Faculté des bioingénieurs

Tree species effects on soil organic carbon stocks and chemical properties of the topsoil in the arboretum of Tervuren

Comparative approach between broadleaves and conifers

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Introduction

Soil carbon storage in forest ecosystems is increasingly recognized as a key ecosystem service for curbing global warming. Forest management plays a major role in the improvement of stable and long-term C storage in soils, in particular by species selection. In recent years, the tree species composition effect on soil organic C stocks has received a considerable attention in literature. A number of researchers have reported on average more important SOC stocks in the forest floor in coniferous species compared to broadleaved species. However, a lack of data about SOC stocks in the mineral material of soil leads to controversial assumptions about the tree species impact on stable C storage in mineral soil compartment. This gap in the field creates a need to quantify SOC stocks in mineral soil horizons to understand to what extend they are influenced by tree species.

In that context, the aim of this master thesis project is to investigate the differences between broadleaves and conifers in terms of carbon storage in the different horizons of soil profile. In the pages that follow, we will attempt to determine if broadleaves are more efficient to store SOC in mineral soil, for a given soil type. A second objective will be to assess the role of soil chemical properties in the SOC dynamics in relationship with species traits. To answer these questions, this study established an experimental design located in the arboretum of Tervuren in Brussels which participate to a network of common garden experiments. Soil samples were collected at several depths in stands of 22 different tree species. The purpose of this experiment is to quantify C stocks from these samples and to analyze their chemical properties to then establish relationships between the different variables.

After a presentation of the context and objectives, the reader will find a description of the experimental design of this work and of the methods used to analyze the data and generate results that would be interpreted. Then, this work contains an overview of the main results of the experiment, exploring the evolution of data with depth and correlations between variables. Tree species effects will be investigated with both discrete and continuous approaches. These results will be finally discussed in the last section of this work.

State of the art

1. The importance of C sequestration in the context of climate changes.

During the last century, forest management has been driven by the optimization of wood production to meet the demand (Vesterdal et al. 2013). But in recent years, there has been an increasing interest for the ecosystem services provided by forests, particularly in a context of climate changes when the focus is put on the need for a more adaptive and more resilient forestry (Lindner et al. 2010). A major function of forests is the capacity of the soil to sequestrate organic carbon. Indeed, on a global scale, there is two-to-three-times as much organic carbon stored in soil as there is carbon in the atmosphere. This huge quantity of soil organic carbon is derived from decay and metabolic activities of living organisms and represents about two thirds of the total soil carbon (Scharlemann et al. 2014). Even if there are considerable variations in the published estimates, most studies report that the global SOC stocks reach approximately a total of 1,500 Petagrams (Scharlemann et al. 2014). In comparison, the total soil carbon stock in the mineral soil is estimated to be around 2,500 Petagrams in the upper 200 cm with a stock of approximatively 700 Petagrams of carbon in the upper 30 cm (Batjes 1996). In 2007, according to a recent study, the total soil carbon stock in temperate forest was estimated at 56.7 Pg in the mineral soil and 12.1 Pg in the litter, with an uncertainty of 6 Pg (Pan et al. 2011). As well as the uncertainty about the SOC stock estimates, efforts must be made to collect more data about the spatial distribution of SOC and carbon emissions from soils (Scharlemann et al. 2014).

The soils of forests play thus a major role in the global carbon cycle. Hence, carbon sequestration capacity of these ecosystems could be optimized for the purpose of mitigating greenhouse gas emissions. The annual global exchange of CO_2 between forests and atmosphere, via photosynthesis and respiration, equals 7 times the anthropogenic C emissions and European forests could be used a sink these emissions (Jandl et al. 2007; Brown 1996). According to the Kyoto Protocol, this sink could be exploited to meet objectives in terms of emission reduction, by improving the carbon stock in forests ecosystem with forestry activities and afforestation, even though this strategy is constrained by few available land area (Jandl et al. 2007). In short to mid-term, carbon sequestration could effectively increase in temperate continental forests, mostly in northern parts of this region. However, in the long-term forests could become a source of carbon because of disturbances caused by climate changes (Lindner et al. 2010). The increasing frequency of events such as forest fires (Vesterdal et al. 2013) is likely to stimulate the release of CO2 from the mineralization of soil organic carbon and therefore diminishing the C stock (Jandl et al. 2007). Moreover, changes in environmental conditions like temperature raising, will impact the soil biota activities and increase soil respiration (Schindlbacher et al. 2010; Jones et al. 2009). A predicted shift in spatial distribution of plant species has also been widely investigated (Augusto et al. 2015). As climate changes will induce a modification of the tree species composition of temperate and boreal forests (Lindner et al. 2010), the soil biota is also likely to change because the living communities in soils are species-specific (Jones et al. 2009). However, this shift to a new equilibrium in carbon stocks concerns only carbon in the non-stable soil organic matter. This type of carbon is found in the forest floor while more stable carbon is stored in the mineral part of the soil (Jandl et al. 2007).

The purpose here is therefore not only to increase the soil organic carbon stock, but also to store more stable carbon by use of management practices that lead to long term C sequestration in forests soils (Jandl et al. 2007). Furthermore, the influence of tree species selection on carbon storage has been widely established(Vesterdal et al. 2013; Mayer et al. 2020) and it can be a key instrument for more appropriate management practices to improve this ecosystem service. However, there is still an unambiguous relationship between tree species and the stability of the C sequestrated in the soil and a need for more significant results has been formulated (Jandl et al. 2007).

2. Carbon storage in soils.

2.1 Carbon storage: a balance between inputs and outputs.

The soil organic carbon stock is determined by the balance between input and output of organic matter fluxes in the soil system. The input mainly consists of dead plant material, from above and belowground tissues. In forest ecosystem, this corresponds to the total litterfall and dead roots from trees. Other sources of soil organic matters are the soil biota, which contributes to carbon input with fecal material and organisms' dead bodies from micro and macro fauna, and the rhizodeposits, referring to exudates from roots and mycorrhizal fungi of the rhizosphere (Mayer et al. 2020). Once in the soil, the fresh organic matter is progressively transformed by soil fauna in the process of decomposition, meaning that the organic matter is fragmented and decomposed into more simple compounds that can be assimilated by microorganisms' enzymes. Microorganisms' respiration is the process by which the soil organic carbon is mineralized and released into the atmosphere as carbon dioxide. This constitutes, with leaching of dissolved organic carbon (DOC), the output of the C. Between the moment organic carbon enters the system and the moment it is released out of the soil, C is stored in the different pools of organic matter in the soil.

Factors thought to be influencing turnover rates of the soil organic matter are the climate, the soil properties and the chemical quality of carbon. Soil properties comprise characteristics such as clay content, soil moisture, pH and nutrient status (Jandl et al. 2003). It has been demonstrated in numerous studies that tree species have an influence on all these elements, except on clay content (Augusto et al. 2015).

2.2 SOC stabilization.

Soil organic carbon is stored in different pools with different levels of protection and stabilization mechanisms (Six et al. 2002). The unprotected C pool is present as non-stable particles from the large particulate organic matter (POM) fraction (Angst et al. 2019). This labile form of C is the most susceptible to disturbances and mineralization because it is easily accessible for soil microorganisms (Desie, Vancampenhout, and Heyens 2019). This C pool comprises also DOC (Zimmermann et al. 2007). Decomposing organic matter can be transformed into stabilized forms, more protected, either physically or chemically, from microorganisms' activities. The physically protected pool is made of microaggregate-associated soil C and chemically protected pool is formed when organic matter is associated with clay minerals or Al-Fe oxides by adsorption (Six et al. 2002). A third stable pool is described in an article in which carbon is biochemically protected by the complex composition of organic matter due to complexation or condensation reactions. This type of C is the most stable and is directly dependent on the litter quality (Six et al. 2002).

The species doesn't have a direct impact on these stabilization processes, except for the recalcitrance of the litter that inhibits the degradation of organic matter, but species traits have an impact on soil properties that have a high importance for soil fauna activity and adsorption processes.

2.3 The role of earthworms in SOC stabilization and decomposition processes.

Several studies have been carried out on a link between soil fauna communities and C in soils. An experiment in afforested post-mining site has shown evidence of the impact of earthworm communities' presence on the carbon in the soil (Frouz et al. 2009). There was a significant correlation between earthworm density and occurrence of cast and C total stock (Frouz et al. 2009). However, investigations on the mechanisms behind this process revealed that the interactions between C and earthworms are context-depend (Jan Frouz 2018). It varies over time and with soil (Hobbie et al. 2007)conditions and litter type (J. Frouz et al. 2014).

An important element for stabilized C storage and involving earthworms is formation of microaggregates and microaggregates (Bossuyt, Six, et Hendrix 2005). A study focused on the effect of incubating soil samples with the earthworm species *Aporrectodea caliginosa* on soil. Results showed that the presence of earthworms stimulates large macroaggregates formation in mineral soil and carbon content of microaggregates occluded into these large macroaggregates, which could lead to a long-term stabilization of the SOC (Bossuyt, Six, et Hendrix 2005). The ingestion of organic matter mixed with inorganic soil material in forest floor and their passage through the earthworm gut results in excretion of cast in topsoil (Bossuyt, Six, et Hendrix 2005). Casts form stable macroaggregate (Blanchart 1992) and can have a protective effect on soil organic matter because organic matter passing through the gut makes bond with mineral components thank to microbial polysaccharides (Martin 1991; Shipitalo et Protz 1989). However, the reduction of carbon mineralization by protection in macroaggregate has been measured in long-term, while in short term earthworms have a positive effect on soil respiration. An experimental study determined that antagonist effects of earthworm *M.anomala*on organic matter dynamic are observed with time (Martin 1991). It showed a reduction of organic matter in cast in the long-term (a few months) while in the short term (a few hours) earthworms increased mineralization of organic matter (Martin 1991). In the short term, earthworms supply microbial communities with a more accessible substrate for respiration Hobbie et al., 2007). But as the soil ages, earthworms' activities promote a shift from labile C in litter to protected POM, with stabilized C, by integration of POC into the mineral pool with bioturbation and physical protection with soil aggregation (Frouz et al. 2014; Vesterdal et al. 2013).

Moreover, these fluxes of soil organic matter from unprotected pool in litter to protected pool in the mineral soil, with formation of cast and aggregates by earthworms, depend on the clay content of the soil (J. Frouz et al. 2014). SOC stabilization is also higher in soil with low SOM content, as well as post-mining sites because organic matter fluxes dynamically depend on the capacity of the different pools (J. Frouz et al. 2014).

3. Impact of tree species traits on SOC stocks: differences between coniferous and broadleaved species.

Several studies have underlined the effect of tree species traits on carbon cycle in forests. This section will resume the trends in C-storage differences between tree species through several experimental studies and highlights the mechanisms behind these results.

3.1 Differences in SOC distribution in the soil profile.

A large body of literature has investigated the effect of tree species on C storage in the forest floor and there is a consensus among scientists that a more important amount of C is stored in the forest floor under coniferous species than under broadleaved species (Augusto et al. 2015; Vesterdal et al. 2013).

A review of several studies on the effect of species on C storage in boreal and temperate forests (Vesterdal et al. 2013) highlighted that among experimental studies focusing on C stored in mineral soil, several didn’t show significant differences. However, other results report a tendency of a higher C stock under broadleaved species than coniferous species. There seems to be a negative correlation between C stock in mineral soil and C stock in forest floor, however the total carbon stock doesn’t seem to differ between species. Some studies illustrated that *Fraxinus*, *Acer*, *Tilia* and *Ulmus* have higher C and better results in terms of C stock than *Picea*, *Pinus* and *Larix*, as well as *Quercus* and *Fagus* (Finzi, Van Breemen, et Canham 1998; Oostra, Majdi, et Olsson 2006; Vesterdal et al. 2008; Langenbruch, Helfrich, et Flessa 2012).

Another study in an afforested post mining site (Jan Frouz et al. 2013) also found a negative correlation between the level of C storage in the forest floor and in the first five centimeters of the topsoil, corresponding to the A horizon. It reported a significantly higher C stock in organic horizons Oe+Oa in coniferous (*Picea* > *Larix* > *Pinus*) than in broadleaves (*Alnus* > *Quercus* > *Tilia*) while C stock in mineral soil is higher in broadleaved stands (*Tilia* = *Alnus* > *Quercus*) than in coniferous stands (*Larix* > *Pinus* > *Picea*). Also, it appeared that the total higher total C stock was correlated with soils with higher C stock in the A horizon.

However, in the review (Vesterdal et al. 2013), the boreal experiments and two studies in temperate forests (Son and Gower 1992; Gurmesa et al. 2013) have the same effect on C storage in mineral soil as in forest floor (Vesterdal et al. 2013). For example, the study of Gursema et al., that took place in 2013 in Danemark identified a higher C stock in mineral soil under *Picea* and *Larix* than under *Quercus* and *Fagus*. Besides, in an experiment in boreal area and a study of Son and Gower (1992, USA), coniferous species have a higher C stock than Quercus and populus and decrease in the order *Picea* > *Pinus* > *Larix*.

Similar results have been found in a most recent study (Desie, Vancampenhout, and Heyens 2019). This experiment investigating the effect of conversion from mixed broadleaved forest to Norway spruce (*Picea*) monocultures identified an increase of 3% to 4% of total C concentration in mineral soil with a conversion from broadleaves to spruce stands (Desie, Vancampenhout, and Heyens 2019).

With these various results among studies, conclusions are thus difficult to reach. This lack of consistent trend probably comes from the context-dependent aspect of species effects on C storage (Mayer et al. 2020). Overall, broadleaved species are expected to store more C in sites with fertile soil and warm climate, whereas coniferous species store more C under less favorable conditions (Mayer et al. 2020). Spatial natural distribution can also bring a bias in results interpretation because coniferous species have tended to settle down on less fertile sites than broadleaves. There is therefore a need in field research for network of common garden experiments to identify tree species effects on C storage in mineral, and thus stable, pools without co-varying effects of site conditions (Jandl et al. 2007; Vesterdal et al. 2013; Mayer et al. 2020).

3.2 Impact of litter inputs and litter quality on decomposition rates and soil conditions.

We can focus on the source of organic dead matter from the tree: above and belowground litter. Litter can differ between tree species in terms of quantity and quality. Concerning quantitative litter characterization, litterfall input flux may not play a significant role in C storage compared to the decomposition and stabilization processes that occur belowground and depend on soil conditions (Jan Frouz et al. 2009) as the amount if litterfall is the same for broadleaves and coniferous species when the climate and site fertility are similar(Augusto et al. 2015; Vesterdal et al. 2008). Furthermore, root exudates may be a factor of variation between species(Augusto et al. 2015). However, few data available on this factor and it will not be considered in this study.

On the other hand, litter quality and chemical composition vary between species, and influence decomposition rates of C in forest floor. Generally, broadleaves litter has a quicker decomposition rate than coniferous litter, resulting in a lower level of labile C accumulation in the forest floor (Augusto et al. 2015; Hobbie et al. 2006). The first factor is the litter lignin content. Lignin is a recalcitrant compound and the more it is present in litter, the lower the substrate use efficiency by microbes (Angst et al. 2019). Overall broadleaved species will have a low lignin content.

Secondly, litter Ca content is an indirect factor influencing decomposition rate. Ca-rich litter correlates with a high decomposition rate (Hobbie et al. 2006). This factor has an impact on soil condition in the forest floor and in particular communities of earthworms, who are reported to be more abundant in forest floor where the Ca content is high (Angst et al. 2019; Reich et al. 2005; Six et al. 2002; Jan Frouz et al. 2009) as it is one of the three factors, with pH and moisture, influencing species-specific soil fauna activities (Vesterdal et al. 2013). As demonstrated by Reich et al. in 2005, forests plots with *A. platanoides*, *A. pseudoplatanus*, and *Tilia* were correlated to higher pH, base saturation and earthworm abundance, whereas the opposite effect was observed on plots with P. sylvestris, P. nigra, and Larix. Broadleaves are nutrient-rich species and have thus a higher Ca content in their litter than conifers (Augusto et al. 2015; Hobbie et al. 2006). This is in line with studies that observed higher soil fauna activities under broadleaved trees (Jan Frouz et al. 2009).

Finally, high C/N, lignin/N, C/P and lignin/P ratios tend to slow down decomposition rates in forest litter and are higher in conifers litter than in broadleaves litter (Augusto et al. 2015).

3.3 The effect of tree species on soil acidity.

These low decomposition rates of low-quality organic litter under coniferous trees result not only in an accumulation of labile carbon, but also in an acidic and nutrient-poor forest floor. As mentioned in the previous section, this will impact the conditions in which soil fauna, and in particular earthworms, evolve (Vesterdal et al. 2013; Reich et al. 2005). There is a large volume of published studies, gathered in a review of Augusto et al. in 2013 (Augusto et al. 2015), that have led to the evidence that coniferous species acidify the topsoil compared to broadleaved species by decreasing the pH of a few one tenth of units and by decreasing base saturation (Ca^{2+},Mg^{2+} and K^{+}). However, this conclusion is a relative effect and comparison between topsoil acidity in coniferous and broadleaved stands is significant only if they are in the same site context(Augusto et al. 2015). Concerning the causes of these pH differences, it can be explained by the difference in organic acids produced during decomposition processes (Mueller et al. 2012), dissimilar nutrients uptake rates, atmospheric pollutants and more acidic and nutrient-poor litterfall in coniferous stands(Augusto et al. 2015).

In addition, if the non-linearity of acidification process is accepted (Chadwick et Chorover 2001), it could be interesting to investigate the relationship between tree species and the shift from one stable state of the soil to another. In 2019, Desie et al. performed in an experimental study focused on the impact of conversion from mixed broadleaved forest stands to monocultures of Norway spruce on the soil acidity. They found that the stands which were on soil in the exchangeable domain (with high pH and high base saturation) shifted to the aluminum domain with the conversion (with low pH and more acid cations), giving another evidence of the effect of a coniferous species on topsoil conditions(Desie, Vancampenhout and Heyens 2019).

3.4 The effect of tree species on SOC stabilization.

As well as C storage distribution in the soil profile, a major element to investigate is the effect of species on the stabilization of SOC. As mentioned in previous sections, stabilization of SOC occurred with the incorporation into stable C pool in the mineral soil. Therefore, we can expect to observe a higher level of stabilized C under broadleaved trees given that these species provided favorable conditions for earthworms, in terms of Ca richness due to the high base saturation and a high pH (see section 3). Bioturbation and aggregates formation are thus encouraged, and the C stability is ensured by material fluxes to mineral and physical protection (see section 2.2).

However, basic conditions with a sufficient level of Ca in the soil can stimulate microbial respiration and accelerate mineralization instead of C incorporation in stable pools (Six et al. 2002). Also, with acid pH and low base saturation conditions, the content in cation Al^{3+} and Fe^{3+} is expected to be high. Although Ca^{2+} is a bond between mineral and organic matter by adsorption on the CEC, Al^{3+} and Fe^{3+} are trivalent cations, so they are capable of stronger binding between clay and humus (Angst et al. 2019; Hobbie et al. 2007). Recent research (Rasmussen et al. 2018) found that when the pH is higher than 5.5, Ca^{2+} is responsible for 10% of the C stabilization by mineral adsorption, but for a pH lower than 4.5, OM complexation with Fe and Al represents 90% of the OM stabilization (Angst et al. 2019; Rasmussen et al.

2018). Indeed, a strong negative correlation has been seen between Al and Fe exchangeable content and C mineralization(Angst et al. 2019; Hobbie et al. 2007), also because in these conditions the low pH inhibit microbial activities, while a positive correlation has been observed between Al and Fe content and mineral soil C pool (Desie, Vancampenhout, and Heyens 2019). These conditions correspond with observations made under coniferous stands in the study performed by Desie et al. (2019).

To draw conclusions about the best soils species-specific conditions for C stability, the study of Desie et al. has compared the ratio of total C in stable pool on total C in labile pool of broadleaved stands, with soil in the exchangeable domain, with this ration for coniferous stands, with a soil in the aluminum domain. The result is a better total C stability associated with broadleaved species. It is explained by the importance of the C in small POM protected in soil aggregate (more important on broadleaved stands) for the total C stability compared to C chemically protected by adsorption (more important on coniferous stands) that contributes less to total C stability (Desie, Vancampenhout, et Heyens 2019). Consequently, for efficient management decisions about species selection, both the C stock capacity and C stability must be investigated, regarding the site context (Angst et al. 2019).

4. Objectives and research question

The main objective of this study is to assess SOC stocks in the topsoil (holorganic + 0-20 cm layers) of 24 monospecific stands, including a wide range of coniferous and broadleaved species, to highlight the impact of tree species traits on carbon storage. A second objective of this experiment is to determine whether tree species traits have an effect on chemical properties of the topsoil such as pH and exchangeable cation contents. We will also determine how these chemical parameters are related to SOC stocks and to what extend they play a role in C dynamic and C stabilization processes.
The main purpose of this master thesis can be expressed with the following research question: *For similar site conditions, does the topsoil of broadleaved stands store more carbon than coniferous stands?*
Two sub-questions will be investigated: (1) *Can we observe significant differences by considering species trait differences? (2) Which species induce more favorable soil conditions for C storage in stable pools in term of acidity and nutrient status?*

These questions rely on the assumption that site conditions, including climate, soil texture and soil pedogenesis, are homogeneous for all the considered stands.

Material and methods

1. Description of the study area

The study was performed in the arboretum of Tervuren, situated in the Sonian forest. This 4400-ha large forest massif is located east and south of Brussels urban area. Eighty percent of the forest cover is even aged and dominated by *Fagus sylvatica L.* (Herbauts, Bayad, and Gruber 1998). The arboretum of Tervuren, created in 1902, covers 120 ha and belongs to the Royal Trust (Royal Trust 2020). The forest expert behind the conception of the arboretum was Professor Charles Bommer, from the Brussels University. The arboretum is a tree collection. Its original objective was to experiment the adaptation capacity of exotic species to local environment to develop intensive forestry to sustain the growing Belgian industry with timber production (Bommer 1930). The arboretum is divided into 40 groups based on the origin area of tree species and gathered into ten geographical zones (Royal Trust 2020). In the context of this study, we focused on eight geographical zones: South West Asia, Pacific West Continental, Pacific West Coast, Northern Atlantic East Coast, North and West Europe, Mediterranean, Atlantic East Coast and Asia.

According to Walloon geological map, the arboretum of Tervuren is located on two different geological layers from the Tertiary era: the sands of the Ledien and the sands of the Tongrien (Sint-Huirbrechts layer). According to Flemish geological map, there is a third layer: the sands of the Brusselien. Between 20,000 and 30,000 years ago, during the Late Pleistocene, a layer of glacial Loess formed deposits on the Tertiary sediments, resulting in the formation of loamy soils.

The study area stands on the following three soil types:

- Abc0: loamy soil with favourable drainage, strongly spotted textural B horizon, thick A horizon (>40cm)
  "Sol limoneux à drainage favourable, à horizon B textural fortement tâcheté et à horizon A épais"
- Aba0 (b): loamy soil with favourable drainage, spotted textural B horizon, thick A horizon (>40 cm)
  "Sol limoneux à drainage favourable, à horizon B textural tâcheté, à horizon A épais"
- ADp: loamy soil with moderate to imperfect drainage, no profile development
  "Sol limoneux à drainage modéré à imparfait, sans développement de profil"

Soils types Abc0 and Aba0 (b) are associated with Luvisols, with respect to the WRB classification system. The soil of the Sonian forest is described as an acidified and desaturated soil with a weak biological activity and low-quality humus (Herbauts, Bayad, and Gruber 1998). It is characterized by an eluvial E horizon that can reach the depth of 40 cm. The clay content is +/- 10%, the water-pH is 3,5-4,5 and apparent density is 1,3 kg/dm^3 with a 51% porosity. There is then a textural and structural discontinuity with the textural B horizon. It is an illuvial horizon that can reach the depth of 120 cm. The clay content is +/- 20% and pH is 4,5-4,9 and apparent density is 1,5 kg/dm^3 with a 42% porosity (Herbauts, Bayad, and Gruber 1998).. This soil type, because of the high density of the Bt horizon, is sensitive to hydromorphic degradation which is associated with a process of pseudogleyfication (Herbauts, Bayad, and Gruber 1998). In degraded Luvisolic soils, crackings form preferential pathways of drainage resulting in the emergence of "glosses", subvertical inclusions in the eluvial horizon continuity (Baize et Girard 2008). Glosses generally shape prismatic patterns in the soil (Langohr 1986). Most of the studied area is situated on degraded Luvisolic soils. A strongly spotted Luvisol (Abc0) is typically associated with old beech forest as the Sonian Forest and it is at a high stage of hydromorphic degradation, while spotted Luvisol (Aba0(b)) is more located on tens or hundreds of hectares large wood (R. Langohr, 1986). A stronger hydromorphic degradation is associated with a larger presence of glosses (Langohr 1986).

Soils in the study area have already known hydromorphic process, according to the Belgian Soil Classification map, with different degrees of impact depending on whether the soil type is Abc0 or Aba0(b). differences exist between E horizon and Bt horizon in terms of texture and acidity. The base saturation is also lower in the E horizon compared to the A and Bt horizons (Baize et Girard 2008).

2. Sampling approach

On the site of the arboretum, 54 monospecific stands have been previously identified and listed in the different geographical zones. Among those 54 stands, 24 have been selected for this experiment, as presented in Figure 1. Several criteria were established for selecting the stands. First, we selected only monospecific stands that were at least 40 years old with a minimum tree circumference of 20 cm, with the center area dominated by the main tree species of the monoculture. Second, similar number of coniferous and broadleaved species was selected, distributed in the different geographical areas as shown in Figure 1 (except in Chili and Argentine and in Boreal America zones). Third, we avoided to select plots standing in valley zones, where the soil type, ADp, differs strongly from the soil types in the rest of the study area because of a limited drainage. The repartition of selected plots regarding soil type is represented in Figure 2. Figure 3 shows how the selected plots are distributed among the three different geological layers, with respect to the Flemish geological map.

