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Management practices during the renewal year affect the carbon balance of a boreal legume grassland

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Evaluating the net ecosystem carbon balance (NECB) of legume-based grasslands is crucial for optimizing grassland management and assessing the sustainability of the milk and beef industries. This study investigated the NECB of a boreal legume grassland in eastern Finland from May 2017 to May 2020, covering the entire three-year rotation cycle. We found that the grassland showed interannual variability in carbon sequestration, fixing 220g C m^{-2} in the first year, 334g C m^{-2} in the second year, and losing 146g C m^{-2} in the last year during the grassland renewal period. The study also examined the effects of mineral nitrogen fertilizer and digestate residue addition on the NECB of the grassland. No significant differences in net ecosystem carbon dioxide exchange were observed between the two treatments, but the application of digestate slurry increased the NECB, suggesting that organic fertilizers could potentially enhance carbon sequestration and sustain ecosystem services. In conclusion, our findings emphasize the importance of developing climate-friendly renovation management practices that maximize the photosynthetic period in boreal legume grasslands. These practices, combined with the use of organic fertilizers, can contribute to improved carbon sequestration and support the sustainability of milk and beef industries that rely on grasslands.

KEYWORDS

agricultural sustainability, boreal environment, climate change, GHG exchange, *Trifolium pratense*

1. Introduction

Grasslands are an essential component of European agriculture covering approximately 31% of the agricultural area (Olesen and Bindi, 2002; Eurostat, 2021). Grassland-based livestock production is the backbone of the Nordic socio-economy (Åby et al., 2014). It is an important source of livelihood and it maintains the population and vitality of rural areas. Production of milk and beef are interconnected in Finland, up to 80% of beef production is coupled with milk production (Åby et al., 2014). The milk and beef industry is currently being challenged for environmental reasons in light of the reduction in the carbon (C) footprint (Olesen and Bindi, 2002; Klumpp and Fornara, 2018). At the farm level, soil C storage enhancing measures that could be used cost-effectively, and without disrupting production, are lacking.

Incorporating legumes into grasslands through practices such as short rotation, intercropping, or mixed planting can potentially improve soil nutrient conditions (Suter et al., 2015), biomass yield (Finn et al., 2013), and ecosystem energy efficiency (Deng et al., 2021). Legumes can significantly affect the nitrogen (N) status through biological dinitrogen fixation (Gylfadóttir et al., 2007; Lüscher et al., 2014). N can be released from decaying biomass above and below ground, and the nodules and root exudates of legumes (Laidlaw et al., 1996; Suter et al., 2015). For example, using ^{15}N individual plant leaf labeling in a legume grassland in Iceland, a field study found that white clover (*Trifolium repens*) cultivated with smooth grass (*Poa pratensis*) provided about 2.5 g N m^{-2}, 50% of the total crop N requirement (Gylfadóttir et al., 2007). A coordinated continental-scale field experiment across 31 European sites reported that the yield of legume grasslands exceeded that of the average grass monoculture with low N fertilization by more than 97% over 3 years (Finn et al., 2013). In addition, a Finnish farm survey found that red clover (*Trifolium Pratense* L.) based grasslands yielded 7.5 ± 1.7 t dry matter (DM) ha^{-1} without N fertilization (Riesinger and Herzon, 2008).

Managed European grasslands are often fertilized with mineral and organic N fertilizers to further optimize the production and profitability of grasslands (Olesen and Bindi, 2002). The application of N fertilizers has been shown to affect soil C storage in grassland ecosystems (Conant et al., 2017). For example, a synthesis analysis comprising 50 studies from different parts of the world reported that fertilization with mineral or organic N fertilizers increased grassland soil C stock at an average rate of 0.57 t C ha^{-1} yr^{-1} (Conant et al., 2017). A study in a Scottish grassland showed that, compared to mineral fertilizers, organic treatment enhanced soil C storage after 6 years of the manure addition, despite increased rates of soil respiration (Jones et al., 2006). However, little is known about the potential impact of legumes on grassland C balance with the addition of mineral or organic fertilizer in the northern regions, where the long winter has a significant effect on C and N turnover in the plant–soil system.

The eddy covariance (EC) method allows continuous measurements of ecosystem C flows for periods of months to years (Baldocchi, 2020). EC-based measurements of CO_2 exchange from grasslands have the potential of providing valuable insights into the impact of management on the net ecosystem C balance (NECB, the net rate of C accumulation in or loss from ecosystems) and exploring opportunities for greenhouse gas mitigation (Chapin et al., 2006; Lind et al., 2016). Thus, as an alternative to monitoring soil organic C (SOC) content over time, changes in NECB can be determined from measured C imports and exports using the EC technique together with measured agronomic parameters, such as harvest and synthetic fertilizer or manure application (Lind et al., 2016; Rutledge et al., 2017; Ammann et al., 2020). Multi-year studies evaluating grassland NECB covering the entire rotation are crucial for understanding the C balance of rotational grasslands (Ammann et al., 2020). Such full-cycle experiments are especially important in the boreal region, characterized by cold climates and short growing seasons, where grasslands are renewed every three to 4 years (Virkajärvi et al., 2015) and wintertime has a significant effect on crop production and nutrient cycling (Maljanen et al., 2009). Currently, however, studies evaluating grassland NECB covering the entire rotation using EC techniques are lacking in the boreal region.

Here, we quantified the NECB of a legume grassland with the EC technique over a three-year rotation cycle in eastern Finland (Figure 1). Our objective in this study was to understand the impact of crop management practices (fertilization, harvesting, and grassland re-establishment) and interannual variability on ecosystem C flows and implications for ecosystem services (e.g., milk and beef production). In this study, we hypothesized that a legume grassland on mineral soil in a boreal environment is a sink for atmospheric C over a three-year rotation cycle and that the effect of fertilizer type on the NECB of legume grasslands varies over the rotation cycle.

2. Materials and methods

2.1. Site description and management

The study site is located in eastern Finland (63°09′ N, 27°140′ E, 89 m a.s.l.; Figure 1). The 30-year (1981–2010) mean annual temperature (MAT) and precipitation (MAP) in the region are 3.2°C and 612 mm, respectively. The soil at the study site is classified as a Haplic Cambisol/Regosol (Hypereutric, Siltic) (IUSS Working Group WRB, 2007) (silt loam; clay 25% ± 6%, silt 53% ± 9% and sand 22% ± 8%) based on the U.S. Department of Agriculture (USDA) textural classification system.

