INFLUENCE OF BIOCHAR APPLICATION ON PLANT NUTRIENTS

A Dissertation Presented

by

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Submitted to the Czech University of Life Sciences
in partial fulfillment
of the requirements for the degree of

ENVIRONMENTAL DATA SCIENCE

April 2022

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DEDICATION

I dedicate this Dissertation to Ouilly, who passed away this week. May he rest in peace.

ACKNOWLEDGMENTS

I want to thank my supervisor doc. Ing. Kateřina Berchová for her immense patience.
I would also like to thank Martin for being such a good friend.

ABSTRACT

The aim of the experiment was to determine the influence of biochar on plant nutrient concentration. Other factors were taken into consideration, such as plant genus and plant components. The experiment was carried out in a greenhouse and the following genera were planted: Deschampsia, Heucera, Hedera, Sedum and Festuca. The plants were submitted to two different soil types, one containing spruce biochar and the other normal soil.

A thorough literature review was made on different types of biochar and their effect on soil chemical and physical properties. The effect of these changes over plant nutrient uptake and content was carefully reviewed.

After a four-month period, the pants were collected and their samples processed in the laboratory, separating the root from the stem. A thorough analysis of their nutritional content was made. The concentration for the following nutrients was observed: Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, S, Se, Sr, Ti, V and Zn. The nutritional variables were selected and divided into three categories: primary nutrients (P and K), secondary nutrients (Ca, Mg and S) and micronutrients (Fe, B, Cu, Cl, Mn, Zn, Co and Ni). The analysis was carried out for both the root and stem nutritional values.

Statistical analysis was made with R (r project). A two-way analysis of variance (ANOVA) was done on individual concentrations, with a significant level of $p<0.05$, followed by Tukey’s HSD test. The statistical significance of biochar application and the different type of genus was tested and reported.

The results showed that biochar significantly affected a few nutrients, both in the root and stem, but with some statistical problems related to the nature of the data. A stronger significance with genus was observed across a larger number of nutrients, hinting at the fact that the different genera interacted differently with specific nutrients.

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LITERATURE REVIEW

Introduction

Biochar is a carbon rich substance produced from the pyrolysis (combustion under low levels of oxygen and high temperatures) of organic matter. Modern industrial bioenergy systems, involving pyrolysis and gasification, focus on the production of combustible gas (syngas) and oil (biooil). The products are usually sold as fuel to generate energy. Biochar comes as a side product (Yao 2018).

Soil organic carbon (SOC) is one of the most important sink and sources of nutrients, and it plays a crucial role in plant development and soil fertility (Pan 2009). In recent years, agricultural lands have been affected by increased climatic stress conditions, such as rise in temperatures, intensified rain and drought seasons and extreme soil erosion and leaching. Such pressures have created a state of nutrient depletion. The default solution has been the addition of organic and inorganic fertilizer (Topoliantz 2005), which improve soil fertility in the short run. Although, this only created a dependence on such products, which need to be amended constantly. In addition, the lack of a solid carbon “back bone” prevents the soil from retaining its nutrients, especially after heavy rainfalls, where the efficiency of inorganic fertilizers is minimal due to leaching of highly mobile nutrients, such as K and NO^{3-}. (Cahn 1993) Furthermore, the application of organic fertilizers is usually short-lived, as they tend to mineralize relatively quick, especially in tropical conditions (Diels 2004,). The application of more stable compounds, such as biochar, that can retain nutrients for a longer period of time and decompose slowly, could be a potential alternative to the continuous and unsustainable application of inorganic and organic fertilizers.

The use of biochar is not new, in fact it has been around for centuries. Charcoal-like organic matter have been discovered in the Amazon basin several feet below ground. In fact it is believed that pre-Colombian farmers used it to amend their nutrient-poor soil more than 2000 yr ago (Lehmann 2006, check). These patches of soil are called “Terra Preta de Indio”, which have been shown to have favorable chemical and physical properties, such as high CEC, WHC and relatively high pH compared to the surrounding soil, which is quite acidic and poor in nutrients (Lehmann 2003, Lehmann 2007). The fertility of these patches has persisted for centuries, despite the lack of anthropogenic activity.

The use of biochar has been studied carefully in the past years, and many benefits have been attributed to its application, such addition nutrients, increase in soil CEC and WHC, alteration of pH and gradual release of nutrients for plant uptake (Chen 2010, Palansooriya 2019). Positive effects of biochar on plant nutrient concentrations have also been observed, with interesting results regarding its total biomass and chemical profile (Lehmann 2003, Chan 2007).

More generally, biochar has been shown to affect agricultural land efficiency and its overall productivity (Dumortier 2020). Reports estimate that by 2050 the global population will reach 9 billion people, followed by a drastic increase in food demand (FOA). An increase in agricultural activities will be inevitable, which will see more land utilized for resource exploitation. Such pressure might take a toll on the out-of-data practices implemented by the agricultural sector. The need for long-term sustainable technologies has never been more urgent. In fact, ensuring a healthy and continuous production of large quantities of crops could be the biggest challenge of this generation. Biochar might be part of the solution.

Characteristics of biochar

The physical and chemical characteristics of biochar are the main drivers behind its effectiveness as a soil additive (Lehman 2009). The conditions under which it is produced determine its physical properties, whereas the type of pyrolytic feedstock influence the chemical characteristics. The interaction of these two determine its overall biological composition (Zhang 2020).

When added to soil, biochar can affect it in two main ways: directly providing nutrients stored on its surface and altering the soil nutrient transformation cycles. Such variations affect plant nutrient uptake and consequently its growth and development (Atkinson 2010).

Different types of biochar exhibit different nutritional profiles. For example, it has been suggested that biochar from feces and sludge contains more micro and macro nutrients than biochar obtained from plant material (Lehmann 2011). In such cases, biochar can also be used as an alternative to modern fertilizers, thanks to its high-nutrient concentration (Chew 2020). Specific biochars must be applied to different soil types in calculated doses, similarly to fertilizers applications. In fact, excessive use can negatively impact plant development and its growth.

Biochar can also improve soil quality by altering its chemistry. Such alterations have been attributed to changes in cation exchange capacity (CEC) and pH (Cheng 2006, Liang 2006 to read). In addition, biochar has been shown to affect availability of both micro and macro nutrients.

On the other hand, some biochars have also been attributed to cause an increase in availability of heavy metals in soil. For example, an excessive amounts of toxic trace elements have been found in biochar produced from sewage and tannery wastes. Their application showed an increase in soil toxicity, inhibiting plants growth (Devi 2014). Such biochars would be considered undesirable for any agricultural application.

Biochar is also characterized by peculiar physical characteristics, such as high surface area and porosity. These traits are strongly influenced by its chemistry and feedstock material, as well as by pyrolytic temperature. Biochar pore structure can be divided into two categories: micropores and macropores. Each category affects soil dynamics in a different way. Micropores, for example, influence soil bulk density (BD), porosity, WHC, soil aggregation (SA), aeration and its biotic population. Macropores, on the other hand, play an important role in root exploration (Ajayi 2016).

The various types of biochar make it extremely difficult to generalize its nature and therefore create a universal model that can predict its effects on soil and plant growth. Therefore, a careful selection of biochars need to be made for specific soil types applications.

Types of biochar

Different biochars can be made from different feedstock materials, pyrolytic techniques, and temperatures. There are essentially two main types of biochar: animal and plant derived. Plant derived biochar tend to have a higher concentration of carbon (C) and lower concentration of nitrogen (N), potassium (K), calcium (P), phosphorous (Ca), magnesium (Mg), copper (Cu), aluminum (Al) and sodium (Na), than the animal type. In addition, it has been observed that plant-based biochar has a lower CEC and pH (Chan 2008).

Studies have found high levels of nitrogen (N) in cow manure and poultry litter-based biochar, potentially making them a valid alternative to modern N-fertilizers (Lang 2005). On the other hand, wood biochar showed high ratios of C/P and C/N ratios and a lower CEC. Thanks to their original fibrous texture, wood biochar was reported to increase saturated hydraulic conductivity (SHC) in a more significant way than its animal-based alternative (Lei and Zhang, 2013).

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Effects of biochar on soil

One of the main reasons behind the increasing interest for biochar is related to its potential land management and agriculture application. In the past years a significant amount of research has been carried out on the effects of biochar on soil dynamics and its fertility (Lehmann 2007). It has been observed that biochar can directly and indirectly alter a series of complex soil nutrient dynamics when amended to specific soils. (Bierderman 2012, Marchner 2006)

The purpose of this section is to summarize the various mechanism through which biochar influences the availability of soil nutrients to plants, focusing on specific nutrition cycles, such as Nitrogen (N), phosphorous (P) and sulfur (S), both in the short and long term.

Soil interactions

Biochar in soil plays an important role in various nutrient transformation dynamics, for example within the N-cycle it has been observed to affect a series of complex mechanism, such as microbial N–fixation, N–mineralization, nitrification, denitrification, and gaseous N losses (Clough 2010). Such alterations can have a short and long-term effect on the overall soil fertility, ultimately affecting plant growth. These cycles also play an important role in the sequestration of greenhouse gasses (GHG), making biochar a potential tool for carbon sequestration (Fatima 2020).

The possible mechanisms through which biochar can influence various nutrient cycles are increase in nutrient pool size and turnover of organic matter, alteration of physical and chemical soil properties and alteration on soil biota (DeLuca 2006)

Pool size and turnover

Biochar was shown to accelerate a series of nutrient cycles by short-term introduction of nutrients to soil ecosystems, increasing its nutrient availability for plant uptake (Dey 2022). The size of organic pools was also observed to increase, depending on biochar type. In fact, during pyrolysis, the different temperatures cause different levels of nutrient volatilization on its surface and different levels of nutrient allocation in the remaining parts of the biochar (DeLuca 2006). Other factors such as feedstock material, retention time, oxygen availability and heating rate can influence its surface residue chemistry (Atkinson 2010).