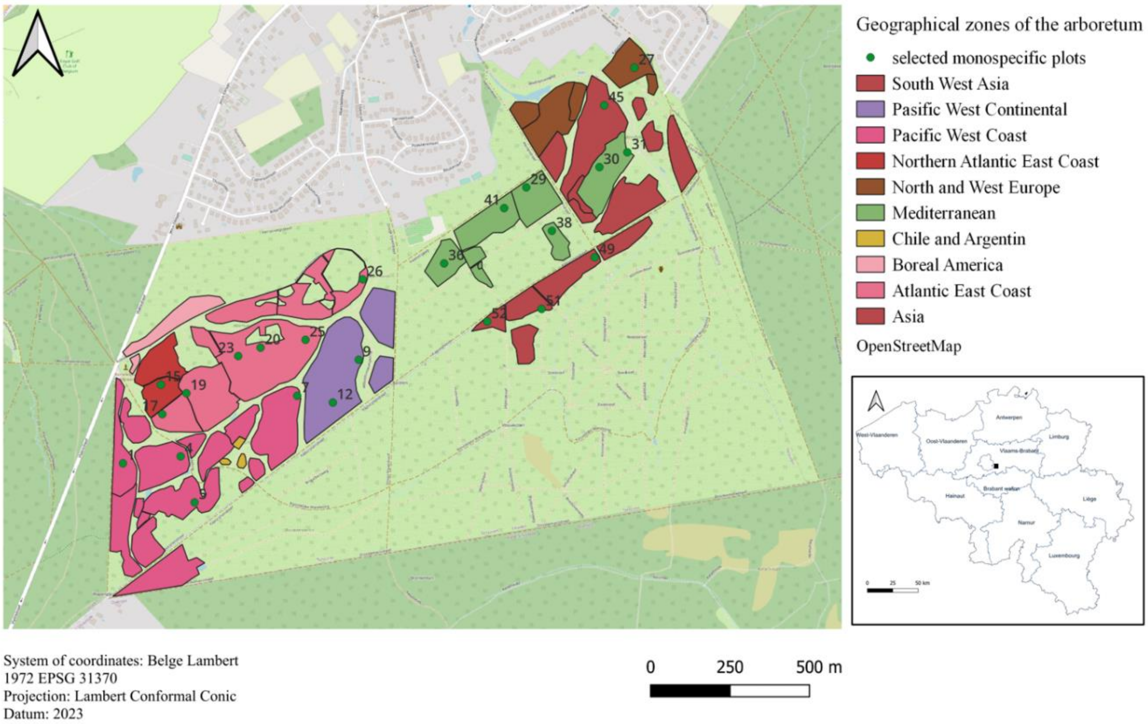

Figure 1: Map of the studied site (arboretum of Tervuren) showing geographical area delimitation and the localization of sampling plots.

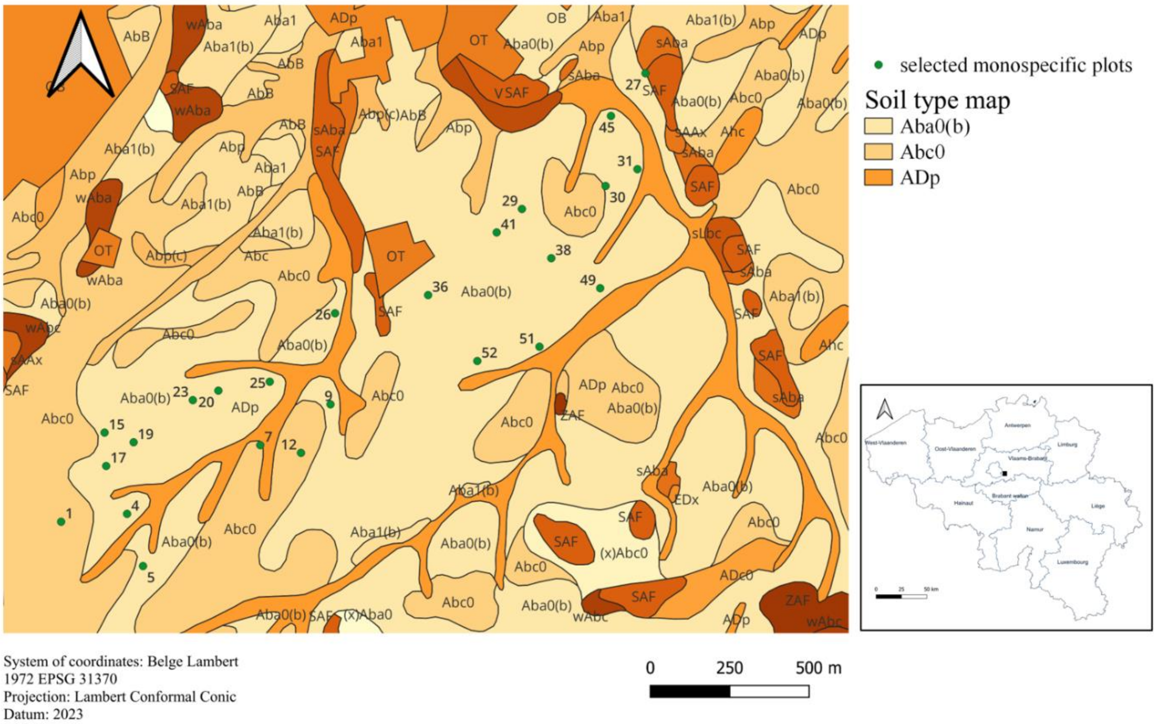

Figure 2: Map of the soil types (Belgian classification) found in the studied site and the localization of sampling plots.

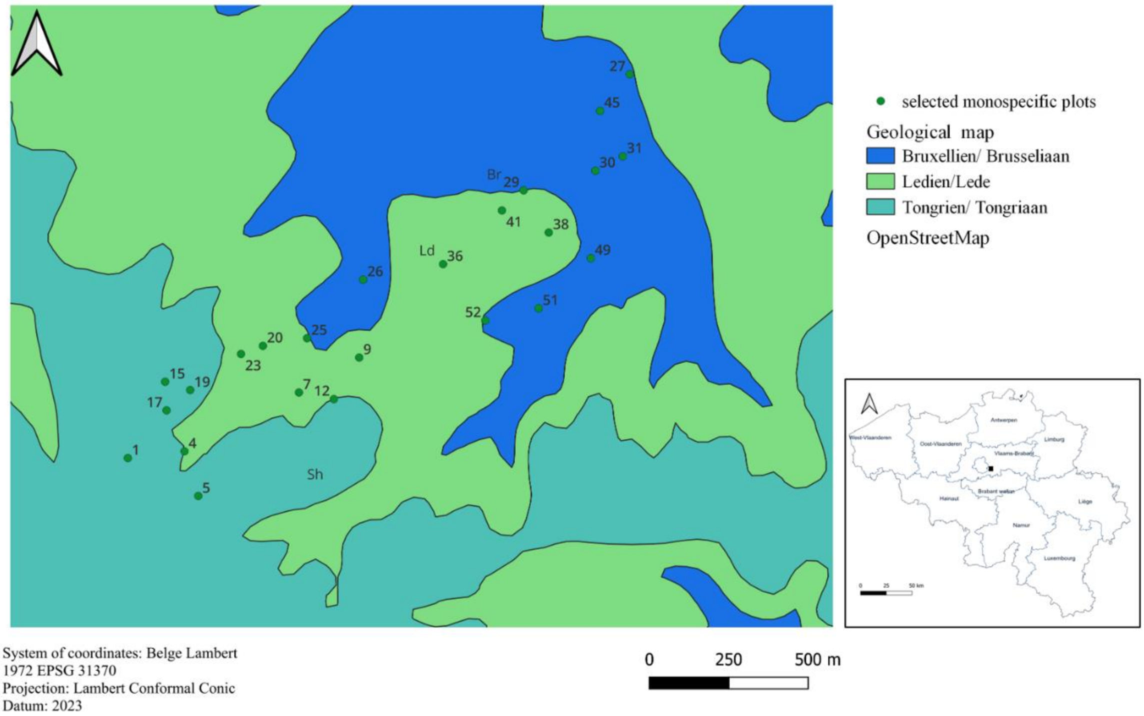

Figure 3: Map of the geological layers found in the studied site and the localization of sampling plots.

On each selected stand, circular plots with a radius of nine meters have been established. The center of each plot has been materialized by a pole planted in the soil. On each plot, the percentage of surface covered by the main tree species and the shrub and herb coverage percentage had been previously reported. Table 1 summarizes the selected plots main tree species, their species category (broadleaves or conifers), their geographical group and information about the plot (coverage percentage). This plots sample contains 22 different tree species, listed in the table below, from 17 different taxonomic groups. Among those tree species, 12 belong to the broadleaves category and 14 belong to the conifers category. *Pseudotsuga menziesii* and *Fagus sylvatica* have replicates because each of these species are presents in two different plots.

Table 1: Plot description: N°, species category (C, conifers; B, broadleaves), herb and shrub coverage (%), soil type, geology layer with respect to Walloon classification (W) and geology layer with respect to Flemish classification (F).

Plot	Tree species (C or B)	Herb coverage	Shrub coverage	Soil type	Geology (W)	Geology (F)
1	*Picea sitchensis* (C)	6,67%	0,00%	Abc0	Tongrien	Tongeriaan
4	*Pseudotsuga menziesii* (C)	77,67%	2-20%	Aba0(b)	Tongrien	Lede
5	*Thuja plicata* (C)	7,33%	0,00%	Aba0(b)	Tongrien	Tongeriaan
7	*Calocedrus decurrens* (C)	22,33%	2,00%	Abc0	Tongrien	Lede

9	*Pseudotsuga menzies* (C)	94,33%	2,00%	Aba0(b)	Tongrien	Lede
12	*Pinus monticola* (C)	53,00%	0,00%	Abc0	Tongrien	Tongeriaan
15	*Juglans cinerea* (D)	63,00%	1-10%	Aba0(b)	Tongrien	Tongeriaan
17	*Tsuga canadensis* (C)	61,00%	6,00%	Aba0(b)	Tongrien	Tongeriaan
19	*Betula alleghaniensis* (B)	17,33%	0,00%	Aba0(b)	Tongrien	Tongeriaan
20	*Acer saccharinum* (B)	54,67%	0,00%	Aba0(b)	Ledien	Lede
23	*Fraxinus americana* (B)	93,33%	38,00%	Aba0(b)	Ledien	Lede
25	*Quercus rubra* (B)	69,00%	1-10%	Aba0(b)	Ledien	Lede
26	*Tilia americana* (B)	78,00%	13-50%	Aba0(b)	Ledien	Brusseliaan
27	*Fagus sylvatica* (B)	7,33%	0,00%	sAba	Ledien	Brusseliaan
29	*Quercus robur* (B)	95,67%	50-70%	Aba0(b)	Ledien	Lede
30	*Acer pseudoplatanus* (B)	79,67%	3,00%	Aba0(b)	Ledien	Brusseliaan
31	*Picea omorika* (C)	0,00%	0,00%	Aba0(b)	Ledien	Brusseliaan
36	*Pinus nigra subsp. Laricio* (C)	89,33%	10-20%	Aba0(b)	Tongrien	Lede
38	*Fagus sylvatica* (B)	5,00%	3,00%	Aba0(b)	Ledien	Lede
41	*Cedrus atlantica* (C)	60,33%	1,00%	Aba0(b)	Tongrien	Lede
45	*Abies nordmanniana* (B)	94,67%	11-30%	Aba0(b)	Ledien	Brusseliaan
49	*Cryptomeria japonica* (R)	34,00%	0,00%	Aba0(b)	Ledien	Brusseliaan
51	*Larix kaempferi* (R)	19,00%	1,00%	Aba0(b)	Ledien	Brusseliaan
52	*Juglans mandshurica* (D)	73,00%	22-50%	Aba0(b)	Ledien	Brusseliaan

Soil samples were collected in the 24 selected plots. When arriving at a plot, the first step was to divide the plots, by eye, into four quadrants following the four cardinal directions. In each quadrant, a sampling spot was randomly chosen, as long as it was an undisturbed location. By collecting four samples at four different locations in the plots, we ruled out the effect of a possible heterogeneity in the plots. On each spot, 6 soil samples are collected corresponding to 6 soil layers from 6 different depth intervals. Three samples have been collected in the topsoil and three other samples have been collected in the deep soil. Prior the soil collection, the organic material of the forest floor, or holorganic layer, has been harvested. A squared timber frame, delimiting a surface of 0,25 m^2, is placed on the litter surface and organic material inside the frame has been extracted and conserved into a paper bag. This operation stops when the first elements of mineral material appear. Following this, the mineral soil samples collecting of the first three mineral topsoil samples has been carried out using a two-piece root auger, a hammer, a gully, a tape measure and a knife. The purpose was to harvest three soil cores at the following depth intervals: from 0 cm to 5 cm below the surface, from 5 cm to 10 cm below the surface and from 10 cm to 20 cm below the surface. Soil cores were extracted from the mineral soil with the cylinder hand auger (and the hammer) and placed in a gully. Cores were then measured and cut with the knife at chosen depths. The three obtained core samples were kept separated into plastic bags. The two first soil samples (0-5 cm and 5-10 cm) were cylindrical cores with a radius avec 4 cm, and a height of 5 cm and the third soil samples (5-20 cm) was a cylindrical core with a radius of 4 cm and a height of 10 cm. Those three samples are quantitative so that their volume can be calculated.

After this step, three other samples of mineral material have been collected in the deep soil using a classical auger, a gully and a tape measure. The purpose was to harvest three soil samples at the following depths: 50 cm below the surface, 75 cm below the surface and 100 cm below the surface. The soil was extracted from the formed cavity with a hand auger and placed in the gully. Soil has been collected using the tape measure at the three chosen depths, in an interval of approximately 5 cm up and below the measured depth (50 cm, 75 cm and 100 cm). The resulting three soil samples were kept separated into plastic bags. Those three samples were non-quantitative because the volume of harvested soil was unknown. The purpose of those samples was to verify the homogeneity of the deep soil properties in the study area.

3. Laboratory analyses

Once soil samples have been collected in the four sampling locations in each plot (6 x 4 x 24), they were stored in a climate room. There, the four samples of the same depth and collected on the same plot were mixed and placed in an aluminum tray. Consequently, a set of 24 x 6 samples were obtained.

All the samples (24 x 6) have been stored in a heat chamber to be air dried at 40°C for at least one week until they reached constant mass. All the samples are then sieved with a 2 mm mesh and put back again into plastic bags. The residues from the sieving (small rocks, branches, roots) were kept separated from soil samples in aluminum tray. The 144 sieved samples were stored in the heat chamber (see annexes 1, protocol 1) After that, several analyses were performed in the laboratory to obtain the following data: carbon and nitrogen content, bulk density of dry soil, effective and potential pH and exchangeable cation content (Al^{3+}, H^{+}, Ca^{2+}, Mg^{2+}, Fe^{3+}, K^{+}, Na^{+}, Mn^{2+}).

First, the carbon and nitrogen content of topsoil soil samples were determined for the depth intervals 0-5 cm, 5-10 cm and 10-20 cm (24 x 3). A sub-sample of each sample was first collected to determine the water content of the soil (see annexes 1, protocol 2). One gram of the air-dried soil sample was weighted in a tared container (FW, fresh weight) with a precision scale Sartorius (0.0001 g) and placed in a heat chamber at the temperature of 105 °C during one night in a desiccator. After the drying period, the desiccator was placed out of the heat chamber and let at room temperature. The containers with dry soils were then weighted at constant weight (tare + DW, dry weight). The humidity percentage has been then obtained by the following equation:

Equation 1:

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H = Humidity content of soil [%]

FW = Fresh weight of air-dried soil [g]

DW = Dry weight of soil [g]

Once the humidity percentage determined for those samples (24 x 3; 0-5 cm, 5-10 cm, 10-20 cm), the next step consisted in the quantification of carbon and nitrogen with a FlashSmart Elemental Analyzer (Flash EA 1112 from Thermo Fisher Scientific) by a high temperature combustion followed by a chromatographic analysis of produced gazes (see annexes 1, protocol 3). A pretreatment of the soil material for this experiment was the grinding (at 20 µm) of a subsample of approximately 5 grams of each air-dried soil sample (24 x3) with a vibratory disc mill RS 200. After the pretreatment of samples, 50 mg of grinded soil were weighted with a precision scale in aluminum wells. The carbon and nitrogen content were directly given in % N and % C. The percentage values correspond to the quantity of C or N (g) contained in 100 g of air-dried soil.

Additionally, the total fresh weight of harvested samples (after air-drying) has been measured (precision 10 g) for depth intervals 0-5 cm, 5-10 cm and 10-20 cm (24 x 3). With the soil humidity percentage (Equation 1) and the fresh weight of the samples, the dry weight has been calculated in Equation 2. The volume of each quantitative sample corresponds to the cumulative volumes of the four soil cylindric cores harvested in each plot, as presented in Equation 3. With the volume of the sample, the bulk density could be found with Equation 4.

Equation 2:

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H = Humidity content of soil [%]

FW = Fresh weight of air-dried soil sample [g]

DW = Dry weight of soil sample [g]

Equation 3:

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V = core sample volume [cm^3]

r = radius of core sample [cm]

h = height of core sample [cm]

Equation 4:

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d = bulk density of dry soil [g/cm³]

V = soil core sample volume [cm³]

DW = dry weight of the soil sample [g]

The bulk density has been used to calculated the carbon content (g) by unit of soil volume (cm³) with Equation 5.

Equation 5:

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c = carbon content by soil volume unit [g/cm³]

d = bulk density of dry soil [g/cm³]

C = carbon content in mass percentage of air-dried soil [% or g/100 g]

These values of carbon content by volume unit could lead to the calculation of carbon stock in each soil layer (in 0-5 cm, in 5-10 cm, in 10-20 cm) with Equation 6. For information, for organic carbon content values in 10-20 cm for plots 17, 25 and 41, it has been decided to use results from an external laboratory (see below).

Equation 6:

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S = carbon stock for a determined depth interval [Mg/ha]

c = carbon content by soil volume unit [g/cm³]

h = height of the depth interval [cm]

Additionally, the percentage of carbon content of the harvested organic material had been previously measured. The carbon stocks in the organic material from holorganic layer (forest floor) were calculated with Equation 7.

Equation 7:

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S = carbon stock in harvested organic material (holorganic layer) [Mg/ha]

DW = dry weight of harvested organic material (holorganic layer) [g]

c = carbon content in harvested organic material (holorganic layer) [%]

s = harvesting surface of organic material (holorganic layer) by sample (0.25 m^2) [m^2]

Concerning the data relative to soil acidity, water-pH and pH in a calcium chloride 0,01 M solution have been determined for all the depths (24 x 6; 0-5 cm, 5-10 cm, 10-20 cm, 50 cm, 75 cm, 100 cm). These measurements followed the procedure referenced ISO 10390:2005 of pH determination as described in annexes 1 (protocol 4). For each sample, five grams of soil have been mixed either with 25 ml of demineralized water or with 25 ml of a solution of $CaCl_2$ 0,01 mol/l. The pH of the two solutions has been measured, after a mixing and rest period, with a pH-meter. Water-pH measures protons present in the soil solution which give an indication of the active acidity (Brady and Weil, 2008). $CaCl_2$ pH represents the exchangeable acidity and measure protons in the soil solution and adsorbed protons on the permanent charges of the clay-humic complex (Brady and Weil, 2008).

Secondly, soil of samples of the depth intervals 0-5 cm, 5-10 cm, 10-20 cm and 100 cm were submitted to a determination of the effective cation exchange capacity and the exchangeable base saturation rate with extraction of a barium chloride solution 0,1 M and the ICP-AES method. These measurements follow the procedure referenced ISO 11260:1994+Cor 1:1996. The protocol (see annexes 1, protocol 5) is described by Karine Henin. As for the CN content experiment, the humidity and dry weight of samples had been previously determined with the same method with a one-night drying period in a heat chamber at 105 °C and the weighting of fresh and dry weights with a precision scale (Sartorius, 0.0001 g). The cations occluded in soil particles were extracted with a $BaCl_2$ 0.1 M solution. For each sample (24 x 4), 30 ml of $BaCl_2$ were added to 2.5 g of soil, of which the exact weight had been noted. After having been agitated for one hour and centrifuged for 10 min, the supernatant is collected into a 100 ml graduated flask. The extraction had to be carried out again two times before making up to the mark with $BaCl_2$ solution and filter the final solution. A blank solution with only 100 ml of $BaCl_2$ was also realized. The solution has been then analyzed by ICP-AES with a previous calibration. With this method, values in ppm were obtained for exchangeable cations (Na, K, Ca, Mg, Fe, Mn, Al). To transform the ppm values into cation content values in cmolc/kg, Equation 8 has been used.

Equation 8:

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$Cation_{content}$ = element content of air-dried soil [cmolc/kg]

ppm = values obtained by ICP [ppm]

V = total volume of $BaCl_2$ used for the extraction (100 ml)

FW = Fresh weight of air-dried soil used for the extraction [g]

DW = Dry weight of soil used for the extraction, calculated with Equation 2 [g]

v = Valence of the element measured out

M = molar mass of the element measured out [g/mol]

Another analysis consisted in the estimation of exchangeable acidity of soil, which corresponds to the sum of exchangeable Al cations and H^+ cations. This variable has been estimated with a NaOH titration of 25 ml of the $BaCl_2$-extracted solution for each sample (24 x 4 + blank sample) (see annexes 1, protocol 5). The concentration of NaOH had been previously determined by a titration of 5 ml of HCl 0.05 M. Before the titration, the electrode of a pH-meter had been immerged into the titrated solution. The titration has been proceeded until the pH of the titrated solution reached a value surrounding 7.8 units. The volume of NaOH used for the titration was noted and the total estimated content of acid cations was calculated using Equation 9.

Equation 9:

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EA = exchangeable acidity (sum of Al^{3+} and H^+) [cmolc/kg]

V_{NaOH} = volume of NaOH used to reach pH 7.8 in the sample

V_{blank} = volume of NaOH used to reach pH 7.8 in blank sample

C_{NaOH} = molar concentration of NaOH solution [mol/l]

V_{BaCl2} = total volume of $BaCl_2$ used for the extraction (100 ml)

FW = Fresh weight of air-dried soil used for the extraction [g]

DW = Dry weight of soil used for the extraction, calculated with Equation 2 [g]

V_{exch} = Volume of the titrated solution (25 ml)

The H^+ concentration could be estimated by subtracting the exchangeable Al content, found with ICP analyses, from the total estimated exchangeable acidity. In case negative H^+ values were obtained, they were considered as being equal to 0.

Once the cation contents had been determined, the effective cation exchange capacity (eCEC) has been calculated. The eCEC corresponds to the sum of the following exchangeable cations: Na, K, Mg, Ca, Al and H. In addition, the base saturation (BS) was also determined. It consists in the ratio between the sum of basic cations (Na, K, Mg and Ca) and the total eCEC. BS is generally multiplied by 100 to have a variable expressed in percentage. The base saturation formula is presented in Equation 10.

Equation 10:

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BS = base saturation [%]

$Cation_b$ = basic exchangeable cation content (Na, K, Mg, Ca) [cmolc/kg]

eCEC = sum of exchange cation content (Na, K, Mg, Ca, Al, H) [cmolc/kg]

Furthermore, texture analyses have been carried out by an external laboratory (Centre provincial de l'agriculture et de la ruralité, La Hulpe). It consisted in analyses of the texture (Clay %, Silt % and Sand %) of soil material from depths 10-20 cm and 100 cm. These analyses have been performed by with respect to the French norm NF X31-107 (September 2003) to determine the distribution of soil particles granulometry – pipette method. Additional data of the total organic carbon (g/kg) and water-pH have also been received from this laboratory (norms: NF EN 15936 and NF ISO 10390).

Finally, the weight data of the harvested leaf (leaves, needles) litterfall over half a year (extrapolated to a yearly litterfall) were supplied. It allowed to estimate the leaf litter turnover rate by dividing this variable by the total weight of collected organic material in the holorganic layer (excluding cones, branches and fruits). The calculation of this variable followed Equation 11. This variable was used to characterized species impact on decomposition process because no other species identity traits related to the foliage were available.

Equation 11:

$$LL_{TR} = \frac{LL_f}{LL_{tot}}$$

LL_{TR} = estimated leaf litter turnover rate

LL_f = estimated yearly litterfall weight (only leaves and needles, excluding cones, fruits and branches) [g/m^2]

LL_{tot} = sampled organic material in the holorganic layer (only leaves and needles, excluding cones, fruits and branches) [g/m^2]

4. Data visualization and analysis

All statistical analyses were performed using Rstudio.

