The nitrogen, carbon and greenhouse gas budget of a grazed, cut and fertilised temperate grassland

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Abstract

Intensively managed grazed grasslands in temperate climates are globally important environments for the exchange of the greenhouse gases (GHGs) carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4). We assessed the N and C budget of a mostly grazed, occasionally cut, and fertilized grassland in SE Scotland by measuring or modelling all relevant imports and exports to the field as well as changes in soil C and N stocks over time. The N budget was dominated by import from inorganic and organic fertilisers (21.9 g N m^{-2} a^{-1}) and losses from leaching (5.3 g N m^{-2} a^{-1}), N_2 emissions (2.9 g N m^{-2} a^{-1}) and NOx and NH_3 volatilisation (3.9 g N m^{-2} a^{-1}), while N_2O emission was only 0.6 g N m^{-2} a^{-1}. The efficiency of N use by animal products (meat and wool) averaged 9.9% of total N input over only-grazed years (2004-2010). On average over nine years (2002-2010) the balance of N fluxes suggested that 6.0 ± 5.9 g N m^{-2} a^{-1} (mean ± confidence interval at p > 0.95) were stored in the soil. The largest component of the C budget was the net ecosystem exchange of CO_2 (NEE), at an average uptake rate of 218 ± 155 g C m^{-2} a^{-1} over the nine years. This sink strength was offset by carbon export from the field mainly as grass offtake for silage (48.9 g C m^{-2} a^{-1}) and leaching (16.4 g C m^{-2} a^{-1}). The other export terms, CH_4 emissions from the soil, from manure applications and enteric fermentation, were negligible and only contributed to 0.02-4.2% of the total C losses. Only a small fraction of C was incorporated into the body of the grazing animals. Inclusion of these C losses in the budget resulted in a C sink strength of 163 ± 140 g C m^{-2} a^{-1}. On the contrary, soil stock measurements taken in May 2004 and May 2011 indicated that the grassland sequestered N in the 0-60 cm soil layer at 4.51 ± 2.64 g N m^{-2} a^{-1} and lost C at a rate of 29.08 ± 38.19 g C m^{-2} a^{-1}, respectively. Potential reasons for the discrepancy between these estimates are probably an underestimation of C losses, especially from leaching fluxes as well as from animal respiration. The average greenhouse gas (GHG) balance of the grassland was -366 ± 601 g CO_2 eq m^{-2} y^{-1} and strongly affected by CH_4 and N_2O emissions. The GHG sink strength of the NEE was reduced by 54% by CH_4 and N_2O emissions. Estimated enteric fermentation from the ruminating sheep proved to be an important CH_4 source, exceeding the contribution of N_2O to the GHG budget in some years.

Keywords: grassland, carbon stocks, carbon sequestration, nitrogen cycling, budget, greenhouse gases

Introduction

Managed grasslands cover an estimated 26 % of earth's land surface (FAOstat, 2008). The impact of reactive nitrogen (Nr) losses, carbon (C) sequestration and greenhouse gas (GHG) emissions (CO_2, CH_4 and N_2O) from these grasslands is therefore of global importance and will become even more relevant in the future as increased standards of living in developing countries are expected to result in a rapid growth of livestock farming (Caro et al, 2014). Carbon and N cycles in grasslands are intricately linked and tightly coupled in extensively managed low N grasslands, with sinks and sources in equilibrium. Converting such systems to intensively managed N fertilised grasslands in the short term may increase the soil organic carbon (SOC) pool from decomposed plant litter and root material as well as through rhizodeposition (Rees et al., 2005) until a new equilibrium is reached (Soussana and Lemaire, 2014). In the case of the long term Broadbalk experiment in the UK, this equilibrium was achieved after 50 years (Powlson et al, 2011). After the conversion to intensive N management, the tight coupling of the N and C cycles becomes disrupted, leading to emissions of N_2O and CH_4 at rates which may outweigh the benefits of C sequestration; increased N input will lead to a decrease in the C/N ratio of the soil resulting in increased nitrification and denitrification processes and thus N_2O losses (Mu et al. 2014), while N fertilisation is a key factor inhibiting CH_4 oxidation in soils. Mosier et al (1991) reported an inhibition of CH_4 uptake on grassland by 41 % after the application of N fertiliser. Several studies indicate that managed grasslands can sequester C (Kim et al., 1992; Jones et al., 2006; Soussana et al., 2004; Ammann et al., 2007) however, uncertainties are high (Janssens et al., 2003). On the contrary, Smith (2014) concluded from long-term experiments and chronosequence studies, that changes in agronomic management may lead to short-term C sequestration, but in the long-term, under constant management and environmental conditions, C stocks are relatively stable. In a grassland ecosystem the C balance is determined by the net biome exchange (the difference between total C input and losses). In managed grassland ecosystems exports through biomass harvesting, the addition of organic manures (from organic fertiliser additions and animal excretion) as well as CO_2

85 and CH$_4$ losses from animal respiration and enteric fermentation can make significant contributions to the C budget.

  Nutrient budgets are a valuable tool to summarise and understand nutrient cycling in agroecoystems and to assess their impact on the environment. As imbalances are not sustainable in the long term, N and C budgets can be used as indicators and regulatory policy instruments for nutrient

90 management in order to reduce losses and increase efficiency. So far, different Nr species have been looked at in separate studies according to their form and impact. Few studies have attempted to calculate N budgets from managed grasslands (e.g. Chen et al., 2004; Ammann et al., 2009; Kramberger et al., 2015), whereas C budgets have been assessed more often and are available for various ecosystems (e.g. Aubinet et al., 2000; Soussana et al., 2007; Ammann et al., 2007, Rytter et al.

95 2015). To calculate the total C and N budget of an ecosystem all import and export processes have to be assessed by measuring or estimating all imports and exports to an ecosystem. The other method is to measure differences in N and C stocks in the soil over time. This approach has the advantage that it requires the measurement of only a single component of the system. However, a large number of samples are needed at an interval of more than 5 years before detectable changes may be statistically

100 significant (Smith, 2004). Moreover this approach does not provide any information about the different processes leading to the final budget.

  In this study we assessed the C and N budget from an intensively managed grassland in Southern Scotland using both approaches. Here we report one of the most detailed analyses of C and N fluxes from a grassland ecosystem over 9 years (2002-2010). This study allowed an analysis of the

105 importance of common grassland management practices such as cutting for silage, grazing of cattle and sheep at different stocking densities, N input by inorganic and organic fertiliser applications, as well as different weather conditions on the N, C and GHG balance. The data provide a unique overview of research undertaken within three European projects GREENGRASS (Soussana et al., 2007), CarboEurope (Schulze et al., 2009) and NitroEurope (Sutton et al., 2007; Skiba et al., 2009).

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2. Methods

2.1 Site description

The experimental site, Easter Bush, is located in South East Scotland, 10 km south of Edinburgh (03°02'W, 55°52' N, 190 m a.s.l) with a mean annual rainfall of 947 ± 234 mm and mean annual temperature of 9.0 ± 0.4 °C (2002-2010). The field (5.424 ha) has been under permanent grassland management for more than 20 years before the start of the experiment with a species composition of >99% perennial ryegrass (*Lolium perenne*) and < 0.5% white clover (*Trifolium repens*). The soil type is an imperfectly drained Macmerry soil series, Rowanhill soil association (Eutric Cambisol) with a pH of 5.1 (in H_2O) and a clay fraction of 20-26%. The ground water table was assumed to be at 0.85 m depth on average and the main rooting zone extends down to 0.31 m below soil surface.

2.2 Grassland management

The grassland was grazed rotationally throughout the experimental period by heifers in calf, ewes and lambs at different stocking densities (Table 1 and Figure 1a). Livestock units used for heifers, ewes and lambs were 0.75, 0.10 and 0.04, respectively (1 livestock unit has a standard live weight of 600 kg head[-1]; SAC, 1995). Lambs were present on the field from April to September only. The grass was cut for silage only in the first two years, on the 1st of June and 8th of August 2002 and on the 29th of May 2003. Ammonium nitrate fertiliser was applied to the field 3-4 times per year, usually between March and July (56 kg N ha^{-1} application[-1] on average). In 2008 an additional fifth mineral N application was added, using urea instead of ammonium nitrate fertiliser. Organic manure was applied on the 28th of September 2004 and 27th of March 2005 as cattle slurry, using a vacuum slurry spreader (Table 4 and 5 and in Fig. 1 a) and b)).

2.3. Annual budget calculations

We assessed the N and C budget by measuring or estimating the import and export of all relevant fluxes to and from the grassland field on an annual basis. Throughout the manuscript all fluxes are presented following the sign convention used in micrometeorology; fluxes from the ecosystem to the atmosphere are positive (exported from the field), while negative values indicate fluxes from the atmosphere to the ecosystem (imported to the field). We set the system boundary for inputs and exports of N and C by the

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field perimeters. The balance of all imports and exports results in the observed changes of N and C on this field over time.

The change in the N balance (ΔN) over time (Δt) of our grassland ecosystem can be written as:

$$\Delta N/\Delta t \quad = \quad FN_{org\ fert.} + FN_{synt\ fert.} + FN_{feed} + FN_{N2\ (biol.\ fixation)} + FN_{dep.} +$$
$$FN_{harvest} + FN_{animal} + FN_{leaching} + FN_{NH3/NOx(fert.,manure,\ animal)} + \quad (1)$$
$$FN_{NOx(soil)} + FN_{N2O} + FN_{N2(denitr.)}$$

N imports include the addition of N from organic and inorganic fertiliser ($FN_{org\ fert.} + FN_{synt\ fert.}$), cake concentrate fed to ewes during lactation (FN_{feed}), the fixation of N_2 through biological fixation ($FN_{N2\ (biol.\ fixation)}$) and the deposition of NH_3, HNO_3, NH_4^+, NO_3^- from dry, and NH_4^+ and NO_3^- from wet deposition (summarised as $FN_{dep.}$). Exports include the N lost from plant biomass at cuts for silage ($FN_{harvest}$), the off-take of N in meat (including bones) and wool from animals (FN_{animal}), the loss of organic and inorganic dissolved N through leaching ($FN_{leaching}$), the NH_3 and NOx emissions from volatilisation of inorganic and organic fertiliser spreading as well as from animal excretion ($FN_{NH3/NOx(fert.,\ manure,\ animal)}$), the emission of NOx from the soil ($FN_{NOx(soil)}$), the emission of N_2O from the soil (FN_{N2O}) and the loss of N_2 from total denitrification ($FN_{N2(denitr.)}$).

