nutrient cycling through feeding on plants, organic substrates and living or dead biota. Their processing of organic material enables greater access by microbes which accelerate decomposition (Paul, 2015). The important bioengineers, earthworms, make nutrients more accessible, improve soil structure, promote aggregation, and increase water infiltration and plant root penetration through their burrows, and also incorporate organic matter into the soil (Rasmussen, 1999).

2.4 Management factors and soil organic carbon

Soil organic matter management is important both for the farmer and for the climate because it influences soil fertility and the global C cycling in the biosphere. In the farmer’s daily life perspective, it controls the release of nutrients in the soil, improves water holding capacity and the workability of the soil, and ultimately, the yield (Loveland & Webb, 2003). The amount of SOC stocks in agricultural soils is dependent on management techniques that affect the long-term evolution. These management techniques influence SOC stocks either by their effect on the amount of C inputs to soil, or on the outputs by a modification of the SOC decomposition rates or soil erosion (Wösten & Kuikman, 2014). For example, management techniques that increase crop yields also implies that the annual amount of C inputs to soil from crop residue increase, while soil disturbance through tillage may have an effect on SOC decomposition rates, and permanent soil cover reduces soil erosion.

2.4.1 Cropping systems

The type of cropping system has a large impact on the potential of SOC storage. Keeping the land covered is the most important strategy to maintain or increase SOC storage. The crop captures carbon from the air, while bare fallow may increase decomposition and exposes the soil to wind and water erosion processes (Kätterer et al., 2012).

The amount of photosynthetically fixed C that is potentially returned to soil as crop residues is proportionally correlated to the agronomic yield (Kätterer et al., 2013; Poeplau et al., 2015b). The applications of amendments (e.g., manures, composts) also have positive effects on SOC contents (Kätterer et al., 2014). In a meta-analysis, Ladha et al. (2011) showed that adding organic amendments increased SOM contents by 37%.

Grasslands generally have higher SOC levels than soils with annual crops (Wiesmeier et al., 2012). SOC storage increase with the proportion of ley in the rotations due to its higher below-ground biomass (Poeplau et al., 2015a) and annual C inputs to soil from the extra-root component (i.e., exudates and fine-root turnover) (Bolinder et al., 2007b). For the same reason, Poeplau et al. (2015c) found that cover crops such as ryegrass also lead to increased C input to the soil. Adding one or more crops to a monoculture crop rotation increases SOC by 3.6% (McDaniel et al., 2014). Kätterer et al. (2013) investigated differences between forage-based crop rotations and exclusively annual cropping systems, concluding that the forage-based crop rotations, on average, retained 0.52 Mg ha^{-1} yr^{-1} more carbon to the soil.

The incorporation of crop residues can also contribute to increased SOC levels and is thereby important for maintaining SOC (Kätterer et al., 2013; Lehtinen et al., 2014). This contribution depends on the type of crop residues. For example, Lupwayi et al. (2004) found that incorporation of grass compared to straw decomposed faster. They also observed that almost 75% of clover residues decomposed during one season, while only roughly 25% of wheat residues decomposed during the same time period. However, the amount remaining after 12 months did not differ much between the different types of crop residues.

2.4.2 Nutrient management

Kätterer et al. (2012) showed that nitrogen fertilization increased SOC stocks by 1.5 to 2.1 kg C per kg N applied in Swedish arable land, where combinations with organic amendments lead to the higher numbers. Conclusions based on data from 135 studies, showed that synthetic N-fertilization increased SOC by 8% (Ladha et al., 2011). The positive effect on SOC from synthetic fertilizers is explained by higher return of above- and belowground residues. However, an American study could not show a general increase in SOC sequestration from N-fertilization (Halvorson et al., 2002). Grandy et al. (2013) also discusses that, even though N-fertilizing increase crop residue inputs, it also alters decomposition dynamics. Nitrogen application significantly accelerates decomposition of labile soil carbon fractions, while promoting stabilization in stable, mineral-associated fractions (Neff et al., 2002).

When discussing N-fertilizing as a way to sequestrate more C into the soil as a carbon sink, Schlesinger (2000) highlights the fact that CO_2 emissions from the production of fertilizers may lead to GHG-emissions that sometimes outnumbers the sequestration.

2.4.3 Tillage practices

Soil tillage is mainly performed to incorporate crop residues and to prepare the soil for appropriate seeding conditions. It affects many soil processes such as the availability and circulation of nutrients, water infiltration, soil structure and aggregate stability. Tillage influences the dynamics of SOC decomposition by the exposure and disruption of aggregates, and also modifies the distribution of organic material in the soil profile (Paul, 2015).

Generally, decomposition rates are slower when residues are left on the surface, compared to when they are incorporated into the soil (Paul, 2015). In a Swedish experiment, however, the conclusions where that incorporation of residues into the deeper soil layers inhibited their decomposition, due to reduced access to water and lower temperatures (Kainiemi et al., 2014).

Conventional tillage leads to more residues being incorporated and a higher surface area becomes available for microbial degradation. No tillage, on the other hand, leaves the residues on the soil surface, leading to lower evapotranspiration, higher water content in the upper soil layers, lower soil temperatures and more stable soil aggregates, ending up in less accessibility for microbes (Rasmussen, 1999). In no tillage systems, decomposition is therefore more affected by atmospheric conditions.

With numerous reviews on the subject, the effect of tillage on SOC stocks is an issue that has been, and still is widely debated in the scientific literature. For instance, in a study on carbon sequestration under different cultivation techniques in the US, Halvorson et al. (2002) found that no tillage systems annually sequestrated much more carbon (233 kg C ha^{-1}) compared to reduced tillage (25 kg C ha^{-1}), while losses occurred with conventional tillage (-141 kg C ha^{-1}). In a commentary on what cumulated knowledge allows us to conclude about the effect of tillage on SOC sequestration by Baker et al. (2007), the analysis suggested that there was no compelling evidence that reduced tillage promoted C to the soil over conventional tillage. These results were derived from studies where samplings exceeded 30 cm depth, suggesting that cultivation technique mostly affected the distribution of carbon in the soil profile, not total carbon loss. In another summary assessment based on published papers on the subject, Kätterer et al. (2012) found that possibilities in decreasing CO_2 emissions through reduced tillage under Nordic conditions were likely quite limited.

2.5 Methodology for decomposition experiments

Decomposition experiments of organic materials in situ are important for understanding nutrient cycling and colonization by soil biota under field conditions. The most common methodology uses litterbags, where the mass loss (or nutrient loss) through time allows researchers to determine the decomposition dynamics of various organic materials (Kampichler & Bruckner, 2009). These types of measurements can also provide complementary information in different parameters used in the SOC simulation models. For example, in the ICBM model that was developed on the SOC data from a long-term field experiment at Uppsala (Andrén & Kätterer, 1997), the decay rate of the labile ("young") SOC pool was based on litter (from straw and roots) bag studies (e.g., Andren & Paustian, 1987; Paustian et al., 1990).

2.5.1 Litterbag experiments

Litterbag studies, where known quantities of organic material are left to decompose, and thereafter retrieved to determine mass loss over time are used to study nutrient cycling and decomposition. It has been a standard procedure used in soil ecology for more than 50 years (Burgess et al., 2002; Kampichler & Bruckner, 2009). Litterbags are useful due to the fact that they enable to study decomposition dynamics under field conditions. The litterbags can be filled with different types of organic material, such as leaves, straw or more complex mixes of organic materials, depending on research topic. Fresh litter of only one species is often used, but a mixture of materials can be used when more realistic experiments are desired (Karberg et al., 2008). The methodology can also be used to investigate the dynamics of decomposition and nutrient cycling through time (i.e., litterbags are retrieved periodically), at different locations or in space (e.g., depth).

Swift et al. (1979) defined three groups of soil animals by their body width, microfauna (< 100 µm), mesofauna 0.1 to 2 mm) and macrofauna (> 2 mm). Through alternation of the mesh-size of the litterbags, researchers therefore can restrict or permit different types of soil fauna from entering the bag. This can be done for optimizing the decomposition or determination of different soil fauna's contribution to the mass loss (Kampichler & Bruckner, 2009). A mesh size of 1-2 mm is the most common.

In order to compare pre- and post-decomposition mass, collected litterbags are often oven dried. Litter decomposition is then estimated through regression equations, using the remaining mass over time to estimate a decomposition rate constant, k (Karberg et al., 2008). Nevertheless, Kampichler & Bruckner (2009) argues that there is a lack of uniformity and comparability between different studies, due to the lack of a standardized analytical protocol and different ways to report the results.

Litterbag studies also have some disadvantages, such as excluding certain organisms and thereby lowering or alter decomposition compared to real conditions (Karberg et al., 2008; Kampichler & Bruckner, 2009). Furthermore, placement of the bags may alter the microclimate or decomposition conditions, and a homogenous mixture of litter is needed in comparative studies (Karberg et al., 2008).

2.5.2 Tea bag index (TBI)

Keuskamp et al., (2013) introduced a new and innovative methodology for studying the decomposition of organic materials *in situ*. The approach is based on the simultaneous use of two organic materials with different decomposability. Teabags are used as litterbags and the approach has the benefits of being a cost-effective and well-standardized method that can be used all over the world. Compared to other litterbags, it does not only gather data to characterize decomposition rates but also allows an estimation of litter stabilization. The teabags (manufactured by Lipton) have different contents, one is Rooibos tea (cortex) and the other is Green tea (leaves). Sarnell[1] explained that Green tea contains more water-soluble carbon and has a C/N-ratio of about 15, while Rooibos tea, with a C/N-ratio of 40, is more recalcitrant and decomposes slower. The tea bags are non-degradable tetrahedron-shaped with a mesh size of 0.25 mm, allowing microorganisms and smaller mesofauna to enter, but excludes macrofauna.

