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Faculty of Forestry and Wood Technology
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Comparison Of Soil Microbial Activity On Sites With Different Forest Management Practices

Diploma Thesis

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DIPLOMA THESIS TOPIC

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Guides to writing a thesis:

1. To evaluate the available literature on the issue of soil organic matter, soil microbial aktivity, processes of decomposition in Central European soils
2. To evaluate 4 sites with different forest management in the area of Masaryk Forest Enterprise (Soběšice)
3. Comparison of organic carbon content and microbial activity in localities with different tree density after forest logging
   Use laboratory analyses of activity of catalase, phosphomonoesterase, Cmic...
   Analyses will be performed 3 times (different phases of vegetation period)
4. Evaluation of the results with respect of different forest management
5. Disccussion (soil management, soil quality, timber production, climate change)
6. The results document graphically and in tabular

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Abstract

Bc. Jiří Volánek

Comparison of soil microbial activity on sites with different forest management practices

Presented thesis is focused on microbial activity of forest soils and aims at characterizing some of its parameters on sites with different silvicultural management practices. Coppice, coppice-with-standard and high forest stands were compared in terms of carbon and nitrogen content, content of microbial biomass, enzymatic activity and selected physical properties of soil. Study was conducted between September 2015 and April 2016 on pre-existing TARMAG II research plot near Soběšice, Brno, Czech Republic. Samples were collected during three different calendar seasons, allowing for seasonal dynamicity assessment of the studied parameters.

Statistical evaluation detected significant effect of management type on potential respiration of studied soil samples as well as significant effect of seasonality on microbial biomass content in incubated samples, phosphatase activity in fresh soil samples, catalase activity in both fresh and incubated samples and potential respiration of studied samples. Results also show that the overall potential activity of urease and catalase was at its highest during the winter season.

Key words: microbial activity, enzymatic activity, soil respiration, soil carbon, forest soil, forest management, seasonal dynamic

Prezentovaná práce je zaměřena na mikrobiální aktivitu lesních půd a má za cíl charakterizovat některé z jejích parametrů na místech s uplatněním různých pěstebních postupů. Hospodářské tvary nízkého, středního a vysokého lesa byly porovnány z hlediska obsahu uhlíku a dusíku, mikrobiální biomasy, enzymatické aktivity a vybraných fyzikálních vlastností půdy. Studie byla provedena v období od září 2015 do dubna 2016 na již existující výzkumné ploše TARMAG II poblíž Soběšic, městské části Brna-Sever, Česká republika. Vzorky byly odebrány v průběhu tří různých kalendářních období, umožňující posouzení sezónní dynamiky studovaných parametrů.

Analýza dat odhalila statisticky významný vliv tvaru lesa na potenciální respiraci zkoumaných vzorků půdy, jakožto i významný vliv sezónní dynamiky na obsah mikrobiální biomasy inkubovaných vzorků, aktivitu fosfatázy v čerstvě odebraných vzorcích, aktivity půdní katalázy a potenciální půdní respiraci. Výsledky také ukazují, že nejvyšší celková potenciální aktivita ureázy a katalázy byla naměřena v průběhu zimy.

Klíčová slova: mikrobiální aktivita, enzymatická aktivita, půdní respirace, půdní organická hmota, lesní půdy, hospodářský tvar lesa, sezónní dynamika

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1 Introduction

Soil is the most essential means of production in forestry and has been included amongst non-renewable resources. The growth and development of aboveground biomass highly depends upon its qualitative parameters. Soil is a very active live ecosystem that is affected by abiotic factors on one side, by biogenic processes of soil fauna and flora on the other side and least, but not last, by the mankind. If one projects all soil forming factors onto a timeline, it becomes obvious that a very lengthy evolution process is dealt with; a process that the gives the soil forming factors another extent, its fourth dimension, the time.

Soil is one of the most important parts of the ecosystem. It stabilizes and affects the sustainability of life on the planet and therefore is classified by the Food and Agriculture Organization (FAO) as the most valuable natural resource. According to FAO, soil has to be protected and its development should be monitored. Perhaps the most important of its functions is the participation in the cycling of matter and the energy flows in nature and the biochemical cycles of the basic components of living organisms (such as carbon, nitrogen and phosphorus).

Soil organic matter is the world's largest reservoir of carbon and energy in terrestrial ecosystems. The carbon stock of the soil is more than twice greater than that of the atmosphere or the plant biomass (Šantrůčková 2014). Forest scientists have long been concerned with soil carbon as it is considered to be a master variable determining soil fertility. Soil carbon has many shapes and forms and largely contributes to biological activity of soil. Moreover, soil carbon and carbon in general, on the global scale, became vital for assessing CO_2 emissions. While a fairly good amount of information is available on above ground carbon shifts, the below ground carbon rate of losses and gains on regional level are still fairly unknown (Marek et al. 2011).

But lets not stop at carbon, since the nutrient cycles of most vital elements in the soil are closely interlinked and can hardly be taken out of context and assessed individually. Soil, possibly more than any other ecosystem, deserves to be examined holistically. Even though the soil plays a central role in the sustainable functioning of ecosystems, in the long term, it receives less attention than monitoring the state of the aquatic environment and atmosphere. The reasons stem mainly from complicated

measurements, high complexity of the ecosystem relationships and slow course of changes in the soil environment.

Processes in the soil and their activity may be evaluated using different criteria. Evaluation of the volume of soil biomass and the assessment of its quality and its forms undoubtedly belongs amongst the most important parameters. Soil quality can also be evaluated on the basis of biochemical transformations of important biogenic compounds by, for example, quantifying mineralization of carbon, nitrogen and phosphorus, that have a direct impact on the supply and circulation of these substances (amounts of nutrients available), while other sensitive parameters are volume of microbial biomass, the diversities of soil microorganisms and their enzymatic activity.

Soil health has been defined by Pankhurst et al. (1997) as: "*The continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal, and human health.*" It has been shown (Powlson et al. 1987, Dick 1994) that any change in land management, by extension of soil health status is reflected in microbial biomass and the activity of soil enzymes in a relatively short period of time, long before the effect may be measurable in terms of changes in soil organic matter.

Soil health, simply summed up as the ability of soil to perform functions that are needed for functioning of all ecosystem components, can be, therefore, also determined by the respiratory or the enzymatic potential of soils by quantifying the biological status of soil or its ability to carry out soil enzyme-catalytic reaction (Dick 1998).

Shifts in soil microbial biomass and enzymatic activity can be attributed to many factors (natural or anthropogenic) and can be also related to the seasonality in temperate zone, global climate change or types of management and land use change. Within forest management practices, logging intensity does belong to this many variables.

The studied research area was last subjected to such variables in terms of manmade interventions into its ecosystem over seven and a half years ago. These interventions are designed to simulate two traditional forms of production forest (Kadavý et al. 2011): coppice forest and coppice with standard forest, which are currently underrepresented, but still offer an attractive alternative for smaller forestry entities regarding, now widely discussed, renewable bioenergy resources.

2 Aims And Objectives

Thesis aims at evaluating **soil microbial activity on sites with different forest management practices**. TARMAG II research plot near Soběšice serves as the foundation for this assay. The research area, primarily set up in order to simulate the effect of coppice and coppice-with-standards forests on biodiversity, was subjected to different intensities of logging and represent two different traditional types of forest management. Selected subplots are closely examined from the angle of their possible impact onto soil quality, while being compared to nearby unaltered high forest stands that can also be viewed as the point zero and attributes the soil properties of the original pre-intervention state.

The evaluation of soil properties is achieved through numerous and extensive laboratory analyses measuring soil microbial activity (respiration and selected enzymatic activity), volume of microbial biomass along with carbon and nitrogen content and their ratio. These variables are considered to be some of the main indicators of the soil state comparative assessment. Basic chemical and physical soil properties are also evaluated and all together juxtaposed in statistical evaluation.

Soil samples were collected within three individual time periods during the years of 2015 and 2016. Each time period was to represent distinct vegetative phase of the temperate forest biome, typical for central Europe, and was to follow vernacular seasonality. This fact should allow for the comparison of the soil characteristics between individual sampling periods and help to portray the microbiological dynamicity throughout the growing season and the period of vegetative dormancy.

3 General Overview

3.1 Basic Concepts

3.1.1 Soil

Just like many run-of-the-mill words, '*soil*' can be used in several connotations. In its traditional sense, soil is understood as the natural medium for the growth of plants, whether or not it has discernible soil horizons (World Reference Base (WRB) Soil Survey Staff 1999).

In the FAO (1998) WRB, soil was defined as: "… *a continuous natural body which has three spatial and one temporal dimension. The three main features governing soil are:*

• *It is formed by mineral and organic constituents and includes solid, liquid and gaseous phases.*

• *The constituents are organized in structures, specific for the pedological medium. These structures form the morphological aspect of the soil cover, equivalent to the anatomy of a living being. They result from the history of the soil cover and from its actual dynamics and properties. Study of the structures of the soil cover facilitates perception of the physical, chemical and biological properties; it permits understanding the past and present of the soil, and predicting its future.*

• *The soil is in constant evolution, thus giving the soil its fourth dimension, time."*

As stated by Jandák et al. in 2001 soil is defined as an independent, natural and historical formation, which is produced due to the complex effects of external factors on the parental rock at a certain time. It is shaped by the soil-forming factors and is a subject to gradual changes due to natural or anthropogenic activities. Soil must be understood holistically, as a component of the natural environment, which together with the atmosphere, hydrosphere and biota forms a functional ecological system.

There are different types of soil and they may differ in many factors. As noted, it is firstly the soil-forming factors that shape soil according to their intensity and combination. Soils also differ because of various land use and type of vegetation cover (e.g. forest soils), where forest management practices can definitely be considered as a certain variable. Soil features are of physical, chemical and biological nature and can be described by soil texture, grain-size structure, color, humidity, mineral composition of soil, amount of organic matter, soil biological activity, etc. Soil pedons (soil profiles)

are vertically divided into horizons. It is again the soil-forming factors (substrate, climatic conditions, soil biota and vegetation) projected in time that affect the formation of individual horizons. Humic horizons are located at the surface (topsoil) followed by great diversity of mineral horizons in lower strata and soil-forming substrate or parent material in the lowest strata (Šarapatka 2014).

Soil basically comprises of two essential components, animate and inanimate. Inanimate component consists of several constituents: **mineral particles, organic matter, water and air**. In addition to the non-living elements soil includes organisms that are vital for most processes in soils. Soil without **living organisms** ceases to be soil and becomes a mere substrate. Representation of individual components in soil, water and air content in particular, is highly variable (Šimek 2007).

A substantial portion of the mineral component in soil is composed of primary a secondary silicates (Jandák et al. 2004). Mineral particles are of various sizes, from colloidal particles to large fragments of inorganic compounds (Šimek 2007). The minerals in the clay fraction generally affect the soil enzymatic activity the most due to their large surface area and ability to act as cationic, and in some cases anionic, ion exchangers (Morra 1997).

Organic matter is composed of vegetation and animal residues and dead cells of microorganisms that are in different stages of decomposition and transformation into new substances. Natural organic matter in soils exist in two different forms: compounds of distinct origin and substances which have been chemically modified in such a way that their source is not determinable (Stevenson 1994). The first group includes polymeric compounds (e.g. cellulose, proteins and lignin), relatively simple molecules of carbohydrates, organic acids and amino acids (Morra 1997). Part of the organic matter in the soil, which has undergone transformation processes involving decomposition and synthetic processes, is called humus. Humic substances are highly polarized, negatively charged, materials and alike most silicates have the capacity of exchanging cations (cation exchange capacity). Humic and non-humic substances exist in soil in association with mineral compounds. These organic-mineral complexes bind enzymes and substrates, thereby influence the extent of catalysis: increase the rate of chemical reactions in soil (Morra 1997)

Organisms representing the living component of the soil are from all three domains: eukaryotes, archaea and bacteria. They are collectively called soil biota. Soil biota is divided into microfauna (e.g. yeasts, bacteria - commonly called actinobacteria,

fungi, protozoa, roundworms, and rotifers), mesofauna (e.g. tardigrades, mites and springtails), macrofauna (e.g. woodlice, earthworms, beetles, centipedes, slugs, snails, ants and harvestmen) and megafauna (e.g. moles) (Šarapatka 2014). Soil microorganisms occur primarily in the form of single-celled organisms, but can also be found in the form of fibers, cyanobacteria or hyphae of fungi (Jandák et al. 2001).

It is soil biota that provides a continuous flow of matter and energy through decomposition and synthetic processes, conversion processes of individual elements and nutrients and interaction between the soil and the environment. The inanimate components of soil constitute the living environment for soil biota and it is these soil organisms make soil a unique natural dynamic entity endowed with distinctive characteristics able to ensure the growth and development of plants (Šimek 2007).

Soil biota with all its components is interconnected within a complex food web. Each link of soil ecosystem plays an important role in such network and is, to some extent, irreplaceable. If one group of soil biota was to be eliminated and another group does not replace its function, the whole food web can be disrupted and can lead to changes in the decomposition of organic matter, its quality or the availability of nutrients (Šantrůčková 2014).

3.1.2 Nutrient Cycles In Soil

The chemistry of all nutrient cycles in soil (such is the complexity soil food webs) is closely interlinked and can hardly be taken out of context and assessed individually (Šantrůčková 2014). All of soil biota, in order to sustain their elemental functions, need energy, carbon, nitrogen, phosphorus and other nutrients for biomass production. Soil biota, plants alike, draw nutrients from their close environment. For example, phosphorus, calcium, potassium, and sulfur are primarily of mineral origin retracted from parent material (Šantrůčková 2014). Nitrogen is, in the first place, bound into biomass from the atmosphere (N_2 fixed by microorganisms) and carbon enters the soil principally as dead wood debris and litter (root exudates set aside) of above ground biomass of green plants (CO_2 being the elemental input of photosynthesis) (Šantrůčková 2014). Various nutrients must undergo a series of transformations (realized mainly through metabolic paths of soil biota and their food webs) to ensure that they come to be reused in an acceptable form by plants (Šantrůčková 2014).

Most transformations of nutrients in forest ecosystems, such as in most terrestrial ecosystems, take place in the soil. It is noted by many (Šantrůčková 2014,

Šarapatka 2014, Šimek 2007) that the speed and the balance of nutrient transformation processes depends on environmental conditions: especially temperature, moisture, aeration, pH; on vegetation cover (type of management) and the activity of soil organisms. However, it must be pointed out, that each process requires its certain specific conditions (Šantrůčková 2014). The imbalance or deficiency of any macro or micronutrient can lead to overall deceleration or seizure of given transformation processes in soil.

Cycling of all elements largely depends on the quality and quantity of dead plant biomass (soil organic matter) input into the soil and the speed at which the biomass is transformed, decomposed and the nutrients released back into the cycle. Mineralization and immobilization microbial processes take place simultaneously with decomposition. Organic matter is progressively converted to inorganic substances and CO_2 within the mineralization process. Conversely, minerals and CO_2 are incorporated into the body of organisms and to soil organic matter in the process of immobilization. Therefore, if mineralization processes prevail over immobilization processes a decline in soil organic matter occurs and can lead to soil structure violations and, in the long term, to soil degradation (Šantrůčková 2014).

Quality of soil organic matter (SOM) and humic substances in the soil (products of transformation processes) can be assessed, for example, by estimating total carbon (C_{tot}) and nitrogen (N_{tot}) content and their ratio (C:N) (Pospíšilová et Tesařová 2009).

It was noted by Tomášek in 2007 that the usual C:N ratio of the temperate zone ranges between 7 – 17:1 and the higher the ratio, the lower the quality SOM and humic substances in the soil is.

Nutrient availability doesn't only play an important role in the growth of plants but also influences the biomass and activity of soil microorganisms (Varma et al. 2004).

3.1.3 Microorganisms In Soil

Microorganisms have adopted different metabolic approaches towards their building material repositories and, by extension, towards the biogeochemical nutrient transformation processes in soil and on that basis can be divided into various functional groups. Some of these groups are directly linked to plant nutrition (e.g. nitrogen fixators and plant symbiotic microorganisms, such as rhizobial bacteria and micorrhizal fungi, methanogenic microbial communities, organic matter decomposers, etc.) (Varma et al. 2004). Many steps in nutrient cycling are performed solely by microorganisms and

some of these microorganisms may take part in more than one biogeochemical cycles (Varma et al. 2004).

Microorganisms utilize three basic energy sources: solar radiation (autotrophic organisms), organic matter (chemoorganotrophic organisms) or inorganic matter (chemoautotrophic organisms). Based on the source of **C**, the basic building component of the cell, are divided to heterotrophic (using organic substances), or autotrophic (when using CO_2). Many microorganisms use CO_2 together with organic substances (mixotrophic organisms). Another physiological division can be made on the grounds of oxygen utilization. While higher plants and animals find oxygen indispensable (aerobic organisms), large groups of microorganisms have developed metabolic pathways to be able to live in conditions of highly reduced oxygen concentrations (miroaerophilic microorganisms) or even total absence of oxygen (anaerobic microorganisms) (Šantrůčková 2014). Anaerobic microorganisms use alternative metabolic pathways (e.g. fermentation, methanogenesis, sulphur oxidation) and oxygen is toxic to them. Facultative aerobic (anaerobic) microorganisms utilize both metabolic pathways (Šantrůčková 2014).

Schloter et al. defined soil microbial biomass (2003) as organisms living in soil that are generally smaller than 10 μm. Soil microbiota contains many species of bacteria, archaea, cyanobacteria, fungi and greater soil organisms, such as yeast, algae or protista. Each species has a specific function but all complement each other and work together as one unit (Brookes et al. 1985). Microbiological species diversity is overwhelming and soil alone is considered to be the largest genetic reservoir on Earth (Šantrůčková 2014). About 100 000 species of soil microorganisms have been identified so far and as suggested it is still a mere fraction (about 1 %) of all expected species. It is not only the interspecies diversity that is considered of importance to balanced functioning of the soil food webs and transformation processes in soil but also the so-called functional diversity (balance in a representation of the functional groups) (Šantrůčková 2014). As the variability of soil biota is too diverse microorganisms are often subdivided according to their ecosystem functions rather than their taxonomy.

Microorganisms are supposed to be the second biggest contributor to living biomass of soil and that is despite their size. (bacteria is mostly smaller than 1 μm). They lag behind roots of plants by 'only' a lower order of magnitude (about 2000 $g.m^{-2}$ on average compared to 400 $g.m^{-2}$) (Šantrůčková 2014).

According to Šantrůčková (2014) the most essential functions of soil microorganisms are:

i. decomposition and mineralization of organic matter in soil and releasing minerals into the environment
ii. bonding nutrients into the biomass (immobilization)
iii. improving soil structure
iv. transformation processes of nutrients in anaerobic conditions (denitrification, fermentation, methanogenesis, oxidation and reduction of Fe, Mn, S and C)
v. fixation of atmospheric nitrogen
vi. metabolite production
vii. production of biologically active substances and the formation of interactions with plants and animals (from symbiosis to pathogenicity)

Soil microorganisms that directly support plant growth and plant development and are an essential part of the decomposer subsystem, which regulates nutrient cycles, are most important for the production and ecological functions of soil (Wardle 1998). This group is involved in many key processes in the soil, contributes to increasing the availability of nutrients for plants and helps to maintain good soil structure. Soil microorganisms are a good indicator of soil quality, especially because of their ubiquity, quantity and irreplaceable role in food webs and nutrient cycles (Mikanová et al. 2010). Wardle had also pointed out on numerous occasions (1990, 1992 and 1998) that soil microedaphon is not a static body, for which it is sometimes considered, and its dynamics are likely to be extremely important to evaluate the degree of release of labile nutrients and their availability to other components of the ecosystem.

The biomass of these microorganisms is a relatively accessible storehouse of nutrients for plants (Šimek 2007). Microbial cells immobilize nutrients during their life and thus prevent their leaching from soil (mainly the case of N) or create inaccessible compounds and complexes (such is the case of P) (Šantrůčková 2014). After the death of the cells, the inner cell content - cytoplasm and the cytoplasmic membrane are easily decomposed and nutrients contained therein are gradually made available to the plants. Since the generation time of microorganisms is short, it can be assumed that the nutrients, which microorganisms immobilized in the spring, are released to the plants

during the growing season. The cell walls decompose more slowly and become part of the soil organic matter (Šantrůčková 2014).

The smallest of microbiota is fully dependable on water content in soil and can only be active in moist conditions (Šantrůčková 2014). It is mainly due to the size and shape of microedaphon as to why it adapted their existence and movement to the liquid phase of soil. Microorganisms in soil are, therefore, largely, if not solely, apt to osmotrophic nutrient intake, obtaining carbon and nutrients from the liquid phase of soil. Soil solution comprises of nutrients from weathered minerals, decomposed organic matter and unbound ions exchanged from the soil sorption complex. The most common way of material exchange of osmotrophic organisms is the transport of solution through membranes. Microorganisms, despite their high amounts are vulnerable due to the their large active surface (Mikanová et al., 2010). In other words, it is their unique competitive advantage (large active surface) that makes them easily vulnerable. Soil microorganisms are considered to be highly susceptible to shifts in their ecological conditions (Mikanová et al. 2010). Seasonal changes of temperature and soil moisture content and perturbations of soil pH, structure and texture or toxic substances can alter the function of the entire ecosystem, that is, inclusive of changes in vegetation cover. Vice versa, changes in vegetation cover (e.g. altering the type of management) can obviously induce functional changes of soil microbiota.

3.1.4 Activity Of Soil Microorganisms

Determination of soil microbial activity is considered to be an important indicator of soil quality and soil health (Nannipieri et al. 2002). The activity of soil microorganisms has a very close relationship to their main functions in the ecosystem and can be assessed, for example, in terms of biomass, volumes of nutrient cycling (nutrient dynamics) or amount of metabolites produced. Soil bacteria are the largest and most biochemically active microbial group in soil. Its great importance for plant nutrition also resides in the ability of binding atmospheric nitrogen (*Rhizobium spp*., *Azotobacter sp*.), as mentioned, but also in releasing phosphorus from soil minerals (P-solubilizing bacteria).

Soil microorganism activity cannot be monitored meaningfully with only a few simple tools. In most countries, **microbial biomass**, **respiration** and **potential nitrogen mineralization** are regarded as part of a minimum data set (WRB 1999).

Soil respiration is the most commonly used measure of the activity of soil

microorganisms (Horáková et al. 2007, Mikanová et al. 2010). It is usually determined through **basal** and **potential respiration** (substrate-induced respiration). Basal respiration (**BR**) is a "qualitative" parameter showing current state respiratory activity of microorganisms and is defined as the amount of released CO_2 per unit of time (Mikanová et al. 2010). It is also the result of the physiological state of microorganisms, their energy requirements, effect of stress factors, inhibitory effects, etc. Substrate induced respiration (**SIR**) measures increased respiration rate immediately after the addition of easily usable substrate. Substrate-induced respiration is according to Mikanová et al. (2010), again, a "qualitative" parameter and shows the maximum potential of soil respiration in substrates that are not limited by nutritional resources. SIR of microorganisms, best reflects their current physiological condition, their energy needs, hence their full mineralization potential.

Carbon content of microbial biomass ($\mathbf{C_{mic}}$ or else mentioned as C_{bio}) belongs to the most often mentioned representatives of microbial activity characteristics (Sáňka et Materna 2004).

It has been noted that we are well past the time when measurement of microbial biomass alone is considered adequate (Dick 1994, Coleman et al. 2004). Still, it is the basic, initial characterization of soil microorganisms. Determination of microbial biomass is considered a good indicator of changes in soil properties, because used methods are not based on the separation or isolation of microorganisms, instead, measured directly in soil samples (Mikanová et al. 2010). Declines in C_{mic} may indicate adverse changes in soil organic matter.

The most important and most frequently assayed activity also includes **enzymatic activity** (Mikanová et al. 2010).

3.1.5 Enzymes In Soil

Only a small proportion of organic substances are simple. Most organic compounds entering the soil are complex, thus must be broken down into simpler substances in order to be of any use to soil biota. The vast majority of organic material must undergo one to several stages of extracellular transformations, to which enzymes significantly contribute.

Chemically speaking, enzymes are protein macromolecules that have the ability to catalyse chemical reactions (lowering activation energy of reactions, without moving the balance in favour of products or reactants). Like all catalysts, enzymes increase the

rate of a reaction by lowering its activation energy, but in comparison with chemical catalysts (also present in soils), enzymes have a much higher specificity to the substrates, work faster and form much more specific products, thereby minimize the formation of by-products (Horáková et al. 2007). Enzymes, within their bio-molecular structure, dispose of one or more catalytic sites, where the catalysed reaction occurs. Catalytic reactions can involve cofactors (non-protein structures consisting of virtually any organic substance or a metal cation) (Hasala 2010).

