Biological communities and ecosystem function in restored and natural prairie wetlands

by

Lauren Elisa Bortolotti

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Abstract

Prairie wetlands provide many important ecosystem services including supporting biodiversity, improving water quality, preventing erosion, recharging groundwater, and attenuating floods. However, more than half of prairie wetlands in North America have been lost, primarily due to drainage for agriculture. Restoration may be able to reestablish lost services, although there remain substantial gaps in our understanding of the recovery of biodiversity and ecosystem function in restored prairie wetlands. Here, I present an investigation characterizing biological communities and ecosystem function in restored and natural prairie wetlands in southeastern Saskatchewan, Canada.

My first objective was to assess recovery of the abiotic environment (water chemistry, sediment organic carbon [OC]) and various biological communities (phytoplankton, sediment diatoms, zooplankton, benthic macroinvertebrates, submersed aquatic vegetation [SAV]) after hydrological restoration. I used a space-for-time study design, surveying “recently restored” (restored 1-3 years before study), “older restored” (restored 7-14 years before study), and “natural” (never drained) prairie wetlands. Recently restored wetlands differed from older restored and natural wetlands in that they had higher total phosphorus (TP) and dissolved carbon dioxide (CO_2) but lower specific conductance, pH, and sediment OC. Phytoplankton, diatom, and zooplankton communities showed little relationship to restoration state, but taxonomic composition of macroinvertebrate and SAV communities were different in recently restored wetlands. The consistent resemblance of older restored wetlands to natural wetlands suggests that recovery of the abiotic environment and many biological communities is possible within ~10

years of restoration, a result with direct implications for management.

I quantified greenhouse gas (GHG) fluxes from the open water of three restored and natural prairie wetlands and used both CO_2 fluxes and net ecosystem production (NEP; measured using the diel oxygen technique) to assess the metabolic status (i.e., net autotrophic or heterotrophic) of prairie wetlands. GHG emissions tended to be high, but variable. The recently restored wetland emitted more CO_2 and methane than either the older restored or natural wetland, and only the latter showed extensive CO_2 uptake. CO_2 supersaturation was a less reliable indicator of wetland metabolic status than NEP, especially at daily timescales, owing to the confounding influence of geochemical processes on CO_2 concentrations.

I measured ecosystem metabolism, including NEP, gross primary production (GPP), and ecosystem respiration (ER) in three restored and natural prairie wetlands and identified the drivers of these rates. Photosynthetically active radiation, temperature, proxies of water column stratification, and SAV abundance were the main drivers of metabolism within wetlands. However, the recently restored wetland differed from the other sites in that chlorophyll *a* (chl *a*) and TP were also drivers of GPP and NEP. Among-wetland differences in NEP rates were determined by a combination of wetland state (i.e., clear water or turbid) and the degree to which emergent vegetation subsidized ER. GPP and ER were highest in the older restored wetland followed by the natural and recently restored wetlands. The GPP gradient across sites was explained by the abundance of SAV whereas the ER gradient by the abundance of substrates for microbial respiration (dissolved organic carbon, sediment OC).

To date, this body of research represents one of the most comprehensive examinations of the recovery of biological communities after wetland restoration in the Canadian Prairie Pothole Region and is the first to look at ecosystem metabolism in this system. My work suggests that many attributes of prairie wetlands recover after restoration, though more work is needed to better characterize the effects of restoration on ecosystem metabolism and to understand how broadly applicable these findings are to the rest of the Prairie Pothole Region.

Preface

The work contained in this thesis reflects a collaborative effort with other scientists. This is reflected by the authorship of published and submitted chapters. For all chapters, I was responsible for study design, data collection, data analysis, and writing, with input from others primarily in the design and writing phases.

Chapter 2 is in press at Freshwater Biology as: Bortolotti LE, Vinebrooke RD, St. Louis VL. Prairie wetland communities recover at different rates following hydrological restoration.

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Chapter 4 will be submitted as: Bortolotti LE, St. Louis VL, Vinebrooke RD. Assessing the drivers of ecosystem metabolism in restored and natural prairie wetlands.

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Chapter 1: General introduction

Wetlands cover between 800 and 1000 million ha of the globe (Lehner and Döll 2004) and provide many important ecosystem services, including supporting biodiversity, purifying water, stabilizing soils and shorelines, attenuating floods, and storing carbon (Zedler and Kercher 2005). The emphasis of many wetland policies on "wise use" as a dimension of wetland conservation reflects their value to humans (e.g., the Ramsar Convention, Canada's Federal Policy on Wetland Conservation). Despite the widely acknowledged value of wetlands, loss and degradation remain a persistent problem (Zedler and Kercher 2005). Canada has 127 million ha of wetlands, although there are ongoing losses of ~30 ha per day on top of historical losses of ~20 million ha (Environment Canada 1991, Watmough and Schmoll 2007). In the Prairie Pothole Region (PPR) of Canada, over half of the wetland area has been lost. Prairie wetlands are afforded little legislative protection compared with other types of aquatic ecosystems owing to their isolation from navigable waters and natural fishless state (Marton et al. 2015).

There are millions of wetlands in the PPR, an area that covers ~71.5 million ha of central North America and spans three provinces and five states (Euliss et al. 1999). These wetlands are also often referred to as potholes, ponds, or sloughs and were formed during Pleistocene glacial retreat. They range from ephemeral basins that hold water only after snowmelt or major precipitation events to permanent features of the landscape (Stewart and Kantrud 1971, Euliss et al. 1999). Drainage for agriculture is responsible for most wetland losses, with small basins disproportionately drained (mean drained wetland area = 0.2 ha; Watmough and Schmoll 2007).

When dealing with total ecosystem loss, as opposed to ecosystem degradation, there are only two possible conservation approaches: 1) prevent further losses; or 2) ecosystem restoration. While the former approach is generally preferable, there are multiple reasons why conservation practitioners increasingly recognize that restoration must be used in concert with wetland retention. First, some parts of the PPR have been so extensively drained (~90 % wetland loss) that there are few wetlands left to conserve. Second, efforts to halt wetland drainage have been unsuccessful and thus restoration is needed to offset ongoing habitat losses. However, despite this important role of restoration in prairie conservation work, very little is known regarding the efficacy of restoration programs. To spend already strained conservation resources wisely, a key question is whether restored wetlands resemble intact wetlands and provide the same types and levels of ecosystem services.

Evaluating restoration success

Most studies that assess the recovery of restored ecosystems measure at least one of three ecosystem attributes: vegetation structure, biodiversity, or ecosystem function (Ruiz-Jaen and Aide 2005). Vegetation structure includes measures such as plant cover or biomass. Biodiversity encompasses species richness, the abundance of organisms, and taxonomic composition of communities. Ecosystem functions are processes that involve the flux of energy or matter in an ecosystem (e.g., nutrient cycling, organic carbon accumulation or remineralization). Measures of ecosystem function are often emphasized as the best approach to assess the recovery of restored sites, yet at the same time are the least measured attributes in restoration ecology (Ruiz-Jaen and Aide 2005, Lake et al. 2007, Palmer 2009, Wortley et al. 2013).

Vegetation structure and biodiversity inventories may provide an incomplete or misleading picture of ecosystem recovery after restoration. The historical emphasis on vegetation and biodiversity stems from the greater ease of measuring these attributes compared to ecosystem function, as well as the assumption that biological characteristics are good indicators of ecosystem functions. However, a meta-analysis of the restoration literature showed that restoration is more effective at reestablishing biodiversity than ecosystem function (Rey Benayas et al. 2009), suggesting that the recovery of these attributes may not go hand-in-hand. Furthermore, multiple studies have documented possible or actual trade-offs between maximizing biodiversity and ecosystem function (Bullock et al. 2011, Doherty et al. 2011, Montoya et al. 2012, Pfeifer-Meister et al. 2012). These trade-offs arise when biodiversity and ecosystem function are not positively correlated; e.g., plant communities dominated by one or two species may be just as or more productive than those with a diverse assemblage of species. Thus, studies of restoration success would ideally measure ecosystem function directly, or even incorporate measures of vegetation structure and biodiversity in addition to function.

Evaluating the success of prairie wetland restoration

Studies evaluating the efficacy of hydrological restoration (hereafter referred to just as ‘restoration’) of prairie wetlands have typically focused on a single bioindicator. In this approach, the success of the restoration is evaluated based on the similarity in biodiversity or taxonomic composition between restored and natural (never-drained) wetlands. The most commonly studied biological communities have been vegetation (Delphey and Dinsmore 1993, Galatowitsch and van der Valk 1996a, 1996b, Puchniak 2002, Seabloom and van der Valk 2003, Zimmer et al. 2003, Aronson and Galatowitsch 2008, Kettenring and Galatowitsch 2011, Fuselier et al. 2012, van der Valk 2013) and birds (Delphey and Dinsmore 1993, Van Rees-Siewart and Dinsmore 1996, Ratti et al. 2001, Begley et al. 2012, Newbrey et al. 2013). Other organisms or wetland characteristics that have been used to evaluate restoration success include: amphibians (Puchniak 2002, Zimmer et al. 2002), invertebrates or invertebrate egg banks (Zimmer et al. 2000, Zimmer et al. 2002, Gleason et al. 2004), algal communities (Mayer and Galatowitsch 1999, Mayer and Galatowitsch 2001, Mayer et al. 2004), water chemistry (Galatowitsch and van der Valk 1996c, Zimmer et al. 2002), soil properties (Card et al. 2010, Card and Quideau 2010, Streeter and Schilling 2015), and carbon storage (Badiou et al. 2011). Thus, the vast majority of studies of recovery of restored prairie wetlands focus on vegetation structure and biodiversity attributes, with little known about the recovery of ecosystem function.

There is also an imbalance in the prairie wetland restoration literature as to where studies have been conducted. Of 24 studies identified as addressing some aspect of the recovery of restored prairie wetlands, only ~20 % of them have been conducted in the northern reaches of the region (i.e., within the Canadian sector). It has been previously noted that there are geographic differences in vegetation recovery after restoration, attributed to different climate and drainage histories between the US and Canada (Puchniak 2002). Tile (subsurface) drainage, which is more difficult to remediate, has been used more extensively in the US; surface drainage ditches are more common in the Canadian PPR (Watmough and Schmoll 2007). Furthermore, the extent of drainage is greater in many parts of the American PPR than in Canada. One may reasonably expect that recovery rates and trajectories could be affected by having fewer intact wetlands on the landscape either via fewer sources of propagules for recolonization of restored wetlands, or greater dispersal distances for propagules owing to lower wetland connectivity.

Prairie wetland function

Relatively little is known about whole-ecosystem functions of prairie wetlands, even outside of the context of restoration. As of May 2016, a search using Thompson Reuters Web of Science for the terms “prairie”, “pothole”, “ecosystem”, and “function” in the title, abstract, or keyword fields returns only 32 results with a collective total of 744 citations. Only two entries have been cited > 100 times (Meyer et al. 1999, 133 citations; Blann et al. 2009, 106 citations), and both of those studies are reviews that encompass wetlands in more than just the PPR. Among the most-studied functions in prairie wetlands are carbon gas and sediment fluxes (Bedard-Haughn et al. 2006, Gleason et al. 2009, Pennock et al. 2010, Badiou et al. 2011, Finocchiaro et al. 2014) and the hydrological response of wetlands to climate (Zhang et al. 2009, Johnson et al. 2010, Liu and Schwartz 2011, 2012, McIntyre et al. 2014, Johnson et al. 2016). There have also been studies of factors controlling dissolved organic carbon (Waiser 2006, Ziegelgruber et al. 2013) and geochemical solute dynamics (Heagle et al. 2007, 2013).

Ecosystem metabolism, which is defined by three components including gross primary production (GPP), ecosystem respiration (ER), and net ecosystem production (NEP; NEP = GPP – ER), has largely gone unquantified in prairie wetlands. Ecosystem metabolism integrates interactions among multiple biological communities and their environment and, as such, can provide a holistic understanding of prairie wetland function. In prairie wetlands, measurements of primary production, and the balance between production and respiration, have been limited to bottle measurements (Waiser and Robarts 2004, Sura et al. 2012). Incubations (bottle measurements) suffer from container artifacts, may miss key processes that operate at the whole-ecosystem scale, and thus may not be suitable for scaling-up to the level of whole ecosystems (Staehr et al. 2012). Open-water methods, based on high-frequency measurement of the dissolved gases involved in production and respiration (i.e., carbon dioxide, oxygen), alleviate many of the issues of incubations, but have yet to be used in prairie wetlands.

Structure of the thesis

The evident knowledge gaps in the prairie wetland restoration literature, as well as shortcomings with respect to a generalized understanding of whole-ecosystem function in prairie wetlands, form the impetus for this thesis. This work falls within a simple conceptual framework wherein drainage and subsequent restoration may affect the abiotic environment or biological communities in prairie wetlands (Fig. 1.1). In turn, ecosystem functions arise from complex interactions between the abiotic environment and wetland organisms. At issue is whether any differences owing to restoration translate to altered ecosystem function (compared to never-drained wetlands). I focus on describing the ways in which restoration influences the abiotic environment and biological communities (arrows 1a,b in Fig. 1.1; Chapter 2), characterizing ecosystem function in restored and natural prairie wetlands (box 2 in Fig. 1.1; Chapter 3), and identifying abiotic and biological drivers of ecosystem function (arrows 3a,b in Fig. 1.1; Chapter 4). All three research chapters concern restored and natural prairie wetlands located in southeastern Saskatchewan (Fig. 1.2). I used a space-for-time study design, sampling wetlands that were categorized as “recently restored”, “older restored”, or “natural”. Recently restored wetlands were restored 1-3 years before the first year of study (2011), older restored wetlands were restored 7-14 years before the first year of study, and natural wetlands had never been drained. Under this experimental design, natural wetlands serve as the benchmark for assessing the recovery of restored wetlands and the two post-restoration age classes indicate whether resemblance to natural systems increases with time.

In my first research chapter, Chapter 2, I present a limnological survey of 24 restored and natural prairie wetlands. The goal of this study was to describe how drainage and restoration affect abiotic and biological characteristics of prairie wetlands (arrows 1a,b in Fig. 1.1), to detect abiotic gradients underlying taxonomic composition of communities (arrow 1c in Fig. 1.1), and to identify a general timeline for the recovery of biological communities in restored wetlands. I characterize water chemistry and taxonomic composition of communities of phytoplankton, benthic diatoms, zooplankton, benthic macroinvertebrates, and submersed aquatic vegetation in restored and natural wetlands. As previously discussed, using biological communities to evaluate restored wetland recovery is a frequently used approach. However, the simultaneous characterization of several disparate biological communities of both producers and consumers is unprecedented for restored prairie wetlands. It is also the first occasion where phytoplankton, benthic diatoms, zooplankton, and benthic macroinvertebrates have been studied in restored wetlands in the Canadian portion of the PPR or, in some instances, anywhere in the PPR. The survey of these wetlands laid the foundation for site selection and interpretation of results as presented in Chapters 3 and 4.

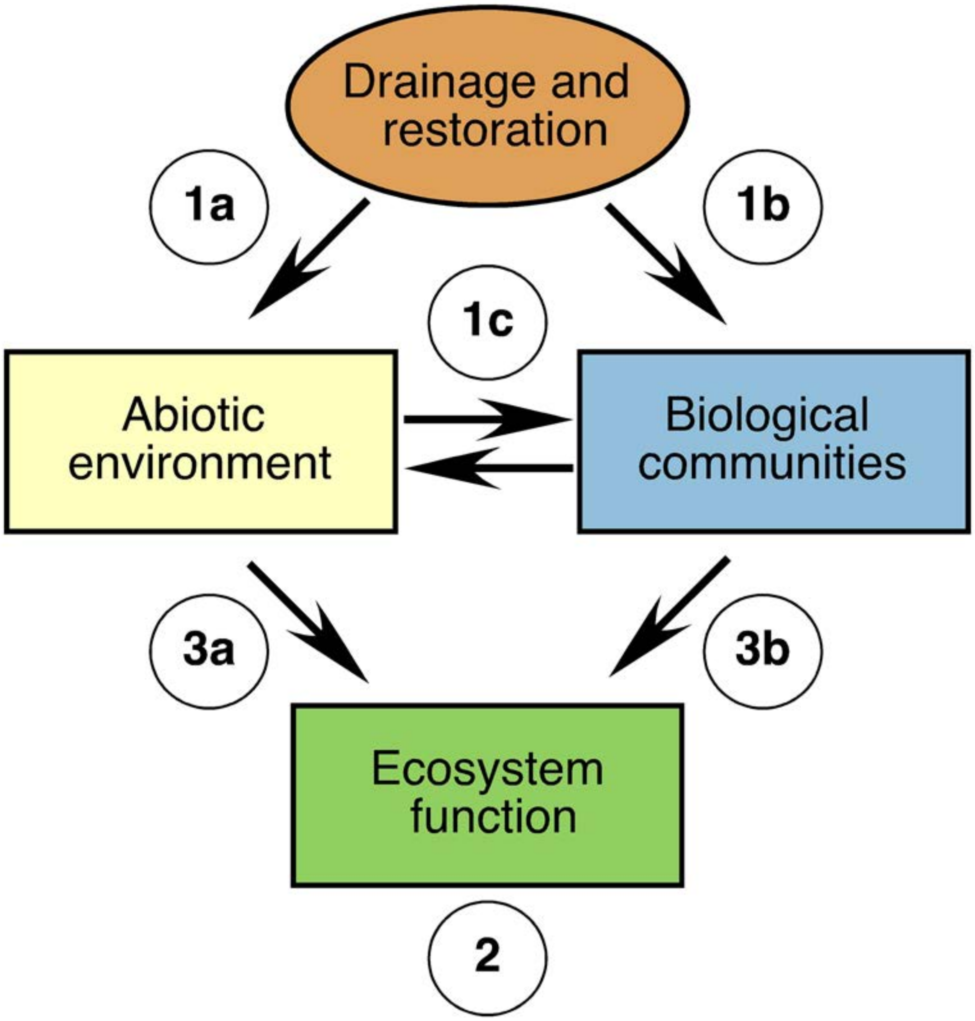

Figure 1.1 A framework for the effects of drainage and restoration on ecosystem attributes including the abiotic environment, biological communities, and ecosystem function. Arrow and box labels (1a-c; 2; 3a,b) are explained in the text.