The study site, a 6.3-hectare agricultural field (280 m × 220 m) cultivated with timothy (*Phleum pratense* L. cv. Nuutti; seed rate 15 kg ha^{-1}) and red clover (cv. Ilte; 5 kg ha^{-1}), was established in 2015, reseeded in May 2017, and renewed in spring 2019 (Supplementary Table S1). In the autumn of 2018, glyphosate was applied to the field using a tractor-mounted sprayer to make the site devoid of any vegetation. Subsequently, the site was plowed using a tractor-mounted plow and left bare for the ensuing winter. In early June 2019, the site was renewed (Supplementary Table S1) with the seeding of a red clover and timothy seed mixture, along with barley (*Hordeum vulgare* L.) as a cover crop, using a tractor-mounted seed drill. Considering the frequency of prevailing wind directions (Supplementary Figure S1), the experimental site was divided into two plots that were treated with either mineral nitrogen (N_{min}) or digestate residue (N_{org}) over a rotation cycle: May 2017–May 2018, June 2018–May 2019, and June 2019–May 2020, hereafter referred to as R_1, R_2, and R_3, respectively. During both grass production years (R_1, R_2), the N_{min} plot was fertilized using a tractor-mounted fertilizer spreader at the start of each growing season (May) and after the 1st cut (mid to late June) with an average annual fertilization rate of 106 kg soluble N, 28 kg P, and 50 kg K ha^{-1}. In contrast, the N_{org} plot was fertilized once after the 1st cut with an average annual fertilization rate of 98 kg N total (of which 53 kg N was soluble), 13 kg P, and 83 kg K ha^{-1}, using a tractor-mounted slurry spreader. In the renovation year (R_3), the whole field received N_{min} plot fertilization with an annual rate of 45 kg N, 20 kg phosphorous, and 38 kg K ha^{-1} using a tractor-mounted fertilizer spreader, while the N_{org} plot did not receive any fertilizers (Supplementary Table S1). The grass was cut using a tractor-mounted mower, followed by a tractor-mounted rake and baler for forage harvesting.

Each treatment was further divided into two sub-plots to assess the grass growth patterns in different parts of the field. The physical and chemical properties of the topsoil (0–15 cm) are given in Supplementary Table S2. Each treatment was harvested typically two times per year, and once during the establishment year (2019) (Supplementary Tables S1, S2). The experimental field was harvested for pre-wilted silage using farm-scale machinery. The sward was cut to 8 cm with a conventional disk mower with conditioner, swathed and baled with farm machinery and the bales were individually weighed for each of the four subplots. Similarly, representative samples were taken from the swaths and oven-dried at 60°C for 40 h to determine DM and the chemical composition of the herbage. Soil and plant C content was analyzed using a Leco TruMac® CN analyzer.

2.2. Eddy covariance and environment measurements

2.2.1. Instrumentation

The EC tower was erected at the center of the study area on the boundary between N_{min} and N_{org} treatments (Figure 1). Measurements of CO_2 and H_2O fluxes were performed using a closed-path EC system with an adjacent weather station for supporting soil climate and meteorological data. The EC system consisted of a Li-7,000 infrared gas analyzer (IRGA, for CO_2 and H_2O mixing ratios, Li-COR Inc., Lincoln, NE, United States), and a sonic anemometer (for wind velocity components, sensible heat flux, and sonic temperature, R3-50, Gill Instruments Ltd., UK) mounted on an instrument tower at a height of 2.5 m above the soil surface. With a flow rate of 10 L min^{-1}, the air samples passed through a heated intake tube (inner diameter 6 mm, length 8 m, PTFE) with two filters (pore size 1.0 μm, PTFE, Gelman®). The IRGA was housed in a climate-controlled cabin and it was calibrated approximately every month during the growing season with a two-point calibration (0 and 399 μL L^{-1} of CO_2, AGA Oy, Finland) and additionally with a dew point generator (Li–610, LI–COR Inc.) for H_2O mixing ratio during conditions when the air temperature (T_a) was above 5°C.

Supporting climatic variables, i.e., net radiation (R_n, CNR1, Kipp & Zonen B.V.), T_a and relative humidity (RH, HMP45C, Vaisala Inc), photosynthetically active radiation (PAR, SKP215, Skye Instruments Ltd.), soil temperature (T_s, 107, Campbell Scientific Inc.), volumetric water content (θ_v, CS616, Campbell Scientific Inc.) at 5 and 20 cm depths, and air pressure (CS106 Vaisala PTB110 Barometer) were measured.

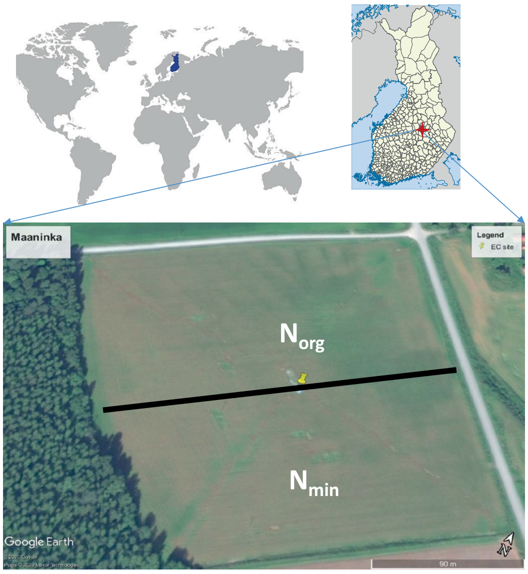

FIGURE 1
Site location at Maaninka (Kuopio, Finland) and experimental set-up.

Eddy covariance raw data were collected at 10 Hz using a data logger (CR3000, Campbell Scientific Inc.). All supporting meteorological and soil climate data were collected as 30 min mean values. Missing T_a, relative humidity, or precipitation data were filled using data from the Maaninka weather station operated by the Finnish Meteorological Institute (FMI), located about 6 km to the southeast of the site.

2.2.2. Processing of flux data

The 30 min EC flux values were calculated from the covariance of scalars and vertical wind velocity. Data processing was performed using EddyUH (Mammarella et al., 2016). Despiking limits were defined for CO_2 at 15 μmol mol^{-1}; 20 mmol mol^{-1} for H_2O; wind components ($u = 10$ m s $^{-1}$, $v = 10$ m s^{-1}, and $w = 5$ m s^{-1}) and temperature (5°C). Detected spikes were replaced by adjacent values or the average of previous values. Point-by-point dilution correction was applied after the despiking. The two-dimensional coordinate rotation was done on the sonic anemometer wind components. The angle of attack correction was not applied. Detrending was done using block averaging. Lag time due to the gas sampling line was calculated by maximizing the covariance. Low-frequency spectral corrections were implemented according to Rannik and Vesala (1999). For high-frequency spectral corrections, empirical transfer function calculations were done based on the procedure introduced by Aubinet et al. (1999). Humidity effects on sonic heat fluxes were corrected according to Schotanus et al. (1983). Additionally, flux values measured when winds were from behind the instrument cabin (85–130°), during rain, and during regular maintenance (e.g., calibration) were discarded.

