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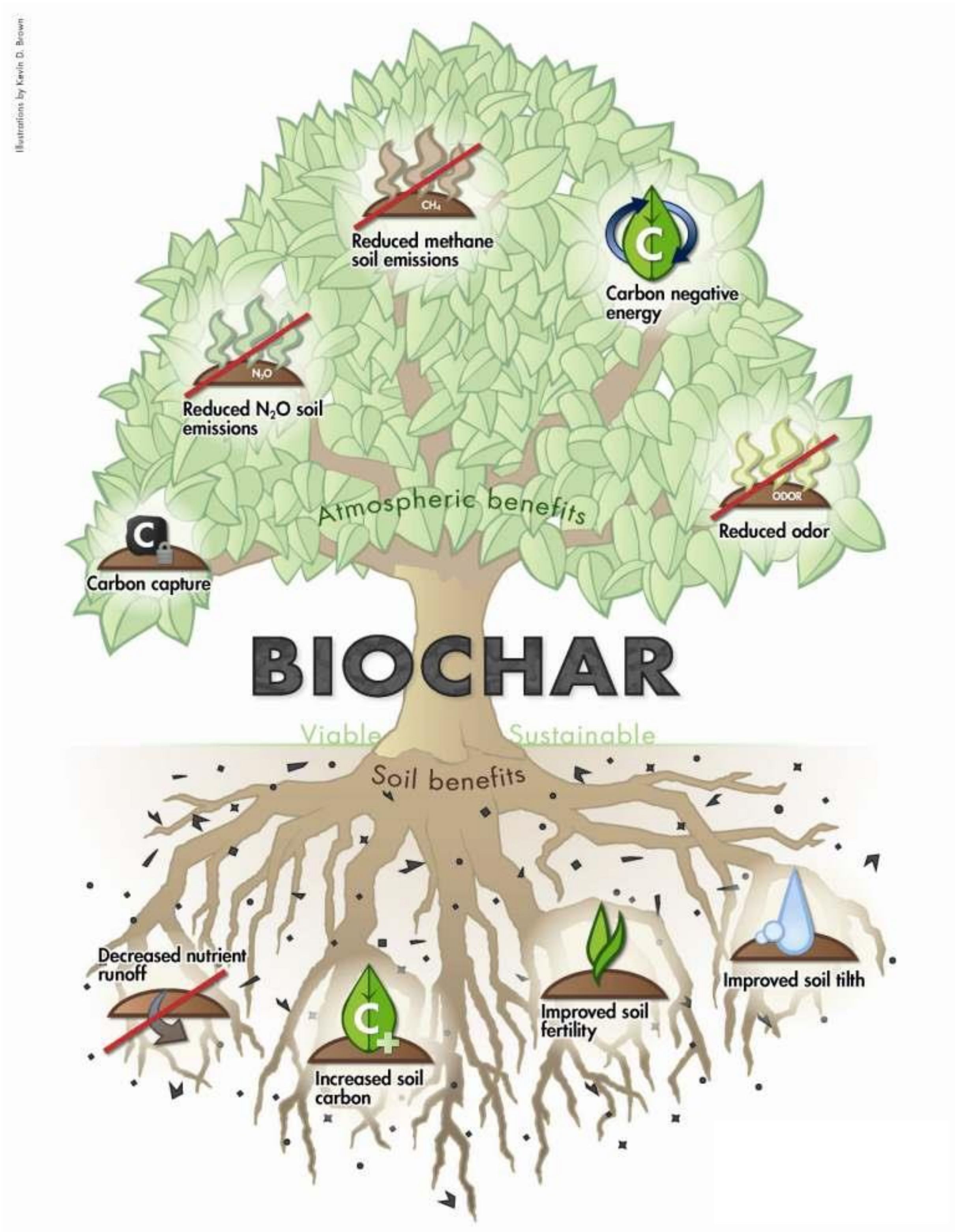
Investigating the impact of aggregate breakdown mechanisms in a Norwegian silty clay loamy soil with Miscanthus amendment

MSc thesis by Anne Schols

April, 2016

Soil Geography and Landscape Group



Source: http://www.biochar-international.org/biochar

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Biochar detachment by water erosion

Investigating the impact of aggregate breakdown mechanisms in a Norwegian silty clay loamy soil with Miscanthus amendment

Thesis report submitted to Wageningen University in partial fulfilment of the requirements for the degree of Master of Science (MSc.) in International Land and Water Management, specialisation Sustainable Land Management.

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Abstract

Loss of soil and organic material from agricultural soils can have severe economic consequences for farmers. The reduction of soil loss can be achieved by increasing the soil aggregate stability. Supposedly, this can be achieved by incorporating biochar into the soil. However, biochar can also be eroded from the soil, further increasing the economic and environmental losses. The mechanisms of biochar incorporation into soil aggregates, and the influence that biochar exercises on soil stability is still poorly understood. This study investigates the main processes involved in the detachment of biochar from the soil by water. Experimental plots on a silty clay loamy soil were used that contain two biochar (pyrolized Miscanthus) application rates (8 and 25 ton per hectare), two soil amendment types (raw Miscanthus and pyrolized Miscanthus), and biochar application in 2010 and 2014 different years. On all biochar treatments, the main aggregate breakdown mechanisms (slaking and clay swelling) were simulated according to the method of Le Bissonnais (1996), by applying fast wetting, slow wetting and shaking after pre-wetting treatments on aggregates of 2 - 6 mm sizes. After dry-sieving the aggregates into fragments sizes of >20 mm, 6 – 20 mm, 2 – 6 mm, 0.6 – 2 mm and <0.6 mm, the Mean Weight Diameter (MWD) was calculated to represent aggregate stability. The aggregate stability was not significantly affected by the rate of biochar added to the soil (p=0.271), nor by the type of soil amendment that was added (p=0.228), nor the amendment duration the biochar has been in the soil (p=0.216). It can be concluded that biochar-amended to a clayey soil does not significantly improve soil aggregate stability. The lack of effect found on aggregate stability by soil amendment in a silty clay loamy soil in this study raises the question whether or not biochar would be worth the investment to improve soil aggregate stability.

Key words: Biochar; Aggregate stability; Aggregate breakdown mechanisms; Detachment; Soil amendment; Norway

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List of abbreviations

ANOVA	Analysis of Variance
FW	Fast wetting
MWD	Mean weighted diameter
SH	Mechanical breakdown by shaking
SW	Slow wetting
TOC	Total Organic Carbon
WSA	Wet-Sieving Apparatus

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1 Introduction

Sustainable soil management to stimulate soil improvement is a necessity in many parts of the world (Lehmann & Joseph, 2009). Biochar amendment to soils is one of the sustainable agro-ecosystem practices that is promoted to have several benefits to improve soil chemical and physical (Ouyang et al., 2013). The book 'The Biochar Solution' written by Albert Bates in 2010 caused a hype due to the promotion of benefits of biochar, such as increase in soil carbon sequestration, while boosting food production due to increased beneficial chemical and physical soil properties. Biochar is a carbon-rich product that is made from heated feedstock (e.g. wood, manure, sewage sludge) at relatively high temperatures (<700°C), under anaerobic conditions or limited oxygen supply (Lehmann & Joseph, 2009). The application of biochar to the soil can have several benefits, such as carbon sequestration (C), improved soil chemical properties, e.g. pH and CEC (Liang et al., 2006), and improved soil physical properties, e.g. soil water retention (Ouyang et al., 2013; Sun & Lu, 2014), hydraulic conductivity (Major et al., 2010), soil fertility enhancement (Lehmann & Joseph, 2009), formation of macro aggregates enhancement (Mukherjee & Lal, 2013).

However, maximizing all benefits simultaneously is not possible, and negative effects may also occur (Jeffery et al., 2013). For example, to prevent that the biochar is transported by either wind or water, soil cover (Lehmann & Joseph, 2009) or mixing of the biochar in the topsoil is required, which may trade-off against the benefits of no-till farming (Jeffery et al., 2013). Few studies exists on the detachment of biochar, and knowledge on the redistribution of biochar is important, especially for soils with biochar addition that are regularly cultivated and therefore vulnerable to soil erosion (Fister et al., 2014). The specific influence that biochar exercises on soil stability is still poorly understood (Sohi et al., 2009). Loss of not only soil but also biochar by erosion can have sincere economic consequences for farmers (Fister et al., 2014). Hence, the main objective of this study is to investigate the main processes involved in the detachment of biochar from the soil by water.

Erosion of cultivated soils follows from soil-aggregate breakdown and detachment of soil fragments by rain (Le Bissonnais, 1996). Therefore, the main mechanisms of aggregate breakdown will be studied in order to determine the degree of detachment of the biochar in soils under different natural processes, namely slaking and breakdown by differential clay swelling. This is the first study that compares the impact of biochar-amended clayey soils on aggregate stability in both short- and long-term, with incubation experiments of 1 and 5 years.

This research was executed in corporation with the Norwegian Institute for Bioeconomy Research (NIBIO), located in Ås, Norway. For the soil sampling, experimental biochar plots were used that contain varying biochar application rates (8 and 25 ton per hectare), varying soil amendment types (raw Miscanthus and pyrolized Miscanthus), and biochar added in different years (2010 and 2014). Three treatments with varying wetting conditions and energies, namely fast wetting, slow wetting, and wet stirring after pre-wetting (further referred to as shaking), that simulate the different mechanisms of aggregate breakdown, were applied on all biochar treatments. This study will address the knowledge gap on how biochar addition to a clayey soil influences aggregate stability, and by which aggregate breakdown mechanisms biochar is detached from the soil.

