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# Lacustrine wetland in an agricultural catchment: nitrogen removal and related biogeochemical processes

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#### Abstract

The role of specific catchment areas, such as the soil-river or lake interfaces, in removing or buffering the flux of N from terrestrial to aquatic ecosystems is globally recognized but the extreme variability of microbiological and hydrological processes make it difficult to predict the extent to which different wetlands function as buffer sys-5 tems. In this paper we evaluate the degree to which biogeochemical processes in a lacustrine wetland are responsible for the nitrate removal from ground waters feeding Candia Lake (Northern Italy). A transect of 18 piezometers was installed perpendicular to the shoreline, in a sub-unit formed by 80 m of poplar plantation, close to a crop field and 30 m of reed swamp. The chemical analysis revealed a drastic NO<sub>3</sub>-N ground 10 water depletion from the crop field to the lake, with concentrations decreasing from 15–18 mg N/I to the detection limit within the reeds. Patterns of CI,  $SO_4$ ,  $O_2$ ,  $NO_2$ -N, HCO<sub>3</sub> and DOC suggest that the metabolic activity of bacterial communities, based on the differential use of electron donors and acceptors in redox reactions is the key function of this system. The significant inverse relationship found between NO<sub>3</sub>-N and 15

<sup>15</sup> Function of this system. The significant inverse relationship found between NO<sub>3</sub>-N and HCO<sub>3</sub> is a valuable indicator of the denitrification activity. The pluviometric regime, the temperature, the organic carbon availability and the hydrogeomorphic properties are the main environmental factors affecting the N transformations in the studied lacustrine ecosystem.

#### 20 **1** Introduction

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Knowledge of the processes controlling the extent and dynamics of nutrient fluxes from diffuse sources in heterogeneous catchments is crucial in predicting and controlling the effect of human activities on the environment. Unlike point source pollution, diffuse pollution cannot easily be controlled, a reduction can only be achieved by appropriate land management techniques. It is well known that modern agricultural practices



significantly contribute to catchment nitrogen losses. In Italy for example, agriculture

accounts for 68% of nitrogen (N) input to the Po river catchment and this percentage increases to 83% if we consider the N input to groundwater (Autorità di Bacino del fiume Po, 2003). In the Po Valley, nitrate (NO<sub>3</sub>) contamination of ground waters considerably constrains the readily available amount of drinking water.

- Over the last few decades, much interest has been focused on specific catchment areas such as riparian zones which are able to reduce or buffer the flux of N from terrestrial to aquatic ecosystems (Peterjohn and Correll, 1984; Haycock et al., 1993; Sabater et al., 2003). The significant role of wetland can be seen in the European Water Framework Directive's (EU, 2000/60), recent integrated approach to river basin management in Europe. In this framework, riparian areas are considered as a significant element
- of the hydrological network in which protection and restoration are required to achieve "good water status" for surface and ground waters (Wetlands Horizontal Guidance, 2003).
- Riparian areas may attenuate nitrogen inputs to aquatic ecosystems through plant
  <sup>15</sup> uptake, microbial denitrification, soil storage and dilution (Hill, 1990; O'Neill and Gordon, 1994; Haycock and Pinay, 1993; Groffman et al., 1996; Devito et al., 2000). Additional attention has focused on denitrification as this process can lead to a real removal of N by the transformation of dissolved N into gaseous forms, creating favourable conditions for denitrifying bacteria to persist. The relative importance of these processes
  <sup>20</sup> seems to be strictly linked to the hydrological properties of soil-water interfaces (Mitsch
- and Gosselink, 2000; Pinay et al., 2000; Burt et al., 2002).

Despite the increasing number of papers over recent years on the role of riparian areas in N removal, the extreme variability of microbiological and hydrological processes make it difficult to predict the extent to which different wetlands function as buffer sys-

tems. In some areas less than 5 m is enough to remove all the NO<sub>3</sub> entering the riparian areas (Balestrini et al., 2007; Blicher-Mathiesen and Hoffman, 1999), while in others, little or no N removal is observed (Devito et al., 1989). Little research has been conducted in large upland aquifers receiving high N inputs and little information is available on the variability of the depth of denitrifying activity in riparian zones (Hill et al., 2000).



In addition, in Italy, with the scarcity of ongoing studies on natural wetlands (Balestrini et al., 2004, Borin et al., 2004), there is a need to observe typologies characteristic of the national territory.

The general aim of the present paper is to evaluate the N buffering capacities of a <sup>5</sup> riparian area adjoining a small lake (Lake Candia) which receives a large nutrient input from agricultural land. We examined spatial and temporal variation in water chemistry and hydrology to identify the main mechanisms (biological, physical, chemical) leading to ground water NO<sub>3</sub> depletion at soil-lake interfaces. Patterns of nitrate, dissolved oxygen, dissolved organic carbon, alkalinity and sulphate concentrations were used to delineate redox conditions and define the occurrence and location of denitrification zones. The effect of some environmental factors, such as organic carbon availability, temperature and hydromorphology, on the N dynamic was investigated.

