



Mitigating Nitrous Oxide Emissions from Grassland Peat Soils

Interactive Effects of Carbon to Nitrogen Ratio Amendments and Clay Addition under Fertilization and Simulated Precipitation

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Abstract

Drained peatlands used for agricultural purposes become greenhouse gas emission hotspots by accelerating the decomposition of organic matter. Nitrous oxide (N₂O) emissions from fertilized peat soils are strongly influenced by soil moisture dynamics; however, the extent to which clay addition modifies the effectiveness of carbon and nitrogen (C/N) amendments (C:woodchips, biochar and N:fertilizer) in mitigating these emissions remains poorly understood. This study investigated the individual and interactive effects of clay addition and C/N amendments on N₂O emissions from grassland peat soils under fertilization and simulated precipitation. A six-week controlled lysimeter experiment was conducted using 18 intact peat soil cores. Natural and clay-amended peat soils received no amendments, woodchips, or biochar, with three replicates per treatment. All lysimeters received calcium ammonium nitrate fertilizer and were subjected to three simulated rainfall events. Nitrous oxide emissions were measured using a closed static chamber technique and analyzed using a linear mixed-effects model. The linear mixed-effects analysis showed significant temporal variability in N₂O fluxes throughout the experimental period ($F_{24} = 2.37$, $p < 0.05$). However, the main effect of amendment type (Control, Biochar and Woodchip) was not significant ($F_2 = 0.58$, $p = 0.57$) and there was no significant main effect of soil type (natural versus clay-treated peat; $F_1 = 0.36$, $p = 0.55$). Furthermore, no significant amendment $\times$ soil type interaction was detected ($F_2 = 1.36$, $p = 0.28$), indicating that the effects of amendments did not differ significantly between natural and clay-treated peat soils. Despite the absence of significant treatment effects, descriptive comparisons showed lower cumulative N₂O emissions following amendment application. In natural peat soil, biochar and woodchips reduced cumulative N₂O emissions by approximately 42% and 47%, respectively, compared to the control. In clay-treated peat soil, wood chips reduced cumulative N₂O emissions by approximately 39%, whereas biochar did not show a comparable reduction; instead, it resulted in a 9.6% increase relative to the control. These patterns suggest that C/N amendments have the potential to mitigate N₂O emissions from peat soil. In conclusion, the results suggest that changes in soil moisture caused by precipitation may act as an important trigger for short-term N₂O pulses in fertilized peat soils. Although wood chips and biochar exhibited lower N₂O emissions than corresponding controls in several treatment comparisons, these differences should be interpreted with caution due to limited replication ($n = 3$ per treatment) and the absence of significant overall amendment effects in the mixed-effects model. The findings highlight the importance of considering interactions between amendment properties, soil conditions, and soil moisture dynamics when developing N₂O mitigation strategies for cultivated peat soils. Long term field studies are needed to evaluate the persistence, and practical relevance of these potential mitigation effects under natural conditions.

Keywords: *Greenhouse gas emission hotspots, Nitrous oxide, Grassland peat soil, Clay addition, C/N amendments*

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Abbreviations

Abbreviation	Description
CH ₄	Methane
CO ₂	Carbon dioxide
GHGs	Greenhouse gasses
N ₂ O	Nitrous oxide
C	Carbon
N	Nitrogen
WFPS	Water filled pore space
WC	Wood chips
BC	Biochar
Ctrl	Control
Nat	Natural
POXC	Permanganate-Oxidizable Carbon
LOI	Loss on ignition
CAN	Calcium ammonium nitrate
C/N	Carbon to Nitrogen ratio
NH ₄ ⁺	Ammonium
NO ₃ ⁻	Nitrate
N ₂	Dinitrogen
RF	Rainfall
KMnO ₄	Potassium Permanganate
SOC	Soil Organic Carbon
ρ	Spearman correlation coefficient
DOC	Dissolved Organic Carbon
WHC	Water Holding Capacity
NO	Nitrogen monoxide
IPCC	Intergovernmental Panel on Climate Change

1. Introduction

Life on Earth is reliant on a temperature range that is regulated by the greenhouse effect. Methane (CH₄), nitrous oxide (N₂O), and carbon dioxide (CO₂) make up approximately 98% of global greenhouse gas (GHG) emissions, and their proportion is projected to increase throughout the twenty-first century due to industrial activities, and the growing global population (López et al., 2013). Rising concentrations of these gases have intensified global warming (Signor and Cerri, 2013), resulting in significant environmental, social, and economic impacts.

The most dominant greenhouse gas, CO₂, has shown an average annual increase of about 0.5% over the past decade, while atmospheric CH₄ concentrations are increasing by about 0.2–1% per year. N₂O, currently considered the most significant stratospheric ozone-depleting substance (Chen et al., 2016), is also increasing at about 0.3% per year (López et al., 2013). Overall, CO₂ accounts for approximately 77% of global greenhouse gas emissions. In comparison, CH₄ and N₂O contribute smaller shares, about 14% and 8%, respectively, but they have global warming potentials that are 23 and 298 times more potent than CO₂, respectively (López et al., 2013). As a result, although N₂O persists in much lower concentrations in the atmosphere, it ranks as the third most significant long-lived greenhouse gas, significantly influencing climate change (Smith, 2017; Zhang et al., 2021). Although the main source of N₂O is microbial processes in the soil of terrestrial ecosystems, it is also driven by increased anthropogenic emissions, including biomass burning and fossil fuels, with a major contribution from agricultural soil management. It is estimated that anthropogenic N₂O emissions contribute 30–45% of total N₂O emissions, with agriculture accounting for more than 80% of anthropogenic emissions (Liimatainen et al., 2018). The main driver of these emissions is the widespread use of synthetic nitrogen fertilizers to meet the growing demand for food and bio-fuel production worldwide (Smith, 2017).

Peatlands are a carbon reservoir that stores about a third of global organic carbon, while covering only 3% of the Earth's surface (Liimatainen et al., 2018). Undisturbed peatlands generally emit minimal or no N₂O and can even function as net sinks. In contrast, drainage and subsequent oxygen exposure can lead to a substantial increase in N₂O emissions. Oxygen exposure accelerates the processes of organic matter decomposition, nitrogen mineralization, and nitrification. This increase in N₂O emissions is more pronounced in nutrient-rich than in nutrient-poor peatlands, while cultivated and fertilized peatlands exhibit the highest emission levels among drained sites (Liimatainen et al., 2018).

Precipitation and nitrogen (N) fertilization are two key drivers of N₂O emissions. Numerous field and laboratory studies have investigated how climate change and soil nitrogen inputs influence N₂O emissions. For instance, in a maize field in China, soil N₂O emissions were found to decrease when deficit irrigation was coupled with lower nitrogen fertilizer application rates (Zhang et al., 2021). A study by Aronson et al., (2019) in the California grasslands found that when drought conditions are followed by heavy rainfall, the additional moisture can trigger periodic pulses of CH₄ consumption and N₂O emissions. These findings suggest that the behavior of soil N₂O emissions is influenced by the interaction between soil moisture levels and nitrogen fertilization. However, while many studies have linked higher precipitation amounts to higher N₂O emissions, the effects of precipitation patterns such as intensity and frequency on these emissions remain unclear (Zhang et al., 2021). The response of N₂O emissions to N inputs is complex, and most research on peatlands has focused on surface N₂O effluents, and the extent of N₂O response to N inputs varies with the amount, type, and duration of N inputs (Shi et al., 2025). Since more N is available for N₂O production, both field/incubation and less than five-year studies showed that higher N₂O emissions from peatlands corresponded to higher N additions. In contrast, studies with N application (100 kg N ha⁻¹ yr⁻¹) for more than 10 years observed no/minimal effect on N₂O emissions. This can be attributed to the anaerobic conditions in peatlands, which are favorable for complete denitrification (the conversion of N₂O to N₂), or to increased soil N content, which facilitates plant growth by absorbing excess N and, in turn, limits the growth and activity of denitrifying microorganisms (Shi et al., 2025).

Peatlands are a distinctive component of both natural and socio-economic systems, providing many ecosystem functions and services (Kløve et al., 2017) while also representing a major source of greenhouse gas emissions. To maximize benefits and minimize adverse effects, improved management of organic soils is essential, as even small changes can have significant impacts. Applying amendments with a high carbon-to-nitrogen (C/N) ratio, such as wood chips or biochar, is a strategy to mitigate N₂O emissions. The addition of biochar, a stable carbon-rich material produced through the pyrolysis of crop residues, has emerged as a potential approach to slow down nitrogen turnover due to its high sorption capacity. Although many studies have confirmed that biochar has a positive effect on soil health, its effect on N₂O emissions is still debated (Gao et. al., 2023). Also, N₂O data from Gao et al.'s (2024) study of wood chip (WC) sizes on emissions revealed that the high fraction of readily accessible organic C in small WC sizes promoted N assimilation or immobilization and contributed to lower emissions. As an emerging non-hydrological measure, several studies have investigated combining peat soil with clay to reduce greenhouse gases (van Agtmaal, and Keuskamp, 2023).

However, little is known about the interactions between clay minerals and peat with carbon amendments, and further studies are needed.

The main objectives of this study were to: 1) assess the effects of biochar and wood chip amendments with and without clay addition on N₂O emissions from grassland peat soils following nitrogen fertilization and simulated rainfall events; 2) characterize the temporal dynamics of N₂O emissions after rainfall events and determine the contribution of rainfall-induced emission pulses to overall N₂O emissions across the different soil amendment treatments; and 3) compare the N₂O mitigation potential of biochar and wood chip amendments between natural and clay-treated peat soils and assess whether clay addition modifies their effectiveness.

The hypotheses tested are therefore,

H1: Biochar and woodchip amendments alter N₂O emissions from fertilized grassland peat soils, and the magnitude of this effects may differ between natural and clay-amended peat soils.

H2: Short term increases in N₂O emissions following simulated precipitation are associated with increased soil water availability and are followed by declining emissions as soils dry.

H3: Clay addition modifies N₂O emissions from fertilized grassland peat soils under simulated precipitation, resulting in different emission patterns between natural and clay-amended peat soils.

2. Literature Review

2.1 Nitrous Oxide (N₂O) and Peat Soils

Peatlands are wetland ecosystems that accumulate dead organic matter over time due to carbon input that outpaces its decomposition rate under anaerobic conditions caused by frequent or continuous waterlogging (Osman, 2018; Page and Baird, 2016). Globally, peatlands store enormous amounts of carbon, equal to or even surpassing amount of carbon contained in all vegetation on the Earth (Page and Baird, 2016) that supports global carbon (C) (Wang et al., 2024) and nitrogen (N) cycles (Soratur et al., 2024). They are found in a wide variety of forms, from frozen palsa mires in the northern subarctic regions to dense tropical swamp forests, with distinct communities of flora and fauna (Page and Baird, 2016). The physical properties of peat soils, including water retention capacity, water storage capacity, water availability, hydraulic conductivity, bulk density, porosity, swelling and shrinkage, etc., vary depending on the degree of decomposition, material sources, bedding, content of mineral substances, and degree of mixing (Osman, 2018).

Since these peatlands deliver a range of beneficial but often underestimated ecosystem services (Page and Baird, 2016), many undisturbed peatlands have been drained, limed and fertilized for agriculture and forestry (Hartmann, 2021). As a consequence, they have increased the oxidation and degradation of organic matter (Hartmann, 2021), making them hotspots for GHG release (Wang et al., 2024). It is estimated that approximately 20% of greenhouse gas emissions from managed peatlands are attributed to N₂O, a potent GHG, primarily produced through microbial nitrification and denitrification processes (Wang et al., 2024).

As illustrated in Figure 1, most microbes and pathways involved in biogeochemical nitrogen cycling can contribute to N₂O production, and these processes are interconnected and interacted to share intermediates or products. Major pathways associated with N₂O production and consumption include ammonia (hydroxylamine) oxidation, nitrifier denitrification, nitrite oxidation, heterotrophic denitrification, anaerobic ammonium oxidation (anammox), and dissimilatory nitrate reduction to ammonium (DNRA, or nitrate ammonification), each mediated by specific microbial groups (Figure 1).

