Answers to the reviewers:

General answer to all four reviews:

We are pleased that our contribution generated four reviews, showing the relevance and importance of the topic. All reviews welcome our critical evaluation of the AIT (acetylene inhibition technique) to determine total denitrification losses, but also expressed severe reservations, judging that we have too positive attitude in favor of the AIT. The second major reservation common to all four reviews concerns our comparison of fluxes measured on soil samples in the laboratory to N_2O chamber flux measurements in the field. Nonetheless, three of the four reviewers share our conviction that the publication of our findings is important in the re-assessment of reported total denitrification values that are based on the AIT method.

As a reaction to the reviewers' justified concerns regarding a number of issues raised in our manuscript, we propose to focus on the limitation of AIT and change the title accordingly to: 'Theoretical and practical limitations of the acetylene inhibition technique to determine total denitrification losses'.

We perfectly agree that a discussion of the relationship between chamber N_2O data and laboratory measurements (without and with C_2H_2) is like comparing apples and oranges, because the necessary soil sampling and preparation for the AIT measurements in the laboratory will have strongly modified soil (sample) conditions before we performed the actual measurements. Still, we would like to recall the original context of this study, which was to contribute to the measurement of all major N fluxes at our grassland site as part of the NitroEurope project. To achieve this goal, measurements of other nitrogen fluxes also had to be upscaled to the field scale. The limitations of upscaling have become obvious, for example, when N_2O chamber flux values were compared to N_2O eddy covariance data (see e.g. Jones et., 2011).

In the case of N_2, our admittedly rather optimistic and implicit hypothesis regarding N_2 flux was to assume that the laboratory measurements on intact soil samples, without C_2H_2 treatment, should give comparable N_2O flux values to those derived from the automated chamber system in the field. If this had been confirmed, the AIT method could have been applied to samples with similar N_2O production and consumption characteristics and would potentially have provided numbers for the total denitrification losses. Clearly, for our grassland site at Oensingen, this hypothesis was not verified. Although our estimation of the range (6 to 26 kg N ha^{-1} yr^{-1}) of N_2 losses were commensurate as the N_2 fluxes necessary to close the N budget of the site (40 +/-30 kg N ha^{-1} yr^{-1}), they cannot reduce the large uncertainty in this budget because of the various limitations we discussed.

Thus the focus of the paper will be shifted from a quantitative objective (what is the N2 flux at our site?) to a more methodological perspective (why the limitations of the AIT technique preclude such quantitative assessments of actual field losses).

Below we indicate in a nutshell how we tackle the major revisions of the manuscript.

- focus on technical aspects of the AIT method
- explain better in the introduction why we have chosen the AIT method.
- we will restructure the presentation of our analysis dividing the observation phase into four different periods with characteristic denitrification activities (see Fig.1).
- we present additional information to underpin the minor contribution of nitrification to the N_2O fluxes at the Oensingen grassland site.
- in conclusion we will clearly express the fact that the AIT method is not suited to measure total denitrification from entire soil samples in heavy soils such as Oensingen.

