Change of soil organic matter composition after spruce afforestation

A density fractionation study conducted in a subalpine region in Switzerland

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As I finished my apprenticeship as a landscape gardener I never would have imagined that five years later I would be writing my MSc-thesis in the unit of Soil & Biogeochemistry at the University of Zurich. The funny thing about that is that I am still dealing with soil and plants but dressed in a lab coat and on the level of carbon fractions and isotopes. So I want to say thank you to all who pushed my interests in this direction and supported me in any way writing this MSc-thesis.

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Abbreviations

a.s.L. Above Sea Level

C carbon

^{13}C stable carbon isotope with atomic mass 13

^{14}C radioactive carbon isotope with atomic mass 14

cHF coarse heavy fraction $>20\mu$m

CO_2 carbon dioxide

DOC dissolved organic carbon

fHF fine heavy fraction $<20\mu$m

fLF free light fraction

HCl hydrochloric acid

HF heavy fraction

LF light fraction

LUC land-use change

N nitrogen

NS not significant

^{15}N stable nitrogen isotope with atomic mass 15

OC organic carbon

oLF occluded light fraction

rcf relative centrifugal force

SD standard deviation

SE standard error

SOC soil organic carbon

SOM soil organic matter

SPT sodiumpolytungstate

TOC total organic carbon

V-PDB Vienna Pee Dee belemnite standard

Abstract

On a global scale, land-use change (LUC) is one of the main sources of human induced CO_2 emissions. In Switzerland, soil organic carbon (SOC) is mainly influenced by the increasing trend of forested areas during the last decades. Especially affected are subalpine and alpine regions.

In the PhD-project of David Hiltbrunner (Hiltbrunner, 2012) the impact of Norway Spruce (Picea abies) afforestation on SOC was studied on a subalpine pasture located at the Jaun-Pass in the canton of Fribourg in Switzerland. Based on the results and data of this project, this MSc-thesis aims in providing a deeper insight into carbon (C) dynamics influenced by afforestation. Soil samples collected by Hiltbrunner (2012) were further fractionated according to their density and particle size (free light fraction (fLF), occluded light fraction (oLF), fine heavy fraction $<20\mu$m (fHF), coarse heavy fraction $>20\mu$m (cHF)). In total, 40 samples of grassland and different forest stand ages (25 yr, 30 yr, 40 yr, 45 yr, 120 yr) were analyzed for C content, nitrogen (N) content and isotopic values(δ^{15}N, δ^{13}C). Beside the analysis of the soil samples, fresh plant compartments (needle, wood, spruce roots, grass roots, grass foliage) were collected and analyzed for the same parameters.

Independent from the land use category (pasture, forest), highest organic carbon (OC) concentrations were found for the oLF, whereas the highest portion of total SOC was found in the fHF. Across sites, SOC stocks declined with depth.

Corresponding with the results of Hiltbrunner (2012), total SOC stocks were only moderately affected by afforestation, while an increase of OC stored in the light fractions (LFs) and a decrease in the heavy fractions (HFs) was found. Differences were mostly pronounced for the topsoil but trends in the subsoil pointed in the same direction. With increasing forest stand age increasing amounts of OC were found to be stored in the LFs, whereas the HFs remained more stable after an initial decrease. The increasing amount of OC stored in the topsoil LFs could be attributed to the increase of the organic layer. The decrease of OC stored in the HFs is supposed to be driven by the cessation of the grass vegetation. Due to the higher portion of the OC stored in the LFs, the SOC stored in the forest is supposed to be less stable.

The shift from grass derived OC to forest derived OC was pronounced for all fractions. The isotopic values and CN-ratios of the fractions were influenced by different input sources but also by decomposition processes. The higher δ^{13}C values found with increasing depth and forest stand age reflects the influence of decomposition. The decrease of soil organic matter (SOM) quality with increasing forest stand age was reflected in the isotopic values and CN-ratios of the LFs but not of the HFs.

1 Introduction

In addition to fossil fuel emission, LUC is the most important source of human induced greenhouse gas emissions (Ciais et al., 2013). Therefore, LUC plays a key role in relation to climate change. According to the findings about C and other biogeochemical cycles, summarized in the fifth assessment report of the IPCC (Ciais et al., 2013), the annual net CO_2 emissions induced by anthropogenic LUC were 0.9 GtC yr^1 on average during 2002-2011. In relation to the total anthropogenic CO_2 emissions, the portion induced by LUC represent about 10%. On a global scale deforestation and the increase in the area cultivated in the tropics, are the most important land-use conversions causing CO_2 emissions (Poeplau and Don, 2013; Don et al., 2011; Houghton and Goodale, 2004; Houghton, 2003). According to the FAO (2010), the total forest area in the world decreased by 0.13% between 2000-2010 especially in South America and in Africa. On the other hand forested areas in Europe slightly increased in the last decade (Figure 1).

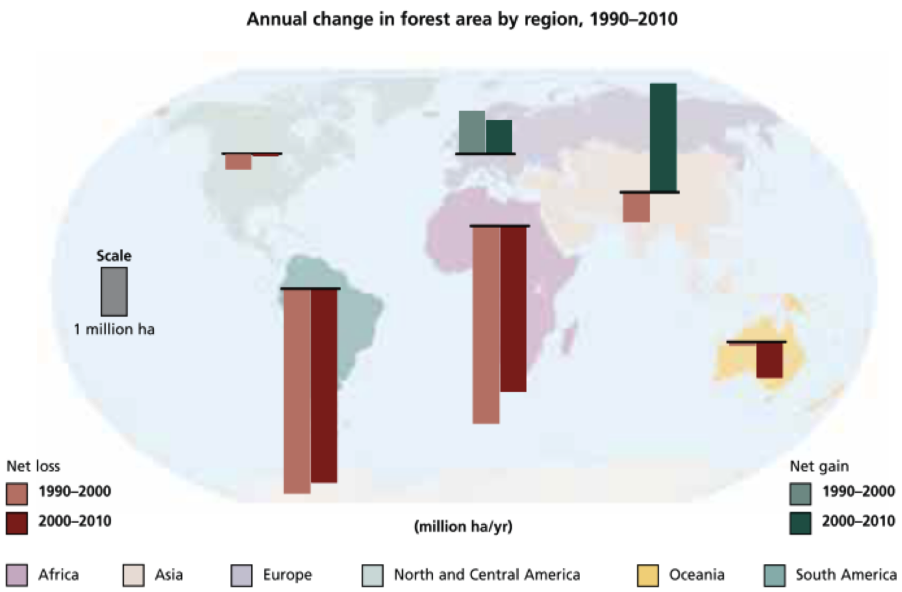

Figure 1: Annual change in forest area by region. FAO (2010)

Due to structural changes in agriculture, forests in Switzerland expanded by about 8% between 1985-2006 (Brändli, 2010). Especially in the Alps afforestation or reforestation of former pasture or grassland is an important phenomenon. In a study of Bolliger et al. (2008), C stocks in Switzerland were calculated based on three different socio-economic scenarios. For a 100-year simulation of the liberalization scenario, which assumes a stop of public support for agriculture, an increase of forest area by 15% meanwhile a decrease of agricultural land by 15.8% was predicted. As a consequence an increase of Cstocks by 12.7% was calculated. Thereof a portion of 74% was stored in forested areas. In contrast, for the extensification scenario, which assumes a support of extensively managed open-land through state-federal subsidies, a loss of C stocks resulted in the simulation. This loss was mainly driven by an increase of extensively managed agricultural soils. Such simulations show the importance of future LUCscenarios on predicting changes in C stocks. According to the BAFU (2012), in Switzerland the trend of C stocks through soil use is negatively rated as a climate indicator. Forest ecosystems are judged as the only CO_2 sinks, whereas other types of soil use (arable land, meadows and pasture, wetlands, settlements, others) act as sources. Due to increased forest management, forest ecosystem can only narrow compensate the emissions from other land-use categories, whereas in the 1990s the compensation rate was much higher.

In the research of LUC, not only the C sequestration in the biomass but also the influence on the SOC plays an important role. Soils enclose more than twice as much C as the atmosphere (Ciais et al., 2013) and are therefore very important for the global C balance. Furthermore, the C in the soil compartments is generally stored for the long-term, whereas C storage in the tree biomass is only for the short-term (Thuille et al., 2000; Harmon et al., 1990; Trumbore et al., 1990). Nevertheless, compared to C fixation in the biomass soil C sequestration is poorly understood and depends on several factors like climate, former land-use, forest type and soil properties (Paul et al., 2002). Considering these facts, it is important for climate change mitigation to understand the influence of LUC and especially the impact of afforestation and deforestation on SOC. In a meta-analysis of 537 studies from Guo and Gifford (2002) the influence of several LUC categories were analyzed. They distinguished between secondary forest and plantation. A secondary forest develops naturally, whereas a plantation is a forest pushed by human activities, meaning trees were planted manually. When there is a conversion of cropland into other land-use categories a gain of SOC results. The opposite way a loss was reported. No clear signal was observed for conversions from forest, secondary forest or plantation to pasture or vice versa. It is important to mention that most of the studies included in this meta-analysis were made in the tropics and therefore, the results might not be representative for Europe. A recently published study of 24 different sites from Poeplau and Don (2013) gives a good overview about the situation in Europe. They distinguish four major types of LUC in Europe: conversion of cropland to grassland or forest and conversion of grassland to cropland or forest. Their results show basically the same tendencies like the ones observed by Guo and Gifford (2002). In addition, they observed a higher influence of LUC on the topsoil compared with the deeper parts and the forest floor was pointed out to be important for C accumulation. The results of these studies are summarized in Table 1.

For Switzerland LUC from pasture or grassland to forest is an important category. Since 90% of the reforested area in Switzerland is located higher than 1000 m a.s.L. and more than 50% even higher than 1500 m a.s.L. (BFS, 2012), subalpine and alpine regions are of special interest. In addition soils in these areas contain a large portion of labile SOC and are therefore supposed to be highly vulnerable (Hagedorn et al., 2010). The PhD-project of David Hiltbrunner (2012) combines these points of interest. The influence of spruce afforestation was studied along a 120-year long chronosequence located on a subalpine pasture at the Jaun-Pass in the canton of Fribourg (Hiltbrunner et al., 2012a,b, 2013) (see section 3.1). Although only a moderate impact on the SOC stocks in the mineral soil was observed, a significant change of the SOM composition was found. The δ^{13}C measurements of the bulk soils showed a shift from grass-derived C towards spruce-derived C. This effect was even stronger with increasing stand age and associated with a decrease of SOM quality. To understand SOC dynamics it is important to study this exchange processes more in detail and on the level of separated fractions. So far, only a few studies were published which used δ^{13}C analysis in a pure C3 environment to study LUC (Grünzweig et al., 2007; Huang et al., 2011). Also the influence of LUC on the different fractions is not well known. Based on the data and soil samples of the PhD-project from Hiltbrunner (2012), this MSc-project has the aim to provide a better understanding of the C dynamics induced by afforestation of former pasture in subalpine regions.

Categories of LUC	**SOC**	**Review**
Cropland to secondary forest (9)	+	Guo and Gifford (2002)
Cropland to plantation (29)	+	
Cropland to pasture (76)	+	
Pasture to cropland (97)	-	
Forest to cropland (37)	-	
Forest to pasture (170)	NS	
Forest to plantation (30)	NS	
Forest to secondary forest (6)	NS	
Pasture to plantation (83)	NS	
Cropland to grassland (6)	+	Poeplau and Don (2013)
Cropland to forest (6)	+	
Grassland to cropland (6)	-	
Grassland to forest (6)	NS	

Table 1: Summary oft the results from Guo and Gifford (2002) and Poeplau and Don (2013). Numbers in brackets=number of studies; +=increase; -=decrease; NS=not significant

This MSc-thesis is structured in eight sections. In the first section, an introduction in the scientific context of the topic is given, which leads over to the objectives presented in section 2. In section 3, the applied methods will be described, followed by the results and the uncertainties presented and discussed in section 4, 5 and 6. The conclusion will be given in section 7 and last but not least a short outlook will complete this thesis.

1.1 Factors influencing SOC following afforestation

The process of C sequestration in soils is not trivial. There are several important factors which have an influence and must be taken into account. According to afforestation a meta analysis of Paul et al. (2002) gives a good overview of the important factors. In their work, they analyzed 43 studies including 204 different sites and determined three main factors which are crucial for changes of SOC after afforestation.

Climate One of the crucial factors is the climate. The decomposition rate of organic material is directly linked to the local climate. The analyzed studies showed an increase of SOC, which was in mainly driven by an increase of mean annual precipitation and in moist regions by an increase of mean annual temperature. The local climate is important for the general conditions of a site but what actually changes after afforestation is the microclimate. According to Kimmins (2004), soils are drier and colder in forests because of higher transpiration and shading. Hiltbrunner et al. (2013) found a 5°C lower average soil temperature under forests than under pasture. Due to this phenomenon Paul et al. (2002) supposed a lower decomposition rate after afforestation, which leads to an increase of detrital C store.

Previous land-use Another important factor is the previous land-use. Three different kinds of land-use categories were distinguished: pasture, crops and agriculture (rotation between crop and pasture). The data-analysis showed an increase of SOC after afforestation of former cropland and agriculture sites, whereas on ex-pasture sites there was a decrease of SOC in the first 10 years. A clear influence of previous land-use was also detected by a meta-analysis of Guo and Gifford (2002) and Poeplau and Don (2013) (Table 1).

Forest type The third factor is the type of forest established. In the study of Paul et al. (2002), SOC was analyzed after the establishment of different forests. It is especially interesting, that the impact on SOC was not the same for all soil depths in the first 10 years. Under hardwoods, SOC increased for all depths. For softwoods, there was an increase for the surface soil (<10 cm) and the layer deeper than 30 cm, whereas in between (10 - 30 cm) there was a decrease. Under Eucalyptus, a decrease for surface soil and an increase for deeper layers (>10 cm) occur. Under Radiata Pine there was a decrease for all depths. These differences are supposed to be driven by the different amounts of litter fall, litter quality, root slough and transfer rates from litter to soil. On a longer timespan, the differences tend to become smaller.

Other factors Beside these three main factors other factors like site management, site preparation (preparation of plantation) and soil properties can have an influence. In particular, soil properties like clay content, texture, pH and the amount of N has an influence on the stability of SOC (Scheffer and Schachtschabel, 2010; Velde and Barré, 2010). Last but not least, time is very important because all the influencing factors described above are dynamic and can change over time. Factors are summarized in Figure 2.

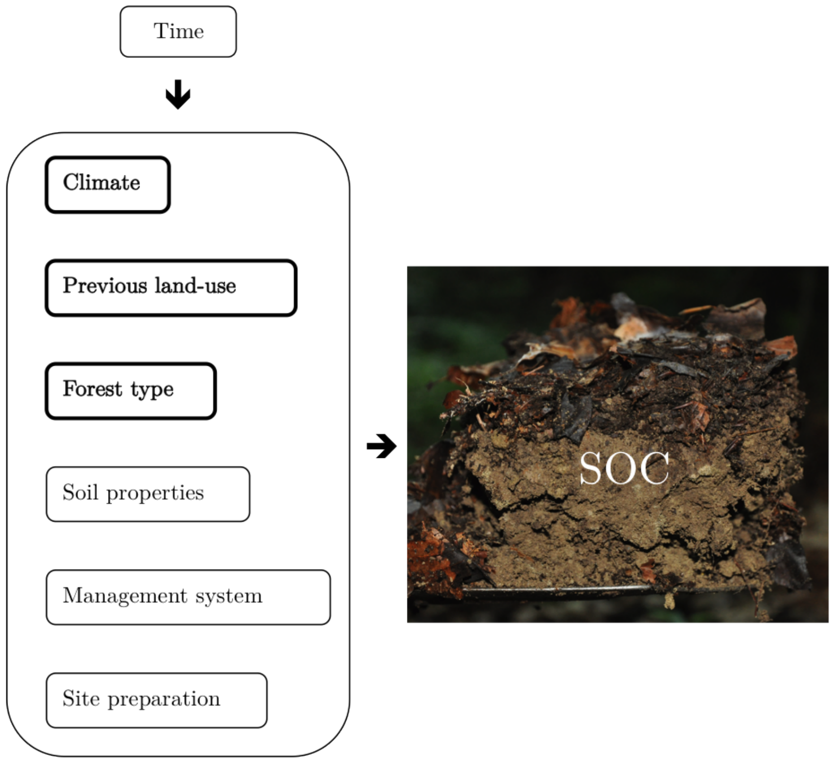

Figure 2: Factors influencing SOC. The bold written factors are the most important ones. Own figure summarized after Paul et al. (2002).