2 Objective and research question

2.1 Problem statement

The assumed beneficial effect of biochar to soil quality will be reduced when the biochar is detached from the soil and transported out of the intended application area. To what extent the biochar is transported out of the field during an erosion event, and which mechanisms play the largest role in this process, is still unknown.

2.2 Objectives

- To understand the influence of the addition of biochar to a Norwegian silty clay loam soil on aggregate stability;
- To understand which mechanisms of aggregate breakdown influence the detachment of biochar particles from the soil during an erosion event.

The following hypothesis will be tested:

1. Biochar amendment to a silty clay loam soil creates a higher soil aggregate stability, where a higher biochar content and longer amendment duration give a higher aggregate stability.

This hypothesis will be tested by comparing aggregate stability of soils with two different types of biochar, different biochar application-rates and different application years.

2. Biochar-amendment reduces aggregate destruction of silty clay loam aggregates (further referred to as clayey aggregates) by reducing the effect of clay swelling and slaking.

This will be tested by applying different methods (fast wetting, slow wetting and shaking) to the aggregates.

3 Background

3.1 What is biochar?

Biochar is a carbon-rich product that is made from heated biomass (e.g. wood, manure, leaves) at temperatures ranging from 200 to 700°C, and under limited oxygen supply (Lehmann & Joseph, 2009; Pratt & Moran, 2010). This process is called pyrolysis, as shown in figure 7. Figure 7 shows that biochar may be the byproduct of energy production.

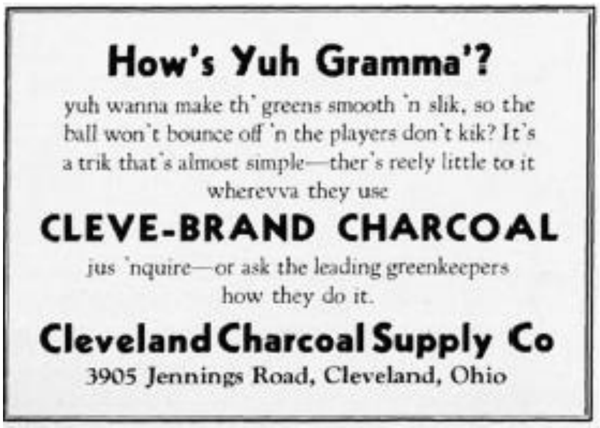

Figure 1 Advertisement for BC to be used as a soil amendment in turf greens. Source: The National Greenkeeper (1933)

The term 'biochar' is relatively new, emerging from Lehmann et al. (2006), where biochar is used to describe the charred organic matter that is applied to the soil with the intention to improve soil properties, as opposed to 'charcoal', which is used for industrial purposes, such as fuel or filter. Nevertheless, the addition of charcoal to soil has been used for a longer time (figure 5). In 1804, Young (1804) finds that farm revenue significantly increased after 'paring and burning' soil with organic matter, but even before this finding ancient Japanese texts on agriculture already described 'fire manure' (Lehmann & Joseph, 2009).

'Biochar is not merely another type of compost or manure that improves soil properties, but is much more efficient at enhancing soil quality than any other organic soil amendment.'

Quote: Lehmann & Joseph, 2009

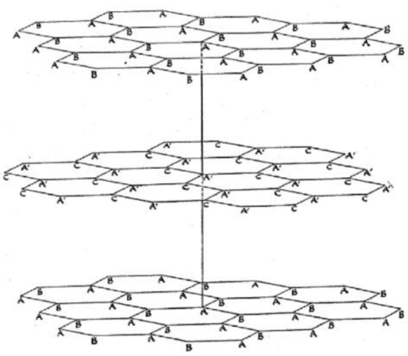

Figure 2 Structure of graphite (Bernal, 1924)

From a chemical point of view, biochar is more difficult to describe. Lehmann and Joseph (2009, p. 1) state that '*the defining property is that the organic portion of biochar has a high C content, which mainly comprises so-called aromatic compounds characterized by rings of six C atoms linked together without oxygen or hydrogen, the otherwise more abundant atoms in living organic matter. If these aromatic rings were arranged in perfectly stacked and aligned sheets, this substance would be called graphite*'. In 1924, J.D. Bernal was the first to characterize the crystal structure of graphite (Figure 6). The temperatures used to create biochar, will form more irregular arrangements of C, and will include O and H, and sometimes mineral compounds (Downie et al., 2009). Biochar has a much higher mean residence time in soils compared to other forms of organic matter (Schmidt et al., 2011), due to the graphite-like layers that contains carbon atoms that are strongly bound to each other (Downie et al., 2009). Ziolkowski and Druffel (2010) assume the residence time of the most stable organic matter fraction to be around 8000 years.

An interest in biochar only developed recently, due to the discovery of 'Terra Preta' soils. The Terra Preta soils contain high amounts of organic C, retain water better, and are more fertile than the surrounding soils of the same base material (Glaser & Birk, 2012). The improved properties of the Terra Preta soils are assumed to come from fire derived organic substances added by humans in pre-Columbian times (Glaser & Birk, 2012; Sagrilo, 2014), which served as a basis for the idea of biochar.

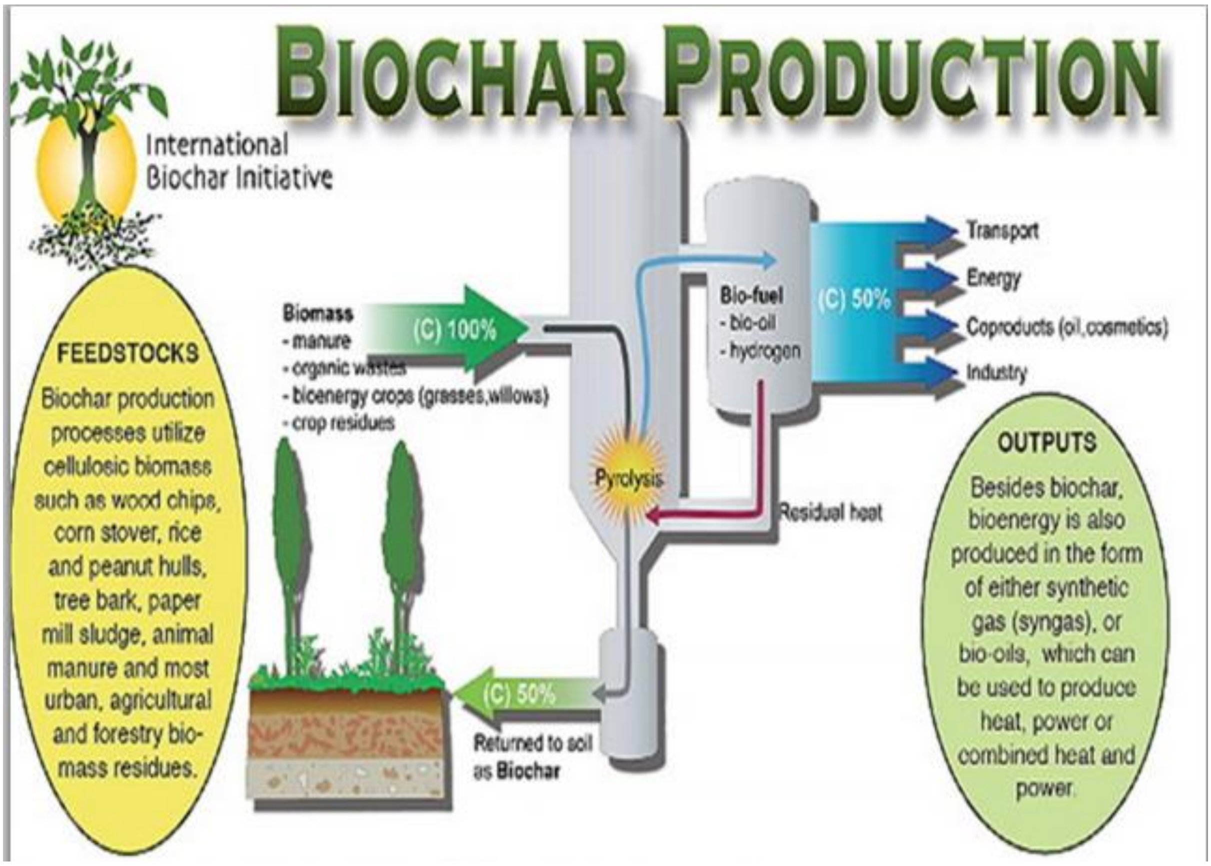

Figure 3 The process of Biochar production. Source: The International Biochar Initiative http://www.biochar-international.org

3.2 Benefits of biochar

The application of biochar to the soil can sequestrate carbon (C) (Jeffery et al., 2013), change soil chemical properties, e.g. pH and CEC (Liang et al., 2006), and change soil physical properties, e.g. soil water retention (Ouyang et al., 2013; Sun & Lu, 2014), hydraulic conductivity (Major et al., 2010), and enhance soil fertility (Jien & Wang, 2013; Lehmann & Joseph, 2009). As explained by Jien and Wang (2013), due to the biochemically protected (recalcitrant) nature of biochar, and its inherent charged surface with organic functional groups, the addition of biochar to the soil can maintain SOM levels for a long extent of time, and increase the aggregate stability of the soil for millennia (Ziolkowski & Druffel, 2010).

