

## Authors' Response to the Editor, Referee #1, Referee #2 and Referee #3

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Reviewer and Editor comments are written in blue

Authors' comments are written in black letters.

Dear Editor,

Thank you for your feedback and for highlighting the most important points which were important to be addressed in the revised version. In the resubmitted version we included your suggestions by the following additions to the manuscript:

**Editor:** You also provide some new, important background info (e.g. figure R2-1) which would benefit from review assessment.

**AC:** As this figure might be of general interest we now included Figure R2-1 in the Supplementary Material as Fig. S2 and refer to it in the revised Manuscript: "We observed moderate diurnal variations in flux origin from the two parcels (Fig. S2). Nevertheless, a similar share of quality-controlled N<sub>2</sub>O fluxes was obtained from the control (48%) and the clover parcel (52%) during the observation period. The net effect in N<sub>2</sub>O emission differences represents a conservative estimate, as N<sub>2</sub>O 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)." (P8 L32 – P9 L3)

**Editor:** The content of your answer to the first issue raised by reviewer 1 isn't really reflected in the proposed change to the manuscript. The "wider perspective" you refer to in your answer seems important in articulating the value of your findings, and could be discussed in more detail in your manuscript.

**AC:** In order to better implement the first issue addressed by reviewer 1 we added a paragraph in the introduction, which should increase clarity about the target of our study and introduce the broader context ("What to do with organic fertilizer?"), right in the beginning of the manuscript.

Inserted text (P3 L 3- 9): "Our mitigation approach investigated the potential for reductions in slurry application accompanied with increased clover proportion in the pasture to reduce N<sub>2</sub>O 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 addition 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."

**Editor:** On a minor note: I agree with the review comment on the bottom of page 9 that your conclusion could easily be misinterpreted: 'In sum, our results indicate that N<sub>2</sub>O can be effectively reduced through the replacement of fertilizer N with N from BNF.' The word "replacement" might suggest that the amounts of external N (additional BNF in clover plot vs. organic fertilizer input) are the same in both treatment. This doesn't seem to be

the case. Lower N<sub>2</sub>O emissions due to lower N inputs aren't all that surprising, so the reader might wonder "why not just cut fertilizer rates"? Perhaps you can rephrase this sentence to avoid confusion? The final sentence of your abstract states your conclusions more clearly.

**AC:** In order to state more clearly what we mean we now wrote, on P 16 L 4-6:

"In summary, our results indicate that N<sub>2</sub>O emissions can be effectively reduced at ecosystem scale through enhancing the clover proportion (and BFN) in permanent grassland while reducing fertilizer inputs and still meeting the N requirements of plants."

Anonymous Referee #1

**Referee #1** General Comments

**R:** The manuscript by Fuchs et al. is well written and easily to follow. The authors report on a 2-year field study of eddy covariance N<sub>2</sub>O flux measurements on two side-by-side grasslands, one managed 'business as usual' (i.e. with frequent additions of organic fertilizer in the form of slurry) and the other with increased proportion of clover and no slurry application (i.e. nitrogen provided by biological fixation instead of organic fertilizer). The authors find that absence of fertilization in the field with increased clover resulted in significant reduction in N<sub>2</sub>O emissions. I agree with the authors' justification of the lack of studies on year-round N<sub>2</sub>O fluxes for grassland systems. The flux measurement methodology used is sound (but see questions below) and the authors have collected a very complete set of supporting measurements to help in the interpretation of fluxes. The study contributes a solid dataset that could be valuable for future modelling efforts. I also liked the use of the GAM models to attribute the N<sub>2</sub>O flux to various covariates.

My main difficulty with the manuscript is the larger context of practicality of the management practice studied. The authors use the term 'fertilizer nitrogen' and 'fertilization' throughout but as far as I understand their experimental setup refers to substituting livestock-derived organic nitrogen with biologically fixed organic nitrogen. Substituting an external input such as synthetic fertilizer with N from biological fixation makes a lot of sense but presumably the dairy/pig farms have slurry that contains N and other nutrients to be recycled back to the soil. The authors used slurry that had been digested in a biogas plant so what will be the fate of this material if not returned to soil? Hence, the proposed mitigation management does not fit within a larger nutrient balance framework: grasslands are producing animal feed but the manure is not returned to fields and instead more nitrogen is added to fields through biological fixation. Perhaps there is some local context that would justify the proposed mitigation practice. If not, I am having a bit of difficulty in identifying the value of the research findings. Overall, I would like to see the dataset published but I think the argument for the value of the findings has to be much better articulated. Perhaps placing their measurements within an N budget framework would help making the manuscript a unique contribution.

**AC:** Thank you for this relevant comment. Indeed, we investigated a system which was conventionally managed by livestock-derived organic fertilizer application, but we see this in the wider perspective and argue that the slurry should replace mineral fertilizer at other sites (e.g. nearby crop sites). While we do argue for direct replacement of mineral N with legumes on fields currently fertilized with mineral N, it is clear that introducing legumes is considered much easier in grasslands than in other crops or would introduce unwanted challenges for

the farmer (i.e. to harvest mixed crop cultures is more challenging compared to grassland). We strongly agree that the slurry digested in a biogas plant should be used, may it be for fertilization as it contains valuable N while industrial N fixation requires high energy input, or another purpose. Implications on the farm level etc. should be investigated but this was not the target of our study. We think that the results of our experiment provide useful insights on N<sub>2</sub>O fluxes from both, clover and control parcel. It needs to be noted that it was not our intention to artificially change the farmers management to mineral fertilization but instead remain as closely as possible to the conventional management as defined by the farmers practice. The wider context of where to apply this strategy and under which conditions it is worthwhile implementing can be investigated in a potential follow-up project. Similarly, we think that the full N budget of both parcels would be worthwhile an investigation in the upcoming years. During our experiment in 2015/16 our focus was on the processes leading to (increased or decreased) N<sub>2</sub>O fluxes and to measure a large dataset of driver variables of N<sub>2</sub>O fluxes, while the N budget considerations would have required additional data acquisition (i.e. NH<sub>3</sub> emissions, dry/wet deposition, N<sub>2</sub> emissions) and was beyond the scope of this project.

We added a sentence in the discussion to make clear that we did not cover these aspects, but think that they are important: (Page 15 L17)

The assessment of the mitigation strategy revealed reductions in N<sub>2</sub>O emissions, an increase in BFN and stable yields under mitigation management. 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.

**R:** Title: Use of legumes is a central theme of the manuscript but does not appear in the title. ‘Management matters’ is catchy but also well-known and a bit vague since it does not specifically identify which management.

**AC:** We strongly agree that stating the specific strategy in the title is useful and change the title to: “Management matters: Testing a mitigation strategy for nitrous oxide emissions using legumes on intensively managed grassland”

**R:** Abstract L. 11: I think the suggested mitigation strategy is for replacing synthetic fertilizer with BNF not animal-derived N with BNF.

**AC:** This seems to be a misunderstanding. Indeed, our aim was to use BNF to reduce the application of animal derived N in form of feces to this grassland and potentially at a wider scale to reduce mineral N application in other fields. Please see also our previous commentary.

**R:** Here and throughout need to identify if referring to organic or synthetic fertilizer

**AC:** We agree with the reviewers comment that the type of fertilizer needs to be introduced in the abstract. The introductory sentence is still valid from a more general viewpoint. The findings on the clover parcel provide a reference to any fertilized field. We would have expected similar or even larger differences when we would have changed the control to field that is characterized by mineral fertilizer amendments. However, this would have implied to artificially change the existing management practices at the control. As a consequence, we added “organically” fertilized in the abstract (L15).

**R:** Check the consistent use of BNF vs. BFN throughout.

**AC:** According to your suggestion we introduced biologically fixed N (BFN) on Page 1 (L9) and used it in a consistent manner throughout the revised manuscript.

**R:** L. 14: Could a broader objective statement be used here (i.e. quantify is a step in trying to answer a more meaningful objective);

**AC:** We agree, that the objective statement should be broad and meaningful and rephrased in order to make our objective clearer: “In order to assess the overall effect of this mitigation strategy on permanent grassland, we performed an in-situ experiment and quantified net N<sub>2</sub>O fluxes and biomass yields in two differently managed grass-clover mixtures.”

**R:** L. 17: ‘To assess the effect of the mitigation strategy’ on what?

**AC:** ... on biomass yields and N<sub>2</sub>O emissions, we found it repetitive to state this twice in the sentence. See also our previous comment.

**R:** L. 18: No results of the 15N method are presented in abstract leaving the reader wondering why it is mentioned here.

**AC:** We thank the reviewer for pointing towards this lack of information and added this information in the revised version (Page 1 L 19):

“The amount of BFN was similar in both parcels in 2015, (control:  $55 \pm 5$  kg N ha<sup>-1</sup> yr<sup>-1</sup> and clover parcel:  $72 \pm 5$  kg N ha<sup>-1</sup> yr<sup>-1</sup>) 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:  $14 \pm 2$  kg N ha<sup>-1</sup> yr<sup>-1</sup> with 4% clover in DM and clover parcel:  $130 \pm 8$  kg N ha<sup>-1</sup> yr<sup>-1</sup> and 44% clover).”

**R:** L. 21: Here and throughout, the authors use ‘no management’ which is not a very clear term. I think the authors mean during background or baseline emission periods (i.e. outside of emission events associated with management). Either the term is defined early on and used or different wording should be used.

**AC:** Thank you for the comment, it is useful to define “no management”. As background or baseline emission might be understood as emissions of a field under no N inputs (neither N fertilizer, nor BFN) we did not want to use these terms. As a consequence, we defined “no management” in in the method section, (Page 10 L9): “no management (here defined as no management during the previous week)”

**R:** L. 24-25: Did the overall N input also decrease in the clover treatment? An overall decrease in N input is different than the argument of ‘replacing fertilizer N with BNF’ used at the start of the abstract. It is not surprising that N<sub>2</sub>O emissions are lower if N inputs decreased.

**AC:** You are making an important point in highlighting the usefulness of N balance considerations, however this is difficult to conclude without measuring NH<sub>3</sub> and N<sub>2</sub> losses. Rough budget considerations (See Table R1-1) with estimation of the overall N input - NH<sub>3</sub> and N<sub>2</sub> (which is especially uncertain), and other gaseous losses resulted in lower overall N input in the clover parcel in 2015 but similar overall N input in the clover parcel in 2016 compared to the control. This was estimated based on literature values, and we are aware of the large uncertainty

in these estimates. Thus, we did not want to include uncertain estimates in our manuscript and suggest to expand the activities at the site to monitor other components of the N cycle in future projects and evaluate changes over a longer period.

Table R1-1. N Budget considerations for both experimental years at parcels. Please note that we do not see a measured N budget within the scope of our study and see this as a rough estimate, which needs to be refined in future projects.

Flux	Uncertainty	2015		2016	
		Control parcel	Clover parcel	Control parcel	Clover parcel
total BNF (above and below ground)	±10%	76	90	18	160
N <sub>fert</sub>	±5%	296	0	181	0
N dep (dry + wet)	± - 10 +50%	28	28	28	28
NO <sub>x</sub> HNO <sub>3</sub> <sup>-</sup> NO <sub>3</sub> <sup>-</sup> NH <sub>3</sub>					
<b>Total input</b>		<b>400</b>	<b>117</b>	<b>226</b>	<b>188</b>
N in Biomass export - 10%		271	238	214	236
NH <sub>3</sub> (30% N fert) Ammann 2009	20-35%	89	0	54	0
NO <sub>3</sub> <sup>-</sup> Leaching 7% of N <sub>fert</sub> + BNF (Ledgard et al. 2009)	(2-40%) of N <sub>fert</sub> + BNF (was below 3.5 kg in Ammann 2009)	26	6	14	11
N <sub>2</sub> O	± see text	4.1	1.9	6.3	3.8
N <sub>2</sub> (5.7* N <sub>2</sub> O)					
Butterbach-Bahl et al. 2013	2-11* N <sub>2</sub> O	23	11	36	22
<b>Total export</b>		<b>413</b>	<b>256</b>	<b>324</b>	<b>272</b>
<b>Budget*</b>		<b>13</b>	<b>139</b>	<b>98</b>	<b>85</b>
<b>Budget (uncertainty range)</b>		<b>13 (-94 - 233)</b>	<b>139 (97-228)</b>	<b>98 (14-258)</b>	<b>85 (25-210)</b>

\*-Input + Export, positive sign indicates soil to atmosphere

**R:** Page 2 L. 13-14: Instruments for EC measurements have been available for a least a decade.

**AC:** The sentence is referring to high frequency in-situ N<sub>2</sub>O measurements that would be continuously possible over the whole year, not basic EC measurements, we specified the sentence to make this clearer “instruments capable of high-frequency continuous N<sub>2</sub>O concentration measurements and steadily deployable in the field have only become available in recent years”.

**R:** L. 17: ‘abiotic factors are generally known’

**AC:** The text was corrected accordingly.

**R:** L. 18-19: N<sub>2</sub>O emissions strongly depend on management practices in most managed systems so I suggest to remove ‘particularly grasslands’.

**AC:** Removed according to your suggestion.

**R:** L. 31: Here the authors refer to ‘external fertilizer amendments’ but slurry would not be considered an external amendment.

**AC:** We agree that there is no reason for using external without definition and deleted it in the revised text.

**R:** Page 3: L. 3-5: This statement seems a bit misleading given that this study is not dealing with synthetic fertilizer.

**AC:** We deleted this statement in order to avoid confusion.

**R:** L. 33: What is the reason for substituting the N fertilizer from organic origin that is already available on the farm?

**AC:** We would like to refer to our initial explanation made at the beginning of this response. In brief, our aim was to test a potential mitigation strategy which could lead to less mineral fertilizer being used in other places.

**R:** Page 4: L. 3: Could the second objective be made more specific? ‘to identify the drivers of N<sub>2</sub>O emissions’ is a bit broad.

**AC:** We rewrote this sentence “... to identify meteorological and soil chemical drivers of N<sub>2</sub>O emissions...”

**R:** L. 21: Is this ‘dairy’ liquid slurry? Why ‘predominantly’? Table 1 refers to ‘organic fertilizer’: was there another source beside slurry?

**AC:** There was no other source besides liquid slurry (originally cattle slurry/manure digested in the biogas plant and then returned to the farm via pipeline) during the experiment, Table 1 specifies “slurry” in the column right to the entry of “organic fertilizer”. To clarify we changed “slurry” to “liquid slurry” in Table 1. It needs to be noted, that during the past ten years there were occasional manure and synthetic fertilizer applications.

**R:** Was the aim to replace all the ~260 kg N/ha with BNF? Table 2 shows a maximum of 130 kg N/ha from BNF. What is the recommended N input to sustain the N removed?

**AC:** Recommendations for Swiss intensively managed grasslands suggest 30 kg available N ( $N_{\text{available}}$ ) per cut (Flisch et al., 2009) (Table 27), whereby  $N_{\text{available}}$  is calculated as 60% (50-70%) from the total slurry N ( $N_{\text{slurry}}$ ) (Table 40 in Flisch et al. 2009). Thus, 50 kg  $N_{\text{slurry}}$  recommended (following Flisch et al. 2009  $N_{\text{slurry}} = N_{\text{available}} / 0.6$ , range of  $N_{\text{slurry}}$ : 43-60 kg ) per cut result in 250 (214 – 300) kg  $N_{\text{slurry}}$  for five cuts (2015) and 200 (171-240) kg  $N_{\text{slurry}}$  for 4 cuts (2016) for intensively managed grassland. For BFN we can estimate that the amount of fixed N total exceeds the presented above-ground BFN in shoot biomass, due to roots biomass N (additional 70% of

shoot biomass (Jørgensen and Ledgard, 1997)) and N transferred to grasses (Nyfeler et al., 2011), which would be e.g. 30 kg N for 2016. The N exports in biomass were on average 180 kg ha over the last 10 years, and ~260 kg N as in the experiment (Table 2) would need to be replaced in the long term. Our aim was therefore to get as much N via BFN as possible, but it was clear that the sward would not be able to replace ~260 kg N with BFN within this short time after over-sowing.