1.2 Stability of SOC

Sources of SOM To study the influence of LUC on SOM composition it is important to have an idea of the organic compounds. According to Scheffer and Schachtschabel (2010), the quality and the origin of organic material in terrestrial ecosystems are mainly from above-ground biomass, dead roots, dead microorganisms and organic exudates of the roots and microorganisms. Under the same climatic conditions, the amount of root-derived C in grassland soils is higher compared to the ones of forest ecosystems (Hiltbrunner et al., 2013; Huang et al., 2011; Scheffer and Schachtschabel, 2010). On the other hand, for forest ecosystems the C input from litter compounds as leafs, bark and branches is important (Hiltbrunner et al., 2013; Scheffer and Schachtschabel, 2010; Paul et al., 2002). If we compare grassland- to forest-ecosystems (vegetation included) > 60% of the organic C in forests is stored above-ground while in grasslands 90% is stored below-ground (Sharrow and Ismail, 2004).

Process of aggregation Since this MSc-thesis focusses on the analysis of the SOM on the level of fractions, it is important to shortly introduce the process of aggregation. When organic material reaches the soil, degradation through microorganisms starts immediately. Crucial for the rapidity of the degradation process is the CN-ratio of the plant residues. Whereas organic compounds with low CN-ratios are rapidly decomposed, organics with high CN-ratios remain more stable and are predisposed for aggregation (Hagedorn et al., 2003). Additional to CN-ratios, the content of lignin and phenolic compounds have an influence. Both compounds are hardly decomposable and can improve long-term aggregation (Blanco-Canqui and Lal, 2004). The aggregation of organic compounds or their conversion products with mineral soil compounds provides protection of further degradation and improves the stability of SOC. Especially clay minerals are highly interactive and therefore very important for aggregation building (Velde and Barré, 2010). Aggregation is especially important for C sequestration in the subsoil. Kaiser et al. (2002) determined that in acidic forest soils 40-50 % of the SOC was stored in the subsoil and bound to clay minerals < 20μm. These findings show in addition, that also the soil depth is an important factor to take into account. In summary, the driving forces influencing stability of SOC are the quality of the organic input, the degree of aggregation and the soil depth.

Fractions Density fractionation is a common method to separate free organic matter from organic matter bound to minerals (Griepentrog et al., 2014; Poeplau and Don, 2013; Schrumpf et al., 2013; Golchin et al., 1994). Through the analysis on the level of fractions, it is possible to study SOC dynamics in the soil. Based on δ^{13}C and ^{14}C measurements, conclusions about the residence time and the pathways of organic C can be made. According to Schrumpf et al. (2013), unaggregated organic material commonly named as free light fraction (fLF) turned out to be unstable with a short residence time. The occluded light fraction (oLF), which is weakly aggregated to minerals shows intermediate Δ^{14}C ages and the most stable fraction turned out to be the organic material bound to minerals (heavy fraction (HF)). In a recently published study from Gunina and Kuzyakov (2014), the pathways of SOM formation towards aggregation showed the following sequence: fLF - oLF - HF. These findings could be determined by alterations of the δ^{13}C values of the fractions, compared to the values of the initial organic material. In matters of LUC, the analysis of the isolated fractions showed an increase of the labile fractions whereas a decrease of the stable fractions was found after afforestation (Poeplau and Don, 2013). The application of this method will be further discussed in subsection 3.2.2.

1.3 ^{13}C and ^{15}N isotopes

Measurements of the natural abundance of stable isotopes can tell us a lot about C and isotopically fractionation dynamics in the soil. Especially the stable isotopes of C (^{13}C) and N (^{15}N) are important for such research. Induced by different physiological processes, the isotopic values vary between different vegetation types and even between different plant tissues (Marshall et al., 2007). So it is possible to distinguish different origins of fresh organic matter in the soil. Based on the phenomenon that most of the biogeochemical processes involved in the decomposition of organic material lead to a depletion of the lighter isotopes (^{12}C, ^{14}N) a relative enrichment of the heavy isotopes results (Garten Jr et al., 2007). Measuring the ratio of $^{13}C/^{12}C$ (δ^{13}C) and $^{15}N/^{14}N$ (δ^{15}N) allows conclusions about the state of decomposition and fractionation processes (Gunina and Kuzyakov, 2014; Hobbie and Högberg, 2012).

1.4 Results of Hiltbrunner et al. (2013)

Since this MSc-thesis is based on the PhD-project of Hiltbrunner (2012) it is important to summarize the findings according to the influence of afforestation on SOC (Hiltbrunner et al., 2013). The key massages are highlighted in bold. For the study, the space for time approach was used to measure SOC dynamics over time. The basis of this approach is the assumption that after a certain timespan the soil reaches equilibrium of SOC content. So if we compare different states of an ecosystem at the same time on the same location, it is possible to make conclusions about the future development. Along 4 transects and a 120-year long chronosequence several soil profiles in different altitudes and for different stand ages (25, 30, 45 year old) of Norway Spruce (Picea abies) were analyzed (Figure 4). The samples were then compared to the soils of the over 150-year old pasture and the mature forest (120 years). Along a fifth transect on the pasture, soils were sampled to avoid a possible bias by the slope. The samples were analyzed for SOC content, ^{13}C and CN-ratios. In addition soil respiration and tree biomass were measured.

Incubation experiments under standard conditions did not show significant differences of mineralization rates between forest and pasture soils. The amount and composition (fungi to bacterial ratio) of the microorganisms in the mineral soil did not significantly change after afforestation. Due to these findings the change of microclimate towards colder soil temperatures and dryer conditions during afforestation is supposed to be the main cause for the lesser amounts of respired CO_2 measured after an incubation of 140 days in the forest. In conclusion, **slower turn-over rates were observed in the Spruce stands**.

The CN-ratio and lignin content of the spruce-vegetation was higher compared to grass. This means that the litter quality is lower in the forest. Changes of the SOM composition were reported through $\delta^{13}C$ measurements of the bulk soils. **A shift from grass-derived C towards spruce-derived C** was found after afforestation.

The consequences for the SOC stock are quite moderate (Figure 3). In the first 30 years there was a small increase of SOC in the mineral soil followed by an abrupt decrease after 40 years and a re-increase up to more or less the same level like the former pasture. The organic layer and the tree biomass increased with stand age. After 40 years, the C stock of the tree biomass exceeds the amount stored in the mineral soil. The mineral soil showed an abrupt decrease of SOC by a forest stand age of 40 years. This was observed to be the point were the grass-vegetation ends due to a decrease of light availability under the spruce stands (highlighted in Figure 3). Differences of the SOC dynamic were also reported between soil depths. Comparing the development in the upper 10 cm with the whole profile down to a depth of 80 cm, the initial decrease and the subsequent re-increase is more pronounced for the whole profile. This indicates that the subsoil was more affected by SOC loss. A possible explanation for this phenomenon is supposed to be some kind of a priming effect due to new root input. As it is illustrated in Figure 3, if only the SOC of the organic layer and the upper 10 cm of the soil are measured, the increase of SOC is approximately 13% higher compared to the results for the soil down to a depth of 80 cm. This indicates the importance of dynamics in the subsoil (Hiltbrunner et al., 2013).

Related studies in alpine regions of Europe indicated similar results. Spruce afforestation in the Southern Italian Alps showed the same initial decrease followed by a re-increase of the organic C stored in the mineral soil (Thuille et al., 2000; Thuille and Schulze, 2006). Even along a chronosequence of reforestation with mixed ash (Fraxinus excelsior) and sycamore

(Acer pseudoplatanus) located in the Eastern Italian Prealps, the same trend curve could be found (Alberti et al., 2008).

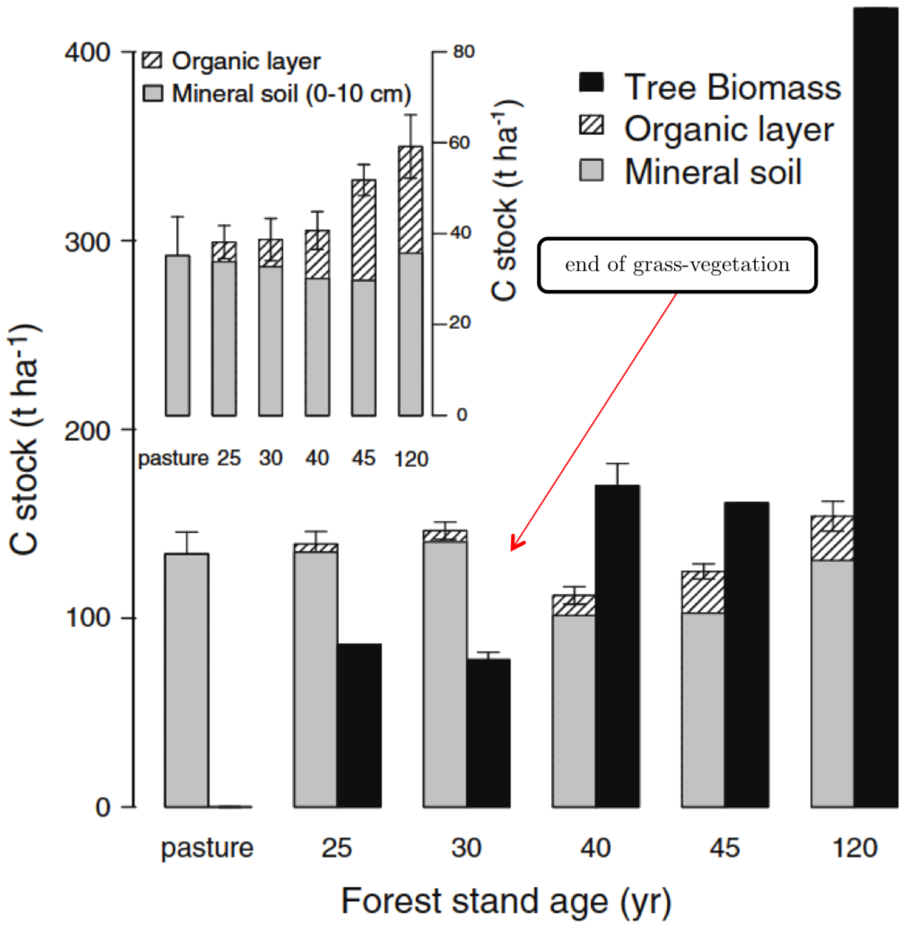

Figure 3: SOC stocks oft the different compartments. Big figure: mineral soil down to a depth of 80 cm. Small figure: mineral soil down to a depth of 10 cm. The Figure has been taken from Hiltbrunner et al. (2013). The point of the abrupt decrease has been highlighted by the author.

2 Objectives

Hiltbrunner et al. (2013) showed that afforestation of pasture has a rather small impact on SOC stock compared with the amounts stored in the above-ground biomass. In addition, they pointed out that there is an influence of afforestation on C cycling rates. The incubation experiment and microclimate observations showed that under forests slower turn-over rates occur due to a combination of lower litter quality and colder microclimate. Why do we have only a small gain of SOC, despite slower turn-over rates and OC accumulation in the organic layer? How are the single fractions influenced by afforestation and what does this mean according to SOC stability? As introduced in section 1, the process of C sequestration is not trivial and there are many factors influencing SOC dynamics. The aim of this MSc-thesis is to provide a better understanding of SOC dynamics influenced by LUC. The focus lies on changes at the level of fractions to gain a more detailed view on the processes going on during afforestation of former pasture. The following hypotheses should be declined or confirmed:

- The heavy fractions (cHF, fHF) are more decomposed and more stable than the light fractions (fLF, oLF).
- The distribution of the different soil-fractions (fLF, oLF, cHF, fHF) change significantly between:
  - grassland and forest: compared to grassland at the forest sites, more OC is stored in the LFs and less in the HFs
  - different forest stand ages: the amount of OC stored in the LFs increases with forest stand age
  - soil depths: the portion of OC stored in the LFs declines whereas for the HFs it increases with soil depth
- The shift from grass-derived OC towards forest-derived OC, is indicated on the level of fractions.

3 Materials and methods

3.1 Sampling design and study site

The study site is located at the Jaun-Pass in the canton of Fribourg in Switzerland. Here, a pasture has been afforested with spruce on a south exposed slope, reaching from 1450 to 1800 m a.s.L.. The mean annual precipitation amounts to 1250 mm and the mean annual air temperature averages in winter to 0.6°C and in summer to 11.4°C. The site is located on calcareous bedrock and the soils are Eutric Cambisols with a clay content of about 50%, a pH between 4-7 and a mean thickness of about 80 cm. Additional information on the location of the study area is given in Table 2.

Location	Community of Jaun Canton of Fribourg, Switzerland
Coordinates	7°15′54 E, 46°37′17 N
Orientation	South-facing slope
Altitude	1450 m to 1800 m a.s.L.
Climate	Mean summer Temp.: 11.4°C, Mean winter Temp.: 0.6°C, Mean annual precipitation: 1250 mm
Soil	Eutric Cambisols pH: 4-7 clay content: ˜50%
Bedrock	Calcareous bedrock C-horizon: ˜60 cm depth

Table 2: Description of the study site

According to Hiltbrunner et al. (2013), the slope has been used as a pasture for at least 150 years. The subalpine pasture was mainly used for cattle grazing. Due to avalanches in the eastern part of the slope, the pasture was afforested with Norway spruce (Picea abies L.).The western part is still used as a pasture. As shown in Figure 4 the afforestation was made sequentially. Therefore different forest stand ages of 25, 30, 40 and 45 years exists. Furthermore, a mature forest of about 120 years is part of the site. Even though there are changes in the altitude, this unique arrangement of different spruce stand ages located at a former pasture might render the site very interesting for LUC studies.

During the PhD-project of David Hiltbrunner soil samples were taken along four transects at different altitudes. The transects T1, T2, T3 and T5 were split into 11 plots with different stand ages and land-use types. In each plot, 5 to 10 soil pits were dug at a distance of 10 m. For the chemical analysis, samples were taken at six different depths (0-5, 5-10, 10-20, 20-40, 40-60, 60-80 cm). Bulk density was measured at two depths (0-10, 10-20 cm) in every second soil pit. The transect T4 which is situated on pasture only was used to identify transversal changes of soils along the slope. For this MSc-project, no additional soil samples were taken but organic material was collected for the analysis of δ^{13}C, total organic carbon (TOC), δ^{15}N and total N in plant compartments and the organic layer. To collect the organic layer in the forest, 20x20 cm frames were used. For each forest stand age and grassland site four frames were collected (in total 48 frames). Frames were taken at the locations, where soil profiles were dug by Hiltbrunner et al. (2013). At the grassland sites, just the grass vegetation within the frame was sampled. Roots were taken with three soil cylinders (0-10 cm) per site (in total 36 cylinders). In addition branches were collected in the forest for the analysis of the wood and bark (Figure 4).

