KU LEUVEN

FACULTEIT BIO-INGENIEURSWETENSCHAPPEN

As above, so below? Influence of overstory-induced acidification on the soil carbon sequestration dynamics in the Gaume forest.

De donkere kant van het bos: hoe beïnvloedt een omvorming naar Fijnspar de koolstofvastlegging in het ondergrondse bosecosysteem van de Gaume?

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In some mysterious way woods have never seemed to me to be static things. In physical terms, I move through them; yet in metaphysical ones, they seem to move through me.

– John Fowles

KEYWORDS

SOM; SOC; carbon sequestration; forest conversion; tree species effect; soil process domains

LIST OF ABBREVIATIONS

C – Carbon

N – Nitrogen

S – Sulphur

Al – Aluminium

Fe - Iron

SOM – Soil Organic Matter

SOC – Soil Organic Carbon

MRT – Mean residence time

ASS – Alternative Stable States

DOC – Dissolved Organic Carbon

S+A – Sand and Stable Aggregates

s+c – Silt and Clay fraction

POM – Particulate organic matter

PCA – Principal component analysis

FAMD – Factor analysis on mixed data

CEC – Cation exchange capacity

BS – Base saturation

C/N – Carbon/nitrogen ratio

LAI – Leaf area index

WRB – World reference base (international soil classification system of the FAO)

SUMMARY

Soils are by far the largest terrestrial carbon reservoir worldwide, and 70% of this carbon is stored under forest. Therefore carbon sequestration in forest soils came on the political agenda as a strategy to mitigate climate change. In this context, a better understanding of soil carbon sequestration and stability in function of forest management options is needed. One of the management options that has an impact on soil carbon is the conversion of broadleaved forests to conifer monocultures for wood production purposes. It is known that these coniferous monocultures may accelerate soil acidification, but their effects on quantity and quality of organic matter are not well understood. The aim of this study was to evaluate the effect of a conversion from ancient woodland to coniferous plantations on the soil carbon dynamics. This study was done by means of a unique twin-plot research set-up in the Gaume forest (southern Belgium). Soil samples were taken from adjacent deciduous and spruce plots and analysed for pH, cation exchange capacity, base saturation and carbon content. A carbon fractionation was performed, which resulted in four separate soil organic carbon fractions. The distribution of carbon over stable and labile pools gives an indication of the carbon stability. A multifactor analysis was performed to evaluate the variation within the natural and conversed study site. Geology is the main determining factor , followed by pH, which strongly reflects the overstory induced soil acidification. This study found evidence that for a specific set of abiotic variables (geology and climate), two alternative stable states in the belowground ecosystem exist. Because pH strongly correlates with overstory tree species, it is not included in this set of abiotic variables. In the two-basin conceptual model, spruce conversions act as a perturbation that induces a shift from the base-dominated soil process domain to the iron-aluminium-dominated soil process domain. The collapse in variability of abiotic and biotic soil properties corresponds with the narrowed biodiversity under spruce ecosystems. Finally this study indicates that although spruce conversion increases the total carbon stocks for topsoils, this extra carbon is stored in more labile carbon pools. Hence, the topsoil carbon stability decreases. This suggest that by spruce conversion the carbon sink becomes more vulnerable and under extreme conditions may even turn into a source of atmospheric carbon.

SAMENVATTING

Bosbodems hebben het potentieel om te fungeren als een natuurlijke stock voor atmosferische koolstof, meer bepaald CO_2. Bijgevolg kwam koolstofopslag in bosbodems op de politieke agenda als mogelijke strategie voor mitigatie van de klimaatopwarming. Een beter begrip van koolstofopslag en stabiliteit in functie van bosbeheer is hiervoor essentieel. Recentelijk werd vaak gekozen voor een conversie van oud bos naar monoculturen van coniferen voor houtproductiedoeleinden. Onderzoek heeft aangetoond dat dergelijke monoculturen bodemverzuring versnellen terwijl hun effect op de kwantiteit en kwaliteit van organische stof in de bodem nog ongekend is. In dit onderzoek wordt dan ook gepoogd om het effect van een conversie van oud bos naar monoculturen van Fijnspar (*Picea abies*) te evalueren. Deze studie werd uitgevoerd met behulp van een unieke "twin-plot" set-up in de Gaume-streek (zuid België). Uit aangrenzende loof- en sparplots werden bodemstalen genomen die vervolgens werden geanalyseerd op pH, kation uitwisselingscapaciteit, basenverzadiging en koofstofgehalte. De stalen werden met behulp van koolstoffractionatie gesplitst over twee labiele en twee stabiele koolstoffracties. Aan de hand van multi-factoranalyse werd de natuurlijke variatie van de site onderzocht. De meeste variatie kan verklaard worden door geologie en pH, waarvan de laatste sterk de conversie geïnduceerde verzuring weergeeft. In dit onderzoek zijn aanwijzingen gevonden dat twee alternatieve stabiele toestanden in het ondergrondse ecosysteem bestaan voor een specifieke set van abiotische variabelen (geologie en klimaat). De variabele pH is niet opgenomen in deze set van abiotische variabelen omdat de correlatie met boomsoort te groot is. In dit conceptueel model fungeert de conversie naar Fijnspar als perturbatie die een verschuiving veroorzaakt van het basen-gedomineerde bodem domein en het ijzer-aluminium-gedomineerde bodem domein. De terugval in variabiliteit van abiotische en biotische bodemeigenschappen veroorzaakt door zulke conversie, komt overeen met de vernauwde biodiversiteit bij monoculturen van Fijnspar. Tenslotte toont deze studie aan dat alhoewel een conversie naar Fijnspar de totale koolstof stocks verhoogt, de extra koolstof wordt opgeslagen in meer labiele pools. Vandaar dat we kunnen besluiten dat de koolstof-stabiliteit in de toplaag afneemt. Dit suggereert dat door de conversie naar Fijnspar koolstof in de toplaag kwetsbaarder wordt voor veranderingen van klimaat, en dat onder extreme omstandigheden bosbodems zelfs een emissiebron van CO_2 kunnen worden.

CONTENT

1 INTRODUCTION

1.1 PROBLEM STATEMENT

Climate change mitigation is one of the toughest challenges for mankind in the coming years. Forests can play here an important role, since soil is by far the largest terrestrial carbon reservoir and 70% of this soil carbon is stored under forests (Dixon et al., 1994; Jandl et al., 2007). Hence, the large carbon storage capacity of forests can be addressed to mitigate increasing atmospheric CO_2 emissions (De Deyn et al., 2008). It is thus evident that carbon sequestration in forest ecosystems recently gained considerable attention in international literature (Lorenz and Lal, 2010).

Forest products have been used as resource for millenia and are still gaining interest (Mantau et al., 2010; Vandekerkhove et al., 2014). However, as the area used for wood production is limited, production needs to be optimized without disturbing other ecosystem services. To satisfy the need for these highly demanded wood products, fast-growing coniferous plantations have been favoured for many years (Verstraeten, 2013). Since the 19th century large patches of Europe's natural deciduous forests were replaced by coniferous plantations (Verstraeten, 2013). In Wallonia e.g., spruce now covers 37% of the total forested area (Verstraeten, 2013) and hence far exceeds its natural range (Klimo et al., 2000).

It has been reported that spruce conversions have adverse effects on forest soils and may accelerate the natural process of forest soil acidification (de Vries and Breeuwsma, 1985; Ulrich and Sumner, 1991; Augusto et al., 2002; de Schrijver et al.,2012). This soil acidification has caused a significant loss of above-ground biodiversity (Barbier et al., 2008). The long life cycles of forests make them far more susceptible to anomalies such as ensuing acidifying litter input, acidifying depositions (de Vries et al., 2003) or long periods of drought (IPCC, 2007). Under extreme conditions soils can even alter from sink to source of atmospheric carbon (Davidson and Janssens, 2006). More understanding to remedy the effects of spruce conversions is therefore necessary.

The effects caused by changes in forest management, such as forest type, on the global carbon cycle are not fully understood (Naudts et al., 2016). A better understanding of soil organic matter (SOM)-cycles, SOM-stability, as well as the link between SOM-stability and overstory tree species is needed. It will allow to take more effective management decisions, ensure sustainable wood-production and to develop better climate mitigation strategies in the future.

This thesis is part of a larger project that aims to clarify the effect of conversion induced acidification on the ecosystem services of biomass production and climate mitigation. It harmonizes with the EU Biodiversity Strategy to 2020, which requires member states to map and assess the state of ecosystems in general and in forests in particular.

1.2 STATE OF THE ART

This chapter begins with section 1.2.1 on forest conversions. The causes of soil acidification, its process and consequences are explained in section 1.2.2. The chapter continues with section 1.2.3 and 1.2.4 which describe the thresholds and alternative stable states in soil acidity. Finally this chapter is concluded with section 1.2.5 which discusses the state of the art on soil organic matter and carbon stability.

1.2.1 FOREST CONVERSIONS

Tree species composition of many forests in Western and Central Europe has changed since the 19th century (Verstraeten, 2013; Klimo et al., 2000; Spiecker et al., 2004). To satisfy the demand for wood products, deciduous tree species in traditional systems were most often replaced by Norway spruce (*Picea abies*) or Scots pine (*Pinus sylvestris*). Nowadays these species far exceed the extent that they would naturally cover (Klimo et al., 2000), as can be seen in figure 1. Norway spruce was favoured because of its good wood quality and the high production potential, even on poor and sandy soils (Verstraeten, 2013). In south Belgium afforestation of mires, bogs, heathlands and poor grasslands, with spruce was a common phenomenon after 1850. In the 1980s the species reached a maximum cover of 200.000 ha corresponding with 37% of the total forested area in Wallonia (Verstraeten, 2013).

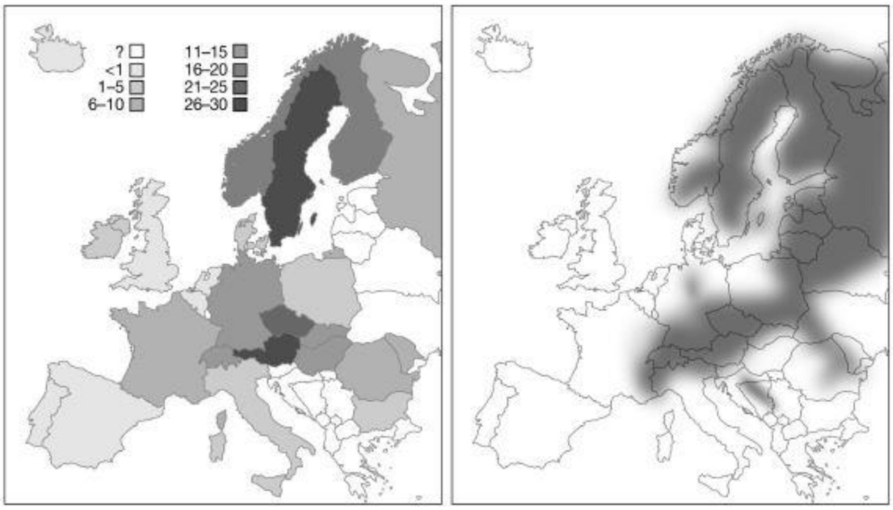


Figure 1: Actual distribution (left, percentage of forest area covered by spruce) and natural range (right) of Norway spruce in Europe (figure from Klimo et al., 2000).

Conversion of deciduous forests to spruce plantations did increase the wood production (Verstraeten, 2013), but also had and still has severe effects on many components of the forest ecosystem (Van Calster et al., 2007). Concerns about soil quality (Augusto et al., 2002; Spiecker, 2003) and biodiversity under these coniferous plantations lead to new insights about planting strategies with native species (Spiecker et al., 2004). Nowadays there is an increasing emphasis on other ecosystem services than wood production and a rising interest in strategies concerning climate change mitigation (Lindner et al., 2010; Vesterdal et al., 2013). Hence information of tree species effects on different ecosystem services is of importance, and acidification by these coniferous tree species is one of the most relevant parameters here.

1.2.2 SOIL ACIDIFICATION

Soil acidification is the accumulation of hydrogen ions in the soil. Hence soil acidity can be expressed as the change in acid neutralizing capacity of the soil system (Ulrich and Sumner, 1991). The most convenient indicator for soil acidity is proton activity, or pH value, of the soil solution (Ulrich and Sumner, 1991). The pH response to increasing input of hydrogen ions is not a linear curve as pH is buffered by several mechanisms. Soil buffering capacity can be described as the resistance of the soil against an effective decrease in pH of the soil solution (de Schrijver et al., 2010). Because of this buffering, new input of H^+ does not immediately result in a lower pH, as can be seen in figure 2 (Bowman et al., 2008).

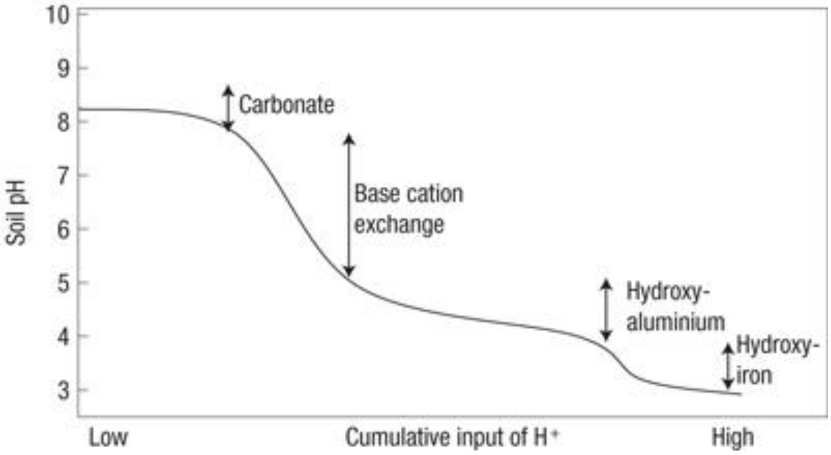


Figure 2: Hypothetical changes in soil buffering systems associated with increasing inputs of protons and associated changes in soil pH (figure from Bowman et al., 2008)

Acidification by natural weathering

There are generally more acidic gases and particles in the atmosphere than basic ones (Galloway, 2001). Accordingly, wet deposition in terrestrial and marine regions away from human influence is already acidic (Galloway et al., 1982). Rainwater is slightly acid due to the dissolved CO_2, which leads to the chemical erosion of inorganic materials (e.g., rocks). On a geological time scale, weak acids such as carbonic acid (dissolved in rainwater) lead to the dissolution of Na, Ca and Mg from soil minerals followed by the leaching of these cations (Ulrich, 1989) and subsequently a decrease in acid neutralizing capacity. The depletion of bases through leaching will induce soils to cascade down the curve of soil pH shown above (figure 2). Organic matter is another potential source of acidity so that most natural soils are acidic due to both organic matter production (with organic acids as itermediate product) and decomposition by soil fauna(with CO_2 as byproduct) (Galloway, 2001; Osman, 2013).

Acidification by tree species

Tree species are known to affect soil chemistry and humus type (Muys and Lust, 1992; Prescott, 2002; Aubert et al., 2004; Reich et al., 2005) as well as nutrient availability (Prescott, 2002; de Schrijver et al., 2007; Hansen et al., 2009), nutrient cycling and physical properties (Binkley and Giardina, 1998; Augusto et al., 2002; Aubert et al., 2010). Tree species also affect the herb layer (Sydes and Grime, 1981; Van Oijen et al., 2005). This herb layer houses a significant biodiversity and ensures a well-functioning forest ecosystem(Verstraeten, 2013). The choice of overstory tree species composition is therefore an important soil forming factor (Jenny, 1994; Hobbie, 1992) which can accelerate acidification. All above described soil properties are influenced by the three major determinants in tree species: (1) the quality and quantity of the litter, (2) canopy interception and filtering capacity and (3) root growth and nitrogen fixation (Verstraeten, 2013).

First determinants are the leaf litter quantity and quality as they affect soil pH and exchangeable cation concentrations (de Schrijver et al., 2012). Litter quantity is mainly determined by forest management, although small variations can be observed between tree species. On the other hand, litter quality differs mostly for tree species rather than management decisions. The quality determines the decomposition rates: low quality litter corresponds with low decomposition rates. Differences in these rates are a consequence of differences in physico-chemical characteristics of the leaves (Berg, 1986; Augusto et al., 2002; Reich et al., 2005; Hobbie et al., 2006; Vesterdal et al., 2008; Vivanco and Austin, 2008). Most important characteristics of the leaves are (1) C/N ratio, (2) lignin concentration, (3) lignin/N ratio and (4) Ca concentration (Verstraeten, 2013). For example, low quality litter is characterized by low Ca concentration with low earthworm activity (Reich et al., 2005; de Schrijver et al., 2012) and high C/N and lignin/N ratios (Melillo et al., 1982; Verstraeten, 2013). As a consequence of these slow decomposition rates, litter accumulates and a thick forest floor is built-up. In this situation the production of organic acids as intermediate decomposition products may enhance soil acidification (de Vries and Breeuwsma, 1985). Hence, coniferous trees have a higher acidifying effect due to their low litter quality (Hobbie et al., 2006).

Secondly, tree species vary in filtering capacity of acidifying atmospheric depositions. Coniferous tree species generally have higher filtering capacities (Nordström and Rundgren, 1974; Ulrich, 1983; Augusto et al., 2002; Rothe et al., 2002; de Schrijver et al., 2007). In other words they accumulate atmospheric depositions faster and thus are characterized by higher soil acidification rates. This is mainly due to the evergreen property and higher leaf area index (LAI) of coniferous trees. Light transmission correspondingly varies with tree species but is first and foremost influenced by forest management (Verstraeten, 2013).

The last driver of the tree species effect is root growth and nitrogen fixation. Nutrient cycling, soil processes and physical soil properties are influenced by root uptake rates, N_2 fixation and mineral weathering through root exudates. Also N_2 fixation can enforce acidification (Van Miegroet and Cole, 1984; de Schrijver et al., 2012). Research on species-specific effects of roots on the forest ecosystem is still lacking (Verstraeten, 2013).

Conversions from deciduous forest to coniferous plantations thus accelerate the process of forest soil acidification via inputs of lower quality litter and by increasing the effect of anthropogenic depositions via higher filtering capacities.

Acidification by anthropogenic depositions

Besides coniferous monocultures also atmospheric depositions are an anthropogenic driver that accelerate the natural process of forest soil acidification. Emissions of sulphur (S) and Nitrogen (N) in Europe increased dramatically since the late 19th century until its peak in the mid-1980s (Schöpp et al. 2003; de Vries et al., 2014).

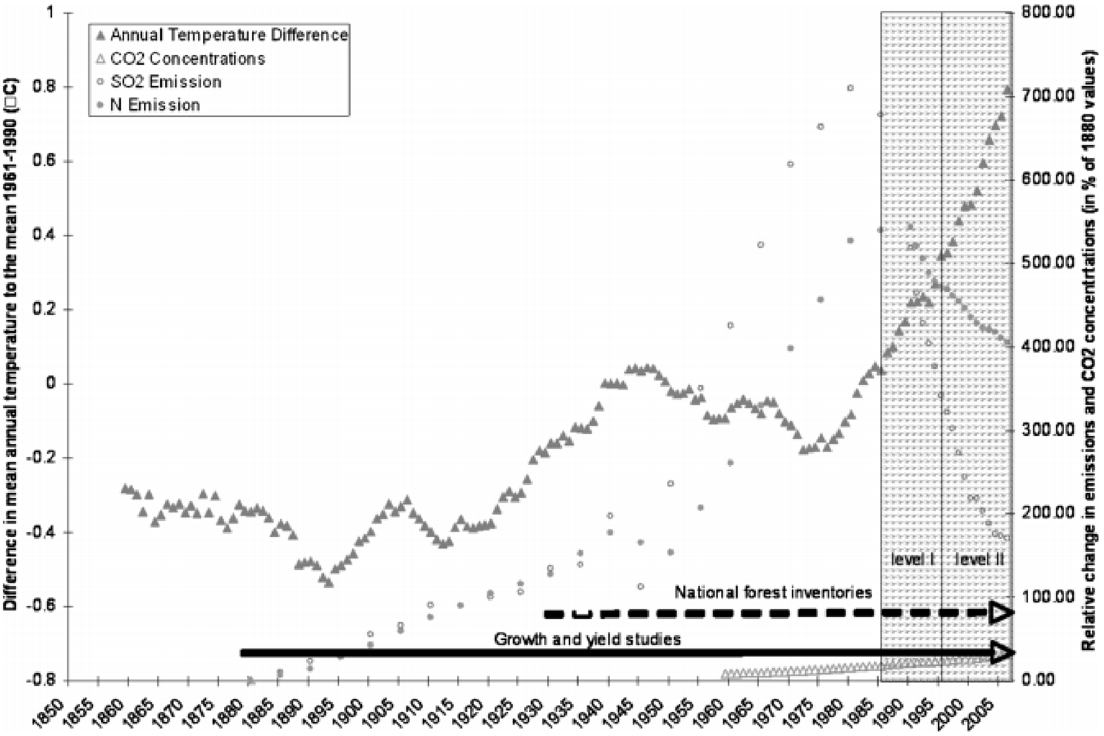


Figure 3: Relative changes in: (i) SO_2 and N emissions in Europe between 1880 and 2007 (data until 1990 based on Schöpp et al. (2003) and EEA data from 1990-2007), (ii) CO2 concentrations since 1960 in comparison to estimated values from 1880 base 292 ppm and (iii) mean annual temperature deviation with respect to the 1961-1990 reference period for the Northern hemisphere averaged using a 10-year moving window. (figure from de Vries et al., 2014)

As sulphur emissions and nitrogen emissions in the 1980s were respectively a factor seven and a factor five higher compared to pre-industrial times (see figure 3), it is clear why more attention to their acidifying effect was given (de Vries et al., 2014). Sulphur was the major contributor to acidification of Europe's terrestrial ecosystems, hence the first steps in emission control were focused on S emissions (Johnson and Reuss, 1984; Fowler et al., 2007). As a consequence of this rising awareness, land based sulphur emissions declined with more than 70% since the end of the 1970s (Fowler et al., 2007). Over the last two decades, emissions of both sulphur and oxidized nitrogen compounds have reduced in Europe (Fowler et al., 2007; de Vries et al., 2014). However, CO_2

concentrations and temperature are still rising (de Vries et al., 2014), as can be seen in figure 3.

The impact of acid depositions on forests evolutions is a complex transient process. As soils respond rather slow and forests themselves have long life cycles, the effects of acid depositions may only manifest after years to decades (Galloway, 2001). Time-scales of forest recovery from acid depositions are even longer, as can be seen in figure 4. Nowadays research concerning acid depositions has shifted focus to long-term resilience of forest ecosystems. Extra knowledge is needed on how forest ecosystems respond to the combination of air pollution and climate change (de Vries et al., 2014). Additionally, insights on how these impacts can be counteracted by forest management are welcome(de Vries et al., 2014).

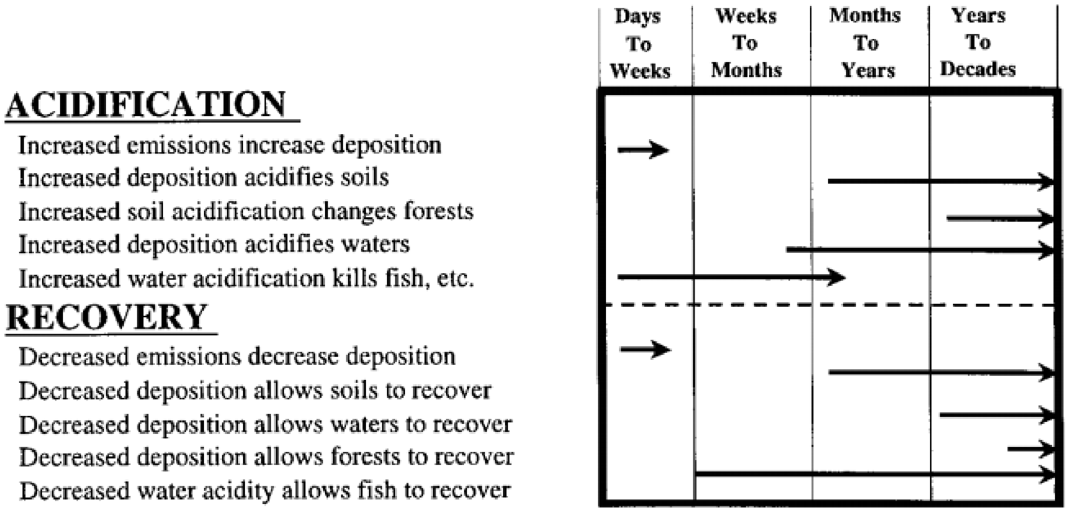


Figure 4: Timescales of acidification and recovery. (figure from de Vries et al., 2014)

Consequences of acidification

Soil acidification in forests has four major consequences. Besides the direct effect of (1) aluminium toxicity and depletion of the base cations, soil acidification has also indirect consequences for: (2) the soil organisms, (3) the forest floor and (4) soil carbon.

Base cations, K^+, Ca^{2+}, Mg^{2+}, are partly replaced on the cation exchange complex (CEC) by H^+, Al^{3+} and Fe^{3+} as a direct consequence of the acidification (Verstraeten, 2013; de Vries and Breeuwsma, 1985). By releasing these base cations the soil neutralizes the increased input of acidity. Free base cations in the soil solution then leach out during rainfall events. Base saturation is a measure for the amount of exchangeable cations present in the soil, in other words the amount of K^+, Ca^{2+}, Mg^{2+} occupying the cation exchange capacity (CEC) (Osman, 2013). The capacity of the cation exchange complex is determined by the amount of clay and organic matter in the soil (de Schrijver et al., 2010), see figure 5. The higher the amount of exchangeable base cations in the soil, the more acidity can be neutralised. As a consequence, soils with a high CEC (and high base saturation) will take longer to acidify (Kumar and Shivay, 2008). However, when the same soil with a high CEC is acidified (and thus base saturation has become very low) it will also take longer to recover from this status (Kumar and Shivay, 2008). After depletion of the base cations, aluminium that potentially toxic is released from the soil (Bowman et al., 2008). The mobilization of Al and other potentially toxic elements as a result of soil acidification directly induces stress to the plant (Osman, 2013).

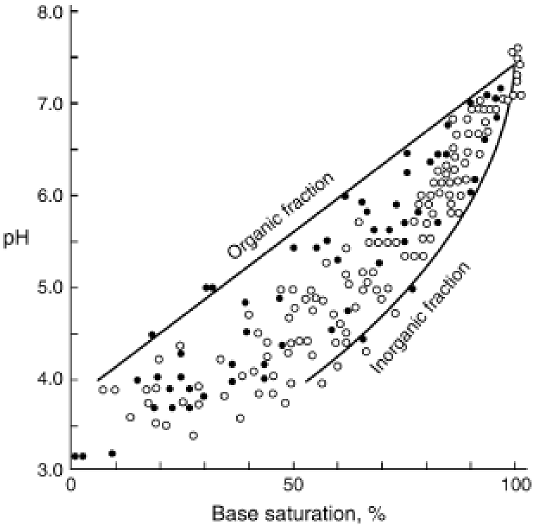


Figure 5: The relationship between pH and base saturation showing the effect of pH on organic and inorganic exchange sites. (figure from Bache, 2008 and Schachtschabel and Renger, 1966).

Acidification therefore influences vegetation (and thus biomass production) directly through nutrient depletion and Al-toxicity (Fuji, 2014). Moreover Al-toxicity also has an impact on soil organisms. Although soil biota only represent a small fraction of the total soil volume (less than 0,5%), their impact on soil properties and soil processes is tremendous (Osman, 2013). By the activity of these organisms, soil becomes a dynamic body (Osman, 2013). Microbial activity is responsible for the biochemical transformations of mineral and organic matter in the soil. Organic matter decomposition, ammonification, nitrification, denitrification, nitrogen fixation, phosphorus and sulphur transformations are all microbiologically regulated processes

(Bolan et al., 2008). Soil pH affects microbial activity and consequently alters these biochemical transformations. In acid soils, fungi outcompete bacteria and actinomycetes (Kooijman et al., 2014). Therefore roots may be affected by fungal diseases and a reduction of processes such as organic matter decomposition, nitrogen mineralization, nitrification, and nitrogen fixation, takes place (Osman, 2013).

Acidification also has an effect on the soil earthworm community (Muys, 1989; van Gestel and Hoogerwerf, 2001; Reich et al., 2005). Several studies have shown the positive effects of earthworms on soil structure, aggregate stability, soil water holding properties and on the stabilization of organic carbon in soil aggregates (Blanchart et al. 1999; Pulleman et al. 2005; Kavdir and I˙lay, 2011). In addition, biological activity and diversity of earthworms leads to higher organic matter turnovers (Ponge et al., 1999) and more homogenisation of the soil profile. Deep burrowing, species can mix organic matter of the forest floor with deep mineral soil. Three ecological groups of earthworms are defined: (1) epigeïc earthworms, that make almost no burrows, (2) endogeïc earthworms, that make shallow burrows, and (3) anecic earthworms, which are the deep-burrowers (figure 6) (Verstraeten, 2013; Schelfhout, 2010). Earthworm communities are impacted by acidification (Muys, 1989; van Gestel and Hoogerwerf, 2001; Reich et al., 2005), litter quality (Reich et al., 2005; Hobbie et al., 2006; de Schrijver et al., 2012) and soil moisture contents (e.g. Nordström and Rundgren, 1974; Grossi and Brun, 1997; Wever et al., 2001; Eggleton et al., 2009). When pH drops below a value of 4 (Muys and Granval, 1997) endogeïc and anecic earthworms disappear from the soil profile.

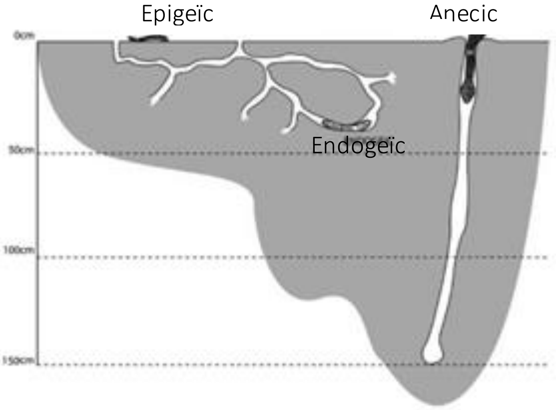


Figure 6: Three ecological earthworm groups: epigeic (small litter dwellers, make almost no burrows); endogeic (shallow soil dwellers) and anecic (deep-burrowers that feed at the surface on litter which they pull into their vertical burrows) (figure from Schelfhout, 2010)

The disappearance of deep-burrowing earthworms as a consequence of the acidification induces a vertical decoupling of different humus layers (Mori et al., 2009; Andreetta et al., 2012). This vertical decoupling is extensively discussed for humus layers but is also relevant for the mineral soil profile (Ponge, 2013; Muys and Lust, 1992; Ulrich, 1992).

Together with microbial- and earthworm activity, overstory tree species composition influences the forest floor (Belote and Jones, 2008). Hence, the forest floor is directly affected by overstory species and indirect by the influence of litter quality and acidification on soil fauna present in the forest floor. Within the effect of tree species a distinction can be made between moder-, mor- and mull-forming tree species (Toutain, 1981; Muys and Granval, 1997; Van Calster, 2008). These humus types each have associated characteristics concerning forest floor and earthworm communities (Jabiol et al., 1995; Zanella et al., 2011), table 1.

Table 1: Characteristics of terrestrial humus forms (after Jabiol et al., 1995; Zanella et al., 2011).

