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} day^{-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.

Keywords (separated by '-') Benthic fluxes - Denitrification - Meromixis - NANI - Nitrogen budget - Nitrogen removal

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

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Introduction

Reactive nitrogen (Nr) inputs to watersheds have increased many fold in the last century, exceeding

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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, 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 and 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 (Pina-Ochoa and 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 persistent anoxia hampers microbial nitrification, which requires oxygen. Furthermore, the end-products of anaerobic metabolism such as sulfides inhibit denitrification and foster DNRA (Burgin and Hamilton 2007; Nizzoli et al. 2010; Azzoni et al. 2015). Persistent stratification in Meromictic lakes deeply affects biogeochemical processes, where deep-water anoxia may last over decades, thus altering redox conditions (Lehmann et al. 2015). Furthermore, deep temperate lakes are undergoing less frequent water turnover, alarmingly shifting toward holo-oligomictic conditions, with the possible onset of meromixis due to the climate changes (Salmaso et al. 2014; Jeppesen et al. 2015; Kraemer et al. 2015; Ficker et al. 2017). For these reasons, Meromictic lakes are especially suited for studying Nr pathways and fate. The coupled assessment of their watershed processes could help to

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evaluate and predict the lake ecosystem responses to altered mixing regime and to increasing anthropogenic pressures in the watershed.

This study has two objectives: (1) to investigate benthic Nr processing in a Meromictic lake and (2) to put such Nr processing in the framework of whole basin N-budgets. We hypothesized that meromictic conditions can depress Nr removal, as the occurrence of anoxia may stimulate NH_4^+ recycling and limit denitrification. Specifically, the aims of this work were: (1) to assess the contribution of denitrification to the lake Nr budget, (2) to evaluate and compare denitrification rates in deep and littoral lake sediments, and (3) to integrate the lake Nr budget into a detailed watershed Nr budget.

We considered Lake Idro, a sub-alpine Meromictic lake, as a model system to assess spatial and temporal variability of microbial N-transformations and to elucidate the role of benthic denitrification as a Nr sink. To put the in-lake Nr removal capacity into a context, we also quantified the total Nr inputs delivered to the lake by its tributaries, the Nr export by the lake outflow and the Net Anthropogenic Nitrogen Input (NANI) to the lake watershed. The NANI approach is widely used to quantify the net anthropogenic Nr load to catchments and to evaluate the spatial and temporal variability of Nr retention within the watershed (Han and Allan 2008; Lassaletta et al. 2012; Hong et al. 2012).

Materials and methods

Study site

Lake Idro is located on the southern slopes of the Alps at 368 m.a.s.l. (Table 1; Fig. 1). The watershed has an area of 609 km^2, an average elevation of 1610 m.a.s.l. and is mostly forested (> 70%). Only one fourth of the lake catchment area is exploited for agriculture, mainly by pastures and rough grazing (17.4%), while cropland agriculture (7.4%) is located only in the lowland (Fig. 1; Table 1). Population density is 40 ind km^{-2}, while livestock units (1 LSU = 1 adult dairy cow) account for 6 LSU km^{-2}. Poultry (96,000 ind), rabbits (16,000 ind) and dairy cows (2300 ind) are the main species farmed (ISTAT 2010). Additionally, 7 trout aquaculture activities operate in the catchment with an annual average production of 1700 t.

Table 1 General characteristics of Lake Idro and its watershed

Average watershed elevation (m)	1600
Average air temperature (°C)	7
Average rainfall (mm $year^{-1}$)	1500
Lake area (km^2)	11.03
Z max (m)	124
Lake volume (km^3)	0.85
Monimolimnion volume (%)	47
Mean TP (μg L^{-1})	110
Mean TN (μg L^{-1})	960
Watershed area (km^2)	609
Agricultural land (km^2)	51
Pastures and meadows (km^2)	106
Inhabitants	18,140
Livestock units	3630
Aquaculture (Mg $year^{-1}$)	1700

Since 1930s, Lake Idro has been regulated by a top releasing dam and used for water supply to lowland irrigation and hydroelectric power generation. The Chiese and Caffaro rivers contribute ~45 and ~41% of the total water inflow to the lake (2.1×10^6 m^3 day^{-1}), respectively. The lake has a total volume of 0.85 km^3, a maximum depth of 124 m and is considered meromictic (Garibaldi et al. 1996). The average residence time of water is about 1 year (Garibaldi et al. 1996). A steep chemocline is present between 40 and 50 m depth, and the monimolimnion accounts for ~50% of the total water volume. The littoral zone (< 10 m depth) accounts for less than ~10% of the total lake surface; the shore is gently sloping on the northern and southern sides, and rather steep on the western and eastern sides. The lake is meso-eutrophic with whole lake total P and N concentration averages of 110 and 960 μg L^{-1}, respectively. In the last 40 years, eutrophication and hypolimnetic anoxia has been exacerbated. Total P concentrations in surficial waters increased from an average of 9 μg L^{-1} in the early 1970s to 21 μg L^{-1} today. NO_3^- concentrations increased steadily from an average of 196 to 868 μg N L^{-1} in the same period (S1 Supplementary material).

