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BACHELOR THESIS

**Nutrient leachability of biochar based
microgranules applied in low organic soils**

2021

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DECLARATION

I declare that I have worked on my bachelor thesis titled "Nutrient leachability of biochar based microgranules applied in low organic soils" by myself and I have used only the sources mentioned at the end of the thesis. As the author of the bachelor thesis, I declare that the thesis does not break any copyrights.

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LIST OF ACRONYMS

BC: pyrogenic black carbon
BP: before present
SOC: soil organic carbon
SOM: soil organic matter
DOC: dissolved organic carbon
TC: total carbon
TN: total nitrates
R: regosol
FS: forest soil
M: microchar
C: carbon
O: oxygen
H: hydrogen
N: nitrogen
K: potassium
Cl: chlorine
Si: silicon
Mg: magnesium
P: phosphorus
S: sulfur
Cu: copper
Zn: zinc
Cr: chromium
Ni: nickel
Al: aluminum
Mo: molybdenum
BLC: biochar loading capacity
t: ton
ha⁻¹: hectare

CEC: cation exchange capacity

HTT: highest treatment temperature

ID: internal diameter

SEM: scanning electron microscope

SR: soil stability ratio

SD: standard deviation

μm: micrometer (=10⁻⁶ m)

nm: nanometer (=10⁻⁹ m)

cmol_c g⁻¹: centimol of charge (1 cmol kg⁻¹ = 1 meq 100g⁻¹) per gram

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1 INTRODUCTION

1.1 Introduction

The application of biochar to soils is considered as a way to improve soil properties while sequestering carbon from the atmosphere. The evidence that supports the application of biochar as a soil amendment to increase crop productivity is growing, its utilization to increase creditworthiness of agricultural lands is related to biochar properties when it is applied to soils. It increases water and nutrient retention in soil, reduces drought episodes in agricultural lands, increases microbial activity of different soil types and improves water and nutrient release to soils.

This thesis is based on the study of different types of microchar (M), defined as microgranules of biochar mixed with manure, and their effect on different soil types. Two different microchars are tested and compared, a standard material industrially produced and an enriched microchar with vermitea. The application of these innovative compounds aims to improve the fertility and to prolong the tenacity of soils to retain nutrients, particularly in soils with low organic matter. In order to analyse the behaviour of microchar in soils, an incubation experiment will be done. The microchar will be tested in two different soils, a regosol (R) from Zvěřínek and a forest soil (FS) from Jevany, SLP.

This thesis includes a review of the properties of biochar, an explanation of its obtention and its effects when applied to soil, as well as other interesting effects of its application as carbon dioxide sequestration or its effect in crop production. In order to understand what biochar is, its definition, background and its most popular application strategies are detailed of the following chapters.

1.2 What is Biochar?

Biochar is a solid and organic material produced by thermal decomposition of carbon-based feedstocks. The decomposition must be made under oxygen-restricted environment, this process is called pyrolysis. The raw matter, or feedstock, used to produce biochar is essentially pre-selected biomass coming from wood, crop residues and manures, among others. The suitability of each feedstock depends on chemical, environmental, physical, economic and logistical factors [1,2].

The best way to describe biochar is as a soil conditioner, although its properties make it distinguished among other natural or produced conditioners. This material is commonly used as a livestock feed supplement, soil amendment, compost additive and for manure treatment [3]. It is also usually applied to improve plant and soil health, to prevent soil erosion, to ensure long-term fertility of agricultural soils and to improve soil water retention and soil carbon content [4,5]. There is evidence that suggest that carbon in biochar has a recalcitrant behavior in soils and can help mitigate climate change by sequestering carbon [6].

The positive effects of biochar in different ecosystems are explained by different characteristics of the material, as the water retention capacity [7], nutrient retention and transformation in soil [5], porosity capacity [4], redox properties [8], liming capacity [9] and its influence in soil structure [10]. These properties can change widely depending on the pyrolysis conditions and the feedstocks characteristics, those factors affect the physicochemical conditions of biochar, and therefore, also affect its suitability for certain applications, define its behavior in soil and determine its transport and fate.

It is important to point that the difference between biochar and charcoal is its utilization purpose, because in a physicochemical comparison those materials are basically the same. In order to use biochar for its basic objectives, enhance greenhouse effect by sequestering carbon in soil and improve soil quality, this C-based material must be treated and impregnated with nutrients. From scientific and practical perspectives, it is useful to distinguish between normal charcoal materials and those modified charcoals in which harmful effects have been reduced and the beneficial ones have been promoted.

1.3 Background

Historically humanity had been enriching soils since thousands of years ago, those man-made soils are called Anthrosols. The first Anthrosols enriched with charcoal are found in small areas throughout Amazonia, covering a total surface from 6,000 to 18,000 km² [11]. These soils were created by indigenous people 10,000 years BP and with depth down to 1 m, the name of these soils is Hortic Anthrosols [12]. Figure 1.1 shows the location of most of the Anthrosols found at the Amazonia, is significant that usually the size of the plots is between 10 and 200 meters in diameter and are placed near actual or historical human settlements.

The first Anthrosols in Europe have been dated to 3,000 years BP and are placed at the German island of Sylt [13]. The largest expanse in Europe, about 3,500 km² dates from the Middle Ages at the soils of The Netherlands, Belgium and Germany; these man-made soils are called Plaggic Anthrosols. The soils of these territories are nutrient poor and dry, the creation of these plots was essential for the survival of the villages [14].

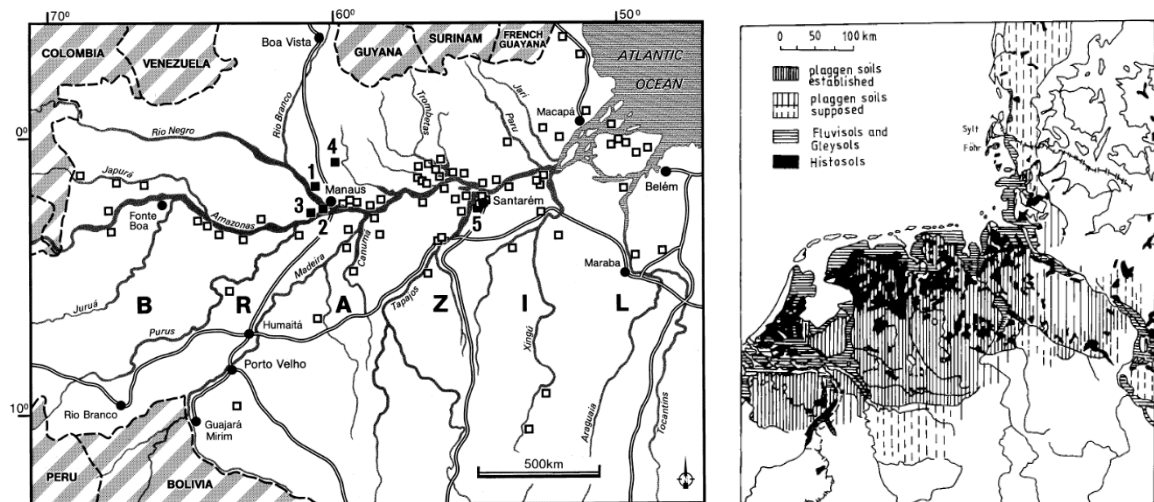


Figure 1.1 Distribution of Anthrosols in Amazonia [95] and Europe [96].

There is an important difference between Hortic Anthrosols and Plaggic Anthrosols, the first ones were made using a wide variety of organic and mineral materials (animal bones, charcoal and pottery fragments) and the second ones were composed by mixing topsoil material, peat and manure. Terra Petra soils are basically really close in composition to the soils from Amazonia, but what differences them from Hortic Anthrosols is the high proportion of charcoal in the first ones, made and added on purpose to improve soil properties [15].



Figure 1.2 Comparison between Terra Preta from Amazonian forests (left; Anthrosol with high quantity of charcoal) and naturally soil Oxisol (right) [15].

Figure 1.2 shows a comparison between soils from the Amazonian Forest, specifically Terra Petra soils (left image) and normal Oxisol soils without any treatment (right image). The color and humidity present in the soil from the left picture make the difference clear between treated and non-treated soils.

The colder climate in Europe contrast with the warm and humid weather of the Amazonia; in cold weather environments the microbial decomposition is much slower and residence times of organic matter are longer, cause of that the recalcitrant behavior of peat and plaggen soil (soil created in parts of northwest Europe) provided the necessary water and nutrient retention benefits to make the investment worthwhile. In tropical soils the recalcitrance of the organic matter needed to be improved and the use of charcoal and other materials to mix with the soil was also a necessity.

1.4 Similar approaches to biochar

A similar approach to biochar is the pyrogenic black carbon (BC), this material is basically charcoal produced by wildfires and can be founded naturally in soils all around the world. The characteristics of BC differ from biochar cause its properties cannot be controlled, even proportion of C could be larger that in some Terra Petra soils. As found in many research papers, the BC is spread around the world and in determined places its proportion referred to the soil organic carbon (SOC) can be potentially high. Skjemstad et al., 2002 found that BC constitutes 10-35% of total SOC from different long-term agricultural soils in the U.S.A., Preston and Schmidt (2006) showed studies on non-forested soils around the world with BC proportions between 1 and 80% of total SOC and Schmidt er al., (1999) analyzed BC quantities of chernozemic soil in Germany and their results of proportion of BC was between 2-45% of total SOC [16].

It is important to emphasize that the properties of BC cannot be controlled like those of biochar, in wildfires the feedstock is compound by aboveground biomass and in biochar the feedstock can be selected based on the suitability of it. Pyrolysis conditions are also important, the temperature, the duration and the rate of temperature increase are parameters impossible to control in a wildfire.

1.5 Application strategies

The way biochar is applied to soils has a considerable impact on the health and functioning of the soil, it can also affect the behavior of biochar and its environment. There are three main different approaches, topsoil incorporation, depth application and top-dressing [17].

Topsoil incorporation is based on applying biochar on its own or by combining it with composts or manures. The degree of mixing depends on the cultivation technique used, in regular tillage systems the mix is done homogeneously through the topsoil till approximately 30 cm depth. Wind and water erosion are responsible of removing biochar among with soil particles.

In depth application the biochar is placed into the rhizosphere, this technique is more beneficial for crop growth and less susceptible to erosion. This application technique can be carried out by pneumatic systems or by applying the biochar in furrows [18].

Top-dressing biochar is based on spreading the biochar, mostly in form of dust, to the soil surface and the incorporation of it to the soil is done by natural processes. The aim for this application form is mainly for those situations where mechanical incorporation is not possible. This application strategy has more risks, this mixture is more susceptible to wind and water erosion, as well as the potential impacts to human health and to other ecosystems [19]. Both techniques, top-dressing and topsoil, can be implemented with a large range of frequencies, it depends on the objectives and conditions of the implemented area.

2 OBJECTIVES AND METHODOLOGY OF THESIS

2.1 Objectives

The main objectives of these work are listed below:

1. Provide a theoretical review of biochar characteristics and its effects in soil, as a carbon dioxide sequestrator and its effect in crop production.
2. Study and determine the characteristics and composition of an industrially produced microchar (protocol without vermitea).
3. Study and determine the characteristics and composition of an enriched microchar (protocol with vermitea).
4. Analyze the effect of microgranuls of biochar in soils with low organic matter by an experiment of incubation. The chosen microchar will be tested in two different soils, a regosol from Zvěříněk and a forest soil from Jevany, SLP.

2.2 Scope of work

To achieve the objectives above, this project is segmented in two parts. The first part consists of a theoretical review of biochar, beginning with its obtention and the most classical methods to produce activated biochar, followed by a review of its physicochemical properties and ending with its effects on soil and the environment. This last section consists of an explanation of the effect of this amendment to soil (explaining parameters as nutrient and water retention, soil stability and the effect on soil diversity), its carbon sequestration potential and its effect in crop production.

The second part of the project consists of an investigation at laboratory of the both microchars mentioned above, in order to determine its composition and characteristics. The effect of microchar in soils with low organic matter is the most important part of the study. The processing of the data obtained at the laboratory is crucial in order to the research of correlations between variables and to analyze its effect in soil.

Is crucial to mention that the scope of this work was reduced by a factor of time, the global pandemic suffered on the last year delayed the laboratory work for months till a point where only the incubation test could be done, and it was done with lots of complications related to governmental restrictions.

3 THEORETICAL PART

3.1 Biochar preparation

The obtention of biochar is based on the process of pyrolysis, depending on the operational conditions of this mechanic the properties of the obtained biochar may differ. The biomass feedstock is also crucial for its production. In this chapter the pyrolysis procedure and how it affects the resulting biochar are explained, as well as the importance of the feedstock material.

3.1.1 Pyrolysis

The process of pyrolysis is described as the chemical decomposition of organic matter by heating in the absence of oxygen, in practice is not possible to make an environment totally free of oxygen, although the degree of oxidation in pyrolysis processes is lower compared to natural combustion [20].

