1 Title: Four meromictic (?) lakes in Itasca State Park, Minnesota,

U.S.A.

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- 31

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34 Abstract

35 Four adjacent lakes (Arco, Budd, Deming, and Josephine) within Itasca State Park in Minnesota, 36 USA are reported to be meromictic in the scientific literature. However, seasonally persistent 37 chemoclines have never been documented. We collected seasonal profiles of temperature and 38 specific conductance and placed temperature sensor chains in two lakes for ~ 1 year to explore 39 whether these lakes remain stratified through seasonal mixing events, and what factors contribute 40 to their stability. The results indicate that all lakes are predominantly thermally stratified and are 41 prone to mixing in isothermal periods during spring and fall. Despite brief, semi-annual erosion 42 of thermal stratification, Deming Lake showed no signs of complete mixing from 2006 to 2009 43 and 2019-2022. Geochemical data indicate that water in Budd Lake, the most dilute lake, is 44 predominantly sourced from precipitation. The water in the other three lakes is calcium-45 magnesium bicarbonate type, reflecting a source of water that has interacted with the landscape. 46 $\delta^{18}O_{H2O}$ and $\delta^{2}H_{H2O}$ measurements indicate the lakes are supplied by precipitation modified by

47 evaporation. The water residence time in meromictic Deming Lake is short (100 days), yet it 48 maintains a large reservoir of dissolved iron. Josephine, Arco, and Deming lakes sit in a valley 49 with likely permeable sediments and may be hydrologically connected through wetlands, and 50 recharged with shallow groundwater, as no streams are present. All four lakes develop 51 subsurface chlorophyll maxima layers during the summer. All lakes also develop subsurface 52 oxygen maxima that may results from oxygen trapping in the spring by rapidly developed 53 thermoclines.

54 Introduction

55 Meromictic lakes, which do not mix seasonally, maintain anoxic bottom waters that 56 minimize sediment resuspension and bioturbation and permit the development of laminated or 57 varved sediments (Anderson et al. 1985; Anderson and Dean 1988). Such sediments can record 58 climatic transitions, vegetation changes, changes in sediment transport, and atmospheric 59 deposition patterns (O'Sullivan 1983). Laminated sediments are also useful for studying the 60 formation, deposition, and diagenetic transformation of chemically precipitated minerals 61 (Wittkop et al. 2020; Ledesma et al. 2022). Such sediments are useful to interpret the origin of 62 minerals in sediments deposited from past stratified marine waters when such conditions no 63 longer exist(Degens and Stoffers 1976; Swanner et al. 2020). Meromictic lakes are present on all 64 continents but are thought to be rare (Walker and Likens 1975; Stewart et al. 2009; Hall and 65 Northcote 2012).

Lakes can develop meromixis when surface wind action does not mix the epilimnion, the upper layer of the lake, with the monimolimnion, the deepest layer of the lake. Meromictic lakes are characterized by a sharp increase in electrical conductivity at a chemocline separating a cold and/or dense monimolimnion from the epilimnion (Boehrer and Schultze 2008). The epilimnion is in contact with the atmosphere, while the monimolimnion is anoxic. This results in changing
sedimentation patterns compared to lakes that mix regularly. A sediment indicator of an anoxic
monimolimnion typical of low sulfur meromictic lakes is enhanced burial of oxygen-sensitive
mineral forming elements, particularly iron and manganese (Dean and Schwalb 2002; Wittkop et
al. 2014).

Lake morphometry is a key factor in the development of meromixis. Lakes that are relatively deep compared to their surface area, which determines the fetch upon which wind can act, are less likely to overturn (Wetzel 2001). The potential for water column stratification is reflected by relative depth (Z_r), which is the ratio of the maximum lake depth (Z_m) to the diameter of a circle of area equal to that of the lake (A^0), expressed as a percentage.

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$$Z_{\rm r}(\%) = \frac{Z_m}{2} \sqrt{\frac{\pi}{A_0}} \times 100$$
 (1)

Most temperate zone lakes prone to meromixis have relative depths exceeding 4% and A⁰ of less than 0.5 km² (Swanner et al. 2020). Meromixis can also result from increased chemical constituents that enrich the water density of monimolimnion, such as from runoff, groundwater inputs, and remineralization processes associated with organic carbon loading (Hakala 2004; Schultze et al. 2017). The identity of the substances causing increased density varies depending on the catchment characteristics but can include dissolved iron and manganese, as well as dissolved inorganic carbon (Kjensmo 1967; Hongve 2002).

Gradients of dissolved oxygen and inorganic nutrients are mediated by primary
productivity and uptake in the epilimnion and sinking and decomposition of biomass in the
monimolimnion (Camacho 2006). Stratification limits the return of nutrients to the epilimnion. A
subsurface chlorophyll maximum layer (SCML) often develops within the metalimnion, the
middle seasonally mixed layer of meromictic lakes (Baker and Brook 1971; Camacho 2006).

Stable stratification, the availability of both light and nutrients, and zooplankton grazing drive
the formation and location of the SCML in meromictic lakes (Klausmeier and Litchman 2001;
Pilati and Wurtsbaugh 2003). Primary productivity is often highest within the SCML (Camacho
2006).

