

Long-term environmental changes in the Canadian boreal zone: Synthesizing temporal trends from lake sediment archives to inform future sustainability

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Abstract

Covering 55% of Canada's total surface area and stretching from coast to coast to coast, the Canadian boreal zone is crucial to the nation's economic and ecological integrity. Although often viewed as relatively underdeveloped, it is vulnerable to numerous stressors such as mining, forestry, and anthropogenic climate change. Natural archives preserved in lake sediments can provide key insights by quantifying pre-disturbance conditions (pre-1850 CE) and the nature, magnitude, direction, and speed of environmental change induced by anthropogenic stressors over the past ~150 years. Here, we paired a review of paleolimnological literature of the Canadian boreal zone with analyses of published sediment core data to highlight the effects of climate change, catchment disturbances, and atmospheric deposition on boreal lakes. Specifically, we conducted quantitative syntheses of two lake health indicators: elemental lead (Pb) and chlorophyll *a*. Segmented regressions and Mann-Kendall trend analysis revealed a generally increasing trend in elemental Pb across the boreal zone until ~1970 CE, followed by a generally decreasing trend to the present. Snapshot comparisons of sedimentary chlorophyll *a* from recent and pre-industrial sediments (i.e., top-bottom sediment core design) revealed that a majority of sites have increased over time, suggesting a general enhancement in lake primary production across the boreal zone. Collectively, this body of work demonstrates that long-term sediment records offer a critical perspective on ecosystem change not accessible through routine

monitoring programs. We advocate using modern datasets in tandem with paleolimnology to establish baseline conditions, measure ecosystem changes, and set meaningful management targets.

Keywords

Paleolimnology, Taiga, Metals, Eutrophication, Environmental Monitoring

Introduction

Stretching across the circumpolar north, the boreal zone is central to the economic and ecological integrity of the eight countries that host this ecosystem (Brandt 2009). Within the boreal zone, both terrestrial and freshwater habitats perform key ecosystem functions, including carbon sequestration on a globally significant scale (Cole et al. 2007; Kurz et al. 2013; Lemprière et al. 2013). Although the boreal zone is best known for its emblematic coniferous forests, it is also covered by extensive freshwater resources and has among the highest densities of lakes in the world (Messenger et al. 2016). Indigenous Peoples, whose cultures, livelihoods, and food security are strongly tied to the freshwater and terrestrial landscapes, maintain traditional homelands in the boreal zone (Kassi 2019).

The urgency to protect, restore, and adjust management practices in the boreal zone is ongoing (Bradshaw et al. 2009; Carlson et al. 2019). Although the boreal zone is widely considered to be pristine, it supports various sectors of activity important to regional and national economies based mainly on natural resources (e.g., forestry, mining), thus exposing both terrestrial and aquatic ecosystems to numerous stressors. The fragmentation of landscapes for resource extraction and other human activities has the potential to increase propagule pressure for non-native taxa and rearrange food webs (With 2004; David et al. 2017; Povoroznyuk et al.

2022). In addition, like other high-latitude regions, the boreal zone is experiencing accelerated climate change (Bush et al. 2019; Berner and Goetz 2022). As a result, drought, insect and disease outbreaks, and forest fires have become more frequent and intense with the changing climate since the beginning of the 20th century, despite such events previously occurring at lower frequencies as naturally occurring phenomena (Price et al. 2013). The increased fire activity, thawing of permafrost, as well as other complex factors, can also lead to aquatic browning, where surface waters become more stained due to enhanced loading of organic matter and iron (Monteith et al. 2007; Finstad et al. 2016), creating pronounced lake-catchment interactions. Understanding how these intensifying stressors affect aquatic systems is critical to the responsible stewardship of inland waters in the boreal zone.

While lake monitoring records are valuable for tracking ecosystem dynamics and informing environmental management actions, most sites are not extensively studied, and sampling techniques often change over time. Furthermore, sites are often remote and pose technical and financial challenges that hinder systematic measurement campaigns. At best, North American monitoring records might span ~50 years, and are often far shorter, initiated after the onset of significant environmental change (e.g., ~1850 following the industrial revolution, ~1950 following the second world war; Räsänen et al. 2006; Bennion et al. 2011; Smol 2019). As a result, knowledge of pre-disturbance conditions is generally lacking. Natural archives preserved in lake sediments (i.e., the focus of paleolimnology) can provide important insights across timescales greatly exceeding the longest monitoring programs (Smol 2019; Figure 1). Paleolimnological records can be used to reconstruct pre-disturbance conditions over hundreds to thousands of years into the past and quantify the magnitude of long-term changes induced by anthropogenic stressors. Furthermore, lake sediments integrate materials from watersheds,

airsheds, and across aquatic habitats (e.g., littoral, limnetic, profundal) year-round, providing comprehensive archives of processes within lakes and their catchments (Catalan et al. 2013). A clear example of the power of paleolimnological records was showcased during the acid rain debates of the 1970-80s, when these data provided key evidence of changes in biological assemblages and even allowed for the quantification of past pH levels that served to inform policy and decision-making (Whitehead et al. 1990; Smol 2019).

A substantial proportion of the boreal zone (28%) is located in Canada (Brandt et al. 2013), covering over half the Canadian landmass and reaching into seven provinces and all three territories. Due to its prominence in Canada, a recent effort, namely the Boreal 2050 project, was launched, which brought together opinions of stakeholders and thought-leaders to assess the risks associated with “failing to achieve the future sustainability of the boreal zone” (Creed and Serran 2019; Creed et al. 2019). The leaders of the Boreal 2050 project identified the major drivers of change in the boreal zone posing potential risks to its future sustainability (i.e., atmospheric change, demands for ecosystem services, demographics and societal values, industrial innovation and infrastructure, and governance) and explored outcomes for the boreal zone along various trajectories (typically, scenarios of reduced impact, business as usual, and increased impact; Creed et al. 2019). However, one noteworthy gap in this initiative was the consideration of long-term data arising from paleolimnological approaches.

Inspired by the aim of the Boreal 2050 project to draw paths toward a sustainable future in the boreal zone, we present the strengths of paleolimnological analyses in the assessment of the drivers of change for this region. Sediment records can anchor future scenarios with a historical perspective that includes consideration of pre-disturbance conditions. Here, we provide a literature review of several known external drivers of change in the boreal zone, explored

through the lens of paleolimnology. We then demonstrate how paleolimnological tools can be used to answer important questions regarding ecosystem dynamics in the boreal zone over the past ~150 years with an analysis of lead (Pb) and sedimentary chlorophyll *a* concentrations across Canada. Finally, we conclude that paleolimnology should be applied to contextualize future environmental change and lake ecosystem dynamics of the Canadian boreal zone.

Boreal lakes: A paleolimnological perspective

The capacity of lakes to capture, accumulate, and preserve settling particles over time enables paleolimnologists to track catchment- or climate-linked drivers of change (externally) as well as shifts in biotic and abiotic interactions within the lake (internally). For example, metal or charcoal concentrations in lake sediments can be measured and directly linked to processes such as mining activities and forest fires, respectively. Numerous biological indicator groups, including the remains of specific algal and animal taxa that are well preserved in lake sediments, may be indirectly associated with ecosystem drivers. These biological proxies have been extensively studied and are often used to infer limnological properties and autochthonous dynamics including nutrients, pH, temperature, depth, and predation, among others (Reavie 2020). Taken together, a suite of paleolimnological proxies can uniquely elucidate the diversity of stressors affecting the Canadian boreal zone and its lakes over centennial time scales (Table 1).

