Management matters: Testing a mitigation strategy for nitrous oxide emissions using legumes on intensively managed grassland

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Abstract. Replacing fertilizer nitrogen with biologically fixed nitrogen (BFN) through legumes has been suggested as a strategy for nitrous oxide (N_2O) mitigation from intensively managed grasslands. While current literature provides evidence for an N_2O emission reduction effect due to reduced fertilizer input, little is known about the effect of increased legume proportions potentially offsetting these reductions, i.e. by increased N_2O emissions from plant residues and root exudates. In order to assess the overall effect of this mitigation strategy on permanent grassland, we performed an *in-situ* experiment and quantified net N_2O fluxes and biomass yields in two differently managed grass-clover mixtures. We measured N_2O fluxes in an unfertilized parcel with high clover proportions vs. an organically fertilized control parcel with low clover proportions using the eddy–covariance (EC) technique over two years. Furthermore, we related the measured N_2O fluxes to management and environmental drivers. To assess the effect of the mitigation strategy, we measured biomass yields and quantified biologically fixed nitrogen using the ^{15}N natural abundance method.

The amount of BFN was similar in both parcels in 2015, (control: 55 ± 5 kg N ha^{-1} yr^{-1} and clover parcel: 72 ± 5 kg N ha^{-1} yr^{-1}) due to similar clover proportions (control: 15% and clover parcel: 21%), whereas in 2016 BFN was substantially higher in the clover parcel compared to the much lower control (control: 14 ± 2 kg N ha^{-1} yr^{-1} with 4% clover in DM and clover parcel: 130 ± 8 kg N ha^{-1} yr^{-1} and 44% clover). The mitigation management effectively reduced N_2O emissions by 54% and 39% in 2015 and 2016, respectively, corresponding to 1.0 and 1.6 t ha^{-1} yr^{-1} CO_2-equivalents. These reductions in N_2O emissions can be attributed to the absence of fertilization on the clover parcel. Differences in clover proportions during periods with no recent management showed no measurable effect on N_2O emissions, indicating that decomposition of plant residues and rhizodeposition did not compensate the effect of fertilizer reduction on N_2O emissions. Annual biomass yields were similar under mitigation management, resulting in a reduction of N_2O emission intensities from 0.42 g N_2O-N kg^{-1} DM (control) to 0.28 g N_2O-N kg^{-1} DM (clover parcel) over the two years observation period. We conclude that N_2O emissions from fertilized grasslands can be effectively reduced without losses in yield by increasing the clover proportion and reducing fertilization.

1 Introduction

Agricultural practices contribute 5.4 Gt CO_2-eq. yr^{-1} (range 11–12%) to global greenhouse gas (GHG) emissions (IPCC, 2014; Tubiello et al., 2015). The technical potential to mitigate GHG emissions from agriculture ranges between 5.5 and 6.0 Gt CO_2-eq. yr^{-1} by 2030 (Smith et al., 2008), exceeding current agricultural GHG emissions. The three major anthropogenic GHGs comprise carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). The agricultural sector is responsible for 84% of global anthropogenic N_2O emissions (Smith et al., 2008). N_2O emissions are primarily attributed to mineral and organic fertilizer applied to soils, manure left on pastures, biomass burning, crop residues and increased mineralization of soil organic matter (SOM) caused by the cultivation of soils (IPCC, 2014; Tubiello et al., 2015). Due to the high global warming potentials of CH_4 and N_2O (GWP, factor 34 and 298, respectively, on a per mass basis compared to CO_2 based on a 100-year time horizon) (IPCC, 2013b), these gases are more important than the CO_2 fluxes from the agricultural sector. However, they remain far less understood than CO_2 fluxes because of interactions between multiple underlying processes that are largely unexplored. In particular, data resolving the dynamics of N_2O fluxes from soils are still scarce, as advances in instruments capable of high-frequency continuous N_2O concentration measurements and steadily deployable in the field have only become available in recent years (Eugster and Merbold, 2015).

Here we test a potential mitigation strategy for nitrous oxide emissions, namely the substitution of fertilizer with biologically fixed nitrogen (BFN) via clover on intensively managed grassland. Processes producing and consuming N_2O are numerous and their complex interactions and dependencies on biotic and abiotic factors are generally known but not yet fully understood (Butterbach-Bahl et al., 2013). Nevertheless, it is known that N_2O emissions in grasslands strongly depend on management practices (Hörtnagl et al., 2018; Li et al., 2013; Snyder et al., 2009) and reducing N_2O emissions while maintaining yields can thus contribute to climate smart agriculture (CSA) (Lipper et al., 2014). For mitigating N_2O emissions from soils, a range of options (e.g. nitrification inhibitors, liming of acid soils, precision fertilizer use, legumes) are available (Bell et al., 2015; Flessa, 2012; de Klein and Eckard, 2008; Li et al., 2013; Luo et al., 2010; Paustian et al., 2016; Smith et al., 2008). The most important strategies focus on increasing the nitrogen use efficiency (NUE) of plants by adjusting the rate, type, timing and placement of organic and inorganic nitrogen fertilizers. With such approaches, the surplus of nitrogen (N) as the substrate for microbial communities producing N_2O, can be reduced or avoided (Flessa, 2012; Galloway et al., 2003; Snyder et al., 2009). Reducing N surplus comes along with other environmental benefits such as reduced ammonia emissions (NH_3) and nitrate (NO_3^-) leaching, both potential sources of indirect (off-site) N_2O emissions. Similar to these mitigation strategies, forage legume species of the Fabaceae family (e.g. white clover, red clover, lucerne, also called alfalfa) grown in grass-legume mixtures have the potential to reduce N_2O emissions as a cost-effective mitigation strategy (Jensen et al., 2012). In legume-rich systems, large parts of the plants' nitrogen (N) demand can be provided from the atmosphere via biological nitrogen fixation (BNF) instead of using fertilizer amendments (Ledgard et al., 2001; Suter et al., 2015). Hence, N input via BNF instead of fertilizers has the potential to avoid large N surpluses by provisioning N in a manner synchronous to plant needs following their growth pattern (Crews and Peoples, 2005). Furthermore, BNF is down-regulated by the plant when demand is low and fixed N is located in the nodules and thus not freely available to microbiota in the soil (Lüscher et al., 2014; Nyfeler et al., 2011).

Our mitigation approach investigated the potential for reductions in slurry application accompanied with increased clover proportion in the pasture to reduce N_2O emissions at the field-scale. Farmers currently use a combination of home-produced slurries and acquired mineral fertilizer. Our suggestion is to apply the slurry in fields which are currently amended with mineral fertilizers, as the home-produced slurry clearly should be used. This would have an additional benefit of reducing the indirect greenhouse gas emissions i.e. those during the manufacture of mineral fertilizers. The quantity of these manufacturing reductions in GHG emissions, which are beyond the field-scale, as well as the full farm nitrogen and GHG budget are well beyond the focus of this study would need further investigation.

Besides the obvious advantage of lower fertilizer amendments, grass–legume mixtures typically achieve higher yields than average grass and legume monocultures ("overyielding effect") and often also higher yields than the best performing monoculture ("transgressive overyielding"), with legume proportions of 40–70% resulting in highest yields (Finn et al., 2013; Lüscher et al., 2014; Nyfeler et al., 2009). In addition, growing selected legumes in mixtures with non-legumes could improve resistance and resilience of forage swards against climatic extremes such as severe drought events (Hofer et al., 2017). Moreover, grass-legume mixtures are beneficial to fodder composition as they are characterized by higher protein contents than grass swards, and show well-balanced feeding values (Phelan et al., 2015). Legume-rich fodder has high crude protein (CP) contents and was shown to increase voluntary intake by 10–20% (Dewhurst et al., 2003), and to increase milk production (Dewhurst et al., 2003; Huhtanen et al., 2007).

Despite the known advantages, introducing legumes causes some challenges for farmers. For instance, maintaining a persistent optimal legume proportion of 30–60% (30–50%, Lüscher et al., 2014; 40–60%, Nyfeler et al., 2011) is not trivial (Guckert and Hay, 2001). Conservation of legumes as hay or silage can be more difficult than for grasses due to lower contents of water-soluble carbohydrates (WSC) and higher pH buffering capacities (Phelan et al., 2015). When protein-rich forage is fed without sufficient WSC, N cannot be used efficiently by livestock and N excretion from the animals increases (Phelan et al., 2015). However, the balance between CP and WSC can be provided by carbohydrates from other plant species in mixtures (Lüscher et al., 2014). Furthermore, exceptionally high legume proportions (> 80%) and legume monocultures can lead to similar N surplus due to high levels of BFN as found in fertilized fields, and consequently to high soil nitrate concentrations (Weisser et al., 2017) which can subsequently lead to enhanced N_2O emissions (Jensen et al., 2012). So far, relatively few in situ measurements at plot scale have been carried out to investigate the effect of legumes and grass-legume mixtures on N_2O emissions (e.g. studies by Klumpp et al., 2011; Virkajärvi et al., 2010; Schmeer et al., 2014; Niklaus et al., 2016; Li et al., 2011). The contribution of legumes to total field-scale N_2O emissions was attributed to decomposition of N-rich plant residues and N from root exudates (Millar et al., 2004; Rochette and Janzen, 2005). Although it was shown that some Rhizobium species are able to produce N_2O via rhizobial denitrification (O'Hara and Daniel, 1985; Rosen and Ljunggren, 1996), direct N_2O emissions from BNF are negligible compared to N_2O from denitrification rates for most investigated species and hence result in no significant effect on field-scale N_2O emissions (Garcia-Plazaola et al., 1993; Rochette and Janzen, 2005).

To date, experimental studies investigating year-round N_2O exchange in grassland systems are scarce (Skinner et al., 2014), and measurements of high temporal resolution in grassland relying on fertilizer input versus grassland based on BFN are missing. Thus, the aim of this study was to test the N_2O mitigation strategy of substituting N fertilizer with BFN by increasing the clover proportion in grassland. Therefore, we measured N_2O exchange and productivity in two adjacent grassland parcels, one with an intensive "business as usual" management compared to a parcel where fertilizer amendments were substituted by over-sowing clover. Our specific objectives were (1) to quantify N_2O emissions from both parcels, (2) to identify the meteorological and soil chemical drivers of N_2O emissions, (3) to assess if substituting N fertilizer with BFN was an effective N_2O mitigation strategy. We hypothesized considerably lower N_2O emissions in the clover parcel, lower soil nutrient availability in the clover parcel and thus no effect of legume proportions on N_2O emissions, and hypothesized fertilization to play the dominant role in driving N_2O emissions in the control parcel. We further expected minor differences in grassland yield between the two parcels, and as a consequence, reduced N_2O emission intensities in the clover parcel.

2 Material and methods

2.1 Site description

The experiment was set up at the Swiss FluxNet site Chamau (CH-Cha), located in the valley of the Reuss river on the Swiss plateau, approximately 30 km southwest of Zurich (47°12′36.8″ N 8°24′37.6″ E, 393 m a.s.l.). The site has been well investigated in terms of CO_2 exchange (Burri et al., 2014 using static chambers (SC); Zeeman et al., 2010 using EC), as well as for N_2O and CH_4 exchange under management that is typical for Swiss grasslands located on the Swiss Plateau (Imer et al., 2013 using SC for N_2O and CH_4 and EC for CO_2; Merbold et al., 2014 using EC for all three gases; Wolf et al., 2015 using EC and SC for N_2O). Two grassland parcels of 2.2 and 2.7 ha, are located adjacent to each other and have a similar management history, i.e. permanent grassland since at least 2002 with a restoration year in 2012 (Merbold et al., 2014). The most abundant species are English ryegrass (*Lolium perenne*) (a mixture of early and late varieties), common meadow-grass (*Poa pratensis*), red fescue (*Festuca rubra*), timothy (*Phleum pratense*), white clover (*Trifolium repens;* small leaf varieties PEPSI, HEBE and big leaf varieties FIONA, BOMBUS), red clover (*Trifolium pratense;* variety BONUS) sown in 2012, complemented by the volunteer species dandelion (*Taraxacum officinale*) and rough meadow-grass (*Poa trivialis*). Each parcel is usually mown four to six times per year for silage or hay production (Table 1). Each harvest is commonly followed by a fertilizer amendment, predominantly in the form of liquid slurry (average ± SD over 11 years (2003–2014) 266 ± 75 kg N ha^{-1} yr^{-1}).

The meteorological conditions at the site are characterized by an average annual temperature of 9.1 °C and an average annual precipitation sum of 1151 mm (Sieber et al., 2011). The soil is a gleysol/cambisol, with bulk densities in 0-0.2 m depth ranging between 0.9 and 1.3 g cm^{-3} (Roth, 2006) and a soil pH of about 6.5 (Labor Ins AG, Kerzers, Switzerland, in 2014).

2.2 Experimental setup and management activities

The field experiment comprised a control and a clover treatment parcel (Fig. 1). The control parcel was managed similarly to previous years, including the common management activities described above (harvest, fertilizer application and occasional grazing, Table 1). The eddy covariance tower, including meteorological sensors, was located at the border between the two parcels (Fig. 1). We used the two years 2013 and 2014 as reference years (no treatment). In order to test the N_2O mitigation option, the treatment parcel was over-sown in March 2015 and April 2016 with clover (*Trifolium pratense L.* and two varieties of *Trifolium repens L.*) to increase the clover proportion of the sward in the clover parcel. In contrast to the control parcel on which 296 and 181 kg N ha^{-1} were added in 2015 and 2016, respectively (Table 1), no fertilizer was applied on the clover parcel during the experiment. To assist clover establishment and increase the clover proportion in the clover parcel, the parcel was grazed with sheep after over-sowing in mid-June and beginning of July 2015 to keep the grass species short and thus reduce competition during the clover establishment phase. The control parcel was mown once instead of being grazed during this time (beginning of July). All other harvests took place at the same day on both parcels (see Table 1 for specific management data including dates, slurry composition and sowing rate).

