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Interactive Comment

Interactive comment on "Influence of hydrological fluxes on bio-geochemical processes in a peatland" by N. Bougon et al.

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Dear editor of Biogeosciences,

We are very grateful to the reviewers that have helped us to provide a more comprehensive and more interesting paper. We have taken all the comments as detailed below and mainly tried to emphasize the comparison between the field and the lab results. We have simplified the technical part of the field monitoring which was sometimes confusing and provided further details about the hydrological important results in order to provide clues to the hydrological contrasts between sites. We thus modified the order of the co-authors. The revised version is added to follow the response to the different comments.



Interactive Discussion



Anonymous Referee #1

General comment: Bougon et al. presents an experimental approach to isolate the physical and chemical factors that might influence the chemical trends in nitrate and sulfate that they observed in their field site. Overall, I agree that such type of experiments are required to obtain better understanding of biogeochemical processes in such complex environment, however, I have some concerns about missing data, some of the interpretation, and its documentation. The topic of this manuscript is definitely appropriate for publication in Biogeosciences. I recommend that the authors give a clearer and more extensive description of the hydrogeology at the field site together with an extended presentation of their microbiological results. The manuscript also needs some improvement in the descriptive and interpretative issues, which are listed below.

Specific comments: Title- I think the word fluxes is not appropriate for this work (and title). The authors do not provide the data to interpret their results in term of fluxes. I suggest to use hydrological conditions instead of hydrological fluxes.

-> Response: We agree with this remark which was thus taken into account in the revised version. We do not study the hydrological fluxes but their impact on the peat, which means the hydrological conditions.

Introduction- The Introduction reviews the literature only until 2004, although many studies were done on the impact of hydrological conditions (including fluxes) on biogeochemical processes since then. It also lack the specific discussion about the main focus of this manuscript- past studies that tested systems that were under variable saturated conditions (e.g., Day and Megonigal, 1993; Phipps and Crumpton, 1994; Spieles and Mitsch, 1999; Ishida et al., 2006).

-> Response: These remarks are true and were thus taken into account in the revised

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version. See page 2, L58-63

Toward the end of the Introduction, the authors state that their aim is to distinguish between physical and chemical conditions on microbial activity. A few sentences later (Material and Methods), the authors mentioned that this will be done using a batch experiments, that will "reproduce their field observations". Although, their laboratory experiments were designed and performed nicely, I don't think that they could relate their results to physical conditions (degree of saturation or fluxes), although the samples were taken from different hydrological regimes. This key point should be more clearly stated throughout the text (title, methods, results and discussion).

-> Response: This remark was taken into account and we have tried to better explain how we tried to distinguish between 'physical' i.e. hydrological condition effects and chemical effects. We have used the proposition of the third reviewer to clearly state the hypothesis which is done from the hydrological conditions to the bacterial community.

Page 4833, section 2.1.2 and Figure 1- I didn't find the description and Figure 1 clear enough to understand the hydrogeological conditions in the field site. There is a clay layer mentioned in the text here (and also later), which is not in the sketch. Since clay layer is a major feature that control the flow in subsurface system it should be described more thoroughly. Also, I didn't understand X sign on the arrow in the sketch of site G, under low-water period. Also, the authors use here the word, river (line 19), while in line 5 they were using the word stream. I suggest using one term in the entire manuscript.

-> Response: These remarks were taken into account. The clay-rich layer was described in the geological section and its permeability is also described in the results section. The clay-rich layer is also presented in hydrological sketches which have been completely redrawn in order to give a clear explanation of the hydrological functioning. Figure 1 was split in 2 figures in the revised version. The revised figure 1 represents the site location and the piezometer map. Hydrological sketches are represented in the revised figure 3 in parallel to the water level dynamics. The word stream has also been

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used throughout the paper.

Page 4834, lines 1-10. In the first line the authors mentioned that "field tests" were done in order to estimate the hydraulic conductivity. I understand that slug tests were performed in the clay piezometers but it is not clear what kind of tests the authors were using in the peat piezometers.