The change in the C balance (ΔC) over time equals the net biome production (NBP) and can be written for our site as:

$$\Delta C/\Delta t \ = \ NBP = FC_{CO2} + FC_{org\ fert} + FC_{animal} + FC_{CH4} + FC_{leaching} + FC_{harvest} \quad (2)$$

FC_{CO2} represents the net ecosystem exchange (NEE) of CO_2 and $FC_{org\ fert}$ is the C input through manure application. Carbon input from animal excretion was not included in the budget as it was assumed to be recycled C from plant and soil uptake. FC_{animal} includes the C off-take through animal weight increase and wool production. As grazing cows were heifers in calf and ewes milk was consumed by their lambs, there was no C off-take through milk to be considered. Methane emissions from enteric fermentation by ruminants, animal excretion and manure application as well as CH_4 fluxes from the soil are included in

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170   FC_{CH4}. $FC_{leaching}$ is the C lost through dissolved organic and inorganic C and dissolved CH_4 leaching and $FC_{harvest}$ represents the C lost from the system through plant biomass export from harvests (cut for silage). Carbon emissions from farm operations (i.e. tractor emissions) or off farm emissions (i.e. fertiliser manufacture) are not included in the C budget.

175   Details of methods to quantify each N and C budget component, as listed in Eq. (1) and (2), are described under Sect. 2.4 to 2.17 and in the Appendix. Some budget components were measured throughout the 9 years presented, while others were only measured in some years or not at all. Missing data were derived from the literature, models or averages from available data from other years.

180   The annual net GHG exchange (NGHGE) was calculated from annual NEE (FC_{CO2}), CH_4 (FC_{CH4}) and N_2O (FN_{N2O}) fluxes using global warming potentials (GWPs) at the 100-year time horizon (1 for CO_2, 298 for N_2O and 25 for CH_4, IPCC, 2013):

$$NGHGE = (FC_{CO2}) + FC_{CH4} * k_{CH4} + FN_{N2O} * k_{N2O} \tag{3}$$

185   Where;

$k_{CH4} = 9.09$, since 1 kg CH_4-C corresponds to 9.09 kg CO_2-C

190    $k_{N2O} = 127$, since 1 kg N_2O-N corresponds to 127 kg CO_2-C

In addition the net annual greenhouse gas balance (NGHGB) was calculated by including the loss of C through animal meat and wool production, harvest off take, C leaching and input by organic fertiliser application:

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$$NGHGB = NGHGE + FC_{org\ fert} + FC_{animal} + FC_{leach} + FC_{harvest} \tag{4}$$

2.4 Nitrogen and carbon import by fertiliser and manure ($FN_{synt\ fert} + FN_{org\ fert.} + FC_{org\ fert}$)

Mineral fertiliser was applied by a spreader as either ammonium nitrate or urea. Six month old cattle
200   slurry was spread by a vacuum slurry tanker. Data of application rates and N content were obtained

from the farmer. Three samples from the slurry tank were taken at each application and analysed for ammoniacal nitrogen (NH_3 and NH_4^+), dry matter content, total N, total C, pH and nitrate. The total N and C import to the field by the slurry was calculated by the volume of the slurry applied and the N and C analyses of the slurry.

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2.5 N import through standard cake concentrate (FN_{feed})

Ewes were given standard cake concentrate "Davidsons super ewe nuts" during lactation, which contained 20 % protein. They were given 1 kg of this additional feed per day for 7 weeks in spring. This resulted in an extra N intake of 0.032 kg N per ewe per day (assuming an N content of 16 % of protein, IPCC 2006a).

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2.6 Nitrogen and carbon export by harvest ($FN_{harvest}$+ $FC_{harvest}$)

The farmer estimated a forage harvest of 15 t fresh weight (FW) ha^{-1} at the first cut and 10 t FW ha^{-1} at the second cut of a year, based on the plant height at the field at the time of cutting and information from harvested plot experiments. As there were two cuts in 2002 and one cut in 2003 the estimated harvest was 25 t FW ha^{-1} y^{-1} for 2002 and 15 t FW ha^{-1} y^{-1} for 2003. A subsample of harvested vegetation was collected and dried at $80^{\circ}C$ for plant N and C analysis using a Carbo-Erba/400 automated N and C analyser.

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2.7 Nitrogen and carbon export by meat and wool (FN_{animal} + FC_{animal})

It was estimated by the farmer that heifers increased in weight by 0.8 kg per day (starting weight of 450 kg). The ewe weight was assumed to be constant (60 kg). They were fed extra protein (standard cake concentrate) to reduce weight loss during lactation, whereas lambs were brought to the field at a weight of 5 kg and removed when they reached a weight of 45 kg. All animals were weighed before they came onto the field at the beginning of the season and again at the end of the season. The total meat export, which includes bones, was calculated from the daily weight increase of heifers and lambs multiplied by the animal number per day. To calculate the N and C export from meat we assumed a N content of meat of 3.5% and a C content of meat of 21% (Flindt, 2002), a N content of bones of 7% and a C

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content of bones of 20% (Marchand, 1842), assuming a total bone content of 20% for sheep (Lambe et al., 2007) and 14% for heifers (Navajas et al., 2010) . Ewes were sheared annually in June, yielding an estimated 2.5 kg of wool per sheep. Wool N and C export was calculated from wool production multiplied by the average sheep number in June, assuming a N and C content of wool of 16.5 and 50%, respectively (Roche, 1995).

2.8 Nitrogen and carbon leaching ($FN_{leaching} + FC_{leaching}$)

Two sets of ten glass suction cups (pore size <1 µm, ecoTech, Bonn, Germany) for soil water and four Teflon suction cups (ecoTech, Bonn, Germany) for soil gas collection were installed in August 2006. One set was located on a slope, another on a hollow. For the budget calculations we only used results from the slope location as the hollow location was frequently water logged. Suction cups were installed horizontally from a soil pit beneath the A horizon (30 cm depth) and at 90 cm depth and connected to 2-l glass bottles in an insulated aluminium box placed into the soil pit. Samples were collected every two to three weeks. For further details and description of dissolved organic and inorganic C (DIC, DOC) and dissolved CH_4 analysis see Kindler et al. (2011). Dissolved inorganic and organic N (DIN, DON) and total N (TN) concentrations in leachate water were analysed by colorimetric analysis (San^{++}, Automated Wet Chemistry Analyzer - Continuous Flow Analyzer (CFA), Skalar, The Netherlands). Leachate C and N concentrations were measured from October 1st 2006 - March 30th 2008. Dissolved C and N were calculated by multiplying concentrations of DIC, DOC and dissolved CH_4 or DIN and DON respectively, with leachate volume. The latter was derived from a soil water model based on balancing daily precipitation and evaporation considering the water holding capacity of the soil (Kindler et al., 2011). For the remaining years N was simulated using the LandscapeDNDC model (Haas et al., 2013), with the model tested and validated with comprehensive measured data. For C leaching linear regression models describing the relationship between calculated C leaching fluxes and leachate volume for the measurement period (DOC; y = 0.0186x - 0.0695, R^2 = 0.8663, DIC; y = 0.021x - 0.0008, R^2 = 0.8056 and dissolved CH_4: y = 0.0019x - 0.0135, R^2 = 0.7623) were used to extrapolate to the remaining years.

2.9 N deposition (FN$_{dep}$)

2.9.1 Wet N deposition

Wet N deposition was determined from daily samples collected by an automatic precipitation sampler (Eigenbrodt$^®$ precipitation collector 181/KS, Königsmoor, D) at Auchencorth Moss (3°14'35 W, 55°47'34 N), 17 km south west of Easter Bush (Skiba et al., 2013, McKenzie et al., 2015). Precipitation samples were analysed for NO_3^- and NH_4^+ by ion chromatography (Methrom AG, Switzerland). Typical detection limits were 0.5 μM for NH_4^+ and 0.4 μM for NO_3^-. Annual inorganic N deposition at this site was then adjusted to annual rainfall amounts measured at Easter Bush.

2.9.2 Dry N deposition

Cumulative monthly concentrations of gaseous and aerosol N species (NH_3, HNO_3, particulate NH_4^+ and NO_3^-) were collected from another field, about 300m distance from our study field, using a DELTA system (DEnuder for Long Term Atmospheric) (Sutton et al., 2001). N dry deposition fluxes were calculated using the average flux from four different inferential models, as described in detail by Flechard et al. (2011).

2.10 N$_2$O fluxes (FN$_{N2O}$)

From June 2002 to July 2003 N_2O fluxes were measured continuously by eddy covariance (EC) using an ultra-sonic anemometer coupled with a Tunable Diode Laser absorption spectrometer (TDL) at a frequency of 10 Hz. Details for the gap filling method of the N_2O–EC data are described in Jones et al., 2011. From August 2006 to November 2010 N_2O fluxes were measured using manual closed static chambers (Clayton et al.,1994, Skiba et al., 2013). Samples were analysed for N_2O using a Hewlett Packard 5890 series II gas chromatograph (Agilent Technologies, Stockport, UK), fitted with an electron capture detector (detection limit: N_2O < 33 ppbV). Fluxes were measured weekly and more frequently during fertilisation. Cumulative fluxes were calculated by gapfilling data for missing days using linear interpolation and summing up all gapfilled data over each calendar year. For the periods where no N_2O fluxes were measured (January-May 2002, July 2003-March 2004, May 2004-July 2006) fluxes were simulated by LandscapeDNDC (Haas et al., 2013). LandscapeDNDC was tested in detail

with available data on plant growth, soil temperature, moisture, inorganic soil N concentration, NO and N_2O, which resulted in general good agreement of simulations and measurements. Results except NO emissions are published in Molina et al., 2016.

2.11 NOx fluxes ($FN_{NOx(soil)}$)

NOx fluxes from the soil were only measured for a short period (June 2009-August 2010). The NOx fluxes were measured using an autochamber system described in detail by Butterbach-Bahl et al. (1997). Measurements were made 4 times per day, every 6 hours for an 8 min period per chamber. We used simulated data from Landscape DNDC for years where no NOx fluxes were measured.

2.12 NH_3 + NOx volatilisation ($FN_{NH3/NOx \text{ (fert.,manure, animal)}}$)

The fraction of nitrogen that volatilises as NH_3 and NOx from applied synthetic fertiliser or cattle slurry application and animal excretion was estimated to be 10% and 20% of total N applied, respectively (IPCC, 2006b). The animal excretion amount was estimated in accordance with the IPCC Guidelines (IPCC, 2006a), for details, please see Appendix.

