Effect of nitrogen availability on the oxygen consumption during mineralization of *Scirpus cubensis* from Infernão Lagoon (São Paulo-Brazil)

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ABSTRACT. This study evaluated the influence of nitrogen additions in the oxygen uptake during decomposition of *Scirpus cubensis*, through laboratory assays. The dissolved oxygen, pH, electrical conductivity (EC) and water temperature were measured. The results showed an initial acidification that decreased gradually, indicating great release of reduced organic matter and subsequent CO_2 formation. The decomposition chambers with greater nitrate concentrations exhibited higher pH mean values. The temporal variation of EC showed a small increase and an abrupt increase with increasing nitrate concentrations. The maximum oxygen uptake increased up to 6.37 mg L^{-1} of nitrate, then decreased. The deoxygenation rate showed an opposite trend from that observed for the oxygen uptake. The coefficients and maximum oxygen consumption related to the particulate detritus mineralization tended to increase with increasing nitrate concentration. The increase in nitrate levels affected the decomposition of each organic matter fraction in different ways, with the particulate organic matter being more easily degraded in richer nitrate concentrations than the dissolved organic matter.

Key words: decomposition, macrophyte, nitrogen, oxygen uptake, POM, DOM.

RESUMO. Efeito da disponibilidade de nitrogênio no consumo de oxigênio durante a mineralização de *Scirpus cubensis* da Lagoa do Infernão (São Paulo, Brasil). Neste estudo, avaliou-se a influência de adições de nitrogênio no consumo de oxigênio durante a decomposição de *Scirpus cubensis*, por meio de ensaios de laboratório. Foram determinados: as concentrações de oxigênio dissolvido, o pH, a condutividade elétrica e a temperatura da água. Os resultados mostraram acidificação inicial que diminuíram gradualmente, indicando grande liberação de matéria orgânica reduzida e formação subseqüente de CO_2. As câmaras de decomposição com maiores concentrações de nitrato apresentaram valores de pH mais alto. A condutividade elétrica mostrou pequeno incremento com o passar do tempo e aumento abrupto com a intensificação das concentrações de nitrato. O consumo de oxigênio máximo global aumentou até a concentração de nitrato de 6,37 mg L^{-1}, depois decresceu. Os coeficientes globais de consumo de oxigênio apresentaram uma tendência oposta às quantidades máximas de oxigênio consumido. Os coeficientes de consumo de oxigênio, relacionados com a mineralização das frações particuladas, tenderam a aumentar com a elevação da concentração de nitrato. O aumento do nível de nitrato afetou diferentemente a decomposição das frações da matéria orgânica; a matéria orgânica particulada foi mais facilmente degradada do que a dissolvida em presença de maiores concentrações de nitrato.

Palavras-chave: decomposição, macrófitas, nitrogênio, consumo de oxigênio, MOP, MOD.

Introduction

The availability of oxygen used by microorganisms during respiration in aquatic environments has great ecological importance, affecting the nature and the final products of decomposition. Under aerobic conditions, most organic matter is reduced to CO_2 (Bertrand *et al.*, 2007), while the remaining fraction contributes to the formation of humic substances and the increment of microorganism biomass, which will also be converted to CO_2 over a longer period of time. Thus, the rates of oxygen uptake in aerobic mineralization are related to CO_2 production, and the quantity of oxygen consumed generally corresponds to the CO_2 released (Berman *et al.*, 2001). Oxygen consumption is also an indirect indication of activity of microorganisms in decomposition (Strauss and Lamberti, 2002). In addition to microorganism activity, physical and chemical parameters affect decomposition, which ultimately determines the rates of mineralization and the cycling of carbon and nutrients, including retention of nutrients in detritus and within the environment (Findlay *et al.*, 2002).