Pyrolysis processes work at temperatures in the range of 400 to 800 °C, depending on the type of pyrolysis and the objective of its utilization. The high temperatures of this process can induce polymerization of the molecules of biomass, larger molecules are also produced, like aromatic or aliphatic compounds. Pyrolysis transforms organic matter into different components, the proportion of solid, liquid and gaseous matter depends on the type of pyrolysis and feedstock used [21]. The solid component obtained after being processed is charcoal, this material can be called biochar if its destination is to be mixed with soil [22].

Chemical industry has adopted pyrolysis to produce a large amount of compounds, among which we find charcoal, activated carbon, methanol and syngas. It is also used to produce lighter hydrocarbons as petrol. Pyrolysis can also occur in a natural environment and produce the already mentioned black carbon [23].

Using pyrolysis to produce charcoal is one of the oldest industries performed by humanity, although, over time, these processes had been continuously evolving to improve in performance, energy efficiency and pollution control [24].

3.1.2 Methods of pyrolysis

Although the basics of pyrolysis process are always the same, there are different methodologies specified to obtain different outputs. The main manipulable variables of pyrolysis are the temperature and the residence time of the feedstock. By changing these variables, the proportions of end products vary, either if the output desired is biochar, bio-oil or syngas [25]. Residence time affects the solid constituents of feedstock and the hot vapor produced under pyrolysis.

There are four types of pyrolysis generally referred in the nomenclature, fast, intermediate, slow pyrolysis and gasification. Slow pyrolysis is commonly referred as carbonization due to the high proportion of solid material produced, also known as biochar. Each type of pyrolysis produces different proportions of liquid, solid (biochar) or gas (syngas) [2,25].

Mode	Liquid (bio-oil)	Solid (biochar)	Gas (syngas)
Fast pyrolysis	75%	12%	13%
Intermediate pyrolysis	50%	20%	30%
Slow pyrolysis (carbonization)	30%	35%	35%
Gasification	5%	10%	85%

Table 3.1 Proportion of liquid, solid (biochar) and gas (syngas) material produced by different methods of pyrolysis. Data obtained from [26] (adapted from [25]).

Table 2.1 shows a comparison between the 4 standardized methods of pyrolysis, the fast pyrolysis produces more liquid content than the others, reaching around a 75% of the total matter. This process is done at moderate temperature, around 500°C and at short hot vapor residence time of approximately 1 second.

Intermediate pyrolysis generates a 50% of liquid, being this its major content, and solid and gas quantities are 20 and 30% respectively. This process is done at moderate temperature, around 500°C and at moderate hot vapor residence time from 10 to 20 seconds.

Slow pyrolysis (carbonization) generates approximately the same quantity of each matter, reaching a 35% in biochar and syngas. Due to this reason, it can be said that this method is optimized for biochar production and it is the most commonly used technology for its production. Carbonization is done at low temperature, around 400°C and with a very long residence time.

Gasification produces around an 85% of syngas material, the name of this technique is directly given from its final product. Gasification is done at high temperature, around 800°C and long vapor residence time [25].

There are other techniques related to the mentioned previously, some modified carbonizations are able to produce more quantities of biochar than the standard procedure quantified by the International Energy Agency (up to 89% of solid output). Hydrothermal carbonization is another process to obtain more proportion of solid material, it is based on pyrolyzing the feedstock at temperatures between 180 and 280°C in water with residence times between 30 minutes to 16 hours, results show high quantities of biochar production (36 – 72% of solid output) [27].

As the pyrolysis conditions can vary in a wide range of possibilities, the scale of the equipment needed can also vary greatly. There are two different scales, industrial scale and small “home-made pyrolysis”. The first one refers to industrial pyrolysis plants, where the biowastes of feedstocks are accumulated and then transformed into biochar. The second one is based on small equipment, even mobile, used in farms where the pyrolysis is done in situ.

3.1.3 Types of biochar

The internal structure of biochar and its properties are different depending on the pyrolysis process used, even with the same feedstock. Low-temperature biochar is formed below 400°C, this C-material has low stability in soil, high cation exchange capacity (CEC) and low pH, it has a lower reactive surface, and its concentration of functional groups is high, as well as its hydrophobicity. High temperature biochar is made at high temperatures, above 600°C, and its characteristics are the opposite as the low-temperature

biochar, high stability in soil, low CEC and high pH, high reactive surface and its concentration of functional groups is low, as well as its hydrophobicity [28].

The internal structure of biochar depends on the type of pyrolysis used to make it. Degradation of cellulose, between 250 and 350 °C, results in a mass loss in form of volatiles. At low temperature of pyrolysis, around 350 - 400 °C, the structure is based on a rigid amorphous C-matrix. At higher temperatures, the structure has a higher content of aromatic carbon due to a higher loss of volatile matter, the alkyl and O-alkyl C turn to aryl C. At biochar formed by middle and high temperature pyrolysis, around 600 °C, the carbonization dominates the process, almost all the remaining non-C atoms are replaced and the C content increases [29].

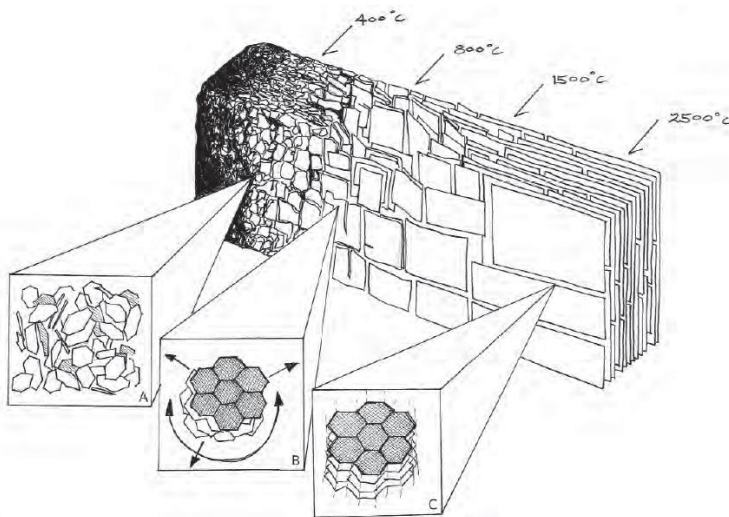


Figure 3.1 Structure of ideal biochar developed with highest treatment temperature (HTT). (A) represents a disordered amorphous C-matrix, increasing in proportion of aromatic C. (B) formation of sheets of aromatic C, remaining non-C atoms are replaced by carbon structures. (C) the structure becomes graphitic. Obtained from [30].

Figure 2.1 shows a representation of the internal structure of biochar at different temperatures of process, this structure is idealized at the HTT point, which is the maximum temperature to which the biomass is subjected to in the pyrolysis reactor. At the beginning the structure is disordered and composed by an amorphous C-matrix, by increasing the temperature the proportion of aromatic C increases. On the next step, carbonization dominates the process and the structure begins to be formed by aromatic sheets of carbon. After that point, the temperature is above 800°C and it is considered as high-temperature biochar, the structure becomes graphitic and it is composed only by carbon structures.

3.1.4 Feedstocks

The biomass selected to be pyrolyzed to turn into biochar is known as feedstock, although any organic matter can be pyrolyzed, the residue (liquid or gas) and the physicochemical characteristics of the biochar may vary from the desired. Pyrolysis conditions of process affect the biochar resultant, but feedstock is the most important factor to control the final properties of biochar. The chemical and structural composition of biochar is inherited from the feedstock [31].

The most important components of feedstocks are the proportion of ash, lignin and cellulose, the quantity of each one determines how the structure of the material is retained during pyrolysis [32]. Biomass with high content of lignin produces the biochar with the highest yields due to the high stability of lignin to thermal decomposition. Even in pyrolysis with comparable conditions, the loss of lignin is half the loss of cellulose [32]. Wood-based feedstocks contain low proportions of ash, although, biomass with high content of mineral (grass, grain husks or straw residues) tend to produce ash-rich biochar. Rice husk may contain up to a 24% by weigh of ash [33] and rice hulls up to a 41% by weigh [34]. The mineral concentration of feedstock material is conserved when transformed into biochar, with the difference that in the resultant product its more concentrated due to the loss of carbon, oxygen and hydrogen [32].

Table 2.2 shows the quantities by weight of ash, cellulose and lignin of some feedstock materials for biochar production. Wood materials have a higher proportion of lignin, it results in coarser and more resistant biochar with high carbon content (up to 80%). The content of cellulose is approximately the same for these four feedstocks, but the difference of ash content is appreciable. Source materials with high content of mineral have high percentages of ash residue.

	Ash	Cellulose (w w⁻¹)	Lignin
Wood (oak, poplar)	0.27 – 1	38 – 45	26 - 30
Wheat straw	11.2	38	14
Maize residue	2.8 – 6.8	39	15
Switchgrass	6	32	18

Table 3.2 Key components by weight in some biochar feedstocks, wood, wheat straw, maize residue, switchgrass. Adaptation of key component quantities obtained from [24].

There is a wide range of materials suitable to be used as feedstock for biochar, some are wood, grain husks, nut shells, crop residues or manure, among others. The materials with higher carbon content, as nut shells or wood, are used for activated carbon production, these also have the advantage of being abundant and having lower associated costs [35,36]. Other materials have been proposed as feedstocks, like biowaste (from municipal waste or sewage sludge) and compost. However, there is an inherent risk with these source materials due to hazardous components as organic pollutants and heavy metals.

Some studies reveal that even using the same type of biomass as feedstock, differences on the growing environmental conditions may produce a variance in the properties and composition [37].

3.2 Biochar fertility

As biochar is only carbon, the impregnation by nutrients is necessary to substitute conventional (mineral) fertilizers by biochar-based products. The addition of biochar to soil affects in a positive way the ecosystem, improving the fertility of soil and the biological activity. However, if untreated biochar is mixed with soil there is the possibility to lead to an inhibition of plant growth and affect negatively to the soil microflora and rhizosphere. There are two characteristics of biochar responsible of this behavior, its porosity structure and its strong adsorption power which takes out the nutrients and water from the soil [38].

Modification of biochar is essential to avoid these effects, there are different mechanics to increase biochar fertility (activate biochar) which are based on chemical or physical modification of the material. Before the application of biochar, it should be loaded with nutrients and water, colonized with microorganisms (to ensure that nutrients are easily available to plants) and aged by oxidation (to bring CEC to its maximum). The most used methods are charging the biochar with compost, with livestock manure, incorporating fertilizers and by lactic acid fermentation [38,39].

The charging of biochar with compost is one of the most extended techniques, is the best way to produce substrates similar to Terra Preta. The stimulation of microbial is high and the availability of nutrients and water increase exponentially. The proportion ideal of

biochar-compost is around 1:1 and be stored for at least two weeks to ensure good performance [39].

Charging biochar with manure from livestock is preferable than using only one type of manure, the yield of biochar using this technique should be better than with the first one. This method is not based on composting, therefore, the manure should be stores at least one year before its utilization [39]. The ratio of biochar to manure should be 4:1, but it can be changed depending on the nutritional needs of the area.

The utilization of fertilizers to activate biochar is another excellent and well implemented technique, usually organic liquid fertilizers are preferable instead of NPK fertilizers due to the availability of other elements besides N, P and K. The period of activation depends on the fertilizer implemented, but it is usually shorter than that of the first technique [39].

The utilization of lactic acid to ferment biochar is the last most common technique used, this technique uses industrial solutions (as EM-A or Brottrunk) to ferment biochar. The process is one of the most laborious, although the activation time is between 3 and 4 weeks.

3.3 Physicochemical properties of biochar

The physicochemical properties of biochar are fundamental for its suitability into a determined soil and for its good performance. This chapter describes a general overview of the chemical and physical properties of biochar before and after its incorporation into a determined soil, focusing on the first step.

3.3.1 Structural composition

The changes of the internal structure of biochar while the process of pyrolysis is done was mentioned at chapter 2.1.3 Types of biochar, nonetheless, this chapter is focused on its external and internal structure when the biochar is already formed.

Each biochar particle is composed by two different structural formations, stacked crystalline graphene sheets and randomly ordered amorphous aromatic structures. Is common to find hydrogen, oxygen, phosphorous and sulfur incorporated with the aromatic rings, those elements help greatly the reactivity of biochar [29]. Figure 2.2

shows a representation of these two structures, the composition and size of them can change from a biochar to another.

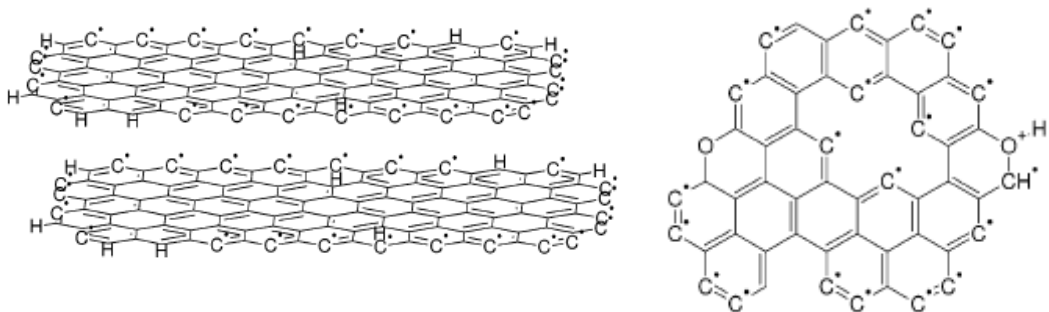


Figure 3.2 Representation of a stacked crystalline graphene structure (left) and an aromatic structure containing hydrogen as free radicals (right). Adapted from [29].