		Mull	**Moder**	**Mor**
Forest floor	➢ **Litter layer**	Possible	Present	Present
	➢ **Fragmentation layer**	Possible, zoögenically transformed	Present, zoögenically transformed	Present, even if sometimes discontinuous; not zoögenically transformed
	➢ **Humus layer**	absent	Present, active, sometimes discontinuous	Present, inactive or partially active
Transition forest floor – mineral soil		Very sharp (<3mm) discontinuity	Not sharp (>= 5mm)	Very sharp (<3mm)
Organo-mineral horizon – A		Biomacro or biomeso	Biomicro or single grain or massive	Absent (=E) or present. If present: not zoogenic of discontinuously biomicro
Horizon of dominant faunal activity		A (anecic and endogenic earthworms)	F (feeding), H (accumulation, droppings)	H (weak or traces or old activity)
Earthworms	➢ **Organic horizons**	Epigeïc and anecic	Epigeïc	Epigeïc rarely present or absent
	➢ **Organo-mineral horizon**	Endogeïc and anecic	Absent	Absent

In mull humus organic matter is intensely mixed with mineral soil. Biological activity thrives: epigeïc, endogeïc and anecic earthworms and bacteria are present to homogenise the soil profile and ensure rapid organic matter decomposition and nutrient cycling (Verstraeten, 2013). As a result the organo-mineral horizon is crumbly and nutrient rich. Species that form mull humus have good quality litter which decomposes fast. Mull is easily distinguished from moder/mor humus types as the latter have a permanent litter layer on top of the mineral soil (see figure 7). Due to the absence of deep burrowing earthworms, the mineral soil under moder/mor humus is less homogenised. Hence, mineral soil within moder humus types is mostly determined by physicochemical processes. Slow transformation rates of litter lead to a gradual accumulation of organic humus near the soil surface. Mor humus types are characterized by an even more abrupt transformation from litter to mineral soil and the accumulation of undecayed plant debris (Den Ouden et al., 2010).

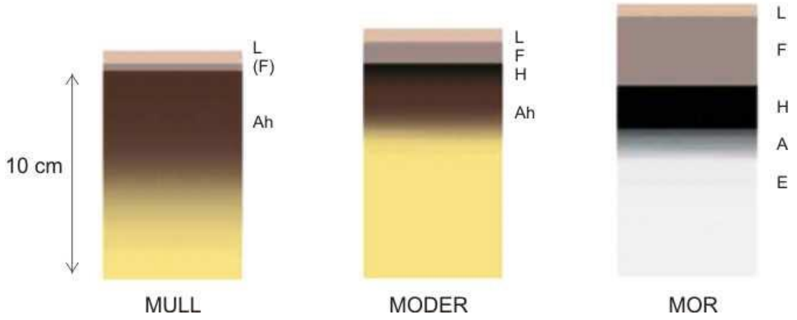


Figure 7: Humus profiles of different humus types: mull humus, moder humus and mor humus. (figure from Den Ouden et al., 2010; Verstraeten, 2013)

Overall, coniferous tree species have poor litter quality and accumulate a large forest floor mass (Hobbie et al., 2006) which could be up to twice that of deciduous species (Augusto et al., 2002). As the forest soil acidifies, biological activity decreases. If earthworms disappear, homogenisation of the soil profile stops and the humuslayer will shift from mull to mor or moder humus type. Thus, as a consequence of acidification, a vertical decoupling of matter between litter layer, the topsoil and the subsoil is induced (Ponge, 2013; Muys and Lust, 1992; Ulrich, 1992).

Finally, also soil carbon is affected by overstory induced acidification. Carbon pools are likely to be influenced by overstory tree species composition (Binkley and Giardina, 1998; Diaz-Pines et al., 2011; Guckland et al., 2009; Miechówka et al., 2011; Vesterdal et al., 2002, Vancampenhout, 2010). Soil organic carbon (SOC) pools are characterized by decomposition and turnover rates of organic matter, which vary under different tree species (Hansson et al., 2011; Kiikkila et al., 2012; Prescott, 2010; Vesterdal et al., 2002). Yet, the key mechanisms for this sequestration are still unclear (Vesterdal et al., 2013). Mueller et al. (2012) found a correlation between SOC and total extractable acidity in the mineral soil, which suggests that tree species might affect SOC content in the mineral soil through species-specific effects on acidification and thus mineral weathering (Vesterdal, 2013). Also Vancampenhout et al. (2010) state that SOC-composition differs in poor and acid systems. Even though many studies concern the role of vegetation in carbon pool formation and stability a general understanding of the effect of tree species is still needed (Jandl et al., 2007). In the next chapter (see 1.2.5) soil organic matter, its stability and decomposition mechanisms are explained in more detail.

This paragraph can be concluded by stating that soil acidification, either naturally or anthropogenic accelerated, has several interacting consequences on the forest ecosystem. Besides the direct effect of depletion of base cations and the mobilization of possible toxic aluminium, microbial- and earthworm communities are also affected by lower pH-values. Overstory induced acidification (by coniferous trees) alters the forest floor and its humus type which subsequently alters SOM-distribution patterns.

1.2.3 THRESHOLDS IN SOIL ACIDIFICATION

In the previous chapters, the general process of soil acidification, its causes (both natural and anthropogenic), and its consequences, were explained. In this chapter, the dynamics of the process are discussed in more detail. The process is non-linear as it is characterized by thresholds corresponding with pH-buffer mechanisms. Next the theory of pedogenic thresholds is introduced and a link to acidification is made.

pH-buffer mechanisms

Soil buffering capacity can be described as the resistance of the soil against an effective decrease in pH of the soil solution (de Schrijver et al., 2010). Because of this buffering, new input of H^+ does not immediately result in a lower pH, as can be seen in figure 8 (Bowman et al., 2008). Buffering capacity is determined by the carbonate content, the content of Mb cations in the silicate minerals, the cation exchange capacity of the soil and the Al content in silicates. (Ulrich and Sumner, 1991) Table 2 summarizes the different buffer mechanisms described by Ulrich and Sumner.

Table 2: Different pH buffer mechanisms and their pH range (Ulrich and Sumner, 1991).

Buffer (Ulrich and Sumner, 1991)	**Buffer capacity (/ha.m) assuming a bulk density of 1,5 (Ulrich and Sumner, 1991)**		**pH(H_2O) range (Verstraeten, 2013)**
Carbonate buffer range	1500 kmol H+ per % CaCO3	Carbonate dissolution will buffer the pH. The buffer rate exceeds any possible acid load as long as the carbonate is distributed within the fine earth. (Ulrich and Sumner, 1991)	6.2-8.6
Silicate buffer range	250-750 kmol H+ per % (primary) silicate	The buffer rate is determined by the weathering rate. (Kauppi et al., 1986)	At any pH
CEC buffer range	70 kmol H+ per % clay (dominance of illite assumed)	Excess protons are buffered by exchangeable bases. CEC processes are rapid as long as diffusion processes into soil aggregates are not rate-limiting.	
Aluminium buffer range	1000-1500 kmol H+ per % clay	When the base saturation of the CEC <5% acid is buffered by dissolution of aluminium silicates.	3.8-4.2
Iron buffer range		Dissolution of iron (Chadwick, 2001)	From pH < 4

The remaining buffer capacity of soil is determined by (1) the parent material and (2) the soils history. Weathering of the parent material results in a certain soil type. Carbonate contents, amount of clay and CEC are intrinsically determined by this soil type. The second determinant is the time that a certain driver has worked upon the soil and how much of the initial buffer capacity that is already depleted.

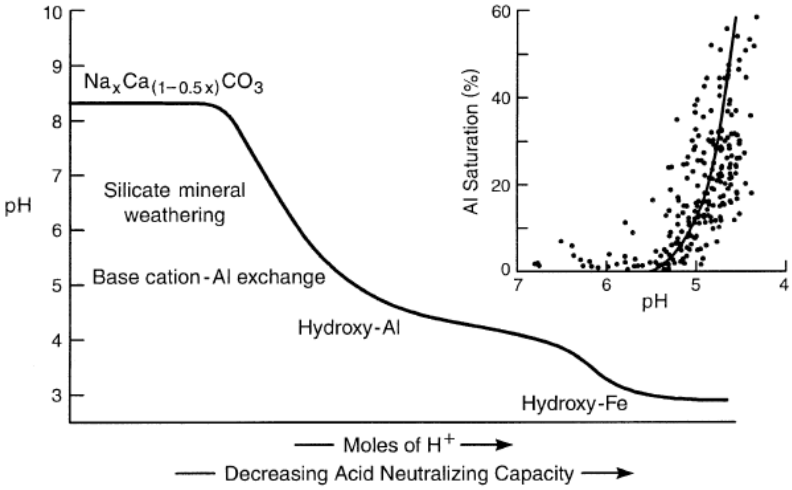


Figure 8: Schematic titration curve showing the loss of soil acid neutralizing capacity with increasing addition of protons and its differential effect on pH depending on the type of chemical buffering reaction (figure from Van Breemen et al., 1983).

Pedogenic thresholds

In 1949, Bryan and Teakle defined pedogenic inertia as "the continuation of a pedogenic process in spite of changes in the pedogenic environment that do not favor the process continuation". These pedogenic inertia were later described by Vitousek and Chadwick (2013) as soil process domains. Soil process domains are separated by pedogenic thresholds, already defined by Muhs in 1984 as "a limit of soil morphology stability that is exceeded either by intrinsic change in soil morphology, chemistry, or mineralogy or by a subtle but progressive change in one of the external soil forming factors". Thus pedogenic thresholds correspond with a small increment in environmental forcing for which there is an abrupt and nonlinear change in soil chemistry (Chadwick and Chorover, 2001). In this thesis pedogenic thresholds affecting pH get the most attention.

Acid buffering reactions predict several pedogenic thresholds and soil process domains corresponding with the different pH-buffering mechanisms (Chadwick and Chorover, 2001). If the buffering capacity of a certain range is depleted there is a rapid transition to the next buffer range, this rapid transition corresponds with a pedogenic threshold. As mentioned earlier, for as long as a certain range is not depleted the soil property responds continuous (this is within its soil process domain).

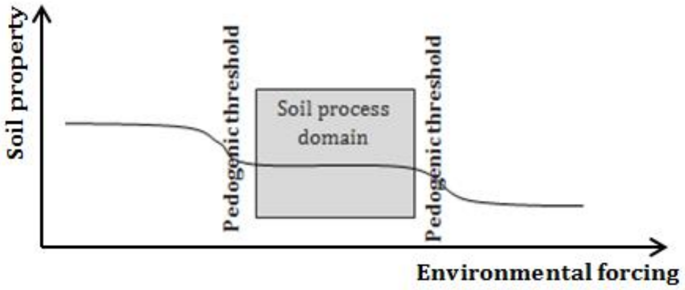


Figure 9: Response of soil property to environmental forcing with several pedogenic thresholds and soil process domains.

Comparing figures 8 and 9, it is evident that there is a similarity between pedogenic thresholds and acidification mechanisms.

1.2.4 ALTERNATIVE STABLE STATES

The concept of alternative stable states (ASS) has already been described and accepted in the field of ecology decades ago (Schroder, 2005; Scheffer et al., 1993; Van Koppel et al., 1997; Carpenter, 2001; Nystrom et al., 2000), but has recently also been introduced in the field of pedology (Vitousek and Chadwick, 2013). However predicting, parameterizing and validating the ASS in natural communities remains challenging (Marzloff et al., 2011). In this chapter the concepts of ASS in soil acidity are clarified.

Theory of alternative stable states in ecology

Factors impacting the state of ecosystems such as climate, nutrient loading, habitat fragmentation or biotic exploitation can change gradually over time.

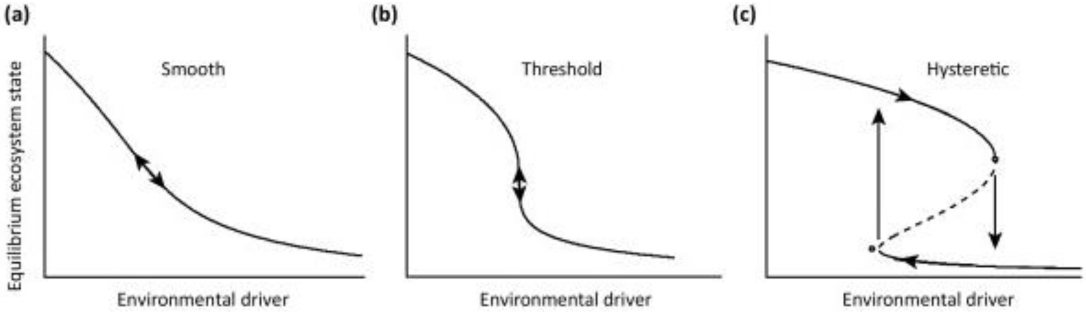


Figure 10: possible ecosystem response curves: (a) a smooth and possible linear response, (b) a threshold characterized curve and (c) an ecosystem response curve with two stable states separated by an unstable equilibrium. (figure from Scheffer et al., 2001)

The response of nature and ecosystems to this change is traditionally viewed as smooth (figure 10a). Nonetheless, several ecological studies have shown that sometimes this smooth change can be disrupted by a sudden drastic drop in the ecosystem state (figure 10b) (Scheffer et al., 2001).

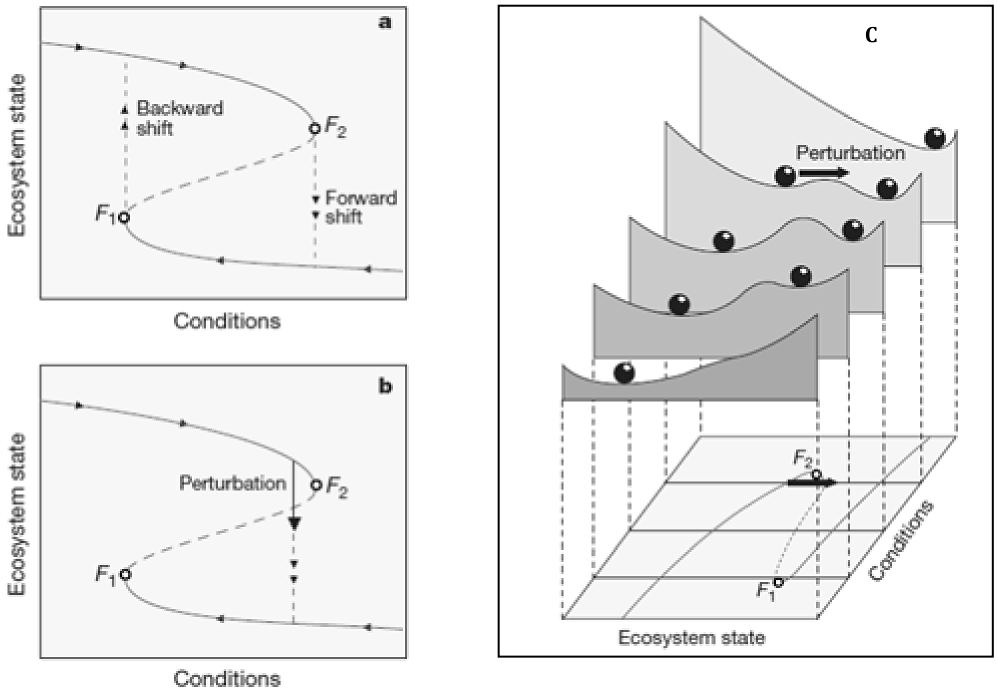


Figure 11: Folded ecosystem response curve with two bifurcation points F1 and F2 (a). Perturbation in ecosystem response curve (b). Perturbation induces a phase shift when large enough to bring the system over the border of the attraction basin (c) (figure from Scheffer et al., 2001).

The theory of ASS corresponds with a folded ecosystem response curve, as can be seen in figure 11 (Scheffer et al., 2001), distinguishing two alternative stable states separated by an unstable equilibrium. Close to the bifurcation point F2 (see figure 11) the shift from the upper stable state to the lower one can be induced by a small change in conditions. This catastrophic shift is called the forward shift. When restoring the former state, a large change in conditions is necessary to reach the point of a backward shift. This introduces the concept of hysteresis: the forward and backward switches occur at different critical conditions (Scheffer et al., 2001). Hysteresis has severe implications for management as it implies that restoration to the former state may require great effort in comparison with preventing the phase shift (Scheffer et al., 2001). Perturbations can also induce a shift if they are large enough to bring the system over the border of the attraction basin, see figure 11b. Two types can be distinguished: (1) perturbation as a sudden stochastic event (e.g., extreme weather event) or (2) perturbation as a sustained external constraint over a longer time scale (Marzloff et al., 2011; Scheffer et al., 2001; Beisner et al., 2003).

Do alternative stable states also exist in soil acidity?

Ponge (2003) hypothesized that the three humustypes mull, moder and mor can be seen as different strategies in which ecosystems evolve over time. Subsequently he introduced a model with two attraction basins (comparable to figure 11) to describe soil-vegetation interactions (Ponge, 2013). According to this theory climate and geology are the most important determinants for the humus form and correspondingly what strategy the ecosystem follows. Ponge (2013) limits his concept to the litter layer. The concept has not yet been expanded to soils and their feedback loops, nor to the relative importance of biotic factors as determinants.

As mentioned above, several soil properties respond to environmental forcing in a non-linear way (Chadwick and Chorover, 2001). Most soil properties are characterized by pedogenic thresholds and follow response-curve b in figure 10. However, for some pH-buffer mechanisms, the response curve could be approximated by figure 10c. For example, when the CEC buffer range releases base cations to the soil solution and aluminium occupies the cation exchange complex. If these base cations are depleted, the soil process domain shifts to the next buffer mechanism. Due to the higher affinity of aluminium for the cation exchange complex it is hard to restore the former state of this buffer range, even if there are new fluxes of base cations present. This inefficient exchange of aluminium by new available base cations on the cation exchange complex is an example of hysteresis. Another example for buffer mechanisms is when all carbonates present in the soil are depleted and a switch to the following pH-buffering mechanism occurs. The soil can never again buffer acidity by carbonates (unless there is an extensive artificial liming operation) and thus can never return to the previous soil process domain. This is an example where hysteresis is not even possible. Biomass production is directly affected by these soil stable states and strong feedback loops exist between soil organisms and their physicochemical environment (Reynolds et al., 2003; Dun et al., 2006; Kopacek et al., 2013; Crowther et al., 2015).

Subsequently there are also thresholds in soil acidity affecting microbial- and earthworm activity. Endogeïc and anecic earthworms can no longer survive in the soil profile once the pH drops below a value of 4 (Muys and Granval, 1997). Together with this decrease in biological and microbial activity (see chapter acidification), the belowground ecosystem shifts from a mesotrophic stable state to an oligotrophic stable state.

Hence the concept of alternative stable states may also apply to soil systems (Vitousek and Chadwick, 2013). In this thesis the hypothesis is made that these alternative stable states in soil acidity affect the carbon sequestration dynamics. In the last chapter, more focus is put on this hypothesis, the general aim and the more specific objectives.

1.2.5 SOIL ORGANIC MATTER AND SOIL ORGANIC CARBON

Soil organic matter (SOM) consists of mineralised and decomposed litter, root and microbial input and is a mixture of different chemical compounds (Piccolo, 2001; Kogel-Knabner, 2008; Kelleher and Simpson, 2006; Vancampenhout, 2010). Within SOM, soil organic carbon (SOC) can be seen as an equilibrium of carbon between aboveground and belowground input of litter and output by decomposition, respiration and leaching of dissolved organic carbon (DOC) (Vesterdal et al., 2013). The amount of SOC in forest soils is determined by climate, slope, aspect, landscape position, soil properties, vegetation and human disturbance (Lal, 2005). SOC itself influences many processes in the forest ecosystem, such as nutrient cycling, cation exchange and soil water storage (Tian et al., 2010) and is vital for several ecosystem services including productivity, climate mitigation, erosion control and hydrological buffering (Smith and Fang, 2010).

Carbon sequestration and SOC

The natural process of 'carbon sequestration' is defined as the uptake of carbon containing substances, in particular CO_2, into a long-lived reservoir (IPCC, 2007; Lorenz and Lal, 2010). More specifically, this transfer and storage of CO_2 into long-lived reservoirs is durable as it would otherwise be emitted or remain in the atmosphere (Lal 2008). Such carbon pools are located in the ocean, biosphere, pedosphere and geosphere (Lorenz and Lal, 2010). In forest ecosystems short-term interactions with the atmospheric CO_2 pool are most important: carbon sequestration in forest ecosystems occurs by uptake of atmospheric CO_2 during photosynthesis followed by the transfer of fixed C into vegetation, detritus and soil pools for secure C storage (Lorenz and Lal, 2010).

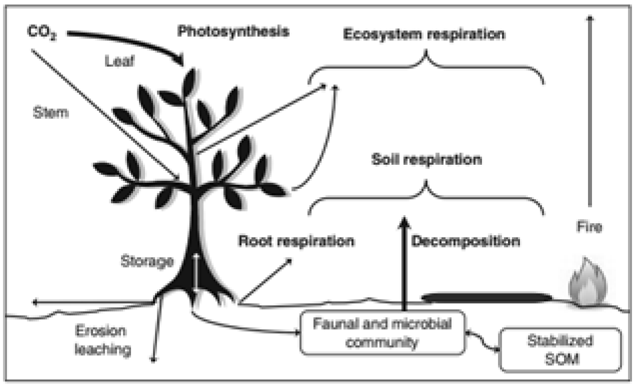


Figure 12: Carbon flow through forest ecosystems (figure from Lorenz and Lal, 2010; modified from Trumbore, 2006).

As forest ecosystems hold 70% of all belowground carbon (Dixon et al., 1994; Jandl et al., 2007; Lal, 2005) emphasis on forest soils and its role on global carbon cycles has increased notably. Part of the soil carbon pool is highly variable in space and time, while a large inert carbon pool may become active when exposed to new environmental conditions (De Deyn et al., 2008). These small changes in the carbon balance of terrestrial ecosystems have significant effects on atmospheric CO_2 (Kirschbaum, 2006; Heimann and Reichstein, 2008; Lal, 2008). Rapid climatic changes or other anomalies can thus alter soils from sinks to sources for atmospheric carbon (Davidson & Janssens 2006). The large carbon storage capacity of soils suggests a potential function for soils to mitigate increasing atmospheric CO_2 concentrations. (De Deyn et al., 2008) As a result, increasing attention is paid to carbon sequestration in forest ecosystems as climate change mitigation strategy (Kondras et al., 2012; Lal, 2005). SOM is known to be the largest terrestrial carbon reservoir, but clear reasons why some SOM can persist for millennia whereas other decompose immediately are not well understood (Schmidt et al, 2011). More insights of SOM stability are necessary to predict how soils will respond to climate change and to better understand the global carbon cycle.

SOM stability

SOM stabilization is a combination of short- and long-term processes (Nair et al., 2010) for which any disturbance may result in decomposition of young and old SOM alike (Ewing et al., 2006; Schmidt et al, 2011). General patterns in the chemical composition of SOM are already evidenced (Vancampenhout et al., 2009, 2010, 2012) but the global mechanisms are still to be identified. Schmidt et al. (2011) describe the stability of the SOM mixture as an ecosystem property, governed by biotic and abiotic factors such as input quality, SOM-mineral interactions, spatial patterns, temperature and water. Better understanding of SOM stability and mean residence time (MRT) is needed to evaluate sustainability and efficiency of carbon sequestration (De Clercq et al., 2015).

Decomposition of organic matter can be divided into three phases, each with different turnover times, see table 3 (von Lutzow et al., 2006). The final SOM phase is responsible for long-term stabilization of carbon in soils (Falloon et al., 2000). Oades (1988) and Hedges et al. (2000) allocated this long-term stability to the highly aromatic structure of humic acid, however other researches proved this theory to be outdated and refer to the importance of organo-mineral complexes (Balesdent et al., 1987; Baldock and Skjemstad, 2000; Kaiser et al., 2002; Ludwig et al., 2003; Kleber et al., 2004).

Table 3: Different decomposition phases of SOM with an indication of the different turnover times. These turnover times can differ drastically depending on the soil type. (von Lutzow et al., 2006)

First phase	25%-33% loss of initial C	1-2 years
Second phase	90% loss of initial C	10-100 years
Final phase	100% loss of initial C	100 to >1000 years

Based on the theory of Sollins et al. (1996), von Lutzow et al (2006) describe three main stability mechanisms: (1) Selective preservation of recalcitrant compounds, (2) spatial inaccessibility to decomposer organisms and (3) interactions between organic matter, minerals and metal ions (Sollins et al., 1996; Six et al., 2002; von Lutzow et al., 2006; Macías and Camps Arbestain, 2010), as can be seen in table 4. These stabilization mechanisms lead to prolonged turnover times in the soil (von Lutzow et al., 2006). The relative importance of these mechanisms for SOM stabilization is frequently debated (e.g., Ekschmitt et al. 2008; Marschner et al. 2008; Lorenz and Lal, 2010). More recent studies indicate the lower importance of selective preservation (1) (Marschner et al.,

2008) compared to interactions (3) with mineral surfaces (Kögel-Knabner et al., 2008; Marschner et al., 2008). This is only the case for long-term stability as selective preservation is of importance in early decomposition phases of surface soils (von Lutwow, 2006). On the contrary , carbon stability in subsoils is most dependent on occlusion of OM due to microstructures and aggregates and organo-mineral interactions (von Lutwow, 2006).

Table 4: Time-scales of stabilization mechanisms and their relevance (from von Lutzow et al., 2006; Sollins et al., 1996).

Mechanisms	Relevant in soil types or soil horizons	Time-scale/year
(1) Selective preservation due to recalcitrance		
Recalcitrance of plant litter and rhizodeposits	Forest floor, A-horizons	1-10
Recalcitrance of microbial products	Forest floor, A-horizons	1-10
Recalcitrance of humic polymers	All soils (Little evidence)	>100
Recalcitrance due to production of charcoal	Soils under vegetation fires	>100
(2) Stabilization by spatial inaccessibility of OM		
Occlusion of OM by aggregation (macroaggregates > 250µm)	Loamy and clayey topsoils	1-10
Occlusion of OM by aggregation (microaggregates 20 – 250 µm)	Loamy and clayey soils	10-100
Occlusion of OM by aggregation (clay microstructures <20 µm)	Loamy and clayey soils	>100
Intercalation within phyllosilicates	Acid soils, little evidence	>100
Hydrophobicity	Acid soils, topsoil, forest floor	1-10 (<100)
Encapsulation in organic macromolecules	More likely in topsoils	>100
(3) Stabilization by interaction with surfaces and		
Ligand exchange	Acid soils, soils rich in oxides, more	>100
Polyvalent cation bridges	Soils with exchange complex dominated	>100
Weak interactions	All soils? Clear evidence is missing	>100
Interaction of metal ions with organic substances (complexation)	Acid soils, calcareous soils, heavy metals contaminated soils	>100

SOM patterns

As mentioned above, decomposition mechanisms of SOM differ for topsoil and subsoil, leading to differences in soil carbon stocks. Some common garden experiments and single tree studies suggest that tree species which accumulate most C in their forest floor have less C in their mineral soil, see figure 13 (Vesterdal et al, 2008). This pattern is a consequence of the better protection of mineral C compared to forest floor C pools (von Lützow et al., 2006; Jandl et al., 2007). Besides the differences in forest floor and mineral soil, also patterns in between species can be observed. More specific, significant differences in forest floor carbon are found between coniferous and deciduous tree species (Vesterdal et al., 2013). The chemical components of coniferous litter are more difficult to decompose so that more litter can accumulate in coniferous forests (Schulp et al., 2008). As a consequence conifers have a thicker forest floor layer and less biological activity to homogenise the soil profile leading to higher topsoil carbon stocks of the forest floor under coniferous trees (Vesterdal et al., 2013). However, less organic matter is transported to the subsoil, leading to lower carbon stocks deeper in the soil. Vesterdal et al. (2013) suggest that some tree species may be better in sequestering carbon in stable forms in the mineral soil (see figure 13).

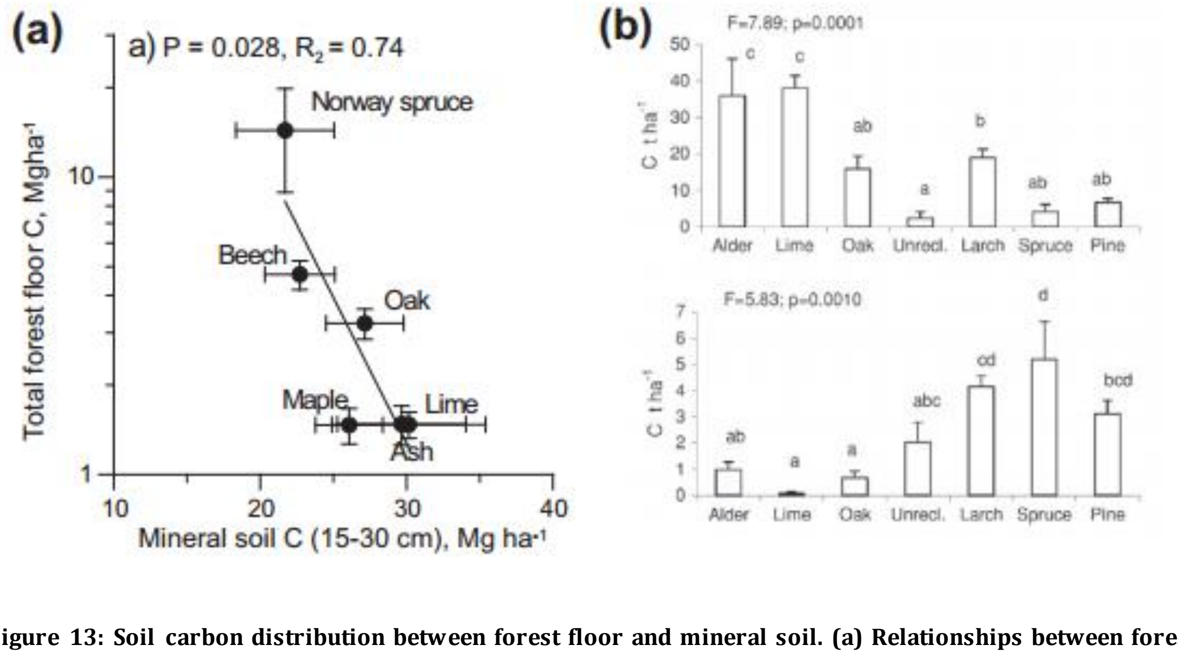


Figure 13: Soil carbon distribution between forest floor and mineral soil. (a) Relationships between forest floor C content and mineral soil C content in the 15-30 cm layer in a Danish common garden experiment (Vesterdal et al., 2008). (b) Carbon stocks in mineral soil (top graph) and forest floor (bottom graph) in reclaimed mine spoil stands after 22-30 years. (figure from Vesterdal et al., 2013)

Besides the differences in total carbon and carbon distribution, also the chemical composition of SOM varies with input type and climate (Vancampenhout et al., 2009) as vegetation, and more specific litter, is an important soil forming factor. Biochemical compounds in plant tissues vary in their susceptibility to decomposition (Suseela et al., 2013) so that there is a gradual transition of residual into stored carbon in SOM. After the decomposition of labile carbon compounds (such as soluble carbohydrates and amino acids), more recalcitrant compounds (lipids, waxes and tannins) are left in the remaining residues (Chapin et al. 2002; Sausen et al., 2014). Also patterns are observed in chemical composition in between mineral soil and topsoil and in between tree species. Brock (2015) states that the amount of lignin-derived compounds is lower for subsoils as well as for coniferous forest soils.

SOM models

Soil organic matter is often divided into pools that vary based on decomposition rates and the characteristic stabilization mechanisms (Zimmerman et al., 2007). Models have been described to map these pools and to better understand site interactions. These models that describe SOM dynamics typically have two to five conceptual C pools defined by their specific turnover rate (Shibu et al. 2006; Vesterdal et al, 2013). This includes one or two labile or dynamic pools, one or two physically and chemically protected pools and a passive or inert pool (Christensen, 1996). As mentioned above, stabile SOC is of importance for long-term carbon sequestration. Elliot and Paustian (1996) and later Skjemstad et al. (2004) introduced the first models to divide SOM over measurable SOC-pools. In this thesis the procedure described by Zimmermann et al. (2007) is used to measure SOC-pools. This fractionation procedure results in five separated fractions which correspond to the SOC-pools of the RothC model (Coleman and Jenkinson, 1999).