Climate

Anthropogenic climate change is a major threat affecting the boreal zone. Although warming trends have been observed throughout the region, northern latitudes have been

particularly susceptible to warming in recent decades (Brandt et al. 2013; Yeung et al. 2019). In contrast, precipitation patterns have changed heterogeneously across the region over the past half century (from 1950-2013; Yeung et al. 2019). General trends suggest an almost 40% increase in precipitation in eastern and northern boreal systems, while some continental areas exhibited a decline (Brandt et al. 2013). Anthropogenic climate changes have already resulted in adverse effects on the boreal zone, including declining wildlife populations, spread of non-native species, increased frequency of cyanobacterial blooms in freshwaters, and increased tree mortality, among other threats which are projected to continue to impact the region as reviewed by Yeung et al. (2019). In lakes, a synthetic examination of paleolimnological records across North America and Europe revealed a common biological response in diatom assemblages (a class of siliceous algae preserved in lake sediments) to anthropogenic climate warming, with earlier shifts observed in high-latitude and high-altitude lakes compared to temperate sites (Rühland et al. 2008; 2015). Looking at periods of past warmth in paleolimnological records (e.g., the Holocene Thermal maximum, the Medieval Climate Anomaly), although not perfectly analogous, can also be useful for predicting the effects of future warming (e.g., Laird and Cumming 2008; Ma et al. 2013; Table 1).

Climate warming can affect lakes via numerous complex interactions including altering water temperatures and ice cover dynamics (Rühland et al. 2008; Leavitt et al. 2009). In turn, the degree/duration of water column stratification, changing water levels, the length of the growing season, and sources and patterns of runoff may all be affected (Fritz 1996; Rühland et al. 2015; Woolway et al. 2022). Given this complexity, paleolimnological studies often incorporate multiple indicators and use several lines of evidence to examine past climatic trends. Based on analyses of primary production metrics, the magnitude of historical changes observed in boreal

lakes is similar to those apparent in temperate regions (Rühland et al. 2008; Labaj et al. 2013; Griffiths et al. 2022). At the southern border of the boreal zone, a multi-proxy paleolimnological study linked climate warming to the novel appearance of nuisance cyanobacterial blooms in a remote, oligotrophic lake (Favot et al. 2019). This trend has also been observed in other boreal-transition lakes, despite stable or even declining surface water nutrient levels (Favot et al. 2019), perhaps driven partly by nitrogen-fixing cyanobacteria that can bloom and/or taxa that can migrate to deeper waters on a diel basis to tap into hypolimnetic nutrient pools (Molot et al. 2014; Rienl et al. 2021). Cyanobacterial blooms are more typical for lakes south of the boreal, but their appearance in the boreal transition zone suggests they may become more common in a warming climate.

Permafrost underlies much of the boreal forest to the north, but with warming these landscapes are becoming increasingly dynamic. A pan-Arctic study found higher lake dissolved organic carbon (DOC) in boreal permafrost sites than in tundra sites, stressing the effects of rapid permafrost thawing on increased lake browning levels in this area (Stolpmann et al. 2021). DOC concentrations in these lakes depend on multiple interacting environmental factors (e.g., permafrost extent, topography, vegetation cover; Stolpmann et al. 2021), hydrological and morphometric factors (e.g., lake water retention time, drainage ratio, precipitation patterns; Houle et al. 2020) as well as anthropogenic factors (e.g., landscape utilization, recovery from acid deposition; Meyer-Jacob et al. 2015; Meyer-Jacob et al. 2019). The shallow lakes that dominate this region are facing unique challenges as a result of anthropogenic climate change (Bouchard et al. 2017). Paleolimnological studies of thermokarst lakes (i.e., lakes formed when thawing permafrost creates surface depressions that are filled with meltwater) have examined responses to warming using many approaches. For example, studies have inferred declines in

snow melt and increased evaporation based on changes in stable oxygen isotopes (Bouchard et al. 2013) and changes in water clarity and mercury transport due to peat subsidence based on shifts in diatoms, plant biomarkers, and mercury concentrations (Coleman et al. 2015; Korosi et al. 2015). Some studies further disentangled the timing and impacts of thaw slumps on lake water quality based on diatoms (Thienpont et al. 2013) and climate-driven lake level declines due to massive drainage events based on organic matter content, stable carbon isotopes and pigment concentrations (MacDonald et al. 2012).

A central feature of the boreal zone is the forest itself, whose establishment and geographic extent has been examined using indicators preserved in sediment cores. For example, pollen, stable isotopes, organic matter content, and diatom assemblages were used to track the northward advance of the central boreal treeline during a mid-Holocene period of climate warming, which was followed by a southward contraction of the treeline (Pienitz et al. 1999). Forest fires are also a major concern in the boreal zone, with a general trend towards increasingly frequent fire events and fire season length (summarized in Yeung et al. 2019). However, there is considerable variation across the region, with charcoal records from the southeastern boreal suggesting decreased fire frequency in the 20th century, attributed to the regional increase in summer moisture (Carcaillet et al. 2001).

In-lake modifications and catchment disturbances

With growing pressures for land and resource use in the boreal zone, lakes are likely to experience changes at the lake or watershed level, such as eutrophication, altered biotic interactions via the introduction of exotic or predator species, and heavy metal contamination from mining activity. Using paleolimnology, we are able to track in-lake modifications using

indicators or measurements to infer, for example, phosphorus levels, pH, algal production, summer deepwater oxygen dynamics, predator-prey dynamics, and other pollutants (Smol 2008).

Eutrophication

Direct human activities such as agriculture and deforestation can increase nutrient inputs (especially phosphorus), which cause an increase in lake primary production, a process known as eutrophication. Eutrophication can cause several effects including harmful algal blooms, depleted deep water oxygen, fish kills, and more (Schindler et al. 1971; Quinlan and Smol, 2001; Carpenter 2005). Warming temperatures can further exacerbate these effects.

Several paleolimnological proxies exist to infer effects of eutrophication following nutrient enrichment. Sedimentary chlorophyll *a* and its diagenetic products can be used to assess total algal production and infer historical lake trophic status (Leavitt and Hodgson 2001; Wolfe et al. 2006; Michelutti et al. 2010; Michelutti and Smol 2016). Many diatoms differ in their affinity to nutrients and have species-specific phosphorus optima. As a result, diatom community composition can be used to model changes in total phosphorus by investigating past assemblages preserved in sediments (Hall and Smol 1992; Reavie et al. 1995). Similarly, the oxygen requirements of chironomid species (non-biting midges, Diptera: Chironomidae) are reasonably well documented, and their subfossil assemblages preserved in sediments can be used to reconstruct deep-water dissolved oxygen conditions (Quinlan et al. 1998; Clerk et al. 2000; Brooks et al. 2001).

An expected outcome of eutrophication is decreased hypolimnetic oxygen due to enhanced oxygen consumption during organic matter decomposition. Therefore, coupling changes in the composition and abundance of diatom and chironomid assemblages strengthens

confidence in an inferred phosphorus increase due to interaction between nutrient and oxygen levels in lakes. For example, Heinrichs et al. (2005) used this coupling approach in a boreal lake to identify human impacts over several decades. They observed an increased diversity among littoral/oxygen-sensitive chironomids while profundal taxa disappeared, and an increased diatom-inferred total phosphorus in response to development in the catchment. Eutrophication and the enrichment of symptomatic cyanobacterial blooms have also been examined in boreal lakes with sedimentary pigment and analyses of cyanobacteria akinetes (Blais et al. 2000; Favot et al. 2019). Furthermore, these proxies have been used to demonstrate the partial success of certain management strategies, such as the implementation of sewage treatment, and at least partial return to pre-disturbance conditions (Little et al. 2000).

