The effects of site preparation on carbon fluxes at two clear-cuts in southern Sweden

Master degree thesis, 30 credits in *Physical Geography and Ecosystem Analysis*

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30 ECTS

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Lund University, Sweden
2014
Abstract

Clear-cutting is a common forest management practice. It alters the carbon balance of forests by eliminating photosynthesis through canopy removal and affecting autotrophic and heterotrophic respiration. In Sweden, almost 200 000 ha of forest is clear-cut every year and site preparation is carried out on more than 80% of it. The effects of different site preparation methods on the soil CO₂ fluxes were studied at two adjacent clear-cuts in southern Sweden from June to October 2013.

The first site was a recently harvested clear-cut, where a block experiment was established to compare soil CO₂ flux of harrowing and mounding site preparation with control plots using chamber measurements. Four treatments were compared: undisturbed soil (control), mineral pit after harrowing (harrowing-mineral), mound with double humus layer (harrowing-mound) and mound with double humus layer capped with mineral soil (mounding). The highest soil CO₂ fluxes were obtained from harrowing-mound plots due to the double humus layer and increased temperatures. Although mounding plots had similar carbon content and high temperatures as harrowing-mound plots, the flux was suppressed by too low soil water content. Control plots showed higher soil respiration than mounding except in July, when the flux was inhibited by very dry conditions. The lowest soil CO₂ flux was found from exposed mineral soil as humus layer had been removed.

The other site was a harrowed 2-year-old clear-cut. Chamber measurements and eddy-covariance measurements were carried out to study the carbon balance of the site. The harrowing-mound plots at the recently harvested site had higher soil respiration than at the 2-year-old site due to fresh substrate available for decomposition. However, the respiration from mineral plots at the 2-year-old site was higher than at the recently harvested site probably due to the enhanced root growth and increased belowground autotrophic respiration. The 2-year-old harrowed site was a source of carbon by 145 g C m⁻² during the study period from the end of May to the end of August. Soil respiration contributed a major part to the ecosystem respiration at a 2-year-old site showing that site preparation has an important effect determining not just soil respiration but carbon balance of the site. The results highlight the importance of studying the carbon balance continuously during stand development to determine the long-term effects of site preparation.

Keywords: geography, physical geography, soil CO₂ flux, site preparation, eddy covariance, chamber measurements
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1 Introduction

Forests cover 30% of the land surface and play a big role in the global carbon cycle by sequestering carbon from the atmosphere (Bonan 2008). They take up carbon through photosynthesis and release it by respiration. Soil respiration is a major component in the carbon balance of terrestrial ecosystems (Subke et al. 2009). Forest soils experience increasing disturbance from climate change and forest management (McDaniel et al. 2014). Even small changes in forest soil CO$_2$ fluxes can have important impacts on the net carbon balance (Pumpanen et al. 2009). Clear-cutting is a common forest management practice. It alters the carbon balance of forests by eliminating photosynthesis through canopy removal and affecting autotrophic and heterotrophic respiration (Zha et al. 2009). As a result, a harvested site is expected to turn into carbon source for several years after harvesting (Mathys et al. 2013; Kolari et al. 2004). The carbon sink or source status of a harvested forest is important to the calculations of regional C balance and for forest management strategies (Giasson et al. 2006).

In Sweden, almost 200 000 ha of forest is clear-cut each year. During the last 10 years, site preparation was carried out in more than 80% of the clear-cut areas (Swedish Forest Agency 2013). Site preparation is commonly done in order to increase the seedling establishment (Johansson et al. 2013). It alters environmental conditions like soil temperature and soil water content which generally results in an increase of microbial activity and decomposition rates (Johansson 1994; Slesak et al. 2010). This leads to an increase in soil respiration (McDaniel et al. 2014). There are a few studies investigating the effect of different site preparation methods on soil CO$_2$ flux (Mallik and Hu 1997; Mojeremane et al. 2012; Pumpanen et al. 2004a; Strömgren and Mjöfors 2012). The soil CO$_2$ flux was found to be the lowest in the exposed mineral soil and the highest in treatments with double humus layer (Pumpanen et al. 2004a; Strömgren and Mjöfors 2012). Mallik and Hu (1997) reported that soil CO$_2$ flux in different site preparation treatments was mainly controlled by modified soil water content and organic matter content, while Mojeremane et al. (2012) found temperature to be the main environmental variable controlling CO$_2$ emissions.

Although the effects of site preparation on soil respiration have been studied before, more detailed information is needed to estimate the carbon emissions caused by different forest management practices. The objective of the present study was 1) to investigate the effects of different site preparation methods on soil respiration and analyse the effects of the environmental factors (temperature, moisture and C and N content) controlling the flux, 2) to compare soil CO$_2$ fluxes from treatments caused by harrowing at a recently harvested site to fluxes from a 2-year-old site 3) to examine the role of soil respiration in the net carbon balance at a 2-year-old clear-cut. The study was conducted at two adjacent sites, one recently harvested and the other 2-year-old clear-cut. Chamber measurements were conducted at both sites to study the soil CO$_2$ flux from different treatment areas. In addition, eddy covariance measurements were carried out on a 2-year-old harrowed clear-cut.
2 Theoretical background

2.1 Carbon cycle

The carbon balance in terrestrial ecosystems is determined by the rate of uptake (photosynthesis) and losses (ecosystem respiration) of carbon (Paul 2007; Valentini et al. 2000). Photosynthesis uses light energy and water to convert inorganic C into organic C. This process is termed gross primary production (GPP). A part of this assimilated carbon is returned back to the atmosphere by plant respiration called autotrophic respiration, which can be divided into aboveground and belowground autotrophic respiration. The other part of assimilated carbon is used for new biomass production, root exudation, transferred to microbes that are symbiotically associated with roots or emitted as volatile compounds for defence against herbivores (Paul 2007; Chapin et al. 1990). Dead woody tissues from plants and dead leaves, flowers, mosses and lichens form a litter layer on the ground surface. The decomposers in the soil break down carbon from both fresh litter and older soil organic matter (SOM) resulting in the release of CO$_2$ which is called heterotrophic respiration (Schulze 2000). The mineralisation process yields also inorganic forms of other nutrients that are taken up by plants (Brady and Weil 2002). Soil respiration is the combined respiration of living roots and rhizosphere organisms (autotrophic respiration) and microbial decomposition of litter and SOM (heterotrophic respiration). Ecosystem respiration is the combined autotrophic and heterotrophic respiration from a whole ecosystem. In forest ecosystems, soil respiration is often dominating the total ecosystem respiration (Valentini et al. 2000).

Forest management by clear-cutting changes the carbon balance of the site. Canopy photosynthesis is eliminated and removal of stem-wood extracts up to 1/4 of C from the ecosystems in the northern temperate forests (Zha et al. 2009; Dixon et al. 1994). Large amounts of fresh litter and decaying roots and stumps enhance heterotrophic respiration. In addition, the increase in temperature and modified soil water content promote the microbial activity and respiration (Slesak et al. 2010). At the same time, growth of root and rhizosphere exudates is reduced and the belowground autotrophic respiration decreases (Buchmann 2000). After harvesting, the carbon balance of the stand is mainly determined by heterotrophic respiration as photosynthesis and autotrophic respiration are minimal. Therefore, post-harvest sites are usually sources of carbon for several years after harvesting (Kolari et al. 2004; Zha et al. 2009). The time for recovery from a source of carbon to sink depends on site productivity and vegetation structure (Hyvonen et al. 2007). Due to enhanced decomposition rates and decreased uptake of nutrients, clear-cutting can result in a loss of soil C and nutrients in the long term as the flow of rhizosphere exudates and annual litter input are reduced until a new stand has established (Hope 2007; Pumpanen et al. 2004b; McDaniel et al. 2014).

2.2 Site preparation

Management practices following harvesting change the soil environment. Site preparation after clear-cutting is commonly used in forestry to promote germination of seeds, seedling growth and to favour the survival of planted trees (Pumpanen et al. 2004b). Competing vegetation is reduced
and therefore competition for water and nutrients is also reduced (Mallik and Hu 1997; Orlander et al. 1996). Another favourable effect of site preparation is decreased insect damages. By planting seedlings directly in mineral soil, the damages related to pine weevils are decreased substantially (Petersson et al. 2005). Soil disturbance improves soil water content and temperature which encourages growth of soil bacteria and decomposition (Chapin et al. 2011; Johansson 1994). The enhanced rate of decomposition and mineralisation mean nutrient release that is needed for the growth of seedlings and planted trees (Lundmark-Thelin and Johansson 1997).

The most common site preparation method in Sweden is harrowing. It is usually done by exposing the mineral soil and mixing organic layers into adjacent ridges (Strömgren and Mjöfors 2012). This method is applicable at sites that are moderately moist (Johansson et al. 2013). Planting is made in the trenches of mineral soil where the humus layer is removed. Exposed mineral soil increases temperatures and therefore decreases the risk of night frosts (Orlander et al. 1996). However, there is a risk of poor nutrient supply (Johansson et al. 2013).

Mounding is one of the other site preparation method used (Johansson et al. 2013). It is done by placing humus patches upside-down next to the mineral pit capped with 10-20 cm mineral soil on top. This leaves patches of elevated mounds and pits of mineral soil (Smolander and Heiskanen 2007). Mounding can be applied on mesic to wet soils with moderate stoniness. Planting is done on elevated spots (Johansson et al. 2013). Mounding enhances nutrient availability, soil temperature and reduces competition. However, it can increase the risk of drought stress in the newly planted seedlings (Nilsson and Orlander 1999).