In the revised manuscript we divide our measurements into four phases (Fig. 1), characterized by different levels of the chamber N$_2$O flux. The first phase July 21st to 15th
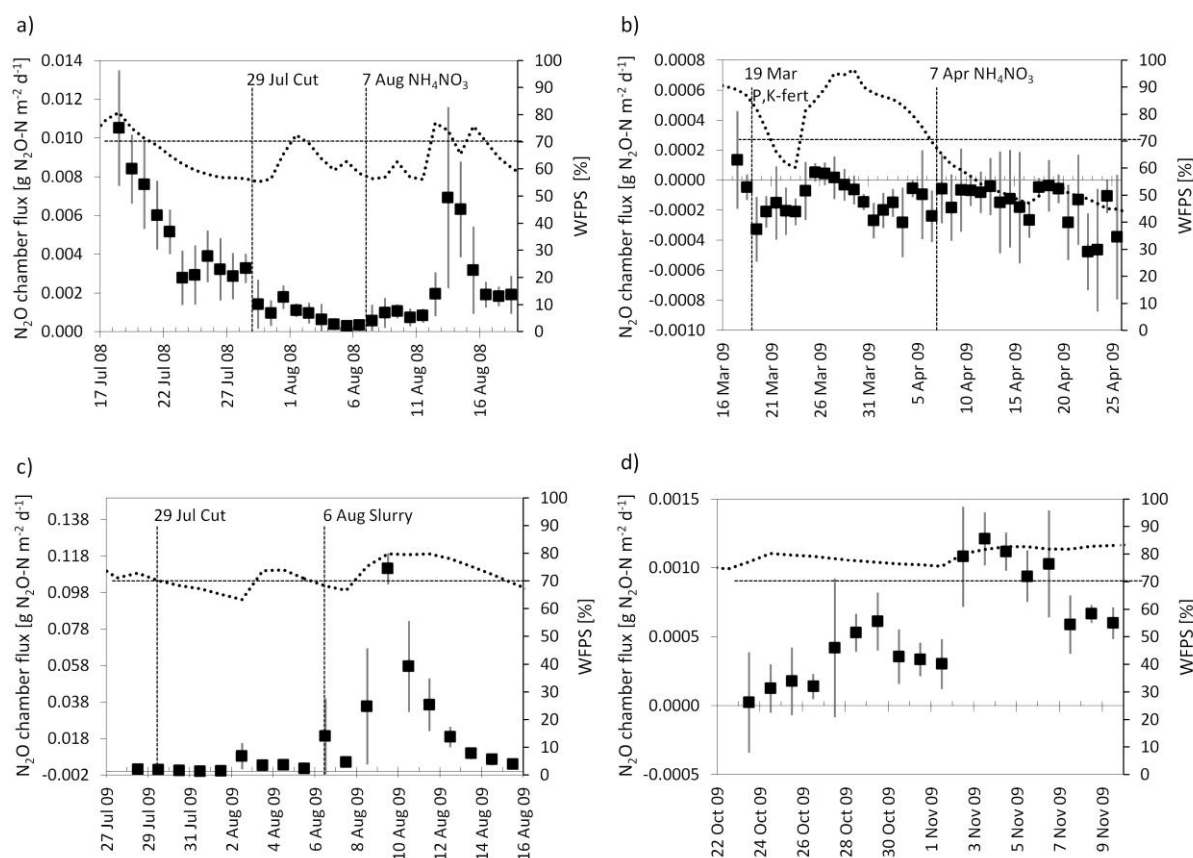
Fig. 1: Four typical measurement phases. a) moderate N$_2$O peak triggered by rain following application of mineral fertilizer. b) background N$_2$O exchange from wet to dry conditions, with indication of small uptake. c) double N$_2$O emission peak after slurry application (first peak nitrification, second peak denitrification). d) background N$_2$O fluxes above field capacity. The horizontal dashed line indicates field capacity (~70%). Note the different scales of the axis.

August 2008 shows a moderate N$_2$O peak triggered by rain after application of mineral fertilizer. The second phase March/April 2009 shows very minor fluxes with indication of a small uptake. The third phase covers a slurry event and shows a double N$_2$O peak (the first peak appeared right after the slurry application, the second one two days later after a rain event). The fourth phase October/November 2009 is again a background phase with very small N$_2$O fluxes. Laboratory determined N$_2$O fluxes without addition of C$_2$H$_2$ for the three phases with small to moderate fluxes show no correspondence at all with the chamber data. This demonstrates that taking the soils samples and their transfer into the measuring apparatus changed the N$_2$O production and consumption processes in a way that the flux recorded with the chambers were changed in an unsystematic way. Only for the time span with a very high N$_2$O production rate the laboratory C$_2$H$_2$ free samples showed a similar value to those measured in the field.

Below we discuss in detail the comments of the four reviewers. Many comments are overlapping. As the review from Reinhard Well (review 1) is the most detailed, we start with his and take the liberty to refer to these answers in our reply the other reviewers.

Answers to the review by Reinhard Well (Reviewer 1):

General comments:

We agree with the statement that there is a clear lack in studies comparing the AIT technique to other approaches. With our laboratory capabilities we were only able to carry out the described approach using the AIT technique. Admittedly a stable isotope approach might have been most informative, but this was not possible in the laboratory at our institute. The isotope method was too costly and we also had reservations on detection limits and how well the labeled fertilizer would be distributed through the soil column. In addition we had no easy way to obtain highly labeled slurry.

