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Biogeosciences
Discussions

Interactive comment on “Kinetics of N_2O production and reduction in a nitrate-contaminated aquifer inferred from laboratory incubation experiments” *by* D. Weymann et al.

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Manuscript BGD-2009-299 by Weymann et al.; published in BGD 7, 503-543: Responses to comments of referee 1

The comments of the reviewer are repeated here and our responses are inserted after each comment. We numbered the comments according to our responses. Responses are marked with R (number).

1. When reading the abstract I already asked myself (the microbiologist) how can a

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kinetic from nitrate to di-nitrogen oxide be modelled without considering nitrite. There is so many nitrate reducing bacterial strains that first (or exclusively) reduce nitrate to nitrite. The prerequisite for a further reduction of nitrite often is nitrate dropping below a certain threshold concentrations or other strains to take over. The authors shortly mention the 'complexly' control of N2O production in the environment (P 505, L: 19-25). Moreover, the authors show (Fig. 3) that N2O accumulates in their batch experiments as long there is plenty of nitrate. From a certain threshold on, N2O starts to disappear. Identical patterns as often observed for nitrate reduction to nitrite and subsequent conversion to N2O/N2. From a microbiological point of view, evaluation of first or zero order kinetics for nitrate reduction to N2O only makes sense if it can be shown that there is no reaction limiting step between nitrate and N2O (e.g. accumulation of nitrite). This information is not found in this manuscript.

R1: We agree that nitrite as an intermediate compound often plays a key role in the denitrification reaction chain and it was taken into account by several modelling approaches (e.g. Almeida et al. 1997). However, nitrite did not accumulate during our incubation experiments and was not detectable in the samples which were simultaneously analyzed for nitrate. We attribute this phenomenon to the pH, which was typically between 4.0 and 5.5 throughout the entire incubation periods (and in a comparable range in the field). Van Cleemput (1998) stated that nitrite accumulation is favoured by high pH and high ammonium concentrations and - vice versa - is not stable in the presence of converse conditions (pH < 5.5-to-6.0). Furthermore, Konrad (2007) showed that nitrite must not necessarily accumulate during incubation of aquifer slurries. We add this important information to the "Material and Methods" section of the revised version by mentioning the nitrite analysis. Second, we introduce this result when Equation 3 is explained (see also Holtan-Hartwig et al., 2000) in order to clarify that no reaction limiting step between nitrate and N2O occurred and Equation 3 is an appropriate assumption as a starting point for the underlying reaction kinetics.

2. A point which confused me, is the differentiation between a 'shallow' heterotrophic

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and a ‘deeper’ autotrophic denitrification zone. The manuscript does, to my opinion, not provide sufficient information to substantiate this differentiation. Indeed, the authors refer to other papers. But as this is a crucial point with respect to the grouping of batch incubations, I would ask for additional information supporting this differentiation given in the paper.

R2: Referee 2 encouraged us to restructure the manuscript with regard to the “Study site” section. Since we feel that recent results obtained from denitrification investigations in the study area are a crucial requirement for the understanding of grouping the batch experiments, we decided to insert this chapter as an “intermediate” one following the introduction (“Denitrification zones and occurrence of N2O in the Fuhrberger Feld Aquifer”). We hope that this structure will better clarify the differentiation between the denitrification zones by adding weight on that point. Moreover, we show the sulfate production rates (in NO3-N equivalents) for the samples from the “deeper” autotrophic zone and compare them qualitatively with the denitrification rates (revised Table 2). Thus, we are able to show that there is strong evidence for autotrophic denitrification as a result from the batch incubations. The method for analyzing sulfate was added to the section “Analytical techniques” (section 3.3 in the revised version).

3. I appreciate that the authors could prove the different potentials for denitrification in the shallow (what they called heterotrophic) and less shallow (‘autotrophic’) zone of the aquifer. But this is not a new finding, as it was already shown earlier (see citations in the manuscript).

