N_2O fluxes as affected by biochar in two soils with contrasting texture and pH

Insights during a wet-dry and freeze-thaw cycle

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Abstract

Nitrous oxide (N_2O) is a potent greenhouse gas and the dominant ozone-depleting substance of the 21st century. Agriculture has been responsible for approximately 74% of anthropogenic N_2O emissions in the 2010s. Large fractions of annual N_2O fluxes occur during short periods with high emissions ("hot moments") such as wet-dry cycles (WDC) and freeze-thaw cycles (FTC), the latter remaining poorly represented in current IPCC emission factors. Biochar amendments have shown mitigation potential for soil N_2O emissions, yet the dependency of this effect on soil type, fertilizer type, biochar dose, and environmental conditions remains insufficiently understood.

This laboratory mesocosm study investigated the effects of woodchip biochar applied at four rates (NBC=0, BC1~6, BC2~15, and BC3~24 t ha^{-1}) on N_2O emissions from a clayey soil (pH 5.7) and a sandy loam (pH 7.1). The soils were fertilized with either synthetic NPKS (Nitrogen, phosphorous, potassium & sulphur) fertilizer or dairy slurry manure. Emissions were measured for 58 days at high temporal resolution using a photoacoustic gas spectrometer (INNOVA 1312) under controlled temperature conditions (15°C). Samples were exposed to one WDC at 70% water-filled pore space (WFPS) followed by one FTC at 80% WFPS (−22°C for 7 days).

During the WDC, biochar significantly reduced cumulative N_2O emissions in the clayey soil, with BC2 reducing emissions under NPKS fertilization by 77% and BC3 reducing emissions under manure by 65%. No significant reductions were observed in the sandy loam, potentially linked to its higher pH, lower organic carbon content, and greater within-treatment moisture variability due to observed soil settling. During the FTC, emissions were substantially higher than during the WDC, accounting for 93.6% of total cumulative emissions in the clay soil and 74.7% in the sandy loam. No significant biochar-induced reductions were observed during the FTC alone, although consistent trends suggested potential effects obscured by high variability and limited replication. Over the full experimental duration, BC2 significantly reduced cumulative N_2O emissions for the manure in the clayey soil by 52.8%.

These findings highlight the importance of soil physicochemical properties (particularly pH, texture, and carbon content) in determining biochar's N_2O mitigation efficacy. The dominance of FTC-induced emissions underscores the need for dedicated studies on biochar effects during freeze-thaw events, incorporating mechanistic measurements to guide future mitigation strategies.

Keywords: nitrous oxide, biochar, freeze-thaw cycle, wet-dry cycle, denitrification, greenhouse gas mitigation

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Abbreviations

Abbreviation	Description
C	Carbon
CO_2	Carbon Dioxide
Corg	Organic carbon
EMMs	Estimated marginal means
FTC	Freezing – thawing cycle
H	Hydrogen
K	Potassium
N	Nitrogen
$N\text{-}N_2O$	Nitrogen bound to nitrous oxide
N_2O	Nitrous Oxide
NH_2OH	Hydroxylamine
NH_3	Ammonia
NH_4	Ammonium
NO	Nitric oxide
NO_2	Nitrite
NO_3^-	Nitrate
NPKS	Nitrogen, phosphorous, potassium, sulphur fertilizer
P	Phosphorous
ppb	Parts per billion
SLU	Sveriges Lantbruksuniversitet
SSA	Specific surface area
WDC	Wet – dry cycle
WFPS	Water-filled pore space

1. Introduction

Nitrous oxide (N_2O) is a long-lived greenhouse gas with an atmospheric lifetime of more than 100 years (Prather et al., 2012). Anthropogenic impacts have caused an increase from 270 to 336 parts per billion (ppb) between 1750 and 2022. 35 ppb of that increase occurred since 1980 under increasing emission rates (Tian et al., 2024). With a global warming potential for 100 years of 273 N_2O has been responsible for about 10% of the reported increase in global temperature (IPCC, 2023). Additionally, N_2O is regarded as the dominating ozone depleting substance of the 21st century (Ravishankara et al., 2009). Agriculture is responsible for a large share of anthropogenic N_2O emissions, causing 74% of the emissions in the 2010s (Tian et al., 2024).

Soil-associated N_2O emissions mainly originate from microbial nitrification and denitrification processes which are governed by biological, chemical and physical conditions in the soil (Kuypers et al., 2018). Emissions are primarily induced globally by an increased substrate availability for these processes through anthropogenic fertilizer application (Firestone & Davidson, 1989; Tian et al., 2019). This effect has been considered by the IPCC in emission scenarios but in the past years it has become apparent that average values tend to be too low and more spatial and temporal variability should be considered (Tian et al., 2020).

Large shares of annual N_2O emissions occur during "hot moments" such as wet-dry (WDC) and freezing thawing cycles (FTC) which are characterized by short term emission increases (Wagner-Riddle et al., 2020). As these "hot moments" are highly dynamic, high measurement frequency is necessary to achieve reliable results (Barton et al., 2015; Wagner-Riddle et al., 2017). Additionally, due to the high spatial variability, multiple replicates are necessary to be able to get reliable, meaningful results (Morris et al., 2013).

Since agriculture is a large contributor to the global anthropogenic N_2O emissions (Tian et al., 2024), targeted nitrogen management strategies try to minimize N_2O losses from agroecosystems. Among others, the use of nitrification inhibitors, adapted crop rotations and the application of biochar have shown to mitigate N_2O emissions from soils (Hassan et al., 2022).

Biochar is a product of pyrolysis of biomass, which is intended for soil application or other environmental management interventions. Biochar can be produced from various materials and under different temperatures, resulting in different chemical and physical properties. It is characterized by a high porosity, alkaline pH, a high specific surface area (SSA) and a highly crystalline structure (Joseph et al., 2021). Biochar produced from wood-based feedstock has been shown to have the highest SSA and to show the highest potential to change soil physical characteristics in comparison to other feedstock types (Ippolito et al., 2020).

Biochar has shown to affect soil conditions and microbial pathways to mitigate N_2O emissions (Borchard et al., 2019; L. Zhong et al., 2025). It was observed that biochar promotes the last step of denitrification through an increase in pH and a so-called “electron shuttle”, facilitating the microbial process. Also, reduced substrate availability through adsorption of nitrate (NO_3^-) and ammonium (NH_4^+) has been observed (Cayuela et al., 2013). This mitigating effect of biochar on N_2O emissions has been summarized in global meta-analyses with reductions ranging from 19 to 38 % (e.g. Borchard et al., 2019; Q. Liu et al., 2018; L. Zhong et al., 2025). However, large differences between soil, fertilizer and biochar types remain and the exact underlying processes to give reliable predictions are not yet fully understood (Joseph et al., 2021). The knowledge of the effects of biochar on N_2O during freezing - thawing cycles is limited and no real mechanistic studies or meta-analyses exist.

Two different soils were either fertilized with a synthetic NPKS fertilizer (containing nitrogen, phosphorous, potassium and sulphur), dairy manure or no fertilizer (control) and amended with three different doses of woodchip biochar or served as the control without biochar. This experimental design was chosen to determine a dose effect of biochar and dependencies on soil and fertilizer types. Emissions were measured at a high temporal frequency with five replicates per treatment to tackle the high temporal and spatial dynamics of N_2O emissions. The experiment was carried out at a controlled temperature of 15°C. During the freezing, the temperature was -22°C for 7 days. All samples were exposed to one WDC at 70% water-filled pore space (WFPS) and after to one FTC 80% WFPS.

The aim of the experiment was to add to the understanding of the role of biochar mitigating N_2O emissions and the interaction with soil and fertilizer type during freeze-thaw events. An increased understanding would allow to draw conclusions on reliable biochar application for N_2O emission mitigation.

2. Literature review

The literature review is structured in two sections: First, nitrous oxide (N_2O) emissions from soils, important processes and influencing factors are described (2.1). Then these processes are linked to biochar properties and proposed pathways on how biochar mitigates N_2O emissions from soils are presented (2.2). Lastly, the defined knowledge gaps are summarized and connected to the experimental design of the present thesis (2.3).

2.1 Nitrous Oxide emissions from soils

Nitrous oxide emissions in soils are the result of biological processes which are performed by a variety of microorganisms (Han et al., 2024). Among others, nitrification and denitrification are the two major processes leading to N_2O emissions in soils (Butterbach-Bahl et al., 2013). These reactions are carried out by a variety of soil microorganism communities that carry out specific or several parts of the transformations (Kuypers et al., 2018). Nitrification + nitrifier denitrification and denitrification as the most important processes (Butterbach-Bahl et al., 2013) will be described in more detail in sections 2.1.1 and 2.1.2. An overview over the involved processes can be seen in Figure 1.

The balance and interaction of these processes determine the potential emissions and pathways of N_2O in soils (H. Wang et al., 2023). Their magnitudes are dependent on soil physicochemical conditions such as oxygen availability (X. Song et al., 2019), soil texture (Z. Zhang et al., 2024), soil structure and substrate availability (Laville et al., 2011). One of the most important predictors of N_2O emissions is the soil moisture or water-filled pore space (WFPS) with emission peaks occurring typically at around 75-80% WFPS (H. Liu et al., 2022). Humans influence these processes by management of soils and input of nitrogen, increasing the level of available substrate the abundance of nitrogen functional genes (Du et al., 2024; Kuypers et al., 2018). These aspects will be described in sections 2.1.3 and 2.1.4.

Large parts of annual N_2O emissions are occurring as short term increased emissions (“hot moments”) namely dry-wet and freeze-thaw cycles (Wagner-Riddle et al., 2020). Freeze-thaw cycles are currently not sufficiently addressed in IPCC emission factors with specific emission factors still to be developed (Hergoualc’h et al., 2019). Freezing-thawing cycles will be further described in section 2.1.5.

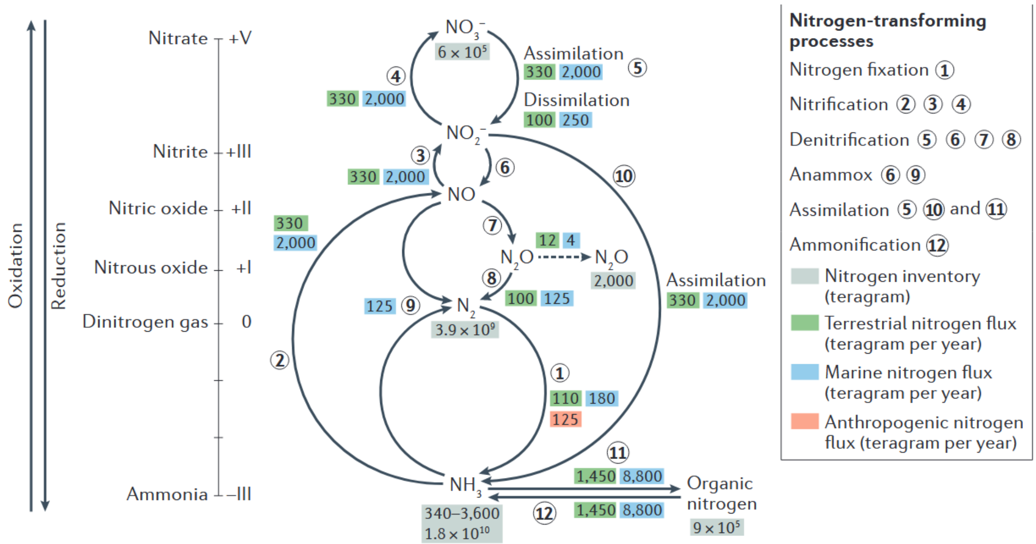

Figure 1 Overview over nitrification and denitrification processes among others (Kuypers et al., 2018)

2.1.1 Nitrification and nitrifier denitrification

Nitrification describes the oxidation of ammonia (NH_3) to nitrate (NO_3^-) through the intermediate stages of hydroxylamine (NH_2OH), nitric oxide (NO) and nitrite (NO_2) under aerobic conditions. These processes are carried out mostly by chemolithotrophic ammonia oxidizing bacteria (AOB) and archaea (AOA). However, also heterotrophic and methanotrophic organisms play a role (Stein & Klotz, 2016).

The first step of NH_3 oxidation to NH_2OH is carried out by AOB and AOA by using the enzyme ammonia monooxygenase (AMO, encoded by *amoA*) (Kuypers et al., 2018; Rotthauwe et al., 1997). The second step from NH_2OH to NO is performed by AOB with the enzyme hydroxylamine oxidoreductase (HAO, encoded by *hao*) (Kuypers et al., 2018). This oxidation is also carried out by AOA, but the enzyme for the AOA process is unknown (Kozlowski et al., 2016). These first two steps are typically carried out by the same microorganism (Kuypers et al., 2018). They are represented by process 2 in Figure 1.

The enzyme carrying out the oxidation of nitric NO to NO_2 (process 3 in Figure 1) remains currently unknown (Caranto & Lancaster, 2017).

The last step from NO_2 to NO_3^- (process 4) is performed via the enzyme nitrite oxidoreductase (NXR) which is encoded by nitrite-oxidizing bacteria (NOB) using the *nxrA* and *nxrB* genes (Kuypers et al., 2018; Stein & Klotz, 2016). Recently also nitrospira bacteria (comammox) which can carry out all four steps (processes 2, 3 and 4) have been discovered (Daims et al., 2015; van Kessel et al., 2015).

Microorganisms responsible for nitrification can also be an important source of N_2O by performing nitrifier-denitrification, which involves the reduction of the formerly oxidized nitrite to NO and N_2O in the same ammonia oxidizing bacteria (processes 6 and 7). This happens under low and varying oxygen conditions, high nitrite (NO_2) availability and possibly low organic carbon availability (Wrage-Mönnig et al., 2018). The process is mostly carried out through several different nitrite reductase enzymes (NiRs) which are mostly encoded by *nirS* and *nirK* genes. This nitrifier denitrification pathway is particularly important for soils with an ammonium nitrogen source as this can lead to increased nitrite concentrations through nitrification (Kool et al., 2010). It differs from the process of denitrification as it is not linked to the oxidation of organic carbon and happens at higher oxygen levels (Stein, 2011).

The most important genes that are used for the detection of nitrification and nitrifier denitrification activity are *amoA, nirS* and *nirK* (Levy-Booth et al., 2014).

The response of this process in combination with denitrification to soil physicochemical conditions will be explained in 2.1.3.

2.1.2 Denitrification

Denitrification describes the reduction of nitrate (NO_3^-) to dinitrogen gas (N_2) with intermediate steps of nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) (Kuypers et al., 2018). Denitrification happens primarily under anaerobic conditions in which nitrogen oxides function as an electron acceptor. It is an important source of N_2O in soils. If fully executed, it can be seen as a reversion of dinitrogen fixation and nitrification. Most denitrifiers are heterotrophic organisms (Zumft, 1997). Under certain conditions under very low oxygen availability with low NO_3^- availability, denitrification process can provide an N_2O sink as it becomes fully denitrified to N_2 (Liang et al., 2025).

