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Zurich**^{UZH}

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Mineralisation of carbon from root residues in agricultural and grassland soils of Switzerland

**Assessment of carbon mineralisation by laboratory
incubation and isotopic analysis**

GEO 511 - Master Thesis

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Abstract

The mineralisation of carbon in the soil is a major player in the global carbon cycle and is assumed both to be influenced by and to contribute to climate change. It is assumed that root residue addition to the soil increases its carbon stocks and thereby has a positive impact in the CO₂ concentration of the atmosphere. The mechanisms that control the mineralisation of carbon are complex and not well understood. To investigate the impact of root residue addition and to examine the influence of the surrounding ecosystem on the CO₂ efflux, sixteen soils from agricultural and grassland areas of Switzerland were selected. ¹³C-labelled ryegrass root residues were added to the soils and incubated for 119 days. The labelled carbon was traced to separate the total carbon mineralisation into the basal mineralisation, the root carbon mineralisation and the priming effect.

The root residue addition caused a significant increase in the total soil CO₂ efflux. The high initial carbon mineralisation rate observed was related mainly to water-soluble carbon. Percolation of the soil samples caused a rapid decrease of the carbon mineralisation. Thereafter, the root C mineralisation rates remained low. The high priming effect during the intensive mineralisation phase resulted from co-metabolism. In transition between the intensive and the slow phase, a low or negative priming effect was observed and is assumed to be due to pool substitution. An increase of the necromass in the slow mineralisation phase must have led to the further increase of the priming effect. The priming effect then decreased again and became negative for some soils towards the end of the experiment, indicating that decomposable carbon was limiting. Although the priming effect significantly contributed to the additional mineralisation of carbon, in total more carbon was added to the soil than was lost at the end of the experiment.

Depending on the soil samples, the total mineralisation of carbon varied from 63 to 215 g CO₂-C kg⁻¹ of soil organic carbon. This large variability was mainly ascribed to differences within the ecosystem properties, of which soil properties were found to have the largest influence, while the climate and the landform were of less importance. Low organic carbon contents in the soil were further found to have a positive impact on the intensity of the priming effect.

In sum, this thesis showed that carbon mineralisation is not only dependent on the quality and quantity of the crop residues but to a larger degree also on the ecosystem properties.

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Abbreviations

APE	Apparent priming effect
C	Carbon
¹²C	Stable carbon isotope with atomic mass 12
¹³C	Stable carbon isotope with atomic mass 13
δ¹³C	Carbon isotope ratio
CaCl₂	Calcium chloride
C_{min}	Carbon mineralisation
CO₂	Carbon dioxide
Fe	Iron
HCl	Hydrochloric acid
Mg	Magnesium
MASL	Meter above sea level
MAT	Mean annual temperature
MRT	Mean residence time
N	Nitrogen
NABO	Soil monitoring network
NaOH	Sodium hydroxide
Na₂CO₃	Sodium carbonate
OC	Organic carbon
OM	Organic matter
PE	Priming effect
RPE	Real priming effect
S	Sulfate
SOM	Soil organic matter
SOC	Soil organic carbon
SrCl₂	Strontium chloride
SrCO₃	Strontium carbonate
SWC	Soil water content
TN	Total Nitrogen
TOC	Total organic carbon

1 Introduction

The global carbon cycle is affected by climate change (Ciais et al., 2013). Anthropogenic activities, such as the burning of fossil fuel, intensive agriculture and an increasing deforestation have led to large shifts in carbon pools (Smith et al., 2014; Ciais et al., 2013). This effect is most evident in the increasing carbon dioxide (CO₂) concentration in the atmosphere and is one of the main problems of climate change (Ciais et al., 2013). However, on a global scale, soil organic matter (SOM) contains about two times more carbon (C) than the atmosphere and the vegetation together (Ciais et al., 2013; Batjes, 1996) and is therefore the largest active terrestrial carbon pool (Schlesinger and Andrews, 2000; Batjes, 1996). At this point, it is uncertain how the SOM and thus the carbon stock in the soil will respond to climate change. This is problematic because soils are a major contributor of CO₂ in the global carbon cycle (Cox et al., 2000). Thus, small changes in the rate of the CO₂ efflux from soils could already have a huge influence on the CO₂ concentration in the atmosphere (Cox et al., 2000).

Climate warming generally leads to higher plant productivity and consequently also to a larger input of carbon from plant residues into the soil (Schimel, 1995; Rustad et al., 2001). It is, however, also expected that climatic warming will cause enhanced mineralisation of soil organic carbon (SOC), which has the opposite effect (Schimel, 1995; Rustad et al., 2001). It is unsure, whether soils will act as a sink or a source of C. This depends on the rates of residue additions and organic matter decomposition (Paustian, 2014; Hagedorn et al., 2010; Davidson and Janssens, 2006a).

Several studies have demonstrated that the biochemical characteristics of plant residues can play a very important role in the mineralisation rate of C and that organic residues with a high lignin content, such as roots, tend to decompose carbon much more slowly than others (Abiven et al., 2005; Trinsoutrot et al., 2000). Studies have also shown that the SOM content is often higher in soils developed under native grasslands than under forests (Weil and Bradley, 2017; Hiederer, 2003; Tate et al., 1995). However, conversion of native grassland to cropland soils and intensification of agricultural management in arable soils have led to significant losses of SOC of 20 to 50 %. (Paustian et al., 2016; Smith et al., 2014; Ciais et al., 2013). Improved land use management could again reduce these emissions and sequester

CO₂ as carbon in the soil (Paustian, 2014; Bardgett, 2011). Therefore agricultural soils have a high potential to store C (Six et al., 2002).

Schmidt et al. (2011) have demonstrated, that not only the biochemical properties of soils play an important role in the mineralisation of C, but also, and probably to a greater extent, the properties of the soil and of the surrounding environment. It is evident that it is important to understand under which circumstances soils serve as a source or as a sink of CO₂ (Smith et al., 2014; Rustad et al., 2000). This means that it is necessary to gain a better understanding about the mechanisms controlling soil organic carbon mineralisation and the extent to which various properties of the soil and the surrounding environment contribute to it (Schmidt et al., 2011). The main interest lies in studying agricultural and grassland soils, as they undergo the largest changes.

The aim of this master thesis is to find out how the addition of root residues affects the C mineralisation of differing soils from agricultural areas and grasslands, especially regarding the origins of the mineralised carbon. The thesis further aims to examine the opposite process, namely how differences in the expressions of soil properties and of the surrounding environment from agricultural areas and grasslands affect the mineralisation of carbon, especially when root residues are added.

1.1 Mineralisation of Soil Organic Matter

Mineralisation is a biochemical process, whereby organic substances in SOM are completely degraded by microorganisms into inorganic compounds, namely carbon dioxide, water (H₂O) and nutrients, such as magnesium (Mg), iron (Fe), sulphate (S) and nitrogen (N) (Blume et al., 2010; Gregorich et al., 2001). These nutrients are the main source for plant growth.

Fresh plant residues can either be directly mineralised after their addition or, be degraded in several decomposition steps before it is mineralised (Stahr et al., 2016; Gregorich et al., 2001). A part of the plant material is also stabilised in the soil and protected from further decomposition (Schmidt et al., 2011). This portion of the plant residues develops to so-called native SOM. The mineralisation can be regarded as the last step in the decomposition process of SOM. The focus of this master thesis will be on the mineralisation of C and no other mineralisation processes will be taken into account.

1.1.1 Concentration, Dynamics and Persistence of Soil Organic Carbon

SOM is a major determinant of the terrestrial carbon cycling (Herrick and Wander, 1997) and the major carbon reservoir of the biosphere-atmosphere system (Falkowski et al., 2000). The concentration of SOM can vary greatly, depending on the geographical location of the soil and its depth under the surface (Blume et al., 2010). Mineral soils consist of 3 to 7 % of organic matter (Hargrove and Luxmore, 1988). The amount of carbon found in SOM most commonly stated in literature is 58 %, a figure that is based on the work of Spengel (1826). More recent research, however, states figures that range from 51 to 62 % of carbon in SOM (Pribyl, 2010). The study of Bird and Pousai (1997) shows that the total organic carbon (TOC) content in soils from temperate grasslands can vary between 1.2 and 38.5 % for surface soils. Fresh plant residues contain about 42% of organic carbon (Martens, 2000; Shaw, 1959).

Organic carbon stocks in soils are determined largely by the balance between residue addition rates to the soil and carbon mineralisation rates to the atmosphere (Paustian, 2014; Jenny, 1941). If the rates of C additions and C losses are equal, the carbon stocks are stable. However, changes in the environment can lead to changes in the input or the loss of carbon, resulting in a movement in C stocks to a new equilibrium level (Paustian, 2014).

SOC consists of a large range of compounds, which can either persist in soils for thousands of years or be decomposed and released to the atmosphere rapidly (Schmidt et al. 2011; von Lützow et al., 2006). About 50 to 80 % of fresh organic residue added to soils is decomposed and returned to the atmosphere as CO₂ within one to two years (Paustian, 2014; von Lützow et al., 2006). The remaining SOC is stabilised through a variety of processes and hence contributes to the longer-term storage of C in soils.

The persistence of SOM is dependent on its vulnerability. The vulnerability of SOM is the likelihood of a soil to lose previously stabilised organic carbon. It is thus a function of stabilisation and destabilisation mechanisms as well as disturbances (Schmidt et al., 2011; Jenny, 1941). SOM protection mechanisms seem to play an important role in explaining differences in the mean residence time (MRT) of SOM (Abiven et al., 2005; Rasse et al., 2005; Balesdent and Balabane, 1996). According to Trumbore and Czimczik (2008) organic matter persists in soil mainly because it is physically isolated and thus protected from decomposition by microbes. Along with physical stabilisation processes, chemical and biochemical processes also play a crucial role when it comes to the persistence of SOM. Chemically stabilised SOM is

protected through chemical association with clay particles. Biochemical stabilisation processes refer to the recalcitrant chemical components of SOM, such as the readily available lignin in roots (Schmidt et al., 2011; Six et al., 2002). Hence, the molecular structure of plant residues plays an important role in the mineralisation of C.

1.1.2 Soil Respiration and Soil Organic Carbon Mineralisation

To simplify matters carbon mineralisation (C_{\min}) can be described as the CO_2 efflux from the soil to the atmosphere (Raich and Schlesinger, 1992). Carbon enters terrestrial ecosystems through a single process, photosynthesis, but is returned to the atmosphere through a variety of processes. In the subsoil these processes are collectively referred to as soil respiration. Soil respiration is divided into CO_2 released by living roots and their associated mycorrhizal fungi (autotrophic respiration) and CO_2 released by decomposition of native SOM by microorganisms (heterotrophic respiration) (Singh and Gupta, 1977). The latter is a metabolic process, in which the described organisms obtain energy for their growth and functioning (Trumbore, 2006; Schlesinger and Andrews, 2000). Making the distinction between autotrophic and heterotrophic respiration can help predict carbon fluxes under changing climate conditions, especially because it is probable that living roots and microorganisms respond differently to these changes (Beverly and Frankin, 2015). The proportion of heterotrophic respiration can vary largely but it is supposed to account for the larger part of the mineralisation (Trolldenier, 1971). Therefore, this master thesis considers the microbial mineralisation of carbon.

The rate of the C_{\min} varies in space and time (Boone et al., 1998) and is controlled by many factors, of which the substrate quality, the TOC content, as well as the properties of the soil and of the surrounding environment, play an important role (Schmidt et al., 2011; Davidson et al., 2006b; Raich and Schlesinger, 1992; Jenkinson et al., 1991). Taking into consideration the large number of stimulating factors that define the C dynamics and mineralisation, one understands why it is complicated to develop an understanding of the mechanisms of the C_{\min} (Metcalf et al., 2011).

Up to date, the interest of studies has clearly focused on the amount of CO_2 mineralised. Little attention has been given to whether the source of the heterotrophically mineralised carbon was fresh plant residues or native SOM and has not been analysed comprehensively

yet (Trumbore, 2006). Obviously, valuable information can be gained from a more detailed analysis and a better understanding of these mineralisation processes that occur in the soil.

1.2 Quality and Decomposition Rate of Plant Residues

The role of the different plant species and organs as a substrate supplier is not well defined with regard to their decomposition and mineralisation. A large variety of plants are used for agricultural purposes and depending on the treatment of the farmer either no parts, all parts or individual parts of the residual plant organs are added to the soil after harvesting. Therefore the fresh organic residues that are added to the soil can have different characteristics (Trinsoutrot et al., 2000).

Studies have shown that different crop residues decompose differently and that this process is controlled by numerous factors (Abiven et al., 2005; Kuzyakov et al., 2000; Trinsoutrot et al., 2000), of which the biochemical composition, the quality of the plant residue, is particularly important (Abiven et al., 2005; Trinsoutrot et al., 2000). The most common compounds of plant residues can be categorised into easily decomposable monomers, such as sugars and amino acids, slowly decomposable and more stable polymers that include hemicellulose and cellulose and recalcitrant polymers such as lignin (Blume et al., 2010).

The study of Trinsoutrot et al. (2000), along with other studies (e.g., Collins et al., 1990), demonstrates that the initial decomposition and thus the C_{\min} rate is strongly related to the mobile component of the organic substances, hence the amount of C initially present in water-soluble form, also called dissolved organic carbon (DOC) (Kalbitz et al., 2000). As decomposition proceeds, the mineralisation of residue C results from more stable C forms, namely cellulose, hemicellulose and lignin. Hence, the concentration of these polymers is assumed to be the key factor in the decomposition of carbon from crop residues in soils (Trinsoutrot et al., 2000). These observations are valid, when decomposition is not controlled by the overall availability of N (Abiven et al., 2005; Trinsoutrot et al., 2000), which is the case in this master thesis. The reason for that is that the N content controls the microbial decomposition because it is needed for their growth and functioning, hence limiting N would most probably reduce the kinetics of the decomposition (Recous et al., 1995).

1.2.1 Variability in the Decomposition Rate of Different Plant Organs

Several studies show that different plant species can significantly influence the C_{min} rate (e.g., Chen and Stark, 2000; Hobbie, 1992). However, the pattern in the C_{min} between different plant species is not very clear. Abiven et al. (2005) have observed a much clearer pattern between the different plant organs.

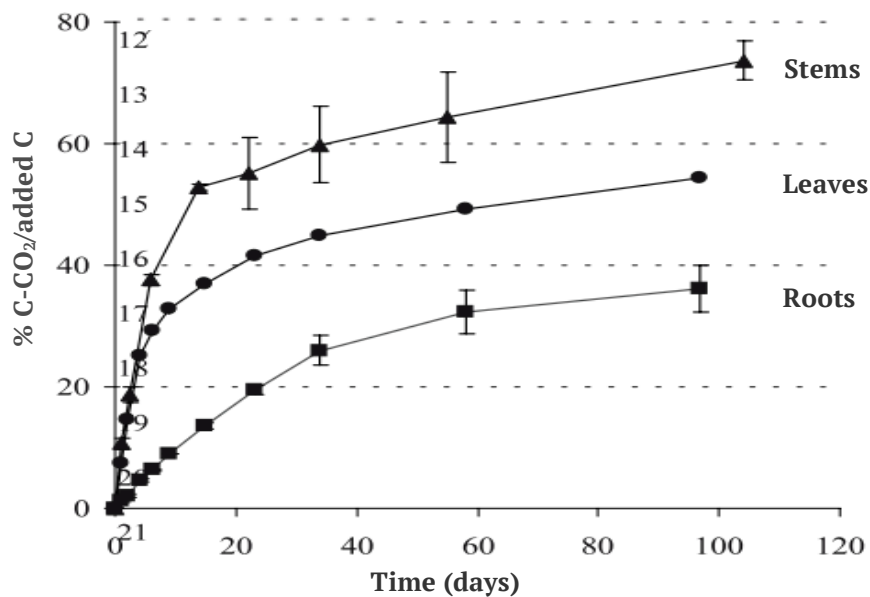


Figure 1: Cumulative C_{min} of the different plant tissues (adapted from Abiven et al., 2005)

Figure 1 is an example from the study of Abiven et al. (2005), which demonstrates the differences between the C_{min} of the different plant organs of rice plants. The study shows that between 20% to 30% less C is mineralised in roots than in leaves or stems within the same plant species. Leaves, stems and roots possess different plant functions, resulting in distinctive biochemical characteristics. Plant leaves, for instance, generally show higher cellulose and hemicellulose contents and roots a higher lignin-like fraction, which consists of a very stable suberin-lignin structure that protects the roots from harmful substances diffused in the soil (Abiven et al., 2005). This structure is one reason, why roots generally decompose more slowly and have a lower C_{min} rate than the aerial parts of the plant (Abiven et al., 2005; Rasse et al., 2005). Roots are thus relatively recalcitrant to decomposition (Shahbaz et al., 2016; Rasse et al., 2005). The slower initial mineralisation in roots is due to a lower water-soluble C content as described earlier in this thesis (Abiven et al., 2005).

The result of the lower C_{min} of roots is a higher contribution of roots to native SOM (Shahbaz et al., 2016; Lian et al., 2016; Abiven et al., 2005; Rasse et al., 2005). Additionally, root-

derived carbon tends to remain in soil much longer than C from the aerial parts of the plants (Rasse et al., 2005; Balesdent and Balabane, 1996).

Nonetheless, it has to be considered that the C_{\min} not only depends on the quality but also on the quantity of the added residues. Shahbaz et al. (2017) have demonstrated that the absolute CO_2 efflux increases with a high residue addition level. Although the cumulative C_{\min} per amount of added residue C remains similar for leaves and stems at the doubled amount of residue addition, the C_{\min} under high root addition in contrast increases up to 15% compared with low additions (Shahbaz et al., 2017).

1.2.2 Potential of Roots for the Carbon Sequestration in the Soil

It was stated earlier in this thesis that a large part of the increasing CO_2 emissions is caused by changes in agriculture and associated land-use changes (Smith et al., 2014; Ciais et al., 2013). Soil C_{\min} is responsible for a major share of the CO_2 emissions stemming from agriculture. Hence, improved soil management could substantially reduce these emissions and sequester some of the CO_2 removed from the atmosphere by plants in form of organic carbon in the soil (Paustian et al., 2016; Lian et al., 2016; Ciais et al., 2013; Smith, 2012). The potential of soils to sequester carbon is large and is applicable at a large scale for a potentially low cost (Ciais et al., 2013; Smith, 2012). Carbon sequestration could therefore be a good strategy to mitigate climate change (Paustian et al., 2016; Smith, 2012).

Rasse et al. (2005) have stated that it is crucial to understand the origin of the carbon stabilised in the soil before exposing management strategies to promote carbon sequestration in soils. The study has revealed that the carbon stored in the soil originates mainly from root carbon because it is stabilised more efficiently than the aerial parts of the plant, and therefore has a larger MRT in the soil (Rasse et al., 2005). Figure 2 shows the most important stabilisation mechanisms which were explained by Trumbore and Czimczik (2008) in chapter 1.1.1 and how they are expressed for root carbon. These mechanisms are especially pronounced for root carbon.

Paustian et al. (2016) revealed different management strategies for agriculture-based soils to sequester carbon, of which roots with enhanced phenotypes, that make root systems grow larger and deeper into the soil, indeed show a specially high potential. Greater root C input is recognised as the main reason for the higher soil C stocks found under perennial grasses than under annual crops (Paustian et al., 2016).

