

Department of Geography, University of Zurich

GEO 511 Master's Thesis

Fragmentation of Charcoal in Soil: Lab and Field Studies

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Abstract

Charcoal is part of the pyrogenic carbon (PyC) continuum that is defined by a range of pyrogenic organic material (PyOM). PyC is considered one of the most abundant organic compounds in the soil and recent estimates show that PyC constitutes on average more than 13% of the total of soil organic carbon (SOC). Due to its aromatic structure, PyC is suggested to have a high recalcitrance to microbial and chemical decomposition and is consequently associated with a high mean residence time in the soil. Generally, this awards PyC a crucial role for potential C sequestration mechanisms and strategies. So far, most of the research concerning PyC has been focusing on methods that discuss the biological and chemical interaction and decomposition of PyC in the soil system. Much less is known about the physical processes that influence the degradation and mobilization of charcoal in the environment. The predominant process among those physical interactions is charcoal fragmentation. In this study, we designed a series of manipulative experiments to stimulate the fragmentation of charcoal, in a set of laboratory and field experiments. We found that fragmentation happened in all our samples independent of substrate material or treatment applied. Among laboratory treatments, crushing the charcoal led to the smallest mean weighted diameter (MWD) ranging between 0.64-1.81mm. The effects of shaking (MWD: 1.42-2.50mm) and freeze/thawing (MWD: 1.12-2.16mm) treatments were less prominent. The feedstock material showed to be the most important parameter explaining differences in the fragmentation patterns. Further, the differences in fragmentation between the laboratory and field experiments depended on the feedstock material; we observed that grass derived charcoal is much more susceptible to fragmentation under field conditions than wood derived charcoal. We suggest that the visual disappearance of grass derived charcoal in our field experiment could be an indication for its stabilization by forming micro-aggregates with the mineral phase of the soil. Therefore, it appears that fragmentation is an important physical process that needs to be investigated to generate knowledge about the persistence of charcoal in the environment.

Table of Contents

Abstract	I
Table of Contents	1
List of Figures	IV
List of Tables	V
1. Introduction	1
1.1. What is Charcoal?.....	2
1.1. Charcoal degradation versus mobilization	3
1.2. The Role of Fragmentation.....	4
1.3 State of Research.....	5
1.4. Research Objectives	6
1.5. Methodological Approach	7
2. Materials and Methods	8
2.1. Sample Material	8
2.2. Sample Preparation	9
2.2.1. Pre-Tests	9
2.2.2. Pyrolysis.....	9
2.3. Laboratory Experiments	10
2.3.1. Experimental Set-up	10
2.3.2. Shaking-Treatment	10
2.3.3. Crushing.....	11
2.3.4. Freeze/Thawing.....	12
2.3.4. Scanning	12
2.4. Field Experiments	14
2.4.1 Experimental Set-up	14
2.4.2. Extraction and Analysis.....	14
2.5. Data Evaluation and Transformation	16
3. Results	17
3.1. Pyrolysis Effect.....	17
3.2. Laboratory Experiments	20
3.2.1. by Substrate Material.....	20
3.2.2 by Treatment.....	20
3.3. Field Experiments	21
3.3.1. by Substrate Material	21
3.3.2. by Matrix	22

3.4. Comparison Lab-Field	22
3.5. Comparison of Methods.....	23
4. Discussion	26
4.1. Fragmentation due to Pyrolysis.....	26
4.2. Laboratory Experiments	29
4.2.1. Effects of used Substrate Material	29
4.2.2. Effects of Laboratory Treatments.....	30
4.3. Field Experiments	32
4.3.1. Effects of used Substrate Material	32
4.3.2. Effects of Matrices.....	33
4.4. Comparison Lab-Field	35
4.5. Limitations and Perspective	36
5. Conclusion.....	39
Acknowledgements	40
References	41
Supplementary Material.....	45
Personal Declaration	50

List of Figures

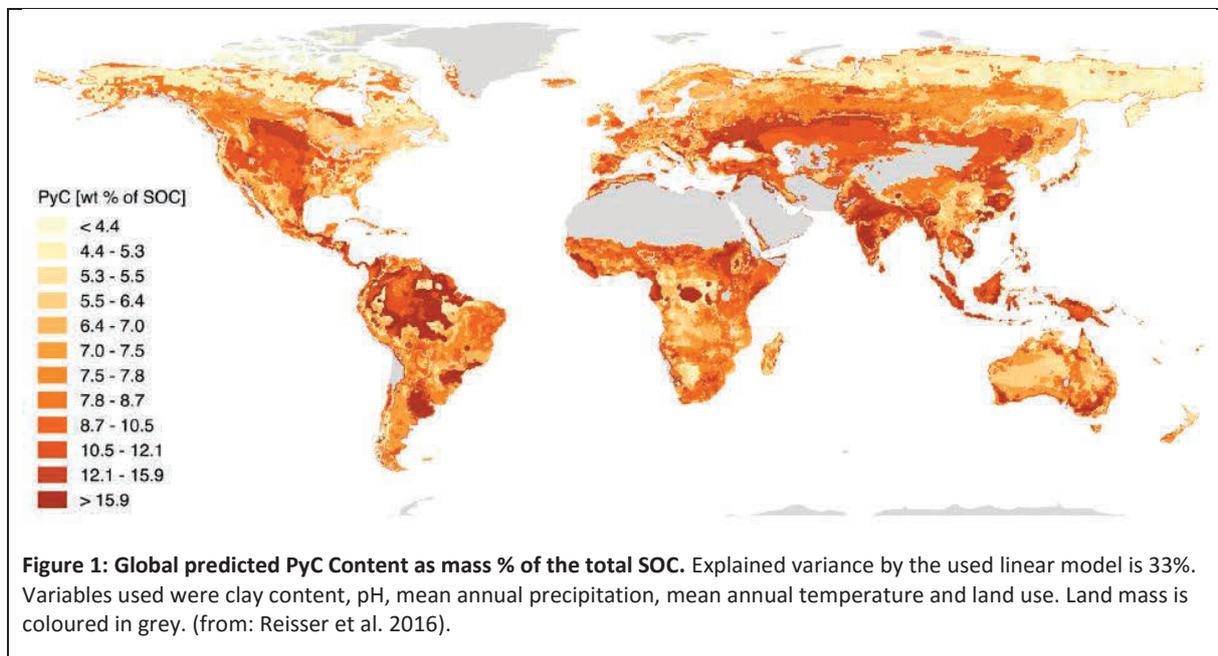
Figure 1: Global predicted PyC Content as mass % of the total SOC (from: Reisser et al., 2016).....	1
Figure 2: Global cycle of pyrogenic C (PyC) from vegetation fires. (from: Santin et al., 2016).....	3
Figure 3: Overview of the conducted experiments and analyses	7
Figure 4: Pyrolysis oven.....	9
Figure 5: Improvised crushing device.....	11
Figure 6: Scans of a grass charcoal sample	13
Figure 7: Field sample preparation	13
Figure 8: Field sample setup	15
Figure 9: Poplar sample after field extraction.....	15
Figure 10: MWD to substrate after pyrolysis	17
Figure 11: Particle size shift after pyrolysis.....	18
Figure 12: Scatterplot of the MWD versus the number of total particles per sample	19
Figure 13: Lab-MWD by substrate	20
Figure 14: Lab-MWD by treatment	21
Figure 15: Field-MWD by substrate	22
Figure 16: Field-MWD by treatment	23
Figure 17: Comparison of lab and field treatments.	24
Figure 18: Scatterplot of the MWD versus the recovery rate of field samples.	25
Figure 19: Scatterplot of the MWD versus the recovery rate after lab treatments..	25
Figure 20: Mechanical resistance of decayed and healthy wood charcoal (<i>Pinus sylvestris</i>). (from Théry-Parisot et al., 2010)	28
Figure 21: PyC Content in % as mass of the total SOC described by different land use types (from: Reisser et al., 2016)	37
Figure S1: Temperature profiles recorded by two TMS data loggers (T1 & T2).	47
Figure S2: Shift of particle size after shaking treatment.....	47
Figure S3: Shift of particle size after shaking treatment.....	48
Figure S4: Shift of particle size after shaking treatment.....	48
Figure S5: Plot of sinking pattern compared to substrate.....	49
Figure S6: Scatterplot of the percentage of sunken particles versus sinking time	49

List of Tables

Table 1: Overview of used substrates	8
Table 2: Composition of feedstock material. (Adapted from: Lohmann, 1993; Lee et al., 2007; Blume et al., 2010).....	8
Table 3: Number of particles before and after the pyrolysis treatment and the associated fragmentation factor	19
Table S1: Output of ANOVA and SNK-Test of lab dataset.....	45
Table S2: Output of ANOVA and SNK-Test of field dataset.....	46

1. Introduction

Climate change will most likely increase the frequency of wildfires in many parts of the world (Santin et al., 2016) which would also modify the input of pyrogenic carbon (PyC) into the environment and subsequently change the global carbon cycle (Lehmann et al., 2008; Singh et al., 2012). Due to its aromatic structure, PyC is suggested to have a high recalcitrance to microbial and chemical decomposition and is consequently associated with a high mean residence time in the soil (Kuzyakov et al., 2009; Major et al., 2010; Noncentini et al., 2010). Generally, this awards PyC a crucial role for potential C sequestration mechanisms and strategies (Lehmann et al., 2008; Kuzyakov et al., 2009; Major et al., 2010; Lehmann et al., 2015).



As reported by Reisser et al. (2016) PyC on average constitutes more than 13% of the total soil organic carbon (SOC). This makes PyC one of the largest identifiable compounds in the soil. Earlier studies reported PyC to account for a smaller portion (1-5%) of the total SOC but supported its spatial omnipresence in the environment (Preston and Schmidt, 2006). Based on a large literature database Reisser et al. (2016) created a global overview of the predicted PyC content as mass of the total SOC (**Figure 1**). The figure shows that PyC can be found almost ubiquitously in the environment.

A variety of studies exist that discuss the persistence of PyC in the soil system and the processes involved in its decomposition and mobilization (Dittmar et al., 2012b; Bird et al., 2015; Pignatello et al., 2015; Santin et al., 2016). Nevertheless, there is still a lack of information about those processes and hence it is difficult to estimate the residence time

of PyC in the soil system (Masiello et al., 2004; Schmidt et al., 2011). This is also reflected by the fact that PyC is yet to be considered in any policy-relevant climate mitigation strategy (Lehmann et al., 2008; Schmidt et al., 2011).

In addition to its importance for the global C cycle, PyC is also associated with a set of positive effects for the soil matrix. PyC is reported to be a powerful adsorbent of organic contaminants (Pignatello et al., 2015) which makes it a useful tool for remediation strategies in polluted soil. Further PyC has been reported to influence positively the nutrient availability and water holding capacity in agricultural soils (Pignatello et al., 2015). In general, physicochemical characteristics of PyC are complex (Bird et al., 2015) and the processes that lead to the chemical alteration of PyC in the soil have to be further investigated (Pignatello et al., 2015).

1.1. What is Charcoal?

Charcoal is the product of an incomplete combustion of organic matter (OM) during natural fire events or controlled pyrolysis (Braadbaart and Poole, 2008; Bird et al., 2015; Santin et al., 2016). The incomplete combustion is the result of anoxic conditions during the thermal degradation of organic material (Maestrini et al., 2015; Pignatello et al., 2015). Charcoal is part of the pyrogenic carbon (PyC) continuum that is defined by a range of pyrogenic organic material (PyOM) (Schmidt and Noack, 2000; Preston and Schmidt, 2006; Pignatello et al., 2015). These compounds include partially charred biomass, charcoal, soot and ultimately graphite (Hedges et al., 2000; Schmidt and Noack, 2000; Preston and Schmidt, 2006). Within this continuum charcoal is defined as visually detectable solid charred residues (Preston and Schmidt, 2006). What all compounds of the PyC continuum have in common is that their chemical structure is characterized by fused aromatic rings that subsequently lead to a high recalcitrance to biological and chemical decomposition (Preston and Schmidt, 2006). Another pyrogenic compound that is discussed in the literature is biochar. The main difference between charcoal and biochar is that the latter is intentionally produced and added as a soil amendment (Pignatello et al., 2015). According to Pignatello et al. (2015) the analogy between wildfire produced charcoal and biochar is limited to a certain extent. They share some chemical and physical features, such as aromatic structure, surface area and porosity. Further, the production conditions are comparable, with a limited oxygen supply and combustion temperatures ranging between 250 to 800°C. As the production of our samples was designed to simulate natural conditions, we will address our pyrogenic carbonaceous material (PCM) as 'charcoal' in the context of this thesis.

1.1. Charcoal degradation versus mobilization

When charcoal is incorporated into the soil matrix it is affected by a variety of biotic and abiotic processes (Cheng et al., 2006; Bird et al., 2015; Pignatello et al., 2015). In general, there exist two different pathways for charcoal in the environment (Santin et al., 2016). The first results in the in situ degradation or mineralization of the charcoal into the soil matrix (Major et al., 2010). The second leads to the mobilization of charcoal into other sinks (Bird et al., 2015; Santin et al., 2016). The study of Santin et al. (2016) provides an overview of the global PyC cycle and the relevant fluxes and pools (**Figure 2**).

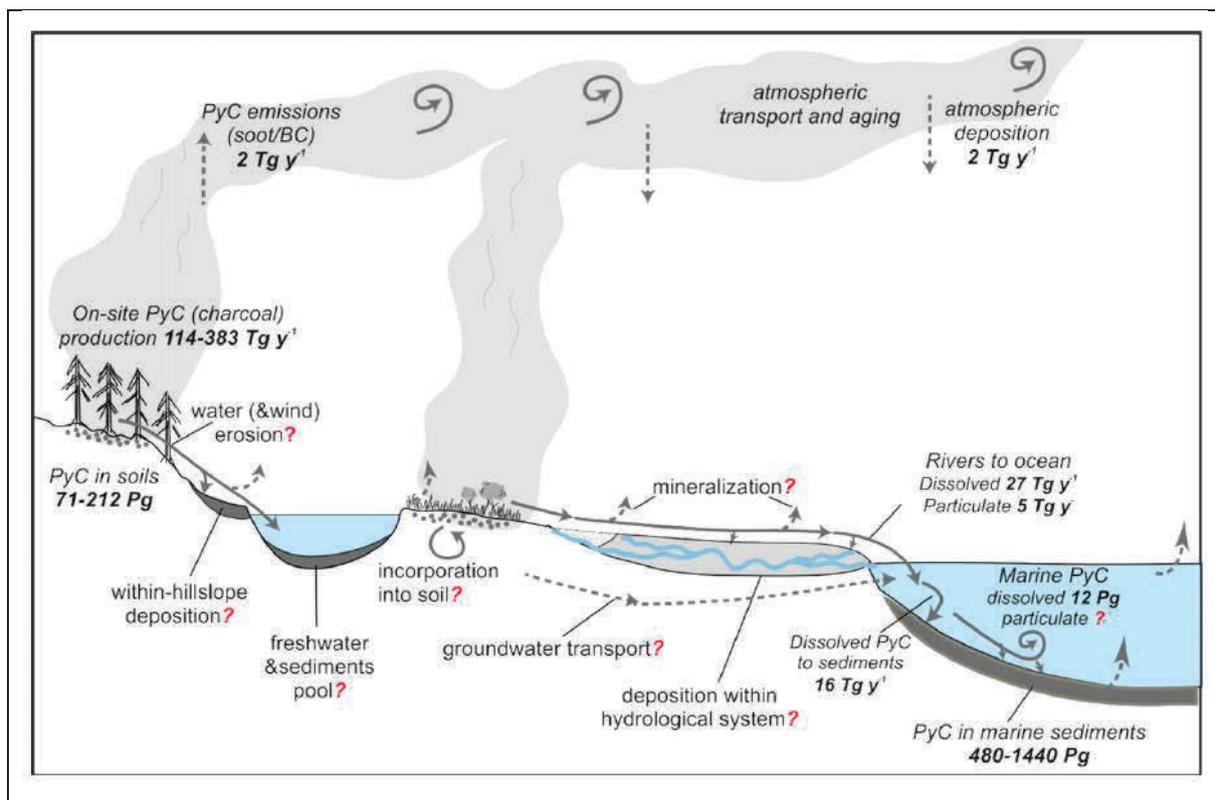


Figure 2: Global cycle of pyrogenic C (PyC) from vegetation fires. PyC production (in Tg C yr⁻¹) is divided in on-site (charcoal) and atmospheric (soot/BC) PyC. Fluxes between atmosphere, terrestrial and marine environments are given in Tg C yr⁻¹. Main PyC pools are given in Pg C. Main uncertainties and unknowns are represented by red question marks. Data derived from Schmidt and Noack, 2000; Hockaday et al., 2007; Elmquist et al., 2008; Dittmar and Paeng, 2009; Jaffé et al., 2013; Coppola et al., 2014; Scharlemann et al., 2014; Bird et al., 2015 and the GFED4 database. These estimates are based on data produced using different approaches which do not account for regional variability and may not distinguish between PyC from different sources. (from: Santin et al., 2016).

The in situ incorporation of charcoal into the soil profile is expected to be controlled by bioturbation, freeze/thaw cycles or gelifluction (Schmidt and Noack, 2000; Gavin, 2003; Preston and Schmidt, 2006). These processes cause vertical movement of PyC along the soil profile into deeper soil layers (**Figure 2**) and can contribute to its preservation but also to potential losses through groundwater transport (Dittmar et al., 2012b; Santin et al., 2016). Therefore, deep soil is considered an intermediate sink in the PyC sequestration

context. The storage capacity of intermediate PyC sinks and their associated fluxes need to be quantified in order to understand how much PyC is mineralized in situ and how much is moving in between the sinks (Jaffé et al., 2013; Santin et al., 2016). This kind of information would be especially valuable for some fire affected ecosystems where an apparent absence of visible char residues prevails (Pignatello et al., 2015).

