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CO_2 fluxes from three different temperate grazed pastures using Eddy covariance measurements

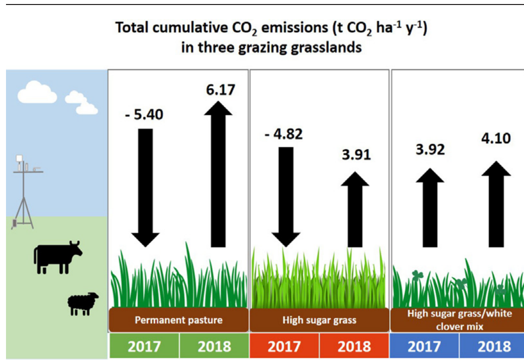
L.M. Cardenas [a], L. Olde [a,*], N. Loick [a], B. Griffith [a], T. Hill [b], J. Evans [c], N. Cowan [d], C. Segura [a], H. Sint [a], P. Harris [a], J. McCalmont [b], S. Zhu [b], A. Dobermann [e], M.R.F. Lee [a,f]

[a] Rothamsted Research, Sustainable Agriculture Sciences, North Wyke, Devon EX20 2SB, UK
[b] University of Exeter, Exeter EX4 4QE, UK
[c] Rothamsted Research, Computational and Analytical Sciences, Harpenden, Hertfordshire AL5 2JQ, UK
[d] UK Centre of Ecology and Hydrology, Bush Estate, Midlothian EH26 0QB, UK
[e] International Fertilizer Association, Paris, France
[f] Harper Adams University, Edgmond, Shropshire, TF10 8NB, UK

HIGHLIGHTS

- Pasture type influenced the atmospheric CO_2 balance in a temperate climate.
- Permanent (PP) and high sugar grass (HS) pastures had similar yearly net CO_2 fluxes.
- PP and HS pastures were a sink for atmospheric CO_2 in 2017 and a source in 2018.
- A pasture of high sugar grass and clover (HSC) acted as a net source of CO_2.
- Night-time CO_2 emissions were influenced by season and pasture type.

GRAPHICAL ABSTRACT



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ABSTRACT

Grasslands cover around 25% of the global ice-free land surface, they are used predominantly for forage and livestock production and are considered to contribute significantly to soil carbon (C) sequestration. Recent investigations into using 'nature-based solutions' to limit warming to <2 °C suggest up to 25% of GHG mitigation might be achieved through changes to grassland management. In this study we evaluate pasture management interventions at the Rothamsted Research North Wyke Farm Platform, under commercial farming conditions, over two years and consider their impacts on net CO_2 exchange. We investigate if our permanent pasture system (PP) is, in the short-term, a net sink for CO_2 and whether reseeding this with deep-rooting, high-sugar grass (HS) or a mix of high-sugar grass and clover (HSC) might increase the net removal of atmospheric CO_2. In general CO_2 fluxes were less variable in 2018 than in 2017 while overall we found that net CO_2 fluxes for the PP treatment changed from a sink in 2017 (-5.40 t CO_2 ha^{-1} y^{-1}) to a source in 2018 (6.17 t CO_2 ha^{-1} y^{-1}), resulting in an overall small source of 0.76 t CO_2 ha^{-1} over the two years for this treatment. HS showed a similar trend, changing from a net sink in 2017 (-4.82 t CO_2 ha^{-1} y^{-1}) to a net source in 2018 (3.91 t CO_2 ha^{-1} y^{-1}) whilst the HSC field was a net source in both years (3.92 and 4.10 t CO_2 ha^{-1} y^{-1}, respectively). These results suggested that pasture type has an influence in the atmospheric CO_2 balance and our regression modelling supported this conclusion, with pasture type and time of the year (and their interaction) being significant factors in predicting fluxes.

* Corresponding author.
E-mail address: louise.olde@rothamsted.ac.uk (L. Olde).

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1. Introduction

Grasslands represent one of the most extensive ecosystems globally, occupying ~25% of the Earth's ice-free land surface (Steinfeld et al., 2006). They are used predominantly for forage and livestock production and are considered to contribute significantly to carbon (C) sequestration (Staerfl et al., 2012) and increased biodiversity (Jerome et al., 2014) particularly in grazing systems. Recent estimates of the potential for mitigating global warming using 'nature climate solutions' (NCS) needed to limit warming to <2 °C, state that up to 25% of mitigation can be achieved from grassland and agriculture measures (Griscom et al., 2017). As emissions from agricultural activities, land-use change and the food chain account for 21–37% of annual global warming emissions (IPCC, 2019), optimal management of grasslands is important in efforts to meet the 2 °C goal of the Paris Agreement and limit climate change in the 21st century.

Below-ground C translocation from leaves to roots from photosynthesis in pastures can be close to twice as much as that from cereals, i.e. 40% vs 25% of total C assimilated, respectively (Kuzyakov and Domanski, 2000). There is an increasing interest in identifying management practices that enhance the photosynthetic input of C into soil or reduce the rate of C loss to increase C sequestration in agricultural soils (Rutledge et al., 2017).

Grasslands under cultivation can also be a large source of N and C losses, to water from leaching and to the atmosphere via pollutants such as ammonia (NH_3) and nitric oxide (NO), and greenhouse gases (GHGs) carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) (IPCC, 2001) which are influenced by soil/climate characteristics and soil, pasture and livestock management. Soil respiration is the second largest C flux after photosynthesis in most ecosystems (Davidson et al., 2002), globally estimated at approximately 75×10^{12} kg C yr^{-1}. It is the primary path by which CO_2, fixed by plants, returns to the atmosphere (Yang et al., 2010). It includes roots and microbial respiration and root C decomposition, both considered to be rhizosphere respiration, and the decomposition of soil organic matter (OM) by microbes (Domanski et al., 2001). Reported values for the contribution of respiration by roots to CO_2 emitted from assimilated C from pastures were typically 41% for perennial ryegrass (*Lolium perenne*) on a fine loamy Gleyic Cambisol (Kuzyakov et al., 1999). In a review by Hanson et al. (2000) values for pastures varied between 10 and 98% of root contribution to total soil respiration; in another compilation of several studies it was reported that 13% of C translocated below ground under *L. perenne* was converted to CO_2. Under a variety of pasture species (including Festuca and Bromus genera) the value was closer to 17% (Kuzyakov and Domanski, 2000).

Carbon dioxide also emanates from respiration of livestock in agricultural systems, with values reported by de la Motte et al. (2019) of 2.0 ± 0.6 kg C LU^{-1} d^{-1}; 3.2 ± 0.5 kg C LU^{-1} d^{-1}, determined by two different approaches. Soil respiration shows a diurnal cycle (Liu et al., 2016) as a response to environmental factors (e.g. temperature), and is influenced by the plant type and growth stage; the soil (type, fertiliser application, tillage) and crop management (species/pasture species mixture, harvest) amongst others. For example, in a grassland site in China under a semi-arid continental climate in 2008, total soil respiration showed strong diurnal patterns in different seasons for, with single maxima at 11:00–15:00 h and the minima at 0:00–6:00 h (Li et al., 2018). (Liu et al., 2016) reported daily maxima and minima for soil respiration under different land uses at 13:00–15:00 and 06:00–08:00, respectively.

Grazed grasslands represent a challenge for measuring emissions due to the presence of animals, particularly when using closed chambers, as these in principle need to remain in place. Chambers can be damaged by the livestock, and the exclusion of excreta deposition in the actual chamber area is also a major limitation of this technique. Micrometeorological techniques such as eddy covariance have been developed to represent larger areas, and provide high temporal resolution and continuous data without chambers. These have some drawbacks, particularly the need to discard data when conditions do not meet the assumptions of the calculations used in the method (due to wind direction and the absence of turbulence, see Baldocchi (2014) for details of pros and cons of this technique). There are also potential problems of under or overestimations of net C gain due to systematic errors in some grassland systems (Twine et al., 2000; Kirschbaum et al., 2020). These techniques additionally assume the existence of a flat homogeneous source, which in grazed systems, represents a challenge, not only as the livestock are point sources violating the assumption of spatial homogeneity (Coates et al., 2017), but also because it is not certain when the animals have been in the footprint area of the tower (Baldocchi, 2014; Stoy et al., 2021).

It is expected that field events such as rainfall, ploughing, fertiliser and manure application, mowing will cause a release of greenhouse gases including CO_2. (Drewer et al., 2017) for example, determined on a UK grassland that 440 g CO_2 m^{-2} were released as CO_2 in the month following ploughing. Measurements 30 days after ploughing showed that the total release of CO_2 was 444–457 g CO_2 m^{-2}, much higher than losses before the ploughing event, which were only 4–17 g CO_2 m^{-2}. However, overall and in the long term there was a net uptake of CO_2 by the grassland. In the study by Griscom et al. (2017), livestock management NCS measures include optimising grazing intensity and the inclusion of forage legumes to reduce nitrogen (N) inputs with potential for mitigation by 2030 of 148 and 147 Tg CO_2e yr^{-1}, respectively. Bossio et al. (2020) state that soil C represents 25% of the potential NCS comprised by the protection of existing soil C and rebuilding existing stocks. In the UK, the same authors estimated the maximum mitigation potential for NCS of 1.31 and 8.53 Tg CO_2e yr^{-1} from optimal grazing intensity and the inclusion of legumes, respectively. This represents about 20% of current emissions (Brown et al., 2021) from agriculture in CO_2e. The C sequestration in grasslands in comparison to arable systems is achieved through better pasture and livestock management, occasional reseeding to rejuvenate swards and increase biodiversity (including legumes), and the return of organic matter from the grazing animals. The study by Bossio et al. (2020) identifies optimal grazing intensity and inclusion of legumes as effective NCS measures to protect and sequester soil organic carbon (SOC).

The typical pasture on grazing land in the South West of England is permanent grassland (not ploughed and reseeded for at least 5 years) dominated by *L. perenne*. This is a productive system but recent improvements in plant genetics offer opportunities for reducing environmental impact whilst retaining production (Ellis et al., 2011). Pasture improvements, either via species bred with specific advantageous properties such as deep rooting, nutritional quality (digestibility, water-soluble carbohydrate content), or by using a grass/clover sward, that can be manipulated to improve productivity (liveweight gain), managing soil hydrology, nutrient retention and generally better nutrient cycling (Orr et al., 2016; Ellis et al., 2011). Pastures species with a high water soluble carbohydrate (sugar) content have been found to be useful in improving N use efficiency (Lee et al., 2002) decreasing enteric CH_4 emissions (Rivero et al., 2020) and if deep rooting they can potentially help sequester C. Additionally, when introducing legumes in the sward, there is a N saving from the exclusion of fertiliser in the management, but they are also considered to increase the potential for C sequestration due to improved root biomass from a beneficial complementarity between plant species (Rutledge et al., 2017). However, when researching on other gases such as N_2O emissions, the inclusion of legumes can be contradictory, as increases may result from the decomposition of plant residues and rhizodeposition (Fuchs et al., 2018).

In our study, we evaluate two specific pasture management interventions under real farming conditions, namely reseeding with a high-sugar grass and reseeding with the same high-sugar grass and introducing clover to enhance animal performance and net C storage. Such measures have generally been proposed as grazing systems innovations for increasing carbon sequestration (Griscom et al., 2017; Bossio et al., 2020). We present for the first time, results of 2 years of continuous CO_2 measurements using eddy covariance (EC) flux towers in three grazed fields under different pastures in a research farm in the South West of England. This data is unique as they supplement the C losses to water, and soil C pool size that have been measured for several years in this UK facility to test productivity and environmental impact of different swards. No other experiment of this scale can assess all of the C pools. We tested the following hypotheses:

1. Permanent pasture systems with intensive grazing are a net sink of CO_2 under good management practices.
2. Reseeding permanent pasture systems with deep-rooting, high-sugar grass varieties or a mix of high-sugar grass and clover increases net sequestration of CO_2 compared to the original pasture.
3. Irrespective of pasture management, environmental conditions result in highly variable, difficult to predict annual CO_2 fluxes.