Comment: N_2O from nitrification was not taken into account.

We considered other pathways for N_2O production, but we unfortunately omitted to indicate the reasons why we believe the major part of N_2O emissions at our field site are from denitrification and why both nitrification and nitrifier-denitrification are only of minor significance. In the revised manuscript we will present the following arguments in more detail:

1) Large N_2O emission events mainly occur when WFPS exceeds field capacity (~70% WFPS), indicative of denitrification being the source process as Reinhard Well suggested. (Fig. 2)
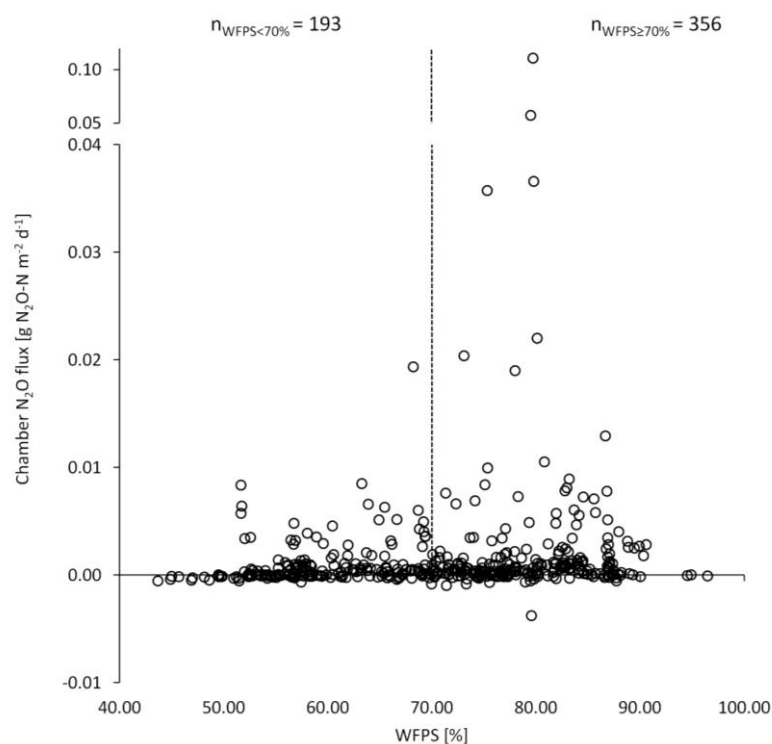
Fig. 2: N_2O fluxes measured in the field in relation to measured water-filled pore space (WFPS) at 5 cm depth.

2) We did not routinely measure ammonium and nitrate concentrations in the soil, but the available measurements in the Oensingen grassland soil generally show a ratio of nitrate to ammonia of 10 to 1 or larger. Only for a short period after application of reduced N fertilizer is this ratio temporarily reversed. On several occasion we traced very rapid nitrification in the field (indicated by a rapid decrease in soil NH_4^+ concentrations, though root uptake could also have contributed part of the NH_4^+ sink)