In terms of sinks a major part of the carbon involved during a fire event is directly emitted as CO₂ into the atmosphere (**Figure 2**) (Druffel, 2004; Scott, 2010). Another important mobilization process is the solubilisation of PyC in water which transports the compounds horizontally above or below ground into river catchments (**Figure 2**) (Dittmar et al., 2012b; Jaffé et al., 2013). This process is accounted for being the main removal mechanism of PyC in soils (Dittmar et al., 2012a) and is estimated to affect 8-27% of annual PyC production (Santin et al., 2016). The fraction of soluble PyC was shown to increase with prolonged residence time in the soil (Abiven et al., 2011). Hydrologic transport of charcoal can also occur due to erosion of fire affected soil profiles (Rumpel et al., 2006; Czimeczik and Masiello, 2007). Marine sediments are considered to be the final PyC sink (**Figure 2**) (Masiello and Druffel, 1998; Masiello, 2004). Ultimately most riverine fluxes end in coastal areas and the anoxic conditions in marine sediments are ideal for the preservation of PyC. Mean residence times are estimated to be in the range of several thousand years (Masiello and Druffel, 1998).

As a result of the ambiguities between degradation and mobilization of charcoal the estimation of mean residence time covers a wide range from decades to millennia (Kuzyakov et al., 2014; Bird et al., 2015).

1.2. The Role of Fragmentation

Despite the fact that atmospheric and hydrologic transport play an important role in the mobilization of PyC, studies have estimated that the majority (over 80%) of the PyC gets incorporated into the soil (Preston and Schmidt, 2006). Physical processes affecting charcoal incorporation and thus persistence involve fragmentation into smaller particles (Théry-Parisot et al., 2010), heteroaggregation with mineral soil particles (Brodowski et al., 2006; Major et al., 2010), deposition of minerals and non-pyrogenic natural organic matter (NOM), and availability of charcoal surfaces and pores to solutes (Pignatello et al., 2015). Especially by forming stabilizing organo-mineral aggregates, the particles are much less susceptible to degradation (Brodowski et al., 2006; Vasilyeva et al., 2011) which in turn increases their persistence in the soil. According to the literature fragmentation, meaning the breakdown into smaller particles, plays a crucial role for these aggregation

processes (Major et al., 2010; Brewer et al., 2014; Pignatello et al., 2015). Studies have shown that smaller charcoal particles were shown to mineralize more rapidly than larger particles (Noncentini et al., 2010; Zimmerman, 2010). As suggested by Noncentini et al. (2010) the particle size and thus the rate of fragmentation plays an important role for the degradation of charcoal and therefore its persistence and residence time in the soil system. The fragmentation of charcoal is reported to happen quite soon after the input into soil (Pignatello et al., 2015) as increased residence time seems to have little effect on the fragmentation (De Lafontaine and Asselin, 2011; Pignatello et al., 2015). Nevertheless, the fragmentation of charcoal is difficult to study due to the wide range of particle sizes resulting from a fire event (Pignatello et al., 2015). This might be the reason why there only exist a handful of studies on charcoal fragmentation.

1.3. State of Research

Most of the research concerning PyC has been focused on methods that discuss the biological and chemical interaction and decomposition of PyC in the soil system (Santin et al., 2013; Brewer et al., 2014; Lehmann et al., 2015). Much less is known about the physical processes that influence the degradation and mobilization of charcoal in the environment (Spokas et al., 2014; Pignatello et al., 2015). The predominant process among those physical degradation processes is charcoal fragmentation. So far this topic has only been a main focus in the field of anthracology and very little direct evidence for fragmentation exists in the literature (Pignatello et al., 2015). Anthracologists are interested in reconstructing archaeological contexts on the basis of charcoal remains. The study of Théry-Parisot et al. (2010) discusses different levels of fragmentation regarding charcoal in the soil. The first level relates to the production properties of charcoal and its feedstock material. The second level discusses post-depositional conditions that affect fragmentation of charcoal.

The role of feedstock material is not yet well defined. Théry-Parisot et al. (2010) suggest, that the same fragmentation patterns could be observed regardless of the initial feedstock. However, this does not account for non-wood feedstock material that is often affected in wildfires (Hammes et al., 2006).

From a soil science perspective there is a lack of comparable studies that address charcoal fragmentation and its implication on the persistence of charcoal in the soil system (Santin et al., 2013; Pignatello et al., 2015).

1.4. Research Objectives

In this study, we designed a series of manipulative experiments to stimulate the fragmentation of charcoal. The main objective was to further elaborate the impact of fragmentation on degradation and mobilization processes of charcoal in the environment. By providing further information about these processes, we attempt to generate additional knowledge about the mean residence time of charcoal in the soil system. These kind of information are needed to elucidate uncertainties about the persistence of charcoal in those ecosystems that have been associated with a rapid disappearance of charcoal residues (Bird et al., 1999; Hammes et al., 2008a; Nguyen et al., 2008; Pignatello et al., 2015). Additional information might also benefit the development of carbon sequestration techniques involving PyC.

The study was divided in a set of laboratory and field experiments. The laboratory experiments addressed physical processes, that according to the literature, cause fragmentation in the charcoal. In a controlled environment the effects of mechanical stress, stress caused by solution in water and stress caused by freeze/thaw cycles were studied. In addition, a short-term (in relation to the discussed mean residence time of charcoal) field experiment was set up to study the effects under close to natural conditions. The experiments were designed to address the role of the feedstock material, the role of field conditions on charcoal stability and the fragmentation that results during the first week of soil incorporation.

In this thesis the following hypotheses were addressed:

- 1) Grass fragments more than wood substrates
- 2) Freeze/thawing cycles cause more fragmentation than physical or water stress based treatments
- 3) Field conditions induce more fragmentation in the charcoal samples than laboratory based treatments

1.5. Methodological Approach

This chapter gives a brief overview of the conducted experiments and analysis (**Figure 3**). A detailed description of the experimental set-ups and methods during this study can be found in **chapter 2**.

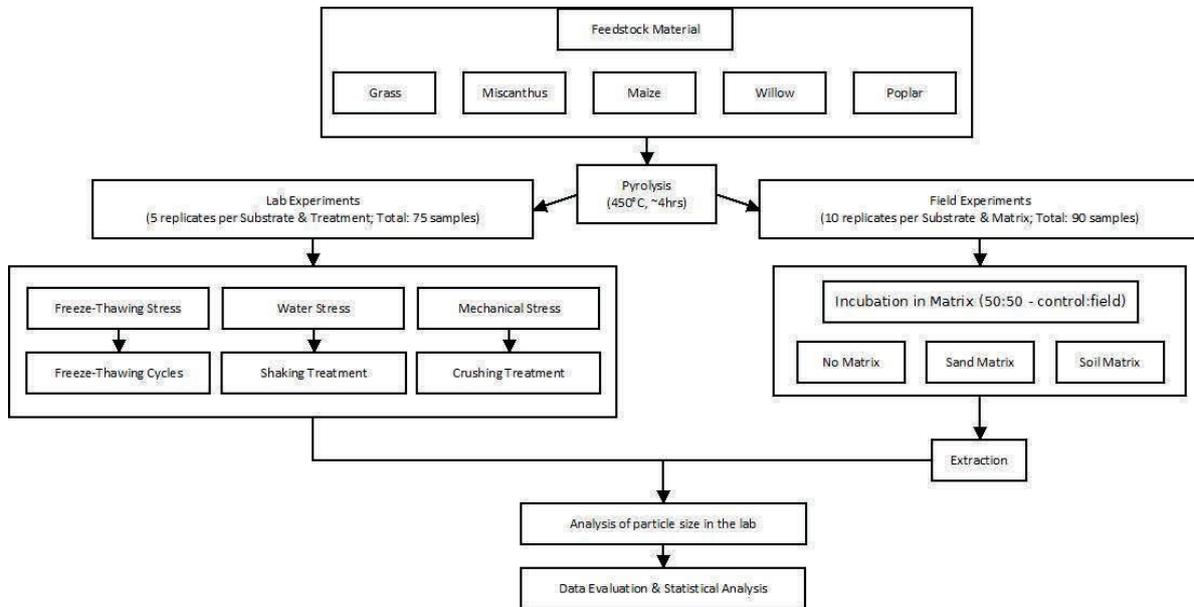


Figure 3: Overview of the conducted experiments and analyses

2. Materials and Methods

2.1. Sample Material

Most of the plant material used in this study was obtained prior to the start by members of the University of Zurich (UZH) and the Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft (WSL). An overview of the used materials is given in **Table 1**. We used two different types of grass. One set of grass samples was labelled with a $\delta^{13}\text{C}$ signature between 3000-5000 ‰. The other samples were collected in the Irchelpark (Zurich) and did not undergo any $\delta^{13}\text{C}$ labelling.

Table 1: Overview of used substrates.

Feedstock	Scientific name	Origin
$\delta^{13}\text{C}$ -labelled Grass	<i>undefined</i>	unknown
Grass	<i>undefined</i>	Irchelpark, Zurich
Miscanthus straw	<i>Miscanthus L.</i>	unknown
Poplar chips	<i>Populus L.</i>	Allmend, CH
Willow chips	<i>Salix L.</i>	Witzwil
Maize straw	<i>Zea mays L.</i>	unknown

In total, five charcoal types were chosen for the investigations in this study. Two types of grass ($\delta^{13}\text{C}$ -labelled and unlabelled; counted as one), miscanthus straw, poplar chips, willow chips and maize straw. **Table 2** shows the cellulose, hemicellulose and lignin contents of the different feedstocks.

Table 2: Composition of feedstock material. Mass percentages (in %) of cellulose, hemicellulose and lignin for the different substrates. (Adapted from: Lohmann, 1993; Lee et al., 2007; Blume et al., 2010)

Substrate	Cellulose	Hemicellulose	Lignin
Poplar	48.4	18.2	21.6
Willow	42.9	21.9	24.7
Miscanthus	43.0	24.0	19.0
Maize	38.0	26.0	19.0
Grass	19-26	16-23	4-6

2.2. Sample Preparation

2.2.1. Pre-Tests

A series of tests were conducted in order to decide on a few key parameters for the main field and laboratory studies. A set of unlabelled grass, poplar and miscanthus samples were combusted at different pyrolysis temperatures. The number of particles was counted before and after the pyrolysis to get an estimate of the fragmentation factor caused by the procedure. The results of these tests were later used to decide on the pyrolysis temperature and amount of feedstock material that was needed for the following experiments.

2.2.2. Pyrolysis

Pyrolysis is the thermochemical decomposition of organic material in the absence of oxygen (Maestrini et al., 2015). In general, the process produces gases, liquids, and results in solid residues that are richer in C content (also known as char).



Figure 4: Pyrolysis oven, with the quartz glass tube partially inserted. On the left is the hose that supplies the samples with N₂.

In total 165 samples consisting of five different substrates were pyrolysed for both the laboratory and field experiments.

Each sample was packaged in tin foil before being inserted into a quartz-glass tube. This tube holds two vents. One vent is used to supply the inside of the tube with a constant N₂ flow, which prevents the material from being completely combusted and results in the desired charring of the material instead. On the other side of the tube a small vent allows

the outflow of gas and pyrolysis oil. The pyrolysis temperature was set at 450°C and all samples were pyrolysed for approximately four hours. According to the literature (Hammes et al., 2006; Turney et al., 2006) this represents a typical temperature for natural fires.

For the field experiments 30 samples for each substrate group, consisting of ¹³C-labelled grass, miscanthus and poplar, were produced (total: 90 samples). The laboratory samples consisted of 15 replicates for each of the substrates (grass (unlabelled), miscanthus, willow, poplar and maize) resulting in a total of 75 samples.

After the pyrolysis the samples were packaged in weighing paper and sealed in airtight plastic bags to prevent the samples from humidification.

2.3. Laboratory Experiments

2.3.1. Experimental Set-up

In the lab experiments three different physical processes were investigated with five different types of charcoal. Per charcoal substrate five replicates were tested.

The first treatment consisted of placing the charcoal into water and putting them into a lab-shaker for 24 hours. The second treatment was to apply a mechanical stress to the charcoal by putting a weight and thus a certain amount of pressure on to the sample. The third treatment was an artificial freeze/thawing of the charcoal samples in a freezer. To simplify the terms of the three treatments, they will be addressed as ‘crushing’, ‘shaking’ and ‘freeze/thawing’ treatments from this point on.

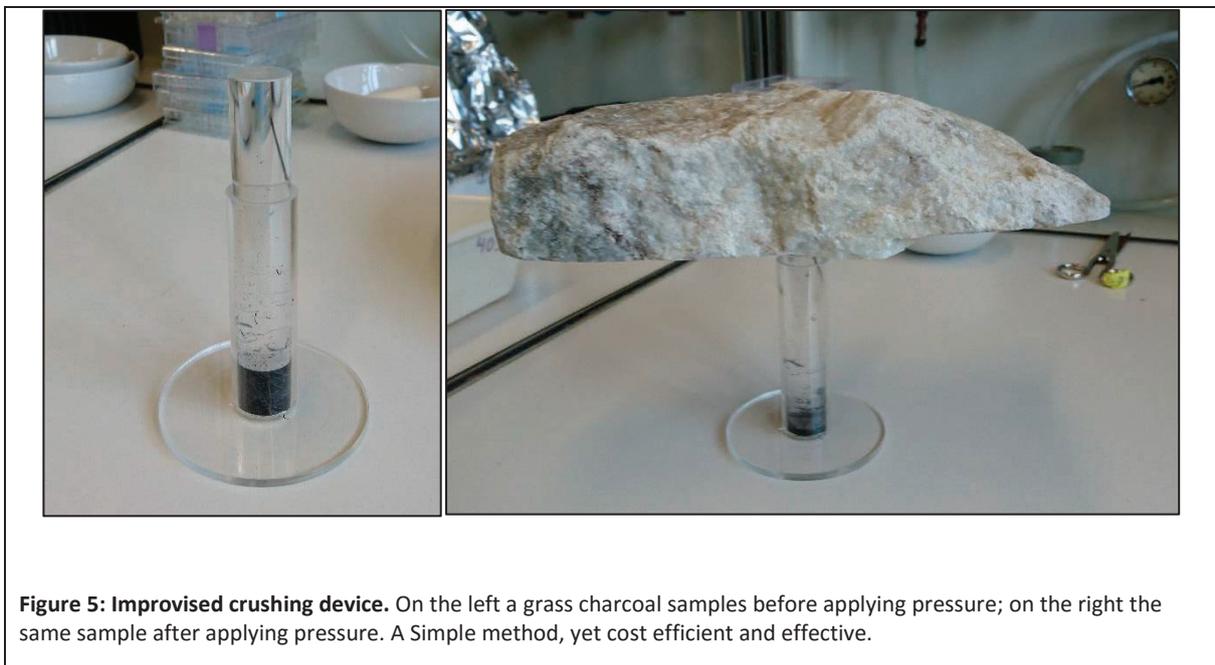
2.3.2. Shaking-Treatment

For the shaking treatment the samples were put into 100ml Erlenmeyer flasks. Rainwater, that was collected in Uster (Switzerland), was then added to the samples in a relation of 1:25. The flasks were then put in a reciprocating shaker at 300 cycles min⁻¹ for 24 hours. This method was adapted from the work of Spokas et al. (2014). In our study we wanted to produce more stress on the charcoal samples and thus increased the number of cycles in the reciprocating shaker compared to 60 cycles min⁻¹ in Spokas et al. (2014).

After the agitation the solution was filtered on a 30 µm SEFAR PET mesh. The samples were dried at 104°C for two days. The dried samples were then dry-sieved based on a sieve-diameter distribution of 3.15/2.0/1.0/0.5/0.25/0.125/0.063mm. After the sieving each fraction got weighed and sealed in a plastic bag to prevent humidification.

2.3.3. Crushing

For this experiment the charcoal samples were fragmented by the application of pressure. The calculation of the needed pressure was done with the web application Terranimo®. This web-tool was designed by the Hochschule für Agrar, Forst- und Lebensmittelwissenschaften (HAFL) in Bern. It is used to calculate the risk of soil compaction and aggregate destruction during tillage by incorporating factors like structural load, soil moisture and clay content. For our purpose a rather dry soil setup was chosen with a low clay content. For the structural load the proposed default settings were chosen. The critical pressure that was needed for a compaction in the created setting was set at 0.89 bar or 8.9 N/cm². As we did not have a hydraulic press to apply pressure in our laboratories, a weight was used to induce pressure on the charcoal samples. Therefore, the samples were put into a polyethylene tube of 1.1cm radius and 7cm height. On top we placed a polyethylene rod to more or less seal the tube. The equivalent of 89 kPa (bar) pressure is 0.9075kg/cm². The tubing had a square area of 3.8cm² which meant that 3.45kg were needed to achieve the desired amount of pressure. In the end, we used a 3.47kg heavy block of marble with a flat underside that was placed on top of the polyethylene rod to crush the samples.



After the application of pressure, the samples were extracted with a brush and sieved dry-sieved based on a sieve-diameter distribution of 3.15/2.0/1.0/0.5/0.25/0.125/0.063mm. After the sieving each fraction got weighed and sealed in a plastic bag to prevent humidification.

2.3.4. Freeze/Thawing

The charcoal samples underwent a treatment prior to being placed into freeze/thawing cycles. The idea was to saturate the hydrophobic charcoal particles in water for several days. Per substrate five charcoal samples were placed in 50ml polyethylene flasks with a height of 10cm. To simulate field conditions rainwater was used as solution in place of distilled water. The flasks were then sealed in order to prevent evaporation. To monitor the process a camera with a recording device was set up to track the time and the number of charcoal particles that would sink during the treatment. The data was later evaluated and profiles for the sinking behaviour of the substrates were created (Supplementary Material: **Figure S5 & S6**).