-> Response: The sentence has been reworded and some details about the number of piezometers tested and type of test has been given. In fact the same method was used for all the piezometers although the monitoring time was longer for the clay-rich piezometers. The clay permeability could also be controlled through the water budget but this is the subject of another paper (Auterives et al., 2009) and seems beyond the scope of this paper. See pages 4 and 5, L122-134

The results from these tests, including data on how the differentiation between horizontal and vertical hydraulic conductivy was done? are not reported later on in the text although this is critical for linking the biochemical results to fluxes (only the total hydrologic budget is reported in Table 1).

-> Response: The results from the tests are now given in the results section as a range of permeability which provides elements for understanding the hydrological functioning. These results provide critical elements to ascertain the reliability of the hydrologic budget as well as the relation between the peat and the sand aquifer. This is a key point of the paper since it provides the basis of the differentiation between the G and S sites, although it is not the focus of the paper but the basic hypothesis. It is now well supported by the hydrologic results.

Page 4835, line 6, typo mistake in the word "throw".

-> Response: This remark was taken into account. It is not throw but through. See page 4,L154

Page 4835, lines 14-17. Text is not clear.

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-> Response: This remark was taken into account and the text modified in order to better explain the section definition. See page 5, L 144-149.

Page 4836, line 11. What is "hydraulic meaning" of intermediate situation?

-> Response: In fact the intermediate situation meaning is the fact that the peatland drainage by the stream period is an intermediate between the G and the S site. This section has been deleted and the definition of the different sites and their hydrological properties have been more clearly defined in the field monitoring section. See page 4-5, L113-134

Page 4836, line 14. Did the experiments were not conducted with the same ratio ? how this might affected the results ?

-> Response: We decided to consider the initial peat saturation and to add the same water volume to each flask. The water/soil ratio is thus slightly different for each experiment, however the range is similar and the results are not affected by this variation. This precision has been added in the text. See page 6, 177-179

Page 4836, line 25 and Page 4837, line 17. Figure 3 is mentioned here, before Figure 2.

-> Response: This remark was taken into account and the order of the figures modified.

Page 4837, line 19. Typo mistake after the word sterilized there are 3 dots?

-> Response: This remark was taken into account.

Page 4837, line 17. The reference mentioned here, Bougon et al. 2007, is not in the reference list).

-> Response: The paper has been published, it is referred as Bougon et al. 2009 in the revised version.

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Results Page 4838, lines 12-13. The authors stated that "The hydrogeological gradients Inducing river fluxes towards the peat were therefore of limited duration". Figure 2, however, shows that for site G the water level in the stream was always higher than that in the adjacent piezometers. My understanding is that hydraulic gradients are therefore from the river to the banks at all time? This is a key point for the whole hypothesis and understanding of the manuscript!

-> Response: We agree with the reviewer, it was difficult to understand this statement from the figure. We have thus modified the results section and provided a more detailed section of the hydrological functioning. A much more complete figure has been added in order to understand the hydrology (See revised figure 3). In fact, even if the stream level is higher, the hydrological gradient in the peat indicates that the peat and the stream are disconnected. See page 8-9&10, L255-295

To my opinion, whole section 3.1.1 should be reconsidered. A key issue here is how fluxes were calculated and the reader has no information about the horizontal and vertical gradients and hydraulic conductivities.

-> Response: As recommended, the section has been modified. The fluxes definition is better given in the text (see page 8-9&10, L255-295) and in the new figure (revised figure 3). The permeability range is also given, which makes the water budget more reliable. (revised table 1).

Page 4840, line 7. Auterives, 2008, is not in the reference list.

-> Response: The paper of Auterives and co authors is currently submitted to Hydro-logical processes.

Page 4840, line 10. The authors stated that "Oxygenation of peat groundwater is promoted by deeper groundwater flow into the sand, and water renewal". Is this means that there is an upward flow from the sand to the peat ? If this is the case, where is the recharge to sand aquifer occurs ? Once again, the hydrogeological patterns are not

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clear.

