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The role of red clover (*Trifolium pratense* L.) in mitigating greenhouse gas emissions: Insights from a temperate agricultural ecosystem

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ABSTRACT

Agriculture, forestry, and land use contribute approximately 22 % of global anthropogenic greenhouse gas emissions, with nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) playing pivotal roles in climate change. This underscores the urgency of adopting sustainable practices such as crop rotation and cover cropping. Red clover, a nitrogen-fixing legume, offers potential for mitigating greenhouse gas emissions by enhancing soil fertility, reducing reliance on synthetic fertilisers, and improving soil health. This study examines the seasonal and management related dynamics of greenhouse gas emissions in temperate agricultural systems and evaluates the short-term and long-term effects of red clover cultivation on soil N_2O, CO_2, and CH_4 fluxes. In addition to crop specific effects, the study identifies key environmental and agronomic factors influencing emission patterns, including soil type, tillage system, farming system, and seasonal temperature and moisture fluctuations. Notably, red clover cultivation initially increased N_2O (7.06 g ha^{-1} d^{-1}) and CO_2 (137.23 kg ha^{-1} d^{-1}) emissions due to biological nitrogen fixation and elevated microbial activity, followed by emission fluctuations linked to organic matter mineralisation. In contrast, CH_4 fluxes remained consistently negative, indicating a methane sink effect (-6.54 g CH_4 ha^{-1} d^{-1} in year three). These findings highlight the complex interplay between management practices and environmental variables in regulating soil greenhouse gas emissions and underscore the need for further research on biomass contributions to support climate resilient agriculture.

1. Introduction

Agriculture, forestry and other land uses are significant contributors to global greenhouse gas emissions, accounting for approximately 22 % of total anthropogenic emissions in 2019 (IPCC, 2022). The primary greenhouse gases emitted from agricultural activities include nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2). Each of these gases has distinct sources and impacts on the environment, making their management crucial for mitigating climate change.

Therefore, sustainable agricultural practices are essential for reducing greenhouse gas emissions from the agricultural sector while ensuring food security and conserving biodiversity. These practices include crop rotation, cover cropping, conservation tillage, and integrated pest management, all of which help to preserve soil fertility, enhance biodiversity, and reduce dependency on synthetic fertilisers and chemical inputs (Tilman et al., 2002). Red clover, a leguminous cover crop, enhances agro-ecological resilience and productivity by fixing nitrogen in the soil. Additionally, it offers various other benefits, including regulating soil temperature and moisture, reducing erosion, runoff, and nutrient leaching, suppressing weeds, and disrupting the cycles of pests and diseases (Wyngaarden et al., 2015).

Legume crops are essential for promoting sustainable agriculture due to their numerous environmental and socio-economic benefits. They are a key source of nutritious food and animal feed globally, while also contributing to lower greenhouse gas emissions compared to other crops. Additionally, legumes support soil carbon sequestration and reduce reliance on fossil energy by decreasing the need for nitrogen fertilisers (Reckling et al., 2014). They improve soil health through nitrogen fixation, increase organic matter, and enhance nutrient cycling and water retention (Stagnari et al., 2017). Due to their environmental

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and socio-economic benefits, legumes play a crucial role in crop rotations, enhancing crop diversity and supporting conservation agriculture, whether as living plants or crop residues. In grassland-based livestock production systems, the environmental and economic value of nitrogen-fixing legumes is increasingly recognised (Hakala et al., 2012).

Despite these advantages, the extent to which legumes reduce greenhouse gas emissions depends heavily on how agro-ecosystems are managed. For instance, Senbayram et al. (2015) found that seasonal and annual N_2O emissions were highest in monocultures of faba beans, but intercropping faba beans with wheat reduced cumulative N_2O emissions by 31 % compared to nitrogen-fertilised wheat. Similarly, Rigon et al. (2021) demonstrated that selecting suitable species for rotation with soybean under no-till practices enhances soil organic carbon and can help mitigate greenhouse gas emissions from tropical soils. However, Nadeem et al. (2012) findings suggest that while legume-grass as green manure has many soil health benefits, its use in heavy clay soils should be carefully managed to avoid increasing greenhouse gas emissions, particularly N_2O. These findings highlight the need for targeted agricultural management practices that consider soil type and crop-specific requirements to optimise both productivity and environmental sustainability.

This study aims to identify the primary drivers of greenhouse gas emissions and explore seasonal and management-related variations, providing insight into the dynamics of soil greenhouse gas fluxes within temperate agricultural ecosystems. Furthermore, the analysis evaluates the effects of red clover cultivation, including the impact one and two years post-cultivation, on soil greenhouse gas emissions.

2. Materials and methods

2.1. Measurement site

The experimental fields where greenhouse gas measurements were conducted are located within the trial fields under the supervision of the Institute of Agricultural Resources and Economics, Stende Research Centre, in Latvia (57.1894, 22.5616). The experimental plots were selected to represent diverse soil types, granulometric compositions, and fertility levels, ensuring the inclusion of conditions that influence greenhouse gas emissions. These fields have been managed under both conventional and organic cropping systems, providing a basis for assessing the impact of long-term management strategies on soil emissions.

The study site includes a variety of soil classifications, primarily *Planosols*, *Alisols*, and *Gleysols* (World Reference Base, WRB), with predominant textures of sandy loam and sandy silt loam (Table 1, Fig. 1). This variability enables a comprehensive assessment of how soil properties influence greenhouse gas fluxes.

2.2. Soil properties and field management

Table 2 presents key soil parameters for each field, including soil pH, soil classification, granulometric composition, phosphorus (P_2O_5, mg/kg), potassium (K_2O, mg/kg), and organic matter content. These factors were critical for field selection, as they regulate microbial activity, soil aeration, and nutrient cycling, all of which influence greenhouse gas emissions. Notably, organic matter content varied widely, reflecting differences in soil carbon storage potential across fields.

The fields were cultivated with various crops under different tillage and management systems over four growing seasons (2021–2024), as detailed in Table 3. Crop types included cereals (spring wheat, barley, winter wheat, rape, rye, buckwheat, and oats), legumes (red clover, field beans, peas), and root crops (potatoes), along with green manure crops. Two soil tillage practices were implemented: conventional tillage, characterised by mouldboard ploughing to a depth of 24 cm, and reduced tillage, which involved disc harrowing to a depth of 10 cm. Yields varied across the plots and years, reflecting differences in crop types, soil properties, and management practices. For greenhouse gas flux analysis, plots 5 and 11 were excluded due to the absence of red clover cultivation during the study period. However, these plots were retained as control sites for evaluating greenhouse gas fluxes in fields without red clover.

Table 1
Coordinates of the fields examined in the study.

No.	Latitude	Longitude	No.	Latitude	Longitude
1	57.2132	22.5506	8	57.2121	22.5549
2	57.2143	22.5522	9	57.1816	22.5399
3	57.2126	22.5520	10	57.1773	22.5438
4	57.2137	22.5535	11	57.1867	22.5653
5	57.1827	22.5403	12	57.2138	22.5492
6	57.2123	22.5539	13	57.2146	22.5506
7	57.1838	22.5411	14	57.1790	22.5478

2.3. Environmental data collection

Fig. 2 illustrates monthly precipitation, air temperature, soil temperature, and soil moisture recorded during the growing seasons from 2021 to 2024. Precipitation and air temperature data were obtained from the Latvian Environment, Geology and Meteorology Centre at the nearest weather station in Stende (57.183363, 22.550719) (LVĢMC, 2020). Soil moisture measurements were conducted using the Lutron PMS-714 Soil Moisture Meter, which measures soil moisture at a depth of 10 cm. Soil temperature was recorded using the Digital Temperature Meter Testo 922, which measures temperature in the upper soil layer. Precipitation levels, represented by bars, peak in July and August, indicating the period of highest rainfall. Soil temperature, shown by a dashed line, rises steadily from March, reaching a maximum in July before declining towards October. Soil moisture, represented by a dotted line, increasing in the early season and reaching its lowest values in July, then gradually increasing. Air temperature, depicted with a solid line, also shows a gradual increase from early spring, peaking around July, and subsequently decreasing in the autumn. These environmental parameters are essential for understanding seasonal variations and their potential effects on greenhouse gas fluxes from agricultural soils.