This study is included among others developed at the Infernão Lagoon, which is an oxbow lake situated in the Mogi-Guaçu River floodplain. It is located at 21° 35' S and 47° 51'W, in São Paulo State, within the area comprising the Jataí Ecological Station. The area of the Ecological Station is still preserved, in spite of increased agro-industrial activities and urban centers in the surrounding area. Both superficial and groundwater pollution are related to the presence of agrochemical substances (*i.e.*, herbicides, insecticides, fertilizers, acaricides and fungicides) from agricultural activities, silviculture and detritus released by sugar cane industries operating in the area of the Mogi-Guaçu River floodplain (Santos and Mozeto, 1992). Thus, the negative consequences of these activities on water quality can be significant. In this context, the knowledge of these ecosystems and how they affect the environment is important in the formulation of proposals to attenuate the effects of diffuse pollution. Emphasizing the eutrophication process, this study investigates, through laboratory assay, the influence of increased nitrogen concentration during decomposition of the emergent aquatic macrophyte *Scirpus cubensis*.

Material and methods

Adult individuals of *Scirpus cubensis* Poepp and Kunth were collected at Infernão Lagoon (9/22/1992) and washed with lagoon water according to the procedures described by Ogburn III *et al.* (1987). In laboratory, the plants were washed with tap water to remove the attached material, dried (at 65°C) until reaching constant weight, and grounded. The fragments were separated in a sieve (0.5 mm mesh), and homogenized and stored in polyethylene bags until the incubation set up. The water was collected in the lagoon with Van Dorn bottles (1/22/93), at three different depths (i.e., surface, middle and bottom of the water column). Then, the sampled water was mixed and stored in polyethylene flasks. In order to remove the coarse particulate organic matter (POM), the sample of water was pre-filtered in laboratory.

In order to estimate the concentration of dissolved oxygen during aerobic decomposition, 10 decomposition chambers were set up with *S. cubensis* fragments and sample water from Infernão Lagoon, in the proportion of 200 mg DW (dry weight) L^{-1}. The water samples were enriched with phosphate (1.32 mg L^{-1}) and different concentrations of nitrate (0, 0.18, 0.36, 0.91, 1.82, 2.73, 4.55, 6.37, 11.83 and 18.2 mg L^{-1}). Control flasks (1 L) with the same nitrogen and phosphorus concentrations and without plant fragments were also prepared, one for each assay condition. The incubations were maintained in the dark and at room temperature (26.1 ± 1.0°C; n = 250) during 81 days. The temporal variations of pH, electrical conductivity (EC) and dissolved oxygen concentration in the incubations were measured. The dissolved oxygen concentration was determined using a DOmeter (Metrohm Herisau AGCH-9100/E-627), the pH was measured with a potentiometer (Digimed DMPH-2) and the EC was obtained with a conductimeter (Digimed CD-2P). At the end of experiments, the water from the incubations was fractionated and the remaining particulate organic matter (POM) was determined by the gravimetric method, after drying at 60°C.

To ensure aerobic conditions (in incubations and control samples), when the dissolved oxygen concentration was below 2.0 mg L^{-1}, the incubations were re-aerated. The results of oxygen consumptions were integrated and the consumption from controls flasks was discounted; these results were used to estimate the maximum values of oxygen consumption and deoxygenation coefficients. These parameters were estimated by fitting the experimental results to a first-order exponential model (Equation 1), according to Bitar and Bianchini Jr. (2002). The fittings were made by non-linear regressions by the iterative algorithm Levenberg-Marquardt method (Press *et al.*, 1993);

$$y_t = y_{MAX}(1 - e^{-kt}) \qquad (1)$$

where: yt = oxygen uptake (mg L^{-1}); y*MAX* = theoretical maximum oxygen consumption (mg L^{-1}); k = deoxygenation coefficient (day^{-1}); t = time (day).

For this study, the mineralization rate constants (k_1 and k_2) of the dissolved organic matter (DOM) and particulate organic matter (POM) and respective maximum oxygen uptake (yMAX(DOM); yMAX(POM)) were estimated. For such estimation, we considered that in the first days of the decomposition, 8.2% of the plant fragments were leached. Thus, the initial amount of particulate organic matter of *S. cubensis* fragments was 91.8% (Bianchini Jr. and Antonio, 2003).