-> Response: This remark was taken into account. Our text was confusing by introducing the idea of groundwater flow. We analysed superficial peat, which is influenced by stream inflow. We modified the sentence as : 'Oxygenation of peat groundwater is promoted by stream water flow into the peat, and water renewal'. (See page 10, L301)

Page 4840, line 15. Where are the higher concentrations, close or far from the stream ?

-> Response: This remark was taken into account. A concentration gradient, dependent on the distance from the stream, was observed in site G with the highest concentration close to the stream. See page 10, L305-307

Page 4841, lines 7-13. The way/format of presenting theses text is not clear.

-> Response: This remark was taken into account and the presentation of the field geochemical results was completely reworded. (See page 10 and 11, L320-332) The changes in nitrate and sulphate concentrations were clearly related to water table dynamics and reflected various redox conditions related to water saturation. However, the field results also showed an obvious variation between sites and with respect to the distance from the stream within each site. An efficient nitrate removal in reference site G and pumping site S - right bank was observed. In pumping site S - left bank, above the abstraction well, the nitrate removal was more limited than in the other sites. Sulphate was produced at high concentration (SO42- > 100 mg/L) throughout pumping site S – left bank, above the abstraction well. In reference site G, extremely high sulphate concentrations (> 1000 mg/L) were observed at the beginning of high-water periods (peat-stream connection), close to the stream. At last, peat sampled close to the stream on the right bank of pumping site S also presented relatively high sulphate concentrations (SO42- = 20-50 mg/L). Moreover, we added a figure representing the field geochemical results. In figure 4 nitrate and sulfate concentration are represented according to the sites and the distance to the stream.

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Page 4842, lines 8-9. The impact of the initial pore water chemical seems to be very important, and it was nicely addressed. Why it is not addresses similarly in the nitrate data although it looks like it has an impact as well (nitrate concentrations over 1 in Figure 4). ?

-> Response: This remark was taken into account. The effect of pore water is much more limited for nitrate, it is only seen in anaerobiosis. It has been described in the text. (result section 3.2.1 and discussion section 4.1)

Page 4843, lines 16-17. Sentence is not clear.

-> Response: This remark was taken into account and the text modified. (see page 12, L372-374)

Page 4843, line 24. Stating here that nitrate removal is due to denitrification is not clear to me especially when this is thoroughly discussed only later on, especially with additional data from Figure 6.

-> Response: This remark was taken into account and nitrate reduction is used until nitrate reduction processes are discussed. (see page 12, L380-382)

Page 4844, line 12. There should be some references mentioned here. The observed reductions here are interpreted as denitrification, although significant decrease in nitrate concentrations was also observed under aerobic conditions. The authors tried to addressed this and explain it by previous observation (such as anaerobic micronisches).

-> Response : This remark was taken into account and the discussion was modified with a more detailed reference to this point. (see page 14, L426-435)

I was wondering why the authors didn't use the "classical" Acetylene Inhibition Method to specifically quantify denitrification ?

-> Response: The quantification of the potential of denitrification by the classical

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Acetylene Inhibition Method was planned in this study. Breaking down of the injection system of the gas chromatography made these analyses impossible.

Section 4.2 Nice discussion.

Section 4.3 Once again, I think the hydrological conditions, including fluxes and stream peat connections are not presented clearly in order to understand their link to the observed water chemistry, and spatial variability.

-> Response: As stated above, the results section has been thoroughly modified and a new figure has been introduced (revised figure 3).

Page 4847 line 7. What are "shallow fluxes"?

-> Response: This remark was taken into account. Typography error it is in fact water fluxes. (see page 17, L522)

End of page 4847 and beginning of page 4848. The authors mentioned toward the end of the text that there is additional data on the microbial community in the study site. I understand that this is a subject of another paper by the authors but I still think that some information should appear also in this manuscript because this is a key issue. The differences that were observed between the peat sites could arise from differences in microbial community structure or simply due to differences in biomass. Any conclusive evidence should be based also on the microbial data (whether you present it in this paper of in another). Summarizing it in 2 sentences toward the end of the discussion is simply not enough (moreover, the reference is not in the reference list ?).