After this pre-treatment, the charcoal samples were filtered on a 30 μm SEFAR PET mesh. The samples were then transferred into glass flasks and 1ml of rainwater was added to each sample. The charcoal samples were then put into freeze/thaw cycles in which they were frozen at $-20 \pm 1^\circ\text{C}$ for approximately 16 hours and thawed at room temperature $25 \pm 0^\circ\text{C}$ for 8 hours. Hence, each freeze/thaw cycle took 24 hours. Per substrate five replicates were treated by 14 of these freeze/thaw cycles. The samples were then extracted and filtered on a 30 μm SEFAR PET mesh. They were transferred into paper bags and dried at 60°C for 5 days and at 104°C for another 24 hours. Next, the samples were sieved based on a sieve-diameter distribution of 3.15/2.0/1.0/0.5/0.25/0.125/0.063mm. After the sieving each fraction got weighed and sealed in a plastic bag to prevent humidification.

2.3.4. Scanning

One third of the lab samples was used for a counting experiment. Before and after the pyrolysis each fraction was placed in a show bag and scanned. The aim was to calculate the number of fragments for each fraction.

Data elaboration was done with the software ImageJ (Version 1.51f). For each scan a background subtraction with a pixel radius of 50 was performed to remove noise. Further the images were binary transformed to apply a threshold value for the charcoal particles. For the image processing an automated Otsu threshold procedure was chosen as this method provides a minimum of intra-class variance while maximizing the inter-class variance. To separate overlapping particles a watershed function was applied before counting the number of particles (**Figure 6**). With this method the number of particles before and after the pyrolysis treatment could be compared.

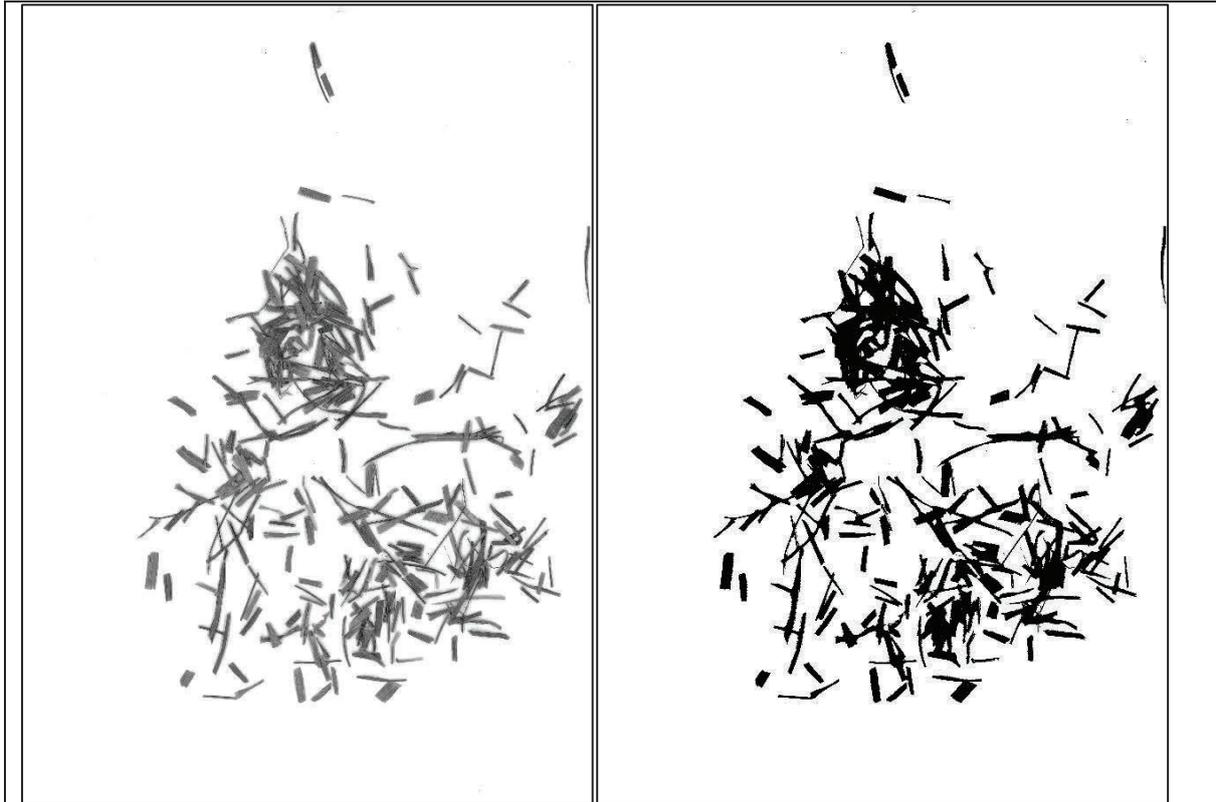


Figure 6: Scan of a grass charcoal sample. The left picture shows the initial scan without any editing; the right picture shows the same sample after the completed binary transformation.



Figure 7: Field sample preparation. The charcoal samples were inserted 2cm below the top of the cylinders. On the left are the samples with a sand matrix; on the right the samples with a soil matrix.

2.4. Field Experiments

2.4.1 Experimental Set-up

The field study was conducted over a time frame of six months (14.01.2016 until 27.06.2016). The time period was chosen to integrate cold weather conditions during winter months and also warmer conditions during spring and early-summer. The total of 90 charcoal samples was divided by substrate (30 each) and were placed into 3 different matrices. The matrices were divided into: (1) no-matrix, (2) sand and (3) soil. The sand was bought from Carlo Bernasconi AG and had a diameter between 1.00-1.70mm. The soil that was used in this study was a loamy soil that was originally extracted from an agricultural soil near Payerne (CH). The samples without a matrix were placed in small empty clay plots. The samples with a sand or soil matrix were inserted into soil cylinders. The charcoal samples were sieved by hand to a diameter >2.0mm. Each sample consisted of 300mg of charcoal. Per matrix 10 replicates of the three substrates (1) grass (2) miscanthus and (3) poplar were inserted into the clay pots or cylinders. In case of the sand or soil matrix the charcoal samples were inserted 2cm below the surface and covered with the respective matrix material (**Figure 7**). In order to retain the smallest charcoal fraction of interest (particles with a 63 μ m diameter) yet still guarantee runoff of residual water, the bottom of each cylinder was covered with a 30 μ m SEFAR mesh. Further two TMS data loggers were inserted into the cylinders with the soil matrix to track the temperature and moisture content of the samples during the time of incubation (Supplementary Material: **Figure S1**). Half of the samples were used as control samples and got stored inside the laboratory building at room temperature. The other half was used as field samples and therefore placed outside on the roof of the Y25 building at University Irchel (**Figure 8**).

While the field samples were exposed to the natural weather conditions, the control samples were watered with the same amount of rainwater that the field samples were exposed. The main difference between the two groups was the temperature regime.

2.4.2. Extraction and Analysis

Prior to the extraction the soil cylinders and clay pots were stored indoors at room temperature to let them dry. In order to extract the charcoal samples from the cylinders the whole samples were emptied out into ceramic bowls. Larger soil aggregates were broken down by hand. Afterwards deionized water was added to the bowls in order to start a flotation.



Figure 8: Field samples setup. The samples were placed on the roof the Y25 building of the University Irchel (UZH)

Depending on the substrate and type it would take several minutes to hours until the desired level of separation between charcoal and soil particles had happened. Thanks to the flotation the charcoal particles could then be separated from the soil matrix by pouring out the water with the floating charcoal particles. Afterwards, any residual free organic matter was removed by hand. The samples were then sieved with cellulose filters to separate the charcoal from the remaining water. The samples were put into paper bags and dried in at 60° for five days, and another 24 hours at 104°C.



Figure 9: Poplar sample after field extraction. The picture shows the whole sample, while the picture in the upper right corner shows some of particles of the 2mm fraction, with a coating of mineral particles.

After the extraction the particles incubated in a soil matrix showed a visible coating with soil particles. Therefore, they were rinsed three times with distilled water and dried for another 24 hours at 104°C. Even after these three rinsing procedures many of the charcoal particles were still coated in soil particles (**Figure 9**).

All the samples were then sieved based on a sieve-diameter distribution of 3.15/2.0/1.0/0.5/0.25/0.125/0.063mm. After the sieving each fraction got weighed and sealed in a plastic bag to prevent humidification.

2.5. Data Evaluation and Transformation

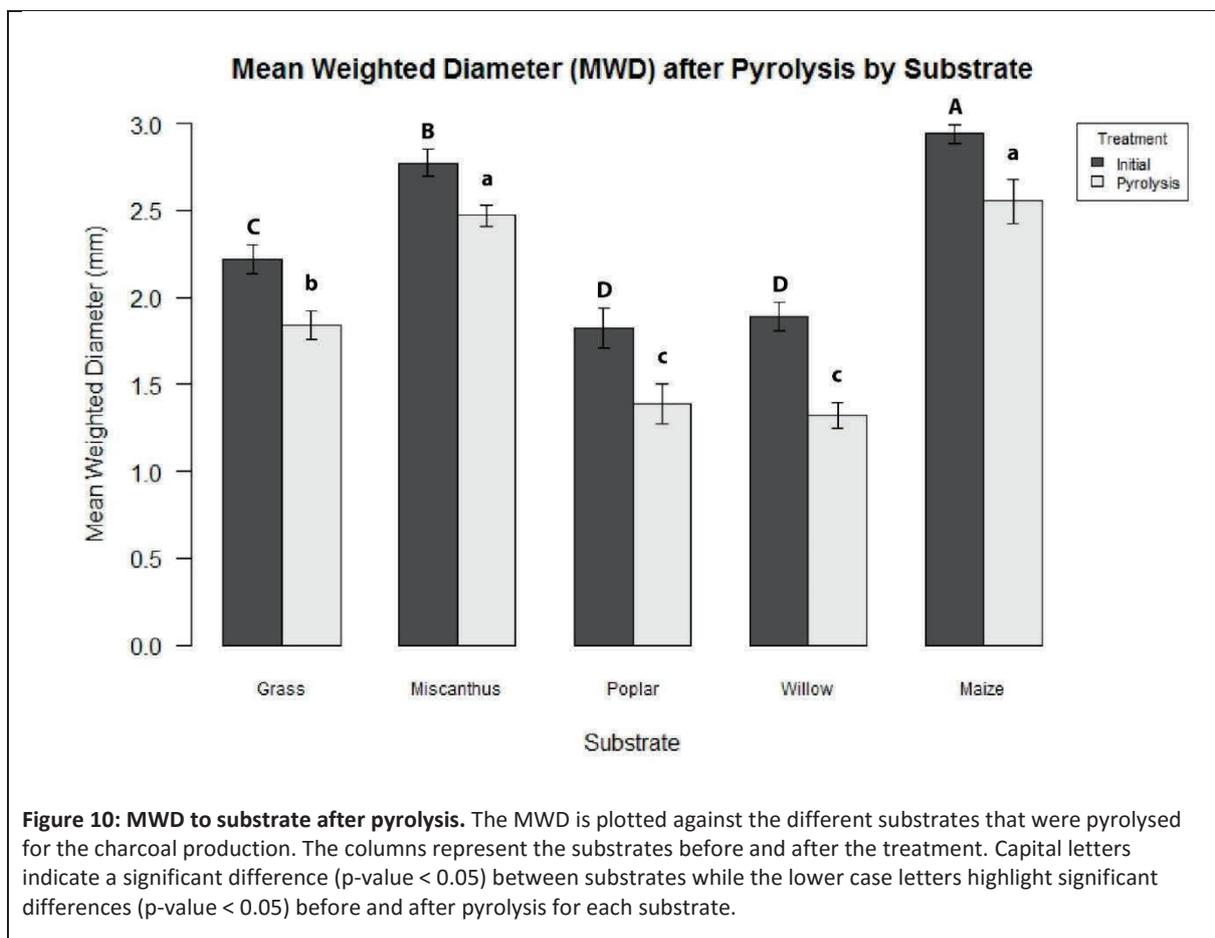
After each step of degradation in the lab and field investigations we recorded the absolute mass of all fractions within one sample. Based on these measurements we could then calculate the mass percentage of each particle size fraction. In combination with the absolute mass of each fraction we then calculated the mean weighted diameter (MWD) for each sample. The MWD values could then be used to compare the differences between the substrates and treatments.

For the statistical analysis the data of the field experiments was exponentially transformed to conform them to normality. The data distribution of the lab experiments was already approximately normal. The MWD values were then tested with One-Way ANOVAs using RStudio® (Version 0.99.451). As a post-hoc test we used the Student-Newman-Keuls method to identify the sample means that are significantly different. A detailed listing of the statistical outputs can be found in the Supplementary Material (**Table S1 & S2**).

3. Results

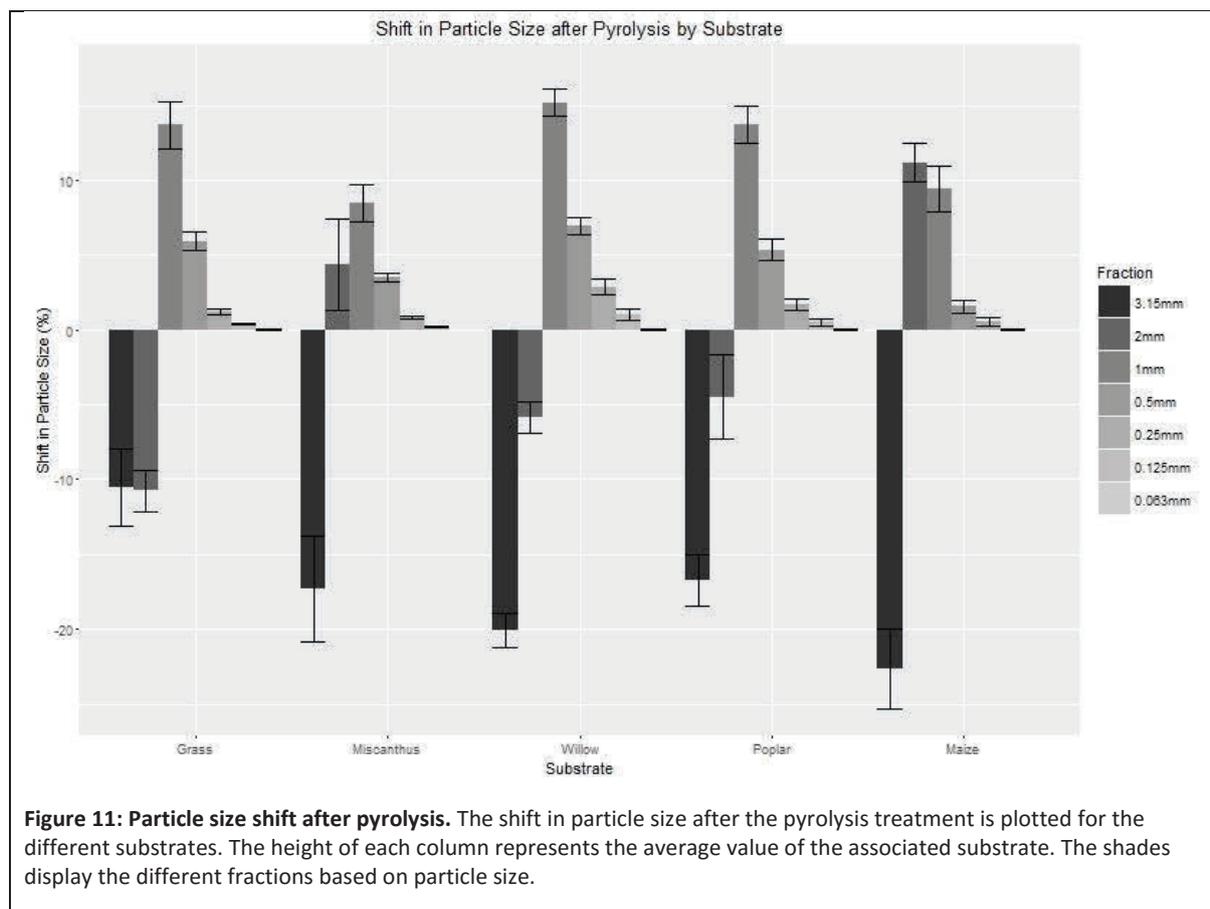
3.1. Pyrolysis Effect

Figure 10 shows the mean weighted diameter (MWD) for the initial and pyrolysis treated samples ordered by their substrate. The MWD after pyrolysis for all the samples ranges between 0.60-3.09mm. When looking at the substrates the smallest MWD values were measured in the poplar (1.39mm) and willow (1.32mm) samples. Maize (2.55mm) and miscanthus (2.47mm) showed the highest MWD values. Grass ranges in between these two groups with an average MWD of (1.84mm).



The pyrolysis did not only reduce the MWD of the samples but also caused various shifts in the different fractions of the samples (**Figure 11**). The 3.15mm and 2mm fraction experienced a decrease in their respective mass percentage while fractions equal or smaller than 1mm received an increase in mass percentage. Maize samples showed the largest decrease in their 3.15mm fraction with a negative shift of -22.7%. The smallest decrease was measured in the grass samples, where the 3.15mm fraction was only reduced by -10.5%. For the 2mm fraction, grass showed a decrease of -10.7%, while poplar (-4.51%)

and willow (-5.86%) showed decreases as well. For miscanthus and maize the 2mm fraction actually showed increases of 4.34% for miscanthus and 11.17% for maize.



These first results after the pyrolysis show a trend for a decrease of MWD and thus a relative increase of smaller fractions. The number of particles is another indicator that is presented in **Figure 12** that shows a similar trend. After the pyrolysis the number of particles was generally increased. The grass samples showed the highest number of particles for their post-treatment samples ranging from 775-1511 particles. The MWD values for the grass and the two wood substrates are below 2mm and are similar to each other. The samples made from miscanthus and maize substrate showed fewer numbers of particles (489-848) and higher MWD values than the wood and grass samples.

Table 3 shows the average number of particles per substrate group before and after the pyrolysis treatment and the resulting fragmentation factor. Grass shows a fragmentation factor of 2.4 induced by the pyrolysis. Therefore, the post-pyrolysis samples of grass have more than twice the number of particles than before the pyrolysis. The fragmentation factor of the other substrates ranges between 1.1-1.4.