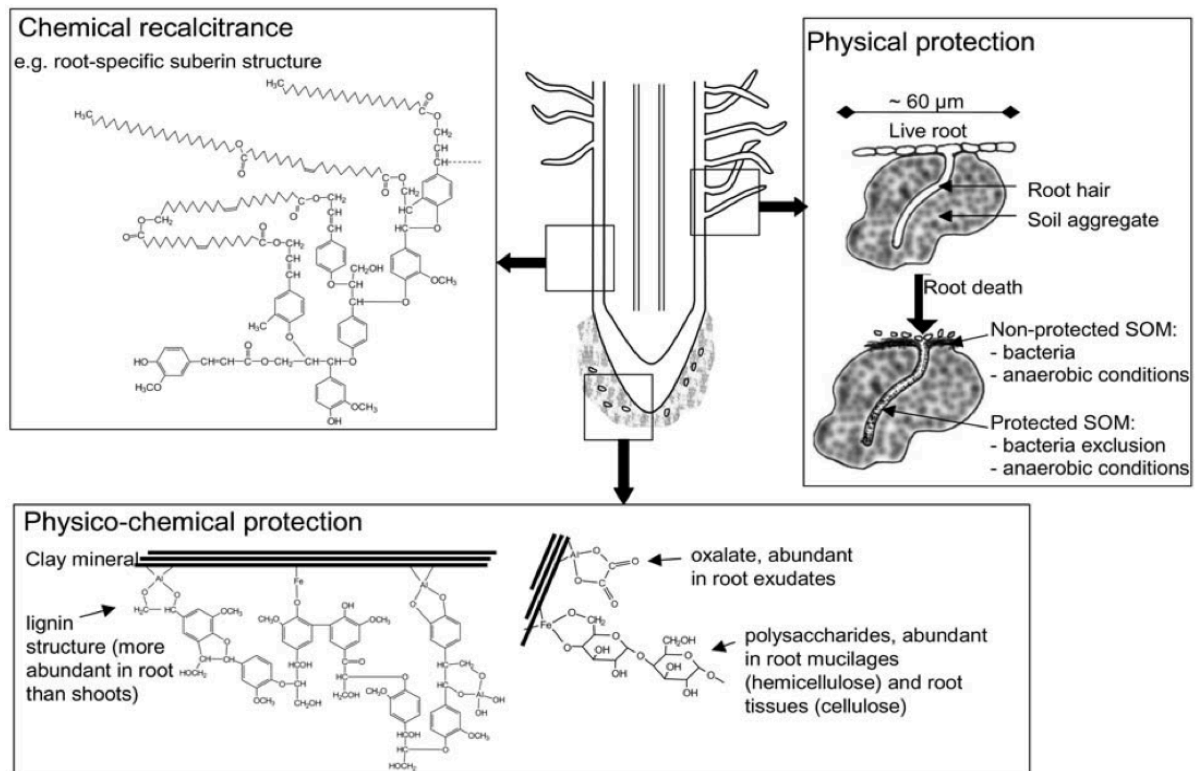


Figure 2: Main protection mechanisms of root C in soils.

It has been shown, that the quality and the quantity of plant residues can strongly affect the mineralisation of carbon. However, environmental factors seem to influence the persistence of SOM and thus the C_{\min} more strongly than the molecular structure of the plant residues, especially on a larger time and space scale (Paustian, 2014; Schmidt et al., 2011). Therefore the C_{\min} of a particular soil can vary substantially from one location to another. Paustian et al. (2014) conclude that climate, soil properties and terrain properties, are amongst the most important environmental factors that influence the C dynamics and mineralisation.

1.3 Influence of Ecosystem Properties on the Mineralisation of Carbon

Organic matter can persist in soils not only because of the intrinsic molecular properties of the SOM itself, but also because of physiochemical and biological influences from the surrounding environment (Stockmann et al., 2013; Schmidt et al. 2011; Jandl et al., 2007). The surrounding ecosystem is assumed to be the driving factor of the soil development and thus has a direct impact on the C dynamics and mineralisation of the soil and therefore also on the vulnerability of SOM (Schmidt et al., 2011; Swift et al., 1979). These drivers might help to explain why even easily decomposable substances can persist in the soil incompletely decomposed for a long time (Stockmann et al., 2013).

According to Torn et al. (2009), a driver is defined as something that has an exceptionally strong influence on a process. For example, considering the decomposition of SOM and thus the C_{\min} , climate can globally be described as a major driver on this process (Davidson and Janssens, 2006a). This thesis, however, investigates a broader range of drivers of the C_{\min} at a regional level, taking as a basis the assumption that climate (temperature and soil moisture), followed by soil (pH and clay content) and terrain properties (slope and Orientation) are the driving forces of the C_{\min} . The selected ecosystem properties differ in their intensity throughout Switzerland, therefore affecting the CO_2 efflux differently across landscapes (Trumbore, 2006; Raich and Schlesinger, 1992). Hence the C dynamics can change significantly from area to area. Additionally, time plays an important role regarding the C_{\min} of the soil (Paustian et al., 1997).

Unfortunately the analysis of the interaction of ecosystem properties has largely been ignored so far. In the following subchapters it will be shown that the different drivers of C_{\min} influence each other. To increase the predictive power of models, it is therefore important to combine several variables into a single model. To gain a better understanding of the stability of SOM and the process of root decomposition, it is important to gain more knowledge about the combined impact of ecosystem properties on the C dynamics.

1.3.1 Influence of Climate Properties on the Mineralisation of Carbon

In literature climate is described as a primary driver of soil respiration. It is furthermore expected that the on-going climate change will increase the global soil respiration (Davidson et al., 2006b; Rustad et al., 2000; Jenkinson et al., 1991). In this study, climate is characterised by the interplay of temperature and moisture.

The climate variable temperature is described as the single best predictor of soil respiration (Reichstein et al., 2000; Kirschbaum, 1995; Raich and Schlesinger, 1992; Singh and Gupta, 1977). It is known that the CO_2 efflux is very sensitive to temperature. Increasing soil temperatures generally lead to a higher CO_2 efflux from the soil because more biomass is produced (Davidson and Janssens, 2006a). When it comes to temperatures above 25 °C, as visible in Figure 4, the amount of SOM will, however, be reduced because the C_{\min} will be stimulated more than the productivity (Rustad et al., 2001). Hence the SOC degradation increases up to two to three times for each 10 °C increase in the mean annual temperature (MAT). Figure 3 on the other hand also shows that biomass production at low temperature

is generally higher than the C_{\min} and SOM can accumulate. Yet, it is assumed that the response of the temperature is greater for the mineralisation of the less stable C fractions than for the stable C fractions (Hobbie et al., 2000). An incubation study of Craine et al. (2010) further reveals that a lower initial mineralisation can be correlated with higher temperature sensitivity, but not with changes in the SOM quality. This shows that several stabilisation mechanisms are temperature sensitive.

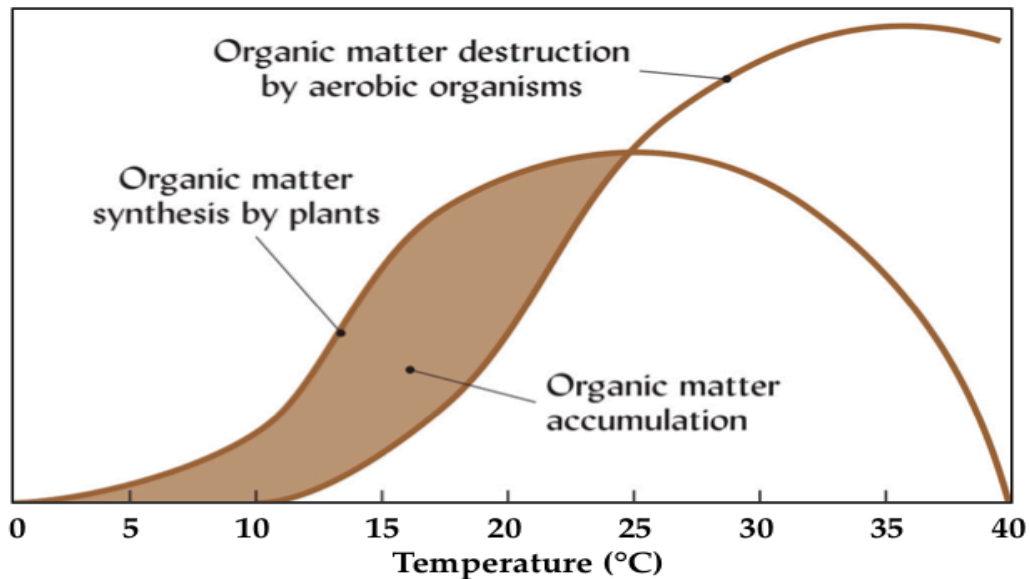


Figure 3: Effect of the temperature on the SOM accumulation. The balance between plant production (OM synthesis by plants) and decomposition (OM destruction by aerobic microorganisms) determines the effect that temperature has upon OM accumulation in soils. The shaded area indicates organic matter accumulation under aerobic conditions (Brady and Weil, 2016).

The effect of the soil water content (SWC) on the C_{\min} is more complex than that of the temperature (Howard and Howard, 1993; Davidson et al., 2000). In incubation experiments the relationship between the soil moisture and the C_{\min} shows a convex form. When water is limiting soil respiration is low and also if the soil becomes so wet that oxygen becomes limiting. Otherwise the soil respiration generally increases when soil moisture increases (Davidson et al., 2000).

It is known that the temperature response on soil respiration interacts with the soil moisture. Basically, high levels of C_{\min} are observed at high temperature and humidity levels and low rates are found in cold and dry regions of the earth (Davidson and Janssens, 2006a; Raich and Schlesinger, 1992). Hence especially high decomposition rates in temperate regions can be observed after rain events in summer (Rey et al., 2002)

1.3.2 Influence of Soil Properties on the Mineralisation of Carbon

The soil C_{\min} and thus also the SOM levels are also influenced by soil properties. However, the effect of soil properties on the C_{\min} in comparison to the effect of the climate is less clear.

Studies show that a high clay content generally results in a lower mineralisation of the SOM (Rutherford and Juma, 1992). There are two mechanisms that are responsible for this. Firstly, clay-rich soils are finely textured and their microorganisms are therefore physically sequestered in the small pores of soil aggregates. This makes them less active because of the relatively anaerobic conditions and better protected against decomposition from the soil fauna (Rutherford and Juma, 1992). Secondly, SOM is physically protected from decomposition because it is bound to large surface areas of clay (Blume et al., 2010). Hence soils high in clay and silt are generally richer in organic matter than sandy soils and therefore tend to store more carbon (Blume et al. 2010; Wang et al., 2003; Jenny, 1941). However, the effect of the clay content in the soil on the C_{\min} is sometimes undetectable (Hassink, 1994). There are several possible reasons for this, such as the variability of the microbial biomass or the availability of substrate (Wang et al., 2003). Furthermore it is unclear how the C_{\min} is affected with regard to the clay content when fresh plant residues are added to the soil.

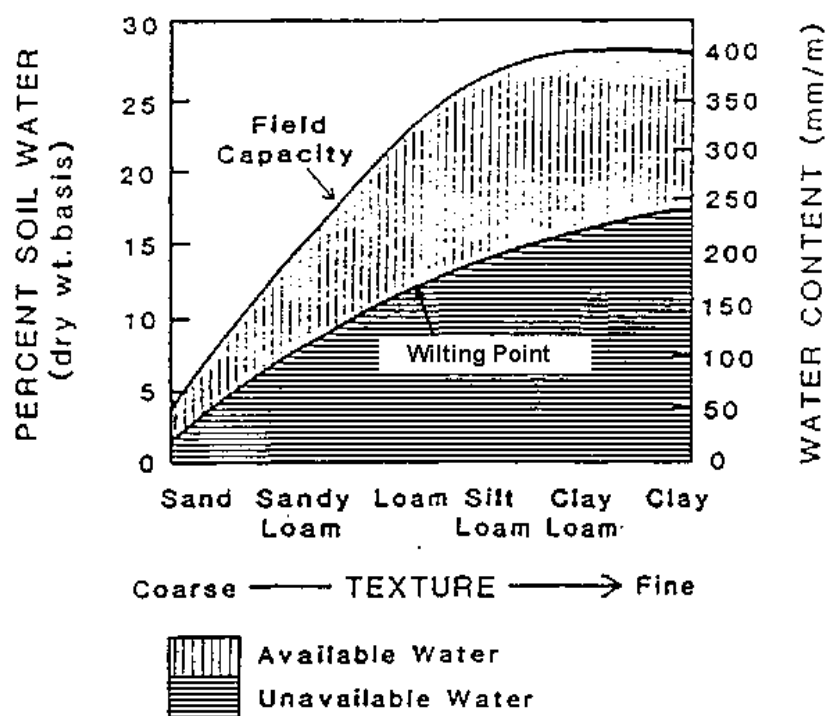


Figure 4: Effect of the soil texture on the soil water content (Salazar et al. 1994).

Soil acidity largely controls the SOM decomposition as it is involved in many states and processes (Leifeld et al., 2013). The pH value of the soil affects the physical, chemical, and biological properties and processes of the soil, such as the availability of certain nutrients, microbial activity, aggregate stability, solubility of heavy metals and plant growth. The pH value is therefore a crucial property to measure whenever soil is investigated (Smith and Doran, 1996). According to Smith and Doran, growth, nutrition and yield of many crops is best at a pH value between 6 and 7.5 (Smith and Doran, 1996). Anderson and Domsch (1993) show that a low pH value reduces the amount and activity of microbes.

Studies show that soil properties are affected by climate properties and vice versa. Figure 4 by Salazar et al. (1994) shows the effect of texture on the SWC. Soil moisture is significantly dependent on soil clay content because small pores are able to hold more water than coarse pores (Balogh et al., 2011).

1.3.3 Influence of Terrain Properties on the Mineralisation of Carbon

High uncertainty lies in the direct effect of the topography of a certain area on the C_{\min} of its soil. Studies, however, have shown that landscape morphology can indirectly affect C_{\min} rates (Kang et al., 2003). The topography, such as the steepness and the orientation of the terrain, therefore contribute to the spatial variability the C_{\min} (Swanson et al., 1992).

The topography can influence a microsite because it affects the microclimate by determining variables such as temperature, light or moisture (Kang et al., 2003). The slope, for instance, has an impact on the distribution of soil water (Riveros-Iregui et al., 2012). Kang et al. (2003) demonstrate that the soil moisture is significantly greater on north-facing slopes compared to south-facing slopes. Biological processes related to the C dynamics such as plant growth or C_{\min} are also affected by these environmental conditions (Mohammadi et al., 2017). The landform further influences geomorphic disturbances. Erosion, for instance, is involved in the lateral distribution of SOC (Doetterl et al., 2016).

Overall, the terrain affects the climate and the geomorphology and therefore plays an indirect but important role in shaping the conditions for the C_{\min} of the soil (Riveros-Iregui et al., 2012). Therefore the slope gradient and orientation are included as drivers of the C_{\min} .

1.4 Priming Effects

In chapter 1.2 about residue quality it has been shown that a low quality of soil carbon limits the amount of available energy for microbes and therefore the C_{\min} rate. As visible in Figure 5b, the addition of fresh organic residues generally stimulates the mineralisation rate of the carbon that was in the soil before fresh residue addition (hereafter native SOC) (Kuzyakov et al., 2000; Bingeman et al., 1953). This is due to the higher availability of energy, which is assumed to lead to an increase in the overall amount and activity of microorganisms (Fig. 5a) (Fontaine et al., 2003; Kuzyakov et al., 2000). This effect is called priming effect (PE) (Kuzyakov et al., 2000).

The PE can either be positive or negative. A positive PE describes an increase in the SOM decomposition through the addition of easily decomposable organic residues compared to the SOM decomposition without any supply of organic substances (Fig. 5b). A negative PE is a reduction in the SOM mineralisation compared to soils without any supply of residues (Fig. 5b). Negative PE may occur due to a change in the preference of microbes, preferring the more easily available substrate instead of native SOM, or because of the inhibition of microbial activity because of changes in the soil environment (Zimmermann et al., 2011; Kuzyakov et al., 2000). Negative PE appears to be of greater significance to the ecosystem, as it can lead to an increase in the carbon stocks in the soil (Kuzyakov et al., 2000).

There is an apparent priming effect (APE) and real priming effect (RPE). The APE is, according to Jenkinson et al. (1985), defined by accelerated CO_2 evolution due to the activation of microbial metabolism and changes in the turnover of the microbial biomass without effects on the SOM decomposition. The APE is unusual for and therefore not further discussed in this study (Kuzyakov et al., 2000). The RPE arises due to the accelerated activity of these microorganisms, which may enhance the degradation of SOM because of co-metabolism and higher enzyme production (Blagodatskaya and Kuzyakov, 2008).

Several studies suggest that the supply of easily decomposable substrate compounds, such as sugars, itself has no or only little effect on the C_{\min} of native SOM compared to the effect of more stable substrate compounds, such as cellulose or lignin, although the former contains more readily available energy (Fontaine et al., 2003). This was also observed in a study of Shahbaz et al. (2017), where the maximum PE was recorded for the mineralisation of root C compared to leaf and stem C. Fontaine et al. (2003) explain this with a competition

for energy and nutrient acquisition between the microorganisms called r-strategists, which are specialized in the decomposition of easy decomposable organic matter (previously dormant microorganisms) and so-called K-strategists, which feed on polymerised SOM (Fig. 5a). Differences in the observed PE can therefore also be related to the structure of the microbial community in the soil (Hamer and Marschner, 2005). Shabaz et al. (2017) further stated that the PE intensity declined, compared to the amount of added substrate, when residue C additions were high, regardless of the residue type. Hence, depending on the quality and quantity of the substrate added, different microbial species can be activated, which consequently changes the microbial community and produce PEs (Kuzyakov and Bol, 2006). Blagodatskaya and Kuzyakov (2008) concluded that if the amount of added substrate C is higher than the microbial biomass C content of the soil, both microbial growth and changes in the community structure could occur.

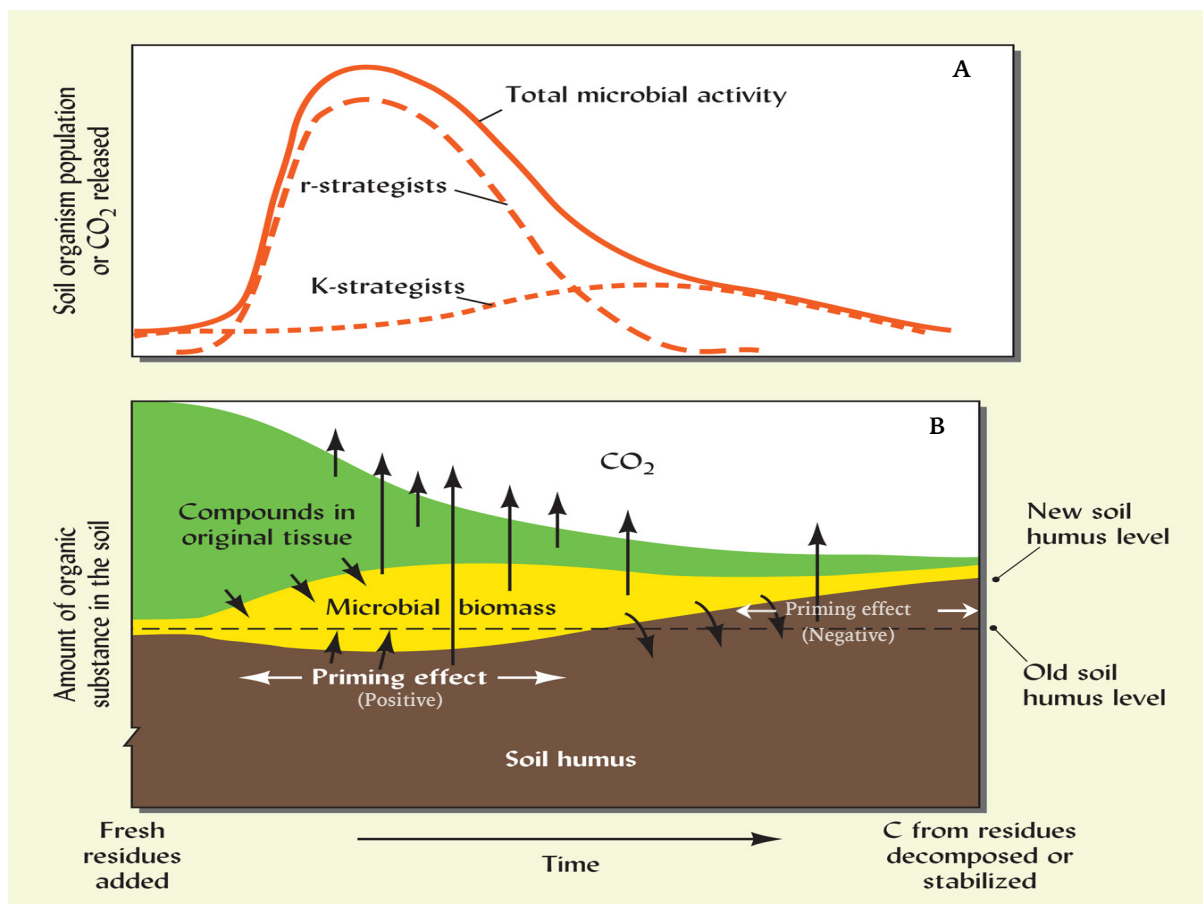


Figure 5: Changes that occur when fresh plant residues are added to a soil. (A) The orange lines show the relative growth and activity of r-strategist and K-strategist microorganisms as well as the total microbial growth and activity (B) The arrows indicate transfers of carbon among compartments. The green area shows the breakdown of the organic residues over time and its conversion into CO₂. The yellow area shows the microbial growth and decay over time. The brown area shows the amount of SOM over time (Brady and Weil, 2016).