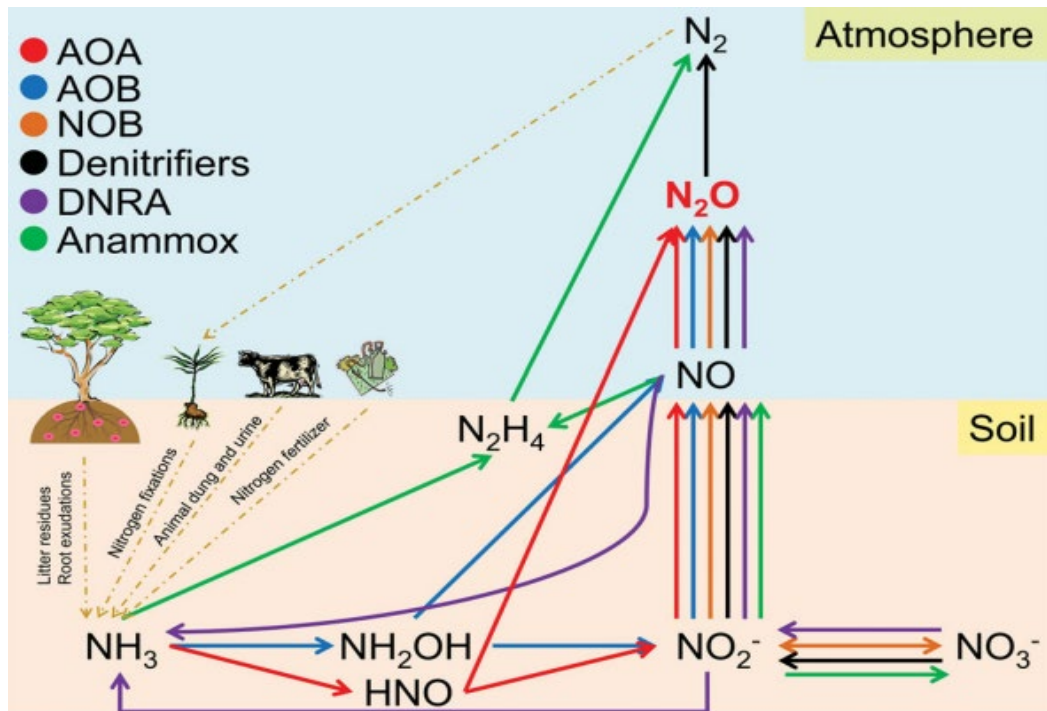


Figure 1: Simplified schematic illustration of the major microbial pathways and microbes involved in global N_2O production and soil nitrogen cycling. The pathways shown include ammonia (hydroxylamine) oxidation and nitrifier denitrification (by ammonia-oxidizing archaea (AOA) and bacteria (AOB)), nitrite oxidation by nitrite-oxidizing bacteria (NOB), heterotrophic denitrification by heterotrophic bacteria, anammox (by anaerobic ammonia oxidizers), and dissimilatory nitrate reduction to ammonium (DNRA, by unknown microorganisms). Different microbial groups and pathways are indicated by different colors (Taken from Hu et. al., 2015)

Despite there is complex and multiple routes for N_2O formation, the nitrification-related processes, particularly ammonia oxidation and nitrifier denitrification, and heterotrophic denitrification, are considered the predominant pathways contributing to soil N_2O emissions (Hu et. al., 2015).

Nitrification is characterized as an aerobic process dependent on the presence of ammonia and oxygen, while denitrification is often considered an anaerobic process governed by the availability of carbon (C), nitrate, and oxygen (Smith, 2017). For instance, nitrification-related processes are assumed to be the major sources of N_2O in water-limited soils, and heterotrophic denitrification becomes a predominant pathway for N_2O emissions under high soil water content (Hu et. al., 2015). However, both mechanisms occur in close spatial relation to one another in the soil, and nitrification or denitrification is dominated by a range of physical, chemical, and biological conditions (Smith, 2017) and their interactions in the soil.

Nitrification involves the microbial oxidation of ammonium (NH_4^+) to nitrate (NO_3^-), with the emission of N_2O as a byproduct. Nitrifier denitrification is a sequential process of reduction of nitrite (NO_2^-), nitrogen monoxide (NO), N_2O , and finally dinitrogen (N_2). In denitrification, NO_3^- is converted to N_2O and then to

the inert N_2 under anaerobic conditions, and in this pathway, NO_2^- , NO , and N_2O are the obligate intermediates (Wang et al., 2021).

2.2 Transformation of Natural Peatlands and N_2O Emissions

During the 19th century, large areas of peatland in Sweden were drained for agriculture to meet the growing population's demand for food production. Today, most of these drained peatlands have been abandoned or converted to forestry, and those still used for agriculture contribute about 6–8% of Sweden's total annual anthropogenic greenhouse gas emissions (Norberg et al., 2021).

Emission factors from the Intergovernmental Panel on Climate Change (IPCC) for temperate and boreal regions suggest that grasslands emit 20–30% less CO_2 than croplands, making leys a promising option for greenhouse gas mitigation (Holzknecht et al., 2025). However, it is doubtful as differences in site characteristics, such as peat quality and drainage may partly explain lower emissions observed in grasslands, causing that converting well-drained, fertile arable land to grassland may not always lead to the expected reduction in emissions (Holzknecht et al., 2025). The systematic review of Holzknecht et al., (2025) showed that the decrease of N_2O fluxes by an average of 7.55 kg/ha/y in grassland compared to cropland. This estimate confirms the difference in IPCC emission factors of cropland and nutrient-rich grasslands with deep drainage in the temperate zone (7.54 kg/ha/y). This may be due to the longer time it takes for grasses to uptake nutrients compared to annual crops, less mineral nitrogen for nitrifying and denitrifying microorganisms (Holzknecht et al., 2025) (Figure 2).

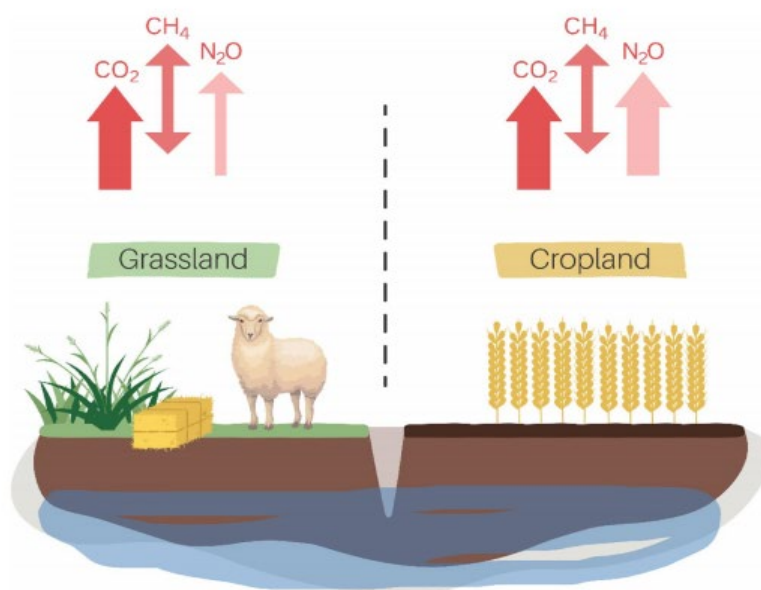


Figure 2: Effects of converting cropland to grassland on greenhouse gas emissions from peat and organic-rich soils in temperate and boreal climates (Taken from Holzknicht et al., 2025)

2.3 Drivers of N₂O Emissions

Several papers have been published based on in situ and ex-situ experiments that classify and describe the key factors affecting N₂O emissions from agricultural sites (Wang et al., 2021). For instance, Li et al. (2022) found that the key factors associated with N₂O emissions across terrestrial ecosystems include soil N content (nitrate, ammonium, and total soil N), microbial biomass, soil moisture, and average annual temperature. In one study by Bouwman et al. (2002), they identified the factors affecting N₂O emissions and categorized them into environmental, management, and measurement categories. A summary of these categorized factors is shown in Table 1 (Wang et al., 2021; Bouwman et al., 2002).

Table 1: Overview of Key Factor Groups Influencing N₂O Emissions (Adapted from Wang et al., 2021; Bouwman et al., 2002)

Environmental	Management	Measurement
Microbial population	Fertilizer application rate	Length of measurement period
Soil available carbon	Tillage systems	Types of measurement
Soil N concentration	Harvest and crop residues	Frequency of measurement
Soil moisture	Irrigation	
Soil texture	Type of fertilizer	
Soil temperature	Type of crop (grass, legumes, annual crops)	
Soil pH and salinity		

2.3.1 Soil moisture

Nitrification and denitrification are the two key biological processes that promote N₂O emissions, and their rates depend on the O₂ supply, which is regulated by the water-filled pore space (WFPS) (Veldkamp et al., 1998). Water-filled pore space (WFPS) is defined as the proportion of soil pore spaces occupied by water (Rabot et al., 2015) rather than air. WFPS plays a critical role in controlling oxygen diffusion, influencing the balance between nitrification and denitrification, and regulating whether nitrogen is emitted as N₂O, a potent greenhouse gas, or as N₂, harmless gas. In peat soils, where water dynamics are particularly sensitive, WFPS serves as a primary predictor of N₂O emissions. Akiyama et al. (2000) found a positive interaction effect that WFPS and soil mineral nitrogen had a greater effect

on N₂O and NO emissions during the summer cropping season in Japan using an automated flux monitoring system. This is further supported by the study by Pihlatie et al. (2004), which shows that N₂O production in peat, loamy sand, and clay soils increases sharply with increasing soil moisture, while emissions from dry soils are relatively low. Organic soils are generally considered to emit more N₂O per unit area than mineral soils. In Pihlatie et al. (2004)'s experiment, under relatively dry conditions (40–60% WFPS), N₂O production was highest in peat soil, followed by clay and then loamy sand. However, under wet conditions (80–100% WFPS), sandy soil exhibited more than twice the N₂O production of peat soil, suggesting that coarse-textured soils have the potential to be significant sources of N₂O when experiencing sudden wetting. However, according to Davidson (1991, 1993), when nitrification is the dominant process, NO production peaks between 30% and 60% WFPS. Above 60% WFPS, denitrification becomes vital and more N₂O is produced than NO. When WFPS exceeds 80% WFPS, N₂O is consumed and N₂ becomes the prime end product. The amount and availability of organic carbon and nitrogen, and temperature can be considered as the causatives for these discrepancies (Pihlatie et al, 2004). It is also confirmed that these results depend on soil texture, as field capacity varies between soils, as shown in their own study.

These studies suggest that in wet environments, e.g., during periods of high rainfall and high soil water content, elevated N₂O emissions are not rare. For instance, in a study of permanent pastures on silty clay loam soils in New Zealand during dry and wet seasons, Choudhury et al. (2001) confirmed this. In another study of grain maize and sunflowers in subtropical Australia, Schwenke et al., (2016) found a fivefold higher rate of N₂O loss in the wet season compared to the dry season. In the dry season, the N₂/N₂O ratio was 43%, while in the wet season the ratio decreased from 29% to 12% as the N fertilizer rate increased (Wang et al., 2021). The Zhang et al., (2021) study on the interactive effect of precipitation and N fertilizer on N₂O emissions revealed that less frequent but high-intensity rainfall lowered soil WFPS by 25.2% and increased N₂O emissions by 45.3%, whereas more frequent, low-intensity rainfall raised soil WFPS by 9.0% and decreased N₂O emissions by 23.9%. At the end of the study, they found that there was a significant interactive effect of precipitation intensity and nitrogen fertilizer application on soil N₂O emissions.

2.3.2 Fertilizer application

N₂O is formed primarily through the natural biogeochemical nitrogen cycle, but it is also released by human activities. Since the onset of the industrial era, the entry of these compounds (N₂O) into the biosphere has almost doubled, resulting in a corresponding increase in N₂O emissions. Today, agriculture is the main driver of these emissions (Smith, 2010), largely due to the widespread use of nitrogen

fertilizer types such as synthetic (mineral) fertilizers (e.g., urea, ammonium nitrate, ammonium sulfate and NPK compound fertilizers, including slow-release fertilizers), solid organic fertilizer (e.g., organic manure, composted municipal soil waste, composted animal manure, and residues of crops), and liquid organic fertilizer (e.g., raw and digested pig slurries) (Wang et al., 2021; Schils et al., 2008; Signor and Cerri, 2013). However, the effect of fertilizers on N₂O emissions is related to the type, amount, and timing of application (Wang et al., 2021). These nitrogen fertilizer application increases the amount of ammonium (NH₄⁺) and nitrate (NO₃⁻) in the soil, which fuels microbial processes such as nitrification and denitrification. More NH₄⁺ leads to more nitrification, while more NO₃⁻ promotes denitrification, both of which can ultimately promote N₂O production (Signor and Cerri, 2013).

The time and type of fertilizer application influences the efficiency of fertilizer use and crop yields. Ammonium has been recognized as the primary source of N₂O following the application of ammonium-based fertilizers (Zhu et al., 2013). However, the conditions that regulate the pathways of N₂O production are still under debate, and the interactive effects of soil conditions and different nitrogen fertilizer sources on N₂O emissions have not yet been systematically explored (Zhu et al., 2013). Zhu et al. (2013) reported that N₂O production increased dramatically by 19-fold as oxygen levels decreased from 21% to 3%. Under low oxygen conditions (3% and 0.5%), nitrifier denitrification accounted for 48–66% of total N₂O emissions in urea-treated soils and 34–57% in ammonium sulfate-amended soils. In comparison, heterotrophic denitrification contributed 34–44% and 43–50%, respectively. Overall, urea application resulted in higher N₂O emissions than ammonium sulfate (Smith, 2017). Furthermore, in a study of the timing of urea and urea-based fertilizer applications on N₂O emissions in rain-fed grain sorghum in Australia, Schwenke and Haigh (2019) found that delayed applications of urea, 3,4-dimethyl pyrazole phosphate (DMPP)-urea, or N-(n-butyl) thiophosphoric triamide (NBP)T-urea at booting reduced the N₂O emission factor (EF, percentage of applied N emissions) by 67–81% compared with urea applied at sowing. However, delayed N application resulted in lower crop N uptake, grain yield, and protein than N applied at sowing due to dry mid-season conditions. Split-N application using urea (33% sowing; 67% booting) reduced N₂O emissions by 59% compared with urea applied at sowing, but maintained crop N uptake, grain yield, and protein content. Also, applying fertilizers before or at sowing in the early stages can lead to a large pool of nitrogen in the soil, which increases N₂O emissions, as the crop cannot efficiently uptake them, and further increases are caused by increased soil moisture due to potential precipitation events (Wang et al., 2021). Zhu et al. (2013) also suggested that improving soil aeration and carefully selecting appropriate nitrogen fertilizer sources or nitrification inhibitors based on specific soil characteristics and environmental conditions could mitigate N₂O emissions. They

also specifically emphasized the need to avoid the use of urea in soils prone to low oxygen levels and acidic conditions (Smith, 2017).