**R:** L. 24-25: Where there any differences in texture, C content, etc. in the two fields monitored?

**AC:** Within the footprint, soil sampling in 0-10 cm depth suggested, that naturally there is some variability (see Table R1. However, this can be seen as typical within field variation and we do not expect that the small soil differences predominantly affected N<sub>2</sub>O exchange.

Table R1. Aggregated data separated into two parcels from soil mapping performed in (Roth, 2006)

Parcel	Corg (kg ha <sup>-1</sup> )		Corg (kg ha <sup>-1</sup> )		Bulk density (kg m <sup>-3</sup> )		Number of samples
	Mean (±sd)	Range	Mean (±sd)	Range	Mean (±sd)	Range	
<b>Control</b>	33.7 (±4.5)	24.9–39.7	34.1(±2.7)	29.3–37.3	1.03 (±0.07)	0.92–1.18	13
<b>Clover</b>	27.4 (±2.5)	24.4–33.7	30.7 (±2.3)	27.3–35.2	1.13 (±0.03)	1.08–1.19	16

**R:** Page 5: L. 3: Was the control plot also grazed? If not, what are the implications of the different grazing regimes?

**AC:** “The control parcel was mown once instead of being grazed during this time of the year (beginning of July).” Furthermore, the control was not grazed in June-July 2015 and cut instead, as this grazing was regarded part of the clover-adapted management, and thus it should be part of the management that we wanted to assess. In contrast, the winter grazing had no clover-related motivation and was just part of the farmers usual practice, and therefore was performed on both parcels (as part of business-as-usual). Overall, we do not expect large effects of grazing due to its low intensity (see also result section 3.6).

**R:** L. 16: better to say ‘digested cattle and pig slurry obtained from a local biogas plant’. If this digestate is not applied to land what is its fate?

**AC:** We rephrased this phrase according to your suggestion and further refer to our explanation of the aim of this study in our first comment.

**R:** Page 6: L. 7-8: Any replicates or just one sensor per depth?

**AC:** This was only one sensor per depth due to limited resources.

**R:** L. 9: How many locations of the 2 ha plots were monitored to determine impacts on microclimate?

**AC:** We installed one representative sensor field plot per parcel, in the main footprint area.

**R:** Page 8: L. 3: How well does EddyPro work for processing N<sub>2</sub>O data? How were the lag times determined given that low N<sub>2</sub>O signal often means the cross correlation method for determining lag times will be off.

**AC:** Processing N<sub>2</sub>O is fluxes in EddyPro works well if the search window for the lag time between eddy covariance wind data and the QCL N<sub>2</sub>O mixing ratios is constrained as much as possible. For the calculation of the time lag between the wind component  $w$  (after coordinate rotation) and the mixing ratio of N<sub>2</sub>O, we followed the covariance maximization method in combination with a pre-determined nominal lag time. This means, we identified the peak in the cross-correlation function between  $w$  and N<sub>2</sub>O in a defined time window of physically possible time lags. As pointed out by Reviewer#1, this cross-correlation function is often noisy due to low signal-to-noise ratios as a consequence of low N<sub>2</sub>O fluxes. In our study, analyzing the frequency distribution of detected lag times over the course of a “low-flux” year yielded clear results that assisted our processing in constraining the lag time search to a relatively short but adequate time window. In order to accurately identify the time lag and avoid systematic bias, we followed a multi-step approach before the calculation of final N<sub>2</sub>O fluxes for each year: Step (1): We first calculated the time lag for the respective year using the covariance maximisation method for a relatively large search window of  $\pm 10$  s, based on despiked and detrended raw data. Next, we analysed the frequency distribution of all found lag times and identified a clear distribution spike between 0.95 and 1.40 s, i.e. most cross-correlation peaks were found in this range (Fig. R1-1 shows results for the year 2013).

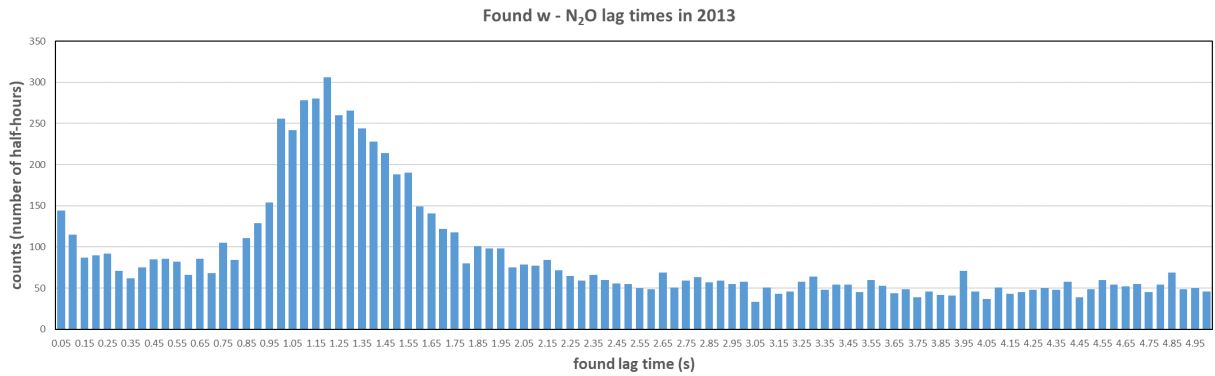


Figure R1-1. Histogram of found lag times between the turbulent wind component  $w$  and N<sub>2</sub>O mixing ratio in a time window of  $\pm 10$  s in 2013. Peak distribution was found at lag time 1.20 s. Shown are only found lag times below 5 s for clarity.

Step (2): Based on the frequency distribution from (1), we defined a narrow lag search window of 0.6 – 1.8 s, i.e. the range in which we found most cross-correlation peaks in (1). In addition, we defined the lag time of peak distribution from (1) as the nominal lag time (1.20 s), that is, whenever the returned time lag was one of the limits of the window (i.e. 0.6 or 1.8 s), the time lag was set to the nominal time lag of 1.20 s. This specific nominal lag time was chosen because it was identified as the most representative lag time over the course of a year. We also analyzed the time series of found lag times to investigate potential seasonal differences. We found that lag times over the course of a year fluctuated slightly but were well within the range of the pre-defined search window of 0.6 – 1.8 s. We are therefore confident that the accurate lag time is found in the defined range.

In addition, we are confident to have adequately constrained the time window due to the used measurement setup at the site. At Chamau, we measured eddy covariance data using a fully digital and real-time logging system that was described previously (Eugster and Plüss, 2010). One major advantage of this system is that wind and scalar data at a specific moment in time are merged immediately in the same data file. As a consequence, the system is



not prone to time drifts between the two instruments (e.g. sonic anemometer and QCL) and the lag time can therefore consistently be found in a relatively narrow time range.

**R:** The same applies to the corrections - if cospectra are being used for any of the corrections they may not be correct for periods with low N<sub>2</sub>O or CH<sub>4</sub> signals.

**AC:** We applied the spectral correction following (Fratini et al., 2012), which builds upon the work by (Ibrom et al., 2007). For each instrument and gas, this method applies spectra correction factors (analytical) in combination with cut-off frequencies (*in-situ*). This method allows to define a threshold between small (in this study: below 2 nmol m<sup>-2</sup> s<sup>-1</sup>) and large (above 2 nmol m<sup>-2</sup> s<sup>-1</sup>) N<sub>2</sub>O fluxes. For CH<sub>4</sub> during the same study period the threshold was set to 10 nmol m<sup>-2</sup> s<sup>-1</sup>. Small fluxes are then corrected following an adjusted model by (Eugster and Merbold, 2015)(Ibrom et al., 2007) (see also Section 2.3, equation 4, and Appendix A in (Fratini et al., 2012), large fluxes are corrected following the approach by (Hollinger et al., 1999) (see Section 2.2, equation 2, and Section 2.3, equation 3, in Fratini *et al.*, 2012). In addition, EddyPro allows the application of the same spectral assessment for different years to improve comparability of flux results between years. Therefore, we first calculated spectral assessments for the year 2012, the year of grassland restoration and the widest range of observed fluxes (from high fluxes during management after restoration to low fluxes later in the year). We then applied these flux assessments to all other years to achieve corrected fluxes. Therefore, to our current best knowledge, we think that the applied corrections are adequate. In addition, we want to acknowledge ongoing discussions within the Integrated Carbon Observation System (ICOS) regarding best-practice calculations of non-CO<sub>2</sub> eddy covariance fluxes, upon which our selected processing steps are based on (Nemitz et al., *in review*).

**R:** L. 16: Are there any biases associated with S or N flow being associated with different kinds of weather (rain occurrence, higher T, etc.)? Please expand on this potential impact.

**AC:** Higher temperatures are associated with higher coverage for the clover parcel fluxes which is more frequently covered during sunny days during daytime. This result in a better representation of fluxes from the clover parcel and less representation of the fluxes from the control. However, both parcels were covered well resulting in a quite low. Precipitation was not especially associated with any wind direction. For the comparison, we minimize this bias by using only days covered on both parcels. We added a sentence to make this clearer (see manuscript Page 9 L5).

“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.”

**R:** L. 24: Were directions always steady over this time period or did you use high frequency data to select the 10 min periods with steady wind direction?

**AC:** On page 8 L15 we wrote “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%).” In other words, from the overall 10-min footprint, our selection criteria were that a minimum of 80% of the footprint weights had to originate from

this respective parcel and thus were representing steady conditions. Wind direction changes during unsteady conditions within <10 minutes largely influence this criterion, thus no further restriction was applied.

**R:** Page 10: L. 34: Could more information be given on 'management'? Which 'Management' aspects? These are not quantitative variables or continuous so how was this handled?

**AC:** We handled them as categorical variables and refer here to page 10 L9 "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 (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."

**R:** Page 12: L. 22: Odd wording: 'later clover parcel'; please edit. Was there a trend for lower yields for the 'clover' parcel before the experiment started? Is this related to differences in soil? L. 24: Was difference consistently <0 for all years? What are the implications for previous C input to soil and N<sub>2</sub>O fluxes during measurement period?

**AC:** We changed "later clover parcel" to "parcel which was transformed into the experimental parcel during the years 2015 and 2016". For the 2007-2013 yields were consistently lower in the parcel which was transformed into the experimental parcel. Differences might occur for multiple reasons: (1) differences in management, i.e. more grazing on the parcel which was transformed into the experimental parcel compared to the other, (2) slight differences in soil properties (i.e. N stocks) due to differences in N inputs (3) a bias in the farmer's field book estimate.

**R:** Page 14: L. 15-16: This interpretation assumes that there was no interaction between factors, i.e. all effects during non-events, called 'times without management' by authors, are due to sward composition while all effects during events was due to slurry application. Please discuss potential interaction effects.

**AC:** This might be a misinterpretation. Effects of other environmental drivers were included. The gam model as used here takes all predictors into account (Table 4). The N<sub>2</sub>O flux in the model is predicted using the effects of management, temperature, soil water content etc. Thus, by including these "confounding variables" they are no "confounding variables", but taken into account as predictors instead. Thus, variability in soil temperature and soil water content is represented in the model and is therefore not confounding this interpretation. The management effects in the model can be interpreted as offsets (resulting in different multipliers for all management categories). What we were not able to include were carry-over effects and land use history effects, including past fertilization on the time of "no management".

**R:** Page 15: L. 23-24: 'In sum, our results indicate that N<sub>2</sub>O can be effectively reduced through the replacement of fertilizer N with N from BNF.' But was all slurry N replaced by BNF? Did the system have an N surplus before that could have been addressed by just matching the Slurry N input to crop needs? IF that is the case then the most achievable mitigation practice would be to adjust the slurry rates to the plant demand instead of using BNF.

**AC:** The N surplus in the soil during and shortly after fertilization is unavoidable and relevant in all fields. It needs to be said, that the farmers in our experiment did not excessively amend slurry, and the site was managed within the framework of regulations. We fully agree with the reviewer, that is crucial to increase NUE as much as possible by optimizing the timing of slurry application, however the steady N input achieved via BNF differs from “bulk” N input via slurry application. Timing and amount of fertilizer application are indeed mitigation practices (but not investigated in this study); As the farmer managed the field according to recommended practices which are based on N balance considerations, there would arise concerns about productivity when reducing fertilizer amounts at no further N input changes. We revised: In summary, our results indicate that N<sub>2</sub>O 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.

**R:** Page 17: L. 31: ‘Thus, we observed lower N<sub>2</sub>O emissions at higher levels of photosynthesis’. Higher levels of photosynthesis also correspond to periods of higher water loss as transpiration and lower water content as seen in your dataset. How can you separate these two confounding effects?

**AC:** An effect of soil water content, may it be due to high transpiration or lack of precipitation or both, would be accounted for in the model as we put soil water content as one of the predictor variables so such an effect would be attributed to the soil water content. Adding NEE as a predictor, however, improves the model (i.e. reduces AIC) and adds explanatory power. Of course, photosynthesis is also correlated with soil water content and temperature, however as we included both in our model they cannot be confounding (i.e. low N<sub>2</sub>O emissions at low water contents are already reflected by the effects of soil water content, if the inclusion of NEE in the model lowers AIC in addition to that, it means that there is an additional effect which was not explained without NEE. Evapotranspiration is not a causal driver of N<sub>2</sub>O production.

**R:** Page 19: L. 24: This brings up an important point. ‘Permanent grasslands’ are temporarily restored as was done with the study area in 2012. Does this restoration involve ploughing or a similar tillage at this site? If so, then authors should discuss the impact of incorporating a larger proportion of legumes into soil on the following N<sub>2</sub>O emissions.

**AC:** Yes, the site was ploughed in 2012 and this was done at both parcels. However, we could not find carry-over effects nor would we have found differences in N<sub>2</sub>O exchange during our experiment as the parcels were not ploughed in 2015/2016. This could become a very nice experiment in a few years when each parcel will have to be restored.

We added in the discussion (Page 15 L 18): “This study covered two years and did not include potential effects of incorporation of clover into the soil during ploughing (which is every 8-10 years).”

**R:** L. 25-26: But what would farmers do with the slurry?

**AC:** Our suggestion is to use the slurry from the biogas plant in fields where currently mineral fertilizers are used. See also our initial statement on this issue.

Technical corrections

**R:** Page 4: L. 5-6: Word missing here: ‘while fertilization to play the dominant role in driving N<sub>2</sub>O emissions in the control parcel’

**AC:** We separated this into two sentences in the revised manuscript: “We hypothesized considerably lower N<sub>2</sub>O emissions in the clover parcel, lower soil nutrient availability in the clover parcel and thus no effect of legume proportions on N<sub>2</sub>O emissions, and hypothesized fertilization to play the dominant role in driving N<sub>2</sub>O emissions in the control parcel” (Page 4: L. 5-6).

**R:** Page 5: L. 7: Mowing is only one of the activities used in harvesting (presumably biomass is also removed). I think it is less confusing if ‘harvest’ is used throughout.

**AC:** We changed this line to “Management activities comprised the regular harvest activities (mowing, swathering and subsequent biomass removal)”. However, we used mowing elsewhere, as this defines clearly the start of the harvest process and gives thus an indication that we refer to the start of the event.

**R:** L. 8-9: Some of this information is already given on L 31 in previous page.

**AC:** We shortened that sentence to avoid redundancy.

**R:** L. 11: Please clarify the study years (2015- 2016, correct). Are the data for 2014 presented?

**AC:** We did not present the biomass data for 2014 and thus changed to 2015-2016.

**R:** L. 17: Check table as ‘herbicide’ not mentioned.

**AC:** The latest herbicide application was in 2013, while no herbicide was applied during the period displayed in the table (2015-2016).

**R:** L. 20: I think you mean with instruments mounted on a mast...

**AC:** We think that in tower is correct terminology here, also for the smaller towers as applied on cropland and grassland sites, see for example Aubinet et al. (2012).

**R:** L. 21: Instead of ‘lying’ use ‘placed’.

**AC:** We changed to “being located”

**R:** L. 26: Instead of ‘The air inlet for N<sub>2</sub>O, CH<sub>4</sub> and H<sub>2</sub>O’ use ‘The air inlet for the absorption spectrometer’.