The tea bags are buried at different locations and recovered after 90 days. They are thereafter dried and weighted and a Tea bag index (TBI), based on the parameters of decomposition rate (k) and litter stabilization factor (S) are derived. The parameters comprising TBI, k and S, are estimators to characterize and compare carbon decomposition, where k is a measurement of short-term effects and S long-term effects (Keuskamp et al., 2013). TBI is responsive to differences in abiotic conditions such as soil moisture and temperature, and the results are in accordance to expectations based on literature about the decomposition process (Keuskamp et al., 2013). According to Sarneel[1], the decomposition rate is not influenced by temperature, while the litter stabilization factor is. The litter stabilization factor S is also more sensitive to soil environmental conditions and differences induced by e.g., tillage treatments can give different outcomes. They also clarified that S showed the strongest

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[1] Judith Sarneel, Researcher at Umeå University and Utrecht University, 2016-02-26, 1st Tea Bag Index (TBI) Workshop.

response. The method has also been confirmed to be sensitive and robust enough to distinguish between different ecosystems and biomes. The TBI is suggested to increase the resolution of decomposition measurements, and can also serve as a reference data point to other decomposition experiments (Keuskamp et al., 2013).

Duddigan[2] found that in vitro experiments showed fast initial decomposition of Green tea that levelled off after 40-60 days. Rooibos tea showed much slower decomposition and started to level off only in the end of the experiment. Higher temperatures lead to faster decomposition. These results were also retrieved from this thesis data (Fig. 3).

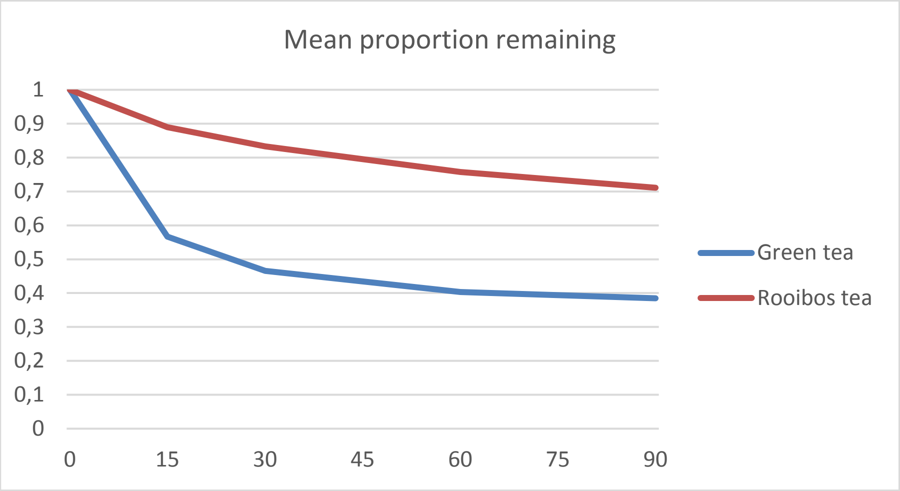

Figure 3. Mean proportion remaining in the ”in situ” experiments performed in this thesis.

In an investigation on the chemical transformations during degradation with the use of spectroscopy, Duddigan[3] observed that in Green tea, aromatic C-O and carbohydrates was reduced after decomposition, while non-polar alkyl groups where increased. In Rooibos tea, on the other hand, carbohydrates and molecules in the O-C-O aromatic spectroscopy region were degraded. The rapid total mass loss in Green tea was suggested to depend on the quick degradation of aromatic C-O, while carbohydrates only showed a steady slope over time. Total carbon decreased fast in the first two weeks in Green tea, and more gradually in Rooibos tea. Nitrogen also decreased rapidly in Green tea, but increased in Rooibos tea due to influx through transport by microorganisms.

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[2] Sarah Duddigan, Postgraduate Research Student at University of Reading, 2016-02-26, 1st Tea Bag Index (TBI) Workshop.

[3] Sarah Duddigan, Postgraduate Research Student at University of Reading, 2016-02-26, 1st Tea Bag Index (TBI) Workshop.

With the results from another study Hefting[4] discussed that the analysis of TBI data was dependent on accurate assumptions. For example, the analysis was only appropriate when a mass loss of maximum 50% had occurred. In practice, this means that an incubation time of three months is reasonable, while a period of six months is too long, making the results less meaningful. Furthermore, tea bags of Rooibos were sometimes destroyed in the field because they were lost to mice and other animals due to the attractive odor. It was also found that the results obtained with Green tea differed by brand (Rooibos tea did not).

Even though the Tea bag method can give appropriate data on decomposition rates and stabilization factors, it cannot substitute the precision and thoroughness of conventional litter bag methods (Keuskamp et al., 2013).

2.6 Objectives

The objective of this thesis was to investigate if different management practices influences *S* and *k* in the soil. We hypothesized that:

- The Tea Bag index approach is sufficiently sensitive to determine the effect of climate, management and soil properties on C dynamics
- *S* is higher under colder and dryer climatic conditions, while *k* is the opposite
- Compared to annual crops, growing forage leads to higher stabilization
- Ploughing has a negative effect on stabilization and SOC and leads to higher decomposition rates than reduced and no tillage systems
- Soil fertility and nutrient management with high inputs increase stabilization
- Soil properties such as pH, C/N ratio and clay content influence both decomposition rates and stabilization

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[4] Mariet Hefting, Assistant professor, University of Utrecht. 2016-02-26. 1st Tea Bag Index (TBI) Workshop.

3 Materials and methods

3.1 In situ experiments with TBI

The experiment used two different types of tea manufactured by Lipton; Green tea Sencha (EAN: 8722700055525) and Rooibos tea (EAN: 8722700188438). The pyramid shaped teabags, were put in the soil at different locations in Swedish long-term field experiments together with temperature logging probes (iButton, manufactured by Maxim). After some minor modifications, the standard protocol for TBI (Appendix 1) was used setting up the experiment, resulting in the following methodology.

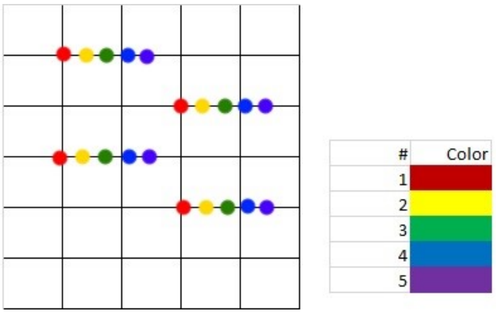
The initial weight of the content in the tea bags and all its components where determined on 20 bags of Green tea and 20 bags of Rooibos tea randomly selected from different boxes and weighed separately after drying in 70^{o}C for 48 hours. Results are shown in Table 1.

Table 1. *Mean initial weight and standard deviation of Green tea and Rooibos tea bag contents.*

Content	Mean weight	Standard deviation
Green tea	1.7174 g	0.0478
Rooibos tea	1.8348 g	0.0270

For each date of measurements in the experimental plots, four tea bags of Green tea, and four bag of Rooibos tea where used per replicate. The tea bags were retrieved after four different time periods; 15, 30, 60 and 90 days. The tea bags from each replicate were harvested at the same time. The four tea bags of each brand were placed at different locations in the experimental plots, within approximately two square meters (Fig. 4).

Each tea bag was identified on its label with a black permanent marker and gently placed in the soil at 8 cm depth in separate holes using a shovel. Each tea bag where located approximately 2-3 cm from each other, in order to not let them have a direct contact with the surrounding soil. The label where still above the ground after the placement. Each row of replicates was marked with a stick.



#	Color
1	🟥
2	🟨
3	🟩
4	🟦
5	🟪

Figure 4. Scheme showing placement of tea bags in a 2 x 2 m grid in field plots (colours indicates sampling dates) Purple was used as a buffer replicate.

After the specified time, the tea bags from each replicate where retrieved and put in a plastic bag, and thereafter in a cool bag (during transport) and refrigerator (storage) to maintain temperatures below 2 °C before drying and weighting. Each tea bag where then dried in a stove for 48 hours at 70 °C. After drying, the tea bags where opened and the contents where weighted separately.

The content from the 90 days-replicate where then mixed together and analyzed for mineral contamination through ashing. The ash content was determined at 550 °C for 16h. The ash content was determined for each of the four sampling dates, but only the results from the time period 90-days are discussed in the present thesis.

