Czech University of Life Sciences Prague

Faculty of Agrobiology, Food and Natural Resources

Department of Microbiology, Nutrition and Dietetics

Bachelor Thesis

The effect of charcoal from historical kiln sites on the structure of soil microbial communities.

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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Agrobiology, Food and Natural Resources

BACHELOR THESIS ASSIGNMENT

Alisa Malakhova

Sustainable Use of Natural Resources
Sustainable Use of Natural Resources

Thesis title

The effect of charcoal from historical kiln sites on the structure of soil microbial communities.

Objectives of thesis

The thesis will aim in determination of microbial communities in relationship to soil characteristics at sites influenced by burned wood in charcoal production.

Methodology

High density of historical kiln sites was discovered during LiDAR mapping and revealed that they are an important structure in forest landscapes. The soil underneath the burning site contains high amounts of carbon, which may serve as food source for specific microbial community. Above that charcoal affects adsorption of particles including bacterial cell and that may also modify community structure and functioning. Methodologically, soil chemical analyses will be performed and complemented by preliminary analyses of microbial community structure. Further work will be performed for the following diploma thesis.

The proposed extent of the thesis

30

Keywords

charcoal, bacteria, forest soil

Recommended information sources

Antal, M.J., Gronli, M. 2003. The art, science, and technology of charcoal production. Industrial and Engineering Chemistry Research, 42, 1619–1640.

Blondel, J. 2006. The "design" of Mediterranean landscapes: a millennial story of humans and ecological systems during the historic period. Human Ecology 34, 713–729.

Carrari, E., Ampoorter, E., Verheyen, K., Coppi, A., Selvi, F. 2016. Former charcoal kiln platforms as microhabitats affecting understorey vegetation in Mediterranean forests. Applied Vegetation Science 19, 486–497.

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The Bachelor Thesis Supervisor

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Dean

Prague on 13. 02. 2020

Declaration

I declare that I have worked on my bachelor thesis titled "The effect of charcoal from historical kiln sites on the structure of soil microbial communities" 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 the copyrights of any person.

In Prague on date of submission _____

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The effect of charcoal from historical kiln sites on the structure of soil microbial communities.

Abstract

This bachelor thesis explores the effects of charcoal left in the soil from historical kiln sites in Moravsky Kras forest, Czech Republic. The effects on soil and microorganisms of the site were compared to the control of uncharred adjacent forest soil. The differences were drawn out by two-part methodology, determination of soil cation exchange capacity (CEC) and extraction of soil microbial DNA. The results were tested using one-way ANOVA and compared between sites with and without charcoal remains. The soil analysis showed that CEC at the charcoal sites is significantly different from the control sites, especially in the FH soil horizon. The ion composition was affected too, the charcoal dominating ion in CEC value was Ca2+, while in the control it is Al^{3+}. The pH of charcoal sites turned out to be slightly more acidic at organic layer, but at mineral layer the pH was increased at charcoal locations, providing a unique mosaic of niches for bacteria and fungi communities. The microbial analysis showed that the total DNA of different quantity and quality is present in every sample. More detailed analysis of the microbial communities by polymerase chain reaction (PCR) and sequencing will be performed in the following diploma thesis. Conclusion of this preliminary study is that the charcoal from historical kiln sites provides the environment of a new niche for microorganisms and it is expected to find altered communities compared to the control sites but also possibly unusual novel bacterial taxa.

Keywords: charcoal, bacteria, forest soil, historical kiln sites.

Vliv uhlí z historických pecí na strukturu mikrobiálních společenstev půdy.

Abstrakt

Tato bakalářská práce zkoumá účinky dřevěného uhlí, které bylo uloženo v půdě na lokalitách historických milířů v Moravském krasu. Účinky na půdu a mikroorganismy byly a dále budou zkoumány ve srovnání s kontrolou přilehlé nenarušené lesní půdy. Výsledky byly získány dvěma metodami, měřením kationtové výměnné kapacity (CEC, KVK) v půdě a extrakcí celkové půdní DNA. Výsledky byly testovány s použitím jednosměrné analýzy rozptylu (ANOVA) a porovnávány mezi místy s a bez dřevěného uhlí. Analýza půdy ukázala, že CEC z míst s dřevěným uhlím se výrazně liší od hodnot kontrolních míst, zejména v horizontu FH. Bylo ovlivněno i složení iontů, protože dominující ionty v půdě s uhlím v CEC jsou Ca^{2+}, zatímco u kontroly je to Al^{3+}. Ukázalo se, že pH v místech s dřevěným uhlím je v organické vrstvě o něco nižší, ale v minerální vrstvě se pH zvyšuje, což vytváří jedinečnou mozaiku životních nik pro bakterie a houby. Podle předběžných výsledků se ukázalo, že v každém vzorku je DNA různé kvality a množství. Další výsledky budou doplněny pomocí polymerázové řetězové reakce (PCR) a sekvenováním amplikonu v navazující diplomové práci. Závěrem této předběžné studie je, že dřevěné uhlí z historických milířišť vytváří nové prostředí pro mikroorganismy a očekáváme, že v tomto prostředí najdeme společenstva odlišná od kontrolních stanovišť, ale možná také neobvyklé nebo neznámé bakterie.

Klíčová slova: uhlí, bakterie, lesní půda, historická milíře

Contents

1. Introduction

The charcoal historical legacy persists in the forests of central Europe. The presence of charcoal kiln sites throughout the Czech Republic confirms the widespread production and hence the use of charcoal as a form of fuel for domestic and industrial activities. Even today, the charcoal finds its usage as soil amendment in agriculture, home medicine and as a tool for artistic expression. Charcoal is produced as a carbon residue during slow-burn pyrolysis. In the case of historical kiln sites, the wood from the surrounding forest was cut, piled up, buried with soil and set on fire. Such construction allowed the pyrolysis to take place and after removal of the charcoal leave a base of charcoal layer on the soil surface. Charcoal persistence in soil altered its properties compared to the surrounding forest soil. The new technology of areal topography laser scanning (LiDAR) enables to localize kiln sites in the landscape and using this method it was discovered that kilns were widespread in the forests. The density of kilns reaches hundreds per square kilometre, which means that it is potentially creating a mosaic of soil alterations with consequences for microorganisms, plants and functioning of the sites.

This thesis focuses on the historical legacy of charcoal in Morava region, Czech Republic and its effect on soil microbiota. The sites of Moravian Karst are located in a mixed forest with Stagnic Luvisol soils. This study adds an insight to how charcoal influences soil, particularly its effect on microorganisms. This is important because currently, the popular form of charcoal, i.e. biochar is used as soil amendments in agriculture aiming in improving the water holding capacity and leaching of nutrients. Even though this study focuses on forest soils, the data would be helpful with identifying microbial changes of undisturbed or arable soils.

The thesis complies with the main subjects studied during the three years of the bachelor programme of the Soil Science with the soil analysis being a part of the study and The Fundamentals of Microbiology relates to the soil DNA extraction and site microbe identification.

2. Objective

The objective of the thesis is at exploring the preliminary effects of charcoal on soil properties and microbial communities in the mixed deciduous forest growing on Stagnic Luvisol.

Hypothesis:

The charcoal kiln sites may increase forest soil diversity due to the enrichment of some soil horizons with carbon. That can modify the living conditions for microorganisms and result in their higher diversity compared to the control sites with no charcoal. The microbial diversity enrichment may be transferred to the whole forest through increased variability of large-scale conditions, thus providing more niches also for other organisms resulting in a changed dynamics of forest structure.

3. Literature Overview

3.1 Background on history

Charcoal is used in various forms and for various purposes. It is a form of fuel in industrial activities requiring high temperatures such as the of smelting cooper, iron and glass production (Deforce et al., 2018) as well as in domestic activities including cooking, artistic drawing medium and a form of medicine (Antal et Grønli, 2003)

Charcoal kiln sites hold the historical legacy of human fuel production and the subtle influence on the forest landscapes in Europe and North America (Hirsch et al., 2017). Much of the studies made on the topic of charcoal deposits in soils mainly showed the changes in an immediate vegetation cover such as in woody species (Carrari, Ampoorter, Verheyen, et al., 2017), forest landscapes (Carrari, Ampoorter, Bottalico, et al., 2017) , tree seedlings (Carrari et al., 2018), Mediterranean forests (Carrari et al., 2016), and Northwest European forests (Carrari et al., 2016). Currently, it seems that microbiome changes due to charcoal present in soil are unique to their geographical locations and environment, yet microbial abundance shows more general patterns and therefore is more prominent (Brieuc Hardy et al., 2019).

Figure 1- Photograph of a charcoal kiln site example in Europe, a wood pile before covering it with soil and burning it to produce charcoal. (Source: Wikimedia Commons, the free media repository. (2019, September 14))

The shape of a usual kiln to ensure pyrolysis was an above ground hearth platform with surrounding ditch borders of a circular shape (Hirsch et al., 2017). Such a shape makes it possible to be detected in topographical laser scans (Ludemann, 2012), where whole forests are scanned to detect hundreds of hearths. Such charcoal kilns date in age from down to the 12th century. However, before that the shape was not above ground but it was made in small pits which date from 12th century to 6ths century (Deforce et al., 2018). Such technique makes it harder to discover the kilns but nonetheless such human interventions influenced the dynamics of medieval and modern forests.

Studying the influence of charcoal kilns on forest dynamics proves to be valuable, as it adds on to the natural history of people as well as new insights on soil management and diversity preservation. Charcoals ability to persist in soil for thousands years, (Abdelrahman et al., 2018), would mean that any charcoal additions to the soil in agriculture influenced the microbial communities and vegetation in those places for the future times. The collection of information on the charcoal long-term effects and behaviour under different soil uses is still lacking and is encouraged to be explored, while the historical kiln sites represent the most valuable option (Brieuc Hardy et al., 2017).

3.2 Differences in Soil Structure

Charcoal in kiln sites is often referred to as biochar due to its soil enhancing properties. Since it stays in the soil for prolonged periods of time the charcoal alters the colouration of horizons, pH, affects nutrient leaching, organic matter content, total carbon deposition, water holding capacity and cation exchange capacity of the soil. These qualities are observed and experimented within agriculture. Larger scale effects of charcoal kiln forest mosaic were and are being explored as well (Biondel, 2014).