-> Response: This remark was taken into account. The microbiological results have been published and the main conclusions are presented in this revised version. (see page 17-18, L547-554)

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General Comment This paper concerns a field study of the effect of hydrology and chemistry on microbial activity leading to nitrate and sulphate reduction. The authors characterize two sites along a river-wetland (peat land) system with respect to transversal gradients in chemistry and hydrology. A two-year monitoring program was set up. At site G there were only short periods during which the water table in the river was higher than in the peat land, whereas at site S there was a more or less continuous flow from the river to the wetland. This difference is believed to explain the higher nitrate removal at site S than at site G. Peat from site G also had a lower initial sulphate content and lower release rates than site S. The diïnËĞAËŸ erences in chloride concentration with distance from the river were significant for both rivers and indicates chemical differences along flowpaths. The authors conclude from the batch experiments that the release of sulphates cannot be attributed to a single process.

An overall comment to the study is that the flow along a river is not dominatingly transversal in the adjacent land and that important gradients in hydrology and geochemistry may exist longitudinally in adjacent wetland parallel to the river. The nature of the problem is likely to be at least two-dimensional. The field sampling does not cover this aspect and this limits interpretation of the results in terms of linking water flow with geochemistry. In fact, the study would have benefited from a more formal identification of two-dimensional flow directions and sampling along flow paths. It is not clear to this reviewer to what extent the geochemical results along transects presents typical gradients in the concentrations of nitrate, sulphate and chloride.

-> Response: The presentation figure has been split in two figures in order to emphasize the global situation of the peatland on one hand and the investigation sites on the other one. The two sites belong to the 'Marais du Cotentin' peatland which constitutes a large area (Fig. 1). This peatland presents an extremely flat surface which ranges between 4 to 5 meters above sea surface. Therefore, no clear gradient parallel to the two main streams have been measured as can be seen in Fig. 3 which compares the water levels in two sites distant from 1.3 km. The upstream/downstream gradi-

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ent remains negligible even during the low-flow period (0.5/1300 m) as regards the stream/peat gradient (1/10-50 m). The peatland hydrology is thus mainly dominated by the exchanges between the peat and the main streams. This has also been explained in the geological section.

Despite the above potential shortcoming of the study, the claims of the paper are moderate and acceptable. Possibly, one may question the news value of the main findings that biological mediation is important for nitrogen removal in water and the considerable effect of hydrological conditions on biological activity in peat.

Specific comments 1. The author should explain why the two-dimensional nature of the flow in adjacent wetland can be neglected in the field sampling program and why the sampling transects are internally representative the geochemical gradients.

2. The author should more clearly describe the news value and contribution of this study.

-> Response: We took into account this comment and we emphasised the fact that : This study tries to relate field hydrological data to microbial investigation through an experimental approach. Using peat samples from sites with different hydrological conditions, we have tried to apply various chemical situations to the microbial communities. With this experimental setting, we try to distinguish the physical from the chemical effects on microbial communities.

3. The estimation of evapotranspiration is a dominating part of the water balance and seems to be estimated only roughly. Please, provide more comments on the accuracy and potential variability of the estimate.