97 Four lakes in Itasca State Park, Minnesota, USA - Arco, Budd, Deming, and Josephine 98 (Figure 1) - have been described as meromictic in the scientific literature since the 1960s (Baker 99 and Brook 1971; Anderson et al. 1985; Stewart et al. 2009). However, there has never been 100 physical and chemical data from all lakes during all seasons - especially spring and fall when 101 mixing would be expected - to document through the maintenance of a chemocline that they are 102 meromictic. The goals of this study are to 1) determine whether these four lakes are meromictic, 103 2) investigate the water type, sources, and reasons for meromixis, and 3) describe the unique 104 biological features of these lakes. To achieve these goals, we conducted fieldwork from 2006-105 2009 and 2019-2022, and integrated data from University of Minnesota students working at the 106 Itasca Biological Station and Laboratories since the 1950s. We hope that this description of the 107 lakes will spur new investigations that utilize their unique geomorphological and biogeochemical 108 features.

109 Methods



111 Figure 1: Map of the four study lakes within Itasca State Park, Minnesota.

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Formation of the four study lakes occurred during the late-Wisconsin glaciation ~12,000 years ago (marine isotope stage 2; Jennings and Johnson 2011). They occupy a tunnel valley that was formed beneath the Wadena lobe of the Laurentide Ice Sheet. Following glacial retreat, the melting of stagnant ice blocks within the tunnel valley left depressions in the landscape nowoccupied by lakes and wetlands (Wright Jr. 1993).

Today, the four lakes investigated in this study sit in the HUC-12 watershed that sources the headwaters of the Mississippi River (U.S. Geological Survey 2017). Budd (478.6 meters above mean sea level; MAMSL) is the highest elevation, while Arco (465.8 MAMSL) and Josephine (465.4 MAMSL) lie at similar elevations. Deming is the lowest elevation of the lakes (464.8 MAMSL).

123 Lake depth measurements were collected using a Garmin Striker 4 dual-beam transducer 124 (sonar) attached to a rowboat or canoe. Depth and GPS measurements were taken every six 125 seconds while the boat was in motion. A Garmin GLO 2 GPS receiver and ArcGIS Collector app 126 was used to navigate, track the boat's course, and ensure even coverage. The shoreline of the 127 lakes was obtained by walking along accessible areas of the shore with the Garmin GLO 2 GPS 128 receiver, or from Lidar-derived digital elevation models. Bathymetry rasters (1 m resolution) 129 were generated from the depth measurements in ArcGIS Pro 3.0 using a 3rd-degree Local 130 Polynomial Interpolation. These rasters were used to calculate lake volumes and contour maps. 131 Rasters and volume data have been deposited with the Environmental Data Initiative (Swanner et 132 al. 2022).

133 Chemical, physical, and biological parameters measured on the four lakes included depth, 134 temperature, specific conductance, salinity, turbidity, pH, oxidation-reduction potential, 135 dissolved oxygen, photosynthetically active radiation, chlorophyll-a, and phycocyanin. Major 136 cations, anions, and isotopes of water (δ^2 H-H₂O and δ^{18} O-H₂O) were determined on lake water 137 retrieved from different depths within the four lakes. Taxon-specific chlorophyll-a fluorescence 138 was collected with a Fluoroporbe (BBE Moldaenke). The data and description of methods are139 available in the Environmental Data Initiative (Swanner et al. 2022).

140 A string of temperature loggers (HOBO Water Temp Pro v2) placed at different depths 141 were deployed into the deep areas of Deming, Arco, and Budd lakes for one year. A conductance 142 logger (HOBO Conductivity Logger) was added near the bottom of the strings in Arco and Budd 143 after six months. These sensors measured temperature every thirty minutes and specific 144 conductivity every 2 hours. The sensor string was not retrieved from Budd, as it could not be 145 located. Conductance measurements with a Yellow Spring Instruments ProDSS 146 temperature/conductivity sensor on deployment and removal were used to check for drift in the 147 HOBO conductance logger. Hourly wind speed data for the duration of sensor deployment 148 utilized the ITCM5 weather station in Itasca State Park. Data was downloaded from MesoWest 149 (https://mesowest.utah.edu/). Plots and analyses were produced in Python or R Studio 150 (2022.07.2) using the RLakeAnalyzer package v.1.11.4.1 (Winslow et al. 2019). Major anions (CO₃²⁻, HCO₃⁻, Cl⁻, and SO₄²⁻) and cations (Na⁺, K⁺, Ca²⁺, Mg²⁺) were used 151 152 to produce a Piper diagram in Geochemist's Workbench 15.0. The concentration of cations and anions was calculated as the percentage of total cations and anions in meq L^{-1} . 153 The isotopes of water ($\delta^2 H_{H2O}$ and $\delta^{18}O_{H2O}$) were measured on spring or seep water that 154 155 had been filtered with 0.45 micron nylon syringe filters and stored at 4 °C with minimal 156 headspace until analysis. Samples were analyzed with a Picarro L1102-i Isotopic Liquid Water 157 Analyzer at the Stable Isotope Laboratory at Iowa State University. The analytical uncertainty and average correction factor for $\delta^{18}O_{H2O}$ are ± 0.05 ‰ and ± 0.30 ‰ for $\delta^{2}H_{H2O}$ relative to V-158 159 SMOW.

