

Interactive comment on “Lacustrine wetland in an agricultural catchment: nitrogen removal and related biogeochemical processes” by R. Balestrini et al.

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Answers to the referee 1

Major comments 1. The temporal variations of the chemical species of interest (nitrate, oxygen, DOC) are widely described in 4.2 paragraph included the effects of the increasing water table in December 2003. We preferred to show some figures with the seasonal mean concentration in all the piezometers instead of several graphs showing the monthly values of species in selected piezometers, since the variations within the season are generally very restricted. The large min-max ranges indicated in the boxwisker plots referred to all the study period. At this regard we underline that data

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obtained from field campaign are affected by a considerable environmental variability not always explainable. We think that this synthesis is a good compromise helping the readers to focus on the main processes. Unfortunately, the available space generally given in a paper do not allowed to describe all the collected data. We decided to describe the variation of nitrate, O₂ and DOC as principal subjects of the processes occurred at the poplar stand. CI did not show any seasonal variations, while HCO₃ concentrations in the piezometers P3-P8 increased from May to July coinciding to the reducing of NO₃ values. But the relationship between NO₃ and HCO₃ is described in 4.1 paragraph. We could add a sentence in the text. 2. In the poplar stand we measure high value of O₂ in some piezometers only in one event (February) in winter and, as written in 4.2 paragraph, we explain this fact with the input of water rich in electron donors, following an intense rainy period. Omitting the O₂ values measured in that date the median concentration are always less than 2 mg/l, excepted for the piezometer 1 with median of 2.56 mg/l. Fig. 11 shows the measured seasonal concentrations. We agree to the referee regarding the incorrect use of 8220;anaerobic8221; instead of 8220;hypoxic8221;. The maximum values of O₂ measured in the Phragmites unit referred to one or at least 2, apparently, random events. The reed swamp unit is a very heterogeneous ecosystem from both spatial and temporal point of view. The O₂ could be present in micro-layer, located at the soil-water interface (Mitsch Gosselink, 2000), whose thickness (sometimes a few millimetres) is variable and related to many physical (rate of oxygen transport, convection currents8230;) and biological factors (presence of aerobic organisms or photosynthetic algae). See also answer to Referee 2. 3. The regressions shown in the paper are estimated from original data. We added the equations in fig. 8 and 9. Regarding the excess of alkalinity inferred from the regression between NO₃-N and HCO₃, we may suppose the overlapping effect of other reactions producing alkalinity, for example manganese and iron reduction, in the sites where the level of nitrate was very low. 4. Dissimilatory reduction of sulfate to hydrogen sulfide is used by a diverse group of heterotrophic strict anaerobes as a sink for electrons generated during oxidation of a carbon source.

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Sulfide is also produced from sulfate during assimilatory sulfate reduction for the synthesis of cysteine and methionine. Unlike dissimilatory sulfate reduction, assimilatory sulphate reduction is tightly regulated so that little or no excess sulfide is produced and secreted from the cell. Furthermore, assimilatory sulfate reduction operates under many growth conditions, such that the strict anaerobic conditions necessary for dissimilatory sulfate reduction are not required (Barton and Tomei, 1995). As indicated in 8220;Results8221; (3.3 paragraph), in the reedswamp the SO_4 concentration linearly diminished with depth ($R=-0.625$, $p<0.00001$). The concentrations measured in the deeper piezometers were always very low with a mean concentration of 0.7 mg/l (table 1). These values can not be explained by dilution (see the SO_4 level in the lake) but they strongly suggest a local consumption. In the reedswamp piezometers we also measured very low O_2 concentration and the release of sulphide was often indicated by the typical smell of rotten eggs. In addition we found an high significant inverse relation between SO_4 and HCO_3 ($R=0.901$, $p<0.00001$; Fig. 9) that could be explained by sulphate reduction. We reasonably think that these data support the hypothesis of the dissimilatory reduction of sulphate occurred in the reedswamp. As shown in fig. 9 in the piezometers where almost sulphate were consumed, the alkalinity continued to increase. Since methanogenesis do not cause an increase of alkalinity and iron and manganese reductions occur before sulphate reduction it seems likely that other processes took place (Stumm and Morgan, 1981). The very restricted amount of nitrate reaching the reed stand drives to suppose that other processes instead of Dissimilative Nitrate Reduction to Ammonium could have a crucial role in the N dynamic in this unit. The great availability of organic matter deriving from plant decomposition suggest the importance of ammonification, i.e. the biological transformations that convert organically bound nitrogen to ammonium nitrogen occurring under both aerobic and anaerobic conditions. 5. We agree to the referee on the questionability of the value of alkalinity as indicator of redox conditions in general. Notwithstanding in our study the good relationships between nitrate vs HCO_3 , and SO_4 vs HCO_3 strongly suggest the occurrence of the reducing reactions sequence expected on a thermodynamic base.