3.2 Calculations of TBI parameters *S* and *k*

The concept of the TBI and the underlying assumptions behind the derivation of the two parameters are described in detail by Keuskamp et al. (2013) and only a short description is given below. Briefly, the Tea bag index consists of the decomposition rate constant *k* and the stabilization factor *S*. *k* is calculated for both the labile and recalcitrant compounds using the equation:

$$W(t) = ae^{-k_1 t} + (1 - a)e^{-k_2 t} \tag{1}$$

Where *W(t)* is the weight of the substrate after incubation time *t*, *a* is the labile and *1-a* is the recalcitrant fraction. k_1 and k_2 are the decomposition rates for the labile and recalcitrant fractions, respectively. The labile fraction *a* is rapidly broken down, and the weight loss is mainly determined by k_1. The recalcitrant fraction *1-a* decomposes slower, whereby k_2 is very low, and assumed negligible during short-time field incubations. The equation can therefore be reduced to the exponential decay function:

$$W(t) = ae^{-k_1 t} + (1 - a) \tag{2}$$

Translated into TBI, the decomposable fractions are Green tea a_g (labile) and Rooibos tea a_r (recalcitrant) (eqn. 3 & 4). To solve the equation above, estimations of a_r is needed. a_r can be estimated from a_g assuming that the relation between a and H, the chemically expected labile fraction, only depends on environmental conditions. During decomposition, some of the labile fractions stabilize and become recalcitrant and the stabilization factor S for the labile compounds can be calculated through the equation:

$$\mathrm{S} = 1 - \frac{a_g}{H_g} \tag{3}$$

Where H_g is the hydrolysable factor of Green tea. Reaching the decomposable factor of Rooibos tea a_r is done by using the hydrolysable fraction of Rooibos tea H_r and the stabilization factor S, giving the equation:

$$\mathrm{a}_r = H_r(1 - S) \tag{4}$$

When knowing $W(t)$ and a_r, these figures can be used in equation (2) to retrieve k for Rooibos tea.

3.3 Presentation of data

Beside some scatterplots, box plot charts were used to present the results. These are constructed as shown in figure 5. Max and min values are the datasets max and min values. The box is constructed from the first (Q1) and the third (Q3) quartile of the dataset, and the line crossing the box shows the median value. The cross shows the mean value.

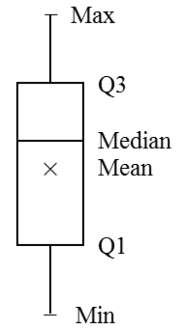


Figure 5. Schematic box plot, describing its components.

3.4 Description of sites

The long-term field experiments were located in five different regions in Sweden, covering a wide range of pedo-climatic conditions (Fig. 6). Soil texture varied from clay to silty sand, while mean annual temperature and total precipitation (standard climate data, 1961-1990) ranged from 2.4 to 8.0 °C and from 528 to 683 mm, respectively (Table 4). Three types of long-term experiments were used: 1) the Swedish soil fertility trials 2) the humus balance trials with forage and cereals, and 3) tillage experiments with different cultivation techniques (Fig. 6 and Table 2 and 3). A total of 13 sites were selected from these three types of experiments and located, from north to south as follows: Umeå (Röbäcksdalen), Uppsala (Säby, Kungsängen, Ultuna), Linköping (Vreta Kloster and Högåsa), Skara (Lanna) and Skåne (Ekebo, Borgeby and Lönnstorp).

The 13 sites allowed us to make specific treatment comparisons. Forage crops could be compared to cereals cropping systems (Forage/Cereal) at 4 sites, different cultivation techniques that involved direct drilling, carrier cultivation to a depth of 5-7 cm, cultivation to a depth of 10-12 cm and 12-15 cm and ploughing to a depth of 20-23 cm (Tillage) could be assessed at 3 sites, and 6 sites allowed a comparison between unfertilized (no NPK) and no manure application plots compared to plots receiving both NPK and manure (Fertility) (Table 2 and 3).

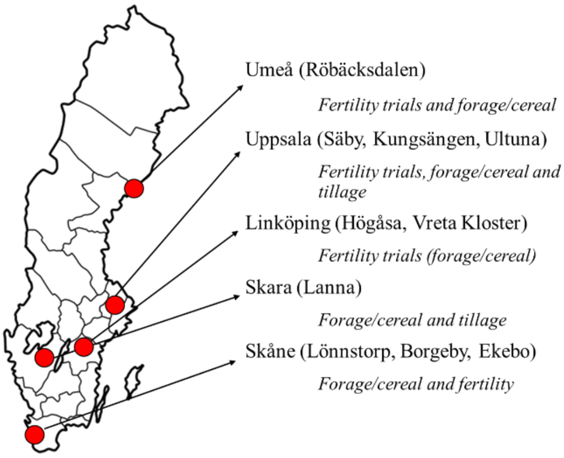


Figure 6. Map showing location of selected sites from the three different types of long-term experiments for five regions in Sweden.

3.5 Climate data and soil properties

The annual climate data where retrieved from the standard climate period (1961-1990) for the Swedish Soil Fertility Experiments described by Carlgren & Mattson, (2001). Daily climate data, such as air temperature and precipitation was gathered through official data from the most nearby Lant-Met climate stations[5]. Daily climate data used was air temperature (1.5 m) from the date of placement of the tea bags to the last date of retrieval, and precipitation data for the time period of one week prior to the placement of tea bags in the soil to the last date of retrieval. A synthetic climate index (air temperature x precipitation) was later calculated through multiplying daily air temperature and precipitation data and then calculating the average index during the experiment. This was done to reflect a comparable and summarized climate for all regions.

Soil properties on the different locations where gathered from the most recent analysis protocols, ranging from 2011 to 2015.

3.6 Statistical analysis

3.6.1 Regression correlation and coefficients of determination

We made linear regression with Excel to determine the correlation (r) between different variables, where the coefficients of determination (R^2) express the proportion of the relationship between variables.

3.6.2 t-test

In order to test the inference about means for each site we made multiple comparisons by conducting a series of *t* test between pairs of means. We also calculated the least significant difference, LSD (which is a form of the *t* test) with a 5% level of probability with Excel to determine whether the two means were significantly different from each other.

3.6.3 “Global analysis”

We also examined the average effect across all the long-term field experiments for the selected categories of management practices (forage/cereal, fertility and tillage). The trials used for each category is then seen to represent a broader possible set of conditions occurring across Sweden. This was done

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[5] http://www.slu.se/fakulteter/nj/om-fakulteten/centrumbildningar-och-storre-forskningsplattformar/faltforsk/vader/lantmet/

with a mixed ANOVA model using the SAS software (SAS Institute Inc, 2001) considering the long-term experiments as being a random effect.

Table 2. *Selected treatments for the sites used in the tillage and forage/cereal comparisons.*

Location	Number	Treatment	Crop	N-fertilization	Straw management	Placement of tea bags
Tillage						
Lanna	R2-4017	Direct drilling	Winter wheat		Chopped	End of April
	R2-4017	Ploughing (20-23 cm)	Winter wheat		Chopped	End of April
Säby	R2-4140	Direct drilling	Barley		Chopped	Mid of May
	R2-4140	Ploughing (20-23 cm)	Barley		Chopped	Mid of May
	R2-4140	Cultivation (5-7 cm)	Barley		Chopped	Mid of May
	R2-4140	Cultivation (10-12 cm)	Barley		Chopped	Mid of May
Ultuna	R2-7115	Ploughing (20-23 cm)	Barley		Chopped	Mid of May
	R2-7115	Cultivation (12-15 cm)	Barley		Chopped	Mid of May
Forage/Cereals						
Röbäcksdalen	R0021	Forage	Sowing in pure stand	150 kg N	Removed	Mid of June
	R0020	Cereals	Barley	120 kg N	Removed	Mid of June
Lönnstorp	R0021	Forage	Barley+undersowing	150 kg N	Removed	End of April
	R0020	Cereals	Barley	120 kg N	Removed	End of April
Lanna	R0021	Forage	Forage III	150 kg N	Removed	End of April
	R0020	Cereals	Oat	120 kg N	Removed	End of April
Säby	R0021	Forage	Barley+undersowing	150 kg N	Removed	Mid of June
	R0020	Cereals	Barley	120 kg N	Removed	Mid of June

Table 3. *Selected treatments for the sites used in the fertility comparisons.*

Location	Number	Treatment	Crop	N-fertilization	Straw management	Placement of tea bags
Fertility trials						
Röbäcksdalen	R3-2037	0 NPK, 0 manure	Barley	0	Removed	Mid of June
		Max NPK, manure	Barley	120 kg N	Chopped and incorporated	Mid of June
Kungsängen	R-9001	0 NPK, 0 manure	Oat	0	Removed	End of May
		Max NPK, manure	Oat	125 kg N	Chopped and incorporated	End of May
Vreta Kloster	R-9001	0 NPK, 0 manure	Spring oilseed	0	Removed	End of April
		Max NPK, 0 manure	Spring oilseed	125 kg N	Removed	End of April
		0 NPK, manure	Forage II	0	Removed	End of April
		Max NPK, manure	Forage II	125 kg N	Removed	End of April
Högåsa	R-9001	0 NPK, 0 manure	Spring oilseed	0	Removed	End of April
		Max NPK, 0 manure	Spring oilseed	125 kg N	Removed	End of April
		0 NPK, manure	Forage II	0	Removed	End of April
		Max NPK, manure	Forage II	125 kg N	Removed	End of April
Borgeby	R-9001	0 NPK, 0 manure	Sugar beet	0	Removed	End of May
		Max NPK, manure	Sugar beet	210 kg N	Chopped and incorporated	End of May
Ekebo	R-9001	0 NPK, 0 manure	Sugar beet	0	Removed	End of May
		Max NPK, manure	Sugar beet	210 kg N	Chopped and incorporated	End of May

Table 4. *Soil properties and standard climatic data (1961-1990) for each of the experimental sites.*

