Leanna Goose Rise and Repair Alliance Organizer (Leanna Goose)

Submitting a letter signed on by Organizations within Minnesota and Individual citizens with 469 signatures. Submitting multiple research articles on wild rice, sulfate, and methylmercury. Submitting the Tribal Task Force on Wild Rice Report. Submitting picture of wild rice on Hay Lake and other healthy rice beds from across the state. Please review each of these closely to understand the importance of upholding the sulfate standard.
Letter is also copied here for visual:
To: Minnesota Pollution Control Agency (MPCA)
Attn: Sign-on Letter from Environmental Organizations, Community Organizations, and Individual Citizens
Re: Enforcement of the 10 mg/L Sulfate Standard in the Keetac Permit and Beyond
The Great Lakes region is home to most of the world's wild rice. In Wisconsin and Michigan, where there is no sulfate standard, wild rice has suffered greatly and is far less abundant than here in Minnesota. That shows us what is at stake. The 10 mg/L sulfate standard is a critical safeguard for wild rice (manoomin), clean water, and public health. We write to thank the MPCA for your commitment to upholding this law and to urge you to remain steadfast in enforcing this standard in the Keetac permit and in all future permitting decisions.
By upholding Minnesota's sulfate standard, we hold companies accountable for their pollution and protect a food that has nourished people here for generations � and, if cared for, will continue to feed generations to come.

Why Enforcement Matters
The 10 mg/L sulfate standard, adopted in 1973, is the only numeric water quality standard in the United States designed specifically to protect wild rice waters (MPCA, Wild Rice Standard, 2011). Courts and the EPA have repeatedly reaffirmed that Minnesota must enforce this standard under the Clean Water Act. The MPCA has no discretion to delay or weaken its application (WaterLegacy, 2024). Scientific evidence shows that sulfate pollution harms wild rice and contributes to methylmercury formation. That, in turn, contaminates fish and endangers human health, especially the health of children (Myrbo, Et Al. 2017; WaterLegacy).

Treaty Rights and Legal Obligations
Minnesota's protection of wild rice is not accidental. In 1837 in exchange for ownership of much of the land that would become Minnesota, the United States "guaranteed" to the Ojibwe their reserved right of "gathering the wild rice, upon the lands, the rivers and the lakes included in the territory ceded," in perpetuity (1837 Treaty with the Chippewa, Art. 5). Wild rice, the only species protected in the treaty by name, is a sacred element of Ojibwe cultural identity. Its protection is central to Indigenous lifeways (Tribal Task Force on Wild Rice). The United States Supreme Court has reaffirmed that this right is federally protected and cannot be infringed by the State of Minnesota because under Article VI of the U.S. Constitution, the treaty is the "supreme law of the land" (U.S. Constitution, Art. VI; Minnesota v. Mille Lacs Band of Chippewa Indians, 526 U.S. 172 (1999)). Minnesota's sulfate standard honors the United States' promise by protecting the rice. Granting sulfate variances known to harm the rice undermines these treaty guarantees (EPA, Stateline, 2024).

Health and Environmental Protection
Elevated sulfate levels do not just harm wild rice. They increase methylmercury in fish, poisoning the fish and the people who depend on those fish as a food source. This risk is especially severe for pregnant women and young children (EPA; Water Legacy) Mining has already caused widespread mercury contamination in Minnesota lakes and rivers. Allowing sulfate pollution from mining to continue to add to this burden would deepen harm to both ecosystems and public health. And respect for the land means respect for our own health. What happens to the land happens to the people who depend on it.

Sustainable Economies
The Minnesota Pollution Control Agency is responsible for controlling pollution in Minnesota, not for protecting the profitability of out-of-state corporations and international conglomerates. U.S. Steel did not provide written evidence that treatment costs to comply with Minnesota law would cause a slowdown or shutdown at Keetac, so the MPCA cannot consider profit impacts as grounds for a variance. Moreover, the idea that mining creates jobs is a myth. Even in the Arrowhead, mining accounts for only 6% of employment� just over 4,000 jobs (Minnesota Employment and Economic Development, Region 3: Northeast Minnesota 2024 Regional Profile). In contrast, leisure and hospitality� industries that depend on clean water for fishing, wild rice stands for fowl habitat, and a steady flow of visitors who want to see the beauty of Minnesota� account for 11.9% of jobs in the region (Minnesota Employment and Economic Development, Current Employment Statistics).
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Minnesota faces multiple new mining proposals that would further threaten critical ecosystems and treaty-reserved resources. Enforcing the sulfate standard now makes clear that the MPCA will enforce the will of Minnesota's elected leaders expressed in state law. And it teaches that corporations must meet state environmental standards, not sidestep them through administrative loopholes. Respecting the standard that the legislature set more than a generation ago respects the democratic will of Minnesotans. It implements the protections that the legislature set to protect the only plant that is named in the treaties that allowed its formation and that is core to so many of our understandings of what it is to be Minnesotan.
Our Call
We call on the MPCA to:
Follow the 10 mg/L wild rice sulfate standard in the Keetac permit and all future permits.

Reject variances that allow out-of-state corporations to pollute for profit at the expense of Minnesotans' treaty rights and public health.

Honor treaty obligations by protecting wild rice, waters, and ecosystems central to Indigenous lifeways.

Demonstrate leadership by holding polluters accountable, ensuring clean water and a healthier future for all Minnesotans.

Sulfate enforcement is not just a matter of environmental protection; it is a matter of law, justice, and respect for both Indigenous Nations and future generations.
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48.) Carol Shimek
49.) Craig Rymer
50.) Denise Marlowe
51.) Duane Franz
52.) Shella Franz
53.) Erica Graen
54.) Fletcher Wolfe
55.) Gail Mulcahey
56.) Great Grandmother Mary Lyons
57.) Harry Greenberg
58.) Janet Tripp
59.) Jona Peters
60.) Julie Boada
61.) Kim Sutton
62.) Mark R Safford
63.) Mark T. Lundholm
64.) Matt Brown
65.) Mr. Thomas Young
66.) Peter Schulze
67.) Richard VandenDolder
68.) Tanya Beyer - wildlife artist
69.) Alexis Racciatti
70.) Thomas Mrotz
71.) Pastor Richard Magnus
72.) Adam Moore
73.) Aleta Borrud, MD MPH
74.) Alex Carroll
75.) Allan Campbell
76.) Allen Gibas
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78.)Andrea Kuenning
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97.) Cameron Roberts
98.) Carol Frechette
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100.) Caroline Miesle
101.) Christina Daniel
102.) Christine A Kwong
103.) Christine Baeumler, Professor, University of Minnesota
104.) Christine Osman
105.) Cindy Angerhofer
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122.) Dennis Olsen
123.) Diana Cumming
124.) Diane Bearman
125.) Diane Haines
126.) Diane Peterson
127.) Don Schuld
128.) Donna E. Clark

129.) Dorothy' Kress
130.) Douglas Heuer
131.) Dr. Cia Sautter
132.) Dr. Emily Hagen
133.) Elizabeth Jarrett Andrew
134.) Ellie Schmidt
135.) Esther Ouray
136.) Gail Morrison
137.) Genevieve Radniecki
138.) Ginger Sisco
139.) Ginny Rudloff
140.) Greg Fynboh
141.) Gretchen Griffin
142.) Gwendolyn Werdin
143.) Henry Walker
144.) Holly J Kellar
145.) Jack Kattner
146.) Jackie Smolen
147.) James Ludowese
148.) Janet Magree
149.) Janice Hallman
150.) Jean A Cleary
151.) Jean Walsh
152.) Jeanne Hoecker
153.) Jeanne Wright
154.) Jeff Stromgren
155.) Jennifer Manner
156.) Jim Niland
157.) Jodi Peterson
158.) Joe Stanley
159.) Juanita Reed-Boniface
160.) Judy Gronquist Grant
161.) Judy Harder
162.) Kaitlyn Walter
163.) Karen Alexander
164.) Kathleen McDonough
165.) Kathryn Gilje
166.) Kathy Brynaert
167.) Katie Gant
168.) Kay M. Randall
170.) Kay Slama
171.) Linda Jokela
172.) Linda Ruetz
173.) Luisa Cichowski
174.) Maggie O'Connor
175.) Malini Basu
176.) Marc Markell
177.) Marcy Leussler

178.) Margaret Michaelson
179.) Marian McKone
180.) Marian Nichols
181.) Marijo Hickok
182.) Mark Holm
183.) Marvin Napgezek, md
184.) Mary Adair
185.) Mary Beckman
186.) Mary Harrison
187.) Mary Jean Mulherin
188.) Mary Krick
189.) Mary Lillo
190.) Matt Rohn
191.) Megan Reich
192.) Melissa Bergstrom
193.) Michelle Hensley
194.) Micki Rasmussen
195.) Misty Butler
196.) ML Wilm
197.) Moe Sadique
198.) Mr David Wee
199.) Mr. James Herther
200.) Ms JL Angell
201.) Nancy Hauer
202.) Nancy K Metz
203.) Nancy Stutzman
204.) Natali
205.) Pam Hauer
206.) Pam Roiger
207.) Pastor Kate Reuer Welton
208.) Patricia Olson
209.) Patty McGrath
210.) Paul Englund
211.) Paul Tuveson
212.) Peder Otterson
213.) Rev Dwight Wagieniw
214.) Rev. Clara Sanders
215.) Rev. Deborah Braun
216.) Rev. Denise Stahura
217.) Rev. Diana McLean
218.) Rev. Dr. Anita L. Bradshaw
219.) Rev. Dr. Karen Smith Selers
220.) Rev. Jane McBride
221.) Rev. Karen R. Larson
222.) Rev. Mark Schlasner
223.) Rev. Paula Bidle
224.) Rev. Richard Sellers
225.) Reverend Roberta M Haskin

226.) Robert Brown
227.) Roger Miller
228.) Sam Blackwell
229.) Sam Tindall
230.) Sarah Gettel
231.) Sarah McCarthy
232.) Scott Tower
233.) Sean MacManus
234.) Sidney Brown
235.) Sister Brenda Lisenby
236.) Stephanie Johnson
237.) Stephanie Putzier, MD
238.) Steven Besser
239.) Steven Reiser, Pastor
240.) Susan Cortilet Jones
241.) Susan S. Austin
242.) Susie Osaki Holm
243.) Susie Petra
244.) Talitha Anderson
245.) Tamar Rubin-Calvert
246.) The Rev. Pamela Fickenscher
247.) Thom Haines
248.) Timothy Leary
249.) Tom Houle
250.) Tom neilson, Ed.D.
251.) Trish Grose
252.) Vernita Kennen
253.) Wallace Wadd, PharmD
254.) Wesley Sisson
255.) Zoe Bird
256.) Rabbi Arielle Lekach-Rosenberg
257.) Jean M. Gilruth
258.) John Bankson
259.) Kathryn Guimaraes
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262.) Kristi Lentz
263.) Don Rideaux-Crenshaw
264.) Jonathan Swoyer
265.) Alexzandra Lucia
266.) Gina Peltier Eele
267.) Peggy Thompson
268.) Jane Wagner Murphy
269.) Alice West
270.) Cathlin Mahal
271.) Alexis Lathrop
272.) Tom Griffin
273.) Anya Stupar

274.) Helen Nikiel
275.) Teena Bolin
276.) Susie George
277.) Heidi Voss
278.) Judy Jungwirth
279.) Torey
280.) Richard McCormick
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282.) Dorothy Williams
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294.) Jeff Richards
295.) Dan Krivit
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297.) Rachel Geissinger
298.) Hannah Logan
299.) Lois Norrgard
300.) Tara Fahey
301.) Steve Clemens
302.) Allan Malkis
303.) Ellen Mueller
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306.) Finley Anderson Newton
307.) Tim Wulling
308.) Margie Hake
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310.) Deacon Alex Theship-Rosales
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349.) Kateri Krantz-Odendahl
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392.) Susan Keller, Environmental Activist
393.) Kurt Schultz
394.) Lauren Kofsky
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397.) Pr Natalia Terfa, Edina Community Lutheran Church
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426.) Susan Roedl
427.) Arlene Mathison | Legislative Team Member
428.) Lynn Shuck
429.) Trevor Roark
430.) Leroy Staples | Leech Lake Band of Ojibwe District 3 Representative
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118.) David Leppik

119.) Dawn Blomgren

120.) Deb Heuwinkel

121.) Debby Evans

122.) Dennis Olsen

123.) Diana Cumming

124.) Diane Bearman

125.) Diane Haines

126.) Diane Peterson

127.) Don Schuld

128.) Donna E. Clark

129.) Dorothy’ Kress

130.) Douglas Heuer

131.) Dr. Cia Sautter

132.) Dr. Emily Hagen

133.) Elizabeth Jarrett Andrew

134.) Ellie Schmidt

135.) Esther Ouray

136.) Gail Morrison

137.) Genevieve Radniecki

138.) Ginger Sisco

139.) Ginny Rudloff

140.) Greg Fynboh

141.) Gretchen Griffin

142.) Gwendolyn Werdin

143.) Henry Walker

144.) Holly J Kellar

145.) Jack Kattner

146.) Jackie Smolen

147.) James Ludowese

148.) Janet Magree

149.) Janice Hallman

150.) Jean A Cleary

151.) Jean Walsh

152.) Jeanne Hoecker

153.) Jeanne Wright

154.) Jeff Stromgren

155.) Jennifer Manner

156.) Jim Niland

157.) Jodi Peterson

158.) Joe Stanley

159.) Juanita Reed-Boniface

160.) Judy Gronquist Grant

161.) Judy Harder

162.) Kaitlyn Walter

163.) Karen Alexander

164.) Kathleen McDonough

165.) Kathryn Gilje

166.) Kathy Brynaert

167.) Katie Gant

168.) Kay M. Randall

170.) Kay Slama

171.) Linda Jokela

172.) Linda Ruetz

173.) Luisa Cichowski

174.) Maggie O'Connor

175.) Malini Basu

176.) Marc Markell

177.) Marcy Leussler

178.) Margaret Michaelson

179.) Marian McKone

180.) Marian Nichols

181.) Marijo Hickok

182.) Mark Holm

183.) Marvin Napgezek, md

184.) Mary Adair

185.) Mary Beckman

186.) Mary Harrison

187.) Mary Jean Mulherin

188.) Mary Krick

189.) Mary Lillo

190.) Matt Rohn

191.) Megan Reich

192.) Melissa Bergstrom

193.) Michelle Hensley

194.) Micki Rasmussen

195.) Misty Butler

196.) ML Wilm

197.) Moe Sadique

198.) Mr David Wee

199.) Mr. James Herther

200.) Ms JL Angell

201.) Nancy Hauer

202.) Nancy K Metz

203.) Nancy Stutzman

204.) Natali

205.) Pam Hauer

206.) Pam Roiger

207.) Pastor Kate Reuer Welton

208.) Patricia Olson

209.) Pattymipl McGrath

210.) Paul Englund

211.) Paul Tuveson

212.) Peder Otterson

213.) Rev Dwight Wagieniw

214.) Rev. Clara Sanders

215.) Rev. Deborah Braun

216.) Rev. Denise Stahura

217.) Rev. Diana McLean

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219.) Rev. Dr. Karen Smith Selers

220.) Rev. Jane McBride

221.) Rev. Karen R. Larson

222.) Rev. Mark Schlasner

223.) Rev. Paula Bidle

224.) Rev. Richard Sellers

225.) Reverend Roberta M Haskin

226.) Robert Brown

227.) Roger Miller

228.) Sam Blackwell

229.) Sam Tindall

230.) Sarah Gettel

231.) Sarah McCarthy

232.) Scott Tower

233.) Sean MacManus

234.) Sidney Brown

235.) Sister Brenda Lisenby

236.) stephanie johnson

237.) Stephanie Putzier, MD

238.) Steven Besser

239.) Steven Reiser, Pastor

240.) Susan Cortilet Jones

241.) Susan S. Austin

242.) Susie Osaki Holm

243.) Susie Petra

244.) Talitha Anderson

245.) Tamar Rubin-Calvert

246.) The Rev. Pamela Fickenscher

247.) Thom Haines

248.) Timothy Leary

249.) Tom Houle

250.) Tom neilson, Ed.D.

251.) Trish Grose

252.) Vernita Kennen

253.) Wallace Wadd, PharmD

254.) Wesley Sisson

255.) Zoe Bird

256.) Rabbi Arielle Lekach-Rosenberg

257.) Jean M. Gilruth

258.) John Bankson

259.) Kathryn Guimaraes

260.) DeeDee Edlund

261.) Gloria Englund

262.) Kristi Lentz

263.) Don Rideaux-Crenshaw

264.) Jonathan Swoyer

265.) alexzandra lucia

266.) Gina Peltier Eele

267.) Peggy Thompson

268.) Jane Wagner Murphy

269.) Alice West

270.) Cathlin Mahal

271.) Alexis Lathrop

272.) Tom Griffin

273.) Anya Stupar

274.) Helen Nikiel

275.) Teena Bolin

276.) Susie George

277.) Heidi Voss

278.) Judy Jungwirth

279.) Torey

280.) Richard McCormick

281.) Kelsie Marie

282.) Dorothy Williams

283.) Hannah Jensen

284.) Hannah Campbell Gustafson

285.) Grant Tillery

286.) Sydney Petersen

287.) Julie A. Sanders

288.) Sandra Hoffman

289.) Shelly Zuzek

290.) Kaydelle Kvarnlov-Leverty

291.) Brian Buxton

292.) Denise Marlowe

293.) Susan Macpherson

294.) Jeff Richards

295.) Dan Krivit

296.) Lynn Indihar

297.) Rachel Geissinger

298.) Hannah Logan

299.) Lois Norrgard

300.) Tara Fahey

301.) Steve Clemens

302.) Allan Malkis

303.) Ellen Mueller

304.) Mary Schiltgen

305.) Orren Fen

306.) Finley Anderson Newton

307.) Tim Wulling

308.) Margie Hake

309.) Minnow Whaley

310.) Deacon Alex Theship-Rosales

311.) Teresa Wasick

312.) Rev. Catherine Malotky

313.) O Stremple

314.) Janet Groenert

315.) Christine nelson

316.) Patricia Eliason

317.) Christine Popowski

318.) Sandra Spieler

319.) Kathy Haskins

320.) Rachael Hanson

321.) Dr Lyn Pegg

322.) Kathryn Palmer

323.) Jerry Blume

324.) Barbara Peterson

325.) Gretchen Goodman

326.) Marcia Lewis

327.) Terry Hokenson

328.) Emily Krumberger

329.) LeEtta Flicker

330.) Margaret Rozycki

331.) Carolyn Hendrixson

332.) Miranda Bryan

333.) Gary Adams

334.) Terry McAnnany

335.) Linda Nelson

336.) Tracy van der Leeuw

337.) Rebecca Cramer

338.) Sonja Peterson

339.) Leah

340.) Dawn M Nothwehr, OSF, Ph.D.

341.) Elizabeth Raasch-Gilman

342.) Alexandra Boese

343.) Dr.Tom Small

344.) Kathleen Harder

345.) Rev. Onryu Kennedy

346.) Beth Aune

347.) Hannah H Mississippi

348.) Sue Lyback LSW Emeritus

349.) Kateri Krantz-Odendahl

350.) Dr. Paula Thomsen

351.) Stephani Schmidt

352.) Janice Wiechman

353.) Janet Tripp

354.) Maren Solheim

355.) Mary Ellen Kavanaugh

356.) Amy Orecchia

357.) Evelyn Kent

358.) Nicole Hill

359.) Emily Thompson

360.) Rachel Crippe

361.) Candace Linares

362.) Bruce Eldevik

363.) Diane Hirigoyen

364.) Elizabeth Andrews

365.) John Kearney

366.) Meg Arnosti

367.) Jennifer Rome

368.) Anna Ebbers

369.) Deb Frank

370.) Karen Wiebe

371.) Bronwen Lu

372.) Isaiah Wanbli Friesen

373.) Jane Carlson

374.) Leah Grunzke

375.) Sarah Weaver

376.) Sally Nankivell

377.) sally downing

378.) Leah Fifield

379.) Ava Corey-Gruenes

380.) Gail Irish

381.) Rev. Chris Bohnhoff

382.) Elizabeth Andress

383.) Justine Wuebold, individual

384.) Peggy Roeske

385.) mary pettigrew

386.) Gloria Karbo

387.) Alice Meadow

388.) Sara Barsel, Ph.D.

389.) Mary Burger

390.) Brian Moe

391.) Nash Edgerton Hall

392.) Susan Keller, Environmental Activist

393.) Kurt Schultz

394.) Lauren Kofsky

395.) Debra Avenido

396.) Pastor Richard Magnus

397.) Pr Natalia Terfa, Edina Community Lutheran Church

398.) Deacon Lauren Morse-Wendt

399.) Mary Vlazny

400.) Keith H. Olstad

401.) Lawrence Edlund

402.) Karen Boyum

403.) Tess Dornfeld

404.) Ariella Tilsen

405. RJ Lawrence

406.) Gina Eele

407.) Donna Perleberg

408.) Nancy Ott-Pinckaers

409.) Max Gries

410.) Dina Johnson

411.) Rabbi Jessica Rosenberg

412.) Sherri Knuth

413.) Mary Ann Van Curs

414.) Cris Nelson

415.) Alice Madden

416.) AP Looze

417.) Nathan Hofstad

418.) Carrie Garcis

419.) Jeanne Landkamer

420.) Elyria Grote

421.) Dr. Kris Gebhard

422.) Rachel Bly

423.) Maggie Pastarr

424.) Sarah Stevenson

425.) Ronna Vinge

426.) Susan Roedl

427.) Arlene Mathison | Legislative Team Member

428.) Lynn Shuck

429.) Trevor Roark

430.) Leroy Staples | Leech Lake Band of Ojibwe District 3 Representative

431.) Amy Elliot

432.) Heather Olson

433.) Christi Kaehn

434.) Claudia Poser

435.) Mr. Thomas Young

436.) Deb Klein

437.) Ronald Lanham

438.) Larry Goose

439.) Lenae Butcher

440.) Amber Whitebird

441.) Pat Goodwin

442.) Savanna Antell

443.) Stephanie Kelley

444.) Nakita Nicazio

445.) Faron Jackson

446.) Paris Nicazio

447.) Ryan White

448.) Kelly Goose

449.) Rebecca Keillor

450.) Nell Schaefer

451.) Shannon Kubenez

452.) Shannon Young

453.) Leanna Goose

454.) Besty LePlatt

455.) Dorothy Hammer

456.) Leslie Forman

457.) Kris Jacobson

458.) Marjorie Huebner

459.) Joshua Spitzner

460.) Rebecca Tkachuk

461.) Margaret Jackson

462.) Roya Damsaz

463.) Adele Lennig

464.) John Byom

465.) Ruth Hiland

466.) Harvey Benson

467.) Claren Sellner

468.) Paula Mohan

469.) Valarian Kingbird

Photos from Hay Lake tell the real story: wild rice here is not abundant; it is dying due to pollution that exceeds the legal limit.

MPCA: The sulfate standard should be enforced and variances rejected; it must be upheld to protect our waters and wild rice from polluting industries.

Photos of Hay Lake vs Healthy Rice beds

Hay Lake September 10, 2025-Photo-Credit Wldflwrgrp

Wild Rice Hay Lake September 10, 2025 Photo Credit: wldflwrgrp

Wild Rice Hay Lake September 10, 2025 Photo Credit: wldflwrgrp
Elevated sulfates lead to fewer seeds, weaker seedlings, and long term wild rice decline (Pastor et al.)

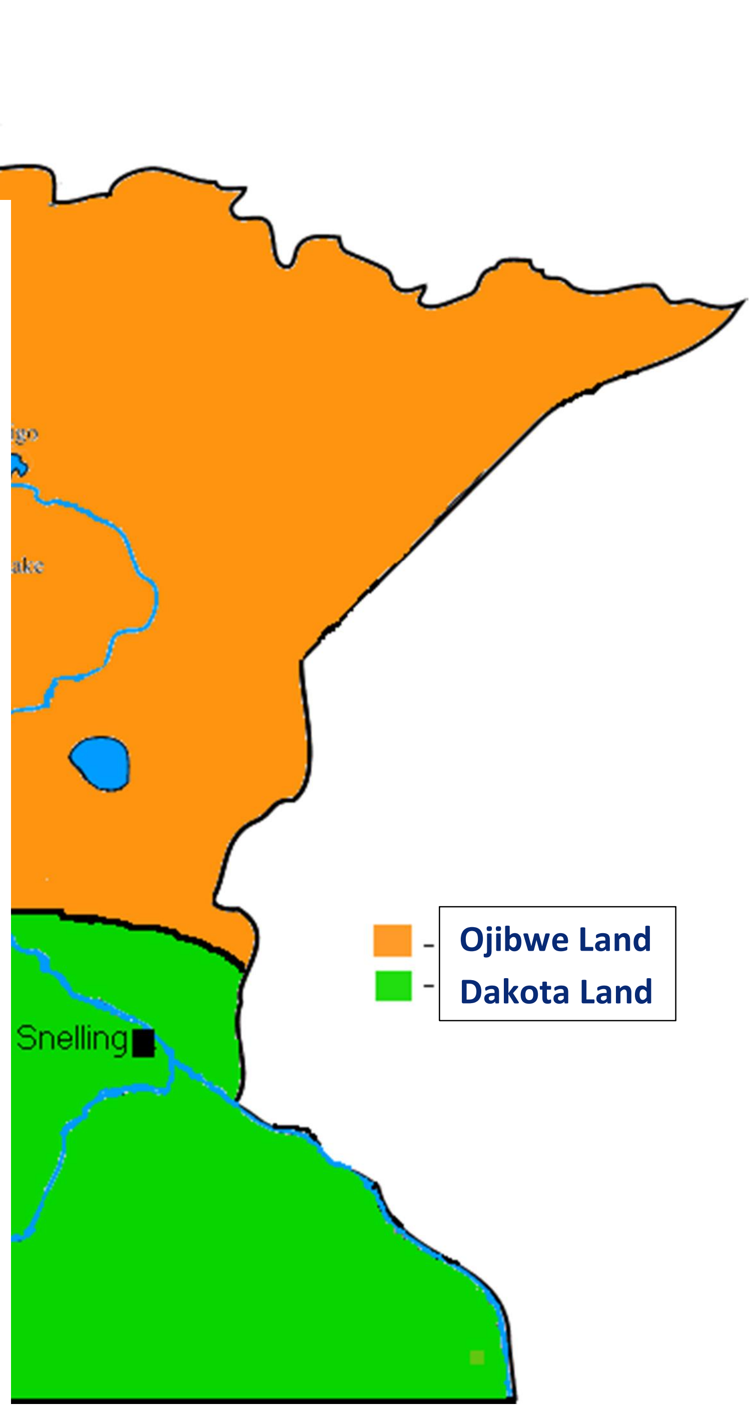
Wild Rice Hay Lake September 10, 2025 Photo Credit: Wldflwrgrp

Hay Lake– aerial footage taken same day as image below September 10, 2025 Photo Credit: Wldflwrgrp

Flowage Lake–Abundant healthy wild rice aerial footage; waters are a mere 9 miles from the proposed Talon Tamarack Mine. September 10, 2025 Photo Taken Same Day As Hay Lake photo above Photo Credit: wldflwrgrp

Leech Lake Wild Rice Harvest–September 7, 2025

2018 Tribal Wild Rice Task Force Report



DECEMBER 15TH, 2018

Prepared by the:

MINNESOTA TRIBAL WILD RICE TASK FORCE

"You will know the chosen ground has been reached when you come to a land where food grows out of the water."

SEVEN FIRES PROPHECY

CONTENTS

EXECUTIVE SUMMARY

This report, and the creation of a Minnesota Tribal Wild Rice Task Force, serves as a response to the 40th Governor of the State of Minnesota creating a "Wild Rice Task Force" that is *disrespectful and contrary to Executive Order 13-10 …* and *directly relegates the Tribes to the status of special interest groups and industry rather than honoring Tribal sovereignty* (*Minnesota Chippewa Tribe Resolution 107-18*).

On May 30th, 2018, Governor Mark Dayton filed Executive Order 18-08 which provided for the establishment of the Governor's Task Force on Wild Rice. The Governor's Task Force on Wild Rice was charged with reviewing scientific literature to identify information related to the impacts of sulfate or other sulfur compounds or habitat conditions on wild rice, and preparing comments that addressed environmental conditions that contribute to wild rice population declines. The proposed composition of the Governor's Task Force on Wild Rice does not respect the sovereignty of the eleven federally-recognized Native American Tribes, Bands, and Communities in the State of Minnesota or the unique status of federally-recognized Tribes that have guaranteed usufructuary rights by Treaties. The proposed Governor's Task Force on Wild Rice composition does not acknowledge that Minnesota's Native American Tribes will be disproportionately affected by the loss of a usufructuary property right directly related to legislation prohibiting enforcement of existing water quality standards and the composition of the task force minimizes the technical expertise, knowledge, and interests of the Tribes.

On May 31st, 2018, the Minnesota Chippewa Tribe (MCT) responded to Executive Order 18-08 by passing resolution 82-18 and sending a correspondence to Governor Dayton informing him that the MCT would support the creation of a wild rice task force provided that each of the member reservations of the MCT be provided a separate seat on the Governor's Task Force on Wild Rice.

On June 28th, 2018, Governor Mark Dayton filed Executive Order 18-09 which amended Executive Order 18-08 and changed the composition of the task force from a representative appointed by the Minnesota Indian Affairs Council to adding a representative nominated by the four Minnesota Dakota Tribes and a representative nominated by the Red Lake Nation, but maintained only one seat available for a nomination by the six Bands of the Minnesota Chippewa Tribe. Furthermore, the proposed composition of the Governor's Task Force on Wild Rice was similar to the Minnesota Pollution Control Agency (MPCA) Wild Rice Advisory Board where during the process and through consultation, the comprehensive comments provided on behalf of the Tribes to the MPCA were generally disregarded and not incorporated into the then proposed wild rice rule.

This resulted in the Tribal Executive Committee of the MCT, comprised of the top two elected officials from each of the MCT Bands, to find that it was in the Tribes' best interest to decline/reject the Governor's offer to participate in the Governor's Task Force on Wild Rice and instead form the Minnesota Tribal Wild Rice Task Force (TWRTF). MCT Resolution 107-18 established the TWRTF which was to be comprised of, provided that such other federally-recognized tribes in Minnesota chose to participate, two representatives from each of the eleven federally-recognized tribes of Minnesota. It also served as an invitation for the other federally-recognized Native American Tribes in Minnesota to participate in gathering and reviewing information, preparing documents, and making recommendations utilizing their own expertise.

The purpose of the TWRTF is to review existing literature, including literature and information based on tradition, culture, and science, that is available to inform the understanding of the impacts of sulfate or other sulfur compounds on habitat conditions on wild rice, identify information gaps, make recommendations on priorities for wild rice research, and prepare a report with recommendations in a similar fashion to that included in Executive Orders 18-08 and 18-09, providing a report to the Governor by December 15th, 2018.

INTRODUCTION

An existing water quality standard for wild rice (10 mg/L sulfate) has been a USEPA federally recognized standard in: Minnesota since 1973, Fond du Lac Reservation since 2001, and Grand Portage Reservation since 2005. The original 1973 rule was promulgated following Minnesota's assumption of Clean Water Act authority and was based upon extensive biological surveys done by state biologist John Moyle in the 1940s. However, while this standard has largely been unenforced by state or federal agencies, the Tribes have fully implemented it. Fond du Lac and Grand Portage have both sponsored basic ecological research and research into the effects of sulfate on wild rice, beginning in 2003 and continuing today. With the concern over the impact discharges with elevated sulfate may have on impact wild rice, Tribes and environmental groups began pushing the Minnesota Pollution Control Agency (MPCA) about 15 years ago to enforce the standard. Concern was also raised from the dischargers (i.e., it would be too expensive to meet standard; is the standard the appropriate number?) who would potentially be regulated.

In 2010 the MPCA was directed by the state legislature to further evaluate the impacts of sulfate and sulfide, and determine if changes to the current standard are needed. MPCA had three goals: to revise the numeric standard to incorporate the latest scientific understanding of the impacts of sulfate; to clarify the beneficial use and which waters support the beneficial use; and to clarify what it means to meet or exceed the standard. The timeline of the process is as follows:

- **Wild Rice Advisory Committee (2011-2017)** – A group of a variety of interests (agencies, tribes, researchers, harvesters, environmental groups, industry, etc.) provided input to MPCA on the standard and scientific studies.
- **Studies (2011-2013)** – State sponsored research programs were completed including field surveys, controlled laboratory experiments, and outdoor container

experiments. Results indicated that sulfate (when converted to sulfide) impacts wild rice.

- **Peer Review Committee (2014)** – Group of independent scientists provided feedback to the MPCA on research projects and results.

- **Minnesota Chippewa Tribe letter to Governor Dayton (2014)** – The letter addressed concerns regarding the definition of "waters used for the production of wild rice" and water quality standards pertinent to wild rice.

- **Legislative Rules (2015, 2016, 2017)** – Rules were passed prohibiting MPCA from identifying impaired wild rice waters and enforcing the existing 10 mg/L wild rice sulfate standard, until a revised rule would take effect. These actions unduly restricted MPCA's regulatory authority, leaving them vulnerable to losing their delegated National Pollutant Discharge Elimination System (NPDES) authority according to the USEPA.

- **MPCA issues proposed rule (2017)** – Instead of the current standard of 10 mg/L sulfate, the proposal was for an equation-based standard (depending on the amount of sulfate, iron, and organic carbon in a system). A unique sulfate standard would be calculated and developed for each system where it applies. A partial list of known wild rice waters, to which the standard would apply, was also published in the revised rule.

- **Minnesota Indian Affairs Council letter to MPCA Commissioner Stine (2017)** – The letter highlighted the deficiencies of MPCA's proposed rule revisions for Minnesota's sulfate standard to protect wild rice.

- **Administrative Law Rudge rulings (2018)** – In January 2018, a report from the Administrative Law Judge was issued disapproving MPCA's repeal of the existing standard and replacing it with the agency's proposed rule revisions. The MPCA asked the judge to reconsider, but the Chief Administrative Law Judge's Order on Review issued in April 2018 confirmed the earlier decision to disapprove MPCA's approach to changing the standard. Some key points of the decision were:

  1) MPCA failed to establish the reasonableness of the repeal of the existing 10 mg/L sulfate standard, and the repeal conflicted with state and federal

statute; 2) the proposed equation-based standard failed to meet the definition of a rule under Minnesota statute, was not rationally related to the MPCA's objective, and was unconstitutionally void for vagueness; 3) the proposed list of wild rice waters was deficient, as it violated federal statutes; 4) the Agency failed to establish need or reasonableness, specifically related to the limited list of wild rice waters that are provided additional protection under narrative standard, in violation of state statute.

- **MPCA withdraws rule (2018)** – Proposed changes to the wild rice sulfate standard were withdrawn by MPCA in May 2018. The existing standard of 10 mg/L sulfate remains in place with legislative restrictions of 2015, 2016 and 2017.

- **Proposed legislation, vetoes, and executive order (2018)** – Attempts were made in the legislature to pass bills removing the existing standard, but the governor vetoed the proposed legislation twice (May 9th letter to Speaker of the House and May 30th letter to Speaker of the House). In May 2018, the governor issued Executive Order 18-08 which established a task force to further evaluate the standard and issue a report by December 15th, 2018. The order also states that no existing permitted facility will be required to install unaffordable equipment to meet existing sulfate standard.

- **Minnesota Chippewa Tribe Resolution 82-18 and letter to Governor Dayton (2018)** – The letter supported the creation of the wild rice task force provided that each member reservation of the Minnesota Chippewa Tribe be provided a seat on the Governor's task force.

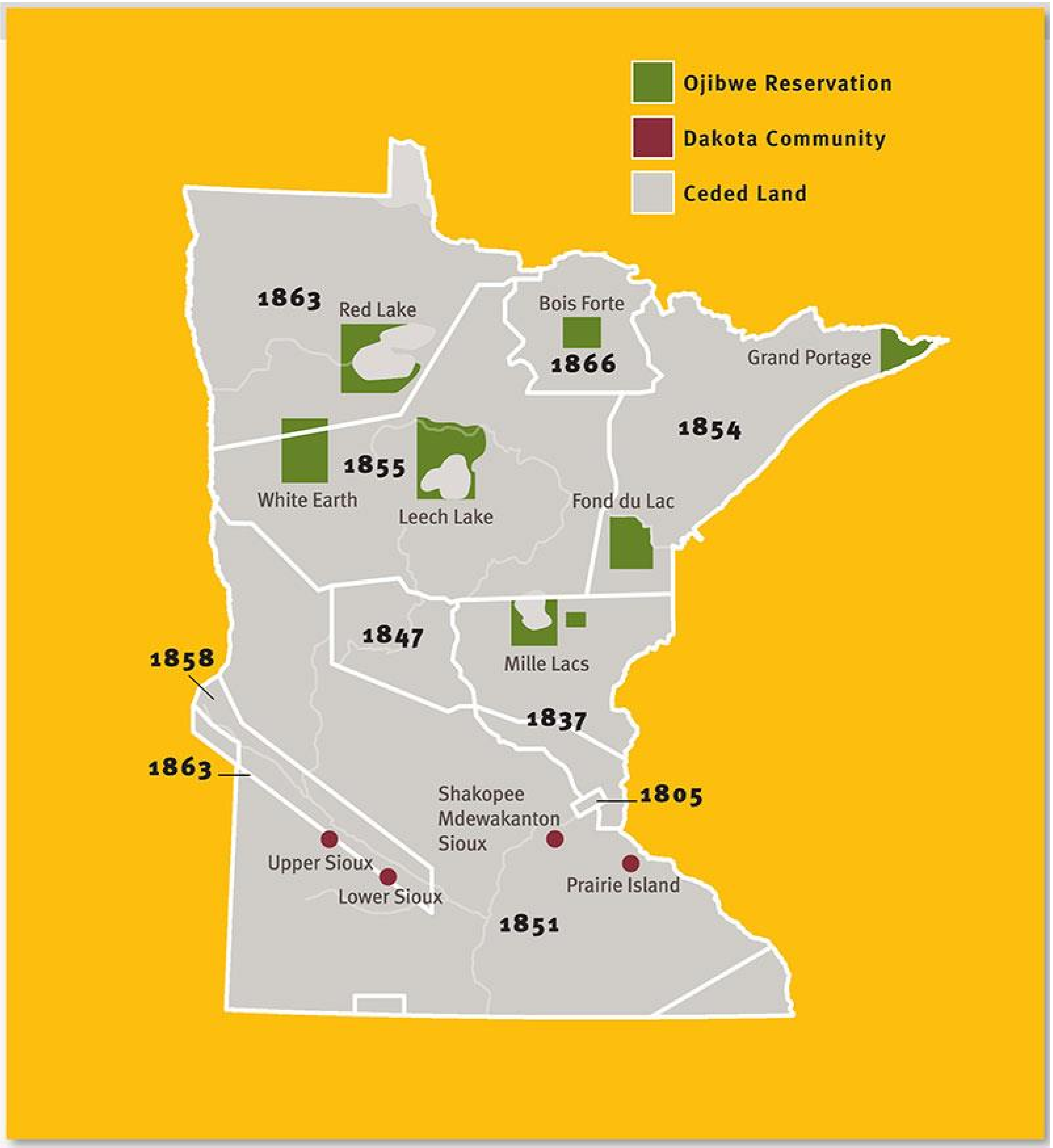
- **Letter from Governor Dayton to Minnesota Tribal Leaders (2018)** – Amendments to be made to Executive Order 18-08 were outlined, which included adding one seat for the four Minnesota Dakota Tribes and one seat for the Red Lake Nation, but maintained the one seat for the six Bands of the Minnesota Chippewa Tribe.

- **Governor Dayton issues Executive Order 18-09 (2018)** – This amended Executive Order 18-08 as described in the governor's letter to the Minnesota Tribal Leaders.

- **Minnesota Chippewa Tribe letter to Governor Dayton (2018)** – This letter respectfully explained the reason for declining the offer to serve on the Governor's Task Force on Wild Rice and subsequently the creation of the Tribal Wild Rice Task Force by the federally-recognized sovereign nations of Minnesota. Furthermore, it stated that the Minnesota Chippewa Tribe will only participate in government-to-government consultation in order to strengthen the relationship between the State and the Tribe, and to ensure that Executive Order 13-10 is implemented properly.
- **Tribal Wild Rice Task Force (2018)** – The formation of the Governor's Task Force on Wild Rice did not allow representation by all tribes in Minnesota. Tribes found this unacceptable as each is a sovereign government and must necessarily represent themselves. In August 2018, the Minnesota Chippewa Tribe passed resolution 107-18 creating a Tribal Wild Rice Task Force charged with developing its own report and recommendations, and communicated its intent by letter to the Governor of Minnesota.
- **Fond du Lac Revised Water Quality Standards (2018)** – In September 2018, Fond du Lac published notice of their revised water quality standards for public comment under their federal Clean Water Act authority. The Band is proposing to maintain their 10 mg/L sulfate standard, as recent research has confirmed it is scientifically defensible, and adding protective narrative standards for wild rice waters.