**AC:** We changed this according to the reviewer’s suggestion.

**R:** Page 6: L. 1: Should refer to Fig. 1 in this section.

**AC:** We agree and added the reference Fig. 1 (Page 6 L6).

**R:** L. 13-15: Is this in addition to the ‘soil sensors for microclimate measurements’ mentioned in L. 9-10?

**AC:** No, with the sentence in L9-10 the soil plots are introduced, while “additional” refers to the fact that we have sensors installed directly at the tower and not in a specific parcel. In order to clarify this, we added a reference to the soil plots in Fig.1, i.e. in L10 we changed to “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”. Then the specification for these follows. Sensor locations are also specified in Table S1.

**R:** Are matric potential and soil heat flux data presented?

**AC:** No, we excluded both from the analysis as they were highly correlated with variables such as soil water content, soil O<sub>2</sub> concentrations, soil temperature and were suspecting problems with multicollinearity if we included them.

**R:** L. 26: Do you mean the ‘average footprint’?

**AC:** Indeed, therefore, we adjusted to average footprint (L. 26).

**R:** Page 7: L. 20: Perhaps give info on clover proportion before (start of methods). The assumption is that proportion of establishment is the same as sowing composition. Did you check the final stand composition?

**AC:** Please see page 7 L4: “Vegetation was separated into legumes and non-legumes (grasses and forbs) to assess the actual legume proportion in the dry biomass“. There is no assumption involved, we directly measured clover biomass weight of the harvested biomass at every harvest (separating harvested biomass in non-legume and legume species), and the resulting percentage is based on the annual biomass. The percentages of clover in dry biomass per harvest are presented in Figure 5c.

We further added in order to clarify that we directly measured clover proportion in biomass (Page L21): “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.”

**R:** L. 23: Use ‘Gauss’.

**AC:** We changed gauss to Gauss.

**R:** L. 29-30: CO<sub>2</sub> molar density refers to the Licor measurements? Since CO<sub>2</sub> was also measured with the QCLAS it may be helpful to inform the reader in EC section as to which data will be resented for CO<sub>2</sub>.

**AC:** CO<sub>2</sub> was not measured with the QCLAS; In the method section 2.3 we described “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<sub>2</sub> and H<sub>2</sub>O concentrations (LI-7500, LiCor Biosciences, Lincoln, NE, USA) and a quantum cascade laser absorption spectrometer (QCLAS) capable to measure N<sub>2</sub>O, CH<sub>4</sub> and H<sub>2</sub>O concentrations (mini-QCLAS, Aerodyne Research Inc., Billerica, MA, USA)”.

**R:** Page 8: L. 6: ‘below’ instead of ‘above’ for N<sub>2</sub>O and CH<sub>4</sub> values.

**AC:** Thanks, we corrected this.

**R:** L.14: fig 1 refers to Kljun et al 2004.

**AC:** The figure was indeed produced with the Kljun et al. (2004) tool for the submitted manuscript since the new 2015 footprint code made available by Kljun et al. (2015) was buggy and provided unrealistic results in our

specific case (we are in discussion with N. Kljun to solve these issues). Thus, we used the cross-wind integrated footprints of her 2004 version of the footprint model for the plot, but we updated the Figure in the revised manuscript with the new (2015) version calculations after having solved the remaining issues.

**R:** L. 15: “Parcel” does not seem to be the correct term here. Do you mean ‘field’ or ‘plot’. Why is footprint so different for South vs North?

**AC:** We checked the wording (“parcel”) and confirm that the terminology corresponds to what we mean. The topography is not symmetric, the Alps are in the South and the Swiss Plateau is in the North. The pressure and temperature differences between the Alps and the Swiss Plateau drive the wind, and hence the wind rose and footprints are not perfectly mirrored between northerly and southerly winds.

**R:** Page 9: L. 13: What was the ‘similar management practice’ in 2014? Please explain earlier in text.

**AC:** We added text earlier in the revised version of the manuscript (page 5 L5):

“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.”

**R:** L.15: I suggest ‘Three management types and one natural event type’ be changed to ‘Three management-derived events and one natural event’

**AC:** Changed similarly to your suggestion to “three management event types”

**R:** Page 11: L. 3: Check citation. What does ‘these’ refer to?

**AC:** Wilks, 2011, page 147 is correct, and this is Copernicus style. “These” refers to the effective sample sizes, we clarified this in the text. “By calculating the effective sample sizes according to (Wilks, 2011:147) and using the effective sample sizes ...”

**R:** L. 23: Sentence not complete.

**AC:** We corrected this. “Volumetric soil water content (at 0.1 m depth) were similar in the control ( $33 \pm 4\%$ ) and the clover parcel ( $31 \pm 5\%$ )“

**R:** Page 12: L. 20: Table has SE as 0.5 not 0.6.

**AC:** The correct version is in the Table thus we changed to 0.5.

**R:** Page 13: L. 8-9: I am not sure what is being compared here (within years or across years): biomass at the clover parcel was lower ( $5.1 \pm 0.3 \text{ t ha}^{-1} \text{ yr}^{-1}$ ) in 2015 and similar ( $4.8 \pm 0.2 \text{ t ha}^{-1} \text{ yr}^{-1}$ ) in 2016.

**AC:** Here we want to compare C in biomass within years. We made the sentence clearer by rearranging and, putting the years together. “C in annual yields at the control parcel was higher ( $5.8 \pm 0.2 \text{ t ha}^{-1}$ ) compared to the clover parcel ( $4.7 \pm 0.3 \text{ t ha}^{-1}$ ) in 2015, while C in biomass was similar for the control parcel ( $5.1 \pm 0.3 \text{ t ha}^{-1}$ ) and the clover parcel ( $4.8 \pm 0.2 \text{ t ha}^{-1} \text{ yr}^{-1}$ ) in 2016 (Table 2).”

**R:** L. 10-11: But values are indicated as ‘ab’ so not different?

**AC:** Your interpretation of the letters is correct, N exported is not significantly lower in the control. We adapted this: ~~“In contrast, N exported was similar across parcels was lower in the control parcel in the second year (control: 238 ± 13 kg ha<sup>-1</sup> yr<sup>-1</sup> ; clover: 262 ± 8 kg ha<sup>-1</sup> yr<sup>-1</sup> ) even though total biomass yields were higher in the control (Table 2).”~~

**R:** Page 14: L. 8: This statement is confusing: ‘periods without management’.

**AC:** Hier war das Ziel das dazuzuschreiben, damit klar ist, dass die Referenz Ctr-0 ist. We revised: “Nitrous oxide emissions significantly increased after fertilizer application (Ctr-F compared to Ctr-0,  $p < 0.05$ ) when compared to N<sub>2</sub>O fluxes during periods of no management on the same (control) parcel (Fig. 8a, Table 4).”

**R:** L. 10-11: ‘a 2.5-fold increase in N<sub>2</sub>O emissions during the seven days following slurry amendment compared to no management (Table 4).’. It is hard to find this information in table; please help reader by giving values...

**AC:** We addressed this by an explicit explanation in brackets. “The effect size showed 2.5-fold N<sub>2</sub>O emissions during the seven days following slurry amendment compared to no management (resulting from applying the back-transformation of to the fertilization effect:  $10^{0.4} = 2.5$ ; Table 4)”. We stick with the straightforward model outputs in the table.

**R:** L. 11: ‘It is important to state that the management effect exists in addition to the effect explained’ is not clear, please edit.

**AC:** With this statement, we wanted to clarify what the reviewer correctly questioned before: At the days after harvest, not all explained variability is necessarily explained by harvest, and rather a combination of the effects of soil temperature and all others driver variables included in the model. We changed the sentence to: The effects of management influence N<sub>2</sub>O fluxes jointly with other measured driver variables (L. 11).

**R:** Page 16: L. 21: I do not consider soil conditions as not ‘external’ factors. . .

**AC:** We agree that the word “external” is misplaced here and replaced this with ‘other’.

**R:** Page 17: L. 11: ‘Clo-0’ is not used in table, please use consistent terminology.

**AC:** Right, we now changed this and put the terms in brackets in the first column of the table. The Ctr-0 is not used, because it was the reference level for the categorical variable management.

**R:** Page 19: L. 24: This brings up an important point. ‘Permanent grasslands’ are temporarily restored as was done with the study area in 2012. Does this restoration involve ploughing or a similar tillage at this site? If so, then authors should discuss the impact of incorporating a larger proportion of legumes into soil on the following N<sub>2</sub>O emissions.

**AC:** Yes, permanent grasslands are restored, here typically every eight to ten years. We clarified the importance of the ploughing

**R:** L. 25-26: But what would farmers do with the slurry?

**AC:** Please see our initial comment on this issue.



**R:** Figure 1: Add explanation of ‘blue dots’ into figure caption.

**AC:** We added “Blue dots represent soil sampling locations.”

**R:** Why are there many more contour lines in the footprint for the N plot vs. S plot?

**AC:** The footprint is shown as a probability density function in 2-d, thus there are two aspects which lead to this: (1) northerly directions are somewhat more frequent than southerly directions; and (2) winds from south vary slightly more in exact wind direction than winds from the north, and hence the probability function is wider (and longer) than when wind is from the north, thus lowering the probability for each unit surface area to be within the footprint. As an example: each meter squared along one contour line has the same weight in the annual flux budget. In other words: the true area covered with measurements when wind is from the north is constrained to a smaller surface than if the wind comes from the south, but since we kept homogeneous conditions in each of the two plots, both directions are representative for the respective plot that measurements belong to.

**R:** Figure 2: Please indicate if symbols are for daily values

**AC:** We corrected the caption as follows: “**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).”

**R:** Figure 6b: not referred to in text.

**AC:** We found that the Figure 6b is referred to on page 13 L23 where we wrote: “... and 39% (36–42%) lower than at the control parcel in 2015 and 2016, respectively (Fig. 6b)”. Still, the Figure 5b was not referred to. We added the sentence: “The living aboveground biomass remaining on the parcel after mowing was  $1.0 \pm 0.3$  t DM ha<sup>-1</sup> on the control parcel and  $0.8 \pm 0.4$  t DM ha<sup>-1</sup> on the clover parcel (Fig. 5b).”

**R:** Figure 8: letter a, b, . . .missing from graphs. X-axis scale for CO<sub>2</sub> flux seems to be wider than dataset.

**AC:** We added the missing letters, which occurred due to the mix-up between earlier versions of this Figure.

**R:** Table 2: Was means test applied to treatments within a year or across two years? The latter (I think) but some of the discussion in text seems to consider ab different than b or a. Letters are missing for last row and 3rd last row year 2016 data.

**AC:** The interpretation of the reviewer is correct, tests were applied across two years and your interpretation of the letters were correct. The missing letters are intention, you can either give a letter to each group (including values that don not go together in a group with others or use only a letter in case of “not significantly different” comparisons in the latter choice. We used the latter option, which means that all values without letter are significantly different from all others. Realizing that your approach might be the more common one, we will change the revised manuscript version accordingly.

**R:** Table 4: Use ‘harvest’ instead of ‘mowing’.



**AC:** We changed this according to your suggestion.

**Anonymous Referee #2**

**R:** This is an important paper, comparing N<sub>2</sub>O emissions from adjacent fields with different proportions of clover content. The paper is well written, and suitable for publication in BG. My main comments are as follows: 1) In the ‘mitigation’ treatment more clover was added, but as it took time to establish, the differences between clover % was rather small in year 2015 (15% & 21%), whereas in year 2016 the differences were large (4% and 44%). Similar differences were observed for the BNF rates (Table 2). These differences and their implications on the yield and N<sub>2</sub>O fluxes is not adequately addressed in this paper. 2) N<sub>2</sub>O was measured using eddy covariance, from 2 adjacent fields. The overall data coverage of both fields was similar (Table 3), but the authors need to demonstrate that the temporal coverage of measurements was similar for both fields. One would not want situations where the airflow is always from field 1 at dawn, for example.

**AC:** With respect to (1), we now address this aspect stating the clover proportions directly when we presented the results for N<sub>2</sub>O fluxes (Abstract and Results) and addressed the implications on our findings in the revised manuscript (see Section 4.2). With respect to point (2), we had checked the data coverage also in dependence of daytime, which covered reasonably well all times of day in both parcels. We will add this information to the Supplementary material (Figure R2-1). In fact, in a broad valley with a typical wind system with up-valley winds during peak daytime and down-valley winds during the second part of the night we get the best share of coverage during dusk and dawn (almost 1:1), whereas the extremes are in the range 1:2 to 2:1 (see Figure R2-1). We added this figure to the Supplementary Material as Figure S2 and refer to it in the text.

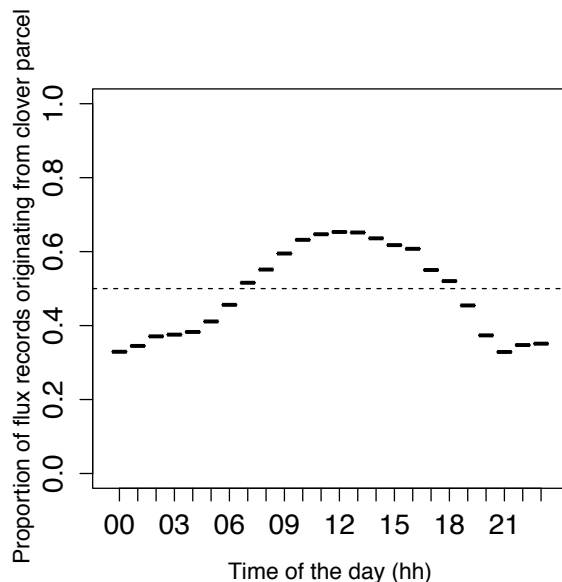


Figure R2-1: Proportion of flux records during the observation period originating from the clover parcel depending on the hour of the day.

**R:** Further suggestions of edits can be found below Abstract: needs to contain the grass clover proportions for the 2 fields

**AC:** We find this a useful suggestion and added the clover proportions and biologically fixed N in the abstract:

“N inputs via BNF were similar in both parcels in 2015, (control: 55 ( $\pm$  5) kg N and clover parcel: 72 ( $\pm$  5) kg N) due to similar clover proportions (control: 15% and clover parcel: 21%), whereas in 2016 the N inputs via BNF were higher in the clover parcel compared to the control (control: 14 ( $\pm$  2) kg N with 4% clover in DM and clover 130 ( $\pm$  8) kg N and 44% clover).”

**R:** Introduction: ‘Apart from the environmental benefits of a reduced N surplus when mineral fertilizer is replaced by BNF, total GHG emissions from fertilizer production of 1.6– 6.4 kg CO<sub>2</sub>-eq per kg fertilizer N, could technically be avoided (Andrews et al., 2007; Brentrup 5 and Pallière, 2008).’ **R:** In which country of climate zone can such GHG reduction rates be achieved?

**AC:** This statement was referring to mineral fertilizer production. We deleted it in the revised manuscript, as this further aspect seems to be misleading and is not directly important for the study.

**R:** Methods: “The site has been well investigated in terms of CO<sub>2</sub> exchange (Burri et al., 2014; Zeeman et al., 2010), as well as for N<sub>2</sub>O and CH<sub>4</sub> exchange under management that is typical for Swiss grasslands located on the Swiss Plateau (Imer et al., 2013; Merbold et al., 2014; Wolf et al., 2015).’ **R:** Add that CO<sub>2</sub> exchange is measured by EC and N<sub>2</sub>O/CH<sub>4</sub> by, presumably, static chambers.

**AC:** According to your suggestion, we addressed the methods specifically for each reference: The site has been well investigated in terms of CO<sub>2</sub> exchange (Burri et al., 2014 using static chambers (SC); Zeeman et al., 2010 using EC), as well as for N<sub>2</sub>O and CH<sub>4</sub> exchange under management that is typical for Swiss grasslands located on the Swiss Plateau (Imer et al., 2013 using SC for N<sub>2</sub>O and CH<sub>4</sub> and EC for CO<sub>2</sub>; Merbold et al., 2014 using EC for all three gases; Wolf et al., 2015 using EC and SC for N<sub>2</sub>O).

