



Balancing productivity and greenhouse gas emissions: Evaluating legume–grass mixtures in managed boreal grasslands

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ABSTRACT

Although the agricultural sector is a major source of greenhouse gas (GHG) emissions, mitigation options exist through altering management practices. One of these options is the selection of forage species, as their varying functional traits may alter the dynamics of carbon and nitrogen in grassland ecosystem. Perennial legumes have a strong potential to reduce nitrogen fertilizer needs by fixing atmospheric nitrogen (N₂) through rhizobia, while deep-rooted species can enhance soil carbon inputs by allocating carbon into deeper soil layers. In this study, we investigated the effects of supplementing a timothy (*Phleum pratense*) grass mixture with either deep-rooted grass species (tall fescue, *Festuca arundinacea* Schreb.), or a legume species (red clover, *Trifolium pratense* L.) on yield, net ecosystem carbon balance (NECB), and the contribution of nitrous oxide (N₂O) in the greenhouse gas balance (GHGB). A two-year field experiment was conducted on a managed boreal grassland with mineral soil. GHG fluxes were measured using manual chambers during snow-free seasons and the snow gradient method during snow-covered periods. Inclusion of legume resulted in lower cumulative N₂O emissions (with legume 1.7 vs. without legume 3.1 kg N₂O-N ha⁻¹) and similar yields compared to the deep-rooted grass mixture, despite requiring 4.2 times less nitrogen fertilization. However, carbon dioxide (CO₂) was the dominant GHG, and legume inclusion led to higher respiration rates, resulting in a higher GHGB. These results highlight the potential of legume mixtures to reduce fertilizer use and N₂O emissions in boreal grasslands.

1. Introduction

Agricultural activities and associated land-use changes are significant contributors to global greenhouse gas (GHG - carbon dioxide CO₂, methane CH₄, nitrous oxide N₂O) emissions, accounting for up to 21% of anthropogenic GHG emissions (55.9 ± 6.1 Gt CO₂-equivalent yr⁻¹) between 2010–2019 (Shukla et al., 2022). The GHG emissions from agriculture are dominated by CO₂ exchange followed by N₂O and CH₄ emissions. On a national scale, Finnish agriculture accounts for 13% of total GHG emissions (Statistics Finland, 2022). Grassland cultivation is a significant part of the Finnish agriculture for animal protein production. Cultivated mineral soils are typically either a small sink or source of GHGs (mostly CO₂ and N₂O with CH₄ emissions being of minor

importance), depending upon the climate, vegetation and management practices (Shurpali et al., 2025).

In Finland, approximately 35% of the cultivated arable land is used for forage production (Official Statistics of Finland, 2024). The length of the growing season in Finland is short, lasting about 120 days (May–September). A typical grass rotation cycle lasts for three to four years. The commonly cultivated grass species include shallow-rooted timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.). For legume grasslands, red clover (*Trifolium pratense* L.) is the most common legume species. In addition, while establishing a new grassland rotation cycle, cereals such as barley (*Hordeum vulgare* L.) or oat (*Avena sativa* L.) are often cultivated as cover crops. Recently, tall fescue (*Festuca arundinacea* Schreb.) is being promoted in Europe due to its deep-rooting

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characteristics. Studies have demonstrated that the species develops a higher root length density and a greater proportion of roots in deeper soil layers compared shallow-rooted species (e.g. Perlikowski et al., 2020).

The common indicators of grassland sustainability are net ecosystem carbon balance (NECB) (e.g. Li et al., 2023) and greenhouse gas balance (GHGB) (e.g. Soussana et al., 2007). The NECB of a vegetated ecosystem (grassland in this study) is the net exchange of carbon (C) between the ecosystem and the atmosphere, accounting for inputs (e.g., photosynthesis, organic fertilizer) and outputs (harvested biomass C, ecosystem respiratory C losses). GHGB is the sum of net C balance, CH₄, and N₂O emissions, expressed in CO₂ equivalents (CO₂-eq). The net C balance is the sum of gross primary productivity (GPP) and ecosystem respiration (TER). While C has a major role in sustainability of agricultural ecosystems, nitrogen (N) cycle is also important from both plant productivity and N₂O emission dynamics perspectives. N₂O is mainly produced in the soils by microbial processes: nitrification and denitrification (Firestone and Davidson, 1989). About 60% of global anthropogenic N₂O emissions originate from agricultural soils (Davidson, 2009; Syakila and Kroeze, 2011) and are mainly due to the application of fertilizers.

Red clover being a perennial forage legume species carries out biological fixation of atmospheric N₂ via root-nodule symbiosis of soil bacteria (e.g. rhizobia). Cultivation of legumes could reduce the need for synthetic mineral N fertilizers and thus reduce N₂O emissions (Carlsson and Huss-Danell, 2003; Fuchs et al., 2018; Senbayram et al., 2016). Moreover, recent studies (Akiyama et al., 2016) hint at a possible role of legumes in the reduction of atmospheric and/or soil N₂O in the process of complete denitrification occurring in the root rhizosphere, further reducing N₂O emissions. While legumes are commonly included in grass mixtures for their multiple agronomic and environmental benefits, incorporating deep-rooted species such as tall fescue is equally important due to their capacity to allocate a larger proportion of C into deeper soil layers (Carter and Gregorich, 2010). Compared with shallow-rooted timothy, tall fescue enhances grassland drought resilience and supports higher primary productivity (Perlikowski et al., 2020).

While benefits of including forage legumes in grass mixtures are well established, these advantages have not yet been verified under boreal conditions due to a lack of high-frequency CO₂, N₂O, and CH₄ flux measurements combined with yield assessments. Therefore, studies are needed to confirm whether such benefits also occur in boreal grasslands. In this study, our objective was to assess how grass species influence NECB and GHGB. We measured GHG exchange from two grass mixture types under boreal climate conditions: red-clover and timothy and tall

fescue and timothy. The measurements were conducted for two years, the grassland establishment year and the first grass year on a mineral agricultural soil in eastern Finland. Specifically, we addressed two research questions:

- (1) Is there a difference in forage yield and NECB between different grass mixtures (RCT vs. TFT)?
- (2) How do different grass mixtures influence N₂O emissions within the GHGB?

2. Material & methods

2.1. Study site

The study was conducted on a 6.3 ha mineral soil field located in Maaninka (63°09'49"N, 27°14'3"E, 89 m.a.s.l.), North Savo region, eastern Finland (Fig. 1). Long-term (1991–2020) annual air temperature in the region is 3.8 °C and annual precipitation is 612 mm, based on observations done at a permanent weather station at Kuopio, Maaninka, maintained by the Finnish Meteorological Institute. The soil is typically frozen and covered with snow from December to April. The soil is characterized as silt loam (25 ± 5.6% clay, 53 ± 9.0% silt, and 22 ± 7.8% sand) based on the US Department of Agriculture (USDA) textural classification system (Lind et al., 2016). The field has previously been cultivated with different grass mixtures and cereals including barley and oat. The year before the initiation of this study, the field was cultivated with timothy, tall fescue, and barley as a cover crop. A single barley harvest occurred during the summer, after which glyphosate was applied. The field was left bare throughout the winter prior to this study.

2.2. Experimental design and field management

In May 2021, the experimental setup was established within the main field, with two treatments with different grass mixtures: red-clover and timothy mixture (hereafter RCT) and tall fescue and timothy mixture (hereafter TFT) (Fig. 1). The experimental setup consisted of three blocks for each treatment and within each block there were three adjacent replicates (n = 18 plots, 1.5 × 10 m) (Fig. 1). Grass mixtures were sown on June 3rd 2021: one containing red clover (4 kg ha⁻¹, 'Ilte') and timothy (15 kg ha⁻¹, 'Nuutti'), and another containing tall fescue (16 kg ha⁻¹, 'Karolina') and timothy (15 kg ha⁻¹, 'Nuutti').

During the establishment year (1.5.2021–30.4.2022, hereafter referred as first year), both treatments were intercropped with barley (180 kg ha⁻¹, 'Vertti') as a cover crop to manage soil fertility and



Fig. 1. Map of the study site located in Maaninka, and experimental design with two grass mixtures (RCT, marked with orange color and TFT, marked with blue color). Spots used for GHG flux measurements on the plots are marked with yellow squares. Online soil loggers used outside growing season for measurements of soil volumetric water content and soil temperature from 5 cm depth are marked as red triangles.

prevent erosion. Barley was harvested as a whole crop on August 2nd 2021. The second year was the first grass year for both treatments (1.5.2022 – 30.4.2023, hereafter referred as the second year), with RCT having 2 harvests (June 21st and August 8th) and TFT 3 harvests (June 16th, July 25th and September 1st). Details on annual fertilization events are given in Table 1; mineral fertilizer was applied using a surface application method.

2.3. Greenhouse gas flux measurements

Greenhouse gas flux measurements were conducted for two years from May 2021 to April 2023. Initially, GHG fluxes from bare soil were measured with static closed chamber method without collars according to Maljanen et al., (2006)). Subsequently, permanent square aluminum collars ($60 \times 60 \times 15 \text{ cm}^3$) were installed on the study plots (Fig. 1). These collars were equipped with a U-shaped frame filled with water to create a seal, preventing gas exchange between the chamber headspace and ambient air. Boardwalks were installed next to collars to minimize soil disturbance during gas measurement.