Each enzyme has its reaction specificity (catalyzes only a specific type of reaction) and substrate specificity (specificity to a specific substrate). Providing the rule of reaction and substrate specificity are fulfiled, the reaction can be expressed by the following general scheme:

Equation 1 General enzymatic reaction scheme, where E stands for enzyme, S for substrate and P for product (Horáková et al. 2007)

$$E + S \leftrightarrow ES \longrightarrow E + P$$

Enzymes can be associated within living cells (intracellular enzymes – endoenzymes). Enzymes can also be bound directly to cell walls or to the slime layer on the surface of cells (ectoenzymes, which in wider terms belong to intracellular) or secreted into the soil environment (extracellular enzymes – exoenzymes). Most extracellular enzymes entering the soil (actively discharged into the environment or released by cell lysis) survive only for a short time and are rapidly decomposed or denatured (Gobat et al. 2004). Exoenzymes can be generally divided into two classes, those freely dispersed and stabilized in soil solution and those bound to complexes of humic colloids or adsorbed onto clay particles (Burns 1982).

It has been evidenced that even the simplest of unicellular organisms produce around 3000 different enzymes (Vodrážka 1996, Bedford et Partridge 2001). Some enzymes are produced by cells continuously (e.g. urease) and are called constitutive; other enzymes are inducible (e.g. cellulose), formed only in the presence of a compatible substrate (Gobat et al. 2004). Enzymatic activities in the soil perforce energy transformations and are indispensable for nutrient cycling. Enzymes also play an important role in organic matter transformation processes as they allow both decomposition and synthesis of organic matter to take place (Gobat et al. 2004). Enzymes that catalyse the exact same reactions (izoenzymes) can be produced by

different organisms (izoenzymes can, for example, differ in their amino acid sequencing). Soil enzyme analysis, therefore, measures the activity of the sum of izoenzymes (Dick 1998).

Enzymes are catalysts of important soil functions (Dick 1998):

i. decomposition of organic compounds
ii. transformation of soil organic matter
iii. releasing of inorganic nutrients for plant growth
iv. N_2 fixation
v. detoxification
vi. nitrification and denitrification

Oxidoreductases, transferases and hydrolases are among the most studied groups of enzymes (Gobat et al. 2004). Oxidoreductase is an enzyme that catalyzes the transfer of electrons from one molecule, the reductant (also called the electron donor) to another, the oxidant (also called the electron acceptor). This group of enzymes usually utilizes NADP or NAD+ as cofactors. Transferases enact the transfer of specific functional groups (e.g. methyl or glycosyl group) from one molecule (called the donor) to another (called the acceptor). Transferases are involved in hundreds of different biochemical pathways throughout biology, and are integral to some of life's most important processes. Hydrolases are enzymes that catalyze the hydrolysis of a chemical bond and under specific circumstance, can also catalyze synthesis. All three groups are involved in the transformation of organic matter and in the making the inorganic nutrients accessible to plants (Gobat et al. 2004).

Amylases, cellulases, xylanases, glucosidases and invertases are essential for the carbon cycle, while proteases, amylases, ureases and deaminases are key enzymes in nitrogen transformations. Phosphatases and arylsulphatases participate in the phosphorus and sulfur compound transformations (Gobat et al. 2004).

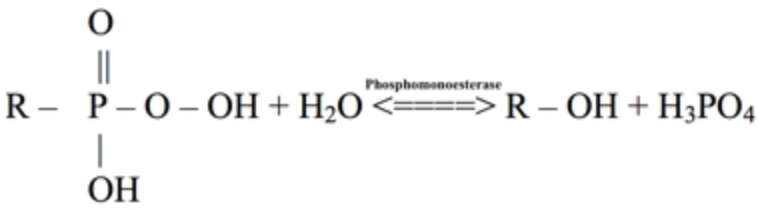
3.1.5.1 Acid Phosphatase (E.C. 3.1.3.2, Acid Phosphomonoesterase)

Phosphatases are amongst the most widely studied enzymes since they are directly related to the nutrient cycle of P. Phosphatases are formed inside many cells of living organisms and are designated for both intercellular and extracellular use (stabilized in soil suspension or bound to colloids) (Vodrážka 1996). Phosphatases

belong to the hydrolase family and together with lipases, tanases and lecithinases form a subgroup of esterases, which are responsible for fatty acid (complex esters) and phosphoric acid molecules breakdown (Horáková et al. 2007). Just as there are many different forms of organophosphates, many types of phosphatases can be identified. Phosphatases are generally divided into acid (pH optimum beween 4 and 6) and alkaline (pH optimum between 8 and 12) (Rejšek 1988). The majority of phosphatases enter the soil suspension after cellular lysis, while the rest is mainly released by living cells of microorganisms, fungi and in a smaller proportion by the root systems of higher plants (Rejšek 1999). Soil pH can largely determine the source of the enzyme, whether it is of bacterial origin (alkaline soil environments) or fungi derived (acidic soils) (Gobal et al. 2004).

Phosphatase can be divided into two groups with respect to their catalyzed substrates: enzymes specific to one type of substrate (e.g. phosphomonoesterases, phosphodiesterase and pyrophosphatase) and enzymes specific to multiple substrates (e.g. adenosine triphosphatase) (Horáková et al. 2007).

Equation 2 Phosphomonoesterases generally catalyze the hydrolysis of monoesters of phosphoric acid as shown in the scheme, where R stands for the organic compound group (e.g. p–NPP)



Limiting factors of phosphatase presence in the soil and its seasonal dynamics activity are: soil moisture; soil temperature; aeration and structure of soil; soil reaction; the content of inorganic and organic colloids in the soil; the sufficient presence of trophically important (for the nutrition of soil organisms) compounds; the quantity and the composition of soil cenosis; the vegetation cover; the quantity and quality of the organic matter in the soil; the presence of inhibitors (e.g. heavy metals, toxic compounds) and activators (Rejšek 1991).

3.1.5.2 Urease, (EC 3.5.1.5, Urea Amidohydrolase)

Urease activity is closely related to the nitrogen cycle and takes part in the decomposition of organic nitrogen in the soil and, therefore, makes nitrogen available to plants (Mikanová et al. 2010). Urease is constitutive, nickel-containing metalloenzyme

of high molecular weight (Horáková et al. 2007). It is a very stable enzyme of the terrestrial environment produced by microbial cells, fungi, algae as well as plant and animal cells (Horáková et al. 2007), which is involved in the hydrolysis of urea to NH_3 and CO_2 (Mikanová et al. 2010). In soil, it is often used in the evaluation of soil fertility. Urease is an enzyme generally specific to multiple substrates (hydroxyurea, dihydroxyurea and semicarbazide) (Horáková et al. 2007), and it hydrolyzes urea via carbamate (Mulvaney et Bremner 1977).

Soil ureases may be exhibited in two different states: intracellular, that is present within ureolytic cells (capable of CaCO3 production) of microorganisms or rarely in extracellular form released from disturbed plant and microbial cells (Mulvaney et Bremner 1977).

Equation 3 The urea hydrolysis scheme catalyzed by urease with ammonia and carbon dioxide being the end products

$$NH_2CONH_2 + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

Urease activity in the soil is very stable and hardly affected by desiccation, direct radiation or storage (at temperatures between -60 °C to + 22 °C). Urease activity in the soil does not correlate significantly with the content of the soil biomass, but can certainly be affected by heavy metals, extreme oxygen concentrations and low nitrogen content in various soil types (Horáková et al. 2007), substrate concentration, temperature, pH, and management type.

3.1.5.3 Catalase (EC 1.11.1.6, Hydrogenperoxide Oxidoreductase)

Catalase is one of the most efficient enzymes known found in nearly all living organisms exposed to oxygen (Guwy et al. 1999) In the soil, it is produced intracellularly by all aerobic bacteria and most facultative aerobes, but never by obligate anaerobes (Alef et Nannipieri 1995). Catalase activity in the soil is, therefore, considered an indicator of aerobic microbial activity and one of the major indicators of biochemical process intensity. Catalase secretion is a defense mechanism of living cells of soil biota to protect their environment from very effective and life threatening oxidant, hydrogen peroxide (H_2O_2). Soil predisposition of sufficient amount of active catalase is of high biological significance as described in detail by Nannipieri (1995). Oxidizer catalysts (responsible for H_2O_2 break down) are mostly bound to sorption

complex. Thus sorption complex of humic colloid particles plays a key role in microbial defence mechanisms (cells would not be able to react fast enough to such strong oxidizers) (Formánek et al. 2006).

Equation 4 The catalase reaction scheme of the decomposition of hydrogen peroxide

$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} 2\,H_2O + O_2$$

Catalase activity is strongly influenced by the sorption complex, soil reaction, iron, calcium and manganese content of the soil, as well as, abiotic and ecological factors (Formánek et al. 2006, Guwy et al. 1999).

3.1.6 Coppice, Coppice With Standard And High Forest Stands According To Kadavý et al. (2011)

3.1.6.1 High Forest Stands

High forest is a type of forest that originated from seeding, seedling planting or from natural regeneration. In contrast to a low forest (also known as a coppice forest), high forest usually consists of large, tall mature trees with a closed canopy characterized by generally long production period (rotation period averages about 100 years). High forests can occur naturally or they can be created and/or maintained by human management. High forest is the most commonly and widely implemented form of management in the forestry sector.

3.1.6.2 Coppice Forest Stands

The term coppicing stands for a traditional and historically widely used method (meeting high fuel wood demands) of woodland management, which takes advantage of the fact that many trees make new vegetative growth from the stump or roots if cut down. The rotation period of such stands is related to the soil properties of the site and can vary from 5 years (e.g. willow pollarding) to 40 years for oak, beech or hornbeam or up 60 years for alder.

The growth on coppice stands is initially faster thanks to available nutrients contained in living root systems, so the height and diameter increment culminates 20-30 years earlier than high forest. Harvested wood is, however, of significantly lower quality; it is knotty, curved at the bottom of the trunk and exhibits lower technical properties. Often repeated and almost complete removal of above ground biomass has a

pronounced effect on nutrient cycles, while short rotations maintain the forest sites in permanent state of regrowth and recruitment.

3.1.6.3 Coppice With Standard (C-W-S) Forest Stands

C-w-s's can be described as multi-storey production type forest stands, where the lower storeys are composed of coppices of vegetative origin and the higher storeys are made of individuals of different age composition of generative origin. C-w-s stands are generally formed by planting new trees (or leaving several selected coppiced standards) on the stand at the end of usual coppice rotation period (30 to 50 years). Resulting effect leads to creating 3 or 4 successive generations of standards (seed trees), each more or less the same age, above the newly established coppice storey of generative origin.

Broadleaf, shade tolerant species with vigorous sprouting potential are usually grown in the lower storey, such as linden, maple, elm, hornbeam, however, species that require more light are also utilized: oaks, horse chestnut, alder or ash. Upper storey is made of economically valuable trees, mostly oaks, but also of maples, elms, cherry trees or larch; possibly poplars and birches, where appropriate or required.

False c-w-s can be crated by leaving the best coppice individuals on the stand, thus false stemwood, allowing them to form even aged upper storey.

C-w-s forest stands are managed by retaining or planting individuals of seed origin at the end of each coppice rotation period. Silvicultural practices of c-w-s stand management are technically difficult and are based on maintaining an optimal relationship between the upper and the lower storeys, controlling the species composition, amount of standards and even canopy, etc. All interventions have to be made according to the economic objectives, with respect to site conditions and interspecies competition relationships.

C-w-s stands have been historically regarded as obsolete forest type as for their incapacity to meet maximum production site potential. However, various applied practices can show highly different outputs ranging from less than100 $m^3.ha^{-1}$ up to 400 $m^3.ha^{-1}$ of stemwood stocking.

3.2 The TARMAG II Research Site

The TARMAG II (Soběšice) site was founded at the turn of 2008 and 2009 near Brno (GPS coordinates: 49°14'42.629" N a 16°35'59.736" E) and is a sister site to it's slightly older sibling TARMAG (Hády) research plot (GPS coordinates: 49°13'29.87"N, 16°40'55.391"E). The TARMAG II site is situated in the Vranov Forest District, part of the Masaryk Forest Křtiny. The Training Forest Enterprise Masaryk Forest Křtiny (TFE), which is an organizational part of Mendel University of Agriculture and Forestry in Brno, manages the surrounding area and both site fall under its administration.

The TARMAG II research area is located circa 2 kilometers southwest off the village of Soběšice in the city of Brno district and geographically falls into the South Moravian Region. From the forestry point of view the plot belongs to Natural Forest Area No. 30 (NFA 30) – Drahanská vrchovina (NFA 30 is closely examined in separate chapter 3.3.3).

The basic purpose of the establishment of this research plot is to simulate the effect of coppice and coppice-with-standards (further referred to as c-w-s) forests on biodiversity. Results obtained by in-situ data collection and further analysis should contribute to the project of Ministry of the Environment of the Czech Republic's (MoE CZ) called 'Biodiversity and Target Management of Endangered and Protected Species in Coppices and Coppices-with-Standards Included in the System of NATURA 2000 (Kadavý et al. 2011).

3.2.1 The Site Before Conversion

The growth was 66 years of age according to applicable Forest Management Plan (2003 – 2012) in 2003, and was then described as even aged fully-stocked, single-storey with closed canopy (Kadavý et al. 2010). The predominant forest cover type on the plot is described according to Czech forest typology as 1B1 (rich hornbeam-oak steppe on plateaus and rounded ridges with mountain sedge: *Carex montana*), which can be described according to Natura 2000 classification as type 91I0 Euro-Siberian steppe oak woods; subtype L6.4: basophilic and xeric oak forest stands. The forest vegetation belongs to management set of stands No. 205 (exposed oak stands of special purpose at lower altitudes) with a rotation period of 130 years and a regeneration period of 30 years. The 80C7 forest stand could be characterized prior to conversion as taking form of false stem wood and was included in the 2003 – 2012 Forest Management Plan as high forest (Kadavý et al. 2010).

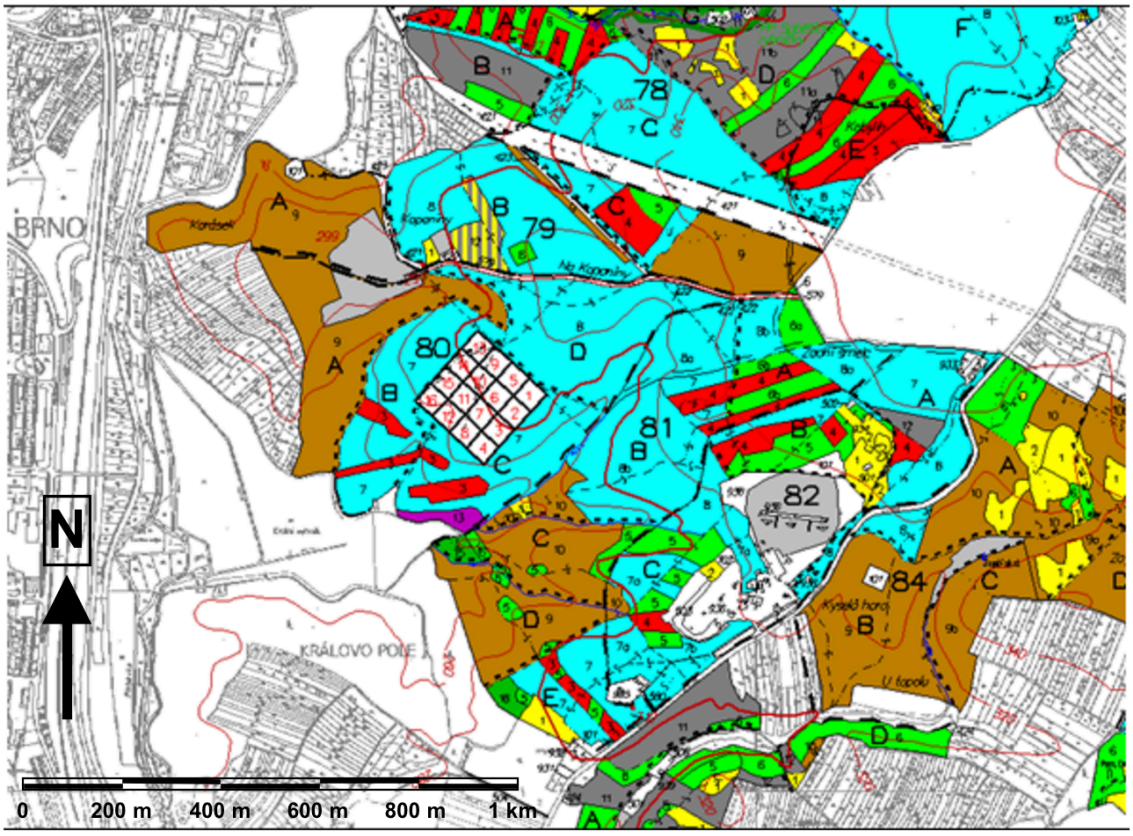


Figure 1 Site location (cell grid) in the vegetation map of the 2003 Forest Management Plan

Table 1 shows individual species composition on the TARMAG II research plot before its conversion. It is evident that sessile oak (*Quercus petraea*) was the dominant species on the site representing 96 % of the total number of trees and also 95,9 % of volume measured over bark. Second most frequently occurring species was Scots pine (*Pinus sylvestris*) with mere 2 – 3 % of standing volume. Fifteen different types of woody species were monitored on the plot. An average of 716 of stems were counted per hectare and an average volume of 232 m^3 was measured over bark (179 m^3 debarked).

The DBH (Diameter at Breast Height) structure of the site is shown in Figure 2. According to Kadavý et al. (2010), data from the period before the intervention show the presence of two strata with diameter frequency peaks at approximately 9 (youngest storey) cm and 23 (mid storey). In addition to the two younger strata older individuals from the former coppice stand can be traced on the site. Their DBH averages about 36 cm (oldest storey). The presence of different age classes was respected when marking potential seed trees. The majority of later selected seed trees (standards) belong to the higher spectrum of DBH classes.

Table 1 Species composition on the TARMAG II plot before conversion (o. b. stands for over bark)

Species	Count (no. of stems)	Occurrence (%)	Volume (m^3 o. b.)	Representation (% of m^3 o. b.)
Quercus petraea	2707	96,0	889,82	95,9
Pinus sylvestris	44	1,6	22,48	2,4
Carpinus betulus	43	1,5	2,38	0,3
Pinus nigra	13	0,5	5,04	0,5
Tilia cordata	13	0,5	2,30	0,2
Larix decidua	13	0,5	3,62	0,4
Quercus robur	9	0,3	1,93	0,2
Acer campestre	7	0,2	0,10	0,0
Prunus avium	4	0,1	0,27	0,0
Sorbus torminalis	3	0,1	0,17	0,0
Acer platanoides	2	0,1	0,07	0,0
Tilia platyphylos	2	0,1	0,02	0,0
Pyrus communis	1	0,0	0,00	0,0
Picea excelsa	1	0,0	0,02	0,0
Pinus strobus	1	0,0	0,01	0,0
SUM	**2863**	**100,00**	**928,23**	**100,00**
Average/ha	**716**	**N.A.**	**232,06**	**N.A.**

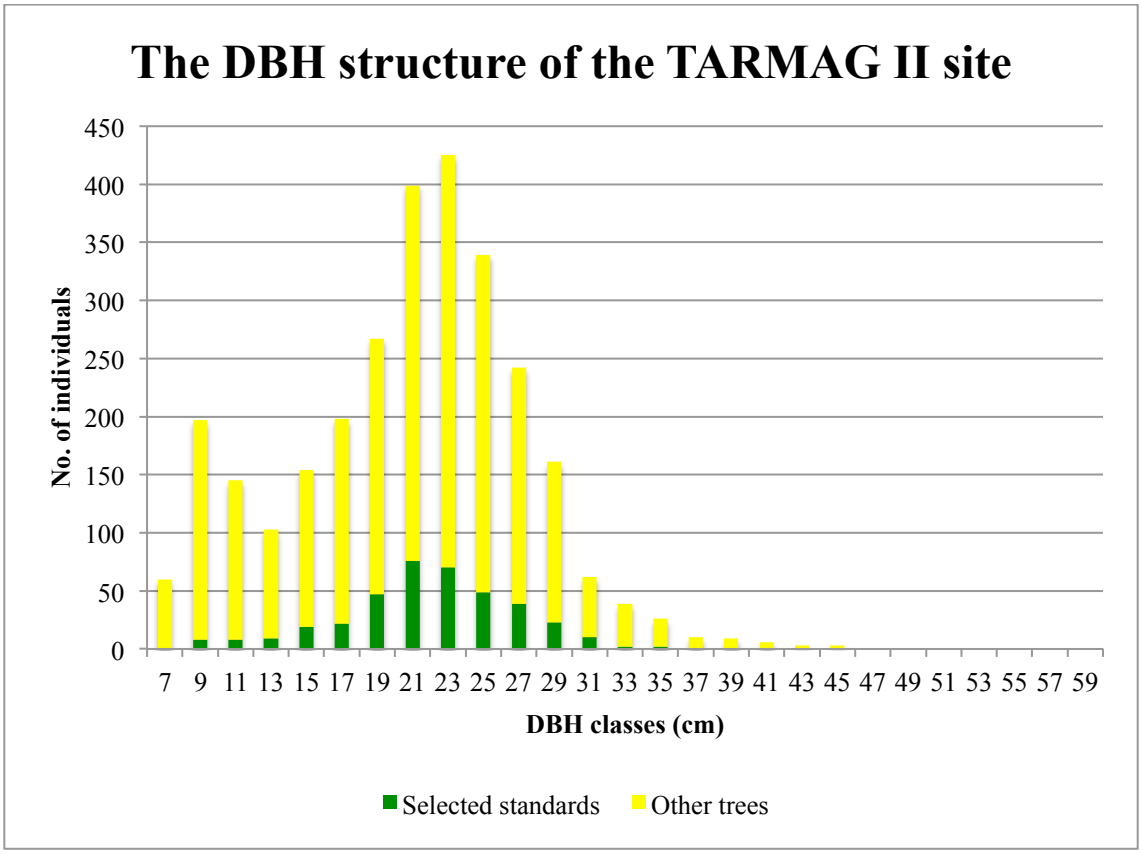


Figure 2 Number of selected standards from all individuals in each DBH class on the TARMAG II site

A standard should meet general qualitative requirements according to Konšel 1931 and Utinek 2004 and those are: (a) perfect health condition (no dead branches in the crown, no surface wounds), (b) at least 6 m long straight trunk with no branches, (c) a dense, long and healthy crown.

3.2.2 Experimental Plot Design And Establishment

The TARMAG II research site is 4 ha in size. The structure of the design replicates the one used in setting up the first TARMAG research area and follows similar pattern. The site covers an area of 200 by 200 m and is divided into 16 cells (50 by 50 m each). Four neighboring cells constitute an area of 100 × 100 m within which four variants of different felling intensity and therefore of varying number of standards are represented.

As shown in Figure 3., yellow color indicates clear cut (cells No. 1, 3, 9 and 11), light green color depicts very high felling intensity (cells No. 6, 8, 14 and 16), mid-green color indicates high felling intensity (cells No. 5, 7, 13 and 15) and dark green color depicts the medium high (lowest applied) felling intensity (cells No. 2, 4, 10 and 12). Furthermore, control plot (black) was established in neighboring forest stand.

The same rules as in the first TARMAG site were also applied to identify the potential standards (seed trees). In all cells, the position of every living tree with DBH larger than 5 cm has been surveyed and recorded in the project dataset along with its species code, DBH, total tree height, and the bottom of the living crown height (Kadavý et al. 2010). The control area, created adjacent to the experimental site, effectively does not have a precisely defined geometric shape since it was only established by selecting and measuring 79 trees of different storey levels suitable for the study (corresponding with the composition of the structure of the tree inventory on the experimental plot and adhering to the rules for the indication of potential standards) (Kadavý et al. 2010).

