

Different performances of independent sediment biological proxies in tracking ecological transitions and tipping points of a small sub-alpine lake since the Little Ice Age

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Abstract

A comparative study of independent geochemical and biological proxies was carried out on a short (83 cm) sediment core collected in 2011 from the deepest point of a small subalpine Lake Ledro (Trentino, N-Italy). The aim of the study is to compare the capability of subfossil photosynthetic pigments, diatoms and Cladocera in tracking lake ecological transitions and tipping points related to major environmental perturbations occurred during the last three centuries, i.e. after the culmination of the Little Ice Age in the Alpine region. In relation to the sparse neo-limnological and climate data available for the lake, the study aims also at defining of the lake trophic and ecological reference conditions, at improving the reconstruction of the nutrient enrichment process during the last decades, and at evaluating the effects of restoration measures initiated in the 1990s. The analysis of the selected proxies outlined a pronounced sensitivity of Lake Ledro to hydrological variability throughout the whole time span considered, especially during the 18th and 19th century, and revealed two major stages in the ecological evolution of the lake, which were mainly controlled by climate related hydrological variability and lake nutrients. The results largely agree with the hypothesis that responses of sediment biological proxies to different natural and human stressors may differ in type, timing and magnitude. Subfossil pigments, diatoms and Cladocera showed a comparable capability in tracking ecological transitions and tipping points related to lake hydrology and nutrient variability, while only diatoms demonstrated a certain capability to track changes in water temperature of the lake studied. The strong response of planktonic organisms to hydrological variability depends on the peculiar catchment and lake morphology, and confirmed that planktonic organism principally respond to climate variability in an indirect way. The reconstruction of the trophic development of Lake Ledro during the last decades revealed that the vulnerability of the lake toward climate and land use driven hydrological variability is congenital for the lake, though at present it is masked by nutrients. This stresses the necessity to maintain and improve the control of nutrient inputs also in reoligotrophicated subalpine lakes, in relation to the present context of human use and climate change, and paying particular attention to the lake-specific sensitivity to local forcings.

Keywords: Paleoecology, Multi-proxy, Diatoms, Cladocera, Subfossil pigments, Ecological transitions, Lake Ledro

Introduction

Sediment studies based on multiple geochemical and biological proxies are considered as a very powerful tool, which is able to strengthen paleoecological reconstructions of past environmental and ecological lake conditions (Birks & Birks, 2006). The study of different subfossil remains reflecting different aspect of the lacustrine ecosystem is particularly important in conditions of scarce historical (e.g. on land use, climate conditions) and limnological information, as it can provide a cross validation of environmental reconstructions through the “weight of evidence approach” (Michelutti & Smol, 2013). However, multi-proxy sediment studies remain less frequent than studies based on a combination of geochemical proxies and one singular biological proxy (Bennion et al., 2015), especially because the taxonomical analysis of subfossil biological remains requires high specialization and is highly time consuming. Moreover, paleolimnological investigations conducted during the last two decades of the 20th centuries were often aimed at reconstructing one environmental variable, especially lake water pH and phosphorus concentrations (Smol, 2008), and at identifying lake reference (i.e. the pre-human impact) conditions in respect to that variable, as required by the European Water Framework Directive (WFD, Bennion & Battarbee, 2007). The flourishing of such paleolimnological reconstructions was tied to the acidification processes experienced by lakes in several regions of the northern Europe and to the anthropogenic eutrophication of the majority of temperate lakes in the northern hemisphere (Smol, 2008).

Past lake pH, nutrients (especially total

phosphorus, TP) and water salinity have traditionally been reconstructed by analyses based on vegetal remains, especially diatoms (Battarbee et al., 2001), while paleolimnological reconstruction of past lake temperature and, more in general, climate conditions, relied on remains of aquatic animals, such as Chironomidae and Cladocera (Lotter et al., 1997; Guilizzoni et al., 2006). Nevertheless, recent studies provided increasing evidence of the intrinsic limits of considering aquatic organisms as preferentially responding to a singular driving factor, even though for applicative purposes. Even the concept of species optima in respect to environmental gradients derived from calibration data sets has been questioned, as they are not optima in an ecological sense but merely weighted average abundances of species occurring along a certain environmental gradient (Juggins, 2013). Moreover, organisms can maintain high growth rates even above the optima, posed that the combination of other key resources remains favorable (Saros & Anderson, 2015).

Though nutrient enrichment still represents the major human threat for the majority of temperate lakes (Smol, 2008), the recovery of several lakes after the launching of restoration measures, contributed to shift the attention of paleolimnologists toward a more ecological approach aimed at understanding the mechanisms controlling the whole-lake evolution over centuries (Bennion et al., 2015). This has been stimulated also by the growing importance of climate variability as a key driving factor (George, 2010; Dokulil, 2014), and by the evidence that climate can interact with other forcing factors (such as nutrients, hydrology, land use) in complex ways producing non-linear, synergistic and hierarchical interactions, which in their turn induce complex lake

ecological responses (Leavitt et al., 2009). Lake responses which mimic a renewed stage of nutrient enrichment are often observed in relation to lake warming (Jeppesen et al., 2005; Tolotti et al., 2012), in relation to the fact that climate and weather variability ultimately affect lake nutrient availability through changes in thermal dynamics (Rühland et al., 2015). The understanding of complex climate-lake interaction is crucial for lake managements, in particular to forecast future lake conditions and services within a context of climate change (Salmaso et al., 2014; Perga et al., 2015). Multi-proxy paleolimnological approaches are particularly useful within this context, as they can contribute to disentangle lake responses to nutrient and climate variability at secular scale, and to discriminate and quantify drivers controlling different lake ecological stages. In addition, independent sediment proxies can contribute at recognizing and interpreting timing and magnitude of ecological transitions, tipping points, and regime shifts (Hobbs et al., 2012; Capon et al., 2015; Wang et al., 2015).

The present work consists of a comparative study of independent geochemical and biological proxies in the sediments of Lake Ledro, a small subalpine lake in N-Italy, and aims at comparing the capability of subfossil photosynthetic pigments, diatoms, and Cladocera in tracking ecological transitions and tipping points related to major environmental perturbations. Though its socio-economic importance at regional level for summer and cultural tourism, Lake Ledro is subjected to multiple natural and human stressors. Its trophic evolution during the last decades is coherent with that of the majority of temperate European Lake and includes a stage of nutrient enrichment between the 1960s and

the 1980s followed by a partial recovery related to lake restoration measures started in the 1980s (Casellato, 1990; Boscaini et al., 2012). Meteorological data are being regularly recorded at Lake Ledro only since 2002, but climate data recorded during the last two centuries over the 5 km distant northern part of Lake Garda show an increment in annual average air temperature in the second half of the 20th century, in particular since the 1970s (Milan et al., 2015; Salmaso et al., 2015).

As the responses of different organisms to major environmental stressors are expected to differ in time and intensity, our hypothesis is that algal sediment proxies should effectively track the nutrient enrichment process since the 1960s, and show an indirect and less defined response to climate change, as recently outlined in the close Lake Garda by Milan et al. (2015). On the other hand, we expect subfossil Cladocera to show a delayed response to lake nutrient enrichment, and to have a good capability to track changes in lake water temperature, especially during periods characterized by low lake nutrient level (Milan et al., 2015; Milan et al., submitted).

Due to its small catchment and the steep altitudinal gradients (Simonneau et al., 2013), Lake Ledro is quite sensitive to hydrological variability and has been affected in the past centuries by frequent floods, which have been recently quantified and demonstrated to be related to both climate conditions and land use (Vannière et al., 2013). As flood events are registered by the lake sediments, the present sediment study provides the possibility to investigate the effects of extreme climate events on lacustrine ecology in the subalpine region. In particular, we expect prompt response and rapid recovery capacity by phytoplankton, and a

smoother, slower and longer lasting response by zooplankters.

Since 1929 Lake Ledro is connected by a penstock to a power plant on the shores of Lake Garda, from which water may be again pumped to Lake Ledro. The limnological investigations started after the 1960s and outlined a certain lake warming after the hydrological connection of the two lakes (Tonolli, 1956; Barbato, 1977), but they did not manage to resolve the debate regarding the possible ecological consequences of the lake regulation on physical, chemical and biological features of Lake Ledro. Therefore, the last purpose of the present study is to look for evidences of possible effects stored in the lake pelagic sediments.

Study site

Lake Ledro (45° 52' N, 10° 45' E, 652 m a.s.l.) is a small lake (area = 3.7 km², Z_{max} = 49 m, Table 1) of glacial origin located in the Italian Alps, close to Lake Garda (Fig. 1). The large catchment area (111 km²) includes mountains culminating at 2254 m a.s.l.. The bedrock is composed of Triassic dolomite and Jurassic and Cretaceous limestones, while alluvial deposits of calcareous and siliceous composition fill the bottom of the tributary valleys. Two temporary tributaries feed the lake: the Massangla and the Pur rivers (Fig. 1). The combination between torrential

regime of the tributaries, steepness of their valleys, and the high ratio between catchment and lake area (~30) make Lake Ledro sensitive to flood events. Recent sedimentary studies (Vanni re et al., 2013) outlined that the lake experienced several stages of enhanced flood frequency during the Holocene, the most recent one extending for the second half of the Little Ice Age.

Table 1 Geo-morphological features of Lake Ledro and its catchment.

Latitude	45° 52' 29" N
Longitude	10° 45' 04" E
Lake altitude	652 m a.s.l. (max. storage level)
Max. catch. altitude	2256 m a.s.l.
Lake Area	3.7 km ²
Lake Volume	0.08 km ³ (max. storage level)
Catchment area	97 km ²
Geology	Dolomite, limestone, morainic and alluvial mixed deposits
Lake origin	Glacial, moraine damming
Z _{max}	49 m
Z _{mean}	~35 m
Retention time	< 1 year
Lake inlets	Massangla, Pur, pipe from Lake Garda
Lake outlets	Ponale (natural, inactive), underwater pipe to Lake Garda

No major event was recorded after the last one in AD 1920, possibly in relation to climate change, land use, and protective interventions on the main lake tributaries (Simonneau et al., 2013). The lake outlet, River Ponale, originally flowed into nearby Lake Garda (65 m a.s.l.), while it is inactive since AD 1929 due to the lake hydroelectric exploitation (Table 2). Lake water enters an underwater pipe located at 25 m depth, is forced through a pumped-storage power plant

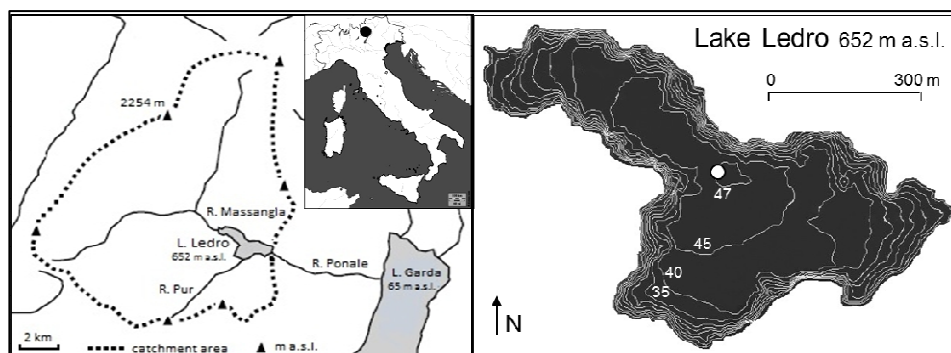


Fig. 1 Left panel: location of Lake Ledro (black circle) in Trentino, Northern Italy, and its catchment area, modified from Magny et al. (2009). Right panel: lake bathymetry and coring location (white circle), modified from Boscaini et al. (2012).