Closed opaque chambers ($60 \times 60 \times 40 \text{ cm}^3$) equipped with an electric fan for continuous mixing of headspace air were used to measure GHG fluxes. Extensions were used when the crop height exceeded the height of the chamber. Chambers were vented by manually waving between measurements. Gas measurements were performed weekly during the growing seasons (May–September, hereafter GS) and bimonthly during the non-growing season (October–April, hereafter NGS). To capture post-fertilization emission peaks, gas fluxes were measured 1, 4, and 6 days after each fertilization event, except for the first event in 2022 due to technical issues, when measurements were conducted only on days 4 and 6.

During the first year, GHG measurements were done with static closed chamber method with permanent collars. With pre-evacuated vials, gas samples were taken from chamber headspace using a syringe at intervals of 5, 15, 25, and 35 min after chamber enclosure. Samples were subsequently analyzed with a gas chromatograph (GC) (Agilent 7890 Agilent Technologies, Inc., Wilmington, DE, USA). Nitrogen (pure N_2) was used as the carrier gas and calibration was conducted using a standard gas mixture with known concentrations of CO_2 , N_2O and CH_4 across seven concentration points.

During the second year, measurements were done with the static closed chamber method, where gas concentration in the chamber was measured in real-time with a LI-COR LI-7820 $\text{N}_2\text{O}/\text{H}_2\text{O}$ and LI-COR LI-7810 CO_2/CH_4 trace gas analyzers (LI-COR Biosciences; Lincoln, NE, USA). Each measurement lasted for 2–4 min depending on the chamber size and extra collars, with gas concentration recorded at an interval of a second. GC and LI-COR methods were tested with in-house method comparison, and the results were found to be comparable (Bhattarai et al. unpublished).

Furthermore, net ecosystem exchange (NEE) was measured weekly during the GS from the same collars, using a transparent chamber ($60 \times 60 \times 30 \text{ cm}^3$). The chamber was equipped with a fan for continuous mixing of headspace air and cooling system to prevent temperature fluctuations inside. Measurements were conducted between 7:00 and

17:00, with clear sky conditions to reduce variability caused by changing cloud cover. Three different light levels were used during the two-minute measurements: one with fully transparent chamber, one with fabric that blocked approximately 50% of the photosynthetically active radiation (PAR) and one with opaque chamber that blocked all PAR. The blocked measurement represented TER measured with dark chamber and was only used for deriving GPP (see Section 2.6).

When snow cover exceeded 10 cm, GHG measurements were conducted by using the snow gradient method (Maljanen et al., 2003). Gas samples (25 ml) were collected using a probe consisting of a steel pipe (3 mm diameter), with a three-way valve and a plastic syringe. The samples were taken just above the snow cover, at the bottom of the snow cover, and at 10 cm intervals throughout the snowpack. The samples were stored in pre-vacuumed vials, and gas concentrations were analyzed with gas chromatograph as described above. Snow porosity was measured by collecting snow samples with a PVC tube (10.2 cm diameter, 60 cm height).

2.4. Ancillary measurements

Comprehensive environmental measurements were conducted during each GHG measurement. Soil temperature (TM-80N, Tenmars electronics co., Ltd, Taiwan) and soil volumetric water content (HH2 Moisture Meter, Delta-T Devices Ltd, UK) were manually measured at a depth of 5 cm directly beside the collar. During the time with snow cover, soil temperature and volumetric water content data was obtained from online soil loggers (TEROS 12, METER Group GmbH, Munich, Germany). PAR (Quantum Sensor SKP 215, Skye Instruments Ltd, UK) and air temperature (109 temperature probe, Campbell Scientific Inc., Logan, UT, USA) inside the chamber were measured during NEE measurement.

Additionally, a continuous series of environmental data including soil temperature (Hydraprobe II, Stevens Water Monitoring Systems Inc., Portland, OR, USA), air temperature, humidity (HMP155, Vaisala, Finland), and PAR (LI-190R Quantum Sensor, LI-COR Biosciences; Lincoln, NE, USA) were collected at 30 min intervals using observations from a weather station located in the middle of the agricultural field parcel. Small data gaps (max 3 h) in continuous data were interpolated, and longer gaps were filled using the data from two additional weather stations located nearby, depending on which one had access to missing data points.

During the time with snow-cover, snow and frost depth were measured weekly from 6 central independent sampling locations in the middle of the experimental site. Frost depth was measured using 1 m long tubes buried vertically in soil, which contained methylene blue solution that turns transparent when frozen.

Biomass inside the collars was manually harvested at 7 cm height, and stubble was left on the ground. The harvested biomass was dried at 60°C for 48 h for dry matter estimation. Biomass carbon content was analyzed from dried biomass using in-house method JOK3016 (LECO). The percentages of red clover, timothy and tall fescue were calculated based on total fresh biomass harvested from the collars. The biomass of individual plant species was measured separately and were divided by total biomass to achieve the percentage. A vegetation index (VI) was derived from weekly measurements of green canopy cover (GCC) obtained using the Canopeo application (Patrignani and Ochsner, 2015). The application quantifies the fraction of green pixels in canopy photographs, resulting in GCC values ranging from 0 (no canopy) to 1 (full canopy). To account for differences in the range of observed values, VI was calculated by:

$$VI = \frac{GCC - GCC_{\min}}{GCC_{\max} - GCC_{\min}} \quad (1)$$

where $GCC_{\max}$ and $GCC_{\min}$ represent the minimum and maximum values observed during the study period. This resulted in VI values varying

Table 1

The fertilization events during the experiment in 2021–2023. Herbicide was added on 2.7.2021 (Basagran 1.7 kg ha^{-1}) and liming was done on 14.5.2021 (3000 kg ha^{-1}).

Activity	Date	kg N ha^{-1}	
Fertilization both	3.6.2021	75	Y6 (15N-6.5P-12.5 K)
Fertilization both	23.5.2022	14.4	Yara 'startti' (12N-22.3P-0K)
Fertilization TFT	23.5.2022	85	NK1 (25N-0P-7K)
Fertilization RCT	23.5.2022	39.6	NK2 (22N-0P-11.6 K)
Fertilization TFT	16.6.2022	100	NK1
Fertilization RCT	22.6.2022	45.1	NK2
Fertilization TFT	25.7.2022	39.6	NK2

between 0 and 1.

For gravimetric water content (GWC) and mineral N content determination, soil samples were taken weekly (at the same time as GHG measurements during GS) from each plot. On each sampling event, four samples were taken from different locations within each plot and combined to form one composite sample per plot. Samples were taken from a depth of 0–10 cm with a soil auger (2.4 cm diameter). Visible plant biomass (e.g., roots) and stones were removed, and soil samples were homogenized manually. Soil GWC was determined by drying the 10 g of the homogenized soil samples at 105 °C for approximately 24 h, and water filled pore space (WFPS%) determined using the percent volumetric water content (θ_v), and the percentage of total soil porosity (TP) calculated from bulk density (g cm^{-3}) and particle density (g cm^{-3}) (Linn and Doran, 1984). Here we used 2.65 g cm^{-3} as particle density as recommended for mineral soil by Linn and Doran (1984). Also, electrical conductivity and pH were measured in milli-Q-H₂O-soil slurry using EC (HQ40d, Hach Co., CO, USA) and pH meters (UB-10, Denver Instrument, CO, USA), respectively. The concentrations of NH_4^+ in soil extracts were analyzed spectrophotometrically at 650 nm with the colorimetric method as described in Fawcett and Scott, (1960)). Similarly, NO_3^- concentration was determined from soil extracts spectrophotometrically at 544 nm using the colorimetric method (Miranda et al., 2001). The spectrophotometric determination of NO_3^- and NH_4^+ was done with FLUOstar Omega microplate reader (BMG Labtech).

2.5. Flux calculation

Soil diffusion GHG fluxes were calculated for each chamber enclosure by fitting a linear regression model of gas concentrations over time. The GHG fluxes by the snow gradient method were calculated using the

equation based on Fick's law of diffusion as described in Maljanen et al., (2003)). The criterion of $R^2 > 0.8$ for the linear fits to the CO_2 concentrations for GC measurements was set as the threshold for acceptable data quality for all the gases. Linearity of the CO_2 concentration in the headspace was inspected for LI-COR measurements also and any deviation in CO_2 concentration was considered an error in the measurement and was removed from the dataset. This resulted in the exclusion of about 2.5% ($n = 15$, GC) and 1.3% ($n = 14$, GC and LI-COR combined) of the measurements from the first and second year, respectively.

For annual balances of N_2O and CH_4 , the gaps between measurement days were filled by using the time weighted average, by multiplying the average daily flux of two consecutive measurements by the number of days between the measurements. The measured and interpolated daily values were then summed up together to get the annual cumulative value.

NEE for growing seasons was calculated with MATLAB (R2021a), by employing regression model to describe the change in the chamber headspace CO_2 concentration over time, with water vapor correction applied (Kutzbach et al., 2007). An exponential fit as proposed in Kutzbach et al., (2007) was used for NEE calculations. Due to possible perturbations from chamber placement, the initial 10 s of the data were discarded, and the subsequent 50 s (100 data points) were used for flux calculation. In case of significant PAR fluctuations due to cloud cover, the most stable 50 s was manually selected Fig. 2.