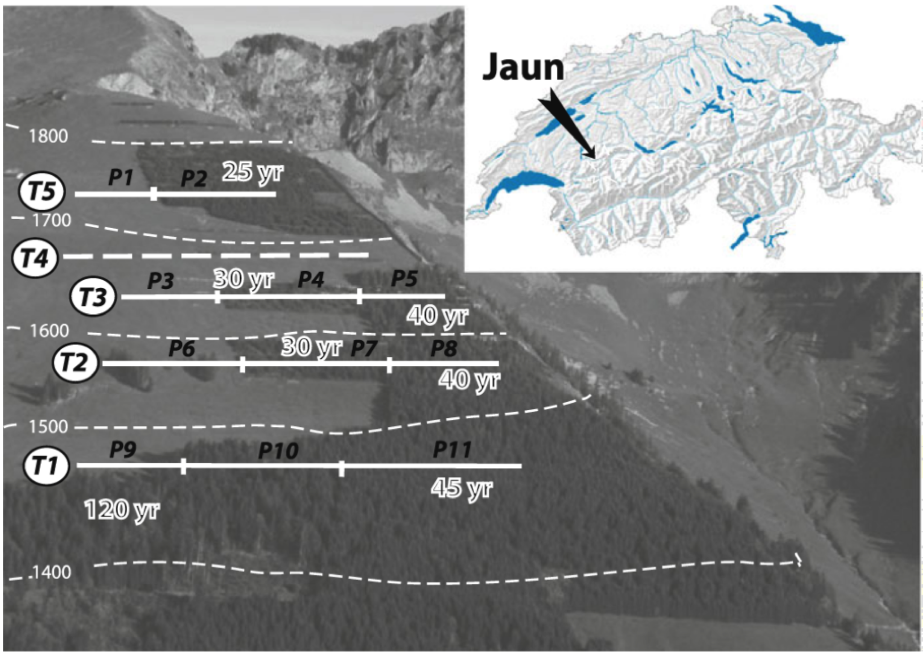

Figure 4: Study site (Hiltbrunner et al., 2013)

3.2 Sample preparation

3.2.1 Plant compartments and organic layer

Water contents and dry weights were determined by weighing all samples before and after drying in the oven by a temperature of 65°C. After drying, subsamples of ca. 2 g were milled for 2 minutes with the ball mill (Retsch, MM200). To extract the roots, the soil samples were washed and sieved. Then, roots were collected by hand, dried and separated according to their diameter in fine roots <1 mm, roots between 1-2 mm and coarse roots >2 mm. All portions were weighted and ball milled.

3.2.2 Density fractionation

Sieved soil samples from the study of Hiltbrunner et al. (2013) were further fractionated according to their density (Golchin et al., 1994). The fractionation was made for each soil depth of two grassland profiles and for the soil samples of each forest stand age (25 yr, 30 yr, 40 yr, 45 yr, 120 yr). In total, the density fractionation was applied to 40 samples. The soil was separated into fLF, oLF and HF. Furthermore the HF were separated by wet sieving into cHF and fHF (Griepentrog et al., 2014). For the fractionation a sodiumpolytungstate (SPT) solution (SPT, TC Tungsten Compounds, Grub am Forst, Germany) adjusted to a density of 1.6 g cm^{-3} was used. According to previous studies, a density cut-off of 1.6 g cm^{-3} has turned out to be the most suitable density to extract a maximum amount of the fLF (Cerli et al., 2012; Crow et al., 2007; Wander, 2004). A fractionation scheme is shown in Figure 5. In our study, a pretest as recommended by various studies (Cerli et al., 2012; Griepentrog and Schmidt, 2013; Schrumpf et al., 2013) was made to determine the adequate dispersion energy for the extraction of the oLF. The adequate dispersion energy is different for every soil and depends on the aggregate stability. The dispersion energy which yields the highest amount of OC in relation to the mineral C was obtained by a stepwise increase of the energy (150 J mL^{-1}, 250 J mL^{-1}, 300 J mL^{-1}, 400 J mL^{-1}). After each step, the oLF was separated and analyzed for the amount of OC. This procedure was made for a topsoil- and a subsoil-sample of a grassland and a forest site. The most appropriate dispersion energy for the soil samples has turned out to be 300 J mL^{-1}. This value is in agreement with the findings of Cerli et al. (2012) where a level of 300 J mL^{-1} was found to be adequate for soils with high clay contents (Grafik preTest). After the whole fractionation procedure, the used SPT solution was recycled according to Six et al. (1999).

Extraction of the fLF To extract the fLF a soil subsample of 10 g was added to a centrifuge-tube and rinsed with 70 mL of SPT solution (mixing ration 1:7). The soil was rinsed with sufficient solution to be able to easily extract the floating material. A mixing ration of at least 1:5 (soil:SPT) seems to be appropriate (Cerli et al., 2012). The solution was allowed to settle for 15 min and then placed in the centrifuge (Megafuge 1.0, Heraeus) for 10 min by a speed of 4000 relative centrifugal force (rcf) (multiples of gravitational acceleration). Afterwards, the floating material (fLF) was collected by vacuum filtration using glass-fibre filters (GF 6, Whatman, Dassel, Germany). Finally, the remaining fLF was rinsed with deionised water until the conductivity of the wash water remained $<50\mu S\ cm^{-1}$.

Extraction of the oLF To extract the oLF the remaining part of the sample was refilled with 80 mL of SPT solution and was allowed to settle for 15 min. Accordingly the solution was dispersed by ultrasound sonification (UW 3400, Bandelin, Berlin, Germany) with the pretested dispersion energy of 300 J mL^{-1}. Before this treatment the disintegrator was calibrated according to Schmidt et al. (1999). To minimize the risk of thermal interferences of the samples, an ice bath was used. After another 15 min of settling, the same centrifuge and filtering procedure as for the extraction of the fLF was applied.

Extraction of the HF The remaining HF with a density >1.6 g m^{-3} was washed with deionised water and centrifuged for three times (Griepentrog et al., 2014). To separate the HF into cHF (> 20μm) and fHF (< 20μm), the samples were wet sieved (200 mm DIA x 5 mm, Retsch). Whereas the fHF was collected from the sieve, the cHF was collected after settling for one week.

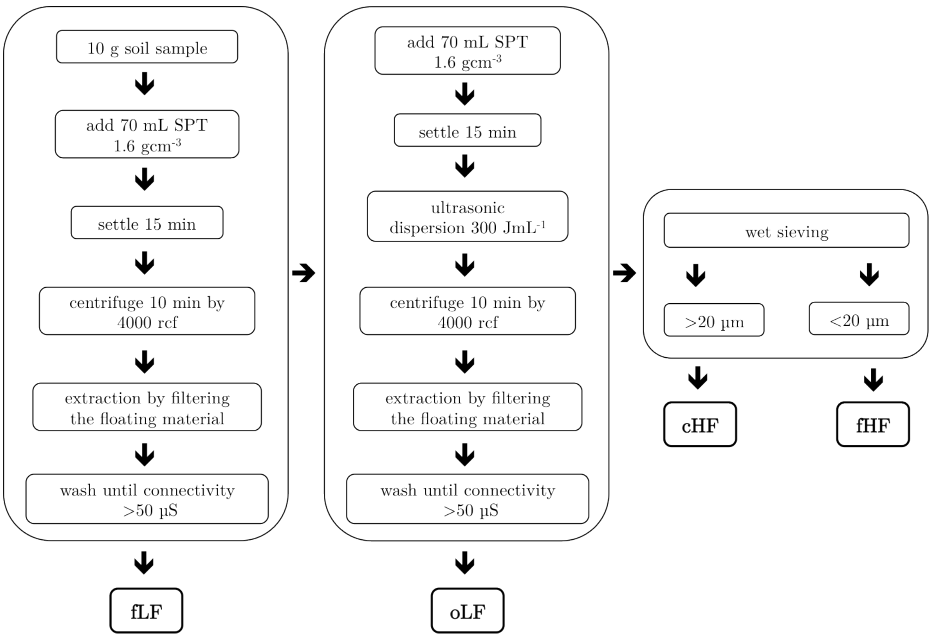

Figure 5: Density fractionation

Freeze drying & milling All the fractions were collected in round flasks and freeze dried (Alpha 2-4 LD plus, Christ) to avoid thermal inferences on the OC. After weighing, all dried soil fractions were milled by hand using a porcelain mortar.

3.3 Chemical analysis

3.3.1 HCl fumigation

Prior the soil C and stable isotope analysis, the inorganic C of samples with a pH>6 was removed by HCl fumigation according to Walthert et al. (2010) and Harris et al. (2001). Aliquots of 25 mg were weighted into silver capsules (9 x 5 mm) and moistened with 50 μL of 1 % HCl solution. Accordingly, the capsules were placed in a desiccator, where they were exposed to HCl vapor (100 mL of 37% HCl solution). After 8 hours in the desiccator, the samples were dried for 24 hours and placed in a vacuum oven for 3 days by a temperature of 40°C applying a vacuum of 200-300 hPa. Finally, the dried samples were packed in larger silver capsules (10 x 10 mm) and formed into balls. Since the N content could be slightly altered through this treatment, N content was measured from untreated samples (Walthert et al., 2010).

3.3.2 Isotope analysis

Measurements of the C and N concentrations and the isotopic ratios of all the samples were done with the automated elemental analyzer continuous flow isotope ratio mass spectrometer (Euro-EA, Hekatech GmbH, Germany, interfaced with a Delta-V Advanced IRMS, Thermo GmbH, Germany). Prior to these measurements, the samples were weighted. For the samples with a pH<6, tin capsules (5 x 8 mm) were used. The initial weight was adjusted according to the expected C to N ratio of the sample material. Isotopic ratios (δ^{13}C and δ^{15}N) were quoted by δ notation and expressed relative to the V-PDB-respectively air-standard. In total 160 samples (4×40) were analyzed.

$$\delta^{13}C = \left(\frac{R_{C_{sample}}}{R_{C_{V-PDB}}} - 1\right) \times 1000‰ \qquad R_C = \frac{^{13}C}{^{12}C} \tag{1}$$

$$\delta^{15}N = \left(\frac{R_{N_{sample}}}{R_{N_{air}}} - 1\right) \times 1000‰ \qquad R_N = \frac{^{15}N}{^{14}N} \tag{2}$$

3.4 Calculating relative stability

The relative stability of the fractions was calculated according to Conen et al. (2008) by considering the changes of the δ^{15}N values and the CN-ratios. The method developed by these authors is based on the fact that decomposition and re-synthesis processes lead to a decrease of the CN-ratio and an increase of the stability of the OC (von Lützow et al., 2006; Kramer et al., 2003; Gleixner et al., 2002). Simultaneously during these processes N is mineralized, which leads to a relative enrichment of ^{15}N (Dijkstra et al., 2006). For the calculations the following equations were used:

$$f_N = 1 - e^{\frac{\delta_h - \delta_l}{\epsilon}} \tag{3}$$

f_N is the proportion of N lost during the transformation an re-synthesis processes (Högberg, 1997; Robinson, 2001). The fractionation taking place during these processes can be described with ϵ (‰) defined as the enrichment factor. Based on studies of Robinson (2001) and Vervaet et al. (2002), the enrichment factor (ϵ) was set to a value of -2.0‰ by Conen et al. (2008). Within this MSc-project, the same value was used. δ_h is defined as the δ^{15}N value of the mineral bound fractions (HFs) whereas the δ_l is defined as the δ^{15}N of the unprotected organic matter (LFs).

$$f_C = f_N + (1 - f_N) \times (1 - \frac{r_h}{r_l}) \tag{4}$$

f_C is defined as the portion of C lost and calculated according to the portion of N lost (f_N). The CN-ratio of the fractions are described as r_h for the HFs and r_l for the LFs.

$$n = \frac{C_h}{C_l \times (1 - f_C)} \tag{5}$$

Based on the amounts of C stored in the HFs (C_h) and LFs (C_l) ($gCkg^{-1}$), a factor n can be calculated by which the age or stability of the HFs is higher relative to the LFs. For this approach by Conen et al. (2008) it is important to consider the assumption that soil remains under steady conditions. Furthermore the approach was applied for grassland in the Swiss Alps and was not tested for forest ecosystems (Conen et al., 2008). In Figure 6, taken from Conen et al. (2008), the method is schematically illustrated. To interpret Figure 6 it is important to consider that an other nomenclature is used. POM stands for particulate organic matter and is comparable with the LFs in this project. mOM stands for mineral-associated organic matter which is defined as HFs in this work.

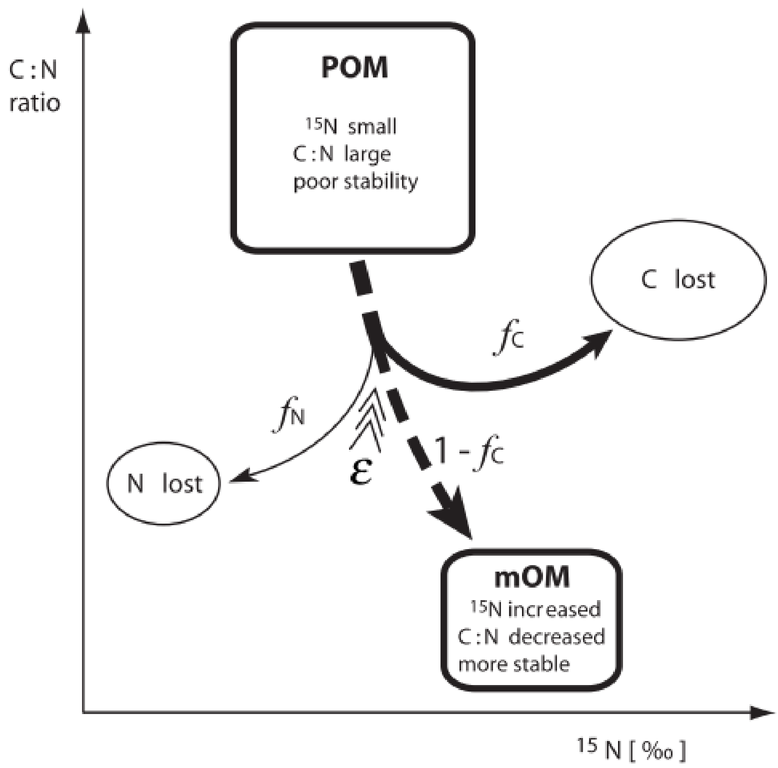

Figure 6: Scheme of the method to calculate the relativ stability by Conen et al. (2008). Particulate organic matter (POM) is defined as LFs and mineral-associated organic matter (mOM) is defined as HFs in this thesis. ϵ=enrichment factor, f_N=proportion of N lost, f_C=proportion of C lost.

3.5 Statistics

All statistics were made with R-Studio (Version 0.97.318). All the data was tested for normal distribution (qqplot; shapiro.test) and for homoscedasticity (leveneTest from package car). Not normally distributed data was transformed by calculating the logarithm. To compare means of different units ANOVA was applied. As post-hoc test Student Newman-Keuls Test was used (SNK.test from package agricolae). Trends were analyzed by fitting linear mixed effect models by maximum likelihood (nlme from package nlme). According to Hiltbrunner et al. (2013), land-use soil depth and stand age were set as fixed effects. As mixed effects plot and in appropriate cases soil depth were set. Results with $P<0.05$ were considered significant. For depth and age effects, all the fractions were tested individually.

4 Results

4.1 Plant compartments and organic layer

4.1.1 Mass distribution

Roots Root biomass in the upper 10 cm of the topsoil is shown in Figure 7. Significantly higher amounts of roots were found under grassland compared to the forest ($P<0.05$). In particular, roots with a diameter <1 mm were 37% more abundant than in the forest topsoils. The 45-year old forest stand even had a root biomass which was 48% lower compared to the grassland. Differences in the diameter class of 1-2 mm were negligible. For the grassland sites, no slope effect was found ($P=0.6$). Roots >2 mm of grassland and forest stand ages of 45 and 120 years were on the same level whereas in the 30 and 40 years stand ages clearly lower amounts were found. Within the different forest stand ages the fine roots <1mm clearly decreased in the 45-year old forest age followed by a re-increase in the 120-year old forest. No pattern existed for the coarse roots >2 mm.