Selected characteristics of each plot are shown in Table 2., which summarizes the initial stocks, felling volumes, post-logging volumes, and timber extraction intensity (derived from stocks). It has to be noted, that during the conversion of the plot all woody vegetation even of shrubby character was removed from the plot unless previously selected as future standards. According to the felling intensities, on average 24, 35 and 45 future standards were marked in cells with very high, high and medium high felling intensity, respectively (Kadavý et al. 2010). Very high felling variant may

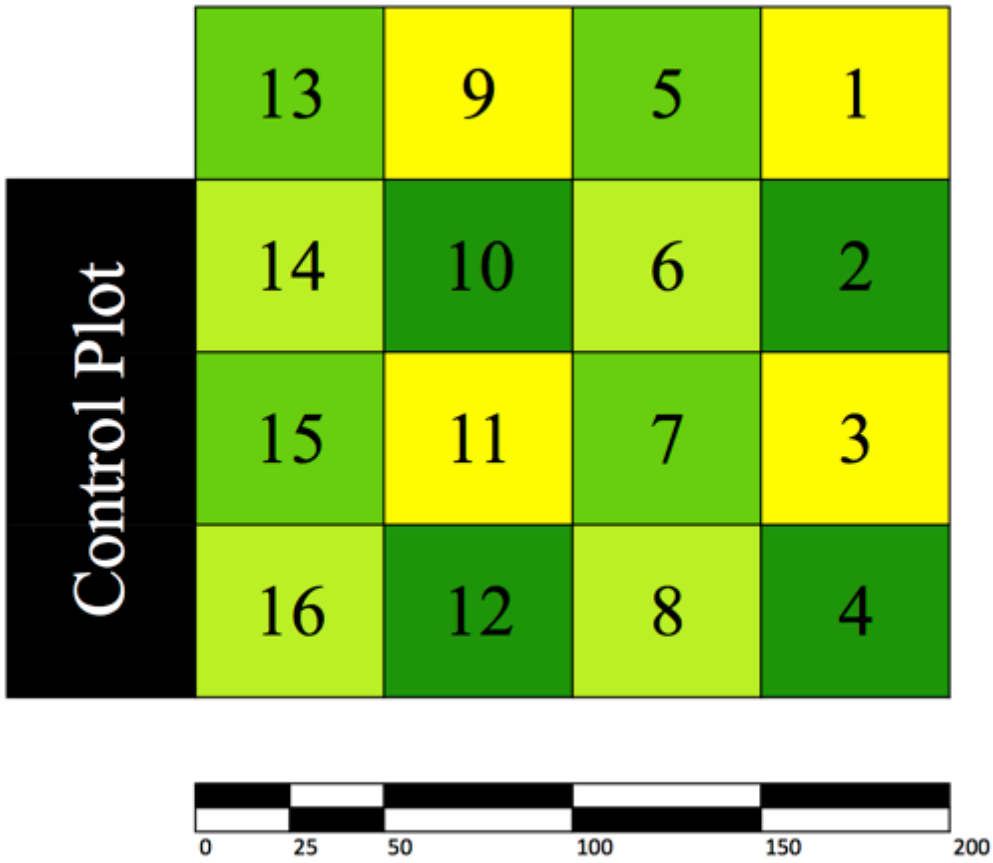


Figure 3 TARMAG II plots with a scale in meters divided in terms of felling intensity where yellow colour represents clear cut, light green colour depicts very high felling intensity, mid-green colour indicates high felling intensity and dark green colour stands for medium high felling intensity. Control plot is marked in black

be described as c-w-s with low standing volume and a small number of standards, high felling variant may be characterized as c-w-s with standard standing volume and an average number of standards and the medium-high felling intensity may be perceived as c-w-s with standard standing volume and a high number of standards (Kadavý et al. 2010).

It was attempted in all cells to achieve a 1:3 ratio between the number of standards in the thicker-older and the thinner-younger storey to mimic a well established c-w-s. Absolute majority of individuals selected (marked with green ribbon prior to felling) as standards were sessile oak – *Quercus petraea.* The logging took place at the turn of 2008 and 2009 as mentioned before and was performed as whole tree harvest. Undesirable trees were cut down and whole trees were transported out of the plot. Logging residue was minimal (Kadavý et al. 2010).

The resulting research area can, therefore, be viewed form two perspectives: as a matrix of plots where each plot was subjected to different logging intensity, but also as an area with two dominant management practice subsets, where two types of forest can be found: namely coppice (clearcut plots) and c-w-s (all other plots). Setting up the other-than-clearcut plots drew on general principles of c-w-s management. The achieved state is described as satisfactory despite the fact that only two storeys were formed after the logging.

Table 2 Selected chracteristics of all TARMAG II plots. Standing volume of all trees was calculated using volume equations published by Petráš and Pajtík (1991). (o. b. stands for over bark and deb. for debarked, logging intensity is defined by the percentage rate of felling from the total volume)

PLOT No.	Initial Volume		Volume of selected standards		Volume of logged timber		Logging intensity
	(m^3 o. b.)	(m^3 deb.)	(m^3 o. b.)	(m^3 deb.)	(m^3 o. b.)	(m^3 deb.)	(%)
1	**68**	**52**	**0**	**0**	**68**	**52**	**100**
2	**61**	**46**	**13**	**10**	**48**	**37**	**79**
3	61	46	0	0	61	46	100
4	59	45	15	11	44	33	75
5	57	45	9	7	48	38	85
6	54	41	11	8	43	33	80
7	54	41	9	6	46	35	84
8	56	42	11	9	44	34	79
9	**64**	**49**	**0**	**0**	**64**	**49**	**100**
10	**52**	**40**	**18**	**14**	**34**	**26**	**65**
11	53	41	0	0	53	41	100
12	64	50	25	20	39	31	61
13	61	49	12	9	49	40	80
14	54	42	12	9	42	33	78
15	53	41	10	8	43	33	81
16	58	45	14	11	44	34	76
Total	**928**	**716**	**159**	**122**	**769**	**594**	**N.A.**

3.3 Wider Territorial Relationships

3.3.1 Climatic Conditions

It is quite difficult to correctly place the site in terms of climate. The studied area lies on the borders of two different climatic regions designated by Quitt (1971). Quitt's climatic classification (widely used in forestry) places the plot right on the edge of climatic region MT11, closely neighboring with climatic region T2.

The MT11 climatic region can be described as moderately warm and its characteristics are: long, dry summers, short transitional periods between seasons, moderately warm springs and autumns, dry and short moderately cold winters with short duration of snow cover (QUITT 1971). The characteristics of the T2 climatic region are very similar and slightly differ in the descriptions of some temperatures and precipitation (T2 is overall slightly warmer with drier winters): summers are long, hot and dry, moderately warm to warm springs and autumns. Winters are short, moderately warm and dry to very dry with a very short duration of snow cover (QUITT 1971).

Table 3 Climatic chart according to Quitt (1971) and with classes applicable to the TARMAG II site where summer days stand for a day in which temperature reaches or exceeds 25 °C, frosty day stands for a day in which temperature reaches or falls bellow 0 °C and icy cold day is a day in which temperature stays bellow 0 °C all day

Climatic region	T2	MT11
Sum of summer days	50 – 60	40 – 50
Sum of days with average temperature of at least 10 ºC	160 – 170	140 – 160
Sum of frosty days	100 – 110	110 – 130
Sum of ice cold days	30 – 40	30 – 40
Average temperature in January (ºC)	-2 to -3	-2 to -3
Average temperature in July (ºC)	18 – 19	17 – 18
Average temperature in April (ºC)	8 – 9	7 – 8
Average temperature in August (ºC)	7 – 9	7 – 8
Average amount of days with at least 1 mm of precipitation (mm)	90 – 100	90 – 100
Total precipitation during the vegetation period (mm)	350 – 400	350 – 400
Total precipitation during the winter period (mm)	200 – 300	200 – 250
Sum of days with snow cover	40 – 50	50 – 60
Sum of overcast days	120 – 140	120 – 150
Sum of days with clear skies	40 – 50	40 – 50

3.3.2 Geomorphology, Geology, Soils And Topography

According to the geomorphological division of the Czech republic the site can be described as follows and belongs to:

System: Hercynian

Province: Bohemian Massif

Sub province: Czech-Moravian System (II, Česko – moravská subprovincie)

Region: Brno uplands (IID, Brněnská vrchovina)

Unit: Drahanská uplands (IID – 3, Drahanská vrchovina)

Subunit: Adamov uplands (IID – 3A, Adamovská vrchovina)

Precinct: Soběšice uplands (IID – 3A11, Soběšická vrchovina)

The site is located within the Brno Massif in terms of geology where the parent materials are mainly amphibolic granodiorites, diorites and sometimes even old matabazites (diabases). The massif is tectonically deformed and differs from the Variscan massifs lying to the west. Devonian conglomerates and grained siliciclastic sedimentary claystones significantly protrude in this area west to the edge of the Moravian Karst. Aeolian blankets are typical for the whole of Brno area and their effective thickness can be of very significant drift depth. Fairly widespread are sandy clay loam deluvial slope sediments. Alluvial sand and gravel drifts are of lesser importance (Culek et al. 2005).

The World Reference Base system of soil classification updated in 2006 by the Food and Agriculture Organization is to be used to describe the soils of the target area: Haplic Luvisols to Chernozems on loess drifts in depressions can be found in the Soběšice region as well as Cambisols and Albeluvisols on slopes and foothills. Rocky valleys and steep slopes of the area are overlaid by a number of soils. These soils are strongly influenced by its parental substrate and can range from lithic Leptosols to Leptosols or rendzic limestone Leptosols. Areas with limestone outcrops, especially on slopes, can feature browned (decalcified fine earth soil segment) rendzic Leptosols crossing over to Cambisols. Haplic Luvisols on loess and loess clay are an important aspect on upland platforms. Relict karst soils such as Terra Fusca and Terra Rossa are good representatives of places where the limestone base is not overlaid with loess (Culek et al. 2005).

The overall declination of the region is from north to south, most slopes are, therefore, south facing. The topography is formed by ridge faults and fault-line gaps, while numerous rocky valleys developed across the fault-line gaps. The deepest river valley is cut out by the Svitava flow, where the elevation difference averages about 300 meters. Other rivers valleys are from 100 m to 200 m deep. Strong valley phenomenon has developed on the Svitava and Svratka watercourses and that, together with diverse geological base and heterogeneous topography, leads to the overall increase in biodiversity of the region. The relief of the region has a character of flat highlands with altitudinal differences of 150 m to 200 m. Some deep cut fault-lines near tall ridges exhibit the nature of rugged highlands with altitudinal segmentation of 200 m to 300 m (Culek et al. 2005).

3.3.3 Natural Forest Area

Natural Forest Area (NFA) represents one of the applications of forestry typology. NFAs are territorial units delimited mainly according to: more substantial differences in parent material conditioning the soil characteristics (geology and pedology), differences in the configuration of the terrain in geomorphologic units (orography), differences in microclimate (climate) and thus in the occurrence of forest communities (phytogeography) (UHUL 2016).

The TARMAG II site falls into NFA No. 30 as mentioned before and is one of the 41 Czech Natural Forest Areas. It spans 157914 hectares of Czech cadastral area (2,74 % of the Czech republic) and its forest cover is estimated to be 55,4 % (UHUL 2016). There are numerous reasons behind having such a high proportion of forest cover. Firstly, it is a hilly region with sharply cut valleys and deep incised glens bearing a character of uplands or highlands. Secondly, it is partially overlapped by Protected Landscape Area Moravský Kras (Moravian Karst) and thirdly it is the home to Training Forest Enterprise Masaryk Forest Křtiny of Mendel University in Brno stretching over 10 265 ha. The NFA is furrowed by hills and its nature is shaped by Drahanská vrchovina, Konická vrchovina as well as Moravian Karst of Devonian origin and also partly by Adamovská vrchovina. In regards to prevailing geomorphological elements broken highlands of folded and faulted structures would have to be mentioned as the dominant feature pieced together by intrusive igneous rocks of Czech highlands. The prevailing soil types are Cambisols, Luvisols and rendzic Leptosols, whereas metamorphosed sediments, such as claystone, slate or limestone, mostly create the base or bedrock. The NFA is clearly distinguished from its surroundings as it is bordered by the Moravian Ravines in the East and the South, and geomorphologically different formations or units (Českomoravské mezihoří, Zábřežské kristalinikum, e.g.) in the North. Its eastern border is formed by Řečkovicko – Kuřimský prolom: a narrow and relatively long fault structure created by sub-parallel decline.

The inner heterogeneity of the region can easily be related to very diverse range of forest communities that can be found within the NFA. Forest vegetation belts range from oak to spruce-beech and that of course has to be substantiated by differences in average annual temperature range (5 – 10 °C) and total yearly precipitation (500 – 750 mm).

3.3.4 Biogeography And Fytogeography

Culek et al. (2005) subdivided the Czech Republic for purposes of national and regional ecological networks. This partitioning has its own hierarchy and consists of typological biogeographical units. Higher units (province, subprovince, bioergion) are of individual character (regional zoning), lower units (biochora, groups of geobiocoene type) are of typological character. Higher units are:

Province - Central European Broadleaf Forests

Subprovince - Hercynian (Hercinská)

Bioregion – Brno (Brněnský)

Floristic composition of the Brno bioregion corresponds with its location. The bioregion is situated close to the edge of the Hercynian subprovince (bordering with the Western-Carpathian and Northern-Pannonian subprovinces). Typical Hercynian (Central European) character is expressed mainly through forest vegetation composition. The basis of undergrowth synusia is typically demonstrated by the presence of sweet woodruff (*Galium odoratum*), bulbiferous coralwort (*Dentaria bulbifera*), european wild ginger (*Asarum europaeum*), greater stilchwort (*Stellaria holostea*), baneberry (*Actaea spicata*), dog's mercury (*Mercurialis perennis*), scotch mist (*Gallium sylvaticum*), wood melick (*Melica uniflora*) and common hepatica (*Hepatica nobilis*) (Culek et al. 2005). Ambroz and Štykar (1999) have also listed other common herbs typically belonging to this bioregion: blackening flat pea (*Lathyrus niger*), Hungarian iris (*Iris variegata*), purple gromwell (*Aegonychon purpurocaeruleum*), touch-me-not balsam (*Impatiens noli-tangere*) and wood sorrel (*Oxalis acetosella*).

Brno bioregion can be influenced, due to its location, by migrant plants from the Carpathian subprovince such as: pendulous sedge (*Carex pendula*), thor (*Hacquetia epipactis*) and wood spurge (*Tithymalus amygdaloides*) or by plants of the Pannonian origin that can be mostly found localized on limestone islets: pubescent oak (*Quercus pubescens*), yellowhead (*Inula oculus-christi*), true narowleaved sedge (*Vignea stenophylla*), golden feather grass (*Stipa pulcherrima*) and yellow flax (*Linum flavum*) (Culek et al. 2005).

4 Materials & Methods

4.1 Studied Area

The TARMAG II research plot is subdivided into 16 subplots of 4 different variable classes of intervention intensity, therefore, each variable is replicated four times over within the studied area (as shown in Figure 3 on page 23). The aim of this thesis was to pinpoint and evaluate only the differences between the two 'extremes' of the current design. That being the subplots with maximal logging intensity (100%) mimicking coppice stands (clearcuts) and the subplots with minimal logging intensity, or else as described by the authors of the design as plots with medium high felling intensity (65 – 79%), simulating coppice-with-standard forest sites.

Two representatives of each class were chosen based on the topology of the site. Topologically most homogenous sites, with similar orientation and least amount of sloping, proved to be subplots 1 and 9, exemplifying the coppice stands, and subplots 2 and 10, chosen as the representatives of c-w-s forest stands. Two new control sampling sites replaced the existing control plot, again, mainly because of the site

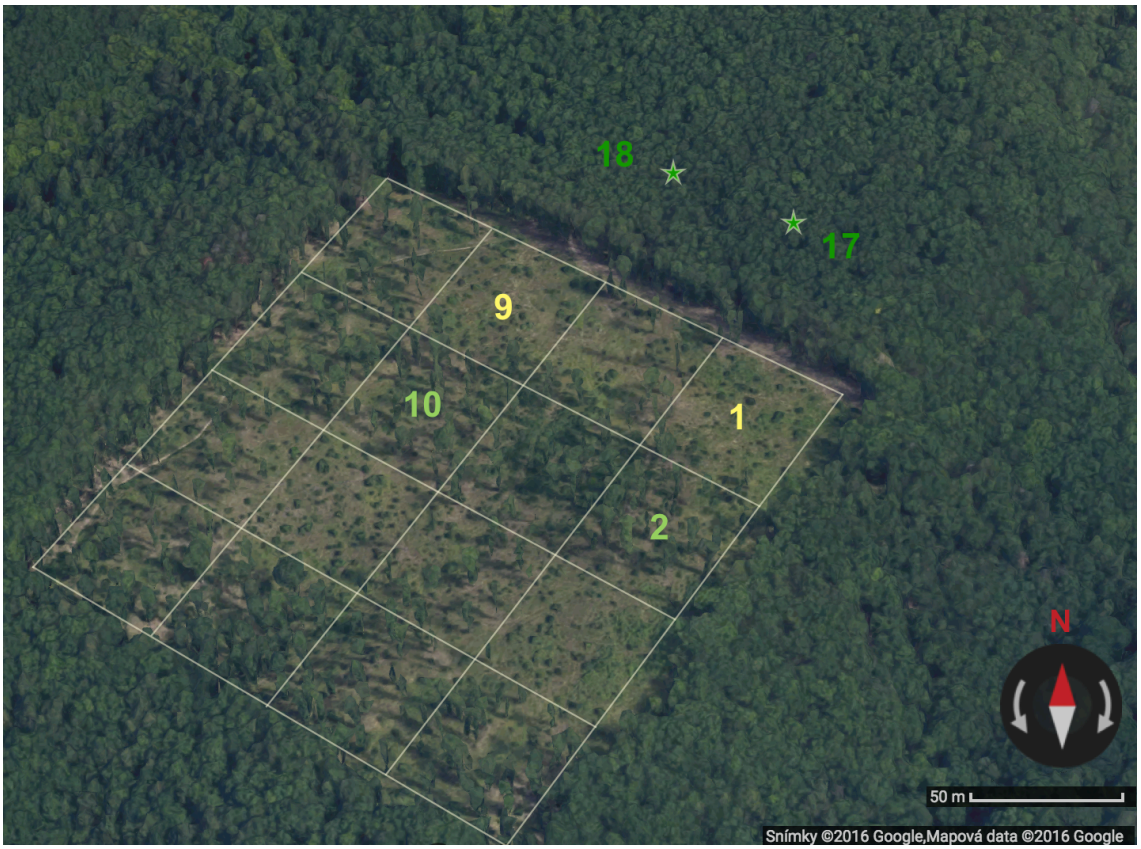


Figure 4 Examined subplots (1,2,9 and 10) of the TARMAG II research plot in spatial relationship to newly set up forest stand control plots (17 and 18)

topology and homogeneity, but also because of vicinity. These new high forest control plots, marked as subplot 17 and 18, are located about 50 m off the current site design in the northeastern direction. Subplots 17 and 18 (17: 49°14'46.4"N 16°36'06.3"E; 18: 49°14'45.5"N 16°36'08.5"E) are intentionally placed further into the neighboring forest stand (not directly adjacent to the TARMAG II site) in order to eliminate the possible edge effect and to offer the closest possible analogy of the pre-intervention state.

4.2 Sample Collection

Soil samples from the TARMAG II research plot were collected within three individual time periods during the years of 2015 and 2016. Each time period was to represent distinct vegetative phase of the temperate forest biome, typical for central Europe, and was to follow vernacular seasonality in order to be able to compare the characteristics of soil microbial biomass and activity dynamics between the beginning and the end of growing season and the period of vegetative dormancy.

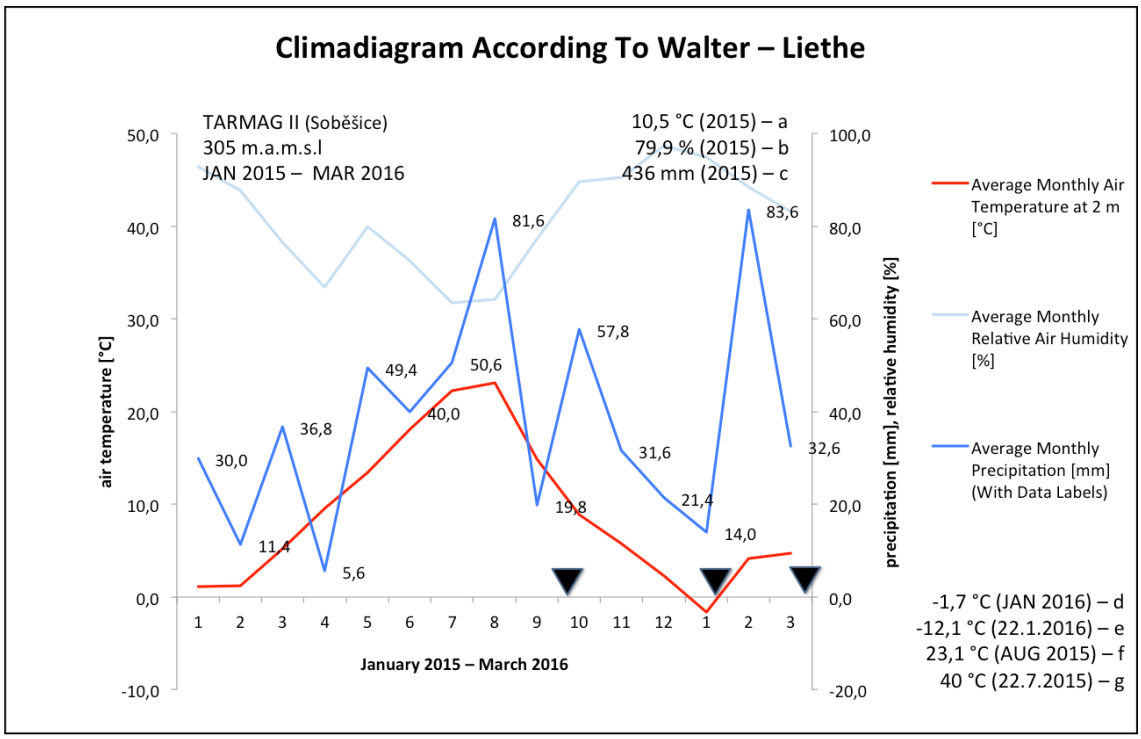


Figure 5 Climadiagram according to Walter – Liethe for the TARMAG II research plot in Soběšice with marked periods of soil sampling (▼) showing climatic data for 2015 and the beginning of 2016, where a stands for the average annual temperature for the year of 2015 [°C], b is for average relative air humidity (2015) [%], c for total annual precipitation (2015) [mm], d for the coldest month with lowest measured average temperature [°C], e for the lowest temperature recorded on the plot [°C], f for the warmest month with the highest monthly average measured [°C] and g for the highest temperature recorded on the plot [°C]

First round of sampling was performed at the beginning October 2015 (12.10.2015 – 13.10.2015) at the end of the growing season. Average daily temperatures preceding the first soil sampling were 13,5 °C (12.9.2015 – 12.10.2015). The first soil sampling followed a pronounced period of drought with minimal amount of precipitation of 4,8 mm (12.9.2015 – 12.10.2015). Second sampling took place on 28.1.2016, during the winter season with average daily temperatures of -2,2 °C (29.12.2015 – 28.1.2016). It should be noted that the topsoil on the TARMAG II site was frozen at the time of collection (forest control plots under thicker canopy cover and thicker litter layer weren't showing signs of freezing through). Third sampling was performed on 24.3.2016 and 25.3.2016 at the start of the growing season after the spring bud break. Average daily temperatures preceding collection were 3,8 °C with daily highs regularly exceeding the 10 °C mark.

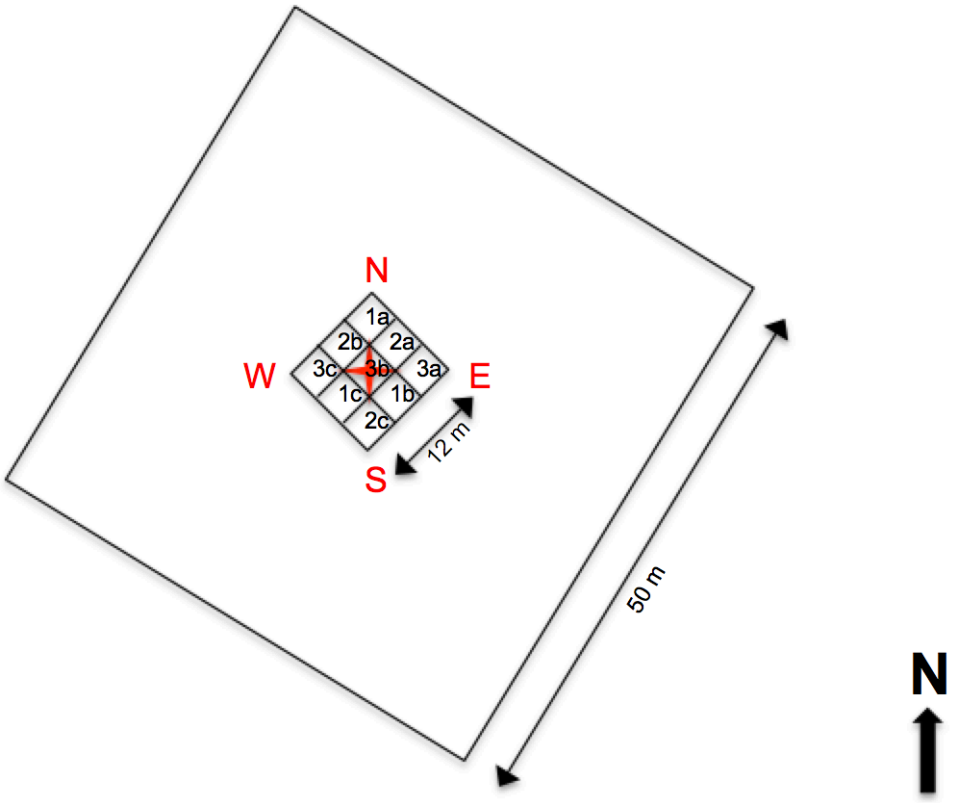


Figure 6 Topographical representation of the systematic approach to sample collection for all studied subplots based on randomized Latin square experimental design, where the large square denotes each subplot and its orientation to north, the subdivided smaller square indicates sampled area oriented according to cardinal directions (numbers in each Latin square stand for sampling periods: 1 autumn, 2 winter, 3 spring and the letters denote sampling points), the red cross is for the subplot center

Samples were collected systematically according to simple randomized Latin square design as shown in Figure 5, where each individual square was 4 by 4 meters of size. The sampling sequence, hence the pattern, was replicated for each studied subplot. Each subplot is marked in situ with a wooden stake. Finding the centers of the Latin squares was simply carried out with a compass (adhering to cardinal directions) and a scaled rope. Soil samples were extracted as close to the center of each Latin square as possible (avoiding trees, stumps and large root systems). Each subplot was sampled at three points marked a, b and c and at two depths (0 cm and 10 cm). The total of four square-representative samples was collected at each square consisting of two metallic cylindrical core samples and two bagged samples (loose soil in airtight plastic bags). Mixed, subplot-representative, soil samples were also taken. Mixed soil samples were created by thoroughly blending equal quantities of soil samples from all three points of each subplot with respect to sample depth. Mixed samples were stored in plastic flasks. All soil samples were taken to the Mendel University pedology lab immediately after the extraction where refrigerated (during their storage) at about 4 °C.

