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Faculty of Forestry and Wood Sciences

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Title: Changes in the Potential for Cellulose Decomposition in Humus Forms After Harvesting or Decline of Norway Spruce Stands

DIPLOMA THESIS

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Tropical Forestry and Agroforestry

Thesis title

Changes of Potential for Cellulose Decomposition in Humus Forms after harvesting or decline of Norway Spruce Stands

Objectives of thesis

Changes of microbiological activity of forest soils are considered as one of the most important effects after forest stand harvesting or decline. Aim of the diploma thesis is represented by the evaluation of differences in the cellulolytic activity in the layers of humus forms (F+H, Ah) after Norway spruce harvesting and clear-cut origin.

Methodology

The research will be performed in laboratory conditions. The soil samples (holorganic layers, organomineral horizons – L+H, Ah) will be taken in standing forest stands and at the clear-cuts of 1 and 3-4 years after clear-cutting, in comparable site conditions. Four localities will be analyzed. The cellulolytic activity will be studied as soil organisms' occupation and decomposition of strips of cellulose (filter paper). Results should document the potential for cellulose decomposition at clear-cuts after Norway spruce harvesting, comparable also with forest decline in consequences. The research is topical considering the large-scale Norway spruce decline and following clear-cuts in the whole Central Europe.

- Review of literature – humus form formation, forest decline, bark beetle calamity – May-December 2021
- Laboratory experiment start – June 2021,
- Soil sampling in stable stands and at clear-cut of differentiated age: 1 and 3 – 4 years – June 2021, each variant including 3 plastic boxes with 10 cellulose strips 10x1 cm,
- Experiment evaluation in 2-week periods – June 2021 to January 2022, determination of color changes and total decomposition in % of the strip area,
- Final project evaluation – February 2022
- Thesis finalization – March 2022.

The proposed extent of the thesis

Min. 60 pages of text (Introduction to Conclusions)

Keywords

Forest harvesting, forest decline, bark-beetle calamity, soil changes, humus decomposition, cellulose decomposition

Recommended information sources

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Declaration

This thesis, titled "Changes in the Potential for Cellulose Decomposition in Humus Forms After Harvesting or Decline of Norway Spruce Stands," is my original work, and all sources have been cited and acknowledged recognized with complete references.

Prague, 12th May 2020

.....

Ernest Gyamfi.

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Abstract

Norway spruce is the most abundant and commonly utilized tree species for timber production in central Europe. In the Czech Republic this species accounts for about 50% of its forest composition which far exceeds the proportion in the natural forest conditions centuries ago. In recent times there has been a decline of Norway spruce stands under the current climatic change (extreme drought, bark beetle outbreak). The changed species composition from broadleaved dominated stands to more coniferous dominated stands affect soil dynamics including the decomposition and transformation of litter into different humus forms. In this study the cellulolytic potential of soil substrates were compared between Norway spruce and clear-cut area (2 years old after Norway spruce harvest) in the same stand and a broadleaved dominated stand in the same forest area. The experiment was performed in laboratory conditions by standard methods to determine the rate of decomposition and coloration of cellulose strips. The highest microbial activity or cellulolytic potential was observed in the broadleaved substrate followed by the clear-cut and the least activity was found in the substrate from the Norway spruce. The decomposition rate between horizons (F+H, Ah) were also compared and similar pattern was observed. There was more intense decomposition and coloration of the cellulose strips in the humus layer than the organomineral layer. Forest management should take into account the effect of species composition on soil properties as they have profound influence on humus formation and nutrient cycling.

Key words: litter decomposition, humus, species composition, microbial activity, soil horizons.

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1. Introduction

Litter decay is an important biological process in forest ecosystems. It plays an important role in the carbon and nutrient cycle. The nature and type of humus found in forest floors are important indicators of the condition, vitality, stability, and growth of forest stands. Litter decomposition is controlled by many factors including the quality of litter, environmental or climatic factors, microbial activity in the soil and soil chemical and physical properties. The most important limiting environmental factors are moisture and temperature dynamics. Decomposition and humification of litter of individual tree species may vary (e.g., Laganière et al. 2010; Marcos et al. 2010), the course of these processes in different habitats may be different (Meier et al. 2005) or modified in the case of mixed growth (Albers et al. 2004).

Norway spruce is one of the most important commercial tree species in forests across Europe. Norway spruce is often grown in even-aged mono-specific stands and managed under a clear cutting system (Slodiacak et al. 2005). Despite the economic importance of this tree species, it is known to accumulate large amounts of humus forms in its stands due to the slow rate decomposition of its litter. Many studies in temperate and boreal forest ecosystems have documented their effect on forest soils as negative (Němec 1928; Pelíšek 1964; Kulhavý 1997). The effects of growing spruce monocultures were observed by a group of forest scientists from the Czech Republic. They estimated humus reserves and forms, soil reaction and carbon/nitrogen (C/N) content of forest floor layers in spruce monocultures, beech and a mixture of spruce and beech stands in the region of Dražanská Upland between 2004 and 2006. They found the highest humus reserves of forest floor in the spruce stand (71.8 t/ha) and the lowest humus reserves were found in the beech stand (46.7 t/ha). The values of soil reaction also indicated higher acidity in the spruce monoculture than in the beech stands (Fabiánek et al. 2009a).

Generally, coniferous trees accumulate more carbon in the O horizons (forest floor) as compared to deciduous trees (Vesterdal & RaulundRasmussen 1998), but little is known about the impact of tree species on chemical composition and reactivity of dissolved organic carbon (DOC) in soil solutions, and the environmental consequences of planting either deciduous or coniferous species in large-scale afforestation programs. It is widely viewed that changes in forest above-ground

diversity and structure indirectly affect the soil microbial community and its functions. Plant litter decomposition is a key process in nutrient recycling and humus formation in forest ecosystems (Kubartová et al. 2009). Decomposition is generally considered to be faster in clear-cuts than in forests, owing to higher temperature and moisture conditions. Microbial activity in soil is also enhanced in warmer and moist conditions in clear-cuts (Edmonds and McColl, 1989; Frazer et al., 1990). Further, clear-cutting may have different influences on decomposition rates at different depths in the forest floor (Binkley 1984). However, slower decomposition has also been reported after clear-cutting in some ecosystems including montane sites on Vancouver Island (Blair & Crossley Jr 1988; Cortina & Vallejo 1994).

Humus formation or decay of organic matter under Norway spruce is known to be slow owing to the recalcitrant nature of its needles and adverse conditions of soil under its stands. Several studies have documented the rate of litter decay under Norway spruce monocultures but have not documented the potential for the decomposition of cellulose alone but almost always all organic components in general. This study seeks to compare the potential for cellulose decomposition in humus forms in spruce stands and after harvesting Norway spruce or after a drastic decline on sites that used to be occupied mainly by this species. Since cellulose is the most abundant polysaccharide synthesized by plants (Gomashe AV 2013) and the building blocks of all plant cell wall studying its rate of decomposition can be an important indicator of microbiological activity in soils.

2. Aims of the thesis

Changes of microbiological activity of forest soils are considered as one of the most important effects after forest stand harvesting or decline. Aim of the diploma thesis is to evaluate the differences in the cellulolytic activity in the layers of humus forms (F+H, Ah) after Norway spruce harvesting and clear-cut origin. The studies will compare cellulolytic activity between substrate from adjacent Norway spruce stand, the clear-cut area from which the Norway spruce was harvested and nearby broadleaved dominated stand.

3. Literature Review

3.1 Litter Decomposition

One of the biological processes essential to the functioning of forest ecosystems is the decomposition of organic waste. Litter is broken down through the processes of decomposition and mineralization, and the carbon and nutrients inside it are released into the forest floor, where they are available for plant uptake (Prescott et al. 2005). Slow decomposition rates result in the accumulation of organic matter and nutrient stocks in the soil; whereas, high decomposition rates assist plants achieve their nutritional needs (Isaac & Nair 2005). Climate, litter quality, and the nature and number of decomposing organisms are all factors that influence litter decomposition. In locations with unfavorable weather, climate is the most important element, whereas in areas with favorable weather, litter quality is the most important regulator. Due to its influence on humus formation, litter quality is nevertheless significant until late in the decomposition process (Berg et al. 1993). The composition and activities of the soil fauna have an impact on the rate and completeness of decomposition. Environmental factors mostly climatic, as well as the chemical and physical characteristics of the litter, influence the population of soil organisms present and their activity. Forestry activities can affect litter decomposition rates in forests by altering any of these parameters (Prescott et al. 2005).

3.2 Factors Affecting Litter Decomposition

Litter decomposition is made up of two processes: the mineralization and humification of lignin, cellulose, and other chemicals by microbes, and the leaching of soluble substances into the soil, where carbon and nitrogen are gradually mineralized. These methods are influenced by abiotic elements such as temperature and humidity, as well as biotic characteristics such as litter chemical composition and soil organisms. As a result, the physico-chemical environment, litter quality, and the composition of the decomposer community are three of the most important factors influencing litter decomposition (Krishna & Mohan, 2017).