**R:** Given that you have reduced the EC averaging time to 10 min from the usual 30 min, I assume that you must have had a relatively equal spread between coverage of both the two plots. You need to demonstrate this, for example by including a graph of N<sub>2</sub>O versus time with different colour dots for the two treatments.

**AC:** We had analyzed in more detail the coverage of both parcels. This included looking at ‘time of day’ versus ‘number of flux measurements’ per parcel binned for time classes as well as of ‘time of day’ versus N<sub>2</sub>O flux per parcel, especially after management events. We added a Figure in the Supplement (Figure R2 and R3).

**R:** Why did you fertilize with 296 kg N/ha/2015 and 181 in 2016?

**AC:** Our aim was to not artificially change the behavior of the farmer and let him choose, according to previous practices, which are a joint outcome of N budget considerations but also practical issues such as weather conditions and how much slurry is available at the time when suitable for field application.

Section 2.6: ‘and a subsample of 5 mg was weighed into tin capsules for further analyses (n = 5 for each parcel per date).’ **R:** You need to add: ‘. . .for further analysis of total C and N and . . . . .’

**AC:** We adjusted this according to the reviewers suggestion (Page 7 L8) “of total C and N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  “

**R:** Figure 1: include the prevailing wind direction, or say what it is in the legend

**AC:** We will include in the caption: “The prevailing wind direction was from the north.”

**R:** Legend to Figure 2 needs to be tidied up.

**AC:** The correct caption, as in the list of figures was inserted.

**R:** Figure 5: Why do you join the dots for graphs b-d, but not for graphs e-f? Looks like there is some inconsistency here.

**AC:** We inserted the line for prediction only for the variables which improved the model fit.  $\text{NO}_3^-$  and DOC did not improve the fit and thus were not included in the reduced model. Consequently, we did not draw the prediction lines (please see also the caption) and only showed the data points.

**R:** Section 3.5: ‘During the reference year 2013’ Add the reference to these 2013 data.

**AC:** The reference data are part of this paper, we realize that we should have introduced the concept of reference years more clearly in the method section.

Therefore we added (Page 4 L27): “We use the two years 2013 and 2014 as reference years (no treatment). In order to test the  $\text{N}_2\text{O}$  mitigation option, the treatment parcel was over-sown in March 2015 and April 2016 with clover.”

**R:** Figure 8 ‘the factor management’ delete ‘the factor’, or place it before ‘(a)’

**AC:** It is now deleted.

**R:** Discussion 1st Parag. ‘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’ **R:** Add the % of clover to remind the reader ‘to an increased clover proportion of x %’

**AC:** We added (i.e. 21% versus 15 % in 2015, 44% versus 4% in 2016) after this sentence.

**R:** Last sentence and elsewhere: change ‘in sum’ to ‘in summary’.

**AC:** Done in both occurrences (L16 and L26)

Section 4.1: ‘than our site, showed typically lower  $\text{N}_2\text{O}$  emissions (0.38–2.28 kg  $\text{N}_2\text{O}$ - N ha<sup>-1</sup> yr<sup>-1</sup>), which can be explained by lower fertilizer inputs compared to our site (Hörtnagl et al. 2018). ‘In sum, our year-round

measurements of N<sub>2</sub>O emissions are higher than multi-site averages due to its fertilizer regime and site conditions, but within plausible ranges compared to other sites. R: Discuss the differences in fertiliser rate and the differences in site composition between the Hortnagl study and yours in greater details, so that the reader also understands why your N<sub>2</sub>O fluxes are larger. Provide more information on the differences between your site and the Hortnagl sites. And, to improve the English change ‘In sum, our year-round measurements of N<sub>2</sub>O’ to ‘In summary, our one-year measurements of N<sub>2</sub>O’

**AC:** Page 15 L26: We elaborated on the discussion of site conditions and fertilizer rates in more detail and refined the paragraph in the revised version. “In summary” was corrected.

**R:** Section 4.2: ‘N<sub>2</sub>O emissions in the clover parcel during our two-year observation period summed up to 1.9 and 3.8 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> in 2015 and 2016, respectively. These values were clearly lower than the values observed from the control parcel.’ R: You need to discuss these observations and others in this section with the fact that the differences in clover proportions between the two fields in 2015 were rather small compared to 2016 (Table 2).

**AC:** We addressed this by adding the clover proportions and the implications in the revised manuscript. “N<sub>2</sub>O emissions in the clover parcel during our two-year observation period summed up to 1.9 and 3.8 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> in 2015 and 2016, respectively. These N<sub>2</sub>O 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<sub>2</sub>O emissions on the clover parcel as in 2015.”

**R:** ‘Jensen et al. (2012) based on site-years.’ R: based on how many site years?

**AC:** Added “Jensen et al. (2012) based on eight site-years.”

**R:** ‘In addition, high total N deposition (NH<sub>3</sub>-N, NO<sub>3</sub>-N, HNO<sub>3</sub>-N, NO<sub>2</sub>-N) on intensively managed Swiss grasslands (15–40 kg N ha<sup>-1</sup> yr<sup>-1</sup>, Seitler et al., 2016)’ **R:** Can you be more specific regarding the N dep rate in your study area. The range you quote is very large.

**AC:** These values were from an available report, in order to get a specific estimate for the study area we contacted B. Rihm who was able to provide spatially explicit estimates in the revised version of the manuscript. We changed to: “In addition, high total N deposition (NH<sub>3</sub>-N, NO<sub>3</sub>-N, HNO<sub>3</sub>-N, NO<sub>2</sub>-N) in the study area (in total 33.8 kg N ha<sup>-1</sup> yr<sup>-1</sup> in 2015, Rihm, personal communication 27<sup>th</sup> June 2018; Rihm and Achermann, 2016) might foster background N<sub>2</sub>O emissions due to increased NH<sub>4</sub>-N and NO<sub>3</sub>-N availability (Butterbach-Bahl et al., 2013)”

**R:** Section 4.3: 1st paragraph: you should qualify phrases such as those shown below ‘N<sub>2</sub>O emissions vary widely across sites’ R: add the ranges of emissions, and presumably the studies for reference are from grasslands? ‘Higher N<sub>2</sub>O fluxes following cutting were similarly observed on a pasture in Central France (Klump et al., 2011).’ R: what is the difference relative to your study? You have done this much better in the 2nd paragraph.

**AC:** Indeed, specific values will add clarity. Therefore, we added how large the effects were in the cited articles. Furthermore “sites” was changed to “grassland sites”. (Page 16: L. 17-20) “Nevertheless, effects of fertilization on N<sub>2</sub>O 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, 1.3 and 3.5% of fertilizer N across years in this study), indicating that fertilization alone is insufficient for explaining N<sub>2</sub>O emissions and highlighting the need to take additional drivers into account.”

Similarly, we added specific values following your suggestion in the sentence on page 16: L. 24: “Higher N<sub>2</sub>O fluxes following cutting were similarly observed on a pasture in Central France (up to 3.7 mg N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup> in Klumpp et al., 2011; up to 7.0 mg N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup> in this study).”

**R:** ‘In agreement with our result, an experiment without seasonal frozen soils at an Irish permanent ryegrass/clover mixture, annual N<sub>2</sub>O emissions between unfertilized ryegrass’ R: ‘change to ‘ In agreement with our result, measurements from permanent grasslands in Ireland, where winter freeze-thaw cycles are very rare, a comparison of a ryegrass/clover mixture, with . . . . .’

**AC:** We changed this similar to your suggestion (Page 17: L.09): “In agreement with our result, measurements from permanent grasslands in Ireland, where winter freeze-thaw cycles are very rare, showed that annual N<sub>2</sub>O emissions in unfertilized ryegrass ( $2.38 \pm 0.12$  kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) were not significantly different from an unfertilized grass–clover sward ( $2.45 \pm 0.85$  kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>)”, ...

**R:** ‘The magnitude of the fertilization effect of 2.5-fold N<sub>2</sub>O emissions on average during the week after fertilization (at 43 kg N amendment per event on average) was comparable to the effect of a 14 °C soil temperature increment if further environmental variables remained constant.’ R: This sentence requires an introduction and significant explanation. It is a bit out of place here.

**AC:** An explanation would be similarly misplaced in the discussion, so we deleted the sentence it in the revised manuscript to avoid confusing the reader. We added an

**R:** Section 4.4 ‘Additionally, high SON content due to previous year’s fertilizer amendments are expected to contribute to the persistently high production levels’ R: I suppose you mean ‘years’ and not year’s’

**AC:** Correct, we meant years’. Thank you for pointing this out.

**R:** ‘the over-sowing was more effective and biologically fixed nitrogen found in shoot biomass in the clover parcel summed up to 130 kg N ha<sup>-1</sup> yr<sup>-1</sup> while only 14 kg N ha<sup>-1</sup> yr<sup>-1</sup> were measured in the control parcel’ R: What is the reason for the legume proportion in the control to decrease between 2015 and 2016?

**AC:** We think that several factors are relevant for the explanation, firstly growth conditions and secondly conditions for germination and establishment. Warmer and dryer conditions in summer 2015 generally favored growth of these clover plants that were already present in both fields (clover proportions were previously were 5–10%, and *T. repens* and *T. pratense* have higher optimum temperatures compared to abundant grass species). Further, but less important, the earlier timing of cutting (quite early 2015-04-22 at lower height, compared to 2016-05-27), and the higher number of cuts in 2015 (5 in 2015 compared to 4 in 2016) might have contributed to

promoted clover growth. Secondly, spring conditions in 2015 were not ideal for clover germination and establishment, i.e. air temperature during the week following the over-sowing in 2015 was low ( $T_{\text{mean}} = 5.5^{\circ}\text{C}$ ), and minimum temperatures were below zero ( $T_{\text{min}} = -4.3^{\circ}\text{C}$ ), there was no precipitation. In contrast, conditions the week after over-sowing were warmer and wetter in 2016 ( $T_{\text{mean}} = 9.1$ ,  $T_{\text{mean}} = 0.03$ , precipitation total 19.1 mm during the week after over-sowing). This is how the relatively small difference between parcels in 2015 and the large differences in 2016 can be explained.

**R:** ‘This indicates that biologically fixed nitrogen at the Chamau could reach higher amounts than observed during our experiment.’ **R:** Can you really deduce this statement from the New Zealand study, where the climate, soil types and perhaps even the grass and clover species used may be rather different?

**AC:** We consider statement sound as the as the cited experiment (Nyfeler et al. 2011) took place in Zurich-Reckenholz (about 30 km from our study site) under similar climatic conditions using the same clover species (*Trifolium repens* and *Trifolium pratense*).

**R:** Section 4.5 ‘due to large springtime emissions (Virkajärvi et al., 2010) indicating that the mitigation strategy is likely to be inappropriate for sites with seasonally frozen soils.’ **R:** Your Swiss soils also experience winter freeze-thaw cycles, but your data suggest that this mitigation strategy works in Switzerland. Please address this discrepancy.

**AC:** We clarified here (P19L17): Much higher  $\text{N}_2\text{O}$  emissions from an unfertilized grass-clover mixture (92% increase) compared to  $\text{N}_2\text{O}$  emissions from a grass sward fertilized with  $220 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  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.

**R:** ‘Due to this effect, temporary grasslands may not reproduce the findings from permanent grassland.’ **R:** You need to provide evidence for this statement. Temporary grasslands are maintained for several years, so are rather different to croplands.

**AC:** We revised to make clearer what we mean: “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  $\text{N}_2\text{O}$  fluxes and thus may not be the best mitigation strategy for croplands and temporary grasslands, where ploughing is done much more frequently.”

**Anonymous Referee #3**

GENERAL QUESTIONS AND COMMENTS

**R:** The paper is well structured and written, it presents a comparison between two differently managed grasslands, to evaluate the impact of the addition of clover as a mitigation strategy for N<sub>2</sub>O emissions. In my opinion, it is suitable for publication in Biogeosciences. See below for some minor comments to the paper.

**R:** The focus of the paper is the evaluation of the integration of legumes as a mitigation strategy, but that does not appear explicitly in the title: I think it should be part of it.

**AC:** We agree and changed the title “Management matters: Testing a mitigation strategy for nitrous oxide emissions using legumes ....”

**R:** To what extent do you think soil properties are needed (frequency of sampling, etc) to interpret flux data? Why daily or bi-weekly? Why many 20cm samples?

**AC:** We assumed large changes in soil nutrients directly after management events, while variation in soil nutrients in times without management were considered much lower following Wolf et al. (2015). We used sampling depth 0-20 cm for soil sampling to replicate the same sampling strategy as applied in Wolf et al. 2015 to assure comparability. Further, the 0-20 cm depth is also represented in biogeochemical models (e.g. DayCent SOC pool, Mineral N) which is relevant as we aim at using our measurements for model validation.

**R:** Would you have any specific suggestions for long term measurements? This is not necessary, of course, but I think it would add value to the discussion to know what you found out to be the most useful variable for your parameterisation, beside the interpretation of your results, of course.

**AC:** Concluding from our data analysis, soil temperature and moisture were crucial and the most important variables. Measurements in 0.1 m depth represented well the microclimatic conditions, while deeper sensors were redundant as these were highly correlated with 0.1 m but less powerful in explaining N<sub>2</sub>O emissions. At sites experiencing regular to freeze-thaw events, in addition to 0.1 m, the 0.05 m soil sensors would be important. O<sub>2</sub> concentrations in 0.1 m improved the prediction in addition to soil moisture, however the effort was quite high as standard sensors were not available and they correlate with soil moisture. The measured matrix potential was not used as it correlated with soil moisture and O<sub>2</sub>, and we therefore consider them less important for long-term measurements. While NH<sub>4</sub> measurements improved the predictions, NO<sub>3</sub> and TOC did not improve predicted N<sub>2</sub>O emissions on a daily resolution, thus less frequent sampling for the latter would be sufficient.

**R:** Could you also report the GWP of the N<sub>2</sub>O measurements to express the mitigation induced by the grassland composition change?

**AC:** We added the difference between both parcels expressed as CO<sub>2</sub>-eq in the abstract of the revised version. “The mitigation management effectively reduced N<sub>2</sub>O emissions by 54% and 39% in 2015 and 2016, respectively, corresponding to 1.0 and 1.6 t ha<sup>-1</sup> yr<sup>-1</sup> CO<sub>2</sub>-equivalents.” We further added in the method section 2.8 the following sentence: “For the calculation of CO<sub>2</sub> equivalents (CO<sub>2</sub>-eq) we used factor 298, which is the current IPCC global warming potential including climate-carbon feedbacks on a 100 year basis (IPCC, 2013).”

**R:** The eddy covariance tower in this experiment is placed at the edge between two differently treated fields. Could you explain a bit better the way you tackled the advection issues between the two treatments/crops? Two years is an impressive duration for such dataset, and I would guess all conditions (time of the day, stability, etc.) have been met in such long time for both fields, but it would be good if it were expressed more clearly through the results. Also, for what concerns the special events, do you have a suitable coverage for both fields in terms of footprint?

**AC:** Quality control (see section 2.7 Eddy covariance flux post-processing) assures that stability criteria were met for all final fluxes, while fluxes were excluded if this was not the case. Furthermore, we analyzed “time of the day” versus N<sub>2</sub>O flux per event, and overall, we had both parcels well represented. Still, there were events at which the coverage from the control parcel was less covered. In our results we compared only cases where both parcels were adequately covered in order to avoid a bias and argue that this direct comparison of both parcels provides a reasonable estimate. We will address this in a supplementary figure.

## BY SECTIONS: MATERIAL AND METHODS

**R:** P5 L1> Replace “fertilised” with “added”.

**AC:** Changed.

**R:** Section 2.2: What is the reason to use two different fertilisation rates in the 2 years? Is it for simulating the business as usual behaviour of the farmer, or did you increase the amount to enhance the effects of the contrast? I see that the clover abundance difference between the two years is quite relevant: is it solely due to the additional grazing? Was the field over sown at the same rates? In connection to the conclusion that up to 44% of clover addition does not lead to further N<sub>2</sub>O emissions, it could be useful to suggest how to achieve such abundances. Perhaps you could expand on this.