Different nutrients volatize at different temperature. For example, carbon starts to volatize at around 100 °C, nitrogen at 200 °C, sulfur above 375 °C, phosphorus and potassium at around 770 °C, whereas magnesium, calcium, and manganese volatize at higher temperatures, usually above 1000 °C (Neary 2005). These differences cause a disproportion in elemental concentration, creating different types of biochar with different chemical profiles. For example, low heat ("slow pyrolysis") biochar (<500 °C) has been shown to have higher concentrations of C and N, which decrease as pyrolytic temperature increases (> 500 °C) (Trompowsky 2005). The concentration of nutrient salts on biochar surface also affects its nutritional input to soil and therefore its bioavailability to plants. An interesting feedback loop occurs where richer nutrient soils produce richer nutrient plants which in turn, after completing their life cycle, start decomposing, returning their nutrients to the environment (Major 2010). It was observed that different components of the plant release such nutrients through different mechanism. For example, the root release of C occurs by exudation and turnover, whereas the above ground tissues recycle their nutrients through senescence.

Physical and chemical properties

Biochar physical characteristics allows it to play an important role in soil. Such characteristics include a high surface area coupled with a complex porous structure (Atkinson 2010).

Biochar can in fact directly alter soil physical and chemical properties. For instance, biochar has been shown to increase WHC, alter CEC, and increase soil surface sorption capacity. It has also been observed that biochar can increase base saturation of acidic mineral soils and alter its pH (Juriga 2019). Most importantly, thanks to its high surface area and porosity, biochar provides an ideal environment for soil microorganism, such as bacteria and fungi (Atkinson 2010/).

These microorganisms require suitable soil water and redox potential to carry out their vital metabolic activities (Joseph 2015). Biochar surface area is scattered with micro (<30 ud) and macro (>75ud) pores (area). Micropores, thanks to their high area/volume ratio, serve as capillary channel for water accumulation and storage (important during dry seasons), whereas macropores serve as gas exchange channels, influencing the redox potential (Lehmann 2011). Such conditions support a more aerobic environment, richer in oxygen, ideal for decomposition of organic material and its mineralization. A more aerobic environment would also affect nitrification and S oxidation, which requires oxygen as an electron donor, making biochar a potential stimulant for such nutrient cycles. (DeLuca 2006). These transformations combined with its physical characteristics (high porosity and surface area), make biochar an ideal environment for soil microorganisms. When a larger microbial population is met with stronger organic inputs (decomposition of organic material), a positive feedback loop is established, which in turn can yield higher volumes of organic material, improving soil fertility in the long run.

Leaching

Different types of soil have different water holding capacity. For example, sandy soils have a smaller WHC than clay soils. Amends of biochar were observed to affect such WHC, depending on biochar and soil type. For example, it has been shown that combining biochar with a sandy soil increased its water content, whereas the opposite was true for loamy-clay soils (Atkinsosn 2018). Lehmann work, combining biochar and a clay-type soil from the Amazon, showed that water percolation decreased in accordance with biochar application, positively affecting plant growth (Lehmnann 2003). Furthermore, despite the high nutrient content found in the biochar-clay mixture, nutrient leaching was minimal, possibly du to biochar strong sorption capacities.

In another study, nitrogen concentration increased with biochar applications, as well as C-N ratio, possibly causing a greater immobilization of inorganic nitrogen. It was also observed that plant uptake of other nutrients, such as P, K, Ca, Zn and Cu increased with increased applications (Lehmann 2003), possibly due to a decrease in leaching of those nutrients.

Other soil properties

Thanks to its unique characteristics, biochar has been shown to alter a series of soil physical properties, such as porosity, hydraulic, and stability (Ouyang 2013). It has also been observed to decrease BD and increase stability (Lal 2013).

Different types of biochar behave differently when applied to soil. Some biochars have been shown to affect specific soil physical properties rather than others. For example, Peng (2011) observed that peanut-shell biochar increased WHC, while rice straw biochar increased AS.

Biochar also been attributed to changes in soil pH, EC and CEC, affecting chemical and biological interactions among nutrients and their availability for plant uptake. One of the main drivers is its pH, which depends on its initial raw material, pyrolytic process, and temperature (Weber 2018). More specifically, studies have shown that biochar pH is positively related to pyrolytic temperature, due to a possible increase in organic acids during pyrolysis (Cheng 2018). Its pH value ranges between 6.5 and 10, however, biochar usually is more alkaline, due to the presence of alkali and alkaline metals the feedstock materials. Other factors affecting biochar pH are the amount of organic functional groups, carbonate content and inorganic alkali concentration in the initial raw material.

Biochar CEC strongly depends on its alkalinity. In the agricultural sector, such characteristics play an important role in acidic soils neutralization, such as the ones found in tropical regions. On the other hand, when biochar is amended to more basic soils, its effect on pH is less obvious, as studies show (Laghari 2016, El-Naggar 2018, Tomczyk 2020). Biochar can increase or decrease soil pH, depending on its initial alkalinity and the type of soil it is amended to. Hence, a general relationship between biochar and its effect on soil pH cannot be drawn.

Another important chemical characteristic influencing plant growth is EC. Soil electrical conductivity is a standard measure of its salinity (amount of salts), which is an indicator of nutrient availability, soil texture and WHC (USDA). Biochar is known to have an EC higher than most traditional agricultural soil types (Igalavithana 2018). In fact, it was shown that biochar applications increased the availability of soluble nutrient ions in soil, such as NO^{3-}, K^{+} and Ca^{2+}.

Even though soil EC does not directly affect plant growth, it indicates nutrient concentration in soil and their availability for plant uptake (SDSHC). Depending on type of soil and crop, high values of EC can negatively affect the overall plant development. Furthermore, recent studies have shown a strong positive relationship between biochar and soil EC (Li 2018), drawing concerns on its application and potential inhibitions on plant growth.

Most types of soils used in agriculture have a net negative charge, especially if amended with material high in carbon content (humus and pit). Similarly, biochar particles are strongly negatively charged (Sohi 2009) and have a high cation exchange capacity. Biochar CEC can vary depending on the amount of carboxylic and hydroxyl groups formed during pyrolysis (Janu 2021). Different types of biochar have different CEC. There are two main factors governing biochar CEC: surface oxidation and surface adsorption of oxidized organic matter (Hossain 2020). Studies have shown that different types of biochar affect soil CEC differently (Peng 2011, Tomczyk 2020). Such changes ultimately affect nutrient availability, soil aggregation and WHC (Yadav 2018). Biochar was also found to suppress long term organic matter turnover (SOM). It was observed that biochar is able to capture large quantities of carbon in the soil and retain them for longer period of timed compared to traditional agricultural soils (Schofield 2019). Combined with its high structural stability and long-term resistance for decomposition, biochar can play an important role in carbon (C) sequestration (Hossain 2020).

Biological properties

Biochar plays an essential role in soil biology and respiration, affecting macrobiotic and enzymatic activity (Hossain 2020). Owning to its high surface area, characterized by a complex porous system, biochar provides a favorable environment for microbial life. Biochar chemical properties also provide ideal conditions to support microorganisms, such as bacteria, mycorrhizal fungi, and actinomycetes (Compant 2010, Prapagdee and Tawinteung 2017). Anderson (20111) found that specific bacteria populations grew according to increased biochar amends, such as Bradyrhizobiaceae (+8%) and Hyphomicrobiaceae (+14%). These two species play an important role in the nitric cycle, denitrifying N oxides (NO^{3-}) to N^2 (Anderson 2011). His work also suggests that biochar has a negative effect on ammonia nitrifying organisms. This destabilizing effect combined with its overall ability to sorb NH_4^+, reduces the overall emissions of N_2O from the soil. Biochar has also shown a positive impact on enzymatic activities (Ouyang 2014), speeding up organic decomposition and increasing nutrient availability for plant uptake. Other organisms were found to be more sensible to biochar amends, such as heartworms (Saleh 1970), which being more susceptible to pH and ammonia concentrations, showed a negative response. Again, different scenarios were studied using different types of biochar; therefore, no general conclusion regarding the overall effect of biochar on soil biological properties can be drawn.

Effect of biochar on nutrients

The interaction between biochar and plant soil can be complex and its agronomical effects unpredictable. The nature of different biochars, their chemical composition and physical characteristics strongly affect soil dynamics. Studies suggested that biochar can alter the availability of plant primary nutrients, especially in nutrient-poor soils, both in the short and long run (Lehmann 2011). The most significant mechanisms were observed to be the direct addition of soluble nutrients from its ash and the mineralization of organically bound nutrients contained in biochar liable portion (Ding 2016). However, these changes depend on specific nutrients and the nature biochar. The following paragraphs will discuss the interaction between biochar and primary, secondary and micronutrients found in soil.

Primary nutrients

Nitrogen

Biochar can directly affect specific nutrient pools by directly providing a nutrient source to the soil. Different types of pyrolytic methods, temperature, and feedstock influence the amount such nutrient content in biochar.

Nitrogen is the primary plant development limiting factor, essential for agricultural crops. Most of the nitrogen added to agricultural soil comes from synthetic fertilizers, high in N concentration, which can strongly destabilize biotic cycles and soil dynamics. Moreover, nitrogen runoff from heavy rains and soil erosion can cause a profound environmental impact to the soil and the surrounding areas, especially in the presence of nearby water courses. In fact, nitrogen leaching can cause severe eutrophication, threatening the stability of multiple ecosystems, especially the aquatic ones.

Biochar could work as an interesting alternative to N-fertilizers, as it can act as a more sustainable source of nitrogen for plants.

In addition to inorganic nitrogen (NH_4^-, NO_3^- , N_2O), biochar is an excellent organic nitrogen source, both in its hydrolyzable and non-hydrolysable forms (Clough 2010). Different types of biochar were observed to have different nitrogen contents. More specifically, studies showed that increasing pyrolytic temperature positively affected N content despite nitrogen's low volatilization temperature (article).

These nitrogen compounds can be found on the surface of biochar, in the form of nitrates, ammonium salts and heterocyclic N- compounds, readily available to be dispersed in the soil for plant uptake (Grieson 2011). Studies have also shown that biochar applications strongly reduce N leeching, in some cases by more than 100%, creating long-term nitrogen pools readily available for plant uptake. Ullah (2020) observed an increase by more than 40% of nitrogen soil concentration in the early and late seasons of plant cycles. He also noticed an increase in plant nitrogen uptake in roots, stems, and leaves by 52.39%, 37.14%, 40.86% respectively.