Management activities comprised the regular harvest activities (mowing, swathering, and subsequent biomass removal) on both parcels, with subsequent slurry applications in the control parcel, besides occasional grazing, plus the over-sowing of the clover parcel. During our reference years 2013 and 2014, management was identical in both parcels in 2013, while in 2014 instead of mowing, cattle were grazing in the control parcel whereas the clover parcel was mown, resulting in similar reference fluxes from both parcels. Yields and exports of C and N were quantified by analysing biomass, sampled destructively during each harvest event (see Sect. 2.7 on vegetation samples), for C and N contents in the years 2015–2016. The fraction of N originating from BNF in the harvested biomass (2015–2016) was quantified *via* the ^{15}N natural abundance method (Unkovich, 2008). Combined with the legume biomass obtained by destructive biomass sampling at all harvest dates, we were able to calculate total amounts of BFN in the harvested biomass. Beyond our own observations, detailed management information for the years 2001–2016 were recorded by the farm staff in a field book. The overall amount of organic and mineral fertilizer applied to the field was documented, subsamples of the applied slurry were taken on the day of application (since 2007) and analysed in an external laboratory (LBU, Eric Schweizer AG, Thun, Switzerland). Slurry applied to the control parcel was digested cattle and pig slurry obtained from a local biogas plant (for chemical composition, see Table 1). Records in the field book also included information on herbicide application, harrowing, rolling and over-sowing (for details, see Table 1).

2.3 Greenhouse gas flux measurements

Greenhouse gas exchange (CO_2, N_2O, CH_4, H_2O) was continuously measured at the site using the eddy covariance (EC) technique, using a mast located at the boundary between the two parcels (Fig. 1). The choice of the EC tower location resulted in the fetch being located most of the time either in one or the other parcel, taking advantage of the two prevailing wind directions. The flux measurement setup consisted of a 3-D sonic anemometer (Solent R3, Gill Instruments, Lymington, UK), an open-path infrared gas analyser for CO_2 and H_2O concentrations (LI-7500, LiCor Biosciences, Lincoln, NE, USA) and a quantum cascade laser absorption spectrometer (QCLAS) capable to measure N_2O, CH_4 and H_2O concentrations (mini-QCLAS, Aerodyne Research Inc., Billerica, MA, USA) (Merbold et al., 2014) at 10 Hz resolution. The air inlet for the laser absorption spectrometer was located at a height of 2.1 m, just below the sonic anemometer head. The air was pulled through a 6 m long tube to the QCLAS located in a temperature-controlled weather proof box. Data acquisition and data storage were conducted according to the setup described in (Eugster and Plüss, 2010). From the high frequency measurements of these sensors, 10 and 30 min flux averages of the respective trace gases were calculated. The basic EC system, measuring CO_2 and H_2O exchange, has been running since 2005 (Eugster and Zeeman, 2006; Zeeman et al., 2010) and was complemented with the field-suitable QCLAS for high frequency (10 Hz) N_2O concentration measurements in 2012 (Merbold et al., 2014). Thus, more than two years of reference fluxes from both parcels under similar management regimes were collected before the beginning of the study presented here.

2.4 Meteorological and soil microclimate measurements

Meteorological variables measured at the Chamau site included air temperature and relative humidity (2 m height; Hydroclip S3 sensor, Rotronic AG, Switzerland), all components of the radiation balance (2 m height; CNR1, Kipp & Zonen B.V., Delft, The Netherlands), incoming and reflected photosynthetic active radiation (2 m height; PARlite sensor, Kipp and Zonen, Delft, the Netherlands) and precipitation (1 m height; tipping bucket rain gauge model 10116, Toss GmbH, Potsdam, Germany) (Table S1, Fig.1). Less than two meters from the tower, basic soil microclimate measurements were carried out. These measurements included volumetric soil water content (at 0.04 and 0.15 m depth; ML2x sensors, Delta-T Devices Ltd., Cambridge, UK) and soil temperature (at 0.01, 0.02, 0.05, 0.10, and 0.15 m depth; TL107 sensors, Markasub AG, Olten, Switzerland). In addition to the sensors close to the tower, each parcel was equipped with a similar set of soil sensors in 2015 (see soil plots, Fig.1) to compare potential differences in soil microclimatic conditions and subsequent effects on GHG fluxes. Soil pH (at 0.1 m depth) and soil oxygen (O_2) concentration (at 0.1, 0.2 m depth) were automatically measured using in-house custom-made sensors (based on ISFET pH-sensor kit, Sentron, Roden, Netherlands and EC410 Oxygen sensors, SGX Sensortech, Chelmsford, UK). In addition, soil water content (at 0.05, 0.1, 0.2, 0.5, 0.8 m soil depth; EC-5, Decagon, Pullman, WA, USA), soil temperature (at 0.05, 0.1, 0.2, 0.5, 0.8 m soil depth; T109, Campbell Scientific Inc., Logan, UT, USA), matrix potential (at 0.1, 0.2 m soil depth; Tensiometer T8, UMS GmbH, Munich, Germany) and soil heat flux (at 0.02 m soil depth; HFP01, Hukseflux B.V., Delft, Netherlands) were recorded. Some of the soil water content sensors stopped functioning on 18th June 2015 (at 0.05, 0.1, 0.2 m) and were thus replaced on 6th August 2015 (Decagon 5TM, Pullman, WA, USA). Signals of these sensors were sampled at 10 s intervals and stored as 10 min averages on a data logger (CR1000; Campbell Scientific Inc., Logan, USA). Sensors at the tower and in its vicinity were previously connected to a CR10X model (Campbell Scientific Inc., Logan, USA), and since March 2016 to a newer data logger (CR1000; Campbell Scientific Inc., Logan, USA).

2.5 Soil nutrient availability

For determining ammonium (NH_4^+), nitrate (NO_3^-) and dissolved organic carbon (DOC) concentrations in the soil, topsoil samples were taken down to 0.2 m depth. The nominally-biweekly sampling was intensified to daily intervals for seven consecutive days following slurry application (see also Wolf et al., 2015). Five samples per parcel were taken along a transect within the average footprint of the EC measurements. Extraction of NH_4^+, NO_3^- and DOC was achieved by shaking 15 g of fresh soil with 50 mL 0.5 M K_2SO_4 for 1 h and subsequent filtering (Whatman no. 42 ashless filter paper, 150 mm diameter, GE Healthcare AG, Glattbrugg, Switzerland) into centrifuge tubes (50 mL tubes, PP, Greiner Bio-One GmbH, St. Gallen, Switzerland). From the extract, a subsample was acidified for the measurement of DOC by combustion in a total organic C and N analyser (multi N/C TOC analyser 2100S, Analytik Jena AG, Jena, Germany). NH_4^+ and NO_3^- were analysed colorimetrically (Vis v-1200, VWR International, Radnor, PA, USA). Thereafter, the remaining soil samples were dried for one week at 105 °C and weighed before and after drying in order to determine the gravimetric soil water content.

2.6 Vegetation sampling and determination of biological nitrogen fixation

Vegetation samples were taken from each parcel at each harvest date by destructive sampling using harvest frames (0.1 m^2; n = 10 for each parcel per date randomly sampled within the EC footprint, clipped at mowing height of 0.05 m, Table S1). Vegetation was separated into legumes and non-legumes (grasses and forbs) to assess the legume proportion in the dry biomass. The only legume species found on site were the sown clover species *Trifolium pratense* L. and *Trifolium repens* L.. Vegetation samples were dried at 70 °C for one week and weighed before and after drying to estimate the water content. Milling of dry biomass samples was done separately for legumes and non-legumes, and a subsample of 5 mg was weighed into tin capsules for further analyses of total C and N, $\delta^{13}C$ and $\delta^{15}N$ (n = 5 for each parcel per date). C and N concentrations, as well as $\delta^{13}C$ and $\delta^{15}N$ values were analysed with a Flash EA 1112 Series elemental analyser (Thermo Italy, former CE Instruments, Rhodano, Italy) coupled to an isotope ratio mass spectrometer (DeltaplusXP, Finnigan MAT, Bremen, Germany). Estimates of BFN were based on the $\delta^{15}N$ measurement. The percentage of shoot N derived from BNF (%N_{dfa}, nitrogen derived from atmosphere) in legume biomass was calculated with the ^{15}N natural abundance method, (Boddey et al., 2000; Unkovich, 2008), following Eq (1):

$$\%N_{dfa} = \frac{(\delta^{15}N_{ref} - \delta^{15}N_{legume})}{(\delta^{15}N_{ref} - B)} \times 100, \tag{1}$$

where %N_{dfa} is the percentage of legume shoot N derived from atmosphere, $\delta^{15}N_{ref}$ is the $\delta^{15}N$ value of a non-fixing reference plant (i.e. grass species) growing in the proximity of the legume and $\delta^{15}N_{legume}$ is the $\delta^{15}N$ value of the legume shoot. The B value is the $\delta^{15}N$ signature of the legume species growing without N available from soil. B was estimated as the weighted mean of B values of *Trifolium repens* L. reported in the literature (–1.48 × ⅔) and *Trifolium pratense* L. (–0.94 × ⅓) (B values from Unkovich, 2008, Appendix 4). Weights were chosen according to the sown legume species composition of ⅔ white clover and ⅓ red clover. The %N_{dfa} in legume shoots was calculated for each legume biomass sample taken. The non-legumes cut within the same harvest frame as the legumes were used as reference delivering the $\delta^{15}N_{ref}$ value (Carlsson and Huss-Danell, 2014). For annual values, harvests and their components, uncertainty estimates were calculated with the Gauss uncertainty propagation (Table 2). Vegetation development was tracked via leaf area index (LAI) measurements (LAI-2000, LiCor Biosciences, Lincoln, NE, USA) carried out on both parcels biweekly as well as before and after mowing or grazing activities. Vegetation height and plant development as well as grazing activities within the footprint were further monitored via standard webcams (IN-5907HD, INSTAR Deutschland GmbH, Huenstetten, Germany).

2.7 Eddy covariance flux post-processing

Net ecosystem fluxes of CO_2, N_2O and CH_4 were quantified by the eddy covariance (EC) method as the covariance between turbulent fluctuations calculated by Reynolds averaging of 10-min blocks of data of vertical wind speeds and trace gas molar densities (CO_2) or mixing ratios (N_2O, CH_4). Molar densities of CO_2 were corrected for water vapour transfer effects (Webb et al., 1980). Frequency response corrections applied to raw fluxes accounted for high-pass (Moncrieff et al., 2004) and low-pass filtering (CO_2: (Horst, 1997); N_2O and CH_4: (Fratini et al., 2012). N_2O and CH_4 fluxes were additionally corrected for spectral losses due to instrument separation (Horst and Lenschow, 2009). All fluxes were calculated using the EddyPro software (v6.1.0, LI-COR Inc., Lincoln, NE, USA).

Before flux calculations, the statistical quality of the raw time series was checked (Vickers and Mahrt, 1997). Raw high-frequency data used in flux calculations were rejected (1) if raw measurements were outside a physically plausible range (vertical wind speed: ± 5 m s^{-1}; CO_2: 200 to 900 ppm, N_2O: below 250 ppb, CH_4: below 1700 ppb), (2) if spikes, defined as data points outside pre-defined sigma (σ) plausibility ranges (vertical wind speed: ± 5σ, CO_2: ± 3.5σ, N_2O and CH_4: ± 8σ), accounted for more than 1% of the respective raw time series, or (3) if more than 10% of available raw data were statistically different from the overall trend in a specific 10-min period. Raw CO_2 measurements were only used for flux calculations if the window dirtiness signal from the open-path infrared gas analyser did not exceed 80% on average per 10-min data block. Half-hourly fluxes were rejected, (1) if fluxes were outside pre-defined ranges (CO_2: ± 50 umol m^{-2} s^{-1}; N_2O: between –50 and 100 nmol m^{-2} s^{-1}; CH_4: between –400 and 800 nmol m^{-2} s^{-1}), (2) if the steady state test (Foken and Wichura, 1996) was outside ± 30%, or (3) if the test on developed turbulent conditions was outside ± 30% (Foken et al., 2004; Foken and Wichura, 1996). The analytical flux footprint model by Kljun et al. (2015) was used for footprint calculations.

The boundary between the two parcels is oriented approximately in East-West direction (75° degrees from north, Fig. 1). Each 10-min flux average was attributed to a parcel only if a minimum of 80% of the flux footprint was in the direction of the respective parcel (i.e. footprint weights from the direction of the respective parcel divided by the total of all flux footprint weights > 80%). Similar methods with EC fluxes from one setup being attributed to certain land use categories according to the respective footprint area were successfully used before (e.g. Biermann et al., 2014; Gourlez de la Motte et al., 2018; Neftel et al., 2008; Rogiers et al., 2005; Sintermann et al., 2011). After quality control, data coverage for N_2O exchange for both years was 62% of the entire period (details in Table 3). We observed moderate diurnal variations in flux origin from the two parcels (Fig. S2). Nevertheless, a similar share of quality-controlled N_2O fluxes was obtained from the control (48%) and the clover parcel (52%) during the observation period. The net effect in N_2O emission differences represents a conservative estimate, as N_2O emissions from the clover parcel are more likely to be overestimated and fluxes from the control parcel are more likely to be slightly underestimated (Fig. S2). Our aim was to analyse flux data originating from either one or the other parcel and avoid mixed GHG fluxes due to wind direction changes during the flux-averaging interval. As the standard 30-min averaging interval often resulted in mixed flux signals, we reduced the averaging period to 10 min, which resulted in a clearer representation of the temporal dynamics of GHG fluxes from each individual parcel. On grassland systems in flat terrain (as the Chamau site), eddies with a time scale of 1–5 minutes are dominating, and thus fluxes based on a 10-min averaging interval adequately represent the atmospheric exchange of GHGs (Lenschow et al., 1994). Our comparison of flux data (full time series) based on 10 and 30 minutes averaging intervals showed that the average of 10-min N_2O fluxes was only 2.3% lower than the 30-min N_2O fluxes. Daily averages were calculated based on all data points per parcel that fulfilled quality criteria 0 (best quality fluxes) or 1 (fluxes suitable for general analysis such as annual budgets) (Mauder and Foken, 2004).

