Nitrogen use efficiency and summer nitrous oxide emissions in a long term organic grass-clover ley system

Implications of anaerobic digestion of cattle slurry on nitrogen management

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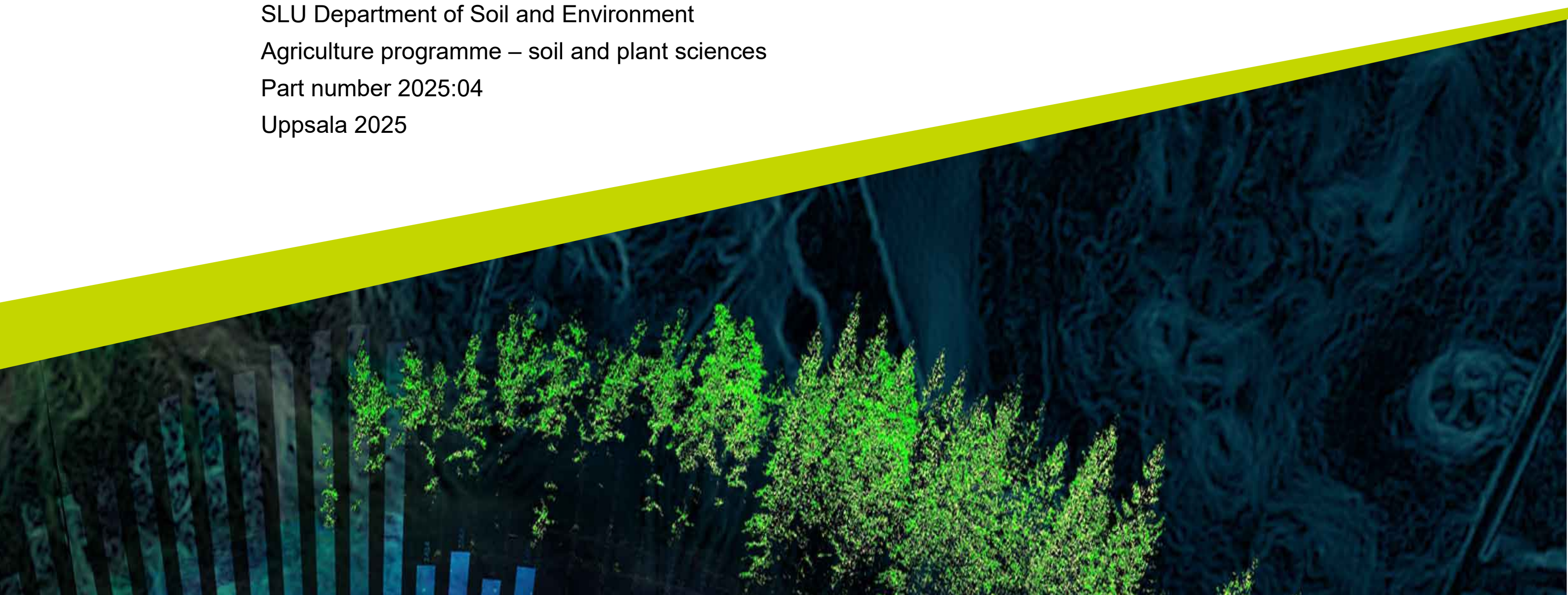

Nitrogen use efficiency and summer nitrous oxide emssions in a long term organic grass-clover ley system - Implications of anaerobic digestion of cattle slurry on nitrogen management

Nitrogeneffektivitet og sommerlystgassutslipp fra et langsiktig økologisk gress-kløver system - Implikasjoner av anaerob utråtning av storfegjødsel på nitrogenforvaltning

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Abstract

This study investigated the long-term impact of anaerobically digested slurry (ADSH) on nitrogen use efficiency (NUE) and nitrous oxide (N_2O) emissions in comparison to untreated slurry (USH) and a control without fertilizer. The hypothesis was that ADSH would improve NUE and reduce N_2O emissions compared to USH but increase emissions relative to the control. The study was conducted on an organic dairy farm in Tingvoll, Norway, where three treatments (control, USH, and ADSH) were applied to experimental plots in a randomized block design. The main outcomes were forage yield, NUE, and N_2O emissions, with yield and NUE assessed over multiple years and N_2O emissions measured on residual effects from long-term application during the 2024 growing season when no fertilizer was applied. The results indicated that both USH and ADSH significantly improved yield compared to the control, with ADSH showing a slight yield increase over USH, although this difference was not significant. Yield variations were more influenced by seasonal factors, such as precipitation and temperature, than by fertilizer treatment. NUE was similar between ADSH and USH, and both treatments showed reduced NUE in the second cut compared to the first. A negative correlation between NUE and clover percentage suggested that biological nitrogen fixation may have reduced the efficiency of the applied organic nitrogen fertilizers. N_2O emissions were primarily driven by soil temperature and lagged precipitation, with no significant treatment effect on emissions. Interestingly, ADSH and USH exhibited similar emissions, with the treatment effect only interacting with temperature. The results suggest that environmental factors, particularly temperature and precipitation, have a stronger influence on N_2O emissions than fertilizer type. Limitations of the study include the short duration of N_2O measurements and potential disturbances from frame placement. Overall, the findings suggest that fertilizer type has a minor impact on NUE and N_2O emissions under cool, wet conditions, highlighting the importance of environmental factors in shaping agricultural emissions. Further research is needed to determine if these results hold in other climates or under different management practices.

Keywords: Nitrous oxide emissions, Anaerobic digestion, Organic fertilizers, Organic farming, Greenhouse gases

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Abbreviations

SLU	Swedish University of Agricultural Sciences
NORSØK	Norges Senter for Økologisk Landbruk / The Norwegian Centre for Organic Agriculture
NUE	Nitrogen use efficiency
N_2O	Nitrous oxide
N	Nitrogen
GHG	Greenhouse gas
US	Undigested slurry
ADS	Anaerobically digested slurry
USH	Undigested cattle slurry (treatment code)
ADSH	Anaerobically digested slurry (treatment code)

1. Introduction

In organic agriculture, there are restrictions on agricultural inputs to sustain healthy soils, ecosystems, and human health, while at the same time ensuring reasonable yields. For example, in Norway, the use of mineral fertilizers in organic agriculture is prohibited. Instead, organic farming relies on natural sources of plant nutrients, such as plant residues, nitrogen-fixing plants, animal manure and urine, compost. Additionally, digestate from biogas production may be used, as long as the feedstock used in the biogas process is certified organic. These alternatives help create a more sustainable nutrient cycle in agricultural systems by reducing external mineral nitrogen (N) inputs. Organic N sources are generally considered more environmentally friendly compared to mineral fertilizers due to their lower energy requirements for production and their contribution to nutrient recycling. A meta-analysis of 133 maize studies found that substituting mineral with organic fertilizer increased yield, reduced N losses, and enhanced soil carbon sequestration, resulting in a net carbon sink despite higher CO_2 emissions (Wei et al., 2020). Moreover, the volatility of the mineral fertilizer market, as demonstrated by the mineral fertilizer price crisis in 2022/2023, can heavily impact farmers who rely on these inputs. To reduce dependence on mineral fertilizers, farmers can adopt organic fertilizers like manure, compost or digestate, which recycle nutrients within the farming system, provided the feedstock for the digestate originates from the farm. An additional strategy to decrease reliance on mineral fertilizers is to improve nitrogen use efficiency (NUE) for organic fertilizers, meaning that the fraction of applied N that is actually utlize by the crop is increased. Enhancing NUE requires precise timing of fertilizer application to match crop nutrient demands, which can be challenging when using organic fertilizers, as the nutrients are generally released more unpredictably than from mineral fertilizers. Optimizing NUE reduces N losses to the environment through gaseous emissions and nitrate leaching, the latter of which contributes to eutrophication of water bodies. The introduction of N, whether from organic or mineral sources, alters the N cycle and increases the risk of N losses. However, N is essential for crop production. Therefore, optimizing NUE is essential to minimize N loss, mitigate environmental damage, and improve economic returns for farmers.

1.1 Slurry management in organic milk production

Animal manure is a valuable plant nutrient resource in organic agriculture, making it beneficial for organic farms to integrate animal husbandry with plant production. In Norwegian organicfarming systems, livestock and crop production are typically integrated on the same farm, meaning that manure from organic dairy operations is applied to on-farm ley pastures rather than being transported to separate crop farms. This localized use of manure optimizes nutrient recycling by creating a local circular nutrient system.

Organic dairy farmers employ various slurry management strategies to optimize nutrient utilization and minimize environmental impact. These practices include composting, anaerobic digestion, and direct field application. Composting involves the aerobic decomposition of manure, resulting in a stable product that enhances soil structure and fertility (Bernal et al. 2017). Anaerobic digestion, on the other hand, breaks down manure in the absence of oxygen, producing biogas for energy and digestate that can be used as fertilizer (Rehman et al. 2019). Direct application of raw slurry (or any kind of fertilizer) to fields is also practiced, though it requires careful timing and management to prevent nutrient runoff and leaching, as well as pathogen exposure.

The choice of manure management strategy significantly influences environmental outcomes. For instance, composting can reduce pathogens and weed seeds (Bernal et al. 2017), but if not managed properly, it may lead to ammonia, methane and N_2O (Cao et al. 2019). Anaerobic digestion has also been shown to mitigate methane emissions from manure storage, thereby reducing the overall greenhouse gas (GHG) footprint of dairy operations, although it also increasesthe risk of ammonia volatilization (El Mashad et al. 2023). Other strategies, such as proper timing and method of manure application, as well as the use of nitrification inhibitors, can also play key roles in minimizing environmental impacts. Understanding the impacts of different manure management strategies on N dynamics is crucial for evaluating their effectiveness in improving NUE and reducing N_2O emissions.

1.2 Nitrogen use efficiency

N is essential for plant growth, being a crucial component of chlorophyll, which is involved in photosynthesis. Therefore, N is a crucial agronomic input for achieving satisfactory crop yields. However, the efficiency with which crops utilize N can vary, as plants do not absorb all the N applied to agricultural soils. When fertilizer application is not properly synchronized with crop nutrient demand, the risk of N losses increase and occur primarily through leaching and gaseous emmisions (N_2O and ammonia) (Ladha et al., 2005). These losses not only reduce crop yield potential but also contribute to environmental issues such as water contamination, increased GHG emissions, and eutrophication in aquatic ecosystems (Ngatia et al., 2019).

