MINE RECLAMATION AND NITROGEN CYCLING; APPLICATION OF MINE SUBSOIL, SOIL AMENDMENTS AND NATIVE VEGETATION AS TOOLS FOR EFFECTIVE ECOSYSTEM RECLAMATION.

by

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Abstract

Mining is a strong contributor to Canadian and British Columbian economies. Mining yields raw materials without which human society would not be able to function. Also, this industry is a source of income for thousands of households. However, because of the large scale of operation, mining has an impact on landscapes and ecosystems including fragile and unique ones such as grasslands. This highlights the need for an effective and ecologically-based reclamation strategy. The reclamation of mine lands is a challenge because mine soils following mining activity are often deprived of organic matter, nutrient-poor, possess adverse physicochemical properties, and can be contaminated by heavy metals. Due to the large size, the capping of reclaimed land by topsoil is often not possible, the attention of reclamation practitioners has focused towards subsoil which is generated as a waste product and often stockpiled in large quantities. It is an open question whether mine subsoil can play a role of starting substrate for mine lands ecological reclamation. This is the overarching question of this thesis: can subsoil be transformed into operational topsoil? It is hypothesized that this process will not be successful if the proper nitrogen cycling is not fully restored as nitrogen is one of six elements called biogenic and is intrinsic to all living organisms. The objectives of this thesis were to investigate (1) whether subsoils collected from New Afton New Gold and Teck Highland Valley Copper mines were suitable to sustain a viable vegetation cover, (2) whether an application of biochar, woodchips, biosolids (nitrogen-rich) and a mixture thereof ameliorate subsoil futures and help in subsoil transformation, (3) whether nitrogen influences revegetation and what transformation this element passes on the way of its cycling during the early stages of reclamation. Two potting experiments were conducted to address the research objectives. The first one took place in the controlled conditions of a greenhouse and worked with native in BC graminoids and legume (nitrogen fixer). The second one was conducted in open-air conditions and worked with three native shrubby species including one non-leguminous nitrogen fixer.

The results indicated that none of the analyzed mine subsoils, due to their low fertility and adverse physicochemical properties, was capable of sustaining viable vegetation when unamended. The situation changed when subsoils were amended by biosolids or a mixture. An addition of 25% of woodchips or 5% of biochar did not help. The vegetation response to nutrient input related to biosolids application was positive in the case of both mines, however, productivity on Teck Highland Valley Copper mine subsoil was significantly larger than productivity on New Afton New Gold mine subsoil in both experiments (experiment 1

Kruskal-Wallis $p < 2.2e-16$; experiment 2 Kruskal-Wallis, $p = 1.7e-08$). Physicochemical tests revealed that New Afton New Gold mine subsoil's high pH and salinity posed a hindrance to overcome for vegetation growth. Additionally, throughout both experiments, the contents of total nitrogen, mineralizable nitrogen, and ammonium decreased, while the nitrate content increased. That in turn might indicate that in the early stage of reclamation, when biosolids are applied as a source of nitrogen, the nitrification step of the nitrogen cycle takes prevalence. Seven native plant species were used in this research and all demonstrated that they can cope with harsh growing conditions created by two mines' subsoils, however, they need to be provided with initial nutrient sources. This research provided valuable information about subsoil, soil amendments, and native vegetation (including nitrogen fixers) to consider when undertaking mine lands reclamation.

Keywords: mine reclamation, subsoil, soil amendments, native plant species, nitrogen cycling, nitrogen fixers

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CHAPTER 1: GENERAL INTRODUCTION

RECLAMATION

Reclamation is a complex process aimed at rebuilding the functionality of areas previously disturbed by either human activity or an occurrence of a forceful natural disaster. Reclamation is a deliberate human intervention to restore an ecosystem to a functioning, self-sustaining, and desirable state (Brown and Amacher, 1999, Garris et al., 2016). Reclamation of disturbed areas usually consists of the reconstruction of the physicochemical and biological features of the soil layer in the first phase, re-vegetated soon after. Therefore the reclamation of soil is generally the first step in the process. Soil is a living space of organisms that form the beginning of most land food chains. When soil structure, fertility, and productivity are restored along with the diversity of microorganisms, it facilitates the colonization of other organisms (Rigby et al., 2016). Environmental disturbance can disrupt soil biophysical processes, such as elemental cycling of nitrogen. Vegetation plays a pivotal role in nitrogen cycle. If, as a result of disturbance, the vegetation cover is destroyed, the cycle becomes strongly interrupted and the pool of nitrogen in the soil quickly decreases (Wanic and Pająk, 2012). In reclamation, plants that enrich the soil with organic matter and biogenic elements are particularly beneficial. In this context, plants from the *Leguminosae* family, such as lupine, pea, alfalfa, and vetch are particularly valued because they fix atmospheric nitrogen (Jefferies et al., 1981 (1); Jefferies et al., 1981 (2); Aschenbach et al., 2012).

Mine Reclamation

British Columbia, Canada, mining regulations require that land disturbed as a result of human mining activity must be reclaimed (MAC, 2018; BC Mines Act, 2021; Ministry of Energy and Mines, 2017). A modern approach to mine reclamation does not begin at the moment of operation termination but much earlier, even before the operation commences. It takes place by planning and ensuring the availability of funds, technical solutions, and specialist knowledge (BC Mines Act, 2021; Ministry of Energy and Mines, 2017; Malaschenko et al., 2017).

Post-mining Soils

Mine soils which are dedicated for reclamation are typically low in fertility (Bradshaw, 1997). Moreover, often such soils are also contaminated with heavy metals such as copper, molybdenum, or zinc (Qiu and Sego, 2001; Wijesekara et al., 2016). In such oligotrophic soils, only a few species of specialized microorganisms can survive and thrive

(Madigan et al., 2012). Nitrogen-fixing bacteria can often cope with such conditions and enrich the soil with essential nitrogen, but their growth is strongly inhibited by the concentration of heavy metals (Wanic and Pająk, 2012).

High pH constitutes a twofold effect: firstly it negatively affects soil microorganisms, and secondly, it influences the solubility of some soil minerals and in this way reduces the availability of some necessary elements for enzymes construction such as iron (Campbell et al., 2008; Freeman et al., 2011; Brown and Chaney, 2016). Excessively high pH cannot be tolerated by many bacteria that play important roles, for example in the decomposition of dead matter. In post-mining soils, adverse pH seems to be one of the most important factors suppressing the composition and function of the microbial community (Bru et al., 2011).

Low soil organic matter is typical for mine subsoil. The use of organic amendments can help remediate this problem (Brown and Chaney, 2016). Organic amendments change pH to more preferable, neutral or slightly acidic levels, provide organic substances which bond with metallic cations, provide nutrients that could be utilized at least at the beginning of the succession process.

Legumes and other symbiotic plants with nitrogen-fixing microorganisms may be excellent tools for post-mining soil restoration (Elias and Chadwick, 1979; Jefferies et al., 1981 (1); Wanic and Pajak, 2012; Santi et al., 2013; Perry et al., 2014 (1); Perry et al., 2014 (2)). For the period when the co-operation with symbiotic microorganisms is being established, legumes use fertilizer as a source of nitrogen. Then, after developing the nodules and N-fixing bacteria activation, they switch to nitrogen provided by hosted diazotrophs (Strzelczyk, 2000). Legumes, though, are rarely good competitors; therefore, after transforming the soil to a more preferable state for other plants, they can be displaced by stronger competitors (Smyth, 1997). That naturally increases biodiversity and drives succession. Moreover, legumes are often good forage for wildlife and livestock, which stimulates the introduction of organic matter and nutrient cycling through feces (Elias and Chadwick, 1979; Jefferies et al., 1981 (2)).

Soil restoration should actively use a range of tools such as soil agricultural preparation, phytomelioration, fertilization, and soil amendments application.

Ecological Background for Mine Reclamation

Organisms sharing the same ecological niche constantly interact. They compete for the same resources and living space, or conversely, form networks of symbionts to co-operate for more efficient resource exploitation (Ratzke et al.; 2020). Therefore, mine soil reclamation should initiate and steer processes targeting the rebuilding of these complex interactions. Microorganisms play a pivotal ecological role. They conduct decomposition of dead matter releasing nutrients for other organisms, they are plant and animal pathogens, but also symbionts e.g. *Frankia* (Pokojska-Burdziej and Strzelczyk, 2000). Therefore, the condition of successful reclamation is to rebuild the complexity of microbial communities and their interactions because the course of most of the processes that take place in the soil depends on microbes (Zornoza et al., 2016).

It is a challenge to reclaim mine soils as an environment for living organisms. Every organism to thrive must find the necessary conditions and resources. These, according to ecological Shelford's law, must be embedded within brackets defining their minimum and maximum of the tolerance. Shelford (1931) presented that each of resources and conditions cannot go beyond either required minimum or maximum of a certain organism. If that happens, that organism would not be able to remain in a given environment. Mine soils are often difficult to colonize for most organisms because many conditions and/or resources go beyond either minimum or maximum level of tolerance. For instance, often the nutrient content is below the minimum, while simultaneously the concentration of heavy metals exceeds the maximum of tolerance. Due to low nitrogen concentrations, only microorganisms able to fix this element from the atmosphere may be able to primarily colonize such soils, but the microbial activity is strongly inhibited by high heavy metal concentration and salinity (Campbell et al., 2008; Freeman et al., 2011; Madigan et al., 2012). A deliberate provision of nitrogen at relatively low amounts at the initial stage of reclamation could act as a succession starting factor and heavy metals/salinity deactivation. The addition of selected soil amendments and/or organic fertilizers target at both aims at once (Larney and Angers, 2012; Zornoza et al., 2016).

Some organisms have special adaptations and abilities to harvest such resources that are unavailable to other organisms. Nitrogen-fixing microorganisms are a good example here. Their unique ability to fix atmospheric nitrogen opens a range of possibilities as they become demanded partners for a symbiotic co-operation with a range of plants. Plants cannot fix nitrogen on their own. Plants may only uptake nitrogen in the mineral forms from soil water solution with an exceptions of some small organic molecules (Carson and Phillips, 2021). The alternative to that is to be provided with nitrogen by microorganisms. Thus, many species of plants and microorganisms established mutual co-operation. Plants that have the ability to co-exist with nitrogen-fixing organisms might play a special ecological role in reclamation because they quickly enrich the soil with organic matter as well as release available nitrogen while decaying (Brożek and Wanic, 2002; Wanic and Pająk, 2012).

In the soil, at any given moment, despite the complex composition of the soil microbiome, most bacteria are present in the inactive forms such as spores or cysts. Many microorganisms can survive the presence of adverse conditions as spores that can persist for many decades (Madigan et al., 2012; Bottos et al., 2014). Factors that inhibit bacterial development are of two natures. The first is anthropogenic, e.g., depleted fertility and productivity, ruined soil structure and heavy metals contamination caused by industrial processes (Wijesekara et al. 2016). The second is natural and seminatural factors such as bacterial phages, adverse climate or weather, lack or excess of oxygen, nutrient-poor soil-forming rock, secreted antibiotics, and many others (Zornoza et al., 2016; Kumar et al., 2010). Therefore, debilitating the negative influence of the above-mentioned factors by reclamation techniques should enhance microbial life and thus increase chances for successful mine soils reclamation.

Applied Reclamation Tools

The upper layer of stripped soil, so-called topsoil, is collected separately from the material excavated from deeper ‘sub-soil’ layers. Topsoil is the preferred material in the soil restoration process because it is rich in plant nutrients and has a developed microbial community, but it is almost always scarce in mine reclamation projects. There is an interest whether subsoil, if not heavily contaminated, might play a role of the basic soil substrate in mine reclamation. This master thesis proposes experiments in which two mine subsoils, various soil amendments, and a range of plant species with nitrogen fixers among them will be used as the tools for effective mine reclamation with the proper nitrogen cycling restoration.

Subsoil

The proposed experiments will be based on the use of subsoil from the two mines: New Afton New Gold [NA] and Teck Highland Valley Copper [HVC]. Subsoils from those two mines have a list of common features, however, they also differ largely.

New Afton New Gold Subsoil

Initial observations were that subsoil collected from New Afton New Gold was light brown, rather dense but with rocks. Rocks tended to break easily and fall apart when hit.

Physicochemical tests show such features of the New Afton New Gold subsoil as pH above 9, very high electroconductivity (EC), rather low organic matter content which translates to low organic carbon and low nitrogen content. Additionally, phosphorus and potassium content was rather low, calcium and boron elevated, but sodium concentration was very high. Additionally, New Afton New Gold subsoil heavy metals content is elevated in terms of copper and molybdenum (See Chapters 2 and 3, Appendices A and B).

Teck Highland Valley Copper Subsoil

Subsoil collected from Teck Highland Valley Copper was grayish in color, rather sandy in texture but with a large amount of rocks. Rocks were solid and did not tend to break apart. Some rocks had intensive green color resulting from a content of oxidized copper.

Physicochemical tests show such features of HVC subsoil as pH above 8, EC low, very low organic matter content that translates to very low organic carbon. HVC subsoil characterizes by extremally low nitrogen content, very low phosphorus content, potassium content low, calcium, sodium not elevated and boron only slightly elevated. Additionally, HVC subsoil heavy metals content is strongly elevated in terms of copper and molybdenum (See Chapters 2 and 3, Appendices A and B).

Soil Amendments

Over the last decades, many ecological restoration experiments have been carried out in which various soil amendments were tested (Pichtel et al., 1994; Gould Gizikoff, 2002; Wanic and Pająk, 2012; Brown et al., 2014; Kelly et al., 2014). One of the important features of soil amendments is its ready availability, in terms of proximity to site and economic feasibility. Often they are produced as waste in many industrial or municipal processes. Thus, reclamation practitioners may easily acquire valuable products useful to them.

Each soil amendment has different properties, therefore, it must be used properly and in a manner well thought through. The selection of soil amendments depends on the objectives. They are often used to enrich the soil with organic matter (Larney and Angers, 2012), provide nutrients, increase water holding capacity, introduce strains of beneficial microorganisms (Zornoza et al., 2016), improve soil buffering, reduce the negative impact of toxic substances such as heavy metals, change pH, and increase cation exchange capacity (Curtis and Claassen, 2009; Brown et al., 2014; Kelly et al., 2014; Aschenbach and Poling, 2015; Hunt et al., 2015; Brown and Chaney, 2016).

The proposed experiments will use three different soil amendments: biosolids, biochar, and wood chips, as well as the mixture thereof. Each of the above soil amendments targets to ameliorate specific subsoil problems.

Vegetation

Vegetation is a crucial element of most reclamation projects. Once successfully established, plants transform reclaimed post-mining soils on their own. When reclamation is intended on soil substrate deeply transformed to unfavorable for vegetation, the process of spontaneous colonization may be unsatisfactorily slow. It might even collapse if additional factor such as harsh climate or herbivores pressure is exerted on plants. On deeply transformed soil substrates, as mines' subsoils are, it is recommended to support revegetation (Smyth, 1997).

With constantly growing interest and shifting cultural values, more attention is being paid to increase our understanding and appreciation of native plants. Scientists and reclamation practitioners highlighted a selection of native plant species that may be successfully used in reclamation (Elias and Chadwick, 1979).

NITROGEN

Nitrogen is an intrinsic part of the most important molecules building organisms' cells (Galloway et al., 2004; Salt, 2004). This element constitutes parts of such biological molecules as DNA, RNA, ATP, amino acids, chlorophyll, and many others. Therefore, it is indispensable in such processes as photosynthesis, respiration, heredity, tissue building, or regulation (Imsande and Touraine, 1994; Salt, 2004; Campbell, 2008; Freeman et al., 2011).

However, the uptake of nitrogen for living organisms is often difficult. Nitrogen is present in the lithosphere in abundance but usually absent in the rock bed on which the soil is formed. Therefore, the only primary source of nitrogen for organisms is the air possessing only 6.2% of Earth's nitrogen (Rosswall, 1981). Moreover, this source of nitrogen is still unavailable for most organisms. Only some microorganisms have the ability to fix atmospheric nitrogen. That element, on one hand, is necessary for all living organisms, but on the other hand, it is difficult to obtain. That is why the low nitrogen content in the topsoil is often the growth limiting factor. Plants deprived of nitrogen grow slowly and are unhealthy (Troeh and Thompson, 2005; Bernhard, 2010).

Nitrogen as an Element and its Cycling in the Environment

Nitrogen may exist in oxidation states from -3 to +5, which allows this element to form a broad array of compounds, both organic or inorganic. The biological uptake of this element is extremely specific. Earth's atmosphere contains $38\text{x}10^8$ Tg of nitrogen, while the entire biosphere maintains only $0.009\ \text{x}10^8$ Tg (Rosswall, 1981). Even though N is present in abundance in the atmosphere, constituting as much as 78% of it, in the atmospheric form (N_2) is unavailable to the vast majority of organisms due to its inertia. Strong triple bonds hold two nitrogen atoms together (Bernhard, 2010). This, in turn, means that a lot of energy and specific enzymes are required to take up this element from the atmosphere and transform it into mineral forms before it can be utilized (Campbell, 2008; Freeman et al., 2011; Madigan et al., 2012). Although every organism needs nitrogen only certain prokaryotes can fix it from atmosphere. Those microorganisms are responsible for the primary provision of nitrogen to the entire animate world (Galloway et al., 2004; Bernhard, 2010; Santi et al., 2013).

To restore nitrogen cycling in reclaimed mine soil substrate it is necessary to know what nitrogen transformations happen at every step of its cycling, and what influences those steps. Knowing this, the mine reclamation practitioners may exert efforts supporting those organisms which are involved in each step of the cycle.

Nitrogen Biogeochemical Cycle

In terms of nitrogen acquisition, all living organisms may be divided into two fundamental groups: nitrogen fixers and non-nitrogen fixers. The first group acquires nitrogen through atmospheric nitrogen fixation. That is called diazotrophy. Non-nitrogen fixers, in turn, divide into two groups again: organisms intaking nitrogen in mineral forms e.g. NH_4^+ or NO_3^- from the soil water solution or organisms feeding on living or dead organic matter containing nitrogen. Organisms that take up nitrogen through the consumption and digestion of organic matter deplete at first the environmental pool of available nitrogen. However, while they carry out their metabolism, they excrete end products of digestion and metabolism which are still rich in organic nitrogen (undigested food residues) or mineral nitrogen (e.g. urea), returning it to the environment. At the end of their life, the whole bodies, which consist of about 15% nitrogen, return it to the environment in which organisms lived (Rosswall, 1981). That is how nitrogen once introduced into the system moves alternately in between living biomass and dead matter, forming a small sub-cycle until it ultimately comes back to the large cycle and gets returned to the atmosphere (Fig. 1.1).

Many organisms that are not able to self-provide the needed nitrogen tackle this problem by entering into symbiosis with organisms that do have the ability to provide them with available nitrogen forms. Such co-symbionts divide into two categories:

- The first category is formed by organisms able to extract intensively the nitrogen compounds from the soil water solution, as in the case of fungi.
- The second category of symbiosis is formed with organisms that have the ability for direct nitrogen fixation from the atmosphere.

The final stage of this multi-step process is usually the conversion of mineral nitrogen back to atmospheric nitrogen. All these processes, starting from the fixation of atmospheric nitrogen, through its transformation, up to the return back to the atmosphere, make up the nitrogen cycling in the environment. In terrestrial ecosystems, most of these processes are carried out by soil microbes (Rosswall, 1981; Campbell, 2008; Zornoza et al., 2016).

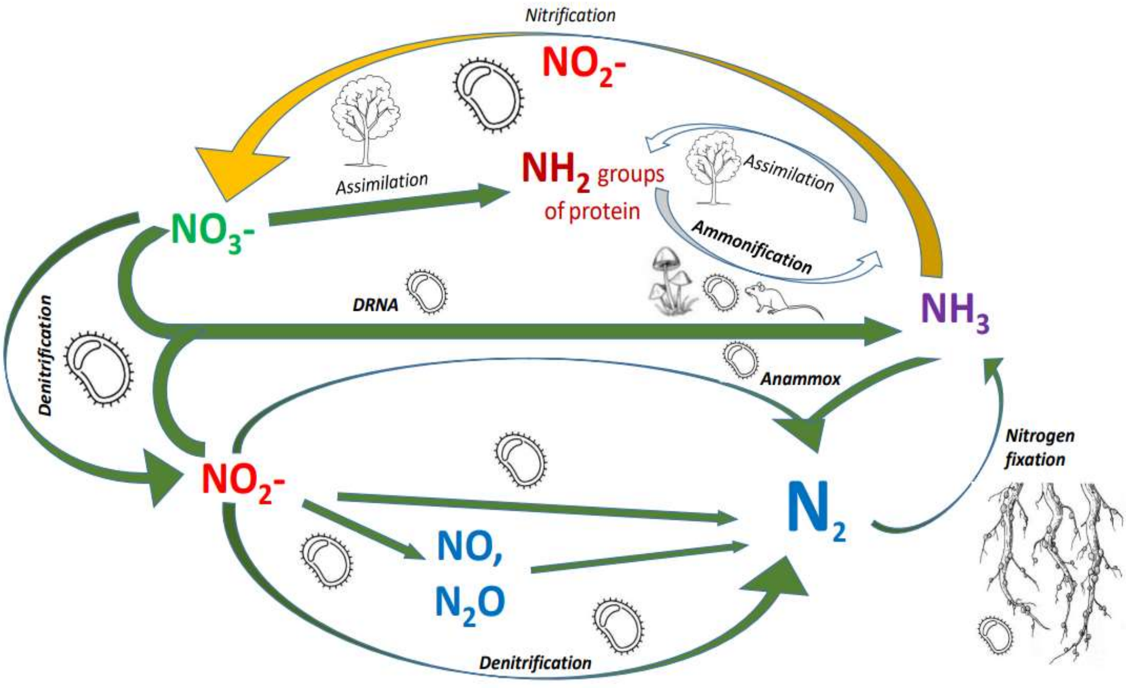

Figure 1.1 *Schematic diagram of biological nitrogen cycle. Yellow arrows stand for oxidation reactions, green arrows stand for reduction. Anammox – anaerobic ammonium oxidation; DRNA – dissimilative nitrate reduction to ammonia.*

Nitrogen Fixation

Introducing nitrogen from the atmosphere into the biosphere by nitrogen fixation is a first step of the cycle (Figure 1.1; Bernhard, 2010). It occurs according to the following reaction: $N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + 16ADP + H_2 + 16P_i$

The *nif* genes encode the enzyme nitrogenase which is responsible for N-fixation. Many varieties of this enzyme are known. N_2 is fixed and transformed into NH_3 or glutamine. NH_3 is either taken up by plants straight away or after the reaction with water in which it is converted into an ammonium ion (NH_4^+) (Imsande and Touraine, 1994).

In the soil, nitrogen-fixing microorganisms live freely or enter into symbiotic relationships with plants or fungi. Many forms of such co-operation have developed during early evolution (Pokojska-Burdziej and Strzelczyk, 2000). The strength of these relationships varies. Bacteria from the families of *Arthrobacter, Azotobacter, Azospirillum* and some others live freely in the soil, but often their accumulations occur in the rhizosphere of the plant roots (rhizospheric bacteria) as plants create preferential living conditions for them (Zornoza et al., 2016). In turn, plants use nitrogen-rich compounds produced by these microorganisms. Other bacteria colonize outer spaces in root tissues. These are still rhizospheric bacteria but their co-operation with plant hosts is stronger. The strongest form of symbiotic relations happens when bacteria penetrate plant root tissues. When some bacteria only colonize the apoplastic root space, the co-evolution of other bacteria along with the host plant led to the penetration and colonization of the interior of the host root cells. The host plant often produces special tissues – nodules (See Figure 1.2). In nodule's cells symbiotic bacteria find a favorable living space. Plants that can co-exist with nitrogen-fixing bacteria often gain a competitive advantage and are first colonists in areas of succession (Santi et al., 2013).

In terrestrial ecosystems, in terms of amounts of fixed atmospheric nitrogen, bacteria have varying efficiency. Bacteria that live freely in the soil are less productive. It is estimated that soil bacteria are able to fix 5 - 50 kg N/ha/year. The intensity of nitrogen fixation depends on many abiotic and biotic factors such as temperature, pH, the presence of competitors, predators, antibiotics, etc. Bacteria that form advanced symbioses with plants can fix up to ten times more nitrogen than free-living ones (Król and Zielewicz-Dukowska, 2005). The efficiency of such endosymbionts in terms of the production of mineral forms of nitrogen is strongly dependent. The performance depends on the species, and even strain of bacterium, plant species, the season, plant physiological state, the presence of other nutrients, climate, etc. It is estimated that some plant species of the *Fabaceae* family fix up to 500 kg N/ha/year (Troeh and Thompson, 2005).

Bacteria that form a symbiosis with vascular plants can be divided into two groups. The first is built by bacteria from the *Rhizobiaceae* family that form a symbiosis with legumes. Those are genera: *Rhizobium, Bradyrhizobium, Azorhizobium* and others (Madigan et al., 2012). The second group is formed by bacteria that form a symbiosis with non-leguminous plants. Representatives of eight plant families form the symbiosis with Actinobacteria from the genus *Frankia.* Such a relationship with Actinobacteria is called actinorrhizae (Huguet et al., 2004; Strzelczyk, 2000; Pokojska-Burdziej and Strzelczyk, 2000; Santi et al., 2013).

In the mine reclamation process supporting nitrogen fixation may play an important role (Jefferies et al., 1981) as this will take over the duty of nitrogen provision to the system when the pool of this element provided initially by fertilization gets eventually depleted.

Figure 1.2 *Nodulation on roots of field locoweed (Oxytropis campestris) on the left and soopolallie (Shepherdia canadensis) on the right.*

Photos credit Piotr Dzumek

Decomposition and Decay

From the mine reclamation point of view decomposition and decay are very important processes that must be supported because they enrich mine soil substrate with strongly needed organic matter and release the entire list of nutritional elements with nitrogen among them. Nitrogen-containing compounds excreted by one organism become available for the uptake and metabolism to other organisms. Substances excreted by many organisms are further decomposed by a range of microorganisms and fungi equipped with the appropriate enzymes (Imsande and Touraine, 1994; Bernhard, 2010; Figure 1.1). Dead animal and plant tissues pass atrophy and end up in the soil, where they undergo rotting and decomposition. Dead organic matter plus excrements build the pool of soil detritus (Biology Online, 2020). There is a broad range of organisms which are soil detritivores, they feed on detritus, decomposing it, to obtain nutrients and energy. The production of mineral forms of nitrogen as a result of decay and decomposition is called nitrogen mineralization. This way nitrogen is mobilized and becomes again available for uptake (Rigby et al., 2016).

As a result of decomposition of organic matter an amine group -NH_2 becomes detached from an organic compound and becomes transformed to ammonia (NH_3). This process is named ammonification (Figure 1.1). In the soil, the ammonia, readily reacts with water forming ammonium ion: $NH_3 + H_2O \rightarrow NH_4^+ + OH^-$. Plants intake nitrogen from the soil mostly in the mineralized forms. These forms, however, account only for around 1 - 2% of total nitrogen in the soil (Carson and Phillips, 2021). Organic nitrogen is gradually mineralized and slowly released. Mineralization might provide 20 to 200 kg N/ha (Curtin and Campbell, 2008). Overall nitrogen uptake may exceed 100 kg N/ha/year (Imsande and Touraine, 1994; Troeh and Thompson, 2005).

NH_4^+ produced primarily by nitrogen fixers and secondarily in a process of decomposition and decay is taken up by organisms e.g. plants through roots for their metabolism or is utilized by nitrification microorganisms to produce energy.

Nitrification

The next group of organisms in the nitrogen cycle are bacteria and archaea carrying out the nitrification process (Figure 1.1; Bernhard, 2010). Nitrification is the oxidation of ammonium ion to nitrogen oxides carried out by obligatory aerobic nitrifiers (Madigan et al., 2012). Therefore, the nitrification process takes place in aerated soils. Nitrification does not occur in soil horizons that are permanently flooded. The nitrification has two major stages. The first stage involves the transformation of ammonium ion and leads to the formation of nitric acid, the second stage involves the conversion of nitric acid and leads to the formation of nitrous acid. Typically, each step is carried out only by one type of nitrifying bacteria. *Nitrosomonas* conduct oxidation of NH_4^+ to NO_2^-. *Nitrobacter* conduct oxidation of NO_2^- to NO_3^-.

The deeper in the ground, the less oxygen. Here the denitrification bacteria begin to manifest their activity (Madigan et al., 2012).

Denitrification

Denitrification consists of the gradual conversion of nitrogen oxides back to nitrogen gas (Figure 1.1; Bernhard, 2010). However, for this process to be energy-efficient, the denitrification stages require different conditions than the nitrification ones. Here, nitrogen oxides are back reduced while the organic compounds are oxidized. Therefore, denitrifying bacteria are usually anaerobic.

Complete denitrification from NO_3^- to N_2 encompasses a series of steps. Passing every step entails certain enzymes which are produced only by certain types of bacteria. Therefore, the entire sequence of various bacteria is needed to complete the entire denitrification. The denitrification takes place in the following stages: 1) $NO_3^- \rightarrow NO_2^-$, 2) $NO_2^- \rightarrow NO$, 3) $NO \rightarrow N_2O$, 4) $N_2O \rightarrow N_2$ (Madigan et al., 2012).

Denitrification leads to nitrogen loss, therefore is not required at the initial steps of mine reclamation. However, it is an intrinsic step closing entire nitrogen cycling.

Dissimilative Nitrate Reduction to Ammonia (DNRA)

DNRA is a process similar to denitrification, but the final product is not atmospheric nitrogen but NH_4^+ (Madigan et al., 2012). DNRA takes place in two stages: 1) $NO_3^- \rightarrow NO_2^-$, 2) $NO_2^- \rightarrow NH_4^+$. Not only prokaryotic organisms conduct DNRA but also some eukaryotes (Putz et al., 2018). As DNRA does not lead to nitrogen loss, it is more preferred than denitrification in the attempt of preserving nitrogen in the reclaimed mine soil substrate.

Anammox

In some ecosystems anammox conducting bacteria are responsible for most of the entire N_2 return to the atmosphere. Those bacteria inhabit anoxic environments. Based on metagenomic studies, it is believed that there may be thousands of species carrying out anammox in specific environments (Long et al., 2013; Solanki et al., 2017). Anammox is the oxidation of NH_4^+ by nitrogen oxides with molecular nitrogen as the final product. Reaction conducted is: $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ (Madigan et al., 2012). Anammox is not demanded in the initial phases of mine reclamation as this process leads to even faster nitrogen loss.

How Nitrogen Transformation Influence on Other Soil Properties

Nitrogen cycling influences a wide spectrum of other soil processes, and vice versa. All soil processes remain in a sort of balance as well as all elements' cycles interact with each other (Rosswall, 1981). Having at least a bit of nitrogen to start, pioneering organisms can begin to influence reclaimed land, exerting further transformation (Ratzke et al., 2020). Nitrogen available in the environment has usually a positive effect on the production of plant biomass. This, in turn, increases the content of organic matter in the soil and the carbon sequestration (Brożek and Wanic, 2002). Thus nitrogen influences the carbon cycle.

Phosphorus has a strong influence on nitrogen cycling because P together with N are important elements of many molecules in organisms' cells. Deficiency of phosphorus limits especially N fixation as well as nitrification (Rosswall, 1981).

The individual stages of the nitrogen cycle often have opposing vectors of bio and physicochemical change in the soil (Ratzke et al., 2020). For instance, nitrogen fixation and denitrification increase pH by uptake of H^+. Protein decay also increases the pH by releasing of OH^- anions. In turn, the nitrification results in acidification due to H^+ release. If nitrogen cycling is balanced, the pH of the soil does not change much, but if any of those processes overweight, the soil pH changes. This is an important hint for mine reclamation practitioners indicating that all steps of the nitrogen cycle have to be rebuilt to maintain the proper pH. Soil pH influences the rate of N-fixation. Nitrogenase has atoms of Fe and Mo in the structure. So Fe and Mo are necessary in the N-fixation (Campbell et al., 2008; Freeman et al., 2011). In soils of high pH, Fe and Mo are not soluble. Thus, high pH may inhibit nitrogenase synthesis.

Apart from plants, some microbes use products of nitrifiers' metabolism: anammox and DNRA bacteria, as well as denitrifiers. Their role in the reclaimed soil should not be forgotten. They prevent leaching of nitrogen oxides to the groundwater, take part in organic matter mineralization, take part in soil microbial food chain, and increase pH (Campbell et al., 2008; Freeman et al., 2011; Madigan et al., 2012).

Importance of Nitrogen in Mine Reclamation

Mine reclamation will not be successful if nitrogen cycling is not rebuilt, often requiring human intervention to increase the speed of recovery. There are two major targets. Firstly, it is to provide an initial nitrogen portion ensuring starting it to cycle, and secondly, it is to prevent nitrogen loss at least at the initial stage of reclamation. Nitrogen may be provided to the reclaimed mine soils in two ways: artificially by fertilization or naturally by nitrogen fixation. Artificial fertilization in the case of deeply transformed mine soils is often necessary. That initial introduction of nitrogen along with other nutrients intends to increase the level of lacking elements above the minimum of tolerance for organisms to colonize. Often microbes are present in the soil substrate in an abundance, however, they try to wait out the adverse conditions in a form of spores (Madigan et al., 2012). Fertilizers may activate them (Suhag, 2016). This may be done with the use of mineral fertilizers, organic ones, or even with the use of nutrient-rich soil amendments. Commercial organic fertilizers or soil amendments seem to be better solution than mineral for initial complex nutrients provision in reclaimed mine soils (Larney et al., 2009, Gardner et al., 2010). Some soil amendments may remediate the problem of lacking soil nutrients.

When initial portions of nutrients are provided, it is a time to introduce organisms that will facilitate further nitrogen provision as these nutrients may be quickly depleted or lost. Plants that co-exist with N-fixing bacteria can fulfill this role. They are used by people in agriculture, in the phytomelioration process, as well as in the bioremediation or the reclamation (Elias and Chadwick, 1979; Jefferies et al., 1981; Santi et al., 2013, Wanic and Pająk, 2012). Legumes such as *Lupinus, Trifolium, Medicago* are most commonly used here. BC flora has many species forming co-existence with N-fixing microbes (Brown and Amacher, 1999; Antos et al., 1996).

In mine reclamation nitrogen cycling may be supported and its loss limited. In the case of mine soils, in which microbiological life is negatively affected (Baker et al., 2011; Larney and Angers, 2012), it is unlikely that the plant will find its symbiotic partner. To increase the chances, bacteria can be inoculated by commercial inoculants. To support plants forming actinorrhizae with *Frankia*, it may be most effective to add a portion of natural soil (Strzelczyk, 2002). Nitrifying bacteria are obligately aerobic. In clay and loamy soils, the ground tends to long water holding. This results in unfavorable aerobic conditions. Therefore, an intervention, such as tillage, aiming in better aeration of reclaimed soils, supports nitrifiers and other topsoil aerobic organisms. Tillage, by oxygen provision, limits denitrifiers, and anammox bacteria in the topsoil (Long et al., 2013). Therefore, if their activity is limited, tillage contributes to available nitrogen lost prevention. Supporting DNRA bacteria also prevents nitrogen loss. Providing of organic matter and sulfide (S^{2-}) also supports DNRA bacteria while inhibiting denitrifiers (Putz et al., 2018).

AREA OF RESEARCH

New Afton New Gold is located just 15 km away from the city of Kamloops, BC. Teck Highland Valley Copper is located around 50 km away. Both mines lie within Thompson-Nicola Regional District of British Columbia. Although not very far from each other both these mines differ strongly (New Gold Inc., 2021; Teck Resources Limited, 2019; "Highland Valley Copper", 2021). Differences encompass such aspects as elevation above the see level, climate, soils, vegetation, bedrock, and others.

New Afton New Gold lies in the area of Bunchgrass Biogeoclimatic Zone which is characterized by high biodiversity and its uniqueness. Bunchgrass covers merely about 1% of the BC area (Province of British Columbia, 1999) but only within this zone 30% of endangered species of BC have their populations.

HVC, on the other hand, is characterized by its large size and, consequently, a significant impact on the environment (Teck Resources Limited, 2019; "Highland Valley Copper", 2021; Malaschenko et al., 2017).

Reclamation undertakings withing both mines are challenging due to several facts: climatic severity, large scale, a vulnerability to species invasiveness, areas' local water retention importance, elevated fire threat and many more.

Mines Characteristics

Both mines extract and pre-treat ores and possess open pits. NA pit is inactive, while HVC has several active and inactive pits. As a result of an ore production within the mines, there is a large scale constant hauling and heavy machinery movement in soil and rock transportation. Earthy waste material is gathered on large stockpiles that transforms the surface creating vast areas for future reclamation. Additionally, both mines possess tailings storage facilities (TSF).

New Afton New Gold Mine

New Afton site is one of New Gold Inc. assets. All New Gold's claims within the New Afton Group span the area of 124.5 km^2. Chiefly it comprises an open pit, underground mining operation, support facilities, a concentrator, and tailings facilities. An underground operation began in 2012, located underneath an open pit which had been exploited under the operation of the historic Afton Mine (New Gold Inc., 2021). The New Afton reserves are estimated for 1.0 million ounces of gold, 2.8 million ounces of silver, and 802 million pounds of copper. The mine produces annually over 75 thousand ounces of gold and over 85 million pounds of copper (New Gold Inc., 2021).

New Afton is located within the Bunchgrass biogeoclimatic zone (Province of British Columbia, 1999). The Bunchgrass zone's principal environmental factor determining its distinctiveness is mean annual precipitation below 300 mm. Most of the precipitation falls within the coldest period of the year. This zone is classified as semi-arid characterized by cool winters and hot, dry summers. Plants native to these growing conditions have developed mechanisms for the effective use of water from the thaw, and to endure long periods of drought. Here grasses are dominating, e.g. bluebunch wheatgrass *Pseudoroegneria spicata* or prairie junegrass *Koeleria macrantha*. Other plants well adapted to these conditions are shrubs with the dominants big sagebrush *Artemisia tridentata* and common rabbitbrush *Ericameria nauseosa*. Their adaptive strategy is intensive root growth. The soils of this zone, mainly due to the abundance of grasses, are more fertile and have a favorable pH. Besides, they are sufficiently airy and are not excessively dense. (Antos et al., 1996; Province of British Columbia, 1999).

Teck Highland Valley Copper Mine

Highland Valley Copper mine is one of many Teck assets located in Canada. It is one of the largest open-pit mines in the world. This site exploits mainly copper and molybdenum. The entire mine area comprises the open pits, the transportation network, rocks grinding and ores concentration facilities, tailings storage facilities and large areas of terrains already subjected to reclamation. HVC produces over 150,000 tonnes of copper annually (Teck Resources Limited, 2019). The Teck Highland Valley Copper mine is one of British Columbia's high-value assets but, being one of the world's largest open-pit production highlights the need for reclamation research. A large number of reclamation projects are conducted presently either on an operational level as well as on experimental (Teck Resources Limited, 2019; "Highland Valley Copper", 2021; Malaschenko et al., 2017).

HVC is located around 1,300 m above sea level, while NA's elevation is only around 700 m. Therefore, in the place of HVC location, there are different climatic conditions. HVC biogeoclimatic zones are Interior Douglas-Fir and Montane Spruce. These zones, in particular Montane Spruce, are characterized by a cool climate, shorter vegetation season, but receiving a significantly larger mean precipitation. These zones are covered with woody vegetation, where douglas fir *Pseudotsuga menziesii*, white spruce *Picea glauca*, and even subalpine fir

Abies lasiocarpa dominate alternately. The nitrogen fixer soopolallie *Shepherdia canadensis* is also typical here. In these zones, the soils are usually characterized as nutrient-poor, stony with an acidic pH. Nitrogen fixers can play an extremely important role here as the suppliers of nitrogen (Imsande and Touraine, 1994; Antos et al., 1996; Province of British Columbia, 1999).

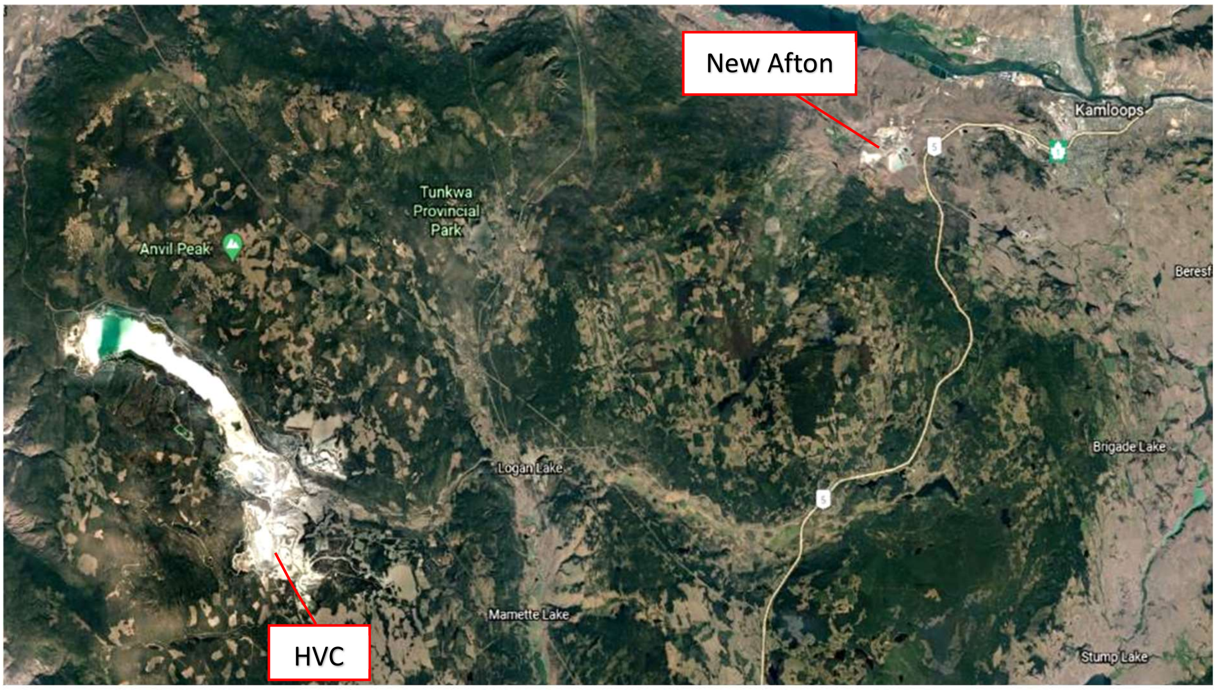

Figure 1.3 *New Afton New Gold and Teck Highland Valley Copper mines location.*
Retrieved from Google Earth.

RESEARCH OBJECTIVES

Two proposed experiments focused on:

I. Testing whether the mine subsoils from New Afton New Gold and Teck Highland Valley Copper have a potential to be applied as a mine reclamation starting medium, without the addition of an amendment, on selected native grasses, forb and shrubs.
II. Testing whether applied soil amendments influence plant productivity and mortality of selected native grasses, forb and shrubs on the New Afton New Gold and Teck Highland Valley Copper subsoil substrates;
III. The qualitative and quantitative change of total nitrogen, mineralizable nitrogen, NH_4^+, NO_3^- in the soil following soil amendments and plant species application.

First study experiment 1 was conducted in optimal plant-growth conditions of the greenhouse. In contrast, second study experiment was conducted in semi-natural conditions in open-air. Eventually, results of both experiments were compared.

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CHAPTER 2: HOW AN ADDITION OF BIOSOLIDS, BIOCHAR, AND WOODCHIPS INFLUENCE SUBSOIL PHYSICOCHEMICAL PROPERTIES, GRASSES AND LEGUME PERFORMANCE, AND NITROGEN CYCLING IN GREENHOUSE CONDITIONS.

INTRODUCTION

As awareness of environmental values increase, an ecosystem-oriented reclamation constantly gains importance (Brown and Amacher, 1999; MAC, 2018). This branch of human activity is complex and interdisciplinary, combining knowledge from many fields of science. Reclamation achieves the best results when it emulates natural processes (Jefferies et al., 1981; Bradshaw, 1997; Bradshaw, 2000). To rebuild an ecosystem, the original ecosystem must be well known. The ecosystem is a very complex network of mutual interdependencies between its biotic and abiotic components. Moreover, organisms occupying a certain niche organize in food webs (McCann, 2007; Egerton, 2007). Energy and matter flow through every level of trophic interactions, allowing for the cycling of elements (Madigan et al., 2012). Restoration of such relations between living organisms and their ecological niche should be the aim of the reclamation process to make it effective (Fraser et al., 2015). Reclaiming areas disturbed by mining is challenging. Mines alter the landscape, transform and remove soils, influence hydrology, increase dust emission, and can leach contaminants into water bodies and soils (Wijesekara et al., 2016). Additionally, mine degraded soils are usually infertile. These issues, and other factors like high salinity or extreme pH, result in the low productivity of these soils (FAO et al, 2020).