2.6. NEE partitioning and gap-filling

We partitioned NEE into its major component fluxes, GPP and TER to calculate seasonal and annual budgets for different treatments. In the study, micrometeorological sign convention is used, where a negative

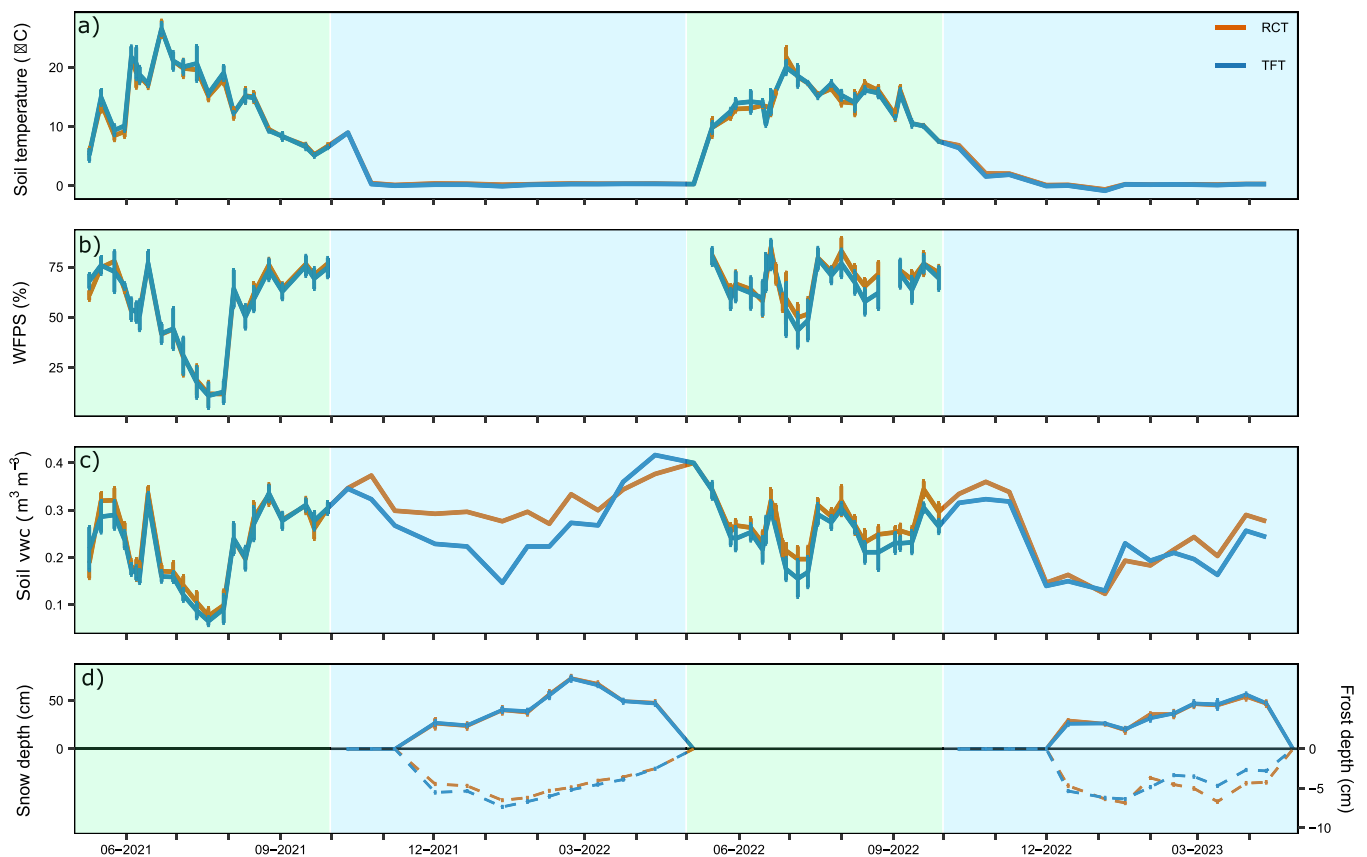


Fig. 2. Environmental and soil variables during the two-year experiment in both treatments: a) soil temperature (°C), b) water filled pore space (WFPS, %), c) soil volumetric water content ($\text{m}^3 \text{ m}^{-3}$), and d) snow height (cm, solid line) and frost penetration (cm, dashed line). In all figures color indicates treatments, orange, RCT and blue, TFT. Background colors indicate growing season (GS, green shade) and non-growing season (NGS, blue shade).

value indicates gas flux from the atmosphere to the plant–soil ecosystem and a positive value indicates gas flux from the plant–soil ecosystem to the atmosphere. Note that many articles based on the eddy covariance data still define GPP and TER as positive-definite quantities while in this study the sign of GPP is negative. Time weighted average method described in previous section was applied for NGS CO₂ fluxes. NEE during GS was derived as the sum of GPP and TER, indicating the overall balance of carbon gain or loss by ecosystem:

$$NEE = GPP + TER \quad (2)$$

TER consists of autotrophic (R_d) i.e., plant respiration and heterotrophic (R_s) i.e., soil respiration. TER was modeled using functions based on Lloyd and Taylor (1994) and modified from Lohila et al., (2003):

$$TER = R_d + R_s \quad (3)$$

$$R_d = VI R_{d0} \exp \left[b_d \left(\frac{1}{T_0 + 273} - \frac{1}{T_1 + 273} \right) \right] \quad (4)$$

$$R_s = R_{s0} \exp \left[308.56 \left(\frac{1}{56.02} - \frac{1}{T_s + 46.02} \right) \right] \quad (5)$$

where VI represents vegetation index (described previously), T₁ is chamber air temperature (°C), T_s is the soil temperature (°C) at a depth of 5 cm and b_d is a constant value (5000), employed by (Lohila et al., 2003). R_{d0} is the rate of plant dark respiration (in mg (CO₂) m⁻² s⁻¹) at the reference temperature T₀ = 10°C and R_{s0} is a parameter describing the soil respiration (in mg (CO₂) m⁻² s⁻¹) at the reference soil temperature of 10°C. Parameters R_{d0} and R_{s0} were estimated by fitting Eqs. 4 and 5 to measured data using nonlinear least squares. The R_{d0} values for the first and second GS were 0.136 and 0.171 (RCT) and 0.136 and 0.160 (TFT), while the corresponding R_{s0} values were 0.133 and 0.0594 (RCT) and 0.880 and 0.0427 (TFT).

GPP was expressed as a function of photosynthetically active radiation, the light response of GPP was estimated for both treatments by pooling the daily data (shading levels of 0%, 50% and 100%) from all the collars with a rectangular hyperbolic saturation curve (Thornley and Johnson, 1990):

$$GPP = \frac{\alpha PAR GPP_{\max} VI}{\alpha PAR + GPP_{\max} VI} \quad (6)$$

where α (g CO₂ μmol per photons in hour) is the initial slope of the photosynthetic light response, and GPP_{max} (g CO₂ m⁻² s⁻¹) is the maximum value of GPP at infinite PAR. By pooling the daily data, only one daily value was obtained to both treatments. GPP_{max} and α were linearly interpolated between measurement days, with both parameters constrained to zero at the onset of the growing season. At the end of the season, interpolation was continued until the point when cumulative autumn heat sums no longer increased. For consistency across years, however, only values up to September 30 were used in the dataset, which was assumed to represent the end of the growing season. The half-hourly GPP, R_d and R_s time series were calculated using daily treatment-specific parameters, including PAR, air temperature, soil temperature and VI. Light response curve fitting was done in Python 3.7.7 with pandas and numpy libraries. All gap-filled NEE values were summed up together to get the annual cumulative NEE value.

2.7. Net ecosystem carbon balance and greenhouse gas balance

NECB and GHGB from the treatments was calculated using equations adapted from Chapin et al., (2006) and Shurpali et al., (2010):

$$NECB = -NEE - C_{\text{yield}} \quad (7)$$

where C_{yield} is biomass-C removal from the collars. In this study CH₄ and leaching were excluded from NECB, since they both are observed to be minimal in this specific site.

$$GHGB = C_{\text{CO}_2} + C_{\text{CH}_4} + C_{\text{N}_2\text{O}} + C_{\text{Yield}} \quad (8)$$

where C_{CO₂} is the annual NEE, and C_{CH₄}, C_{N₂O} are the CO₂- equivalents of annual CH₄ and N₂O emissions. CH₄ and N₂O emissions were converted to CO₂-eq by multiplying with global warming potential (GWP) factors of 27.2 and 273, respectively (100-year period AR6, feedback included, (Shukla et al., 2022)). C_{Yield} was calculated from analyzed carbon content of the harvested biomass (42%). Further on to achieve feed CO₂-eq (emissions per kg dm feed produced) was calculated by dividing GHGB with average harvested yield biomass (t dm ha⁻¹).

2.8. Statistical analysis

All statistical analyses and plotting were done using R statistical software (version 4.3.2). Before statistical testing, data distribution was assessed using histograms, and non-normally distributed data were transformed to normally distributed if needed. Two-way analysis of variance (ANOVA) was used with Tukey's post hoc test to assess significant differences between the treatments and values with *p* < 0.01 were considered statistically significant. To identify the most important variables explaining the variation of environmental, soil and vegetation parameters and gas fluxes, we applied linear regression models. Multi-collinearity of predictor variables was also tested prior to that, by using correlation matrix. Since partitioned CO₂ components only had one value per day, we were not able to calculate significance for some results. However separate uncertainty analysis for NEE, GPP and TER annual budgets were conducted similarly as in Weideveld et al., (2021)). In this approach annual budgets were calculated using multiple models and then the statistical difference based on those was tested with analysis of variance.