Location (Start)	Texture	Clay (%)	C-TOT (%)	C/N	pH	Precipitation (mm)	Temperature (ºC)
Tillage							
Säby (1997)	Silty loam	18	2,78			528	5,5
Ultuna (1997)	Silty clay loam	38	1,16			528	5,5
Lanna (1982)	Heavy clay	47	1,16			558	6,1
Forage/Cereal							
Säby (1981)	Silty clay loam	36	2,55	10,9	5,6	528	5,5
Lanna (1981)	Silty clay loam	36	2,07	11,6	6,1	558	6,1
Lönnstorp (1981)	Sandy clay loam	25	1,69	10,5	6,0	569	7,7
Röbäcksdalen (1980)	Silt loam	<5	1,99	14,2	5,4	582	2,4
Fertility trials							
Kungsängen (1963)	Silty clay	56	2,25	10,1	6,3	528	5,5
Vreta kloster (1966)	Silty clay	50	2,21	10,9	6,9	569	6,8
Högåsa (1966)	Silty sand	10	2,05	13,9	6,3	569	6,8
Borgeby (2010)	Sandy clay loam	15	1,28	12,8	7,7	569	8,0
Ekebo (1957)	Loam	15	2,40	13,5	7,0	683	7,8
Röbäcksdalen (1969)	Silty loam	10	2,54	13,2	6,1	582	2,4

4 Results

4.1 Climate data and soil properties

The climatic conditions during the time period the teabags remained in the soil (~90 days) where variable (Fig. 7). Mean air temperature ranged from 16.5 °C in the Skåne region to 14.1 °C in Skara. Mean soil temperature ranged from 17.4 °C in Skåne to 14.6 °C in the Linköping region. Soil temperature was almost one degree higher than air temperature in the Skåne (R^2 = 0.67) and Skara (R^2 = 0.82) regions, slightly higher in the Umeå region (R^2 = 0.78), the same in the Uppsala region (R^2 = 0.69) and slightly lower in the Linköping region (R^2 = 0.80). Total precipitation showed relatively large differences between regions, with 174 mm precipitation in

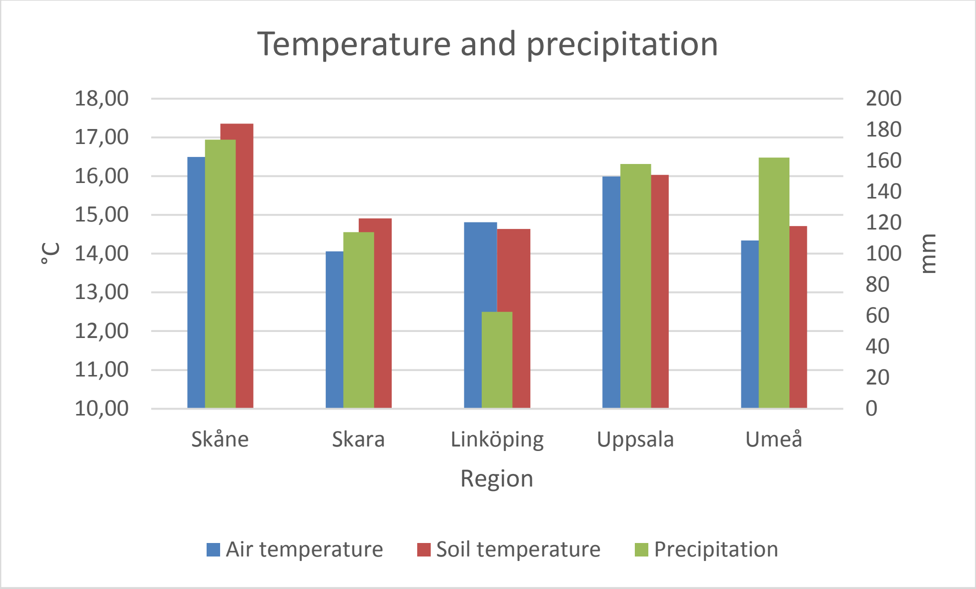

Figure 7. Regional average air- and soil temperature and total precipitation during the time period teabags remained in the soil.

Skåne, 162 mm in Umeå, 158 mm in Uppsala, 114 mm in Skara and 62 mm in Linköping.

We compared the average TBI parameters *S* and *k* (i.e., averaged across the sites) for the five regions with the synthetic climate index of air temperature x precipitation (Fig. 8). There was a strong correlation ($R^2 = 0.92$) between this index and *S*, indicating that the stabilization decreased with increased precipitation and higher air temperatures. However, the relationship between this index and *k*, that describes decomposition rates, was weak ($R^2 = 0.04$), suggesting it is not affected by climatic conditions. Only the value obtained from the Umeå region (0.024) deviated from this flat trend line obtained for *k*. This may be caused by higher influence of precipitation in the synthetic climate index, compared to the other regions, and/or the higher deviation in the *k* values.

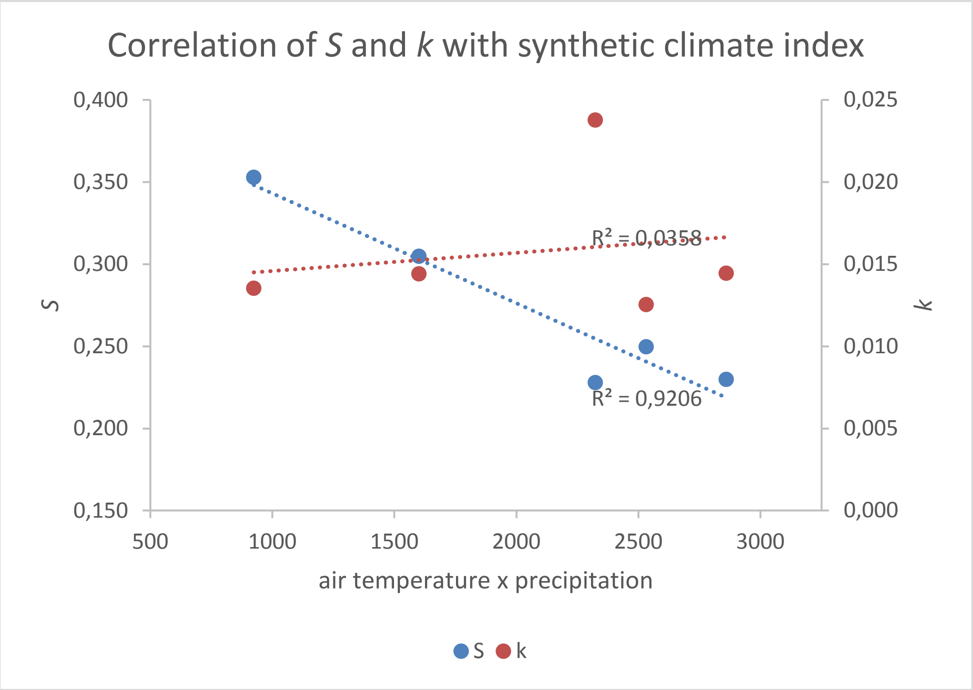

Figure 8. Correlation between the TBI parameters (*S* and *k*) and the synthetic climate index (air temperature x precipitation).

4.2 Forage/Cereal trials

The four trials used for comparing carbon dynamics with the TBI approach between a perennial forage crop with that of an annual cereal crop had been in place for about 35 years, beginning in the early 1980s (Table 4). Except from the site at Röbäcksdalen, the results were always significantly different for *S* ($P < 0.05$), but the results for *k* was only significant ($P < 0.05$) for one of the four sites. However, the significant results showed a variable trend depending on the site locations. Lönnstorp and Säby showed higher *S* in the cereal treatment, while Lanna showed the highest *S* in the forage treatment (Fig. 9).

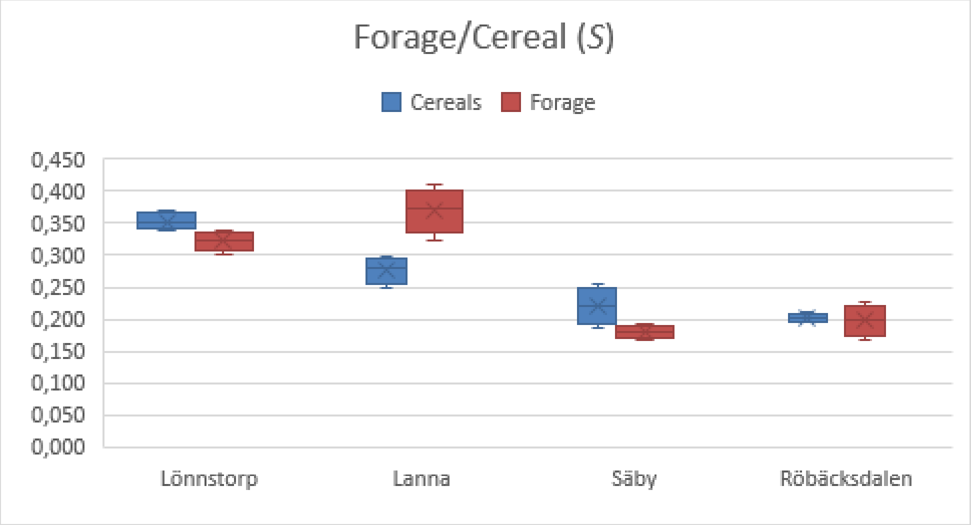

Figure 9. The TBI stabilization factor (*S*) determined at different sites of the long-term forage/cereal experiments ($N = 4$).

It should be considered that these comparisons were not completely identical or uniform, for Lönnstorp and Säby the forage crops where re-established (undersowing in barley) during the season of this study, and for Röbäcksdalen it was re-established in a pure stand, only the forage crop at Lanna was already well (in the third year of production) established (Table 2). Although, a trend for higher *k* values under the annual crop could be observed (Fig. 10), this difference was only significant ($P < 0.05$) at Säby.