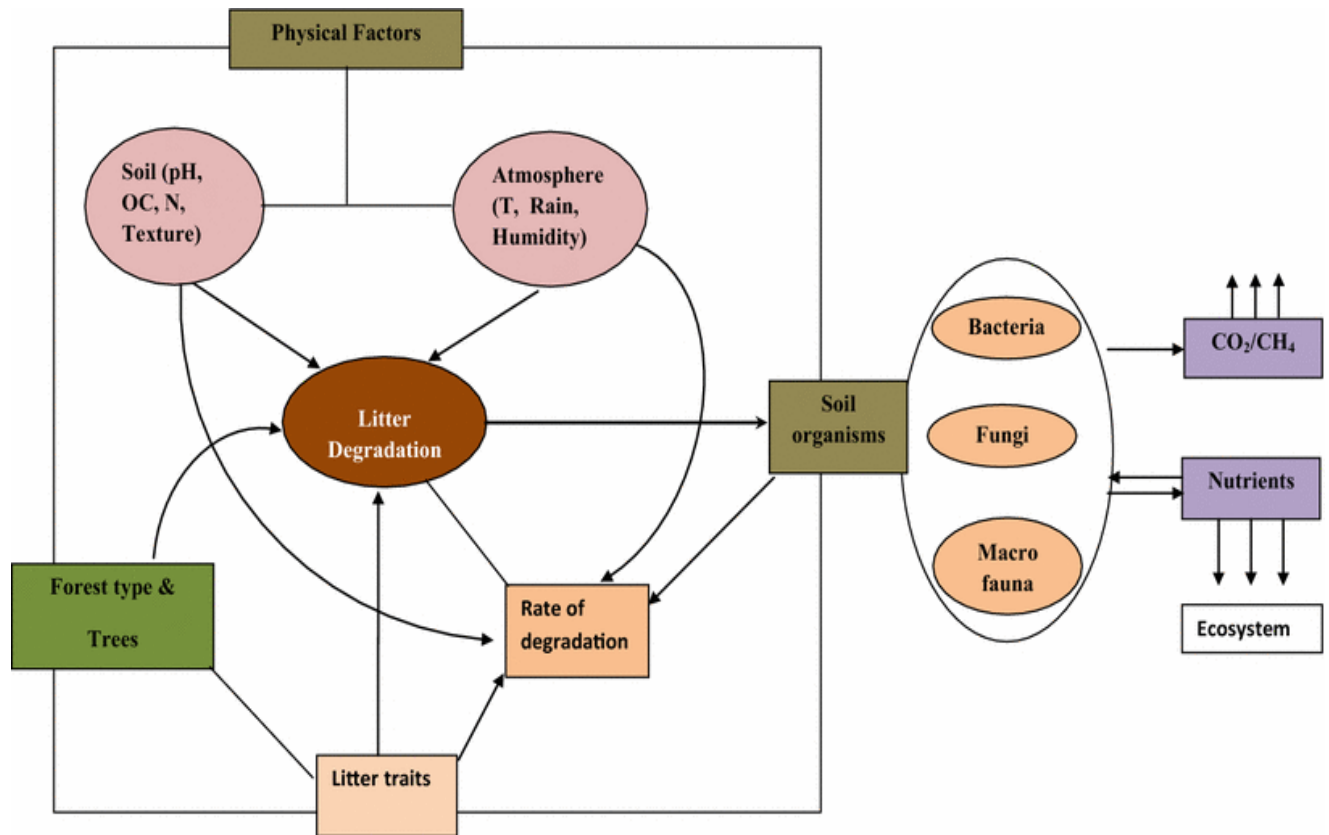


Figure 1. Diagram showing the factors affecting litter degradation (Krishna & Mohan, 2017)

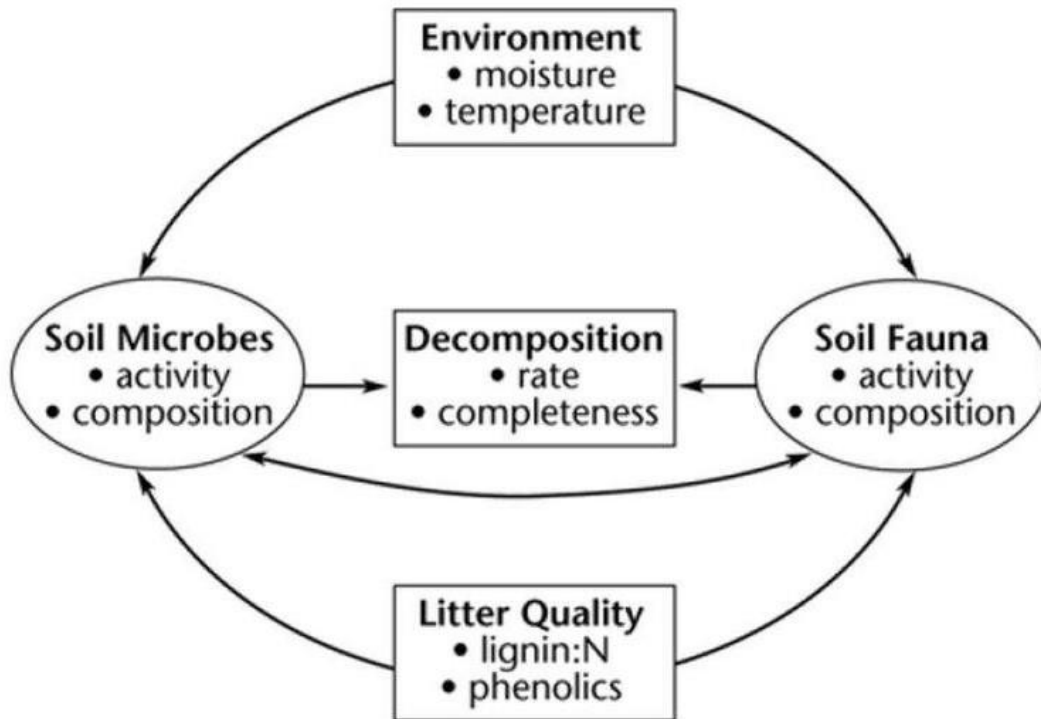


Figure 2. Factors that control the rate and completeness of decomposition (Prescott et al., 2000).

3.2.1 Environmental Factors Affecting Decomposition Rate

Temperature is a major determinant of litter decomposition rates (Hobbie 1992), and decomposition is more temperature sensitive than primary production (Kirschbaum 2000). With increasing soil temperature, microbial activity increases proportionally (Kirschbaum 1995). According to Augusto et al. (2002), microbial communities are primarily determined by environmental variables such as temperature, soil moisture, geology-bedrock, with secondary effects for tree species or litter.

3.2.2 Trees Species and Litter Quality

Changes in tree species in a temperate forest ecosystem, which is typically nutrient-poor (Fisher and Binkley 2000), can cause soil acidification and nutrient depletion by affecting nutrient uptake and storage, atmospheric pollutant interception rates, production of acidic organic matter, and mineralization, nitrification, and weathering rates (Binkley 1995; Binkley & Giardina 1998). As a result, plant species effects may be as important as or more essential than abiotic factors like climate in regulating nutrient cycling and ecosystem fertility (Hobbie 1992; Augusto et al. 2002; Mareschal et al. 2010). The majority of organic material in forest soil comes from vegetation that forms an organic layer (litter) on the soil surface and is partially distributed into the soil (Santa Regina & Tarazona 2001). The chemical composition of nitrogen, phosphorus, potassium, and major cell wall components including lignin, cellulose, and hemicelluloses, which influence litter breakdown and nutrient release, are used to determine the quality of plant litter. Lignin makes from 15–40% of total litter volume. Litter can include as little as 4% lignin or as much as 50% lignin, depending on the circumstances (Krishna & Mohan 2017).

Carbohydrates such as cellulose and hemicelluloses, in addition to lignin, are the most abundant elements in plant litter in terms of quantity. Among these, cellulose (which accounts for 10–50 % of the litter volume) is composed of glucose components linked by β -1-4 bonds to form long chains of molecules that are organized into fibers. Hemicelluloses are sugar polymers such as glucose, and the amount of these polymers varies amongst litter species (Akpor et al. 2005). Litter decomposition rates differ amongst species that degrade under the same ecological conditions (Cornelissen 1996). Different litter properties, such as leaf hardness, nitrogen, lignin, polyphenol concentrations, the C/N ratio, and the lignin/nitrogen ratio, cause these variances in decomposition (Berg et al. 1993; Pérez-Harguindeguy et al. 2000).

Nitrogen and lignin concentration of plant material are the most important factors in controlling decomposition rates among the other variables (Gartner & Cardon 2004). Litter characteristics can be utilized as predictors for decay rates between species because of the close relationship between litter quality and decomposition (Melillo et al. 1989). Due to the loss of easily accessible carbon and the accumulation of recalcitrant chemicals, litter quality often reduces during decomposition

(Gaudinski et al. 2000). Because broad-leaved litter covers more potassium and phosphorus, less lignin, and almost invariably less ether-soluble portions, coniferous tree leaves decompose more slowly than deciduous tree leaves (Gosz et al. 1973).

The overstory species has been proven in several studies to alter the bulk of the forest floor. For example, it was reported that, *Picea abies* has twice the litter weight of hardwood species like *Fagus sylvatica* (Calvaruso et al. 2011). Indeed, factors specific to tree species, such as hardness, shape, lignin/N ratio, foliage lifetime, and the concentration of hydrosoluble components, influence litter decomposition rates. The lignin/nitrogen and C/N ratios are known to be strongly correlated. According to other findings, litters with a low rate of decomposition have a greater C/N ratio than litters with a high rate of decomposition (Augusto et al. 2002). As a result, the tree layer composition affects the rate of litter decomposition significantly.

3.2.3 Soil Microbial Activity and Decomposition

The abundance and arrangement of soil fauna and microbial populations are known to affect the rate of litter breakdown at various stages of decomposition (Dilly et al. 2004). Among the soil microfauna, fungi are the leading decomposer and have more than 75% greater potential to reduce organic matter than other microorganisms (Kjoller & Struwe 1992). Furthermore, their activity will vary seasonally. Besides fungi, litter bacteria are a significant part of the process of organic matter mineralization and accounts for 25–30% of the total soil microbial biomass (Dilly et al. 2001). Leaf decomposition by fungi and bacteria tends to be rapid at nutrient-enriched conditions. The involvement of fungi and bacteria in leaf decomposition could react inversely to stress situations (Pascoal & Cássio 2004). Microbes can also be limited by soil moisture. As the temperature rises, soil moisture has a progressively more significant role in retaining high rates of microbial activity (Peterjohn et al. 1994). As a result, the rate of fresh litter decomposition rises with both increasing temperature and precipitation (Meentemeyer 1978). Even the natural disturbances and silvicultural treatments influence the microbial activities of the soils (Svoboda & Podrázský 2005). In our case, environmental conditions determined by local microclimate were the same and the soil moisture was similar too. The differences in the soil characteristics were, therefore, a result of different tree species (litter) effects dominantly (Podrázský et al. 2020).

3.2.4 Role of Soil Properties in Litter Decomposition

Soil physical and chemical characteristics have a significant role in litter decomposition. Among them, texture is the most significant as it stimulates nutrient and water dynamics, porosity, permeability, and surface area. The major chemical properties include pH, cation exchange capacity, organic matter content and nutrients (Coleman et al. 1999). The organic matter, which influences the different physicochemical factors like bulk density, pH, is the major soil property affecting litter decomposition (Cuevas and Medina 1986). The organic matter can also increase the population density of soil macro-organisms, which plays a significant role in litter mixing and decomposition (Akpoy et al. 2006). Among the mineral nutrients, soil nitrogen status is deliberated as being the primary regulating factor and has received utmost attention, while phosphorous is usually considered as a limiting nutrient because of the low quantity in circulation in major forests. Calcium, nitrogen, and phosphorus are rapidly mineralized in litter (takes several weeks/months), but organic complexes in the soil organic matter pools have much slower turnover times, taking several years or decades (Devi and Yadava 2007). However, while considering an entire decay process, the effects of added nitrogen on the rate of decomposition seem to be irrelevant and can even turn out to be contrary (Krishna & Mohan 2017a).

