Dear editor,

we hereby submit a thoroughly revised version of our manuscript, now titled **"Regulation of N₂O emissions from acid organic soil drained for agriculture"** by Arezoo Taghizadeh-Toosi et al., for further consideration.

We appreciate the constructive comments from both reviewers to the Discussion paper. The reviews acknowledged that novel results are presented, and that overall our conclusions are valid. However, it was also clear that the presentation of this complex story could be improved. The manuscript has therefore been thoroughly revised from the previous submission.

Both reviewers had many specific suggestions for clarification and improvement, which have been addressed. Our actions are described in detail below. Please note that all responses to reviewer comments below are rewritten and extended compared to the preliminary responses uploaded at the web site.

Reviewer #2 had some additional recommendations and concerns. One idea was to disregard aspects related to the role of pyrite oxidation, but since it was a main hypothesis when planning this study, we have kept this element. However, in the Discussion it is now addressed in the first part, before turning to alternative mechanisms. We believe this has given a more logical flow, from the original research questions towards actual findings.

The two neighbouring sites *AR1* and *RG1* were proposed to be part of a split-split plot design. However, these two sites have different cropping histories many years back, and we used the same mixed effects model to analyse results from these and other organic soils in a previous publication (Petersen et al., 2012). On the other hand, we agree that this study was not designed to document land use and season effects, but to study mechanisms under contrasting conditions, and therefore, as proposed by reviewer #2, "Effects of land use and season" was removed from the title. Please also note that in the graphical model analyses each land use and season were analysed separately.

A final recommendation was to restructure the Discussion, and this recommendation we have followed. As a result, much of the text is rewritten and reorganised. The number of subsections was reduced in an attempt to develop a more coherent discussion addressing first environmental controls, and secondly possible pathways.

We hope that these improvements to the presentation, and clarification of various aspects, has made the manuscript suitable for publication.

Kind regards, Arezoo Taghizadeh-Toosi on behalf of all co-authors Anonymous Referee #1 Received and published: 28 February 2019

This manuscript investigated N₂O emissions and concentrations in peat soils under 2 agricultural crops: grassland and potato) at 2 distinct site locations during spring and autumn season of one year only. All combination (site x crop) treatment received different management in terms of fertilisation and harvesting etc. The N₂O production measurements were characterized with static chambers and soil N₂O diffusion probes placed at 5, 10, 20, 50 and 100 cm depths. All potential environmental factors (climatic or edaphic) were also monitored during this period. This manuscript has been resubmitted and is substantially improved and conclusions are now validated. It would appear that a lot of fieldwork and indeed field data have been processed and are not equally discussed here but is focused on the title of the manuscript. It is well written and sufficient information provided to allow their reproduction. Some minor comments below would help clarify some details of the experiment and the results.

Response 1: Thank you for the positive evaluation and helpful comments. We are pleased to learn that you find conclusions supported by the results presented. While this study covers only one year, it builds on a 14-month monitoring study in eight locations, with three land use categories (Petersen et al., 2012), which is why we found it appropriate to focus this study on specific research questions emerging from the previous study. We wanted to keep soil conditions as closely as possible to farming practices at the individual sites, which is why management differed in timing and types of fertilisers, but the effect of fertilisation was an experimental factor at all sites. Below we describe in detail how we have reacted to specific comments [note: line numbers refer to the track-changes copy of the revised manuscript].

Line 11: rephrase or change word 'extensively' or 'intensive'; there is nothing extensive about growing cereals or potatoes on organic soils given the cultivation/fertilisation inputs. Perhaps it was meant to be 'widely' used?

Response 2: We have changed the word 'extensively' to 'widely' to avoid ambiguity.

Line 24: emissions could be given per unit of time, either day or season. Where are those days in terms of season?

Response 3: The sentence was modified for clarity to the following:

"Spring and autumn monitoring periods together represented between 152 and 174 days at the four sites, and during this time the total N_2O emissions were 3-6 kg N_2O -N ha⁻¹ at rotational grass, and 19-21 kg N_2O -N ha⁻¹ at potato sites." (**1. 21**)

Line 79-83 belongs to methods; go straight to your hypothesis questioning the role of crop type and seasonal variation

Response 4: Thank you for this suggestion. The detailed information about measurements (Line 81-83 in the Discussion paper) was deleted, but we have kept the first sentence to establish the context.

Line 130; the fertilisation treatment is different in each site and therefore do not act as replicate but different treatment.

Response 5: Please note that fertilisation was only represented in the statistical analysis as a categorical variable. We chose to follow the actual management at each site, and therefore different fertiliser types, rates and timing of application were used, but all four sites received a significant input of fertiliser N in one of two subplots, which allowed us to test for the effect of N fertilisation on cumulative emissions of N_2O .

L149 each field trip being a day sampling so 2 sites were sampled per day maximum but all were sampled during the same week? Rephrase please.

Response 6: We have rephrased the text as follows:

"Field trips included sampling at two sites, either ARI + RGI or AR2 + RG2, and thus all four sites were visited during two field trips on consecutive days." (**l. 162**)

L258 it is not clear that cumulative N2O emissions are here total or on a daily average.

Response 7: We have revised the wording as follows to clarify:

"The model for daily N_2O emission described above was used to estimate cumulative emissions by integrating the flux curves over time. Treatment effects were then analysed by specially designed linear contrasts as described in detail by Duan et al. (2017), who showed that models with untransformed responses (when using adequate distributions) allow simple statistical inference of the time-integrated N_2O emissions." (**I. 279**)

Line 301 : average deviation of soil temp from air temp is given; could it be better described in terms of sign L304.

Response 8: We report average deviations, but also the largest positive and negative deviations observed. No changes made.

It states in Lin 166 that soil samples were taken at the start of each season April and Sept?

Response 9: The soil characteristics reported in Table 1 were based on analyses of soil cores sampled in April except for AVS and CRS, which were analysed on soil cores sampled in September. This was stated in the Table caption, but now it has been specified also in the text (l. 231). Please note that the description of total reactive Fe analyses (l. 226) was moved up to further emphasize this.

Depth of the total peat layer should be shown in Table 1 as it seems that RG2 is very shallow peat (<25cm). Also von post figure should be given for each peat layer.

Response 10: Unfortunately peat depth was not mapped (but general information is given in **l. 106-108**). It is true, as stated, that the degradation at site *RG2* was extensive, but still met the definition of organic soil. The following sentence was added:

"According to Kandel et al. (2018), the peat at 0-25 cm depth in arable soil in this area has a high degree of humification at H8 on the Von Post scale." (**l. 109**)

Line 350. The WT reported in Figures 5 & 6 is confusing; what are they if you are not showing your continuous measurements (which shows higher WTL?)

Response 11: The WT reported in Figures 5 and 6 were the weekly values measured concurrently with flux measurements. By referring to the continuous measurements, we wanted to stress that soil conditions were highly dynamic, and that the nitrate reduction potential could have been influenced by this. For consistency across seasons, we have chosen to show the values obtained at the time of flux measurements in all contour plots.

Line 407: there in previously in this paragraph, it would help to add the DOY (as per line 410) or else include the month in your Figures.

Response 12: We added this information, so that the last sentence now reads:

"The highest emissions occurred, independent of fertilisation, in June following a WT rise to 35 cm depth on DOY154." (**l. 448**)

Line 415: this is the first time that the monitoring period is mention; this should be explicitly shown in Table 2 at least and therefore rather than total a per day average would be better to compare treatment.

Response 13: The monitoring periods in spring and autumn were defined in **l. 157-160**. We have included information of periods (DOY) in a separate column in Table 2. Also, the cumulative emissions and treatment effects are now shown for both fertiliser treatments also in the autumn.

Figure 3-6: the WT is visible in blue not in grey.

Response 14: The colour of WT lines was changed to bright green, which is visible in black and white reproduction.

Figure 7 : the statistical number on the graphics should be explained in the legend since it is not clear to which lines they apply (especially 7a).

Response 15: We have moved results of the statistical test to the legend; they are referred to by numbers 1-5 i brackets.

Interactive comment on "Regulation of N₂O emissions from acid organic soil drained for agriculture: Effects of land use and season" by Arezoo Taghizadeh-Toosi et al. Anonymous Referee #2 Received and published: 21 March 2019

The authors evaluate subsoil concentrations and emissions of nitrous oxide (N2O) and related soil variables at sites in a raised bog in Denmark which had been identified as having high potential N2O emissions. They conduct measurements over spring and fall, 2015, in four sites (two of which are immediately adjacent to one another): two cropped with potato and two with grasses; each site was also split into fertilized and unfertilized treatments. Using graphical analysis, they determine that in most sites, the concentration of N2O at the capillary fringe was the driver of emissions, though in one season/site combination, N2O emissions were related to sub-surface soil temperature and nitrate concentration. The relationship between water table depth and N2O appeared to vary by site and by season, but included a declining water table depth triggering N2O emissions in spring, and rising water table depth triggering N2O emissions in autumn. Pyrite was largely excluded as making an important contribution to N2O production or emission, and a range of possible mechanisms of N2O production were discussed. Lower emissions from grassland plots were attributed to greater plant uptake of soil N. Annual fluxes were comparable to or higher than IPCC Tier I emission factors for drained organic soils.

This is a concise summary of our study, i.e., based on a previous multi-site study (Petersen et al., 2012) we investigated possible mechanisms of extremely high N2O emission rate during periods with fluctuating groundwater. In the end, our hypothesis regarding involvement of pyrite could not be confirmed, and alternative explanations were explored based on the supporting data obtained. Thank you for the many detailed comments, which we have tried to address below [note: line numbers refer to the track-changes copy of the revised manuscript].

General comments: This is my first reading of the manuscript. This is an impressive field study with a lot of interesting data, and the importance of N2O concentrations in the capillary fringe for N2O emissions is a nice result, as is the finding that pyrite and iron monosulfide are unimportant, and the confirmation of high emissions from these soils. However, I have a number of concerns regarding the experimental design, support for some of the conclusions presented, and organization of the manuscript. The manuscript and discussion in particular could benefit from substantial revision after some contemplation of what the core advances of the study are. There seem to be three main aspects of the study (in no particular order): 1. Understanding the mechanism of N2O production, with a particular examination of the potential roles of pyrite and iron monosulfide. 2. Understanding the variables related to surface N2O fluxes. 3. Understanding the relationship between water table and soil N2O concentrations (and N2O flux). These different aspects are part of a single system, and ideally the paper will weave these aspects together into a single coherent story of the patterns and mechanisms behind N2O dynamics and its drivers. As noted below, I think it may make sense to refocus this manuscript around results related to items 2 and 3, for three reasons: a) it may help to clarify this study, b) results apparently highly relevant to item 1 have been kept for a second manuscript, and c) this study wasn't designed to be a comprehensive investigation of the specific mechanism of N2O production in these soils. Based on the first referee's review, it sounds as though this may be a revised version of an earlier manuscript; if so, sorry to be bringing new

concerns at this stage of the publication process, and apologies for the long comments. Note: I don't have expertise in graphical analysis or in the specific technique used to measure subsurface N2O concentrations.

Response 16: We appreciate the acknowlegdement that interesting results came out of this study. You emphasize that the role of N2O accumulation above the WT for N2O emissions in spring, but also the lack of importance of pyrite and iron monosulfide, are both nice results. We take this as an indication that the study merits publication, and we hope that the extensive revision now makes this possible. It was proposed to omit findings related to pyrite as a possible driver for N2O emissions, which was a hypothesis presented in a previous paper published in Biogeosciences (Petersen et al., 2012). We did not find a proper way to construct a different rationale for the study and therefore present this work along the lines of the original research questions. However, the Discussion section has been completely restructured and partly rewritten in order to address the pyrite hypothesis first, before turning towards alternative mechanisms to explain the extremely high N2O emission rates observed at arable sites. Additional changes were also made in Abstract and Introduction to strengthen the focus of the paper.

Specific comments:

Experimental design:

• The experimental design is unusual, with elements of different types of partly nested designs. Essentially, the first arable potato site (AR1) and first rotational grass site (RG1) are part of what traditionally would be called a split-split-plot design, but RG2 and AR2 are in a split-plot design. What this means is that the role of site in the analysis—there are effectively three sites--varies in its relationship with the treatments. AR1 and RG2 are nested within site, but RG2 and AR2 are not. Additionally, the lack of nesting of AR2 and RG2 within site raises additional issues, since RG2 has such a big difference in organic C content, so treatment is confounded with site in the RG2/AR2 pairing (in addition to the different fertilizer types used). Although differences between the potato and grass treatments are discussed in the results, and the title suggests that these and seasonal differences are the focus of the manuscript, I don't see any methodological description of the analysis that could allow one to compare RG and AR treatments. I'm not strictly sure how it would be done, but I also don't have a problem with softening the wording of the conclusions that can be drawn about the differences between RG and AR treatments—it does look like there may well be treatment differences there, I'm just not sure that with this design that it's possible to establish that statistically. So I think it needs to be made clear that if a strict statistical comparison was not conducted, the conclusions drawn aren't statistically supported (and any related concluding statements should be softened). And if a statistical comparison was conducted, details on how the nesting within site of RG1 and AR1 but not AR2 and RG2 was handled would be helpful. There is also no replication of bog-the results can't technically be generalized beyond the Store Vildmose raised bog. This is not a problem, and the authors don't attempt to extrapolate beyond the Store Vildmose, but it is a limitation that should be explicitly acknowledged. None of these issues should affect the finding that the capillary fringe is often/typically the primary determinant of the magnitude of N2O flux.

Response 17: Although located in neighbouring fields, the sites *RG1* and *AR1* should be considered as independent units, in accordance with the statistical analysis of Petersen et al. (2012). The two sites had different cropping histories many years back, and different crops in the experimental year. Also soil N dynamics and N2O emissions were evidently very different for these two land use categories.

Please note that the statistical analysis did not address land use effects, except that cumulative emissions were compared, and here the differences were evident even without any statistical support. In order to reduce the focus on the factorial setup, the title was changed, as proposed (see reponse 27).

• I am always concerned about field studies the present just one year of data, as it does limit insights into the degree to which patterns observed provide generalizable insights. The manuscript title suggests that comparisons of seasons is one of the central findings of the study, but since only one year of data are included, it is impossible to rigorously compare seasonal differences, as there is no replication of season. There are a lot of varying results in this study, which only strengthen this issue. I don't think these issues affect the finding on the frequent importance of capillary fringe N2O concentrations for surface emissions, though.