The remaining POM of the decomposition provided the final amount of particulate organic matter. The initial and final values of POM were fitted to the exponential model to obtain the mineralization rate of POM (k_2). Subtracting the k_2 value from the overall rate (k), we estimated the mineralization rate of DOM (k_1). With the constant of integration relating to the kinetics of the parallel first-order reactions [$k_1/(k_1+k_2)$], we calculated the maximum oxygen uptake that corresponded to the mineralization of the dissolved fraction (yMAX(DOM)). Subtracting this value from the overall maximum oxygen uptake (yMAX) we obtained the maximum oxygen uptake relating to the particulate fraction (yMAX(POM)). The pH and EC data from incubations were statically analyzed for each treatment (N-enrichment concentration) using the Kruskal Wallis test followed by Dunn's Multiple Comparison test to check for significant differences among treatments ($p < 0.05$).

Results and discussion

The Kruskal Wallis analysis of the time dependence of pH and EC from incubations showed significant differences among treatments ($p < 0.001$). The pH values indicated fast acidification in the early stage of decomposition, followed by a progressive decline with the elapsing time (Figure 1). The control samples followed the same pattern, but the pH values remained higher in the incubations, probably because these decomposition chambers had more organic matter owing to the addition of plant fragments.

The fast initial reduction in pH is attributed to the leaching process (the first stage of the decomposition process), as plant fragments are subjected to water. During this stage there is intensive release of water-soluble substances in the reduced form as well as carbonic acid resulting from the water-carbonic dioxide combination. Carbon dioxide is produced mainly by mineralization of labile, soluble and particulate fractions. The nitrate formation, derived from the ammonia oxidation, also contributes to acidification (Chen *et al.*, 2006). In addition, the oxidation of reduced compounds from respiration causes the pH to increase (Cunha-Santino and Bianchini Jr., 2006), as is the case in the formation of humic compounds (Aguilar and Thibodeaux, 2005). Park and Cho (2003) observed substantial release of soluble organic matter during leaching. Up to 30 to 40% of net production of the submerged freshwater angiosperm *Scirpus subterminalis* can be released as dissolved organic matter on hydrolysis in the first day of decomposition (Otsuki and Wetzel, 1974). Probably drying and fragmentation of the aquatic macrophyte influenced leaching once it increased the rates of release of the hydrosoluble compounds. In fact, the pre-drying of the plants could change the pattern of mass decrease during decomposition (Brock *et al.*, 1982).

The higher pH during decomposition (Figure 1) was consequence of successive aerations to maintain aerobic conditions in the incubations. The oxygenations purged the free CO2 from the system, changing the HCO_3^- equilibrium by increasing the consumption of this ion and leading to a decrease in acidity. The control flasks were also oxygenated only in the beginning of the experiment, since oxidation of the material from the water lagoon consumed oxygen. The initial oxygenation probably stimulated aerobic decomposition of DOM in the control chambers, causing an initial decrease in pH through formation of free CO_2.

The DOM lost during leaching comprises minerals and ions that caused higher EC values in the incubations than in the control flasks (Figure 2). With oxidation of DOM and POM, further ions were released during decomposition, and led to a continuous increase in EC in the incubations. The EC values depend on the budget of ion release (*e.g.* dissolution, chemical oxidations and mineralization) and sink (*e.g.* biological assimilation) processes. The increasing difference between EC for the control flasks and incubations as time elapsed is due to the higher amount of organic matter in the decomposition chambers. This matter is oxidized and releases ions. On the other hand, precipitation and absorption of these ions by organic matter and reutilization and immobilization of the ions by microorganisms could be a competing process. The lowest pH for decomposition chambers probably could increase the influence of precipitation of inorganic nutrients through interaction with the humic acids.

The concentration of ions tended to increase with N-enrichment in the decomposition chambers and in the control flasks (Figures 2 and 3A), which also applies to the pH (Figure 3B). This may be due to the different quantities of inorganic nutrients added to the decomposition chambers and control flasks, as well as to the supplementary addition of ions released during POM and DOM oxidation.

The maximum accumulated oxygen uptake (y*MAX*) tended to increase up to 6.37 mg NO_3 L^{-1} (Figure 4A). In the incubations with 11.83 and 18.82 mg NO_3 L^{-1}, however, y*MAX* decreased while the deoxygenation coefficients (k) increased. The remaining POM was higher in the incubations with lower initial nitrate concentration (Figure 4B).

