Tillage Effects on Soil Respiration in Swedish Arable Soils

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Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2014

Acta Universitatis Agriculturae Sueciae
2014:2

ISSN 1652-6880
ISBN (print version) 978-91-576-7952-2
ISBN (electronic version) 978-91-576-7953-6

Print: SLU Service/Repro, Uppsala 2014

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Abstract

The amount of carbon (C) present in soil is greater than the sum of C present in terrestrial vegetation and the atmosphere combined. Small changes in soils can therefore affect atmospheric CO_2 levels and ultimately the global climate. Soil C is also one of the main soil properties involved in several soil functions critical for soil productivity. Mechanical disturbance of the soil, *e.g.* through tillage, can influence soil C and has been the focus of much research. However, the mechanisms behind C mineralisation are still not completely understood and research results vary. Tillage affects the availability of organic material for decomposers, the soil structure and the activity of soil organisms, which are also affected by changes in soil moisture and temperature.

This thesis examined the effects of different tillage practices on short-term soil respiration and changes in soil structure, moisture and temperature and the effects on C mineralisation in soils. It also quantified potential soil respiration resulting from mechanical disturbance in different Swedish arable soils. In order to unravel the mechanisms involved, experiments were carried out in the field and under controlled conditions in the laboratory. The response of soil respiration to different tillage treatments and plant residue managements was measured in the field and changes in soil structure, moisture and temperature were recorded and related to soil respiration. In the laboratory, soils of different textures were subjected to mechanical disturbance at different water contents and potential soil respiration was measured.

The field studies showed that mouldboard ploughing decreased soil respiration by up to 340 kg ha^{-1} compared with no tillage and 140 kg ha^{-1} compared with shallow tillage during the 10 days following tillage, after which the differences were negligible. Soil temperature and water content did not significantly affect soil respiration in the field. Furthermore, mouldboard ploughing produced most large aggregates (>64 mm), corresponding to about 90% of soil mass in the tilled layer and resulted also in the lowest soil respiration. The potential soil respiration following physical disturbance at a controlled water content and temperature resulted in C losses of up to 74 kg ha^{-1} in the laboratory, indicating that increasing clay content and water content can increase the risk of C losses from soils due to mechanical disturbance. However, under field conditions, the mechanisms behind C mineralisation following tillage are primarily determined by residue management rather than by soil structure or changes in soil moisture and temperature.

Keywords: Tillage, soil respiration, plant residues, organic matter decomposition, soil structure, physical protection, aggregates, soil water content, soil temperature

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Dedication

To my grandmother Sanni.

Contents

List of Publications

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

I Kainiemi, V., Arvidsson, J., Kätterer, T. (2013). Short-term organic matter mineralisation following different types of tillage on a Swedish arable soil. *Biology and Fertility of Soils 49*(5), 495-504.

II Kainiemi, V., Arvidsson, J., Kätterer, T. Effects of autumn tillage and residue management on soil respiration in a Swedish long-term field experiment. (manuscript).

III Kainiemi, V., Kirchmann, H., Kätterer, T. Respiration response to physical disturbance in arable soils of differing texture under controlled conditions. (manuscript).

Paper I is reproduced with the permission of the publishers.

The contribution of Veera Kainiemi to the papers included in this thesis was as follows:

I Planned the experimental work with the co-authors. Performed the practical work, with some assistance. Performed data analyses and wrote the main part with some assistance from the co-authors.

II Planned the experimental work with the co-authors. Performed the practical work, with some assistance. Performed data analyses and wrote the main part with some assistance from the co-authors.

III Planned the experimental work with the co-authors. Performed the practical work, with some assistance. Performed data analyses and wrote the main part with some assistance from the co-authors.

Abbreviations

C	carbon
CO_2	carbon dioxide
DIR	physical disturbance induced respiration
GHG	green house gas
KOH	potassium hydroxide
MP	mouldboard ploughing to 20 cm
NT	no-till
RES	basal soil respiration
SOC	soil organic carbon
SOM	soil organic matter
ST	shallow tillage to 5 cm
W	gravimetric water content
θ	volumetric water content
ψ	water potential

1 Introduction

The amount of carbon (C) present in soils is greater than the sum of C present in living biomass and the atmosphere. Any change in soil organic carbon (SOC) affects atmospheric CO_2 and has an impact on climate change. Loss of SOC is one of the main threats to soils and its protection is listed as one of the main goals in soil protection by the FAO (Bot & Benites, 2005) and the European Commission (Van-Camp *et al.*, 2004; Blum, 2008). A decrease in SOC affects the soil structure, making the soil more sensitive to *e.g.* compaction and reducing its capacity to retain water and nutrients. Moreover, soils provide substrates and habitat for a quarter of all species in the world (Turbé *et al.*, 2010). Anthropogenic influences such land use changes and agricultural management can increase or decrease SOC (Kätterer *et al.*, 2012).

Zero or no tillage and reduced tillage practices have been applied for decades, especially in the USA after the *Dust Bowl* in the 1930s, where large areas were ploughed and left fallow, contributing to significant wind erosion and enormous losses of topsoil. In Northern Europe, where long wet winters are the norm, autumn tillage and winter fallow periods have long been the predominant management practices on clay soils. However, reduced tillage practices are now attracting increasing interest due to the lower work load and fuel costs (Maraseni & Cockfield, 2011) and increased SOC content in the surface soil layers compared with conventional tillage (Franzluebbers, 2008; Virto *et al.*, 2012). In cold temperate regions, the effect of no tillage on C sequestration is small, in contrast to in warm and dry areas (Govaerts *et al.*, 2009; Regina & Alakukku, 2010). Soane *et al.* (2012) reviewed tillage effects on GHG emissions and concluded that the mechanisms behind C mineralisation in relation to tillage are not completely understood.

Tillage modifies soil architecture and thereby changes soil moisture and temperature conditions, but it also cuts up and buries plant residues in the cultivated layer. These changes in physical properties as well as the mechanical disturbance of aggregates influence soil organisms and the decomposition of soil organic matter. In fact, these measures cause complex interactions between water and substrate availability to soil organisms in the surface layers of soils, the activity of which governs C mineralisation. The following questions remain unanswered: What mechanisms and interactions control carbon mineralisation following tillage activities? What is the role played by water availability, temperature change and modification of substrate availability to microorganisms in explaining tillage effects? How do different types of tillage affect these interactions? Is tillage *per se* a significant variable to consider?

2 Aim

The main aim of this thesis work was to improve our understanding of the mechanisms behind soil C turnover as affected by tillage. Strong efforts were made to separate the effects of mechanical disturbance due to tillage from indirect effects, such as changes in soil moisture and temperature, as controlling variables for microbial activity. In order to distinguish between these effects, experiments on mechanical soil disturbance were conducted in the laboratory, where temperature and water availability were controlled. In field studies, the main objective was to investigate how tillage, conducted under different preceding soil conditions, modified soil architecture and interactions with soil water status and temperature. Short-term CO_2 fluxes after different tillage operations were quantified in the field in order to determine the effect of tillage activities on C sequestration in agricultural soils and to provide data useful for planning tillage activities to reduce C losses under Swedish conditions.

The work focused on soil respiration in agroecosystems affected by primary autumn tillage activities. The soil systems were studied without living crops and root respiration from living plants was therefore excluded.

Specific objectives were:

- To study and quantify the short-term effects of autumn tillage practices and tillage timing on carbon mineralisation (Papers I & II)
- To link the changes in soil structure resulting from tillage to soil respiration (Paper II)
- To study the effects of tillage on changes in soil moisture and temperature and connect it to carbon mineralisation (Papers I, II and III)
- To quantify potential soil respiration due to mechanical disturbance in Swedish soils with differing texture and SOC content (Paper III).

3 Background

3.1 Soil Tillage

“When tillage begins, other arts will follow.”
– Daniel Webster

Soil tillage affects many processes in the soil-plant system such as the availability and circulation of plant nutrients, occurrence of weeds, water infiltration and soil structure and aggregate stability. The main objective of tillage is to prepare the soil and provide optimal conditions for plant growth. Tillage can be divided into primary and secondary practices, where the former are considered to be rougher and deeper than the latter. According to common practice, clay soils in Northern Europe are tilled in autumn which is thought to be the best management practice for controlling weeds and loosening the soil, resulting in faster drying after winter. Shallow tillage (ST) is usually referred to as reduced tillage and direct -drilling or non -inversion as no tillage (NT). Such practices have attracted increased interest in Northern Europe since the 1990s, mainly due to the lower workload and fuel costs (Maraseni & Cockfield, 2011). However, the degree to which these tillage practices can be adopted under Nordic conditions depends on the climate, soil and crop type (Rasmussen, 1999). In this thesis, the term ‘soil tillage’ refers to primary tillage practices in the autumn and is divided into three types of tillage: mouldboard ploughing (MP) to a depth of about 20 cm, representing conventional tillage; chisel ploughing and disc cultivation to a depth of about 5 cm, representing ST; and undisturbed soil representing NT.

3.1.1 Tillage – The Plant Residue Manager

In agroecosystems, the dominant soil organic matter (SOM) sources for decomposers are root and shoot residues from the previous crop grown. Different tillage practices affect the incorporation depth of plant residues, which is usually 15-25 cm depth with ploughing and 5-20 cm depth with shallow cultivation. In NT systems, plant residues are mainly left on the soil surface and form a mulch cover, which decreases heat and water exchange to and from the soil.

No tillage systems typically have higher soil water contents than conventional tillage systems (Mielke *et al.,* 1986; Alvarez & Steinbach, 2009) where water evaporation is stimulated through a larger surface area (roughness is increased) and an increased volume of pores exposed to the atmosphere. Soil temperatures can be higher or lower in NT than in conventional tillage systems depending on the climate and weather conditions. During warm periods, residues covering the soil surface decrease the heat flow into the soil, which results in lower soil temperatures than in soils without a surface cover (Azooz *et al.,* 1997). During the autumn, when soil is warmer than air, the heat flow is from soil to air and heat remains in the soil over a longer period under a residue cover than without. In tilled soils, residues are incorporated and do not affect the thermal conductivity as much as in NT and also the diurnal variations are larger (Chatskikh & Olesen, 2007). Higher temperatures are observed in conventional tillage during daytime (Jin *et al.,* 2009), although lower mean daily temperature is observed in conventional tillage than in NT (Chatskikh & Olesen, 2007).

In studies by Breland (1994), Coppens *et al.* (2007) and Rottman *et al.* (2010) it was found that plant residues are decomposed at a lower rate when left on the soil surface. Incorporated residues decompose faster due to enhanced physical contact between soil-microbial systems and more optimal soil moisture (Coppens *et al*., 2007). However, data showing tree litter being decomposed faster when left on the soil surface have also been reported (Tyree *et al.,* 2011). It seems as though decomposition of residues is mainly dependent on prevailing temperature and moisture conditions. In cool temperate climates, temperature limits decomposition and fresh organic material left on the soil surface is decomposed faster due to higher temperature. When residues are incorporated into deeper soil layers, the lower soil temperature and poor aeration limit decomposition compared with that at shallower depths (Christian & Miller, 1986; Ball & Robertson, 1990). Overall, under conditions where water, oxygen and temperature are optimal in the soil, decomposition can be more effective when residues are incorporated (Coppens *et al.,* 2007). Due to the varying results and the lack of data under Northern European conditions, this work focused on quantifying the part of soil respiration resulting from residue management through tillage (Paper II).

3.1.2 Tillage – The Soil Architect

Tillage increases soil surface height and roughness and the amount of air-filled pore space. It also mixes the soil and creates different aggregate size distributions. In Northern Europe, primary tillage conducted in autumn aims to create a larger soil surface area that is prone to frost penetration. The soil structure is thereby affected and the soil becomes easier to modify by secondary tillage practices.