We did not mean that the measure of alkalinity could replace the redox potential measurement.

Other comments The vertical gradient of chloride is not explainable by biological processes (Cl is conservative) or dilution processes (Cl in lake water was about 5 mg/l). In the deeper piezometers (150-220 cm depth) of the reed swamp the sulphate concentration was close to the detection limit and the alkalinity was between 7 and 11 meq/l. We have two hypothesis: i) the occurrence of an anionic adsorption on the soil matrix that, even if it is not so frequent, could occur in a condition characterised by a total consume of sulphate by reducing processes, ii) the presence of older waters at depth, isolated from surface waters, characterised by lower Cl and SO₄ concentrations. The collected data suggested an influence of lake water only in the piezometer R3s and R3d. In this paper we focused on nitrogen transformation that mainly occurred in the poplar unit. In the reed stand, other processes, for example S dynamic seemed more interesting, but they lay outside the scope of the paper. Detailed data on the lake hydrology also in relation to the water table and biochemical processes in the reed stand were reported in Balestrini et al. (2004). The methods generally used to measure denitrification provide the addition of acetylene that inhibits the reduction of N₂O to N₂ and thus the accumulation of N₂O gives a measure of denitrification. For this reason we are used to think to N₂O as the final product of denitrification, but undoubtedly it is an error. We changed the sentence in the conclusion.

Pag. 3505 line 18 -We added a sentence for better explaining the frequency of sampling

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- We changed the title to: A lacustrine wetland 8230;. - We added the charges to the symbol of chemical species. - Modern agricultural practices provide the use of huge amount of nitrogen fertilizers. All the N not consumed by plants, is exported in aquatic systems both groundwater and surface waters, mainly in the nitrate form. - The sen-

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tence at page 3502, line 25 has been wrongly restored by our English reviewer. The proper form is 8220;More attention has focused on denitrification since this process can lead to a real removal of N by transformation of dissolved N in gaseous forms, till favourable conditions for denitrifying bacteria persist8221;. We changed the text in the paper. - One of the main requirement for denitrification is the absence of oxygen (in the case of strict anaerobic bacteria) or low O₂ concentration for facultative anaerobes (for example *Thiomicrospira denitrificans*). The oxygen can inhibits the synthesis and/or the activities of the enzymatic complex responsible of denitrification process. Some old lab studies examining the relationship between O₂ and denitrification rate reported a sharp inhibition effect of O₂ on the microbial process at restricted range of concentration with average of 10 956;mol/l O₂ or less (Tiedje, 1988). Other Authors of the same period (Robertson and Kuenen, 1984) isolated an organism (*Thiosphaera pantotropha*) that uses both O₂ and NO₃ as terminal electron acceptor at concentration of 90of air saturation corresponding to an O₂ concentration of 6.9 mg/l. Most likely the O₂ threshold that may cause the cessation of denitrification varies among organisms. More recent in field studies indicate that denitrification can occur even with detectable O₂ concentration. This may be explainable considering that this bacterial process occur in microsites, often defined 8220;hot spots8221; where O₂ has been completely removed (Mariotti, 1986; Parkin T. B., 1987). From a biogeochemical point of view, 8220;hot spots8221; are patches that show disproportionately high reaction rates relative to the surrounding matrix. For example, hot spots of denitrification may occur along root channels where moisture and organic matter content are high (McClain et al. 2003), or in small patches of organic matter in the C horizon of riparian soils (Jacinthe et al. 1998). Hill et al. (2000) also reported the occurrence of denitrification at depth, at the interfaces between sands and peats or buried channel deposits in a southern Ontario riparian zone. So the samples used for the chemical analysis could have higher O₂ level, representing a synthesis of a larger environment. Many studies on riparian areas reported a sharp decline of NO₃ in groundwater at O₂ concentration of <28211;3 mg/L (Cey et al. 1999, Vidon and Hill, 2004) and this can be interpreted as a condi-

