Denitrification in a meromictic lake and its relevance to nitrogen flows within a moderately impacted forested catchment

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Abstract

We analysed the spatial and temporal variability of benthic nitrogen fluxes and denitrification rates in a sub-alpine meromictic lake (Lake Idro, Italy), and compared in-lake nitrogen retention and loss with the net anthropogenic nitrogen inputs to the watershed. We hypothesized a low nitrogen retention and denitrification capacity due to meromixis. This results from nitrate supply from the epilimnion slowing down during stratification and oxygen deficiency inhibiting nitrification and promoting ammonium recycling and its accumulation. We also hypothesized a steep vertical gradient of sedimentary denitrification capacity, decreasing with depth and oxygen deficiency. These are important and understudied issues in inland waters, as climate change and direct anthropic pressures may increase the extent of meromixis.

Nearshore sediments had high denitrification rates (87 mg m^{-2} d^{-1}) and efficiency (~100%), while in the monimolimnion denitrification was negligible. The littoral zone, covering 10% of the lake surface, contributed ~50% of total denitrification, while the monimolimnion, which covered 70% of the sediment surface, contributed to < 13% of total denitrification. The persistent and expanding meromixis of Lake Idro is expected to further decrease its nitrogen removal capacity (31% of the incoming nitrogen load) compared to what has been measured in other temperate lakes. Values up to 60% are generally reported for other such lakes. Results of this study are relevant as the combination of anthropogenic pressures, climate change and meromixis may threaten the nitrogen processing capacity of lakes.

Introduction

Reactive nitrogen (Nr) inputs to watersheds have increased many fold in the last century, exceeding their uptake and storage capacity and causing large Nr exports with detrimental effects on receiving aquatic ecosystems (Galloway et al. 2003; Howarth et al. 2006). Several assessments have been made of Nr pathways and fate along the terrestrial to aquatic continuum, highlighting knowledge gaps (Howarth et al. 1996; Seitzinger et al. 2006; Bartoli et al. 2012). Namely, the Nr exported by rivers to the coastal zone worldwide averages 25% of the Nr loading generated by human activities in their watersheds, although large differences exist among catchments (Howarth et al. 1996; Howarth et al. 2012). Further effort continues to be needed to elucidate the fate of Nr not reaching coastal waters, the roles played by different landscape and ecosystem components, and the effects of anthropic activities on Nr processing (Seitzinger et al. 2006).

Lakes and reservoirs are biogeochemical reactors where the excess Nr is either retained or permanently removed as N_2 (David et al. 2006; Harrison et al. 2009; Lassaletta et al. 2012). The ratio of Nr retention to Nr removal differs among lakes, depending on a suite of environmental, hydrological, morphological and geographical conditions (Seitzinger et al. 2006; Bruesewitz et al. 2011; Finlay et al. 2013; Rissanen et al. 2013). Ultimately, a wide variety of interlinked biogeochemical processes control Nr (Burgin & Hamilton 2007), with lakes potentially acting as Nr filters in the watershed. In a lake, Nr is assimilated and retained by primary producers, which in turn fuel the food web and, ultimately, deliver organic N (N_{org}) to sediments. Microbial ammonification recycles a fraction of the sedimentary N_{org} as ammonium (NH_4^+). NH_4^+ is in turn assimilated by primary producers or transformed through a sequence of microbial processes comprised of nitrification, anaerobic NH_4^+ oxidation (anammox), denitrification and dissimilative nitrate (NO_3^-) reduction to NH_4^+ (DNRA). These processes have different ecological effects as only denitrification

and anammox permanently remove Nr, whereas DNRA recycles the Nr within the ecosystem. Benthic Nr transformations are intense in both shallow littoral or deep sediments (Nizzoli et al. 2010; 2014), where microbial processes are stimulated by the availability of organic carbon and electron acceptors (David et al. 2006; Revsbech et al. 2006; Wenk et al. 2014).

Although denitrification is recognized as a major Nr sink in lakes, to date only a few studies have assessed the contribution of in-lake denitrification with respect to Nr loadings from the watershed (Mengis et al. 1997; David et al. 2006; Bruesewitz et al. 2011; Rissanen et al. 2013; McCarthy et al. 2016). Factors controlling Nr transformations are mainly inferred from mass balance or simulation models (Harrison et al. 2009; Finlay et al. 2013). Further, processes responsible for Nr removal have not yet been adequately measured. Studies of in-lake denitrification are often biased by the use of the acetylene-inhibition techniques to measure denitrification. This method does not allow for the assessment of the coupling between nitrification and denitrification, because acetylene is a strong inhibitor of nitrification (Groffman et al. 2006). Measurements performed with sediment slurries are also unsuitable, since they may overestimate denitrification rates (Groffman et al. 2006).

The occurrence and persistence of thermal stratification and mixing control in-lake biogeochemical processes. Oxygen and nutrient availability especially limit biological activity. Thermal conditions and availability of oxygen, NO_3^- and organic carbon control benthic denitrification (Piña-Ochoa & Álvarez-Cobelas 2006). Under eutrophic conditions, the stable thermal stratification induces dissolved oxygen depletion in the hypolimnion, which in turn shifts the benthic microbial metabolism from aerobic to anaerobic (Matthews et al., 2008). In the hypolimnion, under anoxic conditions, denitrification depletes NO_3^- in association with two concurrent factors. The NO_3^- supply from the epilimnion slows down during stratification, while

89 persistent anoxia hampers microbial nitrification, which requires oxygen. Furthermore, the end-
90 products of anaerobic metabolism such as sulfides inhibit denitrification and foster DNRA (Burgin
91 & Hamilton 2007; Nizzoli et al. 2010; Azzoni et al. 2013). Persistent stratification in meromictic
92 lakes deeply affects biogeochemical processes, where deep-water anoxia may last over decades,
93 thus altering redox conditions (Lehmann et al. 2015). Furthermore, deep temperate lakes are
94 undergoing less frequent water turnover, alarmingly shifting toward holo-oligomictic conditions,
95 with the possible onset of meromixis due to the climate changes (Salmaso et al., 2014; Jeppesen et
96 al. 2015; Kraemer et al., 2015; Ficker et al. 2017). For these reasons, meromictic lakes are
97 especially suited for studying Nr pathways and fate. The coupled assessment of their watershed
98 processes could help to evaluate and predict the lake ecosystem responses to altered mixing regime
99 and to increasing anthropogenic pressures in the watershed.
100 This study has two objectives: 1) to investigate benthic Nr processing in a meromictic lake and
101 2) to put such Nr processing in the framework of whole basin N-budgets. We hypothesized that
102 meromictic conditions can depress Nr removal, as the occurrence of anoxia may stimulate NH_4^+
103 recycling and limit denitrification. Specifically, the aims of this work were: 1) to assess the
104 contribution of denitrification to the lake Nr budget, 2) to evaluate and compare denitrification rates
105 in deep and littoral lake sediments, and 3) to integrate the lake Nr budget into a detailed watershed
106 Nr budget.
107 We considered Lake Idro, a sub-alpine meromictic lake, as a model system to assess spatial and
108 temporal variability of microbial N-transformations and to elucidate the role of benthic
109 denitrification as a Nr sink. To put the in-lake Nr removal capacity into a context, we also
110 quantified the total Nr inputs delivered to the lake by its tributaries, the Nr export by the lake
111 outflow and the Net Anthropogenic Nitrogen Input (NANI) to the lake watershed. The NANI