The RothC model divides organic matter into decomposable plant material (DPM) and resistant plant material (RPM). The latter decomposes to form CO_2, biomass (BIO) and humified organic matter (HUM). Every pool is again decomposed to CO_2, BIO and HUM (indicated by the 3 arrows splitting up again). Additionally there is a pool of inert organic matter (IOM) which has a radiocarbon age of 50 000 years. Overall five SOC pools can be distinguished, see figure 14 (right).

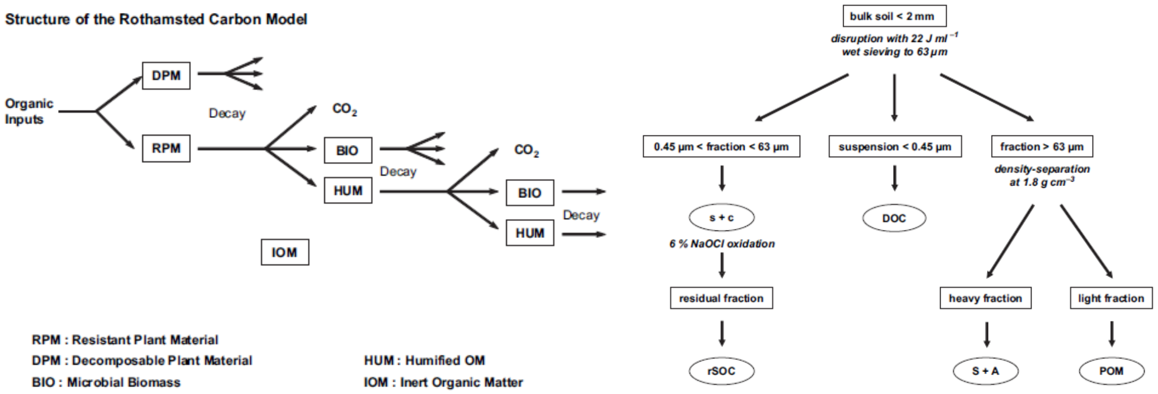


Figure 14: Scheme of Rothamsted carbon model (left) and Zimmermann fractionation scheme (right) (from Zimmermann et al., 2007; Coleman & Jenkinson, 1999).

Zimmermann et al. (2007) differentiate five measurable fractions (figure 14); particulate organic matter (POM), sand and stable aggregates (S+A), dissolved organic carbon (DOC), silt and clay (s+c) and resistant soil organic carbon (rSOC). In figure 15 the link between these five fractions and the RothC model is illustrated. Although many different models exist and can be used, most emphasis in this thesis is given to the model of Zimmermann et al. (2007).

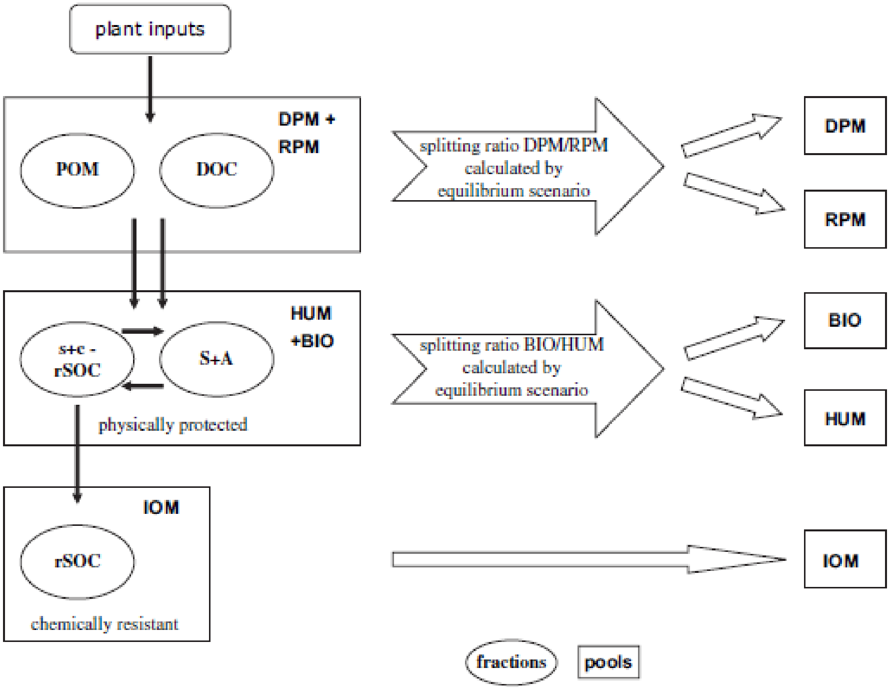


Figure 15: Link between measurable fractions of Zimmermann and SOC-pools of RothC model (Zimmermann et al., 2007).

1.3 HYPOTHESES, OBJECTIVES AND RESEARCH QUESTIONS

As above, so below?

The general objective of this study is to evaluate the effect of a conversion from ancient forest to coniferous plantations on the soil sequestration dynamics. Moreover to understand the effect of overstory induced acidification in relation to SOM stability and whether above ground ecological shifts are mirrored in the belowground ecosystem.

As already mentioned, forest soil acidification is a natural process (through natural weathering and the input of tree life products) which can be accelerated by anthropogenic drivers such as atmospheric depositions and coniferous monocultures (see fig 16, 3 red arrows). In this thesis the following hypotheses are checked: (1) The conversion of ancient forest to coniferous plantation induces soil acidification, (2) This acidification can cause the belowground ecosystem to suddenly shift to another soil process domain and (3) that when a certain pedogenic threshold is reached for this anthropogenic driven acidification, a cascade of thresholds through the soil food web follows. This cascade through the soil food web and the fall into a state that is low in both quality and variability can be seen as the shift to an alternative stable state. After depletion of most of the pH-buffering, biological activity of earthworms disappears which induces a vertical decoupling of topsoil and subsoil. Two alternative stable states in the belowground ecosystem are expected corresponding with two soil process domains: the base dominated domain and the iron and aluminium dominated soil process domain.

As research upon the theory of alternative stable states in forest soils and its link with ecosystem functioning is scarce, a better understanding of these processes, feedback mechanisms, stable states and thresholds will allow more efficient forest management decisions. Also more insight in soil organic matter stability is necessary to predict how soils will respond to climate change, to optimize forest management and to ensure more sustainable wood-production in the future.

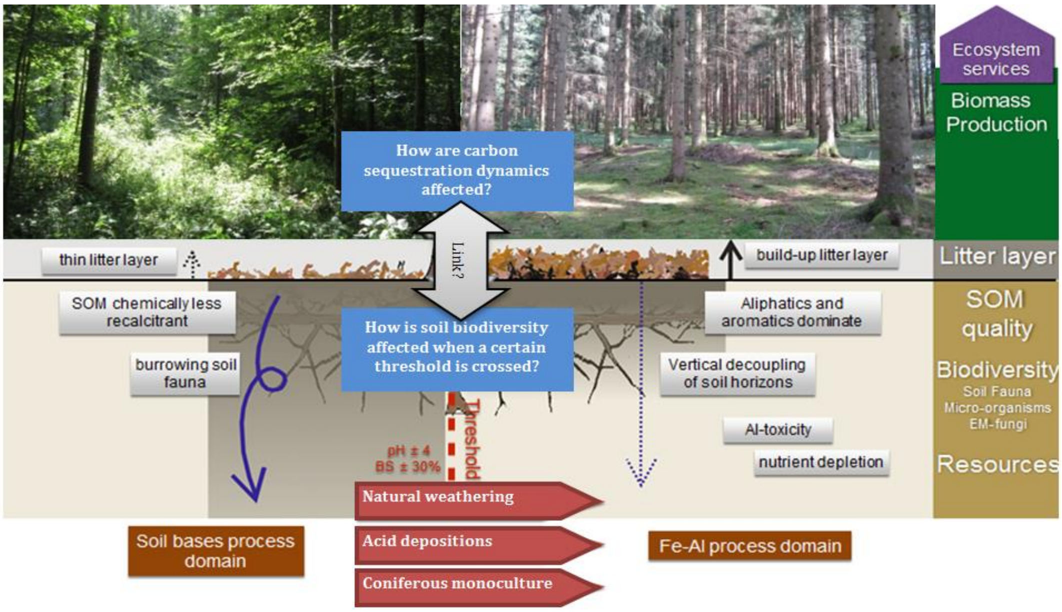


Figure 16: Schematic illustration of hypotheses and objectives. (Adapted from Vancampenhout et al., 2015)

In addition to the general objective, three more specific objectives are delineated:

1. To evaluate whether the effect of the conversion is equivalent for every starting point or modulated by the underlying lithological gradient.
2. To distinguish two soil process domains in the belowground ecosystem. And to demonstrate the possibility of hysteresis by the data.
3. To evaluate the effect of the overstory conversion on the forests ecosystem service of climate mitigation, moreover on the carbon sequestration capacity and SOM stability.

To achieve these objectives the following research questions are defined:

Questions on objective 1:

- Is the difference in pH, base saturation or CEC larger between coniferous plots compared to deciduous plots?
- Does the effect on pH, BS or CEC increase with decreasing fertility of the soil substrate?

Questions on objective 2:

- Does the data of soil properties and/or biological activity indicate two soil process domains?
- Can certain thresholds for the soil properties be observed? Is there a sudden and drastic drop in soil quality along the plots?
- Is there an irreversible (or hardly reversible) decrease in earthworm activity?

Questions on objective 3:

- Is there a significant difference in SOC under coniferous trees compared to deciduous trees? Is this effect the same for topsoils and for subsoils?
- Is there a significant difference in the distribution of SOC over different SOC-pools under coniferous trees compared to deciduous trees? Is this effect the same or reversed for topsoils compared to subsoils?
- If there is a significant difference in SOC, does this difference change along the underlying lithological gradient?

2 MATERIALS AND METHODS

2.1 STUDY AREA

The study area is part of a large forested area in the south of Belgium, the Gaume region (figure 17). This region contains one of the largest complexes of old forest in Belgium, meaning it was already classified as forest on the Ferraris map constructed in 1777. The natural deciduous forest is mostly dominated by *Quercus robur* L., *Fagus sylvatica* L. and *Carpinus betulus* L..

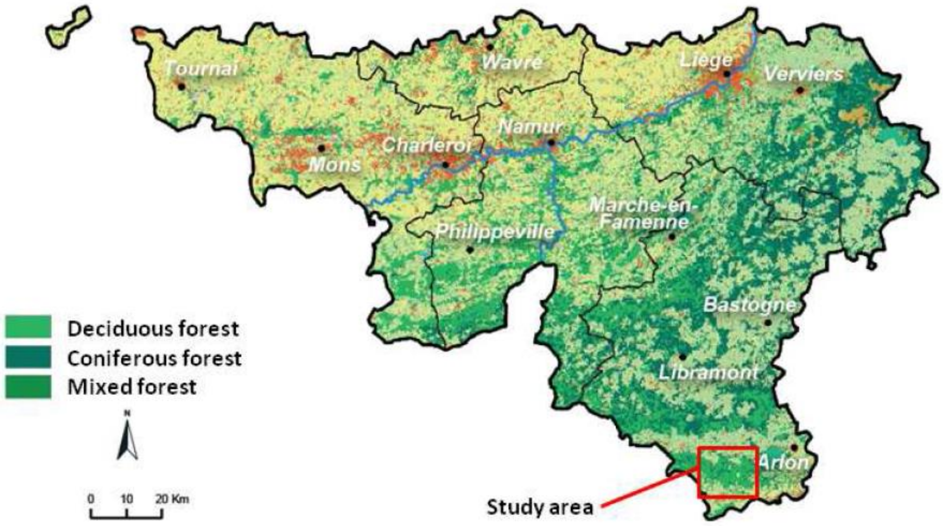


Figure 17: The study area (Gaume region) located in the south of Belgium. (figure fromVerstraeten, 2013)

In the Gaume area two types of geological substrate can be distinguished; the Luxemburg formation and the Arlon formation, as can be seen on the geological map of the area (figure 18). The Luxemburg formation is characterised by red and yellow sands, soft sandstone benches and calcareous sandstone with variable lime contents. The Arlon formation can be described as a collection of different geological layers which mainly consist of sands, decalcified at the surface, and calcareous sandstones (Bouezmarni et al., 2009). The Luxemburg formation results in poorer, sandy soils compared to the Arlon formation, which can result in relatively higher clay contents. However, both substrates result in sandy and poor soils. Due to the intertwining of these two formations, large variability is found in local soil conditions. Hence, a gradient of soil fertility can be observed (Verstraeten, 2013). Also löss deposits can be present on the north faced slopes of the cuestas.

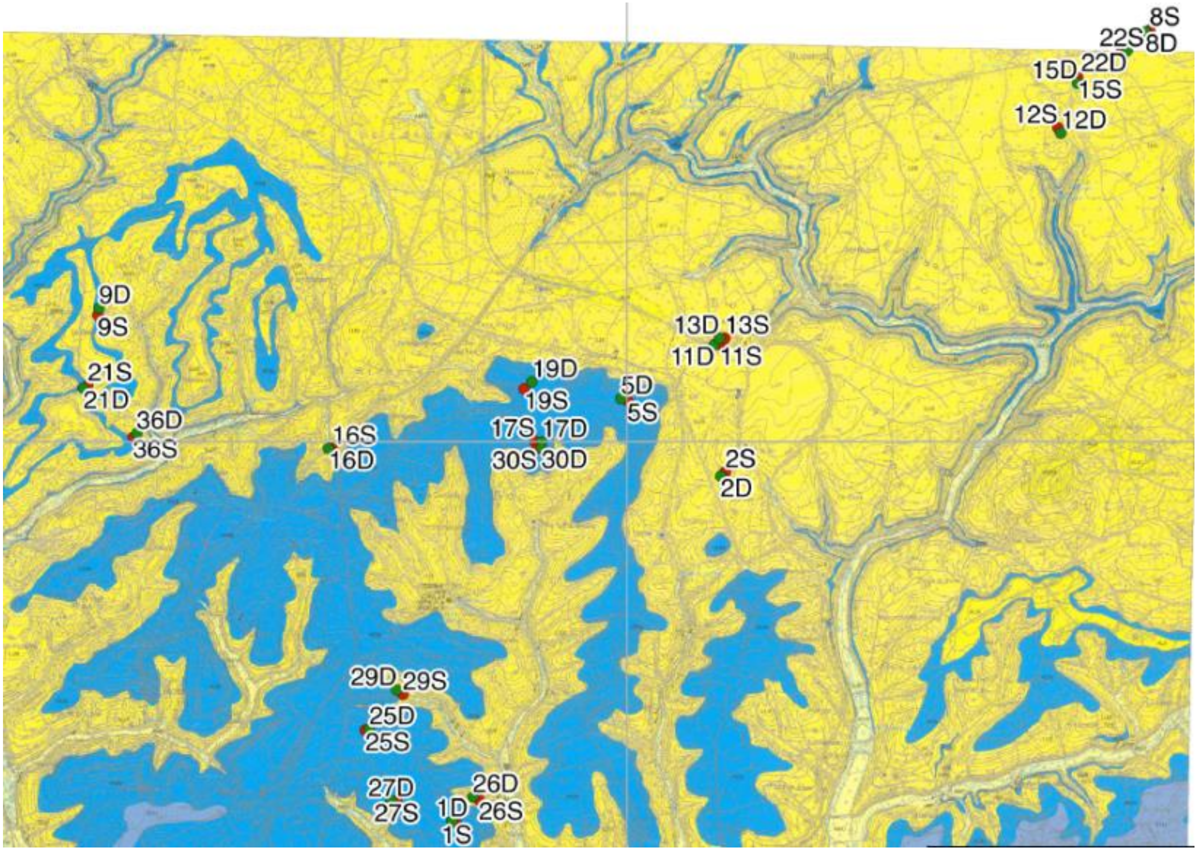


Figure 18: Geological map of the study area with the location of the twin-plots. Two geological substrates are indicated: the formation of Arlon (Blue) and the formation of Luxembourg (Yellow). (from Verstraeten, 2013 adapted from carte hydrologique de Virton)

Between 1950 and 1980 many patches (of 2-15ha) of these deciduous trees were clearcut and replanted with *Picea abies* (L.). This made it possible to establish a set of 20 twin-plots (each 10m x 10m) along a soil fertility gradient (approached by the clay content). For each plot of natural deciduous vegetation an adjoining plot of planted spruce vegetation (of the same age: 35-50 years) exists for the same intrinsic soil fertility. Thus, just before planting the spruce trees, both stands were comparable in forest history, tree species composition and abiotic characteristics. This is an important assumption for the study design (Verstraeten, 2013). Because of this unique study area and the twin-plot set-up it is possible to reduce confounding factors such as influences of tillage, pesticides or contaminants and decrease the bias by the legacy effect as for old forest the site history is well-known.

Previous studies have indicated a significant decrease in soil quality (decrease in pH), species richness and biological activity for the spruce plantations compared to the original vegetation (Verstraeten, 2013; Brock, 2015).

2.2 SOIL SAMPLING

From the 20 existing twin-plots in the Gaume region a selection of 10 was made by analysing previous data of pH, geology, texture, humus classification and earthworm biomass. All plots were aligned to get an idea of the natural gradient for each factor. Also a principal component analysis (PCA) was done on the existing data. This way a set was made that spans the natural gradients that are present.

As in-the-field information is valuable when working with small datasets every plot was evaluated during the field campaign and prominent features such as local topography, extreme rich understories and recent management operations were noted. The field campaign revealed an exceptionally rich understory vegetation for plot 27 and plot 17 as a consequence of local topography. Plot 27 likely receives extra nutrients by subsurface flow coming from the top of the cuesta. Additionally, plot 17 is remarkably richer than plot 30 just 100m away as a consequence of the north faced slope and the extra löss deposits. For every plot a mixture of five samples in the topsoil (0cm-5cm) and a mixture of five samples in the subsoil (25cm-30cm) was taken. These five samples were homogenized into one composite sample. Also at 1m depth a sample was taken, to get a notion of the parent material. Due to stoniness this was not always possible. Afterwards the soil samples were sieved (2mm) and dried at 45°.

The previous set of soil samples (of all 20 twin-plots, taken in the summer of 2013 by Jeroen Buelens) were used to determine pH(H_2O)-values. In addition earthworm activity (collected by Gorik Verstraeten), microbial activity, texture and pH(KCl) were already determined on this set of soil samples (by Jeroen Buelens, Michael van Raemdonck and Olaf Brock).

2.3 LABORATORY ANALYSIS

The soil samples were analysed in the lab division of Soil and Water Management, Department of Earth and Environmental Sciences at the KULeuven University in Heverlee and at the lab division Bioengineering Technology at the KULeuven in Geel.

2.3.1 PH MEASUREMENTS

As pH is typically measured on solutions, soil pH is defined in a similar operational way. Soil pH can be measured in several solutions: 0.01 M $CaCl_2$, water (actual acidity) or 1M KCl (potential acidity) or in deionized water. The soil samples used for pH measurement were taken 2 years ago (by Jeroen Buelens, 2014) on the same plots as the samples taken this year and used for the CEC and carbon analysis.

To measure pH(H_2O) a glass pH-electrode was used. This electrode was first calibrated on two standard pH solutions (respectively pH values of 4 and 7). To make the soil solution, 5g of oven-dried soil was mixed with 25 ml of deionized water and shaken thoroughly for 30 seconds. After 15 minutes (to allow the solution to set properly) the pH was measured 2cm above the soil-solution interface. The electrode was washed with deionized water in between every sample. pH-measurements were done for both subsoil samples as topsoil samples (ISRIC manual).

2.3.2 BASE SATURATION AND CATION EXCHANGE CAPACITY

The cation exchange capacity of a soil represents the capacity of a soil to adsorb exchangeable cations and is expressed in milliequivalents (meq) cations per 100 g soil. The amount of exchangeable cations is determined by replacing the cations by ions with a higher affinity for the charged surface. This can be done by several methods, but cobalthexammine $Co(NH_3)_6^{3+}$ ("cohex"), was chosen in this study (Ciesielski et al., 1997). Cohex has high affinity for negatively charged surfaces, both mineral (clay) and organic (soil organic matter). As the pH is not buffered in the cohex solution, effective CEC (eCEC) is measured. The amount of exchanged Co should ideally be between 10 and 33% of the Co initially added, thus it is of importance to use the correct amount of soil sample. As the soil samples are poor and sandy and CEC are expected to be low, a large mass of 2,5g (and for some even 5g) of oven-dried soil was used. This mass was weighed (accuracy 0,005g) in a centrifuge tube and 25ml of cohex were added. Four blanks and two internal reference samples (Ter Munck) were included in the analysis. All samples were shaken for 1 hour on an end-over-end shaker and afterwards centrifuged for 15 minutes at 3000 rpm. Next, the supernatant was diluted (50x) and acidified with HNO_3 (5M) in a plastic ICP tube. Using ICP-OES the concentrations of Co, Ca, K, Na, Mg, Al, Fe, and Mn are measured.

2.3.3 FRACTIONATION METHOD - ZIMMERMANN PROTOCOL

For the fractionation of SOM in measurable carbon-pools the procedure described by Zimmermann was followed. This procedure divides the soil samples in five different soil organic matter fractions which can be related to pools in the RothC model (Zimmermann et al., 2007).

To thirty grams of soil, sieved to 2mm, 150 ml of deionized water was added. Using an ultrasonic probe (for 3,5 minutes with an energy output of 22J/ml) the mixture was dispersed to break soil aggregates. The suspension was wet sieved over a 63µm sieve for 2 minutes. The particles >63µm, which correspond with sand and stable aggregates (S+A) and the particulate organic matter (POM), were gently removed from the sieve and dried at 45° in an aluminium bin which was weighed before.

From the fraction <63µm, 400 ml was filtered over a 45µm nylon Millipore filter to obtain the dissolved organic matter (DOC): this is the fraction <45µm. The DOC fraction was frozen, and later the amount of dissolved SOC was measured on this fraction. Everything on top of the Millipore filter, corresponding with the silt and clay (s+c) fraction, was dried together with the filter at 45°. To obtain enough silt and clay an extra volume <63µm had to be filtered for some samples. Using sodium polytungstate (SPT) at a density of 1,8g/cm^3 the POM fraction was separated from the S+A fraction. After centrifugation the mixture was frozen and the lighter POM fraction could be scraped of the more heavy S+A fraction. Both fractions were washed with deionized water to remove all residual SPT, dried at 40°C and weighed. Subsequently the amount of carbon was measured with an elemental analyser for both the POM fraction as the S+A fraction.

Due to lack of enough material and because the rSOC fraction is only a minimal percentage of the total s+c fraction, the oxidation step described by Zimmermann was skipped.

2.3.4 SPT RECYCLING

Sodium polytungstate is used in the fractionation method to perform a density separation. As SPT is very costly, recycling after use is advisable (Six et al., 1999). With a column filled with activated carbon and a cation exchange resin, the SPT solution was cleaned. Before starting the recycle step, the column was washed with 2L of deionized water, 2L of NaCl solution and after that again with water. Also in between every batch of 2L SPT the column was washed with deionized water to prevent the column from clogging with dry SPT. The obtained clean SPT was dried at 45° to obtain a high concentration which facilitates forming the density fixed SPT solution as dilution is the most efficient way to do so.

2.4 STATISTICAL ANALYSIS

The statistical analysis is divided into two sections: the first one is done on the 20 twin-plots dataset describing the site, and the second one on the 10 twin-plots dataset concerning the carbon dynamics.

2.4.1 DESCRIPTIVE STATISTICS AND HYPOTHESIS TESTING

For both sections descriptive statistics are tested on all variables: boxplots for all these variables are made using R statistics. A distinction is made between topsoil- and subsoil samples and deciduous- and spruce plots. First the data are tested for normal distributions by a shapiro test in R statistics. Subsequently for each of these variables the difference between topsoil and subsoil and deciduous and spruce is tested with a hypothesis test. Paired t-tests were done on normally distributed data and Mann-Whitney U tests were done on non-parametrical data. If the difference between deciduous plots and spruce plots was significant ($\alpha = 0,05$) an additional test was done to evaluate the covariate ‘clay content’. This was done by testing the correlation between the new variable ‘difference’ (i.e. the difference between deciduous and spruce plots for the specific variable) and the variable clay content. Pearson correlation tests were used for normally distributed data whereas Spearman correlation tests were used for non-parametrical data.

2.4.2 FACTOR ANALYSIS ON MIXED DATA

To further explore the dataset and to highlight important patterns in the data, a factor analysis on mixed data (FAMD) is done using R software. Because the variables geological parent material and humus type are categorical, these variables are defined as factors with respectively two (Luxembourg < Arlon) and six levels (Dysmoder < Eumoder < Hemimoder < Dysmull < Oligomull < Mesomull). For simplicity the FAMD can be seen as a combination of a PCA on the quantitative variables and a multiple correspondence analysis (MCA) on the categorical variables. The FAMD orthogonally transforms variables which are possibly correlated into a set of linearly uncorrelated variables. These uncorrelated variables are the principal components. The first principal component has the largest variance and thus explains most variability in the data. The following principal components each account for more variance than the next and are orthogonal to the precedent principal components. The orthogonality can be explained by the fact that the principal components are eigenvectors from the correlation matrix. This method balances the influence of quantitative and categorical variables. Hence, both types of variables equally attribute to the dimensions of variability (and thus the axes in the plots). The result of the FAMD is a plot of all observations and their scores (factor scores) for the different principal components.

2.4.3 GRAPHICAL ILLUSTRATION OF MOST IMPORTANT RELATIONS IN THE DATASET

Finally, all variables are plotted for increasing soil acidification variables (pH and BS). This is done for both the variables describing the site and for the variables concerning carbon dynamics. The objective of this graphical two-on-two plots is to evaluate the specific relations separately and to observe possible thresholds.

3 RESULTS

This chapter is divided in two major parts: the site characteristics and the carbon dynamics. In the first part variables describing the study site are discussed. These variables are derived from a database of 20 twin-plots (with the exception of the results for CEC and BS, which were only analysed on the 10-twin-plot dataset.) After descriptive statistics on all variables, a principal component analysis is executed to determine the most prominent trends in the dataset. The first part is concluded by scooping in on most important relations and trends. In the second part of this chapter, the data of the Zimmermann fractionation and analyses concerning carbon are shown. This fractionation was executed on the 10-twin-plot dataset as it is an exploratory research.

3.1 SITE CHARACTERISTICS

3.1.1 ABIOTIC VARIABLES

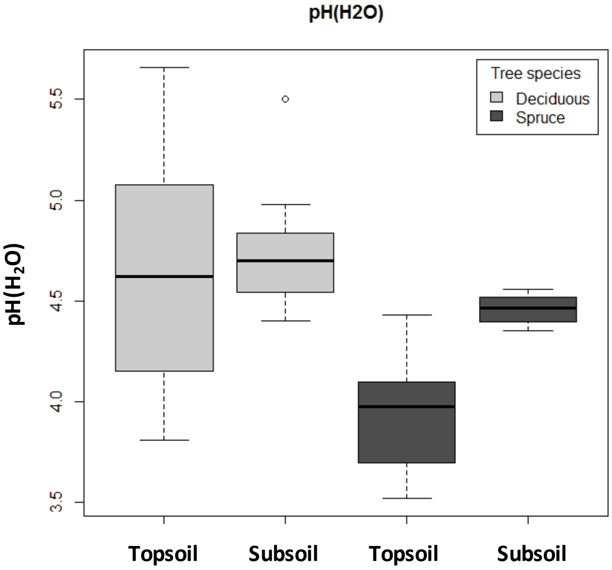


Figure 19: Boxplot of the pH(H_2O)-values for both topsoil and subsoil samples. A distinction is made between spruce plots (dark) and deciduous plots (light).

Figure 19 shows the boxplots of topsoil and subsoil pH(H_2O)–values for both spruce and deciduous plots. The difference between overstory tree species for topsoil pH-values is larger than for subsoil pH-values. R-statistics performed a t-test on these values. The pH-topsoil-values of deciduous plots were significantly higher (t-test, $p < 0,05$) than pH-values of topsoils from plots planted with spruce. As the data for subsoil samples were not normally distributed a Mann-Whitney U test was performed instead of a t-test. Deciduous plots have significantly higher subsoil pH-values (Mann-Whitney U test, $p < 0{,}05$) than spruce plots. The boxplot of subsoil pH-values has one outlier, plot 27. This plot may be a true outlier as it is located in a seepage zone and receives nutrients through subsurface flow coming from the cuesta front.

To test whether clay content is significant as covariate in this paired hypothesis-tests of pH, the correlation of clay and the new variable ($pH_{deciduous} - pH_{spruce}$) is tested with a spearman correlation test in R. For both topsoil as subsoil samples is the difference in pH modestly correlated with clay content of the corresponding twin-plot. The correlation coefficient are $rho_{topsoil} = 0{,}4024$ ($p\text{-value}_{topsoil} < 0{,}1$) and $rho_{subsoil} = 0{,}4040$ ($p\text{-value}_{subsoil} < 0{,}1$).

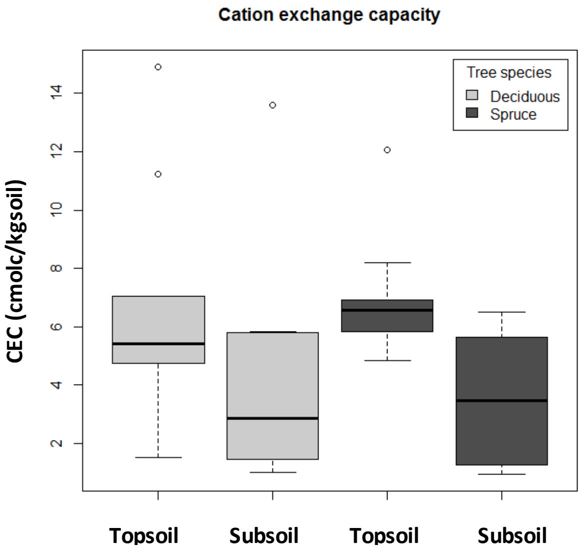


Figure 20: Boxplot of cation exchange capacities for topsoil and subsoil samples. This analyses were executed on the smaller dataset of 10 twin-plots. A distinction is made between spruce plots (dark) and deciduous plots (light).

Boxplots of the CEC's of topsoil and subsoil samples are shown in figure 20. Topsoil CEC values have higher medians than subsoil CEC values. This difference is significant for the spruce plots (Mann-Whitney U test, $p_{spruce} < 0{,}05$) but not significant for deciduous plots (Mann-Whitney U test, $p_{deciduous} > 0{,}05$). For both topsoil and subsoil samples there is no significant difference for tree species (Mann-Whitney U tests).

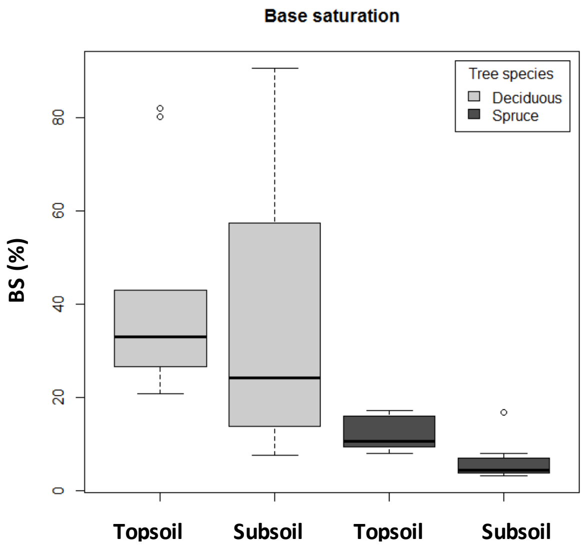


Figure 21: Boxplot of base saturation values for topsoil and subsoil samples. A distinction is made between spruce plots (dark) and deciduous plots (light).