Phosphorous

Phosphorus is another important limiting factor in plant development and growth. Phosphorus has a very low water solubility (one part/300,000 parts water) and it is only accessible by plant in its inorganic forms (HPO_4^{2-} , $H2PO_4^{-}$, H_3PO_4). Modern agricultural practices, especially through the use of heavy fertilizers, have caused severe phosphorous accumulations in soil triggering a series of detrimental mechanisms such as eutrophication of water bodies from soil erosion and runoff (Hooda 2001). Biochar can be used as a valid alternative to such fertilizers. Moreover, studies have shown that biochar can lower the risk of eutrophication and provide sustainable concentrations of phosphorus to the soil is amended to (Bornø 2018). In fact, it was found that biochar adsorbs inorganic (orthophosphate) and organic P compounds reducing their overall leeching rate (Glaser 2019). In addition, biochar application in acidic ($pH < 6.5$) and neutral ($pH = 6.5 – 7.5$) soil was found to increase P availability. This was not true for alkaline soils ($pH > 7.5$).

Different pyrolytic methods, feedstock and temperatures produce different types of biochar, which have different physical and chemical characteristic. This was also true for their P availability.

Potassium

The third primary nutrient for plant development and growth is potassium. Potassium, unlike nitrogen and phosphorus, cannot be synthesized from other chemicals. The principal source of potassium comes from specific minerals such as feldspars and micas, which slowly release quantities potassium in the soil through a series of weathering mechanisms. In the absences of such minerals, potassium must be added in the form of fertilizers.

One of the most common fertilizers used in agriculture is potash. Potash is a common name given to a group of minerals containing potassium (NRCAN). Between 2020 and 2021 potash price increased from 350$ per ton to 600$, a 71% spike. Russia is the second biggest exporter of potash in the world, followed by Belarus. Given the recent political situation in Europe, the global trade of potash has been strongly affected, creating an additional surge in prices which could directly affect the global food market (Politico). Biochar has been suggested as a possible alternative to potash fertilizers in the short and long run (Zhang 2020).

Biochar enhances soil fertility, water holding capacity (WHC) (Atkinson 2010) and AS (Soinne 2014). Unlike nitrogen (N) and carbon (C), potassium (P) has a relatively high volatility temperature, making it more resistant to high pyrolytic temperatures. During pyrolysis, potassium is largely conserved and converted into soluble minerals (Kalicinite), the most important source of potassium in biochar.

Additionally, biochar has been shown to enhance microbial populations and their activity, which affect potassium concentration in soil (Lehmann 2011). Wang (2018) noticed that biochar facilitated the growth of k – dissolving bacteria (KDB), which play an important role in soil K-minerals weathering. Out of all types, clay soil seemed the best option for the promotion of K concentrations.

Secondary nutrients

Calcium

Calcium is an essential nutrient which plays an important role in cell wall formation and membrane stability. It also serves as a messenger in a series of physiological processes, such as plant response to abiotic stress conditions (Thor 2019).

When calcium is deficient, new tissues exhibit morphological abnormalities due to improper cell wall formation. Such deficiencies make the plant more vulnerable to pathogens and infections (Raz 1992).

The effect of biochar on Ca concentration have showed inconsistent results. For example, one study on sweet corn observed that Ca levels in soil increased as biochar was added (Cole 2019). Another study on strawberry showed an increase in calcium uptake and an increase in chlorophyl concentration. Although no overall plant growth was observed, questioning the amening effects of the applied biochar (Amery 2021). However, due to its high sorption properties, biochar can retain cations, such as Ca, decreasing their availability to the soil (Limwikran 2019).

Again, no direct connection can be made between biochar application and calcium availability.

Magnesium

Magnesium is involved in a series of essential processes, such as photosynthesis, which nearly all plants depend on. It is used to produce many enzymes and plays a vital role in the chlorophyl molecule, acting as a central binder in its heterolytic compound (Figure 1).

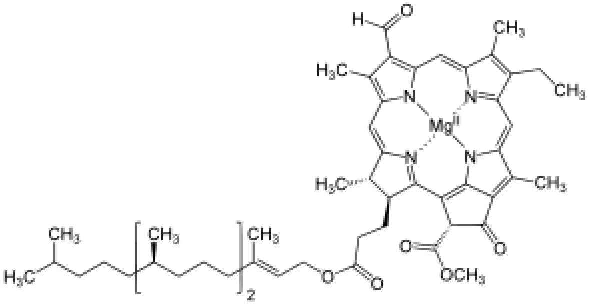


Figure 1 : Chlorophyll molecule

Magnesium is mobile withing the plant, therefore whenever there is a decadency, the first symptoms can be observed on the older leaves. Magnesium availability is influenced by soil pH. It becomes more available as the acidity of soil increases. Magnesium deficiency can also be caused by high concentrations of competing elements, such as potassium, sodium and calcium. (Guo 2016).

Studies have found that biochar can affect nutrient concentration by altering the soil pH and nutrient availability. In the short run, biochar can directly influence soil nutrient pool size by the addition of nutrients. For example, Sadowska (2020) observed an increase in magnesium concentration during the first year of her experiment. However, the increase rate flattened in the following two years. Magnesium concentrations are also susceptible to cation antagonists. High concentrations of NH_4^+, Na^+ and K^+ in soil affect Mg^{2+} uptake by plants (Rietra 2017). Pyrolysis can also affect the extracabiloty of such elements, either through immobilization or volatilization (Ghassemi-Golezani). Different types of biochar from different production methods release cations in different quantities. Their capacity of input nutrients, such as magnesium, ultimately depends on their chemical profile. (Angst 2012).

Sulfur

Sulfur is one of the three secondary nutrients required by plants for their development and growth. There is a dependent relationship between sulfur and nitrogen. Without a proper balance among these two, the plant cannot effectively use nitrogen and other nutrients for a proper function.

Plants obtain sulfur from soil as sulfate and sulfate dioxide (SO_4^{2-}, SO_2), which are reduced by specific amino acids (cysteine) to produce metabolic sulfide donors for the synthesis of various vital molecules, such as glutathione and methionine (Bonner 2005). Sulfur also plays a central role in a series of plant proteins and hormones.

Since the role of sulfur is similar to N in chlorophyll and protein synthesis, its deficiency symptoms resemble those of nitrogen. Sulfur is moderately mobile in plants, therefore young leaves are observed to be the first affected by its deficiency, followed by older ones. After a period of time, the plant becomes uniformly chlorotic, and its development reduces sharply.

Considering that the sulfur cycle works closely to the nitrogen cycle, biochar application can positively affect sulfur mineralization through and enhancement in nitrogen transformation rates (Das 2020). Studies have also found positive relationship between biochar and sulfur-reducing bacteria populations (SRB) (and their ability to metabolize and reduce sulfur) (Sande 2016). This was attributed to biochar positive effect on EC and its ability to facilitate electron transfer (Yang 2020). However, sulfur mineralization is usually favored by acidic environments, therefore biochar strong alkalinity could negatively impact the mineralization rates in soil and therefore S availability. (Binkley 1992). Other factors such as specific chemical profiles influence the direct input of sulfur to soil. On the other hand, studies suggest that the level of mineralization of sulfur depends on its speciation rather than the total concentration of the specific biochar.
The sulfur cycle is an extremely complex mechanism and can be sensible to small environmental variations. For this reason, the influence of biochar on its dynamics is still quite unclear and therefore a general relationship between biochar applications and sulfur soil concentration has not yet been drawn. Further studies are necessary.

Micronutrients

Iron

Iron is an essential micronutrient which plays a critical role in a series of metabolic processes, including cell respiration, DNA synthesis and photosynthesis. Furthermore, multiple metabolic processes are controlled by iron induced enzyme release. It is also involved in the synthesis of chlorophyl and the maintenance of chloroplasts.
Iron availability in soil limits plant species distribution and their crop yield. Iron deficiency inhibits growth and fitness and causes chlorosis. On the other hand, iron

excess can be extremely toxic for cells. Therefore, it is important for plants to be an environment where enough Fe is available but at the same time, in case of excess, it can be immobilized to be later released.

Such circumstances might be provided by biochar which has been observed to affect iron availability in soil (Foereid 2015). It has been proven that Fe concentration increase according to soil acidity due to negatively charged particles desorption and an increase in soil reduction (Wang 2018). Biochar was found to decrease Fe availability, both decreasing soil pH and adsorbing the dissolved Fe^{3+} (Rodriguez – Vila 2016). This can be a desirable effect depending on the status of the soil and plant iron concentration.

Boron

Boron plays a significant role in a multitude of plant functions such as, cell wall formation, movement of sugar in growing tissue, structural stability of biological membranes and seed set and pollination. It is also required for nitrogen fixation in root nodules (Brown 2002).

Multiple studies have been made on the direct addition of boron combines with biochar and their effect on plant growth, but no research has been made on the effect of biochar on boron concentration levels in plant tissues or even soil. Further investigation must be made to establish a connection between biochar application and B in plants.

Copper

Copper is one of the eight essential micronutrients. It affects the plant enzymatic activities and is required for chlorophyl and seed production. Copper can also intensify flavor in vegetables and increase color in flowers.

Deficiency of copper can cause plant susceptibility to multiple diseases, such as ergot, which negatively affects crop yield. Copper is immobile, so the first symptoms of its deficiency are noticed on developing plants of the plant, such as new leaves. Common symptoms are cupping and chlorosis of the whole leaf or its veins. Excess phosphorous or potassium can also indirectly cause copper deficiency. Soil pH can affect copper solubility, decreasing its availability for plant uptake (Bloodnick 2021).

The increased use of fertilizers and pesticides is resulting in a long-term copper contamination of agricultural soils. It is believed that fungicides are the main cause of Cu contamination which can have serious consequences on the crop’s health as well as ours, the consumers (Meier 2017).

Biochar application has been recommended as a potential amendment to contaminated soils, characterized by high concentrations of heavy metals. It was found that biochar affects trace metals concentration in soil and due to its high surface area, porosity, retention and recalcitrance, it can immobilize free moving cations, such as Cu (Gonzaga 2020). Other studies attributed observed low concentration in plant tissues (*Chenopodium quinoa*) to improved water supply in soil as well as great biochar sorption capabilities (Buss 2012). Similarly for other trace metals, biochar sorption capacity depends on pyrolytic temperature and feedstock material. It was found that the higher the pyrolytic temperature the better the biochar could immobilize metals, such as Cu (Cibati 2017).