112 approach is widely used to quantify the net anthropogenic Nr load to catchments and to evaluate the
113 spatial and temporal variability of Nr retention within the watershed (Han & Allan 2008; Lassaletta
114 et al. 2012; Hong et al. 2012).
115
116 **Material and methods**
117 *Study site*
118 Lake Idro is located on the southern slopes of the Alps at 368 m a.s.l. (Table 1 and Figure 1).
119 The watershed has an area of 609 km^2, an average elevation of 1610 m a.s.l. and is mostly forested
120 (>70%). Only one fourth of the lake catchment area is exploited for agriculture, mainly by pastures
121 and rough grazing (17.4%), while cropland agriculture (7.4%) is located only in the lowland (Figure
122 1 and Table 1). Population density is 40 ind km^{-2}, while livestock units (1 LSU = 1 adult dairy cow)
123 account for 6 LSU km^{-2}. Poultry (96000 ind), rabbits (16000 ind) and dairy cows (2300 ind) are the
124 main species farmed (ISTAT 2010). Additionally, 7 trout aquaculture activities operate in the
125 catchment with an annual average production of 1700 t.
126 Since 1930s, Lake Idro has been regulated by a top releasing dam and used for water supply to
127 lowland irrigation and hydroelectric power generation. The Chiese and Caffaro rivers contribute
128 ~45% and ~41% of the total water inflow to the lake (2.1×10^6 m^3 d^{-1}), respectively. The lake has a
129 total volume of 0.85 km^3, a maximum depth of 124 m and is considered meromictic (Garibaldi et al.
130 1996). The average residence time of water is about 1 year (Garibaldi et al. 1996). A steep
131 chemocline is present between 40-50 m depth, and the monimolimnion accounts for ~50% of the
132 total water volume. The littoral zone (<10 m depth) accounts for less than ~10% of the total lake
133 surface; the shore is gently sloping on the northern and southern sides, and rather steep on the

134 western and eastern sides. The lake is meso-eutrophic with whole lake total P and N concentration
135 averages of 110 and 960 µg L^{-1}, respectively. In the last 40 years, eutrophication and hypolimnetic
136 anoxia has been exacerbated. Total P concentrations in surficial waters increased from an average
137 of 9 µg L^{-1} in the early 1970s to 21 µg L^{-1} today. NO_3^- concentrations increased steadily from an
138 average of 196 µg N L^{-1} to 868 µg N L^{-1} in the same period (S1 Supplementary material).
139
140 *Sediment and water features, nutrient fluxes and benthic denitrification*
141 Sediment cores were collected on 24 January, 16 May, 8 August and 21 November 2011 at four
142 sites at 3, 6, 20 and 120 m depth (Figure 1). On each occasion, 3 replicate sediment cores (30 cm
143 length and 4 cm internal diameter) were collected at each site to analyze sediment characteristics,
144 while 4 replicate sediment cores (30 cm length and 8 cm internal diameter) were collected for
145 denitrification rates and dissolved inorganic nitrogen (DIN = $NH_4^+ + NO_2^- + NO_3^-$) flux
146 measurements. In parallel, temperature, oxygen, dissolved sulphides, NH_4^+ and NO_x ($NO_2^- + NO_3^-$)
147 were measured at each site to provide context for flux measurements. Temperature and oxygen
148 were measured directly *in situ* with a multi parameter probe (Idronaut Ocean 316) at 1 m depth
149 intervals. Water column samples for DIN and dissolved sulphides were collected with a Ruttner
150 bottle at surface, 2.5, 10, 20, 30, 40, 50, 60, 90, and 120 m depths at the site of maximum depth and
151 50 cm above the sediment at the other sites. The samples were immediately filtered (0.45 µm) and
152 stored frozen until analysis. Additionally, 25 L of water were collected at each site for core
153 maintenance and incubation.
154 Immediately after collection all sediment cores were placed in a cool box and returned to the
155 laboratory within 6 hours. Cores collected from the maximum depth station, were immediately
156 submerged in anoxic water retrieved at the same depth and closed with a rubber stopper in order to

157 minimize the exposure to atmospheric oxygen. Once in the lab the sediment cores were transferred
158 to separate tanks, submerged in lake water collected at the corresponding sampling site and
159 maintained overnight in the dark in constant temperature rooms at the average temperature
160 measured in the field. Oxic conditions in the tank water collected from the mixolimnion were
161 assured by bubbling air with airstones. Anoxia in the tank water collected from the monimolimnion
162 was assured by covering the incubation tank with a plastic bag and bubbling the water with N_2. The
163 water inside cores was gently stirred avoiding sediment resuspension during the pre-incubation and
164 incubation period with magnetic stirrers driven by a large magnet rotated by an external motor at 40
165 rpm. Measurements began the day after the sampling.
166 The cores for sediment characterization were processed as follows. The upper 0-5 cm section
167 was sliced and rapidly homogenised; 5 cm^3 of the homogenate were subsampled with a cut-off 10
168 cm^3 syringe, transferred into pre-weighed aluminium dishes and weighed to determine sediment
169 density. Sediment porosity (cm^3 H_2O cm^{-3} of sediment) was determined as weight loss from wet
170 sediments after 24 hours at 70 °C. Sedimentary organic matter (OM) was determined from 0.5 g dry
171 sediments as loss on ignition at 450 °C over 3 hours.
172 Fluxes of dissolved inorganic N across the water–sediment interface were quantified via short-
173 term batch incubations in the dark following the protocol described by Dalsgaard (2000; see S2
174 Supplementary material). The water inside the tanks was replaced with new water from the same
175 site prior to initiating the incubation to maintain near *in situ* dissolved nutrient concentrations. To
176 initiate incubations, the water level in the tank was lowered to below the core tops and the cores
177 sealed with floating Plexiglass lids. At the beginning of the incubation period before closing the
178 cores and at the end, water samples were taken from each core for NH_4^+ and NO_x analyses. Water
179 samples were immediately filtered (0.45 μm) and stored frozen until analysis. The incubation time

(from 1 to 3 hours, depending on season) was chosen based on test incubations for oxygen fluxes, to maintain the mean change of water column oxygen concentration within the 20% of the initial saturation (Dalsgaard et al., 2000). Anoxic cores collected from the monimolimnion were incubated for 5 hours at all sampling dates.

In situ denitrification (D_T) rates were measured on the same set of cores used for flux measurements following the Isotope Pairing Technique - IPT - (Nielsen 1992). The IPT is based on the addition of $^{15}NO_3^-$ to the water phase of each core followed by measurements of the produced labelled N_2. After flux measurements the cores were left submerged in the tanks for one hour without lids. To initiate measurements, the water level in the tank was lowered to below the core tops and $^{15}NO_3^-$ was added to the overlying water of each core at a final concentration of 420 µg L^{-1}. Before and 5 min after the addition of $^{15}NO_3^-$, water samples were collected from each core to calculate the $^{15}NO_3^-$ enrichment. The overlying water was then allowed to equilibrate with sediment pore water for 30 min. After this equilibration time the cores were closed with floating Plexiglass lids to avoid gas exchange with the atmosphere. In May 2011, $^{15}NO_3^-$ was added to the water phase of each core to reach 4 concentration levels (420, 980, 1400 and 2100 µg N L^{-1}). Different $^{15}NO_3^-$ additions were used to evaluate the dependency of potential denitrification rates (denitrification of the *in situ* $^{14}NO_3^-$ + denitrification of the $^{15}NO_3^-$) on NO_3^- availability at the 4 different depths. At the end of all incubations 5 ml of 7 M $ZnCl_2$ were added to the water of each core to inhibit further bacterial activity. Just after the addition of $ZnCl_2$, the sediment was slurried and sub-samples were carefully collected with a glass syringe equipped with a 10 cm long gas-tight Tygon® tube. The samples were immediately transferred into 12 ml glass vials (Exetainer, Labco) ensuring that no bubbles formed during sampling. Overflow of at least three Exetainer volumes was assured before

sealing and poisoning with additional $ZnCl_2$ (200 µL 50% w/v) for subsequent analysis of the $^{29}N_2$ and $^{30}N_2$ composition of the dissolved N_2 pool. All of these cores were incubated for the same incubation time as for flux measurements.