2.3.3 Other factors

Managing soil pH is a proposed approach to mitigating N₂O emissions. However, studies conducted so far by several authors on the relationship between N₂O emissions and soil pH have found contradictory results (Bornø et al., 2020). One study examining the effect of increasing soil pH (through liming) on N₂O emissions from silt loam found that autotrophic nitrification was limited at soil pH < 4.5. Furthermore, liming of acidic soils has been shown to stimulate nitrification and affect both nitrification rates and N₂O fluxes. On the other hand, denitrification rates decrease as soil pH decreases (Wang et. al., 2021).

Temperature and moisture play key roles in nitrification and denitrification by regulating microbial activity. They also strongly affect both the production of N₂O and its diffusion from the soil to the atmosphere (Signor and Cerri, 2013). Soil temperature also affects N₂O emissions by influencing the N₂/N₂O ratio. The study by Maag et al. (1996) showed that the N₂/N₂O ratio increases exponentially with increasing temperature, suggesting a linear relationship between log(N₂/N₂O) and temperature. A study by Lai and Denton (2018) on a dairy farm in south west Victoria, Australia with different temperature levels (25 °C, 35 °C, 45 °C) showed the highest N₂O emission rate at 35 °C. However, at 45 °C, the increased N₂ production resulted in a decrease in the N₂O/N₂ ratio from 33% to 85%. This study also highlights that temperature may directly influences the conversion of NO₃⁻ to N₂O due to the decrease in oxygen availability with increasing soil respiration rate, thereby encouraging denitrifying microbes to use NO₃⁻ as a terminal electron acceptor. The literature strongly agrees that the reduction of N₂O to N₂ increases with increasing soil temperature. A study from another perspective found that rising soil temperatures generally stimulate nitrous oxide production up to about 24 °C, which is close to the optimum temperature for bacterial denitrification. Beyond this point, N₂O emissions tend to decline. However, denitrifying microbial communities can adapt to warmer conditions, which can lead to a resurgence of N₂O emissions at higher temperatures (Pärn et al., 2018).

Fine-textured soils tend to produce more N₂O than sandy soils because they contain more capillary pores that hold more water effectively. This promotes the development and persistence of anaerobic conditions, especially in soil aggregates. Some studies show that denitrification increases as soil texture becomes finer and water-filled pore space (WFPS) increases. Conversely, as WFPS decreases, denitrification decreases most sharply in fine-textured soils, followed by medium-

and coarse-textured soils. In clay soils, N₂O emissions increase rapidly once WFPS exceeds 40% and peak at levels above 70% (Wang et. al., 2021).

2.4 Strategies to Mitigate N₂O Emissions

2.4.1 C/N amendments

Nitrogen transformations in soil are primarily driven by two biological processes: immobilization (assimilation), in which microorganisms take up nitrogen and convert it into organic forms, and mineralization (ammonification), in which organic nitrogen is converted to ammonia (NH₃). The relative predominance of these processes is determined mainly by the soil carbon-to-nitrogen (C/N) ratio and the nature of the added residues. When the C/N ratio is below 30:1, mineralization generally dominates over immobilization, resulting in increased nitrogen availability for plant uptake and microbial activity (Baggs et al., 2000; Signor and Cerri, 2013). For instance, straw with a high C/N ratio on the soil surface may increase the immobilization of applied nitrogen fertilizers (Baggs et al., 2000), thereby reducing denitrification and N₂O emissions. In contrast, when no straw or straw with a low C/N ratio is present, immobilization is less likely to occur, leaving more nitrogen available for nitrification and denitrification, which can lead to higher N₂O emissions (Baggs et al., 2000; Signor and Cerri, 2013).

The incorporation of substantial quantities of woody biomass into soil, as practiced in whole orchard recycling (WOR), promotes carbon sequestration, nutrient recycling, and ecosystem health in agricultural fields (Gao et al., 2024). The study by Gao et al., (2024) found that N₂O emissions increased following each fertilizer application, with the lowest total emissions observed in the smallest wood chip (WC) size, indicating significant nitrogen immobilization. These findings suggest that larger WC sizes can delay carbon mineralization and mitigate initial nitrogen immobilization risks, whereas the smallest WC size may have stabilized and enhanced soil organic carbon.

Although some studies have shown a positive correlation between straw addition and reduced N₂O emissions (Yao et al., 2017; Huang et al, 2017), several studies have found that straw promotes N₂O emissions through nitrification and denitrification by increasing the availability of dissolved nitrogen and soil organic matter, providing a suitable environment and substrate for microbes (Duan et al., 2020). Therefore, one possible approach to mitigate N₂O emissions from crop residues is to convert them into biochar and subsequently apply it to the soil. Recent meta-analyses suggest that biochar application can reduce N₂O emissions by an average of around 20% (Abalos et al., 2022). However, other field-based meta-analyses conducted across a variety of cropping systems, including maize, wheat,

rice, vegetables, and pasture, have not found consistent evidence of significant emission reductions, despite observed decreases of up to 17% in some cases (Shakoor et al., 2021; Abalos et al., 2022). Additionally, a recent meta-analysis found that nitrate leaching was reduced by an average of 13% in arable farming and vegetable crops only, with no effect on perennial cropping systems from the addition of biochar (Abalos et al., 2022). However, on other hand, the use of biochar is limited by costs associated with its production, distribution, and application, as well as the loss of nitrogen during its production, which must be compensated from other sources. Although biochar can improve nutrient retention in the soil over time, converting crop residues into biochar reduces the input of readily biodegradable carbon, which negatively affects soil biodiversity and long-term soil health (Abalos et al., 2022). Biochar application rates of $\leq 30 \text{ t ha}^{-1}$ are suggested as the most effective for reducing greenhouse gas emissions while maintaining crop yields. However, its impact on CO_2 , N_2O , and CH_4 emissions depends strongly on the properties of physicochemical properties of biochar (pH, C/N ratio) and soil (pH, textural class). Therefore, factors such as feedstock type, pyrolysis conditions, and application rate must be carefully planned to achieve effective mitigation outcomes (Shakoor et al., 2021).

2.4.2 Mineral Soil (Clay/Sand) addition

Addition of mineral soil (sand/clay) to peatlands has been explored as a strategy to mitigate GHG emissions from drained agricultural peat soils. Research shows that mineral soil cover can affect GHG emissions, but its effectiveness varies depending on specific conditions and practices. A study by Paul et al. (2024) comparing covered and uncovered intensively managed organic grassland soils in Switzerland found that covering the organic soil from a mineral layer did not significantly reduce SOC losses over four years at either site, and CH_4 uptake remained marginal. However, the soil cover lowered the share of N_2O in total GHG emissions from 28% in the reference plots to 7% in the covered plots.

Many studies show that high clay content is associated with slower organic matter decomposition, lower subsidence rates, and higher soil organic carbon (SOC) densities. Due to their small size, shape, and charge, clay minerals are highly reactive and play a key role in organic matter dynamics (Van Agtmaal et al., 2025). Studies on mineral soils have confirmed that clay minerals affect carbon accessibility to microbial communities through mechanisms such as physical protection, adsorption, aggregation, and the formation of organo-mineral complexes (van Agtmaal et al., 2025). Clay can also directly influence heterotrophic activity by adsorbing and immobilizing extracellular enzymes or by altering the composition of microbial communities. In addition, mineral amendments may indirectly affect microbial processes by modifying soil

conditions, including aeration, water retention, pH, and nutrient availability (Van Agtmaal et al., 2025). Van Agtmaal and Keuskamp (2023) studied the effects of adding several different clay types to peat soil and their effects on GHG emissions, particularly CO₂. However, they found that each clay type had different effects on nitrogen emissions (positive/ negative/ neutral).

3. Materials and Methods

The work described in this thesis was carried out as part of the "KLIMAEFFEKTIV" project implemented by SLU (Örjan Berglund; project manager, Sabine Jordan, Kerstin Berglund).

3.1 Site and Soil Sampling

The soil sampling site was located in Hackenstad, approximately 308 kilometers (km) away from Uppsala, and 20 km north of Västervik which belongs to Kalmar county in Sweden at a site with ongoing field trials. This site consists of cultivated peat soil (peat depth about 1 meter), and one part of the site has been amended to a depth of about 5-10 centimeters (cm) by mixing clay into the surface soil.

Eighteen soil samples (soil cores: inner diameter ($\varnothing$) 18 cm, height 30 cm) and 20 small soil samples (small metal cylinders: inner diameter 7.2 cm, height 10 cm) were collected from both clay and clay-free (natural) fields on November 5, 2025. These eighteen soil samples were obtained by first removing the top grass cover and then mechanically penetrating all the lysimeters into the soil (tractor), with the sampling locations positioned as close to each other as practically possible (Figure 3-A). This approach was used to minimize spatial variation in the source soil and to produce relatively comparable soil material among the lysimeters. The 12 small metal cylinders were collected from depths of 5–15 cm and 15–25 cm, corresponding to the soil layers used to fill the lysimeters (Figure 3-B). The remaining 8 metal cylinders were taken from the locations in the field where greenhouse gas emission measurements were conducted. The top and base of the soil samples (soil cores and metal cylinders) were sealed with removable lids. The samples were then transported to the university premises and kept in a covered area until the official commencement of research on January 26, 2026.



Figure 3: (A) Sampling of soil cores; (B) small metal cylinders

3.2 Experimental Design: Laboratory

The soil samples were transferred to the soil physics laboratory, where they were left for several days (4-6 days) to settle and thaw until they reached laboratory environmental conditions. Before initiating the treatments, approximately 5 cm of surface soil was removed from all soil cores to reduce the risk of runoff. A portion of the removed fractions was then air-dried and reserved for subsequent analyses, including Permanganate-Oxidizable Carbon (POXC), pH, and loss on ignition (LOI). The dose and amount of calcium ammonium nitrate (CAN) fertilizer and C/N amendments (biochar and wood chips) to be applied per lysimeter were calculated based on the area of the ring and 150 kg of pure nitrogen per hectare and 1 kg/m³ for C/N amendments, respectively, taking into account that CAN contains 27% nitrogen by weight. Accordingly, the amounts to be applied for the C/N amendment and CAN fertilizer were ~ 5.38 g, ~ 1.5 g in granules, and ~1 g in powder, respectively.

A total of 18 intact soil cores were then installed in a micro-lysimeter in a laboratory facility equipped with a rainfall simulator, with nine cores representing the control and nine representing the clay-amended treatment. The cores were maintained to preserve their structural integrity and arranged within a controlled mesocosm system to enable standardized and reproducible precipitation events (Figure 4A). For each lysimeter group (control and clay-amended), three treatments were established with three replicates each ($n = 3$). The treatments were woodchips-treated soil with (WC-Clay) and without clay addition (WC-Nat), biochar-treated soil with (BC-Clay) and without clay addition (BC-Nat), and no C/N amendment (control) with (Ctrl-Clay) and without clay addition (Ctrl-Nat).

Both wood chips and biochar were then separately incorporated into the soil in the first 10 cm layer at the beginning of the experiment, and CAN fertilizer was applied to the surface as two fertilization events on two different days; detailed descriptions of the fertilization events can be found in Section 1.2.1. The six treatments were randomly assigned, and the layout is shown in Figure 4B. Additionally, all lysimeters were connected to leachate collection bottles to enable further analysis of leachate composition (Figure 4A).