2. Material and methods

2.1. Site description

The study site, the North Wyke Farm Platform (NWFP) is located in the southwest of England (50°46′10″N, 3°54′05″W), a region typically devoted to grazing livestock agriculture. The platform's soil (Harrod and Hogan, 2008) comprises predominantly two similar series, Hallsworth (Dystric Gleysol) and Halstow (Gleyic Cambisol) (Avery, 1980) with about 36% clay in the top-soil. The sub-soil is impermeable to water and is seasonally waterlogged, causing excess water to move laterally. From 1982 to 2018, the average annual precipitation at North Wyke was 1032 mm, with average minimum and maximum daily temperatures of 6.8 and 13.5 °C, respectively. North Wyke has a large and consistent amount of summer rainfall, which is commonplace to agricultural grasslands in this region (Shepherd et al., 2017). The platform is situated on a ridge at 120–180 m above sea level, where its fields slope to the west to the River Taw and to the east to the Cocktree stream (a tributary of the River Taw).

2.2. Experimental setup

The NWFP is a UK National Capability set up to research agricultural productivity and ecosystem responses under different management practices for beef and sheep production in lowland grasslands (Orr et al., 2016). General information about the North Wyke Farm Platform as well

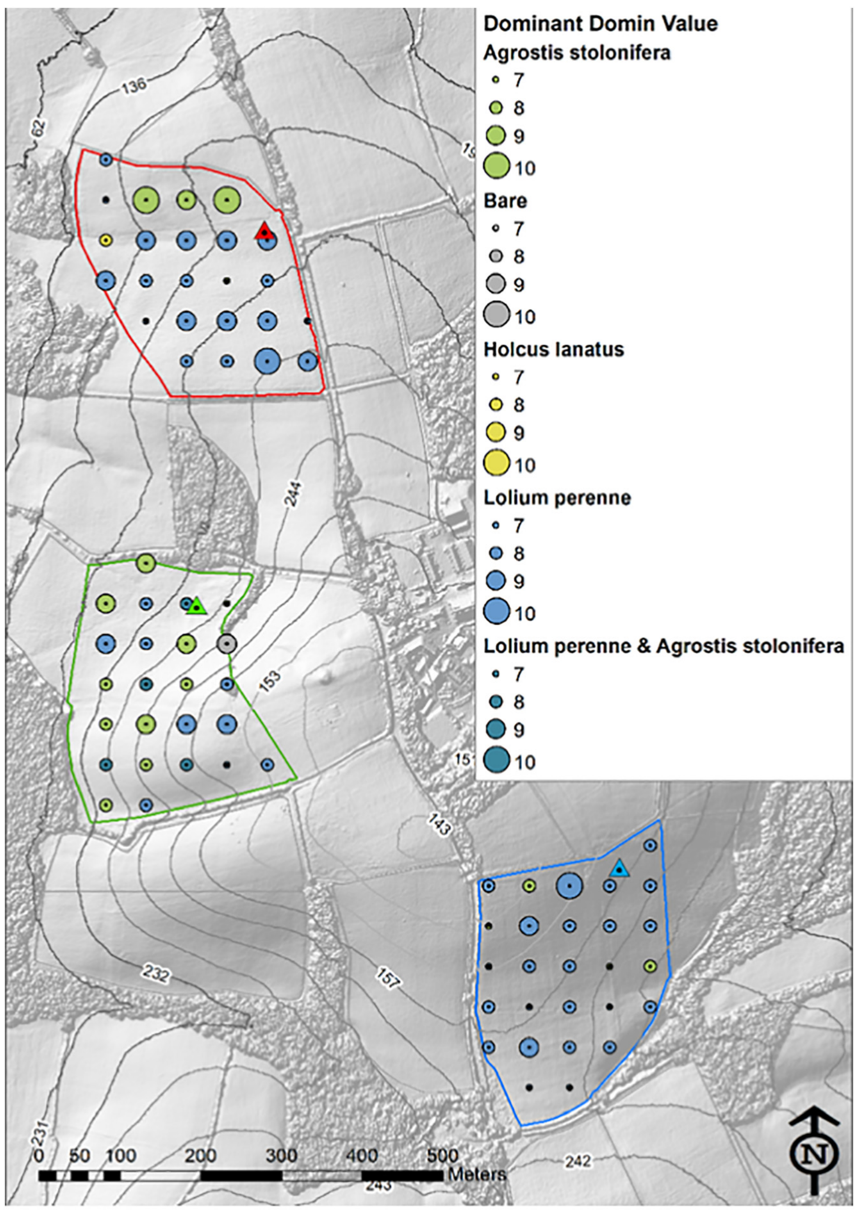

Fig. 1. North Wyke Farm Platform map showing the fields of the three farmlets: Permanent Pasture (PP, green), High Sugar grass (HS, red), High sugar grass/ white clover mix (HSC, blue). Outlined fields are those containing Eddy Covariance towers (triangle). Contour lines show elevation in meters above sea level. Results from the botanical survey carried out in June 2018. Circle colours show Dominant Scale and size the respective dominance score.

as all currently available data can be obtained from https://nwfp.rothamsted.ac.uk/. Three 21-ha farmlets (or 'production systems') based on permanent pasture were established in 2010 to obtain baseline data on hydrology, nutrient cycling and productivity. Each farmlet consists of five catchments ranging in size from 1.62 to 8.08 ha. Each catchment is hydrologically isolated and equipped with monitoring points for rainfall, soil moisture, discharge and water physicochemical properties (see https://nwfp.rothamsted.ac.uk/). The location and topography of the area are displayed in Fig. 1.

Each farmlet is typically grazed from April – October (dictated by annual weather conditions) with finishing beef cattle (from weaning to finish; up to 30) and ewes (typically 75) with their lambs (birth to finish; typically 135 assuming a lambing rate of 1.8). Each herd and flock are rotated between the fields within a farmlet according to pasture availability. The grazing strategy for all farmlets is continuous (variable) stocking with silage cuts in May and July from selected fields, which is used for the housed cattle during the winter. For the purpose of this paper we will use the following LU day^{-1} (livestock units per day) for: cattle = 0.65, sheep = 0.11 and lamb = 0.04 per animal (*pers. comm. North Wyke farm*) for the 2 years of the study. Farmyard manure (FYM) collected during the housing period is stored in farmlet-specific middens until pastures are ready for fertilization between silage cuts. Further details of the management for the platform are described in Orr et al. (2016) and animal performance reported in Orr et al. (2019) and Jones et al. (2021).

After a baseline measurement period (2011 – 13), fields of the two farmlets HS and HSC were ploughed and reseeded. The original pasture was changed to investigate the effect of pasture management on productivity and environmental impact. The third farmlet (PP) was kept as before and is considered a control, i.e. a commonly used grassland management system in the region that had not been ploughed for the previous 20 years. The platform's catchments entered a post-baseline phase at different times, and only from September 2015 to August 2019 has this post-baseline phase been in full operation across all three pasture treatments.

The three farmlets were characterised by the following pastures: (1) "Permanent pasture" (PP) that showed no change from the baseline assessment (botanical composition and more detail regarding the swards can be found in Orr et al. (2016); (2) "High sugar grass" (HS) established after PP by ploughing and reseeding with the *L. perenne* grass variety AberMagic; (3) "White clover/High sugar grass mix" (HSC) established after PP by ploughing and reseeding with a combination of the high sugar grass AberMagic and the white clover variety AberHerald. This was undertaken via a phased transition period from April 2013 to August 2015, where two or three catchments in each of farmlets 2 and 3 were re-seeded each year. The grass and white clover choice of cultivars (cv. AberMagic and AberHerald, respectively) was based on the UK's recommended list of latest germplasm (BGS, 2013).

Fertiliser application, FYM application and grass cutting during the 2-year measurement period for the three EC fields (2017–18), are shown in Table 1. Applied inorganic N fertiliser was as ammonium nitrate on each occasion. The soil characteristics of the fields for 2018 are shown in Supplementary Table 1. The sampling is part of the regular NWFP surveys where soils are collected in each field in a 'W' shape (analytical methods as in McAuliffe et al., 2020).

2.3. Measurements of CO_2 fluxes

In 2016, three catchments (one per farmlet) were selected to install the EC equipment, according to specifications for this technique (details below). Each EC field has slightly different aspects - the chosen PP field has an EC tower in the corner of this North-West sloping field, but with the main wind direction from a relatively flat area at the bottom; the HS field is the most flat one; the HSC field is on a south-east facing slope. An example of the area measured by the tower (footprint) is shown in Fig. 2.

The tower's height was for PP 1.57 m; HSC 1.59 m and HS 1.59 m. Regarding the fetch size, 80% of the footprint was between 3 and 70 m upwind from the tower in all fields. The tower's orientation was South Westerly with prevailing wind shown in Supplementary Fig. 1.

In this paper we include data from 2017 and 2018, as in 2019 the HS field was ploughed and the land use changed to an arable crop. The towers were installed on the largest field (6.45 to 6.65 ha) of each farmlet between April and October 2016 at the following locations: PP on Burrows (50° 46′ 11.28″ N, 3° 54′ 21.96″ W), 154.4 m above sea level and 6.5 ha; HS on Great Field (50° 46′ 26.40″ N, 3° 54′ 18.29″ W), 149.9 m above sea level and 6.65 ha; HSC on Dairy South (50° 46′ 01.20″ N, 3° 53′ 54.78″ W), 168.9 m above sea level and 6.45 ha. Great Field entered its HS post-baseline phase on the

Table 1

Field events: Fertiliser, Lime, Farm Yard Manure (FYM) application or grass cutting (Mow or Top) performed during the given month on the indicated fields: Permanent Pasture (PP), High Sugar grass (HS), High sugar grass/ white clover mix (HSC). Numbers in the 'Field' column give the total fertiliser application in kg per ha. The nutrient composition of the applied fertiliser is given in the last column with numbers giving the percentage of the nutrient (nitrogen applied as N, phosphorus applied as P_2O, potassium applied as K_2O, sulfur applied as SO_3, magnesium applied as MgO).

Date	Operation	Field (numbers give total fertiliser application in kg/ha; tick marks indicate the event having taken place)			Fertiliser nutrient composition (%)				
		PP	HS	HSC	N	P2O	K2O	SO3	MgO
MAR 2017	FYM	26	–	–					
APR 2017	Fertiliser	365	365	–	22	4	14	7	0
	Fertiliser	30	–	–	0	0	60	0	0
		–	100	200	0	20	30	0	0
	Fertiliser	114	115	–	34.5	0	0	0	0
	Mow	✓	✓	✓					
JUN 2017	FYM	103	53	58					
JUL 2017	Fertiliser	–	–	60	0	0	0	50	25
	Lime	–	–	625					
SEP 2017	Top	✓	✓	–					
APR 2018	Fertiliser	–	–	400	22	4	14	7	0
	Fertiliser	–	119	–	33.5	0	0	0	0
MAY 2018	Fertiliser	109	43	108	0	46	0	0	0
	Fertiliser	119	119	–	33.5	0	0	0	0
JUN 2018	Top	–	✓	✓					
JUL 2018	Fertiliser	119	119	–	33.5	0	0	0	0
AUG 2018	Fertiliser	119	119	–	33.5	0	0	0	0
SEP 2018	Mow	–	✓[a]	–					

[a] Half the field (North) was mowed.