Figure 7 Typical preparations for soil sample collection at 10 cm depth. Sample ID clarification (exemplary case 1-9a10 and 1-9s10): first number stands for sampling period (1 – autumn), second number denotes subplot (9), the letters a and s are for sampling point and mixed sample identification respectively and the third letter (10) marks the depth of sampling. Note how dry the soil profiles were during the first sampling

4.3 Soil Description Of Studied Area

All of the six studied subplots of the TARMAG II research area can be classified, in terms of soil type, as **cambisol**, subtype **haplic**. Admixtures of small amounts of aeolian material were found when sampling profiles with hammerhead soil probe. Photos of individual soil profiles are enclosed in APPENDIX 1.

Soil profile description according to doc. Mgr. Aleš Bajer, Ph.D. (Mendel University in Brno, Department of Geology and Pedology):
Type: cambisol
Subtype: haplic

All six sites (4 subplots and 2 control plots) exhibit the same sequence of soil horizons. There are slight differences in the thickness of individual horizons and overall physiological depth according to exposure and microgeomorfology (sloped areas or flats). In all cases, the soil has developed on weathered granodiorite of the Brno massif with more or less distinct aeolian admixture.

Horizon sequence:
Ah - dark gray-brown, organomineral horizon

Bv - brown, significantly brown to rusty brown horizon of soil weathering processes, sandy loam

Bv / IIC - transition of cambic horizon into weathered granodiorite, most probably in situ, or just slightly shifted downslope, limited presence of aeolian admixtures

Figure 8 An example of hammerhead soil probe and recovered sample.

4.4 Sample Handling And Data Harvesting

It was imperative to analyze most samples within the first week after their collection (where applicable) to eliminate possible deterioration of the soil samples or the results. To prevent sample deterioration, collected soil specimens were refrigerated at 4 °C during their whole storage process. Special attention was paid to the possible drying out of the samples (kept in air tight containers or sealed plastic bags) and freezing over (preventing the soil samples from touching the sides of the refrigerator). The principles of sample collection and handling are closely described within the International Standards: ISO 10381-1:2002 and ISO 10381-2:2002

Data for statistical evaluation were obtained through several laboratory methods and analysis.

Physical properties of soil were determined with the application of regular laboratory methods onto metallic core cylinders and (later on for the same samples) with the aid of pycnometers. Both procedures were performed according to general guidelines used at the Faculty of Forestry and Wood Engineering of Mendel University in Brno provided for practical exercises by Rejšek (1999).

Selected properties of soil (pH, loss on ignition, total organic carbon content) were assessed individually for each sampling location utilizing samples collected into airtight plastic bags.

Total microbial carbon content and all studied enzymatic activities (as well as dry matter content essential for result calculation) were examined twice utilizing mixed, subplot-representative, samples. These samples were first analyzed as fresh soil samples, but the same analyses were repeated later, as for the samples were being subjected to one-month period of incubation.

Microbial respiratory activities of soil were determined from the mixed samples (similarly to microbial activity assays) purely after a two-month period of incubation in order to prevent the inclusion of root respiration activity.

Total soil carbon and nitrogen contents (and their ratio) were determined in April 2016 as an additional supportive analysis by RNDr. Ida Drápelová with the use of LECO TruSpec CN soil analyzer (LECO Corp., St. Joseph, MI). These measurements were performed for the first and last sample collection.

4.5 Review Of Applied Laboratory Methods

This chapter offers only principal descriptions of used laboratory methods. Detailed step-to-step procedures of each utilized analysis can be found in APPENDIX 2.

4.5.1 Physical Properties Of Soil

4.5.1.1 Cylindrical Core Method

Cylindrical Core Method is widely used to determine bulk density and other physical properties of soil, but can be also applied to calculating water and air regime of soil. The main device of this analysis is so called Kopecky's physical cylinder usually made from stainless steel of inner volume of 100 ml and maximum height of 5 cm. Cylinders, also known as core cutters, are fully driven (usually hammered) into straightened soil profile with the aid of steel rammers. Cylinders are properly labelled after excavation. Samples should be taken directly to the lab and refrigerated if not being processed straight away.

4.5.1.2 Determination Of Specific Gravity Of Soils

Applied soil analyses of specific gravity is usually described as the ratio of the weight of any volume of soil material to the weight of any volume of water. Calibrated 100 ml pycnometers are utilized in the process of specific gravity determination. Oven dried soil samples are boiled in pycnometers to expel air content and weighed. Resulting weight is subsequently compared to the weight of pycnometer filled with water only.

4.5.2 pH Determination

Soil pH was measured in agreement with Zbíral's methodology published in Analýza půd I (2005) and that is also in accordance with a current international standard ISO 10390:2005 and its Czech counterpart national standard ČSN ISO 10390 (836221). Applied method of routine determination of pH is using a glass electrode in a 1:5 (volume fraction) suspension of soil in water (pH in H_2O) and of potassium chloride solution (pH in 1 M KCl). This procedure should be applicable to all types of dry soil prepared according to ISO 11464:2006 (Soil samples were dried in an oven at temperature not exceeding 40 °C, subsequently crushed and sieved resulting in fraction smaller than 2 mm).

Soil pH is an important measurement to assess potential availability of beneficial nutrients and toxic elements to plants. Soil reaction evaluates the amount of active hydrogen ions (H^+) in soil suspension. According to the nature of the suspension, different kinds of pH can be distinguished: actual (active) soil reaction (pH in H_2O), potential (exchangeable) soil reaction (pH in KCl).

4.5.3 Dry Matter (DM) Determination

The principle of dry matter (DM) determination in soil is based on heating soil samples to temperatures at which water is evaporated (Rejšek 1999). The results are calculated from the weight difference of samples before and after drying and are expressed in grams of dry matter or in percent (dry matter content per fresh soil weight).

4.5.4 Determination Of Loss On Ignition (LOI)

The LOI method is used in accordance with JPP operational methods last updated in 2011 by Central Institute for Supervising and Testing in Agriculture in Brno (ÚKZÚZ) and is based on unified national norm (ČSN EN 15935 (838126) and, not to say, the international European standard EN 15935:2012). It is to estimate the organic matter content in sludge, treated biowaste, soil and waste.

Dried test sample is furnace heated to constant mass at (550 ± 25) °C. The difference in the mass before and after the ignition process is used to calculate the loss on ignition (LOI). LOI is expressed as weight percentage of dry mass (DM).

4.5.5 Determination Of Organic Carbon In Soil (C_{ox})

Method of spectrophotometric determination of organic carbon in soil (C_{ox}) by sulphochromic oxidation applied in the process of estimating the total amount of organic carbon is of normative number 30911 by Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ) (Zbíral et al. 2011).

Soil samples are prepared in accordance with the ÚKZÚZ standardized methods (Zbíral 2002). This involves air drying the samples, sieving out fractions larger than 2 mm, partitioning into smaller amounts (up to 10 g), removing all plant and animal residues and triturating in an agate mortar grinder so as to entirely pass through a sieve of 0,1 mm fraction size.

Oxidizable organically bound carbon in soil is oxidized by the surplus of potassium dichromate solution in sulfuric acid medium at 135 °C. Yellow-orange dichromate ions are reduced to green colour Cr^{3+}. The intensity of green colour is

measured spectrophotometrically. Glucose is used as a standard for calibration. Resulting values represent the volume of oxidizable carbon per one kg of soil dry matter: C_{ox} ($g.kg^{-1}$).

4.5.6 Determination Of Soil Carbon (C_{tot}) And Nitrogen (N_{tot}) Contents

Total of organic soil C content [%], total N content [%] and their ratio (C:N) were determined using LECO TruSpec CN soil analyzer (LECO Corp., St. Joseph, MI) at Mendel University (Department of Geology an Pedology). The LECO TruSpec CN elemental analyzer operates on the principle of dry combustion of samples in an oxygen atmosphere, where helium of a purity of 4,8 % serves as the carrier gas, medical grade oxygen (purity 5,0) as the combustion gas and air as the pneumatic gas. Combustion takes place in a U-shaped quartz tube. Samples are let inside the preheated quartz tube, in which the combustion takes place at high temperature in a stream of pure oxygen. Temperatures range between 950 °C ± 10 °C in the input arm and 850 °C ± 10 °C in the output (burn out) arm. A separation process of ash and combustion gases takes place in the quartz tube. Burnt-out gasses are carried further into the detection system, where C_{tot} is determined form the CO_2 content of the flue gases by an infrared detector and N_{tot} is calculated from the nitrogen oxides content in the flue gas. N_{tot} determination is carried out in a reduction column by a thermal conductivity detector, where NO_x is quantitatively converted to N_2 with the aid of a catalyst after the separation of water vapor and CO_2.

Soil samples, similarly to C_{ox} analysis, are prepared in accordance with the ÚKZÚZ standardized methods (Zbíral 2002) and again, prior to the analysis, dehydrated for 1 hour at 105 °C. Samples are weighed into special tin foils. Initial sample weight of 100 -200 mg has to be increased for lower mineral horizons. Blind samples and standards (with known content of analyzed elements) have to be analyzed simultaneously. Standard samples used for calibration are supplied by the LECO Company. Total Carbon and nitrogen contents of the samples are evaluated according to calibration curves.

4.5.7 Assays On Soil Microbial Carbon (C_{mic})

Used process of soil microbial biomass evaluation comprises of two separate methods by Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ) (Zbíral et al. 2011), where the first serves as a lead-up for the other. Therefore, both procedures, the Chloroform-Fumigation K_2SO_4 extraction method (normative 31010.1)

and the Determination of Oxidizable Organic Carbon in (K_2SO_4) Solution (normative 31020.1), were respectively carried out; hence the division in described laboratory procedure. However, either of applied methods drives at the same result, which is content of extractable carbon of microbial biomass expressed in micrograms per gram of dry matter in soil ($\mu g.g^{-1}$).

Both procedures are to compare the amount of organic carbon in a chloroform-fumigated soil sample to that in a non-fumigated soil sample to determine soil microbial biomass. The Total Organic Carbon content (TOC) will be higher in the chloroform-fumigated sample because the sample contains the cell contents of lysed microbial cells. Hence the difference in extracted TOC between fumigated and non-fumigated examples will provide measure of microbial biomass. Note that you can only assume that this TOC in the soil is of microbial origin. Samples have to be picked free of roots, litter, earthworms, etc., since the microfaunal contribution to TOC is usually less than 5 %.

4.5.7.1 The Chloroform-Fumigation K_2SO_4 Extraction Method

Fumigation of field-moist soil samples (pre-sieved through 2 mm mesh), is carried out for a period of 24 hours in vacuum desiccators with the addition of chloroform. K_2SO_4 is added to each sample and rotary shook for 30 minutes at 200 rpm. It is important to refrigerate soil samples at 4 °C until the fumigation and K_2SO_4 extractions are performed.

4.5.7.2 Spectrophotometric Determination Of Oxidizable Soil Organic Carbon In (K_2SO_4) Solution

K_2SO_4 extracted soil samples are oxidized in strong acid medium with the addition of potassium dichromate and spectrophotometrically analyzed for TOC content. Analysis results need to be recalculated to soil DM and final values are presented as total content of extractable oxidizable carbon in soil: C_{mic} ($\mu g.g^{-1}$).

4.5.8 Urease Activity Determination

Urease activity in soil was examined with the aid of Kandeler's and Gerber's method of colorimetric determination of ammonium published in 1988.

The method comprises of incubation of soil with an aqueous and buffered urea solution at 2 hours at 37 °C in order to release the enzyme from protected locations in soil suspension, extraction of ammonium with KC1/HCI and spectrophotometric

determination of released ammonia. Urease activity expressed as μg of hydrolysed N/g DM/2 h.

4.5.9 Acid Phosphomonoesterase Activity Evaluation

Applied laboratory analysis is based on Tabatabai's and Bremner's procedure (1969) that was later modified by Rejšek (1991) and applies p-nitrophenol phosphate (p-NPP) added to working buffer solution. Enzymatic activity releases p-nitrophenol (p-NP), which is extracted and colored by potassium hydroxide (KOH). The phosphomonoesterase activity is measured spectrophotometrically. Results are determined according to absorbance of analyzed samples at wavelength of 410 nm and the phosphomonoesterase activity is expressed in μg p-NP/g DM/h.

4.5.10 Catalase Activity Evaluation

Determining the activity of catalase in soil is practically the same as quantifying the capacity of soil to decompose hydrogen peroxide (H_2O_2) to water and molecular oxygen. The reason behind measuring the production of O_2 (generated due to the presence of H_2O_2 in the original suspension) and, therefore, the evaluation of the biological activity of soils lies in the presence of microflora capable of producing an enzyme (catalase) catalyzing this reaction. Used procedure was drawn up by Káš in 1954 and gas volumetrically measures the amount of O_2 evolved within 15 minutes period. Catalase activity is expressed in: ml O_2 $5g^{-1}$ 15 min^{-1} and is considered to be very low if lower than 5, low if between 5 –15, medium if 15 –30, high if 30 – 60 and very high if more than 60.

Device called 'Vápnoměr dle Janka' is essential for this procedure. It is a system of two graduated eudiometric measuring tubes 0 –100 ml connected with rubber tubing at their lower ends. The first of the tubes is connected with Erlenmeyer flask (containing an automatic pipette) through the rubber tubing and a three-way stopcock. The system also contains a reservoir bottle connected to both tubes at the lowest part.

4.5.11 Basal (BR) And Potential Respiration Of Soil (SIR)

This methodology is based on the International Standard ISO 16072:2002, which has derived from the German standard DIN 19737 and follows Czech national standards ČSN EN ISO 14240-1&2 (836441). Soil samples were prepared noting Šantrůčková's findings (1993), where prolonged incubation time is recommended due to avoiding excess root respiration. Two types of respiration were measured: basal

respiration (BR), describing current state of soil after the incubation period and potential respiration, utilizing dehydrated glucose infused samples for measurement of the substrate induced respiration (SIR). Microbial soil respiration results from the mineralization of organic substances. In this process, organic substances are oxidized to the end product of carbon dioxide and water, with concurrent uptake of O_2 for aerobic microorganisms. The principle of this method is based on alkaline solution, in this case 1 M NaOH that is placed in Petri dish above the soil surface in a closed plastic container. Carbon dioxide evolution is measured by absorption in the sodium hydroxide for 24 hours. The CO_2 amount absorbed is measured by back-titration of the excess alkali remaining. Results are expressed in units per gram of dry soil per time of incubation to be comparable to other studies (ug C- CO2/g DM/h). It is one of the most commonly used methods of determining metabolic activities of organisms (secondary consumers – mainly microbes) in soil.

4.6 Data Evaluation And Used Statistical Methods

Data were evaluated in April 2016 through the use of Excel (Microsoft Corporation 2011), Stat Plus (Analyst Soft Inc. 2015) and Statistica 6 (StatSoft 2001). Two-factor analysis of variance with repetition (two-factor ANOVA with repetition) was applied. The distribution of harvested data was also tested for normality.

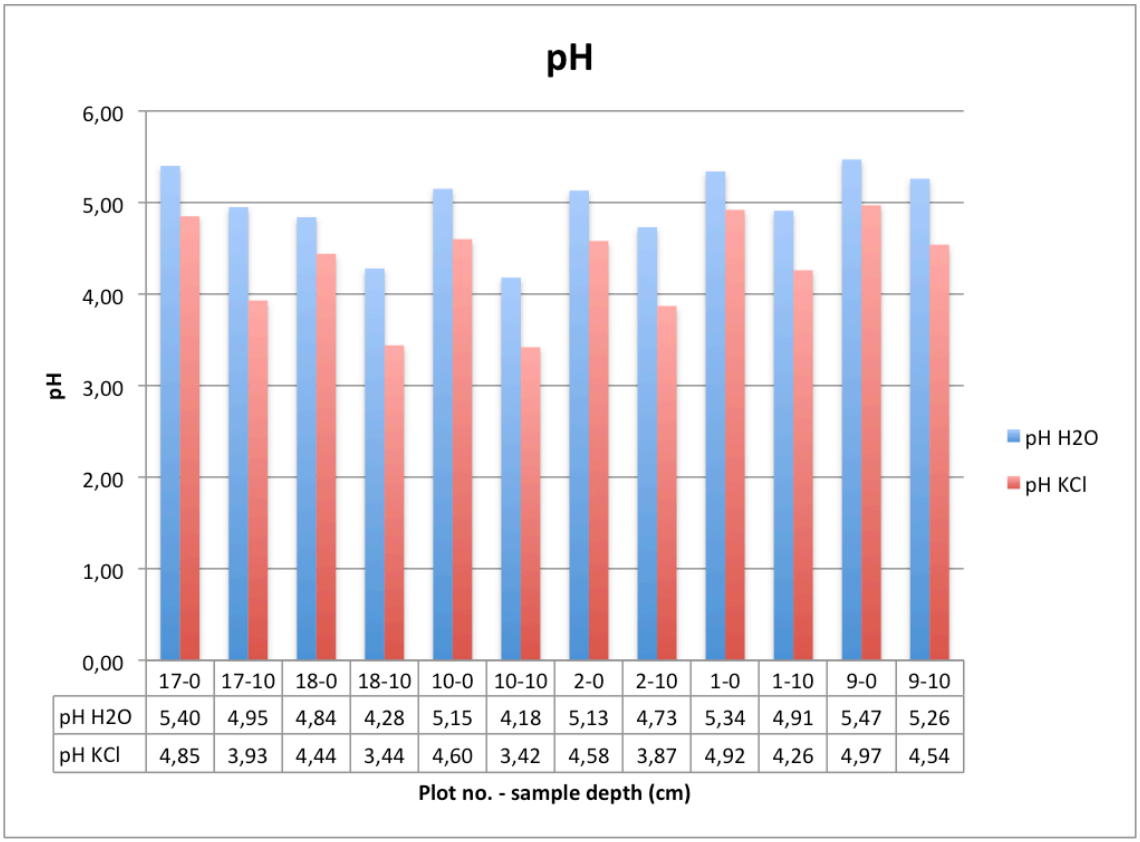
5 Results

Studied plots of different management practices were repeatedly analyzed for several soil properties. Acquired values of soil pH, LOI, $C_{ox,}$ C_{tot}, N_{tot}, C:N, C_{mic}, BR and SIR, as well as soil enzymatic activities, were firstly assessed individually before subjected to statistical evaluation. All supportive data can be found in APPENDIX 3.

Numerous physical properties of soil were determined for each soil sample.

pH

pH was measured from the first samples collected in Autumn. Resulting values suggest strong tendency to acid soil reaction averaging 4,97 for pH in H_2O (actual soil reaction) and 4,32 for pH in KCl (potential soil reaction) with standard deviation (SD) of 0,41 and 0,54 respectively. Figure 9 illustrates that acid soil reaction increases with depth and suggests that the overall pH is the highest on coppiced plots 1 and 9.



	17-0	17-10	18-0	18-10	10-0	10-10	2-0	2-10	1-0	1-10	9-0	9-10
pH H2O	5,40	4,95	4,84	4,28	5,15	4,18	5,13	4,73	5,34	4,91	5,47	5,26
pH KCl	4,85	3,93	4,44	3,44	4,60	3,42	4,58	3,87	4,92	4,26	4,97	4,54

Figure 9 Actual and potential soil reaction of soil samples collected in autumn

LOI (Loss On Ignition)

Direct estimation of organic matter (OM) by the loss on ignition was applied to soil samples collected during the first sampling period in autumn. Results of the test show that average content of OM of sampled topsoil is 11,7 ± 1,45 % and 5,55 ± 0,77 % of samples collected at 10 cm depth. LOI results do not show high variability among plots but the values tend to decrease by about one half with the first ten centimetres of depth.

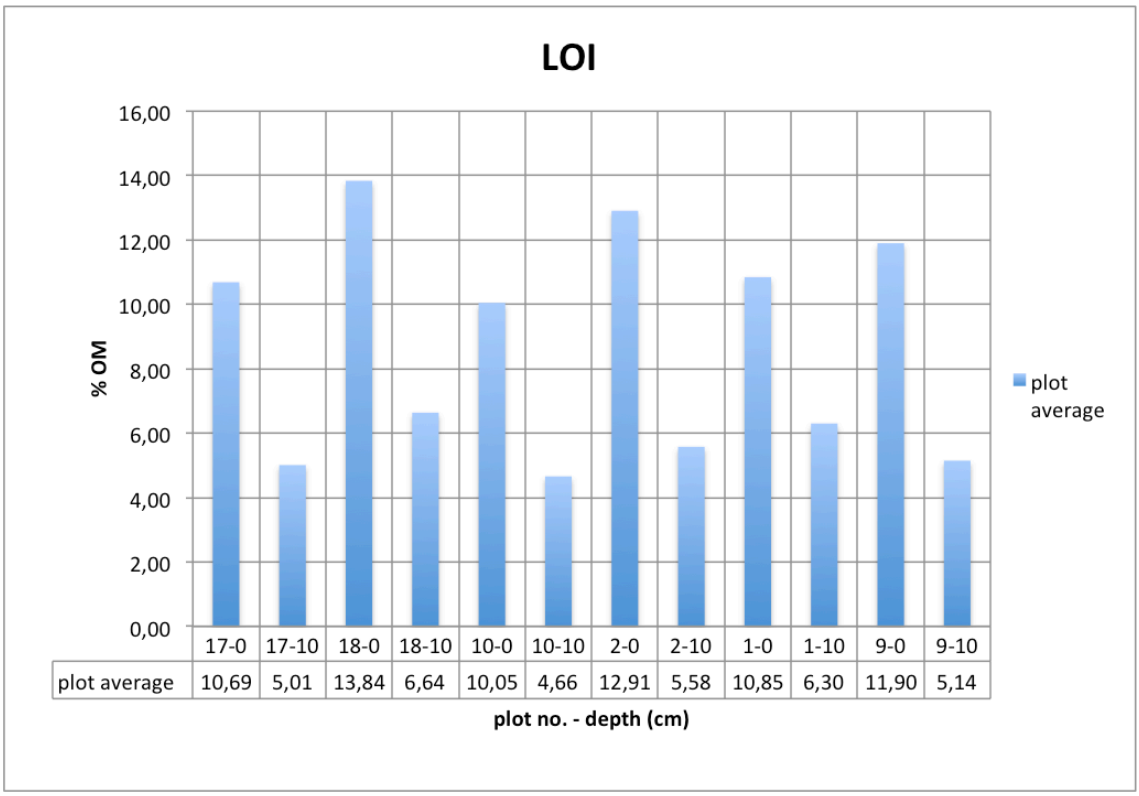


Figure 10 Resulting values of loss on ignition on the TARMAG II plots and high forest control plots

Resulting values were later correlated with soil organic carbon content values (Figure 10) and strong positive interdependency was found: $r(C_{ox}) = 0,98594$; $p = 0,0000000168$; $R = 0,00349$ (r – Pearsons correlation coefficient, p – p value, R – standard error). Thus, loss on ignition was omitted in later data analyses.