#### 2 Methods

- 2.1 Study area
- <sup>15</sup> Lake Candia (45°19′25″ N, 7°54′43″ E) is a small (1.49 km<sup>2</sup>), inter-morainic shallow lake, located in the region of Piedmont in North-West Italy (Fig. 1). The shoreline is almost entirely occupied by a belt of emergent macrophytes in which *Phragmites australis* dominates. The annual floating leaved water chestnut (*Trapa natans*) covers about 20 ha of the littoral zone (Galanti and Romo, 1997). Lake Candia does not have any inflows but is mainly fed from groundwater and rainfall. An unconfined aquifer intercepts the lake (at 226 m asl) from the higher elevation of the moraine (330 m asl) on
- the West towards the North East. A second deeper aquifer occasionally feeds the lake. The climate of the area has been described as humid-temperate and the annual average temperature and precipitation during the 16-year period (1986–2002) was 11.1°C and 912 mm, respectively. About 45% of the Lake Candia basin is occupied by agricul-
- and 912 mm, respectively. About 45% of the Lake Candia basin is occupied by agricultural areas, 40% by forests and grassland and only 3% by urban areas (Ciampittiello



et al., 2005). It receives a large input of nutrients and its ecological status is currently classified as "poor" (DECRETO LEGISLATIVO N. 152/1999).

Field activities were conducted on the north-western shore of the lake in a sub-unit formed by 80 m of poplar (*Populus nigra L.*) plantation, in the upper part close to a crop field and 30 m of reed swamp mainly formed by *Phragmites australis*.

Farmers declared that the synthetic fertilizers regularly applied to the crop field consisted of NPK compound and ammonium nitrate for a total amount of  $200 \text{ kg N ha}^{-1}$ .

#### 2.2 Sampling and analysis

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A transect of piezometers was installed perpendicular to the shoreline, extending from the crop field to the lake. A total of 18 piezometers were positioned at a 5 to 15 m 10 distance from one another and at a depth ranging from 1.5 to 3 m. In the reed swamp and in the transition zone between the two sub-units the piezometers positioned at the same distance were sited in groups of two or three, at different depths: 54-80 cm, surface (s), 143–150 cm, middle (m), and 196–219 cm deep (d) (Fig. 2). The piezometers consisted of PE pipe (4 cm id) with 10 cm slotted ends installed in holes drilled by hand auger. The water table elevation was measured at least once a month, from March 2003 to July 2004 using a suitable sounding probe. Ground water samples were collected simultaneously in the poplar grove and in the reed swamp after at least one volume of the piezometer content had been removed. Lake samples were collected close to the shoreline at a depth of 20-30 cm at the same time as the ground water 20 samples. Water temperature and dissolved oxygen (O<sub>2</sub>) concentration were measured in the field, during the sample collection, from May to July 2004. The scarce rainfall during spring and summer 2003 notably constrained the water availability in the soil

and it was not possible to collect water in a number of the piezometers. In addition, the complete installation of piezometers in the reed swamp ended in November 2003. For these reasons the chemical data of March–November 2003 (10 samplings) is shown separately (Table 2). For a better analysis of the spatial variations we compared only the results available for all the piezometers of the studied transect corresponding to



11 sampling sessions from December 2003 to July 2004.

analyzer.

Soil profiles were obtained from cores collected mainly during piezometer installations. Soil samples were analyzed for organic carbon content using the Walkley-Black method (Gaudette et al., 1974). Hydraulic conductivity was measured in the poplar sub-unit by pumping tests and calculated using the Bower and Rice equation (Bouwer and Jackson, 1976).

Precipitation data collated at the meteorological station located at 2 km away from the experimental area were used.

- The analysis was performed on filtered samples (0.45 μm), except for measurements
   of pH, electrical conductivity and total nitrogen (TN), for which unfiltered samples were used. The alkalinity was measured by two-endpoint potentiometric titration with HCl. N-NH<sub>4</sub> analyzed by molecular absorption spectrometry (Perkin Elmer UV-VISLambda2) using the indophenol-blue method. NO<sub>3</sub>-N, SO<sub>4</sub> and Cl were determined by ion chromatography using a Dionex 2000i equipped with AS4A column. TN was measured us ing molecular absorption spectrometry, following persulphate digestion in an autoclave at 120°C. The organic nitrogen (ON) was estimated by comparing the difference between TN and inorganic N (NH<sub>4</sub>-N+NO<sub>3</sub>-N+NO<sub>2</sub>-N). Dissolved organic carbon (DOC) was assayed by high temperature catalytic oxidation using a Shimadzu TOC-5000 A
- The quality of chemical analysis was checked by including method blanks, repeated measurements of internal and certified reference samples and by regular interlaboratory tests and international intercomparisons (Mosello et al. 2002). The repeatabilities, based on repeated measurements of internal quality controls at different concentrations, were 3% for N-NH<sub>4</sub>, 2% NO<sub>3</sub>-N, 4% for TN, 3% for SO<sub>4</sub>, 6% for Cl. Detection limits were 5 µgl<sup>-1</sup> for N NH<sub>4</sub> = 0.02 mgl<sup>-1</sup> for NO<sub>4</sub>.
- <sup>25</sup> Detection limits were  $5 \mu g l^{-1}$  for N-NH<sub>4</sub>, 0.02 mg l<sup>-1</sup> for NO<sub>3</sub>-N, and Cl, 0.05 mg l<sup>-1</sup> for SO<sub>4</sub> and 0.1 mg l<sup>-1</sup> for TN.