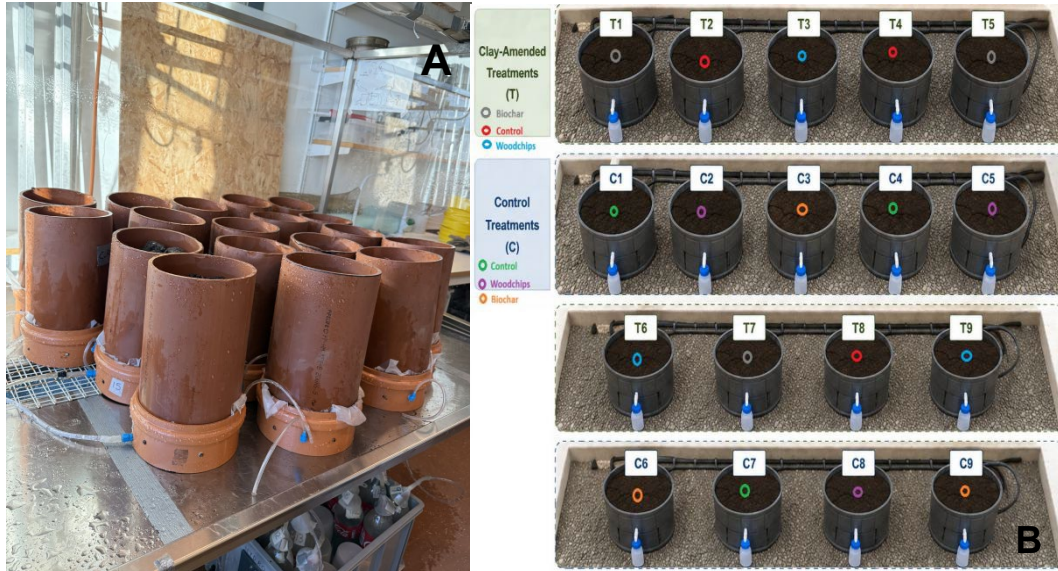


Figure 4: (A) Micro-lysimeter laboratory facility with installed soil cores; (B) Six treatments applied and their random distribution

3.2.1 Rainfall simulation and fertilization

Based on the precipitation (millimeter;mm) and maximum daily precipitation (mm) data for Uppsala for the 30-year periods 1991-2020 and 1961-1990 obtained from Uppsala University, and the focus of the study on N₂O emissions and moisture content, three standardized precipitation events in the range of 25 mm-30 mm (40-48 minutes) were applied to occur in the third week of February (day 16), the first week of March (day 2), and the second week of March (day 9) using the mesocosm rainfall simulator. Since the rainfall distribution was not homogeneous across the 18 lysimeters, a preliminary trial was conducted to see how the distribution varied and thus to attempt to relate emissions to soil moisture content.

Mineral fertilizer (CAN) was applied to the surface in two parts: the first in the second week of February (day 9: granules) and the second in the last week of February (day 23: powder form) to all lysimeters. The amounts applied were described in section 3.2. CAN fertilizer was used to induce N₂O emissions from the soil. The fertilizer provides nitrogen to the soil in the readily available NO₃⁻ and NH₄⁺ forms, which, depending on other conditions, facilitates nitrification or denitrification processes.

3.2.2 GHG emission measurement

Nitrous oxide was quantified using a closed static chamber-based technique. The manual opaque chamber (height 16 cm, ø 18 cm) was fitted onto each lysimeter prior to each measurement, and head-space gas samples were analyzed in real time with a Gasmeter GT5000 Terra portable gas analyzer (3-4 days/week) (Figure 5) for

6 weeks. For each lysimeter, gas concentrations were continuously recorded throughout a 5 minute (min) chamber closure period, generating a time series of gas concentration measurements. The chamber was subsequently flushed with ambient air for 2 min to remove residual gases before the chamber was transferred to the next lysimeter. The same procedure was repeated sequentially for all lysimeters. Gas fluxes were calculated from the slope of concentration changes over the enclosure period, correcting for chamber volume and soil surface area (section 3.4). After each measurement and/or before/after rain, all lysimeters were weighed using a weighing scale to determine soil moisture change.

At the end of the laboratory experiment (week 6), soil samples were collected from each lysimeter and air-dried for analysis of loss on ignition, pH, and POXC. Metal cylinder soil samples were used to determine gravimetric/volumetric water content and bulk density.



Figure 5: Gasmet GT5000 Terra Portable gas analyzer

3.3 Measurement of chemical and physical properties

Soil $\text{pH}_{\text{H}_2\text{O}}$ was determined by weighing 5 g of air-dried soil and adding 10 ml of de-ionized water (1:2 ratio), the slurry was well mixed for 5 min, and pH was measured after 2-3 minutes using a pH electrode (Mettler Toledo SevenEasy S20 pH Meter).

The weighed metal cylinders were oven-dried at 105 °C for 24 hours to determine bulk density (g/cm^3) (Table 2), gravimetric water content (%) and volumetric water

content (%). In addition, water content was measured by weighing the entire lysimeter after each measurement.

The organic matter content (loss on ignition) was determined by drying in an oven at 105 °C for 24 hours, followed by dry combustion at 550 °C for 6 hours. The samples were then cooled in a desiccator and weighed.

Permanganate oxidizable carbon (POXC) was used to study labile C dynamics in soil. Air-dried soil samples from all lysimeters were ground to a fine powder. 0.15 g of weighed soil (0.1 to 0.5 g for organic soils) was placed in an Erlenmeyer flask, and then 20 ml of 0.02 M KMnO₄ solution (pH ~7) was added. The mixture was shaken at 200 RPM for 15 minutes and then filtered. The 0.5 ml of the filtrate (or supernatant) was transferred to a 50 ml volumetric flask and diluted to volume with de-ionized water. Absorbance was measured at 550 nm using a DR 5000 spectrophotometer. Absorbance was calibrated using standard curves and then calculated using Equation 2 as described in Section 3.4.1.

Soil properties in lysimeters, including pH, gravimetric water content, LOI, and POXC, were examined at the beginning and at the end of the experiment (pre- and post-treatment). Table 3 shows the average values with standard deviations of these soil properties for each of the six treatments.

Table 2: Bulk density at the sampling site (Three replicates were obtained for the natural and clay treated and were classified as minimum, median and maximum, and for the locations where GHG measurements were taken at the natural and clay-treated sites, they were classified based on each of the four replicates.)

Bulk Density (g/cm ³)	Minimum	Median	Maximum
Natural (Nat) 5-15cm	0.5733	0.7359	1.2587
Natural (Nat) 15-25cm	0.5638	0.5765	0.8710
Clay treated (Clay) 5-15cm	0.5929	0.7304	0.7658
Clay treated (Clay) 15-25cm	0.4907	0.6167	0.6805
Natural GHG measurement rings	0.5852	0.6782	0.7653
Clay treated GHG measurement rings	0.5434	0.7108	0.9043

Table 3: Soil properties of the lysimeters at the beginning and at the end of experiment (mean/average $\pm$ standard deviation, $n = 3$ in each treatment)

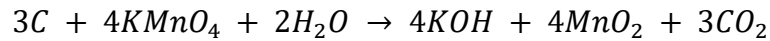
Treatment	Gravimetric water content (%)	pH	% of POXC	Loss on ignition (%)
At the beginning of experiment				
Ctrl-Nat	67.53 $\pm$ 16.81	5.51 $\pm$ 0.01	1.51 $\pm$ 0.18	52.46 $\pm$ 1.19
Ctrl-Clay	58.36 $\pm$ 4.12	5.65 $\pm$ 0.09	0.84 $\pm$ 0.03	33.23 $\pm$ 3.18
WC-Nat	77.18 $\pm$ 8.49	5.48 $\pm$ 0.02	1.42 $\pm$ 0.22	53.12 $\pm$ 0.54
WC-Clay	52.22 $\pm$ 4.59	5.56 $\pm$ 0.03	0.82 $\pm$ 0.24	28.24 $\pm$ 4.03
BC-Nat	75.38 $\pm$ 4.69	5.50 $\pm$ 0.02	1.32 $\pm$ 0.13	53.08 $\pm$ 0.37
BC-Clay	57.41 $\pm$ 3.51	5.58 $\pm$ 0.04	0.89 $\pm$ 0.29	33.05 $\pm$ 2.16
At the end of the experiment				
Ctrl-Nat	39.53 $\pm$ 17.95	5.42 $\pm$ 0.09	1.21 $\pm$ 0.15	52.90 $\pm$ 1.64
Ctrl-Clay	32.87 $\pm$ 7.91	5.35 $\pm$ 0.08	0.86 $\pm$ 0.30	26.74 $\pm$ 10.58
WC-Nat	56.68 $\pm$ 17.03	5.30 $\pm$ 0.07	1.31 $\pm$ 0.13	51.28 $\pm$ 2.29
WC-Clay	54.35 $\pm$ 3.85	5.46 $\pm$ 0.16	0.91 $\pm$ 0.09	33.49 $\pm$ 3.66
BC-Nat	31.27 $\pm$ 3.40	5.47 $\pm$ 0.10	1.21 $\pm$ 0.04	50.67 $\pm$ 1.34
BC-Clay	40.71 $\pm$ 10.55	5.39 $\pm$ 0.11	0.89 $\pm$ 0.18	22.49 $\pm$ 6.63

3.4 Calculation of the results and statistical analysis

3.4.1 POXC measurements

The principle of POXC quantification is the change in the color absorbance of KMnO_4 (a strong oxidizing agent with a dark purple color). When it reacts with soil organic carbon (SOC), carbon (C) is oxidized from C^0 to C^{+4} and Manganese (Mn) is reduced from Mn^{7+} to Mn^{4+} (Equation 1); this reaction changes the color of the solution to a light pink. The reduction in color is proportional to the amount of SOC in the oxidized sample.

Equation 1



The absorbance was calibrated using standard curves and converted to mg of active carbon per g of soil using Equation 2 as follows.

Equation 2

$$POXC \text{ (mg. g}^{-1} \text{ soil)} = \frac{((mM \text{ stock} - (mM \text{ sample} \times DF)) \times 20ml \times 9mg)}{(soil \text{ weight (g)} \times 1000)}$$

Where,

mM stock is concentration of $KMnO_4$ added to the flask (20 mM),

mM sample is concentration of $KMnO_4$ left in the sample after analysis calculated from calibration curve given (mM),

DF is dilution factor (100),

20 ml is volume of $KMnO_4$ solution in the flask,

9 mg is mass of carbon oxidized by 1 mM of $KMnO_4$ (conversion factor),

Soil weight is mass of soil used for analysis (g)

3.4.2 Gas flux measurements

The gas flux (F) was estimated by linearly fitting the sampling time and concentration and calculated as in Equation 3. The first two values (~ 30 seconds) after closure were omitted from the calculation.

Equation 3

$$F = \frac{\frac{\Delta c}{\Delta t} \times \rho \times m \times h}{R \times (273.15 + T)}$$

Where,

$\Delta c/\Delta t$ is the average change in gas concentration during the measuring time (ppm/h),

ρ is the atmospheric pressure (Nm^{-2}),

m is the molecular mass of the gas (MCO_2 : 44.01 g mol^{-1} , MN_2O : 44.01 g mol^{-1}),

R is the ideal gas constant (8.3145 J $mol^{-1} K^{-1}$),

T is the ambient air temperature (K),

h is the total height of the chamber + ring (m)

3.4.3 Statistical Analysis

The obtained data were subjected to statistical analysis using Microsoft Excel and R software (version 4.4.3; R Core Team, 2025) to evaluate the effects of amendments, clay additions, moisture dynamics, and their interactions.

Spearman's rank correlation analysis was conducted to assess the strength and direction of the relationship between the variables. The resulting correlation coefficient (ρ) indicates the magnitude and direction of the relationship, while the corresponding p-value determines whether the relationship is statistically significant. The interpretation of ρ is given in Table 4.

A linear mixed-effects model was fitted using the **lmer()** function in the lme4 package in R (Bates et al., 2015). Treatment, sampling date, and their interaction were specified as fixed effects, while replicate lysimeter was included as a random effect. The model therefore accounted for the non-independence of repeated observations obtained from individual lysimeters. Statistical significance was evaluated at $\alpha = 0.05$.

Cumulative N₂O emissions were calculated by summing the daily N₂O fluxes for each lysimeter over the experimental period. Cumulative emissions at these lysimeter levels were analyzed to assess whether clay addition altered the effectiveness of C/N amendments (biochar and wood chips) in mitigating N₂O emissions. Because the data did not meet the assumptions of a conventional parametric ANOVA, a non-parametric aligned rank transformation (ART) ANOVA was performed. The analysis included soil condition (natural and clay-treated), amendment treatments (control, biochar, and wood chips), and their interaction (soil condition $\times$ amendment) as fixed effects. Each lysimeter was treated as an experimental unit, with three independent lysimeter replicates per treatment combination. Statistical significance was assessed at $\alpha = 0.05$.

Table 4: The table shows the types of correlation, the direction of the relationship (Adapted from Ali Abd Al-Hameed (2022))

Value of correlation coefficient	Description
>1	Completely Positive
0.7 - 0.99	Strong Positive
0.5 - 0.69	Average Positive
0.01 - 0.49	Weak Positive
0	Not a positive relationship

4. Results

4.1 Laboratory Measurements

4.1.1 Temporal dynamics of mean N_2O fluxes and cumulative mean N_2O fluxes

The mean daily N_2O fluxes ($\mu\text{g}/\text{m}^2/\text{day}$) calculated from the laboratory experiment under fertilization and rainfall events over a six-week period (February 03, 2026 to March 12, 2026) are shown in Figure 6. The mean cumulative N_2O fluxes (mg/m^2) over time in the six treatments are shown in Figure 7.

As shown in Figure 6 below, although mean N_2O emission trajectories varied substantially over time and between treatments due to treatment-specific responses to environmental conditions (precipitation) and management events (fertilization, C/N amendments, clay addition), all six treatments followed a similar overall trend.