However, data on the effect of biochar amendment to the soil on the aggregate stability is scarce and different studies give conflicting outcomes (Mukherjee & Lal, 2013). In general, it is assumed that the aggregate stability can be affected by biochar amendment through both direct and indirect effects (Mukherjee & Lal, 2013). Masulili et al. (2010) and Sun and Lu (2014) found that biochar amendment increased the soil water retention, and hypothesized that this effect was caused by an increased aggregate stability, even though they did not measure the aggregate stability. Mukherjee and Lal (2013) summarize that '*the aggregation percentage may decrease with biochar addition*', but they also hypothesize that in the long run the addition of biochar might change the soil properties, and that over time, biochar particles may form complexes that stimulate the formation of aggregates. Ouyang et al. (2013) found that biochar addition does significantly enhance the formation of macro-aggregates (250-2000 µm) in a silty clay soil, and to an even greater extent in a silty loam soil. Jeffery et al. (2015) found no significant effect of the addition of biochar to a silty soil on the aggregate stability three years after the application.

In general, impacts of biochar vary dependent on the soil type and biochar type. The properties of the biochar depend on the feedstock type and the conditions under which the pyrolysis took place (e.g. temperature, timeframe) (Jeffery et al., 2013). Also, the time range varies over which these benefits appear, and benefits may trade-off against each other (Jeffery et al., 2013). For example, increased plant-water availability due to increase in water-holding capacity by the high porosity of biochar particles develops slower, but has a longer effect than direct nutrient availability, which is likely to be used or leached from the system over a short period (Jeffery et al., 2013). In many cases, it is not possible to maximize all benefits at the same time, and even negative effects can occur (Jeffery et al., 2013).

3.3 Biochar and erosion

If biochar is eroded and transported out of the field, the positive effects mentioned above will diminish (Fister et al., 2014). Preferential mobilization and redistribution of biochar is likely due to its low bulk density (Fister et al., 2014). To prevent that the biochar is eroded by either wind or water, soil cover (Lehmann & Joseph, 2009) or mixing of the biochar in the topsoil is required, which again may trade-off against the benefits of no-till farming (Jeffery et al., 2013). Especially for regularly cultivated areas that are vulnerable to soil erosion, it is relevant to know how vulnerable biochar is to be disintegrated and transported from the field (Firster et al., 2014) and which are the main mechanisms for this disintegration. This research will use the concept of aggregate stability and the main mechanism by which aggregate breakdown occurs, to investigate how biochar is detached from the soil and transported out of the field.

3.4 Aggregate breakdown

"Soil aggregates are groups of soil particles that bind to each other more strongly than to adjacent particles" – USDA, 1996, p.1

Because of coherence of soil particles, formed by either aggregation or fragmentation processes (Nimmo & Perkins, 2002), pore space in the soil is created within and between the aggregates (USDA, 1996). The ability of the aggregates to maintain their structure when they are exposed to disruptive forces is called aggregate stability (Amézketa, 1999; USDA, 1996). The amount of present stable aggregates determines the soil structure and pores, and therefore aggregate stability affects the hydrology, aeration, nutrient availability, erosion and biological activity of the soil (Amézketa, 1999).

Measurements of aggregate stability can be an indicator of soil quality and can be used to estimate soil properties like the potential of a soil to erode or crust (Amézketa, 1999; USDA, 1996) under different circumstances or (agricultural) management (Nimmo & Perkins, 2002). Different methods to measure aggregate stability exist because:

1) Destabilization can be produced by mechanisms like slaking, clay dispersion and clay swelling
2) Stability can be determined at different scales
3) Differences in methodology, like the conditions or characteristics of the samples, the choice of treatment, the measurement of the treatment (disaggregation and dispersion are often not clearly distinguished) and/or the choice of stability parameter (Amézketa, 1999).

Measurement of aggregate stability is complicated, due to the interrelationships of the variables influencing aggregate stability. The main problem of measuring aggregate stability is that the destructive force and the aggregate size cannot be measured independently, because the aggregate sizes depend on the destructive force utilized to disrupt them (Nimmo & Perkins, 2002). The methodology can influence the emphasis on either stability or size, but these can never truly be separated. The methodology will depend on the research objectives. This is also the case when simulating conditions, as for example, the stability of wet or dry aggregates is used for different research purposes. The stability of dry aggregates determines wind erosion potential, while the stability of wet aggregates relates to the understanding of the soil-water behaviour, the hydraulic properties like field infiltration, or for example the formation of surface seal (Castellanos-Navarrete et al., 2013; Nimmo & Perkins, 2002).

Also, wetting in vacuum is used for different purposes than for wetting without vacuum. Wetting aggregates in vacuum, and also slow aerosol wetting (Kemper & Rosenau, 1986), reduces the disruptive forces of entrapped air (Nimmo & Perkins, 2002), which can be preferable if the aggregates are being prepared for aggregate stability tests with for example a rainfall simulator or wet-sieving apparatus. If no alternative method to measure aggregate stability is used, it is preferable to wet without vacuum, to simulate natural conditions.

Le Bissonnais (1996) distinguishes the main mechanisms of aggregate breakdown, namely breakdown by compression of trapped air (slaking), breakdown by differential swelling, mechanical breakdown by raindrop impact and physico-chemical dispersion. In order to investigate the main mechanisms of aggregate breakdown, Le Bissonnais (1996) proposes the following three tests, namely fast wetting, slow wetting and wet-stirring (further referred to as shaking). The behaviour of slow wetting a soil, by for example a gentle rain, can be investigated by slow wetting of soils with controlled tension. The fast wetting emphasizes the slaking compared to the other breakdown mechanisms, but in principle, both slaking and clay swelling are dependent on the same properties, including the rate of wetting. The only difference is when you look at the clay content, namely that the breakdown by slaking decreases with an increasing clay content, while the differential swelling increases with an increasing clay content (Le Bissonnais, 1996). Thus, while simulating slaking, the simulation of clay swelling cannot be completely avoided, but the fast wetting does emphasize the slaking mechanisms. Therefore, fast wetting can be used to compare the behaviour of rapid wetting of soils, for example due to a heavy rainstorm event. The shaking can be used to investigate the wet mechanical cohesion of aggregates, without the influence of the slaking mechanism. This means that the air needs to be removed from the aggregates before energy is employed, by either rewetting under vacuum, or by rewetting with a nonpolar liquid (Le Bissonnais, 1996) like ethanol (Hénin et al., 1958).

3.5 Study area

In 2010, a field experiment on silty clay loam soils with pyrolized Miscanthus addition (also referred to as biochar) was started by Adam O'Toole, M. Carnol, C. Moni, H. Silvennionen and D. Rasse, as part of research for Bioforsk, currently called NIBIO. The field experiment is located in Ås, Norway (Figure 1). The soil type is an *Inceptisol*, consisting of silty clay loam (Annex VII), with a total organic carbon content (TOC) of 2.5%.

The main objectives of their research are to: 1) study how stable biochar is under field conditions and how this affects the soil carbon cycling; 2) study the effects on N_2O emissions under field conditions and how this changes after biochar ageing; and 3) investigate the effect of biochar addition on soil and crop.

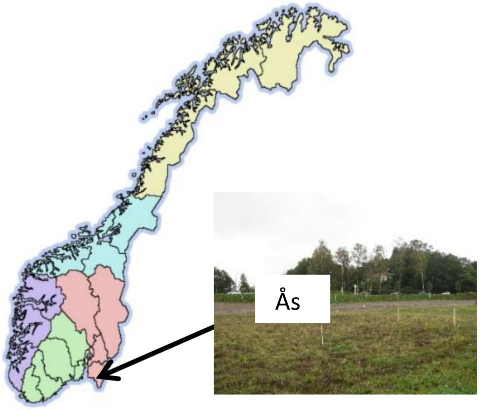

Figure 4 Location indication and photo of Biochar plots in Ås Norway

In 2010, 4 treatments with 4 replica's each were set up. Plots of 8 by 4 meters (Figure 2), have the following application rates of biochar and year of implementation:

- Plots 2, 5, 11, and 16 are control with no biochar
- Plots 3, 8, 9, 14 are biochar 25 t/ha added in 2010
- Plots 1, 6, 12, 15 are biochar 8 t/ha added in 2010
- Plots 4, 7, 10, 13 are plots with 8 t/ha of the raw Miscanthus Giganteus added in 2010

Table 1 Crops produced on the experimental plots in Ås with biochar amendment per year

Year	**Crop production**
2011	Oats
2012	Barley
2013	Wheat
2014	Oats
2015	Fallow

In 2014, four more plots were created within the same field and a biochar application rate of 25 t/ha was applied

- Plots 2a, 4a, 5a, 13a, are biochar 25 t/ha added in 2014

The biochar was produced by Pyreg Gmbh, located in Germany. General information on the biochar production can be found in Table 2. The type of crops produced on the plots with biochar amendment can be found in Table 1.

13 8 t/ha Raw Misc. 2010	**14** 25 t/ha Pyrolized Misc. 2010	**15** 8 t/ha Pyrolized Misc. 2010	**16** Control
9 25 t/ha Pyrolized Misc. 2010	**10** 8 t/ha Raw Misc. 2010	**11** Control	**12** 8 t/ha Pyrolized Misc. 2010
5 Control	**6** 8 t/ha Pyrolized Mics. 2010	**7** 8 t/ha Raw Misc. 2010	**8** 25 t/ha Pyrolized Misc. 2010
1 8 t/ha Pyrolized Misc. 2010	**2** Control	**3** 25 t/ha Pyrolized Misc. 2010	**4** 8 t/ha Raw Misc. 2010

Figure 5 Plots, each of 8m x 4m, with Biochar addition to the soil. Specifics are provided within the blocks.