2.13 N_2 emission by total denitrification ($FN_{N2(denitr.)}$)

Di-nitrogen (N_2) emissions resulting from total denitrification in the soil was not measured in our experiment. We therefore used the N_2 emission rates from LandscapeDNDC simulations.

2.14 Biological N_2 fixation ($FN_{N2 \text{ (biol. fixation)}}$)

The species composition was measured once in 2002 and at monthly intervals in 2003 by the visual estimation method (Braun-Blanquet, 1964), where 50 quadrats of 0.25 m^2 were randomly thrown into the field. As the legume fraction (*Trifolium repens*) was smaller than 0.5% at each measuring point we assumed the nitrogen fixation through plants to be zero.

2.15 Exchange of CO_2 (FC_{CO2})

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NEE was measured continuously from 1.January 2002 till 31.December 2010 by an eddy covariance system consisting of a fast response 3D ultrasonic anemometer (Metek USA-1, Metek GmbH, Elsmhorn, Germany) and a fast closed path CO_2-H_2O analyser (LI-COR 7000 infra-red gas analyzer (IRGA), LI-COR, Lincoln, NE, USA). NEE is the arithmetic sum of the gross primary production (GPP) and total ecosystem respiration (TER). Flux partitioning of measured NEE into GPP and TER was calculated by the same online tool used for gapfilling. In this flux partitioning approach, daytime TER is obtained by extrapolation of a night time parameterisation of NEE on air temperature and GPP is the difference between ecosystem respiration and NEE. Contrarily to unmanaged ecosystem, TER at our site also includes the respiratory loss of CO_2 by grazing animals. Net primary production (NPP), which represents the annual plant growth (difference between GPP and autotrophic respiration) was calculated as 50% of GPP (Amthor 2000, Zhang et al., 2009).

2.16 Methane fluxes (FC_{CH4})

Methane fluxes from the soil were measured with closed static chambers simultaneously with the N_2O measurements (see sect. 2.10). The same GC was fitted with a flame injection detector (detection limit: $CH_4 < 70$ ppbV). The minimal detectable flux was 17 ng CH_4-C m^{-2} s^{-1}. Fluxes were measured weekly and more frequently at fertiliser events. As measured soil CH_4 fluxes were close to zero and did not vary significantly between months, we calculated CH_4 for months where no CH_4 fluxes were measured (January-May 2002, July 2003-March 2004, May 2004-July 2006), as an average monthly cumulative flux from other years.

Methane emissions from grazing animals, i.e. animal excretion and enteric fermentation, were estimated following the IPCC Tier 2 methodology (IPCC, 2006a: Stewart et al., 2009). For details, see Appendix. Methane emissions from slurry applications were assumed to be small. As no chamber measurements were conducted at the time of slurry spreading, the emissions were estimated as 0.07% of the applied assuming that emissions were comparable to those in a related study (Jones et al., 2006), where CH_4 was measured from chambers after slurry application on a nearby field in 2002 and 2003.

2.17 VOC

340 Fluxes of non-methane volatile organic compounds (VOC) were not measured. We assumed similar VOC emissions to those reported by Davison et al. (2008) for an intensively managed grassland in Switzerland, where the daily average flux of methanol, acetaldehyde and acetone over 3 days after cutting were 21.1, 5.1. and 2.6 nmol m^{-2} s^{-1}, respectively. Based on those values, annual VOC emissions from our field were estimated to be in the order of 0.03% of the annual C offtake in harvest
345 and 0.08% of annual C off-take by grazing animals. We therefore assumed VOC emissions to be negligible and did not account for them in the C balance.

2.18 Soil N and C measurements

Total N and C content of the soil were measured in May 2004 and May 2011. One hundred soil cores
350 with an inner diameter of 8.7 (2004) and 8.3 cm (2009, both corers from Eijkelkamp Agrisearch Equipment BV, Giesbeek, The Netherlands) were collected along a regular grid with a distance of 10 m between sampling points on both occasions. The soil sampling grid covered the main footprint area of the site, not the entire field. Cores were separated into layers of 0-5, 5-10, 10-20, 20-30, 30-40, 40-50 and 50-60 cm. Coarse stones of a diameter > 4 mm and roots of a diameter >1 mm were removed from
355 the samples prior to drying at 40 °C. Stone and root samples were air-dried separately. Then, soil samples were sieved to < 2 mm. Particles > 2 mm were combined with the coarse stones. Dry weights of roots and combined stone fractions were determined. Total N and C concentrations in < 2 mm soil separates were determined using dry combustion (VarioMax, Elementar Analysensysteme GmbH, Hanau, Germany). As the site contains no carbonates, total C was assumed to equal organic C. As bulk
360 density varies spatially and over time (e.g. through compaction by livestock), the soil N and C content per unit ground area to a fixed depth will also change, without any change in the mass fraction of N and C in dry soil. Therefore, total N and C stocks were calculated on an equivalent soil mass (ESM) basis, so that comparisons between years were valid (see Gifford and Roderick, 2003, Wendt and Hauser, 2013). A cubic polynomial was fitted to the data, to predict cumulative N and C with cumulative soil
365 mass in the profile. A soil mass of 800 kg m^{-2} was used (Table 7), which corresponds to approximately 60-cm depth, which was the depth of the corer. Uncertainty in the estimates of stock change was based on the prediction intervals in the cubic polynomial at a soil mass of 800 kg m^{-2}.

2.19 Ancillary measurements

370 Soil temperature and volumetric soil moisture were continuously recorded at four depths (3.5, 7.5, 15 and 30 cm) by temperature probes (temperature probe 107, Campbell Scientific, Loughborough, UK) and TDR probes (TDR 100, Campbell Scientific, Loughborough, UK), respectively, the latter installed in June 2002. Rain was measured by a tipping bucket rain gauge, while air temperature and relative humidity were measured by an integrated humidity and temperature transmitter (HUMITTER[®], Vaisala

375 Ltd, Suffolk, UK).

2.20 Statistical and uncertainty analysis

Random error was determined as 2σ-standard error (95% confidence) of the overall mean according to Gaussian statistics. The confidence intervals for group means were used to establish whether or not

380 differences were significantly different from zero. Linear correlations between C and N inputs and outputs were calculated by calendar year. For systematic errors the uncertainty range of measurements as well as of parameterisations and literature based estimates was estimated according to expert judgment. To calculate the combined effect of systematic uncertainties of each flux component on the C and N budget simple Gaussian error propagation rules were used, details are provided in Table S1

385 (Supplementary material). Confidence intervals are given at the 95% confidence level.

Additional details of the methods are provided in the Appendix.

3. Results

390 ### 3.1 Climate and management

The meteorological conditions exhibited substantial inter-annual variability in the study period 2002-2010 (Table 2 and Fig. 2). Annual rainfall ranged from 575 mm to 1238 mm with highest monthly rainfalls of 280 mm month^{-1} in September 2002. Lowest annual reported rainfall was in 2010; this low value was caused by a gap in data from January-March, due to snowfall. Average annual air

395 temperature ranged from 8.3 to 9.6 ºC with highest daily air temperatures of 30.4 ºC in August 2005 and

lowest in December 2010 at -10.3 ºC. Highest average monthly air temperatures were measured in July 2006 at 17 ºC and lowest monthly average air temperatures at 2 ºC in November 2009. In 2003 the highest average annual temperature (9.6º C) and lowest annual rainfall (680 mm) were measured with a correspondingly low annual soil water content of 31%. The duration of the growing season was defined per calendar year as the period bounded by the first and last 5 consecutive days with mean daily air temperature $\geq$ 5 °C. The length of the growing season (LGS) varied between 151 days (2006) and 242 days (2009) (Table 2).

Livestock stocking density exhibited both intra- and inter-annual variability. The average annual stocking densities were lowest in 2002 and 2003 at 0.27 LSU ha^{-1} and 0.54 LSU ha^{-1}, respectively (Table 1), which were the years where the grass was cut for silage and no lambs were present in the field. In 2007, 2008, 2009 and 2010 no heifers were present in the field. Highest annual average stocking densities occurred in 2004 and 2007 at 0.99 LSU ha^{-1} and 0.91 LSU ha^{-1}, respectively. Maximum monthly stocking density occurred in September 2006 at 13.8 LSU ha^{-1}, while interim periods with no grazing at all were observed in all years (Fig. 1a). Mineral N fertiliser was applied split into 3 to 5 applications per year, ranging from 2.5 to 9.6 g N m^{-2} per application (Fig. 1b). Organic manure was applied in 2004 and 2005 as cattle slurry, spread at a rate of 6.9 and 15.8 g N m^{-2} per application, respectively, which resulted in a C input of 55.4 and 171.8 g C m^{-2} per application, respectively (Fig. 1b and c). The grass was only cut in 2002 and 2003. Harvested biomass in 2002 and 2003 ranged from 2.60 to 3.75 t DW ha^{-1} per cut which resulted in an N off-take ranging from 1.7 to 4.7 g N m^{-2} per cut and a C removal from the field ranging from 113.1 to 169.5 g C m^{-2} per cut (Fig. 1c).

3. 2 N budget

In our grassland system the N balance is the difference between the N input through fertiliser and atmospheric deposition and the N output through harvest, animal export, leaching and gaseous emissions. The total resulting balance over the nine years, derived from flux calculations and estimations, showed that N was stored at an average rate of -6.0 ± 5.9 g N m^{-2} a^{-1} ($p<0.05$). From 2003 to 2010, N was stored at a rate of -1.2 to -18.2 g N m^{-2} a^{-1}, whilst in 2002 N was lost at a rate of 13.2 g

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N m^{-2} a^{-1} (Table 3). The major N input consisted of inorganic fertiliser, ranging from -11 to -25.9 g N m^{-2} a^{-1}, averaging at -19.4 g N m^{-2} a^{-1}, while N deposition represented only between 1.9 and 5.9% of the total N input. N input through standard cake feed given to ewes during lactation ranged between 1.1 and 1.9 g N m^{-2} a^{-1}. During the years where N was stored, a significant positive correlation between total N input from fertiliser and N storage was observed (R^2 = 0.55). Largest losses resulted from leaching at an average rate of 5.34 ± 3.1 g N m^{-2} a^{-1} and were estimated to be highest in 2002 at 14.9 g N m^{-2} a^{-1} and lowest in 2003 at 0.09 g N m^2 a^{-1}. The total N off take through meat and wool ranged from 0.15-3.12 g N m^{-2} a^{-1}, while the total annual N offtake from harvest was 11.8 g N m^{-2} a^{-1} in 2002 and 10.4 g N m^{-2} a^{-1} in 2003. Amongst gaseous exchanges, highest losses were estimated from N$_2$ emissions, averaging at 2.9 g N m^{-2} a^{-1} with maximum losses of 4.12 g N m^{-2} a^{-1} in 2009, although in 2004 and 2005 losses from NOx/NH$_3$ volatilisation from excretion and organic fertilisation exceeded losses from N$_2$ emissions. Losses through NOx from the soil were always less than 1% of the total N exchange (0.2 g N m^{-2} y^{-1}). Nitrous oxide emissions ranged from 0.11 to 1.27 g N m^{-2} a^{-1}, representing 1.3-8.4% of the total N export. Annual N$_2$O emissions showed no correlation with precipitation, livestock density or total N input. N$_2$O emission factors (percentage of N lost from total N inputs by mineral and organic fertiliser), ranged between 0.6 and 7.5% (Table 5).