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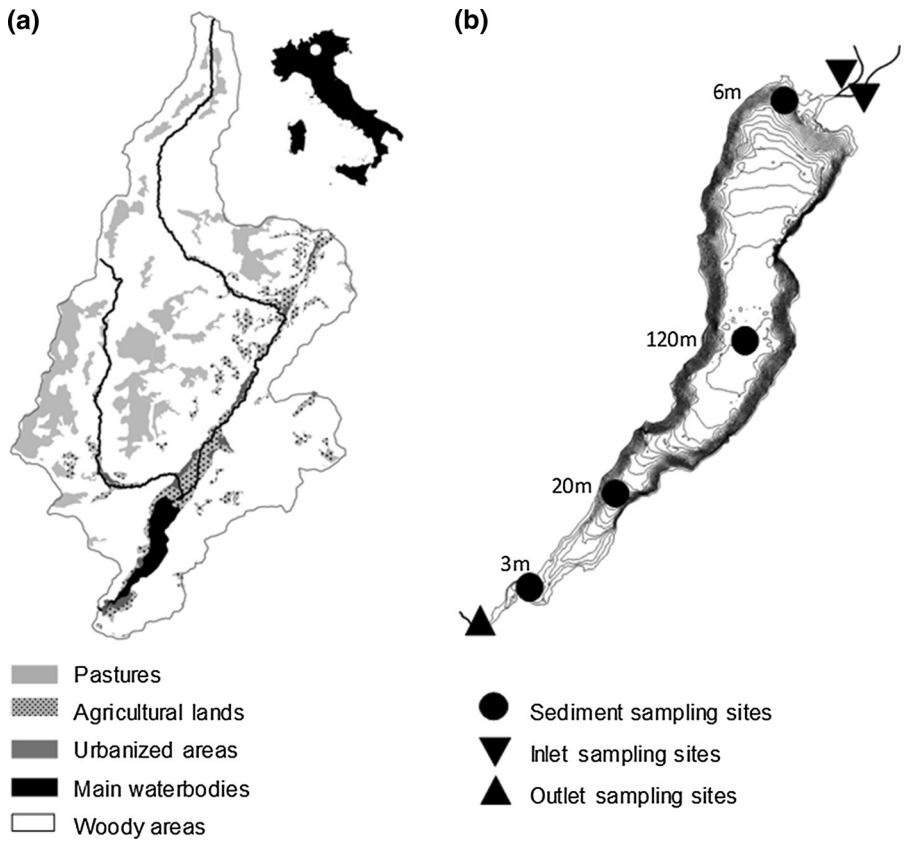

Fig. 1 Location of Lake Idro and land use in its watershed (**a**). Detailed map of the lake showing the sampling sites for sediment and riverine total nitrogen flux estimation (**b**)

Sediment and water features, nutrient fluxes and benthic denitrification

Sediment cores were collected on 24 January, 16 May, 8 August and 21 November 2011 at four sites at 3, 6, 20 and 120 m depth (Fig. 1). On each occasion, 3 replicate sediment cores (30 cm length and 4 cm internal diameter) were collected at each site to analyze sediment characteristics, while 4 replicate sediment cores (30 cm length and 8 cm internal diameter) were collected for denitrification rates and dissolved inorganic nitrogen ($DIN = NH_4^+ + NO_2^- + NO_3^-$) flux measurements. In parallel, temperature, oxygen, dissolved sulphides, NH_4^+ and NO_x ($NO_2^- + NO_3^-$) were measured at each site to provide context for flux measurements. Temperature and oxygen were measured directly in situ with a multi parameter probe (Idronaut Ocean 316) at 1 m depth intervals. Water column samples for DIN and dissolved sulphides were collected with a Ruttner bottle at surface, 2.5, 10, 20, 30, 40, 50, 60, 90, and 120 m depths at the site of maximum depth and 50 cm above the sediment at the other sites. The samples were immediately filtered (0.45 μm) and stored frozen until analysis. Additionally, 25 L of water were collected at each site for core maintenance and incubation.

Immediately after collection all sediment cores were placed in a cool box and returned to the laboratory within 6 h. Cores collected from the maximum depth station, were immediately submerged in anoxic water retrieved at the same depth and closed with a rubber stopper in order to minimize the exposure to atmospheric oxygen. Once in the lab the sediment cores were transferred to separate tanks, submerged in lake water collected at the corresponding sampling site and maintained overnight in the dark in constant temperature rooms at the average temperature measured in the field. Oxic conditions in the tank water collected from the mixolimnion were assured by bubbling air with airstones. Anoxia in the tank water

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collected from the monimolimnion was assured by covering the incubation tank with a plastic bag and bubbling the water with N_2. The water inside cores was gently stirred avoiding sediment resuspension during the pre-incubation and incubation period with magnetic stirrers driven by a large magnet rotated by an external motor at 40 rpm. Measurements began the day after the sampling.

The cores for sediment characterization were processed as follows. The upper 0–5 cm section was sliced and rapidly homogenised; 5 cm^3 of the homogenate were subsampled with a cut-off 10 cm^3 syringe, transferred into pre-weighed aluminium dishes and weighed to determine sediment density. Sediment porosity (cm^3 H_2O cm^{-3} of sediment) was determined as weight loss from wet sediments after 24 h at 70 °C. Sedimentary organic matter (OM) was determined from 0.5 g dry sediments as loss on ignition at 450 °C over 3 h.

Fluxes of dissolved inorganic N across the water–sediment interface were quantified via short-term batch incubations in the dark following the protocol described by Dalsgaard (2000) (see S2 Supplementary material). The water inside the tanks was replaced with new water from the same site prior to initiating the incubation to maintain near in situ dissolved nutrient concentrations. To initiate incubations, the water level in the tank was lowered to below the core tops and the cores sealed with floating Plexiglass lids. At the beginning of the incubation period before closing the cores and at the end, water samples were taken from each core for NH_4^+ and NO_x analyses. Water samples were immediately filtered (0.45 µm) and stored frozen until analysis. The incubation time (from 1 to 3 h, 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 h 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 1 h 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} is the atmospheric Nr deposition, N_{Fert} is the synthetic Nr fertilizer applied to agricultural soils, N_{Fix} is the agricultural N_2 fixation, N_{Trade} is the 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

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catchment scale by weighting each municipality based on the spatial distribution of land use areas in the watershed (Han and 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} - $year^{-1}$ 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 20×20 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} $year^{-1}$ for oxidized and reduced Nr, respectively. We therefore considered an average value of $N_{Dep} = 1900 \pm 760$ mg N m^{-2} - $year^{-1}$, 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 1988; 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 11,800 (meadows) kg N km^{-2} $year^{-1}$, 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 $capita^{-1}$ $year^{-1}$). The latter was calculated assuming a protein consumption of 111 g $capita^{-1}$ - day^{-1} (FAOSTAT 2010), with protein = N × 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

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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 and 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.