Table 2 General information on the biochar production for the experimental design executed in Ås, Norway. From: Rasse et al. (In prep.)

Property	**Units**	**Raw Miscanthus feedstock**	**Pyreg Miscanthus (biochar)**
Pyrolysis temperature	°C	n/a	650-750
Volatile Matter	%	78.0	7.0
Fixed C	%	13.5	70.5
Ash	%	8.5	22.5
C	%	47.9	79.3
H	%	6.1	0.5
N	%	0.2	1.1
O	%	51.0	5.5
BET	M^2/g (N_2)	?	348

The biochar was added to the soil by inverse ploughing in the fall of 2010, creating a layer of biochar particles on a depth of approximately 15 cm (Figure 3). In the autumn of 2011, the biochar was ploughed back to the surface, indicating that the biochar was not well incorporated into the soil (Figure 4).

Figure 6 Biochar incorporation into the soil (fall of 2010)

Figure 7 Biochar ploughed back to the surface (autumn of 2011)

4 Materials and methods

4.1 Soil sampling & preparation

4.1.1 Soil sampling

Soil samples were gathered from the biochar plots created in 2010 and 2014 that have been described in Chapter 3. A spade was used to fill bags of 40 x 50 cm with topsoil (0 – 15 cm) for each of the biochar treatments:

- control: control plot created in 2010;
- mc8: 8 t/ha raw Miscanthus added to the soil in 2010;
- bc8: 8 t/ha Miscanthus biochar added to the soil in 2010;
- bc25: 25 t/ha Miscanthus biochar added to the soil in 2010;
- new: 25 t/ha Miscanthus biochar added to the soil in 2014.

Plant materials, organisms, and soil that were compacted by the spade were excluded or removed from the bags. During this stage, bigger aggregate clumps (> 30 mm) were manually broken into smaller aggregate clumps using as little force as possible.

During the soil sampling, high microbial activity was noticed based on the amount of worms that needed to be removed from the gathered soil.

4.1.2 Drying & sieving

After soil sampling, the aggregates were air-dried in an 'air-drying room' at the NIBIO lab for one week. The samples were dry-sieved into aggregate size fractions of >20 mm, 6 – 20 mm, 2 – 6 mm, 0.6 – 2 mm and <0.6 mm. To understand the influence of the addition of raw and pyrolized Miscanthus to the silty clay loamy soil on aggregate stability (objective 1), each fragment was weighted, to determine the size-distribution of the dry aggregates according to Eq. (1) adjusted from Regelink et al. (2015):

$$MWD_{dry} = \sum_{i=1}^{n} \frac{w_i}{100} \overline{d_\iota} \qquad (1)$$

Where MWD_{dry} = mean diameter (mm)
n = number of aggregate fractions
$\overline{d_\iota}$ = mean diameter of the ith fraction
w_i = weight of soil in the fraction *i* expressed as a percentage of the dry soil mass

To test whether the aggregates were completely dry, 1 sample of 4 grams for each treatment were weighed and put in the oven for 24 hours on 40°C. The weight of the samples after oven-drying was the same as the weight of the samples before over-drying, and therefore it was concluded that the samples were fully dried. The samples were moved to a cool environment (around 4°C) until further analysis.

4.2 Aggregate stability measurement

To understand which mechanisms of aggregate breakdown influence the detachment of biochar particles from the soil during an erosion event (objective 2), the aggregate stability was determined according to the following methods: the method of Le Bissonnais (1996) adjusted by INRA (2015), and the Wet-Sieving Method (Kemper & Rosenau, 1986) adjusted by Eijkelkamp (2008). For all methods aggregate size fractions of 2 – 6 mm were used as the standard test size following Njøs (1967) (Grønsten & Børresen, 2008). The descriptions of the methods can be found in Annex II.

For the drying after the aggregate stability tests, ethanol was used to wash out aggregates left on the sieve to prevent the re-aggregation of the aggregates during drying.

The MWD was calculated after the following aggregate stability tests were executed; fast wetting, slow wetting and shaking. The aggregate stability was classified according to Table 3.

Table 3 Classes of stability and crustability according to MWD values measured with the three treatments offered by Le Bissonnais (1996)

Class	**MWD value (mm)**	**Stability**	**Crustability**
1	< 0.4	Very unstable	Systematic crust formation
2	0.4 – 0.8	Unstable	Crusting frequent
3	0.8 – 1.3	Medium	Crusting moderate
4	1.3 – 2.0	Stable	Crusting rare
5	> 2.0	Very stable	No crusting

4.3 Statistical analysis

4.3.1 One-way ANOVA

One-way ANOVA's (Analysis of Variance) (Field, 2000) were used to analyse whether a significant difference exists between the mean weight diameter while looking at the biochar application rate, the application year and the biochar type (Table 4).

Table 4 Overview of the different treatment types biochar rate, soil amendment type and amendment duration

Biochar rate	**Soil amendment type**	**Amendment duration**
8 tonnes per hectare of biochar added in 2010	8 tonnes per hectare of pyrolized Miscanthus (biochar) added in 2010	25 tonnes per hectare of biochar added in 2010
25 tonnes per hectare of biochar added in 2010	8 tonnes per hectare of raw Miscanthus straws added in 2010	25 tonnes per hectare of biochar added in 2014

A one-way ANOVA only shows whether significant differences exists between the means of two or more independent groups. When looking at multiple groups, the one-way ANOVA does not show between which groups a significant difference exists, or in what way these groups differ from each other (Laerd, 2013). Therefore, a post hoc test was needed to know which groups showed a significant difference with one another.

The results were validated by checking the six assumptions that are required for giving valid results after running a one-way ANOVA (Laerd, 2013).

1) The dependent variable is measured at interval or ratio level.
2) The independent variable consists of two or more categorical, independent groups.
3) The observations in each group are independent.
4) There should be no significant outliers.
5) The dependent variable is approximately normally distributed for each category of the independent variable.
6) There needs to be homogeneity of variance.

When assumptions 1 to 3 were met, assumptions 4 to 6 were checked using the SPSS Statistics Program by creating boxplots for assumption 4, by using the Shapiro-Wilk test for normality for assumption 5 and by using Levene's test for homogeneity of variances to check assumption 6.

4.3.1.1 Shapiro-Wilk test for normality

If the Shapiro-Wilk test for normality is not significant ($p>0.05$), the distribution is probably normal. In most cases, the data was normally distributed. Only the data resulting from the wet-sieving apparatus was not normally distributed ($p<0.05$). Different transformations (logarithmic, exponential, square root and reflection) but none resulted in a normally distributed data set. Therefore, the non-parametric version of ANOVA, the ‘Kruskal Wallis test’ was used.

4.3.1.2 Levene’s test for homogeneity of variances

If the Levene’s test for homogeneity of variances is not significant ($p > 0.05$), the assumption of homogeneity of variances is met. This was the case for all data sets (Annex V).

4.3.2 Pearson’s correlation

To check whether the soil texture differences within the field (Annex VII) have an influence on the aggregate stability, the Pearson’s correlation will be checked on the data for the control plots.

5 Results

5.1 Aggregate stability in biochar-amended soils

Contrary to expectations, aggregate stability measured as Mean Weight Diameter (MWD) after dry-sieving the aggregates into fragments sizes of >20 mm, 6 – 20 mm, 2 – 6 mm, 0.6 – 2 mm and <0.6 mm, was not significantly (p=0.271) affected by the rate of biochar added to the silty clay loamy soil in the field experiment in Ås, Norway, neither by the type of soil amendment that was added (p=0.228), nor the duration the biochar has been in the soil (p=0.216) (Annex VI). Amendments to the soil even caused for slight decreases in MWD after dry-sieving (Figure 8).

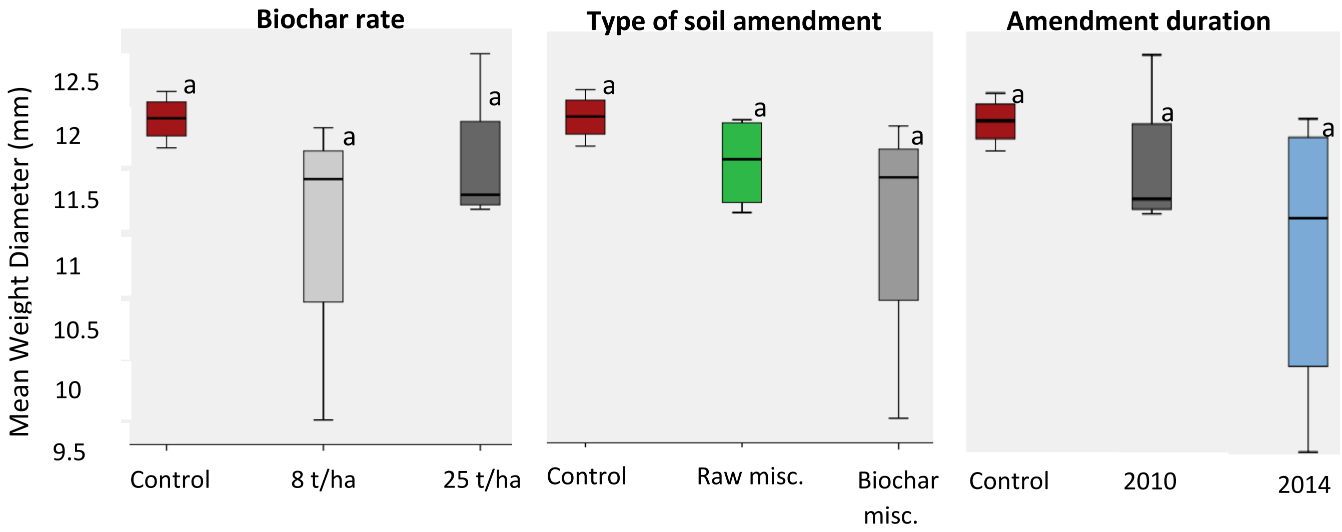

Figure 8 Boxplots of Mean Weight Diameter (mm) of different rates of biochar addition (8 and 25 t/ha), different types of soil amendments (raw Miscanthus and pyrolized Miscanthus) and different years of biochar application (2010 and 2014). Bars with different letters differ significantly (p<0.05).