Table 2 Major environmental and socio-economical events which affected Lake Ledro and its catchment during the study period (1689±9 - 2011).

Period	Event
2009	Massive cyanobacteria bloom (<i>P. rubescens</i>) from autumn 2009 to spring 2010.
Since 1999	Water quality monitoring by the Environmental Agency of Trento
1996-1998	Limnological surveys by E. Mach Foundation (FEM)
1993	Additional underwater pipe (5 m depth) exporting epilimnetic waters to Lake Garda
1987-2000	Decrease of epilimnetic total Phosphorus (TP) level from ~100 µg L ⁻¹ down to 10
1984	First sewage treatment plant on the lake shore
1960-1970	Lake deterioration: nutrient enrichment, phytoplankton (incl. cyanobacteria) blooms, anoxic hypolimnion
1960s	Development of tourism activities
1949	Surface water of Lake Garda pumped overnight in the metalimnion of Lake Ledro
1929	Hydroelectric exploitation: underwater pipe (25 m depth) exporting lake waters to lake Garda
1919	Rapid reconstruction of villages and economic activities
1015-1918	World War I. Ledro Valley almost abandoned
19 th century	Economic depression, famine, epidemics, Italian Independence War, strong emigration
1717-1780	Habsburg rule, economic (agriculture, cattle, handcraft) and demographic development
17 th century	Socio-economic depression, famine, plagues, French invasion

built on the River Ponale, and finally reaches a power plant on the shore of Lake Garda. Water is pumped from Lake Garda back to Lake Ledro since 1949 (Tonolli, 1956).

The modern climate conditions at Lake Ledro are sub-continental, with average minimum and maximum air temperatures around 0°C and 20°C, respectively, and average annual precipitation around 900 mm (Vannièrè et al., 2013). The vegetation around the lake is dominated by *Fagus* and *Abies*, while the forest is dominated by *Picea* between 650 – 1600m a.s.l.. Above 1600 m, alpine grassland replaces forest (Simonneau et al, 2013).

The present-day alluvial plains of the Massangla and Pur rivers correspond to the maximal extension of Lake Ledro after the glacier retreat and, together with the lake shorelines, have been associated with agriculture and human settlements since at least the Bronze Age. During the last ~three centuries, Lake Ledro experienced alternating stages of demographical and economic depression and development, in relation to major historical events (Table 2). After the prosperous 18th century, when agriculture

developed around the lake and pastoral and handcraft activities developed in the higher ranges of the lake catchment, population strongly decreased during the 19th century in relation famines, wars and emigration. During the WWI the whole Ledro Valley was almost uninhabited, but the villages were rapidly reconstructed since 1919. Resident population and tourism activities rapidly developed at Lake Ledro since the early 1960s, also due to the vicinity to Lake Garda, and at present recreational activities represent an important fraction of the local economy (Autonomous Province of Trento, unpubl. data).

Lake Ledro experienced a stage of pronounced anthropogenic eutrophication during the 1970s and the 1980s, with TP average concentrations up to 150 µg L⁻¹ (Casellato, 1990). Lake waters became anoxic up to the metalimnetic layers, while several algal blooms were observed. The lake quality deterioration lead to the activation of a first treatment plant in 1984 and to a rapid decrease of lake TP level since the early 1990s (Autonomous Province of Trento, unpubl. data). The lake is currently in mesotrophic conditions, according to TP

concentrations during the spring mixing around $14 \mu\text{g L}^{-1}$, while the prolonged summer thermal stratification produces a separation of the strongly nutrient depleted epilimnion to the anoxic and nutrient rich hypolimnion (Boscaini et al., 2012). Epilimnetic TP concentration can decrease down to oligotrophic level, and can be further reduced in summer by calcite precipitation (Boscaini et al., 2012). Nitrate ranges from 400 to $1000 \mu\text{g L}^{-1}$, while epilimnetic silica can reach levels potentially limiting for the diatom growth (i.e. 0.5 mg L^{-1}) in late summer. Summer phytoplankton of Lake Ledro is currently dominated by the cyanobacterium *Planktothrix rubescens* Anagnostidis & Komárek, which produces dense metalimnetic populations during the thermal stratification, and can often produce autumnal blooms, as observed in 2009. The presence of *P. rubescens* was first recorded in the late 1960s, but its earlier presence has been hypothesized as observed in several subalpine lakes (D'Alelio et al., 2010). In absence of regular monitoring even during the nutrient enrichment of Lake Ledro in the 1970s, historical data on lake nutrients and phytoplankton abundance and species composition are quite fragmented. Since 2009, four seasonal surveys are being carried out yearly by the Provincial Environmental Agency according to the EU WFD.

Material and methods

Sediment coring and dating

Several short cores were retrieved with a gravity Kajak corer (UWITEC, Austria) from the deepest point of the lake ($45^\circ 52' 44'' \text{ N}$, $10^\circ 45' 10'' \text{ E}$, Fig. 1) in December 2011. This lake region resulted as unaffected by the recent earthquake-related sediment slumps

revealed by recent studies (Vanni re et al., 2013). The 83 cm long master core was extruded vertically and sliced at 0.5 cm intervals from the surface down to 30 cm depth, and at 1 cm intervals from 31 cm down to the core bottom. A parallel core was opened longitudinally in order to examine sediment macroscopic aspect and texture (Fig. 1S, Supplementary Material).

Sediment samples were analyzed for ^{210}Pb , ^{226}Ra , ^{137}Cs and ^{241}Am by direct gamma assay in the Environmental Radiometric Facility at University College London, using an ORTEC HPGe GWL series well-type coaxial low background intrinsic germanium detector. Further details on the analyses are available in Thies et al. (2012). Chronological data were calculated using the constant rate of supply (CRS) dating model (Appleby, 2001).

Three vegetal remains found in the master core at 15, 38 and 82 cm depth could be dated on ^{14}C at the Poznan Radiocarbon Laboratory, Poznan, Poland (<http://www.radiocarbon.pl>), using the age calibration curve r:5, atmospheric data from Reimer et al. (2009), and the software OxCal 4.1.5 (Bronk Ramsey, 2010).

Geochemistry and subfossil pigments

Wet density (WD), water content (H_2O , measured from dry weight, DW) and total organic content (measured as LOI, loss on ignition, at 550°C) were determined for all subsamples. Photosynthetic pigments were determined from every sediment layer. Ca. 0.5 g wet sediment subsamples were extracted in 90% acetone, overnight in the dark, under nitrogen. Sediment was removed by centrifugation (3000 rpm, 10 min) and the extract obtained was used to quantify chlorophyll and their derivatives (Chlorophyll

Derivatives Units, CD) and total carotenoids (TC) by double beam spectrophotometer (SAFAS, UVmc2), and specific carotenoid by Reversed Phase High-Performance Liquid Chromatography using a Thermo Separation HPLC (Ultimate 3000). Details on the HPLC equipment employed and calculations are available in Guilizzoni et al. (2011). Analysis of replicates sediment samples yielded a C.V. of 4.5%- 11.5%, depending on pigments.

Subfossil diatoms and Cladocera

Weighted subsamples (0.7-1.0 g FW) from every core layer were treated with H₂O₂ and HCl according to standard procedures (Battarbee et al., 2001), in order to clean subfossil diatom frustules. The cleaned suspensions were added with 3.5 ml of a calibrated solution of di-vinyl-benzene microspheres (mean diameter = ~8 µm, concentration = 8.02 10⁷ ml⁻¹), in order to estimate the Diatom Accumulation Rate (DAR, Battarbee et al., 2001), permanently mounted in Naphrax® resin (refraction index = 1.7) and counted at 1000 magnification under a light microscope (Leica DM2500, Wetzlar, Germany) equipped with Nomarski differential interference contrast. At least 500 valves were counted for each mount, with the exception of a small set of samples with extremely low diatom concentrations, where six transects were counted. Diatom identification to the lowest possible taxonomic level was based on standard literature (Krammer & Lange-Bertalot, 1986-2004) integrated with recent literature on singular genera (Houk et al., 2010; 2014; Lange-Bertalot & Ulrich, 2014).

Cladocera remains were analyzed every fourth sample along the master core following the methods described by Frey (1986) and Szeroczyńska & Sarmaja-

Korjonen (2007), which include the treatment of about 2 cm³ of wet sediment with KOH (10%) and HCl (10%). Subsamples of 0.1 ml of the cleaned and safranin-stained residue were examined at 100-400x magnification under a light microscope (LEICA DM2500). All Cladocera remains (headshield, shell, postabdomen, postabdominal claws, mandible, caudal furca) were counted, and arranged into number of individuals following Frey (1986). Taxonomical identification was based on Flössner (2000), Margaritora (1983) and Szeroczyńska & Sarmaja-Korjonen (2007). Three to six slides were counted for each samples, in order to obtain a minimum of 100 Cladocera individuals (Kurek et al. 2010). This minimum was not achieved in few samples with extremely scarce Cladocera remains. Further details on Cladocera preparation and counts are available in Milan et al. (submitted).

Environmental variables and data analyses

Homogenized monthly average air temperature and precipitation data for the period 1870-2008 were obtained from the HISTALP webpage (2013) for the station Torbole-Riva del Garda, which is the closest to Lake Ledro (5 km). Data from the weather station Bezzecca (~2.5 km from the lake centre) for the period 2002-2011 were provided by GIS Unit of the Edmund Mach Foundation. Annual, seasonal, and April-September (vegetative period) average air temperature and precipitation were considered in this work (Fig. 2S, Supplementary Material). Historical information on lake nutrients and plankton were collected from scientific literature (Casellato, 1990; Boscaini et al., 2012) and the Environmental Agency of the Autonomous Province of Trento (unpubl. data).

Homogenous pigment, diatom and Cladocera zones along the master core were identified using either the CONISS or the optimal partitioning method as implemented in ZONE (Lotter & Juggins, 1991). The number of significant zones was established through comparison with the broken stick model (Bennett, 1996).

Diversity of subfossil diatom and Cladocera assemblages were determined on the binary logarithm-based Shannon Index (Shannon & Weaver, 1949). The cumulative abundances of benthic and major planktonic diatom groups was computed, as well as the diatom ecological preferences for water phosphorus availability according to Van Dam et al. (1994) and to Houk et al. (2010) for the genus *Cyclotella sensu lato*. Cladocera taxa identified in the core studied were correspondently divided in planktonic and littoral groups (Sarmaja-Korjonen, 2001). *Bosmina longirostris* (O.F. Müller) was excluded from both groups, due to the capacity to live in open water as well as in littoral zone (Szeroczyńska, 1998). Species ecological preferences were defined as in Korhola (1990), Frey (1986), Margaritora (1983).