Night-time NEE and u^* had no significant correlation, hence a default u^* filter of 0.1 m s^{-1} was used. Flux was considered non-stationary following Foken and Wichura (1996). The available flux data were further quality controlled. Both skewness and kurtosis of the data were checked, and the acceptable skewness range was set from −3 to 3 and −2 to 2, and kurtosis from 1 to 14 for CO_2/H_2O. Overall flags higher than 7 were removed (Foken et al., 2004). Finally, the data were visually inspected. From the available data, approximately 53% of the CO_2 and H_2O flux data were retained.

The gap-filling and flux partitioning of NEE were performed using the REddyProc Web online tool.[1] This tool considers both the co-variation of the fluxes with radiation, temperature, and vapor pressure deficit (VPD) and the temporal autocorrelation of the fluxes (Reichstein et al., 2005). The measured and quality-controlled flux data were used as inputs to the Flux partitioning tool. Total ecosystem respiration (R_E) was defined as the night-time measured net ecosystem CO_2 exchange (NEE). The regression between night-time NEE and T_a was calculated using an exponential regression model (Lloyd and Taylor, 1994). Using the model-estimated parameters, the missing half-hour R_E during night and daytime was estimated as a function of the continuous, measured dataset of T_a. Finally, gross photosynthesis

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[1] https://www.bgc-jena.mpg.de/bgi/index.php/Services/REddyProcWeb

(GPP) was calculated as a difference between NEE and R_E. In this paper, CO_2 released into the atmosphere is defined as a positive value and uptake from the atmosphere as negative.

2.3. Net ecosystem carbon balance

Annual and total net ecosystem C balances (NECB) were calculated for each treatment by adding all imports and exports of C to the calculated net ecosystem CO_2 exchange (NEE) (Chapin et al., 2006; Lind et al., 2016).

$$\text{NECB}_1 = \text{NEE} + C_{\text{harvest}} \text{ For the mineral } N \text{ treatment} \quad (1)$$

$$\text{NECB}_2 = \text{NEE} + C_{\text{harvest}} - C_{\text{Norg}} \cdot \text{ For the organic } N \text{ treatment} \quad (2)$$

where C_{Norg} is the C added as organic fertilizer (digestate residue); C_{harvest} is the dry biomass C in aboveground biomass removed by harvesting; Emissions of soil methane–C are ignored in this study as they are likely to be very small (Maljanen et al., 2009; Lind et al., 2016). C_{harvest} was calculated as the total dry matter yield multiplied by the C content, NECB_1 is the total net ecosystem C balances accounting for harvested biomass and NECB_2 is the total C balances accounting also for C addition to the ecosystem in the form of the applied digested residue. Similar to NEE, a negative NECB indicates a (net) C retained in the ecosystem, and a positive sign indicates a (net) C emission or release to the atmosphere.

2.4. Controlling variable analyses

The relationship between daytime ($\text{PAR} > 20\,\mu\text{mol m}^{-2}\ \text{s}^{-1}$) NEE and PAR was examined during periods when the grass growth was at its peak (a week before each grass cutting event during the growing season each year). Prior to the analysis, PAR data were binned at an interval of $10\,\mu\text{mol m}^{-2}\ \text{s}^{-1}$. The values of NEE were plotted against PAR with a rectangular hyperbolic model.

$$\text{NEE} = \frac{P_{\max} \times \text{PAR} \times \pm}{P_{\max} + \text{PAR} \times \pm} + R_d \quad (3)$$

where $P_{\max}$ ($\mu\text{mol m}^{-2}\ \text{s}^{-1}$) is the theoretical maximum rate of photosynthesis at infinite PAR, ± is the apparent quantum yield, and R_d is the rate of dark respiration ($\mu\text{mol m}^{-2}\ \text{s}^{-1}$).

2.5. Statistical analyses

In this study, daily, monthly, seasonal, and annual NEE were calculated using gap-filled data in the R programming environment. Data quality control (see Section 2.2.2) was also conducted using R. The effects of PAR on NEE (Equation 3) were evaluated using the "nlme" package of R (Pinheiro et al., 2014). Multilevel correlations between climatic parameters and CO_2 fluxes were tested using the "correlation" package (Lüdecke et al., 2019). All figures were plotted using the "ggplot2" package (Wickham, 2016) in R.

3. Results

3.1. Climatic conditions during the study period

MAT during R_1, R_2, and R_3 was higher than the 30-year mean (3.2°C), with differences of 0.9°C, 1.6°C, and 1.8°C, respectively (Figure 2). The growing season duration varied across the rotations, with 136 days in R_1, 155 days in R_2, and 142 days in R_3. During R_1, the mean T_a from May to July was lower than the 30-year averages, while August and September values were similar (Figure 3). In contrast, R_2 exhibited a consistently higher mean T_a throughout the growing season compared to the 30-year average. The mean T_a during R_3's growing season was mostly in line with the 30-year averages, except for a higher value in June. The mean topsoil T_s during the growing seasons was 12.6°C for R_1, 14.1°C for R_2, and 12.9°C for R_3 (Figure 3). Corresponding subsoil temperatures were 12.1°C, 13.4°C, and 12.5°C, respectively.

MAP was lower than the 30-year mean (612 mm) during R_2 (542 mm) and R_3 (509 mm), while R_1 (624 mm) was wetter than normal (Figure 2). Precipitation during the growing season of R_3 (173 mm) was lower than that in R_1 (284 mm) and R_2 (252 mm), and precipitation values recorded during the growing seasons over the rotation cycle were all lower than that of the 30-year mean (318 mm, Figure 2). More precipitation was received outside the growing seasons over R_1, R_2, and R_3. Mean topsoil θ_v fluctuated with rain events during the growing season and was 34%, during R_1 and 27%, during R_2 and R_3, respectively, with the corresponding mean subsoil θ_v values of 29, 25, and 26% (Figure 3).

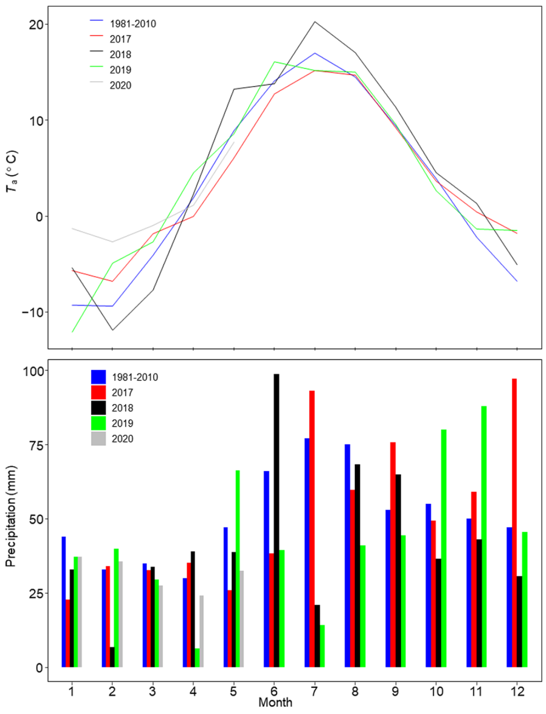

FIGURE 2
Monthly mean air temperature (T_a, °C), and precipitation sum (mm) during 2017–2020 compared with the 30-year average (1981–2010).