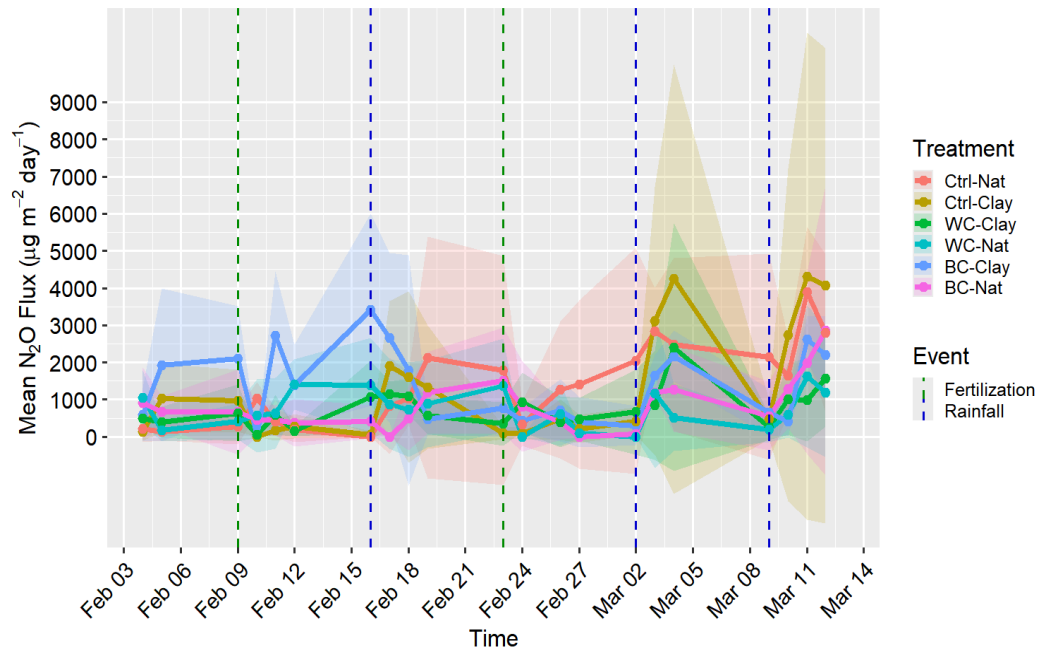


Figure 6: Time series variation of mean N_2O fluxes ($\mu\text{g}/\text{m}^2/\text{day}$) across six soil treatments during the experimental period. Green and blue dashed vertical lines represent the times of fertilization and precipitation events, respectively. Shaded areas represent variability among replicates in each treatment.

In most treatments, N_2O fluxes showed a noticeable increase following the precipitation events, whereas lower fluxes were observed after fertilization events. The strongest emission peaks occurred shortly after the rainfall events in early and

mid-March, and most treatments peaked on the second or third day after the rainfall, indicating that N₂O emissions have short-term episodes.

Following the rainfall event on February 16, N₂O emissions exhibited inconsistent patterns across treatments. Except for Biochar–Clay (BC-Clay), Wood chips–Natural (WC-Nat), and Biochar–Natural (BC-Nat), the remaining three treatments showed a moderate increase in N₂O fluxes. Among all treatments, BC-Nat recorded the lowest N₂O emissions on the day after the rainfall event, followed by a peak in emissions on February 19, three days after the rainfall event. In contrast, Control–Clay (Ctrl-Clay) showed the highest emissions among Control–Natural (Ctrl-Nat) and Wood chips–Clay (WC-Clay), reaching approximately 2000 µg N₂O/m²/day on the day after the rainfall event 1, and gradually declining until the second fertilization event. BC-Clay followed an unusual and relatively higher N₂O emission pattern during the first half of the experiment compared with the other treatments. It was the highest N₂O emitting treatment prior to the rainfall event on February 16 and continued to exhibit fluctuations in emissions over the following days until the second rainfall event (March 2). The second fertilization (February 23), which was applied after the first rainfall event, resulted in variable N₂O responses among treatments. While WC-Clay and Ctrl-Clay showed increased emissions following fertilization, the remaining treatments displayed a decline in N₂O fluxes.

Following the second rainfall event (March 2), N₂O fluxes increased sharply in all treatments, regardless of soil type. Ctrl-Clay exhibited the highest N₂O emission peak, reaching approximately 4000 µg N₂O/m²/day, followed by Ctrl-Nat. Despite this trend remaining similar after the third rainfall event on March 9, N₂O fluxes increased slightly compared to the second rainfall event. Compared with the amended treatments, the controls consistently showed higher and more frequent N₂O emission peaks. While the magnitude of the mitigation effect varied between soil types and amendment treatments, both biochar (BC) and wood chip (WC) amendments were shown to result in lower to moderate N₂O fluxes after both subsequent rainfall events.

A clear temporal variation in N₂O emissions was observed when there was a two-week interval between the first and second rainfall events and a week interval between the second and third rainfall events. During the prolonged dry period before the subsequent rainfall event, most treatments showed a gradual decline in N₂O emissions, along with short-term fluctuations. In particular, N₂O fluxes showed pronounced sudden increases and decreases between March 2 and March 9 before the third rainfall event.

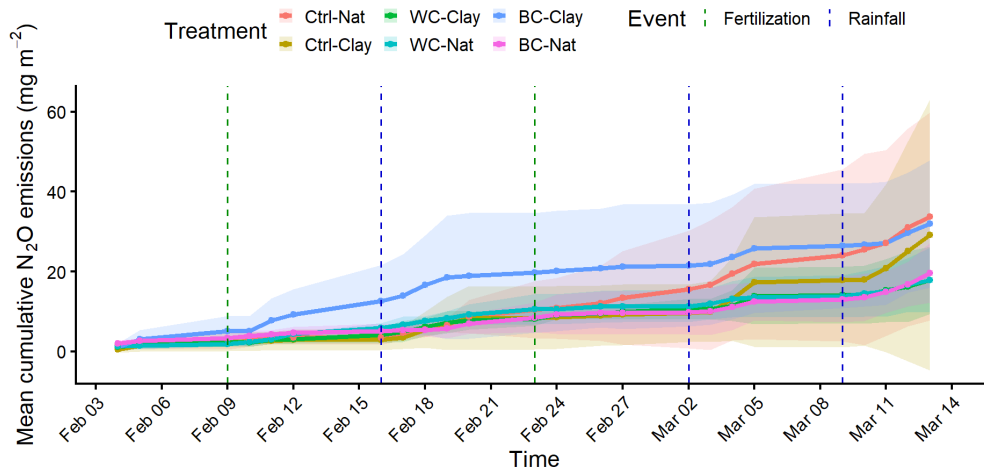


Figure 7: Mean cumulative N_2O fluxes (mg/m^2) over time for control (Ctrl), woodchip (WC), and biochar (BC) treatments in clay and natural soils. Green dashed lines indicate fertilization events, and blue dashed lines indicate rainfall events. Shaded areas represent variability among replicates.

The mean cumulative N_2O emissions were consistent with the temporal patterns observed in the mean flux data, showing that treatments with elevated fluxes over time contributed proportionally to higher mean cumulative emissions (Figure 7). Initially, mean cumulative N_2O emissions increased gradually in all treatments. However, after the first fertilization (February 9) and rainfall (February 16) events occurred in early and mid-February, respectively, differences between treatments became apparent. BC-Clay consistently showed higher mean cumulative N_2O fluxes than other treatments during the early phase of the experiment following the first rainfall event, and this continued until the second rainfall event (March 9), showing a pronounced and significant divergence from the other treatments.

Subsequent rainfall events and fertilization further amplified treatment divergences. The sharp increases in mean N_2O flux in the control treatments after the second and third rainfall events (Figure 6) were also reflected in the corresponding mean cumulative emissions during the same period shown in Figure 7, further highlighting the similar trend. By the end of the experiment, Ctrl-Nat exhibited the highest mean cumulative N_2O emissions (approximately $34 \text{ mg } N_2O/m^2$), followed by BC-Clay (approximately $32 \text{ mg } N_2O/m^2$) and Ctrl-Clay (approximately $29 \text{ mg } N_2O/m^2$) (Figure 7). However, by the end of the experiment, the trajectories of these treatment methods shown in Figure 6 differed from one another; particularly, Ctrl-Clay exhibited the highest value, followed by Ctrl-Nat and BC-Clay, respectively.

In contrast, the amendment treatments showed lower mean cumulative fluxes over time. The mean cumulative emissions at the end of the experiment in WC-Clay, WC-Nat, and BC-Nat remained below $20 \text{ mg } N_2O/m^2$, indicating a substantial

reduction in emissions compared with the controls. These results were consistent with the trends observed in Figure 6.

4.1.2 Cumulative N₂O emissions over 0–2 days following rainfall events for six experimental treatments

Pronounced N₂O emission peaks observed within 2–3 days of precipitation events (Figure 6) led to an increase in mean cumulative emissions over time (Figure 7). Thus, to further characterize the short-term N₂O response following rainfall, Figure 8 presents the sum of daily N₂O emissions over the 2-day period following each precipitation event, calculated separately for each treatment. This allows for a comparison of the magnitude of post-rainfall N₂O emissions across treatments and the three precipitation events.

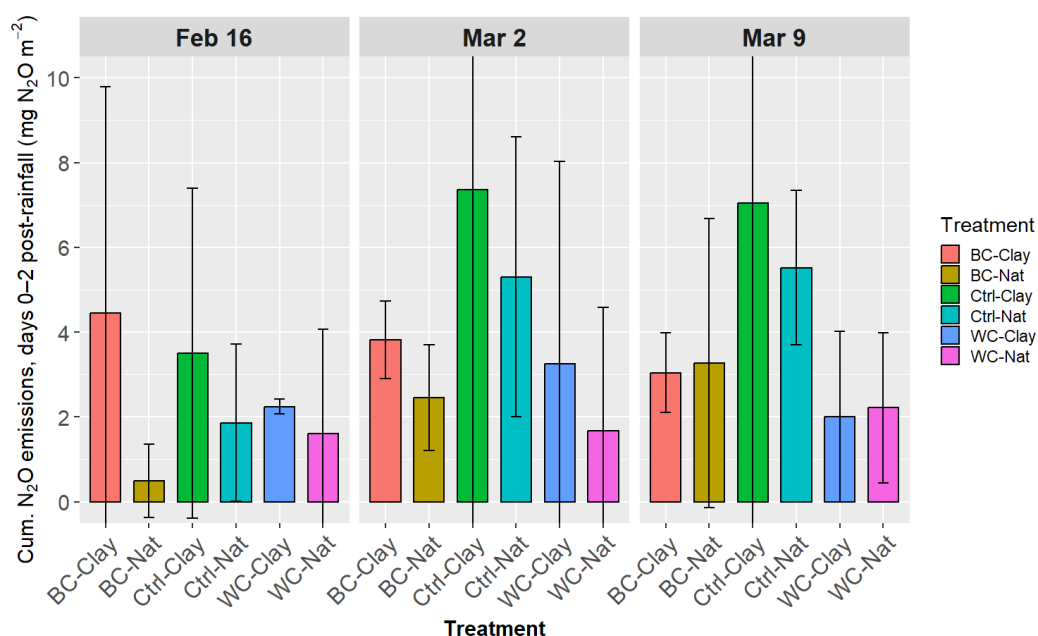


Figure 8: Bars represent cumulative N₂O emissions (mg/m²) measured during the first 48 hours following rainfall events on February 16, March 2, and March 9 across six treatments. Error bars indicate standard deviation (±SD).

The results clearly demonstrate that rainfall events substantially enhanced cumulative N₂O emissions; however, the magnitude of the response differed considerably among treatments over time intervals between precipitation events (Figure 8).

Across all rainfall events, Ctrl-Clay and Ctrl-Nat consistently showed higher cumulative N₂O emissions than the amendment treatments. Ctrl-Clay produced the highest cumulative N₂O emissions, particularly following the rainfall events on March 2 and March 9, reaching approximately 7 mg N₂O/m². Ctrl-Nat ranked

second, with cumulative emissions peaking at approximately 5 mg N₂O/m² following the later rainfall events.

In contrast, the BC-amended (BC-Clay and BC-Nat) and WC-amended (WC-Clay and WC-Nat) treatments exhibited intermediate to lower cumulative N₂O emissions relative to their respective controls. WC-Nat and WC-Clay consistently recorded the lowest cumulative emissions, remaining below approximately 3 and 4 mg N₂O/m² respectively.

The relatively large error bars observed in several treatments indicate substantial variability among the three replicates in post-rainfall N₂O responses. The results of post-rainfall N₂O responses further demonstrated that soil amendments such as BC and WC may help mitigate short-term emission pulses following rainfall, regardless of clay incorporation into peat soil.

4.1.3 Spearman correlation and post rainfall contribution

Spearman correlation analysis revealed strong positive relationships between six-week mean cumulative N₂O emissions and mean cumulative N₂O emissions occurring during the 0-2 and 0-3 days following simulated precipitation events. However, changes in soil properties such as Loss on Ignition (LOI), pH, and Permanganate oxidizable carbon (POXC) showed weak/no correlation with cumulative N₂O emissions (Figure 9).

The contribution of post-rainfall emissions to the total N₂O emissions over the six-week period further demonstrated that a substantial proportion of these emissions occurred during the periods following the three simulated rainfall events (Table 5). The 0-3 post-rainfall windows accounted for 43.65 - 81.59% of cumulative N₂O emissions across treatments, whereas the 0-2 windows accounted for 30.83 - 61.48%. The highest 0-3 post rainfall contribution was observed in Ctrl-Clay (81.59%), followed by Ctrl-Nat (58.47%) and WC-Clay (55.59%). In contrast, WC-Nat and BC-Clay exhibited lower 0-3 post rainfall contributions of 43.65% and 45.86%, respectively. These results show that a substantial proportion of N₂O emissions occurred within the days following the simulated rainfall events.