Charcoals have a porous structure and a large specific surface area, this characteristic is very important as it influences all the essential functions for fertility, as microbial activity and water, nutrient and air retention. The pore-size distribution of carbon materials is also a fundamental feature for biochar behavior in soils, there are 3 different categories according to their internal diameters (ID), macropores ($ID > 50\text{nm}$), mesopores ($2\text{nm} < ID < 50\text{nm}$) and micropores ($ID < 2\text{nm}$) [40]. Macropores help to improve vital soil functions, as aeration and hydrology [41]. Microscope surface areas are larger than macrospore surface in biochar, although macrospore volumes are bigger. A representation of a macropores structure in biochar can be seen at Figure 2.3, this image corresponds to wood-based biochar and was obtained using a scanning electron microscope (SEM) [42].

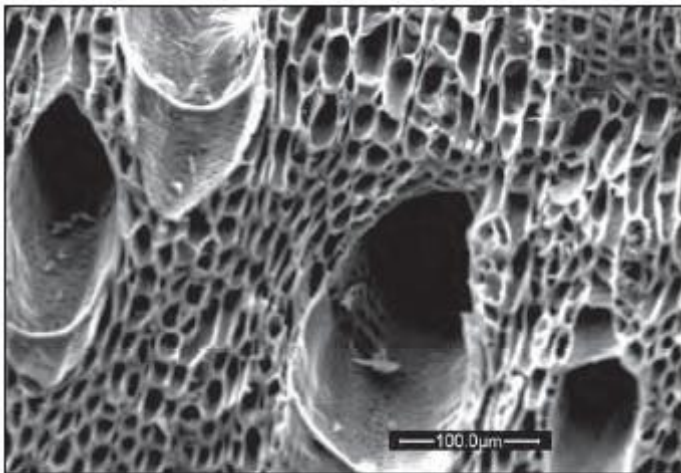


Figure 3.3 Image from Wood-derived biochar produced by slow pyrolysis to show the macroporosity of the sample. Images obtained from [42].

The porosity structure of the feedstock material is retained at the obtained biochar, although, HTT has been found to be one of the most important factors for pore distribution as the majority of physical changes are temperature-dependent and occur while processing the biomass [35,43]. Higher HTT and retention times favor the development of microporosity [35]. These are not the only factors that affect pore size, heating rate and pressure of the pyrolysis process also influence the pore size distribution, as these parameters have an impact on the mass transfer of volatiles [43].

The parameters mentioned before affect the pore distribution greatly but it is important to emphasize that the influence of those parameters in the final char is dependent on the type of feedstock and its behavior at determined processing conditions [31,35]. As an example, Gonzalez et al, 2009 found that increasing the process temperature from 250°C to 500°C at pistachio-nut shells feedstocks the development of micropores is improved, this is due to the increase evolution of volatiles. From the other hand, feedstocks from almond tree pruning have a higher volume of meso and macropores due to the slow decomposition rate of these materials [44].

3.3.2 Particle size distribution

Particle size is determinant for the suitability of each biochar for specific applications, but also for the safety and health issues related to handling, transportation or storage of biochar. The size of biochar's particles is influenced principally by the nature of the biomass feedstock and the process of pyrolysis [45]. As shown in different experiments and discussed by Sohi et al., 2009, wood-based feedstock usually produces more coarser biochar and chars from crop residues generate finer and brittle structures [46].

Pyrolysis procedure also affects particle size, it decreases as the pyrolysis temperature increases (usually at high temperature processes, between 450 °C and 700 °C), this is due to a reduction of the resistance to attrition while the heating process [45].

Temperature is not the only operational condition during pyrolysis that affects biochar physical structure, heating rate, HTT, residence time, pressure, flow rate of inner gas and reactor characteristics are some examples [35,42–44]. Out of all the process characteristics that can affect particle size, heating rate, residence time and pressure during processing showed to be determinant to produce finer particles of char, regardless of the origin of matter [31]. For high heating rates, between 105°C – 500°C sec⁻¹, and short residence times, the result obtained is finer biochar material [31]. On the other hand, slow pyrolysis process with heating rates between 5°C and 50°C min⁻¹ results in much coarser material [45]. For fast pyrolysis the feedstock size must be smaller compared to slow pyrolysis procedures, this also helps to obtain finer or larger and coarser biochar particles.

3.3.3 Chemical composition

The chemical composition of biochar can be described as complex and heterogeneous, it contains stable and labile compounds [46]. Its major constituents are carbon, volatile matter, mineral matter (ash) and moisture [43]. The proportion of these components determines the chemical and physical behavior and function of biochar [24], it also defines if it is suitable for specific applications. The ash content of biochar depends on the ash content of the feedstock. Moisture is also a critical component [43], higher

moisture contents increase the cost of production and transportation of biochar. A reasonable proportion of moisture is about a 10% by weight [37].

While processing biomass numerous chemical bounds break and rearrange, this process results in the formation of different and numerous functional groups as hydroxyl (-OH), amino (-NH₂), nitro (-NO₂), ketone (-OR), carboxyl (-(C=O)OH), aldehyde (-(C=O)H) and ester (-(C=O)OR). These formations predominantly happen on the surface of the graphene sheets and on the surface of pores [47,48]. These groups have different behavior, some act as electron donors while others as electron acceptors, this conduct results on coexisting areas in which properties range from basic to acid and from hydrophilic to hydrophobic [49]. Also, some functional groups can contain other elements as nitrogen or sulfur and these can affect the behavior of biochar, particularly in biochar from manures, rendering wastes and sewage sludge. There is evidence that demonstrates experimentally that the composition, distribution, proportion and reactivity of functional groups are dependent of factors as the source of material and the pyrolysis method used [43].

During the process of pyrolysis, as the temperature rises, the proportion of aromatic carbon also rises and around 300°C the nitrogen content reaches its peak [50]. Low temperature pyrolysis favors the accumulation of large proportion of potassium, chlorine, silicon, magnesium, phosphorus and sulfur. Processing at high temperatures, higher than 500°C, the retention of nutrients is favorized and the yield is equally advantageous [51].

3.3.4 Biochar loading capacity & residence time

Biochar loading capacity (BLC) is related to the maximum amount of C (in form of biochar) that can be added to soils without compromising other soil functions or the environment. Is known that high application rates of biochar produce a loss of its positive effects compared to lower application rates, in which beneficial properties remain longer in time.

In their studies, Rondon et al., (2007) showed positive effects on yields in which biochar rate application was up to 50 t C ha⁻¹, these effects disappeared at 60 t C ha⁻¹ and negative

effects were shown at rates of 150 t C ha^{-1} [52]. These results show that BLC is crop dependent, as well as soil and climate dependent.

In order to calculate the BLC for a specific site, it has to be ensured that future crop productivity is not compromised, soil biology or transport of fine particles are also factors to take into account. As previously mentioned, biochar is created through the pyrolysis of plant materials, this process increases its recalcitrant capacity. This recalcitrant behavior affects directly the residence time, biochar has an estimated residence time in the range of hundreds to thousands of years, while residence time in plant materials is in the range of decades.

3.3.5 Cation exchange capacity & pH

The cation exchange capacity (CEC) is the ability of a soil to hold exchangeable cations, this property influences soil structure stability, nutrient availability, soil pH and the soil reaction to fertilizers and other ameliorants [53]. Mineral and organic matter components of soil have negatively charged sites on their surfaces, these absorb and hold positively charged ions by electrostatic force. This fact is crucial to the supply of nutrients to plants because many nutrients exist as cations, as magnesium, potassium and calcium. In general, a high CEC is related to more fertile soils because they retain more cations, however, low CEC soils are perfectly capable of growing productive crops and pastures[54].

CEC in biochar depends on the type of feedstock, but usually their range is from negligible to approximately $40 \text{ cmol}_c \text{ g}^{-1}$, but it can change after being incorporated to soils [1]. This change may occur by a process of leaching of hydrophobic compounds [55] or by increasing the carboxylation of carbon via abiotic oxidation [56].

Biochar pH values are relatively homogeneous, considering the heterogeneity of its properties. pH values in biochar usually goes from neutral to basic, although is possible to find biochar with pH values from around 5 to 10. Low values of pH in soils (acid pH) often reduce the CEC and nutrient availability. When a biochar is introduced into soil, the pH of the soil-biochar matrix changes, depending on the pH values of both components.

3.4 Biochar in soil

This chapter discusses the effect of biochar once incorporated to soils. First, its effect to soil stability, water and nutrient retention and its effect to microbial activity. Also, some possible negative effects of its application are explained in this chapter, as contamination of soils.

3.4.1 Stability in soil

The physical properties of soil can be modified by incorporating biochar, its texture, structure, pore size distribution and density can be altered. These changes also affect soil aeration, water retention capacity, soil workability and plant growth [45].

Soils are usually considered as a pore system continuum and are characterized by two types of pores, inter-aggregate and intra-aggregate. The first is related to pore spaces between fabric units, as structural pores and macropores, the second type includes pore spaces between particles, referring to textural pores and micropores [57]. Inter-aggregate porosity affects water dynamics (usually in non-structural soils) and intra-aggregate porosity affects water dynamics (usually in structural soils) [58]. The pore distribution in soils depends on the proportion of organic matter.

The addition of biochar to soil can affect the pore network. Biochar particle size, its mechanical strength and pore size distribution can cause the porosity of soil to increase or decrease. The climate characteristics and the methods used for working with soil are also relevant for porosity changes. Biochar application may also increase the net soil surface area [59] and consequently improve soil water retention and soil aeration [45]. An increase in soil specific surface also benefits microbial communities and the sorption capacity of soils. Although, soil hydrology may also be affected by these changes, generating a partial or total blockage of soil pores and generating a decrease in the water infiltration rates of soil.

Many experimental reports expose changes in the soil stability ratio (SR) after the incorporation of biochar, Baiamonte et al., 2015 showed that in sandy-clay soils the SR trends to increase while increasing the amount of biochar implemented [10]. This is due to the cementing effect retrieved by the added organic material [60].

Figure 3.4 shows the SR dependence on the biochar content (f_{bc}), as the biochar content increases, the SR also grows. For this experiment done by Baiamonte et al., 2015, the increment of SR is completely linear until reaching its maximum at a biochar content of 0.33. The biochar content parameter (f_{bc}) used for doing this chart are 0 at the beginning (sole soil), followed by 0.091 (corresponding to 41.6 t ha⁻¹ of biochar), 0.23 (72.2 t ha⁻¹ of biochar) and 0.33 (84.6 t ha⁻¹ of biochar). These results were obtained in a sandy-clay soil [10].

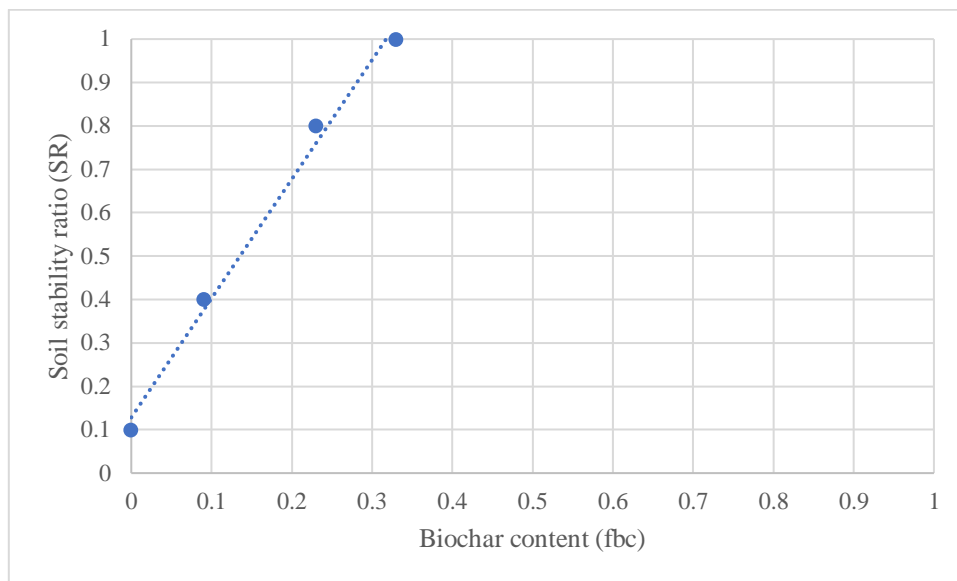


Figure 3.4 Soil stability ratio dependence on biochar content (f_{bc}). Data obtained and adapted from [10].