Standard deviation for N_{Dep} was directly obtained from the variability of data collected for the Lake Idro watershed (coefficient of variation = 40%). Standard deviations for crop yield, Nr content, and livestock Nr excretion were estimated from the average coefficient of variation reported in Soana et al. (2011) which equal to 26, 25, and 14%, respectively (S5 supplementary material). Standard deviations for animal Nr intake were estimated assuming a coefficient of variation equal to Nr excretion. Standard deviations for the parameters for which information about variability were not available (crop fraction distributed to humans, Nr fraction lost in food and feed processing, human Nr intake and fertilizer Nr application), were estimated using an intermediate coefficient of variation (20%) between 14 and 26%, the range of uncertainty of the agricultural coefficients (S5 supplementary material).

Riverine Nr flux estimations

Chemical characteristics of inflowing and outflowing waters were measured monthly from June 2010 to March 2012. At each sampling date water samples were collected for NH_4^+, NO_x, total dissolved (TDN) and total (TN) nitrogen determinations. Samplings and analyses were performed following standard methods (APHA 1998). The daily Nr loadings to and exports from the lake were computed as the product of average daily flow by concentration of the target chemical species. Annual loadings were calculated as the product of the discharge weighted mean concentration by the mean annual discharge of the 2 years (Quilbé et al. 2006). The Chiese Consortium, the Management Authority of lake Idro, and the Autonomous Province of Trento provided the daily average water inflows and outflows. Discharge of minor tributaries was calculated from direct measurements of water velocity, with a water velocity meter (Scubla mod. 2030), and cross sectional area in each sampling day. Total Nr input to the lake was computed as the sum of the river Nr loadings plus atmospheric Nr deposition (wet and dry deposition of both oxidized and reduced Nr) to the lake surface.

Analytical determinations and calculations

NH_4^+ (Bower and Holm-Hansen 1980) and NO_x (APHA 1998) were determined by spectrophotometry. TN and TDN were determined as NO_x after alkaline peroxydisulfate oxidation (Valderrama 1981).

The abundances of $^{29}N_2$ and $^{30}N_2$ in the dissolved N_2 pool were determined with a gas chromatograph in line with a mass spectrometer at the National Environmental Research Agency, Silkeborg (DK) following Risgaard-Petersen and Rysgaard (1995). In brief the N_2 of each sample was extracted from the water in the Exetainer by introducing a helium headspace and shaking vigorously the vial for 5 min. The entire headspace (4 mL) was then carried through the gas chromatograph columns (Roboprep-G-Plus GC) and to a triple-collector mass spectrometer (Europa Scientifica TracerMass) to obtain the isotopic composition of the N_2 by a flow of helium (99.9995% purity). Prior to reaching the mass spectrometer, water vapour,

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CO_2 and O_2 were removed by passing the sample through a drying tube (10 mm × 200 mm packed with $Mg(ClO_4)_2$), a Carbosorb (10–20 mesh) column and a reduction column (15 mm × 300 mm packed with Cu wires at 650 °C) respectively. The increased abundance of $^{29}N_2$ and $^{30}N_2$ in the samples was obtained by subtracting the natural $^{29}N_2$ and $^{30}N_2$ abundance from the signals.

Fluxes of NH_4^+ and NO_x across the sediment–water interface were calculated from the change of solutes concentration over time (Nizzoli et al. 2014) using the following formula:

$$Fx = (Cf - Ci) \times V / A \times t$$

where Fx is the flux of the x species (mg m^{-2} h^{-1}), Cf is the final concentration of x (mg L^{-1}), Ci is the initial concentration of x (mg L^{-1}), V is the volume of the water column (L), t is the incubation time (hours), A is the 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:

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

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

$$D_W = \left(^{14}NO_3^-/^{15}NO_3^-\right) \times D_{15}$$

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

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 and Ferguson (2009):

$$DE = D_T/(DIN + D_T) \times 100$$

The mass transfer coefficient (m $year^{-1}$) 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 depths ($n_{120} = n_{20} = n_6 = n_3 = 16$) and among sampling dates within each depth ($n_{spring} = n_{summer} = n_{autumn} = n_{winter} = 4$). When differences among depth or among seasons were significant, a posteriori comparison of the means was performed using a post hoc Tukey and Kremer test (Quinn and Keough 2002). Differences between daily Nr loads measured at lake inlet and outlet were compared using the non-parametric Mann–Whitney U test. All statistical analyses were performed using the

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statistical computing software R (R Core Team2014). Descriptive statistics are presented within the text as mean ± standard deviation.

Results

Water and sediment characteristics

Temperature, dissolved oxygen and Nr underwent steep variations along the water column (Fig. 2; Table 2). Water temperatures displayed a typical seasonal trend in the shallower sites, changing from 5 °C in winter to 23 °C in summer, and was nearly constant in the deepest waters. Dissolved oxygen concentrations decreased with depth, ranging from 8.6 to 14.1 mg L^{-1} in the surface layer to anoxia below 40–50 m depth. NO_x concentrations followed a similar pattern, decreasing from 868 µg N L^{-1} in the surface to < 100 µg N L^{-1} from 40 m to the bottom. NH_4^+ concentrations followed an opposite trend, increasing from < 20 µg N L^{-1} in the mixolimnion up to ~1000 µg N L^{-1} in the deepest layers. A concurrent accumulation of dissolved sulphides up to 4.3 mg S L^{-1} also occurred from 60 m to the bottom.