Zinc

In the last 30 years our diets have seen a drastical shift. More than 2 billion people turned to diets less diverse than their parents, leading to a series of micronutrient deficiencies (Genc 2005). Moreover, micronutrient malnutrition currently affects 40% of the global populations (Welch), especially in developing countries, mostly due to modern agricultural practices.

Zinc and copper deficiency have been an issue for some time now. Decreasing levels of such micronutrients have been observed in our food for several decades (Sanstead 1991). A wide range of strategies are being studied to tackle this issue, from plant breeding to soil enrichment (Chen 1996). The essential micronutrient in crop production are B, Cu, Fe, Mn, Mo, Zn, Ni and Co in lower concentrations. Intensified cropping, erosion of topsoil, leaching, liming of acid soils and increased use of chemical fertilizers have caused a deficnenty of such nutrients both in soil and plants (Fageria 2002). These practices cannot sustain the increasing demand for more and better food; new approaches need to be developed.

Recent studies have tried to determine biochar effectiveness on micronutrient availability in soil.

For example, Gartler (2013) work with a series of common crops (spinach, beetroot, strawberries) showed an increase in zinc (Zn) bioavailability to plants following a series of biochar applications. He observed an increase of Zn in crops with edible leaves as well as beetroots. The same relationship was observed with cadmium (Cd), although the levels in certain plants (spinach) exceeded the Word's Health Organization maximum permitted concentrations. On the other hand, it was found that micronutrient uptake in wheat corn was negatively affected. Concentrations of iron (Fe), copper (Cu) and zinc (Zn) decreased as biochar was applied (Hartley 2016). This could have been caused by its high potential for nutrient retention, due to biochar high CEC.

CHAPTER 2

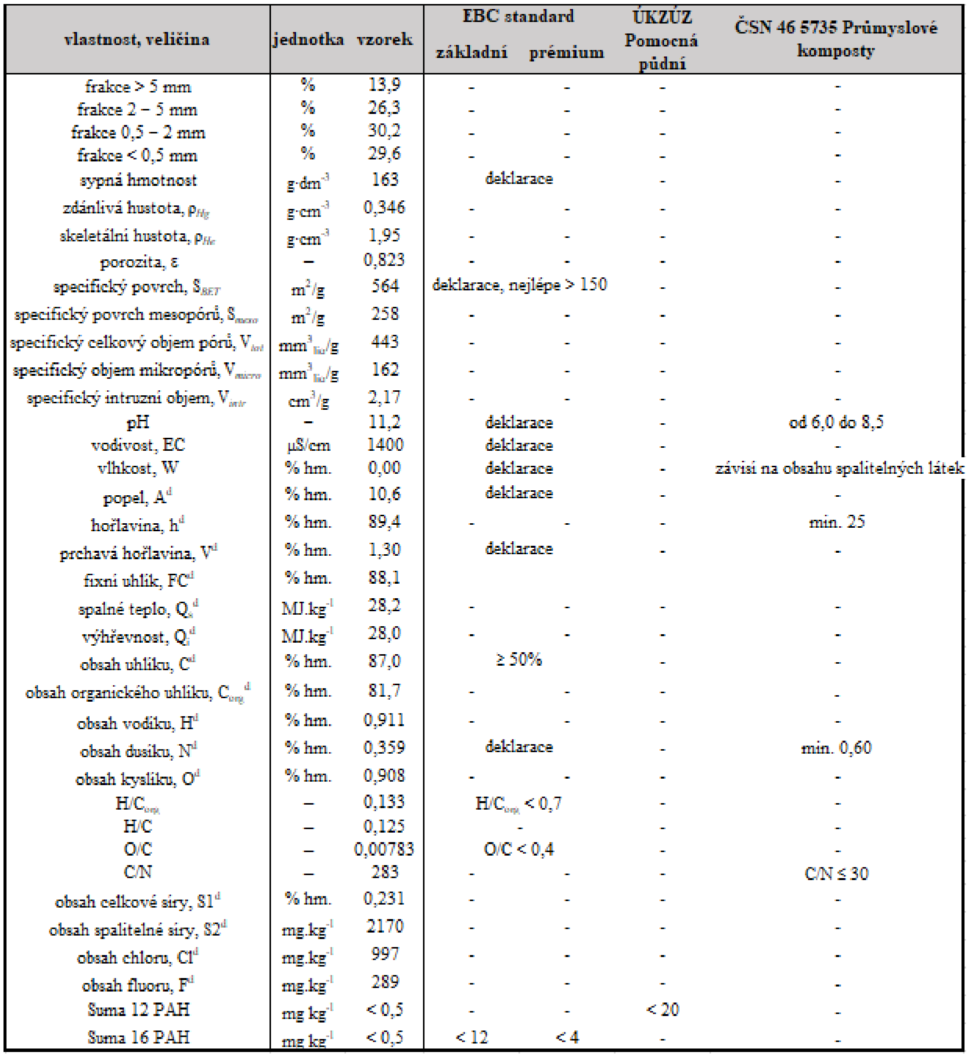
EXPERIMENTAL PART

Methodology

The first part of the experiment took place in a greenhouse. Four genera of plants were planted, with ten exponents for each genus. The genera were the following: Deschampsia, Heucera, Hedera, Sedum and Festuca. Stable conditions were kept for four months. After the development period, four random species from each genus were randomly selected for lab analysis.

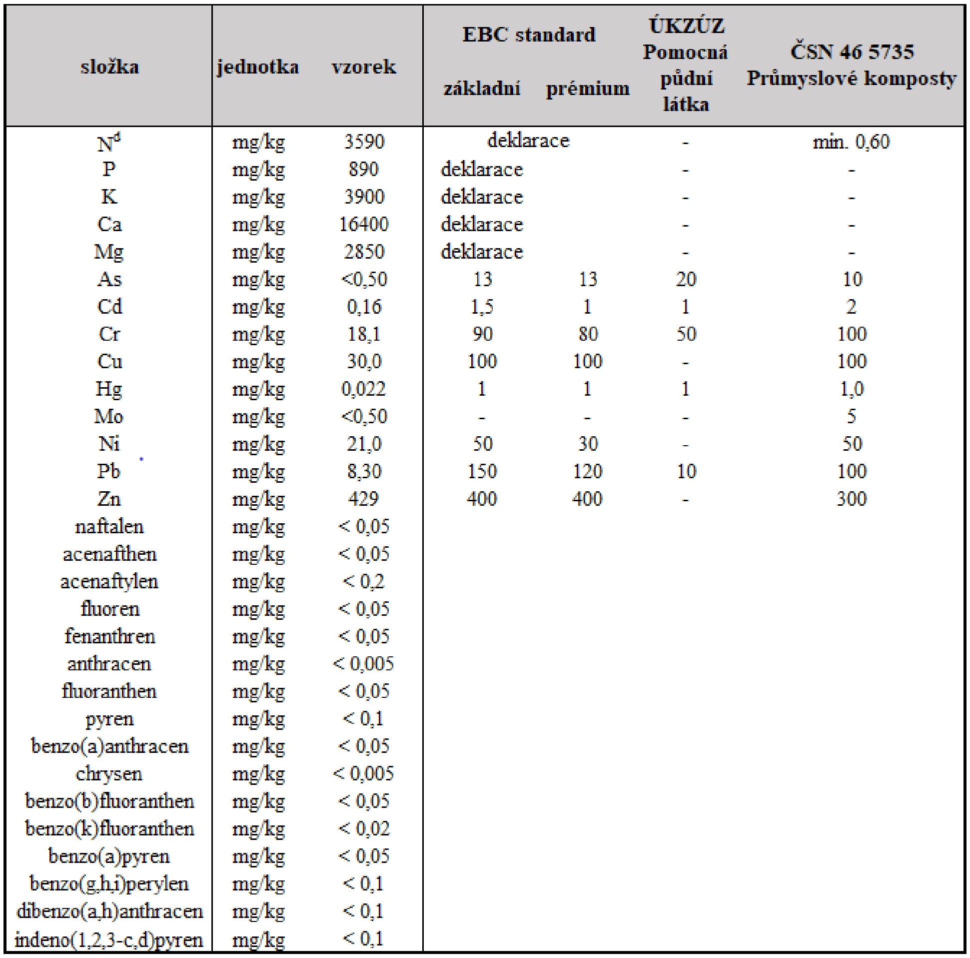
Biochar analysis

This biochar was produced from spruce wood at 600degC by slow pyrolysis in a muffle furnace under 16.7 mL min−1 nitrogen flow rate at atmospheric pressure and retention time of 30 min. The size of its components varies, from pebbles > 5 mm (13.9%) to pebbles < 0.5 mm (29.6%). The most abundant component was characterized by a size of 0.5 – 2mm (30.2%), which explains the small BD (163 g/dm^3). The specific surface area was quite high (564 m^2/g) with a pour density of 0.823. The most interesting characteristic was its extremely high pH (11.2). Also, its EC was quite high, with a value of 1400 μS/cm. The ash content was 10.6 %hm, which could explain the high concentration of primary and secondary nutrients (N = 3590 mg/Kg; P = 890 mg/Kg; K = 3900 mg/Kg; Ca = 16400 mg/Kg; Mg = 2850). The fixed carbon (FC^d) was very high (88.1 %hm), a characteristic of slow pyrolysis biochar, especially for those with a high concentration of cellulose and lignin (Duarte 2019). Fluorine and chlorine content were significantly high, with respective values of 289 mg/kg and 997 mg/kg (Rafiq 2017). Lastly H/C and O/C content were found to be 0,125 and 0.00783, probably due to high contents of carbon (C). The concentration of contaminants and PAH was small, making the biochar suitable for agricultural applications. The only exception was Zn, whose concentration was observed to be quite high (429 mg/Kg). This might not be an issue depending on the type of crop used for cultivation. In fact, some crops are more sensible to high levels of zinc, while other are more resistant. Furthermore, national standards prohibit certain concentration of Zn in some plant tissues (edible leaves), so again, some concern should be directed towards the type of selected crop.