2.4. Greenhouse gas emission measurements and data analysis

Measurements of soil fluxes for N_2O, CO_2, and CH_4 were conducted approximately every two weeks between 10:00 AM and 4:00 PM, covering the period from March to October during the years 2021–2024, when the soil and air temperature had reached + 10 °C degrees and no significant increase in precipitation. Gas emissions were measured using the Picarro G2508, a high-precision analyser based on Cavity Ring-Down Spectroscopy technology. This instrument enables simultaneous, real-time detection of CH_4, CO_2, and N_2O with exceptional sensitivity, stability, and accuracy, providing measurements at one-second intervals (Brown et al., 2023).

For each experimental plot, three separate measurements were taken at different locations, with each session lasting 240 seconds. The measurement time of 240 sec was selected based on previous studies that evaluated optimal durations for gas concentration stabilisation and accurate flux determination (Valujeva et. al., 2017; Grinfelde et al., 2017). Non-transparent chambers with a diameter of 23 cm and a volume of 3 L were used for these measurements. The chamber system consisted of a metal base featuring a sharpened edge, a dome that blocked light, and a rubber seal positioned between the base and the dome to ensure an airtight fit. Connection to the Picarro G2508 was established through a stainless-steel adapter, a Teflon tube (9 m long, 3.175 mm outer diameter, and 1.587 mm inner diameter), and a quick connector with a rubber-insulated seal (Valujeva et al., 2017, 2022, 2023). The metal base was positioned 30 minutes prior to the measurement process, while the dome was attached and connected to the analyser immediately

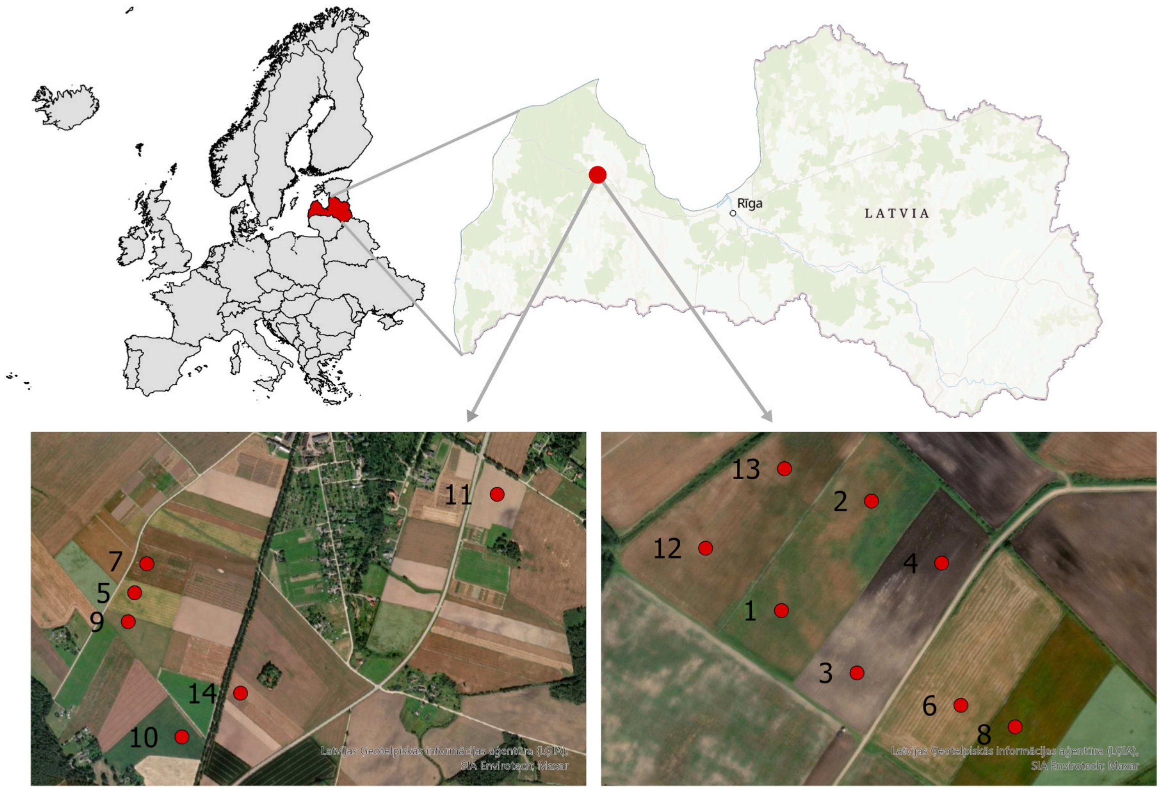

Fig. 1. Location of the Stende Research Centre. Red dots and numbers indicate the locations of the experimental fields.

Table 2
Soil pH, classification (WRB), granulometric composition, and nutrient content for each field.

No.	pH	Soil classification (WRB)	Granulometric composition	P_2O_5 (mg/kg)	K_2O (mg/kg)	Organic content (%)
1	6.8	*Planosols*	Sandy loam	23	66	4.2
2	6.8	*Planosols*	Sandy loam	23	66	4.2
3	6.7	*Planosols*	Sandy loam	39	66	4.5
4	6.9	*Gleysols*	Sandy loam	14	95	34.8
5	5.7	*Alisols*	Sandy loam	133	119	1.6
6	6.7	*Planosols*	Sandy loam	39	66	4.5
7	6.2	*Alisols*	Sandy silt loam	135	133	2.1
8	7	*Planosols*	Sandy loam	14	49	4.6
9	5.8	*Alisols*	Sandy loam	137	98	2
10	6.0	*Alisols*	Sandy loam	140	98	2
11	6.4	*Alisols*	Sandy loam	175	165	1.9
12	6.8	*Planosols*	Sandy loam	21	61	6.8
13	6.8	*Planosols*	Sandy loam	21	61	6.8
14	5.2	*Alisols*	Sandy loam	143	109	1

before data collection began. The chamber base was placed directly onto bare soil whenever possible, except in early spring when crops were still small and could not be entirely avoided. A Diver DI 500 data logger (Eijkelkamp) was also placed inside the chamber to monitor air temperature and pressure during measurements.

The Soil Flux Processor 1.2.05, an analytical software developed by Picarro Inc., was used to process and calculate gas flux measurements, applying a linear model for data analysis (Wagner et al., 1997). The raw data from the Picarro G2508 analyser were integrated into Soil Flux Processor, where fluxes were determined based on the rate of change in gas concentrations within the chamber over the measurement period. Additionally, chamber temperature, soil temperature, and chamber pressure data were manually entered for each measurement according to field observations, ensuring the inclusion of site-specific environmental conditions in the analysis. The calculated emissions were then converted into grams or kilograms per hectare per day, enabling direct comparisons of emissions across different plots and time periods.