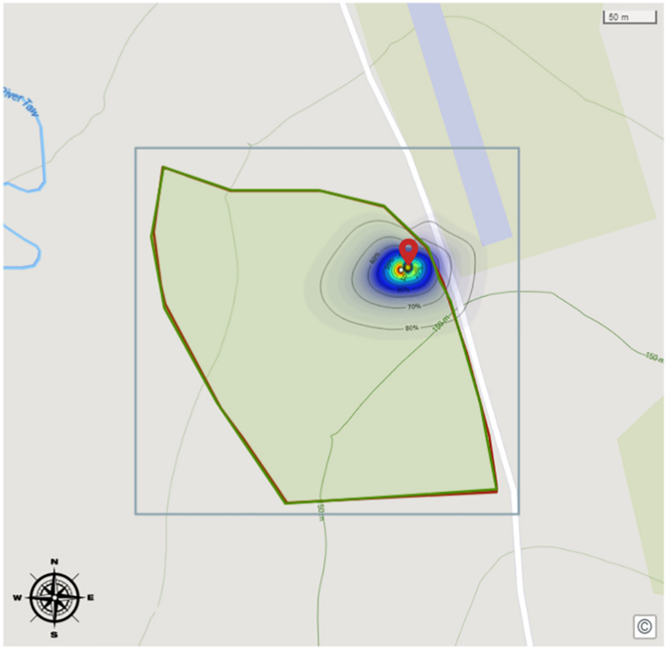

Fig. 2. Footprint of one of the Eddy Covariance towers (HS).

30th July 2013, while Dairy South entered its HSC post-baseline phase on 22nd of August 2014.

Eddy covariance data collected during late 2016 were used to validate tower position and ensure correct instrument operation, site evaluation and data recording. Continuous EC measurements were started in January 2017 and included a fast ultrasonic 3-D anemometer (Gill Windmaster Pro; Gill Instruments Ltd., Lymington, UK) and a closed path CO_2 analyser (LI-7200; Li-Cor Inc., Lincoln, NE, USA). At each site, the following additional data (listed below) were recorded for gap-filling and other purposes (see Section 2.4):

i) photosynthetic active radiation (PAR) (Li-190SL; Li-Cor Inc., Lincoln, NE, USA),
ii) incoming and reflected radiation (CNR4; Kipp&Zonen, Delft, Netherlands),
iii) humidity and air temperature (HMP155, Vaisala, Helsinki, Finland),
iv) precipitation data, obtained from an automated from a weather station nearby and used for all three sites (https://nwfp.rothamsted.ac.uk/),
v) soil heat exchange (HFP01, Hukseflux Thermal Sensors B.V., Delft, Netherlands),
vi) soil moisture and temperature (Hydra Probe II, Stevens Water Monitoring Systems Inc., Portland, OR, USA).

Gas concentrations and wind data were recorded at 20 Hz and logged to the Li-Cor interface Unit (Li-7550, Li-Cor Inc., Lincoln, NE, USA), while other meteorological data were recorded every 5 s onto a data logger (XLite 9210B, Sutron, Sterling, VA, USA). Both data streams were combined using the Li-Cor Smart Flux System.

2.4. Eddy covariance data processing

Raw concentration data were processed into 30-min mean flux rates of NEE using the Eddy Pro Software (Eddy Pro v6.2.2, Li-Cor Inc., Lincoln, NE, USA). Data analysis was performed using the pre-determined processing settings of the "Express Mode" (Li-Cor Inc., 2016) with the following alterations: to account for and minimise the effect of sloping ground, the planar fit correction method after Wilczak et al. (2001) was applied and for the correction of low pass filtering effects the method developed by Fratini et al. (2012) was used.

Data were subsequently filtered and quality controlled using Tovi software version 2.8 (Li-Cor Inc., Lincoln, NE, USA). During filtering, the flux data with Foken flags of 2 were removed, as was data below a calculated u* threshold (Reichstein et al., 2005). Thresholds were for HS 2017: 0.155 m s^{-1}; HS 2018: 0.125 m s^{-1}; PP 2017: 0.108 m s^{-1}; PP 2018: 0.128 m s^{-1}; HSC 2017: 0.090 m s^{-1}; HSC 2018: 0.117 m s^{-1} A footprint analysis was done (Kljun et al., 2015), and any data periods where less than 80% of the footprint was inside the field of interest were removed.

Meteorological data collected by the peripheral sensors were compared to other sensors on the NWFP Data Portal and to COSMOS sensors on the site (the Cosmic-ray soil moisture monitoring network, UK CEH, https://cosmos.ceh.ac.uk/sites/NWYKE). Any faulty data, e.g. due to broken soil sensors, were removed. Meteorological data was then gap-filled (Isaac et al., 2017) using the NWFP and COSMOS data, and ERA-interim forecast data from the ECMWF (Dee et al., 2011) was used where no on-site sensor data was available.

The quality checked and gap-filled meteorological data was used to gap-fill fluxes of CO_2, Latent Heat Turbulent Flux and Sensible Heat Turbulent Flux using the marginal distribution sampling (MDS) Gap Fill tool in Tovi (Reichstein et al., 2005). Half-hourly fluxes were converted to Daily, Monthly and Yearly total sums using Area Under Curve calculations using the DescTools package in R.

Data were gap filled in case of instrument failure or flagged data, to determine cumulative emissions. Due to instrument failures/outages periods based on purely gap filled data longer than 3 days were as follows (longer periods mostly due to instrument sent back for repair):

PP: 14.09.2017 to 30.09.2017 and 11.092018 to 30.11.2018.
HS: 14.09.2018 to 25.09.2018; 18.12.2018 to 31.12.2018.
HSC: 17.01.2018 to 20.02.2018 and 21.09.2018 to 27.09.2018.

NEE was partitioned into ecosystem respiration (ER) and photosynthetic uptake (gross primary productivity, GPP) using the night-time based temperature response of NEE (where GPP = 0 and NEE = ER) following (Reichstein et al., 2005) and implemented in the Tovi software. For the purpose of this study, we consider positive NEE a net source of emissions, and negative values a net sink. The response of GPP to incoming light (PPFD) was then investigated using a rectangular hyperbolic light response curve to estimate light use efficiency (LUE, initial slope of the fitted curve, μmol CO_2 μmol $PPFD^{-1}$) and maximum photosynthetic assimilation (Amax, the asymptote of the curve, μmol CO_2 m^{-2} s^{-1}). Full details of the methodology for this analysis (including data availability and filtering) can be found in supplementary materials. Light response parameters were estimated and compared within a range of management-based time periods of one month each. Due to inter-year variations in farm management timing, it was not possible to capture identical time periods/management events for both 2017 and 2018. Only the winter periods/no grazing events were consistent between the years. The dates of these, and four other management events were considered:

2017

1) winter period/no-grazing (21/2/2017 to 21/3/2017)
2) spring period/no-grazing (25/4/2017 to 25/5/2017)
3) autumn period/grazing (01/10/2017 to 01/11/2017)

2018

4) winter period/no-grazing (21/2/2018 to 21/3/2018)
5) spring period/grazing (15/5/2018 to 15/6/2018)
6) summer period/grazing (28/07/2018 to 28/8/2018)

2.5. Vegetation, soil and livestock characteristics

Plant cover ratio was assessed in 0.5 m × 0.5 m quadrats (area 0.25 m^2). Pasture composition was assessed during a NWFP wide botanical survey carried out in June 2018. Plant species in a 0.5 m × 0.5 m area were visually identified on a 50 × 50 m grid and categorised using the Domin Scale (Rodwell, 2006). Due to a relatively heterogeneous distribution of plant species across the field, botanical data were analysed for an extended footprint area of 100 m around each tower.

The pasture chemical characteristics of the three farmlets are shown in Supplementary Table 1. Snip samples were collected across a 'W' transect of the field using handheld scissors and bulked for analysis. Analysis of Total C and Total N (TC, TN) was done as described in McAuliffe et al. (2020); and pH and SOC as in Cardenas et al. (2016). Water soluble carbohydrate (WSC) concentration was determined by High Performance Liquid Chromatography (HPLC) (Agilent 1260) after extraction with milli-Q H_2O. Crude protein (CP) relative to dry matter content was determined by the calculation: %TN x 6.25 based on the mean N content of amino acids.

Data on the grazing days and general management of the farmlets were sourced from data recorded by farm staff (https://nwfp.rothamsted.ac.uk/). Animal performance data can be found in (Orr et al., 2016) and nutritional quality of the resulting product in Rivero et al. (2020).

Total N and C were measured in 2018 as part of the NWFP annual surveys. Samples were collected to 10 cm depth at 20 points and bulked for analysis using the DUMAS technique. The soil organic matter (SOM) was determined by weight loss on ignition and pH in air-dried soil (soil:water ratio 1:2.5, *w/v*).

2.6. Statistical analysis/data models/uncertainty

Statistical analyses on the gap-filled data were performed in R 4.0.2. Estimation of Monthly and Yearly cumulative values was carried out using the gap-filled data.

Estimates of the uncertainties of the CO_2 fluxes are described in Supplementary material of the annual random error summed by from the mean absolute deviation (MAD) of the half-hour random error.

2.6.1. Modelling daily data

To capture the daily patterns of CO_2 flux rates (μmol CO_2 m^{-2} s^{-1}), Gaussian non-linear models (defined below) were fitted to the CO_2 flux rates for each day per field (921 days × 3 fields = 2763 model units). As the data was not smooth within a day, the Gaussian models aimed to capture smoothed summaries of the bell-shaped daily patterns. These models can then be used to obtain more realistic estimates of features such as the daily minimum and maximum rates (further details below).

After fitting the initial models, extreme values were identified and removed from the dataset (in total 1650 values across all units with a maximum of 4 values removed from any one unit (1650/130740 = 1.26%). An extreme value was defined as a value whose studentised residual from the initial model fit was greater than 3 and also more than twice the interquartile range (IQR) from either the upper or lower quartile of the studentised residuals (i.e. < lower quartile – 2* IQR or > upper quartile + 2*IQR). In most cases it would be a single value but in some cases there were up to 4 removed. These extreme values were likely to have a large influence on the model and so they were removed to improve the overall fit of each model, aiming to increase the number of daily models that fitted well.

After removal of the extreme values, Gaussian models were re-fitted to the cleaned data. The formula for the Gaussian non-linear model is:

$$CO_2\text{–}flux = A + \left(B/\sqrt{(2\pi S^2)}\right) \times exp.\left(-(time - M)^2/(2S^2)\right),$$

with A = the baseline/night-time level, i.e. the level of the flat part of the curve; B/(S*√(2π)) + A = the estimated minimum level reached during the day (also referred to as the trough); S = measure of the "spread" of the Gaussian curve; M = the time at which the minimum level is observed. The area under the curve (AUC) of the fitted curve will give an estimate of the total daily CO_2-flux (CO_2 m^{-2} day^{-1}).

Where measurements did not follow the diurnal Gaussian pattern suggesting something unusual may have occurred, an attempt was made to identify and exclude such days from further analysis. This required some visual inspection of fitted model plots. Only model units with an adjusted R^2 > 0.7 (2532 model units) were considered in the next analysis step where it was of interest to identify which of the other measured variables could explain a significant amount of the variability in the values of the daily Gaussian curve features (night-time level, minimum level). Some model units also had incomplete explanatory data and so the results presented are of the 1546 units that had complete explanatory variable data.