It is obvious that the phenomenon of PE is rather complex and involves multiple mechanisms (Kuzyakov et al., 2000). It is important to note that until 1993 none of the common models of C dynamics took the PE into account, disregarding the fact that the additional C release can be very large and even greater than the amount of C added to the soil (Engel et al., 1993). More recently, there is marked increase in the amount of research done on PE and the C dynamics (Shabaz et al., 2016; Blagodatskaya and Kuzyakov, 2008). This is a clear sign that the current knowledge is insufficient and that further research on the PE and the interaction between plant residues and the soil needs to be done.

1.5 Tracing Carbon Mineralisation with the ^{13}C Labelling Method

There are several methods to trace the C_{\min} . Many methods, however, only manage to distinguish between heterotrophic and autotrophic respiration. Isotopic methods, on the other hand, further allow the distinction between the C_{\min} of plant residues and of native SOM (Hanson et al., 2000).

The two most common (stable) isotopes of carbon are ^{12}C and ^{13}C . The natural abundance is about 98.8 % for ^{12}C and 1.1 % for ^{13}C . During photosynthesis the ^{13}C isotope is discriminated, which leads to a reduction of ^{13}C in plants. This is not only because the isotope ^{13}C is less frequent, but also because it is heavier than the ^{12}C isotope and can therefore be less easily incorporated by the plants (O'Leary, 1981). Depletion in the CO_2 efflux of the lighter isotope ^{12}C , on the other hand, can be seen during organic matter decomposition, which leads to a relative enrichment of ^{13}C in the mineralised carbon (Gunina and Kuzyakov, 2014).

There are two notations to express the amount of the rarer and heavier ^{13}C stable isotope in a sample: $\delta^{13}\text{C}$ or ^{13}C atom % (Staddon, 2004). In this master thesis the notation $\delta^{13}\text{C}$ is used, because it is more common and because the analysis is relatively simple (Staddon, 2004). The $\delta^{13}\text{C}$ expresses the ^{13}C content of a sample relative to the reference standard (V-PDB) with a $^{13}\text{C}/^{12}\text{C}$ ratio of 1.237×10^{-2} (Staddon, 2004). It is calculated as follows:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad [1]$$

where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ ratios of the sample and the standard. The $\delta^{13}\text{C}$ can differ in natural materials, for example in C_3 and C_4 plants and can therefore be used to

trace carbon in the environment (Coleman and Fry, 1991). Using stable isotopes as a tracer is a very helpful tool to detect and study the PE. The knowledge of the $\delta^{13}\text{C}$ signature in different materials for example allows partitioning the total CO_2 efflux from the soil into the mineralisation of residue C, the mineralisation of native SOC when no residues are added and additional mineralisation of native SOC when residues are added (Midwood et al., 2006; Staddon, 2004). The measurement of isotopic ratios also allows an interpretation of the decomposition state (Gunina and Kuzyakov, 2014).

The above-mentioned partitioning can be done with the use of either natural abundance or labelling methodologies (Gunina and Kuzyakov, 2014; Staddon, 2004). It is, however, difficult to trace carbon from different natural materials with natural abundance techniques if the $\delta^{13}\text{C}$ values of the natural materials are too similar. With labelling approaches it is, in contrast, possible to obtain very different $\delta^{13}\text{C}$ signatures between the natural materials in use. In this way the C_{min} can easily be partitioned into its sources of C (Subke et al., 2004). The labelling method is thus more efficient than using the differences in natural abundance because all the carbon pools of interest can be studied individually (Staddon, 2004).

1.6 Related Work

This thesis can be considered as a complement to the on-going PhD project of González Domínguez. The project of González Domínguez focuses on the influence of ecosystem properties on C losses from soils as CO_2 efflux and as DOC in forest soils, whereas this thesis concentrates on the influence of ecosystem properties and root residue addition on the CO_2 efflux from agricultural and grassland soils. For the project González Domínguez also aims to produce a SOC vulnerability ranking of soils (González Domínguez et al., 2014).

For the PhD project, 54 forest sites were selected using a specific selection process. The selected ecosystem properties are the same as in this master thesis. The study sites are distributed over the five biogeographical regions of Switzerland covering the different combinations of the variability of the selected ecosystem properties.

2 Objectives

Schmidt et al. (2011) have shown that ecosystem properties have a large impact on the vulnerability of SOM and thus the mineralisation of carbon. The main drivers of the C_{min} of the soil are the climate properties temperature and moisture (Davidson and Janssens, 2006a). Additionally, the C_{min} is influenced by site-specific properties of the soil, such as the clay content or the pH value, and by the topography of the soil (González Domínguez et al., 2014). The added impact of root residues on the mineralisation has been discussed as well. Studies have shown that roots tend to mineralise more slowly and therefore remain much longer in the soil than the aerial parts of the plant (Abiven et al., 2005). However, much uncertainty exists about the effect of ecosystem properties on the C_{min} and the extent to which they contribute to the C_{min} when root residues are added to the soil.

The aim of this master thesis is to enable a better understanding of the way in which root residue addition to the soil influences the C_{min} in agricultural and grassland soils of Switzerland. It is of particular interest to better understand how the variability of the individual ecosystem properties influences the C_{min} and to monitor the changes that occur when root residues are added to the soil. To achieve this goal a number of soil samples were collected and incubation experiments were designed and carried out to measure the extent to which the C_{min} is altered by adding root residues to the soil samples. Another aim was to find out if PEs in soils enriched with root residues are detectable and how they behave and react to changing manifestations of the ecosystem properties.

A further aim of this thesis is to establish if any patterns between the different contributors of the CO_2 efflux from soil amended with root residues can be detected among agricultural and grassland soils. These different contributors are the mineralisation of residue C (hereafter root C_{min} , or roots), the mineralisation of native SOC without effects of residue addition (hereafter basal mineralisation or control) and the PE, hence the additional mineralisation of native SOC resulting from residue addition. The following research questions and hypotheses derive from these research goals:

1. How does the addition of root residues influence the C_{min} in agricultural and grassland soils of Switzerland?
 - ➔ *Root residue addition increases the total C_{min} .*
 - ➔ *The PEs can be higher than the basal mineralisation.*
 - ➔ *The addition of root residues increases the carbon stocks in the soil.*

2. How do soils with different ecosystem properties influence the C_{min} in agricultural and grassland soils of Switzerland when root residues are added to the soil?
 - ➔ *Climate properties have a larger influence on the root C_{min} and on the PE than soil or terrain properties.*
 - ➔ *The root C_{min} shows a similar dynamics but a different rate in the different soils.*
 - ➔ *The PE shows a different dynamics and rate in the different soils.*

3 Material and Methods

This chapter describes the steps taken and the materials used to answer the research questions. In summary, the influence of the selected ecosystem properties on the C_{\min} of soils without root residues (hereafter also basal mineralisation or control) and on the C_{\min} of soils containing root residues (hereafter also treatment) was analysed. To measure the C_{\min} of the control and the C_{\min} of the treatment, incubations were carried out. The C_{\min} of the soils was analysed on its ^{13}C isotopic signature to further partition the C_{\min} of the treatment into the root C_{\min} , the basal mineralisation and the PE.

3.1 Study Sites

The overall investigation area was limited to the area of Switzerland, which covers 41'285 km². The area of particular interest comprises only the agricultural and grassland areas of Switzerland, which cover 14'817 km². Hence, about one third of the area of Switzerland is defined as agricultural land (BFS, 2009). 10'500 km² of the available agricultural land are currently cultivated. 58 % of the cultivated area consists of natural meadows and grazing land. Thus permanent grasslands make up the major part of the cultivated land (BFS, 2015).

3.1.1 Data Sources

The soils used for the incubation were selected from an already existing Soil Monitoring Network (NABO) database containing 53 grassland and cropland sites. Each profile description provided information on its coordinates, altitude, land-use, soil type, and on selected biochemical properties, such the content of clay in percentage in relation to silt and sand, the pH value, the total nitrogen (TN) content (%) and the TOC content (%). The database was extended with climatic data that was obtained from MeteoSwiss and Meteotest. The climatic characteristics soil temperature and soil moisture, were measured over a time span of 30 years (1981-2010). For the soil temperature the mean monthly temperature of the air in Celsius degree was used, because only the uppermost 20 cm of the soil is targeted in this thesis, which is influenced mainly by the air temperature. The soil moisture in mm m⁻³ was defined by the difference between the mean monthly precipitation and the mean monthly potential evapotranspiration. The database was further extended with data of the orientation in degrees and the slope gradient as a percentage of the maximum slope, which was

gained using a digital elevation model of 1:25'000 from Swisstopo. Selected chemical properties (pH value, TOC, TN) of the existing NABO database were further updated by the analysis of the freshly collected soils. More detailed information about the database with the different variables and their values is given in chapter 4.

3.1.2 Study Site Selection

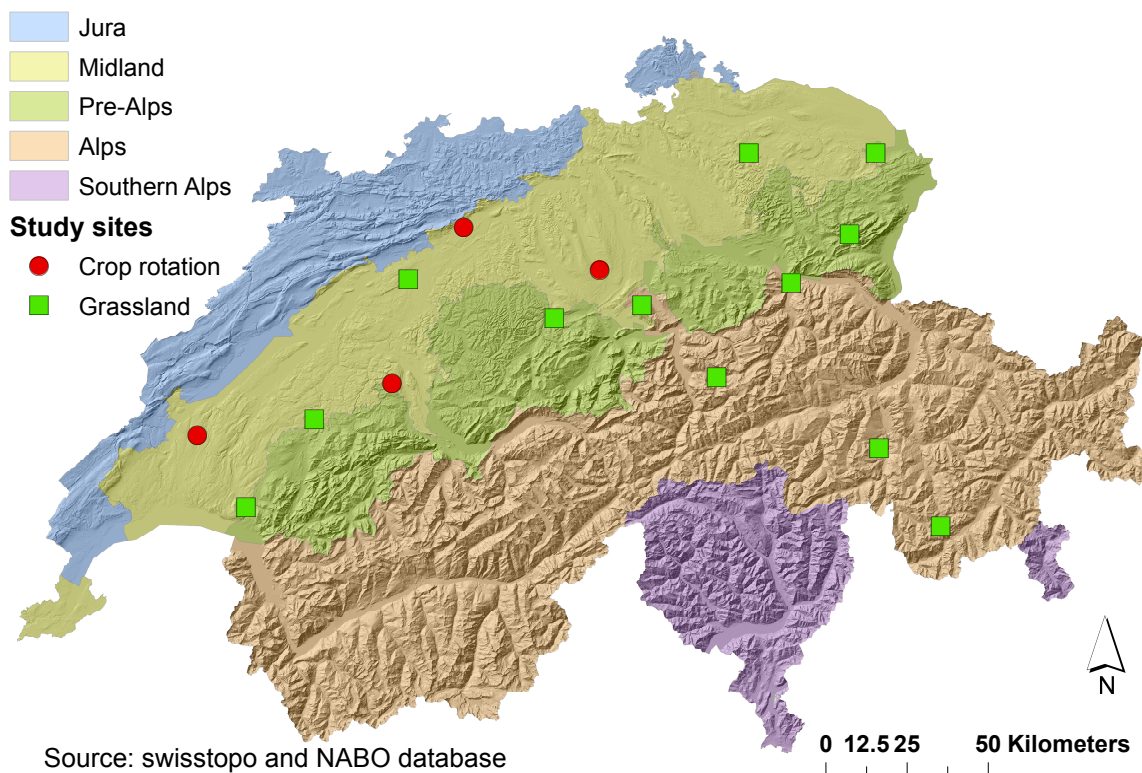


Figure 6: Distribution of the sixteen study sites divided into crop rotation (circles) and grassland sites (squares) (González Domínguez et al., 2014).

Sixteen agricultural sites were selected from the NABO database. The different sites were selected with regard to the hypotheses, in order to be able to compare the influence of the different ecosystem properties on the C_{min} . The selected sites had to be representative for grasslands and croplands in a temperate climate and it was necessary that they represent different combinations of the variables. This meant that they should cover as far as possible the entire range of the ecosystem properties in Switzerland included in the NABO soil database. Furthermore, the selected sites should be distributed over the whole area of Switzerland in order to get a spatial aspect of the ecosystem properties. The selected ecosystem

properties were climate, soil and terrain out of which the two most important characteristics were selected for each property. This resulted in the characteristics temperature and moisture (climate), pH value and clay content (soil), slope and orientation (terrain). Figure 6 shows the distribution of the 16 study sites that were selected according to these criteria. Table 1 presents the values of the different ecosystem properties.

The selected soils are typical for agricultural areas and grasslands. They are mainly cambisols or gleysols. Only one soil belongs to the soil class of stagnosols.

Table 1: Data of the selected ecosystem properties and the TOC on the sixteen selected study sites, including additional information on the site ID, the MASL, the productive region, the location, the soil type and the land use. The TOC contents and the pH values for all the three composites from each site exist but only the means of the three composites are shown in this table.

Site ID	TOC (%)	Temp. (°C)	Moist.	Clay (%)	pH (%)	Slope (%)	Orient. (°)	MASL	Prod. region	Location	Soil type	Land use
55	3.96	8.33	63.87	35	6.38	6.48	355.11	537	Midland	Aadorf (TG)	Cambisol	Grassland - intensive
56	2.04	7.40	67.80	14	5.15	27.02	186.28	945	Midland	Niedermuhlern (BE)	Cambisol	Crop rotation
57	4.21	7.81	92.35	33	5.95	1.28	18.02	431	Alpine	Glarus Nord (GL)	Gleysol	Grassland - intensive
58	2.14	9.33	52.90	26	5.36	11.68	346.36	500	Midland	Hochdorf (LU)	Cambisol	Crop rotation
59	2.49	8.27	52.96	19.3	5.31	4.92	7.04	735	Midland	Ependes (FR)	Cambisol	Grassland - moderate
60	2.81	9.17	52.67	24	4.56	1.12	116.55	464	Midland	Buchegg (SO)	Gleysol	Grassland - moderate
61	4.37	5.57	114.20	33	4.75	33.02	358.39	1100	Alpine	Unterschächen (UR)	Cambisol	Grassland - moderate
62	4.3	3.88	142.05	26.8	5.11	36.23	7.64	1338	Pre-Alps	Nesslau (SG)	Cambisol	Grassland - extensive
63	3.74	7.91	79.62	17	5.41	19.75	68.03	955	Pre-Alps	Werthenstein (LU)	Gleysol	Grassland - intensive
64	2.35	8.89	54.52	35.5	6.32	0.43	54.52	450	Midland	Kestenholz (SO)	Cambisol	Crop rotation
65	3.87	8.90	82.81	26.3	5.31	7.95	238.50	818	Midland	Attalens (FR)	Cambisol	Grassland - intensive
66	4	5.60	52.81	22	4.59	26.25	136.20	1818	Alpine	Lohn (GR)	Cambisol	Grassland - extensive
67	7.75	-0.44	63.15	25.5	4.81	1.94	32.20	2118	Alpine	Bivio (GR)	Cambisol	Grassland - extensive
68	4.58	8.95	51.41	38	6.31	7.30	0.80	526	Pre-Alps	Mörschwil (SG)	Stagnosol	Grassland - intensive
69	0.96	9.19	41.17	16	5.55	4.07	37.03	684	Midland	Pailly (VD)	Cambisol	Crop rotation
70	3.24	5.15	107.02	21.9	4.57	16.66	311.14	1025	Alpine	Küssnacht (SZ)	Cambisol	Grassland - extensive

3.1.3 Variability within the Selected Ecosystem Properties

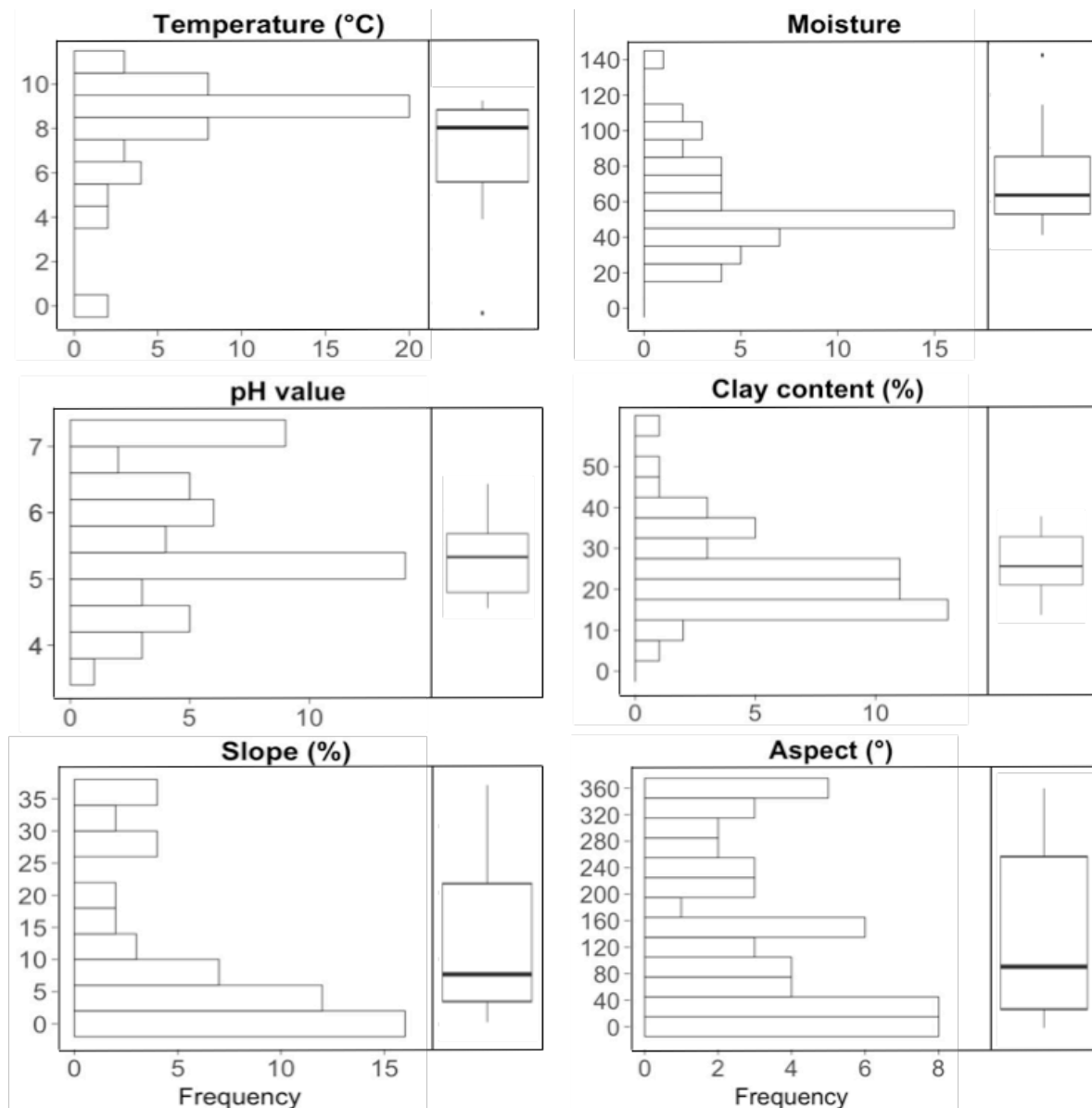


Figure 7: The Variability of the sixteen study sites within the selected ecosystem properties is visible in the boxplots. The Variability of the complete NABO database within the selected ecosystem properties is visible in the histogram.