Tribes did remain engaged with the MPCA throughout the process outlined above. Staff representing some, but not all, Minnesota tribes participated as members of the Wild Rice Advisory Committee. In addition, the MPCA did make efforts to hold additional consultation with all tribes indicating interest, including several Ojibwe Bands from Wisconsin. This consultation did include formal government-to-government meetings and more informal staff-to-staff communications. But despite this involvement and consultation, tribal expertise has not been reflected in the state's policies or rulemaking for wild rice. Tribes have put forth considerable effort in information sharing and commenting, yet most key thoughts and concerns have not been addressed to date. This report reiterates many of the previous concerns. We ask that state and federal regulating agencies meet their responsibilities and work with tribes to protect and maintain natural stands of wild rice for future generations.

Ojibwe Reservations/Dakota Communities and Treaty-Ceded Territories



Cultural Context

The third of seven prophets came to the Anishinaabe people more than one thousand years ago and told them to head west to their chosen land. When they found “the food that grows out of the water,” they would know they were home, and this sacred food would feed their families’ bodies and souls for generations to come. This journey is at the core of the Ojibwe migration story, and the sacred food at the center of their cultural identity, spiritual traditions, and physical well-being is manoomin (Ojibwe word for wild rice). To the many bands of Ojibwe people who have made their homes for centuries around the lakes of Minnesota, manoomin is far more than a crop or a staple food. It is a sacred symbol that represents their journey, their relationship to the land that sustains them, and their very identity as Ojibwe people. Anishinaabe people live by the philosophy “that if we care for the nibi (Ojibwe word for water) and manoomin, the manoomin will care for us”.

While Ojibwe or Anishinaabeg historic and cultural connections to wild rice have been communicated to the public through various media, many people are surprised to learn that ricing also has deep roots in Dakota history. Dakota people used to travel without boundaries around the land which is now the state of Minnesota. Psiŋ (Dakota word for wild rice) was abundant across the state, including in southern Minnesota. Lakes and rivers were clean enough for psiŋ growth then, with unaltered hydrology.

Dakota people were ricing long before the Ojibwe’s prophecy relocated them to the Dakota homelands. Dakota people shared their ricing traditions with the Ojibwe, and these traditional harvest and parching methods are those still used by the native communities today. The settlement era influenced the placement of Dakota people in the southern reaches of Minnesota along the Minnesota and Mississippi Rivers. Dakota people have harvested psiŋ both when it was in the territory they occupied, and when it was in “contested territory” or the middle section of Minnesota that was then a war zone where people weren’t allowed to camp. That territory was often hunted and harvested by both peoples’ groups.

Four Dakota communities now reside in the southern half of Minnesota, with Prairie Island Indian Community lands located along the Mississippi River near Red Wing, Shakopee Mdewakanton Sioux Community located just off the Mississippi River near Prior Lake in Shakopee, and Lower Sioux Indian Community & Upper Sioux Indian Community residing in the Minnesota River valley.

According to Jenks (1901) and many oral history accounts, manoomin/psiŋ used to grow along the reaches of the Mississippi and Minnesota Rivers, as well as the St. Louis river basin. Deloria (1967) gives an account of people in the Red Wing area gathering psiŋ, along with places specifically near Sakpe (now Shakopee) and St. Paul. Oral history tells us Dakota people gathered psiŋ for sustenance along the Mississippi River and backwater lakes on down to Lake Pepin. Psiŋ sustains the Dakota culture to this day, but there is hardship being that psiŋ no longer grows with the same abundance it once did along these rivers.

The Dakota custom of harvesting psiŋ has never stopped since a time immemorial. However, Dakota people now have to travel much farther to reach areas where psiŋ is appropriately abundant for harvest. For many, this means traveling to another Tribe up north because psiŋ has been removed for so long from Dakota people’s current place of residence that the tradition surrounding an annual harvest has been lost. Psiŋ is still deeply embedded in Dakota culture as is evident in ceremonies, gifts, diet, and traditions carried down for generations. The Dakota communities today are working to restore the rice that was once there, and bring back this nutritious resource to their own lands.

This very brief history of the Dakota people tells of a broken connection with something that was abundant in their homelands and is no longer. The Dakota nations must rely on their relatives in the northern half of the state to supply manoomin/psiŋ for restoration seeding, for consumption, and for ceremonies. May this history show us clearly that Minnesotans need to prevent the loss of any more rice in northern regions of Minnesota where manoomin/psiŋ still grows in its native range. Manoomin/Psiŋ is health and life to tribal culture both for the Ojibwe and Dakota people.

Minnesota tribes entered into treaties with the United States in the 1800's to reserve hunting, fishing, and gathering rights in the lands and waters ceded to the United States. The exercise of these rights is fundamental to tribes' cultures and ways of life and maintains religious, ceremonial, medicinal, subsistence, and economic needs.

Every federal agency has a responsibility to these tribes and their treaty rights, and this extends to the protection of the habitats and environmental quality that sustain manoomin/psiŋ. The recognition of sovereign rights is part of any given tribes' ongoing struggle to preserve a culture that is best understood in terms of their relationship with the natural environment. Tribal members continue to harvest and rely upon manoomin/psiŋ for religious purposes including naming ceremonies, funerals, Midewiwin ceremonies, and various seasonal feasts.

These activities are critical components in perpetuating Anishinaabeg/Dakota lifeways and cultural practices. Anishinaabeg/Dakota spiritual beliefs mandate the use of certain plants, animals, and fish in ceremonies attendant to hunting, fishing, and gathering activities. These ceremonies ensure the perpetuation of the resources and the physical, mental, and spiritual well-being of the person. Tribal leaders have noted that elders in their communities reaffirmed the position that traditional foods, including manoomin/psiŋ, are medicine for Anishinaabe and Dakota people. Today, tribes experience higher than average rates of diseases such as diabetes and heart disease. Much of the current state of Native American health can be traced back to historical practices that have displaced tribes and limited access to healthy and traditional foods, such as manoomin/psiŋ. Many tribes are dependent upon manoomin/psiŋ for subsistence needs.

Many Native Americans eat manoomin/psiŋ at least once a month, though historically this rate was much higher. Survey results show that manoomin/psiŋ is the most commonly consumed traditional food, and Native Americans wish to eat it more often. The annual hand-harvest on Minnesota lakes and rivers is a cherished ritual that preserves time-honored traditions and builds tribal community.

Harvesting rice by hand is part of a deeply held belief that this wild gift from the Creator, and the land that sustains it, should be treated with respect and gratitude rather than cultivated and exploited. Hand-harvested rice is frequently offered as gifts and is used as an offering in spiritual ceremonies and funerals.

Health and Subsistence

Despite its cultural significance, Minnesota tribes have experienced challenges in documenting and publicizing the impacts to community health, social cohesion, and access to healthy food that they bear as wild rice resources are being degraded and diminished. The Fond du Lac Band attempted to bring these health and cultural inequities to light in a Health Impact Assessment or HIA, and to clearly and simply articulate the importance of manoomin to the health of the Ojibwe people. This HIA explored historical trauma, grave disparities in health outcomes and access to health care, and socioeconomic inequities (social determinants of health) that shape the lives of traditional people in a modern world. It highlighted the need to protect and support resilient cultural and spiritual practices that connect people to their ancestors, their identity, and future generations. The practices of harvesting, processing, eating, sharing and gifting manoomin; the language associated with these practices and ceremonies that celebrate manoomin are central to the health of tribal communities.

From *Expanding the Narrative of Tribal Health: The Effects of Wild Rice Water Quality Rule Changes on Tribal Health* (Fond du Lac Health Impact Assessment 2018):

> "Food sovereignty is the right of peoples to healthy and culturally appropriate food produced through ecologically sound and sustainable methods, and the right to define their own food and agricultural systems… Harvesting what is naturally occurring and compatible with one's own environment is a key component. When people harvest, process, prepare and serve native foods, they build strong relationships with the land and with each other… The establishment of reservations limited access to traditional foods and replaced them with less nutritious, more expensive store-bought foods, leading to nutritional deficiencies and food insecurity that Native Americans experience today…

A history of displacing tribes and limiting access to traditional foods like manoomin has had profoundly negative and persistent impacts to Native American health and well-being."

To address these health disparities, Prairie Island Indian Community (PIIC) has initiated a movement of food sovereignty in the community. In 2017, PIIC conducted a Food Sovereignty Assessment which strongly revealed a desire by the community to increase the availability, harvesting opportunities and consumption of local psiŋ. PIIC community members classified psiŋ as one of the top five "food(s) that you need or would like to eat that are difficult to get, or are not available, in your community" (Community Assessment Report, 2017). In addition, out of the 75 respondents, 88% felt that "health issues (such as diabetes, heart disease, and obesity) in our community are related to food and diet" and 82% felt that "health issues would improve with access to traditional foods" (Community Assessment Report 2017). This puts a high importance on increasing access to indigenous foods like psiŋ for the health of the community.

Also in the 2017 Food Sovereignty Assessment, the following comments relating to psiŋ were provided in response to the question "if you could tell your tribal or community leaders anything about food and hunger issues in your community, what would you tell them?"

- We need to utilize our land to grow our own foods
- Food is healthcare
- Reconnecting with our land is important to food issues
- Increasing access to traditional foods in order to teach about them
- Providing better access to healthier, fresher food in order to provide people with options
- Becoming as self-sufficient as possible would benefit our community greatly
- More people would eat healthier if they had better access to healthier food
- We need to introduce more traditional foods into community events

A movement in bringing back cultural traditions surrounding wild rice is also taking place at PIIC as multiple educational community events have been happening each year since 2015.
These events, in addition to continued tribal community involvement in psiŋ restoration efforts, include harvesting field-trips, parching, push-poling, and cooking classes.

Similarly to PIIC, the Lower Sioux Indian Community is addressing concerns on food sovereignty. The 'Honoring Little Crow through Healthy and Indigenous Foods Initiative' resolution was adopted by the Lower Sioux Tribal Council in 2016. This policy was developed to implement a system change to increase visibility of and access to healthier indigenous food and beverage choices to support a healthy Lower Sioux Community. Results from the Community's 2018 Food Sovereignty Assessment found that almost half of the respondents considered wild rice the top choice of traditional foods. However, nearly 75% of the respondents stated that they are not able to eat traditional foods as often as they would like. The Lower Sioux Office of the Environment is working on wild rice restoration efforts at four trial sites within the Community (initial seeding in 2015). During the same time, Lower Sioux Recreation department has provided trips during wild rice harvesting season so the Dakota Youth are able to experience ricing "Up North".

Ecology

Wild rice (genus Zizania) is an annual grass that grows in shallow water and slow-flowing streams and produces an edible grain. It is native to Minnesota and can be found in 55 counties in the northern region of the state and few in the southern region, though its range once covered the entire state. Current coverage of wild rice has declined to at least 64,000 acres when growing conditions are favorable.

A fast-growing, aquatic grass, it sustains both migratory and local wildlife, providing critical food and shelter at every stage of its growth and throughout all four seasons. Migrating and resident species alike rely on the plant's nutritious and abundant seeds. In the fall, many species of duck rely on wild rice as a staple food source.

Plant stems provide brood cover for waterfowl and nesting material for species such as common loons, red-necked grebes, and muskrats. Insect larvae that feed on wild rice serve as a rich food source for blackbirds, bobolinks, rails, and wrens. In the spring, decaying rice straw supports a diverse community of invertebrates that in turn supports birds, fish, and amphibians. In the summer, the whole plant provides food for herbivores like Canada geese, trumpeter swans, muskrats, beavers, white-tailed deer, and moose. In the late summer, wild rice provides cover for molting waterfowl and their young. Due to the plant's diverse ecological value, wild rice lakes and streams serve as breeding and nesting areas for at least 17 species listed as "species of greatest conservation need" on MNDNR's Comprehensive Wildlife Conservation Strategy. As an aquatic plant, it also provides habitat for fish. Wild rice provides additional ecological values by improving the quality of ecosystems, allowing for increased ecosystem function. By sequestering nutrients such as phosphorous and nitrogen, wild rice enriches soils while countering the negative effects of nutrient loading in water bodies that can cause algal growth and turbidity. Stands of wild rice form windbreaks and slow water velocity, limiting the mixing of soil nutrients into the water column. They also prevent erosion by stabilizing loose soils.

Management and Restoration

The Stoney Brook watershed encompasses over half of the Fond du Lac Reservation in northeastern Minnesota, at 59,248 acres, and its headwaters include the Reservation's premier wild rice lakes, designated as "Outstanding Reservation Resource Waters" in the Band's federally-approved Water Quality Standards. The watershed was extensively ditched under judicial order in the early 1900's to drain wetlands and open up acreage for crop agriculture, facilitate development, and encourage non-tribal settlement on tribal lands. But the substantial hydro-modification of this ditch system persists, and has resulted in detrimental fluctuating water levels in the wild rice lakes and significant stream and riparian habitat impairment throughout the watershed.

Because of the altered drainage, water level fluctuations in the wild rice lakes, perhaps the single most critical factor affecting natural wild rice productivity, are difficult to moderate during storm events.

Wetlands have been fragmented, and while the direction and flow of shallow ground water between the wild rice lakes is not well understood, it has likely been impacted by the ditch system. The ditch system, which was excavated between 1916 and 1921, lowered the lake levels on Perch, Jaskari, Rice Portage, Miller, and Deadfish Lakes. The total area of these five wild rice lakes prior to the excavation of the drainage ditches was 1,617 acres. The partial drainage of the lakes resulted in the loss of 850 acres of wild rice habitat to competing vegetation such as cattail, pickerel weed, water lily, sedge and horsetail.

The Fond du Lac Band is very committed to protecting, managing and restoring their wild rice lakes. Tribal leadership has expended considerable resources on the restoration of critical habitat on these wild rice lakes, and has directed the Fond du Lac Natural Resources Program (NRP) to manage and restore the wild rice lakes. Over the past twenty years the NRP has planned and implemented projects to accomplish this goal. A series of four water control structures were built to manage water levels for optimizing wild rice growth, and to restore the lakes to their historical size. Restoring lake levels and proper water level management will help the remnant wild rice stands thrive, but lake level management alone cannot restore wild rice in the areas choked with competing vegetation. The restoration of open water habitat favorable for wild rice requires the mechanical removal of many acres of vegetation with a large sedge mat cutter and two aquatic weed harvesters. The benefits from restoring the wild rice lakes include improved wildlife habitat, especially for waterfowl, in addition to providing wild rice for harvesting.

The topography of the White Earth Reservation varies greatly throughout its boundaries and ranges from prairie pothole, transition zones to forests. The landscape supports over sixty-eight thousand acres of surface waters and over three hundred miles of rivers and streams across three watersheds. The soils also range from loam, heavy clay to sandy. Within these zones a multitude of land uses occur, including agriculture. As agriculture practices increase so does the use of fertilizers, pesticides and herbicides, resulting in negative impacts to surface waters and aquatic life including wild rice.

With the added stress of runoff, sedimentation, lack of adequate surface water buffers and accumulation of sulfate, aquatic life is in dire need of protections.

In 1938 the U.S. Army Corps of Engineers built Lock and Dam 3, located in Red Wing, MN, creating Pool 3 of the Mississippi where the Prairie Island Indian Community (PIIC) now resides. The desire to create better shipping lanes along the Mississippi brought about the installation of lock and dams and a 9 foot deep shipping channel along the length of the river. Pool 3 contains both Sturgeon and North Lake, where psiŋ originally grew. The implementation of the lock and dam system drastically changed the function of the river. It created better shipping lanes, but also flooded much of PIIC land. The flooding from the dam increased the size of Sturgeon Lake and North Lake, greatly expanding the backwater areas of the Mississippi. Many isolated lakes and large expanses of marshland important to fish, waterfowl, plants, and other native wildlife were lost. These hydrology changes are thought to be a large reason why psiŋ beds shrank or were extirpated on the Mississippi in the years following the installment of the dams.

PIIC has been working to re-establish psiŋ since 2003 in the Mississippi backwaters and wetlands of Tribal land with a goal to restore 30 acres of wild rice beds. PIIC land sits on about 2,200 acres of backwater lakes, with a band of emergent plants and wetlands encompassing large portions of the Island. PIIC's restoration process includes planting psiŋ in areas of potential growth. Psiŋ is an annual plant, so if flooding prevents growth one year it is not able to re-seed itself for the following year – creating a challenge in the growth cycle. Stocking up a seed bank aids the rice in adapting to its environment, as some rice seed will remain dormant for a number of years before growing. The Land & Environment Department organizes follow-up aquatic plant surveys and appropriate seeding each year to document this re-establishment effort for the Tribe. There have been several years of psiŋ growth on PIIC; 2013, 2015, 2017 being three recent years marking dense rice beds and full growth. Even so, the restored psiŋ beds have totaled just over 7 acres in size and continue to struggle due to extreme spring flooding events. Clearly, there is still more work to be done in restoration on PIIC lands.

Economic Importance, Past and Present

In assessing the importance of manoomin/psiŋ to tribal economies, it is important not to limit the benefit metrics to job and income measures. In regard to tribal manoomin harvests, sales of a portion of the harvest are often used to supplement subsistence (i.e. selling a portion of the manoomin harvest to cover costs for gasoline and other expenses enables tribal members to participate in subsistence activities and provide food for their extended families). Because tribes were forced to participate in a western cash economy by European settlement, and manoomin has been appropriated as a commodity, it has since become a source of material wealth and economic survival for the Ojibwe as well. However, the traditional role of manoomin/psiŋ is still clear today.

Historically, wild rice was the most important grain in Minnesota's economy. Because it was a dietary staple, easily stored for long periods of time, and easy to use, it held considerable economic value for native people and early explorers and settlers. Although other grains became common over time as they were introduced to Minnesota by immigrants, wild rice continued to be popular. Records of state license sales going back to the 1950s clearly show the enduring popularity and value of wild rice. More than 300,000 licenses have been sold since 1957.

Prior to 1970, Minnesota provided half of the global market supply of wild rice; most of which was from hand-harvested natural stands. As cultivation of wild rice increased, by 1990, natural hand-harvested wild rice in Minnesota accounted for less than 10% of the global supply of wild rice. Yet, hand-harvested wild rice remains a vital part of the state's tribal and local economies. In fact, the largest part of the economy revolving around wild rice is the "underground" economy. Much of people's manoomin harvest is gifted or traded and is never tracked in any organized fashion. There is very little accounting or tracking related to wild rice sales, spending, or harvest. Yet, aside from the cultural importance of the activities, this barter and trade system is also important to the economic wellbeing of harvesters by reducing food costs and improving food security.

As part of the Health Impact Assessment, Fond du Lac worked with Earth Economics to develop an economic benefits analysis describing the impact of seasonal manoomin harvest to the tribal and state economies. This analysis estimated impacts on economic activity, food security, and public health, and then estimated changes in those impacts as a result of potential decreases in wild rice productivity and abundance. While the report was not intended to establish any monetary value to the cultural significance of manoomin, recognizing that these values are beyond economic measure, it did make a strong economic case for protecting manoomin and thereby preserving these benefits for future generations.

The effects of wild rice harvesting ripple throughout the economy in obvious and less obvious ways. Some harvesters sell a portion of the wild rice they gather for obvious economic gain. But additional contributions stem from the costs to undertake harvesting, such as gas, drying tarps, or canoes. Those expenditures support other sectors in the Minnesota economy, like retail and service. Wild rice also supports the Minnesota economy in other, less obvious ways. Conservation agencies, tribes, and other groups and organizations invest enormous amounts of money in ecosystem restoration projects that rely on native wild rice as an important plant; and due to their magnetism for waterfowl, wild rice waters serve as popular hunting grounds.

According to the 2006 National Survey of Fishing, Hunting, and Wildlife-Associated Recreation, waterfowl hunters contributed more than 43 million dollars ($43,000,000) to the Minnesota economy. Although hunting numbers on wild rice waters are currently unknown, Ducks Unlimited suggests that no other habitat sees such high concentrations of waterfowl. The shared value that so many Minnesotans place on wild rice habitat is reflected by the widespread efforts of hunting clubs, private citizens, and conservation groups to seed and expand it.

SUPPORTING EVIDENCE OF SULFATE/SULFIDE IMPACTS AND THE NEED TO PROTECT WILD RICE

Hydroponic studies – John Pastor

Dr. Pastor investigated the effects of sulfate and sulfide on the life cycle of wild rice in hydroponic solutions. Sulfate itself had no effect on seed germination or juvenile seedling growth and development, but sulfide greatly reduced juvenile seedling growth and development. The hydroponic experiments demonstrated that the adverse effects to wild rice are an indirect result from sulfide (formed in the low oxygen sediments of mesocosms and natural wild rice ecosystems), not a direct effect of the sulfate.

Mesocosm studies – John Pastor

In outdoor mesocosms (experimental systems that mimic natural ecosystems under controlled conditions), sulfate additions to the water increased sulfide production in the sediments. Wild rice seedling emergence, seedling survival, biomass growth, viable seed production, and seed mass all declined with increasing sulfate concentrations. These adverse effects are a result of the toxicity of the sulfide formed, and the decline in wild rice survival and growth grew steeper over the course of this multi-year experiment. Wild rice grown in mesocosms with higher sulfate concentrations went extinct, at progressively lower concentrations over time. After eight growing seasons of experimental sulfate additions, only the mesocosms with sulfate concentrations of 50 mg/L and the control (no sulfate additions) mesocosms still have wild rice growing and reproducing. This line of research essentially confirms the earlier research by a state biologist, who originally observed that no large populations of wild rice occurs in waters that exceed 10 mg/L sulfate, and wild rice stands are uncommon or absent where sulfate exceeds 50 mg/L.

Iron sulfide formation on roots – Sophia LaFond-Hudson

During the onset of seed production, wild rice root surfaces grown under experimental sulfate-amended treatments developed black iron sulfide plaques on their root surfaces, replacing the typical orange iron hydroxide plaques seen in natural ecosystems and control mesocosms (without sulfate amendments). Iron hydroxides are thought to protect aquatic plants from toxic substances such as sulfide by providing an oxidized barrier around the roots. After these iron sulfide plaques formed on the roots, the wild rice plants ceased uptake of nitrogen, during a point in their life cycle where nitrogen is needed to form seeds. This observed phenomenon may explain the mechanism by which sulfate reduction to sulfide affects seed production and seed biomass, contributing to the decline and extinction of wild rice populations exposed to higher sulfate over time.

Field studies – Amy Myrbo

Comprehensive field surveys led by Dr. Amy Myrbo as part of the state's research program characterized 64 chemical and physical variables over 100 sites across Minnesota. Analysis of the data concluded that, while water temperature and water transparency controlled the suitability of habitat for wild rice, the sulfide in sediment pore water, generated by microbial reduction of sulfate, is the primary control of wild rice occurrence. Anaerobic microbes in lake and river sediments make sulfide from sulfate in the overlying water, and waterbodies that have high concentrations of dissolved sulfide in the sediment have a low probability of hosting wild rice. This research confirms the earlier research by a state biologist, who originally observed that no large populations of wild rice occur in waters that exceed 10 mg/L sulfate, and wild rice stands are uncommon or absent where sulfate exceeds 50 mg/L.

Rooting zone geochemistry – Nate Johnson

Dr. Johnson collected and analyzed rooting zone depth profiles in the experimental mesocosms (Pastor studies) and field sites (Myrbo surveys) to characterize sulfate, sulfide and iron in the rooting zone of wild rice plants. In the mesocosms, a portion of each tank was isolated from plant roots with a sheet of Plexiglass in order to assess the effect of wild rice roots on porewater chemistry (oxidation or reduction). "Peepers" (porewater sensors) were deployed in the plant-free and planted sections of selected mesocosms, and in two field sites where sulfate was elevated (Second Creek, Sandy Lake). He observed a consistent reduction in porewater sulfate as summer progressed, while sulfide increased and was highest just below the sediment-water interface. Lower sulfide concentrations deeper in the sediment layer are likely a result of precipitation with ferrous iron, which had higher concentrations in the deeper sediments, but decreased over the summer season. There was no consistent difference in the porewater of the plant and plant-free portions of the mesocosms, although there were clear differences among the sulfate treatment concentrations.

Temperature dependent diffusion rates of sulfate – Nate Johnson

Dr. Johnson conducted a sediment incubation study to explore the effect that ambient air temperature has on the rate that elevated sulfate concentrations in the water column are converted in the underlying sediment to sulfide, and later release sulfate back into the overlying water. This study was intended to inform whether the seasonal application of the existing sulfate standard was protective (only control sulfate discharges during the growing season). Porewater sulfate decreased over time, as it was reduced to solid-phase sulfide, in both temperature treatments (4.5^{o} C and 23^{o} C), although at a slower rate in the cold treatment; that sulfate reduction rate was calculated, and consistent with observed rates in other studies.

Twin Lakes Monitoring Case Study

A monitoring program has been completed in 2010-2018 at Sandy Lake and Little Sandy Lake. The 1854 Treaty Authority completed the work in support of the Bois Forte Band, and in some years also in cooperation with the United States Steel Corporation. Sandy Lake and Little Sandy Lake, also known locally as the Twin Lakes, historically have produced good stands of wild rice. Wild rice harvesters utilized the lakes when suitable beds for harvest were present, including a history of use by tribal members.

A lake survey in 1966 indicated moderately dense to dense stands covering both lakes. Rice production generally declined through the 1970s and 1980s, with little or no rice found in the lakes during a 1987 survey. Rice production has since remained poor to nearly non-existent. The lakes are located downstream of the tailings basin at the U.S. Steel Minntac iron ore operation. Construction of the tailings basin began in 1966, and the resulting changes to the system have impacted wild rice in the Twin Lakes. Monitoring activities were completed in 2010-2018 to document conditions in the lakes and have included water depth recording, inlet and outlet field surveys, water sampling, vegetation surveys, and aerial surveys.

Under another initiative in 2013, lake sediment cores were collected by University of Minnesota researchers to investigate the historical sulfur inputs to Little Sandy Lake. Their analysis found a significant increase in sulfur counts in only the uppermost 10cm of the sediment core which corresponds with the development and operation of the Minntac mine and tailings basin. This increase in sulfur corresponds with the decline in manoomin. The report "Reconstructing Past Sulfur Loading and Wild Rice Abundance in Little Sandy Lake" summarizes the techniques and findings of their investigation.

Four water sampling locations have been established at the Twin Lakes in a downstream order: at the inlet to Little Sandy Lake, near the center of Little Sandy Lake, near the center of Sandy Lake, and at the outlet of Sandy Lake.
If focusing at water quality entering the lakes from the tailings basin at the inlet to Little Sandy Lake, sulfate has remained well elevated beyond the current standard of 10 mg/L.

Sulfate Concentration at Inlet to Twin Lakes

	Average Sulfate (mg/L)	Sulfate Range (mg/L)
2010	**483**	**360-661**
2011	**357**	**208-561**
2012	**207**	**137-275**
2013	**355**	**215-650**
2014	**301**	**180-419**
2015	**460**	**386-590**
2016	**289**	**217-347**
2017	**379**	**251-589**
2018	**300**	**198-489**

During the monitoring time period of 2010-2018, natural wild rice presence in the lakes has been limited. In general, wild rice has not been observed or a few individual stalks in Little Sandy Lake. In Sandy Lake, sparse stalks of rice have been observed in a few locations. The report *"Sandy Lake and Little Sandy Lake Monitoring (2010-2017)"* referenced in the Appendix summarizes information from the monitoring program. A summary report including information from 2018 has not been completed to date.

Lists of Wild Rice Waters

A piece of the wild rice water quality standard includes a definition of what is a wild rice water. A list of wild rice waters is critical to understand where a numeric water quality standard would apply and be implemented by the state of Minnesota. This list is necessary for treaty areas, but it does not include waters within tribal boundaries. Waters within tribal boundaries are up to the individual Tribes to manage and regulate.

In addition to scientifically determining what is the numeric wild rice water quality standard, it is critical to understand where it would apply. The MPCA was directed by the legislature to answer an important question: what is a wild rice water? From a tribal view, all waters are connected and have importance. Colonization of Minnesota has changed the hydrology of the area with dams and culverts and what once were "rice waters" have changed and new areas now hold wild rice. With the continued exacerbation of climate change it is difficult to predict what waters will continue to hold rice, or what water will need to hold rice for culture and customs to continue. With that in mind, if a lake or river supports, has supported, or could support any wild rice, it is a wild rice water. We do not see any other way to define it.

White Earth continues to express concern regarding how outside agencies define a wild rice water. White Earth contends all surface waters are wild rice waters and therefore no limit(s) should be applied to what constitutes or defines them. Many surface waters were harmed prior to the protections of the Clean Water Act. Numerous historical rice beds have been lost or displaced and these waters also need protection. Due to these reasons, White Earth feels the state's wild rice producing water inventory is incomplete and needs further updating.

Because Minnesota's wild rice waters have not been systematically inventoried, monitored, assessed or protected through regulatory controls for sulfate under the existing standards, many more once-harvestable stands have been degraded or destroyed since the effective date of the Clean Water Act. It is our understanding that the MPCA has utilized a two-acre threshold to initially identify waters where the wild rice sulfate standard would apply. We do not agree with the basis or justification for this criterion to define a wild rice water.

Any wild rice is important and worth protecting. Furthermore, wild rice acreage information is not available for most waters in the state. Monitoring data for waters across the state does not exist for that type of detailed information on wild rice presence. Wild rice is a variable resource throughout the years, and it takes multiple years (and even historic consideration) to understand the potential density and acreage of wild rice in each water. Data collected over an extended period of time may be needed to determine if a water meets the proposed acreage. The MPCA utilized judgement to include or exclude waters, but the acreage criterion they proposed is based on information that largely does not exist, because the state has never invested the resources necessary to establish a baseline inventory of wild rice waters.

The MPCA also proposed to apply an existing narrative standard (Minn. R. 7050.0224), protective of wild rice and the habitat and environmental quality needed to maintain it, *only* to the arbitrary list of 24 wild rice waters identified in Minnesota Rules (Minn. R. 7050.0470) through rulemaking in 1997-98 for waters in the Lake Superior Basin. Tribes had urged the agency to apply that aquatic life use-protective narrative standard to all wild rice waters in the state, but the agency did not do so despite the administrative record that clearly includes commitments by the state to move beyond that initial step.

In the Statement of Need and Reasonableness (SONAR) from 1997, the agency said:

> Finally, the proposed amendments specifically listing the wild rice waters in Minn. R. 7050.0470 and the inclusion of the wild rice narrative language in Minn. R. 7050.0224 are needed because: 1) they are viewed as initial steps in a broader process intended to provide greater public awareness as to the ecological importance of this unique plant species; 2) they provide further support for the study of the physical, chemical and biological factors that are needed to support wild rice development; and 3) the proposed wild rice amendments represent an affirmation of the MPCA's commitment to work in concert with the American Indian Bands on environmental issues of mutual concern.
>
> … The proposed listing of the 24 wild rice waters in Chapter 7050 is specific to a select number of waterbodies within the Lake Superior Basin that have current and/or historic stands of wild rice. No additional numerical standards for wild rice protection purposes are being proposed during the present rulemaking effort. It is the current intent of the MPCA to participate in ongoing studies and assessments of the wild rice plant and wild rice habitat protection issues. MPCA staff also plan to continue to work with the MNDNR and the various Bands to identify additional wild rice waters on a statewide basis.
>
> … The listing of these waters and the proposed narrative wild rice waters standard in Minn. R. 7050, in and of themselves, will not automatically translate into greater protection levels that are afforded to this plant species. Rather, increased protection of natural wild rice stands will happen as a result of a continued dialogue and information exchange between interested and affected parties.

The MPCA has not honored or fulfilled the specific commitments they made with the Tribes in that rulemaking process twenty years ago, to address the overall decline in the number and distribution of wild rice waters in the state, and to continue research and develop best management practices and standards.

A report entitled *"Natural Wild Rice in Minnesota"* was completed in February 2008 by the Minnesota Department of Natural Resources (MNDNR). As part of this report directed by the state legislature, the MNDNR compiled a list of wild rice waters. Although no statewide inventory of wild rice waters can likely be perfect, this MNDNR led effort was well done and completed with input from many partners including tribes and tribal organizations. The MNDNR continues to refine and update this statewide inventory, with additional waters identified and shared with MPCA in 2013.

The 1854 Treaty Authority has developed and maintains with annual updates a list of wild rice waters in the 1854 Ceded Territory. The MPCA proposed list where the standard would apply largely includes the waters from the 2016 updated list (dated 3/24/2016 – 393 locations), but not for most additions made for the current list (dated 3/28/2018 – 512 locations). The procedure for developing and updating the 1854 Treaty Authority inventory of wild rice waters has not changed over time, and reports are utilized from other partners (such as MNDNR) or field observations are recorded. However, the MPCA did not recognize the latest updates in their proposed rule. Analysis shows that the wild rice sulfate standard would not apply at over 100 wild rice locations in the 1854 Ceded Territory.

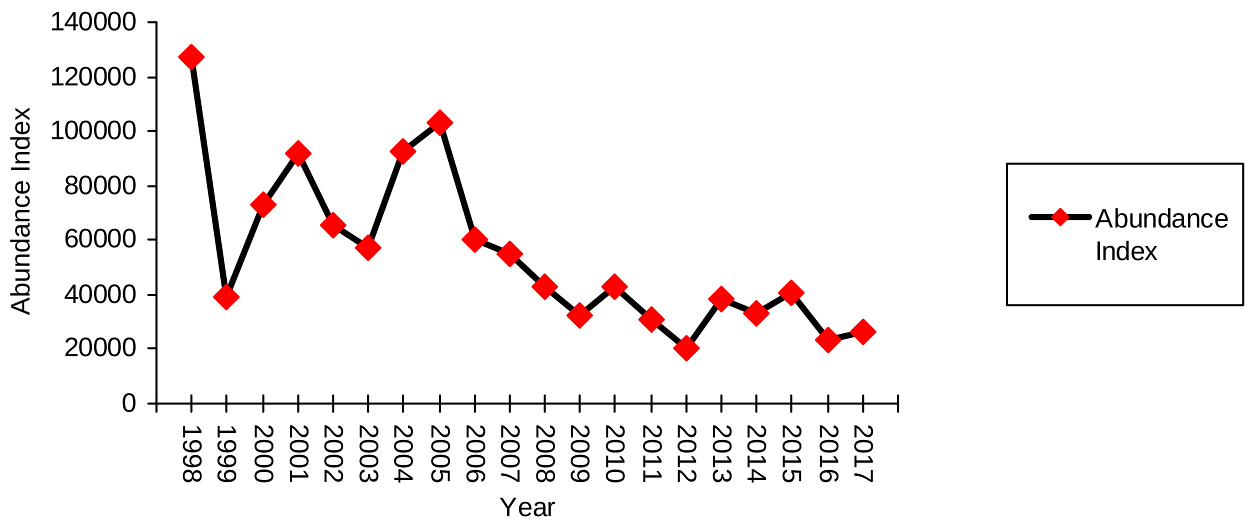
Utilizing available information (2008 MNDNR report, MNDNR updates, 1854 Treaty Authority, other sources) the MPCA compiled a list of wild rice waters in Minnesota. This list included waters with any record or report of wild rice presence. This was a comprehensive exercise, and the best effort to date at compiling wild rice locations across the state. The entire list of wild rice waters developed by the MPCA contains 2,347 locations. This full list is the best statewide inventory that currently exists. However, the MPCA has listed 998 locations as having "insufficient information" where the wild rice water quality standard would not apply. Again, no relevant criteria or long-term monitoring data exists to exclude these waters. The MPCA approach of identifying waters where the wild rice sulfate standard applies is exclusive instead of inclusive, and concern exists over this omission of wild rice waters.

Long-Term Wild Rice Monitoring

In 1998, the 1854 Treaty Authority initiated a wild rice monitoring program on numerous lakes and rivers within the 1854 Ceded Territory in northeastern Minnesota. The 1854 Treaty Authority's monitoring program documents wild rice abundance and identifies trends in production on this group of waters. Monitoring activities have been completed with some variation across years. Seven lakes have been included each year from 1998 to 2018. The monitoring program in 2002-2018 has included the same 10 lakes and rivers.

The focus of the program is to document wild rice biomass each season on a water. This gives a gauge on density, acreage, and plant height each year and ultimately shows changes across time. Protocol has been standardized in the "*Wild Rice Monitoring Handbook"* and "*Wild Rice Monitoring Field Guide"* completed in 2015. In addition to calculating biomass, other activities such as water level monitoring, water sampling, and photography are included in the program. The report *"Wild Rice Monitoring and Abundance in the 1854 Ceded Territory (1998-2017)"* referenced in the Appendix summarizes information from the monitoring program. A summary report including information from 2018 has not been completed to date.

One point to note is the potential long-term decline in wild rice. The summary graph below shows the abundance index (combination of wild rice acreage and density) from 1998-2017 on waters in the 1854 Treaty Authority program. Although it is difficult to determine an exact cause (perhaps climate change and related impacts), this highlights the need to protect a resource that is potentially declining. This decline in "natural" waters is on top of the likely immense amount of wild rice lost statewide due to development, water level changes, water quality issues, etc. since Minnesota statehood.



Total Abundance Index on all Waters in 1854 Treaty Authority Monitoring Program (1998-2017)

This type of monitoring also demonstrates the long-term data needed to begin to understand wild rice presence on a water. This information, along with other sources (oral histories, photographs, etc.) could inform lists of wild rice waters. However, given that long-term monitoring data does not exist on many waters across the state, it is impossible for the MPCA to make a determination to omit wild rice waters where the sulfate standard would apply.

Potentially Affected Dischargers

National Pollution Discharge Elimination System (NPDES) permits are required to include limitations consistent with effluent limitation guidelines for discharges that are causing or contributing to a violation of water quality standards. These limits are not water quality standards themselves, but are calculated so that the permitted discharge effluent will meet water quality standards in the receiving water, and if applicable, must conform to any Total Maximum Daily Load requirement that sets pollutant limits in order to meet water quality standard. 40 C.F.R. § 122.44. Unless end-of-the-pipe discharge concentrations cause or contribute to an exceedance of water quality standards in the *receiving or downstream* water bodies, permit limitations and additional treatment are not required.

In development of the proposed revised wild rice sulfate standard, the MPCA conducted a preliminary analysis on which facilities the new standard might apply. These potentially affected dischargers could adversely impact wild rice waters and if so, would need to comply with the standard. Further analysis of potentially affected dischargers in this section indicates that the wild rice standard would not generally be applied to domestic wastewater treatment plants. Industrial operations upstream of wild rice waters that discharge a much larger effluent volume with higher sulfate concentrations than most domestic discharges would need to add treatment technology to comply with the wild rice sulfate standard.

Water Body Sulfate Concentrations

Water column sulfate concentrations were analyzed to determine which water bodies or water body segments were exceeding the existing 10 milligrams per liter (mg/L) water quality standard. Results from this analysis were then used to identify dischargers to those waters.

Methods

Water column sulfate data was compiled from State and Tribal Agencies. Each dataset was sorted by unique locations. Data from each location was evaluated to determine the average and range of sulfate concentrations. An individual map was then generated for each dataset using the sulfate average or single measurement concentration for every location. The locations of large industrial dischargers were identified on the St. Louis and Itasca County map and the Mississippi River map.

GIS Methods

The maps were created using ESRI's ArcGIS 10.3 software. The power plant locational data was obtained from www.eia.gov, the Reservation boundaries from www.data.gov, and the watershed data from www.usgs.gov. All of the other base data layers came from https://gisdata.mn.gov. The monitoring data and associated locations were brought into ArcMap via Excel spreadsheets and converted to shapefiles. Differently colored and sized symbols were used to display the points based on their average sulfate concentration, with the break values of 5, 10, 30, 50, 100 and 200 mg/L.

As shown on the maps provided below, all of the waters exceeding the existing 10 mg/L sulfate wild rice water quality standard are downstream of mining operations and/or electrical generation power plants in St Louis and Itasca Counties and the Mississippi River.