Following data processing, the compiled dataset was integrated with weather parameters such as precipitation and air temperature, along with soil moisture levels, soil temperature, and soil management information for further statistical analysis. To determine the distribution of soil greenhouse gas emissions, the Shapiro–Wilk test was conducted. Since the data did not follow a normal distribution, the non-parametric Kruskal–Wallis test was applied to assess variations in emissions across different months and years following red clover cultivation, with Dunn's post hoc test used for pairwise comparisons ($p < 0.05$). Furthermore, the General Linear Model analysis was utilised to identify key factors influencing greenhouse gas emissions from soil. Additionally, a General Linear Model was applied to evaluate the influence of environmental and management-related factors on N_2O, CO_2, and CH_4 emissions. The dependent variables were individual greenhouse gas fluxes (analysed separately for each gas), while independent variables included soil type (mineral, organic), tillage system (conventional, reduced), farming system (conventional, organic), month, year, and treatment group (red clover, control). Soil temperature and soil moisture were included as covariates to assess the effect of short-term environmental conditions. The General Linear Model approach was selected due to its flexibility in handling both categorical and continuous predictors and its ability to quantify the relative contribution of each factor to overall variance. Statistical significance was determined at the $p < 0.05$ level. Effect sizes were reported using Partial Eta Squared to assess the strength of associations between predictors and greenhouse gas emissions. All statistical analyses were performed in IBM SPSS Statistics Version 22.

3. Results

3.1. Temporal variations in greenhouse gas emissions

N_2O emissions from soil vary significantly across the years (Table 4).

Table 3
Cultivated crops and yields for each plot. Abbreviations: SW, spring wheat; SB, spring barley; WW, winter wheat; RC, red clover; field beans, FB; R, rye; W*R*, winter rape; P, peas; B, buckwheat; O, oats; PO, potatoes, GM, green manure; TF, varietal trial field; Till, tillage system; CT, conventional tillage; RT, reduced tillage.

No.	2021			2022			2023			2024		
	Crop	Yield ($t\ ha^{-1}$)	Till	Crop	Yield ($t\ ha^{-1}$)	Till	Crop	Yield ($t\ ha^{-1}$)	Till	Crop	Yield ($t\ ha^{-1}$)	Till
1*	SW	2.1	CT	SB + RC	2.1	RT	RC	GM	RT	WW	3.8	CT
2*	SW	2.1	CT	SB + RC	2.1	RT	RC	GM	RT	WW	3.8	CT
3*	SB + RC	1.8	CT	RC	GM	RT	WW	3.4	CT	O	2	CT
4*	SB + RC	1.8	CT	RC	GM	RT	WW	3.4	CT	O	2	CT
5	SB	4	CT	P	4	RT	SW	TF	CT	PO	25	CT
6*	RC	GM	CT	WW	2.8	CT	O	1.22	RT	B	2	CT
7	RC	0.4	CT	SW	4	RT	PO	26.2	CT	SB	TF	CT
8*	WW	3	CT	O	2	RT	B	2	RT	SW	2	CT
9	WW	6.8	CT	SB + RC	3.7	RT	RC	0.24	RT	O	TF	CT
10*	SW + RC	NA	CT	FB	NA	CT	P	NA	RT	R + RC	NA	CT
11	FB	4	CT	B	3.5	CT	W*R*	2.5	CT	WW	6.71	CT
12*	B	1.5	NA	SW	2.2	NA	SB + RC	1.5	RT	RC	GM	CT
13	B	1.5	NA	SW	2.2	NA	SB + RC	1.5	RT	RC	GM	CT
14	B	GM	NA	WW	5.4	NA	SB + RC	2.3	CT	RC	0.5	CT

* Field managed organically

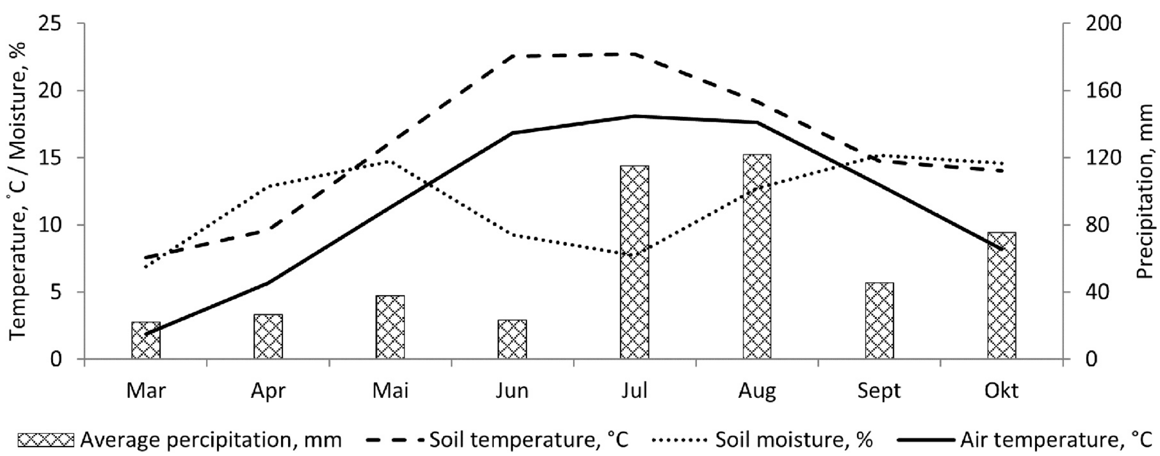

Fig. 2. The sum of monthly precipitation and average monthly air temperature, soil temperature, and soil moisture during the growing seasons from 2021 to 2024.

Table 4
Mean, median, and standard error of the mean (SE) for greenhouse gas emissions during years when red clover is grown in the study period and for control plots.

Year	N_2O, $g\ ha^{-1}\ d^{-1}$			CO_2, $kg\ ha^{-1}\ d^{-1}$			CH_4, $g\ ha^{-1}\ d^{-1}$		
	Mean	Median	SE	Mean	Median	SE	Mean	Median	SE
1	7.06	2.28	0.89	137.23	127.39	2.46	−5.96	−5.06	0.19
2	2.85	1.29	0.27	134.24	118.64	2.90	−5.63	−4.98	0.17
3	4.07	2.28	0.32	144.50	109.52	5.78	−6.54	−6.16	0.20
Control	1.60	1.14	0.25	100.17	93.24	2.90	−3.85	−3.31	0.16

In year 1, when red clover is grown, the mean N_2O emission is significantly higher than in subsequent years (mean = 7.06 g N_2O ha^{-1} day^{-1} in year 1 vs. 2.85 g N_2O ha^{-1} day^{-1} in year 2 and 4.07 g N_2O ha^{-1} day^{-1} in year 3, with $p < 0.01$), indicating notable changes over time. The skewed distribution in year 1 suggests some high values influenced the average, while year 2 shows a decrease in both mean and median emissions, pointing to fewer extreme values. By year 3, the mean and median emissions rise again, indicating an overall increase, but with less skewness than in year 1. Additionally, N_2O emissions in all years are significantly higher than those observed in the control plots ($p < 0.01$), highlighting the impact of red clover cultivation on soil emissions.

Similarly, CO_2 emissions show significant differences between year 1 and year 3, with year 3 (mean = 144.50 kg CO_2 ha^{-1} day^{-1}) being higher than year 1 (mean = 137.23 kg CO_2 ha^{-1} day^{-1}) ($p < 0.01$). In contrast, year 2 (mean = 134.24 kg CO_2 ha^{-1} day^{-1}) does not differ significantly from either year 1 or year 3, suggesting an overall fluctuation in emissions across years. Additionally, CO_2 emissions in all years are significantly higher than those in the control plots ($p < 0.01$), emphasising the influence of cropping systems on soil respiration. CH_4 emissions also show significant differences across the years, with year 3 having a higher CH_4 assimilation rate compared to the other years ($p < 0.01$). Additionally, CH_4 emissions in all years differ significantly from those in the control plots ($p < 0.01$), further emphasising the impact of cropping systems on methane fluxes. These results highlight significant temporal variations in N_2O, CO_2, and CH_4 emissions, suggesting potential changes in the factors influencing these emissions over time.