2.6.2. Variable summaries

Summaries (mean, SEM, min, max) of each model feature (A, trough and AUC) were calculated across all 1546 accepted model units. Other measured variables included: day length, livestock units, soil water content (SWC), soil temperature (TS), net ecosystem productivity (NEP) and PPFD. These were treated as explanatory variables to determine if they could explain the variation in the daily Gaussian model features. As some of the explanatory variables also varied over the course of a day, the mean, range and AUC were calculated as daily summaries of these. A summary (min, max, mean) of the values of the explanatory variables as used in the models (described below) is given in Table 2.

2.6.2.1. Effect of environmental and management factors

2.6.2.1.1. Model selection – main effects of explanatory variables. Linear regression models were identified to describe the variation in each of the three Gaussian model features (night-time level, minimum level and AUC) using the set of explanatory variables listed in Table 2 plus Field and Month. Stepwise selection was applied to identify an appropriate subset of daily summaries of the explanatory variables that could explain a significant proportion of the variation in the responses. Initially a model was selected for each response variable including only main effects of the explanatory variables. To select the models the outputs of forward, backward and full stepwise selection were all considered, inspecting the AIC and adjusted R^2 values.

2.6.2.1.2. Model selection – interactions with field. In a second step of stepwise selection, to further investigate the potential effects of the explanatory variables identified in the first step, regression models were selected to always include "field" and allowed for interactions of the explanatory variables (as selected in the first step) with "field". The final selected models were then used to predict the expected value of each feature at specific values of the explanatory variables. The values of the explanatory variables used for these predictions are given in Supplementary Table 2 and the resulting predicted values are given in Supplementary Table 6.

3. Results

3.1. Climate data

Due to topography, generally stronger winds were detected in the HS field mainly from a westerly and south westerly direction while windspeeds were less strong in the HSC field, but also from a westerly or south westerly

Table 2
Summary of the ranges of explanatory variables as used in the models during the second step of stepwise selection (daily values used to determine ranges, for model explanation see text).

Explanatory variable	Minimum value	Mean value	Maximum value
daylength (h)	7.95	12.34	16.51
livestock units[a]	0.0	5.70	27.86
mean SWC (%)	9.83	40.51	60.21
range SWC (%)	0.0	1.35	24.13
mean TS (°C)	0.95	11.35	20.95
range TS (°C)	0.0	1.24	5.69
mean NEP (μmol CO_2 m^{-2} s^{-1})	−14.62	2.0017	28.43
range NEP (μmol CO_2 m^{-2} s^{-1})	0.031	17.39	88.21
AUC NEP (mol CO_2 m^{-2} d^{-1})	-2	−0.024	0.87
mean PPFD (μmol m^{-2} s^{-1})	−3.80	346.39	1538.15
range PPFD (μmol m^{-2} s^{-1})	0.01	791.03	1859.30
AUC PPFD (mol m^{-2} d^{-1})	−0.238	17.4	64

[a] Livestock units per day are: cattle = 0.65, sheep = 0.11 and lamb = 0.04 per animal (*pers. comm. North Wyke farm).*

direction. The PP field showed strong winds at a slightly lesser frequency, but mainly from a westerly to north westerly direction (Supplementary Fig. 1).

At field level, soil temperature (Fig. 3) was generally similar between the years increasing from around April to the middle of July and decreasing again with lower winter temperatures, reaching 3 °C in 2017, and around 1 °C in 2018. The highest temperatures were 22 °C in 2017 and 24 °C in 2018. PP and HS were similar with smaller ranges (about 17 °C between minimum and maximum), whilst HSC had generally higher temperatures and had more extreme values in both years (22 and 24 °C between minimum and maximum in 2017 and 2018, respectively) with larger variability in the warmer months.

Hourly rainfall is shown in Fig. 4 with few small events in the 2018 summer and more events larger than 2 mm the rest of the year, in 2017 there was a more homogeneous distribution of events and they were mostly less than 2 mm.

The annual rainfall on the site was 815 mm in 2017 and 999 mm in 2018 (from a nearby meteorological station). Although 2018 had more rain, the distribution on a monthly basis (Supplementary Fig. 2) gave more extremes with wetter winter months and drier summer months (May until September), the exception was February with 60 mm in 2017 and 40 mm in 2018. The extreme was in June, i.e. whilst there were 66 mm in 2017, there was only 0.87 mm in 2018.

Soil moisture was similar in the HS and PP fields, but it was generally lower in the HSC in the winter months in 2017 and all of 2018 (Fig. 4) given with field level rainfall. In 2017 soil moisture in HSC and PP were similar and lower than HS in the summer and autumn months. TS was similar in most cases except for HSG in autumn in 2017 and 2018, and spring/summer in 2018 where the TS was higher than in the other 2 treatments (10–23% higher).

Values of PPFD reached 1859.3 μmol m^{-2} s^{-1} (Table 2). The maxima were generally higher in 2018 compared to 2017 in all three pastures. The monthly maximum and minimum values (AUC, Supplementary Table 2) were similar for PP and HSC, but for HS they were higher in 2018.

The response of GPP to incoming PPFD across the range of sites were analysed for one-month periods related to grazing/non-grazing (Fig. 5 and b). Data availability were limited at the HS site for the 2017 spring non-grazing period due to sensor failure (missing PPFD), however, all models fits (sites and periods) remained highly significant ($p < 0.0001$). Data availability for the model fitting (where both GPP and PPFD were present) within each of the sites and study periods can be seen in Supplementary Table 4.

Supplementary Table 3 reports the individual values for LUE and Amax across all sites and study periods. Amax and LUE were generally higher in 2017 than in 2018 with Amax being greater under no-grazing conditions compared to grazing (35.7 ± 5.1 vs 24.6 +/− 3.8 μmol CO_2 m^{-2} s^{-1}). However, LUE was generally higher under grazing conditions compared to non-grazing (0.07 ± 0.01 vs 0.05 ± 0.002 μmol CO_2 $μmol^{-1}$ PPFD). Table 3 shows a summary of Amax and LUE by year and site. Both the highest and lowest Amax was seen at HSC, highest during 2017 (45.7 ± 3.2 μmol CO_2 m^{-2} s^{-1}) where HSC also showed the highest LUE (0.08 ± 0.02 μmol CO_2 $μmol^{-1}$ PPFD) and the lowest during 2017 (17.6 ± 5.3 μmol CO_2 m^{-2} s^{-1}) though the lowest LUE for this year was seen at PP (0.04 ± 0.01 μmol CO_2 $μmol^{-1}$ PPFD).

To make comparisons of photosynthetic activity between plots it is necessary to take account of the large differences in GPP that occur under changing light. To do this, parameters from the individual light response curves were used to predict GPP for PPFD fixed to 600 μmol m^{-2} s^{-1} (GPP_{600}), Fig. 6 shows the results of this. Values (and 95% confidence intervals) can be found inset on corresponding plots in Fig. 5a and b (and in Supplementary

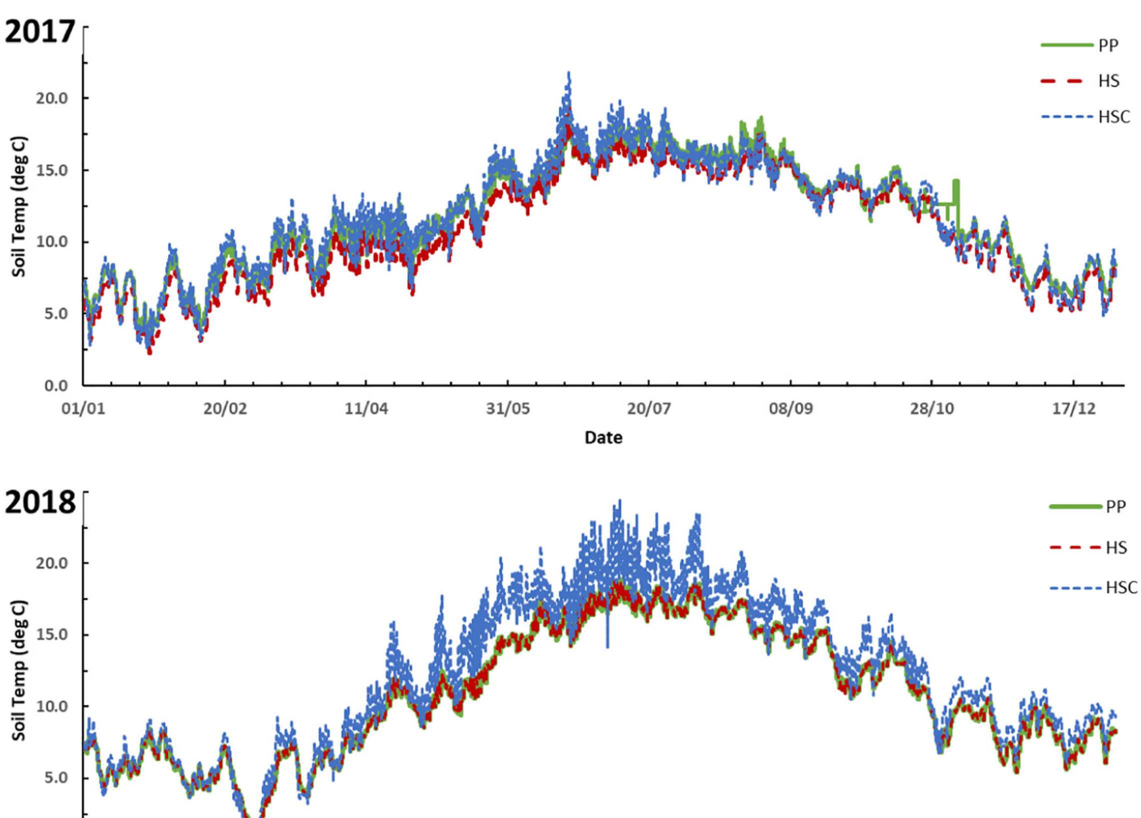

Fig. 3. Soil temperature at 10 cm depth of the three fields Permanent Pasture (PP), High Sugar grass (HS), High sugar grass/ white clover mix (HSC) during 2017 (top graph) and 2018 (bottom graph).