Horizons

Soil profile presents a history of the evolution of the location. The individual soil horizons are distinguishable layers of soil, formed in response to parent material weathering, climatic, geomorphological, vegetational factors and time. From immediate observation, the historical kiln sites have a significant black layer of charcoal preserved in the soil, with topsoil FH layer but

often with no A or B horizons. The top layers in the soil are often spread and transported around with the help of soil organisms. The distinct black colour of charcoal compared to forest or field soils is easy to locate and confirm initial geo findings from laser scan topography. Therefore, the persistence of charcoal is visible on soil horizons as a black ash layer below topsoil (Foth, 1990).

In our study, charcoal appears in organic topsoil and usually includes FH and A horizons, when expected visually. The dark brown-coloured A horizon usually seems most influenced by charcoal mixing with organic matter, but it is assumed that leaching to the lower horizons also occurs. Thus, in comparison of soil profiles the kiln site had a larger black layer than control (Photos 1 and 2 in methodology section).

Organic Matter – Carbon Deposits

Soil carbon includes the organic as well as inorganic carbon forms stored in the soil. As a rule of thumb surface horizons of the soil consist of more organic matter than deeper horizons (Abdelrahman et al., 2018). Generally, SOM volume is influenced by land use and soil layering (Shen et al., 2018). Soil Organic Matter (SOM) is a fertile component of soils and the main niche for soil microorganisms. Usually, it is defined as part of the carbon cycle because its parts are recycled relatively quickly by decomposition (White, 2013). The soils of our study are primarily European forest soils, the SOM of which is influenced primarily by plant litter, animal activities such as bioturbation, and microbial decomposers.

Presence of charcoal in soil showed to influence the quantity and the quality of soil organic matter (Abdelrahman et al., 2018). In more specifically studied forest soils with the goal of detecting the legacy of charcoal production an increase by up to 18%-32% of SOM was measured at relict charcoal hearths (Bonhage et al., 2020). That could be a result of the microenvironment in charcoal sets, where the rate of the organic matter decomposition is affected by changed soil pH, which further affects soil microbial populations. It may also be also explained by charcoal although being a natural carbon compound it persists in soil for many centuries and above that, it has the ability to absorb and store organic carbon so more accumulation can occur. Those effects of charcoal in soil were observed by analysis, which identified charcoal as a rich organic matter (Abdelrahman et al., 2018) (B. Hardy et al., 2017). Although general soil processes affect charcoal sequestration and functioning it was also

observed that the effect of charcoal on soil organic matter depends also strongly on land management practices (Brieuc Hardy et al., 2019).

Water Holding Capacity

The charcoal amendment increases water holding capacity (WHC) of the soil because WHC depends primarily on spaciousness between soil particles and those are modified by charcoal deposits. Charcoal structural property may increase the porosity of the soil and therefore also its capacity for water retention. Thus, charcoal amended soil affects the available water for plant roots as it holds the water after the precipitation event. (Abdelrahman et al., 2018). However, water retention of charcoal also may limit the oxygen circulation between particles and influence the proportion of aerobic and anaerobic bacteria. Actual water availability is fluctuating with seasons and may be influencing in the central European forests with the medium amount of rainfalls (Yan et al., 2015).

CEC Comparison and pH

Cation exchange capacity (CEC) is measured to determine soil ability to hold ions and withstand leaching of those ions. The ions may be the nutrients needed for plant development but may also be toxic elements which may be dangerous for the ecosystem. Usually, the more of the organic matter in the soil the larger is the CEC value. Therefore, charcoal presence in the soil may increase its CEC as it also increases organic matter content. As was found that CEC of charcoal sites was about twice that of non-charcoal sites and that CEC is related to an increase of organic matter in charcoal samples (B. Hardy et al., 2017). In another study, a similar situation was observed because CEC, total organic carbon, pH and other elements were increased with charcoal / biochar amendments to plantation soils (Gao et al., 2017). Increase in available phosphorus and potassium was also observed at charcoal forest sites (Li et al., 2018).

The CEC is pH dependent, which means that is connected to soil source materials, bedrock but also nutrient cycling at the site. The soil pH ranges from 4.0-10.0 and cannot always respond dynamically to the change in vegetation, precipitation and other environmental factors. Forest soils tend to be acidic. Charcoal buffering properties on soil pH have been observed in several studies (B. Hardy et al., 2017) (Carrari, Ampoorter, Bottalico, et al., 2017) and an increase in soil

pH with charcoal was observed and it was connected to an increase in diversity of plant species in the area (Carrari, Ampoorter, Bottalico, et al., 2017).

This leads to a conclusion that increased CEC may help with absorbing toxic materials in the soil, protecting water sources and improving plant health. Possibly charcoal may even be used for mitigation and stabilisation at contaminated sites (Antal et Grønli, 2003).

Larger Scale Effects

As mentioned before (Biondel, 2014) the human influence on nature is much more intricate than just abuse of resources. Mapping of historical charcoal kiln sites opens a forest mosaic of human and nature creating a dynamic surface. Kiln sites throughout Europe played a role into shaping its forests biodiversity and with further investigation the natural history of people and ecology shapes itself in more profound joined history.

Charcoal kiln sites and addition of biochar as soil amendment can also show effects on soil aggregates and quality. As mentioned in (Li et al., 2018) there are up to four different ways of how charcoal/ biochar may affect the structure and preserve aggregates, which are favouring root growth and fungi growth, the carboxyl formation during charcoal oxidation enhances stability, and biochar properties to retain water helps with clay swelling. Important note from the study is that charcoal improving soil aggregates is not always the case, and often depends on predominant soil type, as for example it had no effect on loamy sand soils (Busscher et al., 2010).

3.3 Differences in Soil Microorganisms

Dynamics of microorganisms in soil

The soils nature to change and provide unique niches allows the vast diversity of soil microorganisms to populate its grounds. While searching for DNA fragments in soil samples the main groups of focus are bacteria, archaea, fungi and some eukaryotes such as protozoa and algae. Microbial activities in soil have an associated gene which would make it easier for taxa identification, such metabolic processes include: nitrogen fixation, nitrification, denitrification,

methane production and oxidation, sulphate reduction and degradation of petroleum compounds. When determining soil microorganism diversity vegetation cover and topsoil can be one of the indicators, as the largest diversity is found at root rhizosphere areas. Another factor that may influence bacterial diversity is the mineral composition of the bed rock the soil has developed upon, as was further observed (Vieira et al., 2020). The bacteria can be found around various mineral clusters in soil, it is possible that charcoal particles could provide similar clusters for specific bacteria.

Dynamics of microorganisms in charred soil

Since the studies of charcoal sites are relatively new, the data on changes of the microbial community directly at kiln sites is not available. So, the literature on current biochar applications will be used to propose how the biochar in kiln sites may influence associated microbial communities if a long-term deposition is accounted for. For example, on the plantation field where the biochar was applied (Gao et al., 2017) the differences in soil Actinobacteria populations decreased yet Proteobacteria and Acidobacteria populations increased with charcoal additions comparing to the control plot. An independent of agriculture study of charcoal in soil revealed that the effects of charcoal on microbial communities are often more affected by land management. In addition, it was suggested that it is still an open question whether the specific soil bacteria occurring with charcoal are there due to charcoal ability to provide a novel environment or because the microorganisms use charcoal as food and just participate in its decomposing (Brieuc Hardy et al., 2019).

A report on microbiomes of forest biochar compared forests soils to 4-year-old biochar forest soil (Noyce et al., 2016). Interesting observations are that there were less prokaryotic species and more eukaryotic species at biochar sites. The biochar sites had less Acidobacteria, Planctomycetes and beta-Proteobacteria which is opposite to what have been found in agricultural soil (Gao et al., 2017) were it was said that those taxa have increased. The report also mentions the increase of eukaryotes at biochar sites such as Aveolata superphylum. Another study observed similar mixed results and concluded that charcoal/biochar effect on soil microbial structure does not always follow clear trends (Li et al., 2018).

Due to a sheer number of underlying mechanisms influencing soil microhabitat the field of research is also open to geographical specifics. An interesting observation was made in a Mediterranean kiln site during replanting of the area, (Carrari, Ampoorter, Verheyen, et al., 2017). It was reported than there have been some challenges in replanting woody species on charcoal hearth sites, possibly due to alterations in required nutrients and microhabitats, which not allow the growth of specific mycorrhizal fungi (Warnock et al., 2007). Another explanation was, that for the seedlings of the beech and oak the charcoal sites were too dry (Carrari et al., 2018).

In larger-scale scenarios historical kiln sites bacteria may contribute to the knowledge on forest microbiology during and pose forest fires, as well as its long-term effects, overall improving knowledge of forest dynamics. Even though the studies of charcoal/ biochar effects on microbiota is not abundant yet, some papers note that the comparison effect with control soils may be higher and more effective with agricultural soils rather than adjacent forest soils (Noyce et al., 2016). Additionally, the study of a forest with the kiln sites and the unknown diversity patterns may be interesting to compare with an undisturbed by people forest and compare their dynamics of vegetation and fauna. Currently, however, there is a need for continuous mapping of the microbial interactions with soil not only in production soils but also natural wildlife spaces, creating a better picture for sustainable interaction with nature.

4. Material and Methods

4.1 Soil

The samples were collected from all soil horizons in the forests of Moravian Karst located in Morava, the south-eastern region of the Czech Republic. The soil was developed on Hornblende-biotite granodiorite (amfibol biotitický granodiorit) bedrock. Soil type is the forest Stagnic Luvisol according to WRB 2015 (FAO, 2015). Photos provide a visual comparison of the sites, photo 1 being a charcoal kiln site with visual horizons and photo 2 the non-charred site.