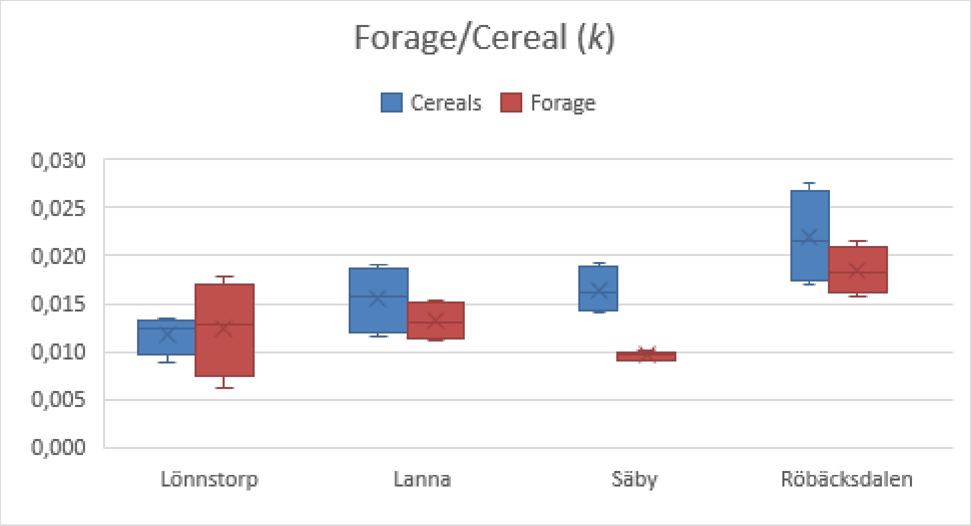

Figure 10. The TBI decomposition rate (*k*) determined at different sites of the long-term forage/cereal experiments (*N* = 4).

4.3 Fertility trials

The tendency is that *S* is higher in full fertilized plots compared to plots with no fertilization (Fig. 11). However, no significant ($P < 0.05$) differences in the sites where found. *k* was not significant and no direct tendency are shown (Fig. 12). There was an error in the amount of fertilizer applied at Kungsängen in 2016, suggesting that results from this site should be treated very carefully.

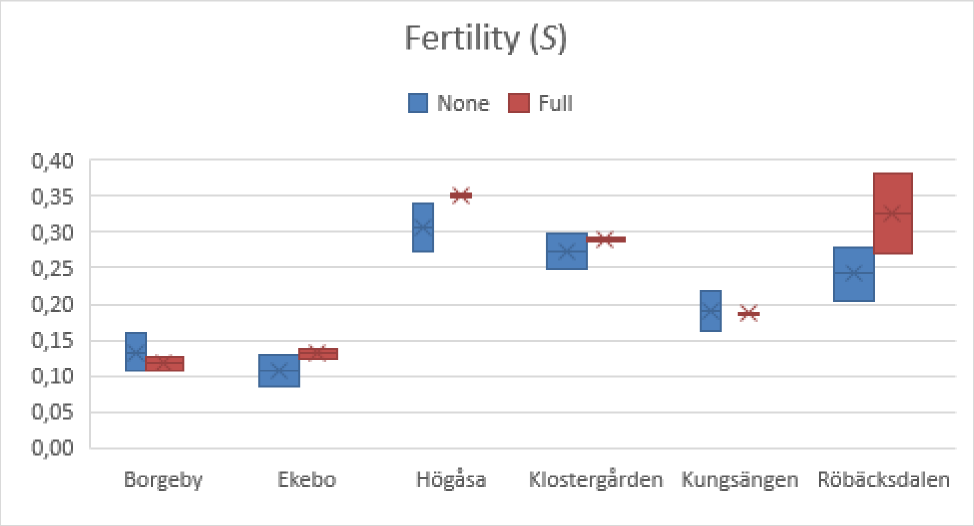

Figure 11. The TBI stabilization factor (*S*) at different sites of the fertility experiments (None = 0 NPK and 0 manure, Full = Max NPK and manure) (*N* = 2).

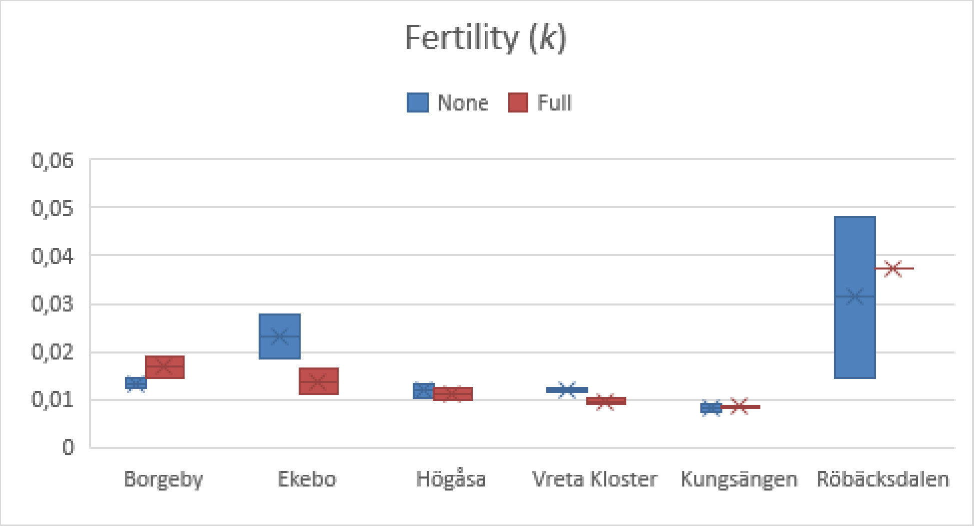

Figure 12. The TBI decomposition rate (*k*) at different sites of the fertility experiments (None = 0 NPK and 0 manure, Full = Max NPK and manure) (*N* = 2).

4.4 Tillage trials

The effect of tillage on the TBI stabilization parameter *S* was only significant different at Ultuna, between ploughing and cultivation to a depth of 12-15 cm ($P < 0.05$) (Fig. 13).

At Lanna and Ultuna, ploughing gave the lowest stabilization, but at Säby (close to Ultuna) it resulted in the highest *S* value. Direct drilling resulted in rather equal values. Shallow cultivation (5-7 cm) was the treatment that showed the highest variations in *S*. Deeper cultivation was similar and rather equal to other treatments (Fig. 14).

The *k* parameter showed only significant differences between cultivation (10-12 cm) and ploughing at the Säby site ($P < 0.05$). The differences between the sites showed an interesting pattern, where ploughing showed a tendency to result in the lowest decomposition rates, compared to the other tillage treatments. Other treatments where rather similar to each other, although results tend to indicate a slightly higher *k* value when deeper cultivation is performed (Fig. 14).

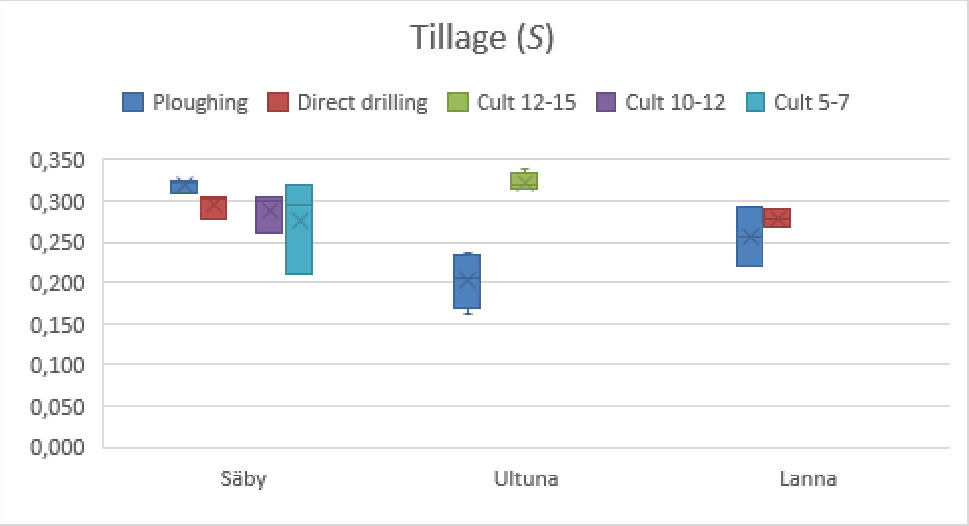

Figure 13. The TBI stabilization factor (*S*) at different sites of the long-term cultivation trials, comparing ploughing, direct drilling and cultivation on different depths (N = 3, 4 and 2, respectively).