Table 1. Different techniques for litter decomposition experiments

Methods used for evaluating litter decomposition	Output	Drawbacks
Mass balance technique	Evaluate litter decay, check on model forecasts	Evaluate litter decay, check on model forecasts
Litter bag technique	Decay at the soil surface	Large mesh size cause mineral loss to the soil and the entry of macrofauna into the bag
Tethered leaves technique	Learning the initial phases of decay	It does not used for microbial litter decomposition; because it allows the entry of macrofauna, whose contact would then controlled by mesh bags
Cohort layered screen technique	Long lasting litter decay studies (more than 3 years)	Fiberglass screen is suggested over aluminum if any chemical or essential properties will be evaluated as well

(Karberg et al. 2008)

3.2.5 Carbon/Nitrogen(C/N) Ratio of the Plant Litter and Decomposition

Reports show that leaf litter decomposition can be calculated from the carbon/nitrogen (C/N) ratio (Melillo et al. 1982). High-quality leaves (nutrient-enriched leaves) will generally decompose more rapidly than low-quality leaves (nutrient-deficient leaves). In general, the decomposition rate is high in species with extreme ash and nitrogen contents and minimum C/N ratios and lignin contents (Singh 1969). Several works showed that the nitrogen concentration of the litter and the C/N ratio is strongly associated with litter decay rates (Berg and Staaf 1981). The concentration of phosphorous and carbon/phosphorus (C/P) ratios appeared to be good predictors of decay rates (Vitousek et al. 1994). Concentrations of lignin and the lignin/nitrogen ratio in plant litter are also good predictors of litter decomposition. These factors and their effects on litter decomposition depend on soil characteristics and plant species (Krishna & Mohan 2017).

3.3 Impact of Clear-cutting on Decomposition Rate

Understanding the impacts of forest practices such as clear-cutting on decomposition processes of organic matter is crucial for the management of post harvested sites. This is because these forest practices often lead to significant loss of nutrients such as nitrogen (N), which can limit soil productivity (Bormann et al. 1974). Effects of clear-cutting on leaf litter decomposition have been examined in a range of deciduous and evergreen broad-leaved forests and coniferous forests. Some studies showed faster decomposition in clear-cut sites than in uncut sites (Klemmedson et al. 1985), whereas others reported either no significant difference (Prescott et al. 2000; Wallace & Freedman 1986) or slower decomposition in clear-cut sites (Prescott 1997; Yin et al. 1989). On the other hand, Zhang and Liang (1995) reported slower decomposition of whole litter and lignin in large gaps (diameter of more than 15 m) than in small gaps (diameter of less than 5 m) in a subtropical forest. Some authors also showed that N release from decomposing leaf litter was enhanced within clear-cut sites compared with uncut sites (Ishikawa et al. 2007).

After forests are clear-cut, increased N availability is frequently recorded, which has been linked to faster decomposition and mineralization of remaining organic matter. The warmer, moister conditions in clear-cuts have been linked to increased microbial activity. However, depending on the regional climate, studies have found that decomposition rates in clear-cuts might be quicker,

slower, or the same as in forests (Yin et al. 1989). Furthermore, at different depths in the forest floor, clear-cutting may have varying effects on decomposition rates (Binkley 1984). Binkley (1984) discovered that cellulose strips incubated in humus decomposed faster in a clear-cut than in a neighboring uncut forest in another coastal montane forest on Vancouver Island. This means that, even if the site's decomposition potential improves, the forest floor will continue to disintegrate slowly due to the nature of the material (Prescott 1997).

In addition, the influence of clean cutting on soil temperature was investigated in another study. The data was collected during a six-year period in the study. The temperature of the soil before and after cutting was compared, as well as between nearby clear-cut and control sites. Soil temperatures at 0.5–3.0m depths increased in the following way because of forest clear-cutting. The temperature of the soil has risen by 2.2–1.4°C. The maximum temperatures in the soil rose by 3.2–1.8°C. The minimum temperature of the soil increased by 0.6–0.2°C. Temperatures climbed by 3.0–1.4°C on a yearly basis. Soil temperatures rose after clear-cutting, owing to insolation, particularly in the summer. On the other hand heat is more easily released from the earth in the cool season (Hashimoto & Suzuki 2004).

3.4 Cellulose in Plant Litter

Cellulose in the plant fiber is organized in a crystal-like form that makes it hard to attack. Cellulose is decomposed using extracellular enzymes by both bacteria and fungi. It is first degraded to monomers, or oligomers of a rare glucose unit, such as cellobiose, which can be engaged into the microbial cell and metabolized (Johansson 1994). Various organisms are capable of degrading the more amorphous kind of cellulose (Eriksson et al. 2012).

It is known thought that cellulolytic potential is limited to certain bacteria groups (Lynd et al. 2002) but this ability is thought to be shared by a variety of fungi and that mostly fungal species dominate the later stages of cellulose degradation in soils (Boer et al. 2005). In plant litter cellulose is the most abundant polysaccharide (Štursová et al. 2012) and hence its decomposition plays an important role in the carbon cycle. In laboratory experiments to evaluate cellulose decomposition in plant litter the cellulose filter method is effective because of the substrate's homogeneity which helps to eliminate differences in the chemical composition of the plant material, a factor that

influences the rate of decomposition. Cellulose filter method is useful because of the homogeneity of the substrate, which helps to exclude differences connected with the chemical composition of the plant material, a factor that affects the decomposition rate (Wasak 2014).

3.5 Humus Forms in Forest Ecosystems

The fertility, the quantity of mineral nutrients, and the physicochemical qualities of soils are all influenced by humus (Rusanov & Anilova 2009). Humus is primarily made up of resistant decomposition products. The substance is partially degraded and chemically stabilized by the time it forms humus. The process of humus creation is not well understood however it is thought to involve microbial alteration of lignin and protein condensation into humus precursors followed by their complexing into humus molecules. The chemical nature of humus is very stable, despite individual humus molecules being quite varied. Humus is low in cellulose and hemicellulose, abundant in big polyphenolic compounds and high in nitrogen when compared to the original plant material (Prescott et al. 2000). The humus form corresponds to the succession of organic (OL, OF, OH, H) and underlying organo-mineral horizons and is the part of the topsoil that is heavily influenced by organic materials (A, AE, Aa). Plant remnants such as leaves, needles, wood, root exudates, and other plant remnants play an important role in forest ecosystems' primary production (A. Zanella & B. Jabiol 2004).

3.5.1 The Humus Form Classification

The sequence and morphological features of organic and organo-mineral horizons observed and documented in the field are used to classify humus forms. These criteria include morphological indications of biological activity. Depending on the forest type, the type, and the rate of decomposition of the various organic components, as well as the assimilation of organic matter into mineral layers differ (Jabiol et al. 2005). In the late 1980s, soil scientists in Europe examined organic matter decomposition in numerous nations, resulting in the classification of three humus types: Mull, Mor, and Mullartiger Torf. The climatic, geological, and biological conditions under which these humus formations formed in Danish beech woods were used to characterize them. It was clear to them that the humus form corresponds to the activity of living organisms within the

topsoil. Many writers contributed to the creation of a humus form classification system based on the importance of living topsoil components (A. Zanella & B. Jabiol 2004).

3.5.2 Humus Formation

Incomplete decomposition results in the formation of humus in an ecosystem. Part of the litter that enters the forest floor is not totally destroyed, but is transformed into humus, which decomposes slowly and accumulates under less favorable conditions for decomposition. To comprehend the formation of humus layers we must first study the decomposition process and the factors that influence its speed and completeness (Prescott et al. 2000). Litter decomposition is often thought to take place in two stages. There is a quick loss of soluble and a slow loss of cellulose in the early stages. Lignin, an insoluble degradation product, is only slightly lost. Carbon is relatively abundant in the early phase, but nutrients are scarce, and the limiting nutrient which is mostly nitrogen is immobilized. When the litter reaches the second stage, it can be classified as humus since the content has stabilized and the degradation of all components has slowed. The beginning of the late stage of decomposition is accompanied by net lignin loss and net nitrogen mineralization (Melillo et al. 1989). How much of the original litter mass remains at the stage where the litter becomes humus and decomposition slows determines whether or not humus will build up on a site. Very little humus will accumulate if most or all of the litter has decomposed by this time but if there is still some litter, humus will form and accumulate (Prescott et al. 2000).

The surface soil, which is recognized as the interface between living organisms and geological deposits, is where humus is formed primarily. Humus is a complex mixture of organic molecules that consists mostly of carbon, oxygen, hydrogen and nitrogen, with organic carbon accounting for 40-60% of the total weight (Andreux 1996). Many hypotheses have been formulated to describe how humus is formed, but none of them adequately explains all the processes involved. The polyphenol theory is the most popular. Varadachari and Ghosh proposed a humus formation theory that integrated existing theories of humus formation. He mentioned that lignin or carbohydrates are the main sources of carbon for soil microorganisms such as fungi and bacteria and serve as precursors for humus formation. Extracellular enzymes break these down into smaller components first. Soluble components are absorbed by microbial cells, and some are transformed into phenols

or quinones. These transformed products together with oxidizing enzymes, are released into the environment where they polymerize via a free radical mechanism to produce various sized humus molecules (Varadachari & Ghosh 1984).

Different species flourish and thrive in different life cycles as a result of substrate quality and abiotic conditions (Wall et al. 2008). Prior to faunal attack, breakdown of low-palatability litter often necessitates pre-conditioning (e.g., bleaching and softening) by white-rot fungi (Daniel et al. 1997; Hafidi et al. 1998). Plant polymers can solubilize phosphorous minerals, and fungal enzymes can breakdown them (Tagger et al. 2008). The rate of decomposition is first influenced by litter quality, and only then, as the litter becomes more edible, does soil fauna play a more important part in the second stage of the decay process (Mori et al. 2009). This supports previous findings that litter decomposition rates are more strongly related to which soil fauna groups are involved and how they succeed each other (e.g., pre-conditioning of litter by bacteria and fungi followed by penetration by microfauna and mesofauna) than to the chemical nature of the litter (Ponge 1991). Microbial attack of litter is also increased by macrofauna digesting, excretion, and enrichment activities. Figure 3 depicts the various theories and mechanisms associated with the formation of humus and humic compounds.