2.3 Factors controlling soil respiration

Most authors rate molecular diffusion and advective flow to be the main transport processes of soil CO₂ flux in air-filled pores (Jassal et al. 2005) with the contribution from liquid phase diffusion in the saturated conditions (Fang and Moncrieff 1999). The diffusion of CO₂ depends on the concentration gradient between the layers, soil water content, porosity of soil layers and layer thickness. However, the flux is also affected by barometric pressure changes, displacement of ground air by infiltrating water (rain) and pressure changes caused by wind gusts (Kutsch 2009).

As heterotrophic respiration is a major process determining the carbon balance after clear-cutting, it is important to investigate the factors controlling the decomposition rates. The efficiency of soil organisms to break down litter and soil organic matter (SOM) and produce CO₂ is mainly controlled by the substrate availability and quality, soil texture and porosity, pH, nutrient concentration and environmental conditions (Paul 2007; Chapin et al. 2011; Kutsch 2009).

The concept of substrate quality is associated with degradability (Kutsch 2009). Residues high in proteins and easily degradable carbon-based compounds decompose rapidly (Kutsch 2009;
Chapin et al. 2011). These are for example nutrient rich leaves from productive sites with a short life span. However, residues high in compounds that require high activation energy (e.g. lignin and polyphenols) decompose slower, e.g. evergreen leaves (Chapin et al. 2011). Decomposition of soil organic matter is affected by its properties. Clay minerals in SOM increase water-holding capacity, restrict oxygen supply and therefore reduce decomposition (Chapin et al. 2011).

High C/N ratio of organic matter plays important role in degradability. Soil organisms metabolize carbonaceous materials to build essential organic compounds and obtain energy for life processes; however they also need sufficient nitrogen to synthesize nitrogen-containing components. Therefore, the C/N ratio of plant residues is important in order to determine the rate of decaying. If the C/N ratio is high, there is a competition between soil microorganisms for the nitrogen (Brady and Weil 2002). Studies have shown that litter with low C/N ratio (high nitrogen concentration) generally decomposes faster than litter with high C/N content (Chapin et al. 2011). Usually the C/N ratios in forests humus horizon are 30/1, but when such soils are cultivated, the enhanced decomposition lowers C/N to near 12/1 (Brady and Weil 2002).

Environmental conditions like soil water content and temperature are regulating soil biological activity critically. Temperature affects many physical, chemical and biological processes in soil. When other parameters remain constant, molecular diffusion always increases with increasing temperature (Paul 2007). Microbial activity and respiration increase exponentially with short-term increases in temperature, enhancing the mineralization of soil organic matter (Chapin et al. 2011). Temperature sensitivity is generally described by \( Q_{10} \), which shows the increase of the rate of the \( \text{CO}_2 \) emissions caused by a 10\(^\circ\)C increase in soil temperature (e.g., if \( Q_{10}=2 \), the \( \text{CO}_2 \) flux doubles for a temperature increase of 10\(^\circ\)C) (Paul 2007; Lloyd and Taylor 1994). In the absence of moisture stress, higher \( Q_{10} \) values mean higher temperature sensitivity (Khomik et al. 2006). The reported values vary between 1.3 and 3.3 (Raich and Schlesinger 1992) but in cool temperate and boreal regions the value can range from 4 to 6 (Davidson et al. 1998; Khomik et al. 2006).

Different microbial communities are likely to be active over a range of soil water content conditions. However, low soil water content levels result in decline of microbial activity due to thinner film of water on soil particles which decreases the rate of diffusion of substrates to microbes (Paul 2007). Osmotic effects also reduce the microbial mobility (Chapin et al. 2011). High soil water content conditions reduce decomposition and therefore heterotrophic respiration. The reason is that water acts as a barrier to oxygen supply and microbial activity is then restricted (Chapin et al. 2011).

The microbial activity in soils, and thus the \( \text{CO}_2 \) production, depends on the interaction of several different factors. Under the same environmental conditions, substrate quality controls the decomposition rate. However, when soils are dry, moisture is controlling the respiration. When water availability is not limited, the microbial activity is controlled mainly by temperature (Paul 2007).
3 Materials and methods

3.1 Study site

The study included two adjacent sites that were located near Sjöbo in Skåne, southern Sweden (Figure 1). The ground vegetation was dominated by mosses and shrubs. The soil had a sandy texture and a thin humus horizon. The average air temperature per month from June to October for the period 1961-2013 was 14.4°C and average precipitation 68.2 mm (measured in Lund, 35 km from the study area) (SMHI 2014). The study period of 2013 was warmer and drier than the average (15.2 °C and 58.7 mm per month from June to October, respectively) (SMHI 2014). The site index was G28, which shows that the site had a relatively high productivity (the potential height of the trees at the age of 100 years is 28 m).

The first site was located at 55° 40' 13.62" N, 13° 39' 10.7274" E and was harvested in February 2013 (called "clear-cut 2013" in Figure 1). Prior to harvest, the dominant vegetation was 69-year-old Norway spruce (Picea Abies) (tree species not confirmed by the forest owner). During harvest 6415 m$^3$ of timber was harvested. A block experiment was established on unplanted and unprepared soil in the beginning of June 2013. Transport of timber and branches was going on during the experiment. After this study, the site was harrowed in April 2014 and Larch (Larix) is planned to be planted there.

The second site was adjacent to the first site (55° 39' 59.508"N, 13° 39' 31.248"E) and was harvested in October 2011 (called clear-cut 2011" in Figure 1). Prior to harvest, the site was covered with 57-year-old Norway Spruce (Picea Abies) (tree species not confirmed by the forest owner). During harvest 3385 m$^3$ of timber was harvested. Harrowing of the site was done at the end of March 2012. It created long ridges of mixed organic layers, surrounded by rows of both undisturbed soil and exposed mineral soil. In autumn 2013, the spatial coverage of the three treatments caused by harrowing were: 33% undisturbed soil, 32% exposed mineral soil and 35% harrowing-mounds on average. Hybrid Larch (Larix × marschkinsii) was planted during summer 2012 (the timing not confirmed by the forest owner). Tree density was 2400 trees/ha. The mean tree height was 99 cm in November 2013. Average thickness of the litter layer was 2.1 cm while the average humus layer thickness was 3.7 cm. Hereafter the site is referred to as harrowing experiment.
3.2 Experimental design and measurements

3.2.1 Block experiment
A block experiment was carried out in order to compare harrowing and mounding site preparations with control plots. A 12x12 m area was chosen at a “clear-cut 2011” and divided into 36 2x2 m blocks, from which 10 were chosen randomly to carry out the experiments on (Figure 3). Three site preparation methods (harrowing, mounding and control) were applied in each block in a random order (Figure 2). Harrowing was done by excavating the humus layer in an area of 0.5x0.5 m and placing it upside down next to the pit with exposed mineral soil. Mounding was performed in a similar way, but 20 cm of mineral soil was added on top of the turned humus layer. The site preparations resulted in four treatments that were classified as: undisturbed soil (control), mineral pit after harrowing (harrowing-mineral), mound with double humus layer (harrowing-mound), mound with double humus layer capped with 20 cm of mineral soil (mounding). The site preparation was carried out manually with shovel. The area of each
treatment was 0.5x0.5 m. Each treatment had 10 replicates. Collars (diameter 16 cm, height 5 cm from the ground surface) were installed in the middle of each treatment. The lower edge of the collar was pushed to a depth of about 5 cm from the surface.

Figure 2. A block of four treatments: harrowing-mineral (front left), harrowing-mounding (far left), control treatment (middle), mounding (right). Each block consisted of four treatments in random order.

Altogether, 40 collars were installed. Once installed, the collars were left in the field for the whole measurement period (June-October). The site preparation and collar installations were done during 1-11 June 2013.

Figure 3. The block experiment site at the clear-cut 2013. Ten blocks were chosen randomly from a 12x12 m area where three site preparation methods were applied resulting in four treatments.
The soil respiration measurements were carried out from 24 June to 31 October 2013. Soil respiration was measured at 5-day long campaigns from June to August. Each plot was measured twice a day between hours 06.00-22.00. In September and October, the measurements were done at 2.5-day long campaigns. CO₂ fluxes from the soil surface were determined by measuring CO₂ evolution in the chamber headspace over time by using a portable infra-red gas analyser (EGM-4, PP-systems, Massachusetts, USA) and a soil respiration chamber (CPY-2, PP-systems, Massachusetts, USA). The EGM-4 had analysing accuracy of <1% of the span concentration over the calibrated range (PP Systems 2010). The chamber had a volume of 3.6 L and the chamber plus collar volume totalled 4.6 L. The chamber was equipped with a fan to ensure sufficient mixing of air in the chamber headspace and was covered with black plastic and aluminium foil in order to shut out sunlight and decrease heating of the chamber during measurements. The measurements started just after the chamber was placed on a collar. Each measurement interval was 124 seconds with measurement taken every 4.2 seconds.

Soil temperature was measured immediately after the soil CO₂ flux measurement with a portable electronic thermometer at a depth of 5 cm inside each collar. Soil water content was measured at 0-6 cm depth (ML2x moisture sensor, Delta-T, Cambridge, UK) in mV. Average value was taken from three consecutive measurements of soil water content at different spots adjacent to each collar.

Soil samples for total C and total N analysis were collected in 10 December 2013 with a soil corer of 5.5 cm diameter. The coring was conducted inside each collar to a depth of 21-26 cm as it was not possible to go to the same depth in all plots (due to stones, big roots etc). The samples were frozen for 1.5 months and then defrosted at 4-5 °C for 5 days. The samples were prepared for soil nutrient analysis in the laboratory and analysed by a Costech ECS4010 elemental analyser (EA) and a VG TripleTrap. For a detailed description see “Soil Sampling” in Appendix 1.