Denitrification starts with the reduction of NO_3^- to NO_2^- (process 5 in Figure 1) (Philippot et al., 2007). The process is carried out by bacteria, archaea, and eukaryotes in anoxic environments with the enzyme nitrate reductase (NAP, encoded by *napA* or NAR, encoded by *narG and narH*). Many organisms contain both (Kuypers et al., 2018). Nitrite is then further reduced to NO by bacteria and archaea with the a variety of the enzyme nitrite reductase (NiRs encoded by *nirS* and *nirK*) (process 6) (Graf et al., 2014). The subsequent reduction of NO to N_2O is catalysed by a variety of different nitric oxide reductases (NOR, encoded by a variety of genes) which occur in a wide range of organisms (process 7). While some bacteria can carry out the full reduction to N_2 using nitrous oxide reductase (NOS, encoded by *nosZ*), most denitrifying organisms are limited to the reduction to N_2O or other parts of the denitrification process (Pold et al., 2025). This imbalance together with different responses of incomplete denitrifiers to environmental conditions (e.g. pH, water content) plays a main role in N_2O formation as its consumption differs from its production (Jones et al., 2014). Full denitrification typically only occurs at very high soil moisture levels (Butterbach-Bahl et al., 2013). The last step of denitrification is represented by process 8 in Figure 1.

The most important genes that are used in the detection of denitrification are *napA, narG, nirS, nirK* and *nosZ* (Levy-Booth et al., 2014).

The response of this process in combination with nitrification to soil physicochemical conditions will be explained in 2.1.3.

2.1.3 Physicochemical controls of N_2O emissions in soils

The main physicochemical control of N_2O emissions in soils is soil oxygen availability which determines the dominance of the involved microbial processes (X. Song et al., 2019). At higher oxygen levels nitrification is induced while at lower oxygen levels denitrification is stimulated (Davidson et al., 2000). Oxygen supply in the soil matrix is dependent on other soil physical and chemical properties which in turn influence N_2O formation. Denitrification hotspots occur at anaerobic microsites in the soil structure, where oxygen is depleted and sufficient energy in the form of organic carbon is present (Schlüter et al., 2025).

Closely connected to the oxygen availability is soil moisture, often expressed in water-filled pore space (WFPS). Depending on the soil type, the highest N_2O emissions occur at WFPS levels of 70-80% (H. Liu et al., 2022). At higher soil moisture levels, N_2O gets further denitrified to N_2 (Butterbach-Bahl et al., 2013). Due to this sensitivity, wet-dry cycles (WDC) have an important influence on emission peaks. This is due to a sharp increase in microbial activity after rewetting, leading to an emission pulse which surpasses emissions from soils kept at constant moisture levels (Borken & Matzner, 2009; Harrison-Kirk et al., 2013). The increase of emissions during WDCs is related to the aerobic conditions necessary for nitrification and the anaerobic conditions necessary for denitrification, which are interchangeably present in WDCs.

Another important determining effect of N_2O emissions is temperature, as it influences microbial activity (Schaufler et al., 2010). N_2O emissions rise not only due to the increased microbial activity at higher temperatures but also due to an increase in soil respiration. This results in increased oxygen depletion in the soil pores, promoting anaerobic conditions for denitrification. This effect indicates a possible increase of N_2O emissions due to climate change (Butterbach-Bahl et al., 2013).

The pH is another important control for N_2O, as the microbial communities involved react differently to different pH levels (Y. Zhong et al., 2023). At lower pH, the reduction of N_2O to N_2 by nitrous oxide reductase (NOS) is gradually inhibited, leading to higher N_2O emissions under moderate soil acidity (B. Liu et al., 2014; Wallenstein et al., 2006). Also, at slightly acidic pH, N_2O emissions are more sensitive to nitrogen application, leading to higher emissions (Y. Wang et al., 2018). In strongly acid soils, the emission potential decreases again (Qiu et al., 2024). Therefore, it has been shown, that an increase in soil pH from slightly acidic soils e.g. by liming or biochar application can reduce N_2O emissions from agricultural soils (Bleken & Rittl, 2022).

The amount and availability of carbon sources further influences N_2O fluxes, with higher peaks being observed with high available C (Harrison-Kirk et al., 2013). As denitrification is a heterotrophic process, carbon is necessary as an energy source (Dlamini et al., 2020). In a metanalysis, soil organic carbon content correlated strongly with the important denitrification genes *nirK*, *nirS* and *nosZ* resulting in subsequent higher denitrification potential if the process substrate (e.g. NH_4^+, NO_3^-) becomes present (Dong et al., 2022)

Soil texture influences N_2O emissions as it has an impact on microbial responses on moisture conditions by influencing oxygen availability and diffusion (Butterbach-Bahl et al., 2013; Singh et al., 2023). Fine-textured soils tend to provide more anaerobic microsites which facilitate denitrification and N_2O formation (Harrison-Kirk et al., 2013). Another effect of texture is that more fine textured soils tend to have a higher carbon content which in turn can support higher microbial activity as described (J. Yang et al., 2025). The pulse in microbial activity after moisture increase tends to be higher in fine-textured soils than in coarse-textured soils as moisture is available for a longer time for microbes due to increased water holding capacity and pore connectivity (Cable et al., 2008; Patel et al., 2021).

The described soil properties not only influence the total N_2O emissions but also the pathways. Denitrification dominates as a N_2O source in very high soil moisture and very low (0%vol/vol) oxygen availability while nitrifier denitrification can dominate under low but relatively higher (0.5 – 3%vol/vol) oxygen conditions (Zhu et al., 2013). Regarding soil moisture, thresholds of 60-70% WFPS have been reported with denitrification dominating at higher WFPS (H. Wang et al., 2023). A decrease in soil pH increases the relative importance of the denitrification pathway in comparison to nitrifier denitrification as enzyme activity for complete denitrification is inhibited as described earlier (Han et al., 2024).

Global ratios between denitrification and nitrification derived N_2O emissions are not fully understood, with analyses ranging from 25.2% (Inatomi et al., 2019 based on a model) to 51.6% (Han et al., 2024, empirical data). In the past decades the earlier view of denitrification as the dominant source of N_2O has been challenged (Zhu et al., 2013).

Due to these various influencing factors, a great spatial variability of N_2O emissions evolves, which makes emission predictions challenging (Harrison-Kirk et al., 2013)

2.1.4 The effect of fertilizer type on N_2O emissions

Given the physicochemical controls explained, the main driver of N_2O emissions in agricultural soils is anthropogenic nitrogen input in form of nitrogen fertilizers.

This increases the substrate availability for the microbial processes involved (Firestone & Davidson, 1989; Tian et al., 2020).

The IPCC takes this effect into account with a tier 1 emission factor of 1% of total N input and more detailed factors for wet (1.6% for synthetic fertilizers and 0.6% for organic) and dry climates (0.5% for all fertilizers) (Hergoualc'h et al., 2019). These values have been criticised for underestimating real N_2O emissions in fertilized soils (Thompson et al., 2019). Actual soil emission factors over all agricultural soils have been estimated at 1.8% (±0.5%) of nitrogen input (Tian et al., 2020).

A meta-analysis by Ouyang et al. (2018) showed that nitrogen fertilization had a strong positive effect on the abundance of the genes involved in nitrification and denitrification processes (as described in section 2.1.1 and 2.1.2). While all fertilizer types had an effect, organic fertilizers showed a significantly stronger influence on the nitrogen cycling genes, supposedly by the general positive effect on microbial biomass by organic fertilizers due to the provision of organic carbon (Kallenbach & Grandy, 2011). This effect of fertilization on N-cycling genes correlated with process rates of nitrification and denitrification, therefore increasing the potential for N_2O emissions when substrate is present (Ouyang et al., 2018).

As NO_3^- is the substrate for the denitrification process, direct NO_3^- fertilizer application can significantly and sharply increase N_2O emissions through the denitrification pathway (Senbayram et al., 2012).

If nitrogen is applied in the form of NH_4^+, the substrate first needs be transformed through the nitrification pathway to provide substrate for denitrification. NH_4^+fertilizers have been reported to decrease emissions in comparison to NO_3^- based fertilizers without yield decreases (Rahman & Forrestal, 2021; Velthof & Mosquera, 2011). While reducing emissions, NH_4^+ fertilizers can lead to strong relative N_2O increases through the nitrifier denitrification pathway as NO_2 levels are increased through microbial transformations (Wrage-Mönnig et al., 2018).

The response of N_2O emissions to nitrogen inputs has been shown to be non-linear, with exponential increases, particularly if crop nitrogen needs are surpassed. This can underestimate emissions in high input systems while overestimating emissions from low-input fields, e.g. in Sub-Saharan Africa (Shcherbak et al., 2014).

If organic amendments are applied, not only the nitrogen content in the system is increased but also the available carbon for microbial processes. The concrete effect of organic amendments depends on the type and its C:N ratio with lower ratios resulting in higher emissions (Charles et al., 2017). Amendments like manure have shown potential to decrease N_2O emissions in comparison to mineral nitrogen compounds (S. Liu et al., 2017; Velthof & Mosquera, 2011; Q. Zhang et al., 2026). This could be due to an increase in N immobilization and a decrease in ammonia oxidizing bacteria due to slower N release (Hei et al., 2023). However, the overall knowledge on the impact of organic fertilizers in comparison to synthetic fertilizers remains inconclusive (Q. Zhang et al., 2026).

2.1.5 Freeze-thaw cycles

Freeze-thaw cycles (FTCs) play an important role in total N_2O emissions, a factor that has been noticed for several decades (Goodroad & Keeney, 1984). However, the guidelines for IPCC emission factors only mention the importance for FTCs but do not include them into their current emission factors (Hergoualc'h et al., 2019). It has been estimated that, by neglecting freeze-thaw induced emissions, global emissions could be underestimated by 17 to 28% (Wagner-Riddle et al., 2017) These observed peaks are the result of different processes. First, an increase of substrate availability due to freezing influences on microbes (solidification and cell membrane ruptures) (Schimel et al., 2007) and on aggregates plays a role (Christensen & Christensen, 1991; Oztas & Fayetorbay, 2003). Further, an increased root turnover and increases in dissolved organic carbon have been observed, possibly stimulating microbial activity (Y. Song et al., 2017).

Another part of the emission peaks is explained by the release of formerly produced N_2O which is then released once soil connectivity is restored during thawing (Teepe et al., 2001). However, this effect is supposedly smaller in comparison to the increase in substrate availability (Risk et al., 2013). Also, different influences on different functional microbes play a role, with denitrifiers showing to be more resilient to cold temperatures than nitrifiers (Smith et al., 2010). Further, it has been hypothesized that N_2O reductase (NOS) might be inhibited under freezing temperatures, hindering the last step of denitrification (Holtan-Hartwig et al., 2002). The typically wet conditions during thawing due to snow melt increase the denitrification potential (Wagner-Riddle et al., 2008). Regarding the N_2O formation pathway, denitrification seems to be dominating in FTCs as a result of increased soil water content and NO_3^- availability (Wagner-Riddle et al., 2008).

However, the exact processes shaping FTCs in soils are not yet fully understood and are also not yet adequately represented in global emission models as many measurements are focussed on the growing season (Wagner-Riddle et al., 2024). To achieve that, accurate and frequent reporting of freezing-thawing process is necessary (Kim et al., 2012).

2.2 Biochar

Biochar is a material resulting from the pyrolysis of organic feedstock under different treatment temperatures. During pyrolysis (thermal decomposition of organic material without oxygen) a part of the carbon content is decomposed, but large parts of the original carbon content remains in the solid product (Lehmann, 2007). It is typically porous, has a low density and a large specific surface area, much higher than the feedstock it is produced from (Weber & Quicker, 2018). The respective chemical properties are highly dependent on the used feedstock and pyrolysis temperature and can be adapted to different use cases (Haghighi Mood et al., 2024). Biochar contains ordered, highly crystalline pyrogenic C, which is persistent to microbiological decomposition as the microbial community typically does not have all enzymes that are required for its decomposition (Lehmann et al., 2024). Biochar has shown to increase the total microbial biomass and enzyme activities in soils through effects on biotic conditions (Pokharel et al., 2020) which might be a function of an increase in pH, water availability and porosity (Joseph et al., 2021). As a soil amendment biochar can increase nutrient availability in degraded soils, sequester carbon and increase yields (Jeffery et al., 2011; Lehmann et al., 2024; Pokharel et al., 2020). Apart from improving soil properties biochar has been shown to mitigate non carbon dioxide (CO_2) greenhouse gas emissions from soils (Cayuela et al., 2014).

2.2.1 Effect of biochar on nitrous oxide emissions

Biochar has shown to reduce N_2O emissions from soils in a variety of environments (Cayuela et al., 2014). While this reduction can be seen in a variety of studies, the underlying mechanisms behind the reduction are not yet fully understood (Borchard et al., 2019). Overall, meta analyses estimated the reduction potential of N_2O through biochar at 19% (L. Zhong et al., 2025), 38% (Borchard et al., 2019) and 54% (Cayuela et al., 2014). These large differences are a result of differences in the included studies (e.g. field vs laboratory study, duration of the experiment, geographic location) and indicate a continued lack of knowledge on the exact mitigation potentials (Borchard et al., 2019; Verhoeven et al., 2017). Overall, laboratory studies showed higher reduction potentials than field studies (Borchard et al., 2019)
One important mechanism is the liming effect of biochar, which is typically alkaline, leading to complete denitrification which is otherwise inhibited at low pH (Cayuela et al., 2014).

Biochar also interacts with the microbial community in soils. It has been observed that the abundances of the *nirK* and *nosZ* gene have increased which promotes complete denitrification. This increase in gene abundance has been reported to be connected to the described increase in pH by biochar application (Aamer et al., 2020). In general, biochar increases total microbial biomass by providing suitable habitats for soil microorganisms and affecting enzyme activity (Pokharel et al., 2020).

Another mechanism is the reduction of N availability by affecting N-cycling processes which reduces inorganic nitrogen contents in solution (Nguyen et al., 2017). This effect is most likely a result of a high biochar C:N ratio, leading to N immobilization and adsorption on surface sites (Brewer, 2024; Van Zwieten et al., 2024). It has also been theorized that NO_3^-, while carried in water, moves into the pore structure of biochar where it then adsorbs to surface sites. These effects reduce the substrate availability for the nitrification and denitrification processes and simultaneously maintain aerobic sites (Sohi et al., 2010).

Biochar mitigates N_2O emissions through the “electron shuttle” which consists in the biochar’s availability to transfer electrons in the carbon matrices to denitrification sites in order to achieve complete denitrification to N_2 (Cayuela et al., 2013; Sun et al., 2018; Yuan et al., 2019).

The dissolved organic carbon pool is affected, as biochar provides organic carbon, particularly shortly after application. This can temporarily stimulate denitrification and N_2O emissions directly but also indirectly by increasing soil respiration and inducing anaerobic sites in soil (Cayuela et al., 2014). This has mostly been observed for biochars with a high labile C content (Weldon et al., 2019).

Another indirect effect is the reduction of substrate availability through increased plant uptake (Q. Liu et al., 2018).

The effect of biochar is dependent on the applied biochar dose. The N_2O mitigation potential tends to increase with increased application rates; however, other benefits (e.g. yield increase) tend to decrease again after application rates exceed 80 t ha^{-1}. Also, the optimal application rate is dependent on the soil pH, as optimal pH can be exceeded by overapplication in neutral to alkaline soils (Q. Liu et al., 2018). L. Zhong et al. (2025) defined a threshold application rate of 20 t ha^{-1} above which significant effects of biochar application on N_2O can be observed, while other analyses exist that did not find a clear dose effect (Kohira et al., 2025).