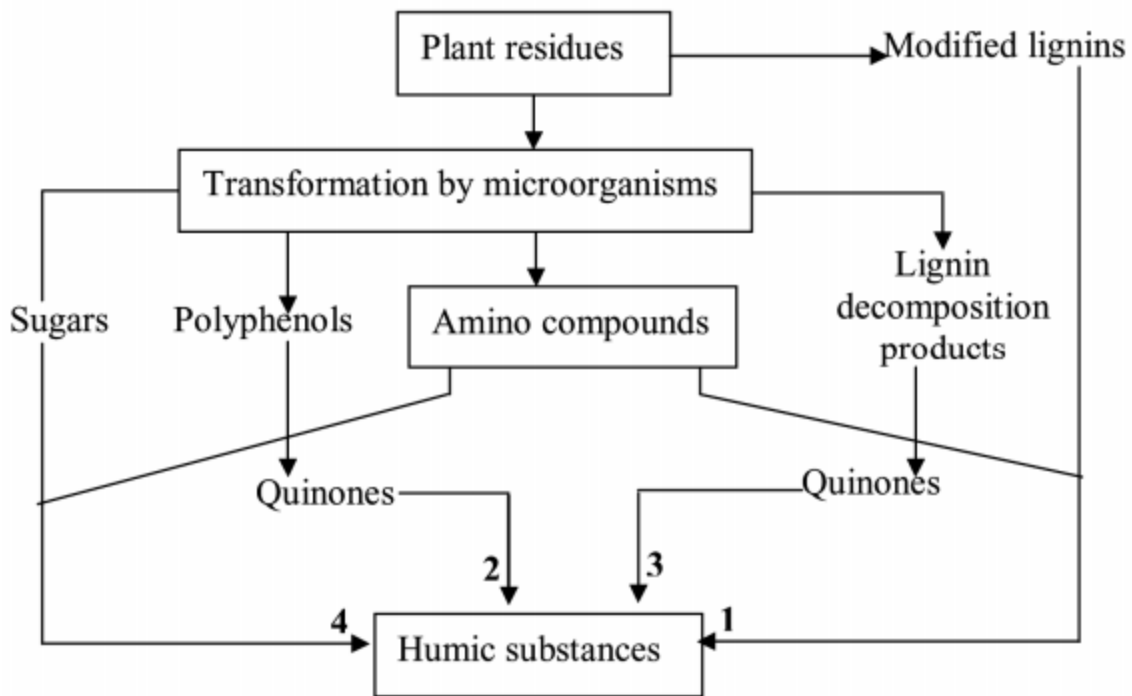


Figure 3. Illustration for the mechanisms involved in the formation of humic substances (Stevenson 1982). (1) theory of Lignin, (2, 3) theory of polyphenols and (4) theory of amino-saccharidic condensation.

3.5.3 Composition of Humus Forms

Humus has a carbon content of about 58% and a nitrogen content of 3 to 6 %, resulting in a C:N ratio of 10–20 (Luo & Zhou 2006). Humic and non-humic components make up soil organic matter (SOM). The non-humic components are unidentifiable plant, animal, and microbial organic wastes. They form about 20% of SOM in most cases. The remaining 80% or more of SOM is made up of humic compounds (i.e., humus), which are created by secondary synthesis reactions. Microorganisms create additional chemicals as litter undergoes metabolic changes, some of which polymerize or condense through chemical or enzymatic reactions. Enzymatic or auto oxidative polymerization processes involving phenolic chemicals believed to be a fundamental mechanism for humus formation (Luo & Zhou 2006).

3.5.4 Factors Controlling the Formation of Humus

The climatic factors; the input of plant residues to the soil; their volume, source, chemical composition; the soil moisture and aeration conditions; the acidity and redox regimes; the microbiological activity, mineralogy, particle-size distribution, and structure of the soil are the major factors regulating the rate and depth of humification process (Aleksandrova 1980).

3.6 Effect of Spruce on Humus Conditions of Forest Soils

The condition and type of humus in forest management are two important aspects that influence the health and growth of forest stands. This phenomenon was brought to the attention of eminent forest pedologists in the previous century. The function of the forest floor within the soil profile, where plant and animal matter decompose and nutrients are released into the soil environment, can be critical. Humification causes the differentiation of forest floor horizons - forest litter, mull, and detritus – where the specific processes of decomposition, mineralization, and humification take place. Decomposition of soil biota and organic excrements occurs in the organo-mineral horizon. Furthermore, surface-derived humus compounds reach there (Samec & Formánek 2007). Chemical and physical features of forest floor layers and the organo-mineral horizon have a significant impact on site trophic qualities and forest ecosystem biodiversity. Thus, we may influence the condition and qualities of the soil environment by changing the composition of forest stands and how they are grown. Plant communities at a given site are directly influenced by the quality of the soil environment. The issue of allochthonous spruce (*Picea abies*) stands grown outside of their natural range has been explored in recent decades. Many authors have mentioned detrimental effects on the soil environment (Fabiánek et al. 2009; Kulhavý 1997; Němec 1928). Even-aged spruce monocultures' humus is known to cause soil acidification. When Ulrich (1983) explained possible harm to forest stands owing to acidity of soils, this phenomenon was first identified and labeled as new forest decline. However, this idea had opponents such as Šály (1978), who regarded acidification as a natural process that cannot be separated from the rest of the soil-forming processes and argued that it was impossible to attribute an absolute effect to the process (Fabiánek et al. 2009).

3.7 Tree Species Composition in European Forests

The expansion of human societies has frequently resulted in the overexploitation of forests and a reduction in their area. During the 18th and 19th centuries, Europe's forest cover was reduced drastically. Policies of afforestation and increased wood production have been imposed from the second part of the 19th century. The planting of huge areas of productive coniferous tree species has been a key feature of these initiatives. Forests of natural deciduous species have been replaced in certain cases by coniferous plantations. The widespread usage of coniferous species has changed the average composition of the temperate forest in western Europe (Rousseau 1990). Norway spruce and Scot's pine are two examples of coniferous species that have been translocated within Europe. Others were brought in from the United States of America (for example Sitka spruce and Douglas fir). In a number of Western European countries, tree species replacement has provoked heated discussion.

Several research on the influence of overstory species composition on forest ecosystems have resulted from these talks. For a long time, the existence of an overstory species effect on soils has been known (Dokuchaev 1995) and observed by various writers. Nonetheless, depending on the researcher, the severity of the species effect is measured in very varied or even contradictory ways. According to Stone and van Goor, the impact of canopy species on soil fertility is insignificant when compared to the effects of soil and forest management (Stone 1975; van Goor 1985). On the other hand, in studies of peatbogs and artificial soils the tree cover composition can be one of the most important factors dictating the features and long-term evolution of forest soils at least for topsoil. Variations in the soils of some of the study locations explain some of the inconsistencies in the results regarding the effect of tree species (Augusto et al. 2002).

3.7.1 Forest Decline in Central European Forests

Given the history of central European forests, it is reasonable to assume that they will be ecologically unstable. Aside from the fact that they are almost all planted or at least managed forests, the majority of them have a long and exciting history dating back centuries (Schimitschek 1969). Many years of land clearing and usage for agriculture, exploitation for the mining industry, woodland grazing, and constructions of various kinds have resulted in the destruction of natural

forests across vast areas of land, permanently deteriorating the majority of the remaining forest sites. The current state of many forest sites demonstrates the catastrophic consequences: soil degradation and compaction, and nutrient exhaustion. The majority of the forest land in densely populated central Europe is relegated to areas that are unsuitable for agricultural development. When the energy shortage became a severe issue in the 19th century, a surge in reforestation effort occurred.

Where deciduous forests once existed, fast-growing conifer species were planted. Nobody cared about where seeds came from. There may be rare exceptions, but the current tree populations' genetic adaptation to site conditions is more than uncertain. Recent forest management methods and wildlife management customs may be contributing to the decline in forest vitality. Most forest ecosystems in central Europe can be regarded severely unstable from an ecological standpoint. Because of the relatively short, and gradually shortened rotation periods in which trees are harvested before early loss of vigor, this issue which is well known to forest ecologists and pathologists went largely undetected. The fast and widespread forest decline in the late 1970s and early 1980s, precipitated by a period of extreme climatic conditions combined with increased air pollution should be seen as a proof of how near central European forests have come to the stress tolerance threshold (Führer 1990).

3.7.2 Decline and Substitution of Norway Spruce

In recent decades, forest reductions in Norway spruce have been drastic, resulting in massive salvage logging. Spruce has started to be replaced in Central Europe, primarily by European beech, as conifer forests are being converted to more natural mixed forests. Climate change, which is commonly addressed, has a significant impact on both species' survival. The Norway spruce (*Picea abies*) and European beech (*Fagus sylvatica*) are two of the most common and economically important species in European forests (Kolář et al. 2017). Norway spruce has been planted well outside its natural range in recent decades, especially in Central Europe, as a fast-growing softwood species (Spiecker 2003).

These constraints might be stated as ecological and growth maxima in forestry. In natural biocenosis, the ecological optimum is a combination of site and climatic conditions that are optimal

for tree species development, existence, and reproduction. The growth optimum is described as the conditions in which a tree species can produce the most wood and have the best quality wood. (Plíva 2000). Natural disturbances such as storms, droughts, and insects have been shown to reduce the resistance of such spruce monocultures (Griess et al. 2012). As a result, even-aged spruce monocultures have been changed to more stable mixed uneven-aged forests, resulting in a higher number of deciduous trees dominated by European beech (Pretzsch et al. 2014). European beech is being planted in Central European areas affected by spruce decline because of its high reproductive and production potential, as well as its relatively wide ecological significance (Ammer et al. 2008).

3.7.3 Effect of Climate Change on European Forests

Climate change, with an increasing incidence of extreme events and changes in temperature and precipitation patterns, is posing new challenges for forest management. Droughts have been predicted to become more frequent, as summers are becoming increasingly warmer and drier, with shifts in seasonality of precipitation (David et al. 2000). In southern Europe, summer droughts have already doubled in intensity and length over recent decades (Zhongming et al. 2012), resulting in growth depressions and increased mortality rates of numerous tree species. Changes are also occurring in seemingly healthy forests (Köhl et al. 2010), which could substantially change species-specific growth potentials and stand composition. To mitigate the effect of changes in climatic patterns on forest ecosystems, there is an urgent need for adaptation strategies to maintain sustainable timber production and important ecosystem services like carbon storage (Albert et al. 2015). Climatic tolerance of tree species and their resilience to extreme events, such as droughts, are two of the main variables used to assess forest vitality (Bolte et al. 2009; Lindner et al. 2010). However, our understanding of the processes that drive species-specific responses to seasonal climate and drought events is still limited. To comprehend biological responses to environmental variability, tree rings have been increasingly used as proxies to assess the effect of climate variables. The annual resolution of tree rings allows for the assessment of the direct effect of stress and disturbances on tree growth, often recorded as narrow rings in the case of droughts. Drought effects have been widely studied and response-indices have been proven to provide important insights into the capability of species to cope with extreme events (Vitali et al. 2017).

The effects of climate change-induced rapid warming and prolonged droughts are increasingly impacting forests in Europe (Stott 2016). Economically valuable tree species have been largely favored and planted beyond their natural (realized) ecological niche but are now under strong pressure (Conedera et al. 2017). For example, drier and warmer climatic conditions increase the vulnerability of Norway spruce (*Picea abies* (L.) Karst.) to bark beetle outbreaks in central Europe (Jakoby et al. 2015), whereas sweet chestnut (*Castanea sativa* Mill.) in southern Europe is suffering from repeated summer drought (Conedera et al. 2010). Silver fir is a key species in Europe for social, economic, and ecological aspects. It is an ecologically valuable species in forests that contributes to recreation landscape and biodiversity and provides protection against gravitational hazards and erosion in mountainous regions (Mauri et al. 2016).