Concentrations of spectrophotometrically determined total carotenoids were used for the reconstruction of past lake pH (Car-pH) and total phosphorus (Car-TP), according to Guilizzoni et al. (2011). Lake TP levels were reconstructed also based on subfossil diatoms. After comparing several model regressions calibrated against different European training sets, weighted-average regressions (WA) with inverse deshrinking and downweighting of species tolerance calibrated against the European Combined (Eu-Comb, Battarbee et al., 2001) and the NW-European (NW-Eu, Bennion et al., 1996) datasets, respectively, were selected on the

basis of: i) the better agreement of diatom-inferred and monitoring lake TP concentrations, ii) higher regression coefficients with lower apparent and prediction errors, and iii) higher cumulative relative abundances of subfossil diatoms represented in the modern training set samples. Diatom abundances $\geq 0.5\%$ in each sample were used for the DI-TP reconstruction, which was computed using the transfer functions implemented in the software C2 version 1.7.2 (Juggins, 2007). Some recently re-named or re-stated taxa were merged prior to reconstruction (e.g. *Cyclotella comensis* Grunow and *Cyclotella pseudocomensis* Scheffler, *Cyclotella costei* Druart & Straub and *C. delicatula* Hustedt), while the correspondence between currently accepted and older nomenclature was accurately checked for the needle-like *Fragilariaceae*.

Major secular changes of pigments, diatoms and Cladocera assemblages were identified by analyzing the sample scores obtained from non-metric multidimensional scaling (NMDS, Kruskal & Wish, 1978) applied to Bray & Curtis dissimilarity matrices of the three proxies considered separately. Pigment and Cladocera data were double rooted, while diatom relative abundances were not transformed. The computation was performed with R 3.2.2. (R Core Team, 2014), *vegan* package version 2.3-0 (Oksanen et al. 2015). The NMDS solution providing the lowest 'stress', which is measure of the configuration stability (Legendre & Legendre, 1998), was selected after 50 trials. A scree plot analysis was performed to identify the final number of NMDS dimensions to be considered (Legendre & Legendre, loc. cit.). Vector fitting analysis was applied to the sample scores of each NMDS configurations in order

to identify the major environmental drivers for the three biological proxies considered, as this analysis correlates the gradients in species composition to the selected variables. Independent sediment variables were used for each proxy considered in order to exploit the whole sediment data set available. The analyses were computed using R 3.2.3 (R Core Team 2014) and variables with a significant correlation ($p < 0.01$) were considered in this work. The temporal variations of pigments, diatoms and Cladocera sample scores along the first two NMDS dimensions were compared with long term climatic variables and milestone environmental events during the time span covered by the present study. In order to attribute ages and sedimentation rates to each subsample within the ^{210}Pb dated core section, a LOWESS interpolation of the non-contiguous radioisotopic ages was performed using R 3.2.3 (R Core Team, 2014).

Correlation between sediment and environmental variables was tested by Spearman rank correlation after application of a Kolmogorov-Smirnov normality test.

Results

Core chronology

Within the top 17.5 cm unsupported ^{210}Pb activity (obtained by subtracting the ^{226}Ra activities from the total ^{210}Pb one) declined almost exponentially with small departures, while a sharp dip was observed between 18 and 21 cm (Fig. 3S, Supplementary Material). ^{210}Pb equilibrium was reached in the core studied at 28 cm depth. The depth profile of ^{137}Cs activity showed a major well resolved peak at 21.25 (coincident with a peak in ^{241}Am), and a minor one at 10.25 cm, which were attributed to the fallout from atmospheric testing of nuclear weapons in

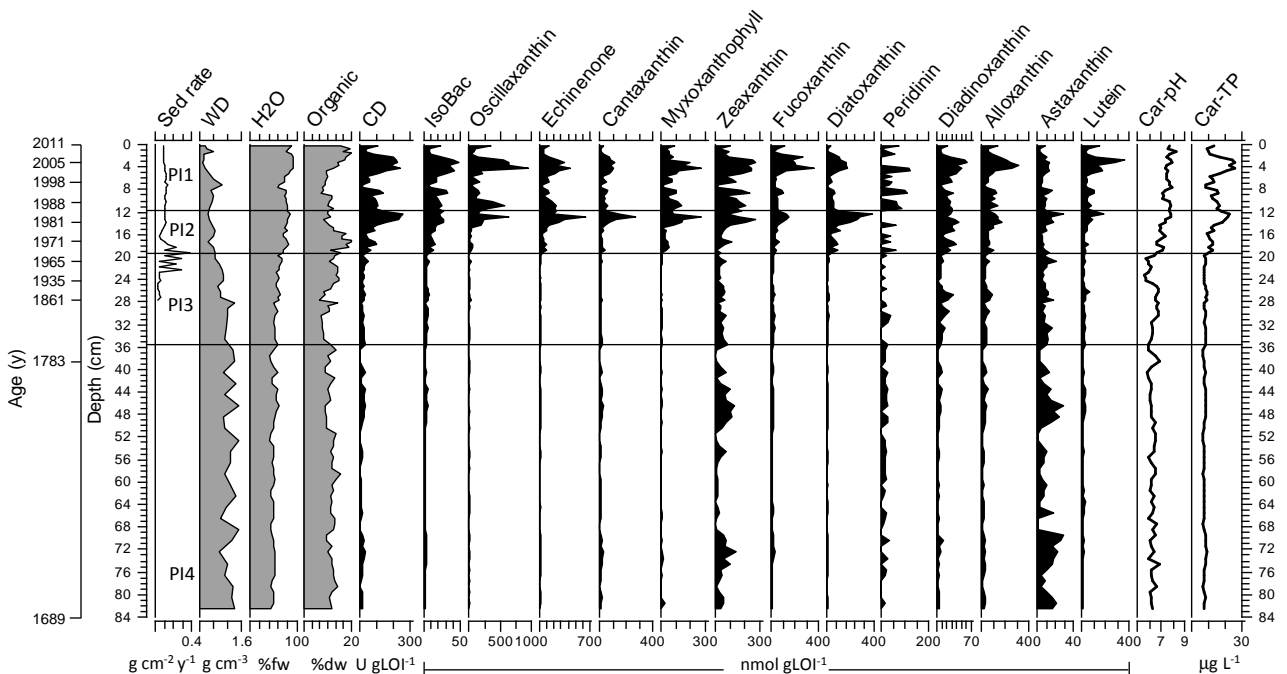


Fig. 2 Depth profiles of geochemical proxies and subfossil pigment concentrations in the master core collected from Lake Ledro in 2011. PI1-PI4 = homogeneous pigment zones, Sed. rate = sedimentation rate, H₂O = water content, WD = wet density, OM = organic content, CD = chlorophyll derivates, IsoBac = γ -carotene (often associated to bacteria), Car-pH and Car-TP = past lake water pH and TP concentrations as inferred from the ratio of spectrophotometric absorbances at 430 and 410 nm, and from total carotenoid concentrations, respectively.

1963 and from the Chernobyl accident in 1986, respectively. The CRS model based core chronology was calculated by using the ^{137}Cs peak at 21.25 as reference level for 1963. Sediment accumulation rates (Fig. 2) showed a gradual increase during the last century and a sharp peak in the middle 1960s which may be derived from a sediment slumping or the “century” flood event which interested the north and central Italy in November 1966 (Malguzzi et al., 2006).

The vegetal remains found in the master core at 15, 38 and 82 cm provided radiocarbon ages of 35 ± 6 , 229 ± 21 and 322 ± 9 years (corresponding respectively to AD 1976 ± 6 , 1782 ± 21 , and 1689 ± 9). The radiocarbon age of sample at 15 cm fully agreed with its ^{210}Pb age (AD 1976 ± 3). However, no depth-age model could be estimated for the entire master core due to numerous interruptions of the regular sedimentation during the last ~3 centuries, which appeared as differently thick brown to grey layers often rich in fine vegetal remains, and were particularly frequent in the core section below 30 cm depth. The major events at 40, 51, 59, 68 and 78 cm depth (Fig. 1S, Supplementary Material), were validated by comparison with the stratigraphy of the longitudinally sliced parallel core and interpreted as flood events through the comparison with the structure of a core collected at 46 m depth in 2008 (Simonneau et al., 2013). Several events related to minor floods (e.g. 1966) or to particularly rainy years (e.g. 1996, 2002, 2007) were detected also in the top 30 cm and could be validated by changes in geochemical and biological proxies (see below).

Geochemistry and subfossil pigments

Wet density of the core investigated oscillated between 1.3 and 1.6 g cm^{-3} from the bottom up to ~28 cm (i.e. AD 1860 ± 36), with highest values occurring in concomitance with the major flood events (Fig. 2). The upper core section showed a gradually decreasing trend, which was also interrupted by a few peaks, as in the late 1990s and 2000s. Water content oscillated around ~50% FW in the deepest 55 cm thick core section, while values steadily increased thereafter, reaching values >80% in the upper 5 cm (Fig. 2). Numerous drops in water content values were evident throughout the core, each one corresponding to enhanced wet densities (Fig. 2). Organic content ranged between 8 and 14% DW in the section up to 28 cm depth, where it started an irregularly increasing stages with maximum values around 18 and 3 cm, which were deposited in the early 1970s and in the second half of the 2000s, respectively (Fig. 2). Wet density, water and organic content were strongly correlated to each other ($-0.91 < r < 0.8$, $p < 0.001$).

The ratio 430:410 (not shown in Fig. 2) oscillated around 0.8 in the core section below ~25 cm, decreased down to 0.7 between 25 and 20 cm, which were deposited between the early 1930s and the middle 1960s, and gradually increased up to 1 close during the last ~5 decades. Concentrations of the principal pigments followed a similar pattern, showing very low values in the deeper core section and a clear increase since the 1960s (Fig. 2). Only the depth profiles of zeaxanthin (marker for cyanobacteria in general) and astaxanthin (marker for N-limited cyanobacteria and aquatic invertebrates, including zooplankton) showed relatively high concentrations also in the deeper core section, and were responsible of the identification of the pigment zone PI1. Cyanobacterial carotenoids, in particular

oscillaxanthin (the specific marker for *Planktothrix rubescens*), echinenone, and canthaxanthin (a marker for colonial taxa) reached concentrations up to 1000 nmol g LOI⁻¹ in the upper 20 cm section, while dinoflagellates carotenoids (i.e peridinin and diadinoxanthin) did not exceed ~130 nmol g LOI⁻¹ even in the most recent core section. The slight increase of these last pigments, and of alloxanthin (Cryptophyceae) between the beginning of the 19th century and the 1960s (Fig. 2) was responsible for the identification of zone PI2. Both the zones PI3 and PI4 were characterized by increased concentrations in cyanobacterial carotenoids, but PI4, which was deposited after AD 1985, showed

enhanced concentration in markers for eucariotic algae (i.e. flagellates and green algae, Fig. 2). Also isorenieratene, a carotenoid commonly associated to photosynthetic sulphurbacteria, increased after the middle 1960s (Fig. 2).