During FTCs, biochar reduced the abundance of *amoA* genes, responsible for NH_4^+ oxidation and reduce the NO_3^- content in soil solution (X. Liu et al., 2016; Z. Yang et al., 2022). It is assumed that possible reduction processes during FTCs follow the same mechanisms as under other conditions, but the current level of understanding is very limited (X. Liu et al., 2016) This is particularly important given the importance of FTC-induced emissions (2.1.5).

2.2.2 Soil and biochar properties influencing the effect of biochar on nitrous oxide emissions

The effect of biochar on N_2O emissions is dependent on how emissions are formed and what physicochemical conditions influence the processes (2.1.3). Soil and biochar properties therefore determine the potential of mitigating N_2O emissions. One observed interaction is the soil moisture content with biochar showing a larger reduction potential at high soil moisture by facilitating complete denitrification (Q. Liu et al., 2018). The largest reduction effects have been observed in loamy soils as these soils provide more anaerobic microsites which favour denitrification processes in which biochar can interact and facilitate complete denitrification. Further, the effect of biochar is suggested to be limited in soils with low carbon contents as carbon is needed for the heterotrophic denitrification with which biochar interacts. Soils with low pH may benefit more from biochar application as they can benefit from its liming effect (Q. Liu et al., 2018). Biochar also showed a higher potential to increase enzymatic activity and microbial biomass in moderately acid soils (Pokharel et al., 2020).

Regarding the biochar feedstock, wood and straw-derived biochar at higher pyrolysis temperatures has shown to have a larger effect on N_2O emissions than biochar produced at lower temperatures and from manure (Q. Liu et al., 2018). At lower pyrolysis temperatures biochar contains higher proportions of labile carbon contents which can in turn increase microbial activity and N_2O formation (Weldon et al., 2019). Higher pH biochar has a larger mitigation effect as increase in soil pH facilitates complete denitrification (Blum et al., 2018). Cayuela et al. (2015) showed that the H:Corg (Hydrogen & organic carbon) ratio is an important indicator of the reduction potential of biochar with low H:Corg ratio resulting in higher reduction potential. This is in turn related to a higher degree of aromatic condensation which is a result of high treatment temperatures (Ippolito et al., 2020).

Regarding the interactions of soil and biochar properties on N_2O emissions during FTC, no conclusive knowledge is currently available.

2.3 Knowledge gaps and hypotheses

As a result of the literature review, the following knowledge gaps have been identified and are being addressed in the presented thesis.

It is still not fully understood when and under which conditions biochar can reduce N_2O emissions. This includes conditions determined by soil and biochar properties but also different environmental cycles (wet-dry and freeze-thaw) that influence emission trajectories. The presented thesis will add to the current knowledge pool by testing the effects of biochar in two different soils and by inducing both, a wet-dry and a freeze-thaw cycle.

Currently no clear knowledge about the dependency of biochar effects on fertilizer types exist. By applying two different fertilizers, the conducted experiment will help understanding existing differences.

Even though some indications of dose thresholds for biochar exist as described by L. Zhong et al. (2025), no information on the dose dependency depending on the fertilizer type is available. Therefore, three different biochar doses are applied to address that. This is particularly important for application efficiency.

While N_2O emissions during freezing-thawing cycles have become an important field of research in the past decades, the exact influence and importance dependent on soils and fertilizers are not fully understood. Also, very limited knowledge is available on the effect of biochar during FTCs which will be addressed in this thesis.

By applying a high temporal resolution in the measurements, it will be ensured not to miss important emission pulses.

To tackle aforementioned knowledge gaps, we hypothesized that:

- Biochar amendments reduce N_2O emissions across different soil and fertilizer types, during wet-dry as well as during freeze-thaw cycles under controlled conditions.
- The effect of biochar application on N_2O emissions differs with the applied dose during wet-dry cycles and the effect is fertilizer-dependent
- Freezing-thawing cycles lead to short lived but high N_2O emissions and their characteristics differ between soils

3. Materials and Methods

3.1 Literature review

The literature report of the present master thesis was compiled using different online sources and search engines such as Google Scholar, Scopus & Research Gate. Also, Claude AI was used to gather important literature. Further, books from the SLU university library in Ultuna were accessed. The snowball method was used to find additional studies about the respective topic. Sources from diverse timeframes were accessed to avoid a time bias by using the snowball system.

Keywords that were used for the literature research are: *Nitrous Oxide* AND *biochar* OR *wetting-drying* OR *freezing-thawing*; *environmental impacts of biochar*; *nitrogen cycle; nitrogen cycling genes*

3.2 Experimental design

Soil samples were collected on 11.02.2026 from two sites near the SLU Campus at Ultuna. The clay soil comes from Kungsängen northeast of the campus, whereas the sandy loam comes from an experimental field north of the campus in Ulleråker (See Figure 2).

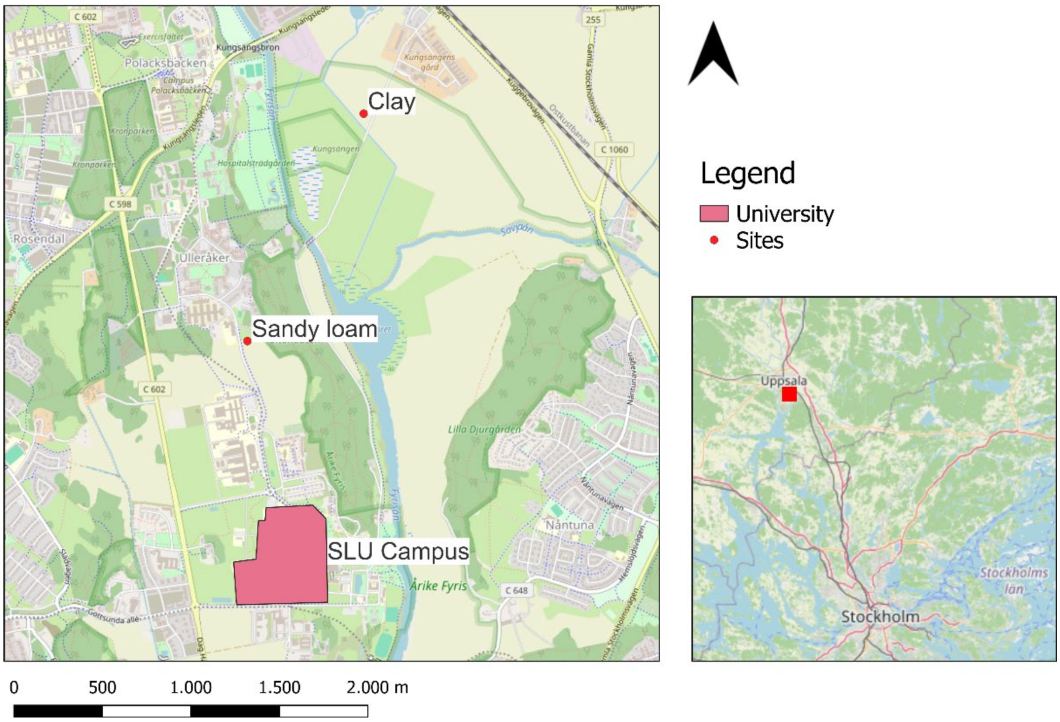

Figure 2: Map showing the locations of soil extraction around the SLU campus in Ultuna/Uppsala with an overview map showing the area in red in relation to Stockholm (OpenStreetMap, n.d.)

To ensure proper homogenization, 50 kg frozen disturbed soil was collected from each experimental site. The two soils were fertilized with two different fertilizers. NPKS fertilizer with 150 kg N ha^{-1} and dairy slurry manure with 87.5 kg N ha^{-1} (25 t ha^{-1} total manure) were applied.

The application rate for the manure was based on an estimated 0.6% N as given by Andersson et al (2026). However, as later laboratory analysis showed that the dairy slurry manure only had 0.35% N, only 87.5 kg N ha^{-1} were applied instead of the 150 kg N ha^{-1} that were aimed for.

The biochar treatment levels were based on 0.5%w/w (BC1), 1.25%w/w (BC2), and 2%w/w (BC3) of the originally applied 550 g of moist soil. The biochar application rates were roughly based on (L. Zhong et al., 2022). However, as the two soils had different original soil moisture contents, differences in the weight percentages occurred if related to the mass of dry soil. Additional differences between the two soils occurred as the sandy loam settled quite substantially during the water application, leading to differences in bulk density between the two soils. This influenced the application rates per hectare, as the values were scaled from the mesocosm height to a typical implementation depth of 10 cm to make them more comparable to other studies. The resulting differences are summarized in Table 1.

Table 1 Weight percentages and t ha^{-1} biochar, different weight percentages are a result of different original moisture contents between the two soils; different t ha^{-1} values between soils are the result of differences in soil bulk density as the height of the soil columns was extrapolated to an average of 10 cm implementation depth in field applications.

	BC1 (%w/w)	**BC2 (%w/w)**	**BC3 (%w/w)**	**BC1 (t ha^{-1})**	**BC2 (t ha^{-1})**	**BC3 (t ha^{-1})**
Clay	0.64%	1.60%	2.56%	5.60	14.01	22.41
Sandy loam	0.58%	1.44%	2.31%	6.32	15.79	25.27
Average	0.61%	1.52%	2.44%	6.00	15.00	24.00

The factorial design approach led to the treatments and replicate numbers in Table 2. The fertilized and non-fertilized without biochar treatments had five replicates to address the high variability of N_2O measurements (Chadwick et al., 2014). The non fertilized samples with biochar treatments had only one replicate as low emissions and therefore smaller effects of biochar were expected. They were included nonetheless to be able to estimate emission factors for the fertilized treatments with biochar. The exact treatment layout with all samples and respective treatments can be found in Appendix 1: Treatment layout. Sample numbers were assigned to the treatments by using random numbers in Excel to prevent a bias because of the spatial and temporal setup during the measurements.

Table 2 Overview of treatments and replicate numbers

Treatment	Replicates	Treatments	Replicates
Clay, No fertilizer, NBC	5	Sandy loam, No fertilizer, NBC	5
Clay, NPKS, NBC	5	Sandy loam, NPKS, NBC	5
Clay, NPKS, BC1	5	Sandy loam, NPKS, BC1	5
Clay, NPKS, BC2	5	Sandy loam, NPKS, BC2	5
Clay, NPKS, BC3	5	Sandy loam, NPKS, BC3	5
Clay, Manure, NBC	5	Sandy loam, Manure, NBC	5
Clay, Manure, BC1	5	Sandy loam, Manure, BC1	5
Clay, Manure, BC2	5	Sandy loam, Manure, BC2	5
Clay, Manure, BC3	5	Sandy loam, Manure, BC3	5
Clay, No fertilizer, BC1	1	Sandy loam, No fertilizer, BC1	1
Clay, No fertilizer, BC2	1	Sandy loam, No fertilizer, BC2	1
Clay, No fertilizer, BC3	1	Sandy loam, No fertilizer, BC3	1
Total amount of samples: 96			

3.3 Sample preparation

After 3 days storage in a drying room to thaw and reduce moisture both soil types were homogenized separately and then filled into the containers according to the treatment layout. Visible rocks and stones were removed, but the soils were not sieved to keep remaining aggregates.

Five samples of about 100 g each were set apart per soil and put in the drying oven to determine an average moisture content of the samples at the start. Initial soil moisture for the two soils was 22.1% for the clay soil and 13.7% for the sandy loam.

The Hünersdorff 1000 cm^3 screw top cans were used for all samples. As for the sample preparation, 550 g of moist soil was filled into the containers, after every third of the 550 g, the soil was compacted by hand using the back of a syringe handle. For the samples that were treated with biochar, one third of the respective biochar treatment amount was applied before every compaction, thus spreading the biochar in the reconstructed profile. For every sample, the exact weight of the container without soil and the final weight with the moist soil was registered. Dry weight of soil per sample was defined with the determined average moisture content of the two soils.

The lids of all containers were perforated with two small holes for the inlet and outlet of the INNOVA measurement device. The weight of every lid after drilling the holes was recorded.

Fertilizers were applied using a pipette for the liquid NPKS fertilizer and a spatula for the solid manure.

After the preparation, the samples were transferred into a temperature-controlled room with 15°C where they remained until the end of the experiment except for the week in which the samples were frozen. The constant temperature was chosen to limit confounding effects on N_2O formation.

3.4 Soil, biochar and fertilizer properties

3.4.1 Soil properties

The soil from Kungsängen is a clayey-textured soil (further called "clay soil") with a clay content of 45.2 %, a pH of 5.7, a total carbon content of 2.9% and very good structure and aggregate stability. The clay soil from Kungsängen has typical characteristics of a "Gyttja" clay soil, indicated by the good structure and low bulk density (Berglund, 1996). The soil from Ulleråker is a sandy-loam soil (further called "sandy-loam soil") with a pH of 7.1, a total carbon content of 1.48% and a weaker structure than the clay soil. The clay soil had a higher total nitrogen content than the sandy loam (0.3% vs 0.13%). Further soil data can be found in Appendix 2: Soil properties. The chemical soil data and the texture was received from a laboratory. The description of the structure is based on a visual and manual estimation.

3.4.2 Fertilizer properties

The liquid synthetic fertilizer was produced in the laboratory and was composed of 1.18M $(NH_4^+)_2SO^4$, 0.236M HK_2PO_4, 0.059M K_2SO_4 (further called "NPKS").
Fresh manure was collected at the SLU experimental farm in Lövsta (further called "manure"). After 7 days of storage in the temperature regulated room the respective samples were fertilized. A homogenized sample was removed on the day of fertilizer application and sent to the laboratory. The manure had a dry substance of 13.1% and C, N, P and K values of 58.9 kg t^{-1}, 3.5 kg t^{-1}, 1.14 kg t^{-1} and 1.09 kg t^{-1}. The C/N ratio was 16.9. The pH of the manure was 7.5. Further chemical data on the applied manure can be found in Appendix 3: Fertilizer properties.

3.4.3 Biochar properties

The analysis of biochar properties was conducted by Eurofins Umwelt Ost GmbH. The biochar was made from woodchips at a temperature of 650°C. The total-C content of the dry biochar was 94.1%, the C-N ratio 196. The molar H/C and H/Corg ratio was 0.19. The complete biochar properties can be found in Appendix 4: Biochar properties.

3.5 Water application

Water was applied according to the bulk density and pore volume of every sample to achieve equal water-filled pore space (WFPS). The water was applied using a spray bottle to ensure uniform application on the soil surface.

The water application events are summarized in Table 3.

Table 3 Water application events and goal WFPS values of every event

Day of water application	**Goal WFPS**
3*	40%
5	60%
6**	70%
30	65%
31	80%

** Originally lower WFPS was applied as the water content of the fertilizers was taken into account for samples that were fertilized after to reach the set WFPS after fertilization. Exact values depend on sample bulk density of each sample and fertilizer type (estimated 80% water content in manure and 100% in synthetic fertilizer)*

*** Samples of the sandy loam eventually experienced higher WFPS as the samples significantly collapsed after the first water applications, therefore the water application was stopped at 70% as some samples of the sandy loam visibly surpassed the 70% WFPS threshold. Bulk density and WFPS was recalculated after as described in 3.6.4*

The repeated water application in the beginning was chosen to let the soil samples stabilize in the temperature-controlled room before the fertilizers were applied.

After the water application the samples remained in the temperature-controlled room (15°C) where they continuously dried, creating a wet-dry cycle. Figure 3 shows the trajectory of WFPS over the whole duration of the experiment separated by soil type.