Biochar has a bulk density lower than mineral soils, therefore, the application of biochar can reduce the bulk density of the soil. As an example, if 100 t ha⁻¹ of biochar with a bulk density of 0.4 g cm⁻³ is applied to a soil with a bulk density of 1.3 g cm⁻³, and the biochar does not fill the pore space of soil, the resultant material will have a bulk density reduction between 0.1 to 1.2 g cm⁻³. Nevertheless, it is possible to increase the bulk density of the mixed material, if the applied biochar has low mechanical strength and disintegrates easily, the existing pore spaces in soil will be filled up, resulting on an increase of the dry bulk density.

Soil compactibility is related to soil bulk density and can be also affected by the application of biochar. Biochar has low elasticity, and this property negatively affects the resilience to compaction [61].

3.4.2 Water and nutrient retention capacity

The application of biochar is beneficial for the agriculture, these benefits are usually related to an improved water and nutrient retention. The addition of biochar to soils has direct and indirect effects on water retention properties, which can last little or long time. Water retention in soils is regulated by the distribution and connectivity of the pore network, which depends on the soil particle size, the aggregation of soil and the soil organic matter (SOM) [2,10].

The water retention of soils is directly affected by the large inner surface area of biochar. This parameter is dependent of the feedstock material and the properties of pyrolysis, a char produced between temperatures of 400 and 1000°C could have a surface area from 200 to 400 m² g⁻¹ [62]. The indirect effects of biochar application are related to an improved aggregation of the resultant soil. Biochar addition can modify the soil structure by interacting with SOM, mineral and microorganisms. Even though high surface area of biochar can increase the retention of water, this effect is dependent on the initial texture of soil. Soil with coarser textures or soils with large amounts of macropores are more prone to improvements in water retention.

The capacity of soils to store water regulates the time and the amount of water available for crop transpiration. Biochar porosity is basically composed of micropores [63], therefore, the additional plant available water depends largely on the biochar feedstock and the texture of the soil in which is applied to. In case the soil starts to dry, the water and nutrients stored in the micropores of biochar may become available. These characteristic increases plant water availability during dry periods and is useful for crop production.

Baiamonte et al., 2014 founded that in sandy-clay soils the addition of biochar results in a pore size diameter increase. By analyzing the differential water capacity of soil at different fractions of biochar, the increment of pore size was confirmed. By increasing the size of pore diameter, the space for water retention also raises and the saturated water content increased from 0.423 to 3.039 kg kg⁻¹. According to these results, the application of biochar to sandy-clay soils results in an increment of the maximum available water content.

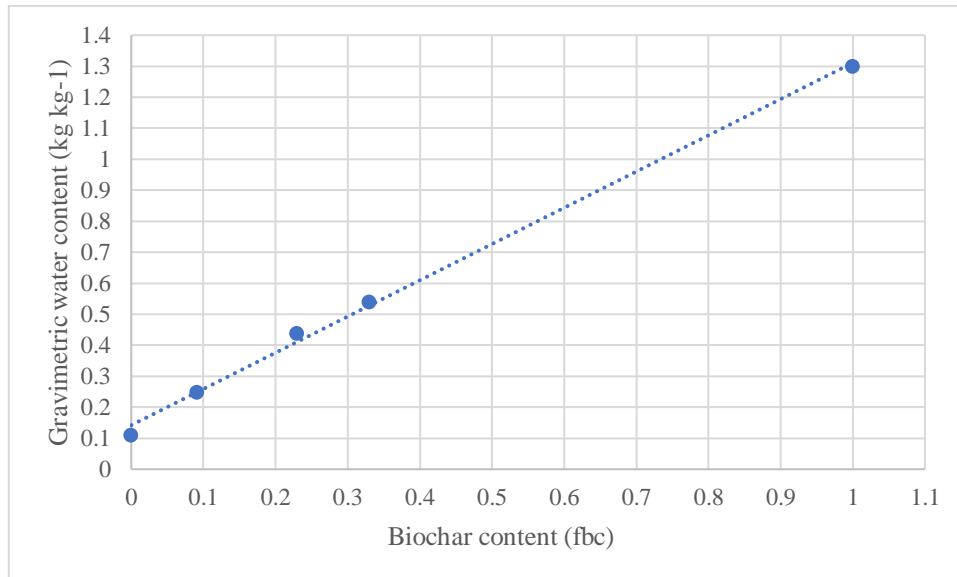


Figure 3.5 Gravimetric water content kg kg^{-1} dependence on biochar content (f_{bc}). The data shown at the chart represents the maximum available water. Data obtained and adapted from [10].

Figure 3.5 shows the gravimetric water content dependence on the biochar content (f_{bc}), as the biochar content increases, the gravimetric water content also grows. The data shown at the chart represents the maximum available water content of the soil, as mentioned before, this parameter confirms that the water retention capacity can increase when applying biochar [10]. This parameter, as almost every already mentioned, may vary according to the soil characteristics and the biochar type, among other factors.

Long term effects on water retention and soil structure are determined by the mechanical stability and the recalcitrance behavior of biochar. Feedstock type, operational conditions, physicochemical conditions and the compaction and compression of biochar determine these properties.

Biochar can help to improve nutrient retention in soil, in addition to enhance nutrient availability in soil it is important to reduce nutrient leaching from agriculture. Several studies indicate that biochar may be also useful to achieve that objective, not only reducing nutrient leaching, but also decreasing the transportation of contaminants below the root zone [26].

Nutrient leaching decreases and nutrient use efficiency improves by increasing water and nutrient retention, these improvements are related to an enhance of the internal reactive surface area of the matrix, a decrease of the water percolation below root zone and an improvement in plant nutrient use through enhanced crop growth. The water percolating

beyond root zone is dependent on the proportion and connections between micro and macro pores.

Soil water retention capacity affects the partitioning of groundwater recharge, surface-water runoff and evapotranspiration. Situations in which the incorporation of biochar improves water retention and plant transpiration lead to scenarios where the percolation can be reduced. The reduction of this parameter helps to improve the retention of nutrients susceptible to leaching (nitrates and base cations).

Biochar contributes directly to nutrient adsorption through charge or covalent interactions on a high surface area. It has been showed that biochar needs to be produced at temperatures higher than 500°C or be activated to increase its surface area and directly improve the sorption of nutrients [64]. Glaser et al., (2002) concluded that biochar help to increase the ion retention in soil and to decrease the leaching of dissolved organic matter and nutrients [65].

It is also reported by [40] that the ratio of uptake to leaching for nutrients increases when biochar is applied, also the water percolation did not decrease in their experiment. This is due to a retention of nutrients by electrostatic adsorption complexes created by charcoal.

3.4.3 Soil microbial activity

Soil is a high complex and dynamic habitat for organisms, it contains the soil biota which is vital to the functioning of soil. The high heterogeneity of soils allows many different niches to be part of the soil biota activity.

On the microscale, the soil can be understood as an aquatic habitat because the micropores are usually full of water. This characteristic is vital for the survival of some microbial species which require an aquatic environment for its existence. Few of these microorganisms could be nematodes and protozoa, the gran majority of these organisms can enter into a state of survival of cryptobiosis. This state guarantees its survival in case of absence of water, when the environmental conditions change and the micropores have water again, the organisms go back to their normal condition [66].

Organisms in soil form complex communities to guarantee their survival, one of the most interesting techniques consists of hiding in safe refuges to avoid becoming preys. Biochar provides high levels of refugia due its porous nature. Another characteristic of these microorganisms is related to energy production, they do not need the decomposition of biochar as an energy source. This is one the mechanics that ensure their survival and is related to the increase in microbial biomass [67]. Kolb et al., (2009) have demonstrated that charcoal addition affects microbial biomass and microbial activity, although the magnitude of microbial organisms depends on the difference in nutrient availability in soil [66]. It is suggested a predictability in the response of soil biota when biochar is applied, this is due to the similar behavior of microorganisms in biochar and in soil.

Currently, there is a lack of data or experimental research between fungi and biochar. Nonetheless, the behavior of these organisms can be predicted according to their performance in similar systems, fungi is most likely to alter the stability and longevity of biochar in soil.

Mycorrhizal (symbiotic association between a plant and a fungus) is also affected by biochar, its abundance increases in the presence of biochar. Plant growth is promoted by applying biochar due to the improved fertility provided by microorganisms as mycorrhizal, this is another mechanism among the already previously mentioned [68]. Biochar also provides mycorrhizal of refuge and increases its live expectancy.

For the most part of soil biota, biochar appears to work more as a mineral constituent of the soil than as organic matter. Although biochar causes a significant increase in microbial efficiency. By adding organic fertilizers among with the biochar, the increase in microbial biomass and its efficiency is even better [67].

The parameter mentioned during this chapter demonstrate that is the interaction between biochar and soil biota which provides the positive effects related to its application.

3.4.4 Contamination by biochar

The increasing interest in biochar puts some focus of interest in its potential for soil contamination. It is decisive to determine that water quality, soil functions and processes are not under risk due to biochar application [37]. The pollutants to consider as dangerous

are not the mineral ones, as salts in some types of biochar may only affect soil functioning; conversely, heavy metals, PAHs and dioxins remain bigger issues of concern for soil contamination and health risk.

The presence of these compounds in biochar depends on the feedstock used (usually contaminated feedstocks) and the operational conditions of pyrolysis. Slow pyrolysis process at temperatures below 500°C may contribute to the accumulation of these contaminants and other species with disinfectant and antibiotic properties (as formaldehydes, xylenols or cresols) [69].

The feedstock used for biochar production is directly related to the presence of heavy metals in the resulting material. Biochar produced from sewage sludge may contain high concentrations of copper (Cu), nickel (Ni), chromium (Cr) and zinc (Zn) [70]. Another example has been reported by Gaskin et al., 2008, low concentrations of molybdenum (Mo), aluminum (Al), Ni and Cr were detected in biochars from poultry litter, pine chips and peanut hull when produced between 400 and 500°C [71].

The safe application rate of biochar is often determined by the metal concentration of the feedstock material, at ordinary application rates the environmental risk derived from metal species is low. It is even comparable to the associated risk of the use of conventional fertilizers. Relevant risk can be expected at high application rates, over 250 t ha⁻¹ [72].

The generation of condensed PAHs is associated to secondary chemical reactions during thermal decomposition of organic matter at temperatures over 700°C. Nonetheless, little concentrations of PAHs can also be produced at temperatures between 350 and 600°C [73]. The possible presence of these molecules in soil and water environments represent a serious public health issue. Until nowadays there are no toxicological reports associated to PAHs in soil due to biochar application, cause of that there are not estimated application rates of biochar related to the presence of PAHs.

Dioxins and furans are other contaminant to consider, fortunately there are no reports about their presence in biochar derived from traditional feedstocks. Pyrolysis at low temperatures (400 – 600°C) does not favor the appearance of these chlorinated aromatic compounds [73].

When biochar is added to soil its sorption capacity increases, this characteristic influences the toxicity, transport and fate of organic compounds and allows biochar to be used as a mitigation strategy for common environmental pollutants.

The mechanism of metal uptake performed by charcoals is based on replacing its existing ions with metal ions. This behavior shows a relationship between the mineral content of charcoal and its mitigation potential for heavy metals. Biochar already shown potential for removing sulphates, benzenes and nitrobenzenes [74], nitrates [75], phosphate and ammonium [40], among other contaminants. The porous matrix of biochar makes it ideal as a microorganism carrier for bioaugmentation programs for specific sites, areas where the native microbial population are in danger or have been suppressed by contaminants [76].

3.5 Other effects of biochar application

Biochar application not only affects the properties of the soil, it can also modify the environment and be useful in many other areas. The sequestration of atmospheric carbon dioxide using biochar is a very promising technique which still needs more research, the same as the replacement of peat in horticulture. This chapter will describe other effects of biochar application, some good and promising techniques and other with negative effects.

3.5.1 Carbon sequestration potential

Emissions from fossil fuels must be reduced in order to meet the challenges of global climate change [77]. In addition to reduce greenhouse-gas emissions, the carbon dioxide present in the atmosphere must be actively withdrawn [78]. This process of carbon sequestration must be long term, substantial, accountable and have low risk of rapid-scale leakage. One strategy that can meet the requirements is based on biochar sequestration. This technique combined with bioenergy production produces clean energy as well as sequesters carbon [1].

There are different approaches to remove carbon from the atmosphere, one of the most standardized is based on growing plants that sequester carbon dioxide in their bodies or in the soil organic matter [78]. These methods related to afforestation have already been accepted under the Kyoto Protocol, but this mechanics can be taken to the next step using biochar.

Even nowadays the duration of biochar's storage is under debate, opinions are pointing to durations from millennial to centennial. Data obtained from naturally occurring biochar suggest the longer duration between these two options, but some field and laboratory trials suggest a shorter duration [79]. Biochar storage capacities are limited by the type of field where the sequestration is performed, for example, agricultural lands converted to non-tillage cease to capture carbon after 20 years and even, eventually, mature forests of decadal and millennial timescales begin to release as much carbon dioxide as they take up.

