Protist diversity and metabolic strategy in freshwater lakes are shaped by trophic state and watershed land use at a continental scale

Continental distributions of freshwater lake protists

Rebecca E. Garner\textsuperscript{a,\#}, Susanne A. Kraemer\textsuperscript{a,\textsuperscript{b,\textsuperscript{c,\textsuperscript{f}}, Vera E. Onana\textsuperscript{a,\textsuperscript{f}}, Yannick Huot\textsuperscript{d,\textsuperscript{f}}, Irene Gregory-Eaves\textsuperscript{e,\textsuperscript{f}}, David A. Walsh\textsuperscript{a,\#}}

\textsuperscript{a}Department of Biology, Concordia University, Montreal, Quebec, Canada
\textsuperscript{b}Genome Center, Department of Microbiology & Immunology, McGill University, Montreal, Quebec, Canada
\textsuperscript{c}Department of Civil Engineering, McGill University, Montreal, Quebec, Canada
\textsuperscript{d}Département de géomatique appliquée, Université de Sherbrooke, Sherbrooke, Quebec, Canada
\textsuperscript{e}Department of Biology, McGill University, Montreal, Quebec, Canada
\textsuperscript{f}Groupe de recherche interuniversitaire en limnologie, Quebec, Canada

Address correspondence to Rebecca E. Garner, rebecca.garner@mail.concordia.ca or David A. Walsh, david.walsh@concordia.ca.

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Abstract

Protists play key roles in aquatic food webs as primary producers, predators, nutrient recyclers, and symbionts. Yet, a comprehensive view of protist diversity in freshwaters has been challenged by the immense environmental heterogeneity among lakes worldwide. We assessed protist diversity in the surface waters of 366 freshwater lakes across a north temperate to subarctic extent covering nearly 8.4 million km² of Canada. Sampled lakes represented broad gradients in size, trophic state, and watershed land use. Hypereutrophic lakes contained the least diverse and most distinct protist communities relative to nutrient-poor lakes. Greater taxonomic variation among eutrophic lakes was mainly a product of heterotroph and mixotroph diversity, whereas phototroph assemblages were more similar under high-nutrient conditions. Overall, local physicochemical factors, particularly ion and nutrient concentrations, elicited the strongest responses in community structure, far outweighing the effects of geographic gradients. Despite their contrasting distribution patterns, obligate phototroph and heterotroph turnover was predicted by an overlapping set of environmental factors, while the metabolic plasticity of mixotrophs may have made them less predictable. Notably, protist diversity was associated with variation in watershed soil pH and agricultural crop coverage, pointing to human impact on the land-water interface that has not been previously identified in studies at smaller scales. Our study exposes the importance of both within-lake and external watershed characteristics in explaining protist diversity and biogeography, critical information in further developing an understanding of how freshwater lakes and their watersheds are impacted by anthropogenic stressors.

Importance

Freshwater lakes are experiencing rapid changes under accelerated anthropogenic stress and a warming climate. Microorganisms underpin aquatic food webs, yet little is known about how freshwater microbial communities are responding to human impact. Here, we assessed the diversity of protists and their myriad ecological roles in lakes varying in size across watersheds experiencing a range of land use pressures by leveraging data from a continental-scale survey of Canadian lakes. We found evidence of human impact on protist assemblages through an association with lake trophic state and extending to agricultural activity and soil characteristics in the surrounding watershed. Furthermore, trophic state
appeared to explain the distributions of phototrophic and heterotrophic protists in contrasting ways. Our findings highlight the vulnerability of lake ecosystems to increased land use and the importance of assessing terrestrial interfaces to elucidate freshwater ecosystem dynamics.

**Keywords**

microbial eukaryotes; trophic state; plankton; phototrophy; heterotrophy; mixotrophy; human impact

**Introduction**

Protists have evolved a vast morphological and ecological diversity (1). In aquatic ecosystems, protists play key roles in the transfer of energy and nutrients by converting sunlight into chemical energy, remineralizing organic matter, controlling microbial biomass, feeding higher trophic levels, and maintaining symbioses, some of which recruit prokaryotic metabolisms (2–4).

Elucidating protist diversity in lakes is relevant for clarifying microbial distributions across a wide array of environmental conditions and for tracking the health of critical freshwaters. Covering <1% of Earth’s surface (5), lakes contribute disproportionately to the global carbon cycle (6–8) and hold essential water resources (9). Lakes display a rich environmental heterogeneity, generated by integrating fluxes of materials and energy from their catchments and airsheds (10, 11). Freshwater lakes are hotspots of biodiversity, collectively containing higher levels of eukaryote richness and endemism (12) and protist community turnover (13) than the marine and terrestrial realms. In the Anthropocene, lakes are increasingly altered by eutrophication (14), warming temperatures (15), deoxygenation (16), salinization (17), and myriad other persistent and emergent stressors (18). There is accumulating evidence that anthropogenic modifications to lake habitats affect protist assemblages (19–21), in turn influencing ecosystem dynamics.

Efforts to study protists from a variety of biomes have unearthed a vast diversity and begun to map their distributions at a global scale (22–24). Such investigations have brought insights into protist environmental preferences and community assembly processes (25–28), food web dynamics (29–33), symbioses (34–36), viruses (37), functional traits (38), and bioindicator values (39, 40). Large-scale lake surveys have shown that protist assemblages are shaped by broad biogeographic patterns (41–43) but...
are also influenced by local environmental factors and interactions with bacteria (44). Phytoplankton surveys recapitulate these observations, while exclusively investigating photosynthetic taxa including Cyanobacteria (45–47). However, there is no clear understanding yet of the distributions of different trophic life history strategies and the environmental drivers underlying their diversity. This knowledge gap is particularly glaring since heterotrophy is likely the most abundant trophic mode (48). Meanwhile, mixotrophs have dramatically altered our view of plankton food webs by combining primary production and prey consumption (49), sometimes surpassing obligate heterotrophs as the leading grazers of bacteria in lakes (50, 51).