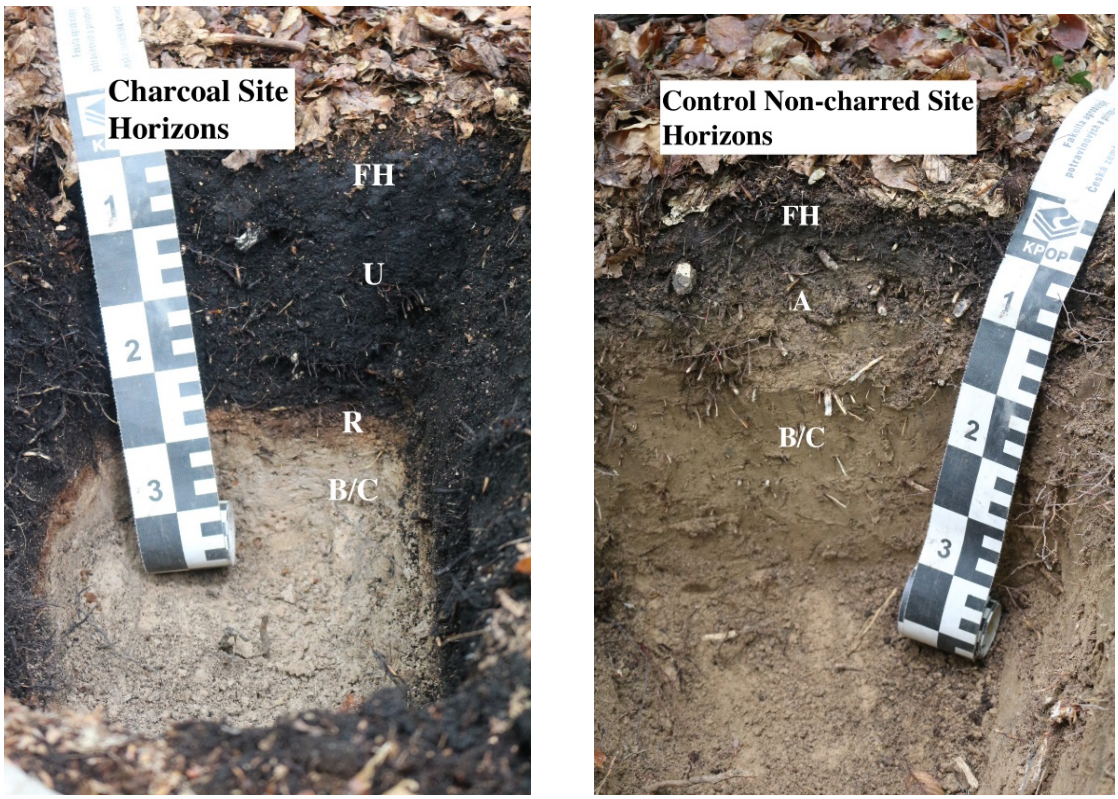


Photo 1 and 2 - Charcoal kiln site horizons (left photo) compared to control non-charred site horizons (right photo) (Photos: RNDr. Václav Tejnecký, Ph.D.)

Determination of the soil CEC, H+ and pH was done following the UNECE Manual on sampling and analysis of soil (Dobbertin M., 2016), following soil analysis method 6 (p. 55-57) for soil pH and soil analysis method 10 (p. 69-74) for determination of exchangeable cations (Al, Ca, Fe, K, Mg, Mn, Na) and free H+.

Determination of Soil pH

Soil Analysis Method 6 (SA06) for measuring soil pH. Using reference method ISO 10390. (Dobbertin M., 2016)

Preparing suspension: The pH will be measured in calcium chloride ($CaCl_2$) and in deionised water H_2O so each soil sample was measured twice. Preparing $CaCl_2$ solution with concentration of 0.01 mol/l will be required to weight 1.47g $CaCl_2 .2H_2O$/ litre water. For each soil sample measure 5ml of air-dried soil (<2mm) soil using volumetric spoon into a 50 ml plastic vials with cups. To the sample vial add five times the volume of calcium chloride solution, meaning 5 ml of soil add 25 ml of solution. Using mechanical shaker mix the suspension for approximately 1 hour, after which let it stand for 1 more hour before measuring pH. The process is repeated for all the samples but for deionised water as solution.

Calibrate the pH-meter before sampling using buffer solutions. Measure the pH while slightly stirring to create a homogeneous suspension. Record the pH value after the number is stabilised. Photo 3 exemplifies laboratory setup during suspension pH measurement. The measured pH table for all sample's pH $CaCl_2$ and pH H_2O can be found in Appendix 1. The results were analysed using one-way ANOVA and graphed in results section under graph 1 and graph 2.

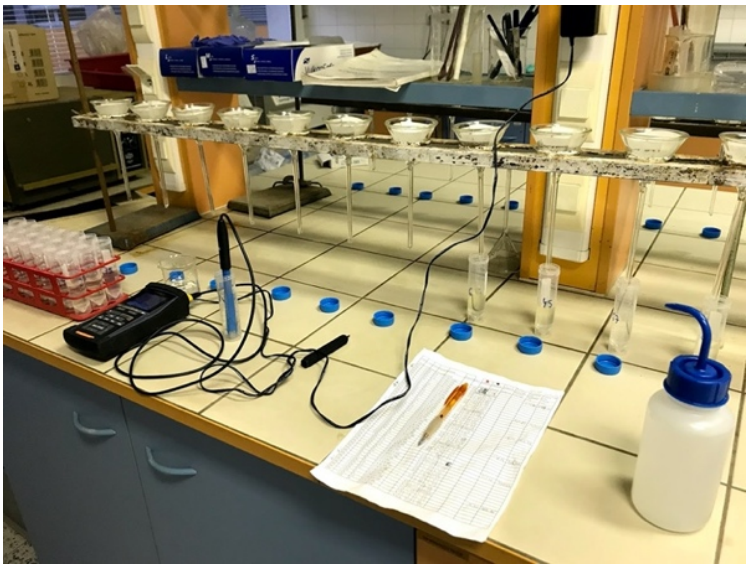


Photo 3 - Method 6 measuring soil pH workplace setup.

Determination of Soil CEC and H+

Soil Analysis Method 10 (SA10) for Determination of Exchangeable Cations (Al, Ca, Fe, K, Mg, Mn, Na) and Free H+. Using reference method ISO 11260 & ISO 14254. (Dobbertin M., 2016).

Creating a sample suspension involves a leaching procedure with barium chloride ($BaCl_2$) solution. Preparing the 0.1 mol/l barium chloride solution takes 24.43g of $BaCl_2$ per 1 litre of distilled water. For each soil type (< 2mm) a 2.5 ± 0.005g weighted sample is placed into a 50 ml centrifuge tube. Adding 30 ml of prepared $BaCl_2$ solution and mix using mechanical shaker for 2 hours and then centrifuge the samples at 4000rpm for 10 minutes. The final result supernatant is then transferred into the plastic vial with a cup through funnel with filter paper. The soil extracts are then ready for analysis.

The cations (Al, Fe, Ca, Na, Mn, Mg, K) are then measured in the 2ml of the extract using spectrometric determination. The ion equivalents per gram of soil for each cation were then calculated using formula $IE = \frac{C \times V}{m \times EQ \times 10}$. The final results table converted to meq/kg can be found in Appendix 2.

In order to determine free H+ 25ml of each sample extract was measured using a pipette and a blank distilled water vial. To each sample add 1.25ml of sodium fluoride (NaF) solution 1 mol/l and then titrate with the sodium hydroxide (NaOH) 0.05 mol/l with pH meter, titrate until pH value is of 7.8. The results table for all samples can be found in Appendix 3.

The total CEC the equals the sum of all ion equivalents of cations and H+ for each soil sample. The full table can be found at Appendix 3. The data was analysed by one-way ANOVA test. The null hypothesis being that the CEC values are the same for charcoal and non-charred soil, and the alternate hypothesis is that the CEC values would be significantly different. All statistical calculations were performed in Microsoft Excel.

4.2 DNA extraction

Composition of microbial communities will be determined using the currently extracted soil DNA. Soil DNA was extracted using a method described by (Sagova-Mareckova et al., 2008). It is an approach based on a cell disruption by bead-beating, followed by a phenol/chloroform extraction. Sterilised 2ml plastic vials with cups are used throughout the process, 250 mg of 0.1mm glass beads and 250 mg of 0.5mm glass beads are added to each vial. The soil then added according to its nature, 0.50g of soil if the sample is from mineral horizons (A, B, B/C, R) and 0.25g of soil if the sample is from organic horizons (FH and charcoal U horizon). In the fume box 600 µl of extraction buffer (50mM NaH_2PO_4 pH 8.0 + 50mM NaCl + 500mM Tris-HCl pH 8.0, 5% SDS) and then added to the sample vial and 300 µl of phenol chloroform mixture (1:1). The mixture is then homogenized by Bead Beater at 2500 rpm for 1 minute 30 seconds. The samples are then placed in centrifuge for 2 minutes at 12000 rpm. The supernatant developed on top of the vial is then collected in the fume box using pipette and transferred to a new sterilized 2ml Eppendorf tube. To the soil sample vial add 300 µl of extraction buffer and repeat the Bead Beater step for 30 seconds and following centrifugation at 12000 rpm for 2 minutes. The additional supernatant is added to the Eppendorf vial with previous supernatant. Labeling on top of the tube and on the side is important because sometimes the Bead Beater and Centrifuge erase top marker. Next follows steps of supernatant extraction. Identifying the volume of a sample and adding 1 volume of phenol/ chloroform 1:1, manually mixing the tube and then centrifuging at 6000 rpm for 5 minutes, transfer the supernatant with pipette to the supernatant-only sample Eppendorf. Again, similar step, the same soil sample vial, measure the volume and add 1 volume of pure chloroform, manually mix and centrifuge again at 6000 rpm for 5 minutes and extract the supernatant for the last time. The soil sample can now be discarded, and the supernatant-only Eppendorf tubes samples are heated till 65℃ in dry bath heater.