HESSD						
4, 3501–3	534, 2007					
Lacustrine wetland in an agricultural catchment						
Title Page						
Abstract	Introduction					
Conclusions	Conclusions References					
Tables Figures						
14	►I.					
•	۰ (۲۰۰۰)					
Back	Back Close					
Full Screen / Esc						
Printer-friendly Version						
Interactive Discussion						
EGU						

#### 3 Results

#### 3.1 Soil features

In the soil profile, the silt layers of lacustrine origin predominate, alternated with sand and deep gravel-sandy layers. In every profile we observed redoximorphic features as grey in colour with red spots and deep grey layers. Organic matter of a very dark colour was often detected within the hydric layers. In the upper part of the transect, the soils were classified as Typic Udorthens, while those close to the lake were Aquic Udorthens. The organic carbon abundance was measured in three soil cores collected at P3, P6 and P8. Within the first 30–40 cm the percentages ranged between 1.8 to 2.5; up to 70–80 cm in depth the values tended to decrease to 0.4–0.8. At a greater depth (to 150–170 cm) the levels increased to 7%. The hydraulic conductivity values gradually diminished from 380 cm d<sup>-1</sup> in the upper part of the transect, to 2 cm d<sup>-1</sup> at the end of the poplar plantation. The measured values fall into the range reported for

15 3.2 Hydrology

fine sands rich in silt (Fetter, 2001).

A relevant seasonal cycle of the water table fluctuations is evident from the measurements conducted from March 2003 to July 2004. 2003 was an atypical year characterized by a very dry spring and summer, minimum water table levels were measured from the March–November period (Fig. 3). Intense precipitation events occurred at the end of November and beginning of December and consequently the water table abruptly increased in all the piezometers, particularly in those located on the hill slope (Fig. 3 and 4). The maximum values, occurred during winter 2004, followed by minimum levels in summer 2004 when many piezometers were empty. The breadth of the cycle is greater upslope and diminishes towards the lake (Fig. 4). At P1 the variations were at least

<sup>25</sup> 3 m; they reduced to 1.8–1.3 m within the poplar plantation, from P2 m to P6, and they further declined to 0.7–0.5 m in the reed swamp. The large range measured on the



hill slope suggests a pattern of winter recharge controlled by conditions occurring upslope. In contrast, the more constrained response observed in the rest of the transect, particularly near the lake, suggests that both the hill slope and the lake exert control over the riparian water table. In mid-summer (27 July) when the slope discharge fell
to under measurable levels, probably because of high evaporation losses and lack of rainfall, the inflow from the lake alone sustained the water table in a limited portion of the riparian zone, from P7 to R3 (Fig. 4).

#### 3.3 Ground water chemistry

We found strong variations in the chemistry of subsurface water within the two subunits sampling regions (poplar and reeds) of the lake Candia riparian zone (Tables 1 and 2).

Box plots of Fig. 5 summarize the concentrations of some variables of interest measured in the poplar sub-unit as a function of the distance from the crop field. A drastic NO<sub>3</sub>-N depletion, from 14.7 mgl<sup>-1</sup> to 0.08 mgl<sup>-1</sup>, is evident from the upslope to the boundary between poplars and reeds. Opposite behaviour was observed in HCO<sub>3</sub> concentrations, which gradually increased from the crop field towards the lake. Within a length of 60 m, the median alkalinity ranged from 0.60 meq l<sup>-1</sup>, at P1, to 7 meq l<sup>-1</sup>, at P8. The Cl concentrations exhibited a bell-shaped distribution with a mean value of 12.6 mg/l at site P1, increasing values to a median of 17.8 mg/l at P4, and decreasing values to 13.7 and 8.5 mgl<sup>-1</sup> at P9s and P9d respectively. DOC levels moderately increased from a median of 2.1 mgl<sup>-1</sup>, upslope, to 5.3 mgl<sup>-1</sup> down slope. Slight N-NH<sub>4</sub> and ON concentrations (0.018–0.027 mgl<sup>-1</sup> and 0.3–0.5 mgl<sup>-1</sup>, respectively) were measured within the poplars.