160Samples for microscopy and water color were collected into amber bottles with a Van161Dorn sampler from three different depths in each lake, including the SCML, if present, as162determined with the YSI ProDSS. Water color was determined on water filtered through a GF-75163(Advantec) glass fiber filter (Cuthbert and del Giorgio 1992). Absorbance was measured at 440164nm and 750 nm. The absorbance at 750 nm was subtracted from the absorbance at 440 nm. The165absorption coefficient was calculated with the equation:166
$$g (m^{-1}) = (2.303 * Absorbance)/(path length)$$
167A conversion was necessary to determine the color of the lake water as seen below.168Color (mg Pt L⁻¹) g₄₄₀ (m⁻¹) = 18.216*[g₄₄₀ (m⁻¹)] - 0.209169Water sampled from the SCML was preserved with 1% Lugol's solution upon returning170to the lab. Fixed samples were settled in the dark for three to seven days.

171 Student reports from courses taking place over several decades at the Itasca Biological

172 Station and Laboratories (IBSL) (Knoll and Cotner 2018), formerly the Itasca Biological Station,

173 were acquired from the library at the University of Minnesota, Twin Cities.

174 **Results & Discussion**

175 Table 1. Morphometric parameters for the four study lakes.

Lake	Volume (m^3)	Surface Area (m^2)	Maximum Depth (m)	Relative Depth (%)	Fetch Distance (m)	Fetch Heading (degrees)
Arco	142,628.73 m ³	$24180 m^2$	12.6 <i>m</i>	7.3%	210.4 m	175 deg.
Budd	183,441.22 <i>m</i> ³	$28184.5 m^2$	16.1 <i>m</i>	8.8%	310.3 m	169 deg.
Deming	242,701.12 m ³	$54325 m^2$	20.8 m	7.3%	346.7 m	188 deg.
Josephine	$315,765.05 m^3$	52711 m^2	14.5 m	5.7%	432.4 m	136 deg.

177 Morphometry and Mixing Status

178 Deming is the largest and deepest of the four study lakes with a 54,325 m² surface area 179 and a maximum depth of 20.8 meters (Table 1). The maximum depth of Deming Lake has 180 previously been reported as 16.5 m (Hooper 1951), 17 m (Baker and Brook 1971), and 17.6 m 181 (Lascu and Plank 2013). Differences in technology and the small surface area (4.3%) with water 182 depths at or below 17 m could account for the variation. Long-term water-level fluctuations may 183 be induced by drought (Lascu et al. 2012). In all lakes, steep-sided deep holes were detected 184 (Figure 2). It is possible that temperature, pressure, and/or salinity gradients present at the tops of 185 these holes could deflect sound beams, causing errors in our depth measurements (Boehrer and 186 Schultze 2008). These factors could have affected all lakes, which were found to be 187 systematically deeper than previously reported. 188 Josephine has the longest fetch and lowest Z_r of all study lakes. The maximum depth of 189 Josephine Lake (14.8 m) has previously been reported as 10.3 m (Baker and Brook 1971), 12-13 190 m (Callis et al. 1976), and 13 m (Gage and Gorham 1985). The surface area with depths at or 191 below 13 m in Josephine Lake is 5.1%. There was the greatest variation between the previously 192 reported maximum depth of Budd Lake (10.8 m; Baker and Brook 1971) and ours (16.1 m). 193 Budd Lake has the highest Z_r (8.8%). Arco Lake has the smallest surface area of 24,180 m² and a 194 maximum depth of 12.8 m. Arco and Budd Lakes have steep banks along their eastern sides 195 (Figure 1). All lakes exceed a Z_r of 4% and have surface areas of less than 500,000 m², typical of 196 meromictic lakes in the temperate zone (Swanner et al. 2020).

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202 Deming Lake has the longest record of seasonal profiles (2006-2009 and 2019-2022), as 203 its sediments have previously been used to investigate Holocene climate variations (Lascu et al. 204 2012; McLauchlan et al. 2013). A chemocline, or sharp increase in specific conductance, persists 205 in Deming Lake at all sampled times (Figure 3), which is expected for a meromictic lake. The 206 chemocline occurs at 11-13 m (Figure 3). The chemocline was below 10 m in 1989 (Church et 207 al. 1989), and from 11-13 m a decade later (Reiter et al. 1998) based on temperature-208 compensated conductance measurements from IBSL student reports, indicating that the 209 chemocline has been at a consistent depth for decades. Earlier observations from numerous 210 student reports indicate a much shallower chemocline in Deming Lake, below 5 m (Engstrom et 211 al. 1974; Karli and Keller 1975; Habedank 1990; Bieter et al. 1991; Condon et al. 1996; Nelson 212 et al. 2011). Many of these measurements were made as conductivity without a standardized cell 213 geometry and may or may not have been corrected to a temperature of 25°C, which is necessary 214 for comparing samples across the thermocline. The temperature-compensated and specific cell 215 geometry measurements in our dataset (i.e., specific conductance) likely better capture the 216 variation in dissolved ions comprising the chemocline.