but we only observed small to moderate N_2O emissions. We will include such an event in the paper (Fig. 3) to illustrate this behavior. On August 6th 2009 slurry was applied bringing 32 kg ammonium-N ha^{-1} and 13 kg organic-N ha^{-1} on the field. Immediately after application soil samples were taken for the AIT measurements, as well as 1 and 4 days later. The N_2O chamber data showed a first N_2O emission peak with a short duration of less than one day, then four days later the start of a second, larger and longer-lasting peak after a rain event. As the laboratory measurements of the samples taken immediately after the slurry application showed a much higher N_2O fluxes without C_2H_2 than with C_2H_2 and the ammonium concentration in the top soil layer exceeded the nitrate concentration we interpret that the first peak in the chamber data has potentially an important contribution from nitrification and/or nitrifier-denitrification and that the second peak is due to denitrification. Note that the first peak contributed a very minor fraction to the cumulated N_2O emission over the 10 days after slurry application. On August 6th the laboratory N_2O fluxes without C_2H_2 were much higher than the values with C_2H_2 but were in the same range as the chamber measurements. This shows that nitrification and/or nitrifier-denitrification was inhibited by the addition of C_2H_2. For the next two sampling dates on August 10th and 12th nitrification should have no major role, as ammonium in the soil was nitrified.
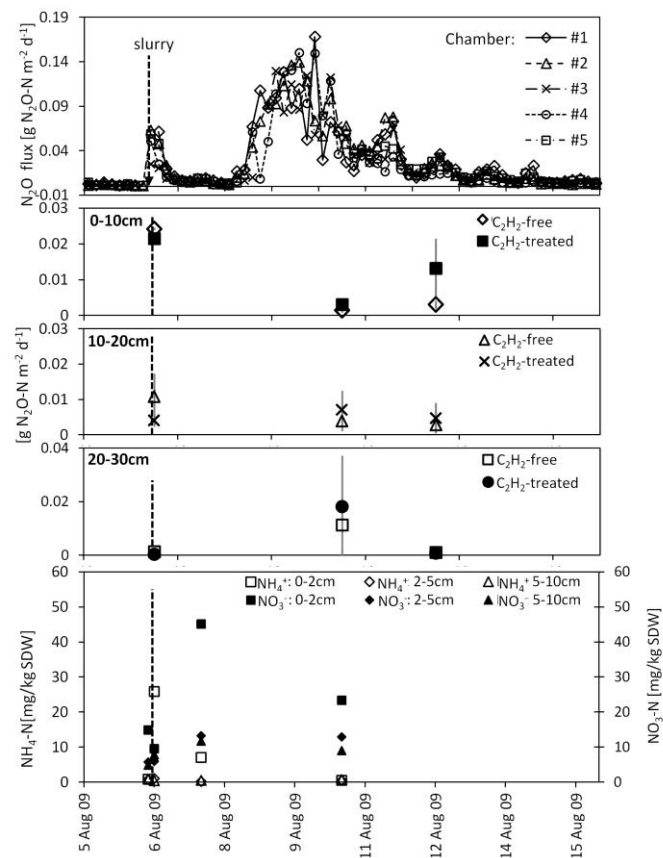
Fig. 3: Time series of N2O fluxes measured in the field (top), from samples measured by AIT from different soil depths, and ammonium and nitrate concentrations (bottom)

Reinhard Well indicated an older review by Becker et al. (1990) that discussed $N_2O/(N_2+N_2O)$ ratios derived by different approaches. In this review the AIT measurements showed systematically lower ratios compared to e.g. isotopic approaches. Unfortunately it is only an extended abstract in the gray literature, leaving a number of questions open.

Specific comments:

Title: We propose to change the title as indicated above.

Line 2: We had in mind a monitoring by an Eddy Covariance approach. We modify this sentence to "e.g. a monitoring based on an Eddy Covariance approach …"

Line 6 to 26: We will revise this sentence. In intensive agricultural systems less than 50% of the imported N in from of fertilizer, N fixation and atmospheric deposition is exported as harvest.

P2853 L3: .. or emitted as N_2O and N_2.

L15-23: we will add the additional biases mentioned by the referee.

P2854: lines 5-10. We will add the indicated reviews.

P2856 L1-4: The auger was used without a liner and consists of a small round cutter at the lower end and a U-shaped tube. After integrally inserting into the soil the auger was turned once before extraction and we assumed that compaction can be neglected. For transportation the soil core was cut into 10 cm sections with a knife and then carefully transferred into plastic bags. Clearly at low water content the soil core could not always be transported in a single piece. But, the lower end of the core (20-30cm) was always intact, hence the rest of the broken core was filled in the incubator above the intact part for the measurement. We will add this information on the sampling device to the manuscript.

P2857: Corrected.

L2858: After addition of C_2H_2 we did not observe a stable N_2O flux after a certain time, but an irregular pattern. In Table 1 both values without and with addition of C_2H_2 will be given in the revised version.

Table 1: Measurement results. Range and log-transformed means of N_2O fluxes from C_2H_2-free and C_2H_2-treated measurements and corresponding daily chamber N_2O fluxes. Highlighted in green: C_2H_2-treated fluxes are larger than C_2H_2-free fluxes.