Nitrogen Use Efficiency (NUE) quantifies how effectively plants utilize applied N. Improving NUE is essential for promoting both agricultural productivity and environmental sustainability. Strategies to enhance NUE include adopting precision agriculture techniques to apply N more accurately, using slow-release or stabilized fertilizers, incorporating organic amendments, and aligning N application with crop growth stages, depending on the specific conditions of the farm (Dobermann & Cassman, 2004). In particular, the use of anaerobically digested cattle slurry, as opposed to raw manure, has shown potential to enhance NUE due to changes in N availability and reduced N losses (Frick et al., 2023). Additionally, biogas treatment of livestock slurry and biowastes has been found to reduce GHG emissions, improve NUE, and decrease N leaching. However, the risks of increased ammonia emissions and methane leakage in digestate needs to be carefully managed to maximize its environmental benefits (Møller et al., 2022).

1.3 Nitrous Oxide (N_2O) emissions

Nitrous oxide (N_2O) is a potent GHG with a long atmospheric lifespan, driving climate change and ozone depletion. Since the industrial revolution, its atmospheric concentration has increased substantially, primarily due to anthropogenic activities, particularly agriculture. With a Global Warming Potential (GWP) 273 times that of CO_2 over 20- and 100-year time frames, and 130 times over 500 years, N_2O plays a major role in global warming and atmospheric chemistry (Forster et al., 2024).

1.3.1 Denitrification

N_2O emissions from soils occur primarily through the microbial processes of nitrification and denitrification, with denitrification being the dominant pathway (Qui et al. 2022). Denitrification is a respiratory process in which nitrate (NO_3^-) is reduced to gaseous N (N_2) under anaerobic conditions (fig 1). In this process, NO_3^- serves as an electron acceptor, while organic carbon compounds act as electron donors and N_2O is produced as an intermediate product during incomplete denitrification.

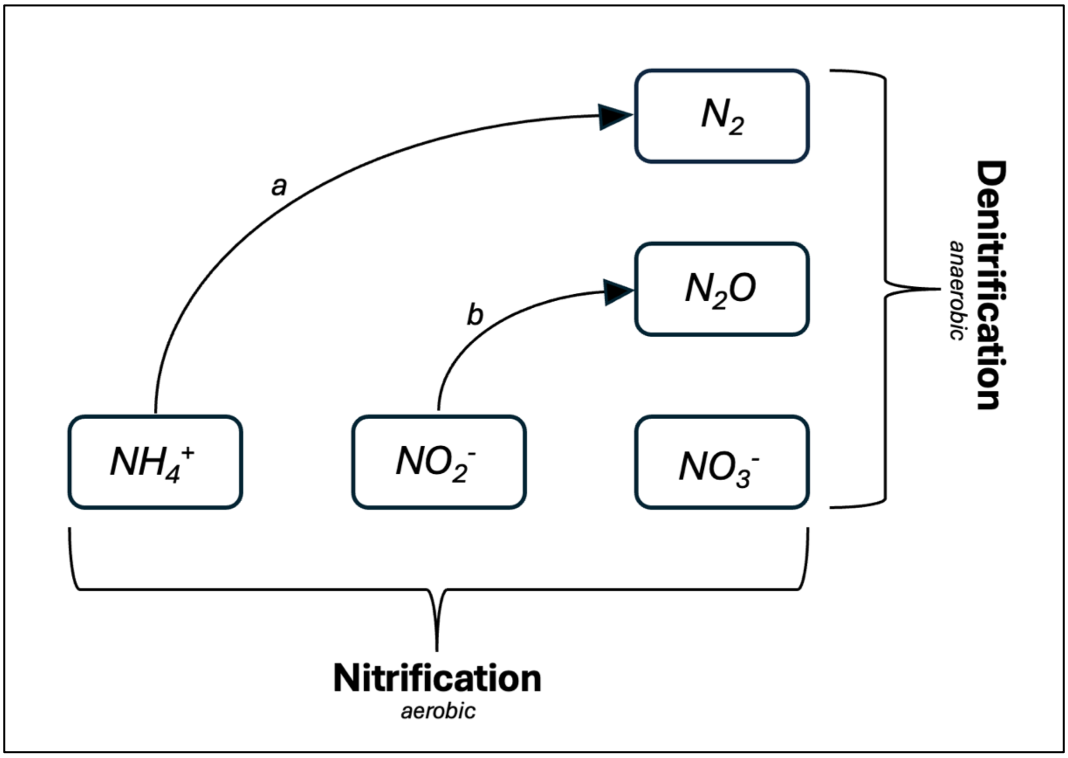

Figure 1: Microbial pathways of N2O-production through nitrification and denitrification. (a) Conversion via hydroxylamine (NH_2OH), an intermediate in ammonia oxidation, into N_2O. (b) Nitrifier denitrification, where nitrifying bacteria reduce nitrite (NO_2^-) to N_2O under oxygen-limited conditions. (Redrawn from Rapson & Dacres, 2013).

Conditions that favor denitrification and N_2O release include high NO_3^- concentrations, abundant organic carbon, and wet or waterlogged (anaerobic) soils (Philippot et al., 2007). These factors are common in agricultural settings where fertilizers, irrigation, organic carbon inputs and soil management practices can amplify N_2O emissions. Quantifying the contribution of each factor to denitrification and N_2O release is critical for designing targeted mitigation strategies to effectively reduce emissions from agricultural soils.

1.3.2 Agricultural Practices and N2O Emissions

Global anthropogenic N_2O emissions have increased significantly between 1980 and 2020, driven primarily by agriculture. The anthropogenic fraction of global N_2O emissions is estimated at 35%, with agriculture contributing 56% of this total (Tian et al., 2024). Manure management and soil emissions, especially from N-based fertilizers, are major contributors. Another study suggest that agricultural practices account for 60-80% of anthropogenic N_2O emissions (Yang et al., 2021).

Several factors contribute to N_2O emissions from agricultural soils, including N inputs, soil moisture and oxygen availability, soil organic carbon (SOC) content, soil pH, and temperature (Butterbach-Bahl et al., 2013). N inputs enhance microbial nitrification and denitrification processes that produce N_2O (Li et al., 2020). Soil moisture content, particularly water-filled pore space (WFPS), plays a key role by creating anaerobic conditions that favor denitrification and aerobic conditions that promote nitrification. When irrigation or precipitation increases WFPS, anaerobic conditions prevail, leading to higher N_2O emissions, especially when soil nitrate levels are elevated

SOC can increase N_2O emissions by providing an energy source for denitrifying bacteria, which enhances microbial activity under anaerobic conditions (Adviento-Borbe et al., 2007). Soil pH also influences microbial processes; higher pH can promote complete denitrification, reducing N_2O production, but it may also increase nitrification and N mineralization, which can offset these reductions. Bleken and Rittl (2022) found that raising soil pH from 4.8 to 5.8 halved N_2O emissions from ploughed leys, likely due to enhanced denitrification efficiency. Liming acidic soils can, therefore, help mitigate emissions.

Temperature is another important factor, as warmer conditions accelerate microbial activity, boosting both nitrification and denitrification (Smith et al., 2018). Temperature also promotes the decomposition of organic matter, releasing more carbon and N into the soil.

Tillage practices influence N_2O emissions by aerating the soil, which increases nitrification and N mineralization, while also raising soil temperature and accelerating decomposition. No-till or low-till practices can reduce N_2O emissions by minimizing soil disturbance, although in compacted or poorly drained soils, these practices may lead to waterlogging and increased denitrification (Six et al., 2006). Therefore, it is crucial for farmers to consider specific field conditions when choosing practices to minimize N_2O emissions.

1.4 Anaerobic digestion as a Sustainable Solution

Anaerobic digestion (AD) is a microbial process that breaks down organic matter such as animal manure, crop residues, or food waste in an oxygen-free environment, producing biogas (methane and carbon dioxide) and a nutrient-rich byproduct known as digestate (Rehman et al., 2019). Digestate contains plant nutrients like N, phosphorus (P), and potassium (K), as well as micronutrients essential for plant growth (García-Lopez et al., 2023). The nutrients in digestate are often more concentrated and bioavailable than in the original substrate due to the breakdown process, which releases nutrients as the organic carbon is converted into gas (Tambone et al., 2010). Additionally, the slightly alkaline nature of digestate implies that a significant portion of N is present as ammonium (NH_4^+), which is readily available for plants.

While digestate has a lower organic carbon content than raw manure, which can lower soil organic carbon (SOC) levels in the short term (Barłóg et al., 2020), it still contains some organic carbon, unlike mineral fertilizers that lack it entirely. Carbon in digestate ias also more stable, meaning theat SOC can increase in the long term due to less carbon breakdown. The lower C/N ratio of digestate also limits the carbon available for denitrifying bacteria, potentially reducing N_2O emissions compared to raw manure (Tambone et al., 2010). These factors not only reduce N losses via denitrification (thereby decreasing N_2O emissions) but also enhance soil structure and nutrient retention, which ensures that a greater proportion of the available nitrogen is in sync with crop demand, ultimately boosting plant nitrogen uptake and overall nitrogen use efficiency NUE. Frick et al. (2023) suggest that anaerobic digestion (AD) of cattle slurry enhances NUE compared to raw slurry when applied to crops like ryegrass.

Although most research on NUE has focused on short-term effects, the long-term impacts of manure management practices remain largely unexplored. In organic dairy farming systems in Norway, two questions can be asked: How do anaerobically digested cattle slurry (ADSH) and undigested cattle slurry (USH) affect long-term NUE, and what are the residual effects of years of slurry application on N_2O gas emissions, particularly in years when no slurry is applied? This study addresses these gaps by (1) quantifying the long-term change in NUE from ADSH versus USH, and (2) measuring the residual N_2O fluxes to provide the essential estimates needed for sustainable manure management strategies.