A systematic increase in the magnitude of specific conductance readings below the chemocline was observed from the 2006-2009 to the 2019-2022 datasets (Figure 3). This could either be an artifact of instrumentation and calibration differences between the two datasets or could represent a temporal change in water column properties. The counties encompassing Itasca State Park experienced extreme drought for several months in the spring of 2006 and exceptional

222 drought for a few weeks in the summer of 2021(National Oceanographic and Atmospheric 223 Administration; Supplementary Figures 2 and 3). This could have increased the lake's salinity 224 through increased evaporation (Jellison and Melack 1993). Alternatively, or in addition, the 225 magnitude of water sources of differing salinities may have been altered (Ludlam and Duval 226 2001). In the second case, a drought would decrease the magnitude of low specific conductance 227 precipitation and increase the proportion of higher specific conductance groundwater to the 228 annual water budget. As Deming Lake does not have a surface inlet, changes in streamflow can 229 be neglected. The period from 2006-2009 was generally dry, and the amount of recharge to the 230 groundwater system may have also decreased over a multi-year period, effectively decreasing 231 groundwater inputs to the lake, and counteracting the effects of evaporation on specific 232 conductance in Deming Lake. In 2019-2022, the exceptional drought was short-lived and 233 specific conductance might better reflect decreases in precipitation than decreases in recharge, 234 resulting in greater specific conductance values. Another possibility is that variations in the 235 amount of in-lake primary productivity could modulate the specific conductance in the 236 monimolimnion (Campbell 1977). Finally, a longer time since a mixing event could also increase 237 the dissolved solute load from 2019-2022 relative to 2006-2009 (Katsev et al. 2010). 238 A chemocline was present at all time points for Arco Lake between 7-10 m except for 239 May 2022 (Figure 3). It was poorly developed in May 2021, and its deepest occurrence was in 240 January 2021. Arco Lake has a maximum depth of 12.8 m, but no specific conductance data was 241 collected below 12 m as part of the profiles acquired during the study period due to the difficulty 242 of finding the small deep spot (1.7% of surface area). The chemocline is shallowest in mid-

summer, consistent with the 7 m chemocline observed in temperature-equilibrated conductivity

readings in 1975 and 1976 (Evans and Bjerklie 1975; Barnes et al. 1976), and 2011 (Harren et al.
2011).

246 Budd Lake had the lowest range of specific conductivity values (23-90 µS cm⁻¹). A 247 weakly demarcated chemocline was observed around 4-5.5 m water depths for May 2021, July 248 2021, October 2021, and May 2022. The January 2021 profile shows a deeper chemocline 249 (Figure 3). 250 Specific conductance values were uniform down to 8 m in Josephine Lake during the 251 current study period (Figure 3). A chemocline was only detected below 11 m in May 2022, while 252 depths below 11 m were not assessed on other dates during the study period due to the difficulty 253 of finding the deepest area before mapping in 2022. Autumnal circulation down to 10 m was 254 reported in Josephine Lake in 1975 (Gage and Gorham 1985).

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Figure 3. Specific conductance profiles of a) Arco Lake, b) Budd Lake, c) Josephine Lake, and
d) Deming Lake. For Deming Lake, seasons were classified according to solstice and equinox
dates.



Figure 4. Hobo sensor data for temperature and conductance from Arco Lake, collected from
May 2021 to May 2022 and from October 2021 to May 2022, respectively (top). Isotherm plot of
the temperature data from the Hobo sensor (bottom).

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Data from the temperature sensors (1.5, 3.5, 5.5, 7.5, 9.1, 9.5 m) and a conductance 265 266 sensor (9.1 m) in Arco Lake from May 2021 to May 2022 are presented in Figure 4. In the 267 summer of 2021, the temperature values in Arco Lake were different at distinct depths, 268 representing the development of a summer thermocline. The temperatures at different depths 269 started to converge in late fall until the lake became isothermal and continued cooling from 6 to 270 4 C in mid-November 2021, representing the erosion of thermal stratification. The Brunt-Väisälä or buoyancy frequency (N), commonly expressed as N² in units s⁻², calculated from the time 271 272 series temperature data is near zero at that time, indicating an erosion of stability, which 273 persisted until the sensors were removed on May 16, 2022, over a week after the ice-off on May

274 8 (Supplementary Figure 4). A drop in the conductance value at 9.1 m in early November 2021 275 preceded isothermal conditions and indicated mixing down to at least 9.1 m (Figure 4). The 276 dimensionless lake number (Imberger and Patterson 1989) was calculated using the time series 277 and hourly wind data from Itasca State Park (Supplementary Figure 5). It started to consistently 278 rise on May 15, 2022 as the epilimnion warmed. In combination with the seasonally variable 279 chemocline depth (Figure 3), these analyses indicate that Arco Lake mixes during isothermal 280 periods and is not a meromictic lake. The data presented is most consistent with Arco being a 281 dimictic lake, although holomixis cannot be ruled out. 282 Data from the temperature sensors (0.5, 2.5, 5.5, 8.5, 11.5, and 14.5 m) in Deming Lake 283 from June 2019 to May 2020 are presented in Supplementary Figure 6. The lake became 284 isothermal in early November 2019. The Brunt-Väisälä frequency calculated from this data 285 dropped to near zero and rebounded slightly due to under-ice thermal stratification, a 286 phenomenon not observed in Arco Lake (Supplementary Figure 4). The specific conductance 287 profiles in Deming consistently show the same trend, although the exact depth and magnitude of 288 the chemocline vary seasonally and on the decadal scale (Figure 3). This indicates partial 289 seasonal mixing in Deming, likely during isothermal periods, but it may be insufficient or of too 290 short a duration to fully mix the lake. Deming was ice-free on April 25, 2020 (Minnesota 291 Department of Natural Resources 2022a), and the lake number started rising immediately due to 292 the onset of thermal stratification (Supplementary Figure 5), likely limiting the opportunity for 293 mixing. Long-term seasonal observation of a chemocline is consistent with Deming being a 294 meromictic lake (Zadereev et al. 2017). 295 Arco presents an end-member of a seasonally mixed lake (dimictic or holomictic) and