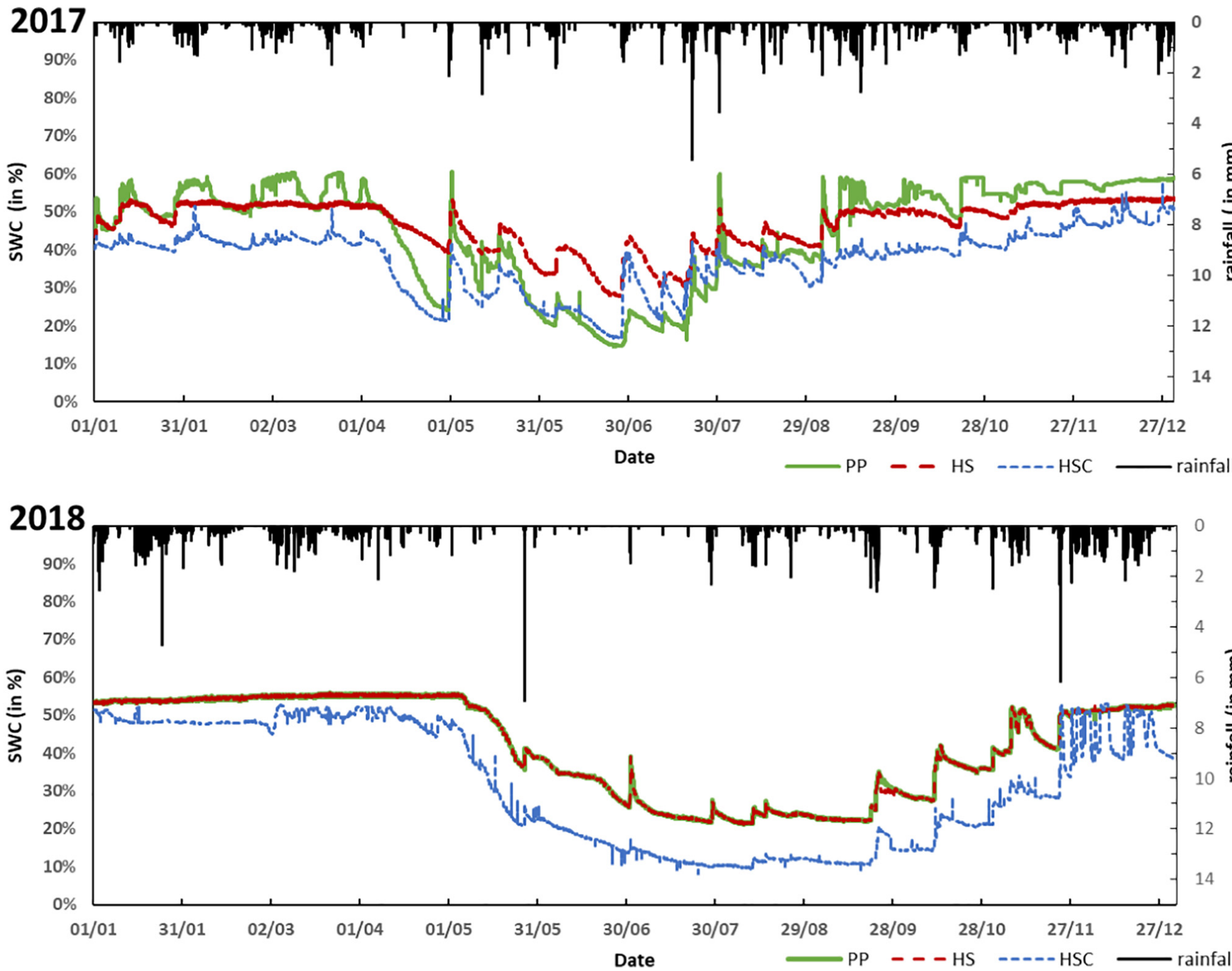

Fig. 4. Soil water content (SWC in %) at 10 cm depth of the three fields Permanent Pasture (PP), High Sugar grass (HS), High sugar grass/ white clover mix (HSC) and hourly total rainfall in mm; 2017 (top graph) and 2018 (bottom graph)

Table 5). Corresponding to the results for Amax, across all sites GPP_{600} was higher in 2017 compared to 2018 (20.0 [19.3, 20.5] vs 11.7 [11.1, 12.1] μmol CO_2 m^{-2} s^{-1}) and higher for non-grazed rather than grazed periods (18.4 [17.5, 18.9] vs 13.4 [12.8, 13.7] μmol CO_2 m^{-2} s^{-1}). Comparing between sites, GPP_{600} was highest for HSC (17.4 [16.7, 17.8] μmol CO_2 m^{-2} s^{-1}) and lowest for HS (14.9 [14.1, 15.3] μmol CO_2 m^{-2} s^{-1}). However, the result for HSC appears to be driven primarily by the winter non-grazing period in 2017 where HSC GPP_{600} (29.8 [28.9, 30.6] μmol CO_2 m^{-2} s^{-1}) was almost double the mean of the other two sites for the same period (15.9 [15.6, 16.2] μmol CO_2 m^{-2} s^{-1}). Comparing between season and grazing status, the lowest overall GPP_{600} was from the summer grazing period (7.8 [7.4, 8.0] μmol CO_2 m^{-2} s^{-1}) while the highest was from spring with no-grazing (22.6 [21.5, 23.2] μmol CO_2 m^{-2} s^{-1}). Grazing reduced the spring period GPP_{600} to 15.5 [14.8, 15.9] μmol CO_2 m^{-2} s^{-1}). Values in square brackets show 95% confidence intervals of the model estimate.

3.2. Soil, Pasture characteristics and grazing management

Supplementary Table 1 shows soil pH was similar in all pastures every season, except HS in the spring that was slightly lower. Soil TC, TN and SOM were always lower in the spring. Soil TC and SOM in PP were up to 34% and 30% higher than the other pastures, respectively. TN was up to 35% higher in PP. All pastures had similar soil C/N ratios (~10), with the lowest in HSC in the autumn.

The results for each study field from the botanical survey carried out in June 2018 are shown in Fig. 1. The percentages of plant species in the three pastures which would have a direct effect on CO_2 fluxes measured by the respective tower are shown in supplementary Fig. 3. There were similar proportions of sown grass in HS and HSC and less in PP; higher proportion of weeds in PP and clover in HSC, as expected.

The chemical composition of the three pastures snip samples showed the WSC percentage was 15.5 ± 5.35, 10.1 ± 3.29 and 11.2 ± 2.83, for HS, HSC and PP, respectively, for samples taken after 2015 until 2017. The CP percentage was 18.4 ± 3.71, 22.5 ± 3.91, 21.2 ± 3.77, respectively. The results in Supplementary Table 1 show that TC was similar in all pastures in spring and summer; values increased in the autumn except in the HS pasture. TN was lowest in the summer, resulting in higher C/N ratios, and between 13 and 17% lower in HSC compared to PP and HS. C/N ratios in spring and autumn were similar in all pastures, except for the HSC pasture in spring which was slightly higher.

Fig. 5. a: Light response curves (PPFD vs. GPP, dotted lines determined as second degree polynomial trendlines) of the three pastures Permanent Pasture (PP), High Sugar grass (HS), High sugar grass/ white clover mix (HSC) determined for three one-month long periods in 2017. None-gap filled data coverage was below 4% for HS during the April/May grazing period and therefore omitted from the analysis b: Light response curves (PPFD vs. GPP, dotted lines determined as second degree polynomial trendlines) of the three pastures Permanent Pasture (PP), High Sugar grass (HS), High sugar grass/ white clover mix (HSC) determined for three one-month long periods in 2018.

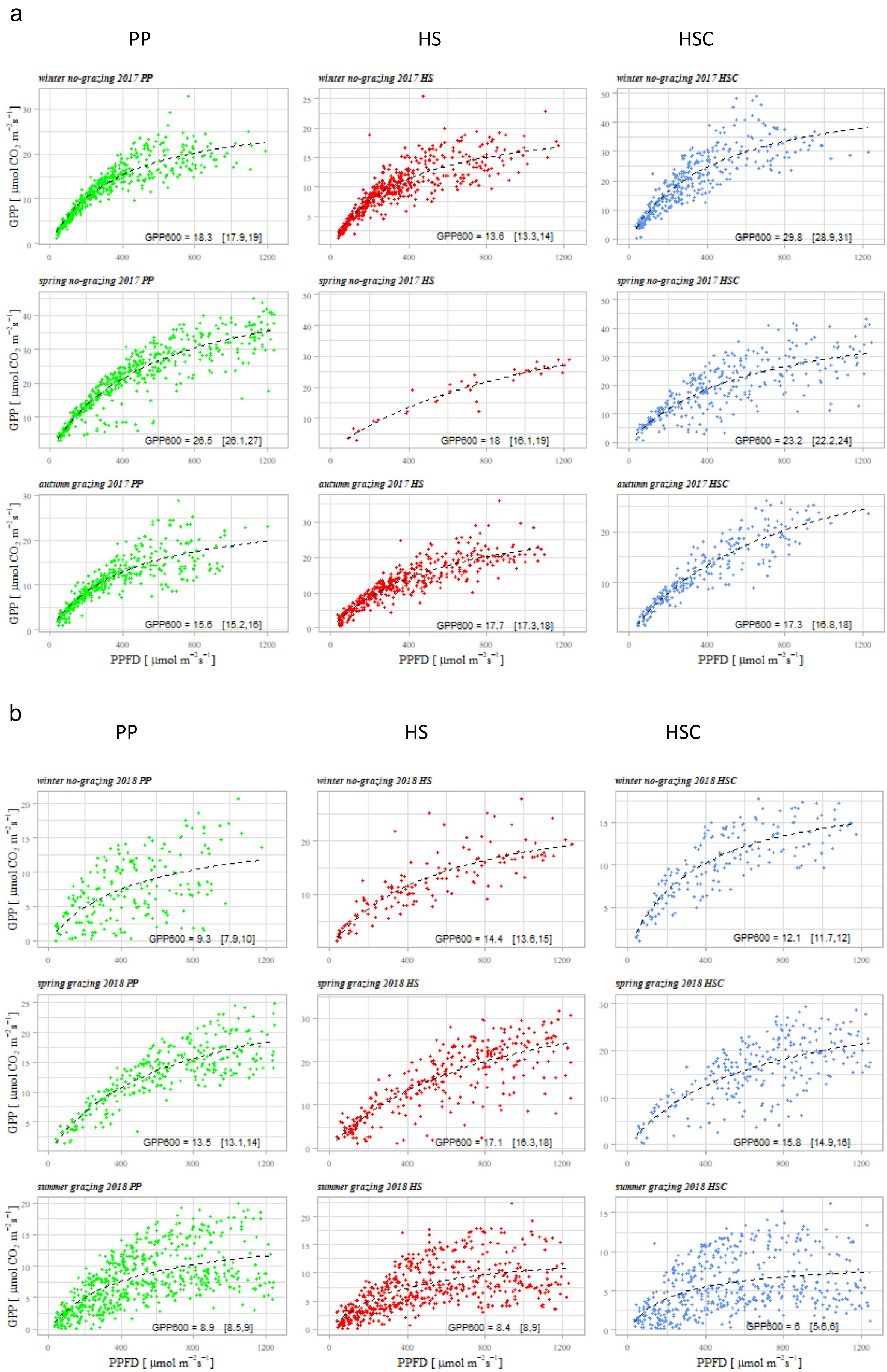
a
PP
HS
HSC
winter no-grazing 2017 PP
GPP600 = 18.3 [17.9,19]
winter no-grazing 2017 HS
GPP600 = 13.6 [13.3,14]
winter no-grazing 2017 HSC
GPP600 = 29.8 [28.9,31]
spring no-grazing 2017 PP
GPP600 = 26.5 [26.1,27]
spring no-grazing 2017 HS
GPP600 = 18 [16.1,19]
spring no-grazing 2017 HSC
GPP600 = 23.2 [22.2,24]
autumn grazing 2017 PP
GPP600 = 15.6 [15.2,16]
autumn grazing 2017 HS
GPP600 = 17.7 [17.3,18]
autumn grazing 2017 HSC
GPP600 = 17.3 [16.8,18]
GPP [µmol CO2 m−2 s−1]
PPFD [µmol m−2 s−1]
b
PP
HS
HSC
winter no-grazing 2018 PP
GPP600 = 9.3 [7.9,10]
winter no-grazing 2018 HS
GPP600 = 14.4 [13.6,15]
winter no-grazing 2018 HSC
GPP600 = 12.1 [11.7,12]
spring grazing 2018 PP
GPP600 = 13.5 [13.1,14]
spring grazing 2018 HS
GPP600 = 17.1 [16.3,18]
spring grazing 2018 HSC
GPP600 = 15.8 [14.9,16]
summer grazing 2018 PP
GPP600 = 8.9 [8.5,9]
summer grazing 2018 HS
GPP600 = 8.4 [8,9]
summer grazing 2018 HSC
GPP600 = 6 [5.6,6]
GPP [µmol CO2 m−2 s−1]
PPFD [µmol m−2 s−1]

Table 3
Summary of maximum assimilation (Amax)[a] and light use efficiency (LUE) by site and year (± S.E.)