Figure 7 shows the variability of the particular drivers within all the study sites of the NABO database for Swiss agricultural and grassland soils as a histogram. The boxplot visualises the variability of the particular drivers within the sixteen selected study sites. The individual values of the drivers for the sixteen selected study sites are listed in Table 1. The variability of the particular drivers within the selected the study sites does not always cover the whole spectrum of the variability found in the NABO database. The variability was, however, representative of the values most commonly found in the NABO database. The range of the

temperature of the selected study sites varied between -0.44 and 9.33 °C and covered almost the whole variability of the database that ranges from -0.44 to 10.84 °C. The range of the temperature is also representative for the most frequent values in the NABO database. The spectrum of the soil moisture of the selected soils was between 41.17 and 142.05 and did not cover the whole range of the moisture found in the NABO database, which reaches from 17.72 to 142.05. Very dry soils were not selected for this thesis. The variability of the selected study sites regarding the pH value (4.56 - 6.38) and the clay content (14 - 38 %) are rather small compared to the total variability of the pH value (3.7 - 7.4) and clay content (5.8 - 59 %) found in the database, but they represent the most frequent values of the database. The range of the slope (0.43 - 36.23 %) and the orientation (0.80 - 358.39 °) within the selected soils covers nearly the whole variety of the slope (0 - 36.44 %) and the orientation (0.80 - 359.39 °) from the database. Both ranges are well represented within the most frequent values in the database.

3.2 Soil Sample Collection

The soil samples from the sixteen sites were collected in summer 2015. For each site, three composites were produced. To this end, soil was taken from within a 40 × 40 m² plot. Because soils can be very heterogeneous within small distances (Tan, 2005), soil was taken from three non-overlapping areas of the plot. From each of these areas, eight soil cores were taken at a depth between 0 and 20 cm and then mixed to form a composite. Thereby each composite constituted an experimental replicate and spatial variability was accounted for. A 5 cm diameter Humax corer was used to collect the soil samples. The samples were then transported in a portable fridge and sieved in the lab to a granularity of ≤ 2 mm. The resulting 48 soil samples were stored at 3.5 °C until the beginning of the experiment in April 2016.

3.3 Soil Sample Preparation and Analysis

Before starting the experiment, the 48 soil samples were analysed on their TOC content, their TN content and their pH value. For these measurements, subsamples of the soil samples were dried at 40 °C and milled. A part of the subsamples was fumigated with HCl to remove the carbonates for the TOC and TN analyses.

The TOC and the TN concentrations were measured at ETH Hönggerberg with an Elemental Analyser (vario MICRO cube, Elementar, Germany). Thereupon, subsamples of 10 - 15 mg were weighed into tin capsules. Then each subsample was burned in the Elemental Analyser at about 950 °C. The resulting CO₂ was measured by means of thermal conductivity.

The pH value was measured with a pH-meter in a 0.01 M CaCl₂ solution. The soil to solution ratio was 1:2.5. CaCl₂ was used as an electrolytic substance, because it is more stable than H₂O. The pH-sensor measures the resistance of this solution and can derive the pH value through calibration with standardised solutions. For this calibration, two solutions with a pH value of 4 and 7, respectively, were used. First, 20 g of the subsamples were weighed into a beaker, then 50 ml of the CaCl₂ solution was added and finally the solution was mixed for 30 minutes. Then the pH value was determined with the pH-sensor (Schmidt et al., 2008).

3.4 Root Sample Preparation and Analysis

The root residues that were added to the soils of the treatment were ryegrass, which is a typical grassland grass (Mawdsley and Bardgett, 1997). To distinguish the C_{min} of the roots from the rest, the stable isotope ¹³C was used as a tracer and labelling methodologies were chosen rather than calculations based on the natural abundance. This was due not only to the reasons described in chapter 1.5, but also because ryegrass is a C₃ plant with natural ¹³C ratios ranging from -40 to -20 ‰ (Staddon, 2004) and the values of the δ¹³C of the soils before incubation were assumed to vary in a similar range (e.g. Gunina and Kuzyakov, 2014; Yu et al., 2010). Tracing the origin of the C mineralised with natural ¹³C abundance would therefore become very difficult, because isotopic methods only work if the isotope signatures of the respired CO₂ sources differ significantly from one another (Trumbore, 2006). In contrast, it is very easy to trace the origin of C in the mineralisation through labelling techniques. Hence, the ryegrass was bred under a ¹³CO₂-enriched atmosphere and labelled with a high factor of ¹³C. The δ¹³C value of the roots was measured to be 2300. As mentioned in chapter 1.5, the labelling was necessary to detect a possible PE.

The carbon content of the roots was measured with the Picarro (see chapter 3.3.5) and was approximately 39 %, which is normal for roots (Syahrinudin, 2005; Hadley and Causton, 1984). The roots were dried and cut into pieces of approximately 5 mm length. This was done in order to homogenise them but also to keep them in a relatively natural condition,

since particle size, according to Angers and Recous (1997) and Fruit et al. (1999), affects the decomposition rate. Also, the roots were not pulverised in this experiment in order to preserve the protection mechanisms of roots described by Rasse et al. (2005). These protection mechanisms seem to have an additional influence on the decomposition rate.

3.5 Incubation Design

Incubations are done to maintain similar conditions, such as a consistent temperature or a uniform humidity, for all the (soil) samples during the experiment, thus allowing a valid comparison of the samples with each other. However, it must be considered that the conditions for incubation experiments are not the same as for in-situ experiments and that artificial effects are more probable to occur. The mineralisation rate can therefore differ substantially between incubation and in-situ experiments (Trumbore, 2006).

For the root residues, the same amount was incorporated in all the soil samples of the treatment to keep the conditions similar. This means that the amount of C in the root residues was not related to the TOC contents of the soils. Instead, the ideal amount of root residues was calculated with the aboveground biomass, the shoot to root ratio and the bulk density, which were all determined by means of literature, visible in Table 2.

Table 2: Reference values for the shoot biomass, the shoot to root ratio and the bulk density for related biome types.

Property	Biome type	Value	Reference
1) Shoot biomass (g m^{-2})	Tallgrass prairie	500	Briggs and Knapp 1995
	Italian ryegrass	600	Baldinger et al., 2013
<i>Mean</i>		550	
2) Shoot to root ratio	Perennial ryegrass	3.8	Lehmeier et al., 2008
	Temperate grassland	4.5	Mokany et al., 2006
<i>Mean</i>		4.15	
3) Bulk density (Mg m^{-3})	Temperate grassland	1.2	Gastine et al., 2003
	Cropland	1.3	Evrendilek et al., 2004
	Cropland with tillage	1.5	Franzluebbers et al., 1995
<i>Mean</i>		1.3	

The percentage of roots in the soil was calculated in Table 3 using the mean values of the soil properties from Table 2. The percentage of roots in the soil is approximately 0.88 %.

Thus for 20 g of soil, 0.18 g of root residues were incorporated into the soil. The calculated percentage of roots was slightly higher than in the studies carried out by with Abiven et al. (2005) (0.32 %) or Johnson et al. (2005) (0.4 %), but still lower than in similar tests carried out by Shahbaz et al. (2017) (1.08 %). The reason for using a relatively high percentage of carbon from root residues was to provide more recognisable patterns (Shahbaz et al., 2017).

Table 3: Calculations and results for the root biomass, the soil mass and the percentage of roots.

Property	Calculation	Result
Root biomass	Mean shoot biomass x mean shoot to root ratio	22'825 kg ha ⁻¹
Soil mass	Mean bulk density x soil depth (20 cm)	2'600'000 kg ha ⁻¹
Percentage of roots	(Root biomass / soil mass) x 100	0.88 %

For the experiment, the three composites of each of the sixteen soils were preincubated at 20°C for nine days, because the earlier sieving and the temperature increase affects the availability of SOM for microorganisms and may cause a respiration flush (Blagodatskaya and Anderson, 1999).

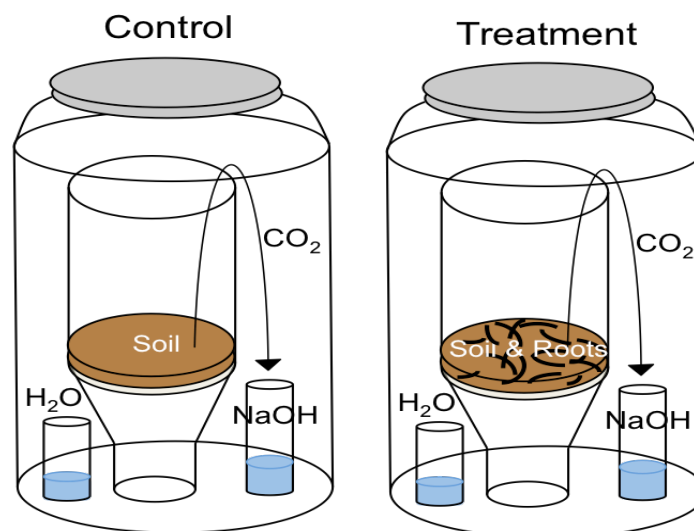


Figure 8: Experimental design for the control and the treatment soils. Se-alable glass jars to measure the CO₂ efflux from the soil respectively from the soil including root residues. The jars also contain NaOH traps and water vials.

From each of the preincubated soil samples two subsamples with a dry equivalent of 20 g were used. One subsample was left without residue addition (control) and the other was enriched with labelled ryegrass root residues (treatment). Therefore the above-mentioned 0.18 g of root residues were homogeneously mixed into each subsample. The soil samples

were then inserted into microlysimeters. The soils were slightly compressed to prevent the soils from being too loose. The soil water tension was adjusted to -20 kPa by percolating the soils with a nutrient solution. The microlysimeters were then placed into sealable glass jars together with a glass vial filled with 20mg of water to keep the air humid and another glass vial with 20 ml of sodium hydroxide (NaOH) (1 M) to trap the mineralised CO₂ (Fig. 8). The jars were then sealed and placed in two incubators, one for the control and one for the treatment samples. The incubators were kept at a constant temperature of 25 °C. The jars were opened periodically, on a total of six sampling dates which occurred after 6, 13, 33, 55, 84 and 119 days. This was done firstly to replenish the jars with fresh atmospheric CO₂ and secondly to replace the NaOH traps to prevent saturation of the NaOH solution. During these samplings the soil samples were also percolated with a nutrient solution.

Three identical jars with the similar experimental construction but containing no soil, so-called blanks, were additionally placed in each of the two incubators. The blanks were used to measure the background concentrations of CO₂. The mean C_{min} of the three blanks of each sampling date were subtracted from the C_{min} of the soil samples of each sampling date

On each sampling date in total three additional vials were filled with 20 ml of fresh NaOH (1 M) solution and immediately sealed. These so-called stocks were taken at the beginning, the middle and the end of the NaOH trap replacement. The stocks were stored and later on used to measure the real conductivity of the soil samples by subtracting the mean conductivity of the three stocks from the conductivity of each soil sample for each sampling date.

3.5.1 Measuring the Soil CO₂ Efflux

The amount of CO₂ in all the NaOH traps of the treatments, controls, blanks and stocks of the six sampling dates (648 samples) was analysed. The following equation shows how the CO₂ efflux from the soil reacts with the NaOH solution in the traps:



The more CO₂ is absorbed into the NaOH traps, the less the solution is depleted with NaOH. The solution becomes less reactive and the electrical conductivity smaller (Wollum and Gomez, 1970). The electrical conductivity is therefore a good tool to measure how much CO₂ was mineralised. To measure the electrical conductivity a conductivity meter was used. The conductivity meter was calibrated before using. The measuring rod was then held in the

NaOH solution until the measured value was stable. Then, the values of the conductivity had to be transformed into CO₂ values. The following transformation algorithm was used:

$$CO_2 = ((-0.1695 * Conductivity) + 29.022) * 20 \quad [3]$$

The mean CO₂ of the three blanks from each sampling date was subtracted from the soil samples of the treatment and the control of the respective sampling date to gain the actual amount of CO₂ resulting from the mineralisation. CO₂ contains 27.29 % of C (Mortimer and Müller, 2007). The CO₂ mineralised is therefore multiplied by the factor 0.2729 to calculate the amount of C mineralised.

3.5.2 Measuring the $\delta^{13}\text{C}$ Isotopic Values

To separate the C_{min} of the treatment into root, control and PE a $\delta^{13}\text{C}$ isotopic analysis was carried out. For this purpose the NaOH traps of the control and of the treatment had to be analysed on their isotopic signature.

In order to analyse the NaOH traps on their ^{13}C isotopic signature, it was necessary to transform the NaOH solution from equation 2 into a powder. Thus, the carbon of the NaOH traps had to be precipitated. For each sample 5 ml of strontium chloride (SrCl₂) was mixed with 5 ml of the NaOH solution in a centrifugation extainer vial and sealed with a cap (according to Harris et al., 1997). The molarity of the SrCl₂ solution was 1 M in order to be in excess with respect to Na₂CO₃. This was necessary to prevent isotopic fractionation from occurring. As a result of the mixing, SrCO₃ with a low solubility was produced. The equation for the precipitation is as follows:



The vials were then centrifuged for 5 minutes at a rate of 2500 rpm to better separate the solution from the precipitation. After centrifugation the resulting water solution was carefully decanted. Then the vials with the remaining SrCO₃ were dried at 60° for about 48 to 96 hours and stored for later $\delta^{13}\text{C}$ analysis (Hagedorn et al., 2004).

The dried SrCO₃ in the vials extracted from all the 576 soil and 36 blank samples were transformed into data using a Picarro automatic stable isotope analyser (Picarro ^{13}C CM-CRDS System). A Picarro is able to measure the concentration of stable isotopes by combusting

the sample in a chamber and separating the fractions of heavy and light isotopes of the molecules of the resulting gas. Subsamples of 7 to 12 mg of dried SrCO₃ from each sample were weighed into tin capsules and analysed on its total C content and $\delta^{13}\text{C}$ value (Hagedorn et al., 2004). The weight was defined in relation to the expected C contents in the samples. The tin capsules (5 x 8 mm) had to be carefully sealed and were stored until there were enough capsules for the analysis in the Picarro. Before and after each run of six samples, a trial run with standards was done in order to avoid procrastination. The standard was a miscanthus grass with a $\delta^{13}\text{C}$ signature of -14.8. The weight of the standard was weighed between 1 and 2 mg according to the same criteria as with the samples. The labelled roots were also examined on their TOC content and $\delta^{13}\text{C}$ value. Therefore five replicates were weighed between 1 and 2 mg according to the same criteria as the samples. On the one hand, the Picarro provided data about the ¹²CO₂ and ¹³CO₂ integral, which served to measure the percentage of SOC in the sample. On the other hand, it measured the $\delta^{13}\text{C}$ values, which served to identify the origin of the carbon and to define the state of decomposition.

3.5.3 Calculations

The $\delta^{13}\text{C}$ value of the mineralised C from the control and from the treatment is needed to calculate the ratio of mineralised C originating from the roots to that stemming from the native SOM in the treatment samples. For the calculation the following linear two-ended mixing model (Bernoux et al., 1998) was used:

$$\% C_{SOC(Treatment)} = \frac{\delta^{13}C_{Treatment} - \delta^{13}C_{Root}}{\delta^{13}C_{Control} - \delta^{13}C_{Root}} \times 100 \quad [5]$$

$$C_{SOC(Treatment)} = \% C_{SOC(Treatment)} \times C_{Treatment} \quad [6]$$

$$C_{Roots(Treatment)} = \% C_{Roots(Treatment)} \times C_{Treatment} \quad [7]$$

In the above equation $\%C_{SOC(Treatment)}$ represents the percentage of C mineralised from the native SOC (SOC that was in the soil before residue addition) in the treatment. $\%C_{Roots(Treatment)}$ represents the percentage of C mineralised from the root C in the treatment and is calculated by subtracting the $\%C_{SOC(Treatment)}$ from 100%. $\delta^{13}C_{Treatment}$ and $\delta^{13}C_{Control}$ are the ¹³C ratios of the C_{min} from the treatment and control samples for each sampling date

and $\delta^{13}\text{C}_{\text{Root}}$ is the ^{13}C ratio of the root residues. The $\delta^{13}\text{C}_{\text{Root}}$ value was always 2300‰. $C_{\text{SOC(Treatment)}}$ is the amount of C mineralised from the native SOC in the treatment. $C_{\text{Roots(Treatment)}}$ is the amount of C mineralised from the root C in the treatment. The linear mixing model approach to determining the relative amount of carbon in the mineralisation originating from the native SOC and from the root C is highly robust and has been successfully used in a variety of other circumstances (Brooks et al., 2002).

To measure the PE, it was necessary to compare the amount of carbon mineralised from the native SOC in the treatment sample (eq. 6) with the amount of carbon mineralised in the control. The PE was calculated according to the following equation by Fontaine et al. (2004):

$$PE = C_{\text{SOC(Treatment)}} - C_{\text{Control}} \quad [8]$$

C_{Control} is the C mineralised from the control sample. If the mineralisation of the native SOC in the treatment is higher than the C_{min} in the control, a positive PE is expected. If the value is smaller, a negative PE is probable. If the value is zero it is likely that no PE occurred.

3.6 Statistical Analysis

All statistical analyses were carried out using the software RStudio (Version 0.99.467, 2009-2015). The data was first visually explored to check the quality of the data and to get an impression of the range and distribution of the data and of potential outliers. When mean values were calculated the standard error was always used in order to indicate how precisely the sample mean value represents values of the three replicates.

The data of the C_{min} was visually checked for the normality of the distribution. A normal distribution is a good indicator for a sufficiently large data collection. The data on the C_{min} was inspected in the original form, in form of square-roots (sqrt) and as logarithms (log), to examine which adaption of the data would show the most opportune normal distribution. The normality was checked with histograms of the frequency and with quantile plots of C_{min} values to visualize the variability. Whenever normal distribution within the samples was observed, a multiple linear regression analysis was done to assess information about the influence of the factors (temperature, soil moisture, clay content, pH value, slope and orientation) on the indicators (cumulative C_{min} of the control, the control+PE the roots and the roots+PE) and to evaluate the relationships among the factors.

For the multiple linear regressions, a multifactorial analysis of variance (ANOVA) was carried

out. In ANOVAs, the effect of the first factor on the indicator is tested in a first step, ignoring the effects of the rest of the factors. Then, after accounting for the effect of the first factor, the effect of the second factor is tested on the remaining unexplained part of the Indicator. This procedure is followed until all the factors in use are tested. The significance codes for the statistical tests that were carried out are: '***' for a significance level of 0 to 0.00099, '**' for a significance level of 0.001 to 0.0099, '*' for a significance level of 0.01 to 0.049, '.' for a significance level of 0.05 to 0.099 and '' for a significance level of 0.1 to 1. The regression models were fitted to the data of the indicators and their MRT was calculated. The MRT of the indicators was used because the normality distribution of the MRT was higher than in the original form. Repeated testing with ANOVA was done to examine the differences in the influence among the factors on the indicators. The influence between and within the factors on the indicators was tested. The aim was to explain as much of the respective indicator as possible and similarly obtain high significance levels for the respective drivers. Therefore, the order and interaction of the drivers was changed in different runs. Some factors did not always appear significant for the indicator and were thus removed from the model. Correlations between the most significant driver of the different indicators were established.

4 Results

The results of the incubation experiment and the statistical analysis are presented in the following sections of this chapter. First, the C_{\min} of the treatment and the control is compared. Then, results about the root C_{\min} and about the native SOC mineralisation are presented for all the soil samples from the different study sites combined and separated into study sites. In a last step the C_{\min} of the root C and of the native SOC are compared with the values of the different ecosystem properties.

4.1 Carbon Mineralisation of the Treatment and the Control

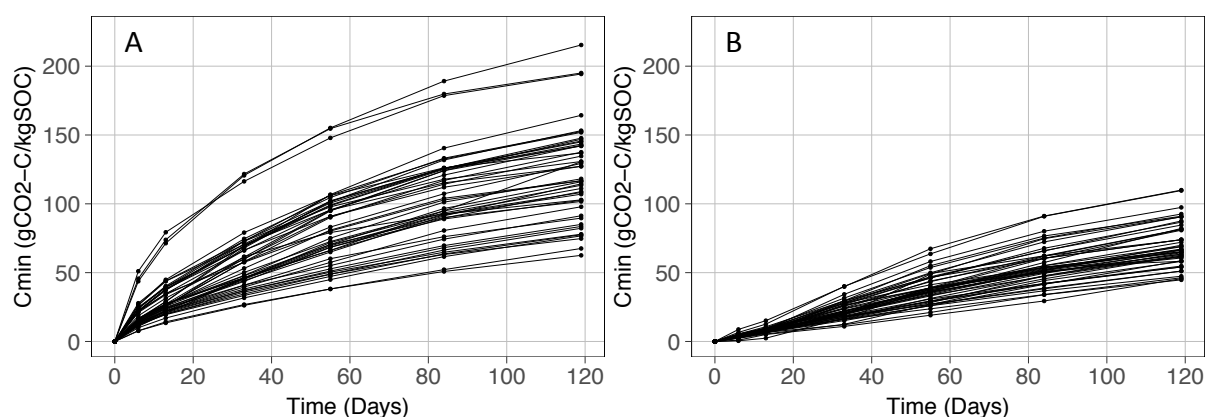


Figure 9: Cumulative C_{\min} in $\text{g CO}_2\text{-C kg}^{-1}\text{SOC}$ over 119 days of incubation from (A) the treatment (B) the control.