Compared to other sequestration methods, biochar is a low-risk strategy. Is difficult to imagine a situation in which the stored carbon can be released; other methods can suffer different incidents or changes that can cause a sudden loss of stored carbon, for example by forest fires, by leaks from geological carbon storage or by converting non-tillage back into a conventional tillage. One of the strategies related to biomass and biochar is grounded on withdraw part of the carbon that cycles annually through plants and sequester it in the biochar cycle. By taken this organic carbon from processes like photosynthesis and decomposition, biochar sequestration directly removes carbon from the atmosphere. Figure 3.6 shows the difference between carbon sequestration by photosynthesis and the carbon sequestration of biochar. The first process is carbon neutral, while the second is carbon negative and consequently it is withdrawing carbon from the atmosphere.

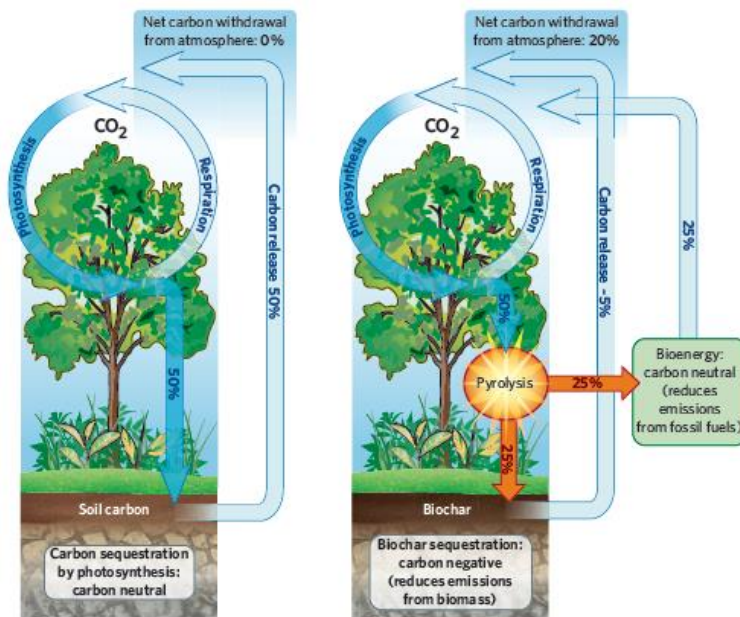


Figure 3.6 Process of carbon sequestration by implementing biochar. Image obtained from [1].

One of the biggest questions about biochar is if this material can be used in national, regional or even at global scale. At local or field scale the use of biochar is convenient, it can be mixed with manures or fertilizers and included in non-tillage methods. To implement biochar in a larger scale, a combination of two important factors must be done: the low-temperature pyrolysis must be complemented with a simultaneous capture of the exhaust gases and those gases must be converted into energy as heat, electricity, biofuel or hydrogen [79].

Nowadays, most companies which use low-temperature pyrolysis to produce bioenergy view biochar as a byproduct and it is usually burned to offset-fuel use and reduce costs. Some studies [80] suggest that emission reductions can be between a 12 and a 84% greater if biochar is returned into the soil instead of burning it, those calculations depend on the feedstock used and the type of bioenergy produced. Biochar sequestration methods offer the possibility to turn back bioenergy into a carbon neutral or carbon-negative industry.

Some examples of carbon sequestration using biochar were done by Lehmann (2007) [1]. Three different approaches were done, each one was able to sequester about the 10% of annual US fossil-fuel emissions (approximately 1.6 billion tons of carbon in 2005). Comparing these results with actual methods for sequestering carbon, like non-tillage methods, the difference is considerable. By converting all US cropland to Conservation

Reserve programs, the result is about a 3.6% of US emissions per year, just a third of what biochar sequestration can offer.

3.5.2 Effect on crop production

There is a large number of studies in which the application of biochar showed significant agronomic benefits, however, some studies reported no significant effects and even some reported negative results. This propose that the efficacy of biochar in crop productivity may vary, due to the different physico-chemical interactions and processes than can occur.

There are three main mechanisms that explain how biochar can benefit crop production. The first is related to the modification of the soil chemistry, the second is associated with the chemically active surfaces of biochar and the third explains how biochar can modify the physical character of soil in a way that benefits root growth and improves nutrient and water retention.

The first (chemically modification of soil chemistry) produces a temporary change in crop productivity, its duration is dependent on biochar weathering and the effects of crop off-take. The other two mechanisms are dependent on the long-term persistence of biochar. Both are related to the impact on water retention and on lowering the soil bulk density of the mixed matrix. These three mechanisms evolve over time as the chemical and physical modification in the matrix affect its properties, gradually the concentration of partially oxidized particles grows and the CEC and pH increase, resulting in a gradual surface oxidation [56].

Actually, there is a lack of experimental studies about the effect of biochar in crop yield, the majority are at small scale, short term and performed without taking into account the possible environmental fluctuations. By analyzing the published studies about crop yield, a major part of them show results with improved results. Table 3.3 shows the results of some experiments assessing the impact of biochar addition on crop yield. Most of results are positive, however, [81] showed a neutral behavior on the development of plants and [82] found negative results on yield. This behavior confirms the statement at the

beginning of this chapter, the biochar efficacy may vary depending on its conditions, the type of soil where applied and the application rates.

Author(s)	Study	Results
Kishimoto & Sugiura (1985)	Soybean on volcanic ash Ioam, Japan	0.5 Mgha ⁻¹ char increased yield 151% 5 Mgha ⁻¹ char decreased yield 63% 15 Mgha ⁻¹ char decreased yield 29%
Kishimoto & Sugiura (1985)	Sugi trees on clay Ioam, Japan	0.5 Mgha ⁻¹ wood charcoal increased biomass 249% 0.5 Mgha ⁻¹ bark charcoal increased biomass 324% 0.5 Mgha ⁻¹ activated charcoal increased biomass 244%
Oguntude et al (2004)	Comparison of maize yields between disused charcoal production sites and adjacent fields. Kotokose watershed, Ghana	Grain yield 91% higher and biomass yield 44% higher on charcoal site than control
Chidumayo (1994)	Bauhinia trees on alfisol/ultisol	Charcoal increased biomass by 13% and height by 24%
Teodoro et al (2019)	Application of co-composted biochar significantly improved plant-growth relevant physical/chemical properties of a metal contaminated soil	The addition of co-composted biochar had no effect on the development of the plant. Positive impact on some characteristics of the soil.
Glaser et al (2002)	Cowpea on xanthic ferrasol	67 Mgha ⁻¹ char increased biomass 150% 135 Mgha ⁻¹ char increased biomass 200%
Iswaran et al (1980)	Pea, India	0.5 Mgha ⁻¹ char increased biomass 160%
Iswaran et al (1980)	Mung bean, India	0.5 Mgha ⁻¹ char increased biomass 122%

Table 3.3 Summary of experiments assessing the impact of biochar addition on crop yield. Adapted from [83] and [81].

3.5.3 Biochar replacing peat extraction

A well-engineered biochar with good plant-available water and nutrient retention properties could be used to replace peat as a growing medium in horticulture and agriculture. If this idea succeeds, the peatlands used for peat extraction could be restored and used for C sequestration, water storage, maintenance of the biodiversity, among others uses. A peatland is able to sequester approximately at a rate of 6 g m^{-2} , while peat extraction causes a C loss up to 36 g m^{-2} [84].

3.5.4 Emissions and atmospheric pollution

During pyrolysis a large quantity of particulate matter are emitted, these emissions are the center of concern about human and environmental health. The issue with this particulate matter is related with its dimensions and the inherent toxicity associated to some types of fine or ultrafine particles [85].

Until a decade ago it was believed that the diseases associated with atmospheric pollution were caused by particles with dimensions up to $10 \mu\text{m}$, nowadays is known that the size of these pollutants is about the nano range. New standards of air quality were regulated, the standard in Europe and U.S.A. is PM_{10} ($< 10 \mu\text{m}$) and $\text{PM}_{2.5}$ ($< 2.5 \mu\text{m}$) depending on the pollutant [86]. The type and quantity of particulate matter produced during pyrolysis depends on the feedstock material and the pyrolysis conditions, as reported by many articles, the pollutants produced at temperature ranges between 30 and 300°C are different than between 300 and 600°C [33,59,86]. PAHs, dioxins and furans are also considered as relevant environmental pollutants, more information about these molecules at chapter 3.4.4.

The emission of atmospheric pollutants during pyrolysis requires further evaluation and studies, not only related with human and environmental health, but also to establish which emissions may cancel possible benefits as carbon sequestration or the retention of determined contaminants.

4 MATERIALS AND METHODS

4.1 Production of microchar

The production of a commercial biochar is usually a secret procedure only known by the producer, although it is possible to know its characteristics and composition due to information provided by the manufacturer. The microgranules analyzed in this work were produced from a commercial biochar, which preparation recipe is hidden. This industrially produced biochar is a ready to market product already certified. Despite not knowing the preparation procedure, some characteristics of this biochar are known and are shown in Table 4.1. From the known information about this original biochar, we know that it is basically composed by carbon (78 %), the pH is basic and the content of nitrogen is around 2%.

Apparent density [g / cm ³]	0.21
Porosity [%]	/
pH [-]	9.1
Conductivity [S / cm]	/
Corg [g / kg]	778
Ntotal [g / kg]	22.7
C / N	34.3
Ptotal [g / kg]	1.15
K [g / kg]	9.65
Ca [g / kg]	20.4
Mg [g / kg]	2.34

Table 4.1: Basic characteristics of biochar [87]

The microchar used in this study is composed by microgranules of the previously mentioned commercial biochar and vermitea, its properties change from the original material. Figure 4.1 shows an image of the microgranules used in this study.



Figure 4.1: Image of the microgranules used in this study

Vermitea is a liquid extract from vermicompost, it has high contents of biological activity and dissolved organic carbon. In order to certificate the product, the microchar was analyzed by an external laboratory (AGRO-LA, spol. S r. o), the results are visible in Table 4.2. As seen in this table, the properties and proportions of the elements that compose the material have change in comparison to the original biochar, a property to have in consideration is the pH, which is not basic anymore. Also, the proportion of P, K and N increased considerably.

	In 100 % dry matter	In original mass	Values of lab. dry matter
Ash (residue after annealing) [%]	43.0	38.7	38.7
P [%]	19.5	17.6	17.6
K [%]	0.810	0.730	0.730
N [%]	3.8	3.4	3.4
NO₃	4.43	3.99	3.99
Organic, burn. Substances (C_{ox}, carbon organic) [%]	57.0	51.4	51.4
Cr [mg/Kg]	10.4	9.34	9.34
Zn [mg/Kg]	17.1	15.4	15.4
pH	-	-	4.60

Table 4.2: Basic characteristics and composition of microchar obtained from the certificate of product done by the laboratory AGRO-LA, spol. S r. o.

4.2 Chemical analysis of microchar samples

Both samples of microchar need to be analyzed to know its properties and composition, depending on the type of analysis to perform, the protocol to follow differs. The content of available nutrients in individual microchar was determined according to the Mehlich III method, electric conductivity (EC) and pH in the extractant were measured using a multimeter and pH meter (Multi 3420 and pH 7310 respectively, WTW, Germany). The amount of carbon and nitrogen was determined using a CHNS analyzer. To determine the DOC, TC, TN a TOC-L CPH machine was used. Major inorganic anions were determined using Dionex ICS-5000 ion chromatography system (Dionex, USA).

In this part both samples analyzed are called “sample 1” and “sample 2” correspond consequently to an industrial biochar (Microchar) and to an enriched biochar (Microchar II with vermitea).

4.2.1 Mehlich III method

The first analysis consists of the measure of available nutrients. For both samples five replicates were made.

First of all, 1 gram of each sample must be weighed on a scale, then 10 ml of a Mehlich III solution must be added to each test tube. Each sample has to be shaken for 10 minutes, the result is a heterogeneous mixture with distinguishable parts after centrifugation. These samples must be filtered with a syringe filter of 0.45 μm , then they must be diluted in a ratio of 1:5 using HNO_3 2%. For every 2 ml of Mehlich III solution are needed 8 ml of the HNO_3 solution.

Both samples of soil used at the incubation experiment were also analyzed by the Mehlich III method, the protocol is the same as for the microgranules of biochar.

4.2.2 DOC, TC, TN and ions in suspension

For these analyses only four replicates of each sample are needed. Also, the measure of pH and conductivity is made for each replicate. The methodology followed to prepare those samples for further analysis is barely the same for each sample, except at the last stage when diluting.

First of all, 1 gram of each sample is weighed on a scale and 10 ml of distilled water are added to each replicate. All the samples must be shaken for one hour, the result is a heterogeneous mixture. The filtering part is done using a syringe filter of 0.45 μm . To finish these preparations, the samples and its replicates must be diluted, the steps to follow are different depending on the sample treated.

For samples 1 and 2 (both biochar samples) two different dilutions are needed, this is because different analysis will be performed for each dilution. The first dilution must be deposited inside glass bottles, the objective of these samples is to analyze the DOC, TC and TN. To prepare these dilutions deposit 2 ml of samples 1 and 2 inside a glass bottle and add 20 ml of demi water.

The second dilution for samples 1 and 2 must be deposited in plastic tubes, the objective of these analysis is to measure ions in suspension. The dilution rate is the same for both samples, 1 ml of microchar samples plus 10 ml of demi water.

Before filtering the samples, a measure of pH and conductivity was made for all six samples. The optimum would be to perform these measures after filtering, but physical impositions due to laboratory equipment ended up resulting in the realization of these measures before filtering.