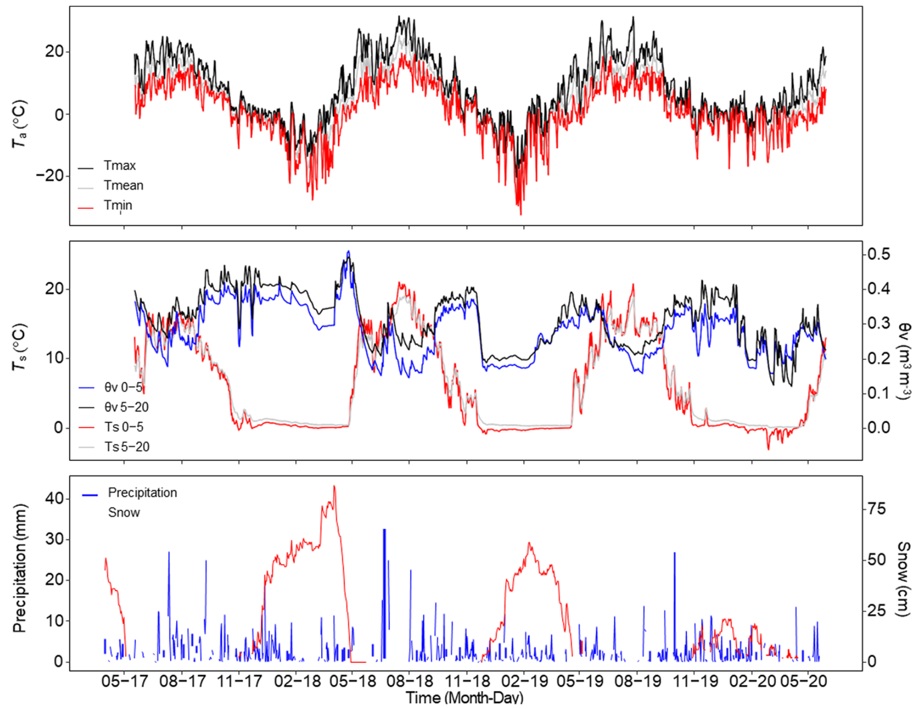

FIGURE 3
Climatic conditions at the study site from May 2017 to May 2020. Daily mean, maximum, and minimum air temperature (T_a, °C); daily averaged soil temperature (T_s, °C) and volumetric water content (θ_v, m^3 m^{-3}) at the top– (0–5cm) and subsoil (5–20cm); daily precipitation sum (mm) and snow depth (cm).

3.2. Biomass yields

The variability of biomass yield among years, treatments, and cuts is noteworthy (Table 1). The maximum annual biomass yield was reported in R_2. The yield from the first cut in R_1 was 25% lower than the second one under the N_{min} treatment, while the yields from the two cuts were about the same under N_{org}. During R_2, however, the yield from the first cut was 2.2 times higher than the second one under N_{min}, while under N_{org}, it was 2.5 times higher.

3.3. Daily net ecosystem CO$_2$ exchange

Daily NEE displayed distinct patterns reflecting the grass phenological development, harvesting impacts, and grassland renewal during the measurement period (Figure 4). In R_1 and R_2, two negative NEE peaks were observed before each grass cut in late June and early August. In contrast, R_3 had only one peak as the grass was cut once during the grassland renewal year (Figure 4).

In R_1, an uptake peak rate of 39 g CO$_2$ m^{-2} d^{-1} occurred in mid-June, followed by a post-cutting CO$_2$ source phase. A second CO$_2$ uptake peak of 22 g CO$_2$ m^{-2} d^{-1} was observed in late July. In R_2, the initial CO$_2$ uptake began 14 days earlier than in R_1, peaking at 48 g CO$_2$ m^{-2} d^{-1} by late May. After the first cut and second fertilizer application, a CO$_2$ source peak rate of 19 g CO$_2$ m^{-2} d^{-1} was observed, followed by a 38 g CO$_2$ m^{-2} d^{-1} uptake peak in late July. The third cut in R_2 was not performed due to low biomass accumulation rates.

TABLE 1 Harvest events and yield as dry matter (kg DM ha^{-1}) and the proportion of clover in grassland added with mineral nitrogen (N_{min}) or digestate residue (N_{org}) over the three-year rotation cycle (May 2017–May 2020).

Rotation cycle	Harvest date	Yield (kg DM ha^{-1})		Proportion of clover in DM (%)	
		N_{min}	N_{org}	N_{min}	N_{org}
R_1	June 29	2,500 ± 90	2,390 ± 296	50 ± 2	43 ± 5
	August 16	3,360 ± 33	2,490 ± 309	64 ± 5	65 ± 7
R_2	June 26	4,346 ± 1,131	4,860 ± 56	37 ± 1	44 ± 4
	August 7	1970 ± 71	1950 ± 6	57 ± 2	78 ± 0
R_3	August 6	3,410 ± 186	3,440 ± 149	na^a	na^a

R_1, R_2, and R_3 indicate three rotation cycles during May 2017–May 2018, June 2018–May 2019, and June 2019–May 2020, respectively. Data shown are mean ± standard error, $n = 3$.
^{a}The yield consisted mainly of whole-crop barley.

In R_3, following grassland re-establishment, the ecosystem reached a peak net uptake of 43 g CO$_2$ m^{-2} d^{-1} in mid-July. From the barley harvest in early August 2019 to the study's end in May 2020, the ecosystem remained a sustained CO$_2$ source (Figure 4).

3.4. Factors controlling CO$_2$ fluxes

A week before the first and second cuts under the N_{min} treatment in 2017 and 2018, and a week before the first cut under the N_{org} treatment in 2019 (Figure 5), high values of estimated P_{max} (potential photosynthetic