An additional map was added to the analysis: "Mean Sulfate Concentrations Downstream of Mine Point Discharges", created by Scott Cardiff (working with the Great Lakes Indian Fish and Wildlife Commission), for the PolyMet Supplemental Draft Environmental Impact Statement, Appendix C, Tribal Cooperating Agencies Cumulative Effects Analysis, 2013.

Eight data sets were used for this analysis. A summary of the agencies that provided data, when the data was collected, the number of locations where measurements were taken, and the number of individual sulfate measurements are listed in the table below.

Table 1. Summary of Datasets Used to Analyze Average Water Body Sulfate Concentrations

Agency	Area of Data Collection	Number of Sulfate Measurements	Number of Discrete Locations	Years of Collection
Minnesota Pollution Control Agency	St. Louis and Itasca Counties	7,198	906	1974-2016
1854 Treaty Authority	1854 Ceded Territories	309	43	2007 - 2017
Fond du Lac Band of Lake Superior Chippewa	Fond du Lac Reservation	741	39	1998 - 2017
Leech Lake Band of Ojibwe	Leech Lake Reservation	644	80	2012 - 2018
Mille Lacs Band of Ojibwe	Mille Lacs Reservation	55	12	2010 - 2017
Grand Portage Band of Ojibwe	Grand Portage Reservation	1,547	32	2000 - 2018
Minnesota Pollution Control Agency	Mississippi River in Minnesota	1,808	87	1973 - 2017
Prairie Island Indian Community	Lower Mississippi River and backwater pools	325	8	2014 - 2017

Approximately seventy-five percent of the of the MPCA data sites in St. Louis and Itasca Counties were below the 10 milligram per liter (10 mg/L) sulfate water quality standard.

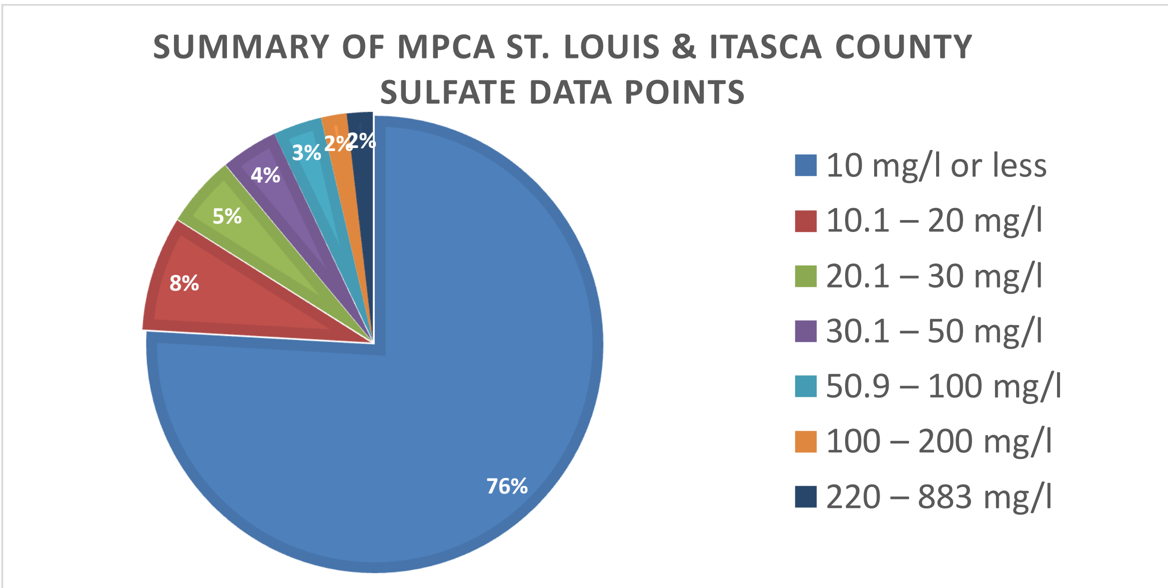


Figure 1. St. Louis and Itasca Counties Average Water Column Sulfate Concentrations

An analysis of sulfate concentrations below 10 mg/L from water column data collected in St. Louis and Itasca Counties demonstrates more than half of the data sites had concentrations of 2.5 mg/L or less.

Table 2. Breakdown of sulfate concentrations 10 mg/L or less (MPCA St. Louis & Itasca County Sulfate Data Points)	
Below Detection	5 %
2.5 mg/L	48 %
2.6 - 5 mg/L	32 %
5.1 - 10 mg/L	15%

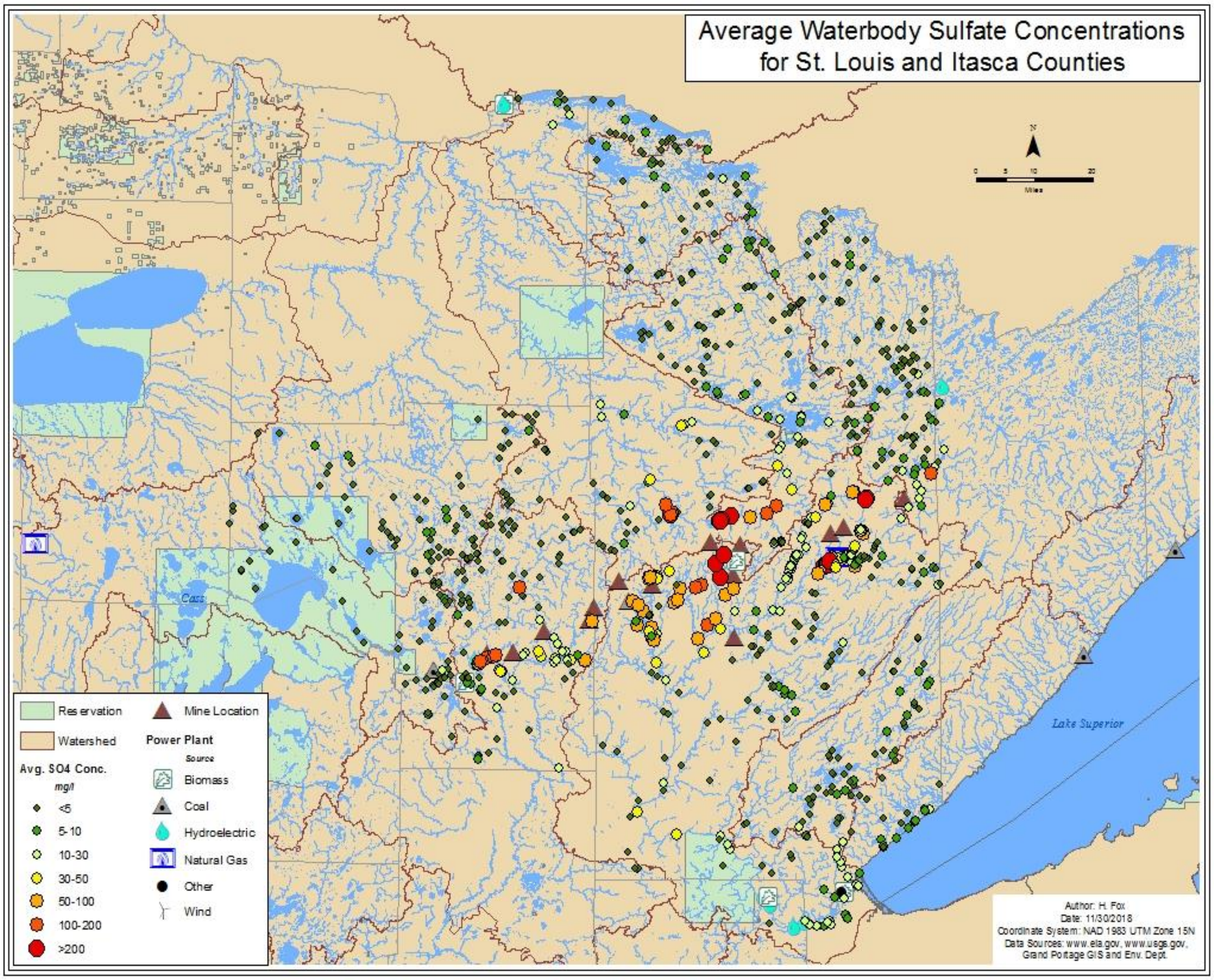


Figure 2. St. Louis and Itasca Counties Average Sulfate Water Column Concentrations

Water column sulfate concentrations are elevated in waters measured downstream of taconite mining operations and natural gas electrical generation facilities. In waters without mining and electrical facility discharges, sulfate concentrations are below 5 mg/L.

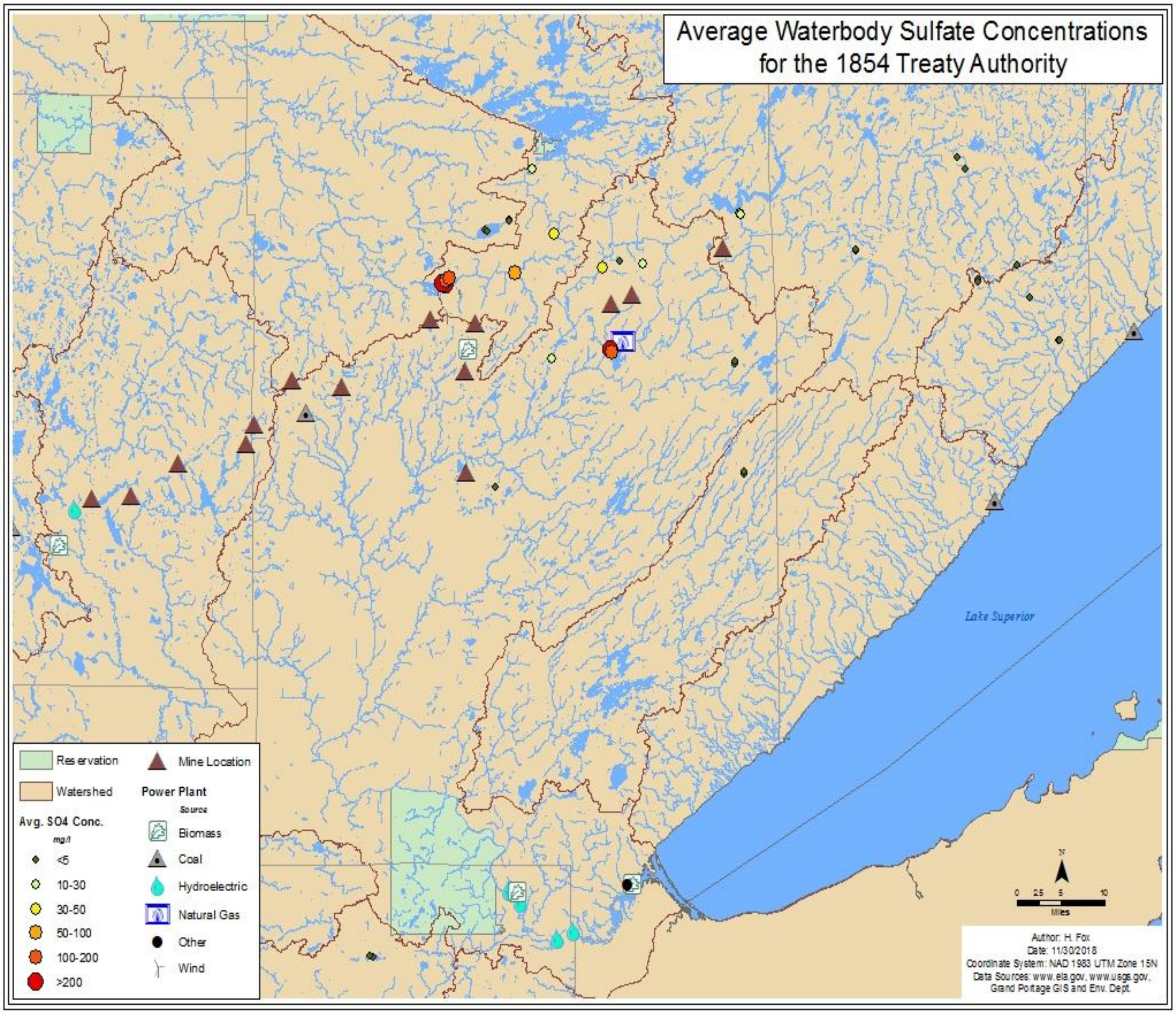


Figure 3. Average Water Column Sulfate Concentrations Measured in the 1854 Ceded Territory by the 1854 Treaty Authority.

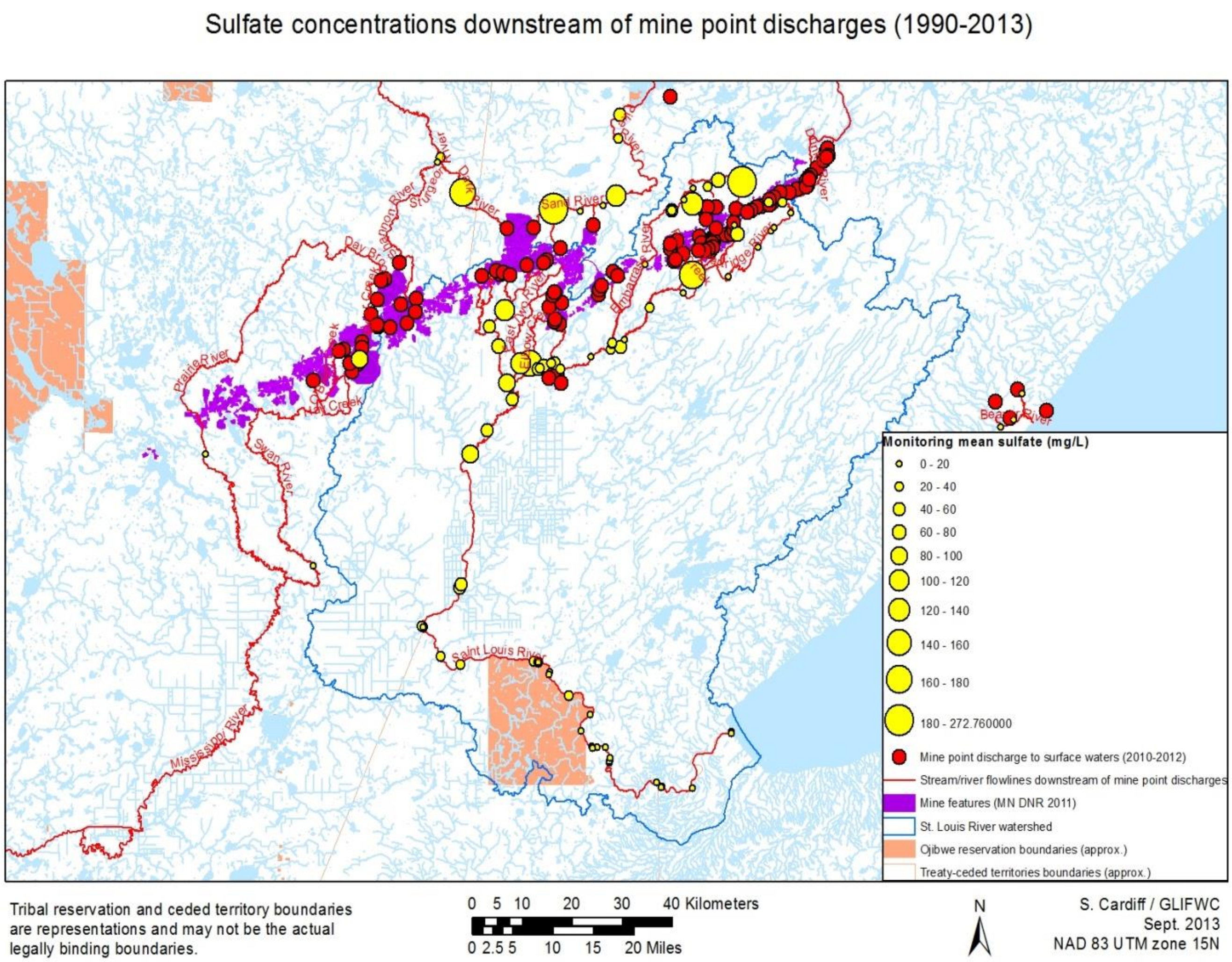


Figure 4. Mean Water Column Sulfate Concentrations Measured Downstream of Taconite Mining Facilities in Northern Minnesota.

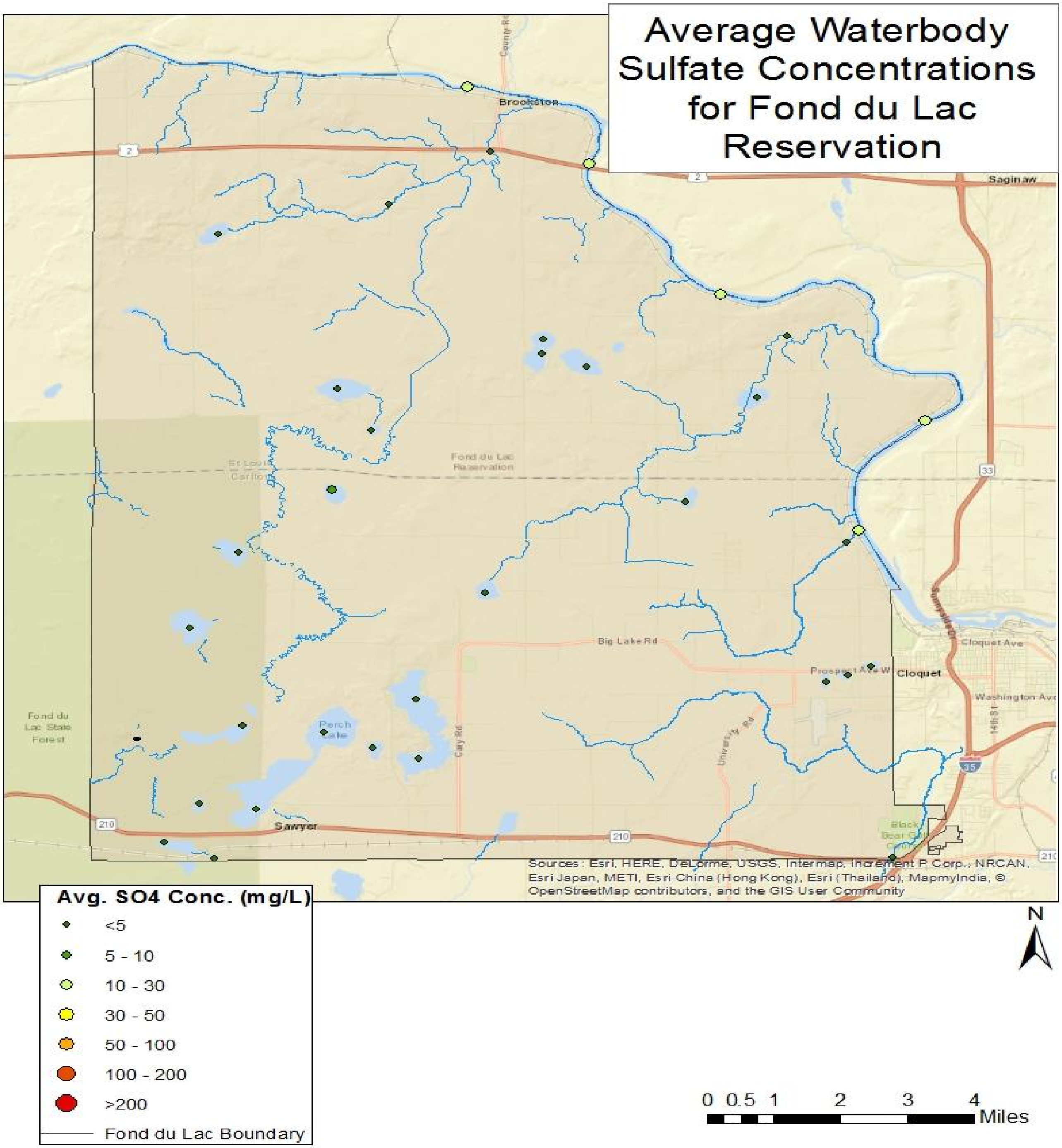


Figure 5. Fond du Lac Reservation Average Waterbody Sulfate Concentrations.

Average sulfate concentrations in reservation lakes and streams are all below 5 mg/L, with the exception of the St. Louis River. The higher sulfate concentrations in the St. Louis River are not naturally occurring; they are a result of high sulfate loadings from upstream facilities. Historic sulfate concentrations in this watershed were consistently below 10 mg/L.

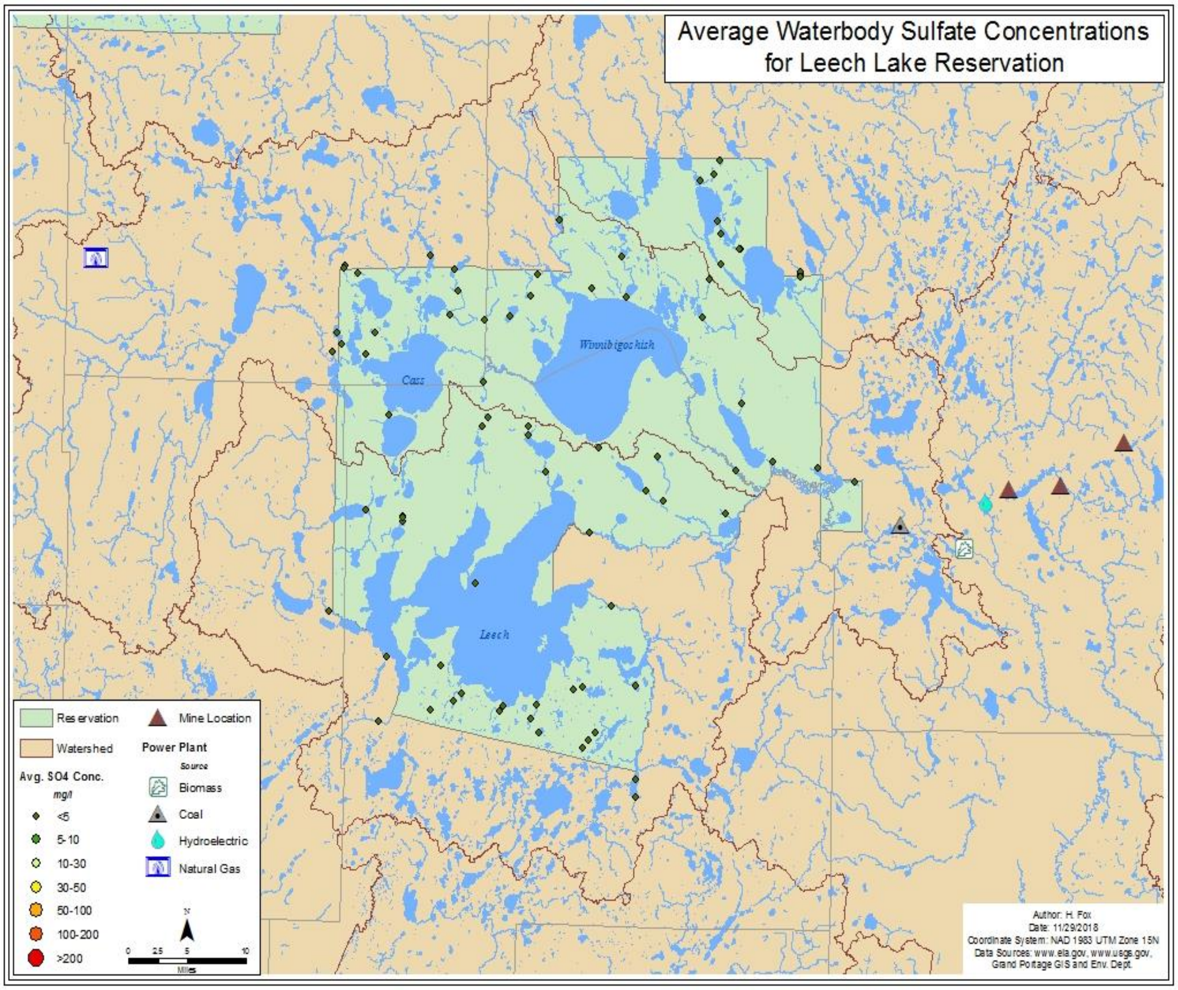


Figure 6. Leech Lake Reservation Average Waterbody Sulfate Concentrations.

All of the average sulfate concentrations measured within Leech Lake Reservation waters are below 5 mg/L.

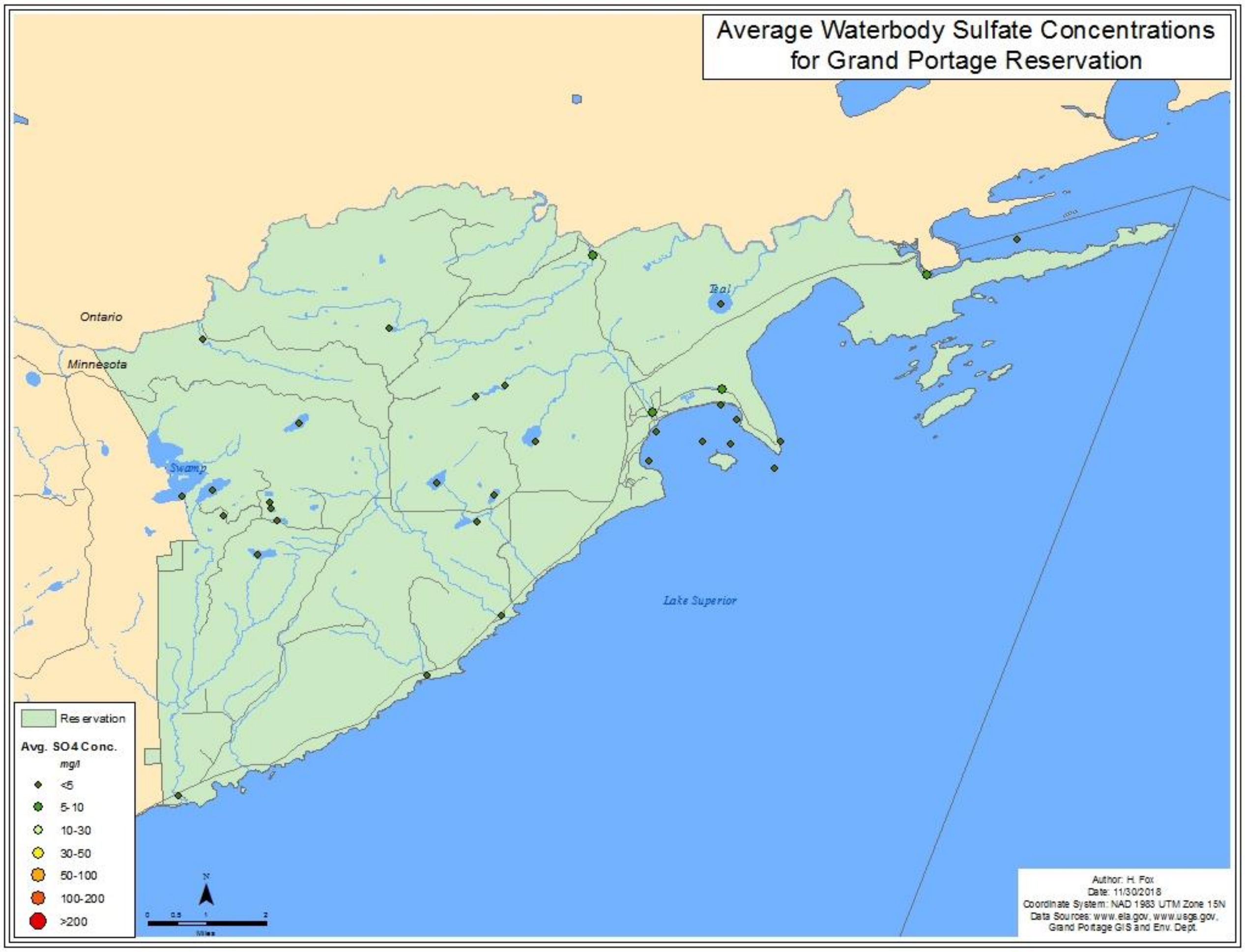


Figure 7. Grand Portage Reservation Average Waterbody Sulfate Concentrations.

The average sulfate concentration in all water bodies within the Grand Portage Reservation are below the federally approved 10 mg/L Grand Portage wild rice sulfate standard. Most waters within the Reservation have an average sulfate concentration below 5 mg/L.

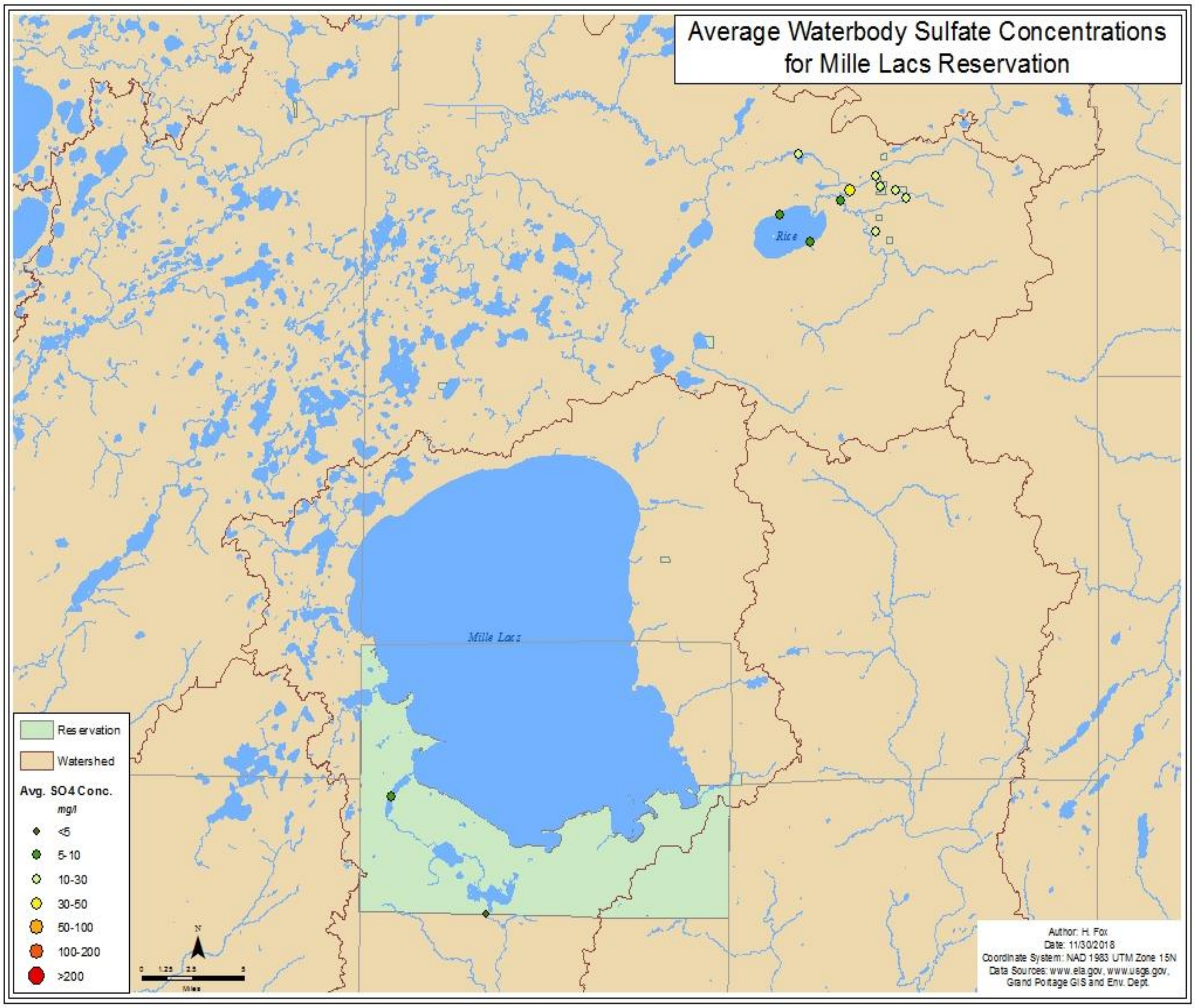
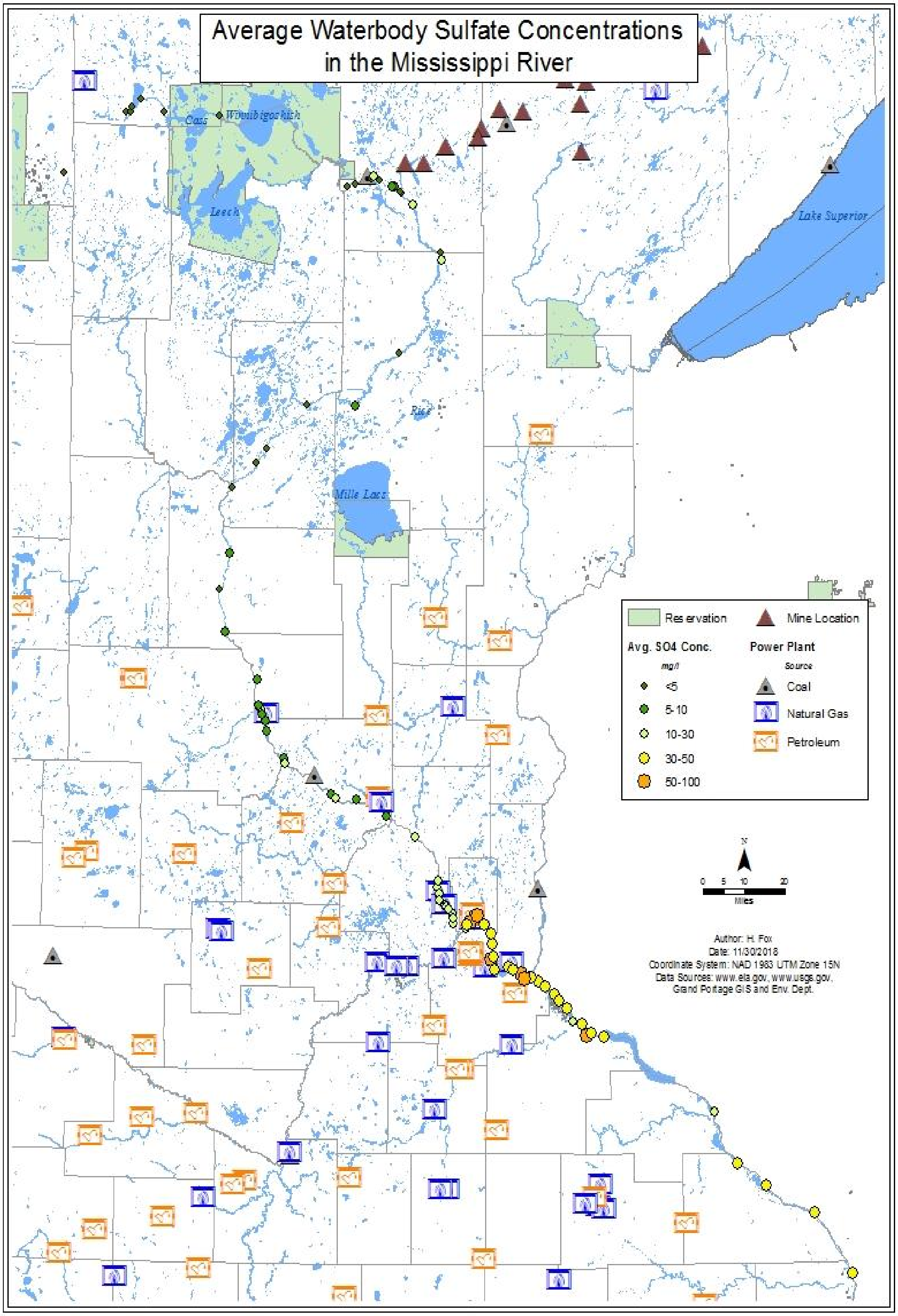


Figure 8. Mille Lacs Reservation Average Waterbody Sulfate Concentrations.

Sulfate concentrations range from less than 5 mg/L to 50 mg/L in waters within Mille Lacs Reservation. Wild rice waters do not exceed the 10 mg/L standard and therefore no treatment would be required for compliance.



Mississippi River sulfate concentrations are below 5 mg/l in the headwaters near the Leech Lake Reservation, and rise to concentrations between 10-30 mg/l as the river passes mine features and a coal-fired electrical generation plant. Sulfate concentrations fall back below 10 mg/l downstream of Grand Rapids. Average sulfate concentrations rise as the river passes inflows from industrial natural gas, coal and petroleum electrical plants between St. Cloud and Otsego to a range between 10-30 mg/l. Near Minneapolis, sulfate increases to concentrations between 30-100 mg/l as the river passes six natural gas and petroleum electrical generation power plants. Downstream of Minneapolis, sulfate concentrations remain between 10-50 mg/l to the southern border of Minnesota.

Figure 9. Mississippi River Average Sulfate Concentrations

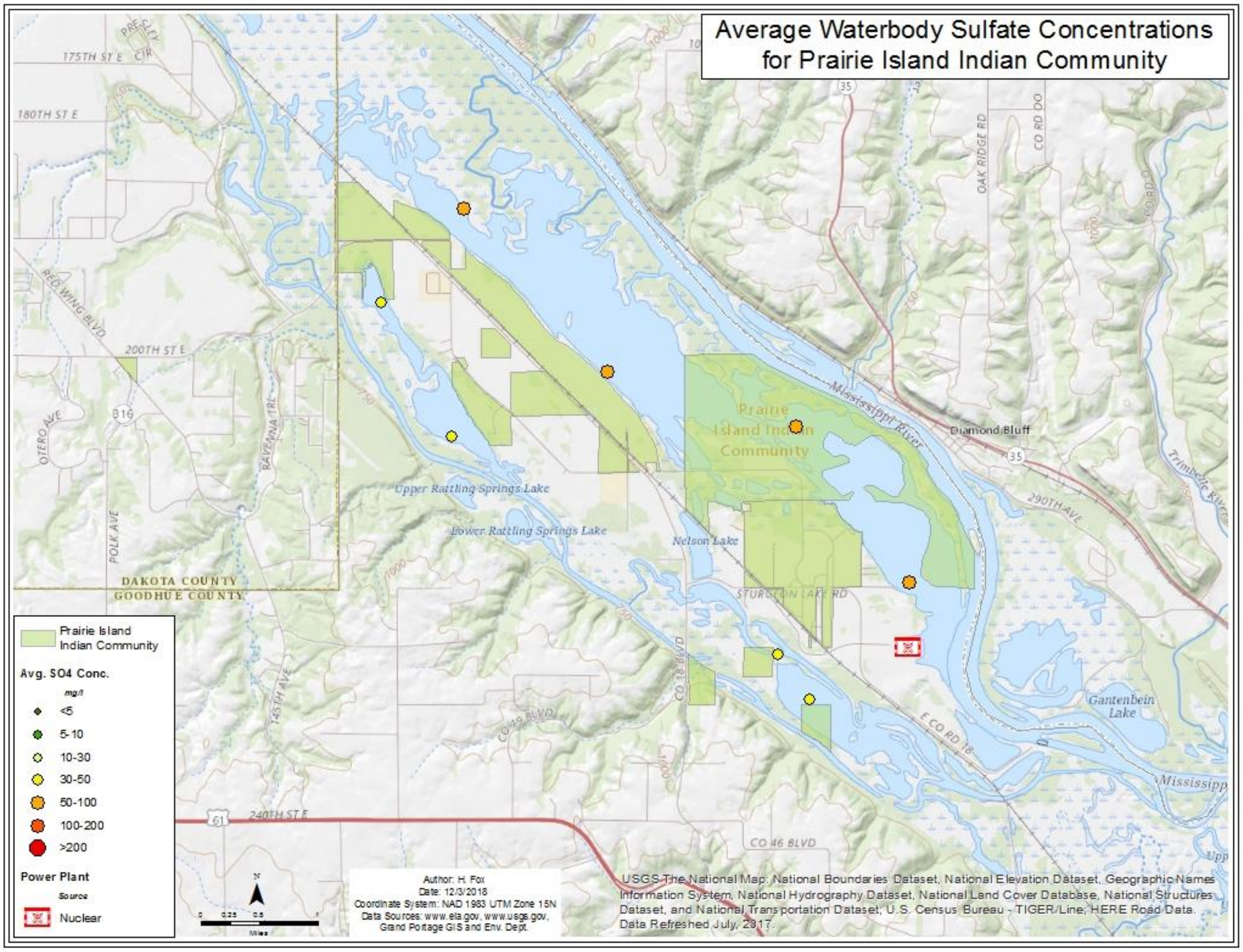


Figure 10. Prairie Island Indian Community Average Waterbody Sulfate Concentrations.