Within the reed swamp the chemistry of subsurface waters also varied strongly <sup>25</sup> mainly with the depth of soils or sediments (Fig. 6). In particular, HCO<sub>3</sub> values increased linearly with depth (R=0.897, p<0,00001), from about 2 to 10 meq l<sup>-1</sup>, between 50 and 220 cm depth. Conversely, Cl, SO4 and N-NH4 linearly diminished with depth (R=-0.933, R=-0.625, R=-0.556, respectively, p<0,00001). Compared to the



poplar sub-unit, the reed subsurface water was really poor in NO<sub>3</sub>-N, with concentrations often being undetectable (<0.02 mg l<sup>-1</sup>), and relatively rich in NH<sub>4</sub>-N ranging between 0,010–0,532 mg l<sup>-1</sup>. Likewise ammonia, ON and DOC were shown in higher concentrations in the reeds than in the poplar sub-unit. In particular at R1s (at lower depth), close to the boundary between poplars and reeds, the median concentrations of ON and DOC reached 2,47 mg l<sup>-1</sup> and 15 mg l<sup>-1</sup>, respectively.

The  $O_2$  measures, indicated low levels at both sub-unit sampling regions (Fig. 7), with median values ranging between 0.5–3.3 mg l<sup>-1</sup>. Maximum values (around 10 mg l<sup>-1</sup>) were measured at upslope (P1 and P3) while the minimum values (about 0.5 mg l<sup>-1</sup>) were measured in the reed swamp at the lower depth.

#### 4 Discussion

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#### 4.1 Denitrification indicators

The results obtained from our study in the Candia Lake basin show that narrow regions of terrestrial-aquatic interfaces are biogeochemically highly variable environments.

<sup>15</sup> The spatial trend of NO<sub>3</sub>-N concentrations observed at the poplar sub-unit, suggests that a portion of riparian region functioned largely as a nitrate sink. The NO<sub>3</sub>-N gradient was very steep, and could range from more than  $6 \text{ mg I}^{-1}$  to the detection limit over distances of less than 5 m.

Both biological and physical processes can produce the N-NO<sub>3</sub> depletion observed in our study area. Changes in NO<sub>3</sub> to CI molar ratios are often used to infer the removal of nitrate by biological processes as chloride is a conservative ion. Large declines in chloride concentrations along a hydrologic flow path can indicate the dilution of a particular water source (e.g. agricultural runoff) by a second water source such as deep groundwater or rainfall. Such a pattern can indicate that associated declines in nitrate

<sup>25</sup> levels are due to the mixing of different water sources rather than actual biological removal. However, in the current paper median chloride concentrations are relatively



constant in the upper transect P1–P3 as are median nitrate values. In contrast, in the central portion P4–P7 median nitrate concentrations decline rapidly whereas, chloride concentrations show small increases. These patterns do not indicate dilution, but instead suggest nitrate removal is occurring.

- <sup>5</sup> The patterns observed for some chemical species suggests that denitrification plays a predominant role in the observed NO<sub>3</sub>-N attenuation. Firstly, the low O<sub>2</sub> levels in the subsurface water indicate conditions generally suitable for denitrifying bacteria (facultative anaerobes) which, in small zones where the O<sub>2</sub> is completely removed, could shift to an anaerobic metabolism. Anaerobic conditions (O<sub>2</sub><2 mg l<sup>-1</sup>) were often observed
- <sup>10</sup> at the locations where a NO<sub>3</sub>-N consumption was evident. Many studies reported a sharp decline in groundwater nitrate concentrations when O<sub>2</sub> fell below approximately  $2 \text{ mg I}^{-1}$  (Cey et al., 1999; Hill et al., 2000). In the present study a significant correlation between O<sub>2</sub> and NO<sub>3</sub> has not been found, in that high nitrate in conjunction with low O<sub>2</sub>, was not observed but a high nitrate even at the O<sub>2</sub> concentration ranging of
- <sup>15</sup> 1.5–2.5 mg l<sup>-1</sup> (P1 site) was seen. This finding suggests that the concurrent presence of nitrate and anaerobic conditions are not sufficient to guarantee that denitrification will take place. On the other hand we found a significant inverse linear relationship between NO<sub>3</sub> and HCO<sub>3</sub> concentrations (R=0.845, p<0.0001) (Fig. 8) that indirectly indicates the occurrence of denitrification. In fact this microbial reduction implies an
- <sup>20</sup> alkalinity increase of 1 eq per mole of nitrate reduced (Stumm and Morgan, 1981). The relationship between  $HCO_3$  and  $NO_3$  is not generally analysed in experimental surveys aimed at studying N dynamic in riparian areas. A link between the loss of  $O_2$  and  $NO_3$ and the production of large amounts of  $HCO_3$  is reported for a glacial outwash aquifer in Minnesota by Puckett and Cowdery (2002). In a study we carried out in a river wet-
- <sup>25</sup> land (Balestrini et al., 2004 and 2007) the declining nitrate found in groundwater along a transect, from a crop field to the stream, was not related to alkalinity. The alkalinity seems to be a sensitive indicator of the extent of denitrification but only in specific types of wetlands. Our data set and literature are not broad enough to identify the environmental features, presumably geological and hydrological, which favour HCO<sub>3</sub>



accumulation in groundwater as a consequence of redox reactions.

