

Peer Reviewer Comments for:

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Full Title: **Nitrous oxide dynamics across nitrogen and pH gradients in headwater streams**

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### **General comments:**

This paper presents the results of a study of stream water N<sub>2</sub>O saturation measured from 72 sites in headwater streams in Denmark. The authors examined additional chemical and physical parameters and found that N<sub>2</sub>O saturation increased with nitrate and was higher at low pH. The authors tied this relationship with pH to potential management of N<sub>2</sub>O emissions from headwater streams in areas with acidic soils. Overall, the study was well presented, and the paper was very well written.

I would have appreciated a few more methodological details. In particular, CO<sub>2</sub> concentrations are shown in the appendix, but the methods section does not explain where these measurements were taken—is this dissolved CO<sub>2</sub> in the stream water or CO<sub>2</sub> in the ambient air?

As mentioned, pH and CO<sub>2</sub> are highly correlated, so I was surprised this information was not included. Based on Figure A5, the clearest relationship is between CO<sub>2</sub> and N<sub>2</sub>O. If the CO<sub>2</sub> was measured in the stream water, it could be very relevant to the stated relationship between N<sub>2</sub>O and pH. For example, aerobic respiration of organic carbon material in sediments could produce CO<sub>2</sub> in the sediments, which is then released to the surface water, decreasing pH. This aerobic respiration also consumes oxygen, leading to anaerobic conditions that could favor heterotrophic denitrification and production of N<sub>2</sub>O. So, I think it is important to include a discussion of CO<sub>2</sub> and why it is related to pH—is the lower pH causing more N<sub>2</sub>O production (or less N<sub>2</sub>O reduction via inhibition of nosZ), or is the lower pH a result of sedimentary reactions that also increase denitrification?

Below are some additional questions related to the study.

### **Specific Comments:**

Line 72: Were samples collected from 72 different streams, or were there some streams with multiple sampling sites?

Line 84: Were these soil types determined 30-60 below the stream bed, or on the banks?

Line 85: What is the resolution of the Danish Area Information System source?

Lines 98-109: For future researchers, it would be helpful to indicate other sampling details, such as if the sampling bottles were triple rinsed with site water, etc. Was stream velocity measured at 0.4d from the streambed, the middle depth, etc.? Was discharge calculated using the area-velocity method? Does “vegetation cover” refer to overhead canopy cover or something else? Where were the iron ochre deposits? What was measured in the ambient air sample? Were samples refrigerated? It would help to clarify how both headspace vials and water sample bottles were stored.

Line 114: What were unfiltered samples used for? Were the samples for SRP filtered twice? (0.45 um and 0.22 um)?

Line 125: What kind of detector was used with the GC?

Line 160: To me, “across individual streams” sounds like there are multiple samples from the same stream that show variation. Using “across streams” might be clearer if you are referring to different streams having different concentrations.

Line 211: CO<sub>2</sub> is reported, but I don’t think I saw this described in the methods. Is this dissolved CO<sub>2</sub> in the stream water or CO<sub>2</sub> in the ambient air sample? Please clarify in the methods and throughout when it is mentioned (Figure A1, A4, Table A2, etc.).

Line 235: Could you explain a “semi-natural stream?”

Line 259: Please clarify the relationship between stream depth and pH. What causes this?

Figure A5: Why is CO<sub>2</sub> not mentioned in the caption? Please specify where CO<sub>2</sub> was measured. Is this dissolved in the stream water?

#### **Technical/Minor Comments:**

Line 40: Capitalize IPCC

Figure 2: Consider changing “identity” in the caption to stream number for clarity. It also might be helpful to indicate the time range of the photos.

Line 135: Dry organic matter content?

Line 143: correlations (plural)

Line 153; Define REML.

Line 154: Is there a word missing after smooth?

Line 158: mean annual precipitation? Could you include total n for all the streams and seasons?

Line 159: subject/verb agreement. Specify that these are stream water N<sub>2</sub>O concentrations.

Line 164: mean saturation measurements...were (subject/verb agreement)

Table 1: Please include n and units for temperature. It might be helpful to list the definitions in the heading in the same order as in the table (and include CO<sub>2</sub>)

Table 2: Consider defining SE and DF in the table heading.

Line 225: This sentence refers to saturation but the numbers shown are concentrations.

Line 232: Please include n. Is it normal for this journal to have a space between a number and the % sign? (Multiple instances)

Line 233: Add comma after 69%, consider rewording to “with a range of ...”

Line 308: change to “undersaturation”

Line 494: Dry organic matter content?

Figures A2-A4: It might be helpful to include the present agriculture for each stream’s watershed in the legend, as in Figure 3.

Table A3: Define SE and DF in heading.