An efficient way to reclaim mine barren land is to cap with a layer of fresh topsoil. The problem is that topsoil is scarce or is deeply transformed after many years of stockpiling (Strohmayer, 1999). In such a case attention is turned toward subsoil, which is abundant on mine sites, also stored as stockpiles. There is interest in whether subsoil can be used as a reclamation starting soil medium. In subsoil characterization, the potential obstacles to ecosystem restoration might be identified, and then these obstacles can be addressed in a planned manner.

Another question is whether soil amendments could help transform subsoil. Knowledge about the properties of soil amendments is increasing, particularly as applied to mine reclamation (Brown et al., 2014; Brown and Chaney, 2016; Gunarathne et al., 2020), nonetheless, still requires deepening, in particular about the proper selection, doses, and methods of application. Little is known about the influence of the soil amendments on the properties of mine subsoils. Three amendments have received particular attention in this study: class A biosolids, biochar, and woodchips, as well as the mixture thereof. Biosolids result from wastewater treatment (Sullivan et al., 2015; Ippolito et al., 2021). Annacis Biosolids used in this study were provided by Metro Vancouver. This class A biosolids, through anaerobic digestion and dewatering, pass very strict requirements for pollutants, pathogens and vector attraction reduction along with others. Biosolids is an amendment very rich in readily available nutrients, as well as with organic matter (Sullivan et al., 2015). Biosolids provide a large portion of available and mineralizable nitrogen. Available nitrogen is predominantly in a form of NH_4^+. Apart from nitrogen, biosolids enrich the soil substrate with phosphorus and zinc, and also increase soil buffering, cation exchange capacity, reduce substrate salinity, as well as the adverse impact of heavy metals (Brown and Chaney, 2016). The biosolids application rate strongly depends on substrate N and P deficiency. It might also vary regarding to the biosolids class, composting time etc. (Sullivan et al., 2015). Cogger and Stahnke (2013) suggest an application of 15 to 20% by volume to land being prepared for new lawn but also inform that the rate for new gardens should be larger. Larney and Angers (2012) state that reclaimed mine soils are usually subjected to the largest organic amendment dosing due to its deep degradation. Woodchips is an amendment rather poor in readily available plant nutrients (Cheng, 2008). Moreover, this material decomposes very slowly, mainly due to lignin content (Datta et al., 2017). Woodchips get produced as a waste in the sawmills processes. This material is rough, contains different sized fractions of woody tissues, mainly coniferous tree cortex. The addition of woodchips lowers the pH of overly alkaline soil substrates, helps in increasing recalcitrant carbon, increases water penetration and aeration, and constitutes a food source for a range of organisms (Yuan et al., 2020). In his work on potting media Bugbee (2008) combined 10, 20 and 30% (v/v) of hardwood sawdust (woodchips) with municipal biosolids compost to observe an influence of this amendment on *Coreopsis grandiflora* and *Rudbeckia hirta* individuals performance, as well as on mineral nitrogen leaching. He found that an application of woodchips in such rates did not affect NH_4^+ leaching from biosolids and did not affect plants performance. Cheng (2008) determined that an application of 30% sawdust applied with NPK fertilizer gave the highest yield. Biochar is a charcoal. It is a slow-release fertilizer rich in phosphorus and potassium and acting beneficially on soil physicochemical properties (Taylor, 2010; Canadian AgriChar, 2020). This soil amendment has a strongly alkaline pH – around 10. It enriches soil substrate with organic matter. Biochar may increase soil buffering, cation exchange capacity, reduce salinity, and an adverse impact of heavy metals (Kelly et al., 2014). AgriChar, which is a manufacturer of biochar product used in this research, suggests using 5% of this product by volume (Canadian AgriChar, 2020). Gunarathne et al. (2020) in their experiment with salt-affected acidic soil used 1.0% - 5.0% detecting that eventually an addition of 5.0% of biochar brought the best results.

Vegetation is one of the key factors in mine reclamation. Intentionally introduced plants species are generally selected according to plant traits such as rate of growth, ecological requirements and function, the potential in competition with other plant species, the ability to enrich the soil substrate with essential nutrients, for example nitrogen (Elias and Chadwick, 1979). The ecosystem will likely not be rebuilt stably if the normal cycle of elements is not restored. Nitrogen-fixing plants can play a special role in reclamation of mine soils that are particularly nutrient-poor. Lupine, clover, or alfalfa are more commonly used nitrogen-fixers for phytomelioration and green fertilization worldwide. However, many of these plants do not belong to the local flora. Four native plant species were selected: three grass species: bluebunch wheatgrass *Pseudoroegneria spicata*, rough fescue *Festuca campestris*, prairie junegrass *Koeleria macrantha,* and one legume field locoweed *Oxytropis campestris*. Three selected graminoids are turf-forming perennial bunchgrasses. They may be found in many ecosystems but are typical and commonly present in grasslands. Those plants are well adapted to semi-arid conditions with hot and dry summers (Antos et al., 1996). That legume may be found in many ecosystems but thrives the best on rocky, gravelly soils with plenty of insolation. It stands well with harsh climate pressure but is not a good competitor (Douglas at al., 1998), which potentially, coupled with an ability for nitrogen fixation, makes this species a good candidate for post-mining reclamation tool.

The study objectives were:

I. To determine similarities and differences between New Afton New Gold and Teck Highland Valley Copper subsoils with or without amendments by observing plant mortality, above-ground biomass productivity, and subsoil physicochemical properties;
II. To test the relative effects of various proportions of the three soil amendments (biosolids, woodchips, biochar) on subsoil physicochemical properties;
III. To measure qualitative and quantitative change of nitrogen compounds related to soil amendments.

METHODS

Study Site

Subsoil substrate was collected from stockpiles at two mine sites: Teck Highland Valley Copper (August 17, 2018) and New Afton New Gold (September 9, 2018). The experiment was located in the Research Greenhouse at Thompson Rivers University. The experiment began on December 22, 2018, and ended on April 13, 2019. It lasted 16 weeks counting from when the seeds were sown to the day of the above-ground biomass harvesting.

Experiment Design

Pots and Seeding

1.2 L square-shaped pots were filled with 10 combinations of subsoils with soil amendments (See Table 2.1). To increase chances for successful germination, seeds were sown in batches instead of sowing just one seed. One batch of seeds (20 seeds in each) was sown in each of the pot corners. This way four one-species monoculture treatments and one four-species mix treatment were created. Grass seeds were sown on the surface of the substrate and legume seeds were covered by a thin layer of soil substrate. After germination, excess seedlings were eliminated such that one individual plant at each pot corner remained. Pots were marked and numbered.

To boost the presence of N-fixing microbes, the legume seeds were inoculated with use of commercial preparation McKENZIE SEEDS GARDEN INOCULANT containing bacteria *Rhizobium leguminosarum* and *Bradyrhizobium sp*. Before seeding, legume seeds were subjected to scarification and 24 h water imbibition (Baskin and Baskin, 1998).

Greenhouse Settings

The greenhouse was on a 14h/10h day/night lighting schedule with three 1,000W lamps operating. 24-hour temperature variation was kept within 15 - 30°C interval.
The substrate was kept moist but not wet. As this experiment did not account for the drought effect, the watering need was visually assessed only. To maintain proper air humidity, the automatic mister was switched on and activated whenever the humidity sensors detected the air humidity below 40%. Apart from that, the mister was activated daily at 7.30 AM for 15-minutes-continuous misting regardless of the sensor readings. The pod was ventilated by the built-in ventilator or by the automatic side and rooftop whenever the temperature approached 30°C or the air humidity exceeded 80%.

Pest Control

The principal adopted was to not influence the physicochemical properties of the soil substrate. Applied: 1. insecticide DOCTOR DOOM GO GREEN TOTAL RELEASE FOGGER containing the active ingredients Pyrethrins 0.4% and Piperonyl Butoxide 2.0%; 2. yellow color sticky traps to fight flying pest; 3. plant protection product NEMASYS, which contains nematodes *Steinernema feltiae,* was applied to the subsoil treatments to fight fungus gnats as this nematode species is a parasite of fungus gnat larvae living in the soil.

Applied Subsoil Substrate Combinations

Subsoils collected from both mines were treated the same way with the addition of soil amendments. Amendments were applied in the volumetric proportions shown in Table 2.1.

Table 2.1 *First study experiment soil medium composition percentage breakdown.*

	SUBSOIL	**BIOSOLIDS**	**WOODCHIPS**	**BIOCHAR**
CONTROL	100%	0%	0%	0%
BIOSOLIDS	75%	25%	0%	0%
WOOD CHIPS	75%	0%	25%	0%
BIOCHAR	95%	0%	0%	5%
MIXTURE OF ALL AMENDMENTS	75%	10%	10%	5%

Regarding low subsoil organic matter content it was decided to apply high volumetric dosing of soil amendments (Larney and Angers, 2012). Biochar was an exception because this material has very high pH (Canadian AgriChar, 2020) and an addition of biochar in larger quantity could potentially even elevate the subsoil pH which was already high (above 8). An addition of 5% biochar followed the AgriChar suggestions of application.

Experiment 1 Overall Treatments Combination

The experiment was a 2 x 5 x 5 factorial design, with 50 treatment combinations:

- 2 subsoil types: 1. New Afton New Gold subsoil, 2. Teck Highland Valley Copper subsoil;
- 5 soil amendment treatments: 1. control [no soil amendments], 2. biosolids alone, 3. woodchips alone, 4. biochar alone, 5. mixture of all three soil amendments (see Table 2.1);
- 5 plant treatments: 1. bluebunch wheatgrass alone, 2. rough fescue alone, 3. prairie junegrass alone, 4. field locoweed alone, 5. mix of all four species.

Replicated 7 times for a total of 350 individual pots. A randomized block design was arranged in the greenhouse.

Soil Sampling

Before seeds were sown, but after subsoil substrate mixing with amendments, and following 7 days for the mix to settle, ten soil samples were collected (2 mine subsoils x 5 subsoil treatments). Additionally, two reference sites were selected near the vicinity of the two mine sites, which served as a source of undisturbed topsoil samples from the natural ecosystem. All samples were collected in labeled 1L plastic bags and frozen at -20°C immediately after sampling. Each time, prior to testing, a portion of the frozen sample was removed from the bag and the rest was deposited back into the freezer to avoid unwanted defrosting. At the experiment termination, the soil substrate from 7 pot-replicates was mixed thoroughly together, and then one sample was collected in a 1L labeled plastic bag. The same procedure was repeated for each of the 50 treatments. Then, the samples were frozen at -20°C immediately after collection. This way all together a pool of 62 1L-samples were obtained: 10 samples of the initial subsoil substrates combinations, 50 treatment-resulting samples at the end of the experiment, and 2 undisturbed topsoil samples for reference. All samples were collected and stored in the same manner until the test was performed.

Tests and Instrumentation Used

Plant Productivity

Plant productivity was measured by harvesting above-ground biomass of each individual plant from each corner of each pot for a total of 1400 specimens. All plants oven-dried in Constant Temperature Oven DKN 812 for 48h at 45°C right after collection. Dried plants were weighed on an analytical scale Fisher Scientific accuSeries accu-225D.

Mortality

Mortality was assessed at the moment of the above-ground biomass clipping and collection.

Soil Physicochemical Tests

Part of the tests was conducted in Dr. Lauchlan Fraser's laboratory at TRU. These were: the soil pH, soil electroconductivity, soil organic matter (SOM) content, as well as total nitrogen and total carbon contents. In addition, soil samples were sent to the Ministry of

Environment and Climate Change Strategy - Analytical Lab in Victoria to analyze them for the content of mineralizable nitrogen and available nitrogen forms: NH_4^+, NO_3^-, as well as basic elements content: Al, B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Zn.

Before testing, according to the procedures, all soil samples were subjected to desiccation in Constant Temperature Oven DKN 812 at 65°C for 24h, then manually crushed in a mortar and sieved through a 0.5 mm laboratory soil sieve. Samples for pH and electroconductivity were excepted from this procedure, as fresh soils are used for these tests. Also, SOM samples were exempted from sieving. In the case of SOM, the desiccation was conducted for 24h at 105°C.

pH and Electroconductivity

Measurements were taken by PALINTEST WATERPROOF 800 pH /CONDUCTIVITY/TDS METER from fresh substrate samples sunk in the distilled water. Prior to measurements taking, the instrument was calibrated.

SOM

Immediately after drying, samples were weighed on analytical scale Fisher Scientific accuSeries accu-225D and results were noted. Samples' weights were between 1.00 – 1.50 g. Then, samples were placed in a muffle oven Furnace 62700 and subjected to 500°C for 5h to volatize all organic matter. After burning samples were weighed again and then the SOM got calculated from the difference between pre-burning and after-burning results.

Total C, Total N

Prior to testing 10 to 15 µg samples got prepared in small tin containers, weighed on analytical scale Sartorius CP 2P, then tightly sealed and weighed again for the weight confirmation. Each of the 62 1L-sample-bags were tested three to five times using FLASHSMART Elemental Analyzer / Flash IRMS EA IsoLink operating with EagerSmart software. Proper levels of gases: N, O, He were checked every time before testing. Technical gases were provided by Praxair. Additionally, proper functioning of the instrumentation was verified every time before and after testing by observing of reactor's temperature and other specific measurements accordingly to the manual. Eventually, the final results were subjected to a comparison with standards provided by the manufacturer.

Mineralizable Nitrogen, Available Nitrogen Forms: NH_4^+, NO_3^-

Mineralizable nitrogen and available nitrogen forms tests were conducted by the Ministry of Environment and Climate Change Strategy - Analytical Lab in Victoria. Tests performed by the lab were KCl Extraction with UV Analysis for mineralizable nitrogen and KCl Extraction with Autoanalyzer Analysis for available nitrogen forms.

Basic Elements

Basic elements test was conducted by the Ministry of Environment and Climate Change Strategy - Analytical Lab in Victoria. Test performed by the lab was Metals via Acid, Microwave Digestion with ICP-OES Analysis.

Statistical Analyses

The software applied was R Studio 1.3.1093 and Excel Microsoft 365. Statistical analyses were divided into 3 sections which align with the study objectives.

Section 1 - mortality and productivity were analyzed to answer the first study question on similarities and differences between two mine subsoils. As a first step, the plant productivity and mortality data sets were tested for following the parametric assumptions (Greenacre and Primicerio, 2013; Lander, 2014). Shapiro-Wilk test followed by residuals plot and Normal Q-Q plot revealed that the data sets violate the normality and homoscedasticity assumptions, therefore, non-parametric models had to be applied. Mortality and productivity y data were subjected to Kruskal-Wallis one-way analysis of variance. To compare subsoil treatments, when the Kruskal-Wallis test was showing a significant difference, populations were subjected to Wilcoxon pairwise comparison. To confirm the revealed overall difference between New Afton New Gold and Teck Highland Valley Copper subsoils the entire sets of both subsoil physicochemical parameters were compared using PERMANOVA test based on Euclidean distance and then visualized by Principal Component Analysis.

Section 2 - After detection that NA and HVC subsoils differ strongly 17 predictors were analyzed one by one to determine which stand behind the detected difference. Tests between two mine subsoils were followed by comparison between subsoil treatments within each mine separately. Analyzed predictors were: pH, electroconductivity, soil organic matter (SOM), total carbon, as well as all the basic elements. All mentioned predictors were analyzed the same way. After revision of results, charts of the most influential predictors were prepared and presented in the main body of the thesis, while all others are presented in the Appendix A.

Section 3 - The remaining 4 predictors related to nitrogen: total nitrogen content, mineralizable nitrogen content, available NH_4^+ and NO_3^- contents were analyzed separately in section 3. Additionally, the last set of analyses takes on qualitative and quantitative change in NH_4^+ and NO_3^- contents throughout the experiment. Firstly the sum of initial NH_4^+ and NO_3^- contents were compared with the final sum and then the initial and final ratios of those two available N-forms were compared as second. This analysis worked only with biosolids-containing subsoil treatments, these were: “75%sub25%bios” and the “mixture”. All other subsoil treatments poor in available nitrogen were disregarded for these tests. Boxplots combine together datapoints from both New Afton New Gold and the Teck Highland Valley Copper. These tests intended to resolve which nitrogen cycling step is prevailing at the initial stage of the mine reclamation with the uncured biosolids application.

RESULTS

Mortality, Productivity, Overall Subsoils’ Difference

Section 1: Mortality

Kruskal-Wallis test showed that mortality of plants growing on New Afton New Gold subsoil combinations was significantly larger than mortality of plants growing on Teck Highland Valley Copper subsoil combinations (Figure 2.1).

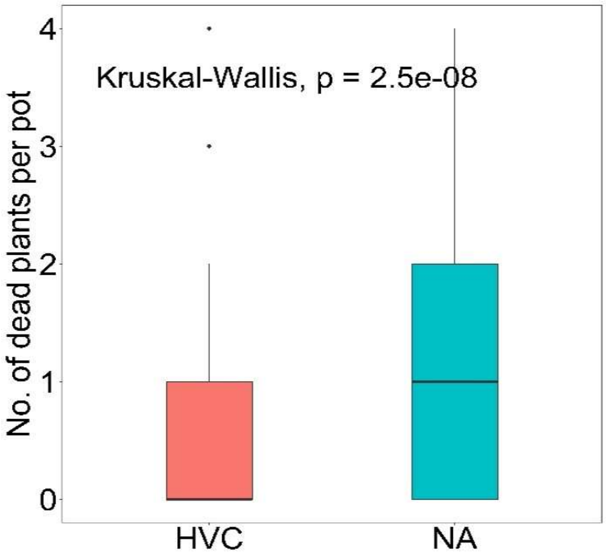

Figure 2.1 *Mortality comparison between two mines’ subsoils: HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Boxplots describe the number of dead plants per pot. Horizontal line indicates the median of plants per pot which did not survive to the end of the experiment. Kruskal-Wallis test was used to compare the medians of plant mortalities. n = 175*

New Afton New Gold Plant Mortality Analysis

Kruskal-Wallis test showed that the mortalities of New Afton New Gold subsoil treatments were not equal (Figure 2.2).

Wilcoxon pairwise comparison test showed that: the treatment with an addition of 25% of biosolids alone resulted in significantly larger mortality than unamended subsoil, than treatment with 25% of woodchips, than treatment with 5% of biochar, as well as than treatment with a mixture of soil amendments. The treatment with a mixture of soil amendments resulted in significantly larger mortality than unamended subsoil, than treatment with 25% of woodchips, than treatment with 5% biochar, as well as significantly lower mortality than treatment with 25% of biosolids. The unamended subsoil treatment resulted in significantly larger mortality than the treatment with 25% of woodchips. There were no significant differences in terms of mortality between unamended subsoil and the treatment with 5% of biochar as well as between the treatment with 5% of biochar and the treatment with 25% of woodchips (Figure 2.2).

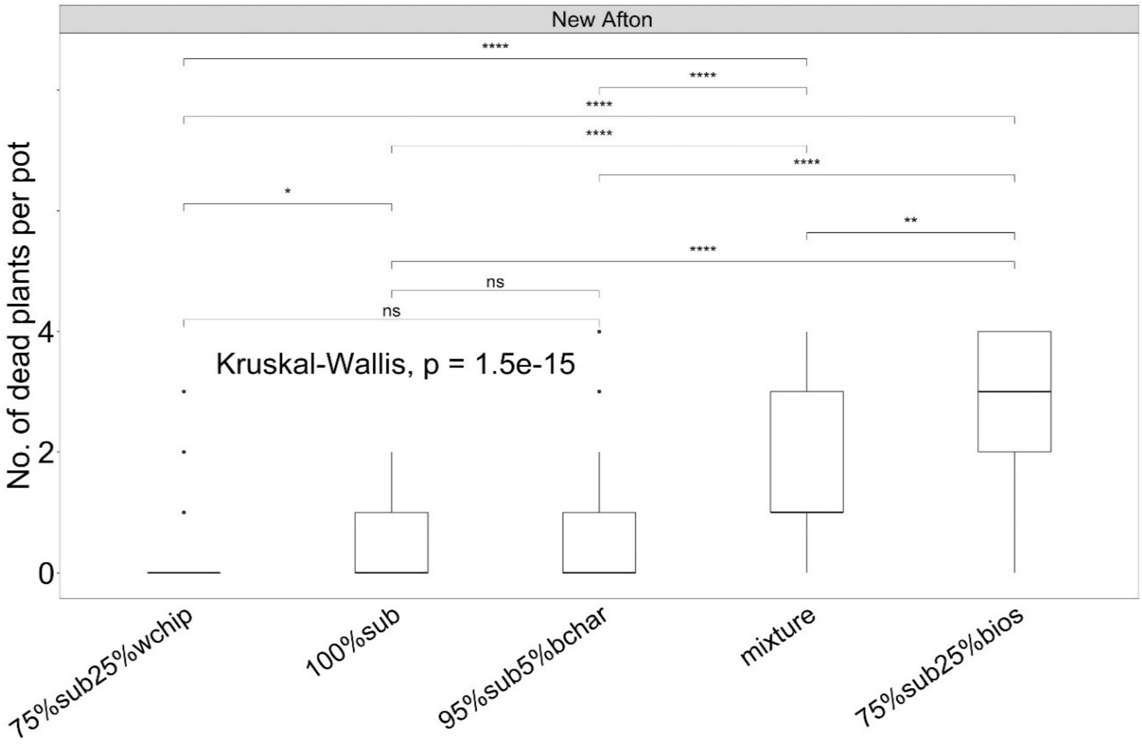

Figure 2.2** Mortality comparison between the New Afton New Gold (New Afton) subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Boxplots describe the number of dead plants per pot. Horizontal line indicates the median of plants per pot which did not survive to the end of the experiment. Kruskal-Wallis test was used to compare the medians of plant mortalities. Wilcoxon test was used for pair-wise comparison: * - $p < 0.05$, ** - $p < 0.01$, * - $p < 0.0001$, ns – difference not significant. n = 35*

Teck Highland Valley Copper Plant Mortality Analysis

Kruskal-Wallis test showed that the mortalities of Teck Highland Valley Copper subsoil treatments were not equal (Figure 2.3).

Wilcoxon pairwise comparison test showed that: the treatment with an addition of 25% of biosolids alone resulted in significantly larger mortality than unamended subsoil, than treatment with 25% of woodchips, than treatment with 5% of biochar, as well as than treatment with a mixture of soil amendments. The treatment with a mixture of soil amendments resulted in significantly larger mortality than unamended subsoil, than treatment with 25% of woodchips, than treatment with 5% biochar, as well as significantly lower mortality than treatment with 25% of biosolids. There were no significant differences in terms of mortality between unamended subsoil, the treatment with 5% of biochar, and the treatment with 25% of woodchips (Figure 2.3).

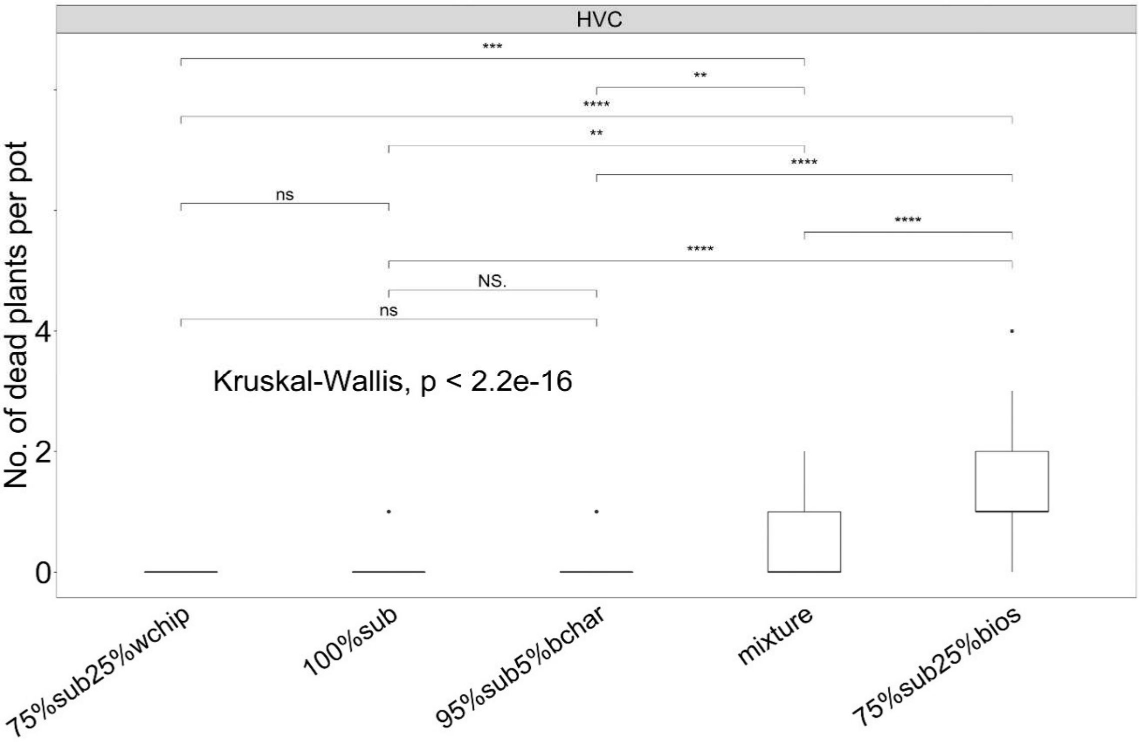

Figure 2.3 *Mortality comparison between the Teck Highland Valley Copper (HVC) subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Boxplots describe the number of dead plants per pot. Horizontal line indicates the median of plants per pot which did not survive to the end of the experiment. Kruskal-Wallis test was used to the medians of plant mortalities. Wilcoxon test was used for pair-wise comparison: ** - p < 0.01, *** - p < 0.001, **** - p < 0.0001, ns – difference not significant. n = 35*

Plant Species Mortality Analysis

Kruskal-Wallis tests showed that individuals of bluebunch wheatgrass, field locoweed, and rough fescue growing on New Afton New Gold subsoil combinations experienced larger mortality than individuals of these species growing on Teck Highland Valley Copper subsoil combinations. Mortalities of prairie junegrass on two mines' subsoils did not differ significantly (Figure 2.4).

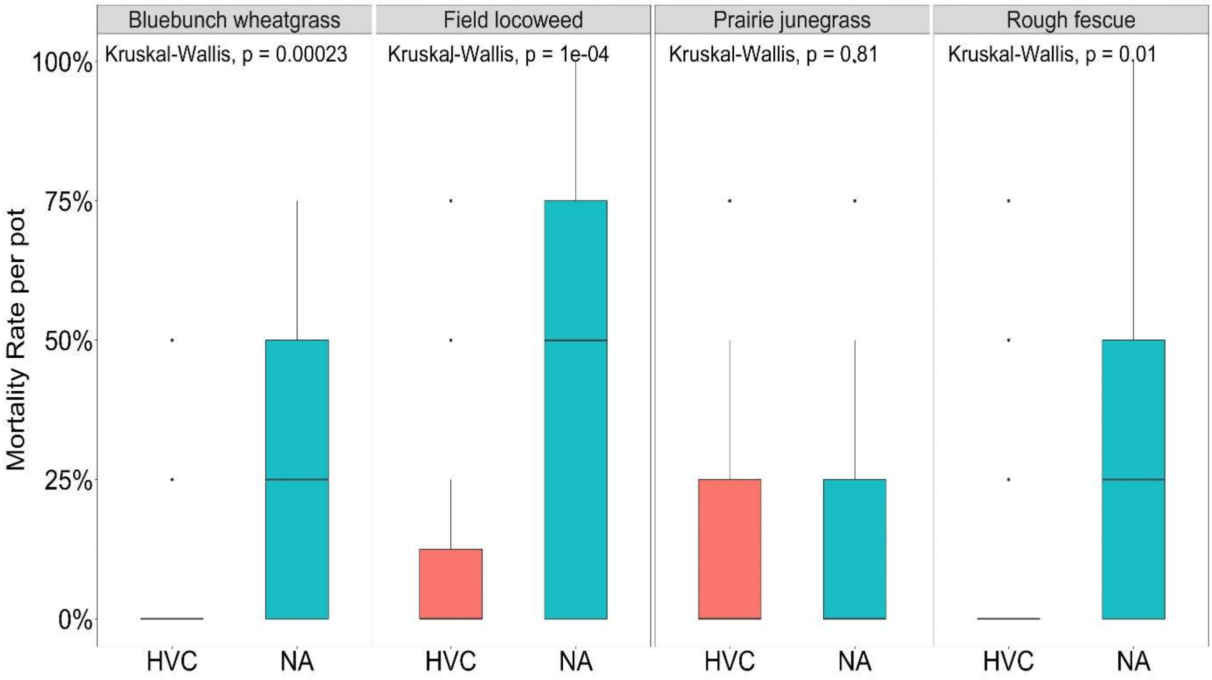

Figure 2.4 *Plant species mortality comparison between two mines' subsoils: HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median mortality rate. Kruskal-Wallis test was used to compare the medians of plant species mortalities. n = 35*

Kruskal-Wallis tests showed that on New Afton New Gold subsoil treatments mortalities of plant species were not equal. Wilcoxon pairwise comparison tests showed that prairie junegrass individuals performed significantly better than bluebuch wheatgrass, field locoweed, and rough fescue. There was no significant difference between all other species in terms of survivorship/mortality on New Afton New Gold subsoil treatments (Figure 2.5 right).

Kruskal-Wallis tests showed that on Teck Highland Valley Copper subsoil treatments all species performed equally in terms of survivorship (Figure 2.5 left).

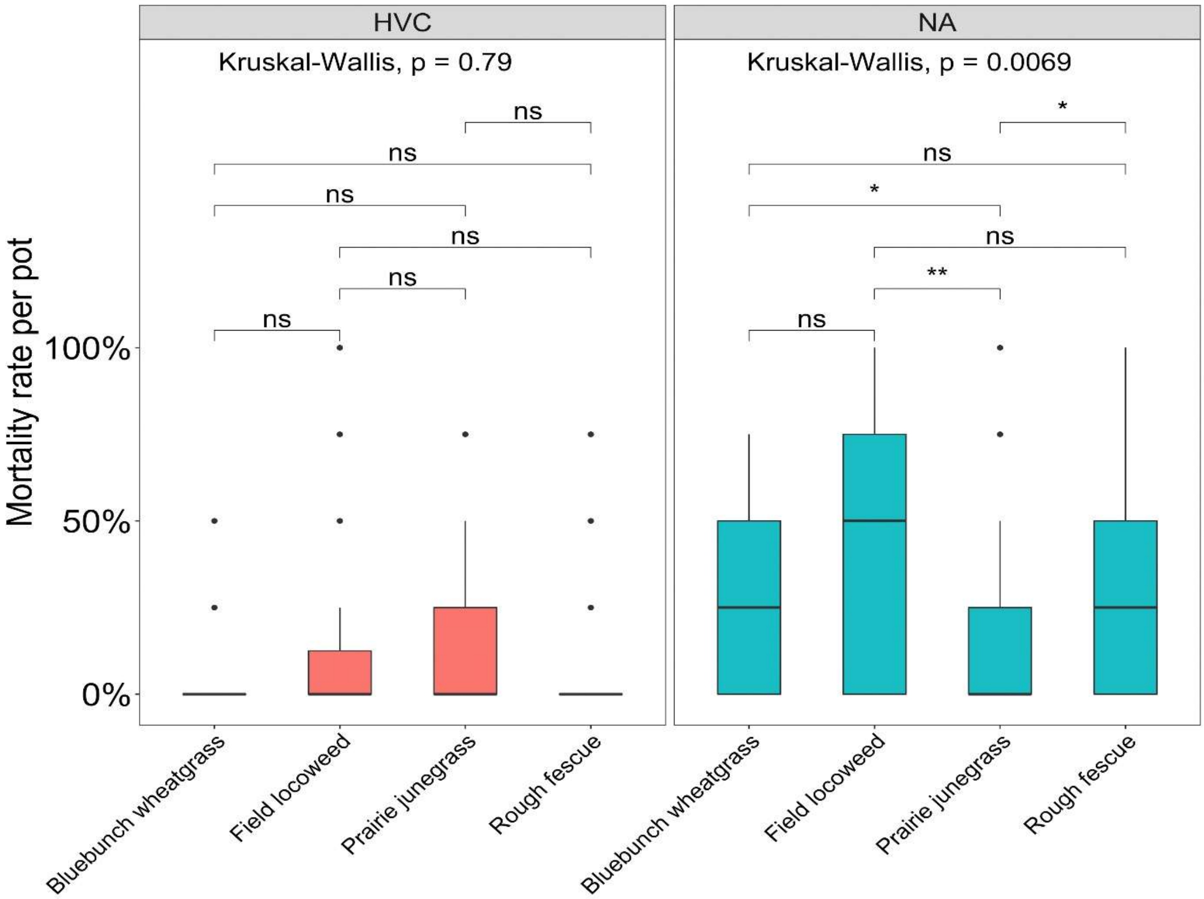

__Figure 2.5__ Plant species mortality comparison on two mines' subsoils: HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median mortality rate. Kruskal-Wallis test was used to compare the medians of plant species mortalities. n = 35

Section 1: Productivity

Kruskal-Wallis test showed that dry above-ground biomass productivity of plants growing on New Afton New Gold subsoil combinations was significantly lower than dry above-ground biomass productivity of plants growing on Teck Highland Valley Copper subsoil combinations (Figure 2.6).

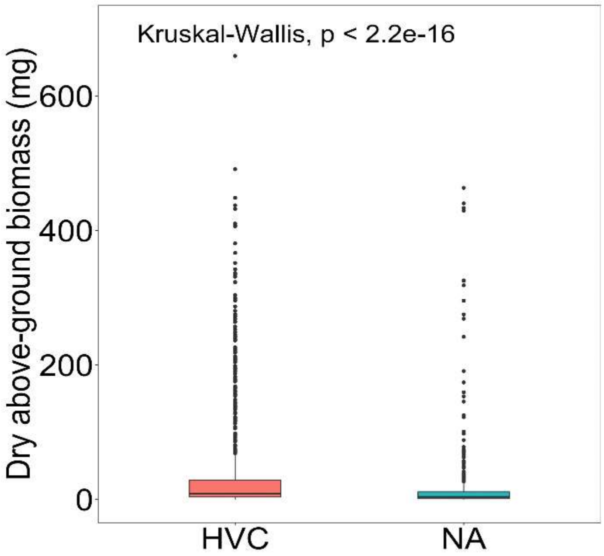

Figure 2.6 *Dry above-ground biomass productivity comparison between two mines' subsoils: HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Boxplots describe dry biomass weight. Horizontal line indicates the median of weights of individual plants at the end of the experiment. Kruskal-Wallis test was used to compare the medians of plant dry above-ground biomass productivity. n = 700*

Average dry above-ground biomass productivity of plants growing on New Afton New Gold subsoil combinations was more than three times lower than average dry above-ground biomass productivity of plants growing on Teck Highland Valley Copper (Figure 2.7).

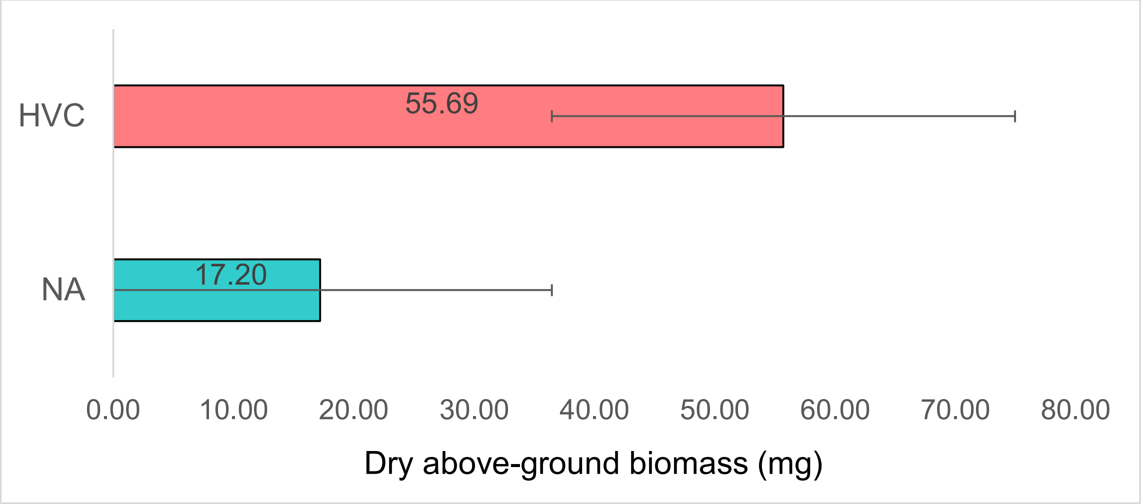

Figure 2.7 *Mean dry above-ground biomass productivity comparison between two mines' subsoils: HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Error bars are standard error of the mean. n = 700*

New Afton New Gold Subsoil Treatments Productivity Analysis

Kruskal-Wallis test showed that the dry above-ground biomass productivities of New Afton New Gold subsoil treatments were not equal (Figure 2.8).

Wilcoxon pairwise comparison test showed that the treatment with a mixture of soil amendments resulted in a significantly larger dry above-ground biomass productivity than unamended subsoil, than treatment with 25% of woodchips, than treatment with 5% of biochar, as well as than the treatment with 25% of biosolids. The treatment amended by 25% of biosolids resulted in a significantly larger dry above-ground biomass productivity than unamended subsoil, than treatment with 25% of woodchips, than treatment with 5% of biochar. The treatment with unamended subsoil resulted in a significantly larger dry above-ground biomass productivity than the treatment with 25% of woodchips only. There was no significant difference in terms of dry above-ground biomass productivity between the treatment with 5% of biochar and the treatment with 25% of woodchips (Figure 2.8).

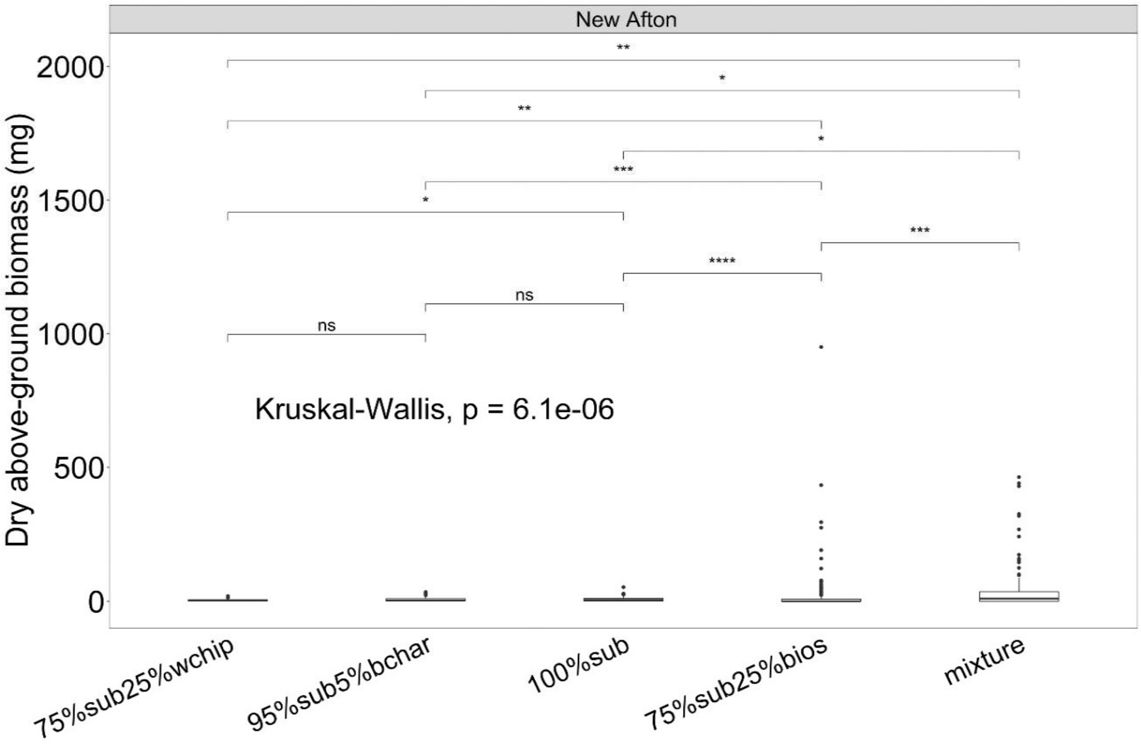

Figure 2.8 *Dry above-ground biomass production comparison between the New Afton New Gold (New Afton) subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Boxplots describe the weights of individual plants' dry above-ground biomass. Horizontal line indicates the median of plants' above-ground biomass. Kruskal-Wallis test was used to compare the medians of plant productivity on particular subsoil treatments. Wilcoxon test was used for pairwise comparison* $*$ *- p < 0.05,* $**$ *- p < 0.01,* $***$ *- p < 0.001,* $****$ *- p < 0.0001, ns – difference not significant. n = 140*

Teck Highland Valley Copper Subsoil Treatments Productivity Analysis

Kruskal-Wallis test showed that the dry above-ground biomass productivities of Teck Highland Valley Copper subsoil treatments were not equal (Figure 2.9).

Wilcoxon pairwise comparison test showed that the treatment with a mixture of soil amendments resulted in a significantly larger dry above-ground biomass productivity than unamended subsoil, than treatment with 25% of woodchips, than treatment with 5% of biochar, as well as than the treatment with 25% of biosolids. The treatment with 25% of biosolids resulted in a significantly larger dry above-ground biomass productivity than unamended subsoil, than treatment with 25% of woodchips, and than the treatment with 5% of biochar. The treatment with unamended subsoil resulted in a significantly larger dry above-ground biomass productivity than the treatment with 25% of woodchips only. There was no significant difference in terms of dry above-ground biomass productivity between the treatment with 5% of biochar and the treatment with 25% of woodchips (Figure 2.9).

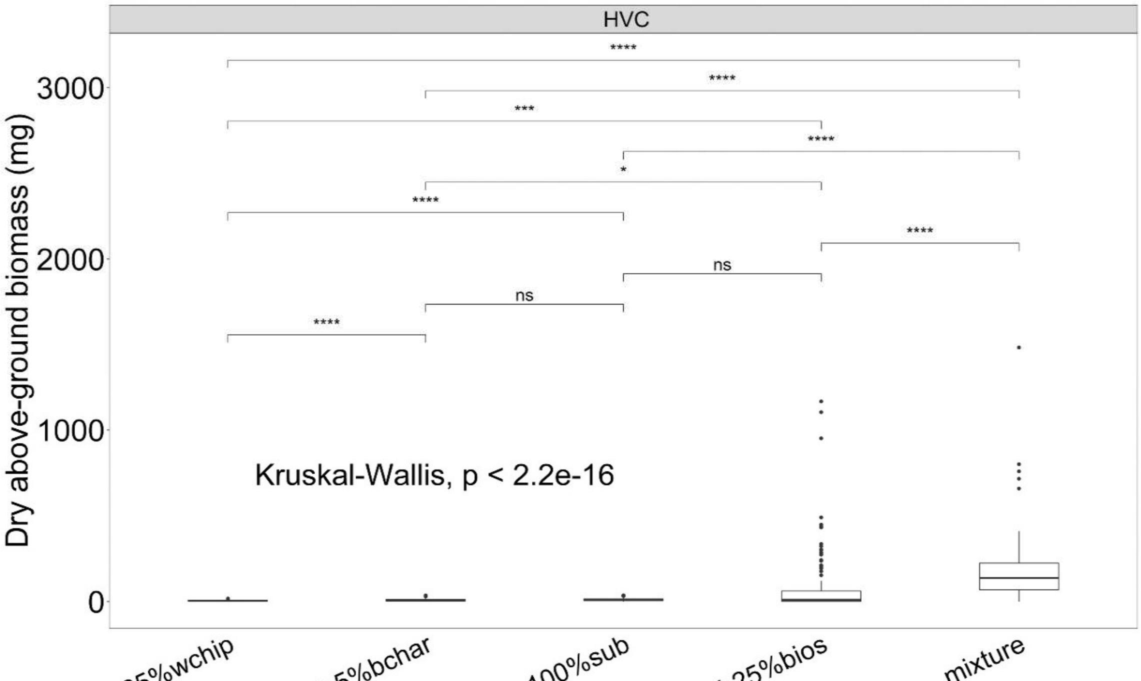

Figure 2.9 *Dry above-ground biomass production comparison between the Teck Highland Valley Copper (HVC) subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Boxplots describe the weights of individual plants' dry above-ground biomass. Horizontal line indicates the median of plants' above-ground biomass. Kruskal-Wallis test was used to compare the medians of plant productivity on particular subsoil treatments. Wilcoxon test was used for pairwise comparison * - p < 0.05, *** - p < 0.001, **** - p < 0.0001, ns – difference not significant. n = 140*

Section 1: Both Mines' Subsoil Overall Difference; Principal Component Analysis

The two mines' data sets of predictors characterizing physicochemical properties were compared and the analysis presented strong evidence that the two subsoils were overall significantly different: Pseudo F = 322.34; $p < 0.001$ (Figure 2.10). Datapoints representing pots containing subsoil with biosolids as the only amendment clustered together separately from all other treatments showing that they distinguished from other treatments. The same with pots containing subsoil with a mixture of amendments. Datapoints representing three other treatments clustered altogether showing that there was similarity between them (Figure 2.11).