4.1 Descriptive analyses

First of all, descriptive analyses were generated for the following variables: carbon content (%), $CaCl_2$ pH, effective cations exchange capacity (eCEC, cmolc/kg), exchangeable acidity (cmolc/kg), base saturation (BS, %) and exchangeable calcium content (Ca^{2+}, cmolc/kg). Those variables are related to chemical properties of the soil, giving an indication on the acidity and the exchangeable cations status. The analyses consisted in a description of the general evolution of those variables with depth. The purpose was to generate one chart for each variable with a depth scale as the y-axis, from the holorganic layer to 20 cm deep for carbon content and from 0 cm to 100 cm deep for the other variables. A graduation of 5 cm was assigned to the depth scale. Values measured in the depth interval 0-5 cm were represented on the scale at the depth 2.5 cm, values measured in the depth interval 5-10 cm were represented on the scale at the depth 7.5 cm and values measured in the depth interval 10-20 cm were represented on the scale at the depth of 15 cm. The holorganic and the depth 100 cm were indicated on the depth scale. The pH data collected at depth of 50 cm and 75 cm were not represented here. In each chart, for each depth, the data harvested in all the plots were summarized by a boxplot, indicating the range, median, first and third quartiles and potential outlier values. The mean value and the standard deviation of data were also generated for each depth (N=24).

The representation of these variables was used to have an idea of the general impact of depth on the soil chemical properties. In a second step, the same analysis was also performed to present the evolution of the carbon stocks (Mg/ha) and the dry soil bulk density (g/cm^3) with depth, from organic material layer to 20 cm for the carbon stock and from 0 cm to 20 cm for bulk density. The same statistics than for previous analyses (range, median, first and third quartiles, potential outlier values, mean value and standard deviation) were generated and a boxplot by depth representing data of all the plots was used. Additionally, a boxplot with associated statistics was used to give an idea of the total carbon stocks for all the plots.

Once the descriptive analyses have been carried out, the correlations between variables were investigated. A first step was to evaluate the correlation between variables by pairs at a given depth. To that end, a bivariate analysis was performed using a correlation matrix to obtain the correlation coefficients between pairs of variables for each depth. A scatter plot matrix was also generated. In addition, a Principal Components Analysis (PCA) of variables by depth was performed to reduce the data set to two dimensions and have a representation of relationship between the data and how it differs between depth intervals. Those analyses have been generated for depths 0-5 cm, 5-10 cm, 10-20 cm and 100 cm considering the following variables: $CaCl_2$ pH, water-pH, base saturation, exchangeable calcium content, exchangeable acidity, eCEC, exchangeable aluminum content, carbon content and silt content in topsoil. For the latter parameter, only for the 10-20 cm and the 100 cm depths.

4.2 Regression analyses: the impact of species composition on topsoil chemical properties.

A second analysis was the investigation of the relationship between the variables related to soil acidity conditions ($CaCl_2$ pH and base saturation in 0-5 cm soil layer) and variables related to tree species identity. Tree species identity factor was represented by discrete approach, where the variable was species category (conifers or broadleaves) and by continuous approach, where the variable was the estimated leaf litter turnover rate.

In the discrete approach, two charts with boxplots were generated with the $CaCl_2$ pH and base saturation (0-5 cm) data for all plots (y-axis) regarding the species category (x-axis). Statistical data (range, median, first and third quartiles, potential outlier values, mean value and standard deviation) were presented for these two charts. To explore how species category influenced the conditions in topsoil, two simple linear regression models were performed with the *lme4* R package. The expected results from this analysis were the coefficients (a and b) of the following equation:

Equation 12:

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where y represented the response dependent variable, either the CaCl2 pH or base saturation, and where x represented the explanatory independent variable, the species category. Regarding the fact that species category is a variable with two discrete values, x took binary values (0 or 1 depend if it is conifers or broadleaves). In case of a continuous explanatory variable (e.g., x = leaf litter turnover rate), the letter b in the equation corresponded to the intercept of the model and a was the slope. In case of a model with a discrete explanatory variable (e.g., x = species category), a indicated the average value for one category, conifers for example, and b was the deviation from a value. The significancy of the regression model is evaluated by to type of statistical metrics. On the one hand, the coefficient of determination R-squared measure the proportion of variance in the response variable that can be explained by the explanatory variable in the regression model (Zach 2021). On the other hand, the analysis of the variance (ANOVA) provides the p-value, which is the significancy test of the F-statistic. F-statistic determines whether or not all the explanatory variables are jointly significant (Cate Jr. et Nelson 1971). In this study, we considered the model as significant is p-value is < 0.05 and moderately significant if p-value is < 0.1. For regression models with several variables, the t-test is also provided. It tests whether each explanatory variable is individually significant. (Cate Jr. et Nelson 1971). The Pr(<|t|) is the p-value of the t-test and its significancy level is fixed at < 0.05 (or <0.1 for a moderate significancy). In simple regression model, there is a unique p-value related to the unique explanatory variable.

This method was repeated for the continuous approach. Two charts, this time point clouds, were generated with the $CaCl_2$ pH and base saturation data (in 0-5 cm) for all plots (y-axis) regarding the estimated leaf litter turnover rate (x-axis), which is a continuous variable. The *lme4* R package was again used to generate two simple linear regression models but, contrary to the previous analysis, with a continuous variable as the explanatory independent variable, the estimated leaf litter turnover rate. Those two models obtained by the linear regression were represented by Equation 12 where y represented, either the $CaCl_2$ pH or base saturation (in 0-5 cm), and where x represented the leaf litter turnover rate. Again, for these two models, the multiple and adjusted R-squared value and p-value were generated.

After obtaining these four simple regression models, multivariate regression models were performed to assess the assumption that the variation of deep soil conditions have no significant influence on the topsoil acidity conditions. Using the *lme* 4 R package, four multivariate regression models were generated similar to the following equation:

Equation 13:

$$y = \sum_{i=1}^{z} a_i x_i + b$$

Where y is the dependent response variable, z is the number of independent explanatory variables used in the model, x is the independent explanatory variable and b is the intercept. In the first model, with the discrete approach, y corresponded to the $CaCl_2$ pH in the 0-5 cm layer and the variables x were the following variables: species category, $CaCl_2$ pH at 100 cm deep, base saturation at 100 cm deep and silt content at 100 cm deep. In the second model, with the continuous approach, y corresponded to the $CaCl_2$ pH and the variables x were the following variables: leaf litter turnover rate, $CaCl_2$ pH in the 0-5 cm layer at 100 cm deep, base saturation at 100 cm deep and silt content at 100 cm deep. These two models are repeated with the response variable y corresponding to the base saturation in 0-5 cm layer. For all four multivariate models, the multiple and adjusted R-squared values were generated. The p-value of each model and of each term of Equation 13 were also estimated. For each model, the stepwise regression was applied, using *FactoMineR* R package, with backward, forward and both directions methods. The anova test was generated to determine which explanatory variables would the most significant in the model, associated with the lowest value of AIC.

A third analysis was added to determine a potential relationship between the calcium and the aluminum contents in the first mineral soil layer and species identity. A simple linear regression model with discrete approach was used for this step, similar to Equation 12. Two charts represented the influence of species category (x, explanatory independent variable) on either calcium content or aluminum content (y; response dependent variable). Multiple and adjusted R-squared and p-value were generated for those two models.

4.3 Regression analyses: impact of species composition on carbon stocks

In the last part of statistical analyses of data, the relationship between measured carbon stocks and species identity has been investigated, both with discrete and continuous approach.

In the discrete approach, the SOC stocks were first studied regarding the depth. For each soil layer (holorganic, 0-5 cm, 5-10 cm, 10-20 cm) a chart was plotted with boxplots presenting carbon stock values, the dependent response variable (y-axis) with the species category as the discrete independent explanatory value. The same method has been applied to total cumulative C stock (holorganic + 5-20 cm layers). The statistics associated with each of the four charts were generated (range, median, first and third quartiles, potential outlier values, mean value and standard deviation). Then, two other charts were plotted presenting the relationship between the carbon stock distribution in soil profile and species category. Boxplots were generated where the x-axis correspond to species category. The y-axis of the first chart corresponded to the ratio between carbon stock in the holorganic layer and the total carbon stock in the profile (holorganic + 0-20 cm) and the y-axis of the second chart corresponded to the ratio between carbon stock in the holorganic layer added to carbon stock in the layer 0-5 cm, and the total carbon stock in soil profile. Those two variables were found according to formula in Equation 14. The statistics associated with each of those two charts were also generated (range, median, first and third quartiles, potential outlier values, mean value and standard deviation). Finally, six simple linear regression models were performed, using *lme4* R package, corresponding to the six charts previously described. Multiple R-squared, adjusted R-squared and p-values data were also presented for each model in order to compare them.

Equation 14:

$$R_1 = \frac{C_{holo}}{C_{tot}} \quad ; \quad R_2 = \frac{C_{holo} + C_{0-5}}{C_{tot}}$$

C_{holo} = carbon stock, measured in holorganic layer [Mg/ha]

C_{0-5} = carbon stock, measured in layer 0-5 cm [Mg/ha]

C_{tot} = carbon stock, measured in whole soil profile (in holorganic layer + 0-20 cm) [Mg/ha]

In the continuous approach, the carbon stocks were first studied regarding the depth, but using the values of estimated leaf litter turnover rate as the variable related to species identity. As for the discrete approach, four charts with point clouds were plotted for the four soil layers where carbon stocks had been measured (organic material layer, 0-5 cm, 5-10 cm, 10-20 cm). The carbon stock was the response dependent variable (y-axis) and the leaf litter turnover rate was the continuous explanatory variable. After that, four simple linear regression model were generated, using *lme4* R package, that linked the carbon stock (for each of the four soil layers) to the leaf litter turnover rate. For each model, multiple R-squared, adjusted R-squared and p-values data were presented.

A second step in the continuous approach was to generate multivariate regression models to investigate the impact of several continuous variables on the two carbon distribution variables presented as R1 and R2 in Equation 14. The used independent explanatory variables are the following variables:

N°	Variables
1.	Estimated leaf litter turnover rate
2.	Calcium (Ca^{2+}) content in 0-5 cm on eCEC in 0-5 cm [cmolc/kg]/ [cmolc/kg]
3.	Aluminum (Al^{3+}) content in 0-5 cm on eCEC in 0-5 cm [cmolc/kg]/ [cmolc/kg]

4.	Calcium (Ca^{2+}) content in 0-5 cm on calcium content in 5-20 cm [cmolc/kg]/ [cmolc/kg]
5.	Aluminum (Al^{3+}) content in 0-5 cm on aluminum content in 5-20 cm [cmolc/kg]/ [cmolc/kg]

Multivariate stepwise regressions were used, with *FactoMineR* R package, and produced two models for the response variable R1 (one with backward method and one with forward or both direction method) and two models for the response variable R2 (one with backward method and one with forward or both direction method) The anova test was generated to determine which explanatory variables would the most significant in the model, associated with the lowest value of AIC. For each of the four obtained models, multiple and adjusted R-squared values and p-values of the model as well as p-values of each variable were generated to compare the significancy of the different models.

Additionally, a last approach consisted in figuring out whether a relationship existed between the carbon stock distribution variables R1 and R2 and exchangeable Al and Ca^{2+}, relatively to total eCEC (variables 2 and 3) and between R1 and R2 and the distribution through topsoil profile of exchangeable aluminum and calcium cations (variables 4 and 5) with simple linear regressions. Four models have been carried out with *lme4* R package with *a* or *b* as response variable and 2, 3, 4 or 5 as explanatory variables and for each model multiple and adjusted R-squared values and p-values were generated. Eight plots were also created to highlights the potential relationship between two variables, with R1 or R2 as values on y-axis and 2, 3, 4 and 5 as variables on x-axis.

Results

1. Overview of soil chemical properties variables.

1.1 Evolution of soil chemical properties variables with depth.

Figure 4 (a) reveals a marked decrease of the mean soil C content from the holorganic layer (mean = 44.1%) to the first mineral soil layer 0-5 cm (mean = 11.7%). On the contrary, variability of values among plots is higher in the 0-5 cm topsoil layer, with a standard deviation of 7.1% than in the holorganic layer, with 3.7%. Below 5 cm deep, C contents are on average lower with a narrow range of values. Mean C content equals 3±2% in the layer 5-10 cm and equals 1.3±0.5% in layer 10-20 cm. It is apparent in Figure 4 (a) that the layer 0-5 cm has the widest range of possible values which extends from 4% to 27% of C. The highest C contents are found in the forest floor, varying from 36.3% to 49.7%. In the 5-10 cm layer, *Pseudotsuga menziesii* (plot 4 and plot 9) and *Larix kaempferi* present C content outlier values of respectively 11%, 5% and 4.7%.

Similarly, the average nitrogen content is decreasing with depth from the forest floor to 20 cm deep, as shown in Figure 4 (b). In the holorganic layer, the mean nitrogen content equals 1.59 % with a standard deviation of 0.25 %. In comparison, the mean value in the first mineral layer 0-5 cm has a less important N content of 0.61 % but a similar a standard deviation value of 0.28 %. Depth intervals 5-10 cm and 10-20 cm present a clear decline of the mean N content with respective values of 0.2±0.1% and 0.1±0.03%). In the holorganic layer, *Juglans mandshurica* is the only species with a N content inferior to 11% (0.93%) and *Pseudotsuga menziesii* (plot 4) is present a distinguishable value N content in the layer 5-10 cm of 0.55 %, which is superior to values for other plots at that depth.

Figure 4 (c) represents C/N data with depth. The mean and standard deviations values C/N seem to show the same trend than Figure 4 (a) and Figure 4 (b). holorganic layer presents an average C/N level of 28.4±4.8, while C/N equals 18.13±3.88 in 0-5 cm and 15.17± 3.73 in 5-10 cm. Results show a slight rise of the average value in the last interval 10-20 cm (16.1±4.43. In the forest floor, particularly high values of C/N are observed in *Picea omorika* (39.13) and in *Juglans mandshurica* (41.42), while *Larix kaempferi* presents extreme C/N values in the layer 5-10 cm (28.36) and in 10-20 cm (29.27). An outlier value of C/N for this depth interval has also been observed on plot 25 with *Quercus rubra* (25.95).

The Figure 4 (d) shows that on average $CaCl_2$ pH increases with depth. Observed mean pH values are similar in 0-5 cm and 5-10 cm layers, around 3.6, but variability of values is more important in the 0-5 cm layer with a standard deviation of 0.6 compared to 0.28 for the 5-10 cm layer. Below 10 cm deep, the $CaCl_2$ pH increases to reach the mean value of 4±0.29 in the 10-20 cm layer and 4.2±0.29 at 100 cm deep. The highest values in the uppermost 0-5 cm topsoil layer are observed in *Tilia americana* (5.3), *Calocedrus decurrens* (4.7) and *Juglans mandshurica* (4.7) and the lowest value is observed in *Larix kaempferi* (2.8)

As illustrated in Figure 4 (e), the average eCEC values, as well as variability among plots, decreases with depth from 0 to 20 cm, as illustrated in with mean values of 18.3±7.6, 8.5±2 and 5.9±1.1 cmolc/kg for respectively the 0-5 cm, 5-10 cm and 10-20 cm layers, while eCEC reaches 9.4±1.5 cmolc/kg at a depth of 100 cm. The uppermost 0-5 cm topsoil layer presents the widest range of eCEC values (from 8.9 to 37.1 cmolc/kg, with the highest value reached in *Pseudotsuga menziesii* (plot 4). *Pseudotsuga menziesii* and *Calocedrus decurrens* are both associated with high outlier values of eCEC in 5-10 cm and 10-20 cm layers.

Figure 4 (f) provides an overview of the evolution of exchange acidity, which is the sum of total Al and H^+ content, with depth. The figure illustrates a slow decline of average exchange acidity from 0 cm to 100 cm deep. In the 0-5 cm topsoil layer, the mean EA value is 6.9±3.5 cmolc/kg with a relatively high variability of values, ranging from 0.8 to 12.8 cmolc/kg, compared to other topsoil layers. The mean values of EA in the 5-10 cm and 10-20 cm layers are respectively 6.5±1.8 and 4.9±1.1 cmolc/kg. At a depth of 100 cm, the average value of 3.4±2.1 cmolc/kg is reported.

Figure 4 (g) shows that the mean value for exchangeable calcium content presents a marked drop with depth, especially between the 0-5 cm and 5-10 cm topsoil layers, with respective values of 9.3±7.1 and 1.4±1 cmolc/kg. The highest variability among species is found in the 0-5 cm topsoil layers, where exchangeable calcium content varies 0.6 to 16.9 cmolc/kg and reaches values of 22.8 cmolc/kg in *Tilia americana*, 23.4 cmolc/kg in *Acer saccharinum* and 24.1 cmolc/kg in *Pseudotsuga Menziesii*. As illustrated in Figure 4 (g), exchangeable calcium content ranges from 0.05 to 3 cmolc/kg in the 5-10 cm, except for *Calocedrus decurrens*, which has a high value of 7.3 cmolc/kg. By contrast, the average Ca^{2+} content and variability of values in deeper soil layer are lower. The mean Ca^{2+} content takes a value of 0.6 ± 0.8 cmolc/kg in the 10-20 cm layer but a rise is observed at the 100 cm depth with the value 4.2±2.3 cmolc/kg.

As presented in Figure 4 (h), There is a steep decline of mean base saturation from 57.2±25.4 % to 22±17.1 % between the 0-5 cm and 5-10 cm topsoil layers. The 10-20 cm topsoil layer, is similar to the upper soil layer in terms of base saturation mean, with a value of 15.2±14.2. Base saturation in 0-5 cm ranges from 14.39 to 96.48 %, which represents a relatively large intervals of values compared to the range in 5-10 cm and 10-20 cm. *Calocedrus decurrens* and Juglans mandshurica present outlier values in the 10-20 cm layers of 47.4 and 58.7%. At 100 cm deep, base saturation results are closed to those in the 0-5 cm layer with a mean value of 62.3±23.7% and a high variability of values is observed in this layer (from 15.7 to 93.52 %)

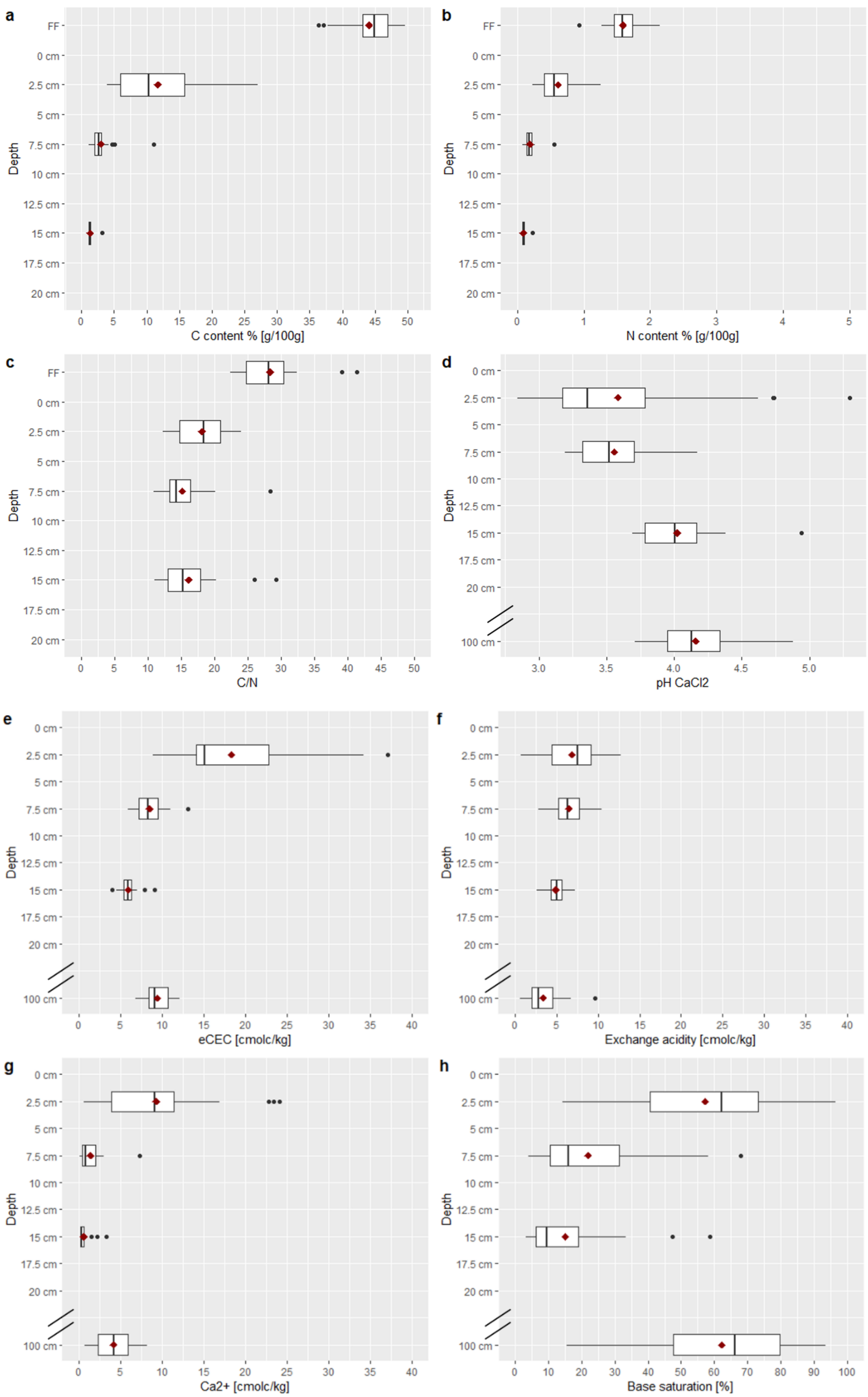

Figure 4: Evolution of values (range, median, mean (red point), first and third quartiles, outliers) of C content (%; a), N content (%; b), C/N (c), $CaCl_2$ pH (d), eCEC (cmolc/kg; e), EA or Al^{3+}+H^{+} (cmolc/kg; f), Ca^{2+}(cmolc/kg; g) and base saturation (cmolc/kg; f) with depth (hologanic layer (FF), 0-5 cm, 5-10 cm and 10-20 cm). (N=24)

The texture data are presented Figure 5 for the 10-20 cm topsoil layer and for 100 cm depth. At both depths, there are no substantial differences between plots. Soils of all studied plots are categorized as loamy soil (category A). There is however an except for plot 27 (*Fagus sylvatica*) which presents a higher sand content and belongs to the soil category L (sandy loam).

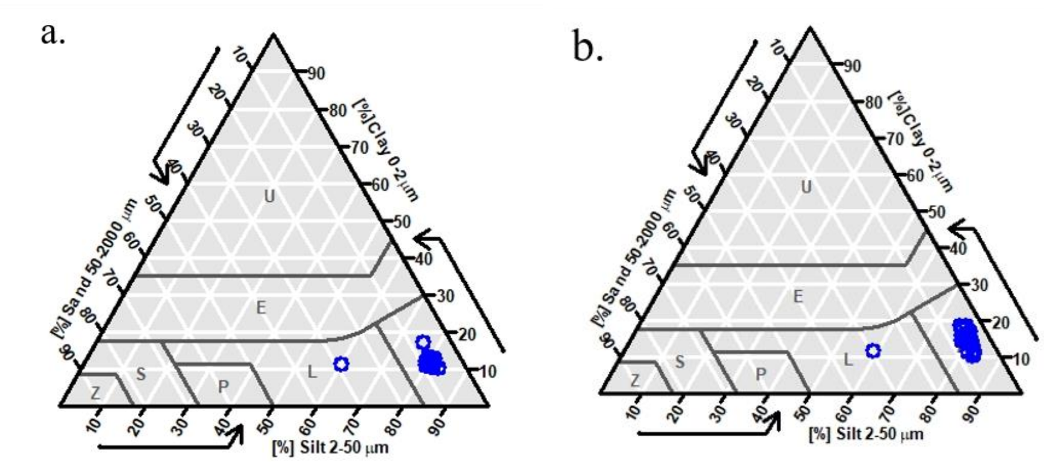

Figure 5: Texture triangles. Measured percentages of clay, sand and silt in each plot in depth interval 10-20 cm (a.) and at a depth of 100 cm (b.). Texture classes in Belgium system: U, heavy clay; E, clay; A, loam; L, sandy loam; P, light sandy loam; S, loamy sand; Z, sand.

1.2 Correlations between soil variables by depth.

The figures of PCA analysis and bivariate correlations by depth are in the annexes (annexes 4).

a. Depth interval 0-5 cm (Annexes 4, figures b and c)

First of all, there is a strong correlation between the two pH values (0.931) that allows us to focus our attention on only the values of pH in a $CaCl_2$ 0.01 M solution. Concerning the relationship between the acidity factors, $CaCl_2$ pH is strongly negatively correlated with the exchange acidity (-0.916) and the aluminum content (-0.771), while it is positively correlated with the base saturation (0.765). The base saturation is predictably positively linked to calcium content (0.805) and is opposed to the exchange acidity and aluminum content (-0.738 and -0.793). The eCEC is strongly correlated with the calcium content (0.898) and significantly correlated with the base saturation (0.527), but there is no evidence of a correlation between the eCEC and EA and Al^{3+} content, but a relevant correlation between Al^{3+} and Ca^{2+} can be pointed out (-0.536). Concerning the carbon content, there is negative correlation with the pH (-0.524) and it increases with the exchange acidity (0.597), and there is a striking correlation with the eCEC (0.689). The N content is strongly linked to the C content (0.962) while the C/N ratio is significantly correlated with acidity variables related to pH (-0.769 and -0.817), EA (0.82) and Al (0.633) and negatively correlated to litter turnover rate (-0.649).