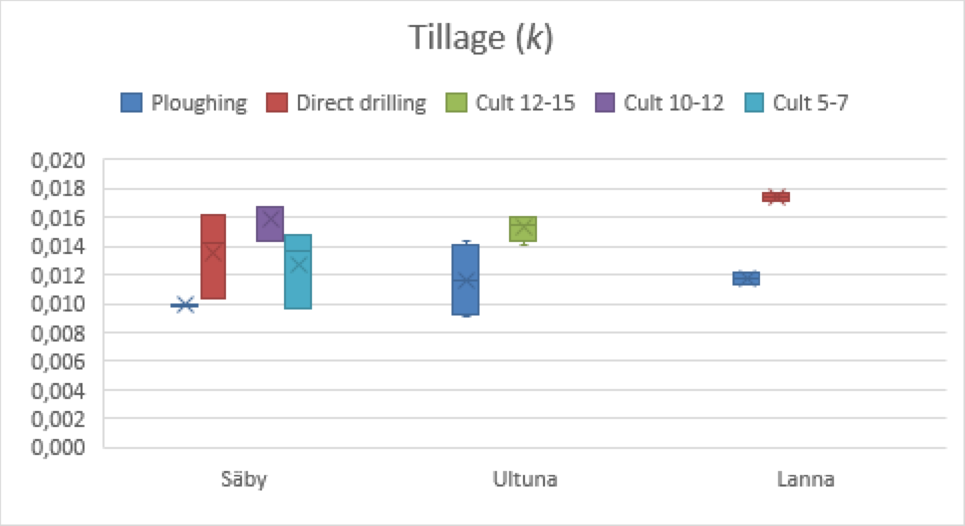

Figure 14. The TBI decomposition rate (k) at different sites of the long-term cultivation trials, comparing ploughing, direct drilling and cultivation on different depths (N = 3, 4 and 2, respectively).

4.5 Established forage compared to annual crops

Considering the results from section 4.2 showing a higher stabilization parameter (S) when the season's crop was an established forage crop (the site at Lanna), we made another comparison including also the data retrieved from the fertility trials in the Linköping region (sites at Högåsa and Vreta Kloster). For that purpose, we used the treatments of Max NPK with 0 manure for spring oil seed compared to Max NPK with manure for Forage II (Table 3), because it reflects the mostly commonly used practice (i.e., no NPK applications almost never occurs in practice) and was rather comparable with the treatments at the Lanna site. For these 6-year soil fertility rotations, the manure is only applied at the end of the rotation in the fall when the forage crop is ploughed under. The last application was made in 2010, so although the rotation with spring oilseed does not receive any manure at all it should not interfere too much in these comparisons.

Results from this analysis shows a clearer (and significant, $P < 0.05$) difference between a forage crop, compared to an annual crop. The established forage crop leads to higher stabilization (Fig. 15). The result for the TBI parameter k is not as clear over all three sites, but it was significantly higher ($P < 0.05$) in the established forage crop both at the Högåsa and Vreta Kloster sites (Fig. 16).

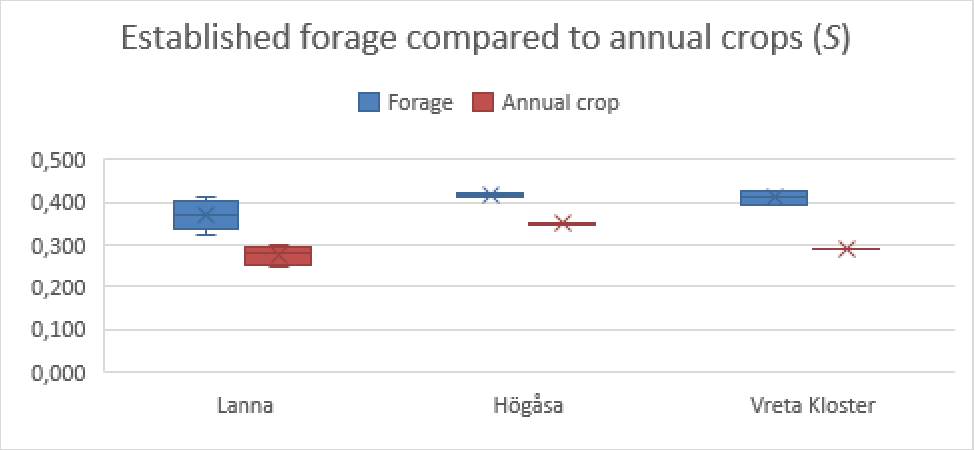

Figure 15. Distribution of stabilization factor (*S*) on different sites where established forage is compared to an annual crop (*N* = 4, 2 and 2, respectively).

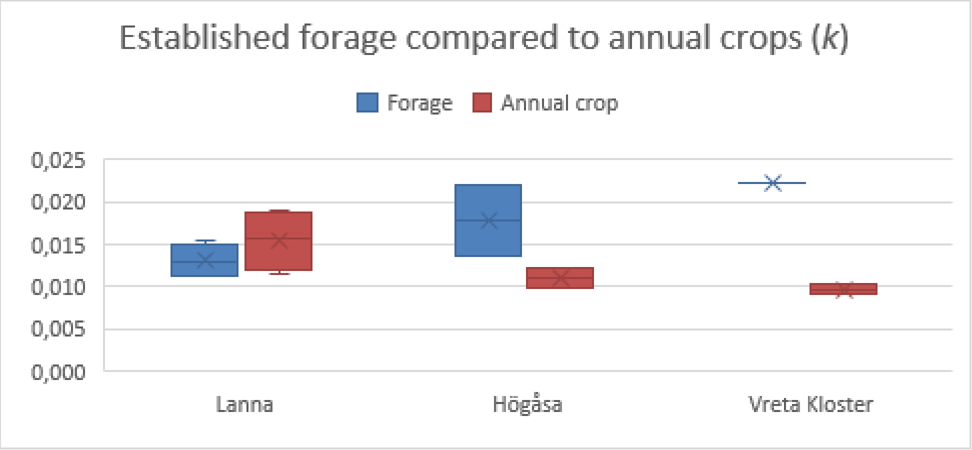

Figure 16. Distribution of decomposition rate (*k*) on different sites where established forage is compared to an annual crop (*N* = 4, 2 and 2, respectively).

4.6 Linear regression

Since the correlation between *S* and the synthetic climate index (air temperature x precipitation) was high on a regional basis (Fig. 8), we investigated this relationship in more detail. For that purpose, we made correlations using data for each of the experimental plots (*N* = 88), and the results showed that the climate index then explained 33% of the variation in *S* (Fig. 17).

The correlations with air temperature and precipitation alone described 21% and 31% of the variation, respectively (Fig. 18), and indicated that *S* decreased both when temperature and precipitation increased. Correlations with soil temperature (*N* = 72) showed no significant ($P < 0.05$) correlation (data not shown).

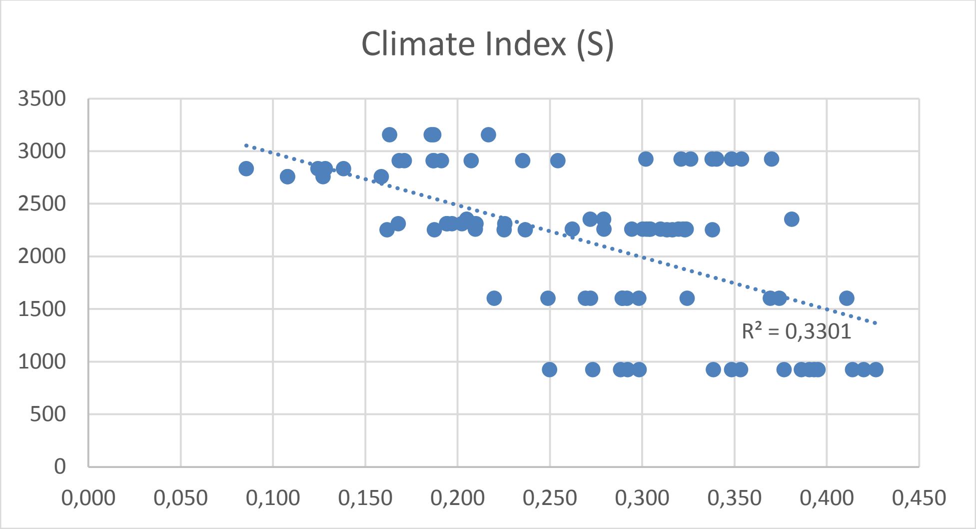

Figure 17. Linear regression between the synthetic climate index (air temperature x precipitation) and the TBI parameter *S* using the data from each of the experimental plots (*N* = 88).

The TBI parameter *k* showed no significant correlation with climatic data (P < 0.05) (data not shown). However, this parameter correlated with the C/N-ratio and clay content, where 13% of the variation in *k* was described by clay content and 19% was explained by C/N-ratio (Fig. 19). The decomposition rate increased with a decrease in clay content, and increased with increasing C/N-ratio.

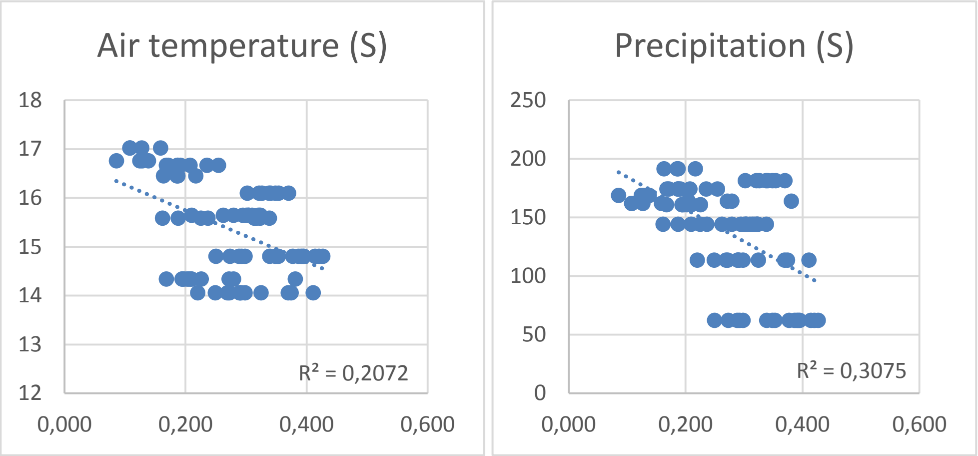

Figure 18. Linear regression between the TBI parameter S and average temperature (left) and total precipitation (right) using the data from each of the experimental plots (*N* = 88).