To investigate the influence of different managements on the N and C budget, we separated experimental years into harvested and grazed (2002 and 2003) and grazed only years (2004-2010, Fig. 2 and 3). During the harvested and grazed years, the main loss of N from the system occurred from the export through harvest (57.6% of total N inputs), followed by leaching (39.2%), while the export from animals (meat and wool) accounted for less than 2% of total N losses (Fig. 2a). The main loss to the atmosphere resulted from total denitrification (N$_2$; 15.4%), followed by NOx/NH$_3$ volatilisation from inorganic N fertiliser applications (9.5%), while N$_2$O emissions accounted for 3.3%, NOx/NH$_3$ volatilisation from excretion for 3.3% and NOx from soil for less than 1%. On average, N was lost from the grassland system during 2002 and 2003; The surplus of 31.3% (all outputs added up in Figure 2a result in 131.3%) represents the N lost from the soil as well as the error in the budget. When grazed-only years were considered (Fig. 2b), N was stored in the grassland system; the residual part, which includes the net accumulation of soil organic nitrogen and all the error in the budget, was 37.7%. Losses

through leaching (18.7%) and N_2 (11.3%) were lower in grazed years compared to harvested years, while the export through grazing animals was considerably higher at 18.8% (sum of N loss through meat, wool and NO_x/NH_3 volatilisation from excretion). An additional loss occurred in grazed years through the volatilisation of NO_x/NH_3 from organic fertiliser applications in 2004 and 2005 (2.6%). Losses through N_2O and NO_x/NH_3 from inorganic fertiliser were comparable to harvested years at 2.4% and 7.9%, respectively.

Cumulative soil N stocks were derived from soil core measurements taken in May 2004 and May 2011 (Table 6). In 2004 N stocks were 840.86 ($\pm$11.89) g N m^{-2} and in 2011 they were 870.02 ($\pm$14.14) g N m^{-2}. Nitrogen storage over the 7 years was calculated from the cumulative equivalent soil mass (ESM) for the soil mass increment of 800 kg m^{-2}, which corresponds to approximate 60 cm depth. The estimated N storage over the 7 years was -4.51 $\pm$ 2.64 g N m^{-2} a^{-1} and was a significant N accumulation to the soil (p < 0.01). The estimated N storage derived from flux calculations between 2004 and 2010 was -9.40 $\pm$ 4.14 g N m^{-2} a^{-1}, which is almost 2 times more than that calculated by sequential soil analysis, however, values were not significantly different from each other.

3.3. C budget

Annual C inputs through photosynthesis (GPP) varied between -982.1 and -2162.9 g C m^{-2}, and losses through autotrophic and heterotrophic respiration (TER) varied between 972.1 and 2183.2 g C m^{-2}, both considerably larger than any other C fluxes (Table 4). If only the NEE was considered (difference between GPP and TER), the grassland acted as a sink for CO_2 at an average of 218 $\pm$ 155 g C m^{-2} a^{-1}, and the CO_2 uptake was significantly different from zero (p < 0.05). The sink strength ranged from -10 g C m^{-2} a^{-1} (2006) to -606 g C m^{-2} a^{-1} (2009), only in 2004, the grassland was a small source of CO_2 (72 g C m^{-2} a^{-1}). Taking into account all C inputs and outputs (NBP), C was sequestered on average at 163 $\pm$ 140 g C m^{-2} a^{-1} over the nine years, although the storage was not significantly different from zero (p<0.05). In 2004 and 2006 C was lost from the ecosystem. The major C import resulted from NEE in all years apart from 2005, when the C input from manure application was larger. Highest C export occurred from harvest in 2002 and 2003 (270.6 and 169.5 g C m^{-2} y^{-1} respectively), while second largest export in 2002 and 2003 and largest exports in other years was leaching (6.8 to 25.1 g C m^{-2} a^{-1}). The

480 measured C leaching value for 2007 (15.4 g C m^{-2} a^{-1}, table 5) differs from the leaching value published for Easter Bush by Kindler et al. (2011), as we only used values of one of the two measured sites in this manuscript (slope, not hollow, as the hollow location was frequently water logged). The third largest C loss consisted of C export from meat in 2004-2010, ranging from 6.4-15.8 g C m^{-2} a^{-1}. In 2002 and 2003, when no lambs were present in the field, C export from meat was exceeded by CH_4 losses from

485 enteric fermentation. Carbon export from wool ranged from 0.5 to 2.1 g C m^{-2} a^{-1}. CH_4 emissions from organic fertilisation, soil processes and animal excretion were always less than 1% of the total C losses. CH_4 losses from enteric fermentation ranged from 1.5 to 5.7 g C m^{-2} a^{-1}, corresponding to 0.5-22.5% of all C losses from the ecosystem. The NBP was dominated by the NEE. A high livestock density tended to reduce the net sink strength. A significant negative correlation of NEE with stocking density could be

490 seen (R^2=0.47). The NBP correlated positively with rainfall (R^2=0.48) and there was only a weak correlation between NEE and rainfall (R^2=0.38).

The net primary production (NPP) in years when grass was harvested and grazed (2002 and 2003) and grazed only (2004– 2010) are presented in Figure 3**.** In both management types most C was lost through ecosystem respiration, (67% and 71% of NPP, respectively). Harvest export represented

495 21% of NPP. Leaching accounted for 1.5% of NPP during harvested years and 2.2% in grazed only years. Animal export (meat and wool) consisted of 1.5% of NPP in grazed only years and was 0.2% of NPP in grazed and harvested years. The sum of all CH_4 emissions (from organic fertilisation, excretion, enteric fermentation and soil) was less than 1% of the NPP. The residual part, which includes the C storage in the soil as well as the uncertainty of the budget, was estimated at 10% and 24% of NPP in

500 harvested and grazed or grazed years, respectively.

The C content for the cumulative soil mass increment 0-800 kg m^{-2} (~ 0-60 cm) was lower in 2011 (12026.05 ± 190.19 g C m^{-2}) compared to 2004 (11824.87 ± 187.84 g C m^{-2}), resulting in a C loss of 29.08 ± 38.19 g C m^{-2} (Table 7). In comparison, based on flux calculations C was stored at 180 ±180 g C m^{-2} y^{-1} over the 7 years. However, neither C loss calculated by sequential soil analysis, nor C

505 storage estimated from flux calculations were significantly different from zero.

3.4. Greenhouse gas budget

Average greenhouse gas fluxes, net GHG exchange (NGHGE) and attributed net GHG balance (NGHGB) for 2002-2010 are shown in Figure 4. The CO_2 storage from the NEE provided the largest term in the annual GHG budget. Carbon dioxide (NEE) was sequestered over the 9 years at a rate of -799 ± 567 g CO_2 m^{-2} a^{-1}. This storage was significantly different from zero ($p < 0.05$). On average, the annual net GHG exchange (NGHGE) was highly correlated with annual NEE (R^2=0.96). On average the grassland was a source of the GHGs CH_4 and N_2O at a rate of 148 ± 30 and 285 ± 131 g CO_2 m^{-2} a^{-1}, respectively, both being significantly different from zero ($p < 0.001$ and $p < 0.01$, respectively). Nitrous oxide losses ranged from 52 g CO_2 eq. m^{-2} a^{-1} (2004) to 588 g CO_2 eq. m^{-2} a^{-1} (2007) (data for each year not shown). Methane from soil processes, manure input as well as animal excretion, accounted for less than 5% of total CH_4 emissions. Methane emissions from enteric fermentation ranged from 53 g CO_2 eq. m^{-2} y^{-1} (2002) to 199 g CO_2 eq. m^{-2} a^{-1}(2004). Annual total CH_4 emissions correlated positively with annual livestock density (R^2=0.99). The CH_4 emissions, which were predominately (> 97%) of ruminant origin weakened the sink strength of NEE by 18 %. If both CH_4 and N_2O were considered the total trade-off of NEE was a substantial 54% and increased to a total of 67 %, if only grazed years were considered. On average the grassland represented a GHG sink of -366 ± 601 g CO_2 m^{-2} a^{-1}, if only NEE, CH_4 and N_2O were included (NGHGE). If all C components ($FC_{org.fert}$, FC_{animal}, $FC_{leaching}$, $FC_{harvest}$) are included, the sink strength of the grassland decreased to -182 ± 560 g CO_2 m^{-2} a^{-1} (NGHGB). This represents a weakening of the sink strength of the NGHGE by 50%, mainly due to the export of harvest. However, it has to be noted that in harvested years the return of the manure, resulting from the grass fed to livestock off -site, would reduce the GHG balance. If only grazed years were considered the sink strength increased slightly by 5.4%, due to the C input from manure in 2004 and 2005. Both, NGHGE and NGHGB were not significantly different from zero.

4. Discussion

4.1. Nitrogen balance

The main N inputs in our study were from inorganic and organic fertilizer additions. The amount of N added through fertilizer was determined by national recommendations (SAC, 2013). Atmospheric N deposition (wet and dry) accounted only for a small fraction of the total N input to our managed

grassland. This is in contrast to semi natural systems, where atmospheric N deposition and biological fixation represents the main N input (Phoenix et al., 2006, Bleeker et al., 2011). Due to high N fertilization rates and low soil pH the legume fraction was less than 1% and biological N_2 fixation therefore a negligible source of N.

