USING PALEOLIMNOLOGY FOR LAKE RESTORATION AND MANAGEMENT

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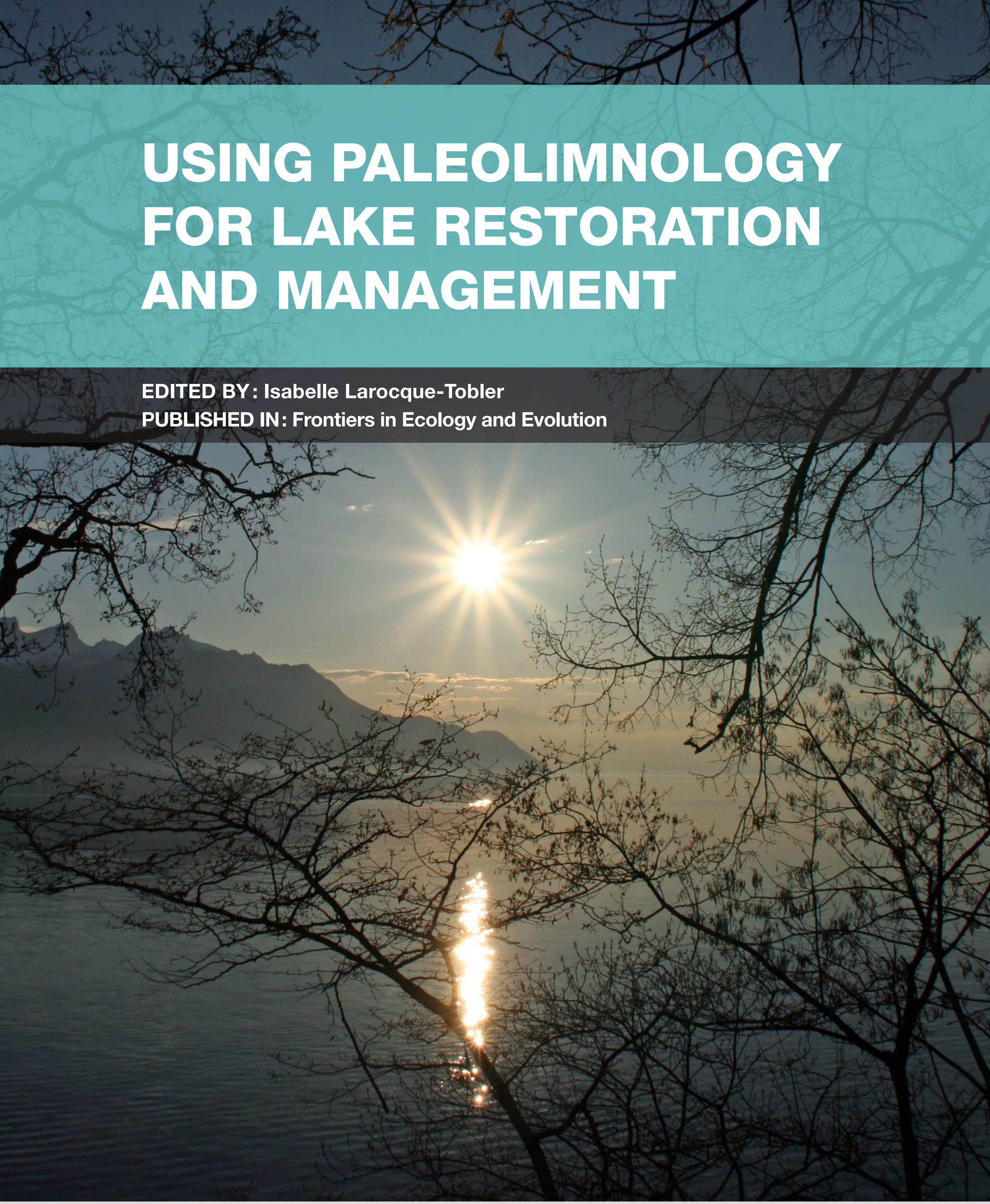

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USING PALEOLIMNOLOGY FOR LAKE RESTORATION AND MANAGEMENT

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This e-book presents state-of-the-art research projects and opinions on using paleolimnology for lake restoration and managment. It illustrates the general idea that proposing adequate restoration and managment solutions must be based on the past. Knowing the natural and anthropic variations a water body went through defines the best ways for maintaining or restoring an ecosystem. By quantifying baseline conditions, paleolimnology provides a definite plan for restoration and managment. This cannot be achieved without studying the past.

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EDITORIAL
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Editorial: Using Paleolimnology for Lake Restoration and Management

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Keywords: paleolimnology, lake sediments, lake restoration, wetlands, lake management

The Editorial on the Research Topic

Using Paleolimnology for Lake Restoration and Management

Paleolimnology has been used for decades to reconstruct past environments and more recently, to quantify the observed changes. Various organisms preserve in sediment of lakes and wetlands and can be used to reconstruct how a water body and the surrounding landscape looked like and has changed through time. For example, pollen, charcoal, and macrofossils can be used to reconstruct vegetation and frequency of fires at the local and regional scale, diatoms, and Cladocera can be used to reconstruct changes in PH and nutrients and insects are indicators of lake level, oxygen concentration, nutrients, and temperature. Thousands of paleostudies have been made worldwide and have exemplified the power of paleolimnology in reconstructing the past at long (more than 100,000 years) and shorter (a few decades; emporal scale).

The areas where paleolimnoogy can have the most impact on human-time scales, i.e., lake restoration and management, still resist its implementation into present and future decision making, in most regions of the world. In the UK (Battarbee et al., 2005), Canada (Rühland et al., 2003), and the USA (Brenner et al., 1993), paleolimnology has been used successfully in case studies to provide the background conditions for restoration and/or to evaluate the state of lakes after restoration. However, these studies are still far from being the norm they should be. Too many restoration and management programs and management programs only refer to scarce historical data as baseline. Consequently, restoration technics applied with poorly defined scientific goals can lead to after-restoration states different from the natural state of the water body and/or failed restoration (Sondergaard et al., 2007).

In this special issue of Frontiers, researchers show how paleolimnology can be used for decision makers in restoration, and management. Saulnier-Talbot sets the scenario with her opinion on how paleodata can be better used for future management. Other papers tackle on different aspects of the use of paleolimnology for lake restoration and management. These topics are enumerated below.

(a) Quantitative methods can be used to define the baseline (or reference) conditions of a lake (i.e., conditions before a lake has been impacted by human activities). These baseline conditions can then be used to propose solutions for lake restoration and/or evaluate if restoration measures have been successful.

Fraser et al. and Whitmore et al. explain the process of developing a transfer function for quantitative TP reconstruction using diatoms, its uses and limits. Quantitative reconstruction is a powerful tool to for reference conditions but, unfortunately, not always straightforward. The two papers explain how and why.

Weckström et al. defines the reference conditions in Finnish and Chinese lakes and show how these lakes have changed in the past 150 years. Although the reference condition is not the

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same in most lakes, Finnish and Chinese lakes seem to have experienced the same directional change over the past 150 years, change created by human and climate impact.

Larocque-Tobler and Pla-Rabès exemplify how diatoms and chironomids can be used to define baseline conditions, study changes which occurred in a highly eutrophied lake in Switzerland and how this data can be used to propose restoration solutions.

Using diatoms preserved in 13 European lakes, Bennion et al. look at the impact of restoration techniques on present and past assemblages to determine if restoration was successful. They also try to determine the time needed for a lake to come back to its reference condition after restoration.

(b) Paleolimnology can be used to define how environmental changes have impacted lakes and identify the parameters to consider for management.

Gell uses multipoxy paleolimnology studies to reconstruct the history and effect of sedimentation on wetlands, and he discuss the implication of these studies on water management in Australia.

Hamilton et al. explore the effects of mining activities on Canadian lakes and evaluate the restoration techniques. They determined that pre-mining conditions were never recovered.

(c) Climate and human activities both have impacts on lakes. Understanding the impacts of both parameters is important for lake management.

Perga et al. proposes solutions on how to decipher the human activities and climate using a multi-proxy approach in three large lakes in France and Switzerland. Their reconstruction of human impact vs. climate can be used for future planning of these ecosystems.

(d) New techniques are being develop to extend the potential of paleolimnology for environmental reconstruction.

Perga et al. tackles the problem of species which usually do not leave hard fossils for reconstruction by using and explaining the potential of DNA.

Wiik et al. used pigments, macrofossils, cladocerans, diatoms, XRF, and stable isotopes in a Tarn in the UK to evaluate the changes occurring during eutrophication.

INCREASED OPPORTUNITIES

Due to its great potential and robust methodologies, paleolimnology in lake management, and restoration programs, as exemplified in this special issue, will be partly conditioned by the ability of paleolimnologists to collaborate with other stakeholders.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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OPINION
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Overcoming the disconnect: are paleolimnologists doing enough to make their science accessible to aquatic managers and conservationists?

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Keywords: Paleolimnological approach, adaptive management, restoration, conservation, sustainability, data bases

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Progress in adaptive management and conservation of ecosystems cannot take place without direct collaboration between academics and practitioners. Hence, one of the most important challenges facing the development of sustainable solutions to current and future environmental problems is finding ways to bridge the gap between fundamental research and applied management, conservation and restoration of ecosystems. Here, I call on the paleolimnological community to help adaptive management move forward by making a more effective contribution of academic advancements on long-term aquatic ecology to the resolution of environmental problems. I present a personal view of how forging more links with environmental managers, conservationists, and the public, and by more effectively adapting and sharing our data and tools, can advance sustainable solutions to the many problems facing aquatic ecosystems all over the world.

Because of their essential nature, freshwaters have always been significantly impacted by human activities. Today, countless lakes, wetlands and rivers require that efforts be made to restore their health and through this, hopefully, the ecosystem services they provide. The task is overwhelming and many lake-types are notoriously difficult to manage and restore (e.g., shallow lakes: Jeppesen et al., 2007; tropical lakes: Lewis, 2000). For this reason, a vast number of people from various fields and with assorted competences must be involved and work together toward common goals. To achieve this, they must find common process and purpose in their interactions.

Over the past decades, the case has been amply made in the scientific literature for the usefulness of paleolimnology in assessing aquatic ecosystem health and aiding the management of lakes (e.g., see Bennion et al., 2011 and references therein). Doubtless the paleolimnological research community is largely convinced of the paramount value of the long-term perspective that the investigation of lake sediment archives can provide to inform sound management practices and develop realistic restoration targets. It is true that paleolimnology has already made great strides over the past 30 years to become more widely accepted, especially within other related scientific disciplines. However, discussions with colleagues and a recent brief incursion into the world of environmental conservation NGOs made me realize that paleolimnology is unfortunately not yet the household word that we would wish it to be within conservation circles. Paleolimnology still remains too infrequently used in aquatic and wildlife management practices. This led me to reflect on how we as paleolimnologists, could find ways to make our tools and data more user-friendly to managers and conservationists of aquatic environments. Moreover, through this process, I asked myself how we can better promote the idea that the integration of the paleolimnological approach is paramount in the development of sound environmental management strategies and policies. If we wish paleolimnology to enable science-based decision making for improving the health of in-land aquatic ecosystems, I believe that we should endeavor to find effective ways to present and promote it with conservation practitioners, managers and policy makers.

In a recent paper, Gillson and Marchant (2014) singled-out the two main issues hindering the application of long-term paleoecological data in management and conservation of ecosystems: (1) management and policy implications are not clearly formulated, and (2) data sets are not accessible or amenable to stakeholders. To this, I should like to add that: the paleoecological approach is insufficiently known and not well understood. So, what can we, as a mostly academic community, do about this? Admittedly, academics (or anyone else doing fundamental research) are seldom consulted on management policy. But instead of waiting to be called upon to explain how our approach can be applied to benefit management, we should aim to be more pro-active and consciously seek-out the people who are directly implicated in management and policy. Discussions with stakeholders can be enlightening and lead us to discover the needs they have singled-out. Attending meetings of community watershed counsels are a good way of making direct contact with the population and with elected representatives. Getting to know the concerns and preoccupations of residents and local officials about their water bodies can contribute to steering research and to the establishment of community-endorsed goals for aquatic ecosystem health. It is also a good way to become better informed and, eventually, of taking part in counseling local management and policy. Identifying restoration and conservation needs in conjunction with managers, conservation practitioners and the public before embarking on a research project with the goal of including them from the start as inherent facets of the research is indeed a promising avenue for academics. Public involvement is paramount to successful conservation, therefore it is also important to follow-up with communities on the advancement and success of restoration and conservation projects and better still, to encourage them to participate in some way, whenever possible.

The problem of the accessibility of data sets to stakeholders is more complex. Even amongst ourselves, we are sometimes reluctant to share data and, consciously or not, we make it difficult for others to use it. Let's take for example, what I like to call "the transfer function problem." With the advent of transfer functions and their statistical refinement over the past 20 plus years, we have developed some powerful and reliable tools that make quantitative inferences of past environmental changes possible. However, many of the published models lack some basic information to make them applicable by researchers other than the ones who developed them. Namely, because they do not include the necessary information for taxonomical harmonization of the sedimentary data sets with the modern (model) set. This shortcoming is a major obstacle to the accessibility of a model to the research community at large and is hindering progress and productivity. As the paleolimnological community grows, more and more research is being carried-out in areas that were once the strongholds of only a few. The accessibility of inference models thus becomes an issue that should be addressed. Then, again, some are of the opinion that many in the paleolimnological community are starting to move away from inference models as we know them. In truth, there are a number of problems with quantitative reconstructions from biological proxies which should not be ignored (see Juggins, 2013), but are beyond the scope of this paper. However, it is worth mentioning that great strides have been made using this approach and its use at present can hardly be discarded as obsolete. As Box and Wilson (1951) famously wrote: "essentially, all models are wrong, but some are useful." Therefore, until we develop more useful hindcasting techniques, I believe inference models will remain an important component of our toolkit.

In my opinion, transfer functions should be made easily available to those who wish to apply them, by including the necessary datasets and taxonomical references in the publications. This can take the form of an appendix or supplementary material in a paper, a web page, or a fully-fledged taxonomic guide. For the sake of accessibility, I would even go so far as to suggest creating an official repository of paleolimnological data, where transfer functions and downcore data could be archived and made available to those who wish to use them. Perhaps the best place to house this archive would be through the website of the International Paleolimnological Association (IPA) (http://paleolim.org/). Also, an updateable list of publications dealing specifically with the use of the paleolimnological approach in management, restoration and conservation could be drawn-up and made available on the IPA website.

That the paleoecological approach still remains insufficiently known and not well understood necessitates still more effort on our part. Of course, many paleolimnologists have already invested immense energy into synthesizing information on tools and techniques [e.g. Developments in Paleoenvironmental Research Book Series, edited by Smol (2001)]. There are also more and more paleolimnologists working outside academia, in government and consulting positions, where they can influence decision-making and promote the approach. But, as a whole, it appears that we have not yet risen up to the challenge issued by Smol (1995) to "continue developing techniques and approaches that can provide these (paleolimnological) data in a form that is meaningful to other scientists and managers." One example of this is the downcore plot. It might appear trivial, but presenting data on a vertical scale instead of the more common horizontal scale can turn many people off, especially when it is accompanied by a host of very unfamiliar species names. There are some examples in the literature of downcore data presented in the more intuitive left-right plots (e.g., Renberg, 1990; Wolfe, 2003) and I think this could be a good way of engaging a non-paleo audience in publications and presentations that are aimed primarily at them. At a more meaningful scale, perhaps the time is ripe for paleolimnologists to attempt to develop indicators specifically aimed at improving and evaluating restoration success. This is being done for terrestrial ecosystems, where protocols and models have been created that help to predict the success or failure of restoration efforts on degraded ecosystems throughout the restoration process (e.g., González et al., 2014). It would also be useful to attempt to develop macroecological restoration strategies based on lake-types and/or at regional scales. Environmental managers and conservationists often use pre-defined ecozones (such as presented in Ménard et al., 2013), which help to guide their efforts at a regional scale. Structuring paleolimnological tools (such as transfer functions, for example) on the same ecozones could to some degree facilitate the application of certain tools by conservation practitioners. It is a question of becoming familiar with the way that managers and practitioners work and incorporating some aspects of their vision into the way we produce knowledge so that it can be useful to everyone. In other words, learning to speak the same language is a good way forward.

Another way to make our science better known and better accepted as an inherent component of aquatic management is assuredly by promoting dialog and engaging many audiences. To this end it is important for those of us interested in making a contribution to the improvement of lake management, to take part in forums and conferences in the applied conservation and management sector. A larger proportion of our community should strive to engage with the media and use social media to share the paleoecological understanding of ecosystem value, whether it be through interviews or blogs or opinion articles (such as this one). Most importantly, for those who have the privilege of mentoring students, include this type of activity in academic training of future paleolimnologists.

Inevitably, many of us will be confronted by resistance to the incorporation of the paleolimnological approach in management strategies (the ever present resistance to change). Paleolimnology and biomonitoring have the reputation of being costly and lengthy, with results that are not always straightforward. To change this mentality, we need to continue to consistently make the point that the results are worth the resources, time and effort invested. Presenting concrete examples of successful restoration projects that have employed the paleolimnological approach, such as the case studies included in Smol (2008), and the papers contained within this special issue, will no doubt be very useful.

Paleolimnologists unquestionably have an important role to play in the development of sustainable adaptation and aquatic ecosystem management strategies. It is high time that we, as a research community, and not just a few among us, start engaging more in the processes that will lead to the effective implementation of these strategies leading to better management, preservation and restoration of aquatic ecosystems. The analysis and interpretation of paleolimnological data remains (and will remain) largely confined to specialists in academia. However, we can engage in a variety of ways to bridge the gap between the needs of management to address "real-world" problems and the orientation of publicly funded fundamental research. I am hopeful that this opinion piece will be thought-provoking and will contribute to move things forward. It is up to us, paleolimnologists, to meet the challenge of making our science, including our knowledge, our data, our techniques and our tools, more widely known, available and palatable to managers, conservationists and the general interested public.

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Tracking past changes in lake-water phosphorus with a 251-lake calibration dataset in British Columbia: tool development and application in a multiproxy assessment of eutrophication and recovery in Osoyoos Lake, a transboundary lake in Western North America

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Recently, there has been an active discussion about the potential and challenges of tracking past lake-water trophic state using paleolimnological methods. Herein, we present analyses of the relationship between modern-day diatom assemblages from the surface sediments of 251 fresh-water lakes from British Columbia and contemporary limnological variables. Total phosphorus (TP) was significantly related to the modern distribution of diatom assemblages. The large size of this new calibration dataset resulted in higher abundances and occurrences of many diatom taxa thereby allowing a more accurate quantification of the optima of diatom taxa to TP in comparison to previous smaller calibration datasets. Robust diatom-based TP inference models with a moderate predictive power were developed using weighted-averaging regression and calibration. Information from the calibration dataset was used to interpret changes in the diatom assemblages from the north and south basins of Osoyoos Lake, in conjunction with fossil pigment analyses. Osoyoos Lake is a large salmon-bearing lake that straddles the British Columbia-Washington border and has undergone cultural eutrophication followed by recovery due to substantial mitigation efforts in managing sources of nutrients. Both diatom assemblages and sedimentary pigments indicate that eutrophication began c. 1950 in the north basin and c. 1960 in the southern basin, reaching peak levels of production between 1960 and 1990, after which decreases in sedimentary pigments occurred, as well as decreases in the relative abundance and concentrations of diatom

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taxa inferred to have high TP optima. Post-1990 changes in the diatom assemblage suggests conditions have become less productive with a shift to taxa more indicative of lower TP optima in concert with measurements of declining TP, although two of these diatom taxa, *Cyclotella comensis* and *Cyclotella gordonensis*, that were previously rare are now abundant.

Keywords: cultural eutrophication, nutrients, phosphorus, proxy methods, diatoms, pigments, inference models

Introduction

Cultural eutrophication is one of the most pervasive environmental issues impacting freshwater ecosystems around the world and is largely the result of anthropogenic loading of excess nutrients. Lake-water phosphorus is the strongest predictor of mean annual phytoplankton biomass, but lake-water temperature is also an important predictor (Elliott et al., 2006). Eutrophication can result in many ecological and environmental changes that are less desirable from a human perspective. This typically includes increases in algal biomass, including cyanobacteria blooms which are recognized as a threat to water quality worldwide (O'Neil et al., 2012). Similar to overall algal production, the factors most strongly related to increases in cyanobacteria are nutrients and temperature (Jöhnk et al., 2008; Kosten et al., 2012; Taranu et al., 2012; Beaulieu et al., 2013), as well as lake depth (Taranu et al., 2015).

It is not a surprise that the economic impact from the adverse ecological and environmental changes associated with cultural eutrophication have been large, estimated to be in excess of $2.2 billion USD per year for freshwaters of the United States (Dodds et al., 2008). Fortunately, the nutrient status of lakes in many parts of North America and Europe are improving due to nutrient management and control, whereas in many developing countries, rapid population growth and watershed development have led to increases in nutrient loads (O'Neil et al., 2012).

Paleolimnology, the science that uses the physical, chemical, and biological characteristics that are preserved in the sedimentary record to reconstruct past ecological and/or environmental conditions, has been useful in studies of lake eutrophication (e.g., Battarbee, 1999). The long-term perspective of paleolimnological approaches, which pre-date high-quality records of water chemistry and/or ecological monitoring, makes paleolimnology well-suited to study cultural eutrophication, a process that typically occurs over decades. Paleolimnological approaches have been developed to study eutrophication (reviewed by Davidson and Jeppesen, 2013) have included changes in: (i) the relative composition and/or abundance of proxies of primary producers from lake sediments, including sub-fossil pigments, diatoms and other algal remains, as well as changes in plant composition from aquatic macrophytes; (ii) changes in the composition, abundance and size structure of many invertebrate groups (e.g., cladocera, and chironomids); and (iii) chemical proxies including direct measurements of total phosphorus (TP), that can be problematic (Ginn et al., 2012), as well as isotopic changes in carbon (C), nitrogen (N), and silica (Si) that can be related to changes in lake production (Davidson and Jeppesen, 2013). Given the limitations of many approaches and the complexities of lake ecosystems, most researchers advocate a multiproxy approach to reconstruct changes in nutrient dynamics, as well as a combination of both limnological and paleolimnological approaches (e.g., Battarbee et al., 2005).

Biological proxies are amongst the most widely used paleolimnological indicators to infer changes associated with eutrophication, with changes in diatom assemblages and pigment concentrations being commonly used. To help interpret changes in biotic assemblages in sediment cores is the use of a modern-day calibration dataset that involves the global estimation of species parameters (primarily species optima to TP). This approach has been termed the calibration-function approach (Birks et al., 1990, 2010; Charles et al., 1990), although there are other approaches including the indicator-species approach, and the assemblage approach (Birks et al., 2010). A large number of calibration datasets, primarily from North America, Europe, Australia, New Zealand, and China have been developed with the goal of understanding how diatom taxa are structured relative to concentrations of lake-water TP concentrations (23 calibration datasets summarized in Hall and Smol, 2010).

The majority of these diatom-based TP transfer functions are constructed from those taxa present in sedimentary diatom assemblages that meet a minimum exclusion criterion (e.g., achieving at least 1 or 2% relative abundance in a small number of lakes), using simple weighted-average (WA) models or models based on WA partial least squares (Hall and Smol, 2010). Weighted-average (WA) regression is the most widely used technique to estimate the relationship of each taxon to lake-water TP. The WA estimates (with the weight being the relative abundance of a taxon in a sample) become parameters of TP optima for the species, which are then used to estimate lake-water TP based on the assemblage composition of sub-fossil assemblages in cores (termed weighted-average calibration, Birks et al., 2010). The weighted-average approach is simple, and is based on the concept that the optima of a particular taxon is highest in the lakes at which it achieves its highest abundance. Over the years, many different calibration approaches have been attempted (e.g., see Birks et al., 2010), but these typically more-complex approaches, have not out-performed the simpler WA approach (Birks and Simpson, 2013). Although changes in diatom assemblages in sediment cores, and the use of diatom-based TP inferences have become widely accepted techniques for tracking trajectories associated with eutrophication (Hall and Smol, 2010), this approach may be misleading, especially if there have been violations made in the assumptions associated with the use of transfer functions (e.g., Juggins, 2013). As an example, there is particular concern over the confounding influence of secondary variables in the training sets, as well as poor or no spatial replicability in diatom optima to lake-water TP across different regional calibration datasets (Juggins, 2013; Juggins et al., 2013).

The measurement of pigments preserved in sediment cores has also been a widely used technique for assessing the extent, magnitude and recovery of nutrient enrichment in lakes, because sedimentary pigments have been shown to be a good proxy for algal biomass (Leavitt and Findlay, 1994; Battarbee, 1999; Leavitt and Hodgson, 2001). Sedimentary pigments are the net result of overall production and degradative processes prior to and after deposition into lake sediments. Degradative processes include herbivorous grazing, photo-oxidation, chemical or microbe-mediated oxidation (Leavitt, 1993; Leavitt and Hodgson, 2001). Fortunately, chlorophylls, carotenoids and derivative products are generally well-preserved and can persist for long periods of time within lake sediments (Leavitt and Hodgson, 2001).

The objectives of this paper are to: (i) develop a large fresh water calibration set based on the abundance of diatom assemblages spanning 251 freshwater lakes (salinity < 0.5 g/L) in British Columbia to understand the relationship between dominant diatom taxa and lake-water TP and other chemical and physical variables; and (ii) use both sedimentary pigments and diatom assemblage data to obtain better information on the historic nutrient status of Osoyoos Lake so that realistic restoration goals can be estimated. Osoyoos Lake was chosen as reductions in nutrient loading have resulted in measured declines in lake-water TP since the early-to-mid 1990s. Although previous studies have been undertaken on sediment cores from Osoyoos Lake addressing a variety of proxies from pollen, diatoms, chironomids and geochemistry (Anderson, 1973; Pinsent and Stockner, 1974; Ryder, 1994), these studies suffered from having insufficiently long cores to capture the last 200 years of sediment accumulation, poor dating, and lower resolution analyses.

Materials and Methods

The British Columbia calibration dataset used in this study consisted of diatom assemblage data and summer water chemistry from 251 freshwater lakes (**Figure 1**). These lakes were sampled to be broadly representative of freshwater lakes across British Columbia, with an effort at collecting samples from deep and shallow lakes across a nutrient concentration gradient. This sampling included several biogeoclimatic zones, from warmer semi-arid regions (<300–400 mm precipitation) on the southern interior plateau (Wilson et al., 1996) to cool sub-humid regions with moderate precipitation in the north (~600 mm/year, Laird and Cumming, 2001), to coastal maritime climates with very high precipitation (2500–5000 mm/year, Laird et al., 2001). The vegetation varied greatly among sampling sites with the semi-arid regions on the southern interior plateau being broadly characterized by Ponderosa pine and bunchgrass, with increasing amounts of interior Douglas fir in the central regions of the interior plateau. Regions farther to the north were

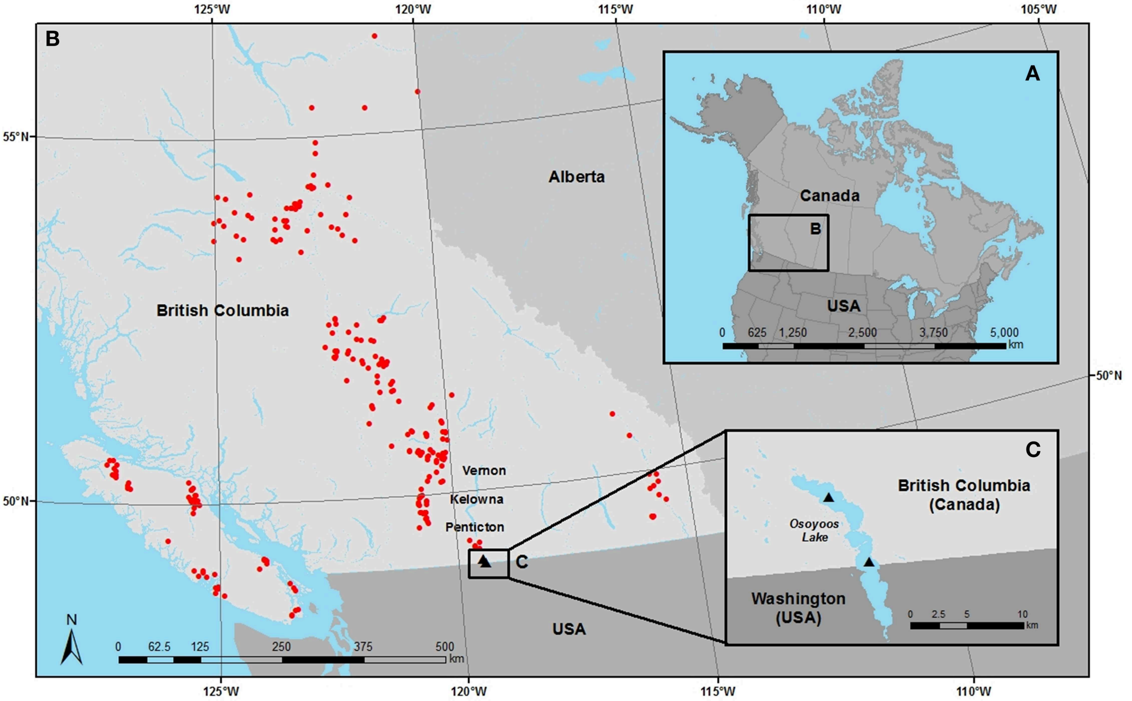

FIGURE 1 | (A) Location of the study region in western Canada, **(B)** the location of the 251 freshwater lakes from the province of British Columbia where surface-sediment samples and contemporary lake-water chemistry were taken to characterize the modern-day diatom assemblages, and **(C)** the location from where sediment cores were removed from the north and south basins of Osoyoos Lake.

dominated by sub-boreal spruce, lodgepole pine, and in some areas trembling aspen and sub-alpine fir. Vancouver Island is characterized by a marine-moderated climate with vegetation dominated by western hemlock and western red cedar (Valentine et al., 1978).

Osoyoos Lake is a relatively large (**Figure 1**; surface area = 23 km^2, maximum depth = 63 m, mean depth = 14 m) salmon-bearing lake with a north-south orientation, and two basins, that straddle the Canada/USA border (Jensen et al., 2012). Osoyoos Lake is the last lake in a chain that drains the Okanagan basin, an area of ~8280 km^2, into the Okanagan River (USA) which is a tributary to the Columbia River and ultimately the Pacific Ocean. The relatively warm climate supports a recreation-based economy, agriculture (mainly orchards and vineyards), cattle ranching and forestry, and the cities of Vernon, Kelowna, West Kelowna, Penticton, and Osoyoos. Estimates of point-source phosphorus inputs from sewage treatment facilities in the Okanagan Valley were ~59,000 kg/year in 1970, which were subsequently reduced by over 10-fold to between 2000 and 4000 kg/year by the mid 1990s (Jensen and Epp, 2002). These reductions are largely due to the introduction of improved sewage treatment plants in Penticton and Kelowna. Nutrient reductions have also resulted from non-point source reductions due to improvements to the agricultural code and Forest Practices Act in British Columbia and other land-management programs; however, these changes were estimated to be small relative to point-source changes (Jensen and Epp, 2002). Measurements of spring TP in the uppermost 10 m of water in the northern basin of Osoyoos Lake between 1969 and 1990 were high, ranging from ~18 to 37 µg/L (mean 27 ± 7 µg/L, $n = 14$). In contrast, between 1990 and 2009, the spring TP was on average ~10 µg/L lower, and the range in TP-values was smaller (TP range 1990–2009 = ~6 to 25 µg/L; mean = 17 ± 5 µg/L, $n = 14$) (Jensen et al., 2012). Similarly, fall epilimnetic TP measurements were ~6 µg/L higher between 1968 and 1990 (TP range ~12–26 µg/L; mean = 16 ± 4 µg/L, $n = 17$), as compared to fall TP measurements between 1990 and 2009 (TP range 4.5–16 µg/L; mean = 9.9 ± 4µg/L, $n = 18$) (Jensen et al., 2012). These changes parallel the measured TP concentration changes in the Okanagan River ranging from approximately 15–40 µg/L in the late 1980s to early 1990s (n = ~25) to between 5 and 20 µg/L from 2004 to 2009 (n >30) (B.C. Ministry of the Environment, unpublished data).

Calibration Dataset

The 251-lake calibration dataset (**Figure 1**) was compiled from summer (June to early September) sampling trips over four different years that included: (i) 1991 and 1993 (92 of the freshwater lakes from the Central Interior Plateau and Rocky Mountain Trench of British Columbia (B.C.) as a part of the larger 219-lake salinity calibration dataset of Wilson et al. (1996), (ii) 1996 (51 freshwater lakes sampled from the mid-to-lower interior plateau of B.C., and 50 lakes from Vancouver Island), and (iii) from 1997, where 53 lakes from a ~250-km radius centered around Prince George, British Columbia were sampled (i.e., the more northern sites on **Figure 1**), as well as five more nutrient-rich lakes from around Victoria, BC. The majority of lakes were sampled by canoe, but a number of lakes were sampled by float plane (Laird and Cumming, 2001; Laird et al., 2001).

The diatom assemblage data for each lake comes from the analysis of the uppermost 1 cm of sediment collected from the bottom of a central large basin of each lake using either a miniature gravity corer (Glew, 1991), or a modified K-B gravity corer (Glew, 1989). Water chemistry samples from a depth of ~0.5 m were also collected from each lake from the same central location in the lake into polyethylene bottles that were rinsed with lake water at least three times prior to obtaining the sample. Water chemistry samples were shipped in coolers on ice, and chemical analyses were normally completed within 96 h of sample collection. Water chemistry analyses for major cations (Ca^{2+}, Mg^{2+}, Na^+, K^+), anions (dissolved inorganic carbon (DIC), Cl^-, SO_4^{2-}), pH, specific conductivity, and nutrients (TP, nitrogen and dissolved silica) followed standard protocols outlined in Wilson et al. (1994). Chemical measurements were not made for DIC and total Kjeldahl nitrogen (TKN) for two lakes, and five lakes additional lakes were missing measurements for TKN. These seven lakes were omitted from the correlation matrix of the standardized environmental variables, but were included in all other analyses.

Slides for diatom analysis were prepared using standard techniques (Cumming et al., 1995). Briefly, a small amount of wet sediment was suspended in a 50:50 (molar) mixture of sulfuric and nitric acid in a 20-ml glass vial for 24 h prior to being submersed at 70°C in a hot water bath for approximately 5 h. The remaining sediment material was settled for a period of 24 h, at which time the acid above the sample was removed. The sample was rinsed with distilled water and allowed to settle once again for 24 h. The procedure was repeated approximately 8 times until the sample was the same pH as distilled water (litmus test). The samples were settled onto coverslips in a series of four 100% dilutions, which when dry, were mounted onto glass slides using the high-resolution mounting medium Naphrax®. For each sample, at least 400 diatom valves were enumerated with a Leica DMRB microscope equipped with differential interference contrast optics at 1000X magnification (numerical aperture of the objective = 1.3). Diatom identifications were based primarily on the references of Krammer and Lange-Bertalot (1986, 1988, 1991a,b); Patrick and Reimer (1966, 1975), and Cumming et al. (1995).

The distributions of all of the physical and chemical environmental variables were visually examined for normality, and transformed if required. A Principal Components Analysis (PCA), in conjunction with a correlation matrix between transformed environmental data, was used to summarize the strength and covariance between the physical and chemical characteristics in the B.C. calibration dataset. The PCA was undertaken on centered and standardized environmental variables, following transformations to better approximate a normal distribution, using the computer program CANOCO v. 5. (ter Braak and Šmilauer, 2012).

Both unconstrained (correspondence analysis, CA) and constrained ordination (canonical correspondence analysis, CCA) were used to identify the main directions of diatom species variation in the 251-lake dataset and to select the minimum number of measured environmental variables that could account for variance in the diatom data, respectively. The strength of the explanatory power of TP was also assessed by the strength of the first eigenvalue of a CCA when TP was the only explanatory variable. All ordinations were undertaken with the computer program CANOCO v. 5.

To reduce the number of potential explanatory variables used to explain the distribution of diatom assemblages in the calibration set, the number of variables was reduced by grouping environmental variables that had correlation coefficients of $>$ 0.7, and one representative environmental variable was chosen from each group. Ordinations were based on: (i) all diatom taxa that achieved at least 2% relative abundance, and (ii) diatom taxa that were represented by at least a Hill's N2 of 5 (i.e., represented equally in the dataset by at least five occurrences). Taxa that achieved a Hill's N2 of at least 5, were further divided into abundant taxa, achieving an abundance of $>10\%$, and subdominant taxa (N2 $>$ 5, but maximum relative abundance $<10\%$). Ordinations were run on all taxa that achieved an N2 $>$ 5 (i.e., the dominant and subdominant taxa) (ter Braak and Šmilauer, 2012).

Diatom-based TP inference models were developed based on the 251-lake dataset using simple weighted-averaging models, on both non-transformed and square-root transformed species relative abundance data following techniques outlined in Birks et al. (1990). In weighted-averaging models, averages are taken twice (once in the regression step and once in the calibration step), which results in the shrinkage of the original gradient (e.g., TP). To correct for this, we used both inverse and classical deshrinking techniques in our models (Birks et al., 1990). The strength of the models was assessed using the coefficient of determination of the inference model after bootstrapping (r^2_{boot}) and the root mean squared error of prediction (RMSEP), and estimates of the optimum and the tolerance of the dominant diatom taxa to lake-water TP were made using the computer program C2 v. 1.7.5 (Juggins, 2003).

Osoyoos Lake Sediment Cores

Two sediment cores were retrieved from Osoyoos Lake with a slide hammer corer (internal diameter ~6.35 cm) on June 17 and 18th, 2009, from a depth of ~50 m in the north basin, and a depth of 23 m in the south basin (**Figure 1C**). The cores from the north and south basins were 90 and 99 cm in length, respectively and were sectioned into ~0.5-cm intervals on site. Core chronologies (i.e., core depth-time profiles) were based on measurement of total ^{210}Pb, ^{214}Bi (a proxy of supported ^{210}Pb) and ^{137}Cs in each core from twenty-one and twenty-two samples from the north and south basin cores, respectively, using gamma spectroscopy (Schelske et al., 1994). Unsupported ^{210}Pb activities were used to estimate the chronology of the cores using the constant rate of supply (CRS) model (Appleby and Oldfield, 1978) using the computer program developed by Binford (1990).

Sedimentary pigments in the cores were analyzed from 32 intervals from each of the two cores from Osoyoos Lake. Sedimentary pigments were extracted from freeze-dried sediments in acetone under an argon atmosphere at -20°C for 24 h. During pigment extraction samples were kept in the dark and on ice at all times to avoid pigment deterioration from light, heat, and oxygen (Leavitt and Hodgson, 2001). Samples were then centrifuged, the supernatant was decanted and filtered (0.2 μm), and finally placed into sealed glass vials. Sample extracts were analyzed using a Waters High Performance Liquid Chromatography (HPLC) system equipped with a photo diode array (Waters model 2996) and a multi wavelength fluorescence detector (Waters Model 2475) following the solvent protocol outlined in Zapata et al. (2000). The HPLC produces chromatograms in a specific sequence based on the properties of the compounds being analyzed. The resulting retention time and absorbance spectra were compared to purified reference materials to quantify the sedimentary pigments. For all pigments, the area under the curve was used to calculate the quantity present in each sample. Pigments were expressed as nmol/g organic dry weight, and percent organic matter (OM) was determined using the standard loss-on-ignition method (Dean, 1974).

Diatoms were analyzed from the same intervals that were sampled for fossil pigments. Diatom valves were enumerated following procedures stated above for the calibration dataset but with the exception that microspheres were added to each sample prior to settling on coverslips to allow for calculations of the diatom-valve concentration following the procedure outlined by Battarbee and Keen (1982). Briefly, to each slurry a known concentration of microspheres was added and were enumerated along with the diatoms and used to calculate estimates of the number of diatoms per gram dry weight. Total diatom concentration provides an additional proxy to assess diatom assemblage changes in sediment cores.

The diatom assemblage changes over the last 200 years in the Osoyoos Lake sediment core were critically assessed to determine: (i) if the TP inferences tracked the main directions of variation in the diatom species assemblages, (ii) if the sub-fossil assemblages in the sediment core were well-represented in the modern 251-lake calibration dataset, and (iii) if the changes in the diatom assemblages were consistent with the known history of eutrophication and recovery of Osoyoos Lake, based on modern water chemistry data, and other eutrophication proxies including changes in diatom concentrations, and changes in sedimentary pigments.

To assess if the extent to which the TP inference tracked the main directions of variation in the diatom assemblages we calculated: (a) the λ_R/λ_P ratio, and (b) correlations between the diatom-inferred TP-values in the core to the axis-1 and -2 scores of a principal components analysis (PCA) of the diatom assemblage (Juggins et al., 2013). The λ_R/λ_P ratio expresses the variance accounted for by the diatom-inferred TP as a fraction of the maximum explainable variance in the core, and is calculated by: dividing the eigenvalue of the first axis of a redundancy analysis (RDA) of the diatom assemblage in the sediment core constrained to the diatom-inferred TP (λ_R) divided by the eigenvalue of the first axis of a PCA on the same downcore diatom assemblage (λ_p). Additionally, greater confidence that the diatom assemblages are tracking the changes related to phosphorus (or correlated variables) is increased when the correlation between the diatom-inferred TP-values to the main direction of variation in the PCA axis-1 and -2 scores are high. If the correlation between the main direction of variation and the diatom-inferred phosphorus values is weak or non-existent, then other environmental variables or interactions between environmental variables, are likely responsible for the observed changes in diatom assemblages, and lead to less confidence in the diatom-based inference. The main directions of variation in the diatom assemblages in the Osoyoos Lake cores were determined from PCA axis-1 and -2 scores using both non-transformed and transformed species abundance data. PCA was chosen to represent the main directions of variation of the diatom assemblages because the gradient length in an initial detrended correspondence analysis was < 1.5 standard deviation units.

To assess the applicability of the modern diatom assemblages to the core assemblages we examined how well each of the dominant taxa in the core was represented in the modern training set. This was assessed by comparing the number of occurrences and the relative abundances of taxa in the core assemblages to the modern assemblages. If core assemblages were not well represented, we re-ran the TP inference models, excluding the influence of the poorly represented taxa, and compared the results of the two WA inference models.

Finally, we assessed changes in the downcore diatom concentrations as an additional proxy to nutrient additions. Data expressed as concentrations (and/or accumulations) can also be used, in addition to percentages, to assess changes in diatom assemblages. Changes in the diatom assemblages and TP inferences were also compared in relation to pigment changes in the core, as well as to historical and water quality information from Osoyoos Lake.

Results

Calibration Data Set

The study lakes span a large portion of the central to southern interior of B.C., the Rocky Mountain Trench, and Vancouver Island, and represent a large diversity of freshwater lakes (salinity < 500 mg/L) with large gradients in lake depth (~1–71 m), pH (6.1–9.7), and nutrients (TP from 2 to 227 µg/L, TKN from 20 to 5000 mg/L, and dissolved silica from 0.1 to 35.4 mg/L) (**Figure 1**, Supplemental 1). The first two axes of a PCA ordination of the physical and chemical lake-water characteristics captured 73% of the variation with the main environmental gradients associated with conductivity, pH and associated variables dominating the first PCA axis (**Figure 2A**). Not surprisingly, pH, specific conductance, salinity, TKN and the base cations are all highly correlated ($r > 0.78$–0.97) (**Table 1**). Other water chemistry gradients were seen in the PCA including those associated with moderately correlated nutrients (TP, TKN, and DOC) which were negatively correlated with lake depth, as well as gradients associated with sulfate and chloride (**Figure 2A**, **Table 1**).

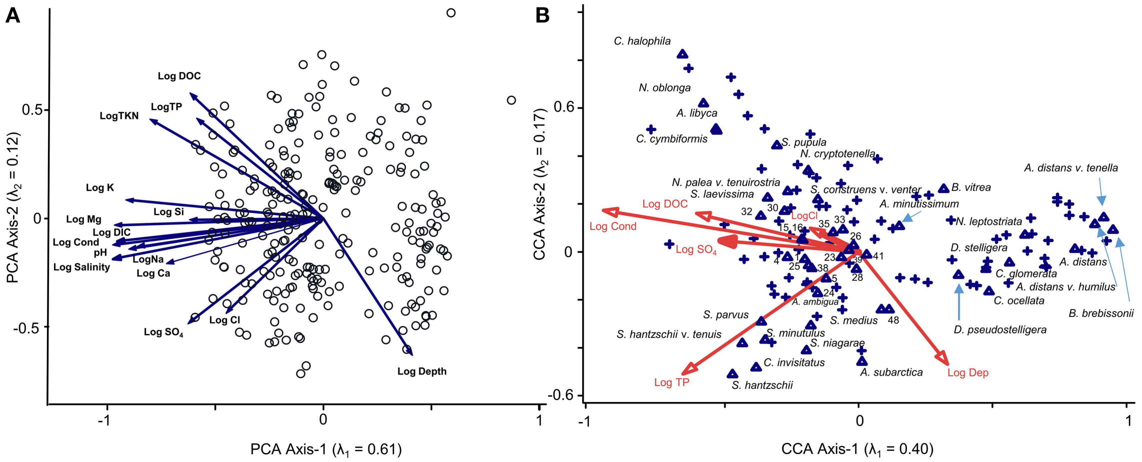

FIGURE 2 | (A) Principal components analysis (PCA) biplot of the location of each of the 251 lakes (open circles) in relation to the standardized physical and chemical variables (mean 0, and unit variance) from these lakes, illustrating the approximate correlations and relative gradient lengths of the chemical and physical environmental variables. **(B)** A canonical correspondence analysis (CCA) biplot, of axis 1 vs. axis 2, showing the relationship between the diatom taxa (Hill's N2, a measure of species evenness across samples, of at least 5) and the forward-selected environmental variables from the surface-sediment diatom assemblages and measured environmental variables from the 251 calibration dataset. The environmental variables to which the species were constrained are shown by solid red arrows. An ordination based on all taxa that achieved a >2% relative abundance, with downweighting of rare taxa, resulted in the selection of the same environmental variables and in the same order of selection, suggesting the robust nature of the results presented above. The labeled diatom taxa (shown as triangles) represents the more abundant taxa (referred to as the dominant diatom taxa, and defined by all taxa that achieved a species evenness (Hill's N2) of at least 5 and a relative abundance of at least 10% in at least one sample). Taxa represented by crosses achieved a Hill's N2 of at least 5, but did not achieve a relative abundance of greater than 10%. Diatom taxa that did not achieve a Hill's N2 of at least 5, and were present at a relative abundance of greater than 2%, were not included in this ordination. Species corresponding to numbers on **(B)** are listed in **Table 2**. Abbreviations: Log Cond, log specific conductance; Log Sal, log salinity; Log TKN, Log total Kjeldahl nitrogen; Log TP, log total phosphorus; Log Cl, log chloride; Log DIC, log dissolved inorganic carbon; Log SO_4, log sulfate; Log DOC, log dissolved organic carbon; Log Ca, log calcium; Log K, log potassium; Log Mg, log magnesium; Log Na, log sodium; and Log Si, log silica. Numbers represent taxa that can be identified from **Table 2**.

TABLE 1 | Correlation matrix of the environmental variables in 244 calibration lakes for which complete chemistry was available.

	Log Depth	pH	Log Cond	Log Sal	Log TKN	Log TP	Log Cl	Log DIC	Log SO_4	Log DOC	Log Ca	Log K	Log Mg	Log Na	Log Si
Log Depth	1.00														
pH	−0.33	1.00													
Log Cond	−0.36	**0.84**	1.00												
Log Sal	−0.34	**0.83**	1.00	1.00											
Log TKN	−0.57	**0.70**	**0.80**	**0.79**	1.00										
Log TP	−0.44	0.52	0.56	0.56	0.68	1.00									
Log Cl	−0.07	0.22	0.32	0.35	0.24	0.17	1.00								
Log DIC	−0.36	**0.84**	**0.98**	**0.98**	**0.78**	0.56	0.24	1.00							
Log SO_4	−0.04	0.48	0.60	0.64	0.38	0.35	0.26	0.54	1.00						
Log DOC	−0.51	0.55	0.64	0.62	**0.81**	0.44	0.17	0.60	0.21	1.00					
Log Ca	−0.14	0.57	**0.74**	**0.73**	0.47	0.51	0.02	**0.75**	0.48	0.27	1.00				
Log K	−0.36	**0.79**	**0.91**	**0.91**	**0.85**	0.60	0.34	**0.88**	0.57	0.68	0.60	1.00			
Log Mg	−0.39	**0.85**	**0.98**	**0.97**	**0.83**	0.56	0.31	**0.97**	0.55	0.68	0.65	**0.91**	1.00		
Log Na	−0.35	**0.74**	**0.89**	**0.89**	**0.81**	0.52	0.52	**0.84**	0.51	0.68	0.47	**0.88**	**0.90**	1.00	
Log Si	−0.32	0.48	0.57	0.55	0.48	0.46	-0.07	0.60	0.27	0.38	0.56	0.52	0.56	0.39	1.00

Seven of the 251 lakes had incomplete chemistry and were omitted prior to calculation of the correlations because dissolved organic carbon values were missing from two lakes, and TKN values were missing from seven lakes (see Supplemental 1). Abbreviations: Log Cond, log specific conductance; Log Sal, log total salinity; Log TKN, log total Kjeldahl nitrogen; Log TP, log total phosphorus; Log Cl, log chloride; Log DIC, log dissolved inorganic carbon; Log SO_4, log sulfate; Log DOC, log dissolved organic carbon; Log Ca, log calcium; Log K, log potassium; Log Mg, log magnesium; Log Na, log sodium; Log Si, log dissolved silica. Correlations > 0.7 are shown in bold.

A total of 209 diatom taxa that achieved a relative abundance of greater than 2% in at least one sample were identified in the 251-lake dataset. These 209 taxa accounted for an average relative abundance of 89% ± 8.8 of the total assemblage across all 251 samples. Of these taxa, 48 achieved a relative abundance of greater than 10% and had a Hill's N2 of greater than 5 (i.e., the dominant taxa), and accounted for an average relative abundance of 72.9% (±20.9) of the assemblage in the 251 lakes. An additional 79 taxa achieved a Hill's N2 of at least 5 (subsequently referred to as the subdominant taxa) and together with the dominant taxa accounted for a majority of the total relative abundance of the assemblage in all of the lakes (82.4% ± 17).

CCA ordinations were undertaken on the full (209 diatom taxa × 251 lake) dataset, as well as on the dominant and subdominant taxa (127 diatom taxa × 251 lake) dataset, both with and without a square-root transformation of the diatom taxa using a set of explanatory variables that included: specific conductance, depth, TP, Cl^-, SO_4^{2-}, DOC, and Si. Specific conductance was chosen to act as a surrogate for the many highly correlated variables associated with this gradient in the dataset including: pH, salinity, TKN, DIC, and base cations due to high correlations among these variables (**Table 1**). In all analyses, similar patterns of species and sites emerged on the ordinations, with specific conductance, TP and log depth, consistently being identified as the environmental variables that could account for the largest amounts of variance in the diatom data. In all CCA ordinations, the eigenvalues of the first axis ranged from 0.4 (transformed dominant and subdominant diatoms) to 0.51 (all 209 taxa with no species transformations), with eigenvalues for axis 2 ranging from 0.17 (transformed dominant and subdominant taxa) to 0.27 (all 209 taxa, with no species transformations). In all cases, placement of the species and sample scores were similar. As an example, we show the CCA based on square-root transformed dominant and subdominant diatom taxa (127 taxa), seven environmental variables (specific conductance, TP, depth, Cl^-, DOC, sulfate, and Si) were forward selected (**Figure 2A**). Deep-water, high-TP diatom taxa including *Cyclostephanos invisitatus, Stephanodiscus hantzschii*, and *Stephanodiscus hantzshii* v. *tenuis* plot in the lower-left quadrant, whereas deep-water, low-TP taxa such as *Aulascoseira distans*, *Cyclotella glomerata,* and *Discostella stelligera* plot in the right quadrants of the CCA ordination (**Figure 2**). Shallow-water, higher-TP benthic taxa including *Navicula oblonga* and *Amphora libyca*, plot in the upper-left quadrant of the ordination.

The single strongest environmental variables in all CCA ordinations was specific conductance (the eigenvalue of the first axis when constrained to specific conductance alone ranged from 0.37 to 0.46 depending on the full or dominant/subdominant dataset, with and without a square-root transformation). TP was the next strongest environmental variable in all ordinations (the eigenvalue of the first axis when constrained to TP alone ranged from 0.17 to 0.29 depending on the full or dominant/subdominant dataset, with and without a square-root transformation).

The dominant diatom taxa showed clear patterns in their estimated TP optima and tolerance across the TP gradient (**Figure 3B**). Only six dominant diatom taxa had an estimated TP optima > 30 μg/L (eutrophic), two taxa had optima >50 μg/L, and 11 had estimated TP optima <10 μg/L (oligo-mesotrophic) (**Figure 3B**). The vast majority of the dominant taxa in this dataset fell within the meso-eutrophic category (10–30 μg/L). The dominant diatom taxa occurred in many lakes (27–200)

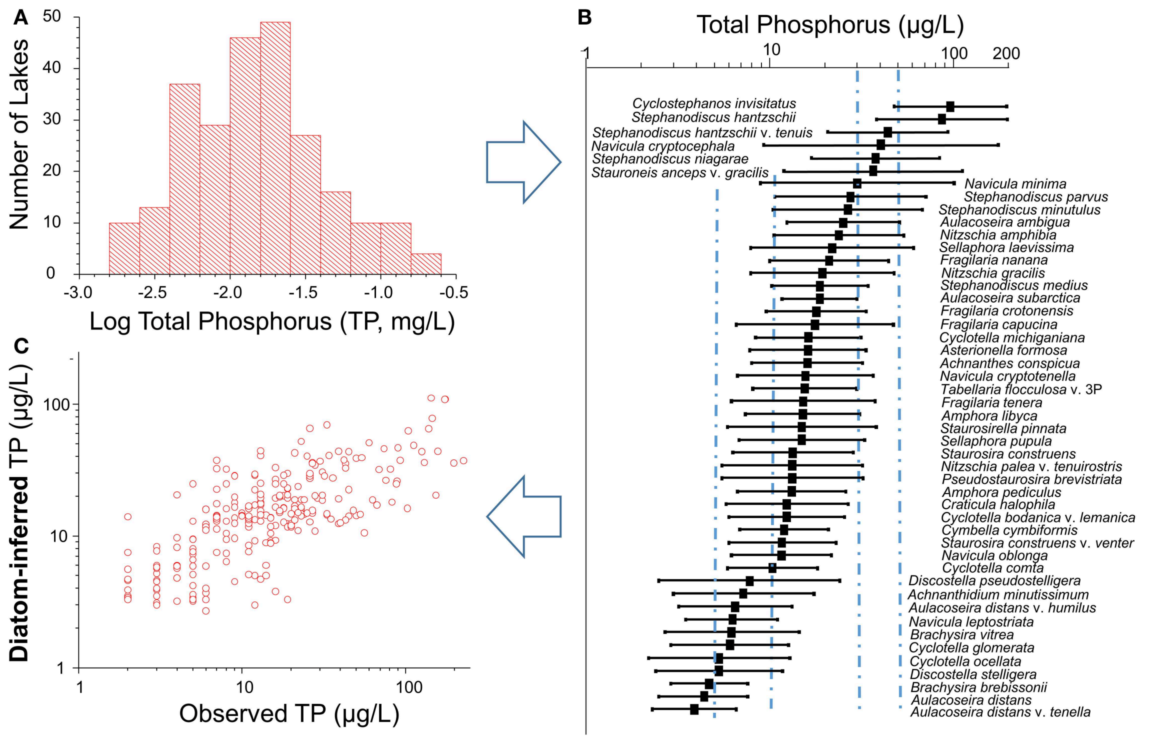

FIGURE 3 | Schematic diagram showing the steps involved in developing a "training" set for inferring lake-water total phosphorus based on the species assemblages preserved in the surface sediments of 251 freshwater lakes from British Columbia. **(A)** Lakes were selected to span a large gradient of total phosphorus (TP) in freshwater lakes (salinity < 0.5 g/L) from which surface sediments were collected and analyzed for diatom assemblages and from which contemporary physical and chemical variables were collected; **(B)** estimates of the bootstrapped weighted-average optima (square) and tolerances (weighted standard deviation) of the dominant diatom taxa (relative abundance >10%; and Hill's N2 of at least 5. Vertical lines are shown at 5, 10, 30, and 50 µg/L TP, representing the boundaries between oligotrophic (<5 µg/L), oligo-mesotrophic (5–10 µg/L), meso-eutrophic (10–30 µg/L) and eutrophic taxa (>30 µg/L) (Wetzel, 2001); **(C)** the relationship between observed TP-values in the 251 calibration lakes vs. the bootstrapped estimate of diatom-inferred TP. The correlation between the observed and diatom-inferred values is 0.74 ($n = 251$, $p < 0.01$).

and at relative abundances ranging from 10 to 92% (average = 36.3% ± 25.3) (**Table 2**). Although a species evenness (Hill's N2) of 5 was one of the criteria that was used to define a dominant taxa, Hill's N2 was much greater than five in the majority of samples, and even higher when calculated species optima were based on square-root transformed species abundances. Estimates of the species TP optima and tolerances for the dominant diatom taxa were highly similar between TP optima based on non-transformed and transformed species abundances ($r > 0.96$) with no apparent bias (**Table 2**).

Models developed to infer lake-water TP based on weighted average calibration had moderate predictive abilities (e.g., **Figure 3C**). These models, were based on: (i) all 209 taxa, with and without square-root species transformations; and ii) on taxa that achieved a Hill's N2 of at least 5 (i.e., 127 dominant and subdominant taxa), with and without square-root species transformations. For all eight models, the apparent coefficient of determination varied only marginally from 0.57 to 0.63, with the bootstrapped coefficient of determination between 0.51 and 0.55. Similarly, the RMSEP varied between 0.31 and 0.32 for the models using inverse deshrinking, and between 0.37 and 0.4 for the models that used classical deshrinking.

Paleolimnological Analysis of Osoyoos Lake

Chronology

The total ^{210}Pb activity in the north-basin core followed a significant exponential decay with core depth ($r^2 = 0.73$, $p < 0.01$, $n = 21$), with a 0.14 Bq/g maximum toward the top of the core declining to ∼ 0.06 Bq/g low at 80-cm depth. Measured ^{137}Cs activity showed a distinct peak between 43 and 58 cm (maximum ^{137}Cs of 0.046 Bq/g at 50.6 cm, approximately 4–5× surface concentrations). The CRS model, based on the unsupported ^{210}Pb concentrations, estimated that the last 200 years were contained in the 90-cm core, and the CRS date of 1963 occurred at a depth of ∼ 42.5 cm, just slightly later than the ^{137}Cs peak. The organic matter (OM) was low throughout the core ranging from 5 to 12%, with higher concentrations in the top 15 cm. Similarly, the total ^{210}Pb activity in the south-basin core followed a significant exponential decay ($r^2 = 0.80$, $p < 0.01$, $n = 22$), with 0.18 Bq/g maximum toward the core top declining to a low of ∼ 0.05 Bq/g below 70 cm in the core. Measured ^{137}Cs activity showed a distinct peak between 39 and 47 cm (maximum ^{137}Cs of 0.043 Bq/g at ∼43.5 cm, approximately 4–5× surface concentrations). The CRS model estimated that the 99-cm core contained at least 200 years of sediment accumulation, and the

TABLE 2 | Estimates of simple weighted-average optima and tolerances for the dominant diatom taxa in the 251-lake calibration dataset, based on both untransformed and square-root transformed species data.

Species number and Species name	N1	Max Abundance (%)	No species transformations				Square-root species transformations			
			N2	Bktr Optimum (µg/L)	Log Optimum (mg/L)	Log Tolerance (mg/L)	N2	Bktr Optimum (µg/L)	Log Optimum (mg/L)	Log Tolerance (mg/L)
1. *Achnanthes* (*Platessa*) *conspicua*	71	10.5	16.0	16.1	−1.79	0.30	51.4	16.9	−1.77	0.35
2. *Achnanthidium minutissimum*	200	50.9	58.5	7.3	−2.14	0.39	132.3	9.2	−2.04	0.41
3. *Amphora libyca*	83	91.8	8.2	15.0	−1.82	0.32	33.4	16.7	−1.78	0.39
4. *Amphora pediculus*	108	14.7	29.4	13.2	−1.88	0.30	72.3	14.3	−1.85	0.34
5. *Asterionella formosa*	177	84.2	56.1	16.2	−1.79	0.32	117.6	15.8	−1.80	0.36
6. *Aulacoseira ambigua*	78	35.0	21.7	25.7	−1.59	0.31	47.6	20.3	−1.69	0.38
7. *Aulacoseira distans*	59	47.7	19.6	4.4	−2.36	0.24	38.1	4.7	−2.33	0.30
8. *Aulacoseira distans* v. *humilus*	27	11.5	10.0	6.5	−2.19	0.33	19.0	6.5	−2.19	0.33
9. *Aulacoseira distans* v. *tenella*	32	15.3	12.7	3.9	−2.41	0.24	23.2	4.1	−2.39	0.25
10. *Aulacoseira subarctica*	37	38.8	14.7	18.8	−1.73	0.20	25.2	18.7	−1.73	0.26
11. *Brachysira brebissonii*	27	11.7	6.4	4.7	−2.32	0.21	18.2	4.5	−2.35	0.24
12. *Brachysira vitrea*	100	19.1	40.6	6.3	−2.21	0.37	77.2	6.8	−2.17	0.38
13. *Craticula halophila*	39	20.6	9.2	12.5	−1.91	0.36	21.3	14.6	−1.84	0.35
14. *Cyclostephanos invisitatus*	19	15.9	7.8	95.6	−1.01	0.32	12.5	76.3	−1.12	0.39
15. *Cyclotella bodanica* v. *lemanica*	143	49.5	33.2	12.4	−1.91	0.32	83.7	12.2	−1.91	0.33
16. *Cyclotella comta*	17	22.4	5.2	10.6	−2.00	0.26	10.2	10.7	−1.97	0.29
17. *Cyclotella glomerata*	57	28.0	20.5	6.0	−2.22	0.33	38.5	6.3	−2.20	0.35
18. *Cyclotella michiganiana*	44	71.0	5.8	16.6	−1.76	0.30	23.7	14.4	−1.84	0.35
19. *Cyclotella ocellata*	39	34.9	11.0	5.3	−2.28	0.40	25.5	6.0	−2.22	0.42
20. *Cymbella cymbiformis*	25	11.9	7.3	11.8	−1.93	0.24	15.8	13.1	−1.88	0.31
21. *Discostella pseudostelligera*	46	25.1	11.5	7.8	−2.12	0.52	28.1	8.8	−2.06	0.50
22. *Discostella stelligera*	94	65.4	40.8	5.3	−2.28	0.36	64.8	6.0	−2.22	0.38
23. *Fragilaria capucina*	118	19.7	30.3	17.7	−1.75	0.43	83.0	15.5	−1.81	0.47
24. *Fragilaria crotonensis*	105	32.2	46.5	18.0	−1.74	0.28	78.1	17.7	−1.75	0.30
25. *Fragilaria nanana*	99	31.0	26.4	21.1	−1.67	0.33	62.3	16.9	−1.77	0.38
26. *Fragilaria tenera*	114	14.4	38.4	15.2	−1.82	0.40	80.7	13.7	−1.86	0.40
27. *Navicula cryptocephala*	62	11.1	11.0	39.5	−1.34	0.67	41.0	20.1	−1.70	0.59
28. *Navicula cryptotenella*	62	16.8	15.7	15.6	−1.79	0.39	41.2	13.2	−1.88	0.39
29. *Navicula leptostriata*	27	10.9	7.0	6.2	−2.19	0.27	17.5	5.9	−2.23	0.29
30. *Navicula minima*	26	15.6	7.5	30.8	−1.47	0.59	18.6	22.1	−1.66	0.56
31. *Navicula oblonga*	48	15.4	13.5	11.4	−1.94	0.28	28.7	13.6	−1.87	0.32
32. *Nitzschia amphibia*	62	39.1	5.4	24.0	−1.64	0.36	30.0	22.0	−1.66	0.40
33. *Nitzschia gracilis*	57	28.4	5.9	19.5	−1.69	0.40	35.7	14.8	−1.83	0.44
34. *Nitzschia palea* v. *tenuirostris*	34	18.5	5.3	13.1	−1.87	0.40	20.2	13.9	−1.86	0.44
35. *Pseudostaurosira brevistriata*	160	79.6	34.0	13.3	−1.88	0.39	100.8	13.1	−1.88	0.41
36. *Sellaphora laevissima*	51	22.9	8.5	21.6	−1.66	0.47	30.6	19.9	−1.70	0.45
37. *Sellaphora pupula*	90	74.7	6.0	15.0	−1.83	0.35	43.5	15.7	−1.81	0.41
38. *Stauroneis anceps* v. *gracilis*	42	45.2	5.1	35.9	−1.42	0.55	17.8	24.1	−1.62	0.56
39. *Staurosira construens*	122	60.0	32.4	13.6	−1.87	0.33	77.0	13.8	−1.86	0.37

(Continued)

TABLE 2 | Continued

Species number and Species name	N1	Max Abundance (%)	No species transformations				Square-root species transformations			
			N2	Bktr Optimum (µg/L)	Log Optimum (mg/L)	Log Tolerance (mg/L)	N2	Bktr Optimum (µg/L)	Log Optimum (mg/L)	Log Tolerance (mg/L)
40. *Staurosira construens* v. *venter*	68	32.7	14.9	11.8	−1.94	0.30	39.4	13.1	−1.88	0.33
41. *Staurosirella pinnata*	188	93.8	70.6	15.1	−1.82	0.41	127.6	14.2	−1.85	0.41
42. *Stephanodiscus hantzschii*	38	42.7	10.4	87.1	−1.05	0.37	22.2	64.7	−1.19	0.41
43. *Stephanodiscus hantzschii* v. *tenuis*	28	20.1	9.3	43.4	−1.36	0.34	18.9	42.8	−1.37	0.34
44. *Stephanodiscus medius*	50	16.5	11.0	18.7	−1.74	0.27	34.2	20.1	−1.70	0.32
45. *Stephanodiscus minutulus*	94	83.2	32.9	26.5	−1.58	0.41	57.8	27.6	−1.56	0.42
46. *Stephanodiscus niagarae*	47	10.5	17.3	38.4	−1.41	0.35	35.5	33.0	−1.48	0.36
47. *Stephanodiscus parvus*	113	92.6	44.4	27.5	−1.56	0.42	71.5	26.6	−1.58	0.42
48. *Tabellaria flocculosa* v. 3P	86	39.6	13.1	15.6	−1.80	0.28	50.5	13.1	−1.88	0.35

Estimates are only shown for the dominant diatom taxa, that were defined as having achieved a relative abundance of greater than 10% in at least one sample, and a Hill's N2 value based on the untransformed species data > 5. The correlation between the optima and tolerances calculated based on untransformed and transformed species abundances is high and significant ($r > 0.96$, $p < 0.01$, $n = 48$). Species numbers correspond to numbers used in ***Figure 2B****.*

CRS date of 1963 occurred at a depth of ∼ 42.5 cm, close to the ^{137}Cs maximum. The OM content is low throughout the core ranging from 3 to 13%, with higher concentrations in the top 15 cm. The age-depth relationship in both cores follows a strong and significant linear relationship in both cores (north core, $r = 0.97$; south core, $r = 0.98$).

Pigment and Diatom Changes

The cores from both the north and south basins indicated large changes in both pigments (**Figure 4**) and diatom assemblages (**Figure 5**) over the last 200 years. The changes through time are described below in three broad time periods: (i) a pre-disturbance period, c. 1800 to 1950; (ii) a period of nutrient enrichment, ∼c. 1950 to 1990; and (iii) a period of recovery, post c. 1990. Throughout the cores, the planktonic taxa were in higher abundances than the benthic taxa.

Pre-disturbance Period (c. 1800–1950)

The pigment data from c. 1800 to 1950 illustrate the high concentrations of the diatom pigment diatoxanthin in both cores, with the core from the shallower southern basin having substantially much higher concentrations of all pigments in comparison to the north-basin core (**Figure 4**). In the north-basin core, pre-1950 levels of the cyanobacterial pigment echinenone, and the cryptophyte-associated pigment alloxanthin were lower in concentration than the pigment associated with green algae, lutein. In contrast, the south-basin core, pigment concentrations were equally represented by echinenone, lutein, and alloxanthin, which were all at higher concentrations in comparison to the north-basin core.

The diatom assemblages in the north- (**Figure 5A**) and south-basin cores (**Figure 5B**) were both indicative of meso-eutrophic conditions between c. 1800 and 1950. During this time, the diatom assemblages in the north-basin core included a number of planktonic taxa that are well-represented in the modern diatom assemblages including: diatom taxa with TP optima <10 µg/L (*D. stelligera* and *Cyclotella ocellata*), taxa with TP optima between 10 and 20 µg/L (*Asterionella formosa* and *Fragilaria crotonensis*) as well as higher abundances of meso-eutrophic taxa (*Aulacoseira subarctica* and *Stephanodiscus minutulus*) and smaller relative abundances of the meso-eutrophic taxon *Stephanodiscus parvus*. In addition, *Stephanodiscus* cf. *vestibulus*, a taxon not represented in the modern calibration dataset, was present at small abundances (i.e., typically less than 5% relative abundance, **Figure 5A**). Diatom-inferred TP inferences from c. 1800 to 1950 generally varied between ∼15 and 20 µg/L in the north-basin core (**Figure 6**). The largest difference in the c. 1800 to 1950 diatom assemblages in the south-basin core relative to the north-basin core, was the high and variable abundances of the meso-eutrophic taxon *Aulacoseira ambigua*. This species varied between 10 and 30% in the south basin, but did not achieve a relative abundance of >5% in the north-basin core (**Figures 5A,B**). The high relative abundance of this taxon is mirrored by its high concentrations, along with the relatively high abundance and concentrations of the meso-eutrophic taxon *F. crotonensis*, and the higher relative abundance and concentrations of *Aulacoseira granulata* v. *angustissima*, a taxon that is not well-represented in the modern calibration dataset (**Figure 5B**). In contrast to the relatively stable diatom-inferred mesotrophic conditions in the c. 1800–1950 period in

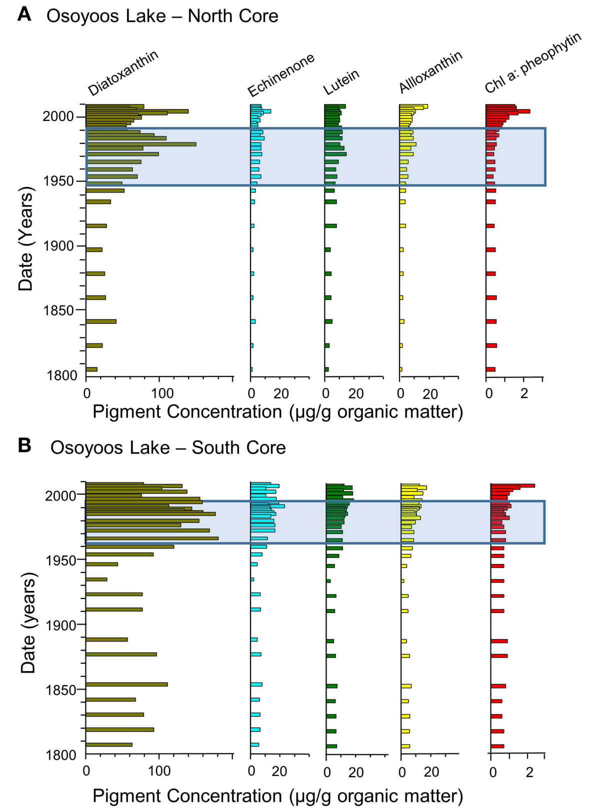

FIGURE 4 | Changes in the pigment concentrations from the dominant algal groups from the dated sediment cores from the north (A) and south (B) basins of Osoyoos Lake over the last 200 years. These pigments include: diatoxanthin (predominantly diatoms), echinenone (cyanobacteria), lutein (green algae), and alloxanthin (cryptophytes). The ratio of chlorophyll *a*: pheophytin is a widely used index of preservation, often an indicator of incomplete post-depositional processes. The blue rectangle represents the zone from which pigment concentrations begin to increase to c. 1990, when the dominant pigment, diatoxanthin, begins to decline, consistent with measured decreases of total phosphorus in modern water chemistry samples.

the north-basin core, the diatom-inferred TP-values from c. 1800 to 1950 varied from ∼15 to 35 µg/L in the south-basin core (**Figure 6**).

Nutrient-enrichment Period (c. 1950–1990)

Starting in the late 1950s in the northern core, and 1960 in the southern core, pigment concentrations began to increase from relatively stable background levels, and increased by ∼2–3 times background concentration for all algal groups (**Figure 4**). Starting in the 1950s in the northern core, there was an increase in the relative abundance and concentration of the meso-eutrophic taxa *S. minutulus* and *S. parvus*, with corresponding decreases in the relative abundance and concentrations of *A. subarctica*, as well as decreases in the relative abundance of the oligo-mesotrophic taxa *D. stelligera* and *C. ocellata* (**Figure 5A**). In the southern core, increases in the relative abundance and concentrations of the more nutrient-rich taxon, *S. minutulus* occurred c. 1960, with a corresponding decrease in the relative abundance of *A. ambigua*, which occurred despite an increase in the concentration of this taxon (**Figure 5B**). These changes resulted in increases in the diatom-inferred TP from ∼15–20 µg/L to 20–40 µg/L between 1950 and 1990 in the northern core (**Figure 6A**), and similar increases at this time in the southern core (**Figure 6B**).

Recovery Period (Post 1990)

Starting in the late 1980s/early 1990s in both cores, there was generally a decrease in the concentration of diatoxanthin, the dominant pigment in both cores, whereas the concentrations of echinenone, lutein, and alloxanthin remain relatively stable (**Figure 4**). The ratio of chlorophyll *a* to pheophytin, a commonly used indicator of preservation (Guilizzoni and Lami, 2003), remained elevated in the post-1990 period in both cores (**Figure 4**). In both cores, there were decreases in the relative abundance and concentrations of the most nutrient-rich diatom taxa (*S. parvus* and *S. minutulus* in the northern core, and *S. minutulus* and *A. ambigua* in the southern core), and resulting increases in the relative abundance and concentrations of many mesotrophic taxa with lower TP optima including *A. subarctica* (**Figure 5**). Taxa that are not well-represented in the modern calibration set, *Cyclotella comensis* and *Cyclotella gordonensis*, increased in both relative abundance and concentration (**Figure 5**). Diatom-inferred TP inferences similarly decrease, with and without the poorly represented taxa in the calibration set (**Figure 6**). The diatom-inferred TP-values between 1950 and 1990 of between 20 and 40 µg/L compare favorably with the measured spring TP between 1969 and 1990 that ranged from ∼18 to 37 µg/L (mean 27 ± 7 µg/L, $n = 14$). Similarly, the post-1990 diatom-inferred values were typically between 15 and 25 µg/L, only slightly higher than the measured values between 1990 and 2009 of between ∼6 and 25 µg/L (mean = 17 ± 5 µg/L, $n = 14$).

Statistical Analyses

The correlations between the main directions of variation in the dominant diatom taxa in the cores from Osoyoos Lake, based on both non-transformed and square-root transformed species abundances, and the diatom-based TP reconstructions (four models: all taxa; all taxa following a square-root transformation; taxa with N2 > 5; and taxa with an N2 > 5 following a square-root transformation) were calculated over the past 200 years, in the north- and south-basin cores. The dominant diatom taxa in the north-basin sediment core accounted for ∼73 ± 4% of the overall abundance of diatom taxa (**Figure 5A**). In the northern core, the PCA axis-1 ($r = 0.93$) and PCA axis-2 scores (0.90) between the non-transformed and transformed diatom taxa were high, as were all of the diatom-based TP reconstructions (correlations between models ranged from 0.86 to 0.99). The diatom-based TP reconstructions were all highly correlated to the PCA axis-2 scores (mean $r = 0.91 \pm 0.04$). However, the λ_R/λ_P ratio was relatively low, ranging from 0.24 to 0.29. Similar patterns were seen in the core from the southern basin. In the southern core, the dominant diatom taxa accounted for ∼79 ± 4% of the overall abundance of diatom taxa (**Figure 5B**). The

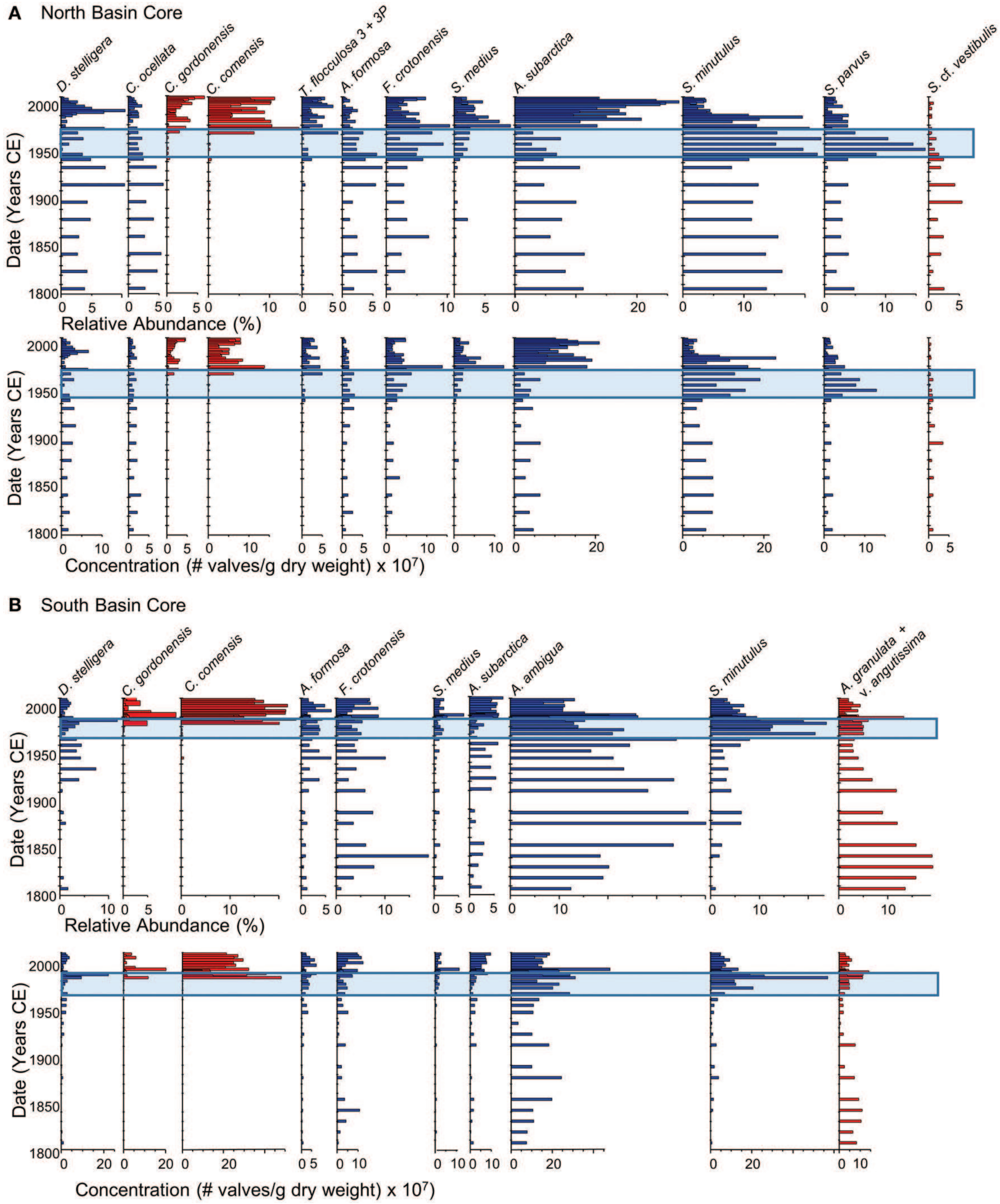

FIGURE 5 | Summary of the changes in the relative abundance and concentrations of the dominant planktonic diatom taxa (>5% relative abundance) over the last 200 years in dated sediment cores from the north (A) and south (B) basins of Osoyoos Lake. Taxa are arranged from left to right based on increasing diatom-inferred total phosphorus optima (**Table 2**). Diatom taxa that were well-represented in the calibration data set are shown in blue (an N2-value of at least 5, and a minimum relative abundance of at least 10%), whereas diatom taxa that were poorly represented (N2 < 5) are shown in red. The blue rectangle represents the period of eutrophication.

correlation between the PCA axis-1 ($r = 0.98$) and PCA axis-2 scores (0.63) between the non-transformed and transformed diatom taxa were high, but with a slightly weaker correlation between the axis-2 scores in comparison to the northern core. As in the northern core, the diatom-based TP reconstructions between the four models were highly correlated (mean $r = 0.68 \pm 0.14$), but the agreement was not as strong as in the northern core. In the southern core, the λ_R/λ_P ratio was relatively low,

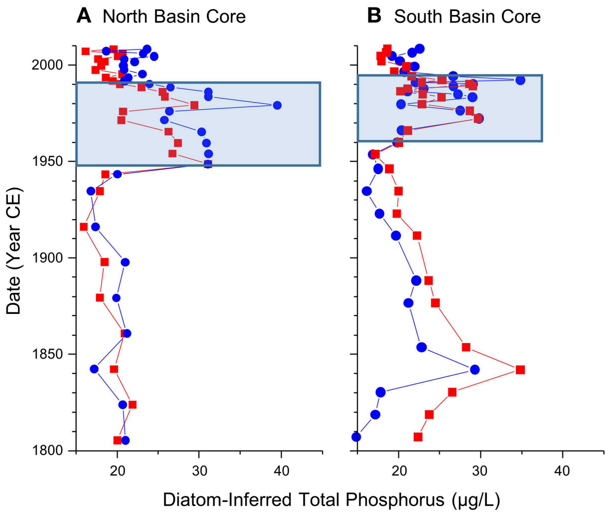

FIGURE 6 | Diatom-inferred total phosphorus values, with (in red) and without (in blue) the poorly represented diatom taxa in the calibration dataset (i.e., taxa shown in red in Figure 5) from the dated sediment cores from the north (A) and south (B) basins of Osoyoos Lake over the last 200 years.

ranged from 0.16 to 0.38, a range much more variable than seen in the northern core.