In the context of organic dairy farming in Norway, this study seeks to explore how AD may hold the key to improving both environmental and economic sustainability by enhancing NUE while reducing N_2O emissions. If proven useful, AD application might provide a breakthrough for farmers looking to mitigate their environmental footprint.

1.5 Objective and Hypothesis

1.5.1 Objective

One objective of this study is to quantify both the long-term effects of anaerobically digested cattle slurry (ADS) on nitrogen use efficiency (NUE) over several years and its residual impact on N_2O emissions—compared with untreated slurry (US)—during the 2024 growth season (May–October), when no fertilizer is applied. In addition, the study will provide essential estimates of how anaerobic digestion can enhance nitrogen cycling, boost NUE, and reduce N_2O emissions, thereby establishing it as a sustainable alternative to applying untreated cattle slurry in agricultural soils.

1.5.2 Hypotheses

The hypotheses are that:

1. anaerobically digested cattle slurry will increase the NUE in comparison to undigested cattle slurry due to nitrogen being more readily available for plant uptake.

2. the residual effect of long-term application of anaerobically digested cattle slurry will reduce N_2O-emissions compared to undigested cattle slurry, but increase in comparison to the control.

2. Materials and methods

2.1 Experimental site

The experimental site is located in Tingvoll research farm (62°54′N,8°11′E), Møre og Romsdal in North-Western Norway. The research farm is owned by the Norwegian Centre for Organic Agriculture (NORSØK) and the experiment SoilEffects has been ongoing since 2011. The area has a Subarctic climate/Temperate oceanic climate (World bank, fig 2) according to the Köppen-Geiger climate classification system. The annual average precipitation between 2015-2024 (April to October) in Tingvoll was 861 mm, and in 2024 (April to October) it was 872 mm (NIBIO), meaning 2024 was a fairly representative year.

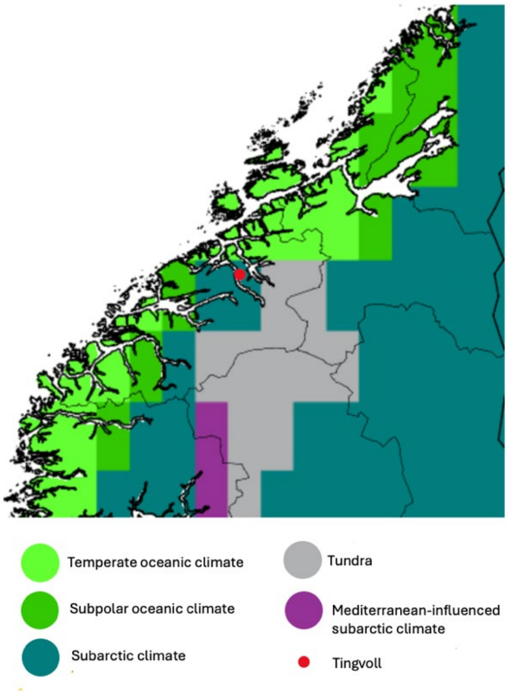

Figure 2: Experimental site with climate zones in the North-West of Norway. Colors represent the different climate zones and the red dot issituated at the experimental site "SoilEffects" in Tingvoll.(Picture reworked from World bank, accessed October 2024, https://climateknowledgeportal.worldbank.org/country/norway)

The experiment was set up in 2011 to study the long-term effects of anaerobically digested (AD) slurry compared to undigested slurry (UD) on soil characteristics and crop yields. The slurry is collected from cattle from NORSØK's own research farm with organic dairy production. The AD slurry is derived from the same UD slurry from the dairy cattle and digested in the farms own biogas reactor. The chemical composition of the AD and UD can be found in Rittl et al. (2023). The system consists of a perennial grass-clover ley harvested 2 times per year, and is re-established every 5 years with a cereal cover crop and harvested as green fodder. During the re-establishment of the ley, no manure is applied (Rittl et al., 2023). In the year 2024 when N_2O emissions were measured, no manure was applied and oats were grown, meaning this study is looking at the residual effects of long-term application.

2.2 Experimental design

The experiment uses a randomized block design consisting of 5 treatments, but here only the following 3 three treatments were used; anaerobically digested slurry (ADSH), undigested slurry (UDSH), and control without any added fertilizer in four (4) blocks which makes 12 plots in total. The fertilized treatments received 220 kg total N per ha and year in the beginning of the growing season between 2011–2023. However, during the years 2014, 2019, 2024, no manure was applied due to re-establishment of the ley and in 2020 due to a problem with the biogas reactor.

2.3 Yield and Nitrogen use efficiency

This study uses data from Rittl et al. (2024) on crop yield, N content, clover percentage collected from the years 2011, 2012, 2013, 2015, 2021, 2022 and 2023. The missing years are due to the cultivation of crops other than ley or the absence of botanical composition evaluations during those years. The botanical composition was conducted using representative samples of 0.5 kg plant material from each plot which was sorted into grass, clover and weeds.

Because of the clover content in the ley, the NUE was calculated two ways; To assess NUEso it is critical to account not only for the N supplied by fertilizer but also for the additional N provided through clover fixation. Therefore, we employ two complementary NUE equations. The first (NUE1) provides a straightforward measure of NUE by comparing the total crop N harvested from fertilized plots to that from unfertilized plots, relative to the rate of fertilizer N applied. The second (NUE2) refines this measure by correcting for the N added through clover fixation. This dual approach enables us to disentangle the effects of direct fertilizer application from the biological contributions of clover

Equation 1

$$NUE1 = \frac{(NF) - (NC)}{R}$$

Equation 2

$$NUE2 = \frac{(NF) - (NC)}{(R + CF) - (CC)}$$

In these equations, **NF** denotes the total crop N harvested from fertilized plots, while **NC** denotes the total crop N harvested from unfertilized plots. The variable **R** represents the rate of fertilizer N applied to the fertilized plots. In the second equation, **CF** represents the amount of N added via clover fixation in fertilized plots, and **CC** represents the N fixed by clover in unfertilized plots.

For estimating clover N-fixation, equations by Nyborg (1995) were used. The equations are adjusted based on the legume proportion in the crop. The rationale for employing two versions is that the efficiency of N fixation by clover can vary with its relative abundance in the ley. When clover makes up less than 50% of the crop, a slightly higher efficiency factor is used, whereas a lower factor is applied when clover dominates (>50%).

Legume proportion < 50 %:

Equation 3

$$Nfix = DS\ crop\ x\ \%\ clover\ x\ 0{,}0345\ x\ (0{,}9/100)$$

Legume proportion > 50 %:

Equation 4

$$Nfix = DS\ crop\ x\ \%\ clover\ x\ 0{,}0345\ x\ (0{,}85/100)$$

Here, DS_crop represents the dry substance of the crop.

2.4 N_2O Flux Measurements

During the growth season of 2024 there was not ley, but oats grown in the experiment. Therefore, no manure was applied this year, and the gas analysis is done on the residual effect of previous slurry and digestate application through several years. The plots were ploughed on April 30th and August 6th. This is not a common occurrence, but due to a high weed pressure, so mechanical weeding had to be done. The plots measured 8 x 3 m and the frames were 51 x 51 cm. One frame was positioned in each plot (Figure 3), and the edges filled with water to avoid gas transfer between outside and inside the chamber. The chamber lids were placed on the frames at the start of gas measurement.

Figure 3: The frames and chambers in some plots in the SoilEffects project. Photo: Kari Løe

Two methods were used for gas flux measurements: the GasMet FTIR analyzer (figure 5) and gas chromatography with manual vial sampling (figure 4) . The GasMet and gas chromatographymeasures a variety of gases, but this study only focuses on N_2O. For both methods gas samples were collected using one manual static chamber per plot. The GasMet system was used in April and May 2024, to provide real-time gas concentration data directly in the field. From June to October 2024, gas samples were collected manually using glass vials for later analysis.

Figure 5:Manual sampling with syringes and vials for later analysis with gas chromatography. Photo: Johanna Maria Zimmermann

Figure 4:Gas measurements using GasMet FTIR analyzer. Photo: Johanna Maria Zimmermann

For vial sampling, glass vials were marked with the date and vial number and evacuated using a vacuum machine before sampling. Gas samples were collected using syringes with 2-Way stopcocks. To ensure proper mixing of the chamber air, the syringe was plunged five times before injecting the sample through the septum into the vial. The vials were over-pressurized to maintain shelf stability. Four gas samples were taken per plot at 10-minute intervals (0, 10, 20, and 30 minutes) after chamber closure to allow for gas flux calculations. These vials were sent to Germany for analysis at Rheinland-Pfälzische Technische Universität Kaiserslautern-Landau (Rhineland-Palatinate Technical University Kaiserslautern-Landau) using a gas chromatograph equipped with a pressure sensor.

For all sampling events, weather conditions, soil temperature at 5 cm depth in each plot, and the time of measurement were recorded. Approximately two measurements were taken per week, depending on weather conditions (preferably after rain), resulting in a total of 27 sampling dates during the 2024 season.

2.5 Statistics

All statistical analyses were conducted in R (version 4.4.2; R Core Team, 2024) within the RStudio IDE (version 2024.09.1+394). The following R packages were used:

- tidyverse (Wickham et al., 2019): Data manipulation and visualization.
- lubridate (Grolemund & Wickham, 2011): Handling date-time data.
- performance (Lüdecke et al., 2021): Model diagnostics.
- lme4 (Bates et al., 2015): Mixed-effects models.
- pbkrtest (Halekoh & Højsgaard, 2014): Bootstrapping and F-tests in mixed models.
- readxl (Wickham & Bryan, 2023): Importing Excel data.
- ggbeeswarm (Clarke et al., 2023): Beeswarm plots.
- ggpubr (Kassambara, 2023): Publication-ready ggplot2 figures.
- lmerTest (Kuznetsova et al., 2017): p-values for mixed-effects models.
- emmeans (Lenth, 2024): Post-hoc analysis of estimated marginal means.
- gasfluxes (Fuss et al., 2024): Gas flux calculations.
- gridExtra (Auguie, 2017): Arranging multiple plots.

Significance was set to $p < 0.05$.

2.5.1 Yield and NUE Analyses

Yield and NUE data from all plots and treatments were combined into a single dataset. Relevant variables were extracted, and the data were processed to ensure consistency. Any missing values were identified and removed using the drop_na() function.