Date	Detector	Unit	C$_2$H$_2$-free N$_2$O			C$_2$H$_2$-treated N$_2$O			Chamber N$_2$O	
			lower	mean*	upper	lower	mean*	upper	mean	sd
21.07.08	pIRS	mg N$_2$O-N m^{-2} d^{-1}	-0.37	-0.67	-1.23	0.95	1.06	1.18	6.0	1.8
29.07.08	pIRS		-0.01	-0.06	-0.32	0.49	0.72	1.04	1.4	1.3
05.08.08	pIRS		1.61	2.41	3.62	4.41	6.48	9.51	0.3	0.2
11.08.08	pIRS		0.01	0.03	0.17	0.49	0.81	1.33	0.8	0.4
14.08.08	pIRS		0.33	0.62	1.17	1.54	2.08	2.81	6.3	2.5
phase 1	**mean**		**0.31**	**0.47**	**0.68**	**1.58**	**2.23**	**3.17**	**2.98**	**3.33**
	median		**0.01**	**0.03**	**0.17**	**0.95**	**1.06**	**1.33**	**1.42**	
19.03.09	pIRS	mg N$_2$O-N m^{-2} d^{-1}	0.60	1.40	3.30	0.36	0.99	2.75	-0.3	0.2
07.04.09	pIRS		0.09	0.20	0.41	0.35	0.58	0.97	-0.1	0.2
17.04.09	pIRS		0.25	0.65	1.68	0.27	0.51	0.95	0.0	0.1
22.04.09	pIRS		0.15	0.53	1.83	0.11	0.19	0.35	-0.5	0.3
phase 2	**mean**		**0.27**	**0.69**	**1.81**	**0.27**	**0.57**	**1.26**	**-0.23**	**0.41**
	median		**0.20**	**0.59**	**1.76**	**0.31**	**0.54**	**0.96**	**-0.19**	
31.07.09	GC	mg N$_2$O-N m^{-2} d^{-1}	3.33	7.28	15.92	5.11	11.03	23.82	-0.009	0.6
03.08.09	GC		13.99	25.05	44.85	6.81	15.17	33.78	3.456	1.0
06.08.09	GC		41.05	67.51	111.03	14.26	21.69	32.99	19.370	20.8
10.08.09	GC		9.31	13.41	19.33	11.28	18.20	29.36	57.593	25.0
12.08.09	GC		6.57	10.06	15.41	10.25	15.46	23.32	19.000	5.6
phase 3	**mean**		**14.85**	**24.66**	**41.31**	**9.54**	**16.31**	**28.65**	**19.88**	**33.05**
	median		**9.31**	**13.41**	**19.33**	**10.25**	**15.46**	**29.36**	**19.00**	
26.10.09	GC	mg N$_2$O-N m^{-2} d^{-1}	0.65	2.00	6.13	0.87	2.66	8.12	0.141	0.1
02.11.09	GC		1.76	4.35	10.76	0.12	0.28	0.66	1.083	0.4
06.11.09	GC		0.36	0.85	2.04	2.36	4.91	10.22	1.030	0.4
phase 4	**mean**		**0.92**	**2.40**	**6.31**	**1.12**	**2.62**	**6.33**	**0.75**	**0.54**
	median		**0.65**	**2.00**	**6.13**	**0.87**	**2.66**	**8.12**	**1.03**	

P2862 L10-14: We decided to remove the part on the isotopic analysis for zero N_2O flux conditions. The paragraph was intended to illustrate the fact that net N_2O fluxes below detection limit do not necessarily correspond to negligible N_2 fluxes. However, we consider this illustration to be of minor relevance in the context of the paper.

P2863: We will add and discuss nitrate and ammonium levels as explained above.

L14-16: Above we specified that for most circumstances the contribution of nitrification to the N_2O fluxes measured with the chamber was minor. For such cases a comparison with field measurements seems useful and necessary to evaluate the laboratory method.

P2865: The entire section on the isotope measurements is removed.

P2865 L19-24: Yes, we have not been entirely correct in our interpretation of enhanced respiration rates measured in the laboratory compared to field respiration. CO_2 concentrations in soil (macro-) pore space rarely exceed a few percent. Accordingly, O_2 concentrations will not be less than a few percent below atmospheric concentrations. The half-saturation constant (k_m) for soil respiration is < 1%. Hence, it makes almost no

difference to respiration when a soil sample was exposed to, say, 18% O_2 in the field and is then measured at 21% in the laboratory.