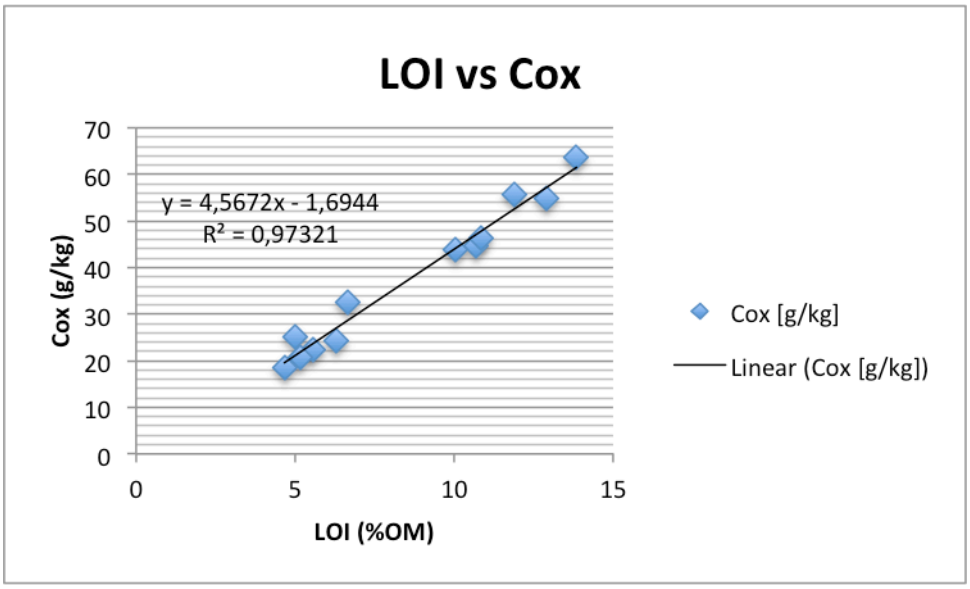
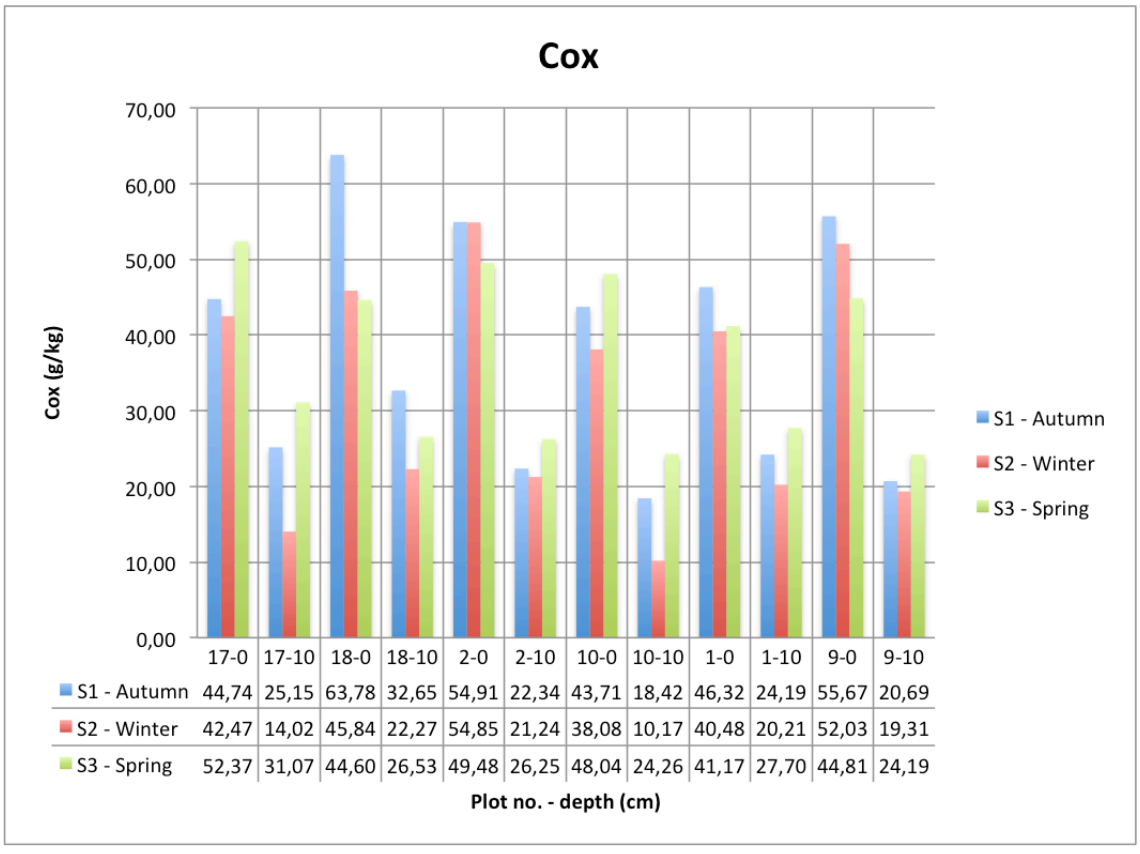


Figure 11 Linear regression model of the LOI and C_{ox} relationship

C_{ox} (Organic Carbon In Soil)



	17-0	17-10	18-0	18-10	2-0	2-10	10-0	10-10	1-0	1-10	9-0	9-10
S1 - Autumn	44,74	25,15	63,78	32,65	54,91	22,34	43,71	18,42	46,32	24,19	55,67	20,69
S2 - Winter	42,47	14,02	45,84	22,27	54,85	21,24	38,08	10,17	40,48	20,21	52,03	19,31
S3 - Spring	52,37	31,07	44,60	26,53	49,48	26,25	48,04	24,26	41,17	27,70	44,81	24,19

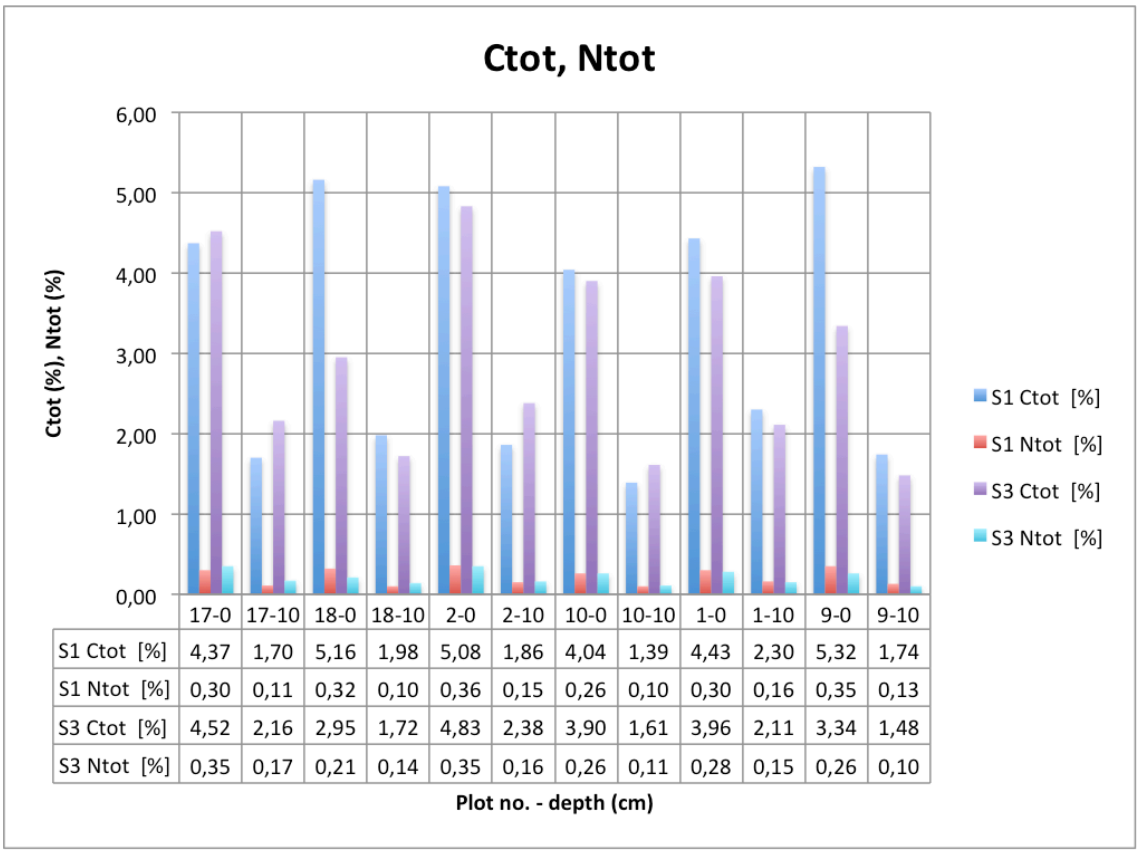
Figure 12 Volumes of organic carbon content in soils sampled in the October 2015, January 2016 and end of March 2016

C_{ox} values acquired through spectrophotometric determination of organic carbon in soil by sulphochromic oxidation come from three vegetation seasons. Highest measured average topsoil value of 51,52 ± 7,91 g/kg was recorded in the autumn, while

the lowest average of 45,63 ± 6,62 g/kg in the winter. The lower soil horizon shows highest numbers in the spring, while the minimum values are again recorded in the winter. Similarly to the LOI figures, the depth of 10 cm exhibits a decrease of organic carbon in soil by about 50 % on average.

C_{tot}, N_{tot}, C:N (Total Soil Carbon And Nitrogen Contents And Their Ratio)

Total C and N contents in soil were measured for samples from the first and last collection period. The actual C_{tot} values are, again, strongly correlated with those of C_{ox} and LOI and show very similar progression trends. Plots 18 and 9 demonstrate a decrease in total C of their organic horizon, such as recorded in the previous analysis, only here is the resulting drop more pronounced.



	17-0	17-10	18-0	18-10	2-0	2-10	10-0	10-10	1-0	1-10	9-0	9-10
S1 Ctot [%]	4,37	1,70	5,16	1,98	5,08	1,86	4,04	1,39	4,43	2,30	5,32	1,74
S1 Ntot [%]	0,30	0,11	0,32	0,10	0,36	0,15	0,26	0,10	0,30	0,16	0,35	0,13
S3 Ctot [%]	4,52	2,16	2,95	1,72	4,83	2,38	3,90	1,61	3,96	2,11	3,34	1,48
S3 Ntot [%]	0,35	0,17	0,21	0,14	0,35	0,16	0,26	0,11	0,28	0,15	0,26	0,10

Figure 13 Resulting values of C_{tot}, N_{tot} of the first and last sampling period (S1 – autumn, S3 – Spring) on the TARMAG II plots and high forest control plots

N_{tot} in analyzed soil samples averages 0,22 % (autumn) and 0,21 % (spring) with an SD of 0,1 and 0,09 respectively. Similarly to carbon analyses the amounts of N deceases considerably with soil profile depth, but is quite evenly distributed among plots and doesn't exhibit much variation.

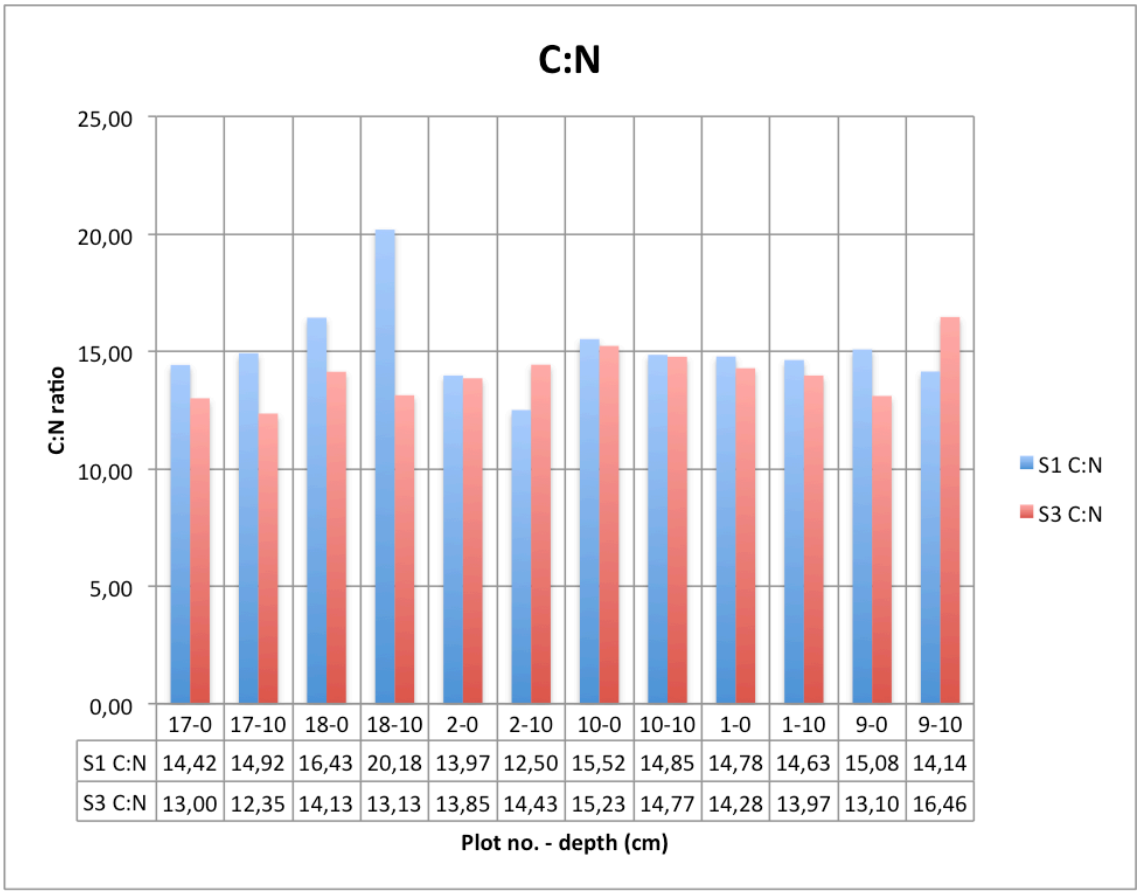


Figure 14 C:N ratio figures of the first and last sampling period

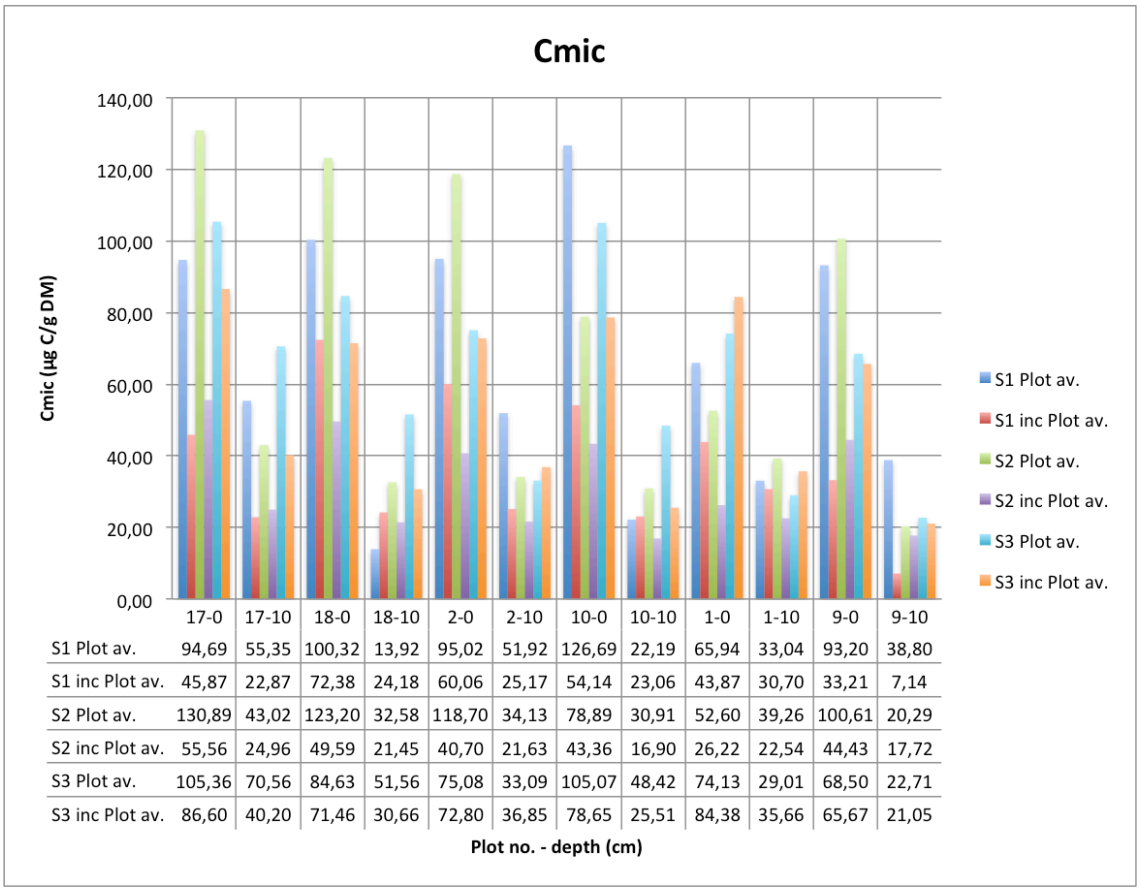
Measurement of the C:N ratio in sampled soil profiles displays fairly even distribution of favorable values averaging about 14:1 to 15:1. At closer examination, it can be noticed that autumn figures show overall higher values than spring figures and that is in both examined layers (Table 4). It can be also noted that the C:N ratio slightly increases with depth. High forest control plot 18, exhibited the highest value of 20,18 % in the autumn. Control plots in general show the biggest drops of C:N ratio over the winter period. Such fluctuations are generally contributed to changes in the N content, but as shown in Figure 13, they are most probably oscillating as a function of both C and N.

Table 4 Examined difference between seasonal averages and examined soil depth in C:N ratio

Soil depth (cm)	S1 C:N	SD (S1 C:N)	S3 C:N	SD (S3 C:N)
0 - 5	15,03	0,87	13,93	0,83
10 - 15	15,20	2,60	14,19	1,42

C_{mic} (Soil Microbial Carbon Content)

All analyses concerning microbiological activity were prepared from samples collected at all sampling periods. Moreover, an additional analysis was carried out after a one-month period of sample incubation. Hence the total of six resulting values for each sample. It is evident that in the vast majority of the cases the incubation led to a decrease in microbial biomass carbon content (by 36 % on average). Collected data are rather complex, but if we were to compare the performance of C_{mic} against seasonality and the management type (logging intensity), we do get to interesting results: the seasonal dynamicity is not displayed in C_{mic} values as they remain almost identical throughout the examined periods (that applies to both, fresh and incubated samples) and is best displayed by the combined (of both horizons) averages of fresh samples (65,92; 67,09; 64,01 all in µg C/g DM). On the other hand, when comparing the values to management type, there is a slight decrease in microbial carbon biomass with increasing logging intensity. This trend can be seen in all different types of samples incubated and fresh and throughout both sampled soil profile depths (e.g. fresh topsoil: 106,52; 99,91; 75,83 or incubated lower soil horizon: 27,39; 24,85; 22,47 all in µg C/g DM).

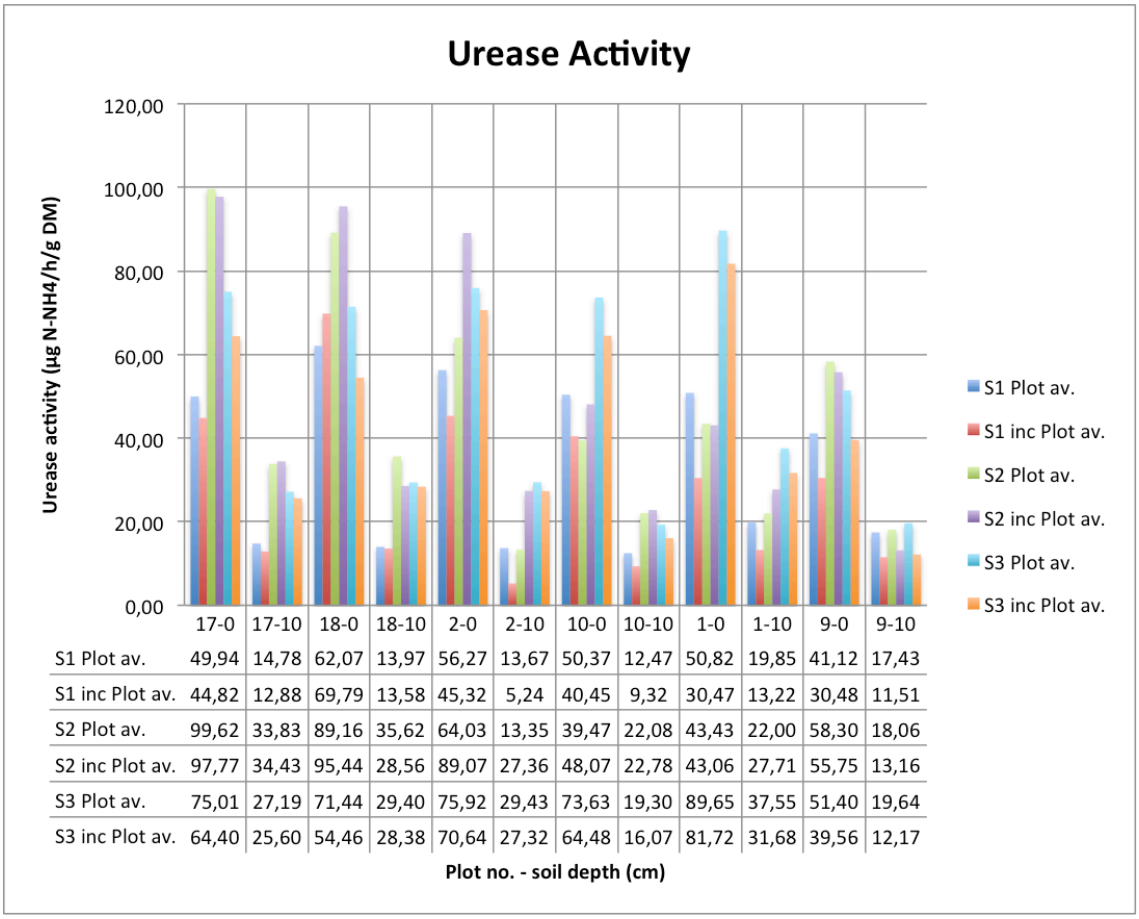


	17-0	17-10	18-0	18-10	2-0	2-10	10-0	10-10	1-0	1-10	9-0	9-10
S1 Plot av.	94,69	55,35	100,32	13,92	95,02	51,92	126,69	22,19	65,94	33,04	93,20	38,80
S1 inc Plot av.	45,87	22,87	72,38	24,18	60,06	25,17	54,14	23,06	43,87	30,70	33,21	7,14
S2 Plot av.	130,89	43,02	123,20	32,58	118,70	34,13	78,89	30,91	52,60	39,26	100,61	20,29
S2 inc Plot av.	55,56	24,96	49,59	21,45	40,70	21,63	43,36	16,90	26,22	22,54	44,43	17,72
S3 Plot av.	105,36	70,56	84,63	51,56	75,08	33,09	105,07	48,42	74,13	29,01	68,50	22,71
S3 inc Plot av.	86,60	40,20	71,46	30,66	72,80	36,85	78,65	25,51	84,38	35,66	65,67	21,05

Figure 15 Measured plot average values of C_{mic} of fresh and incubated (inc) samples

Urease Activity

Resulting values of urea amidohydrolase activity, at first glance, appear to be similar to that of C_{mic}. However, incubation period doesn't seem to have such an unambiguous settling effect as in the previous case. The incubation still does lead to overall cut back of the initial values but, in this case, only by mere 9,43 %. Differences in the enzymatic activity throughout the seasons are, on the other hand, more pronounced. The average activity across all plots is surprisingly at its highest in the winter, driven mainly by plots with denser canopy cover. The distinction between differently managed forest stands based on activity means is still apparent, but the only type that really stands out with the highest overall activity is high forest with balanced average topsoil value of 74,54 µg N-NH4/h/g DM for fresh samples and 71,11 µg N-NH4/h/g DM for incubated samples (sampling at 10 cm resulted in 25,80 and 23,91 µg N-NH4/h/g DM). It is noticeable that the urease activity sharply deceases with profile depth, as the recorded values at 10 cm are on average by 65,3 % lower.