Response 18: Please note that this study does not stand alone. It was preceded by a study (Petersen et al., 2012), where N2O emissions were monitored during 14 months in three regions (including the area investigated in the present study), and including three different land uses, which were arable farming, rotational grass and permanent grass. In total 8 site-years were thus available as background for developing the research questions addressed in this new study.

The previous study also showed strong seasonal dynamics, which is why we found it was acceptable to focus resources on spring and autumn periods, rather than spreading resources across a full year, or across more sites. The results of the previous study are now highlighted in the discussion (**l. 484ff**).

• In the end, these issues might make the conclusions of this study fairly descriptive with respect to the specific questions of the effects of season and land use on N2O emissions and subsurface dynamics. That doesn't mean that these specific results aren't valuable, just that the nature and limitations of the conclusions that can be drawn from the study on these specific questions need to be made very clear in the manuscript. Rethinking what the central finding or findings of the manuscript are may be helpful.

Response 19: We acknowledge the many limitations of a field study kept as closely as possible to the practice of each farmer, but as stated above, this study was developed from a larger, multi-site study with reproduction of land uses and covering more than a year. We agree that this is a predominantly descriptive study. Still, the graphical models did reveal a constrast in the regulation of N2O emissions, which appears to be a novel result.

• The data on the changes in the water table and subsoil concentrations of N2O are great. But the conclusions drawn in section 4.3 could be more compellingly supported (there does not appear to be any quantitative analysis of the relationship) and discussed. Looking at the figures, in some cases N2O concentrations are enhanced above the water table, in other cases, below the water table, and an overall relationship is not immediately obvious. The importance of the capillary fringe concentrations for surface emissions makes this discussion of particular interest, and worth spending some text to guide the reader through your interpretation.

Response 20: Please note that the identification of a significant relationship between N2O concentrations in the gas probe closest to, but above the WT depth and N2O emissions (Figure 7) was the result of a quantitative analysis. The distance between probe depth and WT depth would have varied between samplings, and in periods with rainfall the WT depth may have fluctuated prior to a given sampling. For these reasons it was not possible to analyse the results in greater detail at this time.

• The manuscript often reports whether there was an effect of fertilization (e.g., line 380, lines 404-405, 412-413, and others) or appears to test fertilizer vs site effects (e.g., line 405), and the methods detail how generalized linear mixed models were used to analyze the temporal dynamics of N2O. However, I don't see any reporting of the statistical results of this model or its application to the impact of fertilization on N2O emissions or soil concentrations.

•

Response 21: The statistical results for cumulative emissions are presented in Table 2; we have revised the Table to include DOY periods of monitoring, and cumulative emissions in the autumn are now shown for each fertiliser treatment separately (previously they were pooled because they showed nearly identical N2O emissions).

• A follow-on point is the very nice finding that N2O at the capillary fringe generally controlled N2O emissions, rather than any variables in the topsoil. But this result raises the question of what controls variation in N2O concentration at the capillary fringe. This question seems to be of first order importance in this system, but is not addressed quantitatively in the manuscript. It might help tighten the manuscript if the discussion in section 4.3 is tied more explicitly to N2O concentrations in the capillary fringe.

Response 22: In the new, restructured Discussion, the paragraph referred to is part of section 4.1. There is specific reference to peat decomposition (early spring, with low soil mineral N status) and precipitation (after fertilisation and in the autumn, i.e. situations with high mineral N status) as proposed mechanisms behind N2O accumulation. Actual production pathways are now discussed in section 4.2. Again, the graphical models do represent a quantitative data analysis.

• A general comment: In presenting results, it could be helpful to start each section with a general description of the main results or patterns found instead of starting with detailed information for individual blocks; that detailed information can be presented later, to

support the general patterns or describe deviations from those patterns. Anything you can do to guide readers through the results is great! In addition, the authors occasionally slip interpretation into the results section that would be more appropriate in the discussion section.

Response 23: Text of the Results section has been revised and most subsections amended with an introductory statement to highlight a main aspect of the results.

• One of the main questions addressed in this manuscript is that of the importance of FeS2 oxidation for N2O production. Line 530-531 invokes a separate but presumably related manuscript that presents results showing that FeS2 oxidation is unimportant in this peat soil. It is difficult to say without knowing what the focus of that manuscript is, but my hunch is that it may make more sense to include the FeS2 results from this field study in the separate manuscript (presumably focused on mechanisms of N2O production in these soils), since we are effectively only getting half the story here. Something to consider, anyway. In the end, this manuscript doesn't provide much in the way of firm insights into the mechanisms of N2O production-that's not an inherent problem, it's just not something this study was designed to do--so one idea would be to cut out that part of this manuscript, and make the focus entirely on quantification of fluxes, the nice soil N2O & water table data (and capillary fringe finding), and environmental drivers more generally. It would be easy enough then to include a paragraph on mechanisms of N2O production that cites the other manuscript. Also, just to note, many journals require any related manuscripts that have been submitted elsewhere to be included as part of a manuscript submission, so would be a good idea to check the policy of the journal in question when you submit the separate manuscript.

Response 24: The field study was planned to explore a hypothesis coming out of a previous study (Petersen et al., 2012), and therefore the present study was a direct follow-up. In the restructured Discussion section, the pyrite hypothesis are now addressed first, and rejecting this hypothesis then leads on to a discussion of alternative mechanisms to explain N2O emissions. The unexpected evidence for different sources of N2O in spring and autumn periods in turn leads on to a discussion of biotic and abiotic pathways.

We note in the comment above a statement – "Something to consider, anyway" – and take this to say that a decision not to remove this part of the study is also acceptable.

• The manuscript argues that the water table depth was related to surface N2O emissions (e.g., section 4.1), but that this relationship varied by site and by season, and speculates that soil properties modified the water table depth/N2O relationship. However, there doesn't seem to be any statistical/quantitative analysis to support a water table depth/N2O relationship or how other soil properties modify that relationship (and as noted above, it's not possible to statistically evaluate seasonal differences).

Response 25: The particular statement in the former section 4.1, about soil properties modifying the effect of WT on N2O emissions, was intended as a transition to the next subsections, in which interactions between e.g. soil N status and WT dynamics were

discussed. The graphical model results (Figure 7) did show increasing N2O emissions with declining, as well as increasing WT depth that depended on soil N status, and various other studies were highlighted showing similar results.

The statement referred to above was deleted as part of the rewriting of the Discussion section, for which a more linear story line was developed.

section 4.1 is largely a re-statement of results; much of the actual discussion about the water ٠ table/N2O relationship is sprinkled throughout subsequent sections. A restructuring of the discussion might make the results easier to digest, with one section focused on discussing the capillary fringe result and one focused on understanding the water table/N2O relationship. Some discussion about why the patterns are so variable could be valuable, including some explanation of why water table increases stimulate N2O production at all sites in the autumn (and contrast with the results of other studies, e.g. Maljanen et al. 2003, which saw no effect of rising water table on N2O emission). One possible straw man interpretation could be that in the early spring (or late spring in the case of AR2), N2O production is limited by NH4+ (and/or NO2-/NO3-) availability, which in turn is constrained by the availability of O2. The decline of the water table may release the O2 constraint. In the autumn, in contrast, it is possible that aerobic conditions limit N2O production, and a rising water table or precipitation leads to higher N2O emissions. If indeed the case, why a possible seasonal shift from substrate to O2 limitation happens would be interesting to understand.

Response 26: We appreciate the suggestion to restructure the Discussion and have in fact completely reworked this section. An initial discussion of the hypothesis related to pyrite is now followed by two main subsections follow, which address environmental controls and possible pathways, respectively

The interpretation offered by the reviewer above ("N2O production is limited by NH4+ (and/or NO2-/NO3-) availability") was in fact also proposed in the manuscript (Line 541f in the BGD paper). The study of Maljanen et al. (2003) referred to described a rather different situation, where WT rise never exceeded 50 cm soil depth, and the crop was barley. There would have been less potential for N mineralisation and nitrate accumulation from barley after harvest compared to harvest of a potato crop with intensive tillage, and less potential for interaction between WT and soil mineral N in the top soil. Due to these differences we have not discussed this previous study.

Technical comments:

• Line 1: I would change the title to reflect the focus of the revised manuscript (in addition, as noted above, it seemed to me that neither seasonal nor land use differences were able to be rigorously tested, and so it would be better to exclude phrases like "effects of land use and season" from the title)

Response 27: Thank you for this suggestion – we agree and have changed the title accordingly.

• Line 45: You can check my math, but it seems to me that the global warming potential is still uniformly larger for C than for N2O here. I don't think it is necessary to make the case that N2O fluxes are more important than carbon fluxes, just that the N2O fluxes are large.

Response 28: Please note that we did not compare the importance of C and N2O fluxes, instead we wanted to highlight that N₂O emissions appear to be more influenced by site management compared to soil C losses. The sentence has been changed to:

"Thus, while CO2 emissions are overall more important, site conditions appear to be more critical for N2O." (1. 54)

• Line 44: change ", which" to "that"

Response 29: Has been changed to "and this"

• Line 64: "The sites" : not sure what sites are being referred to. Could you add more context?

Response 30: This has been rephrased to specify that these were the arable sites with extremely high N2O emissions in two regions investigated by Petersen et al. (2012):

"The two regions showing extreme N2O emissions from arable soil had both developed from marine forelands ..." (**l. 74**)

• Line 120: please add a short explanation for why soil gas data were not presented for unfertilized RG2 during Autumn

Response 31: Some probes were damaged during handling (and one visit by heifers) during spring, and replacements were not available. The measurements at *RG2*-NF with unintended slurry application in July were sacrificed. The following was added:

"Due to damage of some probes it was decided to discontinue soil gas sampling in the unfertilised subplot at site RG2, which had by mistake received slurry on DOY 183" (**l. 197**)

• Line 167: was this the fertilized or unfertilized block?

Response 32: Intact soil cores to 100 cm depth were obtained from both fertilised and unfertilised subplots, and the analyses represent both fertiliser treatments. See also reponse no. 37.

• Line 176: remove "quantitatively" (not sure what it is intended to mean)

Response 33: Done.

• Line 210-211: Entirely your choice, but perhaps AVS and CRS don't need to be abbreviated

Response 34: We prefer to use abbreviations.

• Line 197: specify type of filter paper used

Response 35: 1.6 μ m glass microfibre filters was used. The information was added to the text (**l. 213**).

• Line 237: Is the instrument ever checked against a set of standards of varying concentrations?

Response 36: Calibration standards (0 to 2000 ppb) were included before and after each sequence run and used for determination of sample concentrations. Also, extra calibration samples were included after every 10 unknown samples to verify signal stability. We believe these are standard procedures and we have chosen not to spell out the details of analytical runs.

• Line 339: Indicate whether fertilized or unfertilized blocks were sampled

Response 37: Has been rephrased to:

"Nitrite-N concentrations were determined in undisturbed soil collected profiles from the cores sampled at sites RG1 and AR1 on 23 April (DOY 113) and 2 September (DOY 245) 2015. Both fertilised and unfertilised subplots were represented, although at site AR1 the fertilisation had not yet taken place at the time of sampling in April. There was variation at depth in the soil, which could not be linked with fertilisation." (**I. 367**)

• Line 354 change "temporarily" to "temporary" Line 363: change "trends" to "concentrations"

Response 38: Done.

• Line 370: Figure 3 seems to suggest that the N2O concentrations in the top 40cm of soil look to be 1-2 orders of magnitude higher in the fertilized RG1 than fertilized RG2. And unfertilized RG2 looks to be 1-2 orders of magnitude higher than unfertilized RG1 between 60 and 100cm depth for most of the spring. Yet they are described as "generally similar." I wouldn't have thought that would be considered "generally similar"—am I missing something?

Response 39: The color scale for RG2+F was not correct, and concentrations were in general higher at site RG2. When referring to "generally similar", this should be seen in the context of concentrations of several hundred ppm N2O being observed especially at AR sites, but also around DOY150 in RG2+F.

• Line 375-6: Since there are apparently no soil gas data from unfertilized plots in RG2 during autumn, this statement is too strongly worded (even independent of questions of whether the effect of fertilization was tested).

Response 40: Thank you for pointing this out. We have changed the sentence to:

"During autumn, N₂O concentrations in the soil profile at the *RG1* and *RG2* sites varied between 0 and 12 μ L L-1, with a tendency for higher concentrations at 10-20 cm depth (Figure 5). At site *RG1*, where both fertilised and unfertilised subplots could be samples, this was independent of fertilization." (**1. 424**)

• Line 376: The figures are out of order—I think you can swap Figures 4 and 5.

Response 41: We have moved the text relating to *RG* sites in autumn down, and the Figure numbering is now correct.

• Line 380: could you be specific about what soil conditions showed significant within-site heterogeneity? Also, use "substantial" instead of "significant' if this heterogeneity wasn't tested, and if it was, consider providing P values

Response 42: In fact, heterogeneity was inferred from N2O concentrations. Has been rephrased to:

"The soil N2O concentrations suggested that there was considerable within-site heterogeneity in soil conditions, as the highest concentrations were observed in the unfertilised subplot." (**l. 415**)

• Line 384: this is really interpretation, and might be better placed in the discussion.

Response 43: Has been deleted, addressed in the Discussion.

• Line 400: were any measurements made of N in harvested biomass? It could certainly help support the story that differences in uptake could alter N2O emissions from different plots.

Response 44: Unfortunately, we were not able to include manual cuts of the grass before harvest, in order to measure N in harvested biomass.

• Line 405: if specific soil variables cannot be identified as causing the differences, perhaps change "soil conditions" to "site differences"

Response 45: Has been changed to: "site differences other than fertilisation". (l. 447)

• Line 411-12: I'm not sure I see this pattern clearly: emissions are already high when the water table is at 80, and in the fertilized plots of AR1, emissions are 1/3 as large on DOY 259 than DOY 252, even though the water table is at its peak on DOY 259. There's also no apparent effect of the increase in water table starting on DOY 307, and emissions look quite

elevated on DOY 246, which may be before the increase in water table began. A quantitative analysis would be helpful.

Response 46: Thank you for pointing out that the rise in WT was not a strong predictor of N2O emissions in the beginning of September. Previously the precipitation data shown in Figures 5 and 6 started by DOY246, i.e., the first measurement day in the autumn campaign, and this unfortunately left out the information that 10 mm rain fell on DOY244 and 22 mm on DOY245. The two Figures were updated to include this information.