296 Deming an end-member of a meromictic lake among the four lakes studied here. In the absence

297 of high-frequency sensor profiles for Budd and Josephine lakes, the Schmidt stability 298 (Supplementary Figure 7) and the Brunt-Väisälä frequency (Supplementary Figures 8-11) were 299 calculated from seasonal profiles of temperature (Supplementary Figure 12) and salinity for all 300 four lakes. The Schmidt stability indicates that Deming and Budd are the most strongly stratified, 301 followed by Josephine and Arco. It is unknown when ice came off Budd Lake in 2022, but 302 Deming was ice-free on May 7, and Arco and Josephine were ice-free on May 8 (Minnesota 303 Department of Natural Resources 2022a) following strong winds on the evening of May 7. 304 Likely, Budd was also ice-free by May 8. A chemocline consistent with other summer 305 observations was present on May 17, 2022, which could indicate a lack of spring mixing for 306 Budd Lake. However, the January 2021 specific conductance profile for Budd shows a very 307 weak and deep chemocline, which would be expected from an autumn mixing event. Without 308 full profiles of specific conductance in Josephine Lake, it is impossible to evaluate mixing. 309 However, based on existing profiles, if a chemocline persists through spring turnover in 310 Josephine Lake as suggested by the sharp and deep chemocline observed in May 2022, then 311 monimolimnion must be limited to depths below 11 m, representing only 3.6% of total lake 312 volume (11,404.5 m³ of 313,295.5 m³).

Water color is related to the abundance of dissolved organic carbon (DOC) (Pace and Cole 2002). Of the four study lakes, Budd had the most colored water, followed by Deming (Supplementary Table 1). Arco and Josephine had very little color. Visually, Budd and Deming appear brown, while Arco and Josephine appear green. Enhanced water color could lead to a shallower thermocline and stronger stratification in Budd and Deming due to greater light absorption by compounds conferring color (Houser 2006). The Brunt-Väisälä frequencies, calculated from temperature and salinity profiles, are generally highest in the epilimnia during

320	late summer (July 2021 and August 2022; Supplementary Figures 8-11). This indicates that
321	temperature is more important than salinity to the stability of these lakes. In August 2022, when
322	Deming Lake had the highest N ² values observed, the meromictic stability (S') (Walker 1974)
323	was 7.81 J m ⁻² , within the range observed for the meromictic and ferruginous Lake 120 in
324	Canada (Campbell 1977) and Lake Nordbytjernet in Norway (Hongve 1999). These observations
325	suggest that meromixis in Deming Lake is primarily due to thermal stratification, with a small
326	contribution from salinity that could lead to meromixis in this lake but not the others nearby.
327	This phenomenon could be common to dilute meromictic lakes. In fact, a mixing event was
328	recorded for Deming Lake, in the summer of 1997, when a beaver dam west of the lake broke
329	and flooded it (Frane and Walberg 1997), indicating mixing can occur due to catastrophic events.
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% meq/kg

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337 Chemical Characteristics and Water Sources

338 A Piper diagram was used to define the water types of the four lakes based on their major 339 cations and anions. The predominant cation in all four study lakes was calcium (Figure 5). Budd 340 had a greater proportion of milliequivalents from sodium and potassium than the other three 341 lakes, balanced mostly by magnesium. Bicarbonate and carbonate ions represent nearly all anion 342 milliequivalents in Deming, Arco, and Josephine whereas chloride and sulfate contribute to the 343 anion balance in Budd. The water type for Deming, Arco, and Josephine is a calcium-344 bicarbonate type, whereas Budd does not have a dominant water type. The low specific 345 conductance and lack of water type in Budd indicate the water source is predominantly

346 precipitation. Calcium, magnesium, and carbonate/bicarbonate ions source from the calcareous 347 Itasca moraine and are typical for the Itasca region (Megard et al. 1993). This indicates that 348 water supplying Deming, Arco, and Josephine has undergone more water-rock reaction than that 349 supplying Budd, such as might be expected from an increasing proportion of groundwater 350 seepage to a lake's water inputs. Deming, Arco, and Josephine lie in a tunnel valley channel 351 containing coarser sands and gravel while Budd Lake is poorly integrated hydrologically with 352 the other three lakes, and the net seepage in Budd is probably the lowest among all the lakes. 353 Arco (465.8 MAMSL) is located 51 meters north of Josephine (465.4 MAMSL) and Deming 354 (464.8 MAMSL) is located 284 meters north of Arco. The similar lake levels and wetlands 355 between Arco and Deming (Figures 1 and Supplementary Figure 1) indicate these lakes are 356 likely to be hydrologically connected. If the water table mimics the surface topography, 357 groundwater potentially flows from Arco-Josephine to Deming. However, Budd (478.6 358 MAMSL) is perched highest in the watershed, located off-axis of Deming-Arco-Josephine, to the 359 west of a ridge (Figure 1). Because it is perched, the ridge is likely composed of less permeable 360 material than the valley sediments, such as till. The presence of wetlands on the north end of 361 Budd that follow a small valley towards Deming suggests that there could be a surface or near 362 surface hydrological connection from Budd to Deming. Lakes highest in their watershed are 363 more likely to receive a greater proportion of water from precipitation (Kratz et al. 1997), 364 consistent with the dilute nature of Budd Lake.