vlastnost, veličina	jednotka	vzorek	EBC standard		ÚKZÚZ Pomocná půdní	ČSN 46 5735 Průmyslové komposty
			základní	prémium		
frakce > 5 mm	%	13,9	-	-	-	-
frakce 2 – 5 mm	%	26,3	-	-	-	-
frakce 0,5 – 2 mm	%	30,2	-	-	-	-
frakce < 0,5 mm	%	29,6	-	-	-	-
sypná hmotnost	$g \cdot dm^{-3}$	163	deklarace		-	-
zdánlivá hustota, ρ_{Hg}	$g \cdot cm^{-3}$	0,346	-	-	-	-
skeletálni hustota, ρ_{He}	$g \cdot cm^{-3}$	1,95	-	-	-	-
porozita, ε	–	0,823	-	-	-	-
specifický povrch, S_{BET}	m^2/g	564	deklarace, nejlépe > 150		-	-
specifický povrch mesopórů, S_{meso}	m^2/g	258	-	-	-	-
specifický celkový objem pórů, V_{tot}	mm^3_{liq}/g	443	-	-	-	-
specifický objem mikropórů, V_{micro}	mm^3_{liq}/g	162	-	-	-	-
specifický intruzni objem, V_{intr}	cm^3/g	2,17	-	-	-	-
pH	–	11,2	deklarace		-	od 6,0 do 8,5
vodivost, EC	µS/cm	1400	deklarace		-	-
vlhkost, W	% hm.	0,00	deklarace		-	závisi na obsahu spalitelných látek
popel, A^d	% hm.	10,6	deklarace		-	-
hořlavina, h^d	% hm.	89,4	-	-	-	min. 25
prchavá hořlavina, V^d	% hm.	1,30	deklarace		-	-
fixni uhlik, FC^d	% hm.	88,1				
spalné teplo, Q_s^d	$MJ.kg^{-1}$	28,2	-	-	-	-
výhřevnost, Q_i^d	$MJ.kg^{-1}$	28,0	-	-	-	-
obsah uhliku, C^d	% hm.	87,0	≥ 50%		-	-
obsah organického uhliku, C_{org}^d	% hm.	81,7	-	-	-	-
obsah vodiku, H^d	% hm.	0,911	-	-	-	-
obsah dusiku, N^d	% hm.	0,359	deklarace		-	min. 0,60
obsah kysliku, O^d	% hm.	0,908	-	-	-	-
H/C_{org}	–	0,133	$H/C_{org} < 0,7$		-	-
H/C	–	0,125	-		-	-
O/C	–	0,00783	$O/C < 0,4$		-	-
C/N	–	283	-	-	-	$C/N \le 30$
obsah celkové siry, $S1^d$	% hm.	0,231	-	-	-	-
obsah spalitelné siry, $S2^d$	$mg.kg^{-1}$	2170	-	-	-	-
obsah chloru, Cl^d	$mg.kg^{-1}$	997	-	-	-	-
obsah fluoru, F^d	$mg.kg^{-1}$	289	-	-	-	-
Suma 12 PAH	$mg\ kg^{-1}$	< 0,5	-	-	< 20	-
Suma 16 PAH	$mg\ kg^{-1}$	< 0,5	< 12	< 4	-	-

Table 1 : Properties of biochar



složka	jednotka	vzorek	EBC standard		ÚKZÚ Pomocná půdní látka	ČSN 46 5735 Průmyslové komposty
			základní	prémium		
N^d	mg/kg	3590	deklarace		-	min. 0,60
P	mg/kg	890	deklarace		-	-
K	mg/kg	3900	deklarace		-	-
Ca	mg/kg	16400	deklarace		-	-
Mg	mg/kg	2850	deklarace		-	-
As	mg/kg	<0,50	13	13	20	10
Cd	mg/kg	0,16	1,5	1	1	2
Cr	mg/kg	18,1	90	80	50	100
Cu	mg/kg	30,0	100	100	-	100
Hg	mg/kg	0,022	1	1	1	1,0
Mo	mg/kg	<0,50	-	-	-	5
Ni	mg/kg	21,0	50	30	-	50
Pb	mg/kg	8,30	150	120	10	100
Zn	mg/kg	429	400	400	-	300
naftalen	mg/kg	< 0,05				
acenafthen	mg/kg	< 0,05				
acenaftylen	mg/kg	< 0,2				
fluoren	mg/kg	< 0,05				
fenanthren	mg/kg	< 0,05				
anthracen	mg/kg	< 0,005				
fluoranthen	mg/kg	< 0,05				
pyren	mg/kg	< 0,1				
benzo(a)anthracen	mg/kg	< 0,05				
chrysen	mg/kg	< 0,005				
benzo(b)fluoranthen	mg/kg	< 0,05				
benzo(k)fluoranthen	mg/kg	< 0,02				
benzo(a)pyren	mg/kg	< 0,05				
benzo(g,h,i)perylen	mg/kg	< 0,1				
dibenzo(a,h)anthracen	mg/kg	< 0,1				
indeno(1,2,3-c,d)pyren	mg/kg	< 0,1				

Table 2: Properties of biochar

Lab analysis

For mineralization of plant biomass, the sample was properly dried and homogenized with a mortar and pestle. The homogenized sample was then weighed on a laboratory scale into Teflon reaction vessels. The weight of the samples did not exceed 0.2 g. After weighing, the vessels with samples were transferred to a fume hood, where 9 ml of HNO_3 and 1 ml of H_2O_2 were pipetted in them. A valve was placed on the opening of the Teflon vessels, through which the cap was screwed. The program Organic B was selected on the display of the device for mineralization. The whole process of mineralization with preheating and cooling took 40 - 50 minutes. After cooling, the tubes were carefully opened in a fume hood and their content was poured into a 50 ml volumetric flask and the volume adjusted to 50 ml with distilled water.

Due to the sensitivity of the mass spectrometry instrument, the samples needed to be diluted. Dilution was performed into centrifuge tubes.
The samples were measured by ICP-OES spectrometry and inductively coupled plasma optical emission spectrometry. The element content was determined on an iCAP 7000 Series spectrometer (Thermo Scientific).

Data Analysis

The nutrient values were checked for missing values (NA). If the number of NA reached a certain threshold (completion rate), the respective variables containing the excess NA were omitted. This was true for all variables with a completion rate < 0.5 (Equation 1). Following this selection, Be (0.05), Mo (0.23) and Se (0.31) were omitted from further analysis.

$$completion\ rate = 1 - \frac{n.of\ NA\ values}{n.of\ total\ values}$$

Equation 1: Completion rate

Two-ANOVA models were ran for all nutrient variables, testing the significance between nutrient concentration and plant part (genus/stem). In > 90% of the cases, plant part was statistically significant. Therefore, it was decided to divide the nutrient variables in two categories: nutrient concentration in stem and nutrient concentration in root. For every respective category, further two-ANOVA models were carried out, testing for significance between nutrient concentration and both biochar and genus.
Following the models, all nutrient variables were submitted to a Shapiro normality test (shapiro.test) of their residuals (Appendix). If normality was not met, respective normalization techniques were applied (log and min/max) and the normality test ran again for the normalized variables. If normality was still not met, the normalization technique that yielded the lowest p value (shapiro.test) was selected for and a full analysis carried out.

Root

The average root mass with and without biochar was 0.251 g and 0.242 g respectively. The number of NA can be seen in Table 3.

Nutrient	Number of NA	Completion rate
P	8	0.8

Table 3: NA infomration for nutreints in root

Primary nutrients

Multiple normalization techniques were applied to K, with no satisfying results. Even though normalization was not met, a full statistical analysis was carried out (Table 4) The mean, standard deviation and eta squared were calculated (Table 4). Individual models were ran for the nutrient variables. The models showed no significance between biochar and nutrient concentration. The same was true for genus (Table 4).

N		Biochar	Genus		Biochar: Y					Biochar: N				
		F(1,34)	F(4,34)		Des	Heu	Hed	Sed	Fest	Des	Heu	Hed	Sed	Festu
K_L		0.15	2.72	M	1853.30	3148.45	3381.85	3658.12	893.71	1678.63	4458.26	3239.27	3280.86	1503.66
	$\eta 2$	0.0034	0.24	SD	1296.48	2221.73	2356.00	2542.12	610.56	1376.45	3176.91	2318.09	2315.56	1121.37
P		0.32	2.63	M	190.29	360.29	323.79	566.38	175.40	238.11	418.40	358.72	563.35	261.56
	$\eta 2$	0.0070	0.23	SD	133.72	260.32	224.69	398.51	119.84	184.71	293.88	252.34	433.57	189.19

Note. P *p<.05, **p<.01, ***p<.001

Note. N $_L$(log), $_M$(min/max)

Table 4: APA table for primary nutrients in stem

Individual models for the nutrient variables showed no significant difference in concentration among genera.

Positive correlation was observed between P and K with a clear linear relationship (Table 5).

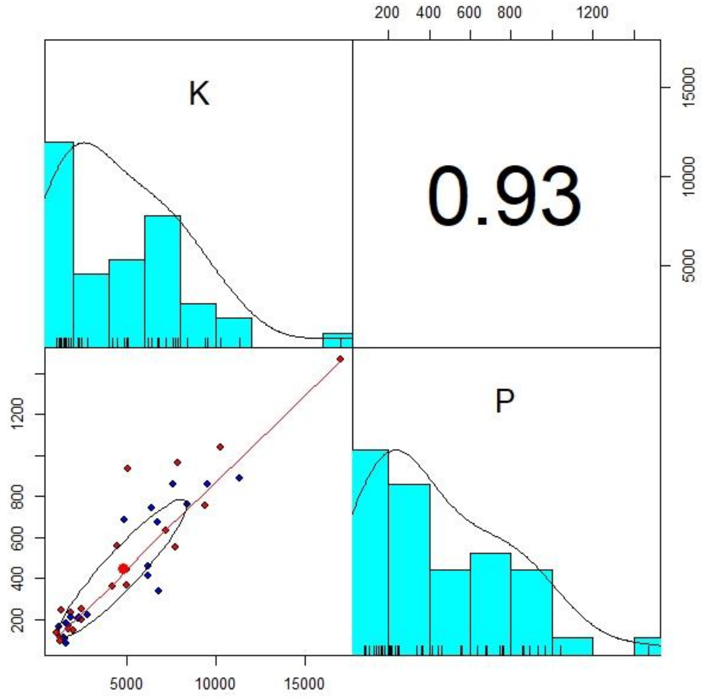


Table 5: Correlation table for primary nutrients in root

Secondary nutrients

Multiple normalization techniques were applied to Mg and S, with no satisfying results. Even though normalization was not met, a full statistical analysis was carried out (Table 6).