2.8 Comparison of N_2O fluxes between parcels

We applied non-parametric bootstrapping in order to estimate the mean annual N_2O fluxes from both parcels and their respective confidence intervals. From all available 10-min fluxes, we took 1000 bootstrapping samples of each day per parcel. Averaging over time results in the bootstrapping estimate of the average annual flux, while the 0.025 and 0.975 percentiles of the bootstrapping distribution reveal the 95% confidence intervals for the mean flux per parcel.

Relative flux differences between parcels were defined as the difference of daily averages between clover and control parcels with respect to the average flux from the control, calculated based on all days for which data from both parcels were available. This was done to minimize potential biases associated with periods of unequal coverage of both parcels. Calculations were done following Eq. (2):

$$\Delta F/F = \frac{\overline{F_{Clover}} - \overline{F_{Control}}}{\overline{F_{Control}}} \tag{2}$$

F_{Clover} and $F_{Control}$ are daily average fluxes from the clover and the control parcels, respectively. Before being able to identify differences in N_2O exchange during the experimental periods, two years of flux data (2013 and 2014) were used to quantify how much the fluxes and the productivity from the two parcels deviated under exactly the same (2013) and similar (2014) management practice. For the calculation of CO_2 equivalents (CO_2-eq) we used factor 298, which is the current IPCC global warming potential including climate-carbon feedbacks on a 100 year basis (IPCC, 2013a).

2.9 Management and rain event specific N_2O exchange

Three management event types and one natural event type were analysed in more detail. These included organic fertilizer application, harvesting (mowing), sheep grazing, and rain events following dry weeks. When fertilization took place less than seven days after harvest, days after fertilization were classified as fertilization and thus not associated with the harvest event. If days after harvest overlapped with days before fertilization, these days were excluded from the fertilization class. In this case, the data displayed and analysed only refer to days after harvest but not to days before fertilization in order to avoid misleading references. A rain event was defined with > 4 mm precipitation following a dry period with < 1.5 mm collected during the 7 days preceding the rain event. When a fertilization event took place at the same time as the rain event (9th August 2015 and 16th July 2016), the event was classified as fertilization event but not as rain event. Grazing overlapped with a rain event on 15th June 2015 and 1st July 2015, thus these days were excluded from the rain event analysis. A pre-analysis was conducted for all these events, comparing N_2O emissions during seven days before the event to seven days after the start of the event (incl. starting date). Grazing showed no significant differences between emissions before and during grazing, nor did rain events. These categories were therefore not considered in the generalized additive model (GAM, see Sect. 2.11).

2.10 Statistical analysis

In order to assess the influence of management and environmental drivers of N_2O fluxes, we used semi-parametric generalized additive modelling (Wood, 2006). We expected non-linear effects of some predictor variables on N_2O emissions, such as soil water content and oxygen concentration. The GAM model is adequate for including these non-linear effects because it prescribes no parametric relationship between predictors and response variable. Instead, the model fits smoothing splines (piecewise defined polynomials) to the relationship between each predictor and the response variable, allowing highly flexible curves if needed (i.e. if improving the goodness of fit), but resulting in the smoothest possible relationship (i.e. linear relationship) if suitable. The response variable was predicted by the sum of all these smooth functions ("additive"). The degree of smoothing for each additive function was determined using generalized cross-validation (GCV).

The response variable was the log-transformed N_2O flux in order to better meet the assumptions of normally distributed residuals. The additive model with a log-transformed response corresponds to a model with multiplicative effects in the original scale. Thus, the predictors' effects influence N_2O fluxes multiplicatively. The influence of management (i.e. fertilization and harvest) and environmental driver variables (e.g. soil meteorological variables, soil chemical variables) on N_2O emissions was investigated based on daily averages of measured 10-min flux data and corresponding environmental variables. For introducing management influence in the regression analysis, dates were labelled according to three *a priori* selected management categories only: post-fertilization (F), post-harvest (H) and no management (here defined as no management during the previous week) (0) in combination with the treatment clover (Clo) or control (Ctr). Thus, five management categories existed (Ctr-F, Ctr-H, Ctr-0, Clo-H, Clo-0). The control parcel without recent management activity (Ctr-0) served as the reference level in comparison to all other management categories. As grazing intensity is low at the site, and grazing did not show any influence on N_2O exchange, we did not include grazing in the GAM analysis. The full set of predictors included soil temperature, soil water content, oxygen concentration, NH_4^-, NO_3^+ and DOC concentration for substrate availability, net ecosystem exchange (NEE) of CO_2 as a proxy for plant activity, and the categorical variable for management activity.

All predictors were included as non-linear terms in the first step, and the basic GAM was fitted using generalized cross-validation as the criterion for the parameter choice resulting in the best fit. This method resulted in several terms being included in the GAM as linear predictors (empirical degrees of freedom, edf = 1). These were finally treated as linear terms in order to obtain their effect sizes. For linear predictors such as soil temperatures, effect sizes can be interpreted as in linear regression models. Soil water content and oxygen concentration showed a non-linear influence on log-N_2O emissions (reverse U-shape), as estimated by the GAM to require more degrees of freedom (edf > 1). These were kept as (nonlinear) smooth terms in the GAM. Stepwise backward elimination was applied for model selection, whereby the number of predictors was reduced until the local minimum value of the Akaike Information Criterion (AIC) was found. Residual analysis showed that the final model residuals were in line with the assumptions of a Gaussian distributed, homoscedastic error term with a mean of zero.

Due to focusing the analysis on in situ measured data only, models that included the soil sampling variables are limited to the observational days on which manually sampled data were available (full model and optimized model). To check consistency of these results (i.e. effect sizes) with results from a wider range of observations (year-round continuous measurements) we built a model ("simple model") based on only the major driver variables soil temperatures, SWC and management as predictors, with the advantage of including more observations due to the wide coverage of these variables. Negative N_2O fluxes were analysed separately, but no significant effects of the same set of predictors on N_2O uptake were found. For auto-correlated time series (i.e. soil microclimatic variables) the t-test on the differences was corrected for autocorrelation by calculating the effective sample sizes according to (Wilks, 2011:147) and using the effective sample sizes in the tests, resulting in adjusted standard errors and p values (se_{adj}; p_{adj}). All statistical analyses were performed with the open source software R (R Core Team, 2016), using the "mgcv" package (Wood, 2011) for generalized additive modelling.

3 Results

3.1 General environmental conditions

Mean annual temperatures in 2015 and 2016 were 10.3 °C and 9.7 °C, respectively (Fig. 2a). Thereby 2015 was 0.2 °C warmer and 2016 was 0.4 °C colder than the previous five years which averaged 10.1 °C. Daily photosynthetically active radiation (PAR) followed the typical seasonal pattern (Fig. 2b). Annual precipitation was 1029 mm in 2015 and 1202 mm in 2016, which is 7% lower and 9% higher, respectively (Fig. 2c), than the 5-year mean annual precipitation (1101 mm). While both years were characterized by a typical wet beginning of the growing season (MAM with 376 mm in 2015 and 379 mm in 2016), similar to the five years prior to our period of analysis, the peak growing season (JJA) in 2015 was considerably drier (260 mm precipitation) than in 2016 (396 mm, Fig. 2c). Growing season, defined by T_{air} exceeding 5 °C for at least five subsequent days, started on 17th March 2015 and 30th January 2016. Starting dates of net CO_2 uptake for at least ten subsequent days, an alternative indicator for start of the growing season, were 27th February 2015 and 8th March 2016, similar to previous years.

3.2 Soil microclimate

An important precondition for the N_2O mitigation experiment is to check for approximately equal soil microclimatic conditions in both parcels, i.e. to exclude the possibility that soil microclimatic variables did act as confounders in the experiment. Soil temperatures were similar in the control (mean 14.5 °C) and the clover parcel (13.6 °C) with measured differences being smaller than the sensor accuracy of ± 1°C. While air temperature fell below 0 °C, soil temperature at 0.1 m depth never fell below 0 °C during the course of the experiment (Fig. 3a). This was also the case for the two reference years 2013 and 2014. Volumetric soil water content (at 0.1 m depth) were similar in the control (33 ± 4%) and the clover parcel (31 ± 5%). The difference between treatments was within the sensor accuracy of ± 3% (Fig. 3b). Oxygen concentration (at 0.1 m depth) ranged between 15 and 21% during three quarters of the measurement period and decreased consistently to 0% during spring in both years (Fig. 3c). Moreover, temporal patterns seen in O_2 concentration were not significantly different in both parcels (measured difference 0.3 ± 0.2% se.adj; p.adj = 0.075). Oxygen concentration during summer (JJA) 2015 was higher compared to 2016 (t= 2.64; p.adj = 0.03), as a consequence of less rainfall compared to summer 2016 (Fig. 2c). Soil oxygen concentration was inversely related to soil water content.

3.3 Soil mineral N and DOC concentration

Ammonium (NH_4^+) concentration in the soil peaked on each day of slurry application in the control parcel and declined during the following few days (Fig. 4a). NH_4^+-N concentration measured in the topsoil ranged between 0.4 and 19.2 mg NH_4^+-N kg^{-1} dry soil in the control parcel during the two years of observations. Significantly lower NH_4^+-N concentration was measured in the clover parcel (0.6–11.1 mg NH_4^+-N kg^{-1} dry soil; paired Wilcoxon-test, $p < 0.01$). While NH_4^+-N concentration peaked after fertilization events in the control parcel, no consistent patterns were observed in the clover parcel where no fertilizer was applied. Soil nitrate (NO_3^-) concentration ranged between 1.7 and 27.7 mg NO_3^--N kg^{-1} dry soil in the control parcel (Fig. 4b). Similar to the observations found for NH_4^+-N, significantly lower soil nitrate levels (0.6–18.9 mg NO_3^--N kg^{-1} dry soil) were found in the clover parcel (paired Wilcoxon-test, $p < 0.01$). NO_3^--N concentration significantly increased over the course of the season in the control parcel (Mann-Kendall-test, 2015 tau = 0.50, $p < 0.001$; 2016 tau = 0.40, $p < 0.001$). Such trend was not observed in the clover parcel in 2015, while it was significant in 2016 (Mann-Kendall-test, 2015: tau = 0.15, $p > 0.05$; 2016: tau = 0.35, $p < 0.01$) (Fig. 4b). Dissolved organic carbon (DOC) measured regularly from soil samples resulted in a range of 42–234 mg C kg^{-1} dry soil in the control parcel (Fig. 4c). Again, significantly lower values were measured for DOC in the clover parcel (0.6–160 mg C kg^{-1} dry soil) (paired Wilcoxon-test, $p < 0.01$) compared to the control. As observed for NO_3^--N, DOC concentration significantly increased with the growing season in the control parcel in both years and in the clover parcel in 2016 (Mann-Kendall-test, control parcel 2015: tau = 0.25, $p < 0.01$, 2016: tau = 0.23, $p < 0.05$; clover parcel 2015: tau = 0.14, $p > 0.5$, 2016: tau = 0.26, $p < 0.05$) (Fig. 4bc). Overall, soil mineral N and DOC concentrations were lower in the clover parcel.

3.4 Sward productivity and vegetation composition

Total annual yields (mean ± SE) of the control parcel were 12.8 ± 0.5 t dry matter (DM) ha^{-1} in 2015 and 11.9 ± 0.4 t DM ha^{-1} in 2016, while yields of the clover parcel were 10.4 ± 0.7 t DM ha^{-1} and 11.0 ± 0.5 t DM ha^{-1} in 2015 and 2016, respectively (Table 2). Previous years' yields of both parcels were 9.3 ± 3.2 t DM ha^{-1} yr^{-1} in the control and 6.6 ± 2.3 t ha^{-1} yr^{-1} in the parcel which was transformed into the experimental parcel during the years 2015 and 2016, based on data of all years with complete records between 2007 and 2013 (mean difference between parcels 2007–2013 of –2.7 t ha^{-1} yr^{-1}; experiment difference 2015/16 –2.4 and –0.9 t ha^{-1}, Tables S2). Thus, yield differences between the two parcels in 2015 and 2016 were in the range of yield differences observed during previous years, with yields being 19% (2015) and 9% (2016) lower at the clover parcel compared to the control parcel (Fig. 5a). The living aboveground biomass remaining on the parcel after mowing was 1.0 ± 0.3 t DM ha^{-1} on the control parcel and 0.8 ± 0.4 t DM ha^{-1} on the clover parcel (measured on 21st April 2015; Fig. 5b). Average clover proportion in harvested biomass in 2015 was 14.5% in the control parcel and 21.4% in the clover parcel. The difference in clover proportion between the two parcels was more visible in 2016, with 4.1% clover proportion in the control parcel and 44.2% in the clover parcel. When analysing individual sampling dates, differences in clover proportion between the control and clover parcel were highly variable in 2015, with substantially higher values for the clover parcel in the months April and June and slightly lower clover proportion in August when compared to the control parcel. In 2016, clover proportions increased and stabilized in the clover parcel, while they decreased in the control parcel with progress of the growing season (Fig. 5c). Leaf area index (LAI) ranged between 0.4 and 5.9, with a maximum at the first harvest each year (Fig. 5d). Average C concentrations in the biomass of all harvests were similar across parcels and plant functional types (legumes 42.9– 45.6%, non-legumes 43.0–45.2% C in biomass across parcels and years, Table 2; Fig. 5e). Average N concentrations in the biomass were always higher in legumes (3.3 ± 0.2%) compared to non-legumes (2.1 ± 0.2%) (Fig. 5f). C/N ratios (data not shown) of total annual yields were slightly higher in the control (19.2 ± 1.7 and 19.8 ± 2.8) than in the clover parcel (17.1 ± 1.0 and 16.7 ± 2.1) for both years, respectively. Vegetation height reflected the vegetation dynamics and reached similar maxima on the control parcel (41 cm and 59 cm) and the clover parcel (44 and 60 cm) in 2015 and 2016, respectively (Fig. 5g). C in annual yields at the control parcel was higher (5.8 ± 0.2 t ha^{-1}) compared to the clover parcel (4.7 ± 0.3 t ha^{-1}) in 2015, while C in biomass was similar for the control parcel (5.1 ± 0.3 t ha^{-1}) and the clover parcel (4.8 ± 0.2 t ha^{-1} yr^{-1}) in 2016 (Table 2). N exported was similar across parcels in the second year (control: 238 ± 13 kg ha^{-1} yr^{-1}; clover: 262 ± 8 kg ha^{-1} yr^{-1};Table 2). Biological nitrogen fixation *via* rhizobia associated with clover (N derived from the atmosphere – N_{dfa}) resulted in BFN in harvested biomass of 55.6 ± 5.3 kg N ha^{-1} yr^{-1} and 14.2 ± 1.7 kg N ha^{-1} yr^{-1} in the control parcel and 71.6 ± 5.0 kg N ha^{-1} yr^{-1} and 130 ± 8.0 kg N ha^{-1} yr^{-1} in the clover parcel during the first and the second year of the experiment, respectively (Table 2, Fig. 5h).