R3: First, the different denitrification potentials were shown only in the field in one study up to now (Weymann et al., 2008). We argue that the recovery of this differentiation under controlled conditions in the lab is a prerequisite for the assessment of the transferability of the lab data and the validity of the respective N2O kinetics. Second, the new finding is the kinetics of N2O production and reduction. Please note that we tried to underline this especially in the title, in the abstract, the objectives and the conclusions in order to add weight to our focus. The kinetics of N2O production and reduction has not been investigated in the Fuhrberger Feld Aquifer and - to our best knowledge - there are only few data from other aquifers. Thus, we argue that our study fulfils the aspect of novelty and the derived rate constants might be used in forthcoming modelling approaches.

4. The data presented and the interpretation are sometimes confusing and misleading. Example given, only in the autotrophic denirtification zone, there is a significant correlation between denitrification rates and organic matter. I am willing to follow the argumentation of the authors that the combination of low (available) organic matter, high nitrate concentrations and a low pH favours N2O accumulation (P 520, L5-20). However, the data presented from the multi-level well do (1) not show a coincidence of high nitrate and high N2O (it is more the other way round, Table 3 also says there is no correlation in the heterotrophic zone between Di and Nt), (2) not show a link to organic matter (DOC data are missing and OM data in Table 1 indicate no clear patterns of OM with respect to N2O), and (3) not provide any spatially resolved pH data.

R4: Since this point was also addressed by referee 2, Figure 1 (data of the multilevel wells) will be substantially revised. We show additionally pH data and DOC concentrations in the revised Figure 1 (DOC is available for the shallow groundwater). We agree that the relation between high NO3- concentrations and N2O accumulation in the near-surface groundwater becomes not necessarily clear with regard to Figure 1. Hence, we changed the expression “high NO3- concentration” to “elevated NO3- concentration”. This more general expression will meet better the depicted concentration gradients. Please note that the NO3- concentrations are shown in mg N L-1, i.e. we interpret NO3- concentrations in the entire near-surface groundwater as “elevated”. Correlations in Table 3 refer to the parameters of the laboratory incubations and the incubated aquifer slurries, i.e. no correlation between Di and Nt found here does not contradict the relation elevated NO3- - high N2O concentrations that was found in groundwater samples from the multilevel wells. However, the title of Table 3 was modified and stronger related to the laboratory experiment to prevent misunderstanding of the data.

5. I also do not clearly see how the calculated denitrification rates fit to the data of groundwater samples from the multi-level wells. At Well B1, most sulphate (originating from autotrophic denitrification coupled to the oxidation of reduced sulphur species) is found in the shallow groundwater. Also in Well I1, where sulphate is more prominent in less shallow areas (the so called 'autotrophic' zone) there is a similar pattern for sulphate and N2O obvious. Is this just by chance?

R5: An explanation for the distribution of sulfate concentrations at well B1 is given (please see P521, L16-21): we assume that mixture of groundwater affects sulfate. The similar pattern for sulfate and N2O at well I1 is due to autotrophic denitrification and characteristic if nitrate enters the zone containing pyrite, so this is not by chance. We described the behaviour of N2O in detail (please see P520, L21-29; P521, L1-10), but did not explained the related pattern of sulfate in this context. Thus, we improve our discussion with regard to this point.

6. I come to the conclusion that the two distinct N2O peaks detected in both vertical profiles at B1 and I1 can not be explained by dentrification rates, organic matter, pH, nitrate concentrations at the moment. Maybe microbial community data could help. Or maybe there are more supportive data not shown so far.

R6: In our opinion, our observations and the references mentioned (van Cleemput, 1998; Hefting et al., 2006) allow a plausible explanation of both N2O peaks. Our argumentation for the heterotrophic zone: van Cleemput (1998) stated (for subsoils), that "all conditions whereby the denitrification process becomes marginal are favourable for N2O formation rather than for N2". We discussed such conditions (which support low denitrification rates) in section 4.1 in the submitted version of the manuscript, e.g. (1) low pH (Deurer et al., 2008) which is shown also in the revised Figure 1, (2) elevated NO3- concentrations (three references are given at P520, L12), and (3) low content and probably low bioavailability of organic carbon. Since we observed simultaneously an N2O peak in the near-surface groundwater accompanied by general considerable accumulation of N2O, why we should not draw the conclusion that the typical occurrence of N2O is explainable by the mentioned governing parameters? However, again encouraged by a remark of referee 2, we decided to show also O2 in the revised Figure 1. Elevated O2 concentrations in the near-surface groundwater are a further argument that supports our explanation of the N2O peak in the near-surface groundwater. Our argumentation for the autotrophic zone: as already discussed in the submitted manuscript, this is a peak occurring at the boundary between heterotrophic and autotrophic zone. Here, groundwater loaded with nitrate meets the aquifer material containing reactive pyrite. We assume that intensification of N2O production causes the observed sharp N2O peak, but this is followed by rapid N2O reduction which prevents an accumulation of N2O in the sense of the heterotrophic zone. However, we weakened the argumentation by replacing “conclude” with “assume” (P521, L3). We agree that microbial community structure can affect the balance between production and reduction of N2O and might thus affect the depth distribution of N2O. In the discussion we will now add this aspect as additional explanation which should be addressed in future studies.