The mean, standard deviation and eta squared were calculated (Table 6). Individual models were carried out for the nutreint variables. The models showed no significance between biochar and nutrient concentration. The same was true for genus. (Table 6). The only exception was Ca, which showed statistical significance between genus (Table 6)

N		Biochar	Genus		Biochar: Y					Biochar: N				
		F(1.34)	F(4,34)		Des	Heu	Hed	Sed	Fest	Des	Heu	Hed	Sed	Fest
Ca_M		0.67	$2×10^{16}$***	M	3661.73	7722.87	7935.25	5031.58	5910.56	4886.02	8111.90	9526.19	6083.32	7295.23
	η2	0.0011	0.87	SD	3147.59	5531.77	5691.42	3719.24	4038.26	3474.32	5698.16	6682.02	4279.82	5319.89
Mg_L		0.35	59.65	M	838.17	1056.40	1033.25	1183.81	663.21	883.83	1185.99	1063.13	1111.25	915.34
	η2	0.0011	0.76	SD	580.14	750.18	762.54	819.18	453.49	665.12	836.90	745.22	802.34	672.92
S		0.49	1.78	M	579.99	603.95	677.89	1093.67	357.74	687.37	757.59	644.75	1169.59	620.93
	η2	0.012	0.17	SD	403.96	430.81	479.13	762.30	245.58	559.21	541.91	471.39	841.39	446.03

Note. P: *p<.05, **p<.01, ***p<.001

Note. N $_L$(log), $_M$(min/max)

Table 6: APA table for secondary nutrients in root

Individual models for the nutrient variables showed significant difference in concentration among genera (Table 7).

Nutrients	**Significant differences**
Ca (not)	Festuca-Deschampsia***
	Hedera-Deschampsia***
	Sedum-Deschampsia***
	Hedera-Festuca***
	Heuchera-Festuca***
	Sedum-Festuca***
	Heuchera-Hedera*
	Sedum-Hedera***
	Sedum-Heuchera***
Mg (not)	Festuca-Deschampsia***
	Hedera-Deschampsia***
	Sedum-Deschampsia***
	Hedera-Festuca***
	Heuchera-Festuca***
	Heuchera-Hedera***
	Sedum-Hedera***
	Sedum-Heuchera***

Note. P*p<.05, **p<.01, ***p<.001

Table 7 : Significant differences among genera for secondary nutreints in root

Positive correlation was observed between a combinations of nutreint varialbes with a clear linear relationship, such as between S and Mg (0.91) and Ca and Mg (0.89) (Table 8).

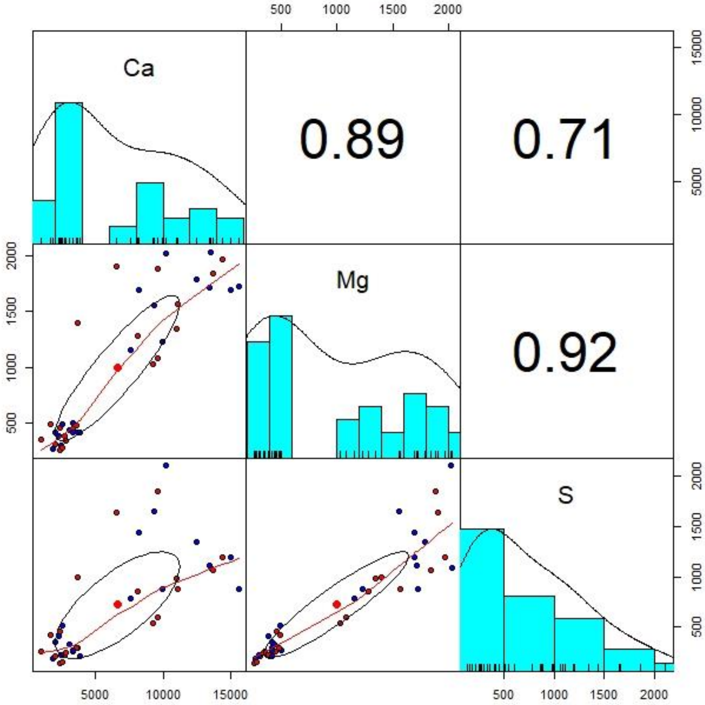


Table 8 : Correlation table for secondary nutreints in root

Micronutrients

Due to an excess number of NA values (NAtable), Cr was omitted from the analysis. Multiple normalization techniques were applied to Mn and Zn, with no satisfying results (Table 9). Even though normalization was not met, the models were applied.

The mean, standard deviation and eta squared were calculated (Table 9). Individual models were carried out for all the response variables. The models showed significance between genus and all nutrient concentration for Fe and Co (Table 9). Additionally, statistical significance between biochar and concentration was observed in Cu (Table 9).

N		Biochar	Genus		Biochar: Y					Biochar: N				
		F(1,34)	F(4,34)		Des	Heu	Hed	Sed	Fest	Des	Heu	Hed	Sed	Fest
Fe_L		1.10	4.68**	M	2409.22	1484.94	1345.84	290.26	2628.84	2895.94	821.71	2208.20	1093.40	2801.60
	η2	0.020	0.35	SD	1875.10	1063.75	1136.32	248.40	1797.04	2086.03	695.71	1790.39	841.14	1974.16
B		0.43	3.76	M	3.47	12.41	5.51	7.26	3.43	4.62	13.87	6.71	7.61	4.92
	η2	0.0089	0.31	SD	2.76	10.01	3.83	5.06	2.36	3.32	9.71	4.99	5.89	3.74
Cu_L		5.93*	0.60	M	12.61	18.78	8.90	7.05	12.22	20.38	36.02	16.83	23.79	20.95
	η2	0.14	0.056	SD	9.38	19.32	6.45	5.13	8.50	15.19	38.54	13.85	21.42	14.84
Mn		0.27	0.97	M	51.39	38.60	40.41	31.29	45.68	49.37	20.14	44.55	28.13	49.01
	η2	0.0072	0.10	SD	38.46	27.38	28.03	24.78	31.51	36.32	15.21	31.39	19.71	36.34
Zn		1.75	0.74	M	30.45	28.47	22.17	26.19	23.63	58.48	37.55	27.56	17.54	55.66
	η2	0.045	0.077	SD	21.04	21.89	17.47	18.19	16.14	68.07	26.33	20.05	14.28	52.18
Co_L		1.02	4.12**	M	1.64	0.83	0.84	0.18	1.50	1.66	0.48	1.13	0.76	1.607
	η2	0.020	0.32	SD	1.20	0.62	0.75	0.22	1.03	1.19	0.42	0.93	0.56	1.12
Ni_L		0.50	0.39	M	6.89	6.14	6.93	2.51	5.39	5.65	5.53	7.08	6.83	5.73
	η2	0.014	0.043	SD	6.24	5.91	5.99	2.14	3.75	4.01	5.14	5.35	5.00	4.03

Note. P: *p<.05, **p<.01, ***p<.001

Note. N $_L$(log), $_M$(min/max)

Table 9: APA table of micronutrients in root

Individual models for concentrations showed some difference in concentration among genus for specific nutrients (Table 10)

Nutrients	Significant differences
Fe	Sedum-Deschampsia*** Sedum-Festuca***
B	Heuchera-Deschampsia* Heuchera-Festuca*
Co	Sedum-Deschampsia* Sedum-Festuca*

Note. P*p<.05, **p<.01, ***p<.001

Table 10 : Significant differences among genus for micronutreints in root

Positive correlation was observed among combinations of micronutrients. Strong linear relationship was found between Co and Fe (0.98), Co and Mn (0.89) and Fe and Mn (0.90) (Table 11)

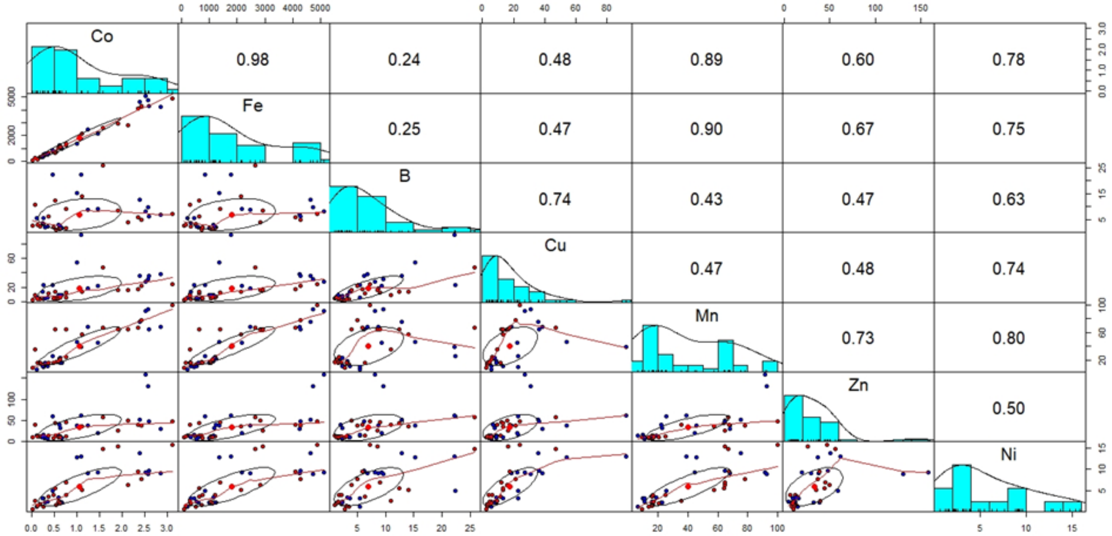


Table 11: Correlation table for micronutreints in root

Stem

The average stem mass with and without biochar was 0.248 g and 0.246 g respectively. The number of NA can be seen in Table 12.