Different tillage practices result in different aggregate size distributions, although the final outcome is also dependent on soil water content at tillage timing. The schematic diagram of this process is illustrated in Figure 1 and is based on data from previous studies conducted on Swedish clay soils (Arvidsson & Bölenius, 2006; Keller *et al.*, 2007). In brief, tillage conducted at higher water content usually produces clods and large aggregates (>32 mm) whereas tillage conducted at lower water contents produces finer aggregates (*e.g.* Tisdall & Adem, 1986). Reduced tillage increases the amount of smaller aggregates (<4mm) which often represent less than 10% of total soil weight (Arvidsson & Bölenius, 2006). Large aggregates and clods (>32 mm) are usually dominant in clay soils after primary tillage in Northern Europe, whereas secondary tillage produces more fine aggregates. An increasing number of tillage operations increases also the amount of fine aggregates (Barzegar *et al.,* 2004).

Soil water content during tillage is important because it controls the amount of force needed to break up the soil. In clay soils and under dry conditions, the force needs to be fairly high, which results in increased fuel consumption and greater friction forces on the equipment. Various figures for the optimal water content for tillage of clay soils have been proposed and are reviewed by Dexter and Bird (2001). When the soil is dry, tillage usually produces high amounts of large aggregates, clods, but also very small aggregates, which can increase the risk of wind erosion (Keller *et al.,* 2007). Given that soil moisture increases after harvest in autumn, ideally tillage timing can be adjusted to target a certain water content, which would result in a certain aggregate size distribution, as described in Figure 1.

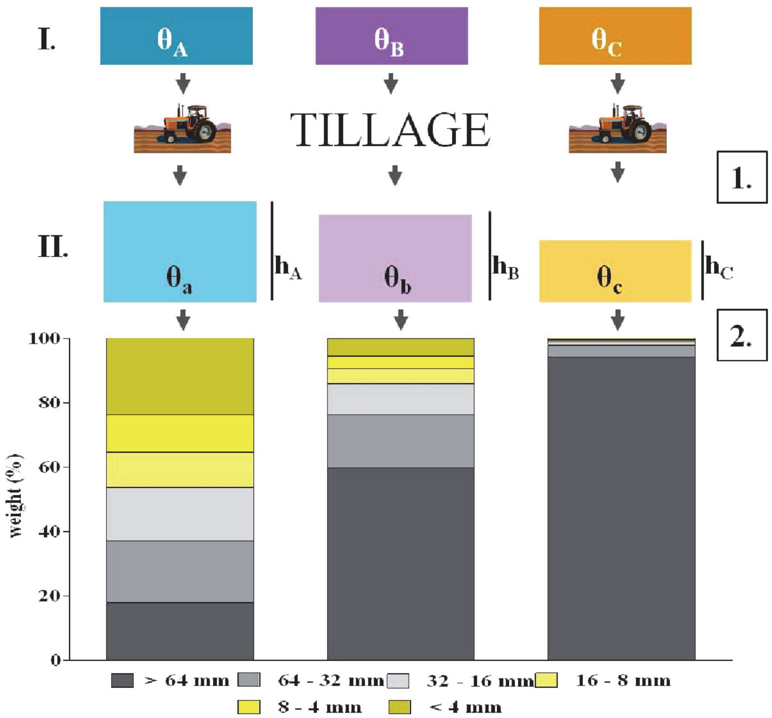

Figure 1. Schematic diagram showing tillage effects on soil structure with three different initial water content values (θ). The example shows shallow autumn tillage conducted on a soil with 58% clay content in Sweden. Data on aggregate size distribution taken from Arvidsson and Bölenius (2006).

I. **Soil before tillage**: Same soil with varying water content: $\theta_A < \theta_B < \theta_C$.

II. **Followed by tillage changes in 1. surface height and 2. aggregate size distribution: 1.** Increased soil surface height in the tilled layer: increase is highest with lowest water content: $h_A > h_B > h_C$. This increases gravitational potential, which leads to a direct decrease in water content in all soils: $\theta_A > \theta_a$, $\theta_B > \theta_b$, $\theta_C > \theta_c$, marked with lighter colour in the image. **2.** Tillage and initial θ affect aggregate size distribution: the amount of large aggregates and clods (>16 mm, marked with grey) is highest with highest water content, whereas the highest amount of small aggregates (<16 mm, marked with green/yellow) is produced in the lowest water content. Furthermore, relative surface area increases with decreasing initial θ.

The preference for a certain aggregate size distribution depends on the function targeted in the soil. Aggregates larger than 20 mm are not optimal (Adem *et al.*, 1984), because breaking these aggregates, when dry, requires a lot of energy. Aggregates larger than 5 mm provide protection against sealing and erosion (Lipiec *et al.*, 2012) and are important for gas exchange to seedlings as well as water infiltration (Heinonen, 1985). The optimal aggregate size for seed germination is between 1 and 5 mm (*e.g.* Carter, 1992).

Tillage affects many soil physical processes through changes in structure. Thermal conductivity is higher in water than air and since soil tillage increases the amount of air space, it limits the ability of the soil to transfer heat. However, a larger air space increases gas diffusion, since gas molecules move faster in air than in water. Increasing the total soil volume by a larger surface height affects the volumetric water content and results in more strongly bound water (higher water suction). Several studies comparing different tillage practices have shown that thermal conductivity is usually higher under NT and reduced tillage and lower under conventional tillage (Abu-Hamdeh *et al.*, 2000). This is most likely due to the larger air space with conventional tillage than with NT. Soil texture affects thermal conductivity; sandy soils have higher conductivity than clay soils, although sandy soils usually retain less water than clay soils.

3.2 Carbon Cycling in Arable Systems

In agroecosystems, the C inputs consist of above- and belowground crop residues, manures and various organic amendments. In general, soil C decreases with soil depth (*e.g.* Mielke *et al.,* 1986; Alvarez *et al.,* 1995; Angers *et al.,* 1997; Miura *et al.*, 2008). Carbon is transported in the soil profile through several mechanisms; *e.g.* in soluble form with mass flow or diffusion and through bioturbation by soil animals such as earthworms. Soil organic matter consists of a wide range of different organic substances and can be classified according to its physical, chemical or biological characteristics. A portion of SOM can be protected from decomposition either chemically or physically. Chemical protection involves organic substances being bound to mineral surfaces or chemically stabilised and not easily degradable (Six *et al.,* 2002). Physical protection involves SOM being situated inside aggregates or in small pores inaccessible to decomposer organisms (Balesdent *et al.*, 2000).

Sampling procedures including only the top 10 cm layer of soil can result in biased interpretations of SOM amounts between tillage treatments. Increases in soil C content are usually observed in the surface soil layer with NT compared with conventional tillage (Franzluebbers, 2008; Virto *et al.*, 2012). Nonetheless, when deeper soil layers and changes in bulk density are taken into account, the differences are often small or even reversed (Balesdent *et al.*, 2000; Govaerts *et al.*, 2009). In fact, the mechanisms behind tillage effects on SOM turnover are not completely understood (*e.g.* Balesdent *et al.*, 2000; Govaerts *et al.*, 2009). In cold temperate regions, positive effects of NT on C sequestration are not consistent (Regina & Alakukku, 2010) and are lower than in warm and dry areas (Govaerts *et al.*, 2009).

In most studies addressing the long-term effects of tillage, SOC content is measured but not soil respiration. Bolinder *et al.* (1999) showed that organic amendments had a greater influence on CO_2 production than tillage practices. VandenBygaart *et al.* (2003) found that converting from conventional tillage to NT increased the amount of SOC in Canadian prairies, but had no influence on soils in more humid regions common in eastern Canada. Furthermore, Lupwayi *et al.* (2004) found that SOM was more slowly decomposed in NT than conventional tillage. The review by Govaerts *et al.* (2009) illustrated the deviating experimental results found in long-term studies comparing conventional tillage and NT. Based on previous studies, it is obvious that detailed mechanistic studies are needed to understand the interactions between tillage operations, SOM decomposition and climate conditions.

3.2.1 Abiotic Factors Controlling SOM Decomposition

Water is vital for bacteria as a habitat, but also for mobility in soil and transport of substrates and enzymes by mass flow and diffusion. Since fungi grow hyphae towards substrates, they are less dependent on transport of compounds with water. Soil fauna in turn are usually more mobile and even less dependent on soil water amount and location. Soil water and temperature are the main drivers of microbial activity and thereby SOM decomposition. Water also has an indirect effect through soil aeration. Under dry conditions, microbial activity is limited by low substrate diffusion, while at high water contents it is limited by oxygen deficiency (Schjønning *et al.,* 2003). A strongly positive correlation between respiration and soil water content has commonly been observed (Roberts & Chan 1990; La Scala *et al.*, 2006; Yoo *et al.*, 2006; Jabro *et al.,* 2008).

Several terms can be used for describing or quantifying soil moisture or soil water content: gravimetric water content (W), volumetric water content (θ), water potential (ψ) and water filled pore space (WFPS). All of these measures have been applied in different studies. Yoo *et al.* (2006) stated that W is not an adequate indicator of biological activity because it does not provide any information about the volumetric water status or saturation level. Changes in bulk density affect the soil volume and the water available for the microbial community and this is not reflected by *W*. Water-filled pore space was proposed as a biologically relevant measure by Linn and Doran (1984). However, when studying undisturbed soil samples, the actual pore volume is often not known when starting an experiment with fresh soil (Schønning *et al.,* 2003). According to Yoo *et al.* (2006), the correlation between C mineralisation and the amount of water-filled pores is higher when pores with diameter less than 30 μm are used, rather than including pores of all sizes. Water potential is a suitable measure especially when comparing soils of different texture with regard to decomposition (Cook & Orchard, 2008).

Although water content and soil respiration are usually thought to be positively correlated, some aspects need to be considered. Scott *et al.* (1996) found a positive correlation between soil water content and respiration up to 80% of WFPS. Similar results are reported in a study by Watts *et al.* (2000), who found a positive correlation between respiration and *W* up to the plastic limit of the soil. In a study by Sey *et al.* (2008), CO_2 peaked at 40% and at 80% of WFPS, with the first peak considered to be from aerobic production and the second from anaerobic production. The optimal range of soil moisture for SOM decomposition seems to vary between soils. Sandy soils have lower water-holding capacity, higher bulk density and are better drained than clay soils. Furthermore, it appears that soil respiration is more sensitive to changes in water content in clay than in sandy soils. A meta-analysis by Moyano *et al.* (2012) concluded that respiration and soil water content are positively correlated in clay soils and negatively in sand soils.

Soil temperature, the other main controller of biological activity (Kätterer *et al.*, 1998), is positively correlated with biological activity (*e.g.* Singh & Gupta, 1977; Davidson *et al.*, 2006). The optimal soil temperature for microbial activity is between 20 and 40°C (Pietikäinen *et al.,* 2005), but the range varies depending on the types of decomposers present. Soil temperature and moisture are independent functions. Thus low soil moisture limits respiration regardless of temperature and low temperature limits respiration despite an optimal water content (Davidson *et al.*, 2006; Flechard *et al.*, 2007; Almagro *et al.,* 2009).

3.2.2 Biological Activity of Soil Aggregates

Soil organic matter and labile organic matter have been reported to be present in macro-aggregates (>0.25 mm and <2 mm) rather than in micro-aggregates (Tisdall & Oades, 1982; Jacobs *et al.*, 2009; Sundermeier *et al.*, 2011). However, contrasting results have been reported by *e.g.* Kristiansen *et al.* (2006). They concluded that the results depend on the sieving method used; studies using wet-sieving generally confirm results presented by Tisdall and

Oades (1982), whereas methods that avoid slaking, such as dry sieving, result in higher SOM amounts in micro-aggregates (<0.25 mm). Moreover, the biologically most active aggregates with the highest respiration rates have been found to be 1-4 mm in diameter (Fernández *et al.*, 2010; Jiang *et al.*, 2011). Only a few studies to date (Kasper *et al.*, 2009; Urbanek *et al.*, 2011) have examined the C and nitrogen (N) content in large aggregates (>2 mm) under conventional tillage and NT.