Subfossil diatoms

The temporal development of the diatom concentrations per units of DW (Fig. 3) reflected the pattern of sediment wet density and water content (Fig. 2). In the core section between 83 and 24 cm depth concentration oscillated between 1 and ~5 10⁸ gDW⁻¹, with minimum values concomitant with the major

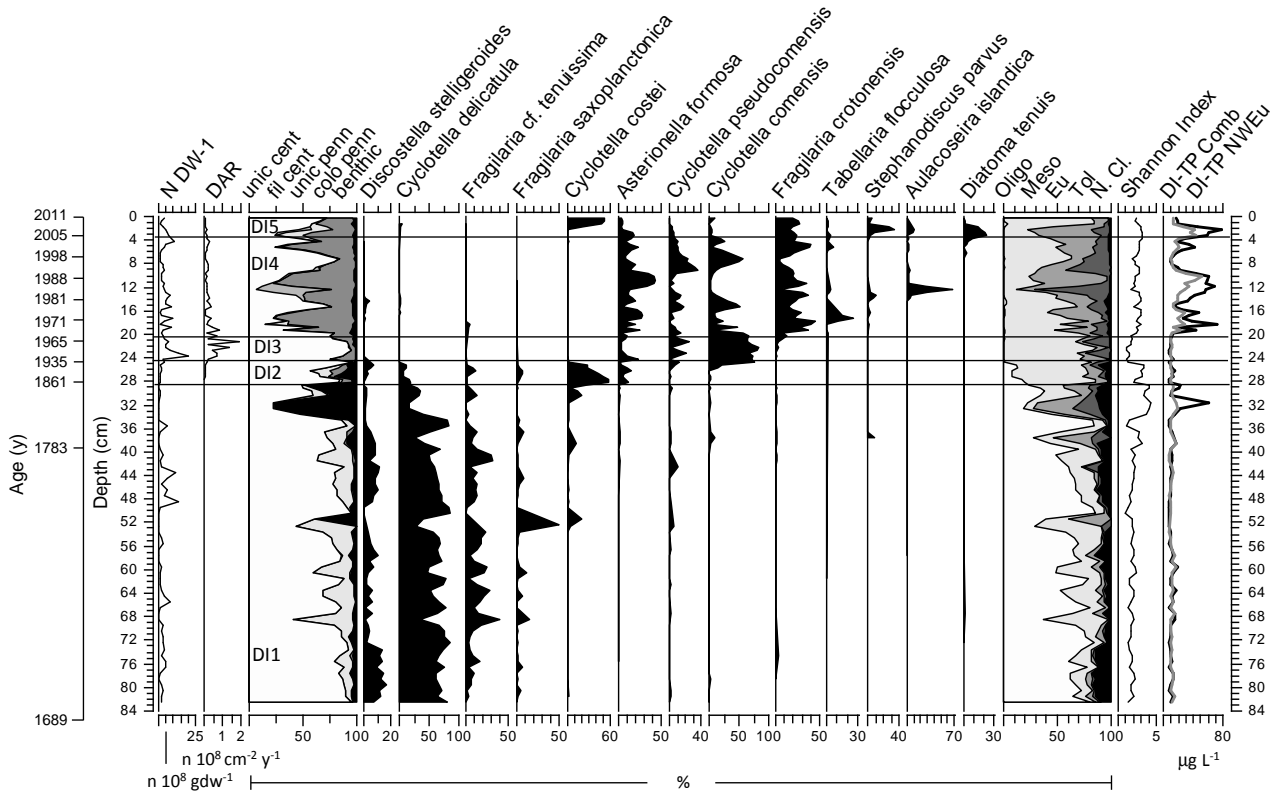


Fig. 3 Depth profiles of diatom concentration, key species, ecological classification, Shannon index and inferred TP concentrations in the master core collected from Lake Ledro in 2011. DI1-DI5 = homogeneous pigment zones, N DW-1 = number of valves per unit of dry matter DAR = diatom accumulation rate, unic cent = unicellular centrics; fil cent = filamentous centrics, unio penn = unicellular pennates, colo penn = colony forming pennates, Oligo = oligotraphentic, Meso = meso to meso-eutraphentic, Eu = eu- to hypertraphentic, Tol = tolerant, N. Cl. = not classified. DI-TP = diatom based lake TP concentrations obtained respectively from the NW-European (NW-Eu, Bennion et al., 1996) and Combined (Comb-eu, Battarbee et al., 2001) calibration sets by applying a weighted average model with inverse deshinking and downweighting of species tolerance. Range standard error of TP reconstruction = 1.2-2.0 µg L⁻¹ for the NW-Eu and 1.2-2.2 µg L⁻¹ for the Comb-Eu models, respectively.

flood events. Almost no diatoms were found at 37.5 and 51-53 cm, which showed increased sediment density and organic content (Fig. 2). Absolute maximum concentrations were recorded between 43 and 49 cm depth (up to $13.4 \cdot 10^8 \text{ gDW}^{-1}$) and at 24 cm ($20.4 \cdot 10^8 \text{ gDW}^{-1}$, Fig. 3). The DAR temporal pattern, referring to the radiometrically dated core section, was highly comparable to the profile of diatom concentration, though the maximum values were recorded some years later, i.e. in the early 1960s (Fig. 3).

Subfossil diatom assemblages were strongly dominated by a few planktonic taxa throughout the core, which accounted to an average relative abundance of 93%. Only between 33 and 25 cm depth, benthic taxa rapidly increased up to 77% (at 31.5 cm) and then decreased more gradually to baseline abundances (Fig. 3). Benthic taxa were represented by numerous (136) but sporadic taxa occurring both in running waters and lake littorals, while around 30 cm they were mainly represented by several small species of the genera *Fragilaria* Lyngbie and *Amphora* Ehrenberg.

Unicellular centric taxa were absolutely dominant in the core section below 20 cm depth, which could be divided into three zones (DI1, DI2 and DI3 in Fig. 3) according to the succession of three species. The alkaliphilic, oligotrophic and cold stenothermic *Cyclotella delicatula* was eudominant in the zone DI1 (82,5-29 cm), where it was accompanied by *Discostella selligeroides* (Hustedt) Houk & Klee, *C. praetermissa* Lund, and by the unicellular planktonic *Fragilaria* cf. *tenuissima* L.-Bertalot & Ulrich (assimilated to *F. tenera* sensu Hustedt in this work) and *F. saxoplanctonica* L.-Bertalot & Ulrich (ex *F. nanana* L.-Bertalot). After the dominance of *C. costei* and the rapid increase

in *Asterionella formosa* Hassall between the middle 19th century and the 1930s (Zone DI2), a rapid increase in *C. comensis* identified zone DI3, which extended up to the layer deposited during the middle 1960s (~20 cm, Fig. 3).

The upper 20 cm thick core section showed an alternative dominance of unicellular centrics and colony forming pennates, while unicellular planktonic *Fragilariaceae* completely disappeared. Zone DI4 was characterized by maximum abundances of *A. formosa*, *Fragilaria crotonensis* Kitton and *C. pseudocomensis*, and by brief and very sharp peaks of *Tabellaria flocculosa* (Roth) Kützing and *Aulacoseira islandica* (Müller) Simonsen. The top zone DI5, which was deposited after the middle 2000s (Fig. 3) showed a decrease of all previously abundant taxa, a rapid succession of *Diatoma tenuis* Agardh, *A. islandica*, *Stephanodiscus parvus* Stoermer & Håkansson, and a recent increase in *C. costei*, which reached again abundances up to 50% since AD 2009.

The trophic classification of the identified taxa (Fig. 3) indicated a dominance of oligotrophic taxa in zone DI1, and increasing proportions of mesotrophic and later eutrophic taxa in zones DI2 to DI4. Zone DI5 showed a clear recovery in mesotrophic species. The Shannon Index depth profile showed a slow increase from the core bottom upward (Fig. 3). Values did not exceed 3 in the deeper 40 cm depth and in the top 20 cm thick section, which were characterized by pronounced dominance by a few planktonic taxa, while values up to 4 were recorded during the benthic stage (20-40 cm).

Subfossil Cladocera

Subfossil Cladocera assemblages of the Ledro core were composed of 33 taxa (6 planktonic and 27 littoral), belonging to the families *Leptodoridae*, *Daphnidae*, *Bosminidae*, *Chydoridae*, *Cercopagidae*. Three major zones were identified (Fig. 4). LC1 (82.5-36.5 cm depth) was mainly characterized by the presence of *Bosmina longirostris* (O.F. Müller). *Bosmina (E.) longispina* Leydig and few individuals of Chydoridae were also found in this zone. Despite the high total Cladocera abundance (Tot Clado) in this zone, the layer at 38.5 cm depth recorded the lowest Cladocera values of the entire core. Only scarce individuals of *B. (E.) longispina*, *B. longirostris*, *Acroperus harpae* (Baird) and *Alona affinis* (Leydig) were identified in this core section. The diversity of Cladocera assemblages, based on Shannon Index, appeared very low in LC1, in relation to the dominance of *B. longirostris* in this zone. LC2 (36.5-29.25 cm, Fig. 4) was

characterized by rapidly increasing littoral species, especially those connected with high water turbidity, such as *Alona rectangula* Sars and *Chydorus sphaericus* (O.F. Müller), or those associated with abundant detritus, like *Disparalona rostrata* (Koch). High densities of *A. harpae* and *A. affinis* were also found in this zone. *B. longirostris* and *B. (E.) longispina* were still present, but with low density in comparison to LC1. This zone showed also the highest diversity (Shannon Index = 3) in Cladocera assemblages.

Zone LC3 appeared to be divided in two subzones in relation to increasing planktonic species density and diversity (Fig. 4) LC3a (29.25-19.25 cm) presented a rapid decrease in total Cladocera abundance, while *B. longirostris* occurred with only sporadic individuals. After the 1930s *Bosmina (E.) coregoni* Baird appeared for the first time, while *Daphnia longispina* O.F. Müller increased gradually. The Shannon Index

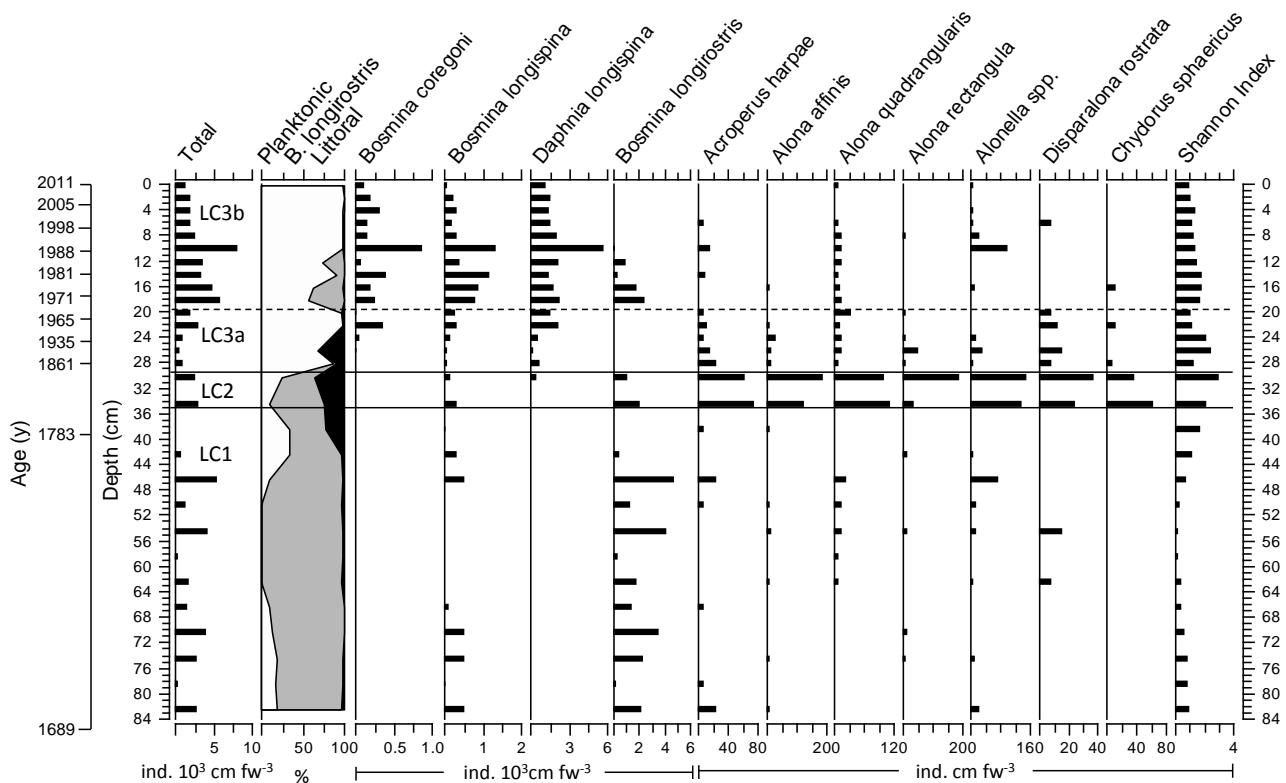


Fig. 4 Depth profiles of subfossil Cladocera total abundance, key species, ecological classification, and Shannon Index in the master core collected from Lake Ledro in 2011. LC1-LC3b = homogeneous Cladocera zones.

