Effect of charred digestate (biochar) and digestate on soil organic carbon and nutrients in temperate bioenergy crop production systems

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Table of Contents

List of Figures

List of Tables

List of Abbreviations and Symbols

a	annum
B	biochar only
BH	digestate:biochar 1:5 treatment
BL	digestate:biochar 1:1 treatment
BS	base saturation
C	control treatment
C/N	carbon nitrogen ratio
CEC	cation exchange capacity
d	day
D	digestate only treatment
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
EC	electrical conductivity
EEG	German Renewable Energy Act (*Erneuerbare Energien Gesetz*)
EU	European Union
GHG	greenhouse gas
GWP	global warming potential
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
n	number of samples
n.a.	not analyzed
p	probability value
SH	Schleswig-Holstein
SMB	soil microbial biomass
TC	total carbon
TN	total nitrogen
TOC	total organic carbon

Summary

In recent years, the interest in bioenergy crop production as a renewable energy source increased substantially because it may contribute to the reduction of greenhouse gas emissions and the satisfaction of the ever increasing worldwide energy demands. Combustion is not the only way of gaining energy from biomass, but also anaerobic digestion or pyrolysis can be used. During the thermochemical decomposition (pyrolysis) of biomass in an oxygen free and high temperature environment, pyrolyzed biomass or biochar is produced together with the two co-products energy and syn-gasses. Biochar, being a carbonaceous material, is known to have the potential to improve soil conditions, reduce greenhouse gas emissions and, most importantly, to improve the long-term carbon storage in soil. However, there still are many unanswered questions about biochar itself, its potential performance as a soil amendment, its influence on terrestrial carbon sequestration, and its behavior in the ecosystems. For this reason, this study was done with the overall objective to better understand the impact of biochar as a soil amendment on terrestrial carbon sequestration, soil quality, crop productivity, and greenhouse gas fluxes.

A field experiment was established at two sites in Drage, Schleswig-Holstein, Germany, with bioenergy crops (crop rotation of *Zea mays* L., *Secale cereale* L. and *Triticum aestivum* L.). The sites represented two different sandy soils: *Gleyic Podzol* and *Gleyic Anthrosol*, which were amended with a total of five different treatments in a randomized complete block design with three replicates. *Gleyic Podzol* was amended with biochar (5 t biochar ha^{-1}), respectively not amended in the control plots. *Gleyic Anthrosol* had already been amended with digestate at 0.15 kg C m^{-2}, and was used unaltered for the control plots. To other plots, biochar was added to establish 1:1 and 1:5 mixtures of the existing digestate and the added biochar (5 and 24 t biochar ha^{-1} resp.). The biochar was produced from dry solid biogas digestate at 650 °C, and was mixed into the topsoils (0 - 0.2 m). Data were collected in all seasons over a two year period. In addition, an aerobic incubation experiment was conducted, investigating the interactions between amended organic matter (digestates and biochar) and inherent soil organic matter, using samples from *Gleyic Podzol* with control, digestate only, biochar only, and 1:1 and 1:5 mixtures of digestate:biochar treatments. During the 133 day incubation period, the CO_2 production was determined, and on selected days samples of the produced CO_2 were analyzed for $\delta^{13}C$.

The results of the field experiment showed that biochar had positive effects on the soil physico-chemical properties in both soils. In the first season after the application, the biochar amended plots had increased soil moisture content, soil reaction, TOC, TN, C/N ratio, inorganic nitrogen, plant available-P and -K, CEC and exchangeable cations (Ca, Mg, Na, K). The values of TOC, CEC, plant available-P and -K, and inorganic nitrogen were significantly higher in biochar amended soils compared to the control soils. Over the next seasons, the positive effects were diminishing, but the effects on CEC and plant available-P and -K remained significant even after two years. In contrast to soil nutrients, a negative effect was detected on soil microbial carbon and nitrogen just after amending the soil with biochar, but in the next seasons the biochar treated plots showed significantly higher microbial carbon, and nonsignificantly higher microbial nitrogen contents. The plots amended with biochar only and with both mixtures of digestate and biochar had a significantly higher crop biomass production in comparison to the control plots. The field experiment did not show a clear relationship between greenhouse gas emissions and organic matter amendments. This may be due to the low application rates of biochar. However, during the whole experimental period the application of biochar to these sandy soils only nonsignificantly increased CO_2 emissions. In the aerobic incubation experiment, all amendments accelerated carbon mineralization during the first phase of the incubation. However, over the whole period of 133 days, the biochar only treatment showed less loss of carbon, thus proving the potential of biochar to increase carbon sequestration.

Overall, the results of this study lead to the conclusion that charred digestate (biochar) is more stable than digestate, and can be used to enhance the sustainability of bioenergy crop production agro-ecosystems with sandy soils, increase their agricultural productivity, and mitigate the impact on climate change.

Zusammenfassung

In den letzten Jahren ist das Interesse an der Biomasseproduktion als Quelle erneuerbarer Energie erheblich gestiegen, da diese zur Reduktion der Freisetzung von klimawirksamen Spurengasen und zur Sicherung des immer weiter ansteigenden globalen Energiebedarfs beitragen kann. Verbrennung ist nicht die einzige Möglichkeit, aus Biomasse Energie zu gewinnen, sondern dies kann auch durch anaerobe Gärung oder durch Pyrolyse geschehen. Bei der thermo-chemischen Zersetzung (Pyrolyse) von Biomasse in einer sauerstofffreien Hochtemperatur-Umgebung wird pyrolisierte Biomasse (Biokohle) erzeugt, sowie als Nebenprodukte Energie und Synthesegase. Es ist bekannt, dass die kohlenstoffreiche Biokohle in der Lage ist, die Bodenqualität zu verbessern, die Emission von Treibhausgasen zu verringern und vor allem auch die langfristige Speicherung von Kohlenstoff im Boden zu verbessern. Dabei gibt es noch viele offene Fragen im Hinblick auf die Biokohle an sich, ihre Wirksamkeit als Bodenverbesserer, ihren Einfluss auf die Kohlenstoffbindung im Boden und ihr Verhalten in Ökosystemen. Diese Arbeit wurde mit der Zielsetzung durchgeführt, den Einfluss von Biokohle als Bodenverbesserer auf die Kohlenstoffbindung im Boden, die allgemeine Bodenqualität, den Ernteertrag und die Freisetzung von klimawirksamen Spurengasen besser zu verstehen.

Dazu wurde ein Feldversuch an zwei Standorten mit Bioenergiepflanzungen in Drage, Schleswig-Holstein, Deutschland durchgeführt (Fruchtfolge mit *Zea mays* L., *Secale cereale* L. und *Triticum aestivum* L.). Die sandigen Böden dieser Standorte (*Gley Podsol*/*Gleyic Podzol* und *Gley-Plaggenesch*/*Gleyic Anthrosol*), erhielten insgesamt fünf verschiedene Be-handlungen in einem vollständig randomisierten Block-Design mit 3 Replikaten. Der Gley Podsol wurde mit 5 t Biokohle ha-1 behandelt, bzw. blieb ohne Zusatz in den Kontrollfeldern. Der Gley-Plaggenesch enthielt einen Zusatz von Gärrückstanden mit 0.15 kg C m-2 bzw. blieb ohne Behandlung in den Kontrollen. Andere Plots erhielten Zusätze von Biokohle, so dass Mischungen der bereits vorhandenen Gärreste mit der hinzugefügten Biokohle im Verhältnis von 1:1 und 1:5 erreicht wurden (5 bzw. 24 t Biokohle ha-1). Die verwendete Biokohle wurde aus trockenen, festen Gärrückständen bei 650 °C hergestellt und in den Oberboden (0 – 0.2 m) eingearbeitet. Daten wurden über einen Zeitraum von zwei Jahren zu jeder Jahreszeit ermittelt. Zu-

sätzlich wurde ein Inkubations-Experiment mit Proben des *Gleyic Podsol* und den fünf Zusätzen Kontrolle, nur Gärreste, nur Biokohle, und 1:1 und 1:5 Mischungen von Gärresten und Biokohle durchgeführt, um die Beziehung zwischen zugesetztem Kohlenstoff und Bodenkohlenstoff zu ermitteln. Die CO_2 Emissionen wurden während der gesamten Inkubationszeit von 133 Tagen ermittelt, und die $\delta^{13}C$-Signaturen des emittierten CO_2 bestimmt.

Die Ergebnisse des Feldversuchs zeigten, dass Biokohle positive Auswirkungen auf die physikalisch-chemischen Eigenschaften beider Böden hatte. In den ersten Monaten nach der Anwendung hatten die Plots mit zugefügter Biokohle höhere Werte für Bodenwassergehalt, pH-Wert, Gesamtkohlenstoff, Gesamtstickstoff, C/N-Verhältnis, anorganischen Stickstoff, pflanzenverfügbares Phosphor (P) und Kalium (K), Kationenaustauschkapazität und austauschbare Kationen (Ca, Mg, Na, K). Dabei waren die Werte für Gesamtkohlenstoff, Kationenaustauschkapazität, pflanzenverfügbares P und K sowie anorganischen Stickstoff in den Böden mit zugefügter Biokohle signifikant höher als in den Kontrollen. In der Folgezeit nahmen diese Effekte ab, aber die Auswirkungen auf Kationenaustauschkapazität und pflanzenverfügbares P und K waren auch nach zwei Jahren noch signifikant. Im Gegensatz zu den Nährstoffgehalten wurde unmittelbar nach dem Zusatz von Biokohle eine negative Auswirkung auf den mikrobiellen Bodenkohlenstoff und -stickstoff festgestellt. Danach hatten die Plots jedoch signifikant höhere Werte für mikrobiellen Bodenkohlenstoff, und nicht-signifikant höhere mikrobielle Stickstoffgehalte. Die mit Biokohle bzw. einer Mischung aus Biokohle und Gärresten behandelten Böden zeigten einen signifikant höheren Ernteertrag als die Kontrollen. Aus dem Feldversuch ergab sich keine klare Beziehung zwischen dem Ausstoß von Treibhausgasen und dem Zusatz von organischer Substanz. Dies kann an den niedrigen Applikationsraten der Biokohle liegen. Während der gesamten Dauer des Experiments erhöhte der Zusatz von Biokohle auf diesen sandigen Böden die CO2 Emissionen. Diese Erhöhung war jedoch nicht signifikant. In dem aeroben Inkubationsexperiment beschleunigten alle Bodenzusätze die Kohlenstoffmineralisation während der ersten Phase der Inkubation. Über die gesamte Dauer von 133 Tagen zeigte der ausschließliche Zusatz von Biokohle geringere Kohlenstoffverluste als die anderen

Behandlungen. Somit konnte die Fähigkeit von Biokohle, die Kohlenstoffbindung im Boden zu erhöhen, bestätigt werden.

Insgesamt führen die Ergebnisse dieser Studie zu dem Schluss, dass Biokohle im Boden stabiler ist als Gärreste und verwendet werden kann, um die Nachhaltigkeit von Agrar-Ökosystemen zur Erzeugung von Bioenergiepflanzen auf sandigen Böden zu verbessern, die Anbauleistung zu erhöhen, und die Auswirkungen auf den Klimawandel zu vermindern.

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1. Introduction

Today, the balance between nature and human is at risk with many problems and challenges which are driven by each other: increasing human population, increasing global food and energy demand, industrial and green revolutions, fossil fuel burning, increasing concentrations of greenhouse gases in the atmosphere, global climate change, demand for renewable energy sources, etc. The renewable energy concept is one of the key issues in the diverse plans which are necessary to cope with those challenges. One of the recent developments in the renewable energy concepts is the use of biomass, which already contributes significantly to the energy supply in the European Union (EU) as a whole, and in Germany in particular. The use of biomass, be it for combustion, or for producing biogas, needs to be developed further with respect to efficient and sustainable land use considering food security, securing soil fertility, and mitigating climate change by reducing greenhouse gas (GHG) emissions and sequestering atmospheric carbon in terrestrial systems (Lal, 2004, 2007, 2010).

To overcome soil nutrient and carbon losses caused by intensive agriculture and to improve soil quality, the addition of organic matter has been a common agricultural practice from the middle ages to the present. Sewage sludge, or digestate can supply soil nutrients and improve soil quality, but traditional organic materials like manures or composts have relatively short half-lives in soil (in compost up to 10 - 14 weeks: Butler and Hooper, 2010, and in sewage sludge 39 - 330 days: Ajwa and Tabatabai, 1994). *Terra-preta* soils, also called Amazonian dark earth or *Terra preta de índio,* are anthropogenic soils rich in organic carbon in the Amazonian basin. Contrary to other highly weathered tropical soils, *Terra-preta* soils remain fertile for several centuries (Lehmann et al., 2003b; Sombroek et al., 2003). After it became clear that the amendment of charcoal and other organic residues from settlements caused the increased organic matter content and the high fertility of these soils, charcoal gained immense scientific interest again, eventually leading to the development of pyrogenic carbon or biochar (Lal, 2005, 2010; Lehmann and Joseph, 2009), and the re-evaluation of traditional organic amendments.

The current study investigates the effect of organic soil amendments on the sustainability of bio-energy crop production agro-ecosystems depending on the type of amendment. Therefore, two different organic amendments were added to arable soils used for bioenergy crop production. One amendment was the waste products from biogas production plants, the digestate, which, under the current land use praxis, is applied to the field in liquid form. The second amendment was thermally treated (pyrolyzed) digestate that was converted to charred digestate or biochar. Rather than just burning the biomass, pyrolysis is a process which produces energy in the form of heat, biosyngases and biochar.

Biochar is chemically similar to charcoal, but has its own distinguished features (Woolf et al., 2010). Biochar is not only important because it contains carbon with a relatively high residence time in soil, which plays a role in soil terrestrial carbon sequestration (Hammes et al., 2009; Johannes and Joseph, 2009), but also because it can have positive effects on soil structure (Jha et al., 2010), water retention (Glaser et al., 2002), soil pH (Major et al., 2010b), availability and concentration of nutrients in soil (Chan et al., 2008; Glaser et al., 2002; Steiner et al., 2009), nutrient retention (Chan et al., 2008; Glaser et al., 2002; Steiner et al., 2009), and the soil micro-fauna (Lehmann et al., 2011), and increases the retention time of soil pollutants and agrochemicals (Graber et al., 2011; Jones et al., 2011a; Mesa and Spokas, 2011; Spokas et al., 2009). Even though biochar has a long list of potential benefits and only few potential risks, all those highly depend on many parameters of both the biochar itself, and of the soil to which biochar is to be applied. Because of this, the results of previous researches were inconsistent, and leave room for many discussions and future research. There still are many knowledge gaps about how biochar is best used as a soil amendment in a specific agro-ecosystem. Thus, the current study is important for better understanding the impact of biochar on soils used for bio-energy crop production in Northern Germany, and for encouraging the use of biochar to meet global climate and carbon sequestration goals.

1.1 Objectives

The general objective of this study is to evaluate how digestate and charred-digestate (biochar) can be used as organic soil amendments to increase bioenergy crop productivity, soil quality, and terrestrial carbon sequestration in bioenergy crop production systems in Northern Germany.

With this main objective, the study focused on evaluating the behavior of both biochar and digestate in soil ecosystems with the following research questions:

1. Does biochar or the combination of biochar and digestate have a synergistic effect on above ground biomass production that is greater than the effect of digestate only?
2. Can biochar or the combination of biochar and digestate sustain soil nutrients over multiple growing seasons?
3. How does biochar or the combination of biochar and digestate affect soil microbial biomass?
4. Do biochar or the combination of biochar and digestate affect soil organic carbon turnover, terrestrial carbon sequestration and greenhouse gas emission?

To answer and evaluate research questions 1 and 2, the following two hypotheses were formulated:

H1. Biochar application will significantly increase above ground biomass production of bioenergy crops.

H2. Biochar application will significantly increase the availability of soil micro- and macro-nutrients.

The following two hypotheses were formulated to evaluate research questions number 3 and 4:

H3. Soil amended with a mixture of digestate and biochar will have a significantly higher soil microbial activity than soil amended with digestate only.

H4. The mineralization of soil inherent organic carbon and the greenhouse gas fluxes will significantly increase with increasing amount of biochar.

To answer the research questions, the above mentioned hypotheses were tested by conducting a field experiment and an aerobic incubation experiment.

The field trial was conducted at two bioenergy crop production sites and used five different treatments containing digestate, biochar (charred digestate), and their mixtures. The chemical parameters of topsoil (0 - 0.2 m) and the emission fluxes were measured monthly, and at the end of each growing season, the above ground biomass was determined. The data was used to evaluate the effects of digestate and biochar on soil quality including the soil micro- and macro-nutrients content and availability, crop productivity, soil microbial biomass carbon and nitrogen, soil respiration and other trace gas emissions.

The aerobic incubation experiment was done with the same treatments that were used in the field trail, and CO_2 production data and the isotope ratio ($\delta^{13}C$) of the produced CO_2 were measured. The data were used to evaluate the effects of digestate and biochar and their mixtures on the potential mineralization of both soil organic matter and amended organic matter.

2. State-of-the-art

2.1 Renewable energy in Germany

In the era of renewable energy, bio energy is considered one of the key options to mitigate climate change and reduce anthropogenic trace gas emissions. Consequently, both the European Union as a whole, and the German government in particular started developing strategies and political agendas to make use of renewable energies for replacing fossil fuels and reducing greenhouse gas emissions in both short- and medium-term planning (Faaij, 2006; Bosch, Johnson, Clément, Mertens & Roubanis, 2009; FNR, 2013). In 2010, the main sources of renewable energy in Germany were wind power, which produced 36.5 billion kilo-watt hours (kWh), followed by hydropower (19.7 billion kWh), biomass (33.5 billion kWh), photovoltaics (12.2 billion kWh), and geothermal energy (< 0.1 billion kWh) (Böhme, *et al.,* 2010).

The United Nations World Commission on Environment and Development (UN-WCED) concept which was published as the Brundtland Report in 1987 (WCED, 1987) demands that the present generation satisfy its needs without endangering the ability of future generations to meet theirs. Following this concept, the sustainable development of the renewable energy sector, and the environmental, social and economic sustainability of the industry became one of the main research areas of the German Federal Ministry of Food, Agriculture and Consumer Protection (*Bundesministerium für Ernährung und Landwirtschaft*, *BMELV*) with the goal to test new approaches in research projects. The results shall be used to develop strategies to ensure the biodiversity of energy crops and ecosystems, to breed new plant varieties, to establish new cultural practices for bioenergy crops reducing the use of pesticide and fertilizer, to provide a year round vegetative cover to reduce soil erosion, to increase the efficiency of energy conversion processes, to improve the cascading use of renewable resources (first being used as a material and then being used as an energy source), and to re-use residual materials as fertilizer (FNR, 2013). In the EU, anaerobic digestion plants or biogas production plants commonly use three different input substrates: landfill waste, sewage sludge, and agricultural residues. But Germany decided to encourage the planting of bioenergy crops to be used as substrate in digestion plants (FNR, 2014). Even though the concept and technique of producing biogas have been familiar for a long time already, energy production from biogas did not play a significant role compared to all other energy sources before the 1990s (FNR, 2013).

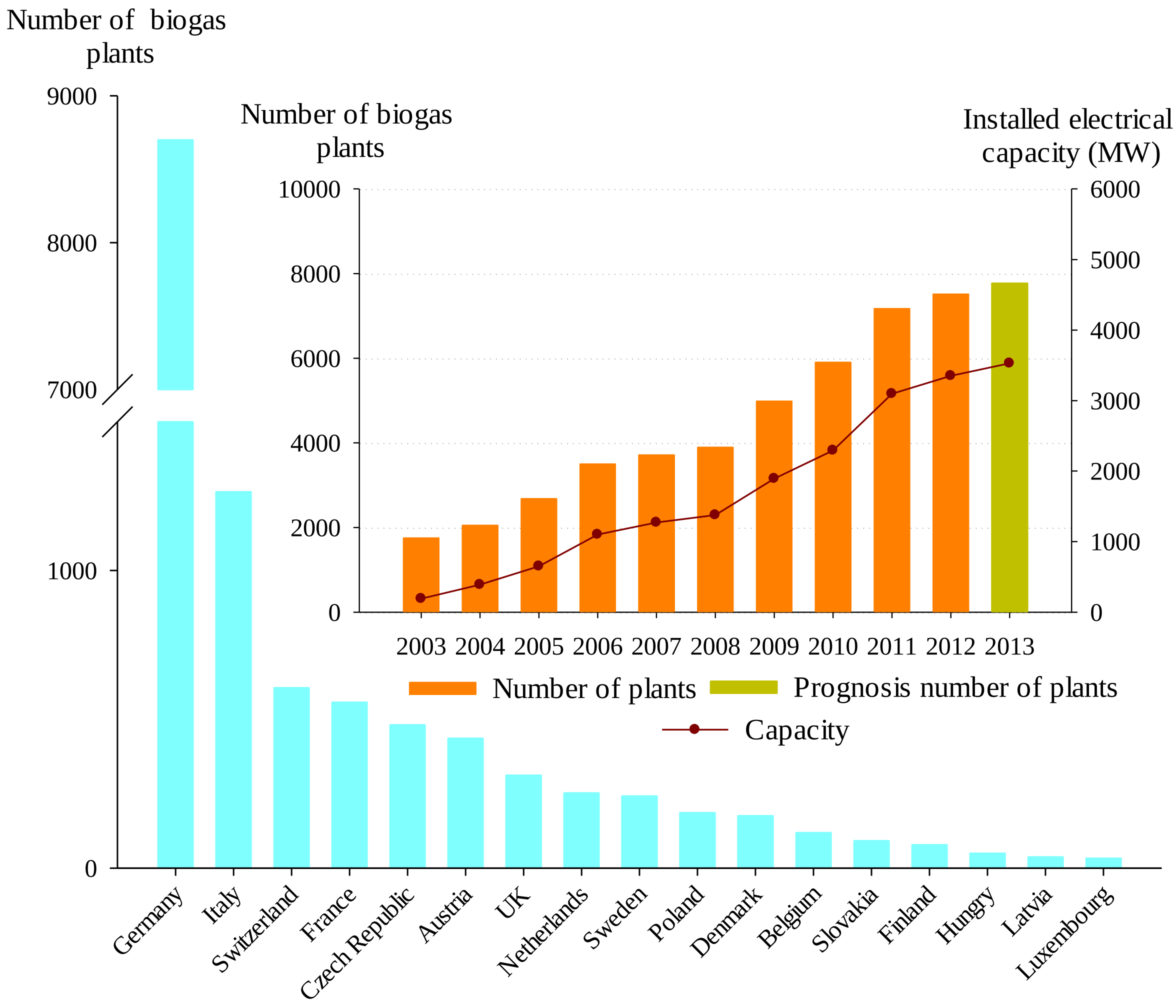


Figure 1: Number of biogas plants in EU member states in 2012 (modified graph from EBA, 2012) and development of the number of biogas plants and their electricity production capacity in Germany (modified graph from FNR, 2015).

2.2 Development of biogas in the German renewable energy sector

According to the German Agency for Renewable Resources (*Fachagentur für nachwachsende Rohstoffe*; FNR, 2014), the agricultural sector, including all bioenergy crops, agricultural by-products, and animal waste, plays the dominant role in Germany's biogas production. According to FNR (2013), over 7,515 biogas plants had the capacity to generate approximately 3,352 megawatts (MW) of electricity (FNR, 2013). The steady increase in agricultural anaerobic digesters in Germany can be directly attributed to a supportive national legal framework coupled with the tariffs paid for renewable energy. The German Renewable Energy Act (*Erneuerbare Energien Gesetz, EEG*) which was introduced in 2000 and its 2004, 2009, and 2012 amendments were able to generate a massive boost in the development of biogas in Germany (FNR, 2014).

In 2012, biogas generated from biomass or agricultural waste and by-products contributed 15 % to the electricity generation, and 8 % to the supply of heat produced by using renewable energies (FNR, 2013). When adding biogas from bio-waste fermentation, landfills, and sewages, the total contribution of biogas was 20 % for electricity and 15 % for heat (FNR, 2013). Biomass, including wood, bioenergy crops, straw and animal excrement, will play a significant role in the future for a sustainable and environmentally friendly energy supply nationwide.

Biogas produced from biomass is suitable for the simultaneous production of electricity and heat, and can be used as a substitute for fuel and natural gas (see Figure 2). The main limitation of biogas to use as a stable energy source is the production fluctuation which depends on the time of the year, time of the day, and weather. In comparison to other renewable energy sources, biogas is flexible to use and easy to store (FNR, 2013). According to FNR (2013) and the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (*Bundesministerium für Umwelt, Naturschutz, Bau und Reaktorsicherheit, BMU*B), in Germany approximately 54 % of biogas is produced by using bioenergy crops, and 50 % of the biogas produced in this way is used for electricity production.

Biogas significantly reduces the burden on the environment. The greatest potential for CO_2 savings lies in the reduced energy expenditure for bioenergy crop production and the installation of anaerobic digesters, compared to the energy gain from anaerobic digestion of the bioenergy crop, slurry, manure and other biomass as input substrates. A list of the environmental advantages of biogas should also include the utilization and reduction of waste and the reduction of methane emissions from slurry and manure, and the usage of digestate as a fertilizer after being processed properly (FNR, 2013). The decentralized nature of energy production from biogas is not only improving the income of rural farmers, but also entails follow-on investments that lead to the strengthening of rural areas in economic terms. In 2012, the renewable energy sector in Germany employed almost 380,000 people, and the biogas sector represented 13 % or 50,000 employees according to FNR (2013).

Biogas offers a diversity of options for use, including the decentralized production of electricity and heat using CHP (Combined Heat and Power) production unit places in the close proximity of the biogas production plant, the feeding into the national power grid using the benefits granted from the EEG, the feeding of upgraded biogas into the natural gas grid, and its use as a substitute for natural gas for energy production, as fuel, or in the chemical industry (Figure 2). However, the main objective is utilizing it as an energy source in an efficient way.

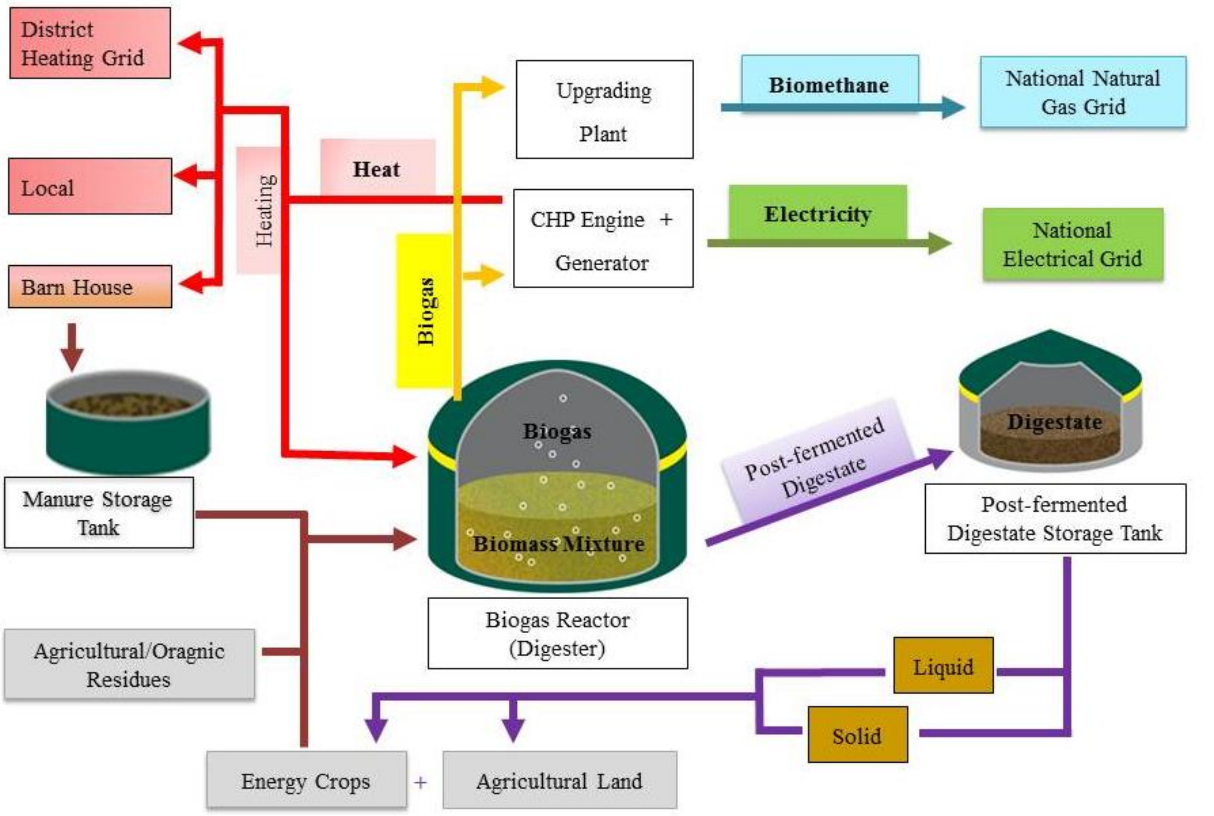


Figure 2: Flow chart of inputs and outputs of agricultural biogas plants in Germany's bioenergy production system.

2.3 Agricultural anaerobic biogas digesters

Anaerobic digestion of organic matter takes place naturally in the absence of oxygen in different habitats: at the bottom of lakes, in swamps, in the stomach of ruminants, in landfills, volcanic hot springs, submerged rice fields, etc. Normally there are four steps in anaerobic digestion: hydrolysis, acidification, acetic acid formation, and methane formation, which all take place simultaneously. During complete anaerobic digestion, different species of microorganisms are involved depending on the processing stage. Those microorganisms produce different kind of gases. Generally, this mixture of gas is known as biogas and includes methane (50 - 75 %), carbon dioxide (25 - 45 %), water vapor (2 - 7 %), oxygen (< 2 %), nitrogen (< 2 %), ammonia (< 1 %), hydrogen sulphide (< 1 %) and other trace gases (< 2 %) (FNR, 2013). Anaerobic digesters in biogas production plants facilitate the anaerobic microbial decomposition of organic matter in a moist environment and temporarily store the biogas. The total biogas yield and the composition of the gas mixture highly depend on several parameters, e.g. the composition of the input substrates (supply of nutrients), and the biological key figures of the fermentation process including sensitivity of the microorganisms to the different environmental conditions of the medium including temperature and pH (Al-Seadi and Lukehurst, 2012).

A large number of organic substrates can be used to obtain biogas, but in an agricultural installation the most commonly used substrates are animal excrements (e. g. slurry from cattle and pigs), by-products from the food and timber processing industries (e.g. vegetable waste from wholesale markets, food waste from restaurants, lawn cuttings, material from landscape conservation, or organic waste from municipal disposals), and bioenergy crops grown especially for this purpose. In 2012, bioenergy crop was the most popular substrate (54 %) in Germany, followed by livestock excrement (41 %), bio-waste (4 %) and industrial and harvest residues (1 %) according to the monitoring report of EEG (FNR, 2013). Among the bioenergy crops, maize is very popular because of its high dry matter and energy yields, and because it provides the best technological suitability and cost structure, and only requires a small amount of fertilizer and plant-protection products. Examples for other substrates are grain, grasses, and sugar beet (FNR, 2013). However, there also are some negative consequences of the usage of maize as a bioenergy crop, for example on soil fertility, biodiversity, and available land for food supply. In Germany, there are numerous ongoing research projects focusing on bioenergy crops for advancing the further development of existing bioenergy crops, investigating the potentials of other bioenergy crop species, introducing new bioenergy crops, and cultivating mixes of bioenergy crops and wild flowers (FNR, 2013).

2.4 Digestate from agricultural anaerobic biogas digesters

A mixture of partially digested or indigestible materials of the biodegradable substances, mineralized or live microbial cells, and mineralized inorganic molecules left after anaerobic digestion is called digestate, which is a nutrient-rich liquid-solid suspension (5 % to 10 % dry mass) (Domínguez, 2012; FNR, 2013; Madan and Mandan, 2015; Makádi et al., 2012; Möller and Müller, 2012). Lignin, which cannot be biodegraded by the microorganisms involved in the anaerobic digestion process, comprises a significant portion of this solid (Madan and Mandan, 2015). Digestate contains more than 90 % of the nutrients entered to the digester (OECD, 2010), but the final quality of the digestate does not only depend on the nutrient value but also on other qualities of the initial substrate including initial C/N ratio, dry matter content, as well as on the micro-environment of the anaerobic digester, and the duration and intensity of the digestion process (Domínguez, 2012; Smith et al., 2007). Due to the production of ammonium substances, the average pH value of the digestate is higher than that of its initial substrate (Brenner, 2008; Domínguez, 2012). Some of the phosphorus is converted into a readily available form, but potassium and magnesium contents do not change significantly during anaerobic digestion (FNR, 2013).

2.5 Application of digestate to agro-ecosystems: limitations and regulations

With the increasing number of large scale agricultural biogas plants, the way of handling and the quality of digestate can have a significant impact on both the human health, and the quality and sustainability of ecosystems especially in the agricultural areas. The digestate produced by most of the agriculture based biogas digesters is directly used as a soil amendment acting as an organic fertilizer with or without pre-application treatments, and bears a low risk of containing hazardous materials like trace metals or harmful pathogens, contrary to the digestate coming out from industrial and other kinds of anaerobic digesters (Evans, 2013; Lukehurst et al., 2010; Möller et al., 2009). The quality of the digestate coming out from the digester solely depends on the micro environment in the digester tank, the original nutrient value of the substrate, and the pre-application handling (Möller et al., 2009). Taking all these facts into account, regulating and monitoring of digestate application to the soil are important. In Germany, there are several regulations about monitoring the quality and disposal process of digestate (BMJV, 2012; Dittrich, 2006; FNR, 2014; Lukehurst et al., 2010; Schneider and Mastel, 2008b).

The use of digestate as an organic soil conditioner is mainly governed by § 3 No. 1 and 2 of the German Fertilizer Law 1977 (*Düngemittelgesetz - 1977*) which cover the impacts on soil fertility, health of humans and domestic animals, food crops, and the sustainability in the ecosystems (Dittrich, 2006). Application is forbidden when the soil is frozen with more than 5 cm snow layer, or when it is flooded and saturated. The maximum application rate of organic fertilizer allowed in the EU is 170 kg N ha^{-1} yr^{-1}, but in Germany there is a maximum permissible excess of 60 kg N ha^{-1} yr^{-1} with 20 kg P_2O_5 ha^{-1} yr^{-1} as long as the soil has less than 20 mg P_2O_5 per 100 g of soil (Domínguez, 2012; German Advisory Council on the Environment, 2013; Schneider and Mastel, 2008b).

2.6 Effect of digestate on agro-ecosystems

In order to understand the effects of digestate on agricultural production, several studies were conducted in different locations in Germany. Most of the studies focused on the effects of wet digestate from agricultural biogas plant as a liquid fertilizer on crops (wheat, maize, rapeseed and rye), which were shown to be quite comparable to the effects of mineral fertilizer (Schneider and Mastel, 2008a; Schneider-Götz, 2007; Wenland et al., 2006). The significantly increased biomass productivity was documented by Stinner et al. (2005; for spring and winter wheat with wet digestate), and Schneider-Götz (2007; winter wheat and maize with wet digestate). However, Pacholski et al. (2011) and Kautz and Rauber (2007) showed in their studies that the dry-matter yield of maize did not significantly increase by only using digestate from agriculture based biogas plants as fertilizer. Besides its effects on biomass yield, digestate from agricultural biogas production also has an effect on the soil quality which is a fundamental component of agro-ecosystems.

Digestate, which is rich in labile organic matter, can create a favorable environment for both macro and micro soil organisms. Several researches showed that the effect of digestate from agricultural biogas production on the population of earthworms, which is a well-known bio-indicator for the assessment of soil quality, is similar to the effect of applying liquid manure (Elste et al., 2010; Hans-Jörg Brauckmann et al., 2009; Sensel et al., 2009).

2.7 Charred digestate (biochar) and its application

2.7.1 What is biochar?

The black to dark brown soil in the Amazon River Basin was discovered in 1870. After Wim Sombroek gave an explanation for that special soil in 1966, biochar gained its first interest from the scientific community (Lehmann and Joseph, 2009; Lehmann, 2007; Lehmann et al., 2006; Schmidt, 2013; Sombroek et al., 2003; Woods and Winklerprins, 2009; World Bank Carbon Finance Unit, 2011). The interest in biochar was uplifted again when soil carbon sequestration was recognized as a potential contribution to mitigating global climate problems. Biochar is a solid carbon rich combusted organic material produced by complete thermal decomposition of biomass by heating it up to a high temperature in the absence of or with only a limited supply of oxygen, in nature or with specialized pyrolysis technologies (Hammes et al., 2009).

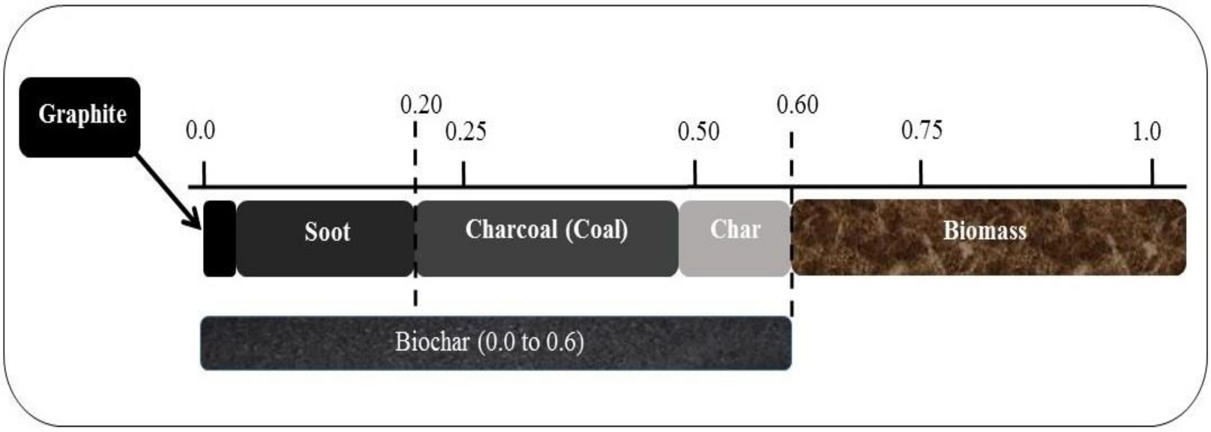


Figure 3: O/C spectrum of the products of thermo-chemical conversion of biomass (modified: Spokas, 2010).

Spokas (2010) summarized how biochar differed from other traditional carbon rich combusted products like graphite, soot, charcoal, char and pure biomass after studying their oxygen to carbon ratio (O/C ratio or black carbon continuum in Figure 3). He further described the relationship between the O/C ratio and the stability of those products in soils. Graphite, the product with the lowest ratio has the highest stability, and the lowest stability is shown by pure biomass.

Several authors tried to give a definition for biochar including chemical and physical characteristics, production conditions and usage (Table 1). The earlier definitions include only chemical and physical characteristics, but the later ones also give details about the usability of biochar and even include specific production conditions for specific uses. Pyrogenic carbonaceous material (PCM), black carbon, and hydrochar are more often discussed together with biochar in the literature, but Lehmann and Joseph (2009) showed those materials are clearly different from each other. PCM is the common term for all materials which are produced by thermochemical conversion and contain some organic carbon, like charcoal, biochar, char, black carbon, soot, or activated carbon. Black carbon is a PCM which originating from wildfires or fossil fuel combustion, and dispersed in the environment. Hydrochar is a solid product produced by hydrothermal carbonization (HTC) and differs from biochar both in the way it is produced and in its properties. Hydrochar has higher hydrogen to carbon (H/C) ratio than biochar, and in contrast to biochar has little or no fused aromatic ring structures.