-> Response: Meteorological data, precipitation and potential evapotranspiration were provided by MétéoFrance. Daily precipitation data were measured at the local meteo-rological station, 5 km north of the sites. Daily potential evapotranspiration data were calculated by MétéoFrance using the Penmann-Monteith equation at the intersections

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of a grid covering France with cells of 0,125° (about 12 kilometres). The equation uses standard climatological records of solar radiation, air temperature, humidity and wind speed. The data were interpolated by an inverse method at squared distances and calculated at knots of the grid including the 5 nearest meteorological stations. Actual evapotranspiration estimation from the Turc formula was controlled using a mass balance approach based on water table hydrograph (Freeze and Cherry, 1979; Healy and Cook, 2002). Both methods agreed within less than 10 %. This result was also confirmed using a chloride mass balance. The precisions about actual evapotranspiration are given in the revised version. (see Section Hydrologic section 2.1.3)

4. Explain more clearly the significance of biological activity in peat on nitrogen removal. For instance, it is stated that "biotic and abiotic conditions indicates the importance of biological mediation in nitrate removal". This is well known and it is not clear exactly what is found in this study in quantitative terms.

-> Response: We did not fully understand the reviewer. Nitrate removal is a major biological process. However part of this process may also interact with chemical reaction. The comparison of biotic and abiotic conditions (Fig. 6) indicates the importance of biological mediation in nitrate removal (deviance analysis, e.g. G site aerobic conditions: P<0.0001). It indicates that the whole nitrate reduction cannot be assigned solely to biological activity. Although the biological mediation is well known, the fact that part of the nitrate reduction is not related to biological processes is not a usual result, especially because our results provide a quantitative estimate of each process. Furthermore, we showed (Bougon et al., 2009) that hydrological conditions presented in field section may structure the microbial community response, which is a new result.

5. I find Table 5 dubious because I'm not convinced that the numbers are representative to the spatial and temporal variability along flow paths. It is also not clear how intermittent changes can be and if the sampling frequency is sufficient. Please, explain.

-> Response: The results presented in Table 5 concerned only results from batch

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experiments. We did not make the statistical analyses on field sites. The sampling frequency was the one presented in the chemical batch analyses: 1, 9, 25, 76 and 240h. The changes are thus the changes of nitrate, chloride and sulphate concentration presented in this paper. We changed also the title of table 5 by: 'Effect of experimental and site parameters on batch evolution'

6. Would soil characteristics be important to differences in chemical processes. For instance, it is well-known that denitrification can be limited by the carbon source in many cases.

-> Response: The impact of carbon source on denitrifcation was also emphasised by another reviewer. This remark was thus taken into account in the revised version and a new section was introduced with some results about the DOC concentrations. (see page 13-14, L406-425)

General comment: Bougon and colleagues presents an experiment research focused on influence of hydrological conditions on nitrate and sulphate fate in peatlands. The topic is appropriate for Biogeosciences journal, however I strongly believe that the manuscript need a thoughtful major revision. Below I provided in detail a list of recommendations but my main comments gravitate around two main aspects: 1) A deep coupling between field data and results from batch experiments; 2) A more exhaustive and convincing explanation of the link between nitrate removal and microbial diversity.

1) Field data and experiments coupling: Authors stated at page 4832 (line 15) that the "Aim of the batch experiments was to reproduce the field observations": According section 2.1.4 data from chemical monitoring included pH, electrical conductivity, redox potential, Cl, SO4 and NO3. However these data are briefly reported in this study (section 3.1.2) then, readers do not have a solid chance to evaluate to what extend the results from batch experiments really help to interpret the field observations. For instance, the authors stated that "changes in nitrate and sulphate concentrations were

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clearly related to water table dynamics and reflected various redox conditions related to water saturation". Is it possible to make more visible these interesting results? More specifically I strongly miss a graph illustrating the NO3 temporal dynamic from the three sites. The authors briefly compared superficially the batch results with filed data in section 3.3. In my opinion, this section is more appropriate in the discussion and it should be explored much more aseriously. This is a crucial aspect because Biogeosciences, strongly supports publication of studies focused on field data interpretation. Therefore authors should take care this piece and include in their manuscript detailed N and S data from field monitoring and to restructure their discussion section analyzing the experimental results from the perspective of the observed field data. A solid and convincing digression about parallelism (and/or dissimilarity) between field data and experiment results will make much more consistent and motivating the manuscript.