My second research chapter, Chapter 3, characterizes the metabolic status (i.e., net autotrophic or net heterotrophic) of three prairie wetlands, quantifies carbon greenhouse gas fluxes from those wetlands, and compares NEP (as measured by the diel oxygen method) to carbon dioxide fluxes as indicators of metabolic status. The latter objective, comparing methods for estimating ecosystem metabolic status, aimed to resolve previous discrepancies in the

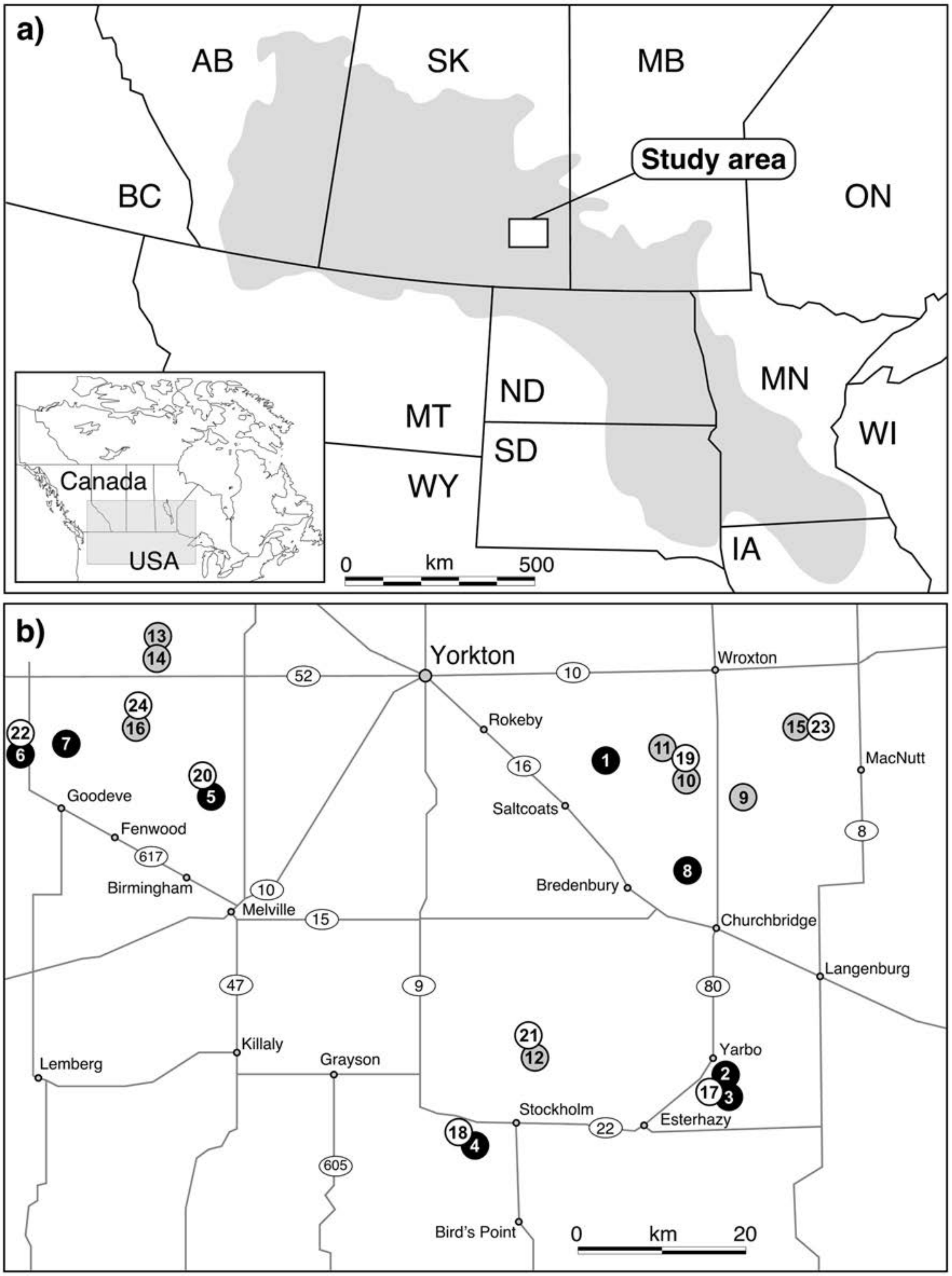

Figure 1.2 Map showing a) the approximate extent of the Prairie Pothole Region (grey shading) and b) the location of study sites in southeastern Saskatchewan. Site numbers correspond to those listed in Table 2.1. Point shading indicates restoration state: black = recently restored, grey = older restored, and white = natural.

literature with respect to the metabolic status of prairie wetlands (Waiser and Robarts 2004). The three studied wetlands are a subset of the 24 wetlands in Chapter 2, and included one recently restored, one older restored, and one natural site. Data were collected for this study during the summers of 2012 and 2013.

In the third research chapter, Chapter 4, I identify drivers of ecosystem metabolism (i.e., arrows 3a,b in Fig. 1.1) in the same three wetlands as in Chapter 3. In addition to identifying drivers of daily variation in NEP, GPP, and ER within wetlands, I also identify factors that drive differences between sites. Although drivers of metabolism have been extensively studied in lakes, streams, and estuaries (Staehr et al. 2012, Hoellein et al. 2013, Solomon et al. 2013), much less is known about metabolic rates and drivers in wetlands.

Finally, Chapter 5 provides general conclusions and recommendations for further study. Here, I synthesize findings from the three research chapters. I also highlight some key challenges regarding research on prairie wetland biodiversity and ecosystem function. Finally, I outline potential areas of future research. These future research topics stem from both my own doctoral thesis as well as perceived areas of need at the interface of academic and applied research.

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Chapter 2: Prairie wetland communities recover at different rates following hydrological restoration

Introduction

Small wetland and pond ecosystems provide important services to humans, including supporting biodiversity, improving water quality, attenuating floods, and sequestering carbon (Costanza et al. 1997, Zedler and Kercher 2005, Downing 2010, Marton et al. 2015). Yet these ecosystems are vulnerable to loss and degradation because they are often not afforded the same legislative protections as lakes and rivers (Marton et al. 2015). Roughly half of the global wetland area has already been lost (Zedler and Kercher 2005). As such, it is imperative that remaining wetlands be protected, and lost or degraded wetlands restored to reestablish their biodiversity and ecosystem functions.

The Prairie Pothole Region (Fig. 1.2a) spans ~715,000 km^2 in Canada and the U.S. and contains millions of wetlands, over half of which have been drained, mainly for agriculture (Euliss et al. 1999). These wetlands formed during Pleistocene glacial retreat and range from ephemeral basins that hold water only after snowmelt or major precipitation events to permanent features on the landscape (Stewart and Kantrud 1971, Euliss et al. 1999). In the Canadian prairies, wetland losses are ongoing, with over 30 hectares lost per day (Watmough and Schmoll 2007). Restoration of drained prairie wetlands may help reestablish lost ecosystem services, though there are gaps in our knowledge of the efficacy of such measures.

Evaluation of restoration efforts is needed to justify their use in management practices and to improve the efficacy of restoration protocols (Wortley et al. 2013). Restoration success is commonly inferred from the degree of similarity between restored and natural ecosystems. In the prairie wetland literature, restoration studies have commonly focused on a single bioindicator, most often vegetation (Delphey and Dinsmore 1993, Galatowitsch and van der Valk 1996a, Puchniak 2002, Aronson and Galatowitsch 2008, Kettenring and Galatowitsch 2011, Fuselier et al. 2012, van der Valk 2013) or birds (Delphey and Dinsmore 1993, Van Rees-Siewart and Dinsmore 1996, Ratti et al. 2001, Begley et al. 2012). There are valid reasons that the prairie wetland restoration literature is biased towards these groups. Plants are common focal group in restoration ecology because vegetation structure can affect other biota and biogeochemical cycling. Also, waterfowl conservation is often a motivating factor for restoration and thus an endpoint worth studying. However, if the overall ecological integrity of restored systems (i.e., whole-ecosystem recovery) is the goal of restoration, more comprehensive metrics of recovery are needed.

We measured abiotic characteristics (water chemistry, greenhouse gas concentrations) and taxonomic composition of several biological communities (phytoplankton, benthic diatoms, crustacean zooplankton, benthic macroinvertebrates, submersed aquatic vegetation) in 24 prairie wetlands. To identify the timeline of wetland recovery after hydrological restoration, we used a space-for-time study design; we sampled wetlands ranging from one to 14 years after restoration and compared them to “natural” sites that had never been drained. We predicted full recovery of water chemistry and biological communities in the restored wetlands within approximately a decade of hydrological restoration as the natural disturbance regime of drying out and reflooding of prairie wetlands (i.e., potentially short hydroperiods) favours fast-growing microorganisms and invertebrates with strong dispersal potential (Euliss et al. 1999).

Methods

Study sites

We sampled 24 naturally fishless prairie wetlands in the aspen parkland ecoregion of southeastern Saskatchewan, Canada (Fig. 1.2b, Table A1.1). Each wetland was sampled three times between May and August 2011 and belonged to one of three restoration states (8 wetlands per state): 1) recently restored wetlands (restored 1-3 years before the study); 2) older restored wetlands (restored 7-14 years before the study); and, 3) natural wetlands that had never been drained. Wetlands were restored by Ducks Unlimited Canada between 1997 and 2010 by building earth berms across drainage ditches and allowing the basin to refill with precipitation and runoff. Because of limitations on the number of wetlands we could sample, we focused our sampling efforts on the extreme ends of the spectrum of possible post-restoration ages (i.e., 1 to 14 years post-restoration) in the study area. We did this to increase our chance of detecting differences among restoration states given the high natural variation in chemical and biological composition of prairie wetlands. We defined the categories of “recently” and “older” restored based on the recovery timelines identified in previous studies of restored wetlands in the northern section of the Prairie Pothole Region (Card and Quideau 2010, Badiou et al. 2011). A shortcoming of this study design is that we could not identify recovery timelines with annual resolution. Consequently, we discuss recovery taking approximately 10 years (i.e., within the 7-14 year age range of the older restored wetlands) when only recently restored wetlands were distinguishable from natural wetlands.

Mean water depth ranged from 0.7 to 2.3 m and surface area from 0.25 to 4.11 ha (Table 2.1). Fields around wetlands were either left idle, grazed by cattle, or cultivated with hay or other crops. All wetlands but the deepest one were semi-permanent, Class IV wetlands (Stewart and Kantrud 1971). Semi-permanent wetlands are characterized by a ring of emergent vegetation (e.g., *Typha*) around an open water zone, though in some recently restored sites this vegetation had not yet established. These wetlands are naturally fishless, however, brook stickleback (*Culaea inconstans* (Kirtland, 1840)) were detected during invertebrate sampling at three sites (Table 2.1). The fish were likely transported from a nearby reservoir during spring flooding as they do not typically survive over winter in these shallow prairie wetlands.

Abiotic wetland characteristics

We measured specific conductance and pH in surface water at the centre of each wetland with a Hach Hydrolab DS5 sonde. Water was collected into HDPE bottles to measure ammonium (NH_4^+), nitrite and nitrate (NO_2+NO_3^-), total dissolved nitrogen (TDN), total phosphorus (TP), total dissolved phosphorus (TDP), dissolved organic carbon (DOC), chlorophyll *a* (chl *a*), and total suspended solids (TSS). Samples were processed and preserved the same day, then stored in the dark at 5°C or frozen until analyzed using standard protocols in the University of Alberta Biogeochemical Analytical Service Laboratory (see Appendix 1 for details of the protocols used). Carbon dioxide (CO_2), methane (CH_4), and dissolved inorganic carbon (DIC) were quantified in July and August only and were analyzed using gas chromatography (see Appendix 1 for detailed sampling and laboratory protocols).

To measure organic carbon (OC) content of sediments we collected five sediment cores from 16 wetlands (5 each of the natural and recently restored wetlands, and 6 of the older restored

Table 2.1. Selected wetland characteristics and water chemistry variables for 24 prairie wetlands. Shown are the site numbers from Figure 1.2 (Map No.), site names, restoration state (recently restored, older restored, or natural), surrounding land use (left idle, grazed by cattle, cultivated with hay, or cultivated with a grain or oilseed crop), presence/absence of fish (Fish), and % cover of submersed aquatic vegetation (SAV). For wetland size and water chemistry, shown are the mean (minimum, maximum) of three sampling periods between May and August 2011 for: maximum wetland depth, wetland area, specific conductance (SpCond), pH, ammonium (NH_4^+), nitrite and nitrate (NO_2+NO_3^-), total dissolved nitrogen (TDN), total phosphorus (TP), total dissolved phosphorus (TDP), dissolved organic carbon (DOC), carbon dioxide (CO_2), dissolved inorganic carbon (DIC), chlorophyll *a* (Chl *a*), and total suspended solids (TSS).

Map No.	Site	Restoration state	Land use	Fish	SAV (% cover)	Depth (m)	Area (ha)	SpCond (µS cm^{-1})	pH	NH_4^+ (µg L^{-1})	NO_2+NO_3^- (µg L^{-1})	TDN (µg L^{-1})	TP (µg L^{-1})	TDP (µg L^{-1})	DOC (mg L^{-1})	CO_2 (µmol L^{-1})	DIC (µmol L^{-1})	Chl *a* (µg L^{-1})	TSS (mg L^{-1})
1	Hines	RR	grazed	absent	100	0.9	0.32	438	7.44	78	2	1550	637	562	15.1	307	3170	12.5	4.9
						(0.8, 1.1)	(0.27, 0.41)	(338, 502)	(7.09, 7.86)	(8, 137)	(2, 2)	(810, 2020)	(418, 765)	(365, 673)	(3.2, 28.5)	(304, 309)	(3099, 3241)	(4.2, 27.1)	(2.0, 7.5)
2	Hood-1	RR	grazed	absent	75	1.3	0.92	580	7.57	8	1	1099	44	36	26.8	418	3485	9.3	1.6
						(1.1, 1.4)	(0.82, 0.99)	(543, 616)	(7.18, 7.96)	(7, 9)	(0. 1)	(792, 1346)	(29, 67)	(29, 40)	(16.1, 40.2)	(361, 475)	(3438, 3532)	(1.3, 15.8)	(0.0, 3.5)
3	Hood-2	RR	grazed	absent	15	1.3	1.45	537	7.83	27	1	999	50	34	16.1	187	3332	8.2	1.5
						(1.1, 1.7)	(1.33, 1.57)	(500, 574)	(7.42, 8.24)	(7, 63)	(0, 2)	(760, 1120)	(29, 75)	(21, 42)	(13.4, 20.7)	(168, 205)	(3253, 3412)	(1.0, 20.8)	(0.0, 4.0)
4	Johanson	RR	grazed	absent	100	0.8	0.31	1114	7.38	25	1	1971	293	185	32.6	427	4185	63.2	4.6
						(0.6, 1.0)	(0.26, 0.36)	(946, 1283)	(7.22, 7.54)	(16, 34)	(0, 2)	(1542, 2400)	(140, 445)	(128, 242)	(25.8, 39.3)			(2.3, 124.1)	(1.6, 7.6)
5	Reinson	RR	idled	present	75	1.0	0.64	1909	7.52	120	1	2415	608	523	35.3	455	4149	74.5	16.7
						(0.9, 1.1)	(0.59, 0.66)	(1690, 2046)	(7.37, 7.67)	(14, 197)	(0, 3)	(1646, 2920)	(487, 700)	(429, 622)	(27.4, 43.2)	(365, 545)	(4111, 4186)	(13.7, 129.0)	(1.2, 41.0)
6	Smith-1	RR	crop	absent	15	1.2	0.56	308	7.09	11	1	769	78	49	11.8	367	2618	17.2	4.3
						(1.1, 1.2)	(0.52, 0.59)	(291, 327)	(6.67, 7.79)	(6, 18)	(1, 2)	(738, 812)	(41, 141)	(33, 61)	(9.3, 14.2)	(319, 414)	(2561, 2676)	(4.2, 38.4)	(0.8, 8.4)
7	Smith-2	RR	crop	absent	38	0.9	1.02	418	6.83	95	2	1133	199	168	18.8	601	3066	4.9	6.8
						(0.8, 1.0)	(0.93, 1.09)	(386, 461)	(6.67, 7.05)	(16, 150)	(1, 3)	(1068, 1248)	(148, 238)	(74, 227)	(17.3, 19.8)	(584, 618)	(3056, 3076)	(4.0, 7.0)	(0.8, 14.0)
8	Sorrell	RR	hay	absent	75	0.9	0.44	1120	7.57	62	1	1767	78	61	22.7	260	3514	7.1	1.2
						(0.8, 1.0)	(0.40, 0.51)	(963, 1200)	(7.16, 8.01)	(9, 149)	(0, 1)	(1214, 2120)	(57, 91)	(41, 73)	(15.3, 33.7)	(256, 265)	(3460, 3569	(3.2, 12.5)	(0.4, 2.5)
			Restoration state mean			1.0	0.71	803	7.40	53	1	1463	248	202	22.4	378	3440	24.6	5.2
9	Adams	OR	idled	absent	75	0.7	1.63	2910	7.39	39	3	1485	33	27	19.6	358	3757	4.6	1.1
						(0.6, 0.9)	(1.43, 1.83)	(2514, 3306)	(7.36, 7.41)	(3, 61)	(0, 9)	(1096, 1750)	(28, 39)	(22, 33)	(14.1, 26.6)	(288, 428)	(3705, 3809)	(4.4, 4.8)	(0.0, 2.0)
10	Penner-1	OR	grazed	absent	38	1.1	0.79	1208	7.49	16	0	1313	42	37	20.4	144	3152	3.9	1.4
						(1.0, 1.2)	(0.70, 0.91)	(1178, 1243)	(7.38, 7.61)	(13, 21)	(0, 0)	(1114, 1560)	(33, 48)	(33, 41)	(17.4, 23.8)	(52, 237)	(3041, 3262)	(2.4, 5.9)	(0.4, 3.0)
11	Penner-2	OR	grazed	absent	75	0.8	0.51	1081	7.30	11	1	1358	57	38	27.9	369	3603	33.7	3.7
						(0.7, 1.0)	(0.48, 0.56)	(1002, 1160)	(7.20, 7.39)	(6, 19)	(0, 2)	(1066, 1584)	(34, 98)	(31, 44)	(21.2, 33.3)	(360, 378)	(3601, 3605)	(4.1, 54.7)	(0.4, 8.0)
12	Rowein	OR	idled	absent	15	2.3	4.11	767	7.36	76	1	1464	266	262	19.5	322	3425	27.8	1.2