Predation and trophic interactions

The Canadian boreal zone is well known for its plentiful freshwater fishing resources. However, increased angling demand and consequent fish introductions have heavily modified endemic fish populations in some lakes of the region. The introduction of these predators has also contributed to the spread of non-native species, altered species interactions across multiple trophic levels, and has overall shifted the balance, stability, and diversity of food webs (Vander Zanden et al. 1999). Fish communities are not always well captured in paleolimnological studies (although novel methods are emerging with considerable promise, e.g., Wang et al. 2021). However, there are several tools based on subfossils that estimate changes in predator-prey interactions and food web stability. For instance, we can track changes in fish predation pressures through time by utilizing the plasticity of certain anti-predation traits and structures of cladoceran zooplankton that appear when top-down pressures are high (Chen et al. 2011; Korosi

et al. 2013). Of note, mucro, carapace, and antennule lengths in Cladocera taxa (namely *Bosmina* spp.) can reflect predation intensity of *Chaoborus* spp., which in turn is heavily influenced by fish introductions and predation within a lake (Labaj et al. 2014a). *Chaoborus* assemblages are commonly studied to infer fish introductions and disappearance as species dominance (particularly *C. americanus*) is often altered due to fish presence or absence, (e.g., Lamontagne and Schindler 1994; Uutala et al. 1994; Sweetman and Smol 2006; Labaj et al. 2013) as well as other important environmental parameters (e.g., lake depth, pH, sodium concentrations, and lake surface area; Kurek et al. 2010). *Chaoborus* spp. may also be valuable indicators of low-oxygen conditions in the hypolimnion (key fish habitat; Quinlan and Smol 2010) as they are mobile and able to oxy-regulate within the haemolymph (Scholz and Zerbst-Boroffka 1998), finding refuge from planktivory in low-oxygen waters. Typically, *Chaoborus* remains are examined in conjunction with other invertebrates (e.g., chironomids; Quinlan and Smol 2010) or with respect to assemblage composition (Kurek et al. 2010).

Some of the above studies highlighted the strengths of leveraging multiple paleolimnological proxies to elucidate multi-trophic interactions among freshwater populations that are sensitive to environmental change. However, preservation of subfossils can sometimes be poor for soft tissues, making it challenging to consider all components of the food web. Sedimentary DNA is emerging as a tool to address this limitation and reconstruct past population and community dynamics in lakes and their catchments (Domaizon et al. 2017; Crump 2021; Gregory-Eaves et al. 2023). DNA from a broad diversity of biota is preserved under the light-shielded, often low-temperature conditions of lake sediments within intact cells or in the form of extracellular molecules adsorbed onto sediment particles. Using molecular genetic techniques (Capo et al. 2021), DNA archived in lake sediments is recovered from across the tree of life and

notably from organisms that are soft-bodied and do not leave morphological remains, including fungi (Talas et al. 2021; von Hippel et al. 2022) and microorganisms (including microbial eukaryotes, cyanobacteria, bacterioplankton, and viruses; Capo et al. 2016; Monchamp et al. 2016; Kisand et al. 2018; Garner et al. 2020; Barouillet et al. 2022; Lemieux et al. 2022).

Sedimentation rates and abiotic changes

Deriving the rate of lake sediment accumulation and establishing chronologies is a required step for numerous paleolimnological investigations to determine the absolute age of sediments, but it also serves as a key metric of the functioning and dynamics of lake ecosystems and their surroundings. Crucially, sedimentation rates are the basis for the calculation of fluxes of matter that enters and is subsequently stored in lake sediments. These fluxes provide yearly estimates for the assessment of elemental budgets (e.g., organic carbon, metals), which in turn anchor systemic changes observed in lakes for several proxies. Owing to their generally lower productivities, lakes set in the boreal region tend to accumulate sediment at a lower rate than the neighbouring and more populated Mixedwood Plains ecozone of Canada (i.e., southern Ontario and Québec; Baud et al. 2022). However, similar to what has been observed globally, lake sedimentation rates in the boreal zone have been increasing since the 1850s. The magnitude of increase since 1850 CE varies across sites and ranges between 1.5 to >10 fold (Baud et al. 2022).

Coincident with increasing sedimentation rates, the accumulation of organic carbon in lakes has also been increasing since the early 20th century (Anderson et al. 2020). Organic carbon accumulation has increased 1.8 fold in boreal lakes since ~1850 CE (Heathcote et al. 2015). Moreover, this increase seems to have accelerated over the second half of the 20th century

(Anderson et al. 2020), similar to the trend observed in sedimentation rates (Baud et al. 2022). These changes might be driven by an increase in soil leaching and eutrophication (Kothawala et al. 2014; Anderson et al. 2020) coupled with higher terrestrial productivity (Drake et al. 2018), due to more intense and frequent precipitation (de Wit et al. 2018) in the context of climate change and warming (Crann et al. 2015). Climate warming leads to the melting of permafrost in boreal regions, increasing soil erosion and material loading in lakes. This can change nutrient supplies, affect carbon sources, and influence the composition of material reaching lake sediments (Hanson et al. 2004). Based on observed increases in terrestrial inputs, researchers have found that allochthonous carbon buried in lake sediments is made up of mainly recalcitrant compounds, leading to lower mineralization (Dean and Gorham, 1998; Sobek et al. 2009). Taken together, these factors may be changing the composition of lake sediments and contributing to increased carbon accumulation.

Metals and acidic precipitation

There is a plethora of precious metal deposits across the boreal zone, exemplified by the presence of ~80% of Canada's active mines located in the region (Wells et al. 2011; 2020). This region also has a relatively high density of retired mines, which can continue to affect metal concentrations in lakes for several decades (Leppänen et al. 2017; Kay et al. 2023). The extraction and refining of these deposits can cause heavy metal contamination of lacustrine systems via effluent runoff, groundwater seepage, and atmospheric deposition. Declines in phytoplankton and zooplankton biodiversity following aquatic metal contamination are common (Roch et al. 1985; Wilk-Woźniak et al. 2011; Winegardner et al. 2017). In addition, significant

toxicological and physiological effects have been reported in fish and humans who contact, consume, or otherwise rely on metal-contaminated freshwater (Amundsen et al. 1997; Wani et al. 2016; Javed and Usmani 2019). Mining activities can also generate substantial emissions of sulfur dioxide and nitrous oxides that contribute to acidic precipitation, the effects of which can be particularly pronounced in boreal shield lakes that have limited buffering capacity (Whitehead et al. 1990; Meyer-Jacob et al. 2019).

Sediment archives are well suited to evaluate heavy metal contamination, with many elements such as chromium, copper, mercury, lead, and zinc being relatively stable following sediment deposition (unlike others such as iron or manganese that are influenced by redox conditions and are therefore mobile in the sediment column; Förstner 1976; Rydberg and Martinez-Cortizas 2014). Several studies have identified post-industrial metal enrichment in boreal lakes linked to human activities such as oil sand developments (Skierszkan et al. 2013; Cooke et al. 2017) and mining (Couillard et al. 2008; Cheney et al. 2020; Jasiak et al. 2021). These studies also included lakes distant from contaminant sources and highlighted a key finding: a significant component of metal enrichment in boreal lakes is atmospherically derived. For example, a study of five undisturbed headwater lakes located > 100 km from large sources of industrial pollution observed moderate to high metal enrichment relative to pre-1860 sediments (Wiklund et al. 2020). Metal enrichment has been observed over even greater distances – a review by Landers et al. (1998) found a widespread, post-industrialization increase in mercury deposition to boreal lakes as far as 2,000 km from an urban or industrial source. Long-range atmospheric deposition is perhaps best exemplified by lead; the introduction and subsequent ban of leaded gasoline has been linked to observable changes in lacustrine sediment lead concentrations over time (Gallon et al. 2005; Ndzangou et al. 2005; Dunnington et al. 2020a).

While the direct measurement of elemental concentrations is a well-established method to track heavy metal pollution, some emerging sediment DNA technologies have been developed to pair with these traditional methods. For example, microbial mercury detoxification encoded in sediment DNA has been used to track historical anthropogenic mercury deposition to lakes (Poulain et al. 2015; Ruuskanen et al. 2020).

In addition to metal contamination, intense mining and industrial activities can generate far-reaching acidic precipitation that may be deposited in lakes. In the heavily industrialized area of Sudbury, Ontario, an estimated 7000 lakes in a 17,000 km² area were acidified to the point where biological damage occurred (Neary et al. 1990; Keller 2009). Widespread national concern about lake acidification during the 1980s led to the creation of the PIRLA projects (Paleoecological Investigation of Recent Lake Acidification), which applied a paleolimnological approach to investigate how North American lakes had changed in response to acidification and sulfur emission reduction programs (Charles and Smol 1990; Whitehead et al. 1990; see Battarbee and Renberg, 1990 for a European perspective). Across much of North America, recent recovery trends have been observed (Stoddard et al. 1999; Garmo et al. 2014), but biological recovery did not necessarily follow (Vinebrooke et al. 2002). For example, depleted calcium concentrations from decades of acidic precipitation can affect crustacean zooplankton populations for decades after precipitation quality has improved (Jeziorski and Smol, 2017). Complicated recovery pathways have also been observed in diatom assemblages due to novel anthropogenic stressors like climate change and road salts (Cheng et al. 2022). Re-acidification pathways can also be mediated by climate-induced drought, which remobilize acid ions stored in wetlands (Faulkenham et al. 2003). Paleolimnologists have used a variety of biological indicators to develop insights into interactions between acidic precipitation and climate change, and this

body of work exemplifies how paleolimnology may be applied to better understand environmental change on a regional scale (Smol 2019).