Figure 9 shows that residue addition caused a significant increase in the total soil CO_2 efflux of the sixteen soil samples and their three composites. The mean value of the cumulated C_{\min} of the 48 soil samples increased from 7 to 12.2 % of the TOC due to residue addition. For both the treatment and the control the cumulative C_{\min} increased over the whole duration of the experiment. The C_{\min} rate in the treatment was especially large at the beginning of the incubation experiment, and then decreased until the end of the experiment. The cumulative C_{\min} of the treatment therefore showed a non-linear trend, while the cumulative C_{\min} of the control showed a relatively linear trend, indicating that the rate of the C_{\min} in the control was relatively stable. The total amount of C mineralised in the 48 control soil samples after 119 days of incubation varied between 45 (site 69, composite 1) and 110 $\text{g CO}_2\text{-C kg}^{-1}\text{SOC}$ (site 57, composite 1). Hence, the range between the lowest and the highest total

C_{\min} in the control soils was $65.2 \text{ g CO}_2\text{-C kg}^{-1} \text{ SOC}$. The total amount of C mineralised in the 48 treatment soil samples varied between 63 (site 67, composite 3) and $215 \text{ g CO}_2\text{-C kg}^{-1} \text{ SOC}$ (site 69, composite 2). Hence, the range between the lowest and the highest total C_{\min} in the treatment soils was $152.82 \text{ g C kg}^{-1} \text{ SOC}$.

4.1.1 $\delta^{13}\text{C}$ Isotopic Signature of the Soil Samples

The mean value of the $\delta^{13}\text{C}$ signature of the soil samples before the incubation was -23 ‰ and varied between -34 and -18.2 ‰ . The $\delta^{13}\text{C}$ signatures of the soil samples were thus indeed, as assumed earlier in this master thesis, in a similar range as the natural $\delta^{13}\text{C}$ signature of the ryegrass plant. This confirms the necessity to label the roots.

During the whole incubation period, the $\delta^{13}\text{C}$ signature differed significantly between the soil samples of the treatment and the control. The lowest signature measured in the soil samples of the control on the first sampling date of the incubation experiment was -34.7 ‰ (site 68, composite 1) and the highest signature was -18.2 ‰ (site 63, composite 1). The lowest signature at the end was -27 ‰ (site 57, composite 1) and the highest was -19.6 ‰ (site 69, composite 2). Hence, the isotopic signature of the control samples did not vary significantly during the whole incubation. The variations that occurred in the control during the experiment were most probably natural fluctuations that can occur due to different air C compositions during the exchange of the NaOH traps. The lowest signature measured in the soil samples of the treatment on the first sampling date of the incubation experiment was 54.4 ‰ (site 55, composite 1) and the highest signature was 1274.9 ‰ (site 69, composite 3). The lowest signature at the end was 12.6 ‰ (site 60, composite 1) and the highest was 216.5 ‰ (site 69, composite 1). The $\delta^{13}\text{C}$ signatures declined in the course of the incubation experiment. In the beginning the $\delta^{13}\text{C}$ signatures declined much faster than towards the end of the incubation experiment.

Due to the ^{13}C labelling of the root residues it was possible to separate the total C_{\min} of the treatment samples into the basal mineralisation, the root C_{\min} and the PE.

4.1.2 Variability in the Mineralisation of Root Carbon

The results for the root C_{\min} are presented both for the root C_{\min} only and for the root C_{\min} including the PE. The PE is included because most experiments to date have not examined the C_{\min} of the roots separately, but have only researched the C_{\min} of the roots including the

PE. The reason for that is that in most studies the root residues were not labelled. In that case the use of control and treatment samples only allowed the distinction between the basal mineralisation and the additional mineralisation due to residue addition, in which the PE is included but can not be distinguished from the root C_{min} . Very few studies so far have included the labelling of the residues and thereby allowing the analysis and the comparison of all three C_{min} types.

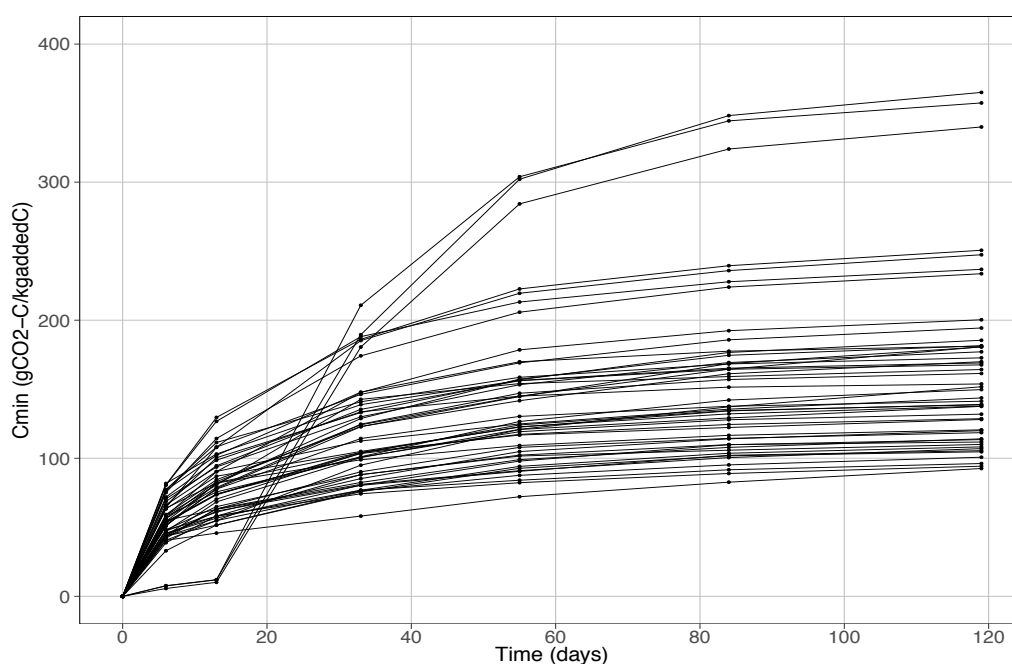


Figure 10: Cumulative root C_{min} in $\text{g CO}_2\text{-C kg}^{-1}$ added C over 119 days of incubation.

Figure 10 shows the cumulative root C_{min} of the 48 soil samples in $\text{g CO}_2\text{-C kg}^{-1}$ added C. In general, the total amount of C mineralised at the end of the incubation varied between 92.6 (site 60, composite 3) and 365 $\text{g CO}_2\text{-C kg}^{-1}$ added C (site 55, composite 2). The pattern of the cumulated root C_{min} of the 48 soil samples was very similar, differing only in the rate of the C_{min} , with three soil samples showing a distinct pattern. The initial rate of the root C_{min} of the 45 soil samples was very high and decreased after the first sampling of the experiment. The three outliers in Figure 10 are the three composites from the soil from study site 55. At the beginning of the incubation experiment almost no mineralisation was visible for these soil samples. Between the second, third and fourth sampling a rapid increase of the C_{min} rate was observed. From this point on the incubation rate of the C_{min} became similar to those of the other soil samples.

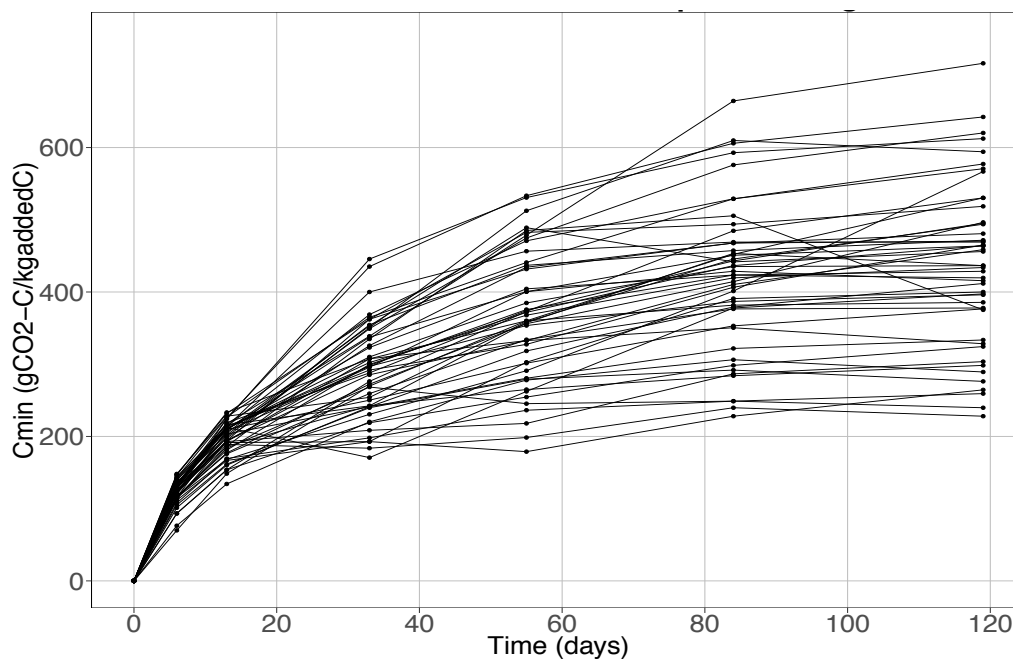


Figure 11: Cumulative root C_{\min} including the PE in $\text{g CO}_2\text{-C kg}^{-1}$ added C over 119 days of incubation.

Figure 11 shows the cumulative root C_{\min} including the PE of the 48 soil samples in $\text{g CO}_2\text{-C kg}^{-1}$ added C. In general, the total amount of C mineralised at the end of incubation varied between 228 (site 61, composite 3) and $717 \text{ g CO}_2\text{-C kg}^{-1}$ added C (site 68, composite 3). With an exception of the high initial rate of the root C_{\min} observed for all the 48 soil samples, the pattern between the soil samples of the cumulated root C_{\min} including the PE was very diverse. After the second sampling some soil samples mineralised within the same rate while other soil samples showed a decline in the rate of the C_{\min} with even negative C_{\min} rates. The soil samples from study site 55 do not appear as outliers in Figure 11 any more. Figure 12 shows the cumulative root C_{\min} (red line) and the cumulative root C_{\min} including the PE (black line) in $\text{g CO}_2\text{-C kg}^{-1}$ added C for the 16 different study sites and their three composites. The pattern and the rate of the cumulated root C_{\min} between the three composites within a study site was very similar. In contrast, the pattern and the rate of the cumulated root C_{\min} including the PE between the three composites within a study site was very different. This was observed, for example, for the study sites 59, 61, 67 and 68. The pattern and the rate of the root C_{\min} of the three composites within a study site was very different to the pattern and the rate of the three composites of the root C_{\min} including the PE.

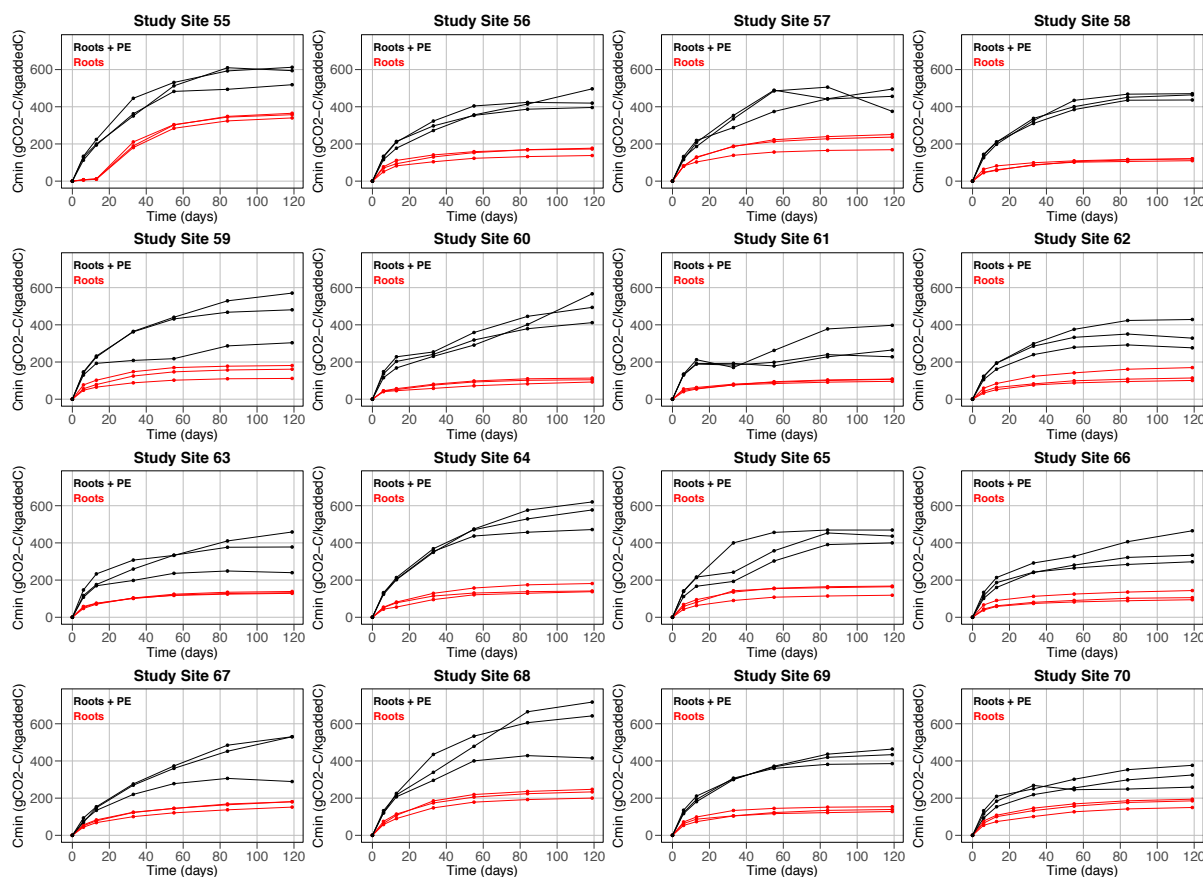


Figure 12: Cumulative root C_{\min} (red line) and root C_{\min} including PE (black line) in $\text{g CO}_2\text{-C kg}^{-1}$ added C over 119 days of incubation for the three composites of the sixteen different study sites.

4.1.3 Variability in the Mineralisation of Native Soil Organic Carbon

Figure 13 shows the cumulative PE of the 48 soil samples in $\text{g CO}_2\text{-C kg}^{-1}$ SOC. In general, the total amount of PE at the end of the incubation varied between 4 (site 67, composite 3) and 123 $\text{g CO}_2\text{-C kg}^{-1}$ SOC (site 69, composite 2). The pattern of the cumulated PE was rather similar for the most soil samples, while the rate between the soil samples showed significant differences. In some soil samples, however, a negative PE was observed for some sections of the incubation period. Surprisingly large rates of the PE were observed for three soil samples. These three outliers are the three composites from the soil from study site 69.

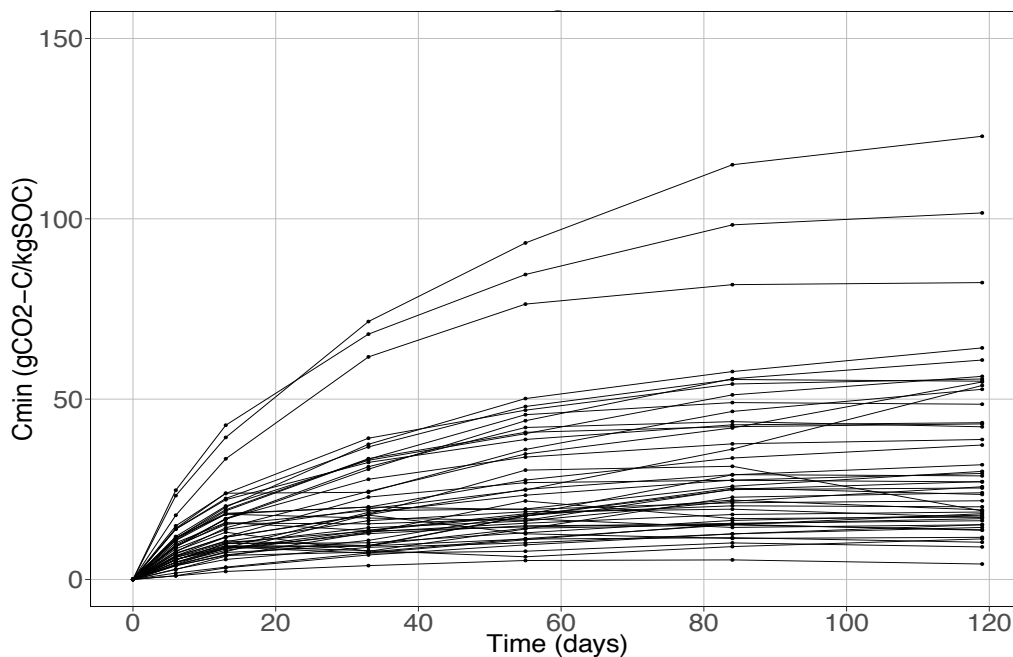


Figure 13: Cumulative PE in $\text{g CO}_2\text{-C kg}^{-1}\text{SOC}$ over 119 days of incubation.

Figure 14 shows the cumulative C_{\min} of the control including the PE of the 48 soil samples in $\text{g CO}_2\text{-C kg}^{-1}\text{SOC}$. The combination of the cumulative C_{\min} of the control and the cumulative PE is the cumulative total C_{\min} of the native SOC. In general, the total amount of C mineralised at the end of the incubation experiment varied between 55 (site 67, composite 3) and 169 $\text{g CO}_2\text{-C kg}^{-1}\text{SOC}$ (site 69, composite 2). For the total C_{\min} of the native SOC no outlier is visible.

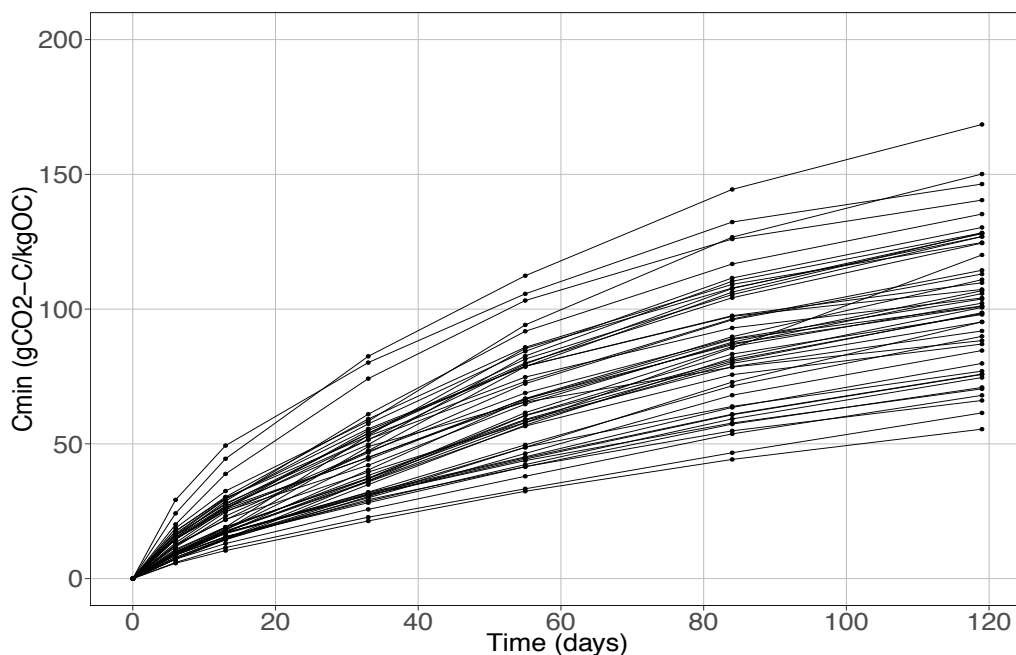


Figure 14 Cumulative C_{\min} of the control including PE in $\text{g CO}_2\text{-C kg}^{-1}\text{SOC}$ over 119 days of incubation.