Year	Site	Amax[a] [μmol CO_2 m^{-2} s^{-1}]	LUE [μmol CO_2 $μmol^{-1}$ PPFD]
2017	PP	35.7 ± 7.5	0.078 ± 0.09
2017	HS	38.0 ± 10.3	0.054 ± 0.005
2017	HSC	45.7 ± 3.2	0.083 ± 0.02
2018	PP	18.3 ± 2.8	0.043 ± 0.006
2018	HS	25.5 ± 6.8	0.049 ± 0.004
2018	HSC	17.6 ± 5.3	0.056 ± 0.006

[a] The PPFD threshold used to compare modelled GPP between management periods is actually relatively arbitrary, it is simply a value to normalise the comparison. A value of 600 μmol CO_2 m^{-2} s^{-1} was chosen as it represents a reasonable value for growing season sunshine in this location and would give a high enough GPP for comparison. This value of 600 μmol CO_2 m^{-2} s^{-1} was used in refitting Eq. (1) (Supplementary Material) with LUE and Amax specific to each management period (previously derived using Eq. (1) (Supplementary Material) from the measured GPP within those periods).

Fig. 7 shows the grazing periods for the 3 pastures and their different treatments. Cattle grazed the longest in 2017 compared to sheep (ewes and lambs) but grazing started late in the season, especially in the PP and HSG pastures. In 2018 cattle started grazing in May but were removed at the end of June and put back on in the autumn, in September for the PP and HSC pastures, and October for the HS pasture. Sheep and lambs grazed for longer in 2018, and in both years they grazed late in winter (Supplementary Fig. 4 shows the grazing densities for the 3 pastures and 2 years).

3.3. CO_2 fluxes

3.3.1. Daily CO_2 fluxes

The daily CO_2 fluxes (g m^{-2} day^{-1}) are shown separated by field and year with shaded areas marking times when animals were grazing (data in Fig. 7). The fluxes were less variable in 2018 and in the winter months in both years. Fig. 8 shows the diurnal cycle of CO_2 fluxes with generally more uptake (negative fluxes) during the day as well as during the summer months (predominantly the grazing season) and more release (positive fluxes) during night times as well as during winter months (predominantly outside of grazing). The amplitude of the daily fluxes was larger in 2017 (range about −30 to 30 μmol CO_2 m^{-2} s^{-1} compared to 2018 (−20 to 20 μmol CO_2 m^{-2} s^{-1}).

3.3.2. Cumulative CO_2 fluxes

Cumulative monthly fluxes during both years (Fig. 9, Table 4) (calculated as sums of AUCs across the fitted models) show similar patterns with negative totals between February and June for PP (−0.343 to −4.165 t CO_2 ha^{-1}) and HSC (−0.236 to −3.148 t CO_2 ha^{-1}) in 2017, and February and July for HS (−0.079 to −3.176 t CO_2 ha^{-1}). A further negative flux appears in September and November in PP (−0.275 t CO_2 ha^{-1}) and HS (−0.594 t CO_2 ha^{-1}), respectively. In 2018, negative fluxes were only between February and April (−0.606 to −3.408 t CO_2 ha^{-1} in all three pastures) except for HS and HSC where a further negative flux appeared in June (−0.624 and − 0.240 t CO_2 ha^{-1}, respectively). HS fluxes had larger negative values in late winter (−1.504 t CO_2 ha^{-1}) and slightly larger in the summer in 2018 (3.611 t CO_2 ha^{-1}), but not in 2017 when HSC was largest (3.125 t CO_2 ha^{-1}) (Table 4).

Annual net CO_2 fluxes varied (Table 5). Summed up over the whole year, PP and HS in 2017 were net carbon sinks of −5.40 and − 4.82 t CO_2 ha^{-1} yr^{-1}, respectively, whereas HSC was a net source (+3.92 t CO_2 ha^{-1} yr^{-1}). In 2018, all three sites were net sources of CO_2, ranging from +3.91 to +6.17 t CO_2 ha^{-1} yr^{-1}. Over both years, PP and HS were nearly carbon neutral, but significantly larger than the estimated uncertainty, whereas HSC was a significant source of CO_2 (+8.02 t CO_2 ha^{-1}).

3.4. Model results

In order to characterise parameters from the flux data, we calculated the mean, standard error of the mean (SEM), minimum and maximum values for A (baseline/night-time level), trough (minimum value) and AUC from the fitted daily Gaussian models. The results for A and trough are below in μmol CO_2 m^{-2} s^{-1} (multiply by 12 × 864 × 10^{-6} to convert to tonnes

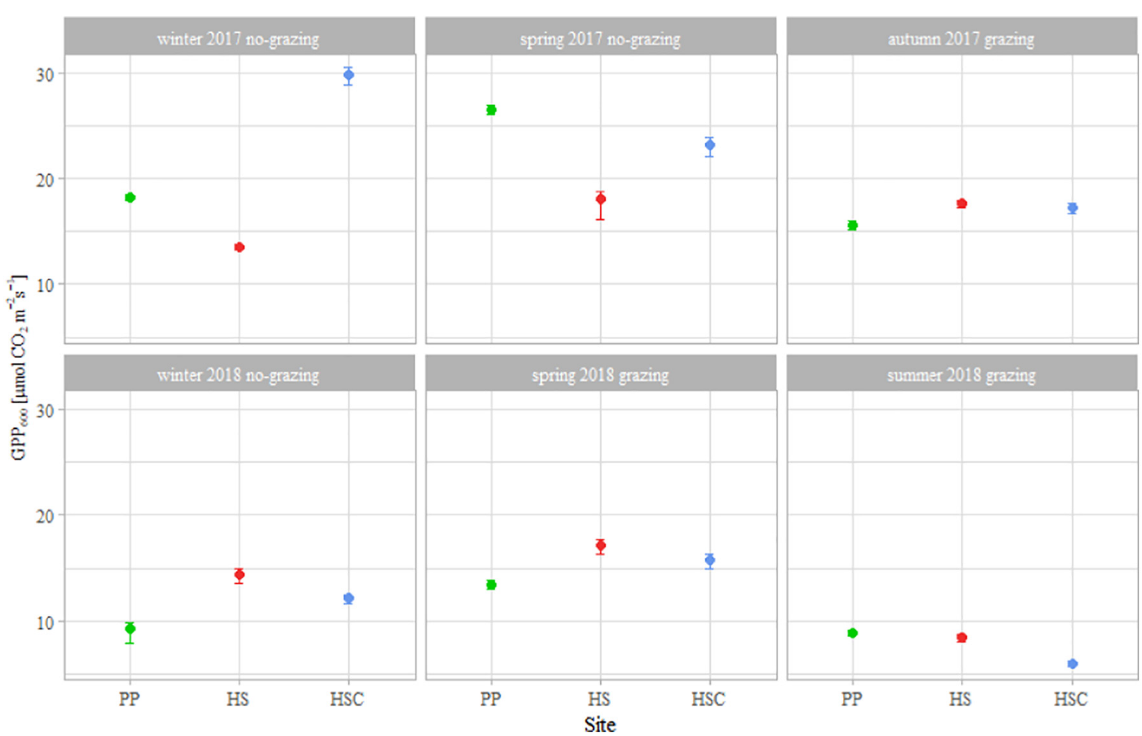

Fig. 6. Comparison across sites and study periods for GPP (μmol CO_2 m^{-2} s^{-1}) estimated at PPFD = 600 μmol m^{-2} s^{-1}. Error bars show 95% confidence intervals. Permanent Pasture (PP), High Sugar grass (HS), High sugar grass/white clover mix (HSC).

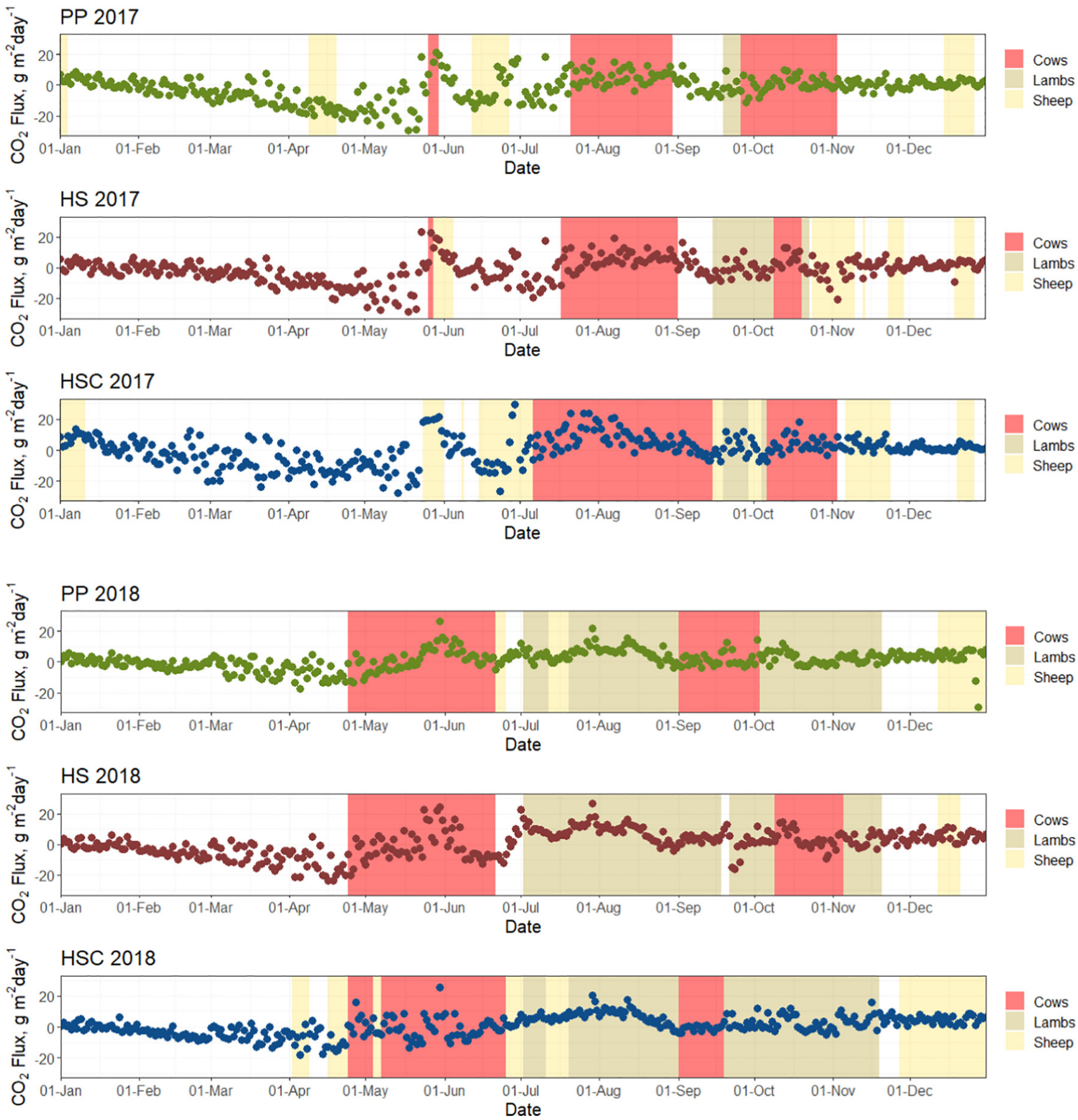

Fig. 7. Daily CO_2 fluxes separated by year and by the three fields Permanent Pasture (PP), High Sugar grass (HS), High sugar grass/ white clover mix (HSC). Shaded areas mark times when animals were on the fields (cows and sheep, including ewes and lambs). Supplementary Fig. 3 shows grazing densities (number of individuals per day) for all pastures and both years.