7. Besides these points, I suffered at several locations in the manuscript from the ‘non-precice’information given. I will highlight some examples in the following. In the ‘Study site’ description, for example, the authors talk about ‘substantial microbially mediated processes’ such as denitrification and desulfurication. Is the later process referring to‘oxidation of reduced sulphur species’? Then this is probably not the right term.

R7: Desulfurication is the heterotrophic reduction of sulfate and occurs in the deeper aquifer when the nitrate is eliminated due to autotrophic denitrification. We add references containing discussions of this process (Korom, 1991, 1992; Böttcher et al. 1991). Moreover, we add a short definition in brackets.

8. Moreover, when talking about autotrophic denitrification with reduced sulphur compounds, I would like to know which compounds are meant. It is like a red line through the paper. The authors continuously talk about reduced sulphur, without mentioning a speciation; e.g. FeS, FeS2, HS-, S0, etc.

R8: We completely agree that the reduced sulphur compounds were not sufficiently specified. Pyrite (FeS2) is the relevant electron donor, exactly spoken, reactive micro-crystalline components of pyrite function as the substrate for nitrate reduction (Kölle et al., 1985). We improve the manuscript with regard to this point.

9. Table 1 provides useful information on the aquifer material sampled. However, the table clearly lacks some detailed information. Abbreviations such as 'Nt' and 'Sulfur' (total sulphur? Or what species?) are not explained in more detail, and units [mgL/1 or μgL/1] are missing.

R9: Corrected.

10. I miss detailed information regarding the pH, as this is an important point of argumentation later in the manuscript. I also miss the information on oxygen concentrations in the aquifer. The authors mention that there is not too much organic carbon, thus limiting heterotrophic denitrification (which may already take place at concentrations of $<$ 2 mgL/1 of dissolved oxygen). It is not clear if the saturated zone of the investigated aquifer is exhibiting reduced conditions already at the groundwater table. DOC concentrations, although available (at least) for the lab incubations are not shown.

R10: As already mentioned, pH, oxygen and O2 were measured and will be shown in the revised Figure 1 (field data). In the case of the lab data, typical ranges will be given in the text, because we feel that addition of these quantities to Figure 2 and 3 would overload these figures. O2 was not found during the entire incubation periods. This will be mentioned in the text.

11. Last but not least, depths 2-3m below the (relatively shallow) groundwater table, I would preferably not call 'deeper aquifer'. The term deeper aquifer is somehow restricted to aquifers or zones of aquifers $<$ 50m below land surface (P 508, L 5). The authors should search for a more appropriate term. R11: Corrected. We clearly define this zone in the "new" section 2 (study site section, see R2) and refer to it later as autotrophic denitrification zone.

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12. Finally, the paper leaves several interesting points unanswered. What do the high N2O values at or close to the groundwater table mean? Is the N2O produced there or transported there, or only accumulating there?

R12: From the low N2O concentrations in the unsaturated zone above the groundwater table (von der Heide et al., 2009) it can be concluded that the high N2O concentrations close to the groundwater table were produced below the groundwater surface. This information is added to the discussion. With respect to the distance between the location of N2O production and the location of maximum concentration we can answer only hypothetically (e.g. data of reactive N2O transport could help but are not available up to now). Furthermore, we argue that these points are not within the main scope of the study (N2O kinetics inferred from the lab incubations). Therefore, we would avoid to add the aspect of reactive transport to the revised version of the manuscript.