The vast majority of the diatom taxa that were present in the Osoyoos Lake cores (i.e., taxa represented in blue in **Figures 5A,B**) are well-represented as dominant diatom taxa in the modern calibration dataset (**Table 2**), with four exceptions. Taxa present but poorly represented in the modern calibration dataset include *C. gordonensis* and *C. comensis*, which together occur at a maximum of 10% relative abundances in the modern calibration dataset, but with a species evenness (Hill's N2) of only 2.8. Another taxon, *A. granulata* v. *angustissima* occurs in the calibration dataset at a maximum abundance of ∼24%, but with a low species evenness (N2 = 3.4). Finally, *S.* cf. *vestibulis* is not represented in the modern calibration dataset. The diatom-based TP reconstructions without these poorly represented taxa, were highly similar (e.g., **Figure 6**).

Discussion

Modern-day Calibration Dataset

Algae have historically been a widely used biological proxy for environmental conditions (e.g., reviewed in Stevenson, 2014). Our 251-lake dataset represents one of the largest calibration datasets developed to examine the relationship between diatom assemblages and important limnological variables, including nutrient concentrations. The large gradients in lake-water chemistry in freshwater lakes (i.e., < 0.5 g/L salinity) in this study reflect the tremendous limnological diversity in British Columbia lakes (**Figure 2A**, Supplemental 1), which range from extremely low-TP waters to lakes with greater than 200 µg/L of TP. Using correlations and PCA analyses, we identified strong associations between dissolved inorganic carbon and base cation concentrations, as well as conductivity and salinity (**Table 1**, **Figure 2A**). Fortunately, TP is only moderately correlated with these variables (**Table 1**, **Figure 2A**), thereby allowing the development of a diatom-based transfer function for TP that is at least partially independent from other key environmental gradients. Our application of this model to diatom stratigraphies in sediment cores from Osoyoos Lake, together with the comparison of these data to sedimentary pigment data and water chemistry measurements from the lake, provide further confidence in the validity of our inferences of past lake-water trophic state.

Within the 251-lake calibration set, the dominant (maximum relative abundance >10%, and represented equally in at least five samples) 48 diatom taxa accounted for a mean of 73% of the total assemblage in all lakes, and together with the subdominant taxa accounted for a mean of 82%. The constrained ordinations undertaken on the full diatom dataset of 209 taxa, and the 127 dominant and subdominant taxa, both with or without a square-root transformations, resulted in similar forward-selected environmental variables, eigenvalues, and placement of taxa and lakes, revealing the robustness of this dataset to species transformations. The importance of specific conductance and related variables (pH, DIC), and TP as environmental variables that maximize the dispersion of diatom taxa in the 251-lake dataset is not surprising given the importance of osmotic stress, pH and nutrients from a physiological perspective. Many previous studies have consistently identified conductivity (e.g., Wilson et al., 1996), pH (e.g., Battarbee et al., 2010) and nutrients (e.g., Hall and Smol, 2010; Reavie et al., 2014) as important limnological variables influencing the distribution of diatom assemblages. Estimates of the optima of the dominant taxa to lake-water TP in this dataset, reveal fewer dominant taxa at the extreme ends of the TP gradient, which is not surprising given the physiological constraint associated with coping with too little, or too much phosphorus, as well as the relative rarity of these oligotrophic and more eutrophic lakes (**Figure 3A**). Of the dominant 48 diatom taxa (**Figure 3B**) half are planktonic species, and half are benthic species, and the estimates of species optima are extremely resilient to species transformations, as similar TP optima were estimated with and without a square-root transformation of the species data (**Table 2**).

There has been an active discussion in the paleolimnological community regarding the strength and validity of tracking past conditions, including phosphorus from modern samples. In this study, estimates of the species optima are provided for the dominant diatom taxa (**Figure 3**, **Table 2**). A comparison of the relative TP optima between the dominant planktonic taxa in our dataset (**Figure 3**) with the dominant planktonic taxa from another large dataset from the Laurentian Great Lakes (Reavie et al., 2014), shows strong and significant correlation for the 17 planktonic taxa that are in common ($r = 0.69$, $p = 0.002$), whereas the correlation of the TP optima of the 11 benthic taxa in common is not significant ($r = 0.15$, $p = 0.66$). In an examination of eight relatively small regional datasets (each dataset between 42 and 152 lakes, median ∼68) Juggins et al. (2013) found only 7 of 28 comparisons (or 25%) yielded significant, but weak correlations between the TP optima of benthic taxa, but 9 of 14 (or 64%) resulted in significant and much stronger correlations for planktonic taxa. These results are consistent with earlier observations of the weaker TP responses of benthic taxa that has been noted especially in shallow lakes (e.g., Bennion et al., 2001; Davidson and Jeppesen, 2013). Lower coherence between common benthic taxa across calibrations sets has been suggested to be related to the higher complexity of the benthic habitats and sedimentary nutrient sources (Bennion et al., 2010; Davidson and Jeppesen, 2013). We suggest that comparing taxa that are in common, but poorly represented between relatively small datasets, can introduce noise into comparisons of optima. The stronger correlations between estimates of TP optima from larger datasets is because such datasets provide more rigorous estimates of optima due to higher abundances and occurrences of the dominant taxa. If we had used the inclusion criterion of Juggins et al. (2013) (i.e., that a taxon must occur in at least 10 samples), we would have increased the number of common taxa in our training set by 100 taxa, which would have caused poor comparison of optima between these poorly represented taxa. Our stricter definition of a dominant taxon in addition to using a large dataset are likely why transformed or non-transformed estimates of TP optima for the dominant taxa are of the same magnitude and highly correlated ($r = 0.96$).

The influence of our 251-lake dataset in determining species optima to TP is further shown by comparing optima from a dataset based on 64 lakes from B.C. (Reavie et al., 1995a). Of the 48 dominant taxa in the 251-lake dataset (**Table 2**), only 22 are present in the smaller 64-lake dataset from B.C. with a range in taxa optima from 6 to 42 μg/L (Reavie et al., 1995a) in comparison to 4 to almost 100 μg/L in the 251-lake dataset. The estimates of taxa with high TP optima in the 251-lake dataset are underestimated in the 64-lake dataset (Reavie et al., 1995a), whereas taxa with low TP optima in the 251-lake dataset are overestimated. Examples of the underestimates of taxa with the highest TP optima in the 251-lake dataset include: 87 vs. 14 μg/L for *S. hantzschii*, 43 vs. 15 μg/L in the smaller dataset for *S. hantzschii* v. tenuis, 44 vs. 17 μg/L for *S. parvus*, 33 vs. 14 μg/L for *S. minutulus*, and 35 vs. 22 μg/L for *A. ambigua*. Similarly, the TP optima with low TP taxa in the 251-lake dataset are overestimated in the smaller dataset (e.g., 4 vs. 15 μg/L for A. distans, 5 vs. 14 μg/L for *D. stelligera*, 5 vs. 20 μg/L for *C. ocellata*). Based on these estimates of TP optima, depending on the taxa present in a core (Reavie et al., 1995b, 2000), past approaches could result in an overestimation of TP-values if more oligotrophic taxa were present, or an underestimation if more eutrophic taxa were present. This bias is likely present in other regions besides British Columbia, since smaller calibration datasets are normally used in the development of lake-water TP models (i.e., of the 23 calibration datasets summarized by Hall and Smol (2010), the number of lakes range from 30 to 238 lakes, with a median dataset size of 53 lakes), and larger datasets appear to be the exception (Dixit et al., 1999; Reavie and Edlund, 2013; Reavie et al., 2014).

In the 251-lake dataset there was little difference in the predictive abilities of the TP inference models developed using all taxa, and/or only the dominant and subdominant taxa, with or without species transformations based on analyses of the 251-lake dataset. All TP inference models had moderate predictive abilities with apparent coefficients of determination of between 0.57 and 0.63 and bootstrapped values between 0.51 and 0.55 (e.g., **Figure 3C**). This is well within the range of 0.31 to 0.82 reported from the 17 studies that reported these values (mean = 0.55; standard deviation = 0.18) (Hall and Smol, 2010). However, the models from the 251-lake dataset represent an improvement over many of these smaller calibration datasets. For example, the 46-lake dataset of Hall and Smol (1992) from B.C. had a relatively high apparent coefficient of determination of 0.78, which declined to 0.28 when bootstrapped (Cumming et al., 1995), due to a lack of redundancy in taxa that could not be reproduced upon bootstrapping. When the Hall and Smol (1992) dataset was augmented to include 18 additional lakes (Reavie et al., 1995a), the coefficient of determination after bootstrapping increased to 0.46.

Much effort has gone into investigating the optimal sample size for diatom-based inference models for inferring pH and salinity, as well as lake-water TP (Wilson et al., 1996; Reavie and Juggins, 2011; Bennett et al., 2014), mainly using model performance parameters including model coefficient of determination, error and bias, and have normally resulted in estimates of at least 50 samples. However, this current study suggests an assessment of the minimum number of lakes to produce good indicators of model performance, is not the same question as developing a calibration dataset with the goal of producing precise and accurate estimates of species parameters (e.g., optima) of the taxa that are present in the cores. The number of lakes required for the latter is much greater than the former, and at this point, our current 251-lake dataset only adequately represents the species optima to TP for 48 taxa, albeit taxa that are common and abundant in many lakes. Modern calibration datasets will need to be much larger, if taxa in the cores occur at a higher abundance than taxa in the modern calibration datasets.

Paleolimnological Analysis of Osoyoos Lake

The present study is based on longer, better-dated sediment cores, allowing a more thorough reconstruction of human impacts on the lake than in the earlier paleoenvironmental studies of Osoyoos Lake (Anderson, 1973; Pinsent and Stockner, 1974; Ryder, 1994). This study employed a multiproxy analysis of primary producers using algal pigments and several metrics based on the modern ecological characteristics of diatom assemblages, including relative abundances and concentration changes, proxies that were not undertaken in earlier studies. These proxies provide estimates of background conditions, evidence of cultural eutrophication starting in the 1950s, and subsequent recovery from eutrophication starting c. 1990, a trend also apparent in the long-term water chemistry measurements. Changes in the planktonic diatom taxa were emphasized because planktonic taxa are better indicators of lake-water TP (see earlier), and also an important component of the diatom flora in the Osoyoos Lake (**Figure 5**). The various metrics that were used to assess the strength of the diatom-based TP reconstructions, indicate that the reconstructions do track a main direction of variation in the diatom assemblages (as indicated by the strong correlation with PCA axis-2 scores), but that other factors are also important. The main direction of variation (the PCA axis-1 scores) tracks the abrupt change in diatom assemblages c. 1990, which is consistent with declines in TP concentration, but is likely also linked to more complex changes in Osoyoos Lake that may be related to climate.

Background conditions of trophic status of the north basin suggest meso-eutrophic background conditions from c. 1800 to c. 1950. These estimates were based on the pigments, the relative abundances and concentrations of diatom taxa, and associated diatom-based TP inferences of meso-eutrophic background conditions (**Figure 6**). Prior to 1950, pigment concentrations in both cores were lower than post-1950 concentrations. All diatom taxa in the north core are well represented by dominant taxa in the calibration dataset, with the exception of S. cf. vestibulus, a taxon that was present at a low abundance, suggesting the taxa optima on which the TP inferences are based are reliable. The background conditions c. 1800 to pre-1960 period in the south basin of Osoyoos Lake suggests meso-eutrophic conditions, but with more variability in the background conditions in comparison to the core from the northern basin. During this period, pigment concentrations in the southern core were also relatively low and relatively stable between 1800 and 1960 in comparison to post-1960 concentrations, but concentrations were approximately double the values seen in the northern core (**Figure 4**). The dominant diatom taxon in the southern basin was *A. ambigua*, a meso-eutrophic diatom (**Figure 3**, **Table 2**), which fluctuated between 10 and 30% relative abundance from c. 1800 to 1960 (**Figure 5**). The diatom taxon, *Aulacoseira granulata* v. *angutissima* had sustained relative abundances of over 10% between c. 1800 and 1850, which contributes to the higher TP inferences due to a high TP optimum for this taxon in the 251-lake calibration (74 μg/L). However, the TP optimum for this taxon is based on data where it did not achieve a species evenness > 5 (Hill's N2-value = 3.4 with 14 occurrences). Nonetheless, the TP inferences remained high once all taxa with Hill's N2-values < 5 were eliminated (**Figure 6**), suggesting that the reconstruction is only partially sensitive to the elimination of *A. granulata* v. *angustissima*. Larger variation in the TP-values prior to 1950 could be due to the shallower nature of the southern basin (maximum depth of ∼23 m, in comparison to the northern basin that has a maximum depth in excess of 60 m) that could make it more sensitive to changes in settlement, climate and/or factors associated with salmon migration. The higher concentrations of pigments in the southern basin in comparison to the northern basin is also likely due to the higher preservation of pigments at shallower sites (Cuddington and Leavitt, 1999).

The pigment and diatom proxy data infer an increase in primary producers in Osoyoos Lake beginning c. 1950 in the northern basin and c. 1960 in the southern core, likely associated with the enhanced loading from point- and non-point sources (Jensen and Epp, 2002) due to the exponential growth of human activities in the Okanagan since the 1940s. Increases in algal pigments occur in both cores, and there were increases in the relative abundance and concentrations of diatom taxa with higher TP optima including *S. minutulus* and *S. parvus* in the northern core, and *S. minutulus* and *A. granulata* v. *angustissima* in the southern core, as well as increases in the concentration of *A. ambigua* (**Figure 5**). The abundances of these taxa remain elevated until c. 1990, as do the diatom-based TP inferences (**Figure 6**). The diatom-based TP increases correspond with increases in the concentrations of all pigments c. 1950 in the northern core and c. 1960 in the southern core, a trend that equates to an approximate doubling in the dominant sedimentary pigment diatoxanthin, a robust diatom indicator (Leavitt and Hodgson, 2001). The increased pigments c. 1950/1960 is not an artifact of changes in pigment preservation with sediment depth because the ratio of chl *a*: total phaeopigments (**Figure 4**), a common and conservative metric for evaluating diagenesis (Guilizzoni and Lami, 2003), was relatively stable from c. 1800 to ∼2000. The earlier onset of eutrophication in the northern and southern basins cannot be discerned with a high degree of certainty, given the relatively low unsupported ^{210}Pb activities in both cores.

Post-1990 changes in the cores are consistent with the controls on nutrient loading, especially the enhanced P removal at municipal sewage treatment facilities (Jensen and Epp, 2002). The diatoxanthin concentrations decrease post-1990, but the chl *a*: total phaeopigment ratio is elevated, suggesting that diagenetic processes are not complete, and that measured pigment concentrations may be an overestimate (**Figure 4**). There were large changes in the diatom assemblage from both basins c. 1990, where there were decreases in the abundances and concentrations of more eutrophic taxa in both cores, and pronounced increases in *C. gordonensis* and *C. comensis*. These two taxa were poorly represented in our 251-lake calibration dataset achieving a Hill's N2 of only 2.8. (i.e., effectively present in only 2.8 lakes, but occurred in 9 lakes). The removal of the taxa with an N2 < 5 from the inference model, resulted in a similar decrease in the diatom-inferred TP-value to generally < 20 μg/L in both the northern and southern basins, consistent with a decrease in the mean spring lake-water TP concentrations between 1969 and 1990 of 10 μg/L.

The abrupt appearance of *C. gordonensis* and *C. comensis*, taxa that did not exist in this lake before the 1990s, suggests that other environmental changes, apart from the decreases in TP, may have occurred. Recent increases in small *Cyclotella* species (including *C. comensis/gordonensis* group) have been noted in many lakes and have been attributed to climate-related changes, including enhanced ice-free periods and stratification (Rühland et al., 2008). However, in the Osoyoos Lake sediment cores *Aulacoseira* taxa increased, a taxon that has been noted to decline in other studies, and thus surmised to further support enhanced stratification as a potential mechanism for the rises in *Cyclotella* (e.g., Rühland et al., 2008). Saros and Anderson (2014) emphasize the importance of the ecology of individual *Cyclotella* taxa and stress the importance of several factors, including nutrients, dissolved organic carbon and light. The present study cannot deduce the actual mechanism behind the increase in *C. comensis/gordonensis*. This new assemblage is likely the result of other limnological changes (including changes in seasonality) perhaps influenced by warmer conditions experienced in the Okanagan over the past several decades and/or changes in precipitation patterns (Pacific Climate Impact

Consortium, 2010) that can impact lake dynamics. However, studies on the seasonality of these taxa would be an important first step to understanding their recent increases in Osoyoos Lake.

In conclusion, the development of strong and appropriate diatom-based inference models inferring lake-water TP is challenging. Large diatom-based datasets allow better estimates of modern relationships than smaller datasets, because more taxa with higher abundances and more even occurrences are encountered. Estimates of species optima to any environmental variable, need to be critically assessed, especially when these estimates are based on low abundances and occurrences, as is typical of many small calibration datasets. Sediment cores from the north and south basins from Osoyoos Lake were investigated using sediment pigments and diatom assemblages to infer background conditions, assess the timing of cultural eutrophication, and assess if biological proxies could track post-1990 trends in improved water quality associated with mitigation efforts in the watershed. Both basins of Osoyoos Lake were inferred to be mesotrophic, and became increasingly eutrophic in the mid-20th century co-incident with the onset of rapid population growth and nutrient loading in the Okanagan. Mitigation efforts have resulted in diatom-inferred TP levels declining to values similar to pre-disturbance values (pre-1950), albeit with a different diatom assemblage. Multiproxy paleolimnological approaches are the only way to provide key insights into changes in lakes over centuries that can be helpful in understanding long-term trophic dynamics which can be helpful in developing realistic mitigation targets.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2015.00084

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Limnetic total phosphorus transfer functions for lake management: considerations about their design, use, and effectiveness

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Regulatory agencies often rely on paleolimnological studies for models that predict variables pertinent to nutrient loading or to public perception. Limitations of statistical approaches often pose significant challenges. We present a case study from Florida USA that involves diatom-based inference models derived from two calibration sets. Spatial autocorrelation conclusions differed with methods and approaches, and h block cross validation was unduly pessimistic. Calibration sets and temporal sets represent fundamentally different populations. The accuracy and precision of temporal inferences for specific lakes can be affected by site-specific factors, and are not likely to be known with the certainty suggested by models. Error terms can provide a false sense of knowledge about the reliability of inferences for temporal samples. Broad error terms for limnetic total phosphorus models have little or no utility in any event. Limnetic total P models can perform poorly when applied to N-limited lakes. Transfer functions should be regarded more as qualitative indicators of past water quality rather than methods with known precision, and more emphasis should be placed on multiple lines of evidence and ecological interpretations.

Keywords: transfer functions, phosphorus, lake management, diatoms, paleolimnology

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Introduction

Transfer functions are common tools for quantifying changes in limnetic nutrient concentrations and primary-producer standing crops in lakes that have been subject to eutrophication (Brooks et al., 2001; Vermaire and Gregory-Eaves, 2007; Guilizzoni et al., 2010). Models are derived that relate recent biological remains in sediments to the measured water quality of overlying waters for a large set of lakes on the landscape. The goal is to construct defensible models that can provide inferences for passive (sediment-core) samples when historic water-quality data are lacking. Models typically are tested with cross-validation procedures that are intended to provide realistic estimates of error terms. Inferences based on assemblages in sediment cores are used to help define reference conditions and restoration goals for lake-management programs (Smol, 1992; Brenner et al., 1993; Battarbee, 1999). This paper focuses on diatoms as historic indicators, but the concepts are applicable to other biological indicators used for eutrophication assessment.

Modern quantitative methods (CCA: ter Braak, 1986; ML and WA: Birks et al., 1990; WA-PLS: ter Braak and Juggins, 1993) were first used to assess water-quality change in acid-rain studies.

Study regions were sometimes remote, and lakes were often similar in water quality (e.g., Davis et al., 1983; Dixit et al., 1988; Charles et al., 1990). Calibration and core lakes were generally acidic, poorly buffered, and low in nutrient content. Lakes were affected by relatively few aspects of human influence, so inferences were subject to minimal covariant influence. Because, pH was never de-transformed from its logarithm expression, error terms were small with respect to inferred means, and models appeared to have high precision. The same modeling approaches have been applied to eutrophication assessment, but this application involves greater limnological complexity, so it merits careful scrutiny.

Several factors contribute to greater environmental complexity in eutrophication studies than in acid-rain studies. Eutrophication is not regionally uniform, but it depends on unique combinations of local influences that include agricultural runoff, storm-water inputs, septic and sewage-treatment inputs, and industrial wastes. Nutrient inputs of nitrogen and phosphorus vary depending on specific sources (Riedinger-Whitmore et al., 2005; Davidson and Jeppesen, 2013). Lakes that undergo eutrophication in urban settings can be subject to simultaneous changes in alkalinity or pH (Ramstack et al., 2003; Brenner et al., 2006; Whitmore et al., 2006). Even within a given study region, factors that induce trophic-state changes can vary.

Lakes of greatest management concern often lie in areas where there is high population density and diverse environmental influences. Lakes in historic studies might have been subject to unexpected influences, such as contaminants, which affected biological indicators in ways that could not be anticipated with a calibration set. The complex nature of urban lakes and eutrophication introduces many sources of variance that potentially create substantial differences between populations of spatial (calibration) and temporal (core) samples.

This paper addresses issues related to the practical aspects of modeling trophic-state changes for lake-management programs. We discuss some statistical approaches, use examples to illustrate potential concerns, and show why knowledge about specific lakes, their history, and ecology can be important for evaluating quantitative inferences. We also examine generalizations about models and eutrophication assessments in regions with shallow lakes.

Purpose-designed Models for Lake Management Programs and Their Challenges

Lake-management agencies typically have well-defined concerns about water-quality issues within their jurisdictions. Paleolimnologists are sometimes called on to construct calibration sets and transfer functions to address criteria within a specific range of water quality. Agencies might intend to regulate water quality for chlorophyll *a* concentrations or cyanobacteria because these factors are evident to the public. In other cases, agencies focus on limnetic nutrient concentrations in order to regulate nutrient loading. Variables of management concern are likely to determine the choice of dependent variables for models. Agencies might have preconceived ideas about reference conditions or calibration designs, and sometimes might not be well-informed about the necessary size or range of calibration sets, or how transfer functions work. Paleolimnologists often must work actively with agencies to inform them about appropriate project design and methods.

A case study from Florida, USA illustrates typical management needs, and how paleolimnological studies often work within constraints imposed by agencies. Florida Department of Environmental Protection (FDEP) asked us to create a calibration set and transfer function to assess nutrient criteria standards proposed by the U.S. Environmental Protection Agency (EPA). FDEP was concerned that the maximum acceptable chlorophyll *a* concentration (20 μg/L) promulgated by the EPA for lakes with color > 40 PCU and alkalinity >20 mg/L CaCO3 was subject to a high Type I error rate, so the proposed federal standard would incorrectly describe naturally productive lakes as impaired.

FDEP requested a model to infer past chlorophyll *a*, but because this is a response variable, we advocated for inclusion of a limnetic total phosphorus (P) inference model. FDEP selected lakes in the color range of 20–100 PCU and alkalinity range of 20–200 mg/L, which mitigated that lakes would have pH values $>\sim 6.5$. We had concern that reference conditions for some lakes might lie outside of this range. FDEP initially proposed 30 calibration lakes and 30 historic application lakes. We advocated for appropriate calibration size (Reavie and Juggins, 2011) by invoking expert opinion through the Paleolimnology Forum listservice, after which the agency agreed to a 70-lake calibration design with a 5-lake application. The agency provided 5-year means of water-quality data from 2005 to 2010 to match sediment surface samples, but our research team was contractually required to collect the surface samples in 2012–2013. Record-low water levels in 2012–2013 changed solute concentrations and introduced a source of error between diatoms in surface samples and the older water-quality values.

As we anticipated, the chlorophyll *a* transfer function ($r^2 = 0.51$, RMSE $= 0.224$ $\log_{10}$ chlorophyll *a*, $n = 72$) had less predictive capability than the total P model ($r^2 = 0.62$, RMSE $=$ 0.183 $\log_{10}$ total P). Analog matching showed that reference conditions for 4 of the 5 historical lakes were outside the calibration range, which substantiated our initial concern. Our report was redeemed somewhat by inclusion of a previous model based on a broader calibration design that had stronger predictive capability ($r^2 = 0.88$, RMSE $= 0.177$ $\log_{10}$ total P, $r^2_{\text{boot}} = 0.77$, $n = 69$). We interpreted lake histories using multiple lines of evidence in addition to water-quality inferences.

This example shows that studies designed to address specific needs of lake-management agencies often must be conducted within imposed constraints that can create practical challenges for paleolimnologists. Investigators must use judgment and advocate for appropriate project design when necessary.

Spatial Autocorrelation, Cross Validation, and the Comparability of Spatial and Temporal Sets

Agencies often seek precision estimates for water-quality inferences. There are inherent problems, however, with defining appropriate error terms for temporal samples based on spatial calibration sets. The following example illustrates potential difficulties.

For needs of the FDEP project, we used expert statistical consultation (Steve Juggins, Newcastle University) for model development and testing. Despite apparent reasonable predictive capability of the total P model ($r^2 = 0.62$), spatial dependency in environmental data was indicated by Mantel correlograms (Oden and Sokal, 1986) at 10-km distances. RMSE estimates increased significantly at 20-km distances, so h block cross validation (Telford and Birks, 2009) was used to exclude samples at a critical cutoff distance of 20 km during cross-validation cycles. As a result, the r^2 of the total P model dropped to 0.27 with an RMSEP of 0.27 $\log_{10}$ μg/L. At this level of performance, a total P inference of 50 μg/L had a 68% confidence interval of 27–93 μg/L, and a 95% confidence interval of 15–169 μg/L, rendering inferences useless for lake-management purposes. The chlorophyll *a* model was deemed to have no practical predictive power.

This assessment proved untenable for the agency. Following the above assessment, the present authors sought other perspectives about model utility. We cross validated the total P model with surface samples from 41 independent lakes that had known water quality. The r^2 was 0.48, less than the ostensible r^2 of 0.62, distinctly better than the h-block estimate of 0.27, and comparable to the bootstrap estimate of 0.46. We interpret that spatial autocorrelation in environmental data alone might not have posed a problem as serious as h block cross validation suggested. Autocorrelation must occur in both response and environmental variables for regression coefficients to be adversely affected (Legendre et al., 2002). In addition, calibration lakes were 1–5 km apart, so the elimination distance of 20 km in h block testing probably led to removal of too many lakes and caused performance estimates to deteriorate. With h block cross validation, there are evident risks of determining that potentially useful models have reduced or little practical utility.

Telford and Birks (2009) showed that pseudoreplication from spatial dependency can lead to underestimation of prediction errors. Similar lakes in one or more regions can create apparent strength in predictive ability, and cause overly optimistic error estimates. If a model is applied to a lake from a dissimilar region, actual error terms for inferences might be larger than the model suggests. By removing pseudoreplicate samples, h block cross validation provides more pessimistic error estimates to compensate for that tendency. If a passive sample is well-centered in the calibration set, however, h block might provide error estimates that are too pessimistic. We question whether potentially overly optimistic or overly pessimistic error estimates are preferable, and suggest that neither might be objectively more correct.

In some calibration sets, regional geology might contribute to spatial relationships in water quality. Judgements about the inclusion of environmental variables might affect conclusions about autocorrelation. For example, Secchi depth and chlorophyll *a* are highly correlated and interdependent, as are alkalinity and specific conductance. All such correlated variables were included in the assessment that found the FDEP models of no value because of spatial environmental patterns. For additional insight about lakes in this region, we tested for spatial autocorrelation in our older calibration set ($r^2 = 0.88$, $n = 69$ lakes) using multivariate Mantel correlograms and multiscale ordination (MSO: Borcard et al., 2011). For these tests, we used *a priori* knowledge based on exploratory analyses to select only environmental variables that exert significant influence on diatom assemblages, and we avoided redundant environmental variables. These tests showed no spatial dependency in diatom or environmental data, including at the 20-km distance. Spatial autocorrelation appears to be influenced by lakes in the calibration set and by the selection of variables. Our conclusions from this experience were that no single method of assessing model performance or error terms seems distinctly more correct or defensible, but the outcome of testing might discount models that could be informative for lake management programs.

Another important concern is the potential distinction between spatial and temporal data sets. Cross validation tests evaluate relationships among environmental and biological data from the landscape, but they don't necessarily provide accurate error estimates for samples in a given sediment core. An RMSEP estimate is determined using separate lakes, whereas samples in a core are temporally correlated. In two lakes for which we have >20 years of modern water-quality values, limnetic total P and pH have no significant correlation ($r = 0.17$, $p = 0.78$; $r = 0.08$, $p = 0.50$) whereas in the calibration set, they are significantly correlated ($r = 0.44$, $p < 0.001$). Samples in calibration and historic sets represent different populations, and can have incomparable types of variance and relationships among environmental variables.

The Site-Specific Nature of Model Response in Complex Systems

Secondary variables might change over time in given study lakes, and might unpredictably exert covariant effects on biological indicators and inferences for variables of interest. As a case example, Lakes Lochloosa and Little Jackson in Florida have been subject to substantial increases in P loading during the twentieth century. Lochloosa experienced an 8-fold increase in P deposition (Kenney et al., 2014), and Little Jackson sustained an approximate 5-fold increase (Whitmore et al., 2006). Diatom-based limnetic total P inferences reflect increased P loading in Lochloosa, but they do not in Little Lake Jackson (**Figure 1**).

The difference in model response might result from effects of pH on diatom assemblages. Little Lake Jackson underwent significant alkalization during the twentieth century because of wetland destruction and ionic loading (Whitmore et al., 2006: **Figure 1**). The lake also was subject to substantial inputs of

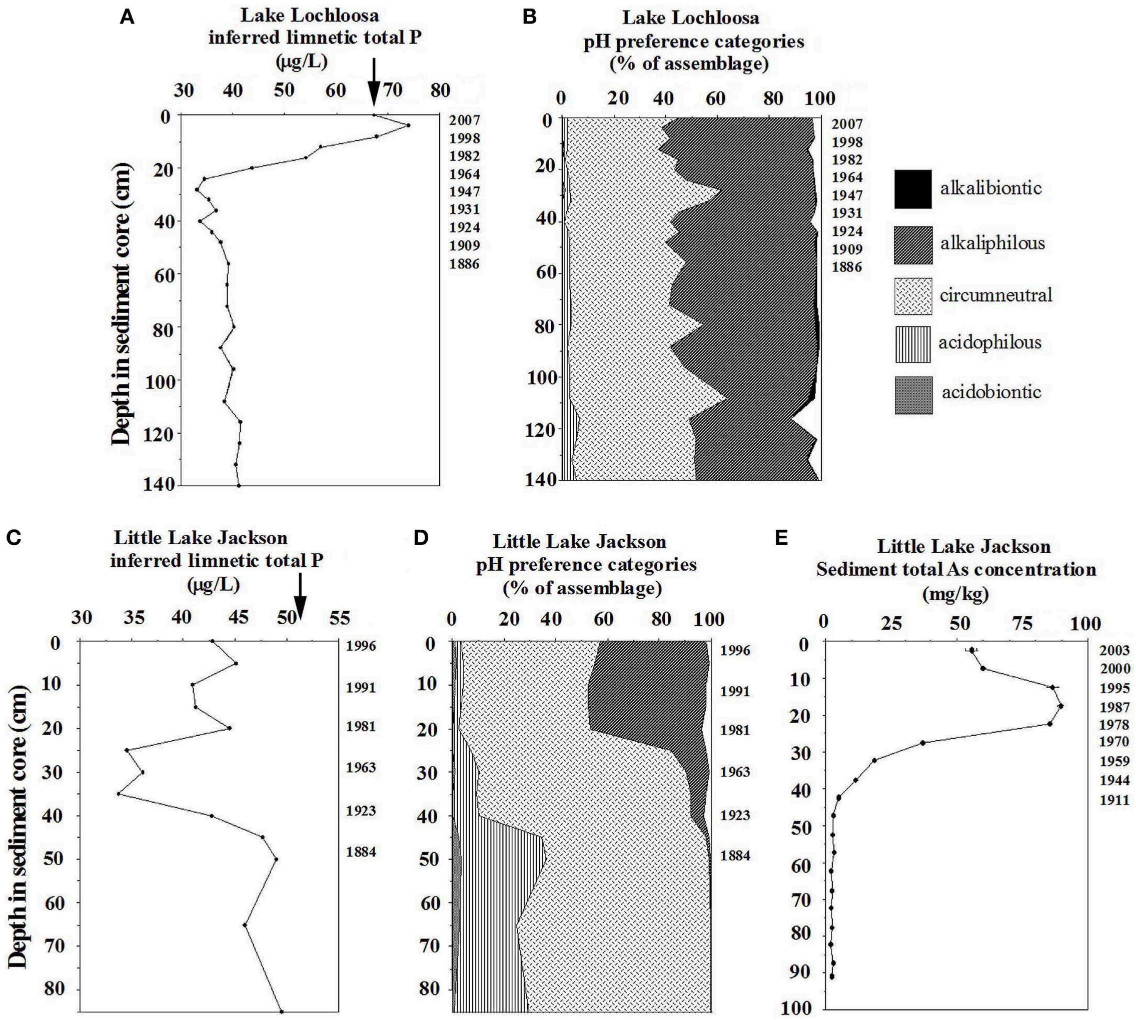

FIGURE 1 | Diatom-based inferred limnetic total P values for Lakes Lochloosa and Little Jackson, with summary pH autecological preference information, and sedimented arsenic concentrations in Little Lake Jackson. Summary pH autecological information was calculated as in Whitmore et al. (1996). Arrows on total P panels indicate means of modern measured water quality. **(A)** Inferred limnetic total P for Lake Lochloosa (modified from Kenney et al., 2014). **(B)** Summary pH preference data for diatoms in Lake Lochloosa. **(C)** Inferred limnetic total P for Little Lake Jackson. **(D)** Summary pH preference data for diatoms in Little Jackson (updated from Whitmore et al., 2006). **(E)** Net sedimented total As concentrations from Little Lake Jackson (modified from Whitmore et al., 2008).

arsenical pesticides (Whitmore et al., 2008; **Figure 1**), which might have affected diatom communities. In contrast, Lake Lochloosa showed no change in pH during increased P loading (Kenney et al., 2014; **Figure 1**). Limnetic total P inferences at the base of the Lochloosa core were consistent with reference conditions for the region, and the modern total P inference (67.2 μg/L) was nearly identical to the modern measured mean (67.4 μg/L, **Figure 1**). Differences in model performance did not result from an abundance of fragilarioid diatoms, an often generalized explanation for model inaccuracy in shallow lakes (Bennion et al., 2001; Sayer, 2001; Heathcote et al., 2015). In surface sediments of both lakes, benthic fragilarioid taxa represent ∼ 30% of the assemblage, and species of *Aulacoseira* represent ∼ 20%, along with various other taxa. Lochloosa, which had more tenable total P inferences, is 44 times larger in size and is shallower (max. depth = 3 m) than Little Jackson (max. depth = 6.9 m). Lochloosa might have been expected to yield worse inferences than Little Jackson because of benthic diatom proliferation or wind-generated mixing of sediments, but this was not the case. In short, we can not generalize whether models are likely to work well or fail based on lake depth or other broad characteristics.

Generalizations about Fragilarioid Taxa in Shallow Lakes

In many of our studies in Florida, fragilarioid taxa are not so much ubiquitous or responding to light in shallow water with indifference to nutrients (e.g., Bennion et al., 2001) as they are indicative of hypereutrophic conditions, where light penetration is poor. Their survival in low-light conditions is probably possible because of tychoplanktonic lifestyles (Sayer, 2001). In our more reliable calibration set, limnetic total P optima of *Pseudostaurosira brevistriata* (127 µg/L), *Staurosira construens* var. *venter* (89 µg/L), and *Staurosirella pinnata* (87 µg/L) are significantly higher, for example, than those of *Aulacoseira ambigua* (49 µg/L), *A. granulata* (44 µg/L), or *A. distans* (50 µg/L). Lakes in the range of fragilarioid optima often are dominated by cyanobacteria. *P. brevistriata* is significantly correlated with $\log_{10}$ limnetic total P ($r = 0.51$, $p < 0.001$, $n = 25$) but not with mean water depth ($r = 0.06$). Temporal shifts to fragilarioid dominance in Florida often mark the onset of advanced eutrophication. Florida lakes in this range tend to be N limited (Kratzer and Brezonik, 1981; Paulic et al., 1996), causing models to underestimate limnetic total P because it is available in excess of biological needs. N-limited lakes often must be eliminated from our total P calibration sets (Brenner et al., 1993; Riedinger-Whitmore et al., 2005; Kenney et al., 2014) because total P is underestimated in those lakes. Elimination of fragilarioid taxa from diatom profiles would remove important ecological information about water-quality change in many cases, and their presence in our region can not be generalized as non-informative.

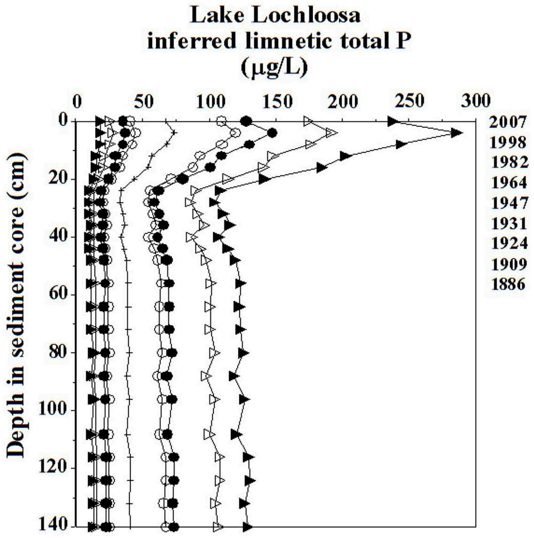

FIGURE 2 | Confidence intervals for diatom-based inferred limnetic total P values for the Lake Lochloosa core. Hatched lines: inferred means. Open circles: 68% confidence intervals (± 1 SE) for the model. Closed circles: 95% confidence intervals (± 1.96 SE) for the model. Open triangles: 68% confidence intervals based on bootstrapping. Closed triangles: 95% confidence intervals based on bootstrapping.

Impractically Large Error Expressions Serve Little Useful Purpose

Because agencies regulate variables such as limnetic total P rather than $\log_{10}$ total P, eutrophication studies don't have the luxury of leaving inferences log transformed, as with acidification studies. When confidence intervals become so broad that they span several trophic-state categories, which often occurs when log total P inferences are de-transformed, they serve no practical use for lake-management purposes. **Figure 2** shows confidence intervals for limnetic total P reconstructions in Lake Lochloosa. Despite accurate inferences with respect to reference and modern conditions and a fairly robust model ($r^2 = 0.88$, RMSE $= 0.177$ $\log_{10}$ total P, $n = 69$, $r^2_{\text{boot}} = 0.77$), confidence intervals are uninformative: it is impossible to demonstrate water-quality change based on error terms from the de-transformed total P inferences. Many published studies report RMSE for limnetic total P models in log-transformed units to avoid unreasonably broad confidence limits. We note that even for strong limnetic P models, investigators might do well to intentionally disregard error terms and treat inferences as qualitative guidelines.

Are Univariate Models the Best We Can Offer?

Statistical approaches for univariate models typically involve removing less common taxa to minimize noise. Eliminating "non-informative" taxa (Juggins et al., 2015) to narrow confidence intervals might provide greater precision, but it can remove information about factors other than the specific variable of interest. Removing taxa shifts the focus to species that serve a singular purpose. Taxa that appear non-informative for a univariate model can reflect other aspects of a lake's history, such as past hydrological influences, macrophyte presence, or changes in water depth. When taxa are removed from an assessment, it narrows the focus of interpretation. Is this potentially "throwing out the baby with the bath water"?

Prediction models typically involve acceptance of basic assumptions that potentially limit the thinking of paleolimnologists about historical changes. One is that system response can be meaningfully understood by focusing on one variable of interest. Another is that by studying the general, we can better understand the specific. A third is that by eliminating less common taxa, we obtain more information about a system. To quote a comment by Deevey (1988) regarding assumptions of hydrological modeling, "If this confidence is misplaced, most limnologists would probably prefer not to hear about it."

Conclusions and Recommendations

Environmental protection and conservation agencies deal with considerable financial and legal obligations related to lake management, so paleolimnological investigators must help address their needs while endeavoring to apply defensible scientific practices. Investigators often are required to work within constraints of project designs, and to actively inform agencies about appropriate study design, optimal approaches, and expected outcomes.

We suggest that calibration sets and temporal sets of data are potentially different populations. The accuracy and precision of temporal inferences for a given lake can be affected largely by site-specific factors, and they are not likely to be known with the objective certainty that modeling usually implies. The effects of local influences can vary over time as well. Using a deductive approach to quantify precision is tenuous for temporal samples in lakes that have unique histories and arrays of environmental influences. Error terms in some cases might underestimate the informativeness of inferences, and in other cases might imply accuracy that is not warranted because of site-specific influences that can't be anticipated through the calibration. As a result, statements about statistical precision can mislead lake managers about the certainty or usefulness of reference estimates. Broad error terms for limnetic total P models generally have little or no utility. In view of these considerations, we recommend that limnetic total P inferences should be regarded as qualitative guidelines rather than values with known precision. To do otherwise involves some degree of misrepresentation.

The accuracy of inferences for reference conditions are best judged in terms of reasonableness with respect to benchmark lakes and edaphic conditions for a given region. Trajectories should be compared with multiple lines of evidence to evaluate patterns and timing, and inferences should be compared with periods of known water quality when data are available. Compelling arguments have been made about the need to incorporate contemporary ecology into assessments of lake ecosystem change (Battarbee et al., 2005; Sayer et al., 2010), and paleolimnologists should consider this advice. The most comprehensive historical reconstructions are those that address multiple system responses (Davidson et al., 2010), and those that combine assessments of floristic change and known ecologies of taxa with quantitative estimates (e.g., Bennion et al., 2011). We recommend that studies addressing trophic-state assessment for lake-management purposes should avoid heavy reliance on univariate models. Statistical modeling does not provide an adequate substitute for a more comprehensive knowledge about the ecology and limnology of the systems being studied.

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Muddied Waters: The Case for Mitigating Sediment and Nutrient Flux to Optimize Restoration Response in the Murray-Darling Basin, Australia

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The waters of the Murray Darling Basin, Australia, have endured multiple stressors for more than a century. Detectable salinization impacts are evident from 1880 CE and elevated fluxes of sediments and nutrients are now widespread. Most wetlands examined paleolimnologically have shown increased sedimentation rates or have lost aquatic plant communities due to the shading effect of increased turbidity, prompting the observation that the waterways of the Murray Darling Basin are among 10 Australian ecosystems most at risk from tipping points. This post-European heightened sediment flux threatens the potential ecological recovery from the application of scarce and expensive environmental water. Nutrients and fine sediments are implicated as drivers of regime shifts that advantage phytoplankton and inhibit the growth of productive macrophyte beds. However, with the river channels identified as likely sources of sediments and sediment-bound phosphorous, it remains possible that the documented ecological changes represent an ongoing response from continued doses from the River. Syntheses of multiple paleolimnological records provide evidence for the management focus to be on sediment supply to maximize the ecological benefit from environmental flow allocations. Here we use paleolimnology to examine in detail the nature and magnitude of the response in a subset of 17 wetlands, to propose means of optimizing the ecological bounce from the release of river waters, encumbered with high doses of sediments and nutrients, to wetlands and floodplains.

Keywords: diatoms, wetlands, floodplain, regime shift, environmental flows, sediments, turbidity

INTRODUCTION

Much research on the wetlands of the Murray Darling Basin, Australia, has taken place over recent decades using contemporary approaches of hydrology and limnology. The high level of abstraction since the 1960s has drawn focus to changes in flow and water volume as principal drivers of wetland change (Jones et al., 2002a). Also, the regulation of the system from the 1920s is regarded as a major driver of change. In concert, these forces have led to reductions in the connectivity between the river and its floodplain which change trophic dynamics, the seasonality of peak flow that alters breeding triggers, the temperature of releases and its impact on fecundity, and the increase in the permanency of wetlands linked to weir pools (Walker and Thoms, 1993). This broad appreciation of the impacts that were brought with twentieth century change has led to a management response dominated by water volume (Maheshwari et al., 1995). So, after more than a decade of vehement contest over water allocations to the environmental reserve, the Murray Darling Basin Plan has allocated 3200 Giga Liters (GL) of water to fulfill obligations under international treaties to the restoration of wetlands across the catchment (MDBA, 2013).

Monitoring of the waters of the Basin commenced in 1951 and this program has been augmented both in breadth and frequency as concern for the ecological condition of the system has become elevated through to the present (Norris et al., 2002; Davies et al., 2012). This research has enabled the production of report cards that have attested to the degraded state of the system and justified calls for the returns of considerable volumes of water from consumptive allocations to the environment. However, no data pre-dating regulation, or the initiation of the principal phase of water resource development (1950–1995), exists. As such, management is largely blind to the baseline ecological condition and the historical range of variability that existed before the peak period of industrialized development. It is also blind to any abrupt changes that may have occurred before regulation. For example, geomorphic evidence of severe gully erosion soon after settlement suggests catchment pressures on wetland condition may have begun as early as the mid-1800s (Prosser et al., 2001). Underlying these anthropogenic drivers, there are also long term trajectories of change that originate from the nascence of wetlands. These remain elements to be considered in the management of these systems but are opaque to those whose focus is on recent changes observed using contemporary approaches.

Ongoing and widespread concerns for the welfare of the aquatic ecosystems of the Murray Darling Basin (Laurance et al., 2011), particularly in the light of the impact of a drying climate (Jones et al., 2002b; Pittock and Finlayson, 2011), have ultimately seen the release and implementation of the Murray Darling Basin Plan, amid great controversy. It is proposed that 3200 GL of water be returned to the natural environment to improve the ecological health of the system (MDBA, 2013). Originally, this was proposed to be borne largely by water buy-backs from the irrigated agriculture industry (at an estimated cost of $5.5 B), however more interest is now on freeing water for the environment by investing in infrastructure designed to minimize evaporative losses (at an estimated cost of $27.5 B; Wittwer and Dixon, 2013). This represents an unprecedented investment in Australia's environmental health and a significant, potential opportunity cost to production from irrigated agriculture through reduced water allocations. There remains little means, other than short term monitoring, to ascertain the ecological benefit from the allocation of such resources. There remains considerable risk that the response from this substantial, and unusual, investment in environmental remediation will be muted, on account of the legacy of stressors poorly identified due to the prevalence of short term research (Gell and Reid, 2014). Politically, the cause for investment in ecosystem health, against that for production from irrigation agriculture, may hinge on the ecosystem science community's credibility in circumscribing the benefit from allocation of scarce and expensive water.

Regime shifts have been a long posited model to explain the shift from a macrophyte-rich state in a wetland to one dominated by phytoplankton (Scheffer et al., 1993; Scheffer and Carpenter, 2003). While Davis et al. (2010) proposed such a mechanism when explaining changes to relatively permanent wetlands in the Swan Coastal Plain (WA), a recent meta-analysis of research claiming to have demonstrated regimes shifts found few revealed a lack of recovery after the removal of the stressor and fewer still demonstrated the shifting feedbacks that would entrench a wetland in an alternate state (Capon et al., 2015). The few that were found to justifiably claim evidence for a threshold change were from shallow lakes. This is supported by widespread evidence for phytoplankton-dominated systems today in wetlands that were once productive, macrophyte-rich waters. The forces of landscape, hydrological and climatic change that may impact upon a wetland are diverse but are likely to have come to bear on the condition of wetlands from early in the industrialized phase, and potentially, early in the sedentarization of people (Bradshaw et al., 2005, 2006). It would be coincidental if the pressures brought by humanity all came to bear on wetlands over the last few decades after reliable monitoring technologies became available. So, it is unlikely that contemporary ecological sampling could reveal regime shifts. Given the absence of historical data, or observations of sufficient detail to show non-linear change, the demonstration of regime shifts must rely heavily on records of long term change available in paleolimnology.

Many paleolimnological studies of shallow lakes have demonstrated abrupt change. For example, Ogden (2000) observed the loss of aquatic macrophytes in large, deep Murray Darling Basin wetlands due to their vulnerability to light attenuation. Sayer et al. (2010) demonstrate gradual regime shifts in constructed wetlands in Norfolk, UK. Wang et al. (2012) revealed abrupt changes in Lake Erhai, China, which followed increasing variability in diatom assemblages in response to increased nutrient loading. Further, Davidson et al. (2013) found that, among 10 billabong sites in the northern and southern Murray Darling Basin, most experienced significant declines in the diversity of benthic diatom communities across the European settlement boundary. Similarly, Kattel et al. (2014) have documented the loss of diverse, littoral cladoceran communities coincident with the rise of phytoplankton in a lower Murray River wetland. They observed however, that it remains uncertain whether this change is due to continued, chronic dosing with river-borne sediment and phosphorus or whether it represents a regime shift reinforced by the establishment of stabilizing feedbacks that lock the wetland into a state rich in pelagic biota, but depauperate in littoral biota.

The resilience of a wetland to reductions in flow relate to its capacity to recover, and retain its identity, after wet conditions return. Intuitively, this resilience could be informed by an understanding of the historic and pre-historic changes a wetland has previously experienced. The contemporary hydrogeomorphic regime of the southern Murray Darling Basin was established after the mid-Holocene pluvial phase. So the broad mechanisms that control the present meander wavelength and the production rate of cutoff meanders has been in place for 7000 years. Jones et al. (1998) revealed that, through this period P/E ratios have varied from 1.2 to 0.7 and Barr et al. (2014) have demonstrated the existence of multi-decadal droughts. Using instrumental data Warner (1987) revealed multi-decadal drought-dominated and flood-dominated regimes. So, while the focus of contemporary society is on shorter frequency, ENSO cycles and their effects, the system has experienced variations of even greater duration and amplitude in the past. Given this natural variability, wetlands and river channels might be expected to have considerably more natural resilience with respect to flow variations than the present report cards suggest. If so, it is likely that other stressors exert a strong influence on condition to the point where they have contributed to the assessment of extensive degradation.

While the impact of industrialized people on Australian ecosystems commenced early in settlement, environmental management programs continue to seek "baseline" information from surveys conducted in the late twentieth century, and even later (Marsland and Nicol, 2007). So the systems we seek to manage, based on recent monitoring were already, and often substantially, impacted, before conditions were first assessed. By these means we are unable to see our real ecological heritage, measure the magnitude of the shift from a real baseline, or attribute the cause (Reid and Ogden, 2006). Without evidence from the past we are unaware of the changes to our natural systems that occur through time (Tibby et al., 2007a,b). The identification of targets for restoration can be largely political and so the past may never be the goal for future investment. Nevertheless, ignorance of the trajectory of change denies scientists, managers and investors alike, evidence of system evolution, cause, and response.

Evidence for the evolution of a wetland is buried away, year upon year, in its sediments. It is somewhat obscured by the variable preservation of key lines of evidence, but emerging technologies are both releasing new lines of evidence, and establishing better timelines of change. Evidence of wetland condition are becoming increasingly accessible as more and more bioindicators, such as eDNA (e.g., Giguet-Covex et al., 2014), are extracted from its archive. While substantial funds are invested to monitor the contemporary condition of impacted systems over long periods of time, relatively few resources can be invested to understand natural assets lost, the magnitude of ecosystem change and the underlying causes of food web simplification.

Many wetlands of the Murray River host continuous sediment records that extend to the time they commenced accumulating sediments—probably at a point after they were lost to the scouring flows of the main channel. Others that regularly dried presumably lost their sediment due to deflation and only commenced continuous sediment accumulation after they became permanent after river regulation. So, some cutoff meanders with large wavelength morphology have records of change that span several millennia (Gell et al., 2005a; Reid et al., 2007) while others have shorter records extending back into the early twentieth century only (Gell et al., 2007). In combination, they provide an opportunity to examine the response of wetlands to many drivers—fewer to the longer term forces such as millennial scale climate variability, and many more to the impact of river regulation and catchment disturbance after European settlement. A typical record shows muted response to climate but an abrupt response to elevated sediment loads and river regulation following industrialization. Attributing these changes to a cause is somewhat limited by the difficulty of establishing a sub-decadal chronology to the sediment record due to challenges faced in the use of radiometric techniques in fluvial settings (Gell et al., 2005a). Nevertheless, it is clear that there has been widespread change in Murray Darling Basin wetlands that are likely due to a greater suite of causes than merely loss of flow and connectivity (Dearing et al., 2015).

This paper draws on existing palaeoecological records from the Murray-Darling Basin to illustrate how floodplain wetlands have been affected by catchment land use change and water resource development. These are used to demonstrate the importance of addressing stressors such as elevated nutrient and sediment fluxes in parallel with hydrological change in efforts to manage and preserve these important ecosystems. Two approaches are used in relation to the existing records: first, a general review and synthesis of the full suite of available records to explore evidence of elevated sediment fluxes and water quality change, especially in relation to nutrients; and second, a reanalysis of a subset of the records to explore patterns in relation to evidence of abrupt loss of submerged macrophytes and potential hydro-geomorphic controls over wetland resilience with respect to macrophyte loss.

MATERIALS AND METHODS

Synthesis of Evidence in Billabong Sediment Records of Sediment Flux and Water Quality Change

Over 50 sediment-based records of change, using fossil diatoms and various dating techniques, have been undertaken in Murray Darling Basin wetlands (Gell and Reid, 2014). Some of these records have been published and interpreted in detail (Gell et al., 2005a,b, 2007; Gell and Little, 2006; Reid et al., 2007; Fluin et al., 2010; Grundell et al., 2012; Kattel et al., 2014), some appear as research dissertations (Silva, 2014) and others are available in unpublished reports. Field and laboratory methods used in each of these studies are detailed in the literature cited above. In broad terms, however, sediment records at each site are derived from sediment cores taken from the deepest sections of each billabong, in most cases supported by multiple cores used to assess general stratigraphy. In addition to diatoms, most studies also include physical, chemical, and other biological indicators such as moisture and organic content, sediment texture, magnetic susceptibility, pollen, macrofossils, and pigments.

While the records in some are underpinned with a range of dating approaches such as ^{14}C AMS, ^{210}Pb, ^{137}Cs, OSL, and exotic pollen others are supported by limited dating control.

Given the carbonate-rich nature of the landscape and high level of fluvial transport of fine sediments few records are supported by clear chronologies and so attributing changes to points in history remains challenging. Nevertheless, broad changes in sedimentation rate can be inferred and clear evidence of broad changes are evident. In many instances, these can be attributed to particular management interventions in the past, such as the establishment of a network of weirs, mostly between 1922 and 1936.

The water quality changes inferred from these records are based on the known water quality preferences of the diatoms preserved in the sediments. Samples are treated using standard techniques (Battarbee et al., 2001) and mounted on microscope slides. Species are identified using standard international (Krammer and Lange-Bertalot, 1986, 1988, 1991a,b) and local floras (Sonneman et al., 2000) usually assisted by microphotography. Taxonomic harmonization between laboratories was assisted by the staging of taxonomic workshops that were central to the production of the local floras.

While these flora formed the basis for the local interpretation of the water quality preferences of common taxa an extensive diatom biomonitoring program across south-eastern Australia, using diatoms, enabled the calibration of species to water quality parameters (Philibert et al., 2006) and the production of an index of stream degradation (Chessman et al., 2007), the Diatom Species Index of Australian Rivers (DSIAR). Ecological interpretations were also based on international indices of water quality, such as Van Dam et al. (1994), as well as the large body of diatom species—water salinity calibration data sets (e.g., Fritz et al., 1991; Gasse et al., 1995; Gell, 1997; Fritz, 1999). Given the changing nature of floodplain wetlands are influenced by the nature of the wetland, but also the river, and the variable interactions between, interpretations of fossil records were aided by insights from river channel diatom samples (Tibby and Reid, 2004) collected routinely by the basin management agency as well as through a diatom-water quality monitoring program of river-connected wetlands (Gell et al., 2002). The complex interactions between the river and its wetlands was considered to preclude the widespread use of transfer functions to quantitatively infer water quality changes although this was attempted in some instances (e.g., Tibby et al., 2003; Gell et al., 2007; Reid and Ogden, 2009). The recent synthesis (Gell and Reid, 2014) summarizes the diatom-inferred change evident in the records reporting on the temporal extent of the record and changes in sedimentation rate since European settlement. It also reports on water quality changes, such as increases in salinity and nutrients and changes in pH, using the known water quality preferences of common diatom taxa. Evidence from these records attests to widespread increases in sedimentation rate and variable evidence for shifts from macrophyte- to phytoplankton-dominance in billabongs (**Figure 1**). Also, they report an increase in eutraphentic taxa and a decline in those known to be competitive in low nutrients conditions on account of them hosting nitrogen fixing endosymbionts (Stancheva et al., 2013) marking a rise in nutrients loads. Importantly, the response varies between sites with some wetlands appearing resistant to change and those more sensitive changing abruptly, particularly in relation to macrophyte loss. This evidence prompts a more thorough investigation into the nature of the changes that have occurred to determine if regime shifts are evident or if the sites are merely continuing to respond to a chronic stressor. Further, it prompts an investigation into the spatial and hydro-geomorphic controls on these sites, to examine whether the changes are predictable according to situation. Cursory observations lead to the identification of four possible wetland response types (Reid and Gell, 2011) which attempt to describe and explain patterns of change in billabongs in relation to shifts from macrophyte-dominated to phytoplankton dominated regimes which are illustrated by the conceptual models in **Figure 2**.

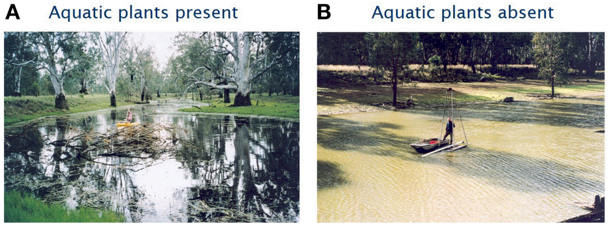

FIGURE 1 | Two billabongs from the Murray Darling Basin (A), clearwater macrophyte-dominated; (B) turbid phytoplankton-dominated.

These records were synthesized in Gell and Reid (2014) and forms part of the review of wetland change described and discussed below.

Selection and Analysis of Key Records

This synthesis enabled the identification of the most appropriate sites to be examined to better understand the nature of change. Seventeen records of wetland change were selected from each of the four response types to examine the nature of the response in detail. The response types comprised the upland tributaries which appeared most resistant to change, the mid-basin, deeper wetlands that appeared to have switched permanently, those in the lower reaches that tended to switch condition and a lowland group that showed little consistent pattern of change perhaps owing to local pollution impacts (**Figure 3**). The nature of the selected wetlands is outlined in **Table 1**.

All but one of these records (Sinclair's Flat) extend well beyond the last two centuries, with several extending more than a 1000 years. Most records therefore, cover the critical pre- to post-European settlement boundary and also provide information on the natural variability of systems. The pre- to post-European boundary used in each record is based on the inferences from the original studies which utilize a range of lines of evidence, including ^{210}Pb dating profiles, appearance of introduced pollen taxa and peaks in magnetic susceptibility, thought to be associated with early post settlement soil erosion (Reid, 2002; Reid et al., 2007).

Because the principal interest of this research is on changes to the trophic structure of billabongs in relation to pelagic vs. littoral productivity the synthesis compares the relative abundances of preserved diatoms grouped according to habitat preference. The major habitat groups in billabongs are planktonic, epipelic

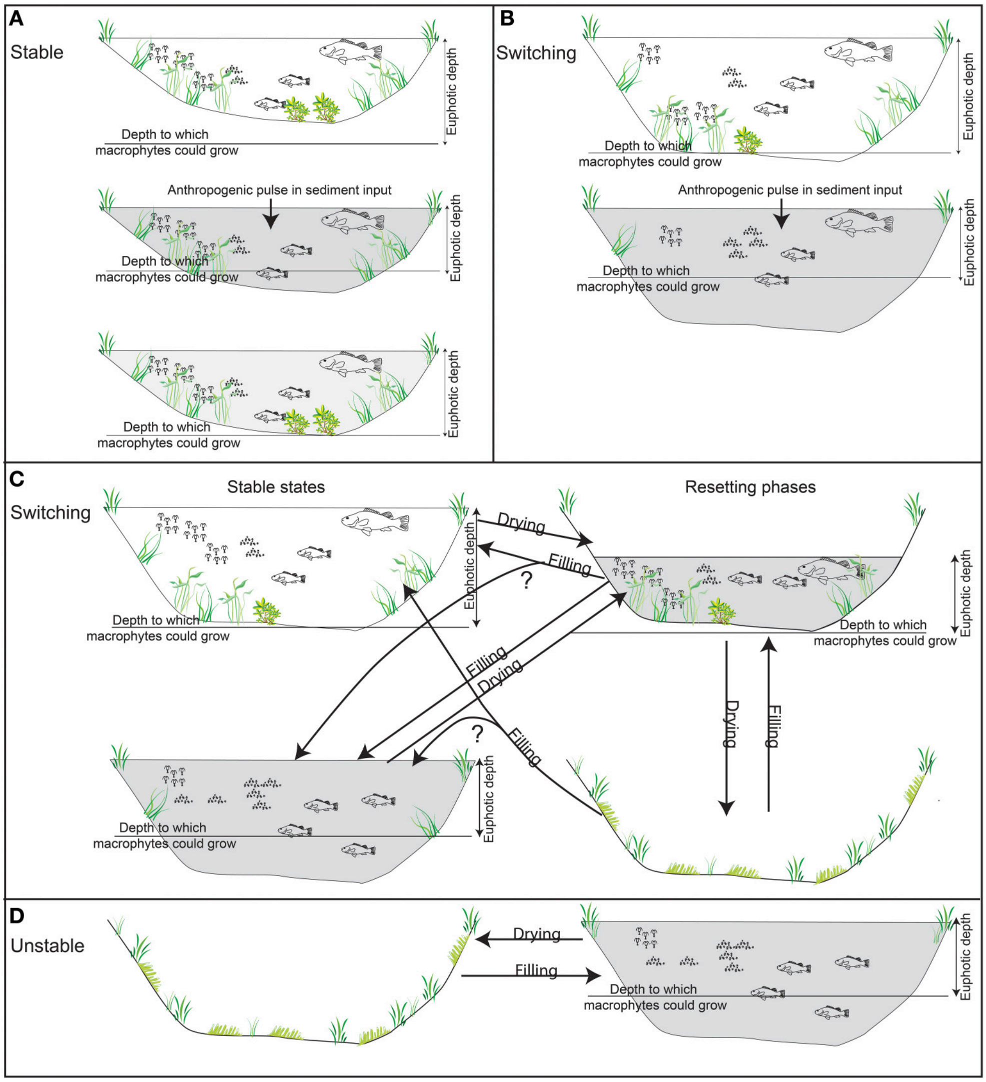

FIGURE 2 | Conceptual models explaining the relationships between billabong geomorphology, hydrology, and the proposed response types. Stable billabongs are resilient to reduced photic depth because pulses in anthropogenic sediment input do not reduce photic depth enough to remove the majority of the bed from the photic zone **(A)**. Switching billabongs are less resilient to reduced photic depth because pulses in sediment input result in the removal of the majority of the bed from the photic zone **(B)**. In both cases, feedback processes may act to strengthen the original or new state once sediment influx is reduced. Switching billabongs may also be reset to either state by drying events **(C)**. Unstable billabongs dry frequently and so fail to develop stable states **(D)**.

(living on mud surfaces), and epiphytic (living attached to plants or other hard surfaces) forms. A further group consists of a suite of genera with poorly defined habitat preferences that are typically associated with variable or frequently disturbed environments (collectively referred to hereafter as "small Fragilaria").

The diatom and dating data then were synthesized for analysis using non-metric multi-dimensional scaling of resemblance matrices based on Bray Curtis Similarity Indices calculated using diatom assemblage (relative abundance of habitat groups) data. This ordination was used to explore patterns in diatom assemblage structure in relation to the abundance of the major habitat groups and change over time in assemblage structure within individual billabong records. Interactions between changes over time in response to European settlement and billabong type were explored by comparing positions of samples on the first axis of the nMDS, the main axis of variation in the data set, in relation to era (pre vs. post) and type (stable, switching, and unstable). Subsets of samples were selected from within individual records for this analysis to reduce the influence of temporal autocorrelation and uncertainty over the timing of the pre- to post-European boundary in the records. Thus, samples were selected to maximize depth gaps between samples and avoid samples immediately above and below the inferred pre- to post-European boundary. The numbers of included and excluded samples for each record and the sample depths for included samples are provided in **Table 2**. Analyses were conducted in Primer v6 (nMDS).

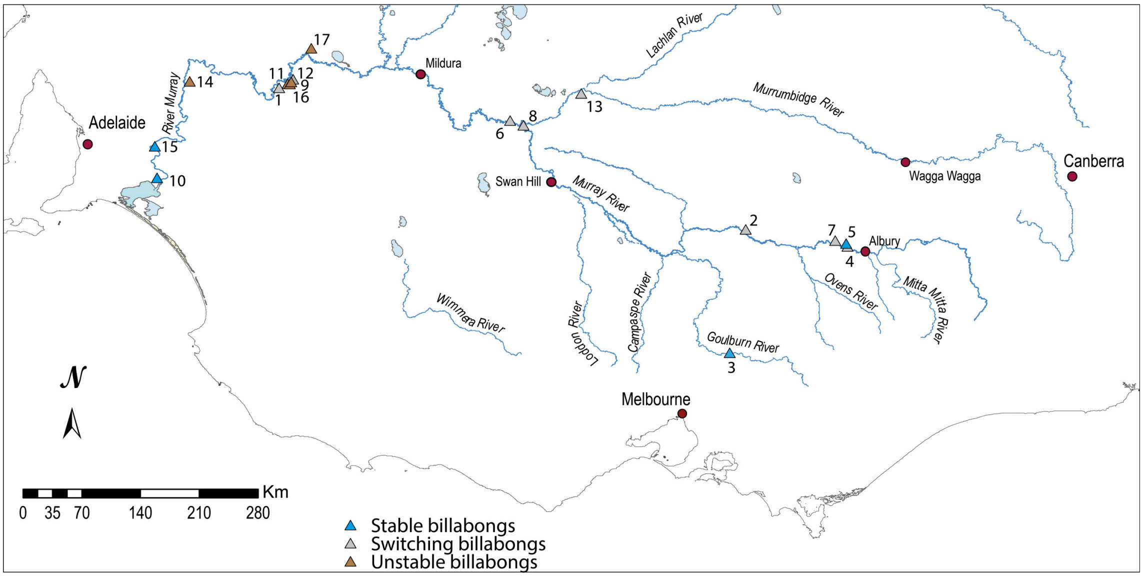

FIGURE 3 | Distribution of the 17 billabongs in the southern Murray Darling Basin classed by response type.

TABLE 1 | Features of the 17 wetlands analyzed.

	Site	River	Maximum water depth (m)	Basal core depth (cm)	Depth to pre-post-European boundary (cm)	Latitude (S)	Longitude (E)
1	Ajax Achilles	Murray	2	249	153	34°19′54″	140°34′51″
2	Billabong 38	Murray	3	265	125	35°50′6″	145°25′25″
3	Callemondah 1	Goulburn	4	210	21	37°10’4″	145°28′23
4	Hogans 1	Murray	3.5	252	176	36°1′20″	146°42′51″
5	Hogans 2	Murray	2.5	174	130	36°1′30″	146°42′39″
6	Hopcrofts Billabong	Murray		240	70	34°42′54″	143°9′9”E
7	Iona 2	Murray	4	260	145	35°58′27″	146°28′36″
8	Junction Park	Murray		180	65	34°46′16″	143°18′5″
9	Mundic Wetland	Murray	2	304	72	34°12′40″	140°47′6″
10	Muroondi Wetland	Murray	0	1440	165	35°18′54″	139°23′
11	Pikes Creek	Murray	0.8	198	86	34°15′35″	140°47′30″
12	Ral Ral Creek	Murray	0.8	82	54	34°04′48″	140°43′32″
13	Russell’s Billabong	Murrumbidgee	1.5	130	88	34°20′9″	143°53′51″
14	Sinclairs Flat	Murray	1.5	70	70	34°16′1″	139°37′58″
15	Swanport Wetland	Murray	0.4	700	100	35°09′	139°18′
16	Tanyaka Creek	Murray	1	276	54	34°13″48″	140°46′
17	Tareena Billabong	Murray	1	427	150	33°57′55″	141°02′6″

RESULTS

Synthesis of Species Assemblage Changes

A synthesis of 51 fossil diatom records from wetlands of the Southern Murray Basin floodplain reveal comprehensive change in condition (Gell and Reid, 2014). Increased sedimentation was observed in 55% of wetlands raising the threat of complete sedimentation in the more shallow systems (Gell et al., 2013). Most wetlands exhibited a shift to true plankton (*Aulacoseira* spp.), or a shift to tychoplanktonic (Sayer, 2001) diatom species (*Pseudostaurosira brevistriata*, *Staurosira construens,* and varieties, *Staurosirella pinnata*) competitive in turbid conditions, reflecting an increase in pelagic forms at the expense of epiphytic (*Cocconeis placentula*, *Gomphonema* spp.) and littoral (*Epithemia* spp., *Eunotia serpentina*) taxa (Sonneman et al., 2000). In particular, large, deep wetlands in the mid-reaches were vulnerable to abrupt shifts to dominance by *Aulacoseira* spp. due to increasing turbidity impacting on the light environment of an extensive area of the benthos at once (Ogden, 2000; Reid et al., 2007).

TABLE 2 | Samples included in exploration of interaction between type and era (pre-European vs. Post-European) illustrated in Figure 8.

Billabong	Post-European samples			Pre-European samples		
	No. of samples not included	**No. of samples included**	**Depths (in cm) samples included**	**No. of samples not included**	**No. of samples included**	**Depths (in cm) samples included**
Ajax	67	5	0, 30, 60, 121	21	4	161, 181, 201, 221
B38	5	5	5, 45, 85, 125, 165	1	3	205, 225, 245
C1	17	5	0, 4, 8, 12, 16	23	5	32, 52, 72, 92, 112
H1	32	5	0, 32, 64, 105, 130	16	4	200, 224, 248, 272
H2	18	5	0, 16, 32, 64, 96	11	2	137, 164
Hopcrofts	4	4	0, 20, 40, 60	12	5	90, 120, 150, 180, 210
I2	20	4	0, 40, 80, 120	2	2	160, 176
JP	9	3	0, 20, 40	7	4	90, 110, 130, 160
Mundic	56	5	0, 15, 30, 45, 60	66	6	90, 120, 152, 180, 212, 272
Murrundi	1	6	25, 45, 65, 85, 105, 125	51	12	205, 305, 405, 505, 605, 705, 805, 905, 1005, 1105, 1205, 1305
Pikes	40	3	0, 20, 60	48	4	106, 126, 146, 166
Ral Ral	90	5	0.5, 10, 20, 30, 40	25	4	60, 66, 72, 78
Russell's	9	4	0, 24, 48, 96	3	1	112
Sinclair	47	4	0, 10, 20, 30	–	–	–
Swanport	5	4	0, 20, 40, 60	9	6	120, 180, 280, 400, 500, 600
Tanyaka	15	3	0, 38, 48	45	5	94, 144, 184, 224, 264
Tareena	18	7	0, 20, 40, 60, 80, 100, 120	24	5	200, 250, 300, 350, 400

Lower in the system the diatom flora in the wetlands shifted from species considered competitive in low nutrient systems, owing to their nitrogen-fixing endosymbionts (Epithemiaceae; Stancheva et al., 2013), to taxa widely known to reflect elevated nutrient concentrations (Van Dam et al., 1994). These variously include *Cyclostephanos dubius*, *Cyclotella meneghiniana*, *Nitzschia* spp. (incl. *N. amphibia*, *N. palea*), and *Stephanodiscus* spp. (Sayer, 2001; Bennion et al., 2004). Substantial declines in *Epithemia* spp. were evident in records from Lake Cullulleraine (Fluin et al., 2010), Loveday Wetland and Loch Luna (Gell et al., 2007), Sinclairs Flat (Grundell et al., 2012), Kings Billabong (Kattel et al., 2014) and upstream at Hogan's Billabong (Reid et al., 2007) while *E. serpentina* was seen to decline in Loch Luna, Pikes Creek, Ral Ral Creek, and Hopcrofts Lagoon (Gell et al., 2005b). Increases in nutrient indicators, including *Stephanodiscus*/*Cyclostephanos* (Sinclairs Flat, Ral Ral Creek, Pikes Creek, Balranald Weir, Loveday Wetland, Coonoocoocabil Lagoon, and the Coorong), *C. meneghiniana* (Tareena Billabong, Gooragool Lagoon, Bomen Lagoon, Psyche Bend Lagoon, and Berry Jerry Lagoon), and *Nitzschia* spp. (Loveday Lagoon, Sinclairs Flat, Ral Ral Creek, Loch Luna, and Hogan's Billabong), were widespread. Large scale hydrological change has seen the onset of both dryland and irrigation salinization which is reflected in the arrival of halobiontic taxa, such as *Amphora coffeaeformis* and *A. veneta*, *Gyrosigma* spp., *Pleurosigma* spp., and *Tryblionella* spp. (Gasse et al., 1995; Gell, 1997), from early after European settlement (e.g., Gell et al., 2005a, 2007). In effect, these records provide evidence for widespread impact on the floodplain wetlands and identify increased flux of sediments, nutrients and salts as major stressors.

Analysis of 17 Billabong Data Set

The nMDS ordination of the diatom assemblages of all samples (sediment depths) from all 17 sites selected for detailed analysis, is shown in **Figure 4**. The position of samples in ordination space reflects the relative abundances of the major habitat groups in each sample, as illustrated by the bubble plots in each of the four panels in **Figure 4**. These show that samples plotting to the lower left typically have abundant planktonic diatoms (**Figure 4A**), those to the lower right typically have abundant epiphytic diatoms (**Figure 4B**) while those plotting to the top have abundant small *Fragilaria* (**Figure 4D**). Samples with abundant epipelic diatoms do not cluster strongly in any one area in relation to axes 1 and 2 (**Figure 4C**). Accordingly nMDS axis 1 reflects variation in the abundance of planktonic (low values) and epiphytic (high values) diatoms in each sample.

The ordination also reveals patterns in the diatom assemblages of billabongs and how these assemblages vary over time (**Figure 5**). The patterns observed largely affirm the classification of response types from Reid and Gell (2011), although uncertain chronology means that separation of the type II and III billabongs proposed in the Reid and Gell (2011) classification is difficult. The modified classification is described below:

(1) **Stable**–records are dominated by epiphytic diatoms and samples remain largely within the lower right quadrant of the ordination (e.g., Callemondah 1, Swanport, and Muroondi wetlands in **Figure 5**);

(2) **Switching**–records are dominated by epiphytic or planktonic diatoms (i.e., samples plot low on axis 2). The planktonic state predominates in the post-European phase and the most recent samples always contain >55%

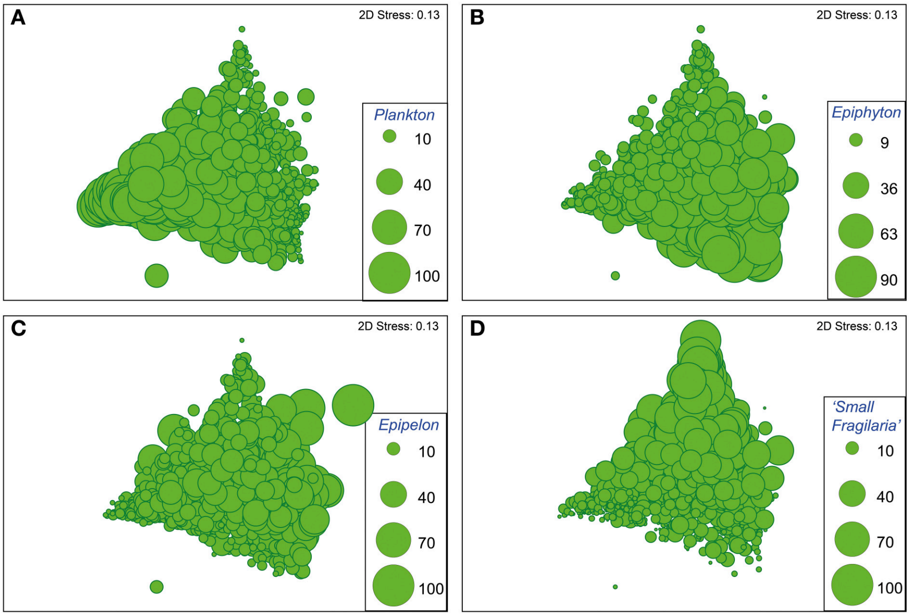

FIGURE 4 | Bubble plots of the full 17-billabong data set (966 samples) on nMDS axes 1 and 2 based on a resemblance matrix of Bray-Curtis similarity measures using relative abundances of diatoms grouped according to preferred habitat (planktonic, epiphytic, epipelic, small *Fragilaria*). Bubble size indicates the relative abundance of planktonic **(A)**, epiphytic **(B)**, epipelic **(C)**, and small *Fragilaria* **(D)** diatoms. 2D stress values indicate the degree that the 2-dimensional ordination represents a distortion of the relationships between samples in the resemblance matrix, with 0 representing perfect representation. Values greater than 0.3 indicate points are close to being arbitrarily placed in the 2-dimensional ordination, thus the 2D stress value of 0.14 indicates the ordination is suitable for illustrating general patterns (Clarke and Warwick, 2001).

planktonic diatoms (e.g., Hogans 1, Hopcrofts Billabong, and Billabong 38 in **Figure 5**). Among switching billabongs, some (e.g., Hopcrofts Billabong in **Figure 5**) appear to have undergone periods of planktonic dominance prior to European settlement. These billabongs are equivalent to the type III billabongs in Reid and Gell (2011) and may switch via mechanisms illustrated in **Figure 2C**. However, these are included within the broader switching group here because uncertain chronology means that the possibility that no phases of planktonic dominance occurred in the pre-European period cannot be ruled out;

(3) **Unstable**–assemblages vary greatly through time and contain relatively high abundances of "small *Fragilaria*" (e.g., Tareena, Mundic, and Tanyaka wetlands in **Figure 5**).