### 3.2.2 Harrowing experiment

Eddy-covariance measurements of the carbon balance were carried out during 30 May - 27 August 2013 in the "clear-cut 2011" area (Figure 1). The CO₂ flux was measured by CPEC200 Closed-Path Eddy-Covariance System (Campbell Scientific,Inc., Logan, UT, USA) (Figure 4), which consisted of a closed-path gas analyser (EC155), a sonic anemometer (CSAT3A) and a datalogger (CR3000). Measurements were done at 10 Hz. Air temperature and relative humidity were measured by Campbell CS215 (Campbell Scientific Inc., Logan, UT , USA). The instruments were mounted on a 3 m high tower (55° 39' 59.569" N, 013° 39' 30.431" E). Soil water content and temperature were measured by Campbell CS655 (Campbell Scientific, Inc., Logan, UT, USA) at 3, 10 and 30 cm depth in two profiles located approximately 20 m from the tower. Another tower was mounted 15 m from the eddy flux tower where a web camera and a net radiometer CNR4 (Kipp & Zonen B.V., Delft, the Netherlands) were placed.
Soil respiration was measured in 11 collars that were installed in the expected main wind direction (between 210 and 220º) 18-44 m from the tower (Figure 5). Four collars were installed in exposed mineral soil, four in the harrowing-mounds and three in the undisturbed soil (control). The technique of chamber measurements and timing were the same as in the block experiment.

Secondary measurements were gathered for background information of the site: coverage area of each treatment, average tree height, soil C/N content and thickness of humus and litter layer. The sample plot locations were selected by dividing wind direction into 16 classes, each 22.5° wide. The frequency of wind direction from each sector was analysed and 5 sectors chosen as the main wind direction. The sectors were lying in the south-west direction. Transects in the middle of sectors were 202.5°, 225°, 247.5°, 270°, 292.5°. In each sector, a 10x10 m plot was marked, called secondary measurements in Figure 1. The plot distance from to tower was chosen randomly between 1 and 80 m, which was the average footprint length from 70% contribution to the total fluxes. All the coordinates were taken by GPS (Magellan eXplorist 510, Magellan Navigation Inc., Santa Clara, CA) with an accuracy of 4.5 m. Coverage area of each treatment and tree height were measured. Two soil samples were taken from the middle of each plot from undisturbed soil and pooled together layer by layer for all plots. Samples were handled the same way as described in Appendix 1, except freezing. Unfortunately the result of soil C and N content of this site was not possible to include in the study due to broken analyser.

Other secondary measurements were gathered about humus and litter layer thickness. It was done in transects drawn from the tower in four directions (north, south, east and west). Measurements were done after every 5 m on the 50 m long transects.
### 3.3 Data treatment

#### 3.3.1 Soil flux calculation

CO₂ fluxes were calculated as:

\[ F = k \cdot \frac{V}{A} \]  \hspace{1cm} (1)

where \( F \) is flux \([\mu \text{mol m}^{-2} s^{-1}]\), \( k \) is rate of change in CO₂ concentration over time \([\mu \text{mol m}^{-3} s^{-1}]\), \( V \) is volume of the chamber and collar \([\text{m}^3]\) and \( A \) is area of the collar \([\text{m}^2]\). A linear regression was fitted on the increasing CO₂ concentration over time. Different starting points and lengths of the fits were considered as it can have a considerable influence on the flux rates (Lai et al. 2012; Koskinen et al. 2014).

Original CO₂ concentration data was given in ppm \((\mu \text{mol mol}^{-1})\). In order to calculate the flux, CO₂ concentration data was converted from ppm to \(\mu \text{mol m}^{-3}\) using the ideal gas law:

\[ C = C_{\text{raw}} \cdot \frac{P_{\text{air}}}{R \cdot (T+273.15)} \]  \hspace{1cm} (2)

where \( C \) is CO₂ concentration \([\mu \text{mol m}^{-3}\] , \( C_{\text{raw}} \) is raw CO₂ concentration \([\mu \text{mol mol}^{-1}]\) , \( P_{\text{air}} \) is ambient air pressure \([\text{Pa}]\), \( R \) is the universal gas constant \([8.3145 \text{ J mol}^{-1} \text{ K}^{-1}]\) and \( T \) is air temperature \([\text{oC}]\) measured in the chamber headspace.

Placing the chamber on the soil alters the concentration gradient between the soil and the atmosphere as gases accumulate in the chamber headspace. The tracing curve of the CO₂ concentration in the headspace starts to flatten out because the diffusion gradient decreases. This can result in underestimation of the flux (Davidson et al. 2002). What is more, the CO₂ concentration measurements can be biased in the beginning of the measurement due to pressure effects and disturbance caused by moving and fixing the chamber (Davidson et al. 2002; Pumpanen et al. 2004a). Therefore, for flux calculations, the data with disturbances in the beginning of measurement should be discarded but the calculation should start as soon as possible after that (Kutzbach et al. 2007). A too short fitting period might underestimate the flux as it is more susceptible for small disturbances (Koskinen et al. 2014; Lai et al. 2012). On the other hand, too long fitting periods might include the flattening of the curve due to saturation and underestimate the flux (Koskinen et al. 2014).

In order to avoid underestimation of fluxes due to saturation in chamber headspace and due to disturbances in the beginning of measurements, the sensitivity of flux calculations to different starting point of the fit and length of the fit was tested. \( R^2 \) (the goodness of fit) and average flux was calculated for different fits.

Mean \( R^2 \) of CO₂ concentration evolution over all measurements was calculated for fitting lengths of 8, 10, 12, 15, 18, 21, 23, 25 and 27 measurement points and flux was calculated for fitting
lengths of 10, 18 and 25 measurement points with starting points in successive order starting from the first measurement point.

Calculated fluxes were accepted only if the linear regression of the concentration evolution was statistically greater than zero, which means that the fit of the regression model to the data was statistically significant. This was determined by testing the $R^2$ value for statistical significance. It showed that $R^2$ should be $> 0.399$ (p<0.05, N=10) (Weinberg and Abramovitz 2002). Therefore data of the measurements was included in the further analysis only if $R^2$ of the concentration evolution was bigger than 0.399.

### 3.3.2 Temperature sensitivity

Temperature sensitivity $Q_{10}$ was calculated as (Lloyd and Taylor 1994):

$$Q_{10} = e^{10\beta}$$  \hspace{1cm} (3)

where $\beta$ is the fitted constant from exponential curve of soil CO$_2$ and temperature relationship.

Statistical analysis was conducted in Microsoft Excel 2010 (Microsoft, MA, USA) and MATLAB R2012a (MathWorks Inc., Natick, MA, USA).

### 3.3.3 Analysis of statistical significance

Statistical significance analysis was carried out in SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). Probability of fit to normal distribution of the data sets was checked with Shapiro-Wilk test (if the significance value of Shapiro-Wilk was $> 0.05$, the data was normal) (Laerd Statistics).

Statistical analysis for the comparison of soil respiration between two sites was carried out in Microsoft Excel (Microsoft, MA, USA) with two-sample t-test assuming unequal variances. The differences were considered significant with a p value of $p<0.05$.

### 3.3.3.1 Soil respiration on block experiment

The differences of the respiration from different site preparations were analysed for each month. Data sets of June and October were transformed logarithmically and analysed with one-way ANOVA Tukey's test. The differences were considered significant with a p value of $<0.05$. Because data transformation for months June, August and October did not produce normal distribution, a paired non-parametric Mann-Whitney U test was used. As multiple comparisons were done in paired Mann Whitney U comparisons, the statistical significant difference was identified according to the Bonferroni correction, which is used to reduce the chances of obtaining type I errors with multiple pair-wise tests (Laerd Statistics). The significance level was divided with the number of tests run, e.g. $0.05/4=0.0125$. It means that for the comparisons done with Mann-Whitney U test p value $<0.0125$ was used.
3.3.3.2 Soil carbon and nitrogen
Soil carbon and nitrogen content, as well the C/N ratio between different site preparation methods were compared with one-way ANOVA. The distributions of the data sets were originally normally distributed (Shapiro-Wilk significance test was > 0.05), therefore no transformations were needed. Tukey’s post-hoc test was applied with p value < 0.05.

3.3.4 Water content conversion from mV to %
The moisture sensor output from mV was converted to volumetric water content by a linear fit equation provided in the manual of the soil water content sensor ThetaProbe ML2x (Delta-T Devices Ltd 1999). The conversion was done according to the soil type where the soil water content was measured. Control and harrowing- mounding plots were considered as organic soils, whereas mineral and mounding as mineral soils.

The conversion equation used for organic soils was:

$$\theta_{\text{organic}} = 0.055x - 2,$$

(4)

where $\theta$ is the soil water content in %, $x$ represents the soil water content value in mV measured with the ThetaProbe sensor.