The heated samples were then mixed with 5M NaCl solution until the final concentration of 1.5M NaCl, the equation was used to properly mix, $\frac{3}{7} \times 1 sample\ volume$ in µl. Then 10% CTAB was added at 1/10 sample volume to achieve 1% concentration. The CTAB was preheated in 40℃ hot bath for at least 30 minutes until completely dissolved before adding. The mixture of supernatant NaCl and CTAB was then heated till 65℃ for 30 minutes using dry bath heater and cooled in cool water till around 20℃. The volume of supernatant was then measured, and 1 volume of pure chloroform was added according to each sample volume. The samples

were then centrifuged for 15 minutes at 4500 rpm, the supernatant from this round collected into a new 2ml Eppendorf tube. To the new supernatant 1/10 of sample volume 3M NaAc was added. Then 0.6 of the new sample volume Isopropanol was added, mixed and left at room temperature for 25 minutes, during which the next step precooled centrifuge was prepared. The samples were centrifuged at 4°C at 10 000 rpm for 20 minutes, this would create a DNA pellet at the bottom of the tube. Next step is discarding the supernatant with pipette so not to lose DNA pellet stuck at the bottom of the tube. Ethanol 70% from freezer is then added to the pellet 50 µl or 200 µl if the samples would be stored in the freezer (at at least -20°C). The samples are then again centrifuged at 4°C for 5 minutes at 10000 rpm, after which again discarded the supernatant carefully not to lose DNA pellet. Open Eppendorf tubes with DNA sample pallets were then dried using CentriVap concentrator at 40°C for at least 8 minutes. Distilled H_2O was dropped on the sample pellets then, 30 µl and heated using dry bath heater at 65°C for 1 hour. After this step 2 µl of the samples can be preliminary tested on DNA presence using gel electrophoresis in order to evaluate the success of the extraction, as some samples had to be adjusted in order to find any fragments (usually with deep mineral horizons).

Final purification step includes a treatment with 1 volume of $CaCl_2$ (1M) mixed with Hepes (1M). The mixture was prepared from 2M $CaCl_2$ and 2M Hepes into 1M in ratio with water 1:1:2. After mixing the samples stood in room temperature for 30 more minutes before column purification step using a GeneClean Turbo kit (MP Biomedicals, Santa Ana, CA)and included instructions of repeated centrifuging of DNA catch tubes, the end result was purified DNA soil sample in volume of 30 µl.

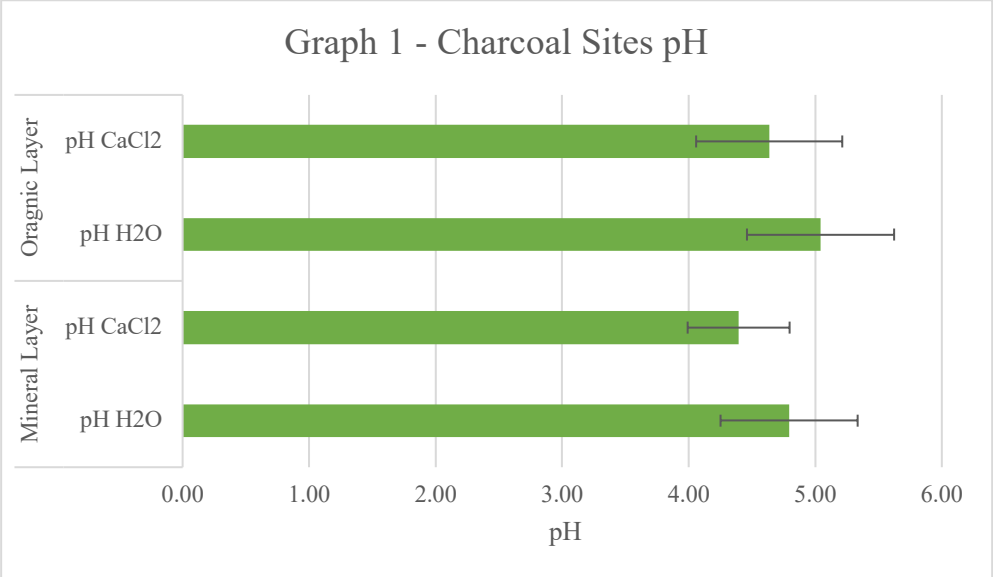
The DNA solution from each site was then tested on gel electrophoresis and screened via GeneSnap UV equipment. During electrophoresis procedure 1% agarose gel was prepared with 5 µl of SYBR Green I and as control marker 5 µl 1kb Plus DNA Marker was used. All samples 2 µl were used to mix with 2 µl marker. Photo 4 in results section – is the snapshot of one of the sets showing successful extraction of some samples. In some samples it was harder to retrieve DNA presence, such as deep mineral layers and the charcoal horizons which often were difficult to purify. After confirmation of the DNA presence the samples go to PCR for multiplication and then to be determined the source, bacterial or otherwise.

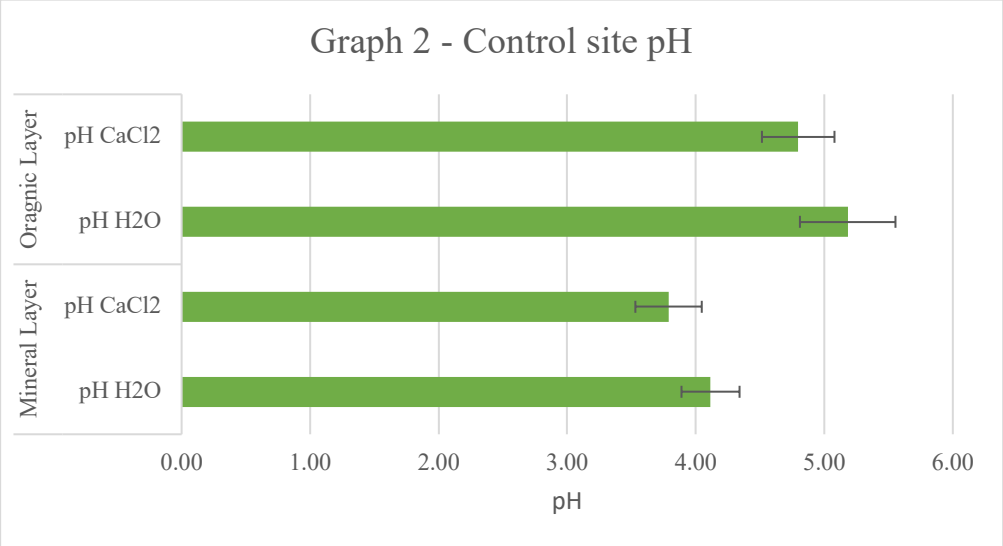
5. Results

5.1 Soil Analysis

Soil pH

The soil pH was organised not by horizons but by layers, due to the inconsistent layering of the sites. Dividing into the organic layer consisting of FH and U horizons and the mineral layer of A, B, B/C, E and R horizons. Raw data collected on soil pH can be found in Appendix 1. Graph 1 – shows the charcoal kiln sites and graph 2 control sites averages with standard deviations.





Graph 1 and 2, Moravian Karst. Comparison of soil pH at sites with and without charcoal. At each site, organic and mineral horizons were separated, and soil pH was determined in $CaCl_2$ and H_2O. The graphs show that the organic layer of the charcoal sites have slightly lower pH than the organic layer in the control but not with as much significant difference as the mineral layer. The standard deviation is higher for charcoal than control. For the mineral layer, the charcoal site has a higher soil pH than in the control sites. The pH H_2O and pH $CaCl_2$ ANOVA test showed that there is a significant difference between charcoal samples and control as both of the tables have values of $p<0.05$.

Table 1- ANOVA summery between charcoal and control sites at pH-H2O

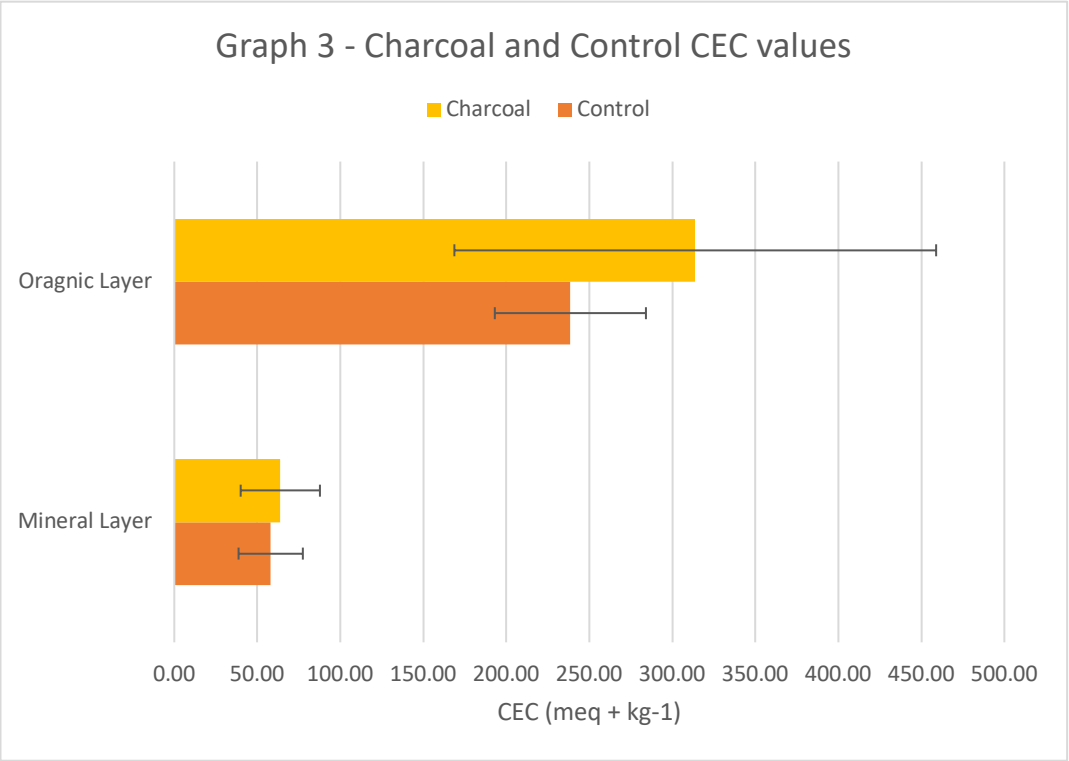
SUMMARY	for H2O pH					
Groups	*Count*	*Sum*	*Average*	*Variance*		
Charcoal Site	38	187.35	4.930263	0.325408		
Control Site	35	154.69	4.419714	0.311844		
ANOVA	One-way					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.749014	1	4.749014	14.89127	0.000248	3.97581
Within Groups	22.64279	71	0.318913			
Total	27.39181	72				

Table 2- ANOVA one-way summery between charcoal and control sites at pH-CaCl2

SUMMARY	for CaCl2 pH					
Groups	*Count*	*Sum*	*Average*	*Variance*		
Charcoal Sites	38	172.05	4.527632	0.265213		
Control Sites	35	142.68	4.076571	0.28187		
ANOVA	one-way					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.706788	1	3.706788	13.56854	0.000446	3.97581
Within Groups	19.39648	71	0.27319			
Total	23.10326	72				

CEC Results

The graph 3 shows an average of the total values of CEC for the respective layers and sites. It shows that the organic layer has significantly higher CEC values than mineral. As well as that charcoal has larger value of CEC than control on both layers, but also higher standard deviation in organic layer. The difference between charcoal and control is especially highlighted at organic layer while at mineral layer the difference is much smaller.