Utilizing multi-year data from reference sites and more disturbed sites seeks to provide a means by which to determine if water quality is different at locations within the lakes as distance from main channels increase. Much of the initial work over the past 10 years has produced data that describes baseline chemical conditions for these ecosystems. Prairie Island started its water quality monitoring program in 2007 which involved monitoring for sulfate annually. In 2014 the program was modified to include bi-weekly sampling for a total of 10 sulfate samples per year at each of the sample sites. This resulted in a more robust data set for sulfate in our backwater areas, providing additional information useful to our wild rice restoration work.

Sturgeon Lake and North Lake are direct backwater lakes of the Mississippi River. Direct flow comes from the Mississippi River into Sturgeon Lake through Brewers Lake inlet, with about 40% of the river flow coming through that inlet during normal water levels, and 60% of the river flow coming into Sturgeon Lake through Brewers Inlet during high water levels. Flow from the Mississippi River also comes directly into North Lake through Jackson Run and Miley Run. This is likely contributing to the higher sulfate levels found on those Mississippi backwaters, since the sulfate levels are comparative to those in the main channel of the river. On the Vermillion River backwaters, sulfate is shown to have higher levels than expected according to the averages of natural occurring sulfate levels in the region. Vermillion River receives surficial groundwater flow from the Mississippi River in a southwesterly direction across the island. This may be contributing to higher sulfate levels in the Vermillion River, in addition to the flashy nature of the river which leads to lower water levels in late summer.

Domestic and Industrial Discharger Assessment

This analysis is used to identify potentially affected dischargers categorized on the MPCA's SONAR list that would likely be affected by enforcement of the wild rice sulfate standard, identify those entities that would not be affected, and identify data gaps.

MPCA's list of "potentially affected dischargers" from the Statement of Need and Reasonableness ("SONAR") was developed solely by calculating which domestic and industrial facilities were within 25 miles of wild rice waters. For this analysis, MPCA provided the NPDES permits for each discharger from the SONAR list along with a spreadsheet that indicates the distance from a facility to wild rice waters, and the wild rice water body names. Some permits were listed two or three times on the MPCA list possibly due to discharges that flow into more than one water body. Therefore, a new spreadsheet tab was created that did not include duplicate permit numbers. Facilities were sorted into three categories based on the distance to wild rice waters: 25 miles; 10 miles; and 5 miles or less.

For each discharger the permitted average wet weather effluent volume was converted to millions of gallons per day and cubic feet per second. When sulfate discharge data was available in an electronic format from MPCA, the average and range of concentrations was calculated. If sulfate data was available from the water body that an entity discharged to, or if there was an average sulfate concentration for the closest wild rice waterbody, that data was also added to the spreadsheet. A column of permit issuance dates were added to the list of potentially affected dischargers.

Notes were taken from each permit regarding the type of discharge. Dischargers were eliminated from the list if the only pollutant added was heat, or if the permit specified that discharges were for pipeline and tank testing and the discharge was to take place in an upland vegetated area. Some potentially affected dischargers were removed from the list based upon GIS analysis, because water would have to flow uphill from the discharge to reach the specified wild rice water. Dischargers were eliminated from the list if the receiving or downstream water bodies were not exceeding the wild rice sulfate of standard of 10 mg/L.

Results

According to MPCA's potentially affected discharger list, thirteen of the top sixteen biggest discharges by volume and sulfate concentration are industrial. These sixteen dischargers are within ten miles of wild rice waters. The remaining three facilities that are not industrial include one facility that treats both industrial and domestic wastewater, and two facilities that appear to treat only domestic wastewater. No sulfate data is available for either of the domestic dischargers or the facility that treats both domestic and industrial wastewater. The range of volume of discharge is 7.29 – 161.8 million gallons per day. The range of average sulfate concentrations is 22.7 – 1054 mg/L.

Table 3. Top 16 Dischargers by Volume from MPCA SONAR

Permit Number	Facility Name	Facility Type	Discharge MGD	Discharge CFS	Average Discharge Sulfate Concentration (mg/l)	Distance to Wild Rice (miles)	Draft Wild Rice Water Name
MN0001007	Minnesota Power – Boswell Energy Center	Industrial	161.80	250.34	**586**	0	Blackwater Lake
MN0000990	Minnesota Power – Laskin Energy Center	Industrial	125.4	194.02	**489**	6	Partridge River
MN0049760	Hibbing Taconite Co – Tails Basin Area	Industrial	4.41 - 65	6.82 - 100.57	**62.6** (Little Fork River) **35** (Mississippi River at Grand Rapids)	2	Shannon Lake
MN0069078	Mesabi Mining Area	Industrial	58.4	90.36	**176**	1	Partridge River
MN0029882	Met Council – Blue Lake WWTP	Domestic	42	64.98		0	Blue Lake
MN0055948	Keewatin Taconite Operations – Tailings	Industrial	32.4	50.13	**177**	10	Hay Lake
MN0042536	Cliffs Erie – Hoyt Lakes Mining Area	Industrial	27.45	42.47	**269**	4	Second Creek
MN0044946	United Taconite LLC - Thunderbird Mine	Industrial	27.37	42.35			St. Louis River
MN0046981	Northshore Mining Co – Peter Mitchell	Industrial	24.11	37.3	**112.3** (Rainy River) **22.7** (St. Louis River)	3	Dunka River
MN0057207	US Steel Corp – Minntac Tailings Basin Area	Industrial	17.11	26.47	**1054**	2	Little Sandy Lake
MN0022080	Grand Rapids WWTP	87% Industrial 13% Domestic	15.2	23.52		1	Mississippi River - Grand Rapids
MN0031879	US Steel Corp – Keetac	Industrial	10.17	15.74	**64.8**	9	Leighton Lake
MN0030147	Winona WWTP	Domestic	9.6	17.84		6	Blue lake
MN0001465	Hibbing Taconite Co	Industrial	1.44 - 7.92	2.28 - 12.25		8	St. Louis River Mississippi River-Brainerd
MN0059633	ArcelorMittal Minorca Mine Inc - Laurentian	Industrial	7.9	12.22	**62.8** (Vermillion River), **274** (St. Louis River)	0	St. Louis River
MN0067687	Mesabi Nugget Delaware LLC	Industrial	7.29	11.28	**437**	7	Partridge River

Twelve major industrial dischargers identified through mapping sulfate concentrations in the Mississippi River between St. Cloud and Otsego and south of Minneapolis were not specified on the MPCA list of potentially affected dischargers. The table above that indicates the largest dischargers by volume and sulfate concentration are electrical utilities. Therefore, it is likely that some, if not all of these dischargers are major contributors to the excursions from the wild rice sulfate water quality standard and are potentially adversely impacting downstream wild rice waters.

Table 4. Major Industrial Dischargers on the Mississippi River between St. Cloud and Otsego Not Included in SONAR List of Potentially Affected Dischargers

Plant Name	Electric Utility Name	City	County	Primary Source	Source Description	Technical Description
Granite City	Northern States Power Co - Minnesota	St. Cloud	Benton	natural gas	Natural Gas = 52 MW	Natural Gas Fired Combustion Turbine
Elk River City of	City of Elk River	Elk River	Sherburne	petroleum	Biomass = 3.2 MW, Petroleum = 9 MW	Landfill Gas; Petroleum Liquids
Elk River	Great River Energy	Elk River	Sherburne	natural gas	Biomass = 34.8 MW, Natural Gas = 190.5 MW	Municipal Solid Waste; Natural Gas Fired Combustion Turbine
Sherburne County	Northern States Power Co - Minnesota	Becker	Sherburne	coal	Coal = 2238 MW	Conventional Steam Coal

Table 5. Major Industrial Dischargers South of Minneapolis on the Mississippi River Not Included in SONAR List of Potentially Affected Dischargers.

Utility Name	Sector Name	City	County	Primary Source	Source Description	Technical Description
Northern States Power Co - Minnesota	Electric Utility	St. Paul	Ramsey	natural gas	Natural Gas = 530 MW	Natural Gas Fired Combined Cycle
Northern States Power Co - Minnesota	Electric Utility	Inver Grove Heights	Dakota	natural gas	Natural Gas = 282 MW, Petroleum = 3.6 MW	Natural Gas Fired Combustion Turbine; Petroleum Liquids;
Northern States Power Co - Minnesota	Electric Utility	Minneapolis	Hennepin	natural gas	Natural Gas = 454 MW	Natural Gas Fired Combined Cycle
Northern States Power Co - Minnesota	Commercial Non-CHP*	St. Paul	Ramsey	petroleum	Petroleum = 4.8 MW	Petroleum Liquids
Cottage Grove Operating Services LLC	IPP CHP*	Cottage Grove	Washington	natural gas	Natural Gas = 251 MW	Natural Gas Fired Combined Cycle
Ziegler Power Systems	Commercial Non-CHP*	St. Paul	Ramsey	petroleum	Petroleum = 1.9 MW	Petroleum Liquids
Veolia Energy	Commercial CHP*	Minneapolis	Hennepin	natural gas	Natural Gas = 0.1 MW	Natural Gas Steam Turbine
Veolia Energy	IPP* CHP**	Minneapolis	Hennepin	natural gas	Natural Gas = 17 MW	Natural Gas Fired Combustion Turbine

*An independent **power** producer (**IPP**) or non-utility generator (NUG) is an entity, which is not a public utility, but which owns facilities to generate electric **power** for sale to utilities and end users.

Combined Heat and Power (CHP**) Combined heat and power (**CHP**) **systems**, also known as cogeneration, generate electricity and useful thermal energy in a single, integrated **system**. **CHP** is not a technology, but an approach to applying technologies.

Community wastewater treatment plants, or domestic dischargers, generally account for the smallest discharges by volume and sulfate concentrations. In fact, on average the volume of discharge water is six times less than industrial discharges and the concentration of sulfate from community waste water discharges are twenty times less concentrated than industrial discharges. The range of the volume of domestic discharges is 0.008 – 42 million gallons per day with an average discharge volume of 2.26 million gallons per day.

The average sulfate concentration of domestic discharges is 15.87 mg/L, with a range of 6.97 – 29.6 mg/L. Where data is available, it appears that domestic dischargers would not be required to provide sulfate treatment unless they discharge effluent to waters already exceeding the wild rice sulfate standard due to industrial discharges.

Table 6. SONAR Listed Domestic Dischargers With Sulfate Water Body Data Indicating Non-Compliance

Permit Number	Facility Name	Facility Type	Discharge MGD	Discharge CFS	Discharge waters	Distance to Wild Rice (miles)	Draft Wild Rice Name	Average Water Body Sulfate Concentration mg/l
MN0051381	Belgrade WWTP	Domestic	0.167	0.26	unnamed creek Middle Fork Crow River	3	Monongalia Lake	16.5 - Middle Fork Crow River @ Lake Monongalia
MN0053279	Biwabik WWTP	Domestic	0.212	0.33	Embarrass Unnamed wetland River	1	Cedar Island Lake	20.6 - Cedar Island Lake
MN0053562	Brownsville WWTP	Domestic	0.055	0.09	Mississippi River	1	Pool 8 at Reno Bottoms	18.1 - Pool 8 @ Reno
MN0022012	Keewatin WWTP	Domestic	0.18	0.28	Welcome Creek	11	Hay Lake	32.9 - Hay Lake
MNG580027	Kellogg WWTP	Domestic	0.06	0.09	Zumbro River	3	Mississippi Pool 5/Spring	32.5 - Mississippi Pool 5/spring
MN0020664	Lake City WWTP	Domestic	1.52	2.35	Lake Pepin	10	Mississippi Pool 4 Robinson Lake	29.6 - Pool 4 Robinson Lake
MN0029904	Met Council – Eagles Point WWTP	Domestic	10	15.47	Mississippi River	19	Sturgeon Lake	58.2 - Sturgeon Lake
MN0045845	Met Council – Empire WWTP	Domestic	28.61	44.27	Mississippi River	25	Sturgeon Lake	58.2 - Sturgeon Lake
MN0029955	Met Council – Hastings WWTP	Domestic	2.69	4.16	Mississippi River	14	Sturgeon Lake	58.2 - Sturgeon Lake
MNG580184	Nashwauk WWTP	Domestic	0.353	0.55	Hanna Reservoir #2	8	Hay Lake	28.4 - Hay Lake
MNG580215	Serpent Lake WWTP	Domestic	0.672	1.04	Rabbit Creek	6	Mississippi River	19 - Mahnomen Lake
MN0025143	Wabasha WWTP	Domestic	0.604	0.94	Mississippi Pool 4 Robinson Lake	0	Mississippi Pool 4 Robinson Lake	29.6 - Pool 4 Robinson Lake
MN0030147	Winona WWTP	Domestic	9.6	17.84	Mississippi River	6	Blue lake	36 above Winona 34 below Winona in Mississippi River

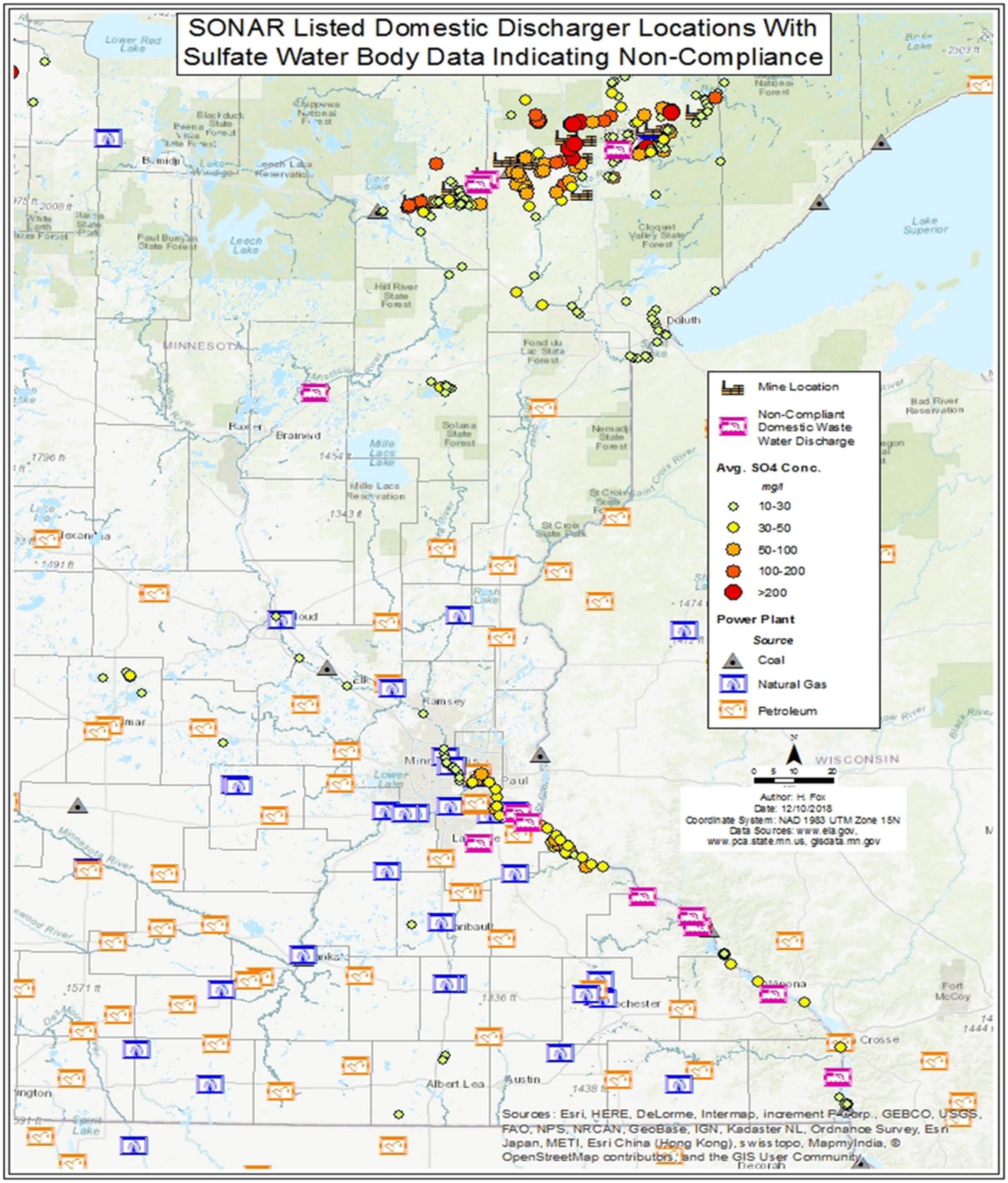


Figure 11. Sulfate Waterbody Data Indicating Non-compliance for SONAR Listed Domestic Dischargers

Table 7. SONAR Listed Domestic Dischargers With Sulfate Water Body Data Indicating Compliance

Permit Number	Facility Name	Facility Type	Discharge MGD	Discharge CFS	Discharge waters	Distance to Wild Rice (miles)	Draft Wild Rice Name	Average Water Body Sulfate Concentration mg/l
MN0020656	Babbitt WWTP	Domestic	0.5	0.77	Hay Lake	0	Hay Lake	6 - Hay Lake
MN0022691	Bagley WWTP	Domestic	0.26	0.41	unnamed wetland Walker Brook Clearwater River	16	Clearwater River	1.5 - Clearwater River
MN0022462	Bemidji WWTP	Domestic	2.5	3.87	Mississippi River	19	Andrusia Lake	2.6 - Ose Lake (3 mi. upstream of Andrusia Lake)
MN0023019	Carlos WWTP	Domestic	0.064	010	unnamed wetland	8	Long Prairie River	7.71 - Long Prairie Rv
MN0066371	Crane Lake WWTP	Domestic	0.053	0.08	Crane Lake	0	Crane Lake	6.1 avg - Crane Lake
MNG580181	Deer River WWTP	Domestic	0.17	0.26	unnamed wetlands	5	White Oak Lake	0.93 - White Oak Lake
MN0020508	Ely WWTP	Domestic	1.5	2.32	Shagawa Lake	5	Fall Lake	4.5 - Shagawa Lake
MN0022080	Grand Rapids WWTP	Domestic	15.2	23.52	Mississippi River	1	Mississippi River - Grand Rapids	Avg. 6 - Mississippi River @ Grand Rapids
MN0023566	Grey Eagle WWTP	Domestic	0.09	0.14	Trace Lake	4	Little Birch Lake	Avg. 5.3 - Little Birch Lake
MN0020869	Jordan WWTP	Domestic	1.29	1.99	Sand Creek	22	Blue Lake	6.9 - Fisher Lake (Blue Lake flows into Fischer Lake) 20+ miles downstream from Jordan
MNG580027	Kellogg WWTP	Domestic	0.06	0.09	Zumbro River	3	Mississippi Pool 5/Spring	Avg. 32.5 - Pool 5/spring
MN0024023	McGregor WWTP	Domestic	0.073	0.11	County ditch #42 Rice Lake Sandy River Steamboat Lake	2	Steamboat Lake	Avg 0.7 - Sandy River Lake (~5 mi N of Steamboat Lake)
MN0064777	Met Council – Blue Lake GW Relief System	Domestic	5.44	8.42	Blue Lake	0	Blue Lake	6.9 - Fisher Lake (Blue Lake flows into Fischer Lake)
MN0029882	Met Council – Blue Lake WWTP	Domestic	42	64.98	Minnesota River	0	Blue Lake	6.9 - Fisher Lake (Blue Lake flows into Fischer Lake)
MN0024155	Miltona WWTP	Domestic	0.008	0.12	unnamed wetland	8	Long Prairie River	7.71 Long Prairie River
MN0024422	Orr WWTP	Domestic	0.099	0.15	unnamed ditch Pelican River Pelican Lake	0	Vermilion River	5.68 - Vermillion River
MNG580187	Winton WWTP	Domestic	0.024	0.37	Shagawa River	2	Fall Lake	3.7 – Shagawa River 1.3 mi SW Winton

No sulfate data is available from MPCA for many of the domestic wastewater dischargers and some of the industrial facilities found on the SONAR list. MPCA was also not able to provide sulfate data for many of the wild rice waters found on the SONAR list.

Table 8. SONAR Listed Domestic Dischargers Without Sulfate Water Body Data Available

Permit Number	Facility Name	Discharge MGD	Discharge CFS	Discharge waters	Distance to Wild Rice (miles)	Draft Wild Rice Name
MNG580148	Audubon WWTP	0.14	0.22	unnamed ditch	No data	Buffalo River
MN0046213	Anchor Bay Mobile Home Park	0.01	0.01	unnamed ditch Rainy River	11	Rainy River
MN0029599	Baudette WWTP	0.24	0.45	Unnamed Stream to Rainy River	14	Rainy River
MNT022985	Callaway WWTP	0.042	0.065	unnamed ditch	No data	Buffalo River
MNG580098	Clearbrook WWTP	0.13	0.19	unnamed tributary	9	Clearwater River
MN0051101	Cromwell WWTP	0.052	0.08	Flower Lake via ditch	0	Flower Lake
MN0020192	Detroit Lakes WWTP	1.64	2.54	unnumbered wetland to peat bog St Clair Lake	12	Pelican Lake
MN0059871	East Gull Lake WWTP	0.14	0.22	Gull River	4	Gull River
MN0023451	Foley WWTP	0.16	0.25	unnamed marsh to Stoney Brook	13	Rice Lake
MN0023515	Garfield WWTP	0.05	0.08	County Ditch #23	2	Ida Lake
MN0025691	Grasston WWTP	0.04	0.06	Snake River	11	Snake River Bay
MN0023701	Hinckley WWTP	0.68	1.06	Grindstone River	4	Kettle River
MN0021458	Hokah WWTP	0.10	0.19	Root River	6	Miss. River Backwater
MN0023736	Houston WWTP	0.25	0.39	Root River	19	Miss. River Backwater
MNG580208	Longville WWTP	0.06	0.09	Unnamed wetland	3	Rice Lake
MNG580032	Menahga WWTP	0.11	0.17	Unnamed stream	7	Yaeger Lake
MN0020699	Moose Lake WWTP	0.50	0.77	Unnamed ditch to Moosehorn River	0	Moose Horn River
MN0021156	Mora WWTP	0.8	1.24	Snake River	2	Rice Creek
MN0024244	Motley WWTP	0.43	0.67	Crow Wing River	1	Placid Lake
MNG580209	Pillager WWTP	0.07	0.11	Crow Wing River	6	Crow Wing River
MN0046388	Pine River Area Sanitary District	0.25	0.38	Pine River Upper White Fish Lake	0	Pine River
MNG580211	Rich Prairie Sewer Treatment Facility	0.23	0.35	Skunk Creek	10	Rice Lake
MNG580213	Sandstone WWTP	0.335	0.5183	unnamed creek	7	Kettle River
MN0024988	Staples WWTP	0.68	1.05	unnamed swamp	16	Placid Lake
MN0064564	Tamarack WWTP	0.01	0.01	Unnamed wetland	12	Flowage Lake

Table 9. Rationale for Domestic Dischargers Removed from SONAR List

Permit Number	Facility Name	Facility Type	Discharge MGD	List Removal Rationale
MN0051381	Belgrade WWTP	Domestic	0.17	Discharge is used as spray irrigation on 3 sites: 130 acres, 39 acres, and 30 acres.
MN0020192	Detroit Lakes WWTP	Domestic	1.64	Discharge is used as spray irrigation over a total of 54 acres
MN0057410	Kettle Falls Hotel & Guest Villas	Domestic	0.01	Spray discharge to 0.63 acre wooded area.
MN0022811	Bigfork WWTP	Domestic	0.08	Rice Creek flows into the Bigfork River and therefore water from the Bigfork would have to flow upstream to impact Rice Creek.
MN0020206	Hoyt Lakes WWTP	Domestic	0.68	Water would have to flow uphill to get to the Partridge River from Whitewater Lake.
MN0020869	Jordan WWTP	Domestic	1.29	Water would have to flow uphill to get to Blue Lake from Sand Creek at Jordan.

Twenty-one industrial facilities were removed from the SONAR list for various reasons listed in the table below.

Table 10. Rationale for Industrial Dischargers Removed from SONAR List

Permit Number	Facility Name	Discharge MGD	Discharge waters	Draft Wild Rice Name	NPDES Permit Removal Rationale
MN0001309	Aggregate Industries – Nelson Plant	13	Mooers Lake (backwaters of Mississippi), Baldwin Lake (backwaters of Mississippi)	Sturgeon Lake	Water is pumped to a sedimentation basin where it percolates into the ground or evaporates. No discharge since 2008. Discharge would only be used as an emergency overflow. Process water is from Mississippi and no chemical additives are used. (permit pg. 3)
MNG250004	Alexandria Light & Power	0.012	Lake Winona	Long Prairie River	This discharge consists solely of once through non-contact cooling water to which the only pollutant added to it is heat. (permit pg. 6)
MN0001431	Sappi Cloquet LLC	0.464	St. Louis River	St Louis River	Authorized discharge consists of non-contact cooling water/industrial stormwater/treated Lake Superior water for St. Louis River augmentation. Does not authorize discharge of process water.(permit pg.12)
MNG255070	Tate & Lyle Ingredients Americas LLC	0.928	Unnamed ditch to St. Louis River	St Louis Estuary (2)	The discharge consists solely of once-through non-contact cooling water to which the only pollutants added are heat and chemical additives consistent with a municipal potable water supply. (permit pg. 6)
MNG250102	USG Interiors LLC – Cloquet	0.13	St. Louis River	St Louis River	The discharge consists solely of once-through non-contact cooling water to which the only pollutant is heat. (permit pg. 6)
MN0070564	Jordan Aggregates LLC	no quantity listed	Sand Creek	Blue Lake	Facility crushes, screens, and washes unconsolidated sand and gravel. The wastewater is routed to a recycling basin. No wastewater expected to leave facility. Stormwater will only leave the site after a two year flood event. (permit pg. 3)
MNG490140	St Louis County Highway Dept	no quantity listed	Various gravel pits and stone quarries	St. Louis River	Stormwater discharges from gravel pits, stone quarries, chrushed rock, concrete mixing, asphalt production. Permit also authorized non-stormwater discharges that do not discharge to surface water. (permit pg. 5)
MNG490177	St Louis County Land Department	no quantity listed	Various gravel pits and stone quarries	Vermilion River	Stormwater discharges from gravel pits, stone quarries, crushed rock, concrete mixing, asphalt production. Permit also authorizes non-stormwater discharges that do not discharge to surface water. (permit pg. 5)

MNG490069	Ulland Brothers Inc	no quantity listed	Various gravel pits and stone quarries	Cloquet River St. Louis River	Stormwater discharges from gravel pits, stone quarries, crushed rock, concrete mixing, asphalt production. Permit also authorized non-stormwater discharges that do not discharge to surface water. (permit pg. 10-11)
MN0000361	Wisconsin Central Ltd – Proctor Railroad Yard	no quantity listed	Kingsbury Creek	St Louis Estuary (2)	Authorized to discharge stormwater associated with industrial activities. (permit pg. 12)
MNG790128	Becker County Sanitary Landfill – Closed	no quantity listed	Unnamed wetland	Big Floyd Lake	Authorized to discharge VOC contaminated groundwater general permit requiring removal of 95% of VOC contamination or greater. (permit pg. 7)
MN0067024	Farmington City of GW Discharges	9	Vermillion River	Fisher Lake	Authorized for short-term seasonal discharge of contaminated groundwater. (permit pg. 2-3) Fischer Lake average sulfate concentration is below the 10 mg/l criteria.
MNG790199	Former Morris Oil Bulk Plant	no quantity listed	Shagawa Lake	Fall Lake	Authorized to discharge VOC contaminated groundwater general permit requiring removal of 95% of VOC contamination or greater. (permit pg. 8) Shagawa Lake average sulfate concentration is below the 10 mg/l criteria.
MN0041556	Calumet Superior LLC – Duluth Petroleum	no quantity listed	unnamed ditch to Mission Creek tributary	St Louis River Estuary	Authorized to discharge stormwater & water used for hydrotesting fuel storage tanks to secondary containment basins. Containment basins are discharged to a grassy area which *could* flow overland eventually reaching unnamed ditch. (permit pg. 3)
MN0052540	Great Lakes Gas Transmission LP	no quantity listed	various locations	Grant Creek	Authorized to discharge waters used to hydrotest pipelines and to dewater pipeline trenches within the permittees right-of-way to upland vegetated areas where possible. Occasional discharges to surface waters with BMPs to control sediment, suspended solids, and erosion. (permit pg. 3-4)
MN0056472	Minnesota Pipe Line Co	no quantity listed	various locations	Sturgeon Lake	Authorized to discharge waters used to hydrotest pipelines and crude oil tanks to well vegetated uplands using BMPs to prevent erosion, sediment transport, and bottom scouring. (permit pg. 3-4)
MN0050041	Northern Natural Gas Co	no quantity listed	various locations	St Louis River Estuary	Permit is for pipeline trench dewatering & to *request* authorization to discharge waters used to test new or existing pipeline structural integrity. (permit pg. 6-7)
MN0060755	Viking Gas Transmission	no quantity listed	various locations	Pelican Lake	Authorized to discharge waters used to hydrotest pipelines and to dewater pipeline trenches within the permittees right-of-way to upland vegetated areas where possible. Occasional discharges to surface waters with BMPs to control sediment, suspended solids, and erosion. (permit pg. 3-4)
MN0067377	Prior Lake Spring Lake Ferric Chloride WTP	no quantity listed	Unnamed Creek to Spring Lake	Blue Lake	This permit authorizes the facility to inject ferric chloride into unnamed creek for the purpose of reducing the phosphorus load reaching Spring Lake. As water passes through the desiltation basin, solid waste by-product (phosphorus flocculent) settles out. The iron flocculent and fine particles are land applied. (permit pg. 3)
MN0068241	Essar Steel Minnesota LLC	5.6	Ann pit Sullivan pit Drapper Annex pit Snowball lake Oxhide lake Pickerel creek	Ox Hide Lake	This project hasn't been fully built yet. Original MN Steel plans included Reverse Osmosis treatment so the facility would not be impacted by wild rice rule.
MN0001007	Minnesota Power – Boswell Energy Center	161.80	Pokegama Reservoir on Mississippi River	Blackwater Lake	Boswell Energy has court-ordered site specific criteria to protect wild rice.

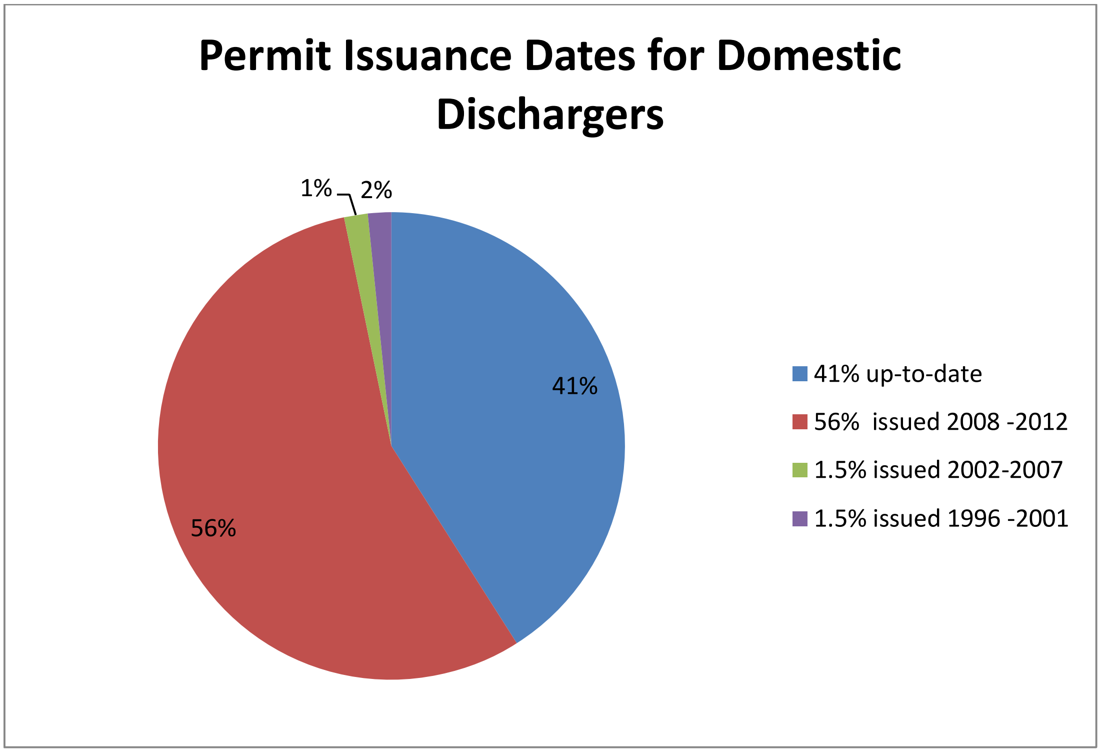


Figure 12. Domestic Dischargers NPDES Permit Issuance Dates

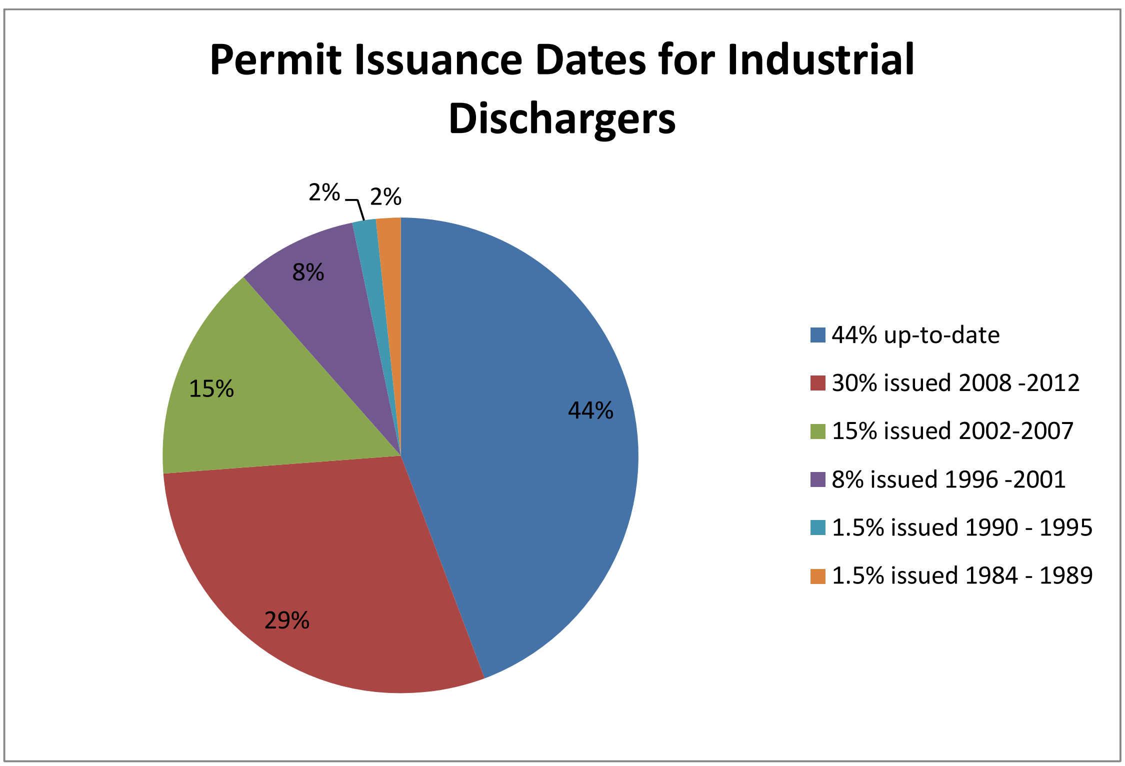


Figure 13. Industrial Dischargers NPDES Permit Issuance Dates

Comparing the proportion of up-to-date permits and those permits issued between 2008 -2012, domestic dischargers' permits comprise ninety-seven percent, demonstrating they are up-to-date or only a few years out of date. Reviewing the oldest two time categories for domestic dischargers indicate that only three percent were issued from 1984 - 2007. Reviewing industrial dischargers' up-to-date permits and those permits issued between 2008 -2012, seventy-three percent are up-to-date or only a few years out of date. Twenty-seven percent of industrial dischargers' permits were issued from 1984 - 2007. This demonstrates that domestic dischargers' are being held to higher permit compliance and/or oversight expectations by the MPCA.

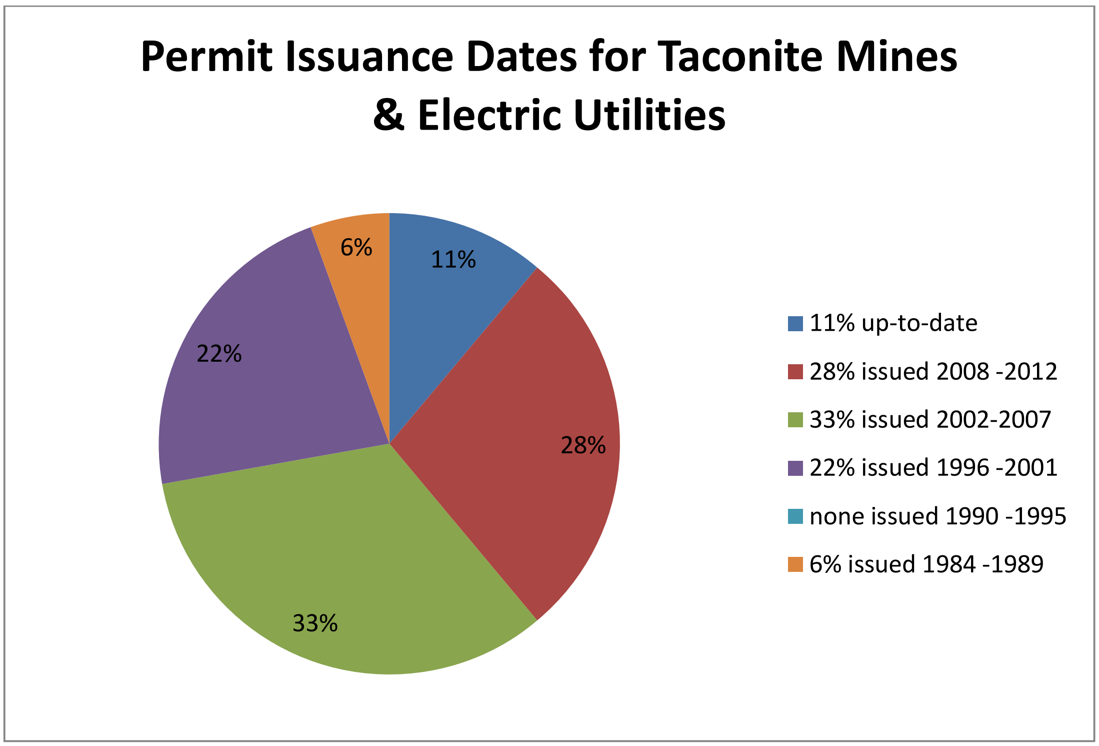


Figure 14. Taconite Mines and Electric Utility NPDES Permit Issuance Dates

By assessing the permit issuance dates for a subset of the industrial facilities, taconite mines and electric utilities included on the SONAR list, only thirty-nine percent are up-to-date or only a few years out of date. However, permits issued from 1984 – 2007 comprise sixty-one percent. This further demonstrates that even amongst industrial dischargers a reduced standard of oversight is applied to taconite and electrical utilities by MPCA. Yet, these are the largest by volume of industrial wastewater discharges and their discharges have the highest concentrations of sulfate.

Conclusions

Domestic dischargers receive more permitting oversight than much larger industrial dischargers. Where data is available, industrial facilities on average discharge six times more wastewater than domestic discharges. Average sulfate concentrations from industrial discharges are at least twenty times more concentrated than domestic discharges.

Table 11. Comparison of Industrial and Domestic Discharge Volumes and Sulfate Concentrations

Type of Facility	Discharge Volume Range (Million Gallons Per Day)	Average Discharge Volume (Million Gallons Per Day)	Average Sulfate Concentration Range (Milligrams per Liter)	Average Sulfate Concentration (Milligrams per Liter)
Industrial	0.0012 - 161.8	12.93	22.7 -1054	301.66
Domestic	0.0008 - 42	2.26	6.97 – 29.6	15.87

Virtually all of Minnesota waters that are not impacted by industrial discharges have sulfate concentrations below the 10 mg/L wild rice sulfate standard. Therefore, if industrial discharges were controlled in accordance with the law to meet Minnesota water quality standards, most domestic wastewater discharges would not require additional treatment to comply with the wild rice sulfate standard. Domestic dischargers that draw drinking water from source water where sulfate concentrations are elevated from industrial activities (e.g. mine pit lakes) could reduce the costs by treating potable water to reduce sulfate instead of adding treatment for wastewater. In addition to reducing costs, treating potable water would have community health benefits.