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Figure 6. Isotope data and fit (dashed line) of an evaporation line ($\delta^2 H_{H2O} = 4.5 * \delta^{18} O_{H2O} - 29.9$, r-squared = 0.93) from the four study lakes plotted with a Local Meteoric Water Line (Stelling et al., 2021 Marcell Exp Forest). Crosses are spring or bog water data from Itasca State Park (Supplementary Table 1). The cross closest to the lake data points is the Deming bog. Analytical precision is within the symbol sizes.

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374 The isotopic composition of water (i.e., $\delta^2 H_{H2O}$ and $\delta^{18}O_{H2O}$ in ‰) in May 2021 varied 375 little with depth in each lake (Supplementary Figure 13). In Arco, Budd, and Deming sampled in 376 June 2019, the epilimnion waters become depleted relative to the deeper water. Additional 377 measurements at Deming Lake in July and October 2021 show enriched values in the epilimnion 378 relative to the surface. Preferential evaporation of light isotopes during the summer and early fall 379 drive the epilimnion to more enriched values. In Figure 6, all lakes data points diverge from a 380 local meteoric water line (LMWL; Stelling et al. 2021) on a lake evaporation line (LEL) with 381 equation $\delta^2 H_{H2O}$ (‰) = 4.5* $\delta^{18}O_{H2O}$ (‰) - 29.9. The intersection of the LMWL and the LEL is 382 the composition of regional groundwater, representing isotopically depleted snowmelt as the 383 predominant pathway for recharging the local aquifers (Krabbenhoft et al. 1994). Historical $\delta^2 H_{H2O}$ and $\delta^{18}O_{H2O}$ data from springs at Elk Lake were retrieved from the 384 385 Minnesota Spring Inventory (Minnesota Department of Natural Resources 2022b). The Elk Lake 386 springs were sampled again in May 2022, as well as a sample from Nicollet Creek and the bog 387 on the southeast side of Deming (Supplementary Table 1). These sites had abundant marsh 388 marigolds (Supplementary Figure 14), which bloom in May at groundwater discharge sites 389 (Rosenberry et al. 2000). The sites at Elk Lake and Nicollet Creek had visible iron 390 mineralization, indicating reducing, iron-bearing water discharge at the surface (Supplementary 391 Figure 14). The Nicollet Creek spring sample lies closest to the intersection of the LEL and 392 LMWL, whereas the Deming bog sample lies closest to the lakes but is more enriched than the 393 lakes (Figure 6). 394 The seasonal amplitude of a lake's isotopes can be used to calculate a lake's water

residence time (Engel and Magner 2019). A time series of temperature data from weather station ITCM5 was used to determine average daily temperatures, and seasonal maximum and minimum values were used to calculate the amplitude in $\delta^{18}O_{H2O}$ inputs of precipitation. The Deming Lake $\delta^{18}O_{H2O}$ values (Supplementary Figure 13) encompass early spring, mid-summer, and late 399 summer data, and were used to determine the seasonal amplitude in δ¹⁸O_{H2O} (Supplementary
400 Figure 15). These two values were used to estimate a mean water residence time of 100 days for
401 Deming Lake.
402
403 *Biological Characteristics*404 Prior work on these four lakes indicated the presence of a lake-wide turbidity maximum
405 layer that was persistently just below 5 m Deming Lake (Baker and Brook 1971). This peak was

406 observable between late May and August from 1968-1970 but was absent in the winter. It was

407 reported in that study that bacteria and phytoplankton both contributed to the turbidity maximum

408 based on microscopic observations. This work indicated the layer was populated by the

409 cyanobacteria Oscillatoria agardhii var. isothrix (this genus is now called Planktothrix).

410





412 Figure 6. Multi-wavelength chlorophyll fluorescence (i.e. Fluoroprobe) measurements at a) Arco,
413 b) Budd, c) Josephine, and d) Deming Lakes, showing seasonal and depth trends in major
414 taxonomic groups. Note that the x scales vary between plots.

415

From 2019 to 2022, chlorophyll-a measured via multi-wavelength fluorescence acquired with a Fluoroprobe allowed for deconvolution of the fluorescence signal so chlorophyll-a could be attributed to one of four taxonomic groups: Cyanobacteria, Chlorophyta, Diatoms and Dinophyta, or Cryptophyta. Taxon-specific concentrations of chlorophyll-a in each lake inform phytoplankton community structures with depth (Figure 6). Deming Lake has a persistent SCML around 5-6 m attributable to Cyanobacteria. The SCML is less distinct in May 2022, a week and a half after ice off. It was also less distinct in May 2021. This suggests that the SCML in Deming Lake is a summer phenomenon that develops at or below the thermocline (Supplementary Figure
12), consistent with the observations from Baker and Brook (1971). In June 2019, a profile of
photosynthetically active radiation was also recorded (Supplementary Figure 19), and showed an
inflection point at 5.5 m, corresponding to the depth of the SCML at that time. These
observations are consistent with cyanobacteria forming a dense accumulation at that depth,
attenuating the light flux into deeper waters.
Samples from the SCML were taken from the four lakes in May 2022: Arco (5 m), Budd

430 (4.5 m), Deming (3.5 m), and Josephine (4.5 m). The most common phytoplankton of the four

431 lakes were the cyanobacteria *Planktothrix* sp. and *Aphanocapsa* sp., and the green alga

432 *Monoraphidium* sp. (Supplementary Figure 17). Some other phytoplankton species identified

were the red alga *Cryptomonas* sp., the cyanobacteria *Planktolyngbya* sp. and *Dolichospermum*sp., as well as other colonial green algae and cyanobacteria species.



