# Nitrogen and phosphorus regulation on nitrification, denitrification and oxygen, pH and nitrate profiles in humic and clear water lake sediment.

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**ABSTRACT: Nitrogen and phosphorus regulation on nitrification, denitrification and oxygen, pH and nitrate profiles in humic and clear water lake sediment.** The aim of this research was to evaluate the influence of nitrate, ammonium and phosphate on the oxygen, pH and nitrate profiles and on nitrification and denitrification processes in the sediment from a clear and a humic lake. Sediment cores were sampled in the littoral zones from a humic brownish and a clear water lake. Some cores from each lake have been enriched with nitrate, ammonium or phosphate. After a pre-incubation time that varied from 24 to 39 hours, pH, nitrate and oxygen profiles were performed with microsensors. Denitrification and nitrification were calculated from the nitrate profiles. Oxygen penetration was higher in the sediment from the humic than from clear lake, indicating a lower activity of the bacterial community in the sediment in the humic one. Oxygen penetration decreased in the sediment of the humic lake after addition of nitrate and ammonium suggesting that the bacterial community in this sediment was nitrogen limited. In clear lake addition of phosphate decreased oxygen penetration in sediment, suggesting that the bacterial community in this sediment was phosphorus limited. Nitrification and denitrification zones were thinner in all treatments in the clear lake sediment. In both lakes and in all treatments, the increase in nitrification rates were followed by an increase in denitrification rates indicating that nitrate availability is the main limiting factor to denitrification in the studied lakes. Nitrification and denitrification rates were lower in the humic lake sediment when no substrate was added, but after addition of ammonium the rates in humic lake increased substantially being higher than in clear lake. It could be concluded that addition of nitrogen and phosphorus does have an effect on nitrification, denitrification and on oxygen and nitrate profiles from clear and humic lake sediments and both processes and profiles will be more affected by the addition of a nutrient that is limiting sediment bacterial community. pH profiles seem not being affected by addition of nitrogen and phosphorus in both lakes.

**Key-words:** nitrification, denitrification, humic substances and sediment.

**RESUMO: Regulação por nitrogênio e fósforo dos processos de nitrificação, desnitrificação e perfis de oxigênio, pH e nitrato no sedimento em lagos com e sem compostos húmicos.** O objetivo desta pesquisa foi avaliar a influência de nitrato, amônia e fosfato sobre os perfis de oxigênio, pH e de nitrato e sobre os processos de nitrificação e desnitrificação no sedimento de um lago com águas claras e outro com águas húmicas. Cores de sedimento foram coletados na zona litorânea em ambos os ecossistemas. Alguns cores foram enriquecidos com nitrato, amônia e fosfato. Após uma pré-incubação que variou de 24 a 39 horas, foram realizados os perfis de pH, nitrato e oxigênio com microeletrodos. As taxas de desnitrificação e nitrificação foram calculadas a partir dos perfis de nitrato no sedimento. A penetração de oxigênio foi maior no sedimento do lago de águas húmicas, indicando uma menor atividade da comunidade bacteriana no sedimento deste ecossistema. A penetração de oxigênio no sedimento do lago de águas húmicas diminuiu após a adição de nitrato e de amônia, sugerindo que nitrogênio limitava esta comunidade bacteriana. A diminuição da penetração de oxigênio no sedimento causada pela adição de fosfato no sedimento do lago de águas claras sugeriu que a comunidade bacteriana no sedimento deste ecossistema era limitada por fósforo. A faixa em que os processos de desnitrificação e nitrificação ocorriam no sedimento do lago de águas claras era mais reduzida do que no sedimento do lago de águas húmicas, em todos os tratamentos. Nos dois ecossistemas, o aumento das taxas de nitrificação foram acompanhadas de um aumento nas taxas de desnitrificação, indicando que a disponibilidade de nitrato é o principal fator regulador do processo de desnitrificação. As taxas de nitrificação e de desnitrificação foram

menores no sedimento do lago de águas húmicas quando não havia adição de substrato. No entanto após a adição de amônia estas taxas aumentaram substancialmente no sedimento do lago de águas húmicas passando a ser mais elevadas do que no sedimento do lago de águas claras. Pode ser concluído que as adições de nitrogênio e fósforo tem um efeito sobre as taxas de nitrificação, desnitrificação e sobre os perfis de oxigênio e nitrato no sedimento e que ambos os processos e perfis serão mais afetados após a adição do nutriente que estiver limitando a comunidade bacteriana no sedimento. Os perfis de pH parecem não ser afetados pela adição de nitrogênio e de fósforo.
**Palavras-chave:** nitrificação, desnitrificação, substância húmicas e sedimento.