Nitrogen mass balance at watershed scale and Nr loading to the lake

The Nr budget of the whole catchment was computed with the Net Anthropogenic Nitrogen Input model (NANI, Howarth et al., 1996):

$$NANI = N_{Dep} + N_{Fert} + N_{Fix} + N_{Trade}$$

Where

N_{Dep} = atmospheric Nr deposition

N_{Fert} = synthetic Nr fertilizer applied to agricultural soils

N_{Fix} =agricultural N_2 fixation

N_{Trade} = net exchange of Nr as food and feed.

NANI was first calculated at the municipal scale, which is the smallest administrative unit at which most of the national statistics are available. To calculate the contribution of each municipality to the Nr budget, municipality-level data were then aggregated at the catchment scale by weighting each municipality based on the spatial distribution of land use areas in the watershed (Han & Allan 2008).

N_{Dep} was estimated using wet and dry deposition of both oxidized and reduced Nr. By convention only oxidized Nr deposition is used in the NANI estimate. NH_3 is short lived in the atmosphere, and deposition of reduced Nr likely reflects local recycling (Hong et al. 2012).

However, NH_3 deposition may be from sources outside the Lake Idro watershed, and thus not locally recycled. This is because of the small size of the watershed and the relatively small apparent contribution of agriculture, a major source of local reduced Nr. To account for local recycling, we subtracted NH_3 volatilization from atmospheric deposition, assuming that all the NH_3 volatilization was redeposited locally. Nr deposition measured at three gauging stations of the CONECOFOR network in the investigated area averages 750±130 and 875±140 mg N m^{-2} y^{-1} for oxidized and reduced Nr respectively (Rogora et al. 2006). These values are in agreement with those estimated with the GAINS-Italy model at a 20x20 km resolution (De Marco personal communication). However, they are lower than estimates extrapolated from the 50×50 km resolution grid of the Co-operative Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutants in Europe (EMEP 2010). The latter are equal to 950 and 1900 mg N m^{-2} y^{-1} for oxidized and reduced Nr, respectively. We therefore considered an average value of N_{Dep} = 1900±760 mg N m^{-2} y^{-1}, we assumed constant over the catchment, and multiplied this estimate by the watershed area. Ammonia volatilization was estimated as the percentage loss of the supplied Nr using published emission values. Nr potentially lost via volatilization in the Lake Idro watershed is primarily related to cattle and chicken manure (30%) and urea fertilizers (15%) (Bussink and Oenema 1998; Misselbrook et al. 2004).

Fertilizer Nr application, agricultural Nr fixation and net Nr food and feed imports were calculated using agricultural and demographic census data from the Italian National Institute of Statistics, year 2010 (ISTAT 2010). Fertilizer Nr application was estimated using available data on fertilizer sales and Nr content for each fertilizer type (ISTAT 2010). The fertilizer sales data were available at the province level divided by form: urea, NH_4^+, NO_3^-, Nr solutions and miscellaneous forms. We assumed that fertilizers were applied in the same province in which they were sold.

However, because the Lake Idro watershed lays within provinces that include intensive agricultural lowland areas outside the investigated catchment, a simple downscaling based on the proportion of watershed area to province area could overestimate the fertilizer input. Therefore, we calculated an average province-level fertilizer application rate by dividing fertilizer sales by the potentially fertilized land area in the province. We then multiplied this average value for the potentially fertilized land area in each municipality included in the watershed. The potentially fertilized area was estimated as the sum of temporary and permanent agricultural crops; excluding N-fixing crops, permanent meadows and pastures.

The amount of fixed N_2 associated with pastures, rough grazing and permanent meadows - which collectively represent more than the 95% of the N-fixing crops in the watershed - was estimated by multiplying the area of the specific N-fixing crop by the areal N-fixation coefficients of each crop type. N-fixation coefficients were estimated as the product of yield and Nr content corrected for the ratio of total biomass produced to harvested biomass (Lassaletta et al. 2012). Average values were 2400 (pastures), 800 (rough grazing) and 11800 (meadows) kg N km^{-2} y^{-1}, comparable to literature values (Hong et al. 2012).

The net Nr imports with food and feed trade were calculated as the difference between Nr production, i.e. sum of crop and livestock Nr production in the catchment, and Nr consumption, calculated as the sum of humans and livestock Nr intake. Human Nr consumption was estimated by multiplying human population by Nr consumption per capita (6.8 kg N $capita^{-1}$ y^{-1}). The latter was calculated assuming a protein consumption of 111 g $capita^{-1}$ d^{-1} (FAOSTAT 2010), with protein = N x 6.25 (Jones, 1941). The inhabitant number in each municipality was obtained from the 2010 Demographic Census (ISTAT 2010). This number was integrated with data on tourist presence (Regional Agency for Environmental Protection).

Livestock Nr consumption and excretion were calculated from livestock numbers, multiplied by the intake and excretion parameters for each livestock type. Per animal Nr intake and excretion parameters of the different livestock classes were obtained from the regional plans for water protection (Lombardy Region 2003). When data were not available, we referred to published values (S3 supplementary material). Livestock Nr production was calculated as the difference between animal Nr intake and excretion (Hong et al. 2012).

The amount of Nr produced by crops was calculated by multiplying the average harvested yield of each crop (ISTAT 2010) by the average Nr content of each harvested crop type (Lombardy Region 2003). Harvested Nr with crops was distributed between humans and livestock, corrected for fractions lost during food and feed production according to the FAO Food Balance Sheet of Italy (FAOSTAT 2010). When data were not available, we referred to published values (Hong et al. 2012). The coefficients used are summarized in the S4 supplementary material.

This estimate of the NANI is based on all the best available information. The accuracy could be limited by the quality of the input data, the accuracy of the coefficients used for converting input data into Nr units and the validity of the assumptions made. The uncertainty associated to the NANI calculation was incorporated into the budget output by performing a Monte Carlo simulation (Han & Allan 2012), which utilized random sampling for all coefficients, atmospheric deposition and fertilizer data. Census data of livestock numbers, human population and the area of each crop type collected from National Statistics were considered well constrained and kept fixed. All the other terms were allowed to vary stochastically and independently around the mean value and were assumed to follow a normal distribution. A total of 1000 models were run as mean and standard deviation settled to constant values after 500 iterations.