Patterns of change over time, within the three response types, are further illustrated by the time series of nMDS axis 1 and 2 scores (**Figures 6**, **7**). These show consistent declines in axis 1 scores from the pre- to post-European periods among switching billabongs. These declines reflect shifts from relatively high abundance of epiphytic diatoms to relatively high abundance of planktonic diatoms. In most cases this decline in axis 1 scores appears to be a step change although the transition is also distinguished by high variability in scores (**Figure 6**). Declines in axis 1 scores are also apparent in several unstable billabongs (Tareena, Mundic, Tanyaka, and Pikes), however these unstable billabongs are distinguished from the switching billabongs by generally higher and more variable axis 2 scores (**Figures 7**, **8**).

Hogans 2, classed as a stable billabong also shows a clear and abrupt drop in axis 1 scores, however, the drop is not as great as in switching billabongs (around 0 compared with around -1), reflecting the lower proportion of planktonic diatoms in the most recent sediments of Hogans 2 (<50%) compared to the switching billabongs (>55%).

There is also a spatial pattern in the distribution of the aforementioned response types (**Figure 3**):

- Stable billabongs are found in the upper middle Murray River, the Goulburn River and in the lower Murray River below the Murray gorge. As noted above, Ogden (2000) also found Ovens River billabongs were relatively stable;
- Switching billabongs are found in the mid-Murray River (Hume Dam to Darling confluence) and lower Murrumbidgee River. Within this group, possible phases of pre-European plankton dominance are apparent further downstream (from around the Murrumbidgee-Murray River confluence) but not upstream;
- Unstable billabongs are confined to the Murray River wetlands situated between the Darling River confluence in south-west NSW and the Murray Gorge in South Australia.

It is perhaps noteworthy that the billabongs that fit less clearly within each of the response types tend to be situated within regions dominated by billabongs with other response types. Hogans 2, for example, lies in a region dominated by switching billabongs and Ajax lies in a region with mostly unstable billabongs (**Figure 3**).

FIGURE 5 | nMDS ordination plots of axes 1 and 2 scores for diatom assemblages from specific billabong sediment records illustrating examples of "stable" (Callemondah 1, Swanport, Muroondi), "switching" (Hogans 1, Hopcrofts, Billabong 38), and "unstable" (Tareena, Tanyaka, Mundic) billabong response types. Samples in each plot are distinguished as being from the pre-European (Pre) and post-European (Post) settlement periods Note that each site plot is a subset of the data points shown in the nMDS ordination of all sites and samples shown in **Figure 4**.

DISCUSSION

Given the high level of natural variability inherent in Australian hydroclimatic systems, and the extended return interval of high impact events, it is insufficient to base the management of a natural system on knowledge derived from recent monitoring. Only an extended record of variability and change coupled with contemporary monitoring is able to quantify the magnitude of change from historical, and clearly reveal the nature of that change. Only then can restoration approaches that target the known shifts in condition be applied.

A recent synthesis of diatom-inferred change (Gell and Reid, 2014) across 51 wetlands highlighted drivers, such as high sedimentation rates and elevated turbidity as well as increased concentrations of nutrients and salts, operating widely across the southern Basin. Importantly, the identified responses varied between sites with some wetlands appearing resistant to change and those more sensitive changing abruptly, particularly in relation to macrophyte loss. This evidence prompted a more thorough investigation into the nature of the changes that have occurred to determine if regime shifts are evident or if the sites are merely continuing to respond to a chronic stressor. Further, it prompted an investigation into the spatial and hydro-geomorphic controls on these sites, to examine whether the changes are predictable according to situation. Cursory observations lead to the identification of four possible wetland response types (Reid and Gell, 2011) which attempted to describe and explain patterns of change in billabongs in relation to shifts from macrophyte-dominated to phytoplankton dominated regimes. This detailed analysis of 17 wetlands allowed only for the identification of three clear response types; those resistant or resilient to stressors (stable), those which tend to frequently change and recover (unstable) and those which appear to have changed into a permanent phytoplankton-dominated state (switching). The tendency of a wetland to conform to one or another type appears to be controlled by the hydromorphic setting with those in the upper reaches stable, those in the lower unstable, and those most sensitive to permanent switching in the middle reaches.

The Magnitude of Change

This recent synthesis of long term records of change from floodplain wetlands across the southern Murray Darling Basin provides evidence of the degree to which wetlands have departed from their long term historical range of variability, and reveals the spatial extent of change across the region. The data presented

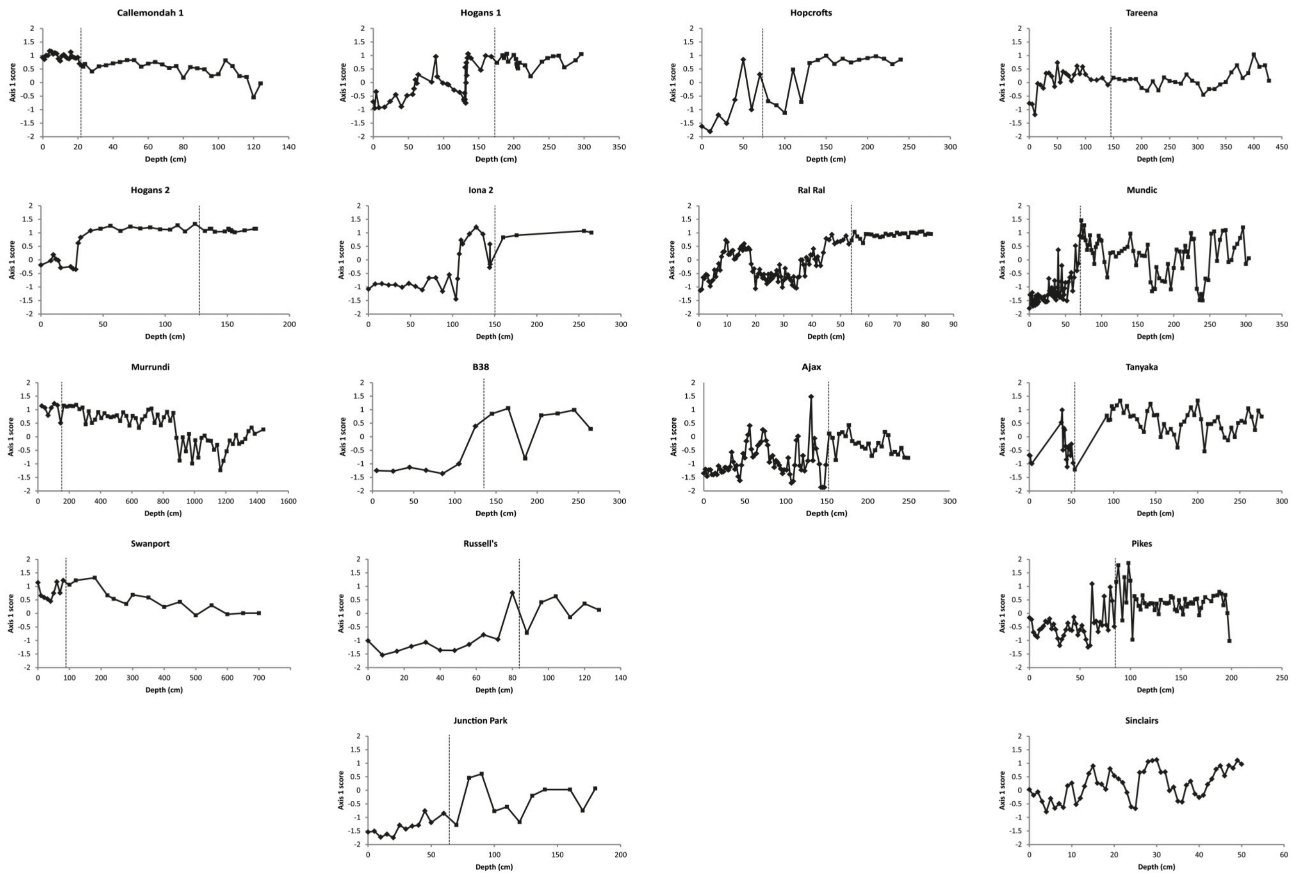

FIGURE 6 | Change in nMDS axis 1 scores over time in stable, switching, and unstable billabongs. Dashed vertical lines indicate inferred timing of European settlement based on the appearance of exotic pollen and/or magnetic susceptibility profiles.

in Gell and Reid (2014), and the detailed analysis presented here, report on records of fossil diatoms that are sensitive to water quality (Reid et al., 1995) and to habitat change (Reid and Ogden, 2009), but are less reflective of broader system health. Also, fossil records are most readily available in more permanent wetlands where sediment accumulation is continuous and so any synthesis may be skewed away from understanding change in seasonal or intermittent sites. The opportunity provided here however, is in the powerful insights that can be gained by monitoring retrospectively into ages beyond the reach of contemporary monitoring approaches, personal recollection and even documents such as diaries and photography.

Contemporary monitoring of waterway condition across the Basin focuses on water quality, fish and waterbird populations and tree health. These data underpin studies that claim the waterways of the basin to be widely degraded (Norris et al., 2002; Davies et al., 2012). Macroinvertebrate monitoring programs rely on comparison of assemblages between test and reference sites. While these approaches are not limited in the sites that may be analyzed, they are greatly limited by the extent of time covered in the data sets and the changing quality of data collection through time. As such, they overlook many decades of change that may have occurred before suitable instrumentation was available and they lack any pre- (European) impact condition for context and assessment of the natural amplitude of variability. Further, it is widely acknowledged that the condition of lowland waterways have changed and few, if any, unimpacted reference sites remain. Monitoring programs that assess impact relative to a “less modified” reference site will inevitably underestimate the magnitude of change that has occurred through time.

An assessment based on a synthesis of long term change from 51 wetlands is a substantial sample by which to assess basin wide change. It represents the largest synthesis of paleolimnological records across any floodplain and so provides the strongest available record of wetland condition change. This record reveals the impact of industrial development on the condition of wetlands across the southern Murray Darling Basin has been comprehensive and greater than the impact of millennial scale climate change.

The Nature of the Change

The combined syntheses of wetland change reveals widespread increased sedimentation rates and the replacement of epiphytic and littoral taxa with planktonic and facultative planktonic forms, particularly in the vulnerable large, deep wetlands of the middle reaches. This is strong evidence for the widespread

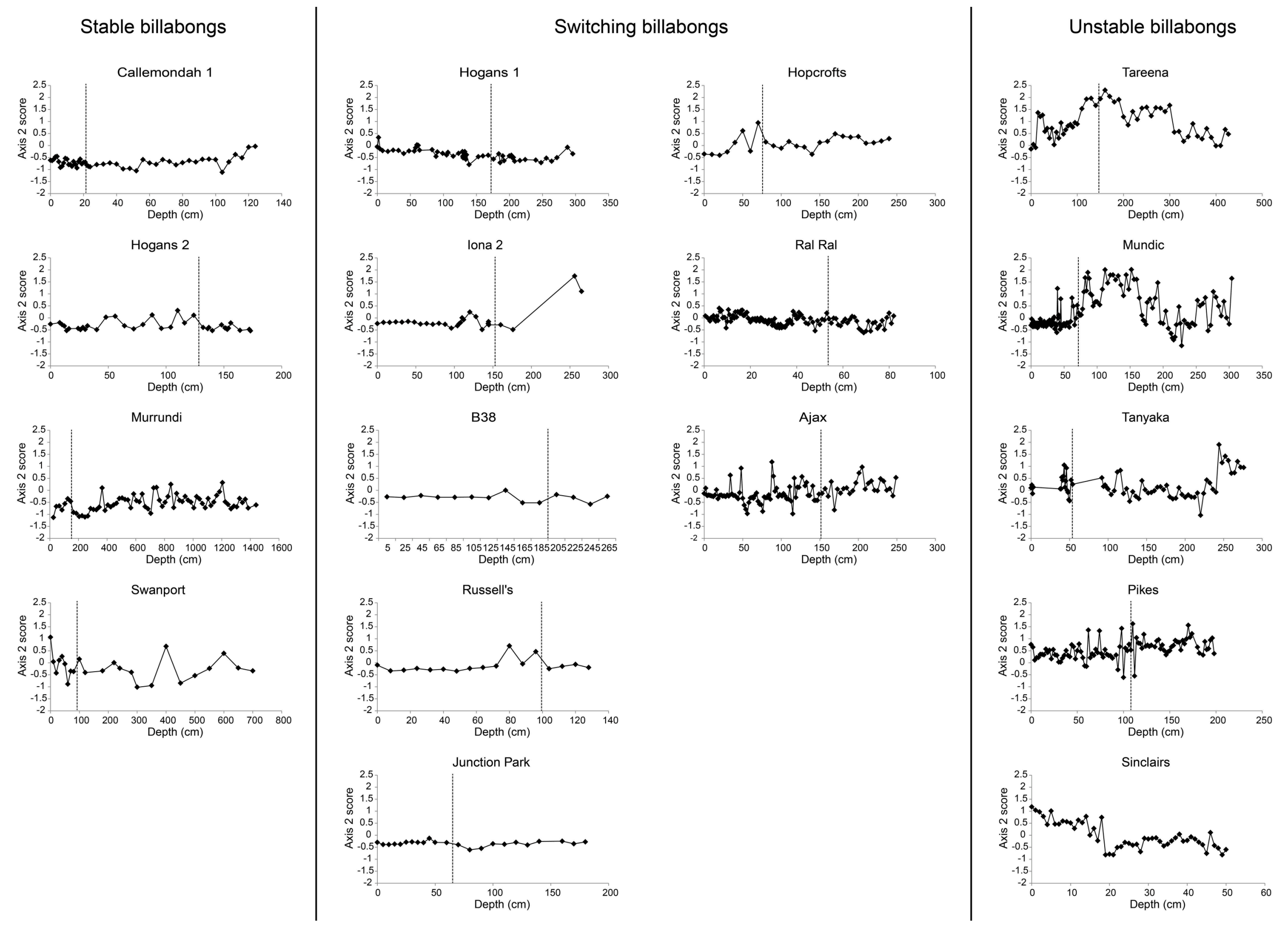

FIGURE 7 | Change in nMDS axis 2 scores over time in stable, switching, and unstable billabongs. Dashed vertical lines indicate inferred timing of European settlement based on the appearance of exotic pollen and/or magnetic susceptibility profiles.

increase in flux of fine sediments from the floodplain or channel banks into rivers and then to wetlands. To date the mix in the source of these sediments remains largely unknown with evidence for subsoil (Olley and Wallbrink, 2004) sources and catchment erosion more broadly (Reid et al., 2007) posited. While government agencies have acted to mitigate this flux through soil conservation measures and the national "Landcare" program, sedimentation rates appear to be increasing despite the likely sediment trapping effect of dams and weirs. It remains that the ongoing flux of sediment is advantaging those diatom taxa that can gain light from within the water body rather than attached to surfaces.

There is widespread increase in key nutrient indicator taxa in many records suggesting the widespread increase in nutrient flux, despite water quality monitoring data showing no particular trend over recent decades. Coincident with the rise of eutraphentic diatom taxa is the widespread decline in the relative abundance of species within the Epithemiaceae. Their decline is evidence in wetland records from Mildura (Kings Billabong; Kattel et al., 2014) to Blanchetown (Sinclairs Flat; Grundell et al., 2012). These forms are known to host nitrogen-fixing endosymbionts and so are competitive in low nitrate waters (Stancheva et al., 2013). While this relationship has not been explored in detail in an Australian context, the widespread decline in *Epithemia* spp. in floodplain records is supporting evidence for a weakening of their competitive advantage after industrial development.

The longer sediment records that extend beyond the arrival of European settlers attest to the natural, elevated levels of salinity relative to many other provinces of the world. The evidence of high numbers of *Gyrosigma* spp. and *Tryblionella* spp. from 3500 years ago in Tareena Billabong (Gell et al., 2005a) is clear evidence of phases of elevated salinity during drier climates. Even so, it is clear that floodplain wetland salinity has increased widely since European settlement even in wetlands considered to be in good ecological condition today (Gell et al., 2007). Several wetlands, including those permanently filled with river water, attest to unprecedented salinity in recent times where modern water quality monitoring reveal no clear change in river salinity. The salinization of many intermittent wetlands that are without long term records of change are clearly evident from the death of fringing aquatic plants and riparian trees (**Figure 9**).

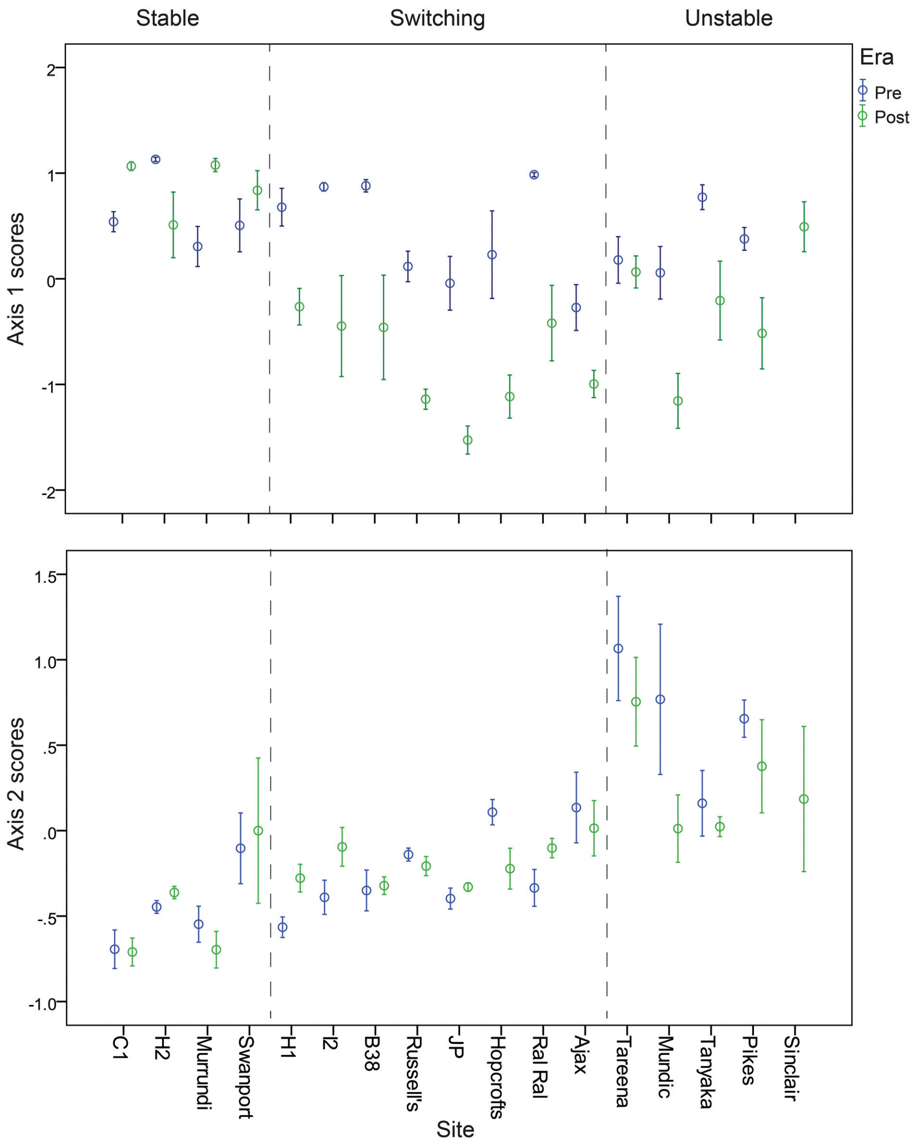

FIGURE 8 | Mean nMDS axis 1 (top) and axis 2 (bottom) scores for subsets of billabong sediment samples from pre- and post-European sediments arranged by billabong and billabong response type.

Shallow lakes, such as floodplain wetlands, have long been identified as systems at risk of non-linear change. These lakes are considered susceptible to regime shifts where increasing loads of nutrients or fine sediments impact on the light environment, limiting the depth to which light energy can penetrate. Under low or moderate nutrient and fine sediment loading, tight nutrient cycling, stabilization of surface sediments and allelopathic effects limit the release of nutrients and sediments into the waterbody, maintaining water clarity and advantaging rooted plants able to access sediment nutrients (Scheffer et al., 1993; Scheffer and Carpenter, 2003). However, with increases in nutrients and/or sediments these stabilizing forces may be overcome leading to declining water clarity and declining macrophyte abundance. The decline in macrophytes allows for the release of nutrients and sediments from the benthos reinforcing a shift to a phytoplankton-dominated state. While monitoring records that demonstrate regime shifts complete with shifts in stabilizing forces remain few (Capon et al., 2015), in the Murray Darling Basin it is possible that regime shifts occurred before the inception if monitoring programs. So, evidence for regime shifts may be limited to long term records of change that are only available using paleolimnological approaches.

Ogden (2000) was the first to invoke regime shifts as a possible mechanism to interpret the decline in fossil chydorid (cladocera) assemblages, and their replacement by pelagic bosminids, in floodplain wetlands in the upper Murray. This evidence has been reinforced by evidence for the rise of river plankton at the expense of epiphytic and benthic diatoms (Reid et al., 2007). This

FIGURE 9 | The recent salinization of Ramco Lagoon in South Australia is clear owing to the presence of dead River Red Gum (*Eucalyptus camaldulensis*) and fringing sedge (*Cyperus* sp.). The irrigation of crops on soils overlying Miocene limestone has driven an increased salt flux to the wetland.

detailed analysis of the diatom assemblages from 17 floodplain wetlands has revealed that many sites across the system spanning more than 1000 river kilometers have shifted to assemblages dominated by euplanktonic and/or tychoplanktonic forms from early in European settlement. Critically, some wetlands also tend to resist this change and yet others exhibit no apparent stability. This analysis classifies wetlands according to their response typology, reveals the distribution of particular response types and interprets the causes of change:

Stable: Resistant or resilient wetlands exist mostly in the upper tributaries that host shallow wetlands that are stable owing to their limited vulnerability to reduced photic depth;

Switching: wetlands that have undergone change from macrophyte dominance to phytoplankton dominance. This is due to their vulnerability to reduced photic depth being large and deep in the middle reaches of the main floodplain. Among these wetlands some do show evidence of regime shifts prior to European settlement. A spatial pattern in the location of apparent repeat switching suggests that variable water levels and perhaps even complete drying, may trigger switches;

Unstable: Variable wetlands in the lower catchment that have been impacted by multiple stressors.

This typology suggests that many wetlands have undergone regime shifts, but that these shifts were often early in European settlement and beyond the instrumental record. The pattern of change reinforces this interpretation in that it is the hydromorphic situation that mediates the individual wetland's response, on account of its vulnerability to a changed light environment. The widespread existence of state switching, albeit with the possibility of resetting in intermittent sites, attests to the basin wide increase in sediment and nutrient flux. While the source of these sediments remains unclear, evidence of elevated accumulation rates of fine sediments near inlets to wetlands (Grundell et al., 2012) suggest that the channel itself is a likely vehicle transferring fine sediment from the catchment to riparian lakes.

Restoration Approaches

The management of the waterways of the Murray Darling Basin has been a national priority for more than a century. Heightened concern for declining condition have culminated in the formulation of the Murray Darling Basin Plan (MDBA, 2013). The principal tenet of the plan is that the natural ecosystem has suffered from the over-allocation of river waters to industrial and domestic use, particularly the use of water to drive a highly productive irrigation industry. The principal mechanism proposed to restore the Basin's waterways is the return of 3200 GL/yr of environmental flows. The benefit of this allocation is being monitored in programs focussing on, but not restricted to, fish populations, waterbirds and macroinvertebrates. Given the 2005 "cap on allocations," and the institution of a water market to direct water allocation, the main mechanism left to government to secure water for flows are buy-backs of user entitlements or the mitigation of evaporative losses through expensive infrastructure. Governments sensitive to the political pressure of disaffected irrigation communities are likely to find the infrustructure approach appealing (Wittwer and Dixon, 2013).

Contemporary monitoring programs based on changes in waterbird and fish population and riparian tree condition have little historical context and so changes are rarely attributed to the stressors that emerged before river regulation. They are also elements of the ecosystem that respond particularly to the availability of water. It follows then that when understanding of a system is derived from these elements recent changes in condition are prominent and restoration programs are focussed on short time solutions based on water volume. From such a short term view it is likely that any understanding of the impacts of abstraction and regulation and the focus of management is on water volume, wetting and drying regimes and river flow. This focus is further emphasized by the historical focus of monitoring on rivers, rather than wetlands, reinforcing the restoration effort to be on river flow rather than wetland condition.

Long term sediment-based records of change overcome the limitations of this approach by providing evidence for the antecedent conditions of wetlands. It is also capable of demonstrating the mechanisms of change allowing for a focus on drivers other than flow. The risk to management of the focus on flow is not only the opportunity costs of the reallocation, but that the waters remain a source of the principal cause of wetland change. So, paleolimnological approaches reveal a greater challenge for management and one which is possibly less palatable owing to the complexity of the drivers of change involved. However, with a longer term perspective we come to understand better the "leakiness" of catchments, the abrupt or gradual nature of change, the present condition in the light of the long term and shifting levels of variability and wetland ontogeny which is largely overlooked under a short term focus.

The contemporary focus on the return of environmental flows and river wetland connectivity invokes a single cause of change—the alteration of hydrological systems. Paleolimnological evidence has documented increased fluxes in elements such as nutrients, salts, and sediment particles and seeks to also manage for these fluxes in concert with water provision. Also, it remains that change may occur through the excedence of some threshold, either due to changing hydrological or water quality drivers. Management then needs to consider non-linear change to fully understand the causal mechanisms. Lastly, wetlands accrete sediments and the basins which hold water become shallower which influences the hydrogeomorphology and light environment. In one site a return to diverse aquatic beds is celebrated as a management outcome when it is in fact an ontogenetic change owing to rapid transformation to a shallow wetland owing to accelerated sedimentation (Gell et al., 2005b). Management by way of water provision, in the absence of investment in managing other drivers, risks the commitment of considerable resources for little, or temporary benefit.

If the pre-impact food web is unknown, then it is not possible to convincingly attest to a restored system. This can only be declared after reference to the paleo-community derived from the archived indicators. If a functional ecosystem is returned, but one that is structurally different, or based on different species, to that in the past, then the site is effectively rehabilitated but different. This circumstance, demonstrable with the evidence of the past, will encourage management to explore the nature of target setting and the expectations of the community to environmental restoration programs. It also will raise awareness of sediment load as an impediment to restoration by watering and so reveal the true complexity of the management challenge. Further in the eFlows debate it will reveal the desire to both allocate flows, and to mitigate sediment and nutrient flux, and so reveal the true ecological benefit, against the costs and sacrifices, of reallocating water. Further it will challenge the focus on water volumes and flow to allow for the implementation of a mix of restorative measures to ensure the biggest ecological bounce from the trade in water. Even despite this declining flows under a warming climate will ensure that even well balanced restoration programs will continue to face great challenges into the future.

CONCLUSION

Programs dedicated to the management of the freshwater ecosystems of the Murray Darling Basin in Australia have evolved into adversarial contests over the allocation of scarce volumes of water that will diminish under climate change (Jones et al., 2002b). The recent plan to recover large volumes of water used for irrigation to restore these ecosystems has been underpinned by monitoring and research programs that have largely spanned recent decades only. These approaches have identified river flow and water allocation as the principal means of restoring the natural systems.

A synthesis and detailed analysis of an array of paleolimnological records of change in the southern basin have revealed that considerable wetland change occurred after European settlement, but well before contemporary monitoring programs commenced. These attest to other, substantial drivers of change and demote water volume as only one of many natural resource management issues. This approach reveals that elevated flux of nutrients and sediments are key drivers of change and that wetlands have exhibited complex response mechanisms attributable to their hydromorphic context. Further, river water is identified as a possible vehicle transporting the sediments and nutrients to the wetlands. These records have also extended the temporal realm of ecological knowledge into the period where more sensitive wetlands may be expected to have responded to early industrialization. In effect, the records do reveal early and abrupt responses and that, in many cases, these conditions have persisted. It stands that reinforcement mechanisms are now in place that may resist restoration attempts.

These studies attest to the ecological and political risk of the ongoing focus on volume as the principal means of securing the restoration of wetland systems. While the quality of river water is compromised the risk remains that the significant investment in water volume under the Basin Plan both alienates the irrigation industry and creates little ecological recovery. Such an outcome threatens the regional community's commitment to the restoration cause given the considerable opportunity cost they have been required to meet through reduced water allocations.

A perspective that incorporates the evidence of ecosystem change drawn from sediment-based studies, in association with contemporary monitoring approaches, would better identify the broader spectrum of drivers of change and direct management to implement a better targeted mix of management measures. This would direct the restoration effort to be as much about the mitigation of nutrient and sediment flux as the withdrawal of valuable water from private entitlements. The funds released from the reduced level of water buy-back can be dedicated to more targeted intervention measures such as riparian watering, nutrient trapping, and bank stabilization. Such an approach would share the load of resource re-allocation, reinstate the commitment of local communities and increase the sustainability of the Plan.

AUTHOR CONTRIBUTIONS

PG drafted the manuscript. MR provided **Figures 1–7** and described the analysis of the 17 test sites. Both authors have undertaken or supervised the analysis of the diatoms records analyzed.

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Using paleolimnology to find restoration solutions: the case of Lake Muzzano, Switzerland

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Lake Muzzano (45°59′50″N 8°55′41″E, 337 m a.s.l.) is a hyper-eutrophied lake located in the Tessin region of Switzerland. Almost every year, algal blooms (*Microcystis*) cover the lake with a thickness of 1–2 cm. These blooms associated with periods of anoxia in summer have led to fish kills in 1967 and 1994. In the hope of avoiding these blooms, a bypass bringing water away from the lake has been established in 1999. This solution was not adequate as blooms kept reoccurring. Sediment removal was then proposed by the Tessin Canton as a possible remediation technique and The L.A.K.E.S Institute had a mandate in 2010 to study the lake (present and past state) to determine the reasons creating anoxia and algal blooms. The present state of the lake shows that anoxia is still occurring when the algal bloom covers the lake's surface. Subfossil diatom and chironomid analyses show that the baseline conditions were those found before 1922 AD when the lake was oligotrophic and supported a diversified community of chironomids suggesting good oxygenation. After 1922 AD, circulation to the lake was cut out and nutrients accumulated in the lake leading to anoxia and the establishment of *Microcystis*. Heavy metal analysis in the sediment shows that the concentration is above the national recommendation and thus sediment should not be removed or should be stored with hazardous material. Based on the present status of the lake and paleolimnological results, two solutions are proposed: to further decrease the nutrients coming in the lake (possibly using filtrating plants) followed by flushing to increase lake water circulation. Physical capping of the sediment to avoid exchange of heavy metals and phosphorus release at the water/sediment interface could also be envisaged once the two prime solutions are in place.

Keywords: diatoms, chironomids, heavy metals, sediment removal, anoxia, algal blooms, capping, filtering plants

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Introduction

In the UK, Scandinavia and Canada, using paleolimnology to determine the baseline limnological conditions to restore a lake has precedents (see papers in this issue). In Switzerland, this is not the case. Restoration solutions are generally based on short term data, with restoration goals which are generally not quantified. Furthermore, most restoration solutions primarily focus on phosphorus control and assume other possible factors are just concomitant. This study is the first one in Switzerland to use paleolimnology to (a) define the baseline conditions for lake restoration and (b) study the past ecosystems to identify the "real" problems leading to eutrophication.

Our study site is Lake Muzzano located in the Italian part (Tessin) of Switzerland. The lake is hyper-eutrophied and suffers from recurrent *Microcystis* algal blooms (Bottinelli, 1999; Bottinelli et al., 2000; Isenburg et al., 2000; Pedrotta, 2009; Studio Blu Progetti Sagl, 2011–2012). Furthermore, anoxia has been measured during summer (Isenburg et al., 2000; Hale et al., 2009; Pedrotta, 2009; Studio Blu Progetti Sagl, 2011–2012). The anoxia-inducing algal blooms have led to fish kills in 1967 and 1994 (Aquarius, 1999). To avoid fish kills, a bypass deriving the major inflow has been installed in 1999. Large pipelines bring the majority of water underground through a small river leading to Lake Lugano. However, water still flows in Lake Muzzano when the system overflows. In theory, overflow should occur only during periods of heavy rain. More than 10 years after the bypass was installed, algal blooms are still occurring. Assuming that the quantity of nutrients coming to the lake was adequately reduced by the bypass, it was suggested that the problem in Lake Muzzano was internal phosphorus loading via sediment and thus sediment removal was proposed. Lake Muzzano is owned by a private non-profit organization called Pro Natura which has the mandate of preserving the lake which houses a unique species of water chestnut (*Trapa natans* var. *muzzanensis).* Before removing the sediment, as suggested, Pro Natura wanted to be sure that sediment removal would not affect this unique water chestnut community. The L.A.K.E.S Institute was then mandated to study Lake Muzzano. The goals of this research are thus to:

(1). Establish the nutrient budget. None of the previous studies had established a comprehensive study of inflows and outflow. Pedrotta (2009) had looked at the inflows and outflow for a few months in 2006 and 2007 but no data were available on a whole year. Isenburg (1998) and Hale et al. (2009) only looked at the nutrients in the lake without comparing with inflows and outflow. Furthermore, the quantity of Phosphorus possibly released from the sediment was never quantified.

(2). Determine the baseline conditions as a target for remediation. Biological and sedimentological analyses will be used to determine the pre-disturbance limnological conditions and study the historical changes of Lake Muzzano through time. This reconstruction should be made from a period of low human impact to reconstruct the natural variability of this ecosystem.

(3). Evaluate sediment removal as a possible restoration solution. The L.A.K.E.S Institute wonders what is contained in the sediment (e.g., heavy metals).

Materials and Methods

The Study Site

Lake Muzzano (45°59′50″N 8°55′41″E, 337 m a.s.l.) is located in the Tessin Canton of Switzerland. The lake's catchment has three main localities contributing to its input of water: Muzzano [population: 837 inhabitants in 2010 (www.muzzano.ch)], Sorengo (1700 inhabitants in 2010 (www.sorengo.ch) and Collina d'Oro (population: 4493 inhabitants in 2011 (www.collinadoro.com). Berganzona, a part of Lugano, also contributes to the input of water to the lake (population: 5375 inhabitants in 2013, http://www.lugano.ch/lugano-politica/quartieri/breganzona.html).

The lake is shallow (3.2 m maximum depth) with an area of 0.22 km^2. The lake has three major and four minor (sporadic) inflows (**Figure 1**). The outflow goes to the major Lake Lugano. Lake Muzzano is turbid with a Secchi depth of 0.6 m measured in March 2012 (**Figure 1**). Between June and October, *Microcystis* blooms are 1–2 cm thick (**Figure 1**).

Lake Muzzano became stratified in the summer of 2012 (**Figure 2A**). In August 10th, 2012, the lake was strongly stratified with temperatures almost 5°C colder below 2.5 m than at the surface. A weak stratification was observed in September and disappeared by October. This stratification has been also recorded in previous years (Isenburg, 1998; Hale et al., 2009; Studio Blu Progetti Sagl, 2011–2012). In such a shallow lake (maximum 3 m depth), polymixis is generally expected. In Lake Muzzano, the stratification is probably due to the presence of algal blooms in the surface of the water column accumulating heat at the surface. When wind occurs, this thermal stratification is destroyed and mixing (uniform temperatures in the water column) occurs. In 2012, the lake was anoxic during summer (**Figure 2A**). Anoxia was also recorded in previous years (Isenburg, 1998; Isenburg et al., 2000; Hale et al., 2009; Studio Blu Progetti Sagl, 2011–2012).

Nutrient Concentration in the Lake and Nutrient Budget in Inflows and Outflow

Water samples (1 l) were taken monthly in the inflows, the outflow and in the lake (surface, 0.5, 1, 2, and 3 m). Nitrates (NO_3), Phosphorus (TP), Phosphates (Orthophosphates), and Ammonium (NH_4) were measured in these water samples using kit tests (concentration in ppm). In June 2012, the Canton of Tessin decided to provide more accurate measurements of these parameters and the monthly water samples from the inflows and outflow were sent to the Laboratorio dell'Ufficio del monitoraggio ambientale in Bellinzona for analysis. The results (in µg/L or mg/L) were used to determine how much of nutrients are still coming in Lake Muzzano and how much comes out through the outflow. The concentrations were also compared to concentrations described as "good," "acceptable," or "mediocre" by the canton (BAFU, 2010).

The debit of each inflow needed to be calculated to know the concentration of nutrients coming into the lake (mg/s) and coming out of the lake. The balance between inflows and outflow provides an understanding of the circulation and the retention time of nutrients. The longer the retention time, more nutrients are available for algal growth. The width and depth of the inflows and outflows have been measured. The flow was measured using a pulse counter measuring counts/minutes. These counts/minutes were transformed into speed following the graph included in the users' manual. The debit (Q) was calculated following this formula:

$$Q = V \times Sm$$

Where Q: debit in m^3/s

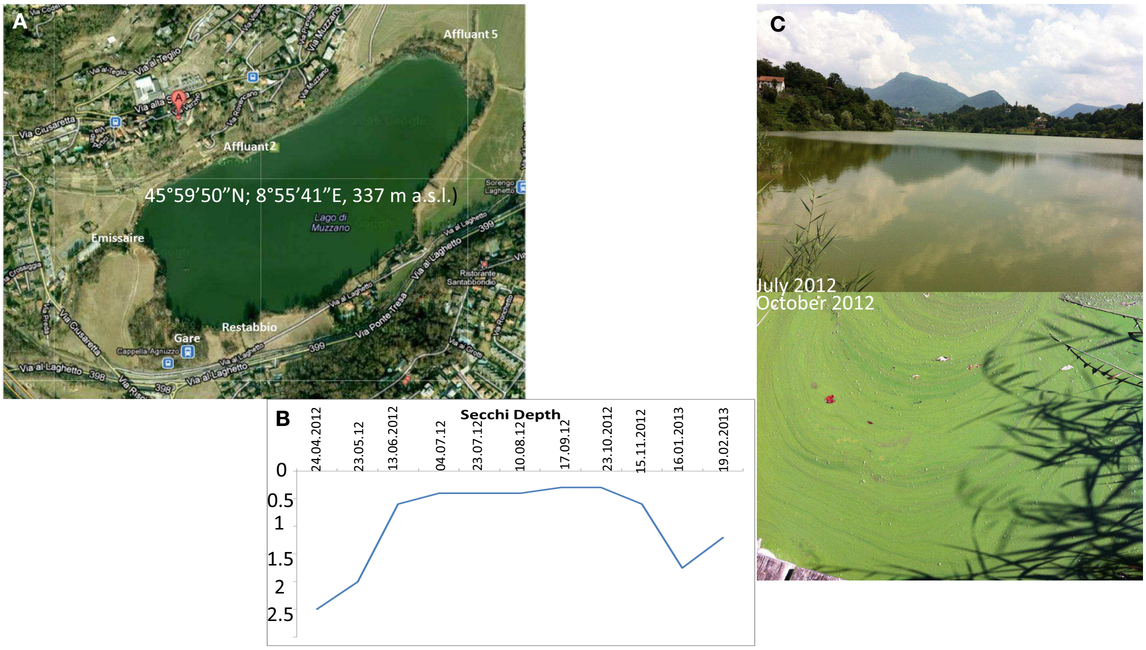

FIGURE 1 | (A) Lake Muzzano and its inflows (Affluant 5 being the larger one, Affluant 2, Restabbio, and Gare being non-permanent ones) and its outflow (Emissaire). **(B)** Secchi depth (m) and **(C)** Pictures of the lake in July and October 2002 showing Microcystis algal blooms.

V: speed of current
Sm: area of the inflow

To calculate the Phosphorus loading per year, the average concentration * the debit for each inflow should be summed (Li et al., 2003) following the formula below:

$$L_1 = K(\frac{1}{n}\sum_{i=1}^{n} C_i Q_i)$$

where C_i is the concentration at time i, Q_i is the flow at time i and K is the time interval (252 days).

Sediment Analyses

Four sediment cores were extracted in March 2012 at the deepest (3.2 m) part of the lake using a UWITEC corer. All cores were transported complete to the laboratory. The longest core (44 cm) was used for most analyses. One half of the core was used for dating and loss-on-ignition measurements, the other half was used for diatom, chironomid and heavy metal analyses.

The half core for dating was cut every 0.5 cm until 15 cm and every cm until 44 cm. All the samples from the surface to 15 cm were freeze-dried and weighted. They were sent to Flett Research (Winnipeg, Canada) for radioisotope (^{210}Pb, ^{137}Cs, ^{226}Ra) measurements. An age-depth model was developed using the Bayesian calibration program OxCal 4.2 (Bronk Ramsey, 2013) and constrained to the know peaks of ^{137}Cesium in 1986 (Chernobyl) and 1963 (Von Gunten et al., 2008).

Loss-on-Ignition (LOI) followed the method exposed in Heiri et al. (2001). First, the water content of all samples is calculated by weighting the wet sediment and subtracting the dry sediment (48 h at 105°C). The percentage of organic matter was calculated after heating the sediment to 550°C for 4 h.

Chironomids were analyzed in all samples at 0.5 and 1 cm for a total of 62 samples. The samples were placed in KOH 10% overnight and filtered in a 90 μm mesh. The sample left in the mesh was poured in a Bogorov counting tray and observed under a magnifier at 10X. Each head capsule was individually picked and mounted in a drop of Hydromatric on a microscope slide and covered with a cover slip. This procedure is very time consuming. To pick one sample can take up to half a day of preparation. The chironomids were identified under a light microscope at 40X-100X. The identification followed Larocque and Rolland (2006) and Brooks et al. (2007). Anoxic taxa were described following Quinlan et al. (1998).

The same samples (62) were analyzed for diatoms following standard procedures (Battarbee et al., 2000). The identification followed Krammer and Lange-Bertalot (1986–1991). The final taxonomy was harmonized to fit the taxonomy and taxonomic resolution of the SWISS-TP dataset in order to apply a TP transfer function using ERNIE software from the EDDI diatom database (http://craticula.ncl.ac.uk/Eddi/jsp/index.jsp). pH was described using the known ecology of diatom species described in the reference manual cited above.

The diatom and chironomid stratigraphies were subdivided into assemblage zones with the technique of optimal splitting by information content (Birks and Gordon, 1985) and the number of statistically significant zones was identified using the Psimpoll 4.27 software (Bennett, 2009).

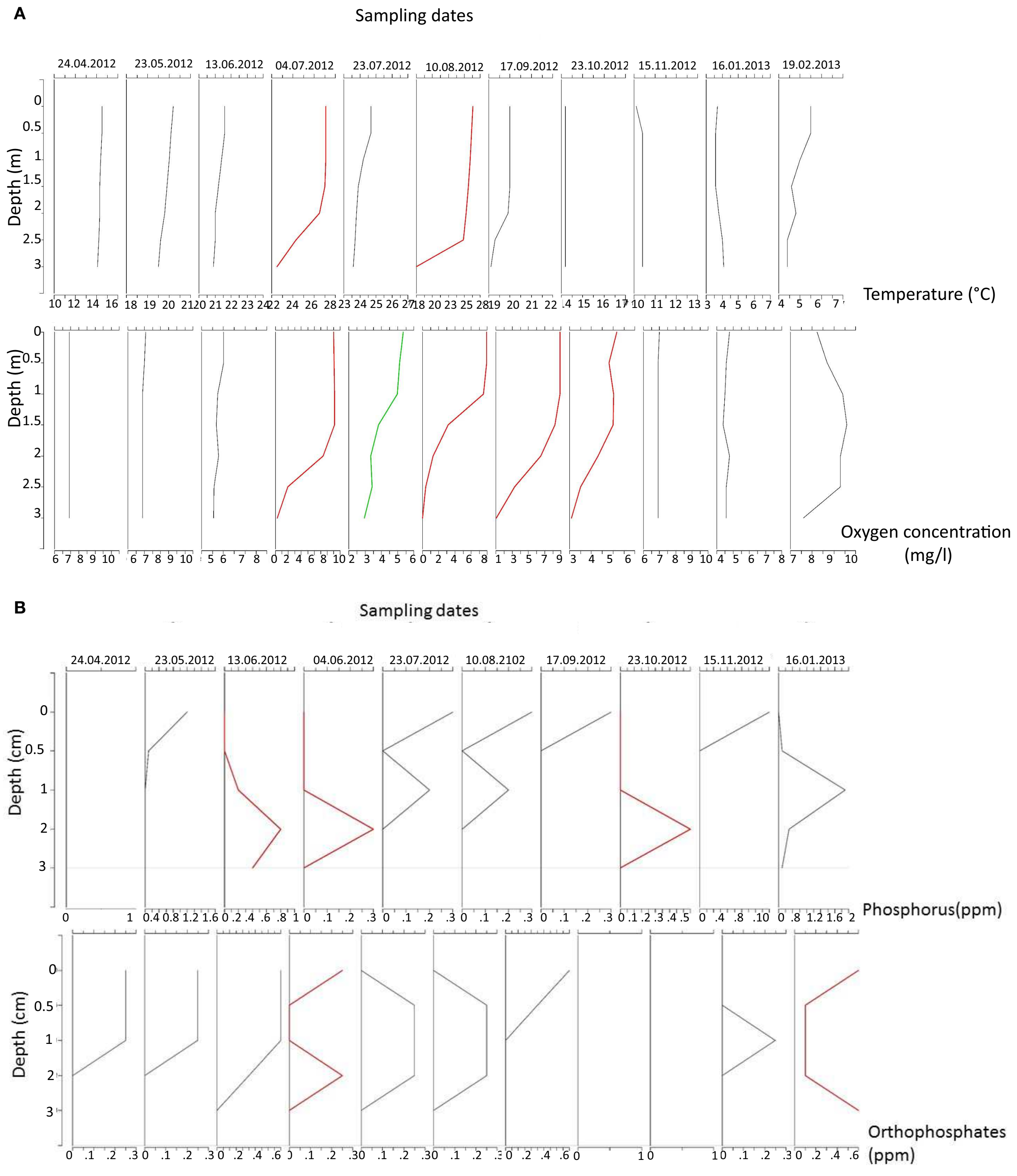

FIGURE 2 | (A) Temperature and oxygen concentration measured during the sampling period. Red lines indicate dates of established thermocline and anoxia. **(B)** Phosphorus and Orthophosphates measured during the sampling period. Red lines indicate dates when the concentration in nutrients was higher at the bottom compared to the surface.

Surface samples (slices 0–1, 1–3, 3–5, and 5–7 cm) were sent to Columbia Analytical Services, Inc. (www.caslab.com) to measure iron-bounded Phosphorus following the method detailed by Psenner et al. (1998). With anoxia, SO_4 increases in the water-sediment interface which oxidizes iron and Phosphorus is released to the water column. The concentration of Iron-bounded Phosphorus is thus important to quantify and determine how much can be released and if this amount significantly exceed the concentration coming into the lake from the inflows.

To calculate the amount of Phosphorus load, the formula from Nürnberg et al. (1994) was be used:

$$\text{Load} = \text{RR} \times \text{AF}$$

RR = areal release rate (mg/m^2/ d)
AF = anoxic factor (d/yr)

$$\text{AF} = \frac{\sum_{i=1} t_i * a_i}{A_0}$$

In this formula, the period of anoxia (t_i in days) is multiplied by the corresponding hypolimnetic area (ai in m^2), and all the products were added up. The grand total id divided by the lake surface area (/lo, mZ) to yield AF.

Sediment at every cm of the core was analyzed in an ICP spectrophotometer from the Institute of Geography, University of Bern. First, each sample was dried in an oven at 100°C overnight to remove the water. The sample was then crushed to obtain fine-grained sediment. The sediment was diluted using a microwave and the following procedure: HF and $NaNO_3$ were added to each sample, the samples were placed in the microwave for 40 min then filtered. The filtered liquid was placed in the ICP-MS spectrophotometer. The concentration of eight different metals [Lead (Pb), Zinc (Zn), Copper (Cu), Cadmium (Cd), Chromium (Cr), Titanium (Ti), and Nickel (Ni) and Arsenic (As)] was measured in each sample. Those are the most common metals found in the Tessin area. They are also the metals usually measured in environmental assessments (BAFU, 2010). The concentration obtained is then multiplied by the dilution (50) and divided by the weight of the sediment (0.25–0.35 g) to obtained μg/kg. Those concentrations were then compared to the contaminated values defined by the Canton (BAFU, 2010).

Results

Nutrients in the Lake

Total Phosphorus was higher at 3 m-depth only at one date in June 2012. Higher concentrations at 2 m were observed on the 4th of July and in October (**Figure 2B**). Only in February 2013 was Phosphorus higher at 3 m than at other levels. Similar results were obtained for Ammonium and Nitrates (Supplementary Image 1).

Nutrient Inflows vs. Outflow

Concentrations of each nutrient at each sampling date are provided as Supplementary Image 3. A summarized debit is provided in **Table 1**. Except for Total Phosphorus, the flows of nutrients are higher in the inflows. Most importantly, the Orthophosphates (PO_4P) coming in is higher than coming out.

TABLE 1 | Flow of nutrients coming from the inflow (SUM IN) and out (OUT) and the balance (OUT-IN).

	P04P μg/s	Ptot μg/s	N02N μg/S	N03N mg/s	NH4N mg/s	Ntot mg/s
SUM IN	1614	3376	1500	98	4	122
OUT	1377	7621	226	32	1	103
OUT-IN	−237	4245	−1273	−66	−3	−19

Phosphorus Loading

Following the formula exposed in the method section, the amount of Orthophosphate (PO_4) coming to the lake is 152 kg/year and Total Phosphorus is 257 kg/year. The amount coming out of the lake is 22.27 Kg/year of PO_4 and 225 kg/year TP.

Possible Release of Phosphorus Form the Sediment

The concentrations of the various forms of phosphorus in the sediment are presented in **Table 2**. From these concentrations, it is possible to calculate the potential amount of phosphorus release from the sediment following the formula presented in the method section. The specific data are presented as Supplementary Image 2. The total phosphorus present in the sediment is 152 kg/year and the phosphorus which can be released from the sediment is 19 kg/year. This possibly released phosphorus represents 12% of the total Phosphorus measured in the sediment.

Dating of Sediment

The activity in ^{210}Pb varied in the first 2 cm suggesting mixing in the very fluid sediment (**Figure 3A**). The activity slowly decreased afterwards which indicates a good stratigraphy and preservation of slices without too much mixing, which is surprising in such fluid sediment until 5 cm. Using an age-depth model (based on the dates of ^{210}Pb and ^{226}Radon **Figure 3B**) and constrained to the ^{137}Cesium peaks of 1963 and 1986, the sediment rate was in average ca. 0.3 cm/year. It was higher (ca. 0.6 cm/year for the first 5 cm), decreased to ca. 0.23 cm/year until 31 cm, increased to ca, 0.5 cm until 40 cm and decreased to ca. 0.25 for the last 3 cm. The 43-cm core, after ^{210}Pb dating represented the last ca. 176 years with an error of about 10 years at the deepest part of the core and about 2 years (date of the model—date of the ^{137}Cesium peak) at the beginning of the core.

Loss-on-ignition

The percentage of water decreases down the core, but is always relatively high (>40%) (**Figure 4A**). In the upper 25 cm, the water content is almost always higher than 88%. The organic carbon varies as well through time with percentages between 8 and 32%. The sediment was typically black with organic matter (**Figure 4B**). After the sediment was heated at 550°C, the sediment remaining had an orange color, suggesting high concentration of iron (**Figure 4C**).

Chironomids

Sixty-two samples were analyzed for chironomids, but many had few head capsules and were merged together. The total of samples

TABLE 2 | Concentration (mg/kgDW) of the diverse form of Phosphorus.

Depth	Total P	Fe-P	Al-P	Biogenic P	Ca-P	Organic P	Mobile P	Available P	Residual	Residual%	DW	DW%
0–1 cm	8000	80	110	330	300	380	1000	4300	1830	22.875	1.3	10.4
1–3 cm	4500	30	120	260	100	390	900	1900	1060	23.55556	0.8	12.5
3–7 cm	3500	20	110	230	100	380	300	1300	1290	36.85714	0.6	23.5

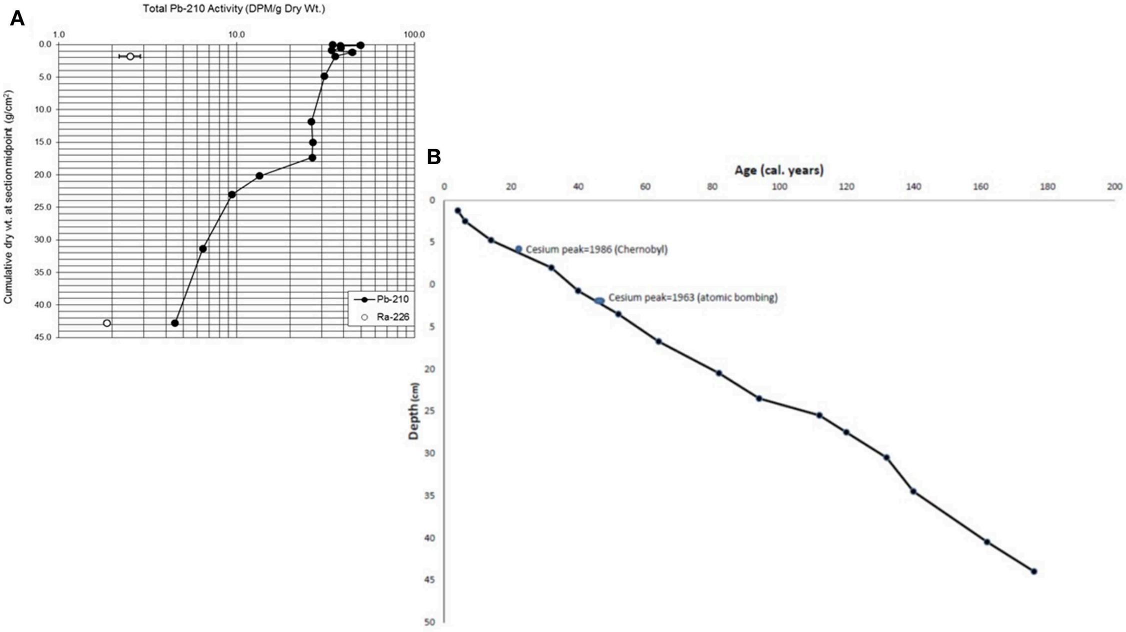

FIGURE 3 | (A) Lead concentration. Graph provided by Flett Research. **(B)** Age-depth model developed using the Bayesian calibration program OxCal 4.2 (Bronk Ramsey, 2013) and constrained to the know peaks of ^{137}Cesium in 1986 (Chernobyl) and 1963 (Von Gunten et al., 2008).

presented here is 36 since ca. 1853 AD (**Figure 5**). Thirty-five taxa were found in the samples. Only those present in at least two levels are presented in the figure.

The chironomid assemblages were significantly different during five episodes between ca. 1880 AD and 2012 (**Figure 5**). Those periods were statistically identified by the technique of optimal splitting by information content (Birks and Gordon, 1985) and the number of statistically significant zones was identified using the Psimpoll 4.27 software (Bennett, 2009).

Between 1880 and 1853 AD (zone 1), the dominating taxa (different *Tanytarsus*, *Psectrocladius sordidellus*-group and *Cladopelma*) are all taxa preferring mesotrophic and acidophilic states. Many of these taxa decrease or even disappear in zone 2 (1880–1922 AD). Zone 2 seems a transitional zone with increases in *Procladius* (meso to eutrophic taxon, often the last to survive anoxia), *Cricotopus* (eutrophic), *Corynoneura* (associated with macrophyte and eutrophic), and *Glyptotendipes* (likes detritus-rich sediment, meso to eutrophic conditions), *Endochironomus tendens*-type (acidophilic, meso) and *Endochironomus impar*-type (eutrophic). In zone 3 (1922–1943 AD), all acidophilic (e.g., *Psectrocladius sordidellus*-group, *Tanytarsus*) disappear. *Procladius, Cricotopus, Endochironomus impar*-type all increase. These are all eutrophic taxa. In zone 4 (1943–1976 AD) *Procladius* dominates with other eutrophic taxa (*Cricotopus*, *Corynoneura*) with some anoxic taxa (*Chironomus*). In zone 5 (1976–1998 AD), many taxa disappear. This is the section where many samples needed to be pulled together as very few head capsules were found. *Endochironomus impar*-type, a eutrophic taxa, dominates. *Procladius* completely disappeared. This is a taxon which can survive under anoxic conditions for a few weeks. In zone 6 (1998–2012), *Procladius* reappears toward the end as well as *Chironomus anthracinus*-group, a taxon surviving anoxia for a few weeks. New taxa (*Limnophyes*, *Smittia*) appear during this zone. *Limnophyes* and *Smittia* are often considered as semi-terrestrial taxa, suggesting an increase in running water coming to the lake.

The taxon *Chironomus* has been shown to be sensitive to heavy metals (Vermeullen et al., 2000) and can be used as an indicator of contamination due to deformation of its mentum caused by exposition. One deformed *C. anthracinus*-type has been found in level 2–3 cm (ca. 2005–2007 AD).

Diatoms

Seven zones were statistically identified in the diatom stratigraphy (**Figure 6**). Zone 1 (1853–1877 AD) was dominated by *Aulacoseira muzzanensis*, *Cyclotella pseudocomensis*,

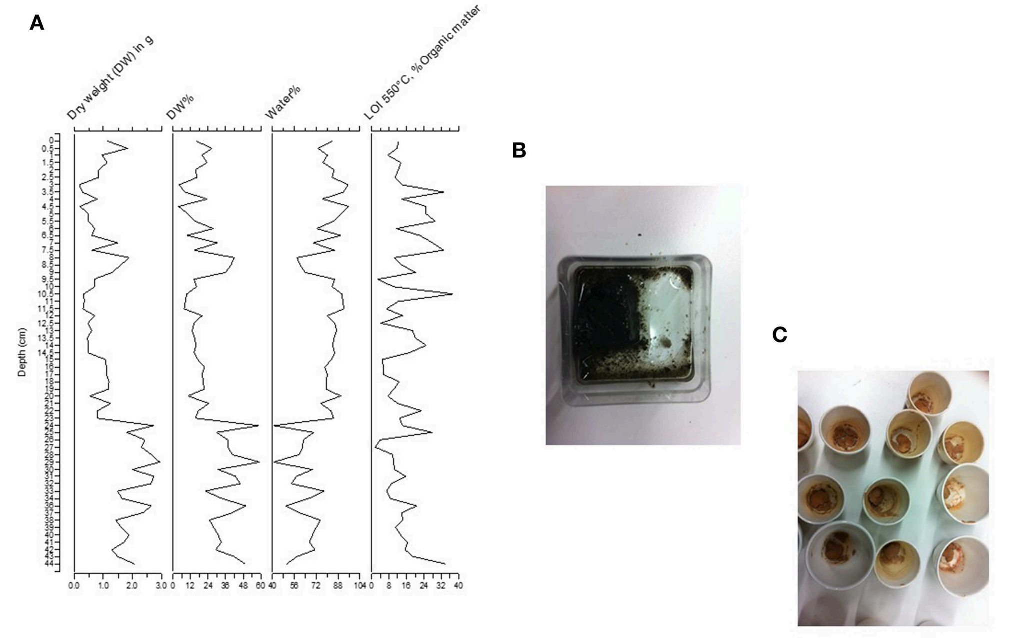

FIGURE 4 | (A) Weight of sediment, water percentage and loss-on-ignition (LOI) percentages. **(B)** Color of wet sediment and **(C)** color of sediment after heating at 550°C. The red color suggests high iron concentration.

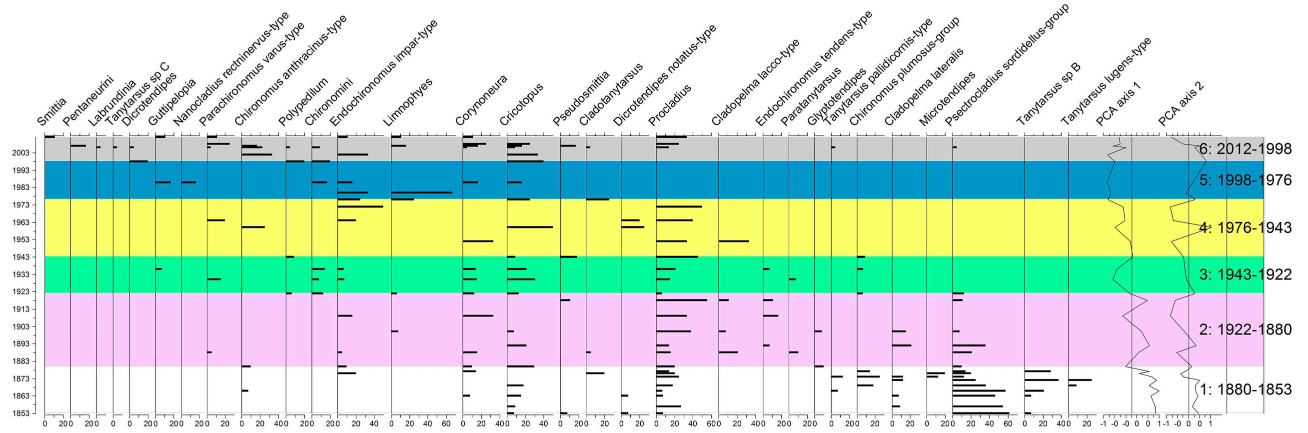

FIGURE 5 | Chironomid diagram. The taxa are in percentages. The zones were created using the technique of optimal splitting by information content (Birks and Gordon, 1985) and the number of statistically significant zones was identified using the Psimpoll 4.27 software (Bennett, 2009).

Aulacoseira ambigua, and *Achnanthidium minutissimum.* The reconstructed TP changed from 30 to <80 µg/l suggesting a change from meso-eutrophic to mesotrophic conditions. The species present suggest low pH conditions. In zone 2 (1877–1922 AD) the same species as zone 1 were present but *A. ambigua* dominated while *A. minutissimum* decreased. These changes were associated with an increase in the reconstructed TP from 70 to 120 µg/l thus to hyper-eutrophic conditions. Based on the species, the pH conditions were still acidic. In zone 3 (1922–1943 AD) the TP first increased to 180 µg/l and decreased to 120 µg/l. The communities changed to a dominance of *Cyclotella meneghiniana*, *Stephanodiscus parvus*, *Stephanodiscus hantzschii,* and *Aulacoseira granulata*. The species present suggest higher pH than in previous zones. This high pH remained through the present. Zone 4 (1942–1964 AD) was marked by a decrease of reconstructed TP to 100 µg/l (hyper-eutrophic) but the species dominating changed to *Fragilaria crotonensis* and *Asterionella formosa*. The taxa present in zone 3 are still there but at lower percentages. In zone 5 (1964–1980 AD), the reconstructed TP oscillated between hyper-eutrophic (120 µg/l) and eutrophic (>80 µg/l) and was marked by a re-increase of *A. ambigua* and *A. muzzanensis.* In zone 6 (1980–1998 AD) one sample (1993)

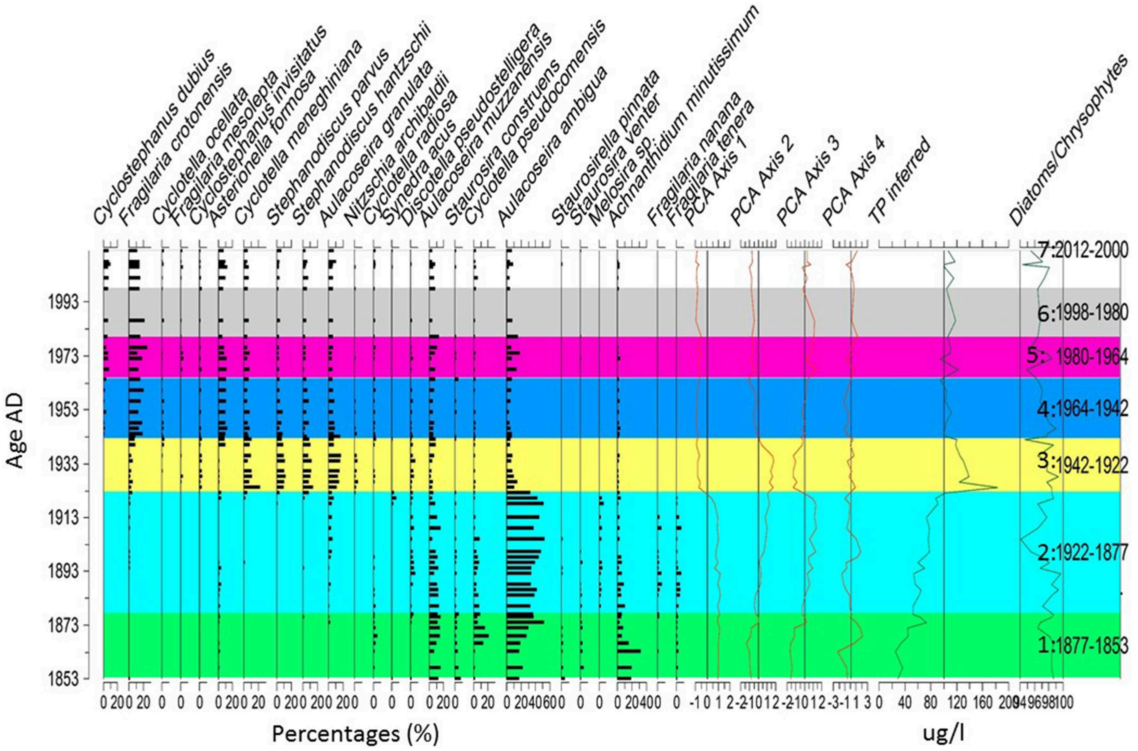

FIGURE 6 | Diatom diagram. The taxa are in percentages. The zones were created using the technique of optimal splitting by information content (Birks and Gordon, 1985) and the number of statistically significant zones was identified using the Psimpoll 4.27 software (Bennett, 2009).

had no diatoms. In zone 7 (1998–2012 AD) the inferred TP oscillated around the average (vertical line in **Figure 6**) but still represented hyper/eutrophic conditions.

Heavy Metals

Generally, the present concentrations (μg/kg) of Cr, Ni, Cu, Zn, Pb are above the "heavily polluted level" (red lines, Giesy and Hoke, 1990) while Cadmium (Cd) was always at an accepted level (**Figure 7**). The concentrations exceed the level of "heavily polluted" since 1895 for Cr, 1950 for Ni, 1860 for Cu and Zn, 1932 for As, 1972 for Ti, and 1875 for Pb.

Discussion

The discussion is made around the three objectives of this research: (1) Establishing the nutrient budget, (2) Determining the baseline conditions as a remediation objective and (3) Evaluating sediment removal as a possible restoration solution.

Establish the Budget of Nutrients

The flow of nutrients was almost always higher in the inflows compared to the outflow except in one date, in November, when *Microcystis* algae left the lake and accumulated in the outflow. For algal bloom development, the most important variables are orthophosphates and nitrates which can be used directly by the algae. The flows of these two nutrients were higher in the inflows compared to the outflow suggesting that a large amount is used by the algae or sediment before they come out of the lake. The external Orthophosphate (PO4) load was evaluated at 152 kg/year and the external load of Total Phosphorus was evaluated at 257 kg/year. In 1999, the bypass was supposed to reduce this charge to below 30 kg/year, as suggested by Vollenweider (1967). Our results suggest that this was not achieved. Pedrotta (2009) evaluated this load at 35 kg/year. However, her evaluation was based only on data from end of summer to October which, as shown by our data, does not include any spring inputs to the lake, which are higher in total nutrients. Furthermore, our estimates are also based on a few heavy rain events, which were never considered in other studies. Our results show that to properly evaluate the load of nutrients, data on a complete year of sampling should be taken into account. The load was measured over 9 months with the precise method from the laboratory. The inflows was much higher in April than at any other months (except during heavy rains) thus our estimate of the external load might even be slightly under-estimated.

The outflow of nutrients was generally higher during periods when the thermocline was broken, suggesting an increase in circulation. Based on these results, two major problems are thus identified:

(a). The amount (concentration and flow) of nutrients coming to the lake is high (257 kg/year) and
(b). These nutrients remain for too long in the lake. They are then used by the algae to develop, especially *Microcystis*.

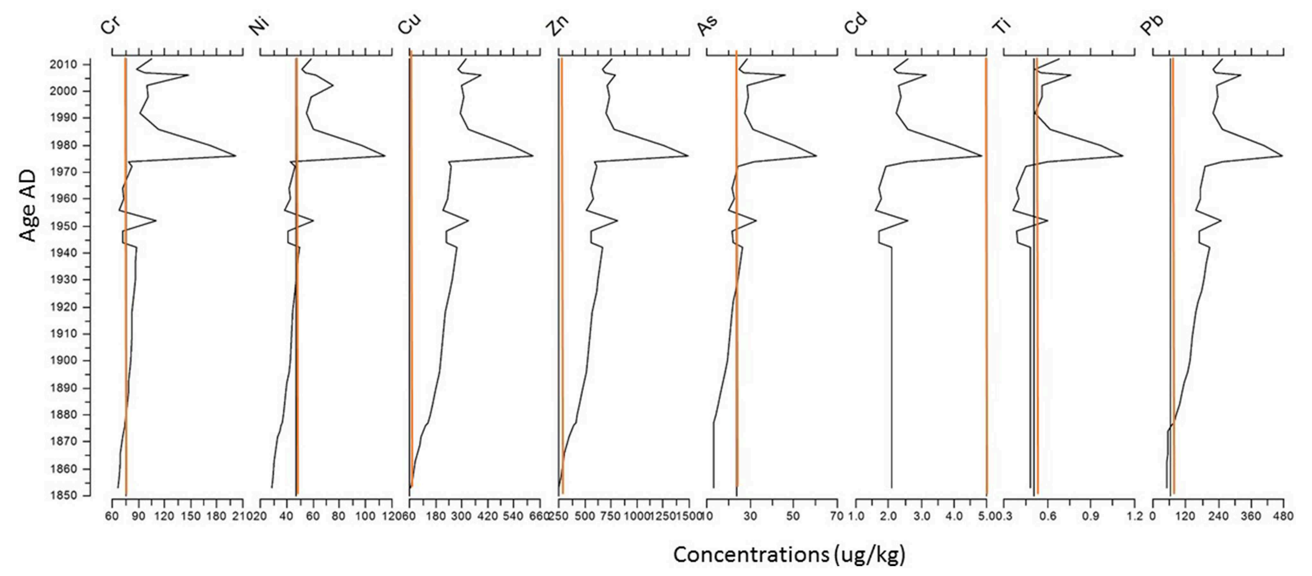

FIGURE 7 | Heavy metal concentrations. The red lines indicate levels quantified as polluted following Giesy and Hoke (1990).

> A stagnant environment favors the establishment of *Microcystis* which floats.

Other parameters influencing the development of *Microcystis* are low N:P ratios, high pH and temperature. These parameters are in Lake Muzzano, all present because they are also dependant on the nutrient budget (higher Phosphorus coming in the lake than Nitrates) and lack of circulation (temperature remains higher at the surface as well as pH).

Phosphorus Release from Sediment

During the sampling in 2012, Phosphorus could have been released from the sediment during two dates with anoxic conditions. The concentrations of Phosphorus in the lake water at the bottom were higher than at the surface during these dates. However, it seems that this Phosphorus release was less important in 2012 than in previous years when Orthophosphate concentrations were constantly higher at 3 m than at the surface (Isenburg, 1998; Hale et al., 2009).

The sediment analysis of the upper 7 cm, the most fluid part of the core, suggests that the whole amount of Mobile Phosphorus (the Phosphorus which can be released from the sediment and used for algal development) is 19.6 kg/year which is much less than the amounts coming in the lake (257 kg TP/year; 152 PO_4 kg/year). Thus, only 7.6% of the Phosphorus could be released from the sediment and used by the algae. In other words, the amount of phosphorus coming from external sources is much larger than the amount possibly released from the sediment. These suggests that, for remediation, the input of nutrients should be treated before investing in "non-permanent" in-lake solutions, such as sediment removal, to avoid resuspension of phosphorus from the sediment.

Determining the Baseline Conditions as a Remediation Objective

Chironomids surviving periods of anoxia are *Chironomini*, *C. anthracinus*-type, *Chironomus plumosus*-type, *Procladius,* and *Corynoneura* (Quinlan et al., 1998). The chironomid analysis showed that anoxia started after ca. 1922 AD when taxa which cannot support low levels of oxygen disappeared and *Chironomini*, *Chironomus plumosus*-type, *Corynoneura,* and *Procladius* dominated the assemblages. The diatoms showed increases of TP to a level of hyper-eutrophy (>120 µg/l). The stronger anoxic period was between 1976 and 1998 with the disappearance of the most anoxia-tolerant taxon (*Procladius*) and many levels during this period had no chironomids. Following this period (1998–2012 AD), *Procladius* started to re-appear, as well as *C. anthracinus*-type, two taxa surviving periods of anoxia. The return of these taxa suggests a slight amelioration of the anoxic condition in the recent years.

The diatom stratigraphy showed seven zones with major changes identified by the zonation program. The most significant changes are concomitant with changes in the chironomid stratigraphy which occurred during five periods (1880–1853; 1853–1877, 1877–1922, 1922–1943, 1943–1976, 1976–1998, and 1998–2010 AD). Considering the errors in dating (±5 years) and the absence of chironomids in many samples, these changes suggest that chironomids and diatoms were influenced by factors influencing the assemblages similarly or concomitantly such as nutrients and oxygen levels. The TP inferences suggest that Lake Muzzano was mesotrophic- slightly eutrophic until 1922 AD with a substantial increase after 1922 AD when the lake became hyper-eutrophic. This hyper-eutrophic state seems to have been maintained until recently. Slight decreases in TP were observed in 2005–2006 AD but re-increased slightly to the present state.

Both indicators showed very concomitant changes. Given the extent of current human activity in the catchment, a reasonable target to which restore the lake is the conditions between 1853 and 1922, where there was human activity although not yet to a point that detrimentally affected the lake. Those are the conditions to which the ecosystem should be brought back to. The chironomids indicate that a good circulation of the lake was present as no anoxia (no taxon suggesting low oxygen levels) was recorded. The diatoms indicate a meso/eutrophic level (<80 μg/l TP) and both indicators suggest acidic conditions based on their taxonomic assemblages. The baseline conditions for nutrients are thus 50 μg/l TP (the average until 1922) with low pH.

The chironomids also show that a major problem since 1922 AD is the change in lake's circulation. At the beginning of the twentieth century, the catchment of Lake Muzzano was substantially changed with draining of the land to create fields and the diversion of many inflows to create only one major inflow and one major outflow. The changes in inflows and outflows created a decrease in the circulation of the lake and started to induce periods of anoxia. This decrease of circulation, and increase in the amount of nutrients to the lake induced a change of state from meso/eutrophic to hyper-eutrophic, which substantially contributed to the decrease of oxygen available, leading to further periods of anoxia.

With circulation, *Microcystis* cannot remain in suspension and reproduce. Thus, increasing the circulation when *Microcystis* appears in the environment would prevent its establishment. *Microcystis* appears generally in spring (May in Lake Muzzano), thus increasing the circulation at that time would help reducing their bloom. Another problem of stagnant environment is the creation of a thermocline blocking the exchange between the air and the deepest part of the lake. In this case, the hypolimnion becomes anoxic. Lake Muzzano is shallow (3-m deep) and is a special case. The thermocline and lack of oxygen is only created when there is no wind or precipitation and when the *Microcystis* are dominating the ecosystem. Our results suggest that this state of anoxia and strong thermocline would not happen in the absence of *Microcystis*. Thus, preventing the *Microcystis* to establish, in spring, before they start to overgrow, would help in reducing the problem of anoxia.

Based on our paleolimnological results, two major changes should be made to the ecosystem of Lake Muzzano.

(a). Reduce further the inflows of nutrients.
(b). Re-establish a circulation which will discourage the development of *Microcystis* and a thermocline blocking oxygen and heat exchanges.

Proposed Reduction of Phosphorus

The proposed reduction of Phosphorus is quantified on the baseline conditions defined by the diatoms. The present phosphorus in the lake was measured in average at 145.3 μg/l in 2011 (from present data, Cantonal Laboratory of Ticino) and inferred by diatoms at 117 μg/l. The difference between measured and inferred values is within the error of the model. Although values are not always exactly the same as the instrumental data, diatom reconstruction in various countries show that the pattern of reconstruction is consistent with instrumental data (e.g., Bigler and Hall, 2003). The baseline conditions (50 μg/l) were inferred by diatoms before 1922 AD. To obtain this concentration in the lake, the amount coming from the inflows should be reduced to 69 kg P/year.

Many solutions to reduce the amount of phosphorus exist. Some are permanent, some need to be re-applied regularly (see editorial). In the case of Lake Muzzano, since the Canton cannot invest to redo the bypass, one solution would be to add filtering plants at the exit of the main inflow. Floating platforms with *Phragmites* and/or *Typha* could be installed. Those filtration systems have shown to decrease the nutrient levels by 30–60% (OIKOS 2000). The goal of this paper is not to discuss the proposed solutions in-depth, and so limited details are given here. The reader should just be aware of the solution proposed to the Tessin Canton and Pro Natura.

Re-establish Circulation

As for phosphorus control, restoring circulation has many solutions, permanent or sporadic (see editorial in this special issue). Increasing the circulation of the lake by dilution/flushing (i.e., increasing the amount of clean water coming to the lake) is a solution which could be envisaged in Lake Muzzano. However, this solution should not be made independently of solution (a) otherwise the high nutrient concentrations will be "flushed" to Lake Lugano.

Increasing circulation would be optimal for *Microcystis* control as they cannot float and establish themselves in turbulent water (Zehnder and Gorham, 1960) and circulation would increase oxygen distribution through the water column preventing anoxia.

Evaluating Sediment Removal as a Possible Restoration Solution

Since the 1980's, the sediments of Lake Muzzano are highly polluted. Restoration techniques envisaged must take into account these high concentrations. Ar (Arsenic), Nickel (Ni), and Titanium (Ti) are elements naturally present in the soils and rocks, and although their concentrations can have increased with mining, the levels measured in the sediment suggest more a source from soils/rocks weathering (Pfeifer et al., 2002). Arsenic, Nickel and Titanium concentrations have been shown to increase, similarly with other heavy metals, suggesting increase weathering and transport to the lake through the inflows.

The peaks of increased concentrations in 2006–2007, 1980, and 1954 correspond to increases in organic matter percentages which also suggest an increase of material transported to the lake by increases in inflows. These periods possibly correspond to major constructions time in the area of Lake Muzzano which possibly increased the transport of organic matter and heavy metals to the lake.