From March to October, N_2O emissions ranged from −0.79–13.64 g N_2O ha^{-1} day^{-1} across the red clover treatment, with negative emissions recorded in April and July, and the lowest positive emissions observed in

June (Fig. 3). The Kruskal-Wallis test indicated statistically significant differences in N_2O emissions across months ($p < 0.01$), with June, July, and September showing the most distinct values compared to other months. Notably, emissions in September (13.64 g N_2O ha^{-1} day^{-1}) were significantly higher than in all other months, while June and July showed significantly lower values.

When compared to the control plots, N_2O emissions under red clover were consistently higher in most months, except April, May, and June, when control plots showed slightly higher values (1.94, 3.62, and 0.96 g N_2O ha^{-1} day^{-1}, respectively). In particular, year 1, corresponding to the period of active red clover cultivation, exhibited the highest peak emissions, with values reaching 13.64 g N_2O ha^{-1} day^{-1} in September and 7.65 g N_2O ha^{-1} day^{-1} in August. Although emissions declined in years 2 and 3 (post-clover cultivation), they remained elevated compared to the control, suggesting residual effects of red clover on soil nitrogen dynamics, likely through increased nitrogen availability from biological fixation and decomposition of organic matter.

Negative emissions were observed in the control plots during March and July (-1.23 and −0.92 g N_2O ha^{-1} day^{-1}, respectively), possibly indicating net N_2O uptake or complete denitrification under low temperatures or elevated soil moisture conditions. In contrast, the red clover plots showed either positive or less negative emissions in these same months, suggesting altered microbial processes due to the influence of red clover on nitrogen cycling.

Environmental conditions may further explain these temporal variations. In April, with a total precipitation of approximately 26.4 mm and an average air temperature of 5.4 °C, negative N_2O emissions suggest conditions favouring N_2O reduction or consumption via denitrification. Similarly, in June, emissions remained low despite higher temperatures (16.8 °C), likely due to limited precipitation (23.3 mm), which may have constrained microbial activity and substrate availability for nitrification and denitrification processes.

CO_2 emissions from soil varied significantly across months and years, ranging from 8.94 to 233.01 kg CO_2 ha^{-1} day^{-1} between March and October (Fig. 4). The Kruskal–Wallis test confirmed significant monthly differences ($p < 0.01$), with post hoc comparisons indicating that emissions in June, July, and August were significantly higher than in cooler months. The peak emission occurred in June of year 3 (233.01 kg CO_2 ha^{-1} day^{-1}), followed by July of year 1 (168.21 kg CO_2 ha^{-1} day^{-1}) and August of year 1 (160.57 kg CO_2 ha^{-1} day^{-1}).

When compared to the control plots, CO_2 emissions were consistently and significantly higher in all three years across most months ($p < 0.01$), particularly during summer. For instance, in July, year 1 emissions (168.21 kg CO_2 ha^{-1} day^{-1}) exceeded the control (88.76 kg CO_2 ha^{-1} day^{-1}) by nearly twofold. In spring (March–April) and autumn (October), emissions were lower across all treatments, reflecting the limiting effects of low temperatures (1.9–8.2°C) on microbial activity.

The seasonal pattern of CO_2 fluxes closely followed air temperature trends, with the highest emissions observed during June to August, when average temperatures ranged from 16.8°C to 18.1°C. Notably, June had relatively low precipitation (23.3 mm), yet exhibited the highest CO_2 fluxes, suggesting that warm temperatures and adequate soil aeration may have enhanced microbial respiration and organic matter decomposition. In contrast, despite high rainfall in July and August (115.3 mm and 121.7 mm), emissions remained high, indicating sufficient oxygen availability or partial drying between rainfall events. These results suggest that red clover cultivation and its residual organic inputs, combined with favourable climatic conditions, promoted elevated CO_2 emissions through enhanced soil respiration.

CH_4 fluxes were consistently negative across all months and treatments, indicating net methane uptake by the soil (Fig. 5). Emissions ranged from −9.01 to −1.80 g CH_4 ha^{-1} day^{-1} , with the strongest uptake observed in April of year 3, and the weakest in October of year 1. The Kruskal–Wallis test revealed statistically significant differences among months and treatments ($p < 0.01$). Post hoc pairwise comparisons showed that CH_4 uptake in April of year 3 was significantly greater than in most other combinations, including the control (adjusted $p < 0.05$). Significant differences were also observed in several other months between red clover treatments and the control, particularly in March, April, September, and October, where red clover plots consistently showed greater CH_4 uptake (more negative values) than the control (e.g., −6.95 in March of year 1 vs. −1.16 in the control, $p < 0.01$).

These findings suggest that red clover cultivation enhances CH_4 uptake, possibly due to increased microbial activity and altered oxygen dynamics in the soil following legume growth. Additionally, seasonal variation was evident, with stronger CH_4 uptake occurring during the spring and early summer, particularly under moderate temperatures (5.6–16.8 °C) and low precipitation (23–37 mm), which are favourable for methanotrophic activity. By contrast, methane uptake declined in October, aligning with cooler temperatures and higher soil moisture (75.3 mm), conditions that may reduce diffusion and methanotroph efficiency.

3.2. Environmental parameter effects

A General Linear Model was applied to identify the factors influencing N_2O emissions across both red clover and control treatments. The model included soil type, tillage, cropping system, month, year, treatment group, and environmental covariates for soil temperature and moisture. The overall model was statistically significant ($F(15, 3381) = 7.911$, $p < 0.001$), with an adjusted R^2 of 0.03, indicating that approximately 3 % of the variance in N_2O emissions could be explained by the included factors.

Among the fixed factors, soil type (F=13.869, $p < 0.001$), month (F

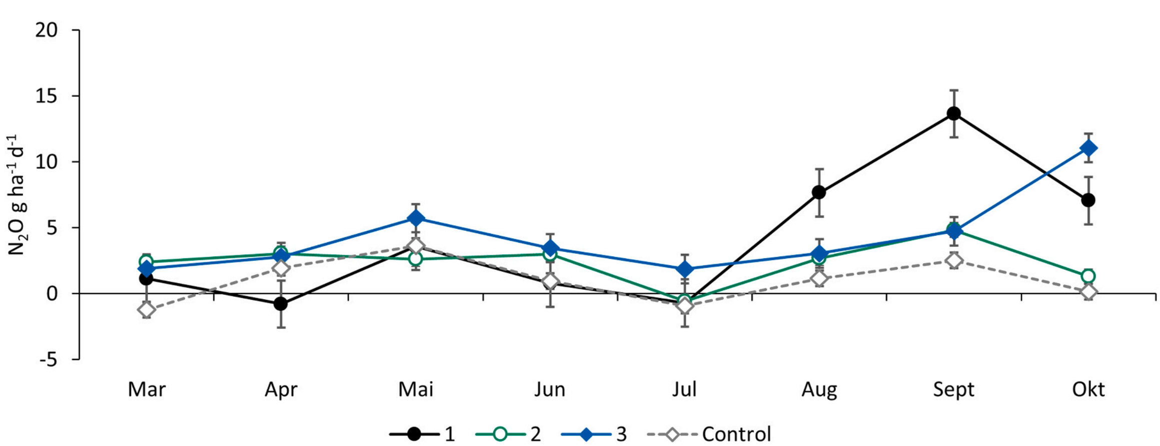

Fig. 3. N_2O emissions during the growing seasons across different stages on red clover cultivation. Bars represent standard error.