In this study, 18S rRNA gene amplicon sequencing was used to investigate the distributions of protists in the surface waters of 366 freshwater lakes across a north temperate to subarctic continental extent. We hypothesized that (1) protist diversity at the local scale and community turnover decrease under high-nutrient conditions, (2) trophic groups respond to different environmental factors, specifically, phototrophs are more sensitive than heterotrophs to bottom-up resource availability, and (3) because lakes integrate their catchments, protist diversity in lakes should reflect watershed conditions. This project was conducted within the LakePulse survey, which sampled hundreds of lakes of different sizes in watersheds under varying levels of human impact with the primary aim to assess lake health through a multidisciplinary lens (52). The current study fills a gap in the mapping of microbial biogeography through this first standardized assessment of protist diversity across Canada, which stewards the greatest abundance of lakes worldwide (53). Our study draws attention to the diversity of protists and the ecological patterns that emerge in a broad collection of newly explored habitats being reshaped by increasing human impact.

Results

Sampled lakes and watersheds display high environmental heterogeneity

Protist assemblages were surveyed in the euphotic zones of 366 freshwater to oligosaline lakes in 12 ecozones across Canada (43 – 68 °N, 53 – 141 °W) (Figure 1). Watersheds ranged widely in area (0.3 – 9,332.3 km$^2$) and were characterized by a variety of human population densities (0 – 3,785 people/km$^2$) and land use, including different proportions of crop agriculture (0 – 81%) and built development (0 –
Lakes had a wide range of surface areas (0.05 – 99.66 km$^2$) and maximum depths (1 – >150 m) and were either vertically mixed (~40% of lakes) or thermally stratified (~60%) at the time of sampling.

Physicochemical conditions differed substantially across lakes, as represented by a broad pH gradient (5.6 – 10.2) and ultraoligotrophic to hypereutrophic states evaluated by total phosphorus (TP) concentrations (2 – 2,484 μg/L) (Figure 1).

Sampled lakes and watersheds reflected regional variation in environmental conditions including anthropogenic gradients (Figure S1). Lakes in northern Canada (Taiga Cordillera, Boreal Cordillera, and Taiga Plains ecozones) were subject to the coldest climates and lowest intensities and proportions of land use within their watersheds. Lakes in western Canada (Montane Cordillera, Pacific Maritime, and Semi-Arid Plateaux) were the deepest on average and located in watersheds with the largest proportions of harvested forests. In central Canada (Boreal Plains and Prairies), a region dominated by plains and prairies with intensive agriculture, lakes were generally shallow, exposed to winds, productive, and high in pH, carbon, ions, and nutrients. Lakes in eastern Canada (Mixedwood Plains, Boreal Shield, Atlantic Highlands, and Atlantic Maritime) had the warmest surface waters and generally the most built-up landscapes, a feature shared with watersheds in the Pacific Maritime. Overall, hypereutrophic, ion- and carbon-rich, and high-pH lake conditions were most often observed in agricultural watersheds with alkaline soils (Figure S2).

Lakes support taxonomically and functionally diverse protist assemblages

Eukaryotic diversity was assessed through the sequencing of 18S rRNA gene fragments amplified from DNA collected in 0.22 – 100 μm surface water particles. A total of 15,848 amplicon sequence variants (ASVs) were inferred in 17,749,930 sequences across 366 lake samples. A final data set of 13,046 putative protist ASVs encompassing 14,622,273 sequences was retained after ASVs assigned to animals, fungi, and plants were removed (Table S1). The rarefaction of pooled samples showed that the sampling of new ASVs plateaued toward 2,000,000 sequences, signaling that the global sequencing effort had exhaustively captured the protist diversity targeted by the primer pair (Figure S3). The most abundant taxonomic groups were Ochrophyta (24% of all sequences), Cryptophyta (18%), Ciliophora
(15%), and Dinoflagellata (11%) (Figure 1). Lineages with the highest ASV richness were Ochrophyta (22% of all ASVs), Dinoflagellata (12%), and Chlorophyta (11%).

We analyzed ASV incidence to assess the contribution of individual assemblages to total landscape diversity. New genotypes accumulated at a high rate in the first ~100 randomly ordered assemblages, followed by a gradual deceleration (Figure 2A). The majority of ASVs were restricted to one or a few lakes (Figure 2B). A smaller number of ASVs were distributed widely, including one ASV assigned to Cryptomonas curvata (Cryptophyta) that was ubiquitous yet highly variable in relative abundance (0.0031 – 64%) across all the lakes sampled.

Trophic functions were assigned to ASVs representing 85% of the total sequence space by leveraging natural history descriptions summarized from the literature (Figure 1; Table S1). Of the functionally annotated ASVs, most were classified as obligate phototrophs (32% of all sequences). The most abundant phototrophs were classified under Chrysophyceae (Ochrophyta; 16% of all sequences), Bacillariophyta (Ochrophyta; 3%), and Chlorophyceae (Chlorophyta; 5%). Bacterivory (20% of all sequences) and mixotrophy (20%) were the next most abundant trophic modes. The most abundant mixotrophs were classified under Cryptophyceae (Cryptophyta; 17% of all sequences) and Dinophyceae (Dinoflagellata; 2%). Cytotrophy (i.e. feeding on other protists), parasitism, commensalism, saprotrophy, and osmotrophy were detected to a lesser extent. Parasites (3% of all sequences) were most abundant in the Coccidiomorphea (Apicomplexa; 2%) and Oomycota (Pseudofungi; 1%). Heterotrophs – which broadly encompassed bacterivores, cytotrophs, saprotrophs, and osmotrophs – comprised 27% of all sequences. Heterotrophy was most abundant in the ciliates Spirotrichea (9% of all sequences), Oligohymenophorea (2%), and Litostomatea (2%) and in other lineages including Bicoecea (Opalozoa; 3%) and Katablepharidaceae (Katablepharidophyta; 2%).