In 2005–2007, the increase in heavy metal concentrations had led to a strong decrease in the diatoms/chrysophytes ratio and one deformed *Chironomus* was found. *Chironomus* is the most robust of the chironomids. If only one head capsule is deformed, it already suggests high impact on the taxon (Brooks et al., 2005). It can survive anoxia and high concentrations of heavy metals (Brooks et al., 2005). It is possible that the creation of protective areas in Lake Muzzano created weathering of the soils and increased leaching of material contained in rocks and soils to the lake. But, the effect was limited to 1–2 years. In 2011, it seems that the building of parking places between the railway and the lake might have possibly increased the amount of heavy metals entering the lake, also by weathering. This is, at the moment, the only explanation The L.A.K.E.S Institute found for the recent increase in all heavy metals. As Lake Muzzano is a protected area, it is (hopefully) unlikely that chemical products were again dumped in the lake. These results suggest that if major construction needs to be done around the lake, the weathering of heavy metals from rock and soils should be considered.

The contamination could be also treated, or at least contained, for the health of the Lake Muzzano ecosystem. There is a possibility for heavy metals to be released during period of anoxia. Although macrophytes can be used to filter heavy metals (phytoremediation), there is surely a level when they start to suffer from the high concentrations (Feiler et al., 2007). Ebadati et al. (2005) found high concentration of zinc, lead, cupper and cadmium in *Phragmites australis*, *Typha angustifolia,* and *Potamogeton crispus* without any problem on their growth. These plants are commonly used for filtering of heavy metals. For Lake Muzzano, the return of *Nympheacea* would be favorable. Their disappearance is certainly linked to the increase in phytoplankton and thus turbid water. *Nympheacea* have been shown to be good filters of heavy metals (Larocque-Tobler, 2013a).

For soils, contaminated sites should legally be decontaminated. There is no such law for lake sediment. The only decision to make is if the effort of decontamination will have an impact on toxicity of the present organisms. Komerek and Zeman (2004) have shown that heavy metals (Cd, Zn, Cu, Hg) are strongly bonded to the sediment and can be released only under acidic conditions in the sediment. Flyhammar and Hakansson (1999) have shown that heavy metals are released only under acidic and anoxic conditions. The presence of iron in the sediment helps the heavy metals to remain bounded. Eggleton and Thomas (2004) suggest that disturbance of the sediment (re-suspension) by benthos or by strong wind could potentially help releasing the heavy metals. For the case of Lake Muzzano, the higher pH and the presence of iron in the sediment (shown in our sediment (color red) and Isenburg et al., 2000) help for the heavy metals to remain in the sediment. With proposed solutions, if anoxia is greatly decreased, the risk of release of heavy metals will be further decreased. Sediment trap data and the Beryllium suggest that there was very little re-suspension of the sediment in 2012 (data not shown here). However, a study of the heavy metals in the water would be important to do, as heavy metals in the water could directly influence the organisms.

Options exist to limit the resuspension of heavy metals which can occur during anoxic periods. The first one is, of course, to avoid anoxic periods and this would be obtained with the solutions proposed for nutrients and circulation. Wildi et al. (2004) have looked at possible restoration techniques and suggested avoiding sediment removal as it often re-suspend the heavy metals back into the system and also contribute to the pollution of groundwater, which is often used for drinking water. In our opinion, the best solution to contain the heavy metals in the sediment to avoid re-contamination would be to use a capping agent, possibly clay material (Helioz-Rybicka and Kyziol, 1992). Discussing this solution (and others) is not part of this paper, but can be found in Larocque-Tobler (2013b). However, capping alone cannot be envisaged as a solution without having made the main solutions which are the reduction of nutrients and the increase of circulation. Capping alone is a non-permanent solution.

Other Problems

Lake Muzzano is a shallow lake (3 m depth). Shallow lakes are generally more resilient to the decrease of external nutrient inputs (Havens and Schelske, 2001) and their recovery time is about 10–15 years. This restoration time can be decreased when other processes (e.g., biomanipulation) are done at the same time as nutrient decrease (Jeppesen et al., 2007; Louette et al., 2009). The problems of restoration in shallow lakes is further discussed in this special issue.

Temperature affects the development of *Microcystis* (Liu et al., 2011). Summer temperature has increased in Lake Muzzano in the past 10 years (Supplementary Image 4). It is thus hypothesized that under climate change, cyanobacterial blooms might become more common in the future in Lake Muzzano. It is thus time to envisage long-term solutions to minimize the effect of warming. This topic is further discussed in other papers of this special issue.

Conclusions

This study showed that Lake Muzzano has become slightly clearer since the bypass was installed in 1999, at least for the months of May–April 2012. The chironomids suggest a slight amelioration of anoxia after 2000 but the diatoms suggest almost no amelioration in the TP level. However, the situation with *Microcystis* blooms and anoxia when *Microcystis* is established were recurrent. The blooms were due to two situations: high nutrient input and lack of circulation. Both situations occurred since ca. 1922 AD as reconstructed by diatoms and chironomids. The input of nutrients to the lake exceeded the amount possibly produced by the sediment and the amount coming out of the lake. The phosphorus analysis from the sediment showed that if it was released under anoxic conditions, the amount would still be substantially lower than the input from inflows. The concentration of heavy metals in the sediment has slightly decreased since the 1970's but are still in concentrations suggesting heavily polluted sediment. Sediment removal is thus prohibited. To bring back the ecosystem to its baseline conditions (low pH, low TP levels (50 μg/l) and oxygen above 4 mg/l, further decrease in nutrients input and increase circulation are proposed. This could be done by filtering plants and flushing/dilution. To avoid re-suspension of heavy metals from the sediment, capping is a solution which can be envisaged.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2015.00070

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Assessing degradation and recovery pathways in lakes impacted by eutrophication using the sediment record

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Efforts to restore enriched lakes have increased yet there remains uncertainty about whether restoration targets can be achieved and over what timescale. Paleoecological techniques, principally diatom analyses, were used to examine the degree of impact and recovery in 12 European lakes subject to eutrophication and subsequent reduction in nutrient loading. Dissimilarity scores showed that all sites experienced progressive deviation from the reference sample (core bottom) prior to nutrient reduction, and principal curves indicated gradual compositional change with enrichment. When additive models were applied to the latter, the changes were statistically significant in 9 of the 12 sites. Shifts in diatom composition following reduction in nutrient loading were more equivocal, with a reversal toward the reference flora seen only in four of the deep lakes and one of the shallow lakes. Of these, only two were significant (Lake Bled and Mjøsa). Alternative nutrient sources seem to explain the lack of apparent recovery in the other deep lakes. In three shallow lakes diatom assemblages were replaced by a community associated with lower productivity but not the one seen prior to enrichment. Internal loading and top down control may influence recovery in shallow lakes and climate change may have confounded recovery in several of the study sites. Hence, ecosystem recovery is not simply a reversal of the degradation pathway and may take several decades to complete or, for some lakes, may not take place at all. By assessing ecological change over a decadal to centennial timescale, the study highlights the important role that paleolimnology can play in establishing a benchmark against which managers can evaluate the degree to which their restoration efforts are successful.

Keywords: diatoms, eutrophication, lakes, paleoecology, management, recovery

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Introduction

Most lakes throughout the world have been modified to some extent by human activity. Excessive nutrient and organic matter loading resulting in eutrophication has affected numerous waterbodies, most notably since the mid-twentieth century associated with the intensification of agriculture and expansion of populations connected to sewage treatment works (Joye et al., 2006; Battarbee et al., 2011). The consequent high algal biomass leads to filtration problems for the water industry, oxygen depletion, recreational impairment, loss of biodiversity, fish mortality, and decline or loss of submerged plants (Smith et al., 1999).

Efforts to restore enriched systems have increased over the last few decades and there are now numerous examples of lakes in recovery (Jeppesen et al., 2005; Verdonschot et al., 2013). Point-source control at sewage treatment works has been particularly effective at reducing external nutrient loads but nutrient pollution from diffuse agricultural sources has proved more difficult to control as it is dispersed over large areas (Carpenter et al., 1998; Schoumans et al., 2014). Nevertheless, restoration schemes that promote use of buffer strips, good agricultural practice, and wetland regeneration have all contributed to the reduction of nutrient loading from agricultural sources (Sharpley et al., 2000). In deep, well flushed lakes, eutrophication is often reversed by the reduction in phosphorus (P) inputs alone, such as in Lake Washington, USA, where P concentrations fell dramatically, phytoplankton biomass declined and there were sustained increases in transparency following effluent diversion and treatment (Edmondson and Lehman, 1981). However, in shallow lakes internal P loading can delay recovery and external P reduction is often combined with other management measures such as dredging or biomanipulation (Søndergaard et al., 2007; Jeppesen et al., 2012), and increasingly geo-engineering techniques which use P-capping agents (Spears et al., 2013; Zamparas and Zacharias, 2014).

Whilst there are many individual success stories, there remains considerable uncertainty about whether restoration targets can be achieved and over what timescales one might expect to see improvement. Recovery may be a slow process as biotic communities tend to exhibit hysteresis and time-lags, and thus ecosystems take time to re-adjust to reduced stress (e.g., Yan et al., 2003; Johnson and Angeler, 2010). In an analysis of long-term datasets from 35 restored lakes, Jeppesen et al. (2005) showed that internal nutrient loading delayed recovery, but in most lakes a new equilibrium for total P (TP) was reached after 10–15 years. Furthermore, new pressures, especially from global warming, may counter restoration strategies. Climate change in combination with land use changes is anticipated to cause increased nutrient loading in lakes, and may increase the frequency and intensity of harmful algal blooms (Jeppesen et al., 2010, 2014). Longer growth seasons, higher water temperature, and more turbid conditions are likely to amplify eutrophication problems (Jeppesen et al., 2010; Moss et al., 2011). Thus the expectation that ecosystems can be returned, following remediation efforts, to conditions prior to enrichment may be a naive one and managers and policy makers may have to accept that "shifting baselines" will limit the ability to meet restoration targets (Duarte et al., 2009; Bennion et al., 2011a; Battarbee et al., 2012). Indeed the concept of "novel ecosystems" has been introduced to describe ecosystems in which the species composition and/or function have been completely transformed from the historic system (Hobbs et al., 2009). It has been argued that in these systems a refocusing of conservation and restoration practices away from existing or historical assemblages may be required though most ecologists advocate management based on the same values that we want from historical ecosystems (Higgs et al., 2014; Corlett, 2015).

Legislative programmes are now in force to reduce pollution and restore aquatic ecosystems to good health in many regions of the world. In Europe, the Water Framework Directive (WFD) with its aim to restore waters to at least good status, has increased the need for effective restoration programmes for all lakes (European Union, 2000). Within the WFD, ecological status is based on the degree to which present day conditions deviate from those expected in the absence of significant anthropogenic influence, termed reference conditions. Consequently there has been a wave of research aimed at defining reference conditions and development of tools for estimating deviation from them. Lake sediment analysis provides unique insights into the history of freshwater ecosystems giving evidence for the nature and timing of ecosystem change, and providing a record of human impact that can be indispensible in developing strategies for ecosystem management (Bennion et al., 2011a). Palaeoecological methods can reveal pre-impact conditions and identify any signs of recovery, and have played a key role in the WFD (Bennion and Battarbee, 2007), particularly in determining pre-enrichment reference conditions and degree of eutrophication (Bennion et al., 2004). Diatom records have proved especially valuable in this respect, largely due to their sensitivity to shifts in trophic status (Bennion and Simpson, 2011; Bennion et al., 2011b). As many restoration programmes progress, there is great potential to employ a combination of limnological and sediment records to track recovery using the pre-eutrophication baseline as a benchmark (Battarbee et al., 2005).

This paper employs palaeoecological techniques to examine the degree of impact and recovery in 12 European lakes that have been subject to eutrophication. Changes in the diatom assemblages, both community composition and diatom-inferred TP (DI-TP) concentrations, in sediment cores from the study lakes are assessed in response to enrichment and subsequent reduction in nutrient loading. The extent to which the diatom assemblages are approaching or deviating from reference conditions is explored using ordination and dissimilarity scores and the identification of the floristic changes is assessed statistically using additive models. The key questions being addressed are: (i) Do the observed changes reflect degradation and recovery?, (ii) Is the recovery pathway simply a reversal of the degradation pathway?, and (iii) Can the lake sediment record be used to track degradation and recovery and thus inform management?

Materials and Methods

Study Sites

The 12 study sites are located in European lowland catchments and represent a range of lake types in terms of lake area, depth, and trophic status (**Table 1**). The lakes are within the temperate climatic zone, located in six countries across Europe from Slovenia to Norway, spanning a latitudinal range from 46.4 to 60.8°N. Most of the lakes lie in lowland (<200 m) catchments, with the exception of Lake Bled in Slovenia which lies at higher elevation. The dataset covers a wide range of lake surface areas

TABLE 1 | Summary characteristics of the 12 study sites.

Site name (year of core collection)	Lat. °N	Long. °E	Country	Alt m asl	Lake area km^2	Max depth m	Mean TP $\mu g\ L^{-1}$	Lake type	Management actions
Barton Broad (1995)	52.7	1.5	England	2	0.77	1.5	74	SN	Reduced external P loading since late 1970s; sediment removal to reduce internal P-loading from 1995 to 2000
Bosherston Central Lake (2001)	51.6	−4.9	Wales	2	0.34	2.0	20	SN	Sewage diversion since 1984, bypass pipeline construction in 1992
Loch Leven (1999)	56.2	−3.4	Scotland	106	13.7	25.5[a]	53	SN	Reduced P loading since 1985 but internal loading issues
Llangorse Lake (1996)	51.9	−3.3	Wales	156	1.4	9.0	118	SN	Sewage diversion in 1981 and 1992
Marsworth Reservoir (1991)	51.8	−0.7	England	115	0.1	4.0	476	SN	Sewage part-diversion and improved sewage treatment works in mid 1980s
Lake Bled (1996)	46.4	14.1	Slovenia	475	1.5	32.0	20	DS	Sewage effluent diversion in 1982
Esthwaite Water (1997)	54.4	−3.0	England	65	1	15.5	28	DS	Reduced P loading since 1986 but internal loading issues and fish farm present until 2009
Gjersjøen (2002)	59.8	10.8	Norway	40	2.4	64.0	15	DS	Sewage effluent diversion in 1971
Kiełpińskie (2010)	53.4	19.8	Poland	120	0.61	11	105	DS	Decrease in fertilizer use and change in land use in early 1990s
Lidzbarskie (2009)	53.3	19.8	Poland	128	1.22	25.5	66	DS	Decrease in fertilizer use and change in land use in early 1990s
Mill Loch (1998)	55.1	−3.4	Scotland	55	0.11	16.8	92	DS	Exact restoration measure and timing unknown
Mjøsa (2006)	60.8	11	Norway	123	362	453.0	4	DS	Improvements to sewage treatment works in late 1970s

Lat., Latitude; Long., Longitude; SN, shallow, non-stratifying; DS, deep, stratifying. [a]the mean depth and coring depth of Loch Leven is 4 m and the lake is therefore classed as SN.

from <1 to 362 km^2 though is comprised mostly of small lakes. Similarly, lakes span a broad range in maximum water depth from 1.5 to 453 m. For data analyses, the sites have been classed as either deep, stratifying (seven lakes) or shallow, non-stratifying (five lakes), in order to explore whether these lake types respond differently to nutrient reduction measures. While Loch Leven has a deep basin of 25.5 m, the majority of the lake is shallow with a mean depth of 4 m, and as the core was collected in a water depth of 4 m, this lake is classed as shallow, non-stratifying.

The lakes cover a wide range of current (annual mean) TP concentrations from 4 to 476 $\mu g\ L^{-1}$, spanning the full trophic gradient from oligotrophic to hypertrophic conditions (**Table 1**). The catchments are largely productive with nutrient loading from either point sources such as sewage treatment works and/or diffuse sources from agriculture. All of the sites have experienced eutrophication within the last ~100 years and have either seen a reduction in external nutrient loading from sewage treatment works since the 1970s and 1980s or have received less diffuse pollution as a result of changes in land use and management (**Table 1**).

Sediment Core Collection and Analyses

A sediment core was collected from the open water area of each lake as part of several different previous studies and, therefore, coring methods and analytical resolution vary from site to site. At least 10 samples from each core spanning the last ~200 years were analyzed for diatoms and, while there were typically >5 samples representing the post-restoration period, in a few cases there were only three samples available. Diatom analysis was carried out using standard methods (Battarbee et al., 2001). A minimum of 300 valves were counted from each sample using a research quality microscope with a 100x oil immersion objective and phase contrast. Krammer and Lange-Bertalot (1986–1991) was the principal flora used in identification. The diatom data are expressed as percentage relative abundances.

Chronologies for the cores included in this study were determined using radiometric methods. Selected sediment samples were analyzed for ^{210}Pb, ^{226}Ra, ^{137}Cs, and ^{241}Am by direct gamma assay using the methods of Appleby et al. (1986). ^{210}Pb chronologies were calculated using either Constant Rate of Supply (CRS) or Constant Initial Concentration (CIC) dating models (Appleby and Oldfield, 1978), based on the method best suited to the data.

Data Analysis

The degree of floristic change in the diatom assemblages between the bottom sample and every other sample in the core was assessed using the squared chord distance (SCD) dissimilarity coefficient (Overpeck et al., 1985) implemented in C2 (Juggins, 2003). The dissimilarity scores range from 0 to 2 whereby 0 indicates that two samples are exactly the same and 2 that they are completely different. This provides a measure of deviation from the reference assemblage. For seven cores, the records extend back to 1800 AD and, therefore, represent a time period prior to major industrialization and/or agricultural intensification (Bennion and Simpson, 2011; Battarbee et al., 2011). For the remaining sites, the cores do not extend back this far but do cover the pre-enrichment period. It is generally considered that a shift from benthic to planktonic dominance occurs with eutrophication (Vadeboncoeur et al., 2003) and such a shift has been noted in the diatom assemblages in several palaeoecological studies (e.g., Battarbee, 1978; Sayer et al., 1999). Hence, the percentage of planktonic taxa vs. non-planktonic taxa was calculated for each sample to assess whether this provides a useful metric for assessing diatom response to degradation and recovery. A simple classification was used such that all centric diatoms, *Asterionella formosa, Tabellaria flocculosa,* and *Fragilaria crotonensis* were classed as planktonic and all others as non-planktonic taxa. This was considered sufficient for revealing the broad habitat shifts.

The first axis scores of a principal components analysis (PCA) or a correspondence analysis (CA or detrended CA - DCA) often fail to capture a long or dominant gradient such as we might expect to be present in temporally-ordered data with progressive change in abundance or composition of organisms. As a result, the time series of axis one scores may be a poor summary of compositional change (Simpson and Birks, 2012). Principal curves (Hastie and Stuetzle, 1989; De'ath, 1999; Simpson and Birks, 2012; Simpson, unpublished; PrC) is a non- or semi-parametric alternative to PCA, CA, DCA, etc. that is particularly suited to the identification of single or dominant gradients within a sediment core sequence. Here we use PrCs to summarize the timing of the major compositional changes in the diatom profiles for the 12 study lakes. We follow Simpson and Birks (2012) and allow the spline degrees of freedom to vary between species. PCA axis 1 was used as the starting curve in the principal curve fit for all sites except Mill Loch and Mjøsa, where a CA axis 1, and Gjersjøen, where PCA axis 2, were used respectively. These changes were needed to achieve a satisfactory fit with simple species response curves along the fitted PrC.

The PrC scores were extracted for each fit and arranged in time order. To determine if statistically significant change in composition (e.g., eutrophication and subsequent recovery) could be identified, we modeled the time series of PrC scores using additive models, with a continuous time first-order autoregressive (CAR(1)) process for the residuals to account for the lack of independence between observations (Simpson and Anderson, 2009). Note that here we use sample age as the sole covariate in the model and therefore, unlike the examples in Simpson and Anderson (2009), we simply wish to estimate the potentially non-linear trend in the PrC scores for each lake, to avoid over-interpretation of the time series of scores. Many of the cores were sampled with strongly-varying density in time, and often considerably more samples were available in the most recent sediments than the reference period. Therefore, we chose to place knots at the deciles of the distribution of sample ages for each core. This allowed the trend splines to adapt to the data in the regions of the cores where more observations were available. Fitting the additive model to the Kiełpińskie data required additional steps beyond those outlined here and in Simpson and Anderson (2009). It was not possible for the optimisation process to automatically distinguish between a wiggly trend or strong autocorrelation and the resulting model fit was highly uncertain owing to poor estimation of both the trend and the CAR(1) term in the covariance matrix of the residuals. To achieve an acceptable fit to the Kiełpińskie data we fixed the CAR(1) parameter, φ, equal to 0.5, allowing for moderate to strong residual autocorrelation, and limited the dimension of the spline basis functions to $k = 8$ ($k = 10$ in the other fits). This achieved a balance between accounting for residual autocorrelation whilst not over-fitting the data.

Approximate significance of the fitted trends can be achieved through the usual methods of statistical inference for additive models (Wood, 2006), however, this only provides a test against the simple null hypothesis of no change in PrC scores with time. Here, our interest is also on where in time the additive models suggest that compositional change takes place if the null hypothesis is rejected. In a linear regression, the slope of the regression line is the first derivative of that curve and, given the standard error of the estimate of the slope, one can determine if the slope differs from 0 (i.e., no effect). With the additive model, the slope of the fitted trend is potentially changing continuously over the time series of PrC scores, and as a result we do not have a single measure of departure from zero slope. Instead, we use the first derivative of the fitted trend spline evaluated at a set of regularly spaced time points over the interval covered by each sediment core.

To estimate the first derivative of the fitted trend spline we use the method of finite differences, in which we predict from the model at the set of regularly-spaced time points and at a second set, shifted relative to the first by a very small amount. The rate of change in the predictions between the original and shifted points is an approximation to first derivative. How well the finite difference method approximates the unknown first derivative of the spline is governed by size of the shift, with smaller values producing more accurate approximations; we used a value of 0.000001 (of a year) as the shift but the results are not sensitive to a range of values around the chosen value. With each first difference estimate we also obtained its standard error and then formed a pointwise 95% confidence interval on estimate. Where this confidence interval excludes 0 (no change, zero slope) we conclude that significant change in PrC scores is observed for that time point. We indicate these periods of statistically significant change using thicker sections of the fitted trend when plotted. Color is also used to convey meaning; blue indicates significant decrease in PrC scores whilst red indicates significant increase. In some plots, we have negated the PrC scores such that enrichment in the diatoms is associated with smaller PrC scores, and less enriched periods with larger PrC scores. This is justified as the PrC score is defined as the arc length along the fitted principal curve from one arbitrarily-chosen end of the curve (Hastie and Stuetzle, 1989). Hence, the sign on the scores is arbitrary and negating the scores for some lakes improves comparison of the extracted PrC scores and the additive models fitted to them.

A diatom-TP transfer function was applied to the diatom data to reconstruct the trophic status of each site using either: (1) a training set of 56 relatively large, deep lakes (>10 m maximum depth) from Scotland, Northern Ireland, Cumbria, southern Norway, and central Europe with annual mean TP concentrations ranging from 1 to 73 μg TP L^{-1} and a median value of 22 μg TP L^{-1} (Bennion et al., 2004); the best model was generated with simple weighted averaging and inverse deshrinking (ter Braak and van Dam, 1989); this model has a coefficient of determination (r^2) between observed and inferred values of 0.75 and a root mean squared error of prediction (RMSEP based on the jack-knifing cross validation method) of 0.25 $\log_{10}$ μg TP L^{-1}, or 2) a Northwest European training set of 152 relatively small, shallow lakes (<10 m maximum depth) with annual mean TP concentrations ranging from 5 to 1190 μg TP L^{-1} and a median value of 104 μg TP L^{-1}, and an r^2 of 0.91 and a RMSEP of 0.21 $\log_{10}$ μg TP L^{-1} for the weighted averaging partial least squares two-component (WA-PLS2) model (Bennion et al., 1996). The most appropriate transfer function was used in each case, i.e., the training set containing the greatest percentage of taxa present in the fossil samples. The DI-TP values for the surface sediment samples were generally in good agreement with the current measured TP concentrations of the study lakes. All reconstructions were implemented using C2 (Juggins, 2003).

The samples of each core were projected passively into a PCA of samples from the modern diatom-TP training sets described above, forming so called timetrack plots. These plots allow the direction of floristic change at each site to be visualized. The sample and species scores were plotted in ordination biplots to illustrate the degree to which the recovery trajectories follow back along the enrichment trajectories. For deep lakes only species that are present in at least two sites and with maximum abundance greater than or equal to 2% are shown and, for shallow lakes, these values were five occurrences and 5% abundance, respectively. These choices were purely for aesthetic reasons to improve the clarity of the plot whilst retaining labeling for the most abundant or frequently occurring taxa. Arrows have been added to the plots to illustrate the enrichment and post-restoration trajectories.

Unless stated otherwise, all analyses were conducted using R (version 3.1.3, R Core Team, 2015) with several additional packages: vegan (version 2.2-1, Oksanen et al., 2014) was used to fit the ordinations, analog (version 0.16-0, Simpson, 2007; Simpson and Oksanen, 2014) was used to fit the principal curves and time tracks, and additive models were fitted using the mgcv package (version 1.8.4, Wood, 2004, 2006, 2011). Additional R functions written by GLS (based on suggestions by Simon Wood, pers. comm.) were used to evaluate the first derivative of the trend splines and form the pointwise confidence interval. R scripts implementing the analyses and reproducing the figures are available online from https://github.com/gavinsimpson/bennion-frontiers-2015.

Results

Dissimilarity Scores

The dissimilarity scores between core bottom and other samples in each core indicate that all sites have experienced deviation from reference condition (core bottom sample) over the period represented by the cores (**Figure 1**). All sites, with the exception of Kiełpińskie and Lidzbarskie where the patterns are less clear, exhibit progressive deviation from the reference sample during the period prior to nutrient reduction, indicating gradual compositional change with enrichment. The diatom assemblages of some sites, most notably the deep lakes, show signs of returning toward the reference flora following reduction of nutrient load. This is most apparent in Lake Bled and Mjøsa, and to a lesser extent in Gjersjøen. Nonetheless, most are still far from reference condition with high dissimilarity scores ranging from 0.38 to 1.57 between the core top and bottom samples.

Percentage Plankton

In four of the five shallow lakes (Barton Broad, Loch Leven, Llangorse Lake, and Marsworth Reservoir) the % plankton increases with enrichment but does not decline during the recovery phase (**Figure 2**). In the other shallow lake, Bosherston Central Lake, the % plankton stays low throughout the record. In the deep lakes % plankton was high throughout the cores (generally >60%) but in Esthwaite Water, Gjersjøen, Mill Loch and Mjøsa slight increases in the planktonic component were observed with enrichment. Only in Mjøsa, and to a lesser extent in Esthwaite Water, was a slight decline in % plankton seen in the recovery period.

Ordination and Transfer Functions

The PrC analysis shows that all sites experienced gradual yet unidirectional shifts in PrC scores during the eutrophication phase, occurring for example in Marsworth Reservoir from 1960 to 1980, in Lake Bled from 1940 to 1970 and in Gjersjøen from 1960 to 1980 (**Figure 3**). However, notwithstanding the slight offset in timing, the scores move in the reverse direction at only four sites (Marsworth Reservoir, Lake Bled, Gjersjøen, and Mjøsa) following reduction in nutrient loading (**Figure 3**). At the remaining sites, there is no clear pattern in the direction of the PrC scores following restoration.

The additive model fits to the PrC scores indicate change in one direction to some degree in all sites except Gjersjøen, where the rapid shifts seen across relatively few data points are not well captured by the model and hence a flat line (no change, with strong residual autocorrelation) is the best fitting model (**Figure 4**). The changes are significant (as indicated by the blue lines in **Figure 4**) in all but Marsworth Reservoir and Lidzbarskie. At Marsworth Reservoir, some of the patterns in the data are explained as temporal autocorrelation rather than as trend. At Lidzbarskie, there is considerable scatter in the points and, once temporal autocorrelation is allowed for, the uncertainty on the fitted trend is sufficiently large that a statistically significant pattern cannot be extracted from the data series. For the deep lakes, the first significant decrease signaling eutrophication occurs at c.1850 in Mjøsa and Mill Loch, c.1910 in

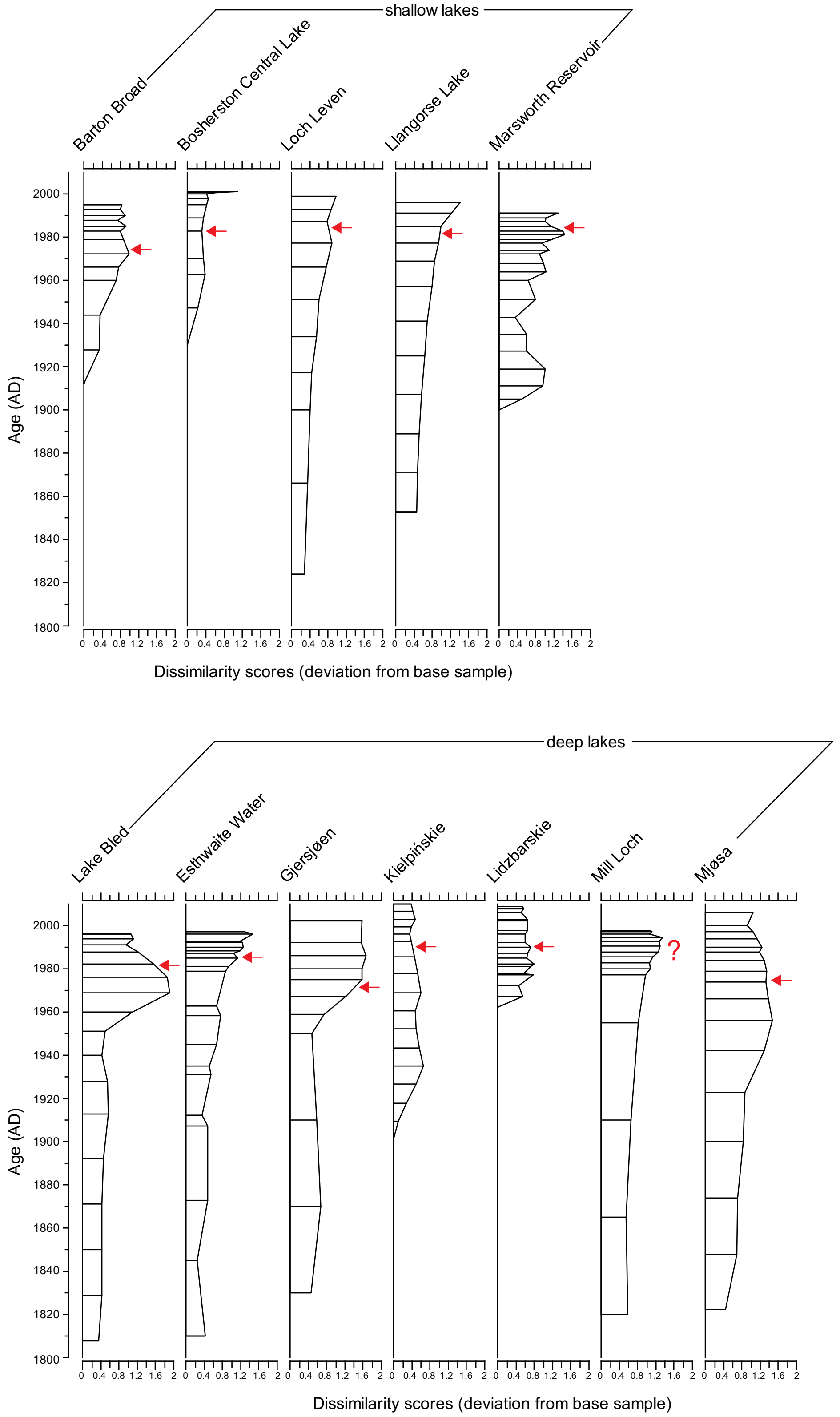

FIGURE 1 | Dissimilarity scores for the 12 study sites. The degree of floristic change in the diatom assemblages between the bottom sample and every other sample in the core; timing of first nutrient reduction is shown by the arrow.

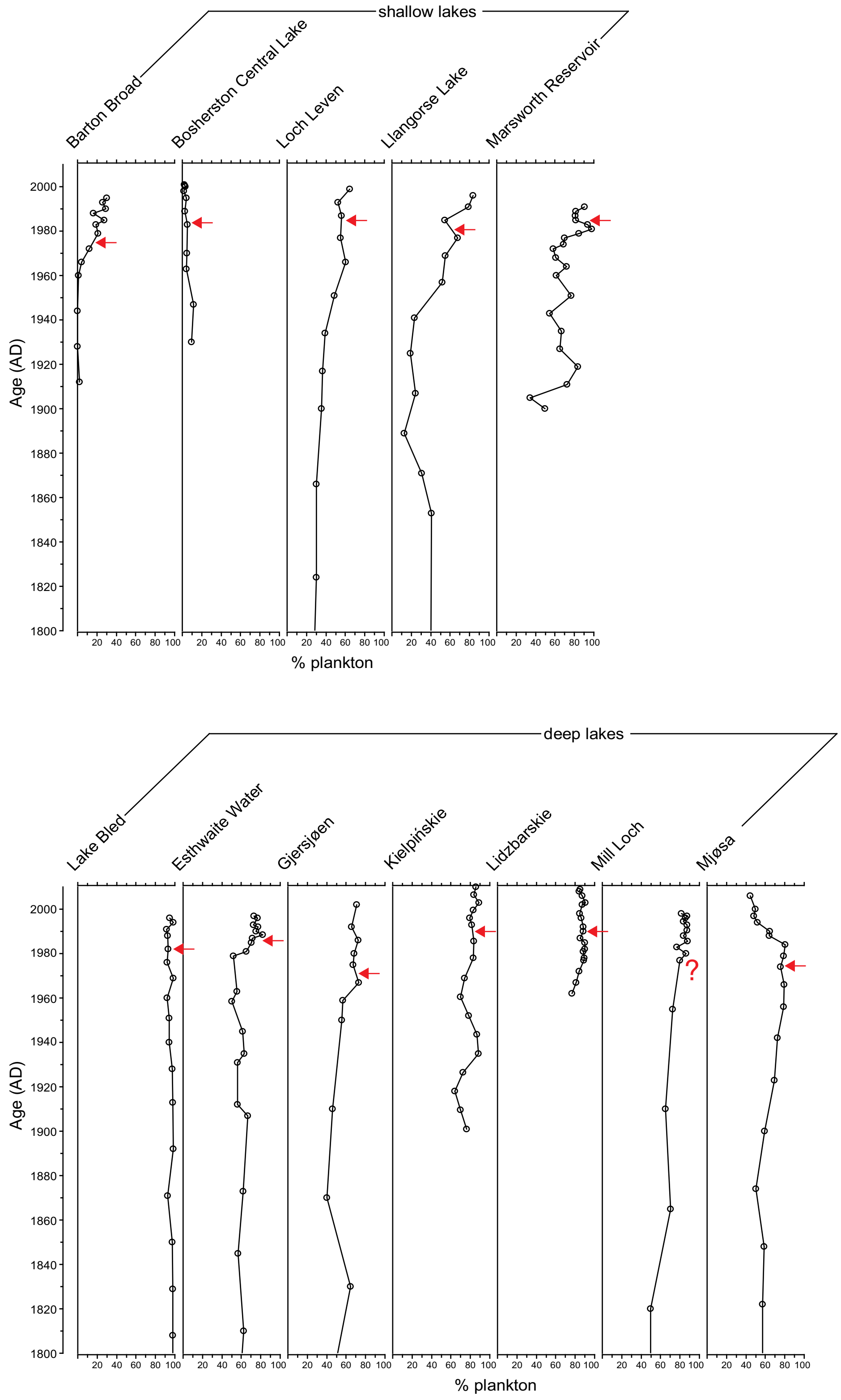

FIGURE 2 | Percentage plankton in the 12 study sites. Timing of first nutrient reduction is shown by the arrow.

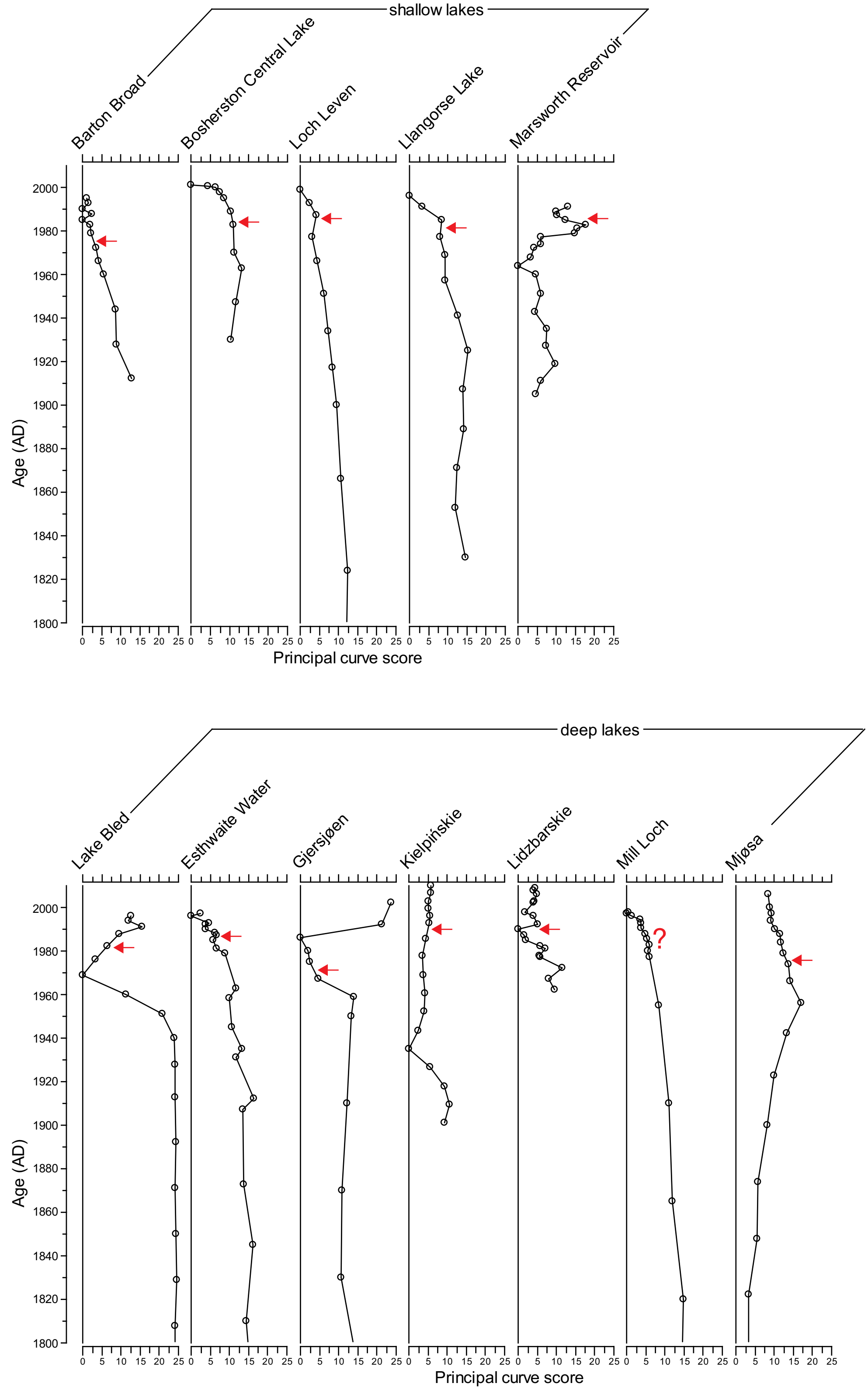

FIGURE 3 | Principal curve scores for the 12 study sites. Timing of first nutrient reduction is shown by the arrow.

Kiełpińskie, c.1930 in Esthwaite Water, and c.1940 in Lake Bled (**Figure 4B**). For the shallow lakes, the first significant decrease occurs at c.1850 in Loch Leven, c.1910 in Barton Broad, c.1940 in Llangorse Lake, and in the early 1980s in Bosherston Central Lake (**Figure 4A**). A statistically significant recovery trend is observed only in Lake Bled and Mjøsa (as indicated by the red line in **Figure 4B**), starting at around the early-1980s and mid-1970s, respectively, and in both cases is approximately coincident with restoration measures although there is a slight offset, with the shifts in the core pre-dating the introduction of measures by around 5 years. At Kiełpińskie, significant compositional change is evident from c.1945 to 1960 (red line in **Figure 4B**) but this reflects modest changes in *Aulacoseira* spp. and *Cyclotella* spp. and does not appear to be associated with recovery from eutrophication as *Stephanodiscus parvus,* typically found in nutrient-rich waters, continues to increase and become dominant during this period.

The diatom transfer functions infer an increase in TP concentrations in seven of the study lakes during the enrichment period (**Figure 5**). Of the shallow lakes, a clear signal was not seen in Barton Broad, Bosherston Central Lake or Llangorse Lake, and of the deep lakes a clear increase was not apparent in Gjersjøen or Kiełpińskie. However, the diatom transfer functions infer a decline in TP concentrations following a reduction in nutrient loading at 11 of the 12 study lakes, the exception being Kiełpińskie where a decrease in DI-TP concentrations is not clearly seen (**Figure 5**). This suggests that at these 11 sites there have been compositional changes toward taxa associated with lower nutrient concentrations following the nutrient reductions. In the case of Kiełpińskie, the shifts in the diatom assemblages were subtle and have resulted in no major change in DI-TP values in recent years.

The PCA of the deep lakes training set revealed that axis 1 (PC1: eigenvalue 10.43) and axis 2 (PC2: eigenvalue 7.76) explained 16% and 12% of the variance in the species data, respectively. These values are relatively large for diatom training sets especially those with very many taxa as here. When the deep lake cores are plotted passively on a PCA of the large, deep lakes training set samples (**Figure 6**) the core samples generally move from the lower right of the plot toward the upper left during the enrichment period. A floristic reversal is most clearly seen in Lake Bled (A), Gjersjøen (C) and Mjøsa (G), where samples move back toward the lower right following a reduction in nutrient loading, and to a lesser extent in Mill Loch (F). However, the additive models indicate that this reversal is statistically significant only in the former two sites. This reverse pattern is not seen in Esthwaite Water (B). Nor is a clear pattern observed for the two Polish lakes: at Kiełpińskie (D) samples move from right to left but there is no subsequent reversal, and at Lidzbarskie (E) there is no clear direction of change. The core trajectories reflect changes in the composition of the diatom flora with taxa associated with lower nutrient concentrations located on the right of the diagram (e.g., *Achnanthes* spp., *Brachysira* spp., *Cymbella* spp., *Eunotia* spp., oligotrophic *Cyclotella* spp., and *Tabellaria flocculosa*), those more typically found in waters with intermediate nutrient concentrations located on the upper left (e.g., *Aulacoseira subarctica*, *Asterionella formosa*, *Fragilaria crotonensis,* and *Cyclotella radiosa*) and those commonly seen in nutrient-rich conditions located on the lower left of the plot (e.g., *Aulacoseira granulata, Cyclostephanos dubius*, *Stephanodiscus hantzschii*, *Stephanodiscus parvus*) (**Figure 7**). The samples for Kiełpińskie and Lidzbarskie are compressed into a small space (**Figure 6**) owing to the dominance of *Stephanodiscus* spp. and *Cyclostephanos* spp. throughout the records.

The PCA of the shallow lakes training set revealed that PC1 (eigenvalue 9.93) and PC2 (eigenvalue 7.15) explained 15% and 11% of the variance in the species data, respectively. When the shallow lake cores are plotted passively on a PCA of the shallow lake training set samples (**Figure 8**) the core samples of all lakes, with the exception of Bosherston Central Lake (B),

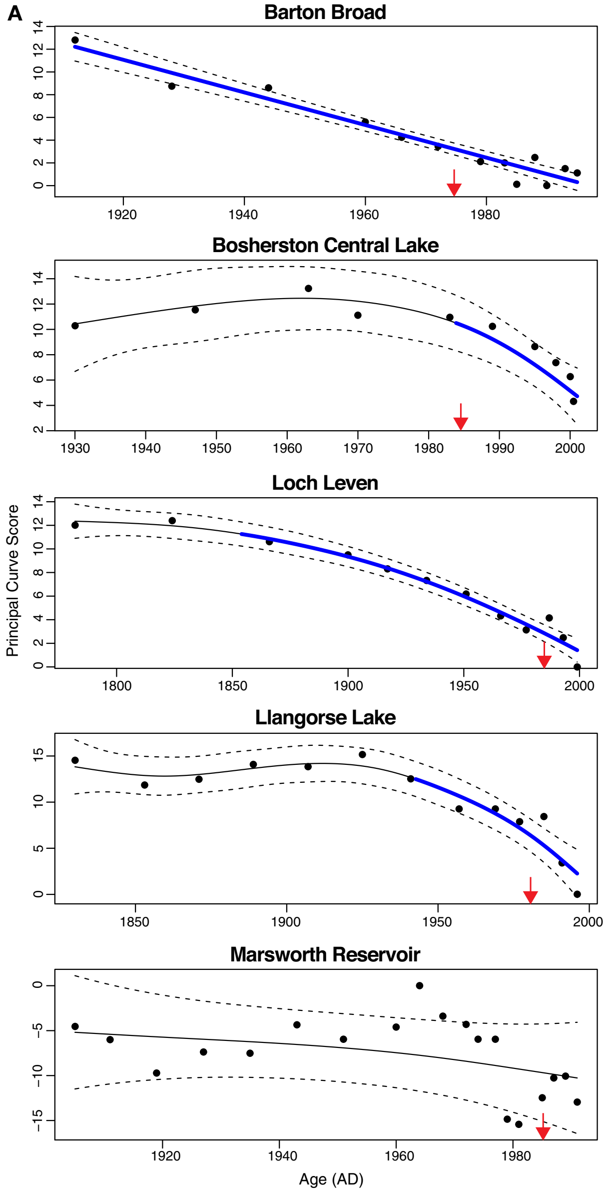

FIGURE 4 | Continued

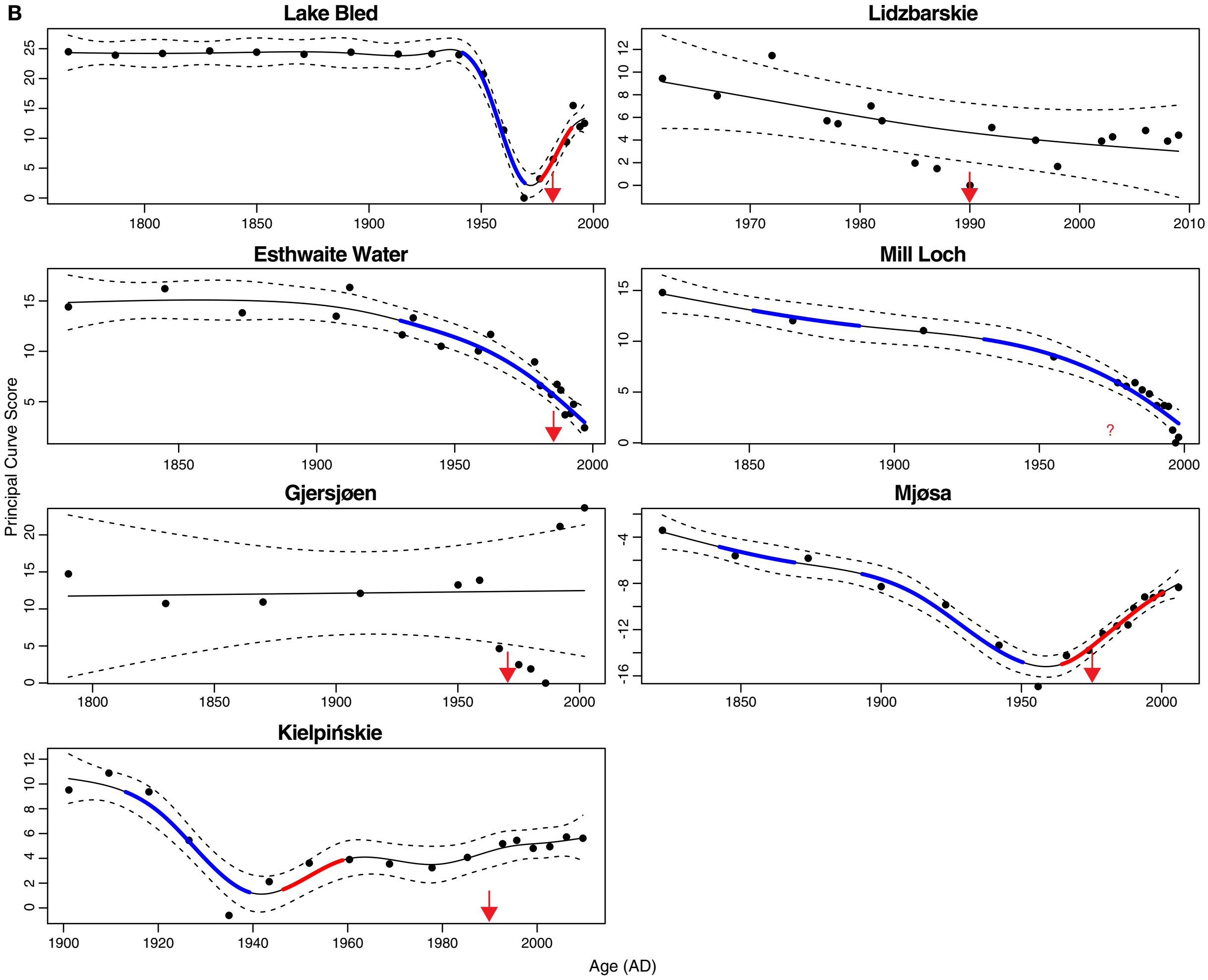

FIGURE 4 | Principal curve fits with derivatives for (A) shallow lakes and (B) deep lakes. Blue and red lines indicate significant decrease and significant increase in PrC scores, respectively. Dashed lines are 95% confidence intervals. Timing of first nutrient reduction is shown by the arrow.

move from the left of the plot toward the right during the enrichment period. A clear floristic reversal is apparent only at Marsworth Reservoir (E) following nutrient reduction, while a slight move back toward the left of the diagram is seen at Loch Leven (C), though additive models indicate that the reversal is not statistically significant in either case. At Barton Broad (A), Bosherston Central Lake (B), and Llangorse Lake (D), the upper core samples move to a new position within the ordination space but do not obviously track back along the enrichment trajectory. As for the deep lakes, the core sample shifts during the eutrophication phase largely reflect a move from taxa associated with relatively nutrient poor conditions located to the left of the diagram (e.g., *Achnanthes* spp., *Brachysira* spp., *Cymbella* spp., oligotrophic *Cyclotella* spp., and *Tabellaria flocculosa*) to those taxa typically found in nutrient rich waters located to the right of the plot (e.g., *Cyclotella meneghiniana*, *Cyclostephanos dubius*, *Cyclostephanos tholiformis*, *Stephanodiscus hantzschii*, *Stephanodiscus parvus*) (**Figure 9**). The benthic *Fragilaria* spp. which are often abundant in shallow lakes, were positioned in the upper left of the diagram.

Discussion

Diatom Response to Changes in Nutrient Loading

One of the challenges for ecologists wishing to track environmental change is to find biological indicators that are sufficiently sensitive to the pressure gradient of interest. Here, a range of diatom metrics were explored as diatoms are sensitive to changes in water quality and are particularly good indicators of lake nutrient concentrations (Hall and Smol, 2010). The most striking changes were observed in diatom composition and were effectively summarized by the PrC and dissimilarity (SCD) scores. The data demonstrate that progressive deviation from the reference condition (here defined as the assemblage at the bottom of the core) occurred at all sites during the eutrophication phase although the trends were statistically significant at only nine of the 12 sites. The diatom shifts were gradual rather than abrupt, reflecting a process of relative decline in taxa associated with low nutrient concentrations and their replacement with taxa typically found in more nutrient-rich waters. The ordination plots illustrate that whilst the reference conditions of the 12

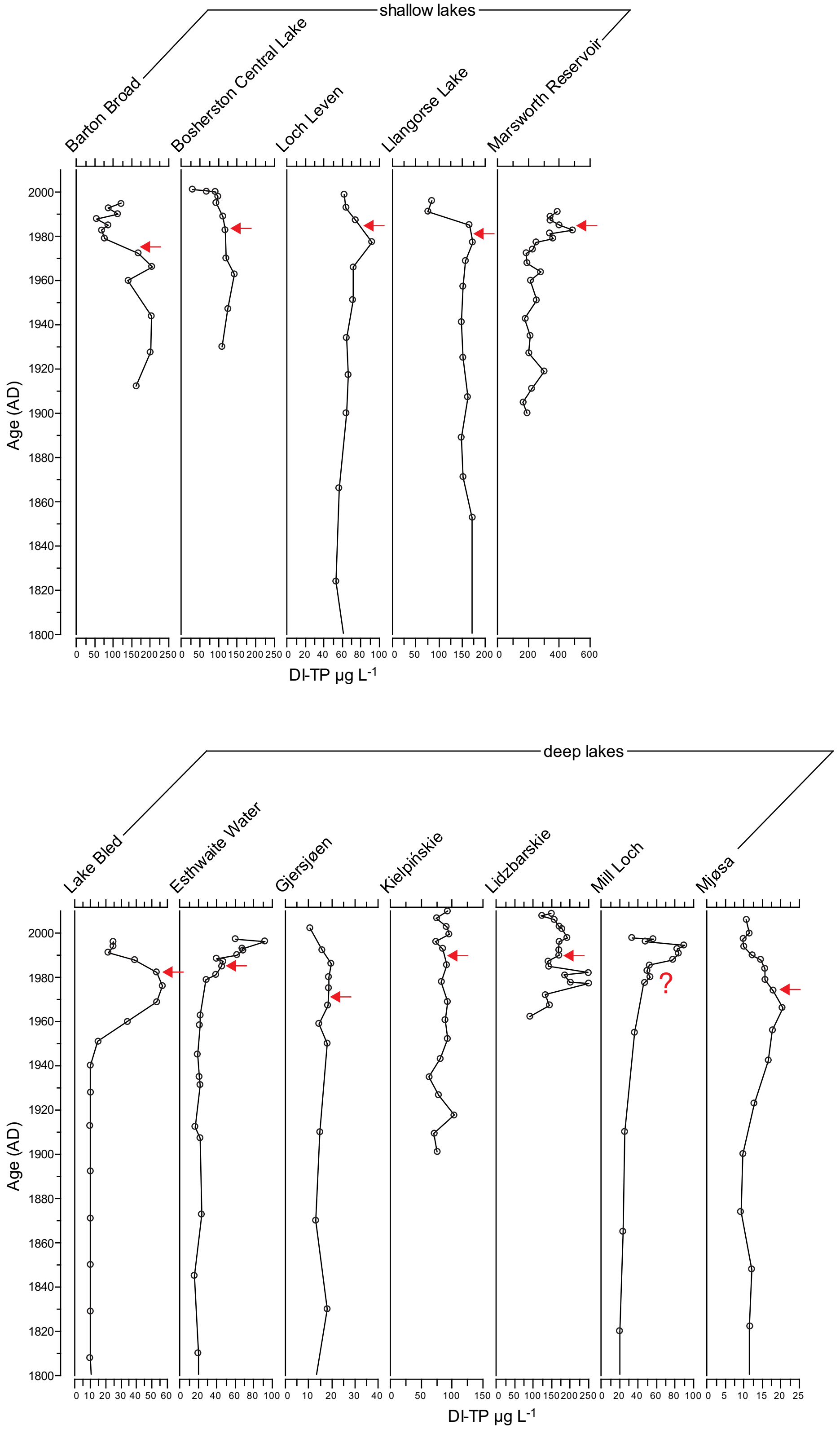

FIGURE 5 | Diatom-inferred TP (DI-TP) reconstructions for the 12 study sites. Timing of first nutrient reduction is shown by the arrow.

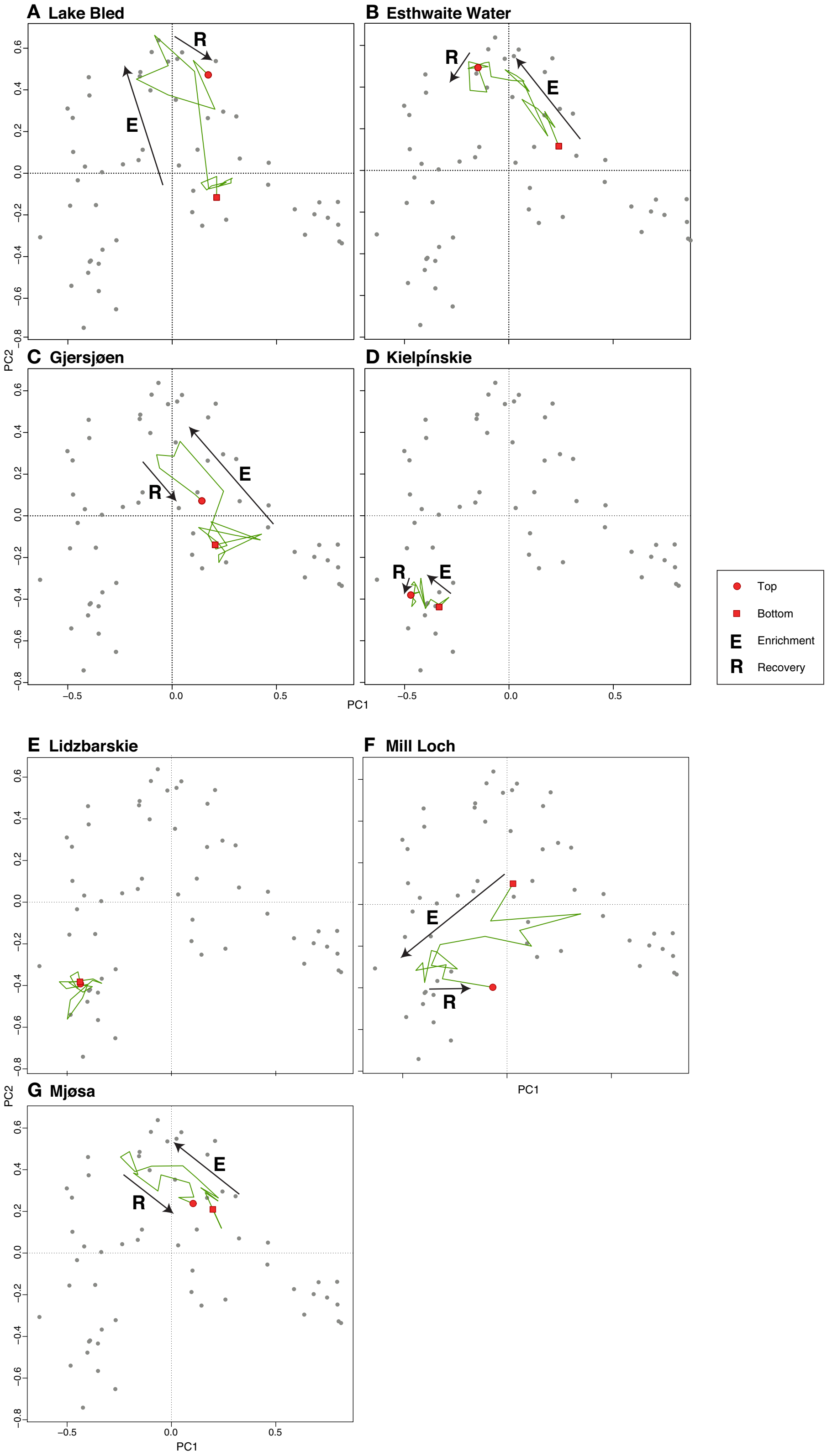

FIGURE 6 | Deep lake cores plotted passively on a PCA of training set samples. The direction of change over time is shown by the arrows. **(A)** Lake Bled, **(B)** Esthwaite Water, **(C)** Gjersjøen, **(D)** Kielpinskie, **(E)** Lidzbarskie, **(F)** Mill Loch, **(G)** Mjøsa.

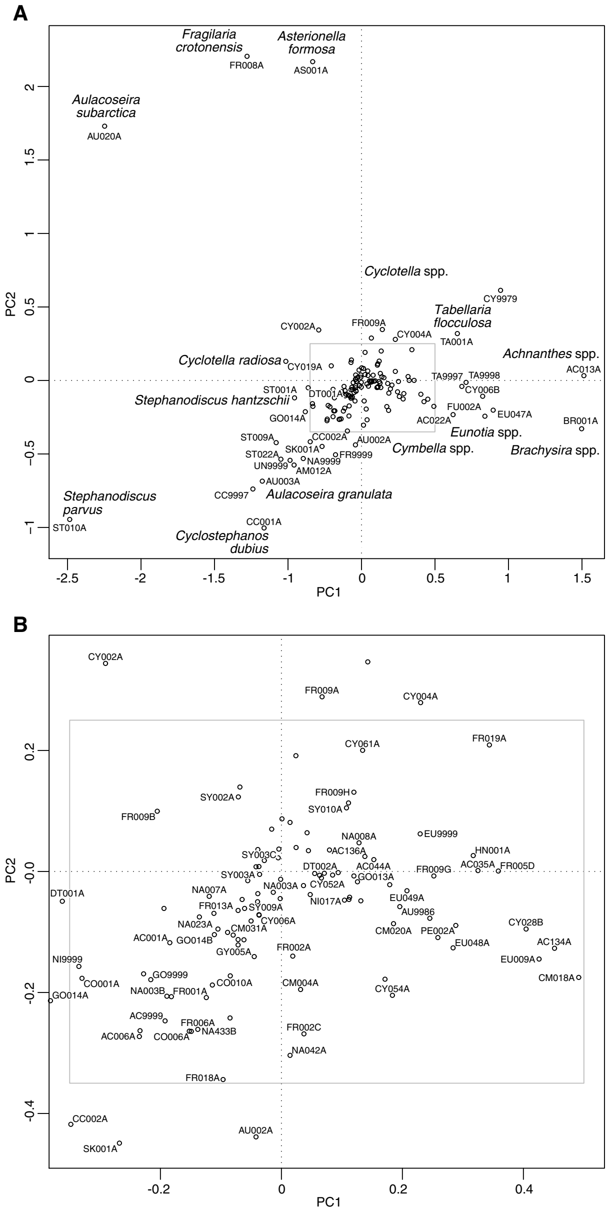

FIGURE 7 | Species plot on a PCA of training set samples for the deep lake cores. (A) All taxa present in at least two sites and with maximum abundance greater than or equal to 2%, **(B)** zoomed in section as indicated by the inset in **(A)**. See Supplementary Material, Appendix 1 for diatom codes and names.

study lakes are site specific there are some common patterns in compositional change with shifts from a flora composed of *Achnanthes* spp., *Brachysira* spp., *Cymbella* spp., *Eunotia* spp., oligotrophic *Cyclotella* spp., and *Tabellaria flocculosa* to one composed of *Aulacoseira subarctica*, *Aulacoseira granulata*, *Asterionella formosa*, *Fragilaria crotonensis,* and *Cyclotella radiosa* as enrichment progresses, and in the most nutrient-rich cases, to an assemblage composed of small centric taxa such as *Cyclostephanos dubius*, *Stephanodiscus hantzschii,* and *Stephanodiscus parvus.* These same shifts have been observed in numerous European lakes during periods of increased nutrient loading (e.g., Anderson, 1997; Lotter, 1998, 2001; Bennion et al., 2004, 2011b) and, therefore, provide a useful indication of ecological change associated with eutrophication.

The shifts in diatom composition following reduction in nutrient loading are more equivocal. A clear reversal toward the reference flora is seen only in three of the deep lakes, Lake Bled, Gjersjøen, and Mjøsa, and to a lesser extent in Mill Loch, a deep lake, and Marsworth Reservoir, a shallow lake, in terms of both the dissimilarity and direction of PrC scores. However, the model fits indicate that the recovery trend is only statistically significant at Lake Bled and Mjøsa. It is noteworthy that the three lakes that show the clearest reversal toward the reference flora are those with the lowest mean TP concentrations and are the deepest lakes in the dataset, supporting observations elsewhere that in deep lakes eutrophication is often reversed by the reduction in P inputs alone (Jeppesen et al., 2005). Nevertheless, as for the degradation phase, the compositional changes after nutrient reduction are gradual rather than sudden suggesting that ecological recovery may take several years to decades to complete. Indeed even these five lakes do not exhibit a return to the pre-enrichment flora over the 20–30 year period since remedial measures were introduced, as dissimilarity scores between the core bottoms and tops remain relatively high, and the core trajectories illustrate that the recent assemblages have not yet returned to those observed in the lower cores. The data for these five lakes suggest that, whilst the diatoms have responded to nutrient reduction and are heading back along the eutrophication pathway, they still have some way to go before they reach reference condition.

For the remaining lakes, the diatom response during the "recovery" period is more difficult to discern. At Esthwaite Water, P loading from a local sewage treatment works was reduced in 1986 but nutrients derived from catchment runoff, a fish farm established in 1981 and sediment P release have negated any potential reduction in lake nutrient concentrations (Bennion et al., 2000; Dong et al., 2012) and thus biological recovery is not apparent. For the two Polish lakes (Kiełpińskie, Lidzbarskie) which are relatively deep, stratifying waterbodies, the reversal toward former assemblages is less striking than for the other deep sites. This may be because there have been no specific restoration measures taken to reduce point sources of nutrients, and any decrease in nutrient loading is due to the reduction of fertilizer use and changes in land use in the catchments caused by the significant economic changes in the country in the early 1990s. Diatom response is, therefore, very recent and appears to be relatively subtle. The sediment records of these two lakes are also rather short, particularly so for Lidzbarskie where the core represents only 50 years. Analysis of a longer sediment core may be necessary to determine the pre-enrichment diatom assemblages and to provide a more comprehensive assessment of timing and degree of change at this site.

The Loch Leven data point to partial recovery of the diatom flora, namely an increase in *Aulacoseira subarctica* relative to *Stephanodiscus* taxa since the mid-1980s, as a result of a catchment management plan introduced in 1985 (Bennion et al., 2012). Long term datasets for the loch show that P concentrations have declined markedly but the trend was non-linear with a

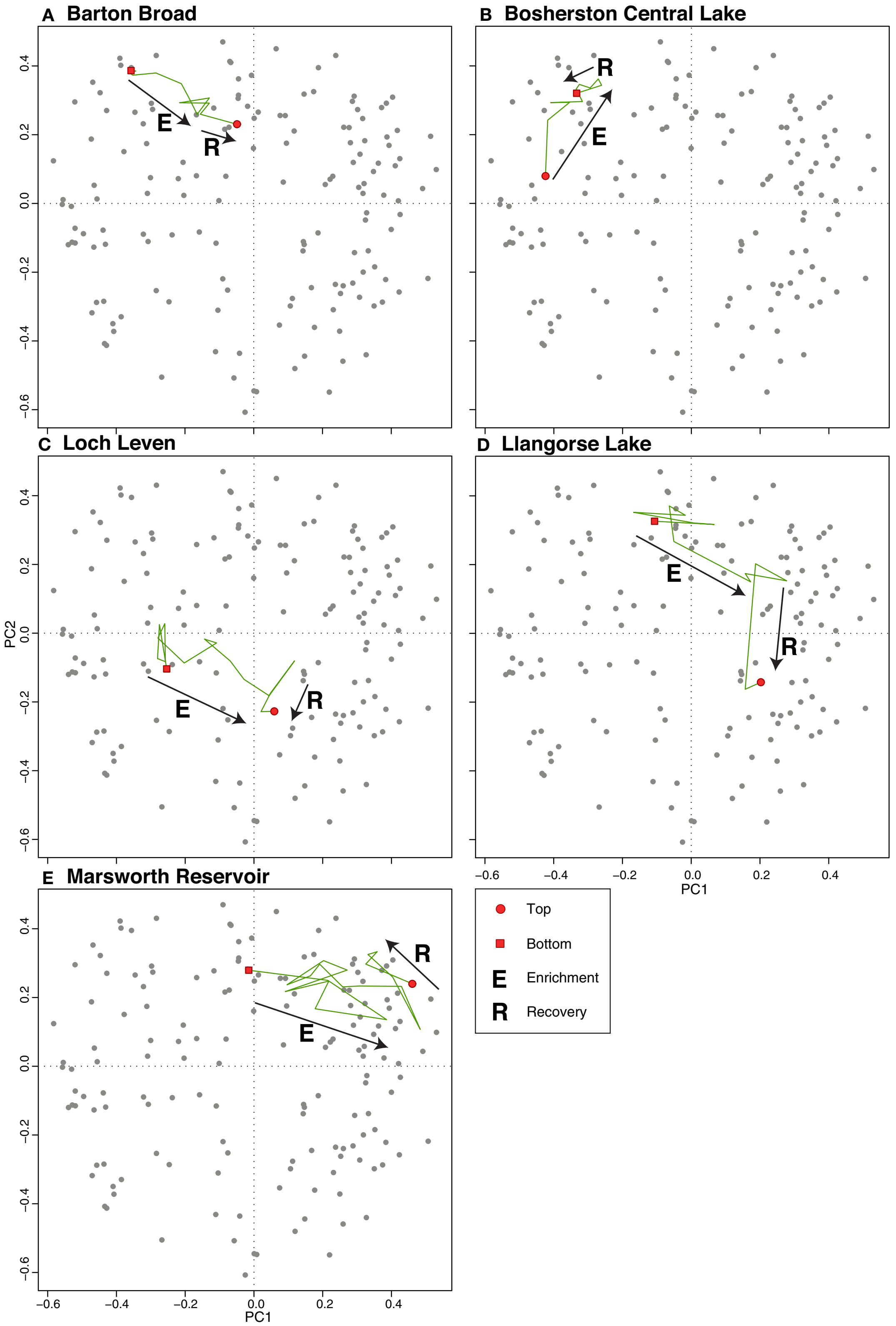

FIGURE 8 | Shallow lake cores plotted passively on a PCA of training set samples. The direction of change over time is shown by the arrows. **(A)** Barton Broad, **(B)** Bosherston Central Lake, **(C)** Loch Leven, **(D)** Llangorse Lake, **(E)** Marsworth Reservoir.

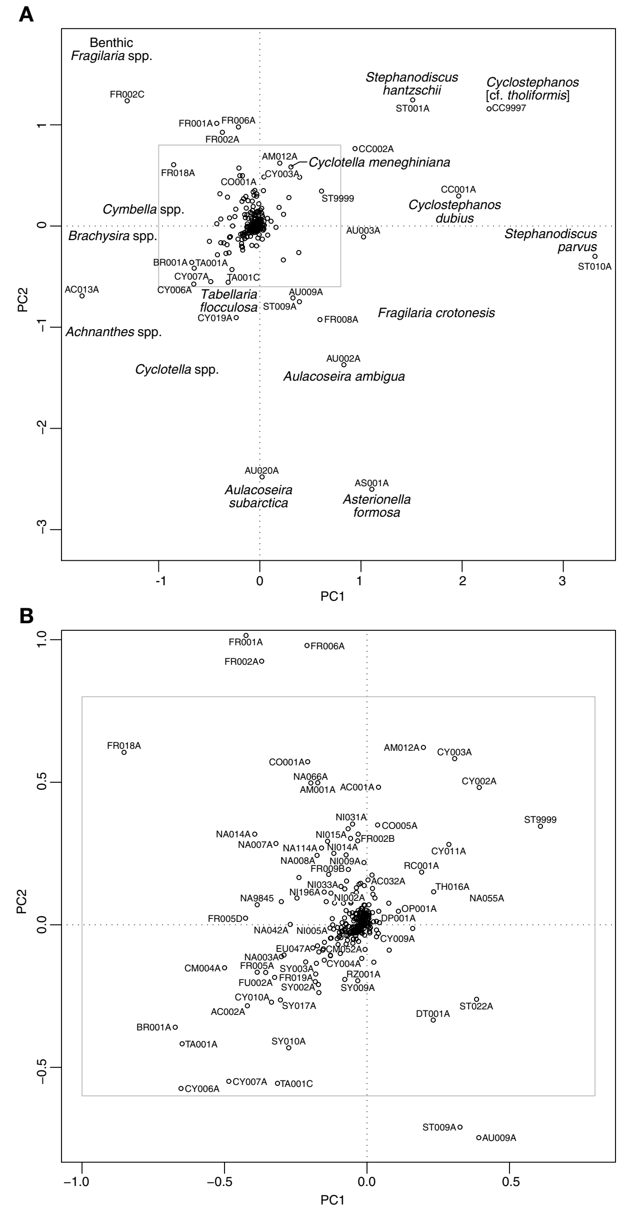

FIGURE 9 | Species plot on a PCA of training set samples for the shallow lake cores. (A) All taxa present in at least five sites and with maximum abundance greater than or equal to 5%, **(B)** zoomed in section as indicated by the inset in **(A)**. See Supplementary Material, Appendix 1 for diatom codes and names.

slight increase in the early 1990s caused by P recycling from the sediments (Carvalho et al., 2012). Additionally agriculture in the Leven catchment remains a significant diffuse source of nutrients to the loch as much of the land is used for arable farming, and rural septic tanks also contribute to the P load (May et al., 2012). The sediment record suggests that those diatom taxa lost during enrichment have not yet returned, most likely because nutrient concentrations remain too high (Bennion et al., 2012). Likewise, at Barton Broad there is little evidence of any recovery in the diatom assemblages. In spite of a substantial reduction in the amount of P entering the rivers from sewage treatment discharges and a consequent progressive decline in lake TP and chlorophyll *a* concentrations since the late 1970s and early 1980s, the reduction in epilimnetic TP was slow due to the continued release of P from the sediments (Phillips et al., 1999, 2005). Barton Broad, therefore, remains dominated by phytoplankton with almost no submerged macrophyte growth and it is perhaps not surprising that the diatoms show minimal response.

At the other two shallow lakes (Bosherston Central Lake and Llangorse Lake) there are compositional changes in the diatoms following remediation efforts but the diatom floras do not appear to revert back toward those seen prior to enrichment but rather move toward a different assemblage. At Llangorse Lake, the effluent from the local sewage treatment works was diverted from the lake in 1981, with a second smaller input diverted in 1992. While the lake appears to be recovering following the remedial measures taken (Bennion and Appleby, 1999), the diatom community in the upper samples is dominated by planktonic forms that were not previously abundant in the record (namely *Aulacoseira subarctica*, *Aulacoseira ambigua,* and *Cyclotella radiosa*) and has not yet returned to the *Fragilaria* spp. dominated assemblage seen prior to enrichment. One explanation for this is that algal productivity is N-limited rather than P-limited for most of the summer and internal P loading from the sediments in summer remains high (May et al., 2010). Furthermore, sub-surface flow has been identified at Llangorse Lake with potential to bring in nutrients from outside the immediate catchment (May et al., 2010). At Bosherston Central Lake monitored total phosphate concentrations have exhibited a decline from the early 1980s following various interventions including diversion of sewage since 1984 and construction of a bypass pipeline in 1992 (Davidson et al., 2002). The principal change in the diatom assemblages from this time, most notably since the early 1990s, has been a shift in life-forms with an overall increase in epiphytic taxa relative to benthic taxa. Interpretation of the diatom species shifts is difficult owing to the subtle nature of the changes and the uncertainties regarding the factors which determine the composition of non-planktonic communities. Nevertheless, changes in the nutrient concentrations, shifts in habitat availability, and plant community structure and alterations in grazing pressures are all possible explanations (Davidson et al., 2002). The importance of both top down and bottom up mechanisms, the role of the trophic cascade, interactions between the pelagic and littoral environments, the potential for alternative stable states (Scheffer et al., 1993), and the numerous pathways of P recycling must all be considered in order to fully understand how shallow lake ecosystems might respond to changes in nutrient loading. It is important to bear this complexity in mind when attempting to interpret the findings for the shallow lakes in the present study.

The data suggest that the percentage of planktonic taxa may be a useful metric for tracking enrichment in shallow lakes as a shift toward higher percentage of plankton was apparent with increased nutrient loading in four of the five shallow sites. An increase was less obviously seen in the deep lakes where percentage of planktonic taxa was generally high throughout the records. The shift from benthic to planktonic production associated with eutrophication in shallow waterbodies has been well documented (e.g., Vadeboncoeur et al., 2003). Benthic algae often become light limited as planktonic forms become more abundant, and as submerged macrophytes are lost so too are potential habitats to support epiphytic taxa. However, a subsequent decline in the planktonic component of the diatom assemblages following nutrient reduction was not evident, indicating that there is a degree of "unhelpful resilience" (Standish et al., 2014) and hysteresis in the systems and the diatom flora does not automatically revert back to that seen prior to enrichment.

Increases in DI-TP were observed in seven lakes during the enrichment period while a decline in DI-TP was seen in 11 lakes following remediation. Hence this metric, which essentially reflects shifts in diatom composition, appears to have some potential for tracking recovery. However, several studies have highlighted the shortcomings of the transfer function technique in certain situations, and this is particularly well documented for shallow lakes where non-planktonic taxa dominate the diatom assemblages. Problems include the influence of factors such as light, substrate, and top-down factors in addition to water chemistry on the distribution of these taxa and their wide tolerance to nutrient concentrations, making them poor indicators of lake trophic status (e.g., Anderson et al., 1993; Bennion, 1995; Bennion et al., 2001; Sayer, 2001; Juggins et al., 2013). Nonetheless, in the absence of other techniques for hindcasting nutrient concentrations, inference models are likely to remain a valuable part of the lake manager's toolkit (Saulnier-Talbot, 2015).

Degradation vs. Recovery Pathways

Our palaeoecological data reveal that whilst in some cases the diatom recovery trajectories do appear to track back along the degradation pathway, in others and the shallow lakes in particular, either little sign of recovery is evident or the assemblages follow a new trajectory. It might be argued that a return to the pre-disturbance diatom flora is less likely than the establishment of a different but ecologically equivalent assemblage i.e., species with a similar autecology. This was seen in several of the shallow lakes (e.g., Bosherston Central Lake and Llangorse Lake) where there were compositional changes toward taxa associated with lower nutrient concentrations following nutrient reduction but these were not the same taxa as those present in the pre-enrichment samples.

Our data accord with the findings of other recovery studies in that ecosystem recovery is shown not simply to be a reversal of the degradation process. Duarte et al. (2009) examined four coastal systems demonstrating that they failed to return to the reference status upon nutrient reduction, offering alternative nutrient sources, internal loading, shifts in limiting nutrients, co-limitation effects of nutrients and light, and decreased filter-feeder activity as potential explanations for failure. Similarly, riverine communities do not necessarily show the anticipated and desired signs of improvement and recovery may lead to endpoints very different from the original undisturbed state (Palmer et al., 1997). Time lags associated with the release of legacy P (the surplus P stored in soils and sediments derived from past land use activities) is one plausible explanation for why P controls may not produce expected improvements in water quality (Jarvie et al., 2013; Sharpley et al., 2013). In UK lakes, Battarbee et al. (2014) demonstrated that recovery of diatom communities from acidification was limited when compared with the pre-acidification reference. Similarly, to the current study, in a few cases the floristic composition of recent samples was different from that observed during and before the acidification phase and, while the reasons for this are not yet clear, nutrient enrichment from atmospheric N deposition and/or climate change are potential factors confounding recovery (Battarbee et al., 2014). Lake ecosystems have been reported to follow convoluted trajectories following nutrient reduction, with internal loading, changes in food webs, the impacts of climate change, and 10–15-year time lags proposed as the causes for the complex lake trajectories observed (Jeppesen et al., 2005). In a palaeoecological study of six Swiss lakes, diatoms did not switch back to the ones characteristic of the early phase of enrichment despite reductions in nutrient loading (Lotter, 2001).