The cation exchange capacity also differed significantly between the charcoal and control sites ($p < 0.05$), table 3 provides a summary of calculation.

Table 3- Total CEC one-way ANOVA summery between charcoal and control CEC values.

SUMMARY for total CEC

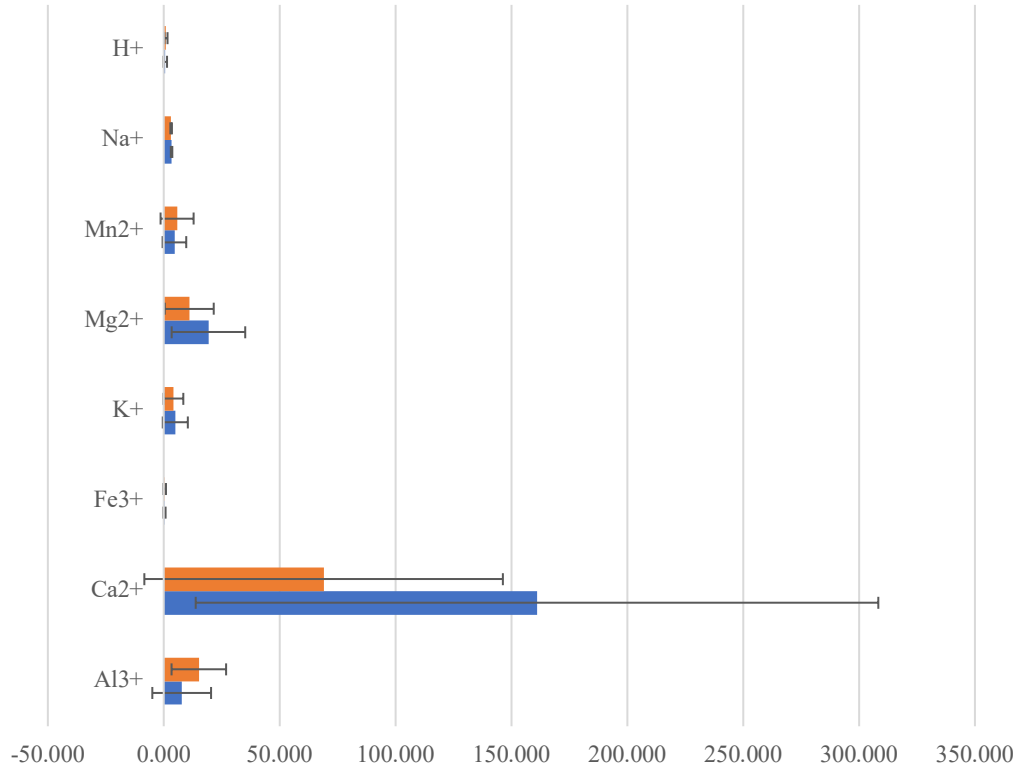
Groups	*Count*	*Sum*	*Average*	*Variance*
CEC Charcoal Soils	38	786.79	20.705	268.5523
CEC Non-charred soils	35	413.25	11.80714	71.41915

ANOVA one way

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1442.446	1	1442.446	8.282756	0.005284	3.97581
Within Groups	12364.69	71	174.1505			
Total	13807.13	72				

Additional results on the ion composition of the CEC for charcoal and control sites are presented in the graph 4. Charcoal has significantly larger amount of Ca^{2+} ion and higher proportion of Mg^{2+} and K^{+} ions. The control has higher contents of Al^{3+}, Fe^{3+}, Mn^{2+} ions.

Graph 4 - Ion Comparison between Charcoal and Control Sites



	Al3+	Ca2+	Fe3+	K+	Mg2+	Mn2+	Na+	H+
Control	15.145	68.983	0.432	4.188	11.006	5.765	3.196	0.964
Charcoal	7.751	161.055	0.340	4.988	19.294	4.611	3.377	0.681

meq + kg-1

5.2 DNA Analysis

The DNA extraction from the soils was performed for 73 samples (Photos 4-12 in the supplement). That had some challenges with the extraction and purification of some samples, so some the samples had to be DNA extracted from the soil again, with modification and changes to the individual components' concentrations. Most of the poor extractions were from mineral horizons (for example Photo 4 –sample n.60, n.45 and n.41) were DNA became too diluted and/or disappeared during purification process and sometimes also form the charcoal topsoil (for

example Photo 4 – sample n.65). The quantity of extracted DNA varies greatly. However, most of the DNA is also of a good quality and the fragments are sufficiently long. Thus, some samples need to be extracted again, as marked in the table 4, mineral layers and charcoal topsoil (sample n. 29) turned out to be too enriched with the DNA inhibitors and preserved the dark colour even after purification. No correlation has been yet observed between charcoal sites and controls in terms of the DNA quantity estimation, mainly because of the yet incomplete collection.

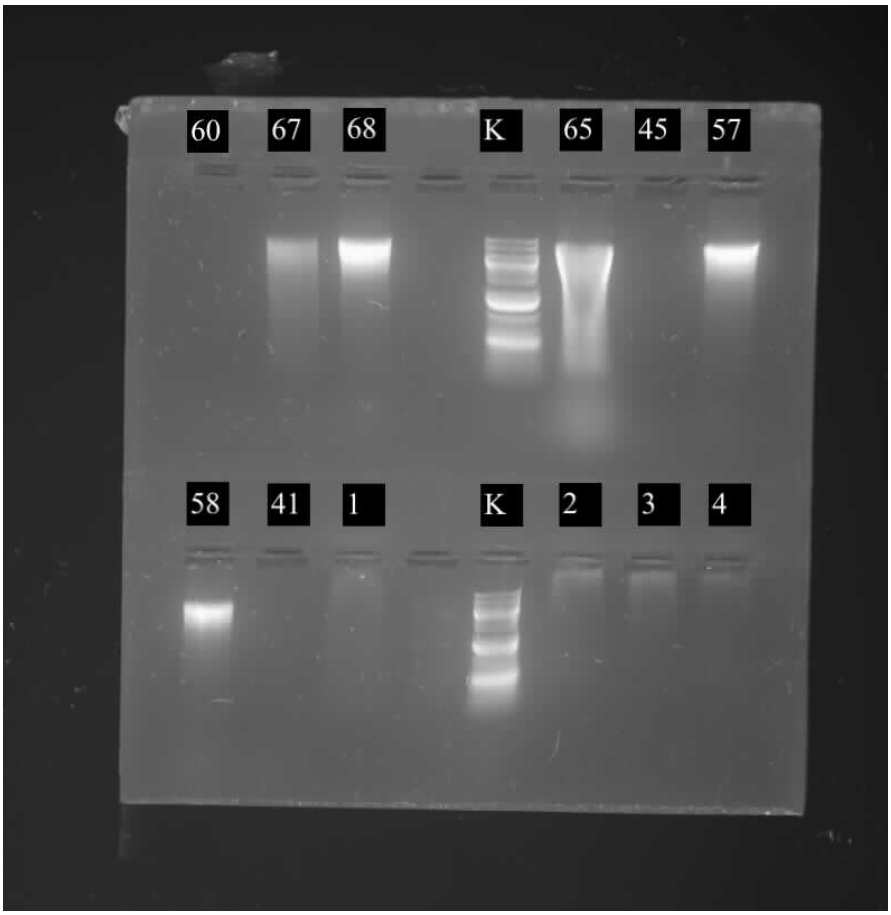


Photo 4 - Electrophoresis gel with 2 µl of DNA with 2 µl of marker and 5 µl of the control ladder.

Table 4- Soil samples DNA estimates with amount of soil used. The charcoal kiln site samples are highlighted in yellow.

n	Sample code	Soil weight (g)	Estimation of DNA concentration (ng/µL)
1	2K FH	0.25	50
2	2K A_{he}	0.25	300
3	2K B_{VS}	0.50	500

4	2M FH	0.25	200
5	2M U	0.5	75
6	2M U	0.5	200
7	2M B	0.50	75
8	2M B/C	To be repeated	To be repeated
9	4K FH	0.5	200
10	4K A	0.51	200
11	4K A/B	0.5	200
12	4K B_{VS}	0.5	200
13	4M FH	0.5	200
14	4M U	0.5	200
15	4M B	0.50	200
16	5K FH	0.5	300
17	5K A	0.5	300
18	5K B_V	0.5	75
19	5M FH	0.25	75
20	5M U	0.5	300
21	5M R	0.50	200
22	5M B/C	1.00	75
23	8K FH	0.25	75
24	8K A	0.5	75
25	8K A	0.5	75
26	8K B	0.50	400
27	8K B/C	To be repeated	To be repeated
29	8M FH	To be repeated	To be repeated
30	8M U	0.5	200
31	8M B	1.00	200
32	11K FH	0.51	300
33	11K A	0.5	300
34	11K B_{VS}	0.51	200
35	11M FH	0.5	300
36	11M U	0.5	300
37	11M R	To be repeated	To be repeated
38	11M B/C	0.50	200
39	12K FH	0.25	75

40	12K A	0.5	200
41	12K B_{vs}	To be repeated	To be repeated
42	12M FH	0.25	300
43	12M U	0.50	75
44	12M U	0.51	200
45	12M R	To be repeated	To be repeated
46	12M B/C	0.5	75
47	12M B/C	0.5	200
48	15K FH	0.5	200
49	15K A_h	0.5	200
50	15K B_{VS}	0.5	200
51	15M FH	0.51	200
52	15M U	0.25	75
53	15M B	0.51	75
54	20K FH	0.5	200
55	20K A	0.5	200
56	20K B_{VS}	0.52	300
57	20M FH	0.5	300
58	20M U	0.51	300
59	20M R	0.51	75
60	20M B/C	To be repeated	To be repeated
61	21K FH	0.5	200
62	21K A_{he}	0.5	200
63	21K B_V	0.51	200
64	21M FH	0.5	200
65	21M FH	0.5	200
66	21M U	0.5	200
67	21M B	0.50	75
68	27K FH	0.5	200
69	27K A	0.51	300
70	27K B_V	0.50	200
71	27M FH	0.5	200
72	27M U	0.5	200
73	27M B	0.50	75

6. Discussion

Soil Analysis

The analysis of soil pH and CEC between charcoal and control sites was in line with the hypothesis that the charcoal would alter soil environment and provide new niches for microorganisms to diversify. It also complied with the literature findings, which provided an information on the overall increase of soil pH and CEC with charcoal additions (B. Hardy et al., 2017) (Gao et al., 2017) (Carrari et al., 2018; Carrari, Ampoorter, Bottalico, et al., 2017).