# Introduction

Eutrophication in aquatic ecosystems caused by nitrogen loading is a worldwide problem (Granell et al, 1990; Caraco & Cole, 1999). Thus, nitrification and denitrification can be considered key processes in the elimination of this excess of nitrogen. Denitrification is mediated by facultative anaerobic bacteria that under anoxic conditions reduces nitrite and nitrate to dinitrogen. Nitrification is mediated by two groups of chemoautotrophic bacteria that oxidize ammonium to nitrite and nitrite to nitrate under oxic conditions. As denitrifiers are supplied by nitrate that diffuses from water or is produced by nitrifiers, denitrification rates are higher at the aerobic-anaerobic interfaces of biofilms, sediments and waterlogged soils (Seitzinger, 1988; Comwell et al, 1999).

The effect of oxygen on nitrification and denitrification have been studied by Jensen et al. (1994), who showed that an increase of oxygen penetration in the sediment promotes an increase in denitrification from nitrate produced by nitrification (coupled nitrification-denitrification) and a decrease in denitrification from nitrate that diffuses from the overlying water. Nitrification rates and nitrate penetration into the sediment also increase with increase in oxygen penetration. Therefore nitrate profiles are regulated by oxygen penetration or production (Jensen et al., 1994; Lorenzen et al., 1998) in the sediment. pH profiles are also influenced by oxygen penetration as the depth of aerobic and anaerobic processes occurrence are determined by this parameter (Kühl et al., 1995).

Oxygen and pH vertical distribution and nitrification and denitrification rates have been investigated in some freshwater and marine sediments, but to my knowledge no research have been done in concern to humic lakes. The classification of humic ecosystems is usually based on their brownish color, being more related to the quantity that on the quality of humic and fulvic compounds. Humic compounds are biomolecules which are yellow to brown or black in color, with molecular weight varying from 500 to 100.000D and biologically recalcitrant (McKnight & Aiken, 1998). The amounts of studies in humic environments increased in the last decade and most of them have been focusing on processes and interactions that occur in the water column. The aim of this research was to evaluate the influence of nitrate, ammonium and phosphate on the oxygen, pH and nitrate profiles and on nitrification and denitrification processes in the sediment of a clear and a humic lake.

# Material and methods

## Study area

Sediment was sampled in the littoral zones from a humic brownish and a clear water lake. The humic lake was located close a cow farm and a cereal plantation. Methane bubbles were observed in the sediment from 1.5cm depth to below. The sediment presented a high vertical and horizontal homogeneity. The presence of some microalgae and copepods over the sediment surface was also noted. The brown color of the water column made it difficult to see the sediment surface at 50cm depth. The clear water lake had a higher presence of microalgae and copepods. The presence of some macroinvertebrates was also observed. The sediment was characterized by having a "fluffy" biofilm (1-2 mm) and a vertical granulometric gradient. After the fluffy part, the sand particles diameter increased with depth. Formation of methane bubbles was also observed, from 1cm to below. The water was completely transparent. Water temperature and pH were 18°C and 17°C and 6,4 and 7,9 in the humic and clear lake respectively.

### Experimental procedure

In each lake, 5 cores (12cm length and 12.8cm$^2$ area) were sampled on May 4$^{th}$ 2000 and brought to the laboratory. Four hours after sampling, they were transferred to bigger chambers with water from the sampling site and stabilized with air bubbling for 20hs. After this period, each core was placed in smaller chambers with 400ml of water from the sampling site. Air has been bubbled in each chamber, to keep the sediment-water interface oxygen saturated. One milliliter of concentrated solutions were added to the cores from two lakes reaching final concentrations of: 500µM $KNO_3$, 500 µM $NH_4Cl$, 50 µM $K_2HPO_4$ + 50 µM $KH_2PO_4$ and 500 µM $NH_4Cl$ + 50 µM $K_2HPO_4$ + 50 µM $KH_2PO_4$. As the fifth core was a control, no addition was made. Cores were incubated in the dark under a temperature of 20°C. pH profiles in the sediment were measured 24 – 28h after addition of nutrients; nitrate profiles after 29 – 36h and oxygen profiles after 37 – 39h.