The rainfall on DOY244 and 245 was probably absorbed by peat in the top soil and therefore did not directly affect WT depth, but very likely gas transport near the soil surface. Hence, the high N2O emissions from sites *AR1* and *AR2* on DOY246 was a result of anoxic conditions in the presence of soil NO3. The text has been modified to clarify this:

"High fluxes were observed on the first sampling day of this monitoring period, DOY246, while WT depth was still at 40 to 80 cm depth. This followed 10 and 22 mm rainfall on the previous two days that was initially absorbed by the peat. Rainfall the following days then led to a rise in WT. The subsequent decline in N2O emissions at AR sites coincided with WT withdrawal." (**1. 453**)

and in the Discussion section:

"Despite 32 mm rainfall on DOY244 and 245, the WT depth was still at 40 to 80 cm and could not account for the very high N2O emissions observed on DOY246 (Figure 6). According to Kandel et al. (2018), the peat of arable soil in this area is highly degraded (H8 at 0-25 cm depth on the von Post scale), and well-degraded peat will release as little as 10% of its water to drainage (Rezanezhad et al., 2016). It is therefore likely that the rain was absorbed by peat above the WT and created conditions suitable for denitrification." (**1. 528**)

• Lines 473-4: could be more specific and change "a short period" to "1 to 2 weeks"

Response 47: Done.

• Line 481 and following: Section 4.3 draws a number of conclusions that don't appear to be supported by any statistical analyses.

Response 48: soil N2O concentrations are equivalent gas phase concentrations, and information about soil bulk density and air-filled porosity at the individual gas sampling positions would be necessary to analyse N2O results. With measurements in only one or two blocks per sites, the discussion is necessarily qualitative.

• Line 516-517: I'm not sure I see this rapid increase in N2O around the water table depth in all the blocks in figure 6?

Response 49: As discussed above, the effect of rainfall was also partly to wet upper soil layers and thereby create conditions supporting denitrification. The text has been modified to emphasize this.

• Lines 533-534: I think it might be better to say that "denitrification in topsoil was the main source. . . " since there is no explanation of how the N2O in the capillary fringe is produced.

Response 50: We have changed the wording to read:

"Bacterial nitrification, denitrification, and nitrifier-denitrification are all potential pathways of N2O formation (Braker and Conrad, 2011), and the). The significant relationship with NO3- at AR sites in the autumn (Figure 7) suggested that denitrification wasactivity in the main source in top soil controlled N2O emissions during this period." (**1. 624**)

• Line 544: I'd change "drive" to "regulate".

Response 51: The sentence was changed in the rewritten Discussion.

• Line 532 and following: this is interesting discussion, but if there are supplementary data that could support application of the ideas to this study (e.g., water filled pore space, acetylene reduction experiments, etc), it would strengthen it considerably. If the manuscript in preparation on FeS2 oxidation includes any detailed examinations of these questions, it may be better to limit the speculation here.

Response 52: It is true that more data or experiments could probably eliminate some of the pathways discussed here. The incubation experiments referred to, however, only look at the potential for iron sulfides to stimulate N2O emissions. And results could not confirm their involvement.

We do find that the differences in environmental controls identified by the quantitative analysis of the graphical models warrant a bit of speculation about possible pathways. They provide some support for the proposed role of ammonia oxidation coupled with either chemodenitrification or nitrifier-denitrification during spring, and of heterotrophic denitrification in the autumn, where nitrate was a strong predictor of N2O emissions in arable soil. The short summary of related literature showed some support for this interpretation, and can hopefully inspire future studies to confirm pathways.

• Line 544-558: looking at Tables S1 and S2, it seems that there is generally more NO3- or NH4+ in these soils on April 22 and/or May 13 than there is NO2- on April 23 (much more if these were the fertilized plots—I could not see any indication of whether the undisturbed core was from fertilized or unfertilized plots). If correct, that suggests that perhaps there is not an imbalance between ammonia oxidation and nitrite oxidation? Perhaps all nitrifier populations are temporarily saturated by the increase in available NH4+?

Response 53: Soil nitrogen pools are not necessarily uniform, and the fact that nitrite accumulated in samples collected in April, but not in September, suggested that there was a difference in the balance between ammonia oxidation and nitrite reduction between the two sampling dates. We have added the following sentence:

"Total concentrations of NH4+ and NO3- at 25-50 cm depth were similar or higher (Tables S1 and S2), but well-decomposed peat is dominated by dead-end pores (Hoag and Price, 1997), and it is likely that N mineralisation and ammonia oxidation to a large extent took place in such pores having a slow exchange of solutes with active pore volumes." (**1. 642**)

• The discussion in Lines 486-489 also seems to suggest that denitrification was cranking along pretty well in the AR sites. And perhaps there's reason to be cautious about inferring processes from snapshots of concentrations, whether a single depth profile or weekly measurements of NH4+ and NO3- . Presumably, high concentrations could indicate anything from slow loss rates of each compound (whatever the pathway may be), or could reflect rapid N mineralization rates. If, by chance, total N concentrations were measured at each sampling date, calculations of net mineralization and net nitrification might be able to provide additional insight into whether and where reactive N might be accumulating.

Response 54: We have in fact tried to be cautious about assigning N2O accumulation and emission to specific processes. Concern is raised about the limitations of point measurements as basis for the interpretation of N transformation processes. It may be argued that the soil gas probe measurements of N2O represent a more time-integrated measure of N transformations, insofar as the equilibration time is in the order of days (cf. Petersen, 2014). High concentrations thus indicate a sustained production and not just transient episodes.

• Line 550-51: This is partly covered in the note immediately above. I see that NH4+ remains at high concentrations, but NO3- does as well, which is why I'm unsure about the suggestion that there is a lack of a mechanism to remove NO2-.

Response 55: Peat soil is an extremely heterogeneous environment and may be dominated by dead-end pores (Hoag R.S., Price J.S., 1997). It is therefore difficult to infer microbial activities from bulk soil concentrations. As mentioned, a sentence has been added to stress this possible spatial heterogeneity (Response 53)

• Figure 2 caption: indicate whether cores were taken from fertilized or unfertilized blocks.

Response 56: It is mentioned in section 2.4.2 'Soil sampling' that soil samples were collected to 1 m depth within 1 m distance from the positions of flux measurements in Block 3 of sites RG1 and AR1. A soil core collected from both fertilised and unfertilised subplots went into the analyses, but at site AR1 the soil was not fertilised until 21 May.

• Figures 3 through 7: I think it might be easier to evaluate these data if the entire year of data are presented in a single plot, rather than separating spring and fall data—I don't think it would make it any more difficult to read the data. An axis break could be included between DOY 167 and DOY 246. You could also explore presenting surface N2O flux in a log scale— there may be a variation that would be visible on a log scale that is difficult to discern on the current linear scale because of the dates with very high fluxes.

Response 57: Thank you for these suggestions. We feel that each Figure already has a lot of information, and we wanted to present results from each land use type side by side. In order to not sacrifice the temporal resolution, we have not revised these Figures.

The manuscript text switches freely between using DOY, month, and terms like "early spring" to describe time, which makes it challenging for the reader to compare the text and figures. Sometimes the DOY is included parenthetically when month names are used, which is great, but this practice should be extended throughout the text. Alternatively, the x axis labels could be changed to month names and days.

Response 58: In order to make time traceable and consistent throughout the manuscript, we have included DOY in parentheses throughout the text, as well as in Figures, but often together with actual dates for reference.

• Tables S1 through S4 would be much easier to read in figure form (possibly in a single figure), though I appreciate the inclusion of the summary data here. Actually, why not explore adding these data as a second y axis in figures 3-6, sharing the panels used for N2O. Since topsoil nitrate is presented as a significant predictor of N2O flux, it could be valuable to be able to compare the data in the figures.

Response 59: It would be useful to show mineral N data along with N2O emissions etc. in Figures 3-6, but they are already very busy, and mineral N data included two N species, two treatments and two depths. We have therefore kept these data in tables as supplementary information.

References

Hoag R.S., Price J.S., 1997. The effects of matrix diffusion on solute transport and retardation in undisturbed peat in laboratory columns. J Contam Hydrol 28:193-205.

Petersen, 2014; Diffusion probe for gas sampling in undisturbed soil. Eur. J. Soil Sci. 65: 663-671.

Petersen et al., 2012. Annual emissions of CH4 and N2O, and ecosystem respiration, from eight organic soils in Western Denmark managed by agriculture. Biogeosciences 8, 403-422.

Regulation of N₂O emissions from acid organic soil drained for

agriculture: Effects of land use and season

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10 Abstract

Drained organicOrganic soils are extensively used for cereal and high-value cash drained for crop production or as grazing land are agroecosystems with potentially high, but variable emissions of nitrous oxide (N₂O) are enhanced by the drainage and cultivation. A). The present study was conducted to investigate investigated the regulation of N₂O emissions in a raised bog area drained for agriculture. The area has been, which was classified as potentially acid

- 15 sulfate soil, and, Here, we hypothesised that pyrite (FeS₂) oxidation was a potential driver of N₂O emissions through microbially mediated reduction of nitrate (NO₃⁻). Two sites with rotational grass, and two sites with a potato crop, were equipped for monitoring of N₂O emissions, as well as sub- and soil N₂O concentrations at 5, 10, 20, 50 and 100 cm depth; during weekly field campaigns in spring and autumn 2015. Precipitation, air and soil Further data acquisition included temperature, precipitation, soil moisture, water table (WT) depth, and soil mineral N were recorded during
- 20 weekly field campaigns. In late April<u>NO3</u>² and early September, intact cores were collected ammonium (NH₄⁺) concentrations. At all sites, the soil was acidic with pH ranging from 4.7 to 5.4. Spring and autumn monitoring periods together represented between 152 and 174 d, with cumulative emissions of 3-6 kg N₂O-N ha⁻¹ m depth at adjacent grassland and sites with rotational grass and 19-21 kg N₂O-N ha⁻¹ at sites with a potato sites for analysis of soil properties, which included acid volatile sulfide (AVS) and chromium reducible sulfur (CRS) to quantify, respectively,
- 25 iron monosulfide (FeS) and FeS₂, as well as total reactive iron (TRFe) and nitrite (NO₂⁻). Soil organic matter composition and total reduction capacity was also determined. The soil pH varied between 4.7 and 5.4.crop. Equivalent soil gas phase concentrations of N₂O ranged from around 10 μL L⁻¹ at grassland sites to several hundred μL L⁻¹ at potato sites, in accordance with lower soil mineral N concentrations at grassland sites. Total N₂O emissions during 152-174 days were 3-6 kg N₂O. N ha⁺ for rotational grass, and 19-21 kg N₂O. N ha⁺ for potato sites. Statistical analyses
- 30 byStatistical analyses using graphical models showed that soil N₂O concentration in the capillary fringe was the strongest predictor forof N₂O emissions in spring; and, for grassland sites, also in the autumn. For potato sites in the autumn, nitrate (the analysis found that NO₃⁻) availability in the top soil, together with temperature, were the main controls on N₂O emissions. Pyrite oxidation coupled with NO₃⁻ reduction could not be dismissed as a source of N₂O,

butChemical analyses of intact soil cores, collected to 1 m depth at adjacent grassland and potato sites in spring and
 autumn, showed that the total reduction capacity of the peat soil (assessed by cerium (IV) reduction) was much higher than explained that represented by the FeS₂-concentration, and the concentrations of total reactive iron (TRFe) were much higher than pyrite concentrations. The potential for chemodenitrification being a source of N₂O during WT drawdown in spring is discussed. In contrast, those of FeS₂. Based on the statistical graphical models and the tentative estimates of reduction capacities, FeS₂ oxidation was found unlikely to be important for N₂O emissions-associated with rapid soil wetting and WT rise. Possible pathways of N₂O production in autumn were consistent with biological denitrification. Soilspring and autumn periods, and the potential sources of N availability and seasonal WT changes were important controls of N₂O emissions.-, are further discussed.

Key words: Drained peat, potentially acid sulfate soil, rotational grass, potato, nitrous oxide, reactive iron

45 1 Introduction

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Worldwide, 25.5 million ha of organic soils have been drained for agricultural use, mainly as cropland (Tubiello et al., 2016), which and this accelerates decomposition of soil organic matter and net carbon (C) and nitrogen (N) mineralisation above the water table (WT) (Schothorst, 1977). Drained organic soils are significant net sources of greenhouse gas (GHG) emissions due to emissions of as carbon dioxide (CO₂) and nitrous oxide (N₂O) (Goldberg et al., 2010; Maljanen et al., 2003). A recent supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories on Wetlands (IPCC, 2014) proposed average annual emission factors of 4.3 and 8.2 kg N₂O-N ha⁻¹ yr⁻¹ for temperate grassland on drained organic soil with low and high nutrient status, respectively, and an emission factor of 13 kg N₂O-N ha⁻¹ yr⁻¹ for cropland. For soil C losses, the emission factors proposed for the three land use categories were between 5.3 and 7.9 Mg CO₂-C ha⁻¹ yr⁻¹ (Hiraishi et al., 2014). This implies that Thus, while CO₂ emissions are overall more important, site conditions are potentiallyappear to be more critical for N₂O than for CO₂ emissions.

Site conditions are defined by land use, management, inherent soil properties and climate (Mander et al., 2010; Leppelt et al., 2014). Both WT drawdown (Aerts and Ludwig, 1997) and WT rise (Goldberg et al., 2010) may enhance N₂O emissions, but such effects depend on soil <u>nitrogenN</u> status (Martikainen et al., 1993; Aerts and Ludwig, 1997). Maljanen et al. (2003) found that WT, CO₂ emissions and temperature at 5 cm depth, together, explained 55% of the observed variability in N₂O emissions during a two-year field study on a drained organic soil, whereas the response to N fertilisation was limited, and they suggested that N released by soil organic matter mineralisation was the main source of N₂O. In a study comparing GHG emissions from organic soil with different land uses in three regions of Denmark; (in total eight site-years), Petersen et al. (2012) also found that site conditions such as WT, pH and precipitation contributed significantly to explain N₂O emission dynamics.