The results of analysis showed that pH of charcoal sites was higher especially in the mineral horizons of the soil. Overall, the soils pH of the locations were acidic below 5.5 pH. It was interesting to observe that on the organic layer pH was not as different as the mineral layer between the charcoal and the control. While the control mineral layer pH was around 4.0 with the charcoal it stayed in the region between 4.0-5.0. There are several possibilities on how the charcoal might have affected it. 1) The charcoal tendency to prevent leaching and hold on to nutrients (Antal et Grønli, 2003) , leaching of the topsoil humus which may have consequence on the mineral layer making it more acidic and thus charcoal may mitigate the effect in comparison with control. 2) The charcoal horizon level U is thicker than control organic layer due to intensity of kiln use, (visible on photos 1 and 2) this thickness may mitigate the pH drop distance from the surface down. 3) The charcoal may stabilize the pH because of carboxylate compounds present in the chemistry of the material (B. Hardy et al., 2017), which helps to buffer the acidity. It is possible that all three of the variants are involved.

Further, the results of CEC soil analysis presented that the charcoal sites had higher CEC values than the control. Similarly, to the pH findings on the horizon level, the organic layer has a larger difference between the charcoal and the control. The CEC of top horizons is greatest and decreases with depth, in this case, the organic layer has a significantly higher value of CEC than mineral layer for both charcoal and control sites. It correlates with the findings of previous papers (Antal et Grønli, 2003; Carrari, Ampoorter, Bottalico, et al., 2017; Gao et al., 2017; B. Hardy et al., 2017) that charcoal carbon nature and nutrient retention contribute to the higher CEC value at charcoal/ biochar amended soils. That might be explained by the influence, which

charcoal has on the CEC value due to its porosity and high surface area, the same was detected in this analysis. Interestingly charcoal kiln history, intensity and temperatures affect the quality of charcoal and its surface area, as mentioned in (Li et al., 2018), higher temperature pyrolysis result in finer particles of charcoal and lower CEC. This may explain why the standard variation of charcoal CEC is so high, possibly due to unequal conditions during pyrolysis on each kiln site and age of sites.

The brief mentioning of ion distribution of CEC values between charcoal and control. Charcoal effect on specific cations (B. Hardy et al., 2017) mentions that charcoal tend to promote the retention of exchangeable Ca^{2+} and Mg^{2+} but not the K^{+} . The cation composition in this study correlates with Hardy (2017) findings, charcoal had larger amounts of exchangeable Ca^{2+}, Mg^{2+} as well as some Na^{+}, the K^{+} values however were not different enough between charcoal and control soils. Control on the other hand had higher values of Al^{3+} and Mn^{2+}. Another unusual observation is the failure to obtain free H+ values for topsoil FH horizon at charcoal kiln sites (marked as absent in Table 7, Appendix 3).

Microorganism Analysis

Unfortunately, because of the incomplete microorganism identification the discussion mainly relies on literature findings. Even though some data on DNA estimates has already been collected, the value does not tell much information about diversity but only that the extraction was successful, and that sample contained some organisms' DNA. On the other hand, in literature, even though it heavily depends on land management and soil type, are present differences regarding microbial biomass and community structures (Li et al., 2018) (Gao et al., 2017; Noyce et al., 2016) and possibly fungi structures (Warnock et al., 2007). The microbial biomass with charcoal additions has increased but soon stabilised and in excess charcoal applications may decrease all together (Li et al., 2018). And as mentioned before in (Li et al., 2018) the effects of charcoal/ biochar on fungi and bacteria populations may not follow clear trends, which leave us to rely on first hand data from local sites and hopefully this study may contribute to larger trend finding.

7. Conclusion

This thesis aimed to identify if charcoals deposited at the historical kiln sites affect the soil properties if those also have a potential to influence the microbial community structure. It was demonstrated that indeed charcoal affects the soil pH, CEC and the cation distributions in soils and therefore hypothesis that charcoal amends soil structure and may contribute to creation of niches and increased biodiversity is correct. Unfortunately, the effect of charcoal on soil microbiology cannot be completely answered as of now without the identification of microorganisms.

Soil analysis brings an additional insight about pH influences, in which the difference between organic and mineral layer pH is less with charcoal soils than in controls. Therefore, this information leads to a conclusion that the different soil structure and chemistry provide novel niches for microorganisms and thus, different structure of microbial communities is expected. The soil DNA was present in all extracted samples; however, the DNA was of differing quality and quantity showing that conditions in the soil are different and influence the DNA extraction. It also seems that different amounts of DNA are present in organic and mineral horizons. The study further contributes to the pool of studies about charcoal and biochar amendment effects on soil.

8. Appendix

Appendix 1 – Soil pH

Table 5 - Sample names, measured pH and dry weight.

Locality	n. locality	station	horizon	n. sample	pH initial	pH H2O	pH BaCl2 (5.34; 5.64) (av.5.49)	weight (g)	weight (g) (without >2mm)
MK	15	Kiln	FH	1	6.6	5.70	5.83	892.05	598.35
MK	15	Kiln	U	2	5.8	5.29	4.51		
MK	15	Kiln	R	4	4.6	4.76	4.17		
MK	15	Kiln	B/C	5	5.5	4.82	4.61		
MK	15	Control	FH	6	/	5.40	5.7		
MK	15	Control	A	7	3.7	4.20	3.67		
MK	15	Control	B/C	8	4.8	4.17	4.24		
MK	17	Kiln	FH	9	/	6.10	6.19	921.20	604.81
MK	17	Kiln	U	10	5.1	5.19	4.42		
MK	17	Kiln	C	12	3.7	5.22	4.72		
MK	17	Control	FH	13	/	5.06	4.48		
MK	17	Control	A	14	4.2	4.04	3.78		
MK	17	Control	B/C	15	4.1	4.28	4.1		
MK	21	Kiln	FH	16	/	6.12	6.18	723.49	518.20
MK	21	Kiln	U	17	5.1	5.13	4.15		
MK	21	Kiln	R	19	4.2	5.02	4.33		
MK	21	Kiln	B/C	20	4.5	4.45	4.37		
MK	21	Control	FH	21	/	5.38	5.04		
MK	21	Control	A	22	4.3	4.24	3.86		
MK	21	Control	B/C	23	4.1	4.37	4.04		
MK	22	Kiln	FH	24	/	5.66	5.8	704.00	508.14
MK	22	Kiln	U	25	5.1	4.84	4.21		
MK	22	Kiln	B/C	27	5.6	4.80	4.42		
MK	22	Control	FH	28	/	5.17	4.78		
MK	22	Control	A	29	4.8	4.08	4.03		
MK	22	Control	B/C	30	4.5	4.01	4.15		
MK	1	Kiln	FH	31	/	5.21	5.5	1530.33	927.31

MK	1	Kiln	U	32	5.1	4.75	4.3		
MK	1	Kiln	A	34	4.7	4.52	4.24		
MK	1	Kiln	B/C	35	4.9	4.69	4.51		
MK	1	Control	FH	36	3.6	4.38	4.14		
MK	1	Control	A	37	3.9	3.83	3.73		
MK	1	Control	B/C	38	4	3.86	3.86		
MK	1	Control	B/C	39	5.2	4.64	4.49		
MK	13	Kiln	FH	40	/	5.56	4.91	782.35	574.20
MK	13	Kiln	U	41	4.5	4.69	4.21		
MK	13	Kiln	E	43	6.5	4.40	4.35		
MK	13	Kiln	B/C	44	4.9	5.58	4.42		
MK	13	Control	FH	45	4.7	5.68	5.15		
MK	13	Control	A	46	4.1	3.97	3.91		
MK	13	Control	E	47	4.5	3.82	4.02		
MK	13	Control	B/C	48	4.2	4.20	4.11		
MK	3	Kiln	FH	49	/	5.47	5.43	675.62	490.54
MK	3	Kiln	A	50	/	4.44	4.14		
MK	3	Kiln	U	51	/	4.55	4.1		
MK	3	Kiln	B/C	53	/	5.10	4.82		
MK	3	Control	FH	54	4.7	5.02	4.58		
MK	3	Control	A	55	4.7	4.20	3.99		
MK	3	Control	B/C	56	4.1	3.95	4.2		
MK	5	Kiln	FH	57	5.5	4.72	4.42	495.72	333.40
MK	5	Kiln	U	58	5.4	4.36	4		
MK	5	Kiln	E	60	5.1	4.82	4.63		
MK	5	Kiln	B/C	61	5.1	6.21	5.03		
MK	5	Control	FH	62	5.4	4.96	4.55		
MK	5	Control	A	63	4.8	4.47	4.23		
MK	5	Control	B/C	64	4.6	4.12	4.06		
MK	5	Control	B/C	65	4.4	4.40	4.09		
MK	20	Kiln	FH	66	4.8	4.94	4.87	560.48	384.77
MK	20	Kiln	U	67	4.6	4.38	4.06		
MK	20	Kiln	R	69	4.2	3.94	3.81		
MK	20	Kiln	E	70	4.7	4.14	4.2		
MK	20	Kiln	B/C	71	4	4.68	4.33		

MK	20	Control	FH	72	5.5	5.17	5.29		
MK	20	Control	A	73	5.5	4.28	4.15		
MK	20	Control	E	74	4.6	3.87	4.1		
MK	20	Control	B/C	75	5.4	4.22	4.06		
MK	18	Kiln	FH	76	5.2	4.81	4.79	503.81	799.33
MK	18	Kiln	U	77	5	3.96	3.5		
MK	18	Kiln	B/C	79	6.5	4.33	4.24		
MK	18	Control	FH	80	5	5.60	5.05		
MK	18	Control	A	81	4.1	3.71	3.86		
MK	18	Control	E	82	4.5	3.92	4.07		
MK	18	Control	B/C	83	4.9	4.02	4.09		

Appendix 2 – Soil Cations

Table 6 - Sample names and corresponding cation value.