3.5 Differences in N_2O exchange between control and clover parcel

Average N_2O fluxes (with 95% confidence interval CI from the bootstrapping given in parentheses) in the control parcel in 2015 were 4.1 kg N_2O-N ha^{-1} yr^{-1} (CI 3.8–4.2 kg N_2O-N ha^{-1} yr^{-1}) and 1.9 kg N_2O-N ha^{-1} yr^{-1} (CI 1.8–2.0 kg N_2O-N ha^{-1} yr^{-1}) in the clover parcel. In 2016, average N_2O fluxes were higher for both parcels (6.3 kg N_2O-N ha^{-1} yr^{-1}, CI 6.0–6.5 kg N_2O-N ha^{-1} yr^{-1} in the control and 3.8 kg N_2O-N ha^{-1} yr^{-1}, CI 3.7–3.9 kg N_2O-N ha^{-1} yr^{-1} in the clover parcel) (Fig. 6a). Annual N_2O fluxes in the clover parcel were 54% (51–57% as 95% confidence intervals) and 39% (36–42%) lower than at the control parcel in 2015 and 2016, respectively (Fig. 6b). During the reference year 2013, average N_2O fluxes in the control parcel were

4.7 kg N_2O-N ha^{-1} yr^{-1} (4.6–4.8 kg N_2O-N ha^{-1} yr^{-1}) and in the clover parcel 4.8 kg N_2O-N ha^{-1} yr^{-1} (4.6–4.9 kg N_2O-N ha^{-1} yr^{-1}) and did thus not differ significantly. N_2O emission intensities (yield-scaled N_2O emissions) during the experiment were 0.31g N_2O-N kg^{-1} DM in the control parcel and thus higher than the 0.18 g N_2O-N kg^{-1} DM observed in the clover parcel in 2015. A similar pattern was observed in 2016, with N_2O emission intensities of 0.53 g N_2O-N kg^{-1} DM versus 0.37 g N_2O-N kg^{-1} DM in 2016 for control and clover parcel, respectively.

3.6 Effects of management activities on N_2O exchange

We observed increased N_2O fluxes after fertilisation in the control parcel, with maximum daily N_2O fluxes reaching 17.4 mg N_2O-N m^{-2} d^{-1} on 25th August 2015 (Fig. S1a), a day of slurry amendment. The effect of fertilizer amendment on N_2O fluxes depended on the environmental conditions during and after the fertilisation event. While several events (e.g. 10th June 2015, 25th August 2015, 16th July 2016 and 17th August 2016, Fig. S1a) were followed by increased N_2O emissions, other events (e.g. 1st June 2016) did not show such an effect (Fig. S1a, inter-quartile range displayed in Fig. 7a). N_2O fluxes decreased to background levels within a few (3–7) days after fertilizer application. Harvest had a moderate influence on N_2O emissions on both parcels (Fig. 7c). Maximum daily N_2O fluxes after harvest were 7.0 mg N_2O-N m^{-2} d^{-1} on 5th July 2016 (Fig. S1a). Average N_2O fluxes on both parcels were significantly higher the weeks after harvest (average of both parcels: 2.0 mg N_2O-N m^{-2} d^{-1}) compared to average fluxes during the pre-harvest weeks (1.4 mg N_2O-N m^{-2} d^{-1}) (Fig. 7b). Neither grazing nor rain events significantly affected N_2O exchange (Fig. 7cd).

3.7 Influence of potential drivers on N_2O exchange

Nitrous oxide emissions significantly increased after fertilizer application (Ctr-F compared to Ctr-0, $p < 0.05$) when compared to N_2O fluxes during periods of no management on the same (control) parcel (Fig. 8a, Table 4). The effect size showed 2.5-fold N_2O emissions during the seven days following slurry amendment compared to no management (resulting from applying the back-transformation to the fertilization effect: $10^{0.4} = 2.5$; Table 4). The effects of management influence N_2O fluxes jointly with other measured driver variables, such as soil moisture, soil temperature, NH_4^+-N, NO_3^--N and DOC concentration in the soil. After mowing no significant increase in N_2O emissions was found for the optimized model in either of the parcels (Table 4b). In contrast a difference in N_2O emissions after harvest was observed for the simple model on the control parcel (Table 4c). If the difference in sward composition itself affected N_2O emissions (e.g. via plant residues or rhizodeposition), we expected a significant effect of the clover treatment compared to the control during times without management (Ctr-0 which was the reference compared to Clo-0, Table 4). Due to the absence of such an effect, we deduce that the increased clover proportions at the clover parcel did not affect N_2O emissions.

Soil microclimate affected N_2O emissions in both parcels. Soil temperature significantly influenced N_2O emissions ($p < 0.05$), indicating a 7% (± 2%) increase in N_2O per °C temperature increase ($p < 0.05$, Table 4, Fig. 8b). Soil temperature had the highest explanatory power ($r^2 = 0.17$) for the prediction of log-transformed N_2O flux as a single explanatory variable (data not shown). Besides soil temperature, volumetric soil water content showed a significant non-linear effect on N_2O emissions (p <

0.05, Fig. 8c). The humpback-shaped functional relationship between volumetric soil water content and log-transformed N_2O emissions (Fig. 8c) shows an increase until 34% and a decrease above 36% volumetric soil water content. Similarly, oxygen concentration significantly affected N_2O emissions ($p < 0.05$, Fig. 8d). Oxygen concentration was non-linearly related to N_2O emissions, showing lowest N_2O emissions (10^{-4} µmol m^{-2} s^{-1}) at 0% oxygen concentration. N_2O emissions increased until a maximum was reached at 17–19% oxygen concentration, and then decreased with further increasing oxygen concentration to atmospheric concentrations of 20.9% (Fig. 8d). Net ecosystem exchange of CO_2, which was used here as a proxy for plant activity, affected N_2O emissions ($p < 0.05$, Fig. 8e) with a 4% (± 2%) decrease of N_2O emissions per µmol m^{-2} s^{-1} net carbon dioxide uptake. Inclusion of NH_4^+-N concentration improved the prediction of N_2O emissions (Table 4, Fig. 8f), leading to an emission increase of 5% (± 3%) per µmol m^{-2} s^{-1}. Note that large NH_4^+-N concentrations only occurred after fertilization, thus the NH_4^+-N effect was mainly influenced by these dates, while it did not play a role for the other management categories. In contrast, NO_3^--N concentration did not improve the prediction of N_2O emissions (Table 4, Fig. 8g). Also, DOC concentrations showed no effect on N_2O emissions (Table 4, Fig. 8h). The slopes of the relationship between drivers and predicted N_2O emission are flatter than expected from visual inspection of the observed values (Fig. 8), as the predictions here depict the dependency of N_2O emissions on the respective driver alone (based on averages of all other drivers), in contrast to observations, which depict combinations of effects of several drivers. The effects of soil temperature, soil water content and management in the full and the optimized model (Tables 4a and 4b) were consistent with the simple model (Table 4c) that included only these three variables and therefore more observations ($n = 891$ versus $n = 93$). Including additional variables (O_2, NH_4^+-N, NEE of CO_2) besides soil temperature and soil water content increased the explained variance in N_2O emissions from 26.3% in the simple model (Table 4c) to 54.5% in the optimized model (Table 4b).

4 Discussion

We quantified ecosystem N_2O exchange at a fertilized control parcel (“business as usual”) and an unfertilized clover parcel where we increased the clover proportion (“mitigation management”). The mitigation management was composed of two major changes compared to the “business as usual” practice; (1) omitted fertilization and (2) over-sowing clover, leading to an increased clover proportion in the experimental sward (i.e. 21% versus 15 % in 2015, 44% versus 4% in 2016). Our analysis showed that the difference in N_2O emissions between both parcels can be attributed to the absence of fertilization on the clover parcel. Increased clover proportion could still have increased N_2O emissions in the clover parcel due to N-rich clover residues and N from root exudates (Rochette and Janzen, 2005), and thereby offset the effect of reduced fertilization. However, we measured similar N_2O fluxes originating from the two parcels of different clover proportion during periods without management, indicating that differences in clover proportion alone (i.e. excluding recent management effects) resulted in unchanged N_2O emissions (i.e. plant residues and root exudates affected N_2O emissions similarly on the clover and the control parcel). We quantified the effects of environmental drivers on N_2O emissions and identified soil temperature, soil oxygen concentration, soil water content and NEE of CO_2 as main environmental drivers of N_2O emissions. The assessment of the mitigation strategy revealed reductions in N_2O emissions, an increase in BFN and stable yields under mitigation management. This study covered two years and did not include potential effects of incorporation of clover into the soil during ploughing (which takes place every 8–10 years). Long-term effects of the mitigation strategy on the N budget of the site, as well as implications on the farm level, (e.g. the feasibility to use the slurry to replace mineral fertilizer elsewhere, fodder composition) should be investigated in future studies. In summary, our results indicate that N_2O emissions can be effectively reduced at ecosystem scale through enhancing the clover proportion (and BFN) in permanent grassland while reducing organic fertilizer inputs and still meeting the N requirements of plants.

4.1 N_2O emissions in the fertilized grassland parcel

N_2O emissions in the control parcel summed up to 4.1 and 6.3 kg N_2O-N ha^{-1} yr^{-1} for the two years, respectively, corresponding to 1.4 and 3.5% of the applied fertilizer N. Annual N_2O emissions are of the same order of magnitude as the values reported from the site in previous years (2010 and 2011) by (Imer et al., 2013), who estimated 2.2–7.4 kg N_2O-N ha^{-1} yr^{-1} based on manual N_2O measurements using static GHG chambers. Similar N_2O emissions of 4.5 kg N_2O-N ha^{-1} yr^{-1} (0.3–18.2 kg N_2O-N ha^{-1} yr^{-1}) from other fertilized grassland sites were reported by Jensen et al. (2012) in a synthesis paper covering 19 site-years. Fertilized grassland sites in Central Europe, and particularly grasslands at higher altitudes, typically gave lower N_2O emissions (0.19–5.28 kg N_2O-N ha^{-1} yr^{-1} across site-years, or 0.1–2.5% of fertilizer input) compared to our site, which showed the highest emissions with respect to both absolute N_2O emissions as well as emissions as a percentage of fertilizer N input (2.55–7.89 kg N_2O-N ha^{-1} yr^{-1} or 1.1–3.6% of fertilizer N input across site-years 2010–2013) as reported by Hörtnagl et al. 2018, compared to 1.4–3.5% of fertilizer N in our study (2015 and 2016). For a more targeted comparison, here we considered only the non-restoration site-years and excluded the 2012 which showed high N_2O emissions particularly related to grasslands restoration. The Hörtnagl et al. (2018) study covered years 2010–2013 of our site but used a different gap-filling method. The high emissions from our site were explained by warm temperatures (~20°C) combined with moist to wet soil moisture conditions after fertilizer events, and therefore particularly favourable conditions for N_2O production compared to conditions at other sites. Hörtnagl et al. (2018) used a conservative method to estimate fluxes during periods without measurement (running-median gap filling, resulting in low estimates when gaps are filled during emission peaks). In this study, gaps for annual estimates were filled with the arithmetic average because this method appropriately represents an average of peak and background emissions, rather than predominantly representing background emissions as with the running median method. In summary, our year-round measurements of N_2O emissions are higher than the multi-site averages due to its fertilizer regime and site conditions, but within plausible ranges compared to other sites.

4.2 N_2O emissions in the unfertilized clover parcel

N_2O emissions in the clover parcel during our two-year observation period summed up to 1.9 and 3.8 kg N_2O-N ha^{-1} yr^{-1} in 2015 and 2016, respectively. These N_2O emissions were clearly lower than the values observed in the control parcel during both years. In 2015, the difference can be attributed to the difference in fertilization between parcels, as the clover proportion was still similar in both parcels (control parcel: 15%; clover parcel: 21% clover). In 2016, large differences in clover proportion (control parcel: 4%; clover parcel: 44% clover) resulted in similarly lower N_2O emissions on the clover parcel as in 2015. However, N_2O emissions in the clover parcel were high compared to other unfertilized grass–clover mixtures with zero or low fertilizer inputs (< 50 kg N) for which average emissions of 0.54 kg N_2O-N ha^{-1} yr^{-1} (0.10–1.30 kg N_2O-N ha^{-1} yr^{-1}) were reported by Jensen et al. (2012) based on eight site-years. Further non-fertilized grass-clover mixtures showed annual N_2O emissions of up to 2.5 kg N_2O-N ha^{-1} yr^{-1} (Li et al. 2011, Table 5). Thus, our measurements exceeded the typical range of values in the second year by 50%. Regular N amendments at the Chamau site in the past might have led to immobilization of N via microbes and subsequent enrichment of the soil organic N (SON) pool (Conant et al., 2005; Ledgard et al., 1998). This in turn is known to lead to higher background N_2O emissions in relation to N_2O emissions observed from sites under long-term extensive management. In addition, high total N deposition (NH_3, NO_3, HNO_3, NO_2) in the study area (in total 33.8 kg N ha^{-1} yr^{-1}in 2015; Rihm and Achermann, 2016) might foster background N_2O emissions due to increased NH_4^{-+} and NO_3^- availability (Butterbach-Bahl et al., 2013). Additionally, NH_3 deposition on the clover parcel originating from NH_3 emissions from the adjacent control parcel is likely to be the cause of increased soil NH_4^{-+} concentrations after the event on 17th August 2016. Furthermore, a possible explanation for the relatively high N_2O emissions from our clover parcel in 2016 were the meteorological conditions which were wetter during summer and therefore more favourable for N_2O production than during 2015. High background N_2O emissions in the clover parcel in 2016 were reflected by similarly high background N_2O emissions in the control parcel, indicating that these were mainly driven by other factors (favourable meteorological conditions, sufficient N substrate availability) and not by the sward composition itself.