The conversion equation used for mineral soils was:

$$\theta_{\text{mineral}} = 0.05x - 5,$$

(5)

3.3.5 Soil carbon and nitrogen content conversion to area basis
The results of the soil carbon and nitrogen content were expressed in mg C g\(^{-1}\) of soil or mg N g\(^{-1}\) of soil. For making comparisons possible across sites, the soil data is usually expressed on per area basis (Robertson 1999). The nutrient content was converted to per area basis by the equation (Ellert et al. 2008):

$$Y = \rho \cdot c \cdot h \cdot 0.01$$

(6)

where $Y$ is the nutrient stock to a fixed depth (kg m\(^{-2}\)), $\rho$ is the density of the whole soil sample (g cm\(^{-3}\)), $c$ is the nutrient concentration (mg g\(^{-1}\) dry soil), $h$ is the length of the core (cm). The assumption of this equation is that soil samples were collected with a fixed surface area (Robertson 1999).
Before applying the equation, the following steps were taken for calculating density. At first the whole sample was dried at 40 °C for a week and then a subsample was taken and dried with 105 °C for 24 hours. As the mass of the subsample was not known (dried at 105 °C) before the drying at 40 °C, a calculation was done:

\[ m_{\text{sub, b40}} = \frac{m_{b40} \cdot m_{\text{sub, b105}}}{m_{a40}} \]  

(7)

where \( m_{\text{sub, b40}} \) is the mass of the subsample before drying at 40 °C (g), \( m_{b40} \) is the mass of the whole sample before drying at 40 °C (g), \( m_{b105} \) is the mass of the subsample before drying at 105°C (g) and \( m_{a40} \) is the mass of the whole sample after drying at 40°C (g).

In the next step, the mass of the whole sample in oven-dry conditions was determined:

\[ m_{a105} = \frac{(m_{b40} - m_{\text{gravel}}) \cdot m_{\text{sub, a105}}}{m_{\text{sub, b40}}} \]  

(8)

where \( m_{a105} \) is the mass of the whole sample in oven-dry conditions (g), \( m_{\text{gravel}} \) is the mass of gravel removed after sieving (g), \( m_{\text{sub, a105}} \) is the mass of the subsample after oven-drying at 105 °C (g).

Then the density of the whole soil sample without gravel was determined:

\[ \rho = \frac{m_{a105}}{\pi \cdot r^2 \cdot h} \]  

(9)

where \( r \) is the radius of the corer (cm) and \( h \) is the length of the core (cm).

3.3.6 Eddy- covariance data processing

The eddy-covariance (EC) technique is most extensively used in ecosystem carbon balance studies of forest ecosystems. It is based on the covariance between turbulent fluctuations of the vertical wind speed and CO₂ concentration (Baldocchi 2003). However, due to assumptions and instrument errors, there are a number of flux errors that need to be corrected during data processing.

Continuous high-frequency data (10Hz) was post-processed by the EddyPro 5.1.1 software (Licor, Inc., Nebraska, USA) in order to calculate CO₂ fluxes at 30-min intervals. Ambient relative humidity and longwave incoming radiation from low frequency ancillary data were included in the ambient measurements for flux corrections and calculation of other parameters.

Raw data was processed in the following steps. The double rotation method for tilt correction was applied in order to correct any misalignment of the sonic anemometer to the local wind streamlines. This method rotates raw wind components to nullify the average cross-stream and vertical wind components. The double rotation method is suggested when canopy height and roughness changes quickly. The turbulent fluctuations were detrended by the block-averaging
method, which calculates the mean value of the variable and turbulence fluctuations as individual departures from the mean. Possible time lags between anemometric variables and variables measured by the gas analyser were compensated by the covariance maximization method, which uses automatic time lag detection within a plausible window of minimum and maximum time lags that are selected for each variable. For flux calculation, gas concentrations were converted into mixing ratios. Air density was used in this conversion. However, the air density fluctuates due to temperature, air pressure and water vapour fluctuations. The Burba et al. (2012) method was used for compensation of density fluctuations as it is appropriate for closed-path systems (LI-COR 2013). Quality check of the calculated fluxes was done according to Mauder and Foken (2006) and footprint estimations according to Kljun et al. (2004).

Different spectral corrections were compared and the method by Ibrom et al. (2007) was chosen for high frequency spectral correction. The raw data was evaluated by 9 statistical tests: spike removal, amplitude resolution, drop-outs, absolute limits, skewness and kurtosis, discontinuities, time lags, angle of attack and steadiness of horizontal wind with the default settings. Flux random uncertainty due to sampling errors was estimated by the Finkelstein and Sims (2001) method.

The flux results were filtered by the CO₂ flux quality and statistical flags for spikes, absolute limits, discontinuities and drop-outs set by EddyPro. Secondly, manual filtering of data was applied by removing unreasonable sudden peaks in data. Gap-filling was done by a method based on Reichstein et al. (2005) and Falge et al. (2001), using an online tool by Max Planck Institute for Biogeochemistry (MPI-BGC Jena, Online eddy covariance gap-filling & flux partitioning tool, accessed 15 April 2014, http://www.bgc-jena.mpg.de/~MDIwork/eddyproc/index.php). The tool is used for gap-filling data, flux partitioning and u* filtering. Net ecosystem exchange, air temperature, relative humidity, global radiation data and u* values were uploaded to the online website. The online tool did u* filtering by splitting the data set into 6 temperature classes and for each temperature class, the data was split into 20 u* classes. The u* threshold was estimated when the night-time flux reached more than 95% of the average flux at higher u* class. The median of the threshold of the 6 temperature classes defined the final u* threshold. Each NEE value measured at low turbulent conditions below the u* threshold was filtered out. 36% of data was filtered out after statistical flags filtering, manual elimination of spikes and u* filtering.

The gap-filling was done by the same online tool. Gaps were filled with an average value at similar meteorological conditions within a time-frame of 7 days, using air temperature and global radiation or by the average NEE value at the same time of the day.

Flux partitioning was applied only for original data with quality indicator 1. Ecosystem respiration was calculated by the Lloyd and Taylor (1994) regression model using night-time data as ecosystem respiration (R_{eco}) and soil/air temperature. For detailed method description, see the above-mentioned website.
4 Results

4.1 Flux calculation

The $R^2$ values varied between 0.91-0.97 and all fit periods showed that $R^2$ was lower when the fit started at the 1st or 2nd measurement point but reached a more stable level after starting the fit at 3rd measurement point (Figure 6). This suggests that the first two measurement points should be discarded from the flux calculation as they might be affected by the disturbance caused by placing chamber on the collar.

The fit length was decided upon which length gave the highest possible flux value. Figure 7 shows the mean flux value for fitting lengths of 10, 18 and 25 measurement points starting in successive order. The later the fit started, the lower was the flux value. It was probably due to saturation in chamber headspace in the end of the measurement period. The flux calculation was chosen to start at 3rd measurement point (at sec 9) with the length of 10 measurement points as it was suggested by $R^2$ and highest flux values.

Figure 6 An example of mean $R^2$ for a fit length of 15 measurement points, starting points in successive order

Figure 7. Mean soil CO$_2$ flux value over all the measurements with fitting lengths 10, 18 and 25 measurement points
4.2 Number of measurements
Before filtering the data by $R^2 > 0.399$ (showing that the curve is statistically significant), there were 1404 measurements at the block experiment site. After $R^2$ filtering, 43 measurements were discarded (7 in September and 36 in October). Discarded data included 60% harrowing-mineral and 30% mounding treatment measurements. Monthly $R^2$ values are shown in Table 1 and the number of measurements per month is shown in Table 2.

Table 1. Mean $R^2$ per month

<table>
<thead>
<tr>
<th></th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
</tr>
</thead>
<tbody>
<tr>
<td>before filtering</td>
<td>0.95</td>
<td>0.98</td>
<td>0.98</td>
<td>0.92</td>
<td>0.75</td>
</tr>
<tr>
<td>after filtering</td>
<td>0.95</td>
<td>0.98</td>
<td>0.98</td>
<td>0.94</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Table 2. Measurements included in data analysis after $R^2$ filtering

<table>
<thead>
<tr>
<th>Treatment</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61</td>
<td>97</td>
<td>93</td>
<td>49</td>
<td>52</td>
<td>352</td>
</tr>
<tr>
<td>Harrowing-Mound</td>
<td>49</td>
<td>95</td>
<td>94</td>
<td>50</td>
<td>56</td>
<td>344</td>
</tr>
<tr>
<td>Mineral</td>
<td>61</td>
<td>95</td>
<td>93</td>
<td>44</td>
<td>24</td>
<td>317</td>
</tr>
<tr>
<td>Mounding</td>
<td>62</td>
<td>96</td>
<td>94</td>
<td>50</td>
<td>46</td>
<td>348</td>
</tr>
<tr>
<td>Total</td>
<td>233</td>
<td>383</td>
<td>374</td>
<td>193</td>
<td>178</td>
<td>1361</td>
</tr>
</tbody>
</table>

4.3 Meteorological conditions from 30 May to 27 August
The measurement period of EC system from 30 May to 27 August was relatively dry. There was a long drought period from 30 June to 27 July (Figure 8). The average daily air temperatures varied between 12 and 24°C. During the first measurement campaign the daily average air temperatures were between 13 and 16°C, however the soil temperatures were between 16 and 19°C. The maximum precipitation per day was 6.6 mm. There were no rainfalls during the second measurement campaign. The daily average air and soil temperatures were more similar, varying between 15 and 20°C. The third measurement campaign was preceded by strong rainfalls; however there were only very low rainfalls during the third campaign. The average daily air temperatures were between 14 and 16°C, soil temperatures varied between 16 and 18°C.
Comparison of average monthly precipitation from June to October 2013 with the 1961-2013 averages shows that July was extremely dry month having 17 mm of rain compared to a long-term average of 72.5 mm (SMHI 2014).

4.4  Block experiment

The following sections show the comparison of soil respiration from different treatments and its correlation to temperature, soil water content and soil C and N content.

4.4.1 Effects of site preparation on soil temperature and soil water content

Site preparation increased soil temperatures by 1 – 4 °C in all of the treated plots in the block experiment compared to the control plot in June, July and August (Figure 9). In those months the soil temperatures ranged between 18 and 22 °C, with mounding having the highest and control plots having the lowest temperatures. In September the differences in temperature between treatments decreased and in October the differences in the temperatures evened out. From June to August, the average temperatures did not change much between months, whereas in September and August, the temperatures started to decrease in all plots.