Genotypic similarity to known protist 18S rRNA gene diversity was assessed through the global alignment of ASVs to V7 region fragments in the Protist Ribosomal Reference database (PR2) (Figure S4). Of the 12,511 ASVs that met the threshold global alignment length, the majority showed high sequence similarity with references in the database. Most ASVs (58%) were ≥96% identical to references. ASVs with 100% similarity to reference sequences occupied 56% of the total sequence space, while ASVs with ≥96% similarity occupied 91% of sequences. The most novel genotypes included the 738...
ASVs with <90% sequence similarity to PR references and were either not assigned to a supergroup (415 ASVs) or primarily classified as Opisthokonta (141), Alveolata (88), or Stramenopiles (30).

Local diversity at each lake was estimated by richness (67 – 1,275 ASVs) (Figure S5), the Shannon index (0.17 – 5.67; Figure 3A), Pielou’s evenness index (0.04 – 0.83), and Faith’s phylogenetic diversity index (8.15 – 76.04). The Shannon index was negatively correlated with magnesium ($r = -0.33$), total nitrogen (TN; $r = -0.32$), dissolved inorganic carbon (DIC; $r = -0.31$), and potassium ($r = -0.30$) concentrations (all correlations had $p < 0.001$). Tukey’s tests of ANOVAs comparing the association of trophic state with local diversity showed that mean richness, Shannon diversity, and evenness were significantly lower in hypereutrophic lakes than in eutrophic, mesoeutrophic, mesotrophic, or oligotrophic lakes (all $p \leq 0.002$) (Figure 3B).

Protist assemblages vary regionally and across lake trophic states

Next, we looked at how communities varied in taxonomic and phylogenetic composition among lakes. A principal component analysis (PCA) of ASV assemblages showed a clear pattern of taxonomic variation by lake trophic state and ecozone along the first dimension (Figure 4A). Assemblages in the typically nutrient- and ion-rich lakes of the Prairies and Boreal Plains were distinguished from assemblages in the lower-nutrient lakes of the Boreal Shield and other eastern regions. Cryptophyte diversity (Cryptomonas, Geminigera, Plagioselmis, and Komma species) contributed the most strongly to the variation among assemblages (Figure 4A). A principal coordinate analysis (PCoA) of generalized UniFrac distances between assemblages showed patterns of phylogenetic variation that were highly congruent with the taxonomic variation observed in the PCA, as evaluated by an RV coefficient correlating the first two dimensions of each ordination (RV = 67%, $p = 1.3 \times 10^{-88}$) (Figure 4B).

Taxonomically distinct assemblages as quantified by local contributions to β-diversity (LCBD) were mostly localized in the Prairies and Boreal Plains, with a few high-LCBD assemblages scattered across other regions (Figure 5). β-diversity partitioning showed that the taxonomic dissimilarities between assemblages were primarily generated through ASV turnover (75% of total variance) and differences in richness to a lesser extent (25%). Highly significant ($p < 0.001$) positive correlations were detected between LCBD and a complement of physicochemical variables including potassium ($r = 0.53$), TN ($r = $...
Physicochemical and watershed conditions predict community turnover

Following the previous observations of high community variability across an environmentally heterogeneous set of lakes, we evaluated the drivers of taxonomic and phylogenetic turnover based on five categories of environmental variables: (1) geography (i.e. latitude, longitude, and altitude), (2) weather, (3) lake morphometry, (4) physicochemistry, and (5) watershed characteristics including land use and edaphic properties.

We employed generalized dissimilarity models (GDMs) to detect nonlinear trends between community turnover and environmental gradients. Physicochemical factors explained the highest deviance in GDMs modeling taxonomic or phylogenetic turnover. In order of decreasing strength, DIC, TP, chlorophyll-a, magnesium, pH, potassium, lake colour, surface temperature, and DOC were statistically significant predictors of taxonomic turnover, whereas chlorophyll-a, magnesium, TN, pH, calcium, and colour were significant predictors of phylogenetic turnover. After physicochemistry, watershed characteristics were the most important predictors of community turnover, with both taxonomic and phylogenetic diversity responding most strongly to soil pH and then proportion of cropland cover. The volumetric fraction of soil coarse fragments and natural landscape coverage were additional predictors of taxonomic and phylogenetic turnover, respectively. Lake morphometry, comprising maximum depth, watershed slope, and shoreline circularity, had relatively weak effects on turnover. Weather and geography did not generate statistically significant GDMs. Model deviances are summarized in Table 1 and the partial effects of individual variables are summarized in Figure S6 and Table S2.

Trophic strategies exhibit contrasting distributions
To examine the distributions of different trophic strategies, we investigated the taxonomic variation of phototroph, heterotroph, and mixotroph communities in separate PCAs (Figure 6). Reflecting the taxonomic variation emerging at the whole-community level, assemblages of each trophic mode were distinguished by lake trophic state and ecozone along the first dimension. However, phototrophs displayed contrasting taxonomic variation patterns to heterotrophs and mixotrophs. To compare the taxonomic turnover of phototrophs, heterotrophs, or mixotrophs among lakes of the same trophic state, we measured the distances of assemblages to the trophic state median within the two-dimensional principal coordinate space. The mean distance of phototroph assemblages to the trophic state median (i.e. turnover) was significantly lower in hypereutrophic lakes than in eutrophic, mesoeutrophic, mesotrophic, or oligotrophic lakes (all \( p < 0.001 \)) (Figure S7). In contrast, the mean distance of heterotroph assemblages was significantly higher in eutrophic lakes than in mesotrophic or oligotrophic lakes (all \( p \leq 0.007 \)) (Figure S7). Compared with either phototroph or heterotroph assemblages, mixotroph assemblages were highly dispersed within trophic state groups (Figure S7).