540   The data obtained from our budget were used to calculate the Nitrogen Use Efficiency (NUE) expressed as the ratio between N in crop and animal products (in this case either the crop harvest or the sum of meat, wool and milk) to the total N inputs to the system (fertilizer, imported manure, standard cake concentrate fed to lactating ewes). The NUE of herbage in cut years (2002 and 2003) of 57.6% (Figure 2a) is comparable to reported N efficiencies of 55-80% in harvested herbage from managed

545   temperate grasslands (Ball and Ryden 1984; Ammann et al., 2009). It has been shown that the NUE in crops is significantly higher compared to the NUE in animal production (Galloway and Cowling, 2002). The NUE of animal products on our grassland system ranged from 5 to 16.6% in grazed only years (2004-2010), with an average of 9.9 %. This is in agreement with the NUE reported for sheep of 6.2% by Van der Hoek (1998) and beef production systems, which reported N efficiencies range from 6 to

550   12% (Whitehead et al., 1986; Tyson et al., 1992) and 5-20% (Ball and Ryden, 1984). Approximately 85% of total harvested and imported N in the EU is used to feed livestock (Sutton 2011, Leip et al. 2011). A measure to reduce N pollution and GHG emissions could therefore be the reduction of meat consumption (Smith et al. 2013).

       Nitrogen was lost from our grassland to the environment through different pathways. Nitrogen

555   leaches from grassland soils in the form of nitrate (NO_3^-), ammonium (NH_4^+) and dissolved organic N (DON). Overall, leaching from our field (5.3 ± 3.1 g N m^{-2} a^{-1}) was comparable to values measured at intensively grazed pastures in Ireland (1.8-6.4 g N m^{-2} a^{-1}, Watson et al., 2007) and England (3.8-13.3 g N m^{-2} a^{-1}, Scholefield et al., 1993) or croplands (e.g. Bechmann et al., 1998, max. leaching losses of 10.4 g N m^{-2} a^{-1}). However, leaching from our study was high compared to the Swiss NitroEurope site,

560   where a maximum loss of 0.35 g N m^{-2} a^{-1} was estimated from an ungrazed grass/clover sward, despite comparable annual rainfall and N inputs from inorganic and organic fertilizer (Ammann et al., 2009). This difference can be explained by the different management of the two sites. Grazed grasslands tend to have higher N leaching rates than cut grasslands since herbage that is not cut and exported from the

field in grazed grasslands but eaten by the grazing animals and returned as urinary N adds N to the system. Furthermore the uneven distribution of excreted organic N further enhances leaching due to the formation of N hotspots, which has been observed at outdoor pig farms (e.g. (Eriksen, 2001). Ryden et al., (1984a) showed a 5.6 times higher leaching loss from grazed compared to cut grassland with 36% of total N inputs lost from grazed compared to 6% lost from cut grassland. On our site 18.7 % of total inputs was lost as leaching in grazed years, compared to 39% in the cut years. However, the higher value in cut years was due to the high rainfall in 2002.

Due to its high background in the atmosphere, N_2 fluxes cannot be measured directly in the field. There are different methods to measure N_2 fluxes indirectly, which have been summarized by Groffman et al. (2006). In our study, we estimated N_2 losses using the process based biogeochemical model LandscapeDNDC (Haas et al., 2013, Molina-Herrera et al., 2016). These losses represented the highest gaseous N losses from our grassland in most years, with an average of 12.6% of total N inputs and 14% of inorganic fertilizer N inputs. This is comparable with an average N_2 loss of 12.5% from inorganic N applications measured by the acetylene inhibition method from a fertilized and cut, but ungrazed grassland in Switzerland (Rudaz et al., 1999). Using the same method, Van der Salm et al. (2007) reported a higher loss of 22% of total N input from a cattle grazed pasture on a heavy clay soil in the Netherlands. In addition to the impact of the heavy clay soil, which could have enhanced denitrification due to reduced oxygen concentrations, grazing is likely to have enhanced denitrification rates in Van der Salm's study. Grazing not only enhances denitrification through soil compaction caused by trampling animals but also due to the formation of N hot spots resulting from unevenly distributed soil N from excretion. In our study N_2 losses simulated by LandscapeDNDC were based on average (per ha^{-1}) changes of the soil N pool instead of the more uneven distribution of soil N in hot spots like urine patches. Therefore is it is likely that N_2 losses in our study have been underestimated and better estimation would contribute to a significant reduction in the uncertainties associated with the overall N budget.

Annual N_2O emissions measured in our study (0.1 to 1.3 g N m^{-2} y^{-1}) are within the range of literature values from reported grazed as well as un-grazed European grasslands (Leahy et al., 2004; Flechard et al., 2007). Generally N_2O losses are higher from grazed grassland compared to cut,

ungrazed pasture (Velthof and Oenema, 1995; Luo et al., 1999) due to a more anaerobic environment as a consequence of soil compaction caused by animal treading and the influence of N and C from the deposition of animal excreta to the soil. We did not observe any correlations between annual N_2O emissions and total N input. We found a relationship between the cumulative precipitation one week before and three weeks after fertilization with N_2O emissions (R^2=0.53) (Skiba et al., 2013). This relationship, together with the influence of stocking density and type of N applied (e.g. Jones et al., 2007; Flechard et al., 2007) needs to be considered when developing Tier 2 N_2O emission factors. In our study EFs were above the uncertainty range (0.3 - 3 %) given by IPCC Tier 1 guidelines (IPCC, 2006b) in four out of nine years.. However, it has been shown that the N_2O emission factor from managed grassland can be higher, especially under wet conditions and with a high soil C content as this is the case for Scottish soils (Jones et al., 2007; Dobbie et al., 1999; Buckingham et al., 2013).

In grazed pastures NH_3 volatilizes from urine patches, decomposing dung as well as from fertilizers containing urea and NH_4^+ (Twigg et al 2011). Increased rates of NH_3 losses have been associated with high stocking densities under a rotational grazing system by Ryden and Mc Neill (1984). In our study, N volatilized as NH_3 and NOx from inorganic and organic fertiliser and animal excretion, accounted for a considerable amount of total N, with losses of 13% in cut and grazed years (2002, 2003) and 19% in grazed only years. In contrast, soil NOx emissions from our grassland were estimated to be negligible, accounting for less than 1% of the total budget. Soil NOx emissions result predominantly from microbial nitrification of either added N fertilizers or following the mineralization of soil organic matter, animal excretions or added manure. Emissions tend to be linked with aerobic soil conditions (Davidson, 1991).

Results from soil analysis taken in May 2004 and May 2011 indicate that our field has stored N (-4.51 ± 2.64 g N m^{-2} a^{-1}). The N budget assessed from the net N flux balance showed that N was stored in the soil of our grassland over the same 7 years at a higher rate (-9.40 ± 4.14 g N m^{-2} y^{-1}), although values were not significantly different from each other. The slight shifts in measurement periods (May 2004 – May 2011) for the soil stock calculations and the period for flux budget calculations (Jan 2004 – Dec 2010), is presumed to be insignificant in this comparison. Results from both methods are within the range of literature values. Neeteson and Hassink (1997) found a N accumulation in SOM of 0-25 g N m^-

620   ^{2} a^{-1} from two cattle-grazed farms in the Netherlands, while Watson et al. (2007) reported a N storage in grazed Irish grasslands ranging from 10-15.2 g N m^{-2} a^{-1}, depending on N inputs. Soil N storage assessed from soil measurements from a cut grassland close to our field, where plots were treated with cattle slurry, stored N over 6 years at a rate of -2.17 g N m^{-2} a^{-1} in the top 10 cm, while, in the same experiment, a N loss was observed from mineral N and urea treatments (4.5 and 8.3 g N m^{-1} a^{-1},

625   respectively) (Jones et al., 2007). In contrast, Schipper et al. (2007) reported an average loss of 9.1 g N m^{-2} a^{-1} in the top 100 cm from managed grasslands over 20 years in New Zealand. The reason for the small difference between methods (flux measurements vs sequential soil sampling) in our study might lie in a possible underestimation of losses from flux measurements. Uncertainties of our estimates are high, especially those for N losses. The largest absolute systematic uncertainty for the N balance was

630   attributed to N leaching as for most years values were modelled using data to valuate the model from only one location. The uncertainty of the leaching estimate would therefore be reduced if the model could be validated with data measured from several locations. The second highest systematic uncertainty was attributed to losses through N$_2$, followed by NOx/NH$_3$ emission from excretion, NOx/NH$_3$ emission from inorganic fertilization and inputs from organic fertilization Combined

635   uncertainties from all components lead to a total systematic uncertainty in the N balance of 2.2 g N m^{-2} y^{-1} (2004-2010).

4.2. Carbon balance

On an annual basis our grassland site was a sink for atmospheric CO$_2$ in most years. NEE was only

640   positive in 2004, which was likely to be due to a high livestock density. Generally, grazing causes a very gradual impact on the CO$_2$ uptake as a part of the field is defoliated each day. The reduced leaf area index (LAI) then leads to a reduced CO$_2$ uptake by plants. In addition to the reduced LAI, grazing presents a source of CO$_2$ from animal respiration, thereby reducing the CO$_2$ sink of the grassland within the field (Levy et al., submitted). The maximum uptake of CO$_2$ measured in our study is close to the

645   upper range of NEE reported for temperate grasslands (100 to 600 g C m^{-2} y^{-1}, (IPCC, 1996). On average over the 9 years the magnitude of the NEE on our grassland (-218.0 ± 154.5 g C m^{-2} a^{-1}) was close to the average NEE measured in a comparison of nine European grasslands over two years (240 ±

70 g C m^{-2} a^{-1}) by Soussana et al. (2007) and comparable to the CO$_2$ sink capacity of managed Irish grasslands measured by Byrne et al. (2007) (290 $\pm$ 50 g C m^{-2} a^{-1}) or Leahy et al. (2004) (257 g C m^{-2} a^{-1}). Despite high variability over the 9 years, the average NEE value was significantly different from zero (p < 0.05). The range of the calculated annual gross primary production (GPP) (-982 to -2163 g C m^{-2} a^{-1}) and terrestrial ecosystem respiration (TER) (972 to 2183 g C m^{-2} a^{-1}) from our field were within reported values for other managed grasslands. Gilmanov et al. (2007) reported the GPP of 18 intensively managed European grasslands ranging from 467 to 1874 g C m^{-2} a^{-1} and TER ranging from 493 to 1541 g C m^{-2} a^{-1}, while Mudge et al. (2011) reported values of 2000 g C m^{-2} a^{-1} for GPP and TER from a intensively grazed dairy pasture in New Zealand.