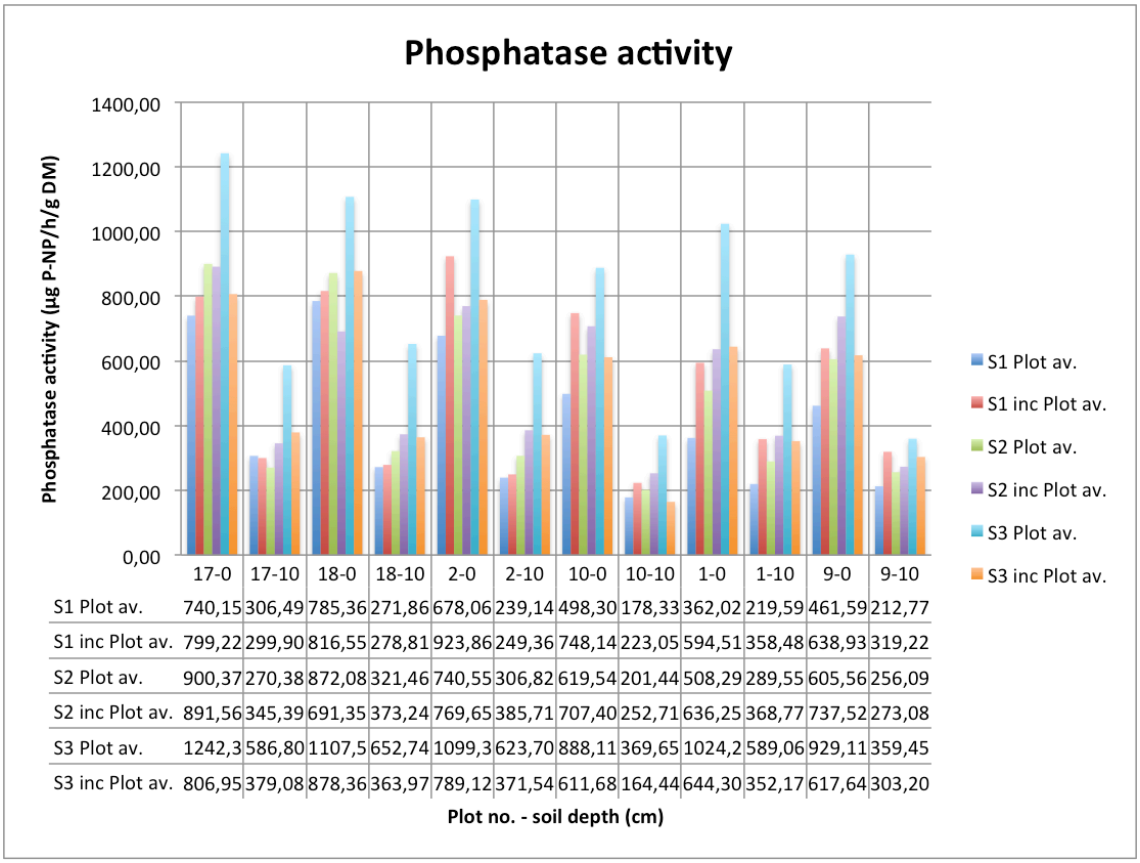


	17-0	17-10	18-0	18-10	2-0	2-10	10-0	10-10	1-0	1-10	9-0	9-10
S1 Plot av.	49,94	14,78	62,07	13,97	56,27	13,67	50,37	12,47	50,82	19,85	41,12	17,43
S1 inc Plot av.	44,82	12,88	69,79	13,58	45,32	5,24	40,45	9,32	30,47	13,22	30,48	11,51
S2 Plot av.	99,62	33,83	89,16	35,62	64,03	13,35	39,47	22,08	43,43	22,00	58,30	18,06
S2 inc Plot av.	97,77	34,43	95,44	28,56	89,07	27,36	48,07	22,78	43,06	27,71	55,75	13,16
S3 Plot av.	75,01	27,19	71,44	29,40	75,92	29,43	73,63	19,30	89,65	37,55	51,40	19,64
S3 inc Plot av.	64,40	25,60	54,46	28,38	70,64	27,32	64,48	16,07	81,72	31,68	39,56	12,17

Figure 16 Urease activity plot average values of fresh and incubated (inc) samples

Phosphatase Activity

Acid phosphomonoesterase activity shows sharp increase in the spring fresh soil analysis. The incubation period of one month, though, brings the values close to the overall average. Despite this, the general prevailing trend is that incubation increases the activity of this enzyme. Seasonal peak of phosphatase activity appears to be in the spring, while the lowest recorded values were obtained in the autumn. Its relationship to the studied types of management on plots with different logging intensity can be demonstrated on the example the post incubation values of soil samples acquired in the spring in coppice, coppice with standard and high forest stands (respectively) at both examined soil layers: 0 –5 cm: 644,86; 758,31; 814,00 and 10 – 15 cm: 274,47; 329,15; 340,07 (all in µg P-NP/h/g DM). Topsoil layer is clearly responsive to the stand type, whereas the lower soil layer does not exhibit the same responsiveness. The overall decrease in acid phosphomonoesterase activity between the two sampled horizons was 56,06 %.

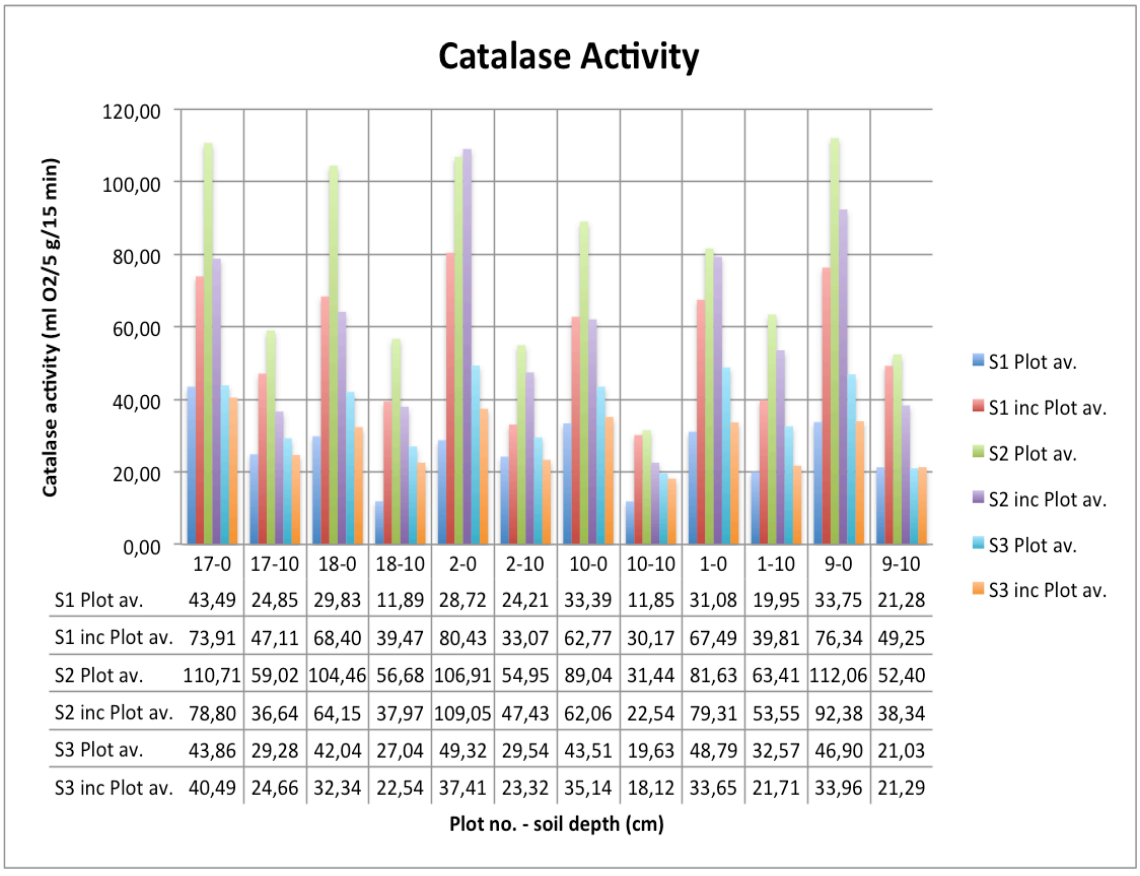


	17-0	17-10	18-0	18-10	2-0	2-10	10-0	10-10	1-0	1-10	9-0	9-10
S1 Plot av.	740,15	306,49	785,36	271,86	678,06	239,14	498,30	178,33	362,02	219,59	461,59	212,77
S1 inc Plot av.	799,22	299,90	816,55	278,81	923,86	249,36	748,14	223,05	594,51	358,48	638,93	319,22
S2 Plot av.	900,37	270,38	872,08	321,46	740,55	306,82	619,54	201,44	508,29	289,55	605,56	256,09
S2 inc Plot av.	891,56	345,39	691,35	373,24	769,65	385,71	707,40	252,71	636,25	368,77	737,52	273,08
S3 Plot av.	1242,3	586,80	1107,5	652,74	1099,3	623,70	888,11	369,65	1024,2	589,06	929,11	359,45
S3 inc Plot av.	806,95	379,08	878,36	363,97	789,12	371,54	611,68	164,44	644,30	352,17	617,64	303,20

Figure 17 Measured values of phosphatase activity in regards to individual plots, seasons, pre and post incubation state

Catalase Activity

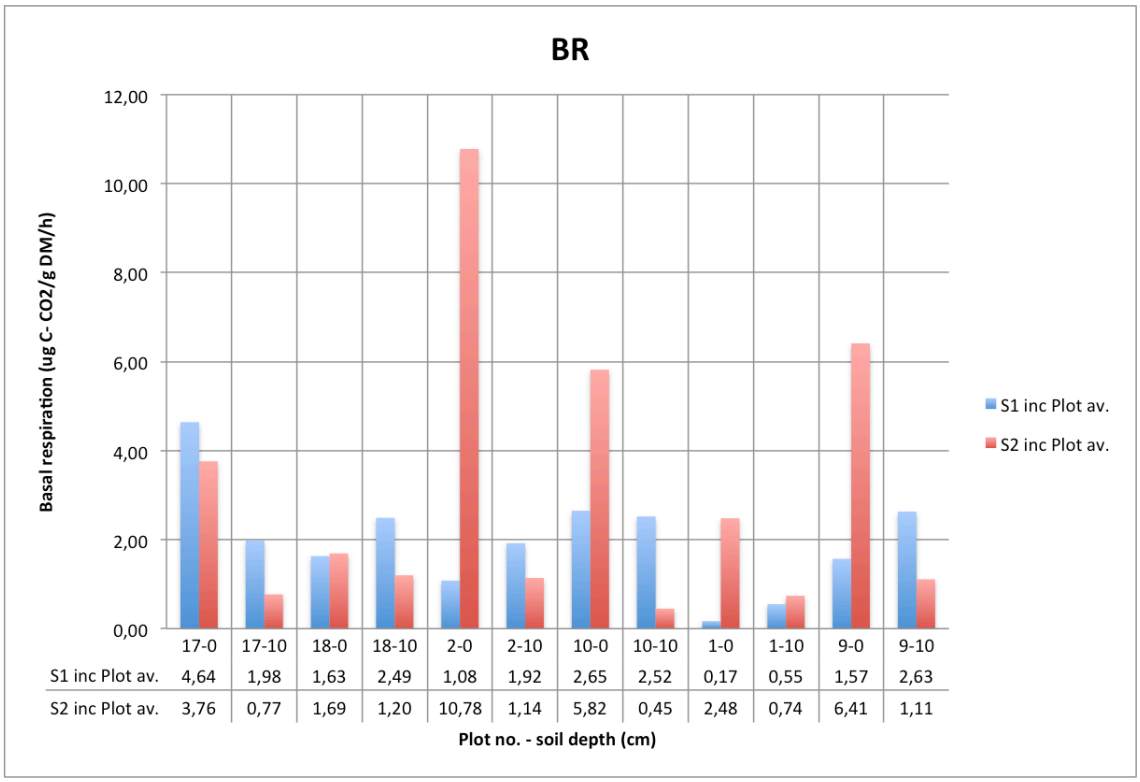
Periodic assessment points out that the highest values were obtained in the winter season. Both, autumn and spring measurements display (similar) lower average values of catalase activity, but differ in the actual effect of incubation onto their activity (steep increase in S1 and about 20% drop in S3). The overall measured activity of hydrogenperoxide oxidoreductase turned out to increase by the incubation by about 20 %. These results are undoubtedly driven by the steep rise of post incubation activity in the first sampling period. Catalase activity shows absolutely no signs of any relationship to logging intensity or management type. Average measured values across various management types (coppice, c-w-s and control plots respectively) are 61,04; 61,48 and 61,45 (all in ml O_2/ 5 g/ 15 min). Averaged results distinguished by the profile depth show higher variation in the lower soil layer. The overall average decrease of catalase activity on the 10 cm depth difference is 45,68 %.



	17-0	17-10	18-0	18-10	2-0	2-10	10-0	10-10	1-0	1-10	9-0	9-10
S1 Plot av.	43,49	24,85	29,83	11,89	28,72	24,21	33,39	11,85	31,08	19,95	33,75	21,28
S1 inc Plot av.	73,91	47,11	68,40	39,47	80,43	33,07	62,77	30,17	67,49	39,81	76,34	49,25
S2 Plot av.	110,71	59,02	104,46	56,68	106,91	54,95	89,04	31,44	81,63	63,41	112,06	52,40
S2 inc Plot av.	78,80	36,64	64,15	37,97	109,05	47,43	62,06	22,54	79,31	53,55	92,38	38,34
S3 Plot av.	43,86	29,28	42,04	27,04	49,32	29,54	43,51	19,63	48,79	32,57	46,90	21,03
S3 inc Plot av.	40,49	24,66	32,34	22,54	37,41	23,32	35,14	18,12	33,65	21,71	33,96	21,29

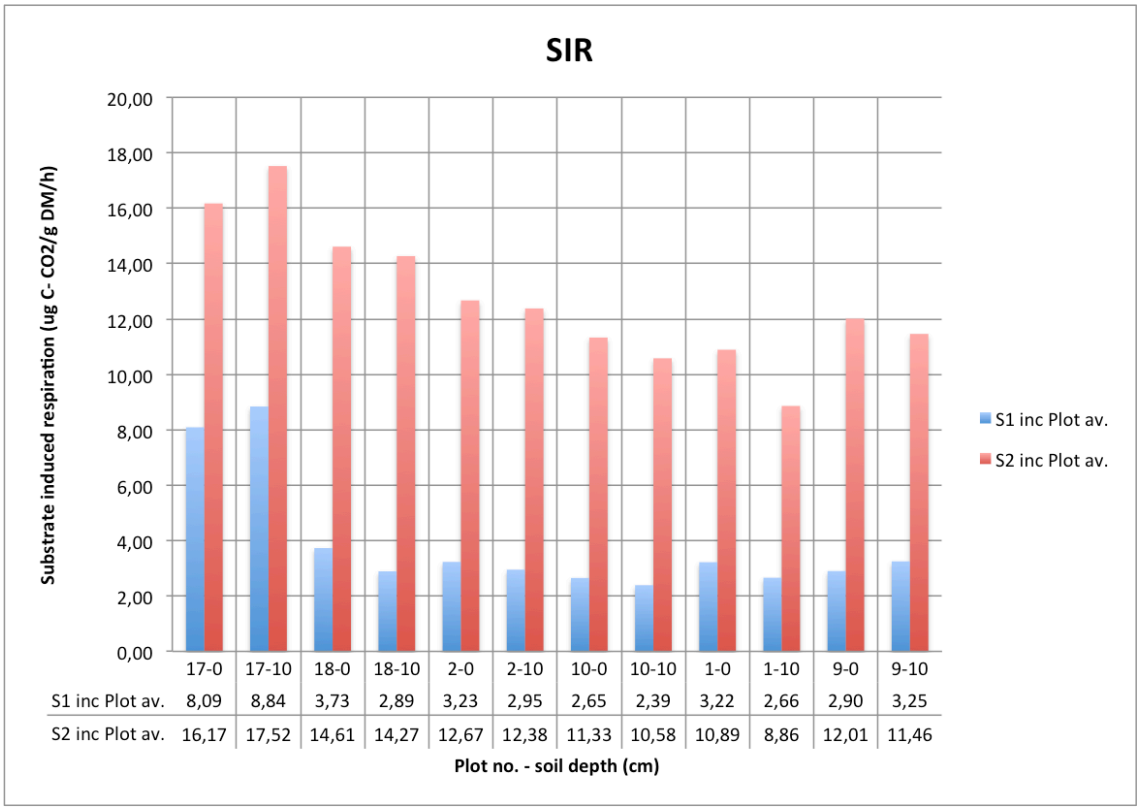
Figure 18 Measured catalase activity values in regards to individual plots, seasons, pre and post incubation state

BR (Basal Respiration) and SIR (Substrate-Induced Respiration)



	17-0	17-10	18-0	18-10	2-0	2-10	10-0	10-10	1-0	1-10	9-0	9-10
S1 inc Plot av.	4,64	1,98	1,63	2,49	1,08	1,92	2,65	2,52	0,17	0,55	1,57	2,63
S2 inc Plot av.	3,76	0,77	1,69	1,20	10,78	1,14	5,82	0,45	2,48	0,74	6,41	1,11

Figure 19 Post-incubation, basal respiration figures of the first and the second sampling period



	17-0	17-10	18-0	18-10	2-0	2-10	10-0	10-10	1-0	1-10	9-0	9-10
S1 inc Plot av.	8,09	8,84	3,73	2,89	3,23	2,95	2,65	2,39	3,22	2,66	2,90	3,25
S2 inc Plot av.	16,17	17,52	14,61	14,27	12,67	12,38	11,33	10,58	10,89	8,86	12,01	11,46

Figure 20 Post-incubation figures of substrate-induced respiration of the first and the second sampling period

Soil respiration was assessed (in all cases) after a two-month period of incubation. The 'missing' results from the spring collection period will be compiled at the end of May. Since only incubated samples were analyzed, the effect of incubation onto the respiratory activity cannot be stipulated. Results form the S1 and S2 periods show that studied soils were more active in the winter period.

Basal respiration shows an overall increase in activity of 54,15 % between the autumn and winter seasons. Resulting values of BR from S1 and S2 are ambiguous in terms of soil profile. S1 displays an increase of respiration activity with depth on coppice and c-w-s plots and a slight decrease on control plots. S2 shows a decline in BR with depth on all sites, hence the overall average for S1 and S2 shows a decline of 27,73 % in respirational activity. If the performance of individual forest classes was compared than in S1 it is the high forest that shows the highest values of BR and in S2 it is the coppice and c-w-s stands.

Substrate-induced respiration activity is similar to BR in its seasonal max (so far). SIR also exhibits higher activity in the winter, with an inter-seasonal growth of 326,4 %. Potential soil respiration levels are highly similar for both sampled horizons and measured values resulted in a slight decrease of activity by only 4,3 %. High forest stand performs the best in terms of SIR and shows the highest mean values in both sampling periods and both sampled soil profile depths c-w-s and coppice (e.g. S2 (0 – 5 cm) high forest, c-w-s and coppice respectively: 15,39; 12,00; 11,45 or S2 (10 – 15 cm): 15,9; 11,48; 10,16 (all in ug C- CO2/g DM/h)).

Statistical data evaluation

Tests of interaction effects were carried out for all the above variables (discussed in the first part of results) as well as selected physical properties of soil related mainly to density, water and air regime characteristics (red listed in APPENDIX 4). Two-factor ANOVA with repetition was applied to highlight statistical significance of the relationships between measured values (measured variables) and two nominal variables: type of management (logging intensity) and seasonal dynamicity. The resulting p-value (a test significance statistic) was compared with the significance level set at 0,05 ($\alpha = 0,05$). P-value < 0,05 demonstrated a statistically significant effect of factors (nominal variables) on the measured variable. P-value > α signaled no statistically significant effect of examined factors onto measured values. Normality tests

of all individual data sets were also performed to highlight data distribution. Detailed results are showcased in APPENDIX 5.

It is apparent that a statistically significant effect of the **management type** (logging intensity) on the examined variables has been established in the cases of:

i. **SIR** (Substrate Induced Respiration – incubated samples)
ii. A (Soil Aeration)
iii. RCM 2 (Relative Capillary Moisture 2 = function of maximum water-bearing capacity and volumetric moisture)
iv. PS (Pore Saturation)

The results also show that a statistically significant effect of **seasonality** onto the examined variables is greater than that of management and has been demonstrated in the cases of:

i. **C_{mic}** (carbon content of microbial biomass – incubated samples)
ii. **Phosphatase** (acid phosphomonoesterase – fresh soil samples)
iii. **Catalase** (hydrogenperoxide oxidoreduktase – fresh soil samples)
iv. **Catalase** (hydrogenperoxide oxidoreduktase – incubated samples)
v. **SIR** (Substrate Induced Respiration – incubated samples)
vi. RCM 2 (Relative Capillary Moisture 2)
vii. PS (Pore Saturation)

Individual datasets of examined variables were tested for normality. Lack of symmetry, excessive (skewness), and pointiness (kurtosis) were considered as two main ways in which data distribution can deviate from normal. The closer the values of these parameters are to zero the closer the data are to standard (normal) distribution. As for that reason an absolute value of ± 1.96 was regarded as indicating asymmetry (significant at $p < 0.05$).

Normality tests revealed that most datasets show normal data distribution. BR and RCM datasets are the only two with skewness larger than selected value. Measured variables of the two datasets can be considered as prone to incorrectly reject a true null hypothesis (type I error).

Discussion

Little is known about the factors that determine the stability of soil of oak coppice forests (Topp et Schlichter 2004). Shifts in soil microbial biomass and activity can be attributed to many abiotic and biotic, natural or anthropogenic factors (e.g. seasonality, stand density, moisture or even the climate change). The main goal of this thesis was to find and somewhat describe the link between such shifts and silvicultural practices represented by three traditional forms of woodland management. One can only just compare a 90-year-old oak stand with recently (7 years ago) established coppice and c-w-s stands (both unmanaged, and left to regenerate naturally). On the other hand the issue can be viewed from the angle of comparing the zero point in time (high forest control plots) to the application of different logging intensities and their effect in time. In that aspect not only plots of different management types are being compared, but also plots with different degree of perturbation. Anthropogenic perturbations with direct effect on soil in managed production forests are of natural occurrence. It should be pointed out that coppiced forest stands are amongst the most intensively managed types. In that instance it would be wise to put forward a few questions: how much of a decline in soil microbial biomass and activity can occur before the quality and sustainability of the ecosystem is compromised? Or: does an increase in such activity lead to an improvement? Which variables are the best to quantify or provide an indication of an impact of management type? All above questions are of course beyond the capacity of this thesis and in order to be answered we must look elsewhere. Yet, this thesis still offers a few interesting insights, which derive form the evaluation of results.

It is known that the cohesion of forest ecosystem largely relies upon the functioning of nutrient cycling (mobilisation and recycling) in the soil (Powers et al. 1998), similarly, that nutrient cycles are largely dependent upon the performance of microbial community (Šantrůčková 2014). Tate (2005) describes that changes in soil carbon stock are usually of very slow progression and such changes can be long preceded by changes of microbial biomass and activity. This upholds and to certain degree explains as to why **no significant variability in the totals of OM and C stock** was established amongst studied plots and yet studied plots **differed in measured values of C_{mic}, phosphatase and urease activity and potential respiration**.

Type of management (logging intensity) had a **statistically significant effect only on potential respiration** although interesting results could be seen throughout the

whole data evaluation process. This could be caused by the fact that **measured values were assessed together for both horizons**. Respiration is the only microbial activity variable that **does not greatly differ between depths** (in all other cases it is mainly the topsoil that seems to be affected by the changes of above ground biomass, stand composition and density) Therefore, it can be stated that **microbial biomass and activity of haplic cambisols decreases significantly with depth** (except for SIR and BR).

Topp and Schlichter (2004) that evaluated microbial activity in oak coppice stands through respiration found out that stand density was the most important limiting factor. Topp and Schlichter (2004) also got to very similar results (2,5 – 5,7 ug C-CO2/g DM/h), where the lowest values were recorded in young dense stands and the highest in young thinned. Those results are highly comparable with values measured on the TARMAG II plot in the winter. High stand density is represented by densely populated subplot 1 (2,48 C- CO2/g DM/h) and low density by subplot 9 with much looser cover (6,41 ug C- CO2/g DM/h).

Abiotic factors in the form of local microclimate and weather patterns preceding sample collection periods **played an important role** in resulting measured values. First collection period (autumn) succeeded a long period of drought with extremely low amount of precipitation and second collection was preceded by a period of sub-zero temperatures. Moisture of soil is often mentioned (Skopp et al. 1990, Zelles et al. 2002, Brockett et al. 2011) as one of the most (if not the most) important factors influencing soil microbiological activity. Temperature is also put forward as one of the main factors limiting microbial processes in the soil (Lin et al. 2016). Substantial microbial activity was recorded in soils even at -6,5 °C, but does significantly decrease with further temperature drop (Flanagan et Bunnell 1980). Quality of organic matter (evaluated by the C:N ratio), often regarded as one of the three most important limiting factors (Tate 2005) showed fairly low variability. Resulting values of urease activity (otherwise known for its stability) as well as soil respiration of the first sampling were considerably lower than those of other sampling periods. Thus the possible explanation of the measured enzymatic activity and respiration levels: lack of moisture in S1; and excessively low temperatures in topsoil during S2.

Winter topsoil samples in coppice and c-w-s stands **were collected partially frozen**. Although this sampling led to interesting findings in terms of catalase and urease activity (peaking in the winter), slightly frozen soil in sampled core cylinders

probably debased some results simply because their were overly saturated by water in their frozen state. This uncommon practice unfortunately subsequently led to the credibility reduction of the physical properties of soil evaluation of the second sampling period.