Table 5: Contribution of cumulative N_2O emissions during the 0–2 and 0–3 days following simulated precipitation events (Feb16, Mar 2, Mar 9) to six-week cumulative N_2O emissions.

Treatment	Total mean N_2O cumulative fluxes (mg /m ²)	0-2 post RF total mean N_2O Cum fluxes (mg /m ²)	0-2 Post-rainfall contribution (%)	0-3 post RF total mean N_2O Cum fluxes (mg /m ²)	0-3 Post-rainfall contribution (%)
Ctrl-Nat	33.79	12.69	37.56	19.75	58.47
WC-Nat	17.87	5.51	30.83	7.79	43.65
BC-Nat	19.66	6.22	31.65	10.81	54.99
BC-Clay	31.91	11.31	35.43	14.64	45.86
Ctrl-Clay	29.12	17.90	61.48	23.76	81.59
WC-Clay	17.80	7.50	42.14	9.89	55.59

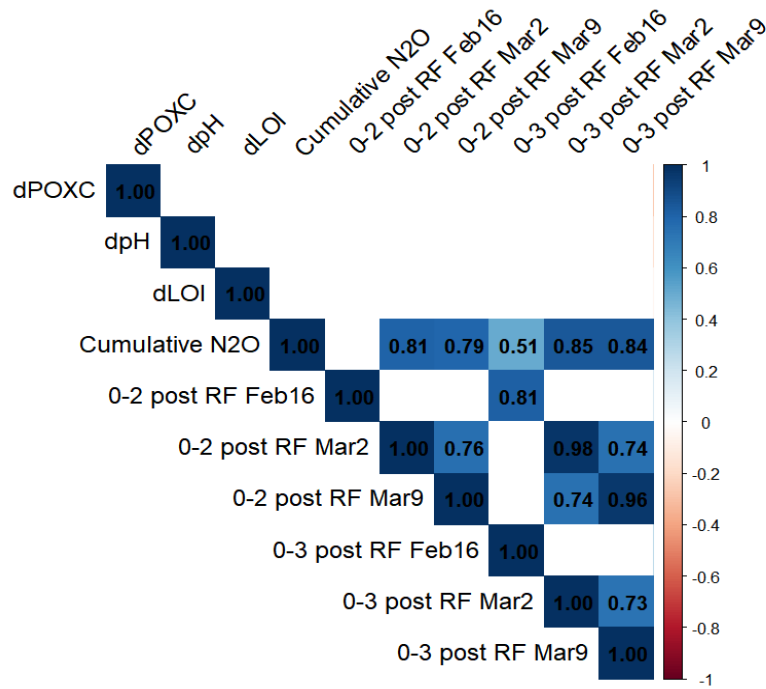


Figure 9: Spearman correlation matrix showing relationships among changes in soil properties, cumulative N_2O emissions, and post-rainfall N_2O emission responses. Only strong Spearman correlation coefficients ($|\rho| \geq 0.5$) are displayed in the boxes. Darker blue shading indicates stronger positive correlations.

This finding supports the interpretation that precipitation is a triggering factor associated with episodic pulses of N_2O emissions from peat soil. However, the

percentage contribution does not quantify the causal effect of rainfall, as the post-rainfall emissions are included in the total cumulative emissions and no independent no-rainfall control was used.

4.1.4 Statistical comparison of mitigation treatment effects on N₂O emissions

The mixed-effects model revealed significant temporal variability in N₂O fluxes, while neither the soil amendment nor the soil type showed a significant effect (Table 6). However, the sampling date significantly influenced N₂O fluxes ($F_{24} = 2.365$, $p = < 0.05$), indicating substantial variation in emissions throughout the experimental period. In contrast, neither the soil amendment ($F_2 = 0.577$, $p = 0.6$) nor the soil type ($F_1 = 0.363$, $p = 0.6$) had a significant effect on N₂O fluxes. The interaction between soil amendment and soil type was also not significant ($F_2 = 1.356$, $p = 0.3$), indicating that the response to the soil amendment did not differ significantly across the different soil types.

The significant effect of the sampling date was consistent with the temporal dynamics observed in N₂O fluxes (Figure 6). N₂O emissions increased following the simulated rainfall events; peak flux values were recorded approximately two to three days after the rain, subsequently declining. This temporal pattern demonstrates a notable, episodic variation in N₂O emissions throughout the experimental period.

Table 6: Statistical results from the linear mixed-effects model evaluating temporal and treatment effects on N₂O emissions.

	Num df	F value	Pr (>F)
Amendment	2	0.58	0.57
Soil type	1	0.36	0.55
Date	24	2.37	0.0003
Amendment:soil type	2	1.36	0.28

4.1.5 Treatment effects on cumulative N₂O emissions in natural and clay-treated peat soils

Figure 10 illustrates the distribution of cumulative N₂O emissions, while Figure 11 provides a detailed overview of daily emissions, highlighting temporal patterns, emission peaks, and replicate variability across treatments and soil types. In the natural peat soil, biochar and wood chip treatments showed lower cumulative N₂O emissions than the control, corresponding to descriptive reductions of

approximately 42% and 47%, respectively. Similarly, in clay-treated peat soil, the incorporation of wood chips reduced cumulative N_2O emissions by approximately 39% compared to the control sample, whereas no such reduction was observed with the incorporation of biochar; conversely, BC-Clay resulted in a 9.6% increase. The box plot showed considerable variability among lysimeter replicates, with the largest variability within treatment variability observed for the clay control, where one replicate ($\sim 68 \text{ mg/m}^2$) exhibited considerably higher cumulative emissions than the other two replicates (~ 5.5 and $\sim 12 \text{ mg/m}^2$) (Figure 10).

Table 7: Observed percentage reduction in cumulative N_2O emissions relative to the corresponding control treatment.

Comparison	Treatment mean (mg /m^2)	Control mean (mg /m^2)	Percent change	Percent reduction
Ctrl-Nat	33.79	33.79	0.00	0.00
BC-Nat	19.66	33.79	-41.81	41.81
WC-Nat	17.87	33.79	-47.12	47.12
Ctrl-Clay	29.12	29.12	0.00	0.00
BC-Clay	31.91	29.12	9.57	-9.57
WC-Clay	17.80	29.12	-38.87	38.87

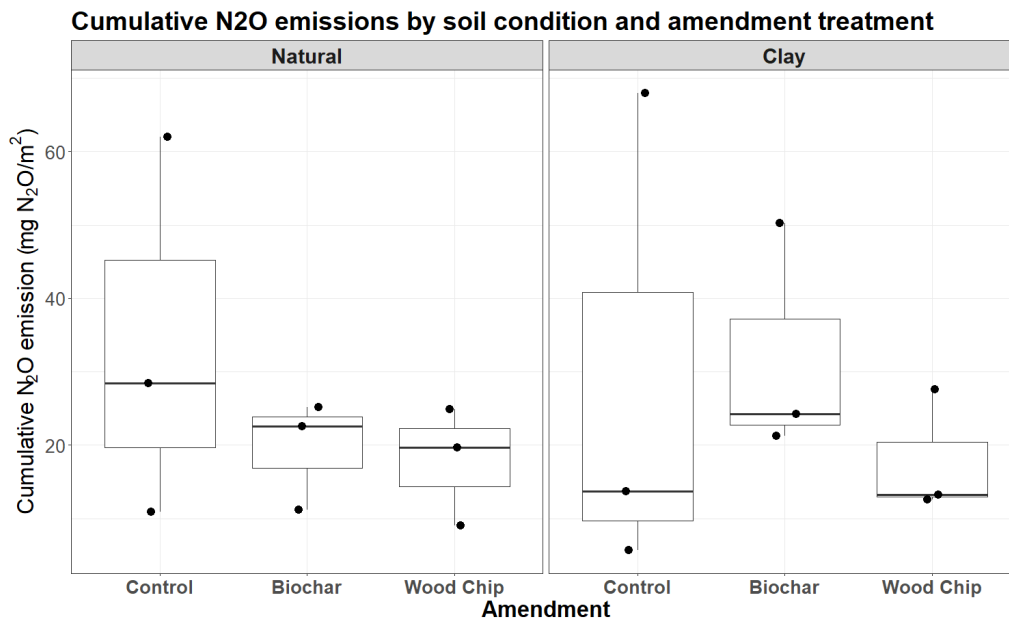


Figure 10: Distribution of mean cumulative N_2O emissions ($\text{mg N}_2\text{O/m}^2$) among amendment treatments in natural and clay-treated peat soils. Box plots display the inter quartile range (25th–75th percentiles), with the horizontal line within each box indicating the median. Whiskers extend to $1.5 \times \text{IQR}$, and black dots represent individual lysimeter replicates ($n = 3$ per treatment).

However, ART ANOVA revealed no significant effect of soil condition (Clay; $F_{1,12} = 0.01$, $p = 0.9$) or amendment treatment (Amendment; $F_{2,12} = 0.56$, $p = 0.6$) on cumulative N_2O emissions. The soil condition $\times$ amendment interaction was also not significant (Clay:Amendment; $F_{2,12} = 0.26$, $p = 0.8$). Thus, although descriptive data showed differences in the magnitude of N_2O reduction among amendment and soil conditions, there was no statistical evidence that amendment effectiveness differed between natural and clay-treated peat soils (Table 8).

Table 8: Results of the aligned rank transform (ART) analysis of the effects of soil condition, amendment, and their interaction on cumulative N_2O emissions.

Term	Df	Df.res	Sum sq.	Sum sq.res	F value	Pr (>F)
Clay	1	12	0.50	458.67	0.01	0.91
Amendment	2	12	40.33	433.33	0.56	0.59
Clay :Amendment	2	12	20.11	456.67	0.26	0.77

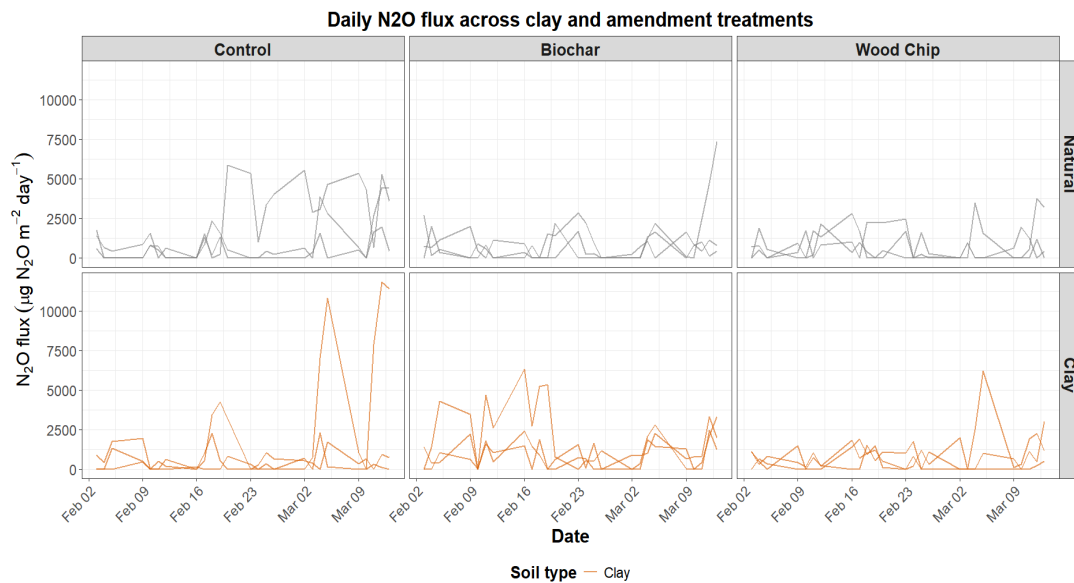


Figure 11: Temporal variation in daily N_2O fluxes ($\mu g N_2O/m^2/day$) across three replicates per treatment following simulated precipitation under wood chip and biochar amendments with and without clay addition

5. Discussion

5.1 N₂O Emission Dynamics Following Fertilization and Precipitation

The study results showed that N₂O fluxes generally increased after rainfall events, peaked within two to three days, and decreased as the soil dried, indicating an episodic emission pattern characterized by short-term N₂O pulses. A similar pattern was observed following subsequent rainfall events, with N₂O fluxes increasing after soil rewetting. This temporal pattern suggests that changes in soil moisture played an important role in regulating N₂O production. Previous studies have shown that soil N₂O emissions can increase substantially following extreme rainfall events or repeated soil wetting–drying cycles (Miller, 2021). Natural variations in soil moisture, driven by environmental and biophysical processes such as precipitation, drainage, and evapotranspiration, can generate wetting–drying cycles (Harrison-Kirk et al., 2013) that influence microbial N transformations and may contribute to episodic N₂O pulses.