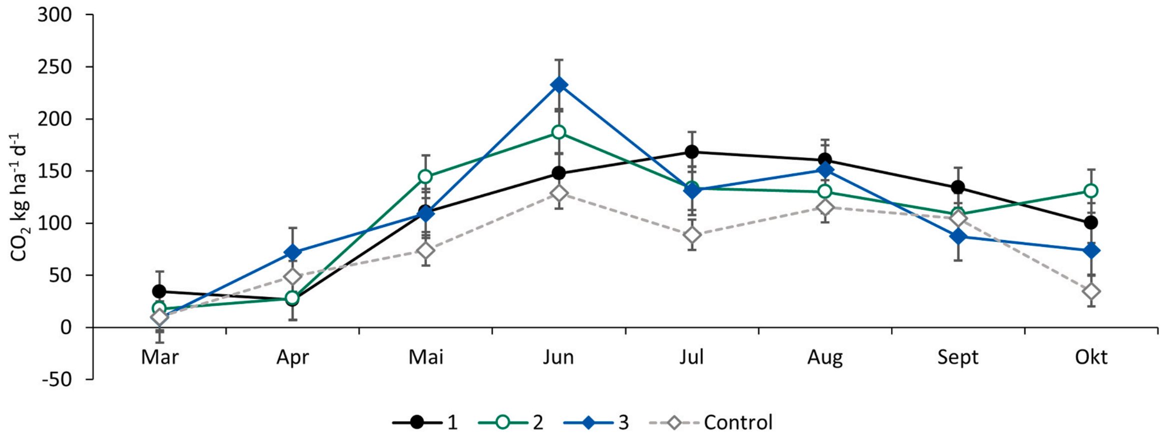

Fig. 4. CO_2 emissions during the growing seasons across different stages on red clover cultivation. Bars represent standard error.

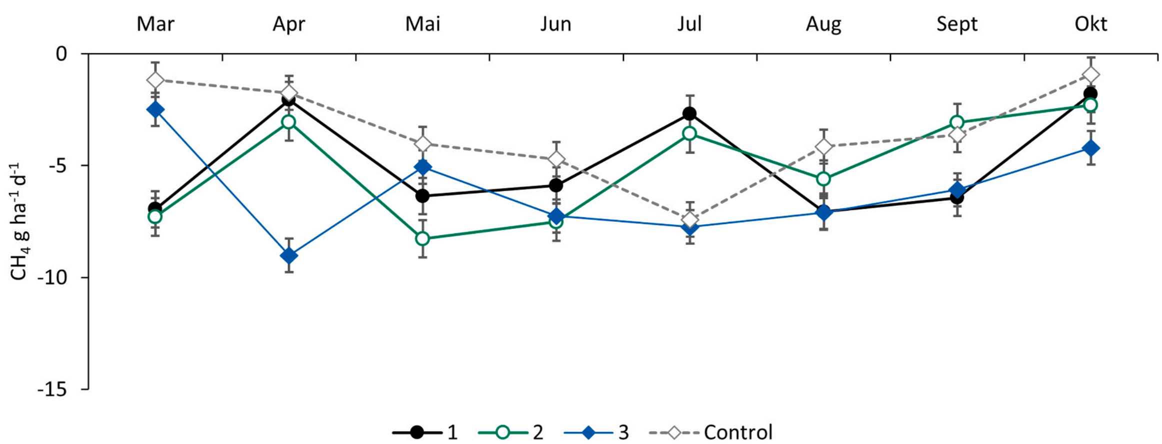

Fig. 5. CH_4 emissions during the growing seasons across different stages on red clover cultivation. Bars represent standard error.

= 7.319, $p < 0.001$), year (F=5.226, p = 0.001), and treatment group (F=6.532, p = 0.011) were statistically significant predictors of N_2O fluxes. Specifically, mineral soils showed significantly lower emissions than organic soils (B=-3.79, $p < 0.001$), and the lowest emissions were recorded in June and July, reflecting seasonal dynamics. The year 2023 showed a significant increase in emissions compared to 2024 (B=4.107, $p < 0.001$).

The treatment group (Redclover vs. Control) showed a statistically significant main effect on N_2O emissions (F = 6.532, p = 0.011), indicating that management practices involving red clover influenced emission levels compared to control plots. The inclusion of both treatment types enabled a meaningful comparison across management systems, revealing that in addition to temporal variation and soil type, the treatment itself contributed to the overall variation in N_2O emissions. Soil temperature and moisture covariates were not significant ($p > 0.2$), suggesting that short-term fluctuations in these physical parameters had limited influence on N_2O fluxes under field conditions. The best-fit model of the General Linear Model analysis for N_2O emissions from soil could be expressed as follows:

$$N_2O = 6.730 - 3.790 \times Soil_{Min} - 3.047 \times Treatment_{Control} + 4.107 \times Year_{2023} - 5.186 \times Month_{May} - 7.093 \times Month_{Jun} - 9.116 \times Month_{Jul} - 4.192 \times Month_{Aug}$$

The variation in CO_2 emissions was significantly influenced by soil type, month, and year, with environmental covariates such as soil temperature and moisture also contributing to the observed patterns. Data from both red clover and control plots were included to enable direct comparison across contrasting management conditions. The overall model was statistically significant (F(15, 3381) = 44.083, $p < 0.001$), with an adjusted R^2 of 0.16, indicating that approximately 16 % of the variance in CO_2 emissions could be explained by the included factors. Among the fixed factors, soil type (F=105.63, $p < 0.001$), month (F=32.037, $p < 0.001$), and year (F=17.708, $p < 0.001$) were statistically significant predictors of CO_2 fluxes. Mineral soils showed significantly lower emissions compared to organic soils (B=-51.725, $p < 0.001$). The highest emissions occurred in June and July, while emissions were significantly lower in April (B=-78.357, $p < 0.001$) and October (B =-30.159, p = 0.001). Compared to 2024, emissions were significantly lower in 2021 (B =-30.137, $p < 0.001$), 2022 (B =-42.065, $p < 0.001$), and 2023 (B =-34.268, $p < 0.001$).

The treatment group (Redclover vs. Control) did not show a statistically significant main effect (F=-7.687, p = 0.192). However, its inclusion in the model allowed for a comparative baseline, emphasising the stronger influence of temporal and soil-related factors. Both environmental covariates were statistically significant: soil temperature had a modest negative effect on CO_2 emissions (B=-2.021, p = 0.003), while soil moisture showed a weak but significant positive effect (B=0.706, p = 0.049). These results indicate that even short-term fluctuations in physical soil conditions may influence CO_2 fluxes under field conditions. The best-fit model of the General Linear Model analysis for CO_2 emissions from soil can be expressed as follows:

$$CO_2 = 220.521 - 51.725 \times Soil_{Min} - 2.021 \times Soil_T + 0.706 \times Soil_M - 30.137 \times Year_{2021} - 42.065 \times Year_{2022} - 34.268 \times Year_{2023} - 78.357 \times Month_{Apr} + 66.771 \times Month_{Jun} + 20.736 \times Month_{Jul} + 18.336 \times Month_{Aug} - 30.159 \times Month_{Okt}$$