3.7.4 Substitution of Norway Spruce by Drought Tolerant Species

Drought susceptibility and accompanying distributional range shifts in spruce could result in significant economic losses. Norway spruce makes up a larger percentage of the planted species in many European forests. For example, spruce currently accounts for 41% of total forest cover in BadenWurtemberg (Hanewinkel et al. 2013), and around 50% in the Czech Republic. As a result, alternative conifer species like the native silver fir (*Abies alba*) and the exotic yet extremely productive Douglas fir (*Pseudotsuga menziesii*) should be explored for forest stabilization. Compared to Norway spruce, both species have shown to be more drought resistant and resilient (Bouriaud & Popa 2009; Nothdurft et al. 2012). The responses of these three conifer species to two major drought years in southwest Germany (2003 and 1976) confirmed silver fir and Douglas fir's superior drought resistance than Norway spruce (Vitali et al. 2017).

The European beech (*Fagus sylvatica*) is the most competitive tree species in Western and Central Europe, as well as in mountain areas of Eastern and Southern Europe, due to its high shade tolerance and growth capacity, as well as its wide climatic and geological amplitude and would naturally dominate far more than half of the forests (Walentowski et al. 2004; Ammer et al. 2005). As a result, European beech has become the most essential tree species to stabilize ecosystems against expected climate change risks in new silvicultural concepts seeking to replace non-natural pure conifer stands with mixed forests (Ammer et al. 2005). With the exception of the Beech Bark

Disease (BBD), *Fagus sylvatica* was once thought to be resistant to pathogens and insect infestations (Weisgerber et al. 2006). However, over the last decade particularly following the wet spring and autumn of 2002 and the severe drought of 2003, an increasing number of European beech trees and stands in Central Europe have declined, displaying symptoms typical of Phytophthora diseases such as small, sparse, and often yellowish leaves, crown dieback, root and collar rot, and aerial bleeding cankers of stems (Jung et al. 2005). Therefore, there is the need to research into species that will be more tolerant and resilient to the current climate change and disease.

In Central European forestry, Norway spruce is one of the most economically valuable tree species. Its extreme susceptibility to droughts, on the other hand, presents a serious challenge to its cultivation in the future, with more frequent and longer droughts and seasonal climate variations likely. More drought-tolerant species are needed to compensate for anticipated losses of forest areas suited for spruce cultivation. When compared to Norway spruce, silver fir and Douglas fir are two viable options that offer lower drought susceptibility and comparable or even higher yield. We can evaluate the impacts of seasonal climate change, including drought stress, on tree-ring width formation of these three commercially important conifer species during the last 60 years using the Black Forest as a regional case study (Vitali et al. 2018).

Furthermore, until 2100, we may forecast probable species-specific growth variations under various climate change scenarios. The study's findings show that in a warmer future climate, both silver and Douglas fir would likely grow more, as projected by the 4.5 and 8.5 Representative Concentration Pathway (RCP) climate change scenarios, and spruce growth will likely diminish. Furthermore, drought susceptibility in silver fir and Douglas fir is lower than in spruce as seen by past drought occurrences and their ability to benefit from milder winters and springs may play a significant part in their ability to accommodate drier summers in the near to mid-term future. This work emphasizes the importance of improving our understanding of the mechanisms that promote drought resistance and resilience in tree species so that management options in the face of climate change can be developed (Vitali et al. 2018).

On the one hand, palaeoecological studies show that silver fir was widely dispersed in a considerably warmer climate, indicating that it has a great ability to adapt to current and future climate change. Species distribution models, on the other hand, anticipate that its climatic appropriateness will deteriorate dramatically in the future. Many academics debate whether this species has the ability to thrive in central and western Europe as a result of expected climate change, based on research from various fields. Some authors say that silver fir has a high potential to survive under warmer circumstances in western and central Europe if there is enough rainfall by 2100, as predicted by climate projections for most places. Dendroecological studies, for example, show that silver fir is more drought resistant and resilient than co-occurring species like Norway spruce, European beech, and larch. Today, the most significant barrier to raising the proportion of fir in mixed forests is ungulate grazing, which frequently limits its growth (Vitasse et al. 2019).

4.0 Material and Methods

The research was performed in laboratory conditions. The cellulolytic activity of substrates was compared in the horizons F+H (holorganic layer) and A (organomineral horizon) at three locations: (1) standing forest stand, (2) clear-cut (2 years age) in the same stand and (3) neighboring broad-leaved stand at the same site.

4.1 Location of Forest Stands

The studied sites are in the stand 118 B7c at the territory of the University Forest Kostelec and Černými lesy. There were three sampling locations:

- Spruce stand aged 76 years (2020), forest type is determined as 3S1, medium-rich oak-beech site (*Querceto fagetum acidophilum* – Viewegh, 2005), soil type Luvisol, altitude is 420 m a.s.l., mean year temperature 8.5°C, mean year precipitation 550–650 mm (*e.g.*, Mondek, Baláš, 2019),
- 2 years old clear-cut in the same stand.
- Mixed broad-leaved stand (oak with hornbeam and lime) 76 years old (2020), site and soil type are comparable. Location of the experimental plot is presented in the Figure 4 below.

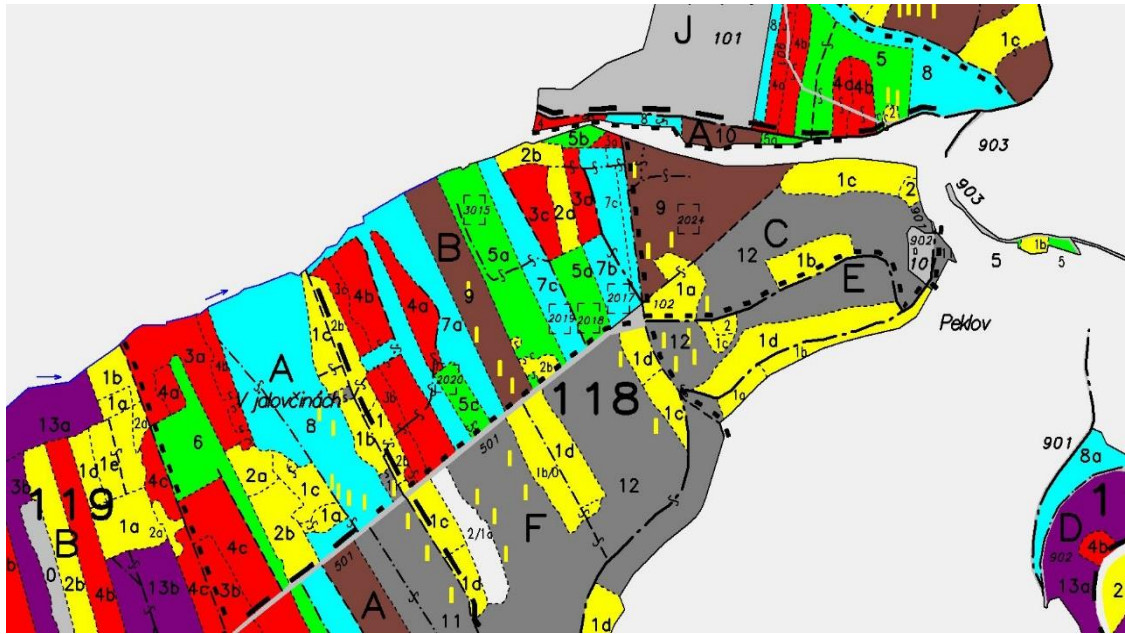


Figure 4. Location of the sampling plots – stand 118B7c



Figure 5. Spruce dominated stand from which substrate was taken



Figure 6. Photo showing clear cut area left bare for 2 years



Figure 7. Photo showing the beech dominated stand from which soil substrate was taken

4.2 Sampling of Soil Substrates from Forest Stands

Samples of holorganic layer (F+H horizon) and organomineral (Ah) horizons were taken at these three localities. At each locality, 5 samples of both horizons were sampled using garden shovel and placed in a mixed or bulk sample. So altogether 6 separate bulk samples were created and transported in the laboratory at the station TRuba near Kostelec and Černými lesy town. Sampling was done on May 19th, 2020.



Figure 8. Clear cut area from which soil substrate was taken

4.3 Establishment and Description of the Experiment

In laboratory conditions, an experiment was established to compare the cellulolytic potential of humus forms in different stand parts with the dominance of Norway spruce, clear-cut and broadleaved species. From each mixed sample, 3 samples of substrate were separated for each horizon (3 boxes for humus layer and 3 for organomineral layer at each location), i.e., 6 boxes for each of the three categories, which were spread on the bottom of the plastic boxes in a layer of 2 cm and their surface was leveled (Figure. 9).

10 strips of cellulose filter paper (80 g/m²) were placed on the surface in each box, divided by a 1 cm pencil drawing. The strips were also 1 cm wide, so that the individual area evaluated was the size of a 1x1 cm square. Thus, a total of 100 individual squares were evaluated in each box. The procedure was evaluated in 3 different time periods.

- color changes of the filter paper, indicating attack of the substrate by cellulolytic organisms,
- gradual and complete decomposition of filter paper, i.e., its absence (Figure.11).

The samples were cultivated in laboratory room conditions with comparable humidity (closing the boxes allows to assume 100% humidity in the boxes and about 20 °C) as shown in figure 10. The samples were spray irrigated at weekly intervals to maintain full moisture.

The samples were evaluated as follows:

- 19.5.2021 - sampling, setting up the experiment, installation in boxes

It took three weeks from the start of the experiment for the strips to begin to color. The experiment was observed, and data recorded for the first time 53 days after the initial set up. The second period of data collection and observation was done 100 days after the initial set up and the last data collection was done 150 days after the setup of the experiment.