Map No.	Site	Restoration state	Land use	Fish	SAV (% cover)	Depth (m)	Area (ha)	SpCond (μS cm^{-1})	pH	NH$_4^+$ (μg L^{-1})	NO$_2$+NO$_3^-$ (μg L^{-1})	TDN (μg L^{-1})	TP (μg L^{-1})	TDP (μg L^{-1})	DOC (mg L^{-1})	CO$_2$ (μmol L^{-1})	DIC (μmol L^{-1})	Chl *a* (μg L^{-1})	TSS (mg L^{-1})
						(2.2, 2.5)	(3.82, 4.44)	(724, 801)	(7.31, 7.44)	(10, 115)	(0, 2)	(1040, 1745)	(154, 393)	(198, 349)	(15.1, 26.4)	(277, 367)	(3291, 3558)	(14.1, 52.7)	(0.0, 2.4)
13	Tataryn-1	OR	hay	present	100	1.2	0.34	1103	7.56	15	1	1062	36	25	11.6	258	2668	9.8	2.1
						(1.0, 1.4)	(0.32, 0.38)	(1061, 1180)	(7.26, 8.03)	(4, 24)	(1, 2)	(707, 1286)	(30, 40)	(22, 30)	(3.1, 19.7)	(208, 308)	(2604, 2731)	(4.1, 19.6)	(0.0, 5.0)
14	Tataryn-2	OR	hay	present	100	1.0	0.85	985	7.70	18	2	981	82	46	13.0	170	3033	17.2	9.1
						(1.0, 1.2)	(0.82, 0.90)	(917, 1083)	(7.27, 7.97)	(4, 34)	(1, 2)	(827, 1068)	(73, 98)	(19, 74)	(9.3, 18.2)	(155, 185)	(2986, 3079)	(12.5, 19.6)	(1.2, 24.4)
15	Toderian	OR	idled	absent	75	0.9	0.84	1917	7.69	76	1	2070	96	74	34.7	130	3041	3.3	1.6
						(0.9, 1.0)	(0.82, 85)	(1840, 1994)	(7.59, 7.79)	(18, 141)	(0. 2)	(1796, 2580)	(53, 141)	(51, 112)	(32.3, 37.2)	(52, 209)	(2738, 3345)	(2.1, 4.6)	(0.0, 4.0)
16	Wilk	OR	idled	absent	75	0.7	0.25	1116	8.11	36	1	1459	106	93	24.2	14	3080	3.1	0.6
						(0.7, 0.8)	(0.22, 0.28)	(986, 1247)	(7.78, 8.44)	(18, 53)	(0, 2)	(1354, 1564)	(96, 115)	(86, 99)	(20.9, 27.5)			(1.6, 4.6)	(0.0, 1.2)
			Restoration state mean			1.1	1.17	1386	7.57	36	1	1399	90	75	21.4	221	3220	12.9	2.6
17	Hood	NAT	grazed	absent	15	1.4	0.87	600	7.47	20	1	1037	42	37	22.4	378	3456	7.4	1.8
						(1.3, 1.5)	(0.77, 0.98)	(570, 630)	(7.23, 7.70)	(7, 33)	(0, 2)	(784, 1220)	(28, 60)	(23, 45)	(14.7, 28.8)	(335, 421)	(3451, 3460)	(1.5, 14.6)	(0.4, 2.5)
18	Johanson	NAT	grazed	absent	100	0.8	0.39	2721	7.67	53	1	1960	71	56	36.1	358	4417	15.4	3.4
						(0.7, 1.0)	(0.33, 0.45)	(2269, 3173)	(7.60, 7.74)	(11, 95)	(0, 2)	(1524, 2396)	(69, 73)	(43, 69)	(28.6, 43.6)			(5.5, 25.3)	(2.4, 4.4)
19	Penner	NAT	grazed	absent	75	1.0	0.63	1766	7.48	67	1	1723	83	75	26.9	214	3666	2.7	1.8
						(0.9, 1.1)	(0.61, 0.64)	(1650, 1847)	(7.34, 7.56)	(58, 72)	(0, 2)	(1034, 2240)	(77, 91)	(67, 86)	(23.6, 30.1)	(201, 228)	(3593, 3739)	(2.6, 2.8)	(0.8, 3.5)
20	Reinson	NAT	idled	absent	100	0.9	0.45	1540	8.41	9	0	1516	98	92	18.4	12	3181	7.4	1.3
						(0.7, 1.0)	(0.40, 0.49)	(1471, 1626)	(7.96, 8.72)	(6, 14)	(0, 0)	(1340, 1620)	(73, 141)	(60, 140)	(4.7, 29.3)	(6, 18)	(2986, 3377)	(4.5, 11.1)	(0.8, 2.0)
21	Rowein	NAT	idled	absent	5	1.2	0.29	2579	7.60	221	1	2381	162	112	29.3	476	4855	85.5	6.7
						(1.1, 1.3)	(0.24, 0.33)	(2267, 2760)	(7.49, 7.74)	(2, 620)	(0, 3)	(1462, 2920)	(107, 239)	(55, 203)	(15.2, 47.4)	(347, 606)	(4776, 4934)	(13.3, 178.2)	(3.2, 10.0)
22	Smith	NAT	crop	absent	50	1.0	0.56	313	7.15	9	1	789	100	51	13.1	302	2496	12.1	3.8
						(0.9, 1.1)	(0.55, 0.56)	(297, 342)	(6.68, 7.72)	(7, 12)	(0. 1)	(654, 928)	(62, 166)	(38, 76)	(11.0, 15.1)	(241, 363)	(2378, 2615)	(3.0, 27.1)	(1.6, 7.0)
23	Toderian	NAT	idled	absent	75	0.9	0.55	774	7.80	13	5	1230	49	34	22.7	194	3160	8.3	1.6
						(0.8, 1.1)	(0.51, 0.59)	(750, 799)	(7.46, 8.14)	(3, 20)	(0, 14)	(1156, 1366)	(42, 62)	(30, 42)	(21.4, 24.6)	(176, 212)	(3160, 3161)	(3.7, 12.0)	(0.0, 4.0)
24	Wilk	NAT	idled	absent	50	0.9	0.89	746	8.31	19	0	1300	40	27	21.9	24	2909	4.1	0.8
						(0.8, 1.0)	(0.86, 0.93)	(710, 783)	(8.30, 8.32)	(6, 31)	(0, 0)	(1140, 1460)	(31, 48)	(24, 30)	(18.5, 25.3)			(4.1, 4.2)	(0.0, 1.6)
			Restoration state mean			1.0	0.58	1380	7.74	51	1	1492	81	61	23.9	245	3518	17.9	2.70

wetlands) in August using a 7.6-cm diameter polycarbonate tube. We sectioned and froze the top two cm of each core. These sections were subsequently freeze-dried, homogenized, and analyzed for OC content by loss on ignition for 4 hours at 550ºC (Heiri et al. 2001).

Biological sampling

We used an integrated tube sampler to collect ~30 L of water. A subsample of this water was preserved with Lugol's solution for phytoplankton identification and enumeration, while the remainder was filtered through a 64-μm mesh sieve to collect crustacean zooplankton, which were then preserved in 95 % ethanol. Phytoplankton from the three sampling periods were pooled and analyzed as composite samples. Aliquots of 50-100 mL were settled and enumerated at a magnification of 400x using the Utermöhl technique (Utermöhl 1958) and a Leica DM IRB inverted microscope. At least 200, though typically over 1000, cells were counted in each sample. Phytoplankton were identified to the highest taxonomic resolution possible, either genus or species, with taxonomic names following Algaebase (www.algaebase.org). Crustacean zooplankton were enumerated using a Leica MZ6 dissecting microscope and identified to species whenever possible following Edmonson (1959). *Alona* spp. were identified to genus and copepod juveniles and harpacticoid copepods to order. At least 200 individuals were identified from each sample, with no more than 50 nauplii contributing to that total. For samples containing fewer than 200 individuals, the entire sample was identified.

We used 15 of the cores collected to analyze OC content (5 cores per restoration state) for identification and enumeration of diatoms and chrysophyte cysts. Sediment subsamples were digested in hot 30 % H_2O_2. Cleaned slurries were then diluted and aliquots were evaporated at room temperature onto coverslips that were then fixed to slides with Naphrax medium. We identified 300 valves (and chrysophyte cysts) under oil immersion at 1000x magnification using an Olympus BX41 microscope equipped with differential interference contrast optics. Diatoms were identified and counted at the finest possible taxonomic resolution, either genus or species, following Patrick and Reimer (1966, 1975), Germain (1981), and Krammer and Lange-Bertalot (1986-1991) with nomenclature updates following Diatoms of the United States (westerndiatoms.colorado.edu). Taxonomic designations were confirmed with field-emission scanning electron microscopy (SEM). For SEM, undiluted slurries were evaporated onto aluminum stubs and sputter-coated with gold, and examined with a Zeiss Sigma 300 VP SEM operating at 10 kV. Micrographs of chrysophyte cysts and diatoms are presented in Plates A1.1 and A1.2.

A D-frame dip net (500-µm mesh-sized, 30-cm maximum aperture) was used to kick sweep and collect macroinvertebrates. On each sampling occasion we collected one sample from the open-water zone and one from within the ring of emergent vegetation. This method captures water column-, benthos-, and vegetation-associated taxa, hereafter referred to collectively as macroinvertebrates. Organisms were identified and counted at the finest possible taxonomic resolution (typically genus), except for chironomid (Diptera) and lepidopteran larvae, ostracods, and oligochaetes, which were identified at coarser taxonomic resolution. Identification followed Clifford (1991) and Merritt et al. (2008). We report and analyzed counts from the open water and vegetation samples combined. Given that these wetlands are naturally fishless, we were surprised to detect fish in three sites while sampling macroinvertebrates. At all three sites, fish were detected during the first sampling visit and observed on all subsequent visits, though by the end of the summer there were often dead fish floating at the wetland surface. To be sure we were not failing to detect fish in other sites, we made extra sweeps with the dip net (five locations per wetland during each of the three sampling periods) and never caught any fish. Thus, given the ease of detection of fish in those three sites, we are confident that the other 21 sites were fishless.

Two years after the initial survey, in September 2013, we returned to all sites to identify percent cover of submersed aquatic vegetation (SAV) within 1 m^2 quadrats along two transects per site. Randomly selected transects extended from the margin of the wetland to the centre. We included in our surveys algal communities that were not captured by other methods including mats of algae that floated on the water surface (metaphyton) and one macroalga (*Chara*). Aquatic plants were identified to species (except for mosses) following Lahring (2003).

Statistical analyses

We used linear mixed-effects models (fitted using the nlme package in R, Pinheiro et al. 2014) with restricted maximum likelihood estimation to evaluate restoration-state-specific trends in water chemistry and sediment OC. Mixed models allow correct prediction of effects, despite autocorrelation owing to repeated sampling of wetlands. We considered the effect of sampling date, time of day, and surrounding land use. Restoration state was included as a fixed effect, site as a random effect, and we included a restoration-state x date interaction. If date, time, land use, or the interaction were not significant, they were not included in the final model. Results are reported as least squares means and 95 % confidence intervals, calculated with the lsmeans package (Lenth and Hervé 2015).

Constrained ordinations were used to identify environmental variables that explained significant ($P < 0.05$) variation in the taxonomic composition of each sampled community. Variance was sufficiently large (i.e., gradients of > 3 standard deviations in the leading axes of exploratory correspondence analyses) to justify use of unimodal ordination techniques (ter Braak and Prentice 1988). We used Hellinger-transformed count data to reduce the influence of species of low abundance and many zeros. SAV species data used in the ordination were the proportion of quadrats containing a species, so were not transformed. Taxa present in less than ~5 % of samples were excluded. Phytoplankton and zooplankton communities were ordinated at the order and genus level, respectively, to improve ordination interpretability. To avoid overparameterizing models and including collinear variables, only a subset of environmental variables were used in the analyses. We included specific conductance, pH, NH_4^+, TP, DOC, chl *a*, as well as date (when appropriate), fish presence, and restoration state. In addition, based on *a priori* hypotheses about environment-species relationships sediment OC was also considered in the constrained ordination of diatoms, and TSS in the constrained ordination of SAV. Environmental variables were square-root transformed, and their significance determined using forward selection and Monte Carlo permutation tests. We present only constrained ordinations where the overall model was significant ($P < 0.05$). For the two cases (phytoplankton and sediment diatom communities) that were not significant, we instead present an unconstrained analysis. All analyses were performed with the vegan package (Oksanen et al. 2015) in R.

Results

Abiotic environment

Wetlands ranged from fresh to moderately brackish (330 - 3300 $\mu S\ cm^{-1}$), and were characterized by pH between 7 and 8, relatively high DOC, low TSS, and variable levels of nutrients and chl

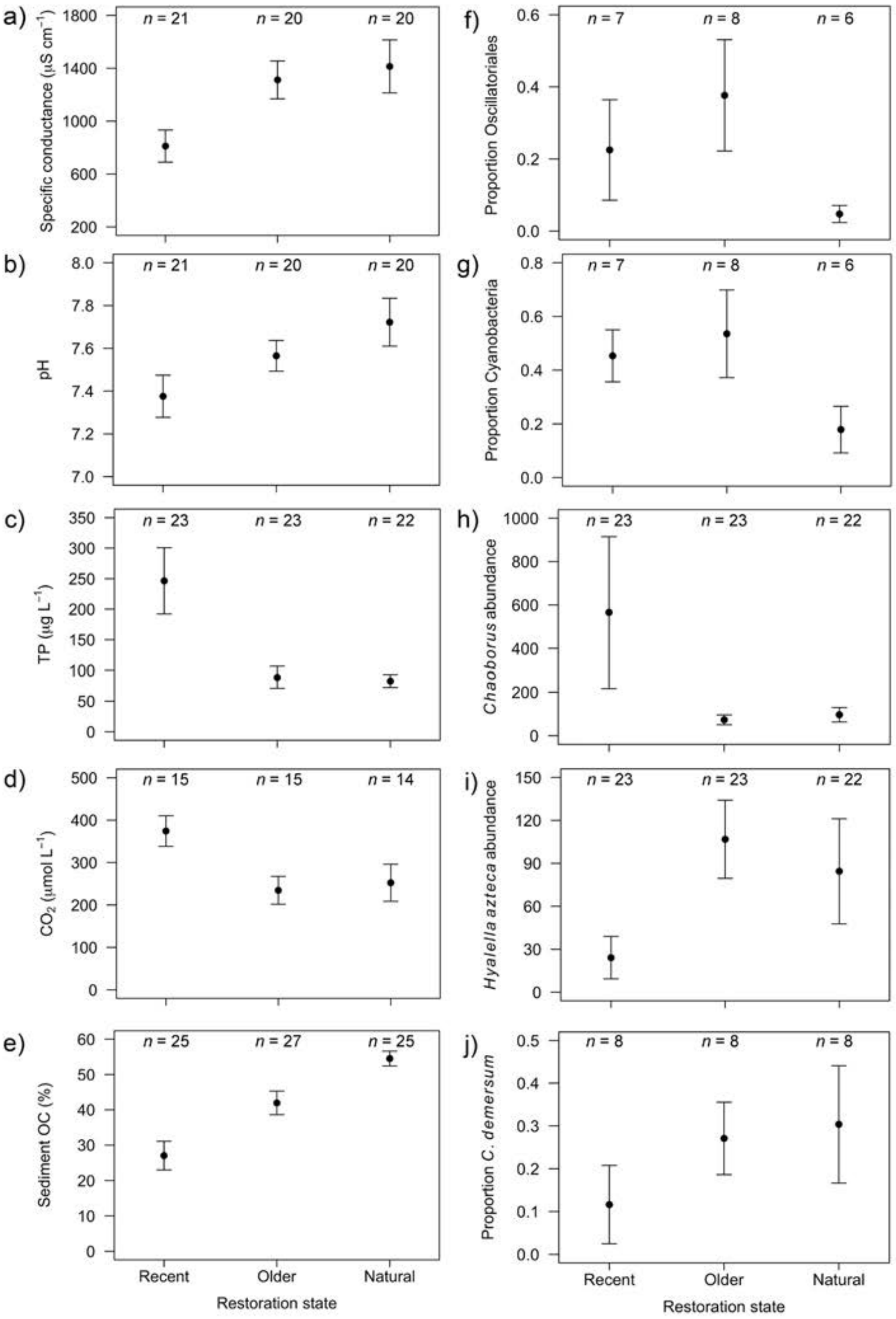

Figure 2.1 Abiotic and biological variables measured in prairie wetlands, summarized by restoration state (recently restored, older restored, and natural). Shown are mean ±1 standard error for: a) specific conductance; b) pH; c) total phosphorus; d) dissolved carbon dioxide; e) sediment organic carbon content; f) the proportion of the phytoplankton community belonging to the order Oscillatoriales; g) the proportion of cyanobacteria in the phytoplankton community; h) the abundance of the larval stage of the dipteran midge *Chaoborus*; i) the abundance of the amphipod *Hyalella azteca*; j) the proportion of sampled quadrats containing the submersed macrophyte *Ceratophyllum demersum*. Specified are the number of samples, rather than the number of sites, analyzed. Phytoplankton samples were a composite of two to three sampling dates each and macroinvertebrate samples were a composite of one open water and one vegetated site each.