293 Standard deviation for N_{Dep} was directly obtained from the variability of data collected for the
294 Lake Idro watershed (coefficient of variation = 40%). Standard deviations for crop yield, Nr content,
295 and livestock Nr excretion were estimated from the average coefficient of variation reported in Soana
296 et al. (2011) which equal to 26%, 25%, and 14%, respectively (S5 supplementary material). Standard
297 deviations for animal Nr intake were estimated assuming a coefficient of variation equal to Nr
298 excretion. Standard deviations for the parameters for which information about variability were not
299 available (crop fraction distributed to humans, Nr fraction lost in food and feed processing, human
300 Nr intake and fertilizer Nr application), were estimated using an intermediate coefficient of variation
301 (20%) between 14 and 26%, the range of uncertainty of the agricultural coefficients (S5
302 supplementary material).
303
304 *Riverine Nr flux estimations*
305 Chemical characteristics of inflowing and outflowing waters were measured monthly from June
306 2010 to March 2012. At each sampling date water samples were collected for NH_4^+, NO_x, total
307 dissolved (TDN) and total (TN) nitrogen determinations. Samplings and analyses were performed
308 following standard methods (APHA 1998). The daily Nr loadings to and exports from the lake were
309 computed as the product of average daily flow by concentration of the target chemical species.
310 Annual loadings were calculated as the product of the discharge weighted mean concentration by
311 the mean annual discharge of the two years (Quilbè et al. 2006). The Chiese Consortium, the
312 Management Authority of lake Idro, and the Autonomous Province of Trento provided the daily
313 average water inflows and outflows. Discharge of minor tributaries was calculated from direct
314 measurements of water velocity, with a water velocity meter (Scubla mod. 2030), and cross
315 sectional area in each sampling day. Total Nr input to the lake was computed as the sum of the river

316 Nr loadings plus atmospheric Nr deposition (wet and dry deposition of both oxidized and reduced
317 Nr) to the lake surface.
318
319 *Analytical determinations and calculations*
320 NH_4^+ (Bower & Holm Hansen 1980) and NO_x (APHA 1998) were determined by
321 spectrophotometry. TN and TDN were determined as NO_x after alkaline peroxydisulfate oxidation
322 (Valderrama 1981).
323 The abundances of $^{29}N_2$ and $^{30}N_2$ in the dissolved N_2 pool were determined with a gas
324 chromatograph in line with a mass spectrometer at the National Environmental Research Agency,
325 Silkeborg (DK) following Risgaard-Petersen & Rysgaard (1995). In brief the N_2 of each sample
326 was extracted from the water in the Exetainer by introducing a helium headspace and shaking
327 vigorously the vial for 5 min. The entire headspace (4 ml) was then carried through the gas
328 chromatograph columns (Roboprep-G-Plus GC) and to a triple-collector mass spectrometer (Europa
329 Scientifica TracerMass) to obtain the isotopic composition of the N_2 by a flow of helium (99.9995%
330 purity). Prior to reaching the mass spectrometer, water vapour, CO_2 and O_2 were removed by
331 passing the sample through a drying tube (10 mm x 200 mm packed with $Mg(ClO_4)_2$), a Carbosorb
332 (10-20 mesh) column and a reduction column (15 mm x 300 mm packed with Cu wires at 650 °C)
333 respectively. The increased abundance of $^{29}N_2$ and $^{30}N_2$ in the samples was obtained by subtracting
334 the natural $^{29}N_2$ and $^{30}N_2$ abundance from the signals.
335 Fluxes of NH_4^+ and NO_x across the sediment-water interface were calculated from the change
336 of solutes concentration over time (Nizzoli et al., 2014) using the following formula:
337
338 $Fx = ((Cf - Ci) * V) / A * t$

Fx = flux of the x species (mg m^{-2} h^{-1})

Cf = final concentration of x (mg L^{-1})

Ci = initial concentration of x (mg L^{-1})

V = volume of the water column (L)

t = incubation time (hours)

A= sediment surface area inside the core (m^2)

Daily rates were calculated by multiplying hourly rates by 24.

D_T, direct denitrification of $^{14}NO_3^-$ diffusing to the sediment from the water column (D_W) and denitrification of $^{14}NO_3^-$ produced by nitrification within the sediment (D_N) were calculated following Nielsen et al. (1992) as follows:

$$D_{15} = p29 + 2p30$$

$$D_T = D_{15} \times (p29/2p30)$$

$$D_W = (^{14}NO_3^- / {}^{15}NO_3^-) * D_{15}$$

$$D_N = D_{14} - D_W$$

where D_{15} represents denitrification of the added $^{15}NO_3^-$, $^{14}NO_3^-$ the ambient unlabelled NO_3^- concentration and $^{15}NO_3^-$ the isotopically-labelled NO_3^- concentration at the start of the incubation, p29 and p30 represent the production rates of $^{29}N_2$ and $^{30}N_2$, respectively. The presence of anammox interferes with IPT calculations resulting in an overestimation of D_T because the N_2 produced by anammox cannot be discriminated from the N_2 produced by denitrification. Therefore, independence of D_T from added $^{15}NO_3^-$ was checked to validate IPT and exclude significant overestimation due to anammox (Risgaard-Petersen et al. 2003).

Benthic denitrification efficiency (DE) was calculated as the percentage of denitrification to ammonification according to Eyre & Ferguson (2009):

$DE = D_T/(DIN+D_T)*100$

The mass transfer coefficient (m y^{-1}) was calculated as the ratio of D_W to the ambient NO_x concentration (David et al. 2006).

The contribution of sediments at different depths to the whole lake denitrification was calculated according to David et al. (2006). The lake sediment was first partitioned into three bathymetric layers on the basis of similarities in the features of the water overlying the sediment and the sediment itself. Then, annual D_T rates were calculated in each of the three layers with a linear integration of the measured daily rates over the sampling year multiplied by the corresponding sediment surface area. Finally, the areal D_T values were summed to estimate the whole lake denitrification rate. This calculation assumes that the change of denitrification rates between two subsequent sampling periods was linear and that denitrification rates were homogeneous within each of the three layers.

Nr retention within the whole basin (terrestrial + lake) was calculated as the difference between NANI and Nr export from the lake outlet. Nr retention within the terrestrial part of the watershed was estimated as the difference between NANI and Nr loading exported by rivers and streams to the lake. The lake Nr retention was estimated as the difference between the Nr loading to the lake and the Nr quantity exported through the lake outlet. Nr retention efficiency (%) relative to Nr input was calculated as 100*(Nr inputs – Nr outputs)/ Nr inputs.