These relationships between variables are summarized on the PCA analysis graph (annexes 4, figure c), which shows a clear opposite trend between the leaf litter turnover and acidity while the carbon content increase with either the acidity and the eCEC, but the graph reveals no relation between the eCEC and the acidity conditions. The N content follows the C content directions. C/N ratio increases with acidity.

b. Depth interval 5-10 cm (Annexes 4, figures d and e)

For that depth interval, the $CaCl_2$ pH and pH H_2O are also correlated. Among the acidity variables, the pH shows a strong correlation with the base saturation (0.720) and is negatively correlated with exchange acidity (-0.789) and aluminum content (-0.653), which are two interdependent variables (0.745). These two variables (EA and Al^{3+}) are also significantly negatively correlated with the base saturation (-0.636 and -0.599). The aluminum content presents a slightly negative correlation with calcium content (-0.454) while a highly significant correlation exists between base saturation and carbon content (0.9). There is a remarkable correlation between the eCEC and the carbon content (0.701) and the exchange capacity (0.584) while the eCEC seems to be mostly linked to calcium content (0.565) and to the exchange acidity (0.510). In this layer, the N content is still correlated with C content (0.935). C/N presents a correlation with acidity data (pH, Al, EA) and is negatively related to leaf litter turnover rate, but those correlations are less significant than for 0-5 cm. To summarize, as shown on PCA 5-10 cm, the leaf litter turnover rate tends to decrease with the acidity, and the carbon content follow the same trends as the acidity variables (EA and Al^{3+}).

c. Depth interval 10-20 cm (annexes 4, figures f and g)

In that depth intervals, the only significant correlations are between the two pH values (0.868) and between the base saturation and the exchange acidity (-0.59). The exchange acidity is correlated with the calcium and aluminum contents (-0.412 and 0.744 respectively) as well as the base saturation is correlated with these two cations (0.93 and -0.51). The eCEC is correlated with the exchange acidity (0.586) and the calcium and aluminum contents (0.47 and 0.507). The carbon content variable presents no significant relationship with other soil variables. In that layer, N and C/N ration are less important variables, as visible on the PCA analysis (annexes 4, figure g).

d. Depth of one meter (Annexes 4, figures h and i)

At that depth, there is an interdependency between acidity factors. That is illustrated by the EA and its close link with the $CaCl_2$ pH (-0.869) and the base saturation (-0.951). EA has also a strong correlation with calcium content (-0.836) and the aluminum content (0.945). Al^{3+} and Ca^{3+} are also quite related to each other (-0.852). The pH shows a strong correlation with cation contents (-0.881 for Al^{3+} and 0.899 for Ca^{2+}) and with the base saturation (0.902). The base saturation is highly dependent of calcium and aluminum content (0.933 and -0.946) and has a significant positive correlation with the eCEC (0.447). The eCEC is correlated with calcium content (0.69) but not with aluminum content. The carbon content is only slightly correlated with the pH (-0.322) and the Al^{3+} content (0.447).

2. Overview of the of carbon stocks and their evolution with depth.

Figure 6 presents the evolution of SOC stocks with depth. The C stock in the organic material layer varies from 1.4 to 44.3 Mg/ha, with a mean value of 9.2±10.6 Mg/ha. The holorganic layer and the depth interval 0-5 cm have a similar values' variability given that the layer 0-5 cm ranges from 14.3 to 51 Mg/ha. Despite this similarity, the C stock is on average higher in the 0-5 cm layer, with a mean value of 26.7± 9.1 Mg/ha, compared to the organic material layer. The highest C stock value is reached in Pseudotsuga *menziesii* in the holorganic layer while the highest value in the 0-5 cm layer is reached in *Abies nordmanina*. Below 5 cm deep, the carbon stock tends to decrease with the depth, as shown in Figure 6. The mean carbon stock value is 14.2± 5.2Mg/ha in the layer 5-10 and 16.1±4.9 Mg/ha in the layer 10-20 cm.

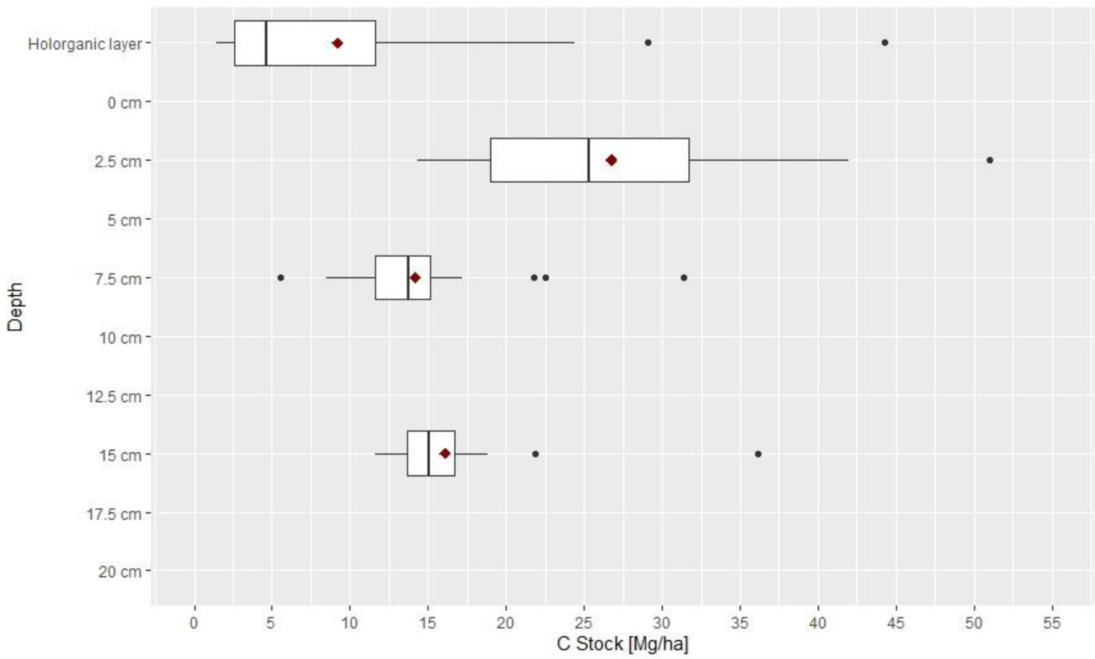

Figure 6: Evolution of carbon stocks (Mg/ha; range, median, mean (red point), first and third quartiles and outliers) with depth (holorganic layer, 0-5 cm, 5-10 cm and 10-20 cm). (N=24)

It is important to point out that this latter value has been calculated for a volume twice as large as the volume of the samples of the 0-5 cm and 5-10 cm layers. The figure also reveals a decline in the variability with the depth. In the 5-10 cm layer, C stock values range from 5 to 17 Mg/ha, with outlier values for following species: *Pseudotsuga Menziesii* (plot 9) (21.7 Mg/ha), *Larix kaempferi* (22.5 Mg/ha) and plot 4 with *Pseudotsuga menziesii* (plot 4) (31.4 Mg/ha). The lowest value of C stock is observed in *Tsuga canadensis* (5.5 Mg/ha). C stocks in the 10-20 cm layer ranges from 11.6 to 8.9 Mg/ha, except for *Quercus rubra* (21.9 Mg/ha) and *Larix Kaempferi* (36.1 Mg/ha).

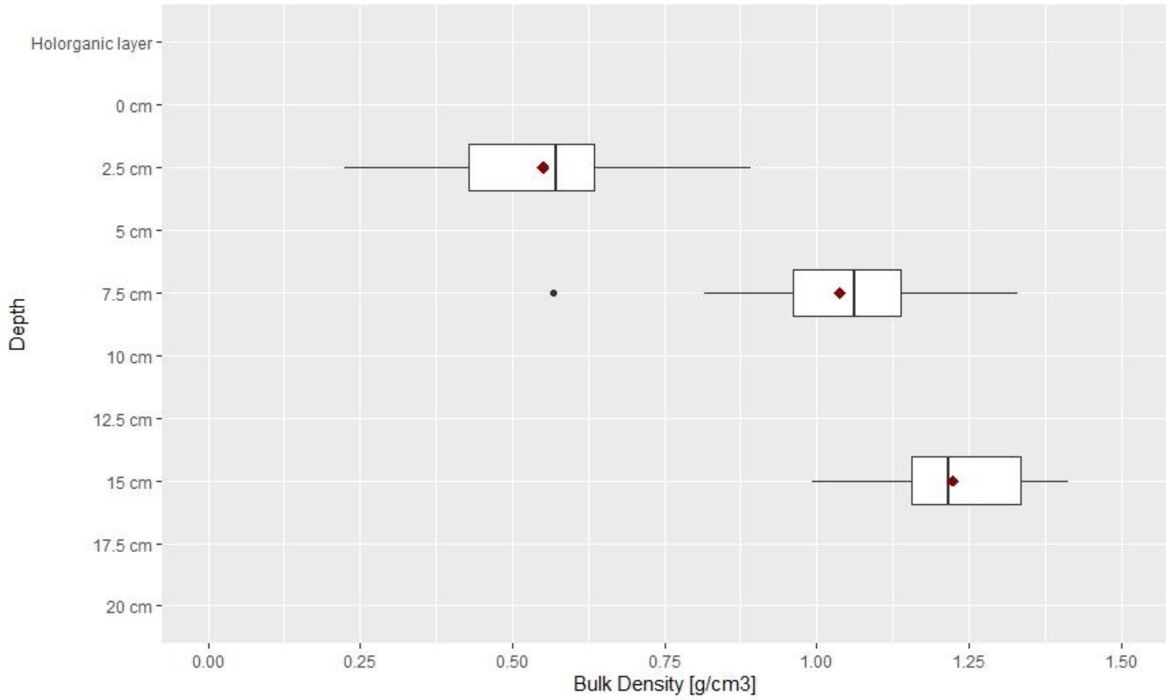

Figure 7: Evolution of bulk density of dry soil (g/ cm^3; range, median, mean (red point), first and third quartiles and outliers) with depth (0-5 cm, 5-10 cm, 10-20 cm). (N=24)

As shown in Figure 7, there is a significant increase of the bulk density with the depth, especially between the 0-5 cm layer and the 5-10 cm layer. The mean bulk density moves from 0.5±0.1893769 g/cm3 in the 0-5 cm layer, to 1± 0.1708773 g/cm3 in the 5-10 layer, and finally equals 1.2± 0.1154187 g/cm3 in the deeper topsoil layer (10-20 cm). The variabilities between bulk density values are similar for the three depth intervals, despite a minor decrease in the standard deviation. The values in the 0-5 cm layer varies from 0.2 to 0.9 Mg/ha, when the bulk density ranges from 0.6 to 1.3 Mg/ha in the 5-10 cm layer and from 1 to 1.4 Mg/ha in the 10-20 cm layer. Figure 7 reveals a particularly low bulk density of 0.6 in 5-10 cm in plot 4 with *Pseudotsuga menziesii*.

Figure 8 gives an overview of the average difference in terms of the total carbon stock, estimated from the organic material layer to one meter deep, between the broadleaved group and the coniferous group. In general, the figure reveals that the total carbon stocks that have been estimated in this study vary between 40 and 93 Mg/ha for whole plots sample, with a global mean value of 59.7 Mg/ha. On average, it seems in Figure 8 that plots belonging to the broadleaves category have a lower total carbon stock than plots belonging to the conifers category but this tendency will be assessed later.

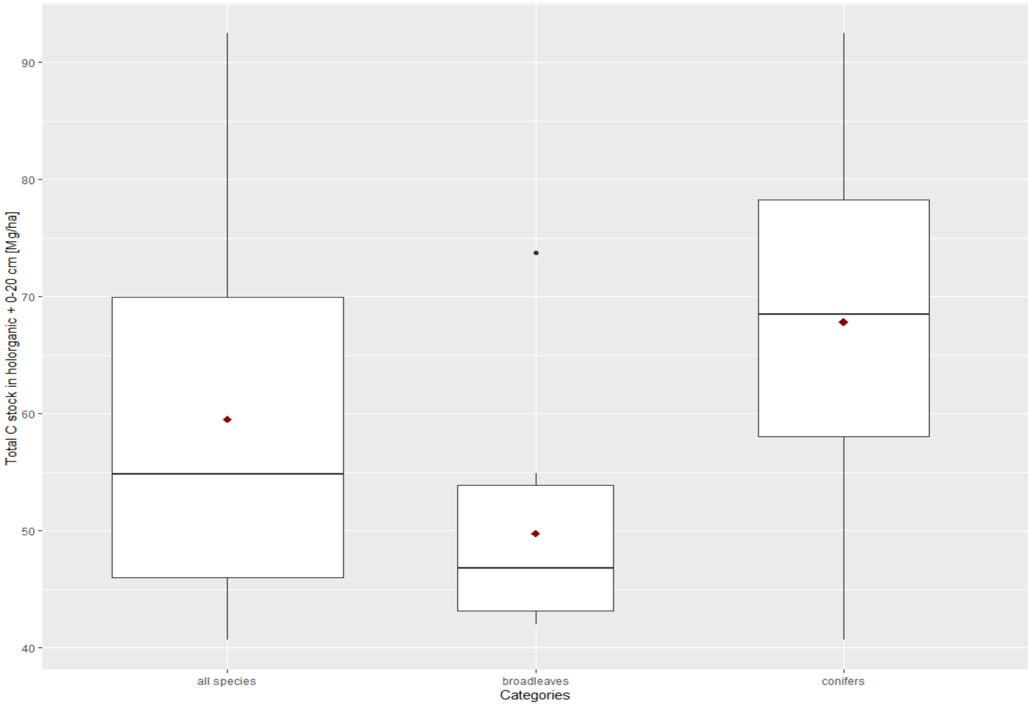

Figure 8: Cumulated (holorganic layer + 0-20 cm) carbon stocks (Mg/ha; range, median, mean (red point), first and third quartiles and outliers) for all plots (N=24) as well as per category (plots with broadleaved species (N=11) and plots with coniferous species (N=13)).

3. Impact of tree species composition on topsoil chemical properties.

3.1 Discrete approach

3.1.1 Simple regression model of the impact of species category on pH in topsoil.

Figure 9 is a view of the effect of the species category, broadleaves or conifers, on the $CaCl_2$ pH in the depth interval 0-5 cm. According to Figure 9 (b), the pH in the 0-5 cm depth interval is on average less acid in plots with broadleaved species, with a mean value of 3.9, than on plots with coniferous species, with a mean value of 3.3. It appears that both conifers and broadleaves are associated with range of pH values relatively close to 2 units. For coniferous species, pH varies from 2.8 to 4.7, while pH for broadleaved species ranges from 3.2 to 5.3. Moreover, the standard deviation of pH values for broadleaves (0.7) is comparable to the standard deviation for conifers (0.5). However, the variability of pH values seems more important for broadleaved plots than for coniferous plots according to Figure 9 (b). Indeed, the distance between the first and the third quantile on the boxplot related to coniferous category equals less than 0.5 pH units but this distance for the broadleaved category is more of the order of one pH unit. The similarity between the range size of the two species category could be thus explained with Figure 9 (a) by the position of *Calocedrus decurrens* on the pH scale, which presents a pH of 4.7 while the other coniferous species do not have pH values exceeding 3.5. *Thuja plicata* is an exception with an outlier pH value of 3.7. Figure 9 (b) reveals that, except for *Thuja plicata* and *Calocedrus decurrens* pH values remain under 3.5 for the coniferous category. Moreover, according to Table *2*, a linear regression between the $CaCl_2$ pH and species category shows significant statistical tests because the p-value is less than 0.05 (0.01411). However, the model leads to low values of multiple R-squared (0.2442) and adjusted R-squared (0.2099).

In conclusion, the first topsoil layer appeared thus to be on average less acid, in terms of pH, under broadleaved species than under coniferous species, except for *Thuja plicata* and *Calocedrus decurrens* which are species presenting relatively high pH values.

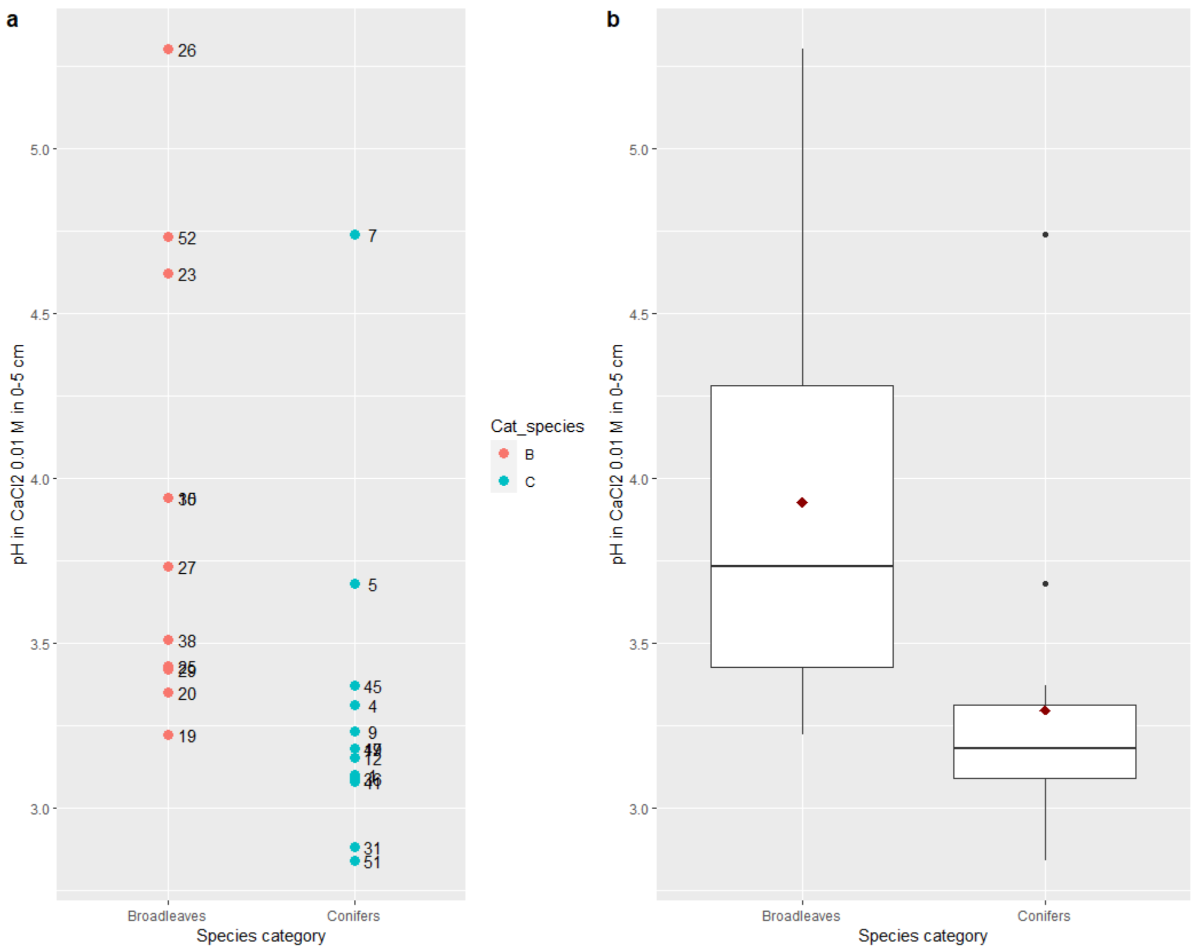

Figure 9: (a) $CaCl_2$ pH in the 0-5 cm topsoil layer as a function of species category (D: broadleaves, R: conifers) with the plot number indicated on the dot (refer to Table 1 for species identification). (b) $CaCl_2$ pH (range, median, mean (red point), first and third quartiles and outliers) in the 0-5 cm topsoil layer per species category (broadleaves (N=11), conifers (N=13)).

Table 2: Parameters coefficients (estimated value, standard error) and statistical test (Pr(>|t|); ANOVA) of a linear regression model predicting $CaCl_2$ pH values in the 0-5 cm topsoil layer as a function of the impact of tree species category (broadleaves; conifers). N=24

Parameters	Estimated coefficient	Standard error of coefficient	Pr(>|t|)

Intercept	3.9264	00.1744	< 2e-16
x1 = Species category (R)	-0.6317	0.2369	0.0141

3.1.2 Multi-variate regression model of the impact of tree species category and deep conditions variables on pH in topsoil.

Table 3 summarizes results of the multivariate regression model where the response variable is the $CaCl_2$ pH in the 0-5 cm topsoil layer and the independent variables are the $CaCl_2$ pH values, the base saturation values and the silt content values at the depth of 100 cm and species category. The purpose of the model is to determine which factor between the tree species effect or the initial deep conditions effect is the most relevant to explain the acidity conditions in the 0-5 cm topsoil layer. As indicated on the table, the statistical tests (Pr(>|t|)) for $CaCl_2$ pH, base saturation and silt content at the depth of 100 cm are less than the significancy level of 0.05. Consequently, those variables are not considered to be relevant explicative factors of the pH values observed in 0-5 cm. The exclusion of those variables is also justified by a stepwise regression analysis because the minimum AIC value is reached when the only explanatory variable in the model is the species category, both for the forward and backward methods. The category species significancy test Pr(>|t|)) is twice as low than in Table *2* and p-value of the model is not significative (0.1432 > 0.1)

Table 3: Parameters coefficients (estimated value, standard error), statistical tests (Pr(>|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of a multivariate regression model predicting values of $CaCl_2$ pH in the 0-5 cm topsoil layer as a function of species category (broadleaves, conifers) and variables ($CaCl_2$ pH; base saturation, %; silt content, %) in the 100 cm deep soil layer. N=24, degrees of freedom = 22.

Parameters	**Estimated coefficient**	**Coefficient standard error**	**Pr(>|t|)**
Intercept	2.413407	4.065174	0.5597
Category species (Conifers)	-0.611385	0.250225	0.0245
$CaCl_2$ pH 100 cm	0.098835	1.020998	0.9239
BS 100 cm	0.004531	0.012394	0.7187
Silt content % 100cm	0.010247	0.026657	0.7049
Statistics	**Multiple R^2**	**Adjusted R^2**	**p-value**
	0.2912	0.1419	0.1432

3.1.3 Simple regression model of the impact of tree species category on base saturation in topsoil.

This statistical analysis is used to evaluate the role of the species on the base saturation in the first soil layer 0-5 cm. Figure 10 reveals that the base saturation in the depth interval 0-5 cm is on average slightly higher with broadleaved species than with coniferous species because the median value and the mean value (65.9 %) for broadleaves are upper on the base saturation scale than the median value and the mean value (49.9 %) for conifers. However, both species categories have similar ranges of values and standard deviations. The base saturation varies from 22.3% to 96.5 % in broadleaved plots while it varies from 14.4 % to 94.5% in broadleaved plots. Besides, the standard deviation for broadleaved category is 24.2 % and it is of 25.1 % for coniferous category. This high variability among plots of the same species category can be observed for both broadleaves and conifers in Figure 10 (a) and (b). As predicted with the stepwise regression, a linear regression between the base saturation in the 0-5 cm top soil layer and species category variable leads to a model with low values of multiple R^2 value (0.1011) and adjusted R^2 (0.06028) and non-significant p-value (0.1299). The estimated parameters and statistical results of this model are shown in Table 4.

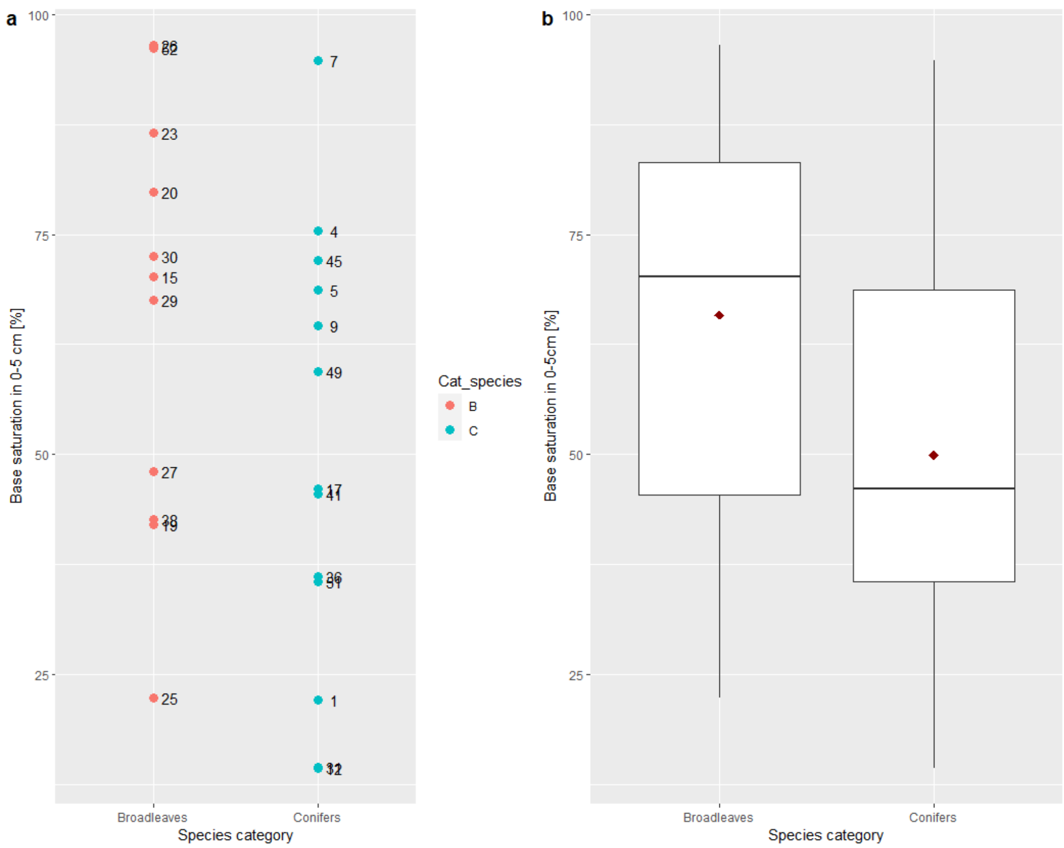

Figure 10: (a) Base saturation (%) in the 0-5 cm topsoil layer as a function of species category (D: broadleaves, R: conifers) with the plot number indicated on the dot (refer to Table 1 for species identification). (b) Base saturation (%; range, median, mean (red point), first and third quartiles and outliers) in the 0-5 cm topsoil layer per species category (broadleaves (N=11), conifers (N=13)).