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ExtremelyIn the study by Petersen et al. (2012), extremely high N₂O emissions corresponding to 38 and 61 kg N ha⁻¹ were observed from arable sites in two of the three regions <u>investigated studied by Petersen et al. (2012)</u>. There are <u>several</u>. <u>Several</u> processes, <u>which</u> can lead to N₂O formation in acid organic soil: <u>Bioticbiotic</u> processes include ammonia oxidation by archaea or bacteria (Herrmann et al., 2012; Herold et al., 2012; Stieglmeier et al., 2014), as well

as nitrifier denitrification and heterotrophic denitrification by bacteria or fungi (Liu et al., 2014; Maeda et al., 2015; Wrage-Mönnig et al., 2018). Abiotic N₂O production can occur through chemodenitrification (Van Cleemput and Samater, 1996; Jones et al., 2015) or abiotic codenitrification (Spott et al., 2011). Characterising-soil profiles with respect to potential electron donors and acceptors of putative chemical or microbial processes may inform about the potential role of individual pathways, which can then be evaluated in controlled experiments (Jørgensen et al., 2009). The sites showing extreme N₂O emissions The two regions showing extreme N₂O emissions from arable soil had both developed from marine forelands and were categorised as potentially acid sulfate soil, i.e., saturated to poorly drained soil containing pyrite (FeS₂) that, upon oxidation, eouldmay lead to acid production in excess of the soil's neutralising capacity (Madsen and Jensen, 1988). The capillary fringe of organic soils represents an interface between saturated and unsaturated soil conditions, and it was speculated that oxidation and reduction of iron sulfides could have interacted with N transformations during periods with changing groundwater level. (Petersen et al., 2012). Drainage will promote oxidation of FeS₂, a process which may be linked to microbially mediated nitrate (NO₃⁻) reduction (Jørgensen et al., 2009; Torrento et al., 2010). The complete reduction of NO₃⁻ to dinitrogen (N₂) can proceed as follows:

$$30NO_3^- + 10FeS_2 + 20H_2O \rightarrow 15N_2 + 20SO_4^{2-} + 10Fe(OH)_3 + 10H^+$$
(1)

However, in the capillary fringe residual oxygen or, alternatively, the acidification produced by FeS_2 oxidation, could favour incomplete denitrification with accumulation of the intermediate N_2O (Torrento et al., 2010):

$$30NO_3^- + 8FeS_2 + 13H_2O \rightarrow 15N_2O + 16SO_4^{2-} + 8Fe(OH)_3 + 2H^+$$
(2)

Nitrate reduction *via* the reaction described in Eq. 2 could potentially have contributed to the very high N₂O emissions reported by Petersen et al. (2012) from the two arable sites, where groundwater sulfate concentrations were also consistently high.

We studied four agricultural sites within one of the regions previously studied by Petersen et al. (2012), i.e., a
raised bog area with acid soil conditions, i.e., The study included two sites with rotational grass and two sites with a potato crop. The, and the study covered spring and autumn periods, where high emissions of N₂O were observedoccurred in previous studies (Petersen et al., 2012; Kandel et al., 2018). Dynamic variables, including N₂O emissions, soil mineral N, temperature and WT level, were monitored weekly, whereas reactive iron and iron sulfides were determined in soil profiles collected in April and/or September. We hypothesised that FeS₂ oxidation coupled with NO₃⁻ reduction was a potentialpossible driver of N₂O emissions, but more so in fields with a potato crop than in undisturbed grasslands with a continuous plant cover, where the plant N uptake is more efficient. It was further hypothesised that seasonal variation in N₂O emissions could be explained as interactions of environmental factors (soil temperature, precipitation, WT depth)would vary with site conditions affecting denitrification (mineral N availability.₃

100 2 Materials and methods

rainfall, WT depth and temperature).

2.1 Study sites

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The sites selected forinvestigated in this study were located in Store Vildmose, which is a 5,000 ha raised bog in northern Jutland, Denmark. The area was, until 150 years ago, the largest raised bog in Denmark, and largely unaffected by human activity. The bog overlies a marine plain formed by the last marine transgression; the sea retreated around 8000 BC, and peat later developed in wet parts of the landscape, attaining a maximum depth of 4.5 to 5.3 m in central parts of the bog (Kristensen, 1945). Between 1880 and 2010, the peat has generally subsided by at least 2 m due to drainage for agriculture or peat excavation (Regina et al., 2016), and today the peat depth is mostly 1-2 m, but in some locations even less (Kandel et al., 2018). The peat and underlying sand is acidic and has been categorised as a potentially acid sulfate soil (Madsen and Jensen, 1988). According to Kandel et al. (2018), the peat at 0-25 cm depth in arable soil in this area has a high degree of humification at H8 on the Von Post scale.

Four sites were selected that were distributed along an east-west transect (Figure 1a). One arable site (*AR1*) was in <u>a field</u> cropped with second-year potato in 2015, while the adjoining an adjacent site (*RG1*) in a neighbouring field had second-year rotational grass; these two sites were also represented in a previous monitoring program (the study of Petersen et al., (2012) as sites *N-AR* and *N-RG*, respectively. Land use treatments (i.e., potato and rotational grass) were replicated at other sites in other fields, referred to as *AR2* and *RG2*; site *AR2* was located 4.6 km to the west, and site *RG2* was located 1.7 km to the east of the paired *AR1-RG1* sites (Figure 1a and S1).

2.2 Experimental design

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In January 2015, an area of 10 m \times 24 m was defined at <u>the location of</u> each site. Sampling positions were georeferenced using a Topcon HiPer SR geopositioning system (Livermore, CA). On 25 February 2015, each site was fenced, and three 10 m \times 8 m experimental blocks <u>were</u> defined (Figure 1b). Each site was further divided along its longitudinal axis to establish two 5 m \times 24 m fertilisation subplots.

For monitoring of WT depth, piezometer tubes (Rotek A/S, Sdr. Felding, Denmark) were installed to 150 cm depth at the centre of each block. On either side of the piezometers, at 2.7 m distance, collars of white PVC (base area: 55 cm \times 55 cm, height: 12 cm [*RG*] or 24 cm [*AR*]) were installed to between 5 and 10 cm depth (Figure 1). The higher collars used at *AR* sites were at level with the ridges during the growth period. The collars, which were fixed to the ground by four 40 cm pegs, had a 4 cm wide flange extending outwards 2 cm from the top to support gas flux chambers. PlatformsTo prevent soil disturbance during gas sampling, platforms (60 cm \times 100 cm) of perforated PVC were placed in front of each collar asto create a boardwalk-to prevent soil disturbance during gas sampling. The exact headspace of each collar was determined from 16 individual measurements of distance from the upper rim; this procedure was repeated whenever collars had been removed and reinstalled in order to enableaccommodate field operations.

Sets of five stainless steel diffusion probes for soil gas sampling at 5, 10, 20, 50 and 100 cm depth were installed vertically within 0.5 m of the flux measurement positions in two blocks (Block 2 and 3) at sites *AR1* and *RG1*, while at sites *AR2* and *RG2* diffusion probes were installed in onenly in block (Block 2). The stainless steel probes were constructed as described in detail by Petersen (2014), with a 10 cm³ diffusion cell connected to the surrounding soil *via* having a 3 mm diameter opening (at the sampling depth) that was covered by a silicone membrane, and which was connected to the soil surface via two 18G steel tubes with Luer Lock fittings (Figure S1).

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A HOBO Pendant Temperature Data Logger (Onset Computer Corp., Bourne, MA) was installed at 5 cm depth in Block 2 at each site. A mobile weather station (Kestrel 4500; Nielsen-Kellerman, Boothwyn, PA) was mounted at 170 cm height at site RG1 for hourly recording of air temperature, barometric pressure, wind speed and direction, and relative humidity. Daily precipitation was recorded at <10 km distance from the monitoring sites at a meteorological station, from where data to fill a gap in air temperature were also obtained.

Management within the fenced experimental sites followed the practices adopted by the respective farmers, e.g., with

2.3 Management

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respect to fertiliser application, grass cuts, potato harvest and soil tillage. One exception to this was N fertilisation, which was only given to one of the two subplots in each block (Figure 1b). Fertilised subplots of the RG1 site received 350 kg ha⁻¹ NS 27-4 fertiliser on 16 April (DOY106), corresponding to 94.5 kg N ha⁻¹. Site RG2 was fertilised with 20-25 Mg ha⁻¹ acidified cattle slurry (pH 6) on 5 May (DOY125), and again on 2 July (DOY183), each time corresponding to 90-110 kg total N ha⁻¹. After the second slurry application, RG2 further received 50 kg N ha⁻¹ as NS 27-4 fertiliser, which was applied by mistake to both fertilisation subplots. The AR1 site received 100 kg N ha⁻¹ as liquid NPS 20-3-3 150 fertiliser on 21 May (DOY141), while the AR2 site received 110 kg N ha⁻¹ as NS 21-24 pelleted fertiliser on 30 April (DOY120). The NS fertilisers contained equal amounts of ammonium (NH4+) and nitrate (NO3-), while N in the NPS fertiliser was mainly as NH4+.

At the RG1 site, the grass was cut in late August, while at the RG2 site the grass was cut in late June and on 9 September (DOY252). Potato harvest at the AR1 site took place in mid-September; (DOY 258), with interruptions due to heavy rainfall. At the AR2 site, the potato harvest took place on 23 September (DOY266).

2.4 Field campaigns

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A monitoring program was conducted during spring, from 3 March (DOY63) to 16 June (DOY169), and during autumn, from 3 September (DOY245) to 10 November (DOY314). Weekly measurement campaigns were conducted at each of the four sites insofar as field operations permitted. Thus, dDuring spring, there were 14, 12, 14 and 15 weekly campaigns at the RG1, AR1, RG2, and AR2 sites, respectively, the differences being due to interruptions for field operations. During autumn, there were 10, 10, 7 and 10 weekly campaigns at the RG1, AR1, RG2, and AR2 sites, respectively. Two Field trips included sampling at two sites, either AR1 + RG1 or AR2 + RG2, and thus all four sites were visited during each two field trip, either ARI + RGI or AR2 + RG2 trips on consecutive days. Campaigns included registration of weather conditions and WT depth, soil sampling, soil gas sampling, and N2O flux measurements. With few exceptions, each campaign was initiated between 9:00 and 12:00; the order of sites visited in each trip alternated from week to week.

2.4.1 Climatic conditions

Air temperature, relative humidity and barometric pressure were logged at the weather station located at RG1. During field campaigns, the WT depth was first determined in each of the three piezometers using a Model 101 water level

170 meter (Solinst; Georgetown, Canada). At AR1 and AR2, WT depth in Block 3 was further recorded at 30-minute time resolution for a period during autumn using MaT Level2000 data loggers (MadgeTech; Warner, NH, USA). Soil temperatures at 5, 10 and 30 cm depth were measured in each block using a high precision thermometer (GMH3710, Omega Newport, Deckenpfronn, Germany), and in addition continuous measurements of soil temperature at 5 cm depth were collected in block 2 at each site using HOBO Pendant Temperature Data Loggers (Onset Computer Corp., Bourne, 127)

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2.4.2 Soil sampling

During all field campaigns, soil samples were collected separately from fertilised and unfertilised subplots by random sampling of six 20 mm-diameter cores to 50 cm depth. Each core was split into 0-25 and 25-50 cm depth, and the six subsamples from each depth were pooled. The pooled samples were transported back to the laboratory in a cooling box and stored at -20°C for later analysis of mineral N and gravimetric water content.

On 23 April (DOY113), and again on 2 September (DOY245), undisturbed soil cores (50 mm diameter, 30 cm segments) were collected to 1 m depth within 1 m distance from the positions of flux measurements in Block 3 of sites *RG1* and *AR1* (cf. Figure 1b). A stainless steel corer (04.15 SA/SB liner sampler, Eijkelkamp, Giesbeek, Netherlands) equipped with a transparent plastic sleeve was used. The steel corer's lower end was capped with a 4 cm long cutting

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head, and hence sampling depths were 0 to 30 cm, 34 to 64 cm and 68 to 98 cm. The intact cores were capped and sealed, and transported in a cooling box to the laboratory, where they were stored at -20°C.

2.4.3 Soil gas sampling

Soil gas samples were collected in 6 mL pre-evacuated Exetainers (Labco Ltd, Lampeter, UK) as described by Petersen (2014) and shown in Figure S2. In brief, the diffusion probes were flushed *via* the inlet tube with 10 mL N₂ containing
50 µL L⁻¹ ethylene (AGA, Enköbing, Sweden) as a tracer. A three-way valve, mounted on the outlet tube, was fitted with a 10 mL glass syringe and an Exetainer. The displaced gas was quantitatively collected in the glass syringe from where the soil gas sample, now partly diluted by the flushing gas, was transferred to the Exetainer. After gas sampling, the probe was flushed with 2 × 60 mL N₂ to remove ethylene, and the Luer Lock fittings were capped. Samples of the N₂/ethylene gas mixture used for sample displacement were also transferred directly to Exetainers for gas

195 chromatographic analysis (n = 3) as reference for the calculation of dilution factors (Petersen, 2014). Sampling for soil gas was done in parallel with flux measurements, but less frequently, sinceexcept when equipment had to be removed during periods with field operations. Due to damage of some probes during spring, it was decided to discontinue soil gas sampling in the unfertilised subplot at site *RG2*, which had by mistake received fertiliser on DOY 183.

2.4.4 Nitrous oxide flux measurements

Gas fluxes were measured with static chambers (60 cm × 60 cm × 40 cm) constructed from 4-mm white PVC and equipped with a <u>closed-cell</u> rubber gasket (Emka Type 1011-34; Megatrade, Hvidovre, Denmark) as seal during chamber deployment. Chambers were further equipped with a 12V fan (RS Components, Copenhagen, Denmark) for

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headspace mixing that was connected to an external battery (Yuasa Battery Inc.; Laureldale, PA), as well as a vent tube with outlet near the ground to minimise effects of wind (Conen and Smith, 1998; Hutchinson and Mosier, 1981). Also, chambers were equipped with an internal temperature sensor (Conrad Electronic SE; Hirschau, Germany), and a butyl rubber septum on top of each chamber for gas sampling. Handles attached to the top were used for straps fixing the chamber firmly against the collar. Gas samples (10 mL) were taken with a syringe and hypodermic needle immediately after chamber deployment, and then 15, 30, 45 and 60 minutes after closure. Gas samples were transferred to 6 mL Exetainer vials, leaving a 4 mL overpressure.

210 2.4.5 Soil analyses

Soil samples collected during the weekly campaigns were sieved (6 mm) and subsampled for determination of soil mineral N and gravimetric water content. Approximately 10 g fresh weightfield moist soil was mixed with 40 mL 1 M potassium chloride (KCl) and shaken for 30 min, and then filtered through 1.6 µm glass microfibre filters, Concentrations of NH4⁺ and NO2⁻ + NO3⁻ in filtered KCl extracts were determined by autoanalyser (Model 3; Bran+Luebbe GmbH, Norderstedt, Germany) using standard colorimetric methods (Keeney and Nelson, 1982). Gravimetric soil water content was determined after drying of soil samples at 80°C for 48 hours.