Comparison of Concentrations between southern and northern Minnesota

Sulfate is naturally higher in the SW part of the state, due to the history of glaciation in Minnesota. Glaciers moved from what are now parts of Canada and upper Minnesota, down and across Minnesota, scraping away large amounts of surface material and leaving behind this higher sulfate glacial till in the areas of SW MN. According to USGS & MNDNR, "*The high concentrations of sulfate in ground water in the west part of the State are probably caused by leaching of sulfate-rich minerals, such as gypsum and iron sulfide, from the drift. These were assimilated and later deposited here by glaciers that moved over Cretaceous [period]…sediments containing sulfate-rich minerals."* PIIC resides on the edge of the driftless region, an area of MN where the last period of glaciers never touched. Areas in MN where glaciers never reached during the last period still have naturally higher sulfate levels from pre-glaciation, such as the parts of SE MN where PIIC resides. USGS reports state in reference to both the St. Peter and Mount Simon-Hinckley aquifers that sulfate in the southwestern potions of the aquifer are naturally higher in sulfate because of the leakage from overlying Cretaceous deposits. This means that the SW portion of Minnesota has naturally higher sulfate levels in the groundwater. It is further important to note that groundwater concentrations of salts may be much higher and get diluted when mixed with surface water.

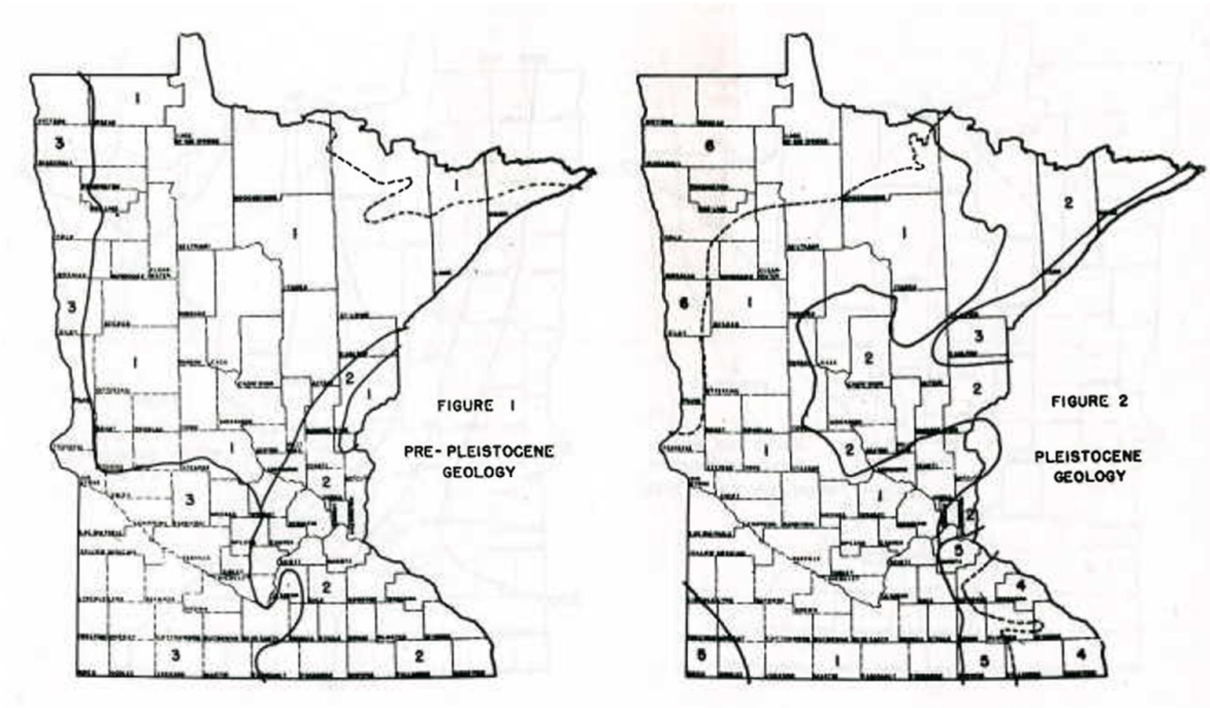


Figure above: Maps depicting geology of MN after last glaciation (Moyle, pg. 32)

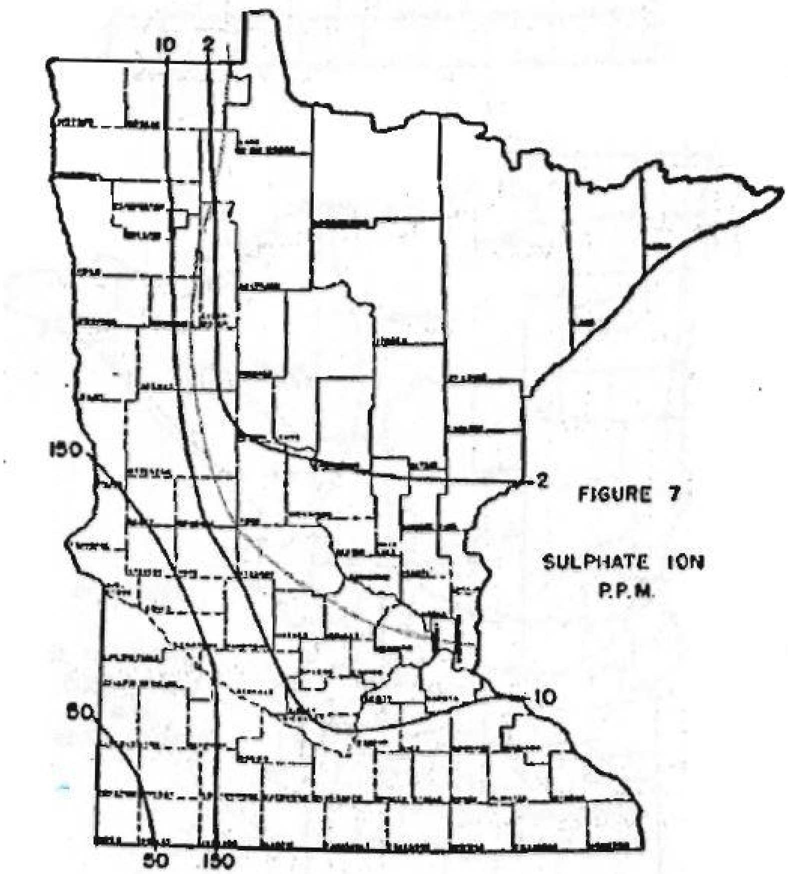


Figure above: Map depicting contours of surface water types in MN based on field measurements (Moyle, pg. 36).

The average surface water sulfate levels of Minnesota were mapped by Myrbo (2017) in a report using MPCA and DNR databases from current research on sulfate concentrations. The map below shows contours of predicted sulfate concentration in surface water using both actual and predicted measurements. Higher sulfate concentrations in southwest Minnesota are attributed to the glacial till deposits discussed previously.

However, in comparison with the map on pg. 44 depicting data from the Mississippi River, predicted sulfate concentrations don't entirely correspond to measured sulfate concentrations. The Mississippi River data shows higher sulfate concentrations in the range 30-50 mg/L in the area just north of, and running through, the Twin Cities. The predicted sulfate concentrations on the Myrbo map estimate this area should be between the 10-30 mg/L range. Records show wild rice grew, and in some places still grows, along the length of the Mississippi River.

However in comparison with the map on pg. 51 of this report depicting dischargers on or near the Mississippi River, there are some concerns about the high sulfate levels seen above and below the Twin Cities area where there are few remaining wild rice waters. Wild rice is not found to grow in the southwest portions of the state where sulfate concentrations are several hundred mg/L due to the naturally higher sulfate content in soils and surface water in that region.

Additionally, in looking at northern Minnesota on the Myrbo map evidence is seen of higher sulfate concentrations in the surface water in the iron range region. This region has sulfate bound along with the iron deposits. Undisturbed watersheds, with sulfate still bound in the glacial and bedrock geology, have low ambient sulfate concentrations. The disturbance of sulfate-rich lobes will cause higher sulfate concentrations to be evident in the surface water. Confirmation of this is shown in the following Myrbo map, where northern Minnesota with naturally low sulfate concentrations has a plume of higher sulfate concentration waters in areas surrounding industrial facilities that disturb the bedrock, releasing the sulfate trapped there.

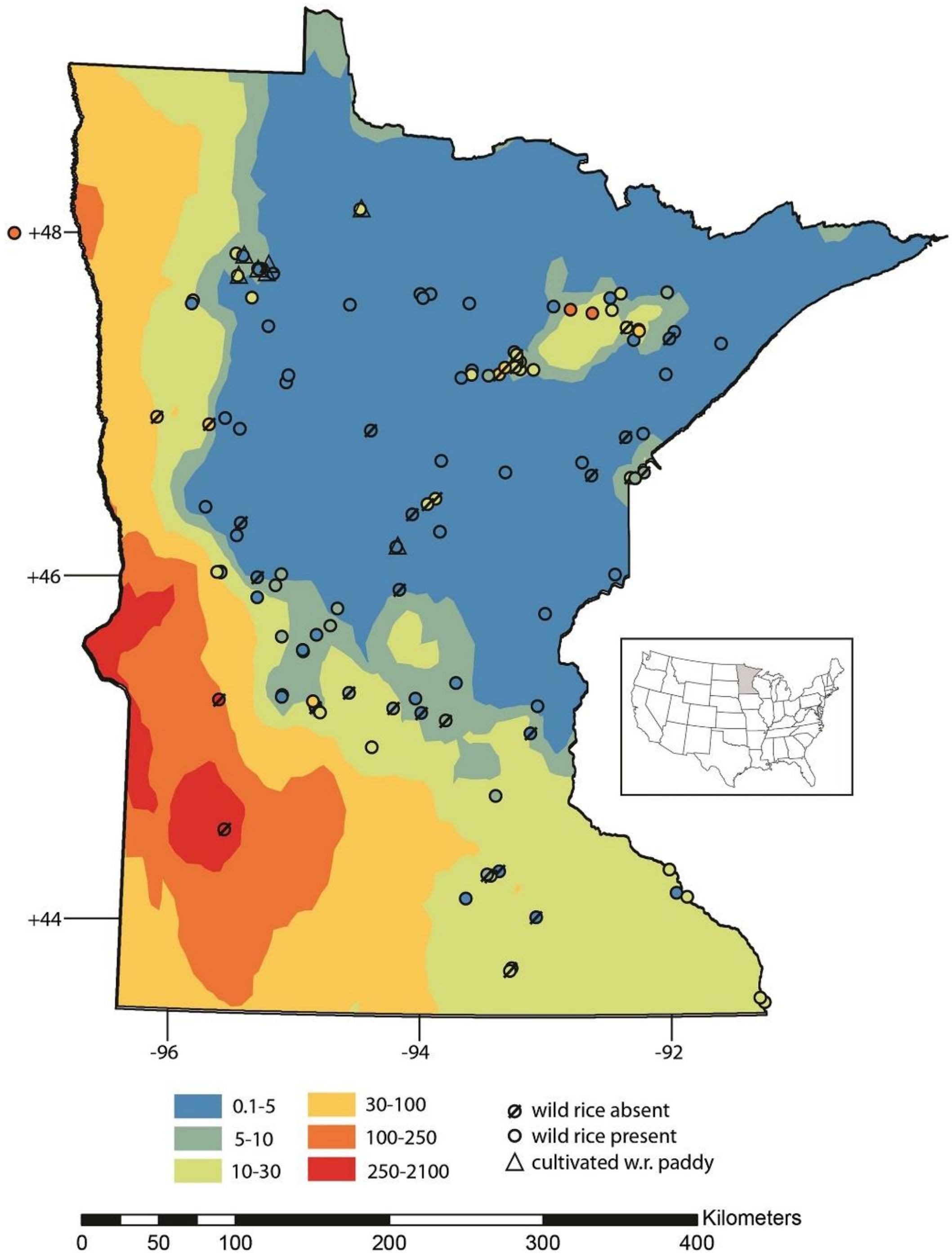


Figure above: Myrbo (2017) "Map of Minnesota showing field sites overlain on kriged contours of average surface water SO4 concentrations from 4,998 waterbodies (data from MPCA and DNR databases). The symbols are filled with the color corresponding to the site's surface water sulfate concentration. Site to the northwest of the Minnesota map is within the state of North Dakota, 40 km west of the border with Minnesota. Sites where wild rice was not found have a diagonal line through the symbol."

RECOMMENDATIONS

Per Minnesota Chippewa Tribe Resolution 107-18, *"the Tribal Wild Rice Task Force will review existing literature, including literature and information based on tradition, culture, and science, that is available to inform the understanding of the impacts of sulfate and other sulfur compounds on habitat conditions on wild rice, identify information gaps, make recommendations on priorities in a similar fashion to that included in Executive Order 18-08, and provide such report to the Governor by December 15, 2018."*

Recommendations are listed in bold, followed by description detailing the recommendation.

Widen the beneficial use of wild rice to include cultural and ecological values. Supporting materials such as the Statement of Need and Reasonableness (SONAR) and the Technical Support Document describe the beneficial use of wild rice as "the harvest and use of grains from wild rice as a food source for wildlife and humans." The scope of this beneficial use is too narrow. Wild rice provides a broad spectrum of services including cultural (importance to tribes and others) and ecological (fishery habitat, water quality, etc.) functions. The way that this can be accomplished for each agency is through MPCA including the wild rice designated use in Class 2 "aquatic life use" and the MNDNR providing a special designation for wild rice, similar to protections for trout streams and calcareous fens.

Include all waters identified by the Tribes, MNDNR, and MPCA as wild rice waters where the standard would apply. The MPCA has done a great job utilizing all information sources to compile a list of wild rice waters. However, the rule it proposed chose to omit approximately 1,000 wild rice waters out of the 2,300 on the list. Unless long-term monitoring data indicates otherwise, all waters on this list should be considered a wild rice water where the wild rice water quality standard applies. The list of wild rice waters should be inclusive instead of exclusive.[1]

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[1] **This recommendation addresses EO 18-08 question a)**

Adopt a more comprehensive wild rice monitoring, assessment, and mapping strategy. Regulatory agencies should promote and advocate for a comprehensive and protective regulatory framework specifically for wild rice waters. A concerted and coordinated effort should be implemented among state, tribal and federal agencies to inventory all existing Minnesota wild rice waters. A coordinated and standardized approach for assessing the condition of wild rice water in Minnesota should also be implemented. Wild rice waters suffer from many risks including hydrological alterations, runoff, fragmentation, lakeshore development, and infrastructure development. These risks need to be quantified and explored so we are proactive in protecting wild rice waters. We recommend using the MN Sea Grant and University of MN "Wild Rice Monitoring Handbook" protocol among state and Tribal agencies.[2] (http://www.seagrant.umn.edu/downloads/sh016.pdf)

Adopt process for adding wild rice waters to list. No effort at identifying wild rice waters is perfect, and new information will feed into this effort. A straight forward and scheduled process for adding waters must be developed and implemented. This should be a collaborative process between tribal and state agencies. At a minimum, additions to the list could be made during the MPCA triennial review process.[3]

Communicate directly with each affected Tribal Government to determine their decision on listing wild rice waters within reservation boundaries. The MPCA has stated that it will not list waters within reservation boundaries if specifically requested by a tribe. Given the sovereignty of each tribe and their jurisdiction over reservation waters, a formal consultation process is required.[4]

Implement and enforce wild rice water quality standard. The current wild rice standard of 10 mg/L sulfate remains in place, but has not been enforced as required by the Clean Water Act. Existing water quality standards must be met and enforced. Regardless of what standard is in place, implementation is the key to preserve and protect wild rice. Previous state legislation that restricts state implementation of upholding the wild rice water quality standard should be rescinded.

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[2] This recommendation addresses EO 18-08 question a) and b)
[3] This recommendation addresses EO 18-08 question a)
[4] This recommendation addresses EO 18-08 question a)

Examine and invest in sulfate reduction research and treatment technologies. Progress towards and ultimately compliance with the water quality standard must be accomplished. We are not opposed to economic development, but environmental standards must be met and enforced.[5]

Establish long-term funding. To accomplish long-term monitoring of wild rice waters, it is necessary to secure adequate long-term funding from general funds for both the MPCA and MNDNR. Additionally, a list of existing funding sources pertaining to wild rice should be created in order to draw from these sources if necessary. However, long-term funding should not rely on grants, as a steady funding stream is necessary to prioritize wild rice protection, management, and restoration.[6]

Seasonal or "flushing" discharges of sulfate should not occur. We agree with the MPCA proposed approach of allowing no seasonal discharge of elevated sulfate, as is allowed in the existing standard. Science has demonstrated that a seasonal application of the standard is not protective. However, the proposed approach that the calculated numeric standard be implemented as an annual average raises concerns. Dischargers could potentially "flush" their systems and release high concentrations of sulfate during certain times of the year, and attempt to reduce or stop discharges during other times. This essentially could function as a seasonal discharge. Annual average sulfate concentrations and permit requirements may be met, but concerns would exist about whether the spirit of the standard is being met and if wild rice and other resources are being adequately protected.

Recognize the value of wild rice and a healthy environment. The state's economic analysis only looks at one side of the equation, namely the economic costs to the regulated community. It does not assign value (or gives a value of zero) to clean water, healthy wild rice, reduced mercury in fish, and health and cultural benefits. These values are immeasurable and can be hard to quantify, but must be considered in regulatory decisions. Documents referenced in this report can be utilized to inform these decisions.

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[5] **This recommendation addresses EO 18-08 question e)**

[6] **This recommendation addresses EO 18-08 question e)**

Address impaired waters of Minnesota. The MPCA maintains a list of impaired waters that do not meet water quality standards in the state. This list is updated and submitted to the USEPA every two years. Wild rice waters impaired from the sulfate standard have not been included to date. Impacted wild rice waters should be added to the Minnesota's impaired waters list, and activities should be implemented to remove impairments. Addressing other impairments will also improve other water quality issues that may be impacting wild rice waters.[7]

Recognize and support tribal sovereignty, culture, and treaty rights. Tribal sovereignty must be recognized, and proper consultation needs to occur on issues impacting natural resources and tribal populations. Tribal culture, and the importance of resources such as wild rice, must be appreciated and respected. Many Bands have signed treaties with the United States retaining rights to hunt, fish, and gather. Treaty rights are the supreme law of the land, and must be recognized and upheld.
For these rights to be exercised, wild rice and other resources must be available (protected and enhanced) to be utilized.

PRESERVE AND PROTECT MANOOMIN/PSIŊ/WILD RICE FOR FUTURE GENERATIONS.

[7] **This recommendation addresses EO 18-08 question b)**

APPENDIX

Appointed members of the Tribal Wild Rice Task Force (by their respective governments):

Deb Dirlam, Director of Environmental Programs, Lower Sioux Indian Community
Justice Wabasha, Environmental Technician, Lower Sioux Indian Community
Margaret Watkins, Water Quality Specialist, Grand Portage Band of Lake Superior Chippewa
John Morrin, Tribal Council Representative, Grand Portage Band of Lake Superior Chippewa
Tara Geshick, DNR Director, Bois Forte Band of Chippewa
Darren Vogt, Resource Management Division Director, 1854 Treaty Authority (representative for Bois Forte Band of Chippewa)
Nancy Schuldt, Water Projects Coordinator, Fond du Lac Band of Lake Superior Chippewa
Thomas Howes, Natural Resources Manager, Fond du Lac Band of Lake Superior Chippewa
Richard Robinson, DRM Director, Leech Lake Band of Ojibwe
Ben Benoit, Environmental Director, Leech Lake Band of Ojibwe
Bradley Harrington, Commissioner of Natural Resources, Mille Lacs Band of Ojibwe
Kelly Applegate, Wildlife Biologist, Mille Lacs Band of Ojibwe
Monica Hedstrom, Natural Resources Director, White Earth Nation
William Bement, Water Division Manager, White Earth Nation
Leya Charles, Water Resources Specialist, Prairie Island Indian Community

Other contributors:

Brandy Toft, Environmental Deputy-Director, Leech Lake Band of Ojibwe
Natalie Boyd, Environmental Technician, Mille Lacs Band of Ojibwe
Tony Swader, Trust Land Administrator, Grand Portage Band of Lake Superior Chippewa
Richard Jackson, GAP Coordinator, White Earth Nation
Michael Northbird, Environmental Program Manager, Minnesota Chippewa Tribe

Arthur Lockwood, Dakota Language Instructor, Prairie Island Indian Community
Franky Jackson, Tribal Historic Preservation Officer, Prairie Island Indian Community
Lars Lidahl, Environmental Technician, Prairie Island Indian Community
Heather Fox, GIS Specialist, Grand Portage Band of Lake Superior Chippewa

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Complete list of Wild Rice Waters developed by MPCA is ***Attachment 5*** in this report

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Pertinent Tribal and State Correspondences with the Governor (2014-2018)
Attachment 1A

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Deloria, E. (1967). Museum News: The W. H. Over Dakota Museum. *University of South Dakota*, pg. 10-12 ***Attachment 3A***

Legislative Rules (2015, 2016, 2017) ***Attachment 4A***

Complete list of Wild Rice Waters developed by MPCA (Oct. 2017) ***Attachment 5A***

PUBLIC COMMENTS

Nancy Beaulieu, Leech Lake Band - when task forces get together we need to protect the issue from all threats. The TWRTF should be considering other pollutants and threats that affect our sacred wild rice. The TWRTF should expand the focus of their task at hand. Reports regarding wild rice should be inclusive and considerate of the importance of protecting it. Effects of climate change should be a part of the overall report. (11/28/18 Open meeting, Mille Lacs Grand Casino)

Michael Connor, Bois Forte - wild rice is not just a substance to eat, it builds relationships within different age group of a community. It's educational, we learn from each other, and all people can relate to the importance of maintaining protections of our culture and history. The diversity of the natural world that depends on this important issue as a long-standing relationship that we all have, from macro-invertebrates to all other species. (11/28/18 Open meeting, Mille Lacs Grand Casino)

Perry Bunting, Mille Lacs - the TWRTF should clarify what the 10mg/L standard really means. That it relates to the sulfate levels of water bodies and not the "end of the pipe". (11/28/18 Open meeting, Mille Lacs Grand Casino)

Debra Topping, Fond du Lac - a baseline, in regards to all pollutants in the lakes within our reservations and treaty-ceded territories, should be included in the report. (11/28/18 Open meeting, Mille Lacs Grand Casino)

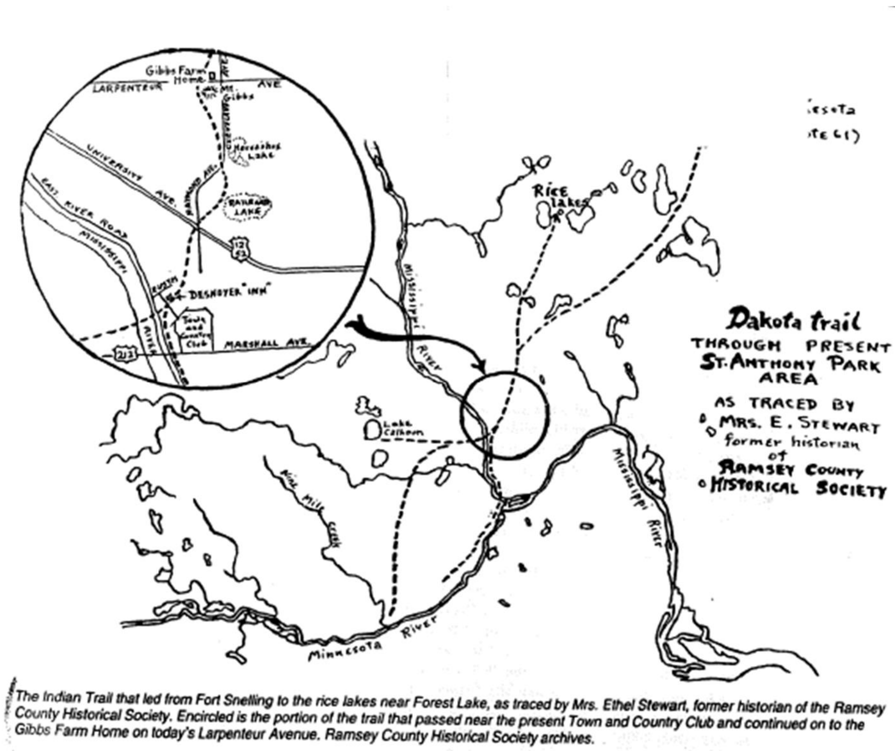
Nicole Buck, Prairie Island Indian Community - I work in land and environment as tribal garden assistant and work with our food sovereignty. Today I'm writing a letter in regards to the growth and production of protecting our wild rice. Wild rice is not only a huge part of my diet but many of our people as well. Wild rice plays many spiritual and physical roles to the Dakota people. From high nutrition for the nourishment of our bodies to the spiritual essence of our ceremonies. Wild rice has been a huge part of our diet prior to colonization, it connects to the land and water ways. Currently as we speak Prairie Island does not have viable wild rice for harvest for our people we have to get it from other tribes in the Northern Territory. Being able to grow and harvest our own wild rice for our people would help us strive with our food sovereignty. My health depends on sustainable wild rice so I hope this letter of support helps us, the people of Prairie Island get a voice in this crucial matter on wild rice. (11/26/18 Email received)

Tina Jefferson, Prairie Island Indian Community - I hope this letter finds its way to a greater cause in protecting our natural resources. In keeping with our traditions and understanding the dilemmas that the dams have created on our waterways on the Mississippi River bottom, we once had a population of wild rice. Since flooding has been prevalent on Prairie Island and decimates our abilities to grow a sustainable crop of wild rice and control of water quality! We have been forced to rely on our other Minnesota Native communities in northern Minnesota to supply our demand for our traditional wild rice and fresh walleye! I am in total support of our communities working together to make this a sustainable food source to our people and our traditions! Though we are not there physically there are many of us that use rice as a staple in our homes and it would be a shame not to have this resource available to us as a people! My father Joseph Campbell worked with and headed many organizations for the condition of our mighty Mississippi and down river alliance! (11/27/18 Email Received)

Cheyanne St. John, Lower Sioux Indian Community -

The Bdewakantunwan Dakota have long been known for their knowledge of harvesting and depending on wild rice. The food source is a staple in a long-existing traditional lifeway, many Dakota elders still make annual pilgrimages to the northern lakes of Minnesota to harvest wild rice, or *pśin*.

As stated by both Prairie Island Dakota Community and Lower Sioux's Office of Environment, numerous historic accounts detail the utility and significance wild rice has to Dakota people as early on as 1600. The image below identifies the Minnesota trails Santee Dakota took to access ricing areas, both Cloudman and Wabasha's Village sites were once situated in areas near present day Minneapolis. Dakota's from both villages actively harvested wild rice in lakes as near as the reclaimed Bde Maka Ska.



A Study of Wildrice in Minnesota. Edman, Robert F. Minnesota Resource Commission (1969)

Lower Sioux's Tribal Historic Preservation Office has conducted numerous interviews will Dakota elders and spiritual leaders over the past decades capturing oral interviews, community histories and landscape/site knowledge. After assessing the responses pertaining specifically to where Lower Sioux/Mdewakanton harvested wild rice most elders replied, "historically, the Dakota of Lower Sioux went north until they reached the furthest south lake and harvested from there."

Overtime the advancement and progression of industry and agriculture resulted in many southern MN waterbodies being drained or tiled, presumably destroying historic-Dakota ricing areas.

Lower Sioux Indian Community is concerned about the potential impact of infrastructure development on the natural resources we depend on for medicinal, cultural, and economic purposes. These concerns extend to proposals and/or permits that might have long-lasting impacts on LSIC's resources.

LSIC wants to prevent environmental degradation and environmental harm in all areas of our ancestral homelands. We do not support projects or policy that risk traditional foods being demolished, poisoned or altered. Wild rice areas (water tributaries, water bodies and adjacent streams) should remain protected and pristine for future access, harvest and establishment.

The State of Minnesota is responsible for issuing many of the permits necessary for infrastructure development to proceed, such as the crude oil Line 3 pipeline. LSIC needs this task force to advocate and evaluate the potential impacts on Treaty rights and our natural resources to ensure the sustainability of pśin for future generations.

On behalf of Lower Sioux Indian Community of Minnesota, we appreciate the opportunity to provide these comments.

Pidamaya ye,

Cheyanne St. John

Cheyanne St. John, THPO/Cultural Dept. Director
Lower Sioux Indian Community
(12/04/2018 Email Received)

Janice Erickson, Prairie Island Indian Community – My name is Janice Erickson. I am an enrolled Tribal member. My husband and my 5 children are all Tribal members too. Our family, friends, & community are connected to Wild Rice for many reasons. The most important reason is we regularly eat wild rice as a part of our natural diet. Our ancestors have been doing the same for countless generations! We also use our wild rice by culture and ceremonies. It is a part of who we are as a people. I am writing this to voice my concern that we need ensure our water is kept clean. The wild rice is dependent on it. It cannot grow or thrive in dirty water. People in general cannot grow or thrive in dirty water! It's really awful that mines aren't cleaning up their waste. Their pollution is deadly & hurting us all. Please make sure your report will fight for what is right! Our future, & future generations are counting on you!
(12/12/2018 Email Received)

AGU PUBLICATIONS

JGR

Journal of Geophysical Research: Biogeosciences

RESEARCH ARTICLE

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Key Points:

- Sulfate addition increased organic matter mineralization in wetland sediment, releasing C, N, P, and Hg to the water column
- Sulfate reduction caused not only higher methylmercury concentrations but higher total mercury concentrations in the surface water
- Increased sulfate loading to freshwaters can cause deleterious effects separate from direct sulfide toxicity to organisms

Supporting Information:

- Supporting Information S1
- Figure S1
- Data Set S1

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Increase in Nutrients, Mercury, and Methylmercury as a Consequence of Elevated Sulfate Reduction to Sulfide in Experimental Wetland Mesocosms

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Abstract Microbial sulfate reduction (MSR) in both freshwater and marine ecosystems is a pathway for the decomposition of sedimentary organic matter (OM) after oxygen has been consumed. In experimental freshwater wetland mesocosms, sulfate additions allowed MSR to mineralize OM that would not otherwise have been decomposed. The mineralization of OM by MSR increased surface water concentrations of ecologically important constituents of OM: dissolved inorganic carbon, dissolved organic carbon, phosphorus, nitrogen, total mercury, and methylmercury. Increases in surface water concentrations, except for methylmercury, were in proportion to cumulative sulfate reduction, which was estimated by sulfate loss from the surface water into the sediments. Stoichiometric analysis shows that the increases were less than would be predicted from ratios with carbon in sediment, indicating that there are processes that limit P, N, and Hg mobilization to, or retention in, surface water. The highest sulfate treatment produced high levels of sulfide that retarded the methylation of mercury but simultaneously mobilized sedimentary inorganic mercury into surface water. As a result, the proportion of mercury in the surface water as methylmercury peaked at intermediate pore water sulfide concentrations. The mesocosms have a relatively high ratio of wall and sediment surfaces to the volume of overlying water, perhaps enhancing the removal of nutrients and mercury to periphyton. The presence of wild rice decreased sediment sulfide concentrations by 30%, which was most likely a result of oxygen release from the wild rice roots. An additional consequence of the enhanced MSR was that sulfate additions produced phytotoxic levels of sulfide in sediment pore water.

Plain Language Summary In the water-saturated soils of wetlands, which are usually anoxic, decomposition of dead plants and other organic matter is greatly retarded by the absence of oxygen. However, the addition of sulfate can allow bacteria that respire sulfate, instead of oxygen, to decompose organic matter that would not otherwise decay. The accelerated decay has multiple consequences that are concerning. The bacteria that respire sulfate "breathe out" hydrogen sulfide (also called sulfide), analogous to the conversion or respiration of oxygen to CO_2. Sulfide is very reactive with metals, which makes it toxic at higher concentrations. In addition to the release of sulfide, the sulfate-accelerated decomposition of plants releases phosphorus and nitrogen, fertilizing the waterbody. Decomposition also mobilizes mercury (which is everywhere, thanks to atmospheric transport) into the surface water. The microbes that convert sulfate to sulfide also methylate mercury, producing methylmercury, the only form of mercury that contaminates fish. This study demonstrates that adding sulfate to a wetland can not only produce toxic levels of sulfide but also increase the surface water concentrations of nitrogen, phosphorus, mercury, and methylmercury.

1. Introduction

Organic matter (OM) accumulates in the sediments of aquatic systems when sediment concentrations of terminal electron acceptors (TEAs) are too low for microbes to completely decompose OM, especially when the supply of the most energy-efficient TEA, oxygen, is low. In water-saturated, organic-rich sediment, microbial sulfate reduction (MSR) can be a dominant pathway for the respiration of OM because oxygen is depleted in the uppermost sediment (Boye et al., 2017). Dissolved sulfate (SO_4) concentrations in continental surface waters are often low (less than 50 mgL^{-1} or 0.5 mmol L^{-1}) (e.g., Gorham et al., 1983) compared to ocean concentrations (2,800 mg L^{-1} or 29 mmol L^{-1}). Because of lower SO_4 concentrations, and because MSR rates can be limited by SO_4 concentrations (Holmer & Storkholm, 2001), the biogeochemical significance of MSR is often considered minimal in freshwater and low-salinity systems (e.g., Capone & Kiene, 1988; Nielsen et al., 2003; Stagg et al., 2017). However, absolute rates of MSR are not clearly lower in freshwater systems than in marine systems (Pallud & Van Cappellen, 2006), and in some cases, rapid cycling between oxidized and reduced forms of S can occur (Hansel et al., 2015).

In this study, we investigated the cascade of biogeochemical effects associated with increased MSR that result from increased surface water SO_4. We simultaneously quantified three different categories of biogeochemical responses related to MSR: (1) mineralization of organic matter and associated release of dissolved C, N, P, and Hg; (2) methylation of Hg; and (3) production of sulfide.

The stoichiometric release of the constituents of OM during MSR, notably C, N, and P, is a phenomenon long recognized by marine scientists. For instance, Boudreau and Westrich (1984) constructed a model of the MSR-mediated decomposition of marine sediment. They showed that SO_4 is reduced to sulfide (H_2S) in stoichiometric proportion to the mineralization of C, N, and P according to the reaction

$$2(CH_2O)_x(NH_3)_y(H_3PO_4)_z + xSO_4{}^{2-} \rightarrow 2xHCO_3{}^{-} + xH_2S + 2yNH_3 + 2zH_3PO_4 \quad (1)$$

C is released as both dissolved inorganic carbon (DIC, from complete oxidation, produced as bicarbonate alkalinity in stoichiometric proportion to sulfide (reaction (1); Boudreau & Westrich, 1984)) and dissolved organic carbon (DOC, from partial oxidation). The nutrients N and P are released in forms that are readily taken up by plants; N is released as ammonia, and P as phosphate. The mineralization of sediment organic matter associated with MSR releases sulfide (S^{2-}) into sediment pore water, which speciates, depending on the pH, into hydrogen sulfide (H_2S) and bisulfide (HS^{-}), henceforth collectively termed sulfide. If reduced S compounds accumulate in the sediment, there may be additional consequences to an aquatic system, such as toxic concentrations of sulfide in pore water (Lamers et al., 2013; Pastor et al., 2017; Myrbo et al., 2017) or conversion of sediment Fe(III) to FeS compounds, which enhances the mobilization of P (Curtis, 1989; Maynard et al., 2011).

The multiple biogeochemical consequences of MSR in freshwater systems have been investigated and documented in more than two dozen publications (Table S1 in the supporting information), which typically address a single issue, such as the production of alkalinity that neutralizes atmospherically deposited H_2SO_4 (Baker et al., 1986; Cook et al., 1986; and others) or the methylation of Hg (Gilmour et al., 1992; Branfireun et al., 1999, 2001; and others). Experimental studies addressing SO_4 reduction, sulfide production, associated OM mineralization, and release of nutrients have been broader (Lamers et al., 2001, 2002; Weston et al., 2006, 2011; and others), but aside from the results reported in this paper, only the experiments of Gilmour, Krabbenhoft, et al. (2007) and Gilmour, Orem, et al. (2007) have investigated all three categories of biogeochemical consequences of SO_4 reduction: OM mineralization, Hg methylation, and sulfide accumulation (Table S1). We also investigated the potential for Hg to be released by mineralization, a phenomenon proposed by Regnell and Hammar (2004).

Sulfate-driven enhanced mineralization of sediment OM and release of dissolved sulfide, N, P, DOC, DIC, and associated increases in alkalinity and pH have the potential to change the nature of an aquatic ecosystem. The immediate release is to the sediment pore water, but these dissolved materials can diffuse into the surface water. Increased internal loading of N and P can drive a system toward eutrophy, which can increase carbon fixation and amplify the cascade of biogeochemical effects associated with increased MSR. Increases in DOC also have the potential to fundamentally change the nature of a waterbody. DOC influences many processes in freshwater ecosystems, including light availability for macrophyte growth, thermal stratification, and bioavailability of metals, P, and C. In addition, DOC interferes with drinking water purification (Williamson et al., 1999). Increases in DIC, alkalinity, and pH can also change the nature of a system. Aquatic macrophyte and algal species often have different optimal alkalinity concentrations (e.g., Moyle, 1945; Vestergaard & Sand-Jensen, 2000), so increases in alkalinity may change aquatic community composition. Because pH is a master variable in aquatic systems (Stumm & Morgan, 2012), increases in pH can cause changes in both aquatic chemistry and the biota that dominate a system, as best documented by changes in diatom assemblages (Patrick et al., 1968).

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The release of sulfide into sediment pore water has multiple biological and geochemical consequences, several of which are related to the reactivity of sulfide with metals. If dissolved sulfide accumulates in pore water, it can negatively affect multicellular organisms inhabiting the sediment because sulfide can denature a range of metal-containing biomolecules, including cytochrome C oxidase, which is essential for respiration by both animals and plants (Bagarinao, 1992). Because aquatic sediment is a primary site of sulfide production, plants that root in sediment are vulnerable to toxic sulfide concentrations (Lamers et al., 2013; Pastor et al., 2017). However, if the watershed supplies sufficiently high loading of reactive Fe or other metals to the sediment, pore water sulfide concentrations may stay below toxic levels even while MSR proceeds as an important mineralization process (Pollman et al., 2017). The formation of FeS compounds effectively detoxifies sulfide (e.g., Marbà et al., 2007; Van der Welle et al., 2007). When Fe availability exceeds the production of sulfide, the accumulation of FeS is a measure of cumulative SO_4 reduction, which can be quantified as acid-volatile sulfide (AVS) (Heijs & van Gemerden, 2000). In addition, phosphorus is mobilized when oxidized Fe compounds with significant capacity to bind phosphate are converted to FeS compounds, which are incapable of binding phosphate (Lamers et al., 1998; Maynard et al., 2011). Thus, MSR mobilizes P both by mineralization of P-containing OM and by changing the form of Fe in sediment.

In addition to releasing C, N, and P, producing potentially toxic concentrations of sulfide, and reducing the solubility of metals, MSR is a primary process leading to the formation of MeHg, the bioaccumulative form of Hg (Gilmour et al., 1992; Hsu-Kim et al., 2013), although other microbial groups can also methylate Hg (Podar et al., 2015). In some cases, MSR can lead to toxic levels of MeHg higher in the food chain. The relationship between SO_4 concentrations and MeHg production is complex, however, and both field and laboratory studies in freshwater and saline ecosystems suggest that there is a dual effect of S on Hg methylation. At low SO_4 concentrations, the addition of SO_4 can stimulate MSR and Hg methylation (Jeremiason et al., 2006). At higher SO_4 concentrations, a greater abundance of inorganic sulfide appears to decrease the availability of inorganic Hg for Hg methylation (Hsu-Kim et al., 2013; Johnson et al., 2016). Because it has been observed that low SO_4 additions often increase Hg methylation and higher SO_4 concentrations decrease methylation, it has been proposed that there is a range of SO_4 and sulfide concentrations are optimal for Hg methylation, above which methylation is inhibited (Hsu-Kim et al., 2013). There is some debate regarding the underlying mechanism, but there is substantial evidence suggesting that dissolved inorganic sulfide above concentrations of 300–3,000 $\mu g\ L^{-1}$ has an inhibitory effect on Hg methylation (Bailey et al., 2017).