	Combined-European		NE-European	
Transfer function	WAT inv. deshrinking		WAT inv. deshrinking	
N. of taxa used	67		69	
Total R.A. used (%)	97 (90-100)		95 (88-100)	
Average S.E. of reconstruction ($\mu\text{g L}^{-1}$)	1.3 (1.2-2.0)		1.5 (1.2-2.2)	
	Apparent	Boot-strapped	Apparent	Boot-strapped
R ²	0.76	0.69	0.81	0.72
RMSE	0.27	0.32	0.26	0.29
Average bias	0.00	0.01	0.00	0.01
Max bias	0.63	0.73	0.65	0.77

Table 3. Statistical performance of the transfer functions used for the diatom based reconstruction of past TP concentration in Lake Ledro as provided by the Combined European (Battarbee et al., 2001) and the NW European (NW-Eu, Bennion et al., 1996) calibration sets. WAT = weighted average with downweighting of species tolerance; R.A. = diatom relative abundance, S.E. = standard error, apparent = errors of estimation, Boot-strapped = error of prediction, RMSE = root mean squared error.

decreased in this zone in association to the density increase in planktonic species. LC3b (19.25-0.0 cm) showed the sporadic presence of littoral species in some samples, while planktonic species definitively became dominant. The rapid increase of planktonic species started in the early 1960s (19.25 cm) and culminated during the 1980s. In the same period total cladocera abundance reached its maximum value. *B. longirostris* was present with only a few individuals and completely disappeared in the top core layers. Since the beginning of the subzone LC3b, the Cladocera diversity decreased gradually down to values around 1. Changes in total Cladocera abundance showed the same pattern of astaxanthin concentration (Fig. 2).

Environmental reconstruction

The past lake pH inferred from the 430:410 ratio (Car-pH, Fig. 2) ranged between 6 and 7 from the core bottom up to 25 cm depth, i.e. till the beginning of the 20th century. After a stage of values around 6 or even lower lasting till the middle 1960s, pH values steadily increased and remained at values around 8 from the middle 1980s to present day (Fig. 2). Carotenoid inferred lake TP concentrations (Car-TP, Fig. 2) oscillated around $8 \mu\text{g L}^{-1}$ from the core bottom up to ~20 cm depth, which was deposited in the middle 1960s. Since then values showed two rapid increases

up to mesotrophic level ($>25 \mu\text{g L}^{-1}$), first in the 1970-1980s and second from the late 1990s to the first half of the 2000s. These two stages were separated by a sharp drop down to base line TP values in the 1990s (Fig. 2). Top Car-TP concentrations between 10 and $15 \mu\text{g L}^{-1}$ agreed with the present mesotrophic status of Lake Ledro.

The NW and the Combined European (NW-Eu and Comb- Eu, respectively in Fig. 3) training sets were selected for reconstructing past TP concentrations of Lake Ledro, as they provided the best estimation of recent average lake TP concentrations during the winter mixing ($14 \mu\text{g L}^{-1}$ in winter 2011, Boscaini et al., 2012) and of the peak TP concentrations within the euphotic zone during the 1980s (up to $100 \mu\text{g L}^{-1}$ in December 1987, Casellato, 1990). Statistical performance of the two transfer functions are reported in Table 3. TP profiles obtained with both the training sets showed values ranging around $10 \mu\text{g L}^{-1}$ from the core bottom up to 20 cm depth, i.e. till the late 1960s. Increased DI-TP concentrations were recorded only around 30 cm depth (middle 19th century) in relation to the dominance of several eutrophic and tolerand benthic *Fragilaria* and *Amphora* species. Though both diatom inferred TP profiles indicated maximum concentration in the second half of the 1970s, around 1988, and again in the second half of the 2000s (Fig. 3), only the values obtained through the NW-

Eu calibration set approach the highest limnological values.

Ecological transitions and environmental drivers

The scree plots indicated two dimensional NMDSs as sufficient to describe the diversity of subfossil pigment, diatom and Cladocera data at secular scale. Sample scores on the two NMDS dimensions (DIM1 and DIM1)

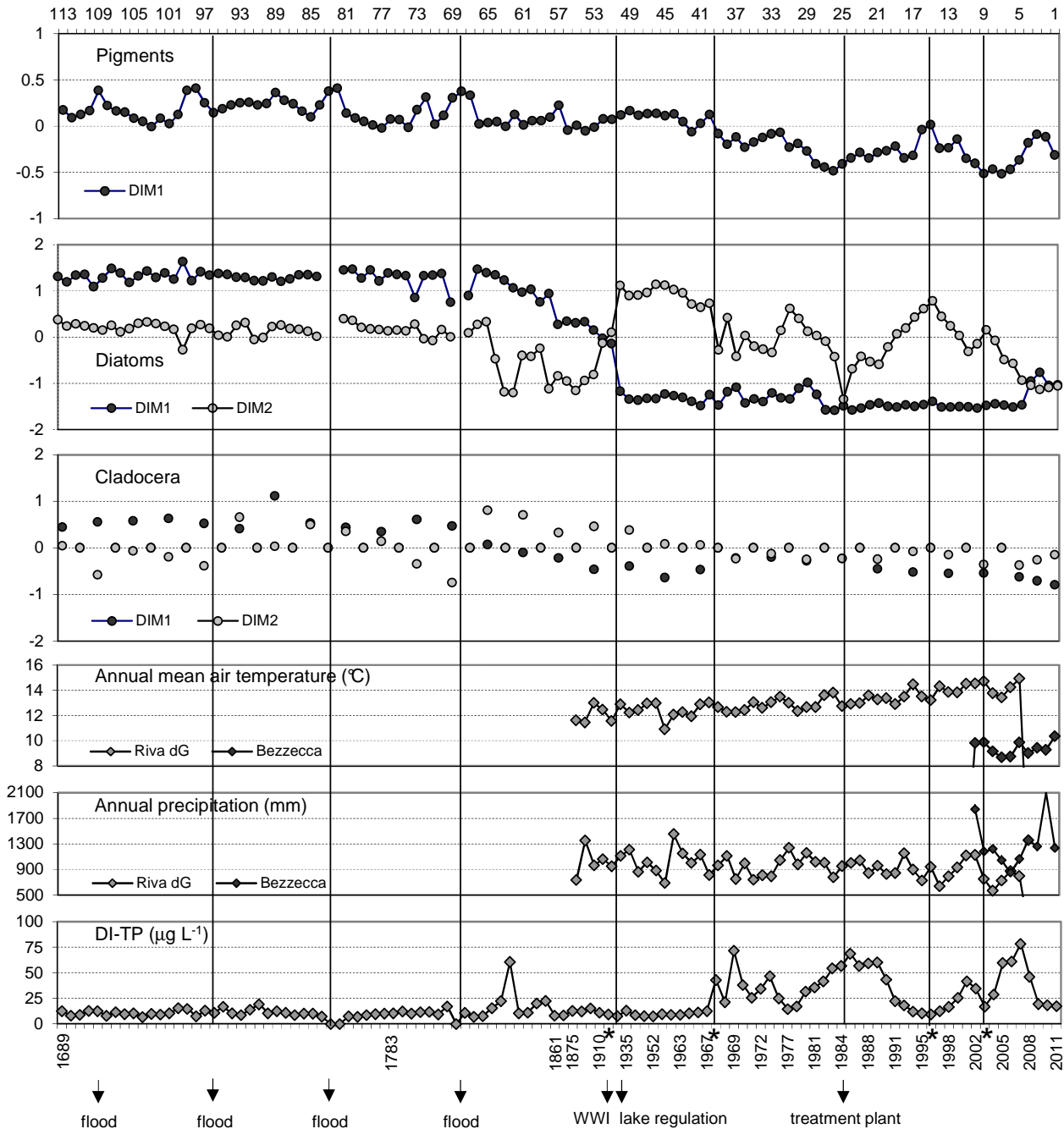


Fig. 5 Samples scores on the two dimensions (DIM1 and DIM2) of the NMDS ordinations based on subfossil pigments, diatoms and Cladocera plotted against air temperature and atmospheric precipitation recorded at the station of Riva del Garda since 1870 and at the station of Bezzecca since 2002. DI-TP = diatom inferred lake TP concentrations. WWI = World War I, Stars indicate the floods event of 1920 and 1966, the rainy 1996 and the hot 2003. Vertical lines mark major changes in NMDS samples scores.

obtained by the analyses of the three biological proxies are represented in Fig. 5. Scores of pigment samples on DIM1 oscillated between 0 and 1 from the late 17th century till the middle 19th century, when they started a slow decrease down to minimum values in the middle 1980s. Score variability was very scarce from the 1920s till the middle 1960s. The distribution of peaks values agreed with the major floods events recorded in the deeper core section, and with the lake TP variations during the last three decades (Tab. 4). Pigment sample scores on DIM2 (data not shown in Fig. 5) oscillated around zero and showed very scarce variability during the whole time span considered.

Diatom samples scores on both the two NMDS dimensions showed very scarce variability from the late 17th till the early 19th century (Fig. 5), when they started to rapidly decrease. The most drastic changes occurred

Table 4. Spearman correlation coefficients between samples score on the two dimensions (DIM1 and DIM2) of the NMDS ordinations relative to subfossil pigments (PI), diatoms (DI) and Cladocera (CL) and independent sediment proxies (n = 113) and climatic variables (n = 50) recorded at the station Riva del Garda (HISTALP webpage, 2013), respectively. WD = wet density, PI-pH = pigment inferred water pH, DI-TP = diatom inferred lake total phosphorus concentration, Ann. Air Temp. = Annual average air temperature, Air Temp MM and AS = mean air temperature for the periods March-May and April-September, Ann. Precipitation = cumulative annual precipitation, Precip. JA = precipitation in the period June-August. ** = p<0.001, * = p<0.1, n.s. = not significant.