**AC:** This was done to interfere as little as possible with the farmers business as usual activities. Thus, we let the farmer chose the amount and timing consistent with how he made his choices previous to the study. We did not want to artificially increase the fertilizer rates, nor artificially change the type of fertilizer, average annual org. fertilizer amounts in previous years (2003-2015) were 260 kg N on average with a range of 184–430 kg. Logically, the 296 kg N in the first year and 181 kg N in the second year are within this range. Sowing rates are given in Table 1, we added a comment on Page 2 L6 “(see Table 1 for specific management dates, slurry composition and sowing rates)”.

**R:** Section 2.7: could you specify and motivate what method you used to calculate the time lag for the different GHG species?

For the calculation of the time lag between the wind component  $w$  (after coordinate rotation) and the mixing ratio of N<sub>2</sub>O (CH<sub>4</sub>), we followed the covariance maximization method in combination with a pre-determined nominal lag time. This means, we identified the peak in the cross-correlation function between  $w$  and N<sub>2</sub>O (CH<sub>4</sub>) in a defined time window of physically possible time lags. This cross-correlation function is often noisy due to low signal-to-noise ratios as a consequence of low N<sub>2</sub>O (CH<sub>4</sub>) fluxes. In our study, analyzing the frequency distribution



of detected lag times over the course of a “low-flux” year yielded clear results that assisted our processing in constraining the lag time search to a relatively short but adequate time window. In order to accurately identify the time lag and avoid systematic bias, we followed a multi-step approach before the calculation of final N<sub>2</sub>O (CH<sub>4</sub>) fluxes for each year:

Step (1): We first calculated the time lag for N<sub>2</sub>O (CH<sub>4</sub>) for the respective year using the covariance maximization method for a relatively large search window of  $\pm 10$  s, based on despiked and detrended raw data. Next, we analyzed the frequency distribution of all found lag times and identified a clear distribution spike between 0.95 and 1.40 s for N<sub>2</sub>O (Figure R3-1 shows results for the year 2013) and between 1.00 and 1.40 s for CH<sub>4</sub> (Figure R3-2), i.e. most cross-correlation peaks where found in these ranges.

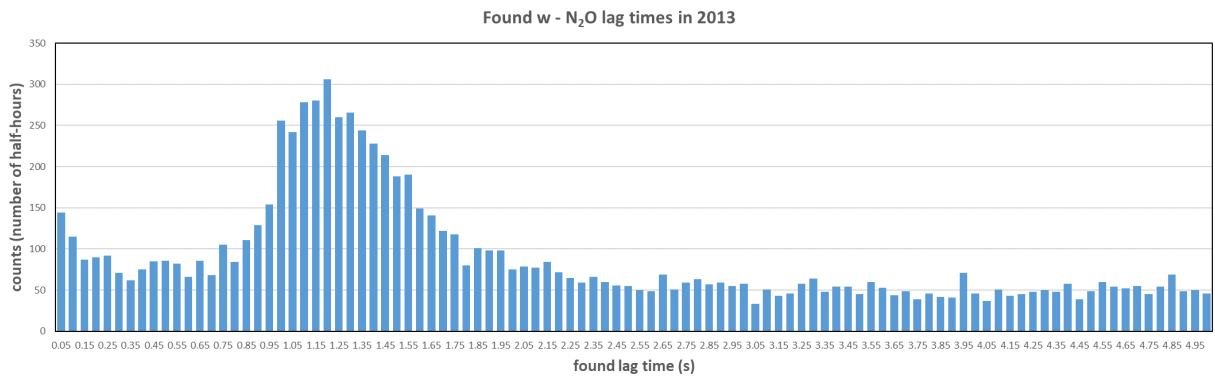


Figure R3-1. Histogram of found lag times between the turbulent wind component w and N<sub>2</sub>O mixing ratios in a time window of  $\pm 10$  s in 2013. Peak distribution was found at lag time 1.20 s. Shown are only found lag times below 5 s for clarity.

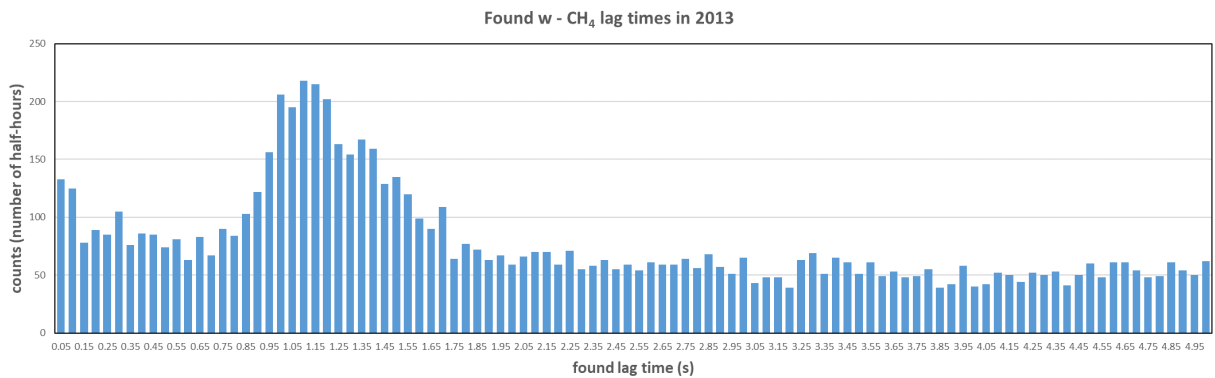


Figure R3-2. Histogram of found lag times between the turbulent wind component w and CH<sub>4</sub> mixing ratios in a time window of  $\pm 10$  s in 2013. Peak distribution was found at lag time 1.10 s. Shown are only found lag times below 5 s for clarity.

Step (2): Based on the frequency distribution from (1), we defined a narrow lag search window of 0.6 – 1.8 s, i.e. the range in which most cross-correlation peaks were found in (1). In addition, we defined the lag times of peak distribution from (1) as the nominal lag times (N<sub>2</sub>O: 1.20 s; CH<sub>4</sub>: 1.10 s), that is, whenever the returned time lag was one of the limits of the window (i.e. 0.6 or 1.8 s), the time lag was set to the respective nominal lag time. These specific nominal lag times were chosen because they were identified as the most representative lag times over the course of a year. We also analyzed the time series of found lag times to investigate potential seasonal

differences. We found that lag times over the course of a year fluctuated slightly but were well within the range of the pre-defined search window of 0.6 – 1.8 s. We are therefore confident that the accurate lag time is found in the defined range.

In addition, we are confident to have adequately constrained the time window due to the used measurement setup at the site. At Chamau, we measured eddy covariance data using a fully digital and real-time logging system that was described previously (Eugster and Plüss, 2010). One major advantage of this system is that wind and scalar data at a specific moment in time are merged immediately in the same data file. As a consequence, the system is not prone to time drifts between two instruments (e.g. sonic anemometer and QCL) and the lag time can therefore consistently be found in a relatively narrow time range (with small fluctuations).

## RESULTS

**R: P12 L6-7.** From figure 4, it almost looks like this is not the case in the last 2 events of 2016, “41,33”. The value of  $\text{NH}_4^+$  after these events seems to be almost double compared to the period before. Could you comment on this, especially addressing the N deposition issue?

**AC:** You refer to the effect of the events on 2016-08-17 and 2016-09-30 taking place in the control affecting soil mineral N in the clover parcel. We agree on your observation for the event on 2016-08-17 and find it worthwhile a closer look. We always took a set of soil samples directly before the respective event in order to get the direct reference. Before the slurry application  $2.5 \text{ mg NH}_4^+\text{-N kg}^{-1}$  increases to  $5.8 \text{ mg NH}_4^+\text{-N kg}^{-1}$  and stays above the reference for seven days. On the last event, the story is not that clear. The reference was  $5.6 \text{ mg NH}_4^+\text{-N kg}^{-1}$ , then we measured lower ( $4.9 \text{ mg NH}_4^+\text{-N kg}^{-1}$ ) at the application date but higher ( $7.2 \text{ mg NH}_4^+\text{-N kg}^{-1}$ ) the day after and lower data compared to the reference afterwards (explicitly 2.5, 5.2, 2.9, 2.7, 2.9 and  $3 \text{ mg NH}_4^+\text{-N kg}^{-1}$ ), thus only the  $\text{NH}_4^+\text{-N}$  measurement of the day following the application date was higher compared to the reference. The overall picture across events was rather diverse.

We therefore changed “no distinct patterns” to “no consistent patterns. We further addressed it in the discussion of the revised manuscript: “Additionally,  $\text{NH}_3$  deposition on the clover parcel originating from  $\text{NH}_3$  emissions from the adjacent control parcel is likely to be the cause of increased soil  $\text{NH}_4^+$  concentrations after the event on 17<sup>th</sup> August 2016.”

**R: P13,L2:** it could be helpful to quantify this similarity, e.g. providing a ratio of C content in biomass between the 2 different treatments directly in the text.

**AC:** We addressed this by directly referring to the values. “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, Tab. 2; Fig. 5e).”

## DISCUSSION

**R: P17,L3-4.** “Grazing had only a minor influence on the overall  $\text{N}_2\text{O}$  budget of the Chamau site and data analysis showed that  $\text{N}_2\text{O}$  fluxes did not significantly respond to the presence of animals (Fig.7c)”. I think that a

quantification would be better here than referring to the plot only, i.e. the relative % in contribution on the total N<sub>2</sub>O budget, for example.

**AC:** According to your suggestion we will add the relative contribution of fluxes during grazing on the total N budget in the revised version of the manuscript (P17, L3-4).

## TABLES AND FIGURES

**R:** Figures 1 and 6 seem to have the appropriate resolution, the others tend to be a bit blurry: would it be possible to increase the image resolution?

**AC:** This effect arises due to the insertion in MS WORD, the original graphics are pdfs and will be submitted as such, in high quality.

**R:** Figure 2. The caption under the image needs correction. “(b) Footprint climatology of the year 2016 with footprint contour lines of 10% to 90% in 10% steps using the Kljun et al. (2004) footprint model” belongs to previous figure; explain panels a, b, and c.

**AC:** The correct caption was added: **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).

**R:** Figure 4. Albeit the treatments with slurry were not applied on the clover field, I think it would be useful to introduce the days of treatment also on the North field charts (perhaps the same dotted lines, no arrows). If no slurry was directly applied, the amount of N in the air during the fertilisation events has certainly changed, and potentially increased the amount of BNF on the clover field.

**AC:** Fertilizer applications are followed by NH<sub>3</sub> emissions (not measured), which can subsequently increase NH<sub>3</sub> deposition on the clover parcel and therefore fertilize the clover parcel. However, this should not affect BNF as BNF is not expected to be limited by N availability.

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# 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<sub>2</sub>O) mitigation from intensively managed grasslands. While current literature provides evidence for an N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O fluxes and biomass yields in two differently managed grass-clover mixtures. We measured N<sub>2</sub>O 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<sub>2</sub>O 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 <sup>15</sup>N natural abundance method.

The amount of BFN was similar in both parcels in 2015, (control:  $55 \pm 5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  and clover parcel:  $72 \pm 5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) 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:  $14 \pm 2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  with 4% clover in DM and clover parcel:  $130 \pm 8 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  and 44% clover). The mitigation management effectively reduced N<sub>2</sub>O emissions by 54% and 39% in 2015 and 2016, respectively, corresponding to 1.0 and 1.6 t ha<sup>-1</sup> yr<sup>-1</sup> CO<sub>2</sub>-equivalents. These reductions in N<sub>2</sub>O 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<sub>2</sub>O emissions, indicating that decomposition of plant residues and rhizodeposition did not compensate the effect of fertilizer reduction on N<sub>2</sub>O emissions. Annual biomass yields were similar under mitigation management, resulting in a reduction of N<sub>2</sub>O emission intensities from 0.42 g N<sub>2</sub>O-N kg<sup>-1</sup> DM (control) to 0.28 g N<sub>2</sub>O-N kg<sup>-1</sup> DM (clover parcel) over the two years observation period. We conclude that N<sub>2</sub>O emissions from fertilized grasslands can be effectively reduced without losses in yield by increasing the clover proportion and reducing fertilization.

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## 1 Introduction

Agricultural practices contribute 5.4 Gt CO<sub>2</sub>-eq. yr<sup>-1</sup> (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<sub>2</sub>-eq. yr<sup>-1</sup> by 2030 (Smith et al., 2008), exceeding current agricultural GHG emissions. The three major anthropogenic GHGs comprise carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O). The agricultural sector is responsible for 84% of global anthropogenic N<sub>2</sub>O emissions (Smith et al., 2008). N<sub>2</sub>O 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<sub>4</sub> and N<sub>2</sub>O (GWP, factor 34 and 298, respectively, on a per mass basis compared to CO<sub>2</sub> based on a 100-year time horizon) (IPCC, 2013b), these gases are more important than the CO<sub>2</sub> fluxes from the agricultural sector. However, they remain far less understood than CO<sub>2</sub> fluxes because of interactions between multiple underlying processes that are largely unexplored. In particular, data resolving the dynamics of N<sub>2</sub>O fluxes from soils are still scarce, as advances in instruments capable of high-frequency continuous N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions in grasslands strongly depend on management practices (Hörtnagl et al., 2018; Li et al., 2013; Snyder et al., 2009) and reducing N<sub>2</sub>O emissions while maintaining yields can thus contribute to climate smart agriculture (CSA) (Lipper et al., 2014). For mitigating N<sub>2</sub>O 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<sub>2</sub>O, 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<sub>3</sub>) and nitrate (NO<sub>3</sub><sup>-</sup>) leaching, both potential sources of indirect (off-site) N<sub>2</sub>O 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<sub>2</sub>O 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

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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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O via rhizobial denitrification (O’Hara and Daniel, 1985; Rosen and Ljunggren, 1996), direct N<sub>2</sub>O emissions from BNF are negligible compared to N<sub>2</sub>O from denitrification rates for most investigated species and hence result in no significant effect on field-scale N<sub>2</sub>O emissions (Garcia-Plazaola et al., 1993; Rochette and Janzen, 2005).

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To date, experimental studies investigating year-round N<sub>2</sub>O 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 **BNF** are missing. Thus, the aim of this study was to test the N<sub>2</sub>O mitigation strategy of substituting N fertilizer with **BNF** by increasing the clover proportion in grassland. Therefore, we measured N<sub>2</sub>O 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<sub>2</sub>O emissions from both parcels, (2) to identify the meteorological and soil chemical drivers of N<sub>2</sub>O emissions, (3) to assess if substituting N fertilizer with **BNF** was an effective N<sub>2</sub>O mitigation strategy. We hypothesized considerably lower N<sub>2</sub>O emissions in the clover parcel, lower soil nutrient availability in the clover parcel and thus no effect of legume proportions on N<sub>2</sub>O emissions, and hypothesized fertilization to play the dominant role in driving N<sub>2</sub>O emissions in the control parcel. We further expected minor differences in grassland yield between the two parcels, and as a consequence, reduced N<sub>2</sub>O emission intensities in the clover parcel.

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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<sub>2</sub> exchange (Burri et al., 2014, using static chambers (SC); Zeeman et al., 2010, using EC), as well as for N<sub>2</sub>O and CH<sub>4</sub> exchange under management that is typical for Swiss grasslands located on the Swiss Plateau (Imer et al., 2013, using SC for N<sub>2</sub>O and CH<sub>4</sub> and EC for CO<sub>2</sub>; Merbold et al., 2014, using EC for all three gases; Wolf et al., 2015, using EC and SC for N<sub>2</sub>O). 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<sup>-1</sup> yr<sup>-1</sup>). 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<sup>-3</sup> (Roth, 2006) and a soil pH of about 6.5 (Labor Ins AG, Kerzers, Switzerland, in 2014).