This study presents results from 30 wetland mesocosms in which the surface waters were treated to maintain a wide range of SO_4 concentrations over the course of 5 years (2011–2015) to assess the impact on wild rice, *Zizania palustris* (Pastor et al., 2017). We took advantage of this experiment to analyze the geochemical conditions in surface and pore water in the mesocosms during late summer 2013, 3 years into the experiment. Pastor et al. (2017) specifically examined the effect of increased SO_4 loading on wild rice, whereas this paper examines the broader biogeochemical impact of augmenting SO_4 to a low-SO_4 system.

2. Materials and Methods

2.1. Experimental Design

The experimental setup (Figure S1 in the supporting information), described in detail by Pastor et al. (2017), consisted of thirty 375 L polyethylene stock tanks containing sediment from a wild rice lake (Rice Portage Lake; +46.6987°, −92.6886°) in which wild rice was grown in self-perpetuating populations at five SO_4 treatment levels (control, 50, 100, 150, and 300 mg L^{-1}). SO_4 concentrations in six replicate mesocosms were routinely monitored, and amendments of SO_4 were added as Na_2SO_4 during the growing season as SO_4 was removed by MSR (Figure 1). Due to MSR, the mesocosm surface waters actually had time-weighted average concentrations of 7, 27, 59, 93, and 207 mg L^{-1}, respectively. Local well water containing an average of 10.6 mg L^{-1} SO_4 was added as needed to compensate for evapotranspiration. Precipitation in the region contains an average of 2.1 mg L^{-1} SO_4, and Rice Portage Lake has an average SO_4 concentration of 2.2 mg L^{-1} (Fond du Lac Band, 2016), so the control was slightly elevated above the ambient SO_4 concentration of the sediment source for the experiment. During the ice-free period (generally May through October), the surface water temperature (T) measured in the morning was correlated with the previous day's mean air temperature (mesocosm T = 0.72 air T + 4.4 °C; R^2 = 0.65). Peak air temperature is reached in July, when the average

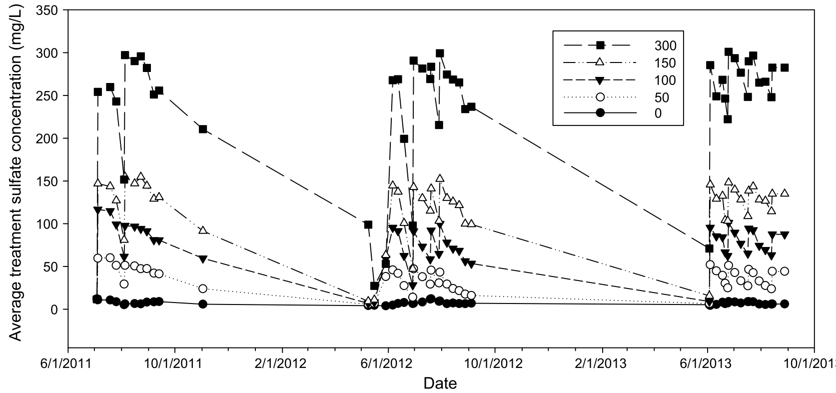

Figure 1. SO_4 concentrations in surface waters of each treatment, showing repetitive depletion and periodic amendment with Na_2SO_4 (average of six mesocosms per treatment on each sampling date).

temperature is 18.8°C (based on 1981–2010 air temperatures measured at the Duluth, Minnesota, airport, 10 km from the experimental site).

The experiments had been in progress for three growing seasons at the time of the sampling for this study, 27 and 28 August 2013, and for five growing seasons at the time of the second, less intensive, sampling (August 2015). The sediment of each mesocosm was divided into two parts for the 2013 growing season by a clear acrylic plate and all wild rice plants removed from one side in order to evaluate the effects of plant root presence on the geochemistry of the sediments. The plate was situated near one end of each mesocosm, such that about 10% of the surface area of 0.6 m^2 was plant-free (Figure S1). The plate was positioned to segregate the sediment without impeding the circulation of the surface water above all of the sediment. Sediment chemistry results presented here are from the side with wild rice plants present, except when analyzing the difference in AVS between the two sides.

2.2. Methods

2.2.1. Sample Collection

Rhizon™ samplers with a 10 cm long, 2.5 mm diameter, cylindrical porous tip (hydrophilic membrane pore size 0.12–0.18 μm (Rhizosphere.com, Netherlands; Shotbolt, 2010)), were connected by Teflon-taped Luer-Lok connectors and silicone tubing to a syringe needle. The sampler was inserted into the sediment, and the needle was then inserted through the 20 mm thick butyl rubber septum of an evacuated serum bottle (Bellco Glass) to initiate pore water draw through the tubing and displace air. After water was observed entering the serum bottle, the needle was removed from the first sacrificial bottle and inserted through the septum of a second evacuated serum bottle to collect the sample. One Rhizon and bottle were used to collect a sample for dissolved iron, preserved with 20% nitric acid. A second Rhizon and evacuated, N_2 gas-flushed sealed bottle, preloaded with 0.2 mL 2 N zinc acetate, 0.5 mL 15 M NaOH, and a stir bar, was used to collect a sample for dissolved sulfide analysis. Each Rhizon was positioned to sample pore water from the top 10 cm of sediment and to avoid collecting water from above the sediment surface. However, it is conceivable that some surface water was able to follow the path of the Rhizon into the sediment and dilute or partially oxidize the pore water sample.

Surface water in each mesocosm was collected for analysis of nitrate + nitrite, TP, TN, DOC, pH, temperature, and alkalinity from 5 cm below the surface of the water. Surface water samples for analysis of total Hg (THg) and MeHg were collected using clean hands/dirty hands protocols in September 2013, filtered through 0.45 μm glass fiber filters, and immediately acidified with 0.5% (by volume) trace metal hydrochloric acid. Samples were stored on ice during transport and at 4°C until analysis.

Pore water P availability was measured with three mixed bed ion exchange bags (Fisher Rexyn 300 resin) placed in the sediment of each tank in spring and harvested at the end of the growing season in 2013. A 3.8 cm diameter piston corer was used to obtain 10 cm long sediment samples for various analyses. Sediment samples for the analysis of AVS were taken monthly from June to October 2013 from replicate mesocosms of four SO_4 treatments (control, 50 150, and 300 mg L^{-1}; no mesocosm was sampled more

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than once). Sediment samples were also taken on 8 October 2013 for the analysis of THg in bulk sediment and on 6 October 2015 for the analysis of total organic carbon (TOC).

2.2.2. Laboratory Analyses

Surface water and pore water analyses were conducted by the Minnesota Department of Health Environmental Laboratory (MDHEL). Total P was measured by in-line ultraviolet/persulfate digestion and flow injection (APHA, 2005, 4500 P-I), DOC by persulfate-ultraviolet oxidation and IR CO_2 detection (APHA, 2005, 5310-C), and alkalinity by automated titration (APHA, 2005, 2320-B). Pore water sulfide samples were prepared for inline distillation and flow injection colorimetric analysis using procedures that avoided exposure to oxygen. The sulfide serum bottle was weighed to determine the amount of sample collected and to adjust for the slight dilution factor of an alkaline antioxidant that was added by injection through the stoppers. The sealed samples were then placed on a stir plate for at least 1 h and subsamples withdrawn for analysis through a needle. Reanalysis of sealed, processed samples 12 months later shows no significant difference in sulfide concentrations, indicating that the sulfide samples were stable prior to analysis (data not shown). SO_4 concentration was measured using a Lachat QuikChem 8000 Autoanalyzer (Lachat Method 10-116-10-1-A). The resin was eluted using a KCl solution and analyzed for PO_4 using a Lachat Autoanalyzer, following the methods of Walker et al. (2006).

An aliquot of the nitrate + nitrite/TP/TN/DOC serum bottle was filtered in the lab within 10 days of sampling using a 0.45 μm filter, preserved to a pH < 2 with 10% sulfuric acid, and transferred to a 250 mL polyethylene bottle for DOC analysis. The remaining sample was preserved to a pH < 2, with 10% sulfuric acid and transferred to 250 mL polyethylene bottle for nitrate + nitrite/TP/TN analysis. The contents of the metal serum bottle were transferred to a 250 mL polyethylene bottle and preserved to a pH < 2 with 10% nitric acid. Analyses were conducted within 30 days of sampling.

THg in surface water and bulk sediment were analyzed with EPA method 1631 by MDHEL, and surface water MeHg was analyzed with EPA method 1630 by Frontier Global Sciences (Bothell, Washington). Inorganic Hg (iHg) was calculated as the difference between THg and MeHg. Sediment AVS was analyzed colorimetrically, as above for pore water sulfide, following acid distillation and in-line alkaline trapping (APHA, 2005; SM 4500-S2). Sediment TOC was analyzed following SM5310C (APHA, 2005), using an OI Analytical Aurora 1030 at Pace Analytical Services, Virginia, Minnesota.

3. Data Analysis

3.1. Sulfate Depletion as the Independent Variable

Because SO_4 is relatively unreactive under oxidized conditions, its loss is attributable to diffusion or transpiration-driven advection (Bachand et al., 2014) into sediment and conversion to sulfide by bacteria. Surface water SO_4 concentrations decreased partly due to dilution by precipitation but largely from loss after movement into the sediment and reduction to sulfide. Sulfide would largely be retained in the sediment as FeS compounds, although some could be lost to the atmosphere as H_2S gas (Bagarinao, 1992) or as volatile organic sulfur compounds (Lomans et al., 2002). The cumulative SO_4 lost from surface water was calculated from a mass balance for each mesocosm from the inception of the experiment in spring 2011 through fall 2013; this quantity, termed here SO_4 depletion, $(SO_4)_{Depl}$, is used as a proxy for net MSR, following Weston et al. (2006). The surface water remained frozen from approximately 1 December to 1 April each winter, and the mesocosms were covered with plastic from November to late April each year and not amended with SO_4. SO_4 reduction was the major biogeochemical process altered by the experimental treatments, and therefore, $(SO_4)_{Depl}$ is the independent variable used in subsequent data analyses. It was only possible to perform a complete mass balance for SO_4, the only parameter consistently quantified in source water, precipitation, and overflow water.

3.2. Calculation of DIC From Measured Alkalinity

Dissolved inorganic carbon (DIC ≡ $[CO_3^{2-}] + [HCO_3^-] + [CO_2^*]$, where $[CO_2^*] = [CO_{2(g)}] + [H_2CO_3]$) was calculated from measured alkalinity and speciated using pH, temperature, and specific conductance of the surface water. At the pH range of the mesocosms (7.60–8.84), 95–98% of DIC is in the form of HCO_3^-, so DIC concentration on a molar basis is nearly the same as alkalinity (ALK) on an equivalent basis (DIC = 0.988 ALK + 0.077, $R^2 = 0.995$). In studies of freshwater, most inorganic carbon data are presented in terms of alkalinity because alkalinity is a familiar metric; however, in comparisons with DOC, inorganic carbon data are presented as DIC so that the units are directly comparable. PHREEQC version 3 geochemical modeling software (Parkhurst & Appelo, 2013) was used to calculate saturation indices for carbonate minerals.

3.3. Statistical Analysis

Statistical analysis was conducted with R version 3.2.3 and STATA (StataCorp, 2015). The effect of increased sulfate availability was assessed through both categorical analysis of the sulfate treatments (Kruskal-Wallis ANOVA test, followed by Dunn's test for multiple comparisons with Holm-Sidak corrections) and through linear regression and nonparametric Spearman rank correlations. We rely primarily on regressions against SO_4 depletion to detect the effects of enhanced sulfate-reduction driven mineralization, rather than categorical analysis of the sulfate treatment results, because (a) biogeochemical changes are not driven directly by SO_4 concentration, but rather by MSR, quantified as SO_4 depletion; (b) although SO_4 depletion may be highly correlated to SO_4 concentration, deviations between experimental mesocosms develop over time, so cumulative SO_4 depletion values eventually no longer align exactly with treatment categories, but rather become continuous variables; and (c) regression provides more statistical power than ANOVA and builds models that allowed us to describe the relationships between SO_4 depletion and response variables (Cottingham et al., 2005). However, when the relationship is not linear, ANOVA and comparison of treatments through Dunn's analysis can help describe the nature of a relationship.

4. Results and Discussion

4.1. The Impact of SO_4 Reduction on Mineralization of Sediment Organic Matter

Increased concentrations of surface water SO_4 resulted in increased sulfate reduction, which necessarily increased the mineralization of organic carbon, as described by reaction (1). Concentrations of surface water DOC and DIC increased in proportion to sulfate reduction, as measured by $(SO_4)_{Depl}$ (Table 1 and Figure 2). The marine literature generally assumes complete mineralization of particulate organic carbon (POC) to DIC in the water column (e.g., Boudreau & Westrich, 1984) (reaction 1), but in freshwater systems and especially wetlands, not all carbon is completely oxidized during decomposition, and a portion of POC may be mobilized as DOC (Howes et al., 1985; Selvendiran et al., 2008). In principle, the constituents of organic matter, such as the nutrients N and P, are mobilized in proportion to the mass of carbon mineralized as a result of MSR-driven decomposition. Surface water DOC and DIC, and the sum DOC + DIC, are therefore used as indicators of OM mineralization in interpreting the mobilization of N, P, and Hg to surface waters (Figure 2 and Tables 2 and 3).

In contrast to many marine systems, it is likely that SO_4 reduction in these sediments was limited more by SO_4 than by organic carbon, given that $(SO_4)_{Depl}$ was linearly proportional to the average SO_4 concentration (Figure S2a; R^2 = 0.87), without any obvious curvature to the relationship that would indicate saturation of MSR.

Regressions of surface water DOC and DIC against SO_4 depletion demonstrate that, on a net basis, about 60% more DIC than DOC was mobilized to the surface water as a result of MSR-driven mineralization (slope of 0.235 mM C per unit SO_4 depletion compared to 0.148; Table 2). The significantly positive slope of the DIC: DOC ratio against SO_4 depletion (Table 2) indicates that increasingly more DIC than DOC was observed in the surface water as sulfate depletion increased. Some mineralization of DOC to DIC likely occurs in the surface water as a result of exposure to oxygen, aerobic bacteria, and sunlight, processes that could have a larger effect as DOC increases.

Not only did surface water DIC and DOC increase in concert with sulfate reduction, but parallel increases occurred in surface water concentrations of constituents of organic matter: N, P, and Hg (Table 1 and Figure 2). DIC, DOC, total P, total N, ammonia, and total Hg in surface water all had increases from the control to the highest SO_4 addition of about twofold, (2.3, 1.7, 1.9, 1.8, 1.7, and 2.6-fold, respectively, Table 1). However, available phosphate in the sediment, an estimate of P availability in pore water, had a larger increase (7.5-fold). MSR consumes acidity as the DIC-based alkalinity is produced (Baker et al., 1986), which increased the average pH from 7.57 to 7.81, a 44% decrease in hydrogen ion concentration (Table 1). If the sulfide subsequently oxidizes (which could happen in a natural system during drought (Laudon et al., 2004) or intentional dewatering), a proportional quantity of alkalinity is consumed as acid is produced

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Table 1
Summary of Effects of Experimentally Increased SO_4 Concentrations on SO_4 Reduction (Quantified as SO_4 Depletion), Organic Matter Mineralization, and Mercury Methylation

		Average of each sulfate treatment (n = 6 for each treatment)						Correlation with SO_4 depletion (Spearman)	
Variable	Matrix	Control	50	100	150	300	Max/Min	Rho	p value
		Variables mainly associated with SO_4 reduction							
SO_4 (T-W mean mg SO_4 L^{-1})	sw	6.7[a]	26.9[ab]	58.5[abc]	93.2[BC]	206.5[c]	31.0	0.93	<0.0001
SO_4 depletion (mg S cm^{-2})	sw	0.14[a]	2.52[ab]	3.63[abc]	4.28[BC]	6.90[c]	48.5	1	
Pore water sulfide (μg S L^{-1})	pw	69[a]	184[a]	224[a]	393[b]	728[b]	10.5	0.81	<0.0001
Pore water iron (μg L^{-1})	pw	12,883[a]	11,122[ab]	6,808[abc]	4,483[BC]	3,032[c]	4.25	−0.82	<0.0001
AVS (mg S kg^{-1})	sed	102[a]	483[ab]	NA	826[ab]	1,413[b]	13.8	0.77	<0.0001
pH	pw	7.57[a]	7.52[a]	7.55[a]	7.75[a]	7.81[a]	1.03	0.39	=0.03
H^+ ion (μmol L^{-1})	pw	0.027	0.030	0.028	0.018	0.015	1.72	0.39	=0.03
		Variables mainly associated with mineralization of organic matter							
TOC (% dry mass)	sed	9.26[a]	7.90[a]	8.18[a]	7.17[a]	8.22[a]	1.29	−0.34	=0.065
DIC (mg C L^{-1})	sw	28.9[a]	47.2[ab]	56.3[BC]	56.7[BC]	66.3[c]	2.30	0.94	<0.0001
DOC (mg C L^{-1})	sw	16.3[a]	21.4[a]	26.8[BC]	24.0[abc]	28.3[bc]	1.74	0.79	<0.0001
Total N (mg N L^{-1})	sw	1.42[a]	1.75[a]	2.35[BC]	2.03[abc]	2.57[BC]	1.81	0.77	<0.0001
Ammonia (mg N L^{-1})	sw	0.09[a]	0.09[a]	0.10[a]	0.10[a]	0.16[a]	1.70	0.38	=0.04
Total P (μg P L^{-1})	sw	13[a]	16[ab]	22[ab]	21[ab]	25[b]	1.92	0.73	<0.0001
Available P (μg P g^{-1} resin)	Resin in sed	0.34[a]	0.40[a]	0.59[ab]	0.92[ab]	2.56[b]	7.45	0.86	<0.0001
Total Hg (ng L^{-1})	sw	1.83[a]	2.09[a]	3.61[ab]	3.25[ab]	4.80[b]	2.63	0.82	<0.0001
		Variables mainly associated with Hg methylation							
Methylmercury (ng Hg L^{-1})	sw	0.20[a]	0.49[ab]	1.21[b]	1.08[b]	1.18[b]	5.91	0.66	<0.0001
Inorganic Hg (ng L^{-1})	sw	1.63[a]	1.60[ab]	2.40[abc]	2.17[BC]	3.62[c]	2.22	0.80	<0.0001
Percent methylmercury	sw	11%[a]	23%[ab]	30%[b]	32%[b]	23%[ab]	2.90	0.45	=0.02

Note. Matrix abbreviations: sw = surface water, pw = pore water, sed = bulk sediment. Averages with superscript letters in common are not significantly different at the 0.05 level.

(Hall et al., 2006). However, the sulfide reoxidation does not reverse the mobilization of the constituents of organic matter (C, N, P, and Hg) or the production of methylmercury (MeHg; see below). Rather, any production of SO_4 from sulfide oxidation creates the potential for additional MSR-driven OM mineralization and Hg methylation (Coleman Wasik et al., 2015; Hansel et al., 2015).

The slope of linear regressions of the C, N, and P in surface water against $(SO_4)_{Depl}$ is an estimate of the increase of that variable in mesocosm surface waters per unit SO_4 reduction (Table 2). The regression slopes provide a basis for estimates of stoichiometric ratios of the constituents mobilized from the sediment solid phase, similar to the calculation that Weston et al. (2006) performed for pore water. The calculation of stoichiometric ratios from the slopes of regressions with $(SO_4)_{Depl}$ is more accurate than calculating ratios from surface water concentrations alone, as the use of slopes accounts for the concentrations of the control (the intercept of the linear regression).

The regression slopes of surface water C versus surface water N, P, and Hg in mesocosms are estimates of the net release of each element relative to that of C (Table 3). These estimates can then be compared to the ratio of these constituents in the primary source material—the sediment—to determine the efficiency of mobilization of sediment N, P, and Hg to surface water, compared to C (Table 3). Although we present efficiency relative to only DOC and only DIC, calculating efficiency relative to the sum of mineralized OM (DOC + DIC) represents the overall net efficiency of mineralization, which ranges from 8% to 38% for the three constituents (Table 3). Although the increases in surface water N, P, and Hg are consistent with the hypothesis that those elements were released to the surface water through sulfate-enhanced mineralization of sediment OM, their lower mobilization efficiencies relative to carbon suggest that other processes were operating to either increase carbon, decrease N, P, and Hg mobilization relative to carbon, and/or increase N, P, and Hg losses. It is likely that some carbon was introduced to the surface waters from sources other than the sediment (e.g., photosynthetic fixation of atmospheric carbon) and that there were losses for N, P, and Hg from the surface water (though adsorption, settling, biological uptake, or atmospheric evasion of N and Hg).

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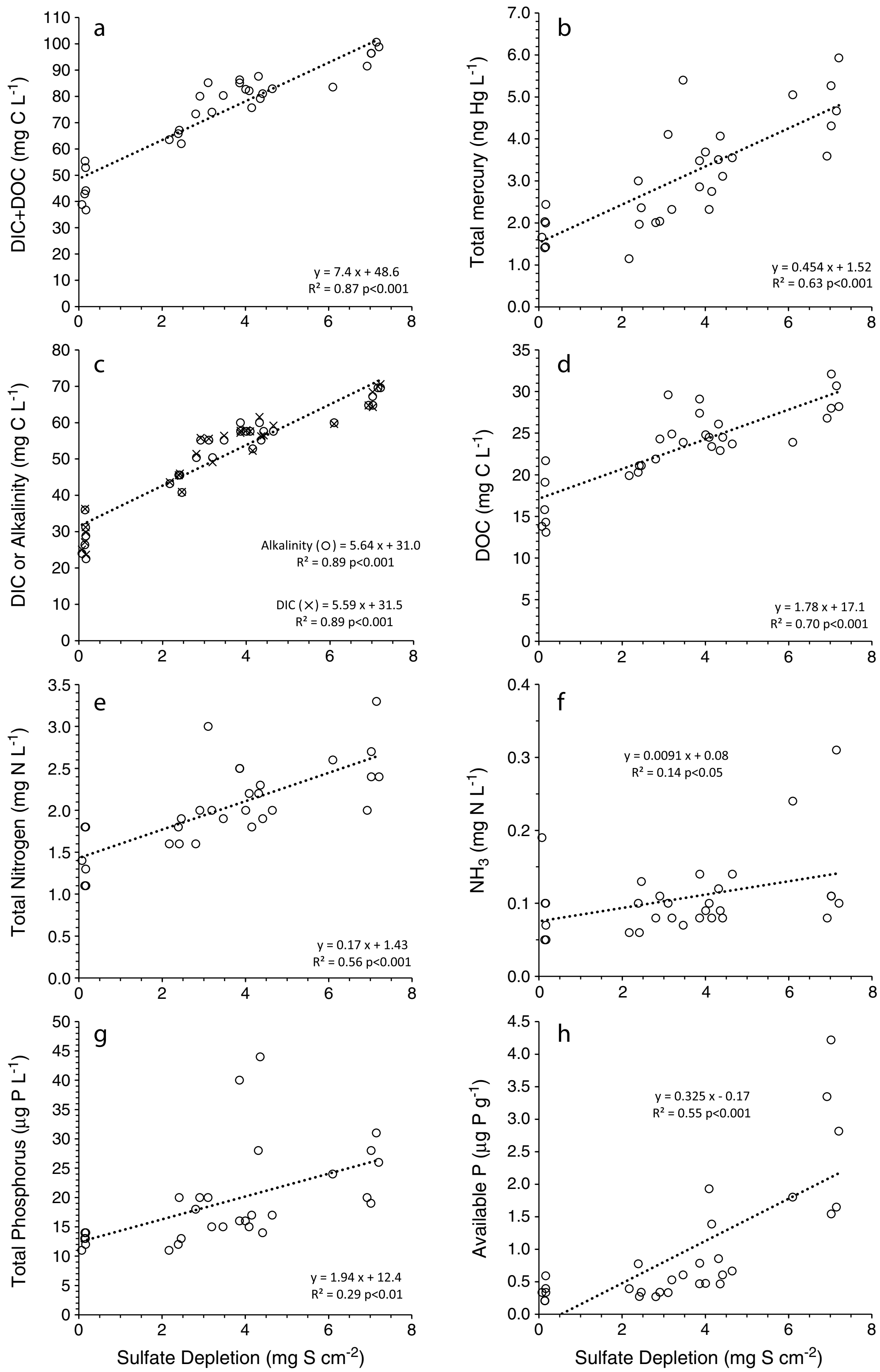

Figure 2. The release of constituents of sedimentary organic matter as a function of SO_4 depletion, showing linear regressions (dotted lines). (a) Sum of surface water DIC and DOC; (b) surface water total mercury; (c) surface water alkalinity and DIC (symbols ○ and ×, respectively; the two regressions are superimposed); (d) surface water DOC; (e) surface water total nitrogen; (f) surface water ammonia; (g) surface water total phosphorus; (h) available phosphate in the sediment, as quantified on ion-exchange resin.

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Table 2
Slopes of Regressions of Surface Water Parameters (mM) Against SO_4 Depletion (mg S cm^{-2})

Surface water variable (molar basis)	Regression against $(SO_4)_{Depl}$ (mg S cm^{-2}) Slope	R^2	*p*
DIC	0.235	0.89	<0.0001
DOC	0.148	0.70	<0.0001
DIC + DOC	0.383	0.84	<0.0001
DIC: DOC	0.044	0.56	<0.0001
TN	0.0121	0.56	<0.0001
TN: DIC	−0.0028	0.25	<0.01
TN: DOC	0.0004	0.01	NS
TN: DIC + DOC	−0.0006	0.08	NS
TP	6.26E−05	0.29	<0.002
TP: DIC	−7.00E−06	0.03	NS
TP: DOC	7.00E−06	0.02	NS
TP: DIC + DOC	−1.00E−07	0.00	NS
THg	2.26E−09	0.63	<0.0001
THg: DIC	9.00E−06	0.46	<0.0001
THg: DOC	6.00E−06	0.23	<0.01
THg: DIC + DOC	2.00E−05	0.42	<0.0001

Note. When a sediment constituent's ratio to DIC or DOC has a significant slope against sulfate depletion, it indicates that the constituent was mobilized to the surface water at a significantly different rate than the DIC or DOC.

In addition to increases of TP in the surface water, the sediment pore water in the highest SO_4 treatment contained 7.5-fold greater available phosphate than the controls, as quantified with ion-exchange resin (Table 1 and Figure 2h). In comparison, the increase in surface water TP was only 1.9-fold (Table 1 and Figure 2g). The difference between phosphorus response in the resin and the surface water may be partly due to (a) loss of TP from the surface water after mobilization or (b) irreversible trapping of mobilized P on the resin. If phosphorus is released from sediment en masse in response to an S-induced shift from iron oxides to iron sulfides, the sediment pore water would experience this release first, while release to surface waters would take longer due to diffusion-limited transport and potentially an iron-oxide barrier at the sediment-water (anoxic-oxic) interface.

DIC in surface water is not conservative, being subject to exchange across the air-water interface, carbonate mineral precipitation, and photosynthetic uptake. Surface water pCO_2 in all mesocosms was above saturation with respect to atmospheric equilibrium by a factor of 1.4–15.5 (based on the DIC speciation calculations discussed earlier; data not shown), so the mesocosms were losing, not gaining, C through gas exchange with the atmosphere. The pCO_2 values in the mesocosms are similar to those reported from epilimnia of small, organic-rich, temperate lakes of low to moderate salinity (Cole et al., 1994; Myrbo & Shapley, 2006). With respect to mineral precipitation, based on geochemical equilibrium calculations, surface waters were undersaturated with respect to all carbonate minerals. Thus, although DIC in surface water is subject to several transport and transformation processes, the sustained presence of CO_2 at quantities significantly above saturation with respect to the atmosphere and the observation of increasing DIC and DOC with increasing $(SO_4)_{Depl}$ (Table 1) provide strong evidence of sulfate-induced increases in net carbon mineralization in the mesocosms.

In addition to the carbon originally present in the sediment, organic carbon was also photosynthetically fixed by wild rice and algae in the mesocosms and subsequently subjected to respiration and some decomposition, adding to the DIC and DOC in surface waters. DOC may also have been released into sediment pore water as an exudate from the wild rice roots (Rothenberg et al., 2014; Windham-Myers et al., 2009). Exudate DOC, however, does not account for the observed increase in DOC, since a negative relationship between the number of wild rice plants and DOC was observed (Spearman's rho = −0.63, $p < 0.001$, Table S2).

4.2. Effects of SO_4 Reduction on Mercury and Methylmercury in Surface Water

We interpret Hg mobilization to the surface water in an analogous manner to C, N, and P, as Hg tends to associate strongly with organic matter in sediment (Feyte et al., 2010). In the mesocosm surface waters,

Table 3
Elemental Ratios in Sediment and Surface Water Across the Range of SO_4 Depletion

Molar ratio in sediment[a]		Molar ratio in surface water[b] DIC	DOC	DOC + DIC	Efficiency of mobilization of sediment N, P, or Hg to surface water, relative to carbon: DIC	DOC	DOC + DIC
C: N	12[a]	19	12	32	63%	100%	38%
C: P	463[a]	3,752	2,366	6,118	12%	20%	8%
C: Hg	1.90E + 07	1.04E + 08	6.5E + 07	1.69E + 08	18%	29%	11%

Note. Together, the ratios are used to calculate the efficiency of mobilization of the constituents of particulate organic matter into the surface water.
[a]Sediment data from Hildebrandt, Pastor, and Dewey (2012), a mesocosm study that obtained sediment from the same natural wild rice stand. [b]Regression slopes of C versus N, P, and Hg in mesocosm surface waters; calculations are made based on surface water DIC alone, surface water DOC alone, and the sum of surface water DOC + DIC.

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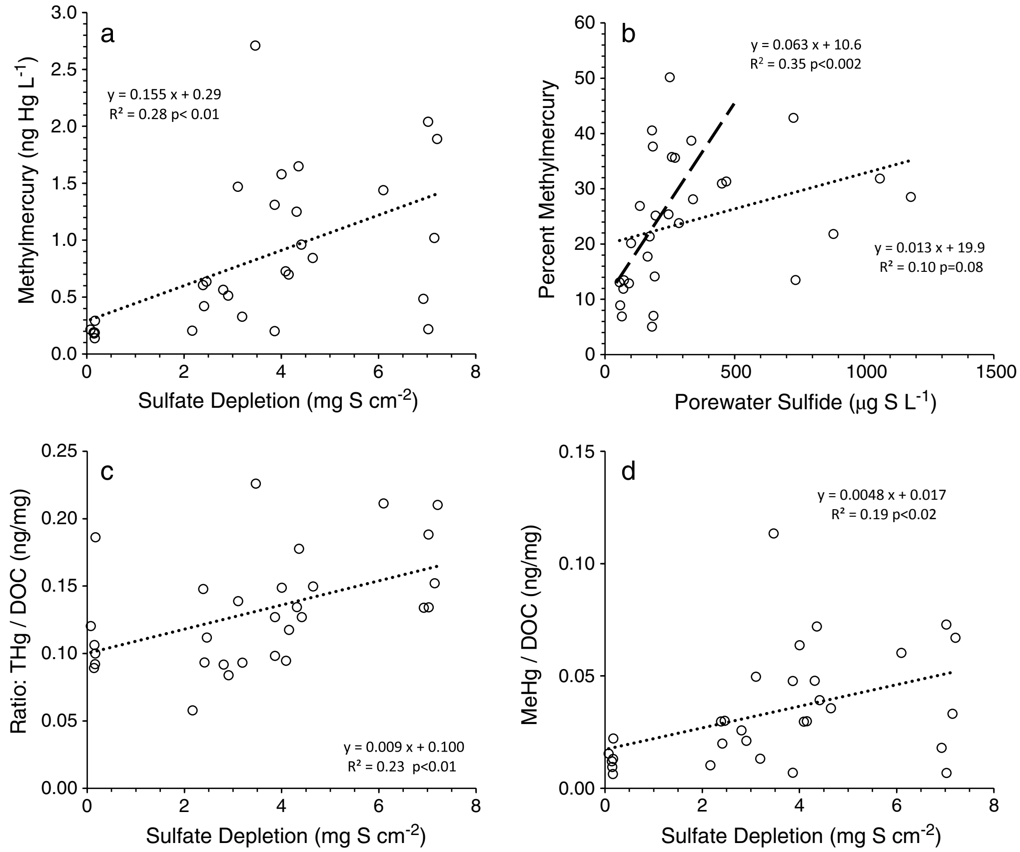

Figure 3. The response of surface water Hg variables to SO_4 depletion and the production of pore water sulfide, showing linear regressions. (a) MeHg as a function of SO_4 depletion; (b) percent MeHg as a function of pore water sulfide, showing regressions for all data (dotted line) and for the subset of data extending only to a pore water sulfide concentration of 468 µg S L^{-1} (dashed line); (c) ratio of THg to DOC as a function of SO_4 depletion; (d) ratio of MeHg to DOC as a function of SO_4 depletion.

THg, inorganic Hg (iHg), and MeHg all increased significantly with increased $(SO_4)_{Depl}$ (Table 1 and Figures 2b and 3a, $p < 0.0001$) and were greater in the highest sulfate amendment by factors of 2.6, 2.2, and 5.9, respectively (Table 1). The relative increase in THg (2.6-fold) is greater than that for DIC, DOC, TN, and TP, which range from 1.7 to 2.3-fold (Table 1). DOC enhances the solubility of both iHg and MeHg and can facilitate the movement of Hg from sediment into surface water (Ravichandran, 2004). The 5.9-fold increase in MeHg indicates that MeHg flux to surface waters was enhanced by sulfate loading disproportionately more than sedimentary release of THg (2.6-fold) and the increase in surface water DOC (1.7-fold).

The genes required to methylate Hg have been found in a wide variety of anaerobic bacteria, including SO_4-reducing bacteria, iron-reducing bacteria, and methanogens (Podar et al., 2015). Though some pure culture and experimental evidence exist for mercury methylation by other bacteria, extensive pure culture, experimental, and landscape-scale observations suggest SO_4-reducing bacteria dominate Hg methylation in many freshwater and marine environments. The relatively large increase in surface water MeHg in response to increased $(SO_4)_{Depl}$ in this experiment supports the assumption that MSR was responsible for most of the observed production of MeHg. It is likely that increased SO_4 loading to low-SO_4 aquatic systems with organic sediment will result in increased Hg methylation even though the relative importance of Hg methylation in the environment by different groups of bacteria is still a subject of debate (Paranjape & Hall, 2017).

If movement of DOC from sediment to surface water were the sole mechanism for the Hg increase in surface water, a constant Hg:DOC ratio would be expected on the $(SO_4)_{Depl}$ gradient. However, THg:DOC, iHg:DOC, and MeHg:DOC ratios in surface water are all significantly correlated with SO_4 depletion (Table S2 and Figures 3c and 3d). Therefore, all forms of Hg (THg, iHg, and MeHg) increase in surface waters more than

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does DOC, indicating that a sulfate-induced enhancement of carbon mineralization may act in combination with either enhanced methylation or an enhanced capacity of DOC to carry Hg. Changes to the binding strength of the DOC in heavily S-impacted mesocosm sediment are possible, as thiol groups on DOC are dominant binding sites for Hg (Skyllberg, 2008). The dual role of organic carbon and sulfur in driving both the production of MeHg and the transport of MeHg could be responsible for the substantially larger maximum increase in MeHg:DOC ratio relative to the increase in the THg:DOC ratio (an average 206% increase relative to a 63% increase, Figures 3c and 3d), as postulated by Bailey et al. (2017).

Regnell and Hammar (2004) identified three MSR-driven processes that might cause mobilization of Hg from sediment in a wetland, (1) mineralization of organic matter; (2) extraction of iHg by reduced S compounds, which could be associated with mobilized DOC; and (3) enhanced production of MeHg, which is more mobile than iHg. They argued that enhanced production of MeHg explained THg mobilization in the minerotrophic peat bog that they studied. However, in this study, increases in surface water MeHg concentrations (Figure 3a) are not sufficient to explain the linear increase in THg observed in this experiment (Figure 2b) because most (67%) of the increase is iHg (Table 1). Some of the increase in surface water iHg could be the result of increased production of MeHg that moved to surface water and was subsequently demethylated. Regardless of the underlying mechanism, our observations clearly show increases in surface water Hg that were greater than the increases in C, N, and P (Table 3); this corroborates other studies (Bouchet et al., 2013; Merritt & Amirbahman, 2007; Regnell & Hammar, 2004) that suggest sediment Hg may be synergistically mobilized to surface waters through mineralization, methylation, and enhanced mobility with DOC.

Recent research has shown that in many ecosystems, higher concentrations of pore water sulfide may inhibit MeHg production through either thermodynamically or kinetically controlled reactions with inorganic Hg (Benoit et al., 2003; Hsu-Kim et al., 2013). We plotted %MeHg, rather than the MeHg concentration, against pore water sulfide because we are interested in identifying the pore water sulfide zone of greatest efficiency for the methylation and mobilization of mercury. In this experiment the MSR-driven mineralization of OM released THg to surface water in addition to producing pore water sulfide. Accordingly, because THg is not constant, plotting %MeHg is the most accurate way to identify peak methylation efficiency. In principle, the restricted bioavailability of Hg to methylating bacteria results in a maximum in MeHg production at intermediate concentrations of pore water sulfide. Consistent with previous research in sulfate-impacted freshwater ecosystems (Gilmour et al., 1998; Gilmour, Krabbenhoft, et al., 2007, Gilmour, Orem, et al., 2007; Bailey et al., 2017), MeHg production was most efficient at intermediate sulfide concentrations. In the control, where average sulfide was 69 μg S L^{-1}, MeHg averaged only 11% of THg in surface waters. In the intermediate SO_4 treatments, which had average sulfide concentrations of 224 and 393 μg S L^{-1}, MeHg production efficiency peaked significantly higher, at averages of 30% and 32%, respectively (Table 1). %MeHg declined to an average of 23% in the highest SO4 treatment, which had an average sulfide concentration of 728 μg S L^{-1}. Given the relatively great scatter in the relationship between %MeHg and sulfide (Figure 3b), it would be most defensible to conclude that the decrease in %MeHg began to occur somewhere between 300 and 700 μg S L^{-1}. There is a strong positive relationship ($p < 0.001$) between sulfide and %MeHg if the five sulfide concentrations greater than 727 μg S L^{-1} are excluded from the regression (which leaves only sulfide concentrations less than 468 μg S L^{-1}, since there is a gap in sulfide concentrations; Figure 3b). Other studies have identified sulfide zones of peak methylation roughly comparable to that found here. In South Florida, Orem et al. (2011) found that sulfide ranging from 5 to 150 μg S L^{-1} did not inhibit methylation but that sulfide concentrations greater than 1,000 μg S L^{-1} did. In a subboreal Minnesota wetland enriched in SO_4 from mining discharge, Bailey et al. (2017) found that sulfide concentrations above ~650 μg S L^{-1} inhibited methylation.

The relationship between surface water SO_4 and Hg methylation can be strongly affected by site-specific conditions. Because of the variable conversion of SO_4 in surface water to sulfide in pore water—primarily due to differences in OM and Fe availability (Pollman et al., 2017)—researchers have found a broad range in the SO_4 concentration associated with maximum efficiency of Hg methylation. For example, Orem et al. (2014) observed that two different areas in the Everglades Protection Area had peak surface water MeHg concentrations at SO_4 concentrations of 2 and 10–15 mg L^{-1}. In the mesocosms presented here peak surface water %MeHg was observed in the two sulfate treatments that averaged 59 and 93 mg L^{-1} (Table 1).