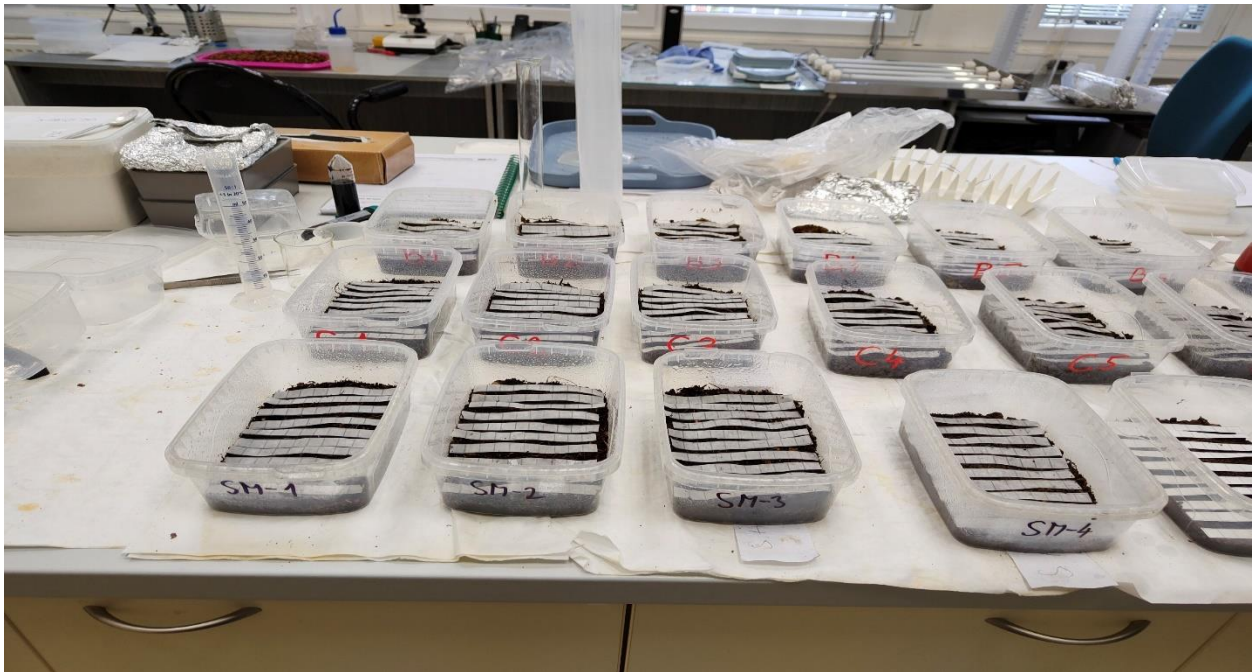


Figure 9. View of the experimental set up on the day of establishment.



Figure 10. Experimental boxes made airtight to assume 100% humidity



Figure 11. Photo showing signs of coloration and decomposition



Figure 12. Photo showing complete decomposition of cellulose strips

4.4 Data Processing and Evaluation

Data was evaluated using SPSS statistical package and Excel. Statistical analysis mainly done with SPSS and charts drawn using Excel. Simple descriptive statistics such as mean, and box and whisker chart were used to summarize the data. The box and whisker charts were used because we have a dataset series for the three soil categories. In this study the Kruskal-Wallis's test was used to analyze the significant difference between color and decomposition rate among spruce, broadleaves, and clear cut. The Kruskal-Wallis's test is nonparametric, which is equivalent to a one-way ANOVA. This test extends the Wilcoxon-Mann-Whitney two-sample test for more than two independent samples. The observations in each category or group come from populations with the same distribution shape, and that the sample is independent and randomly selected. The test statistic for the analysis of one-way variance is calculated based on the ratio of the sum of squares of the treatment to the residual sum of squares. The test uses the same method, but, as with many nonparametric tests, the data ranks are used in place of the raw data. When the Kruskal-Wallis's test shows that results are significant, the samples are not the same. The test does not identify where there are differences among the categories. To check for consistency in the analysis, the one-way ANOVA test was also used to check if there was statistical significance among the three categories of soil.

5.0 Results

50 days after the setup of the experiment the first observation was coloration of the cellulose strips which indicated colonization by cellulolytic organisms (microbes, fungi). Coloration of different shades, colors and intensities began to appear. Coloration was more visible in some strips than others, there was considerable variability between and within boxes. Rate of coloration and decomposition was estimated by area of strips that had been colored and or disappeared or disintegrated. More strips were colored in the boxes containing substrates from broadleaved and clear-cut locations than in the boxes contains substrates from spruce stands. There was no significant coloration or decomposition in all spruce boxes after the first 50 days. Furthermore, intensity of coloration in broadleaved and clear cut was observed and intensity and area of coloration was more in the in the clear cut than the broadleaved in the F+H horizon (difference was not statistically significant) but in the Ah horizon coloration was much higher in the broadleaved than the clear-cut substrate as shown in Table 2 and Figure 10 below. There was much more intense coloration in the F+H horizon than in the Ah horizon in all substrates. Signs of decomposition were also observed in the clear cut and broadleaved as manifested by disintegrating or complete disappearance of marked squares boxes on the cellulose strips. No significant signs of decomposition were observed in the boxes with substrate from spruce stand.

5.1 Comparing Coloration and Decomposition in the Horizons during First Period

In the F+H horizon, coloration was the least in the spruce (22.09%). Coloration was slightly higher in the clear-cut (98.97%) than in the broadleaved (97.25%). In the Ah horizon however, coloration was significantly higher in broadleaved (58.43%) as compared to that in the clear-cut (19.79%) and spruce (19.17%) as shown in table 2 below. Rate of decomposition in the F+H horizon on the other hand was high in both broadleaved (92.82%) and clear cut (91.08%) and least in spruce. In the Ah layer there was a significant difference between broadleaved (37.5%) and Clear cut (8.61%). Spruce had the lowest decomposition rate (1.35%). It was also observed that coloration and decomposition were also faster in the humus layer than the organomineral layer in all substrates or boxes as shown in figures 13 and 14 respectively. However, intensity of coloration did not always correspond to higher decomposition rate. In the whisker box figure boxes 1-3 represent the humus layer and then 4-6 represents the organomineral horizon.

Table 2. Rate of coloration of cellulose strips in the different horizons for all three (3) periods.

Plot	Horizon	19.5.	8.7.	30.8.	8.10
Spruce	F+H	0	22.09 (10.61)	72.69 (19.71)	92.3 (12.83)
	Ah	0	19.17 (21.72)	72.86 (16.89)	87.50 (16.21)
Broadleaved	F+H	0	97.25 (12.14)	100.00 (0.00)	100.00 (0.00)
	Ah	0	58.43 (24.87)	100.00 (0.00)	100.00 (0.00)
Clear-cut	F+H	0	98.97 (3.32)	100.00 (0.00)	100.00 (0.00)
	Ah	0	19.79 (12.69)	100.00 (0.00)	100.00 (0.00)

Note: The standard error is given in parathesis

Table 3. Rate of decomposition of cellulose strips in the different horizons for all three (3) periods

Plot	Horizon	19.5.	8.7.	30.8.	8.10
Spruce	F+H	0	1.18 (4.18)	5.26 (12.72)	5.56 (9.47)
	Ah	0	1.35 (2.64)	16.15 (22.13)	37.63 (34.99)
Broadleaved	F+H	0	92.82 (12.08)	99.46 (2.92)	100.00 (0.00)
	Ah	0	37.5 (31.66)	88.66 (16.09)	92.29 (15.69)
Clear-cut	F+H	0	91.08 (10.72)	95.35 (16.08)	100.00 (0.00)
	Ah	0	8.61 (9.46)	46.15 (35.12)	50.73 (33.23)

Note: The standard error is given in parathesis

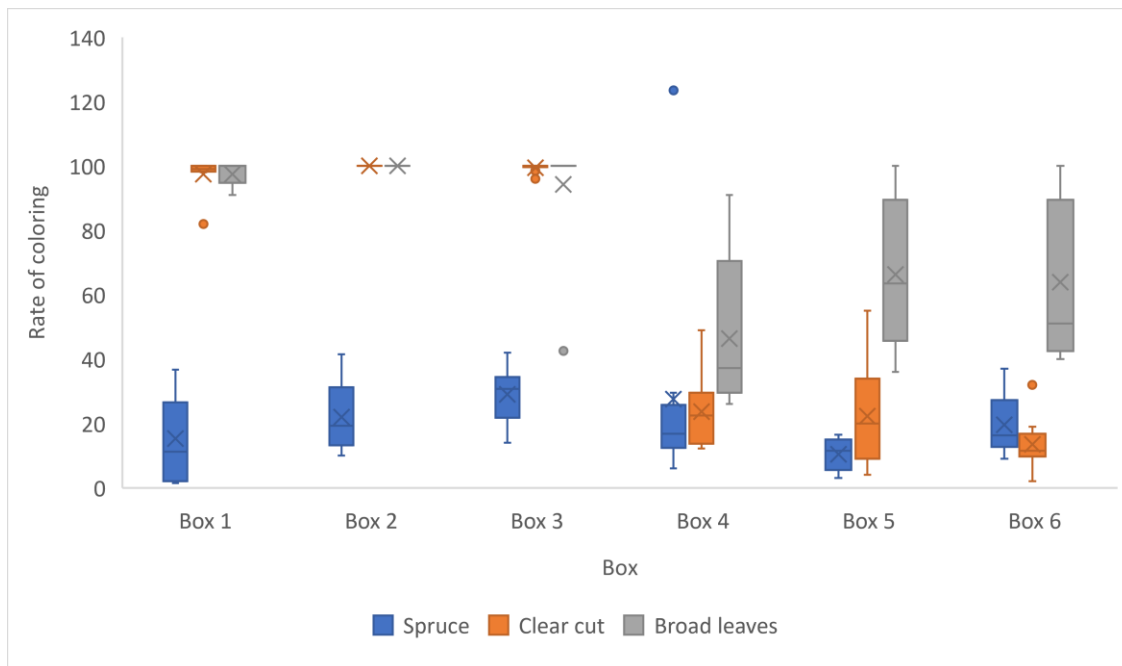


Figure 13. Rates of coloration in all boxes for the first period, Box 1 – 3: holorganic, 4 – 6: organomineral.