Table 1: Available definitions for biochar

Year	Definition	Author/Institute
2009	"*biochar is the C-rich product obtained when biomass, such as wood, manure or leaves, is heated in a closed container with little or no available air*"	Lehmann and Joseph
2010	*"charcoal (biomass that has been pyrolyzed in a zero or low oxygen environment) for which, owing to its inherent properties, scientific consensus exists that application to soil at a specific site is expected to sustainably sequester carbon and concurrently improve soil functions (under current and future management), while avoiding short- and long-term detrimental effects to the wider environment as well as human and animal health"*	Verheijen et al. for European Commission
2010	"*biochar is the porous carbonaceous solid produced by thermo-chemical conversion of organic materials in an oxygen-depleted atmosphere which has physicochemical properties suitable for the safe and long-term storage of C in the environment and, potentially, soil improvement*"	Simon and Sohi
2012	"*biochar is defined as char produced by pyrolysis for use in agriculture (and other non-thermal applications) in an environmentally sustainable manner*"	EBC - European Biochar Certificate
2012	"*biochar is produced by biomass pyrolysis, a process whereby organic substances are broken down at temperatures ranging from 350 to 1000 °C in a low-oxygen (<2 %) thermal process*"	IBI - International Biochar Initiative

2.7.2 Characteristics of biochar

Many authors documented characteristics of biochar in relation to feedstock, production techniques and temperature, retention time, and post-production handling. Both chemical and physical properties of biochar mainly depend on the feedstocks, with the chemical properties having a higher dependency than the physical ones according to available literature (Abdullah et al., 2010; Brewer et al., 2011; Bridgwater, 1999; DeLuca et al., 2006; Liang et al., 2006; Mohan et al., 2006; Novak et al., 2014; Raveendran et al., 1995; Warnock et al., 2007; Yang and Lua, 2003). The chemical characteristics change with the feedstocks (Abdullah et al., 2010), the production technology and conditions (Novak and Busscher, 2013) including temperature and residence time (Bridgwater, 1999), and the cooling technique (Mohan et al., 2006; Yang and Lua, 2003; Zhao et al., 2013).

Biochar produced from woody feedstock rich in lignin has a high carbon and a low ash content compared to switchgrass and corn stove biochar (Brewer et al., 2011; Raveendran et al., 1995). The chemical structure of the carbon in the feedstock decides the final aromatic carbon groups in biochar depending on the production temperature (Brewer et al., 2011; Kwapinski et al., 2010; Luo et al., 2011). The common structural groups in biomass are hemicellulose, cellulose and lignins, which require different temperature ranges in order to be completely pyrolyzed: 200 - 260 °C, 240 - 350 °C, and 280 - 500 °C respectively (Mohan et al., 2006). The surface chemical characteristics of biochar are determined by those aromatic carbon groups which give highly heterogeneous and not well defined surfaces which can have hydrophobic, acidic and basic characteristics (Amonette and Joseph, 2009; Brennan et al., 2001; Hedges et al., 2000; Preston and Schmidt, 2006; Schmidt and Noack, 2000; Spokas, 2010; Trompowsky et al., 2005). Due to its high carbon and low nitrogen content, biochar has highly variable C/N ratio with a maximum value of 400 (Chan and Xu, 2009). Cheng et al. (2008) explained that the high cation exchange capacity (CEC) of biochar is caused by the presence of negatively-charged functional groups. Novak et al. (2009) discovered the relationship between pyrolysis temperature and CEC of biochar: an increase in pyrolysis temperature decreases biochar CEC. This negatively-charged surface of biochar not only adsorbs cations and increases CEC but also is able to adsorb other organic and inorganic compounds (Abebe et al., 2012; Buss et al., 2015; Graber et al., 2011; Kwon and Pignatello, 2005; Lucchini et al., 2014; Mesa and Spokas, 2011; Pignatello et al., 2006; Zhu and Pignatello, 2005). The porosity and surface area of biochar correlates with a mass loss during the pyrolysis (Amonette and Joseph, 2009), and when the production temperature is less than 450 ºC, the porosity is low due to the accumulation of volatile organic compounds inside the pores, and reduces the adsorption capacity (Kwon and Pignatello, 2005; Pignatello et al., 2006).

2.7.3 Significance of applying biochar to soil in agro-ecosystems

The use of charcoal, biochar or biomass-derived black carbon in agriculture is not a new technique, but this has been done by indigenous farmers in many parts of the world including South America, South and South East Asia for a long time already. Besides anthropogenic influences, naturally produced biomass-derived black carbon (e.g. from wild fires) can be found in soil carbon pools (Grossman et al., 2010; Sombroek et al., 2003; Swami et al., 2009; Woods and Winklerprins, 2009). With the developing interest in biochar and all biomass-derived black carbon, those recently started to be manufactured commercially with four main objectives: soil amelioration to improve agricultural productivity, proper waste management, climate change mitigation, and energy production (Johannes and Joseph, 2009). According to Forbes et al. (2006), the worldwide production of biochar was 0.05 - 0.27 Gt per year. Compared to the terrestrial net primary productivity of 60 Gt/yr this was very small (IPCC, 2005, 2007), but all this black carbon makes up one third of soil organic carbon in the majority of soils globally (Major et al., 2010a). Since more than a decade scientists have been discussing the stability of biochar and other char products in soil, and their ability to enhance the carbon sequestration. Those qualities are much higher when biochar is produced at higher temperature (graphite stability: Shneour, 1966, biochar stability: Baldock and Smernik, 2002; Bird et al., 1999; Brodowski et al., 2005; Glaser et al., 2000; Hamer et al., 2004; Kawamoto et al., 2005; Lehmann et al., 2006; Pessenda et al., 2001; Shindo, 1991). On the other hand, higher production temperature will give nutrient losses and a small mass turnover (Bolan et al., 2012; Bruun et al., 2011; Calvelo Pereira et al., 2011; Özçimen and Karaosmanoğlu, 2004).

2.7.3.1 Effects of biochar on soil physical properties

The effects of biochar on soil physical properties are based on its porosity, high surface area, lower bulk density, and water repellency. Only a small number of researches have been conducted on the effect of biochar on soil physical properties compared to researches on biochar's effects on soil chemical properties, but there were some interesting findings. According to Lehmann et al. (2003a, 2003b), Liu et al. (2015) and Novak et al. (2012) biochar increases the water holding capacity of soil because of its increased particle surface area and the water storage within the porous structure, and indirectly increases mycorrhizal fungi and microbial biomass. There was evidence from the studies done by Verheijen et al. (2009) and Liu et al. (2015) that biochar application significantly improves aggregate stability. Atkinson et al. (2010) and Hammes and Schmidt (2009) investigated the influence of biochar on soil erodability. However, there also are studies showing little effect on soil physical properties, and often even conflicting evidences to the above positive effects. For example, Busscher et al. (2010), Peng et al. (2011), and Eastman (2011) reported that biochar had no significant effect on aggregate stability. Many studies and review articles showed the effects of biochar on soil physical properties for specific soils which are of little relevance for agricultural soils such as ancient anthropogenic soils (Atkinson et al., 2010; Ayodele et al., 2009; Glaser et al., 2002; Mukherjee et al., 2014; Sohi et al., 2009, 2010; Verheijen et al., 2010) or with non-agricultural soils (Belyaeva and Haynes, 2012; Jones et al., 2011b; Uzoma et al., 2011). Other studies used disturbed soils, or did not perform *in situ* soil experiments at the fields (Belyaeva and Haynes, 2012; Kameyama et al., 2012; Liu et al., 2012; Pan et al., 2009; Streubel et al., 2011; Tryon, 1948; Uzoma et al., 2011; Van-Zwieten et al., 2010).

2.7.3.2 Effects of biochar on soil chemical properties

Contrary to the soil physical properties, many studies have been done concerning the effects of biochar on soil chemical properties (Busscher et al., 2010; Chan et al., 2007; Deenik et al., 2010; Van-Zwieten et al., 2010). The promising results with highly weathered tropical soils with poor chemical properties (Iswaran et al., 1980; Jeffery et al., 2011; Liang et al., 2006) drew the attention of biochar research to tropical climate (Glaser et al., 2002; Jeffery et al., 2011; Verheijen et al., 2010; Xie et al., 2015), but recently the research interest has extended to subtropical areas with a growing interest in the application of biochar to increase terrestrial carbon sequestration (Kimetu and Lehmann, 2010; Tejada and Gonzalez, 2007; Trompowsky et al., 2005). As an attractive soil amendment, biochar significantly increases pH (EBC, 2012; Glaser et al., 2002; IBI, 2012; Lehmann et al., 2003b; Major et al., 2009, 2010b; Yamato et al., 2006), CEC (Alling et al., 2014; EBC, 2012; Glaser et al., 2002; Hale et al., 2013; IBI, 2012; Lehmann et al., 2003b; Major et al., 2009, 2010b; Yamato et al., 2006), overall sorption capacity (Sohi et al., 2010), availability of major plant nutrients (Jeffery et al., 2011, 2015; Verheijen et al., 2010), base saturation (BS) (Glaser et al., 2002; Lehmann et al., 2003a, 2003b; Major et al., 2009, 2010b; Schulz and Glaser, 2012; Yamato et al., 2006), and available P (Chidumayo, 1994; Yamato et al., 2006).

2.7.3.3 Effects of biochar on agricultural crop productivity

There was much research on the effects of biochar on agricultural soil and agronomic performance. The results of those studies are varied and depend on feedstock, production conditions, rates and procedure of biochar application, soil properties, crop properties, and cultural practices of production systems, but the majority of those studies showed a positive plant response to biochar application (Jeffery et al., 2011; Jha et al., 2010). The literature shows that the influence of biochar on plant biomass can be physical, chemical and biological, by increasing the water holding capacity (Glaser et al., 2002; Laird et al., 2010), affecting the soil pH (Major et al., 2009, 2010b), directly influencing the supply of plant nutrients (Chan et al., 2008), indirectly influencing the availability of plant nutrients (Chan et al., 2007; Pessenda et al., 2001), and affecting the interactions with the soil micro-fauna (Hammes et al., 2009; Ruivo et al., 2009).

2.7.3.4 Effects of biochar on soil microbial biomass

Because the behavior of soil microbial biomass in biochar amended soils highly varied in past studies (Grossman et al., 2010; Khodadad et al., 2011; O'Neill et al., 2009), there are many controversies on how biochar effects soil biological processes, and this still is not well understood (Lehmann et al., 2011). It is clear that biochar application rates and soil type also affect the soil microbial biomass (Lehmann et al., 2011). Bruun et al. (2011), Luo et al. (2011), Steinbeiss et al. (2009), and Zimmerman et al. (2011) documented a higher amount of labile C fractions in biochar amended substrates than in their non-charred feedstocks. Lehmann et al. (2011) explained the positive effect of biochar on the soil microbial biomass (SMB) by its ability to increase the concentration of dissolved organic matter and soil nutrients (N, P, Ca and K), to remove toxic compounds from soil solution by adsorption, and to change the quality of soil water and its pH. Due to fresh biochar's recalcitrance (Kuzyakov et al., 2009), water repellence (Blackwell et al., 2009), and toxicity (Hale et al., 2011; Liu et al., 2008; Zimmerman et al., 2011) some studies report no effect of biochar on soil microbial biomass (Castaldi et al., 2011; Rutigliano et al., 2014; Zavalloni et al., 2011). According to Dempster et al. (2012) and Liu et al. (2008) the toxic effect of biochar due to its polycyclic aromatic hydrocarbons (PAHs) and some highly volatile organic compound substances caused soil microbial biomass to decrease. The ability of biochar to supply a protective environment by avoiding grazers (Pietikainen et al., 2000), and its storage capacity for labile carbon and nutrients (Saito and Marumoto, 2002; Warnock et al., 2007) which is associated with the high internal porosity of biochar, may be the reason for the positive effects of biochar on SMB.

2.7.3.5 Effects of biochar on greenhouse gas emissions and climate change

Applying different organic fertilizers, returning crop residues to the soil, decreased or even zero tillage, and increasing the soil cover are the traditional ways of enhancing the capability of soils in agricultural ecosystems to act as a carbon sink (Lal, 2004, 2007; Lorenz and Lal, 2014). The impacts of these methods on soil carbon storage highly depend on the contribution of the organic amendments to the readily available carbon and to soil nutrients which can boost carbon mineralization, soil respiration and GHG emissions (Oelbermann and Voroney, 2007). Therefore, it is important to find ways to reduce these contributions in order to increase the efficiency and effectiveness of bioenergy production systems as a renewable energy source (Woolf et al., 2010). Incorporating biochar into the soil in bioenergy crop production systems alters the bio-chemical pathways of carbon and other elements, and increases the net withdrawal of atmospheric carbon (Woolf et al., 2010). However, many controversial results were reported by previous studies in both tropical and temperate regions: amending soil with biochar may either increase (Deenik et al., 2010; Luo et al., 2011; Scheer et al., 2011) or decrease (Bolan et al., 2012; Dempster et al., 2012; Knoblauch et al., 2011; Liu et al., 2011; Malghani et al., 2013; Rondon et al., 2005; Zhang et al., 2010a, 2012b; Zimmerman, 2010) emission of CO_2, N_2O and CH_4.

In 2011, an estimate was made by Matovic (2011) claiming that by converting 10 % of the global net primary production to biochar and adding it to the terrestrial carbon sequestration has the potential to reduce fossil fuel emissions by 4.8 Gt C/yr. The Global Warming Potential (GWP) cannot only be reduced by reducing the CO_2 emission, but also by reducing N_2O and CH_4, which have an even higher GWP than CO_2 (Bijay-Singh et al., 2008; Singh and Cowie, 2014; Singh et al., 2010; Spokas et al., 2009). In addition to the direct impacts on GHG emissions, more indirect ways to fix atmospheric carbon into the soil by biochar were documented by researchers: by making soils more resistant to microbial and physical degradation (Major et al., 2010a), by providing a habitat for soil microorganisms which leads to the fixation of carbon in underground fungal networks (Warnock et al., 2007), by producing co-products like bio-oil and bio-syngases which are energy sources and can replace fossil fuel (McCarl et al., 2009). Kammann et al. (2012) and Zhang et al. (2012, 2011) reported no effect on GHG gas emissions after soil was amended with biochar. The internal porosity of biochar together with its water repellence may change soil aeration and water status (Karhu et al., 2011; Zhang et al., 2012a, 2011) and alter the soil microbial community and activity (Feng et al., 2012; Lehmann et al., 2011), which may finally affect soil CH_4 emission.

3. Investigation Area

3.1 Schleswig-Holstein

Schleswig-Holstein (SH) lies between the North Sea in the west and the Baltic Sea in the east. The terrestrial borders of SH are with Denmark in the north, Mecklenburg-Western Pomerania in the east, and Hamburg and Lower Saxony in the south (Figure 4). Looking at the geomorphological landscape, three regions can be identified in SH: eastern, central, and western. The eastern region of SH has bays and fjords along the coastline of the Baltic Sea. This part of SH is rich in natural landscape, including lakes and hilly areas formed during the last European Ice Age (the Weichselian glaciation). Geest, a slightly elevated land surrounded by plains and marshlands, is the common landscape in the central area. The Geest acts as a bridge between the marshes and the hills, and contains poor soils belonging to different ages and diverse origins like Saalian moraines, and fluvio-glacial deposits from the Weichselian period. Drage, where the experimental sites are located, lies on the west side of the central region close to Stapelholm (Figure 4). The area where young marine sediment was deposited during the Holozene represents the marshlands in the western region. This western region has heavy, fertile soils and is intensively used for livestock grazing. It was easily exposed to the high tides and storm floods before those were controlled by manmade dikes (also see land use and vegetation in 3.5). These natural events limit the full utilization of the fertile soil for crop production and the development as an urban area (Becker & Kaster, 2005; Behre, 2008; Schleswig-Holstein, 2014).

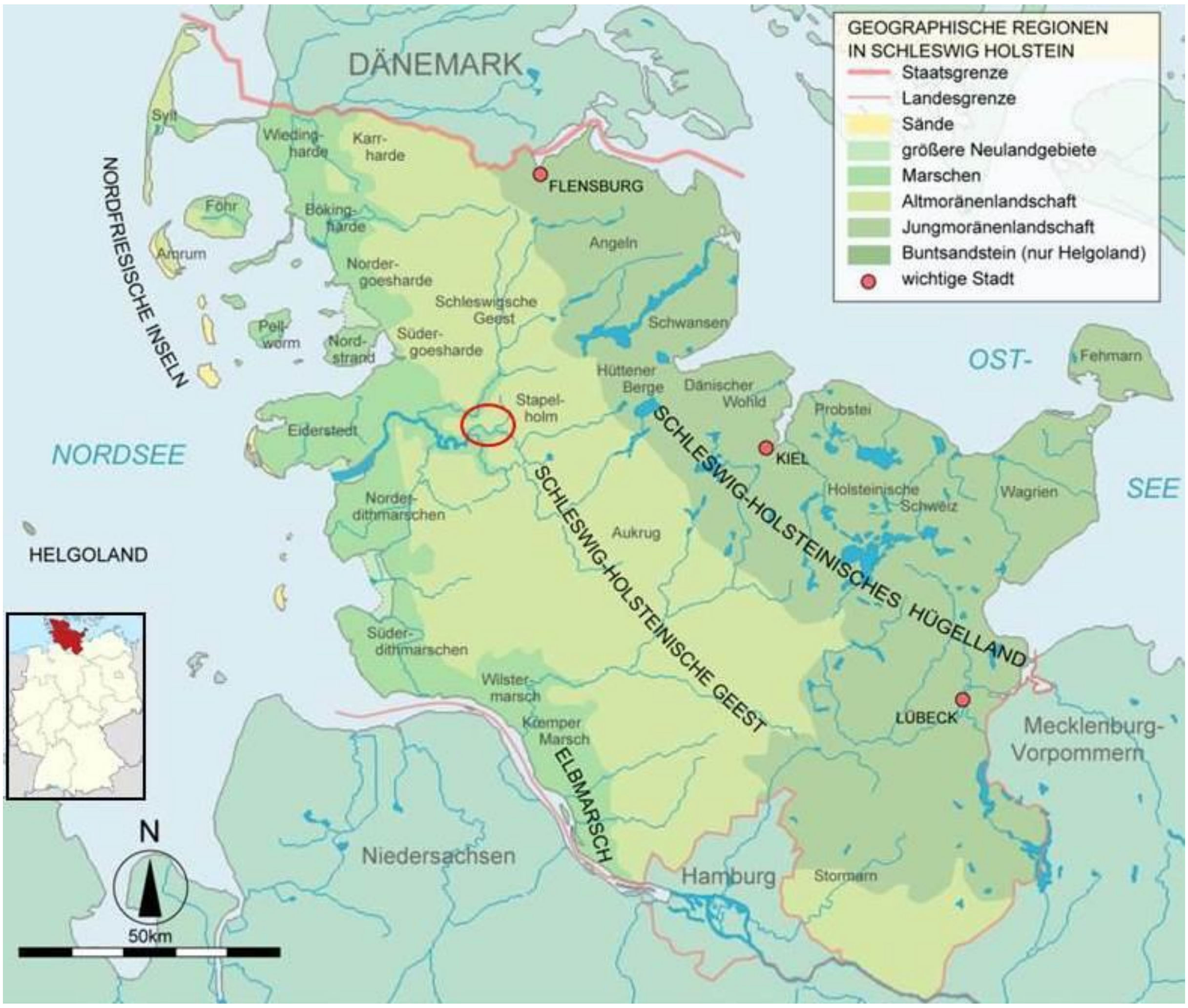


Figure 4: The location of Schleswig-Holstein with its borders and the natural landscapes based on geomorphology: the red circle shows the area of the experimental sites. (Source: www.de.wikipedia.org/wiki/Schleswig-Holstein, accessed on 19.03.2014)

3.2 Regional geomorphology of SH

From a geological perspective, the surface landscape of SH was mainly shaped by the most recent ice age. The greater part of SH, including Stapelholm and Drage where the experimental sites are, has its origins in the ice ages of the Quaternary geological era (2.6 Ma to 12 ka ago) and its interglacial periods (ICS, 2014). The Weichselian (115,000 - 10,600 a ago) and Saalian (300,000 - 130,000 a ago) glacial periods also had an influence on the landscape genesis of SH (Ehlers, 2011). But the development of the SH landscape mainly occurred in the Holocene era (from 11,700 a ago until today; Ehlers, 2011; ICS, 2014; Langheld, 2013; Schmidtke, 1995). The Eemian interglacial period and the Holocene, the two most recent warm periods of the geological history, contributed to the conversion of former glacial valleys of SH into today's marshes and moors (Becker and Kaster, 2005). The development of marshes in the western part of SH was associated with the rising sea levels after the ice age, the continuous influence of the tide and flooding by the North Sea, and the simultaneous rise of the groundwater level. All those activities helped to deposit marine-clay-organic sediments as marine and fluvial deposits in the lowland of west SH. But the Eider-Treene depression (*Eider-Treene-Sorge*) and the mudflats of the North Sea are mainly influenced by marine rather than by river sediments (Becker and Kaster, 2005).

3.3 Soils around Drage and Stapelholm

The parent materials of Stapelholm and Drage are sand and drifted sand formed during the glacial era, which belongs to the Saale glacial period. (Langheld, 2013). Soils of the Stapelholm area, Drage and their immediate neighborhood, are *Podzols*, *Cambisols*, *Gleysols, Anthrosols* and marsh soils (*Fluvisols*) or combinations of two of those soils, or of one of those soils with another soil type. In west Stapelholm, in the Eider-Treene-Depression and directly next to the Geest border, there are *Fluvisols*, and in the far north-eastern part of Stapelholm and Drage there are *Stagnosols* and *Planosols* (Langheld, 2013).

Cambisols (WRB, 2006) can be identified by a cambic diagnostic horizon, but sometimes a mollic horizon also can be found. The low temperature limits the weathering of medium- and fine-textured parent materials, and therefore the young soils still are in the beginning stage of their horizon differentiation. Because of the slow weathering process and the high precipitation throughout the year, the profile is not rich with illuviated clay and other ion compounds like aluminum and iron. Furthermore, the low temperature regime and the reduced microbial activities lead to the accumulation of lignin rich organic litter coming from acidophilic vegetation during the soil formation process (Stahr et al., 2012; USDA, 2010; WRB, 2006). *Podzol* (WRB, 2006) has both eluvial and illuvial horizons as subsurface diagnostic horizons because of the *podsolization* process. The *podsolization* process is intensified by the acidic condition of the soil, high precipitation, little evapotranspiration, low temperatures, acidic parent materials, and acidophillic vegetation including Ericaceae species, oaks and conifers. A natural, non-cemented subsurface layer is common in between the eluvial and the illuvial horizons. Subsurface horizons enriched with the sesquioxides of iron and aluminum are common in *Podzol* (Scheffer & Schachtschabel, 2010; Stahr et al., 2012; Ad-hoc-Arbeitsgruppe Boden, 2005; WRB, 2006).

The Eider-Treene depression is an area rich with *Gleysols* (WRB, 2006). Groundwater governs most of the characteristics of the *Gleysols* profile. A year-round high groundwater level generates an environment lacking oxygen, which leads to the development of *Gleysols* with the typical diagnostic oxidized horizon and the lower adjacent reduced horizon. The oxygen lacking environment induces iron and manganese to stay in their reduced state in which they are easily soluble in water and can therefore move through the soil profile giving it the typical grey and pale grey color (USDA, 2010; WRB, 2006; Scheffer & Schachtschabel, 2010; Stahr et al., 2012). The north-eastern part of Stapelholm has *Pseudogley* (WRB, 2006). In contrast to *Gleysols, Pseudogley* is not governed by the groundwater, but by the water added to the soil from above by rainfall or thawing of snow. *Pseudogley* is characterized by redoximorphic features with or without concretions, and a temporary but periodic water stagnation zone (Ad-hoc-Arbeitsgruppe Boden, 2005; Scheffer and Schachtschabel, 2010; Stahr et al., 2012).

In the area around Drage and Stapelholm, there also are young, organic soils associated with bog, fens, and peat lands (marsh soil). The organic matter content of those soils exceeds 30 % by weight and the profile has features of horizon differentiation, stratification, sometimes a distinct topsoil horizon, and the lower part of the profile can have redoximorphic features. Several types of marshes can be distinguished, depending on their development from their original sediments (Ad-hoc-Arbeitsgruppe Boden, 2005; Scheffer and Schachtschabel, 2010; USDA, 2010; WRB, 2006). The decomposition of organic matter is slowed down due to anaerobic conditions in water saturated soils. Usually the marshes and fens around Stapelholm and Drage (the Eider-Treene depression) are characterized by a low nutrients content which is caused by a rapid flow of groundwater through the sandy materials. Some of those marshes also have an influence from brackish water and the deposition of very fine-grained marine and fluvial sediments (Stahr et al., 2012).

Anthrosol (in German *Plaggenesch;* Ad-hoc-Arbeitsgruppe Boden, 2005; WRB, 2006) can also be found in the vicinity of settlements in the study area. . The diagnostic horizons of *Anthrosol* include an anthropogenic *E*-horizon and a thick *A*-horizon (Ad-hoc-Arbeitsgruppe Boden, 2005; USDA, 2010; WRB, 2006). *Anthrosol* soils have been transformed by anthropogenic activities, which added carbon and nutrients rich animal manure or organic materials to the soil. Organic matter has been regularly added to the topsoil and mixed with the subsurface horizons during the land preparation for crop establishments. This procedure is common in SH where agriculture has a long history, and was intensified after the increased availability of organic waste produced by bioenergy plants (Langheld, 2013; LLUR, 2012).

3.4 Climate

Schleswig-Holstein has a wet humid climate which is affected by the maritime climatic conditions of the Atlantic Ocean and the North Sea, limiting extreme temperature fluctuations, and resulting in mild year-round temperatures. According to Strahler & Strahler (2005), a main meteorological characteristic of SH is sufficient precipitation in all months. The favorable temperature combined with the high precipitation and humidity stimulates a strong development of vegetation (Schmidtke, 1995; Wagner, 1998). According to Wagner (1998), the weather in Stapelholm and Drage, including the experimental sites, is mainly governed by the direction of the wind, the main air flows, and the adjacent higher elevation lands. The main wind direction is from west to east and has a great influence on SH weather. Two prominent air flows, the cold polar air flow, and the tropical warm air flow caused by the Gulf Stream meet over SH (Strahler and Strahler, 2005; Wagner, 1998). Stapelholm and Drage are subject to receive orographic rainfall resulting from the adjacent high Geest area (Wagner, 1998).

For SH, the average monthly air temperature is 11.8 °C (- 4.0 °C to 27.7 °C), and the monthly average precipitation is 74.1 mm (ranging from 1.3 mm to 275.4 mm) based on long term historical climate data (from 1947 to 2014; DWD, 2014). The respective values for the experimental sites, as gathered by the Erfde weather station which is situated 14 km away from the field sites over a 50-year period (1965 - 2014), are a monthly average air temperature of 8.7 °C, and an average monthly precipitation of 70 mm (DWD, 2014).

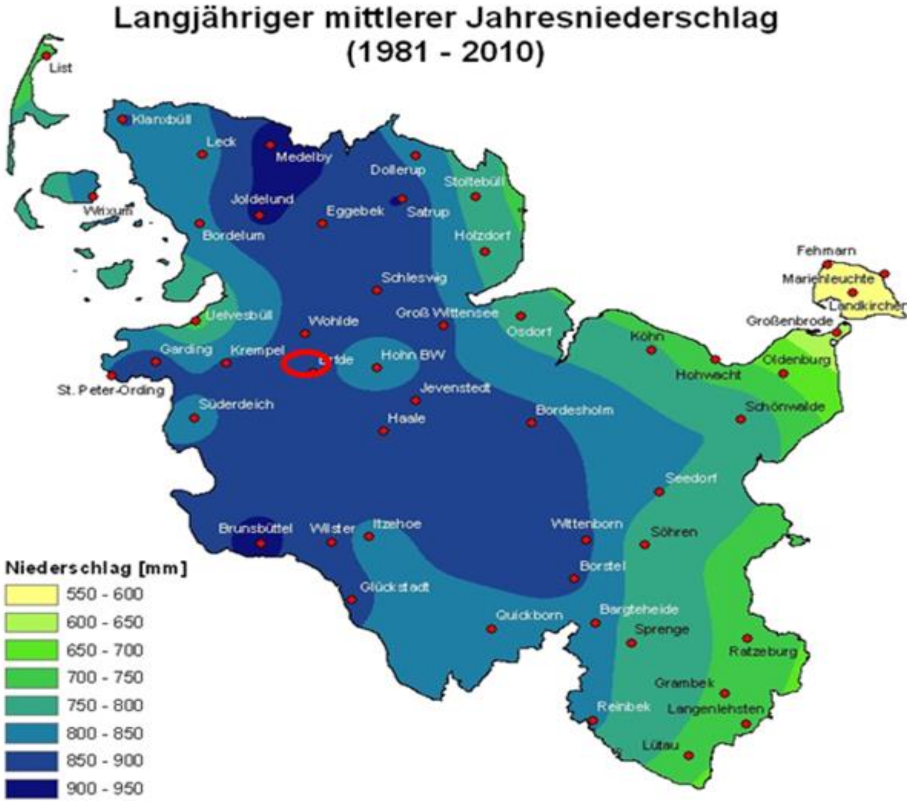


Figure 5: Map of mean annual precipitation (mm) in SH. The red circle indicates the experimental site (source: www.schleswig-holstein.de, accessed on 10.11.2013)

3.5 Land use and vegetation

Except in the salt affected marshes in the south and west and in the fens, initially natural cold-loving arctic woody vegetation developed on the glacial deposits and permafrost soils. Later, with increasing temperatures, this cold-loving vegetation gradually converted into heat-loving trees such as oak, alder, ash, hazel, lime and elm (Behre, 2008). Vegetation changes were not only caused by the thawing of glacial ice and permafrost soils, but also by the competition among vegetation and by human impact from the Neolithic up to the agro-industrial age in the 20th century. In most regions of SH, the natural landscape and original vegetation has been transformed into a man-made cultural landscape (Becker and Kaster, 2005). According to Behre (2008) and Becker & Kaster (2005), Stapelholm and the adjacent high Geests were originally dominated by nutrient-poor acidic soils. Before mid-19th century, there were several attempts to cultivate the marshes and bogs in SH, but the levees were not strong enough to withstand the storm surges. After those unsuccessful attempts in the marshes and bogs, people once again considered the hill Geests for use as arable land (Becker and Kaster, 2005). The original forests in many parts of the high Geest areas in SH were deforested to obtain timber and firewood and later were used as farm land for agriculture. After several years, those lands were no longer productive and were neglected as marginal land with highly degraded soil. Some of the present natural forest areas of SH include the forests originating from re-growth of natural vegetation in those neglected marginal lands (Becker and Kaster, 2005; Behre, 2008). Only small primary forest areas remained untouched including hedges and hedgerows which were originally planted to mark the borders of fields and pasture lands, to procure firewood, and to be used as windbreaks (Becker and Kaster, 2005). Re-forestation activities were started after a comprehensive agrarian reform in 1850, but despite intensive planting of fast growing tree species such as pine and spruce primarily for use as timber, Schleswig-Holstein still is the state with the smallest area of primary forest (10 %) in Germany where the national average of forest land is 31 % (Behre, 2008; BMELV, 2011; Liedtke and Marcinek, 2002; Statistikamt Nord, 2012). In 2011, the land use statistics of SH showed that 70 % of the land in SH was used as agricultural land (BMELV, 2011; Statistikamt Nord, 2012), and 73 % in Stapelholm and Drage (Statistikamt Nord, 2012).

Agriculture was again intensified responding to the demand that was generated by increasing population, which caused the agro-socio-economical soil degradation in northern Germany. With the availability of powered pumping stations, it became easy to drain the diked areas, thus reducing the flooding impacts, and continuous development of increasingly strong and efficient new levees stopped the devastating influence of tides and storm surges on the marshes of Stapelholm during the 20th century (Becker & Kaster, 2005; Behre, 2008). This huge anthropogenic development is still visible in some places in SH, the most prominent example being the development of the city Heide.

3.6 Experimental sites

SH was chosen to carry out the field experiments because of its significant role in Germany’s renewable energy production through biomass. According to the Statistikamt Nord 2012-2, in 2011, 38.6 % of the electricity supplied by SH to the national electricity grid came from renewable energy sources. The highest portion of this contribution is wind energy (25.3 %). Biogas was used to produce 7.7 % of the electricity in SH, while this contribution in all of Germany is only 6 %. SH utilized 19.8 % of its biogas to generate electricity which was higher than the average in Germany: 5.4 % (Statistikamt Nord, 2013). In 2011, SH had 742 biogas plants producing electricity out of biogas, and nearly 24 % of the arable land was used for energy crop production (DBFZ, 2012; MELUR, 2012). The increased demand for biomass by biogas plants converts many pasture lands into bio energy crop production land which is continuously altering the regional agricultural land use pattern.

The field experiments were established at two locations, which were 1.4 km apart from each other in the bioenergy production area in Drage in SH (54° 35′ 41.18″ N, 9° 17′ 27.16″ E and 54° 36′ 43.31″ N, 9°15′25.18″E) between the river Eider in the south and the river Treene in the north and northwest (Figure 6). Site 1 is a flat land very close to the bottom of a sloping land (< ≈ 2 % slope) with a depressed area between the bottom of the slope and the experimental plot. Site 1 did not receive any additional organic matter before, nor was it used for bio-energy crop production or any other agricultural purposes recently. Site 2 is in a bio-energy crop production field, which had regularly received biogas digestate (42 L m^{-2}) at the beginning of each cropping season as organic matter for many years already.

Figure 6: Aerial and ground views of the study sites in Drage. The arrows show where the experimental plots were in the two sites.

4. Materials and Methods

4.1 Production of biochar

The biochar we used in the field experiments was produced by the fast thermal combustion of the dried solid portion of biogas digestate (average particle size ≤ 20 mm) by a bioenergy plant in northern Germany (Biogasvertrieb Nord GmbH & Co.KG in Drage, Germany), which used a mixture of bioenergy crop and animal waste as a substrate for the biogas production. This biogas plant used different crops as feedstock depending on the seasons of the year, e.g. sweet corn, winter wheat and winter rye.

The pyrolysis process was done at PYREG® GmbH in Dörth, Germany, using the PYREG® 500 multi-purpose dry, continuous flow pyrolysis unit at 650 °C temperature. The pyrolysis unit contains a feeder to feed the feedstock to the reactor, a PYREG® reactor where feedstock is pyrolyzed into biochar, and a FLOX® combustion chamber which heats up to 1,250 °C to completely burn synthesis-gases generated during the pyrolyzing process. Some portion of the heat generated in the FLOX® combustion chamber is used by the PYREG® reactor to carry out the pyrolyzing process, and some other portion is used by the combi-dryer to dry the biomass before feeding it to the reactor. The production flow chart of the PYREG® biochar production process is shown in Figure 7.

The thermal decomposition zone operates as an oxygen free environment and under a small to a negative pressure (1.5 mbar or less) to ensure safety by preventing the leaking of flammable gases from the gasification zone. Two contra-rotating, interlocked helical screws are placed inside the PYREG® reactor and are driven by a motor. Therefore, during the pyrolyzing process, both feedstock and biochar are subjected to being physically broken down into smaller particles. The biochar production unit does not have a specific cooling chamber or any air flow going through the warm and fresh biochar. The discharge pipe is equipped with a middle vertical screw leading to an upward flow of the warm fresh biochar. The biochar is cooled down gradually inside this discharge pipe while approaching the collecting container. After that, the biochar gradually is cooled down to room temperature inside the collecting container. The whole retention time of the production process was 30 - 40 minutes.

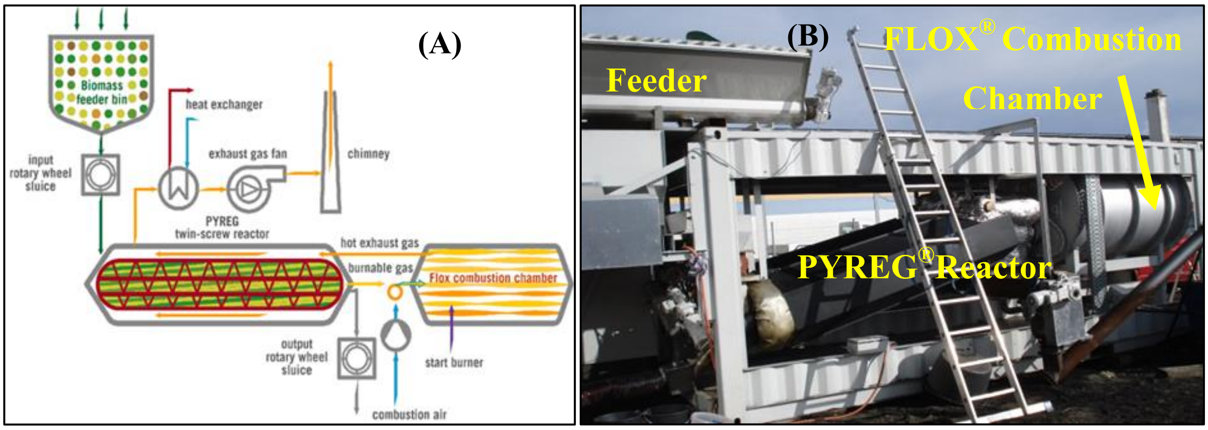


Figure 7: Biochar production process at PYREG® (source for (A)- http://www.pyreg.de, 2012)

4.2 Field experiment: Soil treatments and crop management

A completely randomized block design with three replicates was used in both experimental sites. Site 1 had two treatments, biochar (B) and control (C), and site 2 had three treatments, digestate only (D), and two different mixtures of digestate and biochar: 1:1 (BL) and 1:5 (BH). Table 2 shows more details about those treatments. Each experimental plot was 9 m^2 (3 m x 3 m) in size, separated by 3 m wide buffer zones between the plots, and between the plots and the main field Figure 9.

Table 2: Treatment mixture and resp. soil type and added amount of carbon

Site	Soil type	Treatment	Added amount of carbon
Site 1	*Gleyic Podzol*	Control (C)	No amendment
	Gleyic Podzol	Biochar (B)	0.15 kg C m^{-2}
Site 2	*Gleyic Anthrosol*	Digestate (D)	0.15 kg C m^{-2}
	Gleyic Anthrosol	Digestate:biochar 1:1 (BL)	0.15 + 0.15 kg C m^{-2}
	Gleyic Anthrosol	Digestate:biochar 1:5 (BH)	0.15 + 0.75 kg C m^{-2}

Field site 2 with *Gleyic Anthrosol* was used continuously to produce biomass for bioenergy production with crop rotation between maize (*Zea mays* L.), winter wheat (*Triticum hybernum* L.), and winter rye (*Secale cereale* L.). During our experiment, both site 1 and site 2 had crop rotation with the same crops mentioned above. More details about crop management practices are shown in Figure 8 and Table 3. After harvesting maize (*Zea mays* L.) as a summer crop in October 2012, the soil was chisel ploughed down to 0.20 - 0.30 m depth before seeding winter wheat (*Triticum hybernum* L.) in the 2012/13 winter season.