Few of the above boreal paleolimnological studies extended beyond a regional scale. Indeed, due to the logistical and feasibility challenges associated with conducting large-scale sampling efforts over the Canadian boreal zone, (paleo)limnological studies are often limited in geographic scope. In the following section we present a synthesis of paleolimnological data from sediment records collected within the Canadian boreal zone to illustrate how paleolimnology can characterize trends in key limnological properties across the broader landscape. In particular, we demonstrate how both coordinated sampling efforts and data synthesis of published paleolimnological data can generate an informed perspective on where, how and when environmental change occurred in the Canadian boreal zone over the past ~150 years, with a focus on two key processes of interest that are widely observed and studied across the boreal zone: metal enrichment (as an example of abiotic change) and eutrophication (as an example of biotic change).

Temporal trends in metal enrichment and eutrophication across the Canadian boreal zone

Elemental lead (Pb) contamination is recognized as a major environmental concern, with binational policy actions taken to reduce its impact across North America (Nriagu, 1990). As such, several studies have utilized sediment cores to investigate metal contamination in lacustrine systems across Canada (e.g., Michelutti et al. 2010; Roberts et al. 2019), including a data synthesis approach by Dunnington et al. (2020a). Herein we build on these earlier efforts and synthesize available elemental Pb data across the Canadian boreal zone. In addition, with warming and eutrophication expected to strongly impact higher latitude areas including the

boreal zone, we chose to investigate trends in sedimentary chlorophyll *a* (which includes its main diagenetic products) as an indicator of nutrient enrichment in lakes.

Case study 1: Time series investigation of sedimentary Pb records

To assess how temporal trends in metal enrichment have varied across the Canadian boreal zone, we assembled a dataset of 62 boreal and hemiboreal lake sediment cores from existing paleolimnological literature. We indexed the Web of Science Core Collection in January 2022 with the following search terms:

CU (Country/Region) = **Canada** AND (TS (Topic) = **lake*** OR TS = **lacustrine***) AND TS = **sediment*** AND (TS = **lead contamina*** OR TS = **lead pollut*** OR TS = **lead emission*** OR TS = **element* lead** OR TS = **element* Pb**)

We sought to include studies that presented time series data of elemental Pb concentrations or fluxes for Canadian boreal zone lake sediment records and covered the time period of at least 1900 – 1990 CE (for details on our inclusion process and reasons for exclusion, see supplemental materials S1). Our search identified 487 sources whose titles and abstracts were reviewed by at least two study authors. This narrowed the number of articles down to 118, and then full-text articles were reviewed by a single study author for potential inclusion in our synthesis. Our search ultimately included 37 studies, but we added one more study suggested by a co-author that did not appear in our search (Meyer-Jacob et al. 2019). This additional study focused on dissolved organic carbon rather than elemental Pb, but nevertheless presented Pb concentrations through time.

To extract elemental Pb records from the studies included in our data synthesis, we first looked to the supplemental materials for downloadable data used in the study. In the absence of open data, we used the 'DigitizeIt' software (Bormann 2012; Rakap et al. 2016) to convert Pb records presented in figures into a usable format, a method which shows a high degree of reliability and validity (Rakap et al. 2016). In some cases, digitization was not possible without risking significant error (e.g., due to overlapping data points, data presented as averages or ratios, inconsistent axis labelling) and so these records were removed from our database. In total, we obtained data for 62 boreal zone lake sediment records from our 38 included studies; for further details on their analytical methodologies, please refer to supplemental materials S2 or the original studies.

Our study was limited to the use of Pb concentrations to investigate trends in elemental Pb, rather than Pb flux. Pb flux normalizes elemental concentrations to their respective sedimentation rates, and this approach is recommended for generating mass balances (Engstrom and Rose 2013). However, Dunnington et al. (2020b) recently compared numerous geochemical metrics for two sediment cores (i.e., fluxes, tracer element ratios, enrichment factors, excess measures, and centred log-ratios and concentrations) and found that Pb concentrations were reasonable indicators of Pb pollution in all cases. In our data synthesis, only 12 of the 62 sites presented elemental Pb fluxes. For studies that did not present Pb fluxes, we considered digitizing ^{210}Pb chronologies to calculate flux, but ^{210}Pb sediment chronologies and measurement errors were often absent, making it difficult or impossible to do so with confidence. We therefore opted to focus on concentrations and examined overall trends on a per-site basis.

We used segmented linear regressions to identify significant breakpoints in elemental Pb trends. We subset Pb records to only consider data from 1850 CE to present day, as ^{210}Pb dating

is best suited to recent sediments (Baud et al., 2022). We performed regressions in the R software environment (R Core Team 2022) using the package ‘segmented’ (Muggeo 2022). To determine the ideal number of breakpoints for each record, we assessed segmented models using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) (Akaike 1974; Schwarz 1978). AIC and BIC measure the fit of parameterized models, with BIC more highly penalizing additional model parameters, and are best applied together (Kuha 2004). When the AIC/BIC of two models were within two criterion units (Burnham and Anderson 2002), or in cases of disagreement between AIC and BIC in the number of breakpoints, we selected the model with fewer breakpoints as the best fitting segmented model. For each record, we used analysis of variance (ANOVA) to compare the best fitting segmented model to a simple linear fit (Dunnington et al. 2020a) and in most cases, the segmented model significantly outperformed a simple linear fit ($p < 0.05$). When the segmented model did not provide a significant improvement ($n = 15$ cases), we did not report a breakpoint. Visualized fitted models are available in the supplemental materials S3.

We split our time series into before and after the identified breakpoint (i.e., historical vs. modern trends, respectively) and quantified trends using the nonparametric Mann-Kendall (M-K) test from the package ‘trend’ (Pohlert, 2020). When our breakpoint analysis identified two significant breakpoints, we split the time series into historical and modern trends by the most recently identified breakpoint. The M-K test is based on the Kendall rank correlation and returns a tau (τ) value for each time series between -1 and +1, with -1 showing a monotonic decreasing trend and +1 showing a monotonic increasing trend. In the absence of a statistically significant breakpoint, we applied the M-K test to the entire time series, identified these instances as “Full M-K tests,” and presented them as a modern trend. Occasionally, splitting time series by

breakpoint meant that they did not meet the mandatory 3 data points to apply a M-K test; in these instances, no trend is presented. We visualized the results of our M-K tests using the ArcMap™ 10.8.1 ArcGIS® software by Esri. For further details on M-K trend results and associated p-values see supplemental materials S4.

The mean timing of the most recent breakpoint identified across all of the elemental Pb profiles was 1973 CE $\pm$ 14.6 years ($n = 47$). Trends in elemental Pb before the most recent breakpoint (i.e., historical trends) suggest a widespread increase across the boreal zone around the onset of industrialization, with all lakes demonstrating either a slightly increasing (M-K $\tau = 0.01 - 0.49$, $n = 6$) or strongly increasing (M-K $\tau = 0.5 - 1.0$, $n = 41$) trend (Figure 2). A total of 15 sediment cores had no significant breakpoint detected and thus no historical trend was determined. Conversely, modern trends in elemental Pb (i.e., after the most recent breakpoint) exhibited more heterogeneity relative to historical trends, but many decreased across the boreal zone (Figure 3). A total of 46 lakes had a strongly negative trend (M-K $\tau = -1 - -0.5$; $n = 36$) or a slightly negative trend (M-K $\tau = -0.49 - -0.01$; $n = 10$), while 13 lakes slightly increased during the modern period (M-K $\tau = 0.01 - 0.49$; $n = 7$) or strongly increased (M-K $\tau = 0.5 - 1$; $n = 6$). Three sediment cores have no trend reported because there were not enough points post-breakpoint to calculate the M-K trend.