Microprofiles of oxygen were obtained with a 5-10 µm tip thick Clark-type oxygen microsensor mounted on a micromanipulator connected to a recorder. A 2 point linear calibration was obtained by reading the signal in well-aerated deionized water and in anoxic sediment. Microprofiles of pH were obtained with a 5-10 µm tip thick pHsensor made of pH-sensitive Croning glass with an insulating shaft of lead glass. pH calibration was obtained by reading the signal in standard solutions of pH 7 and 10. Microprofiles of nitrate were obtained with a 30-50 µm tip nitrate microsensor as described in Lorenzen et al. (1998). The advantages in using microsensors to study biogeochemical processes are short response time, high spatial resolution, small disturbance on the substrate and the information one can get just with one simple profile (Kjær, 2000). All sensors were built at the Department of Microbial Ecology from the University of Arhus, but they can be bought at www.unisense.com.

Calculations of the consumption or production of nitrate were performed with the PROFILE 1.0 program (Berg et al., 1998). Consumption and production of nitrate was assumed as being only denitrification and nitrification respectively (Lorenzen et al., 1998).

Nitrate, oxygen and pH profiles were done just once in each core. To apply any kind of statistical test to compare treatments or lakes, at least three profiles for each parameter would be required. The main reason for that no replicates have been made is that this procedure would increase the measuring time in two or threefold and therefore promote changes in the measured profiles. The ideal situation would be to measure all the profiles simultaneously, but this procedure was not possible in this experiment.

## Results and discussion

### Oxygen Profiles

Oxygen penetration was higher in the sediment from the humic lake (Fig. 1) indicating a lower consumption and consequently lower activity or density of the bacterial community in this sediment. Humic lakes are characterized by having low bacterial metabolism and this is mainly due to low availability of labile carbon. As phytoplanktonic primary productivity is reduced due to decrease in light penetration (Lean, 1998), allochthonous and recalcitrant dissolved organic carbon (DOC) formed by humic and fulvic compounds are the main energy source for bacteria (Hessen & Tranvik, 1998).

Addition of nutrients decreased oxygen penetration in humic lake sediment, being the higher oxygen penetration observed in control (Fig. 1B). Due to high DOC pool, C:N and C:P ratios are very high in humic lakes (Jones, 1998). Cimbleris & Kalff (1998) showed that a reduction in the C:N:P ratios in the water column to close to intracelular bacterial ratio caused by addition of nutrients promote an increase in bacterial growth efficiency and metabolism. In this experiment addition of nutrients probably reduced the C:N and C:P ratios in the humic lake sediment, stimulate bacterial growth and metabolism and consequently increased oxygen consumption. Oxygen penetration was lower in the sediment of the humic lake after addition of nitrate and ammonium (Fig. 1B) suggesting that the bacterial community in this sediment was nitrogen limited.

Addition of ammonium did not promote a change in the oxygen profile in the sediment from clear lake in comparison to the control, but addition of nitrate increased oxygen penetration (Fig. 1A). Although nitrate profiles in the sediment were not performed after addition of nitrate in the water, it can be assumed that this ion diffuses and penetrate deeper into the sediment, due to high nitrate concentrations in the water (500 µM). Under anaerobic conditions nitrate is used as electron acceptor by denitrifiers but can also be used by sulphur bacteria, as has been shown for *Thiobacillus denitrificans* (Fenchel et al., 1998). One explanation for the higher penetration of oxygen into the sediment could be that sulfur or other anaerobic bacteria could be using nitrate in the anoxic zone and consequently promoting a deeper penetration of oxygen due to lower availability of reduced compounds closer to the oxic zone. When phosphate or ammonium + phosphate were added, oxygen penetration in clear water sediment decreased, suggesting that the bacterial community in this sediment was phosphorus limited.