Nutrients	Number of NA	Completion rate
Co	22	0.45
Cu	2	0.95
Ni	8	0.8

Table 12: NA infomration for nutreints in stem

Primary nutrients

The first category consisted of the plant’s primary nutrients. Due to lack of data, nitrogen was excluded and only phosphorous (P) and potassium (K) were considered.

Normality was checked for all variables, which was not met. Multiple normalization techniques were applied to the variables, with no satisfying results (Table 13). Even though normalization was not met, the models were applied regardless.

The mean, standard deviation, and eta squared were calculated (Table 13). Furthermore, the models showed no significant interaction between the primary nutrients and biochar. The same was true for genus (Table 13).
Individual models for particular nutrients showed no difference in concentration among genus.

N		Biochar	Genus		Biochar: Y					Biochar: N				
		F(1,34)	F(4,34)		Des	Heu	Hed	Sed	Fest	Des	Heu	Hed	Sed	Festu
K_L		0.079	1.85	M	5407.16	8495.29	2973.21	4672.48	3122.17	4610.89	6478.79	3508.81	4487.77	4036.68
	$\eta2$	0.0019	0.18	SD	3723.91	6612.34	2071.95	3303.44	2176.58	3255.34	4602.46	2550.24	3142.40	2882.82
P		0.32	2.63	M	412.878	780.5450	443.20	496.2925	252.5425	371.93	544.94	467.2800	477.7825	245.98
	$\eta2$	0.0070	0.23	SD	291.3427	578.2392	358.57	376.54	178.8819	291.20	382.13	312.8868	341.25	182.19

Note. P: *p<.05, **p<.01, ***p<.001

Note. N $_L$(log), $_M$(min/max)

Table 13: APA table for primary nutrients in stem

A strong correlation between P and K was observed (Table 14). The Pearson Correlation was 0.95 with a positive and linear relationship between the two variables (Table 14)

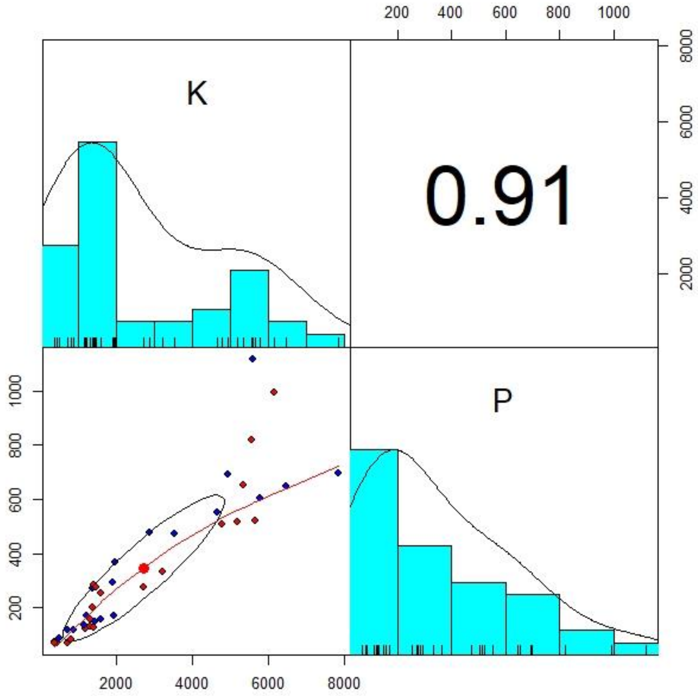


Table 14: Correlation table for primary nutrients in root

Secondary nutrients

The first second category consisted of the plant's secondary nutrients.
Multiple normalization techniques were applied to Mg, with no satisfying results (Table 9). Even though normalization was not met, the models were applied.

The mean, standard deviation and eta squared were calculated (Table 15).
Individual models were carried out for all the response variables. The models showed no significance between biochar and nutrient concentration. The same was true for genus. The only exception was Mg, which showed statistical significance between both biochar and genus (Table 15)

N		Biochar	Genus		Biochar: Y					Biochar: N				
		F(1,34)	F(4,34)		Des	Heu	Hed	Sed	Fest	Des	Heu	Hed	Sed	Fest
Ca		0.69	2.46	M	5900.20	14616.78	6894.25	11919.77	8068.42	5321.26	10324.28	6716.76	13014.57	3674.54
	η2	0.016	0.22	SD	3999.19	10665.69	4790.12	8442.88	5987.73	3857.79	7265.64	4728.02	9053.79	2634.77
Mg		10.77**	21.73***	M	2663.86	2306.68	1252.74	1236.01	2545.07	1574.86	1875.29	1094.93	1338.28	1173.18
	η2	0.063	0.51	SD	1817.18	1633.22	896.73	896.37	1824.00	1072.30	1318.51	772.99	930.23	835.92
S		1.59	2.23	M	1591.17	1482.45	464.27	1203.17	730.00	785.70	959.98	435.58	1343.15	476.36
	η2	0.036	0.20	SD	1085.51	1257.95	342.33	874.43	508.90	543.91	672.94	309.86	946.36	354.96

Note. P: *p<.05, **p<.01, ***p<.001

Note. N $_{L}$(log), $_{M}$(min/max)

Table 15: APA table for xecondary nutrients in stem

Individual models for concentrations showed some difference in concentration among genus for specific nutrients.

Nutrients	Significant difference
Ca	Festuca-Deschampsia*** Sedum-Deschampsia*** Hedera-Festuca*** Heuchera-Festuca*** Sedum-Hedera*** Sedum-Heuchera***
Mg	Festuca-Deschampsia*** Hedera-Deschampsia*** Sedum-Deschampsia*** Heuchera-Festuca*** Heuchera-Hedera*** Sedum-Heuchera***

Note. P*p<.05, **p<.01, ***p<.001

Table 16 : Significant differences among genus for secondary nutreints in stem

Positive correlation was observed among combinations of micronutrients. Although no clear linear relationship was found among any of the nutrients, as shown in (Table 17)

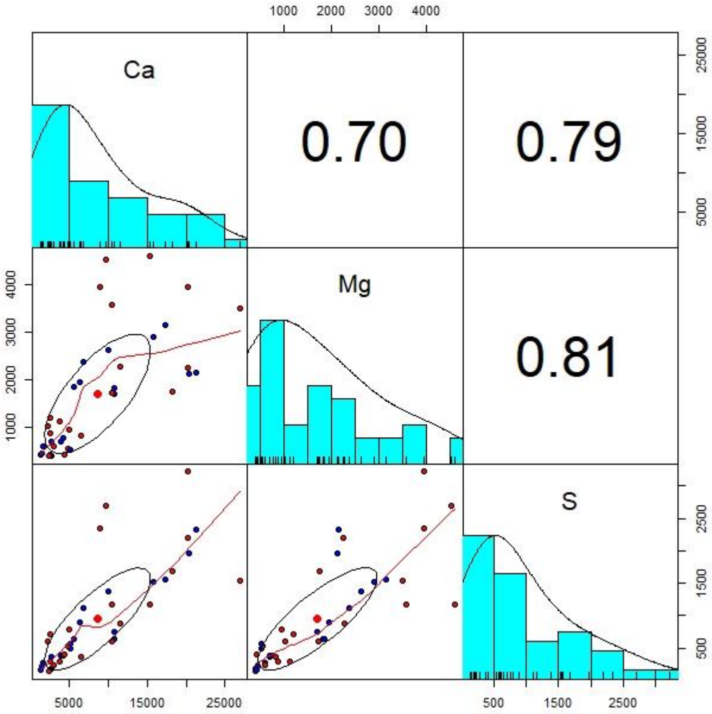


Table 17: Correlation table for secondary nutrients in root

Micronutrients

Multiple normalization techniques were applied (Table 18), with no satisfactory result for some nutrients, such as Cu, Mn and Zn. Even though normalization was not met, the models were applied regardless.

The mean, standard deviation and eta squared were calculated (Table 18). Individual models were carried out for all the response variables. The models showed significance between genus and all nutrient concentration, for all variables (Table 18). Additionally, statistical significance between biochar and concentration was observed for Zn and Mn (Table 18).

N		Biochar	Genus		Biochar: Y					Biochar: N				
		F(1, 34)	F(4, 34)		Des	Heu	Hed	Sed	Fest	Des	Heu	Hed	Sed	Fest
Fe_L		0.33	6.92***	M	256.03	144.41	56.87	8.06	29.88	89.95	121.67	56.61	41.92	18.60
	η2	0.0054	0.45	SD	183.78	130.84	41.53	5.77	25.59	108.43	111.91	40.23	54.94	13.23
B		0.37	3.37*	M	14.38	33.29	19.49	14.05	48.87	18.57	27.13	17.06	13.75	35.94
	η2	0.0077	0.28	SD	9.72	23.62	13.60	10.14	35.80	12.70	19.12	12.07	9.77	26.88
Cu_L		2.92	2.94*	M	4.94	2.77	0.71	0.85	1.77	4.97	2.73	3.36	NA	1.72
	η2	0.047	0.25	SD	3.38	1.98	0.84	0.69	1.52	3.99	2.51	2.41	NA	1.25
Mn		15.07***	0.02*	M	116.14	22.13	32.36	17.83	88.07	21.23	12.94	16.86	10.81	15.29
	η2	0.24	0.21	SD	80.45	17.49	23.21	13.08	64.11	14.74	9.98	13.47	7.62	11.34
Zn		6.38*	12.068***	M	41.33	16.34	31.82	16.86	16.82	25.74	21.19	17.46	14.04	16.02
	η2	0.050	0.38	SD	27.99	11.69	29.81	13.42	11.91	17.62	14.88	12.58	10.32	12.34
Ni		0.87	10.85***	M	6.59	1.74	0.21	NA	0.41	3.31	1.48	0.33	0.20	0.59
	η2	$4.26x10^{-6}$	$6.17x10^{-1}$	SD	4.70	1.35	0.16	NA	0.53	2.24	1.21	0.35	0.17	0.58

Note. P: *p<.05, **p<.01, ***p<.001

Note. N $_L$(log), $_M$(min/max)

Table 18: APA table for micronutrients in stem

Individual models for particular nutrients showed some difference in concentration among genus for specific nutrients.