One of the primary pathways for elemental Pb to enter lacustrine systems is through atmospheric deposition, enabling lead to traverse long distances before entering the landscape (Marx et al. 2016; Dunnington et al. 2020a). Given that leaded gasoline was a primary contributor to atmospheric Pb until the mid-1970s, legislated efforts to remove Pb from gasoline that began in 1975 appear to have contributed to the declining trends observed (Nriagu 1990). Other environmental regulations and policies including the phasing out of coal appear to have

contributed to declining Pb levels in some areas as well (Weiss et al. 2002; Gallon et al. 2005). The increasing historical trends we observed across the boreal zone are congruent with these earlier studies. In addition, the timing of the push toward unleaded gasoline agrees well with the average breakpoint year identified in our lakes that were best described by a segmented linear model (~1973 CE). With 74% of boreal zone lakes exhibiting a decreasing modern trend, it seems likely that elemental Pb concentrations have generally declined across boreal lakes in response to policy efforts to reduce Pb emissions. Still, 13 lakes included in our synthesis exhibited an increasing trend during the modern period, with six of these sites strongly increasing (M-K $\tau = 0.5 - 1.0$).

Strongly increasing modern M-K trends were found in three Northwest Territories lakes (Figure 3, red box), likely caused by nearby gold mining operations at Giant Mine. Wind direction is one possible explanation for why this increase was observed in some, but not all lakes. Several lakes at an equivalent distance from Giant Mine exhibited decreasing trends in Pb enrichment. However, these lakes were in a non-dominant wind direction and researchers posited that the terrestrial supply of legacy metals was therefore lower (Jasiak et al. 2021; Kay et al. 2023). The importance of wind direction is further emphasized in a Pb isotope study from nearby Yellowknife Bay which found that downwind legacy metals stored in the terrestrial environment were responsible for the metal enrichment of modern sediments (Pelletier et al. 2020). Metal enrichment of lacustrine systems can thus persist following the cessation of mining activities, particularly in areas downwind of mining sites. However, of the 42 study lakes that original authors identified as being in close proximity to mines, only 21% showed continued increases in Pb. It is possible that the other 79% of lakes are impacted, but only exhibit elevated levels of other heavy metals; however, as Pb is almost always a co-product of metal mining operations

(Brown et al. 2009), we would generally still expect to see Pb enrichment in response to mining, regardless of the type of mine. This reinforces the importance of local factors, such as wind direction, terrestrial legacy metal supply, and surface and groundwater flow direction, that need to be investigated when evaluating the impact of mining on lake ecosystems. In anticipation of increased resource exploitation across the boreal zone and the need to determine reference conditions, lakes nearby and downwind from mining sites should be studied to adequately assess the impact of mining activities on lake ecosystems, along with modulating factors.

*Case study 2: Snapshot comparison of pre- and post-industrial sedimentary Chlorophyll *a**

To investigate trends in lake primary production across the boreal zone, we leveraged samples that were collected as part of the NSERC Canadian Lake Pulse Network (hereafter referred to as LakePulse; Huot et al. 2019). LakePulse sampled 664 lakes across Canada between 2017-2019; sediments from a 145-lake subset were analyzed for sedimentary chlorophyll *a* as part of a national assessment of primary production (Griffiths et al. 2022). Here, we present a subset of these data for sites within the Canadian boreal zone ($n = 76$; for site details see supplemental materials S2).

Griffiths et al. (2022) measured sedimentary chlorophyll *a* and its diagenetic products as a proxy for historical primary production using visible reflectance spectroscopy (VRS). VRS-inferred chlorophyll *a*, determined according to the methods described in Wolfe et al. (2006) and Michelutti et al. (2010), has been shown to reflect changes in primary production and lake trophic status (Michelutti et al. 2010; Michelutti and Smol 2016). Briefly, sediments were freeze-dried, homogenized, and sieved through a sub-125 μm mesh to attain a relatively even particle

size, before analysing with a FOSS NIRSystem Model 6500 at Queens University in the Paleoecological Environmental Assessment and Research Laboratory (PEARL). Sediment intervals were selected for chlorophyll *a* analysis using a top-bottom approach, whereby the top sample represents recent conditions and the bottom sample represents pre-disturbance conditions. Sediment ages of bottom samples were verified with ^{210}Pb dating to ensure that most cores extended into the pre-industrial era (pre ~1850 CE) (see Griffiths et al. (2022) for a detailed description).

Using the raw spectral data from Griffiths et al. (2022), we inferred sedimentary chlorophyll *a* and its main diagenetic products using natural log-transformation of the following linear equation from Michelutti et al. (2010):

$$\text{Chlorophyll } a + \text{derivatives} = \text{EXP} (0.83784 * \text{LN}(\text{peak area } 650\text{-}700 \text{ nm}) + (-2.48861))$$

To partly account for any minor diagenetic loss or inorganic carbon dilution (Rydberg et al. 2020), we normalized VRS-inferred sedimentary chlorophyll *a* to organic carbon concentrations measured by Griffiths et al. (2022). To assess the magnitude of change in sedimentary chlorophyll *a* since baseline conditions, we calculated percent change in top (modern) chlorophyll *a* relative to bottom (historical) chlorophyll *a* (supplemental materials S4).

We observed a general increase in chlorophyll *a* across the boreal zone since pre-industrial times. VRS-inferred sedimentary chlorophyll *a* (normalized to organic carbon) increased in 76% of lakes, with 29 lakes displaying a marginal increase (0.1-50%), and another 29 that showed a large increase (>50%) (Figure 4). Of the 18 lakes that showed a relative decrease in chlorophyll *a* in the modern interval, 13 were modest decreases (i.e., less than 49.9% declines), and just 5 showed a large decrease (i.e., greater than 50% declines). We found that the

widespread increases in chlorophyll *a* held true without correcting for organic carbon concentration, with 84% of lakes showing an increasing trend (supplemental materials S4). The correction caused 10 records to shift their trajectories from displaying an increasing to a decreasing trend, most of which were modest changes except for one lake. Decreasing trends in chlorophyll *a* may be related to the organic carbon correction, reflecting site specific factors such as the amount and type of allochthonous carbon inputs. For example, an increase in inorganic material entering the lake (possibly driven by increased erosion from land-use, precipitation, or hydrological changes) can relatively decrease the amount of chlorophyll *a* preserved in sediment. Similarly, increased inputs of organic material into the system (possibly driven by increased agricultural and pastoral runoff) can also appear as a decrease in the amount of chlorophyll *a*, as chlorophyll *a* values are normalized to organic carbon. The latter mechanism is possible in lakes of the Boreal Plains and Prairies, whose watersheds have undergone extensive agricultural and pastoral development since pre-industrial times (some observed declines in lakes of these regions disappear when chlorophyll *a* values are left uncorrected; supplemental materials S4). Decreasing trends in chlorophyll *a* may also be explained by pollution or management strategies and policies that mitigate nutrient inputs and algal blooms (Wilkinson et al. 2022). Despite these factors, our study highlights a general increase of primary production across boreal zone lakes since pre-industrial conditions.