The sediment collected at 3 and 6 m depths was composed of fine sands with a high silt content. The average OM content (5.4 ± 1.6%) in the upper sediment horizon was similar for the two sites. By contrast, sediment collected at 20 and 120 m depth was composed of fine textured mud. At 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, $p < 0.001$; Fig. 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} day^{-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} day^{-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, $p < 0.001$).

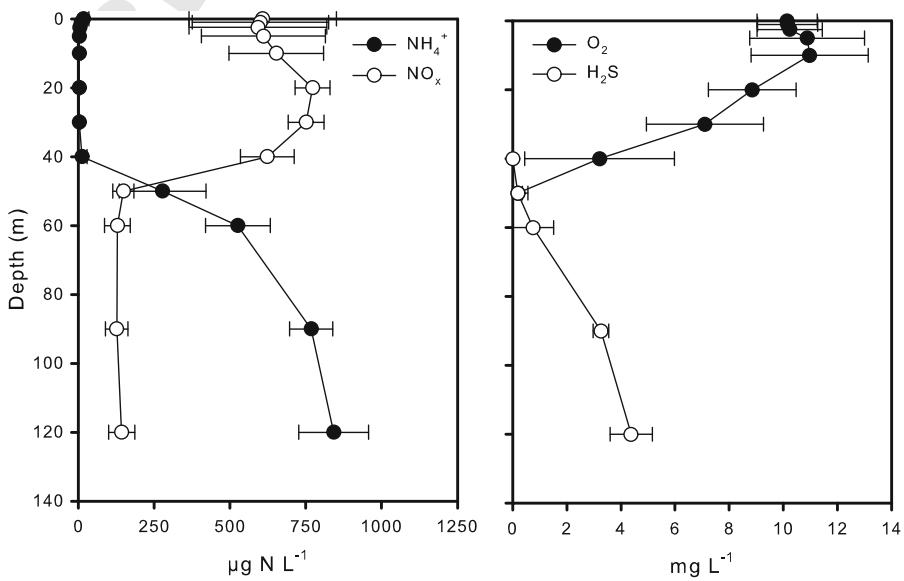

Fig. 2 Water column profiles of NH_4^+ and NO_x concentrations (left), dissolved oxygen (O_2) and dissolved sulphides (H_2S) (right) in the water column of Lake Idro. Each data point is the average of four samplings (January, May, August and November 2011); error bars are standard deviations

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

Sampling depth m	Density g mL^{-1}	OM %	T °C	O_2 mg L^{-1}	NH_4^+ µg L^{-1}	NO_x 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

Nitrification was only a minor source of NO_x for denitrification and D_N rates were not statistically different among the four sites (Fig. 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 (Fig. 4).

Overall, the annual denitrification in the benthic system accounted for a N_2 production of 83 ± 10 t N $year^{-1}$ (7.2 ± 0.4 t N km^{-2} $year^{-1}$). An annual rate of 42 ± 10 t N $year^{-1}$ (69 ± 8.7 t N km^{-2} $year^{-1}$) occurred in the littoral area, while only 11 ± 2 t N $year^{-1}$ (1.3 ± 0.1 t km^{-2} $year^{-1}$) were produced in the monimolimnion sediments (Table 3).

Concurrently, high rates of NO_x consumption were measured in the littoral sediment (− 75.6 ± 77.0 mg m^{-2} day^{-1}) particularly under spring and summer conditions. Much lower NO_x fluxes were detected in the deeper sediments (− 7.0 ± 2.8 mg m^{-2} day^{-1}; Fig. 3b). On average, the benthic system was a source of NH_4^+ to the water column, with significant differences among depths (K–W, $p < 0.01$; Fig. 3c). A clear seasonal pattern was observed in the littoral zone where NH_4^+ release from the sediment peaked in spring and summer (101.1 ± 67.2 mg m^{-2} day^{-1}). At 120 m depth NH_4^+ release was always high (99.4 ± 58.1 mg m^{-2} day^{-1}) with lowest efflux measured in autumn (42.9 ± 4.2 mg m^{-2} day^{-1}) and highest in winter (173.8 ± 23.8 mg m^{-2} day^{-1}). NH_4^+ fluxes were almost undetectable at 20 m depth.

In the littoral benthic system, the net DIN flux was on average slightly into the sediments (− 16.8 ± 48.0 mg m^{-2} day^{-1}) due to the high NO_x consumption, while the monimolimnion sediment was always a net source of DIN (14–224 mg m^{-2} day^{-1}), mainly due to NH_4^+ recycling. Denitrification efficiency was elevated in sediments down to a depth of 50 m depth, while Nr recycling dominated the monimolimnion sediments (Table 3).

Nitrogen fluxes at watershed scale

The estimated NANI was 1785 ± 467 t N $year^{-1}$, corresponding to an areal input of 2929 ± 767 kg N km^{-2} $year^{-1}$ (Fig. 5). Atmospheric Nr deposition accounted for 61% of NANI, equivalent to 1781 ± 763 kg N km^{-2} $year^{-1}$ while the Nr input associated with trade of food and feed was 20% of NANI, equivalent to 574 ± 80 kg N km^{-2} $year^{-1}$. The autotrophic organic Nr production within the catchment (484 ± 85 kg N km^{-2} $year^{-1}$) was not sufficient to meet the Nr needs of the human and livestock population (1057 ± 67 kg N km^{-2} $year^{-1}$); organic Nr was thus imported to the watershed mainly as animal feed (606 ± 78 kg N km^{-2} $year^{-1}$). N_2 fixation associated with crops and pastures (524 ± 112 kg N km^{-2} $year^{-1}$) accounted for an additional 18% Nr input, while fertilizers (51 ± 7 kg N km^{-2} $year^{-1}$) were < 2% of NANI.