4.3 Effects of management and environmental drivers on N_2O emissions

Our aim was to identify the main drivers of N_2O emissions and therefore we investigated the effects of management (fertilization, harvest, grazing, over-sowing leading to increased clover proportion) and environmental variables on N_2O emissions. Fertilization of the control parcel had the largest effect on N_2O emissions. Increased N availability due to fertilization is widely known as a main driver of N_2O emissions, which makes it a key factor for mitigating N_2O emissions (Bouwman et al., 2002; Smith et al., 1997). Nevertheless, effects of fertilization on N_2O emissions vary widely across grassland sites and years (0.01–3.56% in Flechard et al., 2007; 0.1–8.6% in Hörtnagl et al., 2018, 1.4 and 3.5% of fertilizer N across years in this study), indicating that fertilization alone is insufficient for explaining N_2O emissions and highlighting the need to take additional drivers into account. We further observed increased N_2O emissions following harvest events on the control parcel, which may be explained as a consequence of increased rhizodeposition (Bolan et al., 2004; Butenschoen et al., 2008). Subsequently, greater availability of labile C compounds can lead to increased microbial activity, accompanied with increased production of N_2O (Rudaz et al., 1999). Higher N_2O fluxes following cutting were similarly observed on a pasture in Central France (up to 3.7 mg N_2O-N m^{-2} d^{-1}in Klumpp et al., 2011; up to 7.0 mg N_2O-N m^{-2} d^{-1} in this study). Grazing had only a minor influence on the overall N_2O budget of the Chamau site with 3.71% of N_2O-N emitted during grazing periods and data analysis showed that N_2O fluxes did not significantly respond to the presence of animals (Fig. 7c). We attribute this observation to low stocking densities and short duration of grazing (Table 1). Other studies with higher stocking densities have shown that more intensive grazing led to increased N_2O emissions (van Groenigen et al., 2005; Oenema et al., 1997). These were attributed to C and N from animal excreta and to soil compaction by treading and trampling animals, creating anaerobic soil conditions (Flechard et al., 2007; Lampe et al., 2006; Oenema et al., 1997).

An important finding from this study is that increased clover proportion, and subsequently increased BFN, did not increase N_2O emissions, as shown by comparing N_2O emissions between both parcels during periods without management (Table 5c, Clo-0). In other words, substrate from decomposition of plant residues and from root exudates may affect N_2O emissions, but this effect was similar on both parcels, independent of the higher clover proportion and BFN in the clover parcel. This is in contrast to a study on a boreal grass-clover mixture in which significant N_2O emissions were observed in spring, largely exceeding the fertilized grassland control (Virkajärvi et al., 2010). These higher emissions were explained by increased substrate available to microbial communities producing N_2O in the surface layer after spring thaw (Wagner-Riddle et al., 2008). Nitrous oxide emissions from BNF itself (rhizobial denitrification) have been shown to be possible (O'Hara and Daniel, 1985). Nevertheless, due to its small magnitude the contribution to field-scale N_2O emissions is negligible (Rochette and Janzen, 2005). Previous results from a laboratory incubation by Carter and Ambus (2006), who investigated N_2O emissions from unfertilized soils for up to 36 weeks, showed that recently fixed N_2 in a white clover-ryegrass mixture contributed as little as 2.1 ± 0.5% to total N_2O emissions. In agreement with our result, measurements from permanent grasslands in Ireland, where winter freeze-thaw cycles are very rare, showed that annual N_2O emissions in unfertilized ryegrass (2.38 ± 0.12 kg N_2O-N ha^{-1} yr^{-1}) were not significantly different from an unfertilized grass–clover sward (2.45 ± 0.85 kg N_2O-N ha^{-1} yr^{-1}) with clover proportions of 20–25%, hence providing evidence that N_2O emission due to BNF itself and clover residual decomposition were negligible (Li et al., 2011). Our findings are in line with these observations and add the insight that clover proportions of up to 44%, as found in our study, will not result in increased N_2O emissions.

The effects of temperature and soil water content on N_2O emissions as found in our study are in line with established knowledge (Butterbach-Bahl et al., 2013; Flechard et al., 2007). Furthermore, directly measured soil oxygen concentrations, which have hardly been used in field-scale studies before, improved the prediction of N_2O emissions (Table 4). Our data showed that larger plant C uptake (negative NEE) of CO_2 as proxy for plant activity was associated with reduced N_2O emissions, which supports the hypothesis that plant roots are in competition for available N with microbes and often reduce the N availability to microbes (Merbold et al., 2014). Thus, we observed lower N_2O emissions at higher levels of photosynthesis. Our analysis showed that inclusion of NH_4^+-N concentration in the statistical analysis improved the prediction of N_2O emissions, while NO_3^--N and DOC were of less importance for the prediction of N_2O emissions. Comparable results for the influence of NH_4^+ and NO_3^- were found at an Irish grassland (Rafique et al., 2012). In summary, fertilization was the dominant predictor of N_2O emissions, while soil temperature, soil water content, soil oxygen concentration and NEE of CO_2 were significant environmental drivers. Concluding from all management effects, the decrease in annual N_2O emissions under the mitigation strategy was primarily caused by the absence of fertilization, while a potential effect of the increase in clover proportion and increased BFN offsetting these emission reductions was absent.

4.4 Effect of the mitigation strategy on productivity and biological nitrogen fixation

An important precondition for the acceptance of any climate change mitigation strategy is that yields need to be maintained at similar levels as under conventional management. Differences in biomass yields between the control and clover parcels were only minor (19% and 9% lower in the clover parcel in 2015 and 2016, respectively), and comparable to the observed differences between the two parcels prior to the mitigation experiment (Table S2). Maintaining high yields without fertilization can be explained by the increased BFN in the clover parcel and positive interactions between clover and grass ("overyielding effect") (Lüscher et al., 2014; Nyfeler et al., 2009). Additionally, high SON content due to previous year's fertilizer amendments are expected to contribute to the persistently high production levels (Table 2). Similar productivity levels of an unfertilized grass-clover mixture (three cuts, 9% less DM) compared to an adjacent intensive grass-clover mixture (230 kg N fertilizer, 4–5 cuts) were also found at a site 50 km from the Chamau field site in the past (Ammann et al., 2009). Furthermore, our findings are consistent with findings from the more comprehensive study by Nyfeler et al. (2009), who found large overyielding effects in comparable Swiss grassland systems, i.e. grass-clover yields at 50 kg N ha^{-1} yr^{-1} and 50 to 70% clover were as productive as grass monocultures fertilized with 450 kg N ha^{-1} yr^{-1}. The overyielding effect has been reported across a wide range of climates and soil types (Finn et al., 2013; Kirwan et al., 2007), indicating that our result of maintained productivity levels under the mitigation strategy is likely to be reproducible across a wider range of site conditions.

Biologically fixed nitrogen found in shoot biomass was slightly higher in the clover parcel (72 kg N ha^{-1} yr^{-1}) compared to the control parcel (55 kg N ha^{-1} yr^{-1}) in 2015 due to only small differences in clover proportion between both parcels. During the second year, the over-sowing was more effective and BFN found in shoot biomass in the clover parcel summed up to 130 kg N ha^{-1} yr^{-1} while only 14 kg N ha^{-1} yr^{-1} were measured in the control parcel. Previous studies reported similar amounts of BFN for mown and grazed pasture systems (Ledgard and Steele, 1992; Nyfeler et al., 2011), with maxima being as high as 323 kg N ha^{-1} yr^{-1} as observed in a comparable grass-clover mixture (Nyfeler et al., 2011). This indicates that biologically fixed nitrogen at the Chamau could reach higher amounts than observed during our experiment. Clover proportions at our site varied seasonally, with a minimum in spring and maximum in summer in both parcels. Such seasonal cycles in clover proportions occur due to species' developmental cycles, but also competitive advantages/disadvantages of the respective species. Drier conditions, observed for instance in summer (JJA), result in competitive advantages of the clover compared to grasses, as N_2 fixation is less sensitive to dry conditions than uptake of mineral N (Hofer et al., 2017; Lüscher et al., 2005). Furthermore, inter-annual variability of clover proportions can be an additional management challenge for farmers whose aim is to keep a persistent sward composition (Lüscher et al., 2014).

Lower SON content (3490 kg N ha^{-1}) in a grass-clover mixture compared to a 200 kg ha^{-1} yr^{-1} fertilized grassland (4350 kg N ha^{-1}) was observed after 13 years of management comparable to our experiment (Ledgard et al., 1998). It is well-known that N exports exceeding inputs lead to a decreasing SON pool. Potential losses in SON were shown to be closely linked to losses in soil organic C (SOC) (Ammann et al., 2009; Conant et al., 2005) and can therefore compromise the soil's CO_2 sink strength. Thus, detailed investigations on the effect of the clover treatment on SON, SOC content and CO_2 exchange are recommended to comprehensively evaluate the mitigation strategy in the long term.

4.5 Effect of the mitigation strategy on N_2O emissions and emission intensities

We found that the mitigation strategy effectively reduced both N_2O emissions by 54% (51–57%) and 39% (36–42%) in 2015 and 2016 as well as N_2O emission intensities by 41% and 30% in 2015 and 2016, respectively. Past studies carried out in temperate grasslands consistently found reductions in N_2O emissions when reducing fertilizer and increasing BFN through legumes (Table 5). The magnitude of relative N_2O emission reductions ranged from 34% (Šimek et al., 2004) to 100% (Ammann et al., 2009), with absolute N_2O emission reductions of 0.8 kg N ha^{-1} yr^{-1} (Šimek et al., 2004) to 11.1 kg N ha^{-1} yr^{-1} (Schmeer et al., 2014). The variability across studies can be attributed to differences in meteorological and soil conditions as well as variations in the experimental setup (i.e. fertilizer rates applied, realized legume proportions, grass and legume species, Table 5). Much higher N_2O emissions from an unfertilized grass-clover mixture (92% increase) compared to N_2O emissions from a grass sward fertilized with 220 kg N ha^{-1} yr^{-1} were observed under boreal climate conditions in eastern Finland, due to large springtime emissions associated with freeze-thaw cycles (Virkajärvi et al., 2010). Such an effect could not be found at our site, although soils also freeze occasionally during the cold season, but at most in the top few centimetres. Although our tested mitigation strategy seems to be beneficial for permanent grasslands, Basche et al. (2014) and Lugato et al. (2018) have shown that incorporation of clover into the soil may lead to increased N_2O fluxes and thus may not be the best mitigation strategy for croplands and temporary grasslands, where ploughing is done much more frequently.

In summary, the implementation of the mitigation option tested here was found to be effective at permanent grassland in the temperate zone, and is cheap and simple as it requires few management activities, which would favour farmers willingness for implementation (Vellinga et al., 2011).

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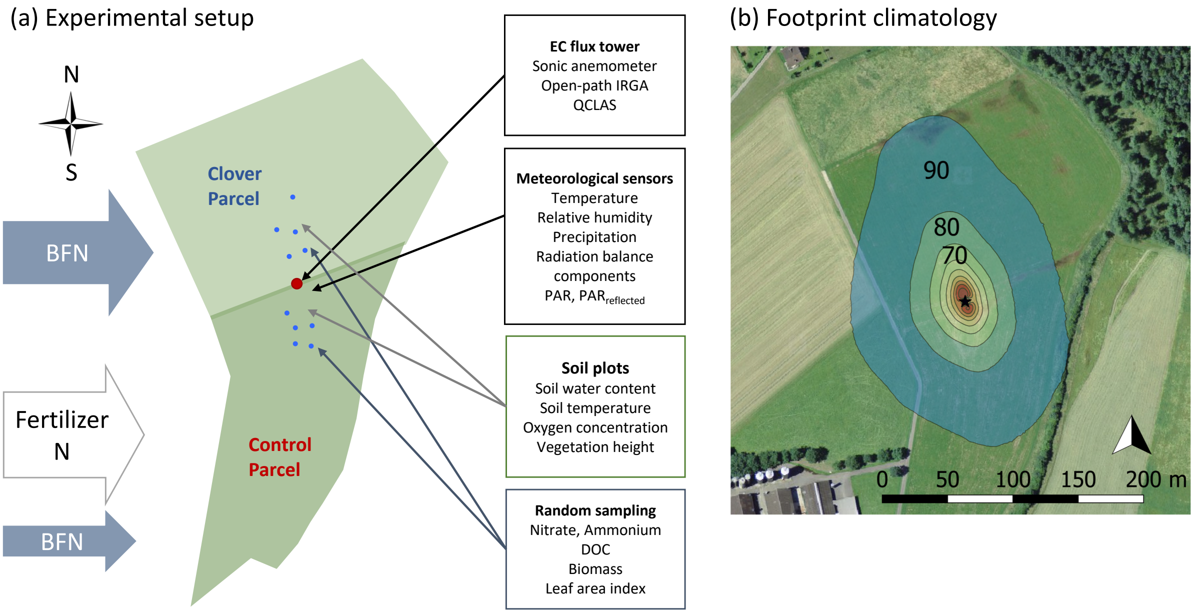

Figure 1. (a) Experimental setup and measured variables at the experimental research site Chamau (CH-Cha). The clover parcel (north) is managed to increase nitrogen inputs from the atmosphere via increased biologically fixed nitrogen (BFN). This was achieved by over-sowing with clover in March 2015 and April 2016. In contrast, the control parcel under conventional management (south) obtains most N in form of organic fertilizer (i.e. slurry) and only small N inputs via BNF. Blue dots represent soil sampling locations. (b) Footprint climatology of the years 2013–2016 with footprint contour lines of 10% to 90% in 10% steps using the Kljun et al. (2015) footprint model (source for background picture: Swisstopo (https://map.geo.admin.ch/). The prevailing wind direction was from the north.