Additional soil characteristics were determined on the intact soil cores collected in April and September at AR1 and RG1. Five cm sections were subsampled from selected depths and analysed for water content, pH, electrical conductivity (EC), total soil organic C and N, and NO2⁻. Soil pH and EC were measured with a Cyberscan PC300 220 (Eutech Instruments; Singapore) in a soil:water solution (1:2.5, w/v). Total soil organic C and total N were measured by high temperature combustion with subsequent gas analysis using a vario MAX cube CN analyser (Elementar Analysensysteme GmbH; Langenselbold, Germany). Soil NO2-N concentrations were analysed in soil:water extracts (1:5, w/v) using a modified Griess-Ilosvay method (Keeney and Nelson, 1982). Total organic C and total N were further determined in bulk soil samples (0-25 cm and 25-50 cm depth) collected at RG2 and AR2 in the same weeks as sampling of intact cores took place at AR1 and RG1.

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The concentration of total reactive Fe (TRFe) at selected depth intervals was determined in the samples from both April and September samplings of intact soil cores. The analysis of TRFe was done using a dithionite-citrate extraction (Carter and Gregorich, 2007; Thamdrup et al., 1994) followed by Fe²⁺ analysis with the colorimetric ferrozine method, which included hydroxylamine as reducing agent (Viollier et al., 2000). The extraction dissolves free (ferric) Fe oxides (except magnetite, Fe3O4), as well as (ferrous) Fe in FeS, but not FeS2.

The intact soil cores, from the September sampling only, were further analysed for acid volatile sulfides (AVS) and chromium reducible sulfur (CRS) as indices of FeS and FeS2, respectively. Quantification of AVS and CRS was based on passive distillation adapted from Ulrich et al. (1997) and Burton et al. (2008). Briefly, 0.5 g soil and a trap with 4 mL alkaline Zn-acetate solution (5%) was placed in 120 mL butyl-stoppered (and crimp-sealed) serum bottles, which were evacuated (1 kPa) and pressurised with N2 (150 kPa) three times to remove O2, eventually leaving the

headspace with N2 at atmospheric pressure. Acid volatile sulfide (primarily FeS) was liberated and trapped as ZnS after injection of 12 mL anoxic 2 M HCl followed by sonication (0.5 h) and incubation (24 h) on a rotary shaker (20°C).

Using the same approach with replicate soil samples, combined AVS and CRS (primarily elemental S and FeS2) was trapped after injection of 12 mL 1 M Cr2+ in 2 M HCl, prepared by reduction of CrCl3 (Røy et al., 2014). Trapped sulfide (ZnS) in the two traps was measured colorimetrically using diamine reagent (Cline, 1969), and CRS was then calculated by difference.

The concentration of total reactive Fe (TRFe) at selected depth intervals was determined in the samples from both April and September samplings of intact soil cores. The analysis of TRFe was done using a dithionite-eitrate extraction (Carter and Gregorich, 2007; Thamdrup et al., 1994) followed by Fe2+ analysis with the colorimetric ferrozine method, which included hydroxylamine as reducing agent (Viollier et al., 2000). The extraction dissolves free (ferric) Fe oxides (except magnetite, Fe₂O₄), as well as (ferrous) Fe in FeS, but not FeS₂-

Finally, the total reduction capacity of the peat at depths of 27-30 cm, 61-64 cm and 95-98 cm was determined. In brief, a suspension (soil:solution, 1:25; w/v) of oven dried (105°C) sieved soil (<2 mm) and 25 mM cerium (IV) sulfate reagent, Ce(SO₄)₂ in 5% sulfuric acid (H₂SO₄)₂ was shaken horizontally for 24 h at 275 rounds per minute (rpm). After centrifugation at 2,000 rpm, residual Ce(IV) was measured by end-point titration using a solution of 5 mM FeSO4 in 5% H_2SO_4 . The amount of reduced compounds was calculated and expressed as meq kg⁻¹.

2.4.6 Gas analyses

Nitrous oxide concentrations were analysed on an Agilent 7890 gas chromatograph (GC) with a CTC CombiPal autosampler (Agilent, Nærum, Denmark). The instrument had a 2 m back-flushed pre-column with Hayesep P connected to 255 a 2 m main column with Poropak Q. From the main column, gas entered an electron capture detector (ECD). The carrier was N2 at a flow rate of 45 mL min⁻¹, and Ar-CH4 (95%/5%) at 40 mL min⁻¹ was used as make-up gas. Temperatures of the injection port, columns and ECD were 80, 80 and 325°C, respectively. Concentrations were quantified with reference to synthetic air and a calibration mixture containing 2013 nL L-1 N2O. Soil profile N2O concentrations were frequently at several hundred µL L-1; linearity of the EC detector response was ascertained up to 1600 µL L-1, but the 260 entire range was not included in analytical runs as a standard practice, and therefore the higher equivalent gas phase concentrations are relatively uncertain.

Ethylene concentrations in soil gas samples and flushing gas were analysed following a separate injection with an extended run time. All GC settings were as described earlierabove, except that run time was different, and gas from the main column was directed to a flame ionisation detector supplied with 45 mL min⁻¹ H₂, 450 mL min⁻¹ air, and 20 mL min⁻¹ N₂; the detector temperature was 200°C.

2.5 Data processing and statistical analyses

Individual N2O fluxes were calculated in R (version 3.2.5, R Core Team, 2016) using the package HMR (Pedersen et al., 2010). This program analyses non-linear concentration-time series with a regression-based extension of the model of Hutchinson and Mosier (1981), and linear concentration-time series by linear regression (Pedersen et al., 2010).

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270 Statistical data (*p* value, 95% confidence limits) are provided by HMR for both categories of fluxes. The choice to use a linear or non-linear flux model was made based on scatter plots and the statistical output.

The temporal dynamics of N₂O fluxes were analysed using a generalised linear mixed model defined with the identity link function, the gamma distribution (see Jørgensen and Labouriau, 2012; McCullagh and Nelder, 1989), and Gaussian random components. The model contained a fixed effect representing the interaction between crop,

275 fertilisation and sampling day, and random effects representing site and sampling position. Standard Gaussian linear mixed models could not be applied, since these models failed to pass standard model control checks (e.g., the Shapiro-Wilk test of normality applied to the residuals and the Bartlett test). Moreover, standard transformations such as logarithm, square root, inverse, and the entire family of Box Cox transformations, also failed to pass the basic model control techniques referred to above. Cumulative N₂O emissions were therefore estimated and treatment effects The
 280 model for daily N₂O emission described above was used to estimate cumulative emissions by integrating the flux curves over time. Treatment effects were then analysed by specially designed linear contrasts as described in detail by Duan et al. (2017), who showed that models with untransformed responses (when using adequate distributions) allow simple statistical inference of the time-integrated N₂O emissions.

The dependence structure of variables that were potential drivers of N₂O fluxes were studied using the a class of multivariate models called "graphical models" (Whittaker, 1990, see also Labouriau et al., 2008a,b; and Lamandé et al., 2011 for applications in soil science). These models represent the dependence of variables using an undirected graph (not to be confounded with the word "graph" used to refer to a plot), i-e-, which is the a mathematical structure composed of *vertices*, represented by points, and *edges* connecting pairs of vertices, represented by lines connecting points, according to the convention explained below. In graphical models, the variables of interest are the vertices of the

- 290 graph (represented as labelled points). Here the variables used were: soil temperature at 5 cm depth (Temp5); soil temperature at 30 cm depth (Temp30); NH₄⁺ and NO₃⁻ concentrations in the top soil (AmmoniumT and NitrateT); N₂O concentration of the soil gas diffusion probe closest to, but above the WT, i.e., in the capillary fringe (N₂OWT); and finally, the N₂O flux (N₂O-flux). The dependence structure of these variables was characterised by the conditional covariances between each pair of variables given the other variables. Those conditional covariances were
- 295 simultaneously estimated using the available data according to a statistical model. The graph representation of the model is constructed by connecting the pairs of vertices (i.e., pairs of variables) by an edge when the conditional correlation of the two corresponding variables, given all the other variables, is different from zero. It is possible to show that two variables directly connected in the graph carry information on each other that is not already contained in the other variables (see Whittaker, 1990, Jørgensen and Labouriau, 2012). Moreover, the absence of an edge connecting
- two vertices indicates that (even a possible) association between the two corresponding variables can be entirely explained by the other variables. According to the general theory of graphical models, if two groups of variables, say A and B, are separated in the graph by a third group of variables, say C (i.e., every path connecting an element of A with an element of B necessarily contains an element of C), then A and B are conditionally uncorrelated given C (see Lauritzen, 1999). This property, called the separation principle, will beas used below to draw non-trivial conclusions on the interrelationship between N₂O-flux related variables. The graphical models were inferred by finding the model that minimised the BIC (Bayesian information criterion, i.e., a penalised version of the likelihood function) as implemented

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in the R package gRapHD (Abreu et al., 2010). This inference procedure yields an optimal representation of the data in the sense that the probability of correct specification of the model, when using this <u>penalizationpenalisation</u>, tends to one as the number of observations increases (see Haughton, 1988). The confidence intervals for the conditional

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one as the number of observations increases (see Haughton, 1988). The confidence intervals for the conditional
 correlations were obtained by a non-parametric bootstrap procedure (Davidson and Hinkley, 1997) with 10,000
 bootstrap samples. Separate analyses were conducted for each combination of season and crop, since different
 dependency patterns appear in those groups.

3 Results

315 3.1 Climatic conditions

In 2015, the annual mean air temperature in the area of this study was 8.7°C, and annual precipitation was 920 mm. This was slightly above the ten-year (2009-2018) average temperature of 8.3°C, and well above the ten-year average annual precipitation of 798 mm. During the spring monitoring period, the daily mean air temperature varied between 1 and 15°C, with an increasing trend over the period, and total rainfall was 220 mm. During the autumn monitoring period, the daily mean air temperature declined from 15 to 5°C, and total rainfall was 148 mm; the most intense daily rain events during spring and autumn were 16.9 and 33.2 mm, respectively. For 2015 as a whole, the annual mean air temperature in the area was 8.7°C, and annual precipitation was 920 mm.

Soil temperature at 5 cm depth showed a clear diurnal pattern (Figure S3), but at all four sites the temperature at the time of chamber deployment was close to the daily mean temperature at this depth. Thus, across the four sites the average deviation ranged from 0.2 to 0.9°C, and the largest deviations on a single day were -2.0 and 2.1°C, respectively.

3.2 Soil characteristics

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Soil characteristics were determined by analyses of intact cores collected in late April (DOY 113) 2015 (Table 1). At all sites the soil was acidic, with pH ranging from 4.7 to 5.4. At the paired sites *AR1* and *RG1*, a weak decline in pH was indicated at 40-50 cm depth. Electrical conductivity at *AR1* and *RG1* sites ranged from 0.15 to 0.91 mS cm⁻¹, with no obvious trends in the data; the highest value (0.91 mS cm⁻¹) occurred at site *AR1* at 93-98 cm in a layer dominated by sand underlying the peat.

The organic matter composition of soil profiles at the four sites varied. Total organic C concentrations at sites *AR1* and *RG1* were 34-43% in the upper 0-40 cm, but then dropped to only 0.3-0.6% at *c*. 1 m depth in the sand. The peat was amorphous and well-decomposed at 0-20 cm depth, while the underlying peat was dominated by intact plant debris. At site *RG2*, the process of peat degradation was evident even at 0-50 cm depth, where TOC concentrations only just met the requirements for being defined as an organic soil. *At RG2*, i.e., the organic C content was below 20 and 10% at

0-25 and 25-50 cm depth, respectively. Site AR2 was characterised by a uniform peat layer (33-38% organic C) at 0-50 cm depth. The Across all sites, the C:N ratios ranged between 14 and 26 in the organic soil layers.

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Two iron sulfide fractions, as well as total reactive iron, were quantified. Acid volatile sulfide ranged from 1.7 to $4.9 \ \mu g \ S \ g^{-1}$ soil across the four sites and showed no clear relationship with soil depth. This was also the case for CRS, which ranged from 24 to 155 μ g S g⁻¹ dry weight soil. Total reactive Fe (TRFe) concentrations in soil profiles from sites ARI and RGI ranged from 1.19 to 4.99 mg g⁻¹ dry weight soil at 0-50 cm depth, and hence concentrations of reactive Fe were up to 25001500 times higher than concentrations of Fe in AVS (assuming this was FeS), and 25-12090 times higher than Fe in CRS results-(assuming this was FeS2). At sites AR1 and RG1, TRFe declined below 20 cm depth and was close to zero in the sand below the peat layer (Table 1).

The highest concentrations of TRFe at sites RG1 (Figure 2b) and AR1 (Figure 2d) occurred at 20 cm depth on 23 April. At site AR1, a sink for TRFe at 40-60 cm depth was indicated. There were only minor differences in the distribution of TRFe between seasons. There was a strong correlation between TRFe and TOC across all sites (r = 0.88, *n* = 16).

The total reduction capacity was determined by a wet oxidation procedure using Ce(SO₄)₂. At both AR1 and RG1, the total reductionreductive capacity of the peat at 27-30 cm depth was outside the range of the analytical method, but at >11,500 meq kg⁻¹. The reduction capacity dropped to around 1000 meq kg⁻¹ at 60 to 65 cm depth with thea declining organic matter content, and 50 to 100 meg kg⁻¹ in the sandy layer at 100 cm depth.

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3.3 Soil mineral N dynamics

Soil concentrations of NH4⁺ and NO3⁻ at 0-25 and 25-50 cm depth were determined in connection with field campaigns (Tables S1-S4). The residence time for mineral N in the soil solution was generally longer at AR compared to RG sites. At AR sites, there was an accumulation of mineral N (Table S2, S4) at both depth intervals during May (Table S2, S4), although, also before N fertilisation. Mineral N concentrations at ARI-were much-greater than at ARI compared to AR2, and at site AR2 only NO3⁻ accumulated; high concentrations in the fertilised subplot in May was due an external input of fertiliser Nr. Fertilisation increased NH4+-N and NO3-N concentrations to generally-100-200 µg g⁻¹ dry weight soil at all sites except RG2 (Table S3), where acidified cattle slurry was surface applied. It is not clear if the slurry infiltrated to >50 cm, or if plant uptake was very effective. applied. The residence time for mineral N in the soil solution was 365 generally longer at AR compared to RG sites. Accumulation of NO3⁻ in the weeks after fertilisation was observed at all sites, and also there was evidence for some transport to 25-50 cm depth.