Data were analysed with nonparametric tests because of violations of the assumptions of normality and homogeneity of variance even after data transformation. The non-parametric Kruskall Wallis H test was used to test differences of denitrification rates and Nr fluxes among

384 depths ($n_{120}=n_{20}=n_6=n_3=16$) and among sampling dates within each depth
385 ($n_{spring}=n_{summer}=n_{autumn}=n_{winter}=4$). When differences among depth or among seasons were
386 significant, *a posteriori* comparison of the means was performed using a post hoc Tukey and
387 Kremer test (Quinn & Keough 2002). Differences between daily Nr loads measured at lake inlet and
388 outlet were compared using the non-parametric Mann-Whitney U test. All statistical analyses were
389 performed using the statistical computing software R (R Core Team, 2014). Descriptive statistics
390 are presented within the text as mean ± standard deviation.
391
392 **Results**
393 *Water and sediment characteristics*
394 Temperature, dissolved oxygen and Nr underwent steep variations along the water column
395 (Figure 2 and Table 2). Water temperatures displayed a typical seasonal trend in the shallower sites,
396 changing from 5 °C in winter to 23 °C in summer, and was nearly constant in the deepest waters.
397 Dissolved oxygen concentrations decreased with depth, ranging from 8.6 to 14.1 mg L^{-1} in the surface
398 layer to anoxia below 40-50 m depth. NO_x concentrations followed a similar pattern, decreasing from
399 868 µg N L^{-1} in the surface to <100 µg N L^{-1} from 40 m to the bottom. NH_4^+ concentrations followed
400 an opposite trend, increasing from <20 µg N L^{-1} in the mixolimnion up to ~1000 µg N L^{-1} in the
401 deepest layers. A concurrent accumulation of dissolved sulphides up to 4.3 mg S L^{-1} also occurred
402 from 60 m to the bottom.
403 The sediment collected at 3 and 6 m depths was composed of fine sands with a high silt
404 content. The average OM content (5.4±1.6 %) in the upper sediment horizon was similar for the two
405 sites. By contrast, sediment collected at 20 and 120 m depth was composed of fine textured mud. At
406 120 m depth the sediment appeared dark black up to the sediment-water interface, with a strong

sulphide smell, whereas at 20 m depth a thick layer of yellowish oxidised sediment was visible at the sediment-water interface at all the sampling dates. The average OM content (9.5±1.7 %) in the upper sediment horizon was higher at 20 m depth compared to the deepest layer (7.2±1.4%).

Benthic Nr fluxes and denitrification rates

Differences between depths 3 and 6 m were not statistically different (K-W, $p>0.05$) for benthic fluxes of NH_4^+, NO_x or D_T rates. Therefore, in the following sections we will refer to these observations collectively as littoral sites (0-10 m bathymetric layer).

D_T rates were significantly different among all sites (K-W, $p<0.001$; Figure 3a). Average D_T rates decreased with depth, being from 2.3 to 24 times greater in the littoral sediment (average 87±85 mg m^{-2} d^{-1}) than in the deeper layers. D_T was especially low to undetectable in the 120 m depth sediment (3.5±2.9 mg m^{-2} d^{-1}). In the 0-10 m layers, benthic D_T underwent a clear seasonal pattern, with spring and summer rates 6 times higher than those measured in autumn and winter (K-W, $p<0.001$).

Nitrification was only a minor source of NO_x for denitrification and D_N rates were not statistically different among the four sites (Figure 3a). D_W accounted on average for more than 90% of D_T and drove the seasonal patterns and spatial distribution of D_T. We also demonstrated that denitrification rates were stimulated by increasing levels of NO_x at all the depths, although the degree of stimulation was one order of magnitude lower in sediments from 20 m to the bottom than in littoral sediments (Figure 4).

Overall, the annual denitrification in the benthic system accounted for a N_2 production of 83±10 t N y^{-1} (7.2±0.4 t N km^{-2} y^{-1}). An annual rate of 42±10 t N y^{-1} (69±8.7 t N km^{-2} y^{-1}) occurred

429 in the littoral area, while only 11±2 t N y^{-1} (1.3±0.1 t km^{-2} y^{-1}) were produced in the
430 monimolimnion sediments (Table 3).
431 Concurrently, high rates of NO_x consumption were measured in the littoral sediment (-
432 75.6±77.0 mg m^{-2} d^{-1}) particularly under spring and summer conditions. Much lower NO_x fluxes
433 were detected in the deeper sediments (-7.0±2.8 mg m^{-2} d^{-1}; Figure 3b). On average, the benthic
434 system was a source of NH_4^+ to the water column, with significant differences among depths (K-W,
435 $p<0.01$; Figure 3c). A clear seasonal pattern was observed in the littoral zone where NH_4^+ release
436 from the sediment peaked in spring and summer (101.1±67.2 mg m^{-2} d^{-1}). At 120 m depth NH_4^+
437 release was always high (99.4±58.1 mg m^{-2} d^{-1}) with lowest efflux measured in autumn (42.9±4.2
438 mg m^{-2} d^{-1}) and highest in winter (173.8±23.8 mg m^{-2} d^{-1}). NH_4^+ fluxes were almost undetectable at
439 20 m depth.
440 In the littoral benthic system, the net DIN flux was on average slightly into the sediments (-
441 16.8±48.0 mg m^{-2} d^{-1}) due to the high NO_x consumption, while the monimolimnion sediment was
442 always a net source of DIN (14-224 mg m^{-2} d^{-1}), mainly due to NH_4^+ recycling. Denitrification
443 efficiency was elevated in sediments down to a depth of 50 m depth, while Nr recycling dominated
444 the monimolimnion sediments (Table 3).
445
446 *Nitrogen fluxes at watershed scale*
447 The estimated NANI was 1785±467 t N y^{-1}, corresponding to an areal input of 2929±767 kg N
448 km^{-2} y^{-1} (Figure 5). Atmospheric Nr deposition accounted for 61% of NANI, equivalent to
449 1781±763 kg N km^{-2} y^{-1} while the Nr input associated with trade of food and feed was 20% of
450 NANI, equivalent to 574±80 kg N km^{-2} y^{-1}. The autotrophic organic Nr production within the
451 catchment (484±85 kg N km^{-2} y^{-1}) was not sufficient to meet the Nr needs of the human and

452 livestock population (1057±67 kg N km^{-2} y^{-1}); organic Nr was thus imported to the watershed
453 mainly as animal feed (606±78 kg N km^{-2} y^{-1}). N_2 fixation associated with crops and pastures
454 (524±112 kg N km^{-2} y^{-1}) accounted for an additional 18% Nr input, while fertilizers (51±7 kg N km^{-2} y^{-1}) were < 2% of NANI.
456 TN concentrations measured in the water at the outlet of the main rivers and small streams that
457 drain into the lake showed large variability, among both sampling dates and sites (499-3469 µg N L^{-1}). The TN pool consisted mainly of NO_x (up to the 80%), with the highest concentrations generally
459 detected in minor creeks (data not shown). The average daily TN load was variable, ranging from a
460 minimum of 860 kg N d^{-1} to a maximum of 6021 kg N d^{-1} (Figure 6). More than 90% of the total Nr
461 load was delivered by the Caffaro and Chiese rivers, mainly as dissolved Nr forms (> 98%). The
462 annual Nr export by rivers to the lake was 846±586 t N y^{-1}, which is equivalent to 47% of NANI.
463 TN concentration measured in river water at the lake outlet (485-1020 µg N L^{-1}) was lower
464 than in the inflowing waters (Figure 6). The daily Nr exported from the lake ranged from 622 kg N
465 d^{-1} to 4065 kg N d^{-1} and was significantly lower than the daily input (Wilcoxon, p=0.013). The
466 annual Nr export from the lake was 598±395 t y^{-1}, equivalent to nearly 70% of the annual inputs.
467 Therefore, the lake either retained or lost nearly 30% of the Nr load (270±706 t N y^{-1}) prior to
468 riverine export.
469
470 **Discussion**
471 Measurements of benthic Nr fluxes and denitrification rates along with Nr mass balances were
472 combined to evaluate the contribution of benthic denitrification to Nr removal under meromictic
473 conditions. These are understudied but important issues in inland waters, as they can provide new
474 insight on the capacity of lake ecosystems to dissipate the Nr load under infrequent to no mixing