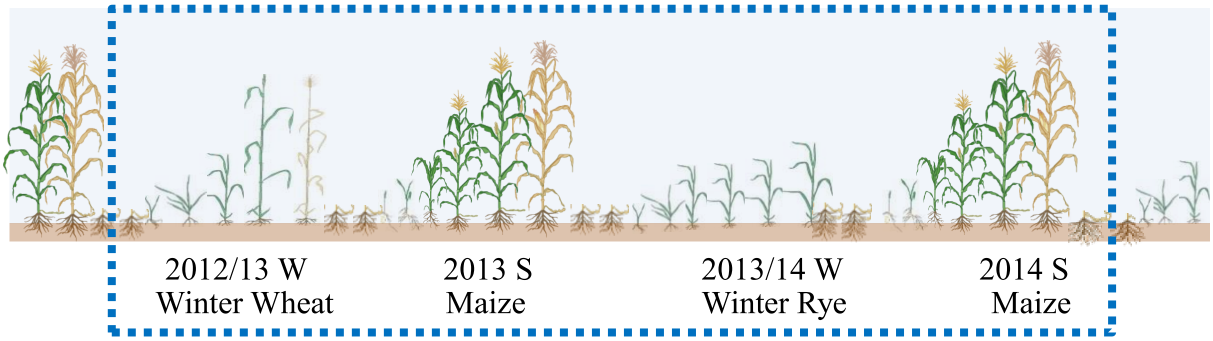


Figure 8: Crop rotation plan

Before seeding was done in the ploughed and pulverized soil, the biochar was incorporated into the soil as a fresh biogas digestate, up to a depth of 20 cm. The lowest application rate used in the experiment (0.15 kg C m-2) represents the amount of carbon applied as digestates by the farmer under normal agricultural praxis. To ensure the equal distribution of biochar, we divided the experimental plot (9 m^2) into 9 smaller sub plots (1 m^2), added the needed amount of biochar onto the soil surface of those small sub plots manually, and distributed the biochar on the soil surface equally by using wooden hand-rakes. Soon after, the biochar was mixed into the 0 - 0.2 m soil layer using a rotary tiller fixed to a single-axle tractor (see Figure A 14 in the annex). More details about the cropping seasons and crops are shown in Table 3.

Table 3: Crop rotation

Crop*	Season	Date		Spacing****
		Seeding	Harvesting	
Winter wheat (*Triticum aestivum* L.)	Winter 2012/13	October 2012	June 2013	100 kg seeds ha^{-1} (10 g m^{-2})
Maize (*Zea mays* L.)	Summer 2013	June 2013**	October 2013	250 kg seeds ha^{-1} (10 seeds m^{-2})
Winter rye (*Secale cereale* L.)	Winter 2013/14	November 2013	May 2014	100 kg seeds ha^{-1} (10 g m^{-2})
Maize (*Zea mays* L.)	Summer 2014	May 2014***	September 2014	250 kg seeds ha^{-1} (10 seeds m^{-2})

* Variety information unavailable ** For site 2: Fertilizer application rate 25 g of Calcium Ammonium Nitrate *(CAN or Kalkammonsalpeter KALKAMON Dünger* in German) m^{-2} *** 25 g of CAN m^{-2} plus liquid digestate 42 L m^{-2} as a surface application ****Maize: row seeding, wheat and rye: broadcasting

After seeding in each cropping season, two soil collars (Ø = 20 cm and height = 20 cm, LI-COR Biosciences) were randomly placed inside each experimental plot to use for future gas emission measurements. The soil collars were inserted into the soil (approximately 15 cm to 18 cm deep), and stayed in the same place for the whole growing season (see Figure 9 and Figure 10).

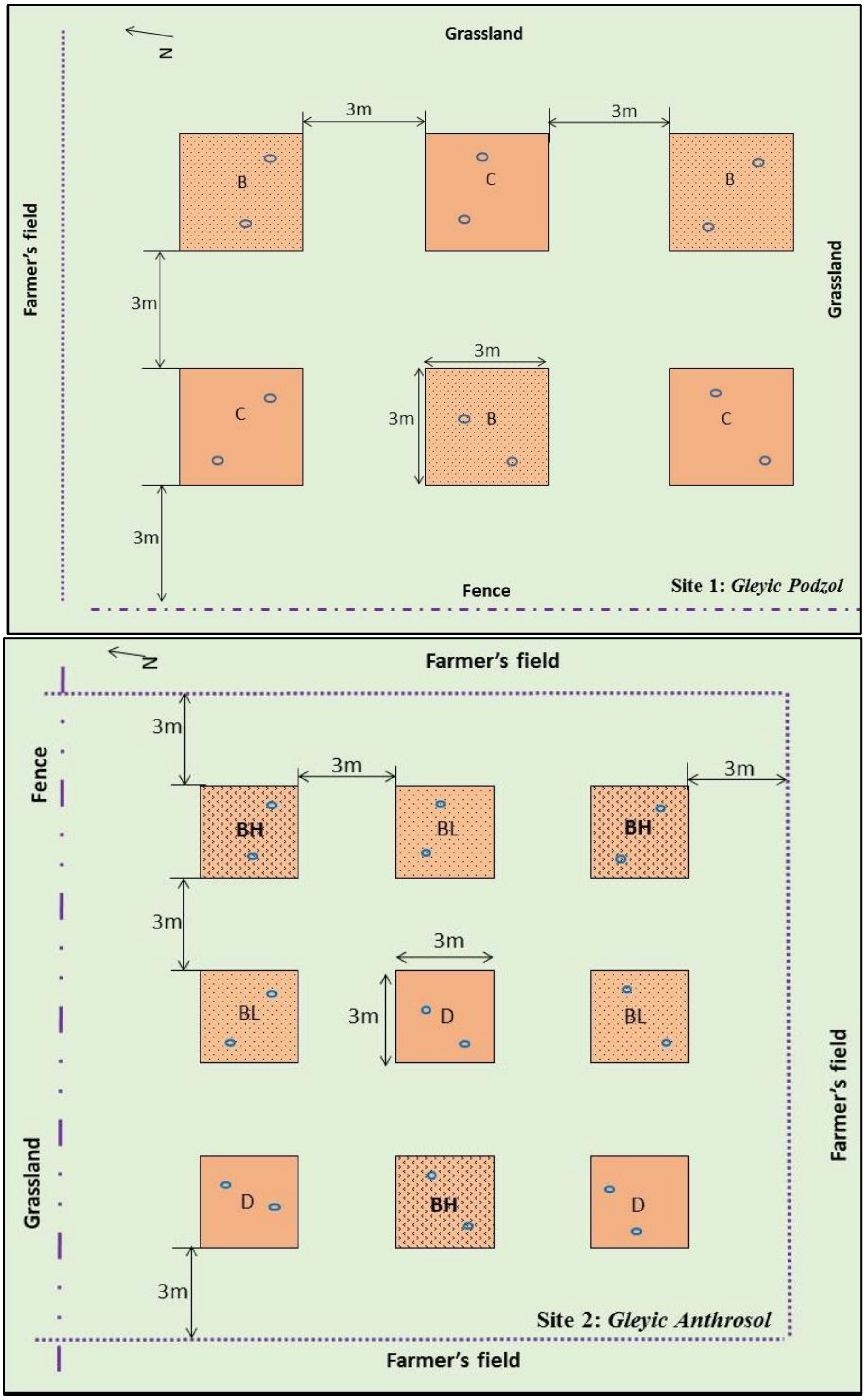


Figure 9: Overview of the plots and the treatment distribution of the field experiment for field sites, site 1 with *Gleyic Podzol* and site 2 with *Gleyic Anthrosol*. The number and the letters indicate the plot number and the treatment for a respective plot. The blue circles indicate the soil collars. Note: the diagram is not to scale.

4.3 Total biomass production

At the end of every growing season, a randomly selected 1 m^2 (1 m x 1 m) area was used to collect a total biomass of the crop. The crop was harvested at a height of $\leq$ 10 cm above the soil surface. The collected samples were weighed, stored in polyethylene bags, and sealed. The root was uprooted with the rest of the stem and the soil particles were removed by shaking. Before obtaining the fresh weight of the root samples, all soil particles were washed with tap water, and excess water was drained at room temperature. Randomly selected, representative above and below ground biomass samples (100 g) were oven dried at 70 ± 1 °C for $\geq$ 48 hours to determine the dry mass of biomass. The same sample was used to determine the total carbon and nitrogen content as described in subsection 4.7.4.

4.4 *In situ* chamber measurements of greenhouse gases

At each treatment plot, soil respiration, N_2O and CH_4 emissions on the soil surface were determined by using the technique of closed chamber (CO_2 by using Automated Soil CO_2 Flux System - LI-8100 survey chamber Ø = 20 cm, LI-COR Biosciences, USA; and N_2O and CH_4 by using Acryl transparent chamber, Ø = 20 cm, University of Hamburg, Germany: Figure 10). The closed chambers were placed on the soil collars (Ø = 20 cm) which were installed at the beginning of each growing season. Both chambers were equipped with a groove together with a rubber sealer to prevent gas exchange with the surrounding environment. Gas flux measurements were carried out over two years, including the crop rotation between winter and summer, from November 2012 to September 2014, except in December 2012 and January 2013 due to the frozen soil collars. Inside the soil collars, the soil surface was cleaned by removing litter material and vegetation at least 30 minutes before starting the monthly chamber measurements to minimize the error caused by soil distraction.

4.4.1 Soil respiration measurements

The emission of CO_2 from the soil surface was measured monthly using a LI-COR® closed chamber. In each plot, measurements were taken at two different places which remained fixed over the whole experimental period. Therefore, for each type of treatment six measurements were taken on each measurement day. The times of the measurements were spread over the day (10:00 to 15:00), but for each plot, the measurements were always taken at the same time of the day.

Many authors discussed the pros and cons of closed chamber measurements. Kutzbach et al. (2007) discussed various errors which are related to chamber measurements that might cause differences to the natural emission fluxes. The placement of the chamber, the pressure gradient between the inside chamber and the ambient atmosphere at the closure time, and the volume of the chamber can alter the result of the chamber measurements (LI-COR Biosciences, 2007; Xu et al., 2006; Conen and Smith 1998). The LI-COR Biosciences Automated Soil CO_2 Flux System was carefully designed to minimize those errors by using the following provisions: a top pressure vent to balance the pressure during the measuring time, an automated slow closing of the chamber to prevent pressure spikes at the chamber closing time and keep equal pressure inside and outside the chamber during chamber closure, a volume of the chamber of 4843 cm^3 which allows to measure small fluxes with higher accuracy, etc. (LI-COR Biosciences, 2007). To further minimize those errors, the automated calculation includes continuous changes of temperature and pressure of the micro-environment inside the chamber during the measurement (Biosciences, 2007).

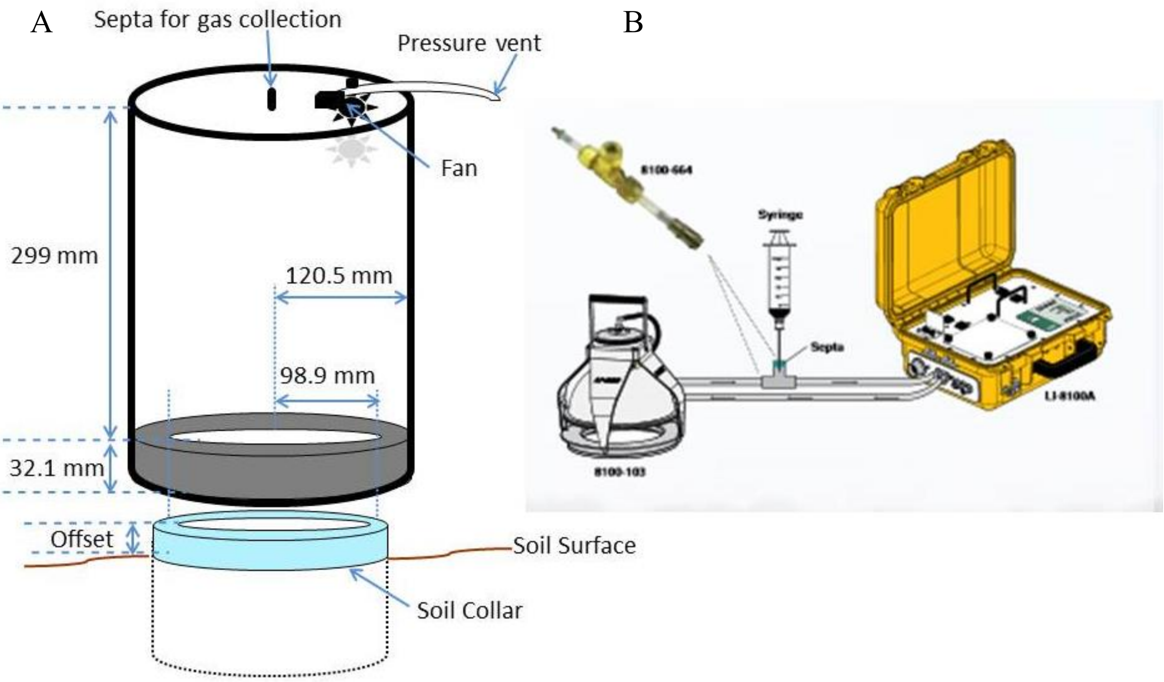


Figure 10: Setup for trace gas measurement and gas sample collection in the field. (A) Acryl transparent chamber and (B) LI-COR Biosciences chamber (Source for (B): www.licor.com).

4.4.2 N_2O and CH_4 measurements

The fluxes of both N_2O and CH_4 were calculated using the gas concentrations measured with gas chromatographs (GC) of gas samples collected from the closed chamber head space via the vent during the chamber closure. Five samples were taken using 60 ml plastic syringes (Omnifix 60 mL, BRAUN Melsungen AG, Melsungen, Germany) equipped with three-way-stopcocks. The first sample was taken just after the chamber closed, and the remaining four samples were taken at intervals of approximately ten minutes. The collected gas samples were analyzed on the following day at the Institute of Soil Science, Hamburg. Both N_2O and CH_4 fluxes were determined using the GC fitted with both a flame ionization detector (FID) and an electron capture detector (ECD) operating at 280 °C and 300 °C respectively (7890, Agilent Technologies, Germany). To ensure the accuracy of the GC measurements, prior to measuring the samples, standard gas samples (0.29, 1.05 and 1.55 ppm for N_2O, and 1.7 and 200 ppm for CH_4) were measured following the same injection procedure. The N_2O and CH_4

fluxes were calculated using the respective gas concentrations and time intervals, pressures, and temperatures of chamber head space with a modified and updated version of the MATLAB script developed by Kutzbach (Kutzbach, et al., 2007). A linear flux calculation was used.

4.5 Soil microbial biomass carbon (MBC) and nitrogen (MBN)

Soil microbial carbon and nitrogen (MBC and MBN resp.) were measured using a fresh soil sample collected from the experimental field in a monthly interval. After removing organic litter materials from the fresh soil sample by hand, and sieving through a 2 mm sieve, the chloroform-fumigation-extraction method (CFE) was performed (Brookes et al, 1985, Vance and Brookes, 1987). For fumigation, 30 g of sieved fresh soil was weighed into a 50 ml glass beaker and fumigated with chloroform for 24 hours at 25 °C under low pressure (- 600 mbar). The fumigated soil sample was shaken for 30 min at 200 rpm with 20 ml of 0.05 M K_2SO_4 solution. The mixture was filtered (Whatman 595 ½ filter papers) before quantifying the carbon and nitrogen content. For another part of the same soil sample, total organic C and N content was determined in the same way but without fumigation. Carbon and nitrogen in the soil microbial biomass were calculated using the following equations (Joergensen, 1995, 1996; Wu et al., 1990).

$$\mathbf{MBC} = \frac{(\mathbf{C_{fum}} - \mathbf{C_{non}})}{\mathbf{0.45}} \text{ and } \mathbf{MBN} = \frac{(\mathbf{N_{fum}} - \mathbf{N_{non}})}{\mathbf{0.54}}$$

where C_{fum} and N_{fum} are the organic carbon and organic nitrogen extracted from the fumigated soil, and C_{non} and N_{non} stand for the respective values of non-fumigated soil, all in µg g^{-1}. The extractable part of the soil microbial biomass is used as the denominator which is 0.45 for carbon and 0.54 for nitrogen (Brookes et al, 1985, Vance and Brookes, 1987). The selected samples from May, August and November 2013, and March, May and July 2014 were analyzed by Paulina Reimers, and the data was used for her bachelor thesis.

4.6 Potential carbon mineralization of digestate and biochar

4.6.1 Aerobic incubation experiment

To study potential mineralization of digestate and biochar, an aerobic incubation experiment was conducted using two different soils and five different treatments with three replicates each as a part of the master thesis work of Magdalena von Leliwa (von Leliwa, 2014). Three *Gleyic Podzol* topsoil (0 - 0.2 m) samples were collected from three different places of the experimental field site 1 in Drage. Those soil samples were collected along the transit which runs diagonally across the site.

For the laboratory incubation experiment, soil treatments similar to the ones used for the field experiment were used (see Table 1 in 4.2.2). Homogenized and litter free soil (25 g) was incubated using airtight glass jars (500 ml) with lids containing butyl rubber stoppers. The soil was treated with the following amendment mixtures: control (C, soil without amendments), biochar only (B), digestate only (D), digestate:biochar 1:1 mixture (D:B 1:1), and digestate:biochar 1:5 mixture (D:B 1:5). The water content of the soil was adjusted to 40-60 % of its maximum water holding capacity by adding the necessary amount of water. The incubation bottles were placed in a dark incubator at a temperature of 22 ± 1 °C. Head space CO_2 concentration was measured by collecting an air sample through the butyl rubber stopper with gradually increasing measuring intervals at the gas chromatograph (Agilent Technologies 7890 A GC system, Böblingen, Germany). When the head space CO_2 concentration exceeded 3 % of the total volume, those samples were flushed with synthetic air to prevent disturbances caused by excess CO_2 in the head space on the new production of CO_2 from the soil. For this purpose, hypodermic needles connected to a vacuum pump were inserted through the butyl rubber stopper in the bottle and the bottle was evacuated for 6 minutes. This was followed by the addition of synthetic air that flowed through the incubation vessels. The synthetic air had a composition of 79.5 % nitrogen, and 20.5 % oxygen. The CO_2 content in the incubation bottles was determined again after one hour.

4.6.2 $\delta^{13}C$ signatures of CO_2

The $\delta^{13}C$ values of CO_2 produced from the incubated soil samples were analyzed twice using a gas chromatograph connected to an isotope ratio mass spectroscope (ThermoQuest Finnigan Delta Plus/GC Combustion III). With the previously measured CO_2 concentration, the injection volume was calculated for each sample, and injected sample volumes varied from 0.01 - 0.3 ml. Values are expressed relative to VPDB using the reference standards LSVEC ($\delta^{13}C$ VPDB -46.6 ‰) and IAEA B7 ($\delta^{13}C$ VPDB -3.0 ‰). The standard deviations of duplicate measurements were less than 0.2 ‰.

4.6.3 $\delta^{13}C$ signatures of soil organic matter, digestate and biochar

The $\delta^{13}C$ signatures of digestate, biochar and soils were determined to quantify the different sources of carbon in the CO_2 produced from the incubated soil. The calculated amount of finely ground and dried samples was weighed into tin cups and measured at a mass spectrometer (Flash 2000, Delta V Plus).

4.7 Analysis of soil, digestate and biochar

4.7.1 Soil profiles and soil sampling

For the soil survey, samples were taken from every diagnostic horizon from soil pits which had been dug in both study sites till meeting the groundwater table. Soil classifications were done with reference to the German soil classification (Ad-hoc-Arbeitsgruppe Boden, 2005), and the World Reference Base for Soil Resources (WRB, 2006). Soils were described according to the characteristics of the diagnostic horizons including soil texture, organic matter content, moisture content, and *'in situ'* observations, with reference to the German Soil Classification System (Ad-hoc-Arbeitsgruppe Boden, 2005). Collected soil samples were stored in polyethylene-bags, and frozen at -5 °C to minimize biological activities prior to performing the laboratory physico-chemical analysis. Undisturbed soil core samples were collected from each diagnostic horizon of the soil pit using a standard metal core (Ø = 5 cm, h =

5 cm) to determine soil water retention (pF). The top and bottom surface of the soil core were trimmed and covered by polyethylene layer and a plastic cap to prevent water losses by evaporation, and disturbances during transportation to the laboratory. The soil core samples were stored at 4 °C temperature in the cool room until starting the laboratory analysis. During the first year of the experiment, mixed soil samples from 0 - 20 cm depth were collected in a monthly interval. During the second year, mixed soil samples were collected seasonally. After the summer crop was harvested in 2013, soil samples were collected from different depths (0 - 0.2, 0.2 - 0.4, 0.4 - 0.6 m) using a soil auger. In September 2014, at the end of the experiment, mixed soil samples and undisturbed soil core samples were collected from all three different treatments in field site 2. Those samples were collected from the uppermost three horizons (Ap, E, GBv) of the soil profiles which were opened inside the treatment plots. All the mixed soil samples were stored in polyethylene-bags under frozen condition until the beginning of the analysis.

4.7.2 Soil texture

The grain size distribution of the collected soil samples was determined according to DIN ISO 11277 (2002). Before the analysis started, the composite samples were dried at room temperature, sieved using a 2 mm sieve, and treated with H_2O_2 when a sample had high organic carbon (> 2%) content. None of the samples needed to be treated for calcium carbonate. For the wet sieving, 30 g of each sample were mixed with 100 ml of water and 25 ml of 0.4 M $Na_4P_2O_7$ (sodium-pyrophosphate) and shaken for 18 hours to disperse the clay minerals. The sand fraction of the pre-treated soil sample was separated from the clay and silt fraction by flushing through a 63 µm sieve and washing with water until the washing water was clear. After drying the sand fraction at 105 °C in the oven, a dry sieve set was used to separate fine, medium and coarse sand. The fraction flushing through the 63 µm sieve was collected into a 1000 ml cylinder and the volume was filled up to 1000 ml before fractioning clay and silt using the Köhn pipette method according to DIN ISO 11277 (2002) using a SEDIMAT 4 - 12 analyzer (UGT GmbH, Müncheberg, Germany).

4.7.3 Soil water retention

The soil water retention curves were determined for the soil horizons of both experimental sites before starting the field experiment in September 2012. Five undisturbed soil core samples were collected from each soil horizon using metal rings (see 4.7.1 on page 43). After determination of the fresh weight, the soil cores were slowly saturated and weighed again. Then, the saturated soil cores were transferred into a pressure plate extractor, the soil water retention was determined gravimetrically at 20, 60, 300, and 1000 mbar pressure, and volumetric water content was calculated using those values (Richards and Fireman, 1943).

4.7.4 Soil chemical properties

Prior to performing the soil chemical analysis, all litter materials, living plants and plant roots, and animals were removed from the mixed samples. The fresh samples were sieved to ≤ 2 mm before getting sub samples, subsequently dried at 105 ± 1 °C for ≥ 24 hours, and milled to use for necessary analysis.

The gravimetric water contents were determined by using 10 g of fresh soil, after keeping them in an oven at 105 ± 1 °C for ≥ 24 hours and then cooling them down to room temperature in a humidity free environment. The amount of water loss due to the oven drying process was used to calculate the gravimetric water content (VDLUFA, 1991).

For the determination of the soil pH and the electrical conductivity (EC), 10 g of fresh, sieved soil samples were mixed with 50 ml distilled water (specific conductivity not higher than 0.2 mS/m at 25 °C) and shaken using a horizontal shaker for 30 minutes. The soil-water suspensions were kept for one hour, then agitated again for five minutes before reading the value using a pH meter (CG 820, Schott, Germany) according to DIN ISO 10390 (2005). The same soil-water suspensions were used to determine the EC according to DIN ISO 11265 (1997), using a glass single-rod electrode conductivity meter (LF 90, WTW, Germany). Both, pH meter and conductivity meter, were calibrated with standard buffer solutions (pH 4 and pH 7 and 0.2 mS m^{-1} at 25 °C) before taking measurements.

The total carbon (TOC) and total nitrogen (TN) content were determined according to DIN ISO 10694 (1996) with an elemental analyzer (Vario MAX Elementar, Hanau, Germany). The soil sample was finely ground using a vibration disk mill with tungsten-carbide-coated walls and oven-dried at 105 ± 1 °C for ≥ 24 hours before using for analysis. The amount of sample that was used for the elemental analyzer depended on the nature of the sample, e.g. 0.3 - 0.7 g for mineral soil samples, 0.245 - 0.280 g for organic rich soil and plant samples. The samples were combusted at a temperature of 900 ºC, and released gases were passed through a copper oxide catalyst after having been dried. During this passage, CO was oxidized to CO_2 and mono-nitrogen oxides (NO_x) were reduced to $N_{2,}$ and both gases were separated using a CO_2 adsorption tube. The quantitative amount of CO_2 and N_2 were measured separately using a thermal conductivity detector.

Following VDLUFA (1991), a double-lactate extraction was used to determine plant available potassium and phosphorus. The samples were shaken with double-lactate solution (0.04 N with respect to Calcium and 0.02 N with respect to HCl; Riehm, 1942) for 90 minutes, and the filtrates were analyzed for potassium in an atomic absorption spectrometer (ASS; type 1100B, Fa. Perkin-Elmer, USA). After adding freshly prepared color indicator solutions, the amount of phosphate molybdenum-complex which was produced in the solution was used to determine the plant available phosphorus using a spectral photometer at 660 nm (DR 5000, Hach Lange).

Depletive extraction with ammonium chloride solution was used to determine the cation exchange capacity (CEC), the amount of exchangeable basic cations, and the base saturation (BS) (DIN ISO 11260, 2011, Blume et al. 2010 SL). Air dried and finely ground 5 g of soil materials were mixed with 25 ml of 1 M ammonium chloride solution. The mixture was shaken for 10 minutes, followed by 10 minutes of centrifugation at *30,000 rpm*. Afterwards, the supernatant was decanted into a 200 ml volumetric flask. After repeating this step three times, 25 ml of 1.25 M NH_4Cl_2 solution was added to the sample and centrifuged and stored overnight. On the following day, the supernatant was decanted into the same volumetric flask. The volume was adjusted of the volumetric flask, the solution was filtered, and the filtrate was used to determine the concentration of the exchangeable cations Na^+, K^+, Mg^{++} and Ca^{++} using an atomic absorption spectrometer (ASS; type 1100B, Fa. Perkin-Elmer, USA). The CEC was calculated as the sum of the concentrations of the exchangeable cations. BS was given as a percentage taken up by the sum of the concentrations of base cations (Na^+, K^+, Mg^{++} and Ca^{++}) in CEC.

Inorganic nitrogen compounds (nitrate/nitrite and ammonium), dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were determined using 0.0125 M $CaCl_2$ extraction. Sieved (2 mm), fresh 30 g of soil were weighed into 100 ml polyethylene bottles, 60 ml of 0.0125 M $CaCl_2$ was added and shaken in a vertical rotating shaker at 120 rpm for 30 minutes, and then filtered through ash free filter paper (Whatman 595 ½, 12-25 µm filter papers). For nitrite and nitrate, 0.6 ml of the extract was used and measured by high-performance liquid chromatography (HPLC, Agilent 1200, Hannover, Germany). In order to determine the amount of ammonium, the sample was analyzed with a spectro-photometer (Hach, Lange, type 5000). DOC and DON in the filtrate were measured using a TOC Analyzer 5050 (Shimadzu TE 2009AH, Japan).

4.7.5 Digestate and biochar

Fresh biochar and dry digestate samples were analyzed to determine water content, pH, EC, bulk density, TOC, TN, C/N ratio, exchangeable cations (K^+, Ca^{++}, Mg^{++}, Na^+), CEC, and BS (TMECC, 2002). pH and EC were determined in an 1:10 (w/v) water extract after shaking the mixture mechanically for one hour, using a pH meter (CG 820, Schott, Germany) and a glass single-rod electrode conductivity meter (LF 90, WTW, Germany). For biochar, the bulk density was calculated by weighing 10 ml of milled sample, and the ash content of biochar was determined by a muffle furnace at 550 °C (TMECC, 2002).

4.8 Statistical analysis

Sigmaplot 12.5® (Systat Software, Inc., USA) was used for performing statistical analysis. The significance of the seasonal variations of gathered data from the field experiments were tested separately for the two soils using one-way ANOVAs. The significance of the differences between treatments were tested using either the Tukey or the Holm-Sidak post-hoc test, depending on the nature of the data sets. Both Sigmaplot 12.5 and OriginPro 8.1G® (OriginLab Corporation, USA) software packages were used to generate the graphs to present the experimental results.

5. Results

5.1 Environmental parameters and crop rotation

The annual total precipitation in 2012, 2013 and the first nine months of 2014 was 967.3, 802.8 and 643.3 mm respectively, according to the weather data collected by Erfde meteorological station (*Deutscher Wetterdienst or DWD*). The average annual rainfall for a 30 year period (1981 - 2010) was 857 mm, hence the average monthly rainfall was 71 mm, ranging from 40 mm (in April) to 95 mm (in October). In all years, February, March and April were dry months compared to the rest of the months of the year (Figure 11and Figure 12). In 2013 from May to June, during the time of the maize crop establishment in the experimental plots, the seedling emergence, and the very young seedling stage, precipitation was less compared to the previous and following years, even though the total annual precipitation value was close to the long term average (Figure 11 and Figure 12).

The long term data gathered from the Erfde weather station shows that the annual average air temperature was 8.7 °C, with monthly values ranging from 1.3 °C (in January) to 17.1 °C (in July). The average annual air temperature in 2013 was 8.7 °C. The coldest month was March (- 0.3 °C), and the warmest month was August (17.5 °C). In comparison to 2012 and 2013, the beginning of 2014 was warmer (Figure 11 and Figure 12).

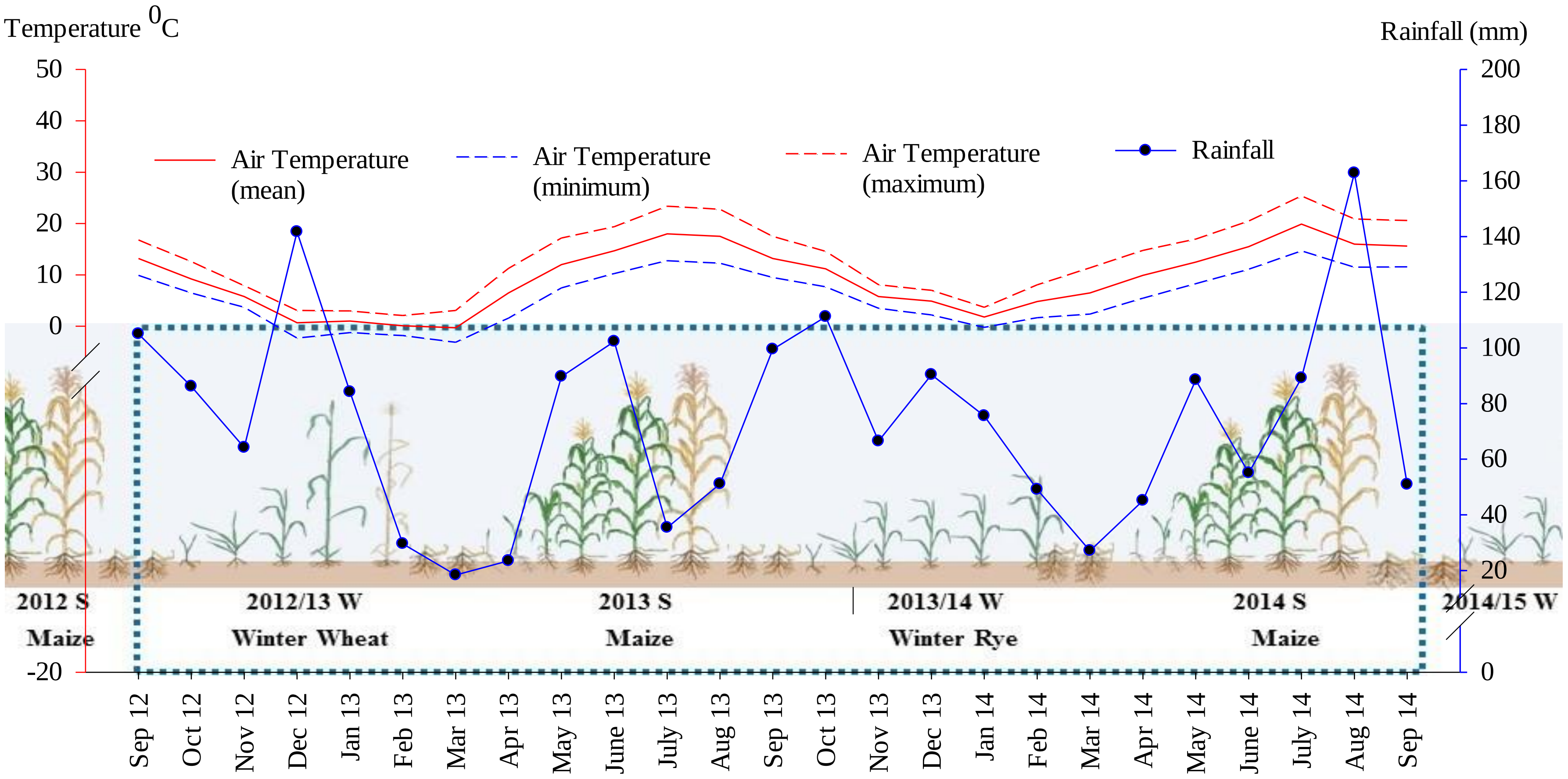


Figure 11: Smoothed monthly mean air temperature, and monthly precipitation, together with changing of crops over the experimental period from September 2012 to September 2014. Also shown are the monthly mean values of maximum and minimum temperatures. Note the different scales for the y-axis. Data source: Erfde meteorological station.

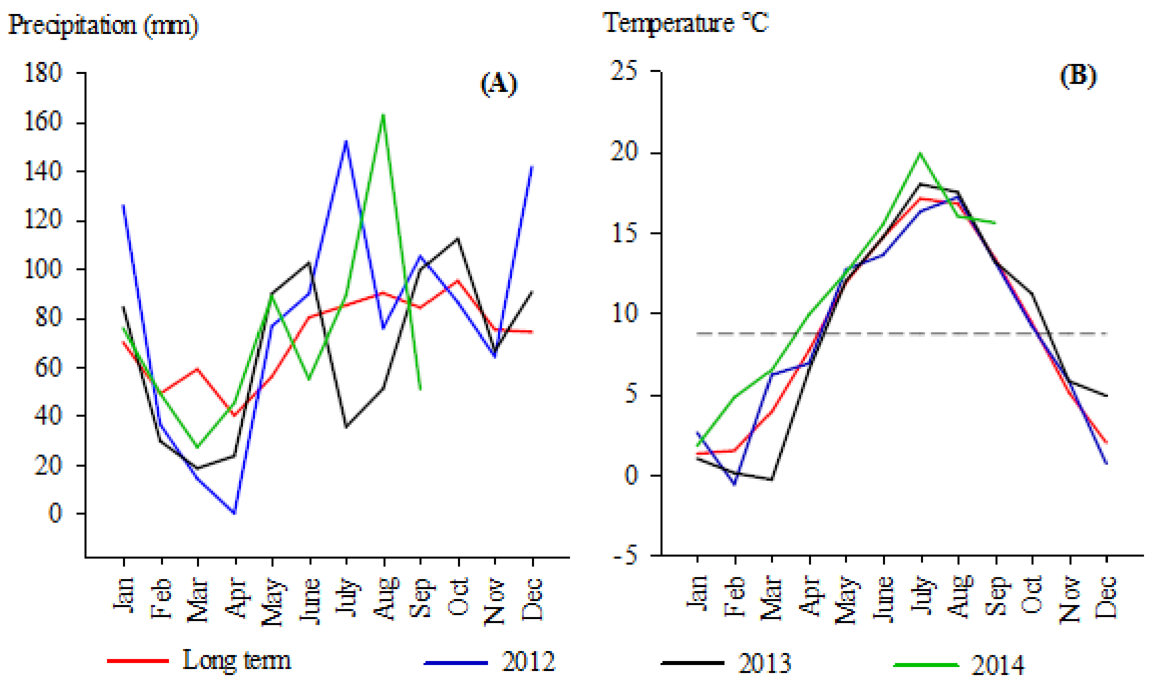


Figure 12: Smoothed monthly precipitation (A) and smoothed mean daily air temperatures (B) in the years 2012, 2013 and 2014, plotted in comparison to the long term values. Data source: Erfde meteorological station.

5.2 Characteristics of biochar and biogas digestate

The biochar was produced by fast thermal combustion at 650 °C and had a finer and more homogeneous texture compared to its original feedstock (Figure 13). The thermal decomposition process resulted in an 87 % weight loss, while the density increased by 20 %. The elemental mass losses of carbon and nitrogen were 86 % and 92 % respectively. The C/N ratio increased from 20 ± 0.2 to 37 ± 1.5 (Table 4). Biochar was more alkaline compared to the original digestate. The elemental compositions of biochar and digestate are shown in Table 4. The total and exchangeable concentrations of Ca^{++}, Mg^{++}, K^{+} and Na^{+} cations were noticeably higher in biochar, but the CEC of digestate was higher compared to biochar.

Table 4: Selected chemical characteristics of digestate and fresh biochar: the mean values with standard deviations (n = 3) and without standard deviations when the number of samples is two or less.

Characteristic	Unit	Digestate	Biochar
pH		6.6	8.00
Bulk Density	g cm^{-3}	n.a.	0.49
Moisture (dry wt.)	%	21.46 ± 1.3	20.66 ± 5.3
TOC	%	39.46 ± 0.3	43.89 ± 2.8
TN	%	2.02 ± 0.04	1.19 ± 0.1
C/N		20 ± 0.4	37 ± 1.5
Total Na*	g kg^{-1}	1.16 ± 0.0	2.63 ± 0.1
Total K*	g kg^{-1}	19.14 ± 0.1	41.67 ± 3.3
Total Ca*	g kg^{-1}	12.48 ± 1.3	38.68 ± 100
Total Mg*	g kg^{-1}	11.14 ± 0.1	29.30 ± 1.6
CEC	mmol$_c$ kg^{-1}	387.73	282.4
BS	%	52.12	100.0
Exch. Na	g kg^{-1}	7.61	9.21
Exch. K	g kg^{-1}	4.37	7.10
Exch. Ca	g kg^{-1}	1.85	2.07
Exch. Mg	g kg^{-1}	1.13	1.45
Ash	%	n.a.	0.45

n.a. = not analyzed, * HNO_3 extraction and n=2

Figure 13: Physical appearance of biochar (left) prepared from dry biogas digestate (right) used as a feedstock; pyrolyzed at 650 °C with a retention time of 30 - 40 minutes.

5.3 Total biomass production

The total biomass productions of each treatment during two winters and two summers are shown in Figure 14 for site1 and site 2 separately. In both site 1 and 2, adding biochar produced higher total above ground biomass. Site 1, which was not used before for continuous agricultural crop production, just after biochar application showed significantly higher total biomass (ANOVA, $p < 0.001$, $n = 3$) without any other additional chemical or organic fertilizer application. The positive effect of biochar on biomass production was most prominent in summer 2014. Site 1 with biochar treatment (B) produced 40.6 % higher biomass which was significantly different from the performance of control treatment (C) in summer 2014 (ANOVA, $p = 0.009$, $n = 3$). Even though the biochar application rate was low, it already enhanced the total biomass production in summer 2013 and winter 2013/14 (26.1 ± 2.4 t ha^{-}1 and 0.74 ± 0.08 t ha^{-1} respectively), but the increments were nonsignificant (ANOVA, summer 2013: $p > 0.05$, n = 3; winter 2013/14: $p = 0.083$, $n = 3$).