13. As a concluding remark, I wonder if the presented manuscript contains sufficient new findings and has the critical mass for publication in BG. There is a pile of papers, dealing with similar and closely related research questions tackled at the same field site from the same authors. To my opinion, it will take the consideration and state of the art analysis of microbial communities and functional genes related to denitrification and sulphide oxidation at the extensively investigated aquifer. This would allow to draw new ‘integrative’ conclusions on what’s going on in this system.

R13: Here, we absolutely disagree with the referee’s remark. Of course, the existing papers deal with closely related questions, but the focus of those studies was either (1) on denitrification (without taking N2O into account) or (2) on occurrence of N2O in the near-surface groundwater and potential indirect emission to the unsaturated zone or to the atmosphere, respectively. There are only two field studies which describe N2O in the whole aquifer (Weymann et al., 2008; von der Heide et al., 2009), but both studies did not investigate the turnover or metabolism of N2O, i.e. its reaction kinetics, they did not couple N2O concentrations to properties of the aquifer material and did not include laboratory incubation of aquifer material to characterise N2O reaction rates.

Until today, there is a lack with regard to the kinetic parameters of N2O production and reduction in the groundwater, and this is not only true for the investigated Fuhrberger Feld Aquifer, it is also a general lack of knowledge. Kinetic parameters are needed to develop more complex models which will be able to predict the indirect emission of N2O, as they project dissolved N2O to the point where it can be emitted to the atmosphere. Therefore, we are convinced that our results are a further step to improve our knowledge of the described kinetics and hopefully of the related indirect N2O emission, which is still a highly uncertain component in the global N2O budget.

14. P 504, L 5: 'in situ' should be given in italic P 504, L 9: I suggest to replace the term 'deeper' groundwater. It is misleading. The authors deal with groundwater from a few meters below land surface. P 504, L 14: Please further specify which 'sulfur' was measured.

R14: We strictly followed the referee's suggestions and corrected these points in the revised version.

15. P 504, L 26: replace the term 'goodness'. P 505, L 8: choose 'up to' or 'more than', but not 'up to more than' P 505, L 9: the term 'surface groundwater' sounds strange to me; consider to use'shallow groundwater', surface near groundwater', etc. P 505, L 16: replace 'By and large', also the 'two crucial subjects' sounds strange - please rephrase.

R15: Corrected. "Surface groundwater" was replaced with the term "near-surface groundwater"

16. P 506, L 9-29: This section should move from the introduction to the discussion section. Moreover it is hard to read and sometimes confusing.

R16: We argue that this section is important as a part of the introduction, because it describes recent lab studies on N2O in aquifers, and prepares and leads to our objectives. However, we restructured the text in the revised version in order to support easier understanding and better reading.

17. P 507, L 3-4: The study investigated the kinetics of N2O production and reduction in‘material’ from an unconsolidated sandy aquifer, not in the aquifer. P 507, L 25: delete ‘its key function for’. P 508, L 1: . . . the other ‘hand’? P 508, L 12: what means ‘schematically’? maybe ‘exemplarily?

R17: All the mentioned points were corrected.

18. P 508, L 17: Where does the high sulphate values in the shallow gw from B1 originate from, if it is assumed that the oxidation of reduced sulphur species is not anymore a relevant process. For high sulphate data from site I1 the authors argue that these are a result of autotrophic denitrification coupled to oxidation of reduced sulphur species. Sulfate values in shallow groundwater from B1 are in the same range than values from groundwater of zones below at I1.

R18: We assume that these high sulfate concentrations found in the near-surface groundwater at B1 are of geogenic origin and due to the influence of intensive use of fertilizers containing sulfate ((NH4)2SO4; (NH4)2SO4âĂćNH4NO3, see Hansen, 2005). This information and the reference are added to the first section of the discussion.

19. P 508, L 18: unconventional formulation: ‘5C’

R19: Corrected.

20. P 509, L 16: I miss NO2- as an important denitrification related parameter.

R20: Nitrite was not measured in the field, but it was monitored during the laboratory incubations. Please see our R1 above.

21. P 510, L 15: I did not get why the sampling of an undefined area of the same depth should reveal samples differing in texture and chemical composition. Was this the intention or the final result (= unexpected spatial heterogeneity) – please explain.