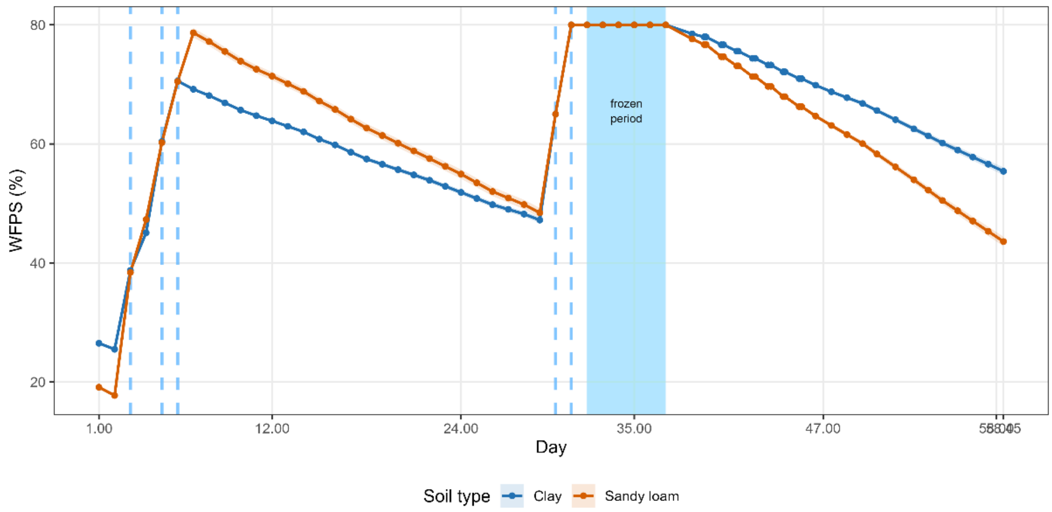

Figure 3 Water-filled pore space (WFPS) in % averaged for the two soils. The data includes all biochar treatments in days 1-31 and NBC and BC2 treatments after day 38. Blue vertical lines indicate water application. The difference in initial WFPS between the two soils in the first part of the experiment is a result of the settling of the sandy loam as described in 3.2

3.6 Laboratory measurements

3.6.1 Temporal measurement layout

The experiment was started on 17th of February 2026. Emissions were measured every second day in the beginning of the experiment (Day 1 – Day 3). After the application of fertilizers and water application, the frequency was increased to daily until the emissions went down to background values (04 – 19) with one break day on day 13. During the transition until the freezing of the samples, emissions were measured every second day (20 – 31) with one two-day break (20 and 21).

After the first part of the experiment (day 1 – 31) only the most promising biochar treatment (BC2) together with the no biochar treatment (NBC) was continued for the freezing-thawing cycle.

The samples were frozen on day 31 after measuring and kept there until day 38. On day 38 one measurement was taken about 4h after removing the samples from the freezer. During the thawing process, two daily measurements were taken until the emissions decreased (39 – 45). After, measurements were taken daily (46 – 50) with a break on day 48 and every second day after (50–58). The measurements were stopped after the emissions returned to background values after the freezing-thawing cycle. The described measurement layout can be seen in Table 4.

*Table 4 Measurement days, light green indicates one daily measurement, dark green indicates two daily measurements. light blue indicates freezing of samples. * indicate water application, # indicates fertilization*

Day	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
Meas			*#		*	*									
Day	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Meas															*
Day	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Meas	*														
Day	46	47	48	49	50	51	52	53	54	55	56	57	58		
Meas															

3.6.2 Emission measurements

The N_2O emissions were measured using a closed chamber technique utilized with the photoacoustic gas spectrometer INNOVA 1312. The needles of the outlet and inlet were poked through sticky tape located in the lid of the containers. The tape was replaced after every measurement to ensure air tightness for the next measurement. To maintain aerobic conditions but minimize drying, the lids were partially closed between measurements with a gap big enough to ensure air exchange. To ensure ambient air conditions in the containers when closing, the lids were removed at least 20 min before the first measurement. Emissions were determined by registering ambient air N_2O concentrations prior to closure time and again approximately 10 minutes after closure of the containers. This process resulted in alternating emission (for a sample that was closed for 10 minutes) and ambient air measurements (for the next sample to be closed). The closure time was decided after the linearity of concentration increase was confirmed as recommended by Chadwick et al. (2014). The N_2O values for ambient air were averaged over all samples of the respective day as no significant fluctuations of ambient air concentrations were expected and sometimes unexpected fluctuations were observed, probably due to uncertainties of the measurement device.

All measurements were transferred manually into an Excel sheet together with the exact time of the closure and the second measurement.

3.6.3 Freezing of the samples

The samples with NBC and BC2 treatment were put into the freezer room at the Department of Soil and Environment at SLU. The temperature of the freezer room was -22°C. The samples remained there from day 31 after measurements until day 38, so a total of 7 days. The samples remained open with the lid tilted on top. Then, the samples were returned into the controlled temperature room at 15°C.

3.6.4 WFPS, Bulk Density and chamber volume measurements

The bulk density was measured for every sample in the beginning and then after the water applications by measuring the height of the soil column from outside the container on at least four points and then averaging this value while taking the thickness of the bottom into account. The soil volume was then used to calculate the remaining chamber volume of every sample.

After the water application, the soil profiles of the sandy loam settled significantly, this was considered in the calculation of the soil and chamber volume with repeated measurements. Measurements of chamber volumes were repeated on days 1, 5, 8, 30 and 31 by measuring the height of the soil column. After day 31, regular test measurements were done which ensured that no need for a repeated measurement of column height was necessary.

Water contents were measured daily by measuring the weight of every container and then subtracting the weight of the container, dry soil, and treatments. The water content was then recalculated into WFPS according to the bulk density and pore volume for every respective sample. For the calculation of the pore volume, an average particle density of 2.65 g/cm^3 was applied.

3.6.5 Data treatment

N_2O and CO_2 values were measured in ppm and transferred to mg/m^3 according to equation (1):

$$(1) \quad \frac{mg}{m^3} = ppm \times \frac{M\ (g\ mol-1)}{22.414\ L\ mol-1} \times \frac{273.15K}{T\ (K)} \times \frac{P\ (hPa)}{1013.25\ hPa}$$

In equation (1), T is 288.15K (15°C), P is the pressure in Uppsala in hPa at the start of the respective measurement (Institutionen för geovetenskaper, 2026) which was considered to be equal to the pressure in the room. M is the molar weight (44.013 g mol^{-1} for N_2O and 44.009 g mol^{-1} for CO_2). The resulting two values (t = 0 and t = 10) were then transformed into flux values using the exact closure time of every sample, the headspace volume, and the area of the soil surface by calculating the linear slope of the concentration change. Emissions for days without measurements were linearly interpolated between adjacent measurements.

Before analysis, all values were upscaled to g ha^{-1} day^{-1} using the surface area of the containers to make values comparable to other existing studies.

For the calculation of cumulative emissions, trapezoidal integration was used. For the second part of the experiment after day 38 with two daily measurements, the average daytime of the measurements was considered for the integration. This was done to avoid bias as the two daily measurements were closer to each other than to the first one on the following day and as fluctuations between measurements were higher.

To calculate emission factors, daily means of non-fertilized treatments were subtracted from every sample. Cumulative values were then calculated by trapezoidal integration. $N\text{-}N_2O$ (nitrogen bound to nitrous oxide) was calculated and then related to the applied N depending on the fertilizer type. This calculation was based on Hergoualc'h et al. (2019).

For some treatments, negative daily emissions were observed which was most likely a result of measurement uncertainties and a delayed effect of high measurements (of the second measurement) on the subsequent ambient air measurement. This conclusion was drawn as negative N_2O emissions are very unlikely to occur at the WFPS levels in the present experiment (Ruser et al., 2006). In order to not confound the statistical analysis, the negative values were included to calculate statistics. However, negative cumulative values are most likely to be interpreted as zero emissions.

3.7 Statistical analysis

RStudio was used for statistical analysis (R Core Team, 2024). All values are expressed as means ± standard errors. A significance level of $\alpha = 0.05$ was used. The analysis for treatment differences was based on cumulative values over the respective timeframe. For comparisons of N_2O emissions between the two soils, a t-test was used with the whole dataset including the non-fertilized treatments after confirming normality and homogeneity of variance. For differences between treatments across soils or soil x fertilizers a two-way ANOVA was used on a reduced dataset without the non-fertilized treatments to achieve a balanced design and as the main interest laid in analysing treatment effects on fertilized soils. If the ANOVA yielded significant results, a Tukey pairwise comparisons based on estimated marginal means (EMMs) of cumulative values was used to determine differences between treatments and to consider the model's error term (Lenth et al., 2026). The compact letter display (CLD) in graphs is a result of the Tukey pairwise comparisons. Relative reductions of treatments in comparison to non-biochar treatments were based on real measured values and can therefore slightly differ from estimated marginal means.

Normality was met for N_2O fluxes for separate cycles and over the whole experiment. As the homogeneity of variances for N_2O was only met for the initial 31 days before freezing, the data for the second part of the experiment and for the analysis of the whole experiment was log-transformed, which confirmed the homogeneity of variances and allowed the use of parametric tests. As negative values existed which would not allow for log transformation, the largest negative value (a) was added to all values (x) before log transformation ($\log(x + a)$). This data transformation is a typical procedure for skewed N_2O measurements (De Klein et al., 2020; Hergoualc'h et al., 2019). The log-transformed data was then used for statistical tests while the original data was used to report emission values as recommended by De Klein et al. (2020).

A linear regression model was fitted for N_2O on CO_2 across all individual measurements. As every measurement was treated as an individual measurement without considering dependencies, the results are only descriptive of overall relationships and to give indications for further analysis.

Differences in peak days and WFPS of peaks were analysed by defining the day/WFPS of highest daily emissions for every sample and then pooling them across fertilizer and biochar treatments for each soil. As the Shapiro and Levene test for the peak days and WFPS across soils were not confirmed a Wilcoxon test was used to determine differences.

4. Results

Figure 4 shows the N_2O emission trajectory of all treatments over the whole experiment. The first part of the experiment started on day 1 with a wet-dry cycle (WDC) and ended on day 31 before freezing the samples. The second part of the experiment started on day 38 after 7 days of freezing when removing the samples from the freezer to induce a freeze-thaw cycle (FTC). As visible in Figure 4, emissions were higher during the FTC than during the WDC (~12x higher peaks for the clay soil, ~5x for the sandy loam; averaged over all fertilizers). Therefore, the result part will be split up in two parts, one focusing the WDC and one on the FTC. At the end, cumulative statistics over the whole duration of the experiment will be summarized. Also, CO_2 emissions averaged over the respective soil x fertilizer treatment are presented in Figure 4.

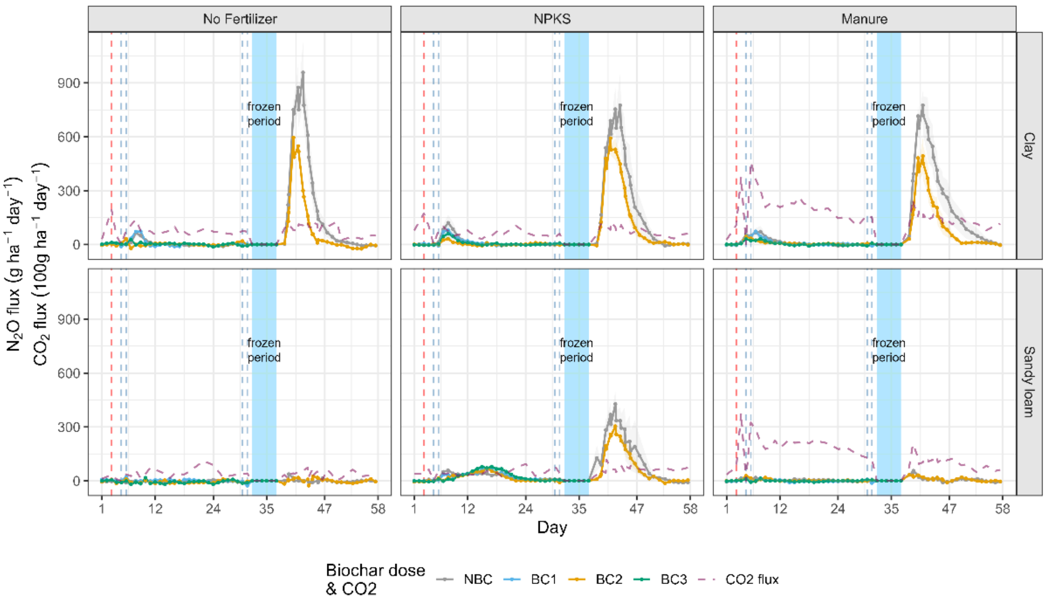

Figure 4 Overview over N_2O emission flux during whole experiment in g ha^{-1} day^{-1} for every soil x fertilizer combination with all biochar treatments. After the frozen period, only NBC and BC2 were measured. The red vertical line indicates water application and fertilization, blue vertical lines indicate water application only, the light blue rectangle represents the time when the samples were frozen, the purple dashed line indicates the soil respiration in 100 g CO_2 ha^{-1} day^{-1} adjusted for readability

4.1 N_2O emissions during wet – dry cycle

4.1.1 N_2O Flux Dynamics

In the first experimental period, emissions were analysed for 31 days until freezing the samples. Figure 5 shows the emission trajectory over the first 31 days of the experiment. The average N_2O emissions of the clay soil were 7.6 ± 0.6 g N_2O ha^{-1} day^{-1} and 8.8 ± 0.6 g N_2O ha^{-1} day^{-1} for the sandy loam. The clay soil had a sharp emission peak around day 8 (max 55.6 ± 8.3 g N_2O ha^{-1} day^{-1}), whereas the sandy loam had a later and broader peak around day 16 (max 24.6 ± 5.1 g N_2O ha^{-1} day^{-1}). The clay soil showed distinct emission peaks in all fertilizer treatments (including "no fertilizer"), whereas the sandy loam only had a distinct peak in combination with the NPKS fertilizer. Soil type had a significant effect on when the peaks occurred averaged over all biochar treatments ($p < 0.01$) with the peak in the clay soil occurring around day 8 while the peak of the sandy loam occurred around day 15. Biochar dose inside soil x fertilizer treatments and fertilizer type inside each soil treatment had no significant effect on when the peaks occurred.