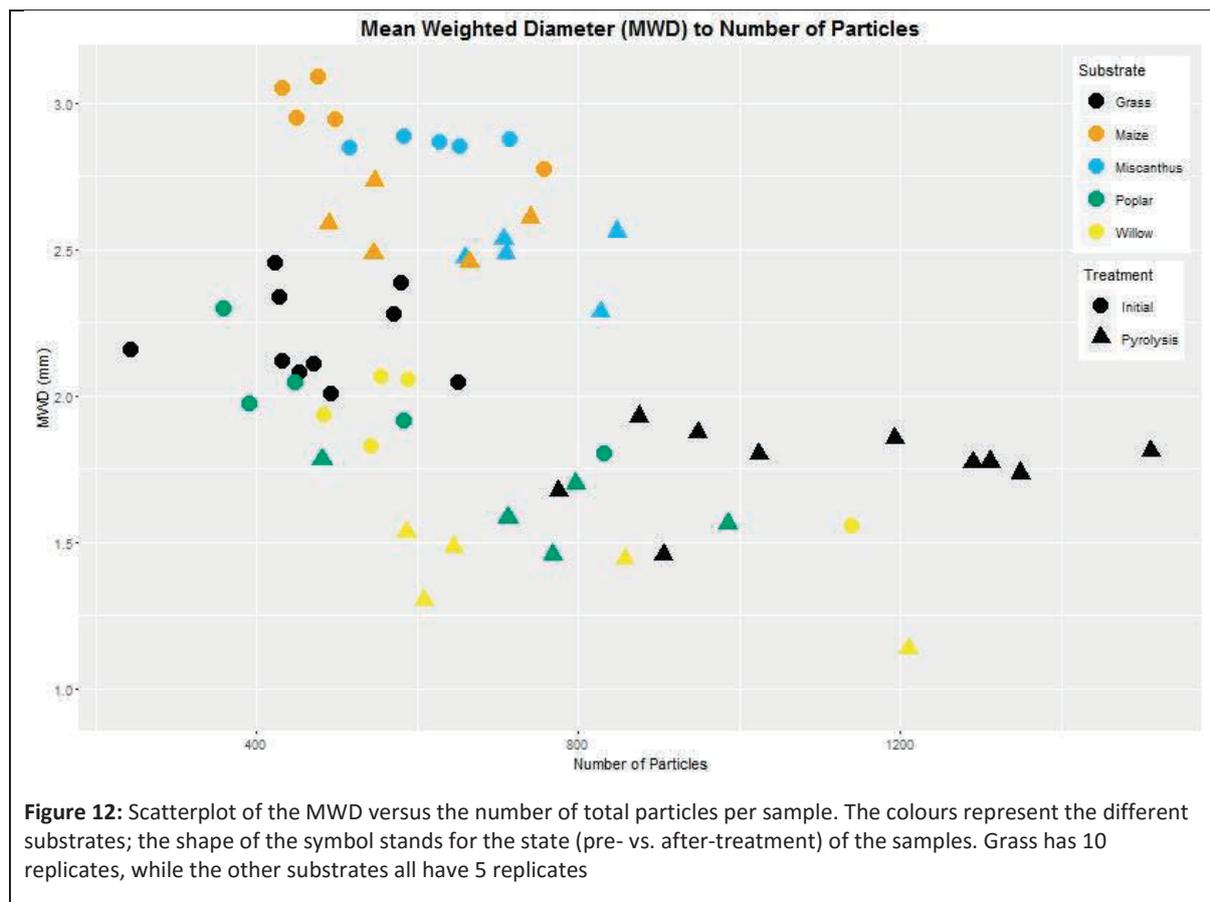


Table 3: Number of particles before and after the pyrolysis treatment and the associated fragmentation factor. The number represent the average of each substrate group.

Substrate	Initial #Particles	#Particles after Pyrolysis	Fragmentation Factor
Grass	474	1118	2.4
Miscanthus	617	750	1.2
Willow	661	781	1.2
Poplar	522	748	1.4
Maize	522	597	1.1

3.2. Laboratory Experiments

In this chapter the results of the different lab treatments are presented. These treatments involve fragmentation induced by shaking, crushing and freeze/thawing of the samples.

3.2.1. by Substrate Material

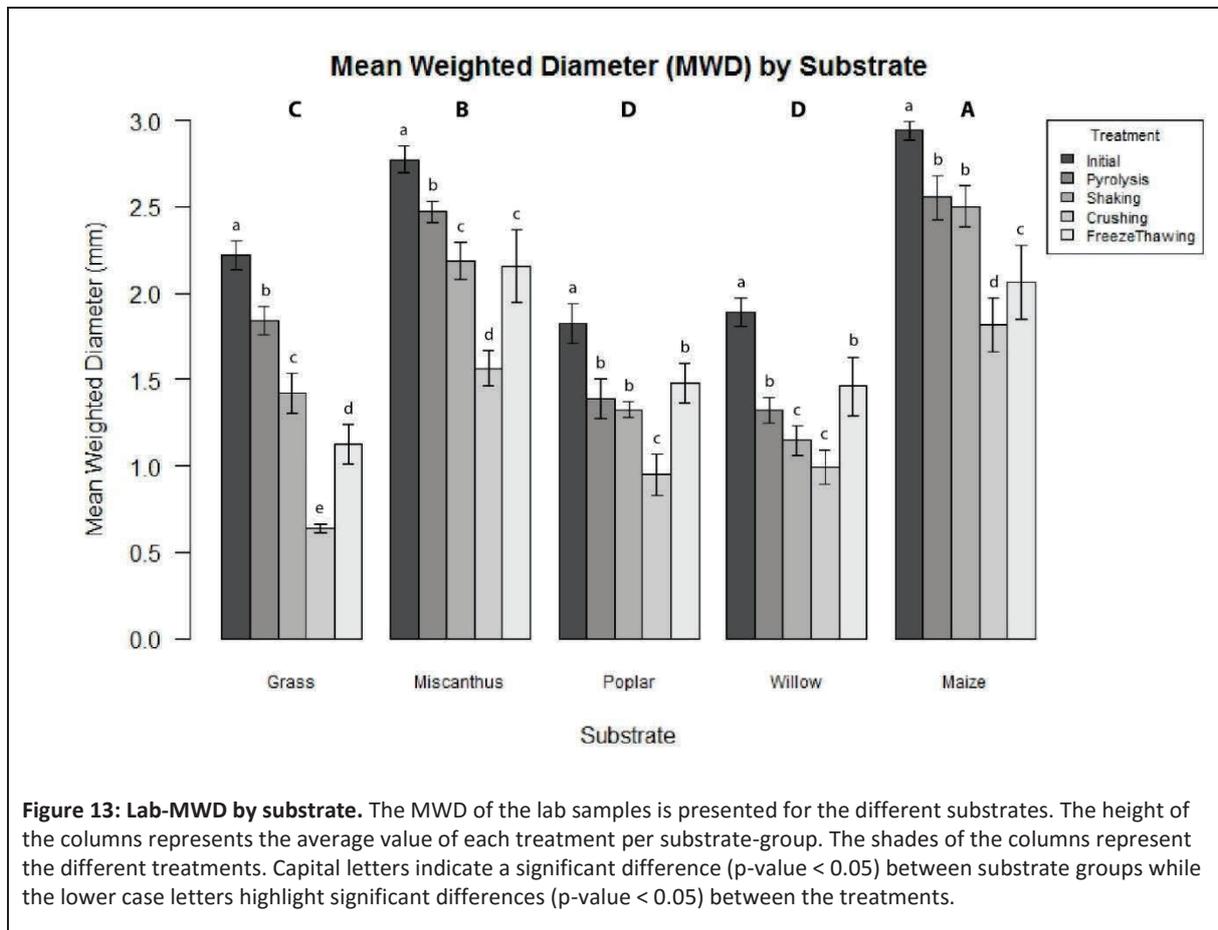
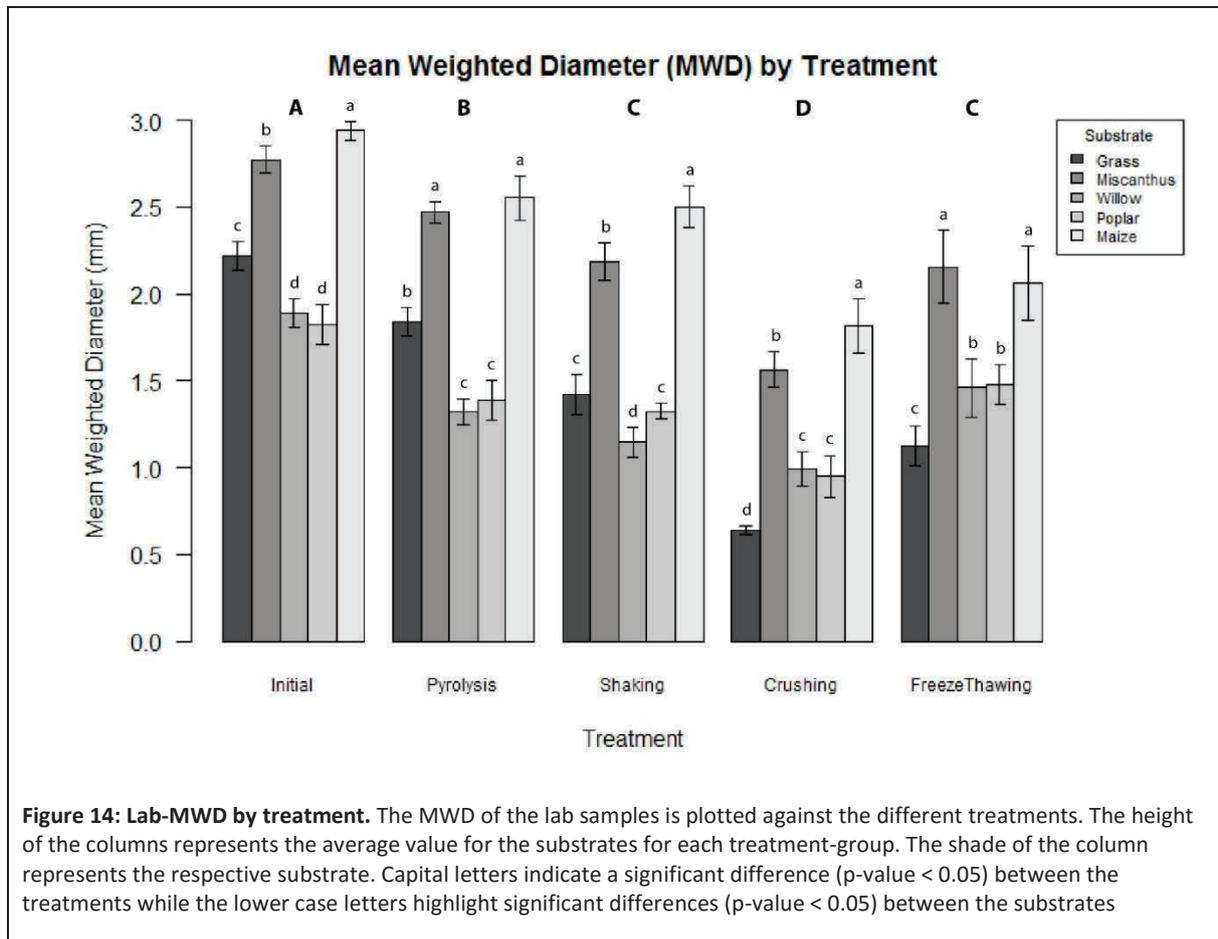


Figure 13 shows the MWD values for the different lab treatments and substrates. With the exception of the two wood substrates, poplar and willow, all the substrates are significantly different from each other ($p < 0.05$). Across all the treatments the highest values were observed in the maize samples, ranging from 1.81-2.94mm. The lowest values were measured in the poplar samples, ranging from 0.94-1.83mm.

3.2.2 by Treatment

The lab treatments decreased the MWD values in all substrates. **Figure 14** shows the MWD values compared to the different lab treatments. The lowest values were measured after the crushing-treatment resulting in an average MWD of 0.64mm for the grass samples and ranging to an MWD of 1.81mm for the maize samples. The crushing

treatment (σ MWD: 0.64-1.81mm) was the only treatment that was significantly ($p < 0.05$) different from the other lab-treatments; shaking (σ MWD: 1.42-2.50mm) and freeze/thawing (σ MWD: 1.12-2.16mm).

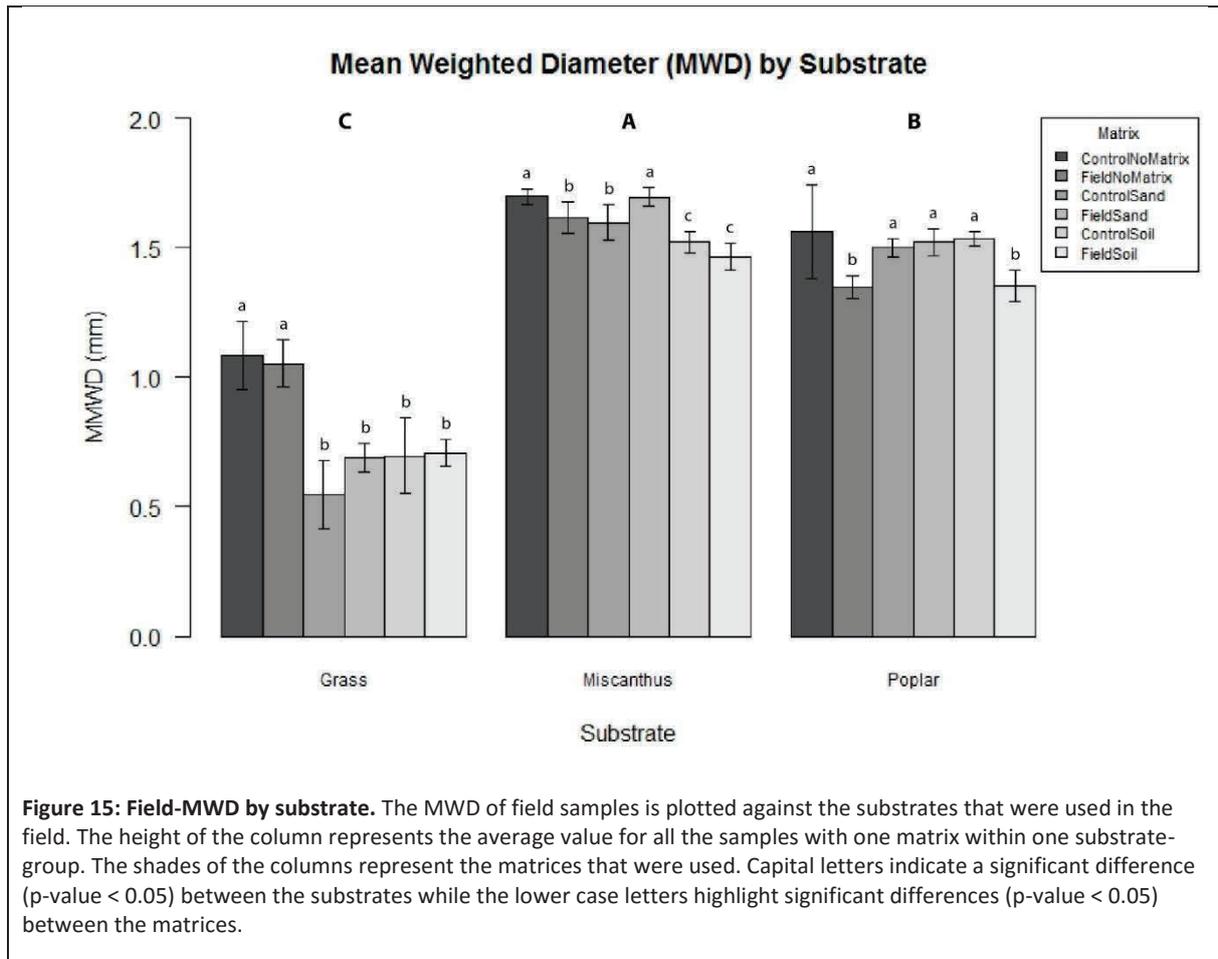


3.3. Field Experiments

In this chapter the results of the field trials are presented and described. The field trials included a total of 90 samples divided into nine sub-categories divided by substrate and matrix.

3.3.1. by Substrate Material

Figure 15 shows the MWD values compared to the different substrates used in the field. Overall, the grass samples show the smallest MWD values for all the different matrices with an average value of 0.79mm across the different treatments. These measurements are significantly ($p < 0.05$) different from the two other substrates. The measurements of the MWD in grass charcoal shows a higher variability than in the other two substrates. The average MWD for poplar samples is 1.47mm and 1.59mm for the miscanthus.