The microclimatic conditions of each site (exposure to sun radiation/shade, possible differences in temperature and moisture) should be highlighted in this instance and as much as they are closely related to the management type, they also play an important role in the **seasonal dynamicity lag** of different sites. This effect can be demonstrated on the example of basal respiration in the winter. BR was peaking on sunlit sites with the highest felling intensity (first sampling shows even distribution with highest values measured in high forest control plots samples). Sunlit sites in several cases show increased microbial activity in the spring. The **effect of seasonal variability was** also **statistically confirmed** for samples tested for phosphatase activity and C_{mic}. Microbial biomass is known to be the highest in the summer and lowest in the winter (Bardgett et al. 1999). Measured values of incubated C_{mic} samples consent to this theory. Eivazi and Tabatabai (1977) found that the phosphate content in soil tends to be higher in the spring. So are measured values of phosphatase activity in fresh soil samples.

Incubation is known and widely used for its settling effect on onto soil samples. The effect of incubation usually closes the gap between samples that are taken out of their natural ecosystem and are exposed to the stress of new closed environment. It is interesting to observe how individual variables reacted to the one-month period of incubation: microbial biomass decreased in the absolute majority of cases, but enzymatic activity showed that it can react in both ways. For example, catalase activity of autumn samples (collected after a period of drought) nearly doubled after incubation, conversely, incubation of spring samples tested for phosphatase led to sharp decline in activity.

Although the results including both examined soil layers show that the potential activity of some enzymes (phosphatase, urease) and microbial biomass (C_{mic}) is considerably lower in areas subjected to higher logging intensity, i.e. areas with higher degree of perturbation, this hypothesis turns out to be statistically unfounded. **Further monitoring** is probably the most realistic approach to obtain objective information about the effect of management and its impact onto the soil ecosystems.

Summary

Extensive monitoring of microbiological, chemical and physical properties of soil samples often denoted distinctive differences between areas with different management practices. This data variability was not always statistically significant, but it is safe to say that the highest values of several studied microbiological parameters (phosphatase and urease activity, potential respiration, microbial carbon content) were consistently obtained in the cases of high forest subplots and declined as a function of logging intensity. The same proclamation cannot be made about Catalase activity, pH, total C_{ox}, C and N contents, where there is neither visible, nor statistically significant relationship between measured values and type of management. Statistical results also pointed out that management type could be an important factor influencing soil aeration, relative capillary moisture and pore saturation.

Seasonal dynamic can also be considered an important factor contributing to shifts in biological activity of soil (e.g. C_{mic}, Phosphatase, Catalase, Respiration), moreover, if seems to significantly affect physical properties of soil related to capillary water (relative capillary moisture and pore saturation). Results also show that the overall potential activity of urease and catalase was at its highest during the winter season.

The depth of haplic cambisol profiles (horizons), from which the samples were collected, plays a critical role in most biochemical and physical properties of soil. Majority of measured values of physical properties change with depth. Both, the potential and actual pH value decreased with the downward distance. Total amount of organic carbon and nitrogen also decreases and most importantly microbiological activity is reduced. The only exception to the rule proved to be soil respiration that, in most cases, showed similar results at both sampled depths (0 – 5 cm and 10 – 15 cm).

References

ALEF, K., NANNIPIERI, P., 1995. Catalase activity. IN: ALEF, K., NANNIPIERI, P., (eds.), Methods in applied soil microbiology and biochemistry, Academic Press, London, 362–363.

ARAUJO, T.M., HIGUCHI, N., ANDRADE DE CARVALHO, J., 1999. Comparison of formulae for biomass content determination in a tropical rain forest site in the state of Pará, Brazil. Forest Ecology and Management (117):43–52.

AMBROZ, Z., ŠTYKAR, J., 1999. Geobiocenologie I. MZLU Brno.

BARDGETT, R.D., LOVELL, R.D., HOBBS, P.J., JARVIS S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biology and Biochemistry, 31 (7):1021–1030.

BEDFORD, M.R., PARTRIDGE G.G., 2001. Enzymes in farm animal nutrition. Cabi publishing. Oxon, UK.

BROCKETT, B.F.T., PRESCOTT, C.E., GRAYSTON, S.J., 2011, Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry 44 (1): 9–20

BROOKES P.C., KRAGT J.F., POWLSON D.S., JENKINSON, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: the effects of fumigation time and temperature. Soil Biology & Biochemistry (17):831–835.

BS EN 15935, Sludge, treated biowaste, soil and waste. Determination of loss on ignition, 2011

BURNS, R.G., 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biology and Biochemistry, (14):423–427

COLEMAN, D.C., CROSSLEY, D.A. Jr., HENDRIX, P.F., 2004. Fundamentals of soil ecology. Elsevier academic press, San Diego, USA.

CULEK, M. et al., 2005. Biogeografické členění České republiky II. díl. AOPK ČR, Praha.

ČSN ISO 10390 (836221), Kvalita půdy - Stanovení pH, 2011

ČSN EN 15935 (838126), Kaly, upravený bioodpad, půdy a odpady - Stanovení ztráty žíháním, 2013

ČSN EN ISO 14240-1 (836441), Kvalita půdy - Stanovení půdní mikrobiální biomasy - Část 1: Metoda substrátem indukované respirace, 2012

ČSN EN ISO 14240-2 (836441), Kvalita půdy - Stanovení půdní mikrobiální biomasy - Část 2: Fumigační extrakční metoda, 2012

DICK, R.P., 1994. Soil enzyme activity as indicators of soil quality. IN: Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A., (eds.), Defining Soil Quality for a Sustainable Environment. Soil Science Society of america, Madison, 107–124.

DICK, R.P., 1998. Soil enzyme activities as integrative indicators of soil health. In:. PANKHURST, C., DOUBE, B.M., GUPTA, V.V.S.R., (eds.) 1998. Biological Indicators of Soil Health. Cab International., 121-156

DIN ISO 16072, Soil quality - Laboratory methods for determination of microbial soil respiration, 2005-06
EIVAZI, F., TABATABAI, M.A., 1977. Phosphates in soils. Soil Biol Biochem (9):167–172

FAO, 1998. WORLD REFERENCE BASE for Soil Resources, World Soil Resources Report No. 84. ISSS–ISRIC–FAO, Rome.

FLANAGAN, P.W., BUNNELL, F.L., 1980 Microflora activities and decomposition in An Arctic Ecosystem: The Coastal Tundra at Barrow, Alaska. Van Nostrand Reinhold, New York, USA. 291–334.

FORMÁNEK, P., VRÁNOVÁ, V., SKOTÁK, V., 2006. Aktivita půdní katalázy v různých formách nadložního humusu Národní přírodní rezervace Kněhyně. Zpravodaj – The Beskides Bulletin: 49 – 52.

GOBAT, J.-M., ARAGNO, M., MATTHEY, W., 2004. The living soil: fundamentals of soil science and soil biology. Enfield Science Publishers, Enfield, USA, 602 p.

GUWY, A.J., MARTINA, S.R., HAWKES, F.R., HAWKES D.L., 1999. Catalase activity measurements in suspended aerobic biomass and soil samples. Enzyme and Microbial Technology (25):669–676

HORÁKOVÁ, D., NĚMEC, M., SZOSTKOVÁ, M., 2007. Laboratorní cvičení z fyziologie bakterií. Masarykova univerzita v Brně, Brno.

HASALA, R., 2010. Enzymové senzory s teplotní regulací, Bachelor thesis. Masarykova univerzita v Brně, Brno.

ISO 10381-1, Soil quality, Sampling, Part 1: Guidance on the design of sampling programmes, 2002

ISO 10381-2, Soil quality, Sampling, Part 2: Guidance on sampling techniques, 2002

ISO 10390, Soil quality -- Determination of pH, 2005

ISO 11464, Soil quality -- Pretreatment of samples for physico-chemical analysis, 2006

ISO 16072, Soil quality -- Laboratory methods for determination of microbial soil respiration, 2002

JANDÁK, J., PRAX, A., POKORNÝ, E., 2004. Půdoznalství. Mendel University in Brno, Brno, 140 p.

KADAVÝ, J., KNEIFL, M., KNOTT, R., 2009. Nízký a střední les: Založení experimentální plochy nízkého a středního lesa projektu TARMAG na území ŠLP Masarykův les Křtiny [online] citation made on 4. March 2016. Available at www: < http://www.nizkyles.cz/content/view/82/91/lang,czech1250/>.

KADAVÝ, J., KNEIFL, M., KNOTT, R., 2010. Nízký a střední les: Založení experimentální plochy nízkého a středního lesa projektu TARMAG na území ŠLP Masarykův les Křtiny (TARMAG II - Soběšice) [online] citation made on 4. March 2016. Available at www:
< http://www.nizkyles.cz/content/view/94/91/lang,czech1250//>.

KADAVÝ, J., KNEIFL, M., SERVUS, M. et al., 2011. Nízký a střední les - plnohodnotná alternativa hospodaření malých a středních vlastníků lesa. Mendel University in Brno, Brno. 83 p.

KANDELER, E., GERBER, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils, 68–72.

KÁŠ, V., 1954, Mikrobiologický průzkum půdy. IN: Praktikum fytocenologie, ekologie, klimatologie a půdoznalství. KLIKA, J., NOVÁK, V., GREGOR A., (eds.). Nakladatelství Československé akademie věd, Praha, 673–742.

KONŠEL, J., 1931. Stručný nástin tvorby a pěstění lesů v biologickém ponětí. Čs. matice lesnická, Písek, 543.

LIN, Q., DE VRIEZE, J., LI, J., LI, X., 2016. Temperature affects microbial abundance, activity and interactions in anaerobic digestion. Bioresource Technology. (209), 228–236.

MAREK, M.V. et al., 2011. Uhlík v ekosystémech České republiky v měnícím se klimatu. Academia, Praha.

MIKANOVÁ, O., ŠIMON, T., CERHANOVÁ, D., 2010. Hodnocení kvality půdy biologickými metodami. Metodika pro praxi. Výzkumný ústav rostlinné výroby, v.v.i., Praha.

MORRA, M.J., 1997. Assessment of extracellular enzymatic activity in soil. In: HURST, C.J., KNUDSEN, G.R., McINERNEY, M.J., STETZENBACH, L.D., WALTER, M.V., (eds) Manual of environmental microbiology. 459-465.

MULVANEY, R.L., BREMNER, J., M., 1977. Evaluation of antimetabolites for retardation of urea hydrolisys in soils. Soil Science Society of America Journal (41):1024–1027.

NANNIPIERI, P., KANDELER, E., RUGGIERO, P., 2002 Enzyme activities and microbial and biochemical processes in soil. In: BURNS, R. G. and DICK, R. P., (eds)

Enzymes in the Environment – Activity, Ecology and Applications. Dekker, New York, USA.

PANKHURST, C.E., DOUBE, B.M., GUPTA, V.V.S.R., 1997. Biological indicators of soil health: synthesis. In: Pankhurst, C., E., Doube, B., M., Gupta, V., V., S., R., (eds). Biological indicators of soil health. CABI, Wallingford, Oxfordshire, 419–435.

PETRÁŠ, R., PAJTÍK, J., 1991. The system of Czech-Slovak volume equations of the main tree species. Lesnícky časopis, 31 (1):49–56.

POSPÍŠILOVÁ, L., TESAŘOVÁ, M., 2009: Organický uhlík obhospodařovaných půd. Mendel University in Brno, Brno, 41 p.

REJŠEK, K., 1988. Fosfatázy v lesních půdách. IN: Acta universitatis agriculturae. Státní pedagogické nakladatelství, Praha., 59–80.

REJŠEK, K., 1991. Vliv antropogenní zátěže lesní půdy na změny aktivity kyselé fosfomonoesterázy. Mendel University in Brno, Brno.

REJŠEK, K., 1999. Lesnická pedologie, practical exercise. Mendel University in Brno, Brno.

SÁŇKA, J., MATERNA M., 2004. Indikátory kvality zeměfělských a lesních půd ČR. Edice Planeta, Odborný časopis pro životní prostředí (12)

SCHLOTER, M., DILLY, O., MUNCH, J.C., 2003. Indicators for evaluating soil quality. Agriculture, Ecosystems and Environment (98): 255–262.

SKOPP, J., JAWSON, M. D., DORAN, J. W., 1990. Steady-State Aerobic Microbial Activity as a Function of Soil Water Content. Soil Science Society of America Journal (23):45–47

SOIL SURVEY STAFF**,** 1999. Soil taxonomy. A basic system of soil classification for making and interpreting soil surveys. United States Department of Agriculture, Washington DC, USA 436 p.

STEVENSON, F.J., 1994. Humus Chemistry, John Wiley and Sons, New York, USA.

ŠANTRŮČKOVÁ, H., 1993. Respirace půdy jako její biologické aktivity. *Rostlinná výroba*, (39):769-778.

ŠANTRŮČKOVÁ, H., 2014. Základy ekologie půdy, skriptum. University of South Bohemia, České Budějovice.

ŠARAPATKA, B., 2014. Pedologie a ochrana půdy. Univerzita Palackého v Olomouci, Olomouc.

ŠIMEK, M., 2007. Základy nauky o půdě. Neživé složky půdy. University of South Bohemia, České Budějovice.

TABATABAI, M.A., BREMNER, J.M., 1969. Use of p-nitrophenol phosphate for assay of soil phosphatase activity. Soil Biology and Biochemistry, (1):301- 307.

TATE, R.L., 2005. Microbiology and enzymology of carbon and nitrogen cycling. In: BURNS, R. G. and DICK, R. P., (eds) Enzymes in the Environment – Activity, Ecology and Applications. Dekker, New York, USA.

TOMÁŠEK M., 2007. Půdy České republiky. Česká geologická služba, Praha 68 s.

TOPP, W., SCHLICHTER, D., 2004. Soil degradation in simple oak coppice forests of the Ahr-Eifel: Implications for forest management derived from soil ecological studies. Cuvilier verlag, Gottingen, Germany

UHUL, 2016. Přírodní lesní oblasti. [online] citation made on 12. March 2016. Available at www:
< http://www.uhul.cz/nase-cinnost/oblastni-plany-rozvoje-lesu/prirodni-lesni-oblasti-plo>. 33

UTÍNEK, D., 2004. Převody pařezin na středni les v městských lesích Moravský Krumlov, disertation thesis. Mendel University in Brno, Brno.

VARMA, A., ABBOTT, L., WERNER, D., HAMPP, R., 2004. Plant Surface Microbiology. Springer-Verlag, Berlin, Germany.

VODRÁŽKA, Z., 1996. Biochemie. Academia, Praha.

WARDLE, D.A., PARKINSON, D., 1990. Comparison of Physiological Techniques for Estimating the Response of the Soil Microbial Biomass to Soil-Moisture. Soil Biology & Biochemistry (22):817-823.

WARDLE, D.A., 1992. A Comparative-Assessment of Factors Which Influence Microbial Biomass Carbon and Nitrogen Levels in Soil. Biological Reviews of the Cambridge Philosophical Society (67):321-358.

WARDLE, D.A., 1998. Controls of temporal variability of the soil microbial biomass: A global-scale synthesis. Soil Biology and Biochemistry (30):1627-1637.

QUITT, E., 1971. Klimatické oblasti Československa. Academia, GÚ ČSAV v Brně, Brno.

ZBÍRAL, J, 2005 Analýza půd I, Jednotné pracovní postupy. Central Institute for Supervising and Testing in Agriculture in Brno (ÚKZÚZ), Brno.

ZBÍRAL, J., MALÝ, S., VÁŇA, M., 2011 Analýza půd III, Jednotné pracovní postupy. Central Institute for Supervising and Testing in Agriculture in Brno (ÚKZÚZ), Brno.

ZELLES, L., ADRIAN, P., BAI, Q.Y., STEPPER, K., ADRIAN, M.V., FISCHER, K., MAIER A., ZIEGLER, A., 2002. Microbial activity measured in soils stored under different temperature and humidity conditions. Soil Biology and Biochemistry 23 (10):955–962

APPENDICES:

Appendix 1 Soil profiles of studied TARMAG II subplots

subplot no. 1

subplot no. 2

subplot no. 9

subplot no. 10

subplot no. 17

subplot no. 18

Appendix 2 Review of Applied Laboratory Methods

A2.1 Physical Properties of Soil

Physical properties of soil were determined with the use of core cylinders and pycnometers according to general procedures used at Mendel University provided for practical exercises by Rejšek (1999).

A2.1.1 Cylindrical Core Method

Cylindrical Core Method is widely used to determine bulk density and other physical properties of soil, but can be also applied to calculating water and air regime of soil. The main device of this analysis is so called Kopecky's physical cylinder usually made from stainless steel of inner volume of 100 ml and maximum height of 5 cm. Cylinders, also known as core cutters, are fully driven (usually hammered) into straightened soil profile with the aid of steel rammers. Soil around the cylinder is excavated and the cylinder is undercut with a knife. Core cutter is carefully lifted and both surfaces of the sample are flat trimmed. The outside surface of the cutter is cleaned. Lids are placed on both cylinder sides and are tightened with rubber bands if necessary. Cylinders are properly labelled. Samples should be taken directly to the lab and refrigerated if not being processed straight away.

1. Take the lids off and weigh the cylinder placed onto small round filter paper to record initial field-moist humidity. Filter paper, the sample is initially placed upon, is included in all further measuring.
2. Cylinders are allowed soak in water till their suspension is saturated (samples are shiny on the top). This procedure takes place for 24 hours and is carried out on wood planks wrapped in filter paper and half submerged into water. Samples should be covered to avoid excessive water of evaporation.
3. When saturated, cylinders are weighed again.
4. Soil samples are set aside onto four times folded sheets of filter paper (or simply four sheets of filter paper) and left to drain for 30 minutes. At this time water content is drained from the greatest non-capillary pores. Samples are put back onto filter papers after weighing.
5. This process is repeated after another two hours. Values at this time describe the state of maximum capillary water capacity and minimum air capacity. Final 'wet' weighing takes place 24 hours after the first saturated measurement. This

is important for distinguishing between capillary and semi-capillary porosity, as well as for obtaining approximate retention water capacity values.

6. Soil samples are oven dried to constant weight at 105 °C. Weighed when cooled down to room temperature.
7. Soil samples are carefully pushed out of the cylinders into a standard enamel laboratory dish and are left to stand together with the core cutters for 7 days to reabsorb atmospheric humidity.
8. Both soil samples and cylinders together with filter papers are weighed, only this time separately. Precautions are taken to include the entire soil sample: each sample is handled with utmost care to avoid losses and spillages; residual soil has to be scraped of the cylinder.

A2.1.2 Determination of Specific Gravity of Soils (D_s)

Applied soil analyses of specific gravity is usually described as the ratio of the weight of any volume of soil material to the weight of any volume of water. Calibrated 100 ml pycnometers are utilized in the process of specific gravity determination.

1. Soil samples are oven dried to constant weight at 105 °C and sieved through 2 mm mesh.
2. Empty pycnometers are weighed (glass stoppers are always included in the weighing)
3. Each pycnometer is filled to about one fifth of its height with soil samples of about 10 g. Weight of sample is recorded.
4. Demineralized water is added to the pycnometer to cover the sample and reach to about one quarter of the height of the pycnometer.
5. Pycnometers are placed on a hot plate and their contents are brought to rolling boil. Boiling the contents for about 5 minutes effectively expels all air content out of the sample. The sample solution must not boil out of the vessel, precautions are taken: wash bottles are used to slow down the boil or flush the sides of the vessel.
6. Pycnometers are cooled and filled to the brim with demineralized water. Stoppers are pushed in with a swift move making sure that there is no residual air in the container.
7. Pycnometers with soil samples and water are weighed and this weight is subsequently compared to the weight of pycnometer filled with water only

according to the equation bellow. Results of specific gravity are given in $g.cm^{-3}$.

Equation A2.1. Calculation of specific gravity (ρ_s), where m_1 stands for the weight of the soil sample (g), m_2 is the weight of pycnometer filled with demineralized water (g) and m_3 represents the weight of pycnometer with boiled soil water suspension

$$\rho_s = \frac{m_1}{m_1 + m_2} - m_3$$

A2.2 ph Determination

Soil pH was measured in agreement with Zbíral's methodology published in Analýza půd I (2005) and that is also in accordance with a current international standard ISO 10390:2005 and its Czech counterpart national standard ČSN ISO 10390 (836221). Applied method of routine determination of pH is using a glass electrode in a 1:5 (volume fraction) suspension of soil in water (pH in H2O) and of potassium chloride solution (pH in 1 M KCl). This procedure should be applicable to all types of dry soil prepared according to ISO 11464:2006 (Soil samples were dried in an oven at temperature not exceeding 40 °C, subsequently crushed and sieved resulting in fraction smaller than 2 mm).

1. Preparation of soil suspension acquires measuring 5 ml of prepared soil sample (or an equivalent of soil in grams) into 125 ml screw-top plastic flask and adding 25 ml of solution medium (demineralized H_2O or 1 M KCl).
2. Extraction is performed on mechanical shaker for 60 ± 10 minutes. Suspension is left to stand for another 60 minutes after shaking and only after than the measurement takes place. The total time of contact of the soil with the solution before measuring the pH must not exceed 4 hours.

A2.3 Dry Matter (DM) Determination

The principle of dry matter (DM) determination in soil is based on heating soil samples to temperatures at which water is evaporated (Rejšek 1999). The results are calculated from the weight difference of samples before and after drying and are expressed in grams of dry matter or in percent (dry matter content per fresh soil weight) (Equation A2.2).

1. Aluminum dishes with imprinted serial numbers were used for the process of drying. The dishes were weighed first and their weight values were recorded against their serial numbers.
2. 10 g of fresh soil was than weighed on laboratory scales with the accuracy of ± 0.01 g and placed in each dish.
3. Prepared samples were dried in an electric oven at constant temperature of 105 ° C for at least 2 hours.
4. The dishes with samples were left to cool down at room temperature and were subsequently weighed.
5. Dry matter content was calculated according to the below equation.

Equation A2.2 Dry matter calculation formula (results are in %), where m_∞ stands for the weight of dish with sample after drying, m_0 stands for the weight of an empty dish and m_1 stands for the weight of a dish with fresh soil sample

$$DM = \frac{m_\infty - m_0}{m_1 - m_0} * 100$$

A2.4 Determination Of Loss On Ignition (LOI)

The LOI method is used in accordance with JPP operational methods last updated in 2011 by Central Institute for Supervising and Testing in Agriculture in Brno (ÚKZÚZ) and is based on unified national norm (ČSN EN 15935 (838126) and, not to say, the international European standard EN 15935:2012).

Dried test sample is furnace heated to constant mass at (550 ± 25) °C. The difference in the mass before and after the ignition process is used to calculate the loss on ignition (LOI). LOI is expressed as weight percentage of dry mass (DM) (Equations A2.3).

1. The determination of LOI is carried out in a numbered flat bottom ceramic crucible. The Crucible has to be empty-prepped by 30 minutes of ignition at 550 °C, cooled down and weighed. After noting down its weight, dried soil sample prepared by oven drying at 105 °C in a shallow aluminum or ceramic dish can be added. Every necessary precaution shall be taken to avoid absorption of atmospheric humidity by the dried sample before weighing.
2. 5 g to the nearest 1 mg (5 ± 0,001 g) of dried soil sample is weighed into the crucible.

3. Crucible is placed in the furnace preheated to 550 °C for at least 60 minutes.
4. Crucible is left to cool down to ambient temperature preferably in a desiccator with silica gel to prevent atmospheric humidity intake. Weighing is carried out immediately after the removal of the crucible from the desiccator and the weighing operation is completed as quickly as possible. The mass of the residue on ignition, and therefore, the LOI shall be regarded as constant, if the mass obtained after a further half-hour period of ignition at 550 C in the pre-heated furnace does not differ by more than 0.5% of the previous value.

Equations A2.3 Loss on ignition (LOI), effectively the content of organic matter expressed in %, where m_1 is the weight of soil sample before ignition, m_2 stands for the weight of the sample after the ignition, M_1 is the weight of the sample together with crucible before ignition and M_2 represents is the weight of the sample together with crucible after ignition. W is the weight of empty crucible

$$LOI = 100 * \frac{m_1 - m_2}{m_1}$$

$$m_1 = M_1 - W$$

$$m_2 = M_2 - W$$

A2.5 Determination Of Organic Carbon In Soil (C_{ox})

Method of spectrophotometric determination of organic carbon in soil (C_{ox}) by sulphochromic oxidation applied in the process of estimating the total amount of organic carbon is of normative number 30911 by Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ) (Zbíral et al. 2011).

Oxidizable organically bound carbon in soil is oxidized by the surplus of potassium dichromate solution in sulfuric acid medium at 135 °C. Yellow-orange dichromate ions are reduced to green colour Cr^{3+}. The intensity of green colour is measured spectrophotometrically. Glucose is used as a standard for calibration.