Boxplots of BS-values are shown in figure 21. Because the variables in this boxplot are not normally distributed Mann-Whitney U tests were executed to investigate the differences. BS-values of deciduous plots are significantly higher ($p_{topsoil} < 0{,}001$ and $p_{subsoil} < 0{,}05$) for topsoil and subsoil samples than plots planted with spruce. Note the wide range of BS in topsoil and especially subsoil deciduous, while variability is much less in spruce. The difference between topsoil and subsoil samples is only significant for spruce plots ($p_{spruce} < 0{,}05$) where subsoil BS is lower than topsoil BS. One outlier for subsoil spruce samples is observed: plot 27. For deciduous plots there is no significant ($p_{deciduous} > 0{,}05$) difference in base saturation of topsoil and subsoil samples. The difference in BS between spruce and deciduous plots is correlated with clay content for topsoil samples with a correlation coefficient rho of 0,7818 ($p < 0{,}05$). Topsoil BS is thus modulated by the underlying lithological gradient. There is no correlation for subsoil samples.

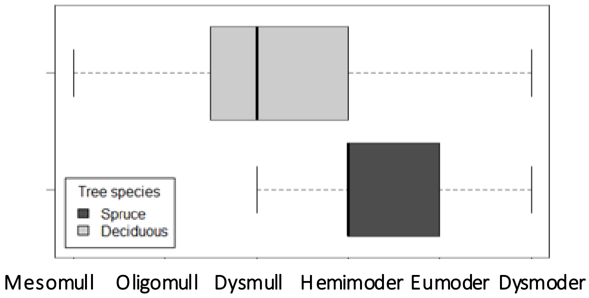


Figure 22: Boxplot for all humustypes within the dataset. A distinction is made between deciduous plots (dark) and spruce plots (light).

Humustypes can be ranked from good quality to relative poor quality: mesomull (2), oligomull (3), dysmull (4), hemimoder (5), eumoder (6) and dysmoder (7). Figure 22 shows two boxplots, respectively of the humustypes of deciduous plots (light) and of spruce plots (dark). Deciduous plots have a higher variation in types and overall a humustype of higher quality than the spruce plots. Spruce plots have significantly higher (Mann-Whitney U test, $p < 0,05$) values of humustype (and thus lower quality). The difference in humustype does not correlate with clay content and thus is seemingly not modulated by the underlying lithological gradient. However, plots with the formation of Arlon as geological substrate have significantly better humustypes compared with plots that lie upon the formation of Luxembourg.

3.1.2 BIOTIC VARIABLES

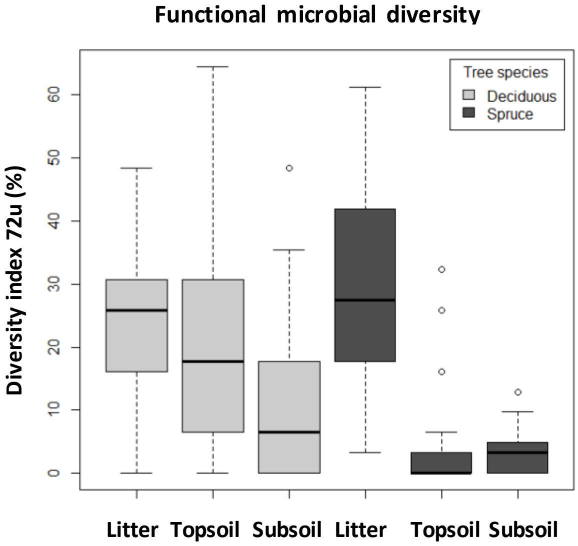


Figure 23: Boxplots for functional microbial diversity (measured by the diversity index of biologs after 72u) for litter, topsoil and subsoil samples. A distinction is made between spruce (dark) and deciduous (light) plots.

Figure 23 shows six boxplots for functional microbial diversity respectively for litter, topsoil and subsoil samples. In the litter layer the median diversity is higher for spruce plots than for deciduous plots whereas in topsoil and subsoil samples the median microbial diversity is higher for deciduous samples. However the difference for litter samples between spruce and deciduous is small in comparison with topsoil and subsoil samples. For the litter samples a t-test could be executed because of the normal distributed data. This test showed that the spruce plots have no significant ($p_{litter} > 0,05$)

higher diversity index for the litter layer. A Mann-Whitney U test was used to research the differences in tree species for topsoil and subsoil samples. For both topsoil and subsoil samples deciduous plots have significantly higher ($p_{topsoil} < 0,01$ and $p_{subsoil} < 0,05$) functional diversity than spruce plots.

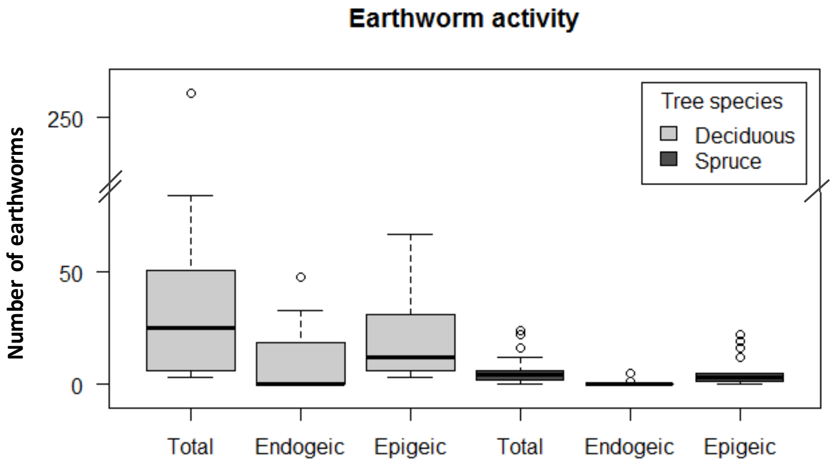


Figure 24: Boxplots of endogeïc, epigeïc and total number of earthworms present in the samples. A distinction is made between spruce plots (dark) and deciduous plots (light).

Figure 24 shows the earthworm abundance (the sum of epigeïc and endogeïc earthworms equals the total number of earthworms present). For spruce plots there is little variation and the amount of earthworms is very low. The median of total number earthworms for deciduous plots is higher as for spruce plots, but also the variation is higher (standard deviation equals 7,09 for spruce plots and 62,26 for deciduous plots). Because the data were not normally distributed a Mann-Whitney U test was executed on the total amount of earthworms and the amount of epigeïc earthworms. Because so little plots have endogeïc earthworms the dataset is too small to do hypothesis-testing for this variable. The amount of epigeïc earthworms is significantly lower ($p_{epigeïc} < 0,05$) for spruce plots than for deciduous plots. The same trend is observed for the total amount of earthworms, these are also significantly lower ($p_{total} < 0,001$) for spruce plots than for deciduous plots.

3.1.3 FACTOR ANALYSIS ON MIXED DATA

To observe how site variables behave together under reference conditions, a factor analysis for mixed data (FAMD) is executed for the deciduous plots only. By iteratively eliminating variables that represent the same variation, eight variables were selected for the final FAMD. This analysis includes six quantitative variables: functional microbial activity, earthworm activity, pH, Ellenberg indicator N, Ellenberg indicator F and percentage sand, and two categorical variables geological parent material and humustype. Because the variables geological parent material and humus type are categorical, these variables are defined as factors with respectively two (Luxembourg < Arlon) and six levels (Dysmoder < Eumoder < Hemimoder < Dysmull < Oligomull < Mesomull).

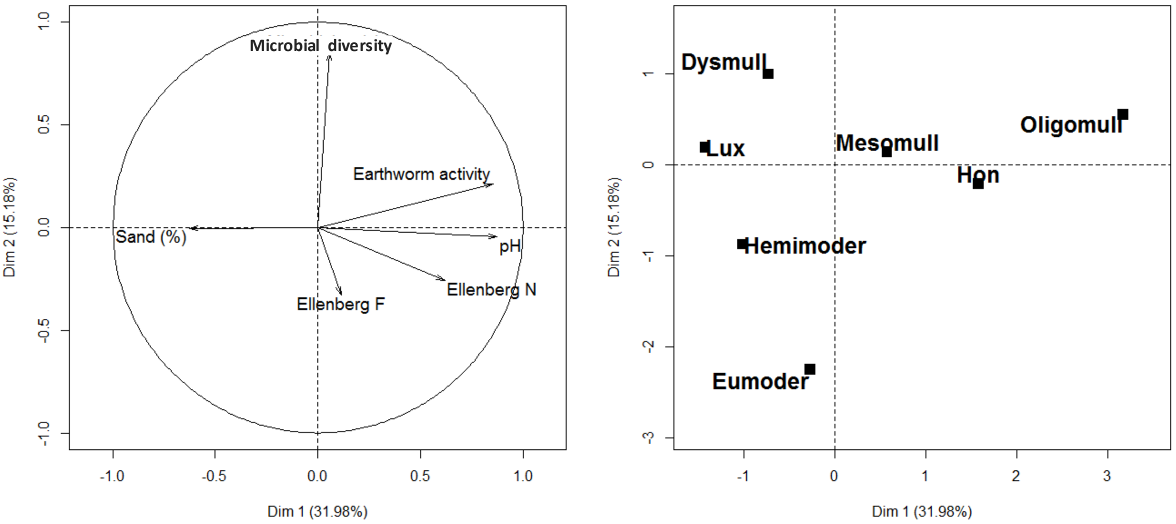


Figure 25: FAMD1: Quantitative (left) and qualitative (right) variables map of FAMD on the deciduous plots. Principal component 1 (x-axis) describes 31,98% of all variability present in the dataset, principal component 2 describes 15,18% of this variability.

Figure 25 shows the factor maps of the quantitative and qualitative variables of the FAMD-analysis. Along the first axis lies the variable pH in the positive direction and texture (sand) in the other direction. The second axis corresponds most with the functional microbial diversity, the moder humustypes and the F Ellenberg indicator value in the other direction. For the qualitative variables: parent material changes from the Luxembourg formation to the Arlon formation along axis one and humustypes decrease in quality in the negative direction of axis two.

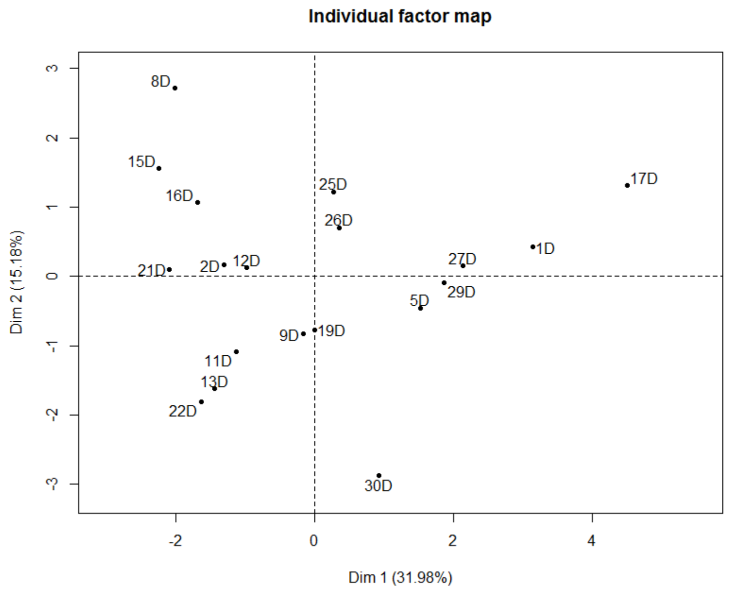


Figure 26: FAMD1: Factor map of the individuals: all individuals (plots) are plotted for their factor scores.

All individual deciduous plots are shown in figure 26. This figure can be interpreted together with the previous figure 25, which shows how to understand the two axes: axis one shows the influence of parent material and axis two the influence of functional microbial diversity. When the BS-values of the 10-twinplot dataset are compared with the FAMD1 results, BS seems to increase corresponding with variable 'earthworm activity' (i.e. in the positive direction of both axes).

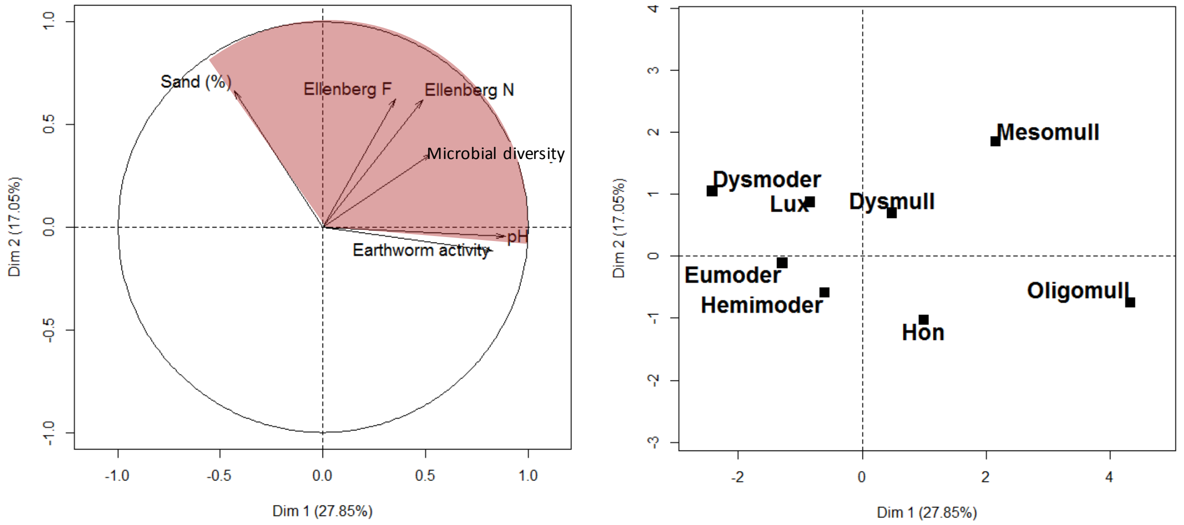


Figure 27: FAMD2: Graph of quantitative variables and qualitative variables of FAMD-analysis on all plots (both spruce and deciduous). Principal component one (x-axis: Dim 1) represents 27,85% of all variability in the dataset and principal component two (y-axis: Dim 2) represents 17,05%. The red polygon shows the range of variation describing the site.

In figure 27 the FAMD-analysis upon both deciduous and spruce plots is shown. Figure 27 shows how the quantitative variables lie along the axes (that represent the two first principal components) and how all different humustypes and parent materials are located along the axes. In comparison with the previous FAMD (figure 25) executed on only deciduous plots, the qualitative variable humustype now contributes more to the first axis instead of the second (as the quality of humustype in figure 27 decreases from right to left). Also the variables microbial activity, percentage sand, Ellenberg F and Ellenberg N have changed direction in the factor map. As a result of the conversion, the variability of the site is described by a smaller range of variation in the different variables. The variables pH and earthworms remain more or less in their position.

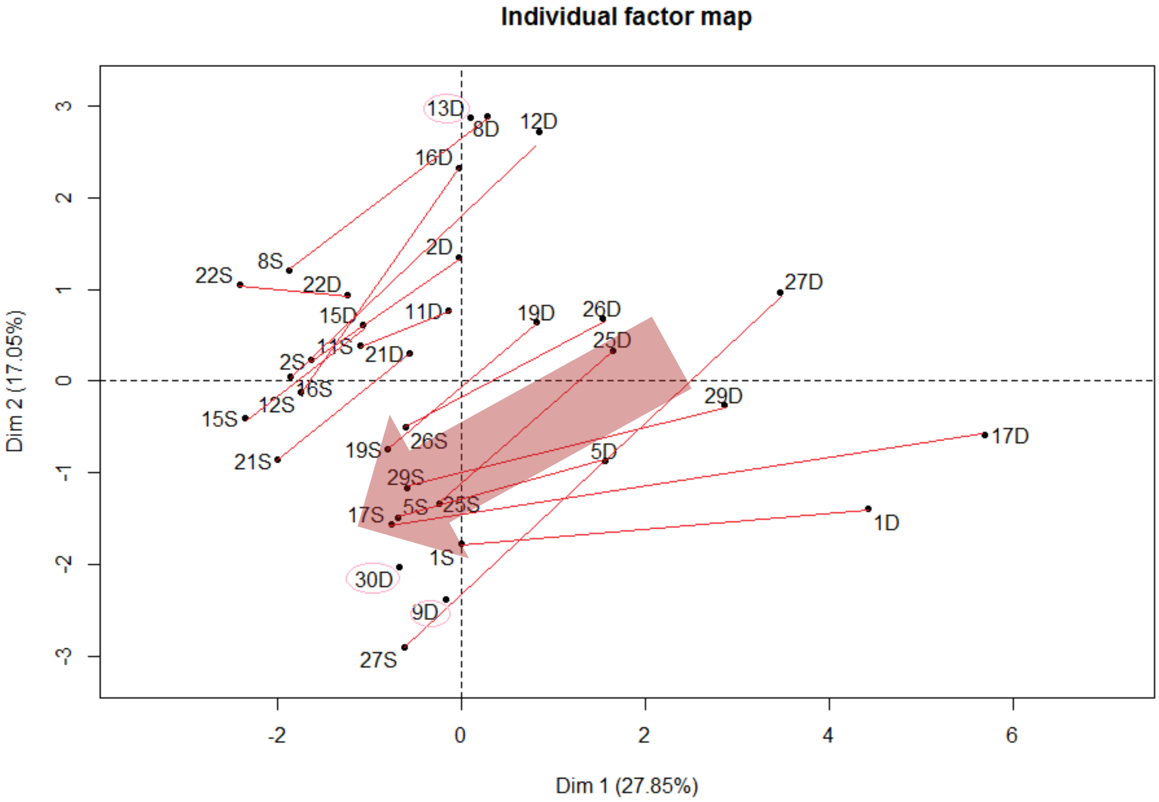


Figure 28: FAMD2: Individual factor map of the FAMD executed on all plots. All twin-plots are illustrated by lines connecting the separate plots. Most deciduous plots change in the direction of the red arrow when spruce is planted. Three spruce plots (36S, 30S and 9S) were not included in the analysis, so could not be paired.

When pairs of plots (twin-plots) are connected on the map of the individuals (figure 28) a general direction of deciduous to spruce plots can be observed. This direction is highlighted by the red arrow in both figure 27 and 28. From all 20 twin-plots, there are three spruce plots (9S, 30S and 36S) that could not be included in the analysis due to missing earthworm data.

3.1.4 ABIOTIC AND BIOTIC RELATIONS

Because in both FAMD's the variable pH is closely related to the first principal component, this variable is looked at in more detail. First the link is made for pH with geological formation and humustype.

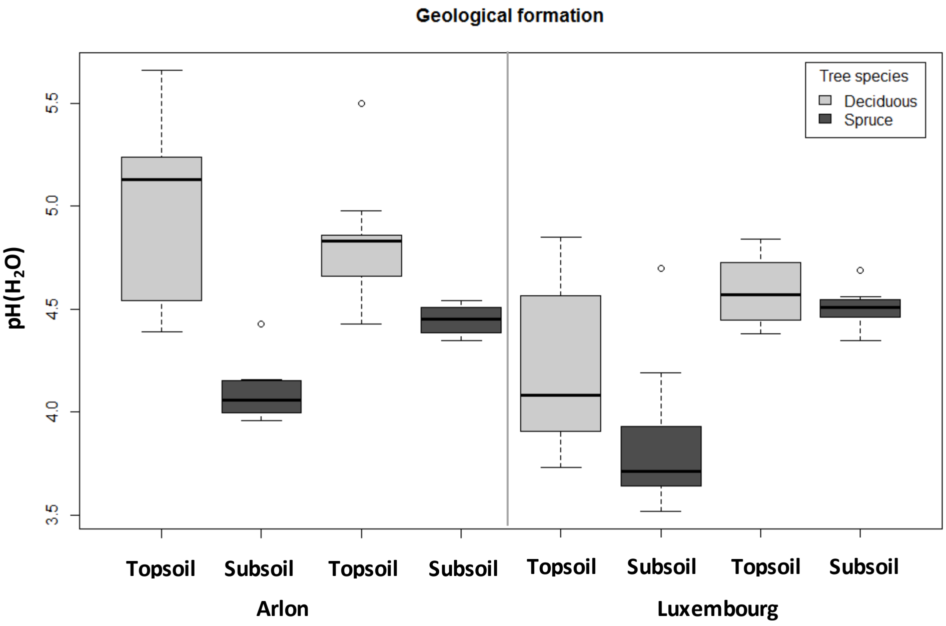


Figure 29: Boxplots of the geological formation Arlon (four most left boxplots) and Luxembourg (four most right boxplots) for topsoil and subsoil pH-values.

Figure 29 shows that for plots with the formation of Arlon as geological substrate, pH values are higher. Within both formations subsoil samples have a higher median pH-value and lower variability.

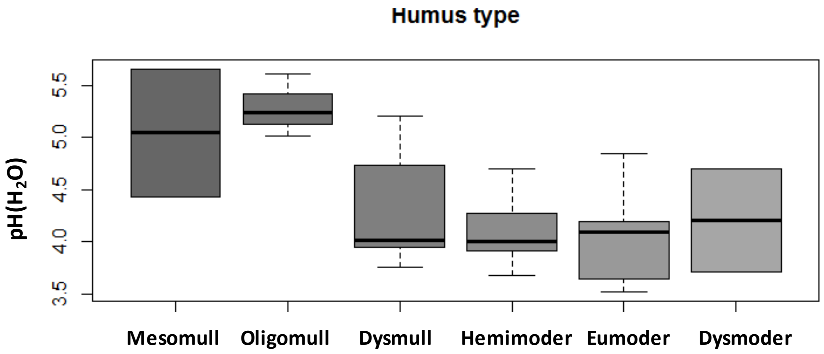


Figure 30: Boxplots of different humustypes (Mesomull, Oligomull, Dysmull, Hemimoder, Eumoder, Dysmoder) for topsoil pH(H20) values.

Figure 30 shows that mull humus types have higher pH-values (taken in the topsoil) than moder humus types. Only two deciduous plots in the dataset have a mesomull humus type and 3 deciduous plots have an oligomull humus type. For these two mull types there are no plots with spruce in the dataset. Plots with spruce have lower pH-values, as mentioned before. Also the difference in humustype between the two geological substrates was tested. Humustypes of plots with the formation of Arlon as geological substrate have significantly lower (Mann-Whitney U, $p < 0,05$) values of humustype (and thus higher quality types).

In the following graphs when pH or clay percentage is plotted, the x-axis represents the increase of acidification (and thus the decrease of pH) or the decrease of intrinsic soil fertility (and thus the decrease of clay percentage). For interesting relations with pH an additional plot of the specific variable in function of base saturation is made. Also the correlation between pH and BS is tested. The graphs of BS are based on 10 twin-plots (20 observations), whereas pH-plots are based on 20 twin-plots (40 observations).

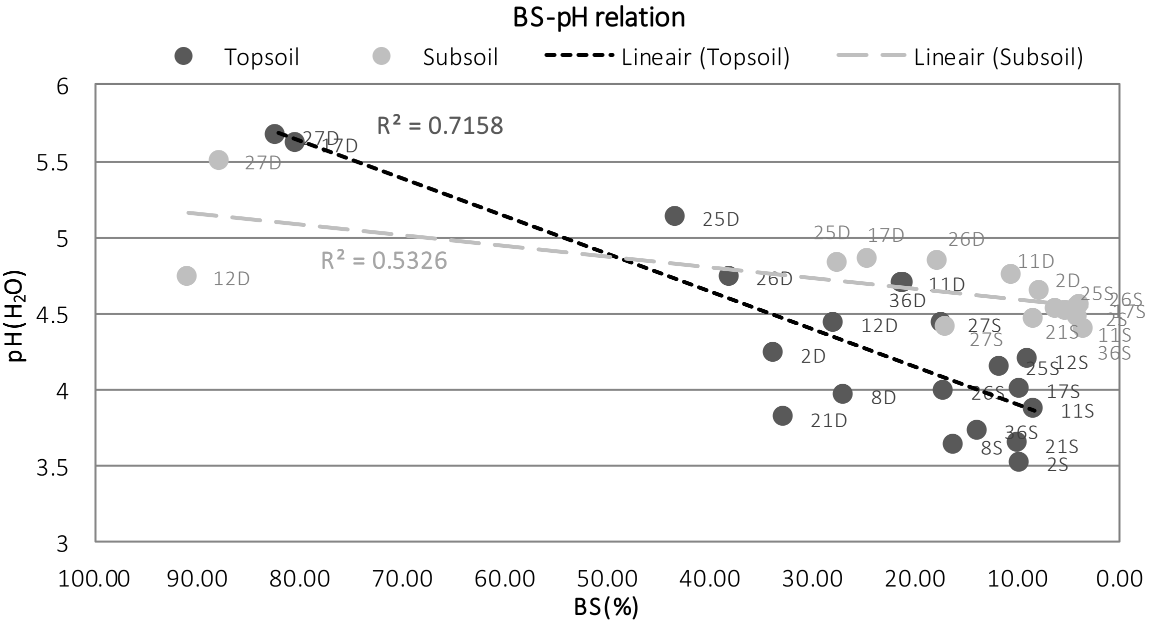


Figure 31: 20 twin-plots are plotted for pH(H_2O) and base saturation. The x-axis shows the increase in acidification (and thus the decrease of BS). Topsoil samples are coloured black and subsoil samples are coloured grey.

In figure 31 the pH-values of topsoil and subsoil samples are plotted for decreasing base saturation. Because the hypothesis is made that with soil acidification pH and BS decrease, the correlation of both variables is tested. The correlation coefficients equal 0,8460 (Spearman test, $p < 0,001$) for topsoil samples and 0,7298 (Spearman test, p <

0,05) for subsoil samples. Note the substrate variation in pH and BS for the deciduous plots, while all spruce plots have a pH below 4,6 and a BS below 17%, both for topsoil and subsoil.

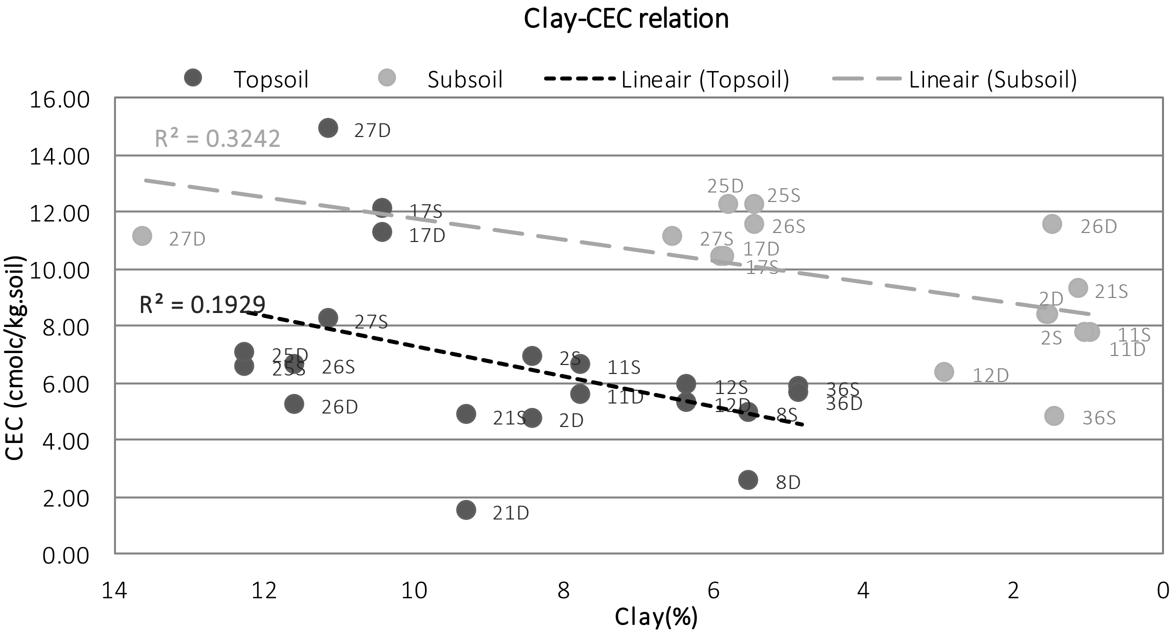


Figure 32: 20 twin-plots are plotted for CEC and clay-percentage. A distinction is made between topsoil samples (dark) and subsoil samples (grey).

With a spearman correlation test the relation between CEC and clay content was tested: for topsoil CECs the correlation coefficient equals 0,4558 ($p < 0,05$) and for topsoil CECs the correlation coefficient equals 0,5727 ($p < 0,05$).

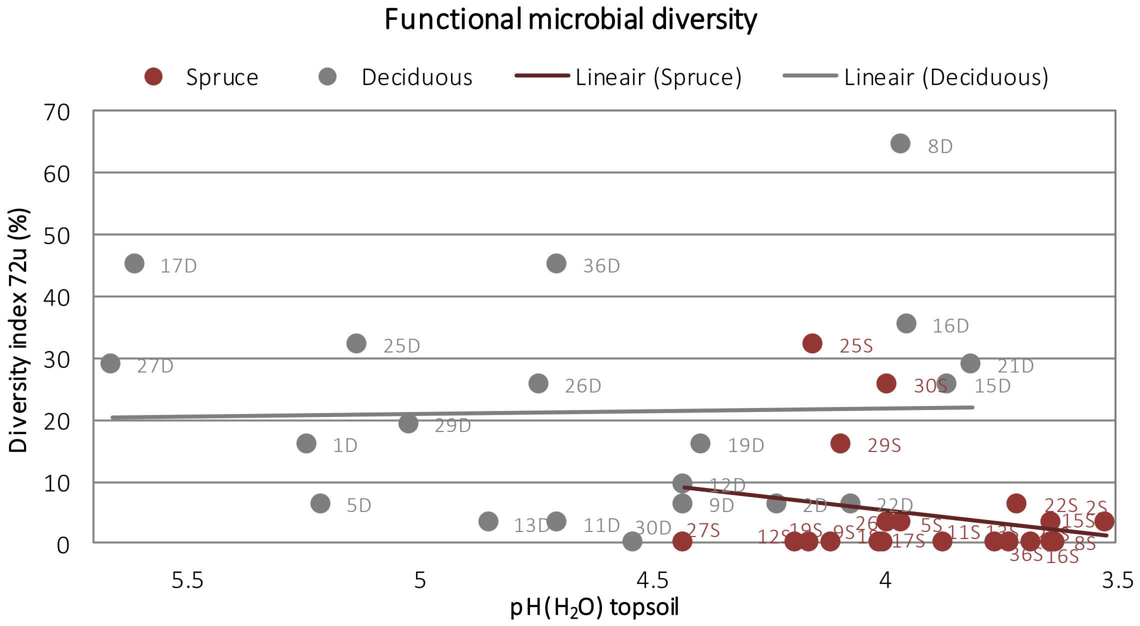


Figure 33: Functional microbial diversity measured by the diversity index of biologs after 72u in function of decreasing pH-values. The x-axis represents the increase in soil acidity.

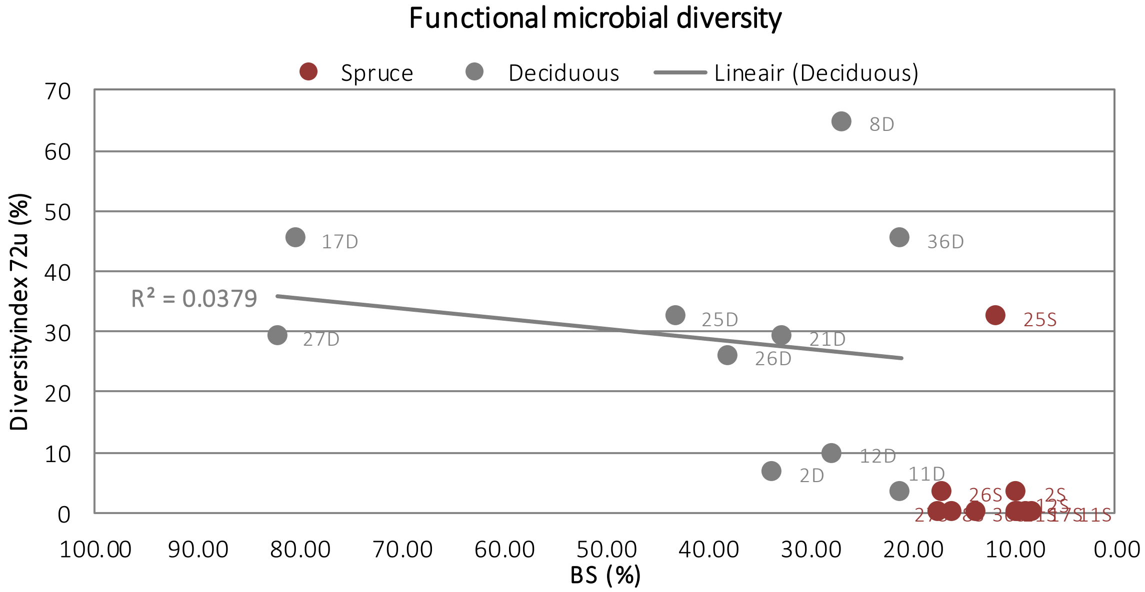


Figure 34: Functional microbial diversity measured by the diversity index of biologs after 72u in function of decreasing base saturation. The x-axis represents the increase in soil acidity.