475 frequencies. A synopsis of the budgets and fluxes resulting from the study highlights that in-lake
476 denitrification was not efficient in removing Nr in most of the lake's sediment. Further, the
477 watershed had a low Nr retention capacity resulting in a high percentage of Nr (47%) exported to
478 the lake system from tributary rivers (Figure 7).
479
480 *Spatial and temporal variability of benthic Nr processes*
481 Different benthic nitrogen processes dominated in deep and littoral sediments of Lake Idro.
482 Nearshore sediments had higher denitrification rates and denitrification efficiency, compared to the
483 monimolimnion sediments where denitrification was almost negligible. Recycling of NH_4^+ from
484 sediments to the water column dominated in the monimolimnion.
485 Spatial heterogeneity of denitrification rates was previously observed in dimictic lakes with
486 rates generally higher in the littoral than in the deeper sediments (Saunders & Kalff 2001;
487 Bruesewitz et al. 2012), but with exceptions due to local conditions (Rissanen et al. 2013; Small et
488 al. 2014). The average D_T of 2.8 mg N m^{-2} d^{-1} measured in the meromictic Lake Idro falls within the
489 lower range of values (1.9 to 84 mg m^{-2} d^{-1}) detected in other deep sediments (Table 4) suggesting a
490 reduced capacity to dissipate Nr. Water column NO_x, as opposed to nitrification, was by far the
491 dominant source of NO_x for denitrification. The incubations of sediment cores with increasing NO_3^-
492 concentrations revealed that in all the investigated sediments denitrification was NO_3^- limited. Low
493 NO_x concentrations in the water column can prevent denitrification by limiting the diffusion of NO_x
494 into the sediment denitrification zone. Furthermore, anoxia precludes nitrification and NO_x
495 production within the sediment. The comparison of denitrification in littoral and deep sediments
496 also evidenced that NO_x addition was less effective in the latter sediments (Figure 4). We argue that
497 anoxia and chemically reducing conditions in the monimolimnion can further inhibit denitrification

498 processes. The mass transfer coefficient, which can be regarded as an index of the efficiency of N_2
499 production relative to NO_x availability (David et al. 2006), was lower in sediments collected from
500 the monimolimnion compared to the other depths (Table 3). Lower values suggest that
501 environmental conditions in the monimolimnion were less suitable to denitrification compared to
502 littoral oxygenated sediments. Denitrification efficiency was less than 5% and anaerobic
503 ammonification was the dominant process of sediment Nr release under these conditions (Figure 8).
504 In contrast, D_T rates of 87 mg N m^{-2} d^{-1} measured in the littoral habitat were within the upper
505 range of values (0 to 238 mg N m^{-2} d^{-1}) measured in other lake ecosystems (Table 4). Here, benthic
506 Nr metabolism followed a typical seasonal pattern with peaks of D_T rates and DIN fluxes in spring
507 and summer. Such high rates likely reflected temperature changes and the consistently high NO_x
508 availability in the water column throughout the year; low D_N indicated that sediment nitrification
509 was a minor source of NO_x. Mineralized Nr was released to the water column as NH_4^+ in littoral
510 sediment due to low nitrification. However, in contrast to the deeper sediments, DIN fluxes across
511 the sediment water interface in the littoral zone were largely driven by microbial NO_x reduction.
512 Here, denitrification of the NO_x bulk diffusing from the water column exceeded the NH_4^+ efflux,
513 and the superficial sediment acted as a net DIN sink (Figure 8).
514
515 *Benthic denitrification and in lake Nr processing*
516 We scaled up denitrification rates to the whole lake to provide context for the contribution of
517 benthic denitrification in the different bathymetric layers to the in lake Nr metabolism (Table 3). To
518 our knowledge, there are only few examples of the concurrent use of mass balances and direct
519 measurements of in-lake processes to evaluate pathways and fate of the Nr loadings from the
520 watershed (Mengis et al., 1997; David et al. 2006; Bruesewitz et al. 2011; McCarthy et al. 2016).

521 Upscaling from core measurements to the whole lake requires addressing both methodological
522 problems and spatial and temporal variability of rates (Groffman et al. 2006). We addressed both
523 kinds of uncertainty. First, we used a well-established technique for measuring denitrification
524 (Groffman et al. 2006). Second, we sampled sediments in three bathymetric layers, from the
525 shallow littoral zone to the deepest monimolimnion, over four seasons to incorporate both the
526 spatial and temporal variability of fluxes and rates (David et al. 2006; Bruesewitz et al. 2012;
527 Nizzoli et al., 2014).

528 The contribution of the three different bathymetric zones to the whole benthic N_2 production
529 was not proportional to the respective sediment area, contrary to what was previously observed in
530 Lake Shelbyville, a low land polimictic reservoir (David et al. 2006) or in the dimictic Gull Lake
531 (Bruesewitz et al. 2012). In Lake Idro 50% of benthic denitrification occurred in the littoral area.
532 This area accounted for only 10% of the lake surface; whereas the monimolimnion sediments,
533 which extended over 70% of the lake surface, contributed only 13% of D_T (Table 3).

534 These data demonstrate that shallow aquatic environments characterized by high NO_x loads can
535 sustain high denitrification rates, and thereby the littoral area has an enormous impact relative to its
536 surface in the control of the external NO_x loads. The disproportionate contribution of the littoral
537 area can be found not only in meromictic lakes but also in eutrophic temperate lakes where water
538 stratification is followed by oxygen and NO_x depletion (Bruesewitz et al. 2011). Under these
539 conditions, the deep benthic system progressively loses the capacity to denitrify (Nizzoli et al. 2010;
540 Bruesewitz et al. 2011), which is preserved instead in the more oxidized littoral sediments (Nizzoli
541 et al. 2014). Yet several anthropic pressures affect littoral zones of lakes (Francis et al. 2007).
542 Among others, human exploitation of water resources and extreme hydrological events affect the

543 magnitude and timing of water-level fluctuations, which in turn may influence benthic metabolism
544 (Hofmann et al. 2008) with possible implications for Nr biogeochemistry.

545 The in-lake D_T (83 ± 5 t y^{-1}) accounted for only 30% of the Nr retained by the lake, much lower
546 than values of 62-100% found in the alpine meromictic Lake Zug, the dimictic eutrophic lake
547 Baldegg and a lowland reservoir (Mengis et al. 1997; David et al. 2006). This discrepancy could be
548 due to the mass balance and acetylene inhibition methods with sediment slurries, which are known
549 to overestimate the *in situ* denitrification rates (Mengis et al. 1997; Groffman et al. 2006).
550 Furthermore, D_T accounted for the removal of only 10% of the Nr load to the lake providing a
551 missing quota of 165 t y^{-1}.

552 Nr retention is the combined result of N_{org} storage in sediment, living biomass, DIN
553 accumulation in the water column and unmeasured N_2 production. In this work we incubated
554 sediment cores under dark conditions. This experimental set up is appropriate for the deeper zones
555 where light does not reach the sediment surface. By contrast light can penetrate to shallow littoral
556 sediments, and part of the Nr is assimilated by benthic primary producers (Nizzoli et al., 2014).
557 Therefore, our approach could have underestimated Nr retention in the littoral zone, especially in
558 the southern part of the lake, colonized by dense submerged meadows of *Lagarosiphon maior*
559 (Bolpagni 2013). Assuming assimilation rates of 0.28-1.26 mg N g d^{-1} (Nizzoli et al. 2014 and
560 references therein) and an average dry biomass of 250 g m^{-2} (Longhi upublished data) Nr
561 assimilation would be in the range of 9-38 t N y^{-1}. This rate is comparable to littoral denitrification.