An additional sign of denitrification activity was the presence of detectable NO<sub>2</sub>-N concentrations at the P2-P7 piezometers of the poplar sub-unit (Table 1). The dynamic of NO<sub>2</sub> in soil and water can be very rapid as it can be produced or consumed by a number of biological or chemical reactions. Some studies report that nitrification and denitrification are the main processes contributing to NO<sub>2</sub> accumulation in soil (Burns

- et al., 1996). In our study area  $NH_4$ -N concentration in groundwater was very low and, conversely, there was a great input of  $NO_3$ -N, consequently we can expect that denitrification was the major  $NO_2$  source process.
- <sup>10</sup> Within the reed swamp, the very low input of NO<sub>3</sub>-N from both the adjacent terrestrial zone and the water of the lake, probably severely constrained the denitrification. In this system, which was very poor in oxygen, SO<sub>4</sub> could become a suitable electron acceptor for the oxidation of the organic C. As sulphate reduction also produces alkalinity (Stumm and Morgan, 1981) the high significant inverse relation found between
- SO<sub>4</sub> and HCO<sub>3</sub> (*R*=0.901, *p*<0.00001; Fig. 9) supported our hypothesis. As shown in Fig. 8 at the higher depth (150–220 cm) where all the sulphate was consumed, the alkalinity continued to increase, from about 6 to 11 meq l<sup>-1</sup>, probably due to iron and/or manganese reduction. The pattern described follows the well-studied thermodynamic sequence reported for lake or marine sediments (Stumm and Morgan, 1981; Kuivila and Murray, 1984; Dillon et al., 1997), but is not often used to interpret the chemical transformation in riparian zones (Hedin et al., 1998; Pukett and Cowdery, 2002; Hill et al., 2000).

In the reed swamp, characterized by still water and abundant plant biomass, the main source of mineralized nitrogen was represented by ammonia derived from the

decomposition of organic matter. Likewise DOC, organic nitrogen also reached relatively high concentrations here, ranging from 0.35 to 4.3 mg l<sup>-1</sup> and it constituted the major nitrogen species.



#### 4.2 Environmental factors affecting nitrogen dynamics

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The results gained from the hydrochemical monitoring over 18 months allowed us to trace the sequence of processes that occurred in the lake Candia wetland over the course of a year and to identify the main environmental variables affecting nitrogen cy-

 cling. The sequence of hydrological and biochemical changes are presented in Figs. 10 and 11 as a series of schematic snapshots.

The long period of drought during spring-summer 2003 caused the progressive lowering of the water table in all the transect resulting in the complete disconnection between the poplar and reed sub-units in September. In these conditions the spring fertilizer applications did not generate the expected rise of NO<sub>3</sub> in the groundwater and the observed levels remained very low during all of 2003 (Fig. 10a).

The abundant precipitation which occurred in December 2003 promoted the prompt development of the shallow aquifer and the consequent hydrological connection of all the piezometers (Fig. 10b). The interaction of the rising water table with the  $NO_3$  rich

- soil waters of the crop field, caused a sharp increase of NO<sub>3</sub> concentrations, simultaneously from P1 to P6 sites. At P7 (67 m from the crop field) and P9s (75 m from the crop field, at 80 cm depth) the NO<sub>3</sub> flush was detected a month later, during the January sampling. Conversely, the NO<sub>3</sub> concentration did not rise at the deeper piezometers, P8 and P9d (157 and 216 cm depth, respectively) and in the whole reed sub-unit. The
- elevated O<sub>2</sub> concentrations measured during the February sampling (Fig. 11b), with levels over 10 mg/l from P1 to P3, indicated the input of water rich in electron donors. In the rest of the transect the NO<sub>3</sub> concentrations gradually decreased along with the oxygen values.

During spring (Fig. 10c), the water table remained quite high and the NO<sub>3</sub> continued to flush from the upland (P1), where the levels were steady around 14–15 mg N l<sup>-1</sup>, thereby ensuring the supply of NO<sub>3</sub> for denitrification in the riparian zone. The NO<sub>3</sub> pattern did not differ significantly from previous months but conditions appeared more suitable for denitrification. In fact the O<sub>2</sub> concentrations were remarkably lower com-

## **HESSD** 4, 3501-3534, 2007 Lacustrine wetland in an agricultural catchment R. Balestrini et al. **Title Page** Introduction Abstract Conclusions References Tables **Figures** 14 Back Close Full Screen / Esc **Printer-friendly Version** Interactive Discussion

pared to the winter period with most of the locations characterized by values less than  $2 \text{ mg I}^{-1}$  (Fig. 11c). In addition, we detected the presence of nitrite, an intermediate product of the denitrification reaction, in locations characterized by low oxygen, and an elevated availability of nitrate (Figs. 10–11c).