	PI-D1	DI-D1	DI-D2	CL-D1	CL-D2
DI-D1	0.69**	---	n.s.	0.79**	n.s.
CL-D1	0.66**	0.79**	n.s.	---	n.s.
WD	0.83**	0.75**	0.24*	0.72**	0.35**
PI-pH	-0.65**	-0.62**	-0.50**	-0.62**	-0.30
DI-TP	-0.59**	-0.59**	-0.36*	-0.59**	-0.35**
Ann. Air Temp	-0.62**	-0.66**	n.s.	n.s.	-0.62**
Air Temp. MM	-0.47**	-0.46**	n.s.	-0.40*	-0.64**
Air Temp. AS	-0.52**	-0.66**	n.s.	-0.44**	-0.54**
Ann. Precip.	n.s.	n.s.	n.s.	n.s.	n.s.
Precip. JA	n.s.	0.35	n.s.	n.s.	n.s.

in the early 1930s, but since then only scores on DIM2 maintained an evident variability, which agreed with lake TP oscillations after the late 1960s. Before the early 19th century variability of Cladocera sample score on DIM2 was larger than on DIM1, despite the evident change around sample 89 in correspondence with changing pigment and diatoms scores (Fig. 5). The most pronounced variation was observed around sample 68, which was deposited between the late 18th and the early 19th century, and agreed with evident changes in the pigment scores and the most pronounced diatom gap (Fig. 5). Since the middle 19th century Cladocera samples scores on both dimensions showed only smoothed changes.

The samples scores of the three biological proxies on DIM1 were positively correlated to each other ($0.66 < r < 0.79$, $p < 0.001$, Tab. 4). They positively correlated with sediment density (and organic content), while showed negative correlation with average annual, spring, and April-September air temperature measured at Riva del Garda, and with the inferred lake pH and TP values (Tab. 4). Scores on DIM2 showed comparable correlation to pH and TP values, while only Cladocera scores were correlated with long term annual temperature. No significant correlation with annual or seasonal precipitations was found.

The vector fitting applied to the three bi-dimensional NMDS ordinations confirmed the strong correlation between the three biological proxies investigated and sediment inorganic and organic content, and inferred pH and TP values (Tab. 5). The analyses outlined that the variability of each proxy was tightly related to changes in key taxa of the remaining two organism groups (Tab. 5) As an example the variability of both pigments and diatoms was positively related with

changes in *B. coregonii*, *B. longimanus* and *D. longispina*, while all the most abundant key diatom species were related to both pigment and Cladocera changes (Tab. 5).

Table 5. Level of significance of environmental and ecological variables (n = 113) used in the NMDS vector fitting relative to the subfossil pigments (PI), diatoms (DI) and Cladocera (CL).

	PI-NMDS	DI-NMDS	CL-NMDS
WD	0.001	0.001	0.001
PI-pH	0.001	0.001	0.001
DI-TP	0.001	0.001	0.001
Total carotenoids	---	0.001	0.001
<i>C. comensis</i>	n.s.	---	0.001
<i>C. delicatula</i>	0.001	---	0.001
<i>D. pseudostelligera</i>	0.001	---	0.001
<i>A. formosa</i>	0.001	---	0.001
<i>F. crotonensis</i>	0.001	---	0.001
<i>F. tenuissima</i>	0.001	---	0.001
<i>B. coregonii</i>	0.001	0.001	---
<i>B. longimanus</i>	0.01	0.01	---
<i>D. longispina</i>	0.01	0.001	---
<i>L. kindtii</i>	0.001	0.001	---

Discussion

The study of the pelagic sediment core from Lake Ledro allowed to outline the environmental and ecological evolution of a small subalpine lake on carbonate rocks during the last ~three centuries, i.e. during the last period of the Little Ice Age (LIA).

The analysis of the sediment stratigraphy showed numerous interruption in the varved structure of the core, which were interpreted as flood records in relation to their aspect and the enhanced wet density and, usually, organic content. The arrangement of the flood records in the cores studied, as well as their higher frequency in the section deposited before the early 20th century, agreed with results of recent investigations focused on the hydrological history of Lake Ledro during the entire Holocene (Simonneau et al., 2013; Vanni re et al., 2014). However, also recent hydrology related perturbations were

recorded in the present study. In particular, one major event associated to enhanced sedimentation rates was interpreted as the century flood of 1966 (Malguzzi et al., 2006), while top sediment layers characterized by higher wet density and organic content could be associated to years with particularly abundant summer (as in 1996 and 2002) or winter (2004-2005, 2008-2009) precipitations.

The analysis of the three selected biological proxies (photosynthetic pigments, diatoms and Cladocera) confirmed the pronounced sensitivity of Lake Ledro to hydrological variability and revealed two major stages in the lake ecological evolution during the last three centuries, which were principally controlled by hydrology and nutrients, respectively.

Hydrology (climate)-driven stage: pre-1860

From the late 17th century to the middle 19th century, Lake Ledro was an oligotrophic and cold lake. Concentrations of photosynthetic pigments suggested low phytoplankton densities throughout the whole period, while algal assemblages were composed by flagellated algae, diatoms, and coccal cyanobacteria containing zeaxanthin. Thanks to their ability to regulate their position within the water column and to integrate their nutrition through mixotrophy, flagellates typically characterize the phytoplankton assemblages of cold and nutrient poor mountain lakes (Tolotti et al., 2009). These groups are often accompanied by planktonic diatoms and small coccal cyanobacteria (Chroococcales) in carbonate mid altitude lakes (Tolotti et al., 2007; 2009). Despite a slight increase in the flagellate pigment markers since the middle 19th century, this general picture did not substantially change till the 1960s, in agreement with stable values

of pigment inferred lake pH and TP. Nevertheless, the analysis of the pigment-NMDS sample scores revealed a variability which perfectly matched the hydrological record of the core, with rapid and short lasting changes coinciding with each major flood event.

During this ecological stage planktonic diatoms were abundant and steadily dominated by *C. delicatula* and other unicellular centrics and pennates species (i.e. *D. stelligeroides*, *C. praetermissa*, *F. cf. tenuissima*, and *F. saxoplanctonica*) typically occurring in nutrient poor alkaline lakes (Lotter et al. 1998; Lotter, 2001). Similarly to pigments, diatom concentrations decreased during each major flood event, likely in relation to dilution by material transported from the catchment. In addition, diatoms showed two drastic increases in the proportion of benthic taxa around 52 cm (early 18th century), and especially in concomitance with the major flood recorded at the beginning of the 19th century. Benthic assemblages were dominated by several abundant *Fragilaria* species, which are characteristic for late-glacial sediments at many sites, possibly in relation with high nutrient availability through soil erosion, or to long ice-cover and reduced water transparency, which hinder the growth of planktonic diatoms (Lotter, 2001; Ampel et al., 2010). The correspondent pulse in diatom inferred TP fits with this picture. As the benthic stage implied a functional change in the diatom assemblages, it can be regarded as a temporary regime shift (Smol et al., 2005; Capon et al., 2015). However, though planktonic diatoms recovered already around the middle 19th century, the major benthonic stage represented a breaking point which introduced a strong reorganization of planktonic diatoms (see below).

Cladocera showed high total abundances in the core section deposited during the LIA, while the diversity was very low in relation to the dominance of *B. longirostris*, which was accompanied by *B. (E.) longispina* and a few individuals of Chydoridae. The dominance of Bosminidae suggests higher lake nutrient levels, apparently in contrast with information on lake trophic level provided by pigment and diatoms, as it (Korosi et al., 2013). However, the higher densities of *B. longirostris* during major hydrological events could be explained by an increase of nutrients entering the lake from the catchment. This hypothesis agrees with the observed depth-profile of sediment organic content, which showed small increments during the major flood events, and could be statistically confirmed by the significant relation between Cladocera sample scores on both NMDS dimensions and WD, and organic content values. The presence of *A. harpae* and *A. affinis* in the deep core section could represent the response of the zooplanktonic community to the low mean air temperatures registered during the Little Ice Age. In fact, these two species are considered as early immigrants after ice retreat (Kamenik et al., 2007).

Similarly to diatoms, also subfossil Cladocera showed an abrupt change in species composition since the major flood at the beginning of the 19th century. Total abundance of Cladocera decreased, while species preferring turbid water, such as *A. rectangula* and *C. sphaericus*, and/or associated with detritus or sand, such as *D. rostrata* and *M. dispar*, rapidly increased. The dominance of these taxa, together with the drastic decrease in planktonic diatoms, suggests a reduction in the planktonic communities of Lake Ledro at least till the middle 19th century, which was likely in

relation to flood related enhanced water turbidity. Comparable, although less pronounced, changes have been reconstructed in Lake Garda for the same period (Milan et al., submitted), suggesting a coherent response of these close lakes to major climate related perturbations. Respect to the rapid recovery of planktonic diatoms since the middle 1800s, Cladocera species indicating turbid and cold waters lingered till the 1960s. This was confirmed by the Cladocera samples scores on both NMDS dimensions, which showed a long and slow decreasing stage after the drastic change occurred in the early 1800s.

This set of geochemical and biological sediment features indicated the evolution of Lake Ledro during the last period of the Little Ice Age as principally driven by climate related hydrological variability. Weather conditions over the Alps were colder and wetter than present during the early 19th century (Casty et al., 2005; Chimani et al., 2013), which may explain the pronounced hydrological variability at Lake Ledro in relations to its large ratio catchment:lake area, and to the steep altitudinal gradients characterizing the top portion of the catchment (Simonneau et al., 2013). This is supported by the recent demonstration that the flood frequency at Lake Ledro during the entire Holocene was positively related to cold periods with increased winter and spring snow precipitations, which were effective in inducing catchment soil erosion (Simonneau et al., 2013). Land use likely did not change during the 19th century, when local economy was quite depressed and population decreased due to famines, wars and emigration.

Transitional stage: middle 19th – middle 20th century

As aforementioned, the plankton changes

occurred during the first half of the 19th century initiated the transition toward a new ecological stage of Lake Ledro, which started in the 1960s and was primarily driven by lake nutrient level. The reorganization was particularly evident for diatoms and, to less extent, for Cladocera between the middle 1800 and the early 1900. Subfossil pigments showed only a slight increase in the flagellate markers, in accordance with the hypothesis of enhanced lake turbidity. The stable pigment and diatom inferred TP values suggested that the community reorganization was not triggered by major changes in lake nutrient level, but by increasing water transparency and temperature recovery. The succession of oligotrophic centric diatoms which are commonly abundant in the summer epilimnion (i.e. *C. costei*, *C. comensis*) suggest lake warming and increasing thermal stratification (Rühland et al., 2015), while the increase in planktonic Cladocera agrees with the improvement of the summer temperatures over the Alps during second half of the 19th century (Casty et al., 2005). Also *A. formosa*, which is considered as performing well also in thermally stratifying lakes due to its star shaped colonies (Rühland et al., 2015), started to increase in Lake Ledro already around 1850. Species composition and abundance of all the three proxies considered were very stable between the 1930s and the 1960s, which was confirmed also by the NMDS analyses.

Nutrient-driven stage: post-1960s

The principal nutrient driven ecological stage of Lake Ledro started in the 1960s, in concomitance with the post war economic development. All the three biological proxies showed coherent changes since then, as also confirmed by the significant correlations

between the samples scores of the respective NMDS configurations. Pigments of all phytoplankton groups rapidly increased, and the specific markers of *Planktothrix rubescens* (oscillaxanthin) and of colony forming, potentially toxic cyanobacteria (echinenone, canthaxanthin) reached the highest concentrations. The increasing concentrations γ -carotene since the 1960s agreed with the extending of anoxic conditions in the lake water column, which have been documented during the maximum lake eutrophication (Casellato, 1990). However, as this carotenoid has been recently related also to cyanobacteria, it cannot be used as a reliable proxy for anoxic conditions in the lake water column (cf. sent by A. Lami).