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R21: Despite we collected the aquifer material from one point (one borehole), this aquifer material was not homogeneous. Repeated sampling included replacing of the “original” sediment with sediment in the vicinity of the borehole. Thus, sediment with different properties from slightly different areas was sampled, causing the observed heterogeneity. We substantiate our phrasing with regard to this point in the revised version.

22. P 510, L 18: which water was used for filling the buckets containing the sediment? Reduced groundwater from the same depth?

R22: Yes! This information was added. The bailer also contained “original” groundwater beside the aquifer material.

23. P 510, L19-20: From an microbiological point of view, storage of aquifer material for a period of 4 weeks is not adequate, if later the intention is to transfer results to in situ conditions. Too many studies have shown fast changes within microbial communities and activities in the order of hours to days during storage. I ask the authors to mention this point in the discussion section and interpret the results (and the transferability) with caution. Related to the incubation time of 350 d, the 30 d of storage may be acceptable.

R23: Done.

24. P 511, L 3: . . . amended with 400 mL of a ‘anoxic’ (oxygen free) test solution?

R24: This test solution was not oxygen free, because we evacuated und purged the samples anyway after closing the bottles in order to establish anaerobic conditions (please see P511, L9 / 10). The test solution that was used for re-injection (P511, L27) was oxygen free. Additional explanations are given also with regard to a comment of reviewer 2.

25. P 512, L 17: you mean ‘gas chromatograph’

R25: Yes. Corrected.

26. P 513, L 18-20: Honestly, I am not very familiar with the modelling approach applied. However, to use the results from an analytical solution as fitting functions sounds to me as a 'double fitting' approach. R26: The described fitting algorithm is the standard approach used in literature. Usually, in linear regression one uses an analytical function: $C(t)= a + bt$, where a and b are the fitting parameters and the linear function is the fitting function. Thus, there is no double fitting. However, if the linear function with the two parameters a and b is not able to fit your data (too large standard deviation or regression coefficient $R2 < 0.5$) than the process kinetics are not described by this analytical linear function. Hence a good process understanding and an appropriate choose of a good fitting function is necessary for a good description of the experimental data. The mean R2 for N2O production (k1) is nearly 1 (Table 4). The heterotrophic N2O reduction has a mean R2 of about 0.7, which is a reasonable agreement. The autotrophic N2O reduction has a mean R2 of about 0.4. Therefore we conclude for the autotrophic case that the analytical fitting function does not properly describe the process. As a consequence we developed a more sophisticated reaction model, which is able to describe autotrophic N2O reduction. A detailed discussion is given in a submitted paper (Geistlinger et al., 2010, Vad. Zone J., under review).

27: P 514, L 17-18: the authors talk about nitrate concentrations and give 'nitrogen' concentrations. Why not just provide the nitrate values. Same is true for P 514, L 26, where N2O concentrations are mentioned.

R27: This was necessary to be able to compare the different N-species. Please see Figures 2 and 3, it is helpful to give "nitrogen" concentrations to get an imagination of the mass balance of nitrate reduction and (N2+N2O) production. To guarantee consistency of the units, we transferred this to the field data.

28: P 515, L 1-2: is there a better term for 'sharp-cut' peak, e.g. pronounced peak, or simply a sharp peak. P 515, L 22: 'inbetween' instead of 'between'?

R28: We followed the suggestions of the referee.

29: P 517, L 15-19. It is interesting that in the autotrophic zone the denitrification rate was positively correlated to organic matter, indicating heterotrophic denitrification? The positive correlation with sediment sulphur is supportive, but it would be good to know what ‘sulfur’ we are talking about. Is it only reduced sulphur species? There is also a lot of sulphate in the system.

R29: It is the total sulfur content of the aquifer sediment. We specified this in the Tables as well as in the text of the revised version. For discussion please see P523, L9-29.

30. P 518, L 6: I am sorry, but the data presented in Fig 4 refer to the chart ‘I1-S2 2.0-2.5’ in Fig 2 not ‘I1-S1 2.0-2.5’ as mentioned in the text as well as in the Fig. 4 legend.

R30: This mistake was corrected.