Figure 33 and 34 show the functional microbial diversity decreases when soil acidity increases (pH and base saturation decrease). The first graph includes 20 twin-plots whereas the graph for BS is based on only 10 twin-plots. Spruce plots (red) are in both graphs furthest along the axis in soil acidity and have low functional microbial diversity. Deciduous plots (grey) have a large variation in functional microbial diversity, relatively unrelated to pH and BS. This high variability is nearly gone when the plots are conversed to spruce. With the exception of plots 25S, 29S and 30S, which are located near the end of the cuesta back. During the fieldtrip high understory growth was observed for these plots. This shows the effect of tree species versus abiotics: the location of the plots may slow the effect of the spruce trees.

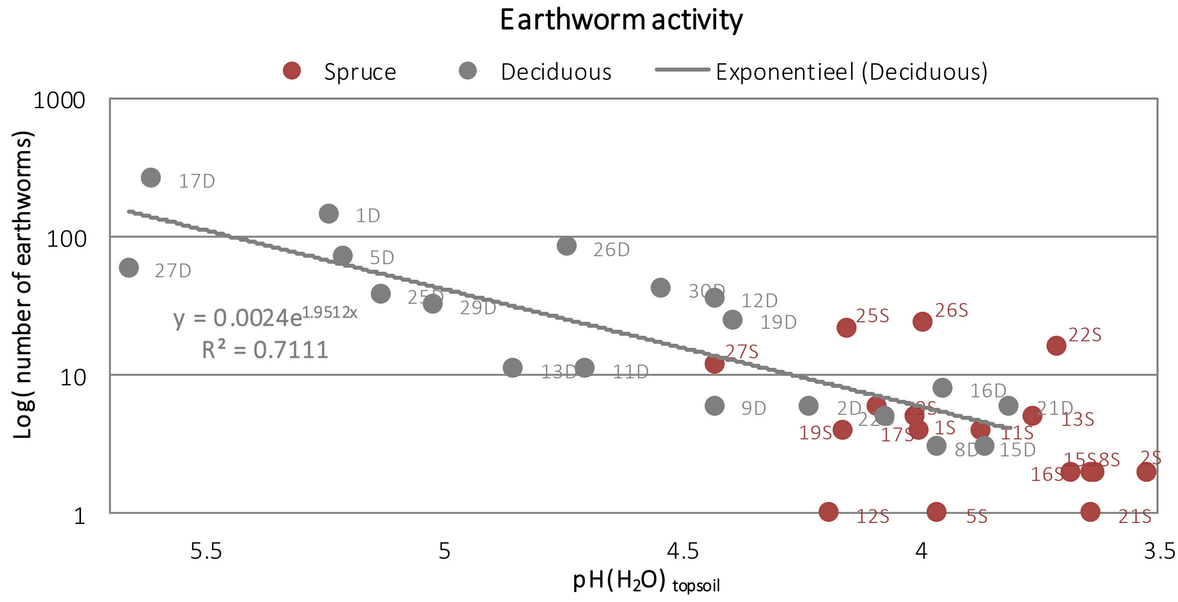


Figure 35: The number of earthworms (log transformed) are potted against decreasing pH-values. The x-axis represents the increase in soil acidity.

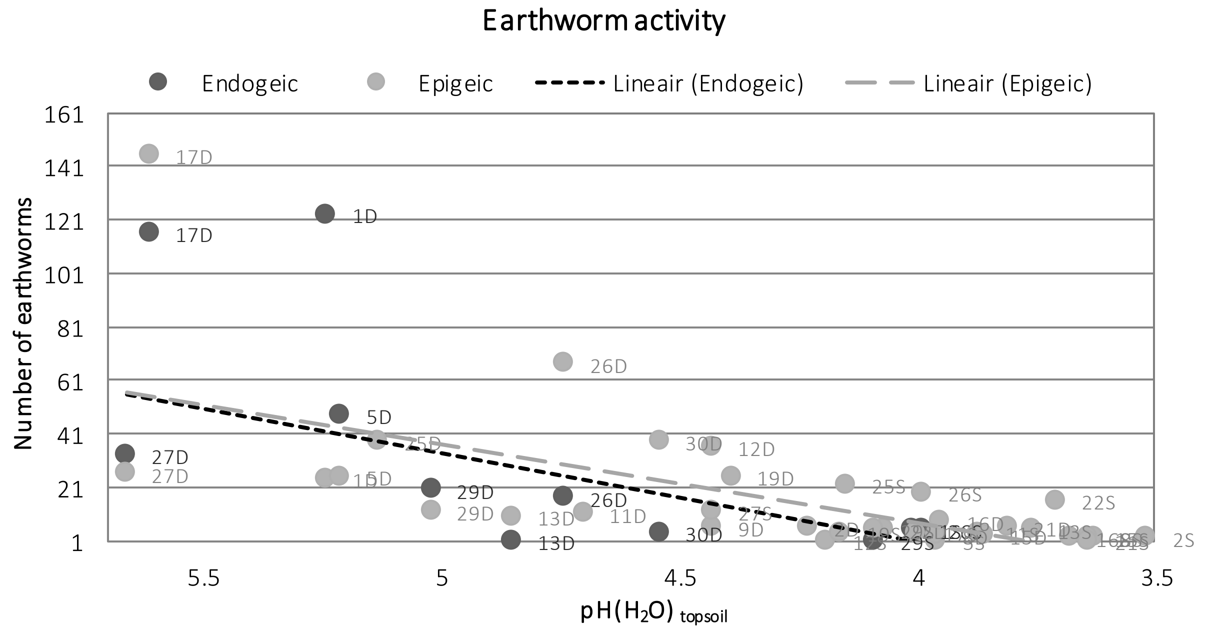


Figure 36: Number of endogeïc and epigeïc earthworms for decreasing pH-values. The x-axis represents the increase in soil acidity.

Figures 35 and 36 show the number of earthworms (log transformed) present for each sample of a plot in function of increasing soil acidity. Figure 36 makes a distinction between endogeïc and epigeïc earthworms. Both figures show that for increasing soil acidity (and thus decreasing pH) the number of earthworms decreases. In figure 35 can be seen that spruce plots (red) have less earthworm activity than their corresponding deciduous plot (green). Endogeïc earthworms approach zero for a pH-value of 4 whereas epigeïc earthworms tolerate the acidification longer.

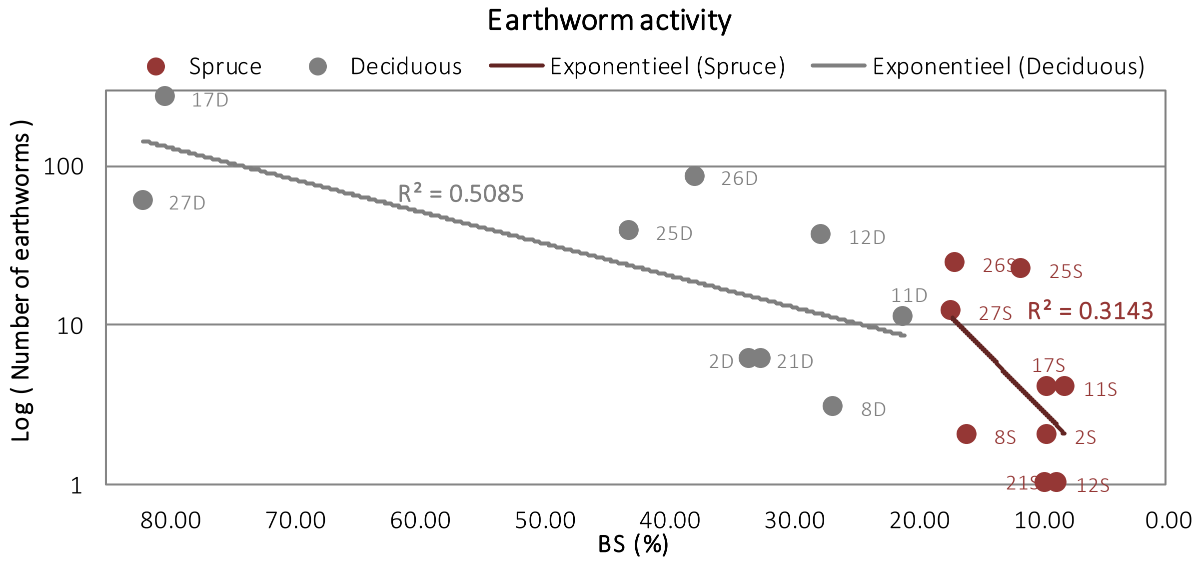


Figure 37: Number of earthworms (log transformed) for decreasing base saturation. The x-axis represents the increase in soil acidity.

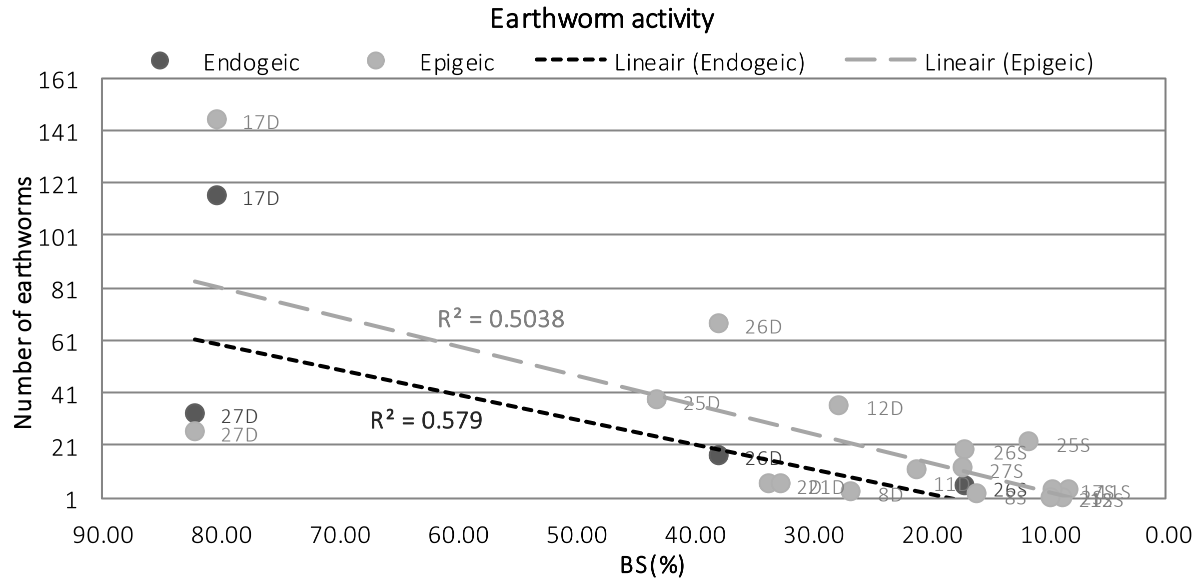


Figure 38: Number of earthworms for decreasing base saturation. The x-axis represents the increase in soil acidity. A distinction is made between endogeïc and epigeïc earthworms.

Figure 37 shows the number of earthworms (log transformed) for decreasing base saturation and figure 38 the number of endogeïc and epigeïc earthworms. In figure 37 spruce plots (red) have low earthworm activities whereas deciduous plots (grey) show a lot of variation: some plots have very low numbers whereas others have high numbers. The same trend as with pH can be observed for endogeïc and epigeïc earthworms: endogeïc earthworms approach zero for a higher base saturation than epigeïc earthworms. The thresholds seem to be at a base saturation of respectively 20% and 10%. Because so few plots have endogeïc earthworms this estimation is based on a very small dataset and thus can in reality be different.

3.2 CARBON ANALYSES

In this second part of the result section the Zimmermann fractionation and the carbon content and stability analyses are discussed.

3.2.1 TOTAL CARBON

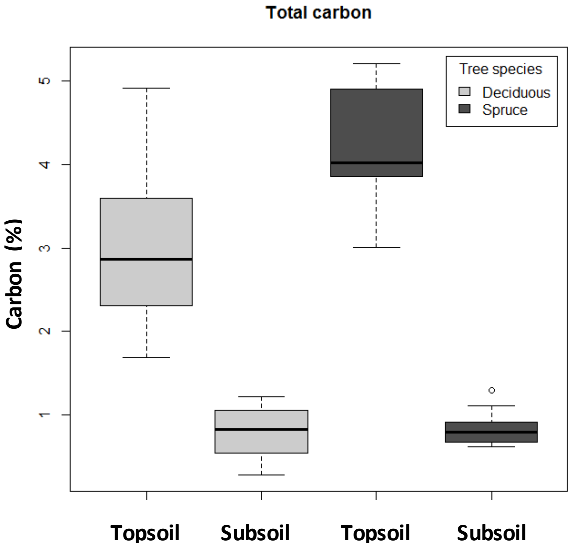


Figure 39: Total carbon contents of topsoil and subsoil samples. A distinction is made between deciduous plots (light) and spruce plots (dark).

In figure 39 the boxplot of total carbon content is shown. Topsoil samples have for both tree species higher median values than subsoil samples. The difference in tree species is larger for topsoil samples as for subsoil samples.

Table 5: results of hypothesis tests on total carbon. The difference between tree species and topsoil and subsoil was tested (H0: difference in means < or = or > zero) with t-test and Mann-Whitney U test. Differences are significant when the p-value is lower than 0,05.

	Tree species effect		Depth effect	
	Topsoil	**Subsoil**	**Spruce**	**Deciduous**
Significant?	**YES** (Spruce > Deciduous) t-test p-value = 0,006447	**NO** (Spruce ≯Deciduous) t-test p-value = 0,3818	**YES** (Topsoil > Subsoil) t-test p-value = 9,349e-09	**YES** (Topsoil > Subsoil) t-test p-value = 2,348e-05

Table 5 shows the result of hypothesis tests on the total carbon contents of topsoil and subsoil samples. Significant differences were found between topsoil and subsoil carbon. The difference between deciduous and spruce carbon contents was only significant for topsoil samples.

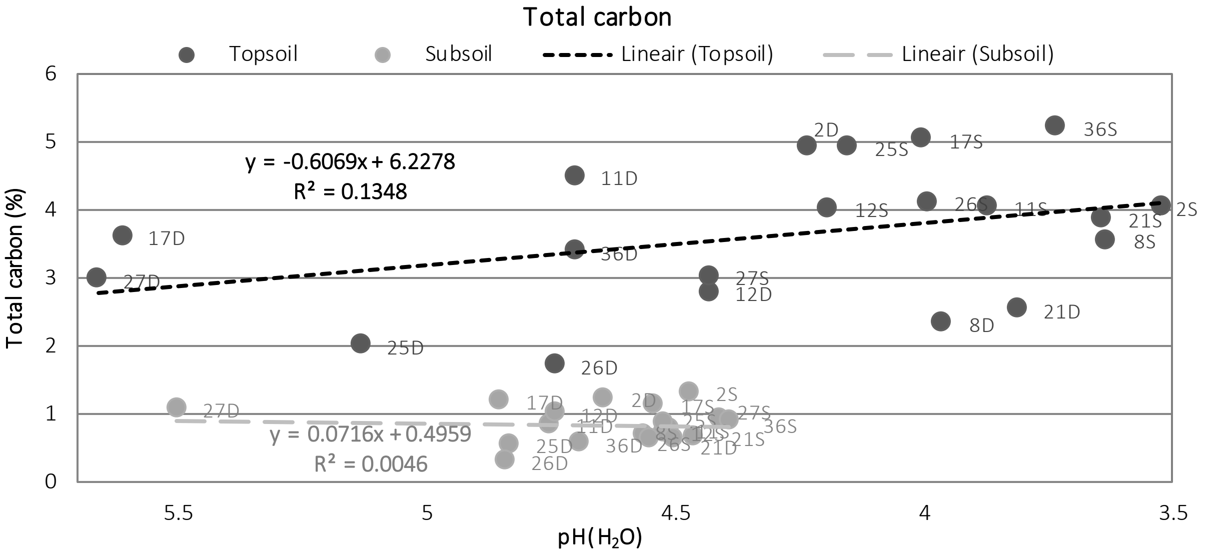


Figure 40: Total carbon of topsoil and subsoil samples for decreasing pH-levels.

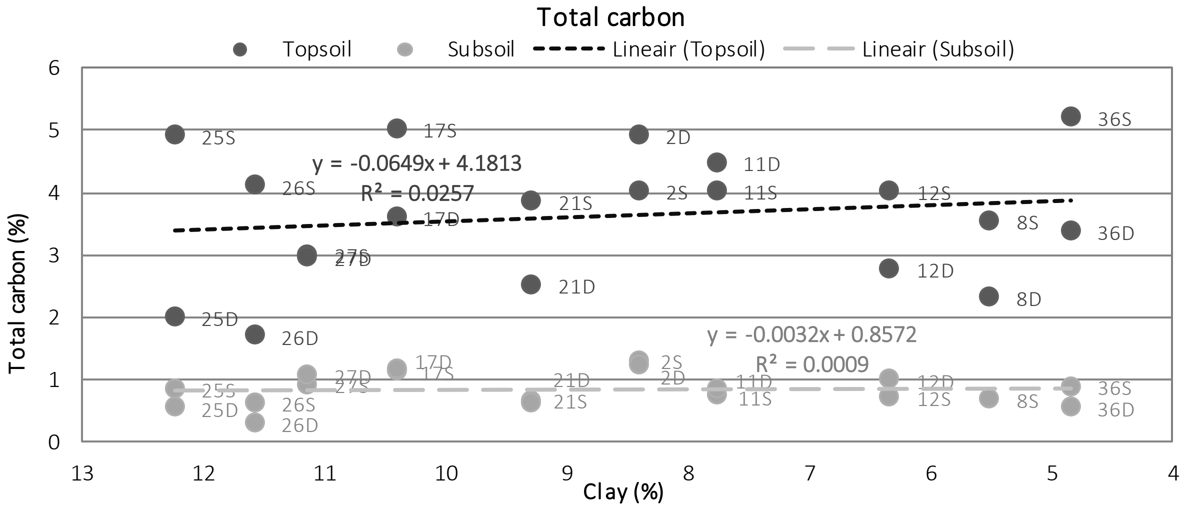


Figure 41: Total carbon of topsoil and subsoil samples for decreasing clay content.

Figure 40 and 41 show that for topsoil samples the total carbon increases with decreasing pH and decreasing clay content. No clear trend can be observed for subsoil samples, however the in comparison small range of subsoil total carbon is noted. Topsoil carbon is correlated with pH and humustype and is not correlated with clay content (correlation coefficients for topsoil carbon: rho $_{pH}$= -0.52 ($p < 0,05$), rho $_{humustype}$ = 0,47 ($p < 0,05$)). Subsoil carbon has low correlation for every abiotic variable.

Proof of concept

Because the earlier observed soil process domains could affect the carbon dynamics, three deciduous plots located in the base domain (plot 17, 25 and 27) and three deciduous plots located in the Fe-Al domain (plot 2, 8 and 21), are selected as a proof of concept. This selection was based on the results of BS and pH described before (see site characteristics). The total carbon stocks for these soil process domains are compared.

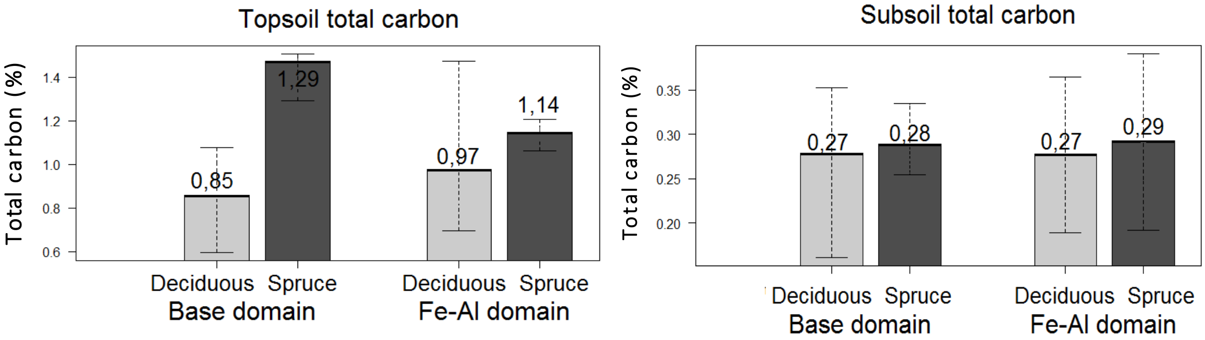


Figure 42: Proof of concept: effect of spruce conversion on topsoil (left) and subsoil (right) carbon stocks for plots initially located in the base domain and in the Fe-Al domain.

Figure 42 shows that from the base-dominated soil process domain (represented by plot 17, 25 and 27) towards the Al-Fe-dominated soil process domain, total carbon stocks increase in the topsoil. For subsoils the effect is minor.

3.2.2 ZIMMERMAN FRACTIONATION

The Zimmermann fractionation executed on 30g of soil sample, resulted in four separate SOC-fractions: sand and stable aggregates (S+A), silt and clay (s+c), particulate organic matter (POM) and dissolved organic carbon (DOC). As dissolved organic carbon weighs very little it is not concluded in the mass percentages (figure 43). From these 4 fractions the percentage carbon (and concentration carbon for DOC) was measured and is indicated in table 7. Using the mass composition and the percentage carbon of each fraction separately, the total carbon present in 30g soil sample was calculated. The percentage of this total carbon stored in each separate fraction is illustrated in figure 44. Because in every step of the fractionation some material could get lost it is important to measure the recoveries after every step. The final recovery of the topsoil samples is 102% and of subsoil samples 94%. These calculated recoveries ([(s+c)+(POM)+(S+A)]/input) can be higher than 100% because some residual SPT may be left and included when weighing the S+A and POM fraction. The carbon content of the SPT was measured to be zero, so that it does not bias the carbon analysis. Without this last step the recoveries ((s+c)+(POM and S+A)/input) equal 99% for topsoil samples and 94% for subsoil samples.

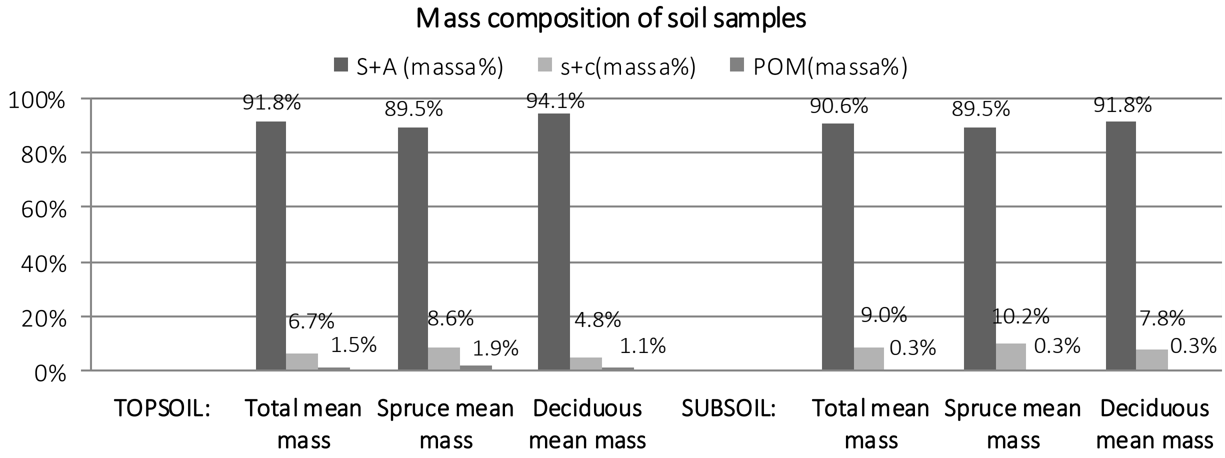


Figure 43: Mass percentages of Zimmermann fractionation for topsoil (left) and subsoil (right) samples.

Figure 43 shows the mass composition of topsoil samples (left) and subsoil samples (right). This histogram is based upon the mean values. An R-statistics shapiro test was used to determine which variables were normally distributed so that subsequently t-tests or Mann-Whitney U tests (non-parametrical test) could be executed.

Table 6: results of hypothesis tests in R on mass composition. The difference in mass was tested (H0: difference in means equals zero) with t-tests and Mann-Whitney U tests. Differences are significant when the p-value is lower than 0,05.

Significantly different from…		**TOPSOIL DECIDUOUS**	
	S+A	**s+c**	**POM**
TOPSOIL SPRUCE? (tree species effect)	**NO** Mann-Whitney U test p-value = 0,08921	**NO** Mann-Whitney U test p-value = 0,315	**YES** t-test p-value = 0,002767
SUBSOIL DECIDUOUS? (depth effect)	**YES** t-test p-value = 0,03722	**YES** t-test p-value = 0,003544	**YES** t-test p-value = 0,0008753

		SUBSOIL SPRUCE	
	S+A	**s+c**	**POM**
TOPSOIL SPRUCE? (depth effect)	**NO** Mann-Whitney U test p-value = 0,393	**NO** Mann-Whitney U test p-value = 0,1051	**YES** Mann-Whitney U test p-value = 1,083e-05
SUBSOIL DECIDUOUS? (tree species effect)	**NO** t-test p-value = 0,1924	**NO** t-test p-value = 0,1878	**NO** Mann-Whitney U test p-value = 0,6305

Table 6 shows the results of hypothesis-testing on the mass composition in R. For topsoil samples there is a significant difference in mass percentage for tree species for the POM-fraction whereas no significant differences for tree species can be observed in subsoil samples. Within deciduous plots all fractions show significant differences between topsoil and subsoil samples. Within spruce plots only for the POM-fraction a significant difference is observed.

Table 7: Carbon percentages of each SOC-fraction separately.

	Topsoil deciduous	Topsoil spruce	Subsoil deciduous	Subsoil spruce
S+A	2,7257 %C	3,4669 %C	0,4447 %C	0,5515 %C
S+c	5,4338 %C	7,3861 %C	2.5808 %C	2,5623 %C
POM	23,4584 %C	26,2643 %C	20,0961 %C	22,5715 %C
DOC	6,6472 mg C/L	9,8538 mg C/L	4,6040 mg C/L	4,6106 mg C/L

By combining the mass composition results with the carbon percentages of each fraction (table 7), the SOC-composition can be calculated. In figure 44 the mean SOC-composition of topsoil and subsoil samples is shown. The carbon percentages are calculated by dividing the carbon in a specific fraction by the sum of carbon in all fractions (the total carbon in the sample). By example: only 1,5% of the mass composition of topsoil samples is POM but of this small mass 24,8% is carbon so that when the total carbon composition is calculated topsoil POM-fractions hold 10% of all carbon.

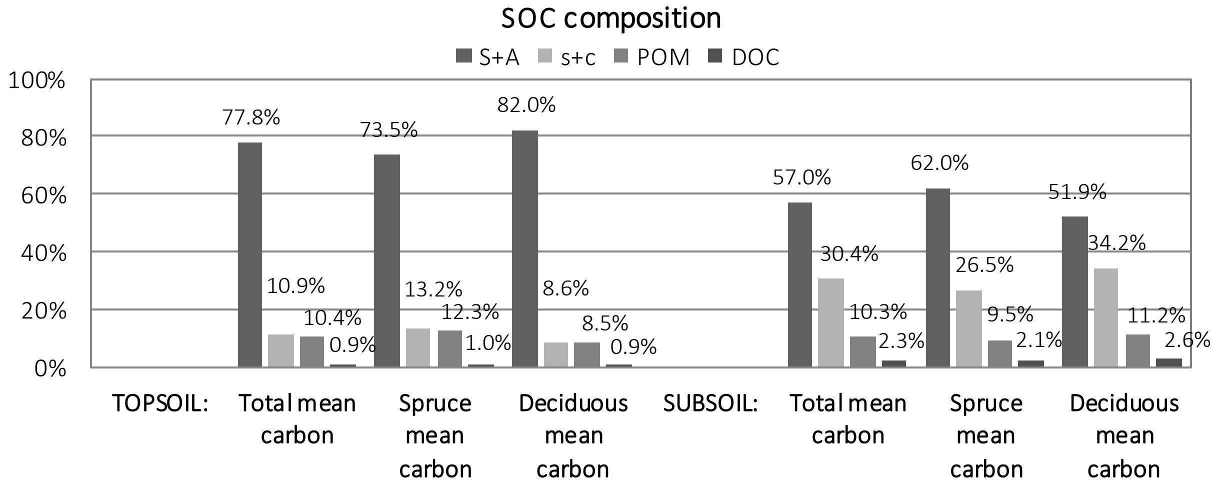


Figure 44: Carbon percentages of Zimmermann fractions for topsoil (left) and subsoil (right) samples. An extra distinction is made between spruce plots and deciduous plots.

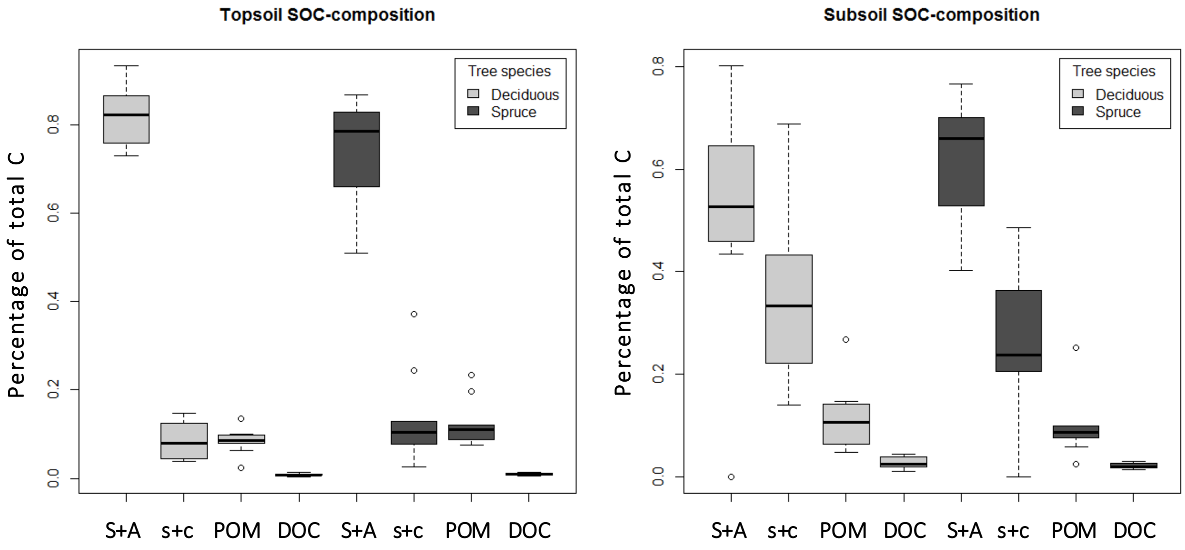


Figure 45: Boxplots of carbon percentages of Zimmermann fractions for topsoil (left) and subsoil (right) samples. An extra distinction is made between spruce plots (red) and deciduous plots (green).