Most of the existing studies on recovery pathways are based on long-term datasets but for the majority of freshwater ecosystems monitoring activities are rather short-term and do not sufficiently account for long time periods required for restoration. The longer timeframe afforded by the sediment record thus lends itself well to studies of lake recovery and by extending back several decades or even centuries is valuable for defining the reference condition against which degree of recovery can be assessed. Nonetheless, palaeoecological data are not without their limitations and in this study it is perhaps the relatively low resolution of the data for the recovery period (i.e., at some sites only two or three samples correspond to the period since nutrient reduction) that is the greatest weakness. Studies are in progress whereby sites that were cored over 20 years ago are being repeat-cored and the sediments fine-sliced to track recovery over the last few decades at a higher resolution than was possible here. Error associated with the chronologies derived from radiometric dating, albeit small in recent sediments of typically only ±2–3 years, can cause difficulty in exactly pinpointing the timing of nutrient reduction in a core and may explain the slight offset between the management actions and diatom response observed in some of our records. Equally, discrepancy in the timings may arise due to changes in land use and management in the study catchments that are not documented and may have occurred prior to the more major and well documented management actions listed in **Table 1**. Furthermore, one has to allow for a good deal of approximation in the models and fits, accepting that the model will interpolate between adjacent time points and the strength of the fitted trend at any point is dependent upon the pattern in the data both prior and subsequent to any particular time point of interest. Caution must also be exercised when interpreting changes seen in surface sediment samples (upper 0–1 cm) in terms of recovery as the reduced period of decomposition experienced by recently deposited diatoms can result in these being over-represented (Sayer, 2001). Varved sequences, offering an annual resolution, lend themselves particularly well to studies of degradation and recovery (e.g., Chandler Rowell et al., 2015). A combination of long-term datasets and palaeolimnological approaches provides a particularly powerful tool for assessing timescales of ecological change (Battarbee et al., 2005; Bennion et al., 2012; Dong et al., 2012).

Management Implications and Factors Confounding Recovery

Several of the study lakes exhibit signs of ecological recovery in terms of reversal in their diatom assemblages but even in these cases the assemblages are still far from reference conditions as much as two to three decades since management measures were taken to reduce nutrient loads. Our findings are in accordance with other studies which report typical timescales of recovery for lakes of 10–20 years lakes (Jeppesen et al., 2005; Jones and Schmitz, 2009; Verdonschot et al., 2013). This has major implications for the WFD which requires waterbodies to be restored to at least good status, over the course of the next two river basin planning cycles in 2021 and 2027, in that the effects of any measures that have recently been introduced could take several decades to be seen. Perhaps even more importantly the data suggest that for some lake systems the assemblages following remedial action may not return back down the degradation pathway at all and, therefore, reference conditions are unlikely ever to be achieved. Factors such as climate change and diffuse, legacy, and emerging pollutants (Deblonde et al., 2011) that are difficult to control may limit the ability to meet restoration targets and managers may have to accept that species assemblages following remediation may not return to those of the past.

In most of our study lakes, the main point source of nutrients, principally P, has been the key focus of management efforts. However, in recent decades diffuse nutrient sources have become relatively more significant than urban wastewater pollution and losses from agricultural land are now the biggest challenge (Schoumans et al., 2014). There has been a growing literature on the need to reduce nitrogen (N) loads as well as P in order to reverse eutrophication (Galloway et al., 2008; Finlay et al., 2013), particularly in shallow lakes with moderate P levels where high summer N concentrations stimulate algal growth and cause loss of submerged plants (e.g., Jeppesen et al., 2007; Moss et al., 2013). Indeed, a recent assessment of nutrient sources to Llangorse Lake revealed the importance of reducing N inputs if restoration targets are to be met (May et al., 2010).

The role of climate change in exacerbating the symptoms of eutrophication and confounding recovery efforts cannot be ignored. Climate change is predicted to result in higher water temperatures, shorter periods of ice-cover and longer summer stratification (Jeppesen et al., 2010). Models suggest that lakes with long residence times may experience higher P levels in the future under warmer temperatures (Malmaeus et al., 2006) and shallow lakes may be particularly susceptible. Ecological consequences might include earlier appearance of spring blooming phytoplankton and increased proportions of cyanobacteria. In some systems, negative effects may be compensated by greater predation pressure by zooplankton which is known to be positively temperature dependent. However, fish activity may also increase in warmer temperatures thereby reducing zooplankton populations through increased predation (Moss et al., 2003). In addition, changes in mixing may influence the availability of nutrients in the photic zone and higher temperatures may enhance sediment-P release, whilst higher winter precipitation is likely to enhance nutrient loss from cultivated fields (Battarbee et al., 2008).

An examination of the role of climate change in explaining the shifts in the diatom assemblages of the 12 lakes is beyond the scope of this study. However, detailed studies on two of the lakes, Esthwaite Water (Dong et al., 2012) and Loch Leven (Bennion et al., 2012) have attempted to explore the ways in which nutrients and climate interact on decadal and inter-annual timescales to affect the diatom communities. In these two lakes, the diatom response has been limited despite significant decrease in external nutrient loading. Dong et al. (2012) conclude that while nutrients have been important at Esthwaite Water during the entire 60-year investigation period, air temperature has become a controlling factor in recent decades during a period when nutrient availability was relatively high. Bennion et al. (2012) showed that at an inter-annual scale the diatom data for Loch Leven exhibit high variability, yet there are several changes in species composition in the recent fossil record that may be attributed to climatic controls. In both of these studies the presence of *Aulacoseira granulata* and *Aulacoseira granulata* var. *angustissma* seems to coincide with warmer temperatures. While the diatoms in Lake Mjøsa have experienced shifts toward the pre-enrichment community, not all the pre-eutrophication taxa have reappeared, and analysis of instrumental records lead Hobæk et al. (2012) to conclude that this is either because nitrate concentrations remain high or because water temperature has increased. Such investigations contribute to a better understanding of the effects of multiple environmental drivers on aquatic ecosystems but equally illustrate the complexity of ecosystem response to synchronous changes in nutrients and climate, and the difficulty of disentangling the effects of these interacting pressures (Battarbee et al., 2012). Models that predict likely outcomes of climate change on nutrient regimes will play a vital role in improving our understanding of future lake response and in guiding management decisions (e.g., Whitehead et al., 2006; Trolle et al., 2015). Whilst sediment records cannot be used in a predictive capacity, they provide an opportunity to validate hindcasts derived from dynamic models (Anderson et al., 2006). They should, therefore, play an increasingly important role in assessing uncertainty associated with future predictions.

Conclusions

In terms of the original questions posed we can conclude that the observed changes in the diatom records do reflect both the degradation and the recovery process. The latter has reached a different stage in each of the study lakes and is more clearly seen in several of the deep lakes where the diatom assemblages have started to revert back toward those seen prior to enrichment. In shallow lakes factors such as internal loading and top down control may influence the recovery process and in this study, whilst the assemblages of several shallow lakes were replaced by ones associated with lower productivity following remediation, they did not track back along the enrichment pathway. It can, therefore, be concluded that the deep stratified lakes tend to follow a more predictable recovery pathway than the shallow lakes. Nevertheless, the recovery process has a long way to go in all cases as the present assemblages remain very different from those seen in the pre-enrichment samples and there are indications that for some lakes reference communities may not return. Dissimilarity and principal curve scores are shown to be useful measures for quantifying the deviation from reference condition and provide a useful method with which to evaluate recovery success in the context of global change.

The study highlights the important role that paleolimnological approaches can play in establishing a benchmark against which managers can evaluate the degree to which their restoration efforts are successful. The decadal to centennial timescale adopted here provides the critical temporal context to inform the difficult decisions that emerge for the management of enriched waterbodies. We recognize that this study is based only on diatom responses and our inferences about biological recovery may therefore be biased, especially as diatoms are arguably one of the most sensitive groups in the system and have short response times relative to other assemblages such as macrophytes and fish. Assessments using multiple assemblages are required to evaluate wider ecosystem responses to environmental stressors, hence multi-proxy palaeoecological techniques have an important role to play in future studies of degradation and recovery pathways. The heterogeneous lake dataset used in this study has enabled the identification of broad-scale ecological patterns in recovering temperate lakes. The next step is to refine our understanding of these patterns for different lake types by analysing groups of lakes with more similar attributes.

Author Contributions

HB led the writing of the paper with contributions from GS and BG. HB and BG counted the diatoms in most of the cores and discussed interpretations. GS carried out the numerical analyses. All authors reviewed and edited the manuscript.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2015.00094

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The coming and going of a marl lake: multi-indicator palaeolimnology reveals abrupt ecological change and alternative views of reference conditions

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Eutrophication is the most pressing threat to highly calcareous (marl) lakes in Europe. Despite their unique chemistry and biology, comprehensive studies into their unimpacted conditions and eutrophication responses are underrepresented in conservation literature. A multi-indicator palaeolimnological study spanning ca. 1260–2009 was undertaken at Cunswick Tarn (UK), a small, presently eutrophic marl lake, in order to capture centennial timescales of impact. Specific aims were to (1) establish temporal patterns of change (gradual/abrupt) across biological groups, thereby testing theories of resistance of marl lake benthic communities to enrichment, and (2) compare the core record of reference condition with prevailing descriptions of high ecological status. Analyses of sediment calcium (Ca), phosphorus (P), pigments, diatoms, testate amoebae, cladocerans, and macrofossils, revealed three abrupt changes in ecosystem structure. The first (1900s), with biomass increases in charophytes and other benthic nutrient-poor indicators, supported ideas of resistance to eutrophication in *Chara* lakes. The second transition (1930s), from charophyte to angiosperm dominance, occurred alongside reductions in macrophyte cover, increases in eutrophic indicators, and a breakdown in marling, in support of ideas of threshold responses to enrichment. Core P increased consistently into the 1990s when rapid transitions into pelagic shallow lake ecology occurred and Cunswick Tarn became biologically unidentifiable as a marl lake. The moderate total P at which these changes occurred suggests high sensitivity of marl lakes to eutrophication. Further, the early record challenges ideas of correlation between ecological condition, charophyte biomass and sediment Ca. Instead, low benthic production, macrophyte cover, and Ca sedimentation, was inferred. Management measures must focus on reducing external nutrient and sediment loads at early stages of impact in order to preserve marl lakes.

Keywords: marl lake, chara, palaeolimnology, eutrophication, lake management, ecological quality, cladocera, macrofossils

Introduction

Globally, freshwater habitats are among the most anthropogenically impacted (Geist, 2011). Increasing population density along with technological advances in food production have increased hydrological modification, landscape homogenization, as well as nutrient loading and sediment losses from terrestrial to aquatic habitats especially over the twentieth century (Stoate et al., 2001; Geist, 2011). The effects on freshwater ecosystems include eutrophication (Hasler, 1947; Ulén et al., 2007), reductions in biodiversity (Baastrup-Spohr et al., 2013), and diminished recreational value (Pretty et al., 2003; Priskin, 2008). The widespread alteration of fresh waters especially in lowland areas where population densities are highest, has likely changed how freshwater habitats are perceived following increasingly few incidences of human encounters with truly natural habitats. Therefore, cultural perceptions of what constitutes natural, undisturbed conditions, may have become biased (i.e., shifting baseline syndrome) (Papworth et al., 2009). In turn, where records of pre-disturbance conditions are lacking, restoration can often be misguided when based on increasingly old and scattered anecdotal evidence (Tibby et al., 2008).

In terms of freshwater biology, it is desirable to characterize lake ecosystems prior to significant human impact, a prerequisite for drafting international legislation and guidelines on environmental protection and restoration (European Commission, 2000; Lake, 2001). Science and legislation, in turn, need to shape the decisions and actions of monitoring agencies, site managers, and owners of private land, in order to have a tangible positive impact on the environment. It is difficult to ascertain pre-impact conditions without historical information, yet in Europe especially, human impact extends beyond the beginning of the Industrial Revolution to Medieval, Roman, and prehistoric times, for which ecological literature is scarce. Consequently, knowledge of the natural state of the environment requires additional methods such as palaeolimnology (Brenner et al., 1993; Bennion et al., 2011). Palaeolimnological methods for tracking changes in ecological structure and function in response to multiple drivers such as eutrophication (Sayer et al., 2010; Davidson and Jeppesen, 2013; Bennion et al., 2015), climate (Last and Smol, 2001; Leng et al., 2006), hydrology, and chemistry (Smol et al., 2001) have developed substantially over the previous decades, allowing for increasingly confident ecosystem reconstructions.

In the UK, most of the highly calcareous (marl) lakes lie in heavily agricultural areas where human impact has a long history (Pentecost, 2009). Marl lakes in their natural state are highly alkaline, carbonate-rich lakes that precipitate calcite and contain distinct macrophyte communities dominated by Characeae and Potamogetonaceae (Pentecost, 2009; Wiik et al., 2013). Further, owing to their geochemistry, the water is naturally of a translucent blue-green color of high transparency (Spence, 1982). Owing to the immobilization of phosphorus (P) via coprecipitation with calcite, which occurs with the highest intensity during summer months, marl lakes have widely been held resistant to eutrophication (Wiik et al., 2013). However, the immobilization of P may be restricted temporally (e.g., dissociation in winter) and spatially (e.g., dissociation in hypolimnion/sediment) (Wiik et al., 2013), and P may be recycled in the sediment via uptake by macrophytes. Therefore, substantial changes in the benthos may occur despite low limnetic P. Indeed, even minor increases in limnetic nutrient availability have been linked to substantial changes in macrophyte communities (Krause and King, 1994; Free et al., 2007) and extirpations of key biodiversity species at a European scale (Stewart, 2001; Blaženèiè et al., 2006). In lakes, moderately clear water combined with dense aquatic flora is frequently considered near-pristine conditions, yet may not reflect truly unimpacted ecology. This may be especially true for marl lakes should further empirical support for sensitivity of their benthic communities arise.

Cunswick Tarn in northern England, despite being listed as a marl lake (Pentecost, 2009), currently contains no marl lake indicator species, does not undergo visible marl precipitation in the summer months, and has brown and turbid rather than clear blue-green, water color. It would not, therefore, be biologically categorized as a marl lake (Wiik et al., 2014) and has been classed as a (naturally) eutrophic water body in legal documentation for Sites of Special Scientific Interest (SSSI) (SD 49/2). Sufficient historical records do not currently exist to characterize the unimpacted "reference status" ecology (European Commission, 2000) of the site. In order to elaborate on concepts of marl lake ecology and -reference status, a multi-indicator palaeolimnological approach was undertaken with specific aims to (1) establish the centennial-scale temporal patterns of change (gradual/abrupt) across biological groups, thereby testing theories of resistance of marl lake benthic communities to enrichment, (2) test whether ecological change was limited to one or two biological groups or whether it was apparent at an ecosystem level (physical–chemical function; multiple biological groups at all observed trophic levels; synchronicity across responses), and (3) compare the core record evidence of reference condition with prevailing descriptions of high ecological status for marl lakes.

Given the steep shelving of the margins of Cunswick Tarn, and the sensitivity of charophytes to light penetration through the water column (Middelboe and Markager, 1997), eutrophication effects were expected to be reflected by noticeable and relatively rapid changes in the charophyte community (as abundance of fossil charophyte oospores; Zhao et al., 2006). These changes were expected to be associated with upcore increases in remains of tall-growing or floating-leaved angiosperms (Blindow, 1992; Ayres et al., 2008; Sayer et al., 2010) as well as of groups of algae and cladocerans that reflect increased nutrient availability and functional change toward increasingly pelagic shallow lake ecology (Vadeboncoeur et al., 2003; Davidson and Jeppesen, 2013). Finally, assuming that marl lakes have distinct biological communities compared with other lake types, and are sensitive to eutrophication, both calcium (Ca) and P were expected to be significant correlates of biological community composition.

Methods

Study Site

Cunswick Tarn (SD489937) is a small (0.8 ha), fishless, relatively shallow ($Z_{\mathrm{max}} = 6.4$ m; $Z_{\mathrm{mean}} = 3.7$ m) kettlehole lake in Cumbria, UK (**Figure 1**). The limestone catchment comprises improved pasture to the north and west, and woodland along a sheltering hillside to the east. A low-gradient outflow lies in the southwest corner (**Figure 1**). The main inflow lies at the southeast corner, in addition to which small, low-nutrient and carbonate-rich streams issue from springs in the east (**Figure 1**). Water high in P seeps from woodland and pastures to the north, and a drain pipe from the north pasture feeds directly into the lake. Further, grain for duck rearing has been deposited at the northern lake margins where soil erosion also occurs. The Tarn is currently eutrophic with mean annual total phosphorus (TP) of 56 μg L^{-1}. Phytoplankton blooms occur in summer months and anoxia develops below 2.5–3 m. Macrophytes are abundant to approximately 4 m depth and dominated by two species (*Nuphar lutea*, *Elodea canadensis*) (Wiik et al., 2014).

Core Collection

Two littoral sediment cores were taken from Cunswick Tarn (**Figure 1**). CUNS1 (74 cm) was taken in January 2008 at a depth of 2.4 m from the southwestern margins using a "fat" Livingstone piston corer (internal diameter 71 mm). CUNS2 (94 cm) was taken in October 2009 from the northern margins at a water depth of 4 m using a "Big Ben" piston corer (internal diameter 140 mm) (Patmore et al., 2014). Most palaeolimnological analyses were undertaken on CUNS2, but analysis of macrofossils and loss-on-ignition (LOI) in CUNS1 confirmed a lakewide signal. The cores were extruded at 1 cm intervals, and sediment samples for pigment analysis (CUNS2) were placed in separate bags and frozen.

Core Chronology and Lithostratigraphy

Core CUNS2 was dated by a combination of ^{210}Pb and ^{14}C accelerator mass spectrometry (AMS) methods. Freeze-dried sediment samples were analyzed for ^{210}Pb, ^{226}Ra, ^{137}Cs, and ^{241}Am by direct gamma assay in the Bloomsbury Environmental Isotope Facility at University College London (UK) (Appleby et al., 1986). The chronology of CUNS2 followed the CRS model (Appleby, 2001), adjusted to a well-resolved ^{137}Cs peak marking 1963. AMS on terrestrial macrofossils was undertaken at the Natural Environment Research Council Radiocarbon Facility (East Kilbride, UK) and the Keck C cycle AMS Lab (University of California, Irvine) (**Table 1**). For samples under 100 μg C, a correction based on small NIST Oxalic acid II was used to account for the effect of old carbon. Further, data were corrected for modern carbon contamination following Santos et al. (2007). The full chronology of CUNS2 was modeled with the Bayesian-based Bacon package (Blaauw and Christen, 2013) of R version 3.1.2 (R Development Core Team, 2010) using 17 ^{210}Pb, and 3 ^{14}C samples (**Table 1**).

Carbonate and organic carbon content of the cores were quantified by LOI following Dean (1974). P and Ca in CUNS2

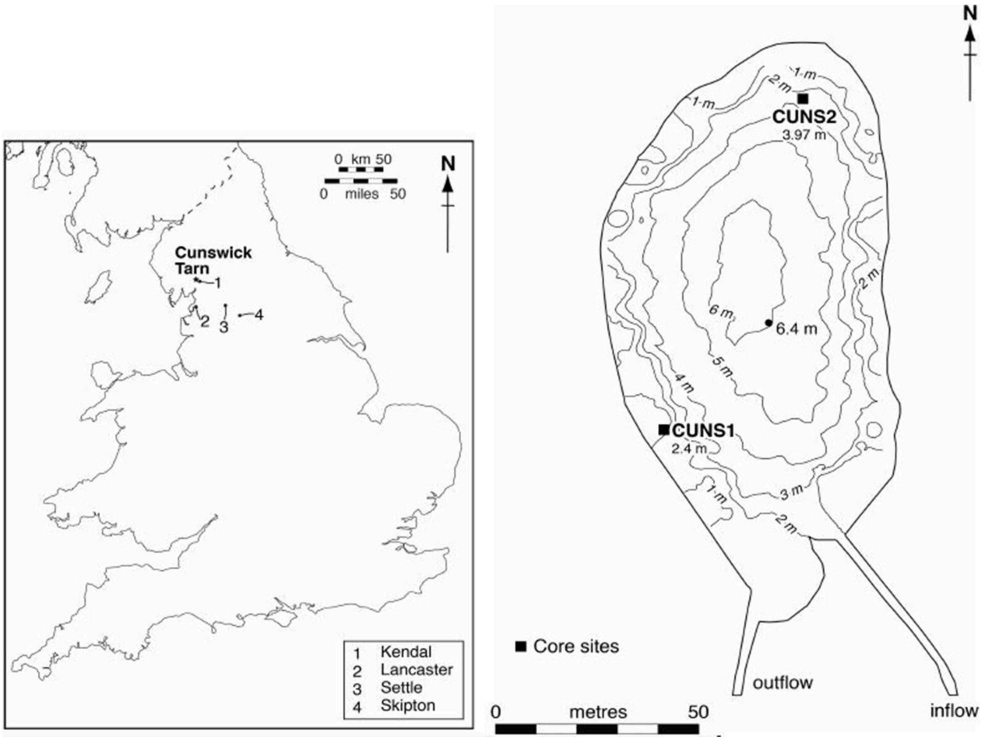

FIGURE 1 | Cunswick Tarn location and bathymetry, showing the core collection sites and water depths.

TABLE 1 | Samples used to create the dating profile of CUNS2.

Depth (cm)	Sample type	Material	Sample ID
0.5, 3.5, 6.5, 10.5, 13.5, 15.5, 17.5, 19.5, 20.5, 22.5, 23.5, 27.5, 30.5, 40.5, 42.5, 44.5, 46.5	^{210}Pb	Bulk sediment	
75.5	^{14}C (AMS)	Terrestrial leaf fragments, *Bidens cernua* seed	UCIAMS-144613
83.5	^{14}C (AMS)	Terrestrial leaf fragments, *Betula* fruit, stems	SUERC-48875
83.5	^{14}C (AMS)	Terrestrial leaf fragments	UCIAMS-144614

Sample ID: Laboratory identifier for radiocarbon samples. (see Appendix 2 in Supplementary Materials for further information on the CUNS2 profile).

were measured by X-ray fluorescence (XRF) (Boyle, 2002) at 1 cm intervals for the uppermost 10 cm, and 2 cm thereafter. In order to track hydrological changes in the Tarn, core CUNS2 carbonate δ^{18}O and δ^{13}C were measured at 5 cm intervals at the Natural Environment Research Council (NERC) Isotope Geosciences Laboratory (Keyworth, UK). Samples were disaggregated in 5% sodium hypochlorite solution (10% chlorox) for 24 h followed by washing and sieving at 85 µm. The residue was filtered, washed, dried (40°C) and ground in agate. The isolated material was reacted with anhydrous phosphoric acid *in vacuo* overnight at a constant 25°C to liberate CO2 for measurement. δ^{18}O and δ^{13}C are reported as per mil (‰) deviations of the isotopic ratios (^{18}O/^{16}O, δ^{13}C/δ^{12}C) calculated to the Vienna Pee Dee Belemnite scale using a within-run laboratory standard calibrated against National Bureau of Standards standards. The patterns in δ^{18}O, and between δ^{18}O and δ^{13}C, were used to determine changes between open and closed conditions in the lake following Leng and Marshall (2004) and Leng et al. (2006), with consideration to disequilibrium values following *Chara* stem and oospore calcification (Andrews et al., 2004).

Biological Groups

Analysis of chlorophyll and carotenoid pigments followed Moorhouse et al. (2014) and was undertaken at all levels of CUNS2. Diatom analysis of CUNS2 followed Battarbee et al. (2001) and was undertaken at 2 cm intervals to a depth of 45 cm, beyond which preservation was poor. Where a strong preference for epiphytic or benthic substrates was absent, percentage abundance by habitat was calculated with an equal weighting for both habitats. Identification was carried out with reference to Krammer and Lange-Bertalot (1986, 1988, 1991, 2004). All identifications were to species level excepting *Gomphonema pumilum* and *Gomphonema angustum* (see Kelly et al., 2008) which were aggregated as *Gomphonema angustum* (agg.).

Testate amoebae were analyzed roughly at 2–3 cm intervals throughout the core. Subsamples of 2 cm^3 were wet sieved through a 250 µm mesh to remove course organic flocs and a 35 µm mesh to remove clay and fine silt. The resulting residues were split into eight aliquots using a wet- splitter (Scott and Hermelin, 1993) and, in accordance with Patterson and Fishbein (1989), were quantified to >150 specimens per sample where abundance was permitting. Species and strains were identified according to lacustrine testate amoeba keys (Medioli and Scott, 1983; Kumar and Dalby, 1998) and published scanning electron micrographs (Roe et al., 2010; Patterson et al., 2012). Due to the significant amount of morphological variability in lacustrine testate amoebae (Medioli and Scott, 1983), a strain-based nomenclature was adopted in accordance with other lacustrine researchers (Escobar et al., 2008; Kihlman and Kauppila, 2012; Patterson et al., 2012), in order to include these "ecophenotypes."

Subfossil cladocerans were analyzed at 3 cm intervals for CUNS2 following Korhola and Rautio (2000). Due to high sediment carbonate concentrations, samples were treated with 10% HCl prior to 10% KOH. All samples were sieved into 150 and 45 µm size fractions and counted following Davidson et al. (2007) to a minimum count of 300 individuals of the most abundant species, or the entire large size fraction where counts were low. Post-abdominal claws other than those of *Daphnia pulex* were aggregated to *Ceriodaphnia* and *Daphnia* spp. Morphological plasticity of *Chydorus* remains was interpreted as variability within *Chydorus sphaericus sensu lato. Bosmina longirostris* was the only species of *Bosmina* confirmed in the core, however the abundance of fragmented and non-diagnostic remains led to aggregation to *Bosmina* sp(p). Identification was undertaken using Frey (1965), Flößner (2000), and Szeroczyńska and Sarmaja-Korjonen (2007).

Macrofossil analysis followed Sayer et al. (2010) and was undertaken at every 6 cm of CUNS2, and for 16 levels of CUNS1. Macrofossils were identified using a dedicated reference collection of plant and animal parts and relevant publications held at UCL Geography. Where species-level detail was not attainable, remains were aggregated. These included *Daphnia hyalina* agg. ephippia (UK species other than *D. magna* and *D. pulex*), *Potamogeton pusillus* + *berchtoldii* leaf tips (*P. pusillus* and *P. berchtoldii*) and Nymphaeaceae trichosclereids, i.e., leaf cells (*Nymphaea alba* and *N. lutea*). Molluscs were mostly identified to family or genus level. Owing to morphological plasticity, charophyte oospores were aggregated as *Chara* spp. for numerical purposes. However, morphotypes were also tentatively identified using reference collection material and an oospore key (Haas, 1994). Uncalcified and calcified oospores were counted separately.

Historical presence of fish in Cunswick Tarn was determined by identification of chaoborid larval mandibles using the cladoceran method. *Chaoborus obscuripes* and *Chaoborus crystallinus* are non-migratory species associated with fishless lakes (Luoto and Nevalainen, 2009; Palm et al., 2012; Tolonen et al., 2012) and therefore their consistent presence in the core record was interpreted as a proxy for fishlessness. Mandibles were identified using Aitken (1954), Uutala (1990), Živić and Marković (2006), and Palm et al. (2011). *Chaoborus obscuripes* and *Chaoborus crystallinus* were not separated and are referred to as *C. obsuripes/crystallinus*.

Historical Evidence

Diagrammatic reconstructions of the macrophyte communities and colonization depths in Cunswick Tarn for the present, the mid-1900s, and the late 1800s/early 1900s were constructed based on (1) the CUNS1 and CUNS2 macrofossil records; (2) historical macrophyte presence and spatial distribution data collated from records held by UCL and Natural England, and other field naturalists; (3) macrophyte community composition and zonation in other marl lakes (Jupp et al., 1974; Spence et al., 1984; Pentecost, 2009; Hilt et al., 2010) or lakes with similar species composition (Spence, 1967, 1982).

Statistical Analysis

All data analysis was performed with R version 3.1.2 (R Development Core Team, 2010) using packages analogue (Simpson, 2007; Simpson and Oksanen, 2011), vegan (Oksanen et al., 2011), and mvpart (De'ath, 2002). Statistical analysis was only undertaken on CUNS2 data owing to the low resolution of the chronology for CUNS1.

Diatom and testate amoeba data were transformed into percentage abundances and taxa with <5% abundance and less than five occurrences in the core profile were omitted. They were also square-root transformed. Pigment and macrofossil data were (log10 + 1), and XRF data (log*ln*)-transformed and normalized with respect to organic matter, respectively. Cladoceran data were transformed into percentage data for principal curves and multivariate regression trees (MRT), and square-root transformed for all analyses. Finally, pigment data were further standardized to abundances between 0 and 1 to reduce the statistical effects of "abundant" pigments (low degradation, high preservation).

Principal Curves

Principal curves (PC) are a multivariate ordination-based method particularly suitable for data sets with a single dominant gradient (Hastie and Stuetzle, 1989; De'ath, 1999) which applied to Cunswick Tarn. For CUNS2 data, PCA axis 1 scores were used for all data sets as a starting point for PCs. The complexity of the smoothing splines fitted to each species was allowed to vary, and the spline degrees of freedom were chosen using the GCV criterion following Simpson and Birks (2012).

Multivariate Regression Trees

Multivariate regression trees (MRT; De'ath, 2002) are a form of constrained cluster method that allow for multiple constraining variables on a response data set. The response data set can be a species abundance table or a dissimilarity matrix, clustered by age to add chronological constraint on temporal series (Simpson and Birks, 2012). MRT was applied to geochemical (P, Ca) and biological data (pigments, diatoms, testate amoebae, cladocerans, macrofossils) for all analyzed levels using age (in years AD) as a constraining parameter in order to determine the timing of major shifts in geochemistry and ecological responses (see Simpson and Birks, 2012; p. 261), and to test for synchronicity in change across indicators (ecosystem change). Analysing each response separately allowed for a high number of data points and therefore confidence in grouping. The number of data points was $n = 94$ (pigments), $n = 54$ (P, Ca), $n = 39$ (testate amoebae), $n = 32$ (cladocerans), $n = 24$ (diatoms), and $n = 16$ (macrofossils).

A previously developed cladoceran-based model capable of semi-quantitative inference of macrophyte and fish abundance, employing MRT (Davidson et al., 2010a) was also applied to the cladoceran data set ($n = 32$) in order to estimate past plant abundance [late summer plant volume infestation (PVI); Davidson et al., 2010b]. The number of *Daphni*a spp. ephippia (counted only at macrofossil resolution) for missing levels was modeled based on the relationship between ephippia (macrofossils) and post-abdominal claws (cladocerans).

Redundancy Analysis

Relationships between P, Ca, and biological data sets were tested using redundancy analysis (RDA). Owing to the varying resolution of the data, RDAs were fitted to matching observations, giving subsets of $n = 54$ (pigments), $n = 18$ (testate amoeba, cladocerans), and $n = 15$ (macrofossils). Analysis of the diatom data set was omitted due to the low number of matching observations. To allow for temporal ordering of the data, significance of variance explained was tested using ANOVA with cyclic permutations. The lowest attainable p-value of such permutations depends on the number of observations tested ($1/n$) and therefore significance was unattainable for most data sets at a 0.05 level (e.g., $1/18 = 0.056$). Therefore, although too liberal, permutation tests were also performed without restrictions.

Results

Core Chronology and Lithostratigraphy

Core CUNS2 spanned a period of approximately 1260–2009 AD (date of core collection) with highest age uncertainties in the middle section of the core (46.5–75.5 cm; **Figure 2**). The gap in measured ages was unavoidable given the constraints of the time spans of ^{210}Pb (to ca. 150 years from present) and radiocarbon dating (minimum age several hundreds of years). Sedimentation rates (SRs), core carbonate (**Figure 3**), and Ca (Supp. 1 in Supplementary Material) followed a similar pattern, the latter two of which were considered equivalent owing to deposition as calcite/marl ($CaCO_3$) and their high correlation (Spearman's rho = 0.97, $p < 0.001$).

Four carbonate zones were apparent in CUNS2 (**Figure 3**) and were used as Zones for all Diagrams in Supplementary Materials, and as sections for presentation of biological data results. Zone 1 encompassed the base of the core (92.5 cm; 1250s) to the early 1400s, characterized by relatively low carbonate content (<10%), low variability, and low SR (ca. 0.12 cm year^{-1}). Carbonate and SR were at their lowest values in Zone 2 (ca. 5% and 0.07 cm year^{-1}, respectively), which encompassed the 1400s (72.5 cm) to the 1890s (42.5 cm). Zone 3 (42.5–22.5 cm) encompassed substantial increases in carbonate and SR (to ca. 1 cm year^{-1}), with two carbonate peaks of 36% and 40% (1920s) followed by a rapid decline (ca. 10 years). Slightly lower carbonate (ca. 20–30%)

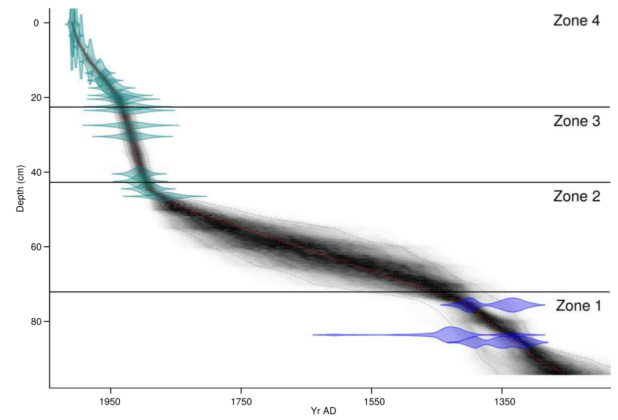

FIGURE 2 | CUNS2 core chronology.

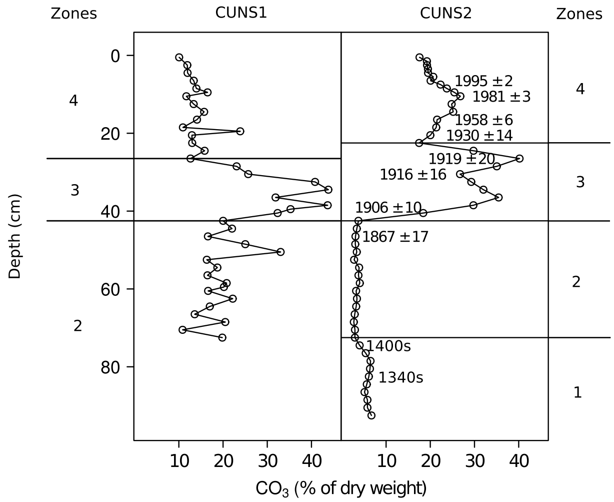

FIGURE 3 | Carbonate concentrations in cores CUNS1 and CUNS2, shown with calendar years obtained by ^{210}Pb-dating of CUNS2 with errors (±), and approximate calendar years based on radiocarbon dates (see Table 1 for details).

and SRs (ca. 0.2–0.5 cm year^{-1}) occurred in Zone 4 from the 1930s (22.5 cm) to 2009 (core top).

CUNS1 followed a similar carbonate pattern to CUNS2, allowing cross-correlation to estimate the chronology of CUNS1 (**Figure 3**). A CUNS1 parallel for CUNS2 Zone 1 was not appropriate owing to the brevity of the CUNS1 sequence and differences in carbonate concentration in this core section. The zone limits for CUNS1, therefore, are Zone 2: core base to 42.5 cm (base to ca. 1890s), Zone 3: 42.5–26.5 cm (1890s–1930s), and Zone 4: 26.5 cm to top (1930s–2008) (**Figure 3**). Higher carbonate concentrations than in CUNS2 were found in Zone 2 (ca. 20% vs. ca. 5%), and similar carbonate peaks (>40%) occurred in Zone 3.

CUNS2 carbonate δ^{18}O could not be measured for most of Zone 2 owing to insufficient carbonate (data not shown). Therefore, only 14 samples were analyzed, five of which were in Zones 1 and 2 and had highly variable δ^{18}O out of equilibrium with rainfall (−3 to −6‰), indicating a hydrologically closed system (Leng and Marshall, 2004; Leng et al., 2006). The uppermost nine samples ranged between −6 and −7‰ and were in equilibrium with rainfall, indicating that Cunswick Tarn became a hydrologically open system between the 1890s and 1900.

CUNS2 P concentrations were expressed relative to core organic matter to correct for dilution by calcite, and are therefore only interpretable as relative values within the core sequence (Supp. 1 in Supplementary Material). Ranged between 0 and 1 for simplicity, the lowest concentrations occurred before the 1530s (0.5–0.6) and between the 1530s and the 1890s (0.4–0.45). Rapid increases occurred in the early 1900s coincident with increases in Ca, with concentrations between 0.55 and 0.8. From the 1930s to 2009, concentrations increased consistently from 0.7 to 1 (Supp. 1 in Supplementary Material).

Zone 1: ca. 1250–1400 (CUNS2 Only; No Diatom Data)

Pigments of algae, higher plants, and cyanobacteria were low in Zone 1 (Supp. 2 in Supplementary Material). Compared with Zone 2, slightly higher concentrations of diatom (diatoxanthin), cyanobacteria (zeaxanthin), and cryptophyte (alloxanthin) pigments occurred throughout. The notable difference between the zones was the concentration of a purple sulfur bacterial pigment (okenone) which was highest at the base of the core and declined to levels below detection at the upper limit of Zone 1 (Supp. 2 in Supplementary Material).

The testate amoeba community (Supp. 3 in Supplementary Material) in this zone included moderate abundances of *Difflugia oblonga* (Ehrenberg, 1832) strain "oblonga" (ca. 15–30%), often associated with organic rich substrates (Kihlman and Kauppila, 2012), and *Centropyxis aculeata* (Ehrenberg, 1832) strain "aculeata" (ca. 10–25%). Lesser proportions consisted of *Arcella vulgaris* (Ehrenberg, 1830) (ca. <10%), *Centropyxis constricta* (Ehrenberg, 1843) "aerophila" and the oligotrophic indicator *Cyclopyxis kahli* (Deflandre, 1929) (Schönborn, 1967; Wall et al., 2010). The eutrophic indicator species *Cucurbitella tricuspis* (Carter, 1856) (Medioli and Scott, 1983) was also present in small amounts (<5%).

Remains of pelagic cladocerans at the base of the core were relatively abundant, and declined slightly toward the upper end of the zone (e.g., *Bosmina longirostris*/spp. 42% to <10%) (Supp. 4 in Supplementary Material). Other filter-feeding and/or pelagic species included *D. pulex*, *Daphnia hyalina* agg. and *Simocephalus* spp. Plant- and mud-associated species included *Alonella exigua*, *Alonella nana*, *Eurycercus lamellatus*, *Graptoleberis testudinaria*, *Alona affinis,* and *Leydigia leydigi*. Species exclusive to Zone 1 included *Pleuroxus truncatus*, *Chydorus piger,* and *Alona rustica*.

Plant macrofossils included terrestrial *Juncus* spp. seeds, fragments of *Nymphaea alba* seeds, and uncalcified oospores of *Chara hispida* agg., *Chara contraria* agg., and *Nitella* cf. *flexilis* (Supp. 5 in Supplementary Material). Oblong

Plumatella sp. statoblasts were particularly abundant in Zone 1 compared to the rest of the core (Supp. 6 in Supplementary Material). Mollusca were largely absent with the exception of Sphaeriidae.

Zone 2: ca. 1400–1890

Okenone remained below detection limits throughout Zone 2 and overall pigment concentrations were lower compared to other zones (Supp. 2 in Supplementary Material). Benthic species (e.g., *Pseudostaurosira brevistriata* ca. 50–60%) dominated the CUNS2 diatom record at the latter end of the Zone (three samples counted), forming 60–80% of the community (Supp. 7 in Supplementary Material). Periphytic taxa formed 10–30% of the community and included *Gomphonema angustum* and *Amphora pediculus*, while planktonic species accounted for <5% (**Figure 4**). *Cyclotella* and *Fragilaria* compositions indicated pre-eutrophication conditions of high light penetration (Moss, 1979; Padisak and Reynolds, 1998; Meriläinen et al., 2000).

Changes among the dominant testate amoeba taxa (Supp. 3 in Supplementary Material) included gradual increases in *Cucurbitella tricuspis* (to ca. 23%), before rapidly declining in the mid nineteenth century, and decreases in *Difflugia oblonga* "oblonga." *Centropyxis constricta* "aerophila" and *Arcella vulgaris* increased in abundance, while *Cyclopyxis kahli* remained in lesser proportions.

Diversity and total abundance of cladocerans were at their lowest in Zone 2, and pelagic taxa were rare (Supp. 4 in Supplementary Material, **Figure 4**). The community was dominated by benthic taxa (e.g., Tremel et al., 2000; Thienpont et al., 2015) including *Chydorus sphaericus*, *Alona guttata/rectangula,* and *Alona quadrangularis*. In contrast, in the macrofossil record of CUNS1 (Supp. 8 in Supplementary Material), planktonic *Ceriodaphnia* spp. ephippia were abundant ($n > 1000 \times 100\,\text{ml}^{-1}$) at the beginning of Zone 2 (ca. 1400–1500s), followed by consistent decreases to the upper end of the zone ($n < 100 \times 100\,\text{ml}^{-1}$).

In CUNS2 Zone 2, there were fewer plant macrofossils of both terrestrial and aquatic origin compared with Zone 1 (Supp. 5 in Supplementary Material). Whilst species composition was similar, Charophyta differed as *Nitella* spp. were absent and *C. hispida* agg. dominated. In CUNS1, there were high abundances of calcified *Chara aspera* oospores in Zone 2, which was not recorded in CUNS2 (Supp. 9 in Supplementary Material, **Figure 4**). One other submerged macrophyte species, *Potamogeton praelongus* (CUNS1), occurred in this Zone. Floating-leaved macrophyte remains included Nymphaeaceae trichosclereids (leaf cells), occurring in CUNS1 and CUNS2 at slightly higher abundance than in Zone 1, and increasing substantially toward the end of Zone 2 in CUNS1 (Supps 5, 9 in Supplementary Material). Only *N. alba* seed remains were recorded, although *N. lutea* occurred in the Tarn in the early 1800s (Baker, 1885). *Potamogeton natans* seeds occurred throughout this Zone (CUNS1). Mollusc remains were more diverse and abundant in Zone 2 compared with Zone 1 (Supp. 6 in Supplementary Material, **Figure 4**). In CUNS2, the Sphaeriidae dominated the record (maximum of $n = 50–100 \times 100\,\text{ml}^{-1}$) whereas in CUNS1 several different taxa became more abundant toward the latter part of the Zone (*Bithynia leachi/tentaculata*, *Planorbis* cf. *albus*, *Valvata* cf. *macrostoma*) (Supp. 8 in Supplementary Material).

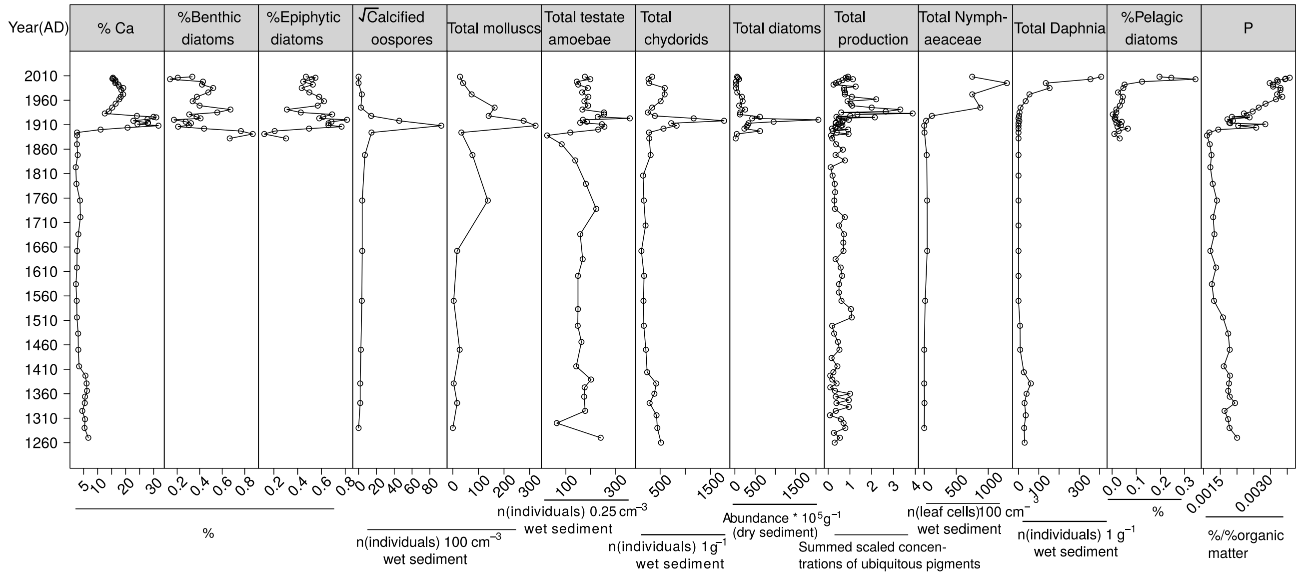

FIGURE 4 | Summary plot of CUNS2 Ca, P, and key absolute and relative abundance changes of biological groups against time.

Zone 3: ca. 1890–1930

The CUNS2 Zone 3 pigment assemblage remained largely similar to Zone 2 with low biomarker concentrations of cyanobacteria (zeaxanthin, canthaxanthin) and cryptophytes (alloxanthin), and purple sulfur bacteria (okenone) below the limit of detection (Supp. 2 in Supplementary Material). Consistent increases toward the top of the Zone occurred in chlorophyte (lutein, pheophytin *b*) and diatom (diatoxanthin) pigments. Increases in biomarkers of all algae (chl *a*, ß-carotene) occurred abruptly at the end of the sequence around the 1920s. In the CUNS1 and CUNS2 macrofossil record, *Gloeotrichia* cf. *pisum*, a nitrogen-fixing colonial cyanobacterium, increased approximately three-fold in abundance throughout the zone (Supps. 4, 9 in Supplementary Material). The proportion of periphytic diatoms in the CUNS2 record increased from ca. 40 to 70%, dominated by *G. angustum* and *A. pediculus*. (Supp. 7 in Supplementary Material). Throughout Zone 3, diatoms were more abundant than in the other zones (**Figure 4**). Persistence of the nutrient-sensitive *Cymbella microcephala* suggested relatively low nutrient concentrations (Schneider et al., 2000) (Supp. 7 in Supplementary Material).

The most substantial and rapid changes in the testate amoeba community occurred in this zone with peaks (at ca. 50%) in *Arcella vulgaris* and *Centropyxis aculeata* "aculeata," taxa often associated with low oxygen environments (Roe et al., 2010; Drljepan et al., 2014). The proportions of *Difflugia oblonga* "oblonga" and *Cucurbitella tricuspis* (to ca. <5%), *Cyclopyxis kahli* and *C. constricta* "aerophila" decreased further. While species diversity was at a minimum in this zone, maximum absolute concentrations increased almost 10-fold (**Figure 4**).

Cladoceran abundances were highest in Zone 3 (**Figure 4**). Benthic taxa remained dominant, however community composition changed toward a dominance of strictly plant-associated species: *Pleuroxus laevis* and *A. harpae* became more abundant than *A. quadrangularis* and *Alonella excisa*, and *Alonella exigua* became absent (Supp. 4 in Supplementary Material). Ephippia of pelagic cladoceran taxa were largely absent or rare in CUNS1 and CUNS2 (Supps. 4, 8 in Supplementary Material).

Two macrophyte phases within Zone 3 were identified in the cores (Supps. 5, 9 in Supplementary Material). Oospores attained maximum abundances in the first phase (CUNS1 $n = 2698\,\mathrm{ml}^{-1}$ and CUNS2 $n = 8707 \times 100\,\mathrm{ml}^{-1}$; Supps. 5, 9 in Supplementary Material; **Figure 4**) which corresponded to high abundances of encrusted stem remains. Six different oospore morphotypes were identified to *C. aspera*, *C. hispida* agg., *Chara* cf. *vulgaris* and *Chara* cf. *globularis*. Historical records further identified *Chara curta* in the Tarn at the end of the 1890s (Stewart, 2001), and *Chara aculeolata*, *Chara desmacantha* [syn *C. curta*] (1890s), and *Chara fragilis* [syn. *Chara globularis*] (1902) (Wilson, 1938). Nymphaeaceae were less abundant compared with Zone 2. In the latter phase (from ca. 1910s) oospores remained numerous, but declined with respect to the earlier phase (CUNS1 $< 400\,\mathrm{ml}^{-1}$, CUNS2 ca. $200\text{–}1600\,\mathrm{ml}^{-1}$). Remains of floating-leaved species (*P. natans*, Nymphaeaceae incl. *N. lutea*) increased and narrow-leaved *Potamogeton* species (*Potamogeton pusillus*/*berchtoldii*, *Potamogeton obtusifolius*) appeared in the record for the first time. Maximum abundances of molluscs occurred in the early part of Zone 3 (Supps. 6, 8 in Supplementary Material; **Figure 4**).

Zone 4: ca. 1930–2000s

Diatom, cyanobacteria, and cryptophyte pigment concentrations in CUNS2 increased substantially from the 1930s to maximum abundances in the 2000s (Supp. 2 in Supplementary Material), indicative of increasing eutrophication (Leavitt, 1993; McGowan et al., 2005). In contrast, biomarkers of all algae (chl *a*, ß-carotene) peaked around the 1930s followed by declines to concentrations similar to the early 1900s (**Figure 4**). In the diatom record, nutrient-sensitive species were succeeded by the more ubiquitous *Amphora pediculus* (Kwandrans et al., 1998; Garcia-Rodriguez et al., 2007; Stenger-Kovács et al., 2007) (Supp. 7 in Supplementary Material). Okenone reappeared in the pigment record and increased from the 1980s, coinciding with a decrease in *G.* cf. *pisum* (Supps. 2, 5, 9 in Supplementary Material) and an increase in the proportion of planktonic diatoms such as *Aulacoseira* spp. and *Stephanodiscus hantzchii* indicative of eutrophication (Reynolds et al., 2002; Negro and de Hoyos, 2005) from ca. 5% to ca. 30% (Supp. 7 in Supplementary Material; **Figure 4**).

Remains of pelagic cladocerans, *Daphnia* spp. in particular, increased consistently from the 1930s to the 2000s in the macrofossil (CUNS1, CUNS2) and cladoceran records (CUNS2) (Supps. 4, 8 in Supplementary Material). *Bosmina* spp., which had been absent from the record after the 1400s, were found in the most recent sediment. Five plant-associated cladoceran species characteristic of Zone 3 declined in abundance in contrast to increases in ubiquitous species (*Alonella nana*), filter feeders (*Sida crystallina*) and sediment-associated species (*Leydigia leydigi*) (Supp. 4 in Supplementary Material).

A substantial increase occurred in the relative abundance of *Difflugia oblonga* "oblonga" in the 1930s (Supp. 3 in Supplementary Material), corresponding with increases in core organic matter. In contrast, relative abundances of *Arcella vulgaris* and *Centropyxis aculeata* "aculeata" decreased. Some recovery in the uppermost part of the core was evident for the aforementioned species with modest increases also in *Cucurbitella tricuspis* (to ca. 9%) in the 1990s. Species diversity and concentrations (**Figure 4**) returned to levels similar to those in Zones 1 and 2.

In the 1930s, *P. natans* seeds disappeared from the core record, and charophyte remains declined substantially (Supps. 5, 9 in Supplementary Material). In CUNS1, oospore numbers fell two-three orders of magnitude from maximal abundances in Zones 2 and 3, while CUNS2 numbers fell to levels comparable with Zone 1. Nymphaeaceae trichosclereids increased to >3 times their previous abundance (CUNS1, CUNS2). Linear-leaved *Potamogeton* remains persisted in the core record (CUNS2). Of the Nymphaeaceae, only seeds of *N. lutea* were found. The Tarn was at the time (1936) described as "very rich in aquatic plants" (Wilson, 1938). The first core record of *Myriophyllum spicatum* (CUNS1) in the 1950s coincided with descriptions of Cunswick Tarn as an "excellent calcareous tarn with *Cladium mariscus*" (NC SSSI citation), however, concerns were raised regarding the spreading of *Phragmites* [*australis*] which dominated the east side (Lewis, 1954).

The abundance of mollusc remains decreased markedly following the 1940s and *Bithynia* spp. became the dominant taxon until the 1980s after which numbers declined to Zone 2 levels (Supps 6, 8 in Supplementary Material).

Constrained Analyses

Ca and P were correlated with between 10 and 37% of the CUNS2 biological data (**Table 2**). Relationships were strongest for P, and for pigments and cladocerans for which the cyclic permutation test was either significant (pigments) or took the smallest p-value possible given the number of samples (cladocerans). Owing to the possibility of Type II errors with relatively low numbers of data points, the significance of all relationships (pigments, testate amoebae, cladocerans, macrofossils) under unconstrained permutation tests suggests that P and Ca were strongly associated with biological change. Macrofossils were the only biological group explained more by Ca (26%) than P (20%) (**Table 2**), however, it is likely that bimodality, which could not be removed, negatively influenced the strength of the test with Ca.

Principal Curves and Multivariate Regression Trees

Chronological MRT splits are indicated as the midpoint between samples either side of a split and therefore are not, for data with differing resolutions, an exact reflection of the temporal relationship across groups. While P and Ca data occurred at identical depths, the splits for pigments (median age gap 6 years) will necessarily be more precise than those for macrofossils (median age gap 40 years). In order to remain unambiguous about split locations given the exact dates put into the model, dating errors were not incorporated into split estimates.

Four significant MRT zones were identified for P (**Figure 5**). For Ca, three or four significant zones were identified by MRT depending on the data transformation used (bimodality in the density distribution of Ca-values interfered with the method). In order to cohere with P and biological data groupings, and to delineate the decline in Ca content in the uppermost section of the core (**Figure 3**), four zones were selected. Relatively small changes occurred in P and Ca concentrations between 1200s and the late 1800s within which one split was found at ca. 1400 (Ca) (Zone 1/2) and 1530 (P). Changes were more synchronous in the latter end of the sequence where splits were identified at 1896 (Ca), 1902 (P), 1930 (Ca), and 1935 (P). The first split marks an increase in the concentration of both elements, and the second a decrease in Ca and continued increase in P.

TABLE 2 | RDA-based correlations and *p*-value of Ca and P over biological data.

Group	*n*	%VarExp		Cyclic *p*		Free *p*	
		Ca	P	Ca	P	Ca	P
Pigments	54	23	37	0.39	0.02*	0.001***	NA
Cladocerans	18	27	30	0.22	0.06^{+}	0.002**	0.002**
Testate ameobae	18	13	15	0.88	0.55	0.02*	0.009**
Macrofossils	15	26	20	0.13	0.40	0.002**	0.003**

$^{+}$*lowest value attainable with given n(samples). The stars refer to standard significance denotations.*

Principal curves identified modest changes in biological communities between the 1200s and the late 1800s. MRTs located shifts of cladocerans and pigments around 1427, and 1370 and 1458, respectively, followed by macrofossils around 1500, and testate amoebae around 1516 (**Figure 5**).

Substantial community change occurred between the 1890s and the 2000s during which splits in biological groups showed clear synchronicity. Split 2 as per P (1902) and Ca (1896) (Zone 2/3), where both elements increased, coincided with splits in pigments (1903), diatoms (1900), cladocerans (1898) and macrofossils (1901). Split 3, where Ca declined (1930) (Zone 3/4) and P continued to increase (1935) coincided with splits in pigments (1936), diatoms (1933), testate amoebae (1933), and macrofossils (1936), with a slight lag in cladocerans (1940).

The highest number of significant splits occurred in primary producers (pigments $n = 8$, diatoms $n = 5$), which also changed significantly in the most recent years (pigments: 2000, diatoms: 1995), coincident with the most recent split in cladocerans (1990) (**Figure 5**). The highest frequency of splits occurred between the 1900s and 1930s. The most important pigment splits are indicated by solid lines (**Figure 5**).

Fish Abundance and the Cladocera-Macrophyte-Fish Model

Absence of fish throughout the core record was indicated by the presence of *C. obscuripes/crystallinus*, and the absence of fish scales and fish leech cocoons (*Piscicola geometra*) (Odgaard and Rasmussen, 2001). Zooplanktivorous fish densities indicated by the cladoceran-based MRT model (Davidson et al., 2010a) were low (<0.001 ZF density m^{-2}) for most data points with the highest density (ca. 0.5) indicated for the lowermost sample in the cladoceran record with high numbers of *Bosmina* spp (**Figure 6**). These values are low compared with the range of zooplanktivorous fish used to construct the model (0–3.26) (Davidson et al., 2010a) and support the idea that fish were absent from the lake particularly through Zones 2–4. Primary producer and cladoceran community changes were therefore assumed to be independent of top-down effects from fish.

Reconstructed August PVI was low ($<10\%$) prior to the 1400s (Zone 1) and following the 1950s (Zone 4). Very low PVI ($<3\%$) was indicated for the uppermost two levels (>2000 AD). The MRT model is semi-quantitative and strictly speaking it is only the changing of the MRT end group that strongly supports the idea of changes in macrophyte abundance (Davidson et al., 2010b). However, the direction of movement in the two-dimensional space of the biplot can also be used to infer past dynamics in macrophyte abundance. This suggests an increase in macrophyte abundance after 1890, which peaked between 1900 and 1915, followed by a consistent decline of plant abundance to the present day where plant abundance is rather low (**Figure 6**).

Discussion

Cunswick Tarn proved particularly suited for detailing bottom-up effects of eutrophication owing to the absence of fish and their top-down influence throughout the core record. With reference to the original research questions, the high correlation of both core P (15–37%) and Ca (13–27%) with all biological data sets (**Table 2**) supported expectations of eutrophication (P) and marling (Ca) as correlates of biological community structure at the whole ecosystem level (**Figure 5**). The two main changes were (1) an abrupt shift between two different benthic phases in the early 1900s (low *Chara* + organic sediment to high *Chara* + marl sediment), and (2) the erosion of benthic primary production pathways as the Tarn became increasingly eutrophic in the mid-late 1900s. While the latter followed initial hypotheses and is widely documented (Bumby, 1977; Carbiener et al., 1990; Vadeboncoeur et al., 2003), the former is a previously undocumented eutrophication-driven switch in marl deposition with important consequences to lake management targets and marl lake reference conditions.

Drivers of Ecosystem Change

Hydrology

The first substantial changes in Ca, P, and ecosystem structure occurred following lake drainage in the 1890s, corresponding to a period of intensifying land management in the area following land enclosures and attempts at drainage of local lakes (Otley, 1830; Walker, 1955; Cousins, 2013). Both lake shallowing and eutrophication can have similar ecological effects and their synchrony in the core record render strict eutrophication responses difficult to identify. For example, increases in calcified *Chara* oospore and stem abundance (Supps. 5, 9 in Supplementary Material), as well as of epiphytic diatoms and macrophyte-associated molluscs (**Figure 4**) may reflect improved light conditions. Higher light availability expands benthic photosynthetic habitat (Leavitt, 1993), and also modulates the optimal depth of calcite deposition (Pukacz et al., 2014), which may confound simplistic responses to nutrients.

Shallowing is supported in the oospore record of CUNS2 by a shift from a dominance of the *Chara hispida* group to the more shore-line associated, lower-growing *Chara aspera*. However, evidence for a predominant effect of eutrophication following an initial pulse of lake shallowing is supported by a few factors. Firstly, the Ca and oospore increase also occurred in CUNS1, the location of which was 1.5 m shallower than that of CUNS2 (**Figure 1**). Secondly, CUNS1 reflected littoral conditions (high carbonate content and *Chara aspera* oospores) throughout the record and therefore did not undergo charophyte community shifts, suggesting that increased nutrient availability was in part responsible for the change. Thirdly, the change in Ca and charophytes was a continuous rather than a stepwise transition, suggesting underlying eutrophication responses independent of a simple water level change. Indeed, the positive relationship between Ca and P in the early 1900s strongly suggest a eutrophication effect on *Chara* biomass and marl deposition.

Internal Drivers: Marl Lake Ecology and Ca-P Cycling

The reasons behind notions of self-sustaining, eutrophication-buffering properties of charophytes include the maintenance of clear water via nutrient uptake from the water column,

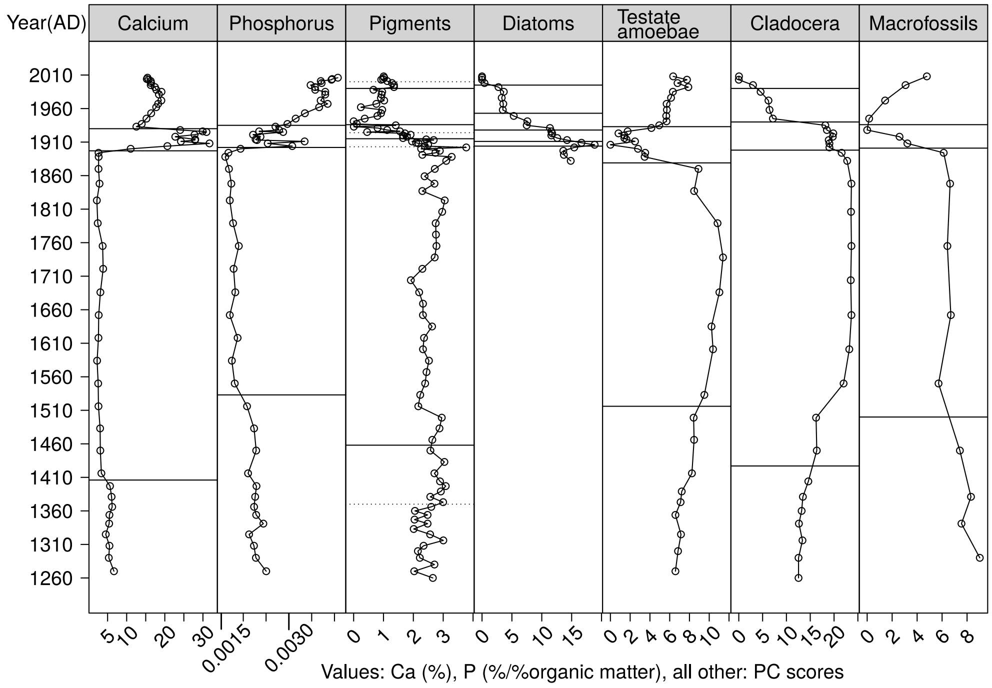

FIGURE 5 | Ca and P, and PCs of biological groups against time. Horizontal lines indicate MRT-derived splits, those dashed to aid inspection indicate least importance.

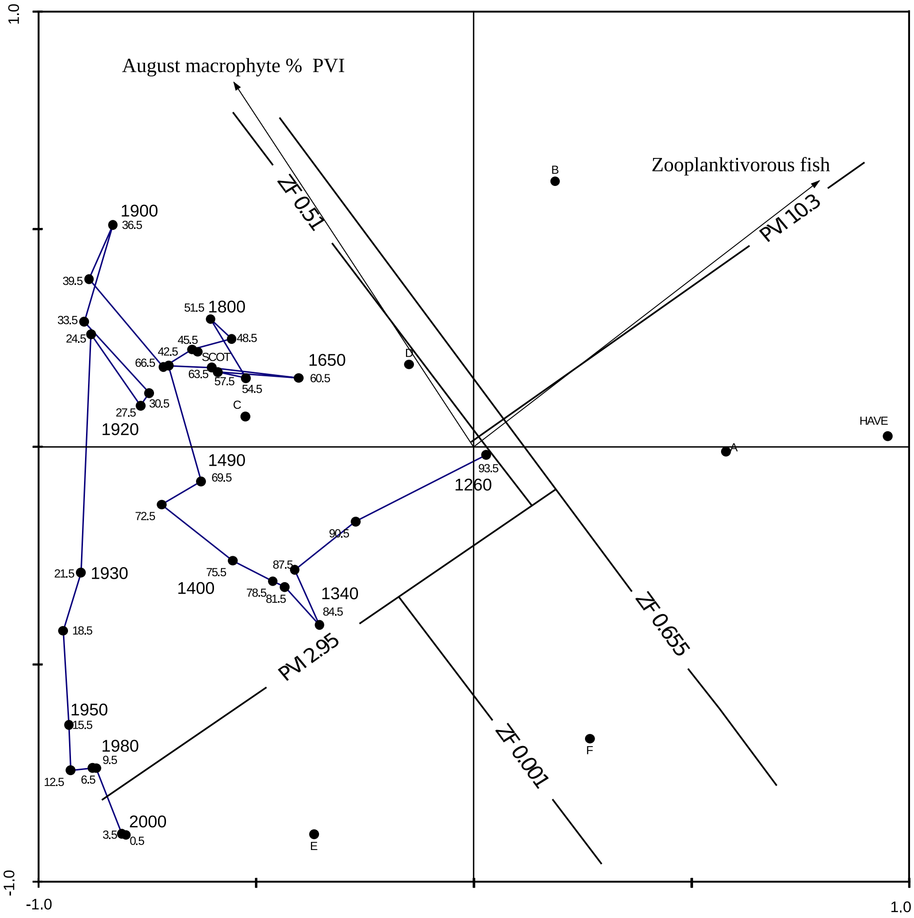

FIGURE 6 | Cladoceran-inferred August plant volume infestation and zooplanktivorous fish abundance of CUNS2 sample depths (cm).

coprecipitation of Ca with P, and sediment stabilization owing to dense growth (Otsuki and Wetzel, 1972; Kufel and Kufel, 2002; Robertson et al., 2007). This so-called inertia to external pressure, and therefore internal driving mechanisms, underlie ideas of occasional abrupt and rapid ecological changes following extended periods of eutrophication (Scheffer et al., 1993; Scheffer and van Nes, 2007). While punctuated change has been largely absent in other palaeolimnological studies of (formerly) *Chara*-dominated lakes (Davidson et al., 2005; Ayres et al., 2008; Sayer et al., 2010), elements of rapid change driven by internal mechanisms could be inferred in the Cunswick Tarn record. These events corresponded to key changes in community composition within a marl lake framework, namely changes in *Chara* abundance, transitions from *Chara* to angiosperm dominance, and finally transitions from benthic to pelagic ecosystem structure (**Figure 7**).

The first major ecological shift in the Cunswick record occurred in the 1890s with sub-decadal changes across all biological groups. The positive relationship between Ca, P, and *Chara* biomass in Cunswick Tarn, mirrored by increased cladoceran-inferred PVI (**Figure 6**), support ideas of self-stabilizing mechanisms especially in the early stages of eutrophication via increased nutrient uptake in the benthos and prevention of phytoplankton increases. Importantly, the structuring role of charophytes was also apparent in the close association of charophyte biomass with abundance responses in other benthic primary producers and consumers (**Figure 4**). Also importantly, these numerical changes were synchronous with strictly qualitative ecosystem change as seen in the multivariate analyses of cladocerans, diatoms, and testate amoebae (**Figure 5**), all of which were transformed to remove effects of total abundance. Indirect effects of high macrophyte biomass, such as local anoxia at the sediment level (Lindholm et al., 2008), may have contributed to the increase in *Arcella vulgaris* and *Centropyxis aculeata* "aculeata" during this time (Roe et al., 2010; Drljepan et al., 2014).

The importance of plant biomass responses as buffers to eutrophication were further supported by the lack of association between P and Ca in CUNS2, which argues against significant chemical defenses against eutrophication via coprecipitation (see also Hobbs et al., 2005; Hutorowicz and Dziedzic, 2008). Further, the second abrupt change in the core record in the 1930s (**Figure 7**) corresponded to the breaking down of marling, substantial declines in macrophyte biomass (**Figure 6**),

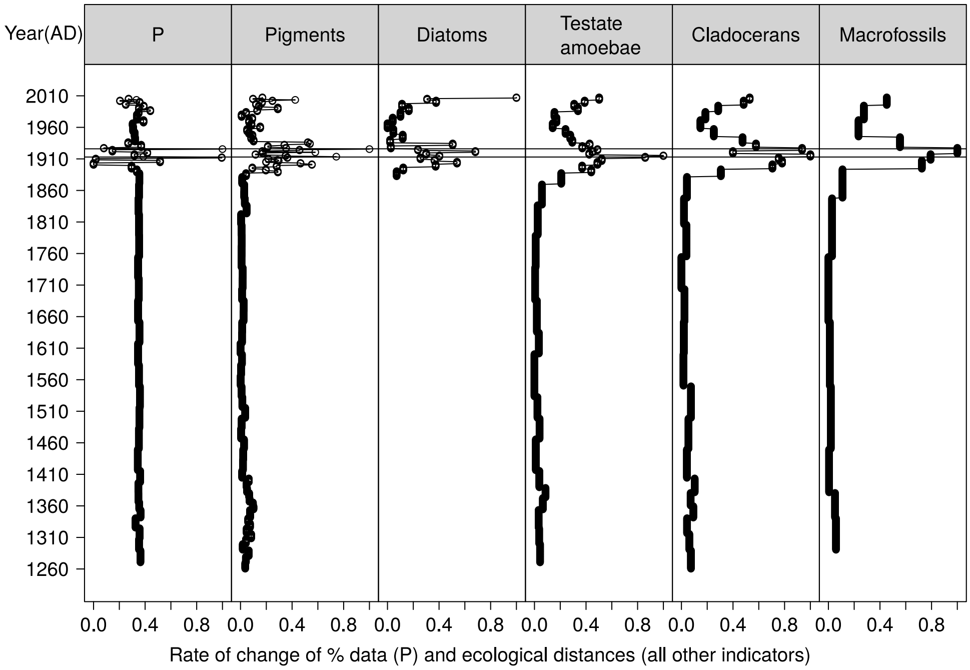

FIGURE 7 | Rate of change in P and ecological distances between consecutive samples of biological groups, ranged between 0 and 1.

particularly that of charophytes (**Figure 4**), and increases in eutrophic indicator taxa, suggesting that high charophyte densities were the main factor in maintaining clear water and ecology reflective of nutrient-poor conditions.

The third marked point of change in the pigments, diatoms, testate amoebae, and cladocera occurred in the 1990s (**Figures 5**, **7**). Increases in eutrophic indicators such as *Cucurbitella tricuspis* (Medioli and Scott, 1983) (Supp. 3 in Supplementary Material), and planktonic diatoms (Supp. 7 in Supplementary Material), cryptophytes (Supp. 2 in Supplementary Material) and *Daphnia* spp. (**Figure 4**) (e.g., Bos and Cumming, 2003; Davidson et al., 2007) together with Nymphaeaceae, combined with associated declines in concentrations of ubiquitous pigments, all support the idea of a larger change in ecosystem structure toward pelagic shallow lake ecology (McGowan et al., 2005). Reciprocally, major declines in cladoceran-inferred PVI (**Figure 6**) and reductions in macrophyte species richness from 9 to 4 between the 1980s and the early 2000s (Wiik et al., 2014) support the idea of a substantial decline in benthic production.

Given the modest concentrations of TP in the lake currently ($56\,\mu g\ L^{-1}$), and increasing P concentrations toward the core top, the decoupling between marling and P loading following the 1930s likely occurred at very low TP concentrations and opened up a "slippery slope" toward the loss of marl lake characteristics in Cunswick Tarn. The buffering capacity of charophytes may function over lower ranges of impact than previously thought. Indeed, sensitivity of charophytes to eutrophication is supported by studies of other marl lakes through time (Krause and King, 1994; Krolikowska, 1997; Hargeby et al., 2007), and also via space-for-time substitution (Kornijów et al., 2002; Free et al., 2007).

Interestingly, there are several marl lakes which have sustained high abundances of precipitating *Chara* meadows, also to considerable water depth (ca. 4 m), despite high nutrient loading and limnetic TP concentrations. These include several lakes in Poland with TP between 40 and $220\,\mu g\ L^{-1}$ (Pełechaty et al., 2004, 2013). There are also charophyte-dominated clear marl lakes with intermediate and variable TP, including Polish Lakes Jasne (TP 30–50) and Słone (TP 10–74) (Peczuła et al., 2014; Pukacz et al., 2014). More detailed studies would be required to establish whether the variability is random, or driven by some other factor such as water hardness, sediment structure, or other spectral characteristics of the water. For example, a TP-independent negative relationship has been found between charophyte abundance and dissolved organic carbon (DOC) (Kłosowski et al., 2006). The relationship does not, however, preclude the existence of marl lakes (such as Cunswick Tarn) in peaty, wetland catchments (Pentecost, 2000; Jones et al., 2011; Peczuła et al., 2014).

Nitrogen

Proxies for biological nitrogen availability were not measured in the sediment record and therefore the ecological impact of changing major nutrient stoichiometry could not be evaluated. However, atmospheric nitrogen deposition, especially in the northwest of England, has been high over the twentieth century, particularly since the 1950s (Pitcairn and Fowler, 1995), which in part reflects increases in nitrogen-rich fertilizer application (Fowler et al., 2005). In Cunswick Tarn, increases in fossil nitrogen-fixing *Gloeotrichia* colonies occurred in both cores around the 1900s and peaked in the 1920s–1930s (Supps. 5, 9 in Supplementary Material), suggestive of modest eutrophication (Laugaste and Lessok, 2004) under conditions of nitrogen limitation (Vis et al., 2008; Hudon et al., 2009).

Concentrations of *Gloeotrichia* colonies soon after declined to pre-1900 levels while *Cocconeis placentula*, a diatom with potential affinity to nitrogen-rich habitats (Werner, 1977; Lebkuecher et al., 2014), increased in abundance. Further, total nitrogen has been shown to display significant controls over testate amoebae populations (Patterson et al., 2012; Ju et al., 2014) in addition to P (Roe et al., 2010), and increasing N availability could be contributing to the 1920s shift in community composition toward a difflugid-dominated assemblage. The transition in algal and testate amoeba indicators corresponded with shifts toward angiosperm dominance and reduced macrophyte biomass in the lake, which may indicate a combined effect of nitrogen and phosphorus in shaping the biological eutrophication responses of Cunswick Tarn.

Climate

The Medieval Climate Anomaly (MCA) was a relatively warm period in the UK, and occurred ca. AD 880–1350 (Guiot et al., 2010; Dong et al., 2012). The MCA was coincident with Zone 1 in the CUNS2 core record (ca. 1260–1400), during which planktonic-dominated foodwebs in Cunswick Tarn were indicated by low macrophyte PVI (**Figure 6**), an abundance of pelagic cladocerans, and relatively high concentrations of cyanobacterial pigments (Supps. 2, 4 in Supplementary Material; **Figure 4**). The presence of considerable concentrations of purple sulfur bacteria pigments further indicated hypolimnetic anoxia at this time. However, high total production was unlikely given low core P and Ca concentrations, the presence of cladoceran oligotrophic indicators *Alonella exigua* (Whiteside, 1970; De Eyto et al., 2003) and *Chydorus piger* (Whiteside, 1970; Bos and Cumming, 2003), the oligotrophic indicator testate amoeba *Cyclopyxis kahli* (Schönborn, 1967; Wall et al., 2010), and the low concentration of Nymphaeaceae trichosclereids.

Warmer climate can promote primary production, thereby effecting carbonate precipitation (Mullins, 1998; Mirosław-Grabowska and Niska, 2007), and favoring high relative abundances of plankton (Jeppesen et al., 2012) as well as high thermal stability, strong stratification, and hypolomnetic anoxia (Viner, 1985; Foley et al., 2012). While the aforementioned were inferred in the "MCA" core record, it is likely that climate was a subsidiary rather than dominant driver of the observed changes. Firstly, interannual temperature variability was considerable through the MCA and the Little Ice Age in the UK (Cage and Austin, 2010; Guiot et al., 2010), implying that a more noisy signal should have been detected in the CUNS2 record if the biology was driven solely by temperature patterns. Secondly, numerical analyses of the biological data sets against variably smoothed April–September temperature reconstructions (Guiot et al., 2010) combined with Central England temperature time series (HadCET) revealed no detectable impact of temperature (data not shown). It remains possible that (1) climate effects were non-linear and (2) climatic conditions interacted with more discrete change within the catchment over the period between zones 1 and 2.

Land Clearance and DOC

Cunswick Tarn lies in a shallow depression in the landscape and would naturally have been surrounded by alder (*Alnus glutinosa*) carr habitat with shading willows in the margin. Under such conditions it is strongly possible that the lake margins would have been heavily shaded, resulting in reduced macrophyte development and periodic local oxygen depletion. Coppicing or marginal clearing for agriculture would have opened up the lake margins to light and increased benthic primary production and water column oxygenation, as suggested by the increase in inferred macrophyte PVI in Zone 2 (**Figure 6**) and the disappearance of okenone (Supp. 2 in in Supplementary Material). While human impact in the catchment was likely, given the long history of the Cunswick Hall estate (Nicholson, 1861), there are unfortunately no detailed records to support the hypothesis.

Some support for shading and DOC as drivers of ecological change can be found in the more recent history of the lake during which okenone has reappeared in the core record. The period coincides with growth of willows (*Salix* spp.) at the lake margins, bank erosion at the north end of the lake which has resulted in high DOC and suspended sediment loads, and strong chemical stratification with hypolimnetic anoxia in summer months.

Implications for Management

Most change in the geochemistry and biology of Cunswick Tarn occurred in the late 1800s when larger-scale land improvement occurred in the catchment, consistent with European agriculture and population trends (Battarbee et al., 2011). Indeed, based on these trends, palaeolimnology-based reference status for lakes under the European Union Water Framework Directive (WFD: European Commission, 2000) is typically derived from the pre-1850s (Bennion et al., 2011). Marl lakes fall under the WFD but are also a designated habitat under the EU Habitats Directive (European Commission, 1992), hence being required to achieve both Good ecological status and Favorable conservation status, respectively. Sites with such dual designation are additionally considered "protected areas" under WFD and are consequently given earlier deadlines for achieving their target objectives.

In order to comply with the targets of river basin management plans as part of the WFD, lake condition assessments, and extensive cross-validation of ecological metrics have been undertaken to ensure that effective measures are taken on correctly and consistently classified sites across Europe (Cardoso et al., 2007; Carvalho et al., 2009; Hering et al., 2013). However, concerns have been raised over the effectiveness of macrophyte and chlorophyll *a* metrics for assessing very shallow ($Z_{\text{mean}} <$ 3 m) and shallow (Z_{mean} 3-15 m) marl lakes (Wiik et al., 2014), with Cunswick Tarn data in particular indicating shifting perceptions of good ecological status, and therefore assessment bias, for marl lakes. Further, this improved understanding of the pre-impact character of Cunswick Tarn has implications for how it is described, protected and restored under national nature conservation designations (SSSI); the Tarn is currently identified as a naturally eutrophic water body, which is in strong disagreement with the palaeolimnological data.

Implications for Marl Lake Conservation Assessment and Reference Status

The uncertainties regarding drivers of biological communities in the lowermost core record (Zones 1 and 2) hinder firm conclusions as to the reference status of Cunswick Tarn. Zone 1, where high DOC loads and/or warmer climate were potentially inferred, depicts the Tarn as an oligotrophic marl lake, with carbonate-precipitating *Chara aspera* and low densities of Nymphaeaceae (especially *N. alba*) in the shallow waters. Abundant organic matter deposition created oxygen gradients in the deeper water, where *Nitella flexilis* agg. grew in low densities. The relatively high volume of open water allowed the development of planktonic foodwebs, with *Daphnia* and chaoborids in the deeper regions and *Bosmina longirostris* in the sublittoral zone (**Figure 8**). Zone 2, in contrast, portrayed oxygenated sediments, higher macrophyte PVI, and foodwebs based on benthic production with planktonic taxa largely absent.

Given the differences between zones 1 and 2, it is important to focus on their similarities to set guidelines for near-pristine status. For example, core carbonate content in both zones was low (<10%) in the deeper water, with low inferred abundances of charophytes and other submerged macrophytes. Higher but still moderate levels of charophytes and carbonate (<20%) occurred in the shallower margins (**Figure 3**; Supp. 9 in Supplementary Material) suggestive of charophyte-dominated marl benches (Jones et al., 2011). The relatively low levels of calcite deposition likely reflected the localized nature of marling in oligotrophic conditions, with calcite supersaturation occurring primarily in the epilimnion and the littoral compared with dissolution in the deeper water (Gonsiorczyk et al., 1997; Ekdahl et al., 2004). Near-pristine conditions in Cunswick Tarn were therefore remarkably "non-marly."

In contrast to prevailing associations of dense charophyte meadows, and high production in the benthos, with high ecological quality, the Cunswick Tarn record has shown these characteristics to be an early response to eutrophication (**Figure 8**). The observation is not completely unexpected, considering that biomass responses to nutrient loading as a self-stabilizing method in *Chara* lakes inherently imply that these characteristics are a symptom of eutrophication. Indeed, particularly dense (and in some cases tall) growth of charophytes has been observed in lakes subject to considerable nutrient loading (Pełechaty et al., 2004; Haycock and Hinton, 2010) in comparison with the more scarce and modest growth of charophytes in lakes of lower impact (Fuller, 1741; Praeger, 1906; Spence, 1982), which was also indicated in the early record of Cunswick Tarn.