TN concentrations measured in the water at the outlet of the main rivers and small streams that 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 detected in minor creeks (data not shown). The average daily TN load was variable, ranging from a minimum of 860 kg N day^{-1} to a maximum of 6021 kg N day^{-1} (Fig. 6). More than 90% of the total Nr load was delivered by the Caffaro and Chiese rivers, mainly as

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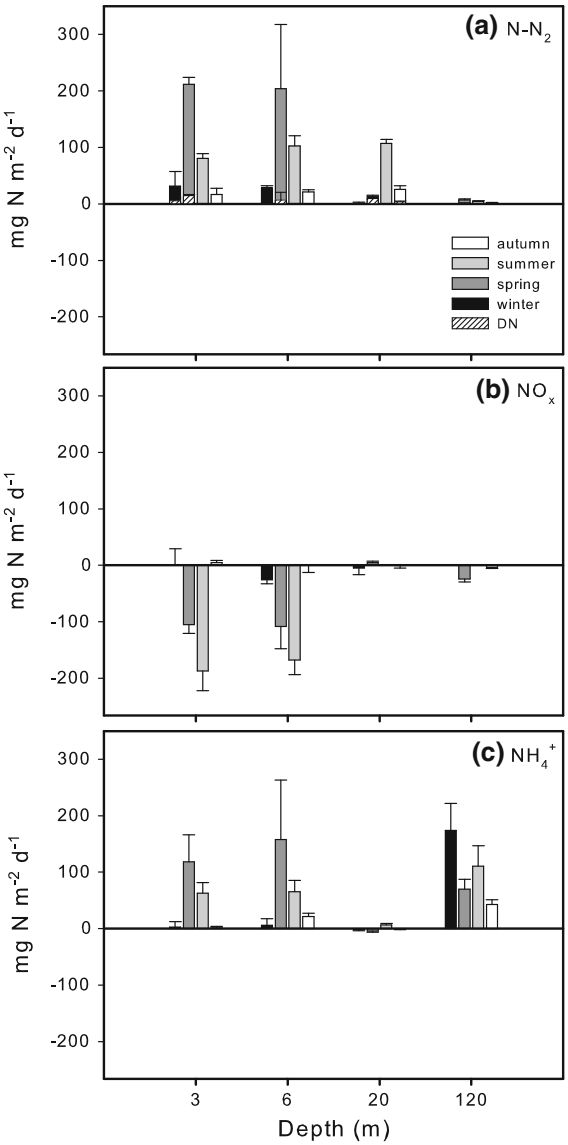

Fig. 3 Rates of total denitrification (D_T), and denitrification coupled to nitrification (D_N), nitrate + nitrite (NO_x) and ammonium (NH_4^+) measured in the lake sediment at the four different depths and in the different seasons. Error bars represent standard deviation (n = 4)

dissolved Nr forms (> 98%). The annual Nr export by rivers to the lake was 846 ± 586 t N year^{-1}, which is equivalent to 47% of NANI.

TN concentration measured in river water at the lake outlet (485–1020 µg N L^{-1}) was lower than in the inflowing waters (Fig. 6). The daily Nr exported from the lake ranged from 622 to 4065 kg N day^{-1} and was significantly lower than the daily input (Wilcoxon, p = 0.013). The annual Nr export from the lake was 598 ± 395 t year^{-1}, equivalent to nearly 70% of the annual inputs. Therefore, the lake either retained or lost nearly 30% of the Nr load (270 ± 706 t N year^{-1}) prior to riverine export.

Discussion

Measurements of benthic Nr fluxes and denitrification rates along with Nr mass balances were combined to evaluate the contribution of benthic denitrification to Nr removal under meromictic conditions. These are understudied but important issues in inland waters, as they can provide new insight on the capacity of lake ecosystems to dissipate the Nr load under infrequent to no mixing frequencies. A synopsis of the budgets and fluxes resulting from the study highlights that in-lake denitrification was not efficient in removing Nr in most of the lake's sediment. Further, the watershed had a low Nr retention capacity resulting in a high percentage of Nr (47%) exported to the lake system from tributary rivers (Fig. 7).

Spatial and temporal variability of benthic Nr processes

Different benthic nitrogen processes dominated in deep and littoral sediments of Lake Idro. Nearshore sediments had higher denitrification rates and denitrification efficiency, compared to the monimolimnion sediments where denitrification was almost negligible. Recycling of NH_4^+ from sediments to the water column dominated in the monimolimnion.

Spatial heterogeneity of denitrification rates was previously observed in dimictic lakes with rates generally higher in the littoral than in the deeper sediments (Saunders and Kalff 2001; Bruesewitz et al. 2012), but with exceptions due to local conditions (Rissanen et al. 2013; Small et al. 2014). The average D_T of 2.8 mg N m^{-2} day^{-1} measured in the Meromictic Lake Idro falls within the lower range of values (1.9–84 mg m^{-2} day^{-1}) detected in other deep sediments (Table 4) suggesting a reduced capacity to dissipate Nr. Water column NO_x, as opposed to nitrification, was by far the dominant source of NO_x for denitrification. The incubations of sediment cores with increasing NO_3^- concentrations revealed that in all the investigated sediments denitrification was

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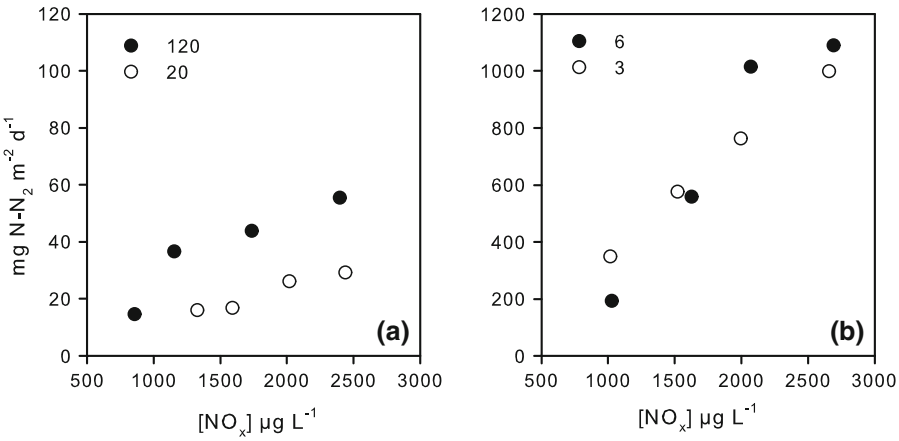