Nitrite-N concentrations were determined in undisturbed soil collectedprofiles from the cores sampled at sites RG1 and ARI on 23 April (DOY 113) and 2 September (DOY 245) 2015. Both fertilised and unfertilised subplots were represented, although at site AR1 the fertilisation had not yet taken place at the time of sampling in April. There was variation at depth in the soil, which could not be explained by fertilisation. In April, the concentration of NO2-N at

both sites was highest (c. $10 \ \mu g g^{-1} dry$ weight soil) around 40 cm depth and declined towards the surface and deeper layers (Figure 2a,c). A decline in NO₂⁻-N concentration was indicated at 50 cm depth at site *AR1* relative to site *RG1*, and also a depletion of TRFe was indicated. However, there was also a lower concentration of peat (cf. TOC in Table 1), which may account for this difference. In September, NO₂⁻-N concentrations were <1 $\mu g g^{-1} dry$ weight soil at both sites, while the much higher concentrations of TRFe were comparable to those in April.

3.4 Groundwater table dynamics

Across the four sites, WT changes ranged from 60 to 100 cm. During spring, WT depth at sites *RG1* and *AR1* ranged varied betweenfrom 17 toand 81 cm, with a steady decline until the end of April (DOY120) that was followed by a period with fluctuations around 60-80 cm depth due to frequent rainfall (Figures 3 and 4). During the first half of September (DOY246 to 259), rainfall caused the WT to rise from 80 to 40 cm depth (Figures 5 and 6). The continuous measurements of WT depth (data not shown) revealed, however, that onOn two occasions (DOY248 and 260) the WT depth rose to 20 cm depth and only gradually declined during the following days: (data not shown). From mid-September there(DOY 258) then followed a period with a gradual WT decline until early November; (DOY 308), where upon the WT rose from 90 to 45 cm depth during a week with intense rainfall.

At site *RG2*, the WT was mostly at 50-60 cm depth during spring, with a temporarilytemporary rise to 30 cm depth by 3 June (DOY139; see Figure 3). In the autumn, sampling campaigns could not be initiated until DOY260 due to harvest. By this time, the WT was close to the surface following intense rainfall, but then declined to 80-100 cm in the sandy subsoil (Figure 5). The WT at site *AR2* was consistently between 45 and 60 cm depth during spring except for a transient increase to 35 cm depth in early June (Figure 4). During autumn, the WT rose to the soil surface in September (DOY260), and then gradually withdrew until early November (DOY 307) when rainfall caused a *c*. 40 cm increase (Figure 6), as also observed at sites *RG1* and *AR1*.

3.5 Soil N₂O concentration profiles

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The distribution and temporal dynamics of N_2O in the soil profiles showed important contrasts between grassland and arable sites. Equivalent gas phase concentrations of N_2O , as determined by in passive diffusion samplers, were determined concurrently with gas sampling, and results are presented as contour plots (Figures 3-6) based on; data compiled in Table S5. A logarithmic grey scale had to be used in order to show trends within both *RG* and *AR* treatments, as concentrations sometimes differed by). Concentrations in many cases varied by several orders of magnitude, this was also true between sites and sampling days, and between depths within individual profiles in many cases. Some, and therefore a logarithmic grey scale was used to show trends. The gaps occurin Figures 3-6 indicate periods, where diffusion probes could not be installed or were temporarily removed due to field operations.

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Under the rotational grass at site RG1, soil N₂O concentrations during spring were mostly between 0.1 and 3 μ L L⁻¹ (Figure 3). A higher concentration (15 μ L L⁻¹) was observed at 40-80 cm depth in the fertilised subplot around DOY139, but only in Block 3 of the field plot. At site RG2, the concentrations of N₂O in the soil during spring were generally similar to those of RG1, although there were more values in the 1-10 μ L L⁻¹ concentration range (Table S5).

405 However, on 3 June (DOY154) a significant increase in N₂O concentration occurred in the fertilised part of the plot with a maximum of 560 µL L⁻¹ at 100 cm depth (i.e., well below the WT). This occurred during a period with frequent rainfall and could have been caused by NO₂⁻ leaching from the top soil. Soil N₂O concentrations in the unfertilised plot also increased around this time, but only to c. 15 µL L⁻¹ and mainly near the soil surface.

During autumn, N₂O concentrations in the soil profile at the *RG1* and *RG2* sites varied between 0 and 12 μ L L⁺ independent of fertilisation and with a tendency for highest concentrations at 10-20 cm depth (Figure 5).

The arable site *AR1*, with sampling positions located in a different field, but only 10-20 m from those of site *RG1*, showed very different soil N₂O concentration dynamics during spring (Figure 4). There was a consistent accumulation of N₂O at 50 and 100 cm depth where seasonal concentrations averaged 340 and 424 μ L L⁻¹, respectively. In contrast, at 5, 10 and 20 cm depth the average N₂O concentrations were 10-30 μ L L⁻¹, and there was no clear response to

fertilisation on DOY141 in terms of soil N₂O accumulation. There The soil N₂O concentrations suggested that there was significant considerable within-site heterogeneity in soil conditions, and as the highest concentrations were observed in the unfertilised subplot. Between DOY75 and DOY100, the concentrations of N₂O peaked at nearly 1500 μ L L⁻¹ at 50 cm depth and were 2-3 fold higher than at 100 cm depth, indicating that N₂O was produced in the capillary fringe as WT in this period was around 60 cm depth. At site *AR2*, the highest soil N₂O concentrations during early spring were consistently observed at 20 cm depth, but then gradually declining to reach the background level of 0.3 μ L L⁻¹ in mid-May (around DOY130). In the unfertilised field plot, the N₂O concentration then increased again at 20 cm depth to reach 272 μ L L⁻¹ following rainfall, and a WT rise to 35 cm depth. With fertilisation, soil N₂O concentrations were even higher at 10 cm depth and reached nearly 400 μ L L⁻¹ in mid-June.

During autumn, N₂O concentrations in the soil profile at the *RG1* and *RG2* sites varied between 0 and 12 μ L L⁻¹, with a tendency for higher concentrations at 10-20 cm depth (Figure 5). At site *RG1*, where both fertilised and unfertilised subplots could be sampled, this was independent of fertilisation.

September was characterised by heavy rainfall (114 mm in total), and at site *AR1* a substantial rise in the WT from 80 to 40 cm depth was observed (Figure 6). Soil N₂O concentrations showed a dual pattern, with maxima at 10 and 100 cm depth through to DOY266 (end of September), and after this time soil N₂O rapidly declined as the WT withdrew.
Nitrous oxide concentrations equivalent to several hundred μL L⁻¹ were measured even at 5 cm depth during this period. During late autumn, the N₂O concentration at 0-50 cm depth varied between 0 and 20 μL L⁻¹, whereas at 100 cm depth it remained high at 100-850 μL L⁻¹. At site *AR2*, the groundwater level was higher than at *AR1* and reached the soil surface by mid-September₅ (DOY 260). Soil N₂O accumulated in both fertilised and unfertilised subplots following saturation of the soil, again with the highest concentrations at 20 cm depth. A secondary increase was observed near the soil surface at the last sampling on DOY314 in November, in response to a period with rainfall and a rapid WT rise.

3.6 Nitrous oxide emissions

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The weekly sampling campaigns during spring and autumn showed much higher N₂O emissions at arable compared to grassland sites independent of season and fertiliser N application. At site *RG1*, N₂O emissions during spring ranged

from 0 to 550 μg N₂O m⁻² h⁻¹, with no effect of fertiliser amendment (Figure 3). Growth of the <u>The</u> grass swardin the
 <u>fertilised subplot</u> showed a strongclear response to fertilisation (not shown), and presumably there was a rapidthat
 <u>indicated</u> uptake of <u>thefertiliser N-added</u>. At site *RG2*, however, a peak in N₂O emissions occurred on DOY154, and the flux was still elevated at the next two samplings. This high flux coincided with the <u>elevated-accumulation of N₂O in the</u> soil profile N₂O concentrations-described above.

At site *AR1*, the N₂O fluxes were generally much higher than at the *RG1* site grassland sites during spring (Figure 4).
Fluxes during early spring reached 2000-6000 μg N₂O m⁻² h⁻¹ and were higher than in late spring where, as for site *RG1*, no effect of N fertilisation was observed. Hence, the higher emissions were associated with soil conditions and notsite differences other than fertilisation. The potato field at site *AR2* showed a different pattern, with N₂O fluxes remaining low during early spring, and for several weeks after fertilisation. The highest emissions occurred, independent of fertilisation, in June whenfollowing a WT rise to 35 cm depth was observed n DOY154.

In the autumn, N₂O fluxes from site *RG1* were consistently low (Figure 5). The first sampling at site *RG2* was on DOY260DOY259 in mid-September, where a high flux of 3000 μg N₂O m⁻² h⁻¹ was seen, which dropped to near zero within 1-2 weeks. Nitrous oxide emissions at site *AR1* were high during September at 4000-10,000 μg N₂O m⁻² h⁻¹ independent of N fertilisation_τ(<u>Table S2</u>), and subsequently declined to near zero (Figure 6). The highHigh fluxes coincided with a rise in were observed on the WT from 80 to 40 first sampling day of this monitoring period, DOY246, while WT depth was still at 40 to 80 cm depth_τ. However, this followed 10 and 22 mm rainfall on the previous two days. Rainfall the following days then was accompanied by a rise in WT. The subsequent decline in fluxes N₂O emissions at *AR* sites coincided with WT withdrawal. At site *AR2* the pattern in N₂O emissions was similar, and again the dynamics of N₂O fluxes aligned with WT dynamics.

Cumulative N₂O emissions were calculated for the 99-105 days of monitoring in spring, and for the 47-69 d period in autumn (Table 2). At *RG* sites, the average N₂O flux from fertilised grassland was significantly higher than from unfertilised grass (7.3 *vs.* 2.0 kg N₂O ha⁻¹) during spring. At *AR* sites with potato, there was no significant effect of N fertilisation, but the cumulative N₂O emissions of 15-17 kg N₂O ha⁻¹ were much higher than from *RG* sites. In the autumn, the there were no residual effects of N fertiliser application in spring, and average cumulative emissions at the *RG* and *AR* sites were 2 and 15 kg N₂O ha⁻¹, respectively.

465 3.7 Interrelationships between driving variables of N₂O production

Graphical models were used to study the dependence structure among selected soil variables and N₂O fluxes.
Interestingly, at *RG* sites in both spring (Figure 7a) and autumn (Figure 7b), and at *AR* sites in spring (Figure 7c), the only variable with a direct link to N₂O flux was soil N₂O concentration in the capillary fringe (N₂O_{WT}), indicating that N₂O_{WT} carried information on the N₂O flux that could not be explained by indirect correlations between the other variables. Moreover, the variable N₂O_{WT} separated N₂O flux from the other variables in the graph which, according to the separation principle (an instance of the general theory of graphical models), indicates that information about this variable rendered all the other variables uninformative with respect to N₂O flux. For example, in the analysis of *AR* sites in spring (Figure 7c), the variables N₂O flux and Temp5 were not directly connected, and therefore any correlation

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between Temp5 and N₂O flux could be completely explained by other variables. The only exception to this pattern was AR sites in the autumn (Figure 7d), where instead two other variables showed a significant relationship with N₂O flux; one variable was NitrateT, i.e., NO₃-N concentration in the top soil, and the other variable was soil temperature at 30 cm depth. All other relationships were unrelated to N₂O flux, or could be accounted for by other variables.

4 Discussion

We<u>This study</u> investigated seasonal dynamics of N₂O emissions and soil conditions in a region in Northern Denmark that wasan area, which has been designated as a hotspot for N₂O emissions in a meta-analysis of organic soils across Europe (Leppelt et al., 2014). Spring and autumn monitoring periods together covered <u>between</u> 152- and 174 d, and cumulative N₂O emissions during these periods were in total 3-6 kg N₂O-N ha⁻¹ for rotational grass, and 19-21 kg N₂O-N ha⁻¹ for arable sites with a potato crop. These numbers indicate, representing <6 month periods, thus confirmed
previous results (Petersen et al., 2012) that annual N₂O emissions werein this area are comparable to (*RG*), or clearly above (*AR*), the IPCC emission factors for drained organic soil of 8 and 13 kg N₂O-N ha⁻¹ yr⁻¹ for nutrient rich grassland and cropland, respectively (IPCC, 2014). Hence, the observations confirmed that organic soil drained for agriculture in this region constitutes a high risk for N₂O emissions, but also showed that this risk depends on land use. The area has been characterised as potentially acid sulfate soil (Madsen and Jensen, 1988), and a previous study showed groundwater sulfate concentrations in excess of 100 mg L⁻¹ (Petersen et al., 2012). We therefore hypothesised that NO₅⁻ reduction coupled with FeS₂ oxidation could be a pathway of N₂O formation in this acid organic soil,

Leppelt et al. (2014) concluded that high N₂O emissions are associated with cropped land having a pH below 4.7, C:N ratios below 30-35, and WT depths of 0.2–0.9 m, and they found a significant positive relationship with annual precipitation. This sites investigated here largely fit this description, but the specific mechanisms behind high N₂O emissions are not easily derived from average annual conditions. The present study was therefore planned to examine high emission periods at higher spatial and temporal resolution to elucidate environmental controls and possible pathways, such as FeS₂ oxidation being a driver of N₂O emissions.

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4.1 Nitrous oxidePyrite, measured as CRS, was quantified at selected depths (Table 1), and with bulk density of the peat varying between 0.15 and 0.3 g cm⁻³ (data not shown), the total amount of CRS at 0 to 50 cm depth would thus be 200-350 mmol FeS₂ m⁻². The N₂O emissions observed during spring and autumn monitoring periods constituted up to 145 mmol N m⁻² in total (site *ARI*), and it is thus theoretically possible that the process described by Eq. 2 contributed to emissions of N₂O. However, the FeS₂ concentration (0.7-2.4 mmol kg⁻¹) represented a minor part of the total reduction capacity (>11,500 meq kg⁻¹ at 27-30 cm depth). Also, the concentration of total reactive Fe was 25-120 times higher than that of FeS₂ (though less in terms of reduction equivalents). Reducing agents other than FeS₂ were therefore likely to be more important, a conclusion that was later supported by a laboratory study in which peat amended with FeS₂ did not show enhanced N₂O production (Taghizadeh-Toosi et al., submitted).