Partitioning protist assemblages by trophic function further allowed us to examine the responses of different groups to environmental conditions. GDMs showed that phototrophs, heterotrophs, and mixotrophs each responded most strongly to physicochemical gradients. Chlorophyll-a, DIC, potassium, and pH were predictors common to the three trophic modes, while calcium, colour, and chloride were additional predictors of phototroph turnover, and TP, surface temperature, sulfate, TN, colour, and chloride were additional predictors of heterotroph turnover. Chlorophyll-a followed by potassium were the top predictors of phototroph turnover, while DIC followed by chlorophyll-a were the top predictors of heterotroph and mixotroph turnover. Watershed characteristics explained an important amount of deviance in phototroph and heterotroph turnover: phototroph turnover was explained by soil pH, crop coverage, soil organic carbon density, and built development, whereas heterotroph turnover was additionally explained by soil coarse fragments. Only phototroph turnover was predicted by lake morphometry variables (watershed slope, lake maximum depth, and circularity). Heterotroph turnover was weakly explained by weather, specifically air temperature and wind speed, and geography, specifically altitude and distances between lakes. Mixotroph turnover was the least predictable from environmental conditions, as physicochemical factors explained less deviance than for obligate phototroph or
heterotroph turnover and GDMs modeled on geography, weather, lake morphometry, and watershed characteristics were not statistically significant. Model deviances are summarized in Table 1 and the partial effects of individual variables are summarized in Figure S8 and Table S2.

Discussion

Protist diversity unveiled at a continental scale

Establishing a comprehensive perspective of freshwater protist diversity is challenging given the substantial environmental heterogeneity of the millions of lakes distributed globally. Our study fills a sizable gap by mapping protist distributions across hundreds of lakes spanning an area covering nearly 8.4 million km$^2$ in the largest study of its kind to employ a standardized sampling scheme (52). Protist diversity was determined in the sunlit surface waters of 366 freshwater lakes varying in size and degree of human impact on the watershed. The sampled biomass bridged cell diameters across four orders of magnitude (0.22 – 100 μm), allowing us to recover pico- to microscale organisms, many of which are not resolved under standard light microscopy and which constitute an expansive microbial diversity encompassing the major eukaryotic lineages and trophic strategies. Our global rarefaction analysis showed that the sequencing effort provided a reasonable estimate of the genotypic variation captured by the primer pair. The rapid accumulation of genotypes across sites indicated that sampling hundreds of lakes was required to assess landscape diversity. The integration of our work with the collection of recent large-scale surveys is leading to a synoptic view of protist ecology (23, 24, 28, 42, 43, 54–57).

Surface water assemblages contain high proportions of phototrophs and heterotrophs

Lacustrine protist diversity was dominated by ochrophytes, which accounted for both the highest sequence abundance and ASV richness. Cryptophytes, ciliates, dinoflagellates, and chlorophytes were also highly represented, reflecting taxonomic profiles typical of freshwater biomes on other continents and identified with primer pairs targeting other gene regions (58). The main taxa contributing to the dissimilarity between assemblages were mixotrophic cryptophytes, whose distributions as major bacterivores may be dependent on the occurrence of specific prey (59).
Phototrophic taxa accounted for the greatest richness and relative abundance of ASVs, perhaps not unexpectedly given that assemblages were sampled from the euphotic zone. Notably, the dominance of phototrophs within the protist fraction appears to be a distinct feature of freshwater photic zones in contrast with the prevalence of heterotrophs in the sunlit ocean and surface soils (13). We showed that heterotrophs and mixotrophs were numerically important groups after phototrophs, which, along with a smaller collection of parasites, illustrates that lake surface waters harbour a broad array of microbial functions linking multiple trophic levels.

Protist communities respond to local environmental conditions

The key environmental drivers of protist diversity in Canadian lakes departed from those observed in previous large-scale surveys. Assemblages showed the strongest responses to physicochemical factors, including nutrient, major ion, and chlorophyll-a concentrations, pH, and lake colour. The environmental drivers in lakes differed from those in marine and soil ecosystems, where protist diversity at large scale is generally predicted by temperature (60) and annual precipitation (28), respectively. The differences in environmental filtering between biomes likely represent fundamental differences in the types of habitats and degrees of ecosystem connectivity. Compared with the more spatially continuous and expansive ocean and soil macroenvironments, lakes are fragmented across the landscape and neighbouring lakes can exhibit widely contrasting physicochemical attributes (10, 61). Lake heterogeneity is amplified by temporal variability and punctuated perturbations. Higher community turnover has previously been measured among lakes than within marine or soil ecosystems (12, 13), which we reassert is linked to physicochemical heterogeneity.

We found that the influence of local environmental conditions far outweighed the effects of geographic variation across the continental extent. Among LakePulse sites, systems with the highest trophic states (typically, Prairies and Boreal Plains lakes) were located at intermediate longitudes, latitudes, and altitudes. A study of Scandinavian boreal lakes spanning a longitudinally-aligned and narrower trophic state gradient (oligotrophic to mesoeutrophic) reported that geography explained more protist community variation than water chemistry (42), complementing our assessment that regional physicochemical heterogeneity is a major determinant of protist diversity. Other surveys identified...
biogeographic patterns of protist diversity structured by the isolation and dispersal limitation of mountain
lake communities (41, 43). Geographic barriers (e.g., the Rocky Mountains) did not appear to generate
strong compositional divisions in our set of protist assemblages but were identified as having an important
influence on the distributions across LakePulse sites of crustacean zooplankton (62), a group with greater
dispersal limitation due to their larger body sizes. Instead, we found the greatest taxonomic and
phylogenetic divisions between regions distinguished by differences in lake trophic state and other local
environmental conditions.