When all components of C import and export were included in addition to the CO$_2$ exchange (NBP) C was stored in our grassland over the 9 years. However, due to the high variability between years, NBP was not significantly different from zero (p = 0.05), suggesting that our site is carbon neutral. The average C storage value on our site (164 $\pm$ 140 g C m^{-2} a^{-1}) is higher than most estimates reported in literature, but due to the high annual variation, still within the range of reported values; Soussana et al. (2007) reported C storage estimates from European grazed and cut grasslands of 104 $\pm$ 73 g C m^{-2} a^{-1}, and Mudge et al. (2011) reported for a grazed and cut grassland in New Zealand fluxes of 59 $\pm$ 56 g C m^{-2} a^{-1} and 90 $\pm$ 56 g C m^{-2} a^{-1} in two consecutive years. NBP estimates from a Swiss grassland cut for silage was shown to sequester C at a rate of 147 $\pm$ 130 g C m^{-2} a^{-1} (Ammann et al., 2007), while estimates from a cut grassland in Germany was shown to vary from being a sink (-28 g C m^{-2} a^{-1}) to being a source of C (+25 g C m^{-2} a^{-1}), depending on years (Prescher et al., 2010). The inclusion of all C imports and exports leads to a weakening of the C sink strength assessed from NEE measurements in five years and even changed the grassland from being a sink to being a source in 2006. Due to the C export from harvest, as well as the stimulation of primary production through grazing, C sequestration tends to be lower in cut compared to grazed systems (Soussana et al 2004). This is represented in our study in the lower residual value of NPP in cut years compared to the residual value from grazed only years (Figure 3), where the residual value represents the C storage in the soil as well as the uncertainty of the budget. However, it has to be kept in mind that the herbage yielded from cuts

675　will end up as animal feed; C will be digested and respired off-site, releasing CO_2 and CH_4 to the atmosphere as well as being returned to the grassland as manure.

Results from soil analysis indicate that our grassland has lost C from 2004-2010 (29 ± 38 g C m^{-2} a^{-1}, Table 6). In the literature, losses as well as storage of C at various rates have been reported from managed grasslands assessed from soil stock measurements. Soil stock change measurements from our
680　field are comparable with values found in the literature. Depending on the study, managed grasslands in Belgium were shown to either loose (90 g C m^{-2} a^{-1}, Lettens et al., 2005a) or sequester carbon (4.4 g C m^{-2} a^{-1} in 0-30 cm, Goidts and Van Wesemael, 2007; 22.5 g C m^{-2} a^{-1} in 0-30 cm, Lettens et al. 2005b). Schipper et al. (2007) reported losses of C from pastures in New Zealand over 20 years at an average rate of 106 g C m^{-2} a^{-1} (top 100 cm), whereas these losses were a result of an earlier land use change
685　from forestry. Schuman, et al., (2002) measured a C sequestration of 10-30 g C m^{-2} a^{-1} from US rangelands (0-60 cm), while Watson et al. (2007) measured a C storage at 112-145 g C m^{-2} a^{-1} in the top 15 cm soil layer from a grazed Irish grassland. Bellamy et al. (2005) showed no evidence of increased C in the topsoil of grasslands in England and Wales and et al. (2009) found no significant change of SOC over time in two UK long term experiments. The above mentioned results are contrasting and
690　inconclusive, because observed C sinks in grasslands are the effect of land management or land use change prior to the beginning of the C stock change measurement. Soussana et al (2014) concluded in a theoretical study that grassland SOC sequestration has a strong potential to partly mitigate the GHG balance of ruminant production systems at low grazing intensities, but not with intensive systems. Smith (2014) examined evidence from repeated soil surveys, long term grassland experiments and simple
695　mass balance calculations and concluded that, although grasslands can act as C sinks, they cannot act as a perpetual C sink and thus could not be used as an offset for GHG emissions.

The comparison of the C storage calculated from the net C flux balance with soil C stock changes show that, the flux balance estimated a C sequestration, while based on C stock changes, C was lost, although neither value was significantly different from zero (Table 6). A literature search by
700　Soussana et al. (2010) showed that generally C sequestration calculations on grassland were lower if derived from SOC stock changes (average -5$\pm$30 g C m^{-2} a^{-1}) compared to C flux balances (average -22$\pm$56 g C m^{-2} a^{-1}), although these estimates were not significantly different from each other. However,

in none of those reviewed studies were C flux and C stock change measured in the same field experiment. A reason for the discrepancy between estimation methods in our study might lie in a possible underestimation of C exports in the flux balance calculation, leading to an overestimation of C storage in the soil. One underestimated flux could be the export of DIC and DOC. Carbon leaching from managed grasslands has not been reported in many studies. Kindler et al. (2011) reported C leaching from various European ecosystems, where the measured data (2007) from our experimental field was part of the study. Our data (30.0 g C m^{-1} a^{-1}, average of two locations as published in Kindler et al. (2011)) were close to the average value (29.4 g C m^{-1} a^{-1}) of the reported European grasslands, which showed a range of C losses of 6.5-42.5 g C m^{-1} a^{-1}. Higher losses have been observed by McTiernan et al. (2001), who measured DOC export from grassland lysimeter plots treated with N fertilizer and slurry over two months. Up-scaled to one year, they measured DOC loss between 25.2 and 70.8 g C m^{-2} a^{-1}, all above what we measured in our study. Important factors controlling the magnitude of C leaching have been shown to be drainage, the topsoil C/N ratio and the saturation of the subsoil's sorption capacity for organic C (Kindler et al., 2011; McTiernan et al., 2001). In waterlogged soils the soil organic matter (SOM) decomposition and groundwater recharge tend to be reduced and thus the amount of C prone to leaching compared to that under more aerobic conditions associated with drainage. Although our field was drained more than 50 years ago, the drainage system does not operate very well, resulting in large puddles of standing water during prolonged periods of rain. The measured data used for the budget were taken at one sampling point, which was not in a waterlogged area. The spatial heterogeneity within the grassland field caused by uneven water management as well as faeces and urine patches requires to sample at more points in order to obtain a representative leaching value. Therefore our leaching estimates are highly uncertain and could be significantly lower and C exports overestimated. Furthermore, leaching was only measured in one year (2008), while values for remaining years were estimated using a simple regression model with an attributed high uncertainty of 32% (5.3 g C m^{-2} a^{-1} of average fluxes). Indeed, Siemens (2003) hypothesized that the underestimation of C leaching from soils can explain a large part of the difference between atmosphere- and land-based estimates of the C uptake of European terrestrial ecosystems. Another underestimated flux could be the loss of CO_2 in the NEE measurements. Gapfilling can introduce uncertainties in the NEE data especially for years with low data

capture. Furthermore, CO_2 losses from animal respiration could be underestimated at times due to the animals moving out of the footprint of the EC mast. Using animal respiration values from chamber experiments of 12.1 g CO_2 kg^{-1} live weight d^{-1} for cows and 11.7 g CO_2 kg^{-1} live weight d^{-1} for sheep and lambs (Shane Troy, SRUC, personal communication), we estimated a maximum CO_2 loss from animal respiration of 53 g C m^{-2} a^{-1} (2002-2010) or 59 g C m^{-2} a^{-1} (2004-2010). So if we assume that all animal respiration has been missed by eddy covariance measurements then the C sink estimated from NEE measurements would be reduced by 24% (2002-2010) or 33% (2004-2010). This theoretical maximum 33% reduction would reduce the net carbon balance to ∼ 122 g C m^{-2} a^{-1} (2004-2010).

740   In addition to uncertainties in the flux budget calculations, uncertainties are also attributed to soil C and N stock measurements. Soil inventory data in our study indicated a loss of C and a storage of N over 7 years, which seems contradictory, although C loss was not significantly different from zero. The uncertainty of soil C and N stock measurements arise from the variability of soil C and N concentrations due to errors from laboratory and to their high spatial variability as well as from the variability in the rock fragment content (Goidts et al., 2009).

4.3 Greenhouse gas budget

In the overall N and C budget N_2O and CH_4 emissions were negligible in terms of N and C losses from the system (1 − 8% of total N losses and 0.6 - 4.5% of total C losses, respectively). However, in terms of CO_2 equivalents, N_2O emissions as well as CH_4 emissions strongly affected the GHG budget. Indeed, the sink strength of the NEE was weakened by N_2O emissions by 29% over all years. Methane emissions from soil processes, manure input and animal excretion were negligible in terms of the C budget as well as in the GHG budget. In contrast, enteric fermentation proved to be an important GHG source. Methane emissions were also measured by eddy covariance technique over several months in 2010 on the same field (Dengel et al., 2011). By dividing CH_4 fluxes by the number of sheep in the field each day, Dengel et al. (2011) calculated CH_4 emissions per head of livestock as 7.4 kg CH_4 head^{-1} a^{-1} for sheep, which is close to the emission factor used in our budget of 7.6 kg CH_4 head^{-1} y^{-1} for ewes, showing that our estimates were realistic. The positive correlation of CH_4 emissions with the stock

density indicates that any changes in animal production will have a major impact on the global CH_4 budget. The weakening of the GHG sink strength of the NEE by N_2O and CH_4 emissions, show the importance of those two gases in terms of global warming. Thus, adapting the management of grasslands by adding fertilizer or manure to increase plant growth and thus improve C sequestration in soils may increase N_2O emissions, while changing land use from cropland to pasture in the attempt to reduce C losses from soils might lead to increased CH_4 losses from grazing animals.

5. Conclusion

In our study only a small proportion of the N inputs from inorganic fertilizer and organic manure were converted to animal outputs or stored in the soil, while the main part was lost through leaching and gaseous emissions. An improvement of the NUE would mean both an economic profit for the farmer as well as an environmental benefit. Estimates from flux budget calculations indicated that our grassland was sequestering C. However, although grasslands can act as C sinks, they cannot act as a perpetual C sink and thus could not be used as an offset for GHG emissions (Smith et al., 2014). Instead, as it is easier and faster for soils to lose than to gain carbon, care must be taken to reduce C loss by management options, rather than trying to increase carbon stocks in grasslands. There was a discrepancy between soil stock measurements and flux budget calculations for the C as well as the N budget. The reason for the discrepancy between C budget estimates might lie in a possible underestimation of C exports such as leaching and animal respiration as well as the uncertainty due to gapfilling in the NEE data. The N accumulation might have been overestimated by the flux calculations through a possible overestimation of N losses, mainly through leaching as well as through N_2 and NO_x/NH_4 emissions. Furthermore, uncertainties are also attributed to soil C and N stock measurements. Our data have shown that the information about the potential of managed grasslands to act as sinks or sources for GHG is important for mitigation and adaption purposes. High plant productivity, stimulated by fertilisation, resulted in high plant CO_2 fixation. However, increased N losses through N_2O emissions counteracted the benefits of C sequestration in terms of GHG emissions. Furthermore, CH_4 emissions from enteric fermentation largely reduced the positive effect of CO_2 uptake, especially in years where NEE rates were small. We therefore conclude that CO_2 exchange alone is not sufficient for the

estimation of the GWP of a managed grassland ecosystem. Only a comprehensive approach, combining C and N cycling will help us to better understand functionalities of ecosystems and to improve modelling by integrating this knowledge.