3. Results

3.1. Meteorological and environmental conditions

The mean annual temperature was 4.4 °C and 4.6 °C and annual precipitation was 622 mm and 578 mm in the first and second year, respectively. The first GS (160 days) was characterized by a dry period from late June to early August, followed by increased precipitation in August. Temperatures exceeded the 30-year regional average throughout the season, except for September (Fig. 3). The second GS (167 days) showed more uniform precipitation patterns throughout the season, with temperatures closer to the 30-year average than in the first GS (Fig. 3).

Snow cover lasted for 164 and 162 days in the first and second year, respectively (Fig. 2). The study site was snow-free on May 5th and April 28th in the first and second year, respectively. Maximum snow depths reached 75 cm and 57 cm in the first and second year, with no significant differences in snow depths between the treatments (Table S1). Maximum frost depths reached 11 cm and 10 cm in the first and second year, and frost depths showed no significant inter-treatment variation. The mean air temperatures in both non-growing seasons were higher than the 30-year average, except for December in the first year, which was almost 5 °C colder than the average (Fig. 3.).

Soil temperatures at 5 cm depth did not differ significantly between the treatments in either of the two years and ranged from −1–29 °C with mean of 9.5 °C in RCT and −1–28 °C with mean of 9.5 °C in TFT (Table S1, Fig. 2). Soil volumetric water content was significantly higher in RCT than in TFT during the first NGS and the second GS (*p* < 0.01) (Table S1) and it ranged from 0.06 to 0.4 m³ m⁻³ with mean of 0.25 m³ m⁻³ in RCT and 0.04–0.4 m³ m⁻³ with mean of 0.23 m³ m⁻³ in TFT. WFPS mostly varied between 40% and 80% throughout the measurement period, except in July of the first GS when it dropped as low as 5% in both treatments (Fig. 2). In statistical analysis, WFPS was used whenever it could be calculated (during the growing seasons) and soil

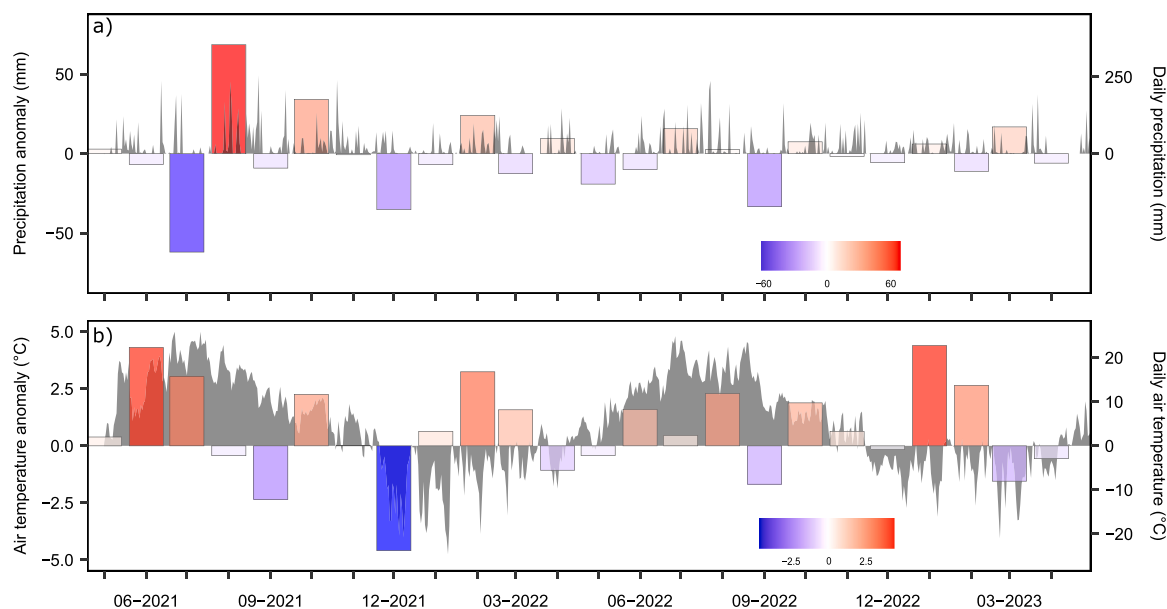


Fig. 3. Monthly anomaly of a) precipitation and b) air temperature from the 30-year average (1991–2020) marked with bars. In secondary y-axis, daily precipitation (mm) and daily air temperature (°C) are marked as gray lines.

volumetric water content was used otherwise. Both variables show very similar behavior (Fig. 2).

3.2. Species composition and yield

During the first GS, GCC peaked on July 5th in both treatments and declined before harvest. During the second GS, GCC in TFT reached maximum on June 31st and declined after the second and third harvest. On RCT GCC reached maximum on July 21st and declined after each harvest.

The barley yield was lower from the first GS compared to the grass yield in the second GS (Table 2). There were no significant differences in total yields between the treatments in either of the years (Table S1). During the first GS, the species composition in both treatments was mainly cover crop (barley, RCT 99%, TFT 99.6%). During the second GS, the species composition in TFT varied between harvests, in the first harvest, tall fescue comprised 48% and timothy 49%; in the second and third harvests, tall fescue increased to 84%, while timothy decreased to 15%. In RCT, the percentage of red clover ranged from 0.1% to 40.4% in the first harvest and from 1.0% to 66.4% in the second harvest, while timothy averaged $73 \pm 12\%$ in the first and $70 \pm 23\%$ in the second harvest.

Table 2

The dry matter yield (kg dw ha⁻¹) averaged per treatment and standard deviations. The dry matter yield was estimated using the biomass harvested from the measurement collars (n = 9). Total means sum of all the harvest events in one season.

Year	Harvest	Treatment	Yield (kg dw ha ⁻¹)
2021	Total	RCT	6620 ± 660
2021	Total	TFT	5870 ± 830
2022	1	RCT	5420 ± 630
2022	2	RCT	4470 ± 730
2022	Total	RCT	9900 ± 1100
2022	1	TFT	3300 ± 1800
2022	2	TFT	3800 ± 1300
2022	3	TFT	3980 ± 470
2022	Total	TFT	10100 ± 3300

3.3. Greenhouse gas flux and seasonal variations

3.3.1. Ecosystem CO₂ exchange

During the first GS, daily TER ranged from 90 to 1090 mg CO₂ m⁻² h⁻¹ in RCT and from 70 to 930 mg CO₂ m⁻² h⁻¹ in TFT, with mean fluxes of 440 ± 260 mg CO₂ m⁻² h⁻¹ and 350 ± 230 mg CO₂ m⁻² h⁻¹, respectively (Fig. 4). No significant differences between treatments were observed in NEE during the first GS (Table S1). However, TER and GPP differed significantly between the two treatments. The daily GPP during the first GS ranged from -1.2 to -1730 mg CO₂ m⁻² h⁻¹ in RCT and from -0.9 to -1550 mg CO₂ m⁻² h⁻¹ in TFT, with mean fluxes of -400 ± 500 mg CO₂ m⁻² h⁻¹ and -340 ± 480 mg CO₂ m⁻² h⁻¹, respectively. GPP reached its negative maximum in July during peak vegetation growth. This was followed by a gradual decrease in GPP until harvest. NEE followed similar temporal patterns as GPP, ranging from -800–430 mg CO₂ m⁻² h⁻¹ in RCT and from -760–330 mg CO₂ m⁻² h⁻¹ in TFT, with mean fluxes of -36 ± 290 mg CO₂ m⁻² h⁻¹ and -14 ± 270 mg CO₂ m⁻² h⁻¹, respectively. The negative NEE values indicate that both treatments acted as net C sinks during the GS. In the first NGS, TER ranged from 4.2 to 660 mg CO₂ m⁻² h⁻¹ in RCT and from 3.4 to 390 mg CO₂ m⁻² h⁻¹ in TFT and was lower compared to the GS.

During the second GS, daily TER ranged from 40 to 840 mg CO₂ m⁻² h⁻¹ in RCT and from 30 to 810 mg CO₂ m⁻² h⁻¹ in TFT, with mean fluxes of 440 ± 180 mg CO₂ m⁻² h⁻¹ and 380 ± 220 mg CO₂ m⁻² h⁻¹, respectively. The daily GPP ranged from -20 to -1490 mg CO₂ m⁻² h⁻¹ in RCT and from -10 to -1460 mg CO₂ m⁻² h⁻¹ in TFT, with mean fluxes of -580 ± 410 mg CO₂ m⁻² h⁻¹ and -550 ± 400 mg CO₂ m⁻² h⁻¹, respectively. RCT exhibited peak C uptake in June and late July. TFT maintained relatively lower GPP throughout the GS, with increases in June and July. NEE during the second GS reflected the trends in GPP, ranging from -800–390 mg CO₂ m⁻² h⁻¹ in RCT and from -980–390 mg CO₂ m⁻² h⁻¹ in TFT. Mean fluxes were -135 ± 300 mg CO₂ m⁻² h⁻¹ in RCT and -180 ± 290 mg CO₂ m⁻² h⁻¹ in TFT, indicating continued C sequestration in both treatments. During the second NGS, TER ranged from 2 to 440 mg CO₂ m⁻² h⁻¹ in RCT and from 1 to 360 mg CO₂ m⁻² h⁻¹ in TFT. On contrary to the first GS, there were no significant treatment differences in NEE or GPP, while TER had significant difference (Table S1).