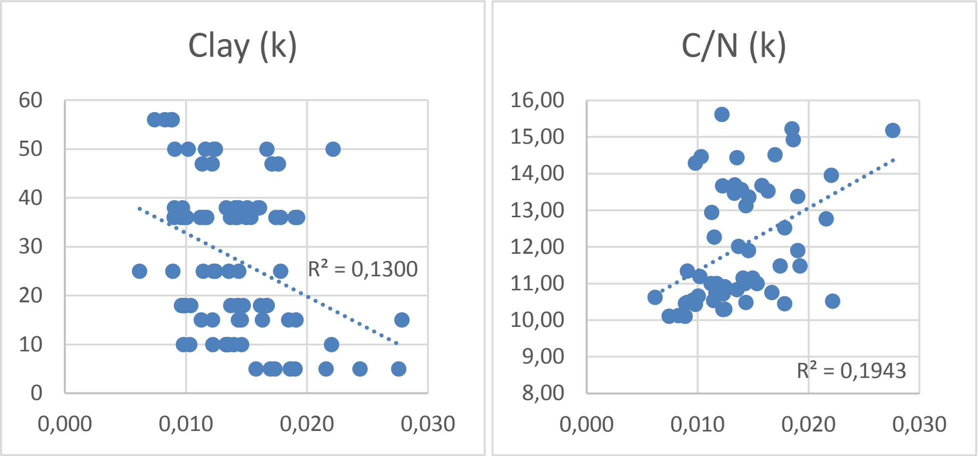

Figure 19. Linear regression between the TBI parameter k and clay content (left) and C/N-ratio (right) using data from each of the experimental plots (except all tillage treatments due to missing C/N data) (*N* = 84 and 56, respectively).

5 Discussion

To summarize the mean effect for each of the specific comparisons on the TBI parameters across Sweden (Fig. 20 and 21), all the sites from each region were also used as replicates in a global analysis of variance using SAS program (data not shown).

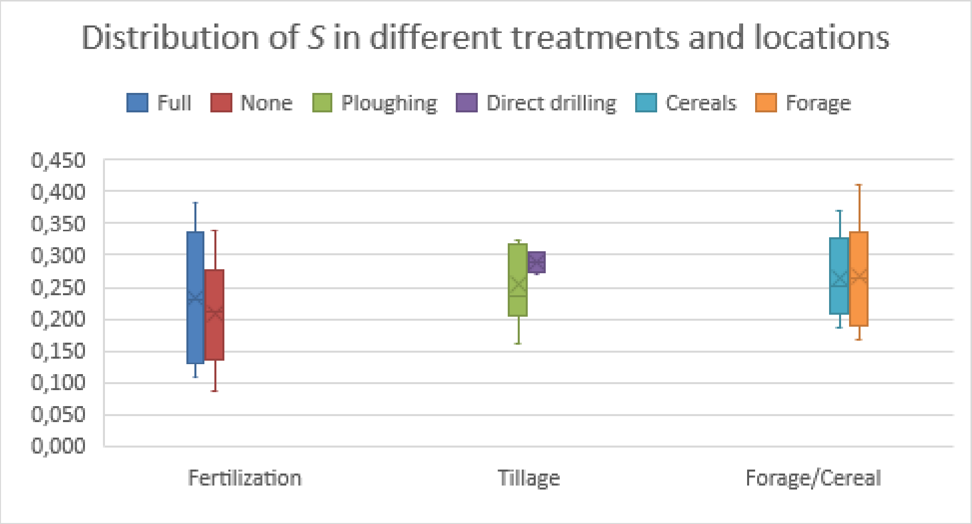

Figure 20. Distribution of the TBI stabilization factor (*S*) in different treatments and locations.

Growing forage instead of an annual crop seemed to be the best practice for maintaining a high stabilization of carbon, as indicated by the TBI parameter *S* (Fig. 20). This effect was particularly strong for the Lanna site where the forage crop was well established. For the remaining sites, where the forage crop was in the year of establishment (Fig. 9), the *S* parameter for the annual crop was more or less equal (Röbäcksdalen), or even higher (Lönnstorp and Säby sites). However, the analysis we made including the two other sites where forage crop was also well established confirmed the results obtained for the Lanna site, as well as this general trend (Fig.

15). The decomposition rate parameter, *k*, showed a pattern of being lower in all long-term forage trials, compared to that for cereals and were significant in the global analysis (Fig. 21)

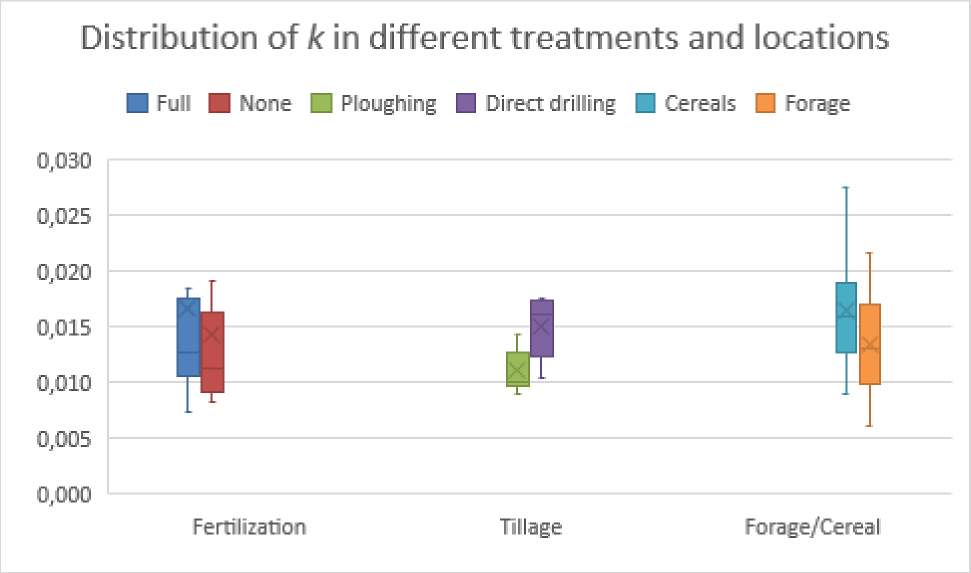

Figure 21. Distribution of the TBI decomposition rate (*k*) in different treatments and locations.

The fertilization trials did not result in any significant differences in either *S* or *k* at the different sites. However, in the global comparison *S* was significantly higher in fertilized than unfertilized plots, *k* was not (Fig. 20 and 21). Kätterer et al. (2014) showed significantly higher SOC levels for fertilized cropping systems, which was explained by higher production of above- and below-ground biomass. Our results show that it may not only be caused by higher inputs, a higher stabilization can also contribute to these differences. This could be due to an increase in total microbial biomass and an alternation of the biological community towards fungi-dominated, caused by favorable conditions (Six et al., 2006).

Ploughing seemed to lead to lower stabilization and lower decomposition rates than direct drilling, but results at all the sites were not significant. However, at the global scale both *S* and *k* were significantly different between the two treatments (Fig. 20 and 21). Shallow and deeper cultivation was rather equal to the other tillage treatments. We could thereby not confirm results such as those obtained by e.g., Halvorson et al. (2002) showing that no tillage sequesters more carbon, and that reduced tillage have an intermediate effect on SOC, while ploughing lead to the lowest stabilization of SOC.

The *k* parameter showed significant differences between ploughing and cultivation (10-12 cm) for the site at Ultuna and between ploughing and direct drilling for the site at Lanna. However, it only showed significant differences between cultivation (10-12 cm) and ploughing for the site at Säby. Nevertheless, the differences observed for this parameter at the sites used in our study showed an interesting pattern, where ploughing tended to result in the lowest decomposition rates, compared to other treatments. The remaining treatments were rather similar to each other, although there was a trend towards slightly higher *k* values when deeper cultivation was performed. Lower decomposition rates from incorporation of residues into the soil were also a finding by Kainiemi et al. (2014), due to reduced access to water and lower temperatures. Our results for the effect of tillage were, in overall, in line with other studies, such as Baker et al. (2007) & Kätterer et al. (2012), where no uniform patterns have been proved.

The climate had a large impact on the SOC dynamics, which is in line with the conclusions of many other studies, e.g., Brockett et al. (2012) & Paul (2015). In particular, the TBI parameter *S* was significantly affected by both temperature and precipitation, *S* was high, when precipitation and temperature was low. This corresponds well to findings by other studies such as those by Bolinder et al. (2007a) & Portillo-Estrada et al. (2016). As explained by Moyano et al. (2012) soil moisture is one of the most determining factors for soil biological activity, and Liu et al. (2009) showed that it was well correlated to mean annual precipitation. Lefèvre et al. (2014) underlined the fact that stable carbon compounds are sensitive to temperature and Gregorich et al. (2016) also highlighted that temperature is the main factor driving the litter decay in soil. Our results suggest that the TBI parameter *S* seems to be sensitive enough to reflect this temperature dependence, since it is a measure of SOC stability. We found no relationship between climate and the TBI parameter *k*, and it was not as sensitive as *S* to the treatments we examined in this study. This could possibly be explained by the fast breakdown of the labile compounds in Green tea.