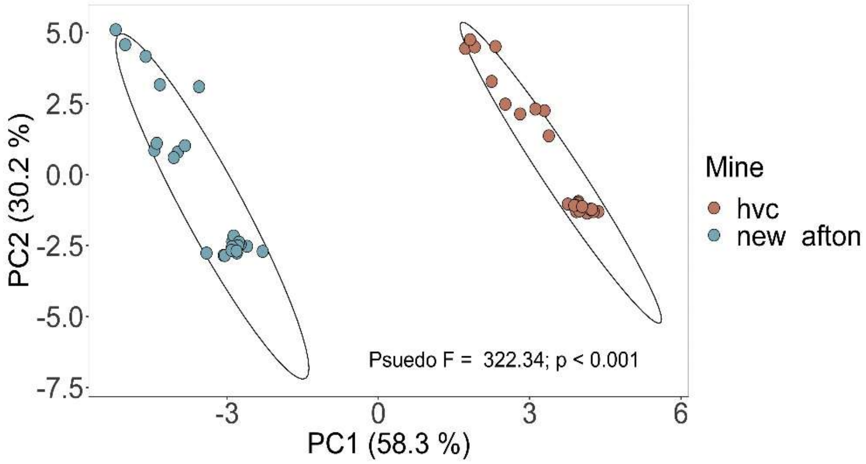

Figure 2.10 *Principal Component Analysis of two mines' subsoils in terms of 21 predictors: pH, EC, SOM, total N and C content, mineralizable N content,* NH_4^+ *and* NO_3^- *contents, as well as the content of 13 basic elements. hvc – Teck Highland Valley Copper, new afton – New Afton New Gold.*

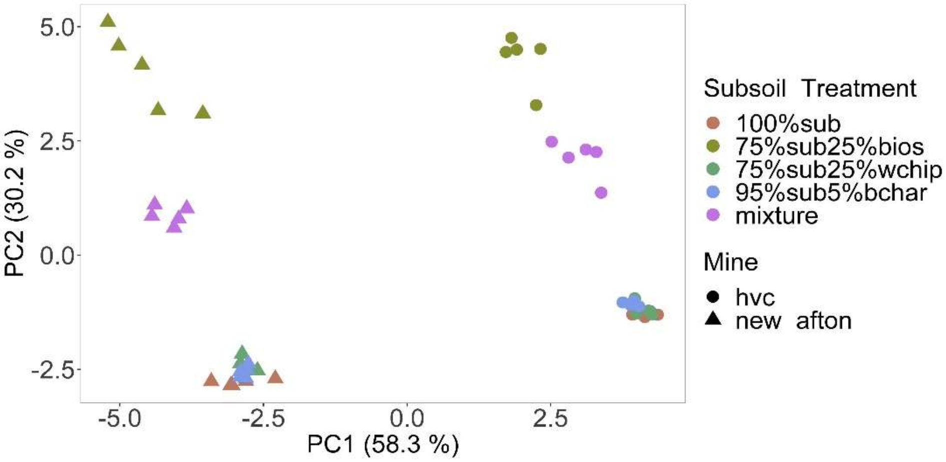

Figure 2.11 *Principal Component Analysis of two mines' subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - Subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. hvc – Teck Highland Valley Copper, new afton – New Afton New Gold.*

Physicochemical Properties of Mines’ Subsoils When Unamended and Amended

Section 2: pH

New Afton New Gold subsoil pH was significantly higher than Teck Highland Valley Copper both in the initial and the final stages (Figure 2.12 left). Within each mine separately there was no significant difference between the initial and the final pH either in the case of New Afton New Gold or in the case of Teck Highland Valley Copper (Figure 2.12 right).

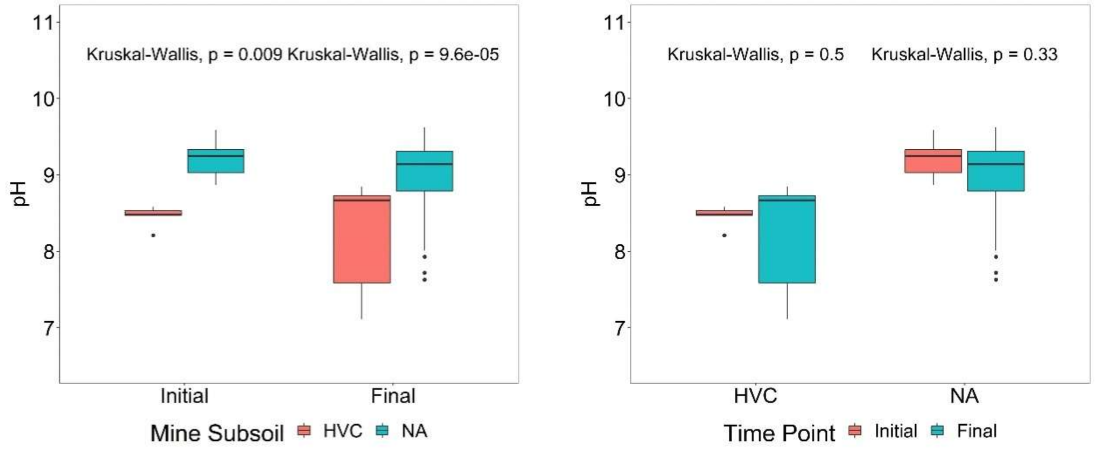

Figure 2.12 *Two mines’ subsoil pH comparison in the initial and final stages of the experiment (left), comparison of the subsoil pH in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

In the case of both mines the final pHs were statistically not equal (Figure 2.13). In both cases, the biosolids-containing treatments had the lowest pH. In both cases as well, pHs seemed to drop throughout the experiment (Figure 2.13).

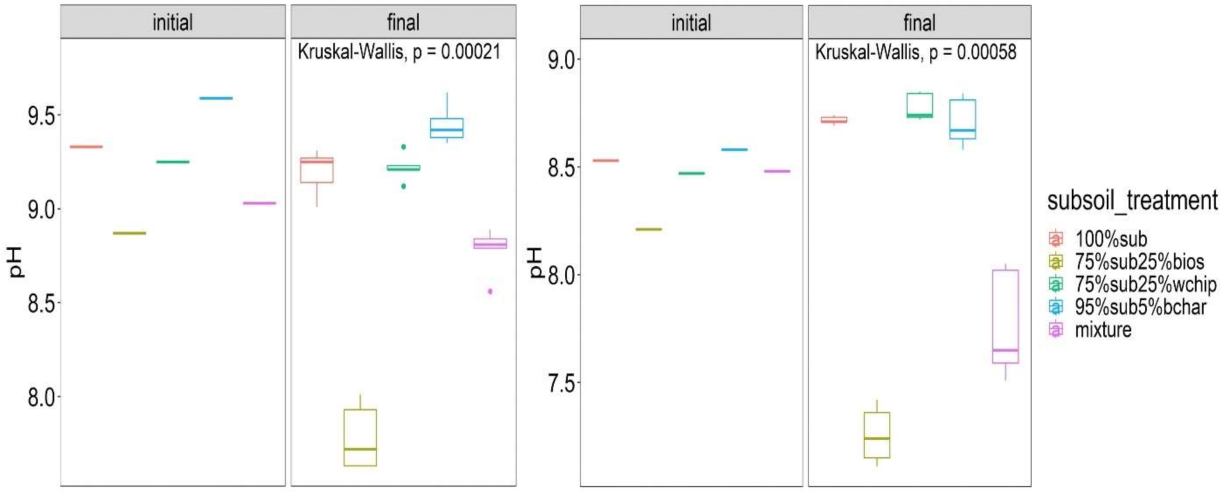

Figure 2.13 *New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments pH comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 5*

Section 2: Electroconductivity (EC)

New Afton New Gold subsoil electroconductivity was significantly higher than Teck Highland Valley Copper both in the initial and the final stages (Figure 2.14 left). Within each mine separately there was no significant difference between the initial and final electroconductivities either in the case of NA or in the case of HVC (Figure 2.14 right).

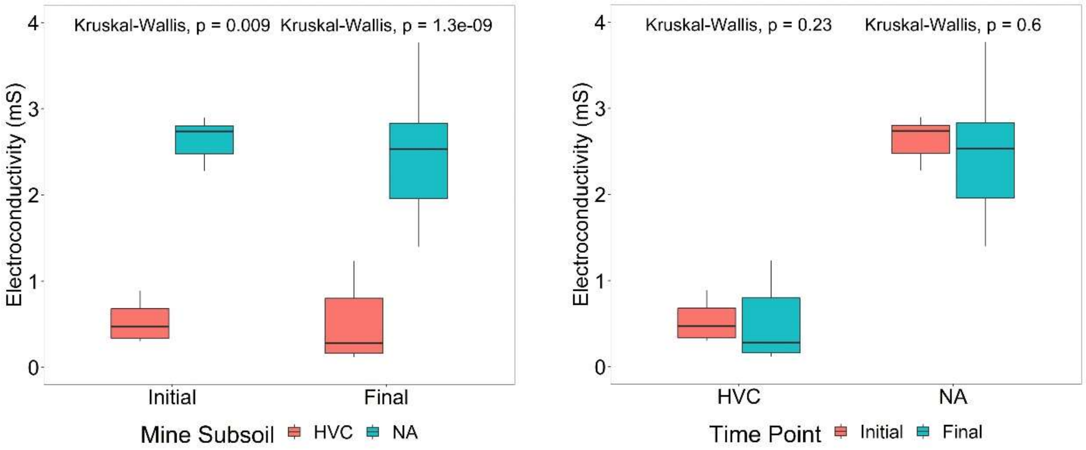

Figure 2.14 *Two mines' subsoil electroconductivities comparison in the initial and final stages of the experiment (left), comparison of the electroconductivities in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

In the case of both mines the final pHs were statistically not equal (Figure 2.15). In the case of Teck Highland Valley Copper subsoil treatments, the addition of biosolids or mixture seemed to increase the EC (Figure 2.15 right). Such a pattern was not visible though in the case of New Afton New Gold subsoil treatments (Figure 2.15 left).

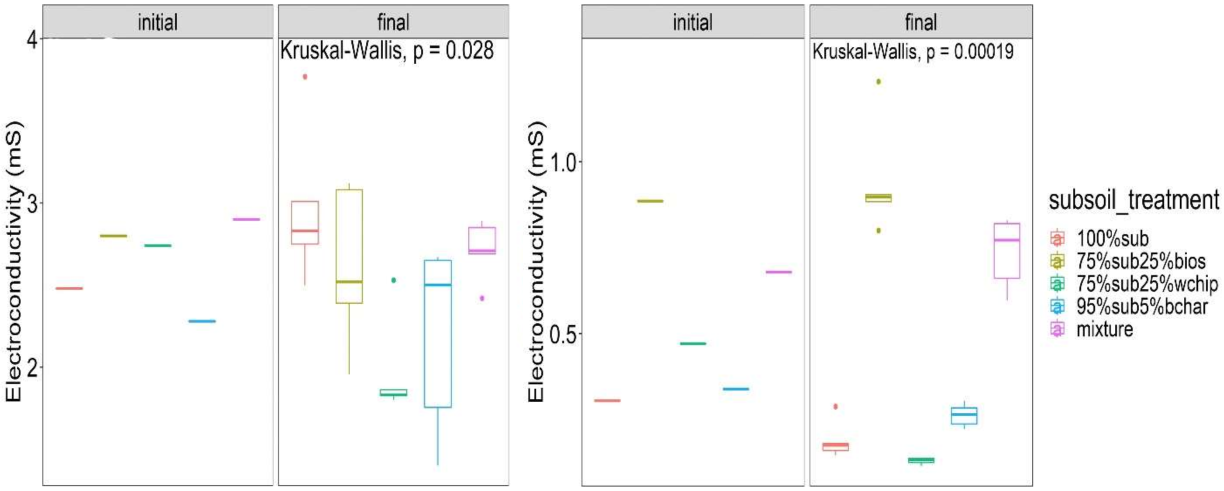

Figure 2.15 *New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments electroconductivities comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 5*

Section 2: Soil Organic Matter (SOM) Content

New Afton New Gold subsoil SOM content was significantly larger than Teck Highland Valley Copper but in the case of the final stage only (Figure 2.16 left). Within each mine separately there was no significant difference between the initial and the final SOM content either in the case of NA or in the case of HVC (Figure 2.16 right).

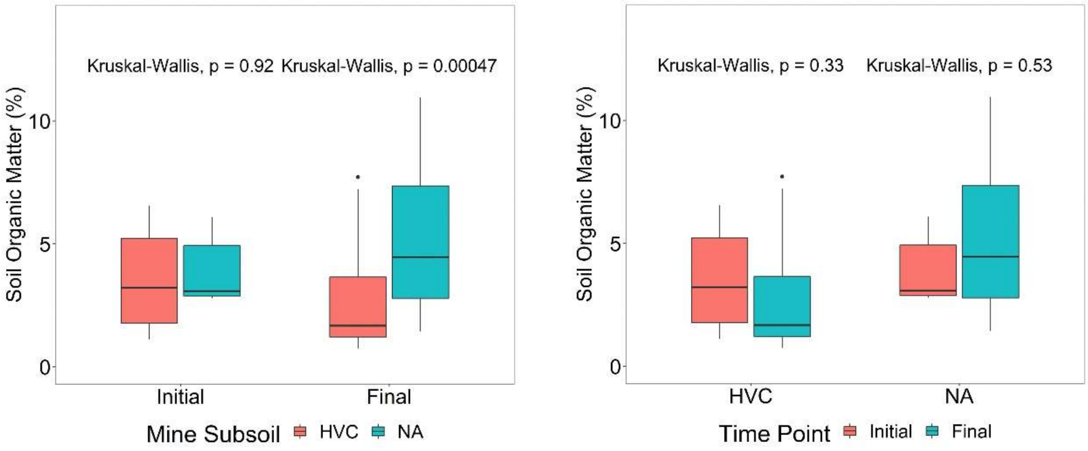

Figure 2.16 *Two mines' subsoil soil organic matter content comparison in the initial and final stages of the experiment (left), comparison of the soil organic matter content in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Initial n= 5, final n= 25*

In the case of both mines the final SOM contents were statistically not equal. In both cases, the biosolids-containing treatments had the largest SOM contents (Figure 2.17).

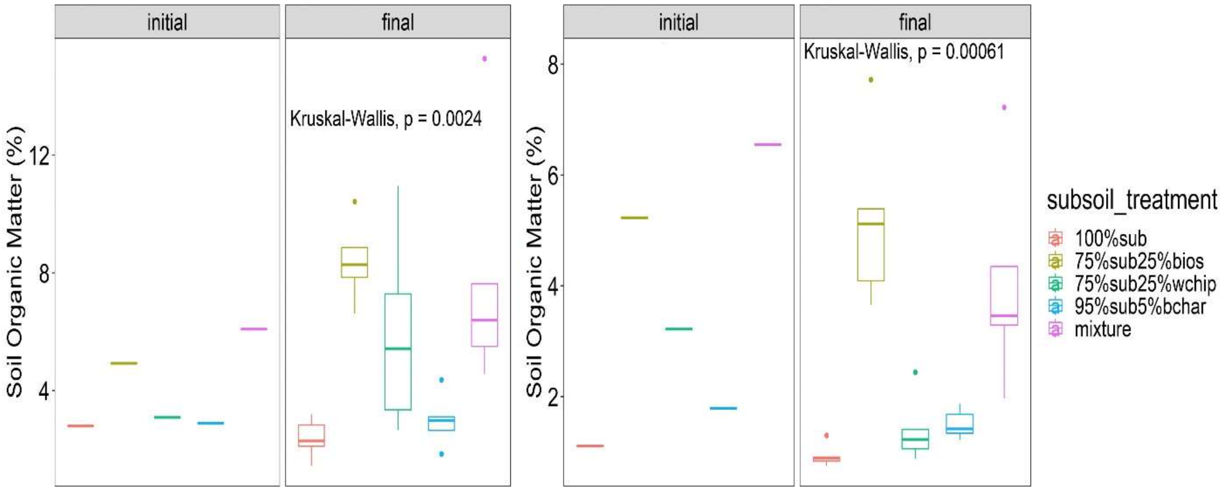

Figure 2.17 *New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments soil organic matter contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians.*
initial n= 1, final n= 5

Section 2: Sodium (Na) Content

New Afton New Gold subsoil Na content was significantly larger than Teck Highland Valley Copper both in the initial and final stages (Figure 2.18 left). Within each mine separately there was a significant difference between the initial and final Na contents both in the case of NA and in the case of HVC (Figure 2.18 right).

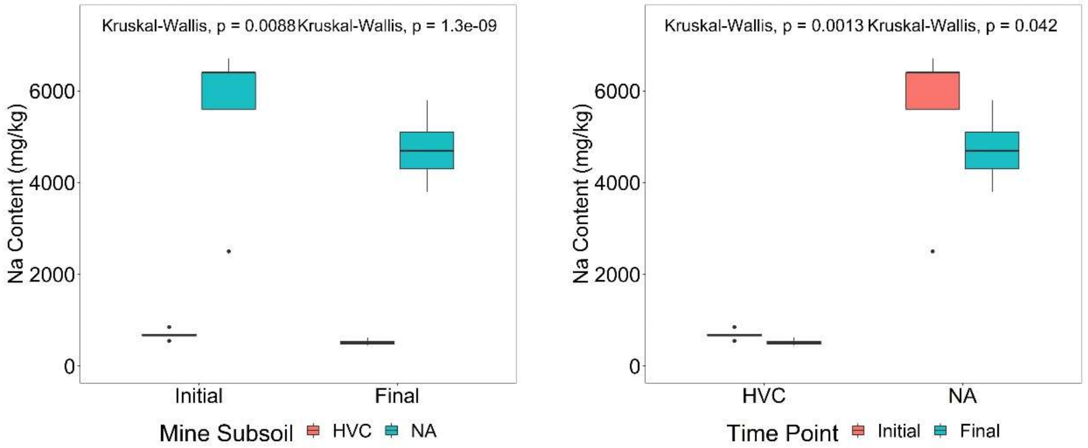

Figure 2.18 *Two mines' subsoil sodium content comparison in the initial and final stages of the experiment (left), comparison of the sodium content in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

Final Na contents were statistically not equal in the case of NA subsoil treatments only (Figure 2.19 left). In the case of both mines, nearly each subsoil treatment contained more sodium in the initial than in the final stage of the experiment (Figure 2.19).

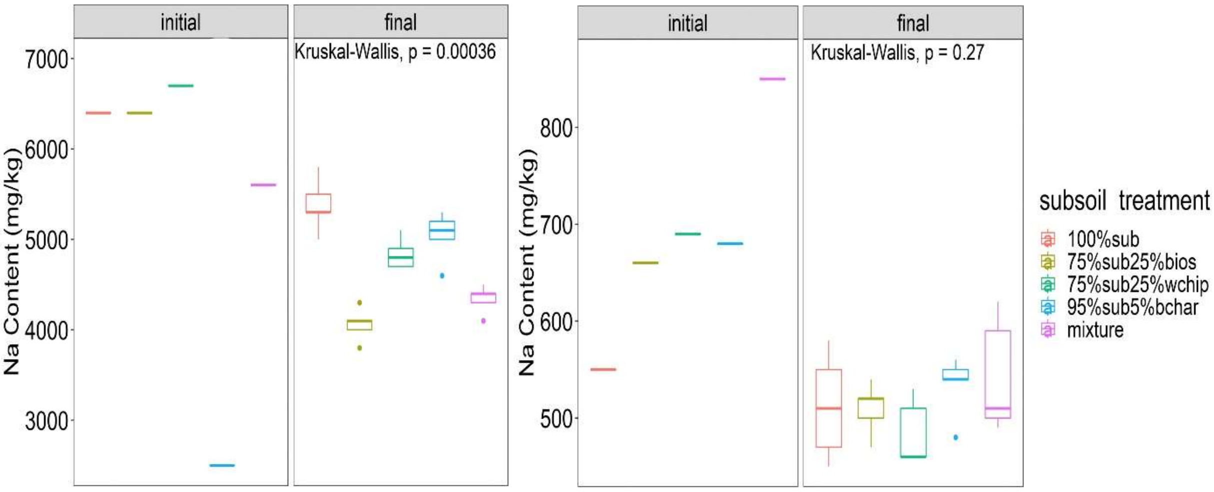

Figure 2.19 *New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments sodium contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 5*

Quantitative and Qualitative Nitrogen Content Analyses

Section 3: Total Nitrogen (N) Content

New Afton New Gold subsoil total nitrogen content was significantly larger than Teck Highland Valley Copper one both in the initial and final stages (Figure 2.20). Within each mine separately there was no significant difference between the initial and the final total nitrogen content either in the case of New Afton New Gold or in the case of Teck Highland Valley Copper (Figure 2.21).

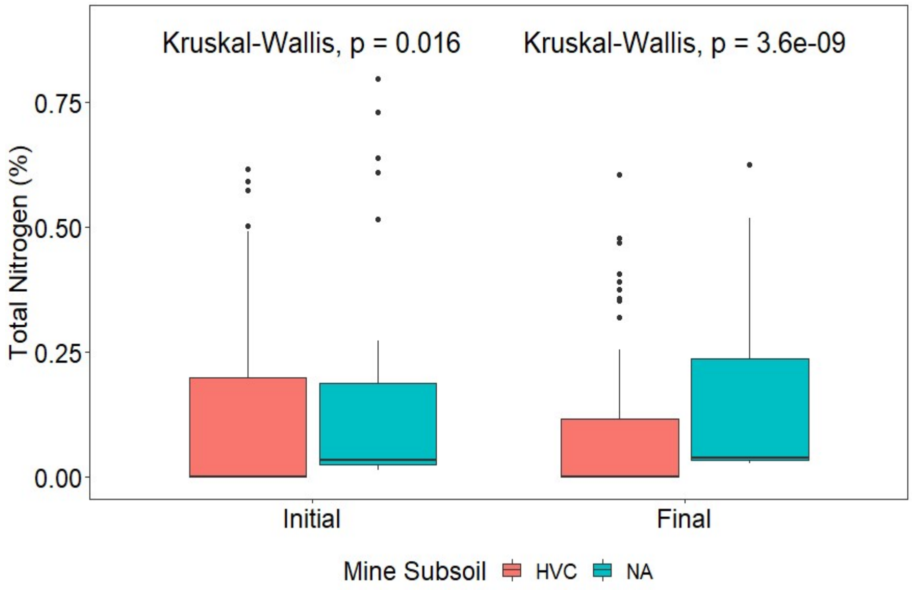

Figure 2.20 *Two mines' subsoil total nitrogen content comparison in the initial and final stages of the experiment. HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 25, final n= 75*

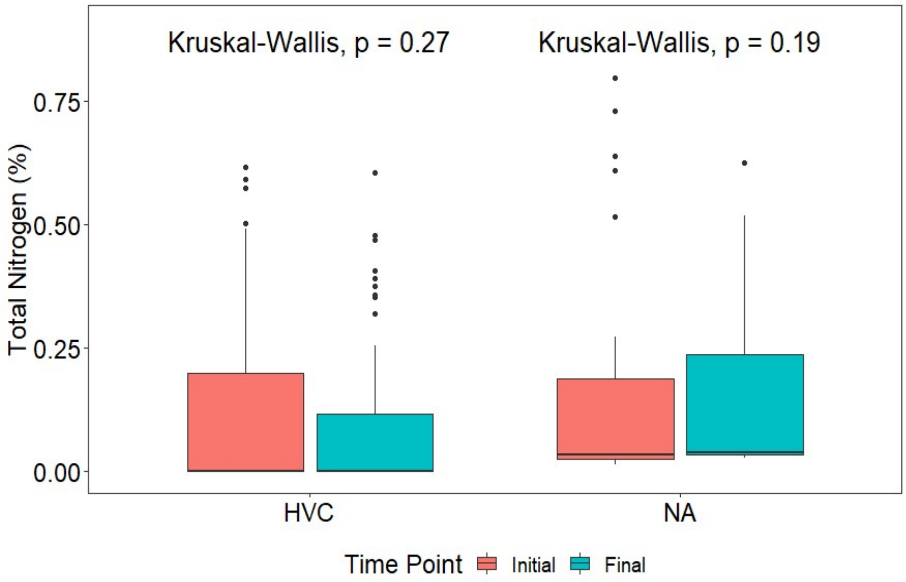

Figure 2.21 *Comparison of the total nitrogen content in the initial and final stages of the experiment within each mine. HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 25, final n= 75*

In the case of New Afton New Gold and the case of Teck Highland Valley Copper subsoil treatments, the initial and final total N contents were statistically not equal (Figures 2.22, 2.23). Both, in the case of New Afton New Gold and the case of Teck Highland Valley Copper subsoil treatments initially there was a statistical difference between the "95%sub5%bios" treatment and the "mixture" treatment. The difference persisted until the end of the experiment. Also, these two treatments differed statistically from all other treatments. This had place in the case of both mines' subsoil treatments. Treatments "75%sub25%wchip", "95%sub5%bchar" and "100%sub" in the case of both mines did not differ statistically either in the initial or final stage (Figures 2.22, 2.23).

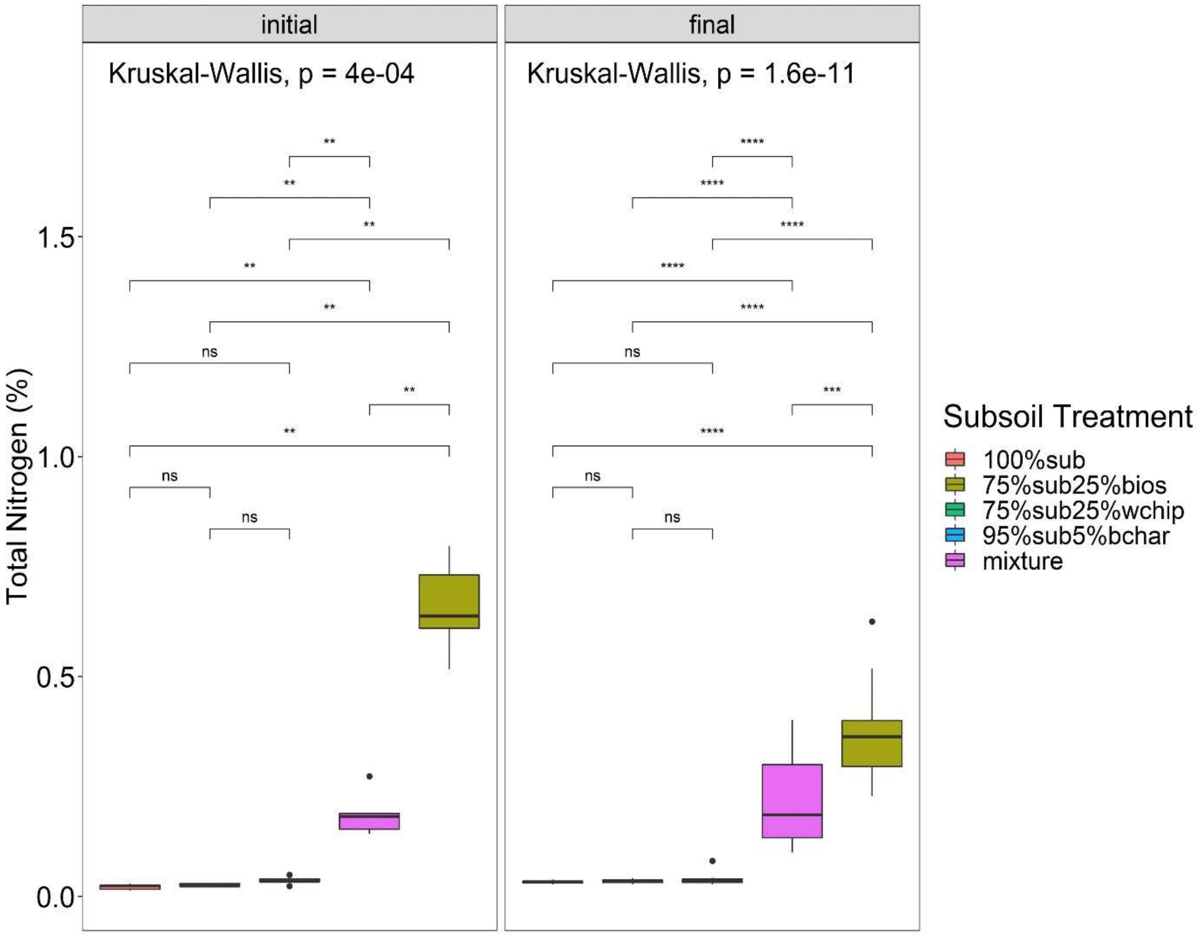

Figure 2.22 *New Afton New Gold subsoil treatments total nitrogen contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison ** - p < 0.01, *** - p < 0.001, **** - p < 0.0001, ns – difference not significant. initial n= 5, final n= 15*

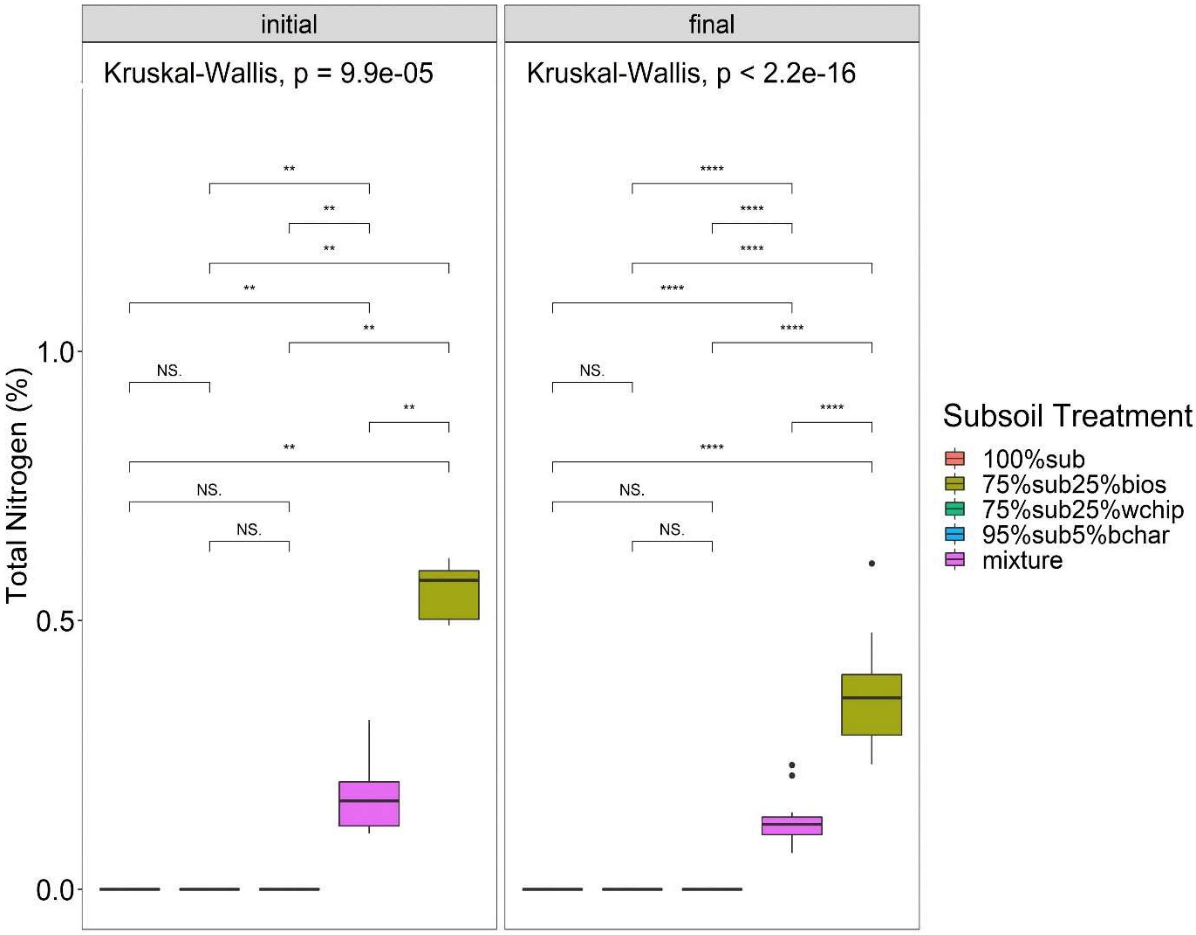

Figure 2.23** Teck Highland Valley Copper subsoil treatments total nitrogen contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison ** - $p < 0.01$, * - $p < 0.0001$, NS. – difference not significant. initial n= 5, final n= 15*

In the case of New Afton New Gold and the case of Teck Highland Valley Copper subsoil treatments only the “mixture” treatment was not significantly different at the end of the experiment from the reference of the undisturbed topsoil in terms of the total nitrogen content (Figures 2.24, 2.25). All other treatments differed significantly from the reference. In the case of “75%sub25%wchip”, “95%sub5%bchar”, and “100%sub” treatments the total nitrogen contents were significantly lower than the reference’s total nitrogen content both in the initial and the final stages of the experiment. “75%sub25%bios” treatment’s total nitrogen content was significantly larger than the reference’s one both in the initial and the final stages of the experiment (Figures 2.24, 2.25).

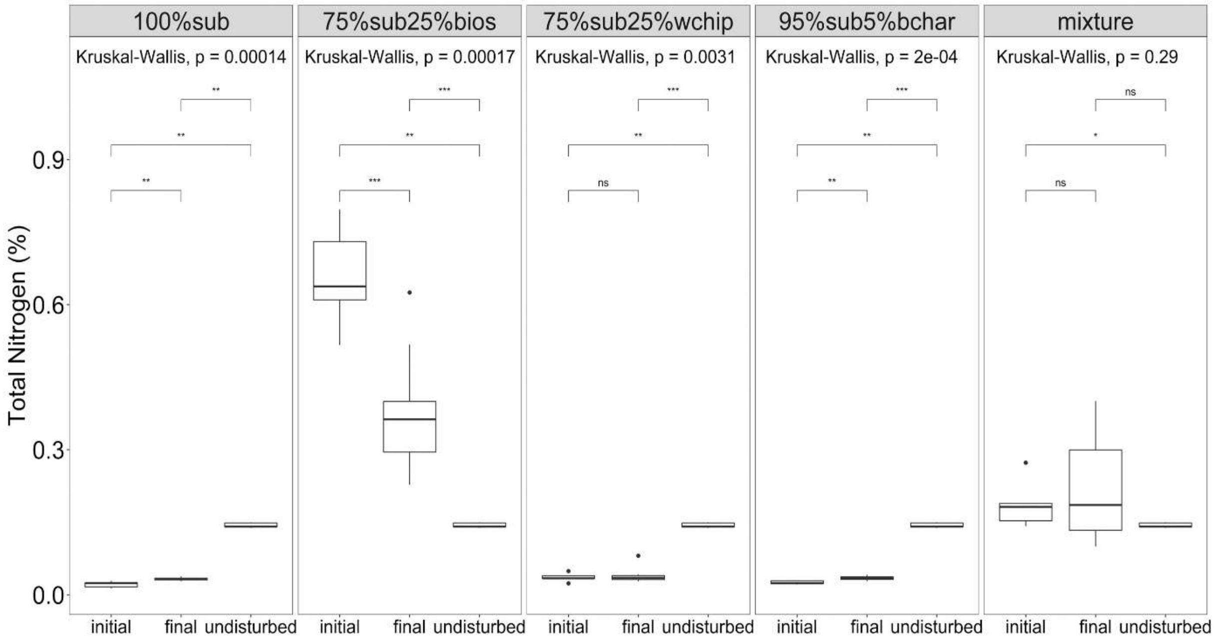

Figure 2.24 *New Afton New Gold subsoil treatments total nitrogen contents comparison in the initial and final stages of the experiment to the reference of undisturbed topsoil. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison * - p < 0.05, ** - p < 0.01, *** - p < 0.001, ns – difference not significant. initial n= 5, final n= 15, undisturbed n=5*

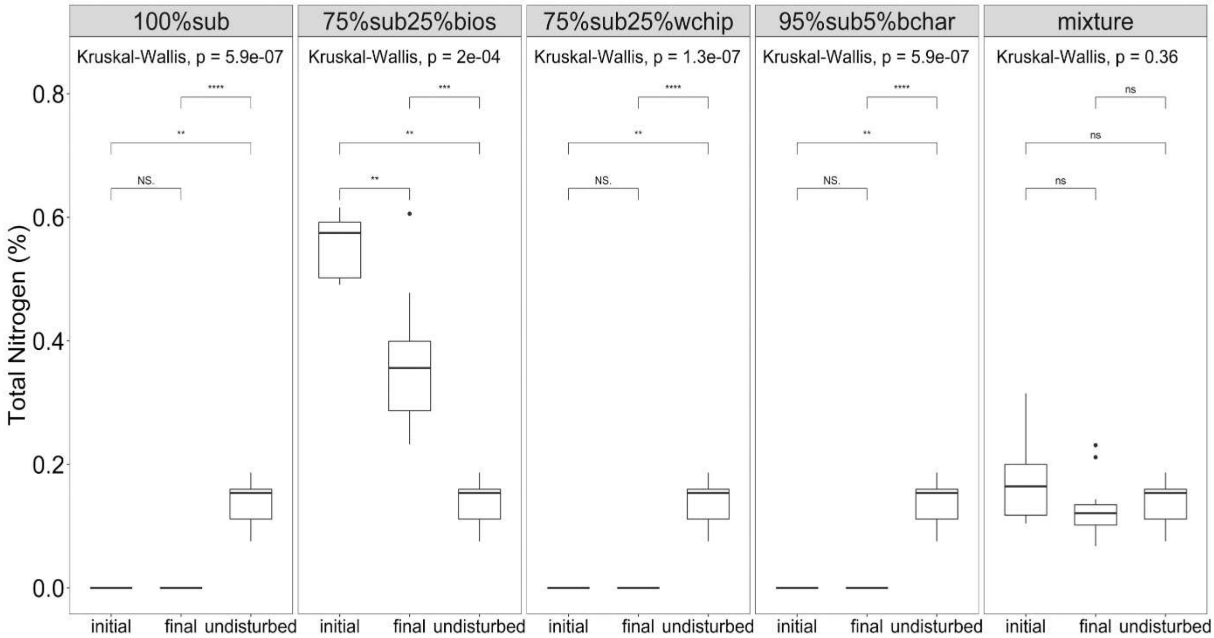

Figure 2.25 *Teck Highland Valley Copper subsoil treatments total nitrogen contents comparison in the initial and final stages of the experiment to the reference of undisturbed topsoil. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison ** - p < 0.01, *** - p < 0.001, ns, NS. – difference not significant. initial n= 5, final n= 15, undisturbed n=5*

Section 3: Mineralizable Nitrogen Content

NA subsoil mineralizable nitrogen content did not differ statistically from HVC subsoil mineralizable nitrogen content in the initial stage of experiment. At the end of experiment the difference became significant (Figure 2.26 left). Within each mine separately there was no significant difference between the initial and final mineralizable N contents either in the case of NA or in the case of HVC (Figure 2.26 right).

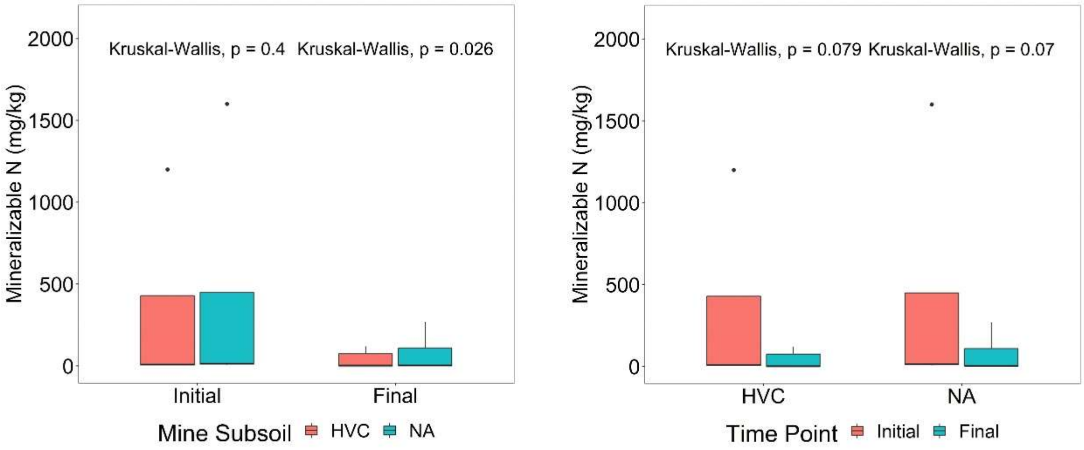

Figure 2.26 *Two mines' subsoil mineralizable nitrogen content comparison in the initial and final stages of the experiment (left), comparison of the mineralizable nitrogen content in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

In the case of NA and the case of HVC subsoil treatments the final mineralizable N contents were statistically not equal (Figures 2.27). In both cases, biosolids-containing treatments had the largest mineralizable N contents. Mineralizable N contents seemed to drop during the experiment (Figures 2.27).

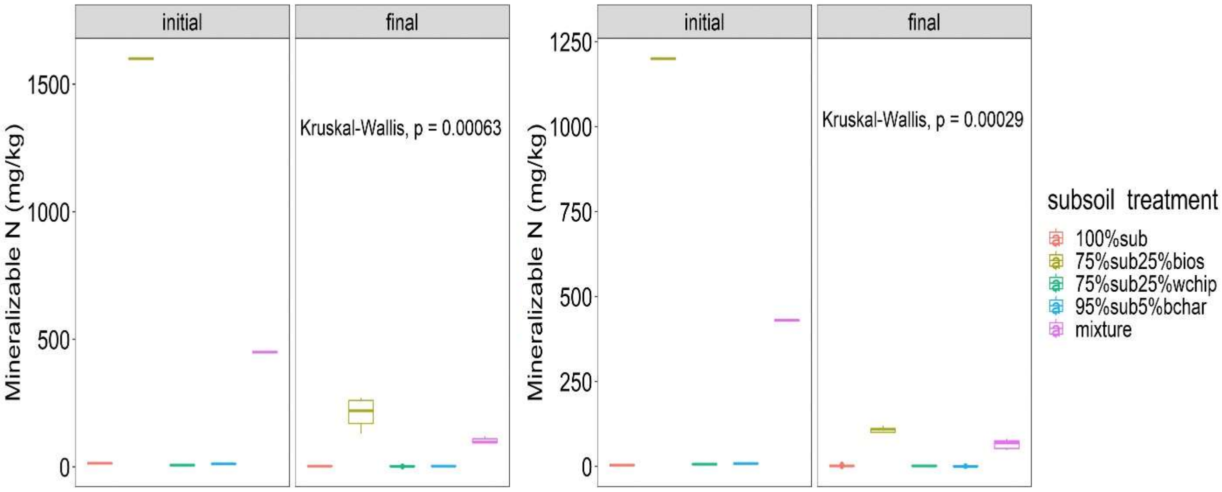

Figure 2.27 *New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments mineralizable nitrogen contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 5*

Section 3: Available Nitrogen Content – Ammonium Cation (NH_4^+)

New Afton New Gold subsoil NH_4^+ content differed statistically from Teck Highland Valley Copper subsoil but only at the final stage (Figure 2.28 left). Within each mine separately there was no significant difference between the initial and final NH_4^+ contents either in the case of NA or in the case of HVC (Figure 2.28 right).