Although previous studies have reported increases in N₂O emissions following fertilizer application, no clear immediate increase in N₂O flux was observed after fertilizer application in the peat soils examined in this study. Instead, the observed rainfall-associated increases suggest that rainfall and the resulting changes in soil moisture may have acted as an important trigger for short-term N₂O pulses, and that fertilizer application alone did not produce an immediate and detectable response. Supporting this observation, Spearman correlation analysis showed strong positive relationships between cumulative N₂O emissions and post-rainfall emissions, with the strongest correlations observed within 2–3 days after the March 2 and March 9 rainfall events (Figure 9). Table 5 further showed that 44–82 % and 31–62 % of total N₂O emissions occurred within the 0–3 day and the 0–2 day post-rainfall periods across treatments, respectively. This indicates that a substantial proportion of N₂O emissions occurred during rainfall-associated periods. As soil water content increases toward saturation, microbial activity may be limited because water-filled pores reduce air-filled pore continuity and limit oxygen diffusion, thereby promoting the expansion of anaerobic soil micro sites (Schlüter et al., 2025). Wang et al. (2020) further concluded that nitrification was the dominant source of N₂O under intermediate soil moisture levels, whereas increasing soil moisture towards saturation shifted the dominant N₂O-producing pathway from nitrification to denitrification. They also reported that fertilization with NH₄⁺ further stimulated nitrification and increased its contribution to N₂O production.

Previous studies have suggested that episodic N₂O pulses can occur when rewetting rapidly activates microbial processes. Schlüter et al. (2025) reported that labile carbon accumulated during dry periods, together with intra-cellular compounds released from microbial cells in response to increase in soil moisture, can be rapidly mineralized following soil wetting, thereby increasing the availability of substrates for microbial N transformations. In addition, wetting can increase the availability and mobility of N substrates by dissolving and redistributing residual fertilizer. In the present study, fertilizer application provided readily available N substrate, and simulated rainfall provided the moisture needed to stimulate microbial activity and N transformations. Therefore, the absence of noticeable and immediate N₂O response after fertilization and elevated emissions after rainfall suggest that fertilizer derived N alone may not be adequate to stimulate substantial N₂O production under the current conditions. Instead, the combination of available N and favourable moisture conditions following rainfall may have created suitable conditions for short-term N₂O production.

The physical form of the applied fertilizer may also have affected the timing and availability of N substrates. The use of both powdered and pelleted fertilizers in the experiment could have resulted differences in dissolution and N availability due to differences in exposed surface area. Powdered fertilizer has a higher surface area and may therefore dissolve more rapidly than pelleted fertilizer, potentially increasing the short-term availability of N for microbial transformations. However, since the form of fertilizer was not investigated independently in this study, its contribution to the observed N₂O pulses cannot be clearly distinguished and therefore should only be considered as a possible contributing factor. Rapid changes in both oxygen supply and oxygen demand can cause abrupt changes in the anaerobic soil volume, creating short-lived periods of enhanced denitrification and N₂O production (Schlüter et al., 2025). Such brief periods of disproportionately high biogeochemical activity relative to long intervening periods are described as “hot moments” (McClain et al., 2003). However, hot moments are more likely to occur when the necessary reactants and environmental conditions coincide; the absence of one or more reactants prevents their formation (Molodovskaya et al., 2012). The observations in this study can also be interpreted within the hot-moment framework described by McClain et al. (2003), where fertilizer-derived N may have represented an available substrate, whereas rainfall may have acted as the critical environmental trigger by increasing soil moisture and altering oxygen availability. The observed increases in N₂O flux following rainfall, followed by rapid declines, are therefore consistent with the concept of rainfall-induced hot moments of N₂O production.

The magnitude of these N₂O pulses varied considerably between treatments and replicates, indicating substantial temporal and among replicate variability in N₂O

emissions. Variations in soil N₂O fluxes result from complex interactions among fertilizer inputs, microbial processes, and soil physical conditions (Wang et al., 2020). Although the rainfall events were simulated under controlled conditions and similar rates of fertilizer were applied, differences in soil moisture dynamics, carbon availability, mineral N availability, and oxygen status may have resulted in different micro site conditions for nitrification and denitrification within the lysimeters. N₂O production is strongly influenced by soil microbial communities, which are highly sensitive to soil aeration conditions (Wang et al., 2020). Under well-aerated, oxic conditions, aerobic microbial processes are favoured, whereas reduced oxygen availability following rainfall can promote anaerobic processes such as denitrification. Thus, even among replicate soils subjected to the same treatment, small differences in moisture distribution and oxygen availability may have created different micro site conditions and consequently resulted in substantial differences in N₂O production.

When compared to N₂O emissions measured during the first 48 hours following rainfall events on February 16, March 2, and March 9 (Figure 8) between clay-treated grassland peat soil (Ctrl-Clay) and natural peat soil (Ctrl-Nat), the highest emissions were consistently observed within the Ctrl-Clay treatment. Similarly, among the C/N-amended treatments, most clay-treated soils exhibited higher N₂O emissions than their corresponding natural peat soils (Figure 8). The potential mechanisms underlying this difference, particularly the contrasting effects of clay on soil moisture, oxygen availability, and organic carbon (C) stabilization, are discussed further in Section 5.2.

5.2 Effects of C/N Amendments on N₂O Mitigation and Their Potential Mechanisms

The linear mixed effects model revealed significant temporal variation in N₂O fluxes during the experimental period ($F_{24} = 2.36$, $p = <0.05$), whereas neither amendment nor soil type had a significant effect on N₂O emissions. The amendment $\times$ soil type interaction was also not significant ($F_2 = 1.36$, $p = 0.3$), indicating that amendment effects did not differ significantly between clay-treated and natural peat soils (Table 6). Although the effects of C/N amendments on N₂O emissions were not statistically significant, descriptive comparisons showed that both biochar and wood chips reduced emissions relative to the corresponding controls, particularly in natural peat soils (Table 7).

The numerical reduction in N₂O emissions following wood chip application in both natural and clay-treated grassland peat soils may be partly explained by the relatively high C/N ratio of the wood chip material. The C/N ratio of organic

substrates can influence the balance between microbial N immobilization and mineralization (Signor and Cerri, 2013), thereby affecting the availability of mineral N for microbial processes, particularly nitrification and denitrification, which contribute to N₂O production. Huang et al. (2004) investigated the effects of plant residue decomposition on N₂O emissions under laboratory conditions using residues with a wide range of C/N ratios (8–118). They found that the incorporation of plant residues enhanced both N₂O and CO₂ emissions, and the magnitude of this enhancement depended on the C/N ratio of the residues. In particular, residues with lower C/N ratios inducing higher concentrations of dissolved organic carbon (DOC) and higher N₂O emissions. Substrates with intermediate C/N ratios (25–75) can be mineralized relatively rapidly; however, their decomposition may be limited by microbial N immobilization and protein complexation by polyphenols during cell lysis. In contrast, substrates with high C/N ratios (>75) are often more resistant to decomposition and are characterized by high proportions of structural compounds, such as lignin, condensed tannin, and terpenes. These substrates also contain relatively little N for decomposer microorganisms, which may favour microbial N immobilization during decomposition (Huang et al., 2004). Thus, following the addition of wood chips, microbes may have immobilized available inorganic N during the decomposition of the C-rich materials, reducing the N available for nitrification and denitrification and consequently mitigating N₂O production. This interpretation is consistent with observations from previous studies showing lower N₂O emissions following the incorporation of high C/N residues compared to low C/N residues. For instance, Rothardt et al. (2021) observed the lowest net N mineralization following the application of winter wheat straw, which had the highest C/N ratio except sawdust. The magnitude of the effect reduced as the C/N ratio of the amendments decreased.

However, Liu et al. (2011) found that a single application of wheat straw (C/N = 78.7) slightly reduced the soil inorganic N content before N-fertilizer application, and significantly increased the inorganic N content of the soil after fertilization. This interactive effect resulted in increased N availability, increased microbial nitrification and denitrification rates, and also increased N₂O and NO production and emissions. In the present study, wood chips reduced N₂O emissions in both soil conditions, despite the application of mineral N fertilizer, suggesting that the high C/N ratio and relatively slow decomposition of wood chips may have promoted microbial N immobilization and reduced the availability of mineral N for nitrification and denitrification. Therefore, these processes may have contributed to the observed mitigation effect of wood chips.

In contrast to wood chips, the N₂O mitigation effect of biochar varied between soil types. Biochar reduced descriptively N₂O emissions in natural peat soils, whereas emissions were higher in clay treated peat soils (BC-Clay), although this

difference was not statistically significant (Table 7). A major proportion of biochar carbon is recalcitrant, often referred to as pyrogenic or black carbon (C). It contains several types of organic structures, including water-soluble organics, high molecular weight aliphatics and a relatively high proportion of insoluble aromatic structures, that may mineralize at different rates (Singh et al., 2012). Unlike many conventional organic materials used in agriculture, the recalcitrant nature of biochar enhances C stability in soil, allowing it to persist for a longer period of time (Grutzmacher et al, 2018). Many studies have suggested different mechanisms and characteristics associated with biochar that may impact N₂O emissions in soil, including its liming effect, enhancement of soil water holding capacity (WHC), interactions with dissolved organic C (DOC), release of toxic/inhibitory compounds, interactions with soil biota, adsorption of inorganic N, improved soil aeration, abiotic reduction of nitric forms to N₂, and increases in the relative abundance of denitrifiers and atypical N₂O reducers (Grutzmacher et al, 2018). However, pyrolysis conditions such as temperature, feedstock residence time, feedstock source, and degree of polymerization and aromaticity are key factors controlling the lability and stability of biochar (Grutzmacher et al., 2018). Among these factors, pyrolysis temperature plays a vital role in determining the quality of biochar-amended soils. Low-temperature biochar, which contains higher proportion of labile aliphatic functional groups carried by volatile matter, may enhance short-term rapid nutrient availability. In contrast, high-temperature biochar can promote the long term binding and stabilization of soil carbon and nutrients. In addition, high-temperature biochars have a higher surface area and micro pore volume than low-temperature biochars, facilitating the formation of more stable complexes (Mukherjee and Lal, 2013). In the present study, a decrease in N₂O emissions was observed in fertilized soils amended with biochar, particularly in natural peat soil. This reduction may be explained by mechanisms reported in the literature, whereby biochar reduces soil NH₄⁺-N and NO₃⁻-N concentrations through its adsorption capacity and high cation exchange capacity, thereby limiting the availability of substrates for nitrification and denitrification and consequently reducing N₂O emissions (Gao et al., 2023). An increase in pH due to oxygen-containing functional groups and ash content may promote the complete reduction of NO₃⁻ during denitrification following biochar amendment, thereby reducing the N₂O:N₂ ratio (Lan, 2017). However, in the present study, soil pH showed a slight decrease across all treatments, from 5.48 - 5.65 before treatment to 5.30 - 5.47 after treatment (Table 3). As no significant change in soil pH was observed among the treatments, the proposed liming effect of biochar is not supported by the present data.

Since the reduction of N₂O emissions from biochar-amended soils may vary with soil type, biochar application may be influenced by changes in: 1) soil physical properties (e.g., air diffusion, aggregation, water retention), 2) soil chemical

properties (e.g., pH, EC, organic and mineral N and dissolved organic C availability, organic-mineral interactions), and 3) soil biological properties (e.g., microbial community structure, microbial biomass and activity, macro-fauna activity, N cycling enzymes) (Lan, 2017). These changes may affect N mineralization and immobilization, as well as N turnover, nitrification, and denitrification processes in biochar-amended soils. Most of these effects on soil properties are related to the chemical composition of biochar, including low molecular weight organic compounds, aliphatic C:aromatic C, intrinsic N content and form, ash content, and acid-neutralizing capacity, as well as its physical properties, such as specific surface area, sorption properties, and particle size. The development of negative charges on the surface of biochar over time and their interaction with native soil organic matter and clay minerals may further facilitate the reduction of soil N₂O emissions (Lan, 2017). However, the present study showed an opposite trend, with the BC-clay treatment showing higher N₂O emissions over time and no significant N₂O mitigation effect. The higher N₂O emissions observed in the BC-Clay treatment may be attributed to interactions between biochar, clay, fertilization, and rainfall-induced changes in soil moisture and oxygen availability. The water retention properties of biochar may have further enhanced the water retention of clay-amended peat soil, promoting oxygen-limited micro sites after rainfall, creating favorable conditions for denitrification. In addition, the availability of fertilizer-derived mineral N and potentially labile C fractions from biochar may have stimulated microbial N transformations, and the fluctuating aerobic and anaerobic micro sites could promote coupled nitrification-denitrification.

Although both woodchips and biochar significantly reduced N₂O emissions from the soil, the magnitude of the reduction differed between the two C/N amendments. This discrepancy may be reflected by the variations in their chemical and physical properties. As a relatively fresh, high C/N organic substrate, wood chip may provide readily available carbon that stimulates microbial growth and promotes the immobilization of fertilizer derived mineral N, thereby limiting the availability of substrates for nitrification and denitrification. In contrast, the predominantly recalcitrant carbon in biochar is less readily utilized by microbes, and its effects on N₂O emissions depend more strongly on its physicochemical properties and interactions with soil conditions.