Annual NEE was positive (indicating the field to be source of C) in both treatments during the first year and negative (indicating the field to

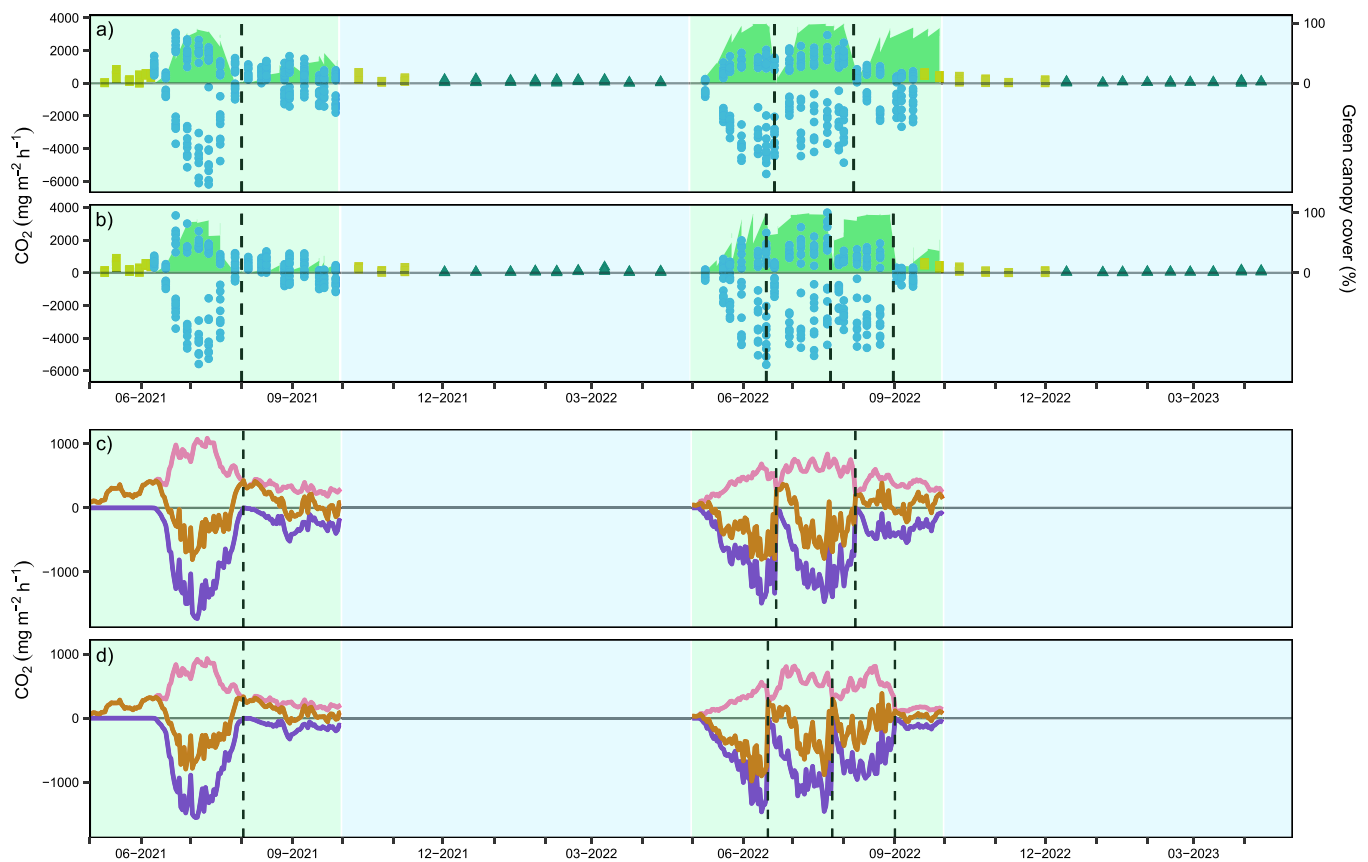


Fig. 4. Measured (a: RCT, b: TFT) and daily modeled (c: RCT, d: TFT) CO₂ fluxes (mg m⁻² h⁻¹) during the two-year study. On secondary y-axis in a and b green values indicate green canopy cover (%). Different symbols in measured values indicate measurement methods: dark chamber (yellow square), opaque chamber (blue circle) and snow gradient (green triangle). Background colors indicate growing season (GS, green shade) and non-growing season (NGS, blue shade). Gap-filled data is partitioned to daily TER (pink), NEE (orange) and GPP (purple). Harvest events are marked as black dashed lines.

be sink of C) during the second year (Table 3). There were no significant differences in GS NEE between the treatments. However, there was a significant difference ($p < 0.01$) in annual NEE between the treatments, during both years (Table S1). TER remained similar between the years, while the amount of biomass and GPP were higher during the second year, resulting in more negative NEE. In addition, CO₂ emissions during the second NGS were lower than during the first NGS.

3.3.2. CH₄ fluxes

CH₄ fluxes remained minimal throughout the measurement period, ranging from -1.8 – 0.9 mg CH₄ m⁻² d⁻¹ in RCT and from -1.7 – 0.5 mg CH₄ m⁻² d⁻¹ in TFT (Fig. 5). The highest and lowest fluxes occurred in July during both years. No significant differences between treatments were observed ($p > 0.01$); both treatments mostly acted as moderate sinks of CH₄.

3.3.3. N₂O dynamics

N₂O fluxes showed different patterns between seasons, with peak emissions occurring during GS following fertilization (Fig. 5). During the first GS, N₂O fluxes ranged from -0.8 – 16 mg m⁻² d⁻¹ in RCT and from -0.5 – 31 mg m⁻² d⁻¹ in TFT, with respective mean fluxes of 1.0 ± 1.9 and 1.4 ± 3.2 mg m⁻² d⁻¹. NGS fluxes were on average lower than during GS (Fig. 5), ranging from -0.02 – 2.1 mg m⁻² d⁻¹ in RCT and from -0.02 – 5.4 mg m⁻² d⁻¹ in TFT, contributing 25% and 30% of annual emissions, respectively. In the second GS, N₂O fluxes ranged from -1.1 – 13 mg m⁻² d⁻¹ in RCT and from -0.4 – 11 mg m⁻² d⁻¹ in TFT, with means of 0.5 ± 1.8 and 1.2 ± 1.8 mg m⁻² d⁻¹, respectively. Similarly, during second NGS fluxes were lower than GS, ranging from -0.2 – 0.4 mg m⁻² d⁻¹ in RCT and from -0.2 – 0.9 mg m⁻² d⁻¹ in TFT, with means of 0.04 ± 0.1 and 0.1 ± 0.12 mg m⁻² d⁻¹, respectively. Second year NGS emissions contributed a smaller proportion to annual emissions compared to the first year (RCT: 13%, TFT: 12%), with both treatments showing decline in emissions compared to the first NGS.

Table 3

The CO₂ flux components from growing seasons (gross primary production, GPP GS, total ecosystem respiration, TER GS, net ecosystem exchange, NEE GS) (g CO₂ m⁻²), non-growing season NEE (NEE NGS), annual NEE and net ecosystem carbon balance (NECB) (t C ha⁻¹ y⁻¹). Uncertainty estimates are given for the growing season GPP, TER and NEE.

Treatment	Year	GPP GS	TER GS	NEE GS	NEE NGS	Annual NEE	NECB
		g CO ₂ m ⁻²				t C ha ⁻¹ y ⁻¹	
RCT	May 2021 –April 2022	-2960 ± 280	3230 ± 240	270 ± 340	810 ± 210	10.8	-21
TFT	May 2021 –April 2022	-2490 ± 340	2590 ± 220	100 ± 360	580 ± 140	6.8	-15.8
RCT	May 2022 –April 2023	-4260 ± 220	3260 ± 290	-1000 ± 340	490 ± 70	-5.0	-10.2
TFT	May 2022 –April 2023	-4050 ± 150	2750 ± 310	-1300 ± 330	390 ± 60	-9.0	-6.5