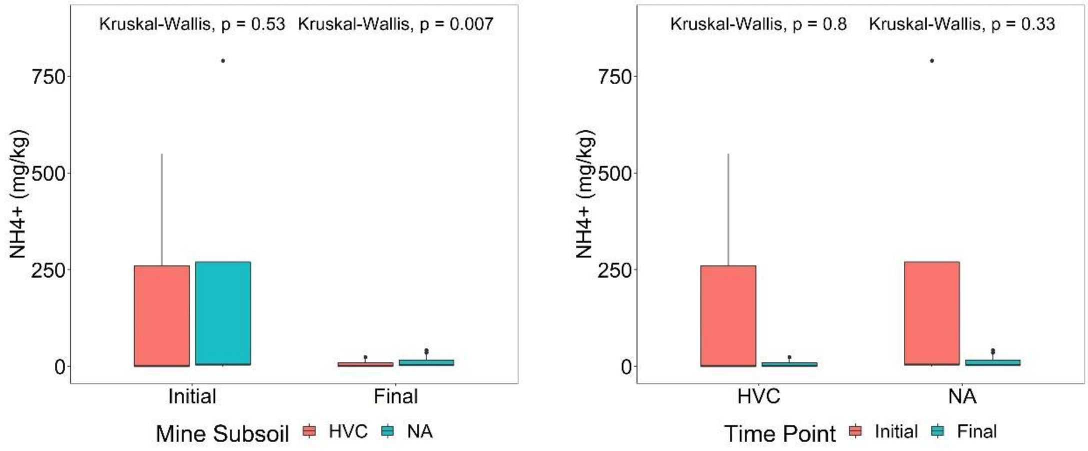

Figure 2.28 *Two mines' subsoil* NH_4^+ *content comparison in the initial and final stages of the experiment (left), comparison of the* NH_4^+*content in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

In the case of NA and the case of HVC subsoil treatments the final NH_4^+ contents were statistically not equal (Figures 2.28). In both cases, biosolids-containing treatments had the largest NH_4^+ contents. NH_4^+ contents seemed to drop throughout the experiment (Figures 2.29).

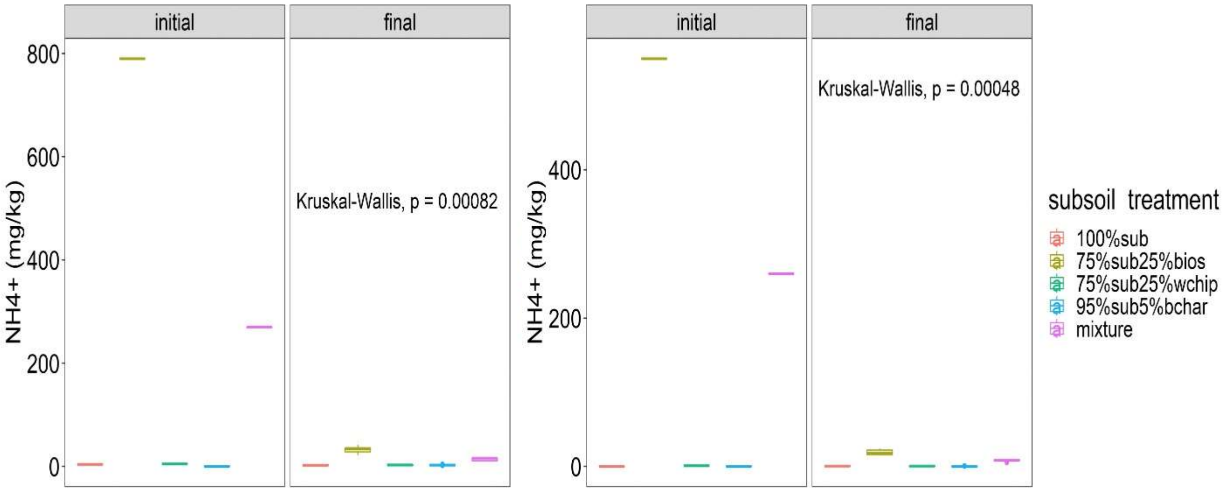

Figure 2.29 *New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments* NH_4^+*contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 5*

Section 3: Available Nitrogen Content – Nitrate Anion (NO_3^-)

NA subsoil NO_3^- content did not differ significantly from HVC NO_3^- content either in the initial or in the final stages (Figure 2.30 left). Within each mine separately there was no significant difference between the initial and final NO_3^- contents either in the case of NA or in the case of HVC (Figure 2.30 right).

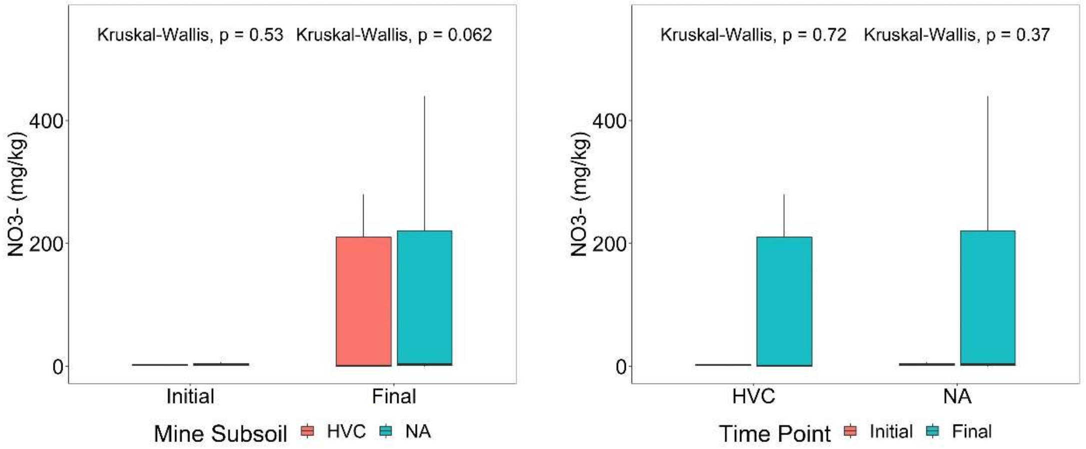

Figure 2.30 *Two mines' subsoil NO_3^- content comparison in the initial and final stages of the experiment (left), comparison of the NO_3^- content in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

In the case of NA and the case of HVC subsoil treatments the final NH_4^+ contents were statistically not equal (Figures 2.31). In both cases, biosolids-containing treatments had the largest NO_3^- contents. NO_3^- contents seemed to increase throughout the experiment (Figures 2.31).

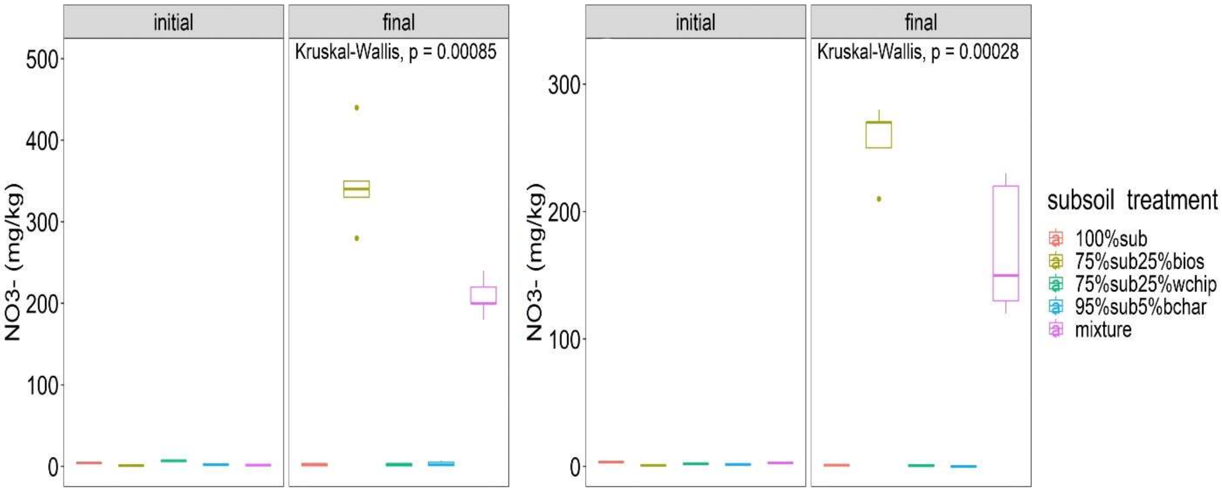

Figure 2.31 *New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments NO_3^- contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 5*

Section 3: Available Nitrogen Content – NH_4^+, NO_3^-; Sum and Ratio

There was no significant difference between the sums of NH_4^+ and NO_3^- in the initial and final stages of the experiment at α= 0.05. However, at α= 0.1 the difference was significant (Figure 2.32).

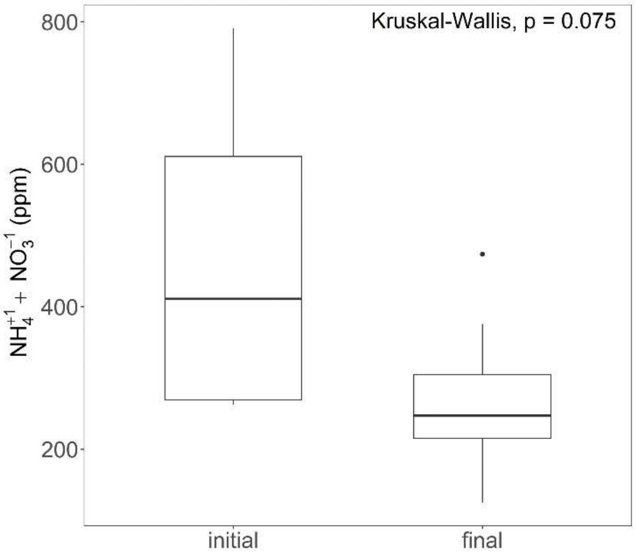

Figure 2.32 *Comparison of the sum of* NH_4^+ *and* NO_3^- *in the initial and final stages of the experiment. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 4, final n= 20*

There was a significant difference between the ratios of NH_4^+ to NO_3^- in the initial and final stages of the experiment at α= 0.05 (Figure 2.33).

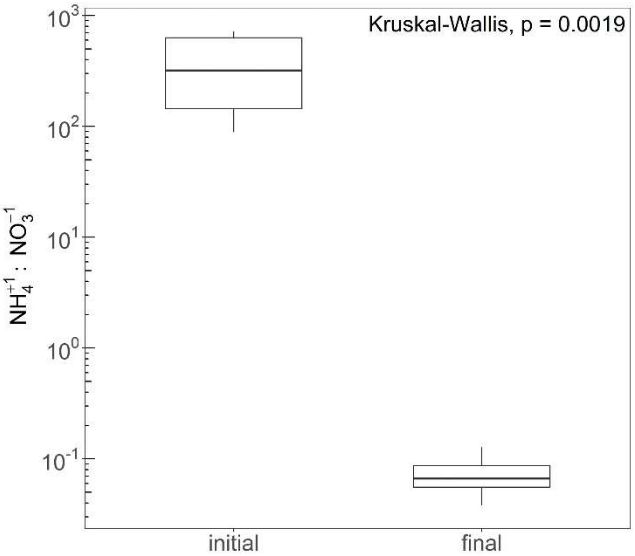

Figure 2.33 *Comparison of the ratio of* NH_4^+ *to* NO_3^- *in the initial and final stages of the experiment. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 4, final n= 20*

DISCUSSION

Results demonstrated that New Afton New Gold and Teck Highland Valley Copper subsoils differed strongly in terms of many physicochemical properties. Differences in physicochemical properties resulted in a broad variability in plant responses such as mortality and above-ground biomass productivity on these two mines' subsoil combinations. The influence of individual soil amendments on subsoil properties also differed strongly.
The nitrogen analyses confirmed that this element's presence and transformation have great importance on the reclaimed subsoil metamorphosis and vegetation response.

Mortality, Productivity, Overall Subsoils' Difference

Mortality

Results showed that the more biosolids added, the higher mortality. Biosolids-free subsoil combinations resulted in significantly lower mortality rates (See Figures 2.2, 2.3). Biosolids contain large amounts of organic but also inorganic, readily available nitrogen (Sullivan et al., 2015; Brown and Chaney, 2016). Nitrogen is essential to plant development, however, overfertilization might be harmful to vegetation by increasing tissue concentration to toxicity levels (Elhanafi et al., 2019). Additionally, overfertilization enhances negative interactions between soil microbial species leading to decrease on biodiversity and elimination of some microbial functions (Ratzke et al., 2020). Thorne et al. (1998) found that overdosing of nitrogen-rich organic amendments can be harmful to arbuscular mycorrhizal fungi. Subsoil, due to low organic matter content, has low potential to bond nutrients such as nitrogen compounds causing vegetation being exposed to high concentration of inorganic nitrogen when large amounts of organic amendments are applied. Castillejo and Castello (2010) pointed out the risk of over-fertilization as an important issue in applying organic amendments to degraded quarry soils. Fenn et al. (1998) reported that observed forests experienced increased mortality and decline in productivity as a result of excessive nitrogen input.

The mortality of the plants growing on New Afton New Gold subsoil treatments was significantly higher than plants growing on Teck Highland Valley Copper subsoil treatments (See Figure 2.1). In terms of mortality rate all applied species except prairie junegrass performed significantly worse on New Afton New Gold than on Teck Highland Valley Copper subsoil treatments (See Figure 2.4). This difference might be associated to the large difference in physicochemical properties of these two mine subsoils. New Afton New Gold subsoil might be named saline and sodic due to high concentrations of salts and sodium, while Teck Highland Valley Copper subsoil is not burdened with this problem. Hanay et al. (2004) demonstrated that vegetation on degraded soils might suffer from high soluble salts and exchangeable sodium content. They proposed gypsum application coupled with organic soil amendments as possible remedy.

Plant mortality in experiment 1 was increased by the infestation of the plant pest fungus gnat. The larvae of this insect feed on dead matter, fungi thallus, but also on tiny living plant roots (Cransaw and Cloyd, 2021). Indeed, the insect attacked in particular these plants which were growing on subsoils amended by biosolids solely or by a mixture, finding in these treatments the best food base. It was noticeable that the more biosolids, the greater the infestation. Additionally, the insects selected those pots in which field locoweed seedlings germinated. Attacked young plants of this species got destroyed quickly after germination causing larger mortality of this species seedlings (See Figure 2.5). Graminoids seemed to be not selected by insects that much. Especially prairie junegrass was avoided by fungus gnats what resulted in significantly larger survivorship of this species individuals growing on New Afton New Gold subsoil treatments (See Figure 2.5). Combinations containing biosolids (lots of SOM and nutrient), field locoweed (preferred plant), and New Afton New Gold subsoil were visibly attacked more than the same combinations but containing Teck Highland Valley Copper subsoil. This is another indicator that subsoils from those two mines differ. The insect control measures gave an unsatisfactory and short-lived effect.

Productivity

The dry above-ground biomass productivity achieved by all plants growing on Teck Highland Valley Copper subsoil combinations was over three times larger than the one achieved by all plants growing on New Afton New Gold subsoil combinations (See Figure 2.7). That demonstrated that physicochemical properties of New Afton New Gold subsoil posed a hindrance for vegetation striving to develop. Plants growing on Teck Highland Valley Copper subsoil combinations did not have to overcome such obstacles to perform. That explains why the above-ground biomass productivity of plants growing on Teck Highland Valley Copper subsoil combinations was significantly larger (See Figure 2.6).

It is striking that, despite the highest mortality rates, biosolids-containing subsoil treatments from both mines were more productive than biosolid-free subsoil treatments.

Biosolids contain large amounts of nutrients in forms ready to take up (Sullivan et al., 2015). Thus, plants growing on subsoils with addition of biosolids reacted immediately (Larney and Angers, 2012). Applied biosolids were provided straight after production, not composted prior to delivery and application. This amendment at the moment of application had an uncured form. Despite the fact that the most of total nitrogen is still in organic form, biosolids in un-composted form contain and release also available nitrogen giving an outburst of fertility (Senesi and Loffredo, 1999; Paschke et al. 2005). Results showed that within a short term of the experiment plants response to organic soil amendments were significant only to this treatments which obtained biosolids. Larney et al. (2000) demonstrated that an addition of organic amendment (manure) rich with available nitrogen and phosphorus to reclaimed soil increased the yield much more than the additions of topsoil or fertilizer. The single addition of organic amendment kept bringing better results over topsoil and fertilizer in terms of the productivity even 16 years after first application (Larney et al. 2009). Shrestha et al. (2009) demonstrated that after 5 years from application nutrient-rich cow manure resulted with significantly larger above-ground biomass productivity on reclaimed coal mine sites than nutrient-poor oat straw. This study presented similar results as an addition of the nutrient-rich biosolids resulted with significantly larger above-ground biomass productivity than nutrient-poor woodchips and biochar (See Figures 2.8, 2.9).

In the case of both Teck Highland Valley Copper and New Afton New Gold subsoils the treatment amended by a mixture of soil amendments resulted in significantly larger above-ground biomass productivity than all other treatments (See Figures 2.8, 2.9). The “mixture” treatment contained biosolids but in lower concentration (10% v/v) than the “75%sub25%bios” treatment (25% v/v). Albornoz (2016) states that high nitrogen fertilization rates are detrimental for crop yield. It seems that the addition of biosolids in a dose of 10% v/v, as in the “mixture” treatment, did not cause the overfertilization problem unlike 25% v/v of “75%sub25%bios” treatment. Additionally, other organic amendments acted in protective way. The addition of woodchips and biochar blended into the mixture bettered the soil physicochemical properties facilitating the plant development. Both woodchips and biochar have potential for exchangeable binding with mineral nutrients slowing down its availability and eventual release. The slower release of nutrients such as nitrogen is important in establishing viable and resilient plant communities on reclaimed disturbed sites (Claassen and Carey, 2007). de Varennes and al. (2010) states that combinations of amendments may work better than each applied singly. In a blend more reluctant to decomposition amendments may provide nutrients and carbon slowly but long lasting, while readily decomposable amendments can act intensively but for shorter time initiating the nutrients cycling (Larney and Angers, 2012).

Overall Difference of Both Mines' Subsoil Physicochemical Properties

Principal Component Analysis confirmed clearly that in terms of physicochemical properties the subsoils from Teck Highland Valley Copper and New Afton New Gold mines differ strongly (See Figure 2.10).

Datapoints representing "75%sub25%bios" as well as the "mixture" treatments tended to cluster together and separately from other treatments which mean that they possessed their specific features different from other treatments. All biosolid-free treatments tended to cluster together which means that an addition of 5% biochar or 25% woodchips did not change enough to cause a distinct difference in terms of physicochemical properties (See Figure 2.11).

Physicochemical Properties of Mines' Subsoils When Unamended and Amended

pH

Both mine subsoils are alkaline. New Afton New Gold unamended subsoil pH (9.3) was much higher than Teck Highland Valley Copper's one (8.50). The difference between both mines' subsoil treatments in terms of pH was statistically significant (See Figure 2.12). Majority of plants perform the best in neutral or slightly acidic pH. That results from nutrients solubility which is optimal in such conditions (Rodriguez, 2020). Elevated pH, especially in the case of New Afton New Gold subsoil, could be a reason of lower above-ground biomass productivity and elevated plant mortality rate. Brown and Chaney (2016) point out the substrate pH as critical variable for metal uptake, availability, and toxicity. While most of heavy metal solubility is limited by high pH, molybdenum acts conversely. Solubility of this element increases along with pH increase (Kaiser et al. 2005; Brown and Chaney, 2016). The Teck Highland Valley Copper subsoil molybdenum content exceeds the CCME guidelines for agricultural, residential and parkland use (CCME, 2021). However, the Teck Highland Valley Copper subsoil's pH was closer to neutral what might result in lesser mobility of molybdenum.

Throughout the experiment pH seemed to drop overall. However, the change was not statistically significant either in the case of New Afton New Gold or Teck Highland Valley Copper subsoils. It seems that 16 weeks of the experiment duration may be a too short period to allow for significant pH change (See Figure 2.12).

Soil amendment addition might influence the substrate pH. The extent of pH change mainly depends on the applied amendment features (Larney and Angers, 2012). That applies to organic amendments as well. Lower pH was adopted by these mine subsoil treatments which contained biosolids. That is because the innate pH of this material is around 8.0 - 8.5. Biosolids contain lots of NH_4^+. The acidifying reaction occurring is $NH_4^+ + H_2O \rightleftharpoons NH_3 + H_3O^+$. The pH of biosolids-containing treatments also dropped the strongest over the time of the experiment (See Figure 2.13). That is attributed to the oxidation of organic N and S compounds (Sullivan et al. 2015). At the same time, the pH of all other subsoil treatments which did not contain biosolids remained high or dropped only slightly.

It is worth adding that the pH of New Afton New Gold undisturbed topsoil was just slightly above 7.0. That indicates that local ecosystems naturally tend to neutral pH. In the case of Teck Highland Valley Copper undisturbed topsoil, the pH was 5.5. That indicates that local flora naturally tends to decrease the pH to acidic levels.

Electroconductivity (EC)

Soil electroconductivity strongly depends on the soluble salts concentration in the soil water solution. In the soil water solution soluble salts are present in a form of inorganic ions (McLachlan et al., 2004). Miller at al. (2017) specify that soluble cations and anions Na, K, Ca, Mg, SO_4-S, Cl are responsible for EC readings. Soils suffering from salinity demonstrate high electroconductivity readings.

While New Afton New Gold and Teck Highland Valley Copper undisturbed topsoils seemed not to differ much in terms of electroconductivity, the ECs of those two mines' subsoils differed hugely (See Figure 2.14). New Afton New Gold subsoil treatments EC median was within the interval 2.5 – 3.0 mS/m, while Teck Highland Valley Copper one's placed within the interval 0.3 – 0.6 mS/m. That is a very large difference indicating that New Afton New Gold subsoil was burdened with a salinity problem. Electroconductivity indicates the level of ions concentration. The higher EC reading the more ions in the soil water solution. This indicator though does not specify which ions are present in the solution. Some ions are desirable being nutrients for plants and microorganisms, while other ions are toxic or act somehow negatively. New Afton New Gold subsoil must contain a significant amount of ions which increased strongly the EC, concurrently suppressing the plants' development.

The highest EC readings of Teck Highland Valley Copper subsoil treatments were lower than the lowest readings of New Afton New Gold subsoil treatments (See Figure 2.15). That strongly suggests that New Afton New Gold subsoil treatments contained lots of adversely-acting ions, while Teck Highland Valley Copper subsoil either did not possess those ions at all or they were present but at the inconspicuous levels. Soil organic amendments may alleviate the salinity problem. Zeynep (2020) successfully used vermicompost to reclaim sodium affected soil. Miller (2017) proposed woodchips as an amendment lowering excessive EC together with associated with it high pH.

It may be noticed as well that throughout the experiment, the electroconductivity dropped (See Figure 2.14). However, the drop was not large enough to cause the difference between the initial and the final electroconductivity readings to be statistically significant. Finally, all that means that the change of reclaimed subsoil in terms of EC is relatively a slow process.

Soil Organic Matter (SOM)

Soil organic matter plays a pivotal role in soil (Brown and Chaney, 2016). Content of this soil fraction decides about such soil features as productivity, nutrient preservation and cycling, water holding capacity, soil microbial activity and many others (Larney and Angers, 2012). Because SOM plays such a great role, mine reclamation efforts need to concentrate on rebuilding this fraction in the top horizon. Organic amendments once again seem to be best tool to achieve this goal. Larney and Angers (2012) state that on degraded substrates an one-off large applications of organic amendments can boost reclamation and lead to sustaining net primary productivity. Gardner et al. (2010) demonstrate that at reclaimed copper mine tailings sites in British Columbia an application of biosolids was more efficient in terms of restoring soils with its functions and productivity than the use of traditional fertilizers. Reid and Naeth (2005) report that an application of organic amendments such as biosolids or composted paper mill sludge gave better results than fertilizers when attending to establish vegetation cover on tundra kimberlite mine tailings in Northwest Territories, Canada. Winter Sydnor and Redente (2002) reported that soil amelioration with organic matter resulted in significant increase of above-ground biomass in reclamation of a high elevation gold mine in Colorado.

Fierro et al. (1999) applied paper sludge supplemented with mineral nitrogen and phosphorus. In their reclamation attempt on an abandoned sandpit they adopted surrounding non-degraded land as a reference. They reported that in the second season such measures as soil carbon and nitrogen neared the natural system levels. In the case of both New Afton New Gold and Teck Highland Valley Copper, the undisturbed topsoil organic matter content measured for this study was near to 4%. Unamended New Afton New Gold subsoil SOM was 3%, constituted though only 3/4 of its topsoil's, while in the case of the Teck Highland Valley Copper unamended subsoil SOM was 1%, constituted therefore not more than 1/4 of its topsoil reference. After amendments addition to mine subsoils the amount of SOM increased, whereof the "mixture" treatment SOM departed the least from its reference of undisturbed topsoil in the case of both mines.

Organic matter provided by organic amendments may have many various forms. Biosolids provide predigested organic matter, well shredded, easy in terms of cation exchange, and prone to further decomposition (Senesi and Loffredo, 1999; Sullivan et al., 2015), while woodchips are built from coarse, not predigested, and difficult for decomposition pieces (Senesi and Loffredo, 1999). Biochar, in turn, is formed of organic matter deeply transformed by high temperature and pressure. Such processes transform organic matter into a material that is reluctant for decomposition (Taylor P, 2010; Canadian AgriChar, 2020).

75%sub25%bios" and "mixture" treatments were the richest in terms of soil organic matter content in the case of both mines' subsoils (Figures 2.17). Concurrently, the same two treatments showed up as the most productive. The treatment "75%sub25%wchip" contained fair amount of organic matter as well. However, this treatment did not result with significant above-ground biomass productivity. The explanation of this discrepancy is that woodchips are mainly built from cellulose and lignin. Both are hard for decomposition, especially lignin (Senesi and Loffredo, 1999; Datta et al., 2017). The "mixture" treatment contains both easy for decomposition and recalcitrant organic matter. This treatment brought the best results in the case of both mines' subsoils. Many researchers suggest blending organic amendments in order to achieve better and longer lasting results (Claassen and Carey, 2007; de Varennes et al. 2010; Larney and Angers, 2012). Cheng (2008) concludes that saw dust gives good results, but when incorporated with suitable amounts of clay, ammonium nitrate and organic amendments.

Basic Elements

Aluminum is one of the most prevailing elements in the Earth's lithosphere (Weil and Brady, 2017). However, it is not important in either plant or animal nutrition. Moreover, large concentrations may cause negative consequences. Its activity increases though only in a low pH environment (Haynes and Mokolobate, 2001). In a higher pH environment aluminum is harmless. New Afton New Gold subsoil, as well as topsoil, contained significantly more aluminum than Teck Highland Valley Copper (See Figure A.4). Nonetheless, regarding the high pH, such concentrations of aluminum should not have any influence on biomass productivity or plant mortality.

In the case of both mines' subsoils, boron content strongly exceeded the CCME guidelines for agricultural land use (CCME, 2021). Boron is a micronutrient for plants. That means that low concentrations of this element are necessary for proper plant development. Biosolids is a good source of boron (Sullivan et al., 2015). However, in larger concentration boron may act negatively on plant development. New Afton New Gold subsoil had significantly larger boron content (See Figure A.5) which may be one of the causes of lower New Afton New Gold subsoil biomass productivity. The boron concentration in NA subsoil and its influence requires more studies.

Calcium is one of the most important nutrients and its roles for plants and animal life is countless (Weil and Brady, 2017). Deficiency of this element may cause improper plant tissue development, cellular membranes deterioration, necroses. Calcium is also responsible for proper soil structure formation. Additionally, Ca has the pH elevation ability (Troeh and Thompson, 2005). The larger concentration of calcium in the New Afton New Gold subsoil (See Figure A.5), may constitute one of the explanations of higher pH and EC. However, both mines' subsoil calcium concentrations seemed to be rather in a norm, and not requiring any additional supplementation.

In the case of both mines' subsoils, copper content exceeded the CCME guidelines for all types of land use (CCME, 2021). Additionally, biosolids provides an additional portion of Cu (Sullivan et al., 2015). Teck Highland Valley Copper subsoil excess of copper content was outstanding. Copper is a heavy metal but also a microelement necessary in plant metabolism. Cu is a cofactor in many enzymes, however in high concentrations might be toxic especially to microorganisms (Trevors and Cotter, 1990). Although the HVC subsoil copper content was significantly larger than the NA subsoil's content (See Figure A.5), and exceeded remarkably the CCME guidelines, it all seemed to not have any negative influence on plants growth and health. Alkalinity of the substrate poses a hindrance for plant Cu uptake (Rodriguez, 2020). High NA subsoil pH could lead to Cu deficiency despite the fact that the NA subsoil had larger Cu content than needed. The copper concentration in NA as well as in HVC subsoils and its influence requires more studies.

Iron plays many important roles in plant physiology (Imsande, 2002). It is worth emphasizing that iron is an intrinsic part of the nitrogenase enzyme. The concentration of iron seemed to be sufficient in the case of both mines' subsoils and having no significant influence on biomass productivity. However, alkalinity of the substrate poses a hindrance for plant Fe uptake (Rodriguez, 2020). High NA subsoil pH could lead to Fe deficiency despite the fact that the NA subsoil had large Fe content. The iron uptake on the NA subsoil requires more studies.

Potassium is one of the most important plant macronutrients (Weil and Brady, 2017). Canadian AgriChar (2020) declares that their biochar is a good source of K. The concentration of potassium in the case of the New Afton New Gold unamended subsoil was low in comparison to New Afton New Gold undisturbed topsoil. Also, there was a strongly significant difference between the NA subsoil and HVC subsoil in terms of potassium content (See Figure A.6). NA subsoil seemed to be lacking with potassium more than the HVC subsoil, which in turn could be one of the reasons why the NA subsoil is less productive. Annacis biosolids were also a good provider with K. This amendment contains around 1,100 mg/kg of an available K in the dried weight.

Magnesium is another important plant nutrient (Shaul, 2002). It plays a crucial role in many processes, for example, photosynthesis by co-building chlorophyll (Madigan et al., 2012). Magnesium concentration was significantly larger in the case of the New Afton New Gold subsoil (See Figure A.6). However, a lower concentration of magnesium in the case of Teck Highland Valley Copper subsoil seemed to not act adversely on plant development.

Manganese is one of the micronutrients. It plays a pivotal role in the reactive centers of many enzymes and chlorophyll (Rodriguez, 2020). Plants may suffer either from deficiency or excess on Mn (Campbell and Nable, 1988). New Afton New Gold subsoil contained significantly larger amounts of manganese (See Figure A.7). However, alkalinity of the substrate poses a hindrance for plant Mn uptake (Rodriguez, 2020). High NA subsoil pH could lead to Mn deficiency despite the fact that the NA subsoil had sufficient Mn content. The influence of Mn in NA subsoil requires more research.

Molybdenum is a heavy metal. Its excess may cause negative consequences on plant development as well as on their consumers. At the same time, molybdenum is also a micronutrient playing a role in many enzymes including nitrogenase (Kaiser et al. 2005). Sullivan et al. (2015) states that biosolids is a good source of this element. Teck Highland Valley Copper subsoil contained significantly more molybdenum than New Afton New Gold subsoil (See Figure A.7). Moreover, the HVC subsoil molybdenum concentration exceeded CCME guidelines for agricultural and parkland land use, but did not exceed for industrial and commercial uses (CCME, 2021). The above indicates that HVC subsoil molybdenum concentration was high but bearable for vegetation.

Sodium is sometimes accounted as a micronutrient. It plays a role in osmotic regulation in tissues because sodium dissociates in water to cations which have a positive charge. Accordingly, when placed on one side of a cellular membrane, this creates a charge potential (Subbarao et al. 2003). Besides its certain roles in living organisms and its non-toxicity, sodium may act adversely on plants and soil-inhabiting organisms. When the concentration of sodium is elevated it leads to the sodic soil problem (Hanay et al., 2004, Clancy, n.d; FAO et al., 2020). First of all, sodium is an antagonistic cation to calcium cation. Calcium plays an important role in the formation of proper soil structure. While sodium overcomes calcium, the soil becomes compacted, difficult for water, and oxygen penetration which eventually acts negatively on plants' root systems (Hanay et al., 2004; Clancy, n.d; FAO et al., 2020). New Afton New Gold subsoil contained several times more sodium than Teck Highland Valley Copper subsoil (See Figures 2.18, 2.19), and indeed during the experiment it was observed that New Afton New Gold subsoil combinations in pots did compacted and did not drain water properly. This element's cations are also highly soluble in water and it is expected that those cations were standing behind the high electroconductivity readings of all NA subsoil combinations. Sodium cations tend to increase pH and this may be also one of the explanations for the NA subsoil elevated pH. When comparing two mines' subsoil departures from its references in terms of sodium content it may be noticed that NA undisturbed topsoil had a pretty low content of sodium in comparison to NA subsoil, while HVC topsoil sodium content was similar to the subsoil's one. Sodium content is one of the largest differences between NA and HVC subsoils.

Phosphorus is one of six biogenic elements. On the molecular level, it consists part of many crucial molecules including DNA or ATP (Madigan et al., 2012). Often soils require supplementation with this element. Sullivan et al. (2015) states that biosolids is a good source of this plant and microorganism essential nutrient. Annacis biosolids contain around 1,900 mg/kg of an available P in the dried weight. New Afton New Gold unamended subsoil contained around three times more phosphorus than the unamended Teck Highland Valley Copper subsoil (See Figure A.7). Limited availability of phosphorus, alike in the nitrogen case, may suppress the vegetation development. However, an addition of biosolids applied with rates adjusted to the nitrogen levels usually cover the phosphorus needs as well (Sullivan et al., 2015). Similar to nitrogen, treatments amended by 25% of biosolids could obtain even too large portion of this element at once which might act rather adversely than positively.

Sulfur is acknowledged as the last biogenic element. This element constitutes part of many vitamins or proteins, therefore, it co-builds enzymes as well (Madigan et al., 2012). Together with iron and molybdenum, sulfur co-builds molecular structures thanks to which nitrogenase can actively fix atmospheric nitrogen (Tanifuji K, Ohki Y. 2020). Even though there may be plenty of sulfur in the soil profile, its elemental form is hydrophobic and unavailable for plants. Soil microorganisms must transform this form before being taken up by plants (Fuentes-Lara et al., 2019). One of the sulfur significations is the fact that this element takes part in the regulation of nitrogen uptake (Salvagiotti et al., 2009). New Afton New Gold unamended subsoil contained a significantly larger amount of sulfur than Teck Highland Valley Copper unamended subsoil (See Figure A.8). Again, an addition of biosolids applied with rates adjusted to the nitrogen levels usually cover the sulfur needs as well (Sullivan et al., 2015). It seems though that plant demand for sulfur is covered by both mine subsoils sufficiently. However, sulfur provided by subsoil may be unavailable or even toxic for plant roots. In terms of sulfur provision, it is important to restore proper soil microflora. By that time elevated sulfur content may act rather adversely than positively.

The last of the analyzed elements - zinc is one of the very important micronutrients. Hundreds of enzymes contain Zn (Weil and Brady, 2017). The deficiency of Zn in plants causes chlorosis and leaf stunting. Nonetheless, zinc is heavy metal as well, therefore, an excess of this element may cause poisoning (Broadley, 2007). When comparing both mines' undisturbed topsoils it is noticeable that both contained similar amounts of zinc. Unamended subsoils contained more zinc than their relevant topsoil, however, the content did not exceed CCME guidelines (CCME, 2021). An elevated amount of zinc, not exceeding the CCME guidelines, came with an addition of biosolids which is a good source of this element (Sullivan et al. 2015). Nonetheless, unamended subsoils contained amounts of zinc similar to the undisturbed topsoil, therefore, it seemed that subsoil alone constitutes sufficient provision of this element without additional supplementation. However, alkalinity of the substrate poses a hindrance for plant Zn uptake (Rodriguez, 2020). High NA subsoil pH could lead to Zn deficiency despite the fact that the NA subsoil had sufficient Zn content. The influence of Zn in NA subsoil requires more research.

Total Carbon

Restoration of carbon cycling is essential in mine reclamation from the perspective of successful revegetation (Larney and Angers, 2012). Soil organic matter contains most of soil carbon. Many researchers proposes organic amendments as the effective tools to rebuild soil organic carbon pool. Shrestha et al. (2009) when conducting experiment on coal mine sites found that the treatment with cow manure was the best in terms of bettering many of substrate properties including the pool of organic carbon. Tian et al. (2009) demonstrated that a long-term application of biosolids to calcareous strip-mined land brough by far better results in terms of soil organic carbon buildup than traditional fertilization.

If comparing two mines ’unamended subsoils in terms of total carbon content it may be noticed that the New Afton New Gold subsoil contained slightly more than 1%, while Teck Highland Valley Copper subsoil contained less than 0.5% (See Figures A.2, A.3). Both readings were low. The pool of organic carbon increased after soil amendments addition.

Even the proper provision of carbon but only carbon is still not sufficient to ensure sustainable vegetation. Such soil amendments as woodchips and biochar provided carbon in decent amounts whereas the above-ground biomass production on such treatments remained still poor. Additionally, the form of provided carbon (easy or reluctant to biological processing) plays a pivotal role. The “mixture” treatment provided both readily degradable and recalcitrant carbon. The complex provision of carbon by the “mixture” treatment might be one of the reasons why this treatment achieved the best results in terms of the above-ground biomass production. Recalcitrant carbon contained in woody amendments will exert long-lasting effect on reclaimed substrate (Senesi and Loffredo, 1999), but for better results should not be applied solo.

Quantitative and Qualitative Nitrogen Content Change

Total Nitrogen

Nitrogen fulfils countless biochemical and physiological functions and is one of the most essential resources for plants (Geng and He, 2020). For its proper development plants require large amounts of N to be supplied (Leghari et al., 2016). Deficiency of nitrogen cause number of malfunctions such as impaired growth, leaves decolouration and chloroses, reduction in flowering and others (Silva and Uchida, 2000). Same as deficiency, the excess of nitrogen can act adversely on plant growth (Fenn et al., 1998, Elhanafi et al., 2019). That refers to reclaimed lands as well (Castillejo and Castello, 2010).

If comparing two mines 'unamended subsoils in terms of total nitrogen content it may be noticed that the New Afton New Gold subsoil contained slightly more than 0.02% (See Figure 2.22), while the Teck Highland Valley Copper subsoil content was below the detection level (See Figure 2.23). Both readings were extremely low. In the case of both mines' referencing topsoils, the total nitrogen content levels were pretty similar to each other and placed around 0.15%. Regarding the above, both unamended subsoils contained by far less total nitrogen than their references (See Figures 2.24, 2.25). On such substrates the vegetation suffers sever nitrogen deficiency. To remediate that problem reclamation researchers propose fertilization, but rather by nitrogen-rich organic amendments than by traditional fertilizers (Bradshaw, 1997; Chambers et al., 2002; Reid and Naeth, 2005; Gardner et al., 2010). Larney et al. (2000) states that the greater the degradation, the better plant response to organic amendments. Various organic amendments were proposed as a source of nitrogen. Some proposed cattle manure (Larney and Janzen 1997; Shrestha et al., 2009), but more recently, researchers attention is turned toward biosolids (Gardner et al., 2010; Sullivan et al., 2015). The biosolids application rates depend on substrate nitrogen supplementation need (Sullivan et al., 2015). Annacis biosolids contain around 5.5% of total nitrogen in the dried weight.

Both initially and finally New Afton New Gold subsoil treatments' total nitrogen content was significantly larger than Teck Highland Valley Copper one (See Figure 2.20). That was because the NA unamended subsoil contained more N overall, however, even NA unamended subsoil total nitrogen content was low. If comparing the initial and the final total N content it may be noticed that they did not differ statistically either in the case of NA subsoil or in the case of HVC. However, in both cases, the final total nitrogen content was lower than the initial one (See Figure 2.21). That suggests that nitrogen gets depleted or lost over time, but the 16 weeks of the experiment duration is too little time to capture that process statistically. That is good information meaning the loss of nitrogen occurs relatively slowly. That gives time to plants and soil microorganisms to acquire more nitrogen and import it to the cycling. Larney et al. (2009) reported the positive effect of nitrogen-rich cattle manure being present 16 years after one-off application.

It is noticeable that the largest provision of total nitrogen was present in subsoils treated by 25% of biosolids. Treatments amended by mixtures came as second. Those two treatments provide a statistically significant amount of total nitrogen when comparing to biosolids-free treatments (See Figures 2.22, 2.23). Other treatments did not provide much nitrogen at all. The "mixture" treatments resulted as the most productive. Its total nitrogen contents were in the interval 0.1% - 0.2%. The "75%sub25%bios" treatment's total N median reached initially nearly 0.6% in the case of HVC and even exceeded 0.6% in the case of the NA. These results indicated that total nitrogen content above 0.2% might start acting adversely. The top level of total nitrogen when it is still harmless to vegetation depends on other factors such as quantity and quality of soil organic matter (Elhanafi et al., 2019).

It is notable that only in the case of both mines' subsoil "mixture" treatments the differences from the references (topsoils) in terms of total N content were not statistically significant (See Figures 2.24, 2.25). That indicates that only these treatments simulated the natural conditions in terms of total nitrogen content.

Mineralizable Nitrogen

Mineralizable nitrogen indicates how much of nitrogen tied up in complex organic residues could be potentially mineralized by soil microbial community to the plant available form of ammonium. Mineralizable nitrogen constitutes a pool of this intrinsic element which is gradually released throughout decomposition. This process depends on an abundance and activity of certain soil microorganisms. Thus, plants and soil microorganisms get provided with required inorganic nitrogen for a prolonged time. Mineralizable nitrogen is a significant fraction of the total nitrogen (van Es et al., 2017). Nitrogen-rich organic amendments, such as biosolids (Sulivan et al., 2015) are good source of mineralizable N. Chambers et al. (2002) reported that the use of biosolids and other organic materials when attempting to reclaim a landfill site in the United Kingdom resulted in eventual increase of readily mineralizable organic nitrogen content.

In this study the amount of mineralizable N dropped throughout the experiment in the case of both mine subsoils (See Figures 2.26, 2.27). Lost mineralizable nitrogen either got mineralized or washed away during watering. In the both mine cases the initial mineralizable nitrogen content did not differ statistically from the final mineralizable nitrogen at $\alpha= 0.05$ (See Figure 2.26). That means that mineralization/loss occurred relatively slowly. The mineralization rate depends among others on the climatic conditions, the microbial community composition, type of substrate and type of soil amendment applied. Cordovil et al. (2007) after conduction of potting experiment with municipal solid waste compost, secondary pulp mill sludge, horn meal, poultry manure, solid phase from pig slurry and composted pig manure reported that poultry manure was the fastest mineralized organic amendment. However, too fast mineralization is unwanted. Organic nitrogen mineralization supposed to be relatively slow and steady (Claassen and Carey, 2007) to help plant and microorganisms benefit the most from released available nitrogen and to limit nitrogen loss by leaching (Brown and Chaney, 2016).

Those subsoil treatments that contain more readily degradable organic matter hold at the same time more mineralizable nitrogen. Regarding the above, here again, those subsoils that were amended by biosolids alone or in a mixture contained noticeably more mineralizable nitrogen than subsoil treatments unamended or amended by biosolids-free amendments (See Figure 2.27).

Initially, the treatments containing 25% of biosolids stood out with an amount of mineralizable nitrogen. In such cases, readings indicated the concentration of mineralizable nitrogen exceeding 1,000 mg/kg. Treatments amended by a mixture of amendments (biochar 5%, biosolids 10%, woodchips 10%) contained much less of mineralizable nitrogen, which was around 450 mg/kg (See Figure 2.27).

Available Nitrogen Forms: NH_4^+ and NO_3^-

Inorganic N includes soluble forms (NO_2^- and NO_3^-), exchangeable NH_4^+, and clay-fixed nonexchangeable NH_4^+ (Rutherford et al., 2008). Therefore, readily available inorganic nitrogen comprises soluble nitrate, nitrite, and exchangeable ammonium. However, the fixed nonexchangeable NH_4^+ starts being slowly released when the pool of available ammonium becomes depleted (Drury and Beauchamp,1991). Available $NO_3^-$and NH_4^+ are among the most essential plant nutrients (Geng and He, 2020). Available NH_4^+ mainly results from organic matter first decomposition and then mineralization (Soon and Liang, 2006).

NO_3^- though in the soil results from the nitrification process conducted by *Nitrobacter*, *Nitrospira*, and *Comammox* bacteria (Madigan et al., 2012). Therefore, in natural soils, the available nitrogen is a fraction of mineralizable nitrogen being released to the soil gradually. However, in the case of an amending with biosolids, the provision with available nitrogen is instant as this amendment contains large amounts of available NH_4^+ (Sulivan et al., 2015). Annacis biosolids contain around 9,500 mg/kg of NH_4^+ and 12.2 mg/kg of NO_3^- in the dried weight. Results of this study are consistent with above as initially measured pool of available nitrogen provided by biosolids was nearly entirely comprised of ammonium (See Figures 2.28, 2.30).