Partitioning protist trophic diversity allowed us to examine how different components of freshwater
food webs respond to the environment. We observed contrasting distribution patterns for each trophic
mode. Phototroph assemblages among hypereutrophic lakes exhibited significantly lower taxonomic
turnover (as evaluated by mean distance to the trophic state median) than lakes at lower nutrient states,
whereas heterotroph assemblages did not follow this trend but turned over significantly more rapidly
among eutrophic lakes than mesotrophic or oligotrophic lakes. Yet, the turnover within each trophic mode
was predicted by a mostly overlapping suite of physicochemical factors, including chlorophyll-a, DIC, ions,
pH, and colour, although rank order of importance varied among groups. Nutrient (TP and TN)
concentrations and surface temperature were exclusive predictors of heterotroph turnover. All of the
environmental predictors of mixotroph turnover were common to both phototrophs and heterotrophs, with
no predictors unique to mixotrophs. Changes in low levels of chlorophyll-a were associated with the most
rapid turnover in phototroph composition, which is to be expected given that chlorophyll-a is linked to
phytoplankton biomass and phototroph diversity shifts along a lake productivity gradient. The next
strongest predictor of phototroph turnover was potassium, which is not a limiting resource (63) but
displayed extreme regional variation peaking in the Prairies, followed by the Boreal Plains. Heterotroph
and mixotroph turnover was primarily predicted by DIC concentrations. Overall, the environmental drivers
of heterotroph diversity mostly reflect the bottom-up controls on primary producers traversing multiple
trophic levels but are rendered further complex by the added effects of nutrient and temperature factors.
Bacterial prey and top-down controls, encompassing predation and parasitism (not measured in this
study), likely also determine trophic functional diversity.
Mixotroph distributions were the least aligned with trophic state and the least predictable from environmental conditions, which should be expected for organisms with the metabolic versatility to occupy variable niche spaces. The balance between primary production and prey consumption is dependent on a mixotroph’s phenotypic plasticity (64). While mixotrophy is competitively advantageous over obligate phototrophy or heterotrophy under low-nutrient conditions (65), primarily phototrophic mixotrophs prevail in oligotrophic lakes and are replaced by primarily heterotrophic mixotrophs as trophic state increases (66). Another variable driving mixotroph diversity in aquatic ecosystems is light availability (67, 68), which is modulated by lake colour, a predictor identified in this study. Here too, bacterial prey and zooplankton predators with a preference for nutritious mixotrophs likely also exert controls (69).

Hypereutrophic lakes are taxonomically distinct

Hypereutrophic lakes, located mostly in agricultural watersheds, contained protist assemblages with the lowest diversity and highest taxonomic distinctness (i.e. LCBD) relative to other lakes in the landscape. Specifically, Shannon diversity was inversely related to ion and nutrient concentrations, while taxonomic distinctness tracked with ion- and nutrient-rich conditions. Hypereutrophic conditions potentially filter protist communities by creating relatively extreme conditions (e.g., light attenuation), tolerated by a small number of taxa assembling into uneven communities distinct from those in lakes at lower nutrient states. Phosphorus is often the limiting nutrient of phytoplankton in numerous freshwater systems (70, 71), yet TP was a predictor of turnover exclusive to heterotrophs. Phosphorus was found to be an important predictor of littoral protist diversity in European lakes (44) and of long-term microeukaryote community turnover evidenced from paleolimnological trends (19). Interactions between protists and Cyanobacteria likely also play a role in determining protist assemblages in hypereutrophic lakes, especially as Cyanobacteria of the order *Microcystis* were found to be associated with high-nutrient conditions across LakePulse sites (72).

Ordinations of separate trophic modes showed that the high community variation among eutrophic lakes was generated by heterotroph diversity, whereas obligate phototroph assemblages were the least varied under hypereutrophic conditions. A positive relationship between compositional heterogeneity and trophic state runs counter to the expectation of reduced community variability (e.g., for
phytoplankton (73)) that is predicted to follow the leveling of abiotic conditions among lakes induced by land use and eutrophication. Biotic homogenization appears to have trended with long-term climate warming and eutrophication in Cyanobacteria (74) and protist (20) assemblages reconstructed from sediment core chronologies. Following our observation of increased compositional heterogeneity among lakes as a function of trophic state, we posit that protist communities in productive lakes are less stable over time, including over the same season as observed in bacterial time series (75). The temporal fluxes in abiotic conditions prompting succession may be induced by allochthonous inputs or nutrient resuspension from sediments accrued at higher rates in regions of extensive lake use and high populations densities (76). Furthermore, given that many Prairies and Boreal Plains lakes are shallow and exposed to winds, temporary stratification followed by destratification is not uncommon (77). We speculate that taxon replacement linked to land use and eutrophication may force a re-evaluation of human impact on biodiversity. In particular, anthropogenic pressures may not inherently decrease diversity but instead increase turnover, possibly at the expense of rare or specialist taxa disappearing from the landscape pool (78).

Watersheds influence lacustrine protist diversity

While the importance of physicochemical factors on lacustrine protist diversity has been described (42, 44) and elaborated upon in this study, the influence of the watershed, in particular soil properties and land use that are often but not entirely correlated with lake physicochemical attributes, until now have not been documented at the continental scale. We found that the taxonomic distinctness of local assemblages (i.e. LCBD) corresponded strongly with the proportion of crop agriculture in the watershed, while surface soil pH was an important predictor of community turnover. Because of their concave topographies and position in the landscape, lakes are recipients of major allochthonous subsidies, with global effects (e.g., carbon storage) that are disproportionate to the spatial extent of lakes (6). The influence of terrestrial catchments on within-lake community dynamics is compelling evidence for why lakes cannot be studied in isolation of their watersheds.