It is generally believed that SOM is more effectively protected from decomposition within microaggregates (Balesdent *et al.*, 2000). Degryze *et al.* (2004) found that most SOC is associated with mineral material and that aggregates between 53 and 250 µm stabilise particulate organic matter (POM) effectively. Dorodnikov *et al.* (2009) found increasing microbial biomass with decreasing soil aggregate size, while Sey *et al.* (2008) also found higher respiration from micro-aggregates than macro-aggregates. However, Yoo *et al.* (2006) did not find any differences in respiration between different aggregate sizes. In contrast, in a study made by Gupta and Germida (1988), the highest C and N mineralization rates were gained from macroaggregates and not from microaggregates. No tillage systems have been found to reduce respiration due to stabilisation of C in micro-aggregates that are occluded in macro-aggregates (Denef *et al.*, 2004). However, contradictory results have been reported for example by Plante and McGill (2002), who found higher respiration from NT than conventional tillage.

Studying soil respiration at the aggregate scale, Yoo *et al.* (2006) found that aggregates between 0 and 4 mm in diameter had the highest respiration at water content of 30% (gravimetric), but that the rate declined when W reached 50%. Larger aggregates showed no correlation between respiration and water content. Differences due to soil texture have also been found, *e.g.* by Scott *et al.* (1996), who found that respiration increased when soil texture changed from coarse to finer particles and that the correlation between respiration and water content was stronger in fine-textured than in coarse-textured soils.

Most previous studies on the effects of tillage on aggregates have focused on aggregate sizes smaller than 2 mm, while larger aggregates have been mostly neglected. From the schematic diagram (Figure 1) can be seen that primary tillage of clay soils in Northern Europe produces mainly large aggregates and clods (>4 mm) that correspond to at minimum 80% of total soil weight in the tillage layer. Furthermore, in the few studies in which all aggregate sizes have been included, the sampling depth has been shallow and it is questionable whether this depth allows comparisons between NT, reduced and conventional tillage. Balesdent *et al.* (2000) and Govaerts *et al.* (2009) state that a sampling depth of 0-30 cm should be used for reliable results. In summary, changes in soil structure due to tillage may explain the differences in respiration rates observed between different tillage systems.

3.3 Soil Respiration – Quantifying C Mineralisation

Soil respiration is normally referred to as CO_2 flux. If plants and carbonates are not present, the CO_2 derives mainly from heterotrophic organisms and measurement of this is considered to be an applicable method for describing biological activity in soil (Rovira & Vallejo, 2002; McLauchlan & Hobbie, 2004). In this thesis, soil respiration refers to CO_2 derived from SOM decomposition.

Respiration is generally stable at constant water content (*ceteris paribus*), but peaks occur after rainfall events (Denef *et al.,* 2001). Drying and wetting cycles seem to affect C dynamics only in the short-term, as respiration does not differ compared with control samples in the long-term (Priemé & Christensen, 2001; Beare *et al.*, 2009). Miller and Johnson (1964) found higher respiration with constant water content than with drying and wetting cycles. Although C mineralisation increased for a couple of days after re–wetting, it reverted thereafter and the increase did not compensate for the very low activity under dry conditions. Furthermore, rainfall has a different effect on soil respiration under different tillage practices. Jabro *et al.* (2008) and Akbolat *et al.* (2008) found higher respiration under conventional tillage than under NT after a rainfall or irrigation event. It seems that rainfall intensifies the effect of tillage by disrupting aggregates, and in this way increasing soil respiration.

A number of studies have reported generally higher soil respiration under conventional tillage than under NT (Alvarez *et al.,* 1995; Prior *et al.*, 2000; La Scala *et al.*, 2006; Reicosky & Archer 2007; Chatskikh *et al.*, 2008; Akbolat *et al.*, 2008). This can be explained by better aeration, enhanced contact between soil microbes and easily decomposable SOM, higher temperature, and exposure of physically protected SOM. Studies reporting higher respiration rates from reduced tillage than conventional tillage have suggested higher water content, increased biological activity in the surface soil and differences in SOM quality as explanatory variables (*e.g.* Kingery *et al.*, 1996; MacDonald *et al.*, 2010). Higher respiration under NT than under conventional tillage has been reported by Gupta and Germida (1988) and Kingery *et al.* (1996). Ball and Robertson (1990) found that respiration was not strongly affected by tillage. This shows that the results obtained to date on tillage effects on respiration are not consistent.

The immediate respiration response to soil tillage seems to be the most significant and is a common effect found in most studies, usually lasting from a few to 24 hours after tillage. Ellert and Jansen (1999) found a 2- to 3-fold increase in CO_2 flux during the first 30 min after tillage, but after 6 hours the flux had returned to the base level. Reicosky *et al.* (2005) also found a large loss of CO_2 during the first five hours following ploughing. Rovira and Greacen (1957) found that tillage treatments resulted in higher microbial activity in all soils investigated, and that this effect generally lasted for 24 hours. However, this immediate increase in CO_2 flux is most likely due to the release of stored CO_2 in soil rather than soil respiration. One should therefore be careful in interpreting short-term CO_2 fluxes following tillage. In this thesis, the CO_2 fluxes during the first hours after tillage treatment were disregarded in the evaluation.

3.4 Outlining Tillage Effects on C Mineralisation

A schematic diagram of tillage effects on soil properties and OM decomposition is presented in Figure 2. For simplicity and within the framework of this thesis, plants and organic amendments were excluded from the system and only plant residues were considered. The main purpose of primary tillage practices is to bury weeds and plant residues (1) and to loosen the soil (2) for secondary tillage practices and sowing; these processes have direct (process 3 and 4) and indirect (process 5 and 6) effects on soil respiration. Tillage directly affects soil structure by breaking down and creating aggregates and thereby increasing air-filled pore spaces. Aggregate breakdown or formation affects SOM decomposition through substrate exposure and changes in soil water status and thermal conductivity (7). Tillage affects the availability of substrates (fresh plant residues) by incorporation into the soil. Fresh plant residues are the main SOM source for decomposition during short-term periods in agroecosystems. Residue placement also affects soil moisture as well as thermal conductivity which, as mentioned previously, controls the activities of soil organisms (7). Mechanical mixing of the soil or tillage leads directly to a flush in CO_2, due to the release of CO_2 stored in deeper soil layers. Moreover, the mechanical disturbance directly affects larger organisms such as soil fungi and fauna and has been found to decrease e.g. earthworm, protozoa and nematode populations (8). In this thesis, the focus is on processes 1-4 and indirect processes 5-7 in Figure 2.

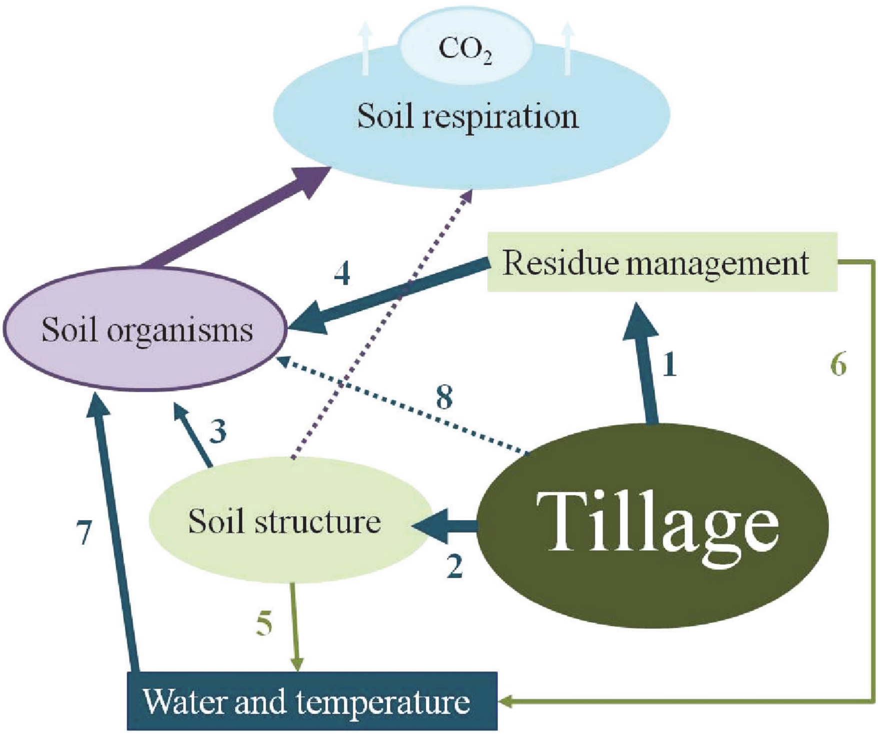

Figure 2. Summary of the processes behind tillage effects on soil respiration included in this thesis. Tillage directly affects crop residues by cutting and burying them (1) and soil structure (2). The residues are substrates for soil organisms (4) and soil structure constitutes the habitat for organisms and soil organic matter storage (3), both of which affect soil respiration. Changes in soil structure and residue management also affect soil water status and temperature (5 & 6), which affect soil organisms (7) and therefore soil respiration. Direct mechanical impacts on soil macrofauna *e.g.* earthworms (8). Purple lines present CO_2 fluxes.

4 Materials and Methods

4.1 Soil Respiration Measurements

4.1.1 Infrared Gas Analyser (IRGA)

All field measurements in this work were conducted with the same equipment allowing unbiased comparisons of results between different sites. A closed chamber (cylinder diameter 0.29 m) and a portable IRGA (GM70, CARBOCAP®, Vaisala) were used to measure CO_2 fluxes in the field. The advantages of this measurement system are its light weight, making it easy to transport between measurement points, and that measurements can be obtained rather quickly. The measurement system is presented in Figure 3. In brief, during a measurement a dark lid is placed on each cylinder, the air in the head space is circulated with a pump and changes in the concentration of CO_2 are measured during a 5-min period. All field respiration measurements were conducted in the afternoon between 13.00 and 16.00 each year. Soil respiration rates were calculated using the ideal gas law, as previously described by Berglund and Berglund (2011).

Weather conditions during autumn can make soil respiration measurements in the field difficult. In Sweden, air temperature together with respiration rates, often decrease rapidly after autumn tillage and the measurement period is narrow. When measuring low CO_2 fluxes a decrease in chamber headspace has been proposed for overcoming too low accumulation of CO_2 (Nagy *et al.*, 2011). However, the experiments in this thesis involved large soil surface roughness and we had to use wide cylinders to obtain representative gas samples. Furthermore, the cylinders had to be high enough to cover the whole tilled layer, in order to minimise the risk of preferential gas flow out of the area covered by the cylinder. All soil respiration-measuring chamber methods have advantages and disadvantages which are discussed in more detail in *e.g.*, Nay *et al.* (1994) and Jensen *et al.* (1996).

Figure 3. Infrared gas analyser (IRGA) in the field. The apparatus consists of a cylinder of various sizes that is placed into the soil. During measurement, a plastic lid is placed on the cylinder. Air is circulated with an air pump (black-silver box in the picture) to the IRGA, which measures the concentration of CO_2 over time.

4.1.2 Automated Respiration Analysis (Respicond IV)

The Respicond IV device (Nordgren Innovations, Djäkneboda, Sweden) is an automated soil respiration meter with which respiration can be measured continuously on a large number of samples at the same time. The complete system is described in detail by Nordgren *et al.* (1988). In brief, the equipment has 96 individual experimental cells that are placed in a water bath with adjustable temperature (Figure 4). In all experiments in this study, the temperature was set to +20 °C. The advantage of the system is that soil respiration can be measured continuously every 20 minutes simultaneously from all samples over long periods.

