

### **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, SO<sub>4</sub> and NO<sub>3</sub>. 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 extent 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 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 NO<sub>3</sub> temporal dynamic from the three sites. The authors briefly compared superficially the batch results with field data in section 3.3. In my opinion, this section is more appropriate in the discussion and it should be explored much more seriously.

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.

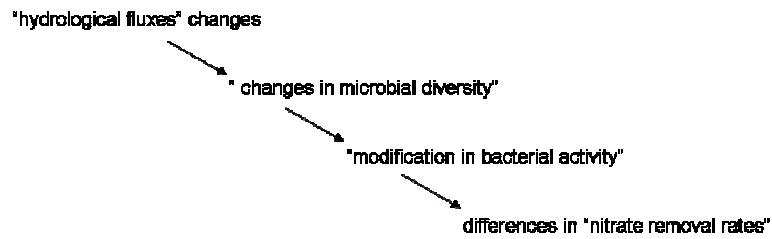
For instance: It is interesting to observe that according section 3.1.2. field data suggested a “*-efficient nitrate removal in reference site G*”, Meanwhile according section 4.3 the batch experiments revealed that “Peat from reference site G showed slower nitrate removal”. Does here we have contradictory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

### **2) Does microbial diversity is really important?**

The discussion emphasize that “heterotrophic denitrification” 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 (site G perhaps?) might show lower denitrification rates (and higher NO<sub>3</sub> 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.

**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.

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?

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.

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.

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 SO<sub>4</sub> production in several anaerobic batches!). ...At this stage I am wondering why nitrate changes are not analyzed with the same approach.....Why?

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), Cl little increase during the incubation, but SO<sub>4</sub> concentration duplicate!!.....Then, the SO<sub>4</sub> production in some anaerobic samples is relevant and this result contradicts the sentence cited previously. Where my mistake is?

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

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

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.

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

***Reference cited in this revision:***

Jaffé et al., 2008 Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 113, G04032, doi:10.1029/2008JG000683

Cumberland SA and Baker A., 2007 The freshwater dissolved organic matter fluorescence-total organic carbon relationship. HYDROLOGICAL PROCESSES, 21 (16): 2093-2099

Sobczak WV and Findlay S, 2002 Variation in bioavailability of dissolved organic carbon among stream hyporheic flowpaths. ECOLOGY, 83 (11): 3194-3209

Vazquez et al., 2007, Effects of the Dry–Wet Hydrological Shift on Dissolved Organic Carbon Dynamics and Fate Across Stream–Riparian Interface in a Mediterranean Catchment. *Ecosystems* (2007) DOI: 10.1007/s10021-007-9016-0

Peduzzi et al., 2008. Dissolved organic matter (DOM) and bacterial growth in floodplains of the Danube River under varying hydrological connectivity. *FUNDAMENTAL AND APPLIED LIMNOLOGY*, 171 (1): 49-61

Clark JM, Chapman PJ, Adamson JK, Lane SN. 2005. Influence of drought-induced acidification on the mobility of dissolved organic carbon in peat soils. *Global Change Biol* 11:791–809.