A linear mixed-effects regression model was developed to predict yield, incorporating treatment as a fixed effect and plot, production year (the age of the ley) and calendar year as random effects. Predictions were generated for all possible combinations of Treatment, Year, and Plot. Final model:

```
Total_yield ~ Treatment + (1 | Produc_year_ley) + (1 |
Block) + (1 | Year)
```

Two separate mixed-effects models were constructed to estimate NUE1 and NUE2. The first model for NUE1 included treatment, harvest cut (first or second harvest), and Clover percent as predictors, with plot and calendar year as random effects. The second model for NUE2 used the same predictors. The difference between the two models is the way of calculating NUE using two different equations (see section 2.4). Interaction terms were assessed using ANOVA comparisons, and non-significant terms were removed. I used PBmodcomp to perform a parametric bootstrap likelihood ratio test comparing nested mixed-effects models, allowing for robust evaluation of the significance of specific fixed effects (e.g., interaction terms). This method yields more reliable p-values than standard tests for mixed models. Final models:

```
NUE1 ~ Treatment + Cut + Clover percent + (1 | Plot) + (1 |
Year)

NUE2 ~ Treatment + Cut + Clover_percent + (1 | Plot) + (1 |
Year)
```

2.5.2 Gas Flux Calculations

Gas flux data were analyzed in R. Data from multiple sampling dates were combined into a single dataframe, and missing values were excluded. A one-day lag for precipitation (dplyr::lag()) was included to assess delayed rainfall effects on N_2O fluxes. To calculate gas flux in mg/m²/min, gas concentrations measured in ppm are first converted to mg/m³ using the ideal gas law. Specifically, the conversion is given by:

Equation 5

$$Cmg/m3 = \frac{Cppm \times MW \times P}{R \times T}$$

where:

- Cppm is the gas concentration in ppm,
- MW is the molecular weight of the gas (g/mol),
- P is the ambient pressure (kPa),
- R is the universal gas constant (8.314 kPa·m³·mol^{-1}·K^{-1}), and
- T is the ambient temperature (in Kelvin).

This mass concentration is then used to calculate the flux by accounting for the chamber geometry and time interval. The flux is calculated as:

Equation 6

$$Flux\ (mg/m^2/min) = \frac{Cmg/m3 \times V}{A\ \times \Delta t}$$

where:

- V is the chamber volume (m³),
- A is the chamber's cross-sectional area (m²), and
- Δt is the time interval (min) over which the concentration change is measured.

Combining the two steps, the overall equation becomes:

Equation 7

$$Flux\ (mg/m^2/min) = \frac{\left(\frac{Cppm \times MW \times P}{R \times T}\right) \times V}{A\ \times \Delta t}$$

Flux calculations were performed using a linear mixed regression model implemented in the gasflux package.

Then, different transformations of the data were tested by fitting different linear models (LMs) to find the form of the data that best meets the model assumptions and provides the most robust and interpretable results.. The transformations included natural logarithmic, base-10 logarithmic, base-2 logarithmic, squre root and cube root transformations. After evaluating the model performances by using model diagnostics the moel using the natural logarithmic transformation was selected. Then, interaction models were built using treatment, soil temperature, and lagged precipitation, testing all pairwise interactions. Non-significant interactions were removed to simplify the model.

The final model for N_2O fluxes included treatment, soil temperature, and lagged precipitation as fixed effects, with plot and sampling date as random effects:

```
N2O_flux_nlog ~ Treatment * soiltemp + lagged precipitation
+ (1|plot) + (1|date)
```

Where N2O_flux_nlog is the natural logarithm of the N_2O flux (mg/m^2/min), soiltemp is the centered soil temperature, lagged precipitation is the centered lagged precipitation (precipitation one day before the sampling), (1|plot) is the random effect of plot and (1|date) is the random effect of the sampling date.

Predictions were generated for varying soil temperature and precipitation scenarios, and visualizations with 95% confidence intervals were created using ggplot2.

3. Results

3.1 Yield

Mean total yields over the years showed that both ADSH and USH consistently produced higher yields than the Control (table 1, figure 6). However, all in all over the years, the differences between ADSH and USH were non-significant (Figure 6, Figure 7).

Table 1: Total ley yield in kg/ha (combined for cut 1 and cut 2) means and SD for each treatment over 11 years.

Treatment	Mean yield	SD
Control	5117	1513
USH	9814	1544
ADSH	10084	1513

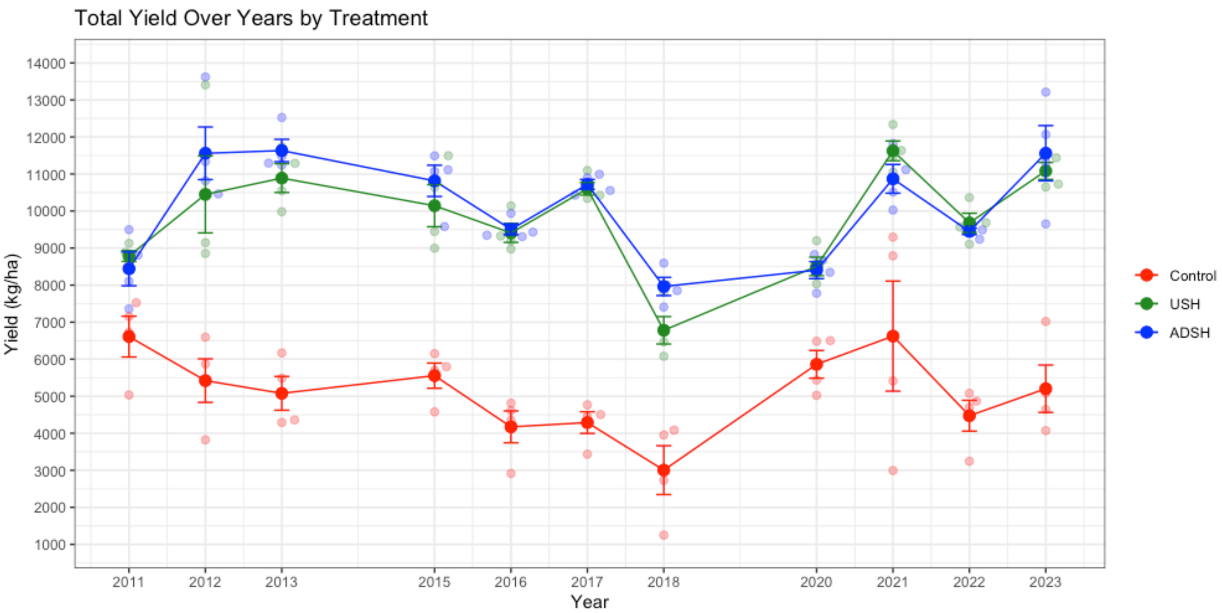

Figure 6: Total ley yield (combined for both cut 1 and cut 2) by year and treatment. Opaque dots are averages with standard errors and translucent dots are observed yield for each treatment replicate. Missing years (2014 & 2019) there were other crops than ley and no fertilizer was applied.

When splitting up the yields into the two yearly harvests (cut 1 and cut 2) Yield differences appear to be more strongly influenced by cut than by treatment, as seen in Figure 7.

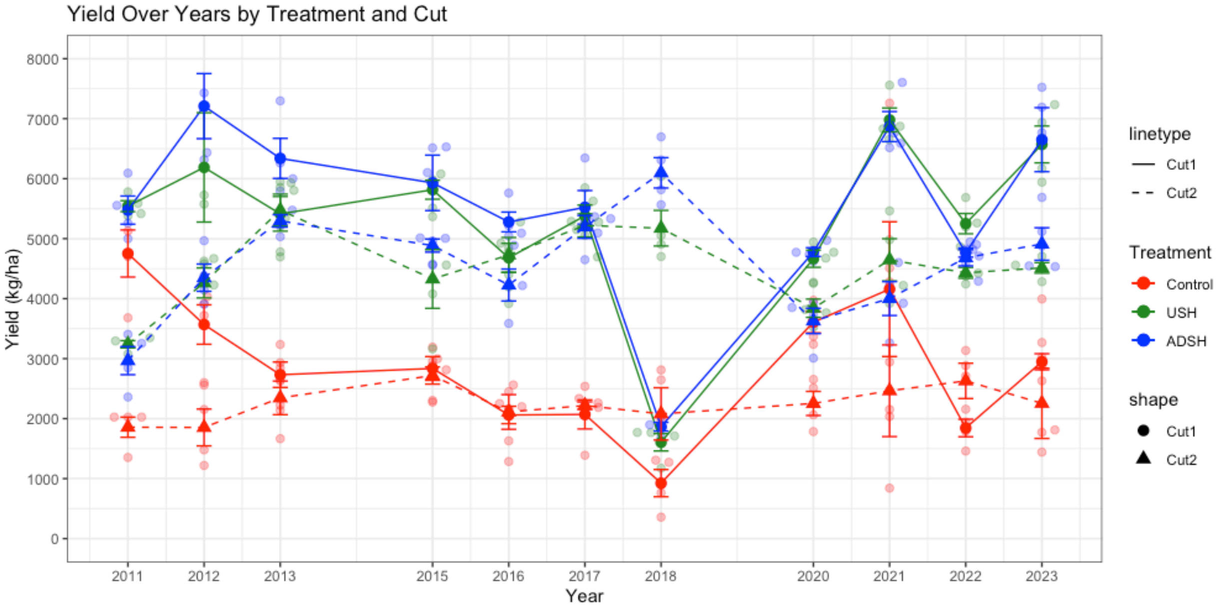

Figure 7: Ley yield for both cut 1 and cut 2 by year and treatment. Opaque dots are averages with standard errors and translucent dots are observed yield for each treatment replicate. Regular lines represent cut 1 and dashed lines represent cut 2. Missing years (2014 & 2019) there were other crops than ley and no fertilizer was applied.