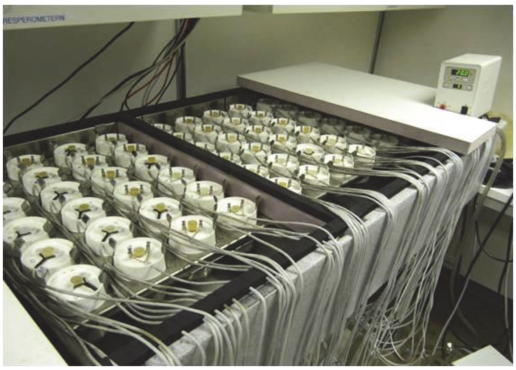

Figure 4. The set-up of the automated respiration analyser (Respicond IV).

The experimental cell (Figure 5) consists of a jar into which the sample is placed and a lid that has a smaller jar attached to it, into which an alkali trap (KOH) is inserted prior to analysis. Two platinum electrodes within each KOH jar are connected to a multiplexer that transfers the signal to a conductometer which detects and measures the change in conductivity and sends the reading to the computer. Carbon dioxide is dissolved into the KOH and forms potassium bicarbonate as in Equation 1.

$$2KOH + CO_2 \leftrightarrow K_2CO_3 + H_2O \qquad \text{Equation (1)}$$

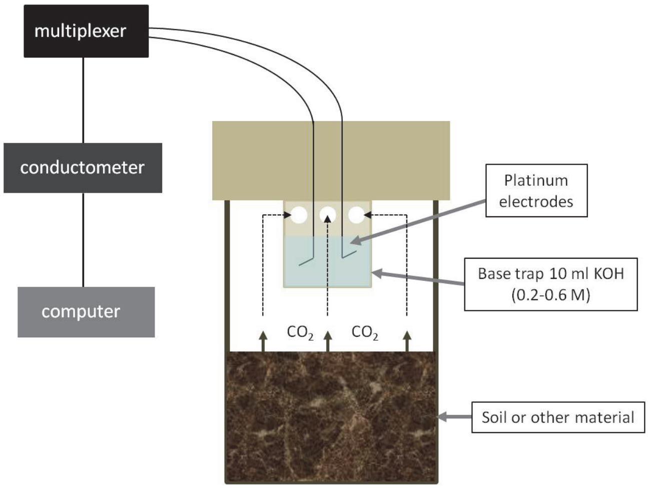

Figure 5. Individual measurement cell in the Respicond IV.

4.2 Field Studies (Paper I & II)

4.2.1 Soils and Sites

Three experimental sites with differing tillage history were selected, all located in Uppsala, Sweden. The main soil properties are presented in Table 1. The soils at all sites were classified as a *Eutric Cambisol*, with 43-58% clay and 1.8-2% SOC content representing typical agricultural soils in this area. At the start of the experiments, MP to 20-22cm or disc cultivation to 5 cm had been carried out since 1974 at Vipängen (Paper II) and chisel ploughing since 1999 at Ultuna. The third site, Säby, had been under MP for at least one century without other previous tillage treatments (Paper I).

Table 1. *Organic C content and texture in the soils used (Papers I and II)*

Site	SOC % (0-30 cm)	Clay content (%)	Silt (%)	Sand (%)
Säby	1.8	43	33	24
Ultuna	1.8	58	33	9
Vipängen	1.9	46	30	24

4.2.2 Experimental Plan

All field experiments had a randomised block design. Tillage treatments were: mouldboard ploughing to 20-22 cm (MP), chisel ploughing or disc cultivation (at Vipängen) to 5-6 cm (ST) and undisturbed plots representing no-till (NT). The Säby site also had a fourth tillage treatment: chisel ploughing to 12 cm (ST12). To minimise respiration effects from weeds, the fields were treated with glyphosate one week prior to tillage at Vipängen and Säby and about 3 months prior to tillage at Ultuna.

At Ultuna, tillage was carried out once, in the middle of September. At Säby and Vipängen, the tillage treatment plots were split and subjected to different sub-plot treatments. At Säby, tillage method was examined in the main plots and tillage timing in the sub-plots, with early and late tillage conducted in September and October, respectively. At Vipängen, long-term tillage method was the treatment in the main plots and residue incorporation in the sub-plots, with incorporation of crop residues to either 6 and 20 cm depth and undisturbed soil with either plant residues removed or left on the surface.

4.2.3 Soil Respiration, Moisture and Temperature Measurements

Emissions of CO_2 were measured with a closed chamber (cylinder diameter 0.29 m) and a portable IRGA (GM70, CARBOCAP®, Vaisala) at all field sites. Soil respiration was measured one day after tillage treatment and installation of cylinders and, thereafter, on 4-5 occasions during a 10-day period. Soil water content and temperature were measured simultaneously at 0-10 cm depth at points adjacent to the cylinders. Soil temperature was determined with a digital thermometer and *W* was determined in samples taken with an auger (3 augers per sampling) and dried at 105 °C.

Immediately after tillage, a dark plastic cylinder (height 15 cm, diameter 29.5 cm, material thickness 1 cm) was pressed into the soil in each sub-plot leaving about 3-5 cm of the cylinder above the surface. The soil around the outer wall of the cylinder was compacted to minimise the risk of air entering from outside into the cylinder. At the Vipängen and Säby sites, steel cylinders (height 40 cm, diameter 29.5 cm, material thickness 2 mm) were inserted into the MP plots to cover the whole tillage layer and therefore minimise the risk of lateral gas flow out from the area of the cylinder. Steel cylinders were needed in MP plots because the plastic cylinders could not withstand the force needed to insert them to about 35 cm. The thinner material also caused fewer disturbances in the soil. These steel cylinders also remained 3-5 cm above the soil surface. Flux -measurements started the day after the cylinders had been installed. Respiration was measured at all sites one day after tillage treatments and thereafter on various occasions and at various intervals depending on the weather conditions. A coefficient of determination of R^2=0.8 was used to exclude unsuccessful measurements. The measurements at all sites were made in a similar way. More detailed information about the measurements can be found in Paper I.

4.3 Soil Aggregates and Respiration (Paper II)

4.3.1 Site and Soil Sampling

The soil used in this study was collected from the Vipängen site described in section 4.1.1. Soil samples were collected from plots after primary tillage: mouldboard ploughing to 20-22 cm (MP) and disc cultivation to 5 cm (ST), conducted 1 month and 1 day, respectively, prior to sampling in the different plots. The experimental set-up had tillage method in the main plots and tillage timing in the sub-plots as at Säby (Paper I). Sampling was conducted by taking shovel samples from the tilled layer, from an area of approximately 0.1 m^2 in MP plots and 0.2 m^2 in ST plots; the larger area in ST was chosen to ensure that enough material was obtained for further analysis.

The field-moist shovel samples were fractionated into different aggregate sizes with the sieving apparatus illustrated in Figure 6. This apparatus has been used to determine aggregate size distributions in the tilled layer in *e.g.* studies by Arvidsson and Bölenius (2006) and Keller *et al.* (2007). The sample was poured onto the first sieve (64 mm) and the large clods were spread gently by hand so that finer material fell into the next sieve (32 mm). Finer aggregates were further transported to the sieves on an assembly line. Aggregates were separated into six different size classes: >64, 32-64, 16-32, 8-16, 4-8, and <4 mm. The sieve apparatus has no shaking force, but the different sized tubes rotate slowly. The main purpose of the apparatus is to separate aggregate size fractions from samples, not to create new aggregates, as would be the case when shaking and sieving equipment is used. Aggregates were stored at +5 °C before further treatments.

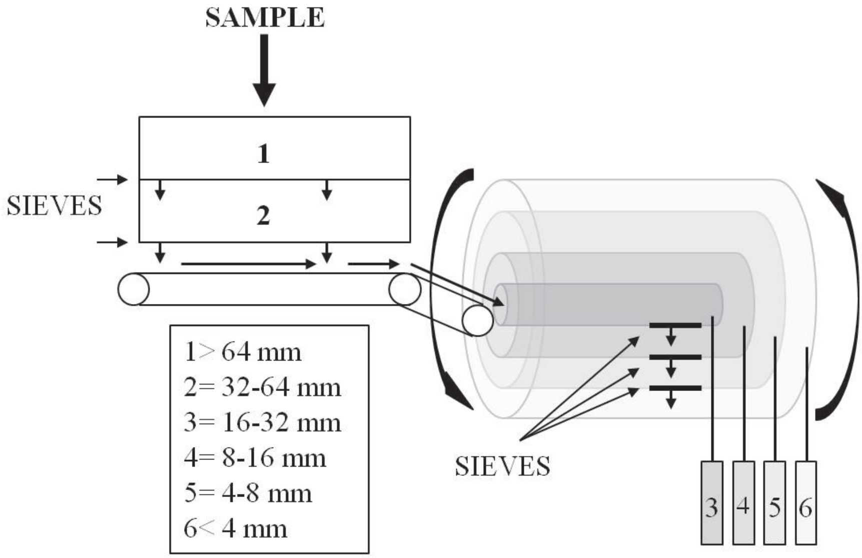

Figure 6. Aggregate fractionation apparatus. Samples are poured into the apparatus, which separates them into different aggregate size classes (1-6).

4.3.2 Experimental Plan

From each size class, aggregates were handpicked (at maximum 200 mL <62 mm, one aggregate >64 mm) and placed on a synthetic fabric so that they could be moved easily to the respiration analysis. The samples were laid on a sand bed adjusted to a water potential of -10 kPa and kept there until stable weight was obtained, after about one week. Thereafter soil respiration was measured at about +20 °C as described below.

For each soil respiration measurement the aggregates were placed in a plastic jar with volume 500 mL and 1000 mL for aggregates <64 mm and >64 mm, respectively (Figure 7). The measurement system is similar to that described by Persson *et al.* (1989). An infrared gas analyser was used and the concentration of CO_2 in the airspace of the jar was recorded manually immediately after closing the lid and thereafter every 10 to 20 min for 90 to 120 minutes.

The slope of the linear regression of CO_2 accumulation over time was used to calculate soil respiration rate. The coefficient of determination was $R^2 \geq 0.85$ for all samples. The flux of CO_2 was calculated using the ideal gas law as in Equation 2 and then divided by dry soil mass, as previously presented by *e.g.* Persson *et al.* (1989). However, the pH dependent solubility of CO_2 in water was excluded from the equation, since the water content was set at a similar level in all samples.

$$m = (res * P * V * M)/(R * T) \qquad \text{Equation (2)}$$

where, m=mg CO_2, res=ppm CO_2 h^{-1}, P=atmospheric pressure=101325 Pa, V=volume of air in the jar (L), M=molecular mass of CO_2 (g mol^{-1}), R= 8.314 J mol^{-1} K^{-1} and T is temperature (K). Air volume (V) was calculated by subtracting the volume of soil and water from the total volume of the jar. Soil solid volume was estimated by dividing dry soil mass with solid density (assuming 2.65 g cm^{-3}) and the volume of water was calculated from gravimetric water content. The mean value of the three measurements divided by dry weight of the aggregates in the sample was used for calculating the respiration rate (mg CO_2 kg^{-1} dry soil h^{-1}).

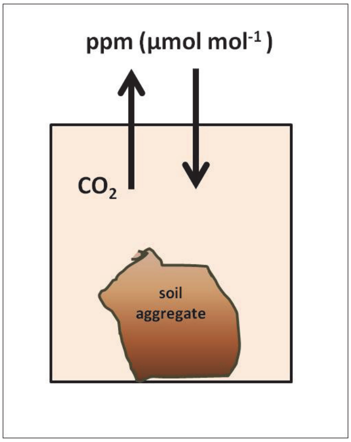

Figure 7. Soil respiration measurement unit in the laboratory. An aggregate is placed in a lidded jar, the air is circulated and the CO_2 concentration in the air is measured using an infrared gas analyser.