Fig. 4 Rates of denitrification measured as a function of NO_x concentration at 120 and 20 m depth (**a**) and at 3 and 6 m depth (**b**) in May 2011. The equations of the regression lines are: $D_T = 0.39 \pm 0.01 \times [NO_x] - 35.4 \pm 31.2$, $R^2 = 0.99$, $p < 0.001$ at 3 m. $D_T = 0.58 \pm 0.16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

Table 3 Distribution of total denitrification (D_T) and denitrification efficiency in the three bathymetric layers in which the lake was partitioned

Bathymetric layer	Sediment surface		Denitrification		Denitrification efficiency	Mass transfer coefficient
	Km^2	%	T N $year^{-1}$	%	%	M $year^{-1}$
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)

Values in parenthesis are standard deviations

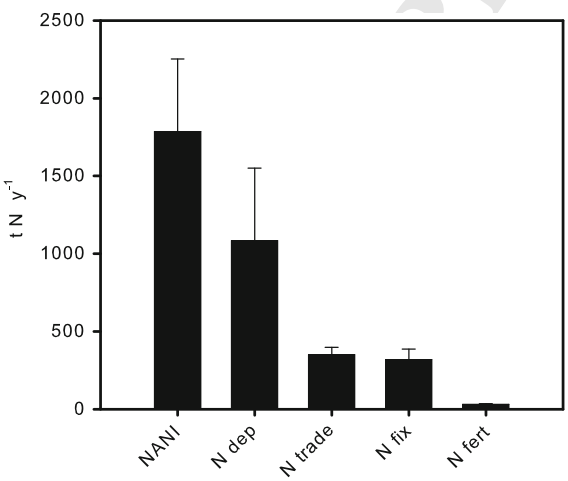

Fig. 5 Net anthropogenic nitrogen inputs—NANI (t N $year^{-1}$) and its components in the lake Idro watershed

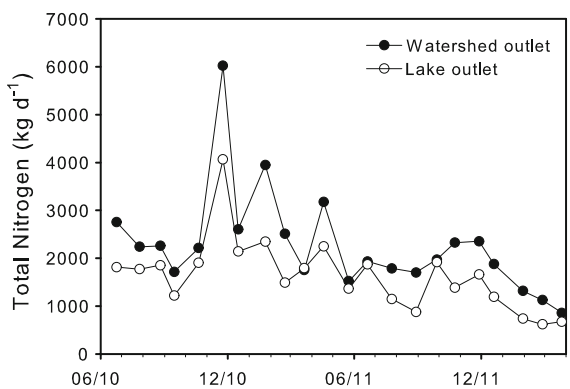

Fig. 6 Daily loads of total nitrogen measured at lake inlet (watershed outlet) and lake outlet from June 2010 to May 2012

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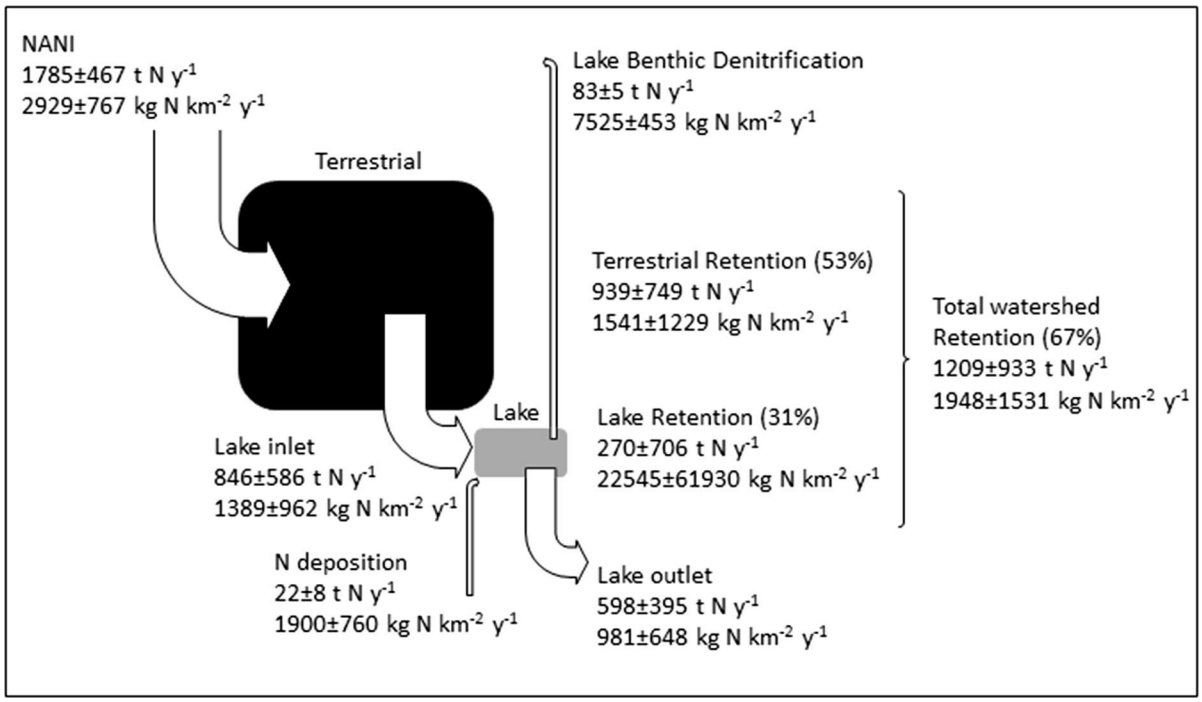