a (Table 2.1). Recently restored wetlands were less brackish than older restored and natural wetlands, had lower pH, and almost 3x higher concentrations of TP and TDP (Table 2.1, Fig. 2.1a-c). They also had more than 50 % higher CO_2 concentrations, but significantly less sediment OC, than older restored and natural wetlands (Table 2.2, Fig. 2.1d-e). NH_4^+, NO_2+NO_3^-, TDN, DOC, DIC, chl *a*, TSS, and dissolved CH_4 did not vary consistently according to restoration state. A land-use effect on TDN and DOC concentrations was driven by the 'crop' land use category (i.e., land cultivated with grains or oilseeds). This land use occurred around only three sites, all of which are in close proximity to each other (Table 2.1, Fig. 1.2b). We have no *a priori* reason to believe that wetlands surrounded by cultivated land would have lower TDN and DOC concentrations than wetlands in other upland matrices. Thus, although every effort was made to equally represent all land uses, we believe this result is attributable to a local area or site effect rather than to land use.

Table 2.2. Results (*F*-statistics, *P*-values, least squares means, 95 % confidence intervals) from linear mixed models examining the effect of wetland restoration state (recently restored, older restored, or natural) on water chemistry variables including specific conductance (SpCond), pH, total and total dissolved phosphorus (TP, TDP), dissolved CO_2 concentrations, and sediment organic carbon content. All models included restoration state as a fixed effect, site as a random effect, and the models of pH and CO_2 also included a sampling date covariate.

		Recently restored	Older restored	Natural
Response variable	**Model statistics**	**lsmeans (95% CI)**	**lsmeans (95% CI)**	**lsmeans (95% CI)**
SpCond (µS cm^{-1})	partial $F_{2,58} = 4.27$	812	1312	1414
	$P = 0.02$	(508, 1115)	(1001, 1623)	(1103, 1725)
pH	partial $F_{2,57} = 3.53$	7.38	7.56	7.73
	$P = 0.04$	(7.20, 7.56)	(7.37, 7.74)	(7.54, 7.91)
TP (µg L^{-1})	partial $F_{2,65} = 7.77$	246	89	83
	$P < 0.0001$	(180, 313)	(23, 155)	(15, 150)
TDP (µg L^{-1})	partial $F_{2,65} = 7.04$	203	74	62
	$P < 0.0001$	(145, 262)	(16, 133)	(3, 122)
CO_2 (µmol L^{-1})	partial $F_{2,21} = 2.83$	377	225	249
	$P = 0.08$	(281, 472)	(129, 320)	(152, 345)
Sediment OC (%)	partial $F_{2,13} = 4.52$	27	42	55
	$P = 0.03$	(14, 40)	(30, 54)	(42, 67)

Algae

A total of 116 phytoplankton taxa were detected with site richness ranging from 1-61 taxa. These taxa span 29 orders representing cryptophytes (Cryptophyta, Katablepharidophyta), green algae (Chlorophyta, Charophyta), cyanobacteria, chrysophytes (Ochrophyta), euglenoids (Euglenophyta), diatoms (Bacillariophyta), and dinoflagellates (Dinoflagellata). Cryptophytes, chlorophytes, and cyanobacteria were present in nearly all sites whereas diatoms and dinoflagellates were relatively rare. Cyanobacteria and its order Oscillatoriales were more abundant in the recently and older restored sites than in the natural wetlands (Fig. 2.1f, g), suggesting that phytoplankton community recovery after drainage and restoration may still be incomplete after 14 years. Ordination revealed that sites were most strongly differentiated along the primary axis based on abundance of members of the cyanobacterial order Oscillatoriales, with natural wetlands showing a narrower range in composition along that axis than restored sites (Fig. 2.2). Natural wetlands were also less distinct along the secondary axis, which represents a composition gradient of small chlorophytes (Sphaeropleales) to picocyanobacteria (Synechococcales, Chroococcales).

We identified 38 diatom taxa (Plates A1.1, A1.2), plus chrysophyte cysts (Plate A1.1), with richness ranging from 15-23 taxa. The majority of taxa were alkaliphilous to circumneutral periphytic diatoms. As such, there were no obvious environmental gradients along either the first or second axis (Fig. 2.3). The three restoration states were interspersed, though recently restored sites showed a larger gradient along both axis 1 and 2 (Fig. 2.3a). Although there were no overall differences in diatom community composition, the relative abundance of some taxa varied with restoration state. Mean % relative abundance ± SD of chrysophyte cysts was greater in recently restored wetlands (36.0 % ± 59.6) than older restored (7.7 % ± 5.7) or natural (16.6 % ± 24.2) wetlands. *Eunotia bilunaris* (Ehrenberg) Schaarschmidt was also more abundant in recently restored wetlands (3.5 % ± 3.9) than in older restored (0.6 % ± 0.5) or natural (0.5 % ± 0.6) wetlands. In contrast, *Cocconeis* spp. were more abundant in natural (7.2 % ± 3.7) and older restored (6.2 % ± 5.9) than in recently restored (1.1 % ± 1.8) wetlands.

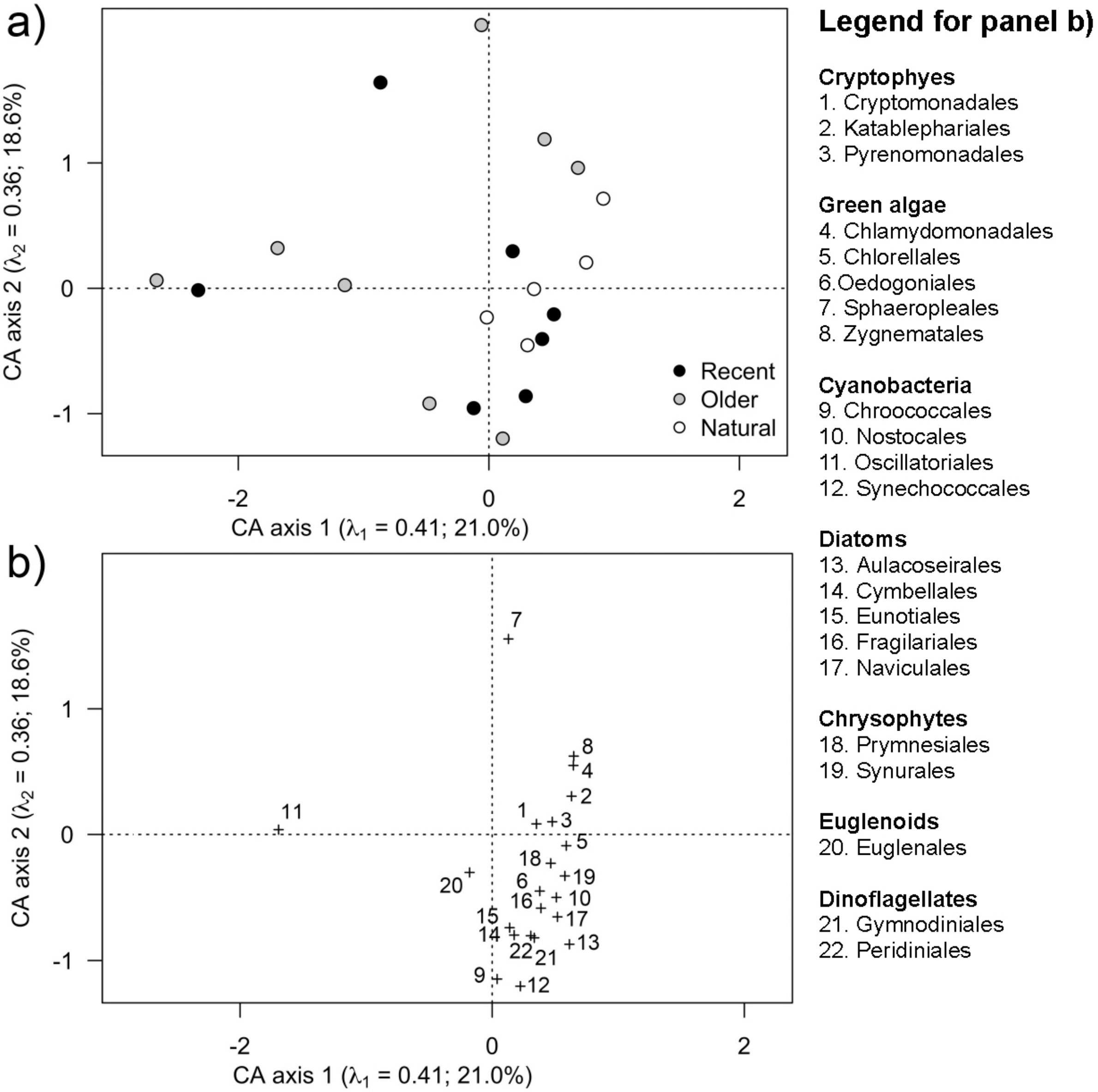

Figure 2.2 Association of a) sites and b) phytoplankton orders in recently restored (RR), older restored (OR) and natural (Nat) prairie wetlands based on correspondence analysis of phytoplankton abundance. The orders associated with the numbers in panel b) are listed in the legend on the right.

Zooplankton

We identified 24 crustacean zooplankton taxa in our study wetlands including 15 cladocerans, three calanoid copepods, five cyclopoid copepods, and one harpacticoid copepod. Site richness ranged from 7-13 taxa. Abundance of *Chaoborus*, a potentially important zooplankton predator, was greater in the recently restored sites than in older restored or natural sites (Fig. 2.1h). There

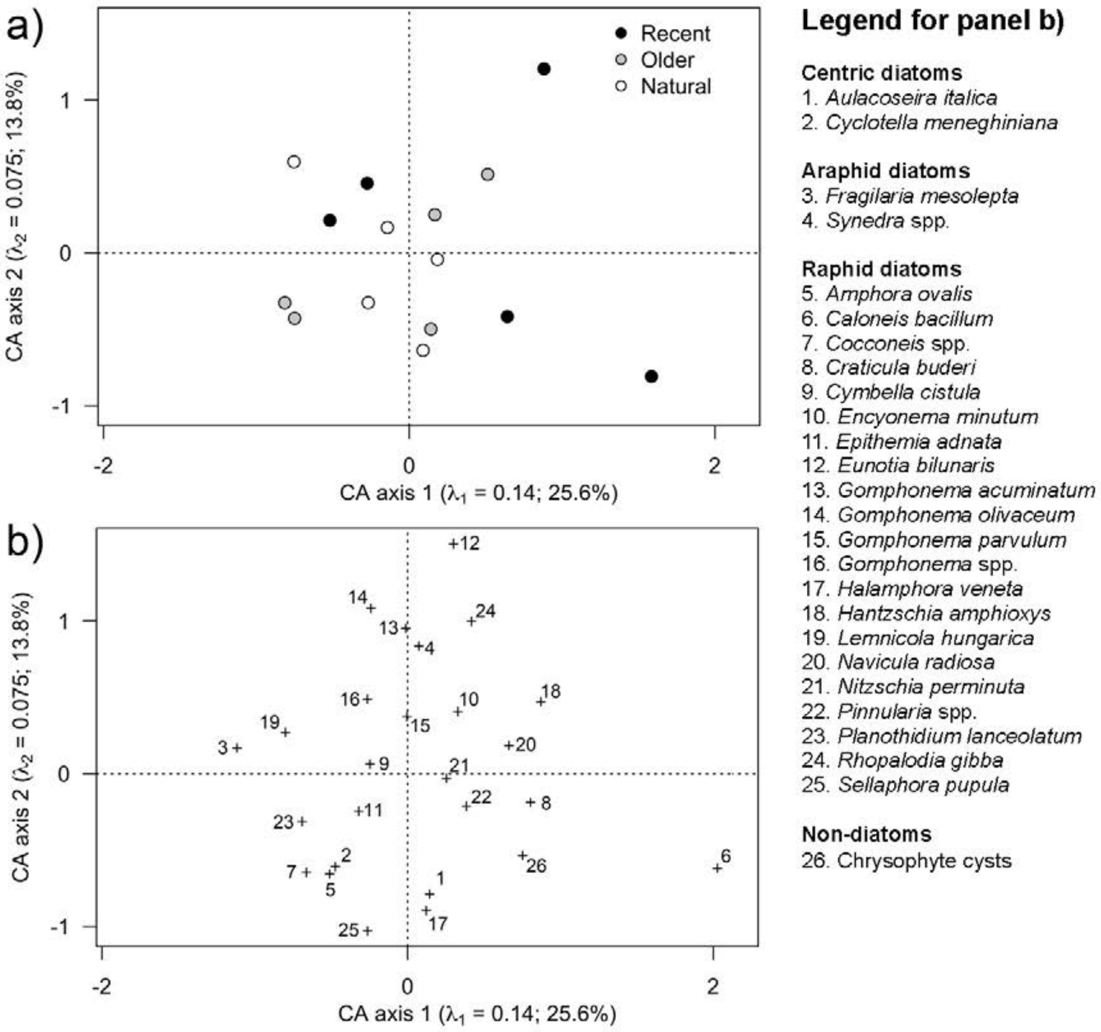

Figure 2.3 Association of a) sites and b) benthic siliceous microfossils in recently restored (RR), older restored (OR) and natural (Nat) prairie wetlands based on correspondence analysis of diatom taxa and chrysophyte cysts. The taxa associated with the numbers in panel b) are listed in the legend on the right. Additional taxonomic information is available in Appendix 2.

was, however, no evidence that zooplankton community composition was related to restoration status (Fig. 2.4a).

DOC and fish presence best explained zooplankton taxonomic variation among the sites (Fig. 2.4). Canonical correspondence analysis (CCA) axis 1 contrasted several relatively high-DOC, fishless wetlands containing higher abundances of large-bodied cladocerans from certain fish-inhabited sites with more copepods and higher chl *a* concentrations. CCA axis 2 represented a less well-defined seasonal gradient along which *Diacyclops navus* (Herrick, 1882) and juvenile calanoid stages were replaced by a greater diversity of both cladocerans and adult copepods.

The overall CCA was statistically significant ($P = 0.001$), and identified environmental factors explained 21 % of total variance.