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Appendix A. Supplementary information

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Table 1. Average annual stocking rates [LSU[*] ha^{-1} a^{-1}].

	2002	2003	2004	2005	2006	2007	2008	2009	2010
Heifers	0.12	0.38	0.05	0.15	0.27	0	0	0	0
Ewes	0.14	0.16	0.82	0.56	0.51	0.68	0.68	0.61	0.53
Lambs	0	0	0.12	0.12	0.12	0.23	0.14	0.11	0.12
all animals	0.27	0.54	0.99	0.83	0.90	0.91	0.83	0.72	0.65

[*]LSU stands for livestock units; 1 LSU has a standard live weight of 600 kg head^{-1} (SAC, 1995)

Table 2. Weather characteristics of each measurement year.

	2002	2003	2004	2005	2006	2007	2008	2009	2010
Annual mean temperature [ºC]	9.2	9.6	8.9	8.8	9.3	9.1	8.6	8.9	8.3
Maximum temperature [ºC]	23.6	29.5	27.4	30.4	26.6	21.4	23.5	28.0	24.0
Minimum temperature [ºC]	-5.1	-8.4	-4.9	-6.6	-5.5	-7.5	-5.8	-7.8	-10.3
Annual rainfall [mm]	1238	680	1169	1028	1120	904	1065	744	575
Soil water content [% by volume]	36.9	31.0	40.3	45.2	36.6	37.7	41.5	39.4	-
Water filled pore space [%]	68.0	57.2	74.3	83.3	67.5	69.5	76.5	72.6	-
Length of growing season[*] [days]	180	196	156	177	151	186	193	242	226

[*]The plant growing season begins and ends with periods of consecutive days, where daily temperatures average more than 5 ºC without any five-day spells of temperatures below 5 ºC.

Table 3. Nitrogen budget and balance for each measurement year and average values, confidence intervals at p > 0.95 (CI) and systematic uncertainties (uncert.) for 2002-2010 [g N m^{-2} a^{-1}]. Negative numbers represent uptake while positive numbers represent loss of N from this grassland ecosystem. Letters indicate data published in previous publications.

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2002-2010 average	CI	uncert.
Organic fertilisation	0	0	-6.9	-15.8	0	0	0	0	0	-2.5	3.6	0.2
Inorganic fertilisation	-20.6	-16.0	-11.0	-17.3	-22.4	-17.3	-25.9	-25.0	-19.0	-19.4	3.1	0.2
cake feed for ewes	0	0	-1.2	-1.4	-1.1	-1.9	-1.1	-1.2	-0.7	-0.9	0.4	0.0
Wet deposition	-0.4	-0.6	-0.6	-0.7	-0.6	-0.6	-0.5	-0.4	-0.4	-0.5	0.1	0.2
[a]Dry deposition	-0.5	-0.4	-0.3	-0.3	-0.2	-0.3	-0.2	-0.2	-0.2	-0.3	0.1	0.2
Harvest	11.8	10.4	0	0	0	0	0	0	0	2.6	3.2	0.4
Meat (incl. bones)	0.2	0.6	2.3	3.1	2.6	2.9	1.8	1.3	1.5	1.80	0.7	0.2
Wool	0	0	0.6	0.5	0.4	0.7	0.7	0.2	0.2	0.4	0.2	0.0
[b]Leaching	14.9	0.1	0.1	4.6	10.6	4.2	5.6	2.6	5.0	5.3	3.1	1.71
N_2	3.7	2.2	1.3	1.7	2.8	3.0	3.3	4.1	3.6	2.9	0.6	0.8
[c]N_2O	1.1	0.1	0.1	0.4	0.9	1.3	0.8	0.4	0.4	0.6	0.3	0.2
NO_x (soil)	0.3	0.1	0	0.1	0.2	0.2	0.3	0.1	0.1	0.2	0.1	0.1
NO_x,NH_3 (inorg.fert.)	2.1	1.6	1.1	1.7	2.2	1.7	2.6	2.5	1.9	1.9	0.3	0.6
NO_x,NH_3 (org.fert.)	0	0	1.4	3.2	0	0	0	0	0	0.5	0.7	0.3
NO_x,NH_3 (excretion)	0.5	0.8	2.3	1.8	1.8	2.4	2.1	1.8	1.6	1.7	0.4	0.8
N balance	13.2	-1.2	-10.8	-18.2	-2.7	-3.7	-10.6	-13.6	-6.1	-6.0	5.9	2.3

[a]Flechard et al. 2011: Dry deposition, modelled average value of the two years 2007/2008
[b]Molina-Herrera et al. 2016: N leaching modelled 2005-2010
[c]Molina-Herrera et al. 2016: N_2O fluxes modelled 2005-2010.
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[c]Flechard et al. 2007: annual N_2O fluxes measured by eddy covariance in 2002/2003 and by chambers in 2004
[c]Skiba et al. 2013: annual N_2O fluxes measured by chambers from Jan. 2007 – Sept. 2010

Table 4. Carbon budget and balance for each measurement year and average values, confidence intervals at p > 0.95 (CI) and systematic uncertainties (uncert.) for 2002-2010 [g C m^{-2} a^{-1}]. Negative numbers represent uptake, while positive numbers represent loss of C from the grassland ecosystem. Letters indicate data published in previous publications.

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2002-2010 average	CI	uncert.
GPP	-2162.9	-1982.0	-2111.4	-1662.4	-982.1	-1722.7	-1441.2	-1722.4	-2015.4	-1755.8	244.4	105.3
TER	1726.9	1725.9	2183.2	1638.5	972.1	1606.7	1324.0	1116.7	1547.0	1537.9	236.2	92.3
NPP	-1081.5	-991.0	-1055.7	-831.2	-491.1	-861.3	-720.6	-861.2	-1007.7	-877.9	122.2	-52.8
[a]CO_2 (NEE)	-436.0	-256.1	71.8	-24.0	-10.0	-115.9	-117.1	-605.7	-468.4	-217.9	154.5	80.0
Organic fert.	0.0	0.0	-55.4	-171.8	0.0	0.0	0.0	0.0	0.0	-25.2	37.8	5.0
Harvest	270.6	169.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	48.9	65.5	5.4
Meat (incl. bones)	0.9	2.9	11.4	15.6	12.9	14.3	9.0	6.3	7.3	9.0	3.3	10
Wool	0.0	0.0	1.7	1.5	1.3	2.1	2.0	0.7	0.5	1.1	0.5	0.1
[b]Leaching	25.1	7.0	22.1	18.7	19.4	15.4	17.0	6.8	14.3	16.4	4.3	5.26
CH_4 (organic fert.)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
[c]CH_4 (soil)	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1
CH_4 (excretion)	0.0	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.0
CH_4 (enteric ferm.)	1.5	3.2	5.7	4.8	5.2	5.2	4.8	4.1	3.8	4.3	0.8	0.9
C balance (NBP)	-137.8	-73.3	57.6	-154.9	29.1	-78.7	-84.1	-587.7	-440.3	-163.3	139.5	16.0

[a]Soussana et al. 2007: NEE July 2002- Dec 2004
[a]Skiba et al. 2013: NEE 2007-2010
[a]Kindler et al. 2011: NEE average multiyear value 2004-2007
[b]Kindler et al. 2011: C leaching losses October 2006- Sept 2008 (Slope value corresponds to data used in this publication).
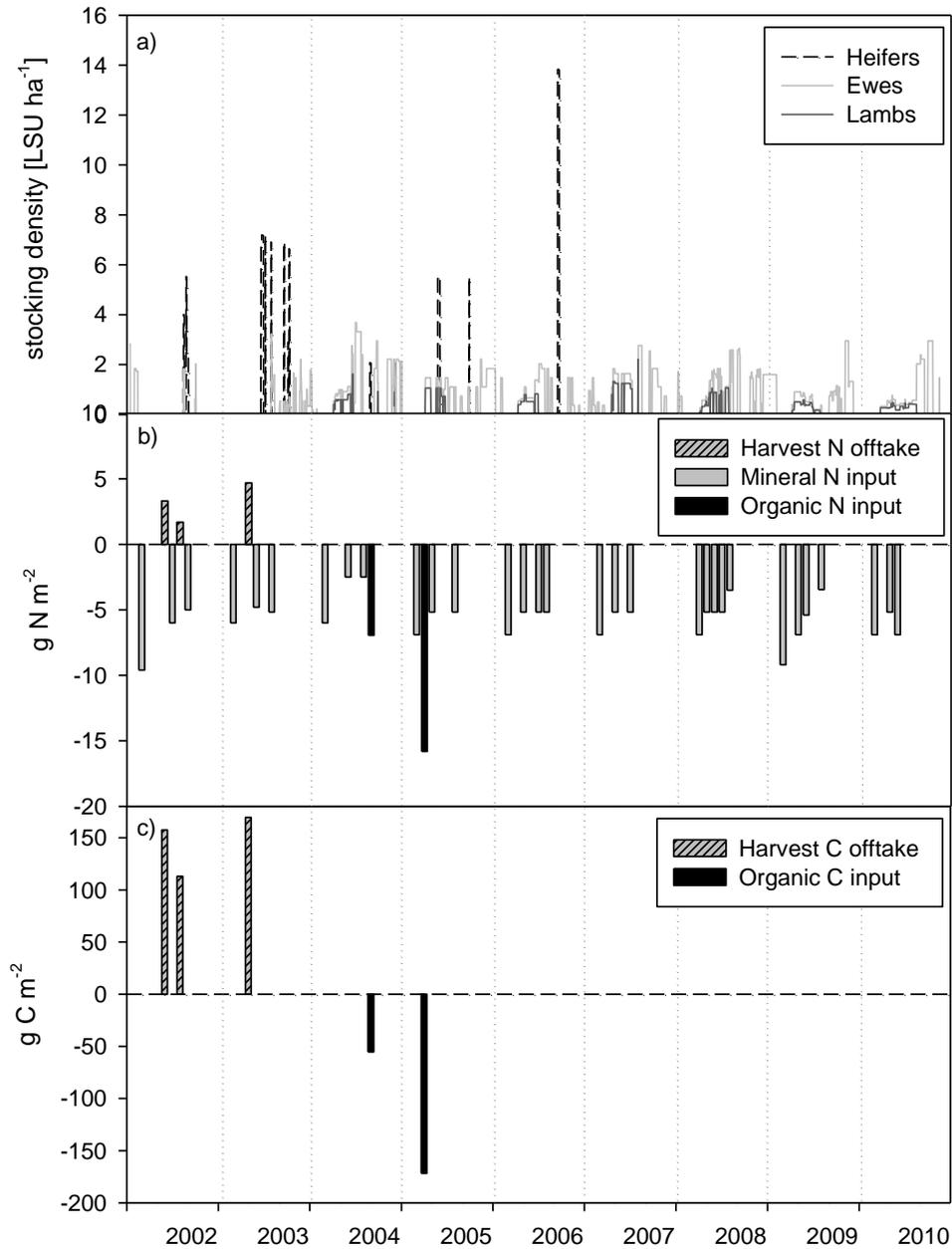[c]Skiba et al. 2013: CH_4 (soil) 2007-2010