Figure 15 shows the cumulative C_{\min} of the control (red line), as well as the cumulative total C_{\min} of the native SOC (black line) in $\text{g CO}_2\text{-C kg}^{-1}\text{ SOC}$ for the 16 different study sites and their three composites. The pattern and the rate of both the cumulated C_{\min} of the control and the cumulated total C_{\min} of the native SOC between the three composites within a study site were very similar. The pattern of the C_{\min} of the control of the three composites from the sixteen study sites was very similar to the pattern of the total C_{\min} of the native SOC of the three composites from the sixteen study sites.

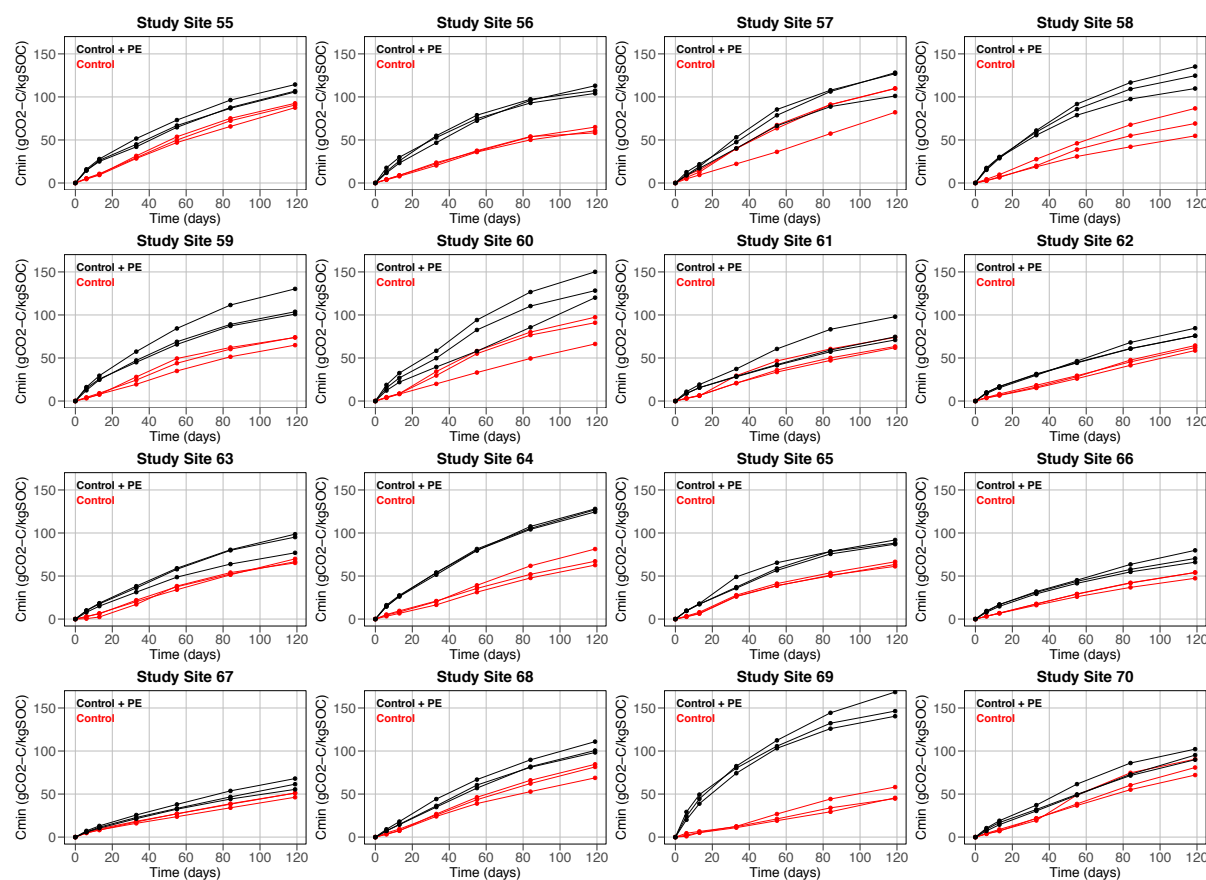


Figure 15: Cumulative C_{\min} of the control (red line) and of the control including the PE (black line) in $\text{g CO}_2\text{-C kg}^{-1}\text{ SOC}$ over 119 days of incubation for the three composites of the sixteen different study sites.

4.1.4 Comparison of the Different Sources of the Carbon Mineralisation

Figure 16 shows the C_{\min} of the treatment (green line) and its partitioning into C_{\min} of the control (blue line), the PE (black line) and the root C_{\min} (red line) in $\text{g CO}_2\text{-C kg}^{-1}\text{ SOC}$ as a mean of the three composites of the 16 different study sites. The rate of the PE and the root C_{\min} was higher than the rate of the C_{\min} of the control in the initial phase of the experiment for most of the soil samples. After an initial increase in the rate of the C_{\min} , a significant de-

crease between sampling one and two was observed for all the different origins of the mineralisation. After an increase between the second and third sampling of the root C_{min} and the PE for most of the sites, a decrease until the end of the experiment occurred. After the second sampling date, the rate of the C_{min} of the control slightly increased for all the soils until the fifth sampling, where a decrease was observed for some soils.

The rates of the root C_{min} and the PE were close to zero at the end of the incubation for most of the study sites. For some study sites the PE became negative. A negative PE was also observed for study sites 55, 61 and 70 in the middle of the experiment. Soils with a high root C_{min} mostly also showed a high PE (sites 56, 58, 59 and 69), whereas soils with a low root C_{min} generally also showed a low PE (sites 61, 62, 66, 67 and 70). If the C_{min} of the roots and the PE were low, the C_{min} of the control was mostly relatively high and if the C_{min} of the roots and the PE both were high, the C_{min} of the control mostly was rather low.

Figure 16 shows, that the root C_{min} and the PE, but also the C_{min} of the control, varied greatly between the different study sites, indicating that they seem to be affected by the different properties of the study sites. Therefore, it is necessary to investigate how ecosystem properties influence the mineralisation of the root C and SOM.

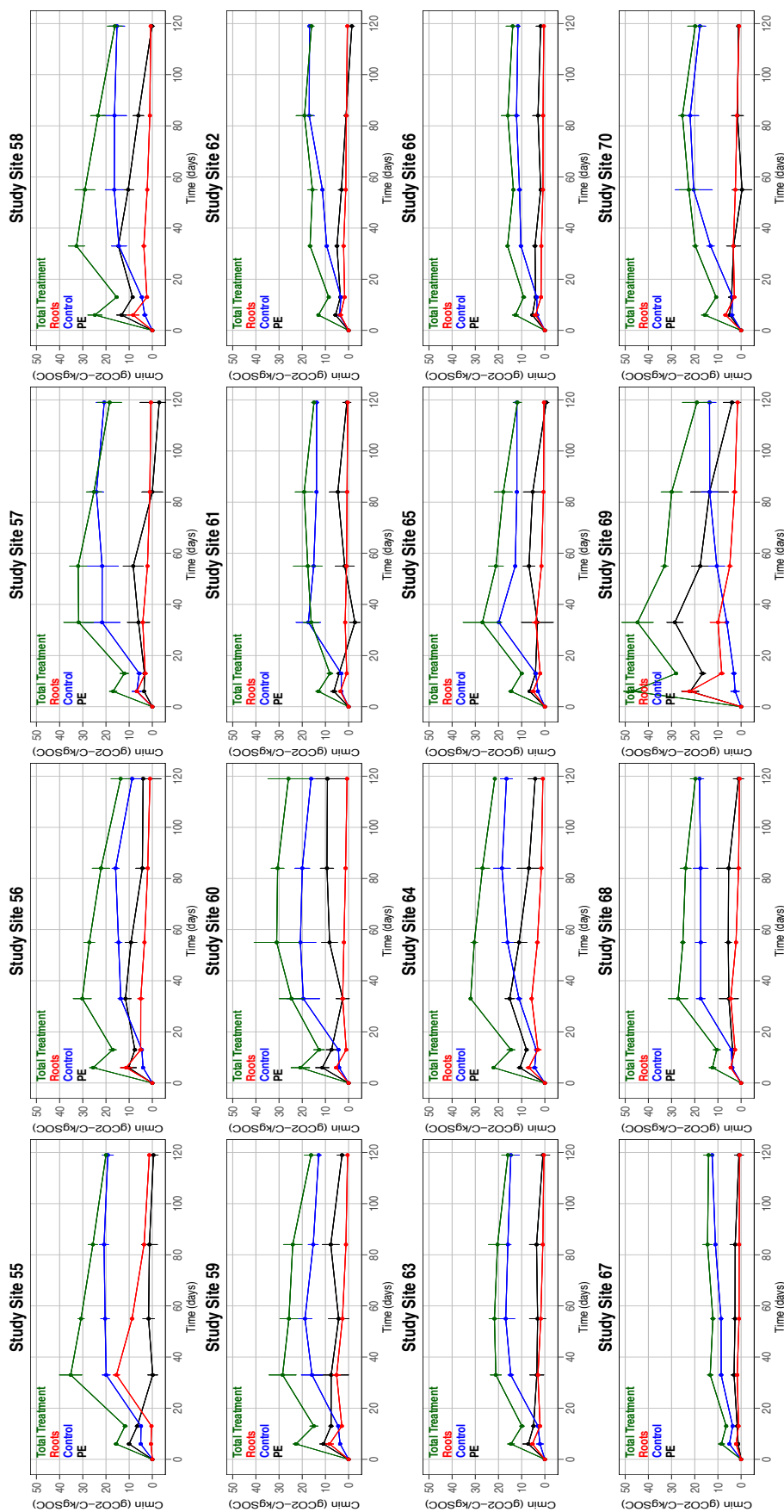


Figure 17: Mean CO₂ efflux of the three composites from the treatment (green line), the control (blue line) and the roots (red line) in gCO₂-C kg⁻¹ SOC over 119 days of incubation for the sixteen different study sites.

4.2 Influence of the Ecosystem Properties on the Carbon Mineralisation

The following multiple linear regression models show the influence of the respective ecosystem properties as factors on the different types of the C_{\min} as indicators. The following equations 10 to 13 are best fits obtained from a stepwise procedure.

4.2.1 Influence of the Ecosystem Properties on the Mineralisation of Root Carbon

The highest significance of the drivers on the root C_{\min} was obtained with the following equation of the multiple linear regression:

$$aov(\sqrt{\text{Roots}}) \sim \text{Clay} + \text{pH} + \text{Temperature} + \text{Orientation} + \text{Slope} + \text{Clay:pH} + \text{Temperature:Moisture} \quad [9]$$

The factor clay content was significant and had a large influence on the variability of the root C_{\min} (Table 4). An even larger part of the remaining unexplained root C_{\min} was explained with the factor pH value. The factors temperature, orientation, slope and the interaction between the clay content and the pH value also explained parts of the remaining unexplained variability of the root C_{\min} but its significance was lower. The factor soil moisture showed no significant influence on the C_{\min} of the roots. This model explained 66.15 % of the total variability of the root C_{\min} .

Table 4: Results of the multiple linear regression on the influence of the drivers on the root C_{\min} .

	Clay	pH	Temperature	Orientation	Slope	Clay:pH
Roots	***	***	**	*	*	**
	0.00035	1.19e-07	0.00138	0.01702	0.2268	0.00329

Figure 17 shows the correlation between the most significant factor pH value and the MRT (y) of the root carbon. In general, the higher the pH, the shorter the MRT and thus the higher the rate of the root C_{\min} . The MRT of the root carbon from the 48 soil samples regarding the whole incubation period was between 0.58 and 2.67 years and the mean of the MRT of all the soil samples was 1.54 years.

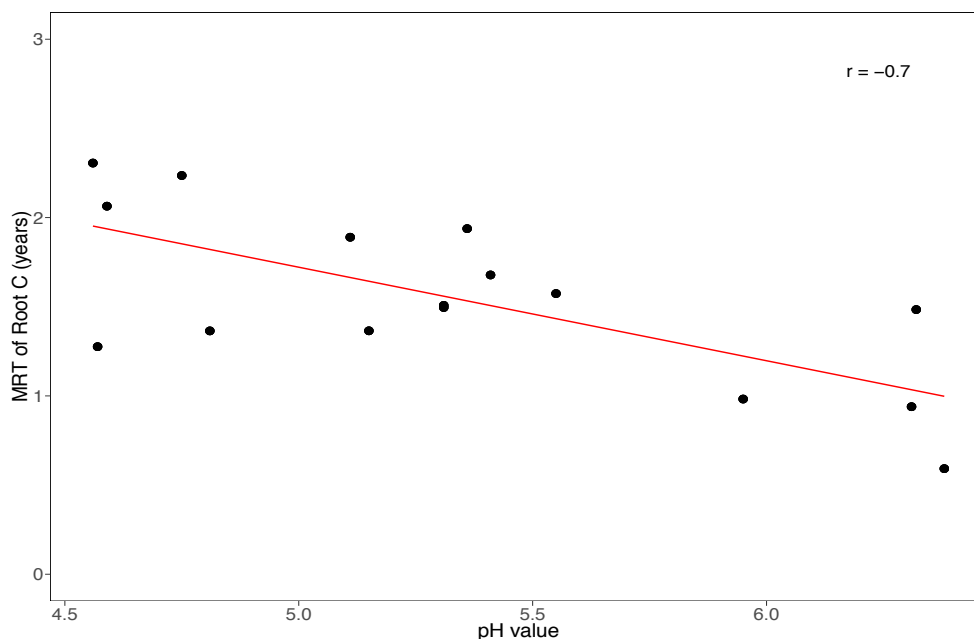


Figure 17: Correlation of the pH value and the MRT of the root C in years when the root C_{\min} is considered.

4.2.2 Influence of the Ecosystem properties on the Mineralisation of Root Carbon Including the Priming Effect

The highest significance of the drivers on the root C_{\min} including the PE was obtained with following equation of the multiple linear regression:

$$aov(\sqrt{\text{Roots} + \text{PE}}) \sim \text{Clay} + \text{Temperature} + \text{pH} + \text{Moisture} \quad [10]$$

The factors clay content, temperature, pH value and soil moisture content influenced the variability of the root C_{\min} including the PE to a rather similar degree (Table 5). But the pH value was able to explain most of the unexplained variability of the C_{\min} compared to the rest of the drivers (Table 5). Although the clay content was in the first place of the regression model, it showed the second lowest significance and therefore this factor did not explain much of the variability of the root C_{\min} including the PE. The factors orientation and slope showed no significant influence on the root C_{\min} including the PE. This model explained 46.89 % of the total variability of the root C_{\min} including the PE.

Table 5: Results of the multiple linear regression on the influence of the drivers on the root C_{\min} including the PE.

	Clay	Temperature	pH	Moisture
Roots + PE	**	***	***	**
	0.004083	0.000989	0.000250	0.007595

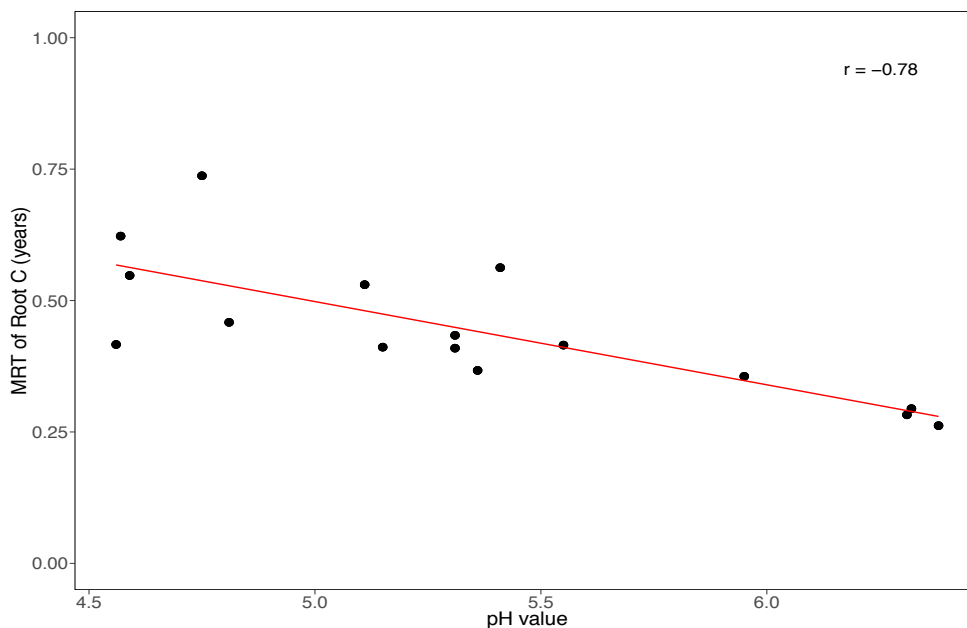


Figure 18: Correlation of the pH value and the MRT of the root C in years when the root C_{\min} including the PE is considered.

Figure 18 shows the correlation between the most significant factor pH value and the MRT (y) of the root C when PE was included in the root C_{\min} . In general, the higher the pH value, the shorter the MRT and thus the higher the rate of the root C_{\min} including the PE. The MRT of the root carbon when the PE was included in the root C_{\min} from the 48 soil samples regarding the whole incubation period was between 0.22 and 0.86 years and the mean of the MRT of all the soil samples was 0.44 years.

4.2.3 Influence of the Ecosystem Properties on the Basal Mineralisation

The highest significance of the drivers on the C_{\min} of the control was obtained with the following equation of the multiple linear regression:

$$aov(\sqrt{\text{Control}}) \sim \text{Clay} + \text{Temperature} + \text{Moisture} + \text{Slope} + \text{Orientation} \quad [11] \\ + \text{Clay:Temperature} + \text{Moisture:Slope}$$

The factor clay content was highly significant and thus had a large influence on the variability of the C_{\min} of the control (Table 6). A large part of the remaining unexplained C_{\min} was attributed to the factor temperature, hence the temperature contributed to the variability of the C_{\min} of the control. The factors soil moisture, slope and orientation and the interac-

tion between the clay content and the temperature and between the soil moisture and the slope also explained parts of the remaining unexplained variability of the C_{min} but their significance was lower, which means that these factors contributed less to the explanation of the remaining unexplained variability of the C_{min} of the control. The factor pH value showed no significant influence on the C_{min} of the control. This regression model explained 59.31 % of the total variability of the C_{min} of the control.

Table 6: Results of the multiple linear regression on the influence of the drivers on the C_{min} of the control.

	Clay	Temperature	Moisture	Slope	Orientation	Clay:Temperature	Moist:Slope
Control	***	***	**	*	.	**	.
	5.19e-05	0.0003	0.0077	0.012	0.0815	0.00806	0.05703

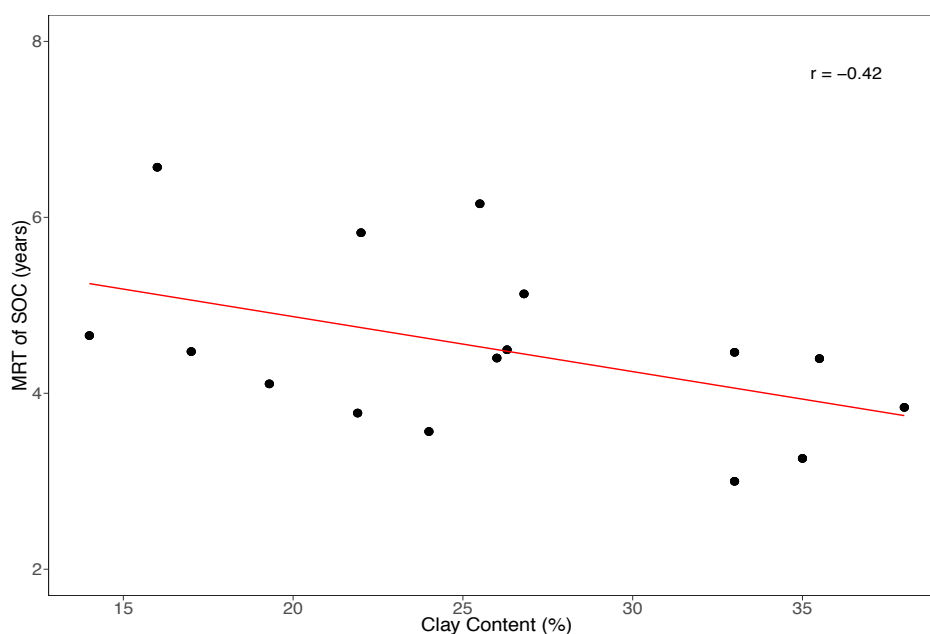


Figure 19: Correlation of the clay content in % and the MRT of the native SOC in years when the C_{min} of the control is considered.