- Later on, over the dry and hot summer season (Fig. 10d), the water table level declined at all locations up to P7, as well as the nitrate concentrations. A marked and abrupt decline of NO<sub>3</sub>-N was evident between P3 and P4 where the concentration decreased from 8.5 to 0.9 mg l<sup>-1</sup> within 10 m. Thus, the nearly complete nitrate removal (NO<sub>3</sub>-N<0.1 mg l<sup>-1</sup>) occurred in a larger area covering 60% of the whole wetland, despite the constant input of nitrate from the P1 site. The O<sub>2</sub> levels further diminished and, conversely, the DOC availability increased mainly in the transition area between poplar and reeds (Fig. 11d). At the end of July when the complete drying out of the shallow aquifer caused a disconnection between upland and the locations closer to the
- reed swamp (P7–P9), the NO<sub>3</sub> source area stopped supplying the riparian zone.
   The seasonal evolution of NO<sub>3</sub>-N in the subsurface waters of the poplar sub-unit suggests the importance of temperature in the biological processes responsible for N removal. The increase in temperature of 5–7°C observed in the ground water from February to the end of May could have stimulated the denitrification. Some studies have reported a significant direct effect of temperature on denitrification rates (Martin et al., 2000; Knowles, 1982). The renewal of the vegetation activity in the spring and
- summer months may also be an important factor in the decreasing nitrate losses from the soil at that time of the year. Higher daily soil respiration rates and an abundant biomass of live roots in spring and early summer were reported for a poplar stand in a riparian area in Central Iowa (Tufekcioglu et al., 1999). The rapid increase of
- DOC concentration observed from May to June at certain sites (P5–P9) may derive from greater organic matter inputs from plants to the soil, therefore providing better conditions for nutrient sequestration within the riparian area. Some studies suggest that denitrification can be limited by a supply of organic carbon (Hedin et al., 1998; Starr and Gillham, 1993; Bradley et al., 1992; Hill et al., 2000) as it provides energy



to the bacterial community and, indirectly, promotes the occurrence of anoxic conditions through O<sub>2</sub> consumption by heterotrophic bacteria. Hedin et al. (1998) reported that NO<sub>3</sub> only accumulated in subsurface waters with DOC concentrations of less than 2 mg l<sup>-1</sup> in Smith Creeck near-stream environments; while other Authors (Starr and Gillham, 1993) suggest that concentrations of 4–5 mg l<sup>-1</sup> are not enough to sustain denitrification. The relationship found between DOC and NO<sub>3</sub>-N using the data relative to P1–P9 sites (Fig. 12), is similar to that reported by Devito et al. (2000) for a sand aquifer in Ontario (Canada) and suggests that denitrification can be limited by a carbon supply of less than 3 mg l<sup>-1</sup>. DOC concentrations always higher than 3 mg l<sup>-1</sup> have been measured, within the poplar sub-unit, starting from P5 to P9 (Fig. 11). The presence of black soil layers rich in organic carbon deposits were also detected at a greater depth (to 150–170 cm) at the same sites. On the contrary, at P1 where the NO<sub>3</sub> level did not decline even in summer, the DOC concentration never exceeded 3 mg l<sup>-1</sup>.

In addition to its role as an organic C supplier, the direct role of vegetation in increas-

- <sup>15</sup> ing the N uptake rate during the growing season has to be expected. In this regard, it is important to note that the temperature increase, typical of the summer months, coincided with soil drying and a lowering of the water table. Some studies have demonstrated that the nutrient plant uptake is controlled by the water table fluctuations, as plant root biomass has been shown to sharply decline with depth (Ehrenfeld et al.,
- <sup>20</sup> 1992; Tufekcioglu et al., 1999). Within the poplar sub-unit, the water table lowered by about 70 cm reaching a depth of 1–3 m during the summer. We did not investigate the distribution of the roots in the soil profile but a number of papers indicate that poplars are generally characterized by a very deep root system. Tufekcioglu et al. (1999) detected the presence of coarse roots of at least 180 cm under poplars in a fine-loamy
- riparian soil; Heilman et al. (1994) reported that roots of 4-year-old hybrids of *Populus trichocarpa* × *Populus deltoides* extended to depths beyond 3.2 m and Gifford (1966) reported a similar rooting depth, about 290 cm, for *Populus tremuloides* in a sandy loam soil. On the base of these findings it is reasonable to hypothesize that poplar trees play a role in lowering the subsurface nitrate concentrations during the growing



season at Candia Lake wetland.

The results obtained indicate that the efficiency of the wetland study in buffering the flux of N is also associated with the topographic features of the area, in that significant  $NO_3$ -N removal occurred in a specific portion of the transect characterized by the di-

- <sup>5</sup> minishing slope. In this area (from P5 to R3) the fluctuations of the water table were more restricted and during several events the ground water reached and rose above the soil surface. Recent research carried out in different riparian areas spread all over Europe demonstrated that the functioning of riparian areas depends on the existence of topographic and soil conditions that produce, at least seasonally, a high water table
- (Burt et al., 2002). The saturation of the upper soil layers rich in organic C creates a suitable condition for denitrification (Burt et al., 1999; Cosandey et al., 2003) and consequently enhances the N buffer effect of riparian zones. The development of soil saturation also depends on the hydraulic conductivity of the soil which is important in optimizing the ground water residence time (Burt et al., 1999 and 2002). The aquifer
- has to be permeable enough to support interactions with the biotic components or allow for the development of anoxic conditions. On the other hand low hydraulic conductivity may give origin to a subsurface flux of NO<sub>3</sub>-N that is too small to be effective. In the upper part of the study area the presence of conductive soil layers (138–328 cm d<sup>-1</sup>) in depth permitted the transport of water rich in NO<sub>3</sub>. The diminishing of the hydraulic

<sup>20</sup> conductivity to values ranging from 67 to 2 cm d<sup>-1</sup>, respectively from P5 to P8 most likely promoted the bacterial processes.