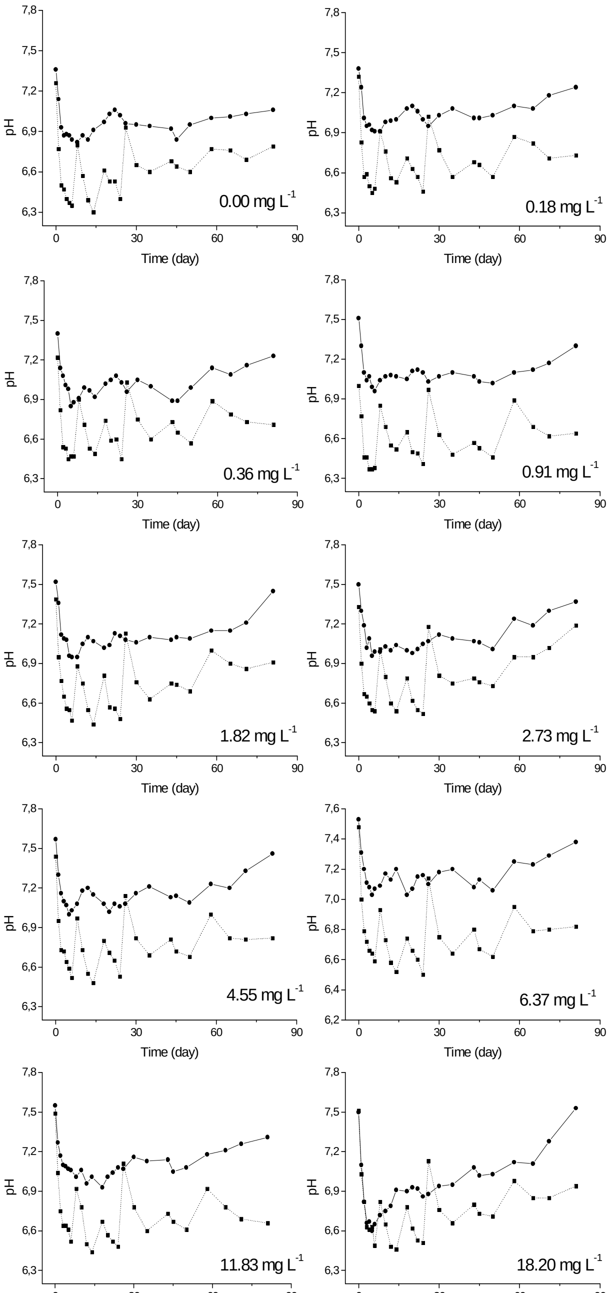

Figure 1. pH temporal variation at different nitrate concentrations (-■- control chambers; - ● - decomposition chambers).