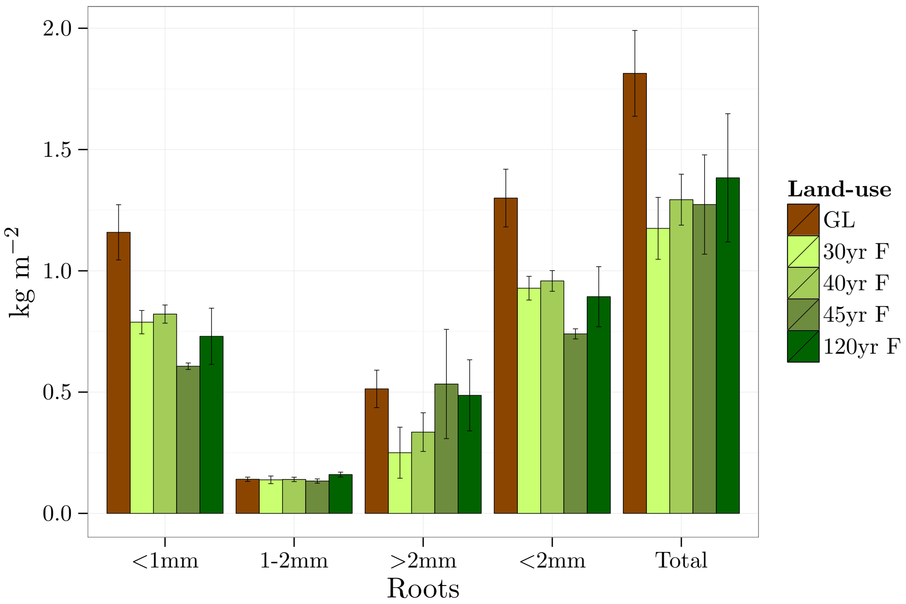

Figure 7: Mass distribution of the roots in the upper 10 cm. The roots are split into different diameter classes (±SE). GL=Grassland; yr=years; F=Forest

Organic layer Measurements by Hiltbrunner et al. (2013) showed a continuous increase with stand ages of organic layer, with 25 t C ha^{-1} being stored in the mature forest (120 yr). Similarly the mass distribution as indicated in Figure 8 significantly increased with increasing stand ages (P<0.001).

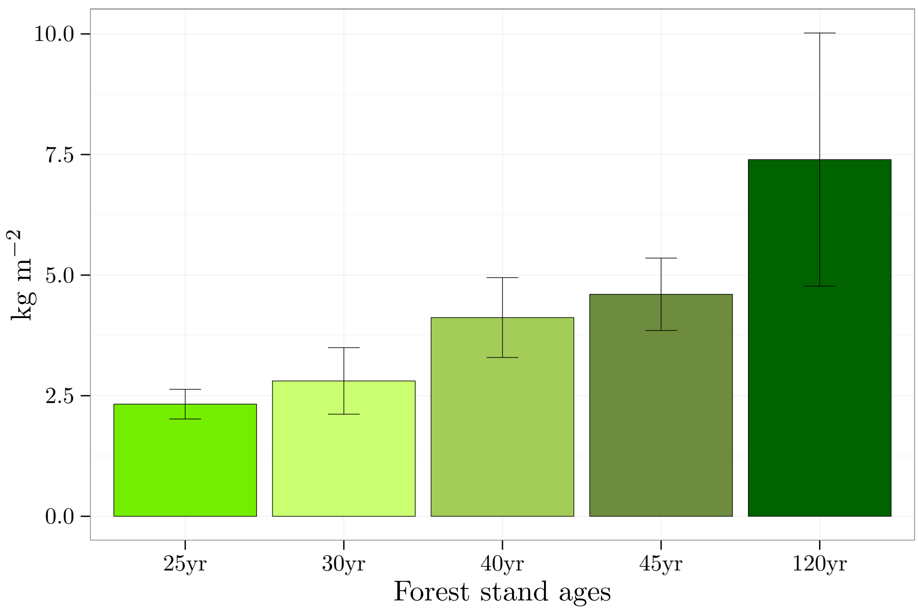

Figure 8: Mass distribution of the organic layer in the different forest stands (±SE). yr=years

4.1.2 Isotopes and CN-ratios

δ^{13}C and δ^{15}N The isotopic values of the plant compartments showed significant differences of δ^{13}C values among the plant compartments (Figure 9; Table 3; P<0.05). Lowest δ^{13}C values were found for needles and the highest for spruce roots. For wood, grass foliage and grass roots, intermediate values were found. The δ^{13}C values of the needles decreased significantly with increasing stand age (P<0.001). For the spruce roots and the branches, no significant influence of the stand age was observed. Furthermore, there was no significant elevation effect for the grassland vegetation.

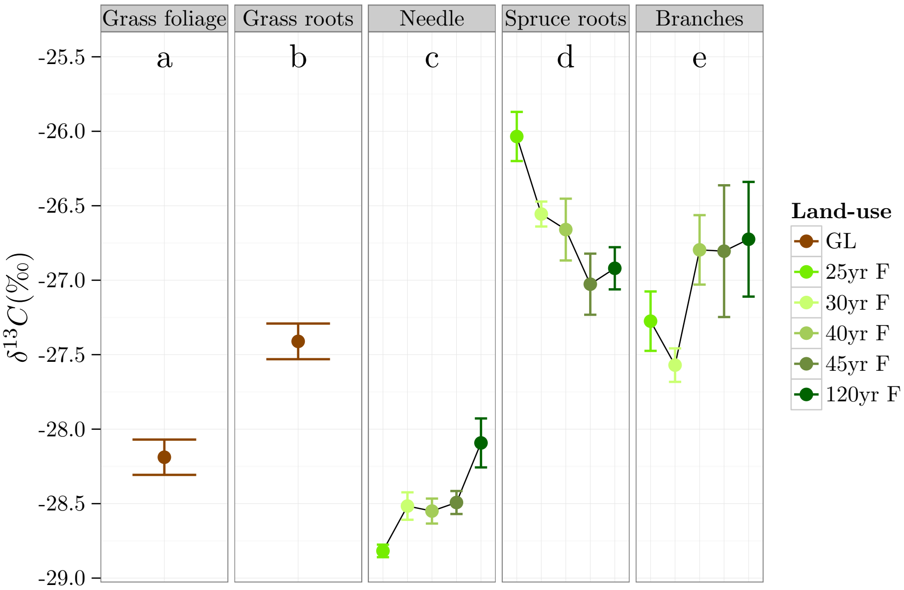

Figure 9: δ^{13}C of the plant compartments. Separated according to the land-use category and forest stand ages (±SE). Letters correspond to the means of each pool. Means with the same letter are not significantly different (P<0.05). GL=Grassland; yr=years; F=Forest

The mean δ^{15}N values of plant compartments differed significantly among each other (Figure 10; Table 3; P<0.05). For all plant compartments of the spruce forest, δ^{15}N values decreased with increasing stand ages (Figure 10; spruce roots, Needle: P<0.001; Branches: P<0.05). The largest range in values was observed for the spruce roots. By fitting a linear regression between stand age and the δ^{15}N values, an adjusted R^2 of 0.67 results for the spruce roots. Comparing the values of the forest with the grassland, the values of needle and spruce roots in the younger forest stand ages were in the same range as the ones of grass foliage and -roots. For needles and roots from the older forest stand ages the δ^{15}N values were lower compared to the grassland.

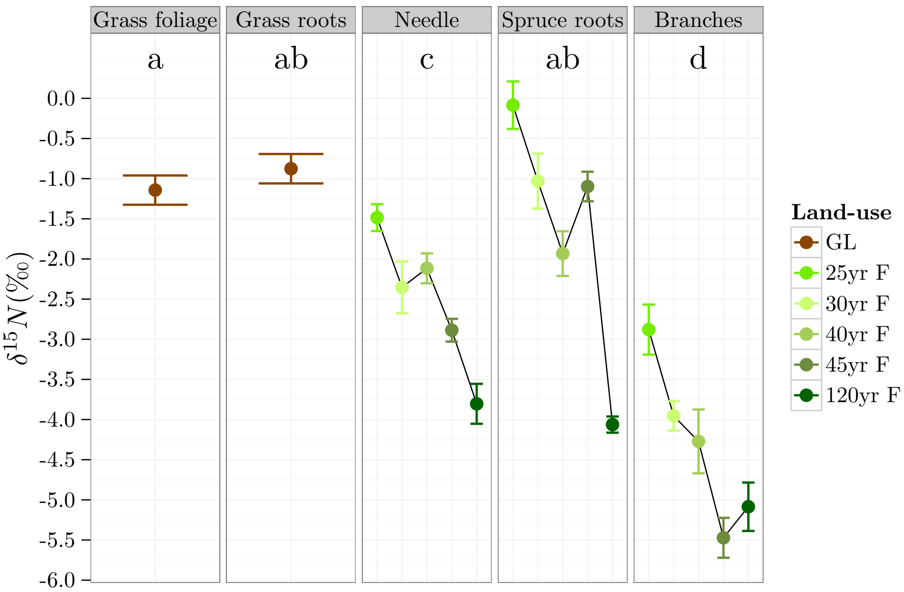

Figure 10: δ^{15}N of the plant compartments. Separated according to the land-use category and forest stand ages (±SE). Letters correspond to the means of each pool. Means with the same letter are not significantly different (P<0.05). GL=Grassland; yr=years; F=Forest

Compartments	**CN-ratio**	δ^{13}**C (‰)**	δ^{15}**N (‰)**	**C (%)**	**N (%)**
Grass foliage	29.0 (1.6)	-28.2 (0.1)	-1.2 (0.8)	33.3 (1.2)	1.9 (0.04)
Grass roots	36.7 (2.2)	-27.4 (0.1)	-0.9 (0.2)	35.9 (0.8)	1.0 (0.05)
Needle	33.9 (1.3)	-28.5 (0.0)	-2.5 (0.2)	37.8 (1.0)	1.1 (0.04)
Spruce roots	57.4 (2.8)	-26.7 (0.0)	-1.6 (0.3)	42.2 (0.5)	0.8 (0.03)
Branches	50.8 (2.2)	-27.1 (0.1)	-4.3 (0.2)	45.9 (0.4)	0.9 (0.04)

Table 3: CN-ratios, δ^{13}C, δ^{15}N, C-content, N-content of the fresh plant compartments (±SE). (roots<2mm)

CN-ratio Comparing the roots of the two land-use types, spruce roots had a significantly higher CN-ratio (P<0.05). The same pattern occurred for the values of needle and grass foliage but it was not statistically significant (Table 3; Figure 11). The highest CN-ratio had the branches although they included bark, whose CN-ratio is lower compared to wood. Within the forest sites only the branches showed a significant increase of CN-ratios, with increasing age (P<0.05). Since the organic litter is a mixture of different plant residues, a mean value per ecosystem was calculated reflecting their relative contribution to the overall organic layer. Accordingly, the litter layer of the spruce forest had a mean CN-ratio of 46.4 (±1.6), whereas the grasslands litter layer had a significantly lower ratio of 32.3 (±1.5; P<0.05).

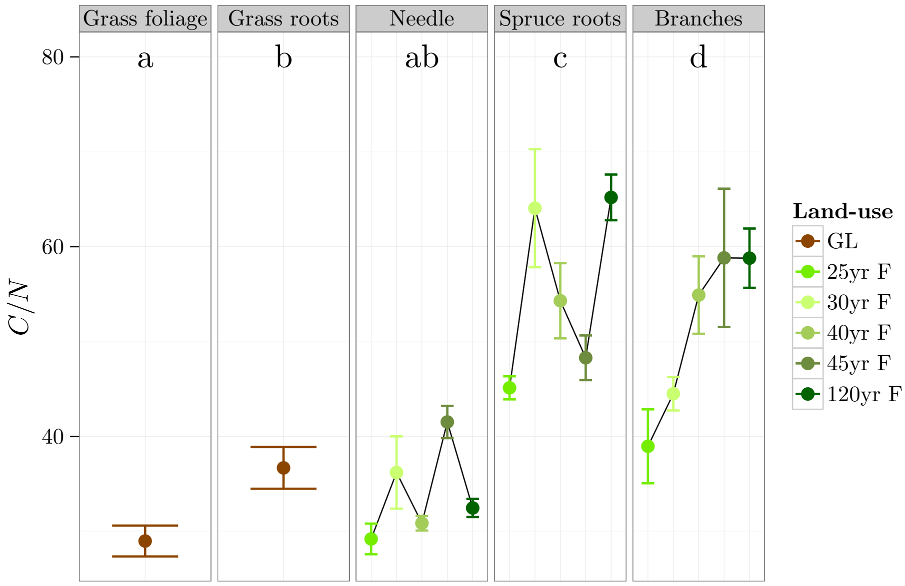

Figure 11: CN-ratios of the plant compartments. Separated according to the land-use category and forest stand ages (±SE). Letters correspond to the means of each pool. Means with the same letter are not significantly different (P<0.05). GL=Grassland; yr=years; F=Forest

The most important results regarding plant compartments and organic layer are summarized as follows:

- Significantly higher amounts of roots were found under grassland.
- The amount of organic layer increased with stand age.
- The isotopic values of the plant compartments differed significantly.
- $\delta^{13}C$ values of the needles and $\delta^{15}N$ values of all forest compartments significantly decreased with increasing stand ages.
- The CN-ratio of the grassland litter is significantly lower compared to the forest.

4.2 Density fractions

4.2.1 Trends for organic C, isotopes and N

Organic C The largest concentration of OC within the soil fractions was found in the oLF (36.6±4.9%) followed by slightly lower concentrations in the fLF (32.0±5.0%). The combined HFs had significantly lower concentrations of OC (P<0.05) with the fHF containing 2.1% C (±1.0%) and the cHF having 1.0% C (±0.7%). No significant difference was observed among land-use types.

The mass distribution of the fractions according to soil depth is shown in Figure 13. The mass recovery after the fractionation procedure was 93% (±0.69%) for all soils. In all profiles the LFs significantly decreased with soil depth (P<0.01), whereas the HFs increased (NS; P=0.8). Within the HFs, there was a relative decrease (mass %) of fHF down to a soil depth of 20-50 cm, whereas in deeper parts an increase occurred. This pattern was found in all profiles except in the 25-year old forest and was significantly influenced by the clay content (P<0.01). As shown in Figure 12, the distribution of the fHF was positively correlated with the clay content.