Table 2: Estimates, standard errors (SE), and p-values (from parametric bootstrapping) for the fixed effects in the total yield model. The effects evaluated include the Intercept (Control treatment), as well as the impacts of Treatment USH and Treatment ADSH compared to the control. The contrast *difference between ADSH and USH is also shown (p-value from pairwise contrast).*

Fixed Effect	Estimate	SE	p-value
Intercept (Control)	5117.16	378.47	< 0.001
Treatment USH	4696.76	244.31	< 0.001
Treatment ADSH	4966.73	244.31	< 0.001
ADSH vs. USH	-270.0	246	0.5174

Table 3: Random effects for the total yield model. This table presents the variance estimates and standard deviations (SD) for the random effects included in the total yield model. The random effects include Calendar year, Block, and Production year, along with the residual variance

Random Effect	Variance Estimate	SD
Calendar year	1 057 000	1027.87
Block	69 390	263.42
Production year	0.158	0.40
Residual	1 313 000	1145.91

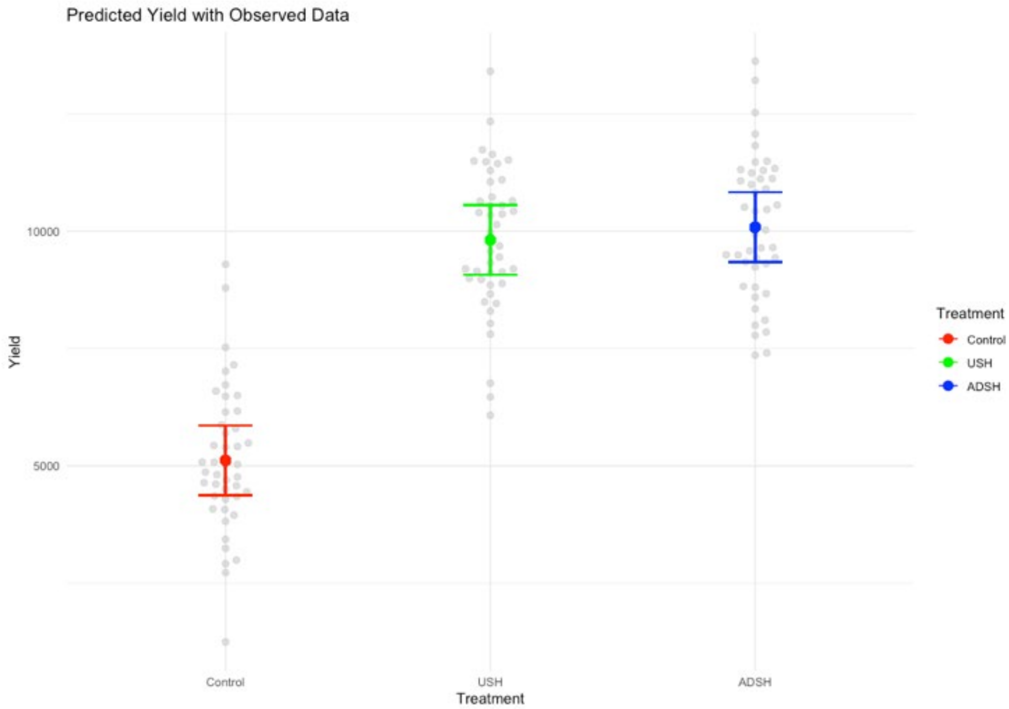

Figure 8:Plot with predicted total (both cut 1 and cut 2) ley yields for 11 years with 95% confidence intervals in color, and beeswarmed (jittered) observed values in grey

The model revealed significant differences in yield among treatments. The intercept, representing the baseline treatment (Control), was estimated at 5117 kg/ha/year ($p < 0.001$). Both USH and ADSH treatments resulted in significantly higher yields relative to the Control, with increases of 4697 kg/ha/year and 4967 kg/ha/year, respectively; however, there was no statistically significant difference between USH and ADSH. These results indicate that the yield differences in the experiment are primarily driven by the treatment effects, while variability associated with production year, block, and year is accounted for as random effects. The model does not include direct measurements of environmental factors such as temperature or soil moisture, so the yield estimates reflect the average conditions captured by the random effects rather than specific scenarios of temperature or moisture extremes.

3.2 NUE

For both treatments (USH and ADSH), the mean values for NUE1 and NUE2 are similar (around 0.4 for both methods). This suggests that both methods yield comparable results for the treatments (table 4). Overall, the data suggests that both treatments (USH and ADSH) have similar NUE values on average, but the degree of variability in NUE1 and NUE2 differs, with ADSH having a bit more variation in both methods compared to USH. The control had most clover percentage followed by ADSH and USH (Table 5)

Table 4: Summary of Nitrogen Use Efficiency (NUE) values for each treatment (USH and ADSH) based on two different methods (see materials and methods), NUE1 and NUE2. The table shows the mean and standard deviation (SD) of NUE for each treatment across all years after pooling data from Cut 1 and Cut 2. Both NUE1 and NUE2 are calculated using two differenct equaions.

Treatment	Mean NUE1	SD1	Mean NUE2	SD2
USH	0.405	0.141	0.382	0.151
ADSH	0.409	0.201	0.391	0.197

Table 5: Average clover % per treatment and cut and total along with standard deviations (SD) for 7 years

Treatment	Cut 1	SD Cut1	Cut 2	SD Cut2	Total	SD Total
Control	32.2	14.4	33.1	16.6	65.3	22.0
USH	23.3	16.3	13.5	11.0	36.9	19.6
ADSH	24.3	18.3	17.4	12.7	41.6	22.3

Mean NUE using two methods (with or without clover N-fixation) is shown for both cuts over the years in figure 9. There was no evidence for differences between the two treatments.

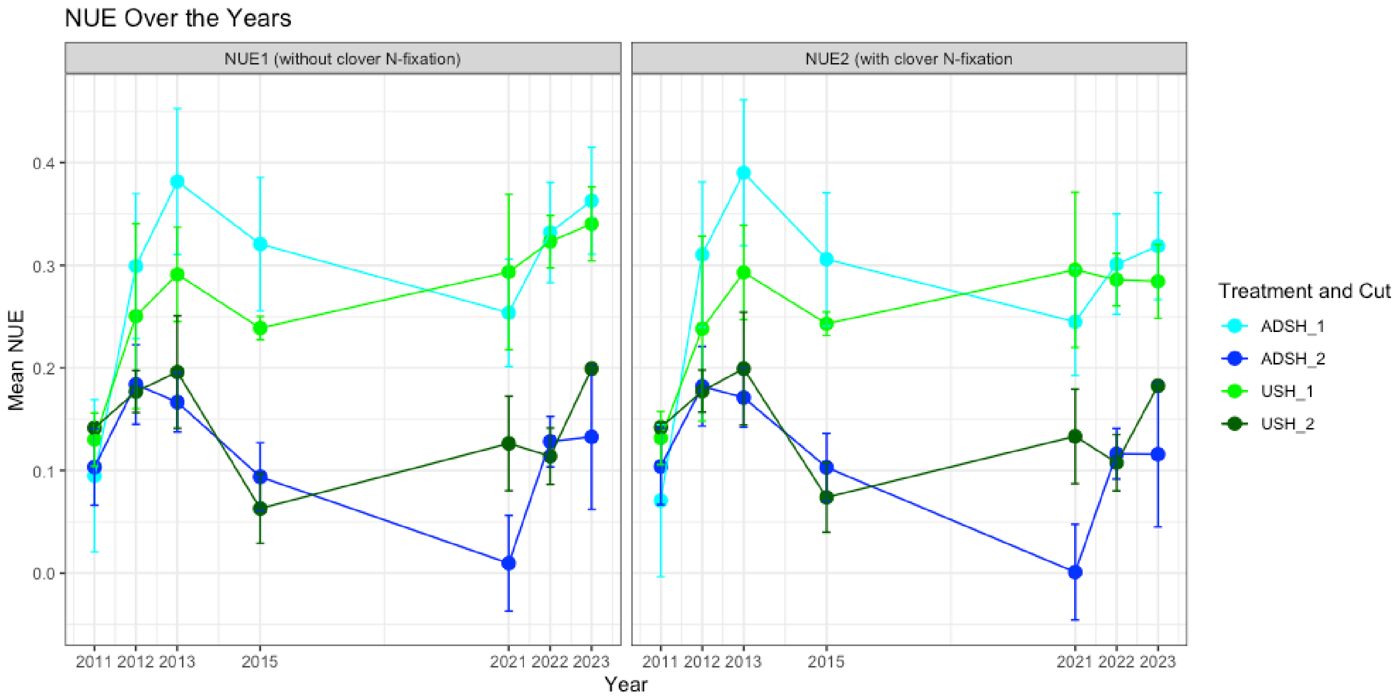

Figure 9: Average NUE1 and NUE2 per treatment and cut over the years standard error bars. Missing years are due to absence of fertilizing that year or unability to quantify the clover percentage

Table 6: Evaluation of fixed effects in the NUE1 model. The table shows the estimated coefficients, standard errors (SE), t-values, and p-values for the fixed effects in the model.

Fixed effect	Estimate	SE	t-value	p-value
Intercept (/Cut1)	0.29019	0.035219	8.240	<0.001
treatmentUSH	-0.0044	0.041426	-0.106	0.919
Cut2	-0.1666	0.017464	-9.538	<0.001

Std. Clover percentage	-0.0317	0.011050	-2.873	0.005

Table 7: Variance estimates for random effects in the NUE1 model. The model includes random intercepts for Plot and Year to account for variation at these levels. The residual variance represents unexplained variation in NUE1.

Random effect	Variance	SD
Plot	0.002888	0.05374
Year	0.002123	0.04607
Residual	0.007468	0.08642

The NUE1 model showed that the intercept for ADSH and Cut 1 was highly significant ($p < 0.001$), with a baseline NUE of 0.29019 (table 6). There was no significant difference between ADSH and USH (estimate = -0.0044, $p = 0.919$). However, Cut 2 had a significantly lower NUE1 compared to Cut 1 (estimate = -0.1666, $p < 0.001$), indicating a decline in N use efficiency in the second harvest. Additionally, Clover percentage had a significant negative effect on NUE1 (estimate = -0.0317, $p = 0.005$), suggesting that higher clover presence was associated with lower NUE1 values. The random effects (table 7) showed that variation across plots ($\sigma^2 = 0.0029$) was slightly higher than across years ($\sigma^2 = 0.0021$), but both were relatively small. The residual variance ($\sigma^2 = 0.0075$) remained the largest source of unexplained variation, suggesting that factors beyond those included in the model contribute to differences in NUE1.