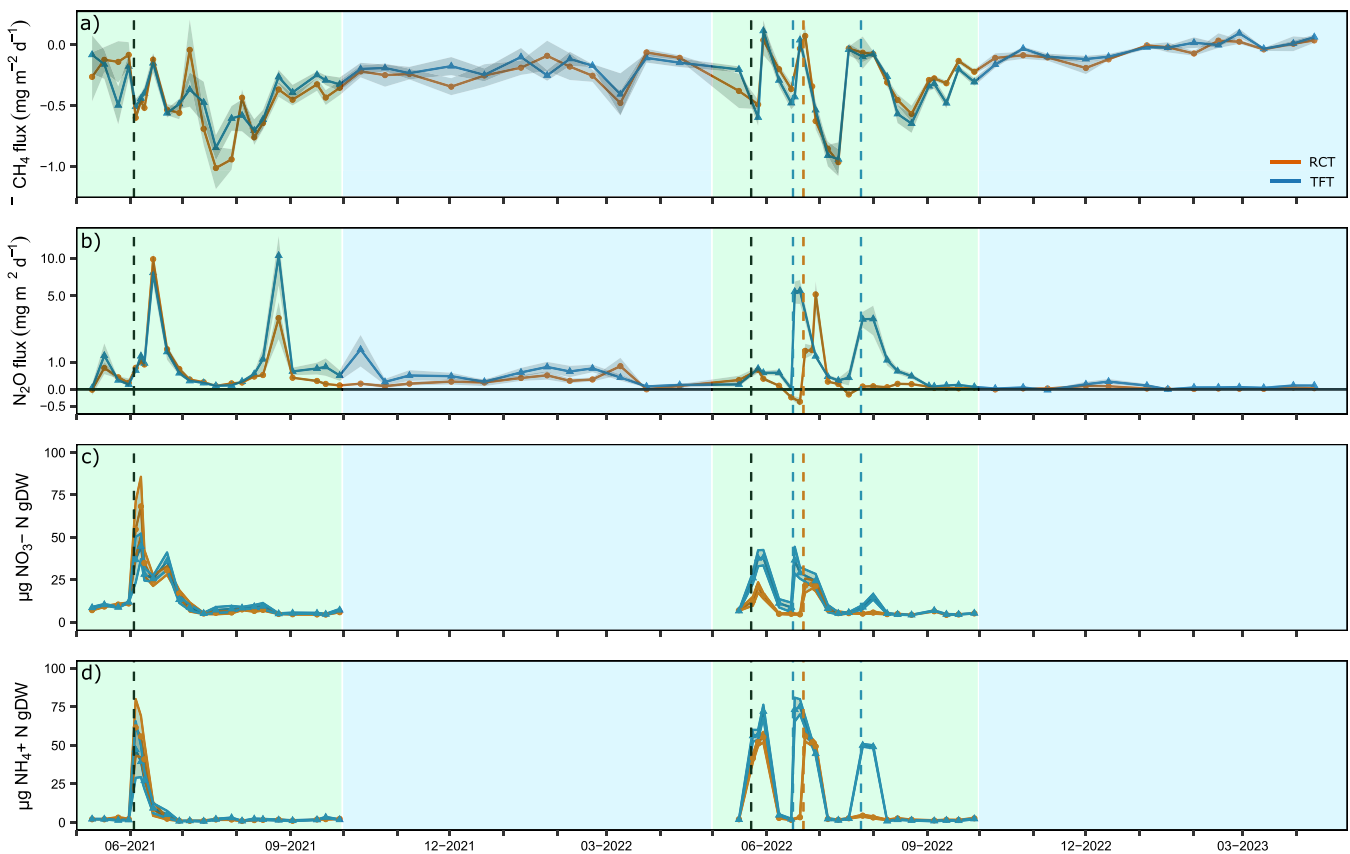


Fig. 5. The measured CH_4 (a, $\text{mg m}^{-2} \text{d}^{-1}$) and N_2O fluxes (b, $\text{mg m}^{-2} \text{d}^{-1}$), NO_3^- (c, $\mu\text{g NO}_3^- \text{N gDW}^{-1}$) and NH_4^+ (d, $\mu\text{g NH}_4^+ \text{N gDW}^{-1}$) concentrations during the two experiment years. Orange lines represent RCT and blue lines TFT. Values represent means and the grey shaded area indicates $\pm$ standard deviation ($n = 9$). Fertilization events are marked by different colored vertical lines: both treatments (black dashed), RCT (orange dashed), and TFT (blue dashed). Background colors indicate growing season (GS, green shade) and non-growing season (NGS, blue shade).

The first GS showed two major emission peaks: one following fertilization on June 14th (RCT: 9.8 ± 3.5 , TFT: $7.7 \pm 4.6 \text{ mg m}^{-2} \text{d}^{-1}$) and another on August 25th (RCT: 3.2 ± 3.7 , TFT: $10.5 \pm 13.2 \text{ mg m}^{-2} \text{d}^{-1}$). In the second GS N_2O fluxes peaked following fertilization on June 29th ($5.1 \pm 4.3 \text{ mg m}^{-2} \text{d}^{-1}$) in RCT, while TFT showed increased fluxes after fertilizer application on June 20th ($5.5 \pm 3.4 \text{ mg m}^{-2} \text{d}^{-1}$) and July 26th ($3.2 \pm 1.5 \text{ mg m}^{-2} \text{d}^{-1}$).

N_2O uptake was observed in RCT in the second year before the first harvest (June 21st). Maximum negative fluxes were recorded on June 15th ($-0.3 \pm 0.2 \text{ mg m}^{-2} \text{d}^{-1}$) and June 20th ($-0.5 \pm 0.4 \text{ mg m}^{-2} \text{d}^{-1}$). Also, in TFT uptake was observed on June 15th ($-0.2 \pm 0.1 \text{ mg m}^{-2} \text{d}^{-1}$). Cumulative N_2O emissions over the entire measurement period were significantly lower ($p < 0.01$) in RCT ($1.7 \pm 0.43 \text{ kg N}_2\text{O-N ha}^{-1}$) than TFT ($3.1 \pm 1.51 \text{ kg N}_2\text{O-N ha}^{-1}$) (Fig. 6).

3.4. NECB and GHGB

Overall, CO_2 and N_2O emissions from the first year were significantly higher than in the second year in both treatments. GHGB (RCT 21.6 , TFT $16.7 \text{ t CO}_2\text{-eq ha}^{-1} \text{y}^{-1}$) and NECB (RCT -21 , TFT $-15.8 \text{ t C ha}^{-1} \text{y}^{-1}$) both indicated emissions to atmosphere in the first year in both treatments (Table 4). During the second year, TFT reached lower GHGB ($6.9 \text{ t CO}_2\text{-eq ha}^{-1} \text{y}^{-1}$) and NECB ($-6.5 \text{ t C ha}^{-1} \text{y}^{-1}$) due to the stronger CO_2 uptake. Similarly, GHGB ($10.3 \text{ t CO}_2\text{-eq ha}^{-1} \text{y}^{-1}$) and NECB ($-10.2 \text{ t C ha}^{-1} \text{y}^{-1}$) from RCT decreased but remained as a source. During the first year, CO_2 dominated the GHGB, accounting for 89% and 77% of emissions in RCT and TFT, respectively, with N_2O contributing the rest (RCT: 11%, TFT: 23%). This pattern was similar also in the second year, with CO_2 representing 92% and 88% of total emissions in RCT and TFT,

respectively, and N_2O contribution (RCT: 7%, TFT: 11%) was lower than the first year. CH_4 contributions were negligible (approximately 0) throughout the study period. Emissions per feed produced (feed $\text{CO}_2\text{-eq}$) in the first year were higher in RCT ($3.3 \text{ kg dm kg}^{-1}$) than in TFT ($2.8 \text{ kg dm kg}^{-1}$), while during the second year it decreased to $1.0 \text{ kg dm kg}^{-1}$ in RCT, and $0.7 \text{ kg dm kg}^{-1}$ in TFT.

3.5. Soil inorganic nitrogen

Soil $\text{NO}_3^- \text{N}$ concentrations ranged from 4 to 183 with the mean of $15 \mu\text{g g}^{-1}$ in RCT and from 4 to 120 with the mean of $13 \mu\text{g g}^{-1}$ in TFT during the first GS both peaking on June 4th (Fig. 5). In the second GS, these concentrations ranged from 4 to 49 with the mean of $9 \mu\text{g g}^{-1}$ in RCT and from 4 to 80 with the mean of $14 \mu\text{g g}^{-1}$ in TFT, peaking on June 23rd in RCT and June 17th in TFT. Soil $\text{NH}_4^+ \text{N}$ concentrations ranged from 0 to 146 with the mean of $9 \mu\text{g g}^{-1}$ in RCT and from 0 to 155 with the mean of $6 \mu\text{g g}^{-1}$ in TFT during the first GS, with a peak in both treatments on June 4th. During the second GS, the range was from 1 to 71 with the mean of $15 \mu\text{g g}^{-1}$ in RCT and from 0 to 118 with the mean of $23 \mu\text{g g}^{-1}$ in TFT. Peaks were measured for RCT in May 30th and June 23rd, and May 30th and June 17th for TFT, following the fertilization events. The temporal response to fertilizer application was different between growing seasons; first-year mineral N concentrations remained elevated for approximately one month after fertilization, while second-year concentrations returned to baseline within two weeks. No significant differences were observed in soil total mineral N, pH, or EC between treatments in the first GS (Table S1). In the second GS, there was a significant difference in all soil variables between the treatments, caused by differently timed management practices, including harvest and