4.1 Environmental drivers of N2O emissions and water table dynamics

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The regulation of N₂O emissions was investigated using a statistical method represented by graphical models. It identified N2O concentration in the capillary fringe as the strongest predictor of N2O emissions from both grassland and 510 arable soil in spring, and from grassland soil in the autumn. The implication is that N transformations at depth in the soil, and not in the top soil, were the main source of N₂O escaping to the atmosphere in these cases. In accordance with this, there was no immediate effect of N fertilisation on emissions of N2O independent of land use. Other studies also found a limited response to fertilisation (Maljanen et al., 2003; Regina et al., 2004), although Regina et al. (2004) later observed a peak in N2O emissions after rainfall. Goldberg et al. (2010) reported that N2O emissions from a 515 minerotrophic fen were produced at 30-50 cm depth, in accordance with the observations presented here, where the highest concentrations of N₂O were mostly observed at 20 or 50 cm depth (Table S5).

Peat decomposing in the capillary fringe during WT drawdown could have been the source of N for N₂O production. It is well established that N₂O emissions from organic soil may be enhanced by drainage (Martikainen et al., 1993; Taft et al., 2017). The), and the response will appear within days, as shown by Aerts and Ludwig (1997) in an 520 incubation study with an oscillating WT. A stimulation of N₂O emissions by WT drawdown was also observed by Goldberg et al. (2010) when simulating drought under field conditions, but in additionalthough a pulse of N₂O also occurred after rewetting. In the present study, the response to WT drawdown was complex, i.e., at sites RG1 and AR1 there was a stimulation of N2O emissions as WT declined in early spring, while this was not evident at sites RG2 and AR2. During autumn there was generally no effect of WT drawdown on N2O emissions. In contrastaccordance with this, 525 rising WT and/or increasing soil wetness in late spring and in the autumn resulted in a consistent increase in N2O emissions at all sites. Hence, the relationship between WT depth and N₂O emission showed seasonal patterns and sitespecific effects, which indicated that other soil properties modified the effect of WT on N2O emissionsearly autumn consistently enhanced N₂O emissions at all sites in the present study. Despite 32 mm rainfall on DOY244 and 245, the WT depth was still at 40 to 80 cm and could not account for the very high N2O emissions observed on DOY246 (Figure

530 6). Well-degraded peat will release as little as 10% of its water to drainage (Rezanezhad et al., 2016). It is therefore likely that the rain was absorbed by peat above the WT and created conditions suitable for denitrification.

4.2 Nutrient status and land use

The repeated-increase in N2O emissions afterduring WT drawdowncycles reported by Aerts and Ludwig (1997) was observed only with eutrophic peat, whereas a mesotrophic peat showed no effect of WT treatmentdynamics on N2O 535 emissions, which were consistently low. A similar interaction between nutrient status and WT depth was observed in field studies comparing N₂O emissions from minerotrophic and ombrotrophic boreal peatlands (Martikainen et al., 1993; Regina et al., 1996). Thus, nutrient status, and N availability in particular, was probably a driver for the higher N2O emissions at AR sites used for potatoes. The RG sites with rotational grass, in contrast, showed much lower N2O emissions despite similar soil conditions and N fertiliser input. In the present study, soil NH4+-N and NO3--N RG1 increased to 133 and 120 µg g⁻¹ dry weight soil upon fertilisation, respectively, concentrations at site but largely returned to the background level of around 5 and 10 μ g g⁻¹ dry weight soil, respectively, within a week (Table S1). In contrast, at site ARI there was significant accumulation of NH₄⁺-N and NO₃⁻-N even before fertilisation on DOY141, and soil mineral N remained high for several weeks (Table S2). This accumulation of soil mineral N

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around the time of potato crop establishment could have stimulated N₂O emissions in the arable soil. Grasslands on

545 organic soil generally show lower emissions of N2O compared to arable organic soil (Eickenscheidt et al., 2015; Petersen et al., 2012), presumably because plants compete successfully with microorganisms for available N. Schothorst (1977) estimated peat decomposition indirectly from the N-content in herbage yield of grassland and concluded that the soil supplied 96 kg N ha⁻¹ when the drainage depth was 25 cm, but 160 and 224 kg N ha⁻¹ with the WT in-drainage ditchesepth at 70 and 80 cm-depth, respectively. In the present study, soil NH4+-N and NO3-N 550 concentrations at RG1 remained mostly below 5 µg g⁺¹ dry weight soil except for a short period after fertilisation (Table S1). In contrast, at site ARI the NO3-N concentrations were mostly at 15-25 µg g⁺¹ dry weight soil during spring, and it declined more slowly after fertilisation, where soil mineral N peaked at c. 500 and 200 µg g⁻¹ dry weight at 0-25 and 25-50 cm depth, respectively (Table S2). This does indicate that the grass sward effectively took up N mineralised from soil organic matter above the WT. Hence, plant uptake of N mineralised from soil organic matter above the WT likely

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Independent of land use there was no immediate effect of N fertiliser application on emissions of N2O. Other studies also found a limited response to fertilisation (Maljanen et al., 2003; Regina et al., 2004), although Regina et al. (2004) observed a peak in N₂O emissions in late spring after rainfall.

4.3 Nitrogen dynamics and N2ONitrous oxide concentration in soil profiles

caused the much lower N2O emissions from rotational grass in this study.

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Only pooled soil samples from 0.25 and 25.50 cm depth were available for characterisation of provided indirect information about soil mineral N dynamics-(Table S1-S4), but additional information can be derived from soil N2O concentration profiles (Goldberg et al., 2008). The soil gas diffusion probes used in this study were installed vertically and thus did not disturb soil stratification prior to monitoring. At RG sites, soil N2O concentrations were generally low and did not provide clear evidence for microbial N transformations. In contrast, at AR sites there was during spring an 565 accumulation of N₂O in, which supports the soil; the highest concentrations at ARI-conclusion above that plant uptake was a main sink for the N released during peat decomposition. At site RG2 an accumulation of N₂O was seen at 1 m depth in late May (Figure 3), which could have been caused by leaching of mineral N from the acidified cattle slurry following extensive rain. In contrast, at AR sites there was significant accumulation of N₂O in the soil; at site AR1 the highest concentrations occurred at 50 to 100 cm depth, while at site AR2 the highest concentrations were at 20 cm 570 depth, in accordance with the higher groundwater table. This suggests that peat decomposition was a significant source

of mineral N, and that biotic or abiotic processes led to extensive N2O accumulation. At site RG2, accumulation of N2O at 1 m depth in late May suggested that mineral N from the acidified cattle slurry had leached from the top soil (Figure 3). Soil N₂O concentration profiles thus These observations indicated that emissions of N₂O at AR sites before fertilisation were due to an interaction betweenwas produced in the capillary fringe, consistent with peat decomposition 575 as a source of mineral N, and declining WT. Inpossibly also in the period followingsaturated zone (see next section). Following N fertilisation, the accumulation of N₂O in the soil profile was mostly associated with precipitation and rising WT.

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Precipitation was high during September 2015, and the rapid rise in WT toward the soil surface resulted in accumulation of N₂O in the top soil at all sites. However, N₂O concentrations peaked at around 10 µL L⁺ at RG sites, as opposed to several hundred µL L⁺ at AR sites. Soil NO3⁻N concentrations at 0-50 cm depth in early September (DOY145) were 5 µg g⁺ dry weight soil at site RG1 (Table S1), but 40-150 µg g⁺ dry weight soil at site AR1 (Table

S2), which could have supported denitrification activity. It is not clear if the source of NO₃⁻ was decomposing potato erop residues or accelerated peat decomposition following harvest, or both.

4.4 Environmental controls

The previous sections have indicated that effects of land use and climate on N₂O emissions (Leppelt et al., 2014; Mu et al., 2014) are modified by soil conditions that may vary across the year. We investigated possible drivers of N₂O emissions using a statistical method represented by graphical models, which identified N₂O concentration in the capillary fringe as the strongest predictor of N₂O emissions from both grassland and arable soil in spring, and from grassland soil in the <u>In the</u> autumn. The implication is that N transformations at depth in the soil, and not in the top soil (despite fertilisation in some treatments) were the main source of N₂O emissions from a minerotrophic fen were produced at 30–50 cm depth. Peat decomposing in the capillary fringe during WT drawdown could thus have been a driver of N₂O production, and indeed the highest concentrations of N₂O were mostly observed at 20 or 50 cm depth (Table S5). On the other hand, at site *AR1* the soil N₂O concentration was high even at 100 cm depth, indicating that N₂O was also

At the arable sites, the regulation of N2O emissions in the autumn was different from that in spring, since the graphical model identified NO3⁻ in the top soil, and soil temperature at 30 cm depth, as significant predictors of N2O emissions at arable sites (Figure 7), although it should be noted that). The accumulation of NO3⁻ was much greater at site AR1 compared to AR2-, suggesting differences in N mineralisation potentials. It is not clear if the source of N was 600 decomposing potato crop residues or accelerated peat decomposition following soil disturbance at harvest, or both. Rainfall most likely triggered denitrification at the AR sites, by rapidly increasing WT depth and soil water-filled pore space, thereby impeding the oxygen supply to much of the soil profile (Barton et al., 2008). This interpretation is supported by N_2O concentrations increasing dramatically around the WT depth N_2O concentrations below, as well as above the WT depth depending on site and block, and in fertilised as well as unfertilised subplots (Figure 6). In an 605 annual study, conducted in other parts of the Store Vildmose bog, Kandel et al. -In an annual study, conducted in other parts of the Store Vildmose bog, Kandel et al. (2018) also measured high peak emissions of N₂O from a potato cropping systemcrop, i.e., around 2000 µg N₂O m⁻² h⁻¹ in October 2014 and 6000 µg N₂O m⁻² h⁻¹ in June 2015, which coincided with NO3⁻ accumulation- and rainfall. Precipitation was also high during September 2015, and the rapid rise in WT toward the soil surface resulted in accumulation of N2O in the top soil at all sites. However, N2O concentrations reached 610 only around 10 µL L⁻¹ at RG sites, as opposed to several hundred µL L⁻¹ at AR sites, confirming that soil mineral N availability was a limiting factor for N2O emissions.

4.5 Possible pathways 2 Pathways of N2O formation

We hypothesised that NO₃⁻ reduction coupled with FeS₂ oxidation could be a pathway of N₂O formation in this acid organic soil, and FeS₂, measured as CRS, was quantified at selected depths (Table 1). Assuming a bulk density of peat
 615 in this area of 0.15 g cm⁻³ (Schäfer et al., 2012), the amount of CRS at 0.50 cm depth at site *AR1* would correspond to around 180 mmol FeS₂ m⁻², whereas the N₂O emission observed during spring and autumn monitoring periods together

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constituted 145 mmol N m². It is thus possible that the process described by Eq. 2 contributed to N₂O emissions; though probably not during spring, where N₂O emissions were unrelated to soil NO₂⁻ dynamics. The total reduction capacity of the peat was much higher than that represented by FeS₂, i.e., >11,500 meq kg⁺ at 27–30 cm depth. Also, the concentration of total reactive Fe was 25–90 times higher than that of CRS. Together this indicates that reducing agents other than FeS₂ were more important. Subsequent incubation experiments with addition of FeS₂ together with different electron acceptors also suggest that FeS₂ oxidation is not a driver of N₂O emissions in this peat soil (manuscript in preparation), and hence alternative pathways should be considered.

 Bacterial nitrification, denitrification, and nitrifier-denitrification are all potential pathways of N₂O formation
 (Braker and Conrad, 2011), and the). The significant relationship with NO₃⁻ at *AR* sites in the autumn (Figure 7) suggested that denitrification wasactivity in the main source in top soil controlled N₂O emissions during this period. <u>This was different</u> in early spring, where however, the emissions soil mineral N concentrations were more strongly related to N₂O accumulatinglow and N₂O accumulated near the WT depth. <u>Here, ammonia oxidation activity may therefore have controlled N₂O emissions either directly, or indirectly *via* production of NO₂⁻ or NO₃⁻. Ammonia
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630 oxidising bacteria (AOB) are scarce in acid peat despite the presence of nitrite oxidising bacteria (NOB) (Regina et al., 1996), and some studies indicate that ammonia oxidising archaea (AOA) predominate in both abundance and activity (Herrmann et al., 2012; Stopnišek et al., 2010). Stieglmeier et al. (2014) isolated an AOA from soil that emitted N₂O at a rate corresponding to 0.09% of the NO₂⁻ produced independent of O₂ availability, but it is not known if this organism is present in acid organic soil-, and at this time an indirect control of denitrification activity seems more plausible.

Stopnišek et al. (2010) found that AOA activity was not stimulated by an external source of NH₄⁺ and concluded that the activity was associated with N released from decomposing soil organic matter. Thus, in early spring the <u>The</u> anaerobic conditions of saturated peat may have been a limiting factor for N mineralisation and in turntherefore ammonia oxidation <u>activity during early spring</u>, a constraint which <u>could have beenwas</u> alleviated as the WT declined and oxygen entered deeper soil layers.

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Ammonia oxidation may drive N₂O emissions indirectly *via* production of NO₂⁻ or NO₃⁻. Nitrite had accumulated at 20-50 cm depth in late April at both *RG1* and *AR1* <u>sites</u> (Figure 2). This), which</u> was consistent with peat decomposition in connection with and ammonia oxidation following WT drawdown. Total concentrations of NH₃⁺ and NO₃⁻ at 25-50 cm depth were significant (Tables S1 and S2), but also suggestswell-decomposed peat is dominated by dead-end pores (Hoag and Price, 1997), and it is likely that ammonia oxidation to a large extent took place in such pores having a slow exchange of solutes with active pore volumes. The accumulation of NO₂⁻ suggested there was an imbalance between ammonia oxidation and nitrite oxidation activity. Estop-Aragonés et al. (2012) found that oxicanoxic interfaces in peat soil were located above the WT depth, and hence the capillary fringe in this study may have been <u>still</u> partly anoxic. Oxygen affinity differs between nitrifiers, with AOA>AOB>NOB (Yin et al., 2018), and thus oxygen limitation could <u>thus</u> have caused the accumulation of NO₂⁻. In acid soil, this would result in product inhibition by HNO₂, if there were no mechanism to remove NO₂⁻; this would be especially true for *AR* sites, where mineral N accumulation was three to four times higher compared to *RG* sites (Tables S3-S6). Nitrifier-denitrification is one mechanism by which ammonia oxidisers can avoid HNO₂ accumulation, and this process leads to N₂O formation (Braker and Conrad, 2011). Another potential sink for NO_2^{-} is chemodenitrification, an abiotic reaction in which NO_2^{-} reacts with Fe²⁺ to produce N₂O (Jones et al., 2015):

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$$4Fe^{2+} + 2NO_2^- + 5H_2O \to 4FeOOH + N_2O + 6H^+$$
(3)

where in Eq. 3 Fe(OH)₃ is shown as anhydrous FeOOH. Some depletion of TRFe was indicated at 50 cm depth at site *AR1*, which coincided with a similar depletion in NO₂⁻ (Figure 2). Nitrifier-denitrification and chemodenitrification are both sinks for NO₂⁻, and therefore both pathways wereare potential sources of N₂O emissions induring early spring.