Table 4: Parameters coefficients (estimated value, standard error) and statistical test (Pr(>|t|); ANOVA) of a linear regression model predicting base saturation values (%) in the 0-5 cm topsoil layer as a function of the impact of species category (broadleaves; conifers). N=24

Parameters	**Estimated coefficient**	**Coefficient standard error**	**Pr(>|t|)**
Intercept	65.847	7.436	1.05e-08
x1 = Species category (C)	-15.896	10.104	0.13

3.1.4 Multivariate regression model of the impact of tree species category and deep conditions variables on base saturation in topsoil.

Table 5 offers a view on the results of multivariate regression in which the response variable is the base saturation in the layer 0-5 cm and the independent variables are the species category and the $CaCl_2$ pH, the base saturation and the silt content at a depth of 100 cm. This regression model reveals the absence of significant correlation between base saturation in 0-5 cm and the acidity factors in the 100 cm deep soil layer. Indeed, the combine effect of these explanatory variables as well as their individual are not relevant to explain the predicted variables because the statistical tests (Pr(>|t|) and p-value) are < 0.05. The category is also not relevant as an explanatory variable (Pr(>|t|) < 0.05). According to the stepwise analysis, with both forward and backward methods, the species category is the only variable in the model that guarantees a minimum AIC value, but the multiple and adjusted R-squared presented in Table 5 confirm that there is not linked statistically important link between the species and the base saturation in 0-5 cm.

Table 5: Parameters coefficients (estimated value, standard error), statistical tests (Pr(>|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of a multivariate regression model predicting values of base saturation (%) in the 0-5 cm topsoil layer as a function of species category (broadleaves, conifers) and variables ($CaCl_2$ pH; base saturation, %; silt content, %) in the 100 cm deep soil layer. N=24, degrees of freedom = 22.

Parameters	**Estimated coefficient**	**Coefficient standard error**	**Pr(>|t|)**
Intercept	142.9877	165.3631	0.398
Category species (C)	-17.5105	10.1786	0.102
$CaCl_2$ pH 100 cm	-53.1165	41.5321	0.216
BS 100 cm	0.7296	0.5042	0.164

Silt content % 100cm	1.2573	1.0843	0.261
Statistics	**Multiple R^2**	**Adjusted R^2**	**p-value**
	0.2328	0.07131	0.259

3.1.5 Impact of tree species category on cation content in topsoil.

Figure 11 shows that the mean aluminum content is on average higher for coniferous species (5.6 cmolc/kg) than for broadleaved species (3.3 cmolc/kg). Additionally, Figure 11 highlights that the coniferous category has a larger range of aluminum content with values from 0.4 to 11.8 cmolc/kg than broadleaved category with values from 0.2 to 8.5 cmolc/kg. The outlier value observable in the figure in the broadleaves category corresponds to *Quercus rubra* (plot 25) and equals 8.5 cmolc.kg. Besides, there is also a difference between the standard deviation of the coniferous category (3.2 cmolc/kg) and the standard deviation of the broadleaved category (2.5 cmolc/kg).

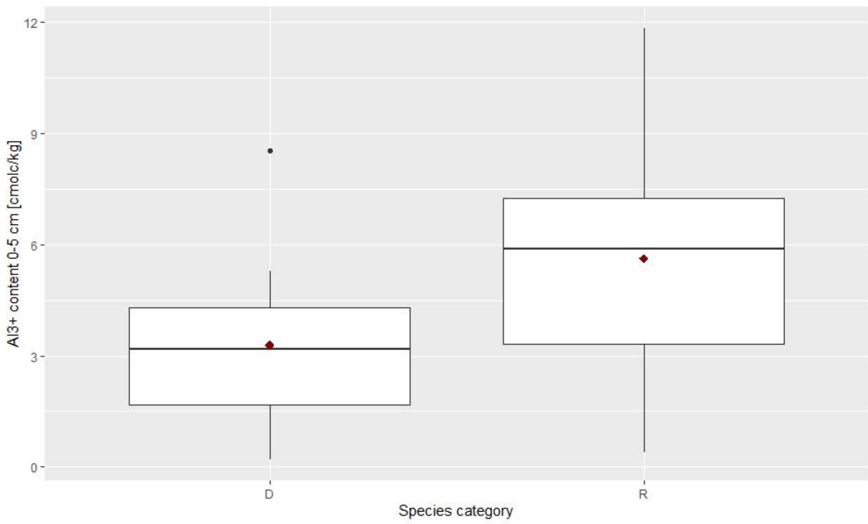

Figure 11: Aluminum content (cmolc/kg; range, median, mean (red point), first and third quartiles and outliers) in 0-5 cm topsoil layer and per category (D: broadleaves (N=11), R: conifers (N=13).

Table 6 presents the results of linear regression model between the species category and aluminum content in 0-5 cm. The model reveals a moderately significant p-value (included in the range 0.1-0.05) with values of multiple and adjusted R-squared equaling 0.152 and 0.1135. Consequently, there is a moderate positive correlation between Al^{3+} in 0-5 cm and the conifers category.

Table 6: Parameters coefficients (estimated value, standard error) and statistical test (Pr(>|t|); ANOVA) of a linear regression model predicting aluminum content (cmolc/kg) in the 0-5 cm topsoil layer as a function of the impact of tree species category (broadleaves; conifers). N=24

Parameters	Estimated coefficient	Coefficient tandard error	Pr(>|t|)
Intercept	3.2911	0.8654	0.000974
x1 = Species category (C)	2.3354	1.1758	0.059613

Unlike Figure 11, Figure 12 reveals no significant differences in terms of calcium content in the depth interval 0-5 cm between the coniferous and broadleaved plots. On the contrary, the two categories present relatively similar median and mean values. On average, the calcium content is of 8.4 cmolc/kg for conifers and of 10.3 cmolc/kg for broadleaves. Similarly, conifers and broadleaves have close standard deviation values of respectively 6.8 and 7.7 cmolc/kg. Moreover, there is no correlation between the species category and the range of calcium values because calcium content of conifers varies from 0.6 to 24.1 cmolc/kg, with the highest value observed for *Pseudotsuga menziesii* in plot 4 and calcium content of broadleaves ranges from 1.3 to 23.4 cmolc/kg.

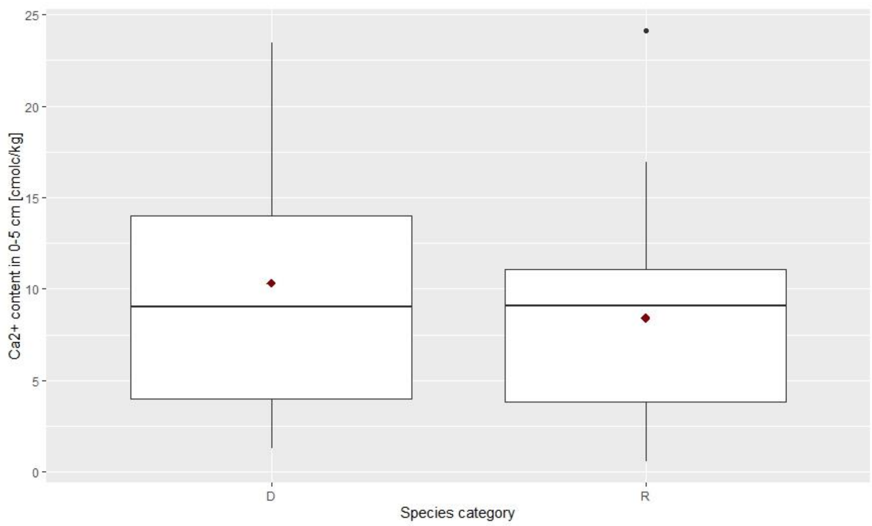

Figure 12: Calcium content (cmolc/kg; range, median, mean (red point), first and third quartiles and outliers) in 0-5 cm topsoil layer per species (D: broadleaves (N=11), R: conifers (N=13)). category.

Table 7 shows that the species category is a non-relevant explanatory variable of the calcium content measured in 0-5 cm because of a p-value of higher than 0.05. The linear model of Ca^{2+} function of category species presents also non-significant values of multiple and adjusted R-squared (0.01854 and -0.02608).

Table 7: Parameters coefficients (estimated value, standard error) and statistical test (Pr(>|t|); ANOVA) of a linear regression model predicting calcium content values (cmolc/kg) in the 0-5 cm topsoil layer as a function of the impact of species category (broadleaves; conifers). N=24

Parameters	**Estimated coefficient**	**Coefficient standard error**	**Pr(>|t|)**
Intercept	10.326	2.169	9.45e-05
x1 = Species category (C)	-1.900	-0.645	0.526

3.2 Continuous approach

3.2.1 Simple regression model of the impact of estimated leaf litter turnover rate on pH in topsoil.

Figure 13 shows the relationship between the estimated leaf litter turnover rate and the $CaCl_2$ pH in 0-5 cm. The data seem to fit the model as multiple R-squared and adjusted R-squared equals 0.4680 and 0.4441 and the p-value is < 0.05, as shown by Table 8. Therefore, the leaf litter turnover rate is significantly correlated with the pH in 0-5 cm.

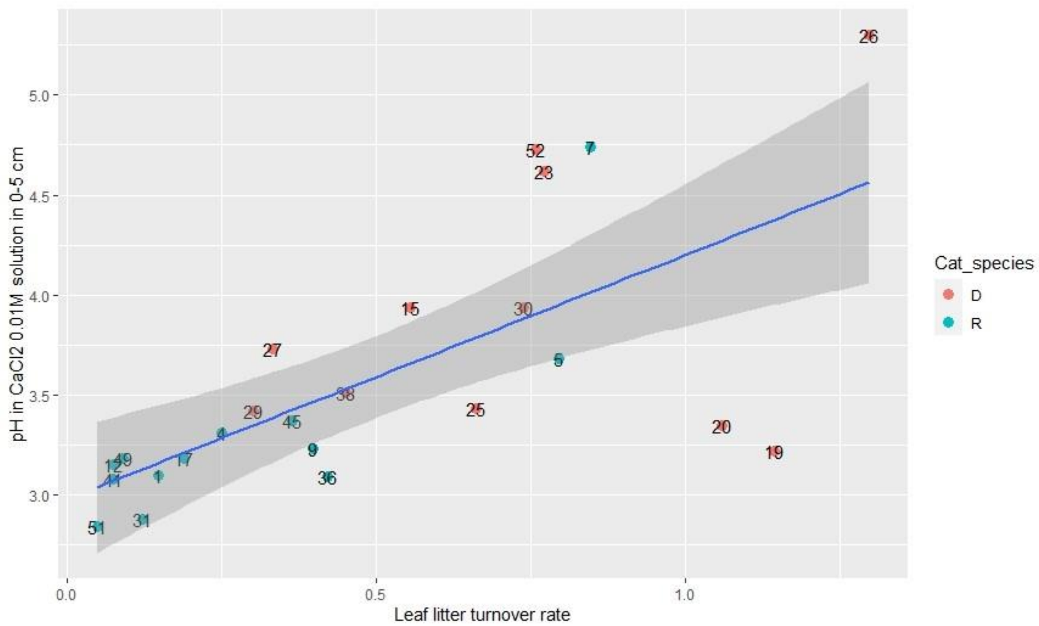

Figure 13: $CaCl_2$ pH in 0-5 cm topsoil layer as a function estimated leaf litter turnover rate and species category (D: broadleaves, R: conifers)

Table 8: Parameters coefficients (estimated value, standard error) and statistical test (Pr(>|t|); ANOVA) of a linear regression model predicting $CaCl_2$ pH values in the 0-5 cm topsoil layer as a function of estimated leaf litter turnover rate.

Parameters	Estimated coefficient	Coefficient standard error	Pr(>|t|)
Intercept	2.9789	0.1694	1.94e-14
x1 = Leaf litter turnover rate	1.2212	0.2774	0.000226

3.2.2 Multi-variables regression model of the impact of leaf litter turnover rate and deep conditions variables on pH in topsoil.

Table 9 presents the results of the multi-variables analysis of the effect of the estimated leaf litter turnover rate and the deep soil conditions on the pH in 0-5 cm. In this model, the only explanatory variable with a p-value < 0.05 is the leaf litter turnover rate. That means that the acidity conditions at one meter deep have no effect on the surface pH and the litter turnover rate, representing species identity, has a strong correlation with the pH. The model presents, as shown in

Table 9, significant values of multiple and adjusted R-squared (0.4791 and 0.4791). Moreover, a stepwise analysis has been realized with all the variables in

Table *9* and the only explanatory variables that guarantees a minimum AIC value is the leaf litter turnover rate, with both backward and forward methods.

Table 9: Parameters coefficients (estimated value, standard error), statistical tests (Pr(>|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of a multivariate regression model predicting values of $CaCl_2$ pH in the 0-5 cm topsoil layer as a function of estimated leaf litter turnover rate and variables ($CaCl_2$ pH; base saturation, %; silt content, %) in the 100 cm deep soil layer.

Parameters	**Estimated coefficient**	**Coefficient standard error**	**Pr(>|t|)**
Intercept	2.570585	3.471460	0.46804
Leaf litter turnover rate	1.181539	0.305258	0.00103
$CaCl_2$ pH 100 cm	0.095635	0.871142	0.91373
BS 100 cm	0.001821	0.010572	0.86507
Silt content % 100cm	-0.001054	0.022973	0.96390

Statistics	Multiple R^2	Adjusted R^2	p-value
	0.4791	0.4791	0.0113

3.2.3 Simple regression model of the impact of estimated leaf litter turnover rate on base saturation in topsoil.

Figure 14 illustrates the distribution of the base saturation values in depth interval 0-5 cm for all plots regarding their values of estimated leaf litter turnover rate. As presented in Table 10, the p-value of the linear regression model between the base saturation and leaf litter turnover rate is particularly low 0.00241 and < 0.05. Besides, the multiple and adjusted R-squared factors of this model are relatively significant and equal respectively 0.348 and 0.3184. These results indicate that the leaf litter turnover rate is a significantly correlated to the base saturation in the first mineral soil layer. However, this relationship is weaker than the correlation between the pH and the leaf litter turnover rate presented in Table 8.

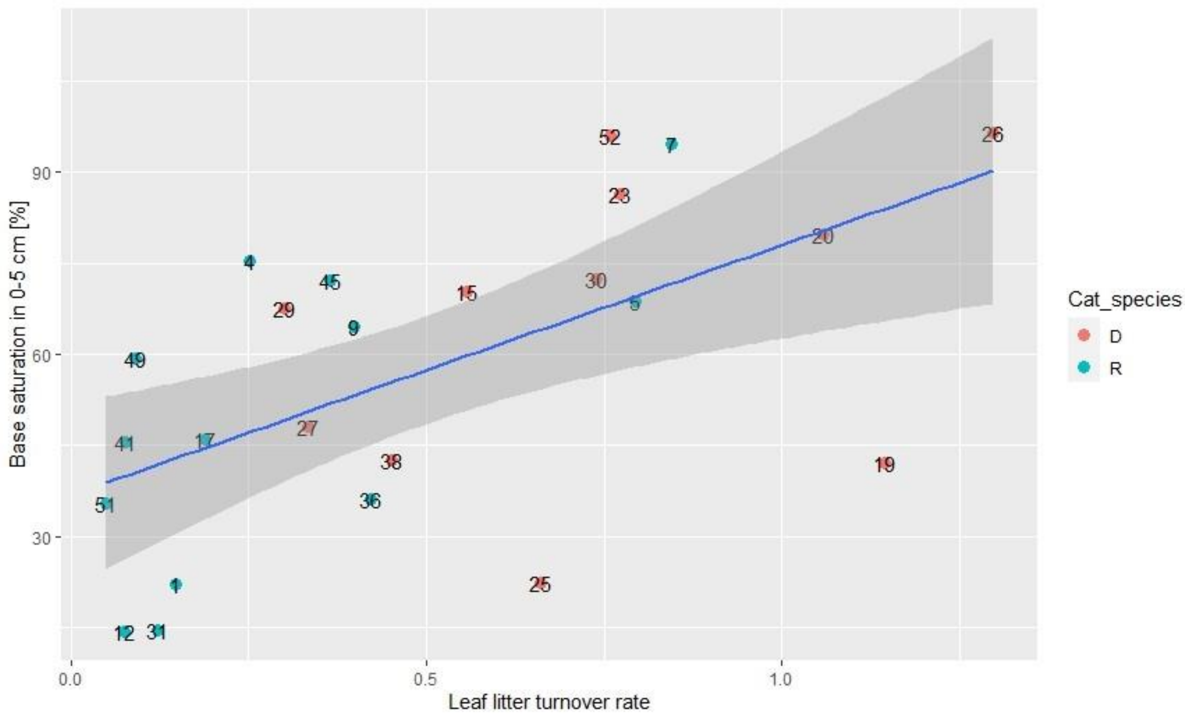

Figure 14: Base saturation (%) in 0-5 cm topsoil layer as a function estimated leaf litter turnover rate and species category (D: broadleaves, R: conifers) and linear regression line (blue).

Table 10: Parameters coefficients (estimated value, standard error) and statistical test (Pr(>|t|); ANOVA) of a linear regression model predicting base saturation values (%) in the 0-5 cm topsoil layer as a function of estimated leaf litter turnover rate.

Parameters	Estimated coefficients	Coefficient standard error	Pr(>|t|)

Intercept	36.835	7.336	5.01e-05
x1 = Leaf litter turnover rate	41.164	12.12	0.00241

3.2.4 Multivariate regression model of the impact of leaf litter turnover rate and deep conditions variables on base saturation in topsoil.

As shown in Table 11, the multi-variables model that explains the base saturation values by the leaf litter turnover rate and the deep conditions is not significant because the multiple and adjusted R-squared have low values (0.2778 and 0.1258) and the p-value of the model is > 0.005. The table also highlights that the base saturation at the surface is not influenced by the variables linked to deep acidity conditions (pH, base saturation at one meter deep) and the texture at one meter deep (silt content). Indeed, the p-values of these variables are > 0.05. The leaf litter turnover rate has the lower p-value. Consequently, this variable is the more significant in the model to explain the base saturation in 0-5 cm, even if its p-value equals 0.0513, which is > 0.05. Besides, a stepwise regression model has confirmed that the leaf litter turnover rate is the only relevant variable in the model, with a minimum AIC value reached when the other independent variables are removed. It can thus be concluded that the chemical properties in the deep soils have no relationship with the base saturation in 0-5 cm and must be ignored. Consequently, their variability among plots must be ignored in the analyses.

Table 11: Parameters coefficients (estimated value, standard error), statistical tests (Pr(>|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of a multivariate regression model predicting values of base saturation (%) in the 0-5 cm topsoil layer as a function of estimated leaf litter turnover rate and variables ($CaCl_2$ pH; base saturation, %; silt content, %) in the 100 cm deep soil layer.

Coefficients	Estimated	Standard error	Pr(>|t|)
Intercept and independent variables of the model			p-value
Intercept	111.3431	158.7044	0.4914
Leaf litter turnover rate	17.0112	8.1771	0.0513
$CaCl_2$ pH 100 cm	-42.1871	39.8778	0.3034
BS 100 cm	0.5287	0.4899	0.2939

Silt content % 100 cm	0.9704	1.0577	0.3704
	Multiple R^2	**Adjusted R^2**	**p-value**
	0.2778	0.1258	0.1653

4. Impact of tree species identity (discrete and continuous approaches) on carbon stocks.

4.1 Discrete approach

4.1.1 Relationship between carbon stock and species category in each soil layers.

Figure 15 (a) highlights the striking difference of carbon stock in holorganic layer that exists on average between the two species categories. It is confirmed by the significant difference between the two mean values as presented in Table 12, with a mean carbon stock in conifers category almost five times higher than the mean carbon stock in broadleaved group. Additionally, both Figure 15 (a) and the standard deviation results (Table 12) show that the variability of carbon stock values is more important for coniferous than for broadleaved species. The carbon stock in plots with broadleaved species varies from 1.4 to 4.9 Mg/ha. This is a narrow range of values compared to the coniferous group with carbon stocks from 2.7 to 29.1 Mg/ha, and even a carbon stock of 44.3 Mg/ha measured for *Pinus monticola*

This tendency of higher mean value and a higher variability in the conifers category compared to the broadleaves category knows a decline while going deeper in the soil, as described in Figure 15 (b), (c), and (d). Table 15 Figure 6 already showed that the carbon stock is decreasing with depth in mineral soil, but Table 12 reveals that the differences between the two species categories is also falling with depth.

In the soil depth interval 0-5 cm, the carbon stock from broadleaved group plots varies from 14.3 to 26.5 Mg/ha while it varies from 19.2 to 42 Mg/ha, with an outlier value of 51 Mg/ha in plot 49 (*Cryptomeria Japonica*). In the interval 5-10 cm, the coniferous group has values varying from 5.513077 to 31.358106 Mg/ha, with three outlier values found in plots 4 (*Pseudotsuga Menziesii*) with 31.4 Mg/ha, 9 (*Pseudotsuga Menziesii*) with 21.7 Mg/ha and 51 (*Larix Kaempferi*) with 22.5 Mg/ha. A particularly low value is observed in plot 17 (*Tsuga canadensis*) with 5.5 Mg/ha. In the interval 10-20 cm, two plots are remarkably standing out from other plots of their respective species' category. That concerns plot 25 with *Quercus* *rubra* for the broadleaves 21.9 Mg/ha, and plot 51 with *Larix kaempferi* for the conifers (36.1 Mg/ha).

As shown in Figure 15 (b) and in Table 12, the median and mean differences between the coniferous and broadleaved plots is non-significant in the layer 10-20 cm. The two categories have a mean carbon stock difference of less than one Mg/ha.

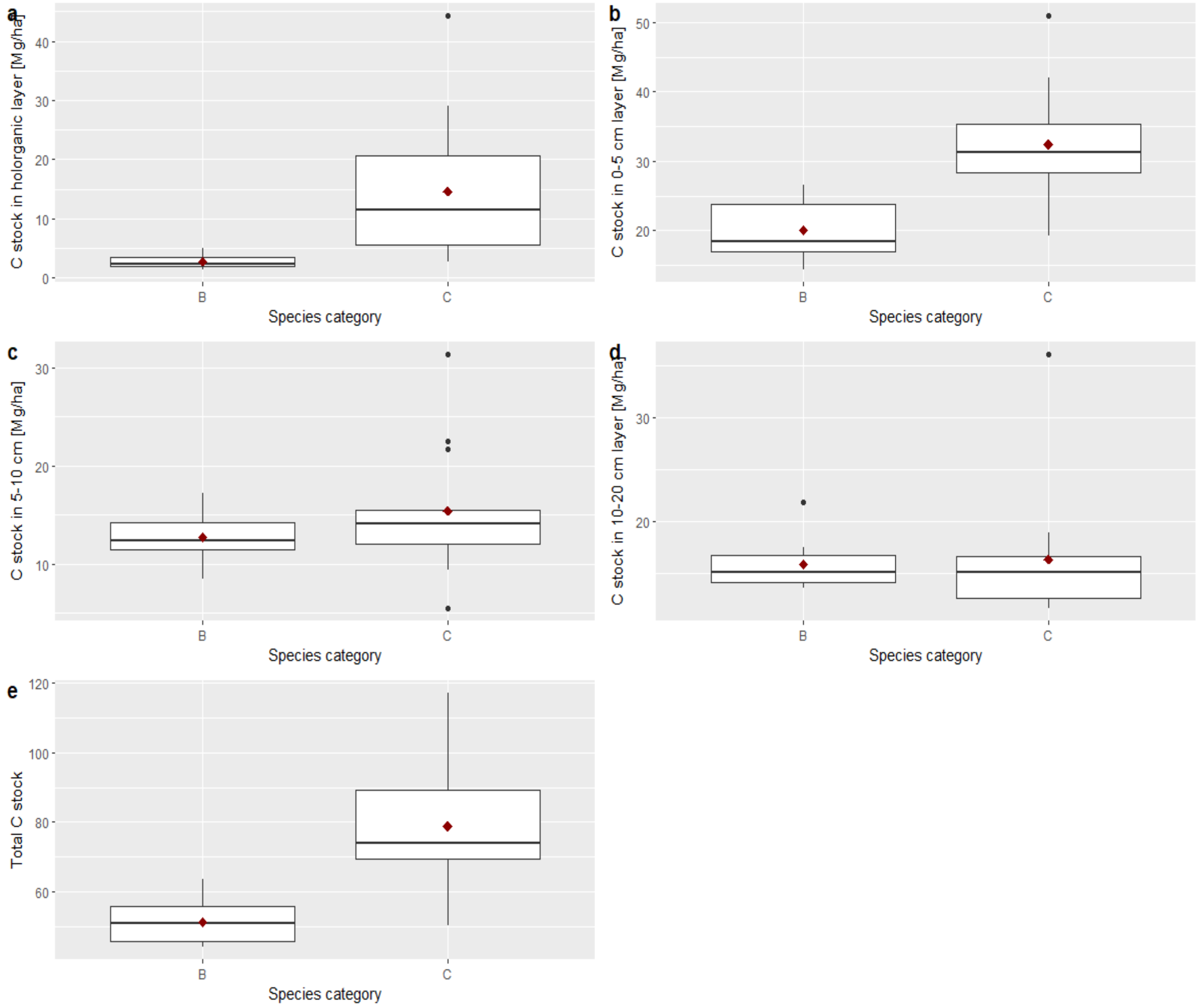

Figure 15: SOC stocks (Mg/ha; range, median, mean (red point), first and third quartiles and outliers) by species (B: broadleaves (N=11), C: conifers (N=13)) in the holorganic layer (a), in the 0-5 cm topsoil layer (b), in the 5-10 cm topsoil layer (c), in the 10-20 cm topsoil layer (d) and in the holorganic layer + 0-20 cm topsoil layer (e).