Both samples of soil used at the incubation experiment were also analyzed to measure their pH and electro conductivity.

4.3 Incubation in soil experiment

In order to study the behavior of the microgranules of biochar, an incubation experiment was performed. This experiment consists of introducing soil with or without microchar into test tubes and add demi water, then the water is extracted and analysed. This process must be done during several weeks to obtain significative results.

The incubation process took 7 weeks to finish, an extraction of samples was done each week. The first extraction took place on the third week of April and the last was on the second week of June.

The experiment was designed to be done with two different soils, the first one was a Regosol from Zvěříněk and the second was a forest soil from Jevany, SLP. The biochar used to do this experiment was the protocol biochar with vermitea. Its composition is shown in Table 5.1.

Four different types of samples were prepared to analyze, a control sample (without microchar) and a sample with microchar for each soil. The samples were prepared by adding 60 g of each soil and 1.2 g of microchar into the test tubes, then 10 cm long rhizons (Eijkelkamp, NED) were placed for the extraction of water. From each sample, 5 replicates were made. Figure 4.1 shows the samples and the extraction equipment.

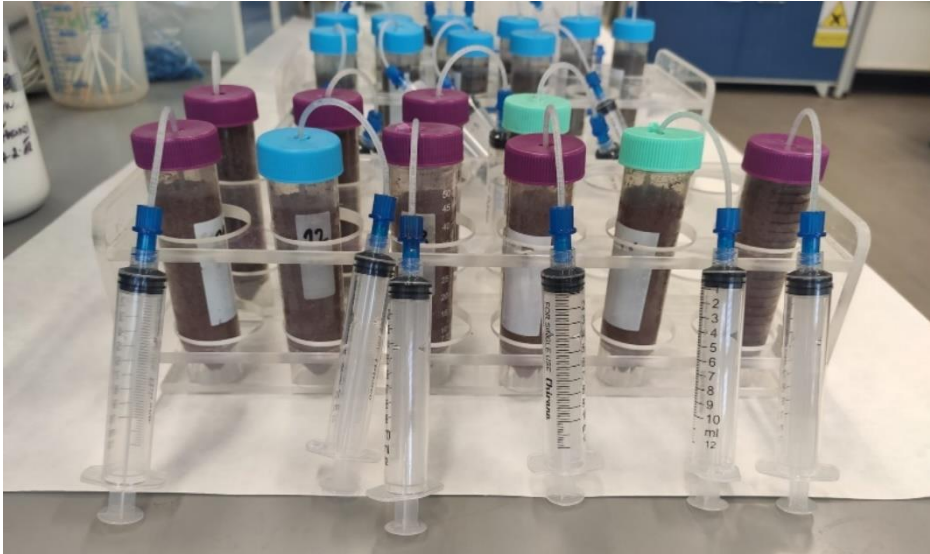


Figure 4.2: Image of the test tubes with the extraction equipment.

The types of samples prepared are:

- Regosol as control
- Regosol + 2 wt% of microchar
- Forest soil as control
- Forest soil + 2 wt% of microchar

The extraction of pore water from the test tubes was done using removable plastic syringes connected to the rhizons and stored at 10 °C until analysis. After the extraction of water from the samples each one needed to be prepared for further analyses, the process followed to prepare each sample depends on the analysis to be performed. Total concentrations of elements in all the analyzed solutions were obtained by inductively coupled plasma optical emission spectrometry (ICP-OES) (720 ES, Varian Inc., CA, USA). Electric conductivity (EC) and pH in the extractant were measured using a multimeter and pH meter (Multi 3420 and pH 7310 respectively, WTW, Germany). Major inorganic anions were determined using Dionex ICS-5000 ion chromatography system (Dionex, USA) and Total organic/inorganic C was determined using the carbon analyzer TOC-L CPH for liquids

Unfortunately, in this report only the results of the first 4 analyses are presented. As mentioned at the section 2.2 Scope of work, several delays have led to not having all the necessary information by the delivery date of this work.

5 RESULTS AND DISCUSSION

5.1 Evaluation of microchar and soil analysis

As described in the previous Materials and Methods section, the Mehlich III method was used to analyze both biochar used in this experiment (microchar and microchar II with vermitae). This section shows the results obtained from the Mehlich III analysis and the CHNS analyzer, which are in Table 5.1. From the results of that performed extraction using Mehlich III, reflecting the contents of nutrients in microchar, it is clear that the results obtained from both samples are different. A comparison using the Anova test shows that most of the compounds show a significant difference, especially carbon, the nitrogen, calcium, potassium and sulfur.

The results of the TOC machine and some anions are shown in Table 5.2, from the outputs it is clear that there is a difference in composition and available nutrients between both microchars. The DOC, TC and TN values are much higher on the first sample (microchar) than on the second (microchar II with vermitea), indicating a possibly better performance of the first sample. The pH and conductivity also were under study, the pH values of both samples are acid, being a bit higher on the second microchar.

The composition of the soils was analyzed, the available nutrients are shown in Table 5.3, as well as the pH and EC. From the results of the performed extraction with Mehlich III, reflecting the contents of acceptable nutrients in the soil, it is clear the difference between both soils analyzed. Regosol has higher quantities of all the elements studied except for K, although its value is similar in both samples. The pH of both is acid, although regosol shows a less acid behavior. The EC of regosol is higher than in forest soil, this behavior is related to the higher content of available nutrients, which indicates a best fertility of the first sample. Despite the results observed in both soils, it is important to mention that these samples have low organic matter levels and not optimal pH or EC values [88].

	Microchar	Microchar II + vermitea
C (mg/kg)	450.72	266.10
N (mg/kg)	31.84	17.32
Ca (mg/kg)	15.78	10.62
K (mg/kg)	4.75	3.85
Mg (mg/kg)	0.31	0.28
Na (mg/kg)	1,31	1.39
P (mg/kg)	7.53	5.40
S (mg/kg)	3.54	2.17

Table 5.1: Concentration of selected substances from both microchar analyzed.

	Microchar	Microchar II + vermitea
DOC (g/L)	2.83	0.17
TC (g/L)	3.15	0.17
TN (g/L)	1.75	0.81
pH	4.98	5.72
Conductivity (mS/cm)	10.37	5.43
Fluoride (mg/L)	2.16	-
Chloride (mg/L)	31.11	45.45
Nitrite (mg/L)	0.12	-
Nitrate (g/L)	3.71	2.01
Phosphate (g/L)	1.16	0.28
Sulphate (g/L)	0.61	0.32

Table 5.2: Content of DOC, TC, TN, different ions in suspension, pH and conductivity of both microchar samples.

	Regosol	Forest soil
Al (g/kg)	-	0.45
Ca (g/kg)	1.10	0.27
Fe (mg/kg)	-	77.55
K (mg/kg)	84.9	91.02
Mg (mg/kg)	220	76.28
Na (g/kg)	-	0.92
P (mg/kg)	41	12.01
S (mg/kg)	24	12.14
pH	4.91	4.4
EC (μS/cm)	82.75	40.8

Table 5.3: Concentration of selected substances, pH and conductivity of soil water from analysed soils.

5.2 Incubation in soil results

The results of the incubation experiment shown in this thesis are not complete, as mentioned before only four measures of TOC and five of composition were obtained from the laboratory. This issue is based on a time problem, although all the samples were ready to be analyzed the last results will be obtained after the deadline date of this paper.

The analysis of soil water was performed on every sample, the results shown in this chapter are always the average value of 5 replicates for each type of sample. The DOC content of each sample can be found at the Appendix section, Table 8.1; the evolution of the changes of DOC content are clearly visible at the chart represented bellow (Figure 5.1). The samples with biochar show higher values of DOC at the beginning of the experiment, this is due to the high content of DOC present at the microchar itself. However, the presence of biochar had a significant effect on reducing the release of this parameter at the end of the experiment, which confirms the fact that the microgranules of biochar are considered a stable organic additive, according to the European Biochar Certificate [3]. In this chart the data is treated logarithmically to see clearly the evolution on the changes in proportion of DOC, both samples with microgranules have higher values at the beginning, but them decrease rapidly. Although all the samples achieve a certain stabilization after that sudden decrease, the samples with microchar ended with lower but more stable values. The factor of releasing of DOC was reduced in comparison with control samples, this behavior was also shown by Schulz H, et al 2013 [89].

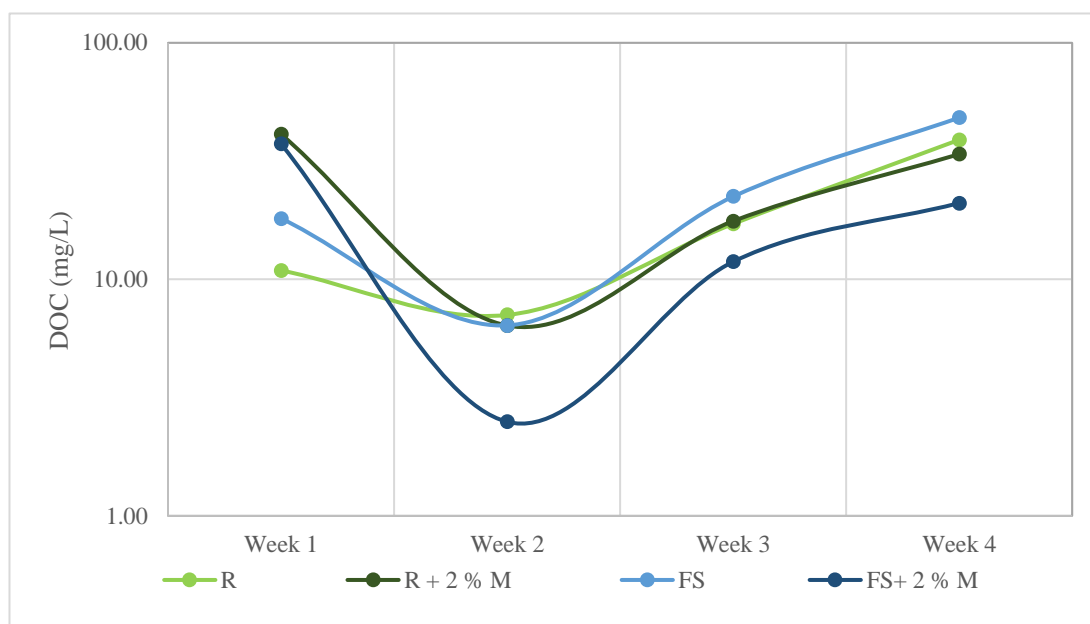


Figure 5.1: Changes in the proportion of DOC content of the samples, this chart is scaled logarithmically.

The content of total carbon (TC) and total nitrates (TN) were also affected by the presence of biochar, as shown in Table 8.2 and Table 8.3 in the Appendix section. Both samples with microchar had a higher content of TC and TN at the beginning due to the high content of them in the microchar. The TC content at the end of the experiment was higher at the samples without microgranules, the behavior of this parameter is really similar to DOC content. Although, the TN content shows a different behavior, at the end of the experiment its concentration was extremely higher compared to only soil samples. Figure 5.2 shows the behavior of TN, at the beginning the samples with microgranules have a much higher content of it compared to control samples of soil, although suffering a decrease in the middle of the experiment, it raises during lasts weeks till reach high and more stable values. Its release rate is also slower at samples with microgranules [89].

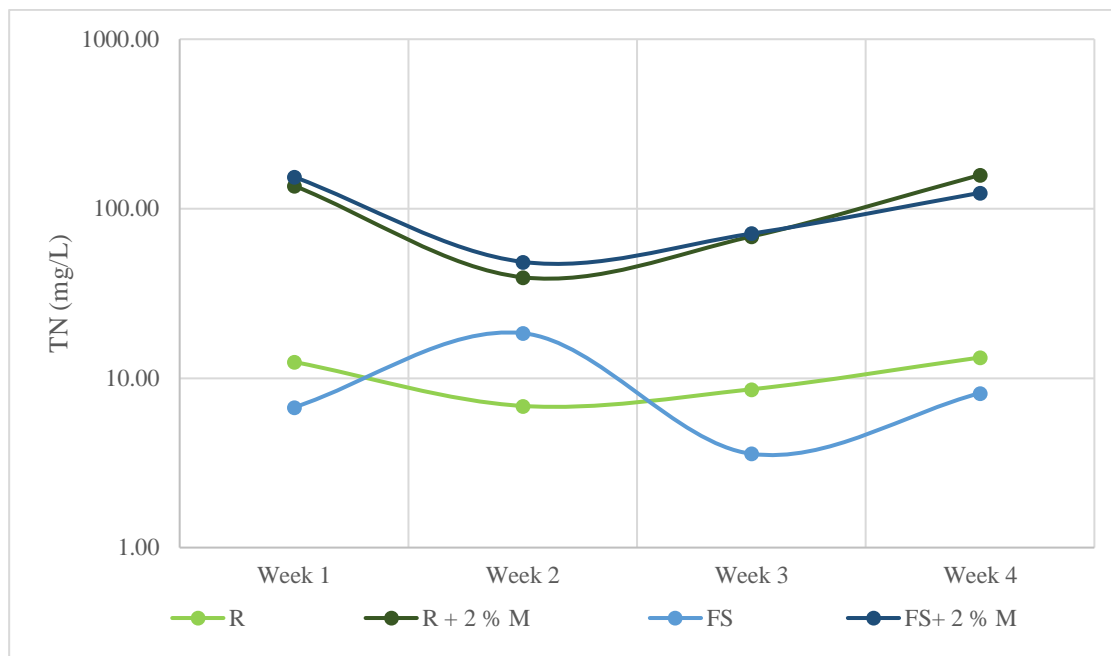


Figure 5.2: Changes in the proportion of TN content of the samples, this chart is scaled logarithmically.