562 In meromictic lakes, persistent water column stratification prevents the upward flux of
563 accumulated nutrients from the monimolimnion. Indeed, in Lake Idro, the monimolimnion is
564 storing ~300 t of NH_4^+. However, the long-term fate of this Nr is unclear. Partial turnover of the
565 upper monimolimnion can potentially occur due to heavy storms or very cold winters. Such events

occurred in the nearby meromictic lakes Lugano and Iseo (Salmaso et al. 2014; Lehmann et al. 2015). Under these circumstances, the accumulated NH_4^+ can migrate upwards into the mixed layer undergoing oxidation to NO_x with denitrification to N_2 (Lehmann et al. 2015).

The water-sediment interface is commonly considered the most reactive zone of aquatic ecosystems, where biogeochemical processes are amplified. However, recent studies suggest that in meromictic lakes the chemocline could also host intense Nr transformations due to the sharp redox transition (Schubert et al. 2006; Hamersley et al. 2009). For example, in Lake Lugano, in summer ~60% of total denitrification was measured in the water column (Wenk et al. 2014). During this study, the chemocline of Lake Idro occurred from approximately 40 to 50 m depth and accounted for about 0.15 km^3. Therefore, even low denitrification rates in this bathymetric layer could greatly contribute to the Nr budget. This needs future investigation given the inability to balance lake imports with exports and denitrification estimates.

The lake as a metabolic regulator of the NANI from the watershed

The Lake Idro watershed is characterized by low anthropogenic pressures, resulting in a NANI far below the average values found for Europe (4000 kg km^{-2} y^{-1}) and worldwide (Boyer et al. 2002; Hong et al. 2012; Gao et al. 2014). Nutrient budgeting is affected by uncertainties due to difficulties in estimating the different budget terms and the assumptions made in model formulation (Oenema et al. 2003; Soana et al. 2011; Hong et al., 2013). Indeed, the NANI of Lake Idro watershed has a relatively high coefficient of variation (CV = 26% of the mean). This CV falls in the upper range of those previously calculated in other watersheds (4 – 20%) with a comparable approach (Yan et al., 2011; Han & Allan 2012; Chen et al. 2016). The variability of the different NANI terms and coefficients can explain this high CV. For example, An et al. (2012) used a uniform 5% coefficient

589 of variation. In contrast we applied, when possible, a specific range for each coefficient, from 14 to
590 40% of the mean. In the Lake Idro basin, as in most catchments with relatively low population
591 density and human activities, atmospheric deposition is the largest NANI component. The
592 variability of NANI is thus due to the large uncertainty associated to this term.

593 We compared NANI and the Nr flux from the watershed throughout the lake inlet. The lake
594 catchment exhibited a low Nr retention efficiency and an average of 47% of the NANI was not
595 retained by the watershed system (Figure 7). Previous studies demonstrated that the Nr flux across
596 watersheds is within 20-25% of the NANI, although large differences have been observed among
597 catchments ranging from < 10% to > 50% (Howarth et al. 2012).

598 The presence of the lake enhances the Nr retention capacity of the whole watershed. Our input-
599 output mass balance indicates that 53% of the NANI is retained by the terrestrial part of the
600 watershed, while the lake retains 13% (Figure 7). This is a high percentage relative to the lake
601 surface which is only 2% of the total watershed area, further confirming that the in-lake
602 biogeochemical processes are quantitatively relevant (Seitzinger et al. 2006; Harrison et al. 2009;
603 Lassaletta et al. 2012). However, the Nr removal efficiency of Lake Idro appears low, when
604 compared to other aquatic ecosystems (Figure 9).

605 Nr removal efficiency of lakes is extremely variable and influenced by latitude, water residence
606 time and trophic status (Seitzinger et al. 2006; Rissanen et al. 2013; Finlay et al. 2013). Water
607 residence time in particular is widely used as an explanatory factor. It represents the time scale for
608 processes that influence Nr removal (Nr uptake, settling of particulate Nr and diffusion of NO_x to
609 anoxic denitrification zones) to occur before flushing downstream (Seitzinger et al. 2006). Lake
610 Idro retained 31% of the external Nr load with an efficiency falling in the lower range of values
611 measured in other temperate lakes with similar water residence time or predicted by means of

612 empirical equations from literature. The latter estimates are much greater and are in the range 40-
613 60% (Figure 9). This low retention efficiency can be explained by the fact that 70% of the benthic
614 system in Lake Idro is affected by anoxia. NO_x depletion and extremely chemically reducing
615 conditions favor Nr recycling rather than denitrification. We added increasing amounts of NO_x to
616 monimolimnic waters above sediments to evaluate denitrification capacity following NO_x supply
617 due to water overturn. At NO_x concentrations between 700-840 µg N L^{-1}, equivalent to those
618 measured at 30 m depth, the theoretical denitrification rates fell to the range of 16.8-21.0 mg m^{-2} d^{-1}
619 corresponding to a Nr dissipation from five to six times higher than that measured *in situ* in this
620 study. Therefore, we can argue that in case of complete overturn, Nr retention efficiency would
621 increase up to ~35% of the external Nr load.

622 In the temperate zone, anoxia and chemically reducing conditions can develop not only in
623 meromictic lakes, but also in the hypolimnion of dimictic eutrophic lakes (Matthews et al., 2008;
624 Nizzoli et al. 2010; Foley et al., 2012). Our results suggest that when the complete turnover
625 becomes less frequent, the denitrification efficiency decreases, especially during the summer
626 stratification period. Eutrophication can accelerate this trend, through organic enrichment and
627 oxygen consumption rates. These impacts are likely to become more severe in the future -
628 exacerbated by the concurrence of eutrophication and climate changes (Foley et al., 2012; Ficker et
629 al. 2017). Numerical thermal lake models (Danis et al. 2004) and direct observations (Salmaso et al.
630 2014; Ficker et al. 2017) suggest that complete turnover could become less frequent with the
631 occurrence of holo-oligomixis and in extreme conditions meromixis. The increased stability of
632 water stratification has been ascribed to altered thermal conditions which are the result of different
633 climate related drivers. Namely the differential increase of water temperature is faster in the surface
634 than deep layers, especially in deep lakes (Rempfer et al. 2010; Kraemer et al., 2015; Ficker et al.

635 2017). Climate change can also cause salinization of many lakes in Mediterranean climate zones
636 (Jeppesen et al. 2015) and hyper-eutrophication of eutrophic lakes with the accumulation and
637 settling of the ungrazed biomass (Moss et al. 2011). Organic matter enrichment coupled with less
638 frequent mixing can induce a positive feedback, since mineralization increases dissolved ions
639 concentration and consequently water density. These processes can amplify the density differences
640 with epilimnic waters, enhancing the stratification stability.

641 In the short term, changes in water circulation along with organic enrichment stimulates
642 denitrification by inducing redox gradients at the water-sediment interface and fueling denitrifiers
643 with an electron source (Seitzinger et al. 2006; Finlay et al. 2013). However, prolonged water
644 stratification could represent a threat for the Nr removal capacity of lakes and their associated
645 ecosystem services. Therefore, factors that impair water mixing and promote the onset of
646 meromictic conditions would indirectly jeopardize the capacity of lake sediments to denitrify and
647 thereby increase Nr delivery to downstream ecosystems.