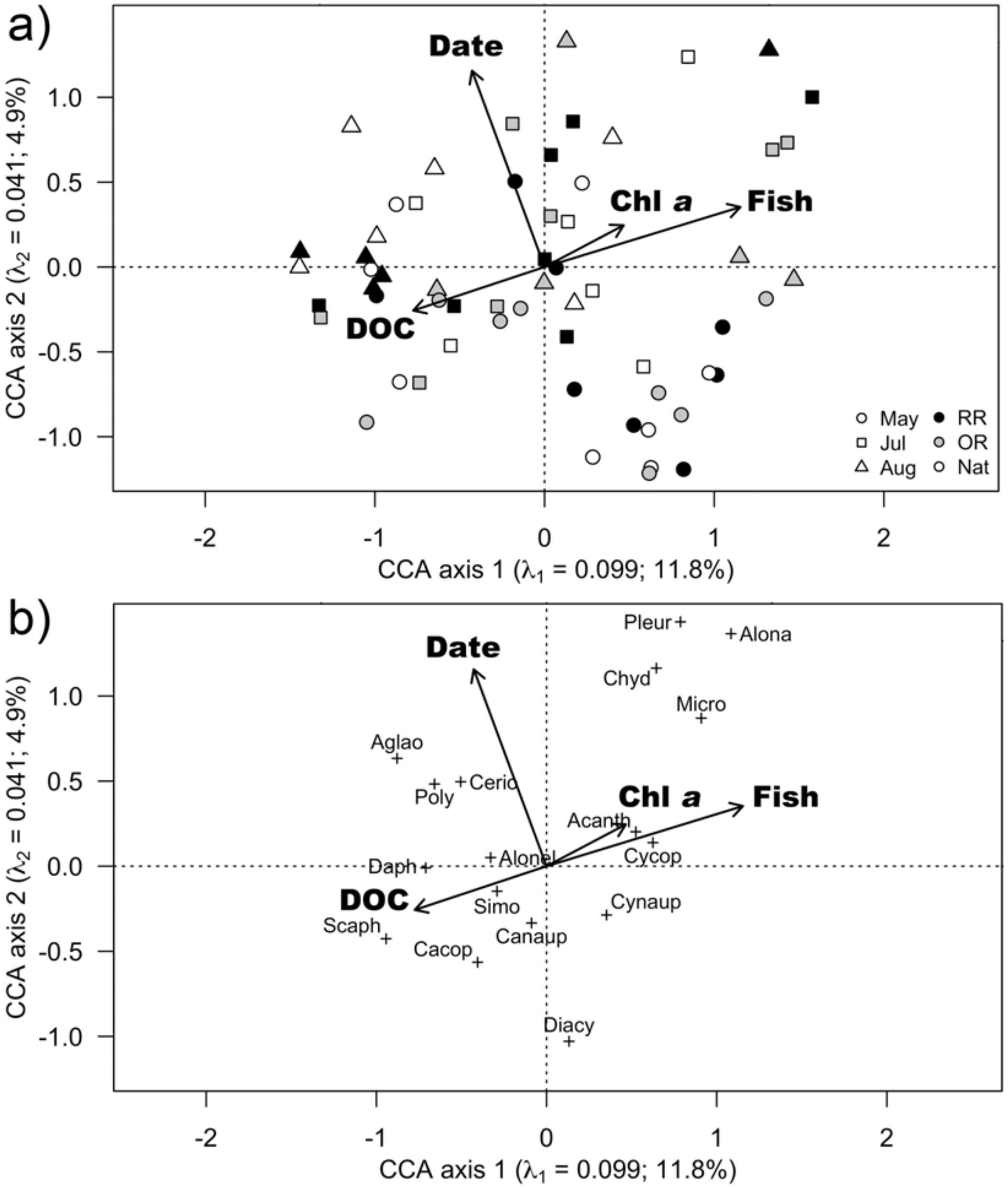

Figure 2.4 Association of a) sites and b) zooplankton genera based on canonical correspondence analysis of crustacean zooplankton abundance and environmental variables in recently restored (RR), older restored (OR) and natural (Nat) prairie wetlands sampled in May, July, and August 2011. Zooplankton taxa include: *Acanthocyclops* (Acanth), *Aglaodiaptomus* (Aglao), *Alona*, *Alonella* (Alonel), calanoid copepodids (Cacop), calanoid nauplii (Canaup), *Ceriodaphnia* (Cerio), *Chydorus* (Chyd), cyclopoid copepodids (Cycop), cyclopoid nauplii (Cynaup), *Daphnia* (Daph), *Diacyclops* (Diacy), *Microcyclops* (Micro), *Pleuroxus* (Pleur), *Polyphemus* (Poly), *Scapholeberis* (Scaph), and *Simocephalus* (Simo). Environmental variables include: date, dissolved organic carbon, chlorophyll *a*, and presence-absence of fish.

Macroinvertebrates

We identified 87 macroinvertebrate taxa including insects (Coleoptera, Diptera, Ephemeroptera, Hemiptera, Lepidoptera, Odonata and Trichoptera), crustaceans (Amphipoda, Anostraca, Ostracoda), molluscs (Gastropoda, Pelecypoda), annelids (Hirudinea, Oligochaeta), and mites (Acari). Site richness ranged from 19-44 taxa. Many (48) taxa were rare, i.e., present in 5% or less of samples. The most common taxa, found in over 50 % of samples, were chironomid larvae (95 %), *Chaoborus* (92 %), *Enallagma* (81 %), *Dasycorixa* (74 %), *Physa* (64 %), and *Hyalella azteca* Saussure, 1858 (58 %).

CCA axis 1 separated wetlands mainly on the basis of restoration state and fish presence (Fig. 2.5). Several older restored and natural wetlands were discriminated from recently restored sites based on the latter containing higher relative abundances of *Chaoborus* (Diptera) larvae (Fig. 2.1h) but fewer amphipods (Fig. 2.1i). Seasonal succession, pH, and increasing chl *a* concentrations characterized the less well-defined CCA axis 2. Specifically, late-summer conditions and greater algal abundance disproportionally favored gastropods and leeches (Hirudinea) over aquatic insects. All together, environmental variables explained 29 % of total variance.

Submersed aquatic vegetation

There were 11 species of SAV detected, including one floating species (*Lemna minor* L.), a species with floating leaves (*Persicaria amphibia* (L.) Gray), and several fully submerged species (*L. trisulca* L., *Utricularia vulgaris* L., *Ceratophyllum demersum* L., *Potamogeton pusillus* L., *Myriophyllum sibiricum* Komarov, unidentified mosses, and the multicellular green alga, *Chara*). Other species, including *Ranunculus gmelinii* DC., and *R. aquatilis* L., possess leaves that may be submerged or floating on the water surface.

CCA axis 1 contrasted several more brackish, DOC-rich natural wetlands and older restored sites from more recently restored, high TP sites (Fig. 2.6). CCA axis 2 further differentiated sites on the basis of *C. demersum* and *M. sibiricum* relative abundance. Recently restored wetlands differed from older restored and natural wetlands in that they contained greater abundance of *Potamogeton pusillus* and *Persicaria amphibia*, but less *C. demersum* (Fig. 2.1j), moss, and metaphyton (Fig. 2.6). Environmental variables explained 31 % of total SAV community variance.

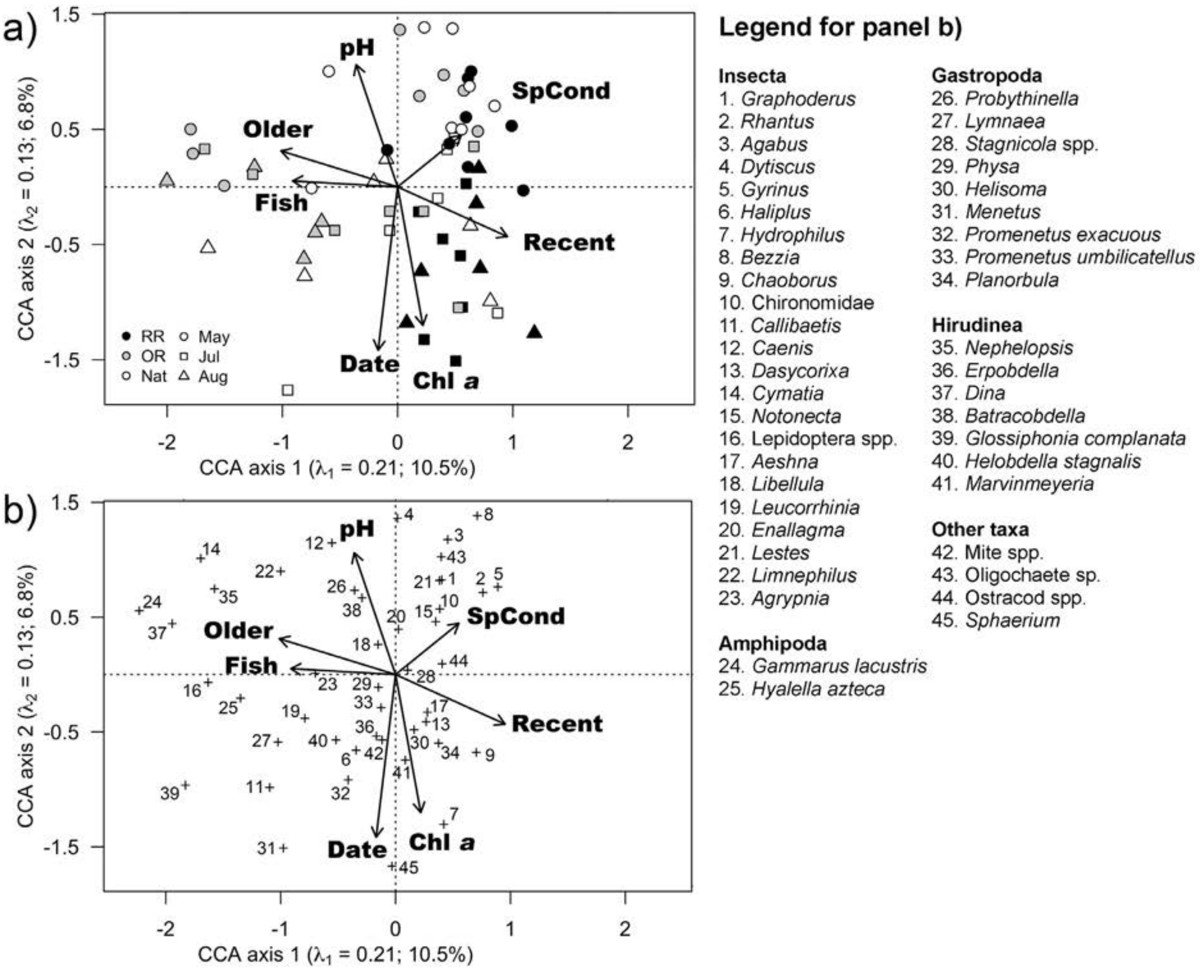

Figure 2.5 Association of a) sites and b) macroinvertebrate taxa based on canonical correspondence analysis of benthic macroinvertebrate abundance in recently restored (RR), older restored (OR) and natural (Nat) prairie wetlands sampled in May, July, and August 2011. The taxa associated with the numbers in panel b) are listed in the legend on the right. Environmental variables include: restoration state (Older/Recent), date, specific conductance, pH, chlorophyll *a*, and presence/absence of fish. Additional taxonomic information is available in Appendix 2.

Discussion

Older restored wetlands more closely resembled natural wetlands than those that were recently restored, supporting our prediction of chemical and biological recovery within approximately a decade following hydrological restoration. This timeline is consistent with studies of the recovery of birds and emergent vegetation (Puchniak 2002), soil properties and microbial communities (Card and Quideau 2010, Card et al. 2010), and greenhouse gas fluxes (Badiou et al. 2011, Chapter 3) in restored prairie wetlands. Taken together, these studies provide an explicit and hitherto unspecified timeline for chemical and biological recovery of restored prairie

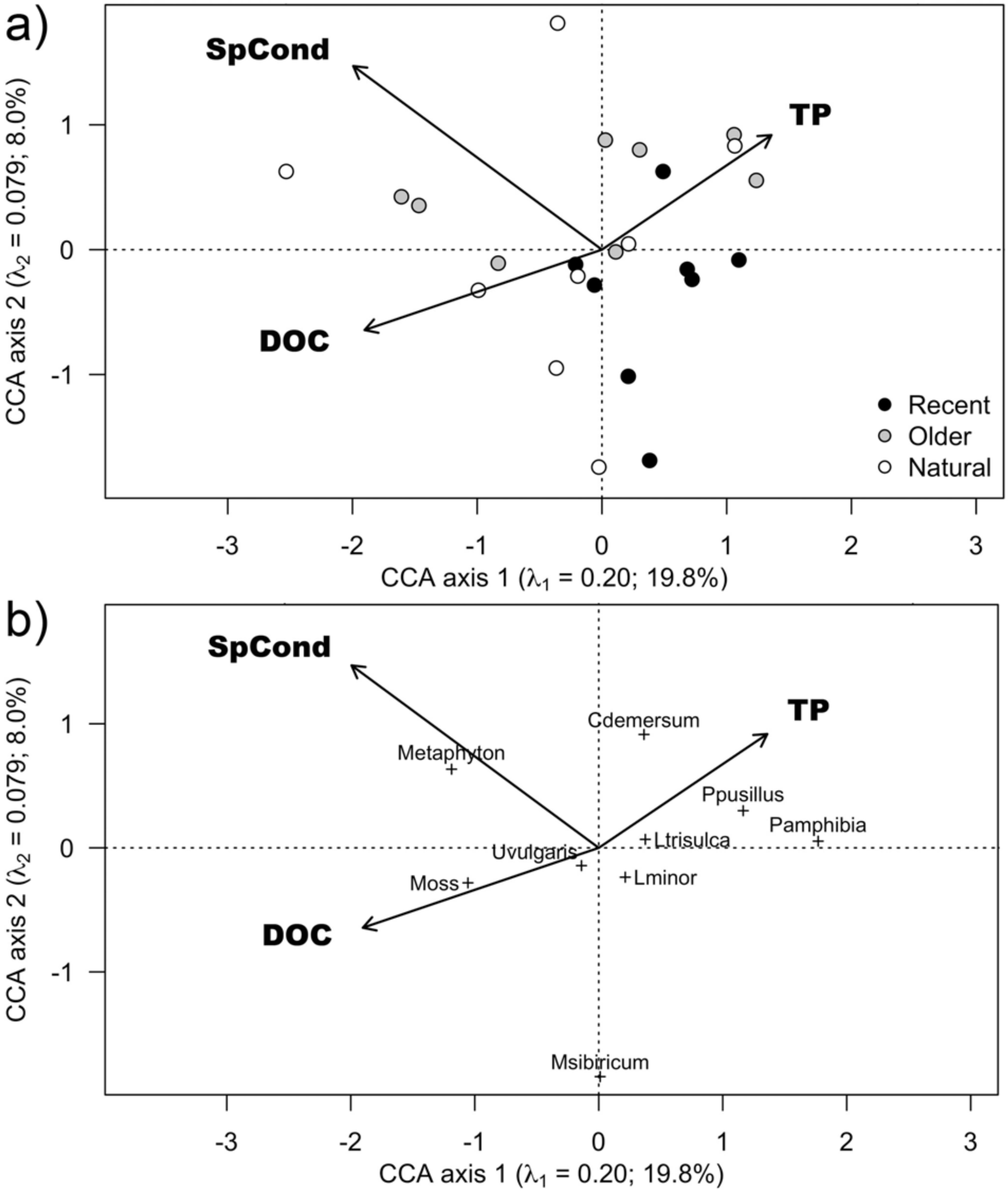

Figure 2.6 Association of a) sites and b) submersed aquatic vegetation (SAV) based on canonical correspondence analysis of the proportional occurrence of SAV along transects in recently restored (RR), older restored (OR) and natural (Nat) prairie wetlands. SAV taxa include: *Ceratophyllum demersum*, *Lemna minor*, *L. trisulca*, Metaphyton, Moss, *Myriophyllum sibiricum*, *Persicaria amphibia*, *Potamogeton pusillus*, and *Utricularia vulgaris*. Environmental variables include: specific conductance, dissolved organic carbon, and total phosphorus.

wetlands. However, there was variation among the quantified chemical and biological recovery timelines. Below, we discuss specific responses to restoration and environmental gradients.

Abiotic environment

Although older restored wetlands were chemically indistinguishable from natural wetlands, those recently restored were distinct in their higher concentrations of TP, TDP, and CO_2, and lower specific conductance, pH, and sediment OC content (Fig. 2.1a-e). Reflooding of wetlands can result in phosphorus release from sediments, likely driven by remobilization of PO_4^{3-} that was previously sorbed to metal (especially iron) oxides (Olila et al. 1997, Kinsman-Costello et al. 2014). Although many processes can affect wetland specific conductance, evapoconcentration is an important factor controlling major ion concentrations in small water bodies (LaBaugh et al. 1987, Heagle et al. 2013). Recently restored wetlands have consistently been observed to have lower specific conductance (Galatowitsch and van der Valk 1996b, Puchniak 2002, Badiou et al. 2011), a pattern possibly attributable to fewer cycles of evaporation during the open water season compared to older restored or natural wetlands. It is more difficult to explain why recently restored wetlands have lower pH. However, several studies have noted a relationship between pH and SAV, with higher pH associated with greater SAV biomass or more productive SAV communities (Carpenter and Lodge 1986, Galatowitsch and van der Valk 1996b, Badosa et al. 2010, Chapter 3). Lower sediment OC content in recently restored prairie wetlands has been observed in other studies (Galatowitsch and van der Valk 1996b, Badiou et al. 2011) and can be attributed to recently restored wetlands having less time to accumulate OC pools and possibly less emergent vegetation to supply sediment OC.

Algae

Surprisingly, there were no distinct differences in algal community composition between restoration states (Fig. 2.2, Fig. 2.3). However, restored wetlands did contain greater relative abundance of cyanobacteria than natural wetlands, especially filamentous taxa of the order Oscillatoriales (Fig. 2.1f-g). Elsewhere, Kinsman-Costello et al. (2014) reported filamentous algal blooms associated with elevated phosphorus concentrations resulting from the reflooding of a drained wetland. Species-specific differences in diatom abundance between restoration states could be related to the lower pH in recently restored wetlands (chrysophytes and *Eunotia bilunaris* are acidophilous) and to differences in SAV composition. Mayer and Galatowitsch (1999) also observed no difference in periphytic diatom composition between restored and natural wetlands.