Soil water content varied more between treatments and between months. The lowest soil water content was in all plots in July and highest in October. Overall, soil water content ranged between 0 - 38%, with the control plots being the wettest and the mounding plots the driest. Soil water content in control plots ranged between 16 and 38%, in mounding plots between 0 and 9%, in harrowing-mound plots between 5 and 17% and in mineral plots between 9 and 22%. Generally, mounds (harrowing-mound and mounding plots) showed the highest temperatures and the lowest soil water content.
Figure 9. a) Average soil temperature for each month per treatment, b) Average soil water content per treatment. Error bars show the standard deviations.

After converting soil water content from mV to volumetric water content (%), soil water content values of mounding treatment got below 0% (minimum -2%). The values were not corrected to 0 although negative soil water content is physically implausible.

There was a clear negative correlation between soil water content and soil temperature in all treatments, meaning that the higher the water content, the lower the temperature (Figure 10). Control plots showed the highest correlation ($R^2=0.44$) and harrowing-mound plots lowest correlation ($R^2=0.22$) between soil water content and temperature. Mounds had higher standard deviation (wider spread) in temperature than control and mineral plots, however control and harrowing-mound plots had highest standard deviation and spread in soil water content (Figure 9 and 10).
4.4.2 Effects of site preparation on soil CO$_2$ flux
The monthly mean CO$_2$ flux was significantly different (p < 0.05) between all treatments from June to October, except for the control and the mounding (p=0.813) plots that were not significantly different in October. The average CO$_2$ flux was highest for harrowing-mound plots, ranging between 5.3 and 9.3 μmol m$^{-2}$ s$^{-1}$ (Figure 11). Mineral plots showed lowest fluxes, ranging between 0.8 and 1.4 μmol m$^{-2}$ s$^{-1}$. Soil respiration from control plots ranged between 2.0 - 4.5 μmol m$^{-2}$ s$^{-1}$ and from mounding plots between 1.7-6.0 μmol m$^{-2}$ s$^{-1}$. Soil respiration of control plots was higher than mounding plots in June, August and September, while mounding plots showed higher values in July.

Mounds (both harrowing-mound and mounding plots) had larger variation between months than control and mineral plots (Figure 11b). In July, mounds had the highest average CO$_2$ flux, whereas control plots had lower flux than in the month before and after.
Figure 11. Monthly averaged CO$_2$ flux in μmol m$^{-2}$ s$^{-1}$ for each treatment from June to October. Error bars present standard deviations.
4.4.3 The relationship between soil respiration and soil temperature

The dependence of soil respiration on soil temperature was assessed with an exponential function which revealed that the relationship was significant (p<0.05) for all treatments. Harrowing-mound and mounding treatments were more correlated to temperature ($R^2=0.35$ and 0.32, respectively) than control and mineral plots ($R^2=0.19$ and 0.15, respectively) (Figure 12).

![Graph showing exponential relationships between soil CO$_2$ flux and soil temperature over the whole range of soil water contents.](image)

Figure 12. Exponential relationships between soil CO$_2$ flux and soil temperature over the whole range of soil water contents.

$Q_{10}$ values (the increase in CO$_2$ flux caused by a 10°C increase in soil temperature) varied from 1.67 to 2.12 across treatments (Table 3). It was calculated using the exponential equations shown in figure 12. Mounds showed higher temperature sensitivity ($Q_{10}$) than control and mineral treatments.

<table>
<thead>
<tr>
<th></th>
<th>Harrowing-Mound</th>
<th>Control</th>
<th>Mounding</th>
<th>Mineral</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{10}$</td>
<td>2.12</td>
<td>1.91</td>
<td>2.03</td>
<td>1.67</td>
</tr>
</tbody>
</table>

Table 3. Calculated $Q_{10}$ values for each treatment.
As soil water content is another important driver of the flux, relationships between soil respiration and soil temperature were analysed in different soil water content classes: <10%, 10-20% and >20% (Figure 13). Low soil water content (<10%) in control plots resulted in lower soil CO₂ flux than at higher soil water content, although higher temperatures were reached. In other treatments, the low soil water content did not result in lower values of soil flux compared to more moist conditions.

Wetter conditions (>20%) resulted in lower temperatures and therefore lower fluxes in all treatments. Lower flux values were the result of the negative correlation between soil water content and soil temperature. There was no clear tendency that high soil water content solely would start to limit the soil CO₂ flux.

Figure 13. Relationship between soil CO₂ flux and soil temperature by soil water content classes: <10%, 10-20% and >20% in each treatment.
Boxplots of soil CO₂ flux and average soil temperature presents more details about the variation of fluxes and average soil temperature between plots within each treatment from June to August (Figure 14). Harrowing-mound plots had the highest variation in soil CO₂ flux between and within plots, whereas harrowing-mineral showed the lowest variation within and between the plots. The difference between minimum and maximum median within treatments was highest for mounding (3.7 μmol m⁻² s⁻¹), followed by harrowing-mound (3.1 μmol m⁻² s⁻¹), control (1.9 μmol m⁻² s⁻¹) and mineral (1.3 μmol m⁻² s⁻¹).

A comparison of mounding plots number 32 and 33 located next to each other (Figure 3) shows that median flux of plot 32 was much higher than median flux of plot 33. Average temperature was 0.6 °C higher for plot 32.

Comparing all treatments, the mounds generally had a wider spatial variation in soil CO₂ flux between different plots and within each plot. The boxplot does not show a clear relationship between the average temperatures and fluxes.

**Figure 14.** Boxplots of soil CO₂ flux and average soil temperature for each plot in June-August. The lower and upper sides of the box represent the 25th and 75th percentiles of the data, the line inside the boxes shows the median fluxes. The whiskers show the 10th and 90th percentiles of the data. The outliers denote the extreme values. The number next to the boxplot indicates the number of plot. The x-axis represents the average soil temperature.
4.4.4 The relationship between soil respiration and soil water content

There were no clear relationships between soil CO$_2$ flux and soil water content in any of the treatments (Figure 15). Harrowing-mound and mounding plots showed a wide range of flux values on a narrow range of soil water content. For example, mounding flux values varied from 0 to 16 μmol m$^{-2}$ s$^{-1}$ on soil water content around 0%. On the contrary, control and mineral plots had a narrow range of flux values over a wide range of soil water content.

![Figure 15. A scatter plot of relationships between soil CO$_2$ flux and soil water content per treatment.](image)

In order to examine the relationship between soil respiration and soil water content more precisely, a temperature range of 13-17°C was chosen to exclude the influence of soil temperature (Figure 16). This temperature range was chosen, because the soil water content variation was the highest on this temperature range (Figure 10). However, the relationship between soil respiration and soil water content did not show a clearer trend on a temperature range of 13-17°C for any treatment. Linear, exponential and quadratic functions were applied to each treatment, but none of them showed R$^2$ values higher than 0.2.
Figure 16. A scatter plot of relationship between soil CO\textsubscript{2} flux and soil water content on a temperature range 13-17°C.

4.4.5 Soil carbon and nitrogen stocks
Total carbon and nitrogen content were analysed for each plot and an average value was calculated for each treatment. For making comparisons possible across sites, results were expressed on a per area basis, therefore converted from mg/g to kg/m\textsuperscript{2} (Table 4).

Table 4. Average carbon and nitrogen stocks and C/N ratio per treatment. The numbers in brackets refer to standard deviations.

<table>
<thead>
<tr>
<th></th>
<th>mg C g\textsuperscript{-1}</th>
<th>mg N g\textsuperscript{-1}</th>
<th>kg C m\textsuperscript{2}</th>
<th>kg N m\textsuperscript{2}</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harrowing-mound</td>
<td>105.0 (44.7)</td>
<td>4.3 (1.6)</td>
<td>6.8 (1.6)</td>
<td>0.3 (0.05)</td>
<td>24.1 (1.8)</td>
</tr>
<tr>
<td>Mounding</td>
<td>72.9 (20.6)</td>
<td>3.2 (0.7)</td>
<td>6.0 (0.9)</td>
<td>0.3 (0.03)</td>
<td>22.9 (1.7)</td>
</tr>
<tr>
<td>Control</td>
<td>38.2 (8.6)</td>
<td>2.0 (0.7)</td>
<td>4.6 (0.8)</td>
<td>0.2 (0.03)</td>
<td>19.3 (2.1)</td>
</tr>
<tr>
<td>Harrowing-Mineral</td>
<td>19.5 (3.1)</td>
<td>1.4 (0.2)</td>
<td>3.2 (0.4)</td>
<td>0.2 (0.02)</td>
<td>14.0 (1.1)</td>
</tr>
</tbody>
</table>

The total carbon content per m\textsuperscript{2} had larger variation (3.2-6.8 kg m\textsuperscript{2}) than total nitrogen content per m\textsuperscript{2} (0.2-0.3 kg m\textsuperscript{2}). The highest mean C content was for harrowing-mound treatment, 6.8 kg C m\textsuperscript{2}. It also showed the highest standard deviation (Table 4). Mounding plots had an average soil C content of 6.0 kg C m\textsuperscript{2}. Average soil C content for control and harrowing-mineral plots was 4.6 and 3.2 kg C m\textsuperscript{2}, respectively. Statistical significance analysis showed that harrowing-mound and mounding treatments were not significantly different in carbon content (p=0.283). Carbon content comparing all other treatments differed significantly (p<0.05).
Nitrogen content differed very little among treatments. The highest average N content was in harrowing-mound plots (0.3 kg N m$^{-2}$) and the lowest in harrowing-mineral soil (0.2 kg N m$^{-2}$). Statistical analysis showed that there was a statistically significant difference between harrowing-mound and mineral treatment (p=0.013), however N content between all the other treatments did not show any statistically significant difference.