Table 8: Evaluation of fixed effects in the NUE2 model. The table shows the estimated coefficients, standard errors (SE), t-values, and p-values for the fixed effects in the model.

Fixed effect	Estimate	SE	t-value	p-value
Intercept (ADSH/Cut1)	0.27886	0.03237	8.615	<0.001
treatmentUSH	-0.00481	0.03927	-0.122	0.907
Cut2	-0.16041	0.01644	-9.758	<0.001
Std. Clover percentage	-0.04400	0.010240	-4.297	<0.001

Table 9: Variance estimates for random effects in the NUE2 model. The model accounts for random effects of Plot and Year, with the residual variance capturing the remaining variability in NUE2.

Random Effect	Variance	SD
Plot	0.002598	0.05097
Year	0.001458	0.03818
Residual	0.006592	0.08119

The NUE2 model showed similar trends, with a significant intercept for ADSH and Cut 1 (0.27886, $p < 0.001$) (Table 8). Again, there was no significant difference between ADSH and USH (estimate = -0.00481, $p = 0.907$). As in NUE1, NUE2 was lower in Cut 2 (estimate = -0.16041, $p < 0.001$), and increasing clover percentage further decreased NUE2 (estimate = -0.04400, $p < 0.001$). Random effects (table 9) showed that variation across plots ($\sigma^2 = 0.0026$) was slightly higher than across years ($\sigma^2 = 0.0015$), but both were relatively minor. The residual variance ($\sigma^2 = 0.0066$) remained the dominant source of variation, similar to NUE1, indicating that unexplained variability is still present in the model.

Both models showed consistent results: USH and ADSH had no significant differences in NUE, while Cut 2 consistently had lower NUE than Cut 1, consistent with the lower biomass yield from Cut 1 to Cut 2. Additionally, higher clover percentages negatively impacted NUE in both models (figure 10). While random effects contributed some variation, residual variance remained the largest source of unexplained differences, highlighting the potential influence of additional factors not included in the model.

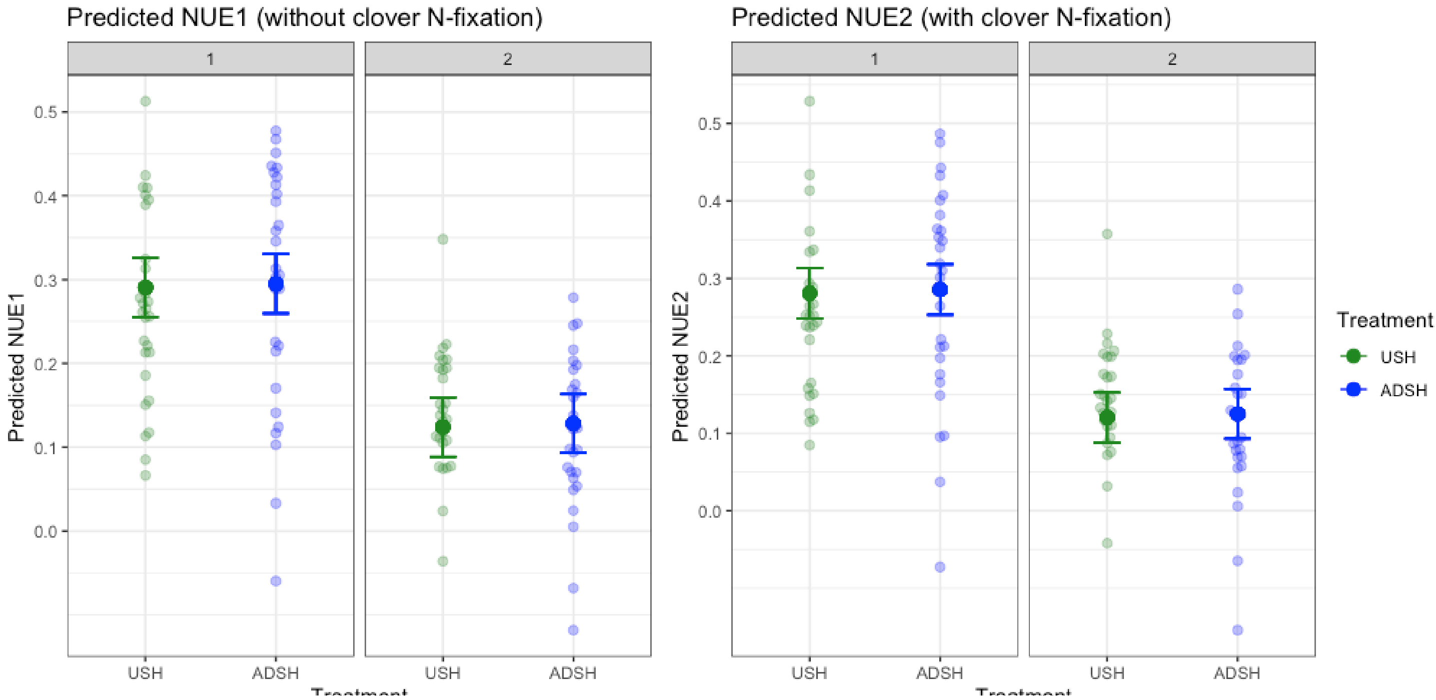

Figure 10: Predicted NUE1 and NUE2 for both cuts for USH and ADSH

3.3 N_2O fluxes

N_2O fluxes varied across treatments, with ADSH showing the lowest mean emissions, while USH and CONT exhibited higher fluxes (table 10). Variability in emissions was highest in the CONT treatment (table 10).

Table 10: Mean and standard deviation (SD) of N_2O fluxes (mg/m²/min) for each treatment.

Treatment	Mean N_2O flux (mg/m^2/min)	SD
Control	0.000716	0.00115
USH	0.000734	0.000644
ADSH	0.000526	0.000517

Figure 11 shows the N_2O-fluxes over the season of 2024 with the mean soil temp measured at 5 cm depth for each treatment..

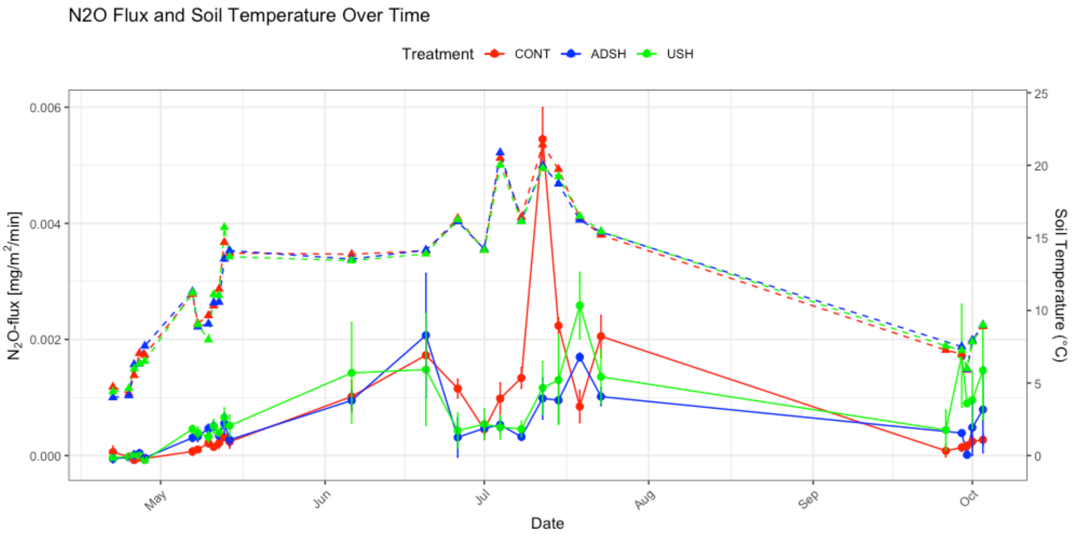

Figure 11: N_2O-fluxes (solid line) over the season of 2024 with soil temperature for treatments above (dashed line). Ploughing occurred April 30th and August 6th.

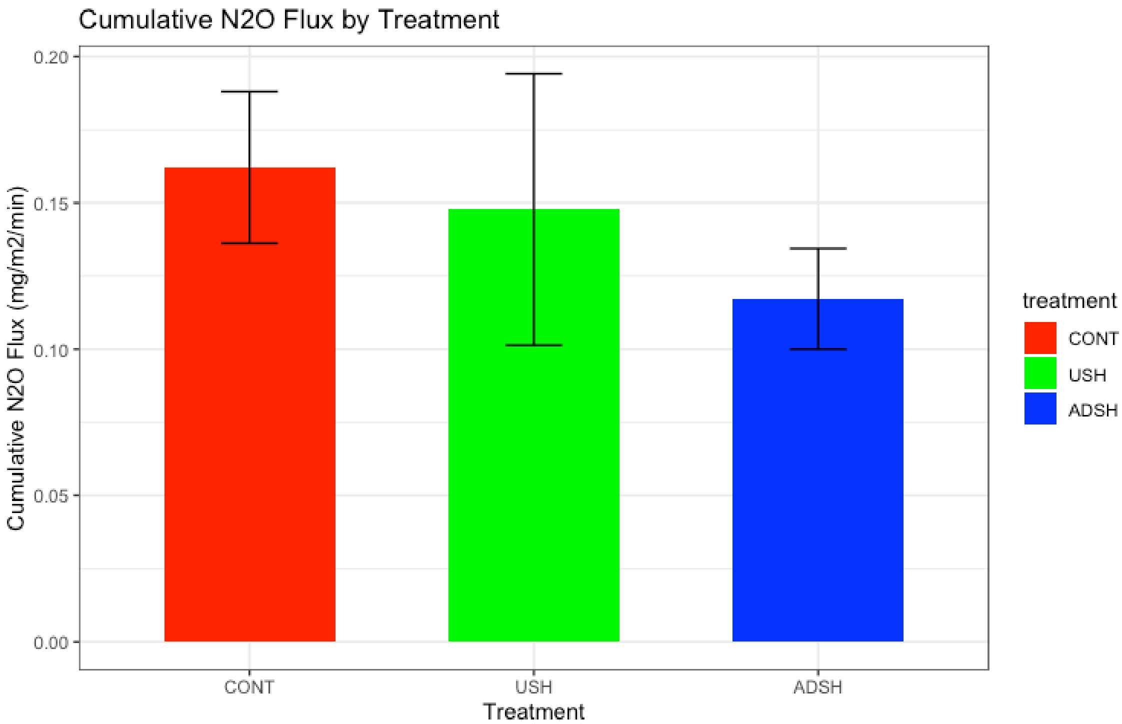

Figure 12: Cumulative N2O-emissions over the 2024 season per treatment. CONT - no fertilizer, USH - undigested cattle slurry, ADSH - anaerobically digested cattle slurry. Error bars represents the 95% confidence intervals.