4.4 Physical Disturbance Induced Respiration (DIR) (Paper III)

4.4.1 Soils and Sampling

Undisturbed soil cores (54 cm^3) were sampled from a depth of 3-6 cm at seven different sites and stored at +5 °C before further treatments. Site properties are presented in Table 2. The sites represented different arable soils with different management histories and varied in soil texture and SOC content.

Table 2. *Soil properties at the different study sites used in the physical disturbance-induced soil respiration experiment (Paper III)*

Site/soil name	Clay (%)	Silt (%)	Sand (%)	SOC (mg kg^{-1})	Use	Location
Pustnäs	2	6	95	1.9	Pasture	59°47N, 17°40E
Röbäcksdalen	8	84	3	3.5	Arable	63°48N, 20°13E
Umeå	13	56	31	1.4	Pasture	63°48N, 20°14E
Sävja	21	47	32	1.9	Arable	59°49N, 17°42E
Säby	43	33	24	1.9	Arable	59°49N, 17°42E
Met-Stat	46	39	15	3.7	Grass	59°48N, 17°38E
Ultuna	46	39	15	2.3	Arable	59°48N, 17°38E

4.4.2 Pre-incubation and basal soil respiration rate (RES)

Water-filled pore space is generally used as a variable for describing the water status in soils in incubation studies. However, Schjønning *et al.* (2003) pointed out that WFPS is not the most optimal variable to use, since it does not include spatial variability in different soils. Water potential is a better water content parameter for undisturbed samples.

The water potential values used in Paper III were -1kPa, -10kPa and -30kPa, which were obtained with sand or ceramic tension plates. Samples were held on the waterbed for 7 days and thereafter respiration was measured for two to three days until a stable respiration rate was observed with Respicond IV (4.3.4). However, stable respiration can be misleading, since in incubation studies the respiration rate usually continues to decrease slowly with time (Winkler *et al.*, 1996). To determine the stabile initial respiration rate, we calculated the slope of observed respiration rates in relation to time and when the slope was equal to zero, the mean value for the last 10 h of measurements was used. All samples used in Paper III fitted into the two -day respiration measurements following nine days of pre-incubation.

4.4.3 Physical Disruption Method and DIR

The suitability of two different methods was tested in a preliminary study. The main aim was to find the method that produced the highest detectable respiration response, was fast and resulted in the lowest soil or water loss from samples. Different methods have been used to create physical disturbance, such as sieving (Hassink, 1992; Yoo *et al.*, 2006) or crushing by hand together with sieving (Roberts & Chan, 1990; Pulleman & Marinissen, 2004). Rovira & Greacen (1957) used a ring-shearing machine, Craswell and Waring (1972) used an electrical mortar to create soil disturbance, and Plante and McGill (2002) treated soil with an electric mixer (egg beater) to simulate soil tillage.

In Paper III, a rotary shaker with sieves (ø 2 mm) and an electric mixer (laboratory homogeniser) were used. In order to simulate different disturbance intensities, the duration was altered between the treatments: For the electric mixer the time scale was 5, 15 and 30 seconds, and for the rotary shaker 5 and 10 minutes with 150 rounds per minute. A sand soil was used (Pustnäs in Table 2) for its easy workability. Figure 8 shows the changes in soil respiration for each method used. Based on these results, we chose to use the mixer 5 s method in further analysis as that resulted in the highest change in respiration and did not result in water or soil losses during the procedure (data not shown).

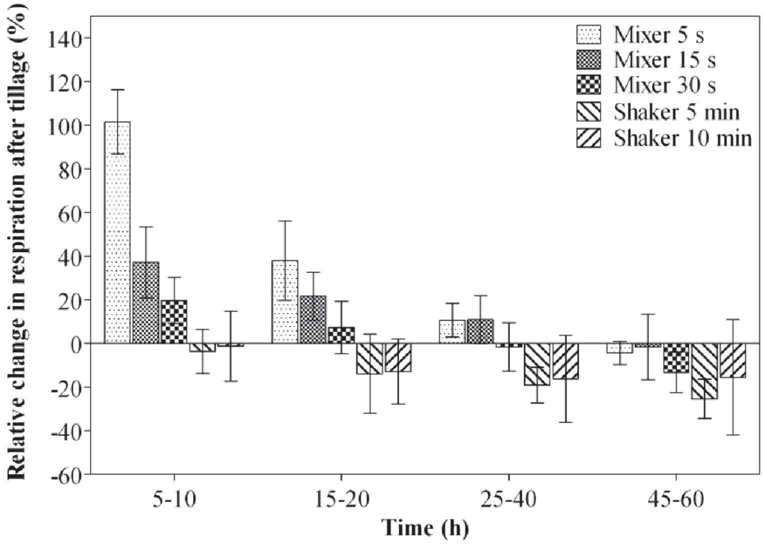

Figure 8. Preliminary study: Relative respiration response (basal respiration/respiration after treatment x 100%) to different physical disturbances under controlled conditions. Mixer = laboratory homogeniser with time span 5, 15 and 30 s, shaker = end-to-end shaker with time span 5 and 10 min. Mean values of 5 h of measurements, with standard error bars (n=5).

After determination of basal respiration (RES), the samples were treated with the mixing apparatus. Soil respiration measurements then continued. At maximum the whole process took 1 h and rates calculated after the treatment were taken to represent DIR rates. The accumulated value of change in respiration, DIR minus RES, was used to calculate the percentage of C respired from the SOC content.

4.5 Basic Soil Properties

Most of the soils used in this thesis were taken from sites for which data on basic soil properties were available. For all field and laboratory studies, SOC was determined by dry combustion on a LECO CHN-932 analyser. Soil gravimetric water content was determined by drying at 105 °C for 2 days. Dry bulk density (BD) was measured after drying and θ was calculated based on W in the incubation study (Paper III).

In Paper I mineral N (NO_{3-}, NO_{2-}, NH^{4+}) was determined on various occasions with a TRAACS 800 AutoAnalyzer (Bran+Luebbe, Germany). Soil sampling was conducted by pooling 4-5 soil cores per plot into one representative sample, described in detail in Paper I. The samples were collected from three depths 0 – 30, 30 – 60, and 60 – 90 cm (presented as >60 cm), and were frozen at − 18 °C within hours of sampling.

4.6 Statistical Analyses

In all experiments ANOVA and Tukey's test were used to test treatment differences, with the significance level set at $p<0.05$. Variables were log-transformed if necessary to obtain a normal distribution. Linear regression and Pearson correlation analysis were used for detecting relationships between two variables. All basic statistical analyses were carried out with GraphPad Prism 5.0.

4.6.1 Physical Properties and Respiration in the Field (Paper I and II)

All field data (Papers I and II) were analysed with a linear mixed model (*lme4* package, R Development Core Team, 2009), treating tillage, air and soil temperature, soil moisture and days after tillage as fixed effects and site and block as random effects. Model residuals were checked for normality. Likelihood ratio tests were performed to compare the model to a null model with only the random effects. Only significant variables were included. Results from the linear mixed effect model are presented as Markov Chain Monte Carlo (MCMC) estimated *p*-values with significance level 0.05.

4.6.2 Soil Respiration in Different Aggregates (Paper II)

Data on respiration from aggregates in Paper II were analysed with a split-plot model (GLM *procedure, SAS Institute, Inc., Cary, NC, software release 9.1. 2002_2004*). The effects of tillage method, residue handling and tillage timing on soil respiration and the interactions between *W*, SOC and aggregate sizes were investigated in the analysis.

4.6.3 Mechanical Disturbance Effects on Respiration - Physical Properties and Soil Texture (Paper III)

Basal soil respiration rate and physical-disturbance induced respiration rate were analysed with a generalised linear model (PROC GLM *procedure, SAS Institute, Inc., Cary, NC, software release 9.1. 2002-2004*) with ψ, *W*, clay content, SOC and BD, and RES as explanatory variables.

5 Results and Discussion

5.1 Soil Respiration Response to Different Tillage Practices in the Field

Soil respiration rates were lower in MP than in the other treatments at all three sites (Figure 9). The highest respiration was observed in NT which was significantly different from the ST and MP treatments at the Säby site. Similar results were observed in the Ultuna site, where respiration decreased significantly in the order NT > ST > MP. No significant differences in respiration rates were observed between tillage treatments at Vipängen. Furthermore, Vipängen and Ultuna had significantly lower respiration rates than Säby. The highest rates were observed in NT at Ultuna and Säby, although at Vipängen ST produced the highest respiration. These results are in agreement with previous studies (Kingery *et al.,* 1996) and indicate that tillage activities, especially ploughing, may reduce decomposition of crop residues moved to deeper soil layers (MacDonald *et al.*, 2010). Crop yields at Vipängen and Ultuna were 200 kg ha^{-1} lower in MP than in ST treatment which might have contributed to the respiration, however the difference is small. The preceding crop was oat at Vipängen and Ultuna and barley at Säby, which has lower straw dry mass than oat (Kim & Dale, 2004) and could not explain the high respiration observed at Säby compared to other sites. However, since Säby and Vipängen were treated with glyphosate only one week prior to tillage to kill weeds and could have contributed to some extent to the respiration rates compared with Ultuna, which was treated with glyphosate three months before the experiment. Glyphosate has also been found to increase microbial activity (*e.g.* Haney *et al.*, 2000).

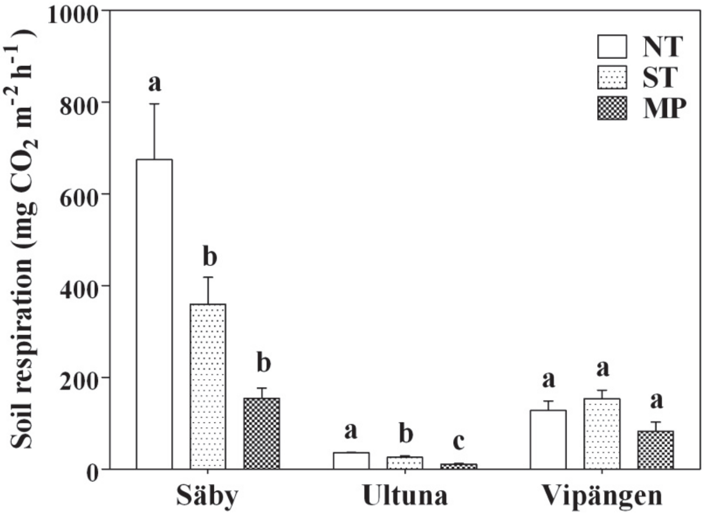

Figure 9. Soil respiration at different field sites. NT = no tillage treatment, ST = shallow tillage to 5 cm, MP = mouldboard ploughing to 20 cm. Mean values during the 10 days after treatment, with standard error lines (n=4).

At Säby, no differences in water content were found between MP and ST treatments during the 10 days following tillage, whereas soil water content decreased after MP at Ultuna and Vipängen (Figure 10). The higher moisture contents at Vipängen and Ultuna were probably due to annual differences. The mean air temperature during the growing season (May-September) was similar at Ultuna and Vipängen (13.7 and 13.8 °C respectively) but higher at Säby (14.3 °C). Furthermore, accumulated precipitation at Ultuna, Säby and Vipängen was 569, 577 and 669 mm, respectively. It is possible that higher clay contents at Vipängen and Ultuna (58% and 48%, respectively) than at Säby (43%) resulted in higher water content, since increasing clay content increases water retention (Emerson, 1995). Since SOC was similar at all sites and could therefore not explain differences in the water content under NT. Furthermore, *e.g.* Moyano *et al.* (2012) have reported that the clay content affects the relationship between soil moisture and respiration and therefore could explain some of the differences in respiration. As seen in Figure 11, soil temperature did not vary greatly between treatments, although differences between the sites were observed.