Our study occurred during a wet period in which wetlands were typically flooded beyond basin margins, with 10 sites (7 restored, 3 natural) connected to other wetland basins during the summer and the possibility that others received overland flow during spring flooding. Hydrologically connected basins often maintain distinct water chemistry (L.E. Bortolotti, unpubl. data), but these connections may augment the already high dispersal potential of small organisms like algae (Shurin et al. 2009). Thus, connectivity may facilitate rapid recovery of algal communities. Alternatively, it is possible that, in the case of phytoplankton, pooling of different sampling dates may have resulted in the loss of important information about seasonal succession (i.e., date effects). In addition, high natural variation in algal community composition may make it difficult to detect community-level responses to either environmental or restoration gradients without considerable sampling effort.

Zooplankton

Zooplankton community composition also did not vary significantly among restoration states (Fig. 2.4). This result is consistent with studies of restored Mediterranean marshes (Badosa et al. 2010) and ponds (Olmo et al. 2012). Such findings may reflect the implicitly high dispersal potential of zooplankton (De Meester et al. 2002), which facilitates rapid recovery and hence minimizes taxonomic differences between restoration states. Furthermore, the previously described high hydrologic connectivity during this study likely augmented aeolian and animal vectors of zooplankton dispersal (Beisner et al. 2006, Frisch et al. 2012) to the restored wetlands.

Date, DOC, chl *a*, and the presence of fish all explained significant taxonomic variation in the wetland zooplankton communities. The influence of date can be explained by communities collected in early June being more similar to those found in July and August than late May. Here, community succession occurred as a result of increases in many taxa that are positively associated with SAV growth (e.g., *Alona* spp., *Chydorus sphaericus* (O.F. Mueller, 1785), *Simocephalus serrulatus* (Koch, 1841); Dodson et al. 2010). Other studies have detected an association between DOC and zooplankton community characteristics (Beisner et al. 2006, Derry et al. 2009, Shurin et al. 2010, Robidoux et al. 2015), though the exact nature of this relationship is unclear. Small cladoceran and cyclopoid taxa, which are generally considered to be weak grazers relative to large cladocerans, were associated with higher concentrations of chl *a*. Chl *a* concentrations may thus reflect the influence of zooplankton grazing on algal abundance or bottom-up effects of food abundance and quality. Fish can shape the size structure of zooplankton communities because large zooplankton are more susceptible to fish predation (Brooks and Dodson 1965). Ultimately, the environmental variables measured somewhat weakly constrained zooplankton community composition, either because we failed to measure some key environmental gradient or because we measured only a narrow range of their tolerance for the selected environmental variables. Alternatively, biological factors such as phytoplankton composition, SAV habitat, and macroinvertebrate predators may be of greater importance in shaping zooplankton community composition.

Macroinvertebrates

Differences in macroinvertebrate community composition among restoration states became evident in July and August (Fig. 2.5). Recently restored wetlands contained higher abundances of *Chaoborus* larvae (Fig. 2.1h), but fewer amphipods (Fig. 2.1i), leeches, and trichopteran larvae. Meyer and Whiles (2008) also observed lower abundance of amphipods and leeches in restored wetlands. There is little consensus among studies of macroinvertebrate recovery in shallow restored wetlands (Zimmer et al. 2002, Meyer and Whiles 2008, Marchetti et al. 2010), though some differences between restored and natural sites may persist for > 10 years after restoration (Meyer and Whiles 2008, Marchetti et al. 2010). We suggest that observed differences between recently restored versus older restored and natural wetlands reflect community succession similar to that which occurs naturally during wet-dry cycles. After reflooding, wetlands are first colonized by taxa that have short generation times and high reproductive output. Such organisms are typically well adapted to temporary, productive habitats (Euliss et al. 1999). Recently restored wetlands contained many species that fit this description including the snails Stagnicola, Helisoma, and Planorbula (Jokinen 1987, Dillon 2010). Similarly, fairy shrimp (Order Anostraca) and mosquito larvae were only found in recently restored sites, likely because they are vulnerable to predation (Euliss et al. 1999). Thus, for macroinvertebrates, drainage and hydrological restoration may mimic the natural disturbance regime of the Prairie Pothole Region.

In addition to restoration state, macroinvertebrate community composition varied with date, specific conductance, chl *a*, the presence of fish, and pH. The effects of date, specific conductance, wetland productivity, and fish on macroinvertebrate communities have all been documented in prairie wetlands or similar ecosystem types (e.g., Euliss et al. 1999, Hanson et al. 2005, Anteau and Afton 2008, Miller et al. 2008), though we did not always observe the same type or direction of effects as previous studies. Over the course of the summer we observed a decline in the abundance of coleopteran and chironomid larvae, but an increase in *Chaoborus*, amphipods, snails, anisopteran odonate nymphs, and Hemiptera. There was evidence of species turnover, but not a change in abundance for Ephemeroptera, Trichoptera, and zygopteran odonates.

We interpret the significance of chl *a* and the presence of fish in the ordination as evidence of both bottom-up and top-down forces structuring the macroinvertebrate community. The significance of pH is more difficult to interpret, though we suggest that it is a proxy for whole wetland productivity, including the influence of SAV. Other work (Chapter 3) suggests that shifts in wetland pH are biologically driven, primarily by SAV. Submersed and emergent vegetation has well-established direct and indirect effects on macroinvertebrate communities (Anteau and Afton 2008, Hentges and Stewart 2010) and such biotic interactions may be of greater importance than abiotic gradients in structuring prairie wetland macroinvertebrate communities (Hanson et al. 2005).

Submersed aquatic vegetation

There was considerable overlap in SAV community composition between restoration states, and restoration state was not significant in the constrained analysis. However, recently restored wetlands tended to lack or have a lower proportion of plants associated with undisturbed SAV communities (e.g., *C. demersum*, moss; Rooney and Bayley 2011). Given that older restored wetlands resemble natural ones (Figs. 2.1j, 2.6), we suggest that SAV community composition takes several years to recover. Other studies have also detected an influence of drainage history on SAV community composition in prairie wetlands (Galatowitsch and van der Valk 1996a, Zimmer et al. 2003, Aronson and Galatowitsch 2008). However, at the more southern latitudes where these studies were conducted, the direction of the effect and the recovery timeline for the SAV community is different. For example, contrary to our study, *C. demersum* was typically more abundant in restored than in natural wetlands (Galatowitsch and van der Valk 1996a, Aronson and Galatowitsch 2008). Geographic differences in prairie wetland vegetation recovery have been previously noted, and attributed to different climate and drainage histories (Puchniak 2002).

Changes in wetland vegetation along a salinity gradient are well documented, with the effects evident even for the relatively short gradient in this study (Fig. 2.6). Consistent with previous descriptions (Stewart and Kantrud 1972), there was a tendency to observe *Potamogeton pusillus* and *L. trisulca* in fresh wetlands and *C. demersum* and moss in more brackish sites. Macrophytes release little phosphorus to the water column and many species take up phosphorus via their roots (Carpenter and Lodge 1986). However, unrooted SAV species that do not have access to nutrients in sediment may either respond to or help shape TP concentrations in the water column. SAV was also likely the driver rather than the consequence of the detected DOC gradient (Fig. 2.6). Much DOC in these wetlands is produced autochthonously, resulting from the decomposition of, or exudation by, macrophytes (Waiser 2006).

Conclusions

Some aspects of prairie wetland water chemistry and zooplankton community composition appeared to be unaffected by drainage and hydrological restoration, or recovered very quickly. There was some indication of persistent changes in the phytoplankton community, most likely stemming from the elevated phosphorus concentrations that typify recently restored wetlands. However, on the whole, restored wetlands generally resembled natural wetlands within approximately 10 years of restoration. These results are encouraging in that they suggest that restoration is an effective tool for reestablishing the capacity of prairie wetlands to sustain biodiversity across multiple trophic levels. However, given that there is a substantial time lag before full reestablishment of these services, it is clearly preferable to protect and retain intact wetlands on the landscape. Although recovery after 10 years is the general timeline we have identified, there was variability among biological communities, highlighting the weakness of using single biological communities as indicators of restoration success. Strong seasonal changes and environmental gradients affecting species composition further complicate the use of biological indicators of recovery after restoration. We suggest that future evaluations of restoration success should focus on ecosystem-level processes that integrate the influence of all biological communities and their abiotic environment for a holistic picture of wetland recovery.

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Chapter 3: Net ecosystem production and carbon greenhouse gas fluxes in three prairie wetlands

Introduction

There are millions of prairie wetlands in the Prairie Pothole Region (PPR; Fig. 3.1a), an area that spans ~715,000 km^2 (Euliss et al. 1999). These wetlands formed during Pleistocene glacial retreat and range from ephemeral basins that hold water only after snowmelt or major precipitation events to permanent features on the landscape (Stewart and Kantrud 1971; Euliss et al. 1999). These wetlands provide many important ecosystem services including attenuating floods, stabilizing soils, improving water quality, and supporting biodiversity including globally important waterfowl populations (Zedler and Kercher 2005). Unfortunately, more than half of the wetlands in the PPR have been lost to human development, primarily drained for agriculture. Despite restoration efforts in many regions, wetland degradation continues to outpace recovery (Bartzen et al. 2010). To guide local management decisions, we need to understand how drainage and restoration affect the functioning of these wetland ecosystems.

Net ecosystem production (NEP) refers to the imbalance between gross primary production (GPP) and ecosystem respiration (ER; Chapin et al. 2006) and may be used to estimate the metabolic status of an ecosystem, i.e., whether an ecosystem is net autotrophic (production exceeds respiration) or net heterotrophic (respiration exceeds production). Although NEP is typically defined with respect to carbon flux, it can be inferred from diel changes in dissolved oxygen (O_2) concentrations. O_2 concentrations rise during daylight hours due to photosynthesis and drop during the night as organic carbon (OC) is respired.

The net flux of carbon dioxide (CO_2) between surface waters and the atmosphere has been commonly used as an indicator of the metabolic status of aquatic ecosystems. Net uptake of atmospheric CO_2 is considered indicative of net autotrophy, the assumption being that photosynthesis should drive dissolved CO_2 concentrations below atmospheric equilibrium, causing CO_2 to diffuse from the atmosphere into the water. In contrast, net release of CO_2 from surface waters is thought to arise when respiration of autochthonous and allochthonous OC exceeds photosynthesis (Duarte and Prairie 2005; Prairie 2008). The prevalence of CO_2 supersaturation in aquatic systems has led to the conclusion that net heterotrophy is widespread,

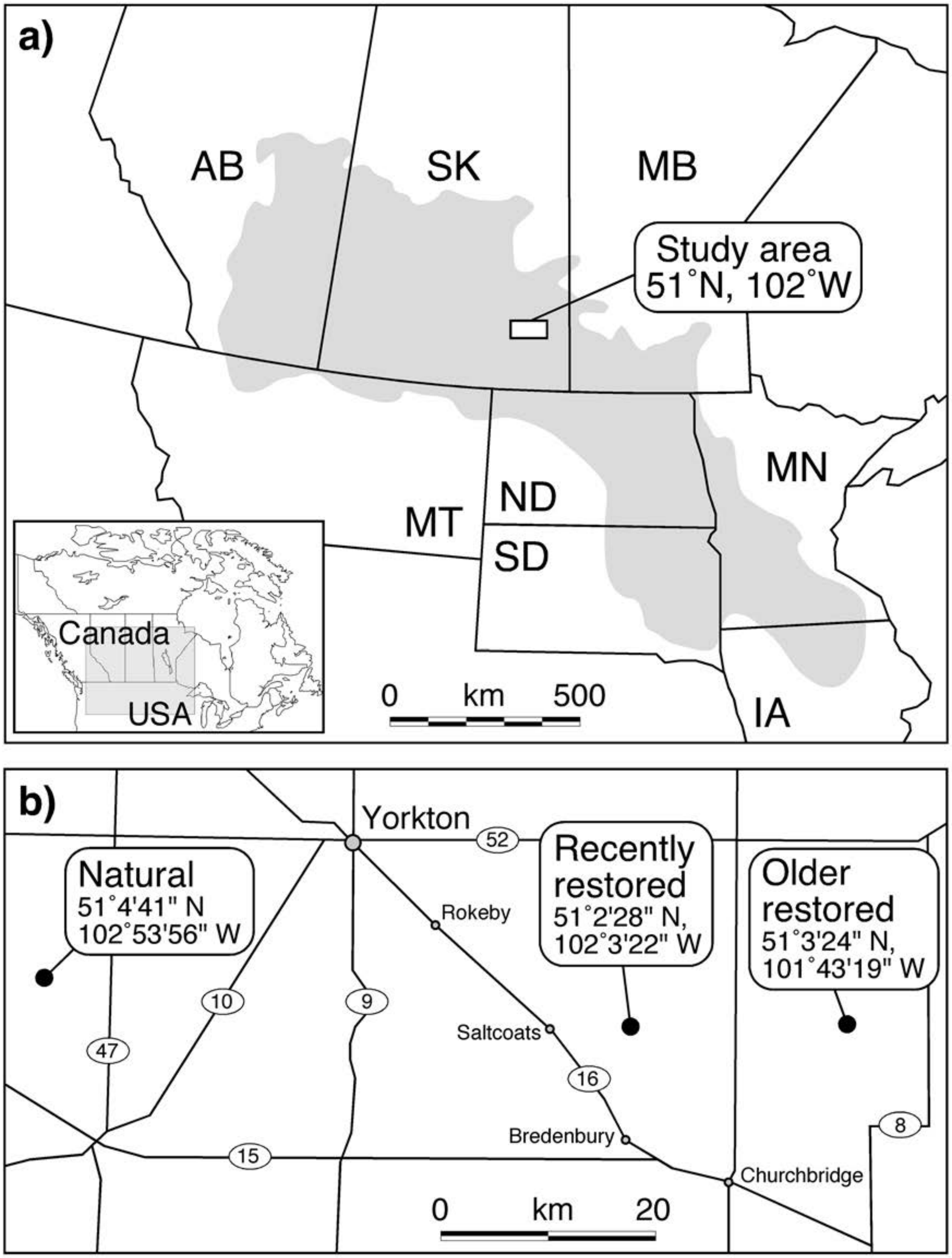

Figure 3.1 Map showing the location of study sites in southeastern Saskatchewan and the approximate extent of the Prairie Pothole Region (grey shading).

at least in oligotrophic and mesotrophic lakes and reservoirs (Duarte and Prairie 2005).

Although both NEP and CO_2 fluxes are used to estimate the metabolic status of aquatic ecosystems, they are not necessarily equivalent. NEP refers strictly to the outcome of biological processes whereas CO_2 fluxes arise from biological and geochemical processes (Chapin et al. 2006). Much emphasis has been placed on the role of allochthonous OC supporting CO_2 supersaturation in lakes (Duarte and Prairie 2005; Prairie 2008). However, CO_2 supersaturation arising from dissolved inorganic carbon (DIC) inputs is increasingly recognized as a common and global phenomenon (e.g., Stets et al. 2009; McDonald et al. 2013; Marcé et al. 2015). These DIC inputs may be of biological or geochemical origin and include CO_2 produced by respiration in adjacent terrestrial ecosystems and carbonate mineral dissolution and precipitation.

The diel O_2 method has often been used to estimate the metabolic status of lakes, but has been seldom employed in shallow freshwater wetlands. In prairie wetlands, the only study of metabolic status found that different methods, including CO_2 fluxes, produced conflicting results (Waiser and Robarts 2004). These authors were unable to resolve this apparent contradiction, highlighting the need for further investigation. Net CO_2 fluxes and the diel O_2 method are both potentially valuable for estimating metabolic status because, as “free-water” techniques, they avoid container artifacts. However, combining carbon and O_2 methodologies may provide additional insight into a system’s biogeochemistry (Obrador and Pretus 2013).

In this study, we characterized the metabolic status and carbon fluxes of the open-water zones of two restored and one natural prairie wetland during the ice-free season across two consecutive years. Our primary objective was to resolve disparities in the estimation of metabolic status in prairie wetlands using a combination of the diel O_2 method and CO_2 flux. By coupling these methods with measurements of total DIC and pH we are able to understand the limitations of and discrepancies between these approaches. Our second objective was to quantify the magnitude and relative importance of CO_2 and methane (CH_4) fluxes from these sites.