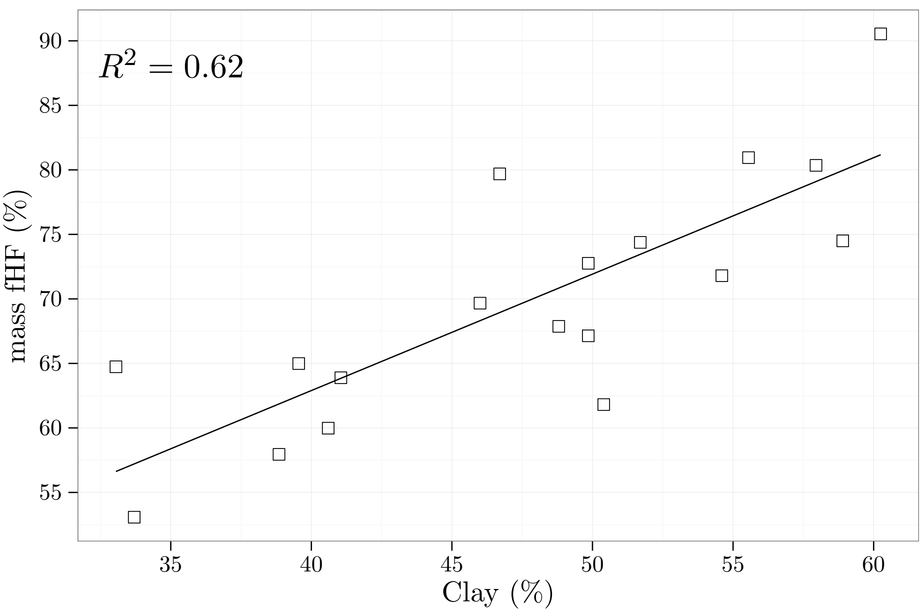

Figure 12: Correlation between the mass distribution of the fHF and the clay content of the soil. Adjusted $R^2 = 0.62$

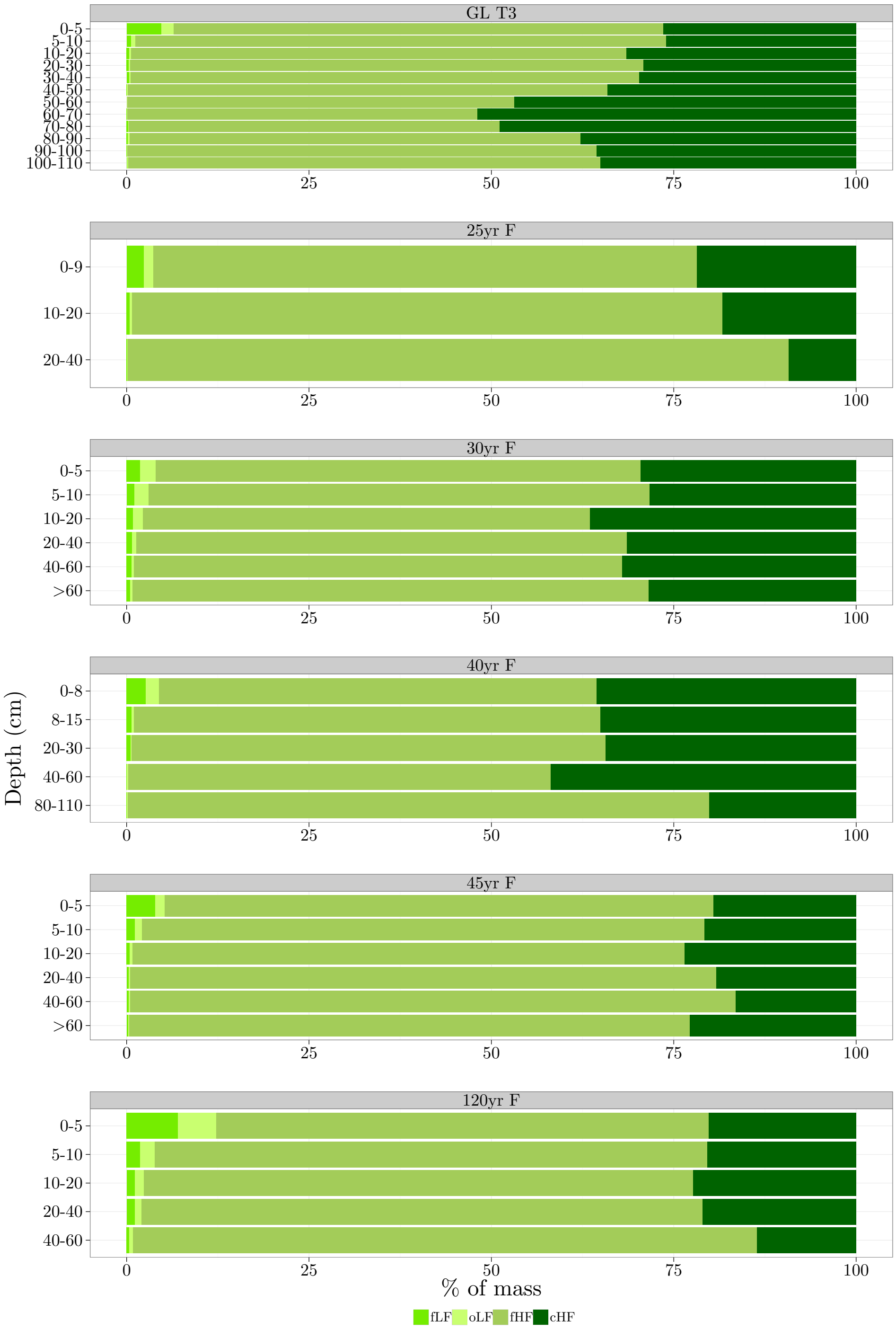

Figure 13: Mass % of the fractions separated according to depth and forest stand ages. GL=Grassland; F=Forest

The OC recovery after fractionation was 89% (±1.16%) compared to the TOC measured by Hiltbrunner et al. (2013). Across all soil depths significantly higher amounts of OC were stored in the fHF (P<0.05), followed by the oLF, fLF and cHF. Thereby, the LFs and the cHF contributed about the same portion of total OC. Within the HFs, mainly the fHF played an important role for C-storage, whereas only small amounts of OC were stored in the cHF. For all fractions significantly higher amounts of OC were found in the upper soil (0-10 cm; P<0.05). With increasing depth a strong decline of OC occurs for all fractions (P<0.001; Figure 14). The decline with depth is most strongly pronounced for the upper 25 cm.

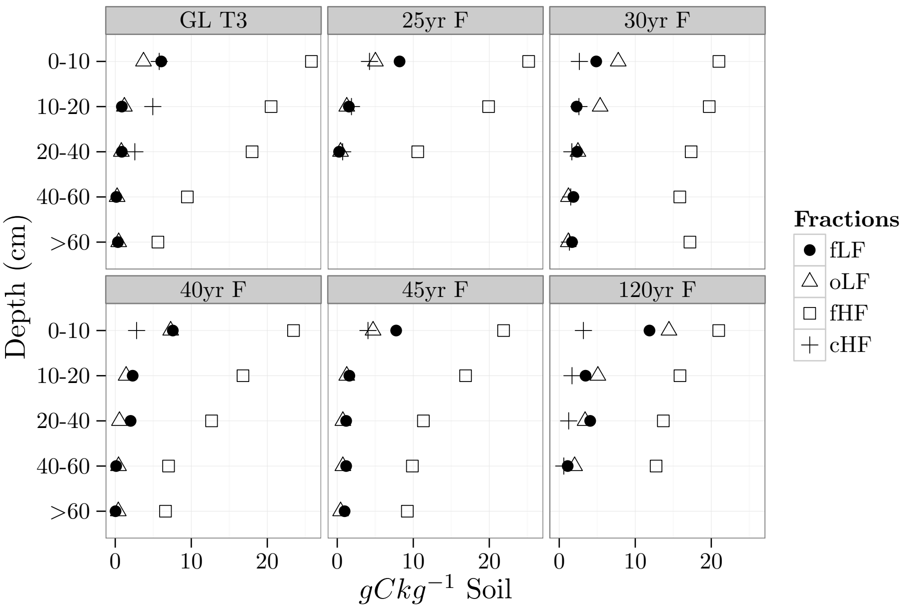

Figure 14: $gCkg^{-1}$ soil stored in the fractions separated according to depth and land-use. GL=Grassland; T=Transect; yr=years; F=Forest

Higher amounts of OC were stored in the LFs and lower amounts in the HFs at forest sites compared to grassland. As shown in Figure 15, this discrepancy was most evident in the topsoil (0-10 cm) and declines as soil depth increases (>10 cm; Table 4). Across all depths, the amounts of OC stored in the oLF of the mature forest (120 yr) were significantly higher (P<0.05) compared to the grassland soils. For the fLF this discrepancy is also pronounced but not statistically significant (Figure 15). Comparing the HFs no significant differences resulted. Comparing the distribution between the different forest stand ages there was an increase of the OC stored in the LFs. This pattern is especially pronounced in the upper soil parts (0-10 cm;

Figure15). This increase with stand age is statistically significant for the LFs (P<0.01) but not for the HFs. For all fractions the decrease with soil depth was significant (LF & fHF: P<0.001; cHfF: P<0.05).

Fractions	**Grassland**		**Forest**	
	0-10cm	>10cm	0-10cm	>10cm
fLF	6.1 (4.2)	0.5 (0.1)	8.1 (1.8)	1.6 (0.3)
oLF	3.7 (1.6)	0.6 (0.1)	8.3 (1.8)	1.7 (0.4)
fHF	25.8 (4.3)	10.4 (2.0)	22.1 (0.8)	13.7 (1.0)
cHF	5.8 (1.8)	3.4 (0.8)	3.4 (0.6)	1.5 (0.2)
LF	4.9 (2.0)	0.6 (0.1)	8.2 (1.2)	1.7 (0.2)
HF	15.8 (6.1)	9.6 (2.0)	12.7 (2.6)	9.5 (1.3)

Table 4: $gCkg^{-1}$ stored in the fractions (±SE).

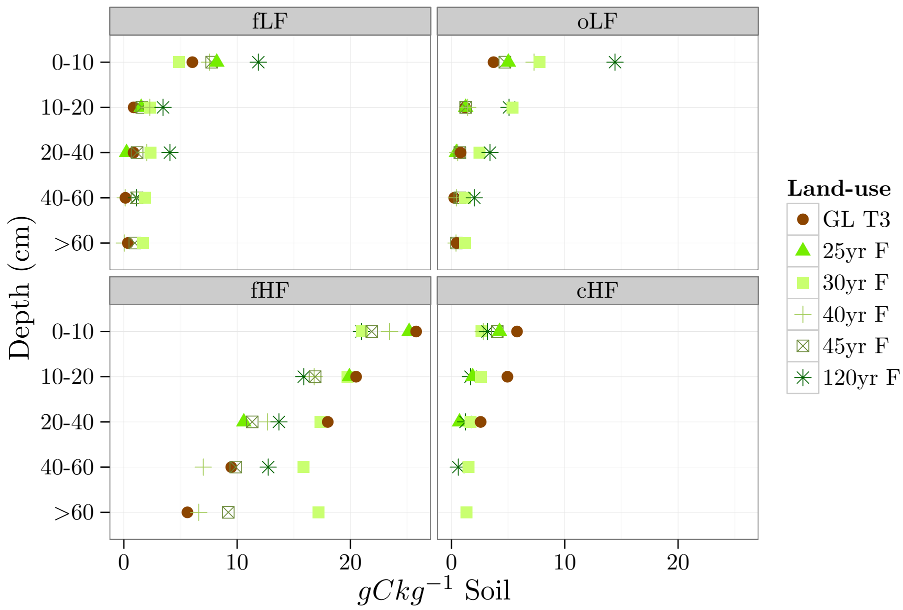

Figure 15: $gCkg^{-1}$ stored in the fractions separated according to depth and land-use. GL=Grassland; T=Transect; yr=years; F=Forest

CN-ratio Across sites, the CN-ratios of the HFs were significantly lower than the ones of the LFs (P<0.05; fHF<cHF<oLF<fLF). As indicated in Figure 16 and Figure 17, the CN-ratios of the HFs decreased with soil depth whereas for the LFs there was an increase (LF: P<0.001; HF: P<0.01). This pattern occurred across all profiles. In the forest sites, CN-ratios of the LFs increased with stand age (P<0.05). This increase was not significant for the HFs. Comparing the topsoil (0-10 cm) between the two land-use categories the LFs of the grassland soils were relatively enriched in N and had therefore lower CN-ratios. For the HFs, differences were less pronounced. At the grassland sites, CN-ratios for the cHF were slightly higher, whereas for the fHF ratios were lower. The values of the subsoil (>10 cm) are lower for the HFs and the oLF of the grassland, whereas the fLF show a slightly higher ratio than in the forest (values are summarized in Table 5).

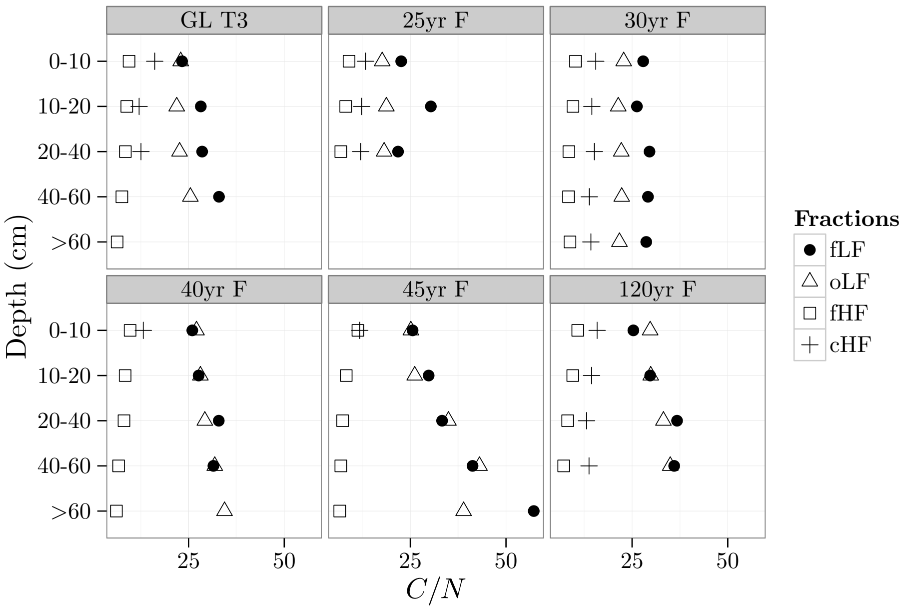

Figure 16: CN-ratios of the different fractions separated according to depth and land-use. GL=Grassland; T=Transect; yr=years; F=Forest

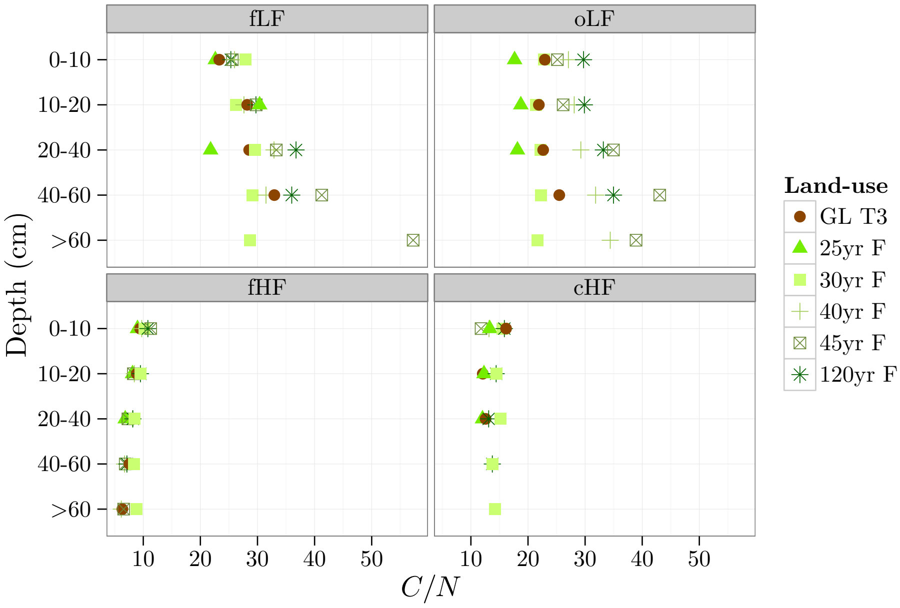

Figure 17: Distribution of the CN-ratios of the fractions separated according to depth and land-use. GL=Grassland; T=Transect; yr=years; F=Forest

Fractions	**Grassland**		**Forest**	
	0-10cm	>10cm	0-10cm	>10cm
fLF	23.3 (0.6)	34.8 (2.8)	25.8 (0.7)	32.6 (2.0)
oLF	22.9 (0.6)	27.3 (2.9)	25.0 (1.4)	28.8 (1.8)
fHF	9.5 (0.1)	7.5 (0.4)	10.4 (0.5)	7.8 (0.2)
cHF	16.2 (1.8)	12.4 (0.2)	14.1 (0.9)	13.7 (0.3)
LF	23.4 (0.8)	31.3 (2.2)	25.4 (0.8)	30.6 (1.4)
HF	12.8 (2.0)	8.8 (0.7)	12.3 (0.7)	9.9 (0.6)

Table 5: CN-ratios of the fractions (±SE).