#### 5 Conclusions

Our study of Lake Candia clearly demonstrates the crucial role of wetlands in the abatement of N flux from agricultural sources to aquatic ecosystems. The metabolic activity of bacterial communities, based on the differential use of electron donors and acceptors in redox reactions to derive energy for growth, is the key function of this system. In a thermodynamic perspective we can identify three different functional units within the



study area: 1) the steep upslope zone adjacent to the crop field where  $NO_3$ -N rich and oxic groundwater supported the aerobic respiration, 2) a flatter zone adjacent to the reed swamp dominated by denitrification that consumed all  $NO_3$ , 3) the reed swamp rich in electron donors, especially DOC, and poor in both oxygen and nitrate; the redox

- <sup>5</sup> condition favoured bacteria capable of deriving energy from sulphate. The biochemical responses occurring in these different units are coupled to the hydrological responses and the different reaction to rainfall inputs over the year. The topography assumes a critical role in regulating the strength of the hydrological connections within the riparian area.
- This successful experimental approach, which comprises both biogeochemical and hydrological monitoring, allows us to gain knowledge of the role played by physical and biological processes in controlling the N dynamic within this specific portion of the Candia Lake catchment. Although the results presented here are obtained from a single case study in a specific climatic environment, they may contribute to developing models on N cycling processes in catchments where N export can be predicted and
- also establish the extent to which riparian zones function as buffer systems.

The mechanistic explanations on denitrification through the analysis of the relationship between alkalinity and nitrate concentrations at soil-lake interfaces represents an innovative approach. In the wetland studied, the alkalinity was shown to be a valuable

indicator of the change in redox conditions Further research will be aimed at analyzing the relationship between N<sub>2</sub>O, as a final product of denitrification, and HCO<sub>3</sub>.

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25

HESSD						
4, 3501–3	534, 2007					
Lacustrine wetland in an agricultural catchment						
R. Balest	trini et al.					
Title Page						
Abstract	Introduction					
Conclusions	Conclusions References					
Tables Figures						
I	►I					
•	• • •					
Back	Back Close					
Full Screen / Esc						
Printer-friendly Version						
Interactive Discussion						
EGU						

#### References

5

20

30

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HESSD				
4, 3501–3534, 2007				
Lacustrine wetland in an agricultural catchment				
R. Bales	trini et al.			
Title	Page			
Abstract	Introduction			
Conclusions References				
Tables	Figures			
14	►I.			
•	•			
Back	Close			
Full Screen / Esc				
Printer-friendly Version				
Interactive Discussion				
FGU				

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15

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HESSD						
4, 3501–3	534, 2007					
Lacustrine wetland in an agricultural catchment R. Balestrini et al.						
Title	Title Page					
Abstract	Introduction					
Conclusions	References					
Tables Figures						
I	►I.					
	•					
Back	Back Close					
Full Screen / Esc						
Printer-friendly Version						
Interactive Discussion						

EGU

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HESSD						
4, 3501–3	534, 2007					
Lacustrine wetland in an agricultural catchment						
R. Balestrini et al.						
Title	Title Page					
Abstract	Introduction					
Conclusions	Conclusions References					
Tables	Tables Figures					
14	۶I					
•	•					
Back	Back Close					
Full Screen / Esc						
Printer-friendly Version						
Interactive Discussion						

## **HESSD**

4, 3501-3534, 2007

#### Lacustrine wetland in an agricultural catchment

R. Balestrini et al.



**Table 1.** Median concentration of major physical and chemical variables in samples collected between December 2003 and July 2004.