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## 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<sub>2</sub>O 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<sup>-1</sup> 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 <sup>15</sup>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<sub>2</sub>, N<sub>2</sub>O, CH<sub>4</sub>, H<sub>2</sub>O) 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),

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an open-path infrared gas analyser for CO<sub>2</sub> and H<sub>2</sub>O concentrations (LI-7500, LiCor Biosciences, Lincoln, NE, USA) and a quantum cascade laser absorption spectrometer (QCLAS) capable to measure N<sub>2</sub>O, CH<sub>4</sub> and H<sub>2</sub>O 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<sub>2</sub> and H<sub>2</sub>O 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<sub>2</sub>O 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.

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#### 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<sub>2</sub>) 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 18<sup>th</sup> June 2015 (at 0.05, 0.1, 0.2 m) and were thus replaced on 6<sup>th</sup> 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<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>) 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<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and DOC was achieved by shaking 15 g of fresh soil with 50 mL 0.5 M K<sub>2</sub>SO<sub>4</sub> 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<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> 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<sup>2</sup>; 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, δ<sup>13</sup>C and δ<sup>15</sup>N (n = 5 for each parcel per date). C and N concentrations, as well as δ<sup>13</sup>C and δ<sup>15</sup>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 BNF were based on the δ<sup>15</sup>N measurement. The percentage of shoot N derived from BNF (%N<sub>difa</sub>, nitrogen derived from atmosphere) in legume biomass was calculated with the <sup>15</sup>N natural abundance method, (Boddey et al., 2000; Unkovich, 2008), following Eq (1):

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

where %N<sub>difa</sub> is the percentage of legume shoot N derived from atmosphere, δ<sup>15</sup>N<sub>ref</sub> is the δ<sup>15</sup>N value of a non-fixing reference plant (i.e. grass species) growing in the proximity of the legume and δ<sup>15</sup>N<sub>legume</sub> is the δ<sup>15</sup>N value of the legume shoot. The B value is the δ<sup>15</sup>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 × 2/3) and *Trifolium pratense* L. (-0.94 × 1/3) (B values from Unkovich, 2008, Appendix 4). Weights were chosen according to the sown legume species composition of 2/3 white clover and 1/3 red clover. The %N<sub>difa</sub> in legume shoots was calculated for each legume biomass sample taken. The non-legumes cut

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within the same harvest frame as the legumes were used as reference delivering the  $\delta^{15}\text{N}_{\text{ref}}$  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.

5 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  $\text{CO}_2$ ,  $\text{N}_2\text{O}$  and  $\text{CH}_4$  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 ( $\text{CO}_2$ ) or mixing ratios ( $\text{N}_2\text{O}$ ,  $\text{CH}_4$ ). Molar densities of  $\text{CO}_2$  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 ( $\text{CO}_2$ : (Horst, 1997);  $\text{N}_2\text{O}$  and  $\text{CH}_4$ : (Fratini et al., 2012).  $\text{N}_2\text{O}$  and  $\text{CH}_4$  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).

15 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:  $\pm 5 \text{ m s}^{-1}$ ;  $\text{CO}_2$ : 200 to 900 ppm,  $\text{N}_2\text{O}$ : below 250 ppb,  $\text{CH}_4$ : below 1700 ppb), (2) if spikes, defined as data points outside pre-defined sigma ( $\sigma$ ) plausibility ranges (vertical wind speed:  $\pm 5\sigma$ ,  $\text{CO}_2$ :  $\pm 3.5\sigma$ ,  $\text{N}_2\text{O}$  and  $\text{CH}_4$ :  $\pm 8\sigma$ ), 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  $\text{CO}_2$  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 ( $\text{CO}_2$ :  $\pm 50 \text{ umol m}^{-2} \text{ s}^{-1}$ ;  $\text{N}_2\text{O}$ : between  $-50$  and  $100 \text{ nmol m}^{-2} \text{ s}^{-1}$ ;  $\text{CH}_4$ : between  $-400$  and  $800 \text{ nmol m}^{-2} \text{ s}^{-1}$ ), (2) if the steady state test (Foken and Wichura, 1996) was outside  $\pm 30\%$ , or (3) if the test on developed turbulent conditions was outside  $\pm 30\%$  (Foken et al., 2004; Foken and Wichura, 1996).

25 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 ( $75^\circ$  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  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  fluxes was obtained from the control (48%) and the clover

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parcel (52%) during the observation period. The net effect in N<sub>2</sub>O emission differences represents a conservative estimate, as N<sub>2</sub>O 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<sub>2</sub>O fluxes was only 2.3% lower than the 30-min N<sub>2</sub>O 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).

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## 2.8 Comparison of N<sub>2</sub>O fluxes between parcels

We applied non-parametric bootstrapping in order to estimate the mean annual N<sub>2</sub>O 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):

$$\Delta F / F = \frac{F_{\text{Clover}} - F_{\text{Control}}}{F_{\text{Control}}} \quad (2)$$

$F_{\text{Clover}}$  and  $F_{\text{Control}}$  are daily average fluxes from the clover and the control parcels, respectively. Before being able to identify differences in N<sub>2</sub>O 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<sub>2</sub> equivalents (CO<sub>2</sub>-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<sub>2</sub>O 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 (9<sup>th</sup> August 2015 and 16<sup>th</sup> July 2016), the event was classified as fertilization event but not as rain event. Grazing overlapped with a rain event on 15<sup>th</sup> June 2015 and 1<sup>st</sup> July 2015, thus these days were excluded from the rain event analysis. A pre-analysis was conducted for all these events, comparing N<sub>2</sub>O 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<sub>2</sub>O fluxes, we used semi-parametric generalized additive modelling (Wood, 2006). We expected non-linear effects of some predictor variables on N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O exchange, we did not include grazing in the GAM analysis. The full set of predictors included soil temperature, soil water content, oxygen concentration, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>+</sup> and DOC concentration for substrate availability, net ecosystem exchange (NEE) of CO<sub>2</sub> 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,  $\text{edf} = 1$ ). 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  $\log\text{-N}_2\text{O}$  emissions (reverse U-shape), as estimated by the GAM to require more degrees of freedom ( $\text{edf} > 1$ ). 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  $\text{N}_2\text{O}$  fluxes were analysed separately, but no significant effects of the same set of predictors on  $\text{N}_2\text{O}$  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 ( $\text{se}_{\text{adj}}$ ;  $\text{p}_{\text{adj}}$ ). 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.

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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  $T_{\text{air}}$  exceeding 5 °C for at least five subsequent days, started on 17<sup>th</sup> March 2015 and 30<sup>th</sup> January 2016. Starting dates of net  $\text{CO}_2$  uptake for at least ten subsequent days, an alternative indicator for start of the growing season, were 27<sup>th</sup> February 2015 and 8<sup>th</sup> March 2016, similar to previous years.

3.2 Soil microclimate

An important precondition for the  $\text{N}_2\text{O}$  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  $\pm 1^\circ\text{C}$ . 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 ( $33 \pm 4\%$ ) and the clover parcel ( $31 \pm 5\%$ ). The difference between treatments was within the sensor accuracy of  $\pm 3\%$  (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  $\text{O}_2$  concentration were not significantly different in both parcels (measured difference  $0.3 \pm 0.2\%$  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 ( $\text{NH}_4^+$ ) concentration in the soil peaked on each day of slurry application in the control parcel and declined during the following few days (Fig. 4a).  $\text{NH}_4^+$ -N concentration measured in the topsoil ranged between 0.4 and 19.2 mg  $\text{NH}_4^+$ -N  $\text{kg}^{-1}$  dry soil in the control parcel during the two years of observations. Significantly lower  $\text{NH}_4^+$ -N concentration was measured in the clover parcel ( $0.6\text{--}11.1$  mg  $\text{NH}_4^+$ -N  $\text{kg}^{-1}$  dry soil; paired Wilcoxon-test,  $p < 0.01$ ). While  $\text{NH}_4^+$ -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 ( $\text{NO}_3^-$ ) concentration ranged between 1.7 and 27.7 mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$  dry soil in the control parcel (Fig. 4b). Similar to the observations found for  $\text{NH}_4^+$ -N, significantly lower soil nitrate levels ( $0.6\text{--}18.9$  mg  $\text{NO}_3^-$ -N  $\text{kg}^{-1}$  dry soil) were found in the clover parcel (paired Wilcoxon-test,  $p < 0.01$ ).  $\text{NO}_3^-$ -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  $\text{kg}^{-1}$  dry soil in the control parcel (Fig. 4c). Again, significantly lower values were measured for DOC in the clover parcel ( $0.6\text{--}160$  mg C  $\text{kg}^{-1}$  dry soil) (paired Wilcoxon-test,  $p < 0.01$ ) compared to the control. As observed for  $\text{NO}_3^-$ -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  $\pm$  SE) of the control parcel were  $12.8 \pm 0.5$  t dry matter (DM)  $\text{ha}^{-1}$  in 2015 and  $11.9 \pm 0.4$  t DM  $\text{ha}^{-1}$  in 2016, while yields of the clover parcel were  $10.4 \pm 0.7$  t DM  $\text{ha}^{-1}$  and  $11.0 \pm 0.5$  t DM  $\text{ha}^{-1}$  in 2015 and 2016, respectively (Table 2). Previous years' yields of both parcels were  $9.3 \pm 3.2$  t DM  $\text{ha}^{-1} \text{yr}^{-1}$  in the control and  $6.6 \pm 2.3$  t  $\text{ha}^{-1} \text{yr}^{-1}$  in the

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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  $-2.7 \text{ t ha}^{-1} \text{ yr}^{-1}$ ; experiment difference 2015/16  $-2.4$  and  $-0.9 \text{ t ha}^{-1}$ , 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  $1.0 \pm 0.3 \text{ t DM ha}^{-1}$  on the control parcel and  $0.8 \pm 0.4 \text{ t DM ha}^{-1}$  on the clover parcel (measured on 21<sup>st</sup> 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 ( $3.3 \pm 0.2\%$ ) compared to non-legumes ( $2.1 \pm 0.2\%$ ) (Fig. 5f). C/N ratios (data not shown) of total annual yields were slightly higher in the control ( $19.2 \pm 1.7$  and  $19.8 \pm 2.8$ ) than in the clover parcel ( $17.1 \pm 1.0$  and  $16.7 \pm 2.1$ ) 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 ( $5.8 \pm 0.2 \text{ t ha}^{-1}$ ) compared to the clover parcel ( $4.7 \pm 0.3 \text{ t ha}^{-1}$ ) in 2015, while C in biomass was similar for the control parcel ( $5.1 \pm 0.3 \text{ t ha}^{-1}$ ) and the clover parcel ( $4.8 \pm 0.2 \text{ t ha}^{-1} \text{ yr}^{-1}$ ) in 2016 (Table 2). N exported was similar across parcels in the second year (control:  $238 \pm 13 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ; clover:  $262 \pm 8 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ; Table 2). Biological nitrogen fixation *via* rhizobia associated with clover (N derived from the atmosphere –  $N_{\text{dfa}}$ ) resulted in BFN in harvested biomass of  $55.6 \pm 5.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  and  $14.2 \pm 1.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in the control parcel and  $71.6 \pm 5.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  and  $130 \pm 8.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in the clover parcel during the first and the second year of the experiment, respectively (Table 2, Fig. 5h).

### 3.5 Differences in N<sub>2</sub>O exchange between control and clover parcel

Average N<sub>2</sub>O fluxes (with 95% confidence interval CI from the bootstrapping given in parentheses) in the control parcel in 2015 were  $4.1 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$  (CI 3.8–4.2  $\text{kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ ) and  $1.9 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$  (CI 1.8–2.0  $\text{kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ ) in the clover parcel. In 2016, average N<sub>2</sub>O fluxes were higher for both parcels (6.3  $\text{kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ , CI 6.0–6.5  $\text{kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$  in the control and 3.8  $\text{kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ , CI 3.7–3.9  $\text{kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$  in the clover parcel) (Fig. 6a). Annual N<sub>2</sub>O 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<sub>2</sub>O fluxes in the control parcel were

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4.7 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> (4.6–4.8 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) and in the clover parcel 4.8 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> (4.6–4.9 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) and did thus not differ significantly. N<sub>2</sub>O emission intensities (yield-scaled N<sub>2</sub>O emissions) during the experiment were 0.31 g N<sub>2</sub>O-N kg<sup>-1</sup> DM in the control parcel and thus higher than the 0.18 g N<sub>2</sub>O-N kg<sup>-1</sup> DM observed in the clover parcel in 2015. A similar pattern was observed in 2016, with N<sub>2</sub>O emission intensities of 0.53 g N<sub>2</sub>O-N kg<sup>-1</sup> DM versus 0.37 g N<sub>2</sub>O-N kg<sup>-1</sup> DM in 2016 for control and clover parcel, respectively.

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### 3.6 Effects of management activities on N<sub>2</sub>O exchange

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

### 3.7 Influence of potential drivers on N<sub>2</sub>O exchange

Nitrous oxide emissions significantly increased after fertilizer application (Ctr-F compared to Ctr-0,  $p < 0.05$ ) when compared to N<sub>2</sub>O fluxes during periods of no management on the same (control) parcel (Fig. 8a, Table 4). The effect size showed 2.5-fold N<sub>2</sub>O 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<sub>2</sub>O fluxes jointly with other measured driver variables, such as soil moisture, soil temperature, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N and DOC concentration in the soil. After mowing no significant increase in N<sub>2</sub>O emissions was found for the optimized model in either of the parcels (Table 4b). In contrast a difference in N<sub>2</sub>O emissions after harvest was observed for the simple model on the control parcel (Table 4c). If the difference in sward composition itself affected N<sub>2</sub>O 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<sub>2</sub>O emissions.

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Soil microclimate affected N<sub>2</sub>O emissions in both parcels. Soil temperature significantly influenced N<sub>2</sub>O emissions ( $p < 0.05$ ), indicating a 7% ( $\pm 2\%$ ) increase in N<sub>2</sub>O 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<sub>2</sub>O flux as a single explanatory variable (data not shown). Besides soil temperature, volumetric soil water content showed a significant non-linear effect on N<sub>2</sub>O emissions ( $p <$

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0.05, Fig. 8c). The humpback-shaped functional relationship between volumetric soil water content and log-transformed N<sub>2</sub>O emissions (Fig. 8c) shows an increase until 34% and a decrease above 36% volumetric soil water content. Similarly, oxygen concentration significantly affected N<sub>2</sub>O emissions ( $p < 0.05$ , Fig. 8d). Oxygen concentration was non-linearly related to N<sub>2</sub>O emissions, showing lowest N<sub>2</sub>O emissions ( $10^{-4} \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 0% oxygen concentration. N<sub>2</sub>O 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<sub>2</sub>, which was used here as a proxy for plant activity, affected N<sub>2</sub>O emissions ( $p < 0.05$ , Fig. 8e) with a 4% ( $\pm 2\%$ ) decrease of N<sub>2</sub>O emissions per  $\mu\text{mol m}^{-2} \text{s}^{-1}$  net carbon dioxide uptake. Inclusion of NH<sub>4</sub><sup>+</sup>-N concentration improved the prediction of N<sub>2</sub>O emissions (Table 4, Fig. 8f), leading to an emission increase of 5% ( $\pm 3\%$ ) per  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Note that large NH<sub>4</sub><sup>+</sup>-N concentrations only occurred after fertilization, thus the NH<sub>4</sub><sup>+</sup>-N effect was mainly influenced by these dates, while it did not play a role for the other management categories. In contrast, NO<sub>3</sub><sup>-</sup>-N concentration did not improve the prediction of N<sub>2</sub>O emissions (Table 4, Fig. 8g). Also, DOC concentrations showed no effect on N<sub>2</sub>O emissions (Table 4, Fig. 8h). The slopes of the relationship between drivers and predicted N<sub>2</sub>O emission are flatter than expected from visual inspection of the observed values (Fig. 8), as the predictions here depict the dependency of N<sub>2</sub>O 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<sub>2</sub>, NH<sub>4</sub><sup>+</sup>-N, NEE of CO<sub>2</sub>) besides soil temperature and soil water content increased the explained variance in N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions between both parcels can be attributed to the absence of fertilization on the clover parcel. Increased clover proportion could still have increased N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions (i.e. plant residues and root exudates affected N<sub>2</sub>O emissions similarly on the clover and the control parcel). We quantified the effects of environmental drivers on N<sub>2</sub>O emissions and identified soil temperature, soil oxygen

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concentration, soil water content and NEE of CO<sub>2</sub> as main environmental drivers of N<sub>2</sub>O emissions. The assessment of the mitigation strategy revealed reductions in N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions in the fertilized grassland parcel

N<sub>2</sub>O emissions in the control parcel summed up to 4.1 and 6.3 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> for the two years, respectively, corresponding to 1.4 and 3.5% of the applied fertilizer N. Annual N<sub>2</sub>O 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<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> based on manual N<sub>2</sub>O measurements using static GHG chambers. Similar N<sub>2</sub>O emissions of 4.5 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> (0.3–18.2 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) 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<sub>2</sub>O emissions (0.19–5.28 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> 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<sub>2</sub>O emissions as well as emissions as a percentage of fertilizer N input (2.55–7.89 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions in the unfertilized clover parcel

N<sub>2</sub>O emissions in the clover parcel during our two-year observation period summed up to 1.9 and 3.8 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> in 2015 and 2016, respectively. These N<sub>2</sub>O emissions were clearly lower than the values observed in the control parcel during

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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<sub>2</sub>O emissions on the clover parcel as in 2015.