The C/N ratio was highest for the harrowing-mound treatment, 24.1 (Table 4). Mounding treatment had C/N ratio of 22.9, control 19.3 and harrowing-mineral plots 14.0. Harrowing-mound and mounding treatments were not statistically different in C/N ratio (p=0.357). The C/N ratio in all the other treatments differed from each other significantly (p<0.05).

The correlation between soil CO$_2$ flux and soil C and N content was analysed including measurements from all treatments (Figure 17). Soil C, soil N content and C/N ratio showed significant correlation with soil CO$_2$ flux (p<0.05). Soil CO$_2$ flux showed stronger correlation to soil C content ($R^2=0.42$) than to soil N content ($R^2=0.13$). There was a strong correlation ($R^2=0.54$) between soil CO$_2$ flux and C/N ratio.
Figure 17. Relationship between soil CO$_2$ flux and a) soil C content in kg m$^{-2}$, b) soil N content in kg m$^{-2}$, c) soil C/N ratio
4.5 Harrowing experiment

The following section gives an overview of the carbon balance of the 2-year-old harrowed clear-cut, where eddy covariance system was measuring CO₂ fluxes from 30 May to 27 August 2013. Soil respiration was measured by chamber measurements during monthly campaigns from June to September.

4.5.1 Carbon balance of the site

Flux partitioning gave an estimation of the main components in the carbon balance: ecosystem respiration (Rₑₑₒₒ) and gross primary production (GPP). Ecosystem respiration describes the autotrophic and heterotrophic respiration. Net ecosystem exchange (NEE) is the carbon balance of the whole ecosystem. If the value is positive, the ecosystem is a source of carbon to the atmosphere.

During the measurement period, ecosystem respiration (Rₑₑₒₒ) was higher than carbon uptake (GPP) and therefore the carbon balance was positive. The site was a net C source of 145 g C m⁻² over the whole measurement period (Figure 18). The cumulative ecosystem respiration was 355 g C m⁻² and gross primary production -210 g C m⁻². The average daily NEE was 1.6 g C m⁻² for the measurement period. The cumulative figure shows that even a small shift in one of the components can have a big influence on the total carbon balance.

![Figure 18. Cumulative carbon balance components: NEE, GPP and Rₑₑₒₒ during 30 May until 27 August.](image)

4.5.2 Comparison of ecosystem and soil respiration

Soil respiration fluxes from chamber measurements were scaled up according to the proportional area of each treatment created by harrowing at the clear-cut site. An average flux was calculated for each treatment (undisturbed, mineral or harrowing-mound) for each of the measurement
campaigns. Each campaign included 11 chamber measurements: 4 on mineral soil, 4 on harrowing-mounds and 3 on undisturbed soil. The proportion of each treatment type on stand level was used to assess the average flux per m².

Comparison of chamber measurements of soil respiration and ecosystem respiration ($R_{eco}$) modelled by eddy-covariance showed a good agreement (Figure 19). Ecosystem respiration measurements ranged between 0.5-7.8 μmol m⁻² s⁻¹ for the period 30 May to 27 August, whereas chamber measurements ranged between 0.7 and 7 μmol m⁻² s⁻¹ measured during three campaigns in June, July and August. All chamber measurements were in the range of ecosystem respiration measurements, whereas the average soil respiration (red dots in figure 19) followed exactly the line of ecosystem respiration on 15 July - 18 July.

![Figure 19](image-url)

**Figure 19.** Chamber measurements compared to eddy-covariance measurements during the measurement period of 30 May to 27 August. Chamber measurements are the average soil respiration from each treatment scaled to its coverage area: 33% undisturbed, 32% mineral soil and 35% harrowing-mounds. The error bars indicate the standard deviation. Soil respiration measurements were only included if the wind direction was from the chamber location area.

It was calculated that without site preparation (site covered only by control plots) the average soil CO₂ flux would have been 18% higher (not tested for significance).
4.5.3 Comparison of soil respiration of 0.5 and 2-year-old clear-cuts

The soil respiration of harrowing-mineral soil, harrowing-mounds and control plots were compared between the two sites. The control plots of the two sites followed a similar pattern of average soil CO$_2$ flux per month from June to September, showing lower respiration in July compared to June and August (Figure 20). Average soil CO$_2$ flux was significantly higher in the harrowing experiment in July (p=0.005). In other months, the soil respiration was similar between sites, the difference was not statistically significant. In August, both sites showed very similar soil respiration, with the average fluxes differing only 0.1 μmol m$^{-2}$ s$^{-1}$. What is more, standard deviation was higher in August than in other months at both sites.

Harrowing-mounds in the block experiment showed a larger variation of soil CO$_2$ flux from June to September than harrowing experiment plots. Harrowing-mounds in the block experiment had higher average fluxes in July and August than in June and September, whereas the harrowing experiment had similar average fluxes in June, July and August and decreased flux in September. In all months, soil respiration from harrowing-mounds in the block experiment was higher than in the harrowing experiment. The soil CO$_2$ flux was significantly higher (p<0.05) in July, August and September for block experiment plots. In June, the difference in flux was not statistically significant (p=0.08).

Comparison of the harrowing-mineral plots in figure 20 shows that the harrowing experiment had more variation in average soil CO$_2$ flux between months than the block experiment. Soil respiration in the harrowing experiment was higher than in the block experiment harrowing-mineral plots in June, July and August. In September, soil CO$_2$ flux at both sites decreased to a similar level of roughly 1 μmol m$^{-2}$ s$^{-1}$. The soil CO$_2$ flux was significantly higher in July and August at harrowing experiment site (p<0.05), whereas in June and September the difference in flux was not statistically significant.
Figure 20. Comparison of soil CO₂ flux from a) control treatment, b) harrowing-mound treatment, c) exposed mineral soil in block experiment (0.5-year-old clear-cut) and harrowing experiment (2-year-old clear-cut). Error bars indicate standard deviation.
5 Discussion

5.1 Quality control of data

Some soil CO$_2$ flux data were removed due to too low R$^2$ values in September and October which might be because of stronger wind speed in the autumn. Strong wind can affect the measurements because it can cause pressure differences between the chamber airspace and surrounding atmosphere (Koskinen et al. 2014). Most of the removed data were from harrowing-mineral and mounding plots. Many collars were damaged by animals in the autumn, mostly collars of harrowing-mineral plots. After repairing the collars, the chamber measurements were more susceptible to stronger winds and leakage as the chamber and collar might not have been properly sealed. Harrowing-mineral plots had a very low flux in October, which means that even small fluctuations played a big role in the CO$_2$ evolution curve. The elevated mounds were more susceptible to stronger winds because of their shape as they were sticking up from the ground surface.

5.2 The effect of site preparation on soil CO$_2$ flux

Soil respiration rates from exposed mineral soil (harrowing-mineral) in the current study from June to October (0.8 - 1.4 μmol m$^{-2}$ s$^{-1}$) were in the same range as reported by Pumpanen et al. (2004b), 0.4-1.1 μmol m$^{-2}$ s$^{-1}$ (units are converted), and Strömgren and Mjöfors (2012), 0.5-2.5 μmol m$^{-2}$ s$^{-1}$. The soil CO$_2$ flux was lowest from exposed mineral soil, because of removed humus layer that contains potentially decomposable soil organic matter (Pumpanen et al. 2004b). Saana et al. (2011) found that the abundance of all decomposer family groups was lower in exposed mineral soil than in other treatments because of shortage of organic material and greater diurnal fluctuations in temperature and moisture than in undisturbed soil (Siira-Pietikainen et al. 2003). Mineral soil had higher temperatures and lower soil water content than control plots on average because of the direct exposure to wind and darker colour. However, the temperatures and soil water content were lower than in mounds because they got less sunlight due to partial shading by mounds in the morning hours. Mounds were more aerated due to the angle to the mean wind.

The highest soil CO$_2$ fluxes were obtained from the harrowing-mounds, ranging between 5.3 and 9.3 μmol m$^{-2}$ s$^{-1}$. Similar to the present study, Strömgren and Mjöfors (2012) reported soil respiration of harrowing-mound plots to peak in July and August, however they measured much lower values of soil respiration on the first summer after harvesting, ranging between 2.0 - 3.2 μmol m$^{-2}$ s$^{-1}$. They argued that the flux was probably inhibited by too dry conditions. The harrowing-mound plots reached higher temperatures than control and mineral plots due to their shape, which changed their angle towards the sun and made them more susceptible to mean wind and aeration. The harrowing-mound plots were darker than the undisturbed soil, therefore reached higher temperatures. This is also found by Giasson et al. (2006). Harrowing-mounds contained double humus layers, therefore there was more substrate available for decomposition. Soil preparation modified the soil structure in the mounds and increased aeration. This is also
supported by Pumpanen et al. (2004b). Due to improved aeration, increased temperatures and double humus layers, the harrowing-mounds had the highest soil respiration.