-> Response: The geochemical results section (pages 10-11, L296-332) has been reworked in order to provide a more clear basis to the field/batch comparison. It helped to discuss the results and their relation to field processes, as suggested by the reviewer. Moreover, we added a figure representing the field geochemical results. In figure 4 nitrate and sulfate concentration are represented according to the sites and the distance to the stream. The changes in nitrate and sulphate concentrations were clearly related to water table dynamics and reflected various redox conditions related to water saturation

For instance: It is interesting to observe that according section 3.1.2. field data suggested a "-efficient nitrate removal in reference site G", Meanwhile according section 4.3 the batch experiments revealed that "Peat from reference site G showed slower nitrate removal". Does here we have contradictory conclusions? This is extremely interesting and should be explored in detail in the discussion!!!

-> Response: This remark was taken into account. We developed the idea in the section 4.3 (see page 15, L470-477): "under anaerobic conditions, peat from reference site G seemed to provide more efficient and rapid nitrate removal due to bacterial

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activity than peat from pumping site S. This effect was seen independently of nitrate concentrations or redox conditions which were similar in the different batchs (Tables 3 & 4). This difference, which agrees with the site observations, argues for the initial hypothesis linking bacterial activity to microbial communities, influenced by the hydrologic conditions."

2) Does microbial diversity is really important? The discussion emphasize that "heterotrophic dentrification" is the most relevant process that modulate the nitrate availability. This conclusion appears coherent. However, strictly reasoning, the batch experiments can not demonstrate that differences in nitrate removal can be attributed to "modification in bacterial activity which are likely related to changes in microbial diversity" (at the end of the abstract) which are, in turn, a consequence of hydrological changes. Then, according this reasoning a four steps cascade effects is suggested to describe the nitrate removal: Denitrification is a respiration process. Then, availability energy (i.e. electrons) source is the main fuel of this microbial respiration. But organic matter availability has not explored in this research. The authors neutralize this aspect stating that "the presence of available dissolved organic matter (>30 mg/l)".....but to date the direct relationship between DOC quantity and quality/bio-availability is theme of research and debate (see for example Jaffé at al., 2008 or Cumberland and Baker, 2007).....and perhaps high DOC content might be indicate low availability (or high refractority). It might be hypotizable that hydrological conditions modulate the DOC quantity/quality (see for instance Sobczak and Findlay, 2002; Vazquez et al., 2007; Peduzzi et al., 2008) and oxygen availability and indirectly the nitrate respiration (i.e. denitrification). Then into the four steps cascade effects described previously, the step "changes in microbial diversity" can be replaced by "changes in organic matter". If we constrain to peatland ecosystems studies the bibliography suggested that drought periods enhance a decrease in DOC concentration in peat waters (Clark et al., 2005) (and probably an increase of aerobic conditions).....If we assume arbitrarily that DOC quantity is synonymous of DOC quality, we can hypothesize that peatland affected by "periodic" shift from saturated to unsaturated conditions

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(site G perhaps?) might show lower denitrification rates (and higher NO3 concentrations) than peatland permanently saturated.....Does the field data and batch experiments corroborate or refute this alternative argument?...it seems that "site G showed slower nitrate removal"..... (Here appears imperative the need to connect field and experimental data!) In any case, if we constrain to data showed in this manuscript, the reader ignore totally if energy source (i.e.DOC) quantity/quality is similar in the different batch treatments and all additional information (but not verifiable, because the reference is missing) is focused of microbial diversity only. In conclusion, the four steps cascade effects described previously appears not convincing because an essential aspect of denitrification is omitted.

-> Response: This remark was taken into account. A new section about the DOC influence has been introduced. We also used the idea of a cascade hypothesis at the beginning of the revised version. (see page 13-14, L406-425)

Additional commentaries: Pag 4832 "Our hypothesis.....": Hypothesis should be stated clearly and argued in the "introduction" section rather than in the "M&M"...In any case this sentence does not really describe an hypothesis.