The widespread increase in inferred lake primary production has been observed globally in several large-scale studies (e.g., Taranu et al. 2015; Summers et al. 2016; Griffiths et al. 2022). Notably, Griffiths et al. (2022) found significant increases in primary production in 80% of their sampled lakes across Canada ($n = 145$). This extensive and increasing trend may be related to a number of factors. Increasing chlorophyll *a* concentrations often reflect

eutrophication, which in many cases can be driven by land-use changes, longer growing seasons and/or changes in water column mixing patterns (Moser et al. 2002; Schindler et al. 2008). Further, eutrophication and its effects are exacerbated by regional climate warming that has been observed in the boreal region (Summers et al. 2016; Favot et al. 2019). Additionally, reduced ice cover, lengthening of the growing season and enhanced thermal stability provide favourable conditions for increased primary production. Detecting temperature-mediated effects in the boreal zone may be easier than in sites further south as their watersheds tend to be less altered and the rate of climate change in the North is accentuated. For example, with the absence of anthropogenic eutrophication, Favot et al. (2019) identified regional climate warming as a crucial factor promoting algal blooms in a remote, oligotrophic lake. Similarly, in Lake of the Woods, Paterson et al. (2017) found that sedimentary chlorophyll *a* continued to rise despite long-term declining nutrient inputs inferred using diatoms. In particular, they noted that increased sedimentary chlorophyll *a* was positively correlated to mean annual air temperature and total precipitation. Furthermore, the widespread increase observed across such a broad geographic area may indicate a larger-scale stressor, such as climate, may be responsible for the apparent trends (Griffiths et al. 2022; Figure 4). Given that higher latitude areas are expected to warm at an increased rate, we might expect to see continued increases in primary production and sedimentary chlorophyll *a* in boreal lakes.

Summary and Perspectives

Leveraging a review of the literature and a data synthesis exercise, we have mapped out the kind of information that can be drawn from paleolimnological studies, and then developed a

comprehensive portrait of long-term trends in eutrophication and metal contamination in the Canadian boreal zone. Although many paleolimnological studies have been undertaken in the boreal zone, this area is understudied relative to its vast number of lakes and compared to more densely populated regions of Canada. There remain numerous opportunities for understanding the expansive and diverse boreal zone in an era of accelerated environmental change.

Paleolimnological techniques offer the possibility to evaluate an extended number of sites where no water quality monitoring has been conducted thus far and therefore help disentangle variation among sites due to geology or morphometry versus environmental stressors caused by human activities. With only a single trip to retrieve a sediment core from a remote boreal lake, we can obtain time series data covering years to millennia. The scalability of such an approach across the boreal region is clear, as limited access to sites can be restrictive to regular monitoring programs. Paleolimnological reconstructions offer immense opportunities for identifying periods of rapid ecosystem change and regime shifts that are unobservable through snapshot sampling approaches. For example, high-resolution scanners such as micro-X-ray fluorescence and hyperspectral instruments are now making it possible to generate very high-frequency time series with relatively little time investment (Croudace et al., 2019). When such analyses are paired with a larger sampling network, it is possible to identify both spatial hotspots and temporal periods of change across the boreal landscape (Baud et al. 2023).

As stated by Brandt (2019), the future of the boreal zone will strongly depend on choices made that “*are largely influenced by the values [populations and governments] hold and their perception of the state of the boreal zone’s terrestrial and aquatic ecosystems.*” In the Boreal 2050 project, atmospheric change, demands for ecosystem services, demographics and societal values, industrial innovation and infrastructure, and governance were identified as potential

threats to the future sustainability of the boreal zone (Creed and Serran 2019). Creed and Serran (2019) broadly describe the potential impacts of these threats, and we expect continued development of the boreal zone to have important effects on lake ecosystems across the landscape. Given that paleolimnological approaches can enhance one's perspective of environmental and ecological changes, such approaches could add substantial value to anchor future scenario building exercises. Specifically, by clearly defining pre-disturbance conditions (Figure 1), we can better contextualize modern environmental conditions and set meaningful targets for ecosystem remediation efforts using the proxies we described above. It is critical to do this now, before long-lasting and sometimes irreversible consequences occur, which can delay lake recovery actions (McGowan et al. 2005). As we strive to protect freshwater resources in a relatively understudied and vulnerable area, we argue it is essential to include a paleolimnological approach.

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Competing Interests Statement

JPS is the Editor-in-Chief of *Environmental Reviews* but recused himself from the consideration of this manuscript. The authors have no other competing interests to declare.

Author Contribution Statement

MG and DRZ contributed equally and share primary authorship of this manuscript. MG, DRZ, KTG, and IGE conceptualized the work. MG, DRZ, KTG, JP, ZET, and HG were responsible for data curation. DRZ was responsible for formal analysis. MG, DRZ, KTG, PWM, AB, REG, ML, M-EM, CP and IGE contributed to writing (original draft). All authors were involved with reviewing and editing. ZET, DA, PF, JPS, and IGE were responsible for supervision and DA, PF, JPS, and IGE were responsible for funding acquisition.

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Data Availability Statement

Data for elemental Pb can be found in the original publications (see supplemental materials S2 for list of data sources). Digitized Pb and chlorophyll *a* data are available on the open science platform Zenodo (<https://zenodo.org/>).

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Tables

Table 1: Commonly used paleolimnological proxies and the processes they are used to investigate. We have included some suggested readings offering additional information beyond what is summarized in-text.

Proxies	Charcoal	Pollen	Elemental Data / Concentration	Diatoms	Cladocera	Isotopes	Physical Sediment Characteristics / Geochemistry	Other indicators	Suggested Readings
CATCHMENT DISTURBANCE									
Fire History	✓	✓		✓					Carcaillet et al. 2001 Philibert et al. 2003
Mining Effluent			✓						Doig et al. 2015 Landers et al. 1998
Metals			✓		✓				Doig et al. 2015 Lockhart et al. 1993 Watmough, 2017 Labaj et al. 2015
Acid Rain / Acidification			✓	✓	✓			Pigments	Faulkenham et al. 2003 Vinebrooke et al. 2002 Labaj et al. 2014b

Terrestrial Organic Matter	✓			✓			<i>Lignins</i>	<i>Moingt et al. 2013</i>
Road Salt		✓		✓				<i>Valleau et al. 2022</i> <i>Cumming and Smol 1993</i>
Hydrological Modifications / Management		✓		✓			✓ <i>Loss-on-ignition & magnetic susceptibility</i>	<i>Serieyssol et al. 2009</i> <i>Wolfe et al. 2006</i>
CLIMATE								
Climate		✓		✓			✓ <i>Chl-a, akinetes</i>	<i>Laird and Cumming 2008</i> <i>Favot et al. 2019</i>
Drought / Precipitation		✓						<i>Faulkenham et al. 2003</i> <i>Ma et al. 2013</i>
Temperature		✓				✓	✓ <i>Chl-a</i>	<i>Hadley et al. 2019</i>
Thermal Regime							✓	<i>Labaj et al. 2013</i>

Proxies	Charcoal	Pollen	Elemental Data / Concentration	Diatoms	Cladocera	Isotopes	Physical Sediment Characteristics / Geochemistry	Other indicators	<i>Suggested Readings</i>
BIOTIC CHANGES									
Eutrophication & O ₂ Levels				✓				<i>Chl-a, cyano blooms, chironomids</i>	<i>Michelutti et al. 2010</i> <i>Blais et al. 2000</i> <i>Heinrichs et al. 2005</i> <i>Favot et al. 2019</i>
Fish Introductions					✓			<i>Chaoborus, Chl-a</i>	<i>Labaj et al. 2013</i> <i>Lamontagne and Schindler, 1994</i>
Invasive Species								<i>Leptodora Scales, eDNA</i>	<i>Navarro et al. 2018</i> <i>Stoof-Leichsenring et al. 2022</i>
Predation				✓	✓			<i>Chaoborus, chrysophytes</i>	<i>Labaj et al. 2014a</i> <i>Uutala et al. 1994</i>

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IN-LAKE MODIFICATIONS						
Erosion & Production	✓	✓		✓	✓	Micro-X-Ray fluorescence spectroscopy Jenny et al. 2019 Baud et al. 2023
C-cycle Disturbances & Sedimentation Rates		✓		✓	✓	Anderson et al. 2020 Baud et al. 2022 Baud et al. 2021 Crann et al. 2015 Jenny et al. 2019

Figure Captions

Figure 1: The strength of paleolimnology is anchored in its ability to generate historical trends. The y-axis denotes variation in a hypothetical paleolimnological proxy. The x-axis represents time. In scenario a (blue line), we observe a fluctuating dynamic that is similar over modern and historical times. In scenario b (green line), we see no significant historical trend, but more pronounced changes over the modern window. In scenario c (red line), we see an increasing historical trend that predates modern monitoring efforts. In all scenarios, paleolimnology can ascertain the reference (i.e., baseline) condition of the lake with respect to the hypothetical proxy. Note that this is a theoretical schematic and not actual data.