Table 12: Mean values ± standard deviations of SOC stocks (Mg/ha) as a function of depth (holorganic layer, 0-5 cm topsoil layer, 5-10 cm topsoil layer, 10-20 cm topsoil layer) and species category (broadleaves (N=11), conifers (N=13)).

Depth	**Broadleaves** C stock Mg/ha	**Conifers** C stock Mg/ha
holorganic layer	2.8 ± 1.2	14.6 ± 12.1
0-5 cm	20.0 ± 4.2	32.4 ± 8.3
5-10 cm	12.7 ± 2.5	15.4 ± 6.6

10-20 cm	15.8 ± 2.4	16.3 ± 6.35

Table 13 shows a significant relationship between the species category (conifers or broadleaves) and the carbon stock in the holorganic layer and in the layer 0-5 cm. The most significant correlation of the table is between species category and the cumulative total carbon stock. However, there is no relevant correlation between the species category and carbon stock measured in the layers 5-10 cm and 10-20 cm. The comparison of total carbon stocks regarding the species category shows an important statistical relevancy given that the of multiple and adjusted R-squared have high values (0.5301 and 0.5087) and the p-value is extremely low (5.507e-05).

Table 13: Statistical test (p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression models predicting carbon stocks (Mg/ha) as a function of species category (broadleaves, conifers) and depth (holorganic layer, 0-5 cm, 5-10 cm, 10-20 cm, holorganic + 0-20 cm (total)).

Statistics tests	**Multiple R2**	**Adjusted R2**	**P-value**
Dependent variables			
holorganic layer	0.3197	0.2888	0.003982
0-5 cm	0.4798	0.4562	0.0001757
5-10 cm	0.06936	0.02706	0.2137
10-20 cm	0.002615	-0.04272	0.8124
Total	0.5301	0.5087	5.507e-05

4.1.2 Relationship between carbon stock distributions in soil profile and species category.

The impact of the species category on the carbon distribution in the soil profile is illustrated Figure 16, representing the carbon stock in the holorganic layer relatively to the total carbon stock and the carbon stock in the holorganic layer and the layer 0-5 cm relatively to the total carbon stock. These two figures have showed similar results. In both cases, the median values and the variability of the carbon distribution seem to be more important for the coniferous group than for the broadleaved group. This observation is confirmed by results in Table 14. The mean and standard value for the distribution are higher for the conifers than for the broadleaves, as well as for the total carbon stock. This conclusion that on coniferous plots, the carbon stock near the soil surface (from the litter until 5 cm deep) is more important relatively to the total carbon stock than for broadleaved plots, where the carbon stock is more equally distributed in the soil profile. It can be pointed out in Figure 16 (a) that *Pinus monticola* (plot 12) has an outlier carbon stock value of 0.521124 Mg/ha which can be explained by a significantly high carbon stock in the holorganic layer in Figure 6.

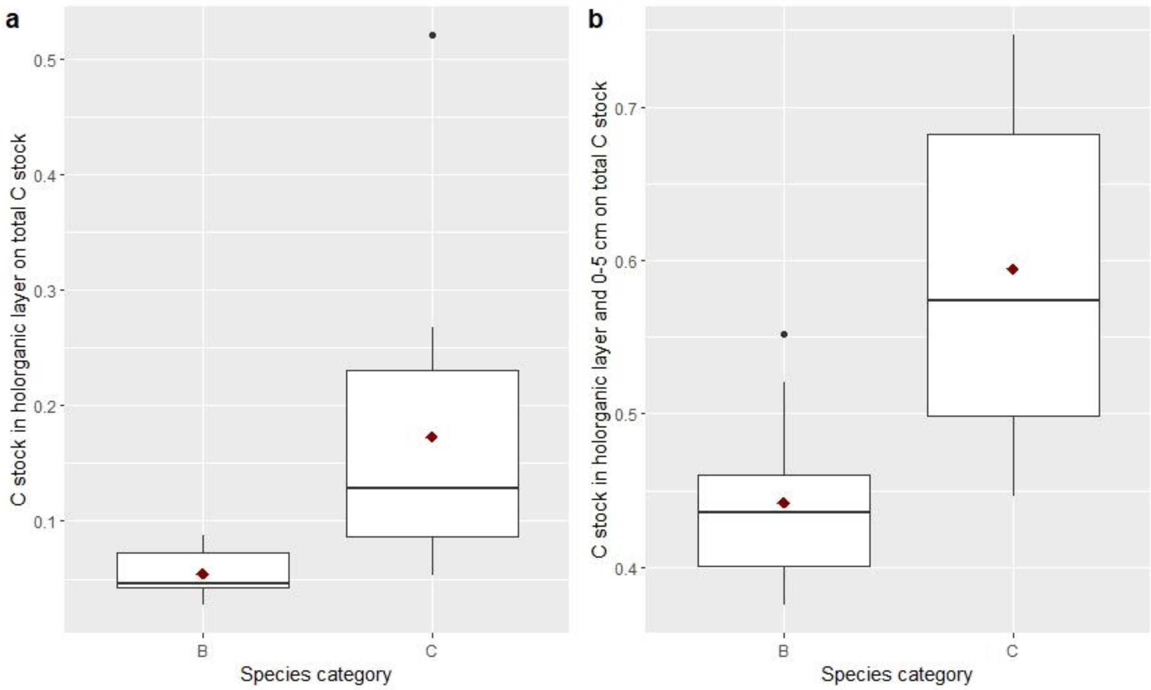

Figure 16: SOC stock (Mg/ha; range, median, mean (red point), first and third quartiles and outliers) by species (B: broadleaves (N=11), C: conifers (N=13)) in the holorganic layer (a) and in the holorganic layer + 0-5 cm topsoil layer (b) on the cumulative carbon stock (holorganic layer + 0-20 topsoil layer).

Table 14: Mean values ± standard deviations of ratio (%) between SOC stocks (Mg/ha) in the holorganic layer (R1) or in holorganic + 0-5 cm layers (R2) and the cumulative total carbon stock (Mg/ha; holorganic + 0-20 cm layers) as a function of species category (broadleaves (N=11), conifers (N=13)).

Depth	**Broadleaves**	**Conifers**
R1	5.5 ± 2.1	17.3 ± 12.8
R2	44.2 ± 5.5	59.4 ± 9.7

and the carbon stock in the forest floor (organic) regarding the total carbon stock of the soil profile and between the category and the topsoil layer (organic + 0-5 cm) regarding the total carbon stock. However, the second distribution model is the most relevant with a low p-value (0.0001374) and relatively high values of multiple and adjusted R-squared (0.4909 and 0.4678) compared to these values for the distribution a. It is presented in Table 15.

Table 15: Statistical test (p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression models predicting the ratio (%) (%) between SOC stocks (Mg/ha) in the holorganic layer (R1) or in holorganic + 0-5 cm layers (R2) and the cumulative total carbon stock (Mg/ha; holorganic + 0-20 cm layers) as a function of species category (broadleaves, conifers).

Statistics tests	**Multiple R2**	**Adjusted R2**	**P-value**
Dependent variables			
R1	0.2932	0.2611	0.006277
R2	0.4909	0.4678	0.0001374

4.2 Continuous approach

4.2.1 Relationship between carbon stocks and leaf litter turnover rate.

Figure 17 (a) shows a tight relationship between carbon stocks in the holorganic layer and the estimated leaf litter turnover rate. The regression is calculated with a nonlinear curve (logarithmic regression) and results are presented in Table 16. As figured in Table 16, carbon stock in the holorganic layer is negatively related to the estimated leaf litter turnover rate. The model presents of the regression presents high values of multiple and adjusted R-squared (0.712 and 0.6995) and a Pr(<|t|) value much lower than 0.05. Figure 17 (b) seems to reveal a negative relationship between carbon stock in the 0-5 cm topsoil layer and the leaf litter turnover rate. However, as presented in Table 16, the nonlinear regression between these variables has low values of multiple and adjusted R-squared (0.303 and 0.2713) but a significant value of Pr(<|t|) (< 0.05). Those results indicate that the carbon stock in 0-5 cm is less related to leaf litter turnover rate compared to the carbon in holorganic layer. Figure 17 (c) and (d) and Table 16 reveal the absence of significant relationship between the leaf litter turnover rate and carbon stocks in 5-10 cm and 10-20 cm topsoil layers. The statistic test Pr(<|t|) for the regression models with those variables is lower than the significancy level of 0.05. Finally, Figure 17 (e) shows that the total cumulative carbon stock (holorganic + 0-20 cm layers) is related to the leaf litter turnover rate. Result in Table 16 confirms that a significant negative correlation exists between these two variables because the correspondent regression model has significant values of determination coefficient and Pr(<|t|) > 0.05. The relationship between carbon stocks in holorganic layer and leaf litter turnover rate is comparable to relationship between the latter parameter and total carbon stocks. Figure 18 presents tendencies to relationship observed in Figure 17 but with a logarithmic scale.

Table 17 confirms the observations of Table 16. Logarithmic scale model shows a more significative relationship between carbon stock in holorganic layer and leaf litter turnover rate (multiple R^2 = 0.8954 and adjusted R^2=0.8906) compared to the previous model.

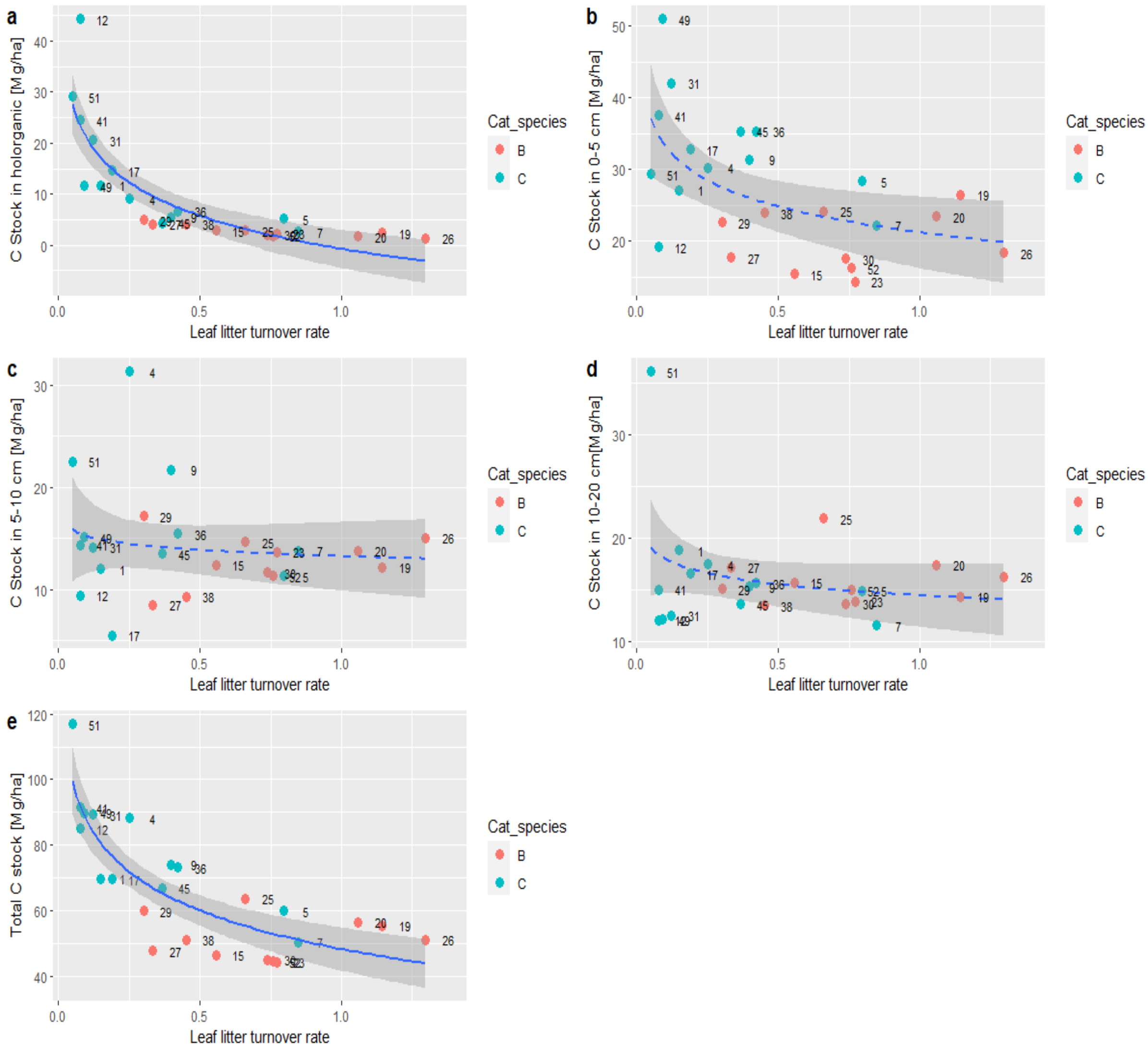

Figure 17: SOC stocks (Mg/ha) as a function of estimated leaf litter turnover rate in the holorganic layer (a), in the 0-5 cm topsoil layer (b), in the 5-10 cm topsoil layer (c), in the 10-20 cm topsoil layer (d) and in the holorganic layer + 0-20 cm topsoil layer (e) with species category (broadleaves, B, red; conifers, C, blue) and with regression curve (y ~ log(x), blue).

Table 16: Parameters coefficients (estimated and standard error (SE)), statistical test (p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of regression models (y ~ log(x)) predicting carbon stocks values (Mg/ha) as a function of estimated leaf litter turnover rate and depth (holorganic layer, 0-5 cm layer, 5-10 cm layer, 10-20 cm layer, holorganic + 0-20 cm layers).

Explanatory variables	**explanatory variable coefficient (and SE)**	**Intercept coefficient (and SE)**	**Multiple R-squared**	**Adjusted R-squared**	**p-value**
holorganic layer	-9.3714 (1.2688)	-0.6947 (1.7910)	0.7126	0.6995	2.165e-07
0-5 cm	-5.245 (1.696)	21.218 (2.394)	0.303	0.2713	0.005314

5-10 cm	-0.8813 (1.1442)	13.2498 (1.6150)	0.02626	-0.018	0.4494
10-20 cm	-1.546 (1.030)	14.459 (1.454)	0.09283	0.05159	0.1477
Total	-17.044 (2.235)	48.232 (3.155)	0.7254	0.713	1.3e-07

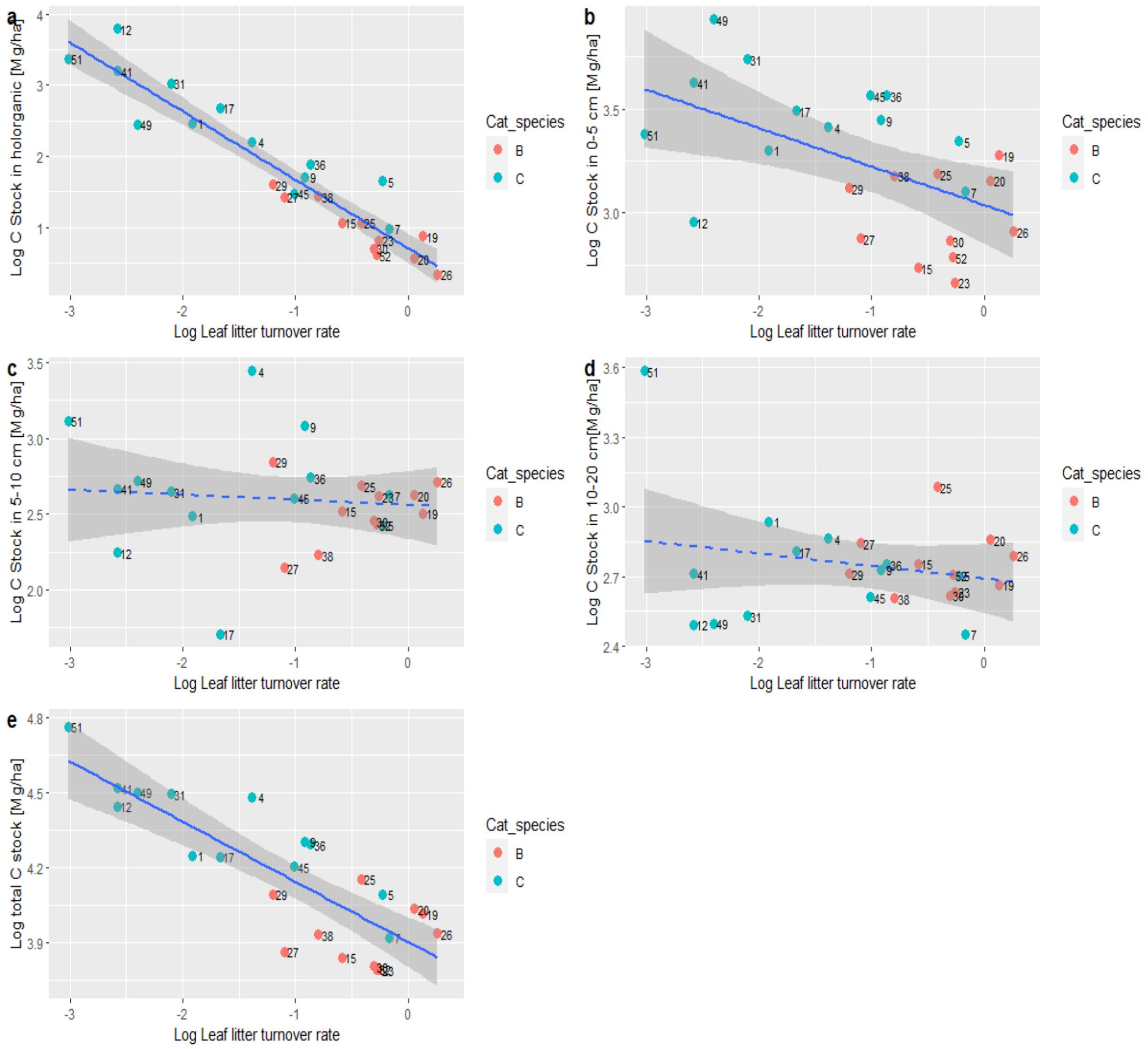

Figure 18: The logarithm of SOC stocks values (Mg/ha) as a function of the logarithm of estimated leaf litter turnover rate values in the holorganic layer (a), in the 0-5 cm topsoil layer (b), in the 5-10 cm topsoil layer (c), in the 10-20 cm topsoil layer (d) and in the holorganic layer + 0-20 cm topsoil layer (e) with species category (broadleaves, B, red; conifers, C, blue) and with linear regression line (blue).

Table 17: Parameters coefficients (estimated and standard error (SE)), statistical test (p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression models predicting the logarithm of carbon stocks values (Mg/ha) as a function of the logarithm of estimated leaf litter turnover rate and depth (holorganic layer, 0-5 cm layer, 5-10 cm layer, 10-20 cm layer, holorganic + 0-20 cm layers).

Explanatory variables	**Explanatory variables coefficient (and SE)**	**Intercept coefficient (and SE)**	**Multiple R-squared**	**Adjusted R-squared**	**p-value**
holorganic layer	-0.96334 (0.07020)	0.70820 (0.09908)	0.8954	0.8906	2.9e-12
0-5 cm	-0.18528 (0.06294)	3.03753 (0.08884)	0.2826,	0.25	0.007512
5-10 cm	-0.03298 (0.07610)	2.55988 (0.10741)	0.008463	-0.03661	0.669
10-20 cm	-0.05377 (0.05039)	2.69117 (0.07112)	0.04922	0.006001	0.2975
Total	-0.24007 (0.03388)	3.90218 (0.04783)	0.6953	0.6814	4.167e-07

4.2.2 Relationship between carbon stock distribution in soil profile and leaf litter turnover rate

As visible in Figure *19* (a) and (b), a negative nonlinear relationship exists between the leaf litter turnover rate variable and the carbon stocks distribution ratio R1 and R2. Values in (a) seems to fit more the logarithmic regression curve than values in (b). Moreover, in Table *18*, both variables R1 and R2, the statistic test Pr(<|t|) lower than the significancy level 0.05 but the value of this latter parameter is much lower for R1 than for R2. It indicates that R1 is more significantly related to leaf litter turnover rate compared to R2. This observation is confirmed by Figure *20* and Table *19* which represent the linear regression model of relationship between R1 and R2 and leaf litter turnover rate but with logarithmic scale. Both variables (R1 and R2) are related to leaf litter turnover rate but the relationship is tighter in the case of R1.

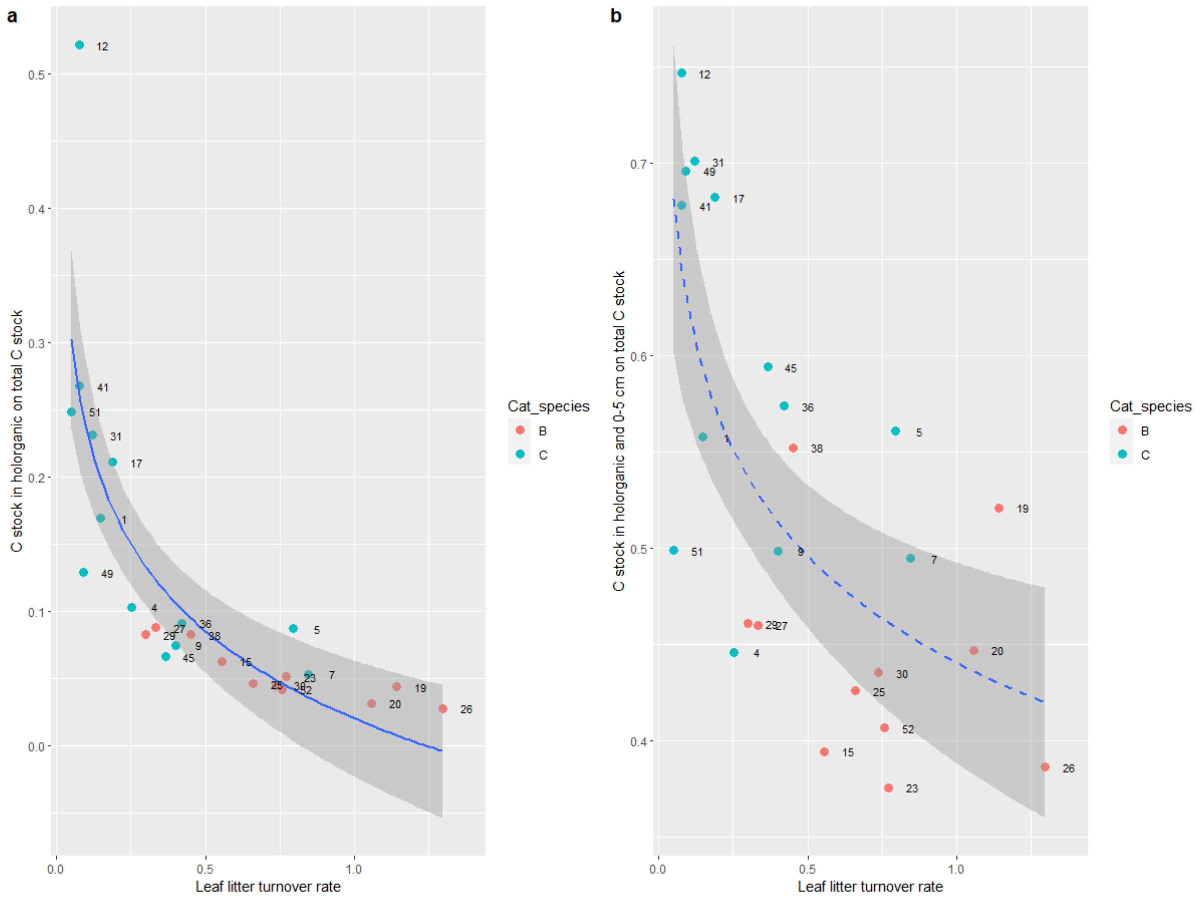

Figure 19: Ratio between SOC stocks (Mg/ha) in holorganic layer (a) or in holorganic + 0-5 cm layers (b) and the total cumulative carbon stock (Mg/ha, holorganic + 0-20 cm layers) as a function of estimated leaf litter turnover rate with species category (broadleaves, B, red; conifers, C, blue) and with regression curve (y ~ log(x), blue).