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tion for denitrification to occur. In addition to the environmental variability, it is worth to consider the uncertainty in measuring oxygen in groundwater. In the reedswamp we measured many samples with concentration of 0.128211;0.30 mg/l of O₂, while, in the poplar, the minimum concentration was 0.32 mg/l. - Page 3511, line 17. We totally agree with the referee comment. The increasing alkalinity observed in the middle and deep piezometer of the reed stand is hard to explain. The occurrence of methanogenesis, expected when all the sulphate is consumed, does not affect alkalinity. We modified the text at page 3511, line 17. - The methods generally used to measure denitrification provide the addition of acetylene to block the reactions till the production of N₂O. For this reason we are used to think to N₂O as the final product of denitrification, but undoubtedly it is an error. We changed the sentence in the conclusion. - As reported in the 8220;Groundwater chemistry8221; paragraph, the N-NH₄⁺ concentrations measured in poplar stand were always very low (0.0188211;0.027 mg/l8722;1) suggesting a rapid biological consumption and/or an immobilisation on the soil matrix. The observed nitrate gradient was not associated to a correspondent increase of ammonia. On the other hand, the restricted amount of nitrate reaching the reed stand (where the NH₄ concentration was higher, see table 1) drives to suppose that other processes instead of Dissimilative Nitrate Reduction to Ammonium could have a crucial role in the N dynamic in this unit. The great availability of organic matter deriving from plant decomposition suggest the importance of ammonification, i.e. the biological transformations that convert organically bound nitrogen to ammonium nitrogen occurring under both aerobic and anaerobic conditions (see page 3511, line 23). - We performed some PCA analysis with explorative intents, but the results were not so informative. So we decided to directly analyse the data, in order to find the expected relationship between ions that may support the hypothesis of the denitrification occurrence, for ex NO₃ vs O₂, HCO₃, DOC.

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- We also studied the dynamic of phosphorous in this project, but we decided to omit

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it in the present paper to avoid the risk of describing in a superficial way both nitrogen and phosphorous behaviour. In addition the P data set was not complete. We started to measure total P, but the irregular presence of fine particulate in the samples caused sporadic high increments in P concentrations. So we decided to measure dissolved total P and to neglect TP analysis (we did not have the resources to perform both analysis). - The dynamic of NH₄ in the reed stand is certainly interesting. We measured higher concentrations in the more superficial piezometers where also we observed a great variability. We did not clearly identified any environmental factor able to explain the observed variations (temperature, degree of reedswamp submersion, etc). The possibility that the Phragmites belt could act a source of NH₄ has to be evaluated, but more specific investigations are necessary to verified this hypothesis. In any case we added a sentence in the text. - We have incorporated most of the suggestions listed in 8220;Some more detailed observations8221;. Regarding the use of soil (Page 3504, lines 25-26), we used the percentages of the total basin included the 12

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