Variation in CH_4 fluxes was significantly explained by multiple environmental and management factors. The model explained 12.2 % of the variance in CH_4 emissions, and was statistically significant overall ($F(15, 3381) = 32.400$, $p < 0.001$), with an adjusted R^2 of 0.122. Among the fixed factors, soil type ($F=48.360$, $p < 0.001$), tillage system ($F=65.454$, $p < 0.001$), farming system ($F=17.240$, $p < 0.001$), month ($F=23.440$, $p < 0.001$), and year ($F=7.971$, $p < 0.001$) had significant effects on CH_4 emissions. Specifically, mineral soils were associated with higher CH_4 uptake ($B=2.014$, $p < 0.001$), while organic farming resulted in significantly more negative CH_4 fluxes ($B = -2.002$, $p < 0.001$). Conventional tillage showed increased CH_4 uptake compared to reduced tillage ($B=1.049$, $p < 0.001$). Temporal variation was also substantial: CH_4 uptake was highest in May, June, and August, while positive fluxes were observed in April ($B=3.931$, $p < 0.001$) and October ($B=3.035$, $p < 0.001$), indicating seasonal shifts in emission patterns. Year 2021 also showed significantly greater CH_4 uptake compared to the reference year 2024 ($B=1.645$, $p < 0.001$), while 2022 and 2023 did not differ significantly. Both environmental covariates were statistically significant: soil temperature had a positive effect on CH_4 emissions ($B=0.213$, $p < 0.001$), as did soil moisture ($B=0.171$, $p < 0.001$), suggesting strong microbial sensitivity to changing physical soil conditions. In contrast, the treatment group (Redclover vs. Control) had no statistically significant effect on CH_4 emissions ($p = 0.405$), indicating that management system alone did not account for differences in methane fluxes.

$$CH_4 = -12.468 + 2.014 \times Soil_{Min} + 1.049 \times Till_{Conv} - 2.002 \times Farm_{Org} + 0.213 \times Soil_T + 0.171 \times Soil_M + 1.645 \times Year_{2021} + 3.931 \times Month_{Apr} - 1.468 \times Month_{Mai} - 1.822 \times Month_{Jun} - 1.871 \times Month_{Aug} + 3.035 \times Month_{Okt}$$

Overall, the explanatory strength of the model varied across gases. The proportion of variance explained was highest for CO_2 ($R^2 = 0.160$) and CH_4 ($R^2 = 0.122$), indicating a moderate fit between predictors and observed fluxes. In contrast, the model accounted for only 3 % of the variance in N_2O emissions ($R^2 = 0.030$), suggesting that key drivers of N_2O variability may lie outside the scope of the included factors. For CO_2, soil type and month were among the strongest predictors, explaining 3.0 % and 5.4 % of the variance, respectively. CH_4 emissions were most strongly influenced by soil moisture (2.0 %), soil type (1.4 %), and tillage system (1.9 %), while the treatment group had negligible influence across all gases. This suggests that soil physical conditions and seasonal patterns play a more consistent role in CO_2 and CH_4 variability, whereas N_2O emissions may be driven by more complex, event-based biological processes not fully captured in the model.

4. Discussion

The cultivation of red clover (*Trifolium pratense* L.) in temperate agroecosystems has a strong effect on soil greenhouse gas (GHG) emissions due to complex interactions among plant derived inputs, microbial processes, and environmental conditions. In the first year of red clover cultivation, higher greenhouse gas emissions are typically observed, primarily due to biological nitrogen fixation by *Rhizobium* bacteria in the root nodules. This process enriches the soil with nitrogen and creates favourable conditions for microbial processes such as nitrification and denitrification (Schipanski and Drinkwater, 2011). Red clover can significantly change the composition and activity of soil microbial communities through several mechanisms. Its symbiotic relationship with *Rhizobium* enhances biological nitrogen fixation, leading to increased availability of reactive nitrogen compounds such as ammonium and nitrate in the soil (Schipanski and Drinkwater, 2011; Bleken et al., 2022). These changes contribute to increased N_2O emissions, mainly as a result of the decomposition of nitrogen rich plant residues and increased microbial activity. This is consistent with findings by Byers et al. (2021), who reported that red clover monocultures lead to greater N_2O build-up and emissions compared to grass-dominated systems. Emissions tend to decrease substantially in the following year but increase again in the second year after cultivation, likely due to the delayed mineralisation of organic matter. Similarly, CO_2 emissions decline after the first year of cultivation but increase again in the third year, reflecting ongoing microbial respiration and soil carbon turnover.

Red clover influences soil microbial communities not only by enhancing nitrogen availability through symbiotic nitrogen fixation but also by altering carbon inputs via root exudates and residue decomposition. Through its symbiotic association with *Rhizobium* bacteria, red clover increases the availability of reactive nitrogen forms such as ammonium and nitrate, which are key substrates for microbial nitrification and denitrification (Schipanski and Drinkwater, 2011; Bleken et al., 2022). These processes are further enhanced by the decomposition of nitrogen rich root and shoot residues and the release of labile carbon through root exudates, which collectively stimulate heterotrophic respiration and contribute to elevated CO_2 emissions (McKenna et al., 2018; Taveira et al., 2020). Although microbial communities were not directly measured in this study, the observed emission patterns align with established evidence that legume cultivation fosters the activity of nitrifiers, denitrifiers, and methanotrophs, which are microbial groups responsible for N_2O production and CH_4 oxidation (Bleken et al., 2022). In particular, the consistent CH_4 uptake observed across all years suggests favourable conditions for methanotrophy, even under increased nitrogen inputs.

Winter conditions introduce additional complexity to microbial greenhouse gas dynamics in red clover systems, primarily through the effects of freeze–thaw cycles and snow cover. These environmental factors create conditions that enhance microbial denitrification, particularly under anaerobic microsites formed by insulated snow layers, leading to substantial N_2O emissions from the decomposition of frost-damaged plant residues (Byers et al., 2021; Badewa et al., 2022). In addition to these natural drivers, management-related factors such as soil compaction and aboveground biomass removal during the vegetation season may further intensify off-season N_2O release (Sturite et al., 2021). It has been estimated that up to 65 % of total annual N_2O emissions can occur during winter and early spring, particularly when snow cover insulates the soil and maintains microbial activity. Moreover, studies have shown that reduced snow cover may lead to even higher N_2O emissions due to increased soil freezing and disruption of nitrogen cycling, resulting in the release of inorganic nitrogen (Groffman et al., 2006; Sanders-DeMott et al., 2018). Manipulated snow cover experiments have demonstrated that winter N_2O emissions alone can account for up to 41 % of total annual emissions, highlighting the critical role of snow depth in regulating nitrogen gas losses (Groffman et al., 2006). These processes are further amplified in soils with high organic matter and moisture content, which favour denitrification during thawing periods (Badewa et al., 2022).