In site 2, which was intensively used for mono-cropping and continuously received biogas digestate during past years, the application of biochar as a soil amendment had a result similar to site 1 (Figure 14). In the BH treatment, the total above ground biomass increased by approximately 18 % in winter 2012/13 ($p = 0.005$, $n = 3$) and 47 % in winter 2013/14 ($p = 0.037$, $n = 3$) compared to the soil with digestate only. In the summer growing seasons, the highest biomass yields were shown with the BH treatment (19.1 ± 2 and 54.6 ± 1 t ha^{-1} for 2013 and 2014 respectively), and was significantly higher compared to digestate only (ANOVA, 2013: $p = 0.006$, 2014: $p < 0.001$, $n = 3$).

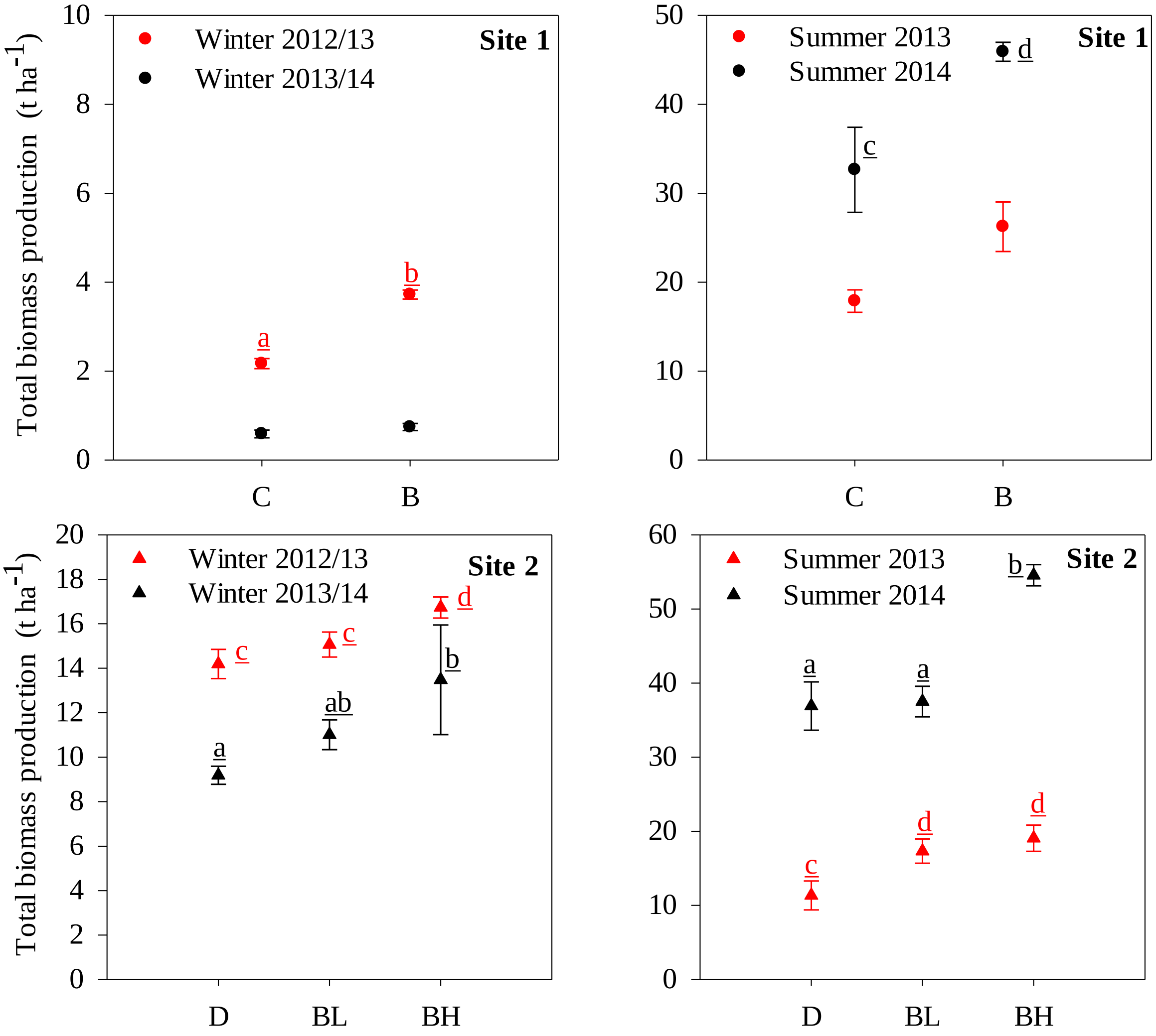


Figure 14: Total biomass production in winter and summer seasons for both experimental sites. Vertical error bars represent ± one standard deviation of the means (n = 3). The different letters near to the error bars indicate significant differences of the mean values. Letters are not shown when differences between means are not significant. C: control, B: biochar, D: digestate only, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5. Note the different scales on the y-axis. Crop specification: winter wheat and winter rye in winter 2012/13 and 2013/14 respectively, maize for all summer seasons.

5.4 Soil characteristics and classification

The soils in both sites developed from glacial sediments, and were later affected by human activities. According to the German soil classification system, the soil in site 1 was classified and described as *Gley-Podsol* (Ad-hoc-Arbeitsgruppe Boden, 2005) and is shown in Table 5. This *Gley Podsol* is named *Gleyic Podzol* according to the World Reference Base soil classification (WRB, 2006). In site 1, the *Gleyic Podzol* did not receive additional organic or inorganic amendments in the past several years and was not used for agricultural crop production.

After modifying the centuries-old soils in order to increase the agricultural productivity, the soil in site 2 developed into a soil with thick Ap and E horizons (≈ 40 cm). The soil in site 2 was classified as *Gley-Plaggenesch* (German classification; Ad-hoc-Arbeitsgruppe Boden, 2005) or *Gleyic Anthrosol* (WRB, 2006) and is shown in Table 6. The soil profile had features of podsolization, providing enough evidence to say that the *Anthrosol* emerged and developed from *Podzol*. According to the visual observation of profiles, both soils displayed old *Podzol* characteristics.

Gleyic Anthrosol had a sandy texture with 3.20 % clay, 3.23 % silt, and 92.59 % sand. *Gleyic Podzol* had 1.96 % clay, 8.11 % silt, and 89.92 % sand, and hence belonged to the same soil textural class as *Gleyic Anthrosol* in the Site 2. The clay fraction of *Gleyic Podzol* decreased with increasing profile depth, but the deepest horizon had an increasing trend again (Figure 15). The clay fraction showed only a very slight increase with increasing depth in the *Gleyic Anthrosol* profile, and the silt fraction of the third horizon (GBv) was noticeably higher compared to the upper horizons (Figure 15). In both sites, the sand fraction dominated in the whole profile. Small stones were randomly distributed among the whole depth of the *Gleyic Podzol* profile.

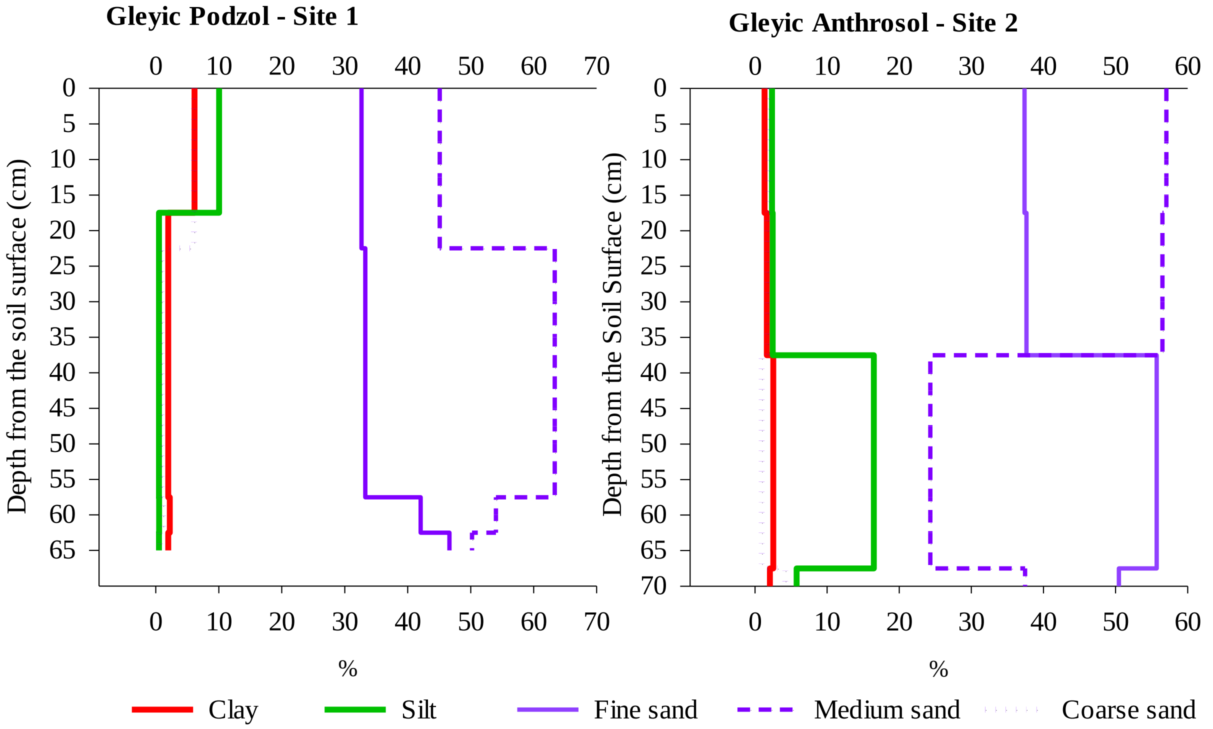
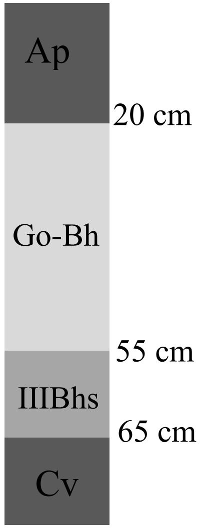


Figure 15: Vertical distribution of clay, silt, and sand fractions in *Gleyic Podzol* and *Gleyic Anthrosol*

In *Gleyic Podzol*, the Ap horizon showed higher medium and fine sand content compared to the lower horizons, and hence had higher soil water content (see Figure 20 in 4.5.1 on page 68). The Ap and E horizons in *Gleyic Anthrosol* had a similarly sandy texture with the same particle density (2.5 g cm^{-3}). The E horizon of *Gleyic Anthrosol* had a slightly lower matrix potential than Ap, which led to quicker drainage of water from the E horizon (see Figure 20 in 4.5.1 on page 68). The last two horizons (GBv and Gro) showed a higher fine sand fraction which resulted in more micro pores compared to the horizons dominated by coarse sand particles.

In their chemical properties, the soil profiles of the two sites were not very similar to each other (Table 7). The upper horizons of the soil which were affected by the vegetation in both sites, in addition to the human activities in site 2, and the water in site 1, differed from each other more in their chemical properties than in their physical ones. In particular, the TOC of the surface Ap horizon of *Gleyic Anthrosol* was more than twice as high compared to TOC in the same horizon in *Gleyic Podzol*. Table 5 and Table 6 show the differences of the TOC, TN and C/N ratios of *Gleyic Anthrosol* and *Gleyic Podzol* throughout the whole profile depth. The lower horizons of both soils had fewer differences compared to the upper two horizons. *Gleyic Anthrosol* was slightly acidic with higher EC compared to *Gleyic Podzol*. The differences between the two soil profiles of the cation concentrations, CEC and BS were not as high as those of the TOC, TN, DOC and DON values. However, the higher cation concentrations, CEC, and BS values for *Gleyic Anthrosol* are giving evidence of the human activities. High root density and surface litter were more prominent in the undisturbed *Gleyic Podzol* compared to *Gleyic Anthrosol* which was used for a monocrop with a deep plough layer during the last several years.

Table 5: Profile characteristics and soil classification of site 1 - *Gleyic Podzol*



Profile details*

Location: Centre of the experimental site in Drage
Geographic coordinates: 54° 36′ 43.31″ N, 9°15′25.18″E
Date of profile acquisition: 25.05.2012
Remarks:

Surface was covered slightly by grass and had signs of recent disturbances in the surface.

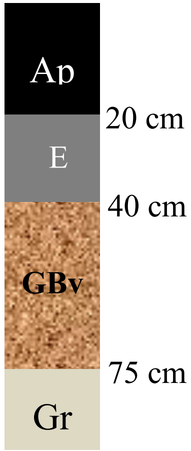
Adjacent to the waterlogged area and the sloping land small stones were scattered randomly among the whole profile.

Depth (cm)	Horizon denotation**	Soil color	TOC (%)	TN (%)	C/N	Bulk density ($g\ cm^{-3}$)	pH	Soil texture
0 - 20	Ap	10YR3/1	0.68	0.07	10	1.54	4.45	Sandy loam
20 - 55	Go-Bh	10YR5/8	0.67	0.01	67	1.67	5.00	Sand
55 - 65	II Bhs	5YR5/2	0.06	0.01	6	1.70	5.29	Sand
> 65	Cv	10YR6/4	0.04	0.01	4	1.74	6.21	Sand

German Classification (Ad-hoc-Arbeitsgruppe Boden, 2005): ***Gley-Podsol***	World Reference Base Classification (WRB, 2006): ***Gleyic Podzol***

*See the profile description sheet (Aufnahmeformblatt) in Table A 1 in the Annex on page 141 **according to US Soil Taxonomy

Table 6: Profile characteristics and soil classification of site 2 - *Gleyic Anthrosol*



Profile details*

Location: Centre of the experimental site in Stapelholm
Geographic coordinates: 54° 35′ 41.18″ N, 9° 17′ 27.16″ E
Date of profile acquisition: 25.05.2012
Remarks:

No surface ground cover vegetation found. Detritus originated from clay bricks.
Well decomposed organic layer.
Much lower root density (3 to 4 fine roots dm^{-3}), and water table at depth 75cm at the end of spring 2012.

Depth (cm)	Horizon denotation**	Soil color	TOC (%)	TN (%)	C/N	Bulk density ($g\ cm^{-3}$)	pH	Soil texture
0 - 20	Ap	10YR2/1	1.37	0.01	137	1.44	5.33	Sand
20 - 40	E	10YR3/1	1.37	0.11	12	1.42	5.78	Sand
40 - 70	GBv	5YR3/2	0.49	0.03	16	1.65	5.73	Sandy loam
> 70	Gro	7.5YR5/8	0.22	0.01	22	1.70	6.08	Sand

German Classification (Ad-hoc-Arbeitsgruppe Boden, 2005): ***Gley-Plaggenesch***

World Reference Base Classification (WRB, 2006): ***Gleyic Anthrosol***

*See the profile description sheet (*Aufnahmeformblatt*) in Table A 2 in the Annex on page 142 **according to US Soil Taxonomy

Table 7: Selected chemical properties of *Gleyic Podzol* and *Gleyic Anthrosol* before applying biochar in October 2012. The average values of the respective horizons are given in the table without standard deviations (n=2).

Soil and site	Depth	Horizon*	EC	DOC	DON	NO_3^--N	NH_4^+-N	Avail. K	Avail. P	Exchangeable Cations				CEC	BS
										Ca	Mg	K	Na		
	(cm)		($\mu S\ cm^{-1}$)	($mg\ kg^{-1}$)		(μM)	($mg\ kg^{-1}$)	($mg\ kg^{-1}$)		($mmol_c\ kg^{-1}$)				($mmol_c\ kg^{-1}$)	(%)
Gleyic Podzol	0 - 20	Ap	37.7	14.0	5.6	86.1	n.a.	40.4	156.8	3.9	0.8	1.5	0.3	22.2	29
(Site 1)	20 - 55	Go-Bh	19.0	12.7	2.5	25.6	n.a.	<15	46.7	1.8	0.3	0.4	0.2	6.3	44
	55 - 65	II Bhs	18.2	8.5	0.7	6.3	n.a.	26.4	38.5	3.3	0.8	0.7	0.3	7.5	66
	> 65	Cv	18.3	11.0	0.6	0.0	n.a.	38.4	14.1	5.5	1.1	0.9	0.2	6.8	100
Gleyic Anthrosol	0 - 20	Ap	76.3	25.5	33.1	481.4	0.50	69.9	147.4	23.9	1.6	1.7	0.2	26.6	100
(Site 2)	20 - 40	E	158.6	27.2	36.7	532.4	0.56	192.9	186.5	32.9	4.6	4.5	0.6	33.8	100
	40 - 70	GBv	58.2	26.7	7.4	113.2	0.19	51.9	49.9	11.3	2.3	1.4	0.2	15.3	99
	> 70	Gro	32.8	25.9	1.3	10.5	0.21	51.9	< 10	4.6	1.0	1.3	0.1	8.3	83

n.a. = not analyzed; pH - 1:1 soil H_2O suspension/slurry; *according to US Soil Taxonomy

5.4.1 Effect of biochar on homogeneity of soil

Though the soils in both sites (*Gleyic Podzol* and *Gleyic Anthrosol*) showed similarities in the soil texture, the huge differences of the chemical properties of the two soil profiles and the different usage of the lands do not allow presenting and discussing the experiment results together. Therefore, the results will be presented and discussed separately for site 1 and site 2.

For site 1, two treatments were applied: control (C) without any soil amendment, and application of biochar at 0.15 kg C m^{-2} (B). Site 2 had three different treatments with 0.15 kg C m^{-2} as a minimum organic carbon input rate: digestate only (D), and two different mixtures of digestate and biochar, 1:1 (BL) and 1:5 (BH). A control site could not be established in site 2 since this site had received digestate regularly over a longer period of time prior to the start of our field experiment. The distribution and identification of the treatment plots in both sites were already shown in Figure 9 (on page 37) in the chapter Materials and Methods.

Before applying biochar to site 1, TOC and TN were not significantly different between the rest of the experimental plots at $p = 0.05$ level, except for plot number 1 (ANOVA, $p = 0.005$, $n= 3$). Plot number 1 showed the lowest TOC level (0.06 ± 0.0008 %), and the rest of the plots had a TOC of 0.08 ± 0.006 % ($n = 15$). The added amount of biochar (application rate was 0.15 kg C m^{-2}) to plots number 2, 4 and 6 was enough to increase TOC significantly compared to the initial TOC in all the plots (ANOVA, $p < 0.001$). After biochar was added, plot number 4 had the highest TOC amount (1.2 ± 0.002 %), which significantly differed from plot number 6's TOC (1.1 ± 0.02 %) at $p = 0.03$. The group of plots used for control (C) continued to exhibit the same significant difference of TOC as in the initial measurements.

Before being amended with biochar, the TN content of *Gleyic Podzol* in site 1 had a similar variation to that of TOC: only TN of plot number 1 significantly differed from the rest of the plots (ANOVA, $p < 0.05$). After applying biochar, TN significantly increased in plots 4 and 6 (4B and 6B in Figure 16) at $p < 0.05$ (ANOVA. $n = 3$).

However, the increase of TN in plot number 2 was non-significant from the initial TN of plot numbers 2, 3, 4 and 5 (ANOVA, $p > 0.05$).

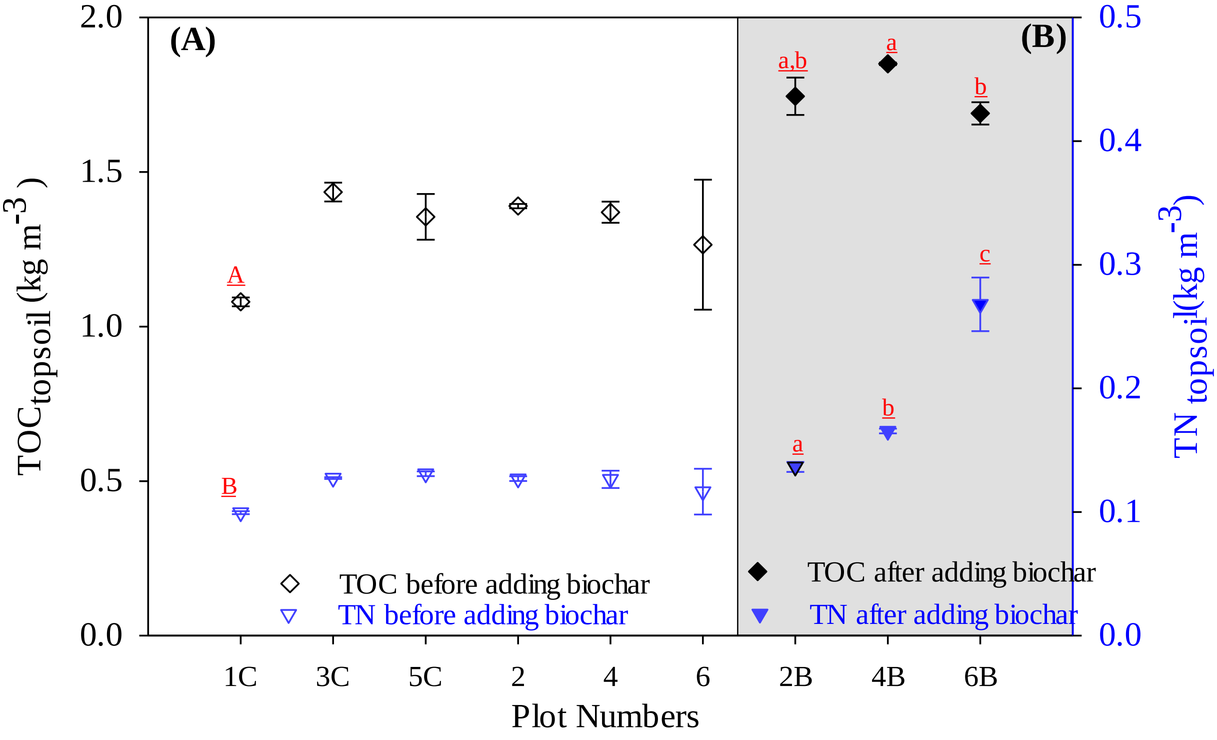


Figure 16: Variation of TOC and TN of *Gleyic Podzol* in site 1 before and after the application of biochar. (A) Variability before applying biochar and (B) variability after applying biochar to plots number 2, 4 and 6. The vertical error bars represent ± one standard deviation of the mean (n = 3); the level of significance between the mean values is indicated by different letters: uppercase letters for before the application, and lowercase letters for after the application. B indicates the biochar amended plots (0.15 kg C m^{-2}).

The TOC contents of the experimental plots in *Gleyic Anthrosol* in site 2 were 58.33 % significantly different before applying biochar and digestate into the surface layer (0 - 0.2 m; ANOVA, Duncan's multiple comparison, $p = 0.05$). These initial significant differences of TOC values were randomly distributed among the plots that were later used for the treatments (symbols without filling color in Figure 17). Within the group treated with digestate only (section A in the graph; plot numbers 7, 11 and 13), there was a significant difference in TOC (ANOVA, $p \leq 0.001$, $n = 3$).

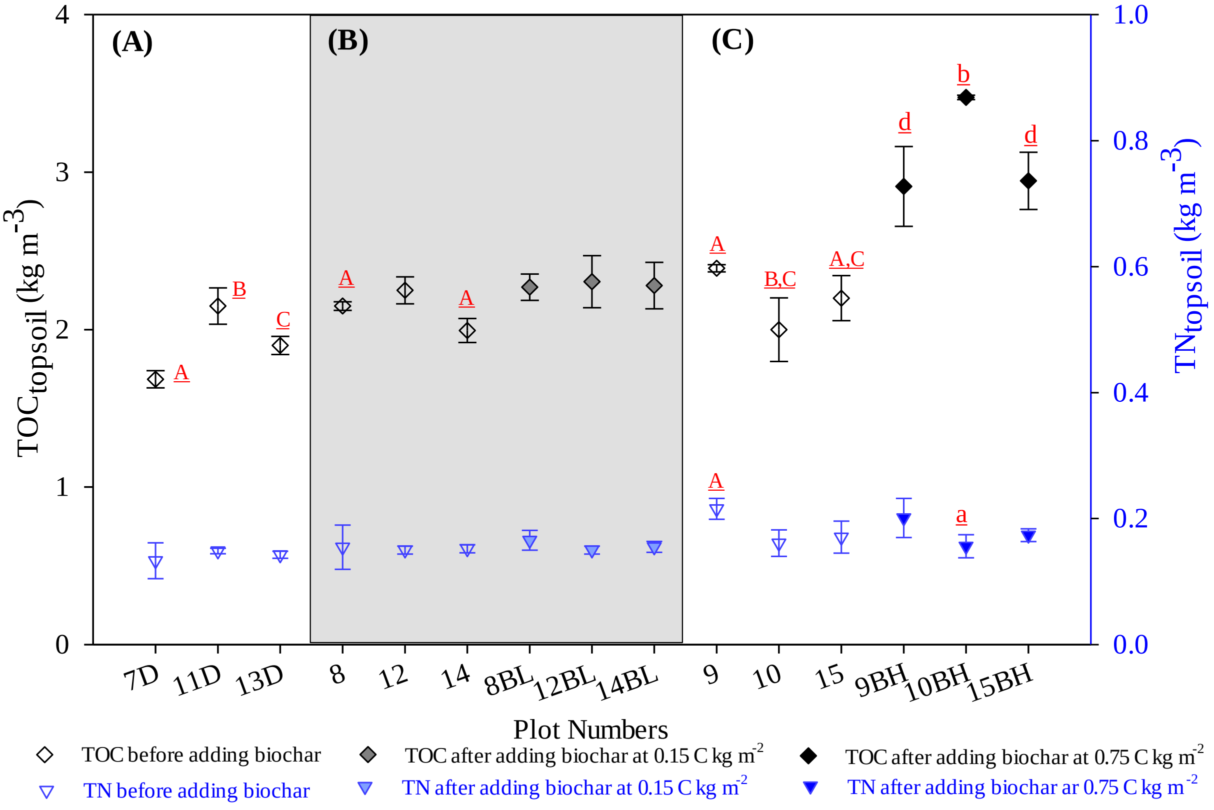


Figure 17: Variation of TOC and TN in *Gleyic Anthrosol* in site 2 before and after application of soil amendments. The symbols without filling color in (A), (B) and (C) show the variability before application, the symbols with filling color in (B) and (C) show the variability after application of digestate:biochar (1:1 for BL, 1:5 for BH). The vertical error bars represent ± one standard deviation of the mean (n = 3); Different letters indicate the level of significance between the mean values - uppercase for before application and lowercase for after application. Letters are not shown when the differences of means are not significant.

Among the plots which received biochar low amount (BL plots), number 12 initially had a significantly higher TOC than the other two plots (8 and 14 plots; ANOVA, $p \leq 0.001$, $n = 3$) but after application the significant difference was gone. Among the plots which received biochar high amount (BH plots), there initially were significant differences between the TOC of all the plots (ANOVA, $p = 0.015$, $n = 3$). After application, there was no significant difference between plots 9 and 15, but plot number 10 had 19 % significantly higher TOC compared to both plots number 9 and 15 (ANOVA, $p = 0.002$, $n = 3$).

Before application of biochar and digestate to the topsoil (0 - 0.2 m) in *Gleyic Anthrosol*, the TN content was not significantly different between plots except for number 9 and 7. Plot number 9 had significantly higher TN compared to all other plots (ANOVA, $p \leq 0.01$, $n = 3$), whereas the number 7 plot had significantly lower TN compared to plot number 15 (ANOVA, $P = 0.047$, $n = 3$). After the soil was amended with biochar and digestate, only the number 10 plot showed significantly lower TN among the plots with high amount of biochar (ANOVA, $p \leq 0.05$, $n = 3$).

The topsoil (0 - 0.2 m) of plot number 5 in site 1 had low DOC (1.45 g DOC per kg soil per m^3) but not significantly different from the rest of the plots (see Table A 4 on page 144 in the Annex). The plots at the edge of the slope where water had accumulated had higher concentration of DON in the surface horizon (0 - 0.2 m) than the ones close to the road. Other chemical properties, including pH, EC, CEC, BS and exchangeable cation concentrations in the topsoil (0 - 0.2 m) of site 1 and 2 showed non-significant variations between mean values of the plots (see Table A 4 on page 144 in the Annex).

Biochar and digestate both are porous media, rich in elements (see Table 4 on page 52 in 5.2). Therefore, adding them to the soil can be expected to considerably alter the chemical composition of soil solid and pore water. In fact, the experiment showed that after adding biochar into the topsoil (0 - 0.2 m) of site 1 and 2, the concentration of some selected soil elements changed (see Table A 5 on page 144 in the Annex). In site 1, all chemical properties increased after biochar was incorporated into the topsoil (0 - 0.2 m) except for DON and Na^+. However, DOC, BS, Ca^{++}, Mg^{++}, and K^+ content did not significantly change after the plots had been treated with biochar (ANOVA, $p > 0.05$, $n = 3$), but CEC significantly increased (ANOVA, $p \leq 0.05$, $n = 3$). Also in site 2, DON did not increase by adding biochar to the soil which already contained digestate (see Table A 5 on page 144 in the Annex). After adding biochar in low concentration to site 2 (BL plots), there were significant differences of Ca^{++}, K^+, and Na^+ cation concentrations, and in the CEC and BS values between the treated plots (ANOVA, $p \leq 0.05$, $n = 3$). With the high biochar application rate (BH plots), only DON and Mg^{++} did not show any significant changes between the treated plots (ANOVA, $p > 0.05$, $n = 3$), while all other parameters showed significant variation (ANOVA, $p \leq 0.05$, $n =$

3; see Table A 5 on page 144 in the Annex).

5.4.2 Mass losses of TOC during the biochar application

Because of the technique we used for biochar incorporation (see 4.2 on page 34 in Materials and Methods), there was a possibility of not having an equal distribution of the added biochar throughout the topsoil (0 - 0.2 m) of the experimental plots. Therefore, assuming that the added biochar was stored in the whole soil mass in the depth of the topsoil (0 - 0.2 m), the application losses were calculated as mass losses of TOC (C kg) to mass of soil (kg of soil in 1 m^3) using the bulk density (*Gleyic Anthrosol*: 1.543 g cm^{-3}; *Gleyic Podzol*: 1.438 g cm^{-3}) and the thickness of the soil layer (0 - 0.2 m).

Before applying digestate and biochar to site 1, except for plot one, TOC did not show significant differences (see Figure 16 and Table A 1 on page 141 in the Annex; ANOVA, $p = 0.45$, $n = 3$), so it was to be expected that the TOC values would not differ significantly after applying biochar either. However, after the application the measured TOC values were significantly different among the various plots (ANOVA, $p = 0.008$, $n = 3$). Plot number 4 had the highest TOC (1.85 ± 0.0 kg C per kg soil per m^3), followed by plot 6 and plot 2 (1.75 ± 0.05, and 1.70 ± 0.05 kg C per kg soil per m^3 resp.). Concerning the mass losses of TOC among the three plots with *Gleyic Podzol* in site 1, there were no significant differences, and the average loss of TOC was 0.35 ± 0.1 kg C kg $soil^{-1}m^3$ (ANOVA, $p = 0.5$, $n = 3$).

The mean values of lost TOC in the BL plots in site 2 were not significantly different, and the average loss of TOC was 0.60 ± 0.15 kg C per kg soil per m^3 (ANOVA, $p > 0.05$). Plot number 12 had the highest loss of TOC, while number 14 showed the lowest one. The BH plots showed a different behavior. The lost amounts of TOC between the BH plots were significantly different, and the loss was higher than in the BL plots (Figure 18). Hence, the application losses increased with increasing amount of biochar applied, but there was no clear and significant correlation between losses and applied amount of biochar.

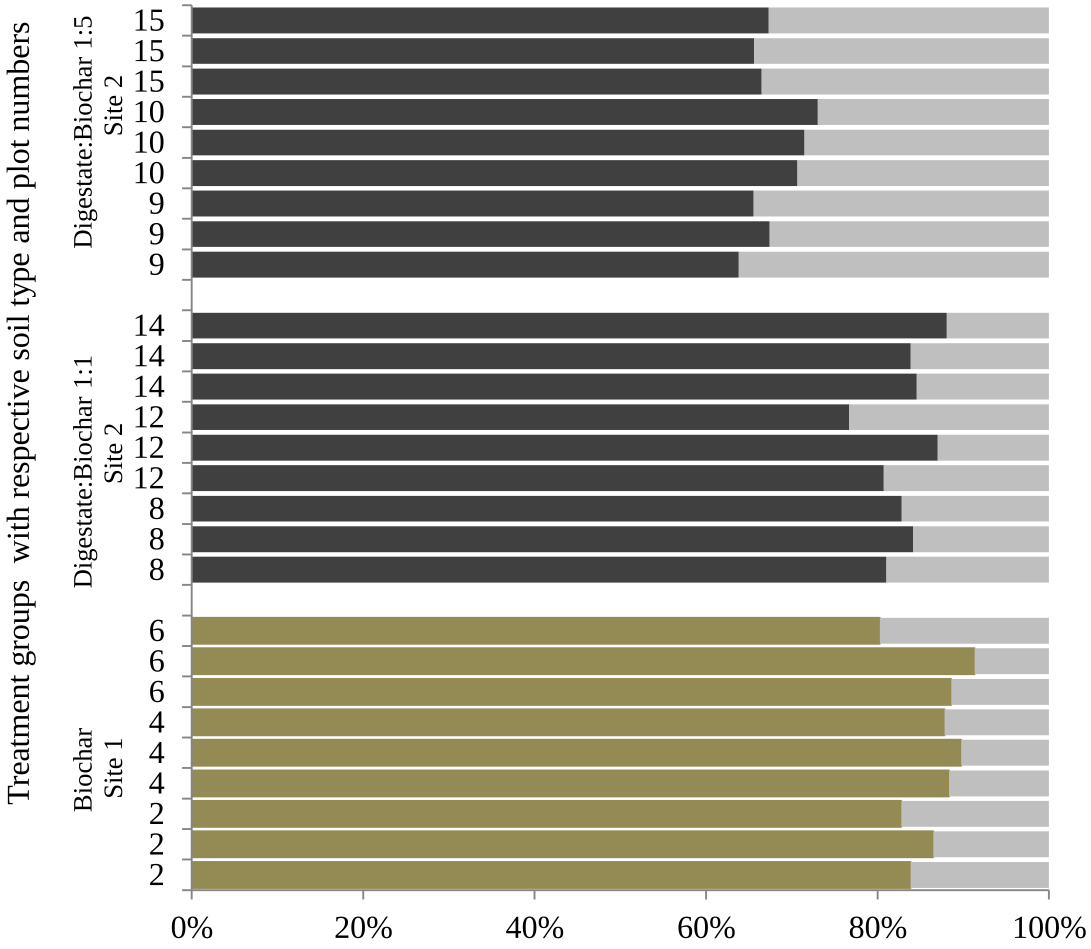


Figure 18: Available and lost TOC during the application of biochar, calculated as % out of excepted TOC content after adding biochar. The olive green and black colored parts of the bars represent available TOC in treated soils in site 1 and site 2 respectively, and the grey parts show the lost TOC.

Adding biochar into *Gleyic Anthrosol* in site 2 showed larger losses compared to *Gleyic Podzol* in site 1 where the experimental plot had a much closer (5.4 ± 0.37 m) thick live fence along the road which acted as a wind-break (see Figure 9 on page 37 in Materials and Methods for the layout of the field and the experimental plots for both sites). Site 2 had an open border along the road, where wind entered to the field without any disturbances. Figure 19 shows how wind and precipitation acted on site 2 during the crop establishment time and the young seedling stage.

Figure 19: The deposited sand particles around the soil collar show the effects of wind and precipitation on the land when there were no or fewer crop canopies and wind-breaks in site 2.

5.5 Effect of biochar and digestate on soil physico-chemical properties

5.5.1 Soil moisture and matrix potential

Soil texture, organic matter content, precipitation rate, and evapotranspiration rate are primary factors which can influence soil moisture, which in turn is a key factor for determining agricultural productivity of a given soil. Hence, adding highly porous organic material like biochar and digestate to the soil may alter the soil water availability. The application of biochar and digestate to *Gleyic Podzol* and *Gleyic Anthrosol* did not significantly effect the seasonal variation in soil water content (ANOVA, $p > 0.05$, $n = 3$; see Table A 6). The BH treatment in site 2 resulted in a slightly, but nonsignificantly higher soil moisture content throughout the experimental period except in summer 2013. The volumetric water content and matrix potential of both sites are shown in Figure 20. Figure 21 shows the same parameters two years after biochar application to the field in site 2.

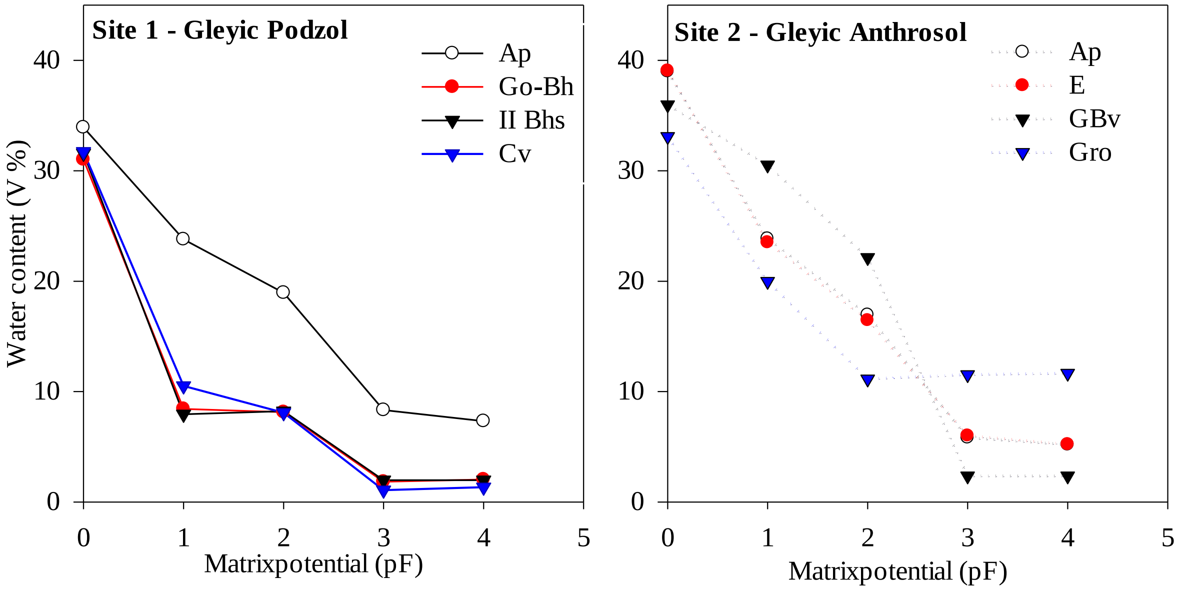


Figure 20: Soil moisture characteristic curves for each soil horizon of both site 1 and site 2 before applying soil amendments in October 2012.