Mean cumulative flux was lowest for ADSH (0.117 mg/m²), followed by USH (0.148 mg/m²) and CONT (0.162 mg/m²) (table 11). However, treatment effects were not statistically significant (figure 12, table 12).

Table 11: Cumulative N2O fluxes and standard errors for each treatment during the season of 2024

Treatment	Cumulative flux ($mg/m^2/min$)	Cumulative SE
Control	0.162	0.0132
USH	0.148	0.0237
ADSH	0.117	0.00877

The model results for N_2O fluxes (Table X) showed that soil temperature had a strong positive effect on N_2O flux (estimate = 0.124, $p < 0.001$), indicating that higher temperatures led to increased emissions. Lagged precipitation also had a strong positive effect (estimate = 0.026, $p = 0.002$). However, there was no evidence for main treatment effects.

Table 12: N2O model summary of fixed effects

Term	Estimate	p-value	Significance
Intercept (control)	-7.213	<0.001	Highly significant

treatmentADSH	-0.053	0.765	Not significant
treatmentUSH	+0.096	0.590	Not significant
cen_soiltemp	0.124	<0.001	Highly significant
cen_precip_lag	0.026	0.002	Significant

A significant interaction between treatment and soil temperature ($p < 0.001$) indicated that the relationship between temperature and N_2O flux varied by treatment (figure 13), meaning that the effect of temperature on N_2O emissions depended on the fertilizer treatment. In contrast, the interaction between treatment and lagged precipitation was not significant ($p = 0.427$), meaning that the effect of precipitation on N_2O flux did not depend on fertilizer treatment (figure 14), thus, this interaction term was not included in the model. Lagged precipitation in this model represents the total precipitation from the previous day, based on the assumption that N_2O flux responses may be delayed rather than immediate.

The model showed greater variation in N_2O fluxes across sampling dates ($\sigma^2 = 0.069$) than between plots ($\sigma^2 = 0.052$), indicating temporal variability had a stronger influence. Residual variance remained high ($\sigma^2 = 0.183$), suggesting other unaccounted factors affected fluxes.

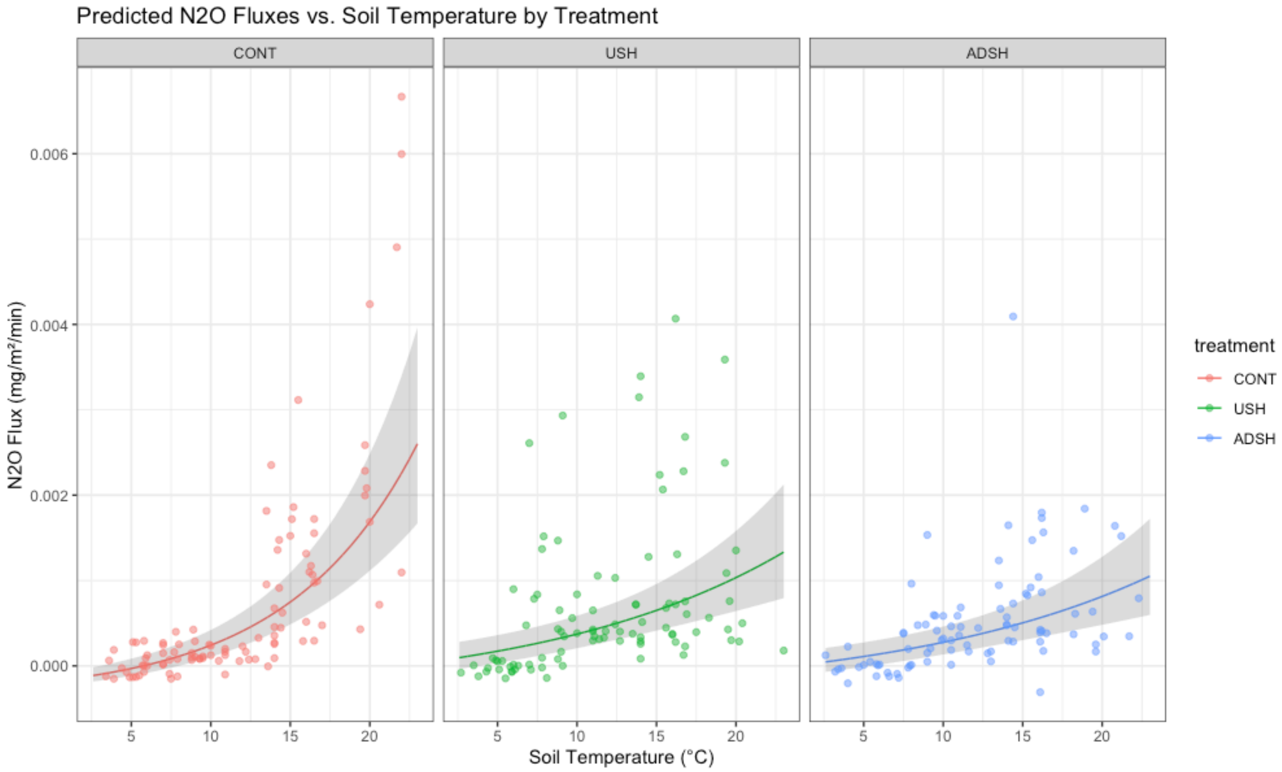

Figure 13: The predicted effect of soil temperature on N2O-fluxes in mg/m2/min by treatment

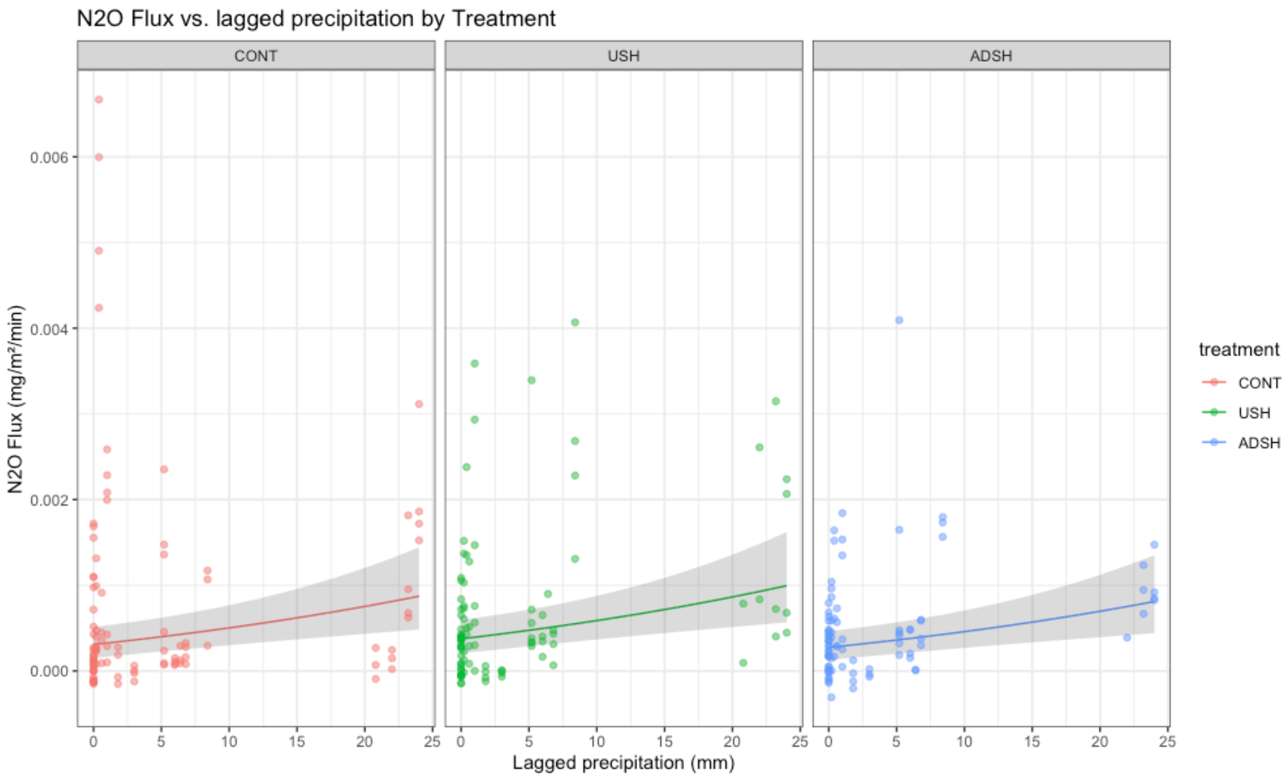

Figure 14: The predicted effect of lagged precipitation on N2O-emissions in mg/m2/min by treatment.

4. Discussion

This study aimed to assess the impact of long-term cattle slurry application of anaerobically digested slurry (ADSH) on NUE as well as N_2O emissions in comparison to untreated slurry (USH). Specifically, we hypothesized that ADSH would enhance NUE due to increased N and decreased organic carbon availability in the digestate and that ADSH would reduce N_2O emissions compared to USH but increase emissions relative to the control.