Locality	station	horizon	n of sample	Al3+ (meq kg^{-1})	Ca2+ (meq kg^{-1})	Fe3+ (meq kg^{-1})	K+ (meq kg^{-1})	Mg2+ (meq kg^{-1})	Mn2+ (meq kg^{-1})	Na+ (meq kg^{-1})
MK	Kiln	FH	1	0.283	380.545	0.199	12.406	34.985	7.030	3.926
MK	Kiln	U	2	4.339	212.008	0.065	1.452	12.604	3.412	3.637
MK	Kiln	R	4	10.050	55.633	0.065	1.973	6.904	1.383	3.091
MK	Kiln	B/C	5	0.948	21.512	0.065	1.294	4.157	0.420	3.157
MK	Control	FH	6	0.283	220.423	0.424	7.548	27.313	11.756	3.591
MK	Control	A	7	35.694	30.135	2.392	2.505	5.422	1.123	3.343
MK	Control	B/C	8	21.733	8.550	0.065	0.829	1.727	0.065	2.787
MK	Kiln	FH	9	0.283	559.707	0.065	12.828	65.748	4.911	3.469
MK	Kiln	U	10	5.664	331.544	0.369	2.750	23.512	8.259	3.262
MK	Kiln	C	12	0.283	54.902	0.065	1.538	12.767	0.960	2.790
MK	Control	FH	13	0.283	208.228	0.065	12.014	23.520	14.736	3.379
MK	Control	A	14	32.885	30.605	0.065	3.328	5.394	6.921	3.388
MK	Control	B/C	15	25.754	9.240	0.228	0.963	2.149	0.896	3.136
MK	Kiln	FH	16	0.283	427.362	0.065	12.559	46.746	4.920	3.797
MK	Kiln	U	17	17.970	198.326	0.065	3.230	15.029	7.337	3.096
MK	Kiln	R	19	5.082	28.004	0.065	3.379	9.290	0.403	3.215

MK	Kiln	B/C	20	3.178	40.286	0.065	4.254	15.540	0.554	3.296
MK	Control	FH	21	0.283	166.208	0.170	10.002	18.088	15.502	4.025
MK	Control	A	22	31.498	26.246	0.822	3.702	4.375	6.077	3.156
MK	Control	B/C	23	27.706	11.871	0.968	1.082	2.410	0.447	2.853
MK	Kiln	FH	24	0.283	426.966	0.822	17.215	49.108	4.820	3.529
MK	Kiln	U	25	11.318	202.418	0.065	3.169	14.669	7.661	3.415
MK	Kiln	B/C	27	5.154	22.856	0.065	1.418	5.303	0.643	3.004
MK	Control	FH	28	0.283	266.073	1.248	15.352	34.521	7.587	3.504
MK	Control	A	29	15.752	41.289	0.065	4.627	5.817	5.833	3.044
MK	Control	B/C	30	15.754	11.662	0.065	1.090	1.938	0.236	3.230
MK	Kiln	FH	31	0.283	365.261	0.065	4.740	37.208	5.536	3.348
MK	Kiln	U	32	9.200	204.199	0.424	2.033	12.519	5.367	3.009
MK	Kiln	A	34	12.934	52.142	0.766	0.950	2.812	1.058	3.094
MK	Kiln	B/C	35	4.443	27.344	0.065	1.126	3.264	0.444	3.225
MK	Control	FH	36	0.283	204.032	0.065	8.509	31.527	33.574	3.569
MK	Control	A	37	22.361	54.328	0.065	3.004	8.366	9.467	3.303
MK	Control	B/C	38	27.588	22.674	0.425	1.232	4.096	4.261	3.145
MK	Control	B/C	39	1.825	31.658	0.065	1.362	4.269	0.295	2.806
MK	Kiln	FH	40	0.283	385.935	0.065	22.324	47.969	7.918	3.614
MK	Kiln	U	41	15.528	232.908	0.065	2.106	15.658	6.887	3.263
MK	Kiln	E	43	1.142	36.555	1.252	2.577	8.017	0.197	3.391
MK	Kiln	B/C	44	0.283	82.944	0.065	5.068	24.071	0.291	4.041
MK	Control	FH	45	0.283	175.627	0.766	10.777	27.524	9.243	3.308
MK	Control	A	46	20.854	15.918	0.065	1.938	3.319	3.816	2.971
MK	Control	E	47	25.077	10.787	0.655	0.643	2.125	0.106	2.800
MK	Control	B/C	48	19.561	21.408	0.065	1.204	4.157	0.229	2.800
MK	Kiln	FH	49	0.283	238.352	0.341	13.328	35.319	5.874	3.563
MK	Kiln	A	50	15.789	93.460	0.797	2.546	10.201	10.390	3.348
MK	Kiln	U	51	14.923	88.611	0.065	1.361	11.401	3.241	3.716
MK	Kiln	B/C	53	0.283	39.678	1.277	2.509	8.178	0.359	3.120
MK	Control	FH	54	0.714	155.468	0.065	13.270	27.128	9.054	3.364
MK	Control	A	55	11.509	19.650	0.597	3.908	4.618	4.268	2.762
MK	Control	B/C	56	13.678	8.334	0.065	0.651	1.897	0.272	2.772
MK	Kiln	FH	57	0.283	174.382	0.065	7.776	28.761	22.287	3.412
MK	Kiln	U	58	25.477	73.608	0.065	2.921	9.484	8.339	3.269

MK	Kiln	E	60	0.283	56.966	0.065	3.000	16.488	0.482	3.137
MK	Kiln	B/C	61	0.283	75.663	0.425	3.352	24.436	0.301	3.414
MK	Control	FH	62	0.283	136.536	0.227	5.853	27.140	18.362	3.425
MK	Control	A	63	9.180	46.641	0.065	2.938	11.153	14.845	2.968
MK	Control	B/C	64	23.440	15.339	0.595	0.970	4.156	2.625	2.988
MK	Control	B/C	65	17.348	22.881	0.065	0.929	8.330	0.427	3.125
MK	Kiln	FH	66	0.283	319.896	0.736	13.409	30.154	16.431	4.029
MK	Kiln	U	67	9.360	96.996	0.881	1.128	5.091	8.024	3.195
MK	Kiln	R	69	22.045	34.898	0.065	1.036	4.203	0.946	3.420
MK	Kiln	E	70	9.661	18.448	0.065	0.681	3.748	0.390	2.802
MK	Kiln	B/C	71	8.230	29.356	1.220	0.762	5.742	0.871	3.054
MK	Control	FH	72	0.283	153.604	0.170	8.154	21.380	8.189	3.288
MK	Control	A	73	7.521	33.116	0.142	2.197	5.505	5.772	3.056
MK	Control	E	74	22.812	10.613	0.455	0.481	2.278	0.269	2.990
MK	Control	B/C	75	24.993	31.161	0.065	1.235	9.644	0.245	3.038
MK	Kiln	FH	76	0.283	290.407	0.065	12.347	46.569	14.973	3.744
MK	Kiln	U	77	71.786	104.058	1.846	1.721	14.299	1.431	4.013
MK	Kiln	B/C	79	6.082	35.967	0.065	1.288	11.227	0.485	3.416
MK	Control	FH	80	0.283	154.647	0.065	10.792	28.729	3.631	3.963
MK	Control	A	81	30.981	26.484	1.334	1.927	5.621	1.529	3.977
MK	Control	E	82	22.569	14.257	1.192	0.816	3.800	0.061	3.047
MK	Control	B/C	83	18.742	18.723	1.277	0.737	5.772	0.052	2.967

Appendix 3 – Soil total CEC and H+

Table 7 - Sample names and corresponding H+ value and total CEC value.

Locality	station	horizon	n of sample	H+ (meq kg^{-1})	CEC (including H+) (meq kg^{-1})
MK	Kiln	FH	1	/	439.37
MK	Kiln	U	2	0.314	237.83
MK	Kiln	R	4	0.809	79.91
MK	Kiln	B/C	5	0.273	31.83
MK	Control	FH	6	/	271.34
MK	Control	A	7	2.793	83.41