The filtering of lacustrine protist diversity by watershed soil chemistry points to the influence of external factors on lake conditions. Soil buffering capacity, determined by soil texture, organic matter
content, and mineral composition, is a main abiotic control on lake water pH (79). Furthermore, soil properties control the mobility of nutrients and their eventual input into lakes. Specifically, soil pH determines the availability and chemical forms of nutrients, and particle size governs the movement of groundwater carrying released nutrients (80). Soil properties also determine the composition and activity of soil microbiomes, which perform biogeochemical transformations modulating the availability of nutrients (81) and seed potential colonists from soils to lakes. Altered precipitation regimes and warming temperatures associated with climate change are expected to increase soil erosion (82) as well as terrestrial nutrient exports (83).

Given the continual increase in land surface transformed by agricultural production and urbanization (84), accelerated watershed land use conversions are widespread. Changes in soils associated with human activities range from increased nutrient loading and acidification by nitrogenous fertilizers in agriculture (85) to shifts in carbon storage precipitated by changes in land management practices or climate (86). Moreover, land use and climate change interact to increase the frequency and magnitude of nutrient and carbon pulses to waterbodies (87). In the interest of securing a healthy future for critical freshwaters, we suggest that current soil chemistry heterogeneity across the landscape can inform predictions about the potential consequences of anthropogenic watershed alterations on lakes. In particular, work can be done to understand the microbial diversity and food web dynamics driven by various soil states under future land use scenarios. Overall, the ability to predict lacustrine protist diversity from watershed conditions, as demonstrated in this study, highlights an expanded potential for monitoring lake ecosystems using remote sensing products (88).

Conclusion
This is the first study to examine the taxonomic and trophic functional variation in protist diversity across the expansive and lake-rich Canadian landscape. We showed that lakes at this continental scale displayed broad environmental heterogeneity including substantial variation in local physicochemical conditions driving taxonomic and phylogenetic community turnover. Watershed soil pH and crop agriculture additionally predicted community turnover and exceptional local-scale diversity. Hypereutrophic lakes were found to contain less diverse and more distinct assemblages than lower-
nutrient lakes, primarily as a product of their variable heterotroph and mixotroph compositions. In contrast, phototroph assemblages were more similar among hypereutrophic lakes. While phototrophy was the prevailing nutritional strategy in lake euphotic zones, heterotrophy was nearly as numerically important; each of these trophic modes was highly predictable from physicochemical and other environmental factors. Our survey and findings serve as a valuable resource for mapping species distributions and provide a basis for future research into the increasing anthropogenic impact on lake microbiomes.

Methods

Lake selection and sampling

Hundreds of lakes were sampled between July and early September in 2017 – 2019 by the Natural Sciences and Engineering Research Council of Canada (NSERC) Canadian Lake Pulse Network (52). Sampling was timed to coincide with the summertime period of water column thermal stratification, where relevant. Lakes were sampled across 12 terrestrial ecozones, regions defined by landform, geology, and vegetation (89). Lake selection was stratified across lake surface area and watershed land use impact categories to capture natural and human-mediated lake heterogeneity. Only natural lakes with a maximum depth of at least 1 m and within 1 km from a road were considered. Freshwater to oligosaline lakes (identified as having conductivity <8 mS/cm and total major ions <4,000 mg/L) were retained for this analysis.

Water was collected using an integrated tube sampler from the euphotic zone over a depth of up to 2 m below the surface at the deepest point in the lake (90). The site of maximum lake depth was located by depth sounding with the aid of bathymetric maps where available. The depth of the euphotic zone was estimated as twice the Secchi disk depth. All water sampling equipment was acid-washed and rinsed three times with lake water before use. Carboys were stored in icepack-chilled coolers until water filtration later in the day. Water was prefiltered through 100 μm nylon mesh and vacuum-filtered on 47 mm-diameter 0.22 μm Durapore membranes through a glass funnel at a maximum pressure of 8 inHg. Up to 500 mL of water was filtered until the filter was nearly clogged. Filters were stored in sterile cryovials at -80 °C.
18S rRNA gene amplification and sequencing

DNA was extracted using the DNeasy PowerWater kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions with the addition of two optional steps: after bead beating and centrifugation, 1 µL ribonuclease A was added to samples, followed by 30 min incubation at 37 °C. DNA was quantified using the Qubit dsDNA BR Assay (Invitrogen, Carlsbad, CA, USA). A ~265 bp fragment of the 18S rRNA gene V7 region was amplified with the primers 960F (5'-GGCTTAATTTGACTCAACRCG-3') (91) and NSR1438 (5'-GGGCATCACAGACCTGTTAT-3') (92) to broadly target microeukaryotes (93). Each PCR reaction contained a total 25 µL mixture of 14.25 µL MilliQ, 5 µL 5X High-Fidelity buffer, 1.25 µL of each 10 µM primer, 0.5 µL 10 mM dNTPs, 0.25 µL DMSO, 0.5 µL Phusion DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), and 2 ng DNA template. PCR conditions followed an initial denaturation at 98 °C for 1 min, 30 cycles of 98 °C for 10 s, 60 °C melting for 30 s, 72 °C for 20 s, and a final extension at 72 °C for 5 min. PCR products were loaded with Orange G dye into ethidium bromide-stained 2% agarose gel and electrophoresed at 40 V for 100 min. DNA bands aligned at the target fragment length against a 100 bp DNA ladder were excised with razor blades and gel-extracted with the QIAquick Gel Extraction kit (QIAGEN, Hilden, Germany), modified by final elution into MilliQ. PCR products were submitted to Genome Quebec for library barcoding and sequencing of 250 bp paired-end reads in three sequencing runs on an Illumina MiSeq platform.