From the results of the performed ICP-OES analysis reflecting the contents of acceptable nutrients in soil it is clear that the presence of the microgranules increase the availability of macronutrients (as potassium, calcium, phosphor, sulfur and magnesium). This is due to the higher contents of these elements in the biochar itself, but it is also a prove that microgranules improve the nutrient retention capacity over time, as also shown by Lehmann et al, 2011 [90]. The concentration of some of these nutrients can be found at Table 8.4, at the Appendix section. The retention of the nutrients depends on each nutrient

itself, some are retained more stable than others. By statistically analyzing the data obtained using a one-way Anova test it is clear that there are significant differences (on some elements) between the control samples and the ones with microgranules. Table 5.4 show the results of Anova test on some macroelements and other elements as Al, Mn and Na. Some present significant difference on both soils, while other only in one or do not have it. “a” means that there is significant difference and “b” that there is not.

Element	Al		K		Ca		Mg		Mn		Na		P		S	
	R	FS	R	FS	R	FS	R	FS	R	FS	R	FS	R	FS	R	FS
Significative Difference	b	b	a	a	a	a	b	a	a	b	a	b	a	a	a	a

Table 5.4: Results from one-way Anova test on some macroelements and other elements, “a” means that there is significant difference on the results and “b” that there is not.

By analyzing the data logarithmically over time is possible to observe the changes in proportions between the different extractions of leaching tests. Figure 5.3 and Figure 5.4 show different charts with the changes in proportion of the concentration of P, K, S, Al, Mg, Mn, Na and Ca, almost all the samples with biochar show more stability on nutrient retention capacity compared to control soils. As mentioned before, the behavior of each element depends on the element itself and its interaction with the soil. The important comparison between the content of these elements is between the control samples of each soil and the soils with microgranules. Ca, K and P concentration is more stable in samples with microgranules, also their content is higher during all the experiment. The other two macronutrients, Mg and S, show higher concentration and stability in regosol than in forest soil, but in this second soil its stability gets worse with the implementation of microchar. The other three elements represented in the charts bellow have different behaviors; Al has the worst values of all the elements represented, with less concentration and stability in both soils after adding microgranules. The stability of Mn and Na is worse in samples with microgranules, and their concentration fluctuates differently depending on the soil analyzed.

Results and discussion

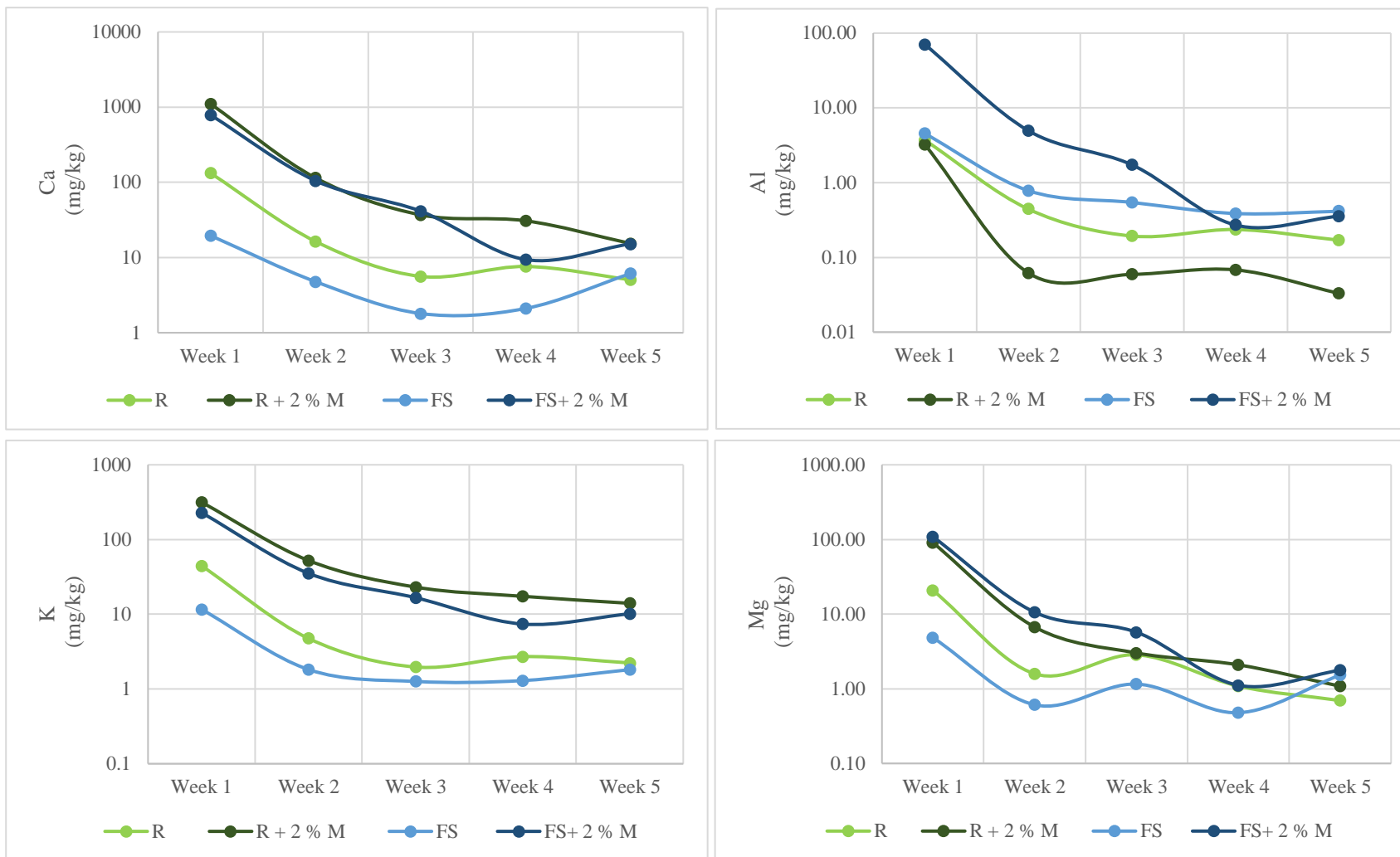


Figure 5.3: Charts showing the evolution of Ca, K, Mg and Al. Charts scaled logarithmically to appreciate the changes in proportion over the duration of the incubation test.

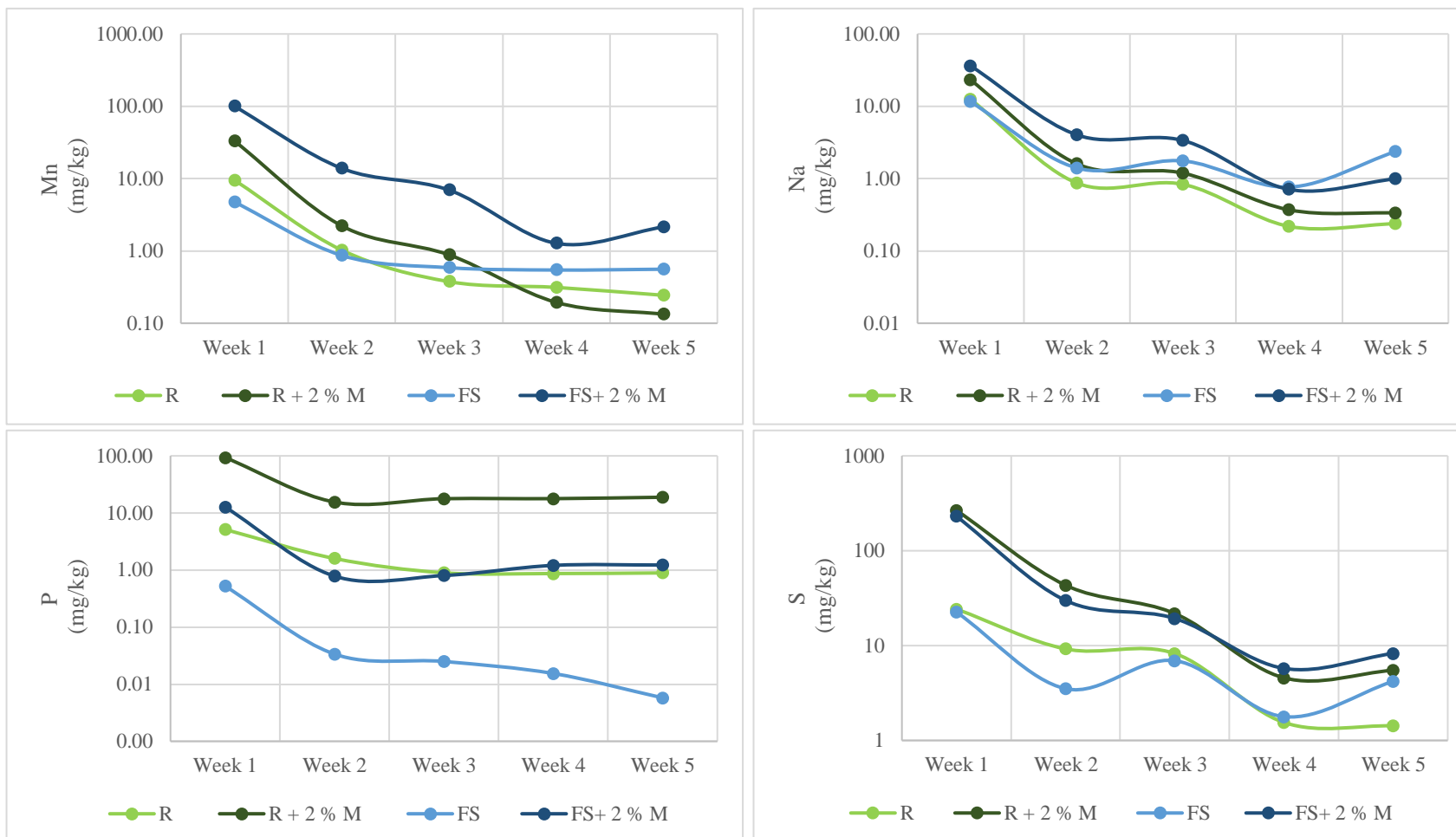


Figure 5.4: Charts showing the evolution of Mn, P, Na and S. Charts scaled logarithmically to appreciate the changes in proportion over the duration of the incubation test.

A measure of the pH and electro conductivity was done to the soil samples before starting to conduct the leaching, the values can be founded at Table 5.5. By statistically analyzing these results performing a one-way Anova test it was found that there are significative differences in both, pH and EC. The pH of the soil is an important factor that affects nutrient availability [91]. The microbial reproduction is also dependent of the pH and the availability of nutrients, although the addition of biochar may help to stimulate this factor, it is largely dependent on the pre-existing soil pH [90].

Sample	pH	EC ($\mu\text{S}/\text{cm}$)	Sample	pH	EC ($\mu\text{S}/\text{cm}$)
R	4.45	47.8	FS	4.88	79.1
R	4.38	37.8	FS	4.68	79
R	4.47	29.8	FS	5.02	74.7
R	4.30	47.8	FS	5.08	98.1
R	4.40	40.8	FS	4.92	82.72

Table 5.5: Results of pH and EC from soil samples.

An analysis of anions was also realized to the samples of the incubation experiment, for this data only three reports are available at the deadline date of this paper. The information available is from the first three weeks of the incubation test. Table 5.6 shows the concentration of anions in soil water, this samples were obtained using rhizones to extract the leaching water. At almost all samples the concentration of anions is higher at the first week, except for nitrite. The samples with microgranules have higher concentrations of anions, even taking into account the clear decrease on the concentrations between the first and second week, microchar samples show a higher stability and a possible higher capacity to retain anions. Comparing the nitrate content obtained in this analysis and the TN values showed before, it is clear that there is an important difference. Taking into account that the content of TN is basically nitrate, the results of the analyses distance from an optimal point of coherence, nonetheless, the evolution of their concentration follows a very similar behavior. The analysis method performed to obtain TN and nitrate concentration were different, despite having different numerical results none of the results should be underestimated. The most consistent results are the ones showed by the anion content, but the TN results are useful to see the evolution in proportion and predict the future behavior of it.