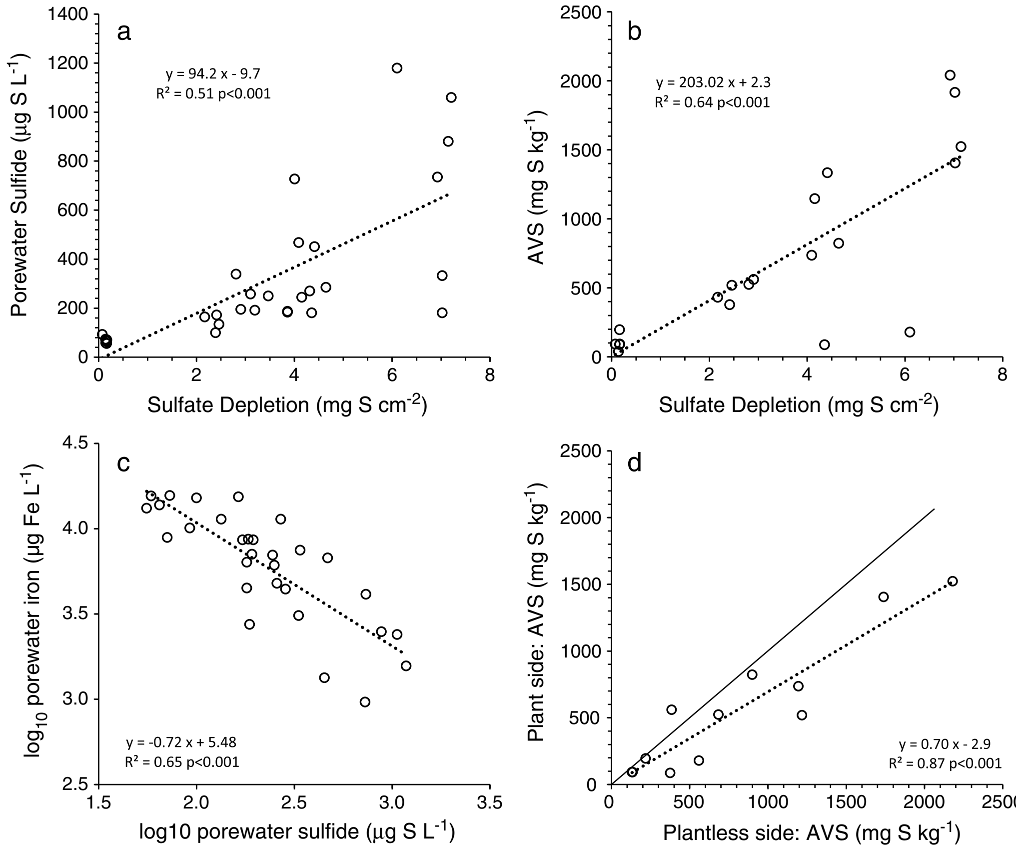

Figure 4. AVS and pore water sulfide, as related to SO_4 depletion, pore water iron, and presence of rooted plants. (a) Pore water sulfide as a function of SO_4 depletion; (b) AVS from the vegetated side of the mesocosms as a function of SO_4 depletion; (c) pore water iron as a function of pore water sulfide; (d) AVS compared between the vegetated side and nonvegetated side. The solid 1:1 line shows that in almost all mesocosms more AVS is found in the side without plants.

4.3. Effects of SO_4 Reduction on Pore Water and Sediment Sulfide

Pore water sulfide increased at higher $(SO_4)_{Depl}$, although with greater variance at higher $(SO_4)_{Depl}$ (Figure 4a), possibly as a result of variable oxidation of sulfide that may depend on the proximity of the Rhizon sampler to plant roots (Schmidt et al., 2011) or of variable bioturbation by invertebrates (Lawrence et al., 1982). When SO_4 is reduced through MSR, the sulfide produced has a number of nonexclusive potential fates: the sulfide could (1) be oxidized within the sediment; (2) remain in the sediment pore water as free sulfide; (3) diffuse into oxygenated surface water, to be oxidized; (4) react with metals in the sediment, forming insoluble precipitates (dominated by iron-sulfide compounds); or (5) be lost to the atmosphere as H_2S gas or as volatile organic sulfur compounds. Because precipitation reactions are fast relative to redox reactions and diffusion, most of the sulfide probably forms metal precipitates if metals are available. When precipitation dominates the fate of sulfide produced from MSR, the continuous reduction of SO_4 and precipitation of iron sulfides form quasi-steady states between surface water SO_4 and pore water sulfide (Figure S2b) and between pore water sulfide and pore water iron (Figures 3 and 4c). The overall mass of sulfide in the mesocosm sediment, quantified through analysis of AVS (from sediment in the vegetated area), is closely correlated with SO_4 depletion (Figure 4b) even though AVS may not include all the reduced sulfide in sediments. It is likely that most of the AVS in these sediments is present as an FeS precipitate because other metals are at low concentrations in these sediments, which came from a relatively pristine (unpolluted) lake (Fond du Lac Band, 2016; Pastor et al., 2017). Note that there are two mesocosms with especially low AVS concentrations (Figure 4b). It is possible that the AVS in the specific location in these mesocosms where sediment core samples were collected was influenced by

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a spatially heterogeneous oxidization process (e.g., root oxygen or benthic invertebrates) that limited the accumulation of sulfide.

AVS was 30% lower in the vegetated side of the mesocosms, suggesting that wild rice released oxygen into the sediment, inhibiting the production of sulfide and/or decreasing sulfide concentrations through oxidation (Figure 4d; Wilcoxon paired test, $p = 0.007$). It is notable that this 30% difference developed in just one growing season, despite the previous 2 years of sulfate treatment. Pore water sulfide showed no statistically significant difference between the two sides owing to high variability within treatments. Numerous investigations have found that rooted aquatic plants release oxygen from their roots, a phenomenon that is usually interpreted as an adaptation to limit the toxicity of reduced chemical species in the pore water, especially sulfide (Lamers et al., 2013). Although oxygen release has been observed in white rice, *Oryza sativa* (Colmer, 2002), it has never been documented in wild rice, which is in the same tribe (Oryzeae) of grasses as white rice, and also develops aerenchyma (Jorgenson et al., 2013), plant structures that provide a low-resistance internal pathway for movement of oxygen to the roots. Since the growth and reproduction of rooted plants can be inhibited by sulfide (Pastor et al., 2017), there may be a tipping point of exposure to sulfide above which oxygen release is insufficient to mitigate phytotoxic effects, and the plant population declines over time, possibly to extirpation. In this experiment, in the third treatment year, the increase in pore water sulfide was the apparent cause of a decrease in the average number of wild rice stems from 17 in the control mesocosms to 3 in the highest-sulfate treatment mesocosms (Pastor et al., 2017).

4.4. Mesocosms as Models for Ecosystem-Scale Effects of SO_4 Reduction

Although mesocosms, as contained ecosystems, are useful because they mimic ecological and biogeochemical processes that occur in the field, extrapolating findings to nature is challenging when plastic walls have prevented exchange of water and materials (Petersen et al., 2009). These wall-based challenges are manifest in three phenomena in this experiment, (1) relatively long surface water residence times due to the lack of a constant throughflow; (2) the presence of the wall itself, which provides a surface for periphyton; and (3) lack of either overland or groundwater loading of external materials:

1. Relatively long surface water residence times: the increased loading of N, P, C, Hg, and MeHg to the surface water of the mesocosms was readily detected because the lack of hydraulic loading from a watershed minimized dilution and loss through the outflow. The impact of an increase in SO_4 loading on surface water concentrations of N, P, C, Hg, DIC, and DOC would be lower in waters with shorter residence times. For instance, Baker and Brezonik (1988), in modeling increases in alkalinity from atmospheric SO_4 loading, noted that net increases in alkalinity would be most important in waters with long residence times ($>$5 years) and that there would be little increase in alkalinity in waters with much shorter residence times ($<$1 year). However, the measured concentrations may not represent the maximum impact of MSR-driven mineralization because the mesocosm wall may enhance removal from the surface water (point number 2, below).
2. Presence of the mesocosm wall: the mesocosms have a relatively high ratio of wall and sediment surfaces to the volume of overlying water, enhancing the removal of surface water nutrients and Hg to periphyton or inorganic sinks such as iron oxyhydroxides. Natural aquatic systems have less proportional loss to surfaces. The quantitative estimates of internal loading of N, P, and Hg in response to MSR-induced carbon mineralization may have been underestimated by the measured surface water concentrations, given that significant loss of these constituents to periphyton may have occurred. In addition, THg was filtered prior to analysis, which would have removed any Hg associated with phytoplankton or other suspended particles.
3. Lack of either overland or groundwater loading of particulate and dissolved material, specifically iron: the availability of iron in sediment is a primary controller of the fate of MSR-produced sulfide (Pollman et al., 2017). In natural aquatic systems, iron would be supplied at a relatively constant rate from the system's watershed over the long term, although varying in magnitude from watershed to watershed (Maranger et al., 2006; Winter, 2001). This experiment was not an accurate long-term mimic of pore water sulfide concentrations because the external supply of iron was cut off at the inception of the experiment. With no loading of iron, but continued loading of SO_4, the continued production of sulfide would be expected to eventually consume all available Fe, allowing pore water sulfide levels to exceed those expected in a natural system at equivalent surface water SO_4 concentrations. This mesocosm experiment provides

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evidence for just such a result. The experiment continued for 2 years after the 2013 sampling presented here. In the fifth year (August 2015) pore water sulfide was much greater than had been observed in 2013, and disproportionately so in the highest SO_4 treatment, which was most likely to consume available Fe. Between the 2013 and 2015, pore water sulfide increased in the control SO_4 treatment (about 7 mg SO_4 L^{-1}) from an average value of 69 μg L^{-1} in 2013 to 116 μg L^{-1} in 2015, a 68% increase. Pore water sulfide in the highest treatment (nominally 300 mg SO_4 L^{-1}, Table 1) increased from an average value of 728 μg L^{-1}in 2013 to 9,350 μg L^{-1} in 2015, a 1,184% increase (Pastor et al., 2017). In a survey of 108 Minnesota waterbodies with a wide range of surface water sulfate, only two exceeded a pore water sulfide level of 3,200 μg L^{-1} (Myrbo et al., 2017).

5. Conclusions

This study demonstrates that increased SO_4 loading to inland waters with organic-rich sediments can significantly increase the decomposition of sedimentary organic matter, which increases internal loading to surface water of the chemical constituents of organic matter, including DIC, DOC, P, N, and Hg. Associated changes include increased production of sulfide and methylmercury and increased alkalinity and pH. Any one of these changes could alone cause significant secondary changes in the structure of an aquatic ecosystem but, taken together, could cause a cascade of primary and secondary environmental changes: increased availability of nutrients (N and P), which can alter dominant plant species, organic carbon production, oxygen consumption, and redox; increased pore water sulfide, which can be toxic to benthic animals and plants; increased MeHg production, which can affect fish and other consumers in the aquatic food web; increased DOC, which can alter light transmission, thermal stratification, and aquatic chemistry; and increased DIC production, which increases alkalinity and pH, affecting aquatic chemistry and biota. Each of these changes resulting from higher surface water SO_4 and consequent increases in MSR has been documented in the literature, but the entire suite of associated changes in aquatic chemistry has not heretofore been demonstrated in an integrated fashion. The degree to which an increase in SO_4 loading affects the ecological structure of the receiving water will depend on the relative increases in N, P, DIC, DOC, Hg, MeHg, pH, and sulfide, which will be a function of background geochemistry and hydrology of the specific system. In this experiment, the changes in these parameters were linearly proportional to SO_4 reduction, which, in turn, was linearly proportional to the time-weighted average SO_4 concentration. The linear responses of the parameters to SO_4 additions suggest that ecologically significant changes may occur even when SO_4 concentrations are elevated only modestly and that dramatic changes may occur with higher sulfate loading.

Acknowledgments
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RESEARCH ARTICLE

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This article is a companion to Myrbo et al. (2017), https://doi.org/10.1002/2017JG003788 and Pollman et al. (2017), https://doi.org/10.1002/2017JG003785.

Key Points:

- Sulfate loading to freshwater ecosystems may alter aquatic plant communities when sulfate is reduced to sulfide in the anoxic rooting zone
- The occurrence of self-sustaining wild rice populations is mainly controlled by pore water sulfide concentrations
- Even if pore water sulfide is low, wild rice is less likely to be found if the surface water is turbid or warm

Supporting Information:

- Table S1
- Table S2
- Table S3
- Data Set S1
- Figure S1

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Sulfide Generated by Sulfate Reduction is a Primary Controller of the Occurrence of Wild Rice (*Zizania palustris*) in Shallow Aquatic Ecosystems

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Abstract Field observations suggest that surface water sulfate concentrations control the distribution of wild rice, an aquatic grass (*Zizania palustris*). However, hydroponic studies show that sulfate is not toxic to wild rice at even unrealistically high concentrations. To determine how sulfate might directly or indirectly affect wild rice, potential wild rice habitat was characterized for 64 chemical and physical variables in over 100 sites spanning a relatively steep climatic and geological gradient in Minnesota. Habitat suitability was assessed by comparing the occurrence of wild rice with the field variables, through binary logistic regression. This analysis demonstrated that sulfide in sediment pore water, generated by the microbial reduction of sulfate that diffuses or advects into the sediment, is the primary control of wild rice occurrence. Water temperature and water transparency independently control the suitability of habitat for wild rice. In addition to generating phytotoxic sulfide, sulfate reduction also supports anaerobic decomposition of organic matter, releasing nutrients that can compound the harm of direct sulfide toxicity. These results are important because they show that increases in sulfate loading to surface water can have multiple negative consequences for ecosystems, even though sulfate itself is relatively benign.

Plain Language Summary Research in the 1940s and 1950s found that wild rice grew best in low-sulfate Minnesota lakes, but it was not known why. The correlation was a puzzle, since sulfate is not very toxic to plants or animals. This study found that the problem is sulfide, not sulfate. Sulfate can be converted into toxic levels of sulfide in the soil in which wild rice germinates and roots. Wild rice is an annual plant that must sprout each spring from seed that was dropped the previous fall into wet soil. Anaerobic microbes in the soil make sulfide from sulfate in the overlying water. Lakes, streams, and wetlands that have high concentrations of dissolved sulfide in the sediment therefore have a low probability of hosting wild rice. The study also found that wild rice prefers high-transparency water and cold winters.

1. Introduction

Minnesota is unique among U.S. states and Canadian provinces in having a water quality standard that regulates sulfate (SO_4) to protect wild rice, *Zizania palustris* and *Zizania aquatica*. The more common wild rice species in Minnesota, *Z. palustris* (northern wild rice), is an annual emergent aquatic grass that forms monocultures in shallow freshwaters (wetlands, lakes, and rivers) in the area of the Laurentian Great Lakes (Minnesota, Wisconsin, Michigan, Ontario, and Manitoba). Wild rice is culturally important to multiple groups in Minnesota, especially Ojibwe, Dakota, and other Native Americans, and also provides habitat and food for waterfowl and other wildlife (Vennum, 1988). In 1977, the Minnesota legislature voted to make wild rice the Minnesota state grain. Minnesota Rule 7050.0224, promulgated in 1973, seeks to limit the exposure of wild rice to SO_4 concentrations exceeding 10 mg L^{-1} (0.1 mmol L^{-1}). This value was based on empirical research that correlated water chemistry to aquatic plant assemblages and included the observations that no large populations of *Z. palustris* occur in waters exceeding 10 mg L^{-1} SO_4 and that stands are uncommon where SO_4 concentrations exceed 50 mg L^{-1} (Moyle, 1944, 1945). In addition, Moyle (1956) noted that plantings of wild rice seed in high-SO_4 regions generally failed. A larger unpublished Minnesota Department of Natural Resources data set also shows that sites with reported wild rice presence (DNR, 2008) are generally correlated with surface water SO_4 below 10 mg L^{-1} (Figure S1 in the supporting information).

We report here on a multiyear field survey that was part of a larger study (Myrbo et al., 2017; Pastor et al., 2017; Pollman et al., 2017) designed to reevaluate the 10 mg L^{-1} SO_4 standard by testing potential mechanisms by which SO_4 might be harmful to wild rice. SO_4 is a relatively nontoxic and unreactive compound under aerobic conditions. Pastor et al. (2017) and Fort et al. (2014) have shown that SO_4 is not directly toxic to wild rice at concentrations up to 1,600 mg L^{-1}, which exceeds concentrations in virtually all natural surface waters of the upper Midwest (Gorham, Dean, & Sanger, 1983). The U.S. Environmental Protection Agency's nonmandatory drinking water standard of 250 mg L^{-1} SO_4 (2.6 mmol L^{-1}) is based on taste rather than toxicity (EPA, 2010).

1.1. Potential Effects of Elevated Sulfate and Sulfide in Freshwater Systems

SO_4 concentrations in most freshwaters are less than a few percent of the mean concentration in seawater (2,800 mg L^{-1} (29.1 mmol L^{-1})). We surveyed 108 different lakes, streams, and wetlands across Minnesota, where the median SO_4 concentration in lakes is 10 mg L^{-1} (10th and 90th percentiles of 0.2 and 285 mg L^{-1} (MPCA, 2016)). In the much higher SO_4 concentrations of marine waters, it is well established that SO_4 can diffuse into sediment and be converted by microbial sulfate reduction (MSR) to potentially toxic sulfide that influences the presence and absence of rooted macrophytes, such as seagrasses (Borum et al., 2005; Ingold & Havill, 1984; Koch & Erskine, 2001; Lamers et al., 2013).

Despite a long-standing assumption that SO_4 is benign (Pester et al., 2012; Schindler, 1986; Urban et al., 1994) and plays a negligible role in freshwater biogeochemistry (e.g., Capone & Kiene, 1988), there is evidence that SO_4 availability in freshwaters can control the concentration and therefore the toxicity of hydrogen sulfide (H_2S) in sediment pore water to plants and animals (Bagarinao, 1992; Kinsman-Costello, O'Brien, & Hamilton, 2015; Lamers et al., 2013; Wang & Chapman, 1999). The chemical species of H_2S varies with pH; below pH 7, H_2S dominates, and above pH 7, the bisulfide ion (HS^-) dominates. For simplicity in this discussion we refer to the sum of the two species as sulfide.

1.1.1. Sulfide Toxicity to Freshwater Plants

Remarkably little attention has been given to the potential toxicity of sulfide in sediment pore water, even after Bagarinao (1992) concluded in a major review that sulfide had been "largely overlooked as an environmental factor for aquatic organisms." In a discussion of sediment toxicity testing, Wang and Chapman (1999) also observed that the biological implications of sulfide in sediments are poorly understood and "all too often ignored." They suggested that sulfide may be more important than ammonia in determining sediment toxicity to organisms and made a suite of recommendations to fill the knowledge gap, including the measurement of sulfide in undisturbed sediments. Kinsman-Costello et al. (2015) measured sulfide in undisturbed sediments and concluded that the potential toxicity of pore water sulfide is likely shaping the plant and animal communities of freshwater ecosystems. Lamers et al. (2013), in a review of sulfide toxicity to aquatic plants, pointed out that traditional toxicity testing generally neglects the chemistry of the rooting zone. Simkin, Bedford, and Weathers (2013) showed that pore water sulfide in a wetland controlled the distribution of plants more than did nutrients.

The toxicity of elevated sulfide to freshwater plants was first recognized in paddy-grown white rice (*Oryza sativa*) in the 1950s (Lamers et al., 2013; Pearsall, 1950). Rice paddies and other water-saturated soils present a profound challenge for rooted plants because of the chemical changes caused by the absence of oxygen and resulting potential toxicity of the pore water (Ponnamperuma, 1972). Anaerobic decomposition of organic matter results in elevated pore water concentrations of ammonia, organic acids, and variable concentrations of sulfide and ferrous Fe, depending on the availability of SO_4 and Fe. The interaction of Fe and the S cycle is complicated (Hansel et al., 2015), but because under anoxic conditions sulfide forms an insoluble precipitate with Fe, elevated pore water sulfide concentrations occur when Fe availability is relatively low (Ponnamperuma, 1972; van der Welle et al., 2006). It is thought that the iron sulfide precipitates are relatively inert and that only sulfide dissolved in pore water is potentially toxic. The concentration of sulfide in pore water is the balance between production and competing fates of sulfide, including precipitation with metals such as Fe, oxidation by oxygen introduced by bioturbation or by release from the roots of macrophytes (Armstrong & Armstrong, 2005), and by downward advection of surface water due to groundwater movement or transpiration by dense macrophyte stands (Bachand et al., 2014).

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1.1.2. Geochemical Consequences of Enhanced Microbial Sulfate Reduction

Increased SO_4 availability can allow increased anaerobic decomposition of organic matter, releasing the inorganic nutrients that generally limit growth of higher plants (N, P, and K) (Lamers, Tomassen, & Roelofs, 1998; Myrbo et al., 2017; Weston et al., 2006, 2011). Enhanced decomposition breaks down particulate and dissolved organic carbon (DOC) in the sediment, which can increase DOC and dissolved inorganic carbon (DIC) in the overlying water (Myrbo et al., 2017).

In addition to supporting organic matter mineralization, MSR production of sulfide causes a cascade of reactions that can alter ecosystem functioning independent of any toxicity to plants and animals. First, sulfide can participate in redox reactions, chemically reducing Fe, which converts Fe from solid phase Fe(III) oxyhydroxides to water-soluble Fe(II) (Hansel et al., 2015). The dissolution of the Fe oxyhydroxides releases sorbed ions into solution, including phosphate and trace metals (Caraco, Cole, & Likens, 1993; Davranche & Bollinger, 2000; Søndergaard, Jensen, & Jeppesen, 2003). Second, sulfide can precipitate dissolved metals, including essential plant nutrients Fe, Cu, and Zn, decreasing their bioavailability and leading to nutrient deficiency (Kirk, 2004; Lamers et al., 1998; Neue & Bloom, 1989; Neue & Lantin, 1994). In systems unpolluted with heavy metals, the sulfide precipitate is overwhelmingly dominated by iron sulfide compounds, consisting of a range of stoichiometries and minerals (Schoonen, 2004), which we here term "FeS compounds." Third, the conversion of SO_4 to sulfide entails the production of DIC, or alkalinity, an effect not fully appreciated in freshwater systems until the mechanistic consequences of acid rain were investigated in the 1980s (Baker, Brezonik, & Pollman, 1986; Cook et al., 1986; Schindler, 1986). Alkalinity is thought to be a major factor influencing the distribution of aquatic species, including macrophyte species (Moyle, 1945; Vestergaard & Sand-Jensen, 2000). In addition, elevated alkalinity may further enhance decomposition, producing a positive feedback to the effects of SO_4-driven mineralization (Geurts et al., 2009; Roelofs, 1991).

1.2. Multiple Plausible Negative Effects of Elevated Sulfate and Sulfide Production on Wild Rice

The purpose of this study was to examine the multiple ways that increases in SO_4 concentration and sulfide production can change the biogeochemical functioning of freshwater ecosystems and potentially negatively affect the growth and reproduction of wild rice populations. Before any field data were collected for this study, alternative hypotheses regarding the most likely negative effects were identified (MPCA, 2011), so that appropriate data would be available to test them. The primary hypotheses of how increases in surface water concentrations of SO_4 could harm wild rice populations include direct toxicity by elevated pore water sulfide; reduced bioavailability of Fe, Cu, or Zn; and increased P bioavailability promoting the growth of phytoplankton and macrophytes that compete for light and space. Additional variables were quantified so that exploratory data analysis could be pursued in addition to evaluation of the primary hypotheses. Surface water, pore water, and sediment physical and chemical properties were collected from 108 different sites, both with and without wild rice present, to inform evaluation of these multiple interrelated hypotheses.

2. Methods

2.1. Study Area

2.1.1. Physical Environment

SO_4 concentrations in surface water vary with geology and climate (Gorham et al., 1983) in a northeast-southwest gradient across Minnesota. Bedrock and bedrock-derived glacial deposits of northeastern Minnesota (the "arrowhead") comprise slowly weathered crystalline materials, generally low in S, and surface waters are dilute (specific conductance of $<$140 and often $<$30 μmho cm^{-1}; Gorham et al., 1983). Within this area of naturally low SO_4, iron-mining activities in the "Iron Range" district of northeastern Minnesota have created an "island" of lakes and streams elevated in SO_4 (Figure 1), a result of the weathering of sulfide minerals in waste rock piles and tailing basins. The surficial geology of southwestern Minnesota, in contrast, is derived from marine shales and carbonates that are relatively S-rich and readily weathered: surface waters often exceed 500 μmho cm^{-1} and may exceed 7,000 μmho cm^{-1} (Gorham et al., 1983). SO_4 concentration is positively correlated with conductivity above about 200 μmho cm^{-1} and 10 mg L^{-1} or 0.1 mmol L^{-1} SO_4 (Figure 7B in Gorham et al., 1983). Overprinting this geological pattern is a strong climatic gradient with warmer, drier conditions toward the southwest (toward the northern Great Plains) and colder, more humid conditions to the northeast (toward the Laurentian Great Lakes), which enhances the conductivity gradient: more evaporative conditions in the southwest serve to concentrate surface waters and further increase ionic

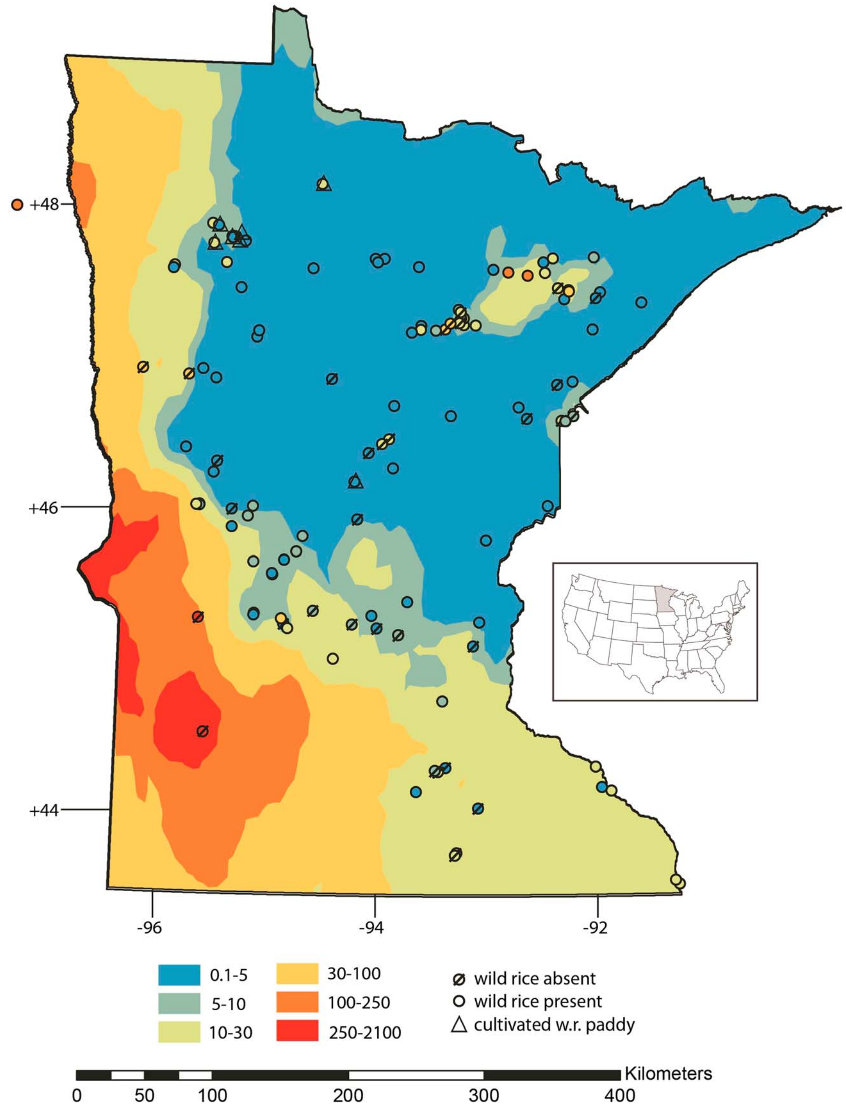

Figure 1. Map of Minnesota showing field sites overlain on kriged contours of average surface water SO_4 concentrations from 4,998 waterbodies (data from MPCA and DNR databases). The symbols are filled with the color corresponding to the site's surface water sulfate concentration. Site to the northwest of the Minnesota map is within the state of North Dakota, 40 km west of the border with Minnesota (see text). Sites where wild rice was not found have a diagonal line through the symbol.

strength, while moister conditions in the northeast maintain low ionic strength in surface waters (Gorham et al., 1983).

2.1.2. Habitat Preferences of Wild Rice

Although the scientific literature contains many assertions concerning the environmental preferences of wild rice regarding water and sediment quality (e.g., DNR, 2008, 2016; Lee, 2002; Moyle, 1944; Moyle & Krueger, 1964; Pillsbury & McGuire, 2009), sometimes little evidence was presented to support the putative preferences. The shallow freshwaters in which wild rice is found are usually relatively transparent because wild rice and other rooted macrophytes do best if they can photosynthesize as they grow each spring from the sediment to the water surface (Scheffer, 1998). Otherwise, any particular habitat preference of wild rice, such as specific chemical ranges of the sediment or surface water, is challenging to identify. Since wild rice is an opportunistic annual plant, a primary habitat requirement is periodic environmental disturbance (Grime, 1977) that keeps perennial plants such as water lilies from controlling the space and light of the shallow waters in which these species cooccur (Pillsbury & McGuire, 2009). Wild rice does not seem to have specific sediment requirements, as it has been observed growing in a variety of substrates (Aiken et al., 1988; Lee,

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1986) within its range. Annual plants are adapted to exploit environments intermittently favorable for rapid plant growth and to maximize seed production (Grime, 1977). Wild rice produces between 25 and 150 relatively large seeds per stem (Eule-Nashoba, Biesboer, & Newman, 2012). Seeds buried in the sediment can survive for up to several decades until conditions are again favorable for germination and growth (DNR, 2008). *Zizania palustris* seeds germinate at low rates unless they have been exposed to near-freezing temperatures for at least 3 months (Kovach & Bradford, 1992), although the environmental cues for subsequent germination are poorly understood, aside from elevated temperature.

Because *Z. palustris* does not normally self-pollinate and is wind-pollinated (which implies that the pollen source must be relatively nearby; Friedman & Barrett, 2009), does not reproduce asexually, and has an annual life cycle (Aiken et al., 1988), in this analysis the presence of wild rice plants is taken to mean that the water-body hosts a successfully reproducing and self-sustaining population. A few of the sites in this study may have experienced recent watershed changes such that steady state has not yet been reached among environmental variables and wild rice reproduction, but the balance of the 108 sites should have been in steady state at the time of sampling. The population of wild rice in a given lake can exhibit large fluctuations from year to year, which has been attributed to disturbances such as abrupt increases in water level (DNR, 2008) and to cyclical changes in N availability (Walker et al., 2010). Wild rice fills a unique ecological niche, in that there are virtually no other annual aquatic macrophytes across the Great Lakes region (Eggers & Reed, 2011).

2.1.3. Site Selection

The goal of the field survey was to identify and sample potential wild rice habitat across a gradient of SO_4 concentrations. Potential wild rice habitat was defined simply as shallow water (30–120 cm deep, though some sampled sites had water as shallow as 5 cm) that was sufficiently transparent to support rooted aquatic macrophytes. Turbidity from dense phytoplankton blooms or suspended solids may effectively exclude rooted macrophytes due to light limitation (Scheffer, 1998).

Most Minnesota lakes that host wild rice populations have low SO_4 concentrations; the median concentration of wild rice lakes is 1.8 mg L^{-1}, and the 75th percentile is 3.6 mg L^{-1} (MPCA, 2014). If only those waters hosting abundant wild rice were sampled, most of those would be low in SO_4, and little would be learned about how elevated SO_4 concentrations (or other important variables) might affect wild rice. Conservation biologists commonly identify important habitat variables through binary (i.e., is the species present or absent at a given site?) logistic regression, which requires the sampling of sites that do not support the species of interest (e.g., Carroll, Zielinski, & Noss, 1999; Peeters & Gardeniers, 1998; van der Heide et al., 2009). Consequently, potential wild rice habitats with a range in SO_4 concentrations were sampled without regard to whether the waterbody hosted or was known to host wild rice. An effort was made to sample waterbodies that covered the range of SO_4 concentrations across Minnesota and especially to sample sites in high-SO_4 regions that had reports of recent or historical presence of wild rice. This strategy also resulted in the sampling of a high-SO_4 wild rice site in the North Branch of the Turtle River (198 mg SO_4 L^{-1}), 40 km into North Dakota from northwestern Minnesota (Figure 1). A gradient of SO_4 concentrations was sampled by identifying potential wild rice habitat in two areas of elevated SO_4, the "island" of elevated SO_4 in the Iron Range region of northeastern Minnesota and waters naturally elevated in SO_4 to the west and south of the known range of wild rice (Figures 1 and S1). Nutrient (N and P) availability (reflecting natural soil fertility as well as agricultural runoff) also increases to the west and south, potentially supporting phytoplankton growth sufficient to exclude macrophytes, including wild rice. Consequently, in western and southern Minnesota, DNR lake databases were screened for the presence of water lilies (*Nuphar variegata* or *Nymphaea odorata*), the occurrence of which indicates transparency sufficient to support rooted macrophytes that also often cooccur with wild rice. In an analysis of DNR aquatic plant surveys from 1,753 shallow lakes, we found that the odds of finding wild rice where there are water lilies are 26 times the odds of finding wild rice where there are no water lilies, with a 95% confidence interval of 20–36 times (neither wild rice nor water lilies = 968; wild rice but not water lilies = 60; no wild rice but water lilies = 272; both wild rice and water lilies = 453).

With these considerations, water bodies were selected for sampling based on preliminary data including average water depth, presence of water lilies, conductivity or SO_4 concentration, and geographic distribution (i.e., to sample widely across the state). Both rivers and lakes were sampled, as were seven different cultivated wild rice paddies. Within a given water body, the field team chose a location for sampling based on a decision tree (Table S1 in the supporting information).

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2.2. Field Methods

Sampling occurred in August–September 2011 and June–September 2012 and 2013. Sampling efforts were focused in late summer to capture physical and chemical conditions when wild rice plants were maturing and when identification of wild rice is most certain; voucher specimens of wild rice were always taken. Samples were collected from an anchored canoe or small boat, except in the cultivated wild rice paddies when the water was too shallow to float a canoe, and samples were collected on foot. A Hach model HQ40d or Hydrolab Quanta sonde, calibrated daily, was used to measure temperature, specific conductance, dissolved oxygen, and pH in the surface water. Water transparency was determined with a 1 m long Secchi-tube (Water Monitoring Equipment & Supply, USA), and apparent color was measured using a Hach model CO-1 color test kit. Surface water samples (later split into separate subsamples; see below) were collected by a technician wearing long nitrile gloves in two 2 L amber Nalgene bottles that had been previously triple rinsed with deionized water and rinsed 3 times with water from the site before filling. Water samples were stored on ice.

Short (~50 cm) sediment cores with ~10–20 cm of overlying water were collected at eight undisturbed locations at least 1 m apart around the boat using an HTH corer (Pylonex, Sweden) with a 7 cm diameter polycarbonate barrel. A piston was inserted in the bottom end of each core as it was retrieved. Cores were kept upright and shaded prior to sample processing.

Aquatic macrophytes were identified and percent cover estimated within a plastic hoop 1 m in diameter placed at 4 locations around the boat. In 2012 and 2013, the number of stems of wild rice inside each hoop was also quantified.

Pore water samples were obtained from cores processed on shore using 10 cm Rhizon™ filters (pore size 0.12–0.18 μm) (Rhizosphere.com, Netherlands; Shotbolt, 2010) inserted vertically into the core tops, following extrusion of overlying water, and connected to evacuated serum bottles with PVC/polyethylene tubing and a stainless steel needle. Three separate cores were sampled, one for nutrients (nitrate + nitrite, TP, and TN) and DOC (70 mL), a second for metals (50 mL), and a third for dissolved silica, Cl, and SO_4 (30 mL). A fourth core was sampled for pore water sulfide, but in this case, the serum bottle was preloaded with 0.2 mL of 2.0 N zinc acetate, 0.5 mL of 15 M sodium hydroxide, and a stir bar, flushed with a nitrogen atmosphere, evacuated, and preweighed. Air was flushed from the Rhizon-tubing assembly with sample pore water using a second evacuated bottle before the needle was inserted into the sulfide sample bottle. In 2011 only pore water samples for sulfide and metals were collected. Pore water pH was measured on a fifth core by inserting the probe of a Hach model HQ40d pH meter into the sediment to a depth of 5 cm and allowing the reading to stabilize.

A composite sediment sample was collected from the uppermost 10 cm of the sixth, seventh, and eighth cores, placed into a stainless steel bowl, and stirred under nitrogen atmosphere to homogenize. A 50-mL subsample was placed in a polypropylene sample bottle along with 1.0 mL of 1.0 N zinc acetate for analysis of acid-volatile sulfide (AVS). The headspace of the AVS sample bottle was flushed with N_2 and the bottle capped; that bottle was placed in a larger glass jar and that jar flushed with N_2 and tightly sealed. The sample was immediately placed in a cooler with dry ice to freeze. The remaining composited sediment was placed into a polycarbonate container and stored on ice for later analysis.

Water subsamples were taken from the large amber Nalgene bottles by a technician wearing nitrile gloves. Sulfuric acid (5 mL of 10%) was immediately added to subsamples for the analysis of P, TKN (total Kjeldahl nitrogen), ammonia, and nitrate + nitrite in 250 mL polyethylene bottles. Nitric acid (2.5 mL of 20%, to acidify to pH < 2) was immediately added to subsamples for the analysis of total metals in 250 mL polyethylene bottles. Samples for dissolved metals were subsampled in 250 polyethylene bottles and subsequently filtered using a 0.45 μm filter and preserved with nitric acid in the laboratory. Samples were stored on ice.

2.3. Laboratory Methods

Surface water, pore water, and most sediment analyses were conducted by the Minnesota Department of Health Environmental Laboratory (MDHEL) in 2012 and 2013, following standard methods. In 2011 other laboratories conducted the analyses (University of Minnesota Soils Laboratory (UMNSL), St. Croix Watershed Research Station (SCWRS), and Gustavus Adolphus College (GAC)); methodological differences are noted, and the laboratory is identified where relevant.

Surface and pore water samples were analyzed for anions (Cl and SO_4) by ion chromatography on a Dionex ICS-3000 (MDHEL), Fe, Ca, Na, Mg, and K by inductively coupled optical emission spectrometry on a Varian 715-ES (MDHEL), Mn, Cu, Zn, Co, Ni, Al, As, and Se by inductively coupled mass spectrometry on a Perkin Elmer Elan DRCE (MDHEL), and DOC by UV-persulfate oxidation on a Tekmar-Dohrmann Phoenix 8000 (SCWRS and MDHEL). N and P were measured by colorimetric methods (on a Lachat Quikchem Flow-Injection Autoanalyzer) at SCWRS and MDHEL following cadmium-reduction (nitrate and ammonia) or dual alkaline-persulfate digestions (TP and TN). Silica was measured colorimetrically at SCWRS on the Lachat Autoanalyzer and at MDHEL on a Beckman Coulter DU 800 UV/VIS spectrophotometer. Pore water sulfide was analyzed colorimetrically on each lab's Lachat Autoanalyzer following in-line acid distillation and NaOH trapping (SM 4500-S2). Alkalinity was measured by potentiometric acid titration (MDHEL) or as DIC by acid digestion and IR detection (SCWRS). DIC was converted to alkalinity using pH, temperature, and specific conductance of the surface water.

Sediment samples from 2011 were analyzed by combustion for total carbon (TC), total nitrogen (TN), and total sulfur (TS) using, respectively, a Tekmar Phoenix 8000 CO_2 analyzer, an Elementar Vario Max N analyzer, and a LECO sulfur analyzer (UMNSL). Samples from 2012 and 2013 were analyzed for CHN on a Costech 4010 Elemental Analyzer (UMN Stable Isotope Laboratory). Total inorganic carbon (carbonate) was analyzed by coulometric titration on a UIC CM5015 CO_2 coulometer, while water and organic matter content were determined by loss-on-ignition methods (Heiri, Lotter, & Lemcke, 2001) in the UMN LacCore facilities. Sediment AVS was analyzed colorimetrically, as above for pore water sulfide, following acid-distillation and in-line alkaline trapping (SM 4500-S2; Hsieh & Shieh, 1997).

Sediment phosphorus was extracted from freeze-dried sediments following methods of Engstrom and Wright (1984) for total-P and Hieltjes and Lijklema (1980) for P fractions (NH_4Cl-extractable, NaOH-extractable, HCl-extractable, and residual (organic)-P). The P extracts were measured colorimetrically by flow-injection autoanalyzer (SCWRS). Extractable iron and trace metals were quantified from a 0.25 g homogenized freeze-dried sediment subsample incubated in 0.5 M HCl for 30 min at 80°C. The samples were centrifuged, decanted, and analyzed by ICP-MS at GAC. This extraction releases metal oxyhydroxides, sulfides, and loosely bound phases from the sediment without appreciably attacking the silicate matrix (Balogh et al., 2009).