ASV inference and annotation

Primer sequences were removed in Cutadapt v. 3.1 (94). Trimmed reads were processed into ASVs through DADA2 v. 1.16 (95). Samples were pooled for ASV inference using otherwise default parameters. Taxonomy was assigned with naïve Bayesian classification trained on PR2 v. 4.12.0 (96). Potentially spurious ASVs were removed by visually inspecting a de novo alignment performed in MAFFT (97). To retain only putative protist ASVs, ASVs assigned to Metazoa, Fungi, and Embryophyceae were removed. Taxa were assigned to trophic functional groups as either photoautotrophs, heterotrophs (bacterivores, cytotrophs, saprotrophs, or osmotrophs), mixotrophs, or parasites according to lineage-specific feeding habits summarized by Adl et al. (98).
Sequence similarities with known diversity

A database of 18S rRNA gene references restricted to the V7 region was constructed by applying the 960F/NSR1438 primer pair to PR v. 4.13.0 in Cutadapt. ASV top hits were queried against the PR² database in BLAST v. 2.6.0+ (99). Sequence identities were reported for ASVs that were globally aligned to references over a length ≥220 nucleotides.

Environmental data collection

Lake trophic states were assigned based on TP concentration thresholds estimated for Canadian freshwater systems: ultraoligotrophic (TP < 4 μg/L), oligotrophic (4 – 10), mesotrophic (10 – 20), mesoeutrophic (20 – 35), eutrophic (35 – 100), and hypereutrophic (>100) (100). Meteorological conditions recorded over seven days leading up to sampling and ice disappearance day data were accessed from ERA5-Land hourly reanalysis (101). Data on watershed slope and lake volume, discharge, and hydraulic residence time were accessed from HydroLAKES v. 1.0 (5). Watershed surface soil properties were accessed from SoilGrids250m (102). Land cover information was compiled as described by Huot et al. (52). Maps were constructed in R with the NAD 83 coordinate reference system and using the coordinates of Canada from the package maps (103) and ecozone shapefiles sourced from the Canada Council of Ecological Areas (89).

Environmental data were categorized into thematic groups of variables. Latitude, longitude, and altitude were categorized as geography variables. Ice disappearance day and meteorological variables (air temperature, precipitations, and net solar radiation) were categorized as weather variables. Lake surface area, circularity, volume, maximum depth, discharge, residence time, watershed slope within 100 m of the shoreline, watershed area, and lake-to-watershed area ratio were categorized as lake morphometry variables. Watershed land use (crop agriculture, pasture, built development, and clear-cut forestry) and natural land cover fractions, human population density, and mean surface soil properties (bulk density of the fine earth fraction, cation exchange capacity, nitrogen, pH, organic carbon density, organic carbon content in the fine earth fraction, volumetric fraction of coarse fragments, clay, sand, and silt) were categorized as watershed variables. Surface water temperature, calcium, magnesium,
potassium, sodium, chloride, sulfate, TP, TN, DIC, DOC, and chlorophyll-\(a\) concentrations, pH, and lake
colour were categorized as lake physicochemical variables. Missing physicochemical data were replaced
with ecozone median values. Highly collinear variables, evaluated by Pearson’s correlation \(r \geq 0.7\), within
all categories except physicochemistry were removed.

Diversity analyses

ASVs were aligned in the SILVA Incremental Aligner v. 1.7.2 (104) against the SILVA 138.1 SSU Ref NR
99 database (2020/08/27 release) (105). A maximum-likelihood phylogeny was constructed in FastTree v.
2.1.11 using the Generalized Time-Reversible model of nucleotide evolution (106). Phylogenetic
dissimilarities between ASV assemblages were calculated as generalized UniFrac distances, which are
sensitive to compositional changes in lineages of intermediate abundance (107). Generalized UniFrac
distances (\(\alpha = 0.5\)) were computed using the GUniFrac package.

To deal with uneven total sequence abundance across samples, sequence count composition
was scaled to relative abundance. Rarefaction analysis was conducted on the total data set by measuring
ASV richness in assemblages randomly subsampled at each 1,000-sequence step. Taxon accumulation
was estimated in a random ordering of lakes using 100 permutations in the package vegan (108). Local
diversity indices (richness, Pielou’s evenness, Shannon diversity, and Faith’s phylogenetic diversity) were
calculated from rarefied community data (i.e. randomly subsampled to the lowest sample abundance
equaling 10,069 sequences) in the R packages vegan (108) and picante (109). PCAs were computed on
Hellinger-transformed community data in vegan.

LCBD and \(\beta\)-diversity partitioning analyses were performed on \(\beta\)-diversity estimated using 100
permutations from Hellinger-transformed community data in the package adespatial (110). RV coefficients
were computed from the first two principal components or coordinates in the package FactoMineR (111).
Nonlinear relationships between \(\beta\)-diversity and untransformed environmental gradients were modeled in
GDMs (112, 113) in the package gdm (114). To create GDM site-pair tables, pairwise dissimilarities
between sites were weighted proportionally to the total number of sequences associated with each
sample. Variable selection for GDMs was performed using backward elimination with 100 permutations
per step. To assess the dispersion in taxonomic composition among lakes of the same trophic state, the
mean Bray-Curtis distances of assemblages to trophic state medians (i.e. centroids) calculated across the first two principal coordinates were compared using Tukey’s tests of ANOVAs.

Data wrangling and statistical analysis were performed in R v. 4.0.2 (115).

Data availability
Sequence data have been deposited in the European Nucleotide Archive under study accession PRJEB42538 (www.ebi.ac.uk). Scripts are accessible from https://github.com/rebeccagarner/lakepulse_protists.