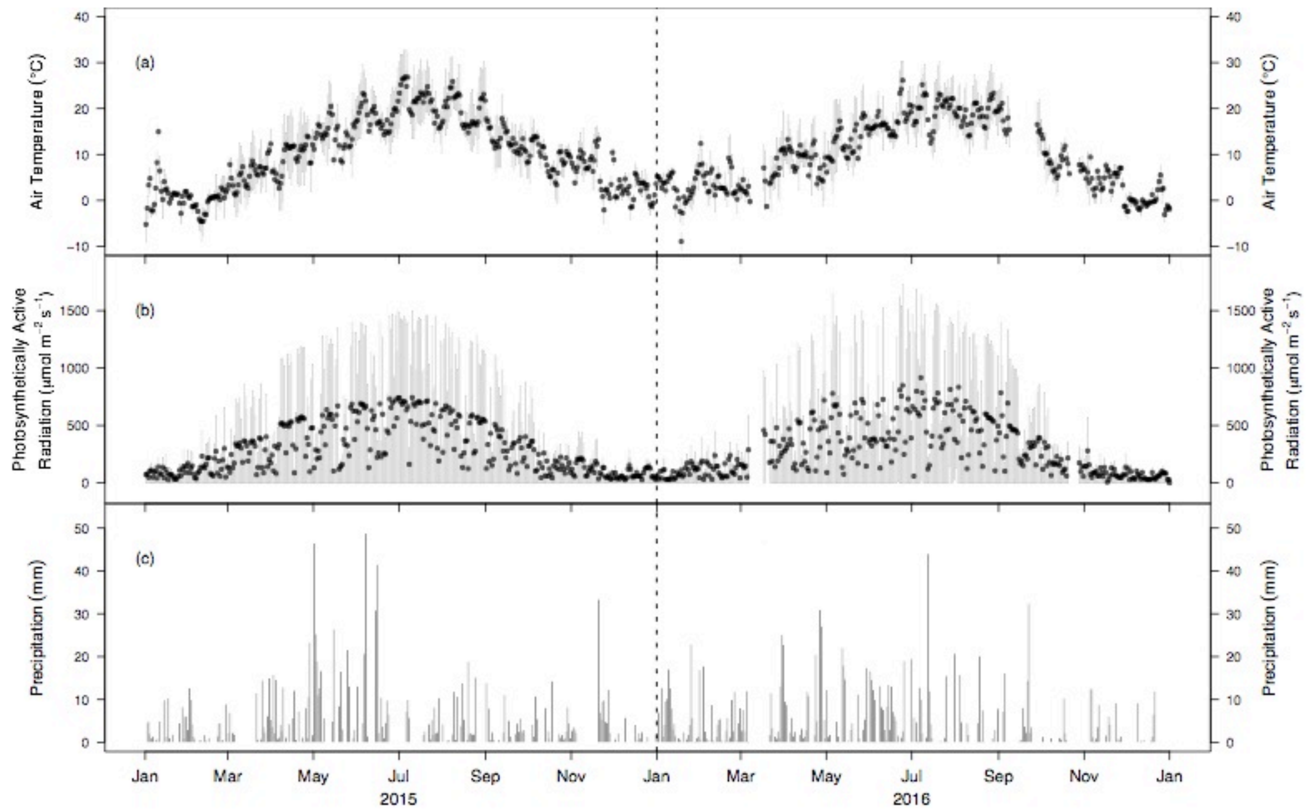

Figure 2. Meteorological conditions during 2015 and 2016. (a) Average daily air temperature (2 m), (b) average daily photosynthetically active radiation (2 m). The grey bars indicate the sub-daily variability (quartiles based on 10 min values). (c) Daily precipitation sums during 2015 and 2016 (1 m).

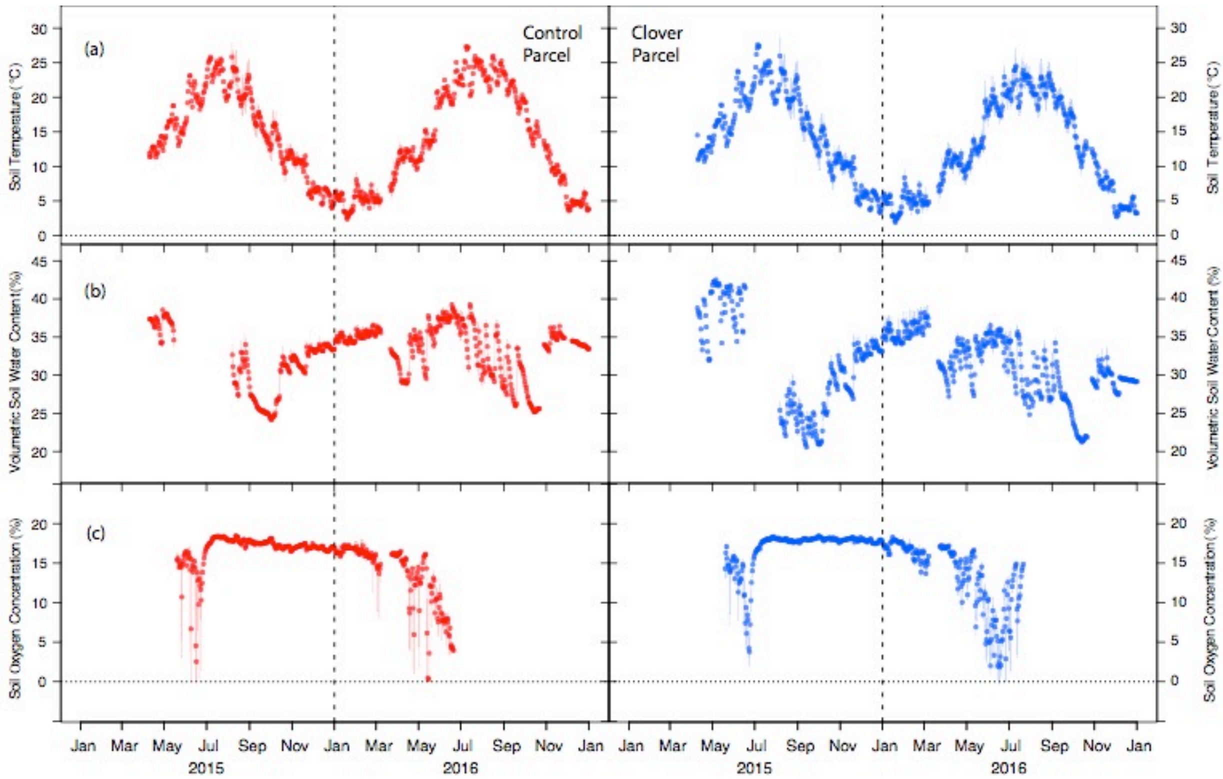

Figure 3. Soil meteorological conditions during 2015 and 2016. (a) Average daily soil temperature (0.1 m depth), (b) average daily soil water content (0.1 m depth), (c) average daily soil oxygen concentration (0.1 m depth) at the control (left, red) and clover parcel (right, blue). The bars indicate the sub-daily variability (ranges of 10 min values).

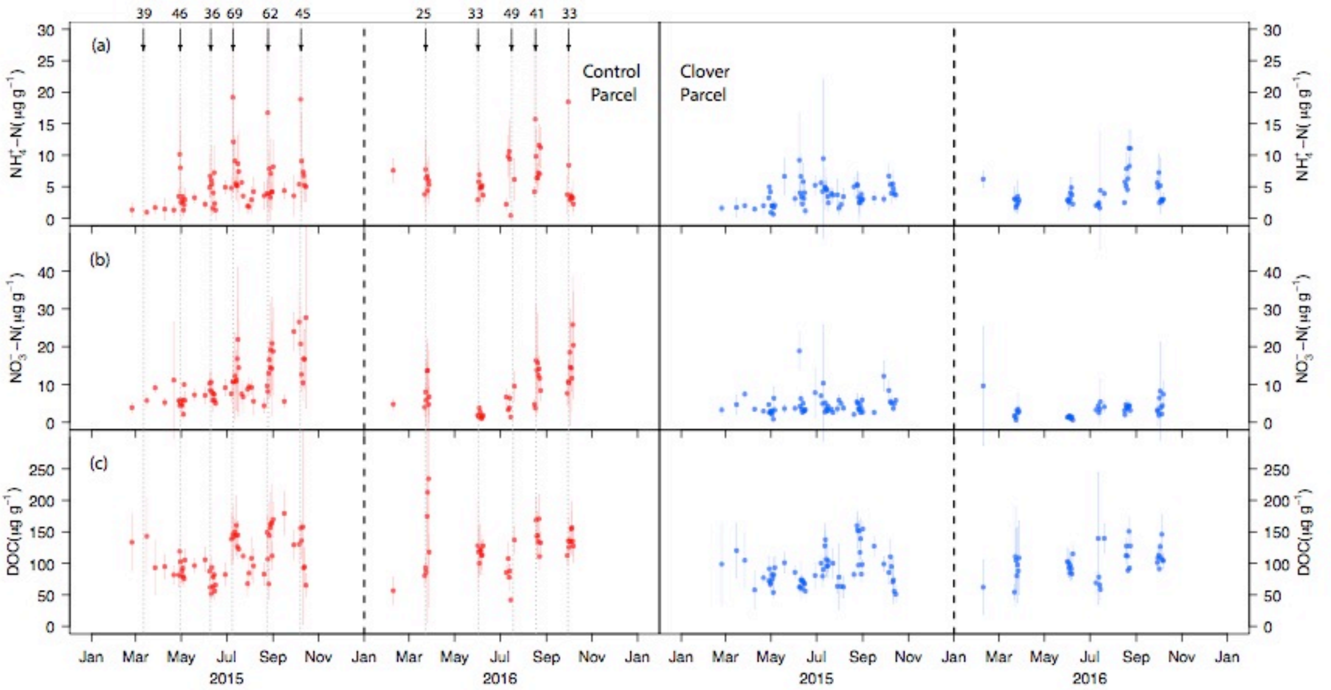

Figure 4. (a) Ammonium-N concentration, (b) nitrate-N concentration, (c) dissolved organic carbon concentration per unit of dry soil at the control (left, red) and clover parcel (right, blue) during 2015 and 2016. Black arrows indicate slurry applications, which only took place in the control parcel. Numbers above the arrows indicate the amount of N (kg ha^{-1}) added to the parcel.

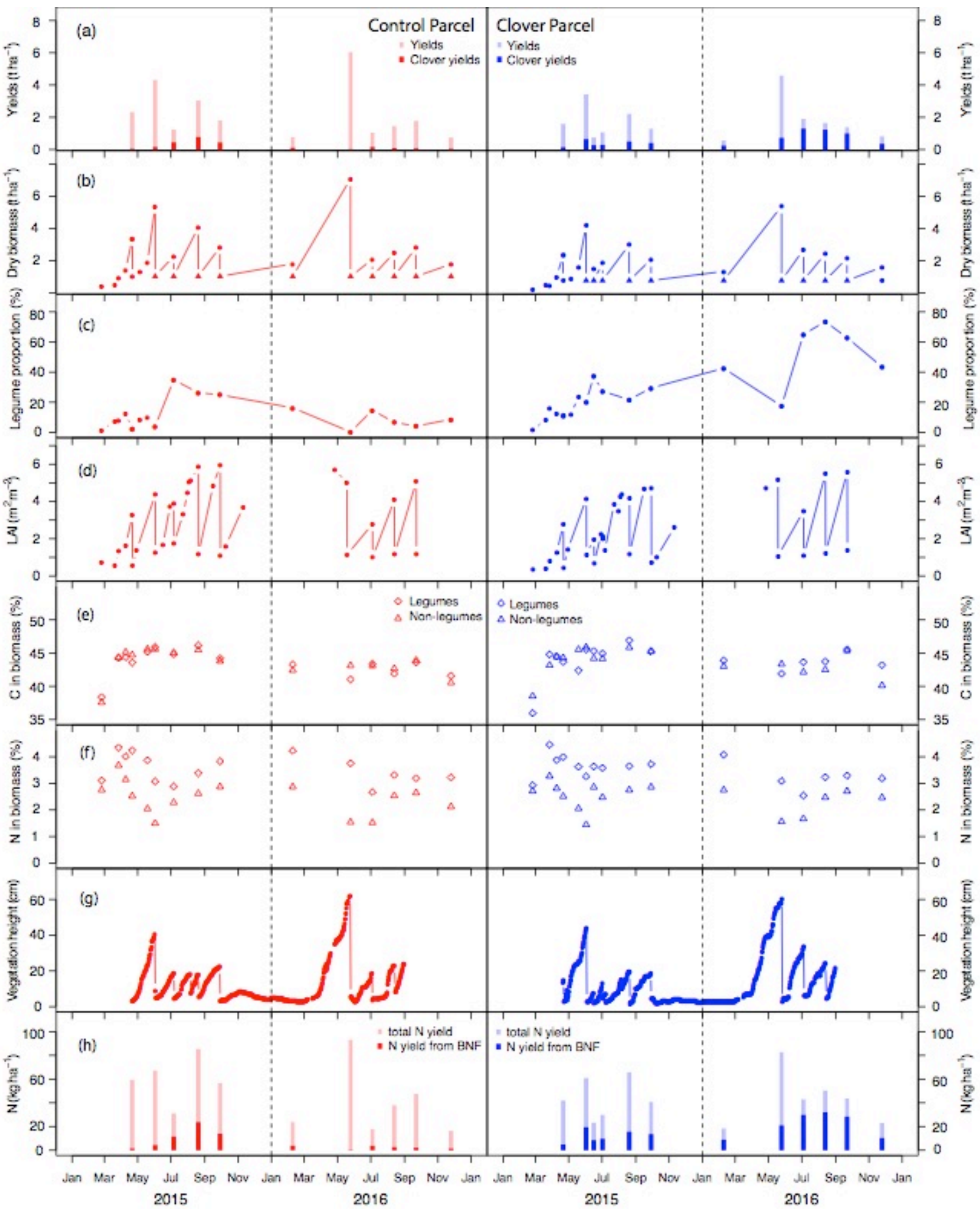

Figure 5. (a) Yields and intake by grazing at the control (left, red) and clover parcel (right, blue), (b) total aboveground biomass. Circles represent the total biomass (legumes and non-legumes), filled triangles are displaying the remaining biomass after harvest (stubble), which was measured once (sampling date 21st April 2015) and assumed to be approximately similar during subsequent harvests. (c) Clover proportion in dry biomass, (d) leaf area index (LAI), (e) C content, and (f) N content in biomass. Diamonds represent the legumes and triangles non-legumes. (g) Vegetation heights derived from webcam images, (h) amounts of total N removal at harvest (semi-transparent), including total amount of BFN in the removed biomass (saturated).