Table 5. Annual N_2O exchange, total N input by fertiliser (mineral and organic) and N_2O emission factors, expressed as percentage of total N inputs in 2002-2010.
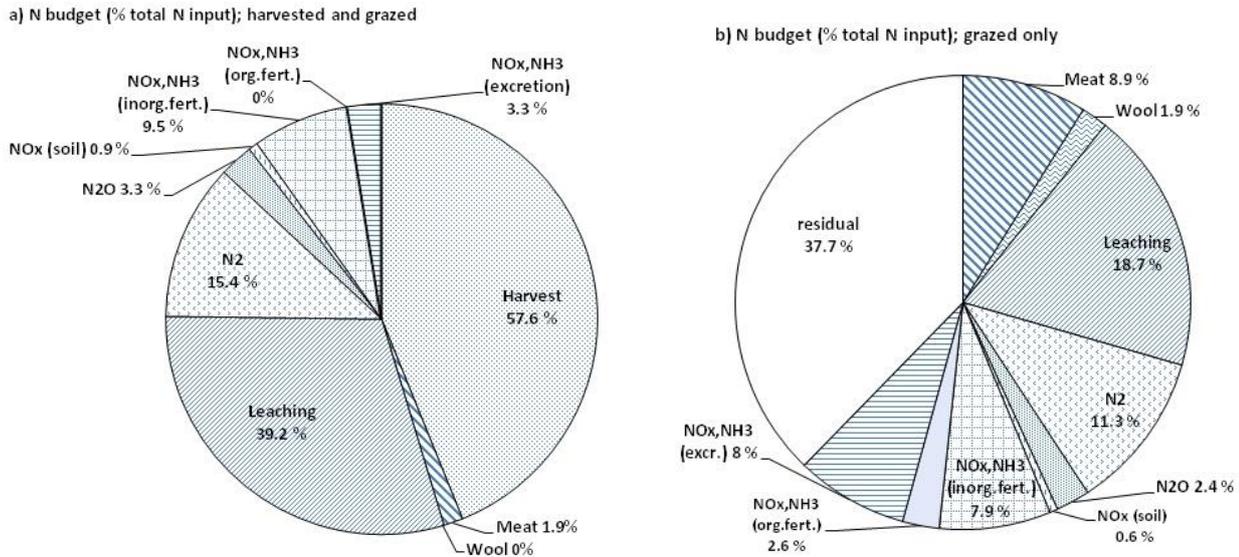
	N_2O flux [g N m^{-2} a^{-1}]	Total N input [g N m^{-2} a^{-1}]	EF [%]
2002	1.14	20.60	5.5
2003	0.14	15.98	0.9
2004	0.11	11.00	0.6
2005	0.36	17.25	1.1
2006	0.88	22.43	3.9
2007	1.25	17.25	7.2
2008	0.84	25.93	3.2
2009	0.41	24.95	1.6
2010	0.35	18.98	1.9

Table 6. N and C soil stocks (g N or C m^{-2}) in May 2004 and May 2011 and budgets (g N or C m^{-2} a^{-1}) over 7 years based on repeated soil N and C stock inventories and flux budget calculations (January 2004 - December 2010). Soil stock changes are based on a soil mass of 800 kg m^{-2}, which corresponds to approximately 60 cm depth. The flux budgets are averages for the years 2004 – 2010 (Table 4 & 5). Numbers in brackets represent confidence intervals. Negative numbers are sinks.
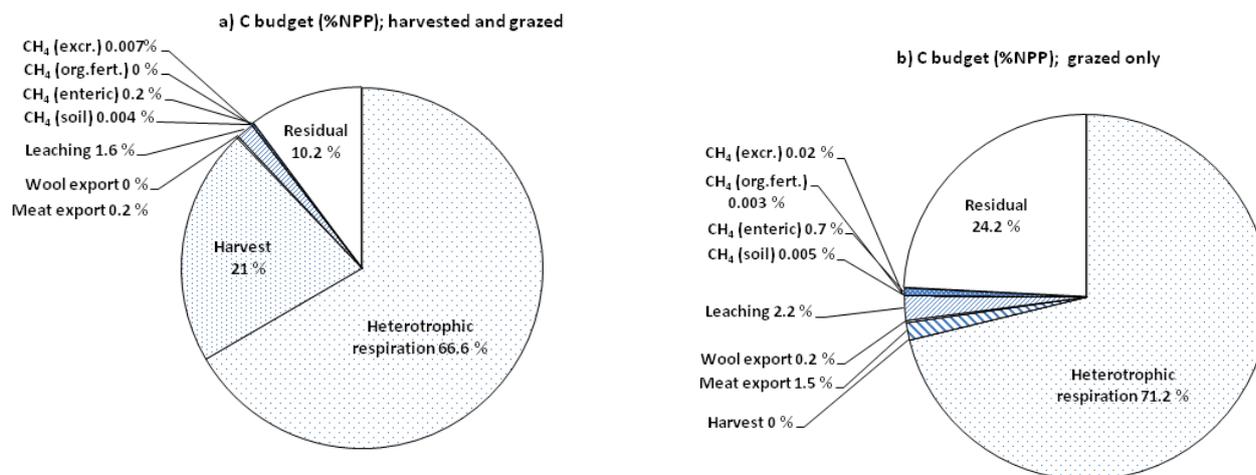
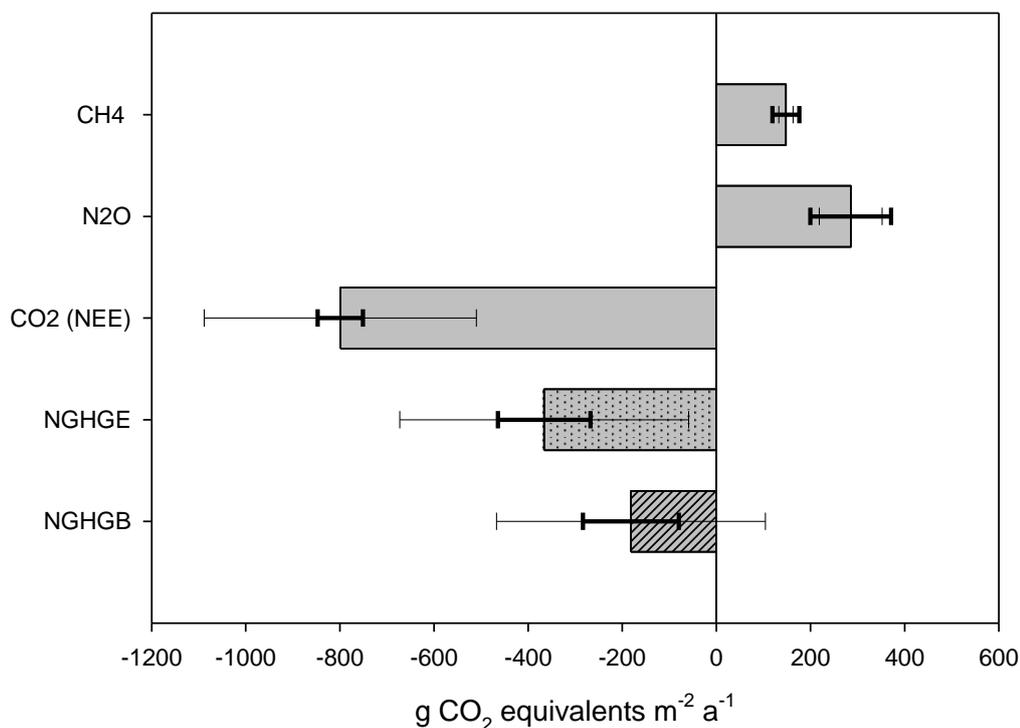|	Nitrogen	Carbon
soil stocks in 2004	840.68 (11.89)	12026.05 (190.19)
soil stocks in 2011	870.02 (14.14)	11824.87 (187.84)
soil stock change	-4.51 (2.64)	29.08 (38.19)
flux budget	-9.40 (4.14)	-179.7 (180)

Figure 1. Stocking density (a), nitrogen (b) and carbon (c) input and export from inorganic and organic fertiliser and harvest from 2002-2010. LSU stands for livestock unit, where 1 livestock unit has a standard live weight of 600 kg head[-1].

Figure 2. Mean annual nitrogen budget for Easter Bush, showing the fate of total N input (fertiliser and deposition) in (a) years when harvested for silage (2002 and 2003) and (b) in years when only grazing took place (2004–2010). The residual term in (b) includes any net accumulation of soil organic nitrogen as well as all the error in the budget calculation.

Figure 3. Mean annual carbon budget for Easter Bush, showing the fate of net primary productivity (NPP) in (a) years when harvested for silage (2002 and 2003) and (b) in years when only grazing took place (2004–2010). Heterotrophic respiration includes the respiration of soil microbes, cows and sheep. The residual term includes all the error in the budget calculation, as well as any net accumulation of soil organic carbon.

Figure 4. Average greenhouse gas fluxes, net GHG exchange (NGHGE) and attributed net GHG balance (NGHGB, includes $FC_{org\ fert}$, FC_{animal}, $FC_{leaching}$, $FC_{harvest}$) for 2002-2010. Positive values correspond to losses and negative values to storage of greenhouse gases to and from the grassland system, respectively. The CH_4 component comprises CH_4 fluxes from enteric fermentation, animal excretion, slurry application and soil exchange, while the N_2O component is the N_2O flux from the soil. CO_2 represent the Net Ecosystem Exchange (NEE). Global warming potentials of 298 and 25 were used for N_2O and CH_4 respectively, using a time horizon of 100 yrs (IPCC, 2013). Thin error bars represent variations (confidence intervals at $p > 0.95$) between years, while thick error bars represent the systematic uncertainty of each value.