Figure 19 shows the correlation between the most significant factor clay content (%) and the MRT (y) of the native SOC when no root residues were added to the soil. In general, the higher the clay content, the shorter the MRT and thus the higher the rate of the C_{min} in the control. The MRT of the SOC when no root residues were added to the soil of the 48 soil samples regarding the whole incubation period was between 2.55 and 7.33 years and the mean of the MRT of all the soil samples was 4.5 years.

4.2.4 Influence of the Ecosystem Properties on the Basal Mineralisation Including the Priming Effect

The highest significance of the drivers on the C_{min} of the control including the PE was obtained with the following equation of the multiple linear regression:

$$aov(\sqrt{Control+PE}) \sim pH + Clay + Moisture + Temperature + Slope + Moisture:Temperature + Clay:Moisture \quad [12]$$

The significance of the temperature was the largest, and thus the temperature explained most of the variability of the C_{min} from the control including the PE although it was only the fourth factor in the regression model. The factor pH value was also highly significant and thus had a large influence on the variability of the C_{min} of the control including the PE (Table 7). A large part of the remaining unexplained C_{min} was also attributed to the factor clay content. The factors soil moisture, slope and the interaction between the temperature and soil moisture and between the clay content and the soil moisture also explained part of the remaining unexplained variability of the C_{min} but its significance was lower. The factor orientation showed no significant influence on the C_{min} of the control including the PE. This model explained 77.24 % of the total variability of the C_{min} of the control including the PE.

Table 7: Results of the multiple linear regression on the influence of the drivers on the C_{min} of the control including the PE.

	pH	Clay	Moist	Temp	Slope	Temp:Moist	Clay:Moist
Control+PE	***	***	**	***	**	**	.
	1.32e-07	9.04e-06	0.00736	3.45e-10	0.00503	0.00180	0.06984

Figure 20 shows the correlation between the most significant factor temperature ($^{\circ}\text{C}$) and the MRT (y) of the native SOC when root residues were added to the soil. In general, the higher the temperature, the shorter the MRT and thus the higher the rate of the C_{min} in the control including the PE. The MRT of the SOC when root residues were added to the soil of the 48 soil samples regarding the whole incubation period was between 1.52 and 5.25 years and the mean of the MRT of all the soil samples was 2.86 years.

Figure 21 shows the correlation between the TOC (%) and the MRT (y) of the native SOC when root residues were added to the soil. In general, the higher the TOC content, the longer the MRT, hence the lower the rate of the C_{min} in the control including the PE.

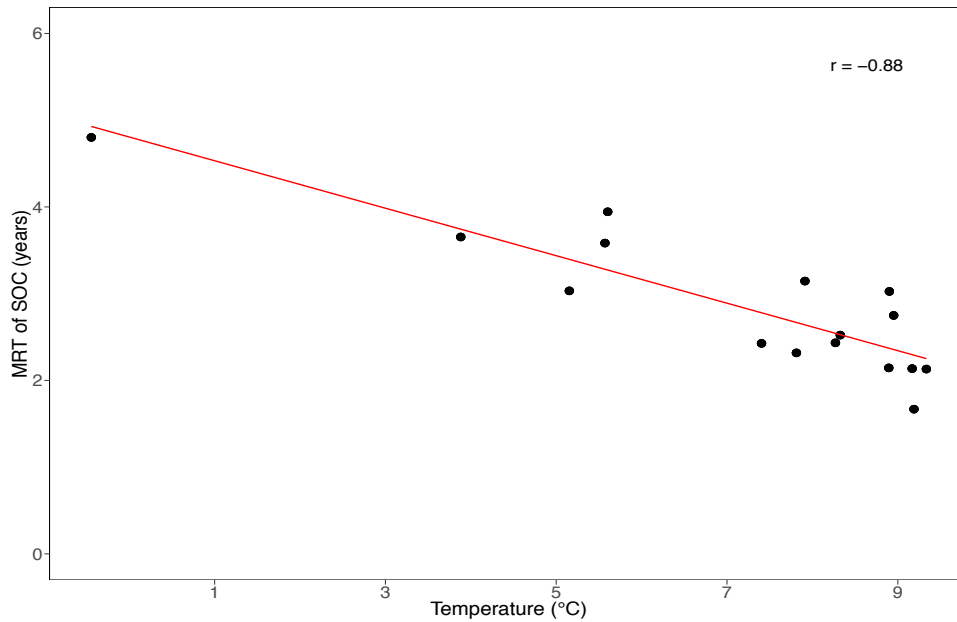


Figure 20: Correlation of the temperature in C° and the MRT of the native SOC in years when the C_{\min} of the control including the PE is considered.

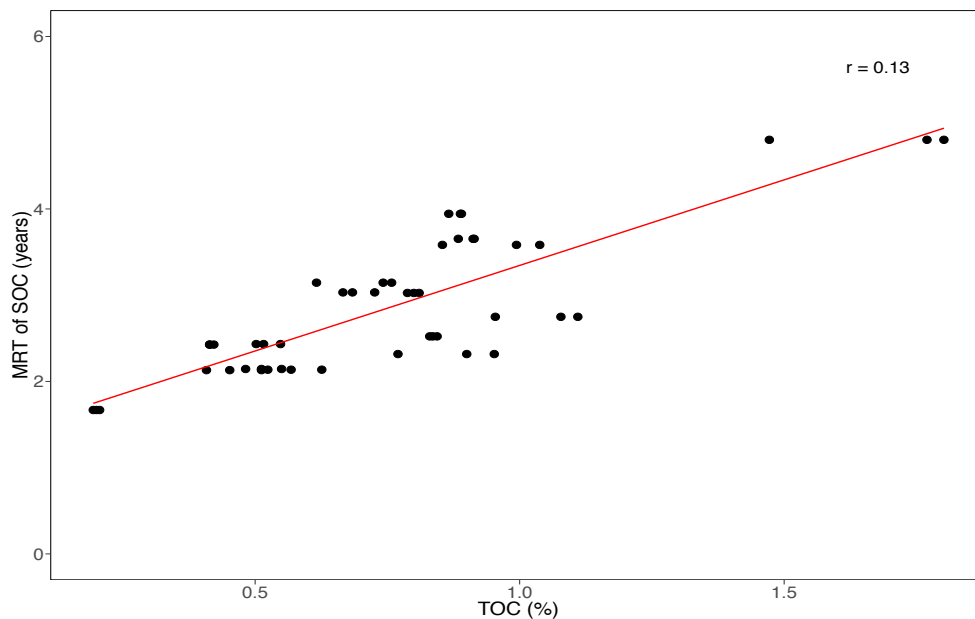


Figure 21: Correlation of the TOC in % and the MRT of the SOC in years when the C_{\min} of the control including the PE is considered.

Only the most significant correlations between the respective drivers and the C_{\min} were plotted in Figures 17 to 21. Further correlations revealed that if the drivers showed a significance, the temperature, the clay content and the pH value were always negatively correlated with the MRT, whereas the TOC, the soil moisture and the slope were always positively correlated with the MRT.

5 Discussion

The aim of this master thesis was to find out how the mineralisation of carbon changes when root residues are added to the soil and how the properties of the soil and the surrounding ecosystem influence the mineralisation. The thesis further focussed on finding out how the carbon stocks are affected due to changes in the C_{\min} by addition of root residues. The most relevant results are discussed with regard to the research questions and hypotheses.

5.1 Dynamics of the Carbon Mineralisation

5.1.1 General Patterns of the Carbon Mineralisation

The results showed that root residue addition caused a significant increase in the total CO_2 efflux from the soil compared to the control without additions. The root residue addition also caused significant positive and negative PEs. These results are not surprising and are strongly supported by other studies (e.g. Shahbaz et al., 2017; Abiven et al., 2005). Surprising is, however, the fact that the variability of the total C_{\min} in the treatment is roughly three times larger than the variability of the total C_{\min} in the control. The total C_{\min} in the treatment varied between 0.53 and 1.81 $\text{g CO}_2\text{-C kg}^{-1}\text{ SOC d}^{-1}$. A similar experiment was done by Shahbaz et al. (2017) and Lian et al. (2016) where the total C_{\min} did not exceed or decrease values between 0.98 and 1.3 $\text{g CO}_2\text{-C kg}^{-1}\text{ SOC d}^{-1}$. Hence, the incubation experiment of this master thesis resulted in a much larger range to what is found in literature. The large variability described in this thesis is due to different rates of the root C_{\min} , of the basal mineralisation and of the PE, which most probably occurred because of differences in the ecosystem properties. This large variability demonstrates why it is so difficult to predict how soils will react to root residue addition. The following discussion provides information on why these differences occurred.

The almost linear trend in the cumulative C_{\min} of the control shows, that almost no disturbances occurred in the soil. The increasing cumulative C_{\min} of the control during the whole incubation experiment also indicates that the soil samples in the control contained carbon until the end of the experiment. However, the rate of the C_{\min} of the control slightly decreased towards the end of the incubation, indicating that the carbon became less accessi-

ble for the active microbes. At the end of the incubation on average still 93 % of the native SOC remained in the soil.

The non-linear increase in the cumulative C_{\min} of the treatment must be due to the residue input, which caused a rapid initial increase of the C_{\min} . According to Abiven et al. (2005) or Trinsoutrot et al. (2000) the high initial C_{\min} rate can be strongly related to the large amount of water-soluble C in the root residues. It can be assumed that the amount of added carbon from root residues (0.071 g) is higher than the microbial biomass C content in the soil. The reason for this is that the microbial biomass C concentration in soils is rarely higher than 5 % of the TOC (Gonzalez-Quiñones et al., 2011) and therefore about 0.003 g of microbial biomass C is assumed to be present in the 20 g of soil used for this experiment. Therefore, both microbial growth and changes in the community structure can occur (Balgodatskaya and Kuzyakov, 2008). It can be assumed that this readily available water-soluble C fraction of the root residues caused a rapid increase in the microbial biomass of r-strategists at the very beginning of the experiment, a phenomenon also described by Fontaine et al. (2003) and Shahbaz et al. (2017). In summary, it can be assumed that the activation of the microorganisms caused the rapid initial increase in the C_{\min} of the treatment.

Trinsoutrot et al. (2000) describe a weakening relationship between the mineralised C and the water-soluble C fraction as decomposition proceeds. This weakening relationship was also observed in this master thesis but it was accelerated by the percolation of the soil on the first sampling date. Hence, changes in the amount of water-soluble C after the first sampling might be the reason why a rapid decrease in the C_{\min} rate between the first and the second sampling was observed for all the different sources of the CO_2 efflux. The percolation on the first sampling date must have flushed away the DOC in the soil, leaving behind less easily decomposable fractions of carbon. Thus, when the DOC was removed after the first percolation, the microorganisms had to adapt to the decomposition of more stable fractions of carbon, which might be the reason why the rates of the root C_{\min} , the basal mineralisation and the PE were reduced. Tipping (1998) explains the reduction of the basal mineralisation by the fact that, similar to the root residues, a pool of potential DOC exists as part of the native SOC and goes into solution when conditions are suitable. This must have been the case for the first percolation of the soil samples of the experiment of this thesis.

When the microorganisms adapted to the less easily decomposable fractions, hence when the more resistant K-strategists were activated, the C_{\min} rate increased again for all the ori-

gins of the CO₂ efflux in most of the soil samples. This was also reviewed by Blagodatskaya and Kuzyakov (2008) who stated that specialised microorganisms are activated when only low substrate quality is available. This explains why the root C_{min} and the basal mineralisation in this experiment increased after the second sampling. Blagodatskaya and Kuzyakov (2008) further stated that the substrate mineralisation is accompanied by co-metabolism of SOM. This explains why the PE in this experiment increased after the second sampling as well.

5.1.2 Variability of the Mineralisation of Root Carbon

From the third sampling until the end of the incubation the rate of the root C_{min} decreased for most of the study sites, indicating that the decomposition of root carbon became more difficult for the microbes and that also the stable fraction became limiting. The lowest root C_{min} rates found during the whole incubation occurred at the end of the experiment and were close to zero for most of the soil samples, indicating that almost only the recalcitrant C, namely lignin, was left (Blagodatskaya and Kuzyakov, 2008).

Table 8: Information on the added root type, the added root C content (g C kg⁻¹ soil) and values of the cumulated root C_{min} and the cumulated root C_{min} including the PE in g CO₂-C kg⁻¹ added C d⁻¹ and in percentage of added C, compared to values found in other studies.

Root type	Root C	root C _{min}	root C _{min} + PE	root C _{min}	root C _{min} + PE	Autor
	gC kg ⁻¹ soil	gCO ₂ -C kg ⁻¹ added C d ⁻¹		% of added C		
Ryegrass	3.55	0.78 – 3.07	1.92 – 6.02	10.4-35.4	22.8-71.7	Abt, 2017
Rice	1.35	-	6.00	-	60	Abiven, 2005
Soybean	1.48	-	3.90	-	39	Abiven, 2005
Wheat	1.61	2.07	-	29	-	Shahbaz, 2017
Wheat	3.22	2.42	-	29	-	Shahbaz, 2017
Grass	0.68	-	1.28	-	12	Neergaard, 2001
Clover	0.77	-	2.13	-	20	Neergaard, 2001
Rape	1.7	1.69	-	30	-	Trinsoutrot,2000

The surprisingly large range of the root C_{min} observed in this study could not be found in other studies so far. Table 8 presents the values of the root C_{min} from this master thesis and values found in other studies and shows that the range of the root C_{min} of this master thesis was more than three times larger than the combination of the values found in other studies. Regarding the relative amount of the total root C_{min} to the total amount of added root C, a

much broader range was also observed in this master thesis (Table 8). It can be assumed that the larger range of the total root C_{min} found in this master thesis compared to values found in literature is due to the broad diversity of stimulating factors and the large variability within these factors, which differs to what has been used in other studies so far. Studies have focused mainly on the effect of the quality and the quantity of the added residue on the root C_{min} (Shahbaz et al., 2017; Abiven, et al., 2005). Some studies may have accounted for the variability of climatic or soil properties but the large number of drivers and the high variability within these drivers has not been used for studies with root residues so far.

Regarding the root C_{min} including the PE, a rather different pattern than that for the root C_{min} was observed. These unclear dynamics observed in the root C_{min} when PE is included was due to the different origins of carbon. The PE was dependent on the carbon from the native SOC, whereas the root C_{min} was dependent on the root carbon.

The additional release of C when PE was included was much larger. The total amount of the additional release was roughly half the amount of the added root C. Although the inclusion of the PE caused a lot of additional C_{min} , the amount was not greater than the amount of added C to the soil in any of the soil samples. This is not in line with Engel et al. (1993), who described that the additional C release can be higher than the amount of added C to the soil. If the PE was included in the root C_{min} , between 22.8 and 71.7 % of the added root C was mineralised at the end of the incubation. The range found in literature has about the same extent (Table 8), but the values from de Neergaard et al. (2001) are below the range of this master thesis. This is probably due to the lower amount of root C added by de Neergaard et al. (2001).

In summary, these findings show that the mineralisation of root C and the mineralisation of root C including PE come to very different results. The inclusion of the PE conveys a wrong impression of the root C_{min} , which highlights the importance of separating the root C_{min} and the PE as firstly described by Engel et al. (1993). It further demonstrates how difficult it is to make any assumptions about the dynamics of the root C_{min} if the PE is included.

5.1.3 Variability of the Mineralisation of Native Soil Organic Carbon

The results show that root residue addition caused a large PE. Without residue addition between 4.95 and 10.05 % of the TOC content was mineralised, whereas with residue addition between 6.16 and 15.17 % of the TOC content was mineralised. Although the total nati-

ve SOC mineralisation (control + PE) was significantly higher than the total root C_{\min} for most of the soils, the rate and the proportion of C mineralised from the native SOM (Table 9) was small compared to the rate and proportion of C mineralised from the roots (Table 8). This indicates that the carbon in the native SOM was less well accessible and decomposable, a finding that is strongly supported by literature (e.g. Van Veen and Paul, 1981).

Table 9: Information on the TOC content (g C kg^{-1} soil) and the duration of the experiment (days) and values of the cumulated PE, the cumulated C_{\min} of the control and the cumulated C_{\min} of the control including the PE in $\text{gCO}_2\text{-C kg}^{-1}\text{SOC d}^{-1}$, compared to values found in other studies.

TOC	Duration	PE	Control	Control + PE	Autor
gC kg^{-1} soil	Days	$\text{gCO}_2\text{-C kg}^{-1}\text{SOC d}^{-1}$			
9.7-90.1	119	0.04-1.03	0.38-0.92	0.47-1.42	Abt, 2017
12.8	64	0.27	0.42	0.68	Shahbaz et al., 2017
12.8	64	0.37	0.42	0.79	Shahbaz et al., 2017
30	14	1.17	0.21	1.38	De Graaff et al., 2010
11	26	0.09	0.48	0.57	Hamer and Marschner, 2005

The broad range observed in the cumulated PE and the cumulated total native SOC mineralisation of the incubation experiment of this master thesis was also observed in other studies on the C_{\min} in arable and grassland soils when root residues were added (Table 9). However, the conditions for incubation experiments of other studies vary largely to the experiment of this thesis. The duration of the incubation was much shorter in the reviewed studies (Table 9). Due to the short incubation periods in other studies, especially in the study of De Graaff et al. (2010), the intensive PE of the initial phase is weighed much more strongly than in the experiment of this master thesis, which also involved the slow incubation phase. In the study of Hamer and Marschner, instead of root residues, fructose was added and thus only the labile fraction of the root residues was represented. In the study of Graaff et al. (2010) no information about the type of added plant residue was given. The most similar conditions to the incubation experiment of this master thesis was found in the study of Shahbaz et al. (2017). This leads to the assumption that the intensity and therefore also the range of the cumulated PE and the cumulated total native SOC mineralisation in other incubation experiments would be smaller if the conditions for the experiment were similar to this experiment. One reason, for the high variability in the PE found in this master thesis is assumed to be the high variability within the different ecosystem properties descri-

bed by Schmidt et al. (2011) and Blagodatskaya and Kuzyakov (2008).

The results further show that the PE arises immediately after the addition of root residues to the soil, which supports the findings of Dalenberg and Jager (1989) and Pascual et al. (1998). The strongest PE for most of the soils was detected in the initial phase of the incubation, a phenomenon which was also observed by Hamer and Marschner (2002). The PE at the beginning of the experiment was even higher than the basal mineralisation for most of the soils. According to Blagodatskaya and Kuzyakov (2008), this immediate increase in the PE can be ascribed to a co-metabolic effect, which occurs because easily decomposable substances are added. Hence the easily decomposable fractions of the added root substrate, most probably water-soluble C, stimulate the microbial growth and trigger the PE. The PE is triggered because an increase in the microbial biomass also stimulates the appetite for the native SOC and thus enhances its degradation. It can therefore be assumed that the PE is associated with changes in the microbial biomass and population.

Hart et al. (1986) have stated that PEs in soils rich in C are larger than those in carbon-poor soils. This effect can, however, not be detected in the results of this master thesis. No clear pattern was observed regarding the PE in $\text{g CO}_2\text{-C kg}^{-1}$ soil. Although for the soil from study site 68, which also contained a lot of carbon (4.58 %), a large PE was observed, an even larger PE was observed for the soil from study site 64, which had a rather low carbon content (2.35 %). With regards to the PE in $\text{g CO}_2\text{-C kg}^{-1}$ of SOC, the results from this master thesis show the opposite effect to what was found by Hart et al. (1986). The highest PEs are observed in soils from study sites with the lowest carbon content (e.g. site 69 or site 58).