Chemical reaction describing the oxidation process:

$$2(Cr_2O_7)^{2-} + 16\ H^+ + 3C \rightarrow 3CO_2 + 4Cr^{3+} + 8H_2O$$

or

$$4Cr0_3 + 6H_2SO_4 + 3C \rightarrow 3CO_2 + 2Cr_2(SO_4)_3 + 6H_2O$$

Soil samples are prepared in accordance with the ÚKZÚZ standardized methods (Zbíral 2002). This involves air drying the samples, sieving out fractions larger than 2

mm, partitioning into smaller amounts (up to 10 g), removing all plant and animal residues and triturating in an agate mortar grinder so as to entirely pass through a sieve of 0,1 mm fraction size.

1. Weigh 0,25 g of modified soil sample into 100 ml combustion flask and pipette in 5 ml of M 0,27 potassium dichromate ($c(K_2Cr_2O_7)$ = 0,27mol/l) and 7,5 ml of concentrated sulphuric acid ($\rho(H_2SO_4)$ = 1,84 g/cm3). Gently stir the contents.
2. Flasks are placed onto preheated laboratory hotplate set to 150 °C for 30 minutes and subsequently left to cool down.
3. Demineralized water is added to flasks to fill them up to the 100 ml mark. Shake the contents gently and let the suspension stand for 60 minutes to allow the sediments to settle.
4. Part of the sample is decanted into centrifuge cuvette and spun for 10 minutes at 2000 g.
5. At least one control (blank) sample is prepared along the whole process.
6. Only perfectly clear samples can be spectrophotometrically measured for absorbance at 585 nm.

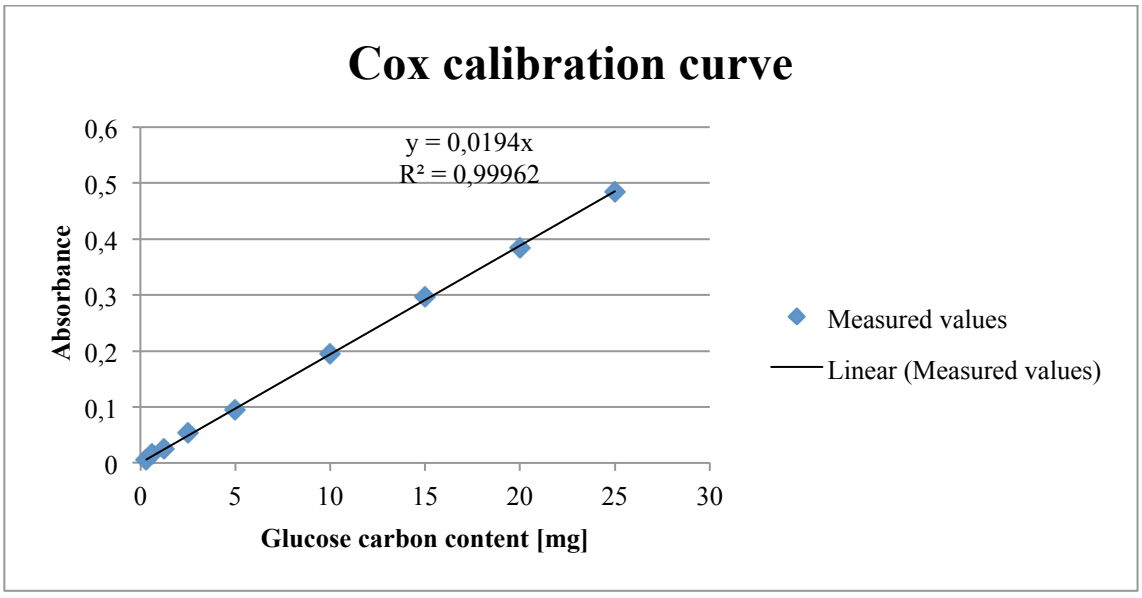


Figure A2.1 Cox calibration curve of spectrophotometrically measured values of pure carbon content of dehydrated glucose that has undergone sulphochromic oxidation

Equation A2.4 Calculation of oxidizable organic carbon in soil (C_{ox}) includes water content (w (%)) related to dry matter content of soil samples and estimated according to ISO 11465, weight of soil sample (m (g)), sample organic carbon content (a (mg)

$$C_{ox} = \left(\frac{a}{m}\right) * \frac{100 + w}{100}$$

Pre-constructed calibration curve (Figure A2.1) was used in the process of final result calculation. It was constructed from spectrophotometric readings obtained by: Dissolving dehydrated glucose of different carbon contents (0 – 20 mg) in dichromate solution in 100 ml flasks topped to the mark. 5 ± 0,05 ml of this solution was then pipetted into 100 ml flasks together with 7,5 ml of H_2SO_4 and stirred. Samples are heated together with the rest of the samples. Remaining part of the oxidation and measuring process is as above.

A2.6 Determination Of Soil Carbon (C_{tot}) And Nitrogen (N_{tot}) Contents

Total of organic soil C content [%], total N content [%] and their ratio (C:N) were determined using LECO TruSpec CN soil analyzer (LECO Corp., St. Joseph, MI) at Mendel University (Department of Geology an Pedology). The LECO TruSpec CN elemental analyzer operates on the principle of dry combustion of samples in an oxygen atmosphere, where helium of a purity of 4,8 % serves as the carrier gas, medical grade oxygen (purity 5,0) as the combustion gas and air as the pneumatic gas. Combustion takes place in a U-shaped quartz tube. Samples are let inside the preheated quartz tube, in which the combustion takes place at high temperature in a stream of pure oxygen. Temperatures range between 950 °C ± 10 °C in the input arm and 850 °C ± 10 °C in the output (burn out) arm. A separation process of ash and combustion gases takes place in the quartz tube. Burnt-out gasses are carried further into the detection system, where C_{tot} is determined form the CO_2 content of the flue gases by an infrared detector and N_{tot} is calculated from the nitrogen oxides content in the flue gas. N_{tot} determination is carried out in a reduction column by a thermal conductivity detector, where NO_x is quantitatively converted to N_2 with the aid of a catalyst after the separation of water vapor and CO_2.

Soil samples, similarly to C_{ox} analysis, are prepared in accordance with the ÚKZÚZ standardized methods (Zbíral 2005) and again, prior to the analysis, dehydrated for 1 hour at 105 °C. Samples are weighed into special tin foils. Initial sample weight of 100 -200 mg has to be increased for lower mineral horizons. Blind samples and standards (with known content of analyzed elements) have to be analyzed simultaneously. Standard samples used for calibration are supplied by the LECO Company. Total Carbon and nitrogen contents of the samples are evaluated according to calibration curves.

A2.7 Assays On Soil Microbial Carbon (C_{mic})

Used process of soil microbial biomass evaluation comprises of two separate methods by Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ) (Zbíral et al. 2011), where the first serves as a lead-up for the other. Therefore, both procedures, the Chloroform-Fumigation K_2SO_4 extraction method (normative 31010.1) and the Determination of Oxidizable Organic Carbon in (K_2SO_4) Solution (normative 31020.1), were respectively carried out; hence the division in described laboratory procedure. However, either of applied methods drives at the same result, which is content of extractable carbon of microbial biomass expressed in micrograms per gram of dry matter in soil ($\mu g.g^{-1}$).

Both procedures are to compare the amount of organic carbon in a chloroform-fumigated soil sample to that in a non-fumigated soil sample to determine soil microbial biomass. The Total Organic Carbon content (TOC) will be higher in the chloroform-fumigated sample because the sample contains the cell contents of lysed microbial cells. Hence the difference in extracted TOC between fumigated and non-fumigated examples will provide measure of microbial biomass. Note that you can only assume that this TOC in the soil is of microbial origin. Samples have to be picked free of roots, litter, earthworms, etc., since the microfaunal contribution to TOC is usually less than 5 %.

Final results are calculated only from the second part of the procedure.

A2.7.1 The Chloroform-Fumigation K_2SO_4 Extraction Method

Fumigation is carried out for a period of 24 hours in vacuum desiccators with the addition of chloroform and NaOH. It is important to refrigerate soil samples at 4 °C until the fumigation and K_2SO_4 extractions are performed.

1. It is preferable to prepare required amount of 0,5 M K_2SO_4 first (Use magnetic stirrer while warming the solution).
2. Field-moist soil samples (pre-sieved through 2 mm mesh), free of visible roots and organic debris, are mixed thoroughly to become homogenous.
3. Plastic 50 ml flasks are to be labeled as to distinguish between non-fumigated (NF) and fumigated (F) samples. All samples are run in triplicates.
4. Weigh 5 g of soil into each flask (water content should be in the 20 to 30 % range for best fumigation results).

5. Chloroform fumigation: vacuum desiccators are cleaned and covered at the bottom with filter paper. Filter paper is moistened with demineralized water and F flasks are placed inside of the desiccator.
6. 100 ml beaker is placed inside the desiccator. Glass beads and alcohol free chloroform are added to the beaker. About 2 g of NaOH drupelets in a smaller beaker are also placed inside the vessel to absorb atmospheric CO_2 during fumigation. Vaseline is applied to all contact areas between the main vessel, the lid and the valve and the desiccator is closed.
7. Vacuum pump is turned on, as is the opening valve. Vacuum created in the desiccator causes the chloroform to boil and evaporate, and of course, to saturate the desiccator's atmosphere. The chloroform is allowed to boil vigorously for five minutes yet it should not spill out of the beaker. Turning off the valve seals the desiccator. The fumigation process should take place for 24 hours.
8. Beaker is removed after upon opening the fumigation vessel and chloroform is allowed to evaporate. It is important that all residual chloroform is removed from the soil samples before proceeding with K_2SO_4 extractions. The flasks may be placed in fume hood with open windows in the room to increase the velocity of the wind flowing over the flasks. This procedure should be allowed to take place for at least 40 minutes.
9. 20 ml of 0,5 M K_2SO_4 is added to each flask. Flasks are closed tightly and rotary shook for 30 minutes at about 200 rpm.

A2.7.2 Spectrophotometric Determination Of Oxidizable Soil Organic Carbon In (K_2SO_4) Solution

K_2SO_4 extracted soil samples are oxidized in strong acid medium with the addition of potassium dichromate and spectrophotometrically analyzed for TOC content. Analysis results need to be adjusted to TOC/g dry soil value.

10. Soil suspension is poured into labeled plastic cuvettes and the samples are readied for centrifugal sedimentation at 4000 rpm for 5 minutes.
11. 1 ml of clear, sedimented F sample is pipetted into labeled 25 ml flasks together with 1 ml of K_2SO_4 (NF samples are prepared only with 2 ml of sample). 3 ml of oxidation reagent consisting of 10 ml of 0,025 M potassium dichromate ($c(K_2Cr_2O_7)$ = 25 mmol/l), 25 ml demineralized H_2O and 73 ml 95% H_2SO_4 is also added to all flasks.

12. Blanks are prepared similarly but only 2 ml of K_2SO_4 solution and 3 ml of oxidation reagent is added to the 25 ml flasks. For the best result triplicate blanks as well.
13. All balloon flasks are put onto preheated hotplate set to 150 °C and the oxidation is allowed to take place for 30 minutes.
14. Samples are set aside to cool down to room temperature and than spectrophotometrically measured (ƛ = 340 nm) in constricted 0,5 cm cuvettes.

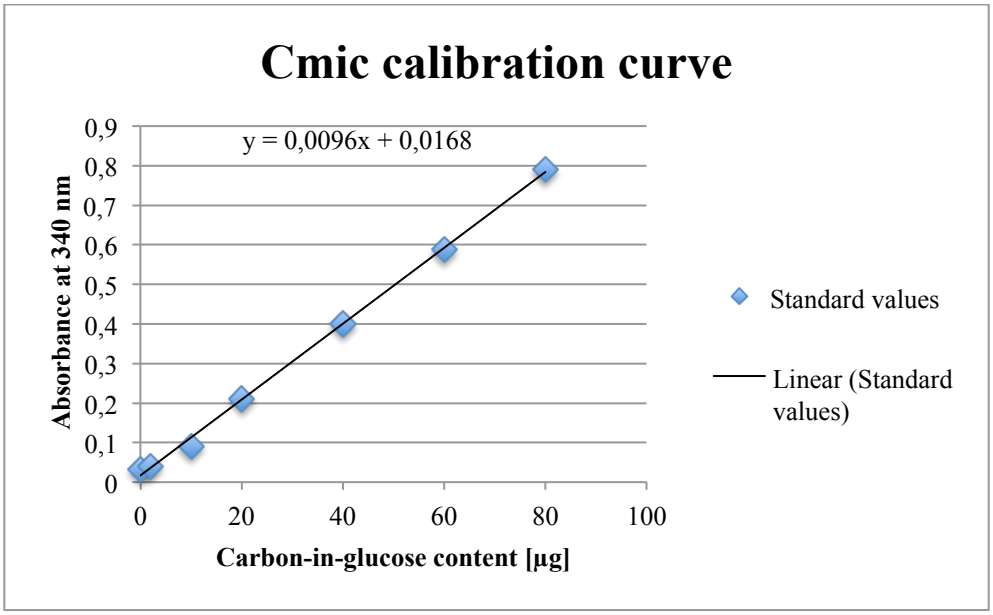


Figure A2.2 Log Inverted graph used for establishing carbon content of microbial biomass (C_{mic}) with projected linear calibration curve of spectrophotometrically measured values of carbon-in-glucose content in K_2SO_4 standard solutions

A2.8 Urease Activity Determination

Urease activity in soil was examined with the aid of Kandeler's and Gerber's method of colorimetric determination of ammonium published in 1988.

The method comprises of incubation of soil with an aqueous and buffered urea solution at 2 hours at 37 °C in order to release the enzyme from protected locations in soil suspension, extraction of ammonium with KC1/HCI and spectrophotometric determination of released ammonia. Urease activity expressed as µg of hydrolysed N/g DM/ 2 h at 37 °C.

1. Field-moist soil is gently triturated before being sieved on a 2 mm screen to separate larger fractions.

2. 2,5 g of soil is placed into 120 ml plastic incubation flasks and wetted with 1.25 ml aqueous urea of M 0,72 solution ($c(CH_4N_2O)$ = 0,72 mol/l). All samples are run in triplicates.
3. The flasks are stoppered with lids and placed into an incubator set to 37 °C, taken out after 2 hours.
4. 15 ml KC1/HC1 (1 M KCl to 0,05 M HCl) is added and the mixtures shaken on a mechanical shaker for 30 min.
5. Control samples were prepared as above with the exception that urea was substituted with demineralized water and added only after incubation immediately before the HCl/KCl adition.
6. The resulting suspension is poured to centrifuge cuvettes and spun at 4000 rpm for 5 minutes.
7. 1 ml of clear sample solution is transferred to 50 ml flasks and 9 ml of demineralized water is added (1 ml is effectively diluted to 10 ml). Successively add 5 ml of Na-salicylate (prepared from: 100ml 0.12% Na-nitroprusside, 100ml 17% Na-salicylate and 100 ml demineralized H_2O) and 2ml 0,1% Na-dichlorisocyanurate.
8. Allow the solution to stand at room temperature for at least 30 min.
9. Absorbancy of all samples was measured at 690 nm (λ = 690 nm).

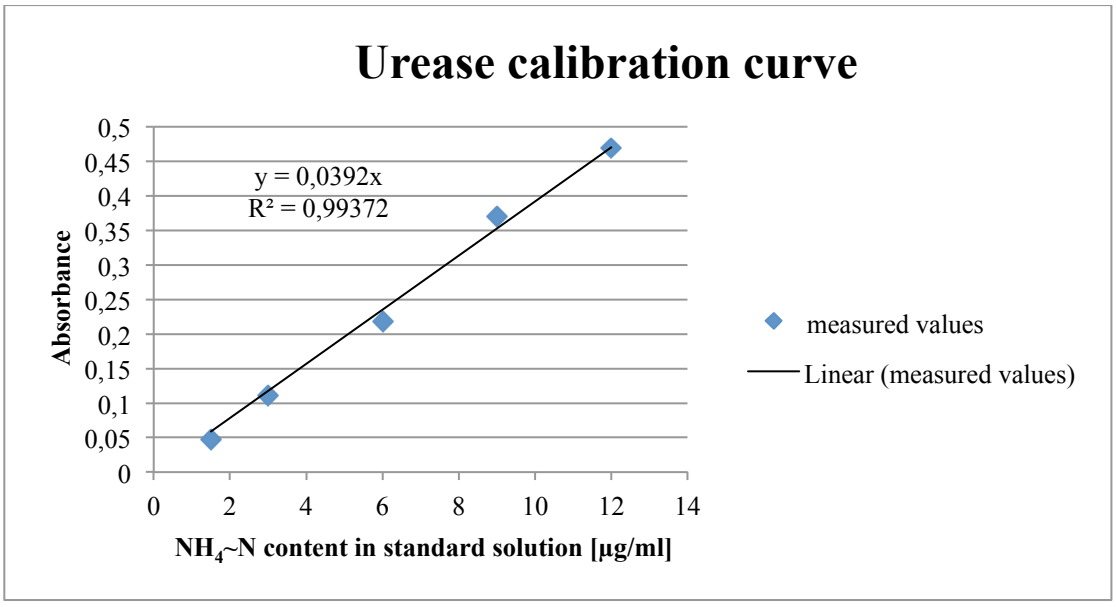


Figure A2.3 Urease calibration curve of spectrophotometrically measured values of NH_4~N in HCl/KCl standard solutions

Pre-constructed calibration curve was, again, used in the process of final results computation. It was constructed from spectrophotometric readings obtained from standard solutions. 3.8207g NH_4C1 was dissolved in 1000ml water to prepare the standards. The NH_4~N content of the solution was calculated by reference to a calibration graph (Figure A2.3) plotted from the results obtained by pipetting 0, 0,15, 0,3, 0,6, 0,9 and 1,2 m1 of 0,382% NH_4Cl into 100 ml flasks, which were additionally filled to the 100 ml mark with the HCl/KCl solution.

Equation A2.5 Extractable content of oxidizable carbon calculated to dry matter content (DM) in soil (µg.g^{-1}), where w is the initial soil sample water content (%), m stands for DM corresponding with the content of fresh soil (g), V is the volume of extraction reagent and C_{sol} stands for the concentration of oxidizable carbon content in the extract derived from calibration curve calculations (µg.ml^{-1})

$$C_{mic} = C_{sol} * \frac{V + \left(\frac{w * m}{100}\right)}{m}$$

A2.9 Acid Phosphomonoesterase Activity Evaluation

Applied laboratory analysis is based on Tabatabai's and Bremner's procedure (1969) that was later modified by Rejšek (1991) and applies p-nitrophenol phosphate (p-NPP) added to working buffer solution. Enzymatic activity releases p-nitrophenol (p-NP), which is extracted and colored by potassium hydroxide (KOH). The acid phosphomonoesterase activity is measured spectrophotometrically. Results are determined according to absorbance of analyzed samples at wavelength of 410 nm and the acid phosphomonoesterase activity is expressed in µg p-NP/g DM/hour, where DM stands for dry matter.

1. 1 g of field-moist soil is weighed into 100 ml Erlenmeyer flask and 12 ml of working buffer solution (of sodium perborate and succinic acid) containing 750 µM/l p-NPP is added. Shake the flasks briefly and incubate for one hour at 37 °C.
2. Filter the solution into plastic cuvettes, add 4 ml of 1 M KOH. Yellow coloration of ions should occur due to strong alkalization. (Alkalized filtrates can be diluted with working buffer solution in 1:1 (1:2) ratio if necessary.
3. Control measuring has to be performed in parallel with each analysis that is carried out. The occurrence of yellow colouring, which did not derive from

p-NP release by acid phosphomonoesterase p-NPP breakdown, has to be considered. Therefore, control samples, prepared similarly, have to contain pure working buffer solution without p-NPP. 12 ml of working buffer solution with p-NPP is added to controls after incubation (just before filtration). Twice the amount of KOH has to be subsequently added to retain the dilution ratio.

4. Absorbance of standard solutions with known p-NP concentration is used for calibration curve construction.
5. Absorbance of analysed samples and standard solutions is measured at 410 nm.

A2.10 Catalase Activity Evaluation

Determining the activity of catalase in soil is practically the same as quantifying the capacity of soil to decompose hydrogen peroxide (H_2O_2) to water and molecular oxygen. The reason behind measuring the production of O_2 (generated due to the presence of H_2O_2 in the original suspension) and, therefore, the evaluation of the biological activity of soils lies in the presence of microflora capable of producing an enzyme (catalase) catalyzing this reaction. Used procedure was drawn up by Káš in 1954 and gas volumetrically measures the amount of O_2 evolved within 15 minutes period. Catalase activity is expressed in: ml O_2 $5g^{-1}$ 15 min^{-1} and is considered to be very low if lower than 5, low if between 5 –15, medium if 15 –30, high if 30 – 60 and very high if more than 60.

Device called '*Vápnoměr dle Janka*' is essential for this procedure. It is a system of two graduated eudiometric measuring tubes 0 –100 ml connected with rubber tubing at their lower ends. The first of the tubes is connected with Erlenmeyer flask (containing an automatic pipette) through the rubber tubing and a three-way stopcock. The system also contains a reservoir bottle connected to both tubes at the lowest part.

1. Weigh 10 g of mineral (or 5 g of organic) field-moist soil into 250 ml Erlenmeyer flask and add 50 ml of demineralized water.
2. Introduce water from the reservoir into the manometer system of two connected eudiometric tubes until it reaches top calibration marks. Lock the system with tightening screw and introduce 20 ml of 3% H_2O_2 into the automatic pipette.
3. Connect the pipette to the Erlenmeyer flask with the rubber stopper. System has to be airtight. Three-way stopcock can be opened. Pour the hydrogen peroxide solution from the automatic pipettes into the flask.

4. Briefly stir the suspension and start measuring time. Stir the contents of the flask every five minutes.
5. Note the level of water in the first eudiometric tube after fifteen minutes (in case of intensive oxygen production pressure in the system can be lowered by releasing excess water from the system into the reservoir bottle).
6. Since each grade on the scale ('0,1') represents 5 ml of newly produced O_2 it is relevant to divide the resulting number on the grade by two providing the initial soil sample was 10 g. Final step is to recalculate the O_2 production to dry matter content in initial soil sample.

A2.11 Basal (BR) And Potential Respiration Of Soil (SIR)

This methodology is based on the International Standard ISO 16072:2002, which has derived from the German standard DIN 19737 and follows Czech national standards ČSN EN ISO 14240-1&2 (836441). Soil samples were prepared noting Šantrůčková's findings (1993), where prolonged incubation time is recommended due to avoiding excess root respiration. Two types of respiration were measured: basal respiration (BR), describing current state of soil after the incubation period and potential, utilizing dehydrated glucose infused samples for measurement of the substrate induced respiration (SIR).

Procedure describes techniques of microbial soil respiration determination in laboratory environment. Microbial soil respiration results from the mineralization of organic substances. In this process, organic substances are oxidized to the end product of carbon dioxide and water, with concurrent uptake of O_2 for aerobic microorganisms. The principle of this method is based on alkaline solution, in this case 1 M NaOH that is placed in Petri dish above the soil surface in a closed plastic container. Carbon dioxide evolution is measured by absorption in the sodium hydroxide for 24 hours. The CO_2 amount absorbed is measured by back-titration of the excess alkali remaining. Results are expressed in units per gram of dry soil per time of incubation to be comparable to other studies. It is one of the most commonly used methods of determining metabolic activities of organisms (secondary consumers – mainly microbes) in soil. The incubation time in this case is two months to minimize CO_2 production of respiring fine roots.

1. Weigh 20 g of organic (40 g of mineral) field-moist soil to 150 ml incubation vessels (airtight plastic containers). Each sample should be made in two repetitions.
2. Add 3,65 mg of carbon contained in glucose per g of dry soil to measure potential respiration. Mix the glucose thoroughly but carefully into soil by stirring with glass rod. Basal respiration is measured without glucose.
3. Bolster a Petri dish with two short glass rods onto each soil sample and add 2 ml of 1 M NaOH. Close the vessels with airtight lids. Containers must be handled with utmost care to avoid spilling the alkaline solution.
4. Incubate for 24 hours exactly. If time period was to be prolonged, record times of incubation and include it in the final calculation.
5. Incubation of at least 3 control samples (empty vessel only with Petri dish and NaOH) must be prepared in parallel with all samples.
6. Vessels are opened after 24 hours of incubation. Alkaline solution is transferred into 50 ml beakers and 1 ml $BaCl_2$ is added into the solution.
7. Perform titration with 0,05 M HCl to phenolphthalein

Equation A2.6 Intensity of respiration calculation integrates the amount of HCl used for titration of controls (A), the amount of HCl used for titration of samples (B), exact concentration of HCl (mol/l), coefficient for recalculation (from V (HCl) to µg C-CO2: 6005), weight of field-moist soil (FMS), dry matter content (DM = weight of 1 g of FMS after drying at 105 °C for 2 hours) and time of incubation (t). Resulting values are in µg C-CO_2 * g^{-1} * hod^{-1}

$$R = \frac{(A - B) * c(HCl) * 6005}{FMS * DM * t}$$