Diatom concentrations and DAR showed a rapid and pronounced increase, as well as the abundance of mesotrophic, and later, eutraphentic taxa (i.e. *A. formosa*, *F. crotonensis*, *S. parvus* in this order). Cladocera showed a progressive increase in total density, with *B. coregonii*, *B. longispina* and *D. longispina* reaching highest density in the late 1980s. Sediment organic content progressively increased independently from the less pronounced hydrological variability, while TP concentrations inferred from both subfossil pigments and diatoms showed a rapid increase up to peak values in the late 1980s and in the middle 2000s. Total Cladocera abundance and species composition did not clearly respond to the recent hydrological perturbations, as they followed the TP increasing and recovery trend till present day.

The NMDS analyses indicated the middle 1960s as a second ecological transition, though changes were not as drastic as in the early 19th century. Though the biological variability of Lake Ledro was

clearly driven by lake nutrient level from the 1960s onwards, the lake sensitivity to the hydrological variability remained recognizable during the last decades. Despite the absence of extreme events, colder and wetter years were sufficient to affect lake phytoplankton communities and nutrient levels. The NMDS sample score analysis suggested the late 1970s and late 1990s, which were locally cold and rainy, as probable responsible for the correspondent drops in pigment concentrations, and in inferred lake TP values. On the other side, the pulse in pigment concentration, in eutraphentic (*S. parvus*), and lake TP concentrations may be considered as related to the cold and snowy winter 2004-2005. Both the nutrient and phytoplankton pulses could be validated by limnological surveys at Lake Ledro (Autonomous Province of Trento, non publ.data), while comparable changes were recorded in 2005 in several lakes of the Alpine region, such as the Italian subalpine lakes (Salmaso et al., 2014; Milan et al., 2015), Lake Geneva (Berthon et al., 2013), Lake Piburger (Tolotti et al., 2012). The 2005 event confirm that late winter snow precipitation are particularly effective in mobilizing the nutrients stored in the catchment soil of Lake Ledro (Simonneau et al., 2013), and indicated that Lake Ledro still maintains a pronounced sensitivity to climate related hydrological variability at present day. However, at present the lake response to hydrological variability is masked by the prevailing nutrient driving role.

The individuation of two major stages, separated by a transition period, in the ecological evolution of Lake Ledro posed the issue of the definition of the lake reference conditions. Though the steady lake oligotrophic status pre-1960s can be easily recognized as the reference trophic

conditions, the definition of ecological reference condition is complicated by the multiple natural and anthropogenic perturbations during the entire time span studied.

The 20th century was strongly affected not only by nutrient enrichment since the 1960s, but also by pronounced changes in land use during its first decades. During the first World War, the entire catchment of Lake Ledro was abandoned, while extended forest clearings took place in relation to the war activities in the region. Villages were repopulated and rapidly reconstructed since 1919, which likely led to further forest clearings and increased soil erosion during the flood event of 1920 (Simonneau et al., 2013). The lake level regulation since 1929 and the connection to Lake Garda since 1949 have also been regarded as responsible of major changes in lake water temperature and biological composition (Tonolli, 1956). However, the present sediment study did not provide robust evidence of major post-regulation environmental or ecological changes. On the contrary, the period between the 1930s and the middle 1960s appeared to be characterized by stable oligotrophic conditions and minimum variability in abundance and taxonomic composition of the three proxies considered, as confirmed also by the multivariate analyses. Similarly, no clear signs of a lake warming induced by the connection with Lake Garda emerge from the sediments records. Between the 1930s and the middle 1960s diatom assemblages were dominated by *C. comensis* which has often been reported as dominating in oligotrophic lakes under warming conditions (Rühland et al., 2015), while Cladocera preferring warm and nutrient rich waters increased as well after the 1930s. However, the warm and dry summers which characterized the 1930s and

1940s (Casty et al., 2005) could have been much more effective in increasing lake water temperature and stratification than the hydrological connection with Lake Garda.

Despite the numerous climate driven hydrological perturbations, lake ecological conditions of Lake Ledro were much more stable during the 18th and 19th centuries. The lake ecological conditions before 1900, i.e. before the major human impact, can be reasonably used as reference for Lake Ledro, considering that planktonic communities responded quantitatively to hydrological variability during this period, but species composition remained almost unaltered. The recent decrease in cyanobacteria carotenoids, in several meso-to eutraphentic diatom and Cladocera species (e.g. *F. crotonensis*, *S. parvus*, *B. coregonii*, *B. longispina*, *D. longispina*), and in the inferred lake TP values agree with the current re-oligotrophication process of Lake Ledro (Boscaini et al., 2012). Its present ecological status, which widely resembles the conditions reconstructed for the period between the middle 19th and the early 20th century, suggests that the lake is currently approaching its reference conditions.

The reconstruction of the evolution of Lake Ledro during the last three centuries outlined differences and common traits in the response of sediment planktonic proxies to major environmental drivers. Pigments responded to hydrological variability during the pre-1960s only in quantitative terms, likely in relation to dilution processes and adverse conditions for plankton growth induced by flood events. Also the recovery after the end of perturbation was rapid for pigments, while changes in the taxonomic composition occurred only as a response to the lake nutrient enrichment since the 1960s. Similarly to pigments, planktonic diatoms responded mainly in quantitative terms to the

hydrological perturbations in the 18th and 19th centuries. Even the dominance of benthic taxa in the early 1800 can be interpreted as the consequence of the extreme dilution of the planktonic diatom community, while the following species succession appeared to be related to decreasing water turbidity and lake warming after the end of the LIA. During the post-1960s period diatoms showed a pronounced response to nutrients, which is recognizable also during the last two decades of major temperature increase.

The Cladocera response to hydrological perturbations during the pre-1960 period was comparable to that showed by pigments and diatoms, while the changes at species level after the middle 1800 appeared to be mainly driven by the progressive recovery of the physical lake properties, in particular by the decrease in water turbidity. In the period post-1960 a prevailing driving role of lake nutrient level was evident, while the smaller hydrological variability was never sufficient to alter the Cladocera assemblages either quantitatively or qualitatively. No clear response to lake water temperature was recognizable, either prior or after the 1960s. In general, Cladocera showed slightly delayed and smoothed responses respect to the phytoplankton proxies, while the recovery after the perturbation was much longer, as recognized both after the flood in the early 1800 and after the maximum eutrophication in the late 1980s.

The three planktonic proxies selected for the present comparative study outlined similar capability in reconstructing both the lake hydrological and trophic variability. Such a pronounced sensitivity to hydrology is quite uncommon in natural lakes (Wetzel, 2001), while at Lake Ledro it depends on the catchment morphology and on the high ratio

catchment: lake area. Effects of hydrological variability were more effectively tracked by pigments and diatoms, while Cladocera provided information only on extreme events which occurred in absence of nutrient enrichment. Nevertheless, the scarce correlations which were found between NMDS sample scores and annual and seasonal precipitation, are possibly explained by the absence of data before 1870. Moreover, this could indicate the climate data recorded at Lake Garda as a poor proxy for climate variability at Lake Ledro.

Pigments and diatoms showed different capability in reconstructing past lake trophic conditions. Pigment inferred TP values were useful to understand timing and direction of the major lake trophic trajectories, but they underestimated the TP concentrations during the maximum nutrient enrichment. As pigment inferred TP values derive from concentrations of total carotenoids in the sediments, underestimations are common in deep and chemically aggressive lakes with poor pigment preservation in the sediments (Guilizzoni et al., 2011). Diatoms provided more realistic lake TP reconstructions, as they better estimated both present and maximum eutrophication (late 1980s) TP level. It is interesting here to mention that the best performing training set for the present study was calibrated on NW Europe (Bennion et al., 1996), while both the central Europe (Wunsam et al., 1995) and the Swiss (Lotter et al., 1998) training sets, which were expected to perform better were both discarded. The first one, which includes large and small lakes of the Alpine region, provided strongly underestimated TP values in relation to very low species optima for all the dominant diatom species. The second one, which includes alkaline lakes in the Swiss

Alps, provided strongly overestimations of both modern and past TP concentrations. This confirms the necessity to handle the available transfer function for TP reconstruction with caution (Juggins et al. 2013).

In Lake Ledro diatoms represented also the best performing proxy for the identification of direct and indirect effects of temperature variability on lake ecological features. In fact, only diatoms showed a rather clear response to the temperature increase occurred after the culmination of the LIA, i.e. in a period which was not affected either by pronounced hydrological variability or nutrient enrichment. This agrees with the observation that lake ecological dynamics directly respond to climate (i.e. through thermal dynamics) in absence of extensive anthropogenic disturbances (Gerten & Adrian, 2002). However, Lake Ledro showed an overall weak response to temperature variability, which was unexpected considering its small lake size. In fact, recent studies outlined that even large and deep subalpine lakes with high thermal inertia strongly respond to temperature changes, which are able to affect the lake circulation dynamics, and, consequently, the mobilization of nutrient stored in the enriched lake hypolimnion (Salmaso et al., 2014; Milan et al., 2015). The pronounced similarity between present and 1850s-1930s plankton abundances and species composition argues in favor of the absence of superimposed temperature effects over the prevailing driving role of nutrients also during recent years. The weak thermal response of Lake Ledro might be explained either by the prevailing role of hydrological variability also after the 1960s, or by local weather dynamics possibly contrasting with the regional and larger scale climate trends. This situation is quite common for mountain lakes located in

secondary Alpine valleys (Tolotti & Thies, 2002).

Conclusions

The present comparative sediment investigation provided an evaluation of the capability of independent biological proxies in tracking the secular scale ecological evolution of a small subalpine lake. The results largely agree with the hypothesis that responses of sediment biological proxies to different natural and human stressors may differ in type, timing and magnitude. The study outlined a comparable capability of subfossil pigments, diatoms and Cladocera in tracking lake hydrological and nutrient variability, and related ecological transitions and tipping points, while only diatoms showed a certain capability to track changes in lake water temperature in the lakes studied. The strong response by planktonic organisms to climate driven hydrological variability confirmed that planktonic organism principally respond to climate variability in an indirect way.

The approach adopted in this study also demonstrated that the combined study of geochemical and biological sediment proxies can substantially improve the reconstructions of recent lake nutrient enrichment processes, the definition of lake trophic and ecological reference conditions, and the evaluation of lake restoration measures within the present climate change context. This is of particular importance for lakes with scarce historical, limnological and climate data, such as small lakes of the alpine region which are mainly not included in regular, long term monitoring programs.

Besides revealing that Lake Ledro is currently approaching its ecological reference conditions, the study revealed a congenital

sensitivity of the lake to climate driven hydrological variability, which is due to the peculiar catchment and lake morphology. This represents a key aspect for the future management of Lake Ledro, considering its socio-economic importance for tourism, the current land use (including agriculture and intensive farming), and the perspective of increasing extreme weather events in the near future. In addition, this poses the attention to the necessity of maintaining, and possibly improving, the control of nutrient inputs not only in impacted lakes but also in recovering and reoligotrophicating lake, paying particular attention to the lake-specific sensitivity to local forcing as conditioned by climate change.