Management and Restoration Priorities

Cunswick Tarn is currently a marl lake only by geology, and is therefore considerably impacted. It is highly unlikely that conditions similar to the thirteenth to eighteenth centuries can be attained given the high load of P in the sediment, low flushing rates, and therefore high potential for internal loading in the lake. However, reductions of external nutrient loads are potentially the most effective means of restoring shallow lakes (Jeppesen et al., 2007), especially lakes such as Cunswick Tarn where fish manipulation is irrelevant. Unless nutrient availability for phytoplankton is restricted, charophytes will not be able to recolonize the Tarn. The most likely sources of nutrients to the Tarn are in the north where a drain pipe leads directly into the lake, and high nutrient concentrations have been measured in the inflow (December TP 44 $\mu g\ L^{-1}$). Streams in the east woodland are likely to contribute less (December TP 14–20 $\mu g\ L^{-1}$). Until 2015, the feeding of artificially high populations of ducks in the lake margins was a further major nutrient source. Given the potential for hydrology to be complex in limestone catchments, an inclusive and larger scale approach to determining nutrient sources may be important.

Given the lags in macrophyte re-establishment in deeper water owing to the high light requirements of germlings and species with low growth forms (Wang et al., 2014), restoration of lake margins is especially important in sites with considerable water depth gradients (Hilt et al., 2010). In this respect, the erosion occurring at the north end of Cunswick Tarn, exacerbated by the duck feeding, is particularly damaging and should be prevented. High external DOC and suspended sediment loads lead to anoxic sediments unfavorable for charophytes (Smith, 2003; Sederias and Colman, 2009) and maintain conditions suitable for reeds and Nymphaeaceae which are currently dominant.

Tree clearance would further open up light-flooded marginal habitat for charophyte re-establishment, however, this kind of management could also be seen as interference with natural succession of trees in the lake surrounds. It is therefore a judgment call of whether moderate interference promoting biodiversity (e.g., Sayer et al., 2012, see also Flöder and Sommer, 1999) is to be preferred over gradual natural successions following neglect (e.g. Biggs et al., 2005) (with the caveat that "natural" succession in this context does not account for grazers prior to major human influence). Considering the large-scale, centennial-scale human alteration of the natural landscape of the UK, including the eradication of top predators, and the high global conservation priority of preventing further biodiversity losses (European Parliament, 2012), coppicing around the lake would be strongly advisable.

Conclusion

Biological community shifts in Cunswick Tarn demonstrated synchronicity in response to gradual eutrophication pressure. Drainage of the lake and land improvement in the lake surrounds in the 1890s led to sub-decadal responses in multiple biological groups (microalgae, macrophytes, molluscs, testate amoebae, cladocerans) and substantial increases in carbonate precipitation. Further abrupt ecosystem shifts occurred in the 1920s, 1930s, and finally the 1990s when the lake changed into its current condition of low macrophyte diversity, high pelagic production, reduced macrophyte colonization depth and predominance of nutrient-tolerant micro- and macrophyte taxa. The patterns of rapid ecosystem-wide change, partly decoupled from the change in core P, supported ideas of abrupt responses to eutrophication as well as the importance of internal dynamics such as the self-sustaining capacity of charophyte beds, in shaping ecological lake structure.

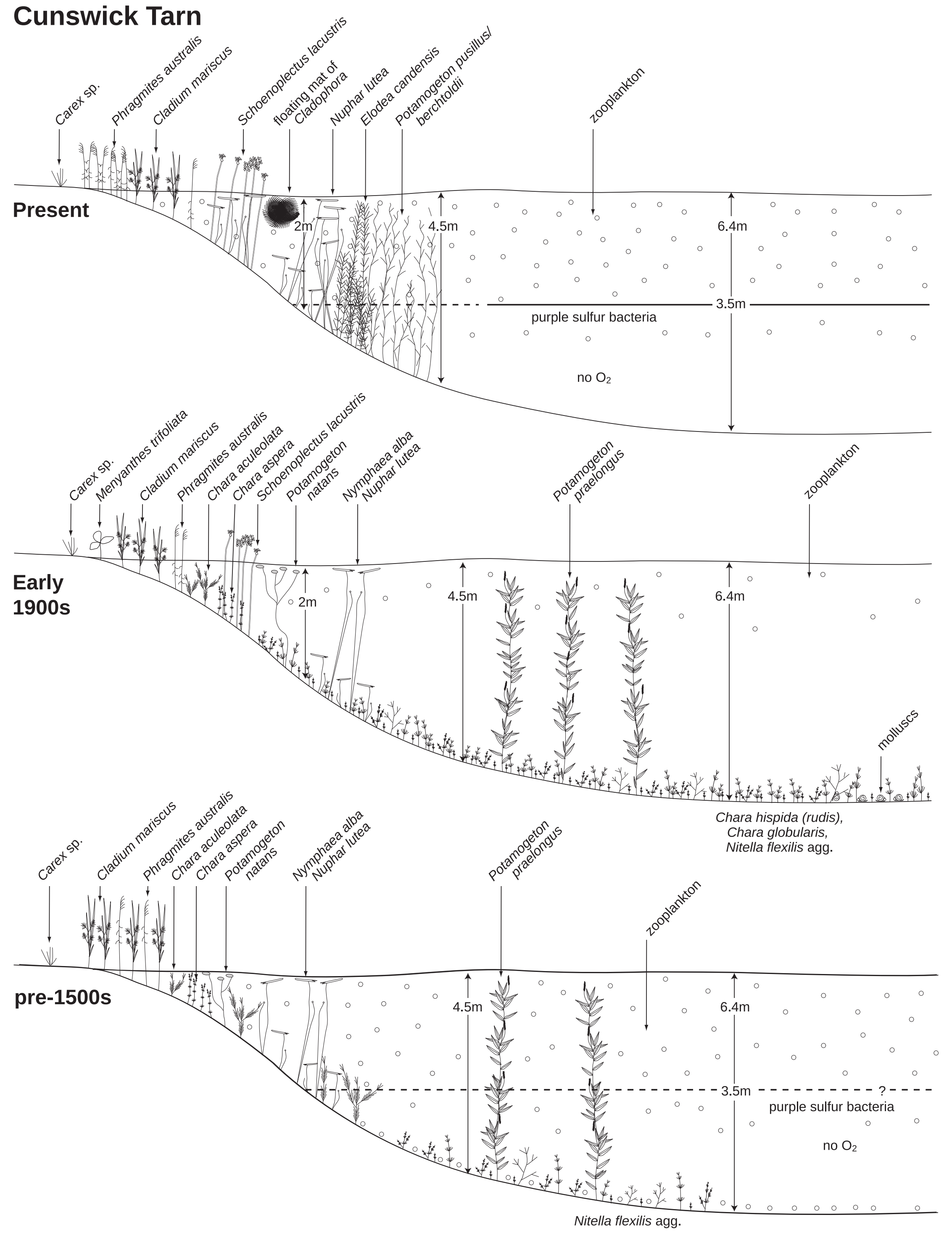

FIGURE 8 | Cross sections of macrophyte cover, showing the currently dominant community, and historical communities based on available historical data.

Variance in marl lake biological communities correlated with both Ca and P as expected for marl lake communities responding to external nutrient load. Somewhat surprisingly, the period of least impact in the Tarn was characterized by very low carbonate precipitation in the deeper water, with marling restricted to the shallower littoral zone where charophyte meadows occurred. It is likely that pristine marl lakes do not attain sufficient levels of photosynthesis for intensive, whole-lake scale authigenic carbonate precipitation, and that macrophyte cover could be quite low.

The detection of two periods of accelerated change over intermediate core P concentrations support hypotheses of high eutrophication sensitivity of benthic communities in marl lakes, especially considering the modest concentration of TP (ca. 56 μg L^{-1}) in Cunswick Tarn currently. Importantly, the association of high benthic biomass and calcite deposition with high ecological condition may be biased, reflecting a shift in perception due to the scarcity of truly high quality examples of marl lakes. The complete loss of marl lake biology in Cunswick Tarn argues strongly for restoration interventions at early stages of eutrophication in order to preserve characteristic marl lake communities. Further, if similar lake type transformations have occurred in other (formerly) marl lakes, perceptions of their geographical distribution and scarcity may be misguided.

Author Contributions

The majority of this publication is the direct result of a PhD by EW, which was supervised and guided by HB, CS, SC, and TD. EW undertook all palaeolimnological analyses, taught variously by HB, CS, SMG, and TD, excepting testate amoebae which are part of a PhD by SP, and diatoms which were part of an undergraduate dissertation by LS. All fieldwork was undertaken by EW and a combination of HB, CS, TD, and LS among others. GS offered crucial guidance during statistical analysis of the data during EW's PhD, and also contributed to the design and execution of statistical methods particular to this publication. EW wrote the manuscript, and all other authors contributed essentially to the interpretation and wording of components falling within their expertise.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2015.00082

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METHODS
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What is the robustness of early warning signals to temporal aggregation?

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A number of methods have recently been developed to identify early warning signals (EWSs) within time-series structure typically characteristic of the rise of critical transitions. Inherent technical constraints often limit the possibility to obtain from sediment both regular and high-resolution time series rather most palaeoecological time series obtained from sediment records represent time-aggregated ecological signals. In this study, the robustness of EWS detection to temporal aggregation was addressed using simulated time series mimicking ecological dynamics. Using a stochastic differential equation based on a deterministic model exhibiting a critical transition between two stable equilibria, two different scenarios were simulated using different combinations of forcing and noise intensities (critical slowing-down and driver-mediated flickering scenarios). The temporal resolution of each simulated time series was progressively decreased by averaging the data from $\Delta t = 1$ up to $\Delta t = 10$ time-unit intervals. EWSs [standard deviation, autocorrelation at lag-1 (AR(1)), skewness and kurtosis] were applied to all time series. Robustness of EWSs to data aggregation was assessed through a block-based approach using Kendall rank correlation Tau. Standard deviation appeared to be robust to data aggregation up to $\Delta t = 10$ for the slowing-down scenario and up to $\Delta t = 5$ for the driver-mediated flickering scenario while autocorrelation remained robust up to $\Delta t = 2$ for the slowing-down scenario and did not support data aggregation for the driver-mediated scenario. Skewness and kurtosis performed poorly for the two scenarios and were not considered as robust EWSs even for the original simulated time series using the block-based approach. Our results suggest that high-resolution palaeoecological time series could be in a large extent suitable to support EWS analyses.

Keywords: early warning signal, time series, temporal aggregation, resilience, stability, lakes

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Introduction

Since the Industrial Revolution, the enhancement of anthropogenic pressures worldwide led to drastic changes in ecosystem functioning (Estes et al., 2011) and species dynamics (Anderson et al., 2008). Ecosystems are complex adaptive systems and species exhibit a large number of biotic and abiotic interactions, their responses to forcings can be linear and progressive but also nonlinear, exhibiting rapid, and abrupt changes (Scheffer et al., 2001, 2012; Dakos et al., 2015). Such catastrophic shifts are related to the existence of multiple attractors and fold bifurcations in response to slow variations in forcing variables (May, 1977; Seekell et al., 2013). In many cases, the human-induced regime shifts of ecosystems or species trigger important alterations of ecosystem services (Folke et al., 2004; Millennium Ecosystem Assessment, 2005). As a consequence, over the last decade, a growing body of research (Scheffer et al., 2001, 2009, 2012; Dakos et al., 2008, 2015; Carpenter et al., 2011) has been developed to define indicators of the rising of a critical transition. Various indicators or early warning signals (EWSs) have been proposed to anticipate such regime shifts (reviewed in Scheffer et al., 2012). The basic assumption is that the structure of the time series of a state variable differs whether the system is close to or far away from an attractor within the attraction basin. EWS methods have been successfully used to identify changes in time series prior of regime shift in various contexts such as climate change (Dakos et al., 2008; Lenton et al., 2012), lakes (Carpenter et al., 2011; Wang et al., 2012), plankton (Veraart et al., 2012) and zooplankton (Drake and Griffen, 2010). Nonetheless, time series required to seek for EWS should be long and recorded at high-resolution. For instance, Dakos et al. (2012a) argued that the frequency of the observations should be higher than the characteristic rate of change of the state variable in the considered dynamic system. This often limits the possibility of using EWS methods to ecological time series and especially palaeoecological time series, which usually have lower temporal resolution than the genuine ecological dynamics it is supposed to describe.

Sediment records have commonly been used to obtain long-term time series of ecosystem's state variables (Dakos et al., 2008) or species (Caswell and Frid, 2013). Spanning specific periods of interest (e.g., late Glacial transition, Little Ice Age, Industrial Revolution), the use of EWSs on reconstructed dynamics would greatly improve our ability to better assess resilience and stability of species or ecosystems. Unfortunately, finest time resolution (i.e., 1–2 years) is seldom to be achieved due to limited or changing sedimentation rate as well as bioturbation that can induce errors in the relative age estimates between successive samples. Interpolation has been regarded has a mean to deal with unevenly spaced empirical time series (e.g., Dakos et al., 2008). Nonetheless, interpolation can spuriously alter the statistical properties of time series (Carstensen et al., 2013). As an alternative, the robustness of EWSs to temporal upscaling or data aggregation may be questioned. What is the lower temporal resolution of data needed for a consistent EWS analysis? Lenton et al. (2012) addressed the effect of data aggregation on their GENIE-2 simulations over different time scales and identify significant effects on the results (specifically temporal autocorrelation) whereas Carstensen et al. (2013) questioned a possible influence of the temporal resolution on EWS detection.

This study aims at assessing the effect of temporal resolution on EWS detection using simulated time series. Specific questions that motivated this study were: (i) To what extent EWS patterns remain consistent after data aggregation? (ii) Is the influence of data aggregation similar when dealing with different kinds of regime shift scenarios?

Four hundred time series were simulated from a stochastic version of the model of Ludwig et al. (1978) using two different parameterizations (details below). This model, widely used in ecological modeling (e.g., May, 1977; Dakos et al., 2012a), exhibits alternative stable states and finely matches abrupt shifts in ecosystem states or in species abundances. Species dynamics greatly differed among species especially due to differences among body size (Yodzis and Innes, 1992). Therefore, to cope with these intrinsic differences, two intensities of Gaussian white noise (i.e., high and low) were used (i) to enable the EWS detection, (ii) to match with patterns found in real data. The different combinations of noise and forcing rate of change aimed at reproducing patterns mostly reported for ecological dynamics prior to a critical transition, namely slowing-down or flickering. Slowing-down is characterized by a slower recovery of the system as long as the forcing variable increases in a context of low noise level (Dakos et al., 2008; Lenton et al., 2012). The flickering phenomenon is characterized by a high noise level which triggers switch of the system state between two basins of attraction due to relatively large disturbances (Guttal and Jayaprakash, 2007; Dakos et al., 2013). In this case, a critical transition can be due to both deterministic and stochastic processes.

Methods

Alternative Stable State Model

The model used to simulate time series is a stochastic differential Equation (1) derived from the deterministic model of Ludwig et al. (1978). The first part of the model provides the classical logistic growth of a population X with maximum per-capita growth rate r and carrying capacity K. For all simulations, $K = 10$ and $r = 1$. The second part of the model represents a harvest function following a type-III S-shaped functional response (Holling, 1959) mimicking nonlinear relationship between a forcing and a response variable (often between a consumer and its resource). The harvest function was retained as it corresponds to a large number of interactions among aquatic species (Morozov, 2010). Ecologically, speaking, c expresses the extent of the forcing intensity or saturation level of the predator for which no more prey can be consumed and h represents a switching value that indicates the scale at which predators switch on new preys under learning process. Tuning this model for $K = 10$ allows producing alternative stable state scenarios intersected by a critical transition (May, 1977). For all analyses, c (hereafter forcing intensity) linearly increased (**Table 1**), and h was set constant at 1. Noise consisted in Gaussian white (Wiener) noise dW centered on 0 with a constant standard deviation (σ, hereafter noise intensity) and was introduced in the model to take into account random variations in forcing pressure. The stochastic equations were simulated using Euler discretization and solved with Ito stochastic calculus (Horsthemke and Lefever, 1984).

$$dX = rX\left(1 - \frac{X}{K}\right)dt - \left(cdt + \sigma dW\right)\frac{X^2}{X^2 + h^2} \qquad (1)$$

Adjusting values for both forcing and noise intensities (c and σ, respectively) led to simulate two different scenarios of time

TABLE 1 | Characteristics of forcing (c) and noise intensities with σ representing standard deviation used in the two different scenarios.

	Critical slowing-down	Driver-mediated flickering
c	1–3	1–3
σ	0.5	1.5

series that mimic different possible scenarios matching empirical time series (**Table 1**). The "slowing-down scenario" was obtained considering a slow linear increase for the forcing variable c and a low and constant amount of noise σ. According to Dakos et al. (2013), the flickering behavior is characterized by a high amount of noise and is thought to express highly stochastic dynamics compared to slowing-down scenario. Therefore, in the "driver-mediated flickering" scenario the forcing variable c increases also linearly with time but a higher constant level of noise is applied. For the slowing-down scenario, the low noise level was adjusted to trigger state shift very close to the actual fold bifurcation (Dakos et al., 2013). By contrast, in the flickering scenario the higher amount of noise was set so as the system could flip from one state to another with forcing facilitation (driver-mediated) (Dakos et al., 2013). For each scenario, 200 time series were simulated.

Time Series Aggregation

The process of aggregation of time series considered all full-length time series simulated. The process produced surrogate time series for which temporal resolution ranges from 1 to 10 time units. Explicitly, for the aggregated time series at $\Delta t = 2$ time units, each two consecutive values were averaged. Similarly, for $\Delta t = 3$ time units, each three consecutive values were averaged and so on until $\Delta t = 10$ time units. The range of temporal aggregation, from 1 to 10 time units, was retained to match the usual time intervals usually reached in high-resolution palaeolimnological studies (e.g., Frossard et al., 2013; Millet et al., 2014).

Early Warning Signals

A large array of EWS methods has been proposed over the last years to detect change in time series structure prior to a critical transition. Four indicators were used in the analyses. (i) The standard deviation (SD) of the state variable X is expected to increase due to wider fluctuations in the system when approaching a tipping point as a consequence of lower recovery rate following disturbances, entraining the system far from the center of the basin of attraction. (ii) The temporal autocorrelation was considered through the autoregressive coefficient of an autoregressive model of order 1 fitted using conditional least-squares method (AR(1)). AR(1) is also expected to increase as the system approaches a critical transition because fluctuations become larger, implying that the successive data become progressively more similar and therefore autocorrelated. (iii) Skewness and (iv) kurtosis, respectively the standardized third and fourth moments around the mean of a distribution of a X were also considered. These two indicators highlight how, as the system reaches a critical transition, it exhibits slower recovery and higher proportion of extreme values. Different patterns can be produced by these two indicators whether the next alternative stable state is lower or higher than the previous one.

All time series were split in two parts using constrained hierarchical clustering based on Euclidean distance between successive time steps to obtain the portions of the simulated time series prior to critical transition on which the analyses were conducted. These portions were then adjusted to the shortest time series in order to compare EWS patterns and detrended using Gaussian kernels (Dakos et al., 2012a).

The use of a moving sliding window can introduce statistical dependence among EWSs that biases statistical estimates of trends and may limit our ability to detect true alarms (Boettiger and Hastings, 2012). To overcome this issue we used a block-based approach similarly to Wouters et al. (2015) to compute independent EWSs. Time series from the critical slowing-down scenario were restricted to 300 time steps (i.e., the shortest length for driver-mediated scenario) so that each block for the two scenarios had equal number of observations. Time series were split in 10 blocks among which Gaussian detrending was applied followed by EWS computation within each block. The length of the time series (300 time steps) could not support aggregation for higher numbers of blocks and restricted EWS computation for aggregation level above $\Delta t = 10$. In the context of this study, it can be considered among the longer ecological time series that can be obtained. Kendall rank correlation Tau then was used to assess the value and the significance of EWS pattern using the block-based approach.

Simulations from the stochastic differential equation were produced with Scilab 5.5.0 (Scilab Enterprises S.A.S). Data aggregation and statistical analyses were performed using R 3.2.0 (R Core Team Development, 2015) as well as the packages "earlywarnings" (Dakos et al., 2012a), "vegan" (Oksanen et al., 2015) and "rioja" (Juggins, 2014).

Results

Two specific scenario patterns were obtained using different combinations of c and σ (**Table 1**, **Figure 1**). Simulated time series were split at the onset of the tipping points using constrained cluster analysis and sections of the time series prior the tipping points were retained to produce consistent EWS comparison for the slowing-down and driver-mediated flickering scenarios. Patterns for the different EWSs were then assessed using Kendall correlations.

EWS Patterns

For each of the two scenarios, the 200 simulated time series allowed accounting for particular behavior of time series due to the stochastic part in Equation (1). Visual inspection indicated that patterns differed among EWSs and that the effect of data aggregation varied both among EWS and scenarios (**Figures 2**, **3**).

For the slowing-down scenario (**Figure 2**), increasing patterns of autocorrelation can be identified at aggregation level of $\Delta t = 1$ and $\Delta t = 2$. At higher aggregation levels, patterns are flattened and even exhibit a decrease in autocorrelation in the last block, especially at $\Delta t = 10$. Differently, standard deviation exhibited

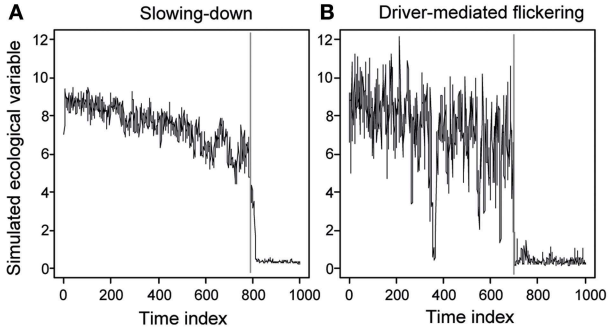

FIGURE 1 | Examples of single time series simulated from Equation (1) producing different patterns according to variations in c and σ. (A) represents a slowing-down scenario for which c increases over time with a low level of noise (small σ). **(B)** refers to a driver-mediated flickering scenario for which c increases over time with a high level of noise (high σ). The gray vertical lines indicate the time at which an observed bifurcation occurs toward a lower alternative stable state using the constrained hierarchical clustering based on Euclidian distance.

unambiguous increasing patterns over the range of aggregation. Its variability among simulations within each block decreased as aggregation increases, which is likely related to our data averaging process. Standard deviation drastically increases in the 10th block, the last sequence before the transition. The time series we used did not integrate data after the transition. Therefore, although an increase in standard deviation can be informative between successive blocks, the last sequence prior to the transition seems to clearly indicate the imminence of the transition. Skewness and kurtosis were mostly characterized by rather flat or decreasing trends at least up to the 8th block. As for standard deviation, these two EWS increased drastically in the last 10th block up to the data aggregation of $\Delta t = 2$. Although, it seems to effectively indicate the rise of the transition, the lack of previous trends limit greatly their ability of anticipate the critical transition in this scenario.

For the driver-mediated flickering scenario, the highest noise intensity compared to the slowing-down scenario produced EWS patterns that were differently conserved over the aggregation range considered (**Figure 3**). For autocorrelation, the within-block variability was rather high as indicated by the large standard error bars. Increasing patterns were not pronounced, except for $\Delta t = 1$. A decrease in autocorrelation within the last two blocks occurs up to $\Delta t = 2$. Differently for standard deviation, increasing trends associated with a marked increase in the last block were conserved up to $\Delta t = 5$. At $\Delta t = 10$, although the 10th block exhibited clearly a high standard deviation, trend prior the 10th block was absent. Neither, skewness nor kurtosis exhibited increasing trends over any level of aggregation for the driver-mediated scenario. In a majority of cases, these two EWS tented to decrease over the last three blocks at aggregation level up to $\Delta t = 2$.

Robustness of EWS to Aggregation

Important differences were found regarding EWS robustness to data aggregation. For standard deviation, Kendall Tau was significantly positive for the slowing-down scenario over the range of aggregation. It increased from ∼0.5 to ∼1 from $\Delta t = 1$ to $\Delta t = 10$, indicating strong increasing pattern when approaching the critical transition (**Figure 4**). For the driver-mediated scenario, the Kendall Tau of the standard deviation was still positive but close to significance at $\Delta t = 1$ and significant at $\Delta t = 2$ and $\Delta t = 5$ (**Figure 4**). Kendall correlations for temporal autocorrelation tended to decrease over the range of data aggregation but were high for the low aggregation level at ∼0.5. They remain significant up to $\Delta t = 2$ for the slowing-down scenario and were close to significance for the driver-mediated scenario only at $\Delta t = 1$ (**Figure 4**). Skewness and kurtosis did not exhibited significant trends even among the original simulated time series (**Figure 4**). The Kendall Tau tended to decrease with date aggregation as expected from the patterns shown in **Figures 2**, **3**. This decreasing pattern becoming more pronounced as data aggregation increases, it can explain the concomitant decrease of the *p*-value of Kendall Tau, especially for skewness in the driver-mediated scenario. For skewness and kurtosis trends tended to decrease with data aggregation. This counterintuitive result could be due to the smaller sample size in blocks over aggregation steps. Nonetheless, Kendall Tau was negative, contrary to our primary expectations. Based on our primary expectations of a positive and significant Kendall Tau, these two EWSs did not meet the required criteria at any level of aggregation to inform about the rising of a critical transition. The robustness of EWSs, assessed using significance of Kendall Tau from the block-based approach, is summarized in **Table 2**.

Discussion

The expanding use of time series obtained from sediment records to address current ecological threats and long-term ecosystem dynamics progressively reconcile palaeoecology and neoecology. A shared *time continuum* recently described by Rull (2014) identifies time scales that can be retrieved from lacustrine archives to reconstruct high-resolution, ecologically relevant dynamics. The unprecedented increase of anthropogenic forcings ongoing since the beginning of the twentieth century (Anthropocene era; Steffen et al., 2011) deeply altered most of natural ecosystems and their associated species fitness leading to possible massive ecosystem or species collapses. In this context, seeking for EWSs that could inform for the rise of critical regime shifts among times series obtained from lake sediments is likely to be a future important demand of conservationists and practitioners to palaeoecologists in a changing environment. Nevertheless, detectability and reliability of EWSs using palaeoecological data were remaining to be tested to develop with confidence their application to a wide array of ecological variables that can be reached from sediment archives. Justifications of model characteristics will be first presented, before comparing EWS advantages in different time series scenarios. The robustness of the EWSs is then discussed prior to provide ecological considerations for ecosystem and species dynamics that could be subjected to EWS analyses.

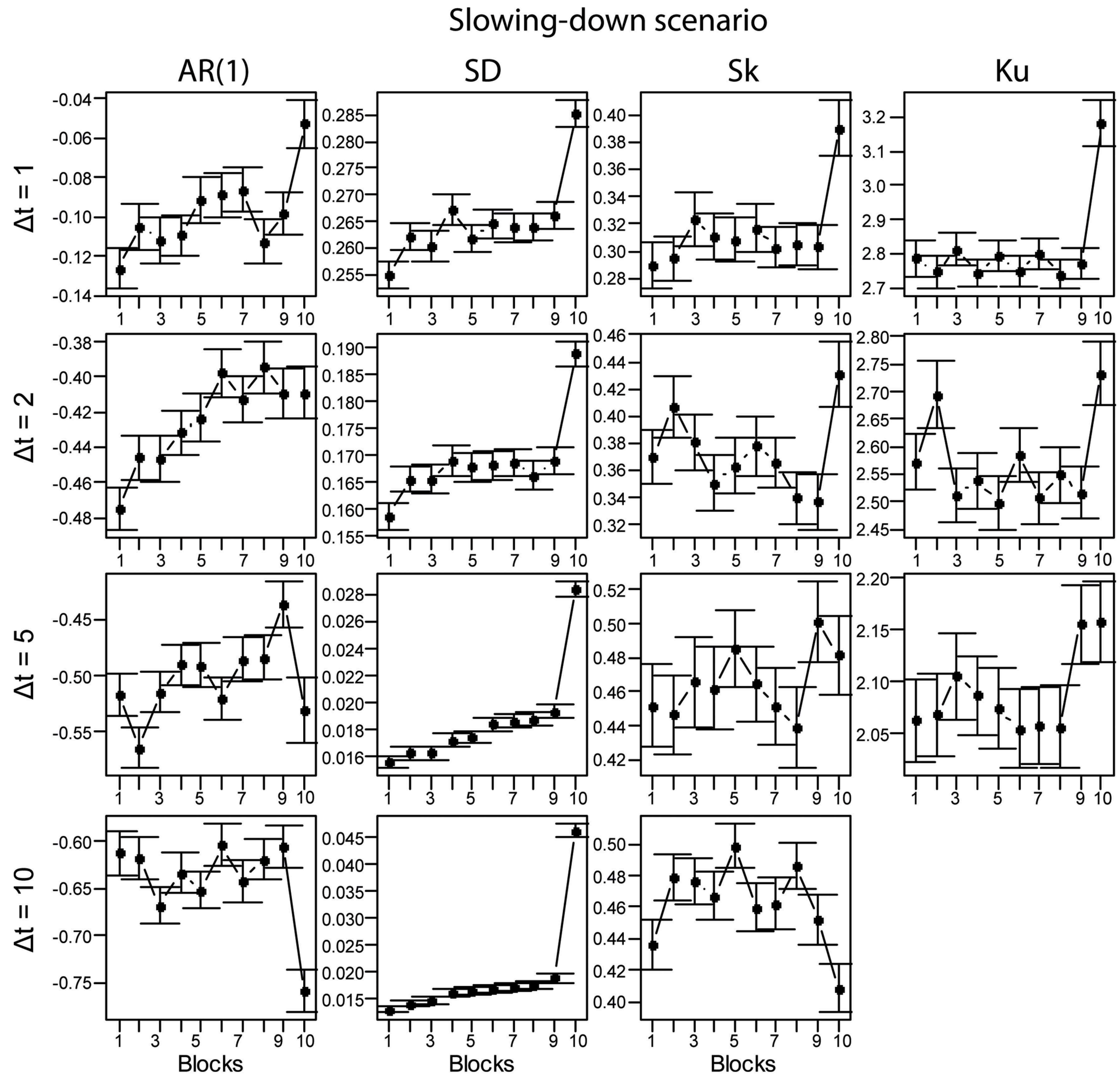

FIGURE 2 | Patterns of four EWSs for the slowing-down scenario from 200 simulated time series using the block-based approach. Time aggregation ranged from Δt = 1 to Δt = 10. Black circles associated with whiskers represent mean values and standard errors of EWSs. As time scale is reversed, the transition occurs at the end of the 10th block. AR(1), autocorrelation; SD, standard deviation; Sk, skewness; Ku, kurtosis. Kurtosis could not be computed for aggregation Δ = 10 because of the lack of data for each block (*n* = 3) for this aggregation level.

Model Choice and Parameterization

Various model formulations exhibiting alternative stable states associated with catastrophic regime shifts have been proposed (e.g., May, 1977; Carpenter et al., 1999). The current model was considered because of its long use in theoretical ecology and especially in recent EWS studies. Moreover, its simplicity allows obtaining highly different scenarios only by varying two parameters (c and σ) related to strong ecological assumptions (forcing intensity, noise on the forcing). Directionality of shift can influence the EWS patterns (Dakos et al., 2012b). For instance, critical slowing-down can be especially detected when the system shifts to basins of attraction from high to low values and more hardly from low to high values. Hence, the model retained was expected to be especially suitable to identify EWS patterns for the different simulated scenarios. In the model formulation, the stochastic component was set to be multiplicative. Although multiplicative noise has been shown to possibly produce spurious patterns leading to difficulties to identify critical transition (Dakos et al., 2012b), this kind of noise (white Gaussian noise applied on c) prevents the occurrence of unrealistic negative results during the simulations especially in a context of important noise.

Early Warning Patterns

The patterns of EWS prior to bifurcation differed among the two studied scenarios. Standard deviation, autocorrelation increased over time prior to the observed bifurcation point for both scenarios. Our results for the driver-mediated flickering scenario

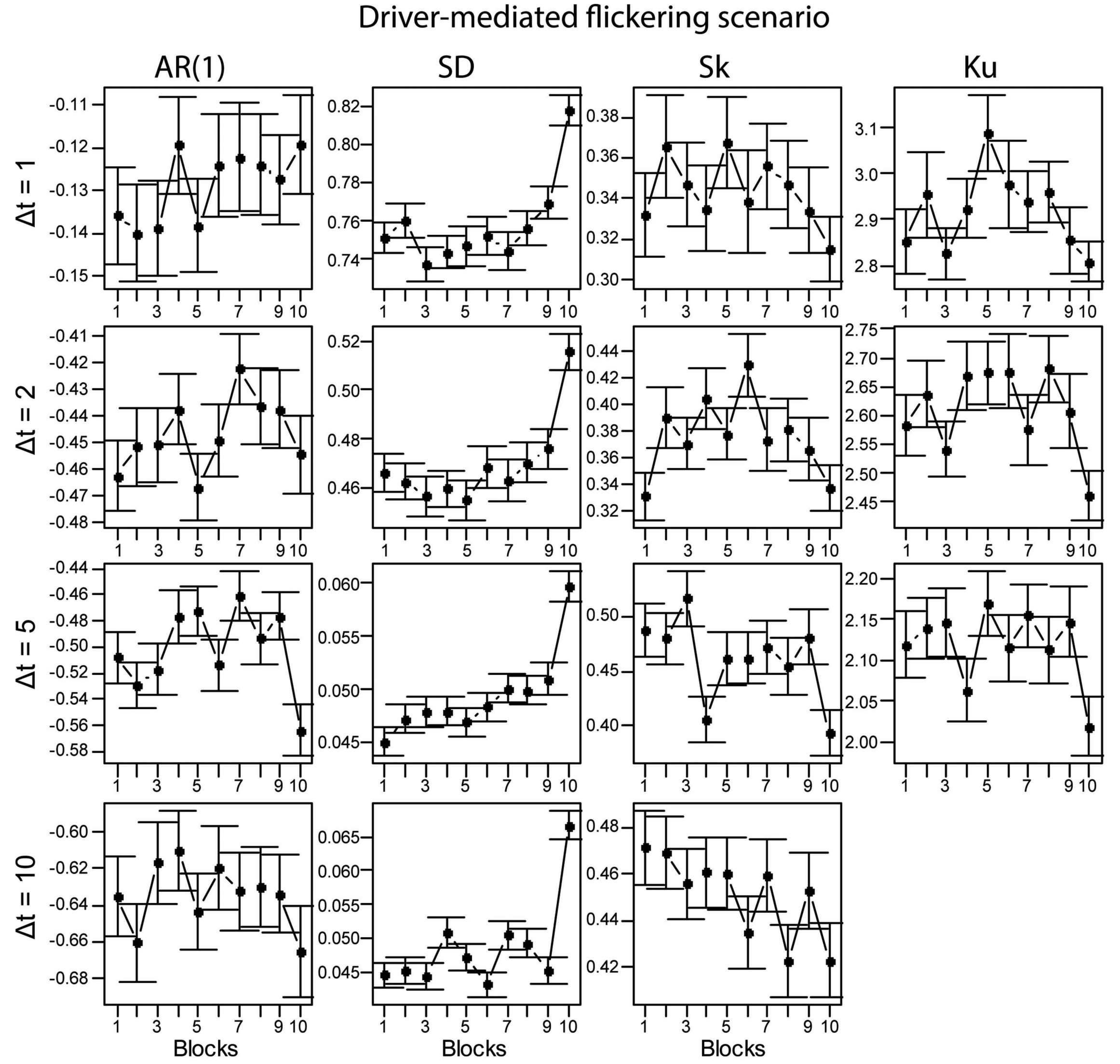

FIGURE 3 | Patterns of four EWSs for the driver-mediated flickering scenario from 200 simulated time series using the block-based approach. Time aggregation ranged from Δt = 1 to Δt = 10. Black circles associated with whiskers represent mean values and standard errors of EWSs. As time scale is reversed, the transition occurs at the end of the 10th block. AR(1), autocorrelation; SD, standard deviation; Sk, skewness; Ku, kurtosis. Kurtosis could not be computed for aggregation Δ = 10 because of the lack of data for each block (*n* = 3) for this aggregation level.

differed from those of Dakos et al. (2013) who used a different model (Carpenter et al., 1999) for which standard deviation and autocorrelation decreased prior to the theoretical critical transition. These authors suggested that a decrease in system's sensitivity to forcings or a reduction of the system's ability to follow high-frequency variations in the environment could explain this pattern. The difference with these results could also be attributable to the fact that we considered only portions of the simulated time series prior the observed critical transition whereas Dakos et al. (2013) accounted the ones that encompass the critical transitions. Nonetheless, by considering time series up to the bifurcation point, we could show that, at least standard deviation and autocorrelation showed an increasing pattern prior a critical transition therefore supporting consistent patterns for the two scenarios. Opposite alternative attractors between the models of Carpenter et al. (1999) and May (1977) as well as the use of portions of time series prior the critical transition are likely to explain the failure to detect clear patterns for skewness whereas Dakos et al. (2013) reported decreasing patterns. Clear patterns for kurtosis were also almost absent prior to observed bifurcation point for the two scenarios suggesting that these two latter EWSs should be considered with caution to identify the rise of a fold bifurcation.

Early Warning Robustness

The robustness of EWSs to data aggregation was addressed using a block-based approach as recently developed by Wouters et al. (2015) in order to produce independent EWS estimates

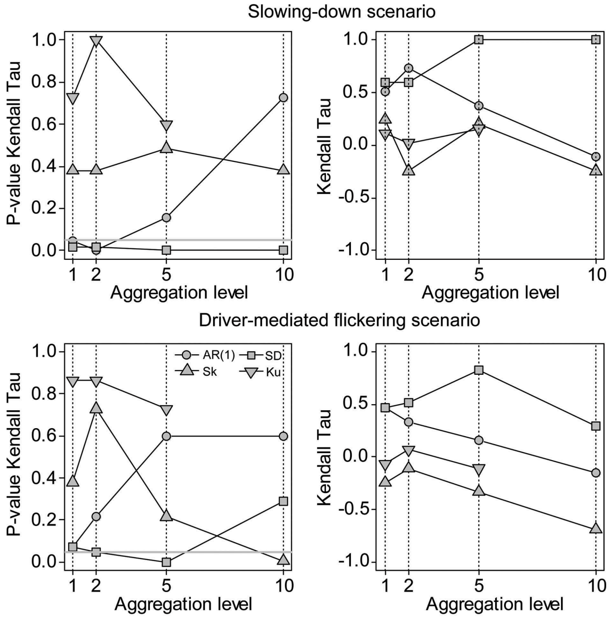

FIGURE 4 | Effect of data aggregation on the detection of four EWS patterns for the two scenarios using the block-based approach. Top: slowing-down scenario. **Bottom**: driver-mediated flickering scenario. For both scenarios, left panels show *p*-values and right panels show the estimates of Kendall rank correlation for mean EWS patterns obtained from 200 simulated time series. The gray lines indicate the threshold of $P = 0.05$ under which correlations are considered significant. AR(1), autocorrelation; SD, standard deviation; Sk, skewness; Ku, kurtosis. Kurtosis could not be computed for aggregation $\Delta = 10$ because of the lack of data for each block ($n = 3$) for this aggregation level.

TABLE 2 | Summary of the robustness of EWSs to data aggregation for the two scenarios according to significance of Kendall Tau using the block-based approach.

	$\Delta t = 1$	$\Delta t = 2$	$\Delta t = 5$	$\Delta t = 10$
SLOWING-DOWN SCENARIO				
SD	×	×	×	×
AR(1)	×	×		
Sk				
Ku				
DRIVER-MEDIATED FLICKERING SCENARIO				
SD	×	×	×	
AR(1)	×			
Sk				
Ku				

Δt indicates the level of data aggregation. SD, AR(1), Sk and Ku refer to standard deviation, autocorrelation, skewness, and kurtosis, respectively.

and prevent overestimation of the ability of EWS signal to identify effective rise of a critical transition (Boettiger and Hastings, 2012). Our analyses revealed clear influence of data aggregation on EWSs, in line with Lenton et al. (2012) who identified a significant influence of data aggregation on temporal autocorrelation for the GENIE-2 simulations. Dakos et al. (2012b) addressed the robustness of the standard deviation and AR(1) on a similar model than ours but in a different perspective. They showed that disturbances had different effects when acting on different parameters of the model. Important differences were found among the robustness of EWS to data aggregation (**Table 2**). Standard deviation was more robust to data aggregation than autocorrelation for the two scenarios considered although is decreased from $\Delta t = 10$ for the slowing-down to $\Delta t = 5$ for the driver-mediated flickering. Autocorrelation was less robust to data aggregation by conserving increasing trends for the slowing-down scenario up to $\Delta t = 2$ and only at $\Delta t = 1$ for the driver-mediated flickering. The higher robustness of standard deviation could originate from the different structure of the aggregated time series compared to the original one. It was likely to be due to the aggregation that merged successive high (or low) data (autocorrelation at lag-1 altered) whereas it can conserve the relative variability structure of the time series. This finding reinforces the interest of standard deviation as generic EWS for different scenarios thriving to a critical transition. Neither, skewness nor kurtosis met the criteria to be robust EWS at any level of data aggregation even for the original time series. Therefore, these two EWSs should be used with caution when applied to ecological time series because different patterns could be expected depending on the structure of the data. As a consequence, the use of a combination of EWSs is suggested to be needed to estimate confidently an approaching regime shift as well as to help at distinguishing between the different possible structures of palaeoecological time series (slowing-down vs. flickering) especially if directionality and extent of environmental fluctuations are unknown.

Ecological Opportunities

To date, reaching suitable ecological time series to perform EWS analyses still remain challenging. Few field data have highlighted changes in the statistical structure of time series prior to sudden shifts exhibited at different time scales. Carpenter et al. (2011) measured chlorophyll at a 5 min time intervals during 3 years in their whole-lake experiment and showed EWS from chlorophyll (change in spectral power) following largemouth bass addition one year prior the food web transition. Hewitt and Thrush (2010) reported an increasing temporal variability (flickering) in the composition of intertidal communities before a trophic and functional change to an alternative community type using bimonthly monitoring data over 20 years. At higher time scale, Beaugrand et al. (2008) identified an increase in the local variance of biological variables (e.g., phytoplankton, zooplancton) prior abrupt climate-driven ecosystem shifts in the North Atlantic using data at an annual scale over around 50 years.

Our results indicated that samples integrating up to 5 years could be suitable for applying EWS among the two scenarios considered (especially for standard deviation; **Table 2**). At higher time scales, only standard deviation for the critical slowing-down scenario appeared to be suitable, up to 10 year time sample, to identify changes in the statistical structure of a time series prior to a critical transition. The implicit assumption that one time unit obtained from the model was equivalent to 1 year could be a bit restrictive in different cases, for instance for plurivoltine species. Apart technical difficulties to reach higher resolution than annual resolution from sediment cores, many lacustrine species are nonetheless univoltine (annual recruitment) and or typically uni-seasonal such as diatoms, meaning that they are also recorded annually (Giguet-Covex et al., 2010). For populations and communities, EWSs can provide relevant information especially for abundant aquatic species whose past abundance can be tracked in sediment archives through biological remains such as chironomids (Frossard et al., 2013) or newly developed molecular analyses (ancient DNA, Savishcheva et al., 2011; Belle et al., 2014).

Species can be expected to intrinsically possess different indicative potential toward EWS analyses because of their different functional responses to environmental changes. For instance, Brodersen et al. (2004, 2008), who studied respiration of midges (Diptera; Chironomidae) in West Greenland and British Columbian lakes, found that species sustained their respiration along an oxygen gradient (forcing equivalent to c) in different ways following linear, break-point and cubic-like patterns. Oxygen being a primary environmental forcing for these species their different physiological respiration is likely to induce different patterns in EWS toward a population critical transition. Additionally, because a critical transition is especially expected in highly connected foodwebs (Scheffer et al., 2012), the position and interaction linking a species to the others could influence the indicative value of EWS for a specific species to the sustainability of the whole foodweb. As a consequence, apical species should be especially suitable to inform of a possible regime shift as they theoretically embed information of basal species (Sugihara et al., 2012). Luckily, they also usually exhibit longer (pluriannual) generation times allowing a fine match using palaeoecological records of their genuine dynamics.

The increasing anthropogenic forcings make the flickering scenarios plausible in the present and future times, especially driver-mediated flickering due to the increase of the number and the strength of forcings (Steffen et al., 2011). Our analyses showed that in this context, EWSs, especially standard deviation, were robust to consistent time aggregation ($\Delta t = 5$). Under highly noisy dynamics (noise-mediated) for which flickering can be induced by different causes (e.g., intrinsic and/or environmental stochasticity), EWS detection could be especially difficult (Contamin and Ellison, 2009; Perretti and Munch, 2012). Additionally, no linear dynamics could be triggered by changes in forcing combinations leading to regime shifts not preceded by EWSs before regime shift (Hastings and Wysham, 2010). Therefore, for the analyses of time series, EWSs should be coupled to nonlinear analyses (Hsieh et al., 2008; Ives and Dakos, 2012) to strengthen the robustness of palaeoecological interpretations.

Conclusions

The detectability of the EWS tested was shown to be robust to data aggregation usually found among high-resolution palaeoecological time series. Nonetheless, the use of a combination of EWSs associated with unbiased methods to estimate statistics of EWS trends such as block-based Kendall Tau should be encouraged to strengthen the robustness of identified patterns. Considered as resilience metrics, EWSs also offer promising decision tools to management ecologists to assess ecological vulnerability under external and internal forcings. They have also been shown to respond to other types of threshold dynamics (e.g., transcritical transitions; Kéfi et al., 2012; Hansen et al., 2013). Hence, their use is expected to be relevant in a large context of ecological dynamics.

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Responses of Aquatic Ecosystems to Environmental Changes in Finland and China

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The concern for the state of global freshwater reservoirs has increased due to deterioration of the water quality during the last decades. This has prompted monitoring and restoration efforts such as the European Water Framework Directive and the national-scale 2nd-investigation and monitoring of the water quality, water volume and biota resources in China. The challenge so far has been the determination of the "natural" state (reference conditions) of freshwater ecosystems. We used the sediment archives of five lakes and one brackish water embayment in Finland and China to assess the impact of selected variables of climatology, hydrology, nutrients, and changes in human population on these ecosystems during the last few centuries. The study sites represent catchment areas with varying land use. Despite the long distance between the sites and their different land-use characteristics, the direction and timing of changes during the last few centuries are well comparable between the high latitudes of Finland and the mid-low latitudes of China. This study reinforces the sensitivity of aquatic ecosystems to environmental change and underlines the usefulness of the palaeolimnological approach as a tool for determining reference conditions.

Keywords: palaeolimnology, european water framework directive, diatoms, lake management, land-use, eutrophication, climate effects

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INTRODUCTION

Water is essential to all life forms and thus its availability and quality are of first order importance. Although it is estimated that freshwaters cover only slightly over 3% of the earth's surface area, the estimated number of lakes is ca. 304 million (Downing et al., 2006). Freshwater lakes are of great value in water storage, flood prevention, and maintenance of biodiversity, in addition to which they are vital resources for settlement pattern, food production, recreation, and tourism (Henriksen et al., 1997). Lakes are dynamic systems that can vary on different time scales (e.g., inter-annual to millennial) due to autogenic processes and natural exogenic stress (e.g., Deevey, 1984). Anthropogenic impact has increased the magnitude and speed of change in freshwater ecosystems deteriorating the water quality drastically. Although humans have affected water quality of lakes for thousands of years (e.g., Renberg, 1990; Bradshaw et al., 2006; Räsänen et al., 2006; Guilizzoni et al., 2011), the most distinctive and intensive changes have happened since ca. 1850 after the period of major industrialization and increased agricultural activities (e.g., Bennion et al., 2004; Battarbee et al., 2011) and at ca. 1950 after the intensified use of artificial fertilizers (e.g., Räsänen et al., 2006). Moreover, it is expected that the growth of global population and the predicted climate warming will increase the pressure on lakes and their ecological state (Millennium Ecosystem Assessment, 2005). These changes will further challenge the protection of aquatic ecosystems and their use for ecosystem services (e.g., Moss et al., 2011; Jeppesen et al., 2012, 2014).

During the last decades, the concern for the state of global freshwater reservoirs has increased significantly resulting in Europe in the Water Framework Directive (WFD), a legislation to secure a good water quality for both surface and groundwater by the year 2015 (European Union, 2000). One of the key issues of the WFD has been the determination of "reference," "pristine," or "baseline" conditions for aquatic ecosystems against which the effects of anthropogenic activities can be measured to achieve the good ecological status required (e.g., European Union, 2000; Bennion and Battarbee, 2007; Bennion et al., 2011). A comparable action has also been undertaken in China, where a national-scale 2nd-investigation and monitoring of the water quality, water volume and biota resources was launched in 2002 including 2693 Chinese lakes within a framework of the Chinese Ministry of Science and Technology (Yang et al., 2010). The aim of this program is to understand the status of Chinese lakes and to improve the Chinese lake database. However, since aquatic ecosystems have been influenced by various anthropogenic pressures for already thousands of years (e.g., Battarbee and Bennion, 2011; Bouleau and Pont, 2015), the defining of the "reference state" can be very challenging. A number of approaches can be used to estimate "reference" conditions including, e.g., expert judgment, modeling, historical data or spatial surveys (Anderson et al., 1995; Bennion et al., 2011). According to the WFD, in the absence of long-term monitoring data, "reference" conditions can be derived using the palaeolimnological approach (Pollard and Huxham, 1998; European Union, 2000).

Palaeolimnological methods offer a powerful tool to reconstruct past environmental conditions and to provide information of the timing, magnitude, rate and direction of ecological change using sediment records (e.g., Smol, 2008). Although the palaeolimnological approach has been used extensively in the context of WFD (Bennion and Battarbee, 2007) it is still too infrequently used in aquatic and wildlife management practices (Saulnier-Talbot, 2015). A wide variety of biological proxies, palaeobioindicators, exist, including the physical remains of biota (e.g., diatoms, dinoflagellate cysts, cladoceran, chironomids, and plant macrofossils) and biochemical markers (e.g., plant pigments and lipids). Diatoms (Bacillariophyceae) are probably the most commonly used group in palaeolimnological analyses (Moser et al., 1996) as they can be identified to species level, they are usually present in diverse, numerically abundant assemblages (Charles et al., 1994), they have a short life cycle reacting fast to changes in their environment, and many species have narrow optima along different environmental gradients (Dixit et al., 1992). Thus, diatoms provide a wealth of environmental information of the environments they live in. They have proven to be a highly useful proxy for research questions such as presented in our study.

In this study we used the sediment archives of five lakes and one brackish water embayment in Finland and China (**Figure 1**) representing catchment areas with varying land use (forests, agriculture, and urban area) to assess the impact of selected variables of climatology (air temperature, precipitation), hydrology (lake-level), nutrient concentrations (total phosphorus, total nitrogen), chlorophyll-*a*, and changes in population on these ecosystems during the last few centuries. The sites comprise large environmental gradients spanning from the Chinese lowland temperate zone to the Finnish northern-boreal zone and from almost pristine catchments to heavily urbanized catchments with 40 million inhabitants (Lake Taihu, Qin et al., 2010). However, we hypothesize that regardless of the geographical location, the impact of similar catchment changes is comparable in the initial timing of lake change, but differs in magnitude and speed. We also hypothesize that the impact of an urban catchment on lake ecosystems is more severe and faster than that of agricultural catchments, the latter being more gradual and long-lasting, and that the impact of global climate warming is stronger at higher latitudes. The main questions to be answered by our study are (1) Are there any detectable changes in the aquatic ecosystems during the last ca. 150 years? If changes can be detected, what was the timing, magnitude, direction and speed of change? (2) Are the possible impacts of changes in agricultural practize, urban pressure and climate change on aquatic ecosystems comparable in Finland and China? (3) Is palaeolimnology well suited for defining "reference" conditions for aquatic ecosystems regardless of the geographical and environmental settings?

Excluding Jerisjärvi and partly Pyhäjärvi, the diatom assemblage data and sediment dates from all other sites have already been published elsewhere Weckström et al. (2004), Dong et al. (2006, 2008), Xiao et al. (2013), Chen et al. (2014), Wang et al. (2014), Cao et al. (2014), Deng et al. (2014), and Ventelä et al. (2011, 2015). In this study, however, the data of single sites is combined and compared in order to answer the research questions described above. This study is part of the Sino-Finnish project "Lakes in Trouble," funded by the Academy of Finland and the Chinese Academy of Sciences, aiming to elucidate the effect of climate change on threatened ecosystem services of eutrophied aquatic ecosystems.

MATERIALS AND METHODS

Pairs of freshwater lakes from Finland and China representing forest catchments, agricultural catchments, and urban catchments (**Figure 1**) were selected for palaeolimnological analyses. The definition of catchment type is based on the main land-use activity of the catchment area. The three catchment types were selected in order to get a larger gradient in the timing, speed and magnitude of change as natural

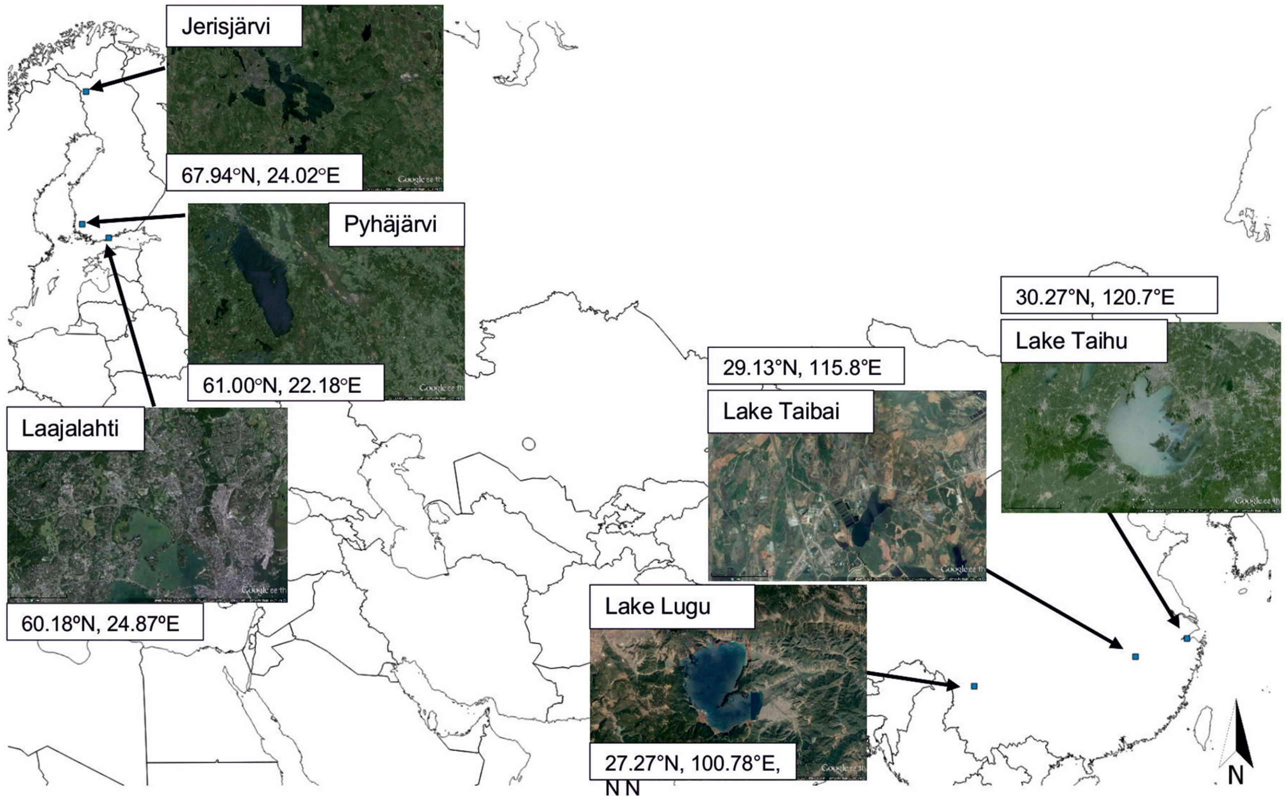


FIGURE 1 | Location of the study sites.

change (forest catchment) is often subtle occurring over centuries or millennia, whereas cultural impact (agricultural catchment) may be seen within decades or even years (urban catchment). The widely applied thresholds for the most distinctive and intensive human-induced changes on lakes (i.e., 1850 and 1950, see above) have been marked as dashed lines in **Figures 2**, **3** for comparison with our lake data.

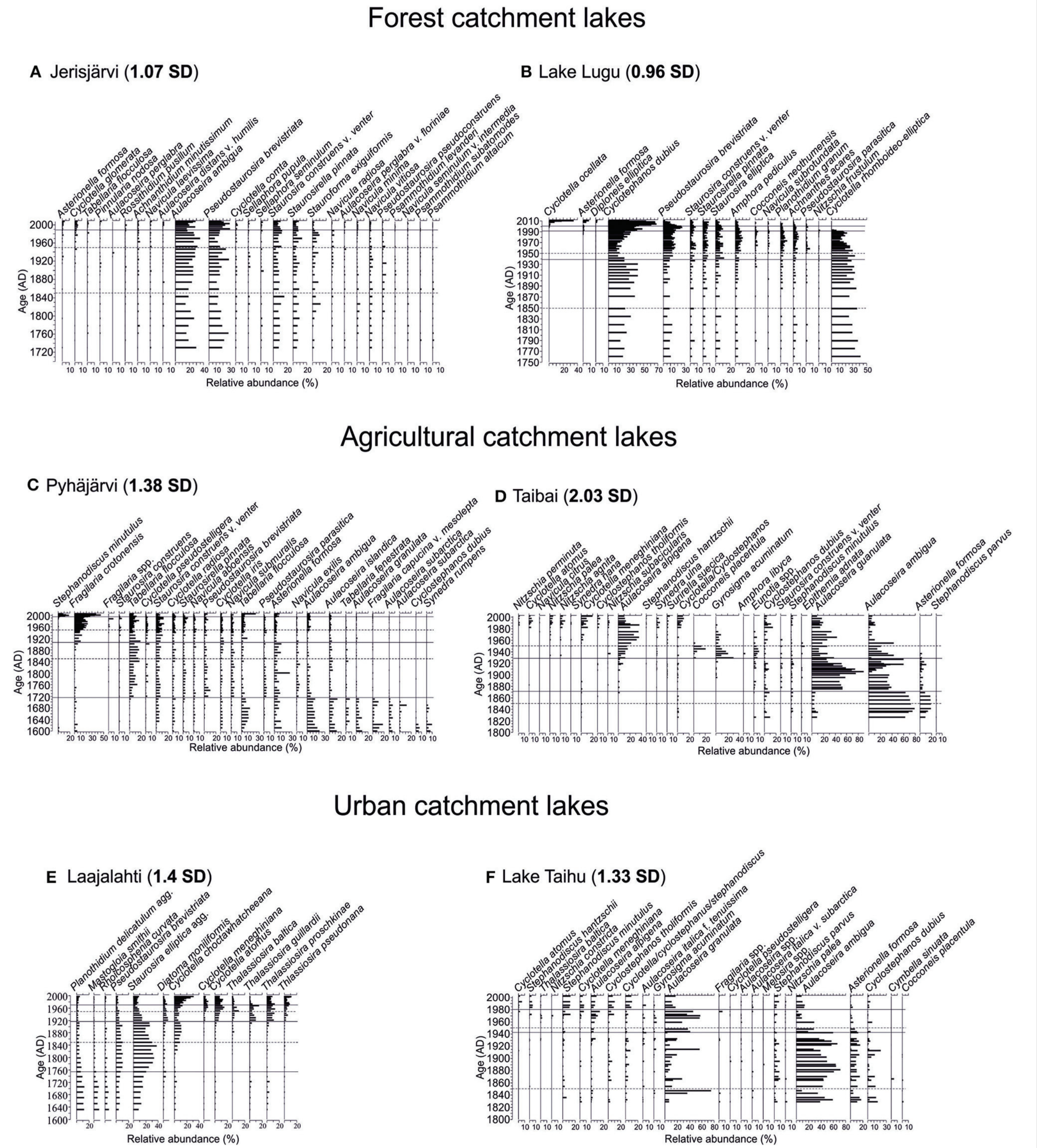
Forest Sites

Jerisjärvi (Finland)

Jerisjärvi (67.94°N, 24.02°E, 258 m a.s.l.) is located in northwestern Finland in the northern boreal zone (**Figure 1**). The lake has a surface area of 23.5 km^2, a maximum depth of 12 m and a mean depth of 3.4 m (OIVA database, 2015). Its catchment area is ca. 109 km^2 of which 83% constitutes of coniferous forests (Corine land cover data from European Environmental Agency). The mean annual average temperature (measured since 1947) decreased between 1947 and 1960 by ca. 2°C, but started to increase again from 1987 toward the present being currently −1.4°C (**Figure 3A**). The mean annual precipitation (measured since 1962) has increased from the beginning of the 1970s toward the present being currently 540 mm (**Figure 3A**; Supplementary 1). Autumnal (mainly August and September) total phosphorus (TP, measured since 1972) has decreased since the peak values of the 1970's, whereas summer TP (July) has slightly increased between 1972 and 1990 to similar levels than the present-day autumnal TP (**Figure 3A**). The lake is ice-covered for up to 8 months of the year (Supplementary 2). A sediment core of 10 cm was derived at a water depth of 4 m using a HTH-corer (Renberg and Hansson, 2008) in April 2007 and subsampled at 0.25 cm intervals representing a temporal resolution of ca. 1–15 years (Supplementary 3).

Lake Lugu (China)

Lake Lugu (27.27°N, 100.78° E, 2691 m a.s.l) is located in Ninglang County, Yunnan Province, southwestern China (**Figure 1**). The lake has a surface area of 48.25 km^2, a maximum depth of 93.5 m and a mean depth of 40.3 m. Its catchment area is ca. 246 km^2 of which 86% constitutes of forest. The mean annual average temperature (measured since 1921) was high between the mid-1920s and the beginning of 1940s, after which it decreased by almost 3°C until 1990. Since then, mean annual temperature has increased toward the present by ca. 1° C being at present 12.7°C (**Figure 3B**). The mean annual precipitation (measured since 1902) has decreased slightly since the 1920s, but more clearly during the last decade being at present 920 mm (**Figure 3B**) (Wang and Dou, 1998; Supplementary 1). Mean annual TP and total nitrogen (TN, measured since 1988) have increased since 1995 from <10 μg/l to 10–20 μg/l, and from ca. 120 μg/l to 120–200 μg/l, respectively (**Figure 3B**) (Wu et al.,



Forest catchment lakes

A Jerisjärvi (**1.07 SD**)

B Lake Lugu (**0.96 SD**)

Agricultural catchment lakes

C Pyhäjärvi (**1.38 SD**)

D Taibai (**2.03 SD**)

Urban catchment lakes

E Laajalahti (**1.4 SD**)

F Lake Taihu (**1.33 SD**)

FIGURE 2 | Stratigraphic diagrams of the most common diatom taxa. The years 1850 and 1950, usually referred to as the timing of most intensive change of aquatic ecosystems (e.g., Bennion et al., 2004; Räsänen et al., 2006) are marked with a dashed line. The statistically significant diatom zones are marked with a solid line. Beta-diversity values (in SD units) are shown in bold next to each site's name. The sites are arranged according to their catchment type: Forested catchments **(A)** Jerisjärvi, **(B)** Lake Lugu; Agricultural catchments, **(C)** Pyhäjärvi, **(D)** Lake Taibai; and Urban catchments, **(E)** Laajalahti, **(F)** Lake Taihu.

2008; Chen et al., 2014) (Supplementary 2). The lake is seasonally strongly stratified. A sediment core of 27.5 cm was derived near the central part of the southern Lake Lugu at a water depth of 41 m using a piston gravity corer in August 2010 and subsampled at 0.5-cm intervals representing a temporal resolution of 1–10 years (Chen et al., 2014).

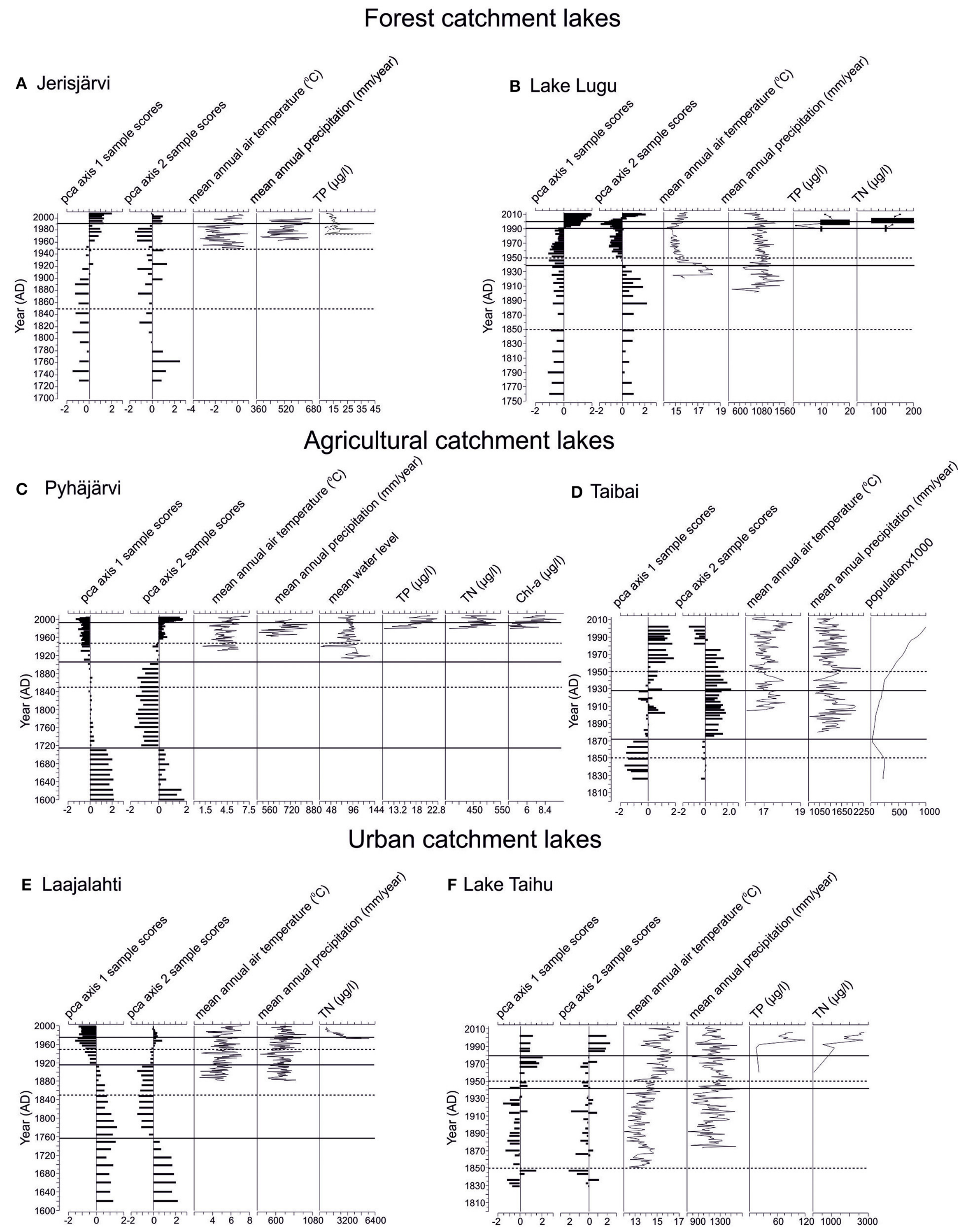


FIGURE 3 | Summary of the PCA 1 and 2 axis sample scores and documented values for annual mean air temperature, annual mean precipitation, water level changes, TP, TN, Chl-*a*, and human population, if available. The years 1850 and 1950, usually referred to as the timing of most intensive change of

(Continued)

FIGURE 3 | Continued
aquatic ecosystems (e.g., Bennion et al., 2004; Räsänen et al., 2006) are marked with a dashed line. The statistically significant diatom zones are marked with a solid line. The black bars indicate roughly the approximate variability of TP and TN values of Lake Lugu according to Whitmore et al. (1997), Wang and Dou (1998), Wu et al. (2008), and Yu et al. (2010). The sites are arranged according to their catchment type: Forested catchments **(A)** Jerisjärvi, **(B)** Lake Lugu; Agricultural catchments, **(C)** Pyhäjärvi, **(D)** Lake Taibai; and Urban catchments, **(E)** Laajalahti, **(F)** Lake Taihu.

Agricultural Sites

Pyhäjärvi (Finland)

Pyhäjärvi (61.00°N, 22.18°E, 45 m a.s.l.) is located in southwestern Finland (**Figure 1**) and is one of Finland's most extensively studied lakes due to its important role in supporting local ecosystem services. The lake has a surface area of 155 km^2, a maximum depth of 26 m and a mean depth of 5.5 m (OIVA database, 2015). Its catchment area is ca. 616 km^2 of which 22% constitutes of cultivated fields (Corine land cover data from European Environmental Agency). This area is one of the main food production areas in Finland, and agriculture as well as food processing industry is very intensive. Thus, the impact of cultivated fields on the lake is significant, although the forested and peatland areas cover a larger proportion of the catchment area than the agricultural area. The mean annual average temperature (measured since 1931) was relatively stable until the end of 1980s after which it started to increase being at present 4.8°C (**Figure 3C**). The mean annual precipitation (measured since 1931) has increased linearly toward the present from ca. 450 mm/year to ca. 650 mm/year (**Figure 3C**) (Supplementary 1). Annual water level measurements (measured since 1914) show distinct annual variability, but no clear trend throughout the measured series. Mean annual TP (measured since 1980) has increased from 1980 to ca. 2001 with some decline and stabilization since then. Mean annual TN (measured since 1980) slightly increased through the 1980s with relatively stable values or some decline since 1987, whereas the amount of chlorophyll a (Chl-*a*) increased through the 1990s with some decline and notable annual variations during the latest years (**Figure 3C**). The lake is ice-covered for ca. 5 months of the year (Supplementary 2). Two sediment cores were derived through the ice from the deepest part of the lake in March 2004 using a HON-Kajak-type corer (Renberg, 1991) for the most recent sediments (0-30 cm) and a modified Kullenberg corer (PP corer; Putkinen and Saarelainen, 1998) for the deeper sections (0–687 cm of which the uppermost 130 cm were used here). The HON-Kajak core was subsampled at 1 cm intervals representing a temporal resolution of 1–10 years (Ventelä et al., 2015), whereas the PP core was subsampled at intervals widening deeper down.

Lake Taibai (China)

Lake Taibai (29.13°N, 115.8°E, 16 m a.s.l) is located in Huanggang city, Hubei Province, in eastern China (**Figure 1**). The lake has a surface area of 25.1 km^2, a maximum depth of 3.9 m and a mean depth of 3.2 m. Its catchment area is 960 km^2 of which 70.4% constitutes of agricultural land. The mean annual average temperature (measured since 1905) has been relatively stable until ca. the mid 1990s after which temperature increased by ca 1°C until ca. 2007, but decreased toward the present being currently 16.7°C (**Figure 3D**). The mean annual precipitation (measured since 1880) has been relatively stable with a slight decrease since the 1970s toward present being currently 1273 mm (Wang and Dou, 1998; Supplementary 1). The water level is significantly impacted by precipitation. No measured nutrient data were available. However, according to Liu et al. (unpublished data) in Cao et al. (2014) the sedimentary TP values have increased since ca. 1650 and increased sharply again at ca. 1970, whereas according to Cao et al. (2014) a major increase in chironomid-inferred limnic TP values occurred since ca. 1940. The population in Huangmei County has grown between ca. 1350 and 1860 from ca. 100,000 to 400,000 inhabitants, after which a sharp decline occurred due to a series of peasant uprising by the Taiping Heavenly Kingdom. Since then the population has increased fast, especially since ca. 1950, being around 1 million at present (Cao et al., 2014) (Supplementary 2). A sediment core of 1.5 m was derived at the deepest part of the lake using a UWITEC sampling system in April 2004 and subsampled at 0.5 cm intervals for the topmost 50 cm representing a temporal resolution of 2–11 years and at 1 cm intervals for the sediment sequence below 50 cm (Dong et al., 2006).

Urban Sites

Laajalahti (Finland)

Laajalahti (60.18°N, 24.87°E, 0 m a.s.l) is a semi-enclosed urban embayment located in Helsinki, southern Finland (**Figure 1**). The embayment has a surface area of 5.3 km^2, a maximum depth of 4 m and a mean depth of 2.4 m. The water exchange of the embayment is restricted, as it is connected to the open archipelago only by two narrow straits and its average salinity is low (4.6 ‰). Its catchment area is ca. 44 km^2 of which 66% constitutes of constructed areas (Corine land cover data from European Environmental Agency). The mean annual average temperature (measured since 1882) is 5.9°C and has increased toward the present from ca. 4.5°C (**Figure 3E**). The mean annual precipitation (measured since 1882) has been relatively stable and is currently 655 mm (Supplementary 1). Mean annual TN (measured since 1972) has decreased drastically toward the present from its peak values at 1972 (**Figure 3E**). The embayment is ice-covered for ca. 4 months of the year. In the 1960s, Laajalahti was one of the most polluted coastal areas of southern Finland, receiving ca. 60 t yr^{-1} of total phosphorus, 300 t yr^{-1} of total nitrogen and 1000 t yr^{-1} of organic matter (measured as BOD$_7$) from a sewage treatment plant built in 1957. At present, the embayment receives only diffuse loading from two brooks discharging 11.6 t yr^{-1} of TN and 0.9 t yr^{-1} of TP (Clarke et al., 2006) (Supplementary 2). A sediment core of 90 cm was derived from the deepest part of the embayment using a Mackereth corer (Mackereth, 1969) in September 1998 and subsampled at 1 cm intervals representing a temporal resolution of 1–20 years (Weckström, 2006).

Lake Taihu (China)

Lake Taihu (30.27°N, 120.7°E, 3.1 m a.s.l) is located in the middle and lower reaches of the Yangtze River, Jiangsu Province, in the eastern China (**Figure 1**). The lake has a surface area of 2338 km^2, a maximum depth of 3.3 m and a mean depth of 2.1 m. Its catchment area is ca. 36500 km^2 of which 28.1% constitutes of constructed area. Although the constructed area does not comprise the major part of the catchment's surface area, the catchment area is heavily populated with estimated inhabitants of 40 million people (Qin et al., 2010). The mean annual average temperature (measured since 1850) has increased from ca. 1920 to 1960 but was lower between ca. 1985 and 1995, after which temperature has increased again toward the present being currently 16°C (**Figure 3F**). The mean annual precipitation (measured since 1873) has been relatively stable with higher values between ca. 1900 and 1920, lower values between 1920 and 1930, after which precipitation increased until the mid-1950s. Since then mean annual precipitation has decreased toward the present being currently ca. 1084 mm (**Figure 3F**; Supplementary 1). Depending on the reference (e.g., Zhang and Qin, 2001; Mao et al., 2009; Deng et al., 2014), the mean annual TP (measured since 1960) has varied substantially between $>20\,\mu$g/l to as high as 250 μg/l. In general, the values have increased until 1997, after which they have fluctuated, but decreased to ca. 100 μg/l at present. The differences in the measured TP values between the different references are most likely caused by the different water sampling points, as the northern part of lake Taihu is the most eutrophied part of the lake (e.g., Deng et al., 2014). The mean annual TN follows very closely the trend of TP described in Mao et al. (2009) with a drastic increase between 1992 and 1997, a decrease until 2002, after which it has increased again toward present (**Figure 3F**) (Supplementary 2). The lake is not stratified due its shallowness, large size and strong impact of wind. A sediment core of 50 cm was derived at a water depth of 2.5 m using a gravity corer in November 2002 and subsampled at 0.5 cm intervals representing a temporal resolution of 2–18 years (Dong et al., 2008).

Sediment Dating

Sediment samples were analysed for ^{210}Pb, ^{226}Ra, and ^{137}Cs by gamma spectrometry in the Environmental Radioactivity Laboratory of Liverpool University (Jerisjärvi, Laajalahti), the State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences (Lake Lugu, Lake Taibai), the Institute of Geological Sciences, Polish Academy of Sciences, Poland (Pyhäjärvi) and in the Bloomsbury Environmental Isotope Facility at the University College London (Lake Taihu). The radiometric dating chronologies were calculated using the Constant Rate of Supply (CRS) and the Constant Initial Concentration (CIC) ^{210}Pb dating models (Appleby and Oldfield, 1978).

Diatom Analyses

Diatoms were prepared using H_2O_2 digestion and HCl-treatment, and cleaned diatoms were mounted in Naphrax®. A minimum of 300 diatom valves from each sample were identified and counted along random transects at 1000x magnification for Jerisjärvi, Pyhäjärvi and Lake Taibai, whereas 600, 500, and 350 diatom valves were counted for Lake Lugu, Laajalahti, and Lake Taihu, respectively. However, due to dissolution of diatom valves in the northern parts of Lake Taihu only 100–200 valves from samples with dissolved and poorly preserved diatoms were counted. Diatom identification was based mainly on Krammer and Lange-Bertalot (1986, 1988, 1991a,b), Witkowski (1994), Qi (1995), Snoeijs (1993), Snoeijs and Vilbaste (1994), Snoeijs and Potapova (1995), Snoeijs and Kasperovièiene (1996), Snoeijs and Balashova (1998), and Witkowski et al. (2000). For more details concerning the procedures and taxonomic literature used, see Weckström et al. (1997a,b, 2004), Dong et al. (2006, 2008), Xiao et al. (2013), Chen et al. (2014), Wang et al. (2014), Cao et al. (2014), Deng et al. (2014), and Ventelä et al. (2015).

Data Analyses

All diatom counts were converted into percentages and plotted as a stratigraphical frequency diagram including only the most common diatom taxa (**Figure 2**), using the program C2 (version 1.7.2; Juggins, 2007). In order to identify periods of the most significant shifts in diatom assemblages constrained optimal sum of squares partitioning was used (Birks and Gordon, 1985). The number of statistically significant zones was calculated using the broken-stick model and the associated approach described in Bennett (1996). Optimal partitioning was implemented using the program ZONE 1.2 (Lotter and Juggins, 1991). Detrended canonical correspondence analysis (DCCA) was used to develop quantitative estimates of compositional turnover as beta-diversity, scaled in SD units (ter Braak and Verdonschot, 1995). The sample ages were added as the sole constraint in DCCA to estimate the total amount of compositional change in each biostratigraphic record. Differences in down-core DCCA sample scores provide an estimate of the amount of compositional change between samples along environmental or temporal gradients (Smol et al., 2005). Principal component analysis (PCA) was used in order to reveal the compositional changes in the biological data. DCCA and PCA were performed using CANOCO for Windows, version 5.01 (ter Braak and Šmilauer, 2007-2012). Time series of annual mean temperature, annual mean precipitation, annual mean water level, TP, TP, Chl-*a*, and population history, if available, were qualitatively compared with the first and second PCA axis sample scores. For more information on the methods used for deriving the environmental variables, see Supplementary 2.

RESULTS

Forest Sites

Lake Jerisjärvi

The diatom stratigraphy of Jerisjärvi consisted of 160 taxa comprising both planktonic and benthic diatoms. The diatom assemblages were divided into two statistically significant zones at ca. 1990 (**Figure 2A**). The diatom assemblages were relatively stable throughout the core dominated by *Aulacoseira ambigua* and *Pseudostaurosira brevistriata* (**Figure 2A**). The first subtle changes can be observed around 1970, when the relative occurrence of *Asterionella formosa* and *Cyclotella glomerata* increased and the proportion of *Aulacoseira ambigua* decreased. The statistically significant change, however, occurred around 1990, where also the proportion of *Pseudostaurosira brevistriata* increased and *Stauroforma exiquiformis* decreased. This change is also demonstrated by the PCA axis 1 and PCA axis 2 sample scores (**Figure 3A**). No distinctive changes can be seen in the diatom assemblages around 1850 or 1950.