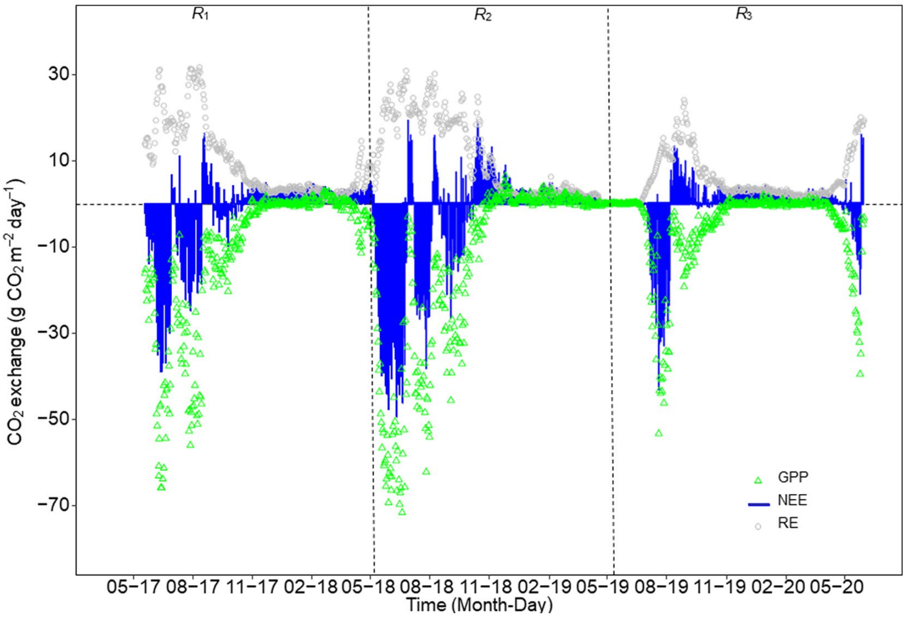

FIGURE 4
Daily CO_2 fluxes (g CO_2 m^{-2} day^{-1}) from grassland from May 2017 to May 2020; Gap-filled CO_2 fluxes were used. Net ecosystem CO_2 exchange (NEE), ecosystem respiration (R_E), and gross primary production (GPP). CO_2 released into the atmosphere is defined as a positive value.

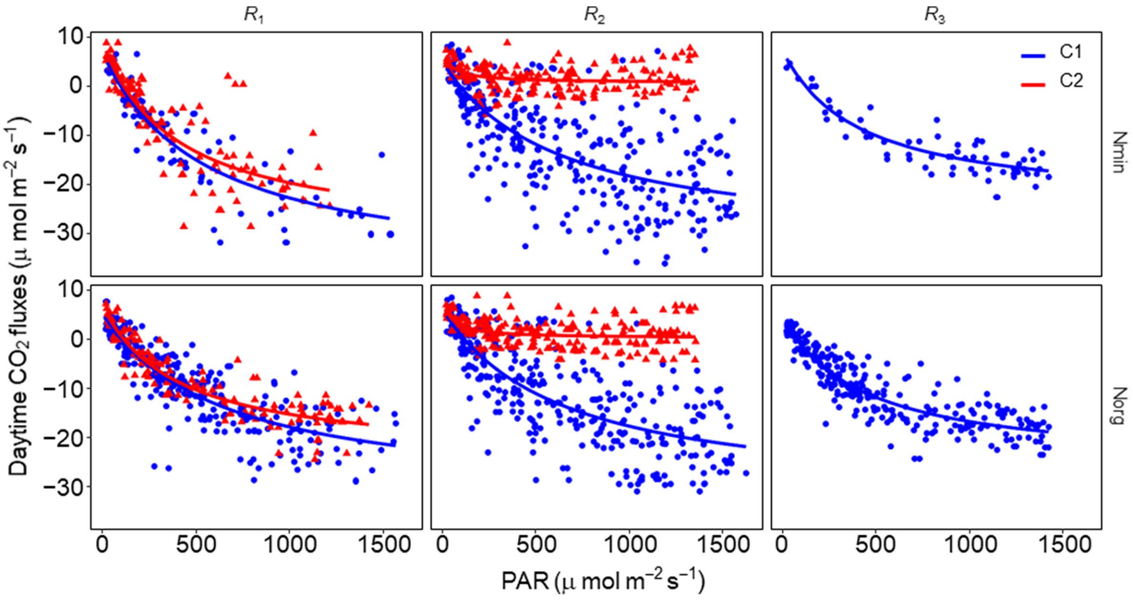

FIGURE 5
Relationship of day−time net ecosystem CO_2 exchange (NEE) with incident photosynthetically active radiation (PAR). Data of NEE were fitted with a nonlinear equation (the estimated parameters are presented in Table 3) with PAR (Section 2.3). R_1, R_2, and R_3 indicate three rotation cycles during May 2017–May 2018, June 2018–May 2019, and June 2019–May 2020, respectively. N_{min} and N_{org} indicate grassland added with mineral nitrogen or digestate residue, respectively. C1 and C2 indicate the first and second grass cuts, respectively. A week's worth of measured 30 min data pairs (NEE and PAR) available before each grass cut were used for the nonlinear regression analysis presented in this figure. Note that there was a single grass cut (C1) made in R3.

capacity) and α (light use efficiency) implied that the climatic conditions and increased leaf area during this time were favorable for high CO_2 uptake ($p < 0.05$, Table 3). The moderate, insignificant differences among light response curves suggested no major differences in NEE under the N_{min} and N_{org} treatments (Table 3, Figure 5).

3.5. Annual C balances

Cumulative R_E and GPP at the study site over the entire three-year study were 2,515 g and 3,061 g C m^{-2}, respectively (Table 2). Thus, based on NEE alone, the ecosystem sequestered 547 g C m^{-2} during

TABLE 2 The annual net ecosystem CO_2 exchange (NEE), ecosystem respiration (R_E), gross primary production (GPP), fertilizer C (C_{Norg}), and dry matter C ($C_{harvest}$) in g C m^{-2}.

	NEE	R_E	GPP	$C_{harvest}$	NECB$_1$	C_{Norg}	NECB$_2$
R_1	−220	965	1,185	257	37	−43	−6
R_2	−334	1,019	1,352	289	−45	−49	−95
R_3	7	531	524	139	146	0	146
Sum	−547	2,515	3,061	685	138	−92	45

Net ecosystem carbon balances (NECB) are presented here with the units of g C m^{-2}. R_1, R_2, and R_3 indicate three rotation cycles during May 2017–May 2018, June 2018–May 2019, and June 2019–May 2020, respectively. A negative NECB indicates C is retained in the ecosystem and while a positive value implies C is lost to the atmosphere. NECB$_1$ indicates the NECB accounting for *C* lost as harvested biomass under mineral *N* application, and the NECB$_2$ is the total net ecosystem *C* balances accounting for harvested biomass and *C* contained in the digested residue.

TABLE 3 Relationship of day–time net ecosystem CO_2 exchange (NEE) with incident photosynthetically active radiation (PAR).