4.1 Yield

The results from the yield analysis show that both USH and ADSH outperform the control, making them both viable alternatives for organic fertilizer. Although ADSH showed a marginal yield increase (4967 kg/ha) compared to USH (4697 kg/ha), this effect could have arisen due to random variation rather than true treatment effect as the difference was not statistically significant. This is consistent with a study that found no significant difference in forage yield between digested and undigested slurry applications over a three-year period (Walsh et al. 2018). In contrast, short-term studies that have reported a pronounced yield increase from digestates attribute this to their higher content of readily available ammonium (Möller & Müller 2012). However, in this long-term study the data suggest that any initial advantage of ADSH diminishes over time, likely due to the mineralization of organically bound N in the USH, resulting in similar yield performances between ADSH and USH long-term. The clover percentage was highest for the control treatment, likely due to the fact that N fixing plants do well without N fertilizer while grasses do not, leading to clover taking over in the control treatment at a higher rate than in the fertilized treatments.

The yield analysis also revealed that the yields differed substantially between cut 1 and cut 2, where yield for cut 1 was larger. This is consistent with previous findings where the first cut is generally larger than the second. A study based in Finland by Niskanen et al. (2006) showed that the yield for the second cut for several Timothy varieties dropped from the first cut. This yield decrease between the first and second harvest is a common occurrence, with lower N availability and shorter growth period. Yield differences were differed more between years than between treatments, suggesting that seasonal factors play a bigger role than these particular fertilizer treatments. For example, 2018 had a large yield dip, likely due to the massive drought that year. The random effects emphasized the importance of accounting for variability across years and blocks, which accounted for considerable yield variation. Furthermore, the residual variance reained high, suggesting that there are other factors influencing yield that were not included in this model analysis. Future studies should investigate whether fertilizer application rate, annual precipitation, or temperature variations significantly contribute to yield differences.

Based on this study, the choice between ADSH and USH may not significantly impact yield performance, and it is therefore no evidence to warrant a fertilizer choice based on yield performance alone.

4.2 NUE

The results did not support the hypothesis that ADSH would significantly improve NUE compared to USH. Both treatments exhibited similar NUE values, indicating that the presumed greater N availability in ADSH did not translate into increased uptake efficiency under these field conditions. Consistent with the yield analysis, NUE was significantly lower in the second cut compared to the first. This suggests a seasonal effect on N uptake efficiency, potentially due to lower N availability later in the growing season. I was unable to find studies who compared NUE between two cuts in a ley system, so this may be an important observation.

Another interesting observation was the negative correlation between NUE and clover percentage, suggesting that increased biological N fixation from clover may have reduced the relative efficiency of applied N fertilizers. This finding aligns with previous studies showing that biological N fixation can contribute to soil N pools but may not necessarily translate into increased crop NUE. A study by Elgersma and Hassink (1997) found that although legume plants can fix atmospheric N, the transfer to non-legume plants is low. In the calculation it can then seem like NUE is lowered, because the legume fixation is regarded as an input, but doesn't necessarily contribute to the grass N uptake.

The choice of NUE calculation method also might have played a role in interpretation. NUE1 provides a straightforward estimate of fertilizer-derived N use, whereas NUE2 accounts for biological N fixation, making it more applicable in systems with substantial legume contributions. However, in this study, the difference between NUE1 and NUE2 was minimal, suggesting that the method of calculation did not substantially alter conclusions.

4.3 N_2O-emissions

The analysis of N_2O emissions indicated that soil temperature and lagged precipitation (precipitation from the day before) were the primary drivers of N_2O fluxes with warmer and wetter conditions leading to increased emissions. This aligns with existing literature that shows temperature as a stimulating factor for microbial activity (Smith et al., 2018), and wet conditions as a driver of denitrification (Philippot et al., 2007).

Contrary to the hypothesis, the residual effect of long term application of ADSH did not significantly reduce N_2O emissions compared to USH and did not increase compared to the control. This suggests that in cold, wet climates such as Norway, environmental factors, specifically temperature and precipitation, may have a stronger influence on emissions than fertilizer type alone. This could differ in warmer climates where microbial activity may be constantly higher due to higher soil temperature. However, interestingly, the effect of temperature depended on fertilizer treatment. This could be because the control treatment had much more scarce foliage, meaning there was less insulation against incoming sun, leading to higher temperature fluctuations and thus higher emissions.

Overall, N_2O fluxes were primarily driven by soil temperature and lagged precipitation, with treatment effects emerging only in interaction with temperature. The absence of a direct treatment effect suggests that ADSH and USH did not independently alter N_2O fluxes but may influence emissions under specific temperature conditions. The significant random effects underscore the need to account for both temporal and spatial variability in emissions. Despite similar temperature measurements across treatments (Figure 11), the significant interaction ($p < 0.001$) indicates that the relationship between soil temperature and N_2O flux differs by treatment. This suggests that even subtle variations in the temperature–flux relationship can result in a statistically significant interaction if the regression slopes differ. Additionally, response variability and a large sample size may contribute to the detected significance, even if visual differences appear minimal. Further diagnostics, such as plotting regression lines with confidence intervals, could help clarify these treatment-specific effects.

The use of a one-day lag for precipitation was based on previous research showing that N_2O fluxes often peak shortly after rainfall due to increased soil moisture and microbial activity. However, it is possible that longer lag periods (e.g., two or more days) could also influence N_2O emissions. A sensitivity analysis testing different lag durations would help assess whether delayed precipitation effects play a larger role than currently estimated.

There was also still a large amount of residual variance in the model, suggesting that additional factors, beyond those considered in the analysis, may be influencing emissions. One major possible contributor to the residal variance could be the ploughing on the 30th of April and 6th of August. Perhaps including “days from last ploughing” in the model could eliminate some of the variance.

Potential other unaccounted factors include soil texture variations, differences in microbial community composition, and localized drainage patterns, all of which could influence N_2O emissions. An additional consideration is the potential impact of frame placement on N_2O flux measurements. Due to the presence of large stones, frames had to be repositioned multiple times before installation, leaving behind small soil disturbances. These disturbances may have influenced N_2O emissions by altering soil aeration and microbial activity in localized areas, potentially contributing to the observed residual variance in emissions.

4.4 Limitations of this study

The N_2O emissions were only measured during a single season (April-October 2024). Measuring over several years could potentially give a more reliable result and perhaps eliminate some residual variance. It may also be wise to stick with one measurement method for gas sampling, as two different methods were used in this study, which could impact the results of the gas fluxes. It would also be interesting to measure N_2O emissions in a year with fertilizer applied to determine whether treatment differences become more pronounced under active fertilization. Since previous literature highlights fertilizer application is a primary driver of N_2O emissions, the absence of fertilizer this year may have masked potential treatment effects even though there has been long-term previous application.

The relationship between NUE and N_2O emissions in organic farming systems is complex and influenced by multiple factors. In general, improving NUE by enhancing N uptake can reduce the amount of N left in the soil, which could in theory lower the potential for N_2O emissions. However, in this study, the role of environmental factors, such as soil temperature and moisture, appears to be more important in driving N_2O emissions. In line with studies by Smith et al. (2018), our results showed that soil temperature and precipitation were the primary drivers of N_2O emissions, with temperature acting as a stimulant for microbial activity and moisture promoting denitrification processes by increasing anaerobic volume in the soil. Therefore, even if NUE improves, N_2O emissions may still be driven by environmental factors, which warrants further research into how we can mitigate these emissions in organic farming.

4.5 Conclusion

This study assessed the impact of anaerobically digested slurry (ADSH) on yield, NUE and N_2O emissions, comparing it to untreated slurry (USH) and a control. Yield and NUE were measured over several years, while N_2O emissions were measured this year, focusing on residual effects as no fertilizer was applied this year.

Soil temperature and precipitation were the primary drivers of N_2O fluxes in agricultural systems, with warmer and wetter conditions leading to increased emissions. Both ADSH and USH significantly improved yield compared to the control, but their effects on N_2O emissions were indirect, influenced more by environmental conditions than the treatments themselves. Contrary to the hypothesis, no significant difference in NUE was found between ADSH and USH, suggesting that long-term application of these fertilizers results in similar N availability. While ADSH has been hypothesized to reduce N_2O emissions due to its altered chemical composition and microbial accessibility, these results do not support this. This raises the question of whether the expected mechanisms—such as differences in nitrification and denitrification rates—are as influential under the cool, humid conditions of this study. It is possible that other factors, such as soil organic matter dynamics or microbial adaptation, play a stronger role in regulating emissions. Further research is needed to clarify whether the hypothesized benefits of anaerobic digestion in reducing N_2O emissions hold under different environmental conditions or whether the treatments genuinely have no meaningful difference in this context.

These findings underline the importance of N management strategies tailored to Norway's climatic conditions, where temperature and precipitation fluctuations strongly influence N_2O emissions. Mitigation measures such as optimizing N application timing, improving drainage, adjusting soil pH, and adopting site-specific tillage practices could help reduce agricultural N_2O emissions while maintaining soil health. Since ADSH and USH performed similarly in terms of yield, NUE, and N_2O emissions, the choice between these fertilizers may depend on other factors, such as access to anaerobic digesters, the demand for biogas, and broader sustainability goals in agriculture.

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Popular science summary

Agriculture is a major source of greenhouse gas emissions, particularly nitrous oxide (N_2O), which has a much stronger warming effect than carbon dioxide. As the world looks for ways to reduce its environmental impact, finding more sustainable farming practices is crucial. Fertilizer management plays a key role in this, as inefficient use of fertilizers can both harm the environment and limit crop growth. In Norway, where organic farming is gaining popularity, understanding how different types of fertilizers affect both crop yield and emissions is essential for sustainable agricultural practices. This study examined the effects of two types of cattle slurry—anaerobically digested slurry (ADSH) and untreated slurry (USH)—on crop yield, nitrogen use efficiency (NUE), and N_2O emissions in organic dairy farming in Norway.

The results showed that both ADSH and USH improved crop yield compared to the control, with no significant difference between the two. N_2O emissions were primarily driven by soil temperature and rainfall, rather than fertilizer type. While the long-term application of ADSH did not reduce N_2O emissions as expected, the findings suggest that environmental factors like temperature and precipitation have a larger influence on emissions than the fertilizer type itself. Additionally, the presence of clover in the field reduced nitrogen efficiency, as clover fixes nitrogen naturally, affecting NUE.

These findings highlight the importance of considering environmental conditions in nitrogen management strategies. Both ADSH and USH can be effective fertilizers, with the choice between them depending on other factors such as access to biogas production or broader sustainability goals in agriculture.

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