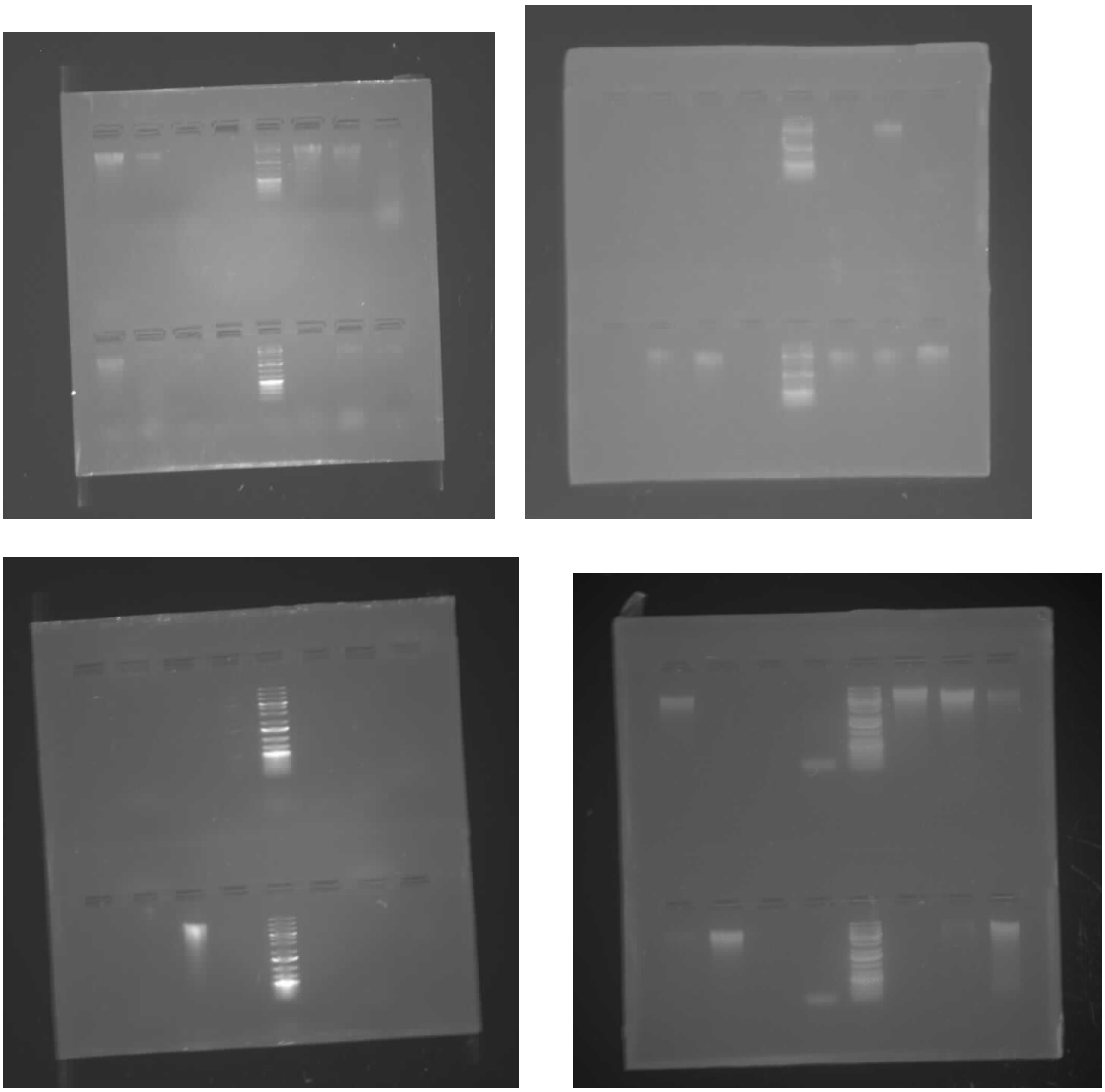
MK	Control	B/C	8	0.567	36.32
MK	Kiln	FH	9	/	647.01
MK	Kiln	U	10	0.407	375.77
MK	Kiln	C	12	0.209	73.51
MK	Control	FH	13	0.404	262.63
MK	Control	A	14	2.126	84.71
MK	Control	B/C	15	0.889	43.26
MK	Kiln	FH	16	/	495.73
MK	Kiln	U	17	0.804	245.86
MK	Kiln	R	19	0.544	49.98
MK	Kiln	B/C	20	0.504	67.68
MK	Control	FH	21	0.068	214.35
MK	Control	A	22	1.732	77.61
MK	Control	B/C	23	1.059	48.39
MK	Kiln	FH	24	/	502.74
MK	Kiln	U	25	0.712	243.43
MK	Kiln	B/C	27	0.413	38.86
MK	Control	FH	28	0.175	328.74
MK	Control	A	29	1.150	77.58
MK	Control	B/C	30	0.819	34.79
MK	Kiln	FH	31	/	416.44
MK	Kiln	U	32	0.555	237.31
MK	Kiln	A	34	0.637	74.39
MK	Kiln	B/C	35	0.313	40.22
MK	Control	FH	36	0.942	282.50
MK	Control	A	37	2.439	103.33
MK	Control	B/C	38	1.745	65.17
MK	Control	B/C	39	0.372	42.65
MK	Kiln	FH	40	0.114	468.22
MK	Kiln	U	41	0.681	277.10
MK	Kiln	E	43	0.553	53.69
MK	Kiln	B/C	44	0.471	117.23

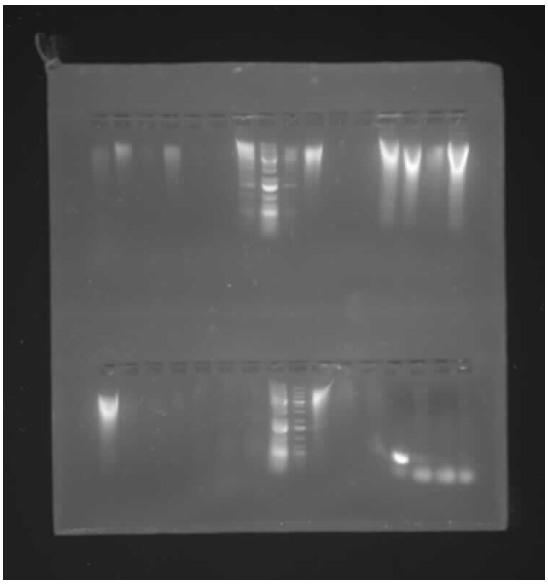
MK	Control	FH	45	0.037	227.56
MK	Control	A	46	1.555	50.44
MK	Control	E	47	1.136	43.33
MK	Control	B/C	48	0.898	50.32
MK	Kiln	FH	49	/	297.06
MK	Kiln	A	50	0.843	137.38
MK	Kiln	U	51	0.952	124.27
MK	Kiln	B/C	53	0.154	55.56
MK	Control	FH	54	0.302	209.37
MK	Control	A	55	1.299	48.61
MK	Control	B/C	56	0.716	28.39
MK	Kiln	FH	57	0.471	237.44
MK	Kiln	U	58	1.201	124.36
MK	Kiln	E	60	0.270	80.69
MK	Kiln	B/C	61	0.071	107.95
MK	Control	FH	62	0.336	192.16
MK	Control	A	63	0.687	88.48
MK	Control	B/C	64	1.019	51.13
MK	Control	B/C	65	0.966	54.07
MK	Kiln	FH	66	0.131	385.07
MK	Kiln	U	67	1.094	125.77
MK	Kiln	R	69	2.002	68.62
MK	Kiln	E	70	0.746	36.54
MK	Kiln	B/C	71	0.513	49.75
MK	Control	FH	72	0.005	195.07
MK	Control	A	73	0.872	58.18
MK	Control	E	74	0.906	40.80
MK	Control	B/C	75	1.011	71.39
MK	Kiln	FH	76	0.169	368.56
MK	Kiln	U	77	4.162	203.31
MK	Kiln	B/C	79	0.692	59.22
MK	Control	FH	80	0.064	202.17

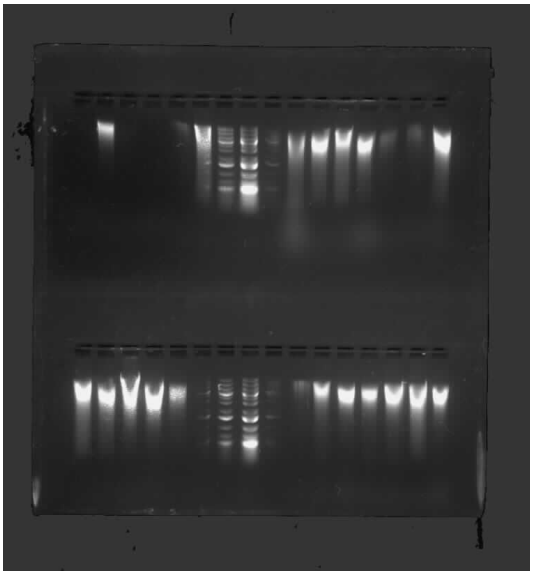
MK	Control	A	81	1.734	73.59
MK	Control	E	82	0.994	46.74
MK	Control	B/C	83	0.958	49.23

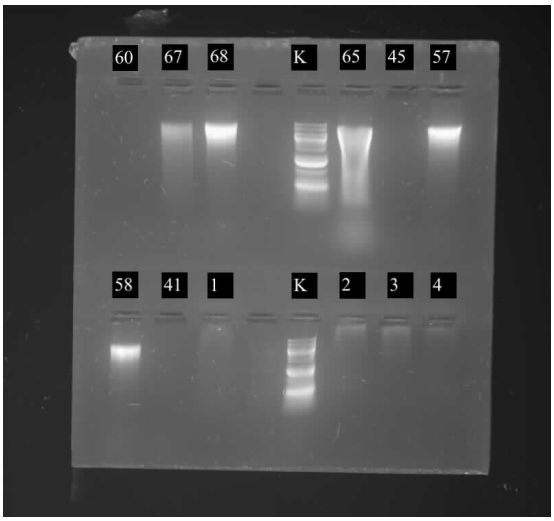
Appendix 4 – Soil DNA Electrophoresis snapshots

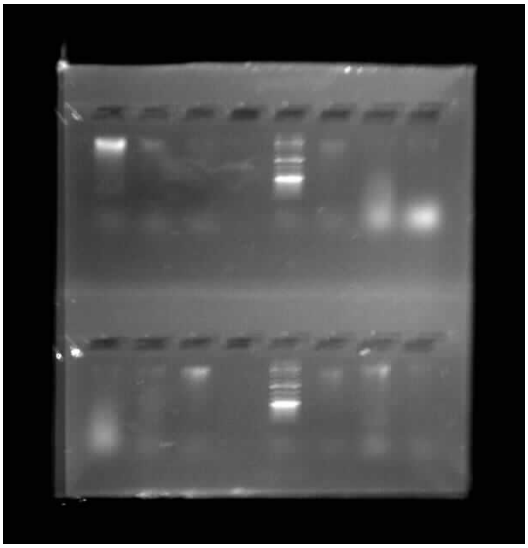
Photo 5-12 - Working with gel electrophoresis to determine DNA in the soil before PCR. As mentioned in results many samples are repeated due to poor or difficult extraction. Everywhere 2 µl of DNA with 2 µl of marker and 5 µl control ladder.








60
67
68
K
65
45
57
58
41
1
K
2
3
4



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