Figure 45 shows the carbon composition of topsoil and subsoil samples. The median percentage carbon in the s+c fraction is higher for subsoil samples than for topsoil samples. In order to execute the proper hypothesis-test, the data were first checked for normal distributions.

Table 8: results of hypothesis tests in R. The difference was tested (H0: difference in means equals zero) with t-test and Mann-Whitney U test. Differences are significant when the p-value is lower than 0,05.

Significantly different from…	**TOPSOIL DECIDUOUS**			
	S+A	**s+c**	**POM**	**DOC**
TOPSOIL SPRUCE? (tree species effect)	**NO** Mann-Whitney test p-value = 0,1655	**NO** Mann-Whitney test p-value = 0,393	**NO** Mann-Whitney p-value = 0,06301	**NO** t-test p-value =
SUBSOIL DECIDUOUS? (depth effect)	**YES** t-test p-value = 0,001636	**YES** t-test p-value =	**NO** t-test p-value = 0,2477	**YES** t-test p-value =

	SUBSOIL SPRUCE			
	S+A	**s+c**	**POM**	**DOC**
TOPSOIL SPRUCE? (depth effect)	**YES** Mann-Whitney test p-value = 0,02881	**YES** Mann-Whitney test p-value = 0,03546	**YES** Mann-Whitney test p-value = 0,03546	**YES** t-test p-value =
SUBSOIL DECIDUOUS? (tree species effect)	**NO** t-test p-value = 0,2203	**NO** t-test p-value = 0,261	**NO** Mann-Whitney test p-value = 0,4359	**NO** t-test p-value =

Table 8 shows the results of the hypothesis tests (Ho: difference in means equals zero) on the SOC-composition data. Within topsoil and subsoil samples there is no fraction with a significant difference in carbon for tree species. For the deciduous plots there are significant differences in topsoil and subsoil samples for all fractions but the POM-fraction and for spruce plots all fractions have significant differences in carbon between topsoil and subsoil samples.

3.2.3 CARBON STABILITY IN THE TOPSOIL

The four different pools discussed above can be simplified into one stable pool (S+A and s+c) and one labile pool (POM and DOC).

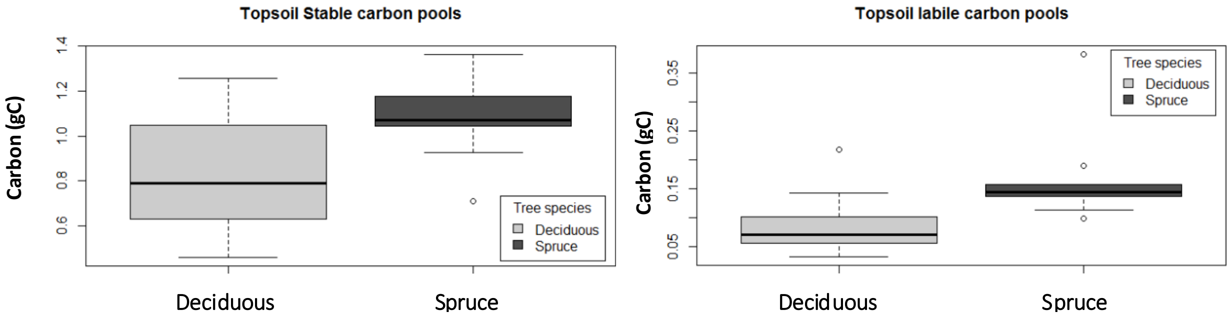


Figure 46: Carbon (gC per 30g soil sample) stored in stable (left) and labile (right) pools of topsoil samples. A distinction is made between deciduous plots (light) and spruce plots (dark).

Figure 46 shows that absolute the amount of carbon is greater in spruce plots. Both for stable pools (t-test: p < 0,05) as for labile pools (Mann-Whitney U test: p < 0,05) there is more carbon in spruce plots than in deciduous plots.

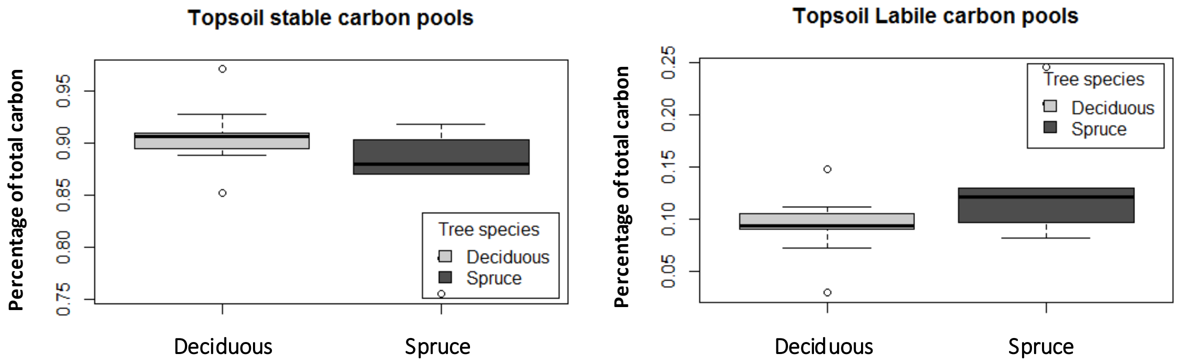


Figure 47: Boxplot of percentages of total carbon in topsoil samples for stable (left) and labile pool (right). A distinction is made between deciduous plots (light) and spruce plots (dark).

Figure 47 shows the percentage of topsoil total carbon that is stored in respectively stable and labile pools (thus, the sum of stable and labile is 100%). The percentage of total carbon stored in stable pools is significantly lower for spruce plots (Mann-Whitney U test: $p < 0,05$) and thus parallel with this trend, the percentage of total carbon stored in labile pools is significantly higher for spruce plots.

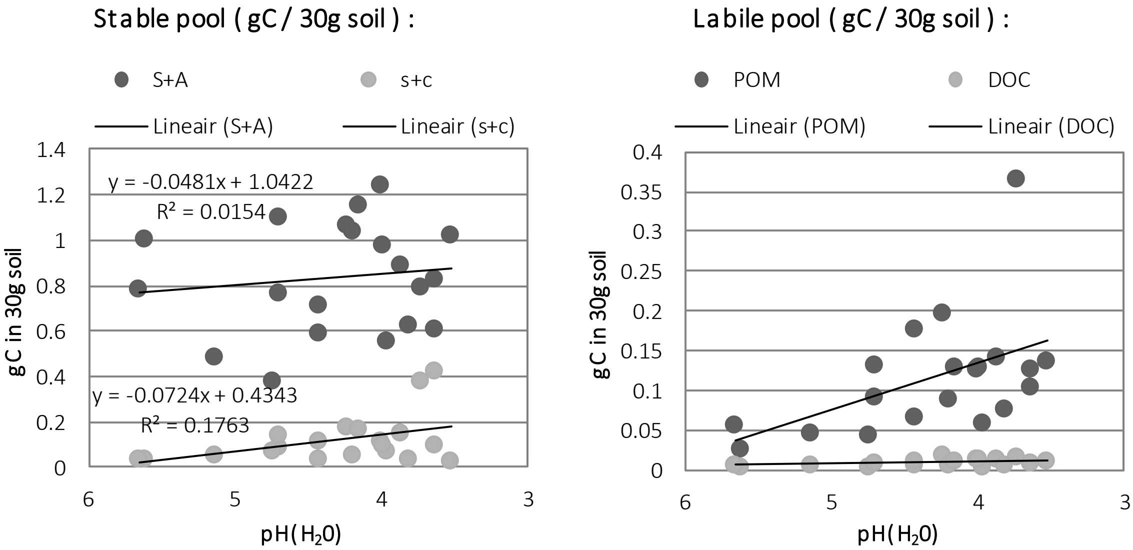


Figure 48: Mass of carbon in fraction for decreasing pH-values. A comparison is made between the stable pools S+A and s+c (left) and labile pools POM and DOC(right).

Figure 48 shows the mass (in g) of carbon present in each fraction (from a 30g soil sample) for decreasing pH-values. Stable carbon fractions S+A and s+c have a small increase in carbon for decreasing pH, whereas the increase in carbon for labile fractions is higher.

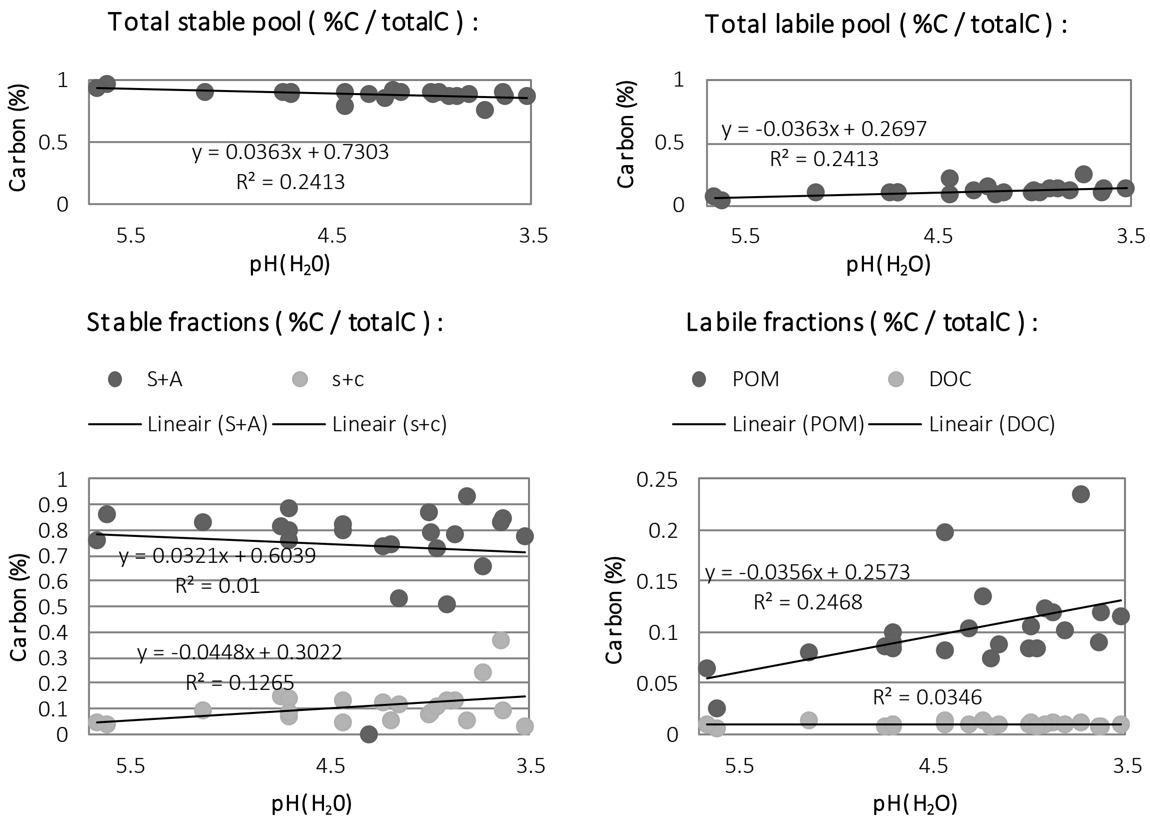


Figure 49: Percentage of total carbon present in fraction for decreasing pH-values. A comparison is made between stable pools S+A and s+c (left) and labile pools POM and DOC (right). The top graphs show the total pools and the bottom graphs show the separate fractions.

In figure 49 the percentage of total carbon stored in stable and labile pools is shown as function of decreasing pH-values. As stable and labile pools together account for 100% the decrease in stable pools for decreasing pH is parallel to the increase in labile pools. When stable pools are divided by labile pools, the carbon stability can be observed.

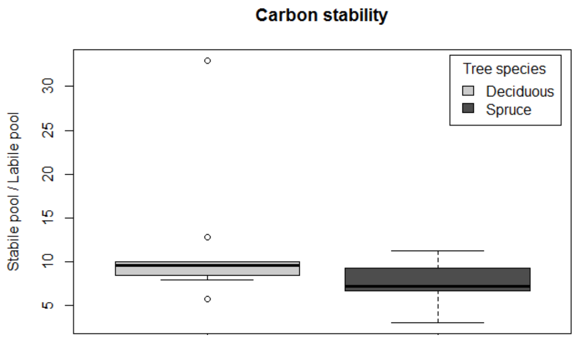


Figure 50: Boxplot of carbon stability (Stable pool / Labile pool) for topsoil deciduous samples (light) and spruce samples (dark).

Figure 50 shows the boxplots of carbon stability for topsoil samples. Spruce plots have significantly lower (Mann-Whitney test: $p < 0,05$) carbon stability than deciduous plots.

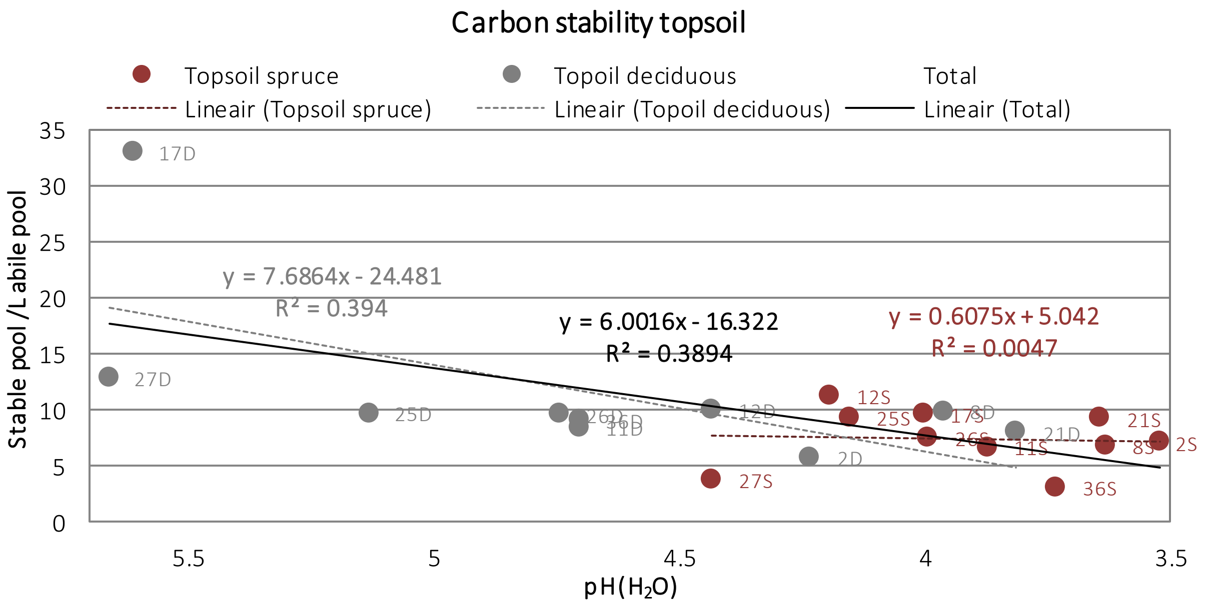


Figure 51: Carbon stability of topsoil samples for decreasing pH-values (and thus increasing soil acidification). A distinction is made between spruce plots (red) and deciduous plots (green).

Figure 51 shows the carbon stability for topsoil samples. Both for deciduous as for subsoil plots the carbon stability decreases with increasing soil acidity (decreasing pH). The total carbon stability is positively correlated with the pH-values (rho_{pH}=0,6240: p < 0,05).

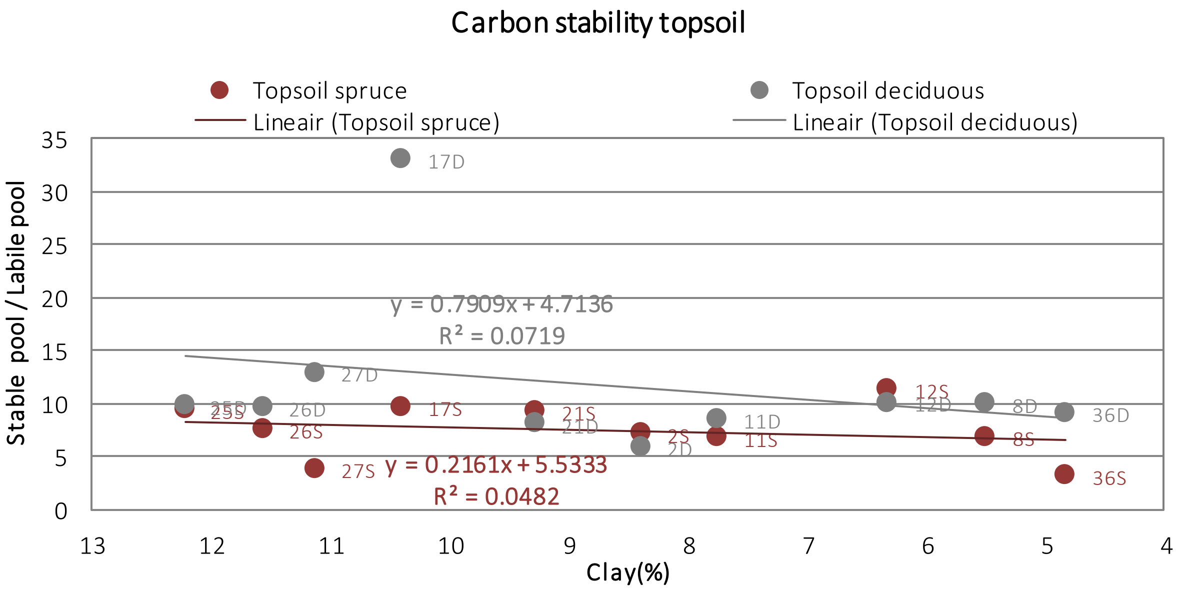
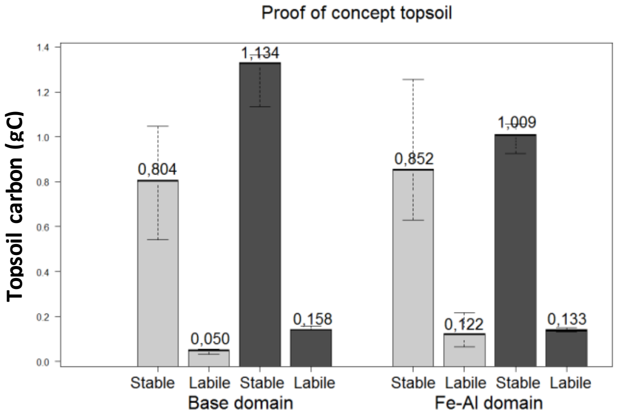


Figure 52: Carbon stability of topsoil samples for decreasing clay content (and thus decreasing soil fertility). A distinction is made between spruce plots (red) and deciduous plots (green).

Figure 52 shows that carbon stability decreases with decreasing clay contents. However the correlation is not significant, this could be due to the extreme low slope of the relation.

Proof of concept



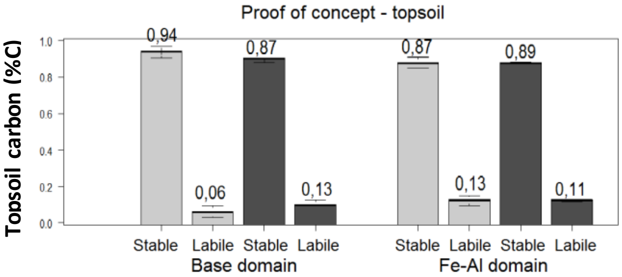


Figure 53: Proof of concept: carbon composition in gC (top) and in % of total carbon (bottom) of plots located initially in the base domain and in the Fe-Al domain. A distinction is made between deciduous plots (light) and spruce plots (dark)

Figure 53 shows the SOC-distribution over stable and labile pools for plots that were initially located in base dominated domain and for plots that are located in the Fe-Al-dominated domain, as a proof of concept. As total carbon storage increases (see figure 42) for the Fe-Al domain, more carbon in absolute values is stored in both stable and labile fractions. However, in the base dominated domain a higher percentage of carbon is stored in stabile fractions. Hence, carbon stability is higher for the base domain (see figure 54).

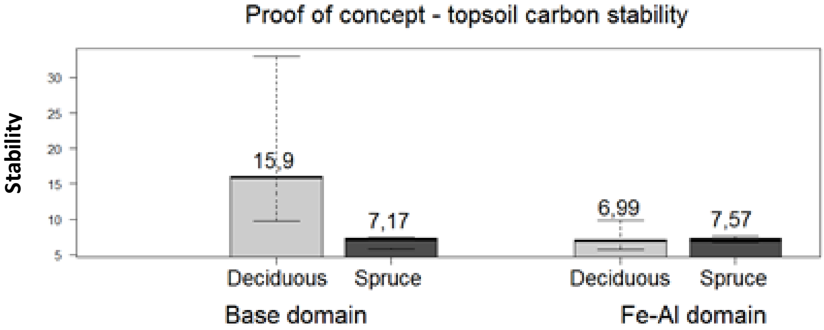


Figure 54: Proof of concept: carbon stability of plots in the base domain and of plots in the Fe-Al domain. A distinction is made between deciduous plots (light) and spruce plots (dark).

3.2.4 CARBON STABILITY IN THE SUBSOIL

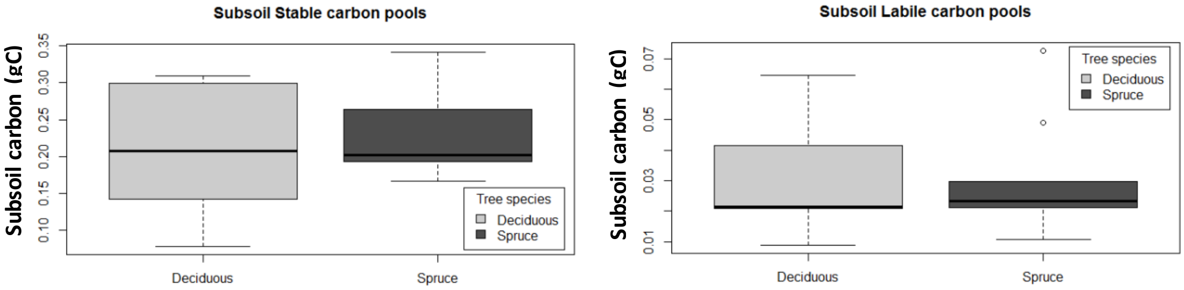


Figure 55: Carbon (gC per 30g soil sample) stored in stable (left) and labile (right) pool of subsoil samples. A distinction is made between deciduous plots (light) and spruce plots (dark).

Figure 55 shows the boxplots of the amount of carbon (in gC) stored in stable and labile pools. Both in stable pools as in labile pools there is no significant difference between spruce plots and deciduous plots. In addition, there is no significant difference (Mann-Whitney U test, p > 0,05) in the division of carbon (in %C) over stable and labile pools between spruce plots and deciduous plots.

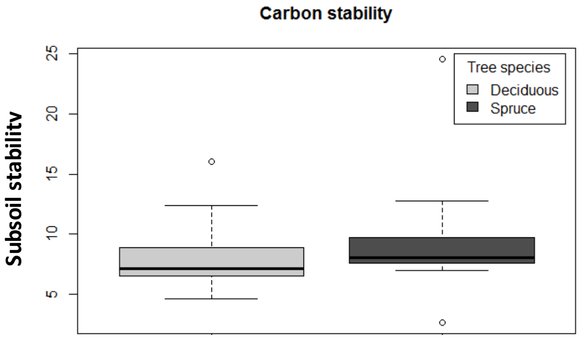


Figure 56: Boxplots of carbon stability in subsoil deciduous samples (light) and spruce samples (dark).

The boxplots in figure 56 show a higher median carbon stability in subsoil spruce samples, however the mean carbon stability of spruce plots is not significantly higher (Mann-Whitney U test, p > 0,05) than the carbon stability of deciduous plots.

4 DISCUSSION

4.1 SITE EVALUATION

4.1.1 LITHOLOGICAL GRADIENT AND PLOT SELECTION

The study site is characterized by two geological parent materials: the Arlon formation on top and the in comparison poorer Luxembourg formation below. Due to topography and river incisions, the Arlon formation is more eroded and at some plot locations even not present anymore. The result is a gradient in soil fertility as a consequence of the intertwining geological formations and the mixture of their weathering products with löss deposits, as Verstraeten (2013) already described. This gradient is confirmed in the study of Brock (2015). The lithological gradient is approximated in the results by the clay content of each twin-plot. The relief of the study site is formed by a cuesta with its front to the north and its cuesta back to the south (figure 57). The cuesta is incised by two river-arms and in these incisions löss deposits can be present on the north faced slopes. Because of this specific relief, plots located in the river incisions of the cuesta back may receive extra nutrients through subsurface flow coming from the top of the cuesta. Plot 27 seems to be located in such a seepage zone and is characterized by higher quality for every abiotic and biotic variable.

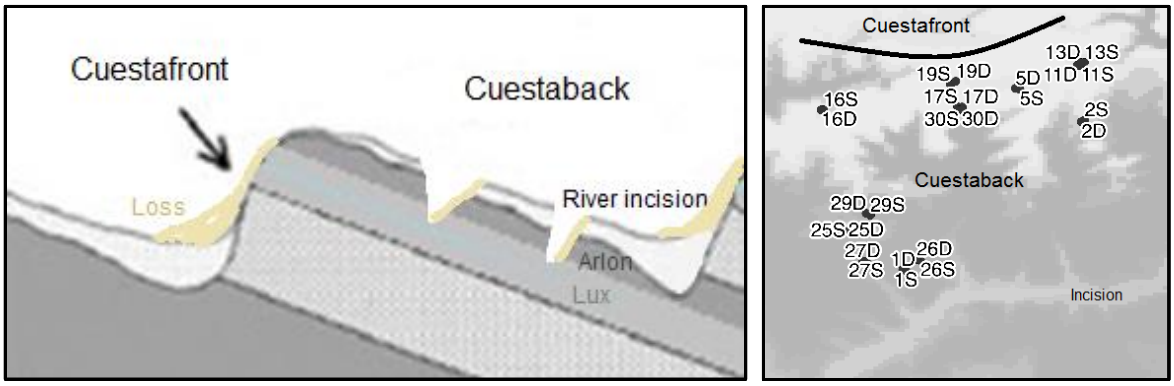


Figure 57: Schematic illustration of cuesta (left) and topographic map of the study site with indication of the cuesta (right).

As the results of the deciduous plots for clay content, CEC, pH and BS show a good distribution along the gradient (see figures 31 and 32), the dataset of 20 twin-plots is a good representation of the natural gradients present in the study site. The subset of 10 twin-plots also spans the natural gradients present.

4.1.2 EFFECTS OF SPRUCE CONVERSION ON SOIL ABIOTIC FACTORS

Previous studies already indicated significant differences in topsoil pH, BS and other nutrients under spruce monocultures (Reich et al., 2005; Hagen-Thorn et al., 2004; Binkley and Valentine; 1991). Hence, spruce is indeed a tree species with strong ecological effects (Ranger ad Nys, 1994; Augusto et al., 2002; de Schrijver et al., 2007; Verstraeten, 2013). Most researches also report the smaller impact of tree species on subsoils (Hagen-Thorn et al., 2004). However, this smaller impact could in fact be a time lag of the acidification as studies on older trees indicate a larger impact in the subsoil (Nordén, 1994).

Corresponding with the literature, this study indicates that spruce conversion results in significant lower pH-values (see figure 19). The overstory tree conversion has the strongest effect on topsoil pH and seemingly does not affect the subsoil dramatically (as the difference for tree species is larger in topsoil samples than in subsoil samples). The underlying lithological gradient co-determines the range of the shift when conversed into spruce, i.e. the highest quality plots acidify more on the same time span. Thus, the hypothesis (1) that the conversion induces soil acidification, can be confirmed.

The CEC is determined by the clay content and the amount of organic matter present in the soil (de Schrijver et al., 2010). This is reflected in the results (figure 20) by the correlation of the CEC of both topsoil and subsoil with the clay content of the plot (i.e the higher the clay content, the higher the CEC). As clay content of the soil remains constant for an overstory conversion, the CEC would only increase for increasing organic matter. The results indicate that the CEC is not affected by the spruce conversion as the CEC does not significantly differ between spruce and deciduous plots. Because the pH was not buffered in this CEC-measurement (eCEC was measured), the drop in pH counteracts the effect of increasing organic carbon under spruce and hence the cation exchange capacity remains the same.

For BS the same trend as for pH is observed: the spruce conversion results in significantly lower BS for both topsoil and subsoil (figure 21). However, the difference between tree species in subsoil samples is larger for BS than for pH. Subsoil BS seems to collapse when spruce is planted: all plots have a BS lower than 8%. This may be a consequence of vertical decoupling, i.e. no more nutrients reach the subsoil (Muys and

Lust, 1992, Ulrich, 1992). One outlier is observed: plot 27 that still receives nutrients through subsurface flow. Thus, base saturation is affected throughout the soil profile, even when the pH still shows limited change. Topsoil BS is modulated by the underlying lithological gradient. In other words, soils with high clay contents, have higher BS for deciduous plots but also have more to lose when the overstory vegetation is conversed.

The lower BS is thus most probably a consequence of the recycling of bases from the litter layer which stops their transfer to the soil (Ponge, 2013). This is confirmed by changes in the litter layer and biological activity (vide infra). Hobbie et al. (2006) mention the higher quality of litter under deciduous trees, which corresponds with better humustypes. Also this research indicates the negative effect of spruce conversion on the humustype as spruce plots have significantly lower humus quality. Ponge et al. (2011) state that geology is a primary factor (together with climate) in determining the humustype. Corresponding with this theory, plots with the formation of Arlon as geological substrate have significantly better humustypes compared with plots that lie upon the formation of Luxembourg. On the other hand, the difference in humustype between spruce and deciduous plots is not correlated with the clay content and thus the underlying lithological gradient.

4.1.3 EFFECTS OF SPRUCE CONVERSION ON SOIL BIOTIC FACTORS

Geological parent material and in particular acidity are important for microbial communities (Brock, 2015). Bacteria thrive in good quality and fast decomposing systems whereas fungi outcompete bacteria when pH and or litter quality is low (Kooijman et al., 2014). This is reflected in the results as microbial diversity in the soil profile decreases when the overstory is conversed (see figure 23). Because of the accumulation of a thick litter layer under spruce trees, microbial activity moves up to the food source and a sharp transition between the litter layer and the topsoil is observed. This sharp transition corresponds with the theory of vertical decoupling of the profile (Ponge, 2013, Muys and Lust, 1992, Ulrich, 1992). Hence, the spruce conversion results in higher functional microbial diversity in the litter layer and a significant decrease of functional microbial diversity in the soil profile. For both acidification fronts (pH and BS), the highly variable deciduous plots collapse into a state of low microbial diversity and low variability when the deciduous forest is conversed into a spruce plantation.

The process of vertical decoupling is also reflected in the earthworm community (Muys and Lust, 1992; Ulrich, 1992). Several studies (Reich et al., 2005; Piearce, 1972; Hendriksen, 1990; Edwards, 1998; Hendrix et al., 1999) reported the high correlation between earthworm abundance and litter quality. Reich et al (2005) specifically attribute this positive effect to the Ca-content of the litter, which is lower for spruce trees. In addition to the effect of bad quality litter, Muys and Granval (1997) report the adverse effect of acidification and the corresponding Al-toxicity for earthworm communities. The results of this study coincide with the literature: the spruce conversion results in a collapse of the number of earthworms. Hence, for increasing soil acidification, the number of earthworms decreases. A distinction is made between endogeïc and epigeïc earthworms. As Muys and Granval (1997) already stated, the number of endogeïc earthworms drastically decreases when pH drops below a value 4. When the acidification continues also epigeïc earthworms disappear from the soil profile. The same trends are observed for BS-values: endogeïc earthworms seem to disappear when BS drops below 20% and epigeïc earthworms show a threshold at 10% BS.