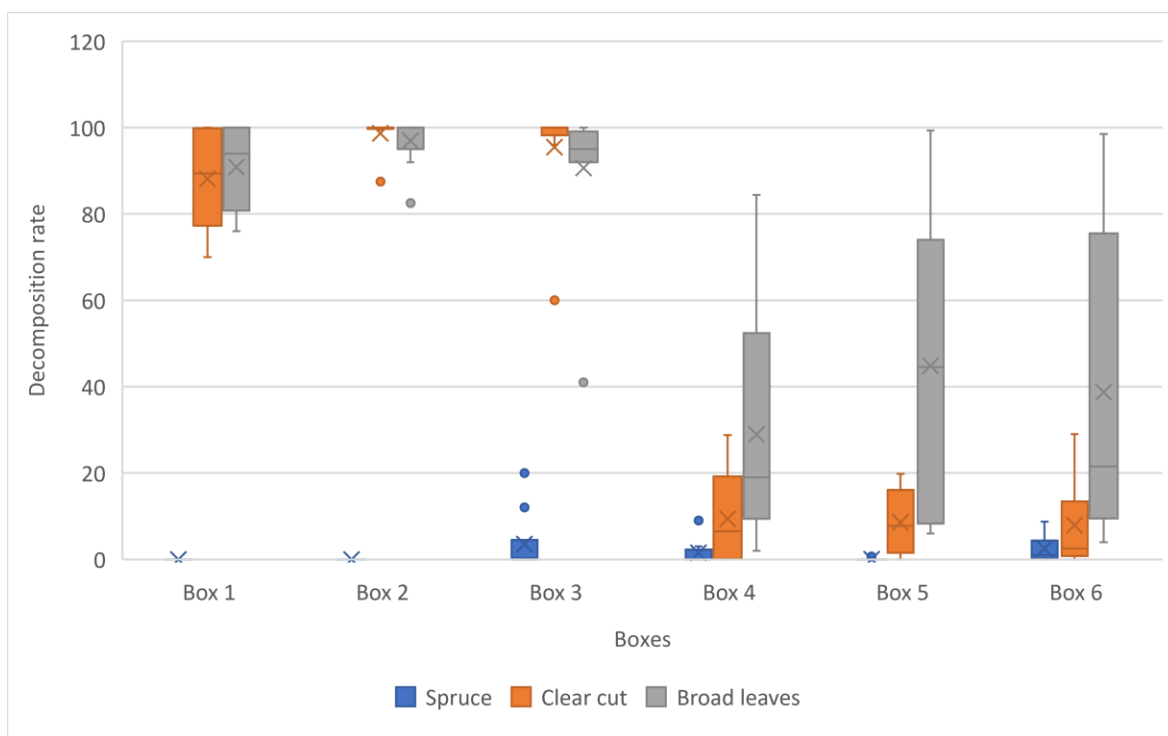


Figure 14. Rates of decomposition in all boxes for the first period, Box 1 – 3: holorganic, 4 – 6: organomineral.

5.2 Comparing Coloration and Decomposition in the Horizons during Second Period

Second period of observation and data collection took place 100 days after the initial set up of the experiment. There was complete (100%) coloration in all horizons for both the broadleaved and clear-cut boxes as shown in Table 2 above. The spruce boxes still had the smallest rate of coloration compared to that of the broadleaved, and clear but had significantly increased after the first period of observation from 22.09% to 72.69 in the F+H horizon and from 19.17% to 72.86% in the Ah horizon. Figures 15 and 16 below shows the data distribution coloration and decomposition rates in all substrates in all horizons. The rate of decomposition however was highest in the broadleaved (99.46%) followed by clear cut (95.35%) and lastly spruce (5.26%) in the F+H horizon. In the Ah horizon it was the same pattern, Broadleaved (88.66%) had the highest rate followed by clear cut (46.15%) and then spruce (16.15%). Contrary to the usual pattern of F+H horizon having higher decomposition rate, it was not so in the spruce, decomposition was rather higher in the organomineral horizon compared to the humus horizon.

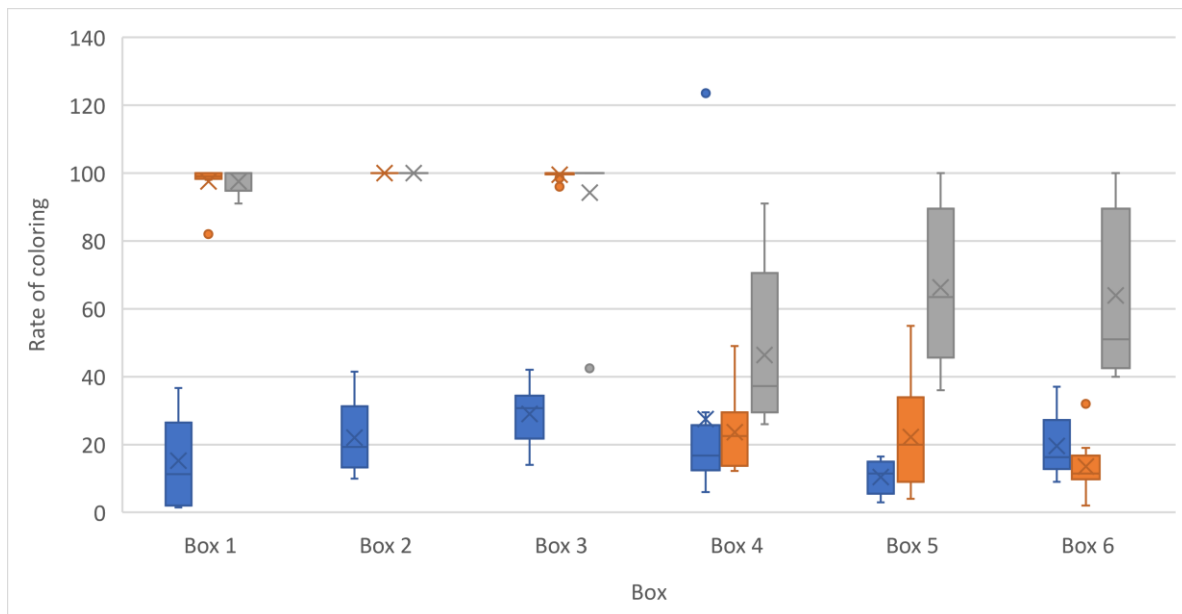


Figure 15. Rates of coloration in all boxes for the first period, Box 1 – 3: holorganic, 4 – 6: organomineral.

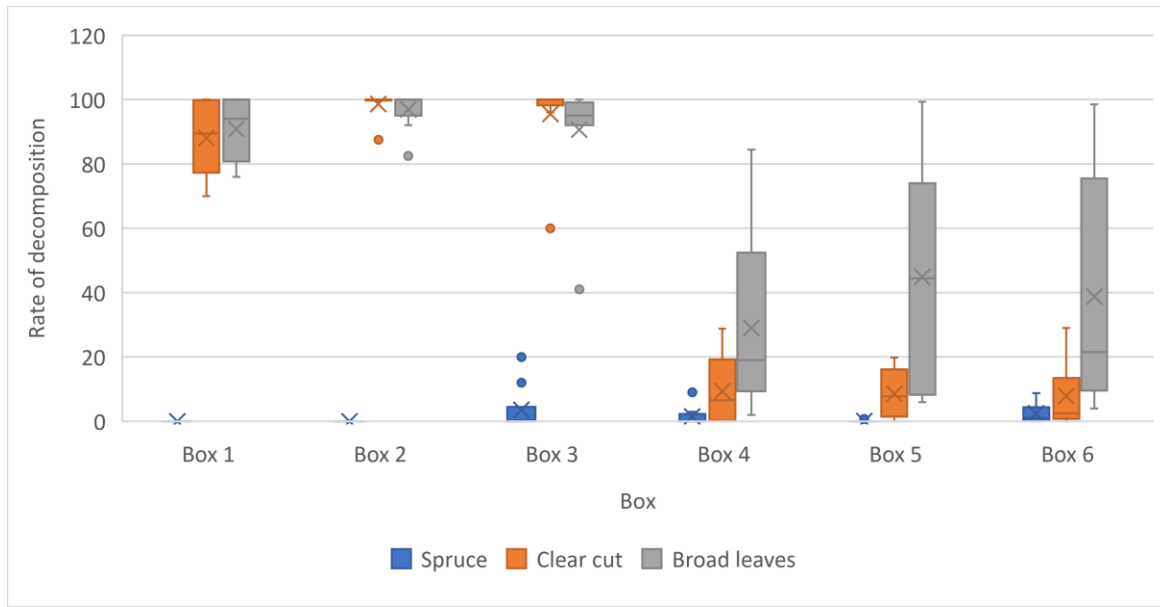


Figure 16. Rates of decomposition in all boxes for the first period, Box 1 – 3: holorganic, 4 – 6: organomineral.

5.3 Comparing Coloration and Decomposition in the Horizons during Third Period

The last period of data collection and observation was done 150 days after the initial experimental set up. Similar pattern of coloration and decomposition was observed in the 2nd period was seen. Rate of coloration had already reached 100% in all horizons (F+H, Ah) in both broadleaved and clear-cut boxes. In the spruce boxes however, coloration continued to increase, and the average value was 92.3% in the humus horizon and 87.5% in the organomineral horizon.

Decomposition rate continued to increase in all boxes. In the F+H horizon both broadleaved and clear cut reached 100% decomposition after 150 days while that in spruce (5.56%) did not significantly increase. In the Ah horizon decomposition increased to 92.29% in broadleaved, 50.73% in clear cut and 37.63% in spruce. Generally, decomposition rate was always higher in the F+H layer compared to the Ah layer in both broadleaved and clear cut but in spruce however rate of decomposition was higher in the humus layer than in the mineral layer throughout all 3 periods of observation. Figures 17 and 18 below shows the distribution, range and average values of coloration and decomposition rates of the cellulose strips in each box for each of the 3 substrates

(spruce, clear-cut and broadleaved). The boxes 1- 3 represent the (F+H) horizon and boxes 4-6 represent substrates taken from the (Ah) horizon.

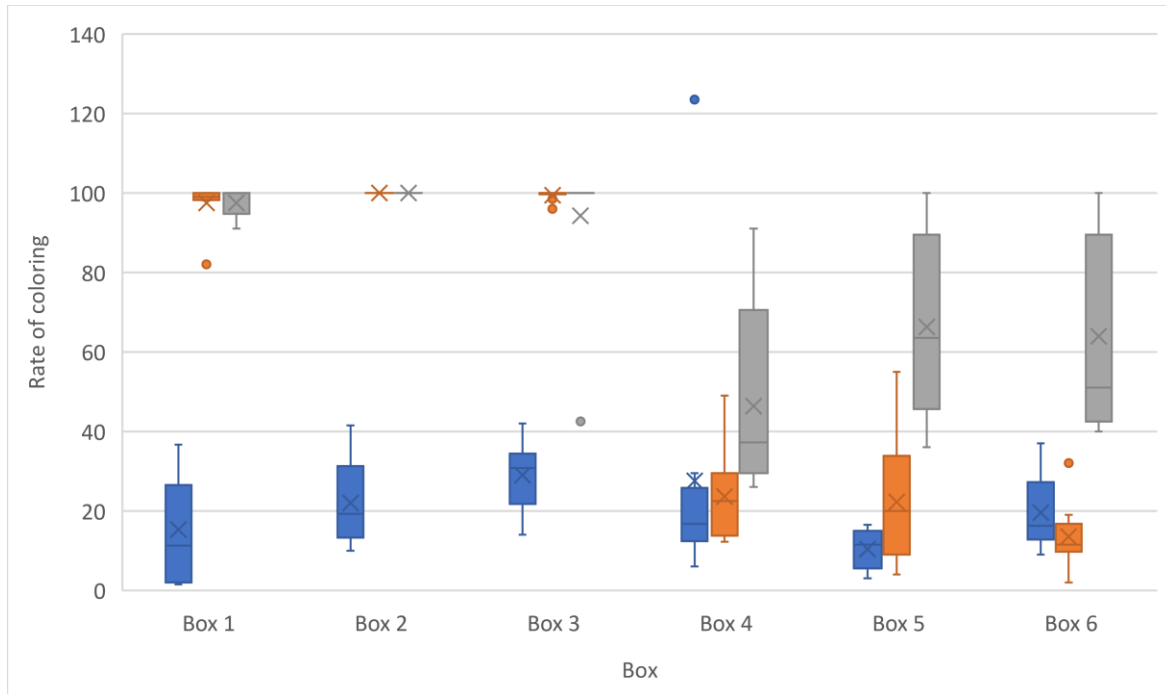


Figure 17. Rates of coloration in all boxes for the first period, Box 1 – 3: holorganic, 4 – 6: organomineral.