C ha^{-1} d^{-1}); and AUC in μmol CO_2 m^{-2} d^{-1} (multiply by 12×10^{-8} to convert to tonnes C ha^{-1} d^{-1}):

i) The baseline/night-time level (A, μmol CO_2 m^{-2} s^{-1}): mean = 6.44, SEM = 0.13, minimum = 0.88, maximum = 108.05;
ii) The minimum value (trough, μmol CO_2 m^{-2} s^{-1}): mean = −10.95, SEM = 0.17, minimum = −41.91, maximum = 3.52;
iii) The area under the curve (AUC, μmol CO_2 m^{-2} d^{-1}): mean = −17,329.19, SEM = 4575.94, minimum = −724,206.3, maximum = 577,328.5.
iv) The time of the day at the mean value (M): mean = 12:25:03, SD = 40 min 49 s, minimum = 05:19:04, maximum = 18:35:19.

Following the stepwise analysis, terms included in the final linear regression models selected for each of A, trough and AUC, the summaries of explanatory variables by field, year and month (used for predictions) and the predictions from final linear models are given in Supplementary material (Supplementary Tables 6, 7, 8 and 9).

The results of the model showed that Field (pasture type), Month and the interaction Field:Month showed highly significant effects ($P < 0.001$) on the values of the night-time level, minimum value and AUC.

4. Discussion

We tested 3 hypotheses in order to assess the potential for different pastures to reduce net losses of CO_2 from a grassland in temperate climate. These are discussed as stated in the introduction section:

Permanent pasture systems with intensive grazing as a net sink of CO_2.

The net CO_2 fluxes for the PP treatment resulted in a change between both years from a sink in 2017 to a source in 2018. The net total at the end of the period was a small source of 0.76 t CO_2 ha^{-1}. Most of the published data corresponds to pasture conversion (Rutledge et al., 2017) but Wall et al. (2019) reports a mean value for a rotationally grazed sward of mainly perennial ryegrass (*Lolium perenne*) of 71 (±77) g C m^{-2} yr^{-1}

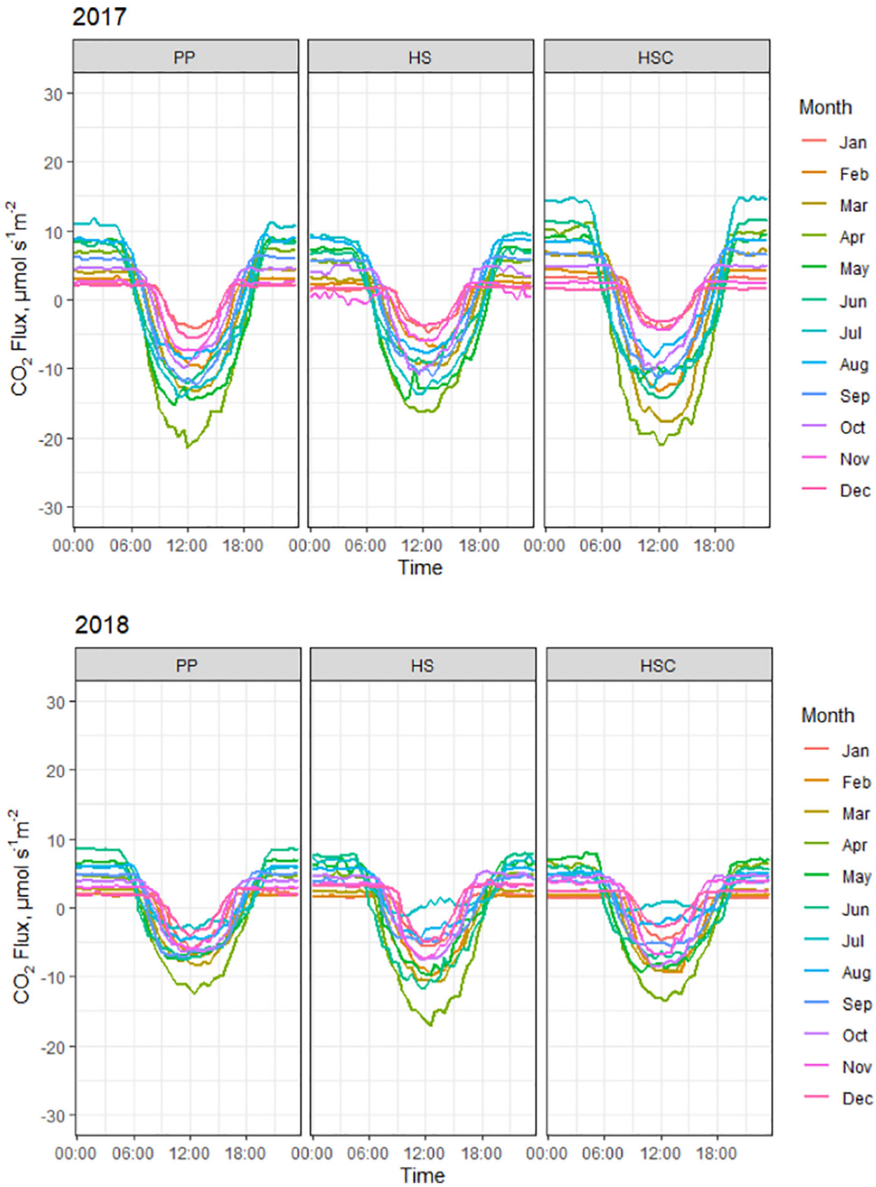

Fig. 8. Average monthly diel curves of CO_2 fluxes separated by year and by the three fields Permanent Pasture (PP), High Sugar grass (HS), High sugar grass/white clover mix (HSC).

(2.82 t CO_2 ha^{-1} yr^{-1}) for 3 years, highly variable but positive in the 3 years. The mean CO_2 flux is within the range of our net fluxes. The mean annual temperature and precipitation for the site were 13.3 °C, and 1250 mm, respectively, based on 30-year (1981–2010) averages.

Reseeded permanent pasture systems with deep-rooting, high-sugar grass varieties or a mix of high-sugar grass and clover effect on net sequestration of CO_2.

Our night-time fluxes of 6.44 μmol CO_2 m^{-2} s^{-1} are within the range of other studies on grassland (Jerome et al., 2014) reporting values between 0 and 15 μmol CO_2 m^{-2} s^{-1}. It agrees with Wohlfahrt et al. (2008) night-time value of 6 μmol CO_2 m^{-2} s^{-1}. The daytime trough in our study was also similar (−10.95 μmol CO_2 m^{-2} s^{-1}) to the study by Wohlfahrt et al. (2008) average midday value of −10 μmol CO_2 m^{-2} s^{-1}. The latter study comprised nine grassland sites that represented the major mountain regions of Europe. These sites encompassed a latitudinal range of 42–68° and an elevation range of 270–1770 m.a.s.l.

The pastures in 2017 were as expected to be a net sink between early spring and summer when the grass is growing; and net sources of emissions the rest of the year due to less or no grass growth, less photosynthesis due to shorter daylight and higher relative respiration. Changes from net sink in the spring to net sources in the summer and autumn have been reported for ryegrass-clover pastures under warm temperate climate, with mean annual temperature and precipitation (1980–2010) of 13.3 °C and 1249 mm respectively (Rutledge et al., 2017). Wall et al. (2019) observed in a dairy farm rotationally grazed during three years the same general pattern of strongest daytime CO_2 uptake in spring to early summer, with a period in summer of minimal CO_2 uptake associated with the much lower soil moisture contents.

In 2018 in our study the fields were sinks only 3–4 months in the year around February–April with the rest of the year as sources most likely as a response to the drought that year (see higher temperatures and lower soil moisture in 2018 in Figs. 3 and 4). The difference between 2017 and 2018, with 2017 having larger and longer period of negative fluxes than 2018 (except for HSC), while in 2018 all sites have a long period of positive fluxes in Autumn/Winter, are likely due to climate affecting crop development.

The trend of annual fluxes as they change from net sink to net source in some pasture year combinations in our study are not generally consistent

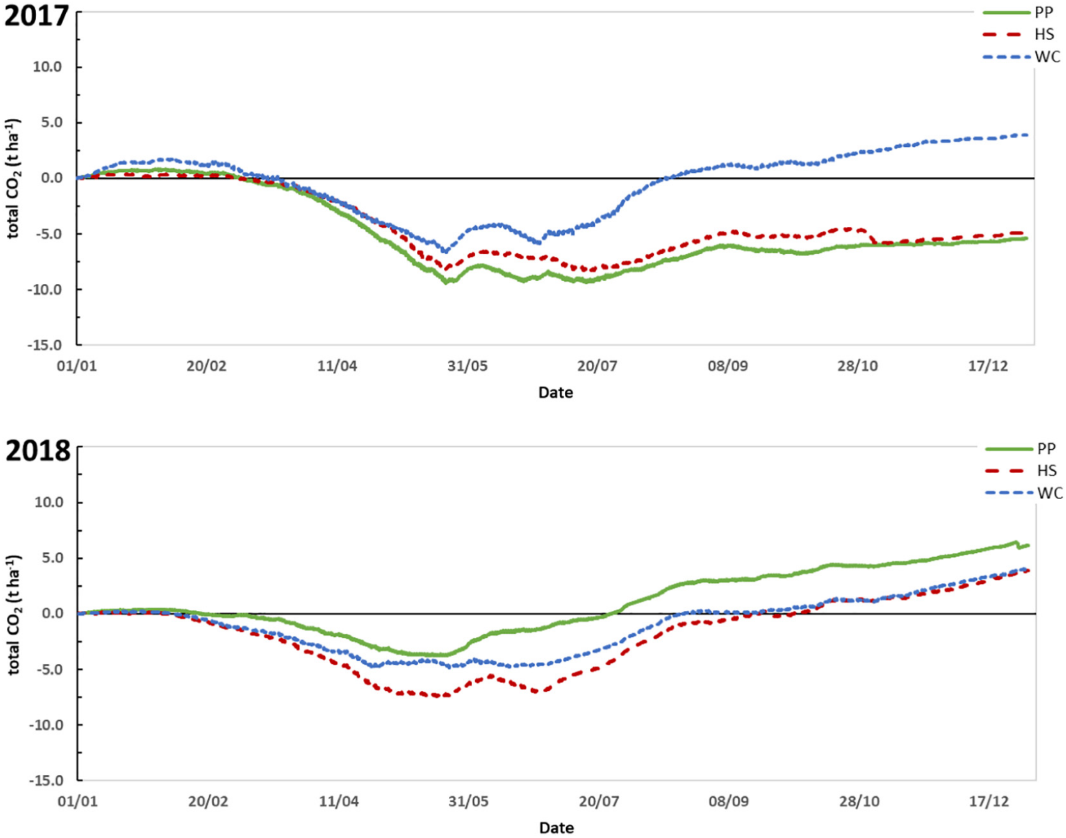

Fig. 9. Cumulative CO_2 fluxes separated by year and by the three fields Permanent Pasture (PP), High Sugar grass (HS), High sugar grass/ white clover mix (HSC).