Isotopes There was a general increase of δ^{13}C values with depth at all sites (P<0.05; Figure 18). Across sites, δ^{13}C values of the HFs were significantly higher than the ones of the LFs (P<0.05; oLF<fLF<cHF<fHF). Comparing grassland with forest soils, a significant increase towards higher δ^{13}C values in the mature forest were found (P<0.01; Figure 19). This pattern was especially pronounced in the upper soil parts. Comparing the values of all fractions the values of the mature forest were significantly higher (P<0.05) compared to the ones found for grassland.

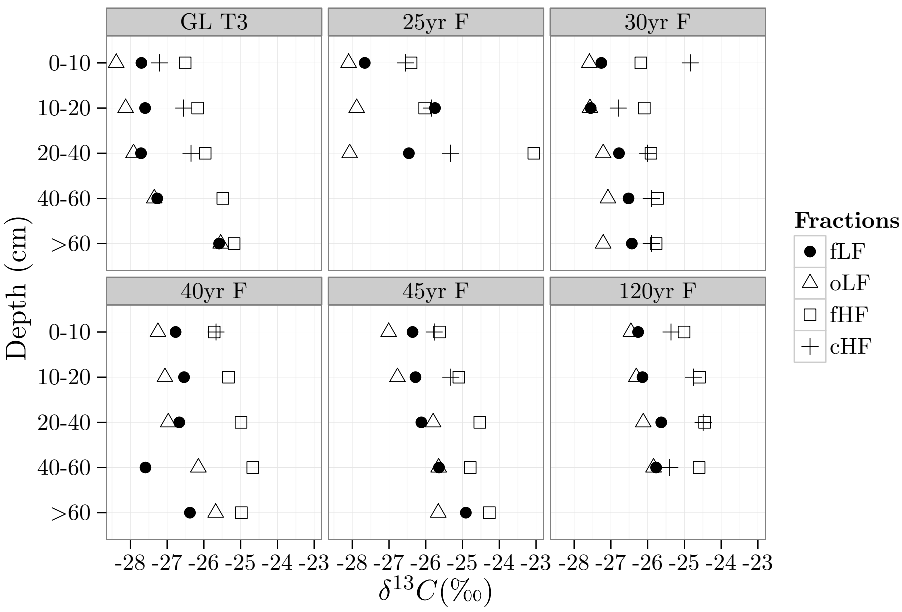

Figure 18: δ^{13}C values of the fractions separated according to depth and land-use. GL=Grassland; T=Transect; yr=years; F=Forest

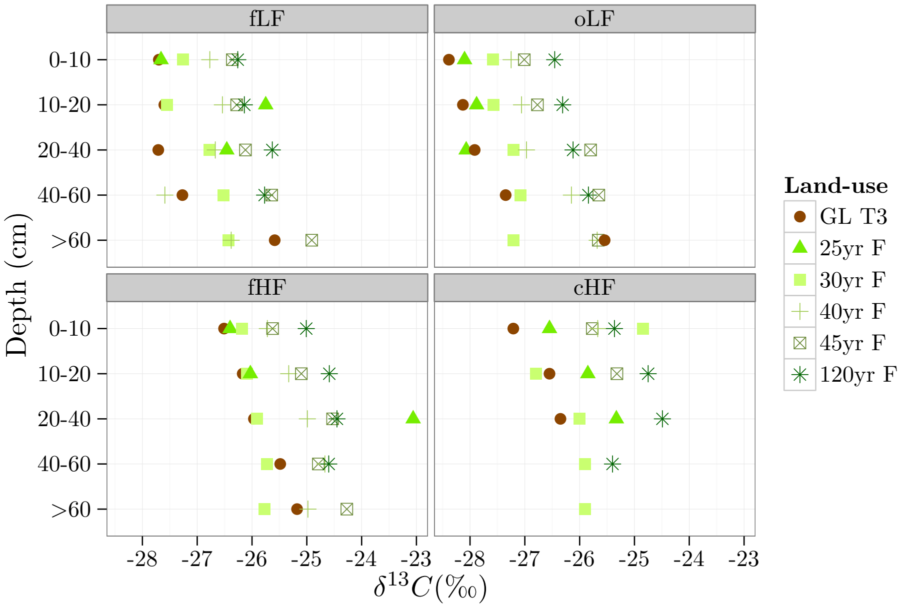

Figure 19: Distribution of the δ^{13}C values of the fractions separated according to depth and land-use. GL=Grassland, T=Transect, yr=years, F=Forest.

The δ^{15}N values are shown in Figure 20. Compared to the LFs, significantly higher values were found for the HFs (P<0.05), whereas the fHF showed significantly higher values than the cHF. Down to a soil depth of about 50 cm, there was an increase of the δ^{15}N values. This increase was followed by a decrease in deeper soil parts. The influence of depth was especially pronounced for the LFs and statistically significant for all fractions (P<0.05). Within the forest δ^{15}N values significantly decreased with forest stand age (P<0.001; Figure 21). Similar to the results found for ^{13}C, this pattern was most strongly pronounced for the upper soil depths. Age and depth influences for all parameters and fractions are summarized in Table 6.

			Forest		
Factor	fLF	oLF	fHF	cHF	Direction
$gCkg^{-1}$	<0.05	<0.05	NS	NS	Age effect: increase
CN-ratio	<0.05	<0.05	NS	NS	Age effect: increase
δ^{13}C	<0.01	<0.01	<0.01	<0.01	Age effect: increase
δ^{15}N	<0.001	<0.001	<0.001	<0.001	Age effect: decrease
			Forest and Grassland		
$gCkg^{-1}$	<0.001	<0.001	<0.001	<0.05	Depth effect: decrease
CN-ratio	<0.001	<0.001	<0.01	<0.01	Depth effect: increase LF, decrease HF
δ^{13}C	<0.05	<0.05	<0.05	<0.05	Depth effect: increase
δ^{15}N	<0.05	<0.05	<0.05	<0.05	Depth effect: increase

Table 6: Depth and age effects, analyzed by fitting linear mixed effect models by maximum likelihood. increase=increasing trend with age or depth; decrease=decreasing trend with age or depth; NS=not significant

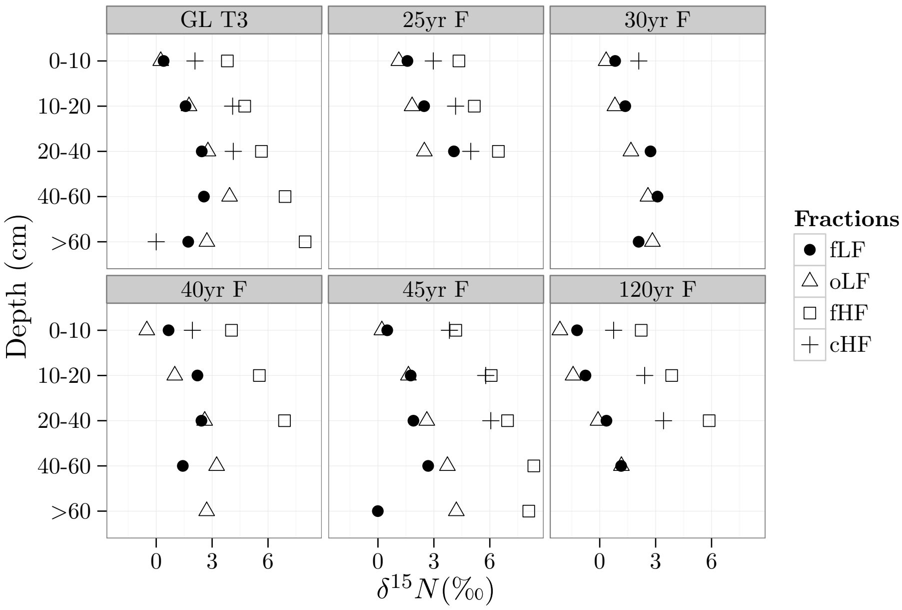

Figure 20: δ^{15}N values of the fractions separated according to depth and land-use. GL=Grassland; T=Transect; yr=years; F=Forest

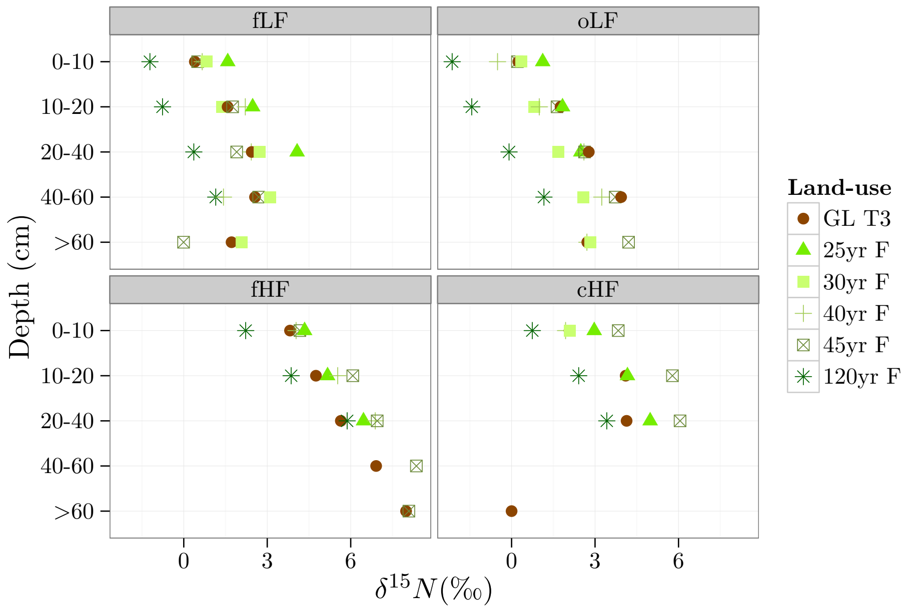

Figure 21: Distribution of the δ^{15}N values of the fractions separated according to depth and land-use. GL=Grassland; T=Transect; yr=years; F=Forest

4.2.2 Relative stability

The calculations of the relative ages (see section 3.4) of soil fractions are summarized in Table 7. The values increased with depth. In the upper 10 cm of the grassland site, the C stored in the HFs was 22 times older than in the LFs. At the forest site, it was 9 times older. At grassland sites, the relative ages of HFs to LFs were generally higher in all soil depths compared to the corresponding forest values.

Depth (cm)	Content ($gCkg^{-1}$)		δ^{15}N (‰)		CN-ratio		Relative age
	LF	HF	LF	HF	LF	HF	LF:HF
Grassland							
0-10	4.9	15.8	0.3	2.9	23.1	12.8	22
10-20	1.0	12.7	1.7	4.4	25.1	10.5	117
20-40	0.8	10.3	2.6	4.9	25.6	10.5	94
Forest							
0-10	13.2	12.1	-1.7	1.5	27.5	13.3	9
10-20	4.3	8.8	-1.0	3.1	29.8	12.0	43
20-40	3.7	7.5	0.1	4.6	35.0	10.6	63

Table 7: Calculation of relative ages based on Conen et al. (2008). Grassland and 120-year old forest.

The most important results regarding density fractions are summarized as follows:

- The mass distribution of the fHF was positively correlated with the clay content.
- Across all soil depths significantly higher amounts of OC were stored in the fHFs followed by the oLF, fLF and cHF.
- Comparing forest to grassland, higher amounts of OC were stored in the LFs and lower amounts in the HFs at the forest sites.
- A significant increase towards higher δ^{13}C values in the mature forest was found.
- Within the forest, δ^{15}N values significantly decreased with forest stand age.
- At the grassland sites, the HFs seem to be generally older in relation to the LFs.

5 Uncertainties

5.1 Methodological uncertainties

The density fractionation was conducted with pre-sieved samples <2 mm. Possible OC amounts stored in the soil fractions >2 mm could not be evaluated. Especially in the LFs, which consist mainly of plant litter, pieces >2 mm can be present. During the whole procedure of soil fractionation, portions of the soil sample may be lost. Especially the wet sieving to fractionate the HF is a source of sample loss. The SPT-solution can react with soluble Ca-ions and form calcium-polytungstate (personal communication by SOMETU (2010), Germany). In some samples clumpy artifacts were found after drying of the HF. These could be due to reactions with Ca-ions (ø=179.64 mmolc kg^{-1}, unpublished data from Hiltbrunner (2012)) and influence the mass-balance. Since this MSc-thesis focusses on the C stored in the fractions and not its mass-balance, these influences are not that relevant. The mass recovery after wet sieving was still >90%. Furthermore, the recovery of OC after fractionation procedure (88.51%, ±1.16) is comparable to other fractionation studies (Griepentrog et al., 2014; Herold et al., 2014; Cerli et al., 2012). The marginal losses could be mainly attributed to compounds dissolving in the SPT solution and material losses during filtering and cleaning procedure (Cerli et al., 2012).

A crucial parameter for density fractionation is the amount of suspension energy for the separation of the oLF. Although dispersion energy was set after a pretest, it could not be tested for each soil sample. As described by Cerli et al. (2012) the adequate dispersion energy can vary among sites and soil depths. Nevertheless, the dispersion energy has to be set to a certain amount to make sure that the procedure is the same for all the samples. Otherwise it is impossible to compare the values.

For each soil depth and fraction, only one sample was analyzed for isotopes and CN content. Further measurements would have been beyond this MSc-project. If more samples could have been included the results would be more founded. For some samples the C and N content of the single fractions was too low to be measured. Therefore, typically for the cHF and for samples of the deeper soil parts, results have been marked as below a certain threshold.

5.2 "Short-comings" of the experimental layout

Possible influences of the slope and East-West gradient on SOC of the grassland sites were previously tested by Hiltbrunner et al. (2013) and were tested on the level of fractions in this work. Neither an impact with altitude nor along the East-West gradient was found. In other studies, significant influences of the slope were found for subalpine grasslands (Hitz et al., 2001;

Leifeld et al., 2005). Although no such influences were found for the grassland soils it cannot be excluded that there is one for the forest. Nevertheless, for the forest sites a possible impact could not be tested because of variations in both altitude and stand ages. The analysis of changes along the forest chronosequence is constrained by a gap between 45 and 120 years old stand ages. Influences of the stand age within this period are interpolated and cannot be confirmed through intermediate stand ages.

Due to poor replicates, results have to be interpreted carefully. Furthermore, many factors like climate, forest type, previous land-use, site management and soil properties can influence C dynamics (Paul et al., 2002). Therefore results can not be transferred to other sites without considering these factors.

6 Discussion

6.1 Plant compartments and organic layer

Amount of roots The distribution of the roots in the upper 10 cm supports the results of Hiltbrunner et al. (2013), who found found about 40% smaller amounts of fine roots of at the forest sites. Since in the study of Hiltbrunner et al. (2013) only fine roots <2 mm were considered, the finer separation in this work showed that mainly the roots <1 mm are responsible for the big differences. These findings support the results of a study by Solly et al. (2013) comparing amounts and mean age of C between fine roots of grassland and forest sites in Germany. They found significantly smaller amounts of fine root biomass in forests but their analysis of root ^{14}C showed older mean ages in forests which indicate slower turn-over rates. ^{14}C measurements were not included in this MSc-project but root biomass can be compared to SOC-stocks. Comparing these parameters (Figure 3 and 7), we see an initial decrease and a re-increase after afforestation. This pattern was mainly pronounced for the roots with a diameter <1 mm, whereas the biomass of the roots between 1-2 mm was almost the same across all sites. Assuming that fine roots are an important factor driving C dynamics after afforestation (Thuille and Schulze, 2006; Paul et al., 2003), the roots <1 mm could play a key role. Following this assumption, the results of this MSc-thesis support the conclusion of Hiltbrunner et al. (2013), that the initial C loss in the topsoil (0-10 cm) is driven by the cessation of the grassland vegetation. The influence of site preparation, which is an important factor influencing C dynamics following afforestation (Paul et al., 2002; Jandl et al., 2007), was not evaluated. Since the spruce saplings were planted manually, it can be assumed that the degree of disturbance was minimal and therefore the influence on C dynamics was only marginal (Hiltbrunner et al., 2013).