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Figure legends

Figure 1. Diversity and distributions of protists across 366 Canadian lakes. Lake trophic states and ecozones are shown on the map of sampling sites as coloured circles and polygons, respectively. The relative sequence abundance of protist taxonomic divisions in each lake is represented in the inner tract of bar plots. The middle tract of bar plots shows the relative sequence abundance of trophic modes. The heatmap on the outer edge illustrates the proportions of land use and land cover associated with the watershed of each lake, whose trophic state is represented in an adjacent coloured circle. Watershed land use proportions are hierarchically clustered to highlight the relationship between agriculture and trophic state.

Figure 2. Accumulation and incidence of genotypes across lakes. A) Accumulation curve of genotypes in a random ordering of lakes. Vertical bars are standard deviations. The accumulation of new genotypes was rapid in the first ~100 lakes, followed by a gradual deceleration. B) Incidence of genotypes across
lakes. Most taxa are distributed across one or a few lakes, whereas a few taxa (magnified in inset plot) are widely distributed. ASV taxonomic classifications are coloured according to the taxonomic divisions in the legend of Figure 1.

**Figure 3.** Local protist diversity of each lake. A) Rarefied Shannon diversity index calculated for each protist assemblage across the Canadian landscape. Ecozones are identified in the map legend of Figure 1. B) Local diversity metrics categorized by lake trophic state. TP concentration thresholds and sample sizes of lakes categorized under each trophic state are: ultraoligotrophic (TP <4 μg/L; \( n = 4 \)), oligotrophic (4 – 10 μg/L; \( n = 48 \)), mesotrophic (10 – 20 μg/L; \( n = 132 \)), mesoeutrophic (20 – 35 μg/L; \( n = 71 \)), eutrophic (35 – 100 μg/L; \( n = 61 \)), and hypereutrophic (>100 μg/L; \( n = 50 \)).

**Figure 4.** Taxonomic and phylogenetic variation of protist assemblages among lakes. A) PCA of the taxonomic variation among protist assemblages at the level of individual ASVs. The relative contributions and taxonomic assignments are shown for the top 7 ASVs (an arbitrary cut-off selected for illustrative clarity) contributing to the variation explained by the first two PC dimensions. B) PCoA of the phylogenetic variation among protist assemblages. Ecozones and trophic state classifications of lakes are represented by letter symbols and colours, respectively.

**Figure 5.** Local contribution to β-diversity (LCBD) of protist assemblages across the Canadian landscape. LCBD describes the taxonomic uniqueness of a given assemblage, i.e. how much the taxonomic composition differs from the rest of the communities in the landscape. Ecozones are identified in the map legend of Figure 1.

**Figure 6.** PCAs of the taxonomic variation among (A) phototroph, (B) heterotroph, and (C) mixotroph assemblages at the level of individual ASVs. The relative contributions and taxonomic assignments are shown for the top 7 ASVs contributing to the variation explained by the first two PC dimensions. Ecozone affiliations and trophic state classifications of lakes are represented by letter symbols and colours, respectively.

**Tables**

**Table 1.** Percent deviance explained by GDMs. GDMs were constructed using various community response data and categories of environmental explanatory variables. Models that were not statistically
significant \((p \geq 0.05)\) are denoted by NS. *Analysis was performed only on taxonomic composition response data.

| Explanatory variables | All protists | Trophic mode* |
|-----------------------|--------------|---------------|
|                       | Taxonomy     | Phylogeny     | Phototrophs | Heterotrophs | Mixotrophs |
| Physicochemistry      | 38           | 33            | 35          | 42           | 18         |
| Watershed             | 15           | 16            | 20          | 19           | NS         |
| Morphometry           | 6            | 8             | 5           | NS           | NS         |
| Weather               | NS           | NS            | NS          | 3            | NS         |
| Geography             | NS           | NS            | NS          | 2            | NS         |

**Supplemental materials**

**Figure S1.** Distributions of A) geography, B) lake morphometry, C) watershed, D) weather, and E) physicochemical variables by ecozone. Dashed lines denote ecozone medians.

**Figure S2.** Principal component analysis of sites based on lake and watershed environmental characteristics.

**Figure S3.** Rarefaction curve showing the number of ASVs detected as a function of sample size across all sites.

**Figure S4.** Sequence similarities of ASVs to known 18S rRNA gene V7 region diversity. A) Frequency of ASVs as a function of sequence identity with the top hit reference in PR². B) Number of sequences associated with the sequence identities of top hits. Bar colours represent the division-rank taxonomic assignment top hit references in PR².

**Figure S5.** Map of rarefied ASV richness.

**Figure S6.** Partial effects of A) physicochemical, B) watershed, and C) lake morphometry variables on the taxonomic and phylogenetic turnover of protist assemblages. Points along the x-axis denote the distribution of explanatory variables and are coloured according to lake trophic state (see map legend in Figure 1).

**Figure S7.** Phototroph, heterotroph, and mixotroph assemblage distances from trophic state centroids.

**Figure S8.** Partial effects of A) physicochemical, B) watershed, C) lake morphometry, D) weather, and E)
geography variables on the taxonomic turnover of phototroph, heterotroph, and mixotroph assemblages.

Points along the x-axis denote the distribution of explanatory variables and are coloured according to lake trophic state (see map legend in Figure 1).

Table S1. ASV taxonomic and trophic functional annotations.

Table S2. Explanatory variables and relative importance of individual predictors in GDMs. Partial effects (i.e. relative importance) of individual predictors within models (parenthesized and presented in descending order of variable importance) were determined as the sum of I-spline coefficients equaling the magnitude of turnover. Non-significant models are denoted NS. *For models of turnover in trophic mode assemblages, analysis was performed only on taxonomic composition response data.
Local contribution to β-diversity:

- 0.0035 – 0.0044
- 0.0029 – 0.0035
- 0.0024 – 0.0029
- 0.0017 – 0.0024