Methods

Study area

Measurements of NEP and carbon greenhouse gas (GHG) flux were made in May-August

2012 and May-September 2013 on three wetlands (Fig. 3.1b, Plate 3.1a-c) in the central aspen parkland ecoregion of Saskatchewan, Canada (Fig. 3.1a). These wetlands were chemically and biologically representative of three “restoration states” and were selected for in-depth study based on the survey of 24 sites described in Chapter 2. Eight of these wetlands had never been drained (“natural” wetlands), eight were restored 7-14 years previously (“older restored”), and eight were restored 1-3 years previously (“recently restored)”. Wetlands were restored by Ducks Unlimited Canada by building earth berms across drainage ditches and allowing the basin to refill with precipitation and runoff. An open-water zone encompassed by a ring of emergent vegetation characterized

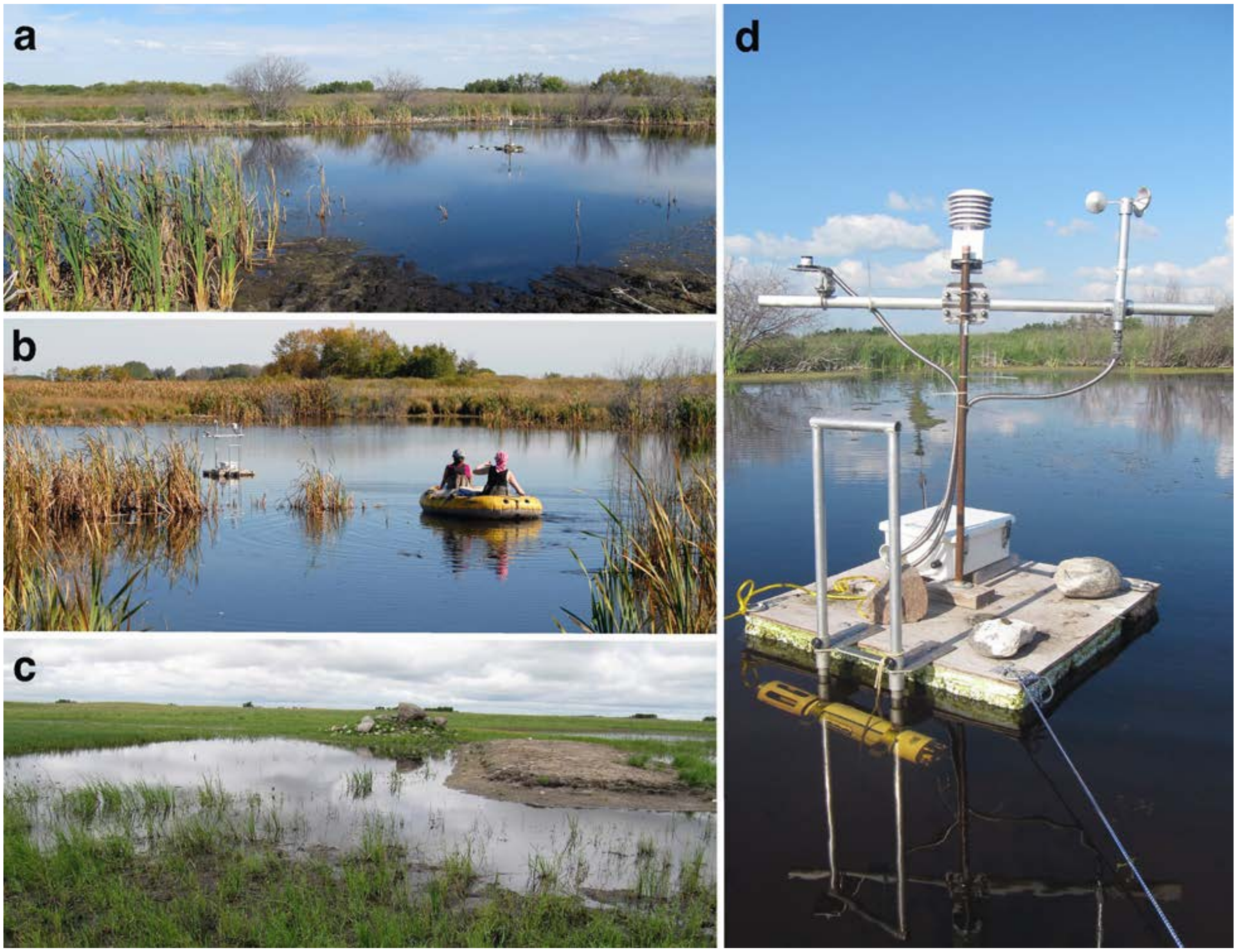

Plate 3.1 Photographs of study sites and equipment. (a) The natural wetland in September 2013. (b) The older restored wetland (restored in 1998) in September 2013. (c) The recently restored wetland (restored in 2009) in July 2011. (d) A raft, holding a multi-parameter sonde and meterological station, deployed in the centre of a wetland.

each site and 19 of 24 sites supported high (50-100 %) submersed aquatic vegetation (SAV) coverage within their basins. The mean depth (over three sampling periods) of the 24 wetlands ranged from 0.70 m to 2.25 m. Mean pH ranged from 6.83 to 8.41 and conductivity from 308 to 2910 µS cm^{-1}. In 2012 and 2013, the natural wetland was located on a 65 ha parcel of uncultivated and ungrazed land. The older restored wetland was also on land that was fallow, except for being lightly grazed by cattle in the autumn of 2012. The recently restored wetland was situated on land lightly grazed by cattle in spring 2012 and cultivated with canola during summer 2013. All three wetlands were classified as semi-permanent (Class IV, characterized by hydroperiods lasting at least 5-6 months per year; Stewart and Kantrud 1971) but retained water during the entire course of this study (Table 3.1). Mean surface areas during the study were 4130 m^2 for the natural wetland, 8750 m^2 for the older restored wetland, and 2670 m^2 for the recently restored wetland. The wetlands were surrounded by a ring of emergent vegetation dominated by cattails (*Typha*), bulrushes (*Scirpus* spp.), and sedges (*Carex* spp.). SAV sometimes covered as much as 100 % of the wetland bottom (Table 3.1) but in the deepest part of the wetland there was always open water above the vegetation from which to collect samples and measurements. The relative cover of SAV within each wetland basin was assessed on a scale of 0-5, but we report results converted to % cover (using the midpoint for values representing a range of % cover; Table 3.1).

Diel oxygen method

We deployed rafts mounted with a sonde and small meteorological station over the deepest point of each wetland (Plate 3.1d). Sondes were equipped with optical dissolved O_2, pH, temperature, conductivity, and oxidation-reduction potential probes and logged every 20 minutes, 25 cm below the water surface. In 2012, we had only one sonde (a Hydrolab DS5) and so it was rotated between the three wetlands every 3-9 days. In 2013, we had two additional sondes (YSI EXO2) enabling continuous deployment at all three wetlands apart from breaks for cleaning and calibration (approximately every two weeks). O_2 probes were calibrated in air-saturated water. The meteorological stations were equipped with a Met One 014A anemometer (at 1 m height), a Young 61302V barometer, a Kipp & Zonen PQS1 photosynthetically active radiation (PAR) sensor, and a Campbell Scientific CR800 or CR10X datalogger.

The diel O_2 method for calculating NEP is based on the premise that during the day, observed changes in O_2 concentrations are the result of two metabolic processes (production of O_2 by autotrophs and consumption of O_2 through respiration by all organisms) and exchange of O_2 with the atmosphere (Odum 1956). Changes in O_2 due to photochemical processes are not modeled (Cole et al. 2000). At night, GPP = 0 so that respiration and atmospheric exchange are the only processes affecting O_2 concentrations. By assuming that daytime and nighttime respiration rates (R_{day} and R_{night}) are equal, it is then possible to: a) calculate ER as the hourly R_{night} rate multiplied by 24 hours; b) infer GPP from the sum of changes in O_2 concentration for each time step (dO_2/dt) during the day plus daytime respiration (the sum of R_{day}); and c) calculate a daily NEP rate as GPP – ER (Cole et al. 2000). Thus, positive NEP indicates net autotrophy and negative NEP net heterotrophy. See Chapter 4 for GPP and ER values used to calculate NEP. It is likely that $R_{day} > R_{night}$ (Pace and Prairie 2005; Tobias et al. 2007; Hotchkiss and Hall 2014; though see Bachmann et al. 2000), which causes an underestimation of GPP and ER, but does not affect

Table 3.1. Water depth and % submersed aquatic vegetation (SAV) cover in a natural wetland that has never been drained, a wetland restored in 1998 (older restored), and a wetland restored in 2009 (recently restored). Wetlands were typically not all sampled on the same date, but always within one week of one another. Sampling dates before the 15th day of a month were designated as an "early" sampling period (e.g., "Early May"), those after the 15th of a month were "late".

		Natural		**Older restored**		**Recently restored**	
Year	**Sampling period**	**Water depth (m)**	**% SAV**	**Water depth (m)**	**% SAV**	**Water depth (m)**	**% SAV**
2012	Late May	0.9	15	0.9	75	0.8	15
	Early July	0.9	75	0.9	75	0.9	75
2013	Early May	0.9	0	0.9	5	0.9	5
	Late May	0.9	5	0.9	5	0.8	5
	Early June	0.9	15	0.9	15	0.9	15
	Early July	0.9	75	1.0	38	0.9	75
	Late July	0.8	100	0.9	75	0.9	75
	Early August	0.8	100	0.9	75	0.9	100
	Late August	0.7	100	0.9	75	0.7	100
	Early September	0.6	75	0.8	75	0.7	100
	Late September	0.6	50	0.8	75	0.7	100

estimates of NEP.

Calculation of metabolic rates followed Cole et al. (2000). Briefly, the change in O_2 concentration over time was considered to be a product of the balance of O_2 production by photosynthesis and O_2 consumption by respiration, and the diffusive exchange of O_2 with the atmosphere (F) in the mixed layer (Z_{mix} = mixed layer depth). Due to the relatively shallow depth of the wetlands, we assumed that $Z_{mix} = Z_{max}$, although this might not have always been the case. F can be calculated as follows:

$$F = k_{O2}(O_{2sat} - O_{2meas})$$

where k_{O2} is the piston velocity (m/s) calculated from k_{600} (Cole and Caraco 1998) and Schmidt coefficient (Jähne et al. 1987), and ($O_{2sat} – O_{2meas}$) is the difference between the concentration of O_2 in equilibrium with the atmosphere (O_{2sat}) and the measured O_2 concentration in water (O_{2meas}). The O_{2sat} term precedes the O_{2meas} term in the flux calculation so as to eliminate the need to change the sign of the flux before using it in metabolism calculations. As a result, positive values denote influx to the wetland whereas negative values indicate efflux. All calculations (Appendix 3) were made in the R programming environment (R Development Core Team 2012).

Dissolved CO_2 and CH_4 collection and analysis

To quantify concentrations of dissolved CO_2 and CH_4 in surface waters, water was collected into evacuated 160 mL Wheaton glass serum bottles capped with butyl rubber stoppers. Each bottle contained 8.9 g of potassium chloride (KCl) preservative and 10 mL of ultrahigh purity dinitrogen (N_2) gas headspace. To collect a sample, bottles were submerged ~10 cm below the water surface and punctured with a needle. Samples were collected three times daily (morning, noon, and evening) on three consecutive days from open water near the raft. These sampling periods occurred three times in 2012 (twice for the natural wetland) and seven times during May-August 2013 plus on four single dates in September.

Immediately prior to analysis, samples were shaken on a wrist-action shaker for 20 minutes to equilibrate dissolved gases with the headspace. CO_2 and CH_4 were analyzed on a Varian 3800 gas chromatograph (GC) equipped with a HayeSep D column (80°C), a ruthenium methanizer to convert CO_2 to CH_4, and a flame-ionization detector (FID; 250°C). We used four gas standards

(Praxair, Linde-Union Carbide), ranging from 75 to 6000 ppm for both CO_2 and CH_4, to calibrate the GC. A Varian Star Workstation program integrated peak areas. Sample gas concentration could then be inferred from headspace gas concentration, ambient and laboratory temperature and pressure, and Henry's Law. Samples were subsequently acidified with 0.5 ml H_3PO_4 to convert all DIC to CO_2, and reanalyzed on the GC.

CO_2 and CH_4 concentrations were used to calculate exchange with the atmosphere as described above for O_2, except that CO_2 influx was corrected for chemical enhancement at high pH using the following equation:

$$F = k_{CO2}\,\alpha(CO_{2meas} - O_{2sat})$$

where α is the enhancement factor for CO_2 diffusion due to the reaction of CO_2 with hydroxide ions, as calculated by Wanninkhof and Knox (1996) and Bade and Cole (2006). For CO_2 and CH_4, positive flux values indicate gas supersaturation (evasion to the atmosphere), and negative values indicate that the gas is undersaturated in water (invasion from the atmosphere).

We measured dissolved species (dissolved organic carbon [DOC] and sulfate [SO_4^{2-}]) that we suspected could be related to dissolved gas concentrations. Water samples were collected into HDPE bottles with each raft deployment in 2012, and ~weekly in 2013. Samples were processed and preserved the same day, then stored in the dark at 5°C until being analyzed at the University of Alberta Biogeochemical Analytical Service Laboratory. DOC was analyzed using a Shimadzu 5000A TOC analyzer; DOC is converted to CO_2 by catalytic combustion and then detected by a non-dispersive infrared detector. SO_4^{2-} was analyzed by ion chromatography wherein the anions are separated and measured using a Dionex IonPac AG9-HC guard column, IonPac AS9-HC analytical column, suppressor device, and conductivity detector.

Organic carbon and carbonate content of sediments

To rule out carbonate mineral precipitation as a major sink of DIC, we quantified carbonate content in wetland sediment. We collected triplicate sediment cores on five occasions between May and mid-July 2013 from each of the three wetlands using a 7.6 cm diameter polycarbonate tube. We sectioned and froze the top two cm of each core. These sections were subsequently freeze-dried, homogenized, and analyzed for OC and carbonate content by loss on ignition for 4 hours at 550 ºC and 2 hours at 950 ºC, respectively (Heiri et al. 2001).

Statistical analyses

To measure the temporal coherence of NEP between sites (i.e., whether seasonal changes in NEP were similar between sites), we calculated intraclass correlation coefficients (r_i; Rusak et al. 1999) pairwise for 2013 data. In two-population comparisons, r_i ranges from -1 to 1 and can be considered statistically different from zero when it exceeds the critical value of r_i (i.e., r_i for $P = 0.05$; Zar 1999). r_i was calculated using R package psych (Revelle 2014). *P*-values were calculated using Monte Carlo permutations. We described the relationship between O_2 and CO_2 fluxes using standardized major axis (SMA) estimation. SMA estimation was chosen because our interest in line-fitting was to describe the relationship, rather than to predict *y* from *x* (Warton et al. 2006). We used linear mixed-effects models (fitted using the nlme package in R; Pinheiro et al. 2014) with restricted maximum likelihood estimation to evaluate restoration-state-specific trends in CO_2, pH, and CH_4 data from the 2011 wetland survey. Mixed models allow correct prediction of effects, despite repeated sampling of wetlands. We considered the effect of date and the time of day the sample was taken. Restoration state was included as a fixed effect, site as a random effect, and we included a restoration-state x date interaction. If date, time, or the interaction were not significant, they were not included in the final model from which mean and confidence intervals were derived. Analysis of covariance (ANCOVA) was used to evaluate temporal and site-specific trends in carbonate content of sediments. Results from the linear mixed models and ANCOVAs are reported as least squares means and 95 % confidence intervals, calculated with the lsmeans package (Lenth and Hervé 2015).

Results

Metabolic status: O_2 and CO_2 methods

Based on the diel O_2 method, the natural wetland showed the greatest net autotrophy of the three sites and the recently restored wetland the least (Fig. 3.2a-c). The natural wetland was net autotrophic on 28 % and 61 % of days in 2012 ($n = 18$) and 2013 ($n = 138$), respectively. The older restored wetland was net autotrophic on 5 % and 34 % of days in 2012 ($n = 22$) and 2013

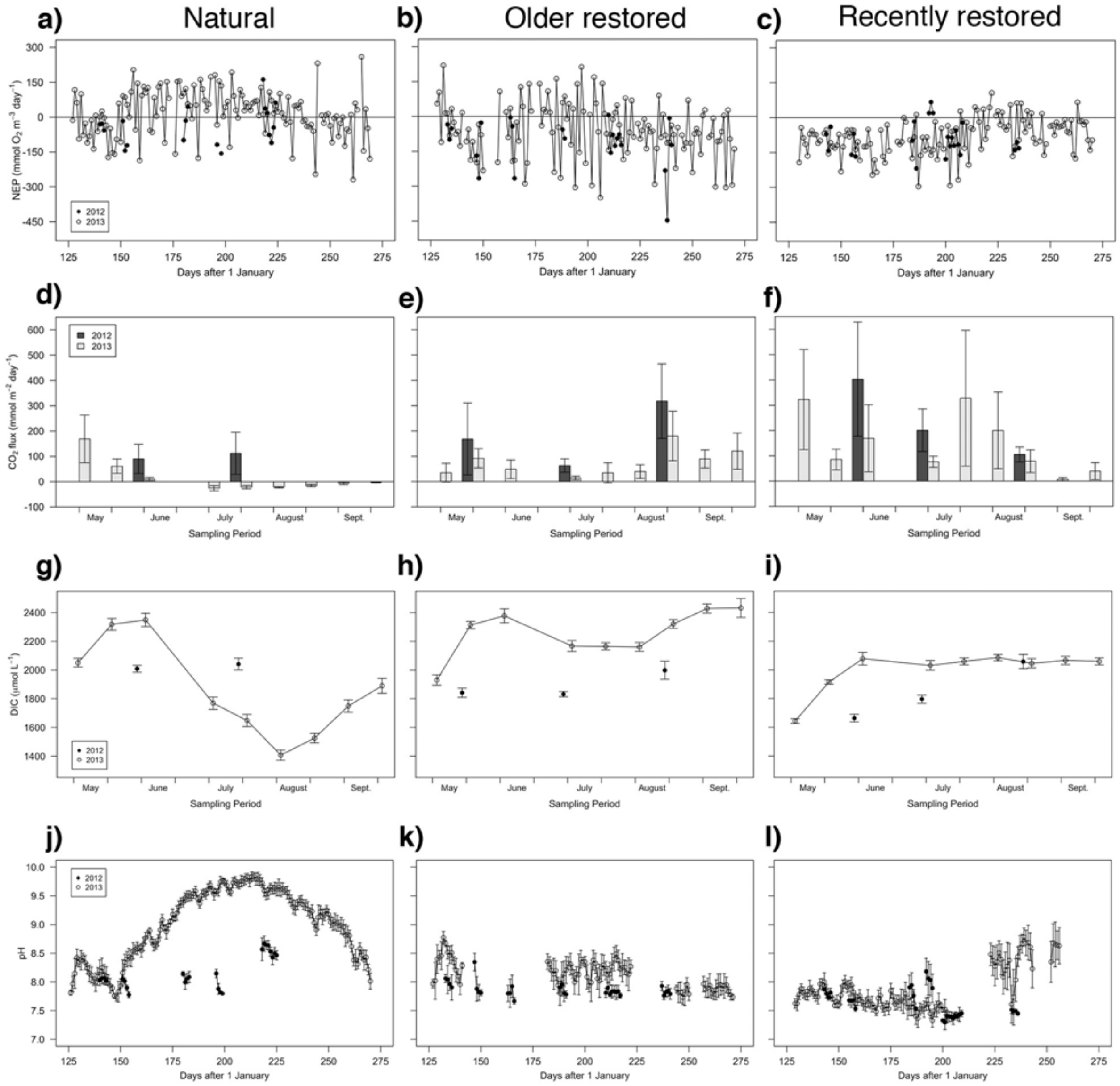

Figure 3.2 Seasonal variations (May-September 2012 and 2013) of selected biogeochemical parameters. The left column of panels corresponds to the natural wetland (i.e., has never been drained), the center column to the wetland restored in 1998 (older restored), and the right column to the wetland restored in 2009 (recently restored). (a-c) Daily estimates of net ecosystem production (NEP; mmol O_2 m^{-3} day^{-1}) derived from the diel oxygen method. Positive NEP values indicate net autotrophy and negative values net heterotrophy. (d-f) Mean and 95 % confidence interval carbon dioxide (CO_2) fluxes (mmol m^{-2} day^{-1}) per sampling period. Sampling periods include nine measurements (three samples per day for three consecutive days at morning, noon, and evening) except in September 2013 when each period includes six measurements from two non-consecutive dates. Positive values indicate efflux to the atmosphere from wetland and negative values influx of CO_2 from atmosphere to wetland. (g-i) Mean and 95 % confidence interval dissolved inorganic carbon (DIC; µmol L^{-1}) per sampling period. (j-l) Daily mean pH (± 1 standard deviation).