Organic layer Beside the roots, C sequestration in forest ecosystems is driven by above ground litter inputs (Hiltbrunner et al., 2013; Poeplau and Don, 2013). The amount of organic layer per m^2 continuously increased with increasing age of the forest (Figure 8). This increase was positively correlated to the increase of OC ($gCkg^{-1}$) stored in the LFs of the topsoil ($P<0.05$; $R^2=0.73$). Comparing the OC stored in LFs of the topsoil (0-10 cm) across the forest sites, the amounts were about 50% higher for the 120-year old forest (Figure 15). Similar results were found by Lajtha et al. (2014) comparing grassland and deciduous forests in Wisconsin. Their analysis of the LFs after 50 years of litter manipulation in a mixed deciduous forest, showed significantly higher OC amounts in the Double Litter plots compared to the Controls. Considering these findings, the significantly higher amounts of OC stored in the topsoil LFs of the mature forest (120 yr) are supposed to be driven by the amount of organic litter input. In addition, a field study of Bird and Torn (2006) with ^{13}C labeled litter in coniferous forests, showed that the incorporation of pine needles into the mineral soil is a very slow process with <1% being incorporated into the mineral soil after 1.5 years. Model calculations showed similar results predicting that less than 3% of the net primary production of a coniferous forest ecosystem being incorporated into the mineral soil within 40 years (Paul et al., 2003). Although there was no labeling involved, the results of this MSc-Project support these findings. The stable distribution of the topsoil HFs along the forest chronosequence is an indication of a slow incorporation rate into the mineral soil.

Isotopes and CN-ratios The isotopic values of the plant compartments corresponds with the values found by Hiltbrunner et al. (2013). Since many fractionation and transformation processes may influence the formation of isotopic values, it is difficult to identify the main process behind it. Therefore the interpretation of isotopic values is associated with a lot of uncertainties. The trends found along the chronosequence of the spruce forest are indications for ^{15}N depletion processes (Hobbie and Högberg, 2012). According to Hobbie and Högberg (2012), a key driver of plant $\delta^{15}N$ besides rooting depth, source differences and fractionation on uptake, is mycorrhizal fungi. N provided to plant hosts by mycorrhizal fungi is usually depleted in ^{15}N and has a lower $\delta^{15}N$ values (Hobbie and Colpaert, 2003; Compton et al., 2007). Hobbie and Colpaert (2003) could show, that this phenomenon leads to lower $\delta^{15}N$ values in plant compartments. This pattern was found along a chronosequence of forest regrowth (Pinus strobus) after agricultural abandonment. Furthermore, Compton et al. (2007) could show, that driven by limited N availability, the mycorrhizal influence on N uptake grows even stronger with increasing stand age. The patterns found in this MSc-project pointed in the same direction (Figure 10). With increasing forest stand age, the $\delta^{15}N$ values decreased. This decrease with stand age could be driven by a decrease of N availability and a stronger fractionation along plant-mycorrhizal uptake.

The CN-ratios of the plant residues are an important indicator for litter quality. The significantly higher ratios of the spruce residues indicate a lower litter quality in the forest which is in line with findings by Hiltbrunner et al. (2013).

6.2 Density fractions

6.2.1 Organic C

General patterns The results of this MSc-thesis are in agreement with previous studies using fractionation as a useful method to separate SOC into fractions with different properties (Herold et al., 2014; Griepentrog et al., 2014; Schrumpf et al., 2013; Poeplau and Don, 2013; Golchin et al., 1994).

The OC recovery after fractionation procedure of 89% (±1.16) is in line with values of Griepentrog et al. (2014) (88%, ±1.6%), Herold et al. (2014) (88%, ±1.1%) and Cerli et al. (2012) (94%).

OC concentrations in soil fractions with the lowest portions in the cHF and the highest in the oLF (cHF<fHF<fLF<oLF) are consistent with Griepentrog et al. (2014). The decline of OC storage with increasing depth corresponds with the patterns for SOC stocks found by Hiltbrunner et al. (2013) and in other density fractionation studies (Cerli et al., 2012; Poeplau and Don, 2013; Schrumpf et al., 2013; Griepentrog et al., 2014) (Figure 23). The depth distribution of OC is supposed to be mainly controlled by the rooting system of a vegetation type (Jobbagy and Jackson, 2000). Beside root distribution, translocation processes of dissolved organic carbon (DOC) contribute to OC storage in the subsoil (Kaiser and Kalbitz, 2012; Kammer et al., 2012; Baisden and Parfitt, 2007). However, disentangling the sources of subsoil SOM was beyond this MSc-project. Mineral surfaces, which are mainly provided by clay minerals, oxides and hydroxides are important for binding and stabilization processes of OC in the soil (Kögel-Knabner et al., 2008). Across all profiles, the highest portion of OC was stored in the fHF. As shown in Figure 12 the depth distribution of the fHF correlates with the clay content of the soil. Considering these findings, the fraction $< 20\mu$m (fHF) plays a key role in the process of OC storage in clay rich soils.

Effect of afforestation Afforestation leads to an initial decrease and a slight re-increase of SOC stocks towards the same amounts stored in the grassland soils. These results correspond with Hiltbrunner et al. (2013) and other chronosequence studies (Poeplau and Don, 2013; Poeplau et al., 2011; Schedlbauer and Kavanagh, 2008; Alberti et al., 2008; Thuille and Schulze, 2006; Conant et al., 2004). On the level of fractions, conversion from grassland to forest lead to an increase of OC storage in the LF and a decrease in the HFs. These results are in agreement with several studies reporting the same patterns (Herold et al., 2014; Poeplau and Don, 2013; Conant et al., 2004). Differences between the two land-use categories were most pronounced for the topsoil (0-10 cm), which is in agreement with Poeplau and Don (2013). Topsoils are most strongly affected because fresh C input and microbial activities are the highest in the upper soil parts (Fontaine et al., 2007). Differences in the subsoil were less pronounced but pointed in the same direction as in the topsoil. After afforestation, the amount of OC in the LFs was stable until a forest stand age of 45 years, whereas the OC of the HF decreased (Figure 23). Taking the 120-year old forest into account, the OC stored in the HF was almost the same as for the 45-year old forest but in the LFs twice as much was stored. This pattern was also reflected by the significant increase with stand age found for the OC stored in LFs of the forest. Comparing the topsoils of grassland and

120-year old forest, in the forest a 56% higher amount of OC ($tCha^{-1}$) is stored in the LFs whereas the OC storage in the HFs is 34% lower (Figure 23). As reported by other studies, the initial OC losses after afforestation are supposed to be driven by the end of the grassland vegetation and by priming of the existing SOC through new root C and DOC (Hiltbrunner et al., 2013; Mobley, 2011; Fontaine et al., 2007). According to the results of this work, the OC stored in the fHF seems to be especially affected by priming. In the topsoil, the OC stored in the fHF was positively correlated with the amount of roots <1 mm ($P<0.05$; $R^2=0.78$). The same correlation was found by Schrumpf et al. (2013). As reported in other studies, roots play a key role as input for OC storage in the soil (Bird and Torn, 2006; Rasse et al., 2005). Based on these findings, the amount of roots could be an explanation for the land-use specific differences of OC stored in the HFs of the soil. According to Conant et al. (2004), the fine and dense grassland roots support the formation of stable aggregates stored in the HFs. Considering these findings, beside a possible priming effect the abrupt decrease of OC stored in the fHF by a stand age of 45 years could be related to the cessation of grassland vegetation (see paragraph 6.1). According to Hiltbrunner et al. (2013), this was observed to be the point where grassland vegetation ends and grass roots as important input for stable aggregates fade away. Since the correlation between roots and topsoil OC storage was only found for the fHF, it can be assumed that the cHF is less affected by changes in the root system. The higher OC portions stored in the LF of the forest topsoils are in agreement with other studies analyzing OC storage in forest soils (Crow et al., 2007; Kaiser et al., 2002; Laganière et al., 2010). These differences according to the LFs are supposed to be driven by the amount and quality of the organic litter input (see paragraph 6.1). According to Poeplau et al. (2011), the accumulation of SOC after afforestation could take more than 100 years. These findings are supported by the results of this work, since the increase of OC stored in the LFs was only pronounced by a forest stand age of 120 years.

Summarizing these results, the LFs are the most vulnerable fractions for afforestation whereas the oLF is most strongly affected. The further separation of the HFs showed that within the HFs the fHF is more affected by afforestation. Within the forest sites, an age effect towards higher amounts could be only found for the OC stored in the LF.

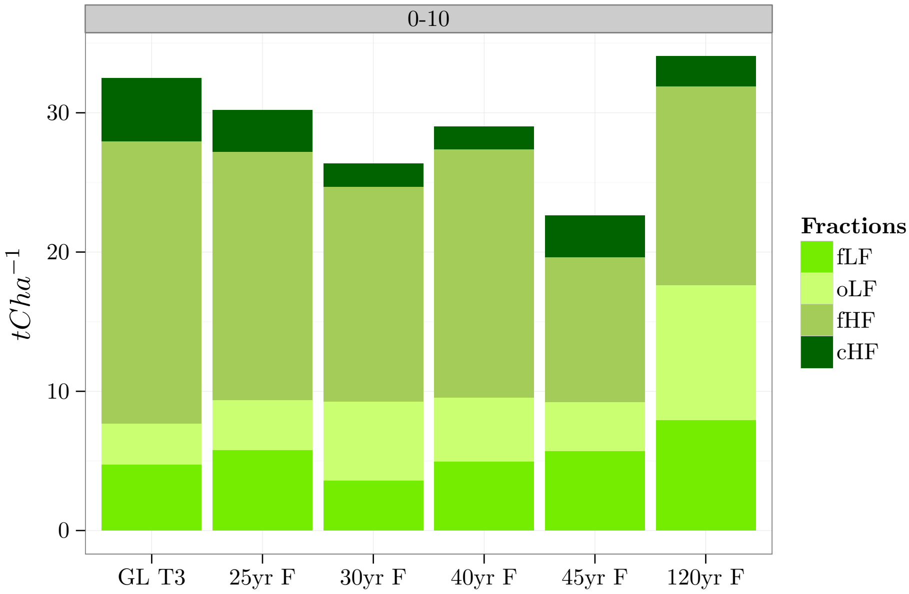

Figure 22: OC content of each site and fraction in the topsoil (0-10cm).

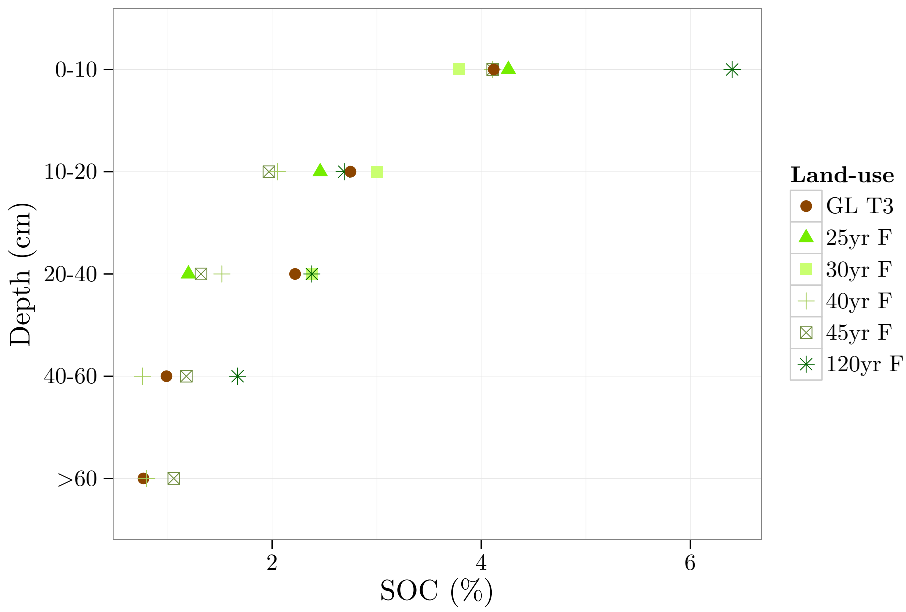

Figure 23: SOC content for all soil depths and sites.

6.2.2 Isotopes and CN ratios

General patterns In general, the results are in line with previous studies analyzing the influence of afforestation on SOC (Hiltbrunner et al., 2013; Poeplau and Don, 2013; Schrumpf et al., 2013; Poeplau et al., 2011; Guo and Gifford, 2002; Paul et al., 2002). Across all sites, the CN-ratios were significantly higher and the δ^{13}C values were significantly lower for the OC stored in the LFs compared to the HFs (Figure 24). Since these parameters can be used to evaluate the decomposition state, these findings indicate that LFs are in a less decomposed state compared with the HFs (Kramer et al., 2003; Poirier et al., 2005; von Lützow et al., 2006; Schrumpf et al., 2013). The results show the following ranking towards advanced decomposition: LFs<cHF<fHF. The same pattern was found by Schrumpf et al. (2013) and Gunina and Kuzyakov (2014). The δ^{15}N and CN-ratios of the fLF and oLFs were rather similar. Therefore no conclusion about their relative decomposition state could be made. The finer separation of the HFs suggested that the OC stored in the fHF is in a more decomposed state when compared to the cHF. In addition the δ^{15}N values of the fractions support these findings. High δ^{15}N values are an even better indicator of advanced decomposition states (Kramer et al., 2003; Compton et al., 2007; Conen et al., 2008; Hobbie and Högberg, 2012) and as illustrated in Figure 20 and 26, trends go in the same direction.

For the HFs, δ^{13}C values increased and CN-ratios decreased with soil depth. The same patterns were found by Schrumpf et al. (2013). In a study of Boström et al. (2007) in which this phenomenon was studied explicitly, this pattern is explained as driven by an increase of microbial processed organic material with soil depth. Drivers of isotopic fractionation processes are difficult to evaluate and therefore isotopic values are difficult to interpret. For example, the lower δ^{13}C values of the fractions in the topsoil, could be also explained by higher amounts of ^{13}C depleted CO_2 in the atmosphere (Boström et al., 2007). For the LFs, δ^{13}C decreased and CN-ratios increased with soil depth. Considering Ugawa et al. (2010), the increase of the CN-ratios in the subsoil LFs could occur due to lower N contents of roots in the deeper soil parts. Schrumpf et al. (2013) could show that the increase of the CN-ratios of the LFs was coupled with slightly smaller $\Delta^{14}C$ values of the subsoil LFs. According to these findings, they suggested less decomposed states and longer mean residence times of the OC stored in the subsoil LFs. Furthermore they suggest a stronger decoupling between the fraction and their source with increasing soil depth. The CN-ratio of the LFs found in this MSc-project support these findings (Figure 17).