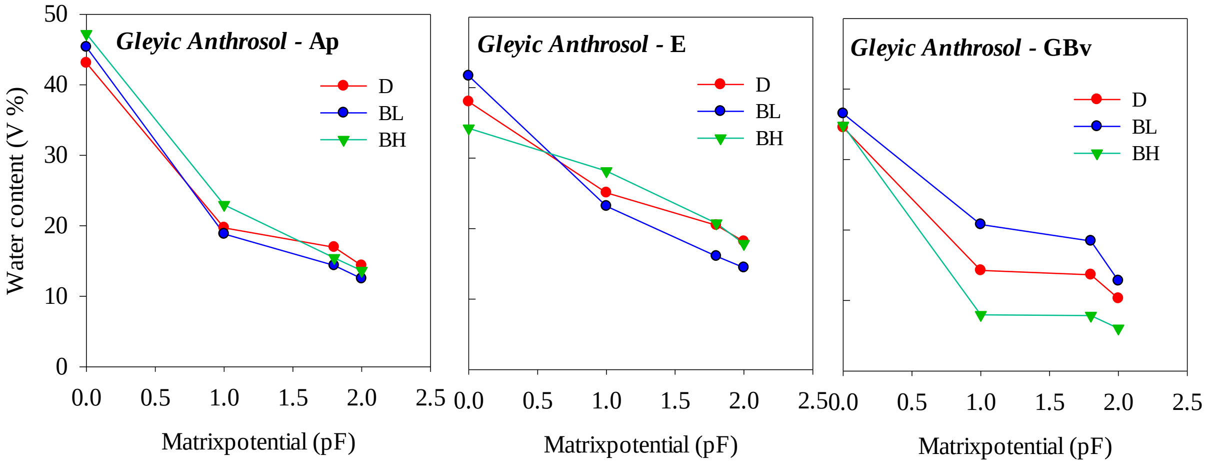


Figure 21: Soil moisture characteristic curves for Ap, E and GBv soil horizons of *Gleyic Anthrosol*, two years after biochar application of D, BL and BH treatments.

5.5.2 Soil reaction (pH) and electrical conductivity (EC)

The effect of the application of digestate and biochar on soil pH and EC of the topsoil (0 - 0.2 m) in both sites is shown in Table 8 and Table A 6 in the Annex on page 145. In site 1, the mean values of soil reaction and EC in the topsoil (0 - 0.2 m) did not significantly increase during the first season after amending the soil with biochar (winter 2012/13; ANOVA, for pH: $p = 0.331$, $n = 6$; for EC: $p = 0.139$, $n = 6$). The first five seasons showed higher EC with biochar amended soil, but after that, EC values of the biochar treated plots and the control plots were quite similar in site 1.

Table 8: Seasonal variation of soil acidity in the topsoil (0 - 0.2 m) in site 1 and site 2 after establishment of the experiment at both sites in October 2012. Shown are mean values and the respective standard deviations (n = 3 to 9).

pH *	**Soil amendments**				
	Site 1		**Site 2**		
	C	**B**	**D**	**BL**	**BH**
Winter 2012/13	4.4 ± 0.6	4.8 ± 0.8	5.2 ± 0.8	5.4 ± 0.8	5.9 ± 0.8
Spring 2013	4.0 ± 0.1	4.4 ± 0.1^a	4.6 ± 0.1	4.7 ± 0.2	5.4 ± 0.6^a
Summer 2013	3.9 ± 0.2	4.2 ± 0.2^a	4.5 ± 0.1	4.5 ± 0.3	5.2 ± 0.5^a
Autumn 2013	4.1 ± 0.1	4.2 ± 0.1^a	4.4 ± 0.1	4.6 ± 0.3	5.2 ± 0.3^a
Winter 2013/14	4.2 ± 0.2	4.3 ± 0.2	4.3 ± 0.1	4.4 ± 0.2	5.0 ± 0.3
Spring 2014	4.1 ± 0.2	4.3 ± 0.1	4.3 ± 0.1	4.5 ± 0.2	4.9 ± 0.2^a
Summer 2014	3.8 ± 0.0	3.9 ± 0.1^a	4.1 ± 0.1	4.2 ± 0.2	4.7 ± 0.3^a

* H_2O suspension. For each field site, values followed by the different letters are significantly different at $p \leq 0.05$. Letters are not shown when differences of means are nonsignificant. C: control, B: biochar, D: digestate only, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5

In site 2, the increase of the soil reaction in the BH treated plots was significant in all seasons, except in both winter seasons (2012/13 and 2013/14). Even after seven seasons since the experiment started in 2012, conditioning *Gleyic Anthrosol* in site 2 with the slightly alkaline biochar in addition to digestate had a positive effect on soil reaction compared to application of digestate only (Table 8). BH treatment also had significantly higher soil pH (ANOVA, $p < 0.05$, $n = 3$ to 6), except in the two winter seasons. However, there were no significant differences between digestate only treatment and BL treatment (ANOVA, $p < 0.05$, $n = 6$ to 9). In the seventh season after starting the experiment in 2012, the soil reaction finally had decreased by 20 % for all the treatments in site 2.

5.5.3 Soil organic carbon and nitrogen

The effects of biochar as a soil amendment to *Gleyic Podzol* in site 1 and as a mixture with digestate to *Gleyic Anthrosol* in site 2 on TOC, TN, C/N, DOC and DON concentration in the topsoil (0 - 0.2 m) are shown in Table 9 and Table A 7 in the Annex on page 145. TOC concentrations of both soils significantly increased after adding biochar ($p < 0.05$). In site 1, after mixing biochar at 0.15 kg C m^{-2} rate, TOC concentration in the topsoil (0 - 0.2 m) increased by 30 % (ANOVA, $p < 0.024$, $n = 6$). In site 2, BH treatment increased TOC by 20.5 % (ANOVA, $p < 0.030$, $n = 6$), and BL treatment increased it by 4.5 % (ANOVA, $p = 0.790$, $n = 6$) compared to the digestate only treatment. At the end of the experiment, TOC in topsoil (0 - 0.2 m) had increased by 3.29 % resp. 1.3 % for BH resp. digestate only treatment, and decreased by 3.2 % for BL treatment.

Site 1 showed only slightly higher TN concentration in the topsoil (0 - 0.2 m) of biochar treatment compared to the control treatment. For site 2, after the application of biochar in October 2012, TN concentration did not clearly change in the first season (winter 2012/13), and the same result showed at the end of the experiment in September 2014.

In site 1, adding 0.15 kg C m^{-2} to the topsoil (0 - 0.2 m) in the form of biochar (B) in September 2012 significantly increased the C/N ratio by 12 % (ANOVA, $p < 0.001$, $n = 6$). The following spring season did not show clear differences of C/N ratios between biochar treated and untreated plots. However, summer and autumn 2013 again showed significant differences (ANOVA; summer: $p = 0.003$, $n = 9$; autumn: $p = 0.016$, $n = 9$). Two years after biochar application to *Gleyic Podzol*, the influences of biochar on the C/N ratio nearly had vanished, and with 5 % there only was an insignificantly higher value in the biochar plots ($p > 0.05$).

Table 9: Seasonal variation of TOC, TN, and C/N in the topsoil (0 - 0.2 m) in site 1 and site 2 after establishment of the experiment in October 2012. Shown are mean values and the respective standard deviations (n = 3 to 9).

	Soil amendments				
	Site 1		Site 2		
	C	B	D	BL	BH
TOC (%)					
Winter 2012/13	0.83 ± 0.1	1.08 ± 0.2^{a}	1.51 ± 0.2^{a}	1.58 ± 0.1^{ab}	1.82 ± 0.3^{b}
Spring 2013	0.84 ± 0.1	1.01 ± 0.2^{a}	1.45 ± 0.1	1.60 ± 0.2	1.89 ± 0.3^{a}
Summer 2013	0.86 ± 0.1	1.02 ± 0.2	1.56 ± 0.1	1.62 ± 0.1	1.86 ± 0.3^{a}
Autumn 2013	0.73 ± 0.1	0.89 ± 0.1^{a}	1.51 ± 0.1	1.57 ± 0.1	1.75 ± 0.3^{a}
Winter 2013/14	0.76 ± 0.0	0.94 ± 0.0^{a}	1.46 ± 0.1	1.56 ± 0.1	1.86 ± 0.3^{a}
Spring 2014	0.83 ± 0.1	0.90 ± 0.1	1.50 ± 0.2	1.51 ± 0.1	1.74 ± 0.2
Summer 2014	0.82 ± 0.1	1.09 ± 0.1^{a}	1.53 ± 0.1	1.53 ± 0.1	1.88 ± 0.2^{a}
TN (%)					
Winter 2012/13	0.08 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Spring 2013	0.08 ± 0.01	0.09 ± 0.03	0.10 ± 0.04	0.11 ± 0.00	0.12 ± 0.01
Summer 2013	0.09 ± 0.01	0.09 ± 0.04	0.12 ± 0.00	0.12 ± 0.01	0.12 ± 0.01
Autumn 2013	0.08 ± 0.01	0.09 ± 0.01^{a}	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.01
Winter 2013/14	0.08 ± 0.01	0.09 ± 0.00^{a}	0.11 ± 0.00	0.11 ± 0.00	0.12 ± 0.01
Spring 2014	0.09 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
Summer 2014	0.09 ± 0.01	0.10 ± 0.01	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.01
C/N					
Winter 2012/13	10.05 ± 0.1	11.25 ± 0.6^{a}	13.5 ± 0.5	14.1 ± 0.5	15.9 ± 1.1^{a}
Spring 2013	10.16 ± 0.9	10.35 ± 1.9	13.3 ± 1.0^{a}	14.2 ± 1.8	15.7 ± 1.3
Summer 2013	9.43 ± 0.7	10.55 ± 0.7^{a}	13.1 ± 0.7	13.7 ± 0.8	15.3 ± 1.2^{a}
Autumn 2013	9.44 ± 0.5	9.96 ± 0.4^{a}	13.0 ± 0.8	13.5 ± 0.4	14.7 ± 1.1^{a}
Winter 2013/14	9.72 ± 0.4	10.04 ± 0.6	13.3 ± 0.7	14.0 ± 0.8	15.2 ± 1.0
Spring 2014	9.47 ± 0.4	9.80 ± 0.4	12.9 ± 1.0	13.6 ± 0.8	13.8 ± 1.1
Summer 2014	9.14 ± 0.9	9.60 ± 0.8	12.5 ± 0.6	15.2 ± 4.2	14.2 ± 1.4

For each field site, values followed by the different letters are significantly different at $p \leq 0.05$. Letters are not shown when differences of means are nonsignificant. C: control, B: biochar, D: digestate only, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5.

In site 2, amending *Gleyic Anthrosol* topsoil (0 - 0.2 m) with biochar and digestate increased the C/N ratio by 17 % for the BH treatment (ANOVA, $p = < 0.001$, $n = 9$), and 4 % for the BL treatment (ANOVA, $p > 0.05$, $n = 9$). In spring 2013, the D treatment had a significantly lower C/N compared to the BL and BH treatments (ANOVA, $p = 0.009$, $n = 9$). During the whole experimental time, the BH treatment showed a higher C/N than the BL and the D treatments, but the differences were only significant in summer 2013 and autumn 2013 (ANOVA; summer: $p = < 0.001$, $n = 9$, autumn: $p = < 0.001$, $n = 9$).

The influences of digestate and biochar on inorganic nitrogen in site 1 and 2 soils are shown in Figure 22. The ammonium nitrogen content in the topsoil (0 - 0.2 m) was not significantly influenced by any of the treatments in site 1 or 2 (ANOVA, $p > 0.05$, $n = 3$ to 9). However, site 1 showed higher ammonium nitrogen with the biochar treatment. Both in site 1 and 2, the ammonium nitrogen data showed high standard deviations caused by the highly varying data inside the treatment group.

Nitrate nitrogen content was significantly influenced by all soil amendments over the whole experimental period (ANOVA, $p < 0.009$, $n = 3$ to 9). Except for the winter 2013/14 season, nitrate nitrogen in topsoil (0 - 0.2 m) of B treatment in site 1 was significantly higher compared to the untreated soil ($p < 0.05$, $n = 3$ to 9). In the first winter (2012/13) after application of biochar in October 2012, 57 % higher nitrate nitrogen showed from the BH treatment (ANOVA; $p < 0.05$, n = 3 to 9), but only a 7 % nonsignificant increase from the BL treatment ($p > 0.05$, $n = 3$ to 9), both compared to treatment D. In the next spring season (2013), nitrate nitrogen content decreased drastically by 69 %, 81 %, and 87 % for the BH, BL and D treatments respectively, and remained like that without big fluctuations during the rest of the seasons (Figure 22). Throughout the whole experimental time period, the BH treatment reported the significantly highest nitrate nitrogen content compared to BL and D treatments (ANOVA; $p < 0.05$, $n = 3$ to 9), but in summer 2013 the difference between BH and D was only small (ANOVA; $p > 0.05$, $n = 3$ to 9).

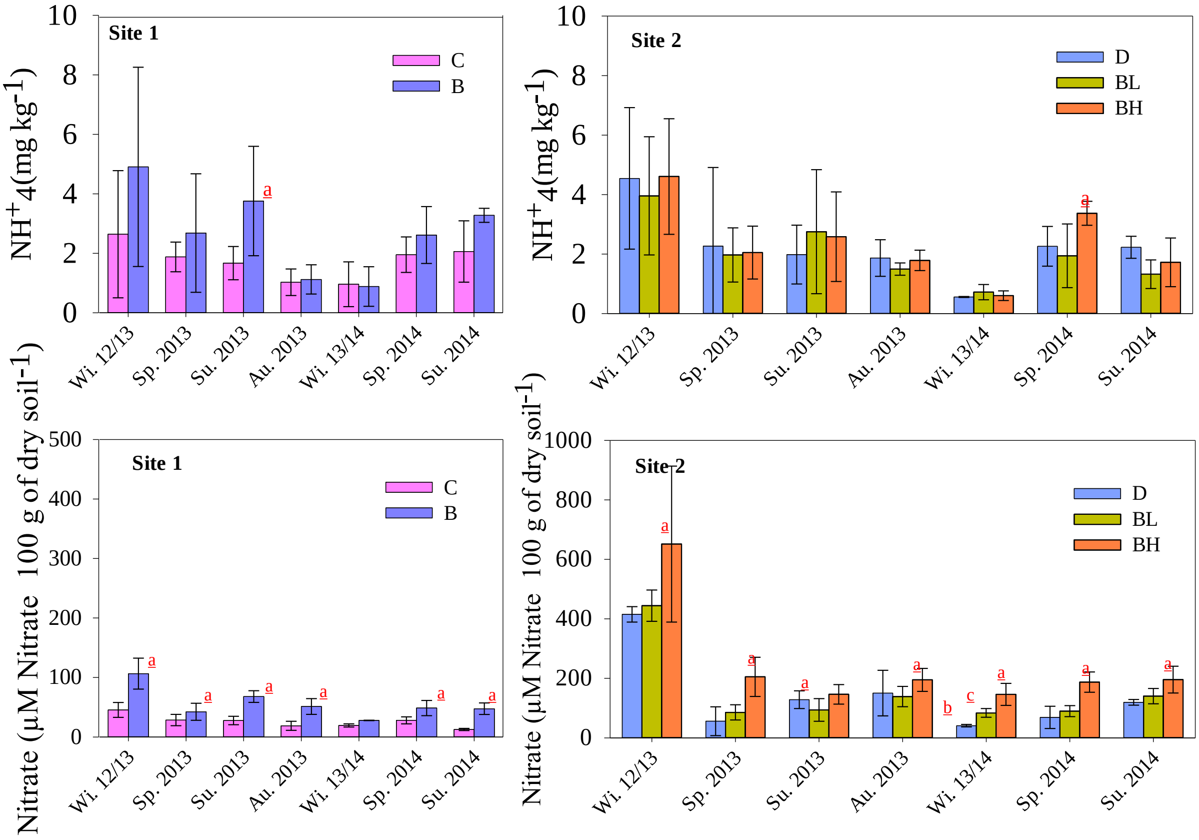


Figure 22: Seasonal variation of ammonium-nitrogen and nitrate-nitrogen concentration in the topsoil (0 - 0.2 m) in site 1 and site 2 after establishment of the experiment in October 2012. Shown are mean values for each treatment. The vertical error bars represent ± one standard deviation of the mean (n = 3 to 9); Different letters of the respective groups indicate the level of significance. Letters are not shown when the differences of means are not significant. Note the different scales in the Y-axis of the Nitrate graphs.

5.5.4 CEC, BS and exchangeable cation concentration

In site 1, most of the seasons showed nonsignificant higher CEC with biochar treated plots (B) (ANOVA, $p > 0.05$; Figure 23). The winter 2012/13 season, right after the application, showed 65 % significant increment of CEC in the B treatment compared to the control (ANOVA, $p < 0.001$, $n = 3$). During the following spring season, CEC in both B and C treatments increased with different rates, but a significant difference was not detected (ANOVA, $p = 0.31$, $n = 3$).

In site 2, which has a higher content of organic carbon than site 1, the result was different. Amending *Gleyic Anthrosol* in site 2 with biochar increased CEC significantly (ANOVA, $p \leq 0.05$; Figure 23). No significant differences were found between the BH and BL treatments ($p > 0.05$, $n = 6$ and $n = 3$) except in autumn 2013 ($p = 0.009$, $n = 6$). Independent of the application rate, adding biochar to site 2 significantly increased CEC by approximately 31 % (33.1 % for BH, and 29.5 % for BL; $p <0.001$, $n = 3$) in winter 2012/13, and very similar values in the following spring 2013 season ($p <0.001$, $n = 3$). After the first main growing season (summer 2013), CEC decreased in the biochar treated plots (BH and BL), and remained the same with little fluctuation. But, one year later, in winter 2013/14, BH treatment showed 36 % higher CEC compared to the D treatment, but because of the high variation of measured values this difference was nonsignificant (ANOVA, $p = 0.20$, $n = 3$). Two years after the application of mixture of digestate and biochar, CEC was still increased in site 2.

In site 1, BS ranged from 14.8 % to 51.4 % over all seasons and both treatments (Figure 23). Treatment B showed nonsignificantly higher BS compared to C from spring 2013 to autumn 2013 ($p > 0.05$, $n = 3$ to 6). In the rest of the seasons, BS values of C and B treatments in site 1 were significantly different (ANOVA, $p < 0.05$, $n = 6$).

Site 2 showed a similar pattern to site 1 (Figure 23), but the BS values had a higher variability within the treatment. Therefore, no statistically significant differences ($p > 0.05$) were observed for BS in site 2 except in winter 2012/13 (ANOVA, $p < 0.002$, $n = 3$) and spring and summer 2014 (spring: $p < 0.001$, $n = 3$; summer: $p = 0.01$, $n = 3$). The spring 2013 season showed 92 % higher BS in the BH treatment than in the D treatment ($p = 0.06$, $n = 3$). At the end of the experiment in summer 2014, BS was still 33 % higher in the BH treatment ($p = 0.01$, $n = 3$).

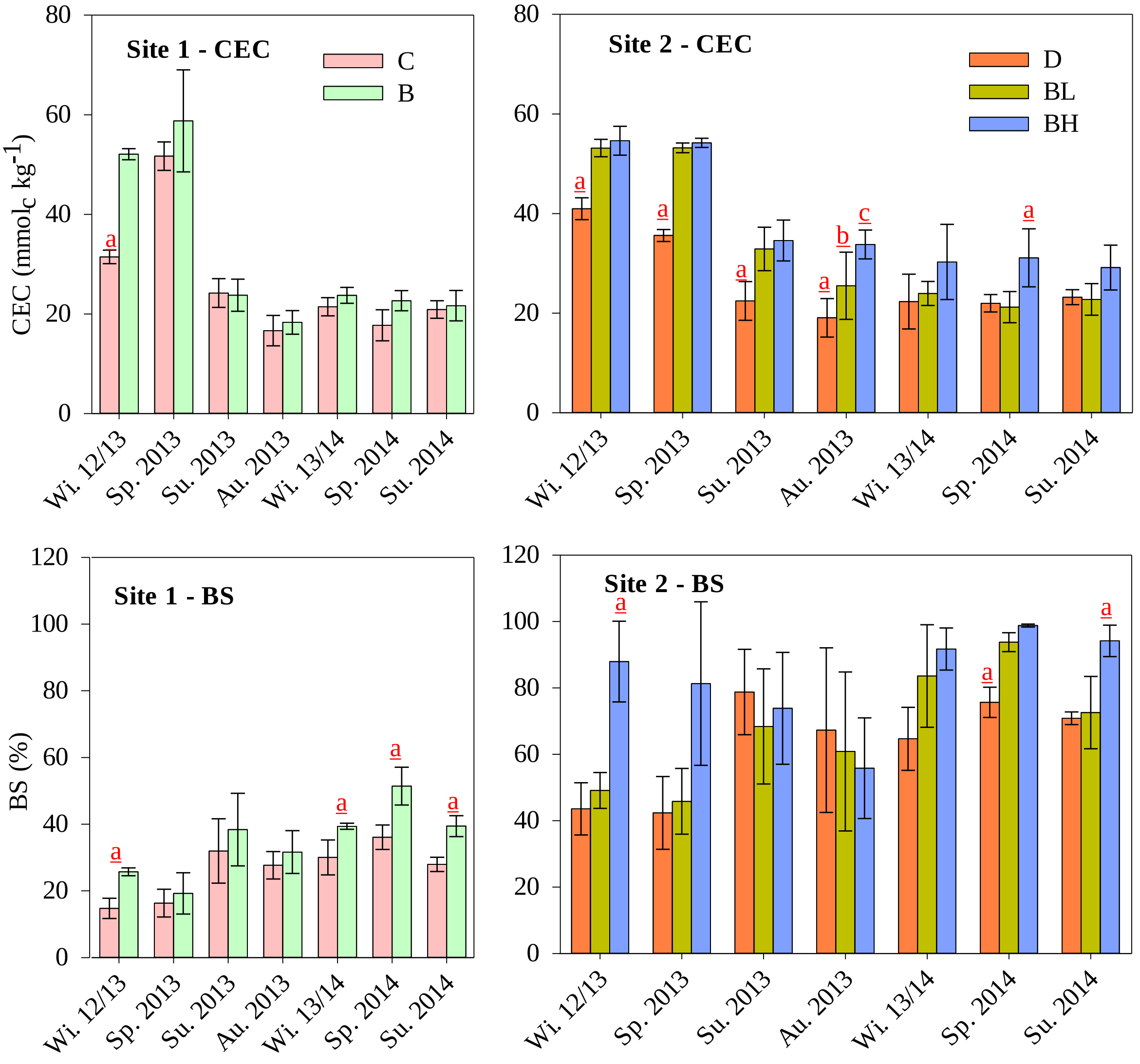


Figure 23: Seasonal variation of CEC and BS in the topsoil (0 - 0.2 m) of site 1 and site 2 after establishment of the experiment in October 2012. Shown are mean values for each treatment for each season (n varies from 3 to 6). Different letters indicate significant differences between means. Letters are not shown when differences of means are not significant.

Table 10: Seasonal variation of the exchangeable cation concentrations in the topsoil (0 - 0.2 m) for selected cations in site 1 and 2 after establishment of the experiment in October 2012. Shown are mean values and the respective standard deviations (n = 3 to 9) for each treatment.

	Soil amendments				
	Site 1		Site 2		
	C	B	D	BL	BH
Ca^{++}					
Winter 2012/13	100.20 ± 4	81.87 ± 71	387.0 ± 30	466.9 ± 47	483.7 ± 72
Spring 2013	152.17 ± 22	155.36 ± 54	384.5 ± 8	514.5 ± 123	575.8 ± 149
Summer 2013	106.84 ± 11	156.39 ± 19^{b}	405.0 ± 83	437.3 ± 128	501.8 ± 150
Autumn 2013	135.27 ± 35	125.80 ± 40	505.7 ± 107	432.1 ± 105	346.3 ± 68
Winter 2013/14	101.47 ± 25	121.58 ± 4	293.7 ± 44	423.6 ± 135	430.8 ± 119
Spring 2014	95.39 ± 17	147.03 ± 27	325.4 ± 23	367.0 ± 61	434.8 ± 124
Summer 2014	100.20 ± 11	120.91 ± 26	329.3 ± 38	332.6 ± 91	432.5 ± 134
Mg^{++}					
Winter 2012/13	15.47 ± 1	49.07 ± 42^{a}	30.80 ± 8	58.67 ± 10	254.40 ± 97^{a}
Spring 2013	17.07 ± 4	43.33 ± 6^{a}	20.00 ± 2	54.27 ± 12	170.27 ± 33^{a}
Summer 2013	12.07 ± 2	70.00 ± 9^{a}	30.13 ± 12	48.07 ± 15	188.53 ± 56^{a}
Autumn 2013	23.20 ± 19	31.13 ± 19	86.93 ± 70	65.40 ± 63	84.87 ± 85
Winter 2013/14	14.26 ± 3	39.37 ± 4^{a}	21.83 ± 7	35.72 ± 12	134.78 ± 63^{a}
Spring 2014	12.47 ± 4	43.34 ± 8^{a}	10.27 ± 11	20.13 ± 22	69.64 ± 79^{a}
Summer 2014	12.03 ± 4	37.38 ± 8^{b}	26.28 ± 7	34.38 ± 13	133.61 ± 4^{a}
K^{+}					
Winter 2012/13	70.57 ± 7	139.44 ± 20^{b}	101.91 ± 65	105.11 ± 33	254.44 ± 24^{a}
Spring 2013	37.81 ± 10	62.81 ± 5^{b}	43.30 ± 10	54.12 ± 5	114.45 ± 31^{a}
Summer 2013	46.87 ± 16	96.35 ± 28^{b}	49.11 ± 16	41.92 ± 7	104.04 ± 26^{a}
Autumn 2013	68.73 ± 21	75.53 ± 14	99.07 ± 42	89.80 ± 34	112.19 ± 49
Winter 2013/14	50.18 ± 4	69.60 ± 33	40.40 ± 3	43.27 ± 6	93.58 ± 21^{a}
Spring 2014	43.27 ± 19	72.47 ± 18	28.93 ± 2	28.54 ± 6	71.55 ± 31^{a}
Summer 2014	40.14 ± 12	47.05 ± 12	61.13 ± 7^{a}	42.23 ± 4	60.61 ± 7
Na^{+}					
Winter 2012/13	3.36 ± 1	2.95 ± 3	5.62 ± 3	6.34 ± 1	8.43 ± 0
Spring 2013	4.88 ± 2	6.34 ± 1	0.29 ± 0^{a}	2.01 ± 1	4.42 ± 3
Summer 2013	17.18 ± 3	19.49 ± 3	18.18 ± 4	16.80 ± 3	21.65 ± 6
Autumn 2013	18.93 ± 2	19.80 ± 2	18.73 ± 5	22.47 ± 11	18.00 ± 1
Winter 2013/14	15.33 ± 2	18.39 ± 1	11.57 ± 0	11.95 ± 1	15.71 ± 3^{a}
Spring 2014	10.27 ± 5	13.79 ± 4	6.21 ± 2	7.05 ± 1	9.73 ± 1^{a}
Summer 2014	7.13 ± 1^{a}	10.19 ± 0^{b}	9.58 ± 3	7.74 ± 1	8.43 ± 2

Values followed by the different letters are significantly different at $P \leq 0.05$. Letters are not shown when differences of means are not significant, C: control, B: biochar, D: digestate only, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5

The seasonal variation of exchangeable cation concentrations in the topsoil (0 - 0.2 m) is shown in Table 10. There was an initial increase of exchangeable Ca^{++} and exchangeable Na^{+} in site 2, which, however, was nonsignificant ($p > 0.05$, $n = 3$ to 6), but no increase in site 1. For exchangeable Mg^{++} and exchangeable K^{+}, there was a clear increase in both soils. Exchangeable Mg^{++} and exchangeable K^{+} concentrations were significantly higher in B treatment compared to C treatment in site 1, and in BH treatment compared to D in site 2 (ANOVA, $p < 0.05$), except Mg^{++} in autumn 2013 ($p = 0.8$ for site 1 and $p = 0.4$ for site 2). For site 2, exchangeable Mg^{++} and K^{+} concentrations increased in the order of D < BL < BH (except for exchangeable K^{+} in spring 2014).

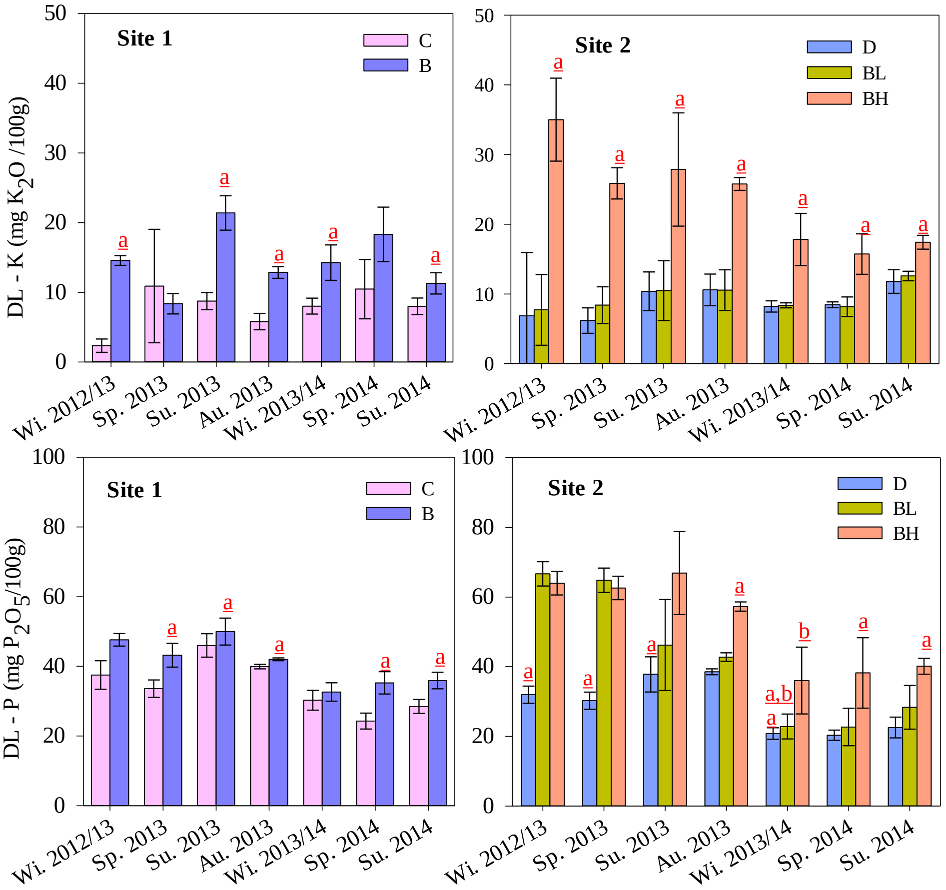


Figure 24: Seasonal variation of plant available K (DL - K in the top row) and P (DL - P in the bottom row) in the topsoil (0 - 0.2 m) of site 1 and site 2 after establishment of the experiment in October 2012. Shown are mean values for each treatment for each season (n varies from 3 to 6). Different letters indicate significant differences between means. Letters are not shown when differences between means are not significant.

5.6 Effect of biochar on soil microbial carbon and nitrogen

Microbial carbon (MBC) and nitrogen (MBN) concentrations showed fluctuations caused by the responses of microbial growth to temperature and crop management activities for both sites. Generally, biochar had a positive effect on MBC and MBN during the whole experimental period, but occasionally also showed a small negative one.

5.6.1 Soil microbial carbon

Except for the winter season 2012/13 just after amending *Gleyic Podzol* in site 1 with biochar, MBC always increased compared to the plots without biochar (Figure 25). In winter 2012/13, MBC was nonsignificantly lower ($p = 0.201$, $n = 6$). Application of biochar stimulated MBC significantly in the subsequent winter season (winter 2013/14; ANOVA, $p = 0.020$, $n = 6$), and in spring 2013 (ANOVA, $p = 0.003$, $n = 6$), autumn 2013 (ANOVA, $p = 0.002$, $n = 6$) and summer 2014 (ANOVA, $p = 0.007$, $n = 6$).

The seasonal variability of MBC in D treatment and with different rates of biochar addition to site 2 is shown in Figure 26. There were significant treatment effects on MBC in all winter and summer seasons (winter 2012/13: $p \leq 0.001$, $n = 6$; winter 2013/14: $p \leq 0.001$, $n = 6$; summer 2013: $p = 0.02$, $n = 6$; summer 2014: $p = 0.016$, $n = 6$). Biochar with higher application rate enriched MBC content in site 2 in all seasons except in autumn 2013 and spring 2014 before reapplication of digestate in summer 2014. In autumn 2013, both D and BH treatments showed the same amount of MBC (62.4 ± 15 and 62.3 ± 11 mg kg^{-1} respectively). In spring 2014, for all treatments in site 2, MBC ranged between 72.39 ± 17 and 79.78 ± 14 mg kg^{-1}. In winter seasons, MBC in the BH treatment significantly increased by 47 % (2012/13: $p < 0.001$, $n = 6$) resp. 82 % (2013/14: $p < 0.001$, $n = 6$), while in the BL plots MBC only showed a nonsignificant increase by 13 % (2012/13: $p = 0.280$, $n = 6$) resp. 28 % (2013/14: $p = 0.162$, $n = 6$) compared to the digestate only treatment.

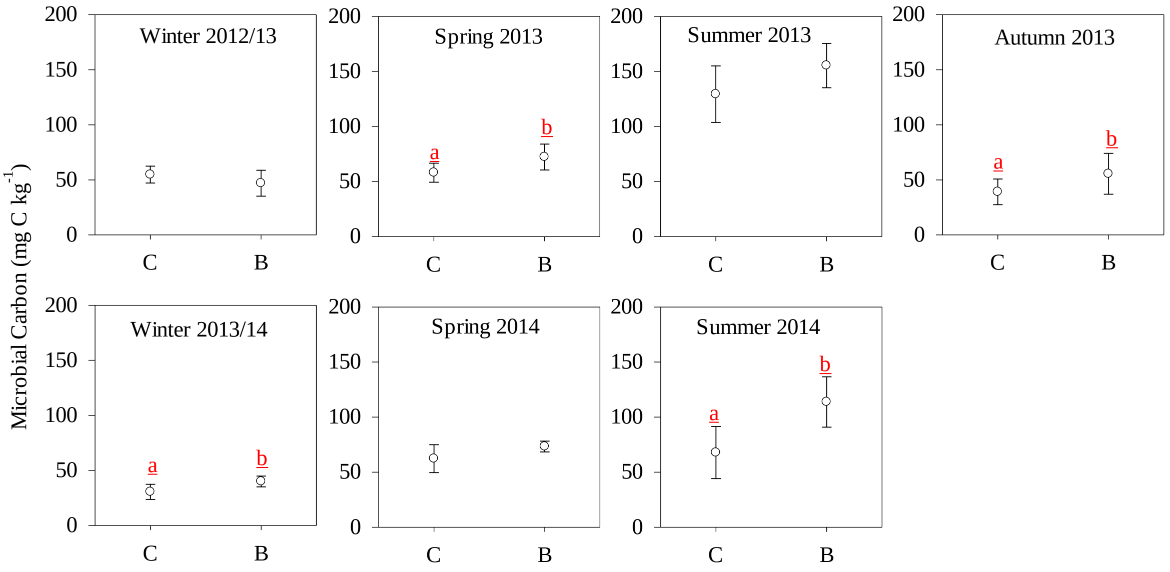


Figure 25: Influence of biochar application on MBC in site 1. Vertical error bars represent ± one standard deviation of the means (*n* varied between 6 and 12). The different letters close to the error bars indicate significant differences of the mean values. Letters are not shown when differences between means are not significant. B: biochar treatment, C: Control.

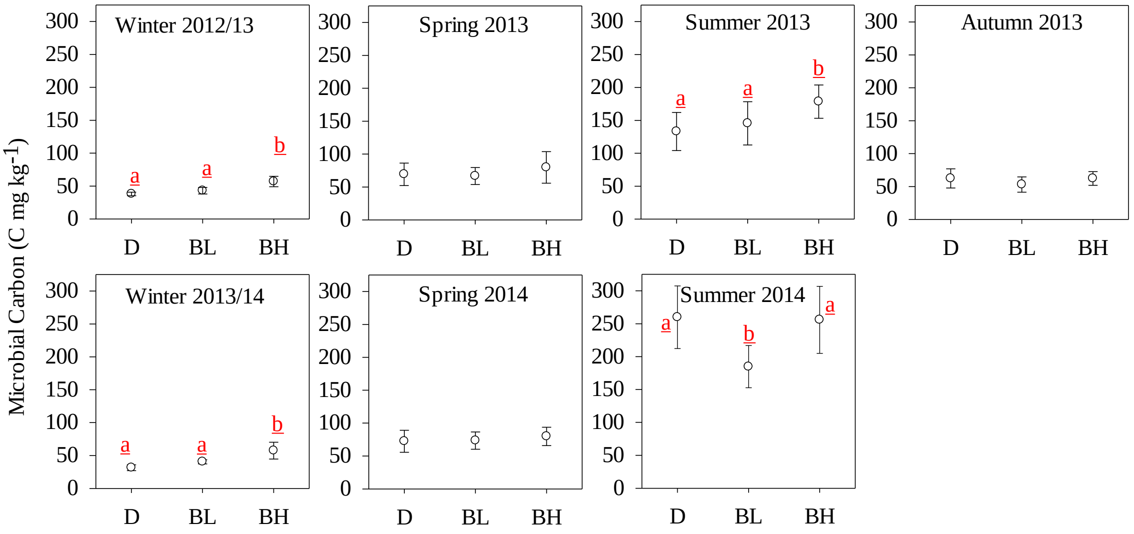


Figure 26: Influence of biochar and digestate application on MBC in site 2. Vertical error bars represent ± one standard deviation of the means (*n* varied between 6 and 12). The different letters near to the error bars indicate significant differences of the mean values. Letters are not shown when differences between means are not significant. D: digestate only, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5.

5.6.2 Soil microbial nitrogen

The amendment of biochar increased MBN in site 1 (Figure 27). However, the first significant increase of MBN only appeared in summer 2013 when MBN increased by 100 % (ANOVA, $p < 0.001$, $n = 12$), followed by 81 % in autumn 2013 (ANOVA, $p < 0.001$, $n = 12$), and 179 % in summer 2014 (ANOVA, $p < 0.001$, $n = 6$). In the rest of the seasons during the total experimental period from October 2012 to September 2014, addition of biochar into site 1 increased MBN only non-significantly.

Higher contents of MBN in site 2 were found in the treatment with digestate only, except in the two summer seasons (Figure 28). In summer 2014, the highest content of MBN was found in the plots treated with BH (7.53 ± 1.8 mg kg^{-1}), while MBN in the D treatment plots was 4.39 ± 1.5 mg kg^{-1}. This 72 % increase significantly differed from the plots which were treated with D (ANOVA, $p = 0.017$, $n = 6$). In summer 2013, the highest MBN was found in the BH treatment (3.9 ± 1.1 mg kg^{-1}), followed by the BL treatment (3.6 ± 0.8 mg kg^{-1}) and the D treatment (3.0 ± 1.1 mg kg^{-1}). In winter 2012/13, the digestate only treated plots (D) contained 24 % and 34 % higher amounts of MBN compared to the BH and BL treatments respectively, but the differences were not significant. In the next winter season (2013/14), there again was a significantly higher MBN in the D treatment, with a 163 % resp. 177 % increase of MBN compared to BH and BL (ANOVA, $p = 0.003$, $n = 6$ and $p = 0.002$, $n = 6$ respectively). During autumn 2013, plots without biochar (D) showed the significantly highest MBN (1.82 ± 1.0 mg kg^{-1}), a 57 % increase compared to the BL plots (ANOVA, $p = 0.012$, $n = 12$), and a nonsignificant 41 % increase compared to the BH plots (ANOVA, $p > 0.05$, $n = 12$).