Year	Harvest	N_{min}				N_{org}			
	Time	P_{max}	α	R_d	R^2	P_{max}	α	R_d	R^2
R_1	C1	−46.10	−0.08	6.93	0.53	−37.90	−0.06	5.15	0.35
	C2	−40.30	−0.10	9.07	0.47	−31.20	−0.08	6.71	0.46
R_2	C1	−37.70	−0.06	4.75	0.62	−38.70	−0.06	5.87	0.59
	C2	−7.33	−0.16	6.76	0.77	−8.60	−0.20	8.55	0.68
R_3	C1	−31.50	−0.08	6.96	0.89	−30.70	−0.07	4.52	0.74

Data of NEE were fitted with a nonlinear equation (Equation 3). The NEE is the measured value of the half-hourly net ecosystem CO_2 exchange, PAR is the corresponding half-hourly value of photosynthetically active radiation, P_{max} is the potential net photosynthetic capacity of the vegetation (μmol CO_2 m^{-2} s^{-1}), R_d is the rate of dark respiration (μmol CO_2 m^{-2} s^{-1}), and α is the slope of the initial, linear increase in NEE with increasing PAR. R_1, R_2, and R_3 indicate three rotation years during May 2017–May 2018, June 2018–May 2019, and June 2019–May 2020, respectively. N_{min} and N_{org} indicate grassland added with mineral nitrogen or digestate residue, respectively. C1 and C2 indicate 1 week before the first or second harvest of aboveground biomass, respectively.

the 3-year measurement period. The three-year cumulative biomass yield was 685 g C m^{-2}. Accounting for C lost as harvested biomass, the NECB was 138 g C m^{-2} with synthetic N fertilization. Accounting for the 92 g C m^{-2} as an input of C to the ecosystem with the application of digestate residue, the NECB was reduced to 45 g C m^{-2} (Table 2).

The NECB of the ecosystem was 37, −45, and 146 g C m^{-2} over R_1, R_2, and R_3, respectively -under the mineral N fertilizer application and 6, −95, and 146 under the organic N application (Table 2). The ecosystem behaved as a source in R_1 and a sink in R_2 under the mineral N treatment, while as a small C sink in R_1, and a greater one in R_2 under organic N application, respectively. The ecosystem lost a large amount of C to the atmosphere in the renovation year under both treatments in R_3.

4. Discussion

Grasslands play a crucial role in agriculture by providing ecosystem services for the milk and beef industries in Nordic countries (Åby et al., 2014; Eurostat, 2021). Few studies have reported the CO_2 balance of grasslands in boreal environments, particularly for an entire rotation cycle including the renovation period. This information is vital for developing best management practices aimed at sustainable and climate-smart land use (Cowan et al., 2016; Li et al., 2021b). Our three-year study assessed the impact of various grassland management practices on the CO_2 balance of a legume grassland in eastern Finland.

The length of the growing season in 2018 was 20 days longer than in 2017, with an early start of 16 days in the spring (Figure 2). This study demonstrated high biomass accrual rates and an enhanced ability to sequester atmospheric CO_2 during early spring, indicative of the changes that can be expected in boreal environments under shifting climatic conditions (Ruosteenoja et al., 2011, 2016). In comparison to a 30-year (1981–2010) average climate, the growing season (May–September 2017) had a cooler mean temperature of 11.6°C, while the average temperature of the growing season in 2018 reached 15.1°C (Figure 2). Similarly, the mean soil temperatures at a 5 cm depth during the growing season in 2018 were 1.7°C higher than the seasonal average in 2017. This difference was mainly due to the warmer temperatures experienced during the 2018 spring. The average temperature difference between mid-April and early June (day 100–157) in the 2 years was 6.1°C, with a maximum difference of 17.7°C on day 136 (Figure 2). Precipitation sums from May to September in 2017 (287 mm) and 2018 (291 mm) were roughly equivalent. The vapor pressure deficit (VPD), an indicator of atmospheric dryness, was also elevated during the 2018 growing season (Figure 2). Higher VPD values under optimal soil moisture conditions promote increased photosynthetic uptake of atmospheric CO_2 by vegetation. The findings presented in this study have important implications for optimizing grassland management practices and identifying opportunities for soil C sequestration in response to the changing climate in boreal regions.

Given the interannual and within-season variability in climatic conditions, various management practices either decreased vegetative cover (due to biomass harvesting or grass cuts, senescence, land preparation—glyphosate application, plowing) or increased it (through phenological development and application of chemical or organic fertilizer). Depending on the management practice followed during a year, the NEE of the legume grassland ranged from being near neutral (R_1) to a large sink (R_2) and eventually to a large source in R_3. When the eddy covariance-based CO_2 exchange measurements were conducted in May 2017, the grassland was already in its second year of rotation, having been established in 2015 and reseeded in 2016. Consequently, September 2018 marked the end of the rotation cycle when the grassland was treated with glyphosate, plowed, and left bare during the subsequent winter. The period from the end of the growing season (late September) in 2018 to late May 2019 represented the typical phase of grassland rotation renewal. These practices led to a sustained soil C loss (Cowan et al., 2016; Li et al., 2021b), offsetting the soil C sequestered during previous seasons. In R_3, following regional practice, barley was cultivated as a cover crop alongside red clover and the grass mixture. The atmospheric CO_2 fixation during the short growth period from sprouting (June 4) to harvest (August 6) is primarily attributable to the vigorous growth of the cover crop (Figure 4). After the first cut, the grassland became a large source of C in R_3, likely due to dry climatic conditions in August and relatively low soil N concentration resulting from a small dose of 45 kg N ha^{-1} applied at planting time. Severe climatic stress and poor grass growth did not warrant a second cut in the season. Following the first cut, the ecosystem remained a sustained source of CO_2 to the atmosphere until the end of this study period (May 2020). Our observations, based on continuous CO_2 exchange measurements, reveal the impact of the renewal phase on the ecosystem balance of the entire 3-year rotation cycle in a boreal environment. These results strongly suggest that greater emphasis should be placed on developing climate-friendly renovation management under the Nordic climate (Klumpp and Fornara, 2018). Such management options could extend the length of production years between renovations, provided that grassland productivity can be maintained, re-seeding is performed rapidly in spring, and sufficient N fertilization is ensured for growth and photosynthesis.

Overall, based on the NECB, the legume grassland was a C source of 45 g C m^{-2} over a three-year rotation cycle (Table 2) considering the mineral N application. However, with additional C input to the ecosystem through the application of the organic fertilizer, the NECB values changed to a small C sink of 6 g C m^{-2} in R_1 and a greater sink of 95 g C m^{-2} in R_2. As no organic fertilizer was added in the last year, the NECB during R_3 remained a large C source. Applying organic N fertilizer has been shown to enhance NECB by indirectly enhancing photosynthesis and directly increasing the soil organic matter (Hirata et al., 2013; Conant et al., 2017; Miao et al., 2019; Zhu et al., 2021). A study at a cool temperate site in Japan reported that more than 80% of the C imported in applied manure remained in the grassland soil (Hirata et al., 2013). Previous studies on European grassland sites have also found that, in general, NECB at the grassland sites without organic matter input was a net loss of CO_2 to the atmosphere or neutral, while it was neutral or a net CO_2 sink at sites with organic matter application (Gilmanov et al., 2007). These observations from previous studies are in line with our results (Table 2), in which C incorporation in soil from the organic fertilizer resulted in the net accumulation of C in grassland soils. A study in a temperate grassland reported that the stimulation of C assimilation was greater than that of ecosystem respiration with the addition of organic N, and eventually increased the ecosystem C sequestration (Gilmanov et al., 2007; Luo et al., 2017). Thus, if NECB of the boreal legume grassland is not N limited, it might largely depend on the C balance between manure input and biomass output (Table 2), which warrants further investigation of the tradeoff between productivity and ecosystem benefits with a combination of mineral and organic N fertilizer. We also computed the hyperbolic light response of NEE to incident PAR from N_{min} and N_{org} treatments for a week before all cutting events over R_1, R_2, and R_3 (Table 3, Figure 5) because P_{max}, α, and R_d (Table 3, Figure 5) are important measures of the ecosystem's ability to exchange CO_2 with the atmosphere. The light response curves from the two treatments were similar in all years with minor differences during R_1 (Figure 5), which suggested that beyond the direct effect on photosynthesis, fertilizer type might affect NECB of boreal legume grassland in different manners, and further study is thus warranted. Additionally, the application of organic manure may enhance N_2O emissions (Jones et al., 2006; Li et al., 2021a), for a complete understanding of the sustainability of managed grasslands, N_2O emissions (CO_2-equivalent) need to be assessed in considering complete net GHG balance.