4.1.4 DAMPENING OF SOIL GRADIENT BY SPRUCE CONVERSION

It has already been evidenced that spruce monocultures are characterized by low above-ground biodiversity compared to mixed deciduous forests (Mitchell and Kirby, 1989; Emmer et al., 1998). The narrowed biodiversity of monocultures (Tesař, 2000) can be observed in the results of this study. Variability for all abiotic and biotic variables collapses when the overstory tree species is conversed to spruce. Spruce systems are thus characterized by a smaller variability in soil properties and correspondingly a narrower ecological niche. Hence, the overstory conversion from deciduous forest to spruce plantations results in a dampening of the natural gradients.

The natural variability of the site is most determined by the variables pH and geology as indicated by FAMD1 (figure 25). When the spruce conversion is included in the FAMD2-

analysis, geology is less important for describing the variation within all plots. Also the positioning of the variables in the factor map (from scattered in FAMD1 to more positively correlated with axis 1 and 2 in FAMD2) indicates a dampening of the variability. All deciduous plots show a decrease in soil quality when conversed into spruce (indicated by a decrease in Humustype, Ellenberg F, Ellenberg N, Functional microbial diversity and pH in FAMD2, figure 27). This degradation from good quality deciduous plots to low quality spruce plots is larger for converted plots with initially higher quality, reflected by their deciduous twin (indicated by high scores on axis one and/or two, figure 27). Thus, the end point in quality after conversion is not affected by the initial soil quality in our study site: both high and moderate quality deciduous plots collapse into the low quality soil process domain when the overstory deciduous trees are conversed to spruce. Hence, hypothesis two, that two soil process domains exist, can be confirmed.

4.1.5 ECOSYSTEM STRATEGY

The observed decrease in humus quality and dampening of humus type variability can be considered adverse for the ecosystem. According to Ponge (2003) the three main humus types mull, moder and mor can be seen as different ecosystem strategies corresponding with different stages in the evolution of the Earth's terrestrial systems. Mor strategies correspond with more primitive Earth conditions and thus less evolved systems: the depauperate fauna and microflora of palaeozoic times corresponded with low litter quality which was enforced by the atmosphere at that time and its acid rains (Ponge, 2003). Mull strategies were introduced in the Cretaceous age together with the explosion of flowering plants and thus when litter reached a better nutrient quality and was able to sustain macroinvertebrate populations (Ponge, 2003). Hence, mull humus results in more complex and more diverse forest ecosystems (Ponge, 2003). In the study of Ponge et al. (2011), based on 3441 plots distributed over the French territory, humus type (and thus the ecosystems strategy) is attributed most to geology and climate and they state that tree species composition has a smaller effect than generally accepted. On the contrary, this local research shows that, for a fixed climate, only the conversion of overstory tree species is enough to shift the entire soil system to a different process domain. Our observations suggest that for a set of independent abiotic variables (geology and climate) defining the condition (figure 58), two soil process domains exist for deciduous tree species. These correspond with the base dominated soil process domain and the iron-aluminium dominated soil process domain, previously described by Vitousek and Chadwick (2013). Thus, the deciduous plots are naturally distributed over both soil process domains, but when the overstory deciduous trees are replaced by spruce trees a shift will occur for the plots that were not yet located in the Fe-Al process domain. The only way to avoid this shift under spruce, seems to be a continuous subsurface input of nutrients which stops the trophic cascade (vide infra) throughout the soil food web (as is the case in plot 27 that lies in a seepage zone). As an argument for ASS, the spruce conversion acts as a perturbation (figure 58) that pushes the system over the border of the attraction basin (according to the theory of Beisner et al., 2003). The corresponding shift from mull to moder humus types suggests a return to more primal and less evolved ecosystems (Ponge, 2003). These primitive ecosystems may be more vulnerable to climatic (or other) anomalies.

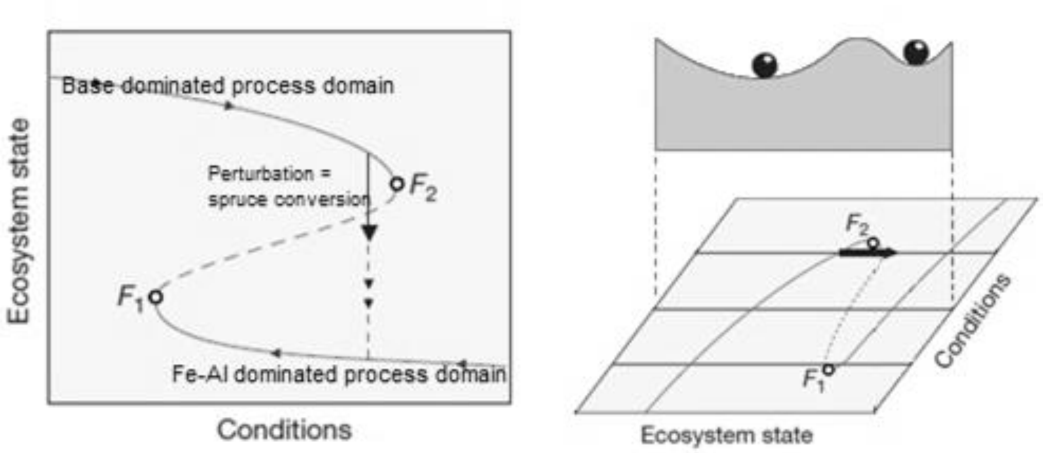


Figure 58: Alternative stable states corresponding with the base dominated soil process domain and Fe-Al dominated soil process domain for a set of conditions (in geology and climate). The perturbation (spruce conversion) is indicated by the black arrow. (adapted from Scheffer et al., 2001)

A second argument for ASS is that as a consequence of the shift in soil process domain, the data indicate a trophic cascade in the soil food web. From the statistics performed on the abiotic variables in this study, it can be concluded that conversion of deciduous forest to spruce plantations induces soil acidification (see figures 19, 20 and 31). This soil acidification acts as a front that moves from the litter layer further down the soil profile, reflected by the vertical differences in pH and BS. Functional microbial diversity and earthworm activity decrease for increasing soil acidification. Because bacterial diversity derived from the biologs decreases, most probably fungi increase. When the threshold of pH 4 (Muys and Granval, 1997) and BS 20% is reached the population of endogeïc earthworms diminishes so that homogenisation of the soil profile stops (Vesterdal et al., 2008). Because the soil is no longer homogenised by soil fauna, there is a vertical decoupling of the soil profile: nutrients are no longer captured and retained in the subsoil (Ponge, 2013; Muys and Lust, 1992; Ulrich, 1992). This is reflected in the collapse of the BS-values for the subsoil under spruce trees (see figure 20).

Many studies have researched the interaction between aboveground and belowground communities (Bardgett et al. 1998, Hooper et al. 2000, Van der Putten et al. 2001, Wardle 2002), moreover how vegetation influences the soil decomposer community. Wardle et al. (1999) state that belowground composition is more affected by the aboveground community characteristics compared to the diversity of this community (i.e. species identity is more important than the diversity of the vegetation). In addition, the relative importance of bacteria versus fungi in the soil system is evidenced to be most determined by overstory plant species (Wardle et al., 2003).

The three hypotheses formulated in the introduction can be confirmed: (1) The conversion of ancient forest to coniferous plantation induces soil acidification, (2) this acidification can cause the belowground ecosystem to suddenly shift to another soil process domain and (3) when a certain pedogenic threshold is reached for this anthropogenic driven acidification, a cascade of thresholds through the soil food web follows, which can be seen as an argument for ASS in the soil system.

4.2 CARBON DYNAMICS

4.2.1 TOTAL CARBON STOCK

The effect of tree species composition has been extensively observed in the organic topsoil layers (Wardle et al., 1997; Alriksson and Eriksson 1998; Reich et al., 2005; Hobbie et al., 2006; Laganière et al., 2009, 2010) whereas few researches studied the effects on mineral soil (Finzi et al., 1998; Giardina et al., 2001; Vesterdal et al., 2008, Laganière et al., 2013). Vesterdal et al. (2013) reported higher topsoil carbon stocks and corresponding lower subsoil carbon stocks under coniferous trees compared to deciduous trees. Also Laganière et al. (2013) found significant differences between topsoil and subsoil SOC-stocks of Canadian forests. However, the tree species effect on SOC-quality was limited to the topsoil in their study (Laganière et al., 2013).

The results of this thesis indicate significantly higher total carbon contents for topsoil spruce samples compared to deciduous samples (corresponding with Vesterdal et al., 2013 and Laganière et al., 2013). However, the difference in subsoil carbon stocks is not significant in the data of this research (as in Laganière et al., 2013). This may be a legacy effect from the previous deciduous vegetation: the period of spruce trees is not long enough to show significant effects. Therefore subsoils under spruce are more similar to the subsoils of deciduous forests (Brock, 2015).

Mueller et al. (2012) found correlations between carbon stocks and total extractable acidity and pH. The increase in total carbon is an indirect consequence of soil acidification: as acidity increases, decomposing organism decrease and thus carbon can accumulate more (Vesterdal et al., 2008). The results of this study correspondingly indicate that topsoil carbon increases with increasing acidity. Topsoil carbon is also correlated with humus type: acid humus types have higher topsoil carbon stocks. Because recalcitrant litter (with moder humus types) is more difficult to decompose, carbon accumulates above the soil. As no correlation is found between topsoil total carbon and clay content, the total carbon content does not seem modulated by the underlying lithological gradient, and is thought to be a pure tree species effect.

4.2.2 FRACTIONATION OF CARBON STOCK

As litter quality is more recalcitrant under coniferous trees, the decomposition rate is slower and fresh organic material can accumulate (Verstraeten, 2013). The largest difference between broadleaved and conifer is found in the POM fraction as transformation into POM is the first step in decomposing organic material. This non-mineral-associated SOC fraction is more labile according to the theory of von Lützow et al., 2007 (Woldeselassie et al., 2012). Although POM remains a small fraction in mass percentage, it holds a lot of carbon.

For the carbon-composition no significant differences were found between tree species. On the contrary, topsoil and subsoil SOC-composition do significantly differ for both deciduous and spruce plots. This could be a consequence of the small dataset or the mix of both states (soil process domains) in the deciduous plots. The boxplots show differences but when tested they are not significant. Hence, as a proof of concept and to investigate the SOC-patterns despite their low significance when statistically tested, three deciduous plots located in the base domain (plot 17, 25 and 27) and three deciduous plots located in the Fe-Al domain (plots 2, 8 and 21) are compared. Deciduous plots that are located in the Fe-Al domain decompose fresh organic material less efficient and have thus higher carbon contents in the fractions. When the overstory is converted to spruce, decomposition slows down even more.

Thus, the carbon fractionation taught us that between tree species, the POM mass-fraction differs the most whereas no significant differences were found between tree species for the other mass-fractions. Within the total carbon distribution no significant differences for the separate fractions were found between deciduous and spruce plots, hence a larger dataset is needed to statistically prove patterns. The combination of fractions into one stable (S+A and s+c) and one labile (POM and DOC) pool could however prove some patterns (vide infra).

4.2.3 CARBON STABILITY

Previous studies (Laganière et al., 2011, 2013; Woldeselassie et al., 2012) found that the high accumulation of SOC in the topsoil under conifers corresponds with less protected storage and hence a system more vulnerable to extra losses when climatic conditions change and favour decomposition.

The same trends are found in this research: spruce plantations have higher absolute carbon contents in both stabile and labile pools. However, 20% of this increase in total carbon is stored in labile pools. In percentage of total carbon, spruce plots have significantly higher labile carbon pools compared to deciduous plots: from 9% of the total carbon for deciduous trees to 13% of the total carbon under spruce. And thus correspondingly spruce plots have significantly lower stable carbon pools (in percentage of total carbon). It can be concluded that although spruce plantations have higher carbon stocks, their carbon is significantly lower in stability (Stable pools / Labile pools) compared to deciduous forests. With increasing soil acidity, more carbon is stored in labile pools, which can mostly be contributed to the increasing POM-fraction. Hence, the carbon stability decreases with increasing soil acidity. Additionally, carbon stability does not correlate with the clay content and is thus not modulated by the underlying lithological gradient.

The proof of concept indicates that higher carbon stabilities are corresponding with the base dominated soil process domain compared to the Fe-Al dominated domain. In addition, the spruce conversion affects carbon stability indirectly through the overstory-induced shift of soil process domains.

4.2.4 IMPLICATIONS FOR THE SOIL FOOD WEB

In this study, a regime shift in the soil food web is observed corresponding with shift from mull-strategy to moder-strategy. The spruce conversion has a negative impact on the -base dominated- soil food web (mull strategy), indicated by the decreasing functional microbial diversity (figure 23), decreasing earthworm activity (figure 24) and less efficient carbon turnover (figure 39). This adverse effect of a spruce conversion can be a consequence of the low litter quality with corresponding slow decomposition (Anderson, 1991; Cornelissen, 1996; Baldock et al., 2004; Hamer et al., 2004; Polyakova and Billor, 2007; Barbier et al., 2008) and the indirect effect of soil acidification on soil organisms (Vesterdal et al., 2008). As a consequence of the acidification earthworm populations decrease (Muys and Granval, 1997) and the effective mixing of organic matter in the soil through bioturbations stops (Vesterdal et al., 2008). This induces a vertical decoupling of the soil profile (Ponge, 2013; Muys and Lust, 1992; Ulrich, 1992) and thus results in less efficient transport of nutrients and carbon to the subsoil. Walse et al. (1998) found that bacterial growth rates are more sensitive to low pH than fungal growth rates (Walse et al., 1998). Because the functional microbial diversity measured by biologs in this study decreases, the fungi most probably increase. Although biolog-methods to measure fungi exist, they are more difficult to cultivate and thus to measure directly. The bacterial dominated soil system thus shifts to a fungi dominated soil system as a consequence of the spruce conversion (Brock, 2015; Kooijman et al., 2014). Vancampenhout et al (2010) report an accumulation of aliphatics and aromatics in the extractable SOC-pools. The decomposition of these more recalcitrant compounds is hampered whereas the relatively more easy decomposable materials such as lignins and cellulose are favoured by the fungi (Sollins et al., 1996; Kiem and Kögel-Knabner, 2003; Hessen et al., 2004; Cross et al., 2007; Marschner et al., 2008; Vancampenhout et al., 2009). The decomposition of lignin and cellulose may even take place in the litter layer and hence limit its transport to the soil (Ponge, 2013).

As a consequence of the conversion induced acidification, soil fauna changes from a bacteria and earthworm rich system to a fungi dominated system. This shift results in different patterns of organic matter decomposition: recalcitrant compounds such as aliphatics and aromatics may accumulate whereas the decomposition of lignin and cellulose is favoured. The different chemical-SOC composition under spruce plantations results in a difference of total carbon stocks. In addition the vertical decoupling results in differences in carbon stocks between topsoil and subsoil.

4.3 SYNTHESIS

We can now conclude that evidence was found to confirm the existence of alternative stable states (ASS) in soils for a specific set of abiotic variables: geology and climate. In other words, geology and climate are the main determinants for the state in which the deciduous plots are located. Here, the ASS coincide with respectively the base dominated soil process domain and Fe-Al dominated soil process domain, described by Vitousek and Chadwick (2013). Ponge (2011) already referred to geology and climate as the main attractors for the two basins of attraction in his theory concerning ecosystem strategies for the litter layer. Contrary to Ponge's theory, overstory tree species proved of major importance in determining the soil systems state, at a local scale. Spruce conversions can be seen as perturbations in the ASS theory and induce a shift from the base domain to the Fe-Al domain when climate and geology remain constant. This shift in the soil system subsequently induces a trophic cascade as evidence was found for an adverse effect on the efficiency of the soil food web (of the mull-strategy). Earthworm activity shows a threshold at pH 4 (corresponding with Muys and Granval, 1997) and BS 20%, from where on all deep-burrowing earthworms disappear from the soil profile. Subsequently the homogenization of the soil profile and the mixing of organic matter stops. Because of this vertical decoupling, nutrients and carbon no longer reach the subsoil (Ponge, 2013; Muys and Lust, 1992; Ulrich, 1992). Functional microbial diversity moved from the soil profile to the litter layer and is outcompeted by fungi. This trophic cascade has consequences for both the total carbon stocks as the SOC-composition. In this study, a higher percentage of total carbon is found for the labile carbon pool under spruce, more specifically in the POM fraction. In addition, in the Fe-Al-domain a higher percentage of total carbon is stored in labile fractions (see figure 49). Correspondingly, the efficiency of the decomposition decreases so that stocks increase and recalcitrant compounds such as aliphatics and aromatics accumulate (Brock, 2015). On the other hand there is an increasing decomposition of fungi-favoured compounds such as lignins and cellulose. Hence, vegetation, through litter input, is the major determinant of the extractable SOC composition (Vancampenhout et al., 2010). The corresponding shift from mull to moder humus types as a consequence of the spruce conversion results in a more primitive forest system (Ponge, 2003) and probably a more vulnerable system. Carbon sequestration also becomes more vulnerable under a spruce monoculture: this study indicated that carbon sequestration increases under coniferous monocultures where at the same time the stability of the carbon storage decreases. Under extreme climatic conditions, carbon stored in labile carbon pools is more easily emitted back into the atmosphere, i.e. the carbon sink can more easily become a carbon source under coniferous monocultures compared to deciduous forests.

4.4 SCOPE FOR FURTHER RESEARCH

The 10 twin-plot dataset is too limited to observe significant patterns in subsoil SOC-composition. Next researches could simplify the Zimmermann protocol with the aim of analysing a larger dataset. As the data of this research suggest that for approaching the labile carbon pool, the POM fraction is relatively more important than the DOC fraction, this DOC analysis could be skipped. Also combining the S+A and s+c into one stable carbon pool would be time-saving. Another simplification of the Zimmermann protocol was already described by Sohi et al., (2001). This method results in three SOC-fractions: free, intra-aggregate and organo-mineral. The analysis of a larger dataset would augment the statistical capacity to prove patterns and could perhaps statistically confirm the proof of concept (that the alternative stable states in soil acidity affect the carbon sequestration) discussed above.

5 CONCLUSION

This study aimed at evaluating the effect of an overstory conversion of deciduous forest to spruce plantations on the soil system and the soil carbon sequestration dynamics. The first specific objective was to check the hypothesis that conversion induces acidification and to evaluate whether the effect of conversion on specific soil variables was modulated by the underlying lithological gradient. The results indicate correlations between parent material and the soil parameters. Plots with the formation of Arlon as geological parent material have generally higher soil quality (pH and BS) and humus types. Hence, geology is an important factor in determining the soil system. It can be concluded that the conversion to spruce has significant negative effects on pH, BS, humus type, functional microbial diversity and earthworm activity. The negative effect on topsoil BS is significantly modulated by the clay content; the negative impact increases with clay content (i.e. plots with high clay content have more to lose when conversed to spruce).

Specific objective two was to observe a shift in soil process domain as a consequence of the overstory conversion. In this study, evidence is found that for a specific set of abiotic variables, geology and climate, two soil process domains exist in the belowground ecosystem. For the constant climate in this study, geology determines the dominating soil process domain. Spruce conversions can be seen as a perturbation that induces a shift from the base-dominated soil process domain to the Fe-Al-dominated soil process domain. This is a first argument for the existence of ASS in the soil. Another argument for ASS is the conversion-induced trophic cascade throughout the soil food web. The collapse of earthworm activities results in a decoupling of nutrient transport, mirrored by the collapse of BS in the subsoil and is followed by an upwards migration to the litter layer of microbial activity. The shift from ASS corresponds with a shift from mull-ecosystem-strategies to moder-ecosystem-strategies and suggest that spruce conversions result in less complex ecosystems with narrowed biodiversity. The narrowed biodiversity is reflected by the dampening of natural gradients in this study.

The third specific objective of this study was to evaluate the effect of spruce conversion on the carbon sequestration dynamics. This study shows that total topsoil carbon stocks increase significantly as a consequence of the conversion. Carbon accumulates in the surface layer as a direct consequence of bad quality litter and an indirect consequence of soil acidification. On the other hand, a decrease in total subsoil carbon stocks under spruce could not be statistically confirmed in this study. However, significant patterns in topsoil carbon stability could be observed. Of the increasing topsoil carbon stored under spruce, a larger percentage is stored in labile fractions resulting in an overall lower carbon stability. Hence, spruce conversion results in higher topsoil carbon stocks but at the same time stocks with lower stability. Thus, under extreme climatic conditions spruce plantations could even release carbon back into the atmosphere.

The dataset of 10 twin-plots is focused on obtaining new information about carbon stability and is as a consequence limited in size due to the time-consuming carbon fractionation. In addition to proving patterns in subsoil carbon storage, a larger dataset could also allow to statistically link the alternative stable states to carbon stability. In this study the proof of concept indicates the relevance of additional studies. Further research could benefit from extra soil variables, for example the measurement of iron and aluminium could support the theory concerning the two soil process domains. Additionally, the dataset could be extended by data of other soil fauna and fungi to investigate the trophic cascade. Further research would however benefit most from more observations, especially on carbon stability data. A simplification of the carbon fractionation method could help to achieve this larger dataset.

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8 APPENDIX

8.1 ADDITIONAL GRAPHS PROOF OF CONCEPT

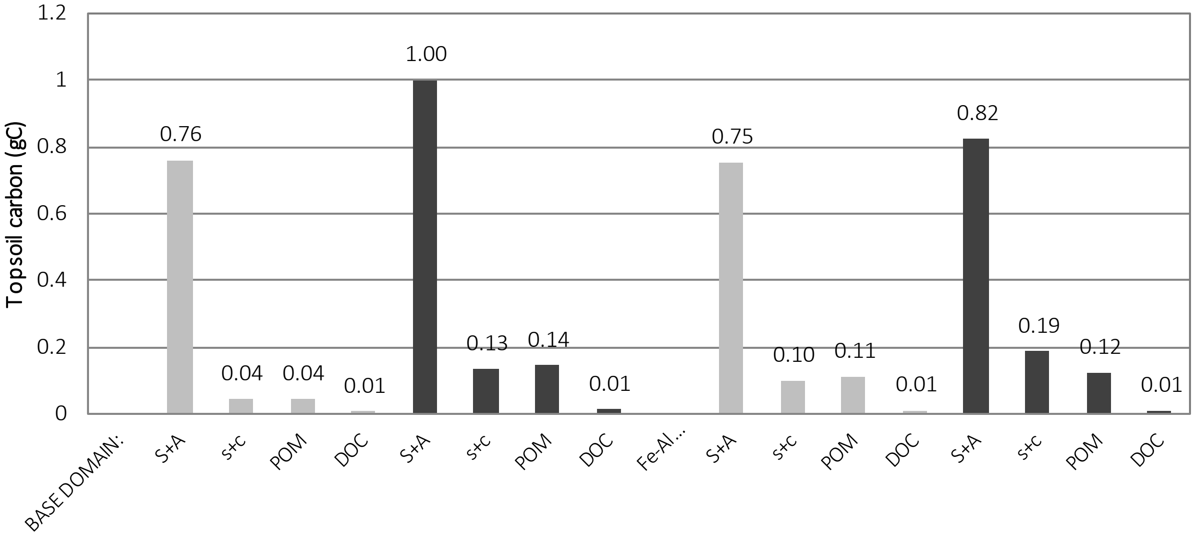

Figure 59: Proof of concept: effect on topsoil carbon fractions (in gC) before (light) and after (dark) a spruce conversion on deciduous plots from the base domain (left) and from the Fe-Al domain (right).

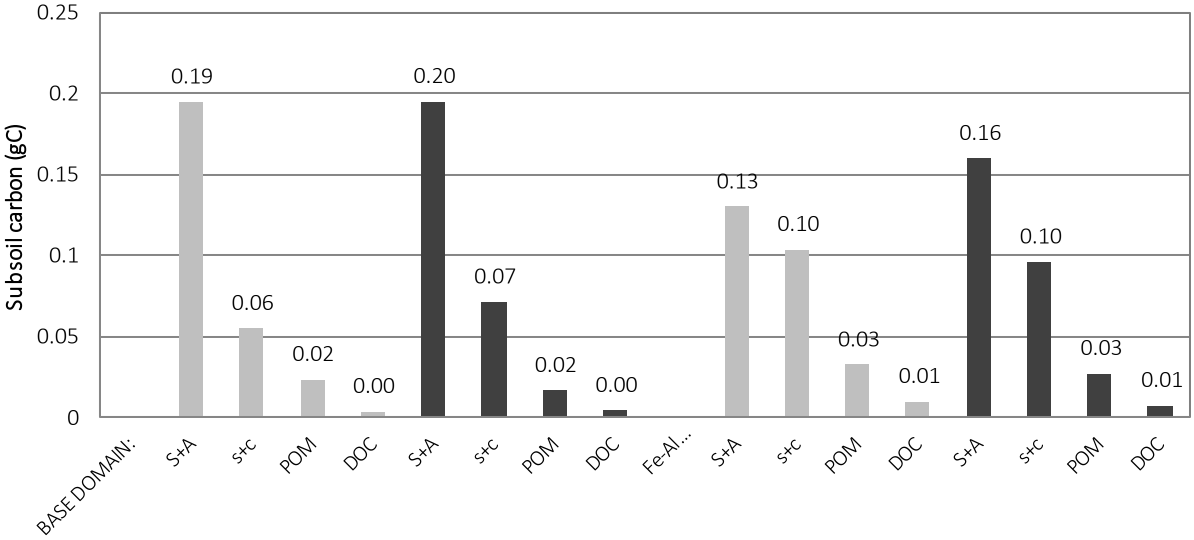

Figure 60: Proof of concept: effect on subsoil carbon fractions (in gC) before (light) and after (dark) a spruce conversion on deciduous plots from the base domain (left) and from the Fe-Al domain (right).

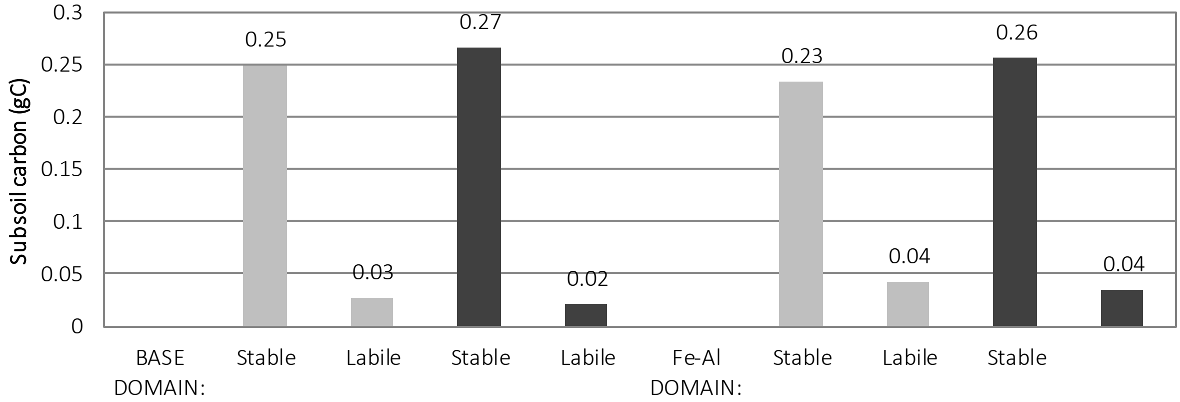

Figure 61: Proof of concept: effect on subsoil labile and stable carbon pools (in gC) of plots from the base domain (left) and from the Fe-Al domain (right). A distinction is made between deciduous plots (light) and spruce plots (dark).

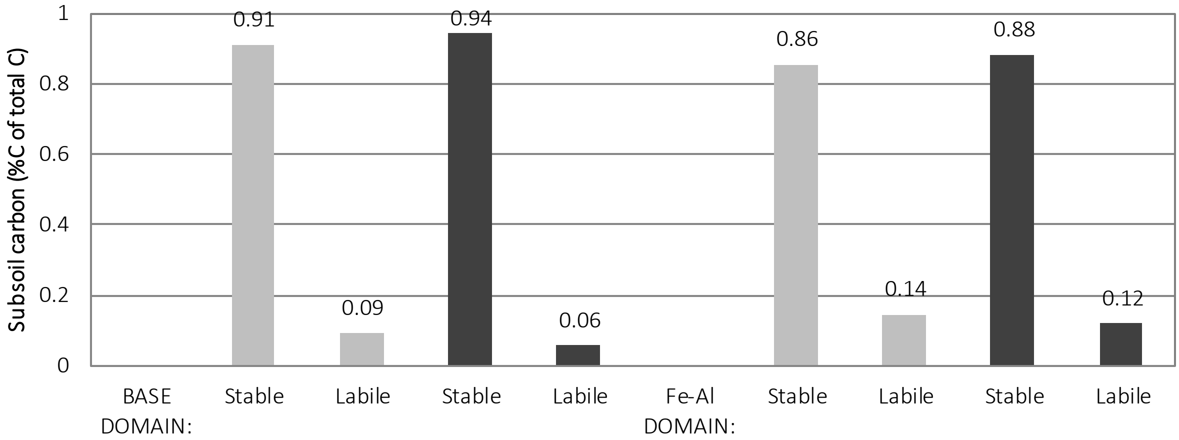

Figure 62: Proof of concept: effect on subsoil labile and stable carbon pools (in % of total carbon) of plots from the base domain (left) and from the Fe-Al domain (right). A distinction is made between deciduous plots (light) and spruce plots (dark).

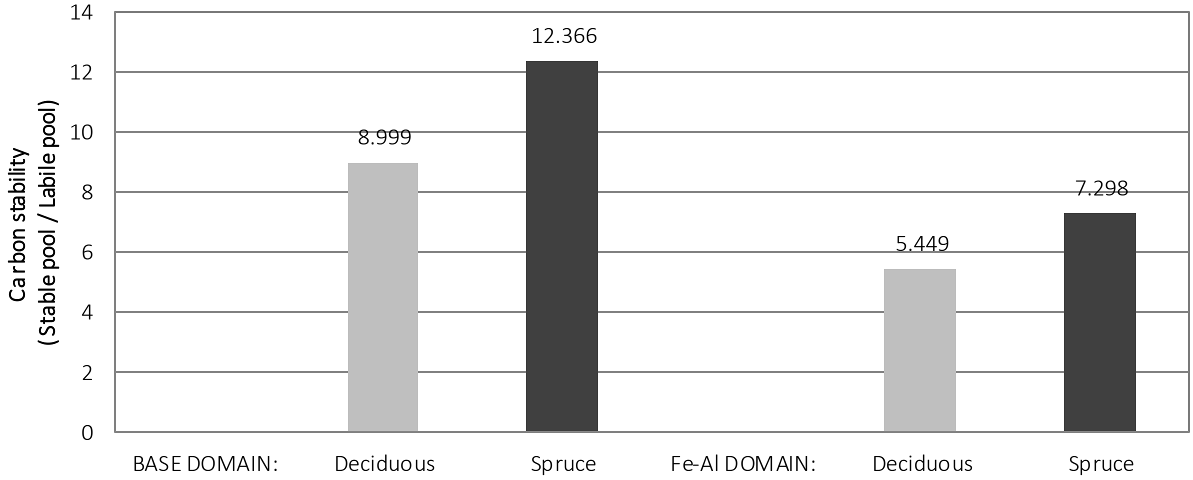

Figure 63: Proof of concept: effect on subsoil carbon stability of plots from the base domain (left) and from the Fe-Al domain (right). A distinction is made between deciduous plots (light) and spruce plots (dark).

8.2 CARBON FRACTIONATION DATA

Table 9: Carbon content of each fraction for a sample of 30g oven-dried soil.