The observation that TRFe concentrations were much higher than those of AVS or CRS (Table 1) was unexpected, but makes it relevant to consider alternative reactions withinvolving iron oxides/hydroxides, which have a potential to produce N₂O that involve iron oxides/hydroxides rather than FeS₂₇₂. One such recently described pathway is Feanmox, a process whereby ammonia oxidation coupled with ferric iron reduction can produce NO₂⁻ below pH 6.5 (Yang et al., 2012):

$$Fe(OH)_3 + 10H^+ + NH_4^+ \rightarrow 6Fe^{2+} + 16H_2O + NO_2^-$$
 (4)

665 Nitrate can also be produced under these conditions (Yang et al., 2012; Guan et al., 2018):

$$8Fe(OH)_3 + 14H^+ + NH_4^+ \to 8Fe^{2+} + 21H_2O + NO_3^-$$
(5)

A shuttle of Fe^{2+} between Feanmox and chemodenitrification (Eq. 3 and Eq. 4) could have caused explain the accumulation of N₂O under anoxic conditions in the saturated zone, where presumably the availability of NH₄⁺ from peat mineralisation would be a limiting factor. The confirmation of pathways will require more detailed investigations that should also involve include molecular analyses targeting microbial communities in the soil profile.

5 Conclusion

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As hypothesised, there was an effect of land use on N₂O-Nitrous oxide emissions, which were clearly higher from arable sites with a potato crop compared to rotational grassland-grass. This was independent of fertilisation, and instead 675 N2O emissions could be associated with soil N mineralisation, rainfall patterns and temperature, as hypothesized. There were strong seasonal dynamics in N2O emissions that were associated with WT dynamics. In spring there was no direct responseConcentrations of pyrite were low compared to the input of fertiliser N, and instead N2O emissions mainly reflected the accumulation of N2O near the WT. At sites used for a potato crop, NO3- accumulated after harvest and was significantly related to N2O emissions. Pyrite was present at low concentrations, and hence some N2O emission from 680 NO3⁻ reduction coupled with FeS2 oxidation could not be dismissed, at least in the autumn. However, the total reduction capacity of the peat-was much higher than that represented by FeS25, and reactive Fe was predominantly in forms other than pyrite, probably as oxyhydroxides. While the hypothesis, that N2O was produced by NO3 reduction coupled with FeS2 oxidation, could not be dismissed, it is likely that other processes were more important. There were strong seasonal dynamics in N2O emissions, and evidence that different pathways were involved. We propose that oxidation of N mineralised from decomposing peat was the main source of N2O during after WT drawdown in spring, where 685

ammonia oxidation together with was followed by chemodenitrification was a likely pathway to N2O formation. In the autumn, (or nitrifier-denitrification of), whereas in the autumn, where NO3 derived from residues or decomposing peat following WT riseaccumulated in arable soil after heavy rainfall was probably harvest, N2O emissions were associated with rising WT and heterotrophic denitrification as the main pathway. Mitigating N2O emissions from the acid organic soil investigated here is challenged by the apparent complexity of underlying processes. However, reducing surplusmineral N in the soil, for example accumulation by ensuring a vegetation cover throughoutoutside the yearmain cropping season, and stabilising the WT depth by effective drainage, are potential mitigation strategies for curbing N2O

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emissions.

695 Author contributions. ATT, LEL, TJ and SOP designed the study. ATT, LEL, VE and SOP carried out sampling and analyses. ATT, RL and SOP were responsible for data analyses. ATT and SOP prepared the manuscript with contributions from all co-authors.

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Table 1. Selected characteristics of soil profiles at the four monitoring sites with rotational grass (RG1, RG2) and arable soil used for potato crop (AR1, AR2). All analyses were done in triplicate; results shown represent mean and standard error of two soil profiles (n = 2). Soils for analyses were collected in late April except for AVS and CRS (early September). Abbreviations: EC, electrical conductivity; TOC, soil organic carbon; TRFe, total reactive iron; AVS, acid volatile sulfide; CRS, chromium reducible sulfur.

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	Depth	pН	EC	TOC	Total N	C:N	TRFe	AVS	CRS
	(cm)			(g 100 g ⁻¹)	(g 100 g ⁻¹)	ratio	(mg Fe g ⁻¹)	(µg S g ⁻¹)	(µg S g ⁻¹)
RG1									
Depth 1	2.5-7.5	5.1 (0.2)	0.26 (0.10)	37.4 (0.2)	1.75 (0.00)	21.3	3.63 (0.11)	2.51 (0.86)	155 (62)
Depth 2	7.5-12.5	5.3 (0.1)	0.15 (0.02)	38.2 (0.2)	1.79 (0.01)	21.3	4.03 (0.44)	NA	NA
Depth 3	17.5-22.5	5.3 (0.5)	0.37 (0.18)	39.7 (0.3)	1.80 (0.04)	22.1	4.14 (0.32)	NA	NA
Depth 4	36-40	4.8 (0.1)	0.55 (0.02)	43.1 (2.7)	1.85 (0.03)	23.3	3.04 (0.26)	2.60 (0.87)	133 (64)
Depth 5	47.5-52.5	5.1 (0.3)	0.42 (0.13)	31.0 (15.6)	1.47 (0.64)	21.1	2.50 (0.55)	4.86 (1.07)	24 (17)
Depth 6	93-98	5.4 (0.0)	0.51 (0.06)	0.6 (0.3)	0.01 (0.01)	ND	0.14 (0.04)	NA	NA
RG2									
Depth 1	0-25	5.0	NA	19.8 (3.4)	1.34 (0.13)	14.8	2.29 (0.56)	NA	NA
Depth 2	25-50	5.1	NA	8.9 (3.0)	0.63 (0.23)	14.2	4.48 (NA)	1.71 (0.00)	33 (7.3)
AR1									
Depth 1	2.5-7.5	5.0 (0.1)	0.45 (0.04)	35.9 (0.1)	1.81 (0.02)	19.9	4.57 (0.09)	1.74 (0.02)	141 (9)
Depth 2	7.5-12.5	5.2 (0.1)	0.42 (0.06)	34.2 (0.2)	1.76 (0.02)	19.4	4.66 (0.15)	NA	NA
Depth 3	17.5-22.5	5.2 (0.1)	0.34 (0.04)	41.0 (2.2)	1.93 (0.11)	21.3	4.99 (0.43)	NA	NA
Depth 4	36-40	4.7 (0.5)	0.37 (0.05)	41.1 (5.8)	1.84 (0.05)	22.4	3.23 (0.41)	2.17 (0.29)	49 (3)
Depth 5	47.5-52.5	4.7 (0.3)	0.48 (0.08)	5.9 (1.7)	0.37 (0.13)	16.3	1.19 (0.19)	1.98 (0.41)	137 (39)
Depth 6	93-98	5.4 (0.2)	0.91 (0.03)	0.3 (0.1)	0.00 (0.00)	ND	0.18 (0.02)	NA	NA
AR2									
Depth 1	0-25	5.1	NA	33.4 (1.2)	1.45 (0.03)	23.1	4.11 (0.03)	NA	NA
Depth 2	25-50	5.1	NA	38.4 (0.2)	1.46 (0.02)	26.2	3.78 (0.14)	1.65 (0.02)	45 (8)

ND - Not determined due to TOC and total N concentrations being at the limit of detection.

NA - Not analysed.

Table 2. Cumulative emissions of N₂O (kg N₂O ha⁻¹) during the spring (99-105 days) and autumn (47-69 days) monitoring period. Estimation for each season was performed using the trapezoidal approximation of the integral of the emission curve. Numbers in parentheses indicate 95% confidence intervals, and significant differences, corrected for multiple testing by the single-step method, are indicated by asterisks. *RG*, rotational grass; *AR*, arable crop (potato); F, fertilised; NF, unfertilised.

	DOY	Cumulative N ₂ O_	RG-NF	RG-F	AR-NF
Spring [#]		kg ha-1			
RG-NF	<u>63-162</u>	2.0_(1.5-2.5)			
RG-F	<u>63-162</u>	7.3_(4.9-9.6)	***\$		
AR-NF	<u>63-167</u>	17.1_(13.9-20.2)	***	***	
AR- F	63-167	15.0_(12.2-17.8)	***	***	NS
Autumn			RG		
RG <u>-NF</u>	252-314	2. <u>20 (1.61</u> -2. <u>79</u>)			
<u>RG-F</u>	252-314	<u>1.9 (1.4-2.4)</u>	NS		
AR <u>-NF</u>	- <u>246-314</u>	14.8<u>13.6</u>(11.6_(10.2 - 17.9 <u>1</u>)	***	_***	-
<u>AR-F</u>	246-314	<u>15.3 (11.2-19.4)</u>	***	***	<u>NS</u>

[§] ***, *p* < 0.001; NS, not significant (*p* > 0.05)

[#]The monitoring periods (spring and autumn) were: DOY63-162 and DOY252-314 (*RG1*); DOY64-169 and DOY260-307 (*RG2*); DOY63-162 and DOY246-308 (*AR1*); DOY64-169 and DOY245-314 (*AR2*).

Figure captions

Figure 1. A. Location of sites *AR1* and *RG1* (both at 57°13'59.7"N, 9°50'40.3E), *RG2* (57°13'55.9"N, 9°52'20.2E) and *AR2* (57°13'7.6"N, 9°46'26.9E). B. Experimental design at each of the four sites, with three blocks centered around piezometers (\bullet) and two subplots, one of which received N fertiliser at the rate of the surrounding field. Six collars for gas flux measurements (S1-S6) were distributed as indicated, and sets of 5 diffusion probes for soil gas sampling were installed near collars in selected positions (see text).

Figure 2. Nitrite-N (a, c) and total reactive iron, TRFe (b, d), in undisturbed soil cores collected at sites *RG1* and *AR1* on 23 April (DOY113; white symbols) and 2 September (DOY245; grey symbols). Results shown are mean and standard error (n = 2). The dotted lines indicate WT level on the two sampling dates.

Figure 3. The top panel shows rainfall, air temperature and management (F – fertilisation) at sites *RG1* (left panels) and *RG2* (right panels) during spring₇. <u>3 March (DOY63) to 16 June (DOY169)</u>. The middle section shows N₂O fluxes (black circles; mean \pm standard error, *n* = <u>3</u>) and contour plots of soil N₂O concentrations in fertilised subplots, and the lower section the corresponding results for unfertilised subplots. A logarithmic grey scale was used in order to show trends within both *RG* and *AR* treatments, and between depths. Soil gas sampling positions are indicated in the contour plots; numbers shown are N₂O concentrations (μ L L⁻¹). Grey Green lines show the WT depth (which varied slightly between blocks). B2 and B3 refer to block number of diffusion probe positions.

Figure 4. The top panel shows rainfall, air temperature and management (T – tillage; F – fertilisation) at sites *AR1* (left panels) and *AR2* (right panels) during spring₇, <u>3 March (DOY63) to 16 June (DOY169)</u>. The middle section shows N₂O fluxes (black circles; mean \pm standard error, *n* = 3) and contour plots of soil N₂O concentrations in fertilised subplots, and the lower section the corresponding results for unfertilised subplots. A logarithmic grey scale was used in order to show trends within both *RG* and *AR* treatments, and between depths. Soil gas sampling positions are indicated in the contour plots; numbers shown are N₂O concentrations (μ L L⁻¹). Gaps are indicated where soil gas sampling probes were installed late, or removed due to field operations. Grey-Green lines show the WT depth (which varied slightly between blocks). B2 and B3 refer to block number of diffusion probe positions.

Figure 5. The top panel shows rainfall, air temperature and management (H - harvest) at sites *RG1* (left panels) and *RG2* (right panels) during autumn₇, <u>3 September (DOY245) to 10 November (DOY314)</u>. The middle section shows N₂O fluxes (black circles; mean \pm standard error, n = 3) and contour plots of soil N₂O concentrations in fertilised subplots, and the lower section the corresponding results for unfertilised subplots. A logarithmic grey scale was used in order to show trends within both *RG* and *AR* treatments, and between depths. Soil gas sampling positions are indicated in the contour plots; numbers shown are N₂O concentrations (μ L L⁻¹); the probes were absent in the unfertilised subplot after harvest. Grey-Green lines show the WT depth (which varied slightly between blocks). B2 and B3 refer to block number of diffusion probe positions.

Figure 6. The top panel shows rainfall, air temperature and management (H - harvest) at sites *AR1* (left panels) and *AR2* (right panels) during autumn., 3 September (DOY245) to 10 November (DOY314). The middle section shows N₂O

fluxes (black circles; mean \pm standard error, n = 3) and contour plots of soil N₂O concentrations in fertilised subplots, and the lower section the corresponding results for unfertilised subplots. A logarithmic grey scale was used in order to show trends within both *RG* and *AR* treatments, and between depths. Soil gas sampling positions are indicated in the contour plots; numbers shown are N₂O concentrations (μ L L⁻¹). Grey Green lines show the WT depth (which varied slightly between blocks). B2 and B3 refer to block number of diffusion probe positions.

Figure 7. Statistical results from Using graphical models, a statistical analysis was conducted for the four eombination of erops (*RG*, *AR*) and season (spring, autumn). a. *RG*, spring; b. *RG*, autumn; c. *AR*, spring; and d. *AR*, autumn. The edges ("lines") connecting vertices ("points") and edges ("lines") indicate significant relationships between explanatory variables and the response variable, i.e., (N₂O flux₇). Statistical results for effects on N₂O flux are: [1] 2.32 (0.12-9.11, *p* = 0.011); [2] 0.74 (0.06-3.05, *p* = 0.034); [3] 0.78 (0.41-2.47, *p* = 0.0002); [4] 1.34 (0.78-4.08, *p* = 0.008); and [5] 2.45 (1.10-9.90, *p* = 0.0002). Key to variables: AmmoniumT – NH₄⁺ at 0-25 cm depth; NitrateT – NO₃⁻ at 0-25 cm depth; N₂O WT – equivalent soil gas phase concentration closest to, but above the water table depth; Temp5 – soil temperature at 5 cm depth; Temp30 – soil temperature at 30 cm depth.













Figure 3















Figure 7