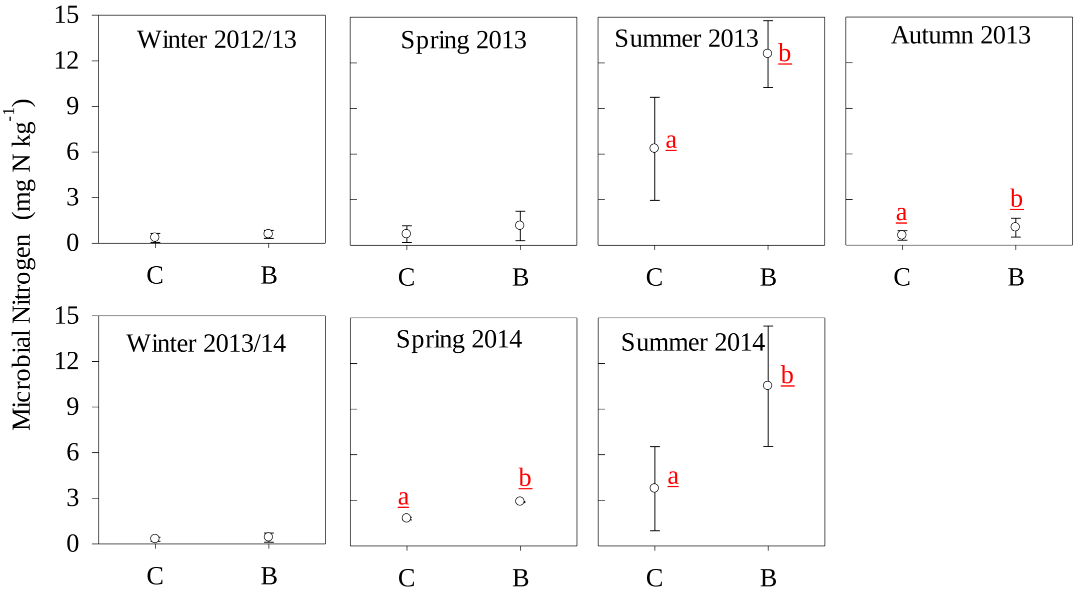


Figure 27: Influence of biochar application on MBN in site 1. Vertical error bars represent ± one standard deviation of the means (*n* varied between 3 and 6). Bars with different letters indicate significant differences of the means. Letters are not shown when differences between means are not significant. C: control, B: biochar application.

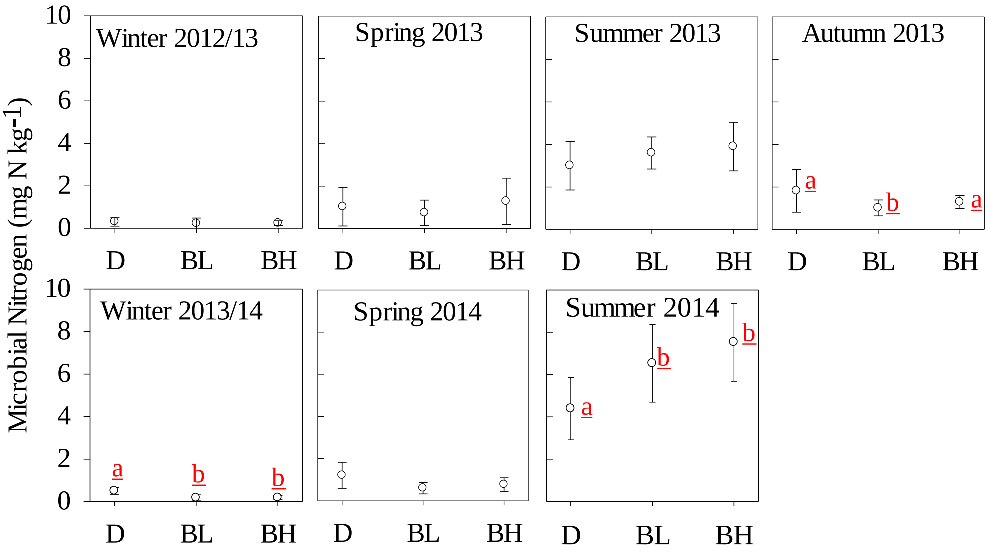


Figure 28: Influence of biochar and digestate application on MBN in site 2. Vertical error bars represent ± one standard deviation of the means (*n* varied between 3 and 6). The different letters near to the error bars indicate significant differences of the mean values. Letters are not shown when differences between means are not significant. D: digestate only, BL: digestate:biochar 1:1, and BH: digestate:biochar 1:5.

5.7 Effect of biochar and digestate on soil carbon turnover

5.7.1 Influence of biochar and digestate on soil carbon mineralization

An aerobic incubation experiment was conducted using the surface soil (0 - 0.2 m) of *Gleyic Podzol* collected from site 1 in 2014. Before collecting the soil samples, a new soil profile was opened for the soil description. The physical and chemical parameters were slightly different from those of the profile opened in May 2012 (see Table 5 on page 58 in 4.4 and Table A 11 on page 148 in the Annex). Non-treated surface soil samples were collected from three different places which were distributed diagonally within the site. These three different sub samples of *Gleyic Podzol* were labelled as 4394-GP, 4395-GP and 4627-GP. For the incubation experiment, the soil was treated with the following amendment mixtures: control (C, soil without amendments), biochar only (B), digestate only (D), digestate:biochar 1:1 mixture (D:B 1:1), and digestate:biochar 1:5 mixture (D:B 1:5). The incubation period was 133 days and $\delta^{13}CO_2$ values were measured on days 16, 52, 93 and 133.

5.7.1.1 Cumulative CO_2 production

The aerobic cumulative CO_2 productions of all three sub samples of the topsoil of *Gleyic Podzol* increased over the whole incubation time (Figure 29). The aerobic cumulative CO_2 production from all treatments of *Gleyic Podzol* varied from 32.2 to 124.5 µmol CO_2 gdw^{-1}. The biochar only treatment (B) produced the lowest cumulative CO_2 emission (54.8 ± 1, 80.7 ± 2 and 33.0 ± 1 µmol CO_2 gdw^{-1} for the three samples 4394-GP, 4395-GP and 4627-GP resp.). The effects of the biochar only treatment (B) on cumulative CO_2 production within the three soil samples were significant (ANOVA, $p \leq 0.016$, $n = 3$). In addition to the treatment effect on cumulative CO_2 productions, there were clear and significant sub soil sample effects and interaction effects between soil and treatment (see Figure A 6 on page 150 in the Annex; two-way ANOVA, $p < 0.001$, $n = 44$). Independent of the particular treatment, sub sample 4395-GP always showed the significantly highest cumulative CO_2 production, followed by sub samples 4394-GP and 4627-GP (95.8 ± 13, 61.1 ± 6, and 38.6 ± 5 µmol CO_2 gdw^{-1} resp.; ANOVA, $p < 0.001$, $n = 15$).

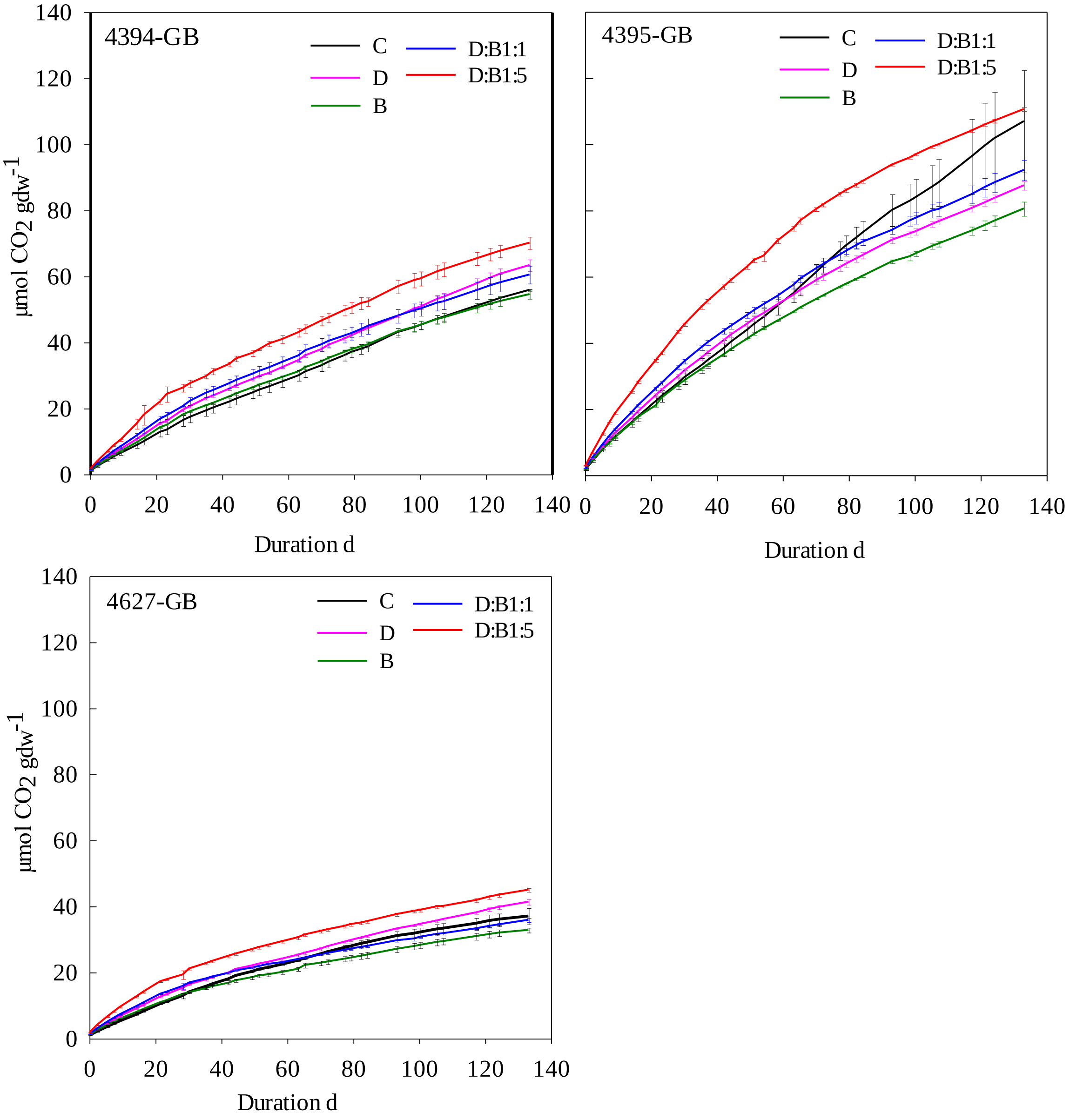


Figure 29: Cumulative CO_2 production during an aerobic soil incubation experiment of *Gleyic Podzol* amended with biochar (B), digestate (D), digestate biochar1:1 (D:B 1:1) and 1:5 (D:B 1:5) mixtures and without any amendments (C). The vertical error bars represent ± one standard deviation of the mean (n = 3).

The carbon turnover rates of the different treatments decreased with incubation time except for the control treatment in the 4395-GP sub sample which had a higher rate after 60 days (Figure 30). The treatments with high organic carbon contents had higher turnover rates than those with a low one and control (see Table A 11 on page 148 in the Annex for TOC data). The effect of added organic carbon disappeared after 60 days, and afterwards the turnover rates were similar for all the treatments except for the control treatment in the 4395-GP sub sample. The highest and the lowest turnover rates were both shown by the D:B 1:5 treatment (2.6 ± 0.1 on day 2 for 4394-GP and 0.07 ± 0.1 on day 105 for 4627-GP).

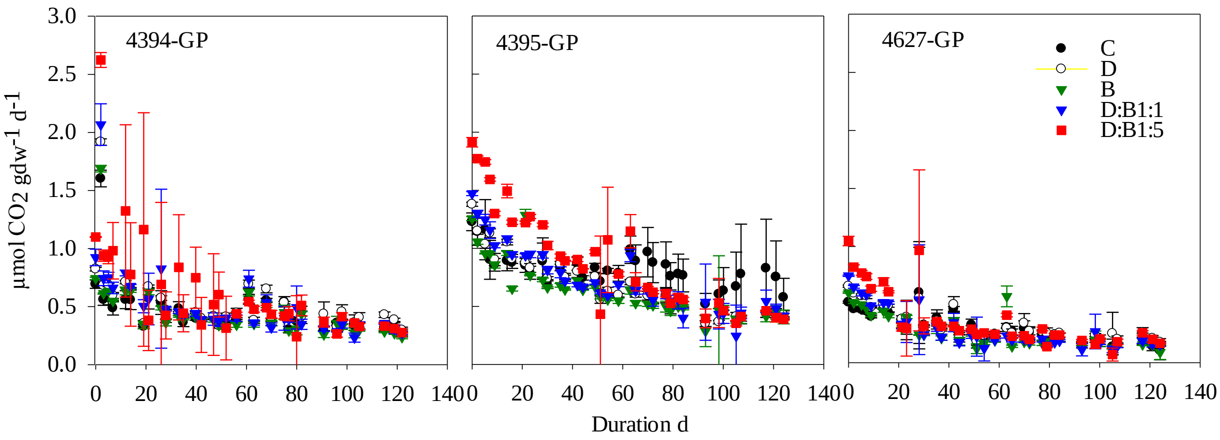


Figure 30: Carbon turnover rates during an aerobic soil incubation experiment of *Gleyic Podzol* amended with biochar (B), digestate (D), digestate biochar 1:1 (D:B 1:1) and 1:5 (D:B 1:5) mixtures and without any amendments (C). The vertical error bars represent ± one standard deviation of the mean (n = 3).

5.7.1.2 Isotopic ratio and fractionation of carbon in the emitted CO_2

The stable carbon isotope values were measured not only for *Gleyic Podzol* but also for pure digestate, pure biochar, and *Gleyic Anthrosol*. The $\delta^{13}C$ value of digestate was -19.2 and was not significantly different from the value -19.5 for biochar (ANOVA, $p = 0.37$, $n = 4$), but significantly different from the $\delta^{13}C$ value of *Gleyic Podzol* (ANOVA, $p < 0.001$, $n = 6$). The highest stable carbon isotope value was found in *Gleyic Anthrosol* and the lowest in *Gleyic Podzol* ($\delta^{13}C$ values: -25.23 ± 0.3 and -27.56 ± 0.6 resp.; ANOVA, $p < 0.001$, $n = 6$).

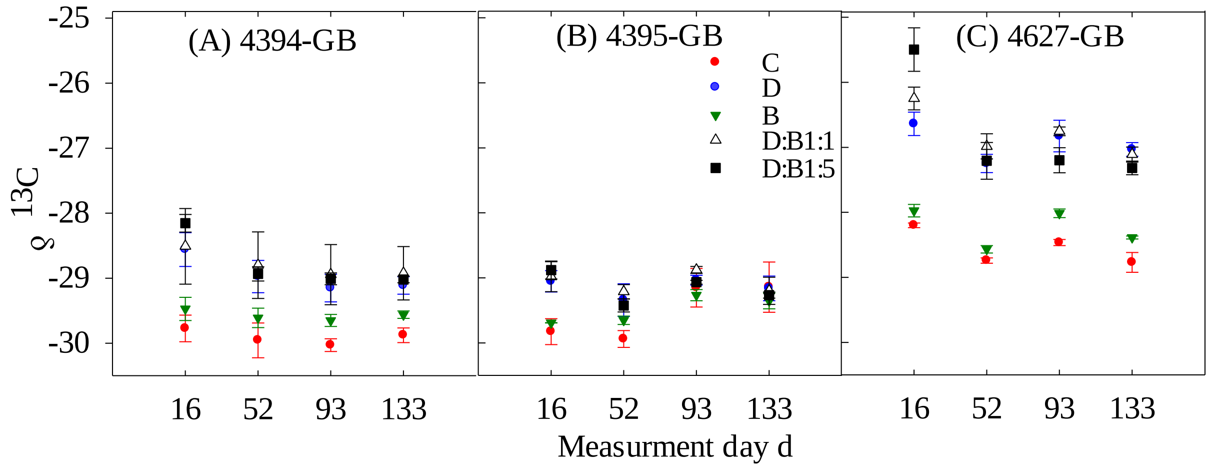


Figure 31: **Variation of δ^{13}C (‰ VPDB) values of produced CO$_2$ from the different treatments for the three different *Gleyic Podzol* topsoil samples (0 - 0.2 m) on days 16, 52, 93 and 133. The vertical error bars represent ± one standard deviation of the mean (n = 3).**

There were significant differences between the δ^{13}C values of the emitted CO$_2$ between the control treatment (C) and the other treatments (B, D, D:B 1:1, D:B 1:5) during the whole incubation experiment (see Table A 12 on page 149 in the Annex). The δ^{13}C signature values of CO$_2$ respired from the control treatment was the lowest with a few exceptions (measurements on days 93 and 133 for the 4395-GP sub sample).

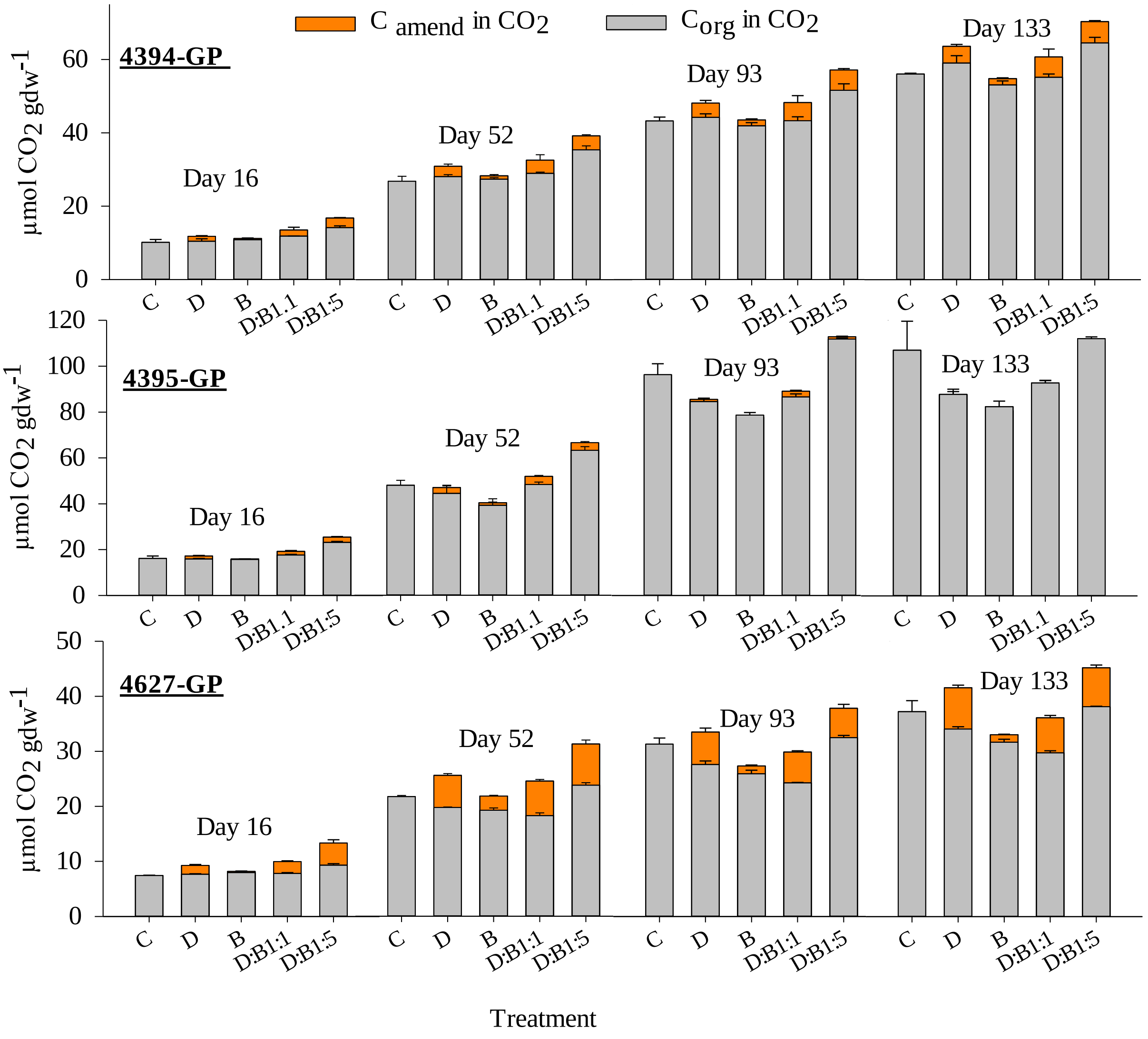


Figure 32: Cumulative amount of CO_2 emitted from specific carbon pools on days 16, 52, 93 and 133 of the aerobic incubation experiment of *Gleyic Podzol* with treatments: control (C), digestate only (D), biochar only (B), digestate:biochar 1:1 (D:B 1:1) and 1:5 (D:B 1:5). The different colors indicate the amount of CO_2 emitted from specific soil carbon pools: grey for the soil inherent carbon (C_{org}), **and orange for the amendments' carbon** (C_{amend}). **Vertical error bars represent ± one standard deviation of the mean (n = 3).**

The proportions of soil organic carbon and amendment carbon in the emitted CO_2 from *Gleyic Podzol* during the incubation experiment are shown in Figure 32. The amount of CO_2 emitted from soil organic carbon was reduced by biochar only in soil samples 4395-GP, and 4394-GP, except on day 16 when the data also showed a lower amount of CO_2 derived from the soil organic carbon pool with the control treatment. At the end of the incubation experiment, the CO_2 produced by soil organic carbon in the digestate:biochar 1:5 treatment did not show a significant difference compared to the values in the control treatment for all three soil samples. The biochar only treatment showed a significantly lower release of amendment's carbon as CO_2 from both sub soil samples 4394-GP and 4627-GP, and a nonsignificantly lower amount in the 4395-GP sub soil sample. The emitted CO_2 from the amendment's carbon in the BL and BH treatments was not significantly different on all four measurement days and for all sub soil samples with the exception of sample 4627-GP on day 16.

5.7.2 Influence of biochar on *in situ* soil respiration and trace gas production

5.7.2.1 Average daily *in situ* soil respiration

During the experimental period from November 2012 to August 2014, the changes of the daily soil respiration values for *Gleyic Podzol* and *Gleyic Anthrosol* followed the changes of the temperature throughout the year (Figure 33). However, the soil temperature had a higher influence on the daily *in situ* CO_2 emission fluxes than the air temperature. The highest emission was in summer, followed by autumn and spring seasons.

For *Gleyic Podzol*, the CO_2 emission from the soil surface ranged from -1.4 ± 1.5 g m^{-2} d^{-1} (March 2014, $n = 4$) to 22.9 ± 5.2 g m^{-2} d^{-1} (May 2013, $n = 4$) for the biochar amended plots and -0.129 ± 0.5 g m^{-2} d^{-1} (February 2014, $n = 4$) to 16.0 ± 0.8 g m^{-2} d^{-1} (May 2013, $n = 4$) for the control plots (Figure 33). For *Gleyic Anthrosol* , the highest amount of CO_2 emitted from the BL plots in August 2014 (27.5 ± 12 g m^{-2} day^{-1}, $n = 4$), and the lowest emission was 54 mg m^{-2} day^{-1} occurring in both the digestate and the BH plots in April 2014.

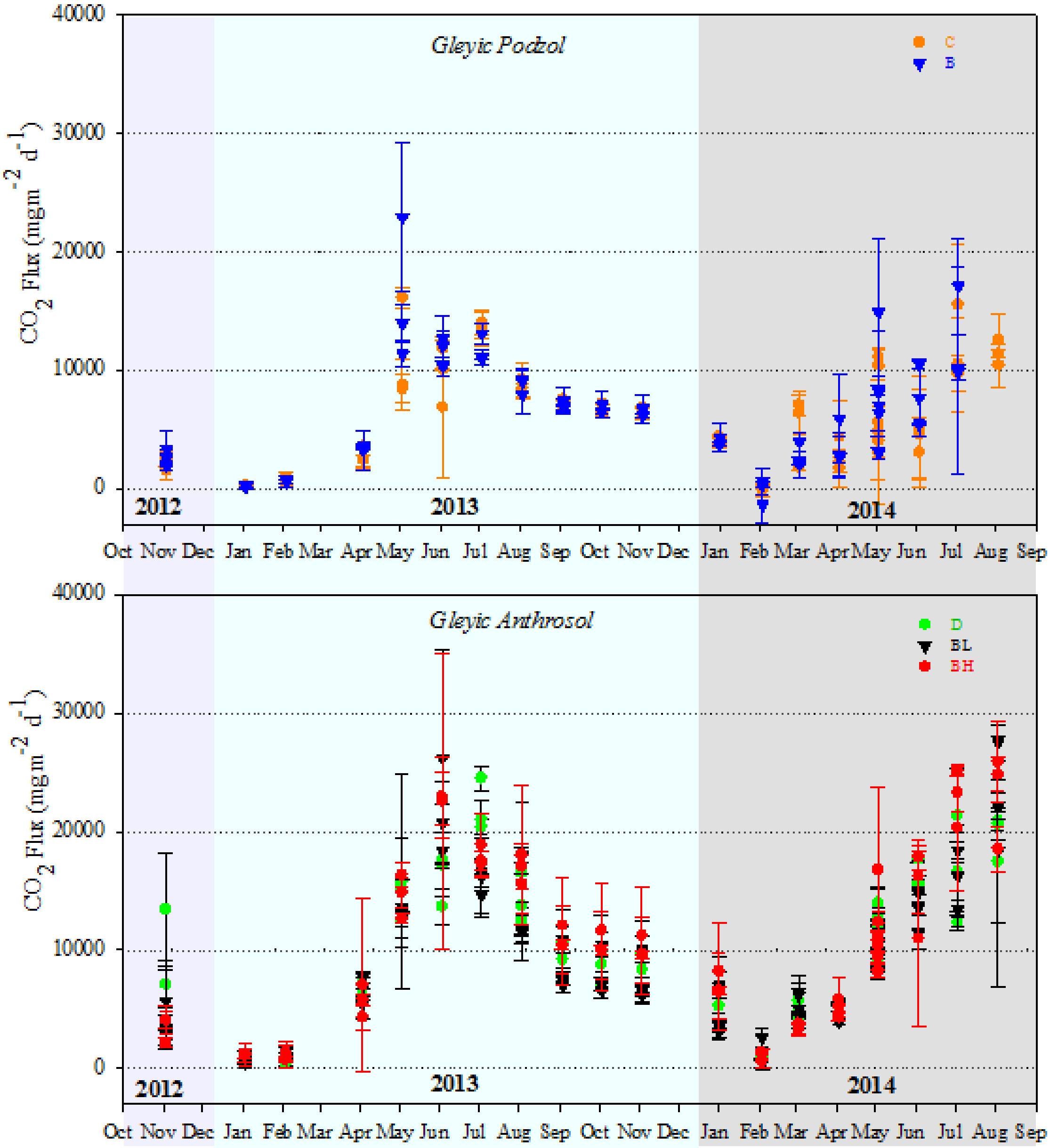


Figure 33: **Daily average of *in situ* soil respiration (CO$_2$ mg m^{-2} day^{-1}) of different soil treatments from November 2012 to August 2014. The vertical error bars represent ± one standard deviation of the mean (n = 12). Treatments are control (C), Biochar (B), digestate (D), digestate:biochar 1:1 (BL) and digestate:biochar 1:5 (BH).**

5.7.2.2 Seasonal variation of *in situ* CO_2 emission

For *Gleyic Podzol*, the seasonal variations of the measured daily values of CO_2 resulting from the soil ecosystem respiration process during the experimental period were not significantly influenced by biochar application (Figure 34; ANOVA, $p > 0.05$, $n = 24$ to 48). However, the addition of biochar produced more CO_2 in the spring and summer seasons of both 2013 and 2014. During winter and autumn 2013, both *Gleyic Podzol* and *Gleyic Anthrosol* with and without biochar emitted approximately equal amounts of CO_2 (Figure 34 and Figure 35). In *Gleyic Podzol*, the amounts of CO_2 produced over the whole experimental period in the biochar treated and the untreated plots were nearly equal (121.8 ± 19 and 122.2 ± 15 g CO_2 m^{-2} resp.). The emitted CO_2 per season was less in 2013 than in 2014, except in spring.

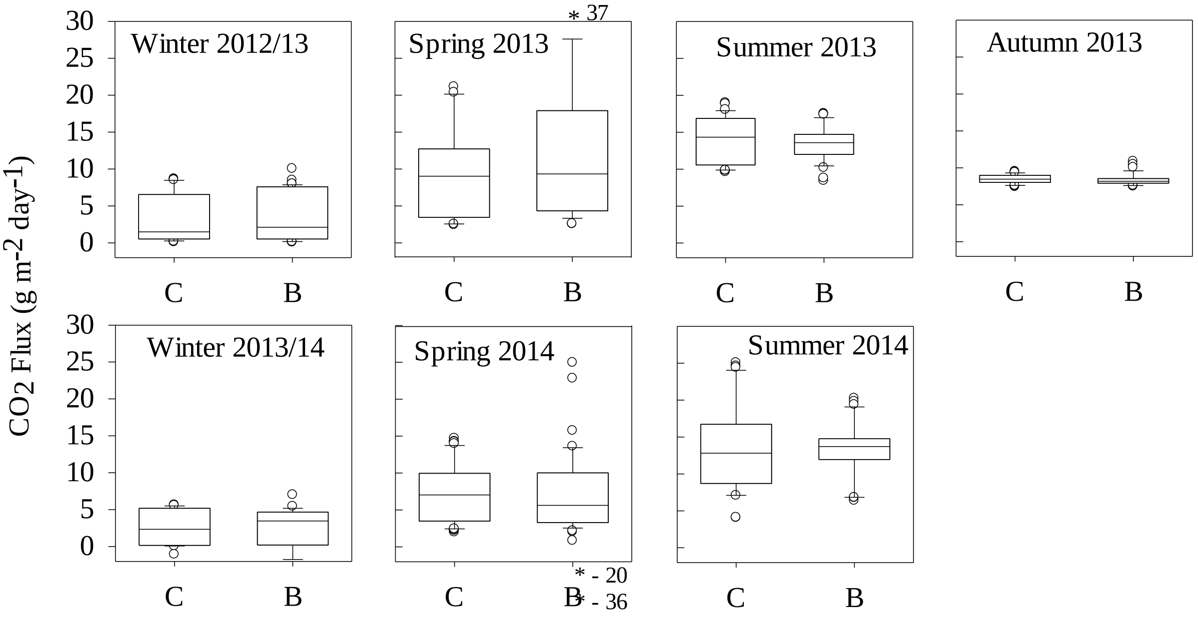


Figure 34: Seasonal variation of *in situ* soil respiration in *Gleyic Podzol* for control (C) and biochar (B) treatments after starting the experiment in September 2012. In the boxplot diagrams, the central line shows the median, the lower/upper horizontal bars are minimum and maximum, outliers (values between 1.5 and 3 times the interquartile range from a quartile) are marked by circles within the plot, and by asterisks together with values outside the plot area. Extreme data points were removed before plotting the data, and n = 24 to 48.

The seasonal soil respiration values for *Gleyic Anthrosol* were not significantly influenced by biochar application (ANOVA, $p > 0.05$, $n = 24$ to 48; Figure 35), except in winter 2012/13 and autumn 2013 (ANOVA, $p = 0.023$, $n = 48$ and $p \leq 0.001$, $n = 48$ resp.; Figure 35). More CO_2 was released from the BH treatment than from the digestate only treatment, except in summer 2014, when after reapplication of digestate to all treatment plots in site 2 the digestate only plots produced a higher amount of CO_2 compared to both BL and BH (29 % and 5 %, respectively).

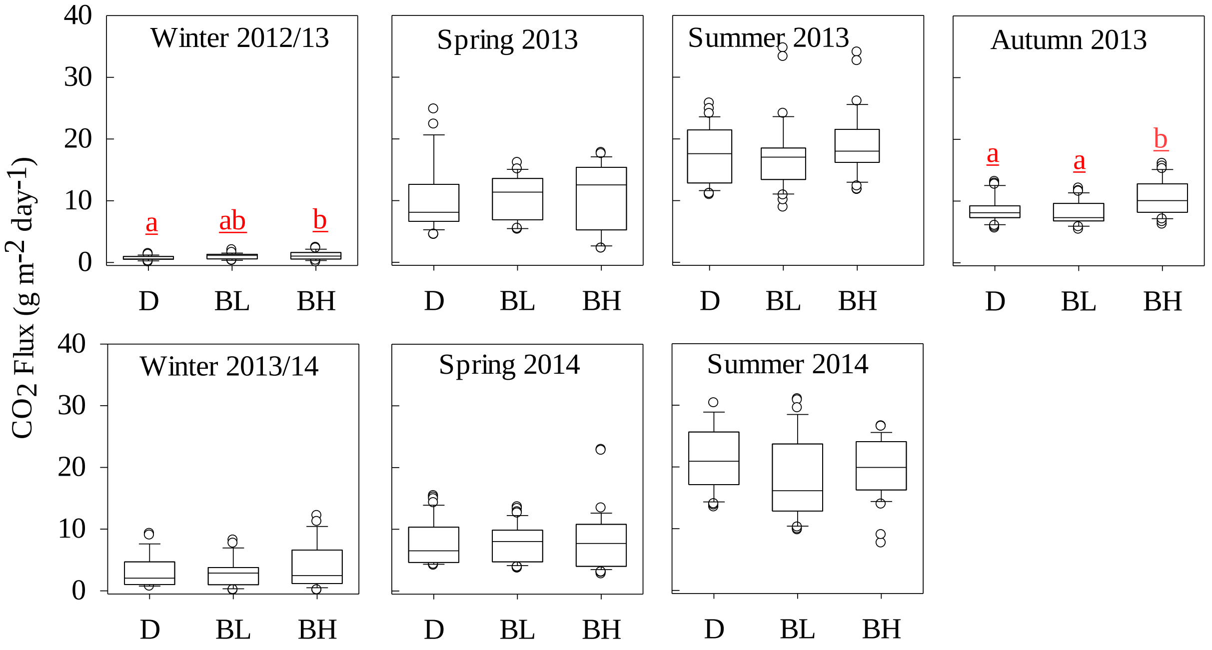


Figure 35: Seasonal variation of *in situ* soil respiration in *Gleyic Anthrosol* after starting the experiment in September 2012 for digestate (D), digestate:biochar 1:1 (BL) and digestate:biochar 1:5 (BH) treatments. In the boxplot diagrams, the central line shows the median, the lower/upper horizontal bars are minimum and maximum, outliers (values between 1.5 and 3 times the interquartile range from a quartile) are marked by circles. Extreme data points were removed before plotting the data. Different letters indicate the level of significance, and n = 73 to 109.

5.7.2.3 Seasonal variation of CO_2 emission rates from the top soil

In summer, the maximum gas emission rates were observed, whereas the winter seasons showed the lowest rates (Figure 36). For both soils, the seasonal mean values of calculated emission rates between treatments did not show significant differences at $p = 0.05$ level, due to high variability within the specific seasons, except in autumn 2013 for *Gleyic Podzol* (ANOVA, $p < 0.002$, $n = 3$). Addition of biochar to *Gleyic Podzol* topsoil (0.2 m) reduced the CO_2 emission rate during the whole experimental period. The CO_2 emission rates ranged from 0.08 to 8.5 mg CO_2 g TOC^{-1} d^{-1} in *Gleyic Podzol* (Figure 36). In summers 2013 and 2014, the control plots showed 10 % and 8 % higher CO_2 emission rates respectively, compared to the biochar amended ones.

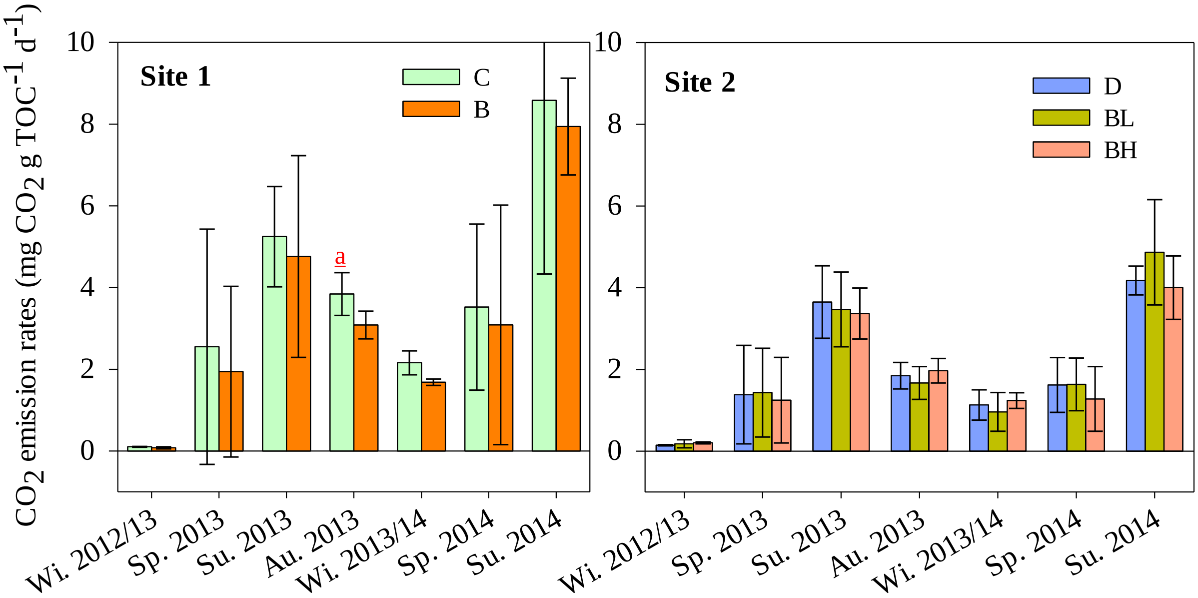


Figure 36: Seasonal variation of *in situ* CO_2 emission rates from *Gleyic Podzol* for control (C) and biochar only (B), and from *Gleyic Anthrosol* for digestate only (D), digestate:biochar 1:1 (BL) and digestate:biochar 1:5 (BH) applications after starting the experiment in September 2012. The vertical error bars represent ± one standard deviation of the mean, and the letter indicates the level of significance of the means (n = 3 to 9).

The positive effect of biochar on CO_2 emission from *Gleyic Anthrosol* which contained digestate was not as clear as the effect on *Gleyic Podzol*. Only in winters 2012/13 and 2013/14 and autumn 2013, BH had higher CO_2 emission rates compared to the BL and D treatments. Winter 2012/13 showed the lowest CO_2 emission for all treatments in *Gleyic Anthrosol*. However, in the following winter 2013/14, the emission rates increased by 675 %, 434 % and 507 % compared to winter 2012/13 for digestate only, BL and BH treatments respectively, and those were significant increases (ANOVA, $p = 0.01$, $p = 0.049$, $p < 0.001$ for digestate only, BL and BH resp., $n = 3$). In both spring seasons (2013 and 2014), the BL treatment showed nonsignificantly higher CO_2 emission rates compared to the two other treatments.