5.1.1 Effect of the rate of biochar addition

MWD was slightly but not significantly (p=0.271) affected by the rate of biochar added to the silty clay loamy soil in the field experiment in Ås (Annex VI). A higher amount of biochar addition did not lead to a higher increase or decrease in MWD (Figure 9).

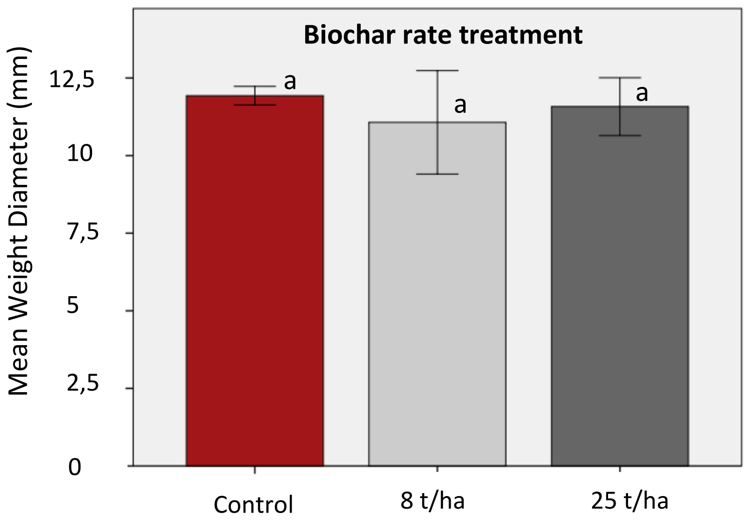

Figure 9 Bar plot of the MWD (mm) for the biochar rate treatment with 8 and 25 t/ha of biochar added to the soil in 2010. Bars with different letters differ significantly (p<0.05). Error bars: 95% CI.

5.1.2 Effect of soil amendment types

MWD was slightly but not significantly (p=0.228) affected by the type of soil amendment (Annex VI). Slight decreases can be observed after 8 t/ha of raw Miscanthus or pyrolized Miscanthus addition to the silty clay loamy soil in the field experiment in Ås (Figure 10), but these were not significant.

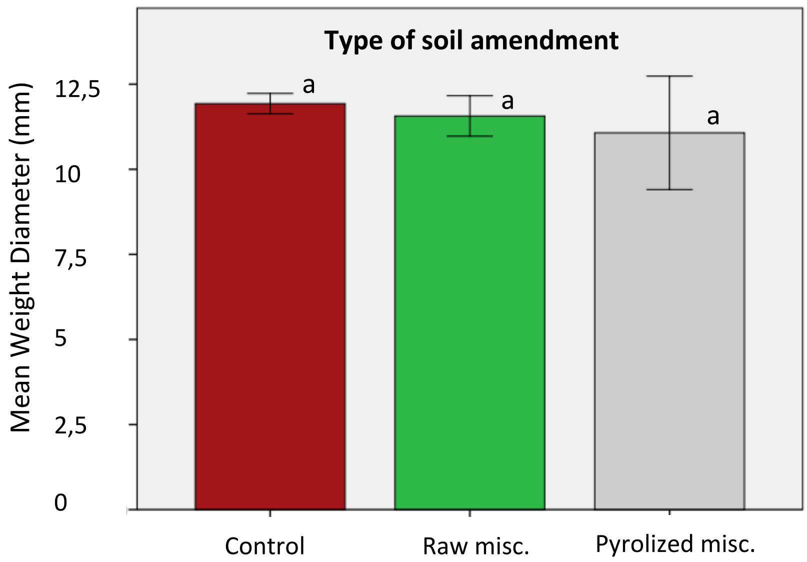

Figure 10 Bar plot of the MWD (mm) for the two types of soil amendment with 8 t/ha of raw Miscanthus and 8 t/ha of pyrolized Miscanthus added to the soil in 2010. Bars with different letters differ significantly (p<0.05)

5.1.3 Effect of amendment duration

MWD was slightly but not significantly (p=0.216) affected by the amendment duration of the 25 t/ha biochar addition to the silty clay loamy soil in the field experiment in Ås. Also, a longer amendment duration did not lead to a higher increase in MWD (Figure 11) as was expected in the hypothesis that a longer biochar amendment duration would increase aggregate stability in a silty clay loamy soil.

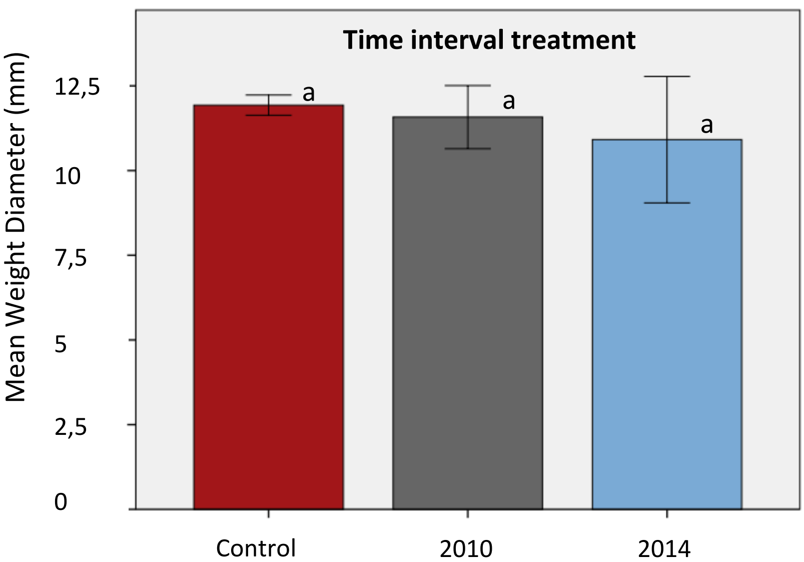

Figure 11 Bar plot of the MWD (mm) for the amendment duration treatment with 25 t/ha of biochar added to the soil in 2010 and in 2014. Bars with different letters differ significantly (p<0.05)

5.2 Effect of aggregate breakdown mechanisms

Significant differences ($p<0.05$) in aggregate stability in aggregates of 2 – 6 mm size were found between fast wetting, slow wetting and shaking within every treatment (biochar rate, soil amendment type and amendment duration). Below, differences between treatments per experiment will be further analysed.

5.2.1 Effect of fast wetting

The presence of biochar the silty clay loamy soil in the field experiment in Ås, Norway, did not lead to significant differences in MWD during fast wetting of the aggregates (Table 5 and Annex VII). Furthermore, higher amounts of biochar did not cause higher stabilization of aggregates, but fresher biochar (2014) did cause for a slight, yet quite insignificant ($p=0.473$) stabilization of aggregates during fast wetting (Table 5a and Table 5c).

Table 5 Effect of biochar rate, soil amendment type and amendment duration on MWD (mm) after fast wetting. Numbers with different letters differ significantly (p<0.05)

a. Biochar rate		
	Mean MWD (mm)	**Std. Error**
Control	0.78^a	0.06
Bc8	0.84^a	0.05
Bc25	0.81^a	0.15

b. Type of soil amendment		
	Mean MWD (mm)	**Std. Error**
Control	0.78^a	0.06
Mis	0.86^a	0.12
Bc	0.84^a	0.05

c. Amendment duration		
	Mean MWD (mm)	**Std. Error**
Control	0.78^a	0.06
2010	0.81^a	0.15
2014	0.95^a	0.06

5.2.2 Effect of slow wetting

The presence of raw Miscanthus or pyrolized Miscanthus in the silty clay loamy soil in the field experiment in Ås, Norway did not lead to a significant increase in MWD during slow wetting of aggregates (Table 6b and Annex VII). Higher amounts of biochar and longer amendment duration only slightly, yet insignificantly ($p>0.05$) increased the MWD (Table 6a, Table 6c and Annex VII).

Table 6 Effect of biochar rate, soil amendment type and amendment duration on MWD (mm) after slow wetting. Numbers with different letters differ significantly (p<0.05)

a. Biochar rate		
	Mean MWD (mm)	**Std. Error**
Control	1.44^a	0.18
Bc8	1.65^a	0.20
Bc25	1.60^a	0.22

b. Type of soil amendment		
	Mean MWD (mm)	**Std. Error**
Control	1.44^a	0.18
Mis	1.72^a	0.17
Bc	1.65^a	0.20

c. Amendment duration		
	Mean MWD (mm)	**Std. Error**
Control	1.44^a	0.18
2010	1.60^a	0.22
2014	1.64^a	0.25

5.2.3 Effect of shaking

The presence of biochar in the silty clay loamy soil in the field experiment in Ås, Norway, did not lead to significant differences ($p>0.05$) in MWD during shaking of the aggregates (Table 7 and Annex VII). Shaking slightly, yet insignificantly ($p>0.05$) decreased MWD for higher amounts of biochar and longer amendment duration (Table 7a and Table 7c).