Fig. 7 Summary of nitrogen budgets for the entire watershed, the terrestrial part and the lake. Data are reported as total (t N year^{-1}) and areal fluxes (kg km^{-2} year^{-1}). Denitrification is treated as a component of the total lake Nr retention

NO_3^- limited. Low NO_x concentrations in the water column can prevent denitrification by limiting the diffusion of NO_x into the sediment denitrification zone. Furthermore, anoxia precludes nitrification and NO_x production within the sediment. The comparison of denitrification in littoral and deep sediments also evidenced that NO_x addition was less effective in the latter sediments (Fig. 4). We argue that anoxia and chemically reducing conditions in the monimolimnion can further inhibit denitrification processes. The mass transfer coefficient, which can be regarded as an index of the efficiency of N_2 production relative to NO_x availability (David et al. 2006), was lower in sediments collected from the monimolimnion compared to the other depths (Table 3). Lower values suggest that environmental conditions in the monimolimnion were less suitable to denitrification compared to littoral oxygenated sediments. Denitrification efficiency was less than 5% and anaerobic ammonification was the dominant process of sediment Nr release under these conditions (Fig. 8).

In contrast, D_T rates of 87 mg N m^{-2} day^{-1} measured in the littoral habitat were within the upper range of values (0–238 mg N m^{-2} day^{-1}) measured in other lake ecosystems (Table 4). Here, benthic Nr metabolism followed a typical seasonal pattern with peaks of D_T rates and DIN fluxes in spring and summer. Such high rates likely reflected temperature changes and the consistently high NO_x availability in the water column throughout the year; low D_N indicated that sediment nitrification was a minor source of NO_x. Mineralized Nr was released to the water column as NH_4^+ in littoral sediment due to low nitrification. However, in contrast to the deeper sediments, DIN fluxes across the sediment water interface in the littoral zone were largely driven by microbial NO_x reduction. Here, denitrification of the NO_x bulk diffusing from the water column exceeded the NH_4^+ efflux, and the superficial sediment acted as a net DIN sink (Fig. 8).

Benthic denitrification and in lake Nr processing

We scaled up denitrification rates to the whole lake to provide context for the contribution of benthic denitrification in the different bathymetric layers to the in lake Nr metabolism (Table 3). To our knowledge, there are only few examples of the concurrent use of

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Table 4 Average daily values of denitrification measured in sediments at different depths in freshwater lakes

Environment	Mix	Ox	Depth	DT	Source
Lake Champlain–Missisquoi bay	PM	OX	1	4.2–238	McCarthy 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 et al. (2013)
Lake Idro	ME	OX	4	16–211	This study
Missisquoi bay–Lake Champlain	PM	S-AN	4.5	0–41	McCarthy et al. (2016)
Lake Ormajarvi	DM	OX	5	60–182	Rissanen et al. (2013)
Lake Suolijarvi	DM	OX	10	67	Rissanen et al. (2013)
Lake Paajarvi	DM	OX	12	85	Rissanen et al. (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)

Values are expressed as mg N m^{-2} day^{-1}. Denitrification rates are measured using IPT during laboratory core incubations (Nizzoli et al. 2010, 2014; Rissanen et al. 2013; Wenk et al. 2014), net N_2 fluxes (Saunders and Kalff 2001; Small et al. 2014) or net N_2 fluxes corrected for N fixation (McCarthy et al. 2016)

PM polymictic, *MO* monomictic, *DM* dimictic, *ME* meromictic, *OX* oxic, *S-AN* seasonally anoxic, *P-AN* permanently anoxic, *n.a.* not known

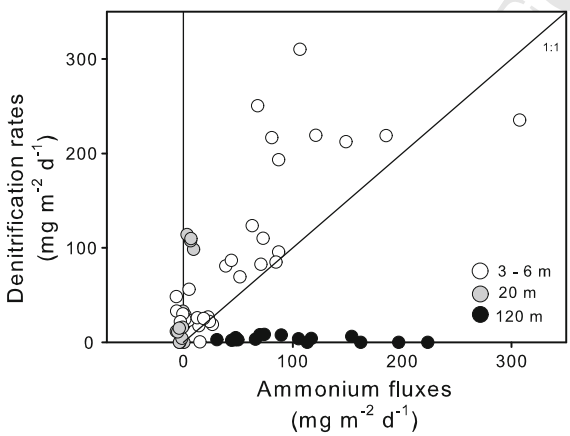

Fig. 8 Relationship between denitrification and net NH_4^+ fluxes across the sediment water interface in the three lake areas

mass balances and direct measurements of in-lake processes to evaluate pathways and fate of the Nr loadings from the watershed (Mengis et al. 1997; David et al. 2006; Bruesewitz et al. 2011; McCarthy et al. 2016). Upscaling from core measurements to the whole lake requires addressing both methodological problems and spatial and temporal variability of rates (Groffman et al. 2006). We addressed both kinds of uncertainty. First, we used a well-established technique for measuring denitrification (Groffman et al. 2006). Second, we sampled sediments in three bathymetric layers, from the shallow littoral zone to the deepest monimolimnion, over four seasons to incorporate both the spatial and temporal variability of fluxes and rates (David et al. 2006; Bruesewitz et al. 2012; Nizzoli et al. 2014).