In the case of both mines, the unamended subsoils were poor with available nitrogen (See Figures 2.29, 2.31). It is not surprising because those substrates did not contain enough organic matter for decomposition and mineralization (See Figure 2.17). Among all three amendments applied in this study a significant amount of available nitrogen was provided only by biosolids, and that was mainly in a form of NH_4^+. Initially the quantity of NH_4^+ was several hundred times larger than NO_3^- (See Figure 2.33). Geng and He (2020) collected 240 field soil samples and measured $NH_4^+ + NO_3^-$ and $NH_4^+ : NO_3^-$. Maximum sum they found was 80 mg/kg, while the ratio was ranging from 100:1 to 1:22.16. For their further experimental proceeding they adopted 36 mg/kg as a high amount of available nitrogen. In this study the maximum sum was near to 800 mg/kg in the case of NA "75%sub25%bios" treatment, while the ratios were ranging from 800:1 to 100:1. In the case of both mine subsoils amended by 25% of biosolids v/v the amount of available nitrogen (mainly NH_4^+) at initial stage of the experiment highly exceeded values which Geng and He (2020) adopted as high. Throughout the experiment though the sum $NH_4^+ + NO_3^-$ dropped (See Figure 2.32), and the ratio $NH_4^+ : NO_3^-$ changed drastically (See Figure 2.33). The ratio converted to nitrate-prevailing over ammonium. This is a result of nitrification. It might indicate that nitrifying bacteria in the reclaimed substrate have become strongly active. On the other side, the drop in sum was not statistically significant at $\alpha=0.05$. That means that the loss of available N forms is relatively slow and vegetation together with microorganisms can profit from available NH_4^+ and NO_3^- for prolonged time.

High levels of available N in the case of both "75%sub25%bios" and "mixture" suggest overfertilization when compared with figures quoted by Geng and He (2020). While it is likely true in the case of the "75%sub25%bios" treatment, the "mixture" treatments' $NH_4^+ + NO_3^-$ levels were below 300 mg/kg. That is still much more than the level which Geng and

He (2020) adopted as high (36 mg/kg), however, they based that value on field soil samples (not reclaimed substrate as in this study). And in the nature the soil inorganic N is among the most limiting nutrients (Geng and He, 2020).

Field Locoweed Performance

Although field locoweed individuals seemed to develop well on some subsoil treatments, at the end of experiment 1 the nodulation was not observed neither in the case of treatments well provided with readily available nitrogen nor on subsoil treatments deprived of nitrogen. That might result from several reasons (Epp, 2015; Midwest Laboratories, 2021):

1) The symbiosis partner was absent in the substrate;
2) The symbiosis partner was inactive due to conditions such as salinity or lack of necessary nutrient;
3) The experiment timing was not proper for the symbiosis formation;
4) The experiment was too short and plants did not manage to form nodules yet.

CONCLUSION

The results identified series of findings and answers to the study objectives. First and foremost, two analyzed mines' subsoils differed strongly in terms of their innate physicochemical properties. That, in turn, affected vegetation mortality and productivity. What they had in common is that in unamended form both subsoils were highly unfertile, and due to that unproductive. Although they possessed satisfactory, or in some cases even excessive, quantity of some micronutrients, they lacked in NPK, which are essential, biogenic elements. Additionally, New Afton New Gold was encumbered with saline and sodic problems additionally hindering vegetation and microorganismal development (Miller et al., 2017). These two mines' unamended subsoils would rather not be a self-sufficient substrate for mine land reclamation when applied alone. The situation could be changed by an addition of organic amendments, however, not all of them. Results showed that an addition of biochar and woodchips alone did not change much. That stemmed from the same reason - both amendments lacked macronutrients, especially nitrogen, and readily degradable organic matter. This study results showed that the simple addition of organic matter (for example by addition of woodchips or biochar alone) is not enough. Organic matter must meet certain conditions such as appropriate degradability and satisfactory nutrient content. Woodchips and biochar can be added mixed with other amendments, but rather not on their own.

From applied organic amendments only biosolids significantly increased above-ground biomass productivity. That resulted from biosolids' large nutrient content such as N, P, and Zn, and predigested organic matter, which is more labile and easier for decomposition and carbon release.

Additionally, as an answer to the first study objective, tests appointed many more differences between the New Afton New Gold and Teck Highland Valley Copper subsoils in terms of physicochemical properties. New Afton New Gold subsoil contained significantly more Ca, but first of all Na cations. Those two elements increased the pH of New Afton New Gold subsoil to highly alkaline which caused organisms to deal with this important hurdle. Moreover, sodium can acts adversely on soil structure and soil solution osmotic features. However, sodium also tends to be washed out. Na levels declined throughout the experiment, which confirmed the washout. It can be expected that over time of reclamation, rainwater will wash away the excess of sodium, and then one of the factors limiting the vegetation growth and development would debilitate. New Afton New Gold subsoil was deprived of K. Potassium is one of the essential elements for vegetation development. Lack of K may be one of reasons for significantly lower New Afton New Gold subsoil productivity. Both subsoils contained an excess of boron, however, New Afton New Gold subsoil went beyond CCME guidance much more than the Teck Highland Valley Copper subsoil. Perhaps, the provision of sulfur was too large too, especially in the case of New Afton New Gold subsoil treatments. S in the soil can form toxic compounds. It requires additional research whether this is the case in New Afton New Gold reclaimed subsoil. Teck Highland Valley Copper subsoil contained increased amounts of Cu and Mo. While this did not affect plant health, it might step up to higher levels in the food chain and accumulate there. This requires additional research.

The best results in terms of plant above-ground biomass production were achieved when mines' subsoils were amended by a mixture of all three soil amendments. This result partially answered the second study objective. An addition of biosolids alone boosted the vegetation strongly, however, an addition of 25% biosolids volumetrically largely increased mortality and brought other signs of overfertilization. In the "mixture" treatment biosolids content was only 10%. This much seemed to cover plant demand for nutrient concurrently not increasing the mortality significantly. It also seems that an application of less stable organic amendments, especially biosolids, is less preferable than the amendments pre-composted (Fierro et al., 1999). Paschke et al. (2005) demonstrated that an addition of a uncured biosolids, by a rapid release of nitrogen, can increase a risk from the unwanted, annual weeds.

The third study objective focuses on an aspect of nitrogen cycling in reclaimed mine subsoil. Results showed clearly that whenever there was nitrogen present in the soil substrate, the biomass productivity was significantly larger than in the treatments in which N was scarce. Significantly larger productivity was also associated with larger provision of carbon. Whenever both N and C were provided in proper quantities, the biomass production was significantly greater. That statement was supported by observations done on treatments amended by biochar and woodchips. Both provided mostly recalcitrant carbon concurrently being poor in nitrogen content and both did not result in satisfactory biomass production. Even within a short time of study 1 experiment lots of nitrogen transformations were observed. Total N, mineralizable N, NH_4^+ content, and $NH_4^+ + NO_3^-$ visibly dropped throughout the experiment. However, the rate of depletion was slow enough for plants to benefit from the nitrogen provision. On the other hand at the end of the experiment, there was significantly more NO_3^- than at the beginning. That means that in the early stage of reclamation with the application of biosolids the nitrification process prevails.

Legumes are not good competitors to grasses especially on phosphorus-rich soils (Smith, 1992). In this study as well the legume field locoweed *Oxytropis campestris* did not do well in terms of competing to other grassy species on subsoils amended with biosolids, nonetheless, its performance on unamended subsoil was better than all other species. The above plus the ability to fix nitrogen makes this species potentially a good candidate to be used in mine reclamation.

Figure 2.34 *The greenhouse trial with graminoids and legume arranged in 7 blocks.*

Photo credit Piotr Dzumek

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CHAPTER 3: CHANGED SOIL AMENDMENTS PROPORTIONS AND ITS INFLUENCE ON SUBSOIL PHYSICOCHEMICAL PROPERTIES, SHRUBBY PLANTS PERFORMANCE, AND NITROGEN CYCLING IN OPEN-AIR CONDITIONS.

INTRODUCTION

In greenhouse conditions plants usually perform better (Vernon, 2019). That is because all aspects of controlled conditions in the greenhouse trials are set for constant optima, while in nature optimal conditions for plant development do not happen often. That explains why the greenhouse experiments possess limited value when extrapolating to field and operational conditions. However, if the results of experiment 2, conducted open-air way in semi-natural conditions, turn out similar to experiment 1, that would strengthen overall research results and the conclusions credibility.

The fertilization has to be done in proper doses adjusted to the nature of fertilizer, vegetation requirements, climatic conditions, substrate properties, etc. The same rule applies when soil amendments are applied. The second study used the same subsoils, the same soil amendments, but in different proportions. This intends to find an optimal dose according to Shelford's law of tolerance (Shelford, 1931).

Reclamation efforts are usually laborious and costly (Bradshaw, 2000; Prach and Hobbs, 2008). That is why mine reclamation practitioners when making decisions on choosing certain reclamation technics need to be supported by solid research results. When laboratory results are backed by experiments conducted in conditions similar to natural, such research gives a stronger basis for practical decisions. The first study has shown that the addition of biosolids in the dose of 25% volumetrically might be too much. It seemed that such a quantity of biosolids increased plant mortality and decreases productivity. A mixture of soil amendments in the study 1 experiment gave much better results than all other treatments. This way a new question emerged: whether that was caused by a lower, but still significant amount of biosolids that provided necessary nutrients in more preferred amounts, or because of the beneficial influence of other soil amendments that were applied in a mixture. To answer this question, in the second study less biosolids was applied - 5% in the case of both the mixture treatment and the treatment with biosolids applied alone. This aimed also at testing whether the lower provision of nitrogen would affect its cycling.

In local environments, shrubby plants play an extremely important ecological role, especially in the lower and middle Bunchgrass zone (Province of British Columbia, 1999).

Three shrub species were selected to the second study: common rabbitbrush *Ericameria nauseosa*, soopolallie *Shepherdia canadensis,* and big sagebrush *Artemisia tridentata*. Common rabbitbrush and big sagebrush are typical and commonly present in lower grasslands (Antos et al., 1996, Province of British Columbia, 1999), where New Afton New Gold mine is located. Soopolallie, in turn, is present in higher elevations (Walkup, 1991; Douglas et al., 1998), being common in Teck Highland Valley Copper mine vicinity. All three species are well adapted to interior BC biogeoclimatic conditions, hence, they may have the potential to be an efficient mine reclamation tool. Soopolallie is a nitrogen fixer forming actinorrhizae with bacteria from various strains of genus *Frankia* (Huguet et al., 2004). This shrub is not a part of typical Bunchgrass zone vegetation, but is commonly present in Interior Douglas-fir and Montane Spruce zones. Soopolallie is often present near water reservoirs, but it also tolerates dry areas with nutritionally poor, mineral soils (Walkup, 1991). It is a species that copes well with various forest disturbances such as fire or stand clear cut because it has the perfect ability to reproduce through root clippings or suckers (Walkup, 1991). Soopolallie produces fruits often consumed by birds and rodents. Besides, this shrub also provides a food base for various ungulates. Seeds of soopolallie experience deep dormancy what is an ecological adaptation to harsh climatic conditions (Baskin and Baskin, 1998). Soopolallie's applicability in mine reclamation might increase due to its nitrogen fixation ability. However, the potential of soopolallie remains still largely unknown, as it is a non-leguminous plant (Diagne et al., 2013). So far, the attention of most reclamation researchers was oriented toward legumes often omitting non-leguminous nitrogen fixers.

The second study objectives were:

I. To confirm similarities and differences between New Afton New Gold and Teck Highland Valley Copper subsoils with or without amendments by observing the physicochemical properties of subsoils subjected to treatments in semi-natural conditions, as well as by observing differences in three shrubby species mortality rates and productivity as responses to the treatments,

II. To test the relative effects of altered proportions of the three soil amendments (biosolids, woodchips, biochar) on two subsoils' physicochemical properties;

III. To compare qualitative and quantitative changes of nitrogen compounds observed in the open-air study with the greenhouse study results.

METHODS

Study Site

The second study was located in a garden within the Aberdeen residential area in Kamloops, BC. Elevation was 866 meters above see level which locates it between New Afton New Gold and Teck Highland Valley Copper elevations. It began on May 23, 2019, and ended on November 7, 2019. It lasted 24 weeks counting from the moment of all pots placing in the experimental site. Subsoil substrate remained the same.

Experiment Design

Pots

2.4 L, round-shaped, perforated at the bottom pots were filled with 10 combinations of subsoils and soil amendments (See Table 3.2). Pots were marked and numbered. Because of 10 replicates of every combination, pots got assigned evenly to 10 blocks. The pot's bottom was lined with weed-blocking fine-perforated textile which was to hold the subsoil material in the pot concurrently allowing water to drain.

Plants and Seeds Sourcing

Table 3.1 *Table presents plant material provenience and the age when transplanted.*

	PLANT SPECIES		
	BIG SAGEBRUSH	COMMON RABBITBRASH	SOOPOLALLIE
PROVENIENCE OF THE PLANT MATERIAL	Splitrock Native Plants Nursery in Lillooet, BC	TRU Research Greenhouse in Kamloops, BC	Splitrock Native Plants Nursery in Lillooet, BC
AGE OF THE PLANT MATERIAL	6 months	5 months	24 months

Common rabbitbrush seeds were sourced from Splitrock Native Plants Nursery. Those seeds were sown 5 months before the experiment commencement to the substrate containing peat and sand in ratio 1:1. Seeds were sown to the substrate similar to the one being used in Splitrock Native Plants Nursery to maintain consistency. The entire process of independent common rabbitbrush growing in the TRU greenhouse emulated the process adopted in professional nursery from Lillooet. That included the same type and size of the styrofoam blocks used (one plaque 140 mL), similar fertilization and the greenhouse settings.

Prior to sowing, seeds were undergone cold-wet stratification (Baskin and Baskin, 1998).

Transplantation of Seedlings

Transplantation of seedlings to the pots containing the subsoil combinations took place on May 9, 2019. To minimize the shock caused to young plants, after transplantation plants were still kept in the greenhouse in controlled conditions for 14 days before transportation to the experiment location. After the transplants' stabilization and ensuring that all plants are in equally good vigor, pots with transplants were moved to the open-air study site. All plants were transplanted within one day.

To increase the transplantation survivorship, seedlings were planted with minimal root system disturbance. That was achieved by transplantation with the entire root system together with the already existing soil substrate in which roots were installed. However, the amount of existing soil substrate was minimal (less than 140 mL) to force quick relying on the treated subsoil.

Pest Control

During the common rabbitbrush germination, to keep greenhouse pests controlled the same measures were taken as in the first study experiment (See Chapter 2; Methods; Experiment Design; Pest Control).

To emulate natural conditions, during the open-air phase of the second study experiment invertebrate pests were not controlled at all. Birds and rodents were not controlled either, however, the study site was fenced to prevent ungulates to encroach.

Settings and Watering

During the common rabbitbrush germination, the greenhouse settings were the same as in the first study experiment (See Chapter 2; Methods; Experiment Design; Greenhouse Settings).

Shrubby plants were watered as required, therefore, in that aspect, the experiment did not emulate the natural conditions. Plants were not left to rely on natural precipitation because once grown in a greenhouse and then radically shifted to the open-air conditions might not be prepared to survive the extended drought periods. Also, plants did not germinate on the mine subsoil which would let them adjust to this substrate. The third reason was that plants were planted in pots that contained a relatively low amount of soil substrate and that in turn could cause increased desiccation.

Applied Subsoil Substrate Combinations

Subsoils collected from both mine stockpiles received the same amounts of soil amendments. Soil amendments were applied in the following proportions as shown in Table 3.2.

Table 3.2 *Second study experiment soil medium composition percentage breakdown.*

	SUBSOIL	**BIOSOLIDS**	**WOODCHIPS**	**BIOCHAR**
CONTROL	**100%**	0%	0%	0%
BIOSOLIDS	**95%**	5%	0%	0%
WOOD CHIPS	**75%**	0%	25%	0%
BIOCHAR	**95%**	0%	0%	5%
MIXTURE OF ALL AMENDMENTS	**75%**	5%	15%	5%

Regarding study 1 experiment results (Chapter 2) in second study it was decided to go with much lower biosolids application to avoid overfertilization. This time the biosolids volumetric application rate was equalized to biochar which did not change and remained on 5%. The application of biosolids was 5 times lower in comparison to first study experiment (Chapter 2) in order to observe whether lowered amount of biosolids would maintain beneficial influence on plant productivity with parallel reduction of plant mortality. While the total amount of organic soil amendments in a mixture remained the same, the amount of biosolids was lowered to 5% and the amount of woodchips was increased to 15%. This was to better use the woodchips feature of limiting an excess of NH4+ brought in by biosolids and to slow down its release.

Experiment 2 Overall Treatments Combination

The experiment was a 2 x 5 x 3 factorial design, with 30 treatment combinations:

- 2 subsoil types: 1. New Afton New Gold subsoil, 2. Teck Highland Valley Copper subsoil;
- 5 soil amendment treatments: 1. control [no soil amendments], 2. biosolids alone, 3. woodchips alone, 4. biochar alone, 5. mixture of all three soil amendments (see Table 3.2);
- 3 plant treatments: 1. big sagebrush, 2. common rabbitbrush, 3. soopolallie.

Replicated 10 times for a total of 300 individual pots. A randomized block design was arranged in the open-air experimental site.

Soil Sampling

Soil samples collection was identical to the one adopted in the study 1 experiment (See Chapter 2; Methods; Soil Sampling). At the experiment termination, the soil substrate from 10 pot-replicates was mixed thoroughly together, and then one sample was collected in a 1L labeled plastic bag and immediately placed in the refrigerator. The same procedure was repeated for each of the 30 subsoil treatments. This way all together a pool of 42 1-L samples was obtained: 30 samples of the final subsoil treatments stage, 10 samples of the initial stage, and two undisturbed topsoil samples for reference.

Tests and Instrumentation Used

In the case of study 2 experiment, the same tests and instrumentation were used as in the study 1 experiment (See Chapter 2; Methods; Tests and Instrumentation Used). The only difference was in the productivity measuring.

Plant Productivity

Because young shrubs were not equal at the beginning of the experiment, the productivity was assessed indirectly by measurement of growth. The lengths of each plant's stem and all twigs were measured and summarized altogether at the beginning of the experiment. The procedure was repeated at the end of the experiment. The total length increment was obtained by subtraction of the initial result from the final one. Lengths of stem and all twigs were measured by a ruler and noted.

Statistical Analyses

In the second study statistical analyses did not differ from those used in the first study. The only difference is in the case of mortality rate presentation as, unlike to experiment 1, in the second study this aspect data has a binary nature. The mortality presenting chart has a "violin" form to depict the 0/1 did-not-survived/survived frequencies.

Once again, statistical analyses got divided into 3 sections (See Chapter 2; Methods; Statistical Analyses).

RESULTS

Mortality, Productivity, Overall Subsoils' Difference

Section 1: Mortality

Kruskal-Wallis test showed that mortality of plants growing on New Afton New Gold subsoil combinations was significantly larger than mortality of plants growing on Teck Highland Valley Copper subsoil combinations (Figure 3.1).

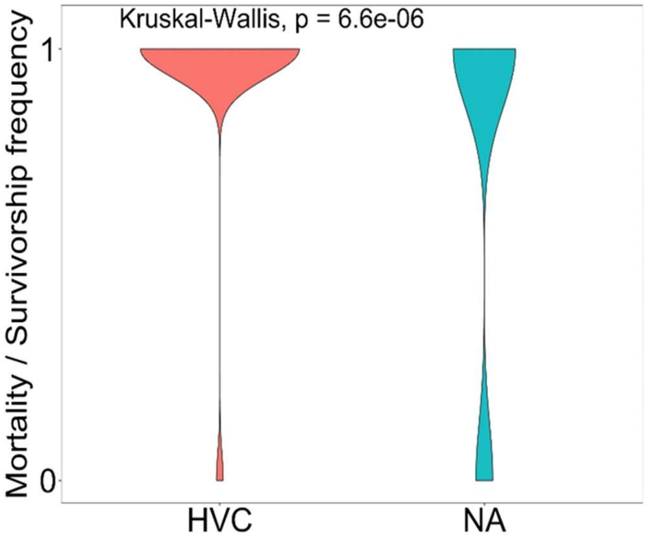

Figure 3.1 *Second study mortality comparison between two mines' subsoils: HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Violin plots describe the frequency of: 0 – plants which did not survive to the end of experiment; 1 - plants which survived to the end of experiment. Kruskal-Wallis test was used to compare the plant mortalities. n = 150*

Kruskal-Wallis tests showed that neither the mortalities on New Afton New Gold subsoil treatments nor on Teck Highland Valley Copper subsoil treatments differed significantly.

Plant Species Mortality Analysis

Kruskal-Wallis tests showed that individuals of common rabbitbrush and soopolallie growing on New Afton New Gold subsoil combinations experienced larger mortality than individuals of these species growing on Teck Highland Valley Copper subsoil combinations. Mortalities of big sagebrush on two mines' subsoils did not differ significantly. On Teck Highland Valley Copper subsoil combinations soopolallie's survivorship was 100% (Figure 3.2).

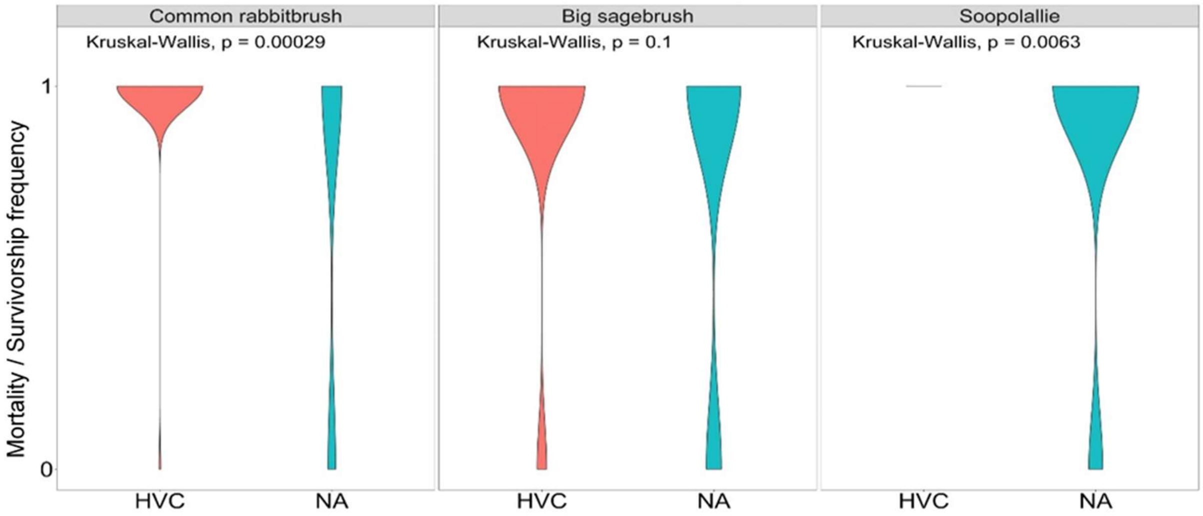

Figure 3.2 *Second study plant species mortality comparison between two mines' subsoils: HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Violin plots describe the frequency of: 0 – plants which did not survive to the end of experiment; 1 - plants which survived to the end of experiment. Kruskal-Wallis test was used to compare the plant mortalities. n = 50*

Kruskal-Wallis tests showed that on Teck Highland Valley Copper subsoil treatments mortalities of plant species were not equal. Wilcoxon pairwise comparison tests showed that soopolallie individuals performed significantly better than big sagebrush (Figure 3.3 left).

Kruskal-Wallis tests showed that on New Afton New Gold subsoil treatments mortalities of all plant species were statistically equal (Figure 3.3 right).

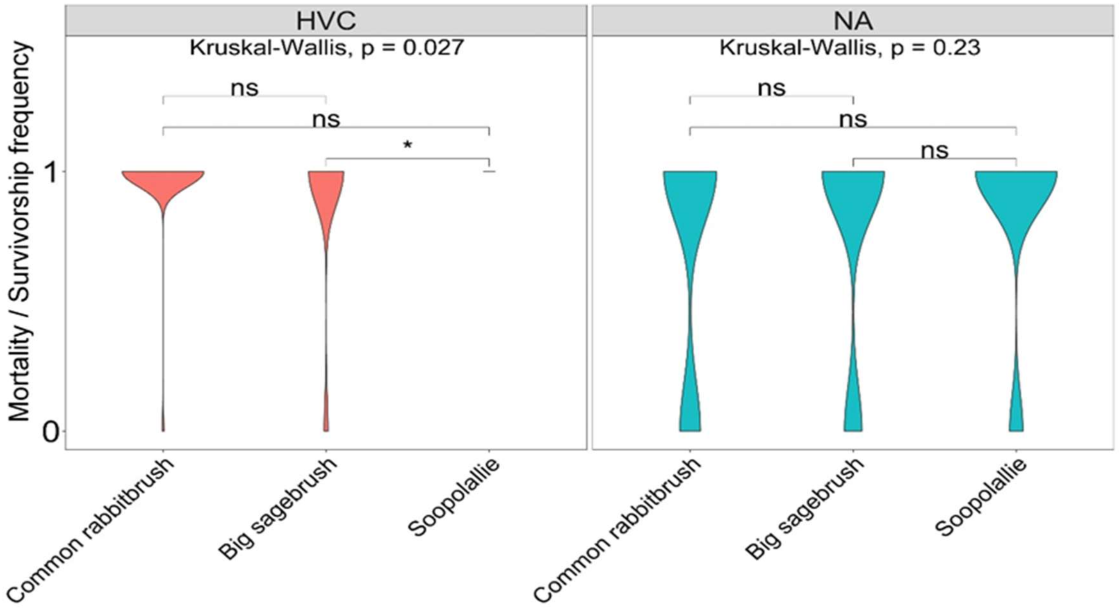

Figure 3.3 *Second study plant species mortality comparison on two mines' subsoils: HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Violin plots describe the frequency of: 0 – plants which did not survive to the end of experiment; 1 - plants which survived to the end of experiment. Kruskal-Wallis test was used to compare the plant mortalities. n = 50*

Section 1: Productivity

Kruskal-Wallis test showed that total stem and twigs elongation of plants growing on New Afton New Gold subsoil combinations was significantly lower than total stem and twigs elongation of plants growing on Teck Highland Valley Copper subsoil combinations (Figure 3.4).

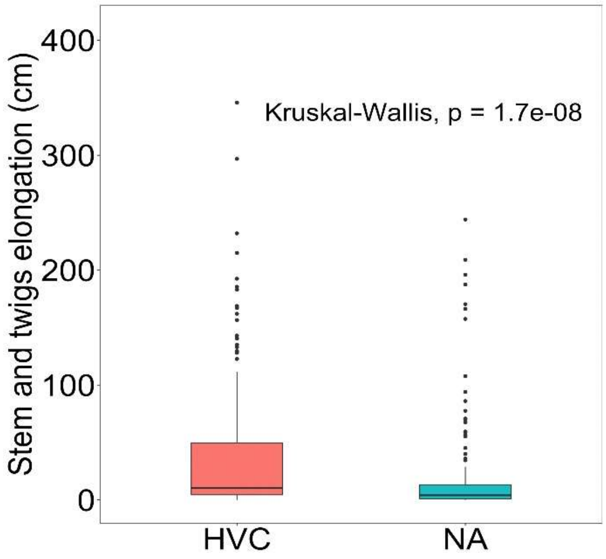

Figure 3.4 *Stem and twigs elongation comparison between two mines' subsoils: HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Boxplots describe stem and twigs elongation of all individual plants per mine subsoil at the end of the experiment. Horizontal line indicates the median of stem and twigs elongation of all individual plants. Kruskal-Wallis test was used to compare the medians. n = 150*

Average stem and twigs elongation of plants growing on New Afton New Gold subsoil combinations was more than two times lower than average stem and twigs elongation of plants growing on Teck Highland Valley Copper (Figure 3.5).

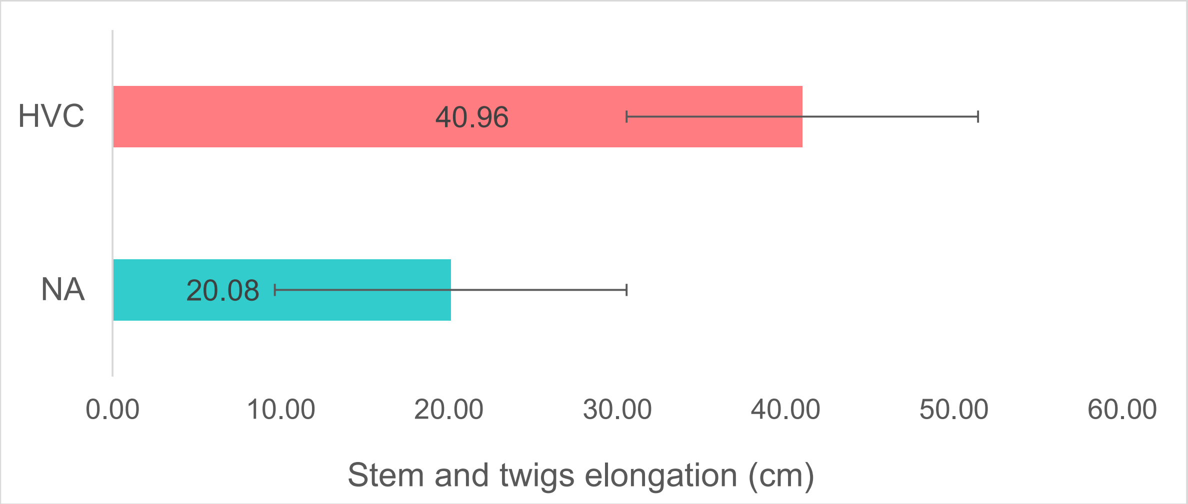

Figure 3.5 *Mean stem and twigs elongation comparison between two mines' subsoils: HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Error bars are standard error of the mean. n = 150*

New Afton New Gold Subsoil Treatments Productivity Analysis

Kruskal-Wallis test showed that the stem and twigs elongations of plants growing on New Afton New Gold subsoil treatments were not equal (Figure 3.6).

Wilcoxon pairwise comparison test showed that: the treatment with a mixture of soil amendments resulted in a significantly larger stem and twigs elongation than treatment with 25% of woodchips only. The treatment with 5% of biosolids resulted in a significantly larger stem and twigs elongation than the treatment with 25% of woodchips and the treatment with 5% of biochar. The treatment with unamended subsoil resulted in a significantly larger stem and twigs elongation than the treatment with 25% of woodchips only (Figure 3.6).

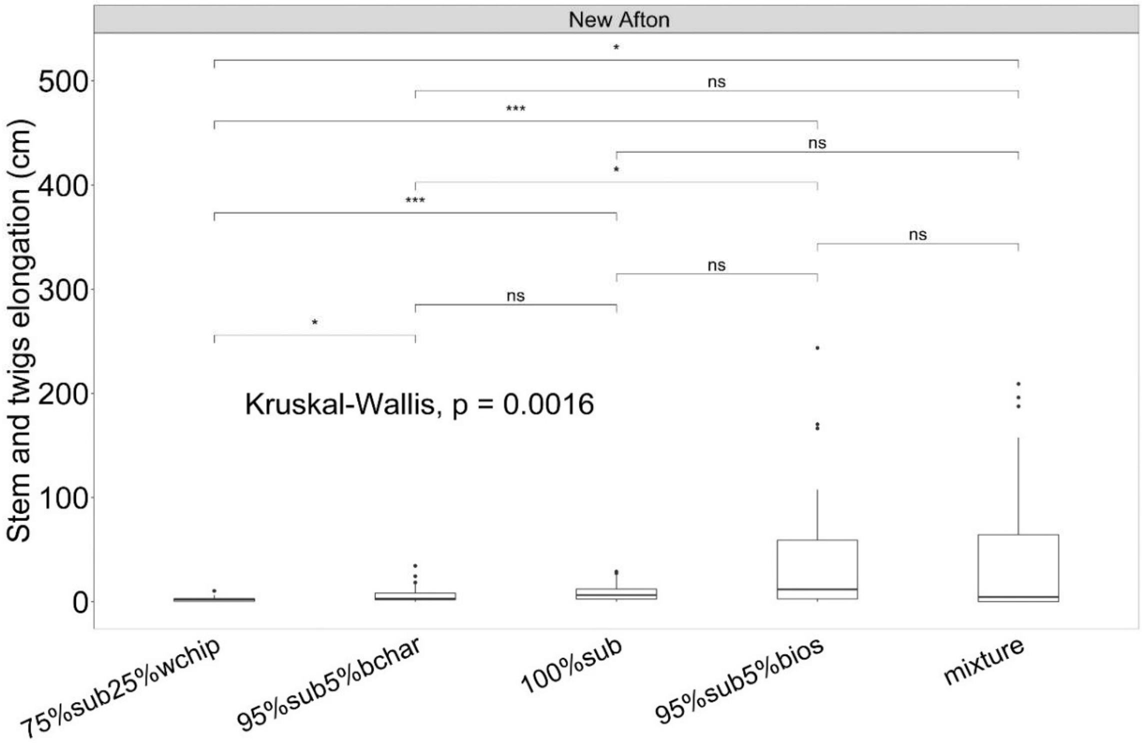

Figure 3.6 *Stem and twigs elongation comparison between the New Afton New Gold (New Afton) subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Boxplots describe stem and twigs elongation of all individual plants per subsoil treatment at the end of the experiment. Horizontal line indicates the median of plants' stem and twigs elongation. Kruskal-Wallis test was used to compare the medians of plant stem and twigs elongation on particular subsoil treatments. Wilcoxon test was used for the pairwise comparison. * - $p < 0.05$,, *** - $p < 0.001$, ns – difference not significant. n = 30*

Kruskal-Wallis test showed that the stem and twigs elongations of plants growing on Teck Highland Valley Copper subsoil treatments were not equal (Figure 3.7).

Wilcoxon pairwise comparison test showed that: the treatment with a mixture of soil amendments resulted in a significantly larger stem and twigs elongation than unamended subsoil, than treatment with 25% of woodchips, and than the treatment with 5% of biochar. The treatment with 5% of biosolids resulted in a significantly larger stem and twigs elongation than unamended subsoil, than treatment with 25% of woodchips, and than the treatment with 5% of biochar. The treatment with unamended subsoil resulted in a significantly larger stem and twigs elongation only than the treatment with 25% of woodchips. The treatment with 5% of biochar resulted in a significantly larger stem and twigs elongation only than the treatment with 25% of woodchips (Figure 3.7).

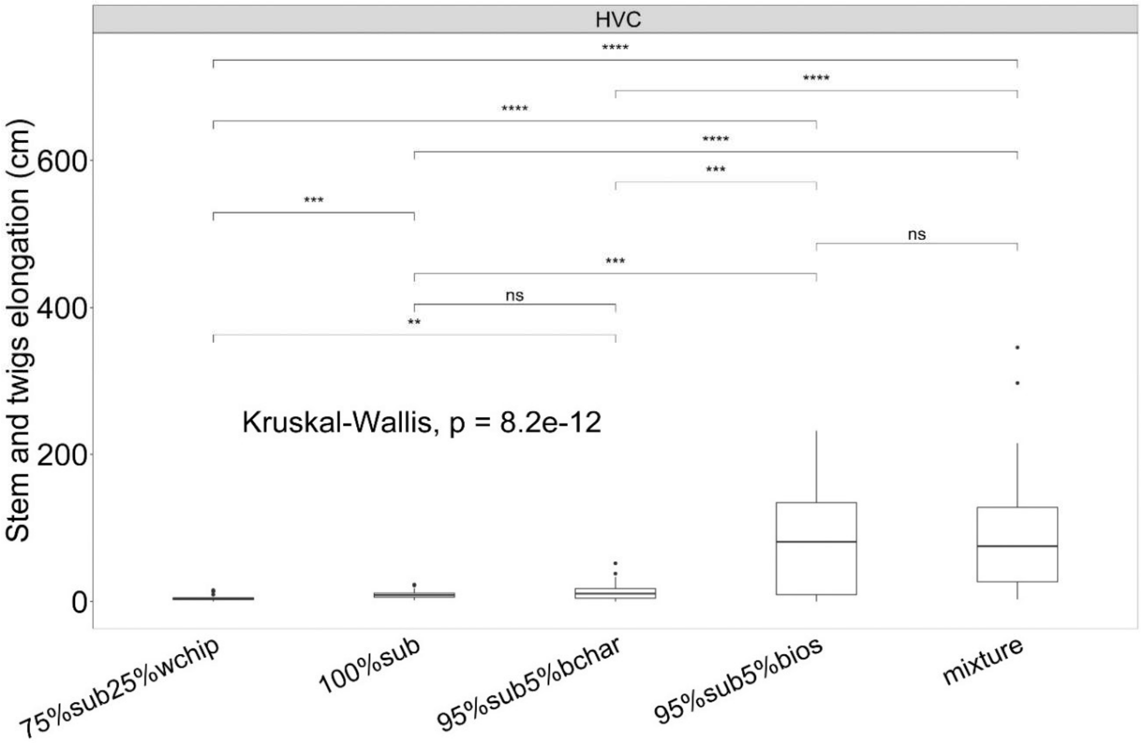

Figure 3.7** Stem and twigs elongation comparison between the Teck Highland Valley Copper (HVC) subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Boxplots describe stem and twigs elongation of all individual plants per subsoil treatment at the end of the experiment. Horizontal line indicates the median of plants' stem and twigs elongation. Kruskal-Wallis test was used to compare the medians of plant stem and twigs elongation on particular subsoil treatments. Wilcoxon test was used for the pairwise comparison. ** - $p < 0.01$, *** - $p < 0.001$, * - $p < 0.0001$, ns – difference not significant. n = 30*

Section 1: Both Mines' Subsoil Overall Difference; Principal Component Analysis

Once again the Principal Component Analysis with 21 predictors presented strong evidence that the two mines' subsoils were overall significantly different Pseudo F= 106.323, $p < 0.001$ (Figure 3.8). Datapoints representing pots containing subsoil with biosolids alone or mixture were distinct from all other (cluster separately). Other treatments cluster together revealing that there were no large differences between them (Figure 3.9)

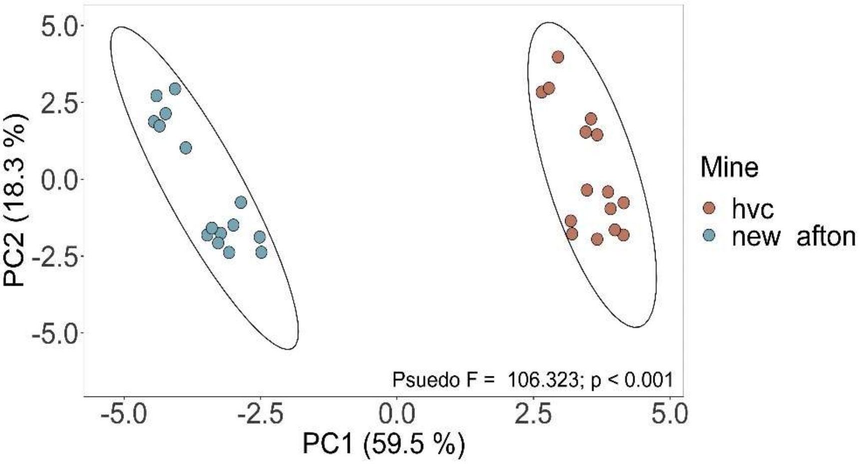

Figure 3.8 *Second study Principal Component Analysis of two mines' subsoils in terms of 21 predictors: pH, EC, SOM, total N and C contents, mineralizable N content,* NH_4^+ *and* NO_3^- *contents, as well as the contents of 13 basic elements. hvc – Teck Highland Valley Copper, new afton – New Afton New Gold.*

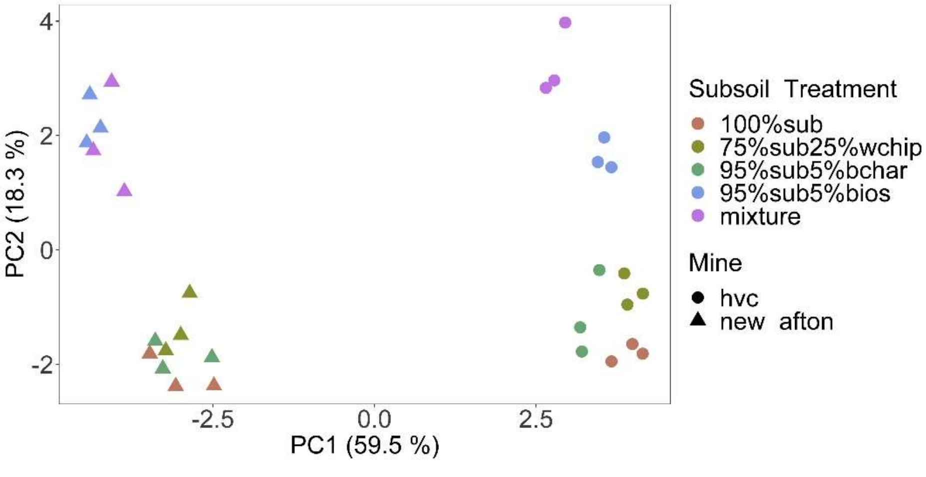

Figure 3.9 *Second study Principal Component Analysis of two mines' subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. hvc – Teck Highland Valley Copper, new afton – New Afton New Gold.*

Physicochemical Properties of Mines' Subsoils When Unamended and Amended

Section 2: pH

New Afton New Gold subsoil pH was significantly higher than Teck Highland Valley Copper both in the initial and the final stages (Figure 3.10 left). Within each mine separately there was no significant difference between the initial and final pH either in the case of New Afton New Gold or in the case of Teck Highland Valley Copper (Figure 3.10 right).

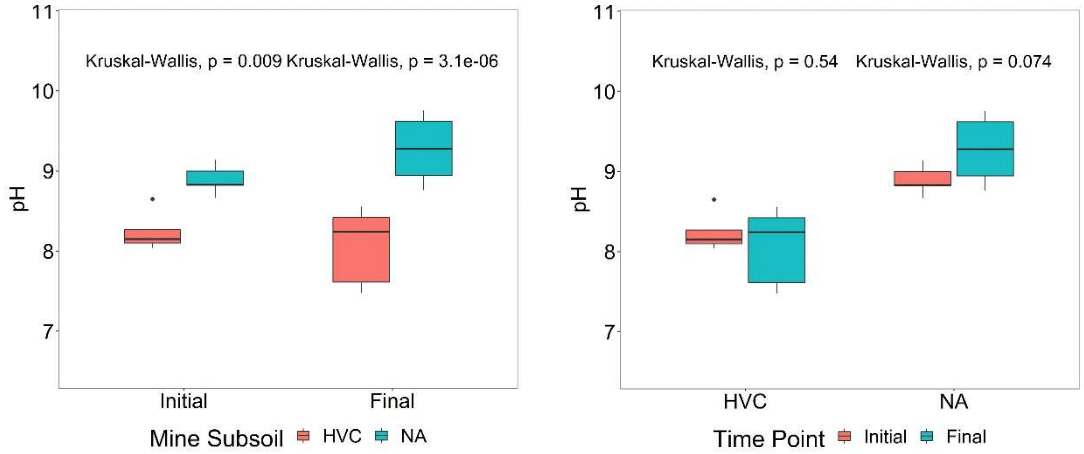

Figure 3.10 *Second study two mines' subsoil pH comparison in the initial and final stages of the experiment (left) Comparison of the subsoil pH in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*

In the case of both mines the final pHs were statistically not equal (Figures 3.11). In both cases, the biosolids-containing treatments had the lowest pH. In both cases as well, pHs seemed to drop throughout the experiment (Figures 3.11).

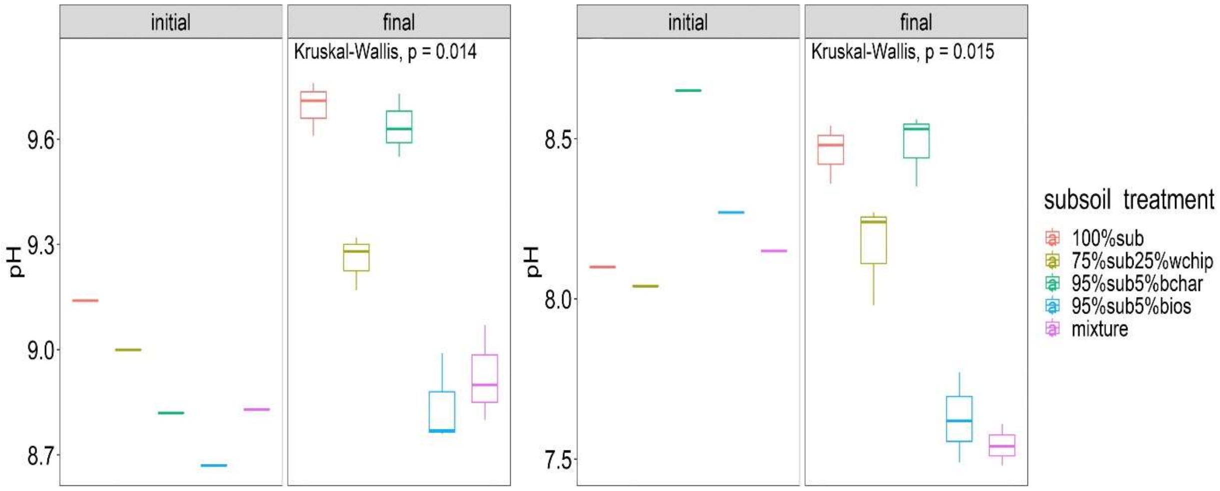

Figure 3.11 *Second study New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments pH comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 3*

Section 2: Electroconductivity (EC)

New Afton New Gold subsoil electroconductivity was significantly higher than Teck Highland Valley Copper both in the initial and final stages (Figure 3.12 left). Within each mine separately there was a significant difference between the initial and final electroconductivities both in the case of NA and in the case of HVC (Figure 3.12 right).