Figure 2: Map of historical M-K trend coefficients for elemental Pb concentration. Historical M-K trends were calculated on pre-breakpoint time series for the most recently identified breakpoint (circles). If no statistically significant breakpoint was detected, then historical M-K trend coefficient was not calculated (white squares). A negative M-K trend coefficient indicates a

decreasing trend in Pb concentration; conversely, a positive M-K trend coefficient indicates an increasing trend. Map produced using Esri ArcMap 10.8.1. 'Canada' and 'Contiguous US' basemap by Esri; 'Boreal' and 'Hemiboreal' basemap by Brandt (2009). Geographic coordinate system 'NAD83'. Projected coordinate system 'Canada Albers Equal Area Conic'.

Figure 3: Map of modern M-K trend coefficients for elemental Pb concentration. Modern M-K trends were calculated on post-breakpoint time series for the most recently identified breakpoint (circles). If no statistically significant breakpoint was detected, the modern M-K trend coefficient was calculated on the entire time series (coloured squares). If there were not enough data points post-breakpoint to calculate M-K trend, no trend is reported (white circles). A negative M-K trend coefficient indicates a decreasing trend in Pb concentration; conversely, a positive M-K trend coefficient indicates an increasing trend. Map produced using Esri ArcMap 10.8.1. 'Canada' and 'Contiguous US' basemap by Esri; 'Boreal' and 'Hemiboreal' basemap by Brandt (2009). Geographic coordinate system 'NAD83'. Projected coordinate system 'Canada Albers Equal Area Conic'.

Figure 4: Map of % change in top-bottom sedimentary chlorophyll *a* and diagenetic products, corrected for organic carbon (OC). A negative % change indicates a decreasing trend in sedimentary chlorophyll *a* and diagenetic products; conversely, a positive % change indicates an increasing trend. Map produced using Esri ArcMap 10.8.1. 'Canada' and 'Contiguous US' basemap by Esri; 'Boreal' and 'Hemiboreal' basemap by Brandt (2009). Geographic coordinate system 'NAD83'. Projected coordinate system 'Canada Albers Equal Area Conic'.

Figures

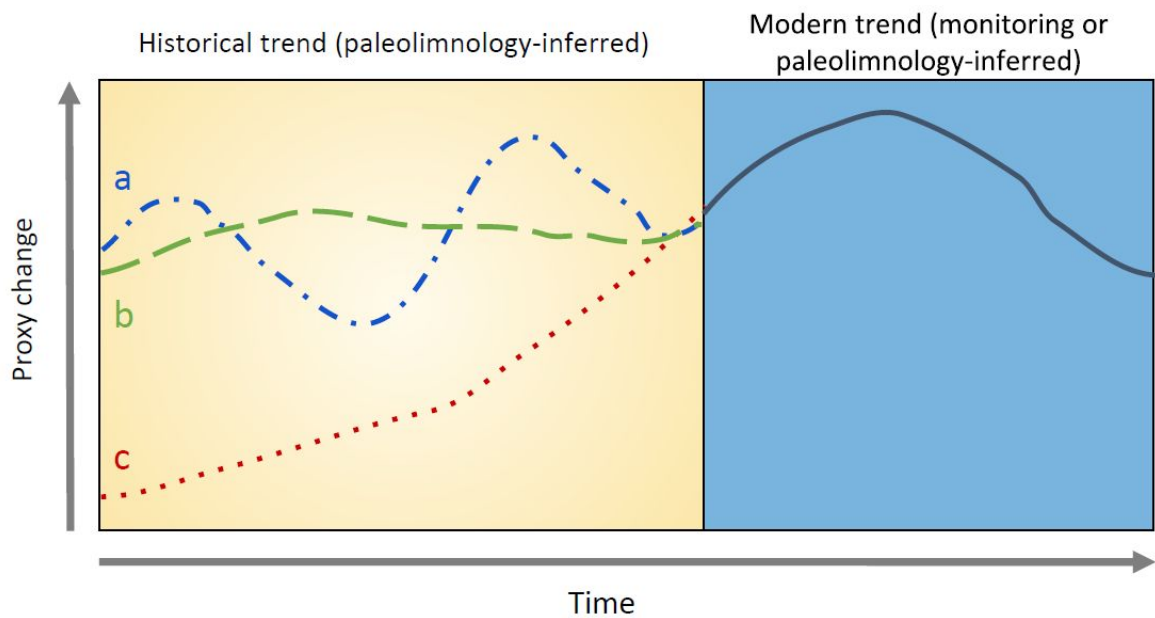


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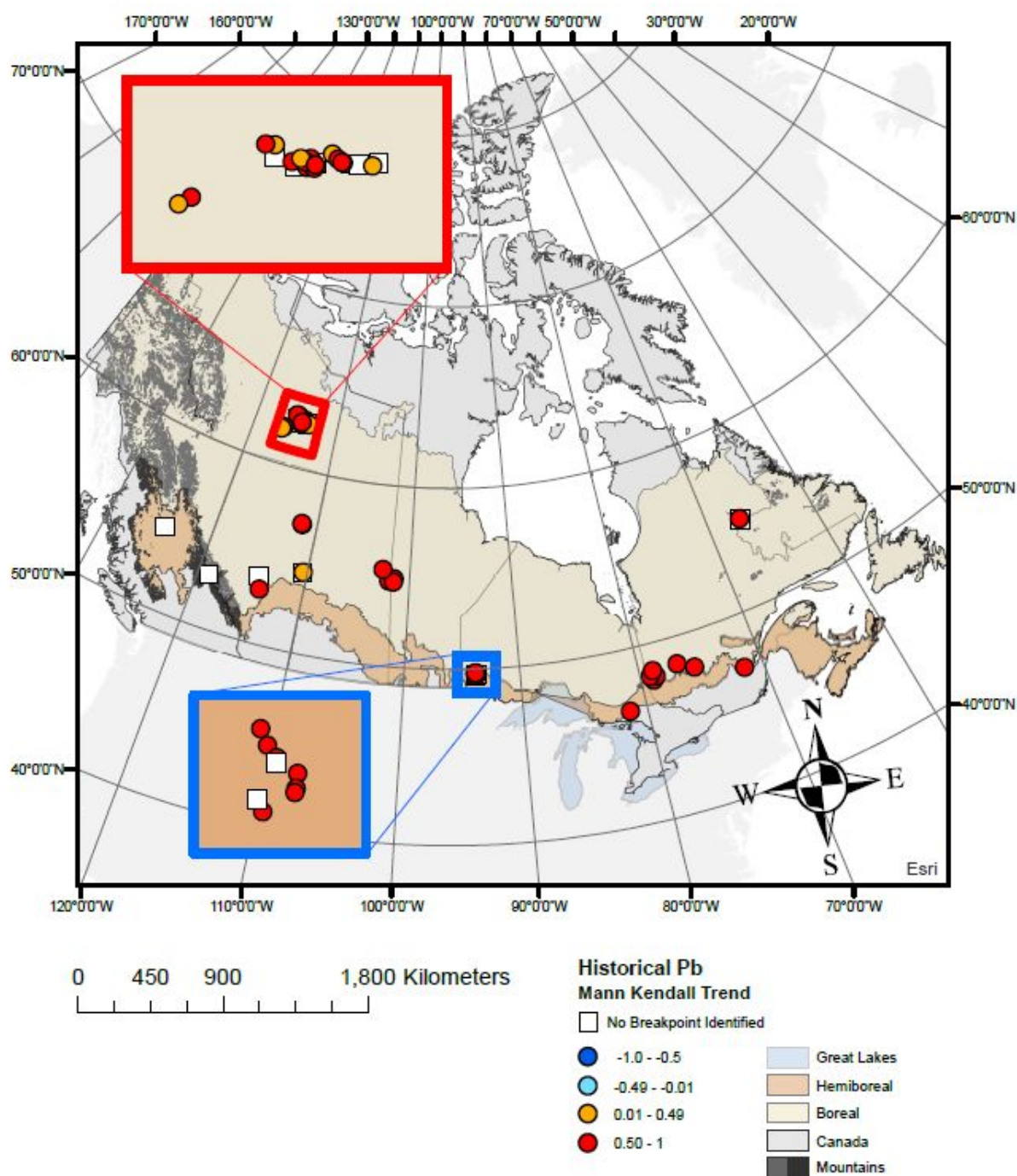