5.7.2.4 Seasonal variation of *in situ* methane (CH_4) and nitrous oxide (N_2O) emissions

Over the whole two year period (September 2012 until August 2014), both N_2O and CH_4 gas emissions from both experimental sites were measured and fluxes were calculated. Analysis of data revealed that the majority of N_2O and CH_4 flux curves was better described by a linear regression than by an exponential model. Therefore, linear fluxes were used for both N_2O and CH_4. Some of the measured data for N_2O and CH_4 emissions from the soil surfaces had a large standard deviation (Figure 37 and Figure 38). Mostly, CH_4 fluxes varied around zero (Figure 38). In winter 2012/13 and in the beginning of spring 2013 for both *Gleyic Podzol* and *Gleyic Anthrosol*, frozen soil, and soil collars covered by an ice layer did not allow to take all monthly measurements. Therefore, there were not enough samples ($n \leq 2$) for statistically analyzing the differences between means. Hence, the seasonal variations of N_2O fluxes are shown in Figure 37 only from spring 2013 to summer 2014.

In *Gleyic Podzol*, the biochar treated plots emitted less N_2O from the soil surface compared to the control plots (Figure 37). However, only spring 2013 showed significantly lower N_2O fluxes in the biochar treated plots (ANOVA, $p = 0.031$, $n = 3$). In all other seasons, the difference of means of the two treatments was not significant at $p = 0.05$. In summer 2014, there was a large difference between the means of emitted N_2O for control and biochar (321.5 ± 182 and 15.7 ± 81 mg N_2O m^{-2} d^{-1} resp.), but the variation of the measured data values was very high (ANOVA, $p = 0.057$, $n = 3$).

Amending *Gleyic Anthrosol* with biochar did not show a significant effect on N_2O emission (ANOVA, $p > 0.05$, $n = 3$). However, there was a reduction of N_2O emissions from both BL and BH treatments compared to the D treatment. Both summers (2013 and 2014) showed a considerable increase of N_2O from the digestate only plots compared to both biochar treatments.

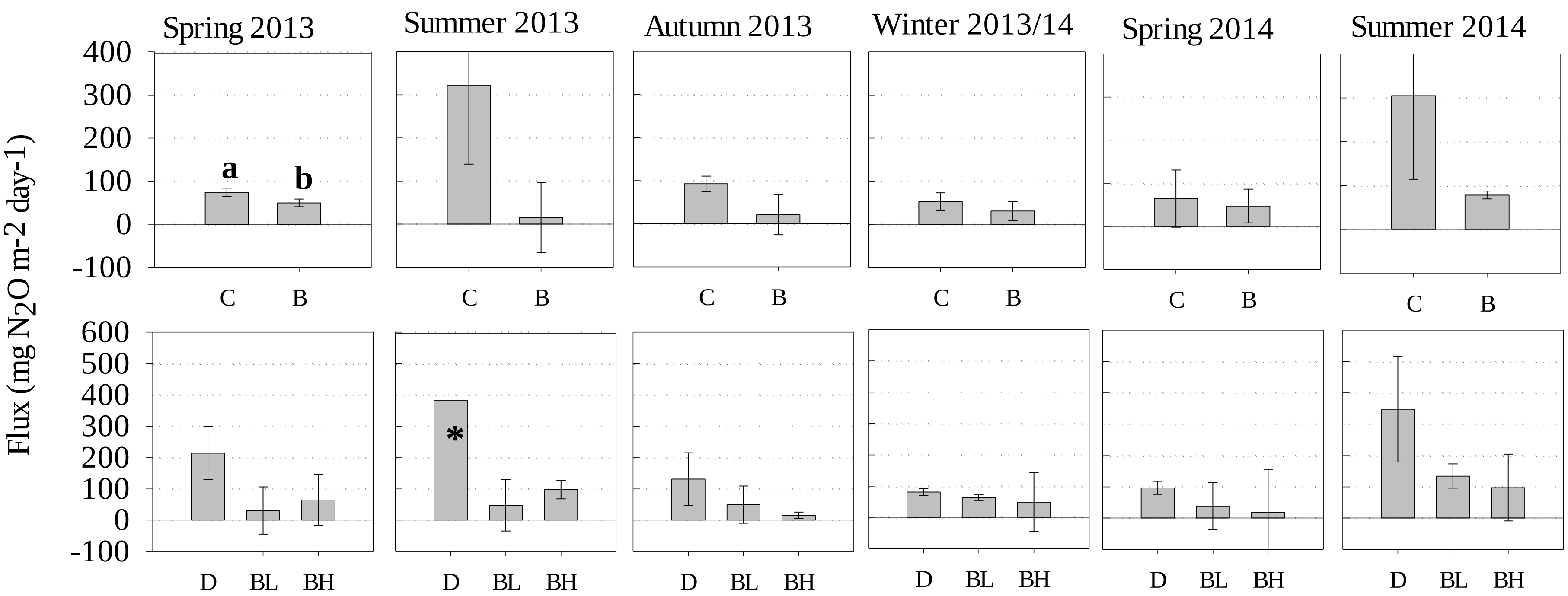


Figure 37: Seasonal variation of *in situ* N_2O emissions in *Gleyic Podzol* (top row) for control (C) and biochar (B), and of *Gleyic Anthrosol* (bottom row) for digestate (D), digestate:biochar 1:1 (BL) and 1:5 (BH) after starting the experiment in September 2012. The vertical error bars represent ± one standard deviation of the mean (n = 6 to 12 measurements per treatment per season). The bars with a * sign indicate that the standard deviation is not shown because n < 3.

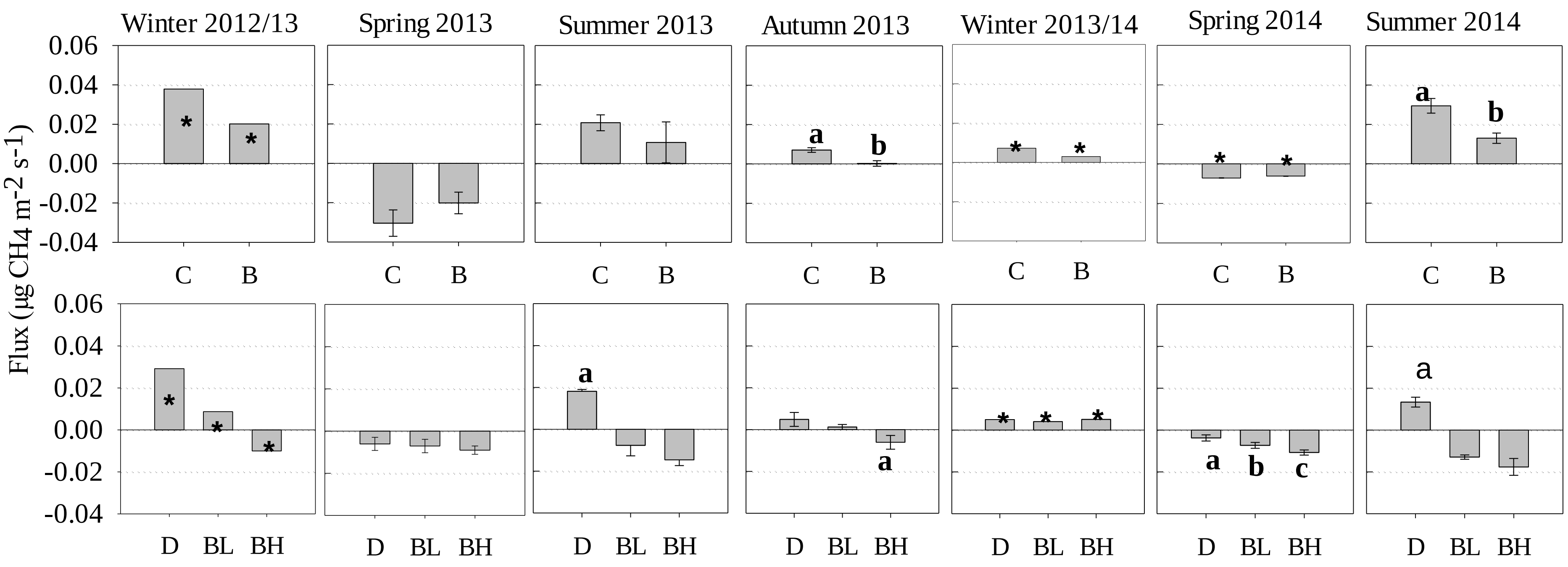


Figure 38: Seasonal variation of *in situ* CH_4 emission in *Gleyic Podzol* (top row) for control (C) and biochar (B), and of *Gleyic Anthrosol* (bottom row) for digestate (D), digestate:biochar 1:1 (BL) and 1:5 (BH) after starting the experiment in September 2012. The vertical error bars represent ± one standard deviation of the mean (n = 6 to 12 measurements per treatment per season). The bars with a * sign indicate that the standard deviation is not shown because n < 3.

Methane showed both negative and positive fluxes for both *Gleyic Podzol* and *Gleyic Anthrosol* during the experimental period (Figure 38). The lowest CH_4 flux was - 0.03 ± 0.0 µg CH_4 m^{-2} s^{-1} in the control treatment in spring 2013. The highest value was 0.04 µg CH_4 m^{-2} s^{-1} in the same treatment in winter 2012/13 (n = 2). When considering all positive CH_4 fluxes, added biochar nonsignificantly reduced the emission of CH_4 in *Gleyic Podzol*, except in autumn 2013 and summer 2014. In both autumn 2013 and summer 2014, this reduction was significant (ANOVA, $p = 0.003$, $n = 3$ and $p = 0.003$, $n = 3$ resp.). Adding biochar to *Gleyic Anthrosol* produced results similar to those in *Gleyic Podzol*. Both positive and negative fluxes were produced, but the majority of fluxes were negative. Most of the time, the BH treatment produced nonsignificantly lower CH_4 fluxes than both D and BL treatments, but differences between fluxes were significant in summer 2013, autumn 2013 and spring 2014 (ANOVA, $p < 0.001$, $p = 0.008$, $p < 0.001$ resp., $n = 3$; Figure 38).

5.8 Carbon balance in the topsoil

During the whole experimental period, TOC in the different soil depths did neither show a consistent accumulation nor a consistent depletion (Table 11). Two years after adding the amendments to *Gleyic Anthrosol*, TOC in the surface layer (0 - 0.2 m) of the digestate only plots had increased by 19 % compared to TOC in the same depth before the start of the experiment (June 2012). The two treatments with biochar in addition to digestate showed higher TOC content in top soil (0 - 0.2 m) than the digestate only treatment (20 % and 34 % for BL and BH resp.). Also the sub-surface soil layer (0.2 - 0.4 m) had higher TOC compared to the value in June 2012. The highest accumulation showed with the BL treatment. The accumulation of TOC in the lower soil layers/depths did not show clear differences even after two years from the biochar application. Considering *Gleyic Podzol*, TOC in the control treatment decreased by 13% after one year of the experiment. The biochar treatment did not show changes of TOC in the surface soil layer, but in the soil depths 0.2 - 0.4 m and 0.4 - 0.6 m where it significantly increased the TOC (by 229 % and 2871 % resp.).

Table 11: Means of TOC in *Gleyic Podzol* and *Gleyic Anthrosol* at the end of main cropping harvests after biochar addition in October 2012 together with the values before applying biochar (June 2012).

Soil	Treatment	Depth (cm)	TOC (%)			
			2012 June*	2013 October	2014 May	2014 October*
Gleyic Anthrosol	D	0-20	1.37	1.46 ± 0.1	1.42 ± 0.3	1.63
		20-40	1.37	1.51 ± 0.2^{a}	0.54 ± 0.1^{b}	1.50
		40-60	0.49	0.62 ± 0.3	0.46 ± 0.3	0.61
	BL	0-20	1.37	1.57 ± 0.1	1.46 ± 0.2	1.65
		20-40	1.37	1.31 ± 0.2	1.04 ± 0.5	1.57
		40-60	0.49	0.63 ± 0.3	0.92 ± 0.3	0.47
	BH	0-20	1.37	1.75 ± 0.1	1.77 ± 0.5	1.83
		20-40	1.37	1.61 ± 0.3	1.20 ± 0.5	1.40
		40-60	0.49	0.82 ± 0.4	0.67 ± 0.2	0.18
Gleyic Podzol	C	0-20	0.68	0.64 ± 0.1	0.59 ± 0.1	-
		20-40	0.07	0.44 ± 0.2	0.25 ± 0.2	-
		40-60	0.06	n.a.	n.a.	-
	B	0-20	0.68	0.79 ± 0.0^{a}	0.68 ± 0.1^{b}	-
		20-40	0.07	0.44 ± 0.3	0.23 ± 0.2	-
		40-60	0.06	0.35 ± 0.3	2.08 ± 2.8	-

Different letters indicate significant differences between treatment means in the same depth (n=3). Letters are not shown when differences are not significant. *Standard deviations are not shown when n < 3. n.a.: not analyzed.

6. Discussion

6.1 Characteristics of digestate and biochar

The solid digestate which was used as feedstock for our biochar was a byproduct of an agricultural biogas plant which uses a mixture of cattle manure and bioenergy crop biomass as feedstock. It had TOC, TN and C/N values of 39 %, 2 % and 20 resp., all of which were close to the value ranges documented by Möller and Müller (2012) for solid digestate originating from cattle manure and agricultural residue (39.6 - 40.0 %, 2.2 - 3.0 % and (11.2 - 19.3 resp.). The biochar had a fine and homogeneous texture, caused by the texture of the fermented and chopped maize biomass feedstock, and due to the way it was produced could easily be broken down into smaller pieces

The biochar produced for this study had a lower TOC content (39 %) and lower carbon recovery (12 %) compared to biochar originating from wood or unfermented grass or waste. Lehmann (2007) discussed the relationship between carbon recovery and production temperature of biochar. According to their data, biochar produced from corn cob at 400 - 800 °C showed 35 to 40 % carbon recovery. The C/N ratios of charred materials can vary within a huge range from 7.3 to 700, depending on TOC in the feedstock and production temperature (Krull et al., 2009; Liu et al., 2015). For our biochar, the low TOC and a comparatively little higher TN content gave a low C/N ratio (20 ± 0.4). This low C/N ratio is favorable for plant growth as was discussed by Haefele et al (2011).

The nutrient content of biochar originating from maize stalk and cob was much lower than in our biochar originating from an anaerobically fermented mix of the whole biomass of maize with animal excretion (Amonette and Joseph, 2009). According to unpublished work done by Schröder (2013), the heavy metals in our biochar did not exceed the values allowed by German authorities in order to protect environmental and human health. All those prove that our biochar and its feedstock were not exceptional, but had normal and acceptable physico-chemical characteristics making them suitable for being used as an organic soil amendment.

6.2 Effect of biochar on above ground biomass production

The mean values of annual precipitation (822 mm) and daily temperature (8.6 °C) within the experimental period were very close to the long term averages from 1981 to 2010 (857 mm and 8.7 °C resp.), so the experimental time represented average climatic conditions for the region. However, the distribution of rainfall was different in 2013 (see Figure 12), which had an influence on the early growth rate and the later development of the maize crop. When later more precipitation occurred, weed flourished in the field. This abiotic stress influenced the total biomass production of the maize in summer 2013 (see Figure 14). The maize in the grower's field did not have a water stress and subsequent weed problem because of an earlier crop establishment and of chemical weeding. Nevertheless our study supported the working hypothesis (H1) that the application of biochar positively influences the production of biomass. All plots with biochar applications yielded higher biomass production in both soils compared to the control plots in site 1 (*Gleyic Podzol*), respectively the digestate only plots in site 2 (*Gleyic Anthrosol*).

The crop response to biochar amended soil is a synergistic effect of various factors: chemical and physical properties of biochar, the rate and frequency of biochar application, the way of application, soil conditions, climate conditions, crop type, and management of the crop, as has been documented by several authors (Asai et al., 2009; Gaskin et al., 2010; Haefele et al., 2011; Major et al., 2010b; Van-Zwieten et al., 2010; Yamato et al., 2006). Although there have been no previous studies that discussed the effect of biochar on the biomass of maize which was grown for bioenergy production in the field trails, there have been many experiments which discussed the responses of grain yield of cereal crops including maize (Grassini and Cassman, 2013; Liu et al., 2015; Major et al., 2010b; Martinsen et al., 2014; Ndor et al., 2015; Zhang et al., 2012b) .

Chan et al. (2007, 2008b) and Zhang et al. (2010a, 2010b, 2011) explained the increased yield of maize after applying biochar derived from greenwaste and wheat straw as a result of increased nutrient availability, decreased soil bulk density and improvement of other soil physical properties. Pan et al. (2009) showed that high crop productivity and nitrogen efficiency result from the accumulation of high amounts of soil organic carbon due to biochar addition. Zhang et al. (2012b) reported higher nitrogen use efficiency, and maize yield increased by 16 resp. 7 % without N fertilizer, and 9 resp. 12 % with N fertilizer when biochar was applied at 20 t biochar ha^{-1} resp. 40 t biochar ha^{-1} for a Calcareous loamy soil with poor soil organic carbon content in the central plain in China. Few discussions exist on below-ground biomass yield, and a meta-analysis was done by Biederman and Harpole (2012), showing that below ground biomass in perennial crop plants did not significantly respond to biochar, but the annual crop did show significant responses with or without fertilizer addition.

Lehmann et al. (2003b) found both positive and negative responses in their study, and also there was no proportional relationship between biochar application rate and increased yield. They also documented that higher rates of biochar application could decrease the nitrogen availability. Similar findings were documented by Major et al. (2010b), showing no change of maize yield in the first year, whereas the following three years had significant yield increases after one time application of wood biochar at 20 t ha^{-1} into an *Oxisol* in the Colombian savanna. However, with the degraded and less productive *Ultisol* in Kenya, biochar increased the cumulative maize yield by 100 % after repeated application in a two year period at 7 t biochar ha^{-1} (Kimetu et al., 2008).

Gleyic Podzol, which had relatively poor physico-chemical properties, showed similar results to *Gleyic Anthrosol*, increasing biomass in the biochar amended plots by by 72 % and 47 % for the winter and summer seasons respectively during the first year of the experiment (see Figure 14). Asai et al. (2009) reported the opposite to our results when studying the effects of biochar on the yield of rice paddy: the yield of upland rice (*Oryza sativa* L.) decreased after application of biochar with no nitrogen fertilization in a nitrogen deficient soil. But Zhang et al. (2010a) reported a higher rice productivity and an increased agronomic nitrogen use efficiency in an acidic organic carbon rich paddy soil with biochar application at 10 t biochar ha^{-1} and 40 t biochar ha^{-1}. In our study, the above ground total biomass of the bioenergy maize crop increased during four consecutive cropping seasons after one time application of biochar at 5 t ha^{-1} with no additional NPK fertilizer to *Gleyic Podzol* with its poor physical properties and low TOC content.

6.3 Effects of biochar on soil physico-chemical properties

Many biochar field trials were carried out all around the world in different climate and agro-ecological zones, however the studies in the tropics were dominating among them (Blackwell et al., 2009; Schulz and Glaser, 2012). Generally, those studies proved positive effects of biochar on the concentration of nutrients, their retention and their availability, which in turn increase soil fertility and thereby plant growth (Bélanger et al., 2004; Glaser et al., 2002; Lehmann et al., 2003b; Major et al., 2010b; Novotny et al., 2009; Rondon et al., 2007). Furthermore, increases in CEC (Glaser et al., 2002; Mikan and Abrams, 1995; Topoliantz et al., 2002), water-holding capacity (Glaser et al., 2002), soil microbial and mycorrhizal activity (Thies et al., 2009; Warnock et al., 2007), soil acidity (Chan and Xu, 2009), and electric conductivity (Asai et al., 2009) have been reported after biochar application to soils. There was one study with negative effects on crop productivity and chemical properties (ryegrass with biochar from biosolids), and some with no significant effect of biochar addition (Jeffery et al., 2011).

The above mentioned studies mainly considered the effect on soil during the main cropping season. This study incorporated the behavior of biochar in the soil ecosystems during both active (summer crop) and inactive (winter crop) times in detail for four consecutive cropping seasons. The second working hypothesis (H2), that the application of biochar increases soil macro- and micro-nutrients, was confirmed by the results of our study.

6.3.1 Soils in the two fields

As mentioned earlier, the soils in the two field experimental sites represent two different soil ecosystems in SH, Germany. Site 1 represents the abandoned land which has not been treated by biogas digestate before, and has not been used for an intensive monoculture system. In contrast, the land utilization and management of site 2 were different: this site has been intensively used to produce biomass for agricultural biogas plants during the past years, and received an amendment of digestate at a rate of 42 Lm^{-2} at the beginning of each cropping season. The chemical and physical properties of *Gleyic Anthrosol* in site 2 have development and chemical characteristics closely similar to those of the uppermost layer of *Plaggic Anthrosol* described by Meuser and Blume (2001) for Osnabrück in Northwest Germany. The soil characteristics of the experimental plots of site 2 were representative for the soils in the adjacent arable land (Langheld, 2013; Rössler, 2013). There are no studies about the soils of the adjacent fields in site 1, and hence there is no information available to compare in how far the experimental plots represent the *Gleyic Podzol* soils of the surrounding area and fields.

6.3.2 Initial changes of soil chemical properties

Before applying biochar to both experimental sites, the experimental plots did not show significant heterogeneity of TOC and TN. Our biochar application rates (5 and 24 t biochar ha^{-1}) were high enough to produce initial significant changes in the topsoil (0 - 0.2 m) of *Gleyic Podzol* and *Gleyic Anthrosol*. The amendment of those sandy soils with biochar derived from anaerobic biogas digestate significantly increased soil pH, EC, TOC, TN, as well as other chemical properties including cations, CEC and BS. As a porous substance with high surface area and many nutrients, biochar can alter the soil nutrient status just after application as well as in the long run. Because our biochar was alkaline compared to its feedstock material, it increased the alkality of the soil. While adding biochar did not significantly influence TN, DON and ammonium, it had significant influences on nitrate in both soils. We found high values of nitrate and ammonium which shows that there is an indirect influence of biochar on the mineralization of organic matter in the soil. All these initial changes of the investigated ecosystems mainly depend on the quality of the biochar and much less on the soil texture and agricultural use of the ecosystem. Our results clearly showed the fertilizing effect of biochar on soils in bio energy crop production ecosystems.

6.3.3 Seasonal changes of soil chemical properties

The initial detrimental effects of biochar on soil chemical properties changed with the following spring season when the vegetation period started. The values of all soil chemical properties which we determined in our study significantly decreased in spring 2013. After that the decline continued with less fluctuation, never totally disappeared, but the differences of soil pH, TOC, TN, DOC and DON were no longer significant. The reason behind these changes may have been the stabilization of biochar within the ecosystems while it was aging in the soil. At the end of the experiment, the plots in *Gleyic Anthrosol* treated with a high amount of biochar showed higher values compared to the other treatments. The rest of the soil chemical properties (CEC, BS, plant available-P and -K) showed clear effects of biochar application in all seasons, but those only were significant for the plots treated with a high amount of biochar in *Gleyic Anthrosol*, and for the biochar treatment in *Gleyic Podzol*. The positive effects of the low application rates of biochar were smaller than those of the high application rate. The ups and downs of our results may have been caused by the fact that biochar undergoes continuous aging and oxidation processes influenced by soil-plant-biochar interactions and other environmental parameters of the soil ecosystems (Cheng et al., 2006; Spokas, 2013).

6.4 Effects of biochar on soil microbial biomass

6.4.1 Review of the $CHCl_3$ extraction procedure

MBC and MBN indirectly reflect soil microbial growth and activities, production of CO_2 in the soil, and soil organic matter degradation (Zhang et al., 2014). We used the $CHCl_3$ fumigation method to determine soil microbial biomass. Badalucco et al. (1990) and Kuzyakov et al. (2009) showed that fumigating soil with $CHCl_3$ can solubilize both living microbial cells and a small amount of dead soil organic matter (0.2 - 0.4 % of total carbon content). Therefore, when the measured MBC is low, the results must be interpreted with special caution. Liang et al. (2010) showed that biochar may adsorb DOC, thereby affecting microbial biomass measurements in biochar amended soil. But Durenkamp et al. (2010) and Dempster et al. (2012) both proved by using the recovery result of ^{14}C experiments that the adsorption of DOC to biochar had no effects on the extraction efficiency of soluble carbon.

6.4.2 Effects of biochar on soil microbial carbon and nitrogen

Generally, our research proved that biochar addition influenced both MBC and MBN in both tested soil ecosystems, showing that under the temperate climatic conditions in Northern Germany, biochar accelerates microbial activities and growth thus confirming our working hypothesis H3. Previous studies showed that the effects of biochar on soil microbial biomass were quite inconsistent: several studies found that soil microbial biomass in biochar amended soils showed no differences from the control soil (Bruun et al., 2011, 2012; Kuzyakov et al., 2009; Zavalloni et al., 2011), other studies reported either an increased (Bruun et al., 2008; Kolb et al., 2009) or a decreased (Dempster et al., 2012) soil microbial biomass after biochar application.

Several explanations for the positive response of soil microbial biomass in biochar amended soils have been given. One explanation is that biochar causes increasing concentrations of labile organic carbon compounds, which derive from biochar (Bailey et al., 2010; Bruun et al., 2011, 2012, 2008; Smith et al., 2010). Furthermore, biochar has been shown to add nutrients directly to the soil nutrient pool, to increase the retention of nutrients because of the larger surface area and highly porous structure of biochar (Cheng et al., 2008; Masiello et al., 2013). Also, biochar applications may increase the habitat for microbes by providing physical protection (Cheng et al., 2008; Pietikainen et al., 2000), or by generating a chemically more favorable environment for the microbes due to the alkalinity of biochar (Lehmann et al. 2011). There also were reports showing the ability of biochar to increase the decomposition of soil organic matter which stimulates microbial activity (Wardle et al., 2008) and the sorption and retention of organic carbon (Lehmann et al., 2011), which helps to increase microbial biomass. However, also negative effects of biochar application on microbial abundance and activity have been reported. Some studies showed that biochar was able to release some harmful highly volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs) which can inhibit microbial growth and activities (Buss et al., 2015;

Deenik et al., 2010). Also, biochar trapped essential nutrients inside of its pores which reduced the availability of nutrients in the soil solution temporarily or permanently (Dempster et al., 2012), and this in turn reduced the activity of soil microbes, soil organic matter decomposition, and N mineralization.

Our research showed that biochar which was produced at high temperatures from nutritionally rich feedstock reduced MBC by 9 % just after applying it to *Gleyic Podzol*. This can be caused by initial fixation of nutrients inside the biochar, and VOCs and PAHs in the biochar (Blackwell et al., 2009; Buss et al., 2015; Downie et al., 2012; Zimmerman et al., 2011). However, MBN in *Gleyic Podzol* did not have an initial depletion effect by applying biochar, but showed an increase in the first winter season. The next three seasons showed both significant and nonsignificant increases of MBC and MBN in the relatively unfertile and coarse-textured *Gleyic Podzol.* This result even remained in the next winter, when *Gleyic Podzol* amended with biochar had a larger active soil microbial community (Figure 25).

Those results suggest that biochar supplied a livable and protective habitat for microorganisms in soil. During the winter season, the growth of microorganisms can be stimulated by biochar because of the albedo effects of biochar (Liang et al., 2014; McCormack et al., 2013) which have a direct influence on the soil temperature. However, with our low application rate of biochar, the albedo effects were less important than the effect on protective habitat.

6.5 Response of soil ecosystems to the application of biochar: soil carbon turnover

In addition to studying the influences of biochar and digestate on soil nutrients, another major goal of this research was to understand how charred (biochar) and non-charred biogas digestate affect the soil carbon pool and soil carbon mineralization. This goal represents the final hypothesis (H4) of our study. The results prove that the CO_2 production did not significantly increase after adding organic carbon as biochar to the soil, but the effects on the other two gases that we measured were not clear from our set of data.

The results of our incubation experiment help to improve the understanding of how biochar alters the potential carbon turnover in *Gleyic Podzol*, and the field gas flux measurements of both *Gleyic Podzol* and *Gleyic Anthrosol* are used to improve the understanding of the behavior of biochar, digestate and their mixture in the soil carbon pool.

6.5.1 Influence of biochar and digestate on soil carbon mineralization: an incubation experiment

In incubation experiments, the preparation of the samples has an effect on the results, especially on the measurements taken on the initial days. During sample preparation, removing plant roots, stones and macro organisms causes physical damages to the soil matrix and soil aggregates, and alters the biological processes (Subke et al., 2010). In addition to the treatment effects, those disturbances can result in initial mineralization of soil carbon (Knoblauch et al., 2013; Subke et al., 2010). In our study, several of the samples had high standard deviations between the replicates. This may be due to the above mentioned disturbances, or to insufficient homogenization of the initial bulk surface soil samples, or of the soil-amendment mixtures, even though we took great care to homogenize the samples.

In an aerobic incubation experiment, CO_2 concentration in the head space can inhibit the activities of aerobic microorganisms. Knoblauch et al. (2013) showed the necessity of flushing the head space with synthetic air when the CO_2 concentration in the head-space reaches ≥ 3 %. On the other hand, there is the possibility to get underestimated cumulative CO_2 and in turn carbon turnover due to the flushing. Also, the flushing can affect the measurements of the post flushing mineralization rates which can occur due to adjusting the equilibrium between partial pressure of CO_2 in the head space and the concentration of CO_2 in the soil solution. However, our soil with sandy texture and with a water holding capacity at 60 % of its field capacity (FC) was not affected as heavily as clay soils with higher water holding capacities.

In the incubation experiment, the substantial differences between the $\delta^{13}C$-signatures of the organic amendments, whose source was a C4 plant like maize, and those of the soil organic matter were used to identify the contribution of the different carbon sources (soil organic matter or organic amendment) to the overall released CO_2. Our amendments (digestate and biochar) had a significantly higher $\delta^{13}C$ value (-19.35 ‰) compared to the soil (-27.56 ‰) used for the incubation experiment. At the beginning of the incubation experiment, the $\delta^{13}C$ values of emitted CO_2 from the amended soil samples were significantly different from the control samples. But at the end of the experiment these differences had disappeared. This gives evidence that a labile fraction of the organic matter that was added to the soil as digestate and biochar was mineralized to CO_2, in particular at the beginning of the experiment.

In all treatments, the CO_2 emission went up just after adding the amendments, showing that carbon mineralization was triggered. In this initial phase, the treatment with the mixture of digestate and a high amount of biochar emitted significantly higher amounts of CO_2 than the digestate only and biochar only treatments.

After this initial phase, biochar had a negative effect on carbon mineralization in both carbon pools, the carbon in the amendments and the carbon in the soil organic matter pool. Some researchers found and discussed both positive and negative priming effects of biochar on the soil organic carbon pool (Knicker et al., 2008; Kuzyakov et al., 2009; Nguyen et al., 2008).

For each treatment, the measured values of produced CO_2 were highly variable between the three soil sub samples; therefore it is difficult to draw a definite conclusion based on those results. However, for each of the sub samples, the CO_2 emissions of the five different treatments basically showed the same behavior. Those three sub samples had differences in their TOC, TN and CEC, but those were not statistically tested for their significance because of lack of data points (Table A 11).

6.5.2 Influence of biochar and digestate on greenhouse gas emissions

Most studies leave the question of GHG emissions from biochar amended soils open, in particular under field conditions, but a few field measurements of GHG emissions from biochar amended soils have been performed (Castaldi et al., 2011; Karhu et al., 2011; Knoblauch et al., 2011; Scheer et al., 2011; Zhang et al., 2012a). In our experiments, we studied the GHG emissions from the soil surface after amending the sandy soil in a bio energy crop production system with biochar and digestate.

Our results showed that adding more carbon in the form of biochar to *Gleyic Podzol* did not increase the CO_2 emission from the soil surface compared to the control. In all seasons, there were no significant differences between the biochar treated plots and the control plots. The emission of CO_2 from *Gleyic Anthrosol* topsoil (0 - 0.2 m) also did not show significant differences, except just after adding biochar to the soil and in autumn 2013. Similar to the incubation experiment, just after adding biochar and in autumn 2013, the plots with a high amount of biochar emitted the significantly highest amount of CO_2, followed by the low amount of biochar plots and the digestate only plots. We also calculated the carbon turnover rates based on CO_2 emission and TOC in the topsoil layer (mg of CO_2 emitted per g of TOC per day). The results showed that the plots with biochar high amount had lower turnover rates compared to the digestate only and low biochar plots. In both soils CH_4 and N_2O emissions decreased after biochar application, but not significantly. Our results showed that it is necessary to study the emission of these two gases from soils in bioenergy production agro-ecosystems more intensively.

Earlier research mainly investigated the influence of biochar on GHG emissions and soil organic matter in soil ecosystems associated with co-metabolic reactions (Cheng et al., 2006; Hamer et al., 2004; Keith et al., 2011; Khodadad et al., 2011; Kuzyakov et al., 2009), and the positive priming effect together with enhanced microbial activity and labile fraction of C in biochar (Czimczik and Masiello, 2007; Wardle et al., 2008). However, most of those works were not done in the field and did not identify the different origins of carbon in the emitted CO_2 and (Lehmann and Sohi, 2008; Wardle et al., 2008).

6.5.3 Influence of biochar and digestate on soil organic carbon balance

After harvesting the 2013 and 2014 summer crops and the 2013 winter crop, we determined TOC in the different soil layers, and later compared the values with those obtained before starting the experiments. The TOC values of the uppermost layer varied with the growing season, probably due to the mineralization of carbon and its transport to lower soil horizons. In *Gleyic Anthrosol*, the lower horizons showed a higher accumulation of TOC in all plots with low and high biochar than in the digestate only plots. The TOC values of lower horizons nonsignificantly increased with time, and with the amount of biochar applied, but we did not analyze the contribution of biochar carbon to this TOC increase. Some authors who studied the stability of biochar in the soil showed that biochar in the subsurface soil layers was more stable than in the topsoil (Major et al., 2010a; Masiello, 1998).

6.6 Methodological limitations in the field experiment

6.6.1 Treatment mixtures and biochar application rates

Site 2 had three different treatments but there was no control treatment without amendment of digestate since the soil had already been enriched with digestate at a rate of 0.15 kg C m^{-2} (roughly 42 L m^{-2}, personal communication with the grower Mr. Kai Spangenberg). Therefore, for *Gleyic Anthrosol* in site 2 the digestate only treatment (D) was used as a basic reference plot.

For our study, two biochar application rates were used: 0.15 and 0.75 kg C m^{-2}, which means roughly 5 and 24 t biochar ha^{-1} respectively. *Gleyic Anthrosol* received both 5 and 24 t biochar ha^{-1}, whereas *Gleyic Podzol* received 5 t biochar ha^{-1} only. As an experiment which was conducted as part of a grower's field, we determined the application rates based on the organic carbon input rate from the grower's application of digestate which was 0.15 kg C m^{-2}. This is the limit allowed by authorities in SH and Germany as regulated by *DüMG 1977* (§1 and §2) (Dittrich, 2006; Domínguez, 2012; Schneider and Mastel, 2008b). Our application rates were low (5 and 24 t biochar ha^{-1} corresponding to 1.5 and 7.5 t C ha^{-1}) compared to the application rates used by previous studies (0.38 - 135 t C ha^{-1} for a pot experiment by Lehmann and

Rondon in 2006; 8 - 30 t C ha^{-1} for a field experiment by Lehmann and Rondon in 2006 and Lehmann et al. in 2006).

According to Haefele et al. (2011) and Lehmann and Joseph (2009), low input rates of biochar allow studying the effects on biomass production together with other effects on the ecosystem. The effect of biochar on crop yield is one of the important aspects when developing recommendations on how to improve the sustainability of agricultural production ecosystems. On the other hand, the low application rates can aggravate the evaluation of the effect of biochar on the terrestrial carbon sequestration of the soil ecosystem (Haefele et al., 2011; Lehmann et al., 2006).

6.6.2 Carbon losses during the application

The biochar used for this study had a low bulk density of 0.49 gcm^{-3} and contained a considerable amount of dust particles. Therefore, the biochar could very easily be blown away by the wind while it was lying on the soil surface before being incorporated into the surface soil layer (0 - 0.2 m). There were significant and high losses of TOC during the application, especially with the high biochar application rate in site 2 where there is no wind barrier along the site. The efficiency and practicability of the field application of biochar are a rarely investigated area in biochar experiments. There were some experiments which studied the effectiveness of solo application, mixed application with compost, animal manure or mineral fertilizer, but very few discussed the effectiveness and practicability of different types of incorporation such as deep vs. surface and banded vs. surface application, or the technical and economic feasibility concerning losses during the application, or safety concerns (Blackwell et al., 2009).

6.6.3 Chamber measurements

The closed chamber is the most commonly and frequently used method in gas emission studies to determine the gas exchange between soil surface and atmosphere. But chamber measurements are greatly influenced by many parameters and sometimes underestimate fluxes. There were several improvements in the chambers which were used for our measurements and flux calculations to minimize the errors associated with the chamber measurements (Davidson et al., 2002; LI-COR Biosciences, 2007; Pihlatie et al., 2013; Xu et al., 2006). In our experiments, the measured concentrations of N_2O and CH_4 were small. Those small concentrations may alter easily during the time between air sample collection from the chamber head space and the laboratory measurements. Those limitations could have some influence on the assessment of the fluxes and the variability of different treatments.

7. Conclusion and Outlook

This study has advanced the understanding of some basic aspects of the soil-plant-biochar interactions after amending soil in bio energy plantation ecosystems in SH, Germany with biochar produced from anaerobic biogas digestate. We used two field sites with different soil types, *Gleyic Podzol* and *Gleyic Anthrosol* which have different physico-chemical attributes and management conditions to investigate the potential benefits of biochar amendment for bioenergy crop production, soil nutrient recycling, climate change mitigation and reduction of greenhouse gas emissions from arable soils.

The soil chemical properties pH, EC, TOC, TN, DOC, DON, inorganic nitrogen, cation concentration, CEC, and plant available-P and -K increased after applying biochar. The positive effects on TOC, inorganic nitrogen, CEC, and plant available-P and -K were significant and remained so over four consecutive growing seasons. Also, biochar significantly increased the microbial growth in the soils. The additional carbon which was received from the biochar increased greenhouse gas emissions only slightly, but this small increase was nonsignificant. The incubation experiment neither showed a clear increasing nor a clear decreasing effect of biochar on the mineralization of the soil organic carbon pool.

The results obtained from this study showed that a single application of biochar from biogas digestate at low rates increased the crop biomass production in both *Gleyic Podzol* (without inorganic fertilizer) and *Gleyic Anthrosol* (with minimal application of inorganic fertilizer) during four consecutive growing seasons. This biomass increase can be explained as a synergy of the effects of biochar on the nutrient concentrations and availability in the soil, which are further increased by the high surface area and porous nature of the biochar which give it the capacity to act as a nutrient holder and medium for microorganisms.

The size of the plots and their position inside the grower’s field were chosen in the best possible way, but there were some unexpected external disturbances which affected the quality of the soil samples a few times and also affected plant growth of some plots.

Those effects could only be noticed after the data were statistically analyzed long after the plot establishment.

While already providing many insights into how biochar can reduce GHG fluxes from arable fields and improve the sustainability of bio energy production agro-ecosystems, our study also raises a set of questions to be answered in the future, and thus opens new room for further research. Additional field and laboratory studies on both short- and long-term basis are needed to answer those questions and further deepen the understanding before being able to gain those benefits at a commercial level and on a large scale.