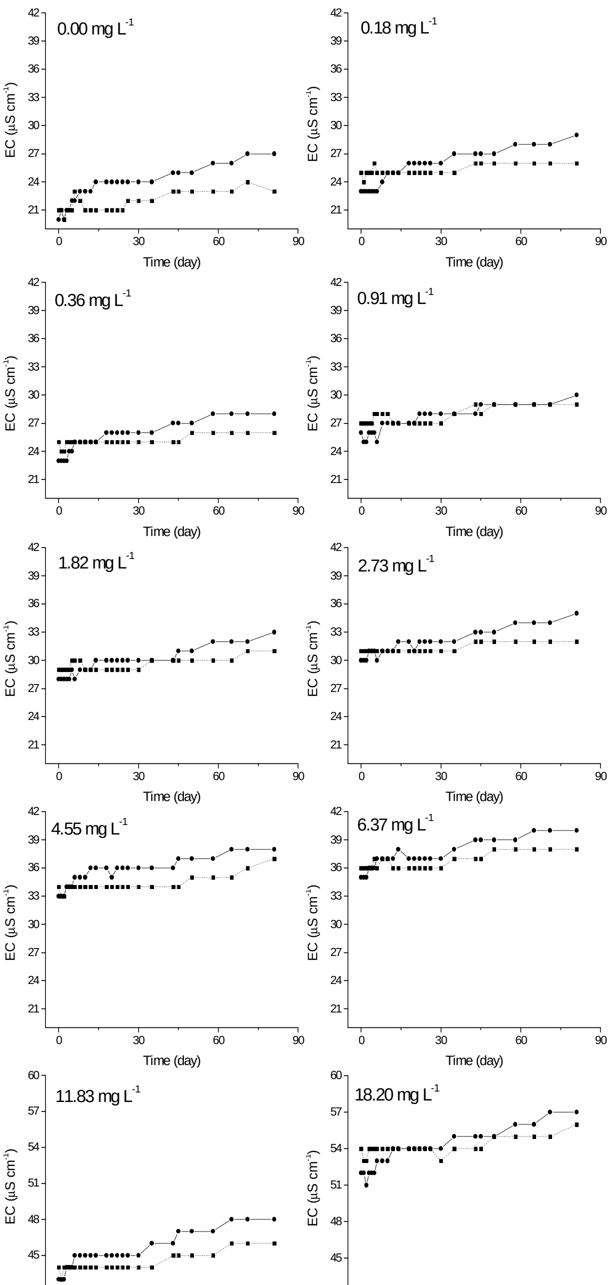

Figure 2. Electrical conductivity temporal variation at different nitrate concentrations (-■- control chambers; - ● - decomposition chambers).

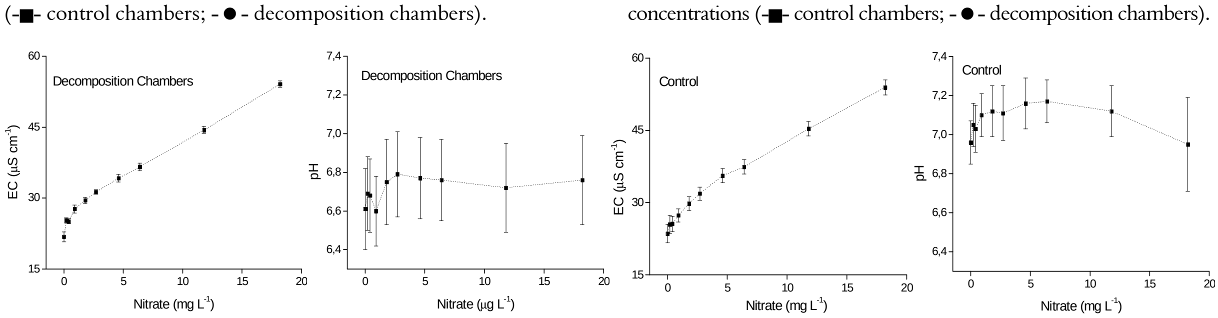

Figure 3. Mean values and standard deviations of electrical conductivity and pH vs. nitrate concentrations.

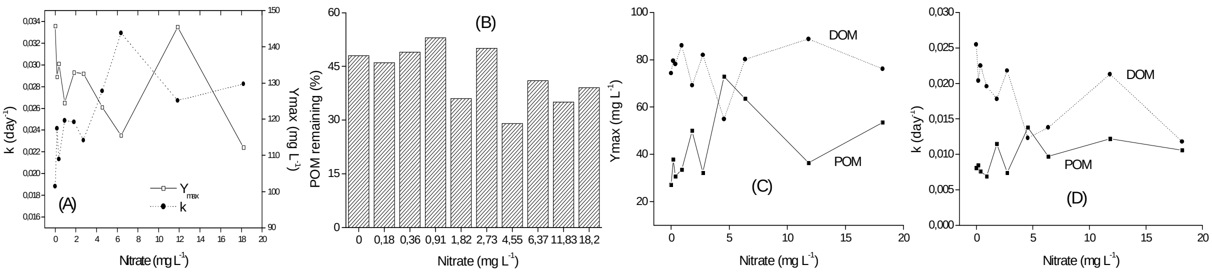

Figure 4. Variation of parameters at different nitrate concentrations: maximum oxygen uptake and deoxygenation rates (A); remaining POM remaining; (B); maximum oxygen uptake (C) and deoxygenation rates (D) from POM and DOM mineralization.