Of course, O_2 concentrations severely affect denitrification. In aerated (non-flooded) soils, O_2 levels in larger pores never drop to a level where denitrification would take place. Nevertheless, denitrification is observed in well aerated soils. It occurs at microsites, such as occurring in water-saturated aggregates, where potential O_2 demand exceeds O_2 supply. O_2 concentrations can drop from atmospheric concentrations at an aggregate surface to zero at a few millimetres within it (e.g. Hojberg et al.,1994). Such a gradient sees little change when the aggregate is taken from the soil profile (e.g. surrounded by 18% O_2) to the laboratory (21%). Hence, we do not expect that any large changes in (anaerobic) microsite aeration occurred between field and laboratory, so that denitrification rates would not have been affected.

Probably the more important change causing enhanced respiration rates is that soil roots needed to be cut during sampling and labile carbon sources were made available by this (we had touched upon this issue briefly on page 2866, first paragraph).

P2868: Reinhard Well added very valuable information to the limitation of the C_2H_2 technique and to the interpretation of contradicting information found in the literature. In combination with all the limitations of the AIT, it is very challenging to clearly differentiate between uncertainty and systematic errors. With our "bulk" method we have limited means to distinguish the different processes yielding changes in the total denitrification rate of the C_2H_2 incubated soil samples from those occurring in the undisturbed soil in the field.

We judge that technical uncertainties are much smaller than the systematic under-estimations that we are unable to quantify, because we cannot compare our results to a well established reference method. We agree with Reinhard Well's comment that Groffman's comment on the reasonable robustness of the AIT method for intensively managed agricultural soils may be misleading, especially when dealing with heavy clayey soils such as that at the Oensingen site.

P2869 L8: most part of this paragraph will be deleted (lines 10 to 15)

L10-22: We would like to keep the comparison with the values given by van der Salm et al. (2007) since they also used the AIT technique in a comparable ecosystem (intensively managed grassland with a high clay content).

P2870: We will add the empirical classification scheme from Germany with the expected 30 to 50 kg N_2 loss per hectare in addition to the estimation given for Switzerland by Braun et al. (1994) based on the N – Budget of Switzerland.

Answers to the review by Michael Dannemann (Reviewer 2)

We are grateful for the valuable additions that are given in the general introduction. They have a high degree of overlapping with the comments by Reinhard Well. In the introduction we will include a more elaborate discussion of the isotopic approach and the information provided by such studies, based on the literature indicated. The potential contribution of nitrification and nitrifier-denitrification to the N_2O flux at our site will be discussed, as already indicated above.

Specific comments:

P2852 L3: We will add information gained from isotopic studies, but it is beyond the scope of this paper to perform a critical review. We chose to use the AIT method, because this approach was within our laboratory capabilities, both from an equipment point of view as well as from the personnel capacity. We take up the suggested change of the title and will modify the abstract accordingly.

P2852 L25: We agree with the comment by the reviewer. In the important publication by Schimel and Benett (2004), it is obvious from Fig. 2 in this paper that relative uptake of N from monomeric organic N depends on the type of ecosystem. As we are working in an intensively fertilized mown grassland system, the N uptake by the plant is largely dominated by the uptake of nitrate and to a lesser extent ammonium, and organic N uptake is likely minor in this ecosystem.

P2853 L21: We have to be more precise in this statement by expressing that either the variability in the method used and/or the variability on the spatial and temporal scale do contribute to the uncertainty of a cumulated flux based e.g. on an annual basis. This uncertainty is of course magnified in case a discontinuous sampling is used.

P2853 L23: See point P2852 L3:

P2854 L5: See also P2852 L3:

P2854 L16: In our setup the samples are exposed to ambient air, therefore oxygen is present in the headspace and consequently also in the macro pores of the soil, unless moisture is near saturation. The Oensingen soil is characterized by a bimodal pore size distribution (Flechard et al, 2007) with mainly large pores and then many fine pores and relatively few pores in the intermediate range. It is the "natural" complexity of the Oensingen soil, that the active denitrification sites will be exposed to a range of oxygen concentration ranging from purely aerobic to fully anaerobic conditions. Consequently it is likely that the failure of the AIT described by Bollman and Conrad (1997) will partially happen, unfortunately in an unpredictable way.