Outlook: Need for future research

There are a number of areas concerning the application of biochar or charred digestate to soil in bio energy production agro-ecosystems which still require further investigation:

- Find out the contribution of different carbon pools to emitted CO_2 under field conditions which are more complex than in an incubation experiment, in order to gain insight into soil-plant-biochar interactions in a more realistic situation.
- Deepen the knowledge about gas emissions. Our study showed interesting data about the emission of N_2O and CH_4 under field conditions, but those need further clarification to be able to draw valid conclusions. Therefore, future experiments should collect a more representative and extensive set of data with more frequent measurements under field conditions.
- Study the influence of the application of biochar on the energy value of biomass in bioenergy crop production systems. This requires interdisciplinary studies and needs to be done before using biochar in commercial agriculture on a large scale.
- Investigate the impact of biochar on the quality of DOC. This research is important to gain the understanding which is necessary for predicting the effects of biochar on the ground water. Also, it is important to evaluate the potential risks associated with the sorption capacity of biochar and certain organic compounds

such as pesticides which are often used in bioenergy crop production agro-ecosystems.

- Deepen the knowledge about soil microbial activities, focusing on diversity and population changes, in particular the changes of ectomycorrhizal fungi.
- Design a more realistic field lysimeter experiment with undisturbed soil which completely covers all the pathways in which biochar can get lost after adding it to the ecosystem, with the goal of developing a model which can be used in future studies.

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Annex

List of Figures in Annex

List of Tables in Annex

Figure A 1: In October 2012: (A) mixing biochar with the soil using a single axle tractor, (B) digestate only plot, (C) digestate:biochar 1:1 plot and (D) digestate:biochar 1:5 plot

Figure A 2: In October 2012 just after winter wheat establishment (A) Site 1 with *Gleyic Podzol* and (B) Site 2 with *Gleyic Anthrosol*

Figure A 3: Vegetative stage of winter season crops: (A) winter rye in April 2014 and (B) winter wheat in April 2013

Figure A 4: Vegetative stage of summer season crops: maize (A) in June 2013 and (B) in June 2014

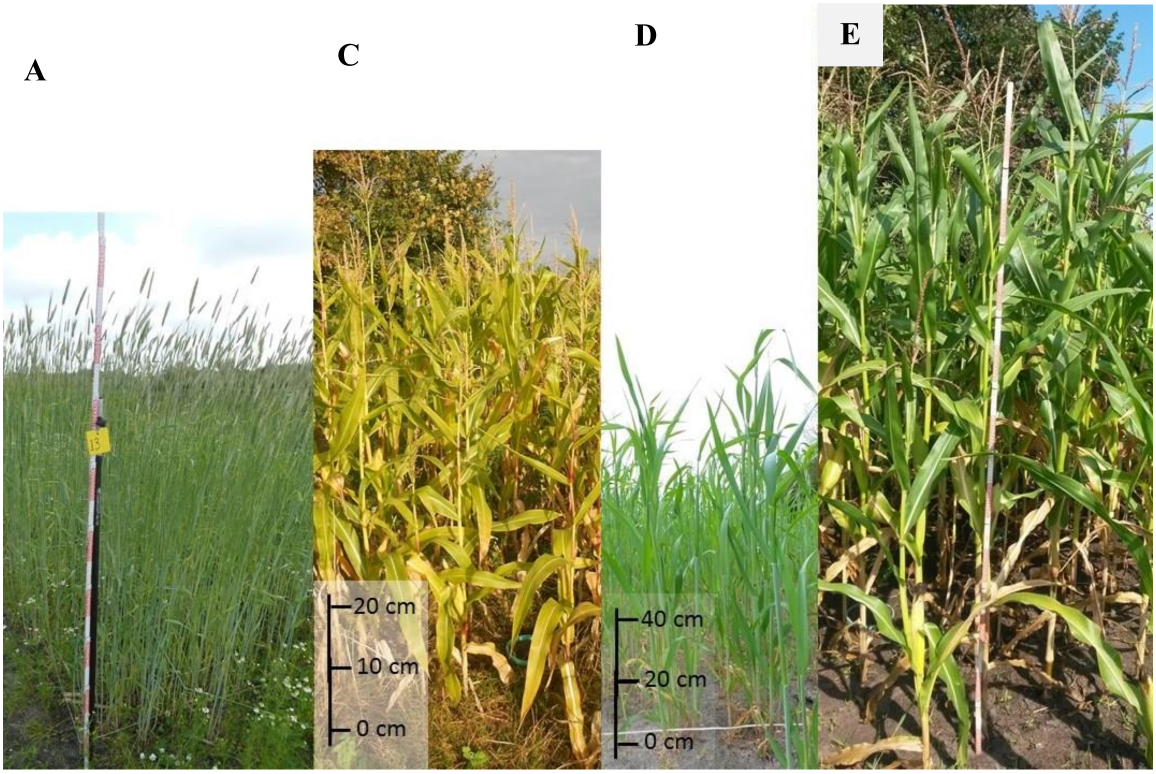

Figure A 5: At harvest time, winter and summer crops: (A) winter wheat in June 2013, (B) maize in October 2013, (C) winter rye in May 2014 and (D) maize in August 2014

Table A 1: The profile description sheet (Aufnahmeformblatt) for *Gleyic Podzol*

Aufnahmeformblatt für bodenkundliche Profilaufnahmen nach KA 5 (mit Seitenzahlenangabe)

Standort: Süderstapel Grünlandfläche von Bauer Spangenberg

Titeldaten	TK-Nr. **55**	Projekt, **55**	Spez.	Profil-Nr., **55**	Datum	Bearbeiter, **55**	Rechtswert, **55**	Hochwert, **55**	Höhe über NN	Aufschl.Art **55,56**	Bemerkungen, **55**
	1620	Dock. Arbeit	-------	S1	15.05.2012	R. Suddapuli	3514181	6025227	7m	GG	---------------

Aufnahmesituation	Neigung **57,58**	Exposition **59**	Wölbung **59**	Reliefformtyp **63**	Mikrorelief **69**	Lage im Relief **69**	Bodenab-/-auftrag **69**	Nutzungsart **71**	Vegetation **73**	Witterung **74**	anthropogene Veränderungen **74**	Bodenorganismen **76**	Bemerkungen **79**
	N 1	NW-SE	WS 2	H	RE	M	----	G	GR	WT 2	O	Lu 2	----------------

Horizontdaten

Lfd Nr.	Horizont/Schicht Ober-/Unter grenze (cm) **79**	Horizontsymbol **83**	**Pedogene und biogene Merkmale**											**Merkmale der Substratzusammensetzung**						
			Bodenart/Torfart **141,148, 157**	Bodenfarbe **108**	Humusgehalt **110**	Hydromorphiemerkmale **112**	Bodenfeuchte **114**	sonstige Merkmale **114**	Gefügeform und Größe **117,121**	Hohlräume (Risse, Poren Röhren) **123**	Lagerungsdichte Zersetzungsstufe **124**	Durchwurzelungsintensität **129**		Substratgenese **135**	Grobbodenfraktionen und Anteilklassen **148**	Summe Skelett (%) **150**	Carbonatgehalt **168**	Bodenausgangsgestein **172**	Stratigraphie **187**	Bemerkungen
1	0 - 20	Ap	Ss	10YR 3/1	h3	--------	feu 2	-----	ein	gri4 f2	Ld2	Wf3 Wg1		pfl	mGf4	5 - 10 %	c0	Sp	qh/qp	-----
2	20 - 55	Go-Bhs	Su2	10YR 5/8	h1	ed f3	feu 2	-----	ein	gri2 f2	Ld1	Wf1		pky	mGf3	5 %	c0	Sgf	qp	-----
3	55 - 65	II Bhs	Su4	5YR 5/2	h3	eh f2	feu 3	-----	ein-kit	gri2 f1	Ld1	W0		fg	0	0 %	c0	Sgf	qp	-----
4	> 65	Cv	Su3	10YR 6/4	h0	eh f1	feu 3	-----	ein-sub	gri2 f2	Ld1	W0		fg	0	0 %	c0	Sgf	qp	-----

Bodensystematische Einheit 190 BB-PP	**Substratsystematische Einheit 132,289,296** pfl/pky-(v)ls(Sp-qp)/fg-ss(Sgf-qp)
Bodenform BB-PP: pfl/pky-(v)ls(Sp-qp)/fg-ss(Sgf-qp))	**Kurzbeschreibung der wichtigsten Prozesse** - Humusanreicherung, verbraunung und vergleyung
Humusform 298 (vmu)	Wasserstand u GOF, **310** / Bemerkungen, **320**

Table A 2: The profile description sheet (Aufnahmeformblatt) for *Gleyic Anthrosol*

Aufnahmeformblatt für bodenkundliche Profilaufnahmen nach KA 5 (mit Seitenzahlenangabe)

Standort: Süderstapel Grünlandfläche von Bauer Spangenberg

Titeldaten	TK-Nr. **55**	Projekt, **55**	Spez.	Profil-Nr., **55**	Datum	Bearbeiter, **55**	Rechtswert, **55**	Hochwert, **55**	Höhe über NN	Aufschl.Art **55,56**	Bemerkungen, **55**
	1621	Dock. arbeit	-------	S2	15.05.2012	R. Suddapuli	3511303	6024891	7m	GS	---------------

Aufnahme-situation	Neigung **57,58**	Exposition **59**	Wölbung **59**	Relieffor-mtyp **63**	Mikro-relief **69**	Lage-im Relief **69**	Bodenab-/-auftrag **69**	Nutzungsart **71**	Vegetation **73**	Witterung **74**	anthropogene Veränderungen **74**	Bodenorganismen **76**	Bemerkungen **79**
	N 1	N	WS 1	KH	RW	K	AY	A	HF	WT 4	DG, O	-----------------	Mais für Bio-gasanlage

Horizontdaten

Lfd Nr.	Hori-zont/ Schicht Ober-/ Unter grenze (cm) **79**	Hori-zontsymbol **83**	**Pedogene und biogene Merkmale**										**Merkmale der Substratzusammensetzung**						
			Boden-art/ Torfart **141,148, 157**	Boden-farbe **108**	Hu-mus-ge-halt **110**	Hydro-mor-phie-merk-male **112**	Bo-den-feuchte **114**	son-stige Merk-male **114**	Gefü-ge-form und Größe **117,121**	Hohl-räume (Risse, Poren Röhren) **123**	Lager-ungs-dichte Zer-setz-ungs-stufe **124**	Durchw-urzelun-gsintens-ität **129**	Substr-atgenese **135**	Grob-boden-frakt-ionen und Anteil-klassen **148**	Summe Skelett (%) **150**	Carbo-nat-gehalt **168**	Boden-aus-gangs-gestein **172**	Strati-graphie **187**	Bemer-kungen
1	0 - 20	Ap	Ss (mSfs)	10YR 2/1	(h4) h2	--------	feu 2	-----	sub-ein	gri4 f3	Ld1	Wf3	om	fGr1	< 2 %	c0	Yj - Sp	qh	----------
2	20 - 40	E	Ss (mSfs)	10YR 3/1	(h3) h2	--------	feu 2	teils sgb	sub-ein	gri4 f2	Ld2	Wf2	om	mG1	< 2 %	c0	Yj - Sp	qh	----------
3	40 - 70	GBv	Su2	5YR 3/2	h1	--------	feu 3	-----	kit-ein	gri2 f3	Ld3-Ld2	Wf0	pfl	fO2 mG2	2 - 10 %	c0	Sp	qp	----------
4	> 70	Gro	Ss (fSms)	7.5YR 5/8	h0	eh / rb	feu 5	-----	ein	gri2 f2	Ld3	Wf0	fg	--------	0 %	c0	Sgf	qp	----------

Bodensystematische Einheit 190 GG-YE	**Substratsystematische Einheit 132,289,296** om-(z1)ss(Yj-Sp-qh)/pfl-(k2/w2)ss(Sp-qp)//fg-ls(Sgf-qp)
Bodenform GG-YE: om-(z1)ss(Yj-Sp-qh)/pfl-(k2/w2)ss(Sp-qp)//fg-ls(Sgf-qp)	**Kurzbeschreibung der wichtigsten Prozesse** - Plaggendüngung (om)

Humusform 298 (mu)	Wasserstand u GOF, **310** GWO ˜75cm	Bemerkungen, **320**

Table A 3: **Variation of TOC and TN content in the top soil (0 - 0.2 m) in *Gleyic Podzol* and *Gleyic Anthrosol* before and after biochar application. Shown are mean values and respective standard deviations (n = 3).**

Soil type	**Plot**	**TOC**	**TN**
		kg m^{-3}	
Gleyic Podzol	1	1.08 ± 0.00	0.10 ± 0.001
	3	1.44 ± 0.05	0.13 ± 0.001
	5	1.36 ± 0.05	0.13 ± 0.002
	2	1.39 ± 0.00	0.13 ± 0.002
	4	1.37 ± 0.05	0.13 ± 0.006
	6	1.27 ± 0.20	0.12 ± 0.019
	2B	1.75 ± 0.05	0.14 ± 0.004
	4B	1.85 ± 0.00	0.17 ± 0.002
	6B	1.69 ± 0.05	0.27 ± 0.022
Gleyic Anthrosol	7	1.69 ± 0.05	0.133 ± 0.030
	11	2.15 ± 0.10	0.149 ± 0.005
	13	1.90 ± 0.05	0.143 ± 0.005
	8	2.15 ± 0.05	0.155 ± 0.035
	12	2.25 ± 0.10	0.150 ± 0.005
	14	2.00 ± 0.10	0.152 ± 0.005
	8BL	2.27 ± 0.10	0.166 ± 0.015
	12BL	2.31 ± 0.15	0.150 ± 0.005
	14BL	2.28 ± 0.15	0.155 ± 0.010
	9	2.39 ± 0.00	0.216 ± 0.015
	10	2.00 ± 0.20	0.161 ± 0.020
	15	2.20 ± 0.15	0.171 ± 0.025
	9BH	2.91 ± 0.25	0.201 ± 0.030
	10BH	3.48 ± 0.00	0.156 ± 0.020
	15BH	2.95 ± 0.20	0.174 ± 0.010

B: biochar, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5.

Table A 4: **Selected soil chemical properties in the topsoil (0 - 0.2 m) for all the treatment plots before applying biochar for both *Gleyic Podzol* and *Gleyic Anthrosol*. Shown are mean values and respective standard deviations (n = 3).**

Soil type	Plot	DOC	DON	Ca^{++}	Mg^{++}	K^{+}	Na^{+}	CEC	BS
		g m^{-3}		mmol$_c$ kg^{-1}					%
GP	1	2.35 ± 0.5	0.45 ± 0.1	18.6 ± 1.5	1.85 ± 0.0	10.4 ± 0.5	0.60 ± 0.0	21.43 ± 1	42.7 ± 2
	2	2.05 ± 0.5	0.50 ± 0.1	19.1 ± 2.5	1.75 ± 0.0	10.5 ± 1.2	1.00 ± 1.0	22.23 ± 0	41.6 ± 3
	3	2.40 ± 0.5	0.60 ± 0.1	21.2 ± 3.0	1.90 ± 0.5	10.0 ± 2.0	0.30 ± 0.0	21.79 ± 1	45.6 ± 6
	4	2.45 ± 0.5	0.45 ± 0.1	32.2 ± 23	2.10 ± 0.5	9.40 ± 0.0	0.30 ± 0.0	22.07 ± 0	60.9 ± 33
	5	1.45 ± 0.0^{a}	0.40 ± 0.1	15.9 ± 4.0	2.00 ± 0.5	9.60 ± 3.0	0.25 ± 0.0	21.58 ± 1	37.0 ± 7
	6	1.95 ± 0.5	0.40 ± 0.0	16.9 ± 3.5	2.05 ± 0.5	9.70 ± 2.5	1.95 ± 2.5	22.18 ± 1	37.8 ± 6
GA	7	3.8 ± 0.5	1.10 ± 0.0	61.70 ± 5	3.25 ± 0.5	7.95 ± 1.0	0.60 ± 0.0	21.64 ± 1	114.6 ± 3
	8	2.4 ± 0.5	1.35 ± 0.0	83.50 ± 5	3.95 ± 0.5	8.50 ± 0.5	1.00 ± 1.0	27.09 ± 1	121.9 ± 4
	9	3.0 ± 0.5	0.95 ± 0.5	102.6 ± 5	5.70 ± 0.5	8.85 ± 1.0	0.30 ± 0.0	33.88 ± 2	119.4 ± 4
	10	2.1 ± 0.0	1.05 ± 0.0	56.95 ± 10	2.65 ± 0.5	6.95 ± 0.5	0.30± 0.0	22.67 ± 1	99.2 ± 13
	11	1.7 ± 0.0 ^{a}	0,95 ± 0.0	65.4 ± 10	3.40 ± 0.5	8.40 ± 1.0	0.25 ± 0.0	24.27 ± 3	107.6 ± 7
	12	2.1 ± 0.5	0.85 ± 0.0	60.3 ± 10	2.85 ± 0.5	7.30 ± 2.0	1.95 ± 2.5	24.77 ± 2	98.4 ± 7
	13	1.8 ± 0.0 ^{b}	1.05 ± 0.0	49.6 ± 10	2.85 ± 0.5	13.8 ± 0.5	0.20 ± 0.0	20.16 ± 1	105.6 ± 11
	14	2.0 ± 0.0	0.85 ± 0.5	58.2 ± 5	3.30 ± 0.5	12.7 ± 1.0	0.55 ± 0.0	23.10 ± 1	105.1 ± 5
	15	2.1 ± 0.0	0.95 ± 0.0	63.1 ± 10	2.80 ± 0.5	13.3 ± 1.0	0.65 ± 0.0	24.41 ± 1	106.4 ± 11

For a chemical property, values followed by the different letters are significantly different at *$P \leq 0.05$*. Letters are not shown when differences of means are nonsignificant. GP: *Gleyic Podzol*, GA: *Gleyic Anthrosol*.

Table A 5: Changes of selected soil chemical properties in the topsoil (0 - 0.2 m) for all treated plots just after applying soil amendments (biochar, and mixtures of digestate and biochar). Shown are mean values and respective standard deviations (n = 3).

Soil Type	Plot	DOC	DON	Ca	Mg	K	Na	CEC	BS
		g m^{-3}		mmol$_c$ kg^{-1}					%
GP	2B	2.80 ± 0.5	0.46 ± 0.1	32 ± 1.5	13.7 ± 2	34.4 ± 6	0.2 ± 0	26.26 ± 1^{a}	92.3 ± 5
	4B	3.55 ± 0.5	0.55 ± 0.1	26 ± 3.0	12.3 ± 6	30.7 ± 11	0.55 ± 0	24.69 ± 1^{b}	83.5 ± 23
	6B	3.40 ± 0.5	0.45 ± 5.1	26 ± 1.5	12.4 ± 2	31.3 ± 1	0.65 ± 0	25.05 ± 1^{ab}	83.2 ± 5
GA	8BL	3.35 ± 1.5	0.55 ± 0.0	84 ± 10^{a}	11.2 ± 3	23.2 ± 10^{a}	1.8 ± 0.1^{a}	32.26 ± 1	124.2 ± 18^{a}
	12BL	2.70 ± 0.5	0.60 ± 0.0	63 ± 5^{b}	7.95 ± 2	102.1 ± 55	6.25 ± 0.6	47.36 ± 4^{a}	169.5 ± 32
	14BL	3.50 ± 0.5	0.70 ± 0.0	71 ± 15^{ab}	11.9 ± 3	106.8 ± 40	6.40 ± 0.4	35.18 ± 5	184.4 ± 31
	9BH	5.45 ± 3.5^{b}	0.65 ± 0.0	104 ± 5^{a}	43.5 ± 22	19.3 ± 3.5	1.90 ± 0.5	27.25 ± 2	111.5 ± 5
	10BH	8.10 ± 0.5	0.80 ± 0.0	64 ± 10	39.9 ± 15	35.1 ± 7	1.70 ± 0.5	28.54 ± 3	132.9 ± 13
	15BH	8.65 ± 1.0	0.90 ± 0.0	68 ± 5	38.4 ± 9	101 ± 25^{a}	5.20 ± 1^{a}	40.26 ± 3^{a}	160.7 ± 16^{a}

For a chemical property, values followed by the different letters are significantly different at *$P \leq 0.05$*. Letters are not shown when differences of means are nonsignificant. GP: *Gleyic Podzol*, B: biochar, GA: *Gleyic Anthrosol*, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5.

Table A 6: Seasonal variation of soil moisture and EC in the topsoil (0 - 0.2 m) in site 1 and site 2 after establishment of the experiment in October 2012. Shown are mean values and the respective standard deviations (n = 3 to 9).

	Soil amendments				
	Site 1		Site 2		
	C	B	D	BL	BH
Soil Moisture (%)					
Winter 2012/13	18.8 ± 9	20.6 ± 8	19.3 ± 4	20.2 ± 6	20.9 ± 9
Spring 2013	16.9 ± 5	16.4 ± 8	13.1 ± 8	14.2 ± 4	14.9 ± 7
Summer 2013	8.2 ± 3	7.0 ± 2	8.6 ± 4	8.5 ± 3	8.3 ± 6
Autumn 2013	13.6 ± 3	16.3 ± 3	13.0 ± 3	12.5 ± 2	13.9 ± 1
Winter 2013/14	22.8 ± 2	23.6 ± 1	17.0 ± 1	17.8 ± 3	19.4 ± 1
Spring 2014	16.7 ± 4	18.4 ± 1	13.2 ± 3	12.2 ± 1	13.8 ± 1
Summer 2014	11.6 ± 1	10.6 ± 1	7.1 ± 1	8.9 ± 2	9.1 ± 5
EC* (µS cm^{-1})					
Winter 2012/13	29.3 ± 5	40.8 ± 17	43.2 ± 7	49.9 ± 23	60.8 ± 14
Spring 2013	18.3 ± 3	23.7 ± 4	33.3 ± 15	35.3 ± 5	73.3 ± 15^{a}
Summer 2013	32.2 ± 19	41.9 ± 23	41.7 ± 8	44.9 ±1 6	59.9 ± 15^{a}
Autumn 2013	16.6 ± 5	19.3 ± 5	34.5 ± 10	32.2 ± 7	44.7 ± 17
Winter 2013/14	16.1 ± 4	23.2 ± 7	24.5 ± 2	23.4 ± 1	31.5 ± 17
Spring 2014	9.6 ± 7	9.5 ± 6	10.8 ± 7	12.8 ± 8	13.2 ± 17
Summer 2014	60.0 ± 2	60.0 ± 13	80.3 ± 15	74.0 ± 3	78.3 ± 15

* H_2O suspension. For each field site, values followed by the different letters are significantly different at *$p \leq 0.05$*. Letters are not shown when differences of means are nonsignificant. C: control, B: biochar, D: digestate only, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5

Table A 7: Seasonal variation of DOC and DON in the topsoil (0 - 0.2 m) in site 1 and site 2 after establishment of the experiment in October 2012. Shown are mean values and the respective standard deviations (n = 3 to 9).

	Soil amendments				
	Site 1		Site 2		
	C	B	D	BL	BH
DOC (mg kg^{-1})					
Winter 2012/13	9.0 ± 6	9.2 ± 8	10.6 ± 10	8.2 ± 7	10.2 ± 10
Spring 2013	13.7 ± 5	13.3 ± 6	13.1 ± 8	12.8 ± 6	20.2 ± 10
Summer 2013	12.0 ± 9	11.8 ± 5	14.7 ± 6	11.9 ± 4	10.9 ± 8
Autumn 2013	7.5 ± 3	6.7 ± 2	9.2 ± 2	10.1 ± 2	9.9 ± 2
Winter 2013/14	8.8 ± 5	8.9 ± 2	7.8 ± 1	8.3 ± 1	7.9 ± 3
Spring 2014	9.6 ± 6	9.3 ± 6	11.9 ± 7	11.1 ± 8	11.8 ± 3
Summer 2014	15.2 ± 0	15.9 ± 1	17.6 ± 3	16.6 ± 2	16.1 ± 5
DON (mg kg^{-1})					
Winter 2012/13	0.7 ± 1	1.0 ± 1	1.3 ± 1	1.1 ± 1	1.4 ± 1
Spring 2013	2.6 ± 1	2.5 ± 1	3.7 ± 2	3.3 ± 1	3.5 ± 1
Summer 2013	1.4 ± 0	2.8 ± 2	2.2 ± 1	2.3 ± 1	2.5 ± 1
Autumn 2013	1.3 ± 1	1.4 ± 1	1.8 ± 0^{a}	2.5 ± 1^{b}	2.5 ± 0^{b}
Winter 2013/14	0.8 ± 1	1.0 ± 0	0.9 ± 0^{a}	1.1 ± 0^{ab}	1.6 ± 1^{b}
Spring 2014	2.1 ± 1	2.1 ± 1	2.7 ± 1	2.4 ± 1	2.8 ± 1
Summer 2014	3.3 ± 1	3.4 ± 1	3.2 ± 1	2.8 ± 0	2.9 ± 0

For each field site, values followed by the different letters are significantly different at *$p \leq 0.05$*. Letters are not shown when differences of means are nonsignificant. C: control, B: biochar, D: digestate only, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5.

Table A 8: Seasonal variation of ammonium nitrogen and nitrate nitrogen in the topsoil (0 - 0.2 m) in *Gleyic Podzol* and *Gleyic Anthrosol* after establishment of the experiment in October 2012. Shown are mean values and the respective standard deviations (n = 3 to 9).

	Soil amendments				
	Site 1		**Site 2**		
	C	**B**	**D**	**BL**	**BH**
NO$_3$ - N (µmol Nitrate 100 g of dry soil^{-1})					
Winter 2012/13	45.48±13^a	106.45±26^b	415.11±26^a	444.26±52^a	651.31±262^b
Spring 2013	28.45±10^a	42.39±14^b	55.88±48^a	85.61±25^a	205.03±66^b
Summer 2013	27.62±7^a	67.89±10^b	128.19±30^a	93.81±38^b	146.30±33^a
Autumn 2013	18.77±8^a	51.28±13^b	150.44±76^a	138.70±34a^b	194.98±39^b
Winter 2013/14	19.38±3	27.83±0	41.00±4^a	83.74±15^a	145.88±37^b
Spring 2014	27.98±6^a	48.65±13^b	68.68±37^a	89.81±19^a	187.31±34^b
Summer 2014	12.60±2^a	47.47±10^b	119.38±10^a	140.12±26^a	195.76±45^b
NH$_4$ - N (mg kg^{-1})					
Winter 2012/13	2.64±2.1	4.91±3.3	4.55±2.4	3.96±2.0	4.61±1.9
Spring 2013	1.88±0.5	2.68±2.0	2.27±2.6	1.97±0.9	2.05±0.9
Summer 2013	1.67±0.6^a	3.76±1.8^b	1.98±1.0	2.75±2.1	2.59±1.5
Autumn 2013	1.03±0.4	1.12±0.5	1.87±0.6	1.50±0.2	1.79±0.3
Winter 2013/14	0.96±0.8	0.89±0.7	0.56±0.0	0.72±0.3	0.60±0.2
Spring 2014	1.95±0.6	2.62±1.0	2.26±0.7^a	1.94±1.1^a	3.37±0.4^b
Summer 2014	2.06±1.0	3.28±0.2	2.23±0.4	1.32±0.5	1.72±0.8

For each soil type, values followed by the different letters are significantly different at $P \leq 0.05$. Letters are not shown when differences of means are nonsignificant. C: control, B: biochar, D: digestate only, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5

Table A 9: **Seasonal variation of CEC and BS in the topsoil (0 - 0.2 m) in *Gleyic Podzol* and *Gleyic Anthrosol* after establishment of the experiment in October 2012. Shown are mean values and the respective standard deviations (n = 3 to 9).**

	Soil amendments				
	Site 1		**Site 2**		
	C	**B**	**D**	**BL**	**BH**
CEC (mmol$_c$ kg^{-1})					
Winter 2012/13	31.5±1^a	52.1±1^b	41.0±2^a	53.1±2^b	54.6±3^b
Spring 2013	51.7±3	58.8±10	35.6±1^a	53.2±1^b	54.2±1^b
Summer 2013	24.2±3	23.8±3	22.5±4^a	32.9±4^b	34.6±1^b
Autumn 2013	16.7±3	18.3±2	19.1±4^a	25.5±7^b	33.8±3^c
Winter 2013/14	21.5±2	23.8±2	22.3±5	24.0±2	30.3±8
Spring 2014	17.7±3	22.7±2	22.0±2^a	21.2±3^a	31.1±6^b
Summer 2014	20.9±2	21.7±3	23.2±1	22.8±3	29.2±5
BS (%)					
Winter 2012/13	14.8±3^a	25.7±1^b	43.6±8^a	49.1±5^a	87.9±12^b
Spring 2013	16.3±4	19.2±6	42.3±11	45.8±10	81.3±25
Summer 2013	32.0±10	38.4±11	78.7±13	68.4±17	73.8±17
Autumn 2013	27.7±4	31.6±6	67.3±25	60.8±24	55.8±15
Winter 2013/14	30.0±5^a	39.4±1^b	64.7±9	83.6±15	91.7±6
Spring 2014	36.1±4^a	51.4±6^b	75.7±5^a	93.8±3^b	98.8±0^b
Summer 2014	27.9±2^a	39.4±3^b	70.8±2^a	72.5±11^a	94.2±5^b

For each soil type, values followed by the different letters are significantly different at $P \leq 0.05$. Letters are not shown when differences of means are nonsignificant. C: control, B: biochar, D: digestate only, BL: digestate:biochar 1:1, BH: digestate:biocha r 1:5

Table A 10: **Seasonal variation of plant available-P and -K in the topsoil (0 - 0.2 m) in *Gleyic Podzol* and *Gleyic Anthrosol* after establishment of the experiment in October 2012. Shown are mean values and the respective standard deviations (n = 3 to 9) .**

	Soil amendments				
	Site 1		Site 2		
	C	**B**	**D**	**BL**	**BH**
DL-P (mg P_2O_5/100 g)					
Winter 2012/13	37.5±4.1	47.6±1.8	32.0±2.5^{a}	66.7±3.5^{b}	64.0±3.4^{b}
Spring 2013	33.6±2.5^{a}	43.2±3.4^{b}	30.2±2.5^{a}	64.8±3.5^{b}	62.6±3.4^{b}
Summer 2013	46.0±3.4^{a}	50.0±3.9^{b}	37.8±5.1^{a}	46.2±13.1^{b}	66.9±11.9^{b}
Autumn 2013	39.9±0.6^{a}	42.0±0.4^{b}	38.5±0.8^{a}	42.8±1.2^{b}	57.3±1.3^{c}
Winter 2013/14	30.2±2.8	32.6±2.6	20.8±1.7^{a}	22.8±3.6^{ab}	36.0±9.6^{b}
Spring 2014	24.3±2.3^{a}	35.3±3.2^{b}	20.3±1.5^{a}	22.7±5.4^{a}	38.2±10.1^{b}
Summer 2014	28.5±2.0^{a}	35.9±2.4^{b}	22.5±3.0^{a}	28.3±6.3^{a}	40.1±2.3^{b}
DL - K (mg K_2O/100g)					
Winter 2012/13	2.3±1.0^{a}	14.6±0.7^{b}	6.9±9.1^{a}	7.7±5.1^{a}	35.0±6.0^{b}
Spring 2013	10.9±8.1	8.4±1.5	6.2±1.8^{a}	8.4±2.6^{a}	25.9±2.2^{b}
Summer 2013	8.7±1.2^{a}	21.4±2.5^{b}	10.4±2.8^{a}	10.5±4.3^{a}	27.9±8.1^{b}
Autumn 2013	5.8±1.2^{a}	12.9±0.8^{b}	10.6±2.3^{a}	10.6±2.9^{a}	25.8±0.9^{b}
Winter 2013/14	8.0±1.1^{a}	14.3±2.5^{b}	8.2±0.8^{a}	8.4±0.4^{a}	17.8±3.7^{b}
Spring 2014	10.5±4.3	18.3±3.9	8.5±0.4^{a}	8.2±1.4^{a}	15.7±2.9^{b}
Summer 2014	8.0±1.2^{a}	11.3±1.5^{b}	11.8±1.7^{a}	12.6±0.7^{a}	17.4±1.0^{b}

For each soil type, values followed by the different letters are significantly different at $P \leq 0.05$. Letters are not shown when differences of means are nonsignificant. C: control, B: biochar, D: digestate only, BL: digestate:biochar 1:1, BH: digestate:biochar 1:5

Table A 11: Selected physico-chemical characteristics of the topsoil (0 - 0.2 m) in *Gleyic Podzol*.

Depth (cm)	Horizon denotation	Sand (%)	Silt (%)	Clay (%)	pH	EC (µS cm^{-1})	TC (%)	TN (%)	C/N	CEC (mmol$_c$ kg^{-1})	BS (%)	δ^{13}C (‰)
0 - 10	Ah	81.1	11.6	7.4	5.1	47	0.91	0.10	9.19	26.7	55	n.a.
10 - 35	rAp	80.5	12.5	7.1	5.1	47	0.69	0.08	8.33	27.6	423	n.a.
35 - 50	Ap-Bv	69.6	21.3	9.1	5.4	40	n.a.	n.a.	n.a.	35.1	84	n.a.
50 - 85	Bv-Go	81.8	10.3	7.9	5.7	40	n.a.	n.a.	n.a.	38.7	81	n.a.
85 - 110	IIGo1	87.2	7.7	5.1	6.3	38	n.a.	n.a.	n.a.	31.0	97	n.a.
110 - 145	Go2	75.6	16	8.4	6.6	57	n.a.	n.a.	n.a.	40.7	99	n.a.
> 145	Gro	88.1	8.7	3.2	6.6	31	n.a.	n.a.	n.a.	26.8	101	n.a.
0 - 20	*4394 (P1)	n.a.	n.a.	n.a.	n.a.	n.a.	1.35	0.13	10.10	26.6	71	-28.8
0 - 20	*4395 (P2)	n.a.	n.a.	n.a.	n.a.	n.a.	1.64	0.16	10.10	34.1	313	-28.8
0 - 20	*4627 (P3)	n.a.	n.a.	n.a.	n.a.	n.a.	0.85	0.09	9.47	24.3	61	-27.8

Source: Magdalina von Leliwa (von Leliwa, 2014)

*Sample notation, n.a: not analyzed

Table A 12: Isotope signature (‰ VPDB) of emitted CO_2 from the different treatments on different days for the aerobic incubation experiment (n = 3).

Sample	Treatment	δ^{13}C (‰)			
		Time (d)			
		16	54	93	133
GP-4394	C	-29.8 ± 0.2^{a}	-30.0 ± 0.3^{a}	-30.0 ± 0.1^{a}	-29.9 ± 0.1^{ac}
	D	-28.6 ± 0.3^{b}	-29.0 ± 0.2^{b}	-29.2 ± 0.2^{b}	-29.1 ± 0.1^{cb}
	B	-29.5 ± 0.2^{a}	-29.6 ± 0.1^{ab}	-29.6 ± 0.1^{ab}	-29.6 ± 0.1^{c}
	D:B 1:1	-28.5 ± 0.6^{b}	-28.8 ± 0.1^{ab}	-28.9 ± 0.5^{b}	-28.9 ± 0.4^{b}
	D:B 1:5	-28.2 ± 0.1^{b}	-28.9 ± 0.1^{b}	-29.0 ± 0.1^{b}	-29.0 ± 0.1^{c}
GP-4395	C	-29.8 ± 0.2^{a}	-29.9 ± 0.1^{a}	-29.2 ± 0.3	-29.1 ± 0.4^{b}
	D	-29.1 ± 0.2^{b}	-29.3 ± 0.3^{b}	-29.0 ± 0.1	-29.2 ± 0.2
	B	-29.7 ± 0.0^{a}	-29.7 ± 0.1^{ab}	-29.3 ± 0.1	-29.4 ± 0.1
	D:B 1:1	-29.0 ± 0.2^{b}	-29.2 ± 0.1^{b}	-28.9 ± 0.1^{a}	-29.2 ± 0.2
	D:B 1:5	-28.9 ± 0.1^{b}	-29.4 ± 0.1^{b}	-29.1 ± 0.0	-29.3 ± 0.0
GP-4627	C	-28.2 ± 0.0^{a}	-28.7 ± 0.0^{a}	-28.5 ± 0.0^{a}	-28.8 ± 0.2^{a}
	D	-26.6 ± 0.2^{b}	-27.2 ± 0.1^{b}	-26.8 ± 0.2^{b}	-27.0 ± 0.1^{b}
	B	-28.0 ± 0.1^{ac}	-28.6 ± 0.1^{a}	-28.0 ± 0.1^{c}	-28.4 ± 0.0^{c}
	D:B 1:1	-26.2 ± 0.2^{bd}	-27.0 ± 0.2^{b}	-26.8 ± 0.1^{b}	-27.1 ± 0.1^{b}
	D:B 1:5	-25.5 ± 0.3^{e}	-27.2 ± 0.3^{b}	-27.2 ± 0.2^{bd}	-27.3 ± 0.1^{b}

Values followed by the different letters are significantly different at $P \leq 0.05$. Letters are not shown when differences of means are nonsignificant. C: control, B: biochar; D: digestate only, D:B 1:1: digestate:biochar 1:1, D:B 1:5: digestate:biochar 1:5

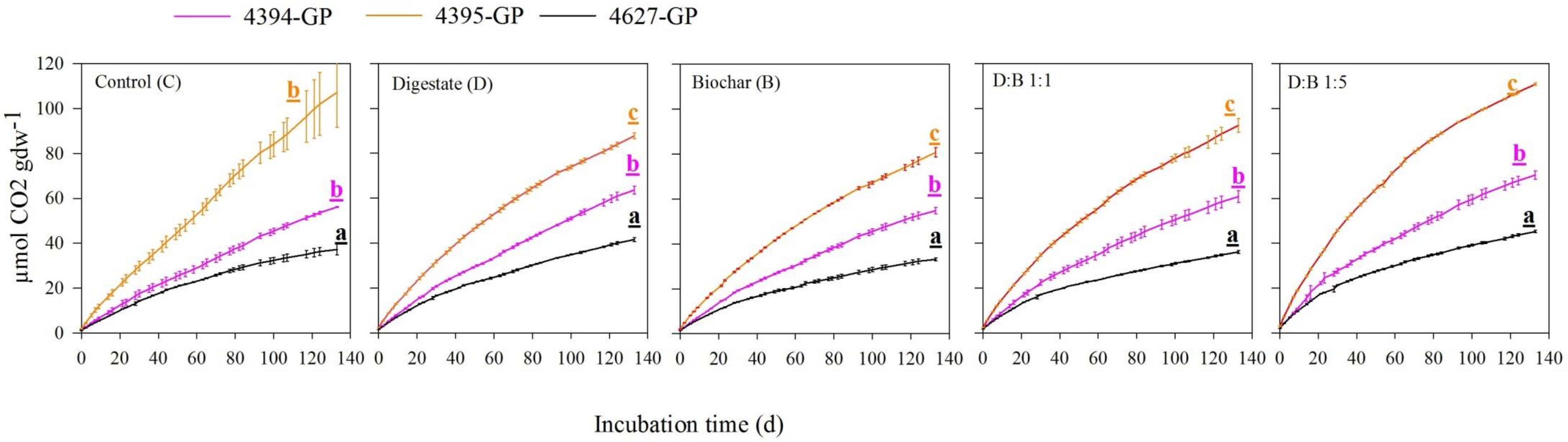

Figure A 6: Cumulative CO$_2$ production during an aerobic soil incubation experiment of *Gleyic Podzol* amended with biochar, digestate, biochar digestate 1:1 and 1:5 mixtures and without any amendments (Control). The vertical error bars represent ± one standard deviation of the mean (n = 3). The letters close to the respective graphs show significant differences of the cumulative CO$_2$ production between the different soil sub samples (von Leliwa, 2014).