Table 7 Effect of biochar rate, soil amendment type and amendment duration on MWD (mm) after mechanical breakdown by shaking. Numbers with different letters differ significantly (p<0.05)

a. Biochar rate		
	Mean MWD (mm)	**Std. Error**
Control	3.33^a	0.09
Bc8	3.18^a	0.06
Bc25	3.03^a	0.27

b. Type of soil amendment		
	Mean MWD (mm)	**Std. Error**
Control	3.33^a	0.09
Mis	3.39^a	0.06
Bc	3.18^a	0.06

c. Amendment duration		
	Mean MWD (mm)	**Std. Error**
Control	3.33^a	0.09
2010	3.03^a	0.27
2014	2.72^a	0.11

5.2.4 Effect of wet-sieving

Contrary to the results found after dry-sieving (Chapter 5.1), significant differences were found after wet-sieving the different biochar rates and the different soil amendment types (Table 8a and Table 8b). A significant decrease (p=0.033) in aggregate stability was found after applying 8 t/ha of biochar (pyrolized Miscanthus) to the silty clay loamy soil in the field experiment in Ås, Norway (Table 8).

Table 8 Effect of biochar rate, soil amendment type and amendment duration on aggregate stability (%) after wet-sieving, with 100% meaning optimal aggregate stability and 0% meaning no aggregate stability. Numbers with different letters differ significantly (p<0.05).

a. Biochar rate		
	AS (%)	**Std. Error**
Control	96.8[a]	0.00
Bc8	93.9[b]	0.02
Bc25	97.1[a]	0.00

b. Type of soil amendment		
	AS (%)	**Std. Error**
Control	96.8[a]	0.00
Mis	97.0[a]	0.00
Bc	93.9[b]	0.02

c. Amendment duration		
	AS (%)	**Std. Error**
Control	96.8[a]	0.00
2010	97.1[a]	0.00
2014	96.7[a]	0.01

5.3 Correlation between clay content and aggregate stability

No significant correlations could be found between the MWD and the percentage of sand, silt or clay (Table 9).

Table 9 Statistics on the Pearson Correlation between the mean MWD and the percentage of sand, silt and clay between the control samples

		Sand	**Silt**	**Clay**
MWD	*Pearson Correlation*	0,234	-0,198	-0,358
	Sig. (2-tailed)	0,766	0,802	0,642

6 Discussion

6.1 Aggregate stability in biochar-amended soils

Contrary to expectations, the addition of biochar did not significantly (p>0.05) improve the aggregate stability of the silty clay loam soil from a field experiment located in Ås, Norway (Figures 9, 10 & 11). The expectations that an increasing biochar-amendment rate or an increasing amendment duration will result in a higher aggregate stability were not found (Figures 9 and 11). Even though different studies give conflicting outcomes on this topic (Chapter 3.4), in general it is concluded that biochar amendment improves aggregate stability (Mukherjee & Lal, 2013). The biological and chemical properties of biochar can bind carbon and the mineral parts of the aggregates, which increases the internal cohesion of aggregates, which increases resistances of aggregates to slaking and differential swelling of clay (Sun & Lu, 2014). Why the biochar-amendment did not significantly improve the aggregate stability will be discussed below.

The aggregate stability of the silty clay loam soil from the experimental field in Ås, Norway, is already high when looking at the results from the wet-sieving (Table 8), with the 8 t/ha biochar amendment having the lowest aggregate stability, namely 93.9% (with 100% as the optimal aggregate stability). The experimental field already almost reached optimal conditions for aggregate stability, which could be caused by three different mechanisms; 1) high amounts of clay minerals (Annex VIII) interact with the organic matter, therefore stimulating aggregation (Angers, 1998); 2) high microbial activity as observed in the field (Chapter 4.1.1) that is likely to replenish the potentially exchangeable C pool through processing of less soluble OM (Sanderman et al., 2008). On top of that, fresh C substrates are probably utilized by microorganisms, and therefore unable to contribute to the Dissolved Organic Carbon (DOC) pool (Sanderman et al., 2008); or 3) drying of the clayey aggregates results in re-aggregation, causing for an overestimation of the aggregate stability. As found by Amézketa (1999), the aggregate stability could be increased by strengthening of the bonds due to drying. The drying of the soil causes for an increased negative pressure in the water, which pulls suspended mineral particles together, breaking the bonds at contact points, while at the same time concentrating soluble compounds in the liquid phase. These solutes (silica, carbonates and organic molecules) are then precipitated around the contact points, stabilizing the aggregates (Amézketa, 1999). In dried soils with higher concentrations of clay or organic material, this effect will be higher, as proven by Six et al. (2004). On the other hand, Lehrsch and Jolley (1992) found that the collapse of aggregates can occur during drying due to air entrapment, which can again cause for a undestimation of the aggregate stability.

Furthermore, whether or not biochar-amendment has an influence on aggregation, is dependent on the soil texture (Ouyang et al., 2013). Ouyang et al. (2013) found that in a silty clay soil, biochar amendment did not significantly improve the aggregate stability. Liu et al. (2012) found that biochar amendment only significantly improved the aggregate stability of silt loam soils, but not of silty loam soils. Jeffery et al. (2015) also found no significant effect of the addition of biochar to a silty soil on the aggregate stability three years after the application. In this research, biochar-amendment to a silty clay loam soil did not influence aggregate stability, which is in agreement with Ouyang et al. (2013).

Another reason why the biochar-amendment did not significantly improve the aggregate stability could be due to the high-temperature pyrolysis (around 600°C) when creating the biochar that was used for this experiment. Biochar properties are dependent on both the pyrolysis temperature (Figure 12) and the feedstock type (Jindo et al., 2014; Lehmann, 2007). A biochar created at 600 °C has a higher recalcitrant character, a higher C content, a larger surface area and higher adsorption characteristics as a biochar obtained at a lower temperature (~400°C) (Jindo et al., 2014). On top of that, high pyrolysis temperatures in general cause for the biochar to have low hydrophobicity (Aston et al., 2014; Gray et al., 2014; Jeffery et al., 2015), yet it is also dependent on the feedstock of the biochar (Aston et al., 2014).

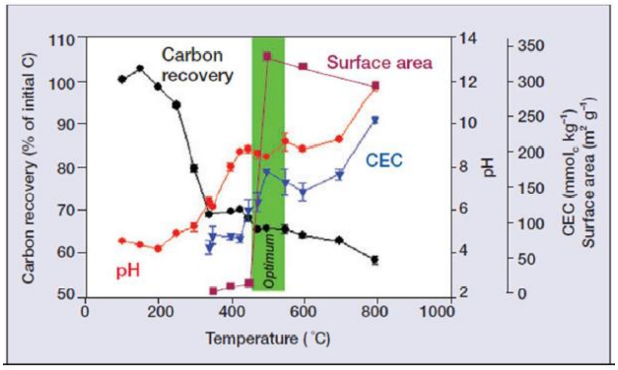

Figure 12 Biochar properties as determined by production temperature (Lehmann, 2007)

The hypothesis that the pyrolized Miscanthus would increase the aggregate stability of the silty clay loamy soil to a higher extent than the raw Miscanthus was not found in the field experiment in Ås (Figure 12). According to multiple researchers (Angers et al., 1993; Bissonnette et al., 2001; Whalen et al., 2003), management systems that increase the C input to a soil also increase the MWD of aggregates. As can be seen in Table 2, the pyrolized Miscanthus has a higher percentage of C and fixed C (79.3% and 70.5%) than the raw Miscanthus (47.9% and 13.5%). Yet, no significant differences were found between the pyrolized Miscanthus, the raw Miscanthus and the control plots. Therefore, no relation between the C input and the aggregate stability was found in this study. This is probably due to the fact that most of the raw Miscanthus is already degraded after an amendment duration of 5 years, decreasing the expected effects. The establishment of aggregates forms over time and is dependent on the interaction of chemical and physical properties of the biochar and of the soil and its biological community (Herath, 2012). Further research needs to be done on the effects of the raw Miscanthus on aggregate stability under different amendment durations, and on different soil properties, in order to draw conclusions on whether or not investing in raw Miscanthus instead of the pyrolized Miscanthus could provide improved soil properties and higher profits on a short-term basis.

6.2 Effect of aggregate breakdown mechanisms

6.2.1 Effect of fast wetting, slow wetting and shaking

In general for this study, the different aggregate breakdown mechanisms (slaking, clay swelling and mechanical breakdown by shaking) impact the aggregate stability to a different extent. The aggregate stability after fast wetting was in all treatments unstable to medium (Table 3 & 5). This is contrary to the very stable aggregate stability after slow wetting (Table 3 & 6) and shaking (Table 3 & 7). The mechanical breakdown by shaking destroyed the aggregates to a lesser extent than slaking and clay swelling. This means that the wet mechanical cohesion of aggregates is strong, and that this gives lower destruction of aggregates then by slaking. The fast wetting emphasizes the slaking compared to the slow wetting, but in principle, both slaking and clay swelling are dependent on the same properties (including rate of wetting). As the aggregate stability is, on average, higher after the slow wetting the aggregates than after fast wetting (Tables 4, 5 and 6), it can be concluded that slaking causes more aggregate destruction than clay swelling in the silty clay loamy soil in the field experiment in Ås, Norway.