However, N<sub>2</sub>O 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<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> (0.10–1.30 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) were reported by Jensen et al. (2012) based on eight site-years. Further non-fertilized grass-clover mixtures showed annual N<sub>2</sub>O emissions of up to 2.5 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> (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<sub>2</sub>O emissions in relation to N<sub>2</sub>O emissions observed from sites under long-term extensive management. In addition, high total N deposition (NH<sub>3</sub>, NO<sub>x</sub>, HNO<sub>3</sub>, NO<sub>3</sub><sup>-</sup>) in the study area (in total 33.8 kg N ha<sup>-1</sup> yr<sup>-1</sup> in 2015; Rihm and Achermann, 2016) might foster background N<sub>2</sub>O emissions due to increased NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> availability (Butterbach-Bahl et al., 2013). Additionally, NH<sub>3</sub> deposition on the clover parcel originating from NH<sub>3</sub> emissions from the adjacent control parcel is likely to be the cause of increased soil NH<sub>4</sub><sup>+</sup> concentrations after the event on 17<sup>th</sup> August 2016. Furthermore, a possible explanation for the relatively high N<sub>2</sub>O emissions from our clover parcel in 2016 were the meteorological conditions which were wetter during summer and therefore more favourable for N<sub>2</sub>O production than during 2015. High background N<sub>2</sub>O emissions in the clover parcel in 2016 were reflected by similarly high background N<sub>2</sub>O 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<sub>2</sub>O emissions

Our aim was to identify the main drivers of N<sub>2</sub>O emissions and therefore we investigated the effects of management (fertilization, harvest, grazing, over-sowing leading to increased clover proportion) and environmental variables on N<sub>2</sub>O emissions. Fertilization of the control parcel had the largest effect on N<sub>2</sub>O emissions. Increased N availability due to fertilization is widely known as a main driver of N<sub>2</sub>O emissions, which makes it a key factor for mitigating N<sub>2</sub>O emissions (Bouwman et al., 2002; Smith et al., 1997). Nevertheless, effects of fertilization on N<sub>2</sub>O 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<sub>2</sub>O emissions and highlighting the need to take additional drivers into account. We further observed increased N<sub>2</sub>O emissions following harvest events on the control parcel, which may be explained as a consequence of increased rhizodeposition (Bolan et al., 2004; Butenschoten et al., 2008). Subsequently, greater availability of labile C compounds can lead to increased microbial activity, accompanied with increased production of N<sub>2</sub>O (Rudaz et al., 1999). Higher N<sub>2</sub>O fluxes following cutting were similarly observed on a pasture in Central France (up to 3.7 mg N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup> in Klumpp et al., 2011; up to 7.0 mg N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup> in this study). Grazing had only a minor influence on the overall N<sub>2</sub>O budget of the Chamau site with 3.71% of N<sub>2</sub>O-N emitted during grazing periods and data analysis

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showed that N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions, as shown by comparing N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O emissions is negligible (Rochette and Janzen, 2005). Previous results from a laboratory incubation by Carter and Ambus (2006), who investigated N<sub>2</sub>O emissions from unfertilized soils for up to 36 weeks, showed that recently fixed N<sub>2</sub> in a white clover-ryegrass mixture contributed as little as 2.1 ± 0.5% to total N<sub>2</sub>O emissions. In agreement with our result, measurements from permanent grasslands in Ireland, where winter freeze-thaw cycles are very rare, showed that annual N<sub>2</sub>O emissions in unfertilized ryegrass (2.38 ± 0.12 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) were not significantly different from an unfertilized grass-clover sward (2.45 ± 0.85 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) with clover proportions of 20–25%, hence providing evidence that N<sub>2</sub>O 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<sub>2</sub>O emissions.

The effects of temperature and soil water content on N<sub>2</sub>O 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<sub>2</sub>O emissions (Table 4). Our data showed that larger plant C uptake (negative NEE) of CO<sub>2</sub> as proxy for plant activity was associated with reduced N<sub>2</sub>O 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<sub>2</sub>O emissions at higher levels of photosynthesis. Our analysis showed that inclusion of NH<sub>4</sub><sup>+</sup>-N concentration in the statistical analysis improved the prediction of N<sub>2</sub>O emissions, while NO<sub>3</sub><sup>-</sup>-N and DOC were of less importance for the prediction of N<sub>2</sub>O emissions. Comparable results for the influence of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were found at an Irish grassland (Rafique et al., 2012). In summary, fertilization was the dominant predictor of N<sub>2</sub>O emissions, while soil temperature, soil water content, soil oxygen concentration and NEE of CO<sub>2</sub> were significant environmental drivers. Concluding from all management effects, the decrease in annual N<sub>2</sub>O emissions under the mitigation strategy was primarily

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	caused by the absence of fertilization, while a potential effect of the increase in clover proportion and increased <b>BFN</b> offsetting these emission reductions was absent.	Deleted: BNF
	<b>4.4 Effect of the mitigation strategy on productivity and biological nitrogen fixation</b>	
5	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 <b>BFN</b> 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 <sup>-1</sup> yr <sup>-1</sup> and 50 to 70% clover were as productive as grass monocultures fertilized with 450 kg N ha <sup>-1</sup> yr <sup>-1</sup> . 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.	Deleted: BNF Field Code Changed
10	Biologically fixed nitrogen found in shoot biomass was slightly higher in the clover parcel (72 kg N ha <sup>-1</sup> yr <sup>-1</sup> ) compared to the control parcel (55 kg N ha <sup>-1</sup> yr <sup>-1</sup> ) in 2015 due to only small differences in clover proportion between both parcels. During the second year, the over-sowing was more effective and <b>BFN</b> found in shoot biomass in the clover parcel summed up to 130 kg N ha <sup>-1</sup> yr <sup>-1</sup> while only 14 kg N ha <sup>-1</sup> yr <sup>-1</sup> were measured in the control parcel. Previous studies reported similar amounts of <b>BFN</b> for mown and grazed pasture systems (Ledgard and Steele, 1992; Nyfeler et al., 2011), with maxima being as high as 323 kg N ha <sup>-1</sup> yr <sup>-1</sup> 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 <b>species’ developmental cycles, but also competitive advantages/disadvantages of the respective species.</b> Drier conditions, <b>observed for instance in summer (JJA)</b> , result in competitive advantages of the clover compared to grasses, as N <sub>2</sub> 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).	Deleted: - Field Code Changed Field Code Changed
20	Lower SON content (3490 kg N ha <sup>-1</sup> ) in a grass-clover mixture compared to a 200 kg ha <sup>-1</sup> yr <sup>-1</sup> fertilized grassland (4350 kg N ha <sup>-1</sup> ) 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	Field Code Changed
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30		Deleted: drier conditions o Deleted: bserved for instance in summer (JJA). Field Code Changed Field Code Changed Deleted: yr <sup>-1</sup> Deleted: yr <sup>-1</sup> Field Code Changed



in soil organic C (SOC) (Ammann et al., 2009; Conant et al., 2005) and can therefore compromise the soil's CO<sub>2</sub> sink strength. Thus, detailed investigations on the effect of the clover treatment on SON, SOC content and CO<sub>2</sub> exchange are recommended to comprehensively evaluate the mitigation strategy in the long term.

#### 4.5 Effect of the mitigation strategy on N<sub>2</sub>O emissions and emission intensities

5 We found that the mitigation strategy effectively reduced both N<sub>2</sub>O emissions by 54% (51–57%) and 39% (36–42%) in 2015 and 2016 as well as N<sub>2</sub>O emission intensities by 41% and 30% in 2015 and 2016, respectively. Past studies carried out in temperate grasslands consistently found reductions in N<sub>2</sub>O emissions when ~~reducing~~ fertilizer ~~and increasing~~ BFN through legumes (Table 5). The magnitude of relative N<sub>2</sub>O emission reductions ranged from 34% (Šimek et al., 2004) to 100% (Ammann et al., 2009), with absolute N<sub>2</sub>O emission reductions of 0.8 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Šimek et al., 2004) to 11.1 kg N ha<sup>-1</sup> yr<sup>-1</sup> (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<sub>2</sub>O emissions from an unfertilized grass-clover mixture (92% increase) compared to N<sub>2</sub>O emissions from a grass sward fertilized with 220 kg N ha<sup>-1</sup> yr<sup>-1</sup> 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<sub>2</sub>O fluxes and thus may not be the best mitigation strategy for croplands and temporary grasslands, where ploughing is done much more frequently.~~

15 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).

#### Acknowledgements

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Deleted: (Basche et al., 2014; Lugato et al., 2018) Nevertheless, contrasting effects to our observations were observed under boreal climate conditions in eastern Finland, with much higher N<sub>2</sub>O emissions (92% higher) from an unfertilized grass-clover mixture compared to N<sub>2</sub>O emissions in a fertilized grass sward (220 kg N ha<sup>-1</sup> yr<sup>-1</sup>) due to large springtime emissions (Virkajärvi et al., 2010) indicating that the mitigation strategy is likely to be inappropriate for sites with seasonally frozen soils. Similarly, the mitigation strategy may have adverse effects in cropland, in contrast to our observations in permanent grassland, as legume cover crops were shown to increase N<sub>2</sub>O emissions following their incorporation into the soil (Basche et al., 2014; Lugato et al., 2018). Due to this effect, temporary grasslands may not reproduce the findings from permanent grassland.

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experimental work and oxygen sensor development. Further thanks go to our colleagues Charlotte Decock and Elisabeth Verhoeven from the Sustainable Agroecosystems research group at ETH for laboratory introductions and valuable advice at the beginning of the experiment. [We thank Natascha Kljun for useful discussions on the footprint parameterization and Beat Rihm for providing N deposition estimates for the study site.](#) Valuable practical support in biomass and soil sampling as well as processing during the experiment was given by the interns Astrid Riemer and Manjunatha Chandregowda and the student assistant Reto Zihlmann.

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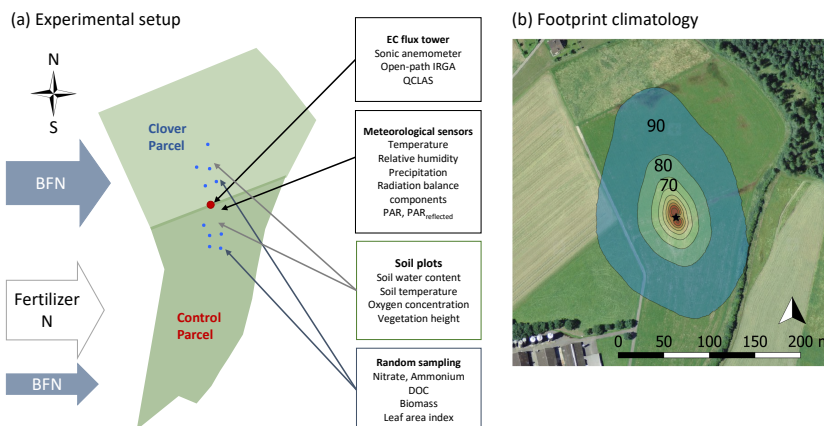
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**Figure 1.** (a) Experimental setup and measured variables at the experimental research site Chamau (CH-Cha). The clover parcel (north) is managed to increase nitrogen inputs from the atmosphere via increased biologically fixed nitrogen (BFN). This 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 Klijn et al. (2015) footprint model (source for background picture: [Swisstopo \(https://map.geo.admin.ch/\)](https://map.geo.admin.ch/)). The prevailing wind direction was from the north.

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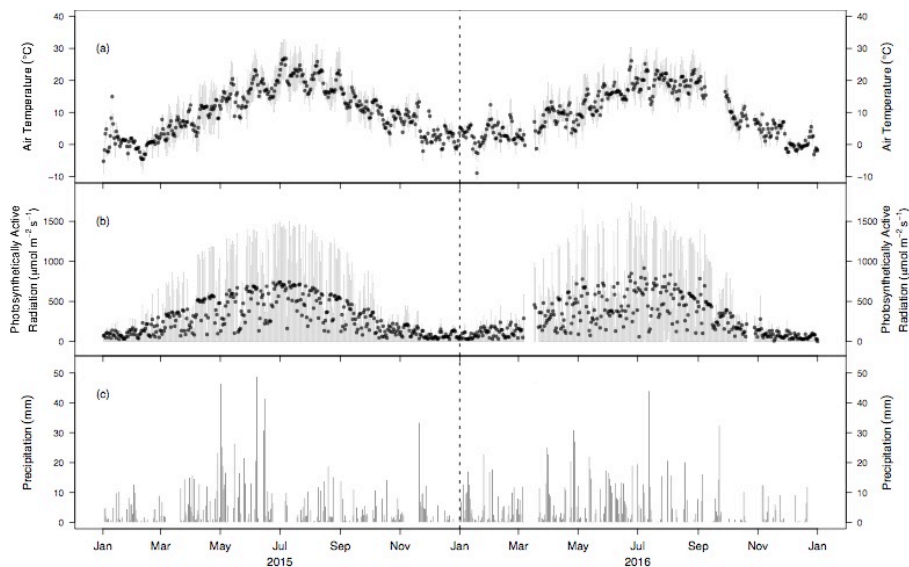
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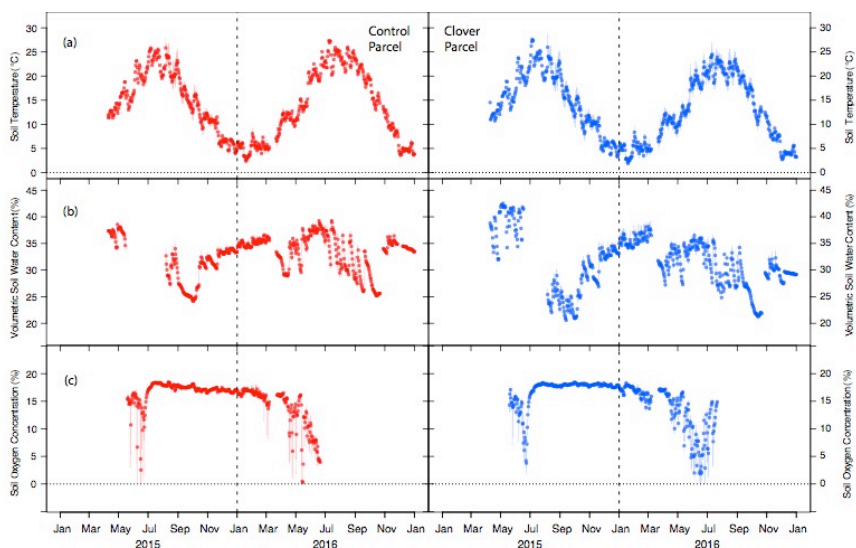
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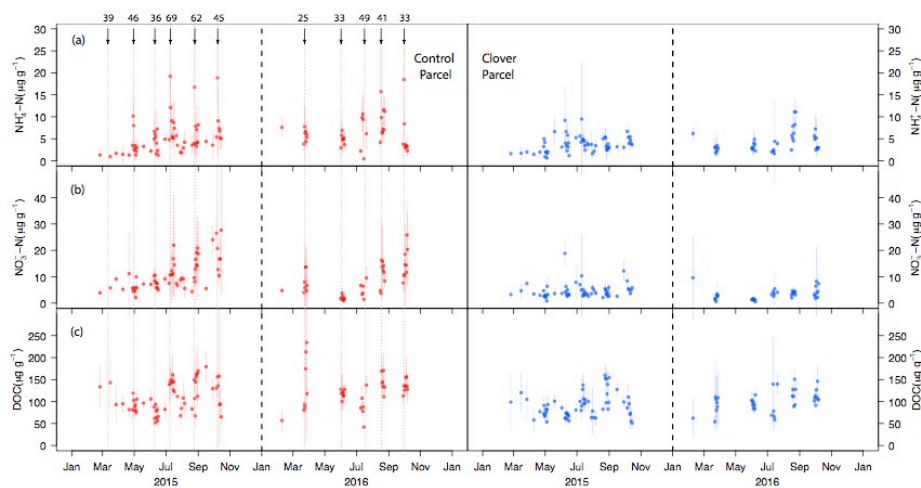


**Figure 2.** Meteorological conditions during 2015 and 2016. (a) Average daily air temperature (2 m). (b) average daily photosynthetically active radiation (2 m). The grey bars indicate the sub-daily variability (quartiles based on 10 min values). (c) Daily precipitation sums during 2015 and 2016 (1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

**Deleted:** Figure 2. (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 biological nitrogen fixation (BNF). 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. (b) Footprint climatology of the year 2016 with footprint contour lines of 10% to 90% in 10% steps using the Kljun et al. (2004) footprint model.