While nitrogen transformations are particularly sensitive to winter soil conditions, carbon dynamics are also affected. Soil CO_2 efflux during winter has been estimated to contribute 11–25 % of the flux observed during the growing season (Shi et al., 2014), with temperature identified as the primary controlling factor (Groffman et al., 2006). As soil temperatures rise during thaw events, microbial processes become more active, resulting in a marked increase in both CO_2 and N_2O emissions (Badewa et al., 2022). However, in contrast to N_2O, CO_2 emissions appear to be less influenced by snow removal, suggesting that microbial respiration may remain relatively stable despite fluctuating freeze–thaw cycles (Groffman et al., 2006). Based on these assumptions, annual CO_2 emissions were estimated by applying a seasonal scaling approach, which accounts for approximately 210 days (seven months) of active vegetation and 120 days (four months) of winter conditions. Winter CO_2 emissions were assumed to represent 18 % of those recorded during the growing season. Using this method, the total annual CO_2 emissions per hectare were calculated for different stages of red clover cultivation: 34.4 t CO_2 ha^{-1} yr^{-1} in the first year of red clover growth, 33.7 t CO_2 ha^{-1} yr^{-1} in the year following red clover cultivation, and 36.2 t CO_2 ha^{-1} yr^{-1} two years after red clover was grown. These results highlight the importance of including winter fluxes in annual greenhouse gas budgets and further underscore the sensitivity of microbial GHG processes to seasonal and management-related factors in legume-based systems. Methane dynamics also exhibit seasonal variation, with winter CH_4 uptake contributing to 13–18 % of the total annual fluxes, indicating that methane oxidation persists even under cold conditions, albeit at a reduced rate (Groffman et al., 2006).

Despite these insights, the current study also has several limitations that must be considered when interpreting the results. Greenhouse gas measurements were restricted to the growing season, with winter fluxes estimated from literature, introducing uncertainty in total emission budgets. While our findings suggest microbial pathways are central to observed greenhouse gas patterns, the absence of direct measurements of microbial community composition or activity constrains the interpretation of underlying mechanisms. Additionally, the use of the Picarro G2508 analyser, while effective in detecting gas fluxes, does not differentiate between emissions derived from aboveground and belowground biomass, and the study lacked quantitative biomass data. This is a critical gap, given that up to 83 % of nitrogen in legume systems can reside in belowground residues (Bleken et al., 2022), while aboveground residues, especially when coupled with mineral nitrogen inputs, are known to increase N_2O emissions (Bleken et al., 2025).

The impact of red clover impact on microbial processes is further complicated by climatic variability; for example, snow depth and soil freezing can significantly alter nitrogen cycling. In manipulated snow cover experiments, N_2O emissions accounted for up to 41 % of annual totals, underscoring the role of snow in moderating microbial activity and nitrogen gas release (Groffman et al., 2006). Freeze–thaw cycles may also inhibit Rhizobium activity, reducing nitrogen fixation and altering nitrogen availability in the soil (Sanders-DeMott et al., 2018). While CO_2 emissions during winter are somewhat stable despite snow removal, microbial activity intensifies upon soil thawing, leading to abrupt increases in both N_2O and CO_2 emissions (Groffman et al., 2006; Badewa et al., 2022). Methane dynamics also exhibit seasonal variation, with winter CH_4 uptake contributing to 13–18 % of total annual fluxes, indicating that methane oxidation persists even under low temperatures.

Finally, the site-specific nature of the study, conducted solely in Latvia, may limit the applicability of the results to other agroecological contexts. Red clover is widely cultivated under diverse conditions, including organic, conventional, reduced-tillage, no-till, mixed cropping, and forage systems, and its ecological effects can vary across soil types, climates, and management practices. This variability, combined with the methodological constraints outlined above, highlights the complexity of accurately quantifying the role of red clover in greenhouse gas emissions across different agroecological contexts.

Therefore, future research should focus on improving the understanding of how both aboveground and belowground biomass influence greenhouse gas emissions in farming systems that include red clover. The current study, using the Picarro G2508 analyser, could not distinguish between these biomass sources, and quantitative data on aboveground biomass were lacking. This represents a critical knowledge gap, as red clover residues, particularly those belowground, play a major role in nitrogen cycling and related emissions. Previous studies have shown that belowground residues can account for 70–83 % of total residual nitrogen in legume systems (Bleken et al., 2022), while aboveground residues may significantly increase N_2O emissions when combined with mineral nitrogen (Bleken et al., 2025). In addition, the interaction between red clover roots, mycorrhizal fungi, and nutrient distribution, such as phosphorus, also needs further investigation (Zhang and Xiao, 2024). Annual, continuous measurement of greenhouse gas fluxes across all seasons, especially during winter periods influenced by freeze–thaw cycles, along with direct microbial analysis, is necessary to more accurately capture seasonal variation and underlying microbial processes. Future studies should also include measurements across different climatic zones, soil types, and management systems, for instance, no-till practices, to better reflect the diversity of conditions under which red clover is cultivated. A deeper understanding of how different plant residues and microbial activity contribute to greenhouse gas emissions will support better residue management and help design sustainable farming systems that make use of the benefits of red clover while reducing environmental impacts.

5. Conclusions

This study provides strong evidence that red clover (*Trifolium pratense* L.) cultivation in temperate agroecosystems affects greenhouse gas emissions, with impacts lasting beyond the active growth period, including significant contributions from winter emissions under freeze–thaw conditions. While red clover improves soil fertility through biological nitrogen fixation and reduces the need for synthetic fertilisers, it can also lead to short-term increases in N_2O and CO_2 emissions due to increased microbial activity and the decomposition of plant residues, particularly through enhanced nitrification, denitrification, and respiration. These patterns are further influenced by environmental factors, soil characteristics, and management practices, including the tillage system, farming system (organic or conventional), and crop rotation.

The results suggest that although legume cover crops benefit long-term soil health, they should be managed carefully to avoid short-term rises in greenhouse gas emissions. This includes proper timing of residue incorporation, matching practices to soil characteristics, and considering methods like reduced tillage or the use of nitrification inhibitors. The consistent methane uptake observed also shows that red clover fields can act as methane sinks under the right conditions.

Applying these findings to greenhouse gas assessment methods and sustainable farming strategies can help improve environmental outcomes in agriculture. Further research should focus on the role of plant biomass and winter-season emissions in shaping greenhouse gas patterns, to enhance prediction models and support more effective land management decisions.

CRediT authorship contribution statement

Kristine Valujeva: Writing – original draft, Visualization, Supervision, Software, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Jovita Pilecka-Ulcugaceva:** Writing – review & editing, Writing – original draft, Validation, Investigation. **Inga Grinfelde:** Writing – review & editing, Validation, Methodology. **Sindija Frienberga:** Writing – review & editing, Methodology. **Olga Skiste:** Writing – review & editing, Writing – original draft, Validation, Investigation. **Lidija Vojevoda:** Writing – review & editing, Methodology. **Kristaps Siltumens:** Writing – review & editing, Writing – original draft, Visualization, Methodology. **Andis Lazdins:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT (OpenAI) to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data will be made available on request.

References

Badewa, E.A., Yeung, C.C., Rezanezhad, F., Whalen, J.K., Oelbermann, M., 2022. Spring Freeze–Thaw stimulates greenhouse gas emissions from agricultural soil. Front. Environ. Sci. 10, 909683. https://doi.org/10.3389/fenvs.2022.909683.

Bleken, M.A., Rittl, T.F., Nadeem, S., 2025. Low N_2O emissions induced by root-derived residues compared to aboveground residues of red clover or grass mixed into soil. Soil Tillage Res. 245, 106309. https://doi.org/10.1016/j.still.2024.106309.

Bleken, M.A., Rittl, T.F., Nadeem, S., Hansen, S., 2022. Roots and other residues from leys with or without red clover: quality and effects on N_2O emission factors in a partly frozen soil following autumn ploughing. Sci. Total Environ. 831, 154582. https://doi.org/10.1016/j.scitotenv.2022.154582.

Brown, I.J., Kitidis, V., Rees, A.P., 2023. Simultaneous high-precision, high-frequency measurements of methane and nitrous oxide in surface seawater by cavity ring-down spectroscopy. Front. Mar. Sci. 10. https://doi.org/10.3389/fmars.2023.1197727.