Acknowledgements

The present investigation has been partially funded by the Autonomous Province of Trento, Italy (Ledro Project, 2011-2013). The authors are grateful to A. Boscaini, and F. Brescancin (FEM) for field activity and recent limnological data, to G. Toller (FEM GIS Unit) for providing meteorological data from the weather station of Bezzecca, and to M. Obrelli for part of the subfossil diatom counts. A special thanks to S. Wirth (Centre for Hydrogeology and Geothermics, Université de Neuchâtel, CH) for the kind validation of the core macroscopic structure, and to C. Bigler, Umeå University, for the constructive comments and suggestions.

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Supplementary material

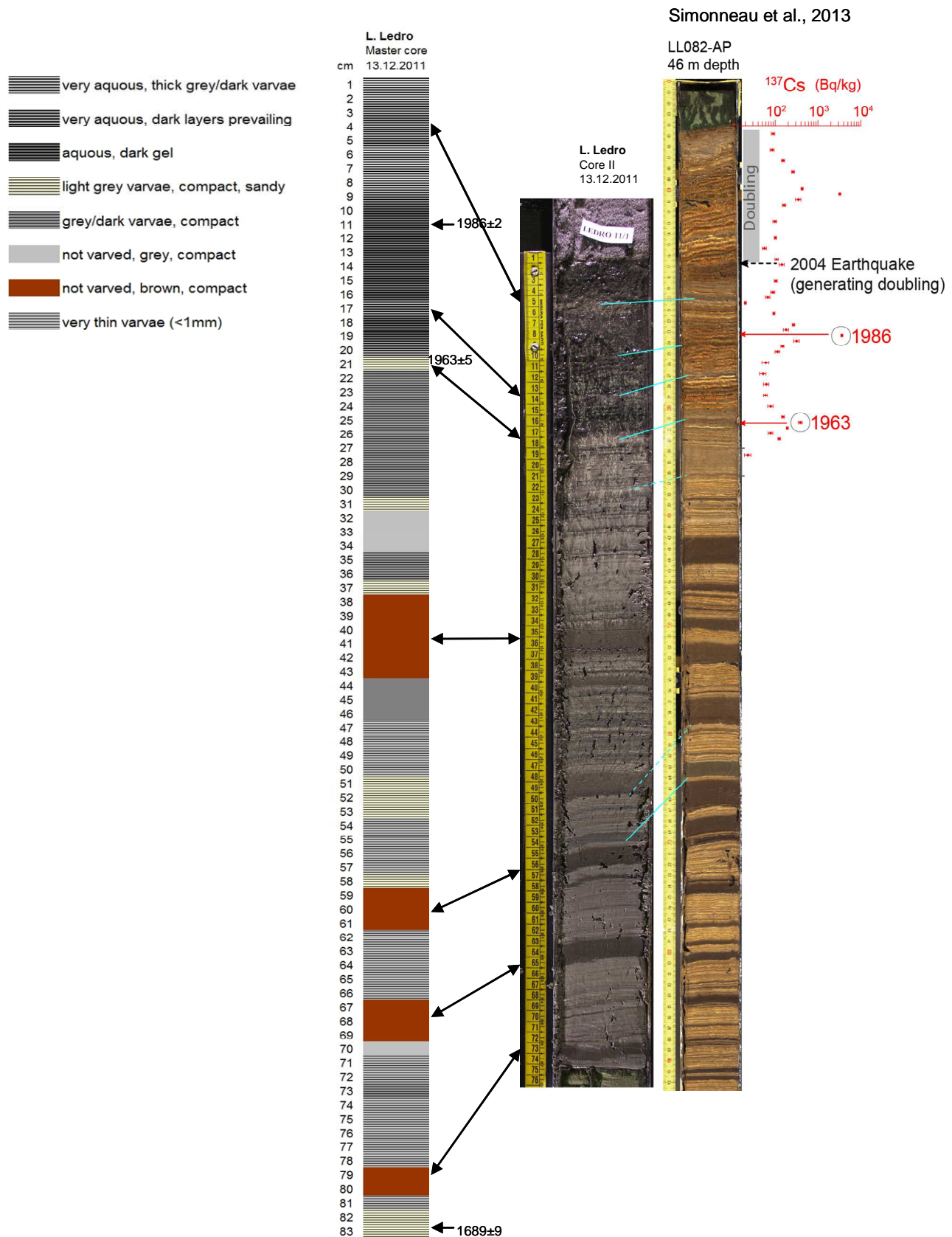


Fig. S1 Visual aspect of the master core collected from the deepest point of Lake Ledro (Trentino, Northern Italy) in December 2011, and comparison with a parallel core collected on the same date and place in 2011, and core LL082-AP, collected from 46 m depth in 2008 (Simonneau et al., 2013). Upper dates provided by ²¹⁰Pb and ¹³⁷Cs radioisotopic dating, deepest dates provided by radiocarbon dating.

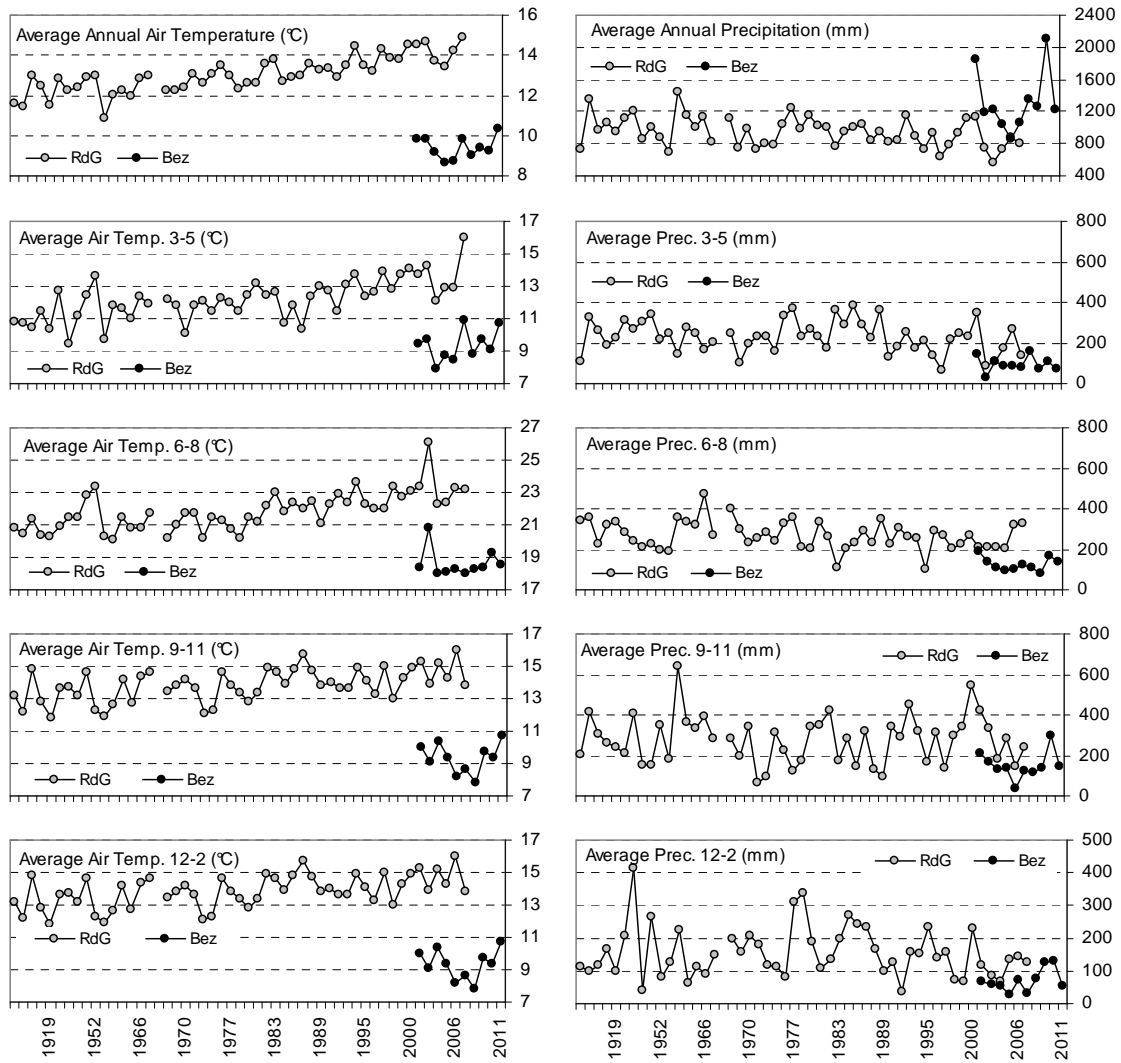


Fig. S2 Annual and seasonal averages of air temperature and atmospheric precipitation data recorded at the weather stations of Riva del Garda (RdG, 65 m a.s.l.) since 1870, and of Bezzecca (Bez., 652 m a.s.l.) since 2002. 3-5, 6-8, 9-11, and 12-2 = average relative to periods March-May, June-August, September-November, and December-February.

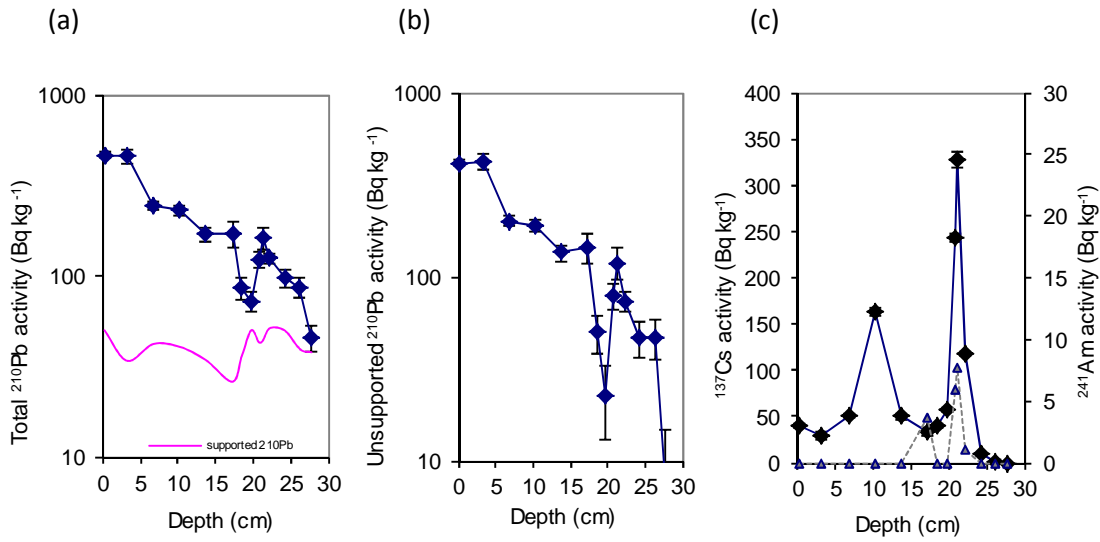


Fig. S3 Fallout radionuclide concentrations in the master core collected from the deepest point of Lake Ledro in December 2011, showing (a) total ^{210}Pb , (b) unsupported ^{210}Pb , and (c) ^{137}Cs and ^{241}Am concentrations versus depth. The supported ^{210}Pb activity fraction corresponds to ^{226}Ra activity assuming secular equilibrium between both radionuclides.