The observed values of soil respiration (2.0 - 4.5 μmol m\(^{-2}\) s\(^{-1}\)) from control plots were consistent with other studies done in harvested northern forests. Strömgren and Mjöfors (2012) reported respiration from undisturbed soil as 2.1 - 3.2 μmol m\(^{-2}\) s\(^{-1}\), Pumpanen et al. (2004b) reported values in the range 1.1 - 6.0 μmol m\(^{-2}\) s\(^{-1}\) (units converted) and Mallik and Hu (1997) found soil respiration fluxes to be in the range 2.2 - 4.1 μmol m\(^{-2}\) s\(^{-1}\) (units converted). Control plots had highest soil water content throughout the measurement period, possibly due to the moss layer and litter layer that insulated against drying and big variations in temperature. This finding is supported by Gastaldello et al. (2007).

Soil respiration from mounding plots was in a similar range as reported by Pumpanen et al. (2004b), 1.1 - 7.5 μmol m\(^{-2}\) s\(^{-1}\) (units converted). The latter study found soil respiration from mounds to be slightly higher than or equal to control plots from June to October. However, mounding plots in this study had a lower soil respiration than control plots, except in July when the respiration was almost double that of control plots. Decomposition in the mounding plots should be favoured due to the buried double humus layers which hinders the drying of buried litter and creates warmer conditions and therefore the decomposition rates are expected to be high (Johansson 1994). Although, the soil C and N content was not significantly different for harrowing-mound and mounding plots, mounding plots had much lower soil CO\(_2\) flux than from harrowing-mound plots. This indicates that the flux was suppressed in the mounding plots. Mounding plots reached the highest temperatures and the lowest soil water content. This is because they were sticking up from the ground, which changed their angle towards the sun and made them more susceptible to mean wind and aeration. However, mounding plots reached even higher temperatures and dried even more than harrowing-mound plots because they were capped with loose mineral soil. This was also found by Giasson et al. (2006).

Spatial variation of soil CO\(_2\) flux between the replicates of treatments was highest in the mounding and harrowing-mound plots probably due to the created mounds that had favourable decomposition rates and therefore were more dependent on the initial variation of C and N content in the humus layer.

5.3 The relationship between soil respiration and soil temperature

There was a statistically significant exponential correlation between soil respiration and soil temperature in all treatments. The temperature sensitivity estimates were in agreement with the values reported in other studies (Mojeremane et al. 2012; Giasson et al. 2006; Kowalski et al. 2003). Mineral plots had the lowest correlation with temperature (R\(^2\)=0.149) and lowest temperature sensitivity (Q\(_{10}\)=1.67). This is in accordance with Mallik and Hu (1997) who found that soils with low organic matter had much lower temperature sensitivity. Mounds had stronger correlations to temperature and higher temperature sensitivity (Q\(_{10}\)>2) probably due to higher organic matter content as both mound types had double humus layer.
5.4 The relationship between soil respiration and soil water content

There was no clear relationship found between soil respiration and soil water content using simple regression models. The range of soil water content varied in different treatments, with control plots being the wettest and the driest (almost 0%). Many authors have found that at low and high moisture ranges, CO$_2$ flux is dependent on moisture rather than temperature, while within an intermediate moisture range, soil temperature is the main controlling factor of CO$_2$ flux (Xu et al. 2004; Gabriel and Kellman 2014; Suseela et al. 2012). Different soil water content threshold values for soil respiration limitation have been reported: Suseela et al. (2012) found the intermediate range where soil water content is not limiting between 15% and 26%, whereas Gabriel and Kellman (2014) found moisture levels between 12 and 35% as optimal to microbial activity in a temperate forest on a shallow podzolic soil. Below a threshold value the diffusion is limited because of discontinuous soil water films which cease the solute diffusion and oxygen transport to decomposers (Suseela et al. 2012; Schjonning et al. 2003). Above an upper threshold, CO$_2$ flux declines as water impedes the gaseous transport and oxygen availability (Gabriel and Kellman 2014). Soil CO$_2$ flux in control plots was lower in July than in the month before and after. It can be associated with the lowest soil water content in that month compared to other months. Pumpanen et al. (2004b) found a similar tendency in average soil CO$_2$ fluxes from undisturbed soil during June to August. One possible reason is that soil water content reached critical value in July and started to inhibit the flux. It was observed in the control plots that fluxes were inhibited when soil water content was below 10% (Figure 13). A more precise investigation on different moisture classes and soil respiration should be conducted for finding specific threshold values.

The results of the present study did not show that any range of soil water content would have inhibited the flux in the mounding plots, although the soil water content was mostly below 10%, which is below the threshold value reported in previous studies that indicate that the flux should be inhibited. It might be possible that as the soil water content was so low all the time, the soil respiration was suppressed throughout the whole measurement period. A lower rate of soil respiration from mounding plots as compared to control plots was reported also by Mojeremane et al. (2012). They reasoned that the decomposition was suppressed by the desiccation which takes place during periods with no rainfall. The precipitation data measured close to the EC system in the present study showed that there was a dry period before measurements in July, but there were rainfalls in June and August before the measurement campaigns (Figure 8). However, the rainfalls were probably not strong enough and the mounds dried fast after that. A similar effect was seen by Giasson et al. (2006), where the dryness of the mounds counterbalanced the effect of increased temperature on soil respiration.

It was not observed in the present study that high soil water content would have started to inhibit the flux rates. It was rather the co-variation between soil water content and temperature that resulted in lower fluxes. Soil water content and temperature often co-vary in field conditions and therefore it is difficult to separate soil CO$_2$ flux responses to changes in individual variables.
(Gabriel and Kellman 2014). Many authors have found that soil respiration is best explained by multiple regression analysis of the combination of soil temperature and soil water content (Suseela et al. 2012; Mojeremane et al. 2012).

5.5 Soil carbon and nitrogen stocks

Soil C and N content proved to be an important factor affecting soil respiration, showing a significant correlation with soil CO2 fluxes. A significant positive correlation has also been reported by Mallik and Hu (1997) and Persson et al. (2000).

The obtained values for soil C and soil N content on control plots are comparable with those found in other studies (Harrington and Schoenholtz 2010; McDaniel et al. 2014). Site preparation increased statistically significantly the carbon content and C/N ratio in the mounds as compared to control plots, which is in agreement with the results by Smolander and Heiskanen (2007). The reason for harrowing-mounds and mounding plots having no significant difference in soil C content and C/N ratio might be due to that the same layers were included in the samples. Harrowing-mounds had double humus and litter layer on top of a mineral layer, whereas the double humus and litter layers in mounds were capped with 20 cm of mineral soil. Therefore the soil samples that were taken from the top soil at these plots included the same layers. The harrowing-mineral plots had the lowest soil C and C/N ratio, probably because the humus layer was removed. The low level of soil C and N after humus layer removal aligns with a study by Yildiz et al. (2010).

Site preparation did not affect the soil N content in different treatments significantly compared to control plots, except significantly different values in harrowing-mound plots and mineral plots. This agrees with findings in studies by Lundmark-Thelin and Johansson (1997) and Smolander and Heiskanen (2007), who reported that the amount of nitrogen did not change significantly in undisturbed soil and mounds during the first year after clear-cutting. The release of N did not start before the decomposition of lignin had started (Lundmark-Thelin and Johansson 1997), which indicates that in the current study the decomposition of lignin had probably not begun yet. However, there can be differences in N dynamics in different treatments in the following years after site preparation.

5.6 Cumulative CO2 fluxes on the 2-year-old site

High ecosystem respiration dominated the ecosystem carbon balance 2 years after clear-cutting, resulting in the site being a source of carbon by 145 g C m\(^{-2}\) from June to August. In comparison, a 50-year-old Douglas-fir stand was a carbon sink between -270 and -420 g C m\(^{-2}\) year\(^{-1}\) (Morgenstern et al. 2004). Different ranges of NEE have been reported for clear-cuts of similar age: a 2-year-old site prepared clear-cut in central Sweden was a source of carbon by 497 g C m\(^{-2}\) year\(^{-1}\) (Grelle et al. 2012) and a 1-year-old French pine forest by 290 g C m\(^{-2}\) year\(^{-1}\) (Kowalski et al. 2003). The present site was a source of carbon by 145 g C m\(^{-2}\) in a 3-month period, whereas 2-year-old clear-cut in a boreal forest in southern Canada had a cumulative NEE of 152 g C m\(^{-2}\) year\(^{-1}\) (Zha et al. 2009). In the current study the cumulative R\(_{eco}\) for the measurement period was
355 g C m$^{-2}$ and GPP -210 g C m$^{-2}$. In the study by Kowalski et al. (2003) the annual $R_{\text{eco}}$ was 996 g C m$^{-2}$ year$^{-1}$ and GPP was -727 g C m$^{-2}$ year$^{-1}$, whereas Zha et al. (2009) estimated annual $R_{\text{eco}}$ 234 g C m$^{-2}$ year$^{-1}$ and GPP -82 g C m$^{-2}$ year$^{-1}$.

From the end of May to the end of August, the average NEE in this study was 1.6 g C m$^{-2}$ day$^{-1}$. Other studies have found similar results: average NEE for growing season (May-to August) at a 2-year-old temperate coastal Douglas-Fir stand was 1.4 g C m$^{-2}$ day$^{-1}$ (Humphreys et al. 2005) and 1.1 g C m$^{-2}$ day$^{-1}$ (unit converted from CO$_2$ to C) at a 5-year-old scarified clear-cut for July-August in southern Finland (Rannik et al. 2002). The latter site showed similar magnitude of $R_{\text{eco}}$ and GPP per day as the results in the present study. Compared to the values found in literature, it can be said that the carbon balance and its components depend strongly on the site conditions and climate.