648

649

650

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845
846

847 **Figure captions**

848

849 **Figure 1** Location of Lake Idro and land use in its watershed (A). Detailed map of the lake showing
850 the sampling sites for sediment and riverine total nitrogen flux estimation (B)

851

852 **Figure 2** Water column profiles of NH_4^+ and NO_x concentrations (left), dissolved oxygen (O_2) and
853 dissolved sulphides (H_2S) (right) in the water column of Lake Idro. Each data point is the average of
854 four samplings (January, May, August and November 2011); error bars are standard deviations

855

856 **Figure 3** Rates of total denitrification (D_T), and denitrification coupled to nitrification (D_N), nitrate
857 + nitrite (NO_x) and ammonium (NH_4^+) measured in the lake sediment at the four different depths
858 and in the different seasons. Error bars represent standard deviation (n=4).

859

860 **Figure 4** Rates of denitrification measured as a function of NO_x concentration at 120 and 20 m
861 depth (A) and at 3 and 6 m depth (B) in May 2011. The equations of the regression lines are
862 $D_T = 0.39 \pm 0.01 * [NO_x] - 35.4 \pm 31.2$, $R^2 = 0.99$, $p < 0.001$ at 3 m.
863 $D_T = 0.58 \pm 0.16 * [NO_x] - 343 \pm 306$, $R^2 = 0.87$; $p = 0.05$ at 6 m
864 $D_T = 0.013 \pm 0.002 * [NO_x] - 2.3 \pm 4.4$, $R^2 = 0.93$, $p < 0.05$ at 20 m
865 $D_T = 0.023 \pm 0.001 * [NO_x] + 1.5 \pm 10.8$, $R^2 = 0.86$, $p = 0.07$ at 120 m
866 Note the difference in scale of the y axes.

867

868 **Figure 5** Net Anthropogenic Nitrogen Inputs - NANI (t N y^{-1}) and its components in the lake Idro
869 watershed

870

871 **Figure 6** Daily loads of total nitrogen measured at lake inlet (watershed outlet) and lake outlet from
872 June 2010 to May 2012

873

874 **Figure 7** Summary of nitrogen budgets for the entire watershed, the terrestrial part and the lake.
875 Data are reported as total (t N y^{-1}) and areal fluxes (kg km^{-2} y^{-1}). Denitrification is treated as a
876 component of the total lake Nr retention.

877

878 **Figure 8** Relationship between denitrification and net NH_4^+ fluxes across the sediment water
879 interface in the three lake areas
880

881 **Figure 9** Proportion (%) of the annual Nr load retained by Lake Idro compared to the predicted Nr
882 retention of three general models. Equations for these relationships are: $\%N = 23.4 \times T^{0.2}$
883 (Seitzinger et al., 2016), $\%N = 22 \times \log_{10} T + 57$ (Finlay et al., 2013), $\%N = 5.6 \times \ln T + 48.3$
884 (Rissanen et al., 2013), with T=Retention Time
885
886

887 **Table 1.** General characteristics of Lake Idro and its watershed.

888

889

890

891

892 Average watershed elevation (m)	1600
893 Average air temperature (°C)	7
894 Average rainfall (mm y^{-1})	1500
895 Lake area (km^2)	11.03
896 Z max (m)	124
897 Lake volume (km^3)	0.85
898 Monimolimnion volume (%)	47
899 Mean TP (µg L^{-1})	110
900 Mean TN (µg L^{-1})	960
901 Watershed area (km^2)	609
902 Agricultural land (km^2)	51
903 Pastures and meadows (km^2)	106
904 Inhabitants	18140
Livestock units	3630
Aquaculture (Mg y^{-1})	1700

905 **Table 2.** Average density and organic matter (OM) content measured in the 0-5 cm sediment
906 horizon and water temperature (T), oxygen (O_2), NH_4^+ and NO_x concentrations measured in the
907 water above the sediment at the 4 sampling depths.

Sampling depth	**Density**	**OM**	**T**	**O_2**	**NH_4^+**	**NO_x**
m	g ml^{-1}	%	°C	mg L^{-1}	µg L^{-1}	µg L^{-1}
3	1.3 - 1.5	3.3 - 4.7	5 - 23	8.6 - 12.1	0 - 42	336 - 868
6	1.1 - 1.2	6.3 - 7.3	5 - 23	8.6 - 14.1	0 - 14	378 - 840
20	1.0 - 1.1	9.1- 10.2	5 - 8	4.0 - 11.5	0 - 56	714 - 826
120	0.9 - 1.0	6.3 - 8.5	7 - 7	0 - 0	672 - 1064	<50 - 100

908

909

910 **Table 3** Distribution of total denitrification (D_T) and denitrification efficiency in the three

911 bathymetric layers in which the lake was partitioned. Values in parenthesis are standard deviations.

912

Bathymetric layer	**Sediment surface**		**Denitrification**		**Denitrification Efficiency**	**Mass transfer coefficient**
	km^2	%	t N y^{-1}	%	%	m y^{-1}
0-10	1.2	10	42 (10)	51	127 (50)	49 (12)
10-50	2.2	20	30 (3)	36	102 (41)	17 (1)
50-120	8.1	70	11 (2)	13	4 (3)	6 (1)
Whole lake	11.5	100	83 (10)	100	21 (15)	16 (3)

913

914

915 **Table 4**

916

917 Average daily values of denitrification measured in sediments at different depths in freshwater

918 lakes. Values are expressed as mg N m^{-2} d^{-1}. Denitrification rates are measured using IPT during

919 laboratory core incubations (Nizzoli et al. 2010, 2014; Rissanen et al. 2013; Wenk et al. 2014), net

920 N_2 fluxes (Saunders and Kalff 2001; Small et al. 2014) or net N_2 fluxes corrected for N fixation

921 (McCarty et al. 2016). PM = polymictic, MO = monomictic, DM = dimictic; ME = meromictic; OX

922 = oxic; S-AN = seasonally anoxic; P-AN = permanently anoxic, n.a. = not known.

923

Environment	Mix	Ox	Depth	DT	Source
Lake Champlain - Missisquoi bay	PM	OX	1	4.2 - 238	McCarty et al. 2016
Lake Stanga	MO	OX	2	17 - 134	Nizzoli et al. 2014
Lake Memphremagog	DM	n.a.	2	2.8 - 113	Saunders and Kalff 2001
Lake Lehee	PM	OX	3	14 - 17	Rissanen 2013
Lake Idro	ME	OX	4	16 - 211	This study
Missisquoi bay - Lake Champlain	PM	S-AN	4.5	0 - 41	McCarty et al. 2016
Lake Ormajarvi	DM	OX	5	60 - 182	Rissanen 2013
Lake Suolijarvi	DM	OX	10	67	Rissanen 2013
Lake Paajarvi	DM	OX	12	85	Rissanen 2013
Lake Stanga	MO	S-AN	20	9.8 - 57	Nizzoli et al. 2010
Lake Memphremagog	DM	n.a.	20	9.8	Saunders and Kalff 2001
Lake Erie	DM	OX	30	17.2	Small 2014
Lake Superior	DM	OX	32	7.8	Small 2014
Lake Huron	DM	OX	85	6.7	Small 2014
Lake Lugano	MO	S-AN	95	1.4 - 20	Wenk et al. 2014
Lake Idro	ME	P-AN	120	0 - 7.0	This study
Lake Superior	DM	OX	180	6.2	Small 2014

924

925

926