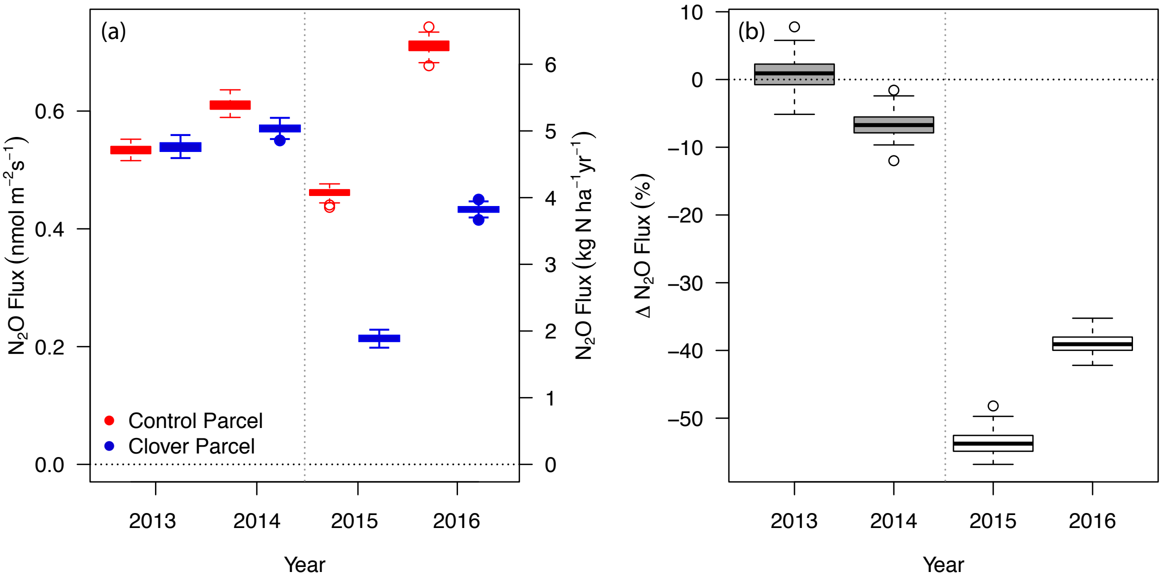

Figure 6. (a) Annual N_2O exchange at control (red) and clover parcels (blue) for the reference years 2013–2014 and the experimental years 2015–2016. (b) Relative differences between N_2O exchange in the control and clover parcels for the reference years (grey) and the experimental years (white). Boxes indicate the inter-quartile range based on nonparametric bootstrapping; bold black lines within boxes indicate the medians.

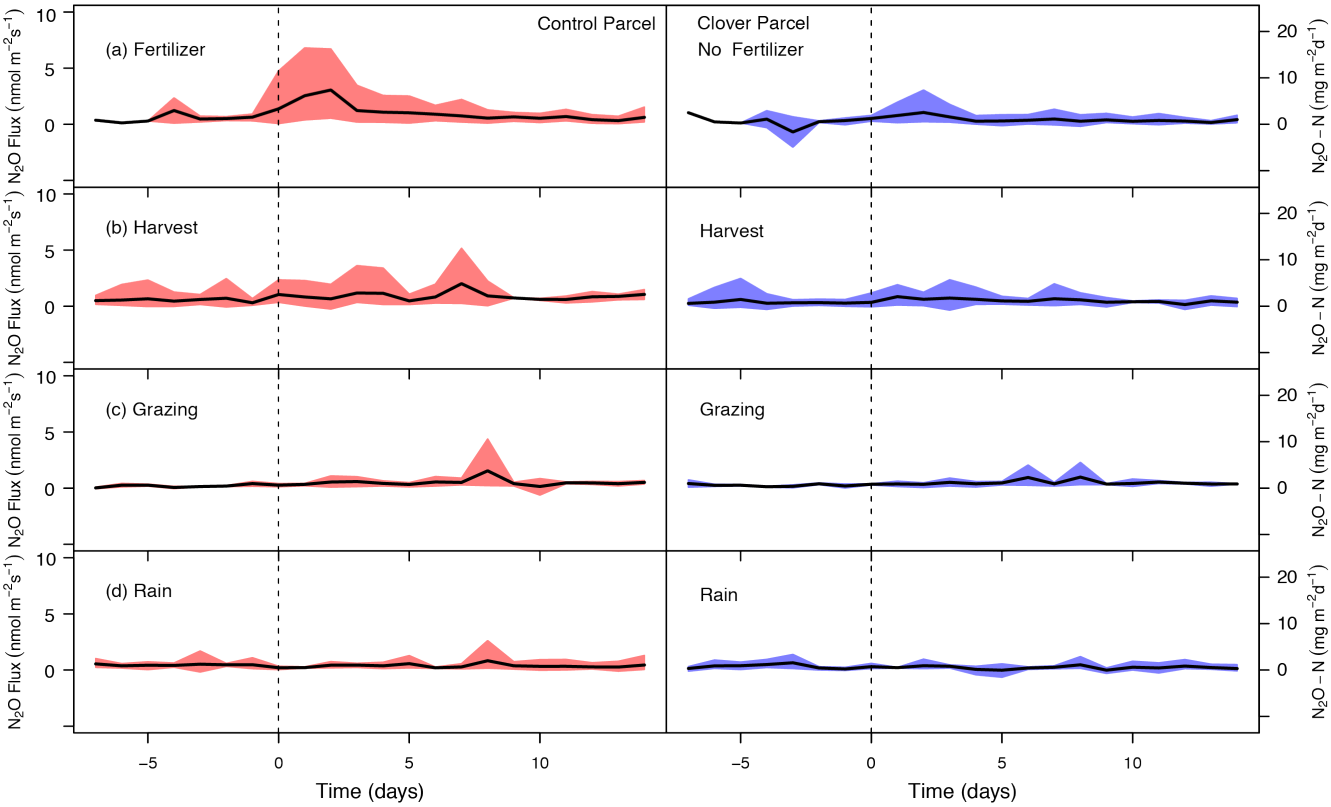

Figure 7. N_2O fluxes (bold lines: average; color bands: inter-quartile range of daily means across all events in 2015 and 2016) in the control and the clover parcels from one week before to two weeks after management events: after (a) organic fertilizer application, (b) harvests, (c) grazing events, and (d) rain events. The black dashed line indicates the start of an event.

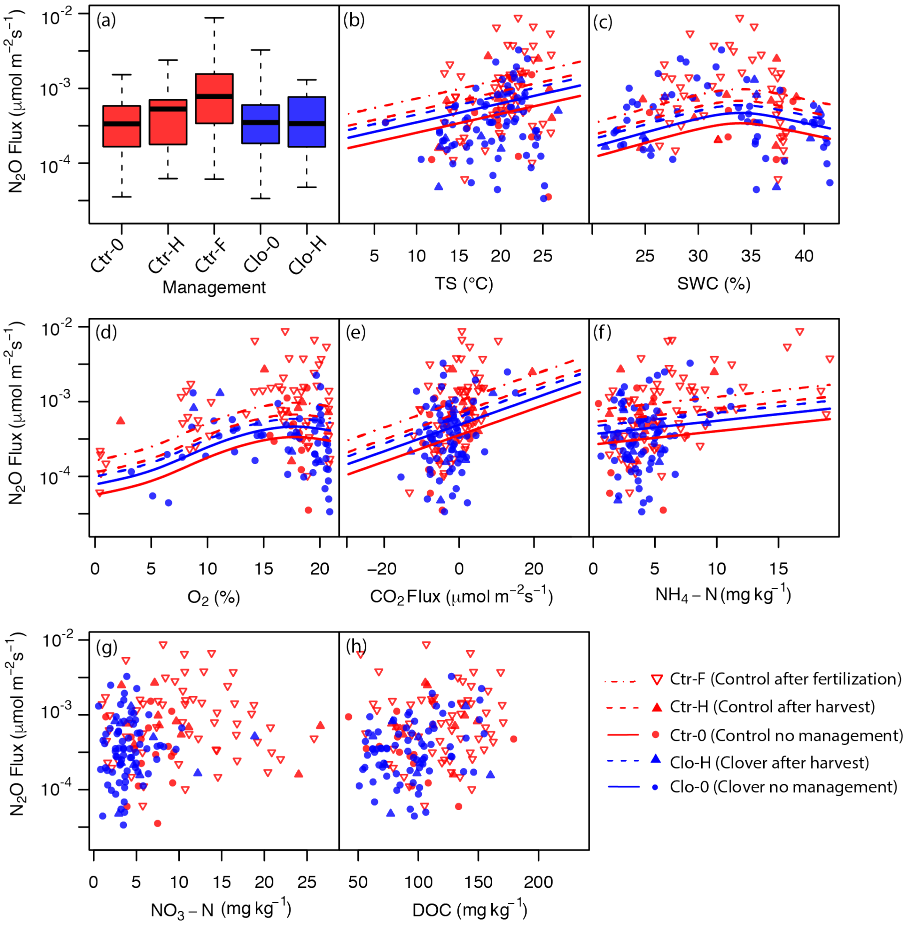

Figure 8. Influence of management and environmental variables on N_2O emissions as predicted by the generalized additive model (GAM). Significant effects were found for (a) management, (b) soil temperature (TS, 0.1 m depth), (c) soil water content (SWC, 0.1 m depth), (d) oxygen concentration (O_2, 0.1 m depth), (e) carbon dioxide (CO_2) flux and, while not significant (f) ammonium-N concentration (NH_4-N, 0–0.2 m depth) still improved the model (lowered the AIC). No significant influence was found for (g) nitrate-N concentration (NO_3-N, 0–0.2 m depth) and (h) dissolved organic carbon concentration (DOC, 0–0.2 m depth). Measurements are displayed as squares for "no management", upward triangles for harvests at the control (red) and clover (blue) parcels, and downward triangles (red) for fertilization (control). Predictions are displayed if lowering AIC as solid lines for the category "no management", as dashed lines for harvests, and as dot-dashed line for fertilization based on average values for all other drivers, respectively.

Table 1. Management activities carried out at the control and clover parcels during the experimental years 2015 and 2016 according to the field book entries of the farmer. For organic fertilizer amendments, the results of laboratory analyses (slurry composition) are given.

*Two varieties of Trifolium repens L., variety HEBE, FIONA, and one variety of Trifolium pratense L. TEDI; 20 kg seeds ha^{-1}; ⅓ of each sort, identical mixture and amounts in both years; aquired from UFA Samen, fenaco Genossenschaft, Winterthur, Switzerland.