Overall, mineralization increased with the nitrate concentration, as observed in y_{MAX} and in remaining POM. The decrease in k up to 6.37 mg NO_3 L^{-1} is probably related to an improvement of refractory organic matter decomposition, as a result of N-enrichment. Some studies with artificial nitrogen enrichment, in laboratory or *in situ*, confirmed the improvement action of nutrients. The weight loss as well as changes in the composition of organic matter in the *Nymphaea alba* mineralization of leaves was fastest in eutrophic conditions. In eutrophic environments there was lignin accumulation in POM while other structural carbohydrates, such as cellulose and hemicellulose, apparently were mineralized (Brock *et al.*, 1985a). In comparative studies of lakes of different trophic levels, Andersen (1978) observed that oxygen uptake and increase of organic nitrogen in eutrophic lake litter was 2-fold higher than that found in lakes low in nutrients.

Experiments developed in laboratory indicated that nitrogen in water can be used by microorganisms to compensate the deficiency of substrates in aquatic plants, which suggests that their decomposition rates would be faster in eutrophic bodies of water than in oligotrophic waters (Almazan and Boyd, 1978). The remaining biomass of *Eleocharis* sp. was 30% higher in wetlands poor in nutrients than in wetlands enriched with nitrogen and phosphorous (Rejmankova and Sirova, 2007). Biomass loss and decomposition dynamics of *Nuphar lutea* leaves were influenced by the trophic conditions (Brock *et al.*, 1985b). Increases in decomposition constant rates were also observed for decomposition of *Typha latifolia* in organic-amended soil (Corstanje *et al.*, 2006). An increase in the microbial growth rate during decomposition of leaves was observed in nutrient-supplied soils (Teklay *et al.*, 2007). In contrast, other studies failed to observe a direct relation between nutrient enrichment and an increase in efficiency of the decomposition process (Triska and Sedell, 1976; Hohmann and Neely, 1993). So the results from supplemental addition of nutrients during the decomposition process are to some extent contradictory and depend on the conditions under which the studies were made. A series of influences on decomposition exists and include: (i) the nature of the organic substrate (qualitative and quantitative aspects of its chemical composition), (ii) the degree of decomposition of the litter, (iii) the range of concentration and nutrient types utilized in the assays, (iv) the interaction with other environmental factors (*e.g.* the presence of nutrients), (v) the relation between litter and nutrients in the water column, and (vi) factors limiting the activity of the microorganisms. In this context, amended nitrate showed differential action on different fractions of organic matter (POM and DOM/labile or refractory). Refractory compounds (*e.g.* lignin) were not affected by fertilization during decomposition (Hohmann and Neely, 1993).

In the present study, from the POM and DOM decomposition coefficient (k) and maximum oxygen uptake (y_{MAX}) (Figure 4C and D), we observed that mineralization of POM tends to be improved with increasing nitrate concentration. However, for DOM mineralization these parameters tend to decrease. These results point to the fact that nitrate availability may affect decomposition of each fraction in different ways, since POM could be more easily degraded in richer nitrate concentrations than DOM. Almazan and Boyd (1978) and Brock *et al.*, 1985a showed greater influence from nitrogen in water when the nitrogen levels in litter are low. This explains the decomposition of *S. cubensis*, since the amount of nitrogen in its tissues ranged between 0.5 to 0.7% dry wt (emergent and submerged parts, respectively, according to Nogueira, 1989) and 1% dry wt (the whole plant, in the present study). These values are considered low. Emergent macrophytes commonly have low-nitrogen and high-fiber content, which has been correlated with a less efficient decomposition process (Godshalk and Wetzel, 1978; Debusk and Dierberg, 1984).

Conclusion

The mineralization of POM was more strongly affected by nitrate enrichment than the DOM mineralization, probably because an optimization of the POM decomposition process exists, since k and y_{MAX} increase with increasing nutrient enrichment. We conclude that in incubations with nitrate concentration up to 6.37 mg NO_3 L^{-1}, the increase in nitrogen stimulated the decomposition process. This inference is corroborated by: (i) the increase in ion amount and in y_{MAX}; (ii) decrease of POM remaining values and, (iii) decrease in k values. The decrease of k points to a possible refractory compound decay and the subsequent decrease of oxygen uptake. The suppression tendency of the decomposition process was probably a consequence of the increasing nitrate availability for the microorganisms. Thus, nitrates would become more easily assimilated than N-organic forms, which undoubtedly require a higher energy output.

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