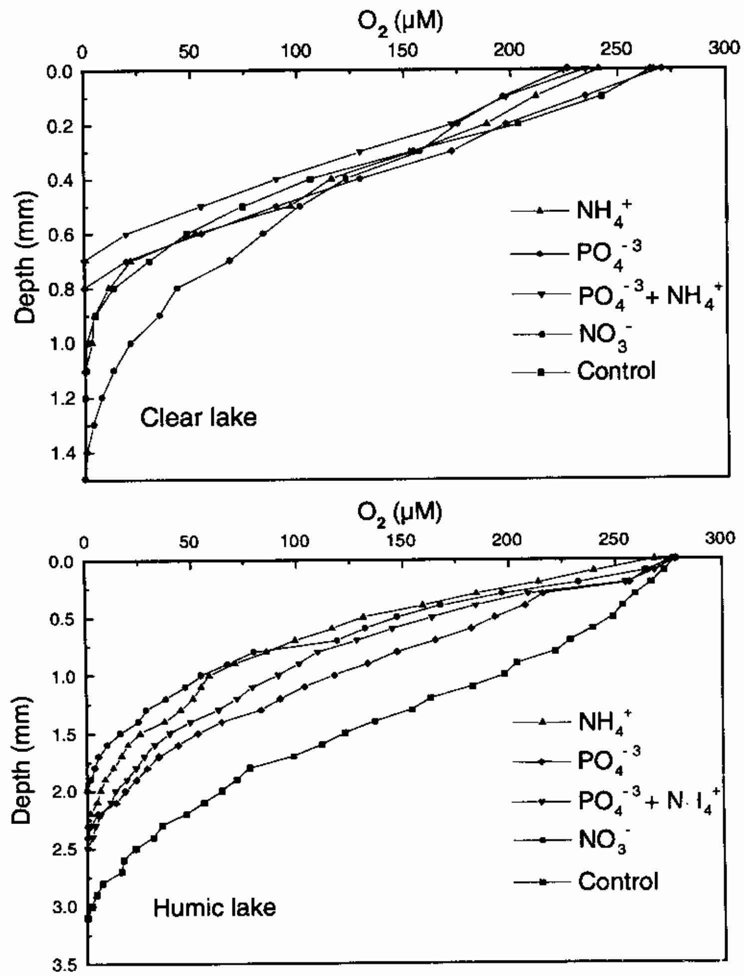


**Figure 1:** Oxygen profiles from clear and humic lake sediment in control and after addition of nitrate ($NO_3^-$), ammonium ($NH_4^+$), phosphate ($PO_4^{-3}$) and $NH_4^+$ + $PO_4^{-3}$. Note differences in scales.

Phosphorus limitation in clear lake sediment was not surprising. Shapiro (1988) point out that the role of phosphorus as the key limiting inorganic nutrient for the productivity of freshwater ecosystems is accepted and little questioned. Fenchel et al. (1998) also consider phosphate as the main limiting nutrient in freshwater environments. However, humic lakes have to be considered an exception, as there is not yet a consensus on limiting nutrients in them. Biological production in humic lakes can be limited by phosphorus, nitrogen or both according to the literature (Jansson, 1998).

## pH Profiles

pH decreased between 1,5 and 2,5 units from the surface to a depth of ca. 2.0mm (Fig. 2A and B) in all treatments in both sediments. This result, together with oxygen profiles (Fig. 1A and B), indicates that these 2.0mm are the most actively metabolic layer in the sediment. Although oxygen does not diffuse until this depth in the clear lake, occurrence of anaerobic processes can promote changes in pH. Below the depth of 2.0mm, availability of electron donors decreased, fermentative processes take place and changes in pH are not so abrupt.