Lake Lugu

The diatom stratigraphy of Lake Lugu consisted of 145 taxa comprising both planktonic and benthic diatoms. The diatom assemblages were divided into four statistically significant zones at ca. 2001, 1991, and 1939 (**Figure 2B**). Overall, the planktonic component was dominated by *Cyclostephanos dubius, Cyclotella rhomboideo-elliptica*, and *Cyclotella ocellata*, whereas the benthic assemblages constituted mainly of *Pseudostaurosira brevistriata* and *Staurosira elliptica* (**Figure 2B**). The first statistically significant change around 1939 is mainly driven by the decrease of *Cyclostephanos dubius*. The clearest change occurred around 1991, when *Cyclotella rhomboideo-elliptica* disappeared from the diatom record. Around 2001, the relative abundance of *Cyclotella ocellata*, *Asterionella formosa* and *Cyclostephanos dubius* increased clearly, whereas the abundance of *Pseudostaurosira brevistriata* decreased (**Figure 2B**). These changes are also clearly demonstrated by the PCA axis 1 and axis 2 sample scores (**Figure 3B**). No distinctive changes in the diatom composition can be seen around 1850 or 1950.

Agricultural Sites

Pyhäjärvi

The diatom stratigraphy of Pyhäjärvi consisted of 295 taxa comprising both planktonic and benthic diatoms (**Figure 2C**). The diatom assemblages were divided into four statistically significant zones at ca. 1994, 1905, and 1715 (**Figure 2C**). The first clear change occurred around 1715, where the amount of periphytic diatoms such as *Tabellaria flocculosa*, *Pseudostaurosira brevistriata*, *Navicula exilis*, and *Cyclotella pseudostelligera* increased and, e.g., *Aulacoseira ambigua, A. islandica, A. granulata,* and *A. subarctica* decreased (**Figure 2C**). A second clear change occurred around 1905, when *Fragilaria crotonensis* increased rapidly and *Tabellaria flocculosa* and *Staurosira construens* f. *venter* maintained high abundances despite the increase in planktonic species. Around 1994 the relative abundance of *Stephanodiscus minutulus* and *Fragilaria crotonensis* increased markedly, but their abundance decreased again during the last decade (**Figure 2C**). These changes are also clearly demonstrated by the PCA axis 1 and 2 sample scores (**Figure 3C**). No distinctive changes in the diatom composition can be seen around 1850 or 1950.

Lake Taibai

The diatom stratigraphy of Lake Taibai consisted of 98 taxa (Dong et al., 2006). The diatom assemblages were divided into three statistically significant zones at ca. 1928 and 1872 (**Figure 2D**). Planktonic species dominated throughout the core and benthic species were rare (**Figure 2D**). Before ca. 1872 the diatom assemblage was dominated by *Aulacoseira ambigua*, *Asterionella formosa*, *Aulacoseira granulata, Cyclotella meneghiniana*, and *Cyclostephanos dubius*. At ca. 1872 a sudden increase of *Aulacoseira granulata* occurred with maximum abundances up to 90%. Around 1928 a second clear change occurred in the diatom assemblages as epiphytic species like *Gyrosigma acuminatum* and *Cocconeis placentula* increased rapidly together with the planktonic *Aulacoseira alpigena* (**Figure 2D**). Since the 1970s small planktonic *Cyclotella* spp., which are favored by nutrient-enriched waters increased and epiphytic species decreased. The change in diatom composition can also be seen in the PCA axis 1 and 2 sample scores (**Figure 3D**). No distinctive changes can be seen in the diatom assemblages around 1850 or 1950.

Urban Sites

Laajalahti

The diatom stratigraphy of Laajalahti consisted of 226 taxa (**Figure 2E**). The diatom assemblages were divided into four statistically significant zones at ca. 1975, 1915, and 1756 (**Figure 2E**). The lower part of the core (ca. 1600–1915) was dominated by benthic diatom assemblages, characterized by small *Fragilaria* spp., but also included several other benthic taxa such as *Mastogloia smithii*, *Rhoicosphenia curvata,* and *Planothidium delicatulum* agg. (**Figure 2E**). The first clear change occurred around 1756 when the abundance of *Staurosira elliptica* increased. The second significant change at 1915 can be seen when the proportion of planktonic diatoms doubled to >40%. At the start of this phase the dominant *Fragilaria* spp. declined and several small planktonic diatoms, such as *Thalassiosira* spp. and *Cyclotella* spp. increased in abundance. There was a further increase in planktonic diatoms in the mid-1950s, characterized by an increase in the common eutrophic taxa *Cyclotella atomus* and *C. meneghiniana* (EDDI, 2001; MOLTEN, 2004). Around 1975 a decline in the eutrophic *Cyclotella meneghiniana* and *C. atomus* and a rise in the less eutrophic taxon *C. choctawhatcheeana* can be observed (**Figure 2E**). These changes are also clearly revealed by PCA axis 1 and axis 2 sample scores (**Figure 3E**). No changes in the diatom composition can be seen around 1850 and 1950.

Lake Taihu

The diatom stratigraphy of Lake Taihu consisted of 81 taxa (**Figure 2F**). The diatom assemblages were divided into three statistically significant zones at ca. 1980 and 1943 (**Figure 2F**). The core was dominated throughout by planktonic diatoms such as *Aulacoseira ambigua, Aulacoseira granulata*, *Asterionella formosa, Cyclostephanos* spp., and *Stephanodiscus* spp. with only a few benthic species. The first clear change in the diatom assemblages occurred around 1943 as the relative abundance of *Aulacoseira ambigua* decreased and that of *A. granulata* increased (**Figure 2F**). A second major shift occurred around 1980 as the proportion of *A. granulata* decreased and, e.g., *Cyclotella atomus, C. meneghiniana, Stephanodiscus hantzschii, S. minutulus*, and *Cyclostephanos tholiformis* increased. These changes can also be observed in the PCA axis 1 and 2 sample scores (**Figure 3F**). No changes in the diatom composition can be seen around 1850 and 1950.

Species Compositional Turnover

The compositional change of the diatom assemblages of the study sites was estimated as beta-diversity. Higher value indicates greater taxonomical change. The total range of the beta-diversity varies from 0.96 SD (Lake Lugu) to 2.3 SD (Lake Taibai) (**Figure 2**). Overall, the forested catchment lakes had the lowest beta-diversity values, whereas, except for Lake Taibai, the values for the agricultural catchment sites and the urban catchment sites were relatively similar.

DISCUSSION

Forest Sites

Jerisjärvi

The remote location of Jerisjärvi in the far north of Fennoscandia, an area with one of the lowest levels of atmospheric deposition in Europe for most environmental contaminants (e.g., sulfur and heavy metals, Hettelingh et al., 1991) and with low anthropogenic impact, has ensured that the lake has remained remarkably stable during the last few centuries (**Figures 2A**, **3A**). Only very minor changes in the diatom composition have occurred highlighting the stability of the aquatic ecosystem and environmental settings except for the last few decades. The relatively subtle change in the diatom composition but clearer change in the PCA axis 1 sample scores around 1990 can mainly be seen as an increase in the abundances of *Asterionella formosa* and *Cyclotella glomerata*. Since the beginning of the 1970s the mean annual precipitation has increased and the duration of the ice cover decreased by ca. 2 weeks (OIVA database, 2015). *Asterionella formosa* is favored by slightly elevated nutrient concentrations (e.g., Anderson et al., 1995), whereas *C. glomerata* is thriving in low TP concentrations (Anderson et al., 1997). The discrepancy between our data and these known ecological preferences could be explained by the observation that *A. formosa* and *C. glomerata* are favored by moderate N enrichment in oligotrophic alpine lakes (Saros et al., 2005; Saros and Anderson, 2015), suggesting that increased TN could have been the reason behind their increase in Jerisjärvi instead of TP. Also, the increase in air temperature (**Figure 3A**) may have strengthened the stratification, thus reducing the mixing of the water column favoring small and/or lightly silicified diatoms (like *C. glomerata* and *A. formosa*) with very slow sinking velocities. Another explanation might be a decrease in silica content as *A. formosa* and *C. glomerata* are lightly silicified species (Olsson et al., 1992).

Lake Lugu

The diatom assemblages of Lake Lugu consisted mainly of oligotrophic taxa before 1990 (**Figure 2B**). Since 1991 the relative abundance of *Cyclotella ocellata* and *Asterionella formosa* increased and *Cyclotella rhomboideo-elliptica* disappeared from the diatom record. *Cyclotella ocellata* is usually found in nutrient poor waters (e.g., Cremer and Wagner, 2003), whereas *Asterionella formosa* thrives in lakes with elevated nutrient concentrations (e.g., Anderson et al., 1995). *Cyclotella rhomboideo-elliptica* is favored by clear and deep-oligo-mesotrophic conditions (Li et al., 2007). This change in the diatom composition is likely caused by the increased mean annual air temperature as the increase in nutrients has been modest before 1995 due to the remote location of the lake, and has been more substantial only after 2003 due to increased tourism (Chen et al., 2014). Increased air temperature will strengthen the stratification, thus reducing the mixing of the water column. This, in turn, enables the increase of the relative abundance of *C. ocellata* and *A. formosa* as they are small diatoms with very slow sinking velocities (e.g., Canter and Lund, 1948) compared to the much bigger and heavier *C. rhomboideo-elliptica*. The effect of the increased nutrient concentrations since 2005 can be seen as a decrease in the abundance of *Pseudostaurosira brevistriata* and an increase in *Cyclostephanus dubius* (e.g., Wu et al., 2008; Chen et al., 2014). As the anthropogenic impact on Lake Lugu has been very modest before the last two decades, climate warming has most probably been the most important environmental variable affecting the lake (Chen et al., 2014).

Agricultural Sites

Pyhäjärvi

The planktonic and meroplanktonic diatom assemblages suggest that Pyhäjärvi was meso-eutrophic before the late 1600s. Since then anthropogenic water level decrease related to the establishment of an iron mill to the outlet river in the 1600s and to a major drawdown in the 1850s had a drastic impact on Pyhäjärvi resulting in lake oligotrophication between ca. 1700 and 1930 (Ventelä et al., 2015). This is supported by an increase of periphytic diatoms and a decline in the proportion of planktonic diatoms, which suggests a reduction in phytoplankton production and improved water clarity. Moreover, a connection of Pyhäjärvi with an aquifer (Rautio and Korkka-Niemi, 2011, 2015) might also have contributed to the oligotrophication. Between ca. 1930 and 1994 major changes in the environmental setting occurred: vendace (*Coregonus albula*) was introduced in the 1950s culminating in high vendace abundances since the 1960s (Sarvala et al., 1998), agriculture intensified due to the increased use of artificial fertilizers after the Second World War (Valkama et al., 2009), and the first signs of climatic change appeared in the early 1990s (Ventelä et al., 2011). Between 1980 and 1994, the measured TP increased from 11 μg/l to 19.3 μg/l. The increase in TP was reflected in a rapid increase of *Fragilaria crotonensis,* a species indicating eutrophic conditions. In 1995, the Pyhäjärvi Restoration Programme was initiated (Kirkkala, 2014) and the Finnish agri-environmental system was established, introducing new resources and tools aimed to reduce external loading. The vendace stock was weaker than earlier and commercial fishermen were (as from 1995) paid to fish also commercially less valuable fish species (smelt, roach, ruffe). Biomanipulation was further intensified after 2002 (Ventelä et al., 2007). These lake management efforts can be seen as decreased abundances of diatoms, which are favored by elevated nutrient levels.

Lake Taibai

Since the beginning of the 1600s the diatom composition in Lake Taibai has been fluctuating (Dong et al., 2006). The quantitatively inferred diatom-based TP values have varied between ca. 50 and

160 μg/l during the last four centuries, and a TP concentration of 50 μg/l has been suggested as a reference/background level of Lake Taibai (Dong et al., 2006; Cao et al., 2014). These values imply that the lake has not been truly oligotrophic for centuries, further supported by the fact that planktonic species have dominated throughout the core and benthic species have been rare (**Figure 2D**). The first change in the diatom assemblages occurred at ca. 1872 as *Aulacoseira granulata* increased suddenly with a relative occurrence up to 90% and *A. ambigua* decreased (**Figure 2D**). This might have been caused by a slight increase in nutrient concentrations or due to increased turbulence as *A. granulata* is more heavily silicified than *A. ambigua* and is more dependent on the mixing of the water column than *A. ambigua*. At ca. 1928 a second clear change occurred in the diatom assemblages as epiphytic species like *Gyrosigma acuminata*, *Eunotia pectinalis* and *Cocconeis placentula* increased rapidly together with the planktonic *Aulacoseira alpigena* (**Figure 2D**). As the epiphytic taxa thrive in shallower environments and *A. alpigena* is distinctly smaller than *A. granulata* and *A. ambigua*, this change might have been due to a decrease in water level. Indeed, according to Liu et al. (2007) and Xiao et al. (2013) the water level has decreased significantly in the 1950s due to land reclamation. Another clear change occurred in the 1970s as small planktonic *Cyclotella* spp., *Cyclostephanos* spp., and *Stephanodiscus* spp., which are favored by nutrient-enriched waters increased and epiphytic species decreased (**Figure 2D**). This change is highlighted also by the significant drop in the PCA axis 2 sample scores (**Figure 3D**). According to Dong et al. (2006), Xiao et al. (2013), and Cao et al. (2014), the nutrient levels of Lake Taibai have increased significantly after the 1950s, and increased even more drastically during the 1970s, reaching values up to 120 μg/l (Dong et al., 2006). Since the 1950s the mean annual air temperature has increase slightly, but the mean annual precipitation instead decreased. The impact of climate change on the lake has been larger before 1900, but after the 1950s has been overridden by the anthropogenic impact. The increase of inhabitants in the catchment area of Lake Taibai during the last 60 years from ca. 400,000 to over a million (Cao et al., 2014) and the intensified agriculture accompanied with large-scale irrigation systems (Xiao et al., 2013) has had a negative impact on the development of the water quality of Lake Taibai.

Urban Sites

Laajalahti

The first notable change in the diatom assemblages occurred in the mid-1700s (**Figures 2E**, **3E**), which can be seen as an increase in *Pseudostaurosira elliptica* agg., *P. brevistriata* and a decrease in the abundances of *Mastogloia smithii* and *Planothidium delicatulum* agg. The predominance of *Fragilaria* spp. is typical for shallow and semi-enclosed coastal embayments as well as recently isolated lakes (Stabell, 1985). The water depth of Laajalahti has decreased over time due to post-glacial isostatic land uplift (at present ca. 0.3 cm yr-1 in the study area) and it may have reached a threshold during the mid-18th century, after which conditions were favorable for small, benthic *Fragilaria* taxa. The largest change in diatom assemblages occurred around 1915, when the proportion of planktonic diatoms doubled to >40% coinciding with a rapid expansion of the urban area (Laakkonen and Lehtonen, 1999). In the mid-1950s, two waste water treatment plants were installed in the watershed of Laajalahti. However, these treatment plants only removed organic matter from sewage (Weckström et al., 2004), which resulted in an increase of the relative abundance of the common eutrophic taxa *Cyclotella atomus* and *C. meneghiniana* (EDDI, 2001; MOLTEN, 2004). In the early 1970s a clear response to improved waste water purification can be seen as a decreased abundance of meso-eutrophic planktonic taxa. In 1986 a further marked reduction in the nutrient load occurred, when the last treatment plant was closed. In response, the eutrophic *Cyclotella meneghiniana* and *C. atomus* declined and the less eutrophic taxon *C. choctawhatcheeana* increased (**Figure 2E**). Although a clear recovery of Laajalahti could be observed after the cessation of waste water loading, there is still a marked difference between the diatom assemblages of the lower part of the core compared to the present diatom assemblages with no sign of a recovery back to more diverse benthic communities.

Lake Taihu

The first notable changes in the diatom assemblages of Lake Taihu can be seen between around 1943, when the dominant species changes from *Aulacoseira ambigua* to *A. granulata* (**Figure 2F**). Both species are favored by turbulent conditions as they are heavily silicified and thus have a high sinking rate (Bradbury, 1975) and thrive in meso- to eutrophic shallow lakes (e.g., Manoylov et al., 2009). Although Lake Taihu has been categorized as oligotrophic before 1960 (Qin et al., 2007), these two *Aulacoseira* taxa, and also the presence of *Asterionella formosa*, which is favored by elevated nutrient concentrations (e.g., Anderson et al., 1995), suggests that the lake has been mesotrophic at least since the 1800s. This finding is in line with, e.g., Dong et al. (2008), who inferred limnic TP values around 50 μg/l before 1900, using a diatom-based quantitative inference model. As precipitation increased slightly during this period the change between *A. ambigua* and *A. granulata* might have been caused by small increase in nutrient concentrations via increased in-wash from the catchment, or increased turbulence as *A. granulata* is heavier than *A. ambigua*. A second major shift occurred around 1980 as the proportion of *A. granulata* decreased and, e.g., *Cyclotella atomus, C. meneghiniana, Stephanodiscus hantzschii, S. minutulus*, and *Cyclostephanos tholiformis* increased. These taxa are known indicators of nutrient-rich waters (e.g., Bradshaw et al., 2002; Dong et al., 2006). This change is caused by the rapid increase in the population density and an increase of in-washed waste water (e.g., Qin et al., 2007). In the 1980s and 1990s the amount of domestic pollution doubled, whereas the input of industrial TP decreased to one third (Qin et al., 2007). This change reflects rapid economic growth and land use alteration. As there are no clear changes in temperature and precipitation at this time, the main cause behind this marked aquatic change in Lake Taihu can be addressed to anthropogenic activity. This is also supported by the measured TN and TP records, which increased clearly in the beginning of the 1990s remaining high until present (e.g., Mao et al., 2009; Deng et al., 2014). The water quality of Lake Taihu has deteriorated significantly from class I before 1970 to class V in the late 1990s (State Environmental Protection Administration, 2000; Dong et al., 2008).

Changes in Aquatic Ecosystems Surrounded by Different Land-use in Finland and China

Clear changes, especially in aquatic ecosystems under anthropogenic pressure were observed. The lakes surrounded by a forested catchment area have remained relatively pristine until the last few decades. Although nutrient concentrations have increased slowly, especially in Lake Lugu, the impact of climate warming and related variables (e.g., changes in stratification and ice cover) seem to be the driving mechanism behind changes in the diatom assemblages of both lakes (**Figures 3A,B**). This is not surprising, as both lakes are located relatively remotely suffering only from minor human disturbance (**Figure 1**). The relatively pristine condition of these lakes is also supported by the lowest beta-diversity values of all lake pairs. The lakes impacted by an agricultural catchment have experienced more changes due to the elevated impact of anthropogenic activities. Both lakes have undergone an artificial water-level decrease, which has altered the diatom composition toward benthic and small-sized planktonic taxa. Climate change has also impacted both lakes, however, a clearer response can be seen in the high latitude Lake Pyhäjärvi, whereas anthropogenic activities have overridden the climate signal in Lake Taibai (**Figures 3C,D**). This lake had also the highest beta-diversity value of all lakes suggesting a significant overall change in the diatom assemblages. Also the aquatic ecosystems surrounded by an urban catchment have undergone several changes related to anthropogenic activities. The changes differ both in time and magnitude as Laajalahti has been affected by human disturbance since the 1920s, whereas Lake Taihu has changed distinctively since the 1980s. As the catchment of Lake Taihu is heavily constructed comprising ca. 40 million inhabitants, the anthropogenic pressure on Lake Taihu is much stronger compared with Laajalahti. This can be seen as a drastic deterioration of the water quality and a drinking water problem (Qin et al., 2010). However, the beta-diversity values are very similar suggesting, that although the anthropogenic stress on Lake Taihu is clearly stronger than in Laajalahti the magnitude of changes in their diatom assemblages is comparable. This points toward an insensitivity of diatom assemblages (no further change in species composition and life forms) at very high nutrient concentrations, which has been discussed previously in, for example, Weckström et al. (2007).

The changes in aquatic ecosystems surrounded by the same catchment type are rather similar regardless of their geographical setting. This suggests that their response to external pressure is comparable. The changes between different catchment types, however, differ in magnitude and timing. Aquatic ecosystems situated in relatively pristine conditions change slowly due to the lack of anthropogenic pressure. The aquatic ecosystems surrounded by human activities, instead, change within years, especially if located in areas with intensive agricultural activities or densely populated urbanized areas.

CONCLUSIONS

Palaeolimnology has been used for decades to reconstruct past environmental conditions, and in the framework of WFD can be used to define "reference" conditions in the absence of long-term monitoring data. However, many of these studies have been spatially restricted. Here were demonstrate the usefulness of the palaeolimnological approach regardless of the different geographical and environmental settings of sites. We used relatively time- and cost-efficient fossil diatom analysis from six radiometrically dated sediment series derived from aquatic ecosystems representing catchment areas with varying land use (forests, urban areas, and agriculture) from Finland and China. By using simple multivariate statistics (DCCA, PCA) we were able to demonstrate the time, magnitude and direction of changes of the study sites, and relate them to different environmental variables. The observed changes in diatom assemblages differed both spatially and temporally and are mostly related to anthropogenic impact and climate forcing. The first distinct changes in the Finnish sites were observed around 1715 in the agricultural site due to an artificial anthropogenic water level decrease, around 1915 in the urban site due to the increasing population of the catchment area, and around 1989 in the forested site due to climate change and due to slightly increased anthropogenic pressure. In China, first clear changes in the diatom assemblages were observed since 1870 in the agricultural site and around 1943 in the urban catchment due to the increased land use activities and dramatic increase in population density especially since the 1980s. First subtle changes in the forested site can be seen around 1939 followed by more marked changes since 1991 due to increased air temperature and tourism.

Despite the long distance between the sites and their different land-use characteristics, the direction, magnitude and timing of changes during the last 150 years are well comparable between the high latitudes of Finland and the mid-low latitudes of China. This study reinforces the sensitivity of aquatic ecosystems to climate change and anthropogenic impact and underlines the usefulness of the palaeolimnological approach as a tool for determining reference conditions for these markedly changed systems.

We also show that the commonly suggested threshold for reference conditions at ca. 1850 for, especially, European lakes and the second major change in aquatic ecosystems at ca. 1950 after the increased use of artificial fertilizers do not necessarily apply to aquatic environments with differing geographical and environmental settings. Thus, instead of relying on such generalizations of the environmental history of aquatic ecosystems, it is of vital importance to assess individual sites using palaeolimnological methods before deciding on management and restoration goals.

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SUPPLEMENTARY MATERIAL

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REVIEW
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High-resolution paleolimnology opens new management perspectives for lakes adaptation to climate warming

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Varved lake sediments provide opportunities for high-resolution paleolimnological investigations that may extend monitoring surveys in order to target priority management actions under climate warming. This paper provides the synthesis of an international research program relying on >150 years-long, varved records for three managed perialpine lakes in Europe (Lakes Geneva, Annecy, and Bourget). The dynamics of the dominant, local human pressures, as well as the ecological responses in the pelagic, benthic, and littoral habitats were reconstructed using classical and newly developed paleo-proxies. Statistical modeling achieved the hierarchization of the drivers of their ecological trajectories. All three lakes underwent different levels of eutrophication in the first half of the XXth century, followed by re-oligotrophication. Climate warming came along with a 2°C increase in air temperature over the last century, to which lakes were unequally thermally vulnerable. Unsurprisingly, phosphorous concentration has been the dominant ecological driver over the last century. Yet, other human-influenced, local environmental drivers (fisheries management practices, river regulations) have also significantly inflected ecological trajectories. Climate change has been impacting all habitats at rates that, in some cases, exceeded those of local factors. The amplitude and ecological responses to similar climate change varied between lakes, but, at least for pelagic habitats, rather depended on the intensity of local human pressures than on the thermal effect of climate change. Deep habitats yet showed higher sensitivity to climate change but substantial influence of river flows. As a consequence, adapted local management strategies, fully integrating nutrient inputs, fisheries management, and hydrological regulations, may enable mitigating the deleterious consequences of ongoing climate change on these ecosystems.

Keywords: vulnerability, mitigation, lakes, varve sediments, climate change, management

Introduction

Lake ecological states and services are compromised by the multiple local human activities they host, and by the complex effects of climate change. Targeting priority management actions requires deciphering which of these environmental pressures are actually responsible for deleterious ecological alterations, while current policies and practices, designed to improve the ecological status of freshwater ecosystems, need to be modified in light of projected future climate change (ter Heerdt et al., 2007; Ulen and Weyhenmeyer, 2007; Reyjol et al., 2014). Such purposes imply that we can assess how current and future climate inflects lake ecological trajectories.

The question of the consequences of climate change on lakes has been intensively tackled over the last decade (Kernan et al., 2010; Dokulil, 2014 for syntheses), with a dominant focus on the detection of common trends by which lakes respond physically, chemically, and biologically to atmospheric warming. Indeed, many of the lake physical responses (increase in surface water temperature, stratification length, timing of ice out) are vertically and spatially coherent across large geographical distances (Livingstone and Dokulil, 2001; Dokulil et al., 2006; Livingstone et al., 2010) while the amplitude of the actual effects shall vary according to geomorphological parameters, such as altitude, shading, surface area, and depth (Fee et al., 1996; Livingstone and Padisak, 2007; Dokulil, 2014). Regional coherence in the lake chemical responses to climate change is somewhat lower (Dokulil and Teubner, 2002; Livingstone et al., 2010) as chemical consequences of climate variability (pH, nutrient concentrations, oxygenation) are modulated by the catchment geological and hydrological characteristics (Schindler et al., 1996; Webster et al., 1996; Blenckner, 2005). For biological parameters, climate change triggers complex cause-effects chains (Shurin et al., 2012), the link between them provided by many interacting parameters that already responded individually to climate change (Woodward et al., 2010). Even though some common biological features have been observed between lakes (Smol et al., 2005; Burgmer et al., 2007), climate-induced changes of the lake biota remain highly idiosyncratic even for lakes within the same catchment (De Stasio et al., 1996; Dokulil, 2014). Overall, biological responses to climate change are less predictable than the response of chemical or hydrological variables (Hering et al., 2010). Even within a single lake, climate-induced changes in water temperature, and in turn in chemical parameters, might not be as pronounced between deep and surface waters, or even occur in opposite directions at different depths (Magnuson et al., 1997; Danis et al., 2004). Therefore the magnitude of the biotic responses to climate change might also be highly variable according to different habitats (surface, deep, or littoral areas). Such uncertainties on lake biodiversity responses to climate change obviously limit forecasting the future of lake ecosystem services (Reyjol et al., 2014).

Although considerable progress has been made in understanding lakes responses to climate change, long-term ecological changes within anthropogenized lakes are probably those for which knowledge is lacking the most. For those systems that have been, and still are, strongly influenced in other ways by human activities over recent decades and last centuries, it is doubly difficult, although vital, to hierarchize the actual contribution of climate change as compared to other, more local, environmental stressors (Leavitt et al., 2009). For instance, nutrient inputs to lakes, from single point or atmospheric sources, and climate change, have evolved synchronously over the last century. Their respective contributions on lake functioning is hardly discernable since both can trigger increasing lake productivity by direct effects on the length of the growing season or indirect effects by altering the bottom-up and top-down control of the food web structure (Jeppesen et al., 2005). The parallel temporal dynamics of human perturbations make difficult to unravel the individual effect of each one in generating eutrophication symptoms (Wolfe et al., 2001; Jeppesen et al., 2005) and the mechanistic pathways by which they act. Instrumental data-series are usually too short to deal with so many degrees of freedom and to attribute unambiguously observed changes to any local and/or climate factors (Leavitt et al., 2009). In addition, lake ecological responses to climate change are so context-dependent that conclusions holding for one lake that has benefited from long-term monitoring can hardly be extrapolated to its neighbor (Dokulil, 2014). Controlled experiments have proven successful in distinguishing interacting effects between nutrient inputs and water warming (Moss et al., 2003; Shurin et al., 2012). Their outcomes suggest, for instance, that controlling nutrient inputs to lakes could help mitigating the impact of climate change (Battarbee, 2010). Yet, they relied on simple systems that necessarily minimize interactive mechanisms occurring at larger temporal and spatial scale. The realism and relevancy of the mechanisms hindcast by such controlled experiment have then to be confronted to long-term observational data (Battarbee, 2010). Paleo-records have provided compelling evidence that current climate conditions have triggered unprecedent ecological changes in high latitudes or altitudes lakes, i.e., ecosystems remote from direct human disturbances (Battarbee, 2010). Yet, for most other freshwaters ecosystems situated in populated regions, the risk of circular reasoning (*sensu* Battarbee, 2010) and the low accuracy and temporal resolution of the sediment records at sub-decadal time scales limit the possibility of disentangling the consequences of climate change from those of local human activities (Battarbee, 2010).

An international research program (which acronym was IPER-RETRO) has been conducted between 2009 and 2013 to precisely tackle the question of the ecological vulnerability of temperate lakes within populated catchments to climate change (i.e., how and how much they have been responding to climate change), for which this paper provides a synthesis of the main outputs. IPER-RETRO relied on the three following hypotheses:

1. Climate warming has overpassed local human activities in driving the ecological trajectories of lakes; i.e., *lakes within populated watersheds are vulnerable to climate change*.
2. Because of interactions between local human activities and climate warming, lakes ecological responses to a similar climate variability depend on the intensity of the local forcings:

lake vulnerability to climate change is conditioned by local forcings.

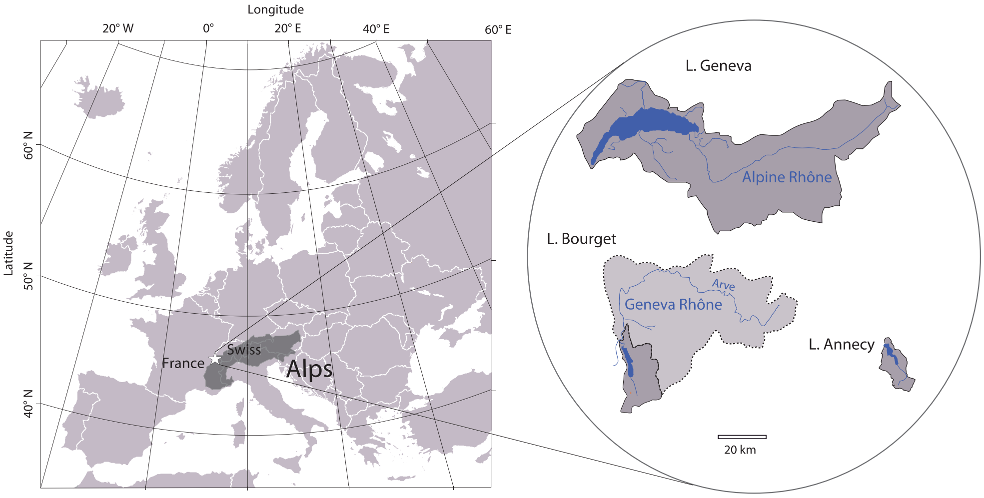
3. *Lake habitats are not equally vulnerable to climate warming.*

Overall, the findings of this research can help providing new perspectives for lake managers (stakeholders) to preserve and orientate lakes ecological trajectories under climate change and promote ecosystem services.

General Rationale

The program was designed in order to evaluate how variations in the intensity of local forcings can affect lake responses to similar climate variability, by comparing the ecological trajectories of different lake habitats for three lakes (Geneva, Annecy, and Bourget, **Figure 1**).

These three subalpine lakes have been carved by the same glacial-tectonic processes 12,000 years ago from which they inherited a comparable geomorphology of large, deep and U-shaped basins as well as a similar geochemical calcareous substratum. All located within 70 km from each other at a similar altitude, they are submitted to a comparable climate variability for which long-term local meteorological records were available (HISTALP, Auer et al., 2007). Otherwise, it was expected that geomorphological similarities minimize differences in their thermal responses to climate change. They also share a common human history over the last 150 years and therefore endured a similar combination of local forcings over time, yet at different intensities. The human population density has increased 3- to 5-fold from the late XIXth century and has triggered eutrophication from the 1930's to the mid-1970's. Maximum Total Phosphorus concentrations (TP) yet differed between lakes but phosphorus inputs from their watersheds have then been successfully abated in the second half of the XXth century and onwards. They host similar fish communities that are yet managed according to slightly different management strategies (Alric et al., 2013).



Parameters	Lake Annecy	Lake Geneva	Lake Bourget
Maximum depth (m)	65	309	147
Total lake area (km^2)	27	582	45
Drainage basin area (km^2)	273	7395	560
Drainage basin area to the total lake area	10.1	12.7	12.4
Volume (km^3)	1.12	89	3.6
River discharge (outlet, m^3.s^{-1})	8	244	15 (+ 30-40 days.yr^{-1} of flow inversion)
Renewal rate (yr)	4	12	8

FIGURE 1 | Lakes location and geomorphological characteristics.

The initial hydrology of their watershed and additional human activities resulted in different flood frequencies for each lake (Jenny et al., 2014a,b). They have been routinely monitored over decadal (Annecy, Bourget) or pluri-decadal time scales (Geneva, **Table 1**). In addition, all three lakes have annually laminated sediments (Jenny et al., 2014a), therefore allowing high-resolution dating (annual up to tri-annual time-steps that were relevant for ecological purposes) and confident correlation between multiple and spatially distributed cores within a single lake (about 150 cores per lake and up to 3000 dated samples; see Jenny et al. (2014a) for methodological details).

All these lakes characteristics promoted a process-oriented comparative design in which annually-dated paleo-records and long-term monitoring data were combined to reconstruct, over two centuries, (i) the dynamics of climate (monitoring data) and local forcings (TP, fisheries management practices, and river discharge; paleo-records calibrated against available monitoring data) and (ii) the multiple biotic (diatoms, cladocerans, cyanobacteria, chironomids) and geochemical (hypoxia, carbon cycling) changes occurring in the pelagic, benthic and littoral habitats (paleo-records). Such reconstructions for local forcings and responses were based on classical and/or newly developed paleo-proxies (**Table 1**). Finally, the respective contribution of climate change vs. local forcings in driving observed ecological changes, such as the time-period at which they contributed to these changes, could be hierarchized, based on Generalized additive models (GAM, Simpson and Anderson, 2009).

TABLE 1 | Summary of neo- and palaeo-ecological data used to infer the dynamics of climate and local forcings and ecological trajectories of lakes Geneva, Annecy, and Bourget over the last 200 years.

Environmental forcings		**Paleo-proxy**	**Available monitoring data**
Climate	Air temperatures		HISTALP (1850–2007) Météo-France (1960)
Local human forcings	Lake TP concentrations (annual averages over 0–20 m depths)	Diatom-inferred TP (Berthon et al., 2013) *Daphnia*-inferred TP* (Berthon et al., 2014)	Geneva (1958–2010) Annecy (1969–1981;1992-) Bourget (1970–1972;2003-)
	Management-induced predation pressure on zooplankton	Size of *Daphnia* post-abdominal claws* (Alric et al., 2013)	Zooplanktivorous fish catches Geneva (1900-) Annecy (1967-) Bourget (1920-)
	Volumes and frequencies of floods from dominant tributaries	Flux of terrigenous elements (Jenny et al., 2014a) 3D distribution of flood event deposits (Jenny et al., 2014b)	Geneva (1800–2010) Annecy (1800–2010) Bourget (1800–2010) Bourget (1700–2010)
Ecological lake trajectories		**Paleo-proxy**	**Available monitoring data**
Community structures	Planktonic diatoms	Diatoms macro-fossil remains (Berthon et al., 2013, 2014)	Geneva (1977-)
	Cladocerans	Cladoceran macro-fossil remains (Alric and Perga, 2011; Alric et al., 2013)	Geneva (1977-)
	Total cyanobacteria and Planktothrix abundances and taxonomic composition	Q-PCR & Sequencing* on 16SrRNA gene +ITS; mcyA gene; Phycocyanine operon (Savichtcheva et al., 2011, 2014)	Bourget (2000–2010) Geneva (1977-) Annecy (1992-)
	Pico-cyanobacteria abundances and taxonomic composition	Q-PCR Sequencing* on 16SrRNA gene +ITS (Domaizon et al., 2013)	
	Littoral and benthic chironomids	Spatially structured chironomid assemblages (Frossard et al., 2013, 2014)	
	Daphnia gene flow	Micro-satelitte analyses of *Daphnia* resting eggs (Alric, 2012)	
Geochemistry	Carbon cycling	δ^{13}C of sub-fossil remains (Perga, 2010, 2011; Frossard et al., 2014, 2015)	
	Hypoxic volumes	3D Distribution of laminated sediments (Jenny et al., 2013, 2014a)*	Geneva (1958-)

**Pinpoints paleo-proxies that were newly developed and validated within the program. Underlined data were provided by © SOERE OLA-IS, INRA Thonon-les-Bains, CISALB, CIPEL, SILA, [02-02-2014], developed by Eco-Informatics ORE INRA Team.*

Environmental Context

Lakes Location and Hydrology

Lakes Geneva, Bourget, and Annecy are young lakes originating from the last deglaciation, located on the northwest edge of the French Alps (**Figure 1**). They belong to a similar hydro-eco-region. They are large, deep temperate lakes (i.e., free from ice during winter) with a relatively fast water renewal rate (**Table 1**).

However, the three lakes present contrasting hydrological conditions because water and sediment supplies from the river systems differ greatly among the three lakes. The supplies are the highest from the large "Alpine-Rhône," with a mean annual discharge of 181 m^3 s^{-1}, which flows directly into Lake Geneva. These supplies are lower from the "Geneva-Rhône," downstream of Lake Geneva, which flows at a rate of from 10 to 100 m^3 s^{-1} and discharges into Lake Bourget through the Savière Channel only during major flooding events (Giguet-Covex et al., 2010). Lake Annecy is only supplied by small local tributaries. Hydrological differences between lakes are well mirrored by outlet flows and the ratio of the drainage basin area to the total lake area (Meybeck, 1995) confirmed the differences in the local river inputs for the three lakes (**Figure 1**).

The Dynamics of Climate and Local Forcings over the Last 150 Years

Climate Warming and Physical Responses from the Water Column

Consistently to the trends observed for the Greater perialpine region (Beniston, 2006), air temperature over the three studied lakes has increased stepwise by almost 2.0°C during the XXth century (**Figure 2A**), with a first warming period between 1940 and 1960 and a second one in the early 1980s (Auer et al., 2007; Alric et al., 2013). Comparison of the data extracted from HISTALP to local meteorological monitoring (Meteo-France 1960–2008) confirmed they adequately captured the interannual variability and trends in air temperatures. This pronounced temperature increase is due to the well-documented northward shift of the subtropical high-pressure system (Auer et al., 2007). Since no trend was observed for mean annual precipitation on the three lakes (**Figure 2A**), only mean air temperature was used in the following and we therefore refer as to "climate warming."

Water temperature monitoring (**Table 1**; Data © SOERE OLA-IS, INRA Thonon-les-Bains, CISALB, CIPEL, SILA, developed by Eco-Informatics ORE INRA Team) covered >30

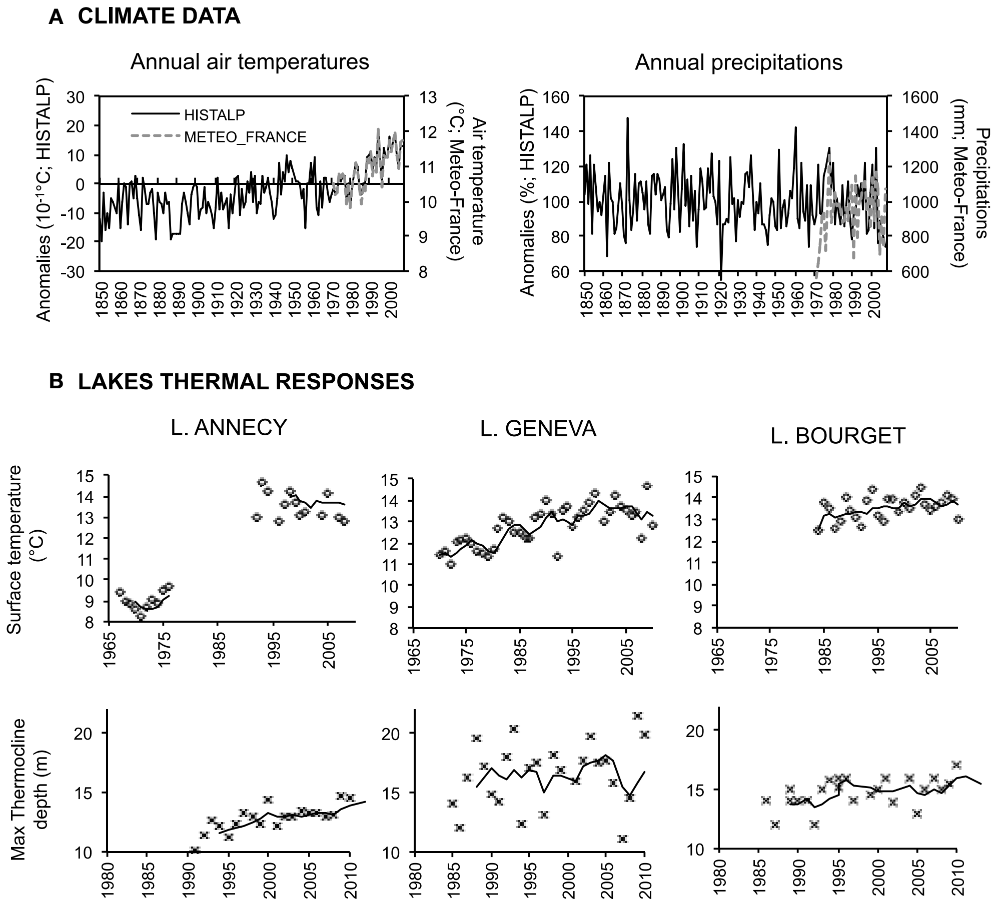


FIGURE 2 | (A) Climate variability over the three lakes for the last 150 years as extrapolated from HISTALP and comparison to locally monitored data (Meteo-France). **(B)** Changes lake key thermal characteristics, upper panel: average lake surface temperature over 0–15 m depths; lower panel: maximum depth of the 14°C layer, as a surrogate for late summer thermocline. Lines represent 5-year moving averages.

years for Lake Annecy (1966–1975 and >1996) and Geneva (>1970) and revealed that the surface temperatures (0–15 m depths) of these two lakes significantly increased between 1970–1975 and 2007–2012 (Lake Annecy, *Mann Kendall's* $\tau = 0.47$, $p = 2.10^{-11}$; Lake Geneva; *Mann Kendall's* $\tau = 0.59$, $p = 1.10^{-4}$; **Figure 2B**). Lake Annecy surface temperatures increased more than those of Lake Geneva between these two periods (+3.5°C for Lake Annecy, +1.1°C for Lake Geneva). Continuous monitoring of Lake Geneva since 1970 revealed that surface temperature warmed suddenly at the end of 1980 (Anneville et al., 2013). However, despite a continuous increase in air temperature, surface temperature did not warm further up over the last 20 years in any of the three lakes (Lake Geneva $\tau = 0.28$; $p = 0.11$; Lake Annecy $\tau = 0.12$; $p = 0.66$; Lake Bourget $\tau = 0.05$; $p = 0.77$). Between the mid 1980s and the current period, Lake Bourget surface water temperature increased less (+0.8°C; $t = 1.8$; $p = 0.13$) than those of Lake Geneva (+1.4°C; $t = 2.8$; $p = 0.03$) over the same period. Data analyses and modeling studies confirmed that stratification duration has increased in all three lakes (Danis et al., 2004; Vincon-Leite et al., 2014; Tapolczai et al., 2015). The thermocline has deepened by 2.5 m in Lake Annecy and by 1.5 m in Lake Bourget (Annecy: *Mann Kendall's* $\tau = 0.61$, $p = 9.10^{-5}$, Bourget: $\tau = 0.32$, $p = 0.02$) over the last 20 years but stayed at similar depth for Lake Geneva (Mann Kendall's $\tau = 0.18$, $p = 0.21$; **Figure 2B**).

In summary, despite their similar geomorphology and geographical proximity, the three studied lakes are not equally thermally vulnerable to climate change. Lake Annecy is the smallest of the three lakes, the most sheltered from winds and the least hydrologically active. Consistently, it is the one for which the thermal effects of climate warming are the strongest (warming of surface waters, thermocline deepening). In lakes Geneva and Bourget, the thermal effects are weaker, with however different physical consequences. In Lake Geneva, surface waters warmed up more than in Lake Bourget, the stratification length increased but with no deepening of the thermocline, in contrast to Lake Bourget (**Figure 2B**).

Climate and Human Alterations of River Discharges

Jenny et al. (2014a) reconstructed the inter-annual changes in river discharge from the main tributaries using terrigenous supplies, (i.e., the detrital inputs from the watershed). Since 1880, terrigenous fluxes have decreased by 50, 40, and 45% for lakes Geneva, Bourget, and Annecy, respectively, indicating a general trend to the decrease in the river discharge regime over the entire studied period (**Figure 3A**). This first order decrease was attributed to local river settlements, i.e., with the introduction of the first dam settlements in 1915, 1901, and 1874 in Lake Geneva, Bourget and Annecy. Despite the absence of any monotonic trend in precipitation change over the last 150 years (**Figure 2**), precipitations presented variations over that period at pluri-annual scales. The second order fluctuations of flood regime were not directly correlated to human activities, and instead attributed to these pluri-annual variations in precipitation regime changes. Recent increases in flood regime were recorded in the 1980s in Lake Bourget and in the early 1990s and the early 2000s in Lake Geneva (**Figure 3A**).

Changes in Nutrient Concentrations

All the three lakes underwent eutrophication in the mid-XXth century, and even for Lake Geneva for which a long-term TP monitoring was available, the temporal dynamics of the early or later eutrophication remained unknown. Therefore, two independent transfer-functions (Diatom-inferred TP; Berthon et al., 2014, and *Daphnia*-inferred TP; Berthon et al., 2013) were applied to reconstruct the TP dynamics over the last two centuries to all three lakes. In contrast to the diatom-based transfer function, which is ultimately based on species assemblages, the *Daphnia*-inferred transfer function relies on the changes in the absolute quantity of *Daphnia* remains. A strong linear relationship between the abundance of *Daphnia* remains (paleo-data) and measured total phosphorus concentrations for the corresponding years (monitoring data) was observed. Although the underlying mechanisms, likely depending on how much *Daphnia* high requirements for P constrain its growth rates (Sterner, 1993; Urabe et al., 1997), are not completely understood, such a strong relationship then permitted to build up solid *Daphnia* inferred total phosphorus concentrations. Inferred TP changes were highly consistent between the two transfer functions and coherent with available TP measurements. However, the *Daphnia* transfer function was more efficient in detecting the earliest changes in nutrient inputs to the lakes ($<10\,\mu$g P.l^{-1}, **Figure 3B**). These two independent inferences were required in order to avoid circularity in the assessment of the contribution of changes in lake trophic status on diatom and cladoceran communities structures.

Although they were oligotrophic at the end of the XIXth century, all three lakes underwent phosphorous enrichment as early as the 1920s, this process intensifying during the 1940s. The three lakes reached varying levels of maximum eutrophication. Lake Annecy did not exceed the oligomesotrophic status (i.e., mean annual concentration over the first 20 m depths: 18 μgP.l^{-1} in 1969). Lakes Geneva and Bourget reached a eutrophic status by the late 1970s (i.e., mean annual concentration over the first 20 m depths: 55–90 μg P.l^{-1}). Following restoration programs during the past three decades, observational data demonstrate that mean TP measured during winter mixing have been successfully reduced to 6 μg P.l^{-1} in Annecy, 19 μg P.l^{-1} in Geneva and 17 μg P.l^{-1} in Bourget (Berthon et al., 2013; **Figure 3B**).

Changes in Fish Management Practices

Fish communities in the three study lakes are similar and typical of subalpine lakes with autochthonous or early-introduced whitefish (*Coregonus lavaretus*), perch (*Perca fluviatilis)*, roach (*Rutilus rutilus*), and pike (*Esox luscious*) as dominant species. Fish communities were also altered through fisheries management practices. Different strategies in the stocking stages of whitefish as well as different authorized fishing size for perch juveniles (YOY) have created varying fish predation pressures over the last 30 years. The dynamics over time in the top-down control of fish on zooplankton were tracked in the sediment from the changing size of *Daphnia* claws over time following the size efficiency hypothesis (Alric et al., 2013). In all three lakes, and consistently with regulation patterns of bottom-up and top-down controls on food webs, fish predation pressure was low under

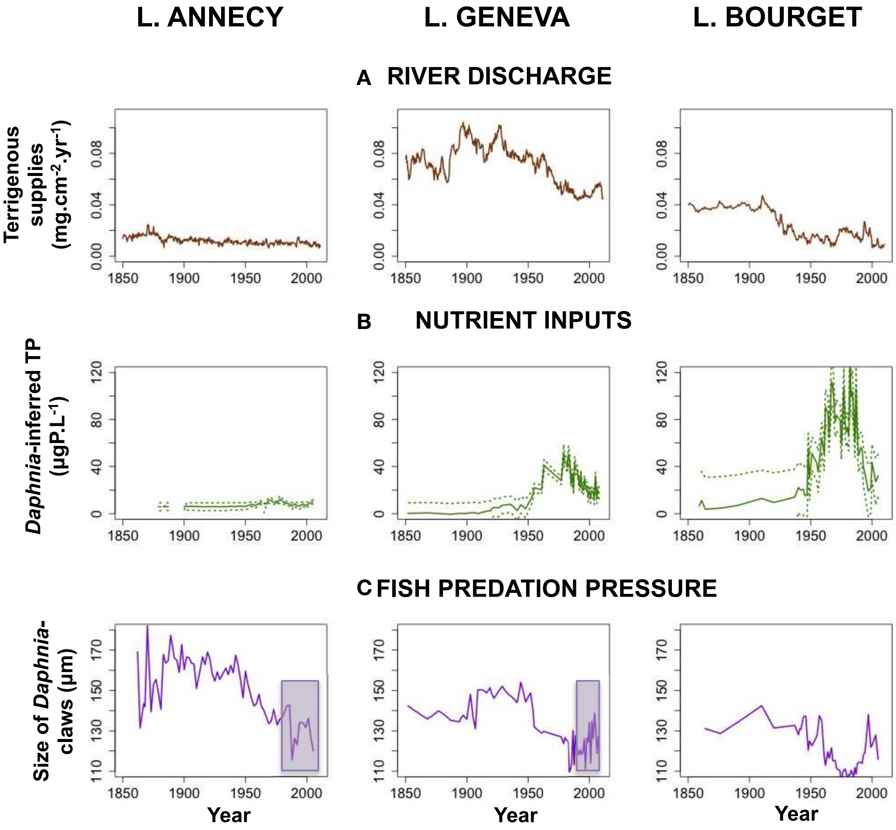


FIGURE 3 | Temporal dynamics of the local human forcings on all three lakes. (A) Terrigenous supplies as a proxy for river discharge (Jenny et al., 2014a); **(B)** Changes in phosphorus inputs as TP (±95% confidence interval) inferred from the abundance of *Daphnia* remains (Berthon et al., 2014); results were highly similar for diatom-inferred TP; **(C)** Changes in fish predation pressure on zooplankton, as inferred from temporal changes in the size of *Daphnia* (Alric et al., 2013). Changes in fish predation pressure that are decoupled from TP (purple rectangles) are considered as resulting from specific fisheries management practices in Lakes Annecy and Geneva.

oligotrophic conditions (and therefore, *Daphnia* were large) but increased during eutrophication. Reversible trajectories (decline in fish predation pressure during re-oligotrophication, mirrored by greater *Daphnia* body sizes) were however observed only in Lake Bourget. In this lake, fish predation pressure is fully coupled to TP, and local fisheries management practices preserve the top-down/bottom-up coupling (**Figure 3**). For the other two lakes, fisheries management practices (whitefish stocking at early stages and interdiction of fishing on Perch YOY) increased fish predation pressure on zooplankton and have disrupted the bottom-up/top-down controls. Fish predation pressure has remained high (Geneva) or even increased (Annecy) over the last 30 years (Alric et al., 2013; **Figure 3C**).

The Vulnerability of the Different Lake Habitats to Climate Warming

Plankton Components and Considered Metrics

"Plankton" designates a vast, diverse, and heterogeneous biological assemblage. It would not have been possible to address the total planktonic biodiversity over time in these three lakes, even relying on molecular methods, as the final purpose was to functionally relate community changes to climate warming. Instead, we targeted key-functional components, representative for biological diversity within plankton. We intended to cover both protozoans and metazoan organisms, from different positions in the food web (primary producers and secondary, tertiary consumers through cladocerans), from pico-(*Synechococcus*), to nano- and micro-plankton (large cyanobacteria and diatoms). Depending on available or developed methods, changes were quantified in term of taxonomic presence/absence (cloning sequencing on specific targets such as for pico-cyanobacteria or diatoms); species relative distribution (microscope countings of remains for diatoms and cladocerans) or abundance (quantitative PCR for total cyanobacteria or *Planktothryx rubescens*).

Climate Warming Impacts on Phytoplankton Communities at Pico-, Nano-, and Micro-planktonic Scales

Diatoms

Fossil remains of diatoms are inescapable paleo-descriptors, and they were used to infer several lake physical and chemical characteristics. Their use as indicator of past temperature is, however, more complex, because physical, chemical, and biological factors can affect diatom assemblage composition independently of water temperature (Battarbee, 2000). In fact, diatom response to climatic forcing can be strongly influenced by the characteristics of the lakes. For example, the comparison of the numerous available diatom paleo-records from remote lakes have already highlighted a general trend to the expansion of *Cyclotella* due to climate warming (Smol et al., 2005; Rühland et al., 2008; Winder et al., 2009), as a structural consequence of their competitive ability in stratified and nutrient-poor waters (Winder et al., 2009). On the contrary, in the deep lakes around the Alps, which are well-stratified because of their morphological features and were oligotrophic for most of their history, *Cyclotella* was the dominant diatom genus in periods of low trophy (e.g., Marchetto and Benedetti, 1995; Marchetto et al., 2004) and its presence cannot be considered as a symptom of recent warming.

Such background knowledge offered a further opportunity to compare diatoms responses to climate warming between those lakes protected from direct human exposure and the urbanized lakes of the program. Yet, paleo-records from lakes situated in densely populated areas are scarcer, and evidence of diatom responses to climate warming was so far equivocal. Changes in nutrient concentrations usually exert such a strong contribution in shaping diatom communities over time that the climate signal is hidden or smothered (Dong et al., 2012). Appearance of so-far not observed diatom species in the uppermost part of the cores are sometimes attributed to climate warming (Thies et al., 2011; Hobæk et al., 2012). Yet, in the absence of a full consideration of potential forcings, the actual contribution of climate warming, as compared to the additional, synchronous local forcings remains uncertain, especially for such a taxonomic algal group, that, as an important grazing resource, is prompted to get also regulated by top-down controls (Perga et al., 2009). The strategic reliance on GAM, that we used all over the program, allows unlocking the methodological bolts by fully considering the temporal dynamics of local forcings (changes in TP as inferred from *Daphnia* abundance; fish predation pressure) and air temperature in order to quantitatively estimate when and how much each of the forcings contributed to observed changes in diatom community structure for the three studied lakes.

Consistently to previous observations of anthropogenized lakes, TP was found to be the main driver of changes in community composition over the last century, but more interestingly, was pinpointed as an important factor modulating diatom sensitivity and responses to climate warming. The slight increase in the contribution of *Cyclotella* in the recent decade in Lake Annecy could not be related unambiguously to warmer air temperature. Diatom communities in this nutrient-limited lake were those for which climate effect was the least detectable, although it is the one on which the physical impacts of a warmer climate are the strongest. In contrast, climate effects on diatom assemblages were much stronger in the two other, mesotrophic lakes, with yet differences in the pathways by which climate shaped their community structures (Berthon et al., 2014). The diatom community of Lake Bourget showed the highest vulnerability and direct responses to climate warming, favoring spring species that can resist earlier stratification in mesotrophic lakes (such as *Asterionella formosa, Aulacoseira islandica,* and *Stephanodiscus parvus*) and summer species that can make the best trade-off between decreasing nutrient concentrations and increased water column stability (*Diatoma tenuis*, *A. islandica*; **Figure 4A**). In Lake Geneva, climate warming also contributed to shaping diatom communities through an indirect pathway; fisheries management practices and climate warming interacted to reinforce top-down control, favoring large, colonial inedible species (*Stephanodiscus binderanus* and *A. formosa*) (Berthon et al., 2014). Therefore, higher nutrient concentrations seem to increase diatom vulnerability to climate warming. In addition, in the two mesotrophic lakes, observed diatom responses to climate change were more diverse suggesting that at moderate nutrient concentrations, there are more direct- and indirect pathways by which diatom life history traits (nutrient requirements, resistance to stratification, growth at low light, edibility…), and therefore species, get selected in a warmer climate.

Cyanobacteria

There are very few specific phytoplanktonic groups that have diagnostic features that are preserved upon sediment archiving (diatoms and chrysophytes), which is so far limited our understanding of long-term phytoplankton response to these micro and nano-algal communities. The recent development and application of molecular tools on DNA archived in lake sediments (Willerslev et al., 2007; Coolen et al., 2008; Boere et al., 2011; Epp et al., 2011) provide the opportunity to enlarge paleo-records to other phytoplankton groups. Herein, a privileged focus was dedicated to cyanobacteria over other taxa. The first motivation was that their proliferation jeopardizes water quality and safety but the lack of long-term data hampers the identification of the trigger of blooms, especially for toxic strains. A second one involves pico-cyanobacteria dominance within picoplankton, a size-range that has long been overlooked within biodiversity monitoring although it might play a crucial role in nutrient and carbon cycling under warmer climates. A first step involved methodological optimization, calibration, and quality control and confirmed that hypoxic and cold bottom waters of these stratified lakes favored DNA preservation, an essential pre-requisite to quantitative molecular approaches (Savichtcheva et al., 2011). Total cyanobacterial abundance over time were at first pre-screened for all three lakes using quantitative PCR (qPCR) on preserved sediment DNA. All three lakes showed temporal changes in total cyanobacterial abundances over time, that were primarily driven by meso- or eutrophic P levels. Warmer temperatures additionally favored higher cyanobacterial abundances in the two lakes that are the most thermally sensitive to climate (Annecy and Bourget; **Figure 4B**). In Lake Annecy through, cyanobacteria were essentially represented by picoplanktonic strains and Lake Bourget presents environmental conditions that favor a high cyanobacterial contribution to the total phytoplankton community (Jacquet et al., 2005; Savichtcheva et al., 2011).

Therefore, following paleo-genetic studies were focused on Lake Bourget. Past abundances of cyanobacteria, including picocyanobacteria (qPCR), and changes in their phylogenetic compositions [sequencing of the 16S rRNA and internally

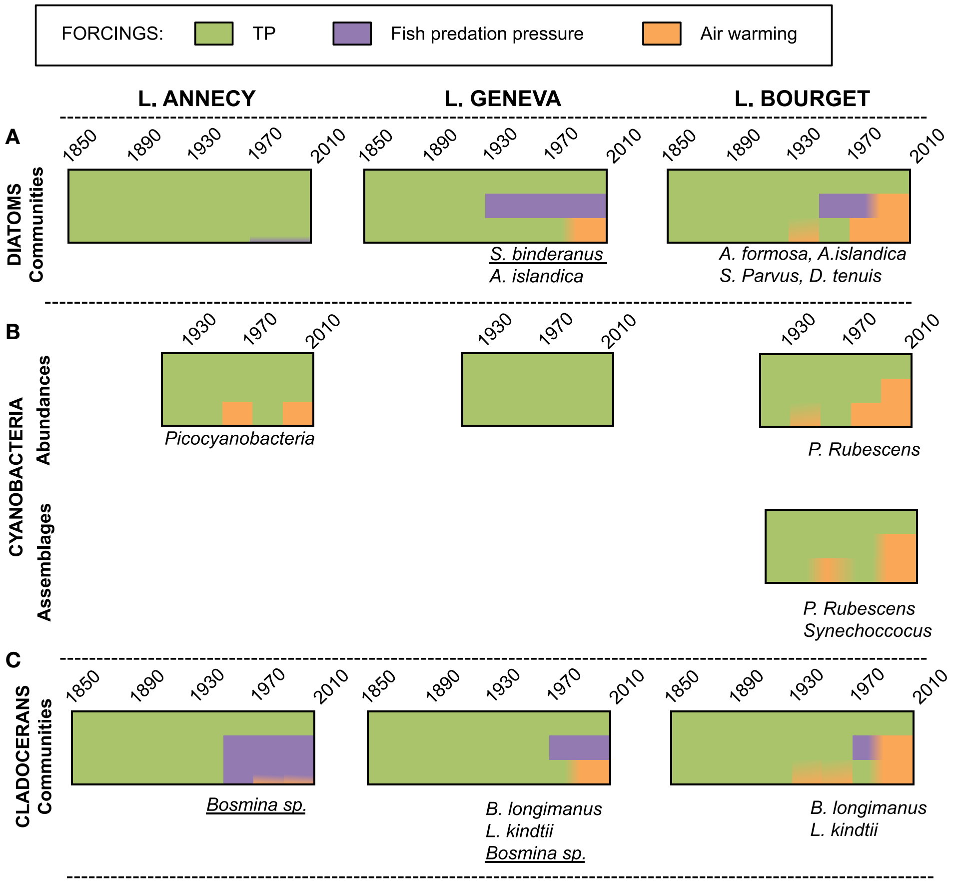


FIGURE 4 | Synthetic view of the relative contribution of local (TP, fish predation pressure) and climate (air temperature warming) forcings to the explained proportion of the planktonic trajectories (A: diatoms, B: cyanobacteria, C: cladocera assemblages) for lakes Annecy, Geneva, and Bourget. Taxa mentioned below the bars are those that are favored by the recent air warming in each lake, either by direct effects, or through a fish-predation mediated effect (when underlined).

transcribed spacer region (Domaizon et al., 2013; Savichtcheva et al., 2014)] were reconstructed over 150 years and the role of climate warming as compared to lake trophic status on the quantitative and qualitative changes was quantified. As for diatoms, changes in TP was the dominant driver of cyanobacteria species successions, even at the scale of picocyanobacterial phylogenetic transitions, while air temperatures had a significant influence on both the colonial/filamentous cyanobacterial and picocyanobacterial abundances (Domaizon et al., 2013; Savichtcheva et al., 2014). Hence, blooming episodes of the toxic *P. rubescens* in Lake Bourget occurred at oligo-mesotrophic state (i.e., in the 1970s and in the 1990s) but warmer waters in the last quarter of the XXth century amplified their abundances as compared to the 1970s. Current re-oligotrophication Lake Bourget has recently operated a transition from *Planktothrix* dominance to *Anabaena* and *Microcystis* within colonial/filamentous cyanobacterial communities which abundances are yet maintained high by still warming climate (Savichtcheva et al., 2014). Therefore, the consequences of climate warming at all cyanobacterial levels have been conditioned by TP levels in the lakes (**Figure 4B**).

Zooplankton

The study of the vulnerability of zooplankters to climate change has been performed through the analysis of parthenogenetic remains (i.e., carapace) for cladocerans and sexual remains (i.e., resting eggs) for *Daphnia*, relying on microscopic observations and molecular analyses. Parthenogenetic remains result from crustacean molts. In these deep and large U-shaped lakes, the cladoceran remains production, sedimentation, and taphonomic processes within the water column do not alter the compositional fidelity of remain assemblages, which accordingly mirror the composition of parental populations in the water column (Alric and Perga, 2011). In contrast, although egg banks do not necessarily always reflect the extant pelagic population over a short-term scale (Jankowski and Straile, 2003; Keller and Spaak, 2004), they provide a useful archive to investigate the taxonomic (e.g., Weider et al., 1997; Duffy et al., 2000) and evolutionary changes over long-term (e.g., Hairston et al., 1999; Frisch et al., 2014). For cyclical parthenogenetic taxa, the production of resting eggs occurred during an environmentally induced sexual reproduction (Hebert, 1980). Moreover, in sympatry or syntopy, the taxonomic and genetic structure of the egg banks depend on the range expansion of species as well as the degree and directionality of gene flow within and between populations (Keller et al., 2008; Cristescu et al., 2012). For cladocerans, both types of remains can be recovered from sediment cores. Following the central position of cladocerans in the food web, as trophic intermediates between primary producers and fish (McQueen et al., 1986, 1989), changes in cladocerans assemblages are likely to respond equally strongly top-down and bottom-up driven local forcings. Otherwise, the opportunity to investigate the temporal genetic architecture of *Daphnia* spp. populations that occurred in syntopy (i.e., *Daphnia longispina* species complex), through mitochondrial and microsatellite markers analyses of the resting egg banks, further reinforces our understanding of how the environmental drivers modify the gene flows and subsequent selecting forces.

The contributions of the local and climate forcings on cladoceran community structure over time were very similar to those already depicted for diatoms, except that the contribution of fisheries management practices on climate vulnerability was greater (Alric et al., 2013). As for diatoms, at low nutrient concentration (Lake Annecy), the cladoceran community was essentially controlled by local stressors, with very limited impact of climate warming. The sole detected impact of climate warming was shown to be indirect, favoring small zooplankton species (*Bosmina* sp.) that can better resist to the high fish predation pressure maintained in this lake by fisheries management practices. In contrast, the more nutrient-loaded lakes Geneva and Bourget were more sensitive to climate warming, but, as for diatoms, magnitude of their responses and the pathways under which climate warming affected their communities varied according to the local context of forcings (**Figure 4C**). Cladoceran assemblages, as for diatoms, are poor paleoclimatic indicators by themselves. Direct impacts of climate warming were indeed only evident for the predatory thermophilic species thriving in warmer, summer waters (*Leptodora kindtii*, *Bythotrephes longimanus*). Other community changes attributable to climate warming in the two mesotrophic lakes occurred at least partly through indirect pathways. Climate warming was shown to favor *Bosmina* species in all three lakes, and this pattern can arise from both strong temperature-dependent growth rates of *Bosmina* (direct impact, as for Lake Bourget) but also their higher abilities to outcompete *Daphnia* facing predation (indirect pathways) when fish predation pressure is maintained high by the interaction between fisheries management practices and climate warming (as observed in Lakes Annecy and Geneva).

Beyond their impact at the community level, environmental forcing factors affect the genetic structure of populations (Alric, 2012). Although not conducted at the same high-resolution scale, paleo-genetics studies on resting eggs recovered from the sediment cores provided interesting insights on underlying mechanisms by which environmental forcings affect *Daphnia* populations. At the gene levels, change in TP was the main, but indirect, cause of modifications in genetic structure of *Daphnia* spp. populations, with TP level that conditioned the magnitude of these changes. Indeed, gene flows within and between populations were higher in the more nutrient-loaded Lakes Geneva and Bourget, compared to Lake Annecy. Environmentally-mediated demographic processes during eutrophication, triggered introgressive hybridization and drastic shift in the genetic structure of the *D. longispina* species complex. The effect of changes in TP on genetic structure varied between the two lakes depending on the fisheries management practices, while climate warming did not seem to act as a selecting force on the genetic structure of populations (Alric, 2012).

The Vulnerability of the Benthic Habitats to Climate Change

Both eutrophication and climate warming are expected to trigger severe modifications of benthic conditions, notably oxygen concentrations, with further consequences on faunal composition and metabolic processes (Wiederholm, 1980; Verneaux et al., 2004; Bastviken et al., 2008). The relative share of each of these environmental forcings on past and current benthic oxic conditions remains hard to quantify in absence of long-term data. Long-term trends of changes in lake hypoxia have been previously reconstructed using chironomid subfossil remains (Millet et al., 2010), organic matter preservation and bioturbation index (Ariztegui et al., 2001) or trace metal accumulation (Tribovillard et al., 2006). These approaches, since they rely on a single-core study, usually detected the onset of hypoxia but did not further inform on its spatial extent, i.e., whether it stays limited to the deepest areas, expands, or retracts. Yet, in absence of quantitative estimates, there are limited in their abilities of hierarchizing the environmental drivers of further changes in hypoxic conditions. The lack of such data also hampers our ability to scale the consequences of hypoxia on benthic faunal for which oxygen concentrations are critical. Therefore, we dedicated a specific attention, in the program, to approach benthic processes through a spatially explicit strategy in order to reach quantitative estimates of temporal changes of hypoxia volumes and address the vulnerability of benthic fauna accounting for depths (habitats).

Volumes of Hypoxic Waters

The preservation of biogeochemical varves, which is favored by the disappearance, following oxygen depletion, of most bioturbating macroorganisms, is a known indicator of the onset of hypoxic conditions (Giguet-Covex et al., 2010). Fine oscillations of hypoxia through complete large lake basins were quantitatively reconstructed by computing the extension of the hypoxic water volumes using multiple sediment cores collected at various depths (40 cores for lake Bourget, 20 for lake Annecy, and 21 for Geneva; Jenny et al., 2013, 2014a).

Even for these lake ecosystems that have been well oxygenated over a millennia-long period, and regardless of past climatic fluctuations, a shift to hypoxic conditions occurred in the 1950s in response to the rise in total phosphorus concentrations above $10 \pm 5\,\mu g\ P\ L^{-1}$. Following this shift, hypoxia never disappeared in any of three lakes despite the fact that environmental policies succeeded in drastically reducing lake phosphorus concentrations. During that period, decadal fluctuations in hypoxic volume were great, ranging between 0.5 and 8% of the total lake volumes. We demonstrated, through statistical modeling, that these fluctuations were essentially driven by river discharge and air temperature (Jenny et al., 2014a). In lakes Geneva and Bourget, which are fed by large river systems, fluctuations in hypoxic volume were negatively correlated with river discharge. In contrast, in Lake Annecy which is fed by a smaller river system, the expansion of hypoxia was only related to warmer air temperatures. These lakes have inherited hypoxia from the eutrophication period in the 1940–1970s and have shifted to a new stable state, in which hypoxic volumes are now controlled by air temperature and river discharges, independently from changes in lakes TP (Jenny et al., 2014a).

Benthic Fauna

Further consequences of changes in oxic conditions at the lake sediment interface on the vulnerability of the benthic fauna in result of climate change have been assessed through chironomid assemblages, which head capsules are well preserved in sediment cores. Chironomids have been extensively used to reconstruct past air temperature (e.g., Heiri et al., 2003; Walker and Cwynar, 2006). Nonetheless, in deep stratified lakes, the influence of temperature on chironomids is rather indirect (e.g., duration of thermal stratification) while oxygen constraint directly influences chironomids at a local-scale (e.g., survival and growth rate; Wiederholm, 1980; Verneaux et al., 2004). In order to reach a comprehensive understanding of benthic processes, including littoral and deep habitats, chironomid paleo-surveys have also been conducted over cores sampled at multiple depths (from the maximum lake depth to half of the maximum lake depth, since hypoxia was not likely to settle closer to the lake surface). Such a strategy required a prior identification of the coring transect presenting the better head capsule deposition, by comparison to modern chironomid communities, and therefore ensuring the best-preserved, depth specific chironomid characteristics in the sediment cores (Frossard et al., 2013). The titanic workload of this preliminary task precludes similar multiple depths-strategy for the very large Lake Geneva. The vulnerability of benthic fauna to climate change has then been studied only on lakes with manageable sizes, i.e., Lakes Annecy and Bourget.

For both lakes, the chironomid assemblages were strongly depth specific and were characterized by oxyphilous taxa (e.g. *Micropsectra*) at all studied depths before the 1940s. Thereafter, dominant changes of the chironomid assemblages occurred through a drift toward dominance of hypoxia-tolerant taxa (e.g., *Sergentia*), confirming that oxygen concentration first structures chironomid communities. The sensitivity of chironomid assemblages to the simultaneous environmental forcings was yet depth dependent. Profundal chironomid assemblages (65 m for Lake Annecy and 145–120 m for Lake Bourget) responded mainly to nutrient enrichment, whereas assemblages at shallower depths (30–56 m for Lake Annecy; 60–90 m for Lake Bourget) were mainly affected by top-down effects of the fish community on the pelagic food web, triggering increasing losses of organic matter from the pelagic to the benthic zone. Interestingly, at sub-profundal depth of Lake Annecy (i.e., 56 m) and at most studied depths in Lake Bourget (i.e., 60, 90, 120, and 145 m), fluxes of *Micropsectra* head capsules increased at the onset of eutrophication for roughly a decade prior to collapse (Frossard, 2013). Such hump-shaped patterns suggested that eutrophication, prior to trigger deleterious decrease in oxygen concentrations, first promotes food availability to chironomids and therefore enhance their recruitment. Despite obvious re-oligotrophication in both lakes during the latest 30 years, benthic habitats still host mostly hypoxic tolerant species, while the abundance of head capsules have kept on decreasing at the greater depths. Since the late 1980s, increased air temperature significantly contributed in increasing the hypoxic pressure at greater depths, potentially through its effect on the strength and duration of thermal stratification, possibly coupled with changes in mixing efficiency during winter. Therefore, local forcings initially triggered the initial shift in chironomid communities at most depths through their impact on bottom oxic conditions. Since the 1980s though, local forcings do not exert any significant control on the deep fauna. Therefore, although remediation measures have been undertaken locally to minimize local forcings, accumulation, and anoxic preservation of organic matter at the bottom seem to have created a feedback loop that may irreversibly alter carbon cycling in deep habitats (Frossard et al., 2015) and, through hypoxia, increased the repercussions of climate warming on the deep chironomid assemblages. In the sublittoral habitats, the occurrence of hypoxia depends less on climate warming than on whether fisheries management practices maintain an important organic matter loss to the benthos by the cascading top-down effects (Frossard et al., 2013, 2014) (**Figure 5**).

Lakes Ecological Vulnerability to Climate Warming

Over the whole century, the lakes ecological trajectories have been predominantly driven by nutrient concentrations. Biodiversity changes due to eutrophication, through direct or indirect (top-down) controls, have been more important than those that could be attributed so far to climate warming, even for Lake Annecy in which eutrophication has been limited. These results echo previous experimental studies showing that the repercussions of climate warming on lakes biodiversity have not yet exceeded those triggered by past and/or current local human activities (Moss et al., 2003; Christoffersen et al., 2006; Ozen et al., 2013). However, all taxonomic groups or processes that we investigated have responded to climate change, in at least two of the three sites, supporting our initial hypotheses that even anthropogenized lakes are vulnerable to climate change. In addition, for most compartments, climatic controls now overpass nutrient controls, suggesting that such a hierarchy in the drivers of lake trajectories might not withstand for long.

Most of what we know about lakes vulnerability to climate change is derived from studies on shallow waterbodies (Mooij et al., 2007; Nielsen et al., 2014). Because of the low heat storage capacity of shallow lakes, water temperatures are not expected to be highly correlated to air temperatures (Adrian et al., 2009; Luoto and Nevalainen, 2013; Winslow et al., 2015), and an increase or imbalance in nutrient concentrations, rather than

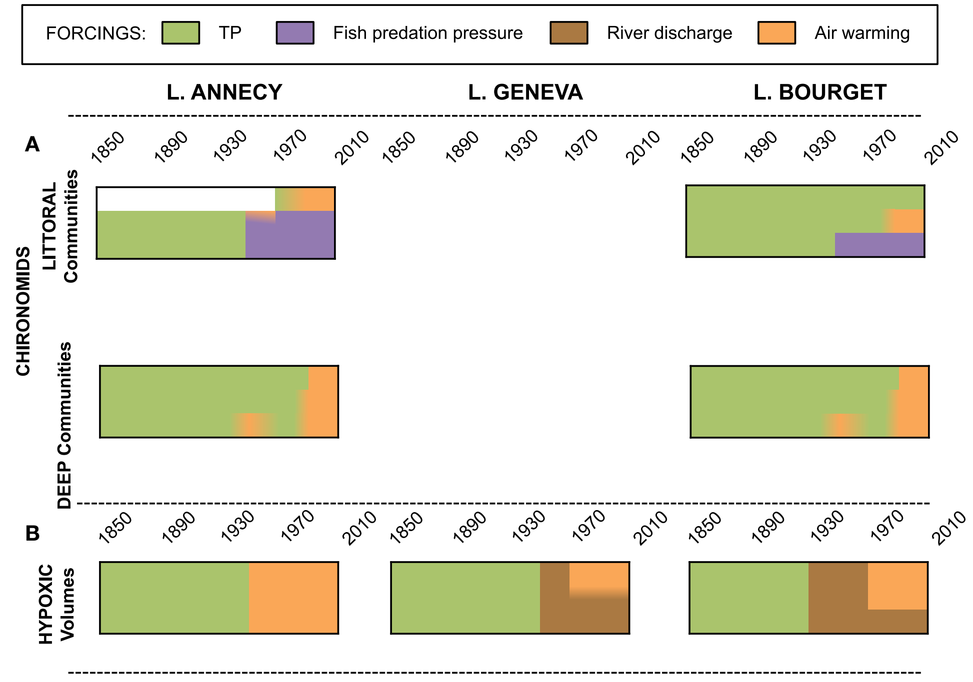


FIGURE 5 | Synthetic view of the relative contribution of local (TP, fish predation pressure, river discharge) and climate (Air warming) forcings to the explained proportion of the benthic trajectories (A: littoral and deep chironomid assemblages; B: hypoxic volumes) for lakes Annecy, Geneva and Bourget.

water warming itself, is the primary pathway by which climate change disturbs the ecological state of these lakes (Mooij et al., 2008). Mesocosm experiments consistently showed that water warming *per se* has only limited repercussions but exacerbates the consequences of nutrient inputs (Christoffersen et al., 2006; Feuchtmayr et al., 2009; Ozen et al., 2013) and this is referred as "warming induced eutrophication." For the large and deep lakes of this study, the general framework is considerably different since phosphorus inputs, which are mostly from domestic and urban origins, have been successfully controlled at the watershed scale, a situation that is similar for many large and deep lakes worldwide (Jeppesen et al., 2005). The absence of warming-induced eutrophication offers the opportunity to evaluate, at an ecosystem-scale and over long-term, how strongly ecological changes attributed to climate warming are actually linked to climate-induced changes in the thermal dynamics.

Of all three lakes, Annecy was the most thermally vulnerable to climate warming, but the one for which planktonic communities were counter-intuitively the least affected. In the two other mesotrophic lakes, the impact of climate change on the ecological trajectories has been strong enough to take over nutrient controls for the last 25 years. Our experimental observations therefore provide strong evidences that climate-induced water warming *per se*, such as further changes in the lake thermal dynamics, generates limited repercussions on plankton communities, similar to shallow lakes where nutrient are the most determinant constrain. Warming effects on planktonic communities get significant at mesotrophic nutrient levels (Christoffersen et al., 2006; Feuchtmayr et al., 2009; Ozen et al., 2013). The most likely explanation lies on the stoichiometry hypothesis that plankton released from nutrient limitation responds to varying thermal conditions (van de Waal et al., 2009). Most of the experimental works so far have focused on changes in total growth rates or total biomass, suggesting that under warmer waters, nutrient use efficiency might be higher (De Senerpont Domis et al., 2013). Long-term cyanobacterial and pico-cyanobacterial transitions in Lake Bourget support these outputs suggesting that temperature-dependent stoichiometric constrains might also control species transitions under warmer climate (Domaizon et al., 2013; Savichtcheva et al., 2014).