5. Conclusion

The net ecosystem CO_2 balance of a legume grassland over an entire rotation cycle in a boreal environment is critical for developing best management practices aimed at sustainable and climate-smart grassland management for the sustainability of dairy and beef farming industries. This study measured the NECB of a legume grassland in eastern Finland over a three-year (2017–2020) rotation cycle. Overall, the entire legume grassland was a carbon source of 45 g C m^{-2} over the rotation cycle. Specifically, it was a weak carbon sink during the first year of the rotation, a stronger carbon sink during the second year, and a large carbon source during the renovation year of the grassland. Management practices for grassland reestablishment, such as the application of glyphosate and plowing in the autumn of 2018, leaving the site bare during the following winter, and having a late and short growing season cover crop, resulted in significant soil carbon loss and offset soil carbon sequestration achieved in earlier grassland rotation years. Our results indicate that the ecosystem's carbon balance can be improved with the application of organic soil amendments. In conclusion, climate-friendly renovation management extending the length of the photosynthetic period under the Nordic climate can lead to higher carbon sequestration in boreal legume grasslands. The application of organic fertilizers can further enhance carbon sequestration, promoting more sustainable and climate-smart grassland management practices that support the sustainability of dairy and beef farming industries.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

YL: designed research, collected data, formal analysis, visualization, and writing—original draft. PK, SK, MM, and PV: designed research, data curation, and writing—review. NS: designed research, collected data, project administration, supervision, and funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1158250/full#supplementary-material

References

Åby, B. A., Kantanen, J., Aass, L., and Meuwissen, T. (2014). Current status of livestock production in the Nordic countries and future challenges with a changing climate and human population growth. *Acta Agricult. Scand. Sect. A Anim. Sci.* 64, 73–97. doi: 10.1080/09064702.2014.950321

Ammann, C., Neftel, A., Jocher, M., Fuhrer, J., and Leifeld, J. (2020). Effect of management and weather variations on the greenhouse gas budget of two grasslands during a 10-year experiment. *Agric. Ecosyst. Environ.* 292:106814. doi: 10.1016/j.agee.2019.106814

Aubinet, M., Grelle, A., Ibrom, A., Rannik, Ü., Moncrieff, J., Foken, T., et al. (1999). "Estimates of the annual net carbon and water exchange of forests: the EUROFLUX methodology" in *Advances in ecological research*. eds. A. H. Fitter and D. G. Raffaelli (Academic Press), 113–175.

Baldocchi, D. D. (2020). How eddy covariance flux measurements have contributed to our understanding of global change biology. *Glob. Chang. Biol.* 26, 242–260. doi: 10.1111/gcb.14807

Chapin, F. S., Woodwell, G. M., Randerson, J. T., Rastetter, E. B., Lovett, G. M., Baldocchi, D. D., et al. (2006). Reconciling carbon-cycle concepts, terminology, and methods. *Ecosystems* 9, 1041–1050. doi: 10.1007/s10021-005-0105-7

Conant, R. T., Cerri, C. E. P., Osborne, B. B., and Paustian, K. (2017). Grassland management impacts on soil carbon stocks: a new synthesis. *Ecol. Appl.* 27, 662–668. doi: 10.1002/eap.1473

Cowan, N. J., Levy, P. E., Famulari, D., Anderson, M., Drewer, J., Carozzi, M., et al. (2016). The influence of tillage on N_2O fluxes from an intensively managed grazed grassland in Scotland. *Biogeosciences* 13, 4811–4821. doi: 10.5194/bg-13-4811-2016

Deng, J., Ni, H., Zhang, Z., Usman, S., Yang, X., Shen, Y., et al. (2021). Designing productive, energy-efficient, and environmentally friendly production systems by replacing fallow period with annual forage cultivation on the loess plateau of China. *J. Clean. Prod.* 320:128660. doi: 10.1016/j.jclepro.2021.128660

Eurostat (2021). *Milk and milk product statistics*.

Finn, J. A., Kirwan, L., Connolly, J., Sebastià, M. T., Helgadottir, A., Baadshaug, O. H., et al. (2013). Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: a 3-year continental-scale field experiment. *J. Appl. Ecol.* 50, 365–375. doi: 10.1111/1365-2664.12041

Foken, T., Göockede, M., Mauder, M., Mahrt, L., Amiro, B., and Munger, W. (2004). "Post-field data quality control" in *Handbook of micrometeorology: A guide for surface flux measurement and analysis*. eds. X. Lee, W. J. Massman and B. E. Law (Dordrecht: Kluwer Academic Publishers), 181–208.

Foken, T., and Wichura, B. (1996). Tools for quality assessment of surface-based flux measurements. *Agric. For. Meteorol.* 78, 83–105. doi: 10.1016/0168-1923(95)02248-1

Gilmanov, T. G., Soussana, J. F., Aires, L., Allard, V., Ammann, C., Balzarolo, M., et al. (2007). Partitioning European grassland net ecosystem CO_2 exchange into gross primary productivity and ecosystem respiration using light response function analysis. *Agric. Ecosyst. Environ.* 121, 93–120. doi: 10.1016/j.agee.2006.12.008

Gylfadóttir, T., Helgadóttir, Á., and Høgh-Jensen, H. (2007). Consequences of including adapted white clover in northern European grassland: transfer and deposition of nitrogen. *Plant Soil* 297, 93–104. doi: 10.1007/s11104-007-9323-4

Hirata, R., Miyata, A., Mano, M., Shimizu, M., Arita, T., Kouda, Y., et al. (2013). Carbon dioxide exchange at four intensively managed grassland sites across different climate zones of Japan and the influence of manure application on ecosystem carbon and greenhouse gas budgets. *Agric. For. Meteorol.* 177, 57–68. doi: 10.1016/j.agrformet.2013.04.007

IUSS Working Group WRB, (2007). *World reference base for soil resources 2006, first update 2007*. FAO, Rome.