PLOT	**TOPSOIL**				**SUBSOIL**			
	S+A	**s+c**	**POM**	**DOC**	**S+A**	**s+c**	**POM**	**DOC**
	gC / 30g soil	**gC / 30g soil**	**gC / 30g soil**	**gC / 30g soil**	**gC / 30g soil**	**gC / 30g soil**	**gC / 30g soil**	**gC / 30g soil**
MEAN	**0.834979**	**0.122327**	**0.116832**	**0.010239**	**0.142242**	**0.069594**	**0.023934**	**0.005399**
2S	1.024191	0.032721	0.138485	0.010888	0.19585	0.145818	0.038448	0.01052
8S	0.828845	0.097669	0.127575	0.008898	0.106977	0.073645	0.015945	0.005776
11S	0.894644	0.155564	0.14348	0.013831	0.151405	0.050516	0.019404	0.003913
12S	1.044191	0.06083	0.090471	0.007895	0.149898	0.044047	0.016151	0.003759
17S	1.246364	0.117692	0.127342	0.014666	0.233248	0.077345	0.019111	0.005188
21S	0.614167	0.429766	0.103968	0.009584	0.124255	0.046684	0.016333	0.004934
25S	1.15895	0.169475	0.129548	0.01282	0.141151	0.083803	0.024931	0.0047
26S	0.981947	0.102642	0.130886	0.014097	0.07549	0.091071	0.01731	0.003699
27S	0.595191	0.116578	0.17802	0.012087	0.210933	0.053449	0.006851	0.003907
36S	0.795491	0.382284	0.366085	0.016938	0.193079	0	0.067012	0.005613
MEAN S	**0.918398**	**0.166522**	**0.153586**	**0.01217**	**0.158228**	**0.066638**	**0.02415**	**0.005201**
2D	1.073174	0.182802	0.198486	0.019117	0.158114	0.14209	0.051413	0.013074
8D	0.556719	0.073761	0.058687	0.005106	0	0.068617	0.026666	0.004379
11D	1.10824	0.093409	0.132959	0.009422	0.128234	0.079631	0.036721	0.00467
12D	0.718492	0.037104	0.068404	0.00708	0.135743	0.138135	0.014194	0.007839
17D	1.005503	0.041454	0.026766	0.004986	0.224519	0.085041	0.036524	0.006685
21D	0.627993	0.043151	0.076441	0.007892	0.102024	0.065455	0.013965	0.007375
25D	0.488254	0.053128	0.047441	0.008261	0.104286	0.035777	0.019343	0.002077
26D	0.384705	0.074834	0.043894	0.004137	0.040577	0.037607	0.005441	0.003342
27D	0.786302	0.037435	0.05638	0.007735	0.255383	0.044502	0.015416	0.003241
36D	0.766225	0.144241	0.091331	0.009346	0.113677	0.028642	0.017498	0.003285
MEAN D	**0.751561**	**0.078132**	**0.080079**	**0.008308**	**0.126256**	**0.07255**	**0.023718**	**0.005597**

Table 10: Carbon content of each fraction in gC per kg soil.

PLOT	TOPSOIL				SUBSOIL			
	S+A gC / kg soil	**s+c** gC / kg soil	**POM** gC / kg soil	**DOC** gC / kg soil	**S+A** gC / kg soil	**s+c** gC / kg soil	**POM** gC / kg soil	**DOC** gC / kg soil
MEAN	**27.83264**	**4.077569**	**3.894416**	**0.341309**	**4.741402**	**2.31979**	**0.797795**	**0.179959**
2S	34.13971	1.090706	4.616181	0.362929	6.528331	4.860614	1.281585	0.350683
8S	27.62817	3.255624	4.252504	0.296589	3.565885	2.454825	0.531515	0.192543
11S	29.82146	5.18548	4.782676	0.46103	5.046817	1.683855	0.646804	0.130449
12S	34.80636	2.027663	3.015704	0.263171	4.99661	1.468226	0.53836	0.125309
17S	41.54547	3.923081	4.244717	0.488868	7.774928	2.578152	0.637028	0.172929
21S	20.47223	14.32554	3.465599	0.319456	4.141824	1.556121	0.544431	0.164455
25S	38.63165	5.64917	4.318274	0.427322	4.705017	2.793446	0.831034	0.156651
26S	32.73156	3.421409	4.362857	0.469886	2.516318	3.035703	0.577012	0.123304
27S	19.83969	3.885924	5.934016	0.402892	7.031109	1.781641	0.228359	0.130224
36S	26.51638	12.74281	12.20284	0.564599	6.435955	0	2.233745	0.187086
MEAN S	**30.61327**	**5.55074**	**5.119536**	**0.405674**	**5.274279**	**2.221258**	**0.804987**	**0.173363**
2D	35.77246	6.093403	6.616188	0.637236	5.270458	4.736327	1.713782	0.435791
8D	18.55731	2.458697	1.956218	0.17021	0	2.287247	0.888862	0.145977
11D	36.94135	3.113628	4.431957	0.314056	4.274478	2.654377	1.224028	0.155653
12D	23.94972	1.236799	2.280127	0.236009	4.524759	4.604509	0.473132	0.261301
17D	33.51676	1.381815	0.892213	0.166212	7.483971	2.834693	1.217467	0.222844
21D	20.93309	1.438364	2.54805	0.263056	3.400806	2.181833	0.465509	0.245841
25D	16.27512	1.770933	1.58137	0.275382	3.4762	1.19256	0.644781	0.069224
26D	12.82351	2.494471	1.463129	0.137913	1.35258	1.253574	0.181359	0.111388
27D	26.21005	1.247839	1.879336	0.257821	8.512778	1.483388	0.513851	0.108029
36D	25.54082	4.808034	3.044363	0.311536	3.789221	0.954717	0.583251	0.109504
MEAN D	**25.05202**	**2.604398**	**2.669295**	**0.276943**	**4.208525**	**2.418323**	**0.790602**	**0.186555**

Table 11: Carbon percentage in each mass fraction (measured on 20mg sample (2mg for POM) with a TOC analyzer) and carbon concentration in the DOC-fraction (measured with a DOC-analyzer).

PLOT	TOPSOIL				SUBSOIL			
	S+A (%C)	s+c (%C)	POM (%C)	DOC mg C / L	S+A (%C)	s+c (%C)	POM (%C)	DOC mg C / L
MEAN	**3.096335**	**6.409976**	**24.8614**	**8.250541**	**0.500967**	**2.571638**	**21.33381**	**4.607374**
2S	4.044122	8.734889	29.45192	9.626406	0.692406	3.038823	19.53354	9.003345
8S	3.694672	7.010106	24.58252	9.189822	0.350171	2.488087	20.10253	5.180071
11S	3.731569	4.728314	22.57281	11.29132	0.621284	1.701543	27.18596	3.90968
12S	3.684056	7.535705	23.66832	6.093238	0.562985	2.122688	23.53565	4.18911
17S	4.998576	7.855342	28.57872	13.03779	0.904202	3.166658	23.76262	5.860569
21S	2.950797	4.863432	23.84314	10.49132	0.419142	1.13766	21.37683	3.561708
25S	3.543257	8.669471	24.75424	8.36548	0.465684	2.351116	23.43281	3.422278
26S	3.571162	3.843713	19.81573	10.07224	0.26519	1.295951	20.51332	2.995587
27S	1.817094	3.915671	28.49438	8.337224	0.807342	1.845402	17.22074	4.251957
36S	2.63434	16.70474	36.88156	12.03345	0.427056	6.475926	29.05102	3.732598
MEAN S	**3.466965**	**7.386138**	**26.26433**	**9.853829**	**0.551546**	**2.562385**	**22.5715**	**4.61069**
2D	2.802889	6.331117	26.41919	11.56826	0.43909	3.038877	20.94075	7.314804
8D	2.256689	4.842701	23.0506	5.643416		3.101392	22.22154	4.424413
11D	4.471815	5.70999	23.36739	7.447046	0.445128	2.689177	28.54076	4.364626
12D	2.590608	5.321983	22.72925	5.302349	0.331387	3.571239	17.33963	5.043416
17D	3.777676	5.171302	21.20409	4.579431	0.65259	2.900428	23.35546	6.830463
21D	1.786658	4.404107	28.08307	4.769822	0.31973	3.317627	19.22027	6.190178
25D	2.187522	5.720175	18.84612	7.640569	0.389861	1.725687	25.20116	1.744911
26D	1.897914	5.501884	23.04221	5.388612	0.149493	1.418833	8.80287	3.629253
27D	1.854104	4.281915	19.33678	4.531103	0.814251	2.223563	17.06122	3.352242
36D	3.631191	7.052963	28.50595	9.601922	0.461385	1.822082	18.27748	3.146263
MEAN D	**2.725706**	**5.433814**	**23.45847**	**6.647253**	**0.444768**	**2.580891**	**20.09611**	**4.604057**

Table 12: Percentage of total carbon of sample stored in the specific fractions.

PLOT	**TOPSOIL**				**SUBSOIL**			
	S+A (% of total C)	**s+c (% of total C)**	**POM (% of total C)**	**DOC (% of total C)**	**S+A (% of total C)**	**s+c (% of total C)**	**POM (% of total C)**	**DOC (% of total C)**
MEAN	**0.777673**	**0.109097**	**0.103794**	**0.009437**	**0.569548**	**0.303586**	**0.10338**	0.023486
2S	0.849045	0.027126	0.114803	0.009026	0.501361	0.373284	0.098423	0.026932
8S	0.779732	0.091881	0.120016	0.00837	0.528689	0.36396	0.078804	0.028547
11S	0.740894	0.12883	0.118822	0.011454	0.672199	0.224277	0.086149	0.017375
12S	0.86771	0.050549	0.07518	0.006561	0.700934	0.205965	0.075522	0.017579
17S	0.827564	0.078146	0.084553	0.009738	0.696489	0.230954	0.057066	0.015491
21S	0.530605	0.371293	0.089822	0.00828	0.64647	0.242885	0.084977	0.025669
25S	0.787976	0.115227	0.088081	0.008716	0.554435	0.329177	0.097928	0.01846
26S	0.798609	0.083478	0.106448	0.011465	0.40246	0.485531	0.092287	0.019721
27S	0.659948	0.129261	0.197389	0.013402	0.76664	0.194262	0.024899	0.014199
36S	0.509669	0.244929	0.23455	0.010852	0.726669		0.252207	0.021123
MEAN S	**0.735175**	**0.132072**	**0.122966**	**0.009786**	**0.619635**	**0.26503**	**0.094826**	**0.02051**
2D	0.728277	0.124053	0.134696	0.012973	0.433556	0.389617	0.140978	0.035849
8D	0.801874	0.106242	0.084529	0.007355		0.688497	0.267561	0.043941
11D	0.824565	0.069499	0.098925	0.00701	0.514468	0.319476	0.147322	0.018734
12D	0.864528	0.044646	0.082307	0.008519	0.458728	0.466814	0.047967	0.026491
17D	0.932134	0.03843	0.024813	0.004623	0.636448	0.241066	0.103535	0.018951
21D	0.831253	0.057117	0.101183	0.010446	0.540326	0.346653	0.073961	0.03906
25D	0.81773	0.088979	0.079455	0.013836	0.645802	0.221552	0.119786	0.01286
26D	0.757934	0.147436	0.086478	0.008151	0.466584	0.432431	0.062561	0.038424
27D	0.885623	0.042164	0.063502	0.008712	0.801727	0.139704	0.048394	0.010174
36D	0.757781	0.142652	0.090324	0.009243	0.696972	0.175606	0.10728	0.020142
MEAN D	**0.82017**	**0.086122**	**0.084621**	**0.009087**	**0.519461**	**0.342142**	**0.111935**	**0.026463**

Table 13: Percentage of carbon stored in stable and labile pools and carbon stability.

PLOT	TOPSOIL			SUBSOIL		
	Stable C pool	**Labile C pool**	**Stability**	**Stable C pool**	**Labile C pool**	**Stability**
MEAN	**0.886769**	**0.113231**	**7.831542**	**0.873133**	**0.126867**	**6.882299**
2S	0.876171	0.123829	7.075645	0.874645	0.125355	6.977376
8S	0.871614	0.128386	6.789	0.892649	0.107351	8.315234
11S	0.869724	0.130276	6.675993	0.896476	0.103524	8.659568
12S	0.918259	0.081741	11.23374	0.906899	0.093101	9.741044
17S	0.905709	0.094291	9.605522	0.927443	0.072557	12.78226
21S	0.901898	0.098102	9.193465	0.889355	0.110645	8.037895
25S	0.903203	0.096797	9.330928	0.883612	0.116388	7.591955
26S	0.882087	0.117913	7.480839	0.887991	0.112009	7.927884
27S	0.789209	0.210791	3.744037	0.960902	0.039098	24.57662
36S	0.754598	0.245402	3.074947	0.726669	0.273331	2.658573
MEAN S	**0.867247**	**0.132753**	**6.532799**	**0.884664**	**0.115336**	**7.670332**
2D	0.85233	0.14767	5.771876	0.823173	0.176827	4.655243
8D	0.908116	0.091884	9.883246	0.688497	0.311503	2.210245
11D	0.894065	0.105935	8.439709	0.833944	0.166056	5.022068
12D	0.909173	0.090827	10.01	0.925542	0.074458	12.43036
17D	0.970564	0.029436	32.97216	0.877514	0.122486	7.164193
21D	0.888371	0.111629	7.958239	0.886979	0.113021	7.84795
25D	0.906709	0.093291	9.719155	0.867354	0.132646	6.538836
26D	0.90537	0.09463	9.567507	0.899015	0.100985	8.902427
27D	0.927787	0.072213	12.84786	0.941432	0.058568	16.07411
36D	0.900432	0.099568	9.043432	0.872578	0.127422	6.847932
MEAN D	**0.906292**	**0.093708**	**9.671421**	**0.861603**	**0.138397**	**6.225577**

8.3 SITE DESCRIPTION DATA

Table 14: Site description variables PART I

PLOT	pH(H20) Topsoil	pH(H20) Subsoil	pH(KCl) Topsoil	pH(KCl) Subsoil	Texture class *	Humus type **	Parent material class ***
1S	4.01	4.37	3.71	3.79	3	4	1
2S	3.52	4.47	2.92	4.03	3	6	2
5S	3.96	4.49	3.31	3.82	3	5	1
8S	3.63	4.56	2.84	4.15	2	6	2
9S	4.11	4.5	3.19	3.97	3	5	1
11S	3.87	4.51	2.97	4.04	3	5	2
12S	4.19	4.52	3.18	4.13	3	6	2
13S	3.76	4.54	3.92	3.84	2	4	2
15S	3.64	4.46	2.85	3.94	2	6	2
16S	3.68	4.35	2.96	3.92	2	5	2
17S	4	4.54	3.22	3.74	3	5	1
19S	4.16	4.4	3.28	3.73	3	6	1
21S	3.64	4.46	2.87	4.07	3	6	2
22S	3.71	4.4	2.8	3.76	2	7	2
25S	4.15	4.52	3.38	3.82	3	5	1
26S	3.99	4.55	3.22	3.82	3	5	2
27S	4.43	4.41	3.61	3.83	3	5	1
29S	4.09	4.38	3.2	3.6	3	6	2
30S	3.99	4.35	3.22	3.71	3	5	1
36S	3.73	4.39	2.81	3.72	2	5	2
MEAN S	**3.913**	**4.4585**	**3.173**	**3.8715**	**2.7**	**5.35**	**1.6**
1D	5.24	4.98	4.37	4.13	3	3	1
2D	4.23	4.64	3.51	4.13	3	4	2
5D	5.21	4.75	4.26	3.94	3	4	1
8D	3.96	4.71	3.19	4.07	2	4	2
9D	4.43	4.59	3.15	3.89	3	5	1
11D	4.7	4.75	3.54	4.16	2	5	2

12D	4.43	4.74	3.38	4.1	2	2	2
13D	4.85	4.49	3.43	3.99	3	6	2
15D	3.86	4.49	3.1	3.96	2	4	2
16D	3.95	4.4	3.13	3.81	3	4	2
17D	5.61	4.85	5.02	3.95	2	3	1
19D	4.39	4.66	3.59	3.84	2	5	1
21D	3.81	4.5	3.13	3.83	3	5	2
22D	4.07	4.65	3.29	4.13	2	5	2
25D	5.13	4.83	4.21	4	3	4	1
26D	4.74	4.84	3.78	3.92	3	4	2
27D	5.66	5.5	5.38	4.9	3	2	1
29D	5.02	4.86	4.19	3.69	4	3	1
30D	4.54	4.43	3.66	3.85	3	6	1
36D	4.7	4.69	3.15	4.19	2	7	2
MEAN D	**4.6265**	**4.7175**	**3.723**	**4.024**	**2.65**	**4.25**	**1.55**

*Texture class: (1) Zand, (2) Lemig zand, (3) Zandleem, (4) Leem,

** Humustype: (1) Eumull, (2) Mesomull, (3) Oligomull, (4) Dysmull, (5) Hemimoder, (6) Eumoder, (7) Dysmoder

*** Parentmaterial: (1) Hondelage, (2) Luxemburg

Table 15: Site description variables PART II

PLOT	Texture			Microbial diversity index 72u (%)		
	% sand	% loam	% clay	Biolog litter	Biolog topsoil	Biolog subsoil
1S	37.2246	50.39	12.3779	29.03226	0	0
2S	55.0654	36.6	8.39291	9.677419	3.225806	0
5S	52.3311	35.68	11.99477	29.03226	3.225806	3.225806
8S	70.19515	24.29	5.49994	32.25806	0	9.677419
9S	41.06606	48.34	10.55077	22.58065	0	0
11S	57.3122	34.94	7.74801	25.80645	0	3.225806
12S	63.8823	29.79	6.31643	58.06452	0	3.225806
13S	71.9843	20.56	7.46528	35.48387	0	0
15S	63.93887	29.5	6.47122	16.12903	3.225806	3.225806
16S	65.15101	28.02	6.83493	61.29032	0	0

17S	53.18925	36.44	10.39184	35.48387	0	0
19S	58.8139	30.68	10.52302	12.90323	0	3.225806
21S	51.896	38.81	9.273	3.225806	0	12.90323
22S	71.68	23.61	4.70046	22.58065	6.451613	6.451613
25S	58.3649	29.43	12.21832	22.58065	32.25806	0
26S	45.60768	42.87	11.55765	16.12903	3.225806	12.90323
27S	46.87221	41.99	11.11994	51.6129	0	0
29S	52.93429	36.64	10.4122	19.35484	16.12903	3.225806
30S	56.4514	31.3	12.26116	51.6129	25.80645	0
36S	70.973	24.21	4.8264	48.3871	0	6.451613
MEAN S	**57.24668**	**33.7045**	**9.046807**	**30.16129**	**4.677419**	**3.387097**
1D	37.2246	50.39	12.3779	22.58065	16.12903	3.225806
2D	55.0654	36.6	8.39291	29.03226	6.451613	16.12903
5D	52.3311	35.68	11.99477	16.12903	6.451613	16.12903
8D	70.19515	24.29	5.49994	16.12903	64.51613	9.677419
9D	41.06606	48.34	10.55077	38.70968	6.451613	0
11D	57.3122	34.94	7.74801	25.80645	3.225806	22.58065
12D	63.8823	29.79	6.31643	25.80645	9.677419	0
13D	71.9843	20.56	7.46528	32.25806	3.225806	0
15D	63.93887	29.5	6.47122	48.3871	25.80645	0
16D	65.15101	28.02	6.83493	25.80645	35.48387	12.90323
17D	53.18925	36.44	10.39184	16.12903	45.16129	22.58065
19D	58.8139	30.68	10.52302	22.58065	16.12903	3.225806
21D	51.896	38.81	9.273	9.677419	29.03226	3.225806
22D	71.68	23.61	4.70046	6.451613	6.451613	3.225806
25D	58.3649	29.43	12.21832	9.677419	32.25806	0
26D	45.60768	42.87	11.55765	38.70968	25.80645	19.35484
27D	46.87221	41.99	11.11994	25.80645	29.03226	35.48387
29D	52.93429	36.64	10.4122	32.25806	19.35484	48.3871
30D	56.4514	31.3	12.26116	0	0	12.90323
36D	70.973	24.21	4.8264	25.80645	45.16129	0

MEAN D	**57.24668**	**33.7045**	**9.046807**	**23.3871**	**21.29032**	**11.45161**

Table 16: Site description variables PART III

PLOT	**Total number**	**Endogeic number**	**Epigeic number**	**Cummulative weight (g)**	**Cummulative weight (g.m2)**
1S	5	5	0	1.264	12.64
2S	2	0	2	0.132	1.32
5S	1	0	1	0.018	0.036
8S	2	0	2	0.492	0.984
9S		0	0		
11S	4	0	4	0.372	2.832
12S	1	0	1	0.081	0.81
13S	5	0	5	0.604	5.4
15S	2	0	2	0.169	1.69
16S	2	0	2	0.486	2.252
17S	4	0	4	0.861	8.258
19S	4	0	4	0.424	4.24
21S	1	0	1	0.022	0.22
22S	16	0	16	3.1	23.528
25S	22	0	22	1.12	8.376
26S	24	5	19	1.408	7.064
27S	12	0	12	0.816	7.616
29S	6	1	5	1.154	10.228
30S					
36S					
MEAN S	**6.647059**	**0.611111**	**5.666667**	**0.736647**	**5.734941**
1D	147	123	24	13.9416	112.9792
2D	6	0	6	0.185	0.77
5D	73	48	25	5.693	36.274
8D	3	0	3	0.196	1.96
9D	6	0	6	0.393	2.698

11D	11	0	11	0.628	3.792
12D	36	0	36	4.404	30.184
13D	11	1	10	1.767	15.582
15D	3	0	3	0.66	6.6
16D	8	0	8	0.522	4.916
17D	261	116	145	34.107	199.846
19D	25	0	25	1.919	14.75
21D	6	0	6	0.384	0.768
22D	5	0	5	0.508	5.08
25D	38	0	38	1.634	10.3
26D	84	17	67	12.892	58.464
27D	59	33	26	6.022	32.22
29D	32	20	12	11.555	108.534
30D	42	4	38	3.54	15.6
36D					
MEAN D	**45.05263**	**19.05263**	**26**	**5.313189**	**34.80617**

Table 17: Site description variables PART IV

PLOT	Ellenberg indicator values					
	AIV_N	CIV_N	AIV_R	CIV_R	AIV_F	CIV_F
1S	5.500	5.959	6.000	6.753	5.273	5.262
2S	5.500	4.115	6.000	6.000	5.333	5.020
5S	6.385	5.422	6.250	6.749	5.500	5.081
8S	5.500	6.513	4.750	4.991	5.143	6.483
9S	5.125	4.317	4.625	4.963	5.100	4.783
11S	5.333	4.132	4.500	4.395	5.375	4.886
12S	5.375	6.594	5.125	5.125	5.100	5.305
13S	5.200	5.324	4.125	4.144	5.125	5.086
15S	5.125	6.559	3.600	3.103	4.875	5.723
16S	5.600	5.823	4.167	4.386	5.600	5.879
17S	6.600	6.714	5.786	6.592	5.429	5.701

19S	6.200	6.129	5.333	5.490	5.071	4.922
21S	4.571	6.246	3.750	3.750	5.571	5.327
22S	6.000	6.000	4.333	4.333	6.000	6.000
25S	5.250	5.665	6.333	6.499	5.200	5.250
26S	5.867	6.378	5.444	5.261	5.625	6.372
27S	6.000	5.303	6.100	6.977	5.375	5.011
29S	5.885	6.382	5.429	6.607	5.480	5.816
30S	6.682	5.986	5.500	5.735	5.565	5.330
36S	4.625	3.817	3.857	3.146	5.250	4.084
MEAN S	**5.616124**	**5.668905**	**5.050377**	**5.249976**	**5.349528**	**5.365921**
1D	6.091	6.826	4.938	4.159	5.286	5.387
2D	6.063	5.746	4.000	4.188	5.467	5.256
5D	5.857	6.944	4.200	4.616	5.619	5.528
8D						
9D	5.500	6.198	3.909	4.797	5.333	6.190
11D	5.625	6.865	3.909	3.785	5.429	5.341
12D	5.300	4.446	3.000	3.000	5.300	4.953
13D	5.810	6.283	4.462	3.739	5.444	5.570
15D	5.333	5.333	4.000	4.000	5.333	5.333
16D	6.250	5.000	4.000	3.263	5.538	5.929
17D	6.500	6.740	4.800	4.243	5.533	5.207
19D	6.105	6.695	4.923	4.634	5.611	5.617
21D	5.556	3.889	4.750	3.924	6.000	7.554
22D	7.000	7.000	6.000	6.000	6.000	6.000
25D	6.063	7.223	4.583	5.013	5.733	5.254
26D	6.421	6.685	4.933	4.109	5.900	5.996
27D	6.150	6.257	5.600	5.700	5.316	5.368
29D	6.233	6.522	5.143	4.473	5.444	5.669
30D	6.333	6.457	4.900	4.083	5.615	5.451
36D	4.909	3.620	3.375	3.205	5.200	4.639

MEAN D	**5.953**	**6.038287**	**4.496043**	**4.259523**	**5.531735**	**5.591649**

Table 18: Cation exchange capacities and base saturation values of the 10-twinplots dataset.

PLOT	CEC topsoil (cmolc/kgsoil)	BS topsoil (%)	CEC subsoil (cmolc/kgsoil)	BS subsoil (%)
2S	6.93	9.54	1.51	3.79
8S	4.94	15.96	0.44	9.20
11S	6.60	8.13	0.94	5.02
12S	5.91	8.73	0.38	10.77
17S	12.07	9.52	5.87	3.88
21S	4.83	9.66	1.10	8.06
25S	6.55	11.54	5.42	5.96
26S	6.62	16.87	5.41	3.67
27S	8.20	17.19	6.51	16.76
36S	5.84	13.60	1.41	3.22
2D	4.74	33.48	1.49	7.63
8D	2.57	26.61	0.25	12.38
11D	5.57	20.99	1.00	10.22
12D	5.27	27.56	2.87	90.75
17D	11.24	80.23	5.82	24.33
21D	1.51	32.49	0.22	43.79
25D	7.03	43.03	5.77	27.25
26D	5.22	37.81	1.43	17.51
27D	14.92	82.06	13.59	87.68
36D	5.67	20.91	0.28	24.50

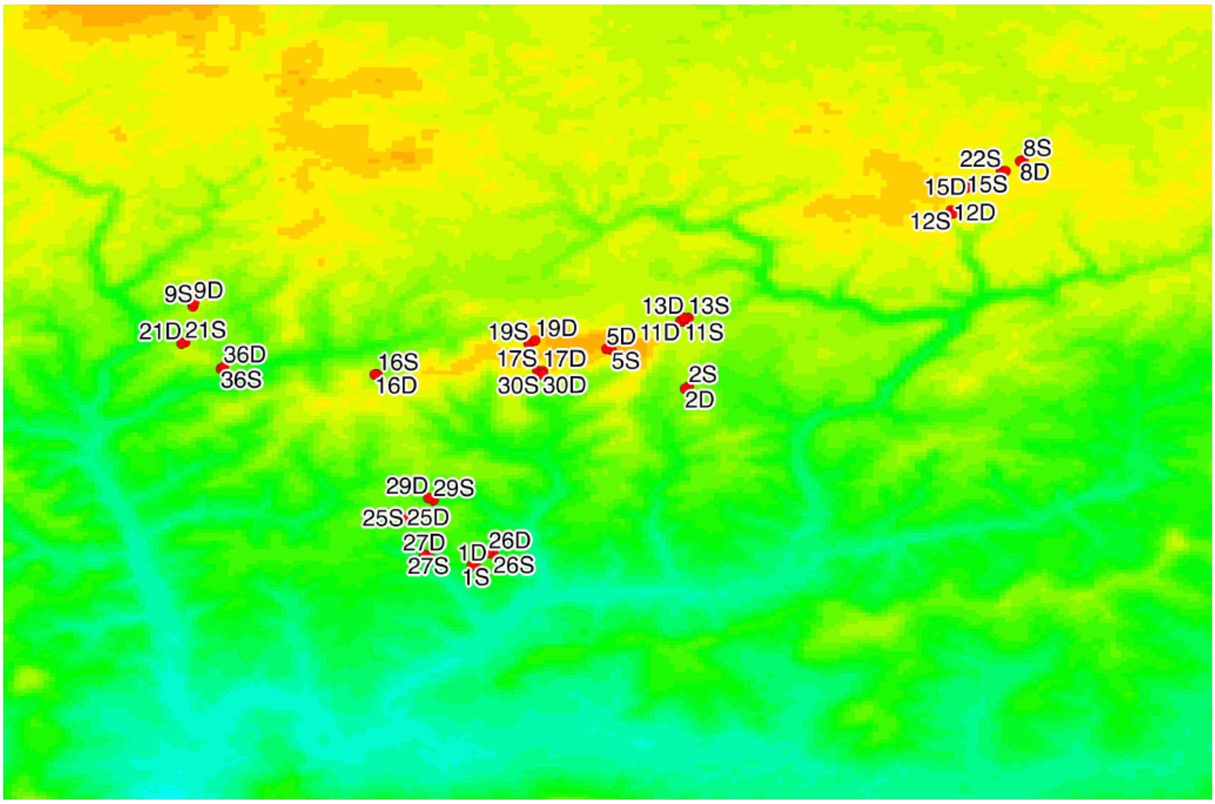

Figure 64: Elevation map of study site.

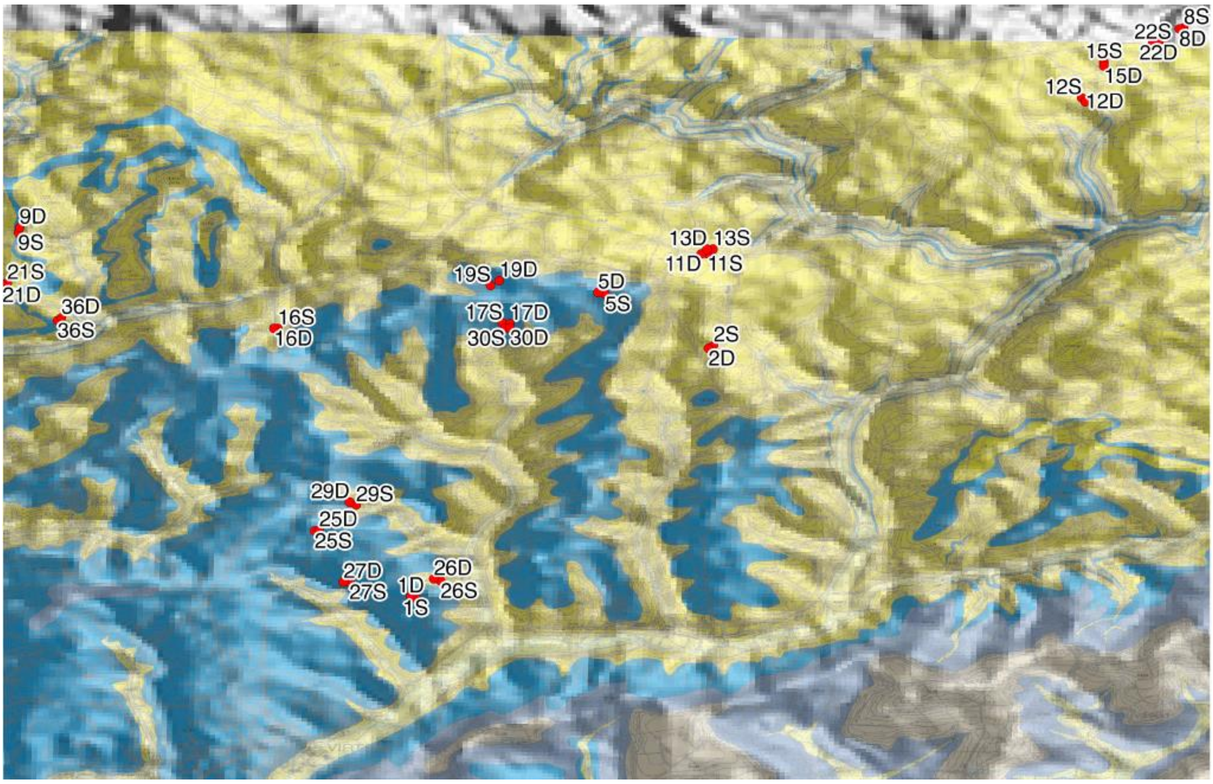

Figure 65: Combination elevation and geological map of the study site.