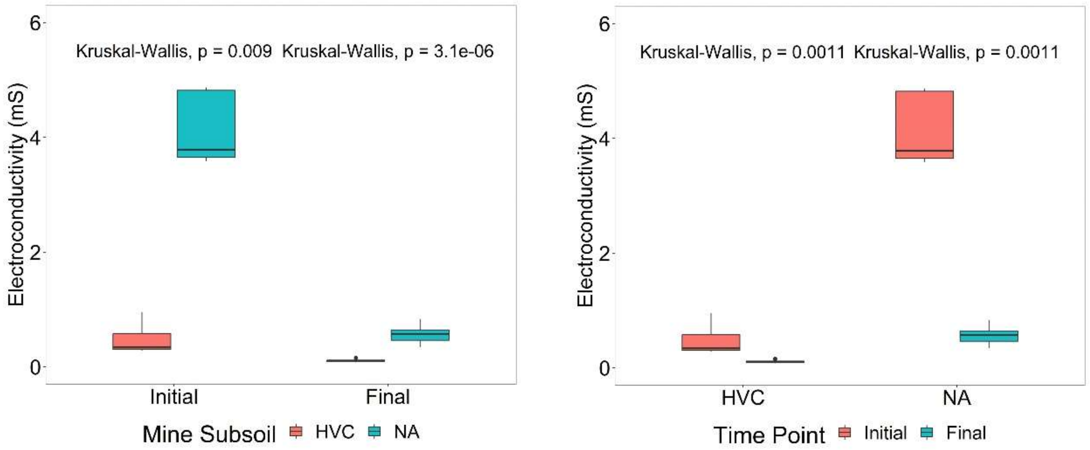

Figure 3.12 *Second study two mines' subsoil electroconductivities comparison in the initial and final stages of the experiment (left) Comparison of the subsoil electroconductivities in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*

In the case of NA subsoil treatments, there was no evidence that the final electroconductivities were statistically not equal (Figure 3.13 left). In the case of HVC subsoil treatments the final electroconductivities were statistically not equal (Figure 3.13 right). Throughout the experiment, the EC dropped.

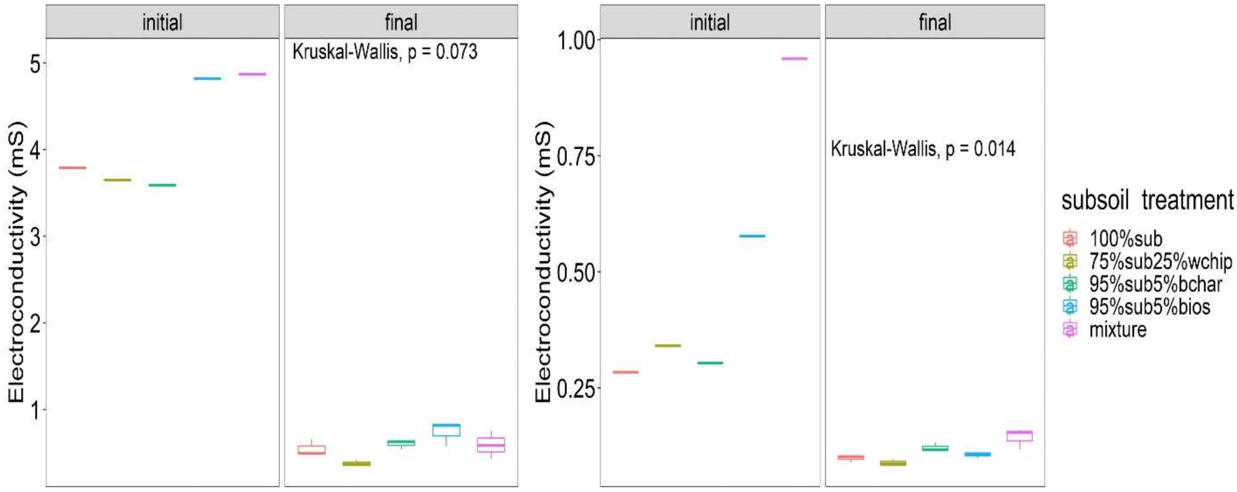

Figure 3.13 *Second study New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments electroconductivities comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 3*

Section 2: Soil Organic Matter (SOM) Content

New Afton New Gold subsoil SOM content was significantly higher than Teck Highland Valley Copper SOM content in both the initial and final stages (Figure 3.14 left). Within each mine separately there was a significant difference between the initial and final SOM contents in the case of New Afton New Gold subsoil only (Figure 3.14 right).

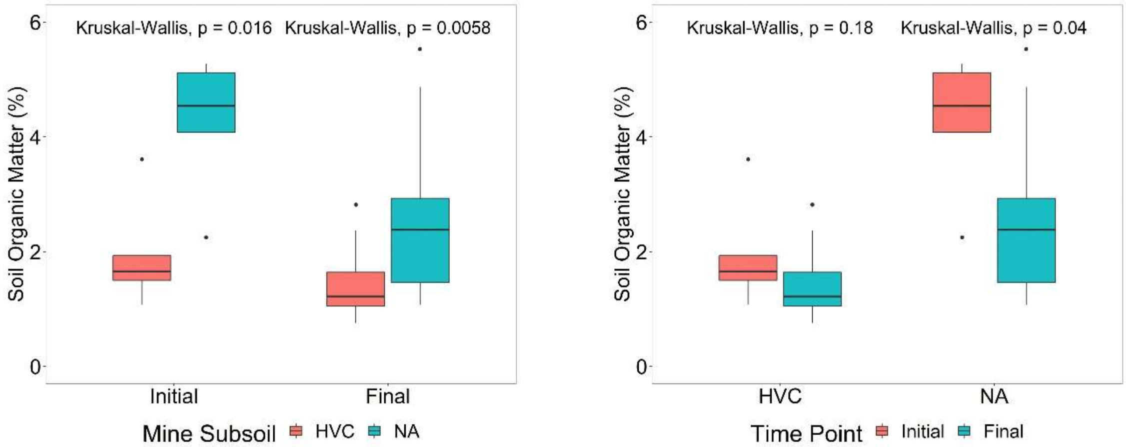

Figure 3.14 *Second study two mines' subsoil organic matter contents comparison in the initial and final stages of the experiment (left) Comparison of the subsoil organic matter contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*

In the case of both mines the final soil organic matter contents were statistically not equal (Figures 3.15). In both cases, the subsoil treatment containing woodchips as an only amendment had finally the second-largest SOM content and the subsoil amended by a mixture had the first largest SOM content (Figures 3.15).

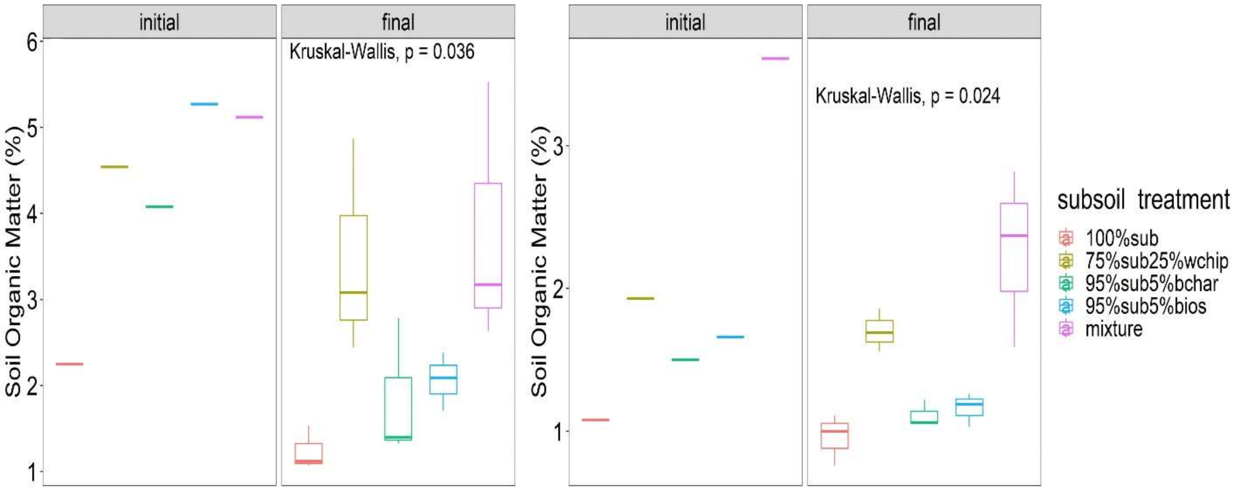

Figure 3.15 *Second study New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments soil organic matter contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 3*

Section 2: Sodium (Na) Content

New Afton New Gold subsoil Na content was significantly larger than Teck Highland Valley Copper Na content both in the initial and final stages (Figure 3.16 left). Within each mine separately there was a significant difference between the initial and final Na content only in the case of the New Afton New Gold subsoil (Figure 3.16 right)

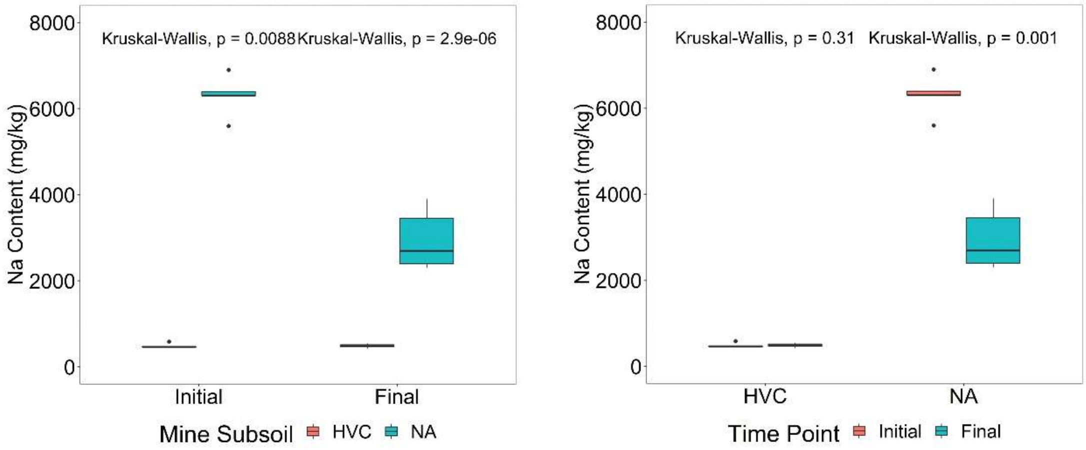

***Figure 3.16** Second study two mines' subsoil sodium content comparison in the initial and final stages of the experiment (left), comparison of the sodium content in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*

Only in the case of NA subsoil treatments, the final Na contents were statistically not equal (Figure 3.17 left). In the case of New Afton New Gold, every subsoil treatment contained more sodium in the initial than in the final stage of the experiment (Figure 3.17 left).

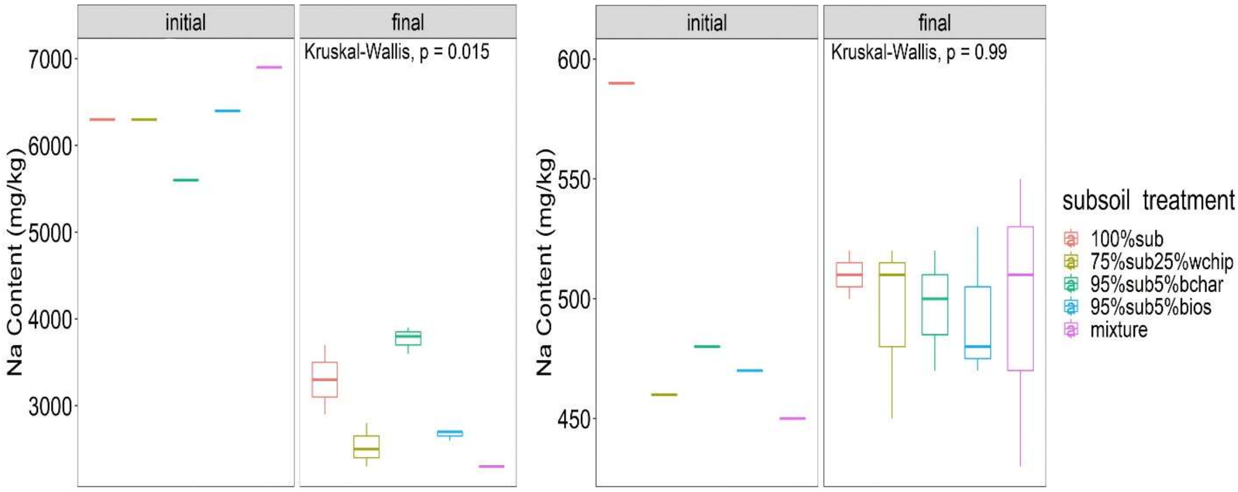

***Figure 3.17** Second study New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments sodium contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 3*

Quantitative and Qualitative Nitrogen Content Analyses

Section 3: Total Nitrogen (N) Content

New Afton New Gold subsoil total N content was significantly larger than Teck Highland Valley Copper total N content both in the initial and final stages (Figure 3.18). Within each mine separately there was no significant difference between the initial and final total N contents either in the case of New Afton New Gold or in the case of Teck Highland Valley Copper (Figure 3.19).

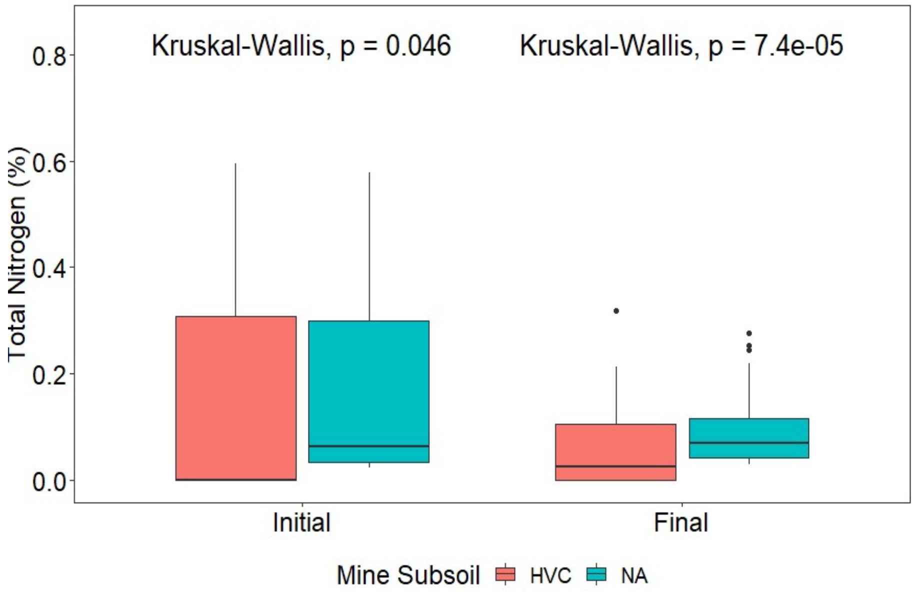

Figure 3.18 *Second study two mines' subsoil total nitrogen content comparison in the initial and final stages of the experiment. HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 25, final n= 75*

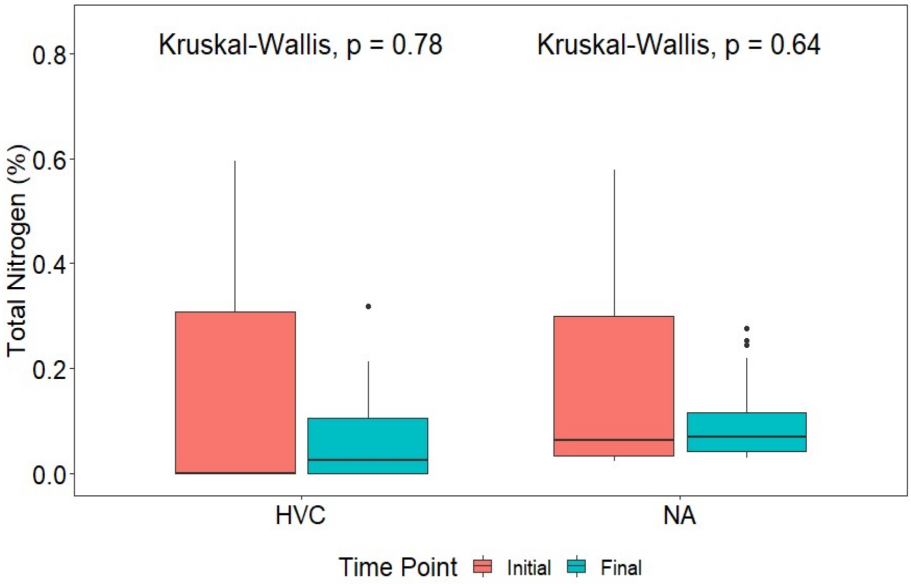

Figure 3.19 *Second study* ***c****omparison of the total nitrogen content in the initial and final stages of the experiment within each mine. HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 25, final n= 75*

In the case of New Afton New Gold and the case of Teck Highland Valley Copper subsoil treatments, the initial and final total N contents were statistically not equal (Figures 3.20, 3.21). Both, in the case of New Afton New Gold and the case of Teck Highland Valley Copper subsoil treatments initially there was no statistical difference between the “95%sub5%bios” and the “mixture” treatments, while those two treatments differed significantly from all other treatments. The situation changed slightly at the end of the experiment. The lack of statistical difference between the “95%sub5%bios” and the “mixture” treatments remained in the case of New Afton New Gold only (Figure 3.20). In the case of Teck Highland Valley Copper, the difference between the “95%sub5%bios” and the “mixture” treatments became significant (Figure 3.21).

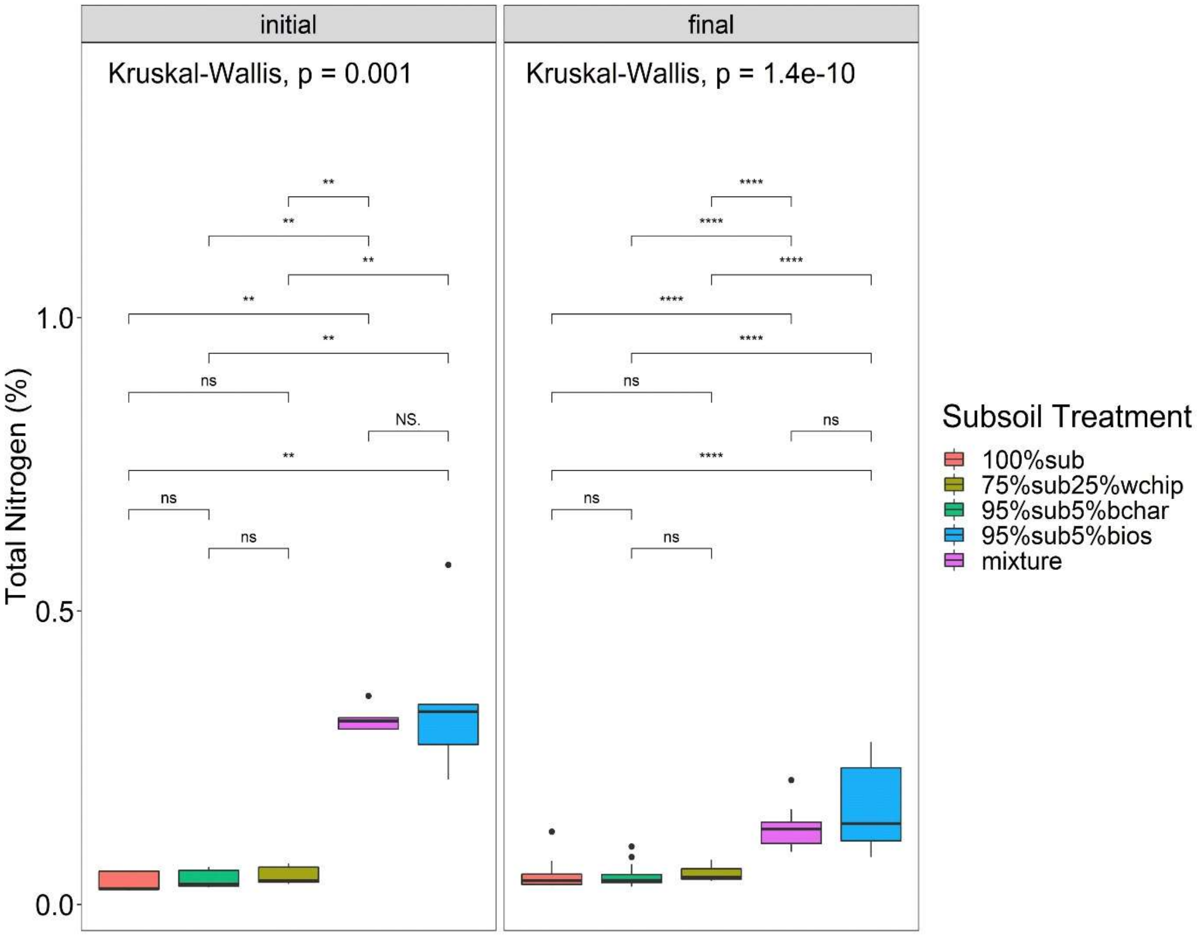

Figure 3.20 *Second study New Afton New Gold subsoil treatments total nitrogen contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison ** - $p < 0.01$, **** - $p < 0.0001$, ns, NS. – difference not significant. initial n= 5, final n= 15*

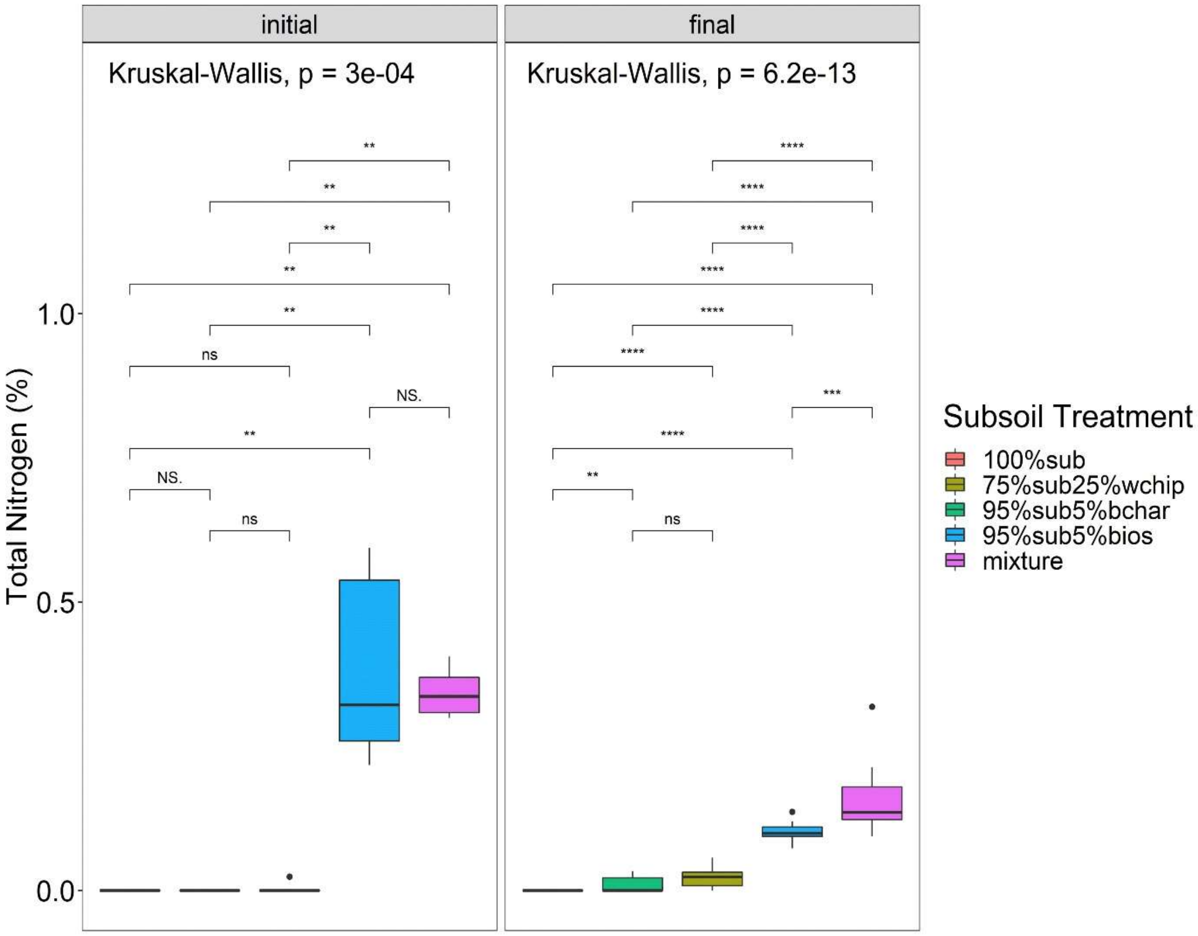

Figure 3.21 *Second study Teck Highland Valley Copper subsoil treatments total nitrogen contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison ** - p < 0.01, *** - p < 0.001, **** - p < 0.0001, ns, NS. – difference not significant. initial n= 5, final n= 15*

Both, in the cases of New Afton New Gold and Teck Highland Valley Copper subsoil treatments only the “mixture” and the “95%sub5%bios” at the end of the experiment were not significantly different from the reference of undisturbed topsoil in terms of total N content. All other treatments differed significantly from the reference (Figures 3.22, 3.23). In these cases, the total N contents were significantly lower than the reference’s one. In the case of both the “mixture” and the “95%sub5%bios” treatments, the initial total N contents were significantly larger than the reference, but finally the total N content dropped and the differences became not significant (Figures 3.22, 3.23).

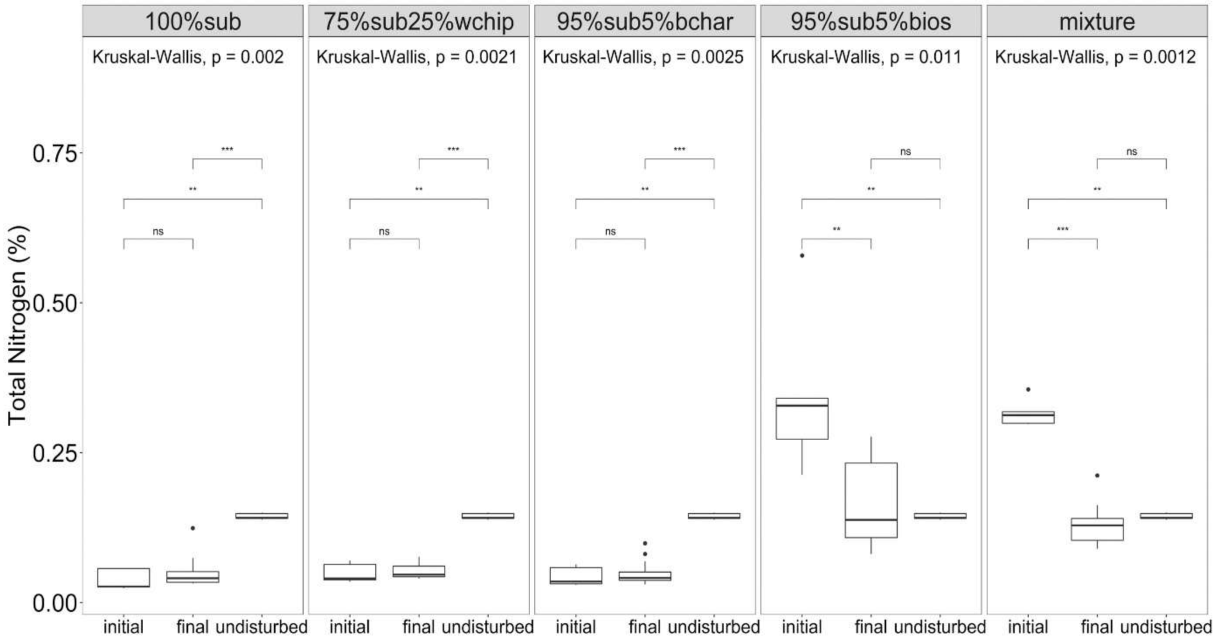

Figure 3.22 *Second study New Afton New Gold subsoil treatments total nitrogen contents comparison in the initial and final stages of the experiment to the reference of undisturbed topsoil. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison ** - p < 0.01, *** - p < 0.001, ns – difference not significant. initial n= 5, final n= 15, undisturbed n=5*

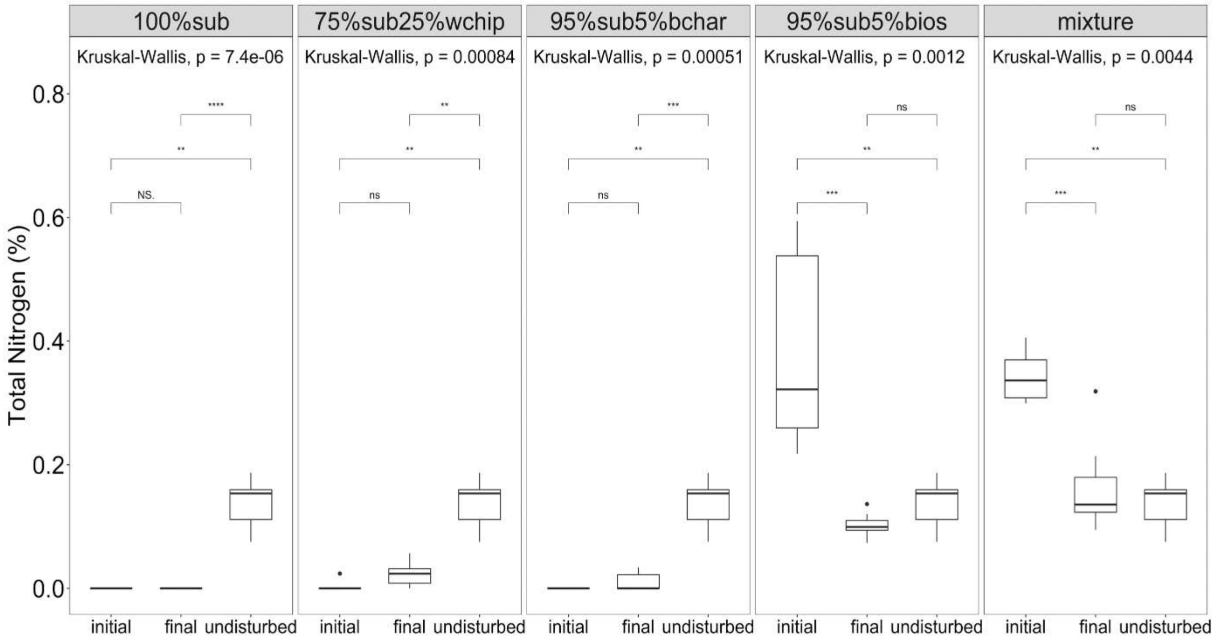

Figure 3.23 *Second study Teck Highland Valley Copper subsoil treatments total nitrogen contents comparison in the initial and final stages of the experiment to the reference of undisturbed topsoil. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison ** - p < 0.01, *** - p < 0.001, **** - p < 0.0001, ns, NS. – difference not significant. initial n= 5, final n= 15, undisturbed n=5*

Section 3: Mineralizable Nitrogen Content

There was no significant difference in terms of mineralizable N content between NA and HVC subsoils either in the initial or final stage (Figure 3.24 left). Within each mine separately there was no significant difference between the initial and final mineralizable N contents either in the case of NA or in the case of HVC (Figure 3.24 right).

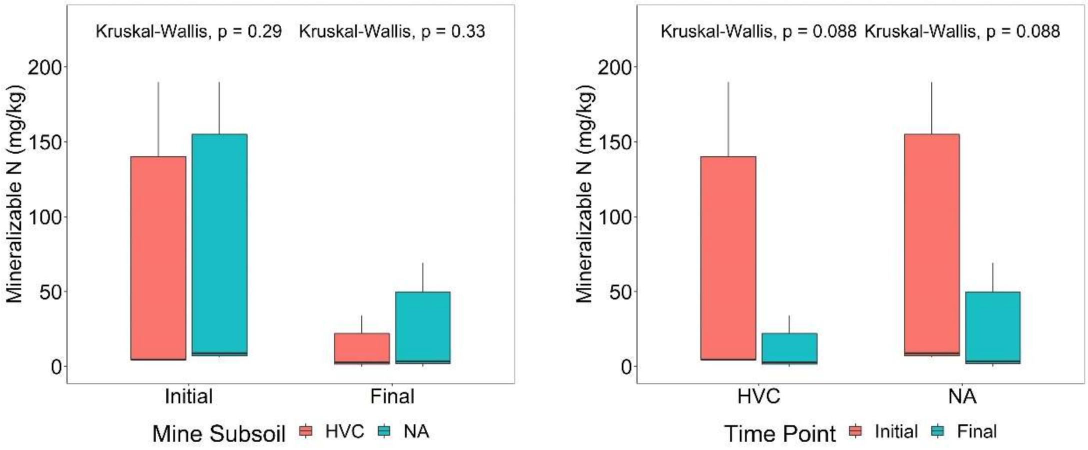

Figure 3.24 *Second study two mines' subsoil mineralizable nitrogen contents comparison in the initial and final stages of the experiment (left), comparison of the mineralizable nitrogen contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*

In the case of NA and the case of HVC subsoil treatments the final mineralizable N contents were statistically not equal (Figures 3.25). In both cases, biosolids-containing treatments had the largest mineralizable N contents. Mineralizable N contents seemed to drop strongly throughout the experiment (Figures 3.25).

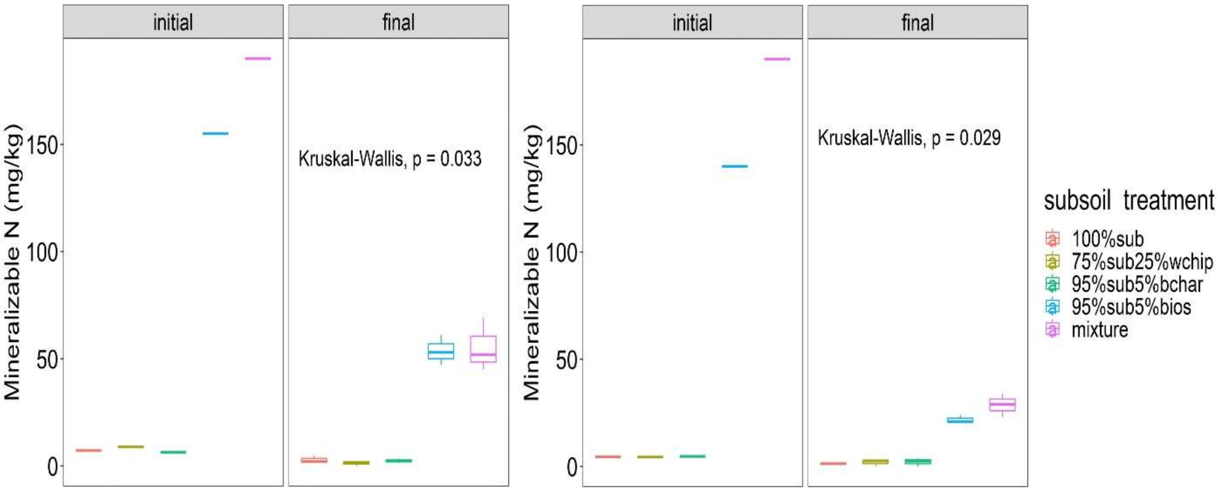

Figure 3.25 *Second study New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments mineralizable nitrogen contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 3*

Section 3: Available Nitrogen Content – Ammonium Cation (NH_4^+)

There was no significant difference in terms of NH_4^+ content between NA and HVC subsoils either in the initial or final stage (Figure 3.26 left). Within each mine separately there was no significant difference between the initial and final NH_4^+ contents either in the case of NA or HVC (Figure 3.26 right).

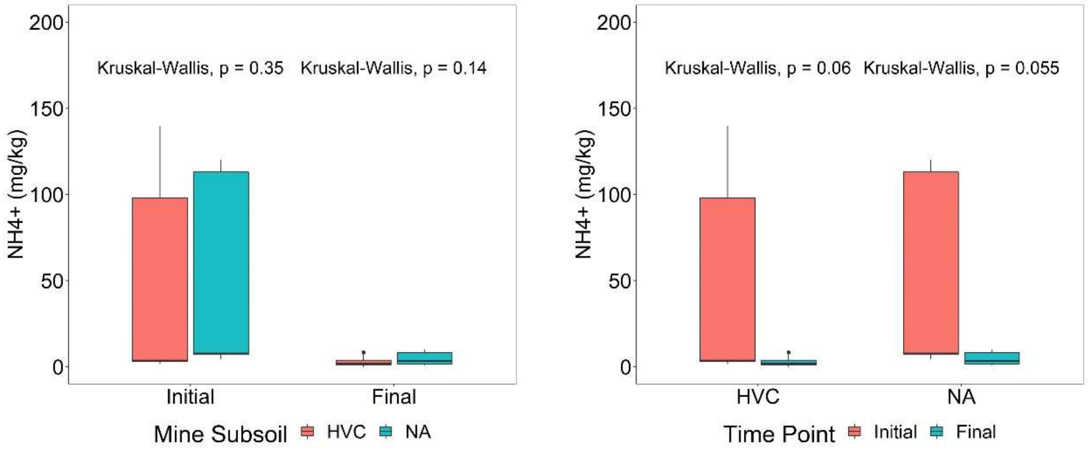

Figure 3.26 *Second study two mines' subsoil NH_4^+contents comparison in the initial and final stages of the experiment (left), comparison of the NH_4^+ contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*

In the case of NA and the case of HVC subsoil treatments the final NH_4^+ contents were statistically not equal (Figure 3.27). In both cases, biosolids-containing treatments had the largest NH_4^+ contents. NH_4^+ contents seemed to drop strongly throughout the experiment (Figure 3.27).

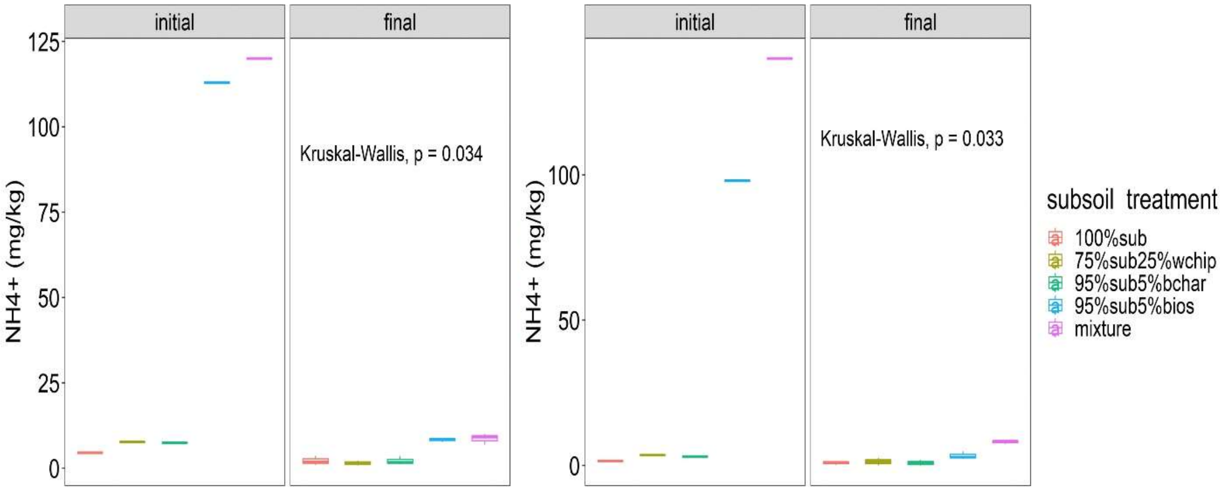

Figure 3.27 *Second study New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments NH_4^+contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 3*

Section 3: Available Nitrogen Content – Nitrate Anion (NO_3^-)

New Afton New Gold subsoil NO_3^- content was significantly larger than Teck Highland Valley Copper subsoil NO_3^- content only in the final stage of the experiment (Figure 3.28 left). Within each mine separately there was a significant difference between the initial and the final NO_3^- content in the case of HVC subsoil only (Figure 3.28 right).

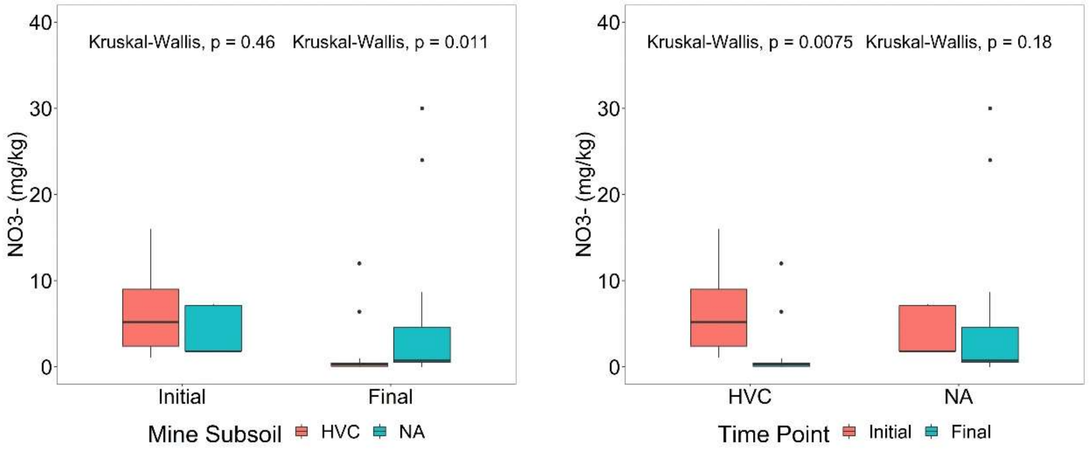

Figure 3.28 *Second study two mines' subsoil NO_3^- contents comparison in the initial and final stages of the experiment (left), comparison of the NO_3^- contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians.*
initial n= 5, final n= 15

Only in the case of NA subsoil treatments the final NO_3^- contents were statistically not equal (Figures 3.29). In the New Afton New Gold case, the subsoil treatment containing biosolids as an only amendment had the largest final NO_3^- content, and the subsoil amended by a mixture had the second-largest NO_3^- content (Figure 3.29 left).

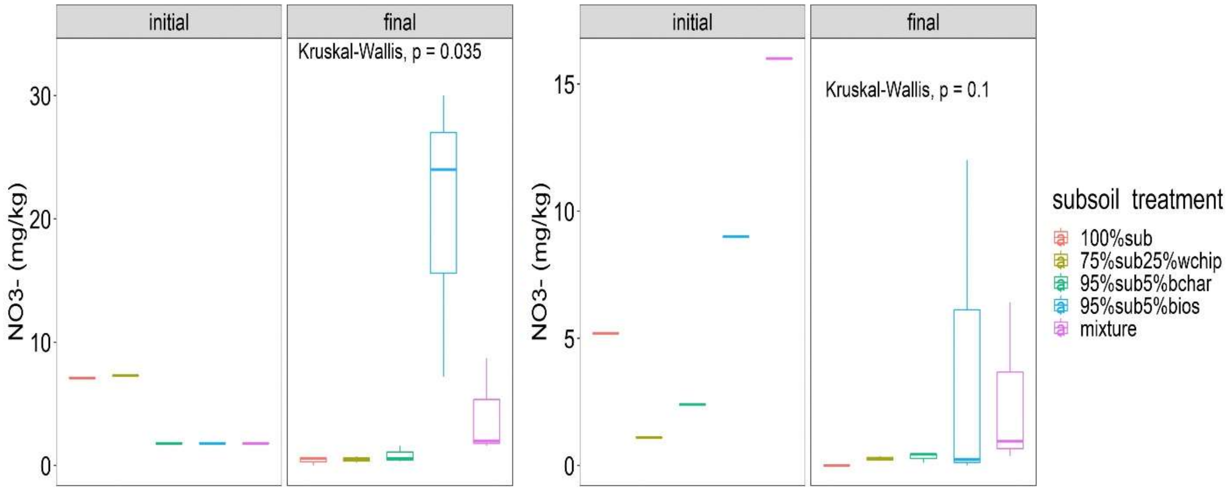

Figure 3.29 *Second study New Afton New Gold (left) and Teck Highland Valley Copper (right) subsoil treatments NO_3^- contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 1, final n= 3*

Section 3: Available Nitrogen Content – NH_4^+, NO_3^-; Sum and Ratio

There was a significant difference between the sums of NH_4^+ and NO_3^- in the initial and the final stages of the experiment at α=0.05 (Figure 3.30).