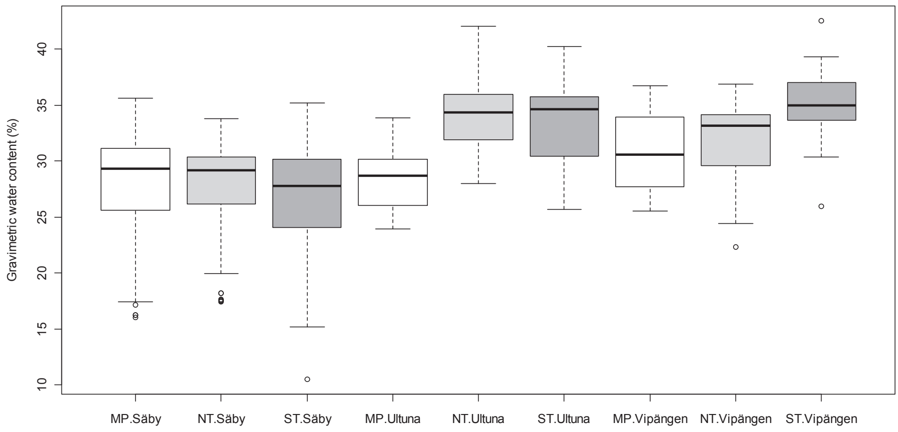

Figure 10. Soil water content at the different field sites. MP = mouldboard ploughing to 20 cm, NT = no tillage treatment, ST = shallow tillage to 5 cm. Mean values during the 10 days after treatment, with standard error lines (*n*=4).

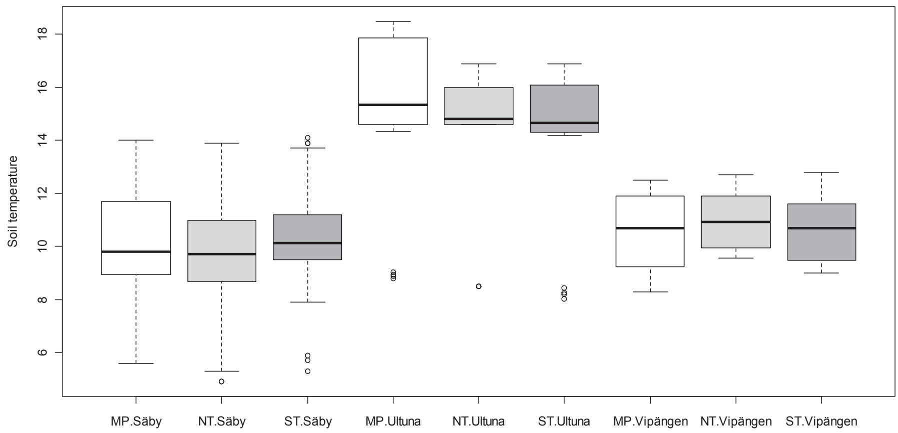

Figure 11. Soil temperature at the different field sites. MP = mouldboard ploughing to 20 cm, NT = no tillage treatment, ST = shallow tillage to 5 cm. Mean values during the 10 days after treatment, with standard error lines (*n*=4).

According to the linear mixed effects model, the most significant variables affecting soil respiration were tillage and days following tillage. The NT and ST treatments significantly increased respiration rate ($p<0.001$) by 8.7 and 4.9 mg CO_2 m^{-2} h^{-1}, respectively, compared with MP. In average over sites and treatments, soil respiration decreased during the first 4 days by 2.6 mg CO_2 m^{-2} h^{-1} ($p=0.07$) and between day 5 and 10 ($p<0.05$) by 5.8 mg CO_2 m^{-2} h^{-1}. The 'days after tillage' variable takes into account the decrease in soil respiration that results from the decrease in fresh plant residues available for decomposition, but this effect cannot be separated from temperature effects on decomposition, since soil temperature decreased with time. However, the model with the 'days after tillage' variable was significantly different from a null model without this variable. Thus, temperature did not fully cover this effect, indicating that a major part of the decline in respiration over time was governed by a decreasing pool of easily decomposable crop residues.

In previous studies, a significant relationship between temperature and soil respiration have been found (Flechard et al., 2007, Almagro et al., 2009). Neither soil nor air temperature could significantly contribute to explain the residual variance ($p=0.24$ and $p=0.53$) in this experiment. Since our study was conducted during a short-term period, the variation in temperature between treatments was low. There was an indication that increased soil moisture increased soil respiration, but the effect was not significant ($p=0.09$). According to the mixed effects analysis, interactions between tillage and soil water content or temperature were not significant. However, soil water content was found to be significantly lower in MP than in ST and NT at Vipängen and Ultuna, respectively. Residue cover in NT and increased surface roughness in ST and MP affected evaporation and resulted in lower W in tilled soil surface than in NT, supporting previous findings by *e.g.* Malhi and O'Sullivan (1990) and MacDonald *et al.* (2010).

Soil respiration was significantly different between sites (Figure 9). These differences were probably related to site properties, such as soil texture, quality of soil organic matter, microbial communities and biomass. The mean daily C loss through respiration during the 10-day period was 43 kg ha^{-1} in NT in Säby. The ST treatment decreased C losses by about 20 kg ha^{-1} and MP decreased them by about 34 kg ha^{-1} compared with NT. At Vipängen and Ultuna, the decrease in C losses brought about by tillage was smaller, between 0.6 and 3 kg ha^{-1} compared with NT. At Vipängen, ST resulted in the highest C losses (about 10 kg ha^{-1}) and the difference compared with NT was 1.7 kg ha^{-1} d^{-1} and to MP 4.6 kg ha^{-1} d^{-1}.

5.2 Linking Soil Structure and Biological Activity in Response to Tillage

Different tillage practices and tillage conducted at different water contents produced different aggregate size distributions (Figure 12). The proportion of large aggregates (>64 mm) was highest in MP, regardless of tillage timing and corresponded to 80- 90% of total soil mass in the tilled layer (Papers I and II). The amount of aggregates measuring 32-64 mm in diameter was significantly higher under ST than under MP regardless of tillage timing and amounted to about 15% of the total soil mass at both Säby and Vipängen. Based on the aggregate size distributions and water content measurements, these results agree with data from September and October tillage presented in the Ultuna site by Arvidsson and Bölenius (2006) in Figure 12. Tillage treatments were performed when *W* was about 29-34% at Vipängen and about 28-30% at Säby. In the study by Arvidsson and Bölenius (2006) *W* was 25-35%.

Since temperature and soil moisture could not explain the variation in respiration between treatments, differences in soil structure were the most probable explanation. One possibility could be that the amount of large clods and aggregates produced by MP decreased soil respiration. However, there was no difference between tillage treatments in the respiration rate for the largest aggregate size class at Vipängen (Paper II), but the biologically most active aggregate size fraction (<16 mm) was most frequent in the ST treatment (Figure 13). These results are partly in contrast to those of earlier studies, where a decrease in respiration with aggregate size has been observed (*e.g.* Tisdall & Oades, 1982; Jacobs *et al.*, 2009; Sundermeier *et al.*, 2011). In some recent studies, small aggregates (<4 mm) have been shown to cause high biological activity (Fernández *et al.*, 2010, Jiang *et al.*, 2011, Urbanek *et al.*, 2011), but this fraction was too small to be analysed in this study.

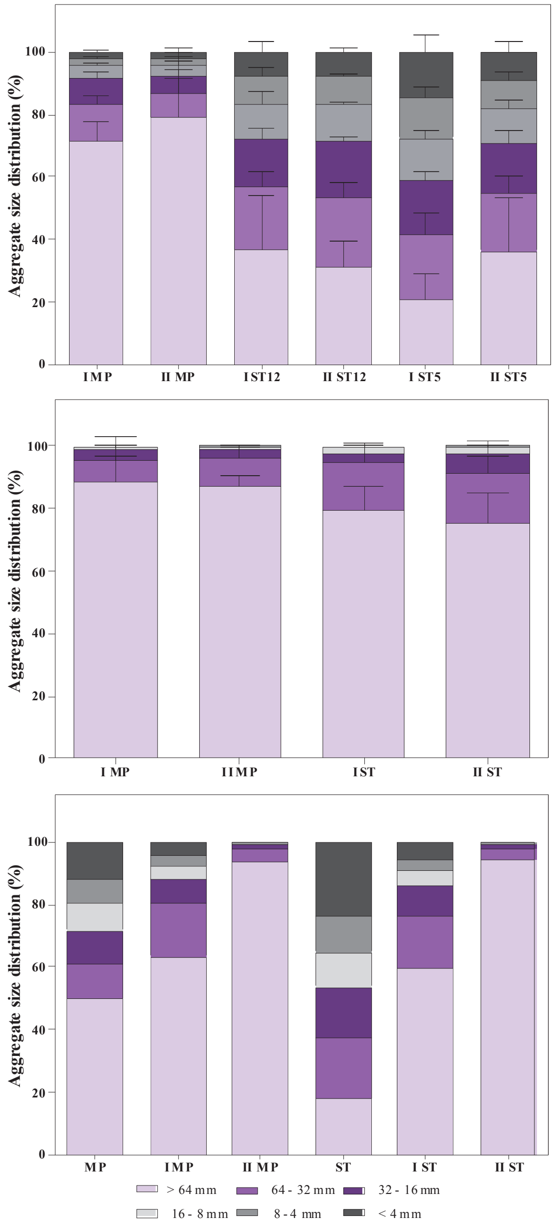

Figure 12. Aggregate size distribution at (top) Säby, (centre) Vipängen and (bottom) converted data from Arvidsson and Bölenius (2006). MP = mouldboard ploughing, ST = shallow tillage, I = September tillage, II = October tillage. Mean values with standard error lines for Säby and Vipängen (n=4).

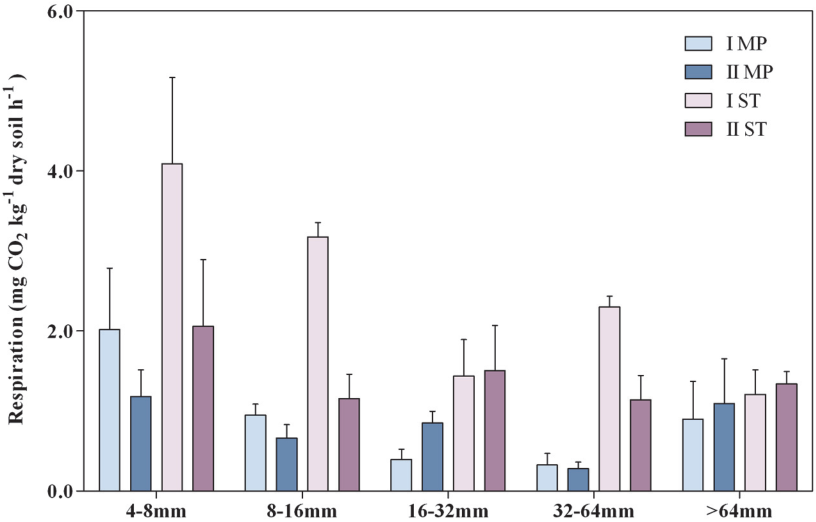

Figure 13. Respiration rate of each aggregate size class. MP = mouldboard ploughing to 20 cm, ST = shallow tillage to 5 cm, I = September tillage, II = October tillage. Mean values with standard error bars (n=4).