436 Figure 7. Dissolved oxygen measurements at a) Arco, b) Budd, c) Josephine, and d) Deming

437 Lakes, showing seasonal and depth trends.

438

439 All four lakes have subsurface oxygen maxima exceeding air saturation during the 440 summer but lack this feature in the fall (Figure 7). The subsurface oxygen maximum in Deming 441 Lake usually occurs at 4 m, near the top of the thermocline (Church et al. 1989; Supplementary 442 Figure 12; Bieter et al. 1991; Balk et al. 2007). Metalimnetic oxygen maxima can result because 443 dissolved oxygen is more soluble in cold water after spring mixing, but as the water warms the 444 gas solubility decreases, causing oxygen to become supersaturated. Stratification induced by the 445 thermocline then limits the discharge of supersaturated oxygen to the atmosphere (Wilkinson et 446 al. 2015). Deming Lake rapidly develops a thermocline after ice-off (Supplementary Figure 6), 447 providing a mechanism for gas trapping. Enhanced biological productivity can also contribute to 448 the oxygen maximum, but is generally a smaller contributor (Craig et al. 1992; Wilkinson et al. 449 2015). The subsurface oxygen maximum is above the SCML in Deming Lake, suggesting that 450 the entrainment of spring oxygen is a larger contributor than photoautotrophy to the oxygen 451 maximum. However, there is also a pH maximum at that depth during the summer 452 (Supplementary Figure 18; Church et al. 1989; Barkow and Habedank 1990; Bieter et al. 1991), 453 as is expected for active photoautotrophy. Itasca Biological Station and Laboratories student 454 experiments that quantified photosynthesis and respiration via dissolved oxygen measurements 455 in bottle experiments generally found that net oxygen release was minimal in the SCML due to 456 vigorous respiration (Engstrom et al. 1974; Barkow and Habedank 1990). Measurements of the 457 O₂/Ar ratio would help to quantify the contribution of the two processes to the subsurface 458 oxygen maximum (Craig et al. 1992).

The occurrence of a summer SCML in Arco, Budd, and Josephine Lakes was more variable than in Deming Lake (Figure 6). Arco had a spring bloom of cyanobacteria in May 2021 and in May 2022, at which time the highest chlorophyll-a of this study was recorded. At both times, the SCML occurred below the oxygen maximum, in a hypoxic zone of the water column.

463 The May 2022 SCML at 5 m in Arco also enhanced light attenuation below that depth

464 (Supplementary Figure 19), as was observed in Deming in June 2019.

Arco, Budd, and Josephine also have subsurface oxygen maxima during summer, although not as consistently as Deming. When the depth of the thermocline is plotted against the depth of the oxygen maximum for all four lakes, there is a significant correlation if October 2021 samples are excluded (Supplementary Figure 20). In the fall, the subsurface oxygen maximum is absent (highest values are at the surface), and the thermocline is deepest (Figure 7). This may reflect that in fall the thermocline has deepened into hypoxic or anoxic waters.

471 Conclusion

472 Although reported to be meromictic, based on seasonal temperature and specific 473 conductance profiles, Arco, Budd and Josephine may be holomictic or even dimictic. Deming 474 has been meromictic in the periods of data collection presented here (2006-2009 and 2019-2022) 475 but experienced a mixing event in 1997 when an adjacent beaver dam broke. Although a 476 seasonally persistent chemocline occurs in Deming Lake, stratification is predominantly a 477 thermal phenomenon, with stability mostly conferred by a thermocline that develops rapidly after 478 isothermal spring and fall periods. Thermal stratification is not typical of meromictic lakes in the 479 temperate zone, but this weak stratification may be common in dilute boreal lakes (Meriläinen 480 1970; Campbell 1977). However, such meromixis may be easily perturbed by hydrographic or 481 land-use changes (Hongve 2002).

The chemical composition of the study lakes reflects their position in the watershed and water sources. The highest-elevation lake, Budd, had no distinguishable water chemical type and likely received most of its water from precipitation. Deming Lake had the highest ionic 485load, consistent with a greater contribution of water that had undergone some water-rock486interaction, likely groundwater. Further work is necessary to characterize groundwater487composition and quantify and localize groundwater input to determine if Deming Lake could be488classified as crenogenic meromictic. The short water residence time in Deming Lake implied by489the analysis of $\delta^2 H_{H2O}$ and $\delta^{18}O_{H2O}$, along with the minimal surface water inputs and likely high490permeability of tunnel valley deposits suggest the shallow groundwater system should be491considered as a major water source.