5.3 Influence of Clay Addition on Amendment Effectiveness and N₂O Mitigation

Since the experimental conditions were standardized across all lysimeters, differences in N₂O emissions between the clay-treated and natural peat soils may partly attribute to the presence of clay and its effects on soil physical properties, in addition to the effects of the C/N amendments. Soil texture influences microbial

activity and N₂O production by regulating nutrient retention, water storage, and gas diffusion through differences in pore-size distribution (Walkiewicz, 2025). In particular, clay-rich soils have increased water holding capacity, and the abundance of micro pores can restrict gas diffusion and oxygen availability, thereby influencing the formation of anaerobic micro sites and microbial processes responsible for GHG emissions. However, clay addition may influence N₂O emissions through both carbon stabilization and changes in soil moisture and aeration. The association of organic matter with clay minerals promotes its stabilization by protecting it from microbial decomposition, thereby slowing organic matter degradation (Singh et al., 2019). As denitrification occurs in anoxic micro sites (“hot spots”) where sufficient NO₃⁻ and carbon (C) are available (Surey et al., 2020), clay addition may reduce N₂O production by limiting the availability of labile C required to sustain microbial N transformations. Although by the end of the experiment the mean cumulative N₂O flux (Figure 7) was higher in Ctrl-Nat (~34 mg N₂O/m²) than in Ctrl-Clay (~29 mg N₂O/m²), the reduction in N₂O emissions associated with clay addition was not statistically significant (Table 8; *p* = 0.9). Given that several clay-treated treatments exhibited higher N₂O emissions following rainfall, the moisture-retention and associated oxygen-limitation effects of clay addition may have partly offset its potential to reduce N₂O production through organic matter stabilization in the present study.

When the interactive effects of C/N amendments and clay addition on N₂O emissions were taken into account, wood chip amended clay and natural peat soils (WC-Clay and WC-Nat) showed similar mean cumulative N₂O emissions (~ 17 mg N₂O/m²). In contrast, biochar-amended clay soil (BC-Clay) exhibited considerably higher mean cumulative N₂O emissions (~32 mg N₂O/m²) than the corresponding natural peat treatment (BC-Nat; ~19 mg N₂O/m²) (Table 7). Along with these differences in mean cumulative emissions among treatments, the Clay × Amendment interaction was not statistically significant (Table 8; *p* = 0.8). Therefore, under the conditions of the present study, there was no statistical evidence that clay addition modified the effectiveness of the C/N amendments in mitigating N₂O emissions. Wood chips may primarily influence microbial N cycle through their relatively high C/N ratio and the availability of decomposable organic matter, while biochar can modify soil processes through changes in sorption, pore structure, water retention, oxygen diffusion, and redox conditions. Consequently, the effectiveness of biochar in mitigating N₂O emissions may be more dependent on its interactions with soil physical properties than that of woodchips. Overall, clay addition did not significantly alter the effectiveness of wood chips or biochar in mitigating N₂O emissions under the experimental conditions. However, the contrasting numerical responses between amendments suggest that their interactions with clay-induced changes in soil moisture, aeration, and N cycling

may be different, highlighting the need for further investigation of the underlying physical and microbial mechanisms.

5.4 Limitations and Further Research

Although this study provides insights into the effects of C/N amendments and clay addition on N₂O emissions from grassland peat soils under fertilization and simulated rainfall conditions, several limitations should be considered when interpreting the results.

The duration of the experiment was limited to six weeks, which allowed for the evaluation of the short-term effects of the amendments, but did not allow for the assessment of their long-term effectiveness and persistence. In particular, the decomposition and stability of wood chips and biochar can vary over time, altering their effects on soil N availability and nitrous oxide emissions. Therefore, long-term experiments are needed to determine whether the observed mitigation effects are sustainable over time before applying directly to the field.

A relatively high variability in N₂O emissions between replicates also represents a limitation of the study. Only three lysimeters were used per treatment, and the considerable temporal and spatial variability in N₂O emissions reduced the statistical power to detect differences between treatments. As a result, non-significant treatment effects should be interpreted with caution, as they may reflect a true lack of effect or a combination of high variability and the limited number of experimental replicates. Given the small sample size and variability of the data, mainly non-parametric statistical methods were used to minimize the influence of outliers and reduce the risk of violating distributional assumptions. Variation in clay distribution between lysimeters may also have contributed to differences in soil physical conditions between experimental units. Although attempts were made to distribute clay as consistently as possible when assigning treatments, variations in its spatial distribution can affect soil structure, water retention, and oxygen diffusion, which may contribute to variability in N₂O emissions. In addition, the distribution of simulated rainfall across the lysimeters may not have been completely uniform due to variations in their position within the mesocosm facility and the performance of the rainfall simulator. Pre-trials were conducted prior to the experiment to assess and improve the water distribution among the lysimeters. Wherever possible, the locations of the lysimeters within the rainfall simulator were rotated.

Furthermore, several mechanisms proposed to explain the observed results in N₂O emissions were not directly measured. Soil mineral N concentrations (NH₄⁺ and

NO_3^-), dissolved organic carbon, redox states, and microbial community characteristics were not monitored throughout the experiment. As a result, the mechanisms underlying the observed N_2O responses can only be inferred from the available data and previous literature. Therefore, it is better to include these measurements to distinguish between nitrification and denitrification pathways and to identify the mechanisms responsible for the mitigation effects of wood chips and biochar.

In addition, controlled lysimeter experiments may not fully represent the complexity of field conditions. Factors such as natural precipitation variability, seasonal temperature fluctuations, vegetation, root activity, and spatial heterogeneity may affect N_2O emissions and amendment efficiency under field conditions. Therefore, field-based experiments conducted over longer periods of time are needed to evaluate the practical relevance and persistence of the observed mitigation effects.

All lysimeters received both fertilizer and simulated rainfall; the experimental design did not include unfertilized or rain-free controls. Since Calcium Ammonium Nitrate (CAN) was applied to the soil surface in dry form, it may not have dissolved and entered the soil solution until the first rainfall event. Consequently, the observed increase in N_2O emissions following rainfall could be attributed to increased soil moisture, the delayed dissolution of the fertilizer, or a combination of both; these mechanisms cannot be distinguished under the present experimental design.

Since both granular and powder forms of fertilizer were applied in this study, the potential impact of fertilizer form on N_2O emissions from peat soils was not specifically assessed. Therefore, future research could investigate how different forms of fertilizer affect N_2O emissions, as well as how wood chip particle size affects its mitigation potential. Smaller wood chip particles have a higher surface area and can decompose more rapidly, which may alter microbial activity, N availability, and N_2O emissions. Therefore, future studies could compare different wood chip sizes and their decomposition rates in relation to N_2O emissions. Similarly, future research on biochar should consider specific and well-defined biochar characteristics, such as feedstock type, pyrolysis temperature, and production process. Alternatively, controlled comparisons of biochar produced at different temperatures from different feed stocks could provide further insight into how these properties affect N_2O emissions. However, including multiple variables may considerably increase experimental complexity and make it more difficult to identify the mechanisms responsible for the observed effects. Therefore, future experiments should focus on clearly defined amendment characteristics to allow for more robust conclusions. Given the economic and energy requirements associated

with biochar production, readily available and locally sourced wood chips or other organic feed stocks may represent more practical and economically feasible options for N₂O mitigation.

6. Conclusion

This six-week laboratory experiment investigated the potential of clay addition and C/N amendments to mitigate N₂O emissions from grassland peat soils under fertilization and simulated rainfall conditions. The results suggest that C/N amendments, particularly wood chips and biochar, may reduce N₂O emissions from grassland peat soils, although their apparent mitigation effects varied among amendment types and soil conditions. Wood chips and biochar showed lower N₂O emissions than the corresponding controls, with reductions of approximately 39 - 47% in the observed comparisons. However, given the limited number of replicates ($n = 3$ per treatment) and the relatively high variability in N₂O emissions, these overall treatment effects were not statistically confirmed.

There was no clear immediate increase in N₂O flux following fertilization alone. Instead, increases in N₂O emissions were associated with simulated rainfall events, indicating that rainfall-induced changes in soil moisture acted as an important trigger for short-term N₂O pulses. Therefore, it can be concluded that fertilization provided a readily available N substrate, and rainfall created favorable moisture conditions that could stimulate microbial activity and induce N transformations that contributed to short-term N₂O emissions.

Despite considerable numerical differences among treatments, clay addition and the clay $\times$ amendment interaction did not significantly affect N₂O mitigation. These findings suggest that clay incorporation did not enhance the effectiveness of C/N amendments under the experimental conditions. However, the contrasting numerical responses of the wood chip and biochar treatments suggest that interactions between amendments and clay-induced changes in soil physical conditions, such as water retention, aeration, and oxygen availability, may affect N₂O production.

To sum up, the numerical reduction associated with wood chips suggests that readily available, high C/N organic materials may offer a practical approach for reducing N₂O emissions from fertilized peat soils. The findings also demonstrate the importance of rainfall-induced emission pulses, suggesting that mitigation strategies should consider not only fertilizer management but also interactions between soil amendments, N availability, and soil moisture dynamics. However, long-term and field-based experiments are needed to determine the persistence, economic feasibility and real world applicability of these mitigation effects under natural environmental conditions.

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Popular science summary

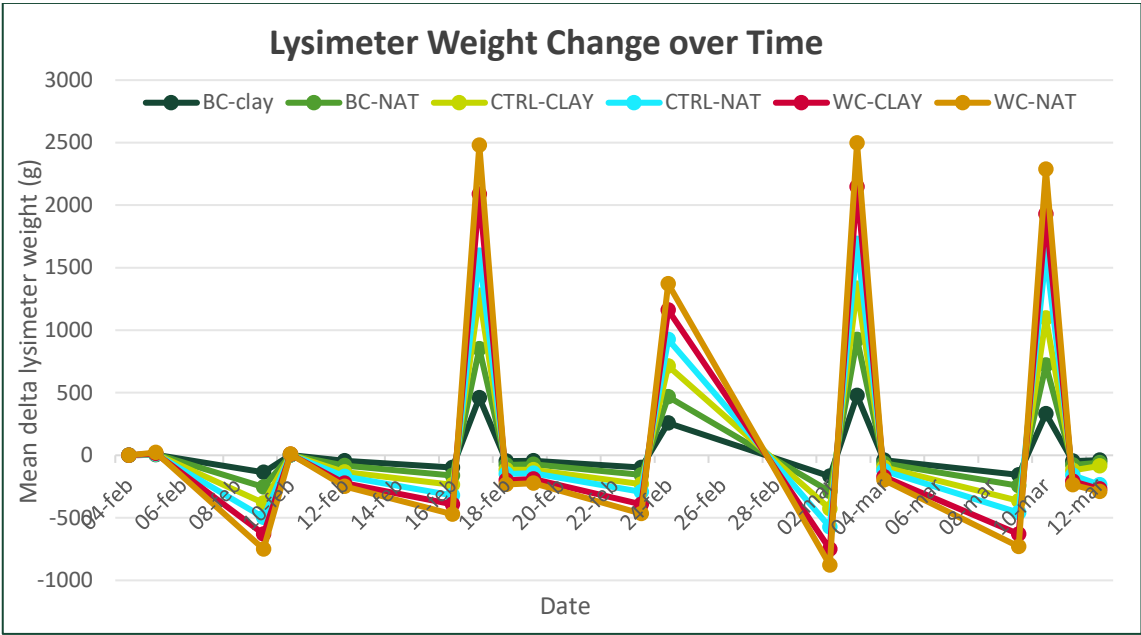
Anthropogenic activities are increasingly affecting the Earth's climate and environment. Peatlands are major global reservoirs of carbon (C) and nitrogen (N), however, many peatlands have been drained for agriculture and other human activities. When drained peat soils are cultivated, the decomposition of organic matter can release large amounts of greenhouse gases, including carbon dioxide (CO₂) and nitrous oxide (N₂O), while fertilization and precipitation events further stimulate N₂O emissions. Therefore, assessing practical measures to reduce greenhouse gas emissions from grassland peat soils is essential for both climate mitigation and sustainable food production.

This study investigated whether adding clay and carbon rich amendments with a high C/N ratio, particularly wood chips and biochar, and their interactions could mitigate N₂O emissions from grassland peat soils. A controlled mesocosm experiment was conducted under fertilization and simulated precipitation conditions. The results showed that fertilization, coupled with favourable moisture conditions following rainfall, created conditions that elevated short-term N₂O emissions.

Neither clay addition alone nor interaction of soil type and amendment did not significantly reduce N₂O emissions. However, both wood chips and biochar showed potential to mitigate N₂O emissions from peat soils, although the treatment effects were not statistically significant ($F_2 = 0.577$, $p = 0.572$). Wood chips, in particular, showed a consistent reduction in N₂O emissions in both natural and clay-treated peat soils. One possible explanation is that the relatively high C/N ratio of the amendments promoted microbial immobilization of available N, thereby reducing the amount of inorganic N available for nitrification and denitrification and, consequently, limiting N₂O production.

In conclusion, the findings suggest that wood chips and biochar have potential as soil amendments for mitigating N₂O emissions from grassland peat soils under simulated rainfall and fertilization. However, further long term studies under field conditions are needed to determine whether these mitigation effects persist over time and under natural environmental conditions.

Appendix 1



Woodchips	Biochar
Source: Pine	Source: Woodchips
	Pyrolysis temperature: 650°C

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