Byers, E., Bleken, M.A., Dörsch, P., 2021. Winter N_2O accumulation and emission in sub-boreal grassland soil depend on clover proportion and soil pH. Environ. Res. Commun. 3 (1), 015001. https://doi.org/10.1088/2515-7620/abd623.

Grinfelde, I., Valujeva, K., Zaharane, K., Berzina, L., 2017. Automated cavity ring down spectroscopy usage for nitrous oxide emission measurements from soil using recirculation system. Eng. Rural Dev. https://doi.org/10.22616/erdev2017.16.n235.

Groffman, P.M., Hardy, J.P., Driscoll, C.T., Fahey, T.J., 2006. Snow depth, soil freezing, and fluxes of carbon dioxide, nitrous oxide and methane in a northern hardwood forest. Glob. Change Biol. 12, 1748–1760. https://doi.org/10.1111/j.1365-2486.2006.01194.x.

Hakala, K., Nikunen, H.M., Sinkko, T., Niemeläinen, O., 2012. Yields and greenhouse gas emissions of cultivation of red clover-grass leys as assessed by LCA when fertilised with organic or mineral fertilisers. Biomass Bioenergy 46, 111–124. https://doi.org/10.1016/j.biombioe.2012.09.021.

IPCC. 2022. Summary for Policymakers [P.R. Shukla, J. Skea, A. Reisinger, R. Slade, R. Fradera, M. Pathak, A. Al Khourdajie, M. Belkacemi, R. van Diemen, A. Hasija, G. Lisboa, S. Luz, J. Malley, D. McCollum, S. Some, P. Vyas, (eds.)]. In: Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [P.R. Shukla, J. Skea, R. Slade, A. Al Khourdajie, R. van Diemen, D. McCollum, M. Pathak, S. Some, P. Vyas, R. Fradera, M. Belkacemi, A. Hasija, G. Lisboa, S. Luz, J. Malley, (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA. doi: 10.1017/9781009157926.001.

LVĢMC. 2020. Meteoroloģiskā stacija Stende [tiešsaiste]. Latvijas Vides ģeoloģijas un meteoroloģijas centrs [skatīts 2025.g. 28.janv.]. (in Latvian) Pieejams: ⟨https://videscentrs.lvgmc.lv/noverojumu-arhivs/meteo/30111⟩.

McKenna, P., Cannon, N., Conway, J., Dooley, J., 2018. The use of red clover (*Trifolium pratense*) in soil fertility-building: a review. Field Crops Res. 221, 38–49. https://doi.org/10.1016/j.fcr.2018.02.006.

Nadeem, S., Hansen, S., Azzaroli Bleken, M., Dörsch, P., 2012. N_2O emission from organic barley cultivation as affected by green manure management. Biogeosciences 9, 2747–2759. https://doi.org/10.5194/bg-9-2747-2012.

Reckling, M., Preissel, S., Zander, P., Topp, C.F.E., Watson, C.A., Murphy-Bokern, D., Stoddard, F.L., 2014. Effects of legume cropping on farming and food systems. Legume Futures Rep. 1, 6. ⟨https://www.legumehub.eu/is_article/effects-of-legume-cropping-on-farming-and-food-systems/⟩.

Rigon, J.P.G., Calonego, J.C., Pivetta, L.A., Castoldi, G., Raphael, J.P.A., Rosolem, C.A., 2021. Using cover crops to offset greenhouse gas emissions from a tropical soil under no-till. Exp. Agric. 57 (4), 217–231. https://doi.org/10.1017/S0014479721000156.

Sanders-DeMott, R., Sorensen, P.O., Reinmann, A.B., et al., 2018. Growing season warming and winter freeze–thaw cycles reduce root nitrogen uptake capacity and increase soil solution nitrogen in a northern forest ecosystem. Biogeochemistry 137, 337–349. https://doi.org/10.1007/s10533-018-0422-5.

Schipanski, M.E., Drinkwater, L.E., 2011. Nitrogen fixation of red clover interseeded with winter cereals across a management-induced fertility gradient. Nutr. Cycl. Agroecosyst. 90, 105–119. https://doi.org/10.1007/s10705-010-9415-z.

Senbayram, M., Wenthe, C., Lingner, A., et al., 2015. Legume-based mixed intercropping systems may lower agricultural born N2O emissions. Energ. Sustain Soc. 6, 2. https://doi.org/10.1186/s13705-015-0067-3.

Shi, W.Y., Yan, M.J., Zhang, J.G., Guan, J.H., Du, S., 2014. Soil CO_2 emissions from five different types of land use on the semiarid Loess Plateau of China, with emphasis on the contribution of winter soil respiration. Atmos. Environ. 88, 74–82. https://doi.org/10.1016/j.atmosenv.2014.01.066.

Stagnari, F., Maggio, A., Galieni, A., Pisante, M., 2017. Multiple benefits of legumes for agriculture sustainability: an overview. Chem. Biol. Technol. Agric. 4, 2. https://doi.org/10.1186/s40538-016-0085-1.

Sturite, I., Rivedal, S., Dörsch, P., 2021. Clover increases N2O emissions in boreal leys during winter. Soil Biol. Biochem. 163, 108459. https://doi.org/10.1016/j.soilbio.2021.108459.

Taveira, C.J., Farrell, R.E., Wagner-Riddle, C., Machado, P.V.F., Deen, B., Congreves, K. A., 2020. Tracing crop residue N into subsequent crops: Insight from long-term crop rotations that vary in diversity. Field Crops Res. 255, 107904. https://doi.org/10.1016/j.fcr.2020.107904.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671–677. https://doi.org/10.1038/nature01014.

Valujeva, K., Pilecka, J., Frolova, O., Berzina, L., Grinfelde, I., 2017. Measurement time estimation of CO_2, CH_4, N_2O and NH_3 in closed chambers and recirculation system with Picarro G2508 analyser. Int Multidiscip. Sci. GeoConference Surv. Geol. Min. Ecol. Manag. SGEM 41 (41), 519–526. https://doi.org/10.5593/sgem2017/41/S19.066.

Valujeva, K., Pilecka-Ulcugaceva, J., Darguza, M., Siltumens, K., Lagzdins, A., Grinfelde, I., 2023. Environmental parameters and management as factors affecting greenhouse gas emissions from clay soil. Acta Agric. Scand. Sect. B — Soil Plant Sci. 74 (1), 2290828. https://doi.org/10.1080/09064710.2023.2290828.

Valujeva, K., Pilecka-Ulcugaceva, J., Skiste, O., Liepa, S., Lagzdins, A., Grinfelde, I., 2022. Soil tillage and agricultural crops affect greenhouse gas emissions from Cambic Calcisol in a temperate climate. Acta Agric. Scand. Sect. B — Soil Plant Sci. 72 (1), 835–846. https://doi.org/10.1080/09064710.2022.2097123.

Wagner, S.W., Reicosky, D.C., Alessi, R.S., 1997. Regression models for calculating gas fluxes measured with a closed chamber. Agron. J. 89, 279–284. https://doi.org/10.2134/agronj1997.00021962008900020021x.

Wyngaarden, S.L., Gaudin, A.C.M., Deen, W., Martin, R.C., 2015. Expanding red clover (*Trifolium pratense*) usage in the corn–soy–wheat rotation. Sustainability 7 (11), 15487–15509. https://doi.org/10.3390/su71115487.

Zhang, H., Xiao, Y., 2024. Contribution of mycorrhizal symbiosis and root strategy to red clover aboveground biomass under nitrogen addition and phosphorus distribution. Mycorrhiza 34, 489–502. https://doi.org/10.1007/s00572-024-01164-6.