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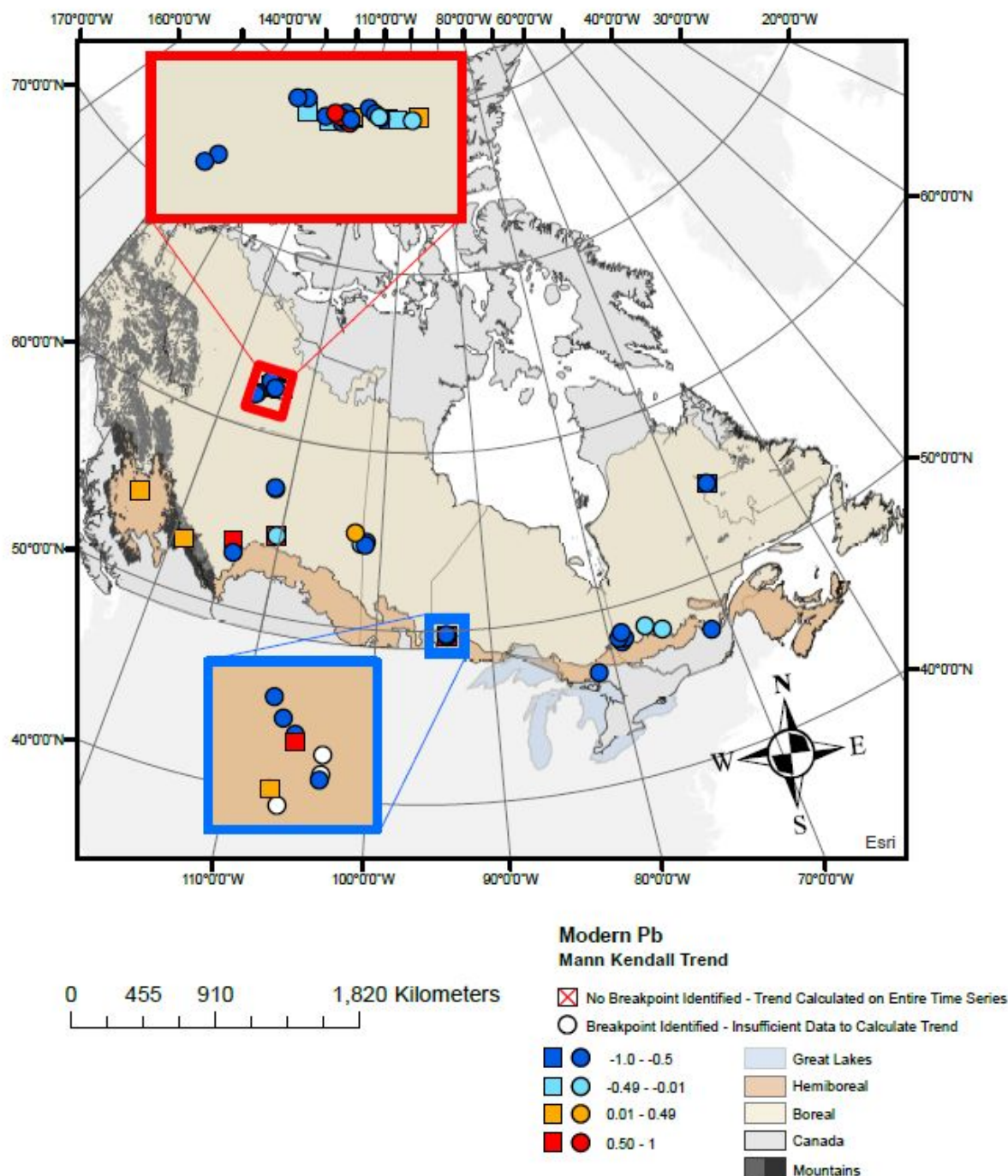


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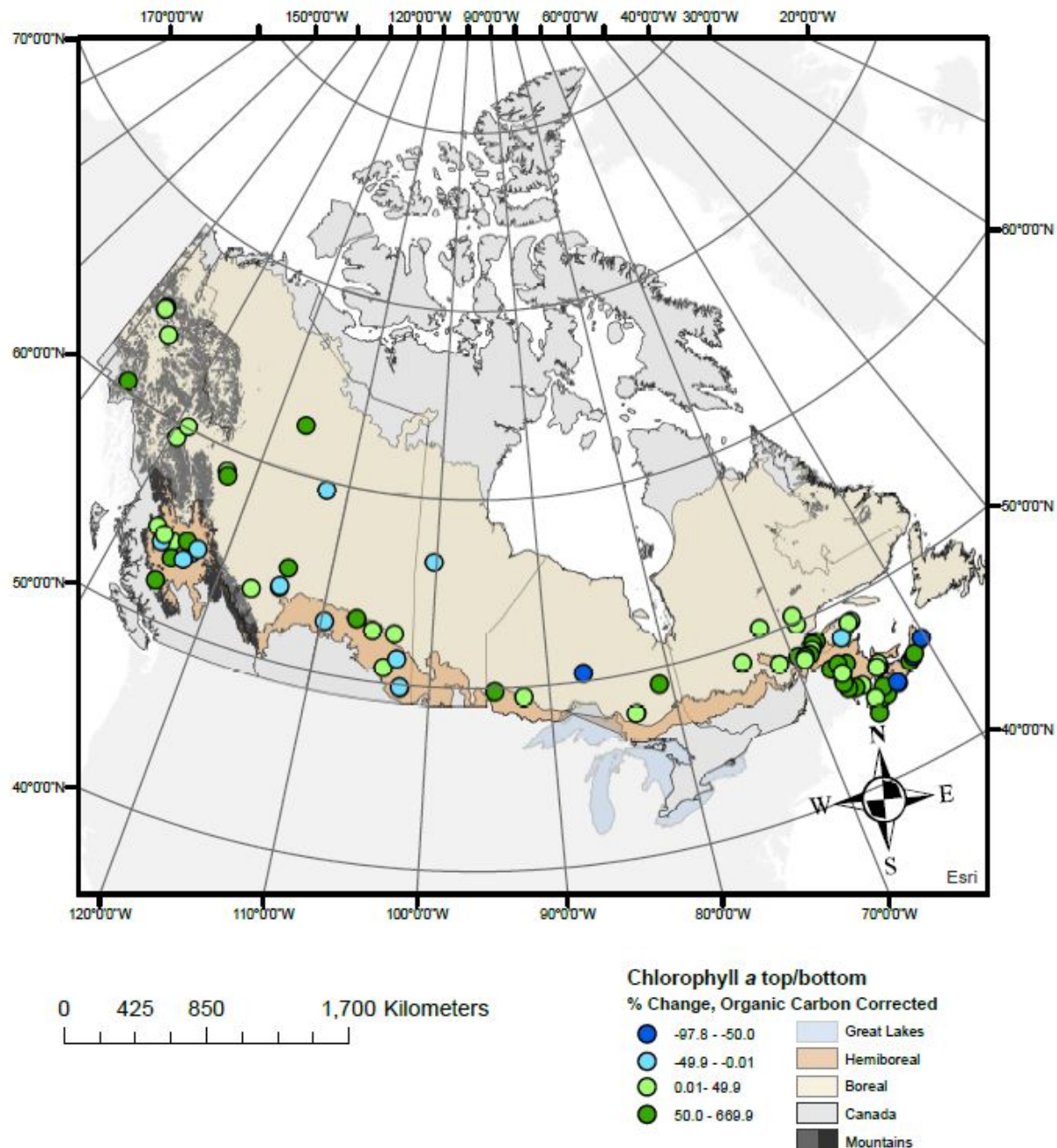


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