Similar as after dry-sieving, raw or pyrolized Miscanthus amendment to a silty clay loam soil did not significantly increase aggregate stability after wetting or shaking. The hypothesis was based on the assumption that the biochar-amended soil have a higher resistance against destructive forces like slaking, clay swelling, and mechanical breakdown. This was confirmed by Herath (2012), who states that the resistance of biochar-amended soils against fast-wetting was significantly higher than the control plots, therefore resulting in a higher aggregate stability. Yet, the expected increase in resistance against the destructive force of fast wetting, slow wetting or shaking due to biochar amendment was not found (Table 5, 6 & 7). Herath (2012) state that less hydrophobicity in biochar-amended soils may have positive implications for soil physical conditions, but Blanco-Canqui et al. (2007) argued that a moderate hydrophobicity could actually improve soil aggregation. This might indicate that the biochar used for this experiment does not have hydrophobic surfaces. Hydrophobicity of the material would reduce water uptake, but not reduce ethanol uptake (Gray et al., 2014). Ethanol is assumed to only be subject to positive capillary forces, therefore nearly saturating all biochar porosity. Depending on the material's surface hydrophobicity, water is subject to positive and negative capillary forces, meaning that different types of biochar have differences in water uptake and accessibility of pores (Gray et al., 2014; Jeffery et al., 2015). Whether the raw Miscanthus and the pyrolized Miscanthus are hydrophobic should be tested in order to confirm whether a lack of hydrophobicity can be a cause for the lack of effect on aggregate stability after soil-amendment of pyrolized biochar or raw Miscanthus.

6.2.2 Effect of wet-sieving

Wet sieving significantly increased the aggregate stability for the raw Miscanthus-amended soil (Table 8) and significantly increased the aggregate stability for the 8 t/ha of biochar-amended soil (Table 8). It is not striking that the wet-sieving apparatus has other outcomes than the fast wetting, slow wetting and wet-sieving proposed by Le Bissonnais (1996), as these former methods distinguish mechanisms of aggregate breakdown, while the wet-sieving apparatus simulates all mechanisms at once. In the wet-sieving apparatus, the resisting influence of the biochar together with the disruptive forces by shaking are combined, either accumulating or mediating effects. When investigating the effect of soil amendment during all aggregate breakdown mechanisms, the positive effects accumulate and cause for the soil to significantly stabilize. Yet, for a higher biochar application rate, or a longer amendment duration, this accumulating effect does not apply (Table 8). This is contrary to expectations, as aggregates form over time and the aggregation is dependent on the interaction of chemical and physical properties of the biochar and of the soil and its biological community (Herath, 2012). A logical explanation for this unexpected result is yet to be found.

6.3 Implications

So is biochar-amendment to the soil really 'The Biochar Solution'? The book written by Bates (2010) caused a hype due to the promotion of benefits of biochar, such as increase in soil carbon sequestration, while boosting food production due to increased beneficial chemical and physical soil properties. The lack of effect found on aggregate stability by soil amendment in this research raises the question whether or not biochar would be worth the investment to improve soil physical properties in the silty clay loamy soil in the field experiment in Ås, Norway, and even whether biochar should be promoted as 'The Biochar Solution'. Of course, a lack of effect on aggregate stability does not mean that biochar amendment has no positive effects at all. Additionally, the lack of effect in a silty clay loam soil does not mean that biochar amendment does not positively affect the aggregate stability of other soil types or on other locations. It also does not mean that other positive effects of the biochar are completely lost (Lehmann & Joseph, 2015), or that the positive effect of biochar amendment on aggregate stability is completely lost, as the horizontal and vertical movement of biochar can also cause for the effects to happen off-site. Horizontal and vertical movement of biochar (and other organic matter types) is complex (Kuhn et al., 2009; Lal, 2003), and could be further studied to support this point. Further research could provide a better understanding of the transport of biochar, and of the effects of biochar on aggregate stability under different site conditions. To stimulate soil carbon sequestration, farmers will need an incentive to apply biochar to their field. Therefore, it will be better to study other possible effects that are assumed to stimulate crop growth, in order to see whether 'The Biochar Solution' does not change into 'Biochar, the Partial Solution'.

7 Conclusions

In general, it can be concluded that biochar applied to a silty clay loam soil does not significantly improve soil aggregate stability after 5 years. The rate of biochar application or the amendment duration did not influence the extent to which the aggregate stability was decreased. The type of material used for the amended soils seems to be of greater importance: raw Miscanthus-amended soils showed more potential to create resistance against disruptive forces than the pyrolized Miscanthus-amended soils, as the aggregate stability was significantly increased after wet-sieving. More research should be done on the influence of the raw and pyrolized Miscanthus on other soil properties to draw conclusions on its profitability for both the soil improvement and crop production. Yet, the lack of effect found on aggregate stability by soil amendment in a silty clay loam soil in this study raises the question whether or not biochar would be worth the investment to improve soil physical properties.

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Appendices

List of Appendices

Annex I: List of equipment

Soil sampling

- Sample boxes
- Plastic bags
- Spade
- Gloves

General for all tests

- Tray
- Pen & marker
- Weight forms
- Sieves of 20, 6, 2, and 0.6 mm

Le Bissonnais (1996) method

- Cups
- Wet-sieving sieves of 0.5mm
- Sieves of 2, 1, 0.5, 0.2 and 0.1
- Erlenmeyer + cork
- Glass beakers
- Ethanol
- Demineralized water

Wet-sieving apparatus

- Wet-sieving apparatus
- Wet-sieving sieves of 0.5mm
- Glass pots
- Aluminium tray to wet sieves
- Ethanol
- Demineralized water

Rainfall simulator

- Rainfall simulator
- Rainfall simulator sieves of 0.5 mm
- Porcelain bowls
- Sedimentation glass
- Extra buckets to catch water
- Filters
- Stopwatch

Annex II: Method descriptions and step-by-step procedure

Method of Le Bissonnais (1996)

This method includes three tests, using various wetting conditions and energies, namely fast wetting (FW), slow wetting (SW) and shaking.

The aggregates will be sieved to 2 - 6 mm and immediately prior to the stability tests, the samples will be put into the oven for 24 hours on 40°C to create uniform test conditions, mainly regarding the soil moisture content. Below, a step-by-step procedure for each test is described. For each test, a sieve of 50 µm will be used, that is placed into a bucket filled with ethanol up to 5 mm above the sieve mesh (Figure 14).

Figure 13 Photo of sieving aggregates on a 5 µm mesh, into bucket filled with ethanol

Fast wetting (FW)

1. Weigh 5 grams of aggregates (6-2mm) into a glass (250 mL). Note the exact weight on a form.
2. Fill the glass up to 50 mL with demineralized water. Make sure not to spray the water directly on the aggregates
3. Let the aggregate immerse for 10 minutes
4. Use a pipette to remove the water from the glass. Be careful not to remove fragments.
5. Put the aggregates on the sieve (mesh size 5 µm), using a flow of ethanol to transport the aggregates from the glass onto the sieve. Be careful not to head the flow of ethanol directly onto the aggregates.
6. Continue to the step-by-step procedure for 'Sieving in ethanol'

Slow wetting (SW)

1. Weigh 5 grams of aggregates (6-2mm) into the sieve. Note the exact weight on a form.
2. Put several filled-up sieves in a can that is located above a (cold-) steam machine.
3. Let the aggregates immerse for several hours, until saturation is achieved. The exact time until saturation depends on soil properties. Check after 1 hour, and spray water 5 times onto the aggregates if no saturation has been reached yet, to stimulate the wetting. Continue the dampening until saturation is reached.
4. Continue to the step-by-step procedure for 'Sieving in ethanol'

Shaking

1. Weigh 5 grams of aggregates (6-2mm) into a glass. Note the exact weight on a form.
2. Put 50 mL of ethanol in a 250 mL glass, and put the aggregates in the ethanol.
3. Let the aggregates immerse for 30 minutes.
4. Use a pipette to remove the ethanol from the glass. Be careful not to remove fragments.
5. Fill an Erlenmeyer with 50 mL of demineralized water.
6. Use the flow of demineralized water to transport the aggregates from the glass into the Erlenmeyer. Be careful not to head the flow of demineralized water directly onto the aggregates.
7. Fill up the Erlenmeyer to 200 mL of demineralized water.
8. Close the Erlenmeyer with a cap or cork.
9. Shake the Erlenmeyer 20 times, as demonstrated by INRA (2015) (http://www6.val-de-loire.inra.fr/ur-sols_eng/content/download/3671/38179/version/1/file/MouvAgitation-anglais.mp4)
10. Let it settle for at least 30 minutes.
11. Use a pipette to remove the demineralized water. Be careful not to remove fragments.
12. Use the flow of ethanol to transport the aggregates from the Erlenmeyer into the sieve. Be careful not to head the flow of ethanol directly onto the aggregates.
13. Continue to the step-by-step procedure for 'Sieving in ethanol'.