Table 18: Parameters coefficients (estimated and standard error (SE)), statistical test (p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of regression models (y ~ log(x)) predicting ratio between SOC stocks (Mg/ha) in holorganic layer (a) or in holorganic + 0-5 cm layers (b) and the total cumulative carbon stock (Mg/ha, holorganic + 0-20 cm layers) as a function of estimated leaf litter turnover rate

Explanatory variables	**explanatory variable coefficient (and SE)**	**Intercept coefficient (and SE)**	**Multiple R-squared**	**Adjusted R-squared**	**p-value**
R1	-0.09355 (0.01475)	0.02001 (0.02081)	0.6466	0.6305	2.2e-06

R2	-0.07982 (0.01782)	0.44047 (0.02515)	0.477	0.4532	0.0001872

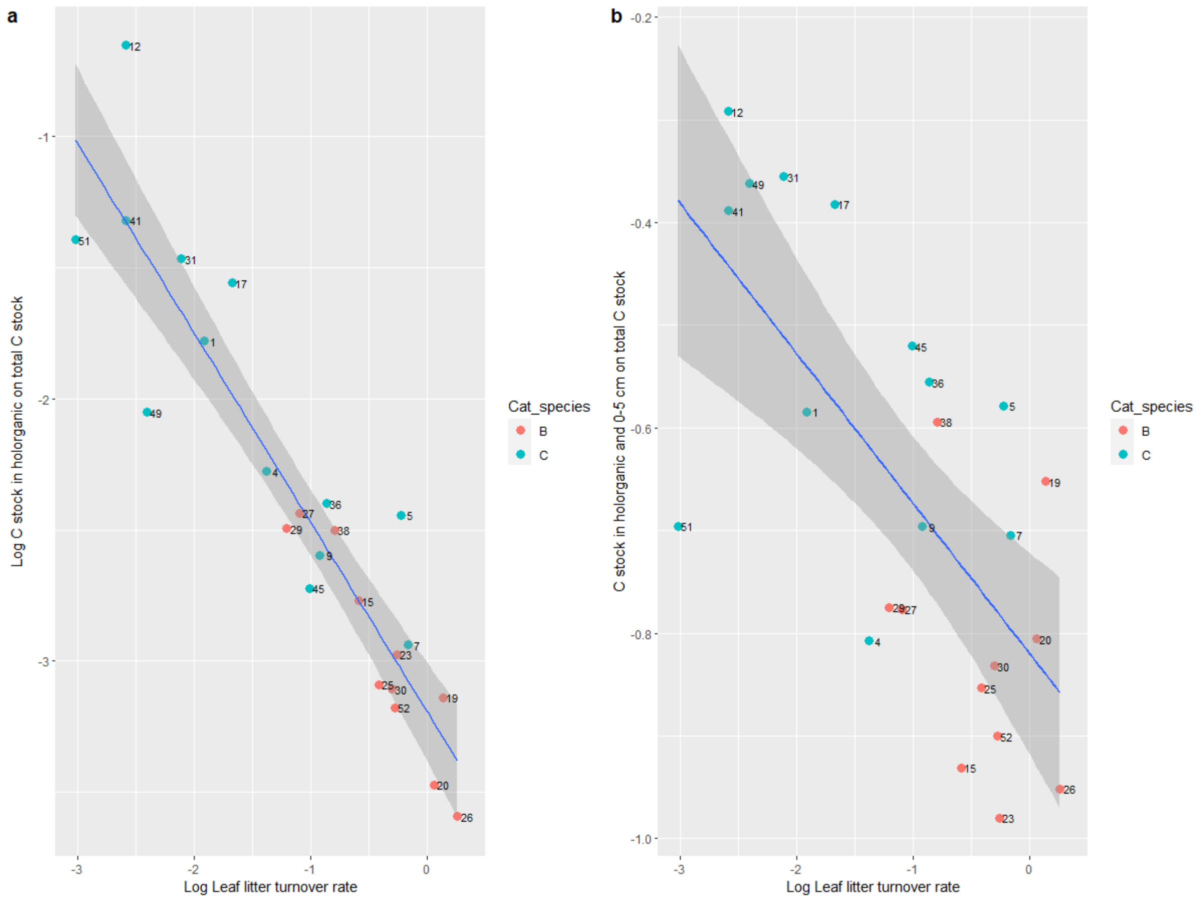

Figure 20: The logarithm of ratio between SOC stocks (Mg/ha) in holorganic layer (a) or in holorganic + 0-5 cm layers (b) and the total cumulative carbon stock (Mg/ha, holorganic + 0-20 cm layers) as a function of the logarithm of estimated leaf litter turnover rate with species category (broadleaves, B, red; conifers, C, blue) and with linear regression line (blue).

Table 19: Parameters coefficients (estimated and standard error (SE)), statistical test (p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression models predicting logarithm of ratio between SOC stocks (Mg/ha) in holorganic layer (a) or in holorganic + 0-5 cm layers (b) and the total cumulative carbon stock (Mg/ha, holorganic + 0-20 cm layers) as a function of the logarithm of estimated leaf litter turnover rate

Explanatory variables	**explanatory variable coefficient (and SE)**	**Intercept coefficient (and SE)**	**Multiple R-squared**	**Adjusted R-squared**	**p-value**
R1	-0.72327 (0.06402)	-3.19398 (0.09037)	0.853	0.8463	1.256e-10

R2	-0.14632 (0.03356)	-0.81991 (0.04737)	0.4635	0.4392	0.0002504

4.2.3 Relationship between carbon stocks distribution in soil profile and chemical properties.

4.2.3.1 Exploration of combined effect of explanatory variables with multivariate regressions.

In this section, the results of two multi-variable regression models are analyzed. These models investigate the effect of the different variables presented in Table 20 (independent variables) on the carbon stock in the holorganic layer on the total carbon stock, referred as ratio R1, and on the carbon stock in the holorganic layer and in the layer 0-5 cm on the total carbon stock, referred as ratio R2. Table 20 contains a continuous variable related to species trait (estimated leaf litter turnover rate) and variables related to soil exchangeable cation content (Ca^{2+} and Al^{3+}), representing chemical properties of topsoil.

Table 20: List of variables used as explanatory variables in multivariate regression models predicting the ratio between carbon stocks (Mg/ha) in holorganic layer (R1) or in holorganic + 0-5 cm layers (R2) and cumulative total carbon stocks (Mg/ha; in organic + 0-5 cm layers).

N°	Variables
1.	Estimated leaf litter turnover rate
2.	Calcium (Ca^{2+}) content in 0-5 cm on eCEC in 0-5 cm [cmolc/kg]/ [cmolc/kg]
3.	Aluminum (Al^{3+}) content in 0-5 cm on eCEC in 0-5 cm [cmolc/kg]/ [cmolc/kg]
4.	Calcium (Ca^{2+}) content in 0-5 cm on calcium content in 5-20 cm [cmolc/kg]/ [cmolc/kg]
5.	Aluminum (Al^{3+}) content in 0-5 cm on aluminum content in 5-20 cm [cmolc/kg]/ [cmolc/kg]

Stepwise regressions have generated similar models with backward, forward and both directions methods. The resulting multivariate model in Table 21 represents the relationship between R1 and the two following variables: (1.) leaf litter turnover rate and (3) $Al^{3+/}$eCEC in 0-5 cm. All parameters in the model are individually significant with values of Pr(>|t|) less than 0.05. Pr(>|t|) equals 0.01140 for variable (1.) and equals 0.04197 for variable (3.). The p-value of the F-statistics is, in comparison, is much lower and equals 0.0002282. It reveals that a combined effect of the two explanatory variables on R1 exists due to a potential correlation between the leaf litter turnover rate and the ratio between Al^{3+} content in 0-5 cm and eCEC in 0-5 cm.

Table 21: Parameters coefficients (estimated and standard error (SE)), statistical test (Pr(>|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of multivariate regression model predicting the (R1)

ratio between SOC stocks (Mg/ha) in holorganic layer and total cumulative carbon stock (Mg/ha; holorganic +0-20 cm layers) as a function of leaf litter turnover rate and the ratio between $Al^{3+/}$ and eCEC in 0-5 cm.

Parameters	Estimated coefficients	Coefficient standard error	Pr(>|t|)
Intercept	0.13586	0.04758	0.00947
Leaf litter turnover rate (1.)	-0.14544	0.05245	0.01140
$Al^{3+/}$eCEC in 0-5 cm (3.)	0.18584	0.08580	0.04197
	Multiple R2	**Adjusted R2**	**p-value**
	0.55	0.5072	0.0002282

Stepwise regressions have generated similar models with backward, forward and both directions methods. The resulting multivariate model in Table *22* represents the relationship between R2 and the two following variables: (1.) leaf litter turnover rate and (5.) the ratio between Al^{3+} content in 0-5 cm and in 5-20 cm. The explanatory variable (5.) is individually significant in the model with values of Pr(>|t|) of 0.00401 (< 0.05). The explanatory variable (1.) is on the contrary not individually significant in the mode because its Pr(>|t|) values equals 0.12806 (> 0.05). However, the p-value of the F-statistics is much lower than parameters statistic tests and equals 6.937e-05. It reveals that a highly important combined effect of the two explanatory variables on R2 exists due to a potential correlation between the leaf litter turnover rate and the ratio between Al^{3+} content in 0-5 cm and in 5-20 cm (5.). This is in line with to previous observations in Table *21*.

Besides, the model predicting R1 values in Table *21* and the model predicting R2 values in Table *22* obtain comparable coefficient of determination (multiple and adjusted), between 0.5 and 0.6. Consequently, both models are similarly significant.

Table 22: Parameters coefficients (estimated and standard error (SE)), statistical test (Pr(<|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of multivariate regression model predicting the (R2) ratio between SOC stocks (Mg/ha) in holorganic + 0-5 cm layers and total cumulative carbon stock (Mg/ha; holorganic + 0-5 cm layers) as a function of leaf litter turnover rate and the ratio between Al^{3+} in the 0-5 cm layer and Al^{3+}in the 5-20 cm layer.

Parameters	**Estimated coefficients**	**Coefficient standard error**	**Pr(>|t|)**
Intercept	0.45855	0.05609	5.8e-08
(1.) leaf litter turnover rate	-0.08470	0.05346	0.12806

(5.) Al^{3+} (0-5 cm) / Al^{3+} (5-20 cm)	0.24520	0.07591	0.00401
	Multiple R2	**Adjusted R2**	**p-value**
	0.5983	0.56	6.937e-05

4.2.3.2 Exploration of individual effect of explanatory variables with simple regression

This section will explore the relationship between each variable related to chemical topsoil properties of Table *20* and carbon stocks distribution variables R1 and R2 using simple linear regression models.

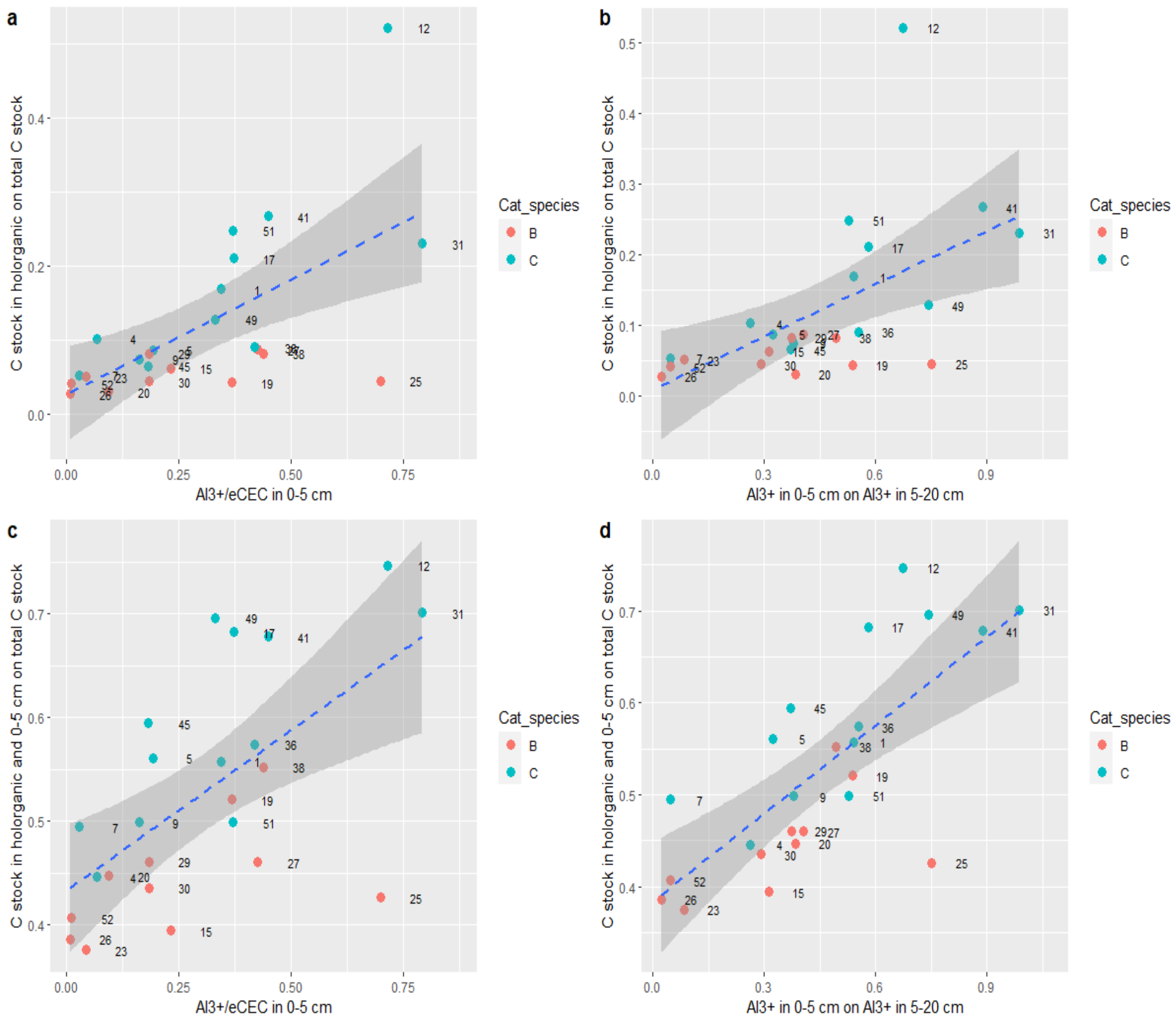

Figure 21: Ratio between SOC stocks (Mg/ha) in holorganic layer (a and b) or holorganic + 0-5 cm layers (c and d) as a function of the ratio between Al^{3+} (cmolc/kg) and eCEC (cmolc/kg) in the 0-5 cm layer (a and c) or as a function of the ratio between Al^{3+} (cmolc/kg) in the 0-5 cm layer and Al^{3+} in the 5-20 cm layer (b and d) with species category (broadleaves, B, red; conifers, C, blue) and with linear regression line (blue).

First, Figure 21 presents relationship between carbon stocks distribution and exchangeable aluminum-related variables. Figures (a), (b), (c) and (d) illustrates linear regression models whose results are presented in respectively

The models of Figure 21 (a), (b) and (c) present similar relationships between the response variable and the explanatory Al-related variable. It is supported the observation in Table 24, Table 25 and Table 26 of similar values of multiple R-squared, which vary between 0.33 and 0.39 and adjuster R-squared, between 0.29 and 0.36. Those models have similar p-values, which vary from 0.001 and 0.003. These values are < 0.05. It indicates that there is an effect of variable (3.) on R1 and R2 and that variable (5.) as an impact on R1. Those observations are less important compared to the tight relationship that exist between the variable (5.) and the R2. Indeed, the regression model between these two variables is illustrated in Figure 21 (d) and presents more significant statistical parameters, presented in Table 28, compared to other models. This model has higher values of R^2, around 0.5, and a much lower p-value, 3.344e-05.

Table 23: Parameters coefficients (estimated and standard error (SE)), statistical test (Pr(<|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression model predicting the (R1) ratio between SOC stocks (Mg/ha) in holorganic layer and total cumulative carbon stock (Mg/ha; holorganic + 0-5 cm layers) as a function of leaf litter turnover rate and the ratio between Al^{3+} and eCEC in the 0-5 cm layer.

Parameters	**Estimated coefficients**	**Coefficient standard error**	**Pr(>|t|)**
Intercept	0.02700	0.03070	0.38856
Al^{3+}/eCEC in 0-5 cm	0.31019	0.08353	0.00121
	Multiple R2	**Adjusted R2**	**p-value**
	0.3853	0.3574	0.00121

Table 24: Parameters coefficients (estimated and standard error (SE)), statistical test (Pr(<|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression model predicting the (R1) ratio between SOC stocks (Mg/ha) in holorganic layer and total cumulative carbon stock (Mg/ha; holorganic + 0-5 cm layers) as a function of leaf litter turnover rate and the ratio between Al^{3+} in the 0-5 cm layer and Al^{3+}in the 5-20 cm layer.

Parameters	**Estimated coefficients**	**Coefficient standard error**	**Pr(>|t|)**
Intercept	0.009026	0.038461	0.81663
Al^{3+}(0-5 cm) /Al^{3+}(5-20 cm)	0.248754	0.075762	0.00339

	Multiple R2	Adjusted R2	p-value
	0.3289	0.2984	0.003393

Table 25: Parameters coefficients (estimated and standard error (SE)), statistical test (Pr(<|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression model predicting the (R2) ratio between SOC stocks (Mg/ha) in holorganic + 0-5 cm layers and total cumulative carbon stock (Mg/ha; holorganic + 0-5 cm layers) as a function of leaf litter turnover rate and the ratio between Al^{3+} and eCEC in the 0-5 cm layer.

Parameters	Estimated coefficients	Coefficient standard error	Pr(>|t|)
Intercept	0.43317	0.03041	1.39e-12
Al^{3+}/eCEC in (0-5 cm) (3.)	0.30956	0.08275	0.00113
	Multiple R2	**Adjusted R2**	**p-value**
	0.3888	0.361	0.001132

Table 26: Parameters coefficients (estimated and standard error (SE)), statistical test (Pr(<|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression model predicting the (R2) ratio between SOC stocks (Mg/ha) in holorganic + 0-5 cm layers and total cumulative carbon stock (Mg/ha; holorganic + 0-5 cm layers) as a function of leaf litter turnover rate and the ratio between Al^{3+} in the 0-5 cm layer and Al^{3+}in the 5-20 cm layer.

Parameters	**Estimated coefficients**	**Coefficient standard error**	**Pr(>|t|)**
Intercept	0.38373	0.03128	2.60e-11
Al^{3+}(0-5 cm) /Al^{3+}(5-20 cm)	0.31967	0.06161	3.34e-05
	Multiple R2	**Adjusted R2**	**p-value**
	0.5503	0.5298	3.344e-05

In a second step, Figure 22 presents relationship between carbon stocks distribution and exchangeable calcium-related variables. Figures (a), (b), (c) and (d) illustrates linear regression models whose results are presented in respectively Table 27, Table 28, Table 29 and Table 30. A relationship between both carbon stocks distribution ratio R1 and R2 and the variable (2.) (Ca^{2+}/eCEC in the 0-5 cm layer) is visible in Figure 22 (a) and (b). Statistical parameters of the regression model predicting R1 as a function of variable (2.) are presented in Table 27 and statistical parameters of regression model predicting R2 as a function of variable (2.) are presented in Table 29. Both models have a highly significant p-value (between 0.001 and 0.005). The significancy of the models are temperate by moderate values of multiple and adjusted R-squared, represents the statistical show significant p-value for these two model (between 0.29 and 0.33), comparable R2 values of models presented in Table 23, Table 24 and Table 25. On the contrary, according to Figure 22 (b) and (d), neither R1 nor R2 are related to the distribution of exchangeable calcium in the topsoil (variable (4.)). Table 28 and Table 30 show significant p-value inferior higher than 0.5 and much lower R-squared value compared to those shown in Table 27 and Table 29.

In conclusion, a highly tight relationship exists between aluminum distribution and carbon stocks distribution in topsoil. Exchangeable calcium and aluminum, relatively to the total eCEC, in 0-5 cm layer has a moderate effect on carbon stocks distribution in topsoil. However, exchangeable calcium distribution in topsoil is not related to carbon stocks distribution in the profile.

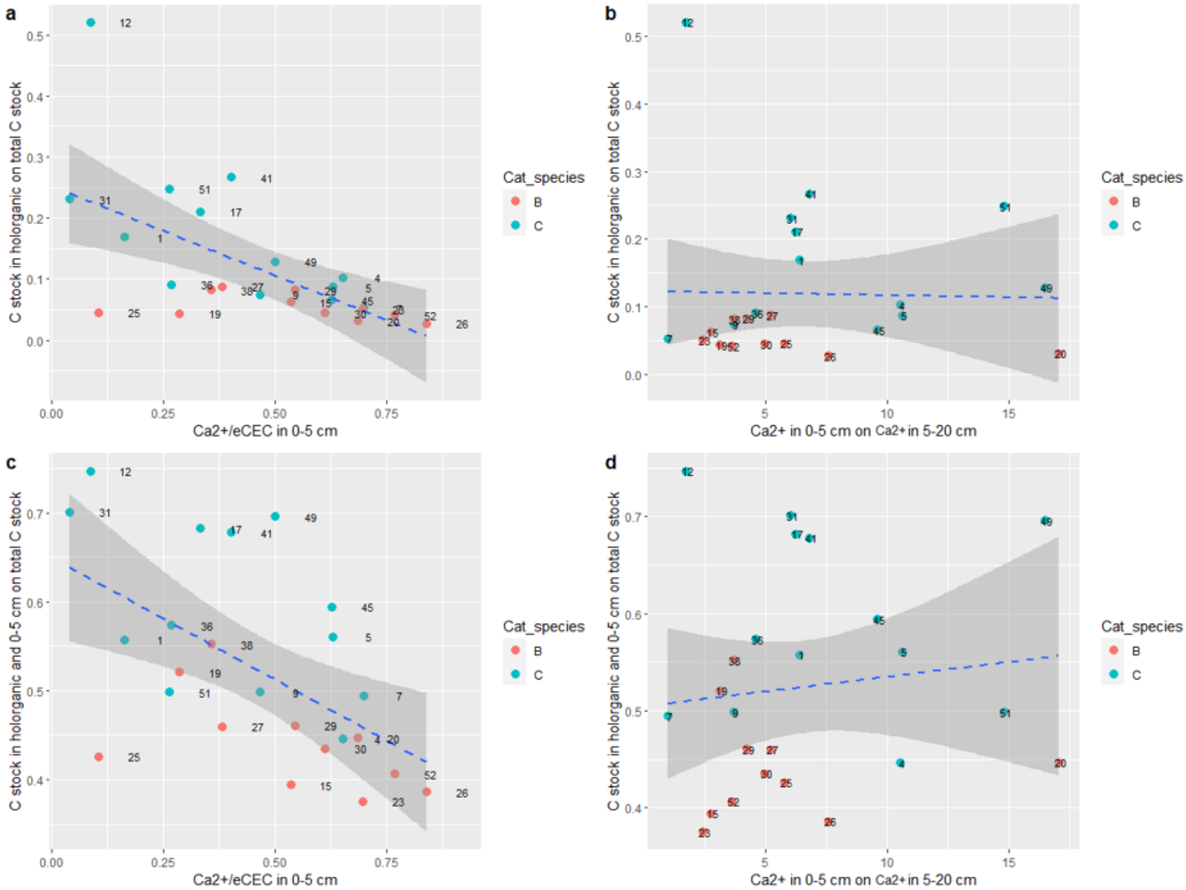

Figure 22: Ratio between SOC stocks (Mg/ha) in holorganic layer (a and b) or holorganic + 0-5 cm layers (c and d) as a function of the ratio between Ca^{2+} (cmolc/kg) and eCEC (cmolc/kg) in the 0-5 cm layer (a and c) or as a function of the ratio between Ca^{2+} (cmolc/kg) in the 0-5 cm layer and Ca^{2+} in the 5-20 cm layer (b and d) with species category (broadleaves, B, red; conifers, C, blue) and with linear regression line (blue).

Table 27: Parameters coefficients (estimated and standard error (SE)), statistical test (Pr(<|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression model predicting the (R1) ratio

between SOC stocks (Mg/ha) in holorganic layer and total cumulative carbon stock (Mg/ha; holorganic + 0-5 cm layers) as a function of leaf litter turnover rate and the ratio between Al^{3+} and eCEC in the 0-5 cm layer.

Parameters	Estimated coefficients	Coefficient standard error	Pr(>|t|)
Intercept	0.25217	0.04206	4.93e-06
Ca^{2+} /eCEC (0-5 cm)	-0.29292	0.08286	0.00186
	Multiple R2	**Adjusted R2**	**p-value**
	0.3623	0.3333	0.00186

Table 28: Parameters coefficients (estimated and standard error (SE)), statistical test (Pr(<|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression model predicting the (R1) ratio between SOC stocks (Mg/ha) in holorganic layer and total cumulative carbon stock (Mg/ha; holorganic + 0-5 cm layers) as a function of leaf litter turnover rate and the ratio between Ca^{2+} in the 0-5 cm layer and Ca^{2+}in the 5-20 cm layer.

Parameters	Estimated coefficients	Coefficient standard error	Pr(>|t|)
Intercept	0.1227193	0.0421214	0.00805
Ca^{2+} (0-5 cm) /Ca^{2+} (5-20 cm)	-0.0006026	0.0053171	0.91080
	Multiple R2	**Adjusted R2**	**p-value**
	0.0005835	-0.04484	0.9108

Table 29: Parameters coefficients (estimated and standard error (SE)), statistical test (Pr(<|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression model predicting the (R2) ratio between SOC stocks (Mg/ha) in holorganic + 0-5 cm layers and total cumulative carbon stock (Mg/ha; holorganic + 0-5 cm layers) as a function of leaf litter turnover rate and the ratio between Ca^{2+} and eCEC in the 0-5 cm layer.

Parameters	Estimated coefficients	Coefficient standard error	Pr(>|t|)
Intercept	0.6496	0.0431	4.45e-13
Ca^{2+} /eCEC (0-5 cm)	-0.2742	0.0849	0.00385
	Multiple R2	**Adjusted R2**	**p-value**
	0.3217	0.2908	0.00385

Table 30: Parameters coefficients (estimated and standard error (SE)), statistical test (Pr(<|t|), p-value; ANOVA) and determination coefficients (multiple R-squared and adjusted R-squared) of linear regression model predicting the (R2) ratio between SOC stocks (Mg/ha) in holorganic + 0-5 cm layers and total cumulative carbon stock (Mg/ha; holorganic + 0-5 cm layers) as a function of leaf litter turnover rate and the ratio between $C2^{+}$ in the 0-5 cm layer and Ca^{2+}in the 5-20 cm layer.