We also made a regression analysis including soil properties such as clay content, C/N-ratio and pH. Unfortunately, we did not have access to data on pH and C/N for the tillage trials (and they were excluded from this analysis). *S* was mostly dependent on air temperature ($R^2 = 0.19$) and precipitation ($R^2 = 0.13$) (Fig. 18), meanwhile no significant correlations with soil temperature where achieved. This may be due to some missing data ($N = 72$ instead of 88) and/or higher deviation in the soil temperatures. Combining air temperature and precipitation, achieving a synthetic climate index, gave higher correlation than the two factors alone ($R^2 = 0.33$) which highlights the interacting effects of these variables on decomposition dynamics. The TBI parameter *S* was not influenced by soil properties, while *k*, on the other hand, was negatively correlated to clay ($R^2 = 0.13$): decreasing with increasing clay content (Fig. 19). It was also positively correlated to the C/N-ratio of the soil ($R^2 = 0.19$).

Clay has large impact on moisture-respiration relationships (Moyano et al., 2012), and positively correlates with SOC (Schimel et al., 1994). Our findings suggest that the higher the clay content, the lower the decomposition rates. This could, with some caution, be translated into that higher clay content leads to higher stabilization and SOC levels.

The C/N-ratio had a positive correlation with *k*, where a higher C/N-ratio resulted in a higher *k* value. This means that when there is more carbon in relation to nitrogen in the soil, then the decomposition rates are increased. This is probably due to the microbial community favoring fungi at high C/N levels, where fungi play a crucial and positive role in SOC stabilization with increases of C/N (Paul, 2015). This may, thereby, lead to lower access of the material by other microorganisms, leading to higher decomposition rates of recently added material.

No significant relationship was found between *S* or *k* and pH. This leaves our hypothesis rejected, although the literature is suggesting that biological activity in the soil tends to decrease with pH (Poeplau et al., 2015a).

The overall results from this study showed that the TBI is a useful approach to investigate the effect of different management practices on SOC dynamics. However, further research is needed where each parameter is investigated in more detail, and where all potential factors influencing *S* and *k* are included. It should also be evaluated if the results derived from the TBI approach are comparable with other approaches used to estimate stabilization and decomposition rates in long-term field experiments.

5.1 Methodology discussion

Overall, the method was derived from the standard TBI protocol. However, we designed the experiment so that we could have sub-replicates with teabags within each of the experimental plots. The size of the area used for this sub-replication was restricted to only a 2 x 2 m grid, which implied that it could not be thoroughly randomized, and, to cover the entire area of the experimental plots (which were much larger). This limitation was made by requests on not disturbing other experiments and field operations made in the trials during the growing season. Furthermore, the degradation of the Green tea went rather far during our 90 days (mass loss of approximately 60%), which according to Hefting[6], could make the results more uncertain.

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[6] Mariet Hefting, Assistant professor, University of Utrecht. 2016-02-26. 1st Tea Bag Index (TBI) Workshop.

6 Conclusions

- The TBI was a useful approach to examine carbon dynamic for different management practices and cropping systems in Swedish long-term field experiments, and it also to some extent reflected differences in climatic conditions between sites.
- Climatic differences between sites were reflected mostly in the TBI stabilization parameter (*S*), whereas differences in soil properties mostly affected the decomposition rate parameter (*k*).
- The *S* parameter increased with lower temperature (R^2 = 0.21) and lower precipitation (R^2 = 0.31), but the best correlation was obtained with a synthetic climate index that reflects the daily interaction between temperature and precipitation (R^2 = 0.33). Variations in *k* was only marginally related to temperature (R^2 = 0.09) and not significant.
- Differences in clay content across sites explained 13% of the variations in *k*, and 19% was explained by variations in C/N-ratios.
- Growing forage leads to higher stabilization than annual crops, except in the year of establishment.
- Ploughing seems to have a negative effect on *S* compared to direct drilling, resulting in lower stabilization in ploughed fields. However, decomposition rates are lower in ploughed fields.
- Generally, a global analysis using the sites as replicates indicates that nutrient management where manure and mineral fertilizers are applied leads to higher S, compared to unfertilized fields.
- Management practices, such as crop choice, cultivation techniques and fertilization, influence both *S* and *k,* although they depend on soil properties and climate. Management practices could therefore be an important way to manage SOC levels in the soil, and thereby the global carbon balance.

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I will also take the opportunity to thank for the hospitality from relatives and friends who on short notice could offer a night’s sleep on my long travels.

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Appendix I – TBI protocol

As described in Keuskamp et al. (2013).

1. Use one bag of Lipton Green tea (EAN: 87 22700 05552 5) and one Lipton Rooibos tea (EAN: 87 22700 18843 8) per replicate. To obtain better estimates of TBI, bury more replicates per site.
2. Measure the initial weight of the tea bag and subtract the weight of an empty bag (see also Table 1) to determine the initial weight of the tea.
3. Mark the tea bags on the white side of the label with a permanent black marker.
4. Bury the tea bags in 8-cm deep, separate holes while keeping the labels visible above the soil and mark the burial site with a stick.
5. Note the date of burial, geographical position, ecotype and experimental conditions of the site.
6. Recover the tea bags after c. 90 days
7. Remove adhered soil particles and dry in a stove for 48 h at 70°C (not warmer!).
8. Remove what is left of the label but leave the string, weigh the bags and subtract the weight of an empty bag without the label to determine the weight after incubation. To get a more precise estimation, open the bag and weigh its content; combust the content at 550°C and subtract what is left from the content weight.
9. Calculate stabilization factor S and decomposition rate k using eqn 1b.
10. More (facultative) instructions and tips on how to incorporate the TBI in scientific experiments can be found on our website: http://www.decolab.org/tbi

Appendix II – Populärvetenskaplig sammanfattning

Tedags för marken

Isarna smälter i nord och söder, regnoväder skapar stora översvämningar i tropikerna och öknarna breder ut sig. Med stor sannolikhet beror pågående klimatförändringar på det moderna samhällets utsläpp av växthusgaser, däribland koldioxid. Jordbruket har potentialen att lösa några av problemen.

Klimatförändringarna måste bromsas och genom Parisavtalet har majoriteten av världens ledare förbundit sig till att den globala temperaturökningen skall hållas under 2 grader. Detta kräver stora samhällsförändringar och för att lyckas behöver utsläppen förmodligen inte bara minska - historiska utsläpp måste också börja samlas in.

Marken innehåller stora mängder kol, nästan dubbelt så mycket som växterna och atmosfären tillsammans. En förändring i detta kolförråd kan därför ha stor påverkan på den globala kolbalansen. Jordbruksmark, som idag täcker ungefär 40 procent av världens landyta har därmed stor påverkan på den globala kolbalansen. Den bedöms kunna bli ett av de mest kostnadseffektiva alternativen för att samla in kol från atmosfären och lagra det i stabilare former. En process som sker genom att växterna tar upp koldioxid från atmosfären som lagras in i biomassan. När växterna vissnat och dött bryts kolet i biomassan ned i marken av markdjur, svampar och bakterier. En del av detta kol blir kvar i marken och kommer att vara borta från atmosfären under lång tid. Genom att gräva ner tepåsar i marken, vilket är en ny metod, kunde jag studera nedbrytningsdynamiken. Syftet var att förstå vad som påverkar den och för att försöka hitta klimatsmarta brukningsmetoder för att minska jordbrukets klimatpåverkan.

Försöket pågick under växtodlingssäsongen 2016 och genomfördes i olika svenska långliggande fältförsök som brukats på samma sätt i minst 30 år. Nämligen de svenska bördighetsförsöken där gödslade led jämförs med ogödslade, växtföljdsförsök där en spannmålsväxtföljd jämförs med vall, samt långliggande jordbearbetningsförsök där plöjning jämförs med kultivering och direktsådd. Jag grävde ner tepåsarna (vanliga Lipton-tepåsar med smakerna Grönt te och Rooibos) strax efter sådd för att tre månader senare samla in dem och väga deras innehåll. Genom att se hur materialet hade brutits ned under denna tid kunde jag räkna ut ett TBI (Tea bag index). TBI kan ses som ett lättanvändbart verktyg för att estimera både nedbrytningshastigheten och andelen av det organiska materialet som stabiliseras i marken.

Resultaten påvisade att stabiliseringen av kol ökade vid odling av vall och i välgödslade led. Troligtvis beror detta på en ökad växtproduktion, ett större rotsystem och förbättrade markförhållanden. Nedbrytningshastigheten i plöjda led jämfört med direktsådda var lägre, förmodligen beroende av att inblandningen av det organiska materialet i jorden sänker temperaturen och vattentillgången, vilket har en sänkande effekt på nedbrytningshastigheten.

Det visade sig vidare att klimatet hade en stor inverkan på stabiliseringen där ökade temperaturer och nederbörd ledde till minskad stabilisering. Av undersökta jordegenskaper gav en hög lerhalt sänkt nedbrytningshastighet. En hög C/N-kvot i marken ledde till ökad nedbrytningshastighet.

Genom en så pass enkel metod som att gräva ner tepåsar i marken kunde jag alltså se skillnader i nedbrytningsdynamiken i olika klimat, odlingssystem och markförhållanden. Jag kunde visa att ett torrt och kallt klimat, vallar och hög växtproduktion ökar stabiliseringen av kol i marken. Dessa faktorer kan vara viktiga för att optimera och öka kolinlagringen i jordbruksmark. Metodiken är intressant för att, på ett standardiserat sätt, karakterisera nedbrytningsdynamiken och utveckla klimatsmarta odlingssystem.