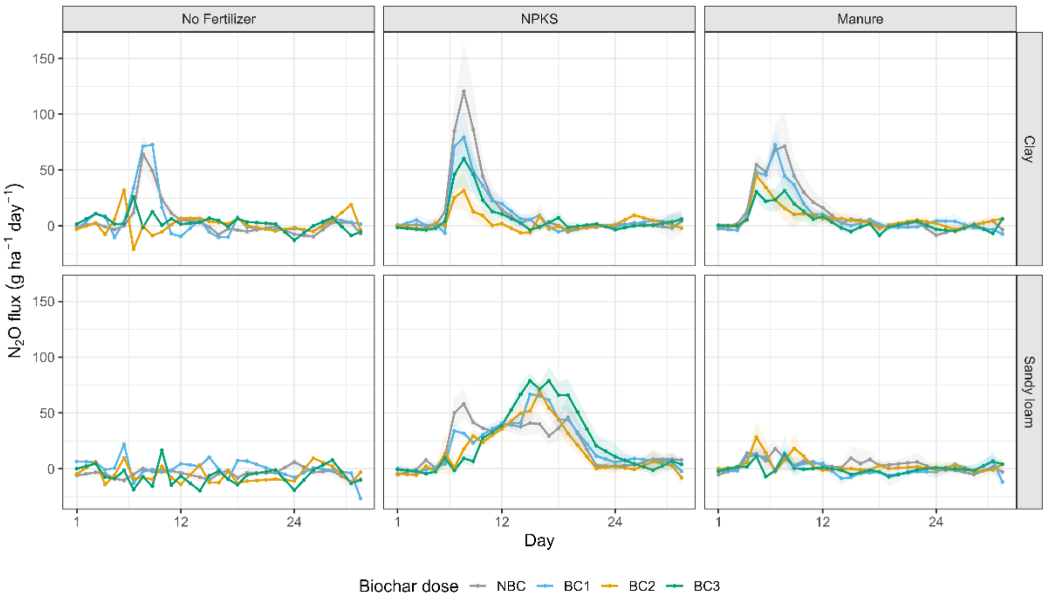

Figure 5 Flux dynamics of N_2O in g ha^{-1} day^{-1} for the first 31 days for every soil x fertilizer combination. Biochar treatments are represented in the indicated colours. The shaded confidence bands represent standard errors.

4.1.2 Cumulative N_2O emissions

No statistically significant difference was observed between soil types ($p = 0.51$) with the average over all treatments being 234.9 ± 32.2 g N_2O ha^{-1} for the clay and 275.0 ± 50.9 g N_2O ha^{-1} for the sandy loam. For the clay soil there was no difference ($p = 1.00$) between the NPKS fertilizer (258.2 ± 59.8 g N_2O ha^{-1}) and the manure (260.5 ± 44.9 g N_2O ha^{-1}). For the sandy loam a significant difference ($p < 0.01$)

between fertilizer types was observed with the NPKS fertilizer (652.2 ± 36.2 g N_2O ha^{-1}) resulting in higher emissions than the manure (55.2 ± 25.7 g N_2O ha^{-1}). The lowest biochar dose (BC1 = 6 t ha^{-1}) showed no significant effect on N_2O emissions in comparison with non-biochar treatment in both soils. Biochar 2 (15 t ha^{-1}) showed a significant reduction for the clay soil fertilized with NPKS (-77.0% compared to NBC treatment, $p = 0.02$). Biochar 3 (24 t ha^{-1}) showed a significant reduction for clay x manure (-64.6%, $p = 0.03$). If averaged over both fertilizers, BC2 (-50.3%, $p = 0.02$) and BC3 (-42.7%, $p = 0.04$) showed significant reductions for the clay soil. In the sandy soil, biochar did not induce a mitigation of N_2O emissions (lowest $p = 0.63$).

The results of the WDC are summarized in a bar chart in Figure 6, underlying values and statistics can be found in Table 13 and Table 14 in Appendix 5: Underlying emission values .

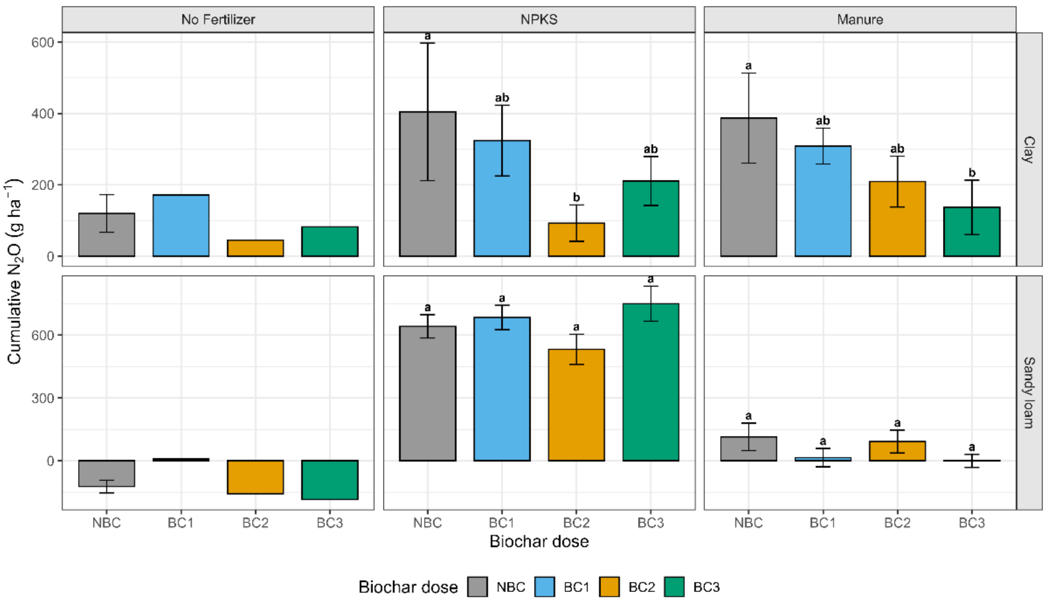

Figure 6 Cumulative N_2O emissions in g ha^{-1} after first 31 days for all soil x fertilizer x biochar combinations. Error bars indicate standard errors. Compact letter display (CLD) is a result of a Tukey-adjusted pairwise comparison of estimated marginal means within soil x fertilizer treatments after an ANOVA displayed in a, ab, b. Underlying values are presented in Appendix 5: Underlying emission values .

4.2 N_2O emissions during freeze – thaw cycle

4.2.1 Flux dynamics of NBC and BC2

In the second experimental period, emissions from NBC and BC2 were analysed between day 38 and 58, a period that included a freeze-thaw cycle. Figure 7 shows the emission trajectory over this timeframe. The average N_2O emission of the clay soil was 232.2 ± 10.6 g N_2O ha^{-1} day^{-1} and 53.3 ± 4.4 g N_2O ha^{-1} day^{-1} for the sandy loam. The clay soil had a sharp emission peak around day 41 (max 679.6 ± 43.4 g

N_2O ha^{-1} day^{-1}), whereas the sandy loam had a broader peak around day 42 (max 143.3 g N_2O ha^{-1} day^{-1}). The clay soil showed distinct emission peaks in all fertilizer treatments (including "no fertilizer"), whereas the sandy loam only had a distinct peak in combination with the NPKS fertilizer. Soil, fertilizer and biochar had no significant effect on when the peak occurred.

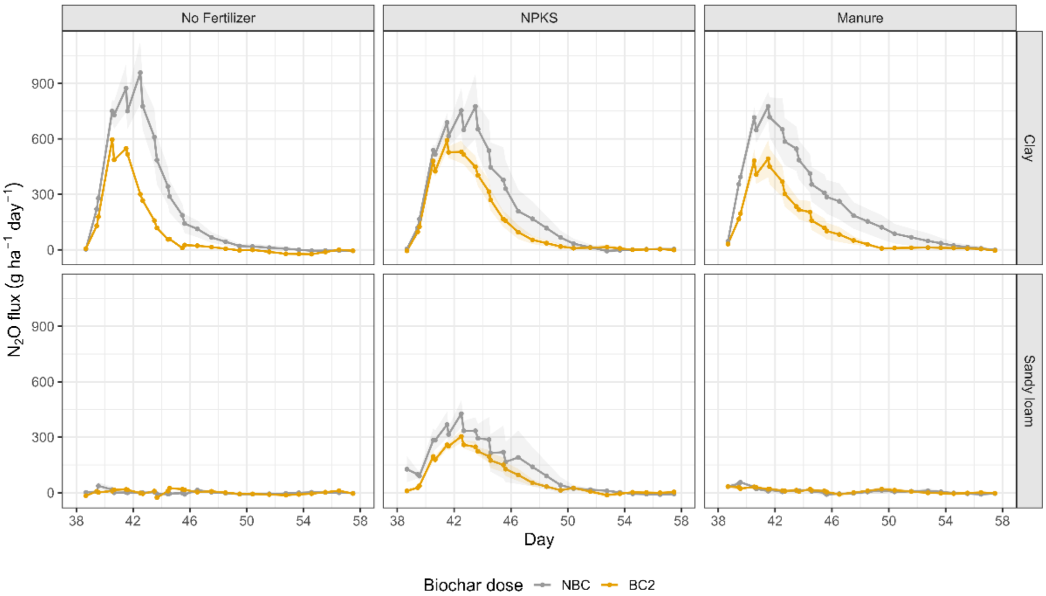

Figure 7 Flux dynamics of N_2O in g ha^{-1} day^{-1} from day 38 till day 58 for every soil x fertilizer combination with NBC and BC2 treatment. Biochar treatments are represented in the indicated colours. The shaded confidence bands represent standard errors.

4.2.2 Cumulative emissions during freeze – thaw cycle

A statistically significant difference was observed between soil types ($p < 0.01$) with the average over all treatments being 3475.9 ± 328.9 g N_2O ha^{-1} for the clay and 809.7 ± 226.3 g N_2O ha^{-1} for the sandy loam. For the clay soil there was no difference ($p = 0.99$) between the NPKS fertilizer (3476.7 ± 466.1 g N_2O ha^{-1}) and the manure (3409.9 ± 641.6 g N_2O ha^{-1}). For the sandy loam, a significant difference ($p < 0.01$) between fertilizer types was observed with the NPKS fertilizer (1958.0 ± 357.9 g N_2O ha^{-1}) resulting in higher emissions than the manure (147.3 ± 52.9 g N_2O ha^{-1}). Observed reductions by BC2 were not significant for emitting treatments (-52.8% for clay x manure, -33.9% for clay x NPKS, -35.5% for sandy loam x NPKS).

The results of the freezing-thawing cycle are summarized in a bar chart in Figure 8, underlying values and statistics can be found in Table 15 and Table 16 in Appendix 5: Underlying emission values .

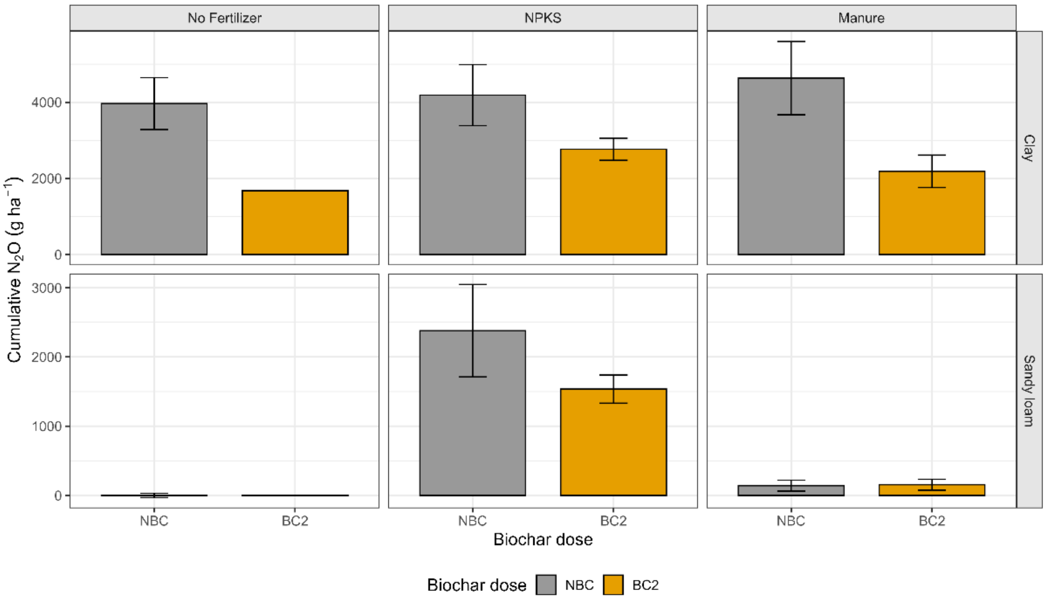

Figure 8 Cumulative N_2O emissions in g ha^{-1} between days 38 and 58 for all soil x fertilizer x biochar combinations. Error bars indicate standard errors. No compact letters are displayed as no significant differences between biochar treatments were observed. Underlying values are presented in Appendix 5: Underlying emission values

4.3 Cumulative N_2O emissions over whole experiment

Results over the whole experiment are dominated by the freezing/thawing cycle as emissions were higher during that phase of the experiment. Only NBC and BC2 are included in the results as the other treatments were not continued in the second phase of the experiment.

A statistically significant difference was observed between soil types ($p < 0.01$) with the average over all treatments being 3723.9 ± 344.9 g N_2O ha^{-1} for the clay and 1077.0 ± 289.4 g N_2O ha^{-1} for the sandy loam. For the clay soil there was no significant difference ($p = 0.99$) between the NPKS fertilizer (3725.9 ± 521.7 g N_2O ha^{-1}) and the manure (3741.3 ± 647.4 g N_2O ha^{-1}). For the sandy loam a significant difference between fertilizer types was observed ($p < 0.01$) with the NPKS fertilizer resulting in higher emissions (2602.4 ± 417 g N_2O ha^{-1} for the NPKS fertilizer and 278.3 ± 59.0 g N_2O ha^{-1} for manure). BC2 showed a significant reduction for clay x manure (-52%, $p = 0.04$). Reductions for clay x NPKS (-37.9%, $p = 0.16$) and for sandy loam x NPKS (-33.8%, $p = 0.40$) were not significant. If averaged over both fertilizers, BC2 showed a significant reduction in the clay soil (-44.2%, $p = 0.04$) and a nonsignificant reduction in the sandy loam (-4%, $p = 0.15$).

The results of the whole experiment are summarized in a bar chart in Figure 9, underlying values and statistics can be found in Table 17 and Table 18 in Appendix 5: Underlying emission values .

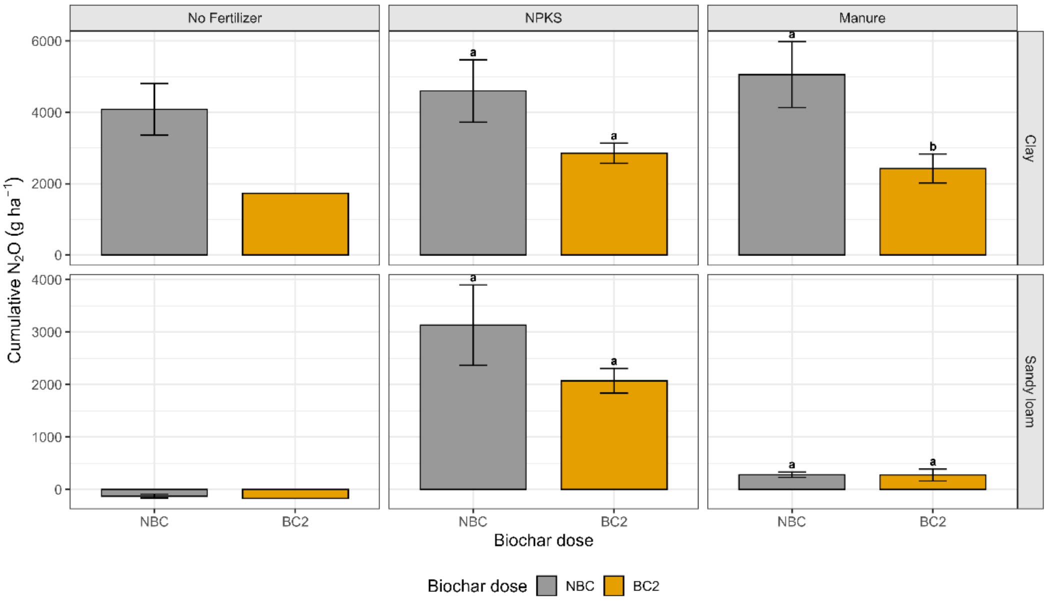

Figure 9 Cumulative N_2O emissions in g ha^{-1} between day 1 and 58 for all soil x fertilizer x biochar combinations. Error bars indicate standard errors. Compact letter display (CLD) is a result of a Tukey-adjusted pairwise comparison of estimated marginal means displayed after an ANOVA in a, b letters. Underlying values are presented in Appendix 5: Underlying emission values .