The carbon balance changes in different growth stages of the stand. It is hard to predict when the site prepared site will turn from carbon source to sink. It has been reported that clear-cuts might be sources of carbon up to 10 to 20 years after harvesting (Zha et al. 2009; Kolari et al. 2004; Humphreys et al. 2005). However, it can be hypothesized that the use of site preparation leads to earlier turn into carbon sink than unprepared site as it favours the growth and survival of regenerating trees and therefore increases carbon sequestration (Giasson et al. 2006). Continuous measurements are needed to investigate the long-term effect of site-preparation.

### 5.7 Comparison of ecosystem respiration and scaled soil respiration

The range of soil respiration measurements were in the range of ecosystem respiration during the study period of 30 May to 27 August. Ecosystem respiration is the sum of soil respiration and aboveground respiration. Typically soil respiration contributes 30-80% to the annual total ecosystem respiration (Davidson et al. 1998), however forest disturbance like harvesting increases the importance of the contribution of soil respiration to ecosystem respiration (Janssens et al. 2001). The good agreement between soil respiration and ecosystem respiration confirms that soil respiration is responsible for most of the ecosystem respiration while aboveground plant respiration is not contributing much to the ecosystem respiration.

The current study showed that without site preparation, the average soil flux would have been 18% higher. One possible reason might be that most of the easily decomposable matter in the harrowing-mound plots had already decomposed during the first year due to favoured conditions for microbial activity. On the other hand, the lower flux values from site prepared site might have been due to too low soil water content in harrowing-mounds that started to inhibit the fluxes. Strömgren and Mjöfors (2012) found that the soil respiration from control plots was the same or even higher than from humus mounds due to dryer conditions in mounds. The effect of site preparation on the soil CO$_2$ fluxes is strongly dependent on the environmental conditions that are controlling the fluxes at the time of the study.
5.8 Comparison of soil respiration at the 0.5 and 2-year old sites

The soil respiration from control plots on both sites showed a similar behaviour over time, having lower CO\(_2\) fluxes in July than in June and August. An extremely dry period in July resulted in low soil water content on control plots at both sites and therefore inhibited the flux. The soil respiration did not differ significantly between the two sites except for in July. The reason might be that there had not been a significant decomposition of organic matter at the 2-year-old site during the previous year. On the other hand, even if most of organic material had decomposed in control plots over time, the autotrophic respiration by roots of growing trees might have increased the soil respiration and therefore the overall flux rates stayed the same.

In July, August and September the soil respiration was much higher from harrowing-mounds in the block experiment. This is probably because there was more fresh organic matter to decompose. Pumpanen et al. (2004b) found that most of the fresh easily decomposable organic matter decayed during the first summer after clear-cutting. Years after clear-cutting, there might be loss in soil C and N pools due to leaching and low soil CO\(_2\) fluxes due to lack of fresh litter for decomposition (Peltoniemi et al. 2004; Piirainen et al. 2007). The declining decomposition rate is also in agreement with findings by Coursolle et al. (2012) who reported that there was a decrease in carbon content from organic layers during the years after clear-cutting.

On the other hand, as plants re-colonise the mineral soil and there is an increase in tree growth with time after harvest, belowground autotrophic respiration increases (Pumpanen et al. 2004b). The proportion of the root and rhizosphere respiration to the soil CO\(_2\) flux increases with time since harvest (Strömgren and Mjöfors 2012). This can be the explanation for significantly higher flux values from mineral soil in harrowing experiment in comparison to the block experiment. Root respiration can contribute to soil respiration in the range between 10-90% depending on the vegetation type and season of the year (Hanson et al. 2000).

5.9 Reliability of the results

Soil respiration measurements were started two weeks after installing the collars. This short time might have caused unreliable results of soil respiration measurements at the first campaign as the flux might have been enhanced due to a disturbed soil structure and due to decomposition of cut roots following the collar placement.

Although the method for flux calculation was chosen carefully to avoid saturation of CO\(_2\) in the chamber headspace, the calculated flux might still be underestimated as a general technique was chosen for all the measurements.

There are big uncertainties in the estimated values of soil water content. It was measured on three places outside the collars in order not to disturb further flux measurements. The average values showed very high standard deviations. This might be due to high soil water content variation around the collar and the probe's sensitivity to stones, roots and soil density.
It has to be taken into account, that there are uncertainties in the NEE estimations due to raw data processing. Different processing options can give different results. The uncertainties of the raw data processing could be estimated by calculating NEE by choosing different methods (e.g. detrending method, spectral correction or tilt correction) and then estimating the standard deviation of the results (Ueyama et al. 2014). What is more, estimations of \( R_{\text{eco}} \) and GPP by flux partitioning include uncertainties as they are modelled by a regression of night-time respiration data versus temperature. There are also uncertainties in the gap-filled data as the gap-filling is based on similar data under same meteorological conditions. The error of gap-filling can be calculated by randomly distributing new gaps, repeating the gap-filling procedure and comparing the results for uncertainty range (Ueyama et al. 2014). Giasson et al. (2006) estimated an error of \( \pm 0.25 \text{ g C m}^{-2} \) per percent gap filled.

While comparing the chamber measurements to the EC data, it has to be taken into account that the chamber measurements were carried out only in a small part of the clear-cut. In order to have more representative soil respiration estimation, more chamber measurements and a wider spread within the main footprint area of eddy flux tower would be needed.

When comparing the treatments of the two sites, it has to be considered that the block experiment was manually prepared whereas the harrowing experiment was prepared by forest machinery. The soil respiration comparison of the two sites includes uncertainties as the number of replicates was much larger in the block experiment than at the 2-year-old site.
6 Conclusion
The results indicate the importance of temperature, soil water content and total carbon content driving the CO$_2$ flux rates. Harrowing-mound had the highest soil CO$_2$ flux due to high temperatures and high organic matter content. Although mounding plots did not differ significantly in carbon content from harrowing-mounds, the flux was suppressed during all the measurement period by low soil water content. Exposed mineral soil had the lowest flux due to removed humus layer. Soil CO$_2$ flux from control plots was inhibited by low soil water content <10% in July. Comparison of a 0.5-year-old site and 2-year-old site showed that in the first year after site preparation the respiration was higher from harrowing-mounds due to fresh substrate available for decomposition. However, the respiration from mineral plots at the 2-year-old site was higher compared to 0.5-year-old site probably due to the enhanced root growth and increased belowground autotrophic respiration.

It was determined that a 2-year-old clear-cut in southern Sweden was a source of carbon. Soil respiration contributed a major part to ecosystem respiration. The 2-year-old harrowed site showed to have lower fluxes than without site preparation; however the effect of site preparation on the soil respiration of the whole area depends on the environmental conditions of the study period. The results highlight the importance of studying the carbon balance continuously during stand development to determine the long-term effects of site preparation.
Acknowledgements
First and foremost, I would like to thank Patrik Vestin for his dedicated support and guidance in every step during the whole process. I owe my gratitude to Anders Lindroth for introducing me to the topic and giving me helpful advice. I would like to thank Övedskloster, who let me carry out the study on their forest land. I am also grateful to Mikhail Mastepanov who helped me with the gas analyser, Åsa Wallin for helping me in the lab and Karl Ljung for setting up the procedure for soil analysis. I would also like to thank my friend Imre Banyasz who kept me company and helped me on the field.
References


Appendix 1

Soil sampling

Soil samples were collected from each collar on 10 December 2013 down to a depth of 21-26 cm. The aim was to determine the C/N content in the whole core because soil had been disturbed by site preparation experiment.

The following steps were taken:

1. Before sampling, plastic bags were labelled with a collar number and treatment type.
2. In the field, a core was extracted from 21-26 cm depth. If a root or stone was hit and the aimed core depth could not be accomplished, the sample was taken at another place inside the collar or adjacent to the collar.
3. The depth of the sample was measured and noted. A picture was taken from each extracted core.
4. The sample was placed in the premarked plastic bag and stored in a cooler. The same procedure was repeated in each plot.
5. In the laboratory, the samples were frozen at -20 °C for 1.5 months because direct laboratory analysis was not possible.
6. After that, the samples were defrosted at 4-5 °C during one week.
7. The samples were weighed with a Sartorius portable scale, then dried in the oven at 40°C for one week.
8. Once samples had dried, each sample was weighed again.
9. Each sample was sieved through two meshes lying on top of each other, the upper one with a mesh size of 5.6 mm and the bottom one with a mesh size of 2.0 mm. The aim was to include all organic material in the sample, therefore the organic material that remained on the meshes after sieving was crushed manually and mixed into the sample.
10. The gravel fraction > 2 mm diameter was removed and weighed.
11. The sieved soil was mixed thoroughly and split using a drop-through sample splitter. The splitting was repeated 6 or 7 times until a sample size of 5 ml was reached.
12. After sample splitting, a 150 ml subsample was taken from the whole sample for soil moisture content determination. The sample was first weighed and then dried in the oven at 105°C for 24 h. After drying, the samples were weighed.
13. The split subsamples were grinded in a ball-and-capsule Retsch Mill 200 for 61 s with 30 Hz.
14. After grinding, the samples were prepared for the C and N analyser. A foil tin was filled with 2-10 mg of a subsample and packed and formed with tweezers into a small ball. Weight of the packed sample was recorded.
15. C and N concentration was analysed using a Costech ECS4010 elemental analyser (EA).
16. The analyser was calibrated using standard material with known carbon and nitrogen content. Four calibration samples 0.5, 1, 2 and 3 mg were used. After each 10 samples, one calibration sample was analysed. The samples were dropped sequentially into the > 1000°C furnace. Oxygen gas promoted the oxidation of the foil capsules. The CO2 and N2 then passed through a gas chromatograph column where they were separated and quantified by thermal conductivity detector. Helium was used as a carrier gas (Brodie et al. 2011).
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