These findings might occur because in carbon-poor soils, a rather low amount of microbes is present initially, which then increases more strongly after residue addition in relation to soils where more carbon is available from the beginning of the incubation. This assumption is based on findings from the study of Dumontet et al. (1985), and other studies (e.g. Mary et al., 1993; Kawaguchi et al., 1986), who have stated that the size of the PE increases with an increasing amount of added organic substances. Hence in this master thesis comparatively much carbon from roots was added in relation to the existing share of carbon in soils with low carbon contents compared to soils with higher carbon contents. Therefore, it is possible that the large increase of the PE was due rather to the comparably high addition of root C than to the low amount of C in soils.

Other findings, which are supported by research from Shahbaz et al. (2017), show that the intensity of the PE during the whole incubation period seems to be positively correlated with the mineralisation rate of the root C. This is, for example, the case for soils from study site 56, 58 or 64, where high PEs and a high mineralisation of the root C was observed. Similarly, soils with a very low root C_{\min} generally also showed very low PEs.

Further findings on the dynamics of the PE and the root C_{\min} show that for some study sites after the second sampling an increase in the PE can be observed, when the root C_{\min} starts decreasing. This is an indication, also described by Shahbaz et al. (2017), that r-strategists might be dying because easily available carbon is limiting. The PE is then caused by reutilisation of the microbial necromass. Hence, the dead microorganisms serve as an SOC primer.

Negative PE was observed for some soil samples during the third sampling and at the end of the incubation period. In some cases, the negative PE at the end of the incubation even caused a larger mineralisation of the control compared to the treatment, which is an indication for increasing C stocks in the soil. It can be assumed that the negative PE on the third sampling was due to the fact that the K-strategist microorganisms, at that point of the incubation were mainly focussed on the mineralisation of relatively stable, but possibly more easily accessible root C, hence a change of the nutrient source from the recalcitrant lignin in the native SOC to the more easily degradable root substrates might have occurred (Blagodatskaya and Kuzyakov, 2008). A possible reason for the negative PE at the end of the experiment might have been the reduced activity of the microbes due to the very low decomposability of the remaining C, as the more easily available C had already been decomposed. This assumption is supported by Kuzyakov (2010) and other studies, who also observed a decreasing PE as decomposition proceeded.

5.1.4 Changes in the Carbon Stocks

At the end of the incubation a large portion of the root C was not mineralised (Table 10) This remaining C is assumed to stem partly from the stable and mostly from the recalcitrant fraction of carbon. According to Haider (1996) root residues contain about 30 % of lignin. Hence from the estimated 42 % of carbon in the plant residues only around 12 % are not lignin. This means that for some soils, such as for the soil from study site 55, at the end of the incubation only carbon from the lignin-structures remained. It can be expected that most of this portion will be incorporated in the soil, because the root C_{\min} at the end of the

experiment was almost zero for most of the soils. Nonetheless, according to Kuzyakov et al. (1997) the degradation of root residues is a constant process, which takes place, to a smaller extent, up to several months to years after the residue addition. Due to time restrictions, it was not possible to gain data from more than four months.

Table 10: Information on the study site ID, the TOC content (g C kg^{-1} soil) and values of the cumulated C_{min} of the control, the cumulated C_{min} of the control including the PE and the cumulated root C_{min} as percentage of the TOC and the added C and as $\text{g CO}_2\text{-C kg}^{-1}$ soil.

ID	TOC	Root C	Control	Control+PE	root C_{min}	Control	Control+PE	root C_{min}	PE
	gC kg^{-1} soil		% of TOC		% of root C	$\text{gCO}_2\text{-C kg}^{-1}$ soil			
55	41.8	3.55	9.02	10.9	35.41	3.77	4.56	1.26	0.79
60	28.6	3.55	8.49	13.28	10.36	2.43	3.8	0.37	1.37
67	90.1	3.55	4.95	6.16	17.1	4.46	5.55	0.61	0.76
69	9.7	3.55	4.95	15.18	14.03	0.48	1.47	0.5	0.99

As pointed out earlier in this thesis, root residue addition also caused a significant increase in the CO_2 efflux from the native SOC, due to PEs. This affected the carbon stocks in the soil as well. Table 10 illustrates, for selected study sites, how much native SOC, with and without PE, and how much root C was mineralised as percentage of TOC and as percentage of carbon added and as $\text{g CO}_2\text{-C kg}^{-1}$ soil at the end of the incubation. In total, more carbon was mineralised than was added for most soils. An exception is soil 69, where in total only $1.97 \text{ g CO}_2\text{-C kg}^{-1}$ soil was mineralised. However, regarding the impact of residue addition on the TOC content of the soil, the basal mineralisation does not have to be accounted for, because basal mineralisation also occurs without residue addition. Considering that, the total root C_{min} and the PE together was smaller than the added amount of root C for all soils. This shows that the addition of root C leads to an increase in the C stocks in the soil. These findings are supported by Lu et al. (2003) and Balesdent and Balabane (1996), who reported that only small parts of the root residues are decomposed. These results are further supported by the study of Abiven et al. (2005), who suggested that the sequestration of root residue C in the soil might be greater than that of aerial parts of the plant and that it is therefore likely that the TOC content in the soil increases when root residues are added.

Furthermore, as described earlier, the PE at the end of the incubation was almost always negative or at least heading towards the negative. Unfortunately, the further course of the PE after the duration of the incubation experiment is unclear, but it can be assumed that

the negative PE would have continued for some time. This negative PE would additionally have had a positive impact on the C stocks of the soil. An increase in the carbon stocks can therefore be expected if the input of root residues occurs regularly enough. This conclusion contradicts the findings of Fontaine et al. (2004) who assume that the input of root carbon may decrease the soil organic carbon content.

Table 11: MRT of the native SOC in years for the soils from each study site without and with addition of root residues.

Study Site ID	MRT of SOC in years without root residues	MRT of SOC in years with root residues
55	3.26	2.52
56	4.66	2.43
57	3.00	2.32
58	4.40	2.13
59	4.11	2.43
60	3.57	2.14
61	4.47	3.58
62	5.13	3.65
63	4.47	3.15
64	4.39	2.14
65	4.50	3.03
66	5.83	3.94
67	6.15	4.80
68	3.84	2.75
69	6.57	1.67
70	3.78	3.03

As a matter of fact, the addition of root residues leads to a decrease in the MRT of native SOC (Table 11), if it is assumed that the negative PE of the further unexamined decomposition process will not compensate the positive PE. A decrease in the MRT does, however, not imply that the carbon content decreases, but it indicates that the carbon circulates faster.

In this context, however, it has to be taken into account that the incorporation of roots in the soil in nature does not only happen for the first 20 cm, as it was the case in this experiment. Roots in nature possess the ability to grow deep and, as demonstrated by Fontaine et al. (2007), their decomposition in deeper layers of the soil might also allow the mineralisation of several thousand years old carbon. Hence, newly cited evidence on the persistence of C has led to legitimate concerns that old C stocks might be more easily degradable than previously thought (Schmidt et al., 2011). It is therefore controversial, whether the potential

of enhanced root phenotypes to sequester carbon in the soil, as described by Paustian et al. (2016), is really as significant as it is claimed to be. It is rather unsure, to which extent more carbon would actually be sequestered in the case of a worldwide stronger root penetration into deeper soil horizons, because, as described by Fontaine et al. (2007), it is possible that root C incorporation into deeper layers of the soil might trigger the inactive microorganisms to a much greater extent than it was the case in this master thesis. If the resulting PE exceeded the amount of residue C incorporated in the soil, it is expected, that the soil would develop into a C source. However, this might not necessarily be the case and therefore, the effect at greater soil depths might be similar to the effect observed in this master thesis. In that case the soil could be expected to develop into a C sink. This uncertainty indicates the importance of investigating the effects of root residue addition at greater soil depths.

5.2 Influence of the Ecosystem Properties on the Carbon Mineralisation

5.2.1 The Mutual Influence of Ecosystem Properties

An analysis of the extended NABO database in Table 1 confirms that, as discussed in the introduction, different ecosystem properties influence each other. This is for example the case between the temperature and the soil moisture. It was found that soils with high temperatures are generally drier (e.g. sites 58 and 69) than soils with low temperatures (e.g. sites 67 and 62). These findings are supported by the study of Davidson and Janssens (2006a). The influence between different properties was also observed between the orientation and the soil moisture. Similarly to the study of Kang et al. (2003), high values of soil moisture were found on north-facing slopes. Table 1 shows that the TOC content is significantly affected by different ecosystem properties. Literature supports the findings, that carbon stocks are lowest in hot and dry biomes (e.g. site 69) and highest in cool and moist biomes (e.g. site 67) (Jenny, 1941; Post et al., 1982). A significant relationship was also observed between the TOC content and the clay content in the soil, which is in line with findings from Balogh et al. (2011) or Lugo et al. (1986). The TOC content was generally higher in soils with a high clay content (e.g. site 68) content and generally lower in soils with a low clay content (e.g. site 69). This confirms the earlier statement that the impact of different drivers on the C_{\min} should be analysed in combination (Raich and Schlesinger, 1992).

5.2.2 General Influence of the Ecosystem Properties on the Carbon Mineralisation

The results have confirmed that the ecosystem properties do indeed, as described by Schmidt et al. (2011), have a direct and even exceptionally high impact on the mineralisation of carbon in agricultural and grassland soils. Most of the selected drivers significantly influenced the C_{\min} and also managed to explain a large part of the variability in the C_{\min} . The large ranges in the mineralisation of root C, the basal mineralisation and the PE that were pointed out earlier in this master thesis can thus mainly be explained with the large differences in the values of the respective ecosystem properties. Depending on what type of mineralisation was considered, different ecosystem properties were the driving force explaining the variability of the C_{\min} , demonstrating that for the root C_{\min} other properties play a key role than for the native SOC mineralisation.

5.2.3 Influence of the Ecosystem Properties on the Mineralisation of Root Carbon

Considering the root carbon, the highest influence on its mineralisation was attributed to the pH value, followed by the clay content. This can be visually observed in the data from Table 1, where high pH values can be put into context with high root C_{\min} . Similar findings occur for the clay content but the association is less significant. Nonetheless, high root C_{\min} rates were more likely to be found in soils with high clay contents than with low clay contents. These results comply with the meta-analysis of Paul et al. (2002), who have found that with increasing clay content the mineralisation increased. These findings are, however, based on forest soils and apply only to the uppermost 10 cm of soil. The results of this thesis are, however, in contrast to studies of Rutherford and Juma (1992) or Lugo et al. (1986), who stated that a high clay content leads to lower C_{\min} rates. The results from Rutherford and Juma (1992), however, might apply for the initial phase of the soil from site 55, which indeed has a high pH value and a high clay content, but shows a much more distinct behaviour in the root C_{\min} than the rest of the soils. Hence, the lag phase of the root C_{\min} of soil 55 at the beginning of the incubation could be explained with the high clay content, because microorganisms are physically sequestered in small pores of aggregates, which makes them less active (Rutherford and Juma, 1992). Only after microorganisms manage to be activated can the decomposition of root C start. The surprisingly high increase after the second sampling might be due to the high pH value of 6.38, which is, according to Smith and Doran (1996), in the optimal range for microbial activity.

The climate property temperature also explained some part of the variability in the root C_{\min} , but this effect was less significant. The climate property soil moisture did not appear to be significant and also the orientation and slope only showed small significance. This indicates that the root C_{\min} seems to be mainly affected by soil properties rather than by climate and terrain properties. This does not correspond completely with the results of Davidson and Janssens (2006a) or Rustad et al. (2000), who stated that climate is a primary driver of the C_{\min} . These different findings, might be due to the fact that Davidson and Janssens (2006a) or Rustad et al. (2000) considered the total C_{\min} and not only the root C_{\min} . A possible explanation for the higher influence of the soil properties on the root C_{\min} is that especially the initial root C_{\min} is dependent on the activity of r-strategist microorganisms, which is influenced more by soil than by climate or terrain properties.

When comparing the root C_{\min} with the root C_{\min} when PE is included, the pH value still had the highest influence on the variability of the root C_{\min} , but the significance of the clay content decreased, while the significance of the temperature increased. The soil moisture also showed some significance. It can therefore be assumed that the PE is influenced to a similar degree by soil and climate properties. Furthermore, a decrease in the explanatory power was observed, when the PE was added to the root C_{\min} , also indicating that the PE is dependent on climate and soil properties, while the main influence of the root C_{\min} are soil properties. The decrease in the explanatory power also shows that it is necessary to separate the root C_{\min} and the PE.

5.2.4 Influence of the Ecosystem Properties on the Mineralisation of Soil Organic Carbon

The clay content, followed by the temperature and the soil moisture had the highest influence on the variability of the basal respiration. A high clay content and a high temperature can be put into relation with a high basal mineralisation, whereas high basal mineralisation was more likely to be found in dry soils. This is in contrast to what was found by Davidson et al. (2000), who observed high basal mineralisation in moist soils. For soils with especially high basal mineralisation (e.g. sites 55 and 57), none of the selected ecosystem properties showed extreme values within the individual ecosystem properties, hence the conditions for C_{\min} with respect to each property were neither very favourable nor very unfavourable. In contrast to that, in soils with very low basal mineralisation (e.g. sites 67 and 69), several extreme values, both favourable and unfavourable values, of the respective eco-

system properties were observed. Therefore, it can be assumed that the combination of only half-suitable values leads to a higher basal respiration than the combination of very well suitable and very badly suitable values.

Results show that the basal mineralisation is dependent on climate properties and to a slightly smaller degree on soil properties and to a minimal degree also on terrain properties. It can be concluded that the rate of the basal mineralisation is influenced by all the chosen drivers but climate properties, as described Davidson and Janssens (2006a) or Rustad et al. (2000), seem to be the main factors influencing the rate of the basal respiration.

Considering the total native SOC mineralisation, hence when PE was added to the basal mineralisation, temperature had the highest influence on the variability of the native SOC mineralisation. Temperature was followed by pH value, clay content, soil moisture and slope. Soils with a high temperature, pH value, clay content and a low soil moisture generally showed high native SOC mineralisation rates. These findings show that the native SOC mineralisation is dependent on climate, soil and to a smaller degree on terrain properties and illustrate the interaction of factors influencing the basal mineralisation and factors influencing the PE. These results are in line with results from Reichstein et al. (2000) or Singh and Gupta (1977), who stated that temperature is the single best predictor of the basal respiration, and also with Blagodatskaya and Kuzyakov (2008), who stated that soil properties, especially the pH value, are the main factors affecting PE.

With regard to the TOC content in the soil, only the total native SOC mineralisation showed a significant improvement in its explanatory, when the TOC content was added to the linear regression model. This indicates that the PE is strongly dependent on the amount of carbon in the soil and underlines the earlier assumption that the more TOC the lower the PE. This effect can be observed for study site 69, with the lowest TOC content and the highest PE. In this soil the PE was always higher than the basal mineralisation.

6 Conclusion

Addition of root residues was found to significantly increase the total mineralisation of carbon. Due to residue addition, the total mean C_{\min} of all samples increased from 7 to 12.2 % of TOC. The PE in the initial phase of the incubation was even higher than the basal respiration for most of the soils. This effect can be ascribed to co-metabolism. In the soil from study site 69, the PE was higher during the whole incubation period. This can be mainly ascribed to the surprisingly low TOC content. Although the PE was large in overall, it was found that the addition of root residues had, at least for the first 20 cm of agricultural and grassland soils, a positive effect on the storage of carbon in the soil, since the addition of the remaining root C into the soil was higher.

An increase in the variability of the total C_{\min} between the study sites was observed when root residues were added to the soil. This large variability was not observed in other studies so far. The mineralisation of root C was found to show very similar dynamics but a very different rate between the soil samples from the different study sites. These differences are due to differences within the individual ecosystem properties. The dynamics and the rate of the PE were rather different in the respective soil samples. The differences in the dynamics were caused by changes in the effects and their intensities in the distinctive soils that occur when root residues are added to the soil. Which effects occurred and at what intensity they occurred was determined mainly by the ecosystem properties. Soil properties were found to be the main drivers of the mineralisation of carbon in agricultural and grassland soils when root residues are added, while climate and terrain characteristics were less important. This was especially the case for the root C_{\min} . The PE was found to be mainly influenced by soil properties as well, but to a smaller degree also by climate properties. Furthermore, a large influence on the dynamics and the rate of the PE can also be ascribed to the TOC content in the soil. It was found that the soil properties alone could account for a very large proportion of the variance in the root C_{\min} and the PE. These findings are not in agreement to what was hypothesised in this master thesis and also are in contrast to what is found in literature, namely that climate properties are the main drivers of the C_{\min} . Climate properties, however, were found to become more important, when it comes to the basal mineralisation.

Depending on the source of C, about one third of the variability of the C_{\min} in the soils from the different study sites remained unexplained. This shows that other drivers than the selected ecosystem properties are influencing the C_{\min} as well and further shows, how difficult it is to predict how soil will react to root residue addition. However, the selected ecosystem properties were able to explain a large part of the variability of the C_{\min} in the soils from the different study sites and can therefore be regarded as key factors driving the C_{\min} in the soil. The high explanatory power of the ecosystem properties regarding the C_{\min} is a clear sign that they should be included in studies, whenever the C_{\min} is thematised.

It can be concluded, that climate change might not necessarily have a negative impact on the carbon stocks in the soil, as it is anticipated in the literature so far. Much more, improved management strategies could increase the sequestration of carbon in the soil. In that case, soils would contribute to a reduction of carbon in the atmosphere. Therefore, it is important to gain a better knowledge on how root residues react in deeper soil layers and in the field.

7 Limitations

A clear limitation of this master thesis was the lack of data on the dissolved organic carbon. Although for each sampling the soil was percolated with a nutrient solution and the percolation was kept to analyse the DOC, there was no time to evaluate these samples. Analyses on the DOC would have been of great value to detect how easily C can be released and to confirm the assumptions made that due to residue addition a large amount of DOC was present initially in the soil and that the percolation caused a dilution of the DOC in the soil.

Furthermore an analysis of the carbon fractions in the root residues would have clarified which fractions (sugar, cellulose, lignin) were limiting at which time of the incubation and the analysis of when which microorganisms would become active or die would have been simplified. In this regard, an analysis of the amount and the composition of the microorganisms would also have been helpful.

In order to obtain a holistic picture of the extent of the PE, it would have been important to continue the incubation for a longer time. The same applies for the root C_{\min} . A longer incubation period would have provided more clarity as to how much carbon can be expected to be sequestered in the soil by adding root residues.

Moreover, a greater variability within the individual ecosystem properties might have shown even more pronounced influences on the C_{\min} . This is especially the case for the soil moisture, which according to the literature has a great influence on the mineralisation but was hardly observed to be influential in this master thesis.

It can be assumed that a small portion of the measured TOC was inorganic, a fact that should be considered but which was not taken into account in this master thesis.

Finally, an analysis of the total nitrogen and the dissolved organic nitrogen content would also have been useful, although in this master thesis nitrogen did not receive much attention. By means of the nitrogen data it would have been possible to make statements about the dynamics of the C/N ratio that has, according to Kuzyakov et al. (2000), a great influence on the PE and therefore also, according to Gunina and Kuzyakov (2014) and von Lützow et al. (2006), on the total mineralisation of carbon and the stability of SOC.

8 Outlook

There are many assumptions with regard on the effect of root residue addition on the mineralisation of carbon in the soil that need to be clarified. Similar experiments, in which the DOC content and the C/N ratio as well as the microbial biomass and composition are additionally analysed, would provide more clarification on the assumptions made.

Further research should also focus evaluating the recalcitrance of the newly formed SOC. In this regard, efforts should also be made in performing similar incubation experiments with deeper soil horizons to clarify the impact of roots on the older carbon in the soil. In addition, field trials would provide better knowledge of the impact of roots residue addition on the mineralisation of carbon in a natural environment. For further studies a higher validity of the results could possibly be achieved if additional drivers, which are expected to influence the C_{\min} in the theory, were accounted for as well. Potential drivers in this regard are the C/N ratio, the altitude, the land use or the bulk density. Likewise, a larger spatial scale might increase the variability within the most important drivers and would contribute to a better explanatory power of the variability of the carbon mineralisation.

Finally, for further investigations on the C_{\min} of root residues, it is of special importance to distinguish between the root C_{\min} and the PE, since a combined consideration can lead to incorrect conclusions regarding the root C_{\min} .

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Personal declaration

I hereby declare that the submitted thesis is the result of my own, independent work. All external sources are explicitly acknowledged in the thesis.

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