The contribution of the three different bathymetric zones to the whole benthic N_2 production was not proportional to the respective sediment area, contrary to what was previously observed in Lake Shelbyville, a low land polimictic reservoir (David et al. 2006) or in the dimictic Gull Lake (Bruesewitz et al. 2012). In Lake Idro 50% of benthic denitrification occurred in the littoral area. This area accounted for only 10% of

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the lake surface; whereas the monimolimnion sediments, which extended over 70% of the lake surface, contributed only 13% of D_T (Table 3).

These data demonstrate that shallow aquatic environments characterized by high NO_x loads can sustain high denitrification rates, and thereby the littoral area has an enormous impact relative to its surface in the control of the external NO_x loads. The disproportionate contribution of the littoral area can be found not only in Meromictic lakes but also in eutrophic temperate lakes where water stratification is followed by oxygen and NO_x depletion (Bruesewitz et al. 2011). Under these conditions, the deep benthic system progressively loses the capacity to denitrify (Nizzoli et al. 2010; Bruesewitz et al. 2011), which is preserved instead in the more oxidized littoral sediments (Nizzoli et al. 2014). Yet several anthropic pressures affect littoral zones of lakes (Francis et al. 2007). Among others, human exploitation of water resources and extreme hydrological events affect the magnitude and timing of water-level fluctuations, which in turn may influence benthic metabolism (Hofmann et al. 2008) with possible implications for Nr biogeochemistry.

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

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

In Meromictic lakes, persistent water column stratification prevents the upward flux of accumulated nutrients from the monimolimnion. Indeed, in Lake Idro, the monimolimnion is storing $\sim$300 t of NH_4^+. However, the long-term fate of this Nr is unclear. Partial turnover of the 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 $\sim$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 40–50 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} year^{-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

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

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

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

Nr removal efficiency of lakes is extremely variable and influenced by latitude, water residence time and trophic status (Seitzinger et al. 2006; Rissanen et al. 2013; Finlay et al. 2013). Water residence time in particular is widely used as an explanatory factor. It represents the time scale for processes that influence Nr removal (Nr uptake, settling of particulate Nr and diffusion of NO_x to anoxic denitrification zones) to occur before flushing downstream (Seitzinger et al. 2006). Lake Idro retained 31% of the external Nr load with an efficiency falling in the lower range of values measured in other temperate lakes with similar water residence time or predicted by means of empirical equations from literature. The latter estimates are much greater and are in the range 40-60% (Fig. 9). This low retention efficiency can be explained by the fact that 70% of the benthic system in Lake Idro is affected by anoxia. NO_x depletion and extremely chemically reducing conditions favor Nr recycling rather than denitrification. We added increasing amounts of NO_x to monimolimnic waters above sediments to evaluate denitrification capacity following NO_x supply due to water overturn. At NO_x concentrations between 700 and 840 μg N L^{-1}, equivalent to those measured at 30 m depth, the theoretical denitrification rates fell to the range of 16.8–21.0 mg m^{-2} day^{-1} corresponding to a Nr dissipation from five to six times higher than that measured in situ in this study. Therefore, we can argue that in case of complete overturn, Nr retention efficiency would increase up to ~35% of the external Nr load.

In the temperate zone, anoxia and chemically reducing conditions can develop not only in Meromictic lakes, but also in the hypolimnion of dimictic eutrophic lakes (Matthews et al. 2008; Nizzoli et al. 2010; Foley et al. 2012). Our results suggest that when the complete turnover becomes less frequent, the denitrification efficiency decreases, especially during the summer stratification period. Eutrophication can accelerate this trend, through organic enrichment and

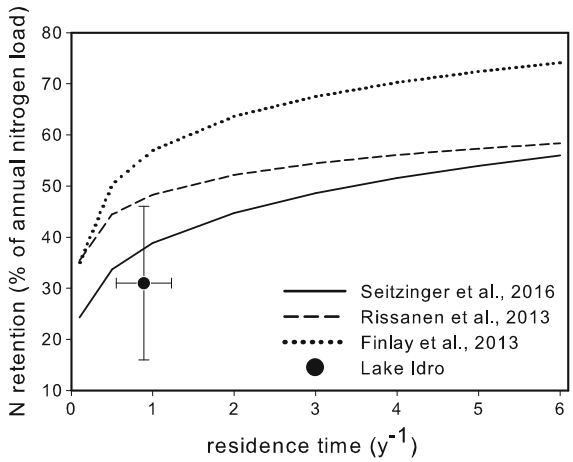

Fig. 9 Proportion (%) of the annual Nr load retained by Lake Idro compared to the predicted Nr retention of three general models. Equations for these relationships are: $\%N = 23.4 \times T^{0.2}$ (Seitzinger et al. 2006), $\%N = 22 \times \log_{10} T + 57$ (Finlay et al. 2013), $\%N = 5.6 \times \ln T + 48.3$ (Rissanen et al. 2013), with *T* retention time

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oxygen consumption rates. These impacts are likely to become more severe in the future—exacerbated by the concurrence of eutrophication and climate changes (Foley et al. 2012; Ficker et al. 2017). Numerical thermal lake models (Danis et al. 2004) and direct observations (Salmaso et al. 2014; Ficker et al. 2017) suggest that complete turnover could become less frequent with the occurrence of holo-oligomixis and in extreme conditions meromixis. The increased stability of water stratification has been ascribed to altered thermal conditions which are the result of different climate related drivers. Namely the differential increase of water temperature is faster in the surface than deep layers, especially in deep lakes (Rempfer et al. 2010; Kraemer et al. 2015; Ficker et al. 2017). Climate change can also cause salinization of many lakes in Mediterranean climate zones (Jeppesen et al. 2015) and hyper-eutrophication of eutrophic lakes with the accumulation and settling of the ungrazed biomass (Moss et al. 2011). Organic matter enrichment coupled with less frequent mixing can induce a positive feedback, since mineralization increases dissolved ions concentration and consequently water density. These processes can amplify the density differences with epilimnic waters, enhancing the stratification stability.

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

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