All four lakes have SCML during summer. The depth of the SCML varies in Arco, Budd,
and Josephine and is sometimes absent, but persists at around 5 m in Deming, corresponding to
the base of the photic zone. Subsurface oxygen maxima above air saturation likely develop by
trapping gas in cold water that then warms with rapid thermocline development in the spring.
Corresponding pH maxima in Arco and Deming indicate that primary productivity could also
contribute to the subsurface oxygen maxima.

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	2					

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- 511

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Supplementary Figure 1. Profiles of the lakes (in meters above mean sea level) along the fence diagrams in Figure 1.



Supplementary Figure 2. Historical drought information for the counties encompassing Itasca State Park during the period of data collection from 2006-2009.



Supplementary Figure 3. Historical drought information for the counties encompassing Itasca State Park during the period of data collection from 2019-2022 (retrieved on October 24, 2022).



Supplementary Figure 4. The Brunt-Väisälä or buoyancy frequency (N², s⁻²), calculated from the temperature time series for Deming Lake from June 2019 to May 2020 (left), and Arco Lake from May 2021 to May 2022 (right). Higher values indicate greater stability.



Supplementary Figure 5. The dimensionless lake number was calculated using wind speeds from the ITCM5 weather station and the temperature time series for Deming Lake from June 2019 to May 2020 (left), and Arco Lake from May 2021 to May 2022 (right). Higher values indicate greater stability.



Supplementary Figure 6. Deming Lake temperatures (C) from June 2019 to May 2020 (left). Arco Lake temperatures (C) from May 2021 to May 2022 (right).



Supplementary Figure 7. Plots of Schmidt Stability for each of the four study lakes from January 2021 to August 2022. Higher values indicate greater stability.



Supplementary Figure 8. The Brunt-Väisälä or buoyancy frequency (N², s⁻²), calculated from the temperature and salinity profiles from Arco Lake from January 2021 to May 2022. Higher values indicate greater stability.



Supplementary Figure 9. The Brunt-Väisälä or buoyancy frequency (N², s⁻²), calculated from the temperature and salinity profiles from Budd Lake from January 2021 to May 2022. Higher values indicate greater stability.



Supplementary Figure 10. The Brunt-Väisälä or buoyancy frequency (N², s⁻²), calculated from the temperature and salinity profiles from Deming Lake from January 2021 to May 2022. Higher values indicate greater stability.



the temperature and salinity profiles from Josephine Lake from January 2021 to May 2022. Higher values indicate greater stability.



Supplementary Figure 12. Temperature profiles of a) Arco Lake, b) Budd Lake, c) Josephine Lake, and d) Deming Lake. For Deming Lake, seasons were classified according to solstice and equinox dates.

Supplementary Table 1. Water color in mg Pt L⁻¹. The depths of individual samples (in meters) are given in parentheses.

Arco		Budd	Deming	Josephine
Depth 1	1.4 (4)	32.87 (2)	8.0 (3.5)	3.1 (2)

Depth 2	3.1 (5)	27.9 (4.5)	8.0 (4.5)	1.4 (4.5)
Depth 3	4.71 (5.5)	24.6 (9)	8.0 (5)	1.4 (9)
Average	3.1	28.4	8.0	2.0
Standard dev.	1.7	4.2	0	1.0



Supplementary Figure 13. Trends in $\delta^{18}O_{H2O}$ and $\delta^{2}H_{H2O}$ with depth in the four study lakes. Deming was the only lake sampled in all seasons. Analytical precision is within the symbol size.



Supplementary Figure 14. Left: marsh marigold in boggy areas around Deming Lake with 3x4" field notebook for scale. Righ: iron mineralization at Elk springs.



Supplementary Figure 15. The range of $\delta^{18}O_{H2O}$ and $\delta^{2}H_{H2O}$ in the four study lakes for the periods specified in Supplementary Figure 13.

Site Name	Latitude	Longitude	δ ¹⁸ O _{H2O} (‰)	$\delta^2 H_{\rm H2O}~(\text{\%})$
Deming Bog	47.169440	-95.167162	-6.83	-10.90
Nicollet Creek	47.193874	-95.230539	-10.90	-81.36
Elk3	47.190478	-95.211350	-10.23	-77.36
Elk4	47.190478	-95.211350	-9.34	-73.92

Supplementary Table 2. Stable isotopes of spring and bog water.

The combined uncertainty (analytical uncertainty and average correction factor) for δ^{18} O is \pm 0.04‰ VSMOW) and δ^{2} H is \pm 0.25‰ (VSMOW), respectively.



Supplementary Figure 16. The range of $\delta^{18}O_{H2O}$ calculated for water vapor based on temperature records from the ITCM5 station calculated according to Engel & Magner, 2019.



Supplementary Figure 17. Cyanobacteria (top row) and eukaryotic algae (bottom row) observed in the study lakes in May 2022.



Supplementary Figure 18. pH profiles of a) Arco Lake, b) Budd Lake, c) Josephine Lake, and d) Deming Lake. For Deming Lake, seasons were classified according to solstice and equinox dates.



Supplementary Figure 19. Photosynthetically active radiation (PAR) profiles of a) Arco Lake, b) Budd Lake, c) Josephine Lake, and d) Deming Lake.



Supplementary Figure 20. Co-variation between the thermocline depth and the depth of the maximum in dissolved oxygen, and the SCML. Panel A includes data from all dates, and panel B lacks data from October 2021. The correlation is significant when October 2021 datapoints are removed.