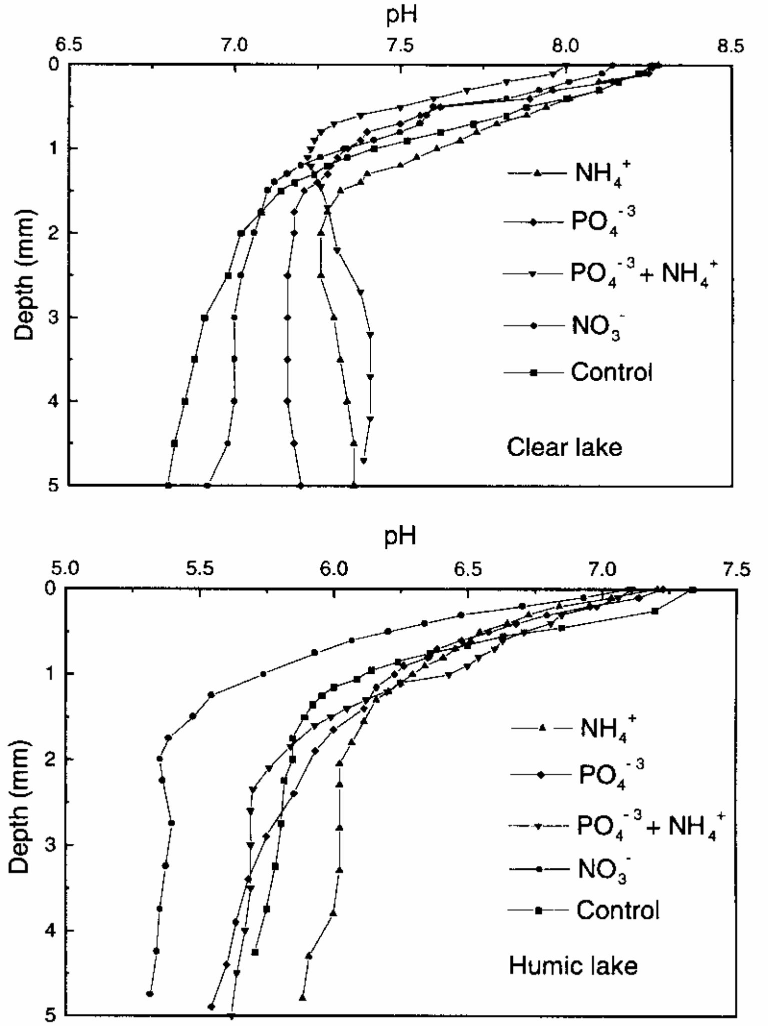


**Figure 2:** pH profiles from clear and humic lake sediment in control and after addition of $NO_3^-$, $NH_4^+$, $PO_4^{-3}$ and $NH_4^+ + PO_4^{-3}$. Note differences in scales.

There was no clear effect of addition of nutrients in pH from clear and humic sediments (Fig. 2A and B). The only exception was the decrease observed of pH in humic lake sediment after nitrate addition, but no explanation could be found for this phenomenon. An increase in oxygen consumption should promote an increase in $CO_2$ production and therefore change pH in the sediment. This effect was expected at least in the sediment in that the limiting nutrient was added. Therefore the absence of changes in pH after addition of nutrients can be attributed to: 1) both sediments have high alcalinities and an eventual increase in $CO_2$ production was not enough to change pH; or 2) changes in pH could just be observed after a longer period of incubation. pH and oxygen measurements have been performed 24 and 36 hours after addition of nutrients. It could have happened that during these 12 hours lack the bacterial community became more active and changes were clearer.

## Nitrate profiles and nitrification and denitrification

In the clear lake addition of ammonium and ammonium + phosphate slightly increased nitrate penetration in the sediment, but onlty addition of phosphate probably promoted a depletion from all nitrate present in the water and sediment and no nitrate profile was observed (Fig. 3A). This result reinforces the conclusion obtained with oxygen profiles (Fig. 1A) that phosphorus limits bacterial community in the clear lake sediment. After addition of phosphate the bacterial community probably increased its metabolism and/or growth and became nitrogen limited, assimilating all ammonium and nitrate available in the water column and sediment. Similar results have been found by Enrich-Prast et al. (in preparation). In the humic lake sediment, addition of ammonium and ammonium + phosphate clearly stimulated nitrification and denitrification and phosphate addition just increased nitrate penetration in the sediment (Fig. 3B). This result also reinforces the conclusion obtained with oxygen profiles (Fig. 1B) that nitrogen limits bacteria community in humic lake sediment.

The zones of production (nitrification) and consumption (denitrification) of nitrate were thinner in all treatments in the clear lake sediment (Fig. 3A and B). As nitrification is an aerobic process, the less oxygen penetration in clear lake sediment restricted the occurrence of this process to a thinner zone. The thinner zone of denitrification in the clear lake sediment can be explained by higher availability of labile carbon that favors heterotrophic processes (i.e. denitrification) as discussed above. Availability of electron donors (organic carbon) can control denitrification (Knowles, 1982) as shown by Brettar & Rheinheimer (1992). In both lakes and in all treatments, the increase in nitrification rates was followed by an increase in denitrification rates too (Tab. I). This indicates that nitrate availability is the main limiting factor to denitrification in the studied lakes.