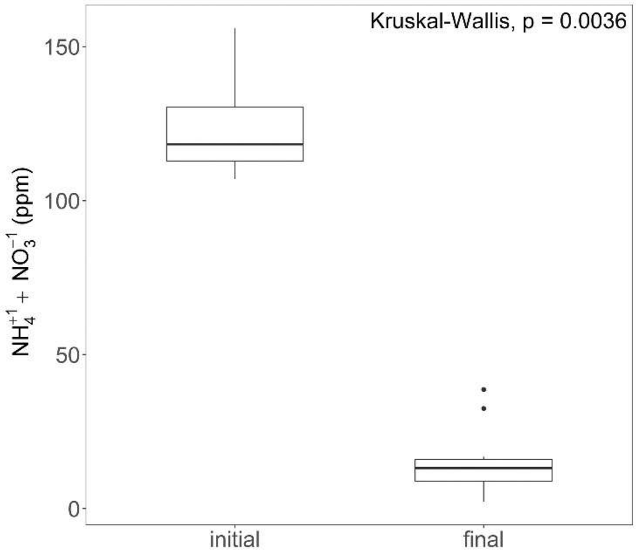

Figure 3.30 *Second study comparison of the sum of NH_4^+ and NO_3^- in the initial and final stages of the experiment. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 4, final n= 12*

There was a significant difference between the ratios of NH_4^+ to NO_3^- in the initial and final stages of the experiment at α=0.05 (Figure 3.31).

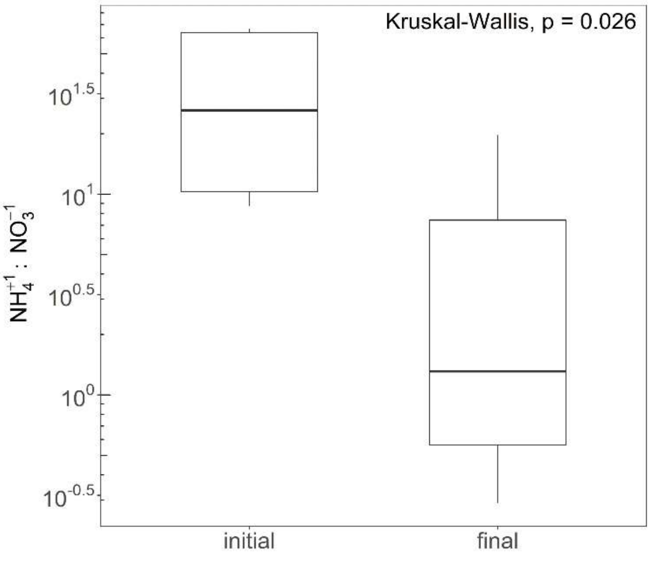

Figure 3.31 *Second study comparison of the ratio of NH_4^+ to NO_3^- in the initial and final stages of the experiment. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 4, final n= 12*

DISCUSSION

Second study experiment confirmed most of the observations on mine subsoils which were revealed in the first study experiment (Chapter 2). Lowering the quantity of biosolids also resulted positively. Open-air conditions did not drive changes in subsoil to other directions than greenhouse conditions of the experiment 1, despite the fact that applied vegetation species were very different in the study 2 experiment. Applied shrubs demonstrated that they were good indicators of reclamation effectiveness as well as they themselves might be a promising tool for mine reclamation.

Mortality, Productivity, Overall Subsoils' Difference

Mortality

In natural conditions, in ecosystems where the woody-stem plants play a dominating role, at the early stage of regeneration young seedlings germinate in abundance but then experience high mortality (Swaine and Hall, 1983). Seedlings are often eliminated by such factors as competition with neighboring vegetation, browsing, extreme climatic events, insect or disease infestation (Collet and Le Moguedec, 2007). On reclaimed mine sites Rizza et al. (2007) demonstrated that overwintering stress kills a large percentage of seedlings. In this study experiment, although insects infestations were observed they did not seem to increase mortality. Adverse soil substrate conditions were rather these which played a major role in causing shrubby seedlings mortality. A large part of deaths resulted from the formation of a film of crystals on New Afton New Gold subsoil (See Figure 3.31). Crystals were debilitating young plants. When windy weather, all plants have been jerked frequently in all directions. After hundreds of wind jerks some plants, debilitated by salt crystals, got torn apart and killed. Salts formation on NA subsoil, with its multifaceted adverse influence on young seedlings, is most probably the explanation of significantly larger mortality among plants growing on NA subsoil versus plants growing on HVC subsoil (See Figure 3.1).

Figure 3.32 *Common rabbitbrush individual covered by a film of white salt crystals debilitating plant's resistance to wind. Plant is torn apart and killed.* *Photo credit Piotr Dzumek.*

However, on both mines' subsoil treatments, the mortality rates were visibly lower than in experiment 1 (Chapter 2). Most probably in can be explained by the lowering of biosolids content to 5% vol. Statistical tests did not detect any significantly larger mortality among seedlings growing on biosolids-containing subsoil combinations.

Productivity

In study 1experiment (Chapter 2) an addition of biosolids increased significantly the grasses and leguminous forb biomass productivity. However, it remained an open question whether the same effect biosolids would have on woody-stem plants. This is an important question as in several reclamation sites in British Columbia shrubs and trees are intended to be re-established. Teck implemented revegetation programs in Highland Valley Copper and in Pinchi Lake Mine in which shrubs were intended for installation on reclaimed sites amended by biosolids (Teck 2012 Sustainability Report; Teck, 2013). Hunt (2017) reports that on reclaimed rock disposal sites in Mount Polley Mine coniferous seedlings grew better on sites where soil substrate was amended with biosolids in comparison to unamended control sites. Additionally, on unamended sites the young trees showed chloroses which indicated nutrient deficiency.

Study 2 experiment results demonstrated that an application of 5% biosolids increased the stem and twigs elongation. However, the differences between the unamended subsoil and the "75%sub25%bios", as well as between the unamended subsoil and the "mixture" treatment were significant only in the case of Teck Highland Valley Copper mine subsoil (See Figures 3.6, 3.7). Again, similarly to study 1 (Chapter 2), the productivity on Teck Highland Valley Copper subsoil combinations was much larger than on New Afton New Gold subsoil combinations (See Figure 3.4). This result confirmed that when amended with nutrient-rich amendment Teck highland Valley Copper subsoil becomes well productive. It is striking that here again the most productive on both mines' subsoils were treatments amended by the mixture of soil amendments. However, in both cases, New Afton New Gold and Teck Highland Valley Copper, the differences between "mixture" treatments and treatments amended by 5% biosolids alone were not statistically significant. That means that an addition of woodchips and biochar in a mixture did not help increase productivity. In turn, similarly to study 1 experiment (Chapter 2), the lowest productivities in the case of both mines were achieved by plants growing on subsoils amended by 25% of woodchips (See Figures 3.6, 3.7).

Overall Difference of Both Mines' Subsoil Physicochemical Properties

Study 2 Principal Component Analysis confirmed the first study (Chapter 2) finding that physicochemical properties of two analyzed mines subsoils differ strongly (See Figure 3.8).

In terms of particular subsoil treatments, the situation in study 2 experiment was similar to the one of the first study (Chapter 2), but not identical. While in experiment 2, both the "95%sub5%bios" and the "mixture" treatments contained the same amounts of biosolids, they did not tend to differ from each other that much as in experiment 1 (See Figure 3.9). That indicates that neither an addition of biochar nor woodchips to the mixture changed much in terms of physicochemical properties.

Physicochemical Properties of Mines' Subsoils When Unamended and Amended

pH

Most plants prefer pH which is around neutral or slightly acidic (Boeckmann, 2019). Also soil bacteria conducting such processes as organic matter decomposition or nitrogen fixation prefer around-neutral soil pH (Griep, 2020).

In the second study experiment the New Afton New Gold subsoil pH is significantly higher than Teck Highland Valley Copper subsoil pH (See Figure 3.10). Both mines' unamended subsoils were alkaline. The further pH departs either way from the value of 7, the larger is the hindrance for plant and microorganisms to develop. New Afton New Gold subsoil pH departed toward alkaline values more than Teck Highland Valley Copper subsoil. Similar to experiment 1 (Chapter 2), pH values of New Afton New Gold subsoil treatments seemed to approach to the top limits of the vegetation tolerance on the strongly alkaline side (Shelford, 1931), while Teck Highland Valley Copper subsoil treatments seemed to adopt pH values closer to preferred by most plant species. Once again, the lowest pH was adopted by biosolids-containing treatments (See Figure 3.11). NH_4^+, provided by biosolids, reacts with molecules of water and forms H_3O^+ which acidify the substrate. This reaction was strongly present especially in study 1 experiment (Chapter 2), while in study 2 experiment this reaction had minor importance as the amounts of biosolids used were several times lower. While in study 2 the acidifying process presented above was limited due to lower amounts of biosolids, the alkalinization remained strong.

Electroconductivity (EC)

In terms of electroconductivity, study 2 experiment confirmed observations from study 1 experiment (Chapter 2) as results from both trials are very similar. From this it might be concluded that even though experiment 2 has been conducted in different conditions, processes deciding about the EC change have carried on similarly. The only difference was that in experiment 2 the change of electroconductivity throughout the experiment was deeper (See Figure 3.12), causing the difference between the initial EC and the final EC to become statistically significant. It could be expected that if the experiment had been conducted for a longer time, the tendency of EC decrease would have been maintained until it approaches the EC level of undisturbed topsoils (references). Electroconductivity indicates the level of ions concentration (Miller at al., 2017). The drop in EC throughout the experiment indicated the decrease in the amount of ions. The deeper drop of ions concentration observed in study 2 experiment may indicate that the loss of certain ions depends on climatic conditions (profuse precipitation happening occasionally, larger temperature amplitudes) as well as on the experiment duration (experiment 2 was 8 weeks longer). That all suggests that not only the use of an organic amendments can help alleviate the substrate salinity problem, but also water wash out can be helpful. Brown and Chaney (2016) suggest that in some cases an irrigation may be required to remove excessive salinity.

Soil Organic Matter (SOM)

Most of soil organic matter concentrates in topsoil. During surface mining entire topsoil is removed. Topsoil removal immediately decreases the soil organic matter budget (Larney and Angers, 2012). Recovery of soil organic matter and nutrient cycling is critical to the success of rehabilitation of deeply disturbed post-mining sites (Banning et al., 2008). In study 2 experiment results, the readings of soil organic matter content in unamended subsoils did not differ much from the readings obtained from study 1 experiment (Chapter 2). It confirms, therefore, the correctness of the test. Again, both New Afton New Gold and Teck Highland Valley Copper unamended subsoils contained less SOM than the reference of undisturbed topsoils, respectively 2 and 4 times. Especially the subsoil from Teck Highland Valley Copper mine contained low amounts of SOM. New Afton New Gold subsoil SOM content was significantly larger than Teck Highland Valley Copper one (Figure 3.14). Poor Teck Highland Valley Copper subsoil SOM content might be one of the explanations why this mine unamended subsoil was not productive. Bauer and Black (1994) reported that in their 4-

year experiment the highest total aerial dry matter and grain yields were associated to these of their study plots which possessed the highest SOM contents. Moreover, the loss of productivity was associated with a depletion of SOM. Bauer and Black (1994) explained that the deplation of SOM was associated with a loss of soil fertility which at the end resulted in decreased productivity. In this study the largest amount of SOM in the case of both mines was noted on subsoils amended by a mixture of soil amendments (See Figure 3.15). It is not surprising as this subsoil treatment contained volumetrically 25% of combined organic soil amendments. Plants growing on the "mixture" treatment produced extensive root system. That increased the final SOM results as well.

Basic Elements

In terms of basic elements, many analyses' results from study 2 experiment were identical or very similar to study 1 experiment results (Chapter 2). Comparison in terms of basic elements contents serves to find more similarities and dissimilarities existing between New Afton New Gold and Teck Highland Valley Copper subsoils. Eventually, the differences found may explain large overall discrepancy in terms of two mines subsoils' vegetation mortality and productivity. Results from both studies identified that New Afton New Gold subsoil contained significantly more: B, Ca, Fe, Mg, Mn, Na, S, Zn. On the other hand, Teck Highland Valley Copper subsoil contained significantly more: Cu, K, Mo (See Chapter 2 and Chapter 3 Results for Na, Appendices A and B for the rest of elements). Some of elements analyzed in both studies were listed by Marschner (1983) as essential mineral plant nutrients, meaning that in plant nutrition these elements cannot be replaced by any other element. These were Fe, Mn, B, Zn, Cu, Mo. From these list none seemed to scarce in analyzed unamended subsoils. Moreover, B in the case of New Afton New Gold subsoil, and Cu, as well as Mo in the case of Teck Highland Valley Copper were exceeding the CCME (2021) guidelines. However, the availability and uptake of nutritional elements is chiefly governed by soil substrate properties (Pandey, 2015), including soil microbial communities presence, activity, and facilitation (Jacoby et al., 2017). Therefore, a simple abundance of certain element in the soil substrate does not guaranty yet a proper elemental nutrition.

On the other hand, such elements as C, N, P, and Zn differentiated subsoil treatments in both experiments. The concentrations of these elements were always higher in subsoil combinations amended by biosolids alone or a mixture (containing biosolids too) (See Chapter 2 and Chapter 3 Results for C and N; Appendices A and B for P and Zn). Since those two subsoil treatments were the most productive as well it may be speculated that the concentration of one or more of these elements influenced positively the productivity.

Analyzing all elements separately, and knowing their potential influence on vegetation, it seemed that a higher concentration of Na dragged the New Afton New Gold subsoil's productivity down, while a higher concentration of K pulled the Teck Highland Valley Copper subsoil's productivity up.

Total Carbon

Soil organic matter is a sink for carbon (Akala and Lal, 2000) and its role in CO_2 sequestration cannot be overestimated. Carbon is stored mainly in a form of organic matter in both living soil organisms and dead organic residues (Insam and Domsch, 1988) Ganjegunte et al. (2009) demonstrate that over years following the mine land reclamation the total carbon content in the soil substrate was shaped by the type of vegetation, substrate texture, as well as by lignine content. Antonelli et al. (2018) revealed that a single biosolids application to reclaimed mine tailings has facilitated plant biomass production and has enhenced the carbon accumulation for more than a decade.

The results of study 2 experiment showed a large difference between both mines' unamended subsoils in terms of total carbon content. While New Afton New Gold unamended subsoil's total carbon content seemed to be low placing around 1.5%, the Teck Highland Valley Copper unamended subsoil's total carbon was even around 6 times lower. The lack of total carbon certainly translates to low microbial carbon as well as low dead organic matter. That might be a reason for little Teck Highland Valley Copper's unamended subsoil productivity. Extreme lack of soil carbon may pose a large hindrance in terms of spontaneous recolonization by soil living organisms. Regarding this, a use of organic soil amendments, which would increase the substrate's pool of carbon, appears as necessary for a successful ecosystem restoration on Teck Highland Valley Copper's subsoil.

Quantitative and Qualitative Nitrogen Content Change

Total Nitrogen

In the second study the amounts of added biosolids were lowered strongly. Biosolids though was the only soil amendment among applied which provided noticeable amounts of total nitrogen. It was revealed in study 1 experiment (Chapter 2) that applied there plants grew the best on substrate possessing 0.10% to 0.20% of total nitrogen. In study 2 experiment all the most productive treatments at the end of the experiment possessed the concentration of total nitrogen which fell into that interval (See Figures 3.20, 3.21). Wang et al. (2017) conducted a large scale mapping of total nitrogen content in Liaoning, a Chinese province. Their results showed that the largest concentration of total nitrogen noted in the 0-5 cm soil layer was 0.16%. Marx et al. (1999) provided an information that a typical agricultural soil in the Willamette Valley, which they analyzed, contained 0.10 to 0.15 % of total N. Therefore, an addition of 5% of biosolids may be close to ideal in terms of total nitrogen provision. What is worth noticing that with an addition of 5% biosolids volumetrically to the subsoil the well-growing plants did not show any negative signs of overfertilization.

New Afton New Gold subsoil total nitrogen content was significantly larger than Teck Highland Valley Copper total nitrogen content (See Figure 3.18). Such results were aligning to study 1 experiment (See Chapter 2 Results). Again the HVC unamended subsoil's concentration of total nitrogen was extremely poor. Insufficiency of this intrinsic element, besides the lack of carbon, may pose an additional and potent hindrance in terms of spontaneous recolonization of reclaimed subsoil by soil living organisms as well as by plants. An availability of N strongly influences soil microorganisms abundance and activity. N deficit causes reduced efficiency of microbial biosynthesis (Blagodatsky and Richter, 1998). Additionally, microorganisms have to compete for nitrogen with plants (Kaye and Hart,1997). It is noticeable that in the experiment these plants that were provided with a decent amount of total C (in a form of biochar and woodchips), but deprived of N remained reluctant to grow. It confirms that nitrogen is crucial for ensuring plant growth. Alike to study 1 (Chapter 2), in study 2 in terms of total nitrogen, the closest treatment to the reference of undisturbed topsoil ("mixture" and "95%sub5%bios") turned out as the most productive (See Figures 3.22, 3.23). What differed study 2 results from the study 1 (Chapter 2) was that the treatments amended by biosolids alone joined to the group of treatments which did not differ significantly from the undisturbed topsoil. That took place thanks to lowering the biosolids concentration to 5% only.

For a second time, in the cases of both mines the final total N contents were lower than the initial ones (See Figure 3.19). However, the initial and final total N contents did not differ statistically either in the case of NA subsoil or HVC. That confirms that nitrogen gets depleted or lost but the process is relatively slow, which is a good news as vegetation and soil microorganisms may profit from the total N pool for a prolonged time.

Mineralizable Nitrogen

Curtin and Campbell (2008) state that the rate of nitrogen mineralization depends on the quantity of mineralizable organic N in the soil and on environmental conditions. They also inform that large amounts of mineralizable N can accumulate under grassland. Only 1 to 4% of total N becomes plant-available during a growing season through mineralization (Marx et al.,1999). Thus, the mineralizable nitrogen plays a pivotal role in maintaining the entire N cycle by the release of mineralized nitrogen on the one hand, and by reception of dead organic matter for mineralization on the other one.

The mineralizable nitrogen concentration in the case of study 2 experiment was remarkably lower in comparison to study 1 experiment (Chapter 2) which corresponds with the lower amount of biosolids applied. Initially, the biosolids-containing treatments stood out with an amount of mineralizable nitrogen among all other treatments, but similarly to total N, the final amount of mineralizable N was lower than the initial one. However, here again in the case of both mines, the differences between the initial and final mineralizable nitrogen contents were not statistically significant (See Figure 3.24).

Initially, the treatments amended with 5% of biosolids alone contained around 140-160 mg of mineralizable N/kg, while the treatments amended by a mixture of soil amendments placed a bit higher, around 160-170 mg/kg (See Figure 3.25). In comparison to the undisturbed topsoil, subsoils amended by biosolids alone or the mixture contained still much more mineralizable N, but only in the initial phase. At the end of the experiment though the amount of mineralizable nitrogen decreased strongly and approached to the reference of undisturbed topsoil. It indicates that the amounts on mineralizable N tend to stabilize over time.

Available Nitrogen Forms: NH_4^+ and NO_3^-

Alike to study 1 (Chapter 2), also in study 2 experiment the levels of available nitrogen strongly exceeded the level which Geng and He (2020) in their study adopted as high. Marx et al. (1999) stated that normal levels of NH_4^+ are ranging from 2 to 10 mg/kg and levels of NO_3^- over 30 mg/kg would be excessive. However, above values refer to natural or agricultural soils, but not necessarily to reclaimed soils. In natural soils the levels of available nitrogen forms stabilize on rather low levels as this forms are quickly taken up and utilized by soil organisms or plants. It is worth to notice that the amounts of available nitrogen forms in undisturbed topsoils collected from vicinities of both mines were also low. Additionally, in these reference soils the NO_3^- prevailed. It testifies that in natural conditions nitrogen cycles fast and living organisms do not let mineralized forms of nitrogen stay unutilized in the soil for long. However, degraded soils are different. Mine reclaimed soils due to its deep degradation supposed receive large organic amendments dozing (Larney and Angers, 2012) providing more of available nutrient.

After a closer look upon the mineralized forms of nitrogen provided by biosolids, it might be noticed that NH_4^+ strongly prevailed over NO_3^- (See Figure 3.31). Other two applied amendments did not provide much of either NH_4^+ or NO_3^- (See Figures 3.27, 3.29). Initially, the treatments amended by 5% of biosolids alone contained around 110-120 mg/kg of mineralized nitrogen forms, whereof NO_3^- was only around 5%. Treatments amended by a mixture of soil amendments contained a bit more of mineralized nitrogen, which was around 120-160 mg/kg, but again, initially NO_3^- constituted a lesser fraction. At the end of study 2 experiment though, unlike to the first study, the level of available mineral nitrogen forms dropped significantly (See Figure 3.30). Additionally, the proportion NH_4^+: NO_3^- changed, but not as drastically as in the study 1 experiment (See Figures 2.33, 3.31). However, NO_3^- started to play a more important role. This again confirms that in the open-air conditions, same as in the greenhouse, nitrification process prevails at the initial stage of reclamation after biosolids application to the reclaimed subsoil.

Soopolallie Performance

On both subsoils amended by woodchips alone, soopolallie experienced the largest average growth rate among all species. The same situation took place on Teck Highland Valley Copper subsoil amended by biochar. That may suggest that soopolallie can cope with harsh conditions of reclaimed mine unamended soils. Surprisingly, soopolallie didn't take on on New Afton New Gold subsoil combinations that contained biosolids but did better on biosolids-free New Afton New Gold subsoils combinations. Many of soopolallie individuals produced nodulation which indicates that the process of nitrogen fixation was at least initiated. All above testifies that *Shepherdia canadensis* can be considered as a good candidate to become an efficient tool in deeply degraded mine soils. The ability for nitrogen fixation adds value to this species. Hendrickson and Burgess (1989) provided that in a regenerating lodgepole pine *Pinus contorta* stand in southern British Columbia *Shepherdia canadensis* fixed 0.78 kg/ha/year. It might seem not too much, however, once installed, soopolallie can have a long lasting positive effect on reclaimed land by its constant nitrogen provision to the ecosystem. Rhoades et al. (2008) presented results showing that about half the 600 g total N/m^2 accumulated across the newly formed river terrace chronosequence occurred during the 120 years when *Shepherdia canadensis* was dominant. Moreover, they informed that over years *Shepherdia canadensis* has enriched soil nitrogen pool several times more than willow *Salix sp.*

CONCLUSION

The second study experiment confirmed most of the observations from the first experiment which strengthen the credibility of both studies findings. The first study objective focused on differences and similarities between New Afton New Gold and Teck Highland Valley Copper subsoils. When experiment 2 confirmed that these two subsoils physicochemical properties differ strongly, one aspect is worth emphasizing – electroconductivity value of New Afton New Gold subsoil at the end of the experiment 2 dropped much more than in the study 1 (Chapter 2) case. This means that in natural conditions the elution of sodium excess happened quickly. Observing the sodium leaching rate, it could be speculated that thanks to the wash out it rather would not take longer than two vegetation seasons that the Na concentration approaches to the undisturbed topsoil level.

The second study objective focuses on soil amendments' influence on mines' subsoils. In experiment 2, unlike to experiment 1 (Chapter 2), the same amounts of biosolids (5% vol.) were used in two subsoil treatments (1. - "95%sub5%bios", 2. - "mixture"). This aimed at:

- verification whether smaller amounts of biosolids support plant growth to a similar extent as larger amounts applied in experiment 1 (Chapter 2),
- verification whether the greater productivity of the "mixture" treatment over the "95%sub5%bios" treatment in experiment 1 (Chapter 2) was because other soil amendments (biochar, woodchips) were applied in the mixture and they supported the growth, or because an application of 25% of biosolids alone in "75%sub25%bios" treatment was simply a too large dose eventually reducing the productivity and increasing the plant mortality.

Results from study 2 experiment have answered to both these questions. Firstly, the addition of only 5% of biosolids in both "95%sub5%bios" and "mixture" treatments resulted in much larger plant growth in comparison to all biosolids-free subsoil treatments, and secondly, the fact that there was no statistical difference in productivity and mortality between

“95%sub5%bios” and “mixture” treatments showed that the other two soil amendments in a mixture did not contribute to elevated productivity at least at the early stage of the reclamation.

The third study objective focused on nitrogen cycling aspect. In study 2 experiment on one hand the dynamics of nitrogen compounds changes over time of the experiment were intensified by the open-air weather conditions such as high summer temperatures or occurring heavy downpours, and limited on the other hand by the simple fact of much lower biosolids application. The most important finding from both studies is that nitrogen cycling phases contributing to nitrogen loss pace relatively slowly allowing vegetation to profit from elevated nitrogen compounds concentration for longer time.

In the second study three shrubby plant species were used, including a non-legume nitrogen fixer. Additionally, all three species are native plants of great ecological function fulfilled by blooming in late summer and early fall, being grazed by wildlife, providing shede for seedlings etc. It was observed that all three species did well on mine subsoils. That is why all three species seem to be good candidates to become valuable tools for the usage in mine reclamation.

Figure 3.33 *The second study open-air trial with three shrubby species: big sagebrush (Artemisia tridentata), common rabbitbrush (Ericameria nauseosa), and soopolallie (Shepherdia canadensis). Plants are randomized in 10 blocks.*

Photo credit Piotr Dzumek

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CHAPTER 4: RESEARCH CONCLUSIONS

RESEARCH SYNTHESIS

Fraser et al. (2015) state that mine reclamation should rebuild the entire ecosystem with its biodiversity rather than just restoring vegetation cover. Only restoration of a complete and fully functional ecosystem may bring sustainable and meaningful results. Simple revegetation by seeding agronomics, often non-native, might achieve temporary results only but lead to eventual collapse of the reclamation endeavor. It is clear that to restore a functional ecosystem all its components including ecological interactions and elements cycling need to be rebuilt. Usually, degraded mine substrates are not able to facilitate the sustainable and resistant ecosystem (Gardner et al., 2010). What makes the situation worse are the improper physicochemical properties of post-mining soils (Sheoran et al., 2010) that often contain high concentrations of heavy metals (Brown and Chaney, 2016). Both study experiments from chapters 2 and 3 demonstrated that the subsoils from New Afton New Gold mine and Teck Highland Valley Copper mine differed strongly. However, what they had in common was a deep lack of organic matter as well as plant and microorganisms nutrients. As both are essential for vegetation and microbiota, bare mine subsoils from two analyzed mines are rather not suitable to fulfill the role of topsoil restoration substrate in mine reclamation projects. This answers the first research objective. Nonetheless, both experiments demonstrated as well that the unfavorable features of analyzed mines' subsoils could be bettered by soil organic amendments application.

Three organic soil amendments were applied in both research studies. From biochar, woodchips, and biosolids only the latter have brought positive, statistically significant results in both short-term experiments. Biochar and woodchips applied alone, as low in nutritional value and containing mostly the recalcitrant carbon, did not show any positive effect on either plant survivorship or productivity. Only biosolids demonstrated a positive effect on plant productivity, but when applied in excess demonstrated a negative influence on plant survivorship especially on New Afton New Gold subsoil. This may be attributed to the fact that biosolids is an organic material containing large concentrations of essential plant and microorganisms nutrients such as nitrogen, phosphorus, zinc, and sulfur. Additionally, these elements are present in chemical forms easy for biological uptake (Sullivan et al., 2015). However, when the dose is too large, an excess of the above elements can act adversely on plant tissues (Elhanafi et al., 2019). When woodchips and biochar applied alone did not act beneficially in short term due to their low elemental nutrients content and recalcitrant form of carbon, they seemed to play important roles as overfertilization prevention and favorable substrate structure supporters when applied together with biosolids in the soil amendments mixtures. Results of this research confirm that neither biochar (Canadian AgriChar, 2020) nor woodchips (Cheng, 2008) should be applied on their own only but they act supportively to other organic or inorganic fertilizers. The above findings brought answers to the second objective of this research.

The third research objective focused on the qualitative and quantitative change of nitrogen in the mine subsoil subdued to reclamation treatments. Nitrogen is essential for all living organisms (Campbell et al., 2008). Therefore, the constant provision of this element and restoration of its cycling is necessary for mine reclamation to be successful. The main observation from both studies was that unamended subsoils from both mines were strongly deprived of nitrogen. None of the applied organic amendments but biosolids only was a good source of this intrinsic element. Plants take up nitrogen mainly in the mineral forms of NH_4^+ and NO_3^- (Geng and He, 2020). However, biosolids provide large amounts of ammonium only but hardly any nitrate. Some plats exhibit a preference for either mineral nitrogen forms (Tylova-Munzarova et al., 2005; Tang et al., 2020). This suggests that nitrate-oriented plant species would not perform well on either of mine subsoils even when amended by biosolids. Chalk and Smith (2020) demonstrate though that plants can be flexible in respect to the use of mineral nitrogen forms. Both studies' results of this research showed that throughout the length of experiments the total nitrogen content, along with mineralizable nitrogen, and NH_4^+ content dropped, while NO_3^- content increased. That in turn showed that nitrate-preferring plants can be applied in mine reclamation projects using subsoils amended by biosolids. Such plats would obtain their more suitable mineral nitrogen which would eventually result from the process of nitrogen transformation.

MANAGEMENT IMPLICATIONS & FUTURE RESEARCH

As topsoil is scarce, there is an interest in whether the subsoil could be an effective substitute for topsoil in mine reclamation. However, as demonstrated in this research mine subsoils can differ strongly. Subsoils from one location may possess strongly negative physicochemical features that would eliminate such material from usage in mine reclamation. In other instances, a simple amelioration could alter subsoil features to the extent that such material might become a very valuable mine reclamation material. Concluding from this, subsoils vary strongly from place to place and because of this need to be well analyzed prior to application in mine reclamation. Results from one mine might be not representative for another mine.

Organic amendments often turn out to be better than commercial fertilizers. Moreover, they can be combined bringing various beneficial features into the system. A combination of biochar, woodchips, and biosolids resulted in the largest plant productivity. Moreover, organic soil amendments result often as waste from local industry e.g. woodchips or communities habitation, and day-to-day functioning e.g. gardens' waste compost or biosolids. That makes these potential soil amendments cheap and available locally which in turn minimizes the transportation needs lowering the cost (Larney and Angers, 2012; Piorkowski et al., 2015) and CO_2 emission. However, organic soil amendments have also their downsides. For example, biosolids come as a by-product from municipal wastewater treatment (Sullivan et al., 2015). As such, this product might contain various unwanted substances like antibiotics, traces of household chemicals, and metabolites of medicines just to mention a few. There is no study which has demonstrated negative effects of biosolids to human health, nonetheless, above-mentioned substances may possess ecotoxic features. Waterhouse et al. (2014) demonstrate in their study that despite plant productivity's strong increase an application of biosolids caused elevated mortality among native earthworms. Earthworm mortality in biosolids-amended soils was 100%, compared with 42% and 25% in stockpiled and unmodified soils respectively. This demonstrates a need for more research on soil amendments' ecotoxicity, bioaccumulation, the influence of composting times on organic amendments' adverse features, proper proportions and dosing, as well as on potential substances leaching problem.

Until recently, in British Columbia, agronomics were domint plants used in revegetation. This resulted from several facts: such plants had adaptability to a wide range of conditions, grew fast, prevented soil erosion quickly after being sown, and often presented high forage value, their propagules were easily available and significantly cheaper than native plants. Non-native graminoids or forbs such as legumes (e.g. alfalfa or clover) have been widely used for example as cover crops (Elias and Chadwick, 1979; Jefferies et al., 1981; Tribouillois et al., 2014). On the other hand, the importance and potential use of native plants in the field of revegetation was not well recognized and, therefore, few economic entities have dealt with the propagation of these plants. However, non-native plants have their disadvantages too. Often, these plants did not give way to native plants during the succession process (Davis et al., 2005). Besides, some of the introduced species could change their character to invaders. Another consequence of the presence of non-native plants is the possible resistance to locally present population limiting factors such as diseases, or herbivory, which builds their competitive advantage over native plants. Yet, the non-native organisms are accused of bringing new, previously unknown diseases or other harmful organisms, such as non-native herbivorous insects, to these areas. With scientific investigation and shifting cultural values, more attention is being paid to increase our understanding and appreciation of native plants. Regarding the above further research on native plant species applicability in mine reclamation, especially species involved in nitrogen fixation such as field locoweed or soopolallie, would be a continuation of a good trend of transition from introduced agronomics to species native in British Columbia.

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Appendix A – First Study Remaining Elements Results

Carbon

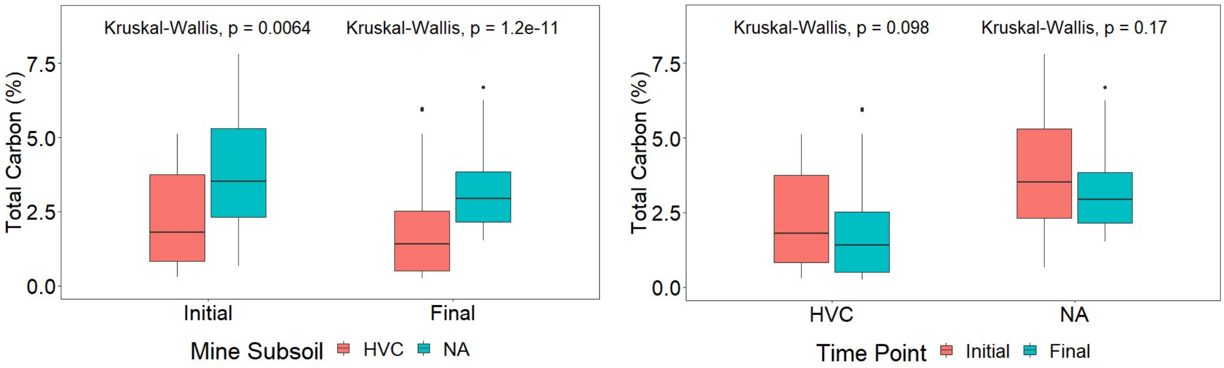


Figure A.1 *Two mines' subsoil total carbon content comparison in the initial and final stages of the experiment (left), comparison of the total carbon content in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 25, final n= 75*

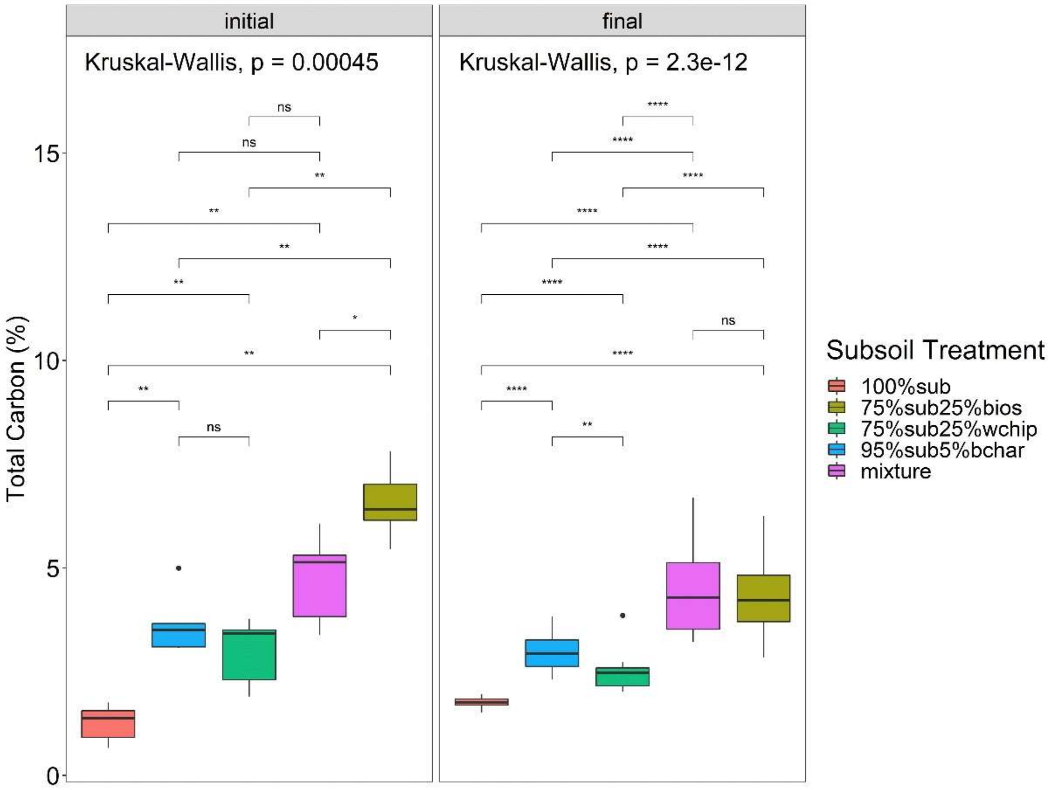


Figure A.2 *New Afton New Gold subsoil treatments total carbon contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison * - $p < 0.05$, ** - $p < 0.01$, **** - $p < 0.0001$, ns – difference not significant. initial n= 5, final n= 15*

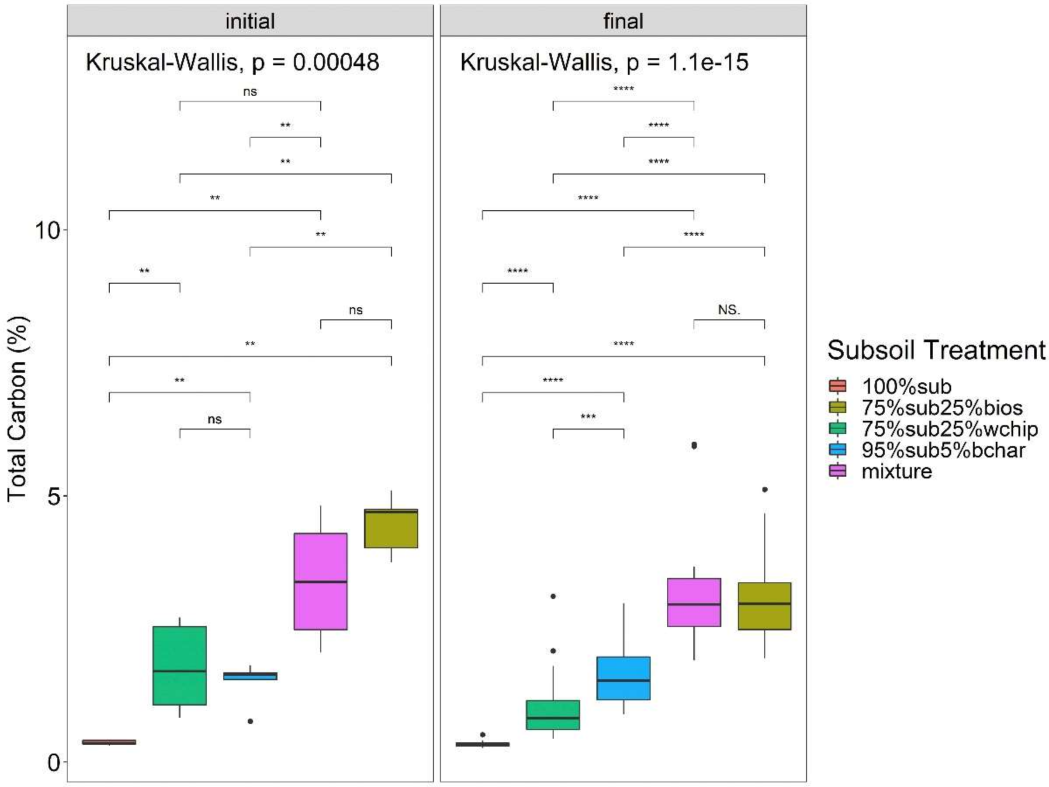


Figure A.3 *Teck Highland Valley Copper subsoil treatments total carbon contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 75%sub25%bios – subsoil amended by 25% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison ** - p < 0.01, *** - p < 0.001, **** - p < 0.0001, ns, NS. – difference not significant. initial n= 5, final n= 15*

Aluminum

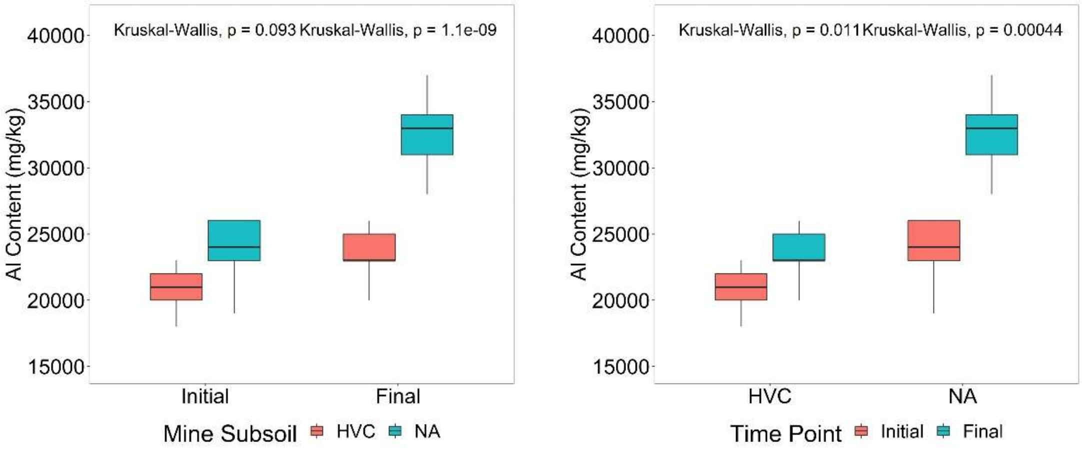
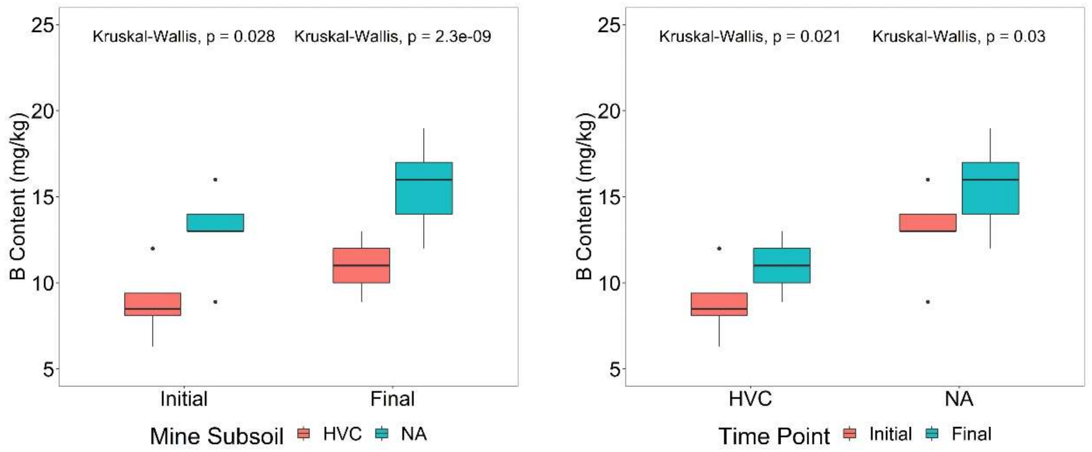
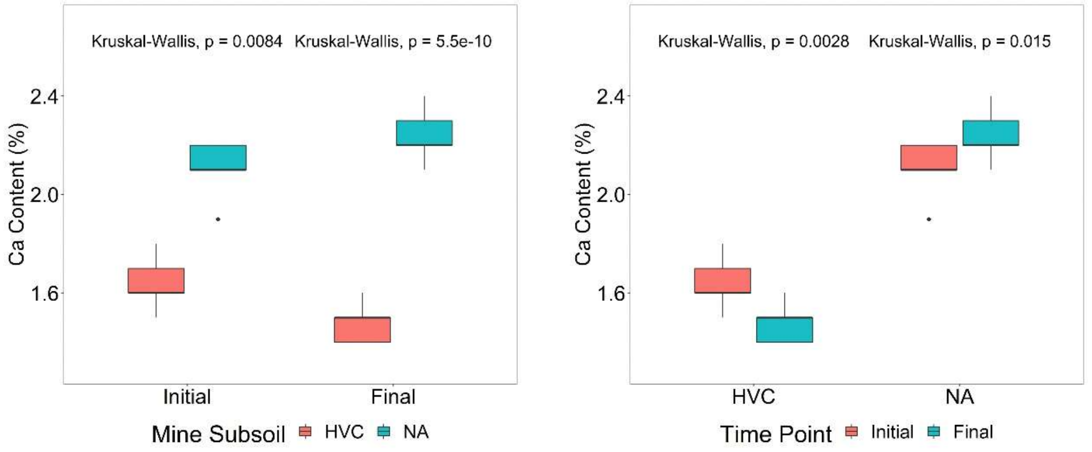