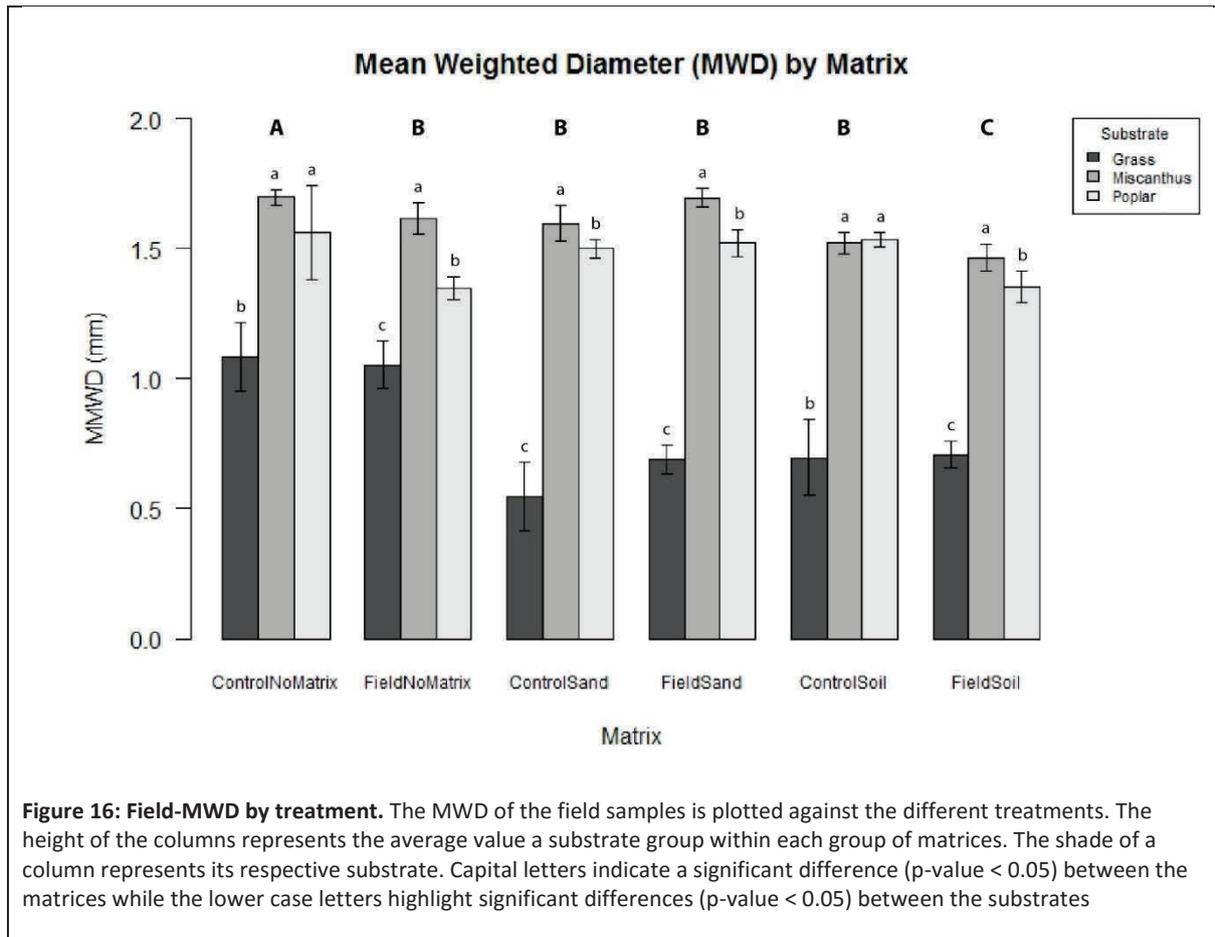


3.3.2. by Matrix

Figure 16 presents the MWD values for the field samples compared to the matrix they were incubated in. The highest values were obtained from the control samples without a soil matrix (MWD = 1.08-1.69mm). These values are significantly higher ($p < 0.05$) than for the other treatment groups. The smallest MWD values were measured in the field samples with a soil matrix (MWD = 0.70-1.46mm) and were significantly lower than the measurements of the other treatments ($p < 0.05$).

3.4. Comparison Lab-Field

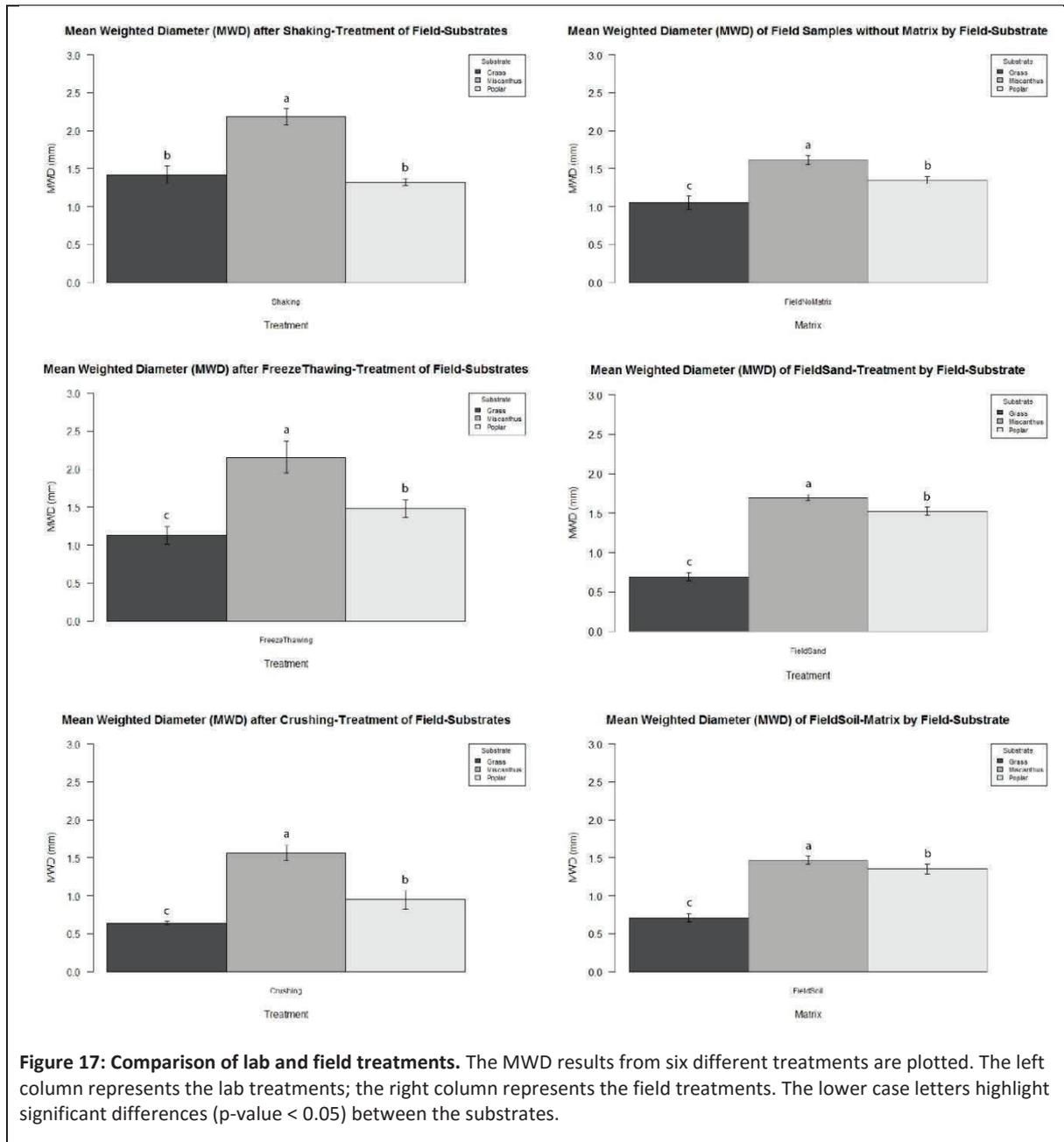
Figure 17 shows a comparison between lab and field treatments. The lab treatments should basically be comparable to the field treatments without a soil matrix (top-right). The MWD values after the freeze/thawing treatment (grass = 1.13mm, miscanthus = 2.16mm, pop = 1.48mm) in the lab resemble the field treatment without a matrix (grass = 1.05mm, miscanthus = 1.62mm, pop = 1.35mm) the most.



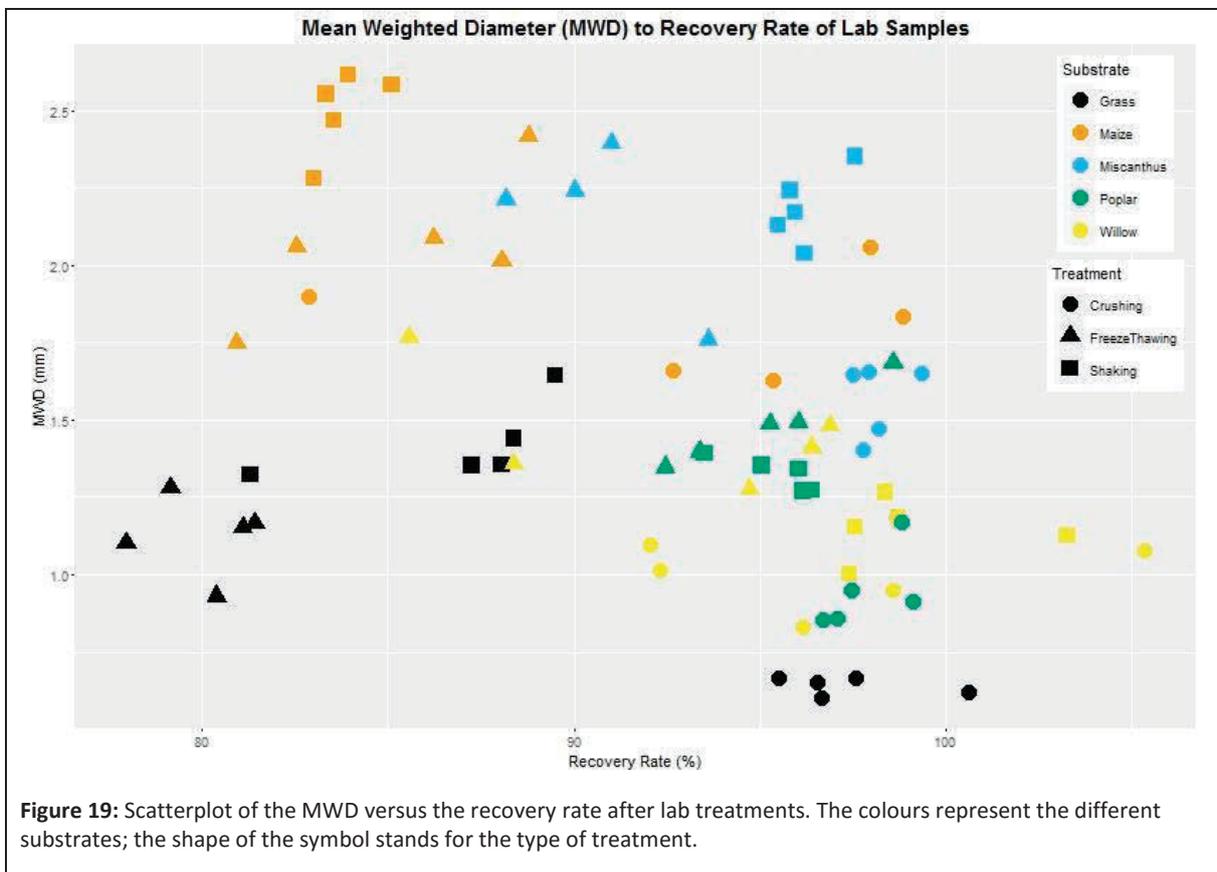
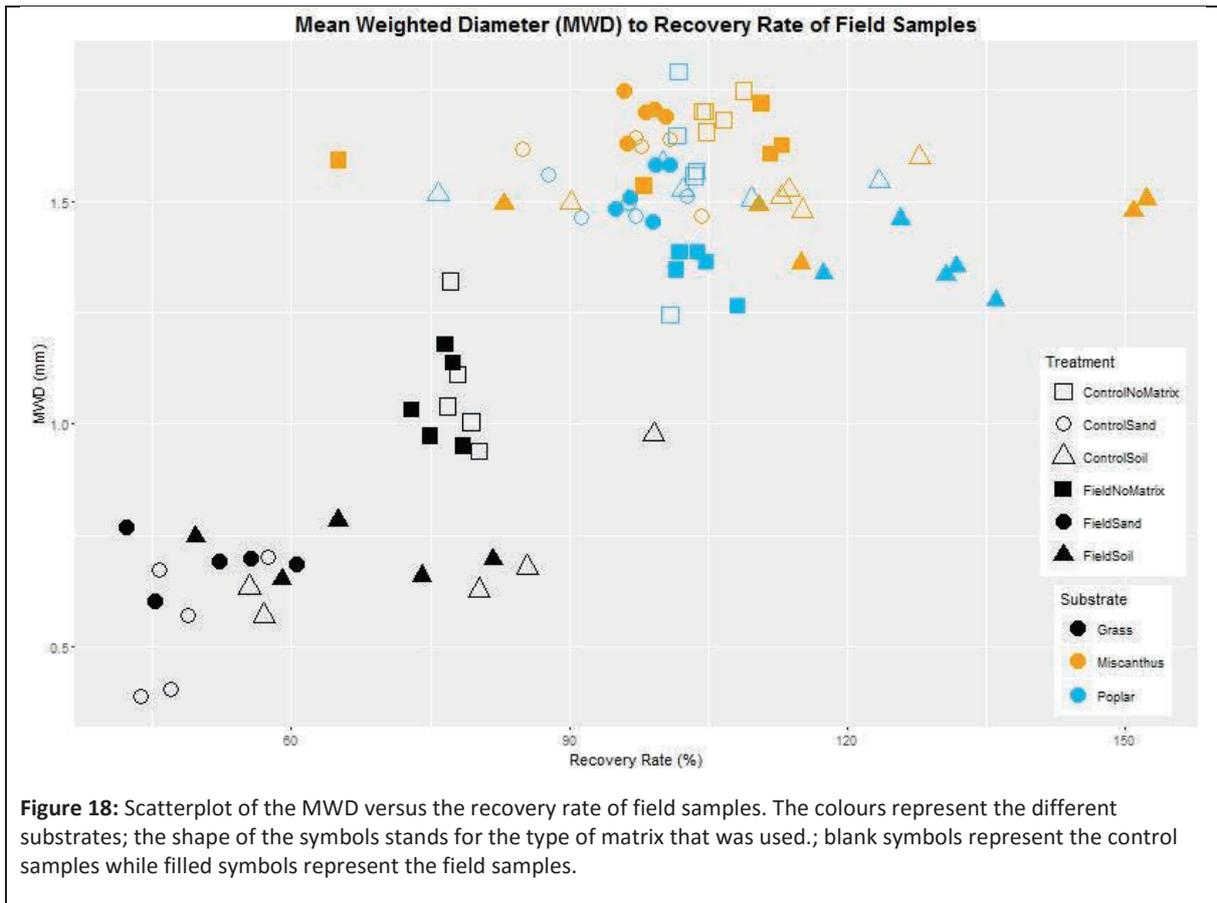
3.5. Comparison of Methods

This chapter presents the results that have a supporting function for the results of the different lab treatments and/or field treatment.

Figure 18 shows the MWD values compared to the recovery rate of the field samples. The lowest recovery rates were achieved in the grass samples. The recovery rate of incubated samples ranged from 42-85%, with one extreme value at 99%. The field samples without a soil matrix showed a recovery rate of 73-80%. The recovery rates for miscanthus showed the biggest range, from 65% in a non-matrix sample up to over 100% (max = 152%) in the soil incubated samples. The poplar samples show a similar range. The lowest recovery rate was achieved in a soil-incubated control sample at 75%. The highest recovery rate was retrieved from a soil-incubated field sample at 136%. Generally, the MWD values for the grass samples is much smaller than for the poplar and miscanthus samples.



The recovery rate was calculated for the samples after each lab treatment (**Figure 19**). Similar to the field extractions the grass samples show the lowest recovery rates. They range from 78% up to 100%. In miscanthus the recovery rates range from 88-99%. Maize showed slightly lower recovery rates, ranging from 81-99%. On average the highest recovery rate was found in poplar and ranged between 92-99%. As for willow the recovery rates were a little smaller on the lower end 85%, yet ranged up to 98%. Two willow samples showed a recovery rate >100% (103% and 105%) after the freeze/thawing treatment.



4. Discussion

The results from this study are in line with the previous assumptions that charcoal particles fragment due to certain mechanical influences. Our results further show that with the exception of the shaking treatment all the other lab treatments caused a significant fragmentation in all the used substrates. Mechanical pressure is thereby the outstanding treatment as it produced the smallest MWD while freeze/thaw cycles surprisingly did not induce major fragmentation. Concerning the substrates, the two wood samples surprisingly showed smaller MWDs after the lab treatments than the grass samples. Interestingly, the results from the field experiment were quite different, as after the field incubations almost half of grass charcoal particles could not be recovered; the assumption was that the charcoal particles were fragmented so much that they were no longer visibly detectable. This result may provide further insight in post-fire processes in grasslands. The miscanthus and poplar charcoal on the contrary hardly underwent any fragmentation during field incubation. Based on our hypothesis that grass would fragment more than wood substrates due to treatments, the results from the lab and field experiments are rather contradictory.

4.1. Fragmentation due to pyrolysis

The results of the pyrolysis indicate that the properties of the feedstock material play a crucial role for the fragmentation of charcoal. Whilst the grass substrate did not show the smallest MWD (1.84mm), which instead was found in the two wood species (poplar = 1.39mm, willow = 1.32mm), the grass fragmented almost twice as much (**Table 3**) as the two wood species. Interestingly the strong fragmentation did not substantially affect the MWD of the samples (**Figure 12**). The strong fragmentation observed in grass is in accordance with our hypothesis that grass substrates are more susceptible to fragmentation than wood substrates.