The results in Paper II indicate that the differences between the respiration rates of small aggregates represent differences in the activity of the decomposer community. In larger aggregates, the biological activity may have been limited by either lower gas exchange from the intra-aggregate space or, a lower concentration of oxygen inside large aggregates, as found by Smith (1980). However, the lowest gravimetric water contents were found in largest aggregates (Paper II), although the water potential was at the same level, which could have decreased respiration. The higher amount of large pores in large aggregates could also explain the lower gravimetric water content, as postulated by Usowicz *et al.* (2013), who found lower gravimetric water content with increasing aggregate size even though the water potential was at the same level.

Respiration from the tilled layer was calculated by scaling the respiration rate for each aggregate size according to the corresponding mass fractions (Figure 14). No significant differences were observed between tillage treatments or for tillage timing, though ST had slightly higher SOC contents especially in the size class 32-64 mm (Figure 14). The large size fractions (>32 mm) contributed more than 90% of total respiration from the tilled layer in both treatments due to a higher proportion of clods (>64 mm) in MP than in

ST. Total respiration was higher in ST than in MP because of the high amount of small aggregates and the high respiration in them. These results indicate that MP results in lower respiration in the field because of a higher proportion of large aggregates, which have less active SOM decomposition. However, physical disturbances such as frost penetration and slaking due to wet con4ditions during the winter and spring most likely lead to breakdown of these aggregates and can possibly result in higher respiration rates before or after secondary tillage.

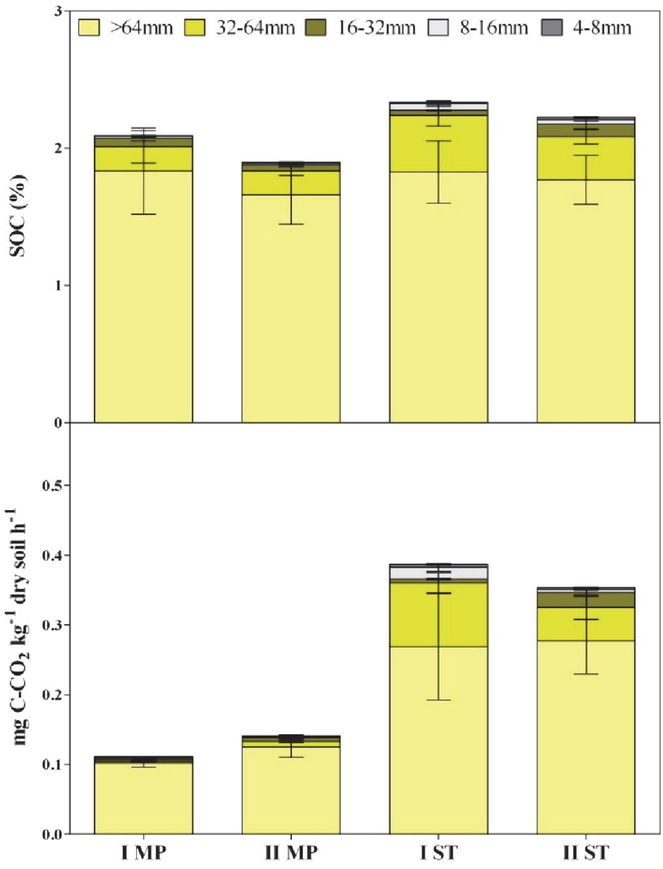

Figure 14. Soil organic carbon content (SOC, %) and soil respiration rate corresponding to an aggregate mass proportion of 1 kg soil. MP = mouldboard ploughing to 20 cm, ST = shallow tillage to 5 cm, I = September tillage, II = October tillage. Mean values with standard error bars (*n=4*).

5.3 Tillage Retarded Soil Respiration – A Residue Incorporation Effect?

Incorporation of residues decreased soil respiration compared with when residues were left on the soil surface (Figure 15). These results are in agreement with those by Christian and Miller (1986) and Ball and Robertson (1990), although contrasting results have been reported by Coppens *et al.* (2007) and Rottmann et al. (2010). Soil respiration decreased with increasing incorporation depth and was significantly lower in MP than in ST. This is in agreement with previous studies by Malhi and O'Sullivan (1990), Kingery *et al.* (1996), MacDonald *et al.* (2010) and Tyree *et al.* (2011).

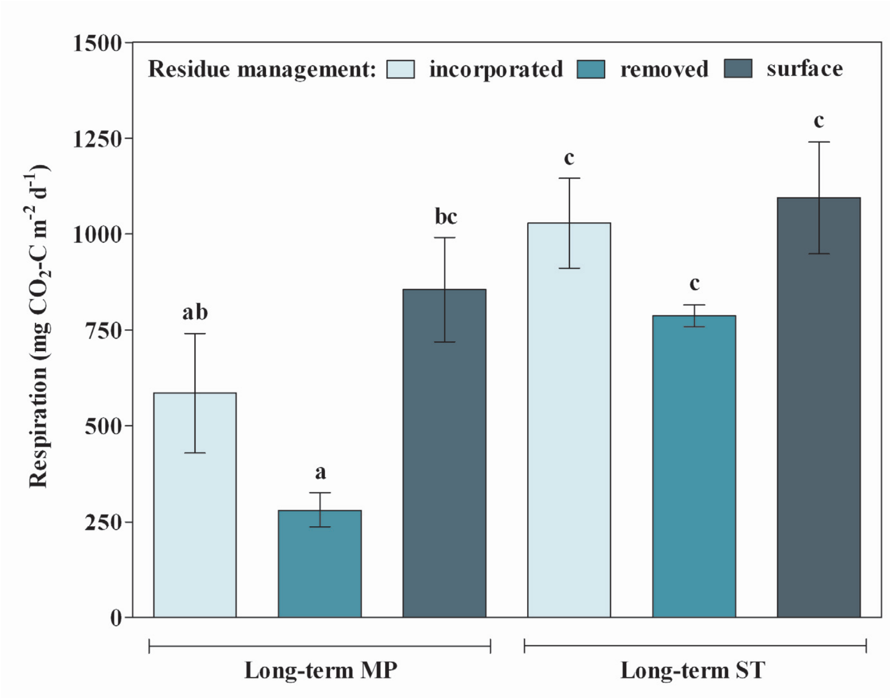

Figure 15. Soil respiration rates in different residue management treatments with long-term mouldboard ploughing (MP) or shallow tillage (ST). Residue treatments: Incorporation of residues with MP (20 cm) or ST (5 cm), residues removed and residues left on the soil surface. Mean daily values over a 10-day period with standard error bars ($n=4$). Different letters indicate significant differences ($p<0.05$).

Soil temperature was lower in the residue incorporation treatments, especially in MP, where residues were incorporated to greater depth (Paper II). Soil water content was significantly higher in ST (34-38%) than in MP (28-36%) throughout almost the entire experimental period (Figure 2 in Paper II). This is in agreement with several previous studies (Malhi & O'Sullivan, 1990, MacDonald *et al.*, 2010). Thus, lower soil temperature and lower water content with increasing soil depth can explain the difference in residue decomposition between MP and ST. Leaving residues on the soil surface probably lowered the thermal conductivity, which explains why tillage and residue removal from the surface decreased soil temperature, as has also been reported in previous studies (Azooz *et al.*, 1997; Abu-Hamdeh, 2000). A residue cover also prevents water evaporation from the soil and results in wetter conditions (van Donk *et al.*, 2010). Furthermore, the soil surface roughness in tilled soils increases the total surface area from which water can evaporate (Batey, 2009; Larsbo *et al.*, 2009). Residue incorporation (removal from the soil surface), regardless of tillage method, results in significantly lower soil water contents and, consequently, lower respiration rates (Almagro *et al.*, 2009; Moyano *et al.*, 2012).

Different residue management regimes in combination with tillage were also tested in the field plots. The idea was to compare whether surface cover and incorporation of residues resulted in similar decomposition under different tillage systems (Figure 15). On removing residues manually before ploughing and replacing them on the soil surface afterwards, the differences in soil respiration between MP and ST were not significant. This is in agreement with the findings by Bolinder *et al.* (1999) and MacDonald *et al.* (2010) who concluded that plant residues explain differences in SOM decomposition to a greater extent than the tillage method used. The results in this thesis showed that MP can significantly decrease the short-term decomposition of plant residues. Long-term tillage treatment and the short-term residue management explained about 78% of the variation in soil respiration.

The differences between NT treatments without residue cover and MP and ST were most likely related to differences in crop yield between the treatments during the previous year, when ST resulted in 35% higher yield than the other treatments. Moreover, crop yield is positively correlated with the amount of roots and stubble present in the soil, and therefore there were most likely higher inputs of fresh organic materials in the ST treatment. This further supports the view that the amount and management of plant residues play a greater role in explaining organic matter turnover than tillage *per se*. The increased biological activity, especially fungal activity, found in reduced tillage systems (*e.g.* Frey *et al.*, 1999; Jacobs *et al.*, 2011) is therefore probably caused by residue management.

5.4 Potential Short-Term C Mineralisation due to Physical Disturbance

Soil respiration in undisturbed soil cores under controlled conditions could be explained by the SOC, clay content and ψ corresponding to 63% of the variation measured (Paper III). Respiration rates decreased with water potential and were highest at -1 kPa in the Röbäcksdalen and Met-Stat soils, which also had the highest SOC contents (Figure 16). The interaction between clay content and ψ was a significant variable according to the generalised linear model. Higher volumetric water content was observed at the same ψ with increasing clay content. This result supports previous findings by Hassink (1992) and Wang *et al.* (2003), who stated that the increase in respiration due to water content is dependent on the clay content. Furthermore, a positive relationship between SOC and soil respiration was observed (Figure 17), confirming findings by *e.g.* Brooks *et al.* (2005) and Regina and Alakukku (2010). Both high clay and high SOC content resulted in lower soil bulk density and higher soil respiration. Therefore, the relationship between water content and respiration varies between soils and supports the results by *e.g.* Moyano *et al.* (2012).

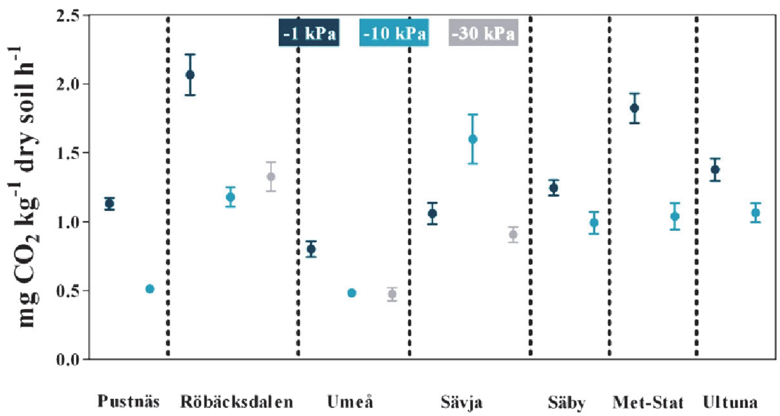

Figure 16. Basal soil respiration at different sites, with increasing clay content from left to right, and soil water contents. Mean values with standard error bars.

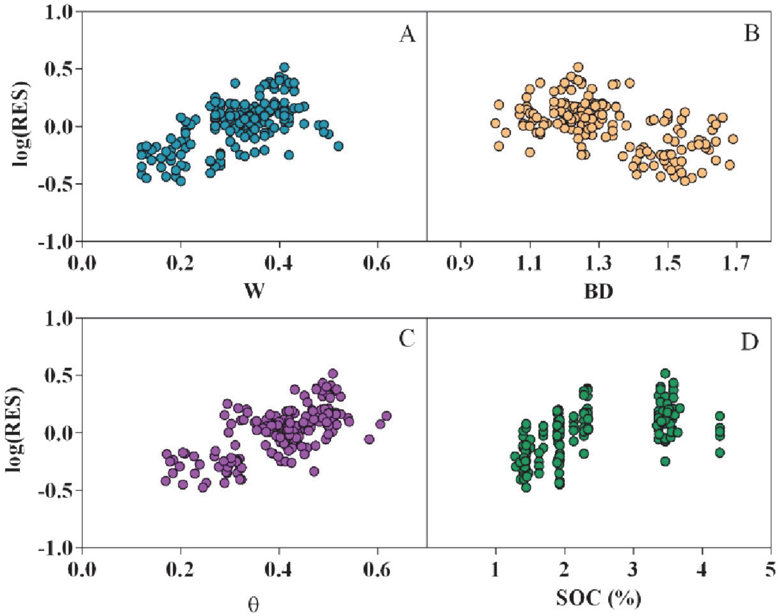

Figure 17. Basal soil respiration (log(RES)) in relation to A. gravimetric water content (*W*), B. volumetric water content (θ), C. bulk density (BD) and D. soil organic carbon (SOC).