4.4 N_2O emission factors over whole experiment

The emission factors equal to N- N_2O per applied nitrogen. It is important to note that the values for the unfertilized treatments were based on only one replicate, therefore no statistical tests were performed. The results are summarized in Table 5.

Table 5 Emission factors per equivalent of applied nitrogen in % for all fertilized treatments. Values are based on 150 kg ha^{-1} for the NPKS and 87.5 kg ha^{-1} for the manure as described in 3.2

Soil type	**Fertilizer type**	**Biochar dose**	**N_2O emissions fertilizer – No fertilizer (g ha^{-1})**	**Equals g N (g N ha^{-1})**	**Emission factor (% N/applied N**
Clay	NPKS	NBC	515.0	327.1	0.22%
Clay	NPKS	BC2	1125.5	716.2	0.48%
Clay	Manure	NBC	973.0	619.2	0.71%
Clay	Manure	BC2	697.3	443.7	0.51%
Sandy loam	NPKS	NBC	3259.7	2074.4	1.38%
Sandy loam	NPKS	BC2	2241.6	1426.5	0.95%
Sandy loam	Manure	NBC	408.1	259.7	0.30%
Sandy loam	Manure	BC2	445.2	283.3	0.32%

4.5 Relationship of N_2O with CO_2 and WFPS

Running a regression analysis for CO_2 as a predictor for N_2O emissions, the regression was positively significant for samples with the NPKS fertilizer ($p < 0.01$ for both soils) and not significant for the manure fertilizer. For the non-fertilized samples, the relationship was significant for the clay soil ($p < 0.01$) and non-significant for the sandy loam ($p = 0.90$). The results of the regression analysis are summarized in Figure 10.

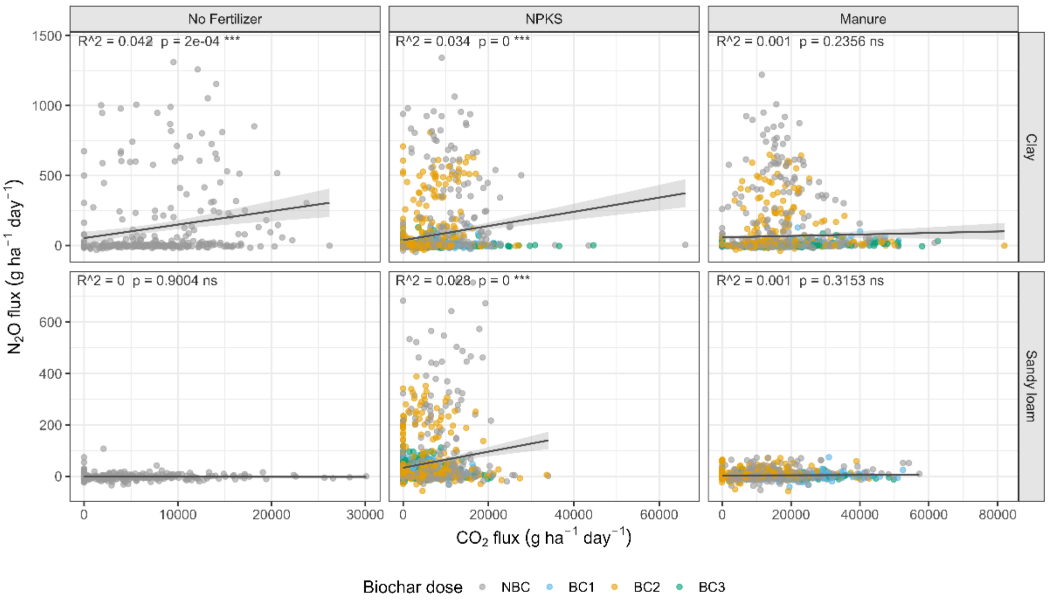

Figure 10 Regression analysis with N_2O over CO_2 for all soil x fertilizer combinations including all biochar treatments, each point represents one measurement day. The regression is pooled across all biochar levels. P values are based on a linear regression analysis. Colours of datapoints indicate biochar types.

A scatterplot was done for N_2O emissions over WFPS to visually assess differences of the relationships across different soil x fertilizer treatments. The scatterplot is shown in Figure 11.

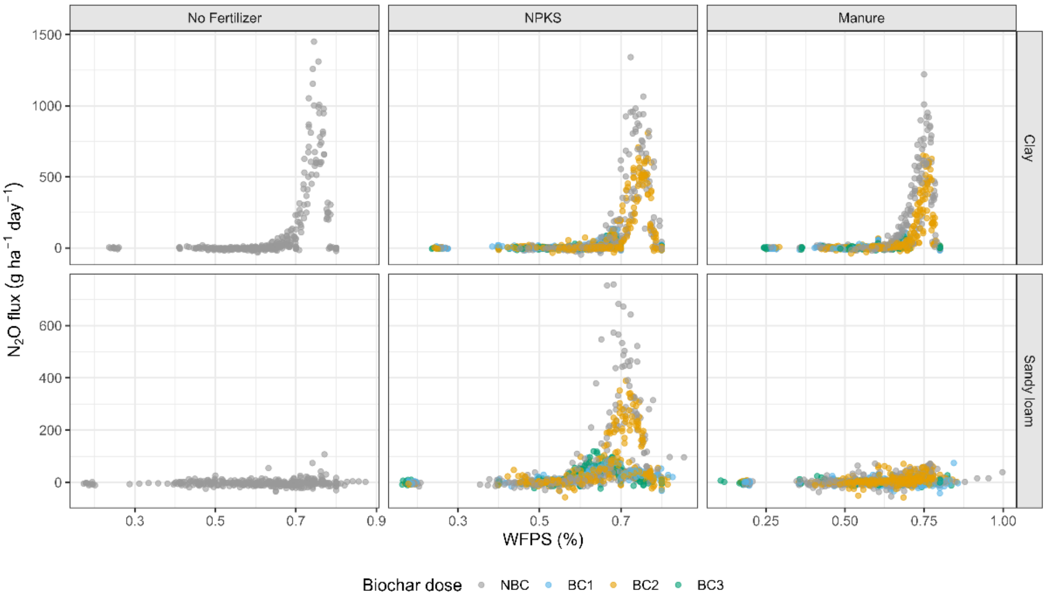

Figure 11 Scatterplot with N_2O over WFPS for all soil x fertilizer combinations including all biochar treatments, each point represents one measurement day.

After visually assessing the scatterplot, an analysis of the WFPS of the highest emissions measurement depending on the fertilizer and soil type was done. The results showed that there were no significant differences between soils and fertilizers for the first peak during the WDC.

For the second peak there was a significant difference in peak WFPS between the NPKS and manure in the clay soil ($p = 0.02$). The peak for the NPKS occurred at an average of 74.66 % WFPS while the peak for the manure occurred at an average of 75.90% WFPS. Also, the soil type had a significant influence on the WFPS of peak emissions ($p < 0.01$). The second peak in the clay soil occurred at 74.66 % WFPS while the peak in the sandy loam occurred at 71.52% WFPS.

5. Discussion

The aim of this thesis was to measure nitrous oxide (N_2O) in two different soils, one sandy and one clayey soil, under a wet-dry (WDC) and freeze-thaw cycle (FTC). Two different fertilizers were applied (NPKS, manure + control). Biochar was tested at four different rates (NBC = 0 t ha^{-1}, BC1 = 6 t ha^{-1}, BC2 = 15 t ha^{-1}, BC3 = 24 t ha^{-1}) in the first part of the experiment to observe differences in N_2O trajectories and cumulative emissions and be able to relate them to treatment effects. The most promising treatment (BC2) was then continued into the second part of the experiment during the freezing-thawing cycle.

5.1 General observations during the experiment

Emissions were mostly driven by the addition of the fertilizers in the clay soil during the first part of the experiment and during the whole experiment in the sandy loam. In the second part, soil induced emissions dominated for the clay soil with no difference between fertilized and non-fertilized treatments. A linear regression analysis indicated a positive relationship of N_2O with CO_2 in NPKS treatments. The lack of relationship between manure fertilized treatments and N_2O could be because of increased CO_2 emissions directly after manure application as labile carbon and microorganisms are present which respire carbon (L. Liu et al., 2024). This would confound CO_2 as a predictor for microbial activity in relation to N_2O formation after manure application.

5.2 Nitrous oxide during wet-dry cycle

During the first part of the experiment a WDC was induced by raising the WFPS to >70% and then measuring the emissions while the samples were drying. Distinct emission peaks were observed for all fertilizer treatments in the clay soil with the highest peaks occurring with the synthetic NPKS fertilizer. No significant difference in cumulative emissions between fertilizers in the clay soil was observed. The manure caused a visibly broader peak in the clay soil than the NPKS fertilizer. This could be a result of differences in the microbial community between the two fertilizers, resulting in different emission trajectories (Lazcano et al., 2021).

In the sandy loam only the NPKS fertilizer caused a peak which was broader, and later than the peak of the NPKS fertilizer in the clay soil. That the peak occurred later, was most likely a result of the described soil settling in the sandy loam. That caused the peak to occur later but at the same WFPS as in the clay. The longer duration of the peak could be a result of increased nitrifier denitrification at lower moisture levels (Bateman & Baggs, 2005). The higher pH of the sandy loam would facilitate N_2O formation through that pathway at lower moisture levels in comparison to the clay soil (Han et al., 2024). However, this relationship cannot be validated as N_2O emission pathways were not analysed in the present study.

The manure did not cause a distinct emission peak in the sandy loam and there was a significant difference between the two fertilizers. The very low emissions for the manure in the sandy loam could be because of increased ammonia (NH_3) volatilization of manure in the sandy soil due to a lower CEC and adsorption potential (Ma et al., 2021; Zhenghu & Honglang, 2000). Also, the higher pH of the sandy loam increases the risk of NH_3 volatilization (Pan et al., 2016). However, this cannot be confirmed as no NH_3 volatilization measurements were conducted.

There was no significant difference between total emissions of the two soils. Emissions were driven by fertilizer input, which is in agreement by the importance of nitrogen application for N_2O emissions (Reay et al., 2012).

In the clay soil, biochar treatments showed significant reduction effects with BC2 mitigating cumulative N_2O emissions of the NPKS fertilizer by 77% while BC3 lowered emissions of the manure by 64.6%. These findings challenge the first hypothesis as biochar reductions were not detectable across soil types. However, the differences in the effective biochar dose supports the second hypothesis of varying effects dependent on dose and fertilizer. The reduction values are higher than average values in meta-analyses (e.g. -38% in Borchard et al., 2019 and -32% in Q. Liu et al., 2018 both based on field and laboratory studies). The high clay content of the clay soil (46%) caused originally lower expectations for the biochar mitigation potential. This is due to a lack of capillary pores in very fine textured soils that hinders the formation of anaerobic microsites where biochar can interact. However, the higher organic C content, the good structure and the lower pH may have been the reason, the biochar amendments still showed an effect. That is due to sufficient carbon supply for the microbial processes, formation of microsites in aggregates and potential for a liming effect (Q. Liu et al., 2018). Another possible reason is that laboratory studies showed higher reduction rates than field studies which are included in the mentioned meta-analyses (Borchard et al., 2019). Also, wood-based biochars produced at high treatment temperature (>500°C) have shown to reduce emissions more effectively than biochar produced at lower temperature and from other feedstock (Q. Liu et al., 2018). This has been attributed to weaker aromatic and surface structures in low temperature biochars (Mandal et al., 2016). Regarding the reduction pathways, the adsorption of substrate, the facilitation of complete denitrification through electron shuttling, liming and the increase of the *nirK* and *nosZ* genes are the current state of knowledge (Aamer et al., 2020; Q. Liu et al., 2018) but the pathways responsible in this study were not analysed.

BC1 did not reduce N_2O emissions which is in line with a certain observed threshold application rate of biochar (L. Zhong et al., 2025). BC3 did not reduce emissions for the NPKS fertilizer in the clay soil while BC2 did. This effect of a lack of reduction at higher doses has been observed in other studies, while the underlying reasons are not fully understood (Shakoor et al., 2021; Y. Zhang et al., 2025).

The need of a higher biochar dose for effective emission reduction for the dairy manure could be a result of the increased microbial activity through organic amendments (Lazcano et al., 2021). Due to the higher activity a subsequent additional need for substrate adsorption, gene abundance increase and electron shuttling would be necessary (Cayuela et al., 2014). However, this theory cannot be validated with the available data, as the reduction pathways were not analysed in the present study and currently no meta-analyses are available comparing the necessary biochar dose depending on the nitrogen source. The need for a higher biochar dose for the organic fertilizer was additionally interesting, as a lower amount of N ha^{-1} was applied as described in 3.2.

The application rate of BC2 with 15 t ha^{-1} for the NPKS fertilizer lies below the threshold application rate of 20 t ha^{-1} observed in a meta-analysis by L. Zhong et al. (2025). However, the effectiveness of the lower application rate could be a result of the more controlled conditions in the laboratory study in comparison to the field studies that L. Zhong et al. (2025) was based on. For the organic fertilizer, the effective application rate of BC3 with 24 t ha^{-1} lies above this threshold.

For the sandy loam no significant reductions and no visible trends through biochar amendments were observed for either of the two fertilizers during the WDC. This is partly consistent with the findings that biochar had a larger effect in more acid soils in comparison to alkaline soils (Q. Liu et al., 2018; Pokharel et al., 2020). The sandy loam had a pH of 7.1 in comparison to the clay soil with 5.7, which could explain the reduced effect of biochar in the sandy loam to a certain extent. However, even though the mentioned meta-analyses found a pH dependent effect, it was not completely absent in alkaline soils. Therefore, the difference in pH might not fully explain the lack of effect. Biochar has shown to have a larger effect in soils with a higher soil organic carbon content as carbon is necessary for the heterotrophic denitrification process with which biochar interacts (Q. Liu et al., 2018). The lower carbon content in the sandy loam could be a reason for the lack of biochar effect. A meta-analysis by Cayuela et al. (2013) has shown that biochar promotes the last step of denitrification mostly in fine textured soils, however there was no observed relationship between texture and total mitigation capacity. Therefore, the reason for the lack of effect in the sandy loam remains inconclusive.

5.3 Nitrous oxide during freeze-thaw cycle

During the second part of the experiment the WFPS was first increased to 80% and then the soils were frozen at -22°C for 7 days. During the thawing process emissions were measured with a higher temporal resolution for the samples with NBC and BC2 treatments. Both soils showed much higher total N_2O emissions during the freeze-thaw cycle (FTC) than during the WDC (93.6% of total observed emissions for the clay soil and 74.7% for the sandy loam during the FTC). The clay soil had significantly higher total emissions than the sandy loam. The clay soil showed high emissions for all fertilizer treatments including the non-fertilized treatments which indicates soil-derived emissions instead of fertilizer-induced. This increase in soil-derived emissions is in line with the current theory of how FTC-induced emissions are formed. The clay soil had a high carbon and nitrogen content and a very good soil structure. With the destruction of aggregates and cells, nitrogen which is present in soil aggregates becomes available for microbes which is linked to increased soil derived emissions (Christensen & Christensen, 1991; Oztas & Fayetorbay, 2003; Schimel et al., 2007). This dynamic differs to the WDC in which emissions were mostly induced by fertilizers in both soils.