In the literature, the properties of fresh charcoal mainly depend on two variables. The first variable is the heating process that includes the heat source, the temperature, the time of exposure and the heating rate (Braadbaart, 2008; Lehmann et al., 2008; Nguyen et al., 2010). Our findings were independent of these factors as all samples underwent the same pyrolysis treatment, with a temperature of 450°C, an exposure of 4 hours and the same heating rate. The second variable are the physical and chemical properties of the feedstock material. According to the literature, the taxon, size, density and porosity of the initial material play a crucial role on the charcoalification process (Braadbaart and Poole,

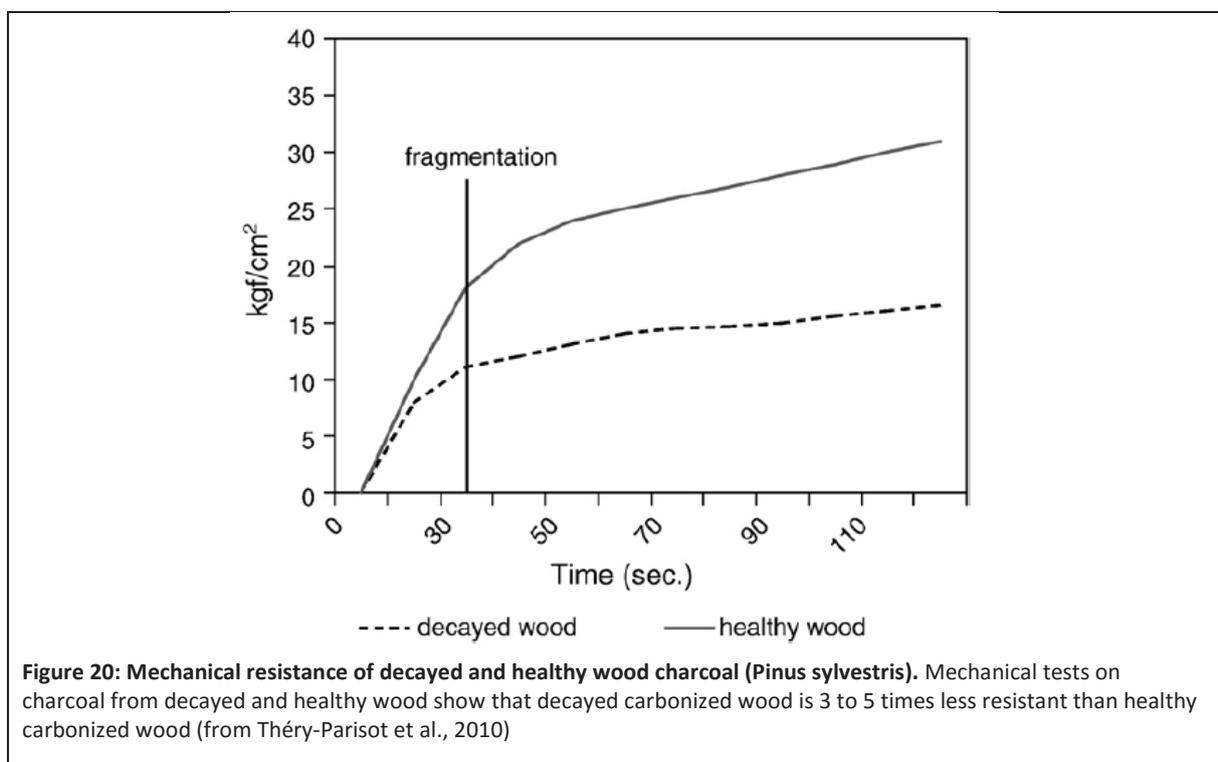
2008; Brewer et al., 2014) and the surface properties of the end products (Kloss et al., 2011). In the study of Keiluweit et al. (2010), it was shown in Fourier Transformed Infra-Red (FTIR) spectra that grass and wood charcoal displayed differences in their physical structure when produced at the same temperature. In the frame of this study we did not examine the physical structure of the charcoal in detail. Still, the results from the pyrolysis treatment indicate that the differences in fragmentation between wood and grass charcoal can be explained by the inherent chemical and physical properties of their respective feedstock material. One possible important factor for the fragmentation during charcoalification could be the lignin, cellulose and hemicellulose contents of the feedstock materials. Compared to the wood species used in this experiment, grass features smaller contents of cellulose, hemicellulose and lignin (**Table 2**). While the thermal decomposition of hemicellulose occurs at temperatures between 220-315°C and for cellulose at temperatures between 315-400°C (Yang et al., 2007; Brebu and Vasile, 2010). The thermal decomposition of lignin is much slower (Brebun and Vasile, 2010) and covers a wider temperature range (160-900°C) (Yang et al., 2007). According to Branca et al. (2005) the temperature needed for a complete thermal decomposition of lignin lies at 700°C. This indicates that the thermal decomposition of lignin might have been incomplete in the samples produced in this study, due to the pyrolysis temperature set at 450°C. This incomplete combustion could in turn positively influence the physical stability of charcoal derived from ligneous material and diminish their fragmentation. Still, the study of Théry-Parisot et al. (2010) suggests that the chemical and physical structure of charcoal is neither correlated nor predictable by the structure of its initial wood feedstock. This is in accordance to the results from this study, as the two wood substrates show similar values for the MWD (**Figure 10**) and for the fragmentation factor induced by the pyrolysis (**Table 3**).

The shift in particle size after the pyrolysis treatment (**Figure 11**) revealed that grass, willow and poplar samples had a larger number of smaller particles compared to the miscanthus and maize samples. Further, the shifts in the grass samples were spread over a wider range of fractions, which might explain the fact that the MWD did not get affected as strongly by the fragmentation (**Figure 12**).

These findings lead us to two realizations: Firstly, the results of the pyrolysis treatment might have been crucial for the subsequent lab treatments as the differences in MWD were passed on. Secondly, these results were rather surprising as we expected these two straw samples to build intermediate charcoal compounds in terms of particle size and fragmentation rate. In general, miscanthus and maize show smaller lignin contents than the two wood species (**Table 2**). Therefore, the fragmentation rate caused by pyrolysis

cannot be explained by the lignin induced stability of the charcoal. However, these findings could be an indication that the heterogeneity of the feedstock material might play a more important role for the stability of the charcoal particles than the composition of the substrate (Théry-Parisot et al. 2010). The initial wood material showed this heterogeneity, as each sample contained visually distinguishable stem and bark material. In addition, we were able to observe different stages of decay in the feedstock material.

In the study of Théry-Parisot et al. (2010), the mechanical resistance and thus the fragmentation of wood charcoal was dependant on the decomposition rate of the initial feedstock. Healthy carbonized wood was 3-5 times more resistant than decayed carbonized wood (Figure 20).



The particles of the miscanthus and straw samples showed much more homogenous characteristics. Therefore, the heterogeneity observed in the wood samples could explain the stronger fragmentation of wood samples compared to maize and miscanthus straw samples. As for the grass samples, the chemical and physical composition of its feedstock material might be the main influence for the fragmentation properties of its charcoal particles.

4.2. Laboratory Experiments

The lab experiments were based on the idea that the charcoal undergoes a variety of physical degradation processes in the soil (Preston and Schmidt, 2006; Pignatello et al., 2015). In this study three different physical treatments were applied. The crushing treatment produced the smallest MWD for all the different substrates (**Figure 14**). The shaking and freeze/thawing treatment produced similar MWD that were higher than the MWD produced by crushing. Therefore, our hypothesis that the freeze/thawing treatment would induce the highest rate of fragmentation must be rejected.

4.2.1. Effects of used Substrate Material

Out of all the lab treatments there are distinct differences observable between the substrates. The maize and miscanthus particles show the highest MWD across all treatments (**Figure 13**). However, those two substrates also show the highest variability among all substrates. A potential explanation for this variability could be the fact that the treatments mainly affect the biggest particle sizes (3.15mm and 2.0mm fractions) in terms of fragmentation (Supplementary Material: **Figures S2-S4**). Since these two materials inherited a larger amount of big particles (**Figure 10**) after the pyrolysis, they were in turn affected the most by the fragmentation induced by the lab treatments.

Charcoal produced from grass substrate showed similar fragmentation patterns due to lab treatments as the two straw materials. The two fractions with biggest particle sizes were affected the most and resulted in a reduction of the MWD (**Figure 13**).

Similar to the results of the pyrolysis treatment the MWDs of the wood samples resemble each other across all the lab treatments. As the wood samples inherited already smaller particles from the pyrolysis treatment it is not surprising that the lab treatments, which seemed to mainly affect bigger particles, did not induce such a strong fragmentation in these samples. When looking at the shift in particle size in the two wood samples (Supplementary Material: **Figures S2-S4**) the magnitudes never exceed shifts greater than $\pm 10\%$ for all the different particle size fractions. Nevertheless, a large percentage of the wood charcoal was present in form of bigger particles. The fact that these fractions did not undergo any major fragmentation may be caused by their feedstock composition (Czimczik et al., 2002; Noncentini et al., 2010). The results from Brewer et al. (2014) show that the density of wood derived charcoal is higher than the one of grass derived charcoal. They attributed these differences to the cell structure of the feedstock material that was most likely preserved during pyrolysis (cf. **chapter 4.1**). Generally, these studies suggest

that charred wood results in more stable compounds and is thus more resistant to fragmentation than charcoal produced of grass.

In general, the results from this study and the study of Théry-Parisot et al. (2010) indicate that the chance of fragmentation is much higher if the initial substrate material is not built out of strong physical structures (Pignatello et al., 2015).

4.2.2. Effects of Laboratory Treatment

In the literature (Preston and Schmidt, 2006; Spokas et al., 2014; Lehmann et al., 2015), it has been discussed whether physical processes such as cryoturbation, gelifluction, wetting/drying and mechanical stress (e.g. by tillage) play an important role in the degradation of PyC. Therefore, we suggest that the simulation of such processes in this study might provide additional information in this regard. All the mentioned processes are reputed to cause fragmentation in the charcoal samples (Preston and Schmidt, 2006; Lehmann et al., 2015) which in turn is itself an important initial step for the oxidation and degradation of charcoal particles (Noncentini et al., 2010).

The MWDs of all the substrates were significantly reduced by every lab treatment. This holds true for the pyrolysis treatment and the three subsequent lab treatments. The crushing treatment produced the smallest MWD in all five substrates (**Figure 14**). The shaking and freeze/thawing treatment produced similar MWDs when all substrates are grouped. These result led us to reject our hypothesis that freeze/thawing cycles would induce the strongest fragmentation in the charcoal samples.

The fact that the crushing treatment produced the smallest MWD is rather surprising. According to Skjemstad et al. (1999), the compaction of charcoal particles was reported primarily to cause a movement within the soil profile. Other processes such as trampling or bioturbation (Théry-Parisot et al., 2010) can affect the charcoal deposits mechanically as well. In this more recent study by Théry-Parisot et al. (2010) it was however suggested, that the vertical and horizontal movement of charcoal would consequently lead to its fragmentation and ultimately to its visual disappearance. A recent study by Chrzazvez et al. (2014) showed that charcoal can be very resistant to pressure (up to 22.5 MPa) until it was completely destroyed. In our study a pressure of approximately 0.1 MPa was applied to the charcoal samples which was already sufficient to induce fragmentation in the charcoal samples. As stated by Chrzazvez et al. (2014) the structure of the substrate is primarily responsible for the fragmentation of the charcoal samples under pressure. This is in accordance with the results from our study as charcoal derived from ligneous material

was much more resistant to fragmentation induced by pressure than grass derived charcoal samples.

The importance of the substrate material was also represented in the results that concerning the effects of water stress. While the effects on the willow and poplar samples were still rather small (**Figure 14**), the shaking treatment induced a lot of fragmentation in the grass and miscanthus samples. Still, the effect of the shaking treatment was much smaller than in the crushing treatment. In the literature it is assumed that especially aged charcoal samples show a larger soluble fraction than fresh charcoal, mainly due to an increased level of oxidation (Braadbaart et al., 2009; Abiven et al., 2011; Dittmar et al., 2012a). Since our samples represent fresh, unoxidized charcoal this might partially explain the absent impact of fragmentation caused by water stress. Naisse et al. (2015) stated that the exposure of biochar to wetting/drying and freezing/thawing cycles caused a substantial loss by leaching of small dissolved particles. According to their study, the feedstock significantly influenced these results, as less particles were leached from wood derived charcoal than from maize derived charcoal. The study of Spokas et al. (2014) showed different results, as their substrates with a high lignin content were shown to disintegrate and dissolve more readily than feedstocks with a high cellulose content (e.g. grasses). The results from our study do not correspond with the findings of Spokas et al. (2014), as the highest rate of fragmentation could be observed in grass derived samples. This is in accordance with our hypothesis that fragmentation is induced more strongly in charcoal produced from grass substrate. Nevertheless, there remain many uncertainties concerning the quantification of charcoal solubilisation effects and the subsequent fragmentation regarding these processes.

Based on our hypothesis, we expected freeze/thawing cycles to induce a stronger fragmentation effect than the other two lab treatments. However, only in grass and maize samples a significantly smaller MWD could be observed compared to the results of the shaking treatment. Charcoal is reputed to be hydrophobic and to have a complex nanoporous structure (Hammes et al., 2008b; Gray et al., 2014; Pignatello et al., 2015). Prior to the freeze/thawing cycles we therefore incubated the charcoal particles in water and performed a sinking experiment (Supplementary Material: **Figure S5 & S6**). The data from this sinking experiment implies that most of the grass particles got water saturated during the incubation, whereas only a small portion of maize particles were supposedly saturated by this process. Therefore, it is surprising that these two substrates showed a significant fragmentation induced by the freeze/thaw treatment. Apart from the hydrophobicity, another effect that might have been relevant for the water saturation in this experiment could have been sealed internal pores that were not accessible to solutes

(Brewer et al. 2014, Gao and Masiello, in revision). Since we did not perform any density or porosity measurements on the charcoal we could not further elucidate these findings. Still, the absent impact of freeze/thawing based reported in this study could be the result of an insufficient water saturation. Further, our findings in accordance with the work of Théry-Parisot et al. (2010). Their results indicate that freeze/thaw cycles seem to induce fragmentation only in restricted situations (Pignatello et al., 2015). In their study, charcoal originating from decayed wood was more porous and fragmented more, into smaller particles, and faster than their healthy counterparts during freeze/thawing. As we did not collect precise information about the state of decay in our samples, the results are hard to compare. Nevertheless, it shows that further research is needed to understand the processes involved in the fragmentation induced by water and freeze/thawing stress mechanisms.

4.3. Field Experiments

The lab experiments were designed to simulate physical stress occurring in a natural setting. However, such approaches are always limited in their comparability to the actual processes in the environment. Hence we also conducted a field experiment that was set up to test the behaviour of the charcoal in a setting as close as possible to natural. Poplar, miscanthus and grass samples were used to represent wood, straw and grass types of charcoal. The matrices were chosen to simulate surface properties (clay pots), incubation in a sandy matrix, and a soil matrix. While the grass was prone to a strong fragmentation pattern, the miscanthus and poplar samples hardly showed any fragmentation (**Figure 15**). These results are in accordance with our first hypothesis that grass samples are prone to stronger fragmentation than wood derived charcoal. Yet, for our third hypothesis (that field incubation would induce a stronger fragmentation than the artificial lab treatments) this only holds true for the grass substrate and therefore we have to reject this hypothesis.

4.3.1. Effects of used Substrate Material

As mentioned in **chapter 4.2.1** the initial substrate structure plays an important role for the fragmentation properties of the resulting charcoal samples (Théry-Parisot et al., 2010; Pignatello et al., 2015). The grass samples were fragmented much more and showed MWD values between 0.6-1.0mm (**Figure 15**), which is less than half their initial MWD (2.0mm). The effect of fragmentation during soil incubation was much less prominent in the miscanthus and poplar samples, where the post depositional MWD values were comparable and ranged between 1.35-1.7mm. As suggested by Spokas et al. (2014),

charcoal produced from high-lignin feedstocks is disintegrated more readily in the soil and especially in sandy textured soils. This contradicts our results, where the charcoal produced from high cellulose feedstock (grass) showed a faster and stronger fragmentation. The fact that the recovery rate for the grass samples was partly below 50% (**Figure 18**) limits the impact of these findings. The question arises whether the missing portion of particles was lost due to transportation (Major et al., 2010) or whether the particles were already integrated into the soil system (Braadbaart et al., 2009).

A potential explanation for the faster degradation of grass charcoal in the soil, or rather the slower degradation of wood derived charcoal, may be the lignin content of the feedstock material. Brebu and Vasile (2010) suggest that chars produced from biomass with a high lignin content show a low reactivity compared to charcoals produced from biomass with lower lignin contents. Whereas, grass derived charcoal shows a higher reactivity in the soil matrix. Further the study of Zimmerman (2010) shows that smaller particles are more susceptible to undergo heteroaggregation when incubated in the soil. By forming small organo-mineral aggregates (microaggregates) (Brodowski et al., 2006; Vasilyeva et al., 2011) the aggregated charcoal particles would consequently no longer be visually detectable. As our extraction method in this study was based on a visual separation of charcoal and soil particles, this effect might explain the low recovery rate we observed.

4.3.2. Effects of Matrix

When comparing the control with the field samples (**Figure 16**), only a few treatments showed clear differences between the two set-ups. This can be attributed to the lack of colder conditions for the outside samples. Due to the El Niño Southern Oscillation, winter 2015/2016 was rather mild in Zurich and the temperature anomaly for central Europe ranged between +2-4°C (NOAA, Climate.gov). With the temperature probes that we installed we could only observe one cold event that fell below the -5°C mark (Supplementary Material: **Figure S1**). Freeze/thawing has been shown to be a potential effect for fragmentation (Théry-Parisot et al., 2010; Pignatello et al., 2015). We address the lack of stronger differences between control and field samples to the lack of freeze/thawing events in the field incubations.

Between the different matrices, the field samples without a matrix and the field samples with a soil matrix showed the biggest differences compared to the control samples (**Figure 16**). The fragmentation induced in the samples without a matrix might be explained by the stronger exposure to abiotic processes (rain, wind, temperature). Even though charcoal degradation is enhanced in a soil matrix, the heteroaggregation with

mineral particles may also contribute to the stabilization of the charcoal particles (Brodowski et al., 2006; Vasilyeva et al., 2011). Therefore, the lack of a soil matrix might have led to increased fragmentation due to a missing protective layer, in both a physical and chemical way (Skjemstad et al., 1999; Cheng and Lehmann, 2009).

The observed differences between the fragmentation in sand and soil matrices can be attributed to the influence microbial communities and enzymatic activities (Kuzyakov et al., 2009; Singh et al., 2012). The higher microbial activity in the soil usually leads to faster decomposition and thus fragmentation in the soil, compared to sand (Kuzyakov et al., 2009). These decomposition rates are believed to be reduced over time due to aggregation or the preferential mineralization of charcoal compounds (Kuzyakov et al., 2009). However, the decomposition of char by microbial communities is still poorly understood and requires further investigation (Czimczik and Masiello, 2007). In general, there is a lack of field observations covering the fragmentation, degradation or transportation of charcoal (Major et al., 2010).