In few cases, planktonic species that have benefited from climate warming are considered as thermophilic species (i.e., that would outcompete other species due to higher growth rates in warmer waters, Lürling et al., 2013). This is indeed the case for the large predatory cladoceran species that now thrive in the waters of Lakes Bourget and Geneva. Otherwise, most planktonic taxa promoted by climate warming are not typical summer species (Alric et al., 2013; Berthon et al., 2014). They instead show life-history traits that more substantially relate to the observed impacts of climate warming on the thermal structure of the water column (either resistance to increased stratification as for Lake Geneva or ability to grow at low light at the end of summer due to a deeper thermocline, i.e., *P. rubescens* and *A. formosa* in Lake Bourget) or through predation-mediated pathways cascading down to phytoplankton

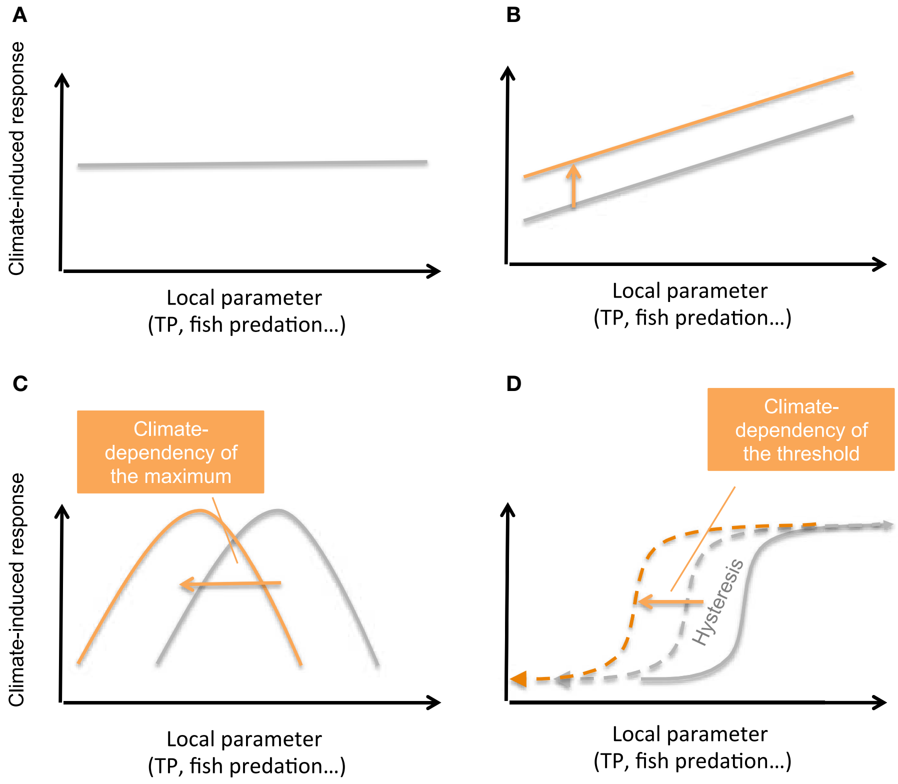


FIGURE 6 | Hypotheses about the link between lake ecological vulnerability to climate warming (amplitude or types of the ecological changes attributed to climate warming) and local environmental forcings under management (TP, fish predation pressure, river discharge…). (A) Null hypothesis. **(B)** Linear relationship. **(C)**. Hump-shaped dependency (exhibiting maximum value). **(D)** Regime shift dependency (exhibiting threshold value). Gray and orange lines depict relationships for current and projected climate change.

(cladoceran or diatom tolerance to grazing). The role of top-down controls on plankton seasonal (Sommer et al., 2012), and interannual (Jeppesen et al., 2005) dynamics is currently revised and theoretical or experimental works point to a higher than initially expected contribution to plankton dynamics (Kratina et al., 2012). Climate warming shall, especially in shallow lakes, even reinforce top-down controls by increasing the length of the growing season, per-capita feeding rates and recruitment of zooplanktivorous larval stages (Kratina et al., 2012). Such beneficial consequences of climate warming have also been documented or suggested for the two fish species under management in these lakes: Whitefish *Coregonus lavaretus*; Anneville et al., 2009, and Perch *Perca fluviatilis*; Gillet and Dubois, 2007). The paleo-ecological approach provided solid, long-term ecosystem-scale evidence of fish predation pressure controlling zooplankton community structure, cascading down to phytoplankton assemblages and even organic matter export to the sediments. The predation-mediated pathways by which climate structures planktonic communities and at some point littoral benthic communities, were even reinforced by local fisheries management practices in Lakes Geneva and Annecy.

The vulnerability of planktonic communities to climate warming in these large and deep lakes was therefore rather independent from their thermal vulnerability. The direct physiological impacts of warmer waters on plankton life cycle and species transitions are not likely the most significant pathways by which climate warming alters planktonic communities in these lakes. Instead, nutrient constrains and fish predation pressure interplay with climate change, regulating the sensitivity of the communities to water warming and subsequent climate-induced hydrodynamic changes and creating multiple, indirect pathways which exert a dominant structuring impact on surface (planktonic and littoral) communities structures.

In contrast to planktonic communities on which TP drove continuous species transitions over the last century, benthic community changes were abrupt. Depth-changes in benthic communities mirrored the onset and expansion of hypoxia at the lake bottom. TP acted as a trigger of hypoxia, but above a relatively low threshold (around $10\,\mu g$ $P.L^{-1}$, i.e., upper limit of oligotrophy), further expansion or contraction of hypoxic volumes have not been anymore controlled by further changes in TP. There have been, instead, driven by climatic and hydrological forcings, that regulate oxygen replenishment at the bottom by winter mixing or river inputs. Therefore, benthic trajectories are discontinuous and the legacy of past eutrophication, even limited, has triggered significant regime shifts that are mediated by the accumulation and respiration of organic matter. While surface (pelagic and sub-littoral) communities are still responding to changes in local forcings, deep biodiversity, and oxic conditions seem currently almost only regulated by climatic factors (river discharges being under both local and climatic controls). Surface and deep habitats are therefore currently uncoupled in these three lakes since they now respond to a different set of forcings along varying dynamical regimes.

Operational conclusions of the program would be that local management strategies could help minimizing the impacts of climate warming on lake ecological processes and biodiversity. Surface habitats still respond to both local and climate forcings. Therefore, pursuing efforts to reduce TP inputs to the lake and adaptation of management practices could help to limit the climate impacts on the pelagic food web. For instance, adapted stocking practices, schedule or fishing regulation could significantly limit cascading effects of fish predation pressure on the food web and avoid amplification of these effects by climate change in these lakes, i.e., the development of large cyanobacteria or unedible phytoplankton species, and further counter organic matter export to the sediment and maintenance of littoral hypoxic conditions. Further research is yet required to test the robustness of such conclusions on a larger set of sites (**Figure 6**). What remains unclear is the shape (discontinuous, continuous, or gradual; Kefi et al., 2013) of the relationships linking the local parameter (TP, fish predation pressure) to the qualitative and quantitative response of the surface communities or processes under climate warming. Such information would be crucial to help managers to set target TP or fisheries management practices in order to limit lake vulnerability to climate change and ultimately promote ecosystem services. For benthic, deep habitats, hydrologic regulation of river discharge might be, for lakes fed by large river systems, a control lever on which managers might act to limit deep-water hypoxia. Preservation of part of the natural river flow is long known as a powerful way to protect the ecological integrity of river ecosystems (Richter et al., 2003) but has only recently been considered within some still rare lake management plans. Although this kind of initiative could benefit bottom oxygenation by releasing its dependency to climate warming, the relationship between TP and hypoxic volumes clearly follows a regime shift dynamics which first threshold was at $10\,\mu\text{g P.L}^{-1}$. Lack of recovery of bottom oxic conditions for Lake Annecy, although TP has crossed the threshold line already a decade ago, suggests that accumulation of organic matter is a legacy that generates hysteretic dynamics. The value of the next threshold of recovery is therefore uncertain and might also further decrease depending on further warming scenarios (**Figures 6C,D**), as already modeled for shallow lakes (Nielsen et al., 2014). A next step and perspective of this IPER-RETRO research program, that is currently undertaken, is to compare multiple trajectories of similar peri-alpine lakes in order to detect and test these hypothetic relationships and investigate potential thresholds.

Author Contributions

All authors contributed to the acquisition, analysis and interpretation of data for the presented work and approved the present manuscript. MP, CP, VF, JJ, and BA substantially contributed to build up this synthesis.

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Using diatom assemblages and sulfur in sediments to uncover the effects of historical mining on Lake Arnoux (Quebec, Canada): a retrospective of economic benefits vs. environmental debt

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Monitoring changes in environmental conditions is increasingly important as the Canadian economic infrastructure ramps up exploration and mining development in the more inaccessible northern regions of Canada. Governments are concurrently assessing effects from past mining activities and absorbing the economic cost to society with on-going remediation and monitoring initiatives. The abandoned Aldermac mine in northwestern Quebec, mined from 1932 to 1943, is an excellent case study for assessing the state of environmental and economic effects of past mining operations. A paleolimnological approach, using diatoms as environmental proxies, was used to evaluate the spatial and temporal impacts on aquatic receiving environments. Based on the inferences drawn from diatom assemblages in Lake Arnoux, prior to mining activity, lake water pH was similar to that of surrounding lakes (circumneutral to weakly acidic). After mining operations terminated, changes in pH and alkalinity in Lake Arnoux coincided with distinct increases in sediment sulfur content. Across a 30- to 40-year span (circa 1940–1970s) a significant decline in phytoplankton flora coincided with lake acidification and increased clarity of the water column. This resulted in an increase in the benthic diatom population (>90%), replacing the planktonic diatoms. Observed shifts in environmental proxies are concurrent with one, and possibly two, reported tailings pond breaches at the abandoned mine site. Adverse effects of the abandoned Aldermac mine on nearby ecosystems, combined with pressure from local citizens and environmental groups, forced responsible accountability for site restoration led by the Quebec government. Based on the historical period of economic growth, the financial benefits of the Aldermac mine were significant and justify the current pay-it-backward costs for environmental remediation. However, it has now been documented that the pay-it-backward model is not sustainable in the modern economy. New pay-it-forward approaches, addressing our gray water footprint, are required to merge economic and environmental sustainability for future prosperity.

Keywords: acid mine drainage, Aldermac mine, biomonitoring, diatoms, Lake Arnoux–Dasserat system, paleolimnology, pH, sulfur

Introduction

In the early 1900s, resource development and exploration were key drivers for economic development in Canada. At the same time, the Great Depression of North America (1929–1939) had a significant negative effect on resource development. The gross national product (GNP) dropped by 40% before the end of the Depression era and the beginning of the Second World War (Amaral, 2002). Despite the difficult economic period, the mining sector still developed in the province of Quebec during the 1920s and 1930s. In particular, mining development in the Abitibi-Timiskaming region significantly expanded after gold was discovered in the 1920s. This lead to the opening of numerous mines and the creation of two major mining towns: Val-d'Or ("valley of gold") and Rouyn-Noranda. The mining, pulp and paper, and tourism industries continue to be key economic activities in the region, with gold, as well as copper, zinc, lead, and other metal commodities as important resources. In 2013, mining investment in the Abitibi-Timiskaming region totalled US$0.8 B (Institut de la Statistique du Québec, 2014). Despite being an active mining region, a number of mines established in the mid-1900s were abandoned, leaving a vast legacy of acid-generating tailings [Ministère de l'Énergie et des Ressources Naturelles, 2015]. For example, the Aldermac mine site (1932–1943) had the second largest tailing facility and was one of the most contaminated sites in the area (Ministère de l'Énergie et des Ressources Naturelles, 2015). During its operation, the Aldermac mine exploited a group of massive sulfide lenses which produced copper, gold, silver, silica, and pyrite (Ministère de l'Énergie et des Ressources Naturelles, 2015). To generate these products, 1,500,000 tons of tailings were created and deposited over 76 hectares (Ministère de l'Énergie et des Ressources Naturelles, 2015). This was at a time when there were no guidelines for environmental risk management. The tailings were composed of approximately 50% sulfide minerals with high concentrations of sulfur, arsenic, cadmium, copper, molybdenum, and zinc (Ministère de l'Énergie et des Ressources Naturelles, 2015). The low pH observed in the region was caused by exposing sulfide minerals (e.g., pyrite FeS_2) to air and water and generating sulfuric acid: acid mine drainage. The two common chemical reactions in pyrite degradation are (Chandra and Gerson, 2010):

$$2FeS_{2(s)} + 7\,O_{2(aq)} + 2H_2O_{(aq)} \rightarrow 2Fe^{2+} + 4SO_4^{2-}{}_{(aq)} + 4H^+_{(aq)}, \text{ and}$$

$$FeS_{2(s)} + 14Fe^{3+}{}_{(aq)} + 8H_2O_{(aq)} \rightarrow 15Fe^{2+}{}_{(aq)} + 2SO_4^{2-}{}_{(aq)} + 16H^+_{(aq)}.$$

Production of H^+ decreases pH and increases acidity. Atmospheric sulfur deposition (e.g., from smelter stacks in Rouyn-Noranda and Sudbury, or from long range atmospheric transport) also adds to the production of sulfuric acid affecting aquatic and terrestrial ecosystems.

Past mining activities and residual tailings left on site have seriously affected nearby aquatic receiving environments, principally the Arnoux River and Lake Arnoux. Restoration activities at the Aldermac site were initiated in 2008, 65 years after mine closure, and have since amounted to an estimated cost of more than US$16.5M (Ministère de l'Énergie et des Ressources Naturelles, 2015). Despite rehabilitation, the Arnoux River and one of its small tributaries (directly flowing through the tailings) were still acidic, with high concentrations of dissolved metals and high conductivities (measurements taken in July 2012; Leguay et al., in press). However, in the surface water of Lake Arnoux, pH seemed to recover during the course of this study from measured values of 4.03–5.37 in 2011 and 5.30–6.25 in 2012 to 6.58–6.77 in the fall of 2013 (unpublished data).

As more economic initiatives look to exploration and mining development in Canada's north, spatial, and temporal monitoring will be of increasing importance to assess environmental conditions. Governments are compelled to assess the environmental legacy of past mining practices in addition to the economic cost to society for remediation, while promoting mining in relatively pristine northern regions (e.g., the Quebec Plan Nord, the Ontario Ring of Fire, and the Northwest Territories Mining Initiative). As of 1995, the contemporary mining industry in Quebec must provide financial guarantees to ensure environmental remediation post-closure. In the province of Quebec alone, more than 100 abandoned mining sites are under the responsibility of the government, which has already injected millions of dollars into restoration (Ministère de l'Énergie et des Ressources Naturelles, 2015). In 2010, the mining legacy to the province reached an environmental debt estimated to be over US$600M (Ministère des Ressources Naturelles et Faune, 2011). With extensive new mining developments in northern Quebec expected in the near future, it is fundamental to understand their potential effects on aquatic ecosystems and to develop performance evaluation techniques for mine site rehabilitation in advance. The amount of public money invested in resource development should include scientific evidence of whether or not an adversely affected ecosystem has recovered or is recovering from mining contamination post-remediation.

Algae, particularly diatoms, are susceptible to changes in environmental conditions and are excellent indicators of past and present ecosystem integrity (Stoermer and Smol, 1999). Diatom assemblages, preserved in lake sediments, can provide valuable information about reference conditions and reference assemblages prior to mining activities. Studies from northern Quebec examined the impact of mining on diatoms and water quality and consistently recorded elevated metal concentrations in the sediments with altered diatom assemblages (Cattaneo et al., 2004, 2008; Laperrière et al., 2008). Diatom-based models, developed in northwestern Quebec, examined correlations between water quality and diatom assemblages (Fallu et al., 2000; Enache and Prairie, 2002; Dixit et al., 2007). The studies by Dixit et al. (2007) and Enache and Prairie (2002) specifically found strong correlations between diatoms and pH. Fallu et al. (2000) demonstrated strong linkages between diatom assemblages, alkalinity, and dissolved organic carbon in northern Quebec and Labrador. Environmental shifts can also have significant impacts on the higher trophic levels and the overall biological health and stability of freshwater systems.

The objective of this study was to investigate the downstream response of diatom assemblages to water quality changes caused by mining activities at the Aldermac mine. A sediment core was collected from Lake Arnoux in 2011, which is the most proximal lentic environment to the Arnoux River, directly downstream from the Aldermac site (**Figure 1**). Temporal changes in the fossil diatom assemblage composition were assessed in the Lake Arnoux core. The diatom profile from Lake Arnoux was also compared with the assemblages from sediment cores sampled at three sampling stations farther downstream to assess spatial changes with distance from the Aldermac site. In addition, the economic benefits of the Aldermac mine were examined with specific reference to profits gleaned vs. the costs of environmental damage and remediation. The sustainability of this model of government-led rehabilitation of abandoned mines in the public interest was explored.

Methods

Study Area

The mining region of Abitibi-Timiskaming in northwestern Quebec (along the Ontario border) is underlain by the Precambrian Shield, covered by the boreal forest in Abitibi and a mixed forest in Timiskaming. Generally, natural waters in this region are low in nutrients, slightly acidic, and have high concentrations of dissolved organic carbon (DOC). The Aldermac mining site (derived from the names of the two prospectors Alderson and Mackay) is located near Arntfield, 25 km west of Rouyn-Noranda. Seventy-six hectares of tailings were left exposed on the site and were drained by small effluents, which reached the Arnoux River. The Arnoux River discharges into Lake Arnoux, a shallow water body (<5 m deep). In turn, Lake Arnoux feeds into Bay Arnoux and Lake Dasserat, a well-known lake in the area for recreational fishing, hunting, boating, and cottages. In the immediate vicinity, there were also eight smaller mining operations at varying times during the 1900s.

Sediment cores from Lake Arnoux, Northern Lake Dasserat, Bay Renault, and Confluence Bay Arnoux were collected in September 2011 (**Figure 1**). The Lake Arnoux coring station is the most proximal to the Aldermac site near the mouth of the Arnoux River (48°14.2843′N/79°19.0407′W). The entire lake is about 4.5 m deep and has no discernable depocentre for sediment focusing. The station was selected for its proximity to the mouth of the Arnoux River, the shores of which remain rich in tailings post-restoration. The other three sites are located in Lake Dasserat and have been named Northern Lake Dasserat (48°16.2658′N/79°25.6293′W), Bay Renault (48°12.5763′N/79°24.6881′W), and Confluence Bay Arnoux (48°14.7120′N/79°24.1266′W). Northern Lake Dasserat is the deepest point in Lake Dasserat (15.2 m). Confluence Bay Arnoux (approximately 9.5 m water depth) is just downstream from where Bay Arnoux drains into Lake Dasserat. Finally, Bay Renault is the deepest site in the southwestern quadrant of Lake Dasserat (approximately 14.5 m).

Sample Collection

At the three sampling stations in Lake Dasserat (Bay Renault, Confluence Bay Arnoux, and Northern Lake Dasserat), scuba divers from Environment Canada retrieved sediment cores

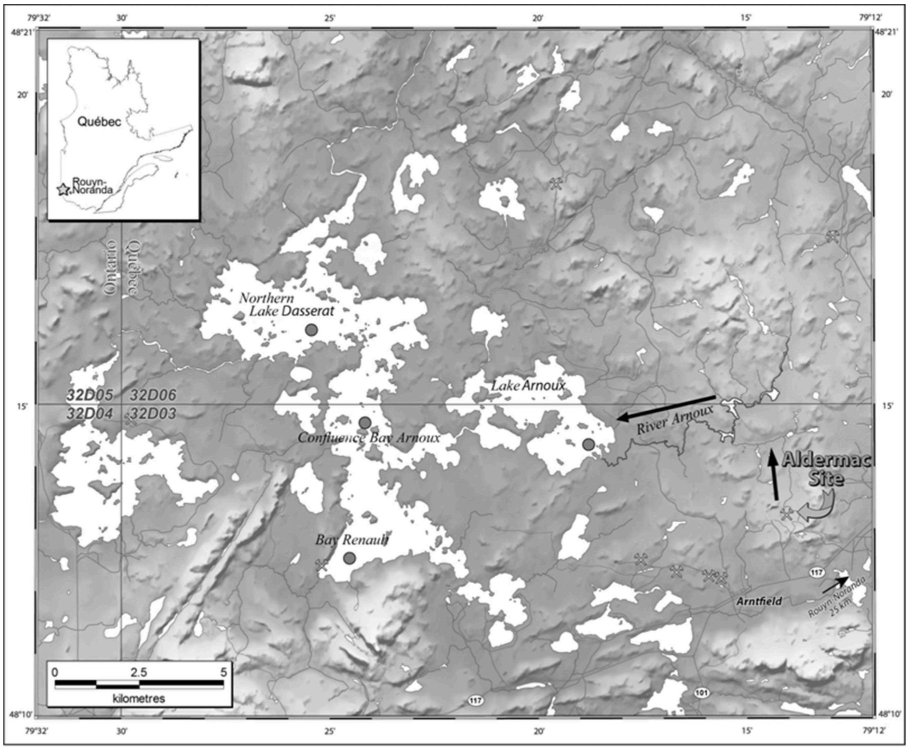

FIGURE 1 | Locations of the former Aldermac mine and the sampling stations (gray dots) downstream in Lake Arnoux and Lake Dasserat. Black arrows identify surface water drainage flow from the abandoned mining site. Map sheets for this figure are 32D03, 04, 05, 06.

(Mudroch and MacKnight, 1991). The cores were hand-taken by the divers to avoid or minimize compaction, smearing, tilting, and mixing of unconsolidated hydrous sediments, and to preserve the sediment–water interface (e.g., Baxter et al., 1981; Glew et al., 2001). Core lengths varied from 40 to 54 cm. The sediment core at Lake Arnoux was recovered by a modified gravity corer due to poor water quality conditions for diving. A 10-cm diameter gravity corer gently penetrated the sediments to permit preservation of both the core integrity and the sediment-water interface. The technique was appropriate for this shallow site and closely simulated the high quality of diver-taken cores. The length of the recovered core in Lake Arnoux was approximately 40 cm. Several cores were taken adjacent to each other at every station with each dedicated to complementary analyses after sub-sampling. Cores were extruded on site and sectioned at 0.5–1.0 cm depth intervals using either a mechanical extruder or a hydraulic extruder with water pressure advancing the core. Sediments cored in this study were either organic-rich (e.g., Lake Arnoux gyttja) or mixed glaciolacustrine clays from glacial Lake Barlow-Ojibway (e.g., Lake Dasserat; Veillette, 1994).

Samples were stored at 4°C on site, during transport, and upon return to headquarters. They were frozen at −60°C before they were transferred into a freeze-dryer and dried under a vacuum of less than 170×10^{-3} mBar for approximately 2–3 days. Samples from the same core were divided for diatom analyses and ^{210}Pb dating. Adjacent cores taken at the same time and sites were treated similarly before submission for analyses of sulfur content.

Diatom Sampling, Processing, and Analyses

Diatoms collected from the four sediment cores were processed following the procedure of Gajewski et al. (1997); a minimum of 600 valves were counted from subsamples, documenting core sections of known dry weights. Taxonomic identifications were made to the lowest possible level. In this study, selected depths from each core were identified based on a general timeline of pre- and post-mining activity. Core samples were selected mainly from recent times (>1930s) with one sample from the early-1900s and one sample from the bottom of each core dating back to before 1850.

Diatom data were expressed as relative abundances to reflect changes in the assemblage structure, indicating potential fluctuations in water quality. Absolute values were also available and were used to support observations from the relative abundances. Taxonomic diatom species identifications were performed by comparison with photomicrographs published in floras from similar geographic regions and environmental conditions (Camburn and Charles, 2000; Fallu et al., 2000; Siver et al., 2005). Additional references were also used to assist in the identifications of rarer species (e.g., Krammer and Lange-Bertalot, 1985, 1986, 1988, 1991a,b). Photomicrographs of the prominent diatoms in this study are presented in **Figure S1**. Diatom profiles were generated with the software C2 version 1.7.6 (Juggins, 2014), including only the species present with relative abundance ≥ 5% in at least two core depth intervals.

Determinations of past pH and alkalinity in this study were based on the development of transfer function models using C2 and data from Siver et al. (2004, 2005). In their studies, Siver et al. (2004, 2005) developed weighted average models with boot-strapping validation using pH and alkalinity data (non-transformed) from 56 lakes. Taxa not present in at least two lakes were excluded, and in total, 218 taxa were included in the refined models used in this study. Although calibration models are optimal when developed from the region of study, Siver et al.'s (2004, 2005) data were used because they contained more taxa optima estimates for lower pH ranges (<5.5), had key taxa found in this study (photographically documented), and were publically available. For discussion, additional taxonomic comparisons were made with average weighted mean optima developed from the eastern USA and northwestern Quebec (Camburn and Charles, 2000; Fallu et al., 2000; Gaiser and Johansen, 2000; Dixit et al., 2007). These studies were selected because of their locations, photomicrographic validations of taxa, and similar environmental conditions.

Sediment Dating

A total of 40 freeze-dried sediment samples (10 from each of the four cores) was analyzed for ^{210}Pb (46.5 keV), ^{137}Cs (661 keV), ^{214}Bi (609 keV), and ^{214}Pb (295 and 351 keV) by low-background HPGE gamma spectrometry (Appleby et al., 1986). Analysis was completed after storage for one month in sealed polypropylene vials. Sample storage prior to analysis was to achieve equilibration between ^{222}Rn, ^{214}Bi, and ^{214}Pb with ^{226}Ra. The excess ^{210}Pb activity (^{210}Pb$_{ex}$) was calculated by subtracting the ^{226}Ra activity, as determined by the gamma emissions of ^{214}Bi and ^{214}Pb (its daughter isotopes), from that of measured ^{210}Pb. Counting efficiencies were determined using standard solutions of mixed nuclides (QCYB40 from AEA Technology) and sediment material certified for ^{226}Ra activity (IAEA-314 from the International Atomic Energy Agency). The measured activities were corrected for sample geometry and self-absorption (Appleby et al., 1992). In all cores, the vertical profiles of ^{210}Pb$_{ex}$ activity were characterized by an exponential decrease as a function of depth in the sediment (**Figure 2**; **Figure S2**). Assuming no mixing, constant sedimentation rate, and constant input flux of unsupported Pb (CF:CS model; Appleby and Oldfield, 1978), the sedimentation rate, ω, can be calculated using the following relationships:

$$A = A_o e^{-\beta z}, \text{ and}$$
$$\beta = \gamma/\omega,$$

where A and A_o are the activities of the excess ^{210}Pb at depth z and at the top of the core, respectively, β is the slope of ln A vs. z, and γ is the decay constant for ^{210}Pb (i.e., 0.03114 a^{-1}). Sedimentation rates thus determined range between 0.16 and 0.29 cm a^{-1} depending on the sample site (**Figure 2**). **Figure 3** shows ^{137}Cs profiles for each core with peaks corresponding to the maximum atmospheric fallout of ^{137}Cs as a result of nuclear weapons testing (1963–1964). The well-defined ^{137}Cs peaks observed in all the cores coincide closely to the time period at which the atmospheric fallout of ^{137}Cs reached a maximum (1963–1964).

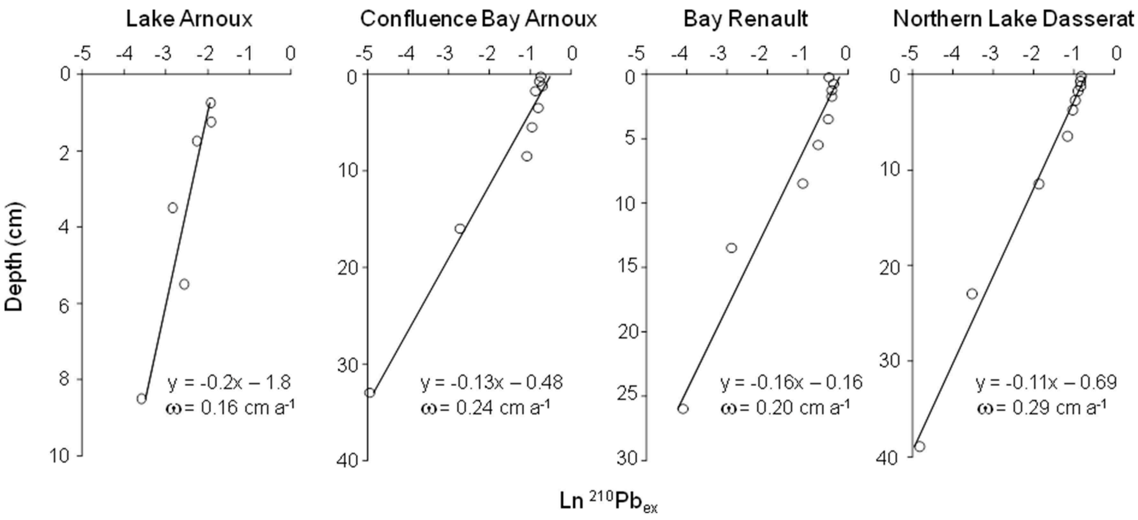

FIGURE 2 | Ln^{210}Pb$_{ex}$ as a function of depth (cm) for each of the four cores. Due to some variability in the ^{210}Pb profiles from Lake Arnoux, only the values that followed the expected decreasing trend in concentration as a function of depth were included in the model (4 data points excluded).

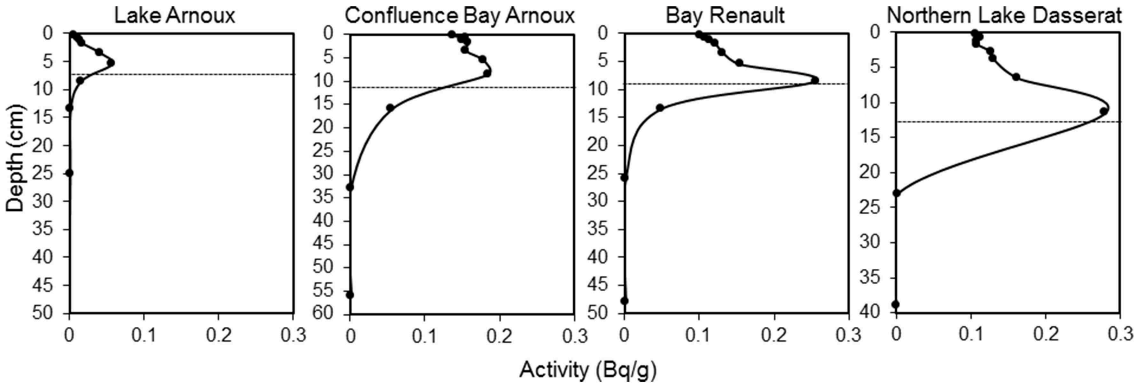

FIGURE 3 | Plots of ^{137}Cs activity as a function of depth (cm) for each of the four cores. The dotted line corresponds to the year 1963–1964 based on sedimentation rates calculated from ^{210}Pb data.

Sulfur Content

After sediment samples were frozen and freeze-dried, they were sieved through an 80-mesh (177 μm) screen. Sediment dissolution was a modified aqua-regia treatment (1F Exploration Geochemistry aqua-regia ultra-trace analysis; Acme Analytical Laboratories, Limited, Vancouver, British Columbia; currently Bureau Veritas Mineral Labs AQ250/AR250 analytical package). Pulp-splits of 0.5 g samples were leached with a 6 ml mixture of HCl, HNO_3, and distilled/deionized water (2:2:2 ratio by volume) at 95°C for 1 h. The sample solution was diluted to 20 ml and analyzed by inductively-coupled plasma mass spectrometry (ICP-MS; Perkin–Elmer Elan instrument) for sulfur contents with a detection limit of 0.02%. Certified reference materials (CRMs; LKSD-1 and LKSD-4), in addition to blind duplicates, were inserted into sediment sample runs for QA/QC. Sulfur (S) contents were expressed as % weight.

Results from aqua-regia digestion are considered a partial digest and were compared with those from multi-acid digestion, which represent near-total dissolution (1T Exploration Geochemistry multi-acid ultra-trace analysis by Acme Labs; equivalent to the current Bureau Veritas Mineral Labs MA250 analytical package; detection limit of 0.04%). Sediments splits of 0.25 g were heated in 10 ml of HNO_3-$HClO_4$-HF solution until fuming and were dried. The resulting residue was dissolved in 4 ml of 50% HCl and heated. After cooling, the solution was loaded into polypropylene test tubes and filled to make a 10 ml volume with 5% HCl before analysis by ICP-MS.

Although measurements of S content after the 4-acid digestion (near total) is consistently higher than those for aqua-regia, the mean difference in S content between the two methods is small (0.02% weight). The two datasets show identical trends in S content with depth at all sediment coring stations. For the purpose of this study, ICP-MS measurements from the aqua-regia digestion are presented.

Relevant Temporal Data Retrieved from Archives

Citizen letters of complaint reported tailings containment failure and environmental degradation of lakes and water courses downstream of the Aldermac mine to different branches of the Quebec government (Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2015; obtained by access to information request). Two letters reported dead fish in Lake Arnoux dating back to 1948. In addition, two letters reported development of a rusty brown-colored bathtub ring along rocky shorelines of Lake Arnoux, which were observed during low water levels and are still evident today. One letter cites pH measurements in the Arnoux River and the center of Lake Arnoux, which were acidic in 1950 (pH of 3.6 and 4.5, respectively). Water analyses, also conducted in 1957 at different sites in the region, measured surface water pH as low as 2.4 in small tributaries of the Arnoux River (on the mine property; Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2015). Additional pH data from 1957 to 1961, reported in citizen letters (Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2015), record pH measurements ranging from 3.3 in Lake Arnoux (near Provencher Creek) to 3.9 in the northern portion of the lake. For this time period, surface water of Lake Dasserat (unspecified location) had a pH between 5.8 and 7.1, whereas Bay Arnoux (before discharging into Lake Dasserat) had low pH of 4.2–5, suggesting acidification downstream of Lake Arnoux (**Figure 1**). Another citizen, in a letter written subsequently in the 1970s, refers to Lake Arnoux as a dead lake, devoid of fish (Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2015). Apart from reported fish observations, there was little to no evidence that the biology and ecosystem health of water systems discharging from the mining site were monitored.

Results

Sediment Sulfur Content

Sediments below 20 cm depth had consistent sulfur (S) values of < 0.06%S. The S content profiles for the three cores in Lake Dasserat showed consistent patterns with %S peaks observed at sediment depths of 8–10 cm (**Figure 4**), occurring after 1965. In all three cores from Lake Dasserat, the initial increase in %S occurred after 1943. The S peak in Lake Arnoux occurred at a depth of 4–6 cm after 1965, but %S started to increase at ∼15–17 cm (early 1900s). The highest %S (2.5%) was observed in Lake Arnoux. Peak %S contents correspond to 7- to 64-fold increases from the late 1950s to the 1970s. All profiles indicated an increase

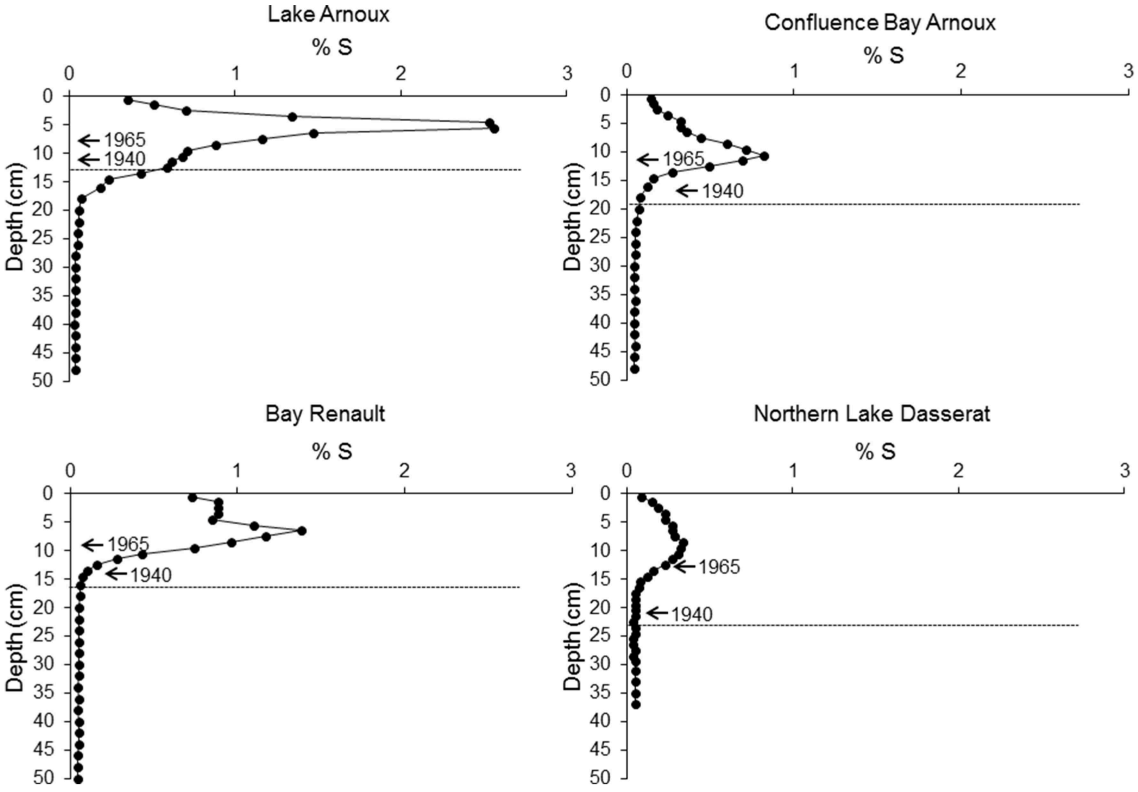

FIGURE 4 | Profiles of total percent sulfur concentrations in sediments for each of the four cores. ^{210}Pb dates aligned with sediment depth are presented with arrows. The dotted line corresponds to the pre-mining period based on sedimentation rates determined with ^{210}Pb data.

and subsequent decline in %S over a 60-year period. Current S content of approximately 0.1% is evident in Northern Lake Dasserat and Confluence Bay Arnoux. Concentrations in Bay Renault persisted at 0.7% and Lake Arnoux registered 0.4%S. At all sites, the rate of decline was equal to the rate of the initial increase, with the steepest increase and decrease in Lake Arnoux.

Diatom Assemblages through Time and Space

The total number of taxa found in all four core sediments combined was 327, representing 64 diatom genera. The genera with the most species were benthic, represented by *Navicula* Bory, *Nitzschia* Hassall, and *Pinnularia* Ehrenberg. Sixteen percent of the taxa were planktonic, predominantly from the genera *Aulacoseira* Thwaites and *Fragilaria* Lyngbye. Calculated valve densities, indicative of diatom productivity, ranged from 159 to >1650 million cells g dry weight^{-1}. The lowest average cell densities were observed in Lake Arnoux at 251 million cells g dry weight^{-1}. The highest average densities were 671 million cells g dry weight^{-1} recorded from Bay Renault.

Overall, *Asterionella formosa* Hassall, *Tabellaria flocculosa* (Roth) Kütz. *sensu lato*, and *Aulacoseira subarctica* (O.Müller) Haworth were the dominant taxa, in terms of occurrence and relative abundance, in all four cores (**Figure S1** and **Data Sheet 1**). However, these taxa were absent or *quasi* absent in the top 6 cm of Lake Arnoux sediments and did not reach a relative abundance of ≥5% in this core. *Aulacoseira pusilla* (Meister) Tuji and Houli was present in all four cores (**Figure S1** and **Data Sheet 1**) but only found with abundances ≥12% in Lake Arnoux with the exception at the top 6 cm where it was completely absent (**Figures 5–8**). *Cyclotella lemanensis* O.Müller *sensu lato* and *Discostella stelligera* (Cleve and Grunow) Houk and Klee *sensu lato* were also present in all four cores with abundances generally ≤10% (**Figures 5–8**). These two taxa were also absent in the top 6 cm of the Lake Arnoux sediments (**Figure 5**). The abundance of *T. flocculosa* increased in Lake Arnoux from 16 to 7 cm and then disappeared (**Figure 5**). In Confluence Bay Arnoux and Bay Renault, *T. flocculosa* increased after the initiation of the mine and stabilized (**Figures 6**, **7**). In contrast, *T. flocculosa* decreased from 13 cm depth to present in Northern Lake Dasserat (**Figure 8**). *A. formosa* was present in larger numbers (>5%) at all the Lake Dasserat sites and was <5% in Lake Arnoux. Prior to 1940, *A. formosa* relative abundances in Northern Lake Dasserat, Bay Renault, and Confluence Bay Arnoux were lower with an increase during the mining and post-mining period. Recently there has been a reduction in *A. formosa* similar to pre 1940s levels (**Figures 6–8**). In Lake Arnoux, *A. formosa* was absent in the counts after 1975 (**Figure 5**). The proportion of benthic diatom species was relatively constant throughout the cores (20–30% of the assemblages), with the exception of Lake Arnoux, where a sharp increase, up to 99%, was observed in the top 6 cm (**Figure 5**). *Brachysira microcephala* and *Fragilaria crotonensis* were prominent spatially-distributed tychoplanktonic/benthic species in Lake Dasserat (Confluence Bay Arnoux, Bay Renault, and Northern Lake Dasserat;

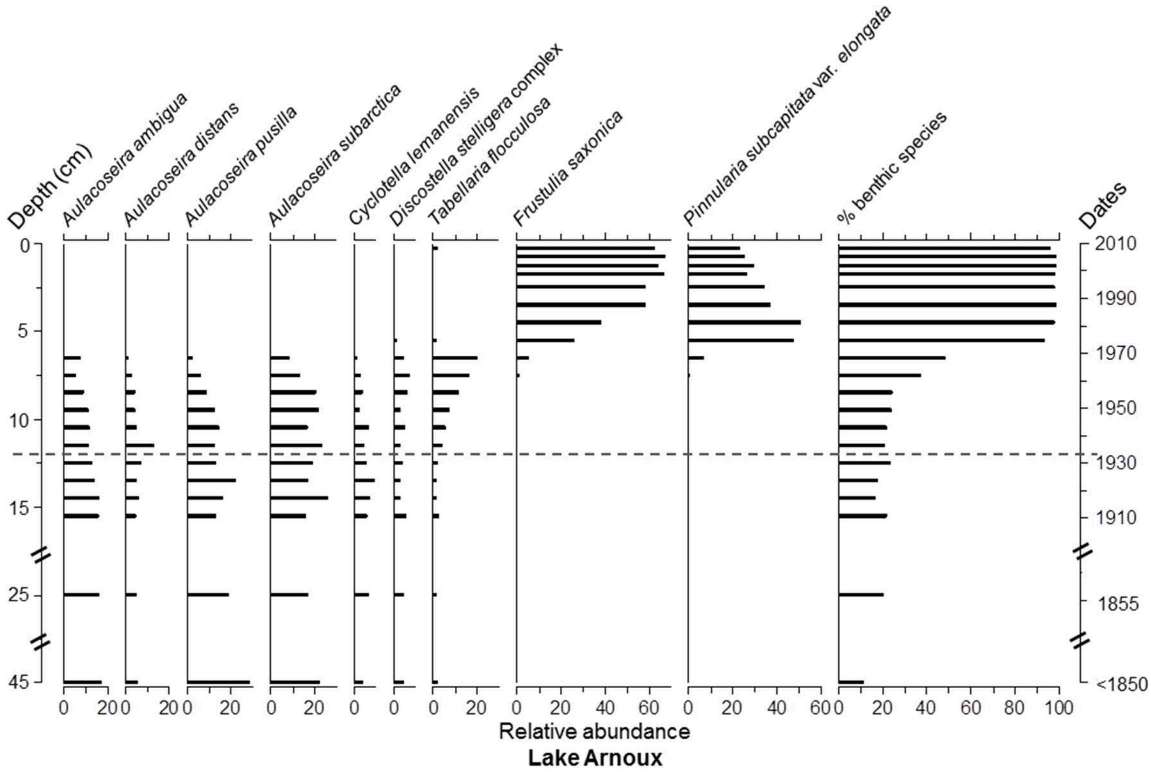

FIGURE 5 | Diatom taxa paleolimnological profiles represented as % abundance in the benthic sediments of Lake Arnoux.

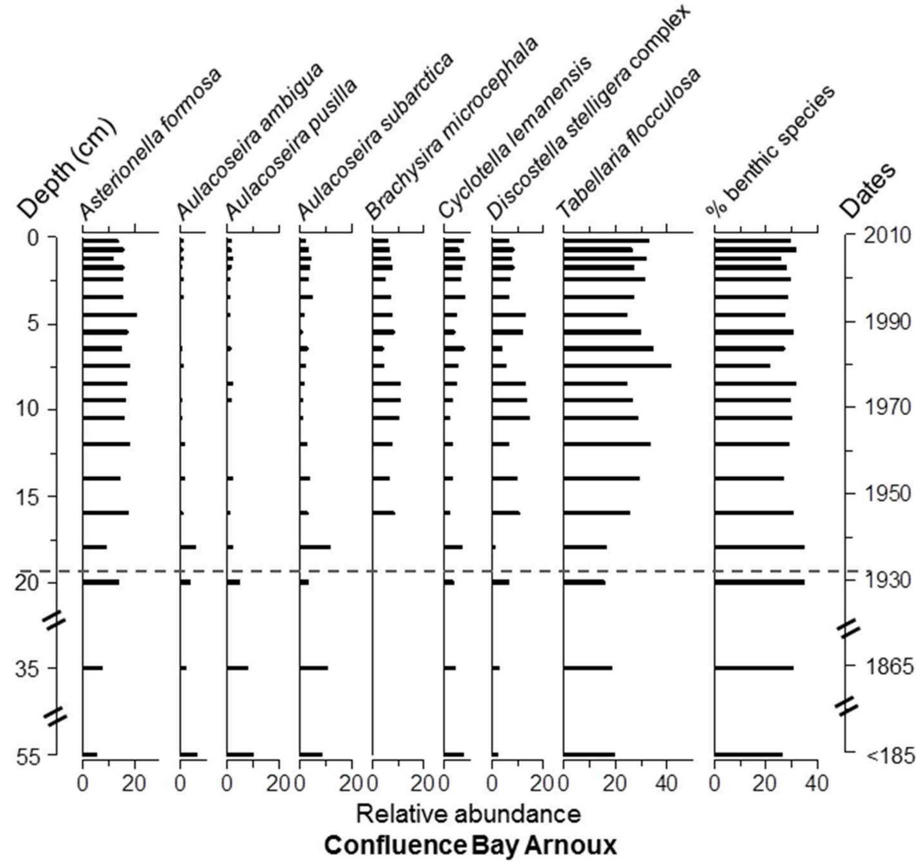

FIGURE 6 | Diatom taxa paleolimnological profiles represented as % abundance in the benthic sediments of Confluence Bay Arnoux. The dotted line corresponds to he pre-mining period based on sedimentation rates determined with ^{210}Pb data.

Figure S1 and **Data Sheet 1** and **Figures 6–8**), whereas *Frustulia saxonica* and *Pinnularia subcapitata* var. *elongata* dominated in Lake Arnoux (**Figure 5**). Numerous other benthic species were observed in this study, but their relative abundances were <5%.

The assemblages from Lake Arnoux showed an abrupt change in taxonomic composition and life form in the top 6 cm of the core (circa 1965), switching from a dominance of planktonic *Aulacoseira* species to a dominance of benthic acidophilic taxa, including *F. saxonica* and *P. subcapitata* var. *elongata* (**Figure 5**). This marked shift in the assemblages was preceded by a gradual increase in *T. flocculosa* and a subtle decrease in *A. ambigua* and *A. pusilla* between 12 and 7 cm depth. Chrysophyte cysts (not shown) decreased gradually from 68.4 million at the bottom to 7.9 million cysts g dry weight^{-1} at the top of the core.

The three cores from Lake Dasserat recorded similar and less variable profiles in the diatom assemblages. Nevertheless, some changes in the structure of the assemblages are worth noting. For example, in the Confluence Bay Arnoux core, the benthic species, *B. microcephala,* appeared at 16 cm and remained present to the top of the core with relative abundances between 5 and 12% (**Figure 6**). This observation was accompanied by a decrease in *Aulacoseira* species, as well as an increase in *A. formosa*, *D. stelligera*, and *T. flocculosa*. These changes correspond to the estimated period of approximately 1940. Despite the sudden appearance of *B. microcephala*, the overall proportion of benthic species did not increase to a large extent in the core. However, from 16 cm to the top of the core, this species dominated the benthic assemblage, whereas more diverse benthic assemblages were present in the older sediments. In Bay Renault, the most significant modification in the diatom assemblage structure happened circa 1965 showing an increase in *T. flocculosa* with abundances between 15 and 30% down-core and 30–55% from a depth of 10 cm and up (**Figure 7**). This increase in *T. flocculosa* was preceded by the appearance of *B. microcephala* circa 1945, but with abundances never ≥8%. In Northern Lake Dasserat, the most significant changes through time were the increase in *Aulacoseira* spp. (particularly of *A. subarctica*) and *F. crotonensis*, and the overall general decline of *A. formosa* and *T. flocculosa* circa 1990 (**Figure 8**).

Diatom pH and Alkalinity Reconstructions

The weighted averaging model for pH (data not transformed) using inverse deshrinking was:

$$\text{Estimated pH} = 0.852\ \text{pH} + 0.918$$

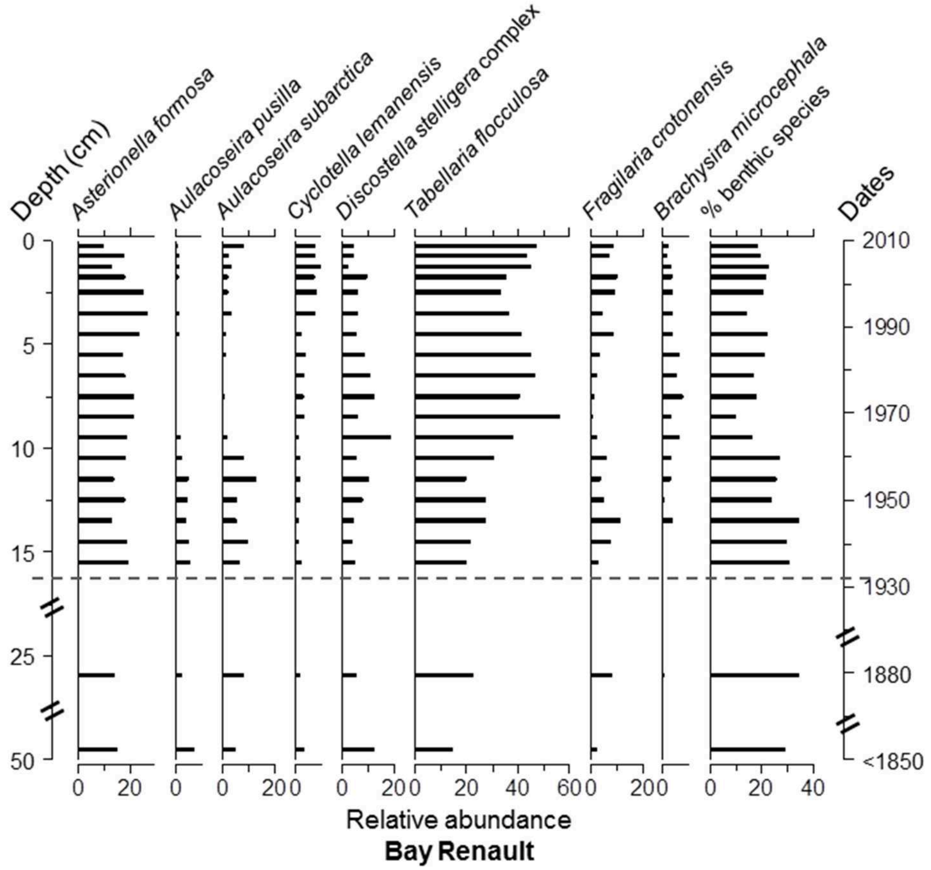

FIGURE 7 | Diatom taxa paleolimnological profiles represented as % abundance in the benthic sediments of Bay Renault. The dotted line corresponds to the pre-mining period based on sedimentation rates determined with ^{210}Pb data.

with an R^2 of 0.85, a root mean square error (RMSE) of 0.35 and a root mean square error of prediction (RMSEP) of 0.44. A selection of the prominent taxa in this study showed a broad spectrum of pH optima estimates (**Data Sheet 2**). *P. capitata* var. *elongata*, *B. serians,* and *F. saxonica* had the lowest pH optima, 4.8, 4.82, and 4.92, respectively. In contrast *Ulnaria ulna*, *Stephanodiscus niagare*, and *A. formosa* had higher pH optima determinations of 7.34, 7.29, and 7.00, respectively.

Estimates of pH in Lake Arnoux from <1850 to present varied from 4.09 to 7.17 (**Figure 9**). Eight diatom taxa, representing 60–95% of the community, were central to these pH determinations. There was a significant drop in pH between 5.5 and 8.5 cm (circa 1965). Below 8.5 cm the estimated pH ranged from 6.74 to 7.34, whereas between 5.5 cm and 0.25 cm, the estimated pH ranged from 4.09 to 4.42. Between 7 and 31 taxa were used in the pH reconstructions. In contrast, Bay Renault, Confluence Bay Arnoux, and Northern Lake Dasserat had estimated circumneutral pH-values throughout the core sections (pH ~7.0). Confluence Bay Arnoux from 0.25 to 15 cm had estimated pH-values at or below 7.0 (**Figure 9**), whereas Bay Renault and Northern Lake Dasserat had values at and slightly above 7.0. Confluence Bay Arnoux, Bay Renault, and Northern Lake Dasserat used 19–30, 15–32, and 14–27 taxa in the down-core reconstructions, respectively.

The weighted averaging model for alkalinity (data not transformed) was:

$$\text{Estimated alkalinity} = 0.728\ \text{alkalinty} + 10.713$$

with an R^2 of 0.73, a RMSE of 33.15 and a RMSEP of 41.9. Estimates of alkalinity from Lake Arnoux ranged from −72 to 109 μeqL^{-1} (standard errors varied from ± 42 to 90 μeqL^{-1}), and showed consistent values up to the 1950s with estimated concentrations ranging from 73 to 103 μeqL^{-1} (graph not shown). After the 1950s, alkalinity dropped to negative estimates, with the lowest values between the depths of 0.25 and 5.5 cm. The low buffering capacity of the lake prior to the 1950s changed to no buffering capacity after the reported tailings pond breach. Alkalinity levels were stable and improving slightly at the top of the core (2011). Lake water alkalinity in August 2013 was measured at 160 μeqL^{-1} (unpublished data), indicating recent improvements in water quality and buffering capacity. In contrast, alkalinity estimates from <1850 to present from Confluence Lake Arnoux, Bay Renault, and Northern Lake Dasserat ranged from 69 to 107 μeqL^{-1} with estimated standard

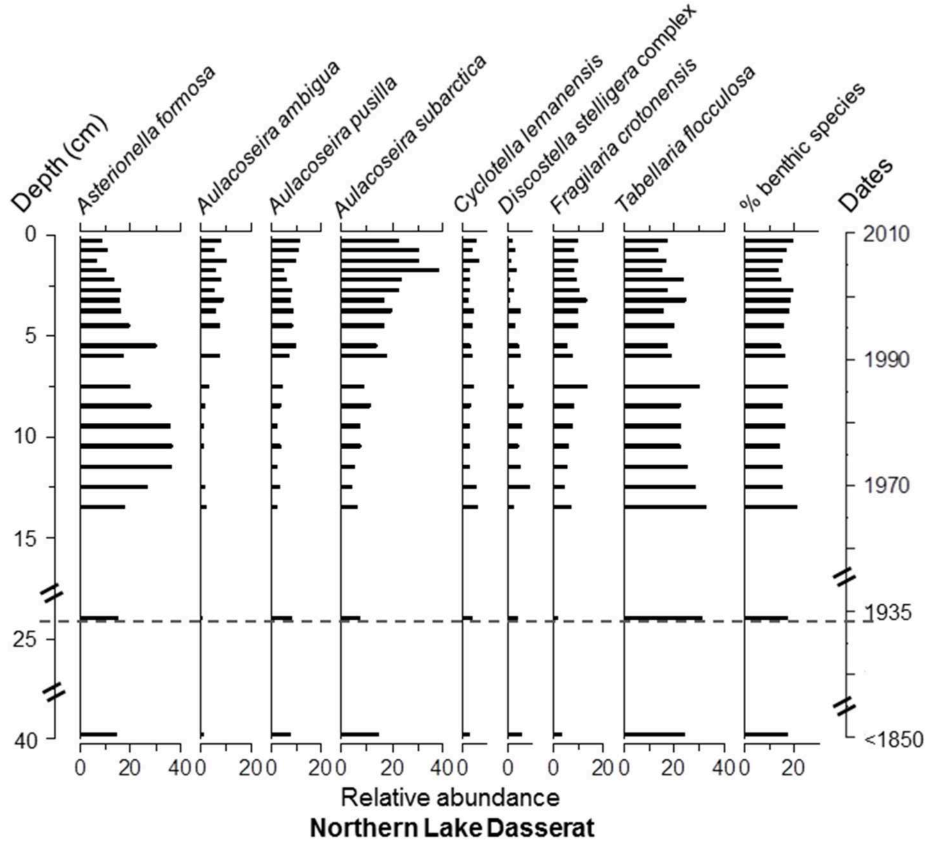

FIGURE 8 | Diatom taxa paleolimnological profiles represented as % abundance in the benthic sediments of Northern Lake Dasserat. The dotted line corresponds to the pre-mining period based on sedimentation rates determined with ^{210}Pb data.

errors ± 41 to 44 μeqL^{-1}. The estimated alkalinity profiles were strongly correlated with the estimated pH profiles and, therefore, are not presented here.

Discussion

Sedimentation Rates and Sulfur Content

The sedimentation rates (0.16–0.29 cm a^{-1}) calculated for the Lake Arnoux–Dasserat system were comparable with published studies for other boreal lakes in the region (Moingt et al., 2014). Sedimentation rates estimated using the ^{137}Cs profiles and the dating horizon were relatively similar to values obtained based on the CF:CS model. This indicates that the chronology for this study is reliable at the stations in Lake Dasserat. The ^{210}Pb sedimentation rates for all three sites in Lake Dasserat were similar (0.20–0.29 cm a^{-1}).

In contrast, the coring station in Lake Arnoux has an estimated sedimentation rate of 0.16 cm a^{-1}. Considerations include the nearly uniform shallow bathymetry of Lake Arnoux (4–5 m) and lack of a clear sediment depocentre, so that sedimentation would be spatially distributed in the immediate area of the mouth of the Arnoux River. The approximate correlation of the ^{137}Cs peak with a sediment date of 1963–1964 (**Figure 3**) further indicates that the higher sedimentation rates recorded at stations located in zones of sediment focussing in Lake Dasserat were not possible at the coring station in Lake Arnoux. However, it is reasonable to expect that sediment mixing (e.g., by wind action or bioturbation) in Lake Arnoux could have compromised the dating to some degree.

Marked increases in S content were observed after 1950 in Lake Arnoux, with the initial increases staring at about 16 cm (15–17 cm sediment section). The age error associated with this 2 cm sediment section represents ± 12 years, which would place the initiation of mining operation at about 1917. This is before the opening of the mine and the difference in chronologies could be associated with some uncertainties with the ^{210}Pb dating for Lake Arnoux (low number of data points). The uncertainties in dating could be simply a problem of sediment mixing in the bottom of Lake Arnoux, which could also account for the presence of increasing %S lower in the sediments (15–17 cm). In addition, there could be a possibility of soluble S migration downward in the sediments resulting in a subtle %S gradient. Finally, we have no information about the occurrence and duration of pre-mining activities which could account for some early increases in %S.

During the mining operation (1932–1943), there was a consistent increase in %S in the sediments of Lake Arnoux which halted briefly before the sharp increase after 1965 peaking at 2.5 %S. The immediate drop in S from the 1960s to present was at

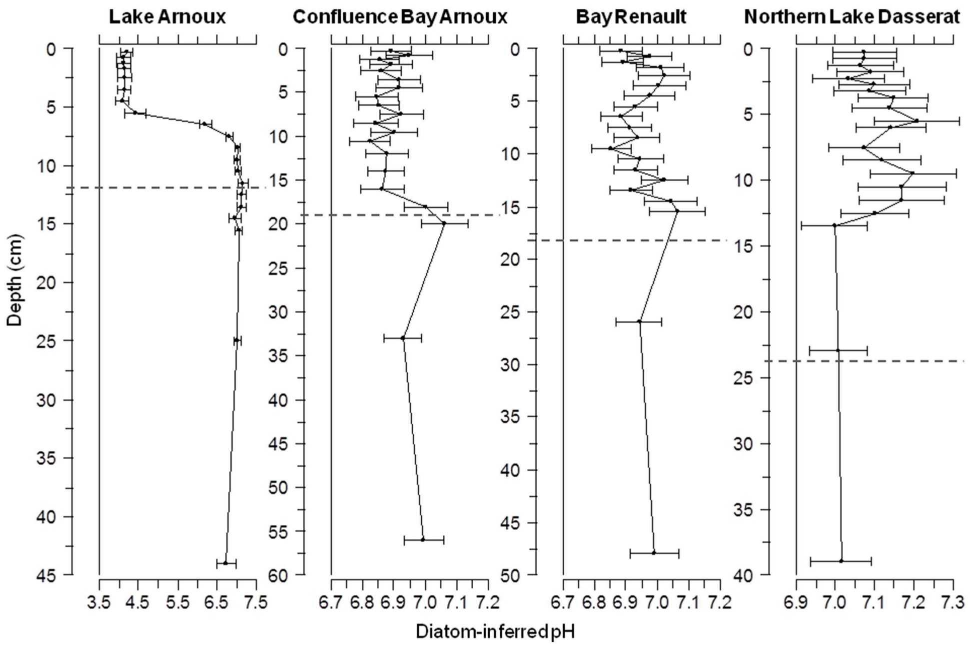

FIGURE 9 | Profiles of diatom-inferred pH-values for Lake Arnoux, Confluence Bay Arnoux, Bay Renault and Northern Lake Dasserat. The dotted line corresponds to the pre-mining period based on sedimentation rates determined with ^{210}Pb data. The dotted line corresponds to the pre-mining period based on sedimentation rates determined with ^{210}Pb data.

the same rate as the increase, which suggests that there was a single large pulse of S influx in the late 1960s and early 1970s. Coupled with recent findings of high S levels in tributaries of the Arnoux River near the Aldermac site ($>10000\ \mu M\ SO_4^{2-}$, Leguay et al., in press), it appears that the primary source of S is the Aldermac site. Bay Renault had 46%, Confluence Bay Arnoux 60% and Northern Lake Dasserat 85% less S, relative to Lake Arnoux (based on peak concentrations). Sediments from Lake Arnoux recorded increases in S content before 1940, whereas increases at the Confluence Bay Arnoux and Bay Renault stations started after circa 1950. As for Northern Lake Dasserat, increases in S content occurred after 1960, but were much lower. These results support an aquatic vector for the migration of S away from the Aldermac mining site. Although these findings seem logical, S can also be influenced by other vectors (e.g., westerly winds, water discharge rates). The Lake Dasserat region is also within the wind circulation and zones of influence of smelters in Sudbury (Ontario) and Rouyn-Noranda (the Horne smelter; e.g., Olsen et al., 1982; Mayer et al., 2007). Therefore, other industrial sources could contribute to S deposition in the study lakes by atmospheric transport.

Evidence for a tailings pond breach on the Aldermac property was reported by Bédard (2000), highlighting that a mining waste containment structure broke in 1942. Water contamination and dead fish were also reported from Lake Arnoux in 1948 (Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2015) in addition to a citizen's letter of complaint from 1959 (Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2015) of a tailings containment failure on the Aldermac property and resultant release of contaminated waters into Lake Arnoux and beyond. A citizen's letter also mentioned that the plume of contaminated waters from Lake Arnoux discharging into Lake Dasserat was visible from the air and looked like the "tentacles of an octopus" (Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2015). It is not discernible whether these reports were independent records of the same tailings pond breach or were two separate breaches. The change in accumulation rates and sharp increases in S in Lake Arnoux over a short period are consistent with the tailings pond rupture explicitly reported. The sharp decline in %S after the peak indicated a discrete pulse of S input, which abruptly ceased. High ^{210}Pb dating errors limit the precise determination of breach year(s). Similar patterns in S content distributions were also evident at the more distal Lake Dasserat coring stations, but were not as distinctive. In addition to atmospheric transport and other local mining activities, the surface water flow in the Lake Arnoux–Dasserat system was also altered. For example, the regional drainage divide was shifted by damming to accommodate the early logging industry, which relied on wood floating for timber transportation to the province of Ontario, causing a flow reversal in Lake Dasserat (Organisme de Bassin Versant Abitibi-Jamésie, 2015). This reorganization of the watershed became problematic for the hydropower station situated to the north because of reduced flow. A decision was later made to return the drainage divide line to its natural position in 1942 with the construction of multiple dams still in place today (Organisme de Bassin Versant Abitibi-Jamésie, 2015). The surface flow reversals could have affected aquatic transport of S into Bay Renault after mining operations ended. However, flow reversal in Lake Dasserat did not affect S content and diatom composition in Lake Arnoux.

Past Mining Activities and Diatom Assemblages

The striking turnover in diatom composition (planktonic to benthic assemblages) observed in the Lake Arnoux core clearly demonstrates major temporal environmental changes related to a gradual, then rapid decline in pH. The reconstruction of pH from the diatom transfer function model showed pre-industrial pH of about 7.0, similar to the three sampling stations in Lake Dasserat. This level was maintained up to the 1950s, then dropped dramatically in alignment with the pulse of S input from the reported rupture of a tailings pond on the abandoned mine site (Citizen report, Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2015). pH-values of 3.3 and 3.9 reported in Lake Arnoux between 1957 and 1961 coincide with the reported tailings pond rupture (Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2015). The pH reconstruction at the top of the core (2011) was 4.2, whereas the modern measured pH value for Lake Arnoux was 4.5, which is within error of the model. Increases in surface water pH from an average of 4.5 in 2011 to 5.7 and 6.7 in 2012 and 2013, respectively, in addition to the early reappearance of the planktonic *T. flocculosa*, are early indicators of a temporal trend toward less acidic waters.

The changes in diatom assemblages and water quality in Lake Arnoux started from 1930–1940, when the Aldermac mine was operating. However, increases in %S in the sediments peaked in the mid to late 1960s and followed the same increasing trend as *T. flocculosa*. The peak in %S matched the abrupt changes observed in the diatom community from planktonic to benthic species. Changes in lake water pH were significant from circum-neutral or slightly acidic waters before mining to strongly acidic waters after mining. Lake acidification was reported by local citizens (Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2015) and by inferred pH from the diatom transfer function model. The similar rates of increase and decrease at all the sampling stations, up to 10 km from the Aldermac site, indicate that there was a regional effect farther downstream. The slow increase and decrease in %S and lower maximum S content at the Northern Lake Dasserat site also suggest a lag response compared to more proximal sites.

The change of the diatom community in Lake Arnoux, after mining operation terminated, and the coincident peak in S levels, indicates a tolerance of the diatom community to stressors. The pulse of S-rich sediment input into Lake Arnoux during the tailings breach could have blocked the sunlight because of increased suspended particulate matter, reduced buffering capacity and altered pH. In reaction to this stress, the plankton community declined, and more tolerant acid-loving benthic diatoms appeared, like acidophilic *F. saxonica* and *P. subcapitata* var. *elongata*. The new light condition favored benthic diatom development, resulting in a suitable environment for the dominance of acidophilic species like *F. saxonica* and *P. subcapitata* var. *elongata*. Lower abundances of chrysophyte cysts toward the top of the core suggest lower numbers of living planktonic chrysophytes, which is consistent with the disappearance of prominent planktonic diatoms during the same period. The total disappearance of planktonic taxa and abrupt transition to benthic taxa show that primary productivity in Lake Arnoux switched from planktonic to benthic. However, the lake sediment record indicates that the change in diatoms was not likely instantaneous but developed over a period of up to 5 years, which is consistent with a biological lag response. The paleolimnological transitions in diatom composition were in two phases: (1) an increase in the planktonic taxa *T. flocculosa* (circa 1942–1960); and (2) a change from planktonic to benthic species (circa 1960–1970).

In other sectors of the Abitibi mining region, pH has been highlighted as a significant environmental stressor. The fossil diatom assemblages of Lake Perron (current pH 3.72) and Lake de la Pépinière (current pH 4.7) included the benthic *F. saxonica*, but not *P. subcapitata* var. *elongata* (Dixit et al., 2007). Overall the three lake systems (Arnoux–Dasserat, Perron, and de la Pépinière) had different fossil diatom assemblages, although all had predominantly benthic acidophilic species, indicating clear-water lakes.

Although valve deformities have been observed in low pH and metal-contaminated waters (e.g., Cattaneo et al., 2004; Falasco et al., 2009; Morin et al., 2012), they were rare in this study. Some species are tolerant of high metal concentrations and can be good indicators of elevated metal levels (Morin et al., 2012). For example, a number of taxa within the genus *Pinnularia* have been reported as acid-tolerant organisms occurring in acidic pit ponds (e.g., *P. ferroindulgentissima*) and bogs (*P. subcapitata* var. *elongata*) (Czarnecki and Cawley, 1997; Krammer, 2000). This can explain, in part, why deformed valves were not observed in Lake Arnoux. Further, Verb and Vis (2000) reported that *P. subcapitata* was present in acid mine drainage waters and tolerant of elevated metal concentrations (e.g., Al, Fe, Mn). Verb and Vis (2000) also reported that *Frustulia rhomboides*, a closely aligned taxon to *F. saxonica,* was dominant in acid mine drainage waters. Finally, the low species richness observed in Lake Arnoux after the mid-1960s can be explained by the highly acidic and elevated metals environment, suggesting that species richness or diversity can also represent an indicator of mining effects.

The paleolimnological diatom assemblages at all three sites in Lake Dasserat were generally invariant over the last 200^{+} years, with planktonic diatoms dominating the assemblages. The open water site of Northern Lake Dasserat showed some changes in the diatom composition (from *A. formosa* and *T. flocculosa* to *A. subarctica* and *F. crotonensis*; two more circum-neutral species living in similar nutrient conditions) from the early 1990s to present, but the changes were not evidently linked to pH. Both Bay Renault and Confluence Bay Arnoux sites demonstrated an increase in *T. flocculosa* from the early 1900s up to 1965 and then became stable to present day. There was also an introduction of the benthic species *Brachysira microcephala,* an acid-loving species, at these sites (<10% abundance). These subtle changes in the diatom community possibly suggest minor shifts in lake water alkalinity or pH, but there was no statistical significance to the change. Confluence Bay Arnoux is distal enough from the immediate discharge of Lake Arnoux, and within the buffering capacity of Lake Dasserat, to minimize the environmental effects of S input. Other mining activities near Bay Renault may have affected water quality and induced the presence of *B. microcephala*.

Mining Economics vs. Environmental Debt

Mining, both past and present, has been a significant contributor to the Canadian economy. Today, mining and associated processing industries contribute an estimated US \$34 B a^{-1} to the economy (Marshall, 2014). Over the last 5 years this represented an average contribution of 5% to the Canadian Gross Domestic Product with tax estimates to federal and provincial/territorial governments at US \$7.6 B a^{-1} (Marshall, 2014). The mining industry directly accounts for approximately one in every 46 Canadian jobs and, indirectly through goods producing sectors, contributes one in every 10 jobs. In 2011, mining added US \$10.3 B to Quebec's economy by employing 85,000 in 32 mines and plants (GEreports, 2015). The region of Rouyn-Noranda and Val d'Or in Quebec is considered one of the seven major Canadian frontiers, which were developed through mining.

Since 1995, mining companies operating in Quebec are obligated to submit both a mine rehabilitation plan and closure plan to the government of Quebec for approval (Ministère de l'Énergie et des Ressources Naturelles, 2015). The 1995 Mining Act was intended to protect the government of Quebec from unforeseen site restoration expenditures and ensure that mining companies followed socially and environmentally acceptable practices. A modified version of the Act was enacted in 2013, increasing the liability of the mining company to 100% of the projected rehabilitation costs. This, along with increased royalties and taxation, has dropped Quebec from 1st to 21st in the world's ranking for mining exploration (GEreports, 2015). Both a mine remediation plan and closure plan must be approved before mining leases can be granted. This is in stark contrast to the early 1900s, when no such legislation or environmental risk assessment existed. It is no surprise that mining companies, like the proprietors of the Aldermac mine, abandoned the site after operations ceased, without any notion or obligation in site rehabilitation and further environmental restorations. With respect to older abandoned or orphaned mines in Quebec, it becomes the responsibility of the government to deal with the legacy of environmental degradation and accompanying debt. Large amounts of money need to be invested to reclaim the sites. In the case of the Aldermac property, to date over US \$16.5 M in restoration costs were spent since 2008. Adjusted for inflation, this government expenditure for site remediation in 1942 would have been equivalent to circa US\$ 1.3 M (United States Department of Labor, 2012). If known at the time, the magnitude of the rehabilitation expense would have been weighed by past governments against revenues from taxes, economic development, and employment.

As a cost-benefit analysis exercise, an estimate of the time-equivalent value of resources extracted from the Aldermac mine can be compared with costs of environmental restoration. Over the 11-year period of operation, the Aldermac mine produced circa 200,000 tons of extracted resources (Ministère de l'Énergie et des Ressources Naturelles, 2015). This grossed an estimated US \$130 M based on metals market values of 1943 (Schodde, 2010; Macrotrends, 2015). Presented at today's market values, (inflation adjusted, not market adjusted) these commodities of copper, silver, and gold would represent circa US \$1.7 B (United States Department of Labor, 2012). Based on a 7% corporate taxation rate for the mining industry in 1935 (Vallières, 2012), the Aldermac mine returned circa US \$9.1 M to the government of Quebec; inflation adjusted to 2010, this represents US \$121.7 M. The inflation-adjusted tax, averaged over the lifespan of Aldermac operations, would represent 1.2% of the current mining tax revenue generated for Quebec per year (based on 2011 taxation data). In today's economy, the Adermac mine would be an important contributor to the GDP of Quebec. A simple calculation of profit vs. rehabilitation expenses (\$121.7–16.5 M) would further suggest that the government of Quebec ultimately profited from the Aldermac mine even after pay-it-backward remediation activities. It is important to also recognize the unaccounted economics of mining during the development of a young country. In the 1930s, Canada was in the middle of the Great Depression of North America. People needed jobs to survive and governments needed tax dollars. Based on the cost to mine copper in 1943 (Schodde, 2010), the primary metal commodity produced at the Aldermac mine, it is estimated that US \$68 M was spent in mining costs to build and operate the mine; a large proportion of this amount in support of both local and Quebec-based business. No additional estimates of economic benefits can be made credibly because data are not available. In total, even with the pay-it-backward environmental costs, the economic benefits justified the extraction of these mineral resources during the 1930s using the technology and ethical values of the period.

However, the results here show a significant environmental alteration in Lake Arnoux with substantive S loading, along with drastic changes in pH and alkalinity. These changes in Lake Arnoux have biologically altered lake health. Remediation expenses of \$16.5 M represent costs toward environment stabilization (shoreline reconstruction, containment of seepage, etc.) but not directly toward removal of the problem. The proposed remediation plan is containment and stabilization on the mining property itself, with natural biological (e.g., ecosystem recovery) and physical process (e.g., sediment capping) doing the work of "cleaning the environment" on the site and farther downstream. The cost to remove the contaminants would be prohibitive and change the economic balance-sheet.

Based on modern economics, paying-it-backward in rehabilitation costs and unforeseen future socio-economic costs is not socially or financially acceptable (Ministère du Développement Durable de l'Environnement et de la Lutte Contre les Changements Climatiques, 2012). To assess the cost to global and human sustainability, the economics of conserving and recycling resources must also be considered along with the value of restored ecosystems, including goods and services they supply. New approaches in assessing the economics of our terrestrial and aquatic footprints will effectively merge sustainable environmental conservation with industrial development (e.g., Hoekstra et al., 2011; Zhi et al., 2015). For example, the restoration of the Solbec-Cupra mining site in Quebec during the 1990s, at a cost of US $4.8 M, resulted in a high-quality environment. The restoration created goods and services (tourism, recreation, new development, refugia development for wildlife) with an estimated worth ca. US $0.75–$1 M per year in 2009 (Vittet, 2011). Estimating goods and services of an ecosystem after mine site restoration (sustainable economics) represents an interesting argument for site remediation investments and improved rehabilitation plans.

Future-proof mining represents a global initiative of the mining industry to streamline costs, centralize knowledge, and optimize efficiencies (GEreports, 2015). Mining will not be for new resource prospects but for the best new prospects. The new business model will optimize productivity and limit environmental liabilities under the economics of lower profit margins (GEreports, 2015). Investments in post-restoration monitoring will be a fundamental component of future mine site remediation plans. As standard planning, secondary economic benefits should be incorporated into mining initiatives, whereby high-quality environments will be created with accompanying goods and services. This *ad hoc* assessment should not only be restricted to the directly affected site, but also extend to adjacent receiving environments. The social license to operate is generally perceived to be more crucial in areas downstream of mining operations (e.g., tourism, recreation, local community health). In the current study of the abandoned Aldermac mine, adjacent lake environments (Lake Arnoux, Lake Dasserat) were economically impacted with respect to hunting, fishing, tourism, and recreation. Micro-economics, along with more stringent environmental legislation, will drive the new model of mining economics.

Conclusions

A multi-proxy assessment was used to evaluate the water quality and ecosystem health of lakes downstream of the abandoned Aldermac mine in northwestern Quebec: (1) diatoms were used as paleolimnological bio-indicators to evaluate spatial and temporal environmental effects of mining massive sulfide deposits nearly eight decades ago, (2) sulfur content of lake sediments was analyzed as a pH driving vector, and (3) paleolimnological findings supported testimonial observations, which provided documented evidence for contaminant releases by on-going acid mine discharge and discrete tailings breach(es) after the mine was abandoned. Diatom assemblages in lake sediments indicate that Lake Arnoux was similar in pH, water quality (oligotrophic to weakly mesotrophic) and diatom composition as other lakes in the region before the onset of mining. Post-mining, the surface water of Lake Arnoux became increasingly acidic; after 1940 a shift from circumneutral to strongly acidic conditions were inferred from diatom assemblages. Once acidified, the water column allowed for the development of a poorly-diverse benthic diatom community, dominated by acid-loving metal tolerant species, in place of the planktonic species.

There were potential early signs that restoration activities (initiated in 2008) were starting to return receiving aquatic environments to pre-mining states, from a marked rise in surface water pH in Lake Arnoux and the early appearance of a planktonic diatom. However, it is clear that increased investments in post-restoration environmental monitoring are required. Monitoring, both on the abandoned mine property and in receiving environments downstream, serves as a tool for performance measurement of restoration strategies and adaptive management. The ultimate success of remedial restoration initiatives necessitates performance measurement in both on-going economic costs and long-term health of the environment.

During the Great American Depression, mining initiatives, like the Adermac mine, supported the economy of Quebec and Canada. Based on the historical period of economic growth for Quebec and time-equivalent value, revenues from the Aldermac mine were significant and justify the current costs for environmental remediation. However, this model is not sustainable in the modern economy and does not meet the requirements for environmental sustainability of resource development. New approaches, like the gray water footprint modeling (e.g., Zhi et al., 2015) are required to merge economic and environmental sustainability for future prosperity.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2015.00099

Figure S1 | SEM images of the prominent diatoms in Lake Arnoux. 1, *Frustulia saxonica*, whole valve external view; 2, *Frustulia saxonica*, valve apex internal view; 3, *Pinnularia subcapitata* var. *elongata*, internal view; 4, *Pinnularia subcapitata* var. *elongata*, apex internal view; 5, *Tabellaria flocculosa*, external view; 6, *Asterionella formosa*, external view; 7, *Aulacoseira subarctica*, external view; 8, *Aulacoseira pusilla*, external view. Scale bars: 1 = 20 μm; 3, 5, 6 = 10 μm; 2 = 5 μm; 4, 7, 8 = 2 μm.

Figure S2 | $^{210}Pb_{ex}$ as a function of depth (cm) in the sediments for each core.

Data Sheet 1 | Diatom counts (relative abundances) for each of the four cores.

Data Sheet 2 | Spectrum of pH optima estimates for the prominent taxa observed in this study.

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