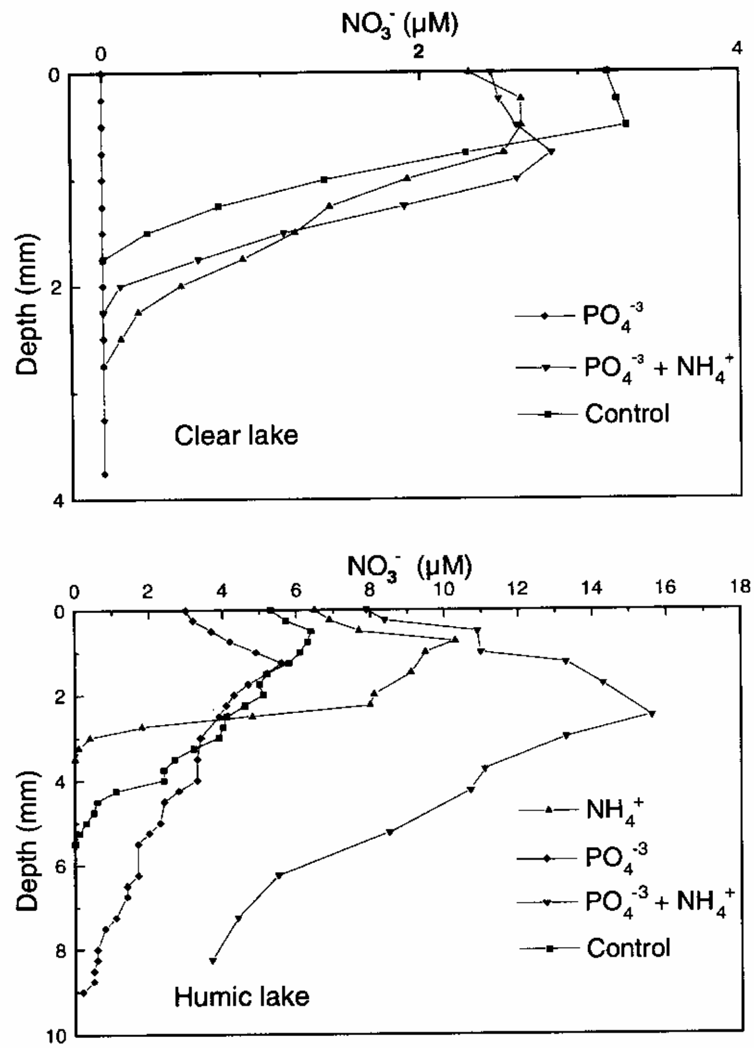


**Figure 3:** Nitrate profiles from clear and humic lake sediment in control and after addition of $NH_4^+$, $PO_4^{-3}$ and $NH_4^+ + PO_4^{-3}$. Note differences in scales.

Table I: Nitrification and denitrification rates (µmol. $m^{2}.h^{-1}$) from clear and humic lake sediment in control and after addition of $NH_4^+$, $PO_4^{-3}$ and $NH_4^+ + PO_4^{-3}$.

| Treatment | Nitrification | | Denitrification | |
|---|---|---|---|---|
| | Clear | Humic | Clear | Humic |
| Control | 38.2 | 21.2 | 23.8 | 13.0 |
| $PO_4^{-3}$ | 0.0 | 9.0 | 0.0 | 5.4 |
| $NH_4^+$ | 15.8 | 103.7 | 10.8 | 43.2 |
| $PO_4^{-3} + NH_4^+$ | 36.7 | 53.3 | 18.4 | 23.0 |

Nitrification and denitrification rates were lower in the humic than in the clear water sediment in control cores, but in cores with addition of ammonium and phosphate + ammonium the rates in humic lake increased and were higher than in the clear lake sediment. As the bacterial community was nitrogen limited in the humic lake, heterotrophic bacteria probably compete with nitrifiers for the ammonium and with denitrifiers for the nitrate available. This certain limit nitrification and denitrification in this sediment. After addition of ammonium the bacterial community in the sediment are not limited by nitrogen any more and availability of ammonium and nitrate will be higher for nitrifiers and denitrifiers respectively.

## Conclusion

Addition of nitrogen and phosphorus does have an effect on nitrification, denitrification and on oxygen and nitrate profiles from clear and humic lake sediments and both processes and profiles will be more affected by the addition of a nutrient that is limiting sediment bacterial community. Addition of nitrogen and phosphorus seems not have an effect on pH profiles in the sediment from both lakes.

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