Regarding the incubations in a soil matrix the work of Brodowski et al. (2005) showed charcoal in three forms in soil matrix: (1) a free form, (2) small particles that were aggregated to bigger mineral particles, and (3) larger particles attached to fine mineral matter. In our study we observed all three of these forms. Most of the charcoal particles were still in their free form (1). Charcoal particles produced from poplar and miscanthus material were covered by a fine mineral layer (3). The extracted charcoal particles (**Figure 9**) showed a visually detectable aggregation with mineral parts of the soil matrix. Even after thoroughly rinsing the particles with water, the aggregates would still persist, implying strong chemical bonds. Another result that points towards heteroaggregation in the soil were the recovery rates observed after the field incubation. Usually recovery rates are expected to be <100%, while in our experiment they exceeded 100%. This indicates that heavier mineral fractions were aggregated with the lighter organic charcoal. Further we assume that a large part of the grass charcoal particles was aggregated with mineral particles (form (2) in Brodowski et al. (2005)) and was thereby no longer visually detectable. The fragmentation during the soil incubation might have promoted the formation of these organo-mineral aggregations (Brodowski et al., 2006; Czimczik and Masiello, 2007; Zimmerman, 2010). As suggested in other studies, the aggregation between charcoal and the mineral phase of the soil can occur very rapidly (Brodowski et al., 2006), in situ within less than one year (Singh et al., 2014). This is supported by other existing field observations where a rapid disappearance of charcoal particles could be observed (Bird et al., 1999; Hammes et al., 2008a; Nguyen et al., 2008). In our study, the effects of organo-mineral interactions could be detected after an incubation of just six

months. In general, these organo-mineral aggregations are seen as stabilizing process that protects the charcoal from further decomposition (Czimczik and Masiello, 2007; Brodowski et al. 2006; Zimmerman, 2010). By aggregating the charcoal in these invisible microaggregates this process potentially decreased the recovery rate of our grass samples, as our extraction method was based on a visual separation of charcoal and soil particles. However, we could not provide evidence that the grass charcoal particles were still present in the soil. The measurement of the $\delta^{13}\text{C}$ signature of the soil would give insight if the charcoal particles were still present in an aggregated, yet invisible form in the soil matrix (Braadbaart et al., 2009).

The fact that a large amount of the grass charcoal was not retrievable after an incubation period of only six months presents further implications about the processes occurring after a fire event in a grassland or savanna ecosystem. In those natural settings, the charcoal was not visibly detectable after less than a year (Pignatello et al. 2015) and early losses were substantial (Nguyen et al., 2008, Ding et al., 2013).

Of course additional experiments would have to be conducted in order to study the potential fragmentation of charcoal. Larger wood derived charcoal particles are supposed to be less prone to being incorporated into the soil and thus persist longer (De Lafontaine and Asselin, 2011; Santin et al., 2016). The study from Théry-Parisot et al. (2010) suggests that post-depositional processes act as a homogenization agent for different substrates with increased residence time in the soil. Therefore, it would be interesting to conduct a long-term study and track the fragmentation pattern of charcoal substrates that showed less fragmentation within the six months of this experiment.

4.4. Comparison Lab-Field

When comparing the results from the lab experiments with the field experiments it shows that the main differences can be observed in the fragmentation properties of the wood and straw charcoal substrates. While grass charcoal was prone to strong fragmentation in both experiments, wood derived charcoal was only susceptible to strong fragmentation in the lab experiments (**Figure 13 & 15**).

As described in **chapter 4.1**, the heterogeneity of the wood derived charcoal (Théry-Parisot et al., 2010) might play crucial role on the fragmentation and thus the MWD of those samples. Due to the composition of the initial feedstock material the produced charcoal inherited a certain fragmentation pattern during the pyrolysis which can most likely be accounted for resulting in smaller MWD values after the different treatments as well. The big discrepancy between the fragmentation patterns of the lab and field

experiment might thereby be explained by the initial particle size of the charcoal samples. For the field experiment 300mg of charcoal particles with a diameter >2mm were used. This could indicate that the parts of the wood feedstock with a higher state of decay were not included in the field samples, which in turn led to less fragmentation (Théry-Parisot et al., 2010).

Another interesting difference that could be observed, was that the recovery rates that were calculated after each lab treatment ranged below the 100% mark, as normally expected. However, the shaking and freeze/thawing treatments, that involved water as a solution during the experiment, showed lower recovery rates than the crushing treatment (**Figure 19**). These results could indicate that small charcoal particles were lost in the solution during the experiment or the extraction of the charcoal particles (Bird et al. 1999; Braadbaart et al., 2009; Naisse et al., 2015).

When looking at the different fragmentation processes, it is difficult to correlate the isolated processes in the laboratory that were conducted under controlled conditions with the processes taking place in the field experiment (**Figure 17**). In field incubations the conditions are much more complex and unaccounted biotic processes could influence the fragmentation. We therefore have to reject the hypothesis that field conditions induce more fragmentation in charcoal particles. Field conditions induced more fragmentation in grass derived charcoal, whereas the observed fragmentation in charcoal derived from poplar and miscanthus feedstock were less striking.

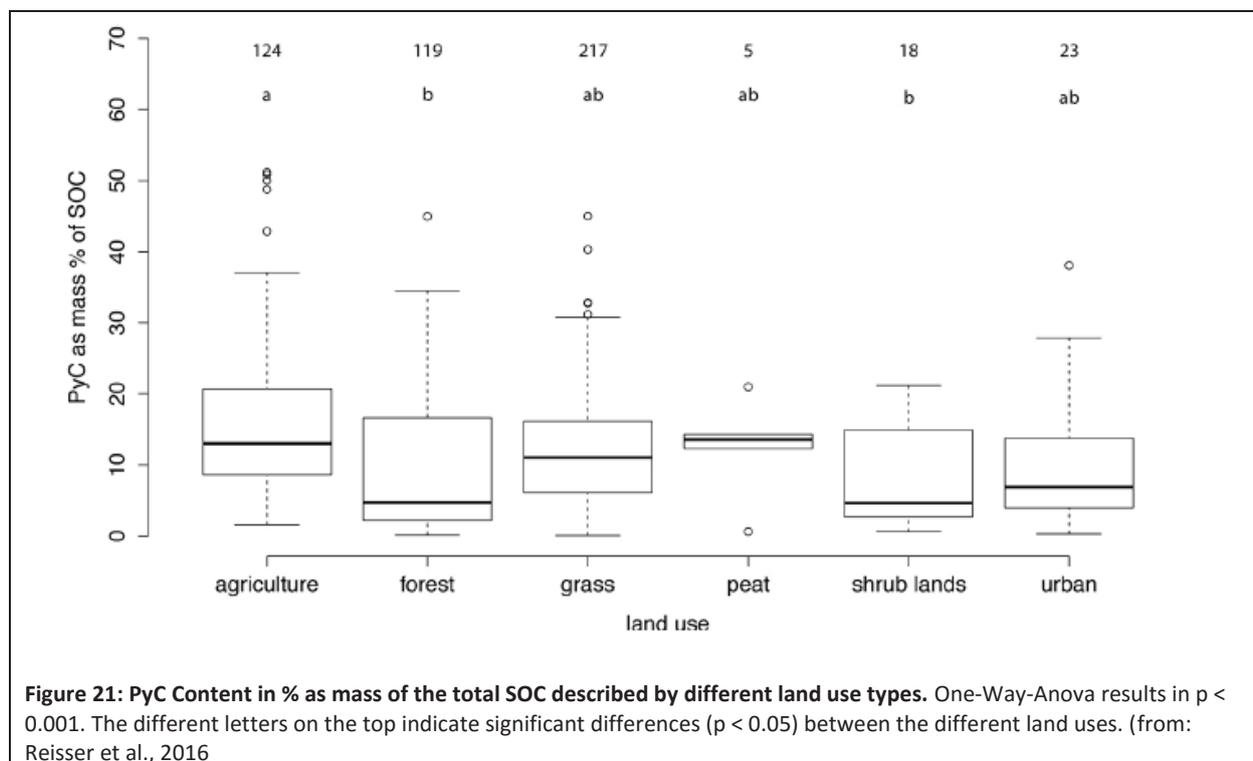
4.5. Limitations and Perspective

Based on the experiments conducted in this study we observed fragmentation induced by a series of physical processes. The differences observed between the fragmentation processes in the lab and field experiments were discussed in the previous chapters. Still the methods used in this thesis show a series of limitations.

In general, the series of experiments conducted in this study indicate that laboratory studies under controlled conditions can provide further information about mechanical processes causing charcoal fragmentation during its incorporation into the soil matrix. Still, there is always a limitation to experiments conducted under controlled lab conditions and the combination of field or glasshouse studies is highly suggested. Especially long-term field experiments and settings simulating ecosystem-conditions similar to savannas, grasslands or forests, that are mostly affected by fire events, could prove to be beneficiary for the further understanding of the involved processes.

As suggested in the literature (Brewer et al., 2014; Gao and Masiello, in press) the porosity and density of charcoal are two important characteristics, that determine the persistence of charcoal in the soil system. In our study we did not address either of those two properties to the results from the shaking and freeze/thawing treatments. Combining these results with porosity measurements might have provided further insight about the water holding capacity and the weaker fragmentation effects (compared to other treatments) observed in these experiments.

Concerning the field experiments, it has been shown in anthracology studies that the pH plays a crucial role in the fragmentation of charcoal in the soil. These studies show that alkaline conditions are more favourable for inducing charcoal fragmentation (Cohen-Ofri et al., 2006; Braadbart et al., 2009). Based on the concept of enhanced heteroaggregation with smaller charcoal particles, alkaline soils are consequently associated with an increased stabilization of charcoal (Noncentini et al., 2010). The results of Reisser et al. (2016) support these findings, as the estimates for alkaline soils were shown to contain at least 50% more PyC than acidic soils. Therefore, it would have been interesting to measure the pH values of the soil that was used in our experiments.



The results of our study suggest that wood derived charcoal is generally expected to show a higher recalcitrance to fragmentation in the environment. These findings would indicate that wood derived charcoal would also have a longer mean residence time in the soil matrix. Therefore, we would expect to find larger amounts of charcoal residues in forest soils, compared to other fire affected ecosystems like grasslands or savannas. However, the results of Reisser et al. (2016) show, that the PyC content of grasslands is slightly higher with a mean of 12.1%, compared to the mean PyC content of 9.7% in forests (**Figure 21**). Forest soils even showed the lowest content of PyC in SOC for all the land use types. At first these findings might be surprising, yet the fragmentation of charcoal into smaller particles might actually promote their persistence in the soil (Braadbaart et al., 2009; Noncentini et al., 2010). As discussed in chapter 4.3.2., smaller particles are more readily aggregated with fine mineral particles and clay particles. Such aggregations would cause a stabilization of the charcoal in the soil system. This indicates that fragmentation might lead to a visual disappearance of charcoal. Yet on a chemical level, it would still be present in the soil matrix in an aggregated and more stabilized form. As the visual disappearance was reported in the literature (Pignatello et al., 2015), we used $\delta^{13}\text{C}$ labelled grass as a feedstock material for the field experiments to measure the $\delta^{13}\text{C}$ signature of the soil after the extraction of the charcoal. Due to a lack of time we were unable to conduct these measurements and can therefore not explain the fate of the charcoal at this point in time.

5. Conclusion

For the conduction of this study we produced charcoal by combusting a variety of different feedstock material under close to natural conditions. Based on a series of laboratory and field experiments we then studied the influence of physical processes that cause fragmentation in charcoal. Our key findings are as follows:

- We observed fragmentation in all charcoal samples, independent of their substrate material or undergone treatment.
- The feedstock material shows to be the most important parameter explaining differences in combustion and fragmentation patterns.
- To a certain extent laboratory experiments are a viable method to simulate natural processes that lead to a fragmentation of charcoal.
- Applying mechanical stress to charcoal particles was the most significant process to induce fragmentation in charcoal; shaking charcoal in a water solution and applying freeze/thaw cycles to the samples did not induce strong fragmentation.
- Substrate dependency of fragmentation varies between laboratory and field experiments. Grass and wood derived charcoal showed similar fragmentation patterns in laboratory treatments. During the field experiments however, grass derived charcoal was much more susceptible to fragmentation than wood derived charcoal.
- The quick visual disappearance of grass derived charcoal in the soil incubations is either an indication for the loss of charcoal from the soil profile or an indication for its stabilization by forming micro-aggregates.
- Turnover behaviour of charcoal in fire affected ecosystems is complex. We suggest that fragmentation studies can provide further knowledge about the persistence of charcoal in such ecosystems.

The assessment of charcoal fragmentation helps to further understand the translocation and/or incorporation of charcoal in the soil profile. The progressive fragmentation of incorporated charcoal might result in its visual disappearance. Thus, it is especially important to distinguish between visual and chemical presence of charcoal in the soil. Fragmentation in the environment is associated with a series of physical and chemical processes. This study mainly focused on the physical processes that lead to the fragmentation of charcoal in the soil. Nevertheless, we suggest that the entirety of parameters need to be investigated to produce estimations of the mean residence time of charcoal and to advance its potential role for C sequestration.

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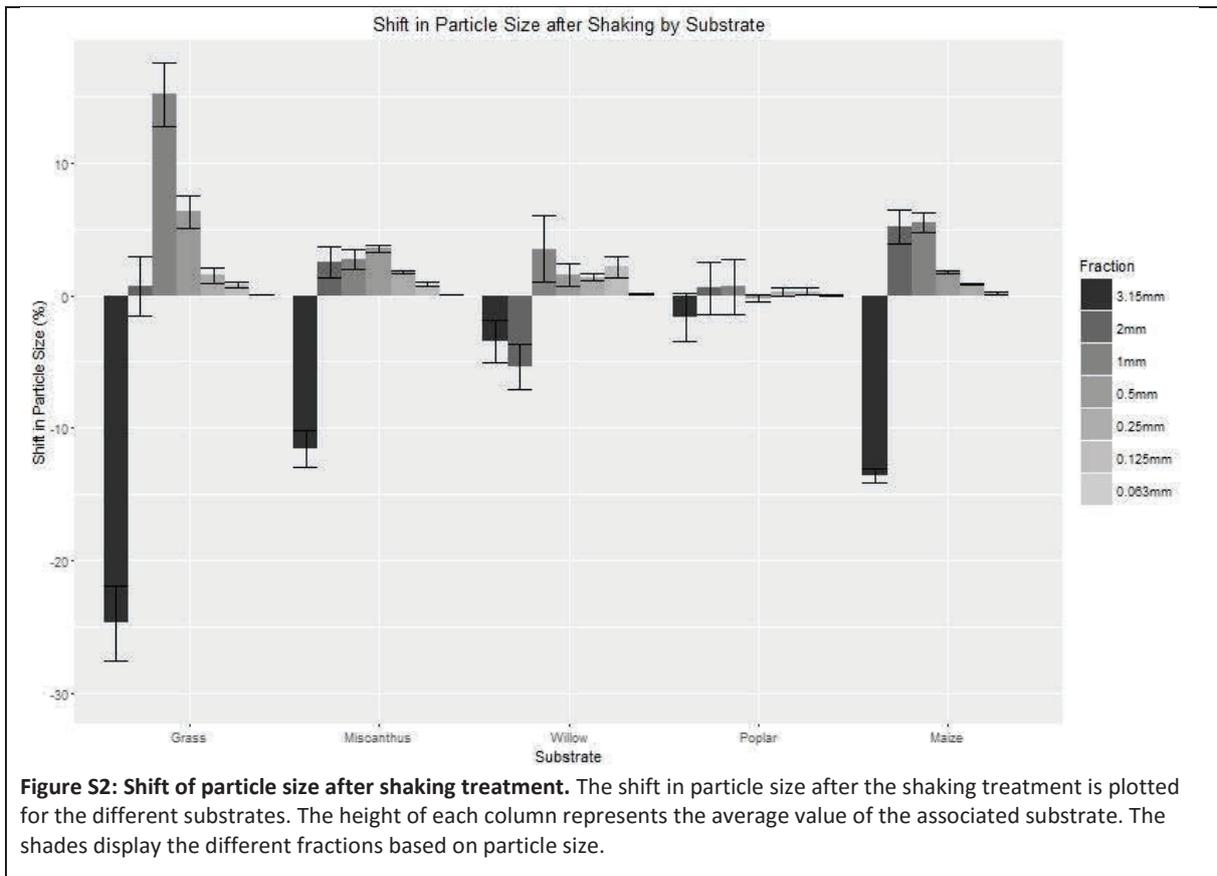
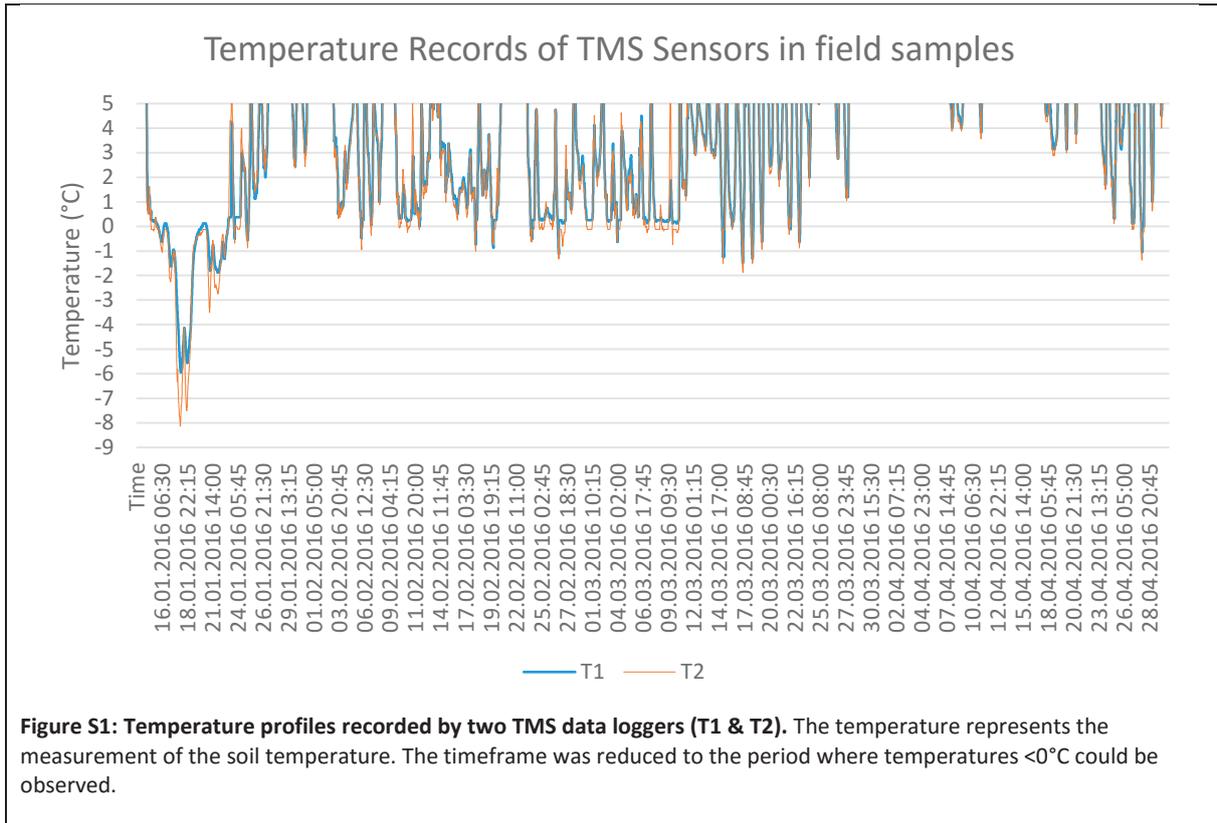
Supplementary Material

Table S1: Output of ANOVA and SNK-Test of lab dataset.