Jones, S. K., Rees, R. M., Kosmas, D., Ball, B. C., and Skiba, U. M. (2006). Carbon sequestration in a temperate grassland; management and climatic controls. *Soil Use Manag.* 22, 132–142. doi: 10.1111/j.1475-2743.2006.00036.x

Klumpp, K., and Fornara, D. A. (2018). "The carbon sequestration of grassland soils-climate change and mitigation strategies" in *27th general meeting of the European grassland federation* (Cork: Teagasc, Animal and Grassland Research and innovation Centre)

Laidlaw, A. S., Christie, P., and Lee, H. W. (1996). Effect of white clover cultivar on apparent transfer of nitrogen from clover to grass and estimation of relative turnover rates of nitrogen in roots. *Plant Soil* 179, 243–253. doi: 10.1007/BF00009334

Li, Y., Clough, T. J., Moinet, G. Y. K., and Whitehead, D. (2021a). Emissions of nitrous oxide, dinitrogen and carbon dioxide from three soils amended with carbon substrates under varying soil matric potentials. *Eur. J. Soil Sci.* 72, 2261–2275. doi: 10.1111/ejss.13124

Li, Y., Li, Z., Cui, S., Liang, G., and Zhang, Q. (2021b). Microbial-derived carbon components are critical for enhancing soil organic carbon in no-tillage croplands: a global perspective. *Soil Tillage Res.* 205:104758. doi: 10.1016/j.still.2020.104758

Lind, S. E., Shurpali, N. J., Peltola, O., Mammarella, I., Hyvönen, N., Maljanen, M., et al. (2016). Carbon dioxide exchange of a perennial bioenergy crop cultivation on a mineral soil. *Biogeosciences* 13, 1255–1268. doi: 10.5194/bg-13-1255-2016

Lloyd, J., and Taylor, J. A. (1994). On the temperature dependence of soil respiration. *Funct. Ecol.* 8, 315–323. doi: 10.2307/2389824

Lüdecke, D., Waggoner, P., and Makowski, D. (2019). Insight: a unified interface to access information from model objects in R. *J. Open Source Softw.* 4:1412. doi: 10.21105/joss.01412

Luo, Q., Gong, J., Yang, L., Li, X., Pan, Y., Liu, M., et al. (2017). Impacts of nitrogen addition on the carbon balance in a temperate semiarid grassland ecosystem. *Biol. Fertil. Soils* 53, 911–927. doi: 10.1007/s00374-017-1233-x

Lüscher, A., Mueller-Harvey, I., Soussana, J. F., Rees, R. M., and Peyraud, J. L. (2014). Potential of legume-based grassland–livestock systems in Europe: a review. *Grass Forage Sci.* 69, 206–228. doi: 10.1111/gfs.12124

Maljanen, M., Virkajärvi, P., Hytönen, J., Öquist, M., Sparrman, T., and Martikainen, P. J. (2009). Nitrous oxide production in boreal soils with variable organic matter content at low temperature – snow manipulation experiment. *Biogeosciences* 6, 2461–2473. doi: 10.5194/bg-6-2461-2009

Mammarella, I., Peltola, O., Nordbo, A., Järvi, L., and Rannik, Ü. (2016). Quantifying the uncertainty of eddy covariance fluxes due to the use of different software packages and combinations of processing steps in two contrasting ecosystems. *Atmos. Meas. Tech.* 9, 4915–4933. doi: 10.5194/amt-9-4915-2016

Miao, F., Li, Y., Cui, S., Jagadamma, S., Yang, G., and Zhang, Q. (2019). Soil extracellular enzyme activities under long-term fertilization management in the croplands of China: a meta-analysis. *Nutr. Cycl. Agroecosyst.* 114, 125–138. doi: 10.1007/s10705-019-09991-2

Olesen, J. E., and Bindi, M. (2002). Consequences of climate change for European agricultural productivity, land use and policy. *Eur. J. Agron.* 16, 239–262. doi: 10.1016/S1161-0301(02)00004-7

Pinheiro, J., Bates, D., DebRoy, S., and Sarkar, D., (2014). Nlme: linear and nonlinear mixed effects models. R package version 3.1–117.

Rannik, Ü., and Vesala, T. (1999). Autoregressive filtering versus linear detrending in estimation of fluxes by the eddy covariance method. *Bound.-Layer Meteorol.* 91, 259–280. doi: 10.1023/A:1001840416858

Reichstein, M., Falge, E., Baldocchi, D., Papale, D., Aubinet, M., Berbigier, P., et al. (2005). On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm. *Glob. Chang. Biol.* 11, 1424–1439. doi: 10.1111/j.1365-2486.2005.001002.x

Riesinger, P., and Herzon, I. (2008). Variability of herbage production in mixed leys as related to ley age and environmental factors: a farm survey. *Agric. Food Sci.* 17:394. doi: 10.2137/145960608787235522

Ruosteenoja, K., Jylhä, K., and Kämäräinen, M. (2016). Climate projections for Finland under the RCP forcing scenarios. *Geophysica* 51, 17–50.

Ruosteenoja, K., Räisänen, J., and Pirinen, P. (2011). Projected changes in thermal seasons and the growing season in Finland. *Int. J. Climatol.* 31, 1473–1487. doi: 10.1002/joc.2171

Rutledge, S., Wall, A. M., Mudge, P. L., Troughton, B., Campbell, D. I., Pronger, J., et al. (2017). The carbon balance of temperate grasslands part I: the impact of increased species diversity. *Agric. Ecosyst. Environ.* 239, 310–323. doi: 10.1016/j.agee.2017.01.039

Schotanus, P., Nieuwstadt, F. T. M., and De Bruin, H. A. R. (1983). Temperature measurement with a sonic anemometer and its application to heat and moisture fluxes. *Bound.-Layer Meteorol.* 26, 81–93. doi: 10.1007/BF00164332

Suter, M., Connolly, J., Finn, J. A., Loges, R., Kirwan, L., Sebastià, M. T., et al. (2015). Nitrogen yield advantage from grass–legume mixtures is robust over a wide range of legume proportions and environmental conditions. *Glob. Chang. Biol.* 21, 2424–2438. doi: 10.1111/gcb.12880

Virkajärvi, P., Rinne, M., Mononen, J., Niskanen, O., Järvenranta, K., and Sairanen, A. (2015). Dairy production systems in Finland. *Grassland Sci. in Eur.* 20, 51–66.

Wickham, H., (2016). *ggplot2: Elegant graphics for data analysis*. Springer, New York.

Zhu, Y., Merbold, L., Leitner, S., Wolf, B., Pelster, D., Goopy, J., et al. (2021). Interactive effects of dung deposited onto urine patches on greenhouse gas fluxes from tropical pastures in Kenya. *Sci. Total Environ.* 761:143184. doi: 10.1016/j.scitotenv.2020.143184