31. P 518, L 12-24: Can the reason for the ‘bad’ fit be caused by the different kinetic steps being presents when going from nitrate to dinitrogen oxide? R31: We do not think so. Please see R1 for explanation.

32. P 519, L 9-10: To my opinion, this separation is not sufficiently supported with data; sulphate data at least partly do not support this argumentation and DOC values are not shown. Organic carbon in the sediment material seems on average higher in the autotrophic zone.

R32: As already mentioned, Figure 1 will be completely revised and extended. Furthermore, sulfate production rates are shown for the samples from the autotrophic zone in the revised Table 2. This will also support the differentiation of the process zones.

33: P 521, L 16-18: . . . indicate that the aquifer is recharged by water that infiltrated at forest or pasture areas.

R33: Corrected.

34. P 521, L 16-18: Can the dilution of sulphate be estimated based on potassium

concentrations by a simple dilution model? This would support the argumentation.

R34: We argue that the additional introduction of such a dilution model is not within the main scope of our study. However, we give ranges for potassium concentrations in the text which support a qualitatively imagination of the dilution with groundwater originated from forest / pasture areas.

35: P 521, L 25-27: what is meant: ‘O2 had been kept constant in the anaerobic incubations’?

R35: We agree that this formulation could be misunderstood. Anaerobic conditions had been kept constant, there was no constant elevated O2 concentration. We corrected the phrase.

36: P 522, L 13-17: It is from a microbiological point of view quite strange that no microbial activity (consumption of DOC and nitrate) was measureable at these high DOC concentrations over a period of 350 days. There is no DOC which is ‘not’ bioavailable. To my opinion, it is only a matter of time.

R36: Perhaps this statement is valid for (surface) soils, but we doubt whether it is generally valid for the groundwater. It is possible that labile, bioavailable DOC fractions are consumed in the unsaturated soil before entering the groundwater. Marginal reactivity of DOC in groundwater and subsoils was also reported by several authors (McCarty and Bremner, 1992; Jacinthe et al., 1998; Siemens et al., 2003). We improved our argumentation in the revised version with regard to this point.

37: P 523, L 2: What reduced sulphur species are we talking about? The authors mentioned that the test site is under investigation since more than two decades. There should be some information available what sulphur species are present in the aquifer. I ask the authors to be more precise.

R37: Done. See also R8 please.

38: P 524, L 8: replace the word ‘goodness’

R38: The phrase “goodness of fit” is a very conventional phrase which is often used to describe the “quality” of a fitting function compared to experimental data. We do not change this expression.

39. P 525, L 16-17: Rephrase the sentence.

R39: Corrected.

40. P 525, L 24-25: The field data shown do not tell anything about denitrification rates in situ.

R40: This is true, but it is possible to take into account the nitrate removal efficiency of the different denitrification zones, i.e. low nitrate removal efficiency in the near-surface groundwater and a considerable nitrate reduction in the autotrophic zone. We rephrased to ensure that no denitrification capacities are compared, but the nitrate removal efficiencies. However, our conclusion that lab and field data are in satisfactory agreement regarding that point is still valid.

41: P 526, L 19: replace ‘is exhibited by’ by ‘ is evident from’ Fig. 1: unconventional labelling of the axes (mg SO4 L-1 = SO4 [mgL-1]); follow the style of the journal. The paper would clearly benefit from incorporating DOC and pH values in Fig 1.

R41: The expression was replaced and the Figure is revised by incorporating DOC and pH data.

42. Fig. 2: It would be great to have DOC and sulphate data included. Only these can provide evidence if the dentrification was ‘heterotrophic’ or ‘autotrophic’.

R42: Sulfate was measured only for the samples from the autotrophic denitrification zone. We show the sulfate production rate in the revised Table 2 to prove that autotrophic denitrification occurred. The fact that heterotrophic denitrification replaced the autotrophic process in the near-surface groundwater was shown by von der Heide et al. (2008). As already mentioned and stated in the manuscript, we did not observe significant decomposition of DOC in the heterotrophic samples. Thus, DOC dynamics gave no hint on heterotrophic denitrification. We assume that organic C used by the heterotrophic denitrifiers originated mainly from particulate organic C in the aquifer material.

References (as far as not cited in the manuscript)

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