ANOVA ^{lab}						
	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	Sig.
lab\$Substrate	4	47.44	11.861	433.107	< 2e-16	***
lab\$Treatment	4	28.57	7.143	260.812	< 2e-16	***
lab\$Substrate:lab\$Treatment	16	3.85	0.24	8.777	4.65E-16	***
Residuals	200	5.48	0.027			
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						
Student-Newman-Keuls TEST						
SNK Parameters	Df	ntr	alpha	test	name.t	
	72	6	0.05	SNK	lab\$Treatment	
Groups	means	std	r	M		
1. Initial	2.329861	0.4843507	75	a		
2. Pyrolysis	1.916249	0.5531488	75	b		
3. Shaking	1.716732	0.5492802	25	c		
4. FreezeThawing	1.657974	0.4366103	25	c		
5. Crushing	1.192728	0.4541414	25	d		
SNK Parameters	Df	ntr	alpha	test	name.t	
	200	5	0.05	SNK	lab\$Substrate	
Groups	means	std	r	M		
1. Maize	2.54069	0.4149651	45	a		
2. Miscanthus	2.40576	0.4001337	45	b		
3. Grass	1.709494	0.5401936	45	c		
4. Poplar	1.489223	0.3364835	45	d		
5. Willow	1.469147	0.3519878	45	d		

Table S2: Output of ANOVA and SNK-Test of field dataset. The dataset was exponentially transformed before the analysis.

ANOVA ^{field}						
	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	Sig.
field\$Substrate	2	120.37	60.19	537.515	< 2e ⁻¹⁶	***
field\$Treatment	5	8.68	1.74	15.507	2.48E ⁻¹⁰	***
field\$Substrate:field\$Treatment	10	7.53	0.75	6.726	2.72E ⁻⁰⁷	***
Residuals	72	8.06	0.11			
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						
Student-Newman-Keuls TEST						
SNK Parameters	Df	ntr	alpha	test	name.t	
	72	6	0.05	SNK	field\$Treatment	
Groups	means	std	r	M		
1. ControlNoMatrix	4.430011	1.2256184	15	a		
2. FieldSand	4.0127	1.533157	15	b		
3. FieldNoMatrix	3.926329	0.9501677	15	b		
4. ControlSoil	3.753324	1.282927	15	b		
5. ControlSand	3.726519	1.4856648	15	b		
6. FieldSoil	3.414215	1.0503338	15	c		
SNK Parameters	Df	ntr	alpha	test	name.t	
	72	3	0.05	SNK	field\$Substrate	
Groups	means	std	r	M		
1. Miscanthus	4.971889	0.4879705	30	a		
2. Poplar	4.382171	0.5440148	30	b		
3. Grass	2.277489	0.5504537	30	c		



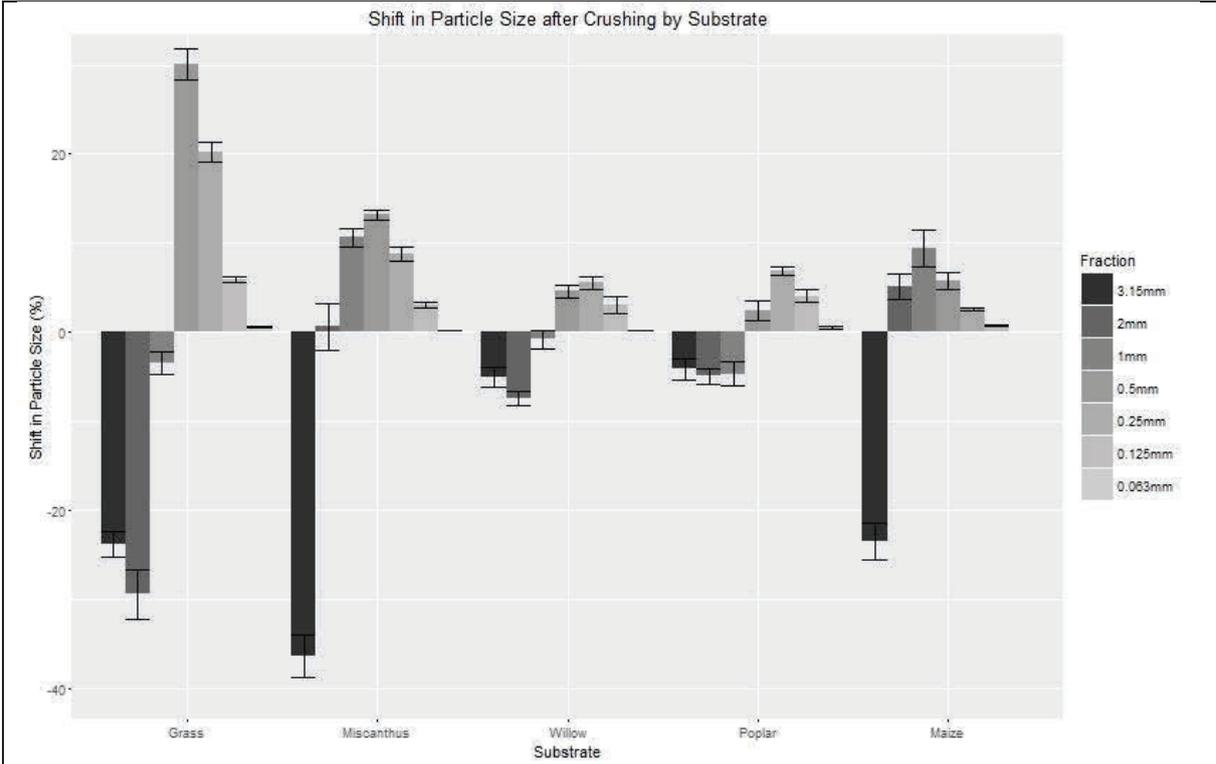


Figure S3: Shift of particle size after crushing treatment. The shift in particle size after the crushing treatment is plotted for the different substrates. The height of each column represents the average value of the associated substrate. The shades display the different fractions based on particle size.

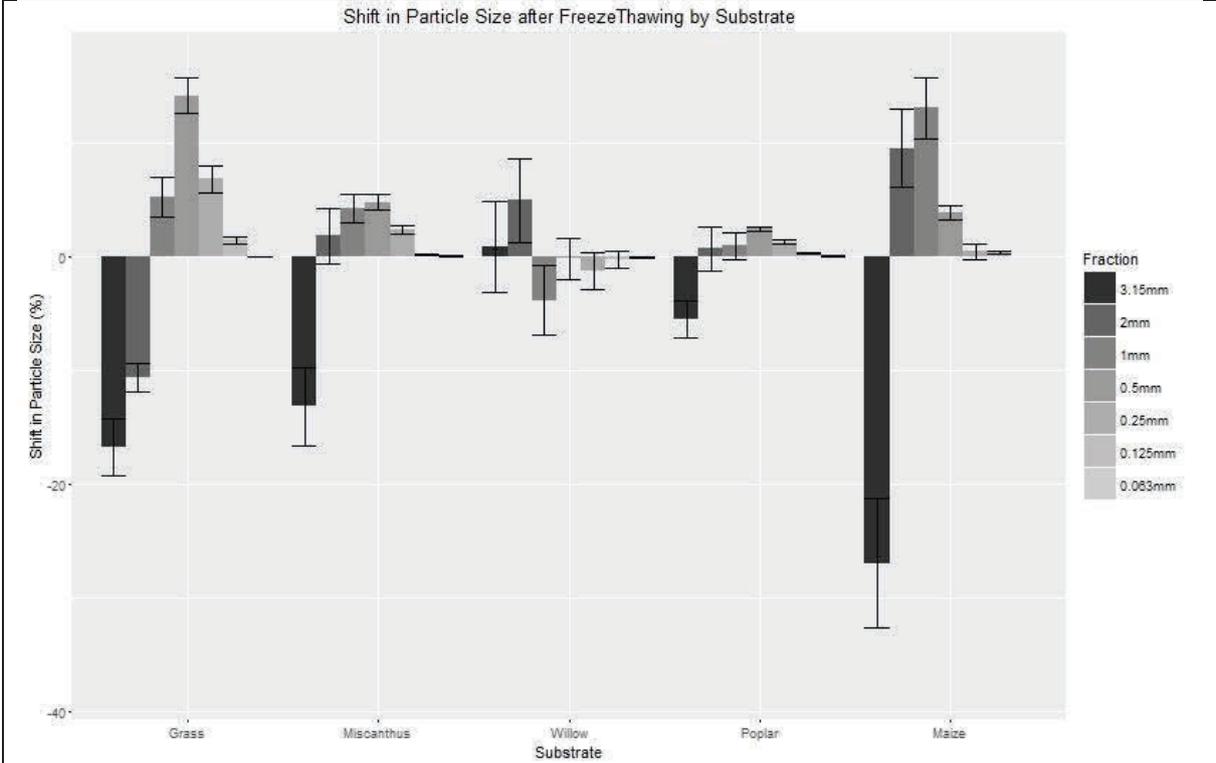
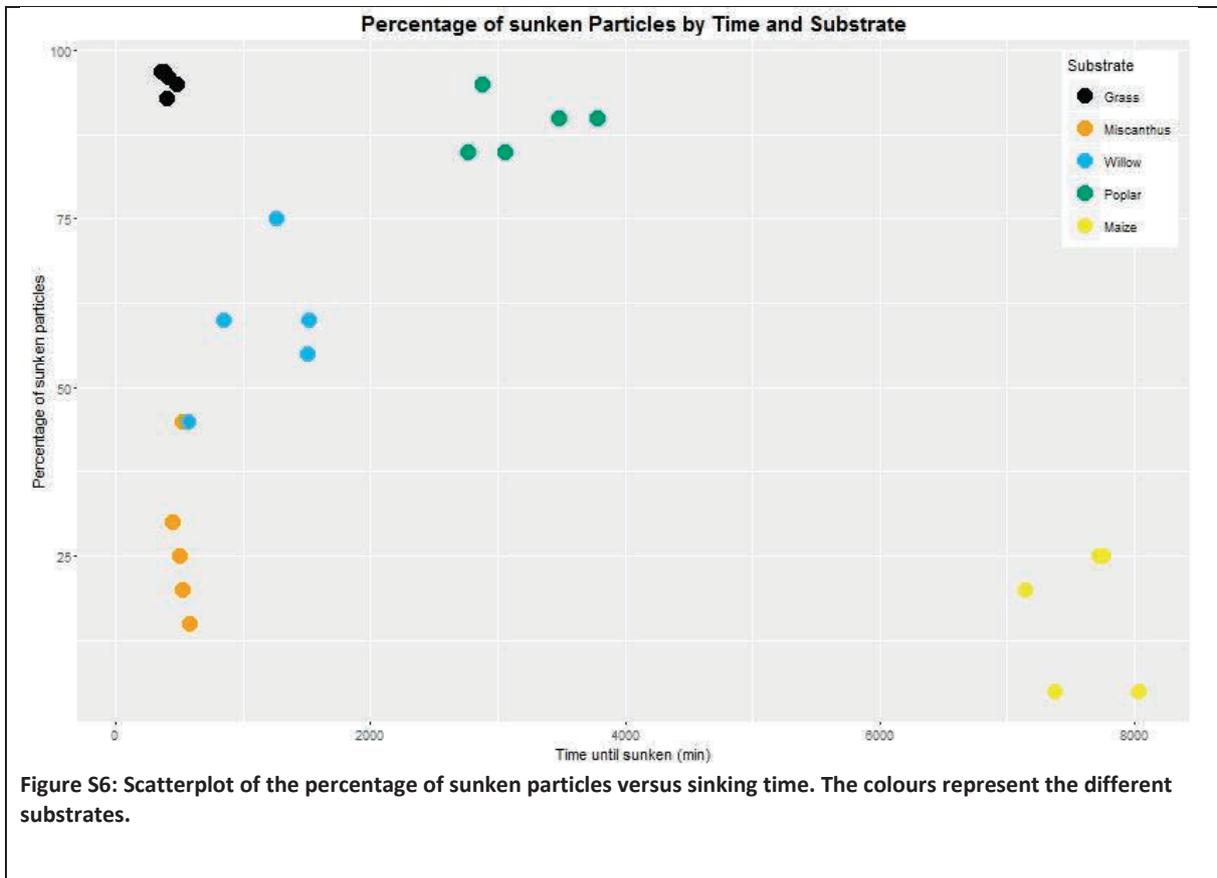
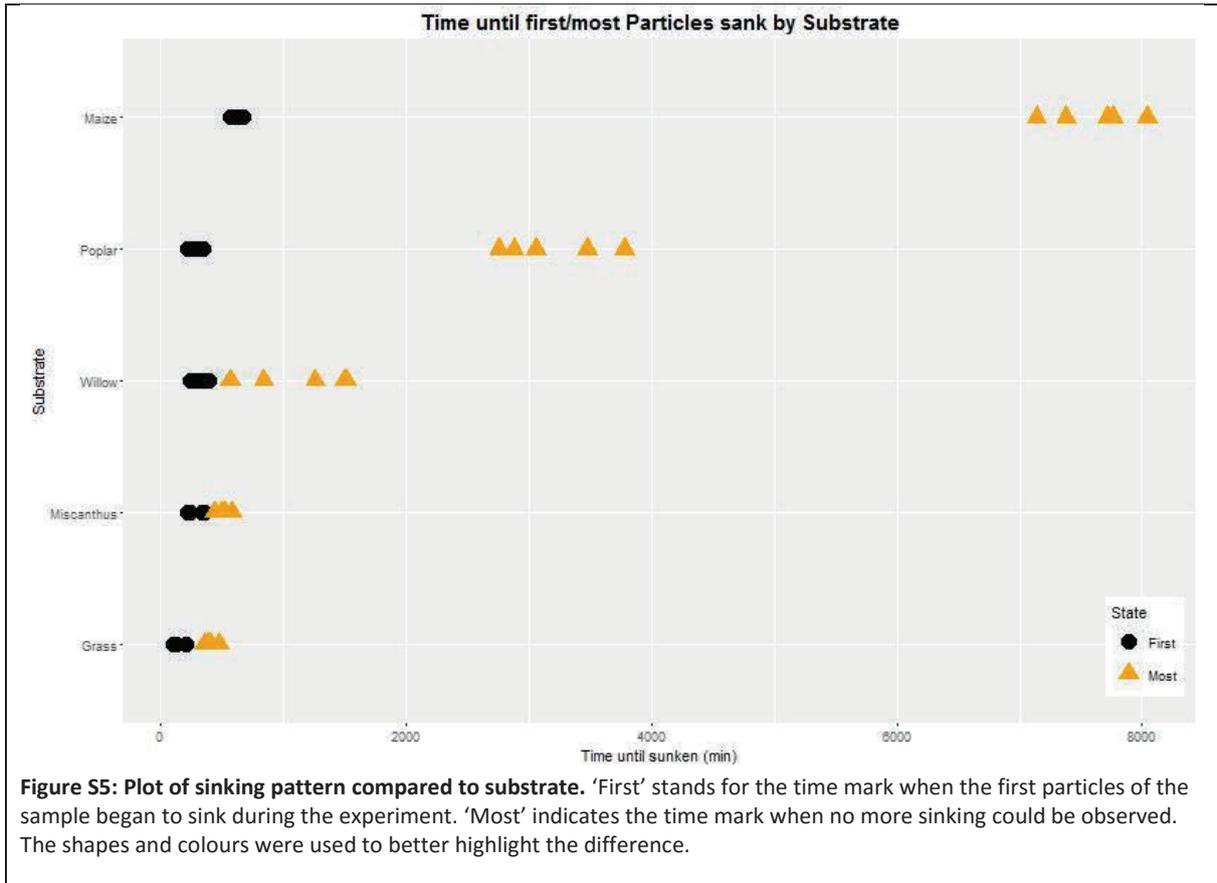


Figure S4: Shift of particle size after freeze/thawing treatment. The shift in particle size after the freeze/thawing treatment is plotted for the different substrates. The height of each column represents the average value of the associated substrate. The shades display the different fractions based on particle size.



Personal Declaration

I hereby declare that the submitted thesis is the result of my own, independent work. All external sources are explicitly acknowledged in the thesis.

Zürich, 30. September 2016

Lukas Gmünder