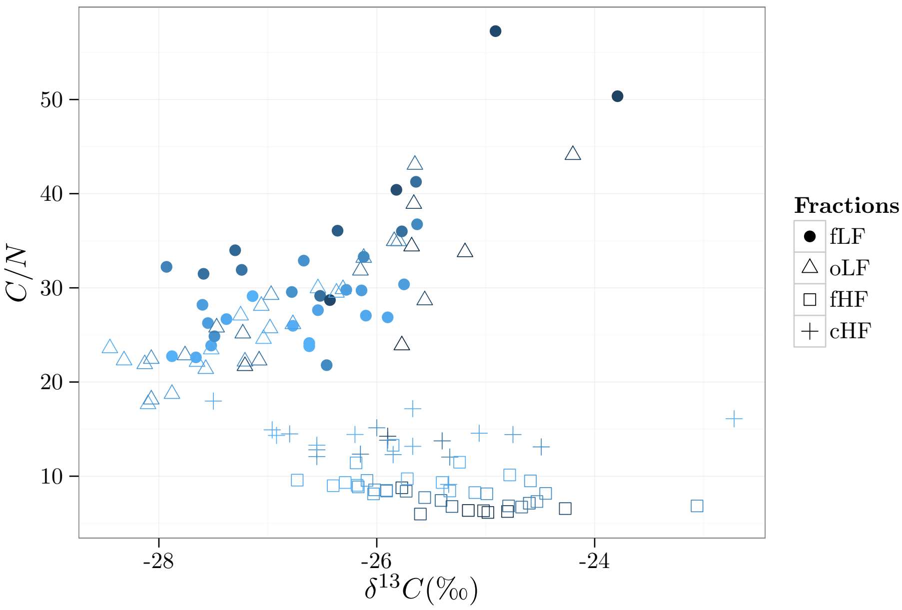

Figure 24: δ^{13}C plotted against CN-ratios of the fractions. Colored according to soil depth. The darker the color, the deeper the soil depth.

Effect of afforestation Comparing the two land-use categories, the significant increase of δ^{13}C values and decrease of δ^{15}N values with stand age suggest a shift towards forest-derived OC. For the HFs the CN-ratios remained stable. This pattern is supported by higher CN-ratios of the LFs, reflecting the decrease of litter quality following afforestation (Hiltbrunner et al., 2013; Schrumpf et al., 2013). In Figure 25, values of the fractions are compared to the δ^{13}C values of spruce and grass roots. Assuming that roots play a key role for OC storage in the soil (Bird and Torn, 2006; Rasse et al., 2005), the shift towards forest-derived OC was clearly shown for the LFs. The HFs showed the same pattern but their δ^{13}C are generally higher than the ones of the roots. As pointed out by Hiltbrunner et al. (2013), this stronger decoupling could be attributed to more advanced decomposition states and changes of the decomposition process. According to Chen et al. (2005), there is a stronger discrimination during the decomposition process in forests which results in higher δ^{13}C. This might also be an explanation for the significant increase of the δ^{13}C values with stand age. These results support the findings of Hiltbrunner et al. (2013) who found the same patterns for the isotopic values of the TOC and concluded that afforestation leads to changes of SOM composition. In general, the δ^{15}N values of the fLF continuously decreased along the forest chronosequence, indicating a coupling with the decrease found for δ^{15}N values of the plant compartments (Figure 26).

Since fLF representing unaggregated organic material, these results can be compared to Compton et al. (2007) who found the same patters comparing δ^{15}N values between the organic layer and trees. The δ^{15}N of the mineral associated HFs generally increased until a forest stand age of 45 years and decreased by a stand age of 120 years (Figure 26). These results support the patters found by Compton et al. (2007), analyzing the development of δ^{15}N values along a century of forest regrowth after agricultural abandonment. They found an increasing decoupling of δ^{15}N values in soils and plants after afforestation. The results of this MSc-thesis showed the same for the HFs until a forest stand age of 45 years. Taking the δ^{15}N values of mature forest (120 yr) into account, the decoupling trend ends. Long-term observations of δ^{15}N showed that plant and soil δ^{15}N are strongly coupled considering time spans over thousands of years (Högberg, 1997; Compton et al., 2007). Since the mature forest involved in this study represents a permanent ecosystem, the decreasing values of the HF could support these observations.

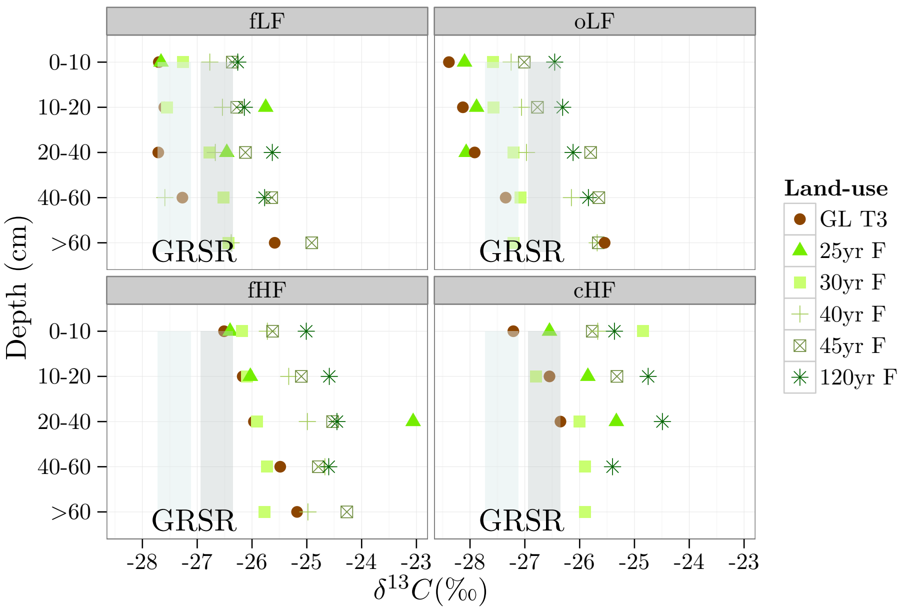

Figure 25: δ^{13}C values of the fractions separated according to depth and land-use. GL=Grassland, T=Transect, yr=years, F=Forest. Grey bars represent the 50%-quantiles of the δ^{13}C values from the spruce roots (SR) and the grass roots (GR).

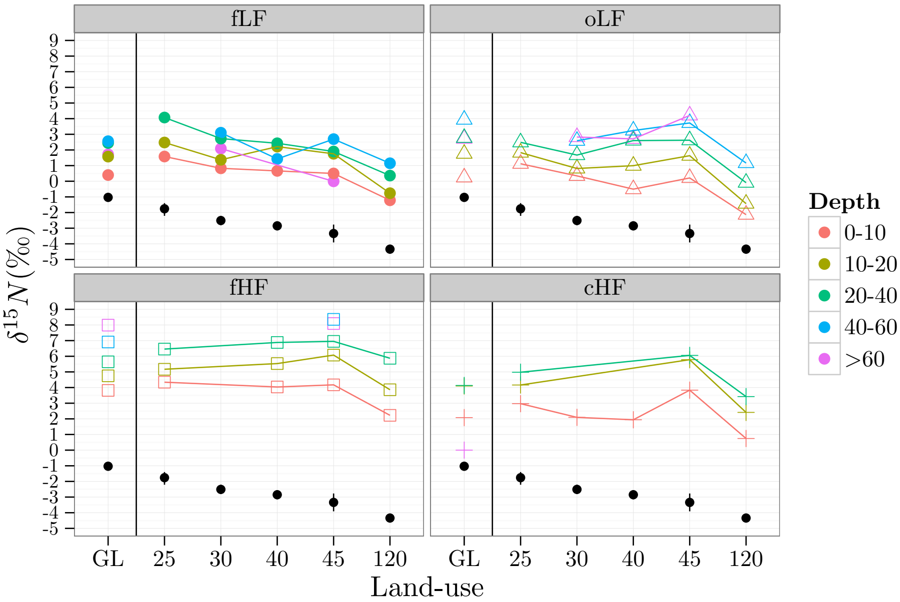

Figure 26: δ^{15}N values of the fractions separated according to depth and land-use. GL=Grassland, numbers=forest stand age. Black point-ranges represent the mean δ^{15}N values (±SE) of the plant compartments. For grassland sites foliage and roots (0-10 cm) are included. For the forest sites needles, branches (with bark) and roots (0-10 cm) are included.

Relative stability The calculated values of the relative stability of the fractions correspond with the findings of Conen et al. (2008). In Figure 27, δ^{15}N values are plotted against the CN-ratios of the soil fractions. Under consideration of the assumption, that low CN-ratios combined with high δ^{15}N values indicate a high age and stability of the OC (Conen et al. (2008); see Figure 6), the oldest OC is stored in the fHF followed by the cHF and the LFs (Figure 27). With increasing depth older relative ages were calculated (Table 7). These results are supported by other studies where $\Delta^{14}C$ values were measured (Schrumpf et al., 2013; Kögel-Knabner et al., 2008). The applied method by Conen et al. (2008) was only tested to calculate the relative age of the HF and not for further separation into fHF and cHF like it was made in this work. Therefore, calculations were only made for the combined HFs. Nevertheless, the generally higher δ^{15}N values and lower CN-ratios of the fHF compared to the cHF (Figure 27), confirms that it would be relevant to calculate the relative age for the fractions separately. It would be especially interesting for the fHF since it turned out to be a very important fraction regarding SOC storage. Comparing the relative ages between grassland and the 120-year old forest, HFs were on average 78 times older than the LFs at the grassland site, whereas in the forest, HFs were only 38 times older (Table 7). At the forest site, the age of the fractions were narrower, which may be connected to C turn-over rates. Hiltbrunner et al. (2013) measured a 50% lower soil respiration rate in the forest for the same site. This results were mainly explained by colder and dryer soil conditions, reduced root respiration and lower litter quality. Soil respiration can be used as an indicator for biological activity and C turn-over rates (Scheffer and Schachtschabel, 2010; Hiltbrunner et al., 2013). In a recent study of Herold et al. (2014), turn-over times on the level of fractions were evaluated using $\Delta^{14}C$ measurements. They found significant differences between the $\Delta^{14}C$ of the LFs but not for the HFs, indicating faster turn-over rates for the LFs at the grassland sites. Combining these findings with the results of this work, the lower relative ages of the forest fractions could be driven by the slower turn-over rates within the forest. This suggests that the LFs in the forest are older and more persistent than the ones at the grassland sites and have therefore narrower ages. To confirm theses results and to compare absolute ages between the two land-use types, $\Delta^{14}C$ measurements are needed. In a study of Mills et al. (2014) who compared ^{14}C data of non-forested and forested soils on a global scale at 114 sites, younger SOM was found in the forest soils.

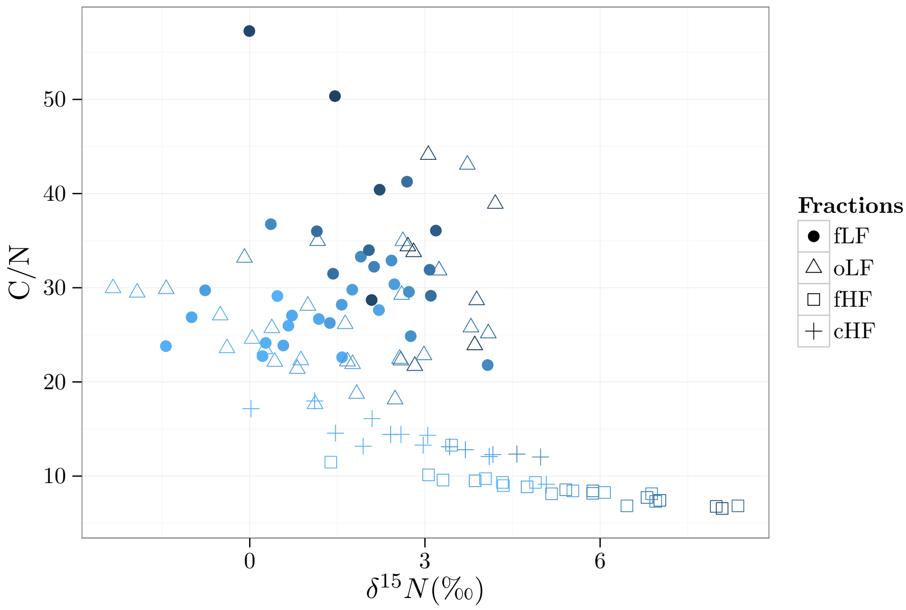

Figure 27: δ^{15}N plotted against CN-ratios of the fractions. Colored according to soil depth. The darker the color, the deeper the soil depth.

7 Conclusion

The density fractionation of the samples from the PhD-project of David Hiltbrunner (Hiltbrunner, 2012) provided a deeper insight into C dynamics following afforestation. Coming back to the initial hypotheses, the herein presented results show that SOC can be separated into fractions with different isotopic signatures, OC and N content, independent of the land-use category. The oLF had the highest OC concentrations, whereas the highest portion of total SOC was stored in the fHF. Across sites, SOC storage declines with depth. Interpreting the isotopic signatures and CN-ratios, LFs were in a less decomposed state than the HFs. Within the HFs, the fHF was in a more advanced decomposition state. The decomposition state of the HFs was supposed to be more advanced in deeper soil parts. The increasing CN-ratios of the LFs with soil depth were difficult to interpret. Possible explanations could be the less decomposed states and lower N content of deep soil roots. The HFs were relatively older than the LFs and therefore more stable. Within the HFs, the OC stored in the fHF was supposed to be more stable. Caused by the high OC storage and stability, the fHF is a very important fraction for C dynamics. Since the distribution of the fHF is linked to the clay content of the soil, this fraction especially plays a key role in clay rich soils. Evaluating the effect of afforestation and coming back to the initial hypotheses (see section 2), the following key conclusions can be drawn:

- Afforestation only moderately affects total SOC stocks but leads to an increase of OC stored in the LFs and a decrease in the HFs. Differences are mostly pronounced for the topsoil but trends in the subsoil point in the same direction. With increasing forest stand age, increasing amounts of OC are stored in the LFs, whereas the HFs remain more stable after an initial decrease. The age-related increase of OC stored in the LFs in the topsoil could be attributed to the increase of the organic layer and the slow incorporation into the mineral soil. The decrease of the OC stored in the HFs by a forest stand age of 45 years is supposed to be driven by the cessation of the grass vegetation and a possible priming effect due to new root C and DOC. Due to the higher portion of the OC stored in the LF, the SOC stored in the forest is supposed to be less stable but the stability of the LFs has to be interpreted in a land-use specific context. Changes in microclimate and lower litter quality leads to slower turn-over rates in the forest which could enhance the stability of the LFs.

- The shift from grass-derived OC to forest-derived OC is pronounced for all fractions. The isotopic signature and CN-ratios of the fractions are not only influenced by different input sources but also by decomposition processes. The higher $\delta^{13}C$ values with depth and along the forest chronosequence reflects SOM turn-over and decomposition. The decrease of SOM quality with increasing forest stand age is reflected in the LFs but not in the HFs.

8 Outlook

The source of OC stored in the fractions still remains unclear. Since roots play a key role for OC storage in the soil (Bird and Torn, 2006; Rasse et al., 2005), it would make sense to include roots distribution in the subsoil in LUC studies. To evaluate the role of roots for OC storage in the fractions, it would be interesting to develop a method which makes it possible to distinguish between root-derived and shoot-derived SOC. According to Mendez-Millan et al. (2010), the detection with cutin and suberin can be used. Cutin is a waxy polymer, which is characteristic for shoots whereas suberin occurs in roots. Including such measurements should make it possible to better evaluate the role of roots according to OC storage in the soil.

To get a better estimate of the turn-over times and stability of the fractions, labeling technics and $\Delta^{14}C$ measurements are the most powerful approaches. To use such methods would improve the profoundness of the results. Processes influencing isotopic values in the soil are still difficult to evaluate. Further research within these fields would help to interpret δ^{13}C and δ^{15}N values. So far the influence of mycorrhizal fungi on fractionation processes remains rather unclear.

Since by now the chronosequence has a gab between 45 and 120-year old forest stand ages, it would make sense to evaluate intermediate dynamics trough repeated soil sampling and C/N measurements at regular intervals. Such a monitoring could also be beneficial as a means to analyze the influence of climate change.

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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.