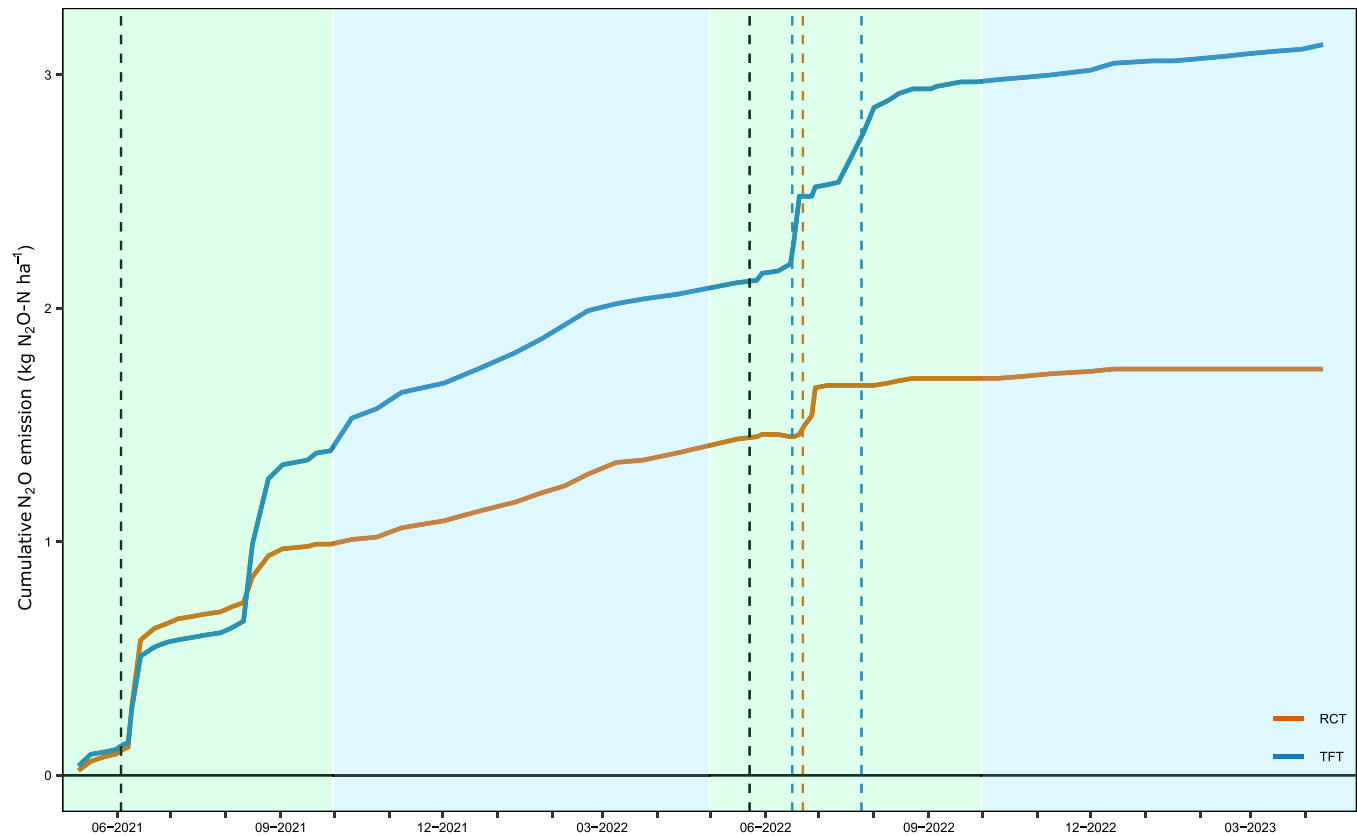


Fig. 6. Daily cumulative N₂O (N₂O–N kg ha^{−1}) emissions from the two experiment years. Orange line represents RCT and blue line TFT. Fertilization events are marked by different colored vertical lines: both treatments (black dashed), RCT (orange dashed), and TFT (blue dashed). Background colors indicate growing season (GS, green shade) and non-growing season (NGS, blue shade).

Table 4
The contributions of CH₄, N₂O in CO₂-eq per treatment, annual net ecosystem exchange (NEE), biomass C (*biomass carbon content 42%), greenhouse gas balance (GHGB) and feed CO₂-eq. Standard deviation calculated when possible.

Treatment	Year	CH ₄ -C	N ₂ O-N	Annual NEE	Biomass C *	GHGB	Feed CO ₂ -eq
		kg ha ^{−1}			t ha ^{−1} y ^{−1}	t CO ₂ -eq ha ^{−1} y ^{−1}	kg dm kg ^{−1}
RCT	May 2021 –April 2022	-0.08	1.3 ± 0.4	10.8	10.2	21.6	3.3
TFT	May 2021 –April 2022	-0.08	2.0 ± 1.2	6.8	9.0	16.7	2.8
RCT	May 2022 –April 2023	-0.04	0.4 ± 0.2	-5.0	15.2	10.3	1.0
TFT	May 2022 –April 2023	-0.05	1.1 ± 0.5	-9.0	15.5	6.9	0.7

fertilization events.

3.6. Relationship with environmental factors and fluxes

During the first GS, TER was positively correlated with soil temperature, WFPS, and VI (Table S2). First GS N₂O fluxes were positively correlated with WFPS, and soil pH. During the first NGS, CO₂ fluxes had a positive correlation with soil temperature and frost depth and N₂O fluxes had a negative correlation with soil volumetric water content. During the second GS, TER was again positively correlated with soil temperature, WFPS, and VI. N₂O fluxes had a positive relation with soil temperature and total mineral N. During the second NGS CO₂ fluxes had a positive relation with soil temperature, soil volumetric water content and negative with frost depth. Both soil volumetric water content and soil temperature had a negative relation with N₂O fluxes.

4. Discussion

This study examined inter- and intra-annual variation in GHG fluxes and vegetation dynamics under two forage crop treatments, one of which included a legume species and other a deep-rooted grass species. While the general effects of legumes on reducing N₂O emissions and maintaining yields have been reported in previous studies, the novelty of our study lies in its focus on mineral soils under boreal conditions, where high-frequency measurements of CO₂, N₂O, and CH₄ fluxes together with yield assessments are scarce.

The results revealed distinct differences in CO₂ exchange and N₂O dynamics between the treatments, particularly influenced by species composition. We found CO₂ to dominate the total GHG balances, with N₂O having a small role and CH₄ fluxes being negligible compared to the CO₂ and N₂O fluxes.

4.1. Plant species composition affects the carbon exchange and N₂O flux dynamics in boreal mineral soil

4.1.1. Carbon exchange

The CO₂ flux rates in this study are close to the other studies in boreal mineral soils; although we achieved higher TER values than previous studies (Lind et al., 2020). CO₂ flux rates were similar between the treatments and followed the same trend in the beginning of the study and started to differ after barley harvest in the end of the first GS. Overall, the CO₂ dynamics were strongly driven by the management schedule in both treatments, with stronger CO₂ uptake with more photosynthetically active biomass and decreased carbon sequestration after harvest events as reported in previous studies (Heimisch et al., 2021; Lind et al., 2020).

The presence of barley as a cover crop during most of the first GS likely masked treatment-specific differences, which only became apparent after cover crop harvest. RCT displayed better growth than TFT after the cover crop was harvested, resulting in stronger CO₂ uptake at the end of the first GS (Fig. 4). GPP levels were comparable between the treatments in the second GS, although RCT maintained a higher TER, resulting in smaller NEE (less carbon sequestration). Non-growing season CO₂ fluxes were low from both treatments during both years, although higher in RCT. Following the cover crop harvest in the first GS, RCT exhibited consistently higher CO₂ emissions than TFT across all measurement periods. Higher respiration rates in RCT can be partly explained with plant species behavior: the N fixation process of legumes has been calculated to consume 12 g of organic carbon per g of N₂ fixed (Heytler et al., 1984), leading to higher root respiration compared to grasses (Jensen et al., 2012). This likely contributed to the greater TER observed in RCT compared with TFT. Soil moisture also regulates microbial respiration, both very wet and very dry conditions suppress activity, while optimal respiration occurs within a soil volumetric water content of 0.2–0.5 m³ m⁻³ (Moyano et al., 2013). Moisture levels in the RCT during the first NGS and the second GS fell within this range, supporting the possibility that soil moisture contributed to the higher TER in RCT. Although our study did not quantify the relative importance of plant traits versus soil conditions, future work should focus on disentangling these factors, particularly in legume–grass mixtures.

There was a major difference in the annual NEE between the study years, as both treatments were sources of CO₂ in the first year and sinks in the second year. As the NGS CO₂ emissions were similar between the study years, the difference occurred from the sites acting as a source of CO₂ in the first GS and as a sink in the second GS. The most likely explanation for this is the presence of cover crops in the first year. However, weather conditions are also known to influence grassland carbon dynamics, even though in this study the weather was quite similar between the two years. In addition to the major difference in annual NEE, the carbon removal from the ecosystems via biomass affected the NECB considerably. In the second year, more carbon was removed as the yields were higher than in the first year, which made the NECB higher both in RCT (−10.2 t ha⁻¹ y⁻¹) and TFT (−6.5 t ha⁻¹ y⁻¹) compared to the first year. Similarly, the carbon removed via biomass in the first year made both treatments stronger sources to the atmosphere when looking at NECB (RCT −21, TFT −15.8 t ha⁻¹ y⁻¹). In addition, the dry period during the first growing season might have affected the yield level of barley, and therefore the NECB. NECB in the second year being higher is lining with other studies where annual crops have been reported to emit more GHG compared to perennial crops (Lind et al., 2020). This is mainly linked to longer period of vegetation cover, as perennial grasses can begin to utilize the nutrients and start carbon sequestration earlier in the season and continue later into autumn, whereas annual crops often lose valuable weeks at both ends of the growing season.

CH₄ fluxes were close to zero or slightly negative during the whole measurement period at both treatments. The small variation in CH₄ fluxes in boreal mineral soil has been recorded in multiple studies (e.g.

Jones et al., 2005), and thus it is many times seen as negligible in mineral soil.