The sandy loam only showed high emissions for the NPKS fertilizer with emissions for the manure being similar to the non-fertilized samples. The sandy loam had a weaker structure and lower carbon and nitrogen content, which could explain the continuous importance of fertilizer induced emissions as the breakdown of aggregates was supposedly of minor importance in comparison to the clay soil. However, the nonetheless increased emissions in comparison to the WDC could be a result of cell breakdown, increased emissions through higher soil moisture and release of formerly produced N_2O (Schimel et al., 2007; Wagner-Riddle et al., 2008). These results support the third hypothesis of the importance of FTC induced emissions and its difference between different soils. This finding is in accordance with a number of existing studies. Wagner-Riddle et al. (2007) showed that between 30 & 90% of yearly emissions occurred during the non-growing season mostly due to freeze-thaw induced emissions. In years with important thawing events, thaw induced emissions have accounted for 70% of annual emissions in a northern prairie cropland (Wagner-Riddle et al., 2024). In another field experiment, the frost depth and intensity were strongly correlated to freeze-thaw emission increases (Yanai et al., 2011). Priemé & Christensen (2001) observed up to 2.5x higher emissions after a freezing-thawing cycle than after a wet-dry cycle. One possible explanation for the high peaks in the present experiment (~12x higher peaks for the clay soil, ~5x for the sandy loam; in comparison to peaks observed during WDC, averaged over all fertilizers) was the very low freezing temperature because of the freezing room availability. Pelster et al. (2019) tested freezing temperatures between -3 and -1°C and showed that emissions were highest when freezing intensity and thawing rates were highest. Koponen & Martikainen (2004) showed that a deep frost (-15°C) greatly increased emissions after formerly, milder FTCs were applied. In the present experiment, freezing intensity (-22°C) and thawing rate (direct transfer to the 15°C room) were higher than in the mentioned studies which could possibly explain the high peaks.

Observed reductions (-52.8% for clay x manure, -33.9% for clay x NPKS, -35.5% for sandy loam x NPKS) by biochar application were not significant during the FTC. This finding did not support the first hypothesis of biochar effects throughout different cycles. It is theorized that the lack of significance was due to high data variability during the FTC. This differs from a number of conducted studies which found important reduction potentials by applying biochar during FTCs (e. g. Kettunen & Saarnio, 2013; X. Liu et al., 2016; X. Yang et al., 2023; Z. Yang et al., 2022). There are currently no meta studies available comparing reduction potentials of biochar during FTCs depending on soil properties. Also, no process-based measurements, e.g. substrate & enzyme measurements, were conducted in the present study which limits interpretability of why emissions were not reduced. Therefore, further research is of additional importance as the greater part of total fluxes was registered during the FTC and the underlying processes and mitigation potentials are not fully understood.

5.4 Nitrous oxide during whole experiment and emission factors

Over the course of the whole experiment overall emissions were strongly dominated by emissions that occurred during the freezing-thawing period. A significant difference was observed between soil types over the whole experiment with the clay soil having significantly higher average total emissions. This is mostly due to the sandy loam almost not showing emissions under the manure treatment and very high soil induced emissions of the clay soil during the FTC. This difference for the two soils is in agreement with the higher emissions in fine textured soils due to increased pore connectivity (Patel et al., 2021). Another part of the higher emissions of the clay soil could be explained by the higher carbon content and therefore possibly higher microbial activity (Harrison-Kirk et al., 2013) and by the higher soil nitrogen content. The clay soil also has a lower pH (5.7) possibly inhibiting the last step of denitrification. B. Liu et al. (2014) observed an inhibition of N_2O reductase below pH 6.1 leading to higher denitrification induced emissions. Possible reasons for the strong differences between the two soils after the FTC were given in 5.3.

For the clay soil, no difference between fertilizer types was observed while the NPKS fertilizer had higher emissions than the manure for the sandy loam. Possible reasons are strong ammonia volatilization of the manure in the sandy soil due to a higher pH and coarser texture as discussed earlier (5.2).

BC2 significantly reduced emissions for the manure in the clay soil (-52.8%). This reduction was only significant for the whole experimental duration, not if only the WDC or FTC was considered. This indicates that the determination of reduction potentials of BC2 for the manure during the separate cycles was less accurate due to high variability and fewer observations. Including the whole dataset (58 days) increased statistical power and showed significant reductions. This is supported by observed (even though non-significant) reductions for separate cycles in clay x manure x BC2 of 45.9% (WDC) and 52.8% (FTC). As potential reduction pathways during FTCs, increased substrate adsorption have been proposed but the current knowledge is limited (X. Yang et al., 2023). This result opens room for further studies particularly on biochar effects during FTCs to mitigate these important emissions as described earlier.

Observed emission factors during the experiment ranged from 0.22 to 1.38% for NPKS and from 0.30 to 0.71% for manure. For wet climates, the IPCC proposes a 1.6% tier 2 emission factor for synthetic fertilizers and a 0.6% emission factor for manure. The manure caused higher emission factors in the clay soil while the NPKS caused higher emission factors in the sandy loam indicating soil and fertilizer specific factors. During the limited duration of the experiment most of the observed emission factors fell below these values. Only the clay soil with manure had a higher EF (0.71%).

5.5 Limitations of the study

Important limitations of this study stem from the experimental design as important interactions, e. g. temperature change, root activity, plant growth, lateral water movement, were excluded which are present in field studies. This approach was chosen to have more controlled conditions to get indicative results for future field studies. Also, only one WDC and one FTC was applied, simulating different conditions in contrast to reality with multiple cycles per season. This is a particularly important limitation for the interpretation of the calculated emission factors. Another limitation was the low freezing temperature (-22°C) which was a result of freezing room availability at the department. The impacts of this low temperature were discussed in 5.3.

There was only one replicate for non-fertilized treatments with biochar to calculate emission factors. This limitation to one replicate limited the statistical interpretability of these results. They were included nonetheless to be able to estimate emission factors and differentiate fertilizer induced emissions from soil induced emissions.

The settling of the sandy loam in first part of the study (3.2) might have had an influence on the results and might have reduced precision to determine a treatment effect. This settling caused a significantly higher variance ($p < 0.01$) of WFPS in samples of the sandy loam in comparison to the clay. As WFPS proved to be a rather good predictor for N_2O emissions, potential effects of biochar might have been obscured by the higher inner treatment variance of WFPS. This would open room for additional studies with more careful sample preparation.

During the increased temporal measurement frequency during the FTC, a decrease in N_2O emissions for the second daily measurement was observed on every day on which two measurements were taken (visible in Figure 7). It was hypothesized that this was the result of a depletion of N_2O in the soil pores because of the air flow created by the INNOVA measurement device. Due to this depletion in the soil pores, the partial pressure of N_2O in the soil pores could have been reduced and the flux rate lowered at the second measurement. This observation possibly puts a limitation on the maximum temporal frequency of measurements using devices that create a strong airflow.

Another confounding effect was due to the different amounts of applied nitrogen for the two fertilizer types. This was a result of greatly differing nitrogen content estimations (0.6%) as given by Andersson et al. (2026) and actual nitrogen contents (0.35%) in the manure. This limits the comparison of the two different fertilizers.

No mechanistic explanation of the biochar effect was possible as indicating properties such as NH_4^+/NO_3^- concentrations or enzyme values were not measured due to time and resource restrictions. However, the goal of the study was not to provide mechanistic explanations for the observed effects but to observe general trends which can give future research options. In future studies mechanistic measurements should be included to achieve a more process-based understanding of underlying processes. This is important to develop reliable biochar application programs, taking biochar production and soil properties into account.

6. Conclusion and outlook

This study demonstrated that biochar's capacity to mitigate N_2O emissions is strongly dependent on soil and fertilizer properties as well as on environmental conditions. Significant reductions were found for the clay soil during the WDC. BC2 (15 t ha^{-1}) reduced emissions by 77% for the NPKS fertilizer while BC3 (24 t ha^{-1}) reduced emissions by 65% for the manure. During the FTC no significant reductions were observed despite consistent trends. The non-significant effect in the sandy loam could be partly explained by a higher soil pH, limiting the liming potential of biochar and a lower carbon content, limiting denitrification activity. Also, observed soil settling during the WDC in the sandy loam leading to differences in WFPS possibly played a role.

The findings showed differences in the effective biochar dose dependent on the fertilizer type during the WDC, an interaction that is currently not fully understood with the need of further research. Furthermore, a certain threshold effect was observed, with BC1 (6 t ha^{-1}) not reducing emissions while higher doses did. Further research is needed to provide reliable application regimes, taking interactions of soil, fertilizer and biochar types into account. This would allow to efficiently mitigate N_2O from agricultural fields with the appropriate biochar doses.

In the FTC only the most promising biochar treatment (BC2) was continued and showed no significant reductions despite visible trends for all emitting treatments (-34% to -53%). Emissions were much higher in the FTC with 93.6% (clay) and 74.7% (sandy loam) of total emissions occurring during that cycle. The very high shares of N_2O emissions during the FTC underscore the importance of FTC induced emissions and call for further research on the mitigation potential of biochar under these environmental conditions. Further studies should include mechanistic measurements on the mitigation pathways of biochar during FTCs and focus on the mentioned dose effects. Mechanistic measurements should include repeated measurements of substrate availability, pH and enzyme activity. The results also accentuate the need for a more detailed inclusion of FTC induced emissions into the IPCC emission factors, a field currently not sufficiently addressed.

While BC2 did not reduce emissions during the FTC or WDC in clay x manure, it reduced emissions by 52.8% if the whole experimental duration is considered. This indicates effects during both cycles that possibly became significant by pooling measurements and increasing statistical power by including more datapoints. This showed the high variability of N_2O emissions during FTCs and underlines the necessity of high replicate numbers to achieve significant results.

The results of the present thesis showed important interactions between soils, fertilizers, biochar doses and environmental interactions. With further research, context-adapted and dose-optimized biochar recommendations can be achieved.

This is particularly important in the context of accelerating climate change, addressing emissions of an important greenhouse gas.

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Looking back at the past years of studies I am extremely glad about all the people I met on the way, and I am really happy to have shared such an amazing time together. To come to Uppsala together with so many great friends was a particularly great experience, and I will always remember this year as one of my best throughout my education!

A special mention at this point goes to the Türkenschanzchampion and of course to the Bengelsburg.

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Popular science summary

Nitrous oxide is a gas that is nearly 300 times more potent as a greenhouse gas than carbon dioxide. Most of these emissions come from agricultural soil, where microorganisms convert fertilizer nitrogen into nitrous oxide. Finding ways to reduce these emissions without sacrificing agricultural productivity is one of the important challenges of our time.

One promising approach is the addition of biochar to agricultural soils. Biochar is a charcoal-like material produced by heating wood or other organic materials at high temperatures with little to no oxygen (pyrolysis). The resulting material is porous, carbon-rich, and very stable in the soil. When mixed into agricultural soils, biochar can change the soil environment in ways that reduce the production of nitrous oxide by soil microorganisms.

This study tested how different amounts of woodchip biochar affect nitrous oxide emissions in two different soils under controlled laboratory conditions. One soil was a heavy clay soil, the other a sandy loam. Both were fertilized with either synthetic NPKS fertilizer or dairy manure. Three doses of biochar were tested alongside an untreated control.

The experiment also simulated two cycles that are important for nitrous oxide formation: a wet-dry cycle, where soil moisture is raised and then allowed to decrease, and a freeze-thaw cycle, where soils are frozen and then thaw.

The results showed that biochar was effective in the heavy clay soil, reducing nitrous oxide emissions by up to 77% during the wet-dry cycle depending on the fertilizer type and biochar dose applied. The sandy soil showed no significant response to biochar, possibly because of its higher pH and lower carbon content.

Importantly, emissions during the freeze-thaw cycle were far higher than during the wet-dry phase, accounting for 94% and 75% of total emissions for the clay respectively the sandy loam. This confirms that freeze-thaw events are critically important contributors to agricultural greenhouse gas budgets, yet they are currently not adequately accounted for in international emission guidelines. Biochar did not significantly reduce emissions during freezing and thawing. However, reductions were observed when taking the whole experiment into account, opening room for more studies which include freezing-thawing cycles. Over the course of the whole experiment emissions induced by the manure in the clay soil were reduced by 52.8% after biochar treatment.

This study shows that biochar can be a useful tool for reducing nitrous oxide emissions, but that its effectiveness strongly depends on the soil it is applied to, the fertilizer and the conditions under which emissions occur. More research is needed, particularly on how biochar behaves during freeze-thaw events and dose dependent effects. By improving the understanding of the underlying processes, reliable recommendations for agricultural management are achievable in the future.

Appendix 1: Treatment layout

Table 6 Overview over all sample numbers with respective treatment

Nr.	Treatment	Nr.	Treatment	Nr.	Treatment
1	Sandy_loam_Manure_NBC	33	Sandy_loam_NPKS_BC2	65	Clay_NPKS_BC2
2	Clay_NPKS_NBC	34	Sandy_loam_NPKS_BC3	66	Sandy_loam_BC2
3	Sandy_loam_NPKS_NBC	35	Clay_BC2	67	Clay_No_fertilizer_NBC
4	Clay_BC1	36	Sandy_loam_Manure_NBC	68	Clay_NPKS_NBC
5	Sandy_loam_BC1	37	Clay_Manure_NBC	69	Sandy_loam_Manure_BC3
6	Clay_No_fertilizer_NBC	38	Sandy_loam_NPKS_NBC	70	Sandy_loam_NPKS_BC1
7	Clay_BC3	39	Sandy_loam_NPKS_NBC	71	Sandy_loam_NPKS_BC3
8	Clay_NPKS_BC1	40	Clay_NPKS_BC1	72	Sandy_loam_Manure_NBC
9	Clay_No_fertilizer_NBC	41	Sandy_loam_NPKS_BC2	73	Sandy_loam_NPKS_BC1
10	Clay_Manure_BC3	42	Clay_Manure_BC2	74	Sandy_loam_NPKS_BC3
11	Clay_Manure_NBC	43	Sandy_loam_NPKS_BC3	75	Clay_Manure_BC1
12	Sandy_loam_Manure_BC2	44	Clay_Manure_BC1	76	Clay_Manure_NBC
13	Clay_Manure_BC2	45	Clay_Manure_BC2	77	Clay_No_fertilizer_NBC
14	Sandy_loam_NPKS_NBC	46	Sandy_loam_NPKS_BC2	78	Clay_NPKS_BC1
15	Sandy_loam_NPKS_BC1	47	Sandy_loam_Manure_BC2	79	Clay_NPKS_BC3
16	Sandy_loam_NPKS_BC3	48	Sandy_loam_NPKS_NBC	80	Clay_NPKS_BC3
17	Clay_NPKS_NBC	49	Sandy_loam_Manure_BC2	81	Clay_NPKS_BC1
18	Clay_Manure_BC2	50	Clay_No_fertilizer_NBC	82	Sandy_loam_Manure_BC1

19	Clay_NPKS_NBC	51	Sandy_loam_No_fertili zer_NBC	83	Clay_NPKS_NBC
20	Sandy_loam_NPKS _BC1	52	Sandy_loam_No_fertili zer_NBC	84	Clay_NPKS_BC2
21	Sandy_loam_Manu re_BC2	53	Sandy_loam_NPKS_B C1	85	Sandy_loam_Manure_ BC1
22	Sandy_loam_Manu re_BC3	54	Sandy_loam_No_fertili zer_NBC	86	Clay_Manure_BC3
23	Clay_NPKS_BC2	55	Sandy_loam_Manure_ BC2	87	Sandy_loam_Manure_ BC3
24	Sandy_loam_NPKS _BC2	56	Clay_Manure_BC1	88	Sandy_loam_BC3
25	Sandy_loam_Manu re_NBC	57	Sandy_loam_Manure_ BC1	89	Clay_Manure_NBC
26	Sandy_loam_Manu re_BC3	58	Clay_NPKS_BC1	90	Clay_NPKS_BC2
27	Sandy_loam_Manu re_NBC	59	Clay_NPKS_BC3	91	Clay_Manure_BC3
28	Sandy_loam_Manu re_BC1	60	Sandy_loam_Manure_ BC3	92	Sandy_loam_NPKS_B C2
29	Clay_NPKS_BC3	61	Clay_NPKS_BC3	93	Sandy_loam_No_fertili zer_NBC
30	Clay_Manure_NBC	62	Clay_Manure_BC1	94	Clay_Manure_BC3
31	Clay_Manure_BC2	63	Clay_Manure_BC3	95	Sandy_loam_No_fertili zer_NBC
32	Sandy_loam_Manu re_BC1	64	Clay_Manure_BC1	96	Clay_Ammoniu_sulfat e_BC2

Appendix 2: Soil properties

Table 7 Soil properties as results of laboratory measurements. Exact methods are mentioned when known. Other results were received from an external laboratory and measured by standard methods.