-> Response: This remark was taken into account and the hypothesis was more clearly described (see end of the introduction pages 3-4, L75-97)

Pag. 4832 (line 19): "water fluxes and peat moisture which were both higher in the S site". This is an important point: How do the authors discern the role of water flux from that of the soil moisture? According the manuscript title, discerning the role of "water flux" on N and S fate is the main objective of this study. However, since "water fluxes and peat moisture which were both higher in the S site" what kind of information the authors managed to conclude that the water flux prevails over water moisture as the main hydrological driver for chemical processes?

-> Response: This remark was taken into account. We do not and cannot determine the part of water fluxes and peat soil moisture on chemical process in this study. What

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we can say is that difference in hydrological condition will induce by the fact difference in peat moisture. By this lab experiment, we can conclude that the environmental perturbation presented in the field section can modify ecological and chemical processes. (see end of the introduction pages 3-4, L75-97)

In section 2.2 (and in more detail in section 3.1.1) the authors stated that the "left bank of site S is influenced by a permanent water influx (river to peat)" while "Influx from the stream to site G only occurs during very high water periods". Nevertheless the figure 2 suggests the opposite interpretation because the differences in water levels are clearer in panel A (reference site G) than in panel B (left bank site S). I suspect that the panel A describes the site S and not the site G (and vice versa). It is urgent to verify this figure, otherwise it introduces serious doubts to the reader.

-> Response: See comment of reviewer 1 which asked the same question. (see section 3.1; pages 8 -9 - 10 and 11)

Section 3.2.2. The first paragraphs of this section are a discussion rather than a results description. I suggest shifting these sentences to the discussion.

-> Response: This remark was taken into account and the paragraphs sent to discussion (see page 14, L437-447).

The formula used to describe the corrected sulphate concentrations should be described in the M&M section. It is important to state this formula in the M&M otherwise the reader does not interpret properly figure 5 and table 4. (...a rapid inspection of table 4 reveals SO4 production in several anaerobic batchs!).

-> Response: This remark was taken into account and the sulphate formula introduced in the method section. (see page 8, L228-235)

... At this stage I am wondering why nitrate changes are not analyzed with the same approach...... Why?

-> Response: As stated above, the pore water influence is limited for nitrate which are

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only reduced and never produced in the experiments.

In any case I am not sure of the precision of the following sentence: "No significant sulphate production was observed during the same period under anaerobic conditions". For instance, in table 4 the anaerobic sample "Site S left bank", "close" to the stream, (treatment with nitrate), CI little increase during the incubation, but SO4 concentration duplicate!!.....Then, the SO4 production in some anaerobic samples is relevant and this result contradicts the sentence cited previously. Where my mistake is?

-> Response: There is no sulphate production in anaerobic samples without nitrate addition, which has been indicated. In fact, the value of 395 is a typographic mistake. It is explained why we do not argue about the sulphate production in this case. The real value is :215,7mg/L.

Enlarge the legend text of figures 4, 5 and 6.

-> Response: This remarks was taken into account.

Page 4844 (line 17). "....denitrifiers use nitrate as final electron donor under anoxic conditions".....electron "acceptor", not "donor".

-> Response: This remark was taken into account.

The reference "Autevires et al., 2008" (Pag. 4832 (line 4)) and "Bougon et al. 2009" (Pag. 4837 (line 26) and page 4848 (line 7)) are missing in the reference list.

-> Response: This remark was taken into account and the references modified.

Figure 2. What does the "NGF" acronym in the y-axis stand for?

-> Response: This remark was taken into account. NGF is the acronym of 'nivellement general francais' which means French Standard Levelling. It has been changed to meter above sea level.

Please also note the Supplement to this comment.

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Anaerobic -+ NO3-

Anaerobic -+ NO3-

- Anaerobic - + NO₃

Aerobic

- Aerobic

Aerobic

 $-+NO_3$

 $- + NO_3^-$

-+ NO3





Control : abiotic samples

Pumping

Reference

site G

Site S

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Full Screen / Esc

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Fig. 6.

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