P2854 L24: The comparison of AIT total denitrification values to chamber data is hampered by the potential influence of nitrification and nitrifier-denitrification. However, we believe nitrification is a minor contributor to total N_2O fluxes at our site. We add the evidence based on experimental data to the manuscript, as shown in our reply to Reviewer 1. The minor contribution of nitrification to the N_2O fluxes at our intensively managed grassland site makes a comparison of chamber N_2O fluxes with the AIT more meaningful, or at least less fraught with inconsistencies. The lack of expected similarity in the measured N_2O fluxes from the chambers in the field and from the laboratory samples demonstrates that on top of the difficulties and limitations specific to the AIT method, soil sampling probably strongly affected the denitrification activity of the samples, thereby undermining their representativeness for soil under field conditions.

P2861 L1ff: We will change the evaluation and the presentation of the data gathered with the AIT, providing all results, fluxes with and without addition of C_2H_2 (Tab. 1). The total denitrification losses will be recalculated taking always the values with addition of C_2H_2. As we had no means to conduct alternative analysis with either stable isotopes or N_2 replacement technique, it is unfortunately not possible to make the desired comparison of the AIT with such a method.

P2862 L10: This paragraph will be removed. For completeness we will hereby nonetheless provide information of the sampling and measurement technique. The $d^{18}O$ and $d^{15}N$ measurements were done at the Physics Institute at the University of Bern by the former group of Thomas Blunier. The method is described in e.g. Vieten et al. (2007). For each measurement, 120ml gas were sampled with a syringe from the head space of the closed static chamber where the headspace gas was circulated in a closed loop between chamber and an N_2O analyser.

P2864 L20ff: We argue above, using additional data of ammonium and nitrate concentrations in the soil as well as detailed temporal data, that only a small fraction of the

total N_2O fluxes can be attributed to nitrification. But we do agree with Michael Dannemann that the discussion and consequently the criticisms of the AIT method has to be sharpened along the line that we indicated above.

Answers to reviewer 3:

Title: we changed the title to a more appropriate one.

Abstract: We will write the abstract more concisely. The original goal was to determine the total denitrification loss of our grassland, as well as its uncertainty related to the AIT method. The new focus is clearly on the methodological discussion, as it is also reflected in the changed title.

All other points by the third reviewer are dealt with in the answers given above.

Answers to the review by Nicole Wrage (Reviewer 4):

We believe that in the above replies we have addressed most of the concerns of Nicole Wrage that caused her to recommend rejection of the manuscript in the present form. As we explained above, the AIT methodology did not deliver successful results for our Oensingen soil. We unwittingly over-simplified the N_2O production processes in the discussion and did not mention nitrifier-denitrification, although we are well aware of this process, well examined in the thesis and papers by Nicole Wrage. C_2H_2 will inhibit nitrification as well as nitrifier-denitrification. Both processes can clearly contribute to N_2O either measured in the static chamber or in the laboratory incubation method without C_2H_2, but as demonstrated above, this contribution is likely very minor at our site

Specific comments:

P2856: Taking of soil cores: Our sown grassland has about 40% bare soil, which is where the soil cores were taken. We did not remove or clip any plants. The soil cores were measured at field temperature, at which the samples were collected. The C_2H_2 concentration was 5%.

P2858: Switch to measurement with GC: The photoacoustic measurements are faster (1 sample per minute versus one sample every 5 min for the GC measurements), but the broadband IR spectroscopy used in the Innova analyzers are prone to severe biases and interferences as described in the manuscript. The time measuring one sample was always 30 minutes.

P2859: comparison C_2H_2-free and -treated samples: This figure will be removed in the revised manuscript as it does not correspond to our standard procedure where we first measure the N_2O fluxes without C_2H_2 and afterwards the N_2O flux with addition of 5% C_2H_2.

P2861 L2: How often was 'occasionally'? We reorganized the presentation of the data and have divided them into four phases (Fig. 1) that show distinct N_2O chamber fluxes.

P2862: isotopic measurements: The isotope section will be removed as suggested by Reinhard Well.

All four authors thank again for the constructive reviews.

R. Felber, F. Conen, C. Flechard, A. Neftel

Literature:

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