A significant positive relationship between soil respiration, *W* and θ was found (Figure 17), in agreement with studies by Yoo *et al.* (2006), Jabro *et al.* (2008) and Almagro *et al.* (2009). The results in Paper III showed no evidence of decreased SOM decomposition due to oxygen limitation even at water contents near saturated conditions. Similarly, Schjønning *et al.* (2003) found that anoxic conditions take a long time to develop under laboratory conditions when the sample size is small.

Physical disturbance of soils induced up to 100 % higher respiration which lasted up to 320 h. The magnitude of the increase in respiration upon disturbance varied between sites and water contents (Figures 2a and 2b in Paper III). Several studies have shown that soil disturbance increases respiration, to more than twice as high as that in undisturbed soil (Powlson, 1980; Roberts & Chan, 1990; Pulleman & Marinissen, 2004). In Paper III, the increases in respiration were highest during the first day after treatment, after which the rate decreased again and returned to that of the untreated soil after about one week. The duration of enhanced respiration varied between sites and treatments (Table 3). Furthermore, the lowest water potential (-1 kPa) with mixing and re-compaction gave the highest respiration rate of all soils.

Accumulated respiration rate during 200 h after mixing the soils was calculated as C losses from SOC, as presented in Table 3. In general, the effects of mixing were greater under wetter than under dryer conditions. Low water contents resulted in decreased C losses, indicating that water content was limiting for soil respiration, regardless of substrate exposure by mixing. However, in the Röbäcksdalen and Umeå soils, the optimal water potential for the highest respiration was -10 kPa, and there were no statistically significant differences between water contents at these sites.

The relative C loss through respiration due to physical mixing was explained by clay content, water potential, SOC and physical mixing. According to the generalised linear model, these variables explained 86% of the variation (F-value 20.23, $p<0.0001$). A higher respiration response to mixing can be expected in soils with higher clay content due to the higher physical protection of SOM in such soils (Hassink, 1992; Wang *et al.*, 2003). Clay content was also the most significant variable affecting C losses via physical disturbance (F-value 20.72, $p<0.0001$). Water potential was another significant explanatory variable (F-value 16.00, $p<0.0001$). Physical disturbance treatment, D or DR, affected C losses, but exclusively through the interaction with clay and water content (F-value 3.15, $p<0.0001$). Furthermore, high soil water content and mixing resulted in a higher respiration response. However, in soils with high bulk density (1.5-1.6 g dm^{-3}), the response was the opposite.

Table 3. *Proportion of C respired from SOC following physical disturbance of soils at different water potential values (ψ). D = mixing, DR = mixing with re-compaction. Sites are presented with increasing clay content (Pustnäs < Met-Stat). Mean values, with standard error (n=5, *n=3). Minimum and maximum duration of respiration response in each treatment is also shown*

Site	ψ (-kPa)	D (%)	Duration (h_{min}-h_{max})	DR (%)	Duration (h_{min}-h_{max})
Pustnäs	1	0.12±0.06	30-200	0.18±0.05	3-177
	10	0.02±0.00	18-57	0.07±0.01	31-84
Röbäcksdalen	1	0.01±0.00	0-250	0.02±0.00	5-230
	10	0.02±0.00*	68-189*	0.07±0.02*	172-214*
	30	0.01±0.00*	44-60*	0.03±0.01*	146-214*
Umeå	1	0.00±0.00	0-22	0.00±0.00	0-30
	10	0.04±0.03*	22-215*	0.09±0.03*	101-214*
	30	0.00±0.00*	13-17*	0.02±0.01*	18-177*
Sävja	1	0.11±0.02	200-200	0.13±0.02	200-200
	10	0.04±0.02	17-74	0.02±0.01	28-135
	30	0.01±0.00	5-91	0.06±0.02	67-108
Säby	1	0.00±0.00	7-7	0.00±0.00	5-5
	10	0.00±0.00	14-39	0.00±0.00	14-26
Ultuna	1	0.08±0.03	0-320	0.09±0.01	250-320
	10	0.03±0.01	3-305	0.01±0.00	40-129
Met-Stat	1	0.09±0.03	134-320	0.19±0.07	295-320
	10	0.03±0.01	5-255	0.02±0.00	78-201

Physical disturbance resulted in a C loss of 74 kg ha^{-1} in the Met-Stat soil, with SOC content 3.4%, which was the highest value measured. Similar losses through physical disturbance have been found by Roberts and Chan (1990) and Rovira and Greacen (1957), who observed physical disturbance-induced C losses of between 20-60 kg ha^{-1}. The amount of SOC in the soil did not seem to affect the C losses, since at sites with less than 2% SOC the C losses varied between 30 and 50 kg ha^{-1}, which was also observed by analysis of variance (Paper III). The Röbäcksdalen soil, with SOC content 3.5%, resulted in C losses of 8.5 kg ha^{-1}, which was lower than from the other soils tested. This indicates that the organic material in this soil is highly recalcitrant and not greatly affected by physical disturbance. The results showed that potential C losses through physical disturbance cannot be estimated from SOC content, although variable including SOM quality may improve predictions.

Although C loss through soil respiration due to physical disturbance was observed under controlled conditions, this mechanism is most likely limited or hidden by other mechanisms under field conditions. Firstly, the absolute changes due to physical disturbance are too small to be measurable in the field even over a timescale of decades. Secondly, in the field, physical disturbance of the soil by tillage decreased soil respiration, which indicated that tillage affects soil respiration by inhibiting plant residue decomposition. Furthermore, during the 10 day period, when differences between tillage treatments were observed, the decrease in C loss by MP corresponded to roughly 340 kg ha^{-1}, which is significantly higher and also in the opposite direction of what was observed in the laboratory experiment. This result supports previous studies that found increases in soil respiration due to physical disturbance (*e.g.* Alvarez *et al.*, 1995; La Scala *et al.*, 2006; Reicosky & Archer, 2007) but also findings that residue management controls soil respiration to a greater extent than physical disturbance (*e.g.* Gupta & Germida, 1988; Kingery *et al.*, 1996; MacDonald *et al.*, 2010).

6 Conclusions

The main aim of this thesis work was to improve our understanding of the mechanisms behind soil C turnover as affected by primary tillage. In summary, tillage effects on soil respiration can mainly be attributed to residue management rather than changes in physical properties, soil water content, soil temperature, or structural changes in Swedish arable soils. Results from this work help to answer a part of the questions addressed in the introduction:

What mechanisms and interactions control C mineralisation following tillage activities?

- Residue incorporation by tillage inhibits their decomposition and is the main explanation for low respiration rates following tillage. This is due to lower soil water content and temperature in deeper soil layers.
- The production of large aggregates and clods in mouldboard ploughing results in lower respiration rates because large aggregates have low respiration rates and correspond to the largest mass fraction produced by tillage.

Is tillage *per se* a significant variable to consider?

- Carbon losses due to physical disturbance under controlled conditions can occur in different Swedish arable soils. This confirms previous findings that part of the unavailable SOM is exposed to decomposition by mechanical disturbance.
- Mechanical disturbance *in situ* by tillage activities should increase soil respiration, according to results obtained under laboratory conditions. However, the absolute changes are too small to be measurable in the field even over a timescale of decades.

What is the role played by water availability, temperature change and modification of substrate availability to microorganisms in explaining tillage effects?

- Even a small decrease in water content decreases the physical disturbance-induced increase in soil respiration and basal soil respiration rates.

How do different types of tillage affect these interactions?

- Early autumn mouldboard ploughing reduces soil respiration rates compared with shallow tillage or no tillage regardless of the tillage history.
- Increasing tillage depth decreases the decomposition of plant residues.

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Acknowledgements

This work was partly funded by the Swedish Farmers' Foundation for Agricultural Research within the project 'Effects of tillage on soil biological activity, nitrogen mineralisation and soil organic matter balance'.

I am grateful to many people who have helped me during this process and this work would have not come to conclusion without your contributions. Especially I want to thank my supervisors for giving me this chance to develop myself and expand my knowledge and several other peoples contributions for making this work possible:

- I am grateful for my main supervisor Thomas Kätterer for the discussions and patience while guiding me, for keeping my feet on the ground, for always being available and helping me when in need. I appreciate a lot your expertise, advices and attitude that one can still have a laugh once in a while even though the situation is serious.
- I want to also thank Holger Kirchmann for supervision, your guidance and feedback has made me a significantly better researcher and writer, also your thrive for research, constructive criticism and fearless attitude towards new projects inspires me.
- Thanks to my supervisor, Johan Arvidsson, for being a great help not just at the desk job but also in the field, I appreciate your patience and help in solving the challenges I have met.
- I would have not done this thesis without the help of the technical staff in the field and laboratory. Thank you Mulle and Aaron for doing the most important work in the field - the tillage, I could have not done the experiments without you. Also, spending little time at your office reminded me to always have in mind the applied aspect of soil science, what is the purpose of it and who does it serve in the end. I want to thank Jerker and Liselott for helping me with the field sampling and

measurements in 2012 after my surgery, I am not understating if I say I would have bled to death without you. Big thanks to the ladies in the laboratory: Inger, Rose-Marie, Christina and Annika for the basic soil analysis and helping me find all the needed equipment for my experiments.

- Thanks to everyone in the soil biology and plant nutrition group for your support and all the nice “skördefester” we have had together.
- BIG UPS for my homies at the department Carina, Maja, Martin and Stefan for all the laughs and support.
- I am grateful for Mary McAfee for the great language editing, Claudia and Ulf for the statistical support. Anna Mårtensson and Jon Petter Gustafsson for good comments for the thesis. Kristin Boye and Anke Herrmann for reading my thesis and giving it a quality lift.
- Iso kiitos mun perheelle Äiti, Isä, Tylle, Eero ja Airi kaikesta tuesta ja jaksamisesta, ilojen ja surujen jakamisesta.
- My wingwomen Inka, Maija ja Riina. En mä mitään terapiaa tarvitse kun mulla on teidät. Piditte mua pystyssä tosi vaikeina aikoina ja tuun aina ja ikuisesti olemaan kiitollinen teille ja rakastamaan teitä. Mä voitin kaverilotossa kun sain teidät - v*eni vidi vici*
- My ”Swedish” family, the Boye family, thank you so much for everything and taking me as a part of your family during my time in Sweden. I miss you guys so much.
- Suuret kiitokset kaikille ihanille suomi-ystäville mm. Antti, Jaakko, Eija, Emmi, Anna-Kaisa, Mina, Minna, Jonna ja Jode, Late, Mikko, Pauliina, Niina T., Niina ja Tuukka, Laura ja Mikke, Fagerholmit, Kainiemen ja Kalliaisen suku.
- I also want to thank the day when I started climbing that has had a huge impact on me and my development as a person. Most of all I am grateful for meeting all the fantastic and inspiring people here in Sweden, thanks for sharing all the experiences with me and for all the good times: Olle, Elina, Isabelle, Magnus, Annax2, Lara, Kalle, Francis, Ry, Conny, Joel, Anders, Jenny och Johan, Micke, Calle, Jonas and many more.