**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).

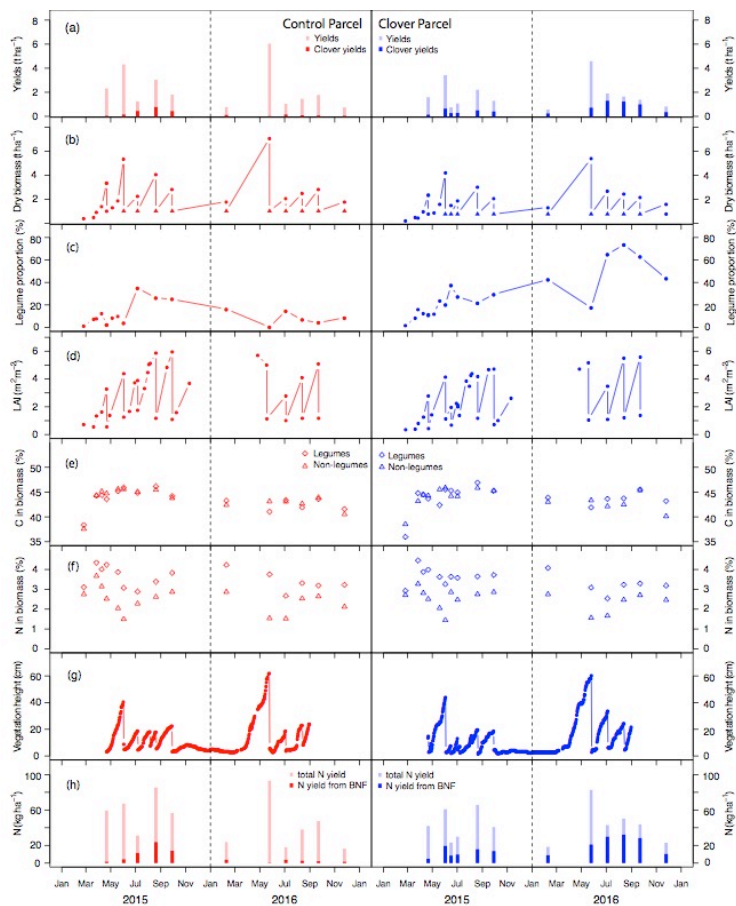


**Figure 4.** (a) Ammonium-N concentration, (b) nitrate-N concentration, (c) dissolved organic carbon concentration per unit of dry soil at the control (left, red) and clover parcel (right, blue) during 2015 and 2016. Black arrows indicate slurry applications, which only took place in the control parcel.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

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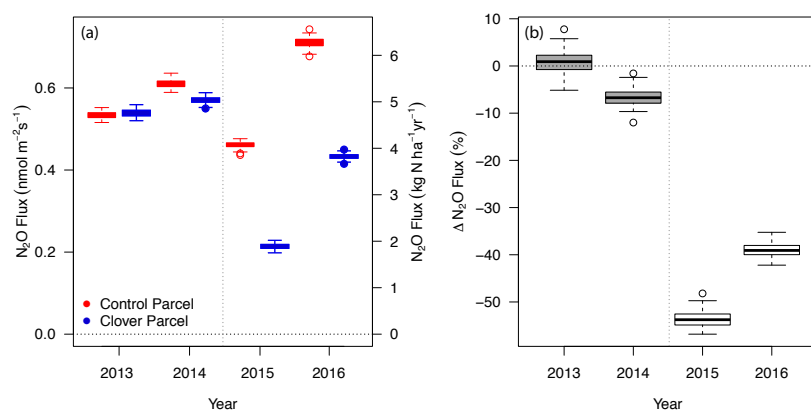
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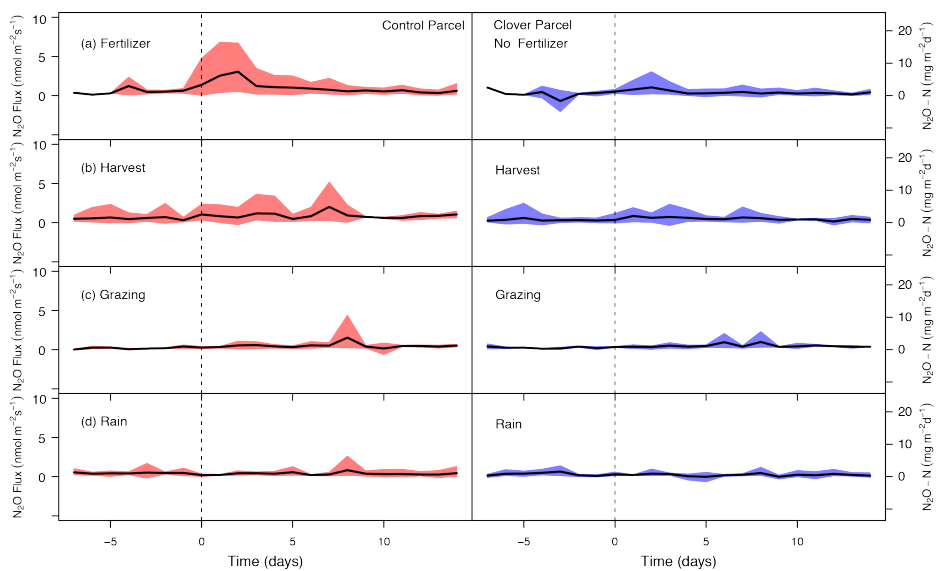
**Figure 5.** (a) Yields and intake by grazing at the control (left, red) and clover parcel (right, blue), (b) total aboveground biomass. Circles represent the total biomass (legumes and non-legumes), filled triangles are displaying the remaining biomass after harvest (stubble), which was measured once (sampling date 21<sup>st</sup> 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 **BNF** in the removed biomass (saturated).

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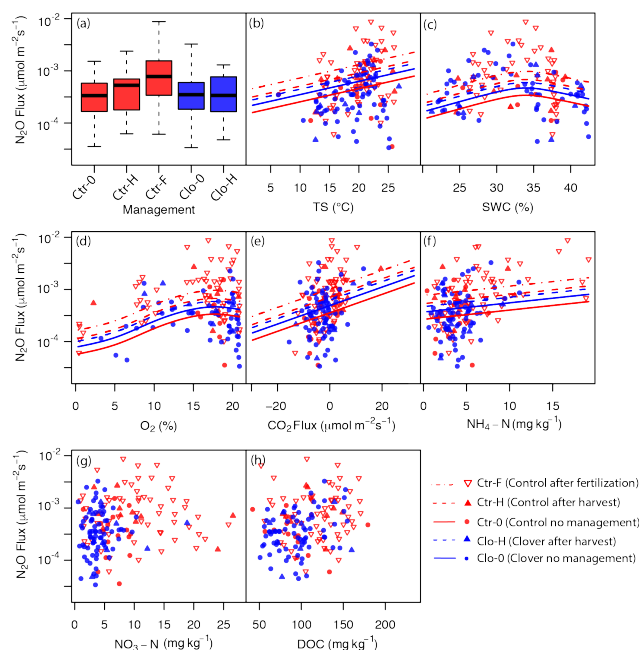
**Figure 6.** (a) Annual N<sub>2</sub>O exchange at control (red) and clover parcels (blue) for the reference years 2013–2014 and the experimental years 2015–2016. (b) Relative differences between N<sub>2</sub>O exchange in the control and clover parcels for the reference years (grey) and the experimental years (white). Boxes indicate the inter-quartile range based on nonparametric bootstrapping; bold black lines within boxes indicate the medians.

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**Figure 7.**  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  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 ( $\text{O}_2$ , 0.1 m depth), (e) carbon dioxide ( $\text{CO}_2$ ) flux and, while not significant (f) ammonium-N concentration ( $\text{NH}_4\text{-N}$ , 0–0.2 m depth) still improved the model (lowered the AIC). No significant influence was found for (g) nitrate-N concentration ( $\text{NO}_3\text{-N}$ , 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.

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**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. TED1; 20 kg seeds ha<sup>-1</sup>; ½ 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	Day	Organic matter	pH	total N	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	C/N	P	K <sub>2</sub> O	Ca	Mg	S	Total DM	
						Unit ha <sup>-1</sup>		(%)		g kg <sup>-1</sup> DM	g kg <sup>-1</sup> DM	g kg <sup>-1</sup> DM		g kg <sup>-1</sup> DM	g kg <sup>-1</sup> DM	g kg <sup>-1</sup> DM	g kg <sup>-1</sup> DM	g kg <sup>-1</sup> DM	g kg <sup>-1</sup> DM	
2015	Clover	2015-04-21	2015-06-22	Overwintering pasture	Grass + sludge	200	15.04	67.6	79.2	7.8	82.3	40.3	0.3	4.8	11.0	25.7	58.9	67.2	35.4	6.6
		2015-04-21	2015-06-22	Mowing	Grass + sludge	44.4	15.06	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
		2015-06-02	2015-06-03	Moving	Grass + sludge	28.1	15.06	66.6	386.2	7.5	69.4	47.8	<0.001	5.6	11.2	25.7	85.1	102.2	29.3	9.0
		2015-06-15	2015-06-19	Grazing	Sheep	31.1	15.06	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
		2015-07-01	2015-07-06	Drainage	Grass + sludge	22.2	15.07	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
		2015-08-20	2015-08-20	Moving	Grass + sludge	25.0	15.08	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
		2015-09-28	2015-09-28	Moving	Grass + sludge	27.5	15.09	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
		2015-10-08	2015-10-08	Moving	Grass + sludge	40.9	15.10	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
		2015-10-08	2015-10-08	Moving	Grass + sludge	28.2	15.10	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
		2015-10-08	2015-10-08	Moving	Grass + sludge	28.2	15.10	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
		2015-10-08	2015-10-08	Moving	Grass + sludge	28.2	15.10	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
		2015-10-08	2015-10-08	Moving	Grass + sludge	28.2	15.10	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
		2015-10-08	2015-10-08	Moving	Grass + sludge	28.2	15.10	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
		2015-10-08	2015-10-08	Moving	Grass + sludge	28.2	15.10	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
2016	Clover	2016-04-06	2016-06-27	Overwintering pasture	Grass + sludge	20.0	16.04	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
		2016-04-06	2016-06-27	Overwintering pasture	Grass + sludge	20.0	16.04	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
		2016-04-06	2016-06-27	Overwintering pasture	Grass + sludge	20.0	16.04	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
		2016-04-06	2016-06-27	Overwintering pasture	Grass + sludge	20.0	16.04	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
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**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  $\delta^{15}\text{N}$  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 <sup>-1</sup> )	Total	12.8 (± 0.5) <sup>a</sup>	10.4 (± 0.7) <sup>b</sup>	11.9 (± 0.4) <sup>ab</sup>	11.0 (± 0.5) <sup>ab</sup>
Biomass export (DM kg ha <sup>-1</sup> )	Legumes	1860 (± 176) <sup>a</sup>	2240 (± 141) <sup>b</sup>	503 (± 80) <sup>ab</sup>	4840 (± 355) <sup>ab</sup>
	Non-Legumes	11000 (± 541) <sup>a</sup>	8170 (± 666) <sup>b</sup>	11400 (± 462) <sup>a</sup>	6150 (± 493) <sup>b</sup>
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)
$\delta^{15}\text{N}$ (‰)	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 <sup>-1</sup> )	Total	5780 (± 222) <sup>a</sup>	4720 (± 289) <sup>b</sup>	5120 (± 221) <sup>ab</sup>	4760 (± 228) <sup>b</sup>
	Legumes	843 (± 78) <sup>a</sup>	1020 (± 70) <sup>a</sup>	216 (± 24) <sup>a</sup>	2120 (± 123) <sup>b</sup>
	Non-Legumes	4940 (± 235) <sup>a</sup>	3700 (± 295) <sup>b</sup>	4900 (± 220) <sup>a</sup>	2640 (± 275) <sup>b</sup>
N (kg ha <sup>-1</sup> )	Total	301 (± 10) <sup>a</sup>	264 (± 13) <sup>b</sup>	238 (± 13) <sup>ab</sup>	262 (± 8) <sup>b</sup>
	Legumes	63 (± 6) <sup>a</sup>	80 (± 5) <sup>a</sup>	17 (± 2) <sup>b</sup>	149 (± 9) <sup>b</sup>
	Non-Legumes	238 (± 9) <sup>a</sup>	184 (± 13) <sup>a</sup>	221 (± 11) <sup>a</sup>	113 (± 9) <sup>a</sup>
BFN (kg ha <sup>-1</sup> )	Legumes	55 (± 5) <sup>a</sup>	72 (± 5) <sup>a</sup>	14 (± 2) <sup>b</sup>	130 (± 8) <sup>b</sup>

**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 <sub>2</sub> Flux	N <sub>2</sub> O Flux	CH <sub>4</sub> Flux	CO <sub>2</sub> Flux	N <sub>2</sub> O Flux	CH <sub>4</sub> 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 <sub>2</sub> Flux	N <sub>2</sub> O Flux	CH <sub>4</sub> Flux	CO <sub>2</sub> Flux	N <sub>2</sub> O Flux	CH <sub>4</sub> 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

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**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 <sub>2</sub> O 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-P)	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 <sub>2</sub> Flux (μmol m <sup>-2</sup> s <sup>-1</sup> )	0.02 (± 0.01)	0.018*	0.02 (± 0.01)	0.025*		
NH <sub>4</sub> -N (μg g <sup>-1</sup> )	0.02 (± 0.01)	0.167	0.02 (± 0.01)	0.074		
NO <sub>3</sub> -N (μg g <sup>-1</sup> )	-0.01 (± 0.01)	0.231				
DOC (μg g <sup>-1</sup> )	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 <sub>2</sub> concentration	2.81	0.0001***	2.72	0.0003***		
Observations	90		93		891	
Adjusted r <sup>2</sup>	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

Deleted: (Ctr-H)

Deleted: (Ctr-F)

Deleted: (Clo-0)

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**Table 5.** Summary of studies investigating N<sub>2</sub>O 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<sub>2</sub>O emissions, or if discontinuously sampled included a sensible strategy used by the authors in order to estimate annual fluxes.

Source	Treatment	N <sub>fert</sub> (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	Clover %	N <sub>2</sub> O (kg N <sub>2</sub> O-N ha <sup>-1</sup> yr <sup>-1</sup> )
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
Klump et al. 2012	low clover	157	19	1.72
Klump 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