Sieving in ethanol

1. Slowly shake both the bucket with the sieve filled with aggregates for a few seconds. The shaking duration is proportional to the amount of fragments to be sieved.
2. Remove the sieve mesh from the ethanol and let the ethanol flow from the sieve into the bucket.
3. Repeat step 1 and 2 a few times.
4. Use ethanol to transport the fragments left in the sieves into a glass.
5. Let it rest for a few minutes.
6. Use a pipette to remove the ethanol from the glass. Be careful not to remove fragments.
7. Put the glass in the oven for 24 hours on 40 °C.
8. After drying, weigh the aggregates. Note the weight on a form (do not forget to note the weight of the glass).
9. Use a soft brush to get the aggregates on a pile of sieves (2mm, 1mm, 0.5mm, 0.2mm, 0.1mm, and 0.05mm)
10. Shake the pile of sieves for a few seconds.
11. Remove the fragments sieve by sieve with a hard brush from the sieves and weigh the amount of soil per fragment size (>2mm, 2-1mm, 1-0.5mm, 0.5-0.2mm, 0.2-0.1, 0.1-0.05mm and <0.05mm).

The method of determining aggregate stability by a single sieve method was first described by Kemper and Rosenau (1986). Combining this single sieve method with the theory of wet-sieving by multiple sieves (De Leenheer & De Boodt, 1959; Hofman, 1973), resulted in the modified multiple wet-sieving method. De Leenheer and De Boodt (1959) used the concept of the Mean Weight Diameter (MWD) that was first presented by Van Bavel (1950) .

$$MWD = \frac{\sum_{i=1}^{i=n} m_i \times d_i}{\sum_{i=1}^{i=n} m_i} \qquad (2)$$

Where m_i = mass of the fraction i (g)
d_i = mean diameter of the fraction i (mm)
n = the total number of the fractions

When MWD values are obtained, the stability and crustability of the soil can also be categorized into the classes as shown in table 3, provided by Le Bissonnais (1996). Le Bissonnais (1996) describes the MWD as '*the sum of the mass fraction of soil remaining on each sieve after sieving multiplied by the mean aperture of the adjacent mesh*'.

Wet-Sieving Apparatus

The method of the Wet-Sieving Apparatus to determine the aggregate stability was first described by Kemper and Rosenau (1986). The method as described below is an adjusted version as the method described by Eijkelkamp (2008). The method as described by Eijkelkamp (2008) involves a second sieving in a chosen solution dependent on the pH, to disrupt all aggregates, leaving stones and organic material on the sieve. After the second sieving, the ratio unstable aggregates (disrupted by the first sieve) versus stable aggregates (disrupted by the second sieve) can be determined, excluding the weight of the stones and organic material. Within this research, the second sieving will not be executed, as it is preferred to keep the stable aggregates in one piece, in order to be able to separate loose biochar particles from these stable aggregates. Some biochar particles will be too big to flow through the sieve, but will be regarded as they have been gone through the sieve, in order to establish the biochar left within the aggregates and the biochar that loosened from the soil after the treatment. The following steps will be executed:

Weighing and preparation

1. Locate the required number of round plastic boxes, and weigh them
2. Way 25 g soil (2-6 mm aggregates) from each cylinder in the cup, and note the weight of the cup + soil
3. Air-dry the samples for two days
4. Locate the numbered sieves with 0.5 mm mesh size (there are also 0.26 mm sieves) and write down the weight of each sieve on the form
5. Moisture the sieves and allow them to drain for one minute
6. Write down the weight of the metal containers on the form

Stability Test

7. Way 4 g of the 2-6 mm aggregates in the sieves, and note the weight on form (remember to subtract the weight of the sieve afterwards)
8. Number the glass beakers, and note the weight of glass on the form
9. Moisture the aggregates with damp until the desired water content. If it takes too long (check after 1 hour), spray 5 times with demineralized water to stimulate the wetting process.
10. Put about 87 ml of demineralized water in the weighed and numbered metal containers, enough to cover the soil when the sieve is at the bottom of its stroke
11. Place the containers in the sieving apparatus
12. Place the numbered sieves in the sieve holder directly over metal container
13. Lower the sieves so they reach into the container
14. Starter the motor, and let it move up and down for 3 min and 45 sec
15. Wait for a few minutes, then take the containers on a tray and note the weight on the form
16. Flush the content of the containers into a glass beaker with demineralized water
17. Flush the content left in the sieve into a glass beaker with ethanol
18. Air-dry the content in the beakers to evaporate most of the water
19. Put the glass beaker in the oven on a temperature of 105 ° C until the soil is dry (about one day)
20. Way the glass + soil, and note the weight on the form
21. Save the content from both glass beakers for further analysis with the Picarro13 to establish the biochar content. This procedure will be explained further below.

The aggregate stability will be expressed as the percentage of dry material remaining on the sieve after the stability test relative to the initial amount of dry soil.

Weight of wet sieve soil sample (g):	J
Weight of glass (g):	G
Weight of dry residue in glass + glass (g):	GR
Weight of dry soil before test (g):	STF J = - (J *% V / 100)
Weight of dry residue in the glass (g):	RT = GR - G
Weight of dry residue in the long term after test (g):	STE = STF - RT
Aggregate Stability (%):	% A = STE * 100 / STF

Annex III: Forms for Le Bissonnais method

Fast wetting/slow wetting/shaking

Date												
Sample code fw/sw/sh	Weight aggr. (≈ 4g)	Start time immersion (dw)	End time immersion (10m)	Start time oven-drying	End time oven-drying (24h)	Weight > 2mm a	Weight 2–1mm b	Weight 1–0.5mm c	Weight 0.5-0.2mm d	Weight 0.2-0.1mm e	Weight 0.1-0.05mm f	Weight <0.5mm g
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1.												
1.												
1.												
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Annex IV: Form for Wet-Sieving Apparatus (WSA)

Wet-sieving apparatus (2-6 mm aggr. size)														
Date									Fragments left in sieve A			Soil in metal can B		
Sample code (plot.method)	Weight cup	Weight cup + soil	Nr sieve + can	Weight sieve	Weight metal can	Weight of sieve + soil	Weight of soil in sieve (g)	Weight of can + soil after WSA	Nr glass	Weight of glass (g)	W dried soil + glass (g)	Nr glass	Weight of glass (g)	W dried soil + glass (g)
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Annex V: Significance of Levene's test for homogeneity of variances

Aggregate stability all fraction sizes

	Sig.
Rate	0,099
Material	0,056
Year	0,051

Annex VI: Descriptive statistics MWD after dry-sieving

Treatment	Sig. test normality	Mean	95% confidence interval for mean		Median	Std. deviation	Minimum	Maximum	Range
			Lower bound	Upper bound					
Control	0,992	11,93	11,63	12,23	11,94	0,19	11,70	12,15	0,45
bc8	0,090	11,07	9,41	12,74	11,45	1,05	9,53	11,86	2,33
bc25	0,026	11,58	10,65	12,51	11,33	0,59	11,21	12,45	1,24

Treatment	Sig. test normality	Mean	95% confidence interval for mean		Median	Std. deviation	Minimum	Maximum	Range
			Lower bound	Upper bound					
Control	0,992	11,93	11,63	12,23	11,94	0,19	11,70	12,15	0,45
mc8	0,237	11,57	10,97	12,16	11,60	0,37	11,17	11,91	0,74
bc8	0,090	11,07	9,41	12,74	11,45	1,05	9,53	11,86	2,33

Treatment	Sig. test normality	Mean	95% confidence interval for mean		Median	Std. deviation	Minimum	Maximum	Range
			Lower bound	Upper bound					
Control	0,992	11,93	11,63	12,23	11,94	0,19	11,70	12,15	0,45
bc25	0,026	11,58	10,65	12,51	11,33	0,59	11,21	12,45	1,24
new	0,536	10,91	9,05	12,78	11,18	1,17	9,35	11,95	2,60

Annex VII: Outcomes of one-way ANOVA's after wetting procedures

		Normality Sig.	ANOVA between groups	
			F	Sig
FW	Rate	0,123	0,084	0,920
	Material	0,250	0,244	0,788
	Year	0,127	0,815	0,473
SH	Rate	0,726	0,653	0,544
	Material	0,713	0,751	0,499
	Year	0,585	1,124	0,367
SW	Rate	0,475	1,803	0,220
	Material	0,740	4,197	**0,052**
	Year	0,521	1,533	0,267

Annex VIII: Classification of soil texture per control plot

			%		
	3	2 - 0.6	7		
Plot 2 - Control, 1/8/2012		0.6 - 0.2	20		
		0.2 - 0.063	14	41	sand
Plot 2 Loam		60 - 20 µm	8		
		20 - 6 µm	14		
		6 - 2 µm	12	34	silt
		< 2 µm	25	25	clay
	4	2 - 0.6	4		
Plot - 11 - Control, 1/8/2012		0.6 - 0.2	11		
		0.2 - 0.063	8	23	sand
Plot 11 Silty Clay Loam		60 - 20 µm	11		
		20 - 6 µm	20		
		6 - 2 µm	18	49	silt
		< 2 µm	28	28	clay
	5	2 - 0.6	5		
Plot 16 - Control, 1/8/2012		0.6 - 0.2	11		
		0.2 - 0.063	6	22	sand
Plot 16 Silty Clay Loam		60 - 20 µm	11		
		20 - 6 µm	20		
		6 - 2 µm	18	49	silt
		< 2 µm	29	29	clay
	6	2 - 0.6	9		
Mat Plot 5 - Control, 1/8/2012		0.6 - 0.2	18		
		0.2 - 0.063	10	37	sand
Plot 5 Clay Loam		60 - 20 µm	14		
		20 - 6 µm	11		
		6 - 2 µm	13	38	silt
		< 2 µm	25	25	clay