Parameters	**Estimated coefficients**	**Coefficient standard error**	**Pr(>|t|)**
Intercept	0.504693	12.148	3.14e-11
Ca^{2+} (0-5 cm) /Ca^{2+} (5-20 cm)	0.003030	0.005244	0.569
	Multiple R2	**Adjusted R2**	**p-value**
	0.01495	-0.02982	0.5692

Discussion

1. Homogeneity of site conditions.

This study was a priori considered as a common garden experiment. Like this current study, common garden experiments generally occur in a unique site with several randomly planted monocultures of different tree species with, in ideal conditions, several replicates for each species (Vesterdal et al. 2013). This experiment design was supposed to eliminate the effects of cofounding factors related to the initial environment conditions variability. Therefore, we first aimed to assess that the hypothesis of site conditions homogeneity holds true and to demonstrate that initial site conditions are not interfering with studied tree species effects. The data visualization showed important variabilities between plots in terms of acidity and cation contents in the deep soil. By contrast, texture appears to be quite homogeneous between plots, except for *Fagus sylvatica* (plot 27). Moreover, analyses revealed no detectable impact of deep soil chemical properties on the acidity conditions in the upper mineral top soil, which seems to be mainly influenced by species composition and forest floor characteristics. Consequently, the factors linked to initial environment conditions can be removed from the study of species effects on top soil.

This conclusion could be related to the soil type in the arboretum. As mentioned previously, the soil in study area is referred as Luvisolic soil. This type of loamy soil presents a leached eluvial horizon spreading from approximately 10 to 40 cm deep. Beyond this depth, the illuvial horizon, with a higher density, accumulated leached elements from the upper soil layers. According to a local study focusing about the pedogenesis of these Luvisolic soils, the process of illuviation and the hydromorphic event occurred during the periglacial era, ante Holocene (Langohr 1986). After this event, the biologically activity could have changed soil conditions to a depth of 40 cm. Below this depth, the illuviation horizon is supposed to have been remained unchanged since the periglacial era, because of its high density (Langohr 1986). As a results, the impact of surface vegetation should be mostly concentrated in the first 40 cm of the topsoil, even if root exudates may have an impact more deeply. This assumption could support the interpretation of empirical data but we must nevertheless stay careful as it is based on small amount of literature. On the one side, the low variability of chemical properties, in particular cation-related variables, between plots in the 10-20 cm layer could be explained by the previous leaching of elements. On the other side, the large variability between plots of cation contents in the layer 0-5 cm could be more influenced by external organic matter supply and, consequently, by tree species.

Moreover, the presence of “glosses” implies that eluvial soil characteristics could be found in deeper layers (Baize et Girard 2008). We formulate the hypothesis that the important differences observed in cation contents at the depth of 100 cm could depend on whether the soil sample was harvested in a “glosse” or not. However, the weak variability in the clay content data between plots at that depth interferes with the hypothesis.

In conclusion, inherent site conditions do not influence the assessment of species impact on mineral topsoil properties and the study site can be considered as an adequate site for a common garden experiment.

2. Evolution of carbon stock, carbon content, and chemical properties with depth.

The carbon content measures show a strong variability among plots in the holorganic layer and in the 0-5 cm topsoil layer. Underneath this depth, there is no major difference, except for *Larix kaempferi* and both *Pseudotsuga menziesii* plots. Those present relatively high carbon content in the layer 5-10 cm. Results of carbon content in the 0-5 cm horizon are compared to those of a common garden study in Poland (Mueller et al. 2012). That latter study found low carbon content values in uppermost horizons, ranging from 0.77 % to 1.67%, compared to the current study (11% on average).

Carbon stocks variability among species declines similarly with depth. Total carbon stocks cover a wide range of values, from 40 to more than 90 Mg/ha. The same study (Mueller et al. 2012) obtained total carbon stocks (in a 0-20 cm topsoil layer) varying from 14 to 28.8 Mg/ha. These total values are less important than in the current study but they do not include carbon stocks in forest floor. In a recent study in Denmark (Gurmesa et al. 2013), total carbon stocks are included in a range from approximately 50 to 90 Mg/ha while forest floor carbon stock is in the order of 1 to 20 Mg/ha and total 30 cm deep soil stock varies from 50 to 70 Mg/ha. Total values of carbon stocks of this study are closer to our results, even if these stocks values were calculated in a thicker topsoil layer of 30 cm instead of 20 cm, which might therefore explain why those are higher. However, both studies found a similar range of values for forest floor carbon stocks, without considering the case of *Pseudotsuga menziesii* and *Larix Kaempferi*. Those two species present very high carbon stocks in the forest floor.

Variability evolutions with depth for $CaCl_2$ pH, eCEC, exchangeable calcium and acid exchangeable cations reveal a substantial effect of species composition on topsoil chemical properties mostly observable in uppermost soil horizon (0-5 cm). These chemical properties tend to become homogeneous among species with depth. Moreover, the range of pH values suggests all studied plots present acidic surface conditions.

3. Differences in soil chemical properties with species.

The analyses revealed that the $CaCl_2$ pH in 0-5 cm is on average higher under broadleaved stand than under coniferous stand. Indeed, while broadleaves cover wide range of pH values, conifers are associated with more acidic pH in the uppermost topsoil, except for *Calocedrus decurrens* and *Thuja plicata*. In addition to this species impact, the $CaCl_2$ pH in 0-5 cm is highly correlated with the leaf litter turnover rate. As shown by the results, the pH increases with the leaf litter turnover rate, apart from *Betula alleghaniensis* and *Acer saccharinum*, which have relatively low pH for a high value of leaf litter turnover rate. Concerning exchangeable aluminum in 0-5 cm, the concentrations are higher under coniferous stand than under broadleaved stands. Bivariate correlation matrix of variables and PCA in 0-5 cm also reveals high negative correlation between acidity variables (aluminum content, EA, pH) and the leaf litter turnover rate. It can be assumed that differences of leaf litter turnover rate explain the acidity differences in uppermost topsoil (0-5 cm) between broadleaves and conifers. This study confirms what is described in the current literature: conifers have a lower litter decomposition rate and accumulate more organic matter in the forest floor and consequently, they have more acidic surface conditions (Augusto et al. 2015; Hobbie et al. 2006). Therefore, we conclude that the relationship observed between acidity conditions in the 0-5 cm topsoil layer and species composition is due to differences of litter accumulation capacity between broadleaves and conifers.

By contrast, there was no evidence of a species effect on the base saturation and Ca^{2+} content in the uppermost topsoil layer 0-5 cm, compared to the strength of the relationship between species and surface acidity. Moreover, the relationship between BS and leaf litter turnover rate seemed relatively weak compared to the influence of leaf litter turnover rate on acidity variables. However, as discussed in previous section, an important variability among plots exists for the base saturation and Ca^{2+} data in 0-5 cm. Moreover, it has been shown that this variability was not related to the parental material or the deep soil conditions. The calcium content in 0-5 cm layer may thus have been modified with time in different ways under the different stands, but not specifically depending on whether it was broadleaves or coniferous species. Some studies from a common garden experiment in Poland reported correlation between species identity (broadleaves or conifers) and calcium content, but only calcium content of the foliage litterfall or of the forest floor layer, but not especially in the mineral soil (Mueller et al. 2012; Reich et al. 2005).

A way to explain the special case of *Calocedrus decurrens* is that this coniferous species has a lower lignin content in fresh foliar compared to other conifers (Keenan et al. 1996), resulting in a less recalcitrant litter, which might result in a surprisingly high litter turnover rate for this species category. Moreover, Giant sequoia are on average associated to higher pH and base cation content in comparison with mixed-conifer species (Carey et al. 2020). It is due to particularly high calcium content of their leaves (Zinke et Crocker 1962). Yet, *Sequoiadendron* *giganteum* (giant sequoia) and *Calocedrus decurrens* are both member of the same family of Cupressaceae, and share similar characteristic. This could explain why *Calocedrus decurrens* presents relatively high pH (Figure 9), Ca^{2+} content (Figure 4), leaf litter turnover rate and low carbon stock in the forest floor compared to other studied coniferous species.

4. Species impact on carbon stocks.

One of the main objectives of this study was to quantify potential differences of carbon stocks between coniferous and broadleaved stands and describe how these differences evolve with the depth. The starting hypothesis was that the observed differences would not be found in the total carbon stocks but in spatial C distribution in the soil profile. Indeed, the conifers were supposed to have higher soil C stocks in the forest floor and the uppermost mineral soil compared to the total stock, while broadleaves were supposed to have a lower C stock in the forest floor and uppermost soil layer and more carbon in deeper topsoil layers. This assumption is not completely confirmed by our results. As expected, carbon stocks in forest floor and in the 0-5 cm topsoil layer are on average higher under coniferous species than under broadleaved species. However, no C stocks differences were observed between species in mineral 5-20 cm soil layer. Moreover, the estimated total carbon stocks appear to be higher in the case of conifers. The soil C stocks distribution (in the forest floor and 0-5 cm compared to the total stock) is also higher in coniferous species compared to broadleaved species. It confirms that coniferous species accumulate more organic soil C in the forest floor and in the uppermost horizon but there is no evidence of a more efficient C storage in mineral soil in broadleaves.

On the other hand, a negative correlation between carbon stocks and leaf litter turnover rate exists for the forest floor and the 0-5 cm topsoil layer but there is no significant correlation between these two variables in 5-10 cm and 10- 20 cm layers. Besides, the leaf litter turnover rate appears to be related to C stocks distribution and total C stocks. Furthermore, a low leaf litter turnover rate corresponds to a low rate of organic matter decomposition. By consequences, carbon accumulates in the forest floor, resulting in more carbon in 0-5 cm layer. Yet, our result showed that coniferous species have higher carbon stock in forest floor and 0-5 cm topsoil than broadleaved species. Consequently, the leaf litter turnover rate might be a species identity trait. Coniferous species have low leaf litter turnover rate and, by consequences, accumulate more carbon stocks (Mayer et al. 2020). This last assumption is confirmed in literature by the observation of thicker forest floor layer under coniferous species (Desie, Vancampenhout, et Heyens 2019). In conclusion, the important carbon stocks observed with conifers in the forest floor and topsoil layer 0-5 cm may be related to the tendency of these species to accumulate organic matter in the forest floor due to a low decomposition rate.

There is a consensus among common garden experiment studies about the higher carbon stocks in forest floor in coniferous stands compared to broadleaved stands. A small amount of these studies reported similar trends in terms of C storage in mineral topsoil for broadleaves and conifers (Reich et al. 2005; Mueller et al. 2012; Alriksson et Eriksson 1998) (Ovington et al., 1954). Others authors supported our start hypothesis. Indeed, in these common garden studies, carbon stocks are higher in the mineral topsoil layer of broadleaved stands compared to coniferous stands (Oostra, Majdi, et Olsson 2006; Vesterdal et al. 2008)

Using a common garden experiment, Gursema et al., 2013, found out that organic C stocks were more important in coniferous stands than in broadleaved stands in the forest floor (*Quercus* < *Fagus* < *Picea* < *Larix*) and in the 0-30 cm mineral topsoil layer (*Quercus* < *Fagus* < *Larix* < *Picea*) and total stocks (*Quercus* < *Fagus* < < *Picea* < *Larix*). They explain the higher C stocks in conifers with both higher carbon concentrations and higher soil bulk density.

The differences between our results and results of other common garden studies in terms of species effects on soil C stocks may be explained by soil chemical conditions effect on C stocks. Some studies indicated that broadleaved species are more likely to be efficient in carbon sequestration in the stable pool of mineral soil when environmental conditions are favorable (Mayer et al. 2020). In the meantime, coniferous stands are expected to present better results in terms of carbon storage compared to broadleaved stands when they are established in sites with non-favorable conditions (Gurmesa et al. 2013).

Besides, this current study occurred in a site associated with “poor” surface conditions. This was first assumed with the common characteristics of the soil type (Luvisol) as found in literature, which we described previously. Secondly, results showed exchangeable Al and pH values associated to the acidic conditions of the aluminum buffer domain, a soil process domain described in literature (Desie, Vancampenhout, et Heyens 2019; Chadwick et Chorover 2001). Therefore, it may be interesting to assess the relationship between carbon stocks and other variables related soil conditions (pH, exchangeable cation content, base saturation) in order to investigate the role of soil environment in C storage dynamics, regarding species composition, to understand the mechanisms behind.

5. Interactions between carbon stocks and soil chemical properties.

The impact of the cations-related and acidity-related variables on carbon stocks and mechanisms behind will be discussed in this section.

The multivariate analyses (section 4.2.3.2 in results) associated the carbon stocks distributions with exchangeable aluminum, with the leaf litter turnover rate as an explanatory co-factor, but excluded exchangeable calcium. The relationship between exchangeable aluminum and C stock distribution is supported by the exploration of individual effect of each of those variables. In particular, the ratio between C stock in holorganic + 0-5 cm layers and total stock (R2) appears to have a highly strong positive correlation with the ratio of aluminum distribution in the profile. Considering that the variables measured in mineral soil layer 0-5 cm mirrored conditions in the forest floor, this result may confirm that an accumulation of organic C in forest floor or at a certain depth of mineral topsoil is associated with acidic conditions, represented here by the exchangeable aluminum content.

An uncertainty remains concerning the mechanisms under this correlation. At this stage, we do not know whether carbon accumulation is the cause of acidity parameters raising or vice versa. The relationship between these two variables is probably a positive feedback loop. High acidity level inhibits the decomposer communities' activities, which are negatively impacted by a low pH and high Al contents and acidic cations can make SOM less accessible for microbial decomposers (Hobbie et al. 2007). Consequently, SOM, and by consequences C stocks, accumulate in the forest floor or in the uppermost mineral soil layer (0-5 cm). On the other hand, the accumulation of SOM in forest floor is reported to be favorable to organic acid accumulation (Mueller et al. 2012) and to the acidification of the environment, especially under coniferous species with "poor" litterfall (Augusto et al. 2015), as previously.

Secondly, the analyses of linear regressions between C stocks' distribution

Even though exchangeable calcium-related variables were considered as non-significant in the multivariate regression, as compared to exchangeable aluminum, the simple regression models with calcium and C stocks showed some correlations. The ratio between Ca^{2+} and eCEC in the 0-5 cm layer is related to C stocks' distribution. The significancy level of these correlations is comparable as those between Al/eCEC in 0-5 cm and C stocks' distribution. However, this relationship might reflect the correlation which exists between C content and eCEC measured in 0-5 cm, more than a real effect of exchangeable calcium content on C distribution in soil profile. Besides, the exchangeable calcium distribution in soil profile appeared to have no impact on C stocks' distribution.

In literature, exchangeable calcium content in the forest floor and in the foliage is generally identified as a main explanatory factor for C storage in the forest floor (Reich et al. 2005; Hobbie et al. 2006). Our study has neither data about foliage calcium content, considered as an essential species identity trait (Reich et al. 2005), nor data about calcium content in the litter, but calcium content in 0-5 cm may represent calcium content in the forest floor. The mechanism explaining the relationship between Ca^{2+} content and C stock in forest floor is related to the presence of earthworms (Reich et al. 2005; Hobbie et al. 2006; Gurmesa et al. 2013; Angst et al. 2019). By fractioning litter and ingesting organic material in the forest floor, earthworms occupy two main functions. First, after a passage through earthworm gut, organic material is more accessible for microbial communities and the SOM mineralization is enhanced. Secondly, earthworms' presence increases the incorporation of organic carbon in the mineral soil. An enhanced activity of earthworms' community might therefore result in a decrease of the forest floor thickness and the carbon stocks in the forest floor. Considering earthworms activities are positively correlated with Ca^{2+} content of the leaf litter, concentration of Ca^{2+} in forest floor and uppermost soil layer (0-5 cm in our case) should be negatively correlated with carbon stocks in those soil compartments. Yet, our study doesn't support that hypothesis.

This may be explained by a hypothetical absence of earthworms in the study site regarding the acidic conditions in the surface environment. Temperate species of earthworm cannot tolerate a water-pH less than 3.5 and are scarce when water-pH values are inferior to 4.5 (Edwards 2004). In that case, neither Ca^{2+} content in forest floor nor earthworm activities have an impact on the carbon stocks in uppermost soil layers (forest floor and 0-5 cm mineral soil). When earthworms are absent from forest floor, the decomposition rate of litter is mostly affected by species traits related to foliage litter quality other than Ca^{2+}, such as the foliage lignin concentration for example (Mueller et al. 2012).

Moreover, a study in particular (Gurmesa et al. 2013) suggested that differences between the tree species effect on mineral carbon stocks among studies could be link to earthworm presence. Studies that observe higher carbon stocks in mineral topsoil for broadleaves than for coniferous species may be associated with a high earthworm diversity and activity, compared to studies where the carbon stocks in mineral topsoil of broadleaves are lower or equal to those of coniferous species. Earthworm activities are more enhanced under broadleaves than under conifers plots due to litter quality differences (Ca^{2+}), resulting in better C incorporation into mineral soil in broadleaved species. In sites with earthworm low activity, C fluxes between soil horizons do not differ between broadleaves and conifers. Hence, differences or absence of differences in terms of carbon stocks in mineral soil layer are only led by carbon stock accumulation in uppermost soil horizons (forest floor + 0-5 cm). Our study supports this last example.

In conclusion, the impact of tree species identity on carbon stocks is significant in the forest floor and 0-5 cm by differences between coniferous and broadleaved stands. These differences are related to litter quality differences between coniferous and broadleaved species. In absence of earthworms, foliage litter recalcitrancy is the dominant explanatory factor. Coniferous species are more likely to accumulate carbon stocks in the forest floor and 0-5 cm mineral soil layer because of a lower leaf litter turnover rate and it is correlated with higher Al^{3+} content in those organic and mineral soil layer.

If no differences in terms of stocks in mineral material between coniferous and broadleaved species, we can raise the following question: how current surface conditions, which present differences between conifers and broadleaves, influence the stability of carbon stored in mineral soil? Carbon is stored in mineral material in to different compartment: labile carbon pool and stable carbon pool. As coniferous species accumulate more carbon in the forest floor, more carbon is stored in the labile pool of large POM (Desie, Vancampenhout, et Heyens 2019). In the context of this current study, the stable carbon pool draws our interest. Carbon in stable pool is inaccessible for microbial respiration and is stored either by occlusion in soil aggregates (S + A fraction) or by chemical-binding with mineral material, like clay minerals or oxides (s+c fraction) (Desie, Vancampenhout, et Heyens 2019).

The occlusion of SOM in aggregates is enhanced by earthworm activity (see section State of the art). As earthworm activity is not considered in the current study because of a hypothetical low activity, we will not consider the carbon in S + A fraction. However, our results report an important impact of species category on the aluminum cation content. A high content of Al^{3+} in the 0-5 cm soil layer under coniferous species may be related to a higher proportion of SOM stabilized by binding with mineral clay (s+c). Yet, in the absence of evidence to prove this hypothesis, we are unable to affirm if the higher total SOC stocks in conifers are stored in stable or labile pool.

6. Management options

The assumption that accumulation of carbon is forest floor and 0-5 cm under coniferous stands is compensated for in broadleaved stands by higher stocks in mineral soil is not supported by our study. Moreover, the higher total stocks in case of coniferous species only mirrored the higher carbon stocks in forest floor. Consequently, based on current data we cannot claim whether or not broadleaved species planting is an adequate management option to increase carbon stocks in mineral topsoil.

We previously made the assumption that the high aluminum cation content in coniferous plots could potentially be related to a higher stability of the carbon stocks in the layer 0-5 cm by the complexation of SOM with mineral compounds. However, the important forest floor carbon stocks under coniferous species are less protected again rapid changes of the environment or disturbances such as forest fires. If that kind of event occurs, a huge amount of labile carbon could be mineralized, releasing a more important amount of carbon dioxide into the atmosphere compared to broadleaved species forest floor. Additionally, the high aluminum content under coniferous species may represent a risk of aluminum toxicity.

7. Cautions, restrictions and suggestions for further research

The results interpretation may have been restricted by the experimental design and must be considered with cautions. First, the experiment didn't have replicates for tree species and only one plot by species were considered, except for *Pseudotsuga menziesii* and *Fagus sylvatica*. I would have been interesting to investigate the variability between plots with same tree species to strengthen the analysis of tree species effect. Carbon stocks obtained for plot 4 and 9 with *Pseudotsuga menziesii* are significantly close for all depths. Similar results have also been observed for the two plots associated with *Fagus sylvatica*, except for a different of

5 Mg/ha for the carbon stocks in the soil layer 0-5 cm. Both species have also similar values of base saturation and pH in the two plots.

Secondly, we didn't take into account the edge effects. There was no evaluation of the proximity of established plots with stands from other tree species that could have an influence on litterfall. Then, a potential lack of precision in the measures may be possible due to lack of precision in the soil samples collecting process. There could be a bias linked to the person that collected the samples when that person had to distinguish and separate the organic material from the mineral soil. It is possible that the way the forest floor has been removed before soil sampling differs between different plots. Moreover, the material used to harvest the soil may also be a source of imprecision. The precision with which the exact defined volume of soil was collected depended on the soil moisture: if the soil was dry, it crumbled but if the soil was moist, it was easier to collect a complete cylinder. Soil lost during manipulation of samples is also a factor to take in account in the precision of the method. This may have induced a bias in the carbon stocks calculation. The season of litterfall harvesting compared to the moment when forest floor was collected may also impact the estimation of the leaf litter turnover rate. Finally, the interpretation of results may also be incorrect because we didn't consider the potential impact of human activity and forest management that disturbs the site environment.

For further research, it could be interesting to collect data of the chemical composition of foliage litter (in terms of calcium, lignin, carbon and nitrogen content). These data could be used as variables representing species identity and related to litter quality. It could also be useful to collect data about the quantity of carbon or SOM released by roots because roots are non-negligible underground carbon source. Furthermore, it would be interesting to have an estimation of the part of carbon in soil present as DOC, because this fraction of carbon stocks constitutes the most labile part. Another lack of data in this current study was the absence of information about earthworm populations and frequency of earthworm observation to confirm the assumption that earthworms do not play an important role here in the carbon dynamic. Finally, to take management decisions about tree species, it would be helpful to study the stability of carbon stored in the mineral soil.

Conclusion

In the context of this master thesis, the aim was to assess the species composition effect on SOC storage, in particular in mineral soil, for a certain soil type and in relationship with observed chemical soil properties. The first hypothesis concerning the homogeneity of site inherent conditions has been validated. As a result, cofounding factors could be neglected in the assessment of species impact.

As expected, the tree species effect on soil properties' parameters was by far less significant in the deepest mineral horizons of the topsoil than in the forest floor and the uppermost topsoil layer. The study also showed a dramatic decline of absolute SOC stock values between the forest floor and the uppermost topsoil layer and the mineral topsoil. The study revealed that coniferous forest floor is mostly associated with acidic pH, high exchangeable aluminum contents and low litter turnover rate. However, some exceptions to the general trend were observed (e.g., *Calocedrus deccurens*). A correlation between SOC stock and high level of exchangeable Al was also established.

The current study also confirmed that the SOC stocks in the forest floor and the uppermost topsoil layer are higher in conifers compared to broadleaves species, as commonly described in literature. The main goal of the current study was to provide significant findings about differences between broadleaves and conifers in terms of SOC stocks in mineral soils. As a result, species composition had no substantial effect on SOC stocks in the 5-20 cm topsoil layer. We made the assumption that this unexpected result might be related to acidic soil conditions, unfavorable for soil living communities, that could lead potentially to a low earthworm activity. In case this hypothesis is valid, this result may be in line with the following assumption reported by literature: conifers are more efficient to accumulate SOC than broadleaves species under unfavorable conditions. However, we have no consistent evidence to prove that the carbon accumulated under conifers stand will be stored in the long term in stable SOC pools.

For a better understanding of the tree species effect on C dynamic in soils in the study site, further investigation might associate the obtained SOC stocks with a study of the soil biological communities and foliar traits of different species.

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Tree species effects on soil organic carbon stocks and chemical properties of the topsoil in the arboretum of Tervuren

Abstract

In the context of climate changes, forest management can improve the long-term storage of soil organic carbon by tree species selection. In literature, it is often reported that coniferous species are more likely to store a high quantity of SOC in the forest floor compared to broadleaves species. However, there is a lack of data about the impact of species on SOC stocks in the mineral soil. In the context of this master thesis, soil samples were collected in the arboretum of Tervuren in stands of 22 different species. The aim of the study was to identify a potential difference of SOC stocks in mineral topsoil between broadleaves and conifers. A second objective was to determine in which soil conditions SOC stocks were higher, and to relate these soil chemical properties to species traits. The results show higher SOC stocks in the forest floor and in the uppermost topsoil layer in conifers, due to OM accumulation. Besides, a relationship is established between high carbon contents and high contents of exchangeable Al, and by extend acidic conditions. By contrast, we observed no substantial impact of species on SOC stocks in the mineral topsoil below 5 cm. An explanation to this unexpected observation may rely on acidic conditions in the topsoil. Indeed, we suggest that topsoil conditions in the study area are unfavorable for earthworm activity and that might have an impact on SOC fluxes between the forest floor and the mineral topsoil, independently from species effect. This hypothesis needs to be confirmed by further investigation. It may be in line with the assumption in literature that conifers are more efficient to accumulate SOC than broadleaves species under unfavorable conditions, but not necessary in SOC stable pools.

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