Property and unit	**Clay**	**Sandy loam**	**Method**
Clay content (%)	45.2		Note: samples were given to the laboratory, but results were not received at the time of the hand in. The classification as sandy loam is based on a personal communication (E. Karltun, personal communication, 2026)
Silt content (%)	38.6		
Sand content (%)	6.5		
LOI	9.7		
pH	5.7	7.1	
Tot-N (%)	0.3	0.13	LECO TruMac CN
Tot-C (%)	2.9	1.48	LECO TruMac CN
P-AL mg/100 g	1.9	26	
Kl	I	V	
K-AL mg/100 g	22.4	17.7	
Kl	IV	IV	
Mg-AL mg/100 g	26.1	6.1	
K/Mg-AL	0.9	2.9	
Ca-AL mg/100 g	265	240	
Al-AL mg/100 g	51	24	
Fe-AL mg/100 g	67	16	
K-HCl mg/100 g	415.7	182.6	
Kl	5	3	
P-HCl mg/100 g	71.6	75	
Kl	4	4	
Cu-HCl mg/kg	34.3	16.1	
B mg/kg	1.5	0.6	

Appendix 3: Fertilizer properties

Table 8 Properties of the applied manure as received from an external laboratory.

Property	**Value**	**Unit**
Dry substance	13.1	%
Total Nitrogen	3.5	kg/(ton)
Organic Nitrogen	3.1	kg/(ton)
Ammonium Nitrogen	0.4	kg/(ton)
Total Carbon	58.9	kg/(ton)
Total C/Total N	16.9	-
Total Phosphorus	1.14	kg/(ton)
Total Potassium	1.09	kg/(ton)
Total Magnesium	1.00	kg/(ton)
Total Calcium	2.64	kg/(ton)
Total Sodium	0.23	kg/(ton)
Total Sulphur	0.45	kg/(ton)
pH	7.5	-

Appendix 4: Biochar properties

Table 9: Biochar properties of woodchip biochar. [a] DIN 51719: 1997-07, [b] DIN 51732: 2014-07, [c] DIN 51724-3: 2012-07, [d] DIN 51733: 2016-04, [e] DIN 51726: 2004-06, [f] DIN 51720: 2001-03, [g] BGK III. C2: 2006-09

Parameter	**Dry base**	**Unit**
Ash content (550°C) [a]	2.2	% (w/w)
Total carbon [b]	94.1	% (w/w)
carbon (organic)	93.6	% (w/w)
Hydrogen [b]	1.5	% (w/w)
Total nitrogen [b]	0.48	% (w/w)
Sulphur (S), total [c]	0.03	% (w/w)
Oxygen [d]	2.3	% (w/w)
Total inorganic carbon (TIC) [e]	0.5	% (w/w)
carbonate- CO_2 [e]	1.7	% (w/w)
H/C ratio (molar)	0.19	
H/Corg ratio (molar)	0.19	
O/C ratio (molar)	0.018	
Volatile Compounds [f]	4.0	% (w/w)

Table 10: Elements from the borate digestion of ash 550 °C acc. to DIN 51729-11:1998-11(AR); all elements analysed according to DIN EN ISO 11885 (E22):2009-09

Element	**Value**	**Unit**
Calcium as CaO	31.0	% (w/w)
Iron as Fe2O3	2.9	% (w/w)
Potassium as K2O	15.3	% (w/w)
Magnesium as MgO	5.0	% (w/w)
Sodium as Na2O	0.7	% (w/w)
Phosphorus as P2O5	1.9	% (w/w)
sulphur as SO3	1.1	% (w/w)
Silicon as SiO2	3.9	% (w/w)

Table 11: Macronutrients-LiBO2/Li2B4O7/LiBr-melt of ash 550°C acc. to DIN 51729-11:1998-11(OS), all macronutrients analysed according to DIN EN ISO 11885 (E22):2009-09

Macronutrient	Value	Unit
Phosphorus as P_2O_5	0.4	g/kg
Potassium as K_2O	3.3	g/kg
Calcium as CaO	6.7	g/kg
Magnesium as MgO	1.1	g/kg
Sodium as Na_2O	0.2	g/kg
sulphur as SO_3	0.2	g/kg

Table 12: Elements from the borate digestion of ash 550°C acc. to DIN 51729-11:1998-11(OS) all elements analysed according to DIN EN ISO 11885 (E22):2009-09

Element	Value	Unit
Iron (Fe)	0.4	g/kg
Silicon (Si)	0.4	g/kg

Appendix 5: Underlying emission values and statistics

Table 13 Differences in N_2O emissions during the wet-dry-cycle between NBC and BC treatments with p values as a result of Tukey pairwise comparisons on estimated marginal means. Positive values in the estimate column indicate reductions

contrast	**Soil type**	**Fertilizer type**	**estimate**	**SE**	**df**	**t**	**p**
NBC - BC1	Clay	NPKS	44.75	100.80	67	0.44	.971
NBC - BC2	Clay	NPKS	300.03	100.80	67	2.98	.021*
NBC - BC3	Clay	NPKS	151.98	100.80	67	1.51	.439
NBC - BC1	Sandy loam	NPKS	-6.50	100.80	67	-0.06	1.00
NBC - BC2	Sandy loam	NPKS	121.31	100.80	67	1.20	.627
NBC - BC3	Sandy loam	NPKS	-66.72	100.80	67	-0.66	.911
NBC - BC1	Clay	Manure	114.41	100.80	67	1.14	.669
NBC - BC2	Clay	Manure	189.58	100.80	67	1.88	.246
NBC - BC3	Clay	Manure	291.72	100.80	67	2.89	.026*
NBC - BC1	Sandy loam	Manure	63.16	100.80	67	0.63	.923
NBC - BC2	Sandy loam	Manure	10.86	100.80	67	0.11	1.00
NBC - BC3	Sandy loam	Manure	73.01	100.80	67	0.72	.887

Table 14 Cumulative N_2O emissions in g ha^{-1} from day 1 until day 31 with standard errors (n = 5), values without standard errors only had one repetition. Compact letter display (CLD) is a result of a Tukey-adjusted pairwise comparison of estimated marginal means after an ANOVA displayed in a, ab & b. Values are calculated over Soil x Fertilizer x Biochar treatment and Soil x Biochar to show effects independent of the Fertilizer type.

Soil type	Fertilizer type	Biochar dose	Mean ± SE	CLD soil x fert
Clay	No fertilizer	NBC	119.9 ± 52.6	NA
		BC1	171.7	excluded (n<2)
		BC2	45.1	excluded (n<2)
		BC3	82.8	excluded (n<2)
	NPKS	NBC	404.6 ± 192.5	a
		BC1	324.1 ± 99.0	ab
		BC2	92.9 ± 51.0	b
		BC3	211.1 ± 68.6	ab
	Manure	NBC	387.2 ± 126.2	a
		BC1	308.6 ± 50.3	ab
		BC2	209.3 ± 71.2	ab
		BC3	137.1 ± 76.1	b
Sandy loam	No fertilizer	NBC	-123 ± 30.1*	NA
		BC1	8.6	excluded (n<2)
		BC2	-157.2*	excluded (n<2)
		BC3	-184.5*	excluded (n<2)
	NPKS	NBC	641.9 ± 55.8	a
		BC1	684.2 ± 58.2	a
		BC2	532.3 ± 72	a
		BC3	750.2 ± 83.4	a
	Manure	NBC	114.2 ± 65.3	a
		BC1	15.2 ± 44	a
		BC2	91.6 ± 54.5	a
		BC3	-0.4 ± 31.3*	a
Soil type	**Biochar dose**		**Mean ± SE**	**CLD soil**
Clay	NBC		303.9 ± 80.8	a
	BC1		303.2 ± 49.2	ab
	BC2		141.5 ± 42.4	b
	BC3		165.8 ± 45.8	b
Sandy loam	NBC		211 ± 90	a
	BC1		318.7 ± 110	a
	BC2		269.3 ± 87.8	a
	BC3		324 ± 129.7	a

** Negative total emission values are most likely a result of fluctuations of measurements due to the natural inaccuracy of the measurement device. Negative emissions are very unlikely to occur at the WFPS levels in the current experiment (Ruser et al., 2006). Therefore, the values most probably represent zero N2O fluxes.*

Table 15 Differences in N_2O emissions during the freeze-thaw-cycle between NBC and BC treatments with p values as a result of Tukey pairwise comparisons on estimated marginal means. Values are based on log-transformed values as described in 3.7. Positive values in the estimate column indicate reductions

contrast	**Soil type**	**Fertilizer type**	**estimate**	**SE**	**df**	**t**	**p**
NBC - BC2	Clay	NPKS	0.70	0.58	33	1.22	.232
NBC - BC2	Sandy loam	NPKS	-0.04	0.58	33	-0.07	.945
NBC - BC2	Clay	Manure	0.41	0.58	33	0.70	.486
NBC - BC2	Sandy loam	Manure	-0.34	0.58	33	-0.58	.564

Table 16: Cumulative N_2O emissions in g ha^{-1} from day 38 until day 58 with standard errors (n = 5), values without standard errors only had one repetition. Compact letter display (CLD) is not displayed as there were no significant differences between biochar treatments.

<table>
<tr><th>Soil type</th><th>Fertilizer type</th><th>Biochar dose</th><th>Mean ± SE</th></tr>
<tr><td rowspan="6">Clay</td><td rowspan="2">No fertilizer</td><td>NBC</td><td>3965.2 ± 680.3</td></tr>
<tr><td>BC2</td><td>1679.3</td></tr>
<tr><td rowspan="2">NPKS</td><td>NBC</td><td>4187.1 ± 800.3</td></tr>
<tr><td>BC2</td><td>2766.3 ± 290.8</td></tr>
<tr><td rowspan="2">Manure</td><td>NBC</td><td>4633.1 ± 960.8</td></tr>
<tr><td>BC2</td><td>2186.7 ± 425.8</td></tr>
<tr><td rowspan="6">Sandy loam</td><td rowspan="2">No fertilizer</td><td>NBC</td><td>-0.6 ± 30.5*</td></tr>
<tr><td>BC2</td><td>3.2</td></tr>
<tr><td rowspan="2">NPKS</td><td>NBC</td><td>2380.0 ± 667.7</td></tr>
<tr><td>BC2</td><td>1535.9 ± 203.4</td></tr>
<tr><td rowspan="2">Manure</td><td>NBC</td><td>140.3 ± 79.8</td></tr>
<tr><td>BC2</td><td>154.3 ± 78.8</td></tr>
<tr><th>Soil type</th><th colspan="2">Biochar dose</th><th>Mean ± SE</th></tr>
<tr><td rowspan="2">Clay</td><td colspan="2">NBC</td><td>4261.8 ± 445.5</td></tr>
<tr><td colspan="2">BC2</td><td>2404.0 ± 247.5</td></tr>
<tr><td rowspan="2">Sandy loam</td><td colspan="2">NBC</td><td>839.9 ± 357.9</td></tr>
<tr><td colspan="2">BC2</td><td>768.6 ± 240.6</td></tr>
</table>

** Negative total emission values are most likely a result of fluctuations of measurements due to the natural inaccuracy of the measurement device. Negative emissions are very unlikely to occur at the WFPS levels in the current experiment (Ruser et al., 2006). Therefore, the valued most probably represent zero N2O fluxes.*

Table 17 Differences in N_2O emissions during the whole experiment between NBC and BC treatments with p values as a result of Tukey pairwise comparisons on estimated marginal means. Values are based on log-transformed values as described in section 3.7. Positive values in the estimate column indicate reductions

contrast	Soil type	Fertilizer type	estimate	SE	df	t	p
NBC - BC2	Clay	NPKS	0.47	0.33	33	1.42	.164
NBC - BC2	Sandy loam	NPKS	0.28	0.33	33	0.85	.401
NBC - BC2	Clay	Manure	0.70	0.33	33	2.12	.041*
NBC - BC2	Sandy loam	Manure	0.51	0.33	33	1.55	.131

Table 18: Cumulative N_2O emissions over the whole experiment with standard errors (n = 5), values without standard errors only had one repetition. Compact letter display (CLD) is a result of a Tukey-adjusted pairwise comparison of estimated marginal means after an ANOVA displayed in a & b. Values are summarized averaged over Soil x Fertilizer x Biochar treatment and also Soil x Biochar to show effects independent of the Fertilizer type.

Soil type	Fertilizer type	Biochar dose	Mean ± SE	CLD soil x fert
Clay	No fertilizer	NBC	4084.2 ± 722.7	NA
		BC2	1728.1	excluded (n<2)
	NPKS	NBC	4598.2 ± 874.5	a
		BC2	2853.6 ± 282.3	a
	Manure	NBC	5057.2 ± 925.0	a
		BC2	2425.4 ± 405.8	b
Sandy loam	No fertilizer	NBC	-127.2 ± 35.9*	NA
		BC2	-169.3*	excluded (n<2)
	NPKS	NBC	3132.5 ± 766.0	a
		BC2	2072.3 ± 235.0	a
	Manure	NBC	280.9 ± 51.5	a
		BC2	275.8 ± 114.1	a
Soil type	**Biochar dose**		**Mean ± SE**	**CLD soil**
Clay	NBC		4579.9 ± 464.1	a
Clay	BC2		2556.6 ± 235.5	b
Sandy loam	NBC		1095.4 ± 454.4	a
Sandy loam	BC2		1051.9 ± 317.3	a

** Negative total emission values are most likely a result of fluctuations of measurements due to the natural inaccuracy of the measurement device. Negative emissions are very unlikely to occur at the WFPS levels in the current experiment (Ruser et al., 2006). Therefore, the valued most probably represent zero N2O fluxes.*

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