Figure A.4 *Two mines' subsoil aluminum content comparison in the initial and final stages of the experiment (left), comparison of the aluminum content in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

Boron



Calcium



Copper

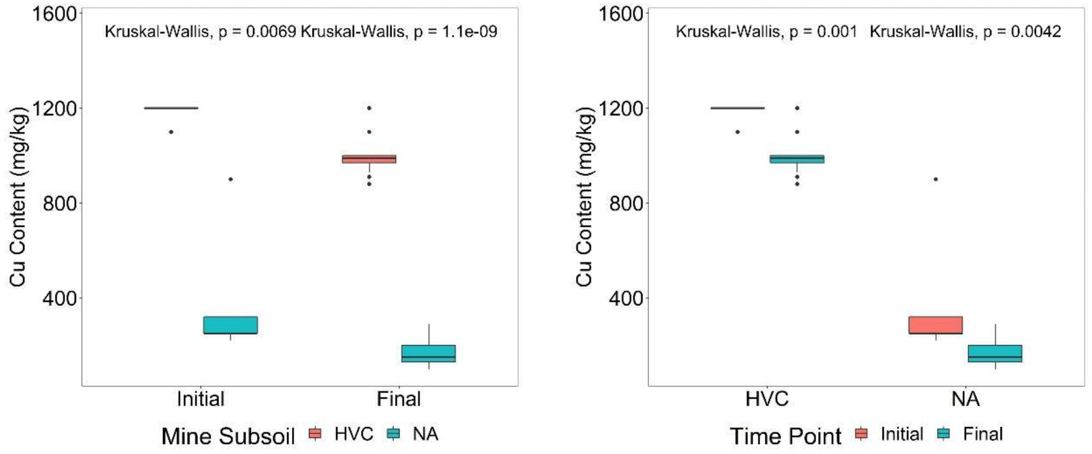
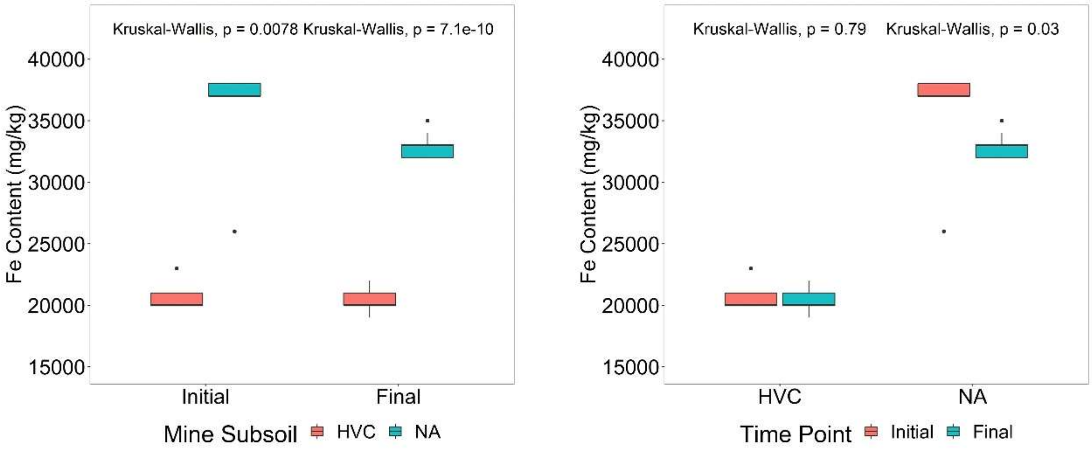
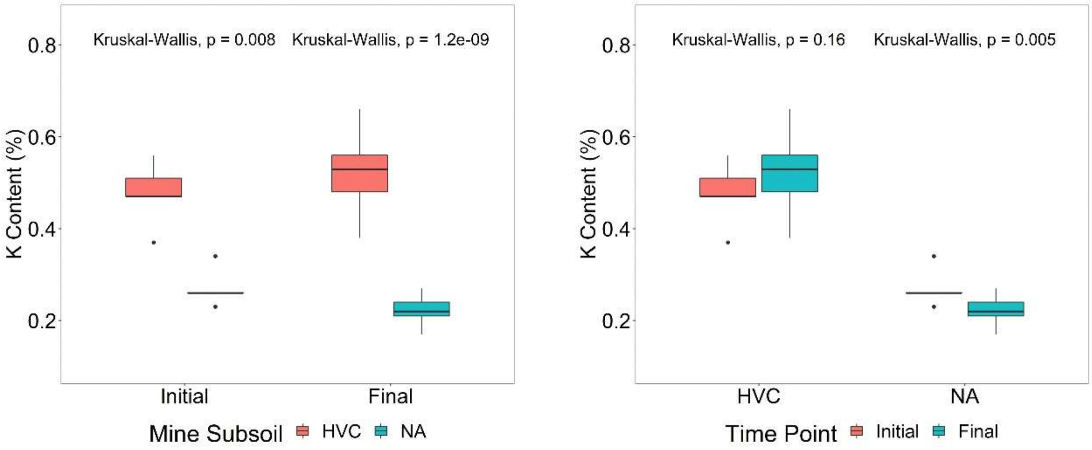


Figure A.5 *Two mines' subsoil boron, calcium, copper contents comparison in the initial and final stages of the experiment (left), comparison of the boron, calcium, copper contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

Iron



Potassium



Magnesium

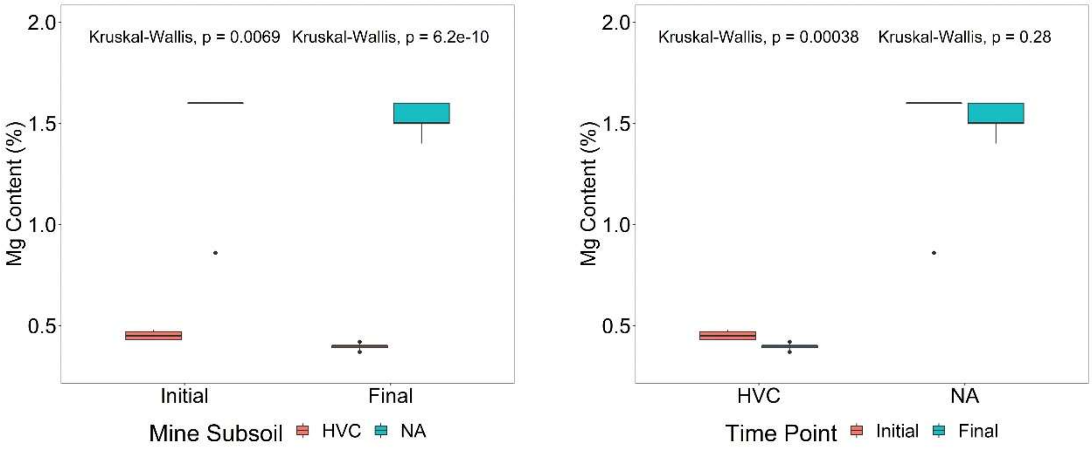
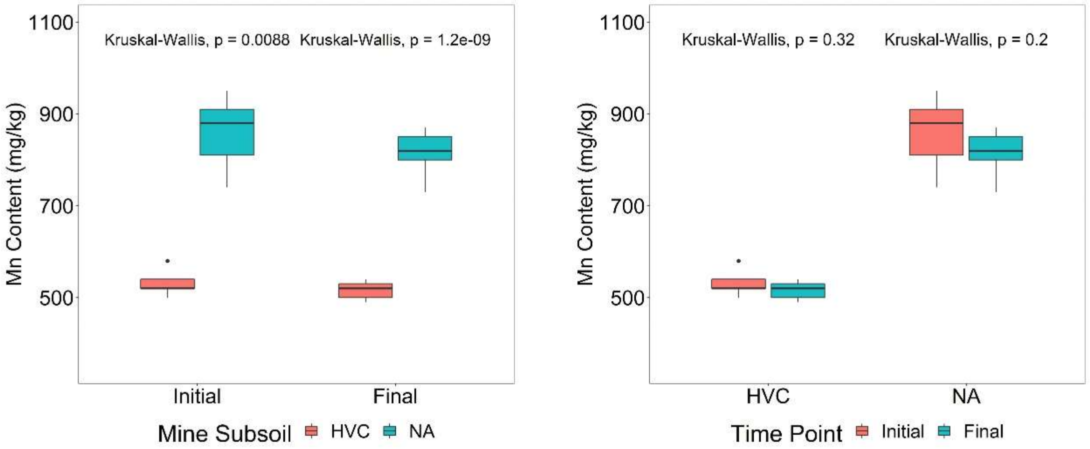
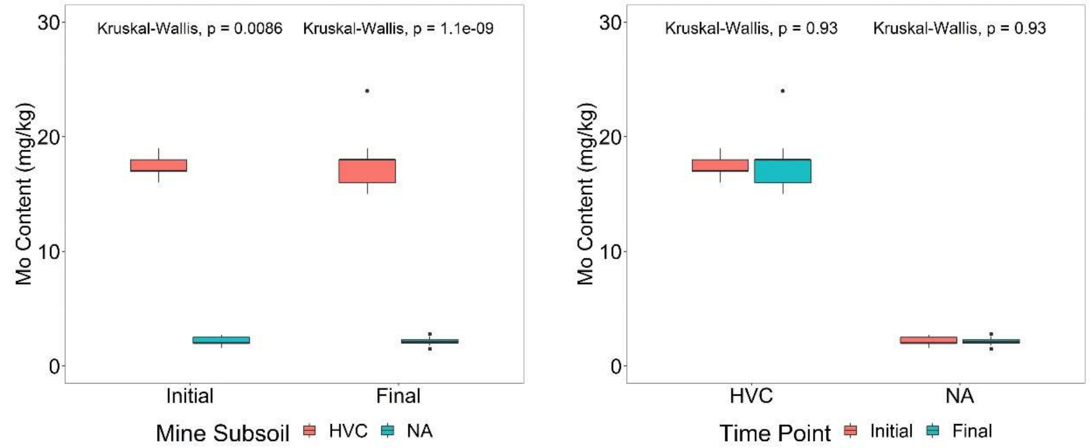


Figure A.6 *Two mines' subsoil iron, potassium, magnesium contents comparison in the initial and final stages of the experiment (left), comparison of the iron, potassium, magnesium contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

Manganese



Molybdenum



Phosphorus

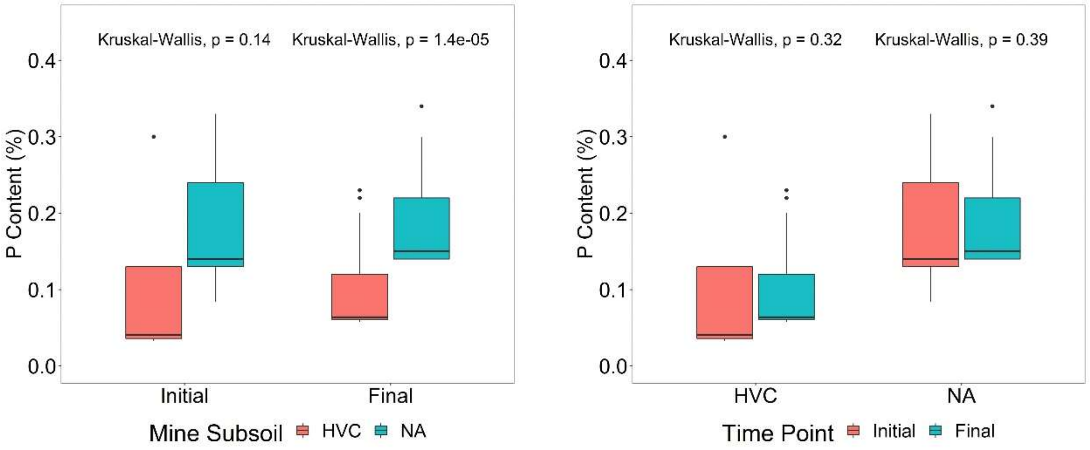
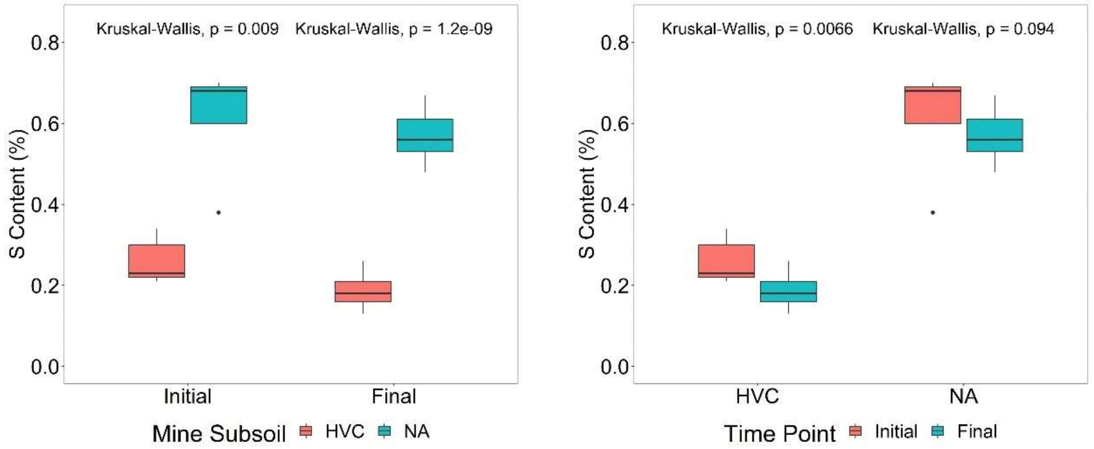


Figure A.7 *Two mines' subsoil manganese, molybdenum, phosphorus contents comparison in the initial and final stages of the experiment (left), comparison of the manganese, molybdenum, phosphorus contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

Sulfur



Zinc

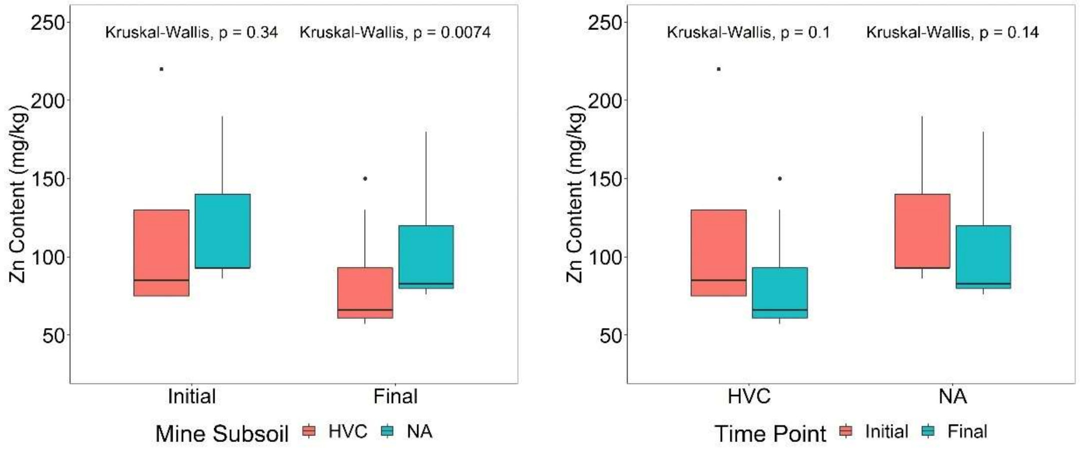


Figure A.8 *Two mines' subsoil sulfur, zinc contents comparison in the initial and final stages of the experiment (left), comparison of the sulfur, zinc contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 25*

Appendix B – Second Study Remaining Elements Results

Carbon

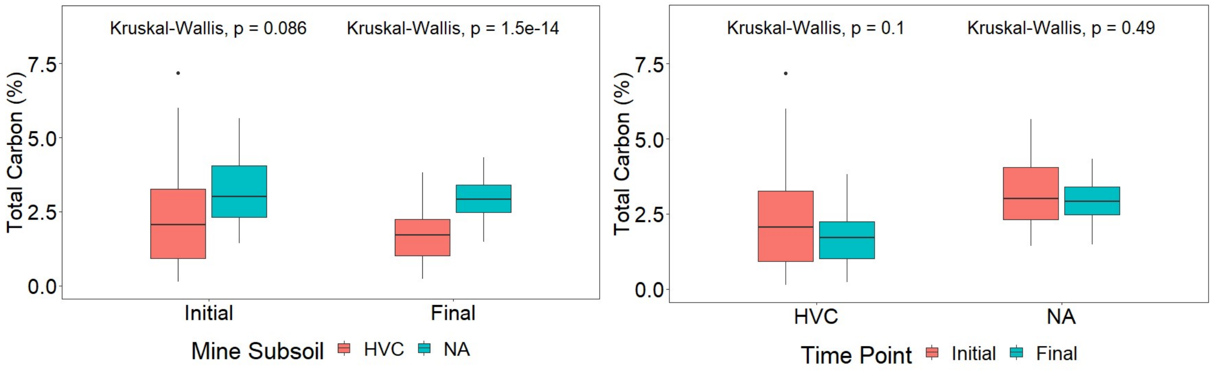


Figure B.1 *Second study two mines' subsoil total carbon content comparison in the initial and final stages of the experiment (left), comparison of the total carbon content in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 25, final n= 75*

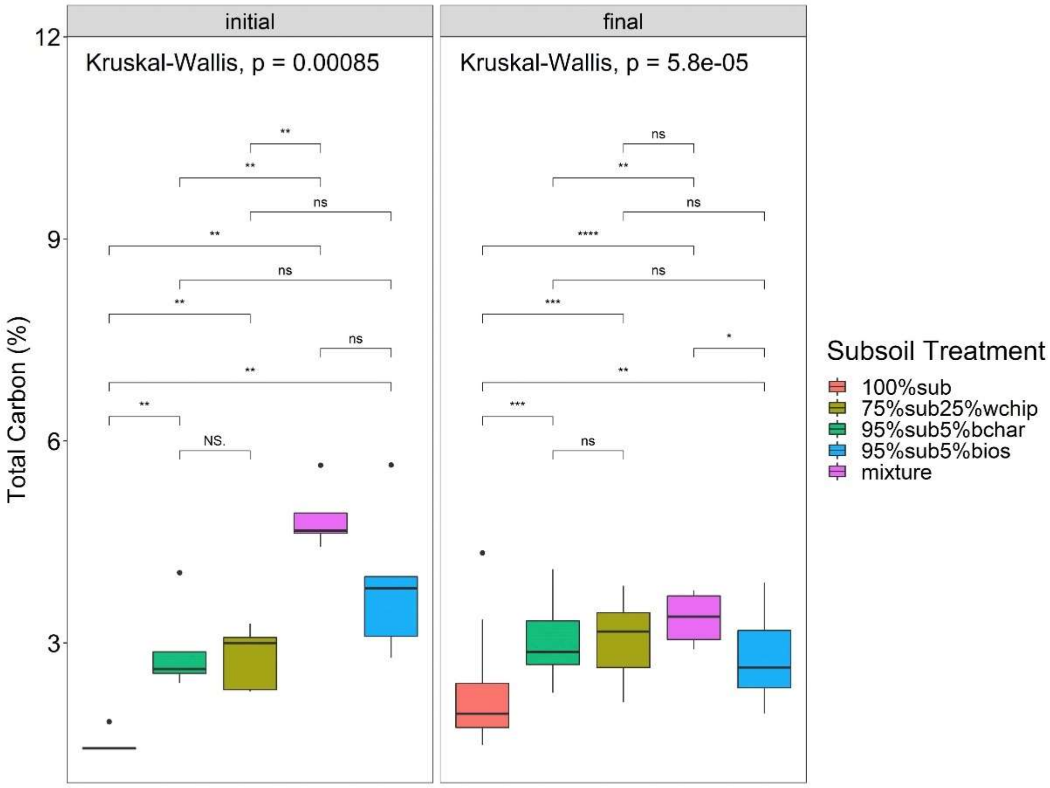


Figure B.2 *Second study New Afton New Gold subsoil treatments total carbon contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, **** - $p < 0.0001$, ns – difference not significant.*
initial n= 5, final n= 15

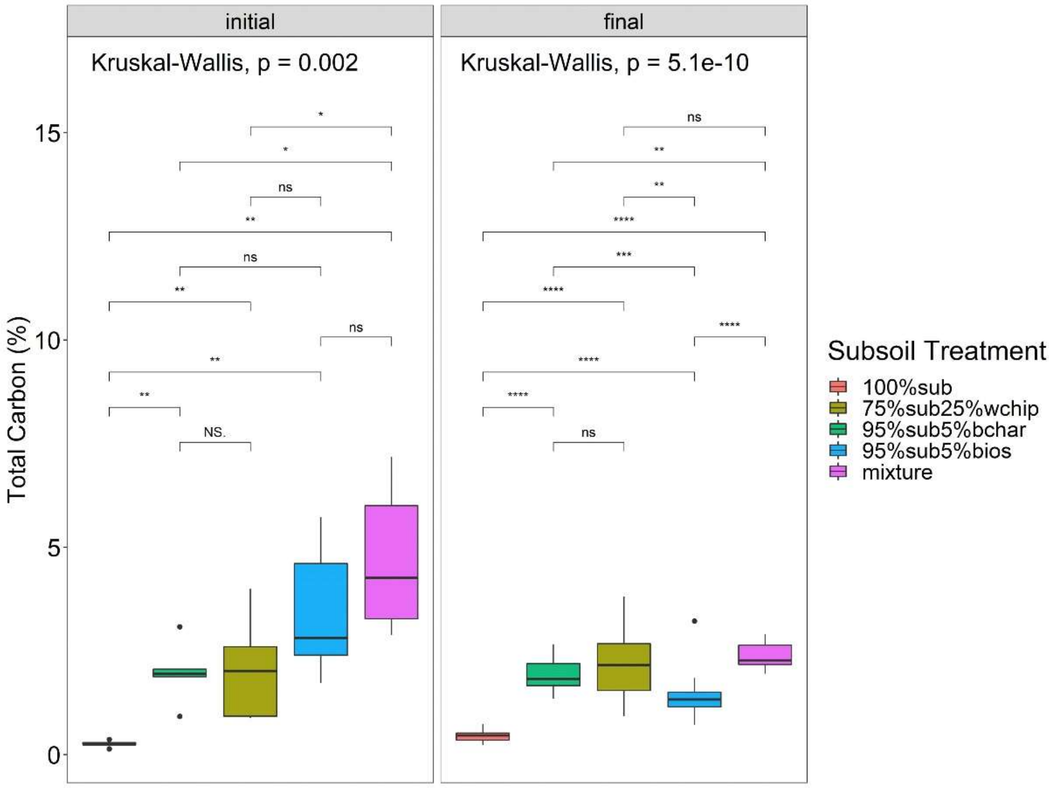


Figure B.3 *Second study Teck Highland Valley Copper subsoil treatments total carbon contents comparison in the initial and final stages of the experiment. Subsoil treatments: 100%sub – unamended subsoil, 95%sub5%bios – subsoil amended by 5% of biosolids alone, 75%sub25%wchip - subsoil amended by 25% of woodchips alone, 95%sub5%bchar – subsoil amended by 5% of biochar alone, mixture - subsoil amended by a mixture of amendments. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. Wilcoxon test was used for pairwise comparison * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, **** - $p < 0.0001$, ns, NS. – difference not significant. initial n= 5, final n= 15*

Aluminum

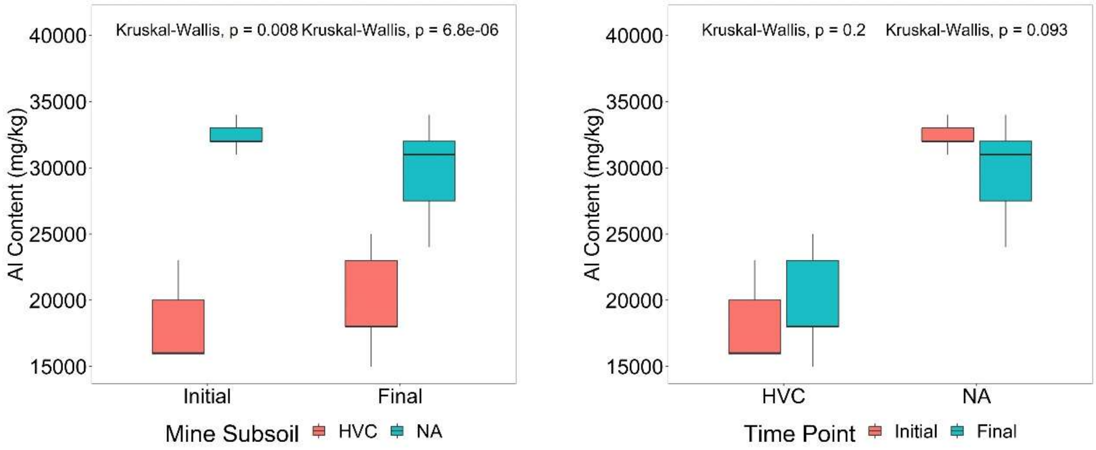
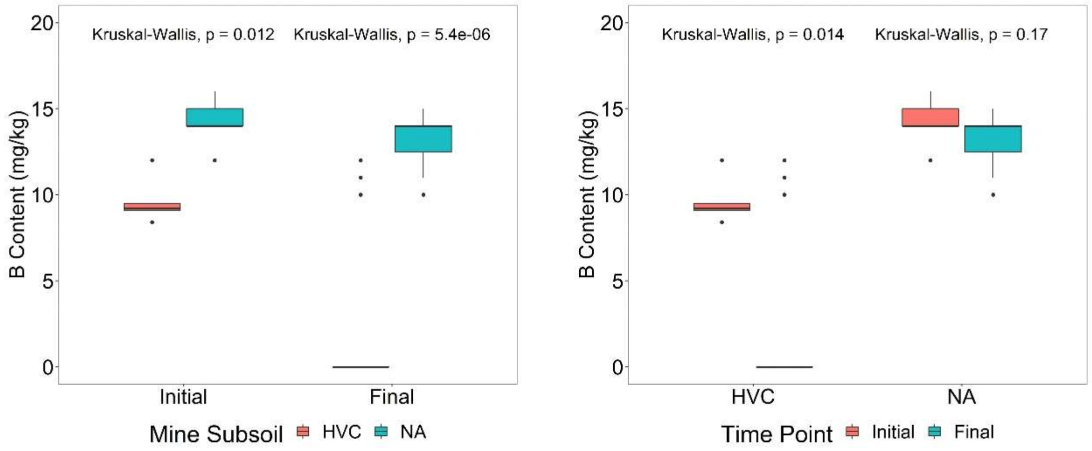
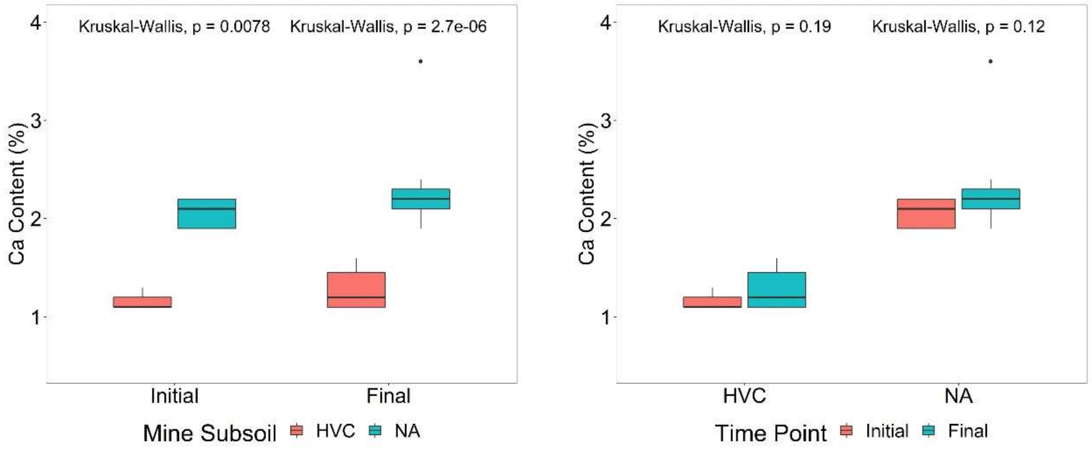


Figure B.4 *Second study two mines' subsoil aluminum content comparison in the initial and final stages of the experiment (left), comparison of the aluminum content in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*

Boron



Calcium



Copper

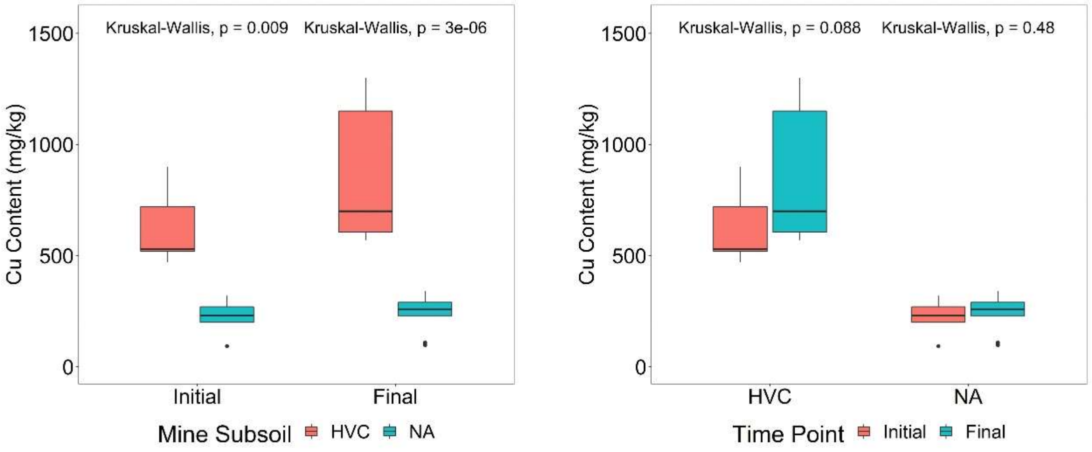
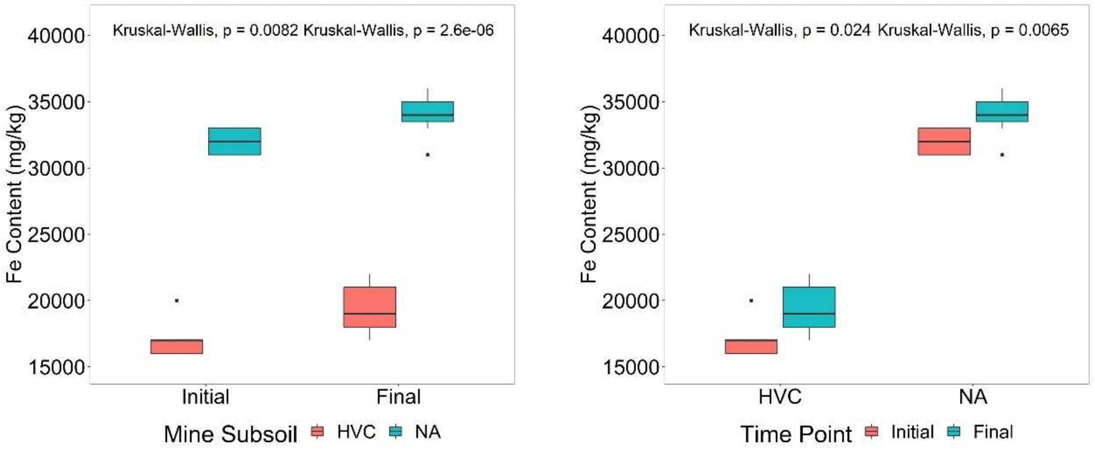
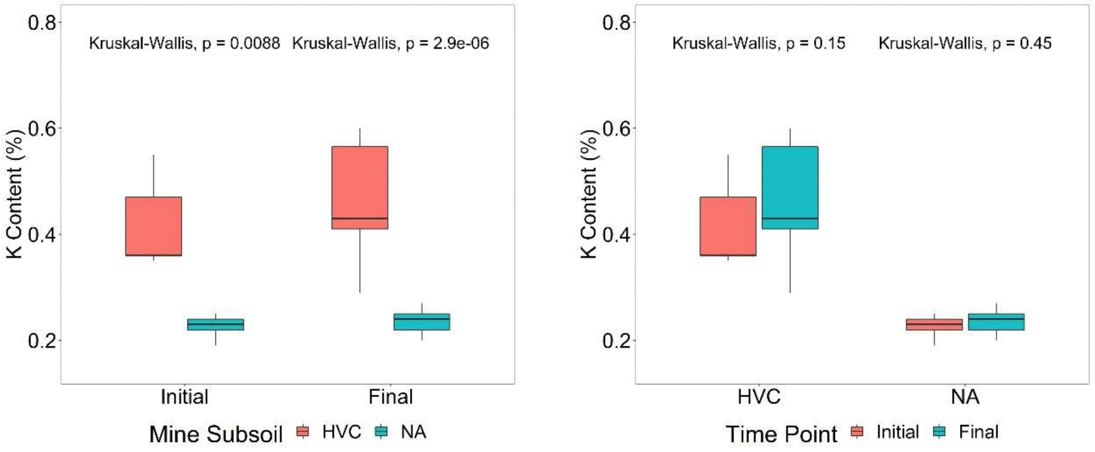


Figure B.5 *Second study two mines' subsoil boron, calcium, copper contents comparison in the initial and final stages of the experiment (left), comparison of the boron, calcium, copper contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*

Iron



Potassium



Magnesium

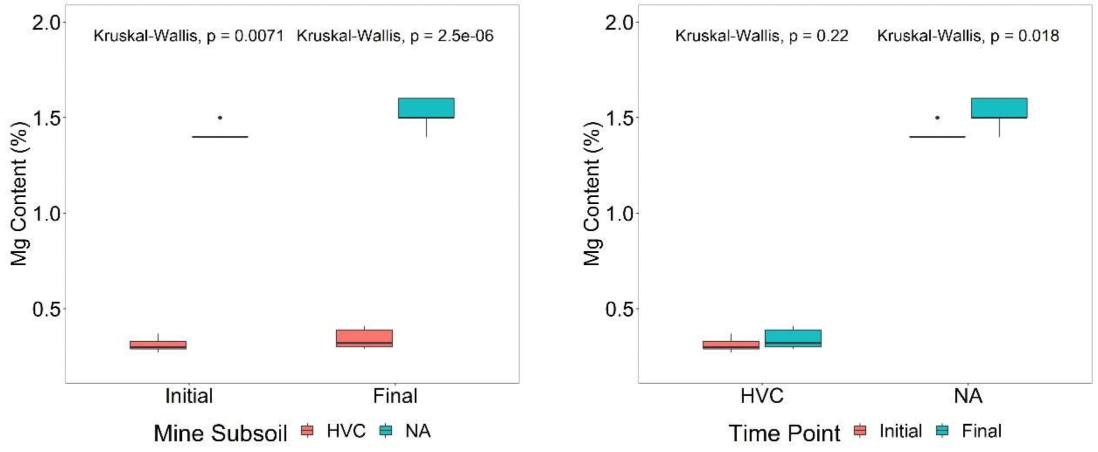
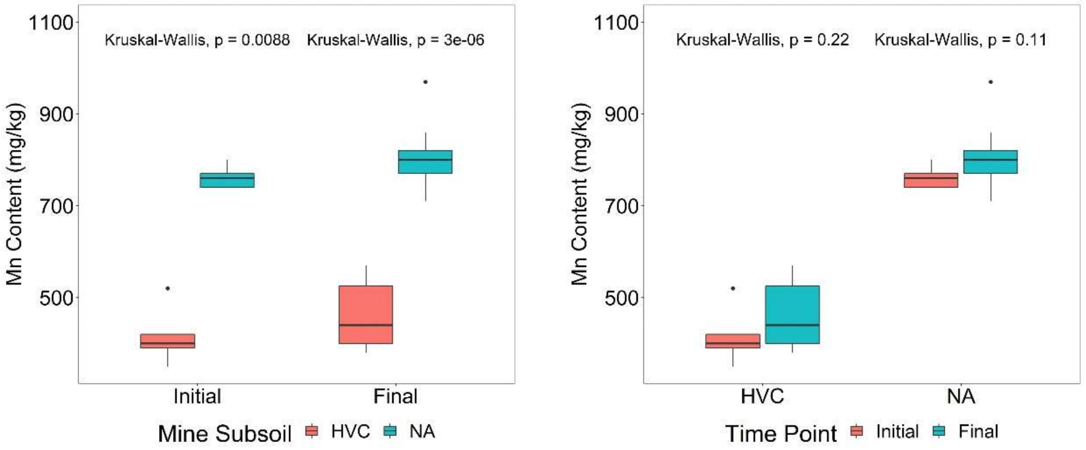
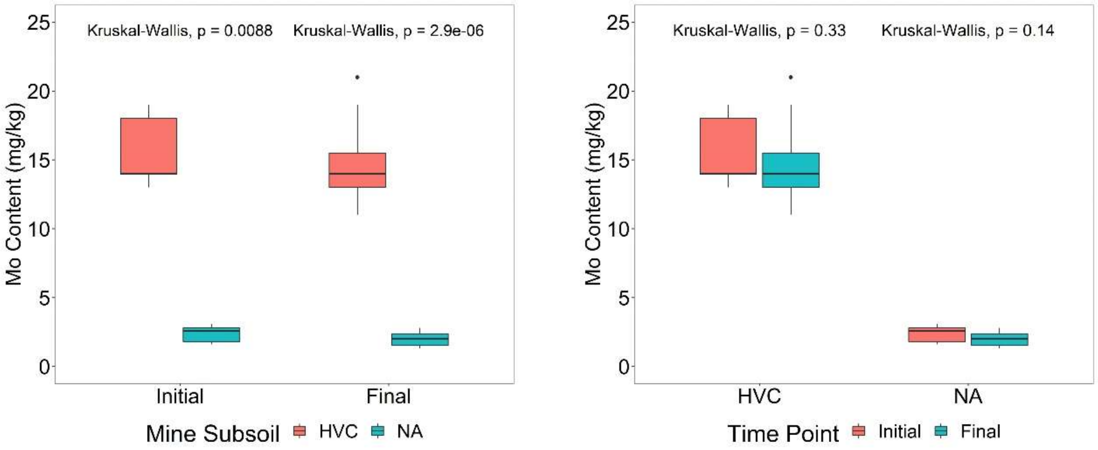


Figure B.6 *Second study two mines' subsoil iron, potassium, magnesium contents comparison in the initial and final stages of the experiment (left), comparison of the iron, potassium, magnesium contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*

Manganese



Molybdenum



Phosphorus

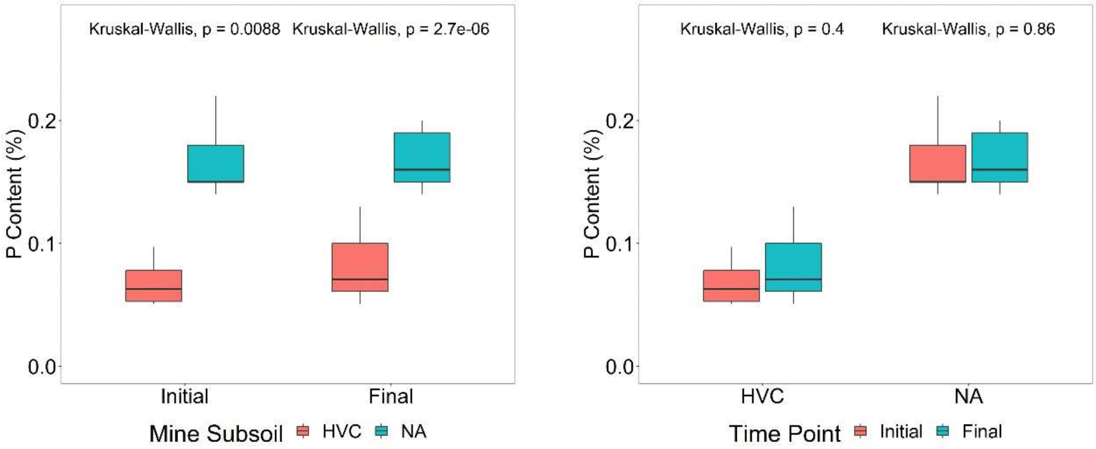
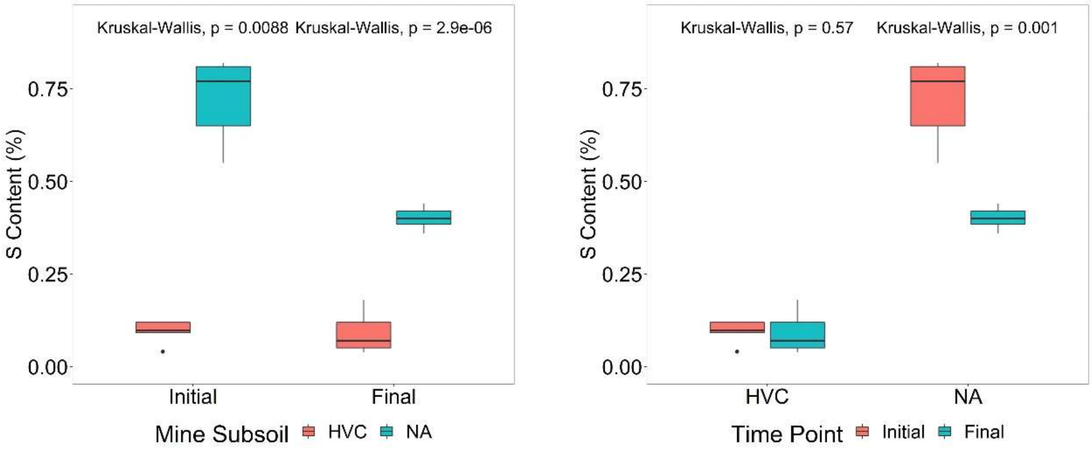


Figure B.7 *Second study two mines' subsoil manganese, molybdenum, phosphorus contents comparison in the initial and final stages of the experiment (left), comparison of the manganese, molybdenum, phosphorus contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*

Sulfur



Zinc

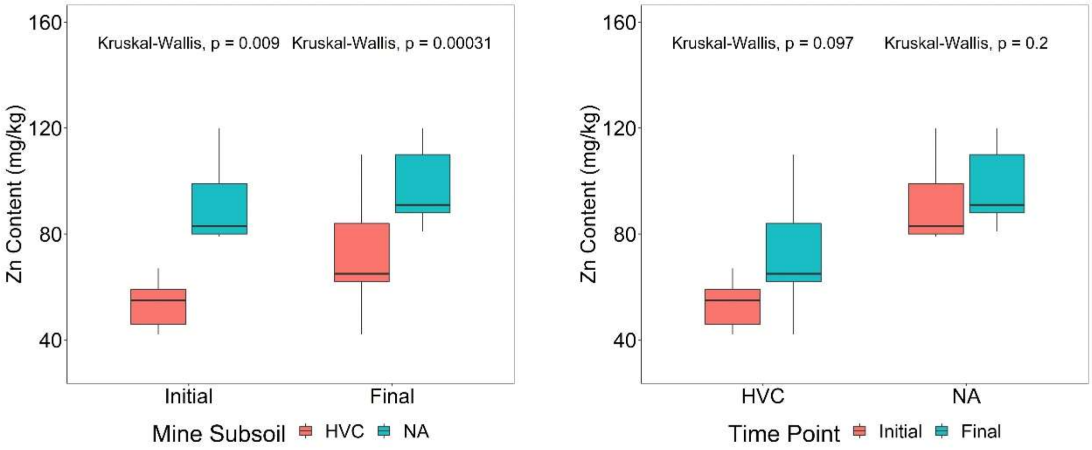


Figure B.8 *Second study two mines' subsoil sulfur, zinc contents comparison in the initial and final stages of the experiment (left), comparison of the sulfur, zinc contents in the initial and final stages of the experiment within each mine (right). HVC – Teck Highland Valley Copper, NA – New Afton New Gold. Horizontal line indicates the median. Kruskal-Wallis test was used to compare the medians. initial n= 5, final n= 15*