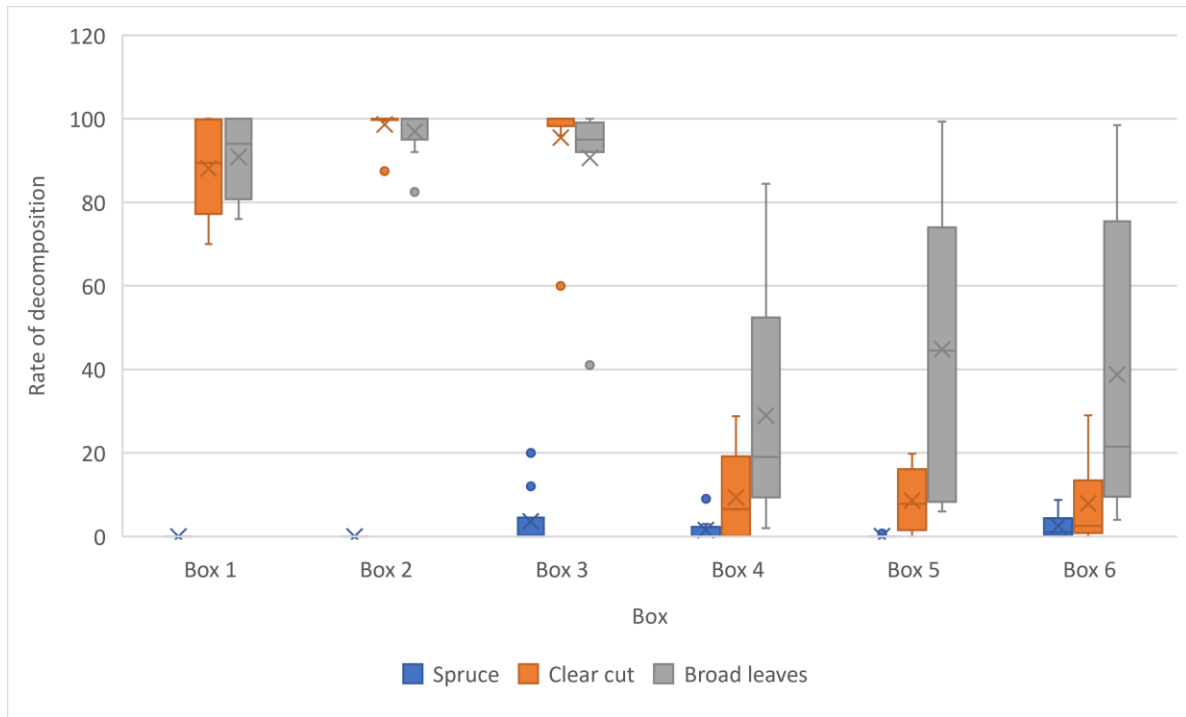


Figure 18. Rates of decomposition in all boxes for the first period, Box 1 – 3: holorganic, 4 – 6: organomineral.

6.0 Discussion

The result from this study demonstrates the potential of substrates from different localities in the same forest environment to decompose cellulose which is the most abundant component of plant litter. The results obtained reveals that there is a much higher rate of decomposition or cellulolytic activity in the substrates from the broadleaved dominated stand compared to the substrates from the Norway spruce stand and the clear-cut area after the harvest of the Norway spruce. The substrate from the Norway spruce showed the lowest potential for cellulolytic. This was shown by the extent of coloration and decomposition, or disintegration of the cellulose paper embedded on the various substrates in the experiment. The data from the experiment also shows a significant difference between the cellulolytic potential of the substrates from the different horizons of the soil profile. Substrates from the holoorganic layer showed a higher cellulolytic activity or decomposition rate than the substrates from the organomineral layer in both the clear-cut locality and the broadleaved dominated stands.

However, the horizons in the Norway spruce locality showed a different pattern, there was higher cellulolytic activity in the organomineral layer as compared to its holo-organic layer. This reason for this occurrence is not very clear. Perhaps this may be due to extreme character of the Norway spruce litter and the shifting of larger decomposers into deeper horizons with more transformed organic matter. After clear-cutting and in the broadleaved humus the conditions for these larger decomposers become favorable also in the top organic horizons. The acid and poor litter of Norway spruce is after clear-cutting primarily enriched and transformed into more favorable substrate in which large decomposers (worms, Nematodes, mites etc. can thrive. This so-called lag-phase is supposed in the case of extreme organic substrates and its decomposition (Binkley 1986).

To explain the faster decomposition rate or higher cellulolytic activity by the substrates from the broadleaved stand we need to understand the factors that contribute to forest floor litter decomposition. Plants affect the physical, chemical, and biological properties of their soil environment through litter breakdown and the generation of nutrients and protons in their rhizodeposits (Ayres et al. 2009). In this research the abiotic factors were kept constant as experiment was kept in laboratory conditions of constant temperature around 20 and 100%

humidity in all boxes so the differences in cellulolytic potential could only be explained by the biotic factors which is the microbial population in the substrates as well as the chemical composition of the substrates. The higher intensity of coloration of the cellulose strips was due to the higher microbial population in the substrates and hence contributed to higher decomposition rate in the substrates from the broad leaves and the clear cut.

This results agrees with outcome of the studies that investigated the impact of beech and Norway spruce on the structure and diversity of the rhizosphere and soil microbial communities by (Uroz et al. 2016) who found significant changes in the abundance, composition, and structure of microbial communities associated with two different tree species of the same age, deciduous European beech and coniferous Norway spruce planted in the same soil. Their research to showed that different tree species growing in the same soil qualitatively and quantitatively affects their soil microbiota. Their work supports the hypothesis that tree species differentially impact soil microbial communities. They found archaea and fungi in the soil of the forest to be more strongly determined by tree species and bacteria showing a stronger rhizosphere effect. Observation and results from this work also supports well the conclusions obtained by (Urbanová et al. 2015) who suggested that fungal communities are more affected by tree species than are communities of bacteria. This confirms the data and results from this work that showed higher microbial population in the broadleaved and clear-cut substrates than that of the Norway spruce substrate as demonstrated by their ability to degrade cellulose.

The other possible explanation for the faster decomposition is the chemical nature of the material forming the substrate in each category. It is well documented there is a strong correlation between lignin/N and C/N ratios (Augusto et al. 2002). It is known that Norway spruce litter have higher C/N ratio and more lignin content making decomposition of its litter slower. This usually contribute to acidification of the soil and hence not favorable for many microbes to thrive. This explains why there is accumulation of humus forms or litter under the stands of Norway spruce and other coniferous species. Broadleaves on the other hand have lower C/N ratio and lower lignin content and hence making it easier for microbes to decompose its litter and hence less accumulation of litter under forest stands dominated by broadleaves.

Substrates from the clear-cut site showed faster decomposition rate compared to the substrates from the Norway spruce stand. In this work the higher cellulolytic activity in the clear-cut substrates may be probably due to the fact that with time the soil became less acidic after the removal of Norway spruce trees from the stand. A less acidic soil together with high temperature because of no crown cover promotes the growth of microbial communities and new vegetation. Similar observations have been made in other studies where clear-cut increased decomposition rates of litter. For example (Klemmedson et al. 1985), reported that needles in cleared cut treatments lost weight more quickly than those in forested treatments when ponderosa pine forest was compared to adjacent clear cut site. Indicating a higher rate of decomposition in the clear-cut sites. However (Ishikawa et al. 2007) reported that the remaining mass of litter was generally lower in the clear-cut plot than in the adjacent uncut site. But it must be mentioned that the control site was dominated by four broadleaved species and just one coniferous species. Other studies have reported no significant differences (Prescott et al. 2000) and a few reporting slower decomposition of litter after clear cut (Zhang & Liang 1995). This shows that there has not been a consensus yet and more investigation needs to be done on the effect of clear cutting on decomposition of litter.

The cellulolytic potential of the substrates was also compared between the humus layer and the organomineral layer. In all the substrate types there was higher coloration and faster decomposition in the surface humus layer than in the organomineral layer. However, they were much more intense in the broadleaves and clear-cut substrates than the Norway spruce. This was much expected in a more conducive environment supporting the growth of microbial communities in the humus layer compared to the organomineral layer. The humus layer contained more organic matter components which serve as feed for microbes and other litter decomposers which live closer to the soil surface. This work agrees with other authors who found similar patterns. The soil layer had a greater impact on soil microbial populations than the gap size, according to (Yu et al. 2018). Their findings revealed that enzyme (alkaline phosphatase) activity increased in the organic layers in a positive relationship with gram-negative bacteria and fungus concentrations ($p < 0.05$). Only gram-negative bacteria, however, are thought to play a significant influence in enzyme activity in the mineral layer ($p < 0.01$). They proposed that depending on substrate availability, comparable microbial populations in different soil levels may play different roles in nutrient cycling. They also found that increased relative abundance of soil microbial community in organic layers could be

due to a variety of factors. Litter inputs, root exudate, and turnover in the upper soil layer, according to (Zhang et al. 2018) would offer microorganisms with a considerable amount of fresh substrates and energy.

7.0 Conclusion

The aim of this research work was to evaluate the potential of cellulose decomposition within the humus forms after harvesting or decline of Norway Spruce in comparison with neighboring stand dominated by broadleaved trees. In laboratory conditions, the decomposition and color changes of filter paper strips as a source of cellulose in contact with substrates of humus forms from two soil horizons (F + H, Ah) of Norway spruce stand, Clear cut area and broadleaved stand were monitored. The results obtained confirmed a higher microbiological activity under the substrates from the broadleaves and clear-cut stands than from the Norway spruce substrates. This was visually manifested by color changes and disintegration of the cellulose strips in both soil horizons. In addition, the humus layer had a much higher cellulolytic potential than the organomineral horizons in all substrates. It is clear from the results of this work and from other authors that forest species composition has an influence on the physical, chemical, and most importantly the biological activity of the soil. Hence care should be taken when establishing forest stands considering the effect of Norway spruce effect of humus form formation. Perhaps in the territory of the Czech Republic and central Europe, an approach where there is a balance between coniferous species like Norway spruce and soil improving species (broadleaves) to mimic natural stand composition as it were before should be considered. Long term studied may be needed to confirm the effect of such admixture of species on the soil properties.

8.0 References

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