	Т	pН	HCO <sub>3</sub>	NH <sub>4</sub> -N	NO <sub>3</sub> -N	NO <sub>2</sub> -N	ON	$SO_4$	CI	0 <sub>2</sub>	DOC
	°C		meq I <sup>-1</sup>	mg l <sup>-1</sup>	$mgl^{-1}$	mg I <sup>-1</sup>	mg l <sup>-1</sup>	mg l <sup>-1</sup>	mg l <sup>-1</sup>	mg I <sup>-1</sup>	mg l <sup>-1</sup>
P1	11.7	6.17	0.60	0.018	14.76		0.69	16.9	12.6	2.35	2.04
P2	11.7	6.61	1.97	0.015	10.29	0.13	0.31	20.8	14.0	1.90	2.11
P3	10.5	7.00	3.20	0.012	13.06	0.13	0.81	36.2	16.5	1.68	2.93
P4	10.9	6.90	3.72	0.012	8.06	0.11	0.35	45.4	17.8	1.31	3.89
P5	11.3	7.02	5.03	0.012	4.85	0.15	0.51	56.7	17.5	0.85	4.35
P6	10.8	7.00	4.49	0.010	5.76	0.16	0.46	52.5	16.2	0.62	4.51
P7	11.7	7.15	5.04	0.027	3.69	0.10	0.35	51.5	15.2	1.39	3.82
P8	14.2	7.41	7.00	0.015	0.08		0.30	92.1	14.9	2.39	4.34
P9s	13.5	6.86	4.16	0.063	0.33		0.50	77.2	13.7	1.55	5.03
P9d	13.9	7.17	7.71	0.031	0.00		0.24	34.0	9.5	1.17	3.89
R1s	12.2	6.22	2.37	0.356	0.03		2.47	197.7	12.6	0.69	15.01
R1m	11.6	6.89	9.24	0.047	0.05		1.27	31.3	4.4	2.82	5.90
R1d	12.2	6.85	10.19	0.029	0.00		0.71	0.7	2.8	1.55	5.10
R2s	12.0	6.48	2.71	0.138	0.05		1.07	276.4	10.5	0.50	8.29
R2m	12.9	6.90	8.75	0.041	0.05		0.86	6.7	4.6	3.25	6.63
R2d	11.4	6.93	9.92	0.016	0.00		0.51	0.7	1.5	1.16	6.22
R3s	11.8	6.60	4.41	0.532	0.05		0.96	84.2	9.6	0.78	7.90
R3d	11.5	6.75	7.68	0.198	0.05		1.08	0.7	8.3	0.91	6.46
lake	17.0	7.95	1.08	0.088	0.18		0.64	6.2	4.8	10.60	7.04

## HESSD

4, 3501–3534, 2007

#### Lacustrine wetland in an agricultural catchment

R. Balestrini et al.

Title Page					
Abstract	Introduction				
Conclusions References					
Tables Figures					
Back Close					
Full Screen / Esc					
Printer-friendly Version					
Interactive Discussion					

EGU

**Table 2.** Mean concentration of major chemical variables in samples collected between March and November 2003.

	pН	HCO <sub>3</sub>	$NH_4$ -N	NO <sub>3</sub> -N	ON	$SO_4$	CI	DOC
		meq l <sup>-1</sup>	mg l <sup>-1</sup>					
P2	7.07			0.56		24.5	10.4	
P3	7.32	4.86	0.029	2.67		33.3	13.8	2.21
P4	7.34	3.45	0.065	0.34	1.17	27.2	11.6	3.38
P5	7.42	4.70	0.201	0.04	0.77	26.8	13.0	6.39
P6	7.62	4.38	0.100	0.10	1.77	26.3	12.6	4.95
P8	7.52	4.79	0.145	0.06	1.34	32.3	11.3	6.10
P9s	7.38	5.26	0.224	0.06	2.97	5.7	9.7	7.65
R1s	6.77	3.74	1.212	0.02	3.56	174.2	11.2	30.9
lake	8.07	1.11	0.217	0.17	0.95	4.8	4.5	6.70



Fig. 1. Map location of the Lake Candia catchment.



## **HESSD**

4, 3501–3534, 2007

Lacustrine wetland in an agricultural catchment

R. Balestrini et al.





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Fig. 2. Vertical cross section of the experimental field. The maximum and minimum recorded water levels are shown. Codes represent piezometer nest locations.  $\bullet$  = indicate position of the piezometers screen.

### HESSD

4, 3501–3534, 2007

Lacustrine wetland in an agricultural catchment







Fig. 3. Monthly variation of the water table in selected piezometers and monthly rainfall amount collected close to the study area.



# **Fig. 4.** Fluctuations of water table profile, averaged by row, during the study period. Codes above represent piezometer nest locations . $\bullet$ = indicate position of the piezometers screen.







Fig. 5. Boxplot showing the distribution of NO<sub>3</sub>-N, HCO<sub>3</sub>, CI and DOC in the reed sub-unit.

## HESSD

4, 3501-3534, 2007

#### Lacustrine wetland in an agricultural catchment

R. Balestrini et al.





Fig. 6. Boxplot showing the distribution of HCO<sub>3</sub>, SO<sub>4</sub>, CI and NH<sub>4</sub>-N in the reed sub-unit.

### **HESSD**

4, 3501-3534, 2007

#### Lacustrine wetland in an agricultural catchment

R. Balestrini et al.





Fig. 7. Boxplot showing the distribution of  $O_2$  in the poplar (a) and in the reed sub-unit (b).



















**Fig. 10.** Mean nitrate and nitrite (star symbol) concentrations (mg N I-1) along the studied transect during: **(a)** March–September 2003, **(b)** December–March 2004, **(c)** April–May 2004 and **(d)** June–July 2004.





HESSD						
4, 3501–3	3534, 2007					
Lacustrine wetland in an agricultural catchment R. Balestrini et al.						
Title	Title Page					
Abstract	Introduction					
Conclusions	References					
Tables	Tables Figures					
I	۶I					
•	•					
Back	Back Close					
Full Screen / Esc						
Printer-friendly Version						
Interactive Discussion						

EGU