Table 4
Monthly cumulative fluxes (NEE of CO_2) per treatment (Area under the curve [AUC] calculations from gap-filled flux data).

Month	Cumulative PP 2017 [t CO_2/ha]	Cumulative HS 2017 [t CO_2/ha]	Cumulative HSC 2017 [t CO_2/ha]	Cumulative PP 2018 [t CO_2/ha]	Cumulative HS 2018 [t CO_2/ha]	Cumulative HSC 2018 [t CO_2/ha]
Jan	0.789	0.296	1.656	0.391	0.095	0.142
Feb	−0.500	−0.160	−0.675	−0.606	−1.504	−1.363
Mar	−1.866	−1.341	−2.058	−1.120	−2.072	−1.537
Apr	−4.165	−3.176	−3.148	−1.925	−3.408	−1.681
May	−2.384	−2.579	−0.416	0.691	0.763	0.191
Jun	−0.343	−0.079	−0.236	1.384	−0.624	−0.240
Jul	0.123	−0.593	3.125	2.022	3.611	2.469
Aug	1.977	2.322	2.588	2.096	2.318	2.201
Sep	−0.275	0.103	0.541	0.476	0.679	0.187
Oct	0.589	0.284	0.931	0.830	1.266	0.775
Nov	0.094	−0.594	0.968	0.869	0.969	1.389
Dec	0.508	0.652	0.583	1.020	1.776	1.527

with Wall et al. (2020) in New Zealand pastures with mean annual temperature and precipitation of 13.3 °C, and 1249 mm, respectively as they report seven years of data with positive mean NEP values between 108 and 436 g C m^{-2} yr^{-1}. In that study, the grazing intensity was much larger (> 150 cows ha^{-1}) compared to our study (30 cows ha^{-1} and 90 sheep ha^{-1}) but for a shorter period (< month). In another study, Wall et al. (2019) observed for a 3-year period, fluxes between 164 and 364 g C m^{-2} yr^{-1} when excluding grazing. Contrasting results are presented from a study in 14 European grasslands using EC and chambers, where it was found that grasslands were on average a CO_2 sink (−1783 to −91 g CO_2 m^{-2} yr^{-1}), with CO_2 dominating the annual budget when also considering CH_4 and N_2O (Hortnagl et al., 2018). Our negative yearly fluxes (−540 to −91 g CO_2 m^{-2} yr^{-1}) fit in this range. The release values in 2018 agree with expected values for grasslands of 5 t CO_2 m^{-2} yr^{-1} (Raich and Schlesinger, 1992).

Table 5
Total cumulative CO_2 emissions (NEE of CO_2) (t CO_2 ha^{-1} y^{-1} and field three fields Permanent Pasture (PP), High Sugar grass (HS), High sugar grass/ white clover mix (HSC) (uncertainties in brackets).

	2017	2018	Total
PP	−5.40 (±0.08)	6.17 (±0.08)	0.76
HS	−4.82 (±0.11)	3.91 (±0.09)	−0.91
HSC	3.92 (±0.11)	4.10 (±0.11)	8.02

It is well established that leaf level photosynthetic characteristics vary within and between individuals, species and functional types (Oberbauer and Oechel, 1989; Shaver and Chapin, 1995; Street et al., 2007). Generally, the emissions from the 3 pastures in our study behaved similarly in the winter months, probably due to the lack of plant growth not affecting respiration and photosynthesis, so the effect of the pasture type was not relevant. Seneviratne et al. (2010) reported that in central and Northern Europe in contrast to the Mediterranean region, radiation limits evapotranspiration and not soil moisture which might explain these results. In the spring and summer months there were differences between pastures, particularly the HS treatment in 2018 which was a larger sink compared to the other swards in the spring, and larger source in late summer, probably due to the effect of this high sugar grass on the atmospheric CO_2 balance in soil. In fact, we found that HS treatment contained about 50% more WSC than the other 2 pastures. C translocation by pastures can reach up to 80% of assimilates (Kuzyakov and Domanski, 2000) and from several studies with *L. perenne* it is reported that an average of 40% of all below ground C is converted to CO_2. It is possible that the HS pasture releases more C rhizo-deposits in soil, increasing the potential for CO_2 losses. This did not happen in 2017 possibly as this year was wetter in the summer months, resulting in slower diffusion rates decreasing the soil-atmosphere fluxes. Also, the higher temperatures in 2018 could have promoted higher microbial decomposition of OM and mineralisation rates due to drier weather (Rex et al., 2021).

The overall net flux for HSC was the largest of the three pastures, even though it received the least N, in disagreement with previous studies in arable crops that report that soil respiration was higher with N application, explained as the result of larger biomass and plant N concentrations (Johansson, 1992; Yang et al., 2010). We have no direct measure of biomass, but animal performance at the same stocking density was comparable (Orr et al., 2019), and the N content agrees with lower values in the HSC pasture. However, the results in Yang et al. (2010) from diurnal variation of the fluxes differed between arable crops and grasslands, where higher fluxes were recorded in the day and lower at night on the former indicating that the balance between nutrients and soil moisture differ between arable crops and grasslands.

The lower CP values measured in HS agree with published data where higher WSC in pasture corresponded to lower CP (Staerfl et al., 2012). Although the botanical survey carried out in 2018 generally did not seem to show significant differences in % coverage between PP and HS for some major species; there were some differences in minor species that could have been reflected in the resulting differences in chemical composition. The % coverage for *Trifolium repens* in HSC agrees with the target of 30%, and no *T. repens* was found in the other 2 pastures as expected. The TN of the HSC was between 13 and 21% lower than the other 2 pastures for the 3 seasons (Supplementary Table 1) potentially reflecting the lack of fertiliser N applied and less N uptake by the plant but its CP content was higher (6 and 19% higher compared to PP and HS, respectively) in disagreement with previous studies such as Cunha et al. (2001) who found that increase in N fertiliser increases CP in the plant. However, the study by Cacan (2018) showed the highest CP was not on the highest N rate treatment, but the rates were small and there was effect from the phosphorus applied.

The different trends in the annual net fluxes between PP and HS fields (from a net sink in 2017 to a net source in 2018) and the HSC field (net source both years), would suggest that the pastures had an influence in the atmospheric CO_2 balance. The model results also confirm an effect of pasture, as well as time of the year (month) and the interaction between these 2 factors. Some feedback mechanisms can influence the effect of pasture, as for example soil moisture in the HSC was lowest in both years and all year (Fig. 4), with lower soil moisture promoting mineralisation and potential resulting in increased emissions.

The differences between treatments, although not consistent, i.e. no site stands out in terms of consistently being “better” than the others, show a small sink potential for the HS pasture when taking the net of both years.

Predictability of annual carbon fluxes according to environmental conditions and pasture management

The model showed that pasture type and time of the year and their interaction influenced the fluxes. In addition, the climate data did not show great differences between the three study fields as expected – only soil temperature was slightly higher in HSC, especially on 2018, most likely due to the fact that this field is on a south facing slope. The trend in SWC (opposite to soil temperature and lower in HSC especially in 2018), is most likely due to the same reason.

Drought has been linked to ecosystems behaving as net sources of CO_2 but this depends on the physiological response to limited plant available water and on structural changes in the vegetation during the drought (van der Molen et al., 2011). Physiological responses of the vegetation to drought include reductions in enzymatic activities as well as stomatal closure to prevent water loss (van der Molen et al., 2011).

In addition, the smaller variability in the fluxes observed in 2018 does not correspond to the larger temperature range (~25 °C) compared to 2017 (~17.5 °C), nor to the range in SWC values experienced that year (Figs. 3, 4). Although the total rainfall in 2018 was larger than 2017, the contrasting rainfall pattern, with larger monthly totals in the winter months and early spring compared, with the summer months having much less or almost no rain, can explain these differences in the fluxes.

The lower SWC in the HSC pasture (except in the summer months) with higher ST in 2017 could have increased mineralisation resulting in consistent higher fluxes compared to the other 2 pastures (which resulted in net sinks). In 2018, HSC was drier all year, reaching very low SWC (10%) due to the drought that year but the resulting net fluxes were similar to HS, probably due to compensation between lower negative fluxes, and larger positive fluxes in HS. The larger net flux in PP cannot be explained by SWC in 2018, as SWC values were similar to HS. The overall inclusion of clover resulting in a net source for the 2 years disagrees with findings in ungrazed grasslands where plant diversity is expected to increase C sequestration as a result of increased plant production and C inputs to the soil via roots, see Rutledge et al. (2017). Their study stated that periodic pasture renewal temporarily reduces the CO_2 sink strength (or can become a CO_2 source). They also found that a large proportion of CO_2 taken up from the atmosphere was respired back during the post renewal year compared to the year before.

We are not able to confirm whether the livestock were within the footprint of the tower. Although, previous studies have used various methods to account for cows respirations including the use of GPS sensors, measuring fluxes with the animals under confinement, and measuring C intake (Jerome et al., 2014); having accurate records for the whole grazing period is labour intensive and in the case of beef cattle, it requires bringing the animals to handling facilities to change batteries or download data if using sensors, ideally we would try to increase the footprint to enclose the livestock in that area. Jerome et al. (2014) estimated that respiration from cows comprised ~8% of the total ecosystem respiration, and so it is not a major component of the flux.

5. Conclusions

We present 2 years of CO_2 measurements on three pastures, namely a permanent pasture (PP), a high sugar grass (HS) and the same high sugar grass mixed with clover (HSC). The net CO_2 fluxes for the PP and HS treatments resulted in a sink in 2017 and a source in 2018, resulting with a total net at the end of the period as a small source for PP and small sink for HS. HSC in contrast was a net source both years, suggesting that the pastures had an influence in the atmospheric CO_2 balance.

The detailed statistical modelling revealed that Field (pasture type), Month and the interaction Field:Month had highly significant effects ($P < 0.001$) in the night time CO_2 rate, minimum rate at approximately midday and the area under the curve (daily fluxes). The soil temperature was generally warmer in 2018 with larger rainfall overall but with more extremes and larger PPFD values which seemed to have influenced the atmospheric CO_2 balance resulting in net CO_2 loss. The light use efficiency and GPP were higher in 2017 which combined with slightly cooler weather might have caused a net C gain for 2 of the pastures, but still a loss for HSC.

The results don't confirm that in the short-term permanent pastures neither that reseeding these pastures result in a sink of CO_2 and it is more likely that the seasonal and inter annual variability are more important on the net fluxes.

This study deals with one component of the C cycle. Other losses as mentioned earlier should also be considered when trying to mitigate global warming, particularly N_2O and CH_4 due to their larger global warming potential.

CRediT authorship contribution statement

L Cardenas: Conceptualization, Project administration, Supervision, Methodology, Investigation, Writing full draft– review & editing.
N Loick: Data curation; Formal analysis, Software, Validation.
L Olde: Data curation; Formal analysis, Software, Validation, Writing – review & editing.
B Griffith: Resources, Writing –editing.
T Hill: Writing – review & editing.
J Evans: Formal analysis, Writing – review & editing.
N Cowan: Writing – review & editing.
C Segura: Writing – review & editing.
H Sint: Writing – review & editing.
P Harris: Resources, Writing –editing.
J McCalmont: Software, Formal analysis, Writing – editing.
Z Songyan: Writing –editing.
A Dobermann: Conceptualization, Funding acquisition, Writing – review & editing.
M.R.F. Lee: Funding acquisition, Resources, Writing – review & editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

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