4.1.2. N₂O flux dynamics

The annual N₂O emissions from this study (0.4–2.0 kg N₂O–N ha⁻¹ y⁻¹) are within the range of reported N₂O emissions from boreal mineral soils (−0.1–13.3 kg N₂O–N ha⁻¹ y⁻¹, Fuchs et al., 2018). Also, the strength of N₂O uptake (maximum uptake flux −1.1 mg m⁻² d⁻¹) reported in this study is similar to those measured on other studies on boreal mineral soil (Glatzel and Stahr, 2001; Sheehy et al., 2013; Syakila and Kroeze, 2011). The maximum uptake of N₂O occurred before the first harvest in second GS, when the plants were the most active based on photosynthetic activity (Fig. 4). Total cumulative N₂O emissions were 54% lower in RCT (1.7 kg N₂O–N ha⁻¹) than in TFT (3.1 kg N₂O–N ha⁻¹), aligning with previous findings that legume crops tend to emit less N₂O than other crop types (Jeuffroy et al., 2013). NGS N₂O emissions contributed 25% and 30% in the first year and 13% and 12% in the second year to the annual emissions of RCT and TFT, respectively. In previous studies in similar conditions, it has been observed that wintertime emissions of N₂O could cover large part of yearly budget (Lind et al., 2019; Maljanen et al., 2009). The vegetation status in the second NGS (end of the GS VI 0.6) likely reduced the emissions compared to first NGS (end of the GS VI 0.3), since well-established vegetation affects the dynamics of mineral N and reduces wintertime emissions (Lind et al., 2019; Maljanen et al., 2009).

In the beginning of the study, N₂O flux rates were similar between the treatments and followed the same trend, indicating that the experimental site was in balance between the treatments. However, after barley harvest at the end of the first GS, emissions from TFT started to increase more than in RCT (Figs. 5, 6). This trend mostly continued throughout the study, until the end of second GS, and could be explained by the differences in grass species composition. Red clover has been seen to reduce N₂O emissions from grasslands through its ability to fix nitrogen and reduced need for synthetic fertilizers (Carlsson and Huss-Danell, 2003), which could explain the lower fluxes from RCT compared to TFT from cover crop removal until the end of the study.

We observed peaks in N₂O emissions mainly after the application of fertilizers, which contributed largely to the annual balances, as has been reported in several studies; fertilization increases the peak by rapidly enlarging the pool of mineral N (NH₄⁺ and NO₃⁻) in the soil, which accelerates both nitrification and denitrification, leading to increased N₂O production (Bouwman et al., 2002). In the first year, the treatments received the same amount of fertilizer and showed a similar peak in emissions 11 days after (Fig. 5). On the contrary, in the second year, the fertilization induced peaks in TFT were stronger compared to RCT, which is most likely because TFT received 2.4 times higher amount of fertilizer N than RCT (Table 1). This difference in peak magnitude led to significant differences in cumulative N₂O emissions between treatments, as background emission levels were low for the majority of the year. We had one fertilization event during the study, the first fertilization in the second year, that did not cause a peak in N₂O emissions in either of the treatments, although the soil mineral N concentrations were elevated. This could be explained by the efficient uptake of applied N by actively growing overwintered vegetation after a non-growing season with limited N availability in the soil (Shurpali et al., 2025).

The first fertilization during the second growing season did not lead to a peak in N₂O emissions in either treatment. Instead, emissions followed a declining trend. This pattern may be attributed to limited accessibility of mineral N—particularly from the native mineral N pool by N₂O-producing microbes. This limitation was likely caused by plant growth with progressing summer (Shurpali et al., 2025), as well as a delay in the incorporation of fertilizer N into the soil which is also supported by elevated soil mineral N concentrations after the fertilization (Fig. 5).

In addition to the fertilization-induced peaks, we observed a pronounced increase in N₂O emissions following a precipitation event in

August during the first GS. The associated rise in soil volumetric water content likely enhanced microbial activity and induced physical changes in soil properties, such as diffusivity and infiltration. Similar responses have been reported in previous studies, although the underlying mechanisms remain poorly understood (Keane et al., 2019; Kim et al., 2012; Rudaz et al., 1991; Ruser et al., 2001; Schmeer et al., 2014). The peak was visible in both treatments, although higher in TFT ($10.5 \pm 13.9 \text{ mg N}_2\text{O m}^{-2} \text{ d}^{-1}$). As there were no differences in soil mineral N between the treatments, one explanation for the different magnitude of the peaks could be the soil volumetric water content conditions prior to the precipitation event: TFT had drier soil conditions compared to RCT (WFPS $6 \pm 0.4\%$), possibly leading to stronger changes in soil physical conditions.

4.2. GHGB and emissions per feed

The total GHG balances in our study were dominated by the NECB components (CO_2 and biomass C), accounting 77–92% of the total GHG emissions during both measurement years in both treatments, which is a common finding in mineral grasslands (Heimsch et al., 2021; Lind et al., 2020). There was a major difference in the total GHG balance between the study years, which was largely explained by the high CO_2 emissions from the first year with cover crop and the site acting as a carbon sink in the second year with pure grass sward. The treatment effect remained minimal during the first year, most likely as the cover crop (barley) was dominating the vegetation composition. Although N_2O had smaller role than CO_2 in total GHG balance in this study, it is vital to minimize N_2O emissions due to its high global warming potential and long atmospheric lifetime (Shukla et al., 2022).

While looking at the GHGB calculated per area (Table 4), both treatments seem to be strong or moderate sources during both years. However, when calculating the emissions per produced feed (feed $\text{CO}_2\text{-eq}$) rather than per area, both treatments were a small source in the first year and close to net zero in the second year (Table 4). This indicates that grass cultivation during the establishment year on boreal mineral soil can be considered climate-smart, particularly in terms of emissions efficiency. In agricultural studies, it is also important to investigate the production efficiency in addition to GHG emissions to get better overall picture of the climatic footprint of an agricultural site.

4.3. Sustainable forage production through legume-grass mixtures

In our study, the inclusion of red clover in the forage mixture resulted in yield levels ($9900 \text{ kg dw ha}^{-1}$) comparable to those of the pure grass mixture ($10070 \text{ kg dw ha}^{-1}$), despite lower N fertilization (Table 2). This finding is particularly relevant when developing climate-smart agricultural practices, as strategies aiming to reduce GHG emissions must also simultaneously maintain high agronomic productivity. The potential to reduce fertilizer input without compromising the yield has been shown in legume-based systems previously also (Finn et al., 2013; Nyfeler et al., 2009; Snyder et al., 2009). We could not maintain the optimal legume percentage that has been suggested to be between 30% and 60% (Lüscher et al., 2014; Nyfeler et al., 2011), as in our study it ranged from 0.1% to 40% in the first harvest and from 1.0% to 66% in the second harvest (first year is not considered here because of cover crop). Therefore, we were most likely not able to determine the full potential of red clover, although our findings do indicate several benefits (e.g. reduced need of N fertilizer) associated with its use. In addition, by decreasing the fertilizer amounts via legume addition in the grass mixture, also the indirect emissions decrease, as fertilizer production has been calculated to be $4.0 \text{ kg CO}_2\text{-eq per kg N}$ and fertilizer application $4.2 \text{ CO}_2\text{-eq per kg N}$ (Kajava and Sairanen, 2024).

4.4. Study limitations

We acknowledge that with weekly or even biweekly manual

chamber measurements, we only have access to limited spatial and temporal data. Thus, we might not have been able to catch all the temporal variability in GHG fluxes. Studies also suggest there might be emission peaks at the end of spring (e.g. Maljanen et al., 2007), that could be vital part of the annual balance. Due to harsh spring conditions, conducting GHG flux measurements using chambers can be challenging. This may result in the exclusion of emission hotspots, such as freeze–thaw events, which are known to produce elevated GHG fluxes, particularly N_2O (Maljanen et al., 2007).

We also acknowledge that the study period of two years is relatively short compared to the typical rotation cycle (3–4 years) of boreal grasslands. Consequently, dynamic changes in soil carbon pools, vegetation community stability, and GHG balances during the middle and late stages of the rotation cycle are not captured. This limitation should be considered when interpreting the long-term ecological and productive effects of the two grass mixture treatments.

In addition, the use of gap-filling methods to compensate for missing data points may also result in over- or underestimation of the fluxes and is vital study point for further research (Barton et al., 2015). In boreal region the non-growing seasons and winters are long, so it is vital to consider the whole year while calculating and reporting the annual balances.

5. Conclusions

Legume-based forage mixtures such as red clover–timothy present a viable strategy to reduce N_2O emissions in boreal grasslands, largely due to their capacity to replace synthetic fertilizer inputs with biologically fixed nitrogen. Despite lower mineral fertilizer rates, total forage yields remained similar with non-legume mixtures, indicating that agronomic performance was not compromised. However, higher respiration and gross primary productivity from the legume stands suggest trade-offs in the net C balance, possibly linked to greater respiration demands for N fixation. Over the long term, careful management of fertilizer timing, harvest regimes, and species composition can help optimize both greenhouse gas mitigation and productivity. While these initial results highlight the potential of legume-based systems for reducing emissions and sustaining yields, full grass rotation cycle studies (3–4 years) with high-frequency measurements are needed to fully capture the seasonal and annual flux variability and to elucidate potential gains in soil carbon sequestration.

CCRediT authorship contribution statement

Sanni Semberg: Writing – review & editing, Investigation. **Petra Manninen:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Narasinha Shurpali:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Perttu Virkajärvi:** Supervision, Funding acquisition, Conceptualization. **Annalea Lohila:** Writing – review & editing, Supervision. **Hem Raj Bhattarai:** Writing – review & editing, Supervision, Conceptualization. **Yuan Li:** Writing – review & editing. **Henriikka Vekuri:** Writing – review & editing, Methodology. **Olli Peltola:** Writing – review & editing, Methodology. **Timo Vesala:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Perttu Virkajärvi reports financial support was provided by Finnish Ministry of Agriculture and Forestry. Narasinha Shurpali reports financial support was provided by Research Council of Finland. If there are other authors, they 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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2026.110481](https://doi.org/10.1016/j.agee.2026.110481).

Data availability

Data will be made available on request.

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