Year	Parcel	Start	End	Management	Specification	Amount Unit ha^{-1}	Unit	Dry matter (%)	Organic matter (%)	Organic C (g kg^{-1} DM)	pH	total N (g kg^{-1} DM)	NH_4-N (g kg^{-1} DM)	NO_3-N (g kg^{-1} DM)	C/N	P (g kg^{-1} DM)	P_2O_5-P (g kg^{-1} DM)	K (g kg^{-1} DM)	K_2O-K (g kg^{-1} DM)	Ca (g kg^{-1} DM)	Mg (g kg^{-1} DM)	S (g kg^{-1} DM)	Total DM (kg ha^{-1})	Total N (kg ha^{-1})
2015	Clover	2015-03-13	2015-03-13	Oversowing, rolling	Seed mixture OH HEBE, FIONA, TEDI*	20.0	kg																	
		2015-04-21	2015-04-22	Mowing, swathing, bringing in silage	Grass silage	11.1	dt FS																	
		2015-06-02	2015-06-03	Mowing, swathing, put hay on wagon	Hay	44.4	dt FS																	
		2015-06-15	2015-06-19	Grazing	Sheep	28.1																		
		2015-06-30	2015-06-30	Drainage grubber																				
		2015-07-01	2015-07-06	Grazing	Sheep	31.1																		
		2015-08-20	2015-08-21	Mowing, swathing, spinning, bringing in silage	Grass silage	25.0	dt FS																	
		2015-09-28	2015-09-28	Mowing, swathing, put hay on wagon	Hay	22.2	dt FS																	
	Control	2015-03-11	2015-03-11	Organic fertilizer, trail hose	Slurry	21.5	m³	2.2	67.6	392.1	7.8	82.3	49.3	0.3	4.8	11.0	25.1	55.7	67.2	33.4	6.6	5.5	474.0	39.0
		2015-04-21	2015-04-22	Mowing, swathing, bringing in silage	Grass silage	23.9	dt FS																	
		2015-04-29	2015-04-29	Organic fertilizer	Slurry	28.6	m³	2.6	71.5	414.5	7.7	61.2	37.1	<0.001	6.8	9.5	21.7	64.6	77.8	27.8	7.2	5.3	744.5	45.6
		2015-06-02	2015-06-03	Mowing, swathing, put hay on wagon	Hay	50.0	dt FS																	
		2015-06-09	2015-06-09	Organic fertilizer, trail hose	Slurry	30.5	m³	1.7	66.6	386.2	7.5	69.4	47.8	<0.001	5.6	11.2	25.7	85.1	102.5	29.3	9.0	5.4	517.7	35.9
		2015-06-30	2015-06-30	Drainage grubber																				
		2015-07-06	2015-07-06	Mowing, swathing, spinning, bringing in silage	Grass silage	34.1	dt FS																	
		2015-07-09	2015-07-09	Organic fertilizer, trail hose	Slurry	35.5	m³	2.6	65.8	381.2	8.0	74.5	47.7	<0.001	5.1	15.1	34.6	72.8	87.7	41.8	9.1	6.6	921.8	68.7
		2015-08-20	2015-08-21	Mowing, swathing, spinning, bringing in silage	Grass silage	37.5	dt FS																	
		2015-08-25	2015-08-25	Organic fertilizer, trail hose	Slurry	27.3	m³	2.6	65.8	381.5	8.0	87.9	55.8	5.7	4.3	15.6	35.8	67.2	81.0	45.8	8.1	6.3	709.1	62.3
		2015-09-28	2015-09-28	Mowing, swathing, put hay on wagon	Hay	40.9	dt FS																	
		2015-10-08	2015-10-08	Organic fertilizer, trail hose	Slurry	28.2	m³	2.0	63.8	370.1	8.1	79.0	45.7	3.5	4.7	14.5	33.2	78.2	94.3	42.8	8.0	6.5	563.6	44.5
2016	Clover	2016-01-26	2016-02-10	Grazing	Sheep	5.5																		
		2016-04-06	2016-04-06	Oversowing, rolling	Seed mixture OH HEBE, FIONA, TEDI*	20.0	kg																	
		2016-05-25	2016-05-27	Mowing, swathing, spinning, bringing in hay	Hay	66.7	dt FS																	
		2016-07-04	2016-07-04	Mowing, swathing, spinning, bringing in silage	Grass silage	22.2	dt FS																	
		2016-08-13	2016-08-14	Mowing, swathing, spinning, bringing in silage	Grass silage	15.3	dt FS																	
		2016-09-22	2016-09-24	Mowing, swathing, spinning, silage bales	Grass silage	20.0	dt FS																	
		2016-11-22	2016-11-30	Grazing	Sheep	5.5																		
	Control	2016-01-26	2016-02-10	Grazing	Sheep	5.5																		
		2016-03-23	2016-03-23	Organic fertilizer, trail hose	Slurry	21.5	m³	1.6	66.9	387.6	8.0	72.8	43.4	1.0	5.3	10.7	24.5	84.5	101.8	29.5	7.1	5.7	343.7	25.0
		2016-05-25	2016-05-27	Mowing, swathing, spinning, bringing in hay	Hay	81.8	dt FS																	
		2016-06-01	2016-06-01	Organic fertilizer, trail hose	Slurry	25.5	m³	1.6	65.3	378.8	8.0	80.6	45.9	1.5	4.7	12.4	28.4	79.9	96.3	36.0	8.1	6.9	407.3	32.8
		2016-07-04	2016-07-04	Mowing, swathing, spinning, bringing in silage	Grass silage	23.9	dt FS																	
		2016-07-16	2016-07-16	Organic fertilizer, trail hose	Slurry	24.5	m³	2.8	68.8	398.9	8.2	71.2	49.8	<0.001	5.6	12.0	27.4	66.5	80.1	35.3	8.0	6.2	687.3	48.9
		2016-08-13	2016-08-14	Mowing, swathing, spinning, bringing in silage	Grass silage	23.9	dt FS																	
		2016-08-17	2016-08-17	Organic fertilizer, trail hose	Slurry	23.2	m³	1.6	67.0	388.4	8.0	110.0	60.3	<0.001	3.5	13.7	31.4	72.8	87.7	42.2	9.2	6.7	370.9	40.8
		2016-09-22	2016-09-24	Mowing, swathing, spinning, silage bales	Grass silage	24.5	dt FS																	
		2016-09-30	2016-09-30	Organic fertilizer, trail hose	Slurry	26.8	m³	1.2	66.5	385.3	8.0	103.0	55.0	<0.001	3.7	13.8	31.7	80.3	96.7	39.3	9.5	6.8	321.8	33.1
		2016-11-22	2016-11-30	Grazing	Sheep	5.5																		

Table 2. Characteristics of the exported biomass from the control and clover parcels in 2015 and 2016 for legumes, non-legumes and total biomass (legumes and non-legumes). Numbers in brackets give the respective standard errors. The legume proportion is based on the annual biomass exported. C and N content and $\delta^{15}N$ values refer to mean values across all samples. BFN refers to N derived from the atmosphere in harvested clover biomass. Means sharing the same superscript (per row) are not significantly different from each other (Tukey's HSD, $p < 0.05$); No significance tests were applied for percentages and ratios.

		2015		2016	
Variable (Unit)		Control	Clover	Control	Clover
Biomass export (DM t ha^{-1})	Total	12.8 (± 0.5)^{a}	10.4 (± 0.7)^{b}	11.9 (± 0.4)^{ab}	11.0 (± 0.5)^{ab}
Biomass export (DM kg ha^{-1})	Legumes	1860 (± 176)^{a}	2240 (± 141)^{b}	503 (± 80)^{ab}	4840 (± 355)^{ab}
	Non-Legumes	11000 (± 541)^{a}	8170 (± 666)^{b}	11400 (± 462)^{a}	6150 (± 493)^{b}
Legume proportion (%)	Total	15 (± 12)	21 (± 8)	4 (± 5)	44 (± 20)
C content (%)	Legumes	45.3 (± 1.1)	45.6 (± 0.3)	42.9 (± 0.9)	43.8 (± 0.6)
	Non-Legumes	45.1 (± 1.4)	45.2 (± 0.4)	43.0 (± 1.0)	43.0 (± 1.0)
N content (%)	Legumes	3.36 (± 0.24)	3.56 (± 0.14)	3.30 (± 0.14)	3.08 (± 0.18)
	Non-Legumes	2.18 (± 0.12)	2.25 (± 0.16)	1.94 (± 0.19)	1.85 (± 0.17)
$\delta^{15}N$ (‰)	Legumes	-0.47 (± 0.54)	-0.72 (± 0.21)	-0.37 (± 0.55)	-0.76 (± 0.24)
	Non-Legumes	4.77 (± 0.83)	4.48 (± 0.42)	5.10 (± 0.94)	3.45 (± 0.55)
C (kg ha^{-1})	Total	5780 (± 222)^{a}	4720 (± 289)^{b}	5120 (± 221)^{ab}	4760 (± 228)^{b}
	Legumes	843 (± 78)^{a}	1020 (± 70)^{a}	216 (± 24)^{b}	2120 (± 123)^{c}
	Non-Legumes	4940 (± 235)^{a}	3700 (± 295)^{b}	4900 (± 220)^{a}	2640 (± 275)^{c}
N (kg ha^{-1})	Total	301 (± 10)^{a}	264 (± 13)^{b}	238 (± 13)^{ab}	262 (± 8)^{b}
	Legumes	63 (± 6)^{a}	80 (± 5)^{a}	17 (± 2)^{b}	149 (± 9)^{c}
	Non-Legumes	238 (± 9)^{a}	184 (± 13)^{a}	221 (± 11)^{a}	113 (± 9)^{a}
BFN (kg ha^{-1})	Legumes	55 (± 5)^{a}	72 (± 5)^{a}	14 (± 2)^{b}	130 (± 8)^{c}

Table 3. Data availability of the GHG flux measurements over the two years experimental period (a) before quality assessment and quality control (QAQC) (flagged 0, 1 and 2; after Foken et al., 2004) and (b) after QAQC (acceptable quality flagged 0 and 1; after Foken et al., 2004). The reference for 100% is a year without data gaps.

(a)		Acquired measurement hours before QAQC (h)			Data coverage before QAQC (%)		
		CO_2 Flux	N_2O Flux	CH_4 Flux	CO_2 Flux	N_2O Flux	CH_4 Flux
2015	Both Parcels	6958	7969	7964	79	91	91
	Control Parcel	4089	4826	4823	47	55	55
	Clover Parcel	2869	3143	3141	33	36	36
2016	Both Parcels	7456	7734	7734	85	88	88
	Control Parcel	3911	4485	4485	45	51	51
	Clover Parcel	2302	2518	2518	26	29	29

(b)		Acquired measurement hours after QAQC (h)			Data coverage after QAQC (%)		
		CO_2 Flux	N_2O Flux	CH_4 Flux	CO_2 Flux	N_2O Flux	CH_4 Flux
2015	Both Parcels	4930	5984	5223	56	68	60
	Control Parcel	1418	2120	1837	16	24	21
	Clover Parcel	2298	2395	2091	26	27	24
2016	Both Parcels	3787	5040	4250	43	58	49
	Control Parcel	1081	1895	1581	12	22	18
	Clover Parcel	1548	1921	1615	18	22	18

Table 4. Results of generalized additive models (GAM) (a) including all variables (full model), (b) reduced after stepwise backward elimination, dismissing DOC and nitrate (optimized model); (c) simplified including only management, soil temperature (TS) and volumetric soil water content (SWC). The control parcel without recent management (Ctr-0) was used as the reference level for the categorical variable management, thus the constant represents predictions for Ctr-0 and the effect sizes of all other management categories depict differences compared to Ctr-0. The effect sizes are displayed with their standard errors and p values for all linear terms. For the non-linear terms soil water content and oxygen concentration, the respective empirical degrees of freedom (edf) and p values are shown. The effect sizes are direct model outputs, while the values used in the text were back-transformed to increase comprehensibility.

Dependent variable: log N_2O Flux						
	(a) full model		(b) optimized model		(c) simple model	
Covariates	effect size (± se)	p-value	effect size (± se)	p-value	effect size (± se)	p-value
Parametric coefficients:						
Control after harvest (Ctr-H)	0.30 (± 0.24)	0.223	0.13 (± 0.22)	0.567	0.17 (± 0.07)	0.012*
Control after fertilization (Ctr-F)	0.46 (± 0.19)	0.016*	0.40 (± 0.17)	0.025*	0.31 (± 0.06)	<0.0001***
Clover no management (Clo-0)	0.14 (± 0.18)	0.432	0.11 (± 0.18)	0.529	-0.02 (± 0.03)	0.567
Clover after harvest (Clo-H)	0.24 (± 0.22)	0.269	0.20 (± 0.22)	0.359	0.10 (± 0.07)	0.129
TS (°C)	0.03 (± 0.01)	0.023*	0.03 (± 0.01)	0.004**	0.03 (± 0.002)	<0.0001***
CO_2 Flux (μmol m^{-2} s^{-1})	0.02 (± 0.01)	0.018*	0.02 (± 0.01)	0.025*		
NH_4-N (μg g^{-1})	0.02 (± 0.01)	0.167	0.02 (± 0.01)	0.074		
NO_3-N (μg g^{-1})	-0.01 (± 0.01)	0.231				
DOC (μg g^{-1})	0.002 (± 0.001)	0.303				
Constant	-4.22 (± 0.25)	<0.0001***	-4.17 (± 0.23)	<0.0001***	-3.97 (± 0.04)	<0.0001***
Approximate significance of smooth terms:						
	edf	p-value	edf	p-value	edf	p-value
SWC	2.33	0.119	1.87	0.048*	1.98	<0.0001 ***
O_2 concentration	2.81	0.0001***	2.72	0.0003***		
Observations	90		93		891	
Adjusted r^2	53.5%		54.5%		26.3%	
Explained deviance	60.9%		60.2%		26.9%	
GCV score	0.1183		0.1152		0.1761	

$^{*}p<0.05$ $^{**}p<0.01$ $^{***}p<0.001$

Table 5. Summary of studies investigating N_2O emissions simultaneously in permanent grasslands of at least two different clover proportions. We included studies with > 200 days temporal coverage and at least biweekly sampling of N_2O emissions, or if discontinuously sampled included a sensible strategy used by the authors in order to estimate annual fluxes.

Source	Treatment	N_{fert} (kg N ha^{-1} yr^{-1})	Clover %	N_2O (kg N_2O-N ha^{-1} yr^{-1})
Ammann et al. 2009	low clover	230	21	1.60
Ammann et al. 2009	high clover	0	32	-0.10
Jensen et al. 2012	fertilized pasture	NA	0	4.49
Jensen et al. 2012	unfertilized grass	0	0	1.20
Jensen et al. 2012	grass-clover	0	NA	0.54
Jensen et al. 2012	pure clover	0	100	0.79
Klumpp et al. 2012	low clover	157	19	1.72
Klumpp et al. 2012	high clover	157	35	1.52
Li et al. 2011	rhyegrass grazed	226	0	7.82
Li et al. 2011	fertilized rhyegrass-white clover grazed	58	20-25	6.35
Li et al. 2011	unfertilized rhyegrass-white clover grazed	0	20-25	6.54
Li et al. 2011	rhyegrass-background	0	0	2.38
Li et al. 2011	grass-clover background	0	20-25	2.45
Schmeer et al. 2014	uncompacted grass	360	15	8.74
Schmeer et al. 2014	compacted grass	360	15	13.31
Schmeer et al. 2014	uncompacted lucerne-grass	0	70	2.46
Schmeer et al. 2014	compacted lucerne-grass	0	70	2.22
Simek et al. 2004	no clover	210	0	2.28
Simek et al. 2004	high clover	20	60	1.50
Simek et al. 2004	pure clover	20	100	1.50
This study 2015	low clover	296	15	3.82
This study 2016	low clover	181	4	6.27
This study 2015	high clover	0	21	1.89
This study 2016	high clover	0	44	4.07
Virkajärvi et al. 2010	no clover	220	0	3.65
Virkajärvi et al. 2010	high clover	0	75	7.00