Nutreints	**Significant differences**
Fe	Festuca-Deschampsia*
	Sedum-Deschampsia*
	Heuchera-Festuca*
	Sedum-Heuchera*
B	Sedum-Festuca*
Mn	Sedum-Deschampsia*
Zn	Festuca-Deschampsia*
	Hedera-Deschampsia***
	Sedum-Deschampsia***
	Heuchera-Festuca***
	Heuchera-Hedera***
Ni	Sedum-Heuchera***
	Festuca-Deschampsia***
	Hedera-Deschampsia***
	Sedum-Deschampsia***
	Heuchera-Hedera*

Note. P*p<.05, **p<.01, ***p<.001

Table 19 : Significant differences among genus for micronutreints in stem

(Zn if values kept with no normality, smallest residuals, but when applied min max, strong significance on bio and genu)

Positive correlation was observed among combinations of micronutrients. For example, strong linear relationship was found between Cu and Fe (0.68), Zn and Fe (0.63), Ni and Fe (0.83) and Ni and Mn (0.67), as shown in Table 20.

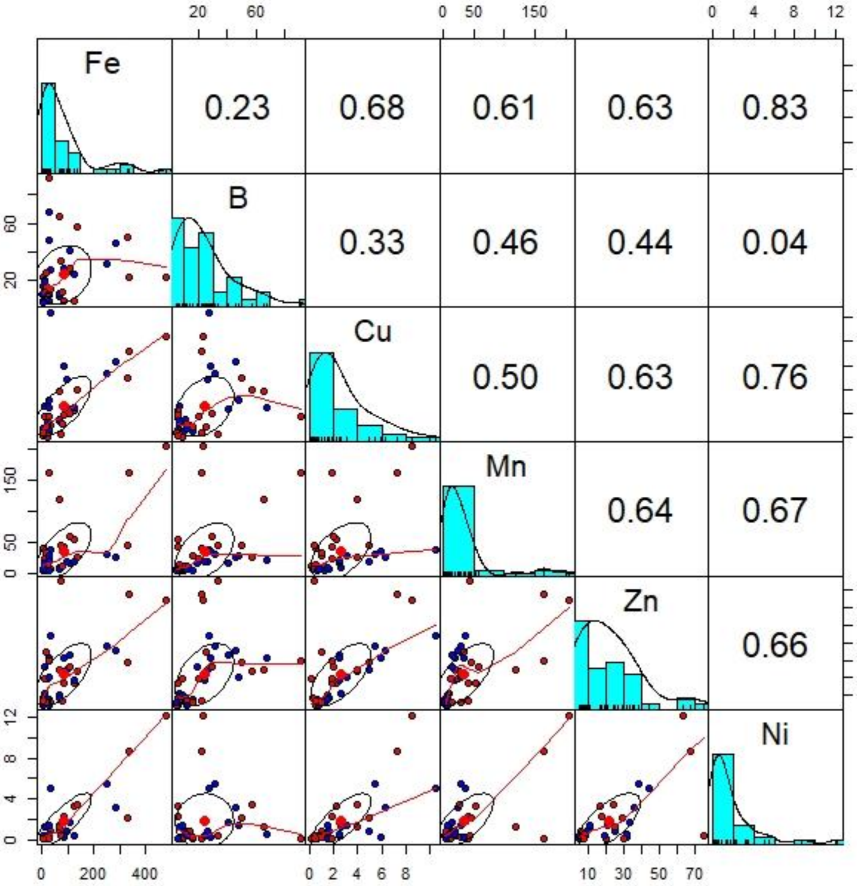


Table 20: Correlation table for micronutrients in root

CHAPTER 3

DISCUSSION AND CONCLUSION

Discussion

The two main components in a plant are its roots and stem. The root anchors the plant to the ground and absorbs resources from the soil, such as water and nutrients. The stem provides support and carries those resources to the rest of the plant. They play very different roles and is not surprising if their nutritional composition differs.

Such assumption was confirmed in this experiment. Through a series of statistical models, it was determined that these two parts interacted differently with soil nutrient concentrations. For this reason, two parallel analyses were carried, one for the root and for the stem and their interaction with both biochar and different genera was observed.

Thanks to its chemical and physical properties, biochar can affect nutrient concentration in soil and therefore alter its nutrient availability for plant uptake. Such properties play different roles depending on the nature of the different nutrients. Wood based biochar is usually high in pH (8 – 10). The batch used for this experiment was found to be extremely alkaline (11.3). The strong alkalinity affects the solubility of many nutrients, increasing their availability for plant uptake. On the other hand, its high cation exchange capacity could have had the opposite effect. The applied biochar had a moderately high EC (1400 μS/cm), which affects CEC. This might have played an important role in the retention of free moving cations in soil, decreasing both their leaching and availability for plant uptake.

The concentration of the following categories were analyzed and their behavior modeled through a series of statistical approaches. The chosen categories were: primary nutrients, secondary nutrients and micronutrients.

Primary nutrients:

Wood biochar usually has acceptable levels of phosphorus (P) and high levels of potassium (K) (Jahromi 2018). This assumption was true also for this biochar (Table 1,2). However, despite the high levels of K and P concentrations, no significant difference was observed in plant uptake relative to biochar application for both the root and stem (Table 4 and 13)

This might have been due to biochar high pH (Table 1), which could have decreased the solubility of both K and P. On the other hand, studies have shown that biochar can increase potassium solubility by decreasing its capacity to exchange sites in clay interlayers, ultimately increasing its availability (Rasuli 2021). The lack of significance was probably due to the small number of values for each nutrient variable, hence the inconsistency.

Positive correlation was observed between K and P, both in the root (0.93) and the stem (0.91). For what regards genus, no significant relationship was found between primary nutrients and different genera, for both the root and stem (Table 5 and 14).

Secondary nutrients:

The biochar applied was found to have very high concentration of Ca and Mg (Table 2). Despite the increased levels of such nutrients, no significance difference was found following biochar application in both the root and the stem (Table 6 and 15). The only exception was Mg, where a significant interaction was observed between its concentration in the stem and both biochar and genus (Table 15). Lots of significant differences were found among genera, in both stem and root concentrations (Table 7 and 16).

In the root, strong positive correlation was found between Mg and S (0.92) and Ca and Mg (0.89), which was not the case for root nutrient concentration. Again, this might be due to a lack of data.

Results showed to be inconsistent, and no clear patterns could not be drawn. This might be due physiological and biochemical differences between the different genera of plants and almost certainly due to the lack of sufficient data.

Micronutrients:

Macronutrients play a vital role in plant growth and development. Their concentration in soil should not exceed certain levels and the same is true for their concentration in plant tissues, hence the name.

Biochar has been found to be a great source of micronutrients. Their concentration mostly depends on feedstock material, pyrolytic temperature, and methodology. Wood biochar was found to be less nutrient dense than other types of biochar, especially when it comes to its micronutrient composition.

The batch of applied biochar was made from spruce wood and had a quite high hash percentage (Table 1), hence its high nutritional concentration (Table 2). However, micronutrient values were found to be moderate, except for Zn. Zinc is an essential element for both plant and human health, but in high concentration it can be toxic.

Significant interactions were observed between some micronutrients and biochar. For example, in the roots, significant statistical interaction was found between Cu and biochar, with an increase in concentration following biochar applications for all genus with the exception of Sedum (Table 9). In addition, genera was found to be significant for Fe and Co (Table 9). Similar results were observed for the stem concentration.

Significant interactions between micronutrients and biochar were found in Mn and Zn (Table 18). However, both these variables did not meet normality, thus compromising the results of the statistical models. Significant interactions with genus type were found in both the root and stem concentrations. For example, for the root, such significance was observed for Fe and Co (Table 10), while in the stem it was found for multiple nutrients, such as Fe, Cu, Mn, Zn, Ni and B (Table 19). However, Fe, Mn and Cu did not meet normality, thus their statistical relevance might be compromising.

Biochar is an extremely versatile material, with a wide range of application. Thanks to its peculiar physical and chemical properties, its applicability and functionality surpasses most agricultural colloids and fertilizers. It can be a great source of nutrients to the soil as well as a great amendment for amelioration of contaminated soils. Again, it is truly a versatile material. Although, there are a series of problems laying behind its production and compatibility with different soil types.

Biochar can be produced from a series of different raw materials, under different pyrolytic conditions. These are important factors that determine the overall nature of the final product. Special attention needs to be paid towards the composition of different biochar and their application to specific soil types. In addition, as today, the cost of production of biochar is relatively high compared to modern fertilizers and soil additives. However, our current global situation is shifting, both socially and environmentally. More pressures are felt on a multitude of crucial markets, such as the agricultural sector. The need for development and progress is pushing out the “old ways” of dealing with crop production and slowly embracing new ideas and techniques. I believe that biochar could play an important role in the upcoming future as an efficient and reliable technology to deal with a series of soil related issues, such as increased erosion, leaching and nutrient deficiency.

Conclusion

The purpose of the experiment was to find a connection between nutrient concentration and biochar application. The results showed no true significant interaction between the different nutrient categories and biochar. This was true for both the concentrations in the root and stem. Even though some nutrients showed some degree of significance (Mg and Mn in stem), their data was not reliable and could have compromised the results. The lack of significant interaction between plant nutrients and biochar application might have been due to the limited number of values for each category. In fact, only 40 data points per nutrient were collected for both nutrient variables for the root and stem.

On the other hand, a stronger statistical significance was found between different plant genera and their nutrient concentration. This was true for both the root and stem. The interaction was more evident in the stem, which saw significance in a series of nutrients, such as Mg, Fe, B, Cu, Zn and Ni. In the stem, significance among genera was only found in Ca, Fe and Co. There results did not come as a surprise, as different plant genus probably interact with soil nutrients in a different way.

Despite the ambiguity of the results, I believe that further investigations should be carried out. Biochar is an extremely interesting material with a range of different application, in the short run and more importantly in the long run. I believe that if I had extended the length of the experiment, I would have noticed more concrete and reliable results. Biochar has been observed to be an incredible tool for the retention of nutrients in the long run, a property that could play an important role in upcoming scenarios. As mentioned above, we are entering a new environmental area, characterized by harsher and more extreme climatic patterns. Longer periods of rain and drought will occur more frequently, as they already are, and the protection of soil fertility will become a priority.

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