2.4. Data Subsets and Statistical Analysis

From 2011 to 2013, 260 site visits were conducted in 108 different natural waterbodies, including lakes, small streams, backwaters of the Mississippi River, and wetlands, plus 7 different cultivated wild rice paddies. For a variety of logistical reasons, the full suite of samples could not be collected on some site visits. Three subsets of the field data were identified for the analyses reported here. A subset that consists of all of the samples from natural waterbodies (excluding the cultivated paddies) with virtually complete analyses (surface water, pore water, and bulk sediment; $n = 194$) was termed for internal purposes Class D. Pollman et al. (2017) used Class D to develop a structural equation model to elucidate key variables that govern the concentration of sulfide in sediment pore water. A second subset, Class S, of 51 samples from 7 stream and 8 lake sites was each sampled 3 to 5 times from 27 May to 19 September 2013, to provide a data set to assess seasonality in variables. A third subset, Class B, was used for conducting probabilistic analyses to identify the most likely parameters controlling the presence and absence of wild rice and to examine Spearman nonparametric correlations among field variables. Class B consists of one sample from each site ($n = 108$) and excludes samples collected in 2011, which were analyzed with slightly different lab methods. Although Class B was not created as a random sample of Minnesota waterbodies, the frequency distribution of SO_4 concentrations is intermediate between a probability-based survey conducted by USEPA (MPCA, 2016) and a list of known wild rice water bodies, which are overwhelmingly low-SO_4 lakes (MPCA, 2014). Therefore, the Class B data set is reasonably representative of potential wild rice habitat, and binary logistic regressions can be used to approximate the probability of wild rice occurrence as a function of field variables such as pore water sulfide. The Class B data set is used in all analyses presented here, with two exceptions: (1) Figures 3a–3c present the full Class D data set, plus for comparison, data from the cultivated wild rice paddies; (2) Statistical analysis was performed with the software package R version 3.2.3.

To identify variables associated with the presence and absence of wild rice, we relied on binary logistic regressions (BLR), using the glm function in R. BLR does not require normally distributed data, but

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Table 1
Field Variable Correlation With the Presence-Absence of Wild Rice, Assessed Through Binary Logistic Regression (BLR), Plus Spearman Correlations Between Variables[a]

	Binary logistic regression (BLR) correlation with field variable			Spearman correlation with field variable (rho)				
Field variable	Log transformed?	*p* value	Correlation direction	Wild rice density (only wild rice sites) *n* = 67	Pore water sulfide	Sediment AVS	Water transparency	Water temperature
pw K	Y	0.0008***	Negative	−0.27*	0.46***	0.11	−0.10	0.33***
pw sulfide	Y	0.0012***	Negative	−0.31**	1.00	0.29**	−0.07	0.17
Water depth (m)	N	0.0028***	Negative	0.07	0.11	0.08	0.22*	0.19*
Transparency (cm)	N	0.0031***	Positive	0.11	−0.07	−0.13	1.00	−0.08
sw TN	Y	0.0054**	Negative	−0.12	0.22*	0.08	−0.61***	0.23*
sed Se % dry	N	0.0059**	Negative	0.12	0.08	0.27**	−0.21*	0.13
sw Temp	N	0.0077**	Negative	0.08	0.17	0.11	−0.08	1.00
pw Fe	Y	0.0109*	Positive	0.20	−0.58***	0.00	0.04	0.09
sw pH	N	0.0200*	Negative	−0.14	0.28**	0.08	−0.05	0.35***
sw TP	Y	0.0353*	Negative	0.15	0.05	0.29**	−0.58***	0.27**
Latitude	N	0.0376*	Positive	−0.04	−0.06	−0.09	0.13	−0.51***
sed TS % dry	Y	0.0483*	Negative	0.20	0.40***	0.42***	0.03	−0.08
sw K	Y	0.0922		−0.03	0.29**	0.21*	−0.18	−0.08
sed AVS % dry	Y	0.1317		0.02	0.29**	1.00	−0.13	0.11
sw sulfate	Y	0.1475		−0.10	0.44***	0.45***	−0.07	0.04
sed TP % dry	N	0.2697		−010	0.07	0.30**	−0.14	0.14
sw alkalinity	Y	0.2786		0.25*	0.22*	0.26**	0.11	0.17
pw TN	Y	0.2963		−0.30	0.31***	0.14	−0.20*	0.34***
pw NH4	Y	0.4505		−0.33**	0.33***	0.22*	−0.17	0.26**
sed Fe % dry	Y	0.4795		0.16	−0.35***	0.38***	−0.10	−0.06
pw DOC	Y	0.4865		0.08	−0.05	−0.1	−0.21*	0.09
pw Si	N	0.5548		0.03	0.33***	0.18	0.07	0.29**
pw TP	Y	0.6341		0.02	0.12	0.30**	−0.26**	0.26**
sed TN % dry	N	0.6807		−0.06	0.14	0.04	0.06	−0.06
sed water content	N	0.7274		−0.07	0.15	0.10	0.10	0.02
sed TOC % dry	N	0.7854		−0.10	0.10	0.02	0.10	−0.06

[a]Note. The variables are ordered by the significance of the BLR. The first 12 variables have BLR significance of $p < 0.05$. The additional 14 variables are listed because of their correlation with pore water sulfide, sediment AVS, or surface water transparency or temperature—or their notable lack of correlation with wild rice presence-absence (pw = pore water; sw = surface water; sed = sediment).
*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

nevertheless, we transformed some variables (Table 1) to achieve approximately normal distributions. BLR also yields the probability of occurrence at a given value of the variable. Spearman nonparametric correlations (rho) between field variables in Class B were examined as part of the effort to identify the major biogeochemical interactions in these shallow-water systems. Seasonality in a variable was assessed using linear mixed effects models, with time (fraction of a year) as a fixed variable and site as a random factor (to account for multiple samples per site). In the model developed for each field variable, we accounted for the seasonal cycle using the following equation (Crawley, 2007), where *A*, *B*, and *C* are fitted model coefficients:

$$y = A + B\ \sin(2\pi\ \text{time}) + C\ \cos(2\pi\ \text{time}) \quad (1)$$

3. Results and Discussion

3.1. Field Variables Associated With Wild Rice Presence and Absence

Of the 64 field quantified variables, BLR identified 12 that are associated with the presence/absence of wild rice at the 0.05 probability level or better (Table 1): pore water sulfide, K, and Fe (Figures 2a, 2b, and 2f); surface water temperature, TP, TN, and pH (Figures 2d, 2m, 2n, and 2o); sediment Se and TS (Figures 2c and 2p); water depth and transparency (not shown; Figure 2e); and latitude of the site (Figure 2g). These variables may be important in controlling the presence or absence of wild rice or may merely be correlated with one or more actual causative factors. Because we are primarily interested in factors that control presence or absence of wild rice, in contrast to the density of wild rice, we place primacy on the BLR results and use the Spearman

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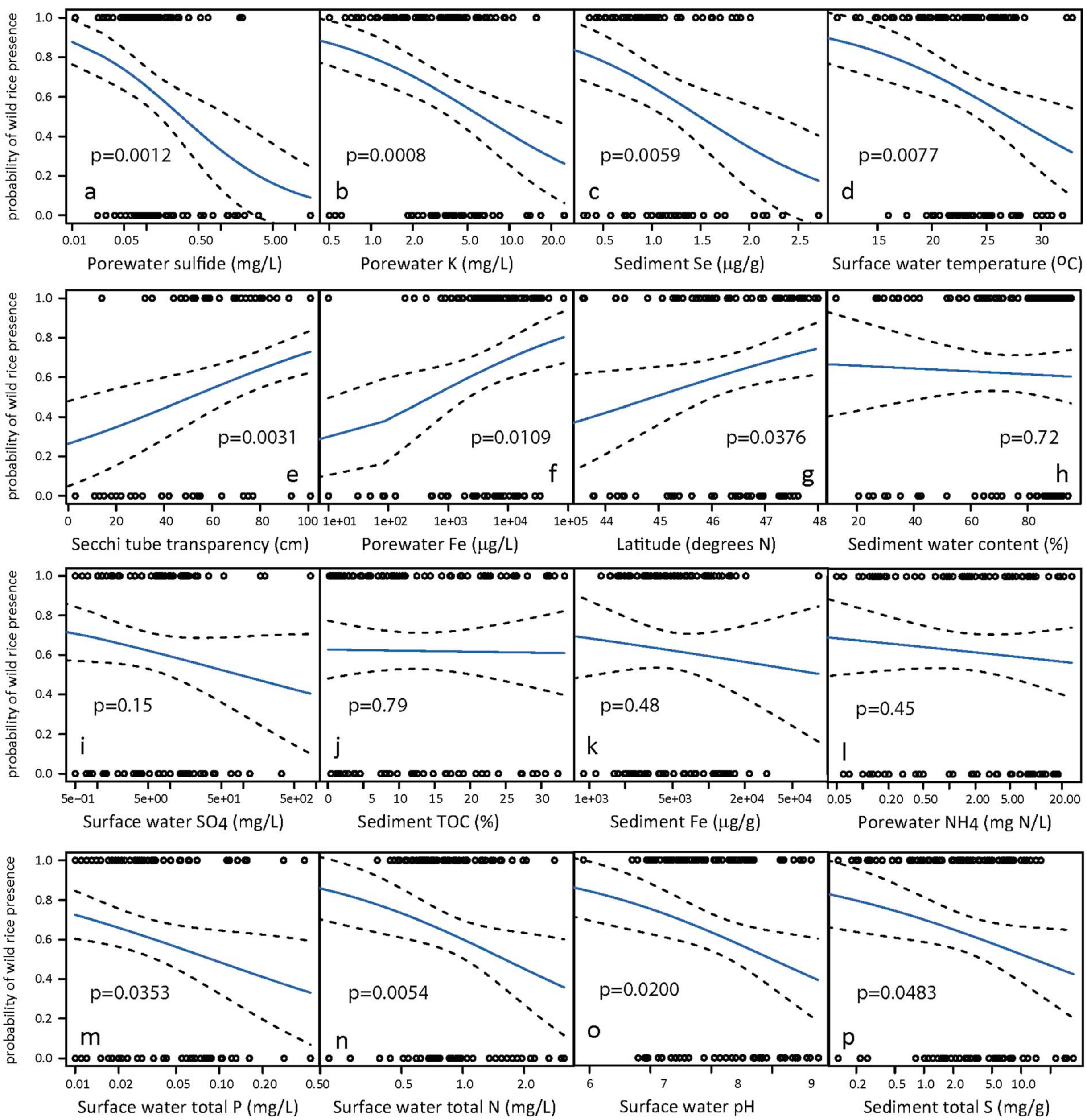

Figure 2. Binary logistic regressions for 16 variables found to be statistically significant in this study or indicated in the literature as important for wild rice habitat. The open circles indicate the value for a given parameter of each site used in the analysis; the circles at the top of a plot indicate sites with wild rice present; the circles at the bottom of a plot indicate sites with wild rice absent. The span of the dashed lines around the solid line indicates the 95% confidence interval.

correlations to help understand the relationships between environmental factors of interest. Wild rice density is negatively correlated with pore water sulfide, potassium (K), and NH_4 and positively correlated with surface water alkalinity (Table 1). There are likely factors controlling the density of wild rice at any particular location in addition to the variables measured, such as herbivory and hydrological disturbances.

One should be cautious in the interpretation of statistically significant associations between wild rice and field variables, in that true cause and effect are not necessarily obvious. For instance, is the toxic quality of pore water sulfide sufficient explanation for its negative associations with both the presence and density of wild rice? Do sites with greater density of wild rice have lower pore water sulfide because low sulfide allows wild rice to grow, or because plants release oxygen from their roots, oxidizing sulfide? The true explanation is likely a combination of the two mechanisms: elevated pore water sulfide can eventually extirpate a wild rice

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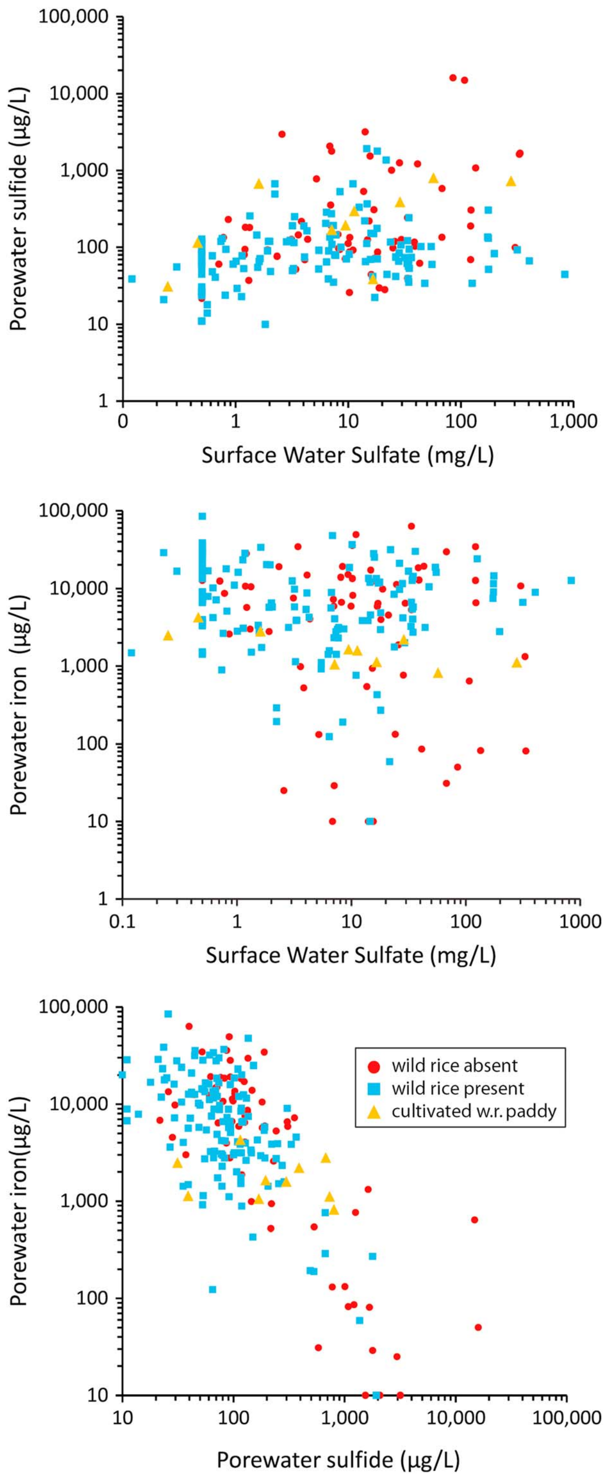

Figure 3. Class D data plus cultivated wild rice paddies (yellow triangles) showing sites with wild rice present (blue squares) and absent (red circles). Note that (a) pore water sulfide is not linearly related to surface water SO_4, indicating that additional factors besides SO_4 concentration contribute to the sulfide concentration in pore water; (b) wild rice can occur in sites over a wide range of surface water SO_4 values as long as pore water Fe is relatively high; and (c) pore water Fe and sulfide have a strong negative relationship, due to the energetically favorable formation of FeS compounds, and that wild rice mainly occurs where pore water sulfide is <1,000 μg L^{-1}.

population by reducing seedling survival and seed production, weight, and viability (Pastor et al., 2017), but oxygen release from roots when sulfide concentrations are low enough to allow wild rice to successfully reproduce drives sulfide to even lower concentrations than would have occurred without the plants (Myrbo et al., 2017). Although the negative correlation between pore water sulfide and wild rice density (rho = -0.31, $p < 0.01$, Table 1) could be interpreted to mean that lower sulfide allows a larger population to grow, it is also possible the correlation is partly caused by oxygen release from the wild rice roots, decreasing sulfide concentrations. This hypothesis is supported by the observation from a controlled experiment that acid-volatile sulfide (AVS) sediment concentrations were 30% lower when wild rice was present (Myrbo et al., 2017).

Of the 12 variables significantly associated with the occurrence of wild rice, we propose that nine are noncausal and merely related to one or more of three truly causative factors: (1) pore water sulfide, (2) water transparency, and (3) water temperature. These three variables are not significantly correlated with one another (Table 1), and there are plausible mechanisms for each to independently exclude wild rice from otherwise suitable habitat: (1) elevated sulfide reduces the growth of wild rice (Pastor et al., 2017); (2) low water transparency plausibly constrains the ability of a germinated wild rice seed to reach sufficient light before it runs out of endosperm energy (Aiken et al., 1988; DNR, 2008); and (3) warmer winter water temperature at lower latitudes may limit seed germination, as the seeds of *Z. palustris* must experience at least 3 months in cold water to break seed dormancy (Kovach & Bradford, 1992) (although summer temperatures were measured in this study, winter and summer temperatures would be correlated).

3.2. Pore Water Sulfide as a Causal Variable and Associated Correlated Variables

Our primary a priori hypothesis was that elevated surface water SO_4 can produce elevated pore water sulfide concentrations which, in turn, negatively affect potential wild rice habitat. A plot of pore water sulfide against SO_4, with wild rice presence noted (Figure 3a), suggests that wild rice is generally not found at sites with elevated sulfide. Of the sites with pore water sulfide greater than 1,000 μg L^{-1}, wild rice was present in 23% (3 of 13, with only a few plants found at 2 of those 3 sites). In contrast, wild rice was present at 74% of the sites where sulfide was less than 1,000 μg L^{-1}. Despite historical reports of *Zizania aquatica* in Minnesota, inspection of voucher specimens, following the taxonomy of Terrell et al. (1997), found only *Z. palustris* at all sites sampled. *Zizania aquatica* (Southern wild rice) is rare in Minnesota, but its range extends east through Wisconsin and the southern part of the Great Lakes region to New England and occurs in freshwater tidal marshes south to Florida and west to Louisiana (Terrell et al., 1997). The findings of this study, especially those related to temperature, may not apply to *Z. aquatica*.

Despite a strong relationship between sulfide and wild rice, the relationship between SO_4 and pore water sulfide among the 108 field sites was not a simple linear function. High surface water SO_4 did not always result in high pore water sulfide (above 1,000 μg L^{-1}), and some samples with surface water SO_4 less than 10 mg L^{-1} had pore water sulfide greater

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than 1,000 μg L^{-1} (Figure 3a). The 33 samples with surface water SO_4 below 1.0 mg L^{-1} all had pore water sulfide less than 200 μg L^{-1}, suggesting that surface water SO_4 might limit maximum sulfide production.

Wild rice was present at high and low SO_4 concentrations as long as pore water Fe was high and sulfide was low (Figures 3b and 3c): pore water Fe and sulfide each exert antipathetic control over the concentration of the other (Figure 3c; Pollman et al., 2017), and wild rice mainly persists in sites where pore waters are low in sulfide and high in Fe. Pollman et al. (2017) developed a structural equation model showing that variations in three variables (SO_4, sediment TOC, and sediment extractable Fe) contribute nearly equally to the observed variations in pore water sulfide among the sites in this study. Sulfate-reducing bacteria are simultaneously limited in their production of pore water sulfide by surface water SO_4 and sediment TOC (which are roughly proportional to available SO_4 and labile organic matter, respectively). Concentrations of sulfide in pore water are then constrained by the availability of pore water Fe, which is in turn controlled by the overall supply of Fe in the sediment (Pollman et al., 2017).

If pore water sulfide concentration is a causative factor controlling the presence of wild rice, as hypothesized, then other variables may be statistically significant because they either vary with the process of SO_4 reduction or with the sulfide itself. Pastor et al. (2017) showed that wild rice growth declines in proportion to sulfide concentrations in both hydroponic and outdoor mesocosm experiments. In a study of the Pastor et al. mesocosms, Myrbo et al. (2017) showed that the enhanced mineralization of plant litter associated with SO_4 reduction produced increases in surface water P and N, and increases in pore water sulfide caused decreases in pore water Fe. These effects from the controlled mesocosm experiment are consistent with correlations observed among sites in the field data between pore water sulfide and surface water N and pore water Fe (Table 1). Surface water P is not significantly correlated with pore water sulfide in the field data, despite dual mechanisms of P mobilization from sediment, (1) the interaction between sulfide and Fe (Caraco et al., 1993; Maynard, Dahlgren, & O'Geen, 2011; Smolders & Roelofs, 1993) and (2) mineralization of organic matter. The lack of correlation of surface water P with pore water sulfide in this field study is not surprising because given the wide variety of landscapes sampled, there is no reason that pore water sulfide would be proportional to the mass of Fe that has reacted with sulfide and released sorbed P. In SO_4 addition experiments where the P and Fe content of the sediment is held constant, there is often a significant correlation between pore water sulfide and mobilized P (Myrbo et al., 2017). In contrast to the lack of correlation of pore water sulfide with surface water P in this field study, AVS is significantly correlated with surface water P (rho = 0.30, $p < 0.01$, Table 1), presumably because AVS is proportional to the iron that has reacted with sulfide.

Elevated pore water sulfide is a product of SO_4 reduction-driven mineralization of sediment organic matter, which also releases the constituents of the decaying plant material, N, P, K, silica, and C (either as DIC, which increases alkalinity, or DOC) into sediment pore water and the overlying surface water (Myrbo et al., 2017). Consequently, the occurrence of wild rice is negatively associated not only with pore water sulfide ($p = 0.001$) but also with elevated pore water K ($p = 0.0008$) and surface water TN ($p = 0.005$, Table 1). Median concentrations of these variables are lower in wild rice waters compared to waters with no observed wild rice (88 versus 126 μg L^{-1} sulfide, 2.4 versus 4.3 mg L^{-1} pore water K, and 0.74 versus 0.95 mg L^{-1} TN, Table S3). Pore water sulfide is itself positively correlated with pore water K, TN, NH_4, and silica, surface water K, alkalinity, and pH, and sediment TS and AVS (Tables 1 and S4).

The strong evidence for the association of elevated pore water K with the absence of wild rice (Table 1) is interesting, as K is an essential plant nutrient, and therefore, it is unlikely that the association is based on toxicity to wild rice. Rather, it is likely that the association is a result of the simultaneous mobilization of K with the production of sulfide as plant matter is mineralized. Potassium does not bond covalently with organic compounds and is readily leached out of dead organic matter (Troeh & Thompson, 2005). Silica phytoliths dissolve as plant matter is mineralized, allowing additional K that had been trapped within the phytoliths to be released into sediment pore water (Nguyen et al., 2015). Wild rice and other wetland macrophytes develop abundant phytoliths that release dissolved silica upon decomposition (Struyf & Conley, 2009). Additional dissolved silica is likely released to pore water as epiphytic diatoms are mineralized. Pore water silica, K, and sulfide are all significantly correlated with each other (Tables 1 and S5). The negative correlation of pore water K with wild rice may be magnified by its additional positive correlation with elevated water temperature (Table 1), which plausibly accelerates dissolution of silica in organic matter (Gudasz et al., 2010; Kamatani, 1982).

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AVS largely consists of solid-phase sulfide, which is not available to plants and therefore is not significantly associated with wild rice presence/absence ($p > 0.10$, Table 1). AVS is a measure of cumulative sulfide production, which is proportional to past mineralization of organic matter, consistent with significant positive correlations between AVS and pore water TP and NH_4, and surface water TP and alkalinity ($p < 0.01$, 0.05, 0.01, and 0.01, respectively, Table 1).

The negative association of sediment total-S (TS) with wild rice presence (BLR $p = 0.048$, Table 1) is likely the result of both TS and wild rice being controlled by sulfide production; the median TS concentration at sites with wild rice is 2.6 mg g^{-1}, compared to 4.1 mg g^{-1} at sites without wild rice (Table S3). Sediment TS is correlated with both pore water sulfide and AVS. The negative association of sediment Se with wild rice (BLR $p = 0.006$) is surprising, given that median Se concentrations are very low (0.9 and 1.2 μg g^{-1} in sites with and without wild rice, respectively). The slightly higher Se at sites without wild rice may be caused by the coprecipitation of Se and S by SRB, as shown by Hockin and Gadd (2003). Selenium is correlated with sediment total S (rho = 0.35; $p < 0.001$).

3.3. Water Transparency as a Causal Variable and Associated Correlated Variables

Lower water transparency is associated with a lower probability of wild rice occurrence (BLR $p = 0.003$). Transparency is not related to wild rice density (rho = 0.11, $p > 0.20$, Table 1); however, low transparency can apparently exclude wild rice (11 of the 12 sites with transparency <30 cm had no wild rice), but above that threshold, other variables control wild rice density. Low water transparency decreases photosynthesis of wild rice seedlings while growing to the water surface, which (a) decreases oxygen production that could otherwise be used to detoxify sulfide internally (Krüssel et al., 2014), or externally if released into pore water from the roots (Colmer, 2003); and (b) decreases the energy available for root development, enhancing vulnerability to a sudden increase in water depth, which can uproot an entire year's cohort (DNR, 2008). Wild rice is unusual among grasses in that the stem develops before the root, probably because the seedling may have to grow over 50 cm before reaching the water surface, at which time floating leaves are developed that can supply energy for root development (Aiken, 1986). If transparency is too low, or the water too deep, the energy stored in the seed can be insufficient for the seedling to reach the water surface.

Water transparency can be controlled by the density of phytoplankton, which was not measured in this study. However, transparency is highly correlated with the nutrient concentrations that control phytoplankton growth, total phosphorus (rho = −0.58, $p < 0.001$), and total nitrogen (rho = −0.61, $p < 0.001$). Transparency can also be controlled by colored dissolved organic matter, consistent with the observed significant correlation between transparency and water color (rho = −0.68, $p < 0.001$, Table S4).

3.4. Temperature as a Causal Variable and Associated Correlated Variables

Wild rice germinates at higher rates following longer exposure at temperatures closer to freezing (Kovach & Bradford, 1992), consistent with the significant association of wild rice absence with lower latitudes (BLR $p = 0.04$) and warmer summer water temperature (BLR $p = 0.008$; medians of 21.9° and 23.7°C with and without wild rice, respectively). Sites with warmer summer water temperatures generally also have warmer winter temperatures. Warmer and shorter winters would cause lower germination rates, ultimately reducing the probability that a population could persist over the long term. For this reason, the Minnesota Department of Natural Resources hypothesizes that climate change may push the range of wild rice farther north (DNR, 2008, 2016). However, the temperature control of wild rice germination has not been adequately investigated to rigorously assess this hypothesis. The observed association of wild rice with lower pH surface waters (BLR $p = 0.04$; median pH values of 7.8 and 8.5 at sites with and without wild rice, respectively) may be due simply to the correlation of wild rice with cooler surface waters: the solubility of CO_2 is higher in colder water, leading to lower pH (Spearman correlation between temperature and pH = 0.35, $p < 0.0001$).

Lower latitude sites are negatively associated with wild rice presence and density (Table 1). Lower latitude sites are correlated with higher water temperature (rho = −0.51, $p < 0.001$). Geological and land use gradients also correlate with latitude, producing correlations with surface water N and P (rho = −0.25 and −0.31, $p < 0.01$). Despite these correlations with nutrients, latitude is not significantly correlated with water transparency (rho = 0.13), so the general unsuitability of lower latitudes for wild rice may be a combination of warmer winters and some reduced transparency as a result of nutrient enrichment. Latitude is not significantly correlated with pore water sulfide (rho = −0.06).

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3.5. Synergy Among the Three Causal Variables of Sulfide, Temperature, and Transparency

While temperature may control wild rice presence/absence through winter temperatures too warm to achieve high rates of seed germination in the spring, higher summer temperatures may act synergistically on variables correlated with pore water sulfide. Elevated summer temperatures likely enhance microbial activity, no matter which electron acceptor is respired by the dominant microbes (Gudasz et al., 2010). Water temperature is indeed correlated with pore water concentrations of the plant nutrients N, P, and K released by decomposition, which are also significantly correlated with either pore water sulfide or AVS (Table 1). Although sulfide production may be enhanced by elevated temperature, the correlation between sulfide and temperature is weak (rho = 0.17; $p > 0.05$).

The production of sulfide plausibly contributes to the significant correlation of six of the nine variables with wild rice occurrence: P and N in surface water, Fe and K in pore water, and S and Se in sediment. Water temperature is significantly correlated with three of these variables (P, N, and K), and therefore, synergistically reinforces their associations with the absence of wild rice. Elevated surface water P and N are further synergistically associated with the absence of wild rice because their release via MSR-driven mineralization also enhances phytoplankton growth, reducing light available to wild rice seedlings; P and N are highly correlated with reduced transparency ($p < 0.001$, Table 1).

In this data set greater water depth is negatively associated with the occurrence of wild rice, not because wild rice grows better in shallower water, which it may, but because of where field crews took samples when wild rice was not present. Following a decision tree (Table S1), when wild rice was not present, field crews usually sampled among water lilies, which, on average, were observed in slightly deeper water than wild rice (67 cm compared to 52 cm).

Finally, *multiple* BLR was employed to investigate the question of whether any of the correlated variables provided additional explanatory power for the occurrence of wild rice beyond the models with sulfide, transparency, or temperature as base predictors. Surface water temperature is predictive of wild rice presence independent of sulfide ($p = 0.03$). Pore water K improves models based on sulfide ($p = 0.02$) or temperature ($p = 0.004$) alone but provides no significant additional explanatory power beyond a model built on both sulfide and temperature ($p = 0.07$). Overall, multiple BLR analysis confirms that other variables provide no additional explanatory power and that the three base predictors are independent of each other.

Multiple BLR was used to model the probability of wild rice occurrence (WR presence) as a function of pore water sulfide (pw sulfide, in mg L^{-1}), water transparency as measured by the Secchi tube (trans, in cm), and water temperature (Temp, in °C):

$$\text{Logit}\,(\text{WR presence}) = 0.532 + 0.0183\,(\text{trans}) - 1.169\,(\log10\ \text{pw sulfide}) - 0.107\,(\text{Temp}) \quad (2)$$

where odds = exp(logit) and probability = (odds/(1 + odds)).

Pore water sulfide and water transparency are significant variables in the multiple BLR ($p = 0.012$ and $p = 0.016$, respectively), whereas water temperature is only marginally significant ($p = 0.056$). To visualize the interaction of the variables, a 3-D plot was constructed that predicts the probability of wild rice occurrence as a function of sulfide and transparency, while holding constant the marginally significant variable, temperature, at the median value of the Class B data set (23.2 C; Figure 4). Within the range of variables in Class B, modeled probabilities of wild rice occurrence range from a high of 89.8% (trans = 100 cm, which was the maximum of the measurement device, and pw sulfide = 11 μg L^{-1}, which was the reporting limit) to a low of 9.6% (trans = 3 cm, which was the minimum observed, and pw sulfide = 2,000 μg L^{-1}). Modeling was cut off at a sulfide concentration of 2,000 μg L^{-1} because only three Class B sites had pore water sulfide greater than 2,000 μg L^{-1} and none of the three hosted wild rice.

3.6. Field Variables not Evidently Associated With Wild Rice Presence and Absence

Some variables included among the initial hypotheses, or in the past cited as important attributes of wild rice habitat, were not found to be significantly associated with wild rice presence and absence. These include sediment TOC ($p = 0.79$, Figure 2j; DNR, 2008; Lee, 1986; Moyle & Krueger, 1964), flocculent sediment (quantified here as the sediment water content; $p = 0.72$, Figure 2h; DNR, 2008; Lee, 1986; Moyle & Krueger, 1964), sediment N ($p = 0.68$, Table 1; Carson, 2002; Walker et al., 2010), sediment P ($p = 0.27$, Table 1; Carson, 2002; DNR, 2008), and surface water alkalinity ($p = 0.28$, Table 1; Moyle, 1944).

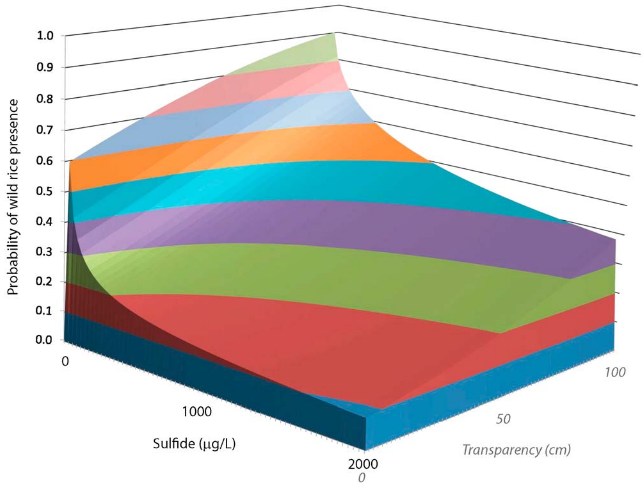

Figure 4. Visualization of the relationship, determined by multiple binary logistic regression, between water transparency and pore water sulfide in controlling the probability of wild rice occurrence at 23.2°C, the median temperature for all sites in the class B data set.

Reduced sediments are generally regarded as producing pore water constituents that are potentially toxic to rooted plants; potentially toxic agents, in addition to sulfide, include ammonia and Fe (Pezeshki & DeLaune, 2012). Analysis of the binary logistic regressions showed no evidence for ammonia toxicity (BLR $p = 0.45$, Figure 2l) or Fe toxicity (wild rice presence is positively correlated with pore water Fe, BLR $p = 0.01$, Figure 2f). As a product of decomposition, ammonia is correlated with pore water sulfide, K, and silica (Spearman correlations $p < 0.001$, Table S4).

The field data provide little evidence that elevated sulfide production excludes wild rice by producing Fe, Cu, or Zn deficiency (Table S3). The possibility that *low* pore water Fe limits wild rice growth is difficult to distinguish from sulfide toxicity because of the strong negative correlation between pore water Fe and pore water sulfide (rho = −0.58, $p < 0.0001$, Table 1). Hydroponic studies using chelated Fe in combination with elevated sulfide (Pastor et al., 2017) show reduced growth, implying that the mechanism is sulfide toxicity, rather than Fe limitation, assuming that the chelated Fe remains bioavailable in the presence of sulfide (Li et al., 2009). The median pore water Fe levels at sites with and without wild rice are similar, 5.7 and 5.9 mg L^{-1}, respectively; average levels are 11.0 and 7.0 mg L^{-1}, respectively, which produced the significant BLR positive correlation between pore water Fe and wild rice presence. On balance, there is little evidence to support the hypothesis that elevated sulfide reduces wild rice growth through Fe deficiency rather than by direct sulfide toxicity. Pore water Cu concentrations were often below the detection limit in the field sites sampled, precluding analysis of their correlation with wild rice presence and absence. The BLR for pore water Zn concentrations with wild rice presence and absence was not statistically significant (data not shown). Neither Cu nor Zn had a significant Spearman correlation with pore water sulfide (data not shown), unlike Fe.

3.7. Seasonality in Field Variables

Fifteen natural wild rice sites were sampled 3 to 5 times in 2013 to determine which field variables exhibit statistically significant seasonal trends that might be important to consider in the overall analysis, given that different sites were sampled in different months. Some variables conformed to expected seasonal trends, such as water temperature ($p = 0.001$), which increased in early summer, peaked about 1 August, and then declined. Some of the surface water variables exhibited monotonic increased concentrations over the summer (alkalinity, Na, Mg, and SO_4; $p = 0.0009$, 0.008, 0.021, and 0.05, respectively). One hypothesis that explains this observation is that waterbodies in Minnesota, after being diluted by spring snowmelt, in general become more concentrated as the summer progresses due to evaporation and, for waterbodies with shorter residence times, greater influence of groundwater flux. Cl and Ca had nonsignificant positive slopes ($p = 0.29$ and 0.37, respectively), whereas K exhibited no trend ($p = 0.48$). As expected, none of the solid-phase variables of the homogenized 10 cm long sediment cores showed any significant seasonal trends (e.g., sediment extractable Fe $p = 0.93$, sediment TOC $p = 0.96$). Among the pore water variables, only K and pH exhibited significant trends. Pore water K values decreased over the summer ($p = 0.006$), likely as a result of diffusion into surface water or uptake by growing rooted macrophytes. Pore water pH generally increased over the summer ($p = 0.003$), which may reflect loss of CO_2 as the sediment warmed over the summer, reducing the solubility of gases. The lack of seasonality in pore water sulfide ($p = 0.62$) indicates that sulfide concentrations were in steady state with the variables that exert primary control over its concentration, surface water SO_4, extractable Fe, and sediment TOC (Pollman et al., 2017). Of the variables controlling pore water sulfide, only SO_4 exhibited even marginally significant seasonal trends ($p = 0.05$, 0.93, and 0.97, for SO_4, extractable Fe, and sediment TOC, respectively). Despite the finding of seasonal variation in SO_4, Pollman et al. (2017) found that SO_4 is one of the primary variables that control pore water sulfide concentrations. Seasonal variation in SO_4 concentrations undoubtedly contributes to noise in the statistical relationship documented by Pollman et al. (2017). Myrbo et al. (2017) found that the microbial production of pore water sulfide in a wild rice sediment is proportional to the long-term average SO_4 surface water concentration.

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3.8. Cultivated Wild Rice Paddies

Commercial wild rice paddies have been reported with healthy stands of wild rice growing under surface water SO_4 levels as high as 170 mg L^{-1} (Aiken et al., 1988). Our limited measurements in seven different paddies ranged from 0.3 mg L^{-1} to 279 mg L^{-1} SO_4, with a median of 8.3 mg L^{-1} (n = 7, Table S3). Natural wild rice waters in our study had a median SO_4 concentration of 4.1 mg L^{-1}. Surprisingly, median pore water sulfide in paddies was 182 μg L^{-1}, greater than the medians of natural wild rice waters (88 μg L^{-1}; n = 67) and waters without wild rice (126 μg L^{-1}; n = 41). The median sediment extractable Fe concentration of paddies (4.5 mg g^{-1}) is similar to that of natural wild rice waters (4.8 mg g^{-1}). In contrast, median sediment TOC is much higher in paddies (25.2%) than natural wild rice waters (9.1%). It thus appears that the greater TOC driving enhanced SO_4 reduction (Pollman et al., 2017) and driving median pore water Fe lower than in natural wild rice sediments (1.6 and 5.7 mg L^{-1}, respectively), despite the similar reservoirs of Fe in the sediment. Elevated production of sulfide coupled with the consumption of the available Fe may put some paddies on the brink of sulfide toxicity to wild rice.

However, the physical setting of cultivated wild rice paddies differs from that of natural lakes and streams in a number of important ways. Often paddies and their surface sediments are dewatered during the growing season through use of buried drainage tiles, allowing tillage after harvesting and enhanced aerobic decomposition of rice straw and roots, and possibly reoxidation of sulfide and reduced Fe. Prior to drainage tile installation in the 1980s, failure of wild rice crops was sometimes attributed to elevated sulfide (Grava & Rose, 1975; Gunvalson, personal communication, 2016). Water depth in paddies is also typically shallower (median of 30 cm) than wild rice habitat in natural lakes and rivers (median of 52 cm), allowing seedlings to emerge above water with less energy expended, photosynthesize sooner, and release oxygen from roots to oxidize sulfide. The use of nitrogen fertilizers may further enable seedlings to elongate more quickly through the water and into the air. Fertilized plants have been shown to be more resistant to sulfide toxicity than are control plants (Geurts et al., 2009). The production of sulfide would be inhibited if N fertilizers were applied as nitrate—but N fertilizers are applied as ammonia or urea (Oelke et al., 1997).

3.9. Conclusions and Implications

Analysis of an extensive suite of physical and chemical parameters from 108 different sites with potential wild rice habitat shows that pore water sulfide toxicity is a primary biogeochemical factor controlling the occurrence of wild rice populations in otherwise favorable habitat. High concentrations of pore water sulfide greatly decrease the probability that a wild rice population will be found in a waterbody. When pore water sulfide is low enough to support reproducing wild rice populations, however, it is likely that the relationship between sulfide and wild rice becomes more complicated and analysis of cause and effect more ambiguous. The variation in sulfide concentration is correlated to the density of wild rice, at least partially as a result of oxygen release from roots, and thus, not only does sulfide affect wild rice but wild rice affects sulfide.

Aside from low pore water sulfide, favorable wild rice habitat has long, cold winters and transparent surface water in the ice-free season. *Zizania palustris* seeds germinate at low rates if the winter is too warm or too short. The probability that wild rice seedlings successfully grow to maturity is reduced if photosynthesis is inhibited by low water clarity. Thus, pore water sulfide (itself a function of surface water SO_4, sediment Fe, and sediment organic matter (Pollman et al., 2017)), water temperature, and water transparency together largely determine wild rice presence and absence. These three factors are independent of one another but may act synergistically on other related processes.

In addition to generating sulfide, SO_4 reduction supports organic matter mineralization that releases nutrients and alkalinity to the surface water, which has the potential to change plant community structure even if Fe is high enough to keep pore water sulfide from reaching phytotoxic levels (Myrbo et al., 2017). Natural and anthropogenic SO_4 loading to freshwaters may thus strongly affect ecosystem composition and function, despite the low direct toxicity of SO_4 under oxic conditions.

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