Fluoride (mg/kg)	Week 1	Week 2	Week 3
R	0.47 ± 0.14	0.30 ± 0.05	0.23 ± 0.13
R + 2 % M	0.37 ± 0.08	0.08 ± 0.01	0.12 ± 0.05
FS	0.13 ± 0.01	0.12 ± 0.007	0.1 ± 0
FS+ 2 % M	1.98 ± 0.32	0.71 ± 0.09	0.36 ± 0.07
Chloride (mg/kg)			
R	3.56 ± 3.24	1.90 ± 1.16	0.89 ± 0.67
R + 2 % M	9.57 ± 6.74	3.70 ± 1.64	2.14 ± 0.66
FS	1.15 ± 0.12	0.43 ± 0.09	0.42 ± 0.06
FS+ 2 % M	5.97 ± 0.39	3.10 ± 0.19	1.54 ± 0.32
Nitrite (mg/kg)			
R	0.5 ± 0.08	0.04	0.02 ± 0.01
R + 2 % M	0.25 ± 0.09	8,32 ± 6.26	0.20 ± 0.09
FS	0.01 ± 0.006	-	-
FS+ 2 % M	-	-	-
Nitrate (mg/kg)			
R	90.57 ± 8.69	26.18 ± 16.50	16.51 ± 10.02
R + 2 % M	231.64 ± 22.38	133,47 ± 34.07	194.93 ± 123.48
FS	5.64 ± 1.88	8.40 ± 13.68	3.13 ± 0.68
FS+ 2 % M	191.77 ± 13.24	109.02 ± 13.72	244.85 ± 64.21
Phosphate (mg/kg)			
R	1.45 ± 0.12	3.33 ± 1.26	2.05 ± 1.31
R + 2 % M	57.80 ± 19.20	45.43 ± 17.95	57.87 ± 21.61
FS	0.20 ± 0.09	0.13	0.06 ± 0.08
FS+ 2 % M	5.86 ± 5.64	1.10 ± 0.62	1.59 ± 0.90
Sulphate (mg/kg)			
R	9.17 ± 2.61	25.27 ± 34.95	43.39 ± 76.3
R + 2 % M	160.63 ± 28.93	129.17 ± 29.54	51.85 ± 17.36
FS	9.98 ± 1.01	8.39 ± 0.53	27.54 ± 48.73
FS+ 2 % M	137.6 ± 11.66	92.32 ± 8.63	56.96 ± 11.11

Table 5.6: Concentration of selected anions in soil water, obtained using rhizones at the first two weeks of incubation.

5.3 Discussion of the results

The results shown in this section are an evidence of some of the statements mentioned at the theoretical part of this thesis. An appropriate biochar may improve the chemical and physical characteristics of determined soils, properties as nutrient or water availability, pH or aeration [90]. The pH of soils is one of the most important factors that affect soil fertility [91], the addition of biochar to low organic matter soils may improve its properties and make them more suitable for plant growth. By the addition of biochar, the improvement of soil fertility can be explained by a pH increase in acid soils [9] and an improvement in nutrient retention [92].

The physical and chemical properties of biochar are keys to understand the performance of this material in the improvement of soil's fertility, one of these mechanisms is related to an increase in water holding capacity [17]. In addition to this feature, some authors have indicated that biochar also significantly improves the cation exchange capacity of soils [93]. The increase in amount of exchangeable cations in soils leads to an improvement in soil fertility and nutrient retention; as reviewed at the incubation experiment, the increment of extractable nutrients and the retention and stability of macronutrients in soil was enhanced after the application of microgranules of biochar. In general, the improvements of soil properties could directly or indirectly improve the nutrient content and decrease nutrient leaching, mechanisms responsible of soil fertility [94].

Biochar has shown not only to improve soil physicochemical properties, but also is able to change soil biological properties [79], this feature indicates a possible stimulation of microbial production when microgranules of biochar are added to acid and low organic matter soils. The increment of pH, the enhance of nutrients availability, the improved water retention capacity and the porous structure of biochar are some of the characteristics that lead to an improvement of the microbial community [90]

6 CONCLUSIONS

After analyzing the results obtained in the development of this project and recalling the objectives set at the beginning of this thesis, a series of conclusions are drawn:

The first analysis of both microchars and soils were only a part of the second experiment, the incubation test. Analyzing the results obtained from that last experiment, the presence of biochar in the soil improved its retention properties and reduced the release of DOC, TC and TN. The presence of biochar in both samples also had a positive effect on the amount of anions in soil, this factor is also due to the high content of ions in the biochar used for preparing the microgranules; nonetheless, it seems that the capacity to retain those anions was improved by the application of biochar.

The application of microgranules also increased the accessibility of macronutrients, which is to some extent caused by the significant contents of these nutrients in the microchar itself, but it has also shown a higher stability on the nutrient retention capacity due to the application of it.

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8 APPENDIX

8.1 Results of DOC, TC and TN from the incubation experiment

	Week 1	Week 2	Week 3	Week 4
R	10.91 ± 2.94	7.07 ± 4.18	17.16 ± 5.33	38.91 ± 28.05
R + 2 % M	41.09 ± 21.75	6.38 ± 5.51	17.63 ± 10.41	33.83 ± 16.28
FS	18.1 ± 5.10	6.39 ± 4.01	22.38 ± 2.24	48.25 ± 5.66
FS+ 2 % M	37.37 ± 25.29	2.5 ± 2.05	11.87 ± 3.44	21.02 ± 4.60

Table 8.1: DOC results from the incubation experiment, results obtained using a TOC-L CPH machine.

	Week 1	Week 2	Week 3	Week 4
R	10.99 ± 2.94	7.17 ± 4.18	17.16 ± 5.33	38.91 ± 28.05
R + 2 % M	41.17 ± 21.75	6.14 ± 6.03	17.63 ± 10.41	33.83 ± 16.28
FS	18.18 ± 5.10	6.49 ± 4.01	22.38 ± 2.24	48.25 ± 5.66
FS+ 2 % M	37.37 ± 25.29	2.38 ± 2.43	11.87 ± 3.44	21.02 ± 4.61

Table 8.2: TC results from the incubation experiment, results obtained using a TOC-L CPH machine.

	Week 1	Week 2	Week 3	Week 4
R	12.46 ± 1.79	6.86 ± 1.59	8.59 ± 4.04	13.24 ± 3.56
R + 2 % M	136.64 ± 60.21	39.37 ± 19.63	68.5 ± 39.71	157.98 ± 69.13
FS	6.72 ± 3.56	18.42 ± 30.27	3.58 ± 0.45	8.16 ± 5.78
FS+ 2 % M	154.13 ± 83.18	48.53 ± 4.27	71.23 ± 19.51	124.01 ± 13.77

Table 8.3: TN results from the incubation experiment, results obtained using a TOC-L CPH machine.

8.2 Results from ICP-EOS analysis from the incubation experiment

	Week 1		Week 2		Week 3		Week 4		Week 5	
P (mg/kg)	Main	SD	Main	SD	Main	SD	Main	SD	Main	SD
R	5.13	0.48	1.59	0.48	0.89	0.48	0.87	0.31	0.89	0.30
R + 2 % M	93.25	5.54	15.50	5.54	17.76	6.36	17.80	3.95	18.85	4.04
FS	0.52	0.01	0.03	0.01	0.03	0.01	0.02	0.00	0.01	0.01
FS+ 2 % M	12.68	0.46	0.78	0.46	0.80	0.36	1.21	0.28	1.22	0.63
K (mg/kg)										
R	44.539	5.3112	4.75	1.25	1.97	0.78	2.71	1.22	2.23	0.94
R + 2 % M	315.81	33.185	52.39	8.91	23.08	7.37	17.39	3.00	14.02	1.59
FS	11.646	0.2137	1.82	0.12	1.26	0.12	1.30	0.16	1.82	1.17
FS+ 2 % M	228.89	12.283	35.30	3.33	16.66	3.44	7.40	1.57	10.15	1.80
S (mg/kg)										
R	24.244	5.2645	9.22	10.59	8.25	10.23	1.55	0.38	1.42	0.60
R + 2 % M	266.43	38.608	43.27	9.92	21.77	5.79	4.57	2.32	5.49	3.51
FS	22.542	0.6713	3.51	0.27	6.94	10.15	1.78	0.24	4.20	4.80
FS+ 2 % M	230.44	19.107	29.88	3.37	19.32	4.13	5.75	1.68	8.27	2.02
Ca (mg/kg)										
R	132.73	15.82	16.36	10.32	5.61	2.73	7.60	1.72	5.06	0.90
R + 2 % M	1106.7	143.85	114.64	31.81	36.95	20.13	30.82	6.54	15.36	4.83
FS	19.6	3.58	4.78	4.76	1.80	0.18	2.10	1.00	6.14	9.12
FS+ 2 % M	788.32	63.09	104.81	11.84	41.39	11.42	9.40	2.52	15.23	4.14
Al (mg/kg)										
R	3.69	0.08	0.44	0.03	0.19	0.13	0.24	0.12	0.17	0.08
R + 2 % M	3.21	0.46	0.06	0.04	0.06	0.02	0.07	0.03	0.03	0.02
FS	4.52	0.15	0.78	0.05	0.54	0.05	0.39	0.03	0.41	0.02
FS+ 2 % M	70.31	17.03	4.94	1.18	1.73	0.61	0.27	0.07	0.36	0.10
Fe (mg/kg)										
R	0.31	0.00	0.61	0.84	0.04	0.04	0.00	0.00	0.00	0.00
R + 2 % M	0.21	0.04	0.02	0.03	0.02	0.02	0.00	0.00	0.00	0.00
FS	1.46	0.07	0.24	0.04	0.19	0.03	0.04	0.02	0.11	0.09
FS+ 2 % M	0.73	0.11	0.06	0.07	0.04	0.04	0.00	0.00	0.00	0.00
Mg (mg/kg)										
R	20.82	3.91	1.59	0.82	2.85	4.41	1.09	0.31	0.70	0.17
R + 2 % M	90.67	2.94	6.71	1.62	3.02	1.52	2.09	0.43	1.08	0.27
FS	4.85	0.22	0.61	0.07	1.16	1.53	0.48	0.13	1.53	2.45
FS+ 2 % M	108.84	8.20	10.59	0.88	5.71	1.36	1.11	0.36	1.77	0.47
Mn (mg/kg)										
R	9.50	0.45	1.02	0.65	0.38	0.21	0.31	0.12	0.24	0.12
R + 2 % M	33.07	2.77	2.22	0.74	0.88	0.50	0.19	0.12	0.13	0.10
FS	4.74	0.14	0.87	0.11	0.59	0.11	0.54	0.10	0.56	0.05
FS+ 2 % M	99.90	6.84	13.86	1.13	6.94	1.60	1.27	0.52	2.15	0.54
Na (mg/kg)										

R	12.50	9.16	0.87	0.39	0.83	0.54	0.22	0.13	0.24	0.16
R + 2 % M	23.15	17.91	1.60	0.32	1.19	0.38	0.37	0.15	0.33	0.13
FS	11.53	0.35	1.40	0.11	1.76	0.55	0.76	0.10	2.36	3.40
FS+ 2 % M	36.23	1.33	4.03	0.23	3.37	0.62	0.71	0.18	0.99	0.17
As (mg/kg)										
R	0.04	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R + 2 % M	0.14	0.03	0.04	0.02	0.01	0.01	0.02	0.01	0.02	0.00
FS	0.01	0.02	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00
FS+ 2 % M	0.03	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00
B (mg/kg)										
R	0.05	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R + 2 % M	2.78	0.33	0.28	0.03	0.16	0.04	0.09	0.03	0.09	0.03
FS	0.10	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FS+ 2 % M	2.94	0.19	0.29	0.02	0.14	0.02	0.04	0.02	0.07	0.02
Co (mg/kg)										
R	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R + 2 % M	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FS	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FS+ 2 % M	0.27	0.02	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Cu (mg/kg)										
R	0.12	0.06	0.05	0.03	0.00	0.00	0.00	0.00	0.00	0.01
R + 2 % M	0.05	0.04	0.07	0.02	0.00	0.00	0.01	0.01	0.00	0.01
FS	0.06	0.03	0.06	0.02	0.00	0.00	0.00	0.01	0.00	0.00
FS+ 2 % M	0.11	0.04	0.04	0.04	0.00	0.00	0.01	0.01	0.00	0.00
Li (mg/kg)										
R	0.07	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R + 2 % M	0.11	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FS	0.05	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FS+ 2 % M	0.16	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sr (mg/kg)										
R	0.51	0.05	0.04	0.03	0.01	0.01	0.02	0.01	0.01	0.00
R + 2 % M	2.80	0.18	0.16	0.05	0.06	0.04	0.04	0.01	0.01	0.01
FS	0.08	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.05
FS+ 2 % M	1.90	0.15	0.18	0.02	0.09	0.02	0.01	0.01	0.02	0.01
Zn (mg/kg)										
R	1.61	0.23	0.24	0.09	0.22	0.01	0.18	0.03	0.08	0.03
R + 2 % M	4.12	0.29	0.32	0.20	0.09	0.04	0.02	0.06	0.04	0.02
FS	0.51	0.22	0.08	0.04	0.05	0.00	0.06	0.02	0.14	0.26
FS+ 2 % M	1.41	0.58	0.15	0.06	0.06	0.02	0.03	0.04	0.03	0.02

Table 8.4: Concentration of different nutrients of water soil over time, obtained using ICP-OES analysis method.