(*n* = 127), respectively. The recently restored wetland was net autotrophic on 13 % of days in both 2012 (*n* = 24) and 2013 (*n* = 130). Although we observed a greater proportion of autotrophic days in 2013, there was good agreement between years for NEP estimates on similar dates (Fig. 3.2a-c). Thus, it is possible that with greater sampling frequency in 2012, the proportion of autotrophic days could have been similar between years. The mean ± standard deviation of NEP over the sampled dates in 2013 was 16.9 ± 98.7 mmol O_2 m^{-3} day^{-1} in the natural wetland, and -59.7 ± 118.1 mmol O_2 m^{-3} day^{-1} and -79.6 ± 76.3 mmol O_2 m^{-3} day^{-1} in the older and recently restored wetlands, respectively. Though the restored wetlands more closely resembled each other in terms of metabolic status (i.e., both were net heterotrophic), seasonal changes in NEP were more synchronous in the older restored and natural wetlands ($r_i = 0.66$) than between the restored wetlands ($r_i = 0.44$) or natural and recently restored wetlands ($r_i = 0.30$). These correlations were all statistically significant, with $P < 0.001$. Peak net autotrophy occurred earlier in the natural and older restored wetlands (Fig. 3.2a, b) compared with the recently restored wetland (Fig. 3.2c).

Based on CO_2 fluxes (Fig. 3.2d-f), the natural wetland was never net autotrophic (i.e., CO_2 flux < 0) in 2012 (*n* = 6) but was net autotrophic on 64 % of days in 2013 (*n* = 25). The older and recently restored wetlands were never net autotrophic in 2012 (*n* = 9) but were net autotrophic on 4 % of days in 2013 (*n* = 27 and 25, respectively). When estimates of metabolic status from the two methods are compared on a date-by-date basis, they agree on all dates in 2012 and 48 % of dates in 2013 in the natural wetland. In the older restored wetland, estimates agree on all dates in 2012 and 70 % of dates in 2013. In the recently restored wetland, estimates agree on 67 % and 83 % of dates in 2012 and 2013, respectively.

Carbon fluxes

Chemical enhancement of CO_2 uptake ranged from 1.04 to 8.36, with an average α of 3.72 times the base rate. CO_2 fluxes ranged from uptake of 60 mmol CO_2 m^{-2} day^{-1} in the natural wetland to efflux of 1350 mmol CO_2 m^{-2} day^{-1} in the recently restored wetland. Mean ± SD of CO_2 fluxes in 2013 were: 19.4 ± 82.4 mmol CO_2 m^{-2} day^{-1} from the natural wetland, 67.5 ± 85.3 mmol CO_2 m^{-2} day^{-1} from the older restored wetland, and 162.0 ± 231.7 mmol CO_2 m^{-2} day^{-1} from the recently restored wetland. Although collection of fewer samples in 2012 limited interpretation, CO_2 fluxes appeared to follow a similar seasonal pattern in both years, except in the natural wetland (Fig.

3.2d-f). CO_2 concentration declined with increasing pH ($\log_{10}CO_2 = 11.17(2.02\ SE) - 1.14(0.03\ SE)pH$; $F_{1,241} = 2176.37$, $R^2 = 0.90$, $P < 0.001$), with no observations of CO_{2meas} exceeding CO_{2sat} when pH ≥ 9.0 (Fig. 3.3a). CO_2 concentrations were unrelated to DOC ($F_{1,33} = 0.99$, $R^2 = 0.03$, $P = 0.33$).

Like CO_2 fluxes, total DIC followed a similar seasonal pattern in both years in the restored wetlands (Fig. 3.2h,i). The natural wetland (Fig. 3.2g) showed inter-annual variation, with DIC declining strongly between June and August 2013. DIC and pH were correlated in the natural wetland ($r = -0.83$, $P < 0.001$, $n = 88$) but showed no relationship in the older ($r = -0.05$, $P = 0.68$, $n = 72$) or recently ($r = -0.07$, $P = 0.54$, $n = 82$) restored wetlands (see also Fig. 3.3b).

Higher CO_2 fluxes in the recently restored wetland and different seasonal changes in pH among wetlands (Fig. 3.2j-l) were consistent with the results from the survey of 24 wetlands (sampled 2-3 times each) in 2011. In 2011, CO_2 concentrations varied with restoration state (partial $F_{2,21} = 2.83$, $P = 0.08$) and in 2011 were: natural = 248.7 µmol L^{-1} (151.9, 345.4); older restored = 224.7 µmol L^{-1} (128.9, 320.4); and, recently restored = 376.7 µmol L^{-1} (280.9, 472.4). pH did not vary with restoration state (partial $F_{2,21} = 1.99$, $P = 0.16$), but varied with date (partial $F_{1,33} = 10.25$, $P = 0.003$) and the effect of date was different among restoration states (for restoration-state x date interaction, partial $F_{2,33} = 3.36$, $P = 0.047$). pH was highest in the natural wetlands (7.72 [7.48, 7.96]) followed by the older (7.57 [7.33, 7.80]) and recently (7.39 [7.16, 7.63]) restored wetlands.

The recently restored wetland consistently displayed greater CH_4 fluxes than the other wetlands (Fig. 3.4). Mean ± SD CH_4 fluxes in 2013 were: 0.8 ± 1.1 mmol CH_4 m^{-2} day^{-1} from the natural wetland, 0.7 ± 1.2 mmol CH_4 m^{-2} day^{-1} from the older restored wetland, and 13.3 ± 20.1 mmol CH_4 m^{-2} day^{-1} from the recently restored wetland. There was little temporal coherence between the wetlands with peak CH_4 fluxes occurring at different times during the open-water season. CH_4 concentrations were higher in recently restored wetlands in the 2011 survey: natural = 10.5 µmol L^{-1} (2.8, 18.3); older restored = 6.1 µmol L^{-1} (-1.6, 13.8); recently restored = 15.7 µmol L^{-1} (8.0, 23.4). However, this result was not statistically significant (partial $F_{2,21} = 1.29$, $P = 0.30$), likely owing to high temporal variability of CH_4 fluxes.

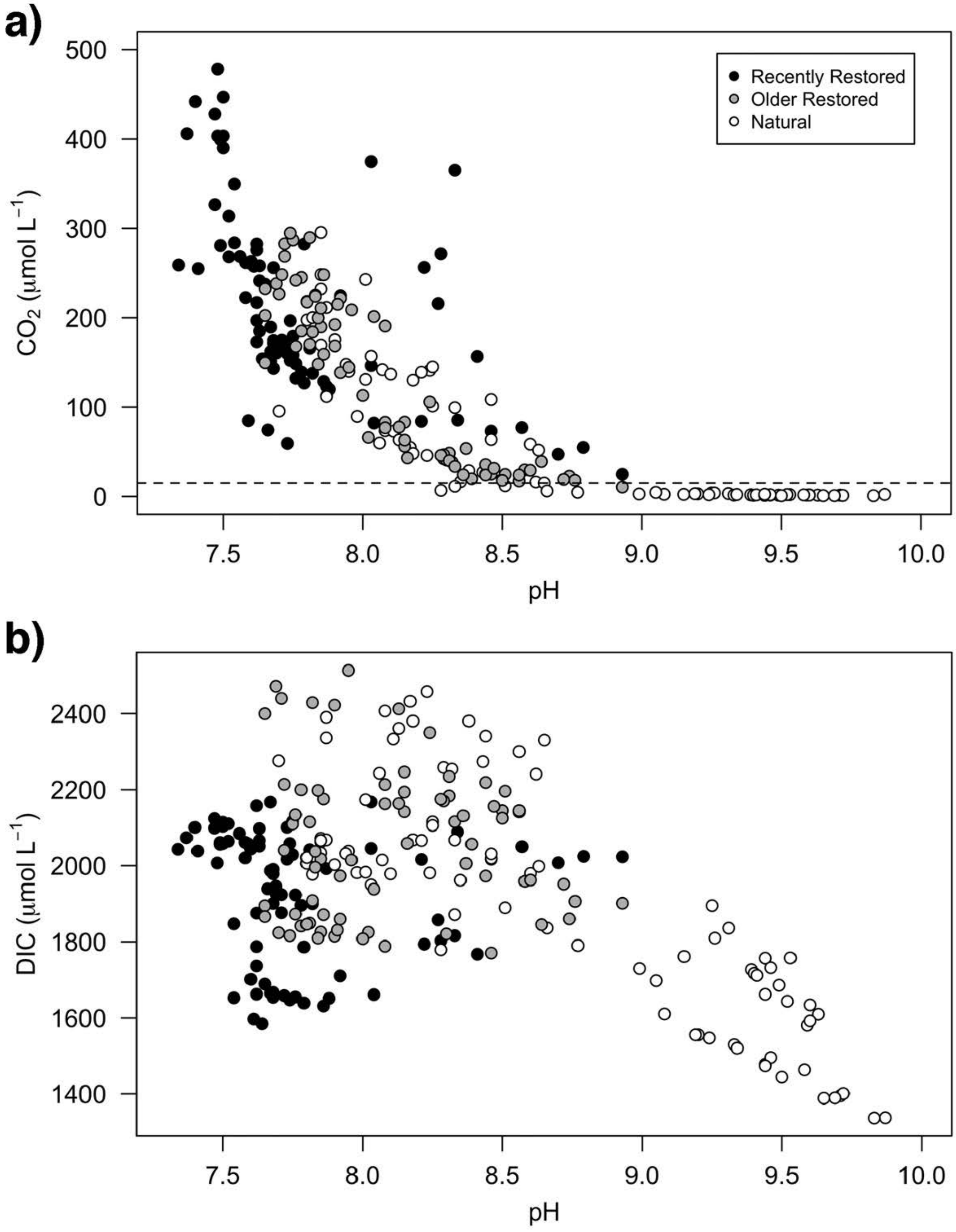

Figure 3.3 a) Relationship between CO_2 (µmol L^{-1}) and pH and b) total dissolved inorganic carbon (DIC; µmol L^{-1}) and pH in 2013 in the three studied wetlands. The dashed line in a) represents the average atmospheric equilibrium concentration of CO_2 for the study period. Thus, points below the dashed line represent CO_2 undersaturation, resulting in CO_2 influx from the atmosphere to wetland.

Organic carbon and carbonate content of sediments

OC content was highest in the sediments of the older restored wetland (%OC = 60.2 [53.8, 66.7]), followed by the natural (%OC = 39.1 [32.9, 45.3]) and recently restored (%OC = 27.0 [20.8, 33.2]) wetlands. OC content varied with site ($F_{2,35} = 28.39$, $P < 0.001$), date ($F_{1,35} = 3.98$, $P = 0.054$), and there was a site x date interaction ($F_{2,35} = 6.10$, $P = 0.0053$). Carbonate content (expressed as % $CaCO_3$) in sediment was highest in the older restored wetland at 17.2 % (15.2 %, 19.2 %). The natural and recently restored wetlands were similar with 7.5 % (5.4 %, 9.5 %) and 7.7 % (5.7 %, 9.7 %) carbonate, respectively. Carbonate content varied with site ($F_{2,38} = 31.54$, $P < 0.001$) and date ($F_{1,38} = 5.34$, $P = 0.026$), but temporal changes were similar among wetlands ($F_{2,38} = 0.36$, $P = 0.70$). Carbonate content declined seasonally, though changed < 3 % between May and July.

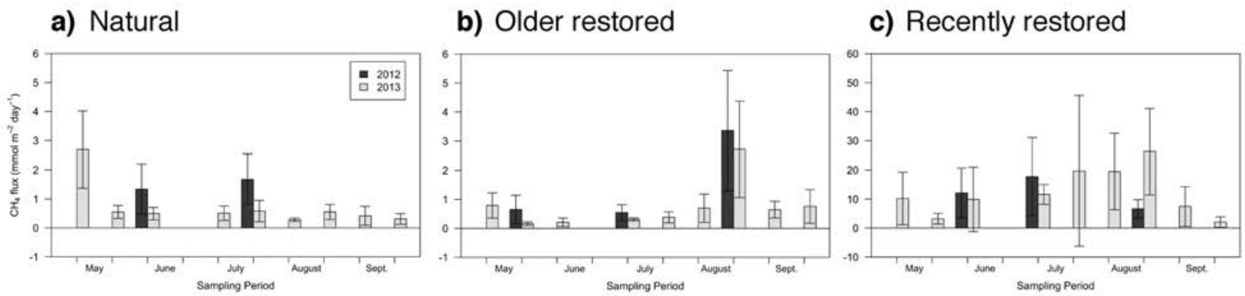

Figure 3.4 Mean and 95 % confidence interval methane (CH_4) fluxes (mmol m^{-2} day^{-1}) for the three studied wetlands. Positive values indicate efflux to the atmosphere from wetland. Means are per sampling period and each sampling period includes nine measurements (three samples per day for three consecutive days at morning, noon, and evening) except in September 2013 when each sampling period includes six measurements from two non-consecutive dates.

Discussion

The prairie wetlands we studied were mainly net heterotrophic, which does not support earlier predictions for littoral, macrophyte-dominated ecosystems (Duarte and Prairie 2005). However, we also found that the wetlands displayed substantial spatial and temporal variation in their metabolic status. Although there was poor concordance between the diel O_2 method and net CO_2 fluxes on shorter time scales (days to weeks), at the seasonal scale both methods suggested that the restored wetlands were net heterotrophic during the ice-free season in both years, whereas the natural wetland was net autotrophic in 2013. CO_2 and CH_4 emissions were greatest from the recently restored wetland. Although that site also had the greatest net heterotrophy, we suggest that CO_2 emissions here are supported by geochemical processes and cannot be solely explained by respiration of OC. Below, we offer potential explanations of these key findings.

Assessing the metabolic status of prairie wetlands

The degree of net heterotrophy observed in these wetlands was surprising. The diel O_2 method integrates O_2 fluxes owing to the production, respiration, and eventual decomposition of SAV and algae. In contrast, because emergent macrophyte leaves exchange gases directly with the atmosphere, the production and respiration of these plants is not captured in estimates of NEP in the open-water zone. However, the diel O_2 method does partially capture the decomposition of these plants, thereby inflating open-water ER rates. Thus, had we been able to incorporate the metabolic processes of emergent vegetation, it is likely we would have detected greater net autotrophy at the entire wetland level. Given that much of the organic matter in the sediment is autochthonous in origin (Roehm 2005), OC content could serve as a rough proxy for the degree of influence of emergent vegetation on ER. ER rates were, on average, highest in the older restored wetland (Chapter 4), the site with the greatest sediment OC content (60 %) and most emergent vegetation (L.E. Bortolotti, *pers*. *obs*.). Conversely, ER was lowest in the recently restored wetland, the site with the lowest sediment OC content (27 %) and least emergent vegetation.

The diel O_2 method revealed high temporal variability of NEP; net heterotrophic and net autotrophic days were often interspersed within a single ice-free season (Fig. 3.2a-c). In contrast, CO_2 fluxes suggested little day-to-day variation in metabolic status. Accordingly, daily estimates of metabolic status often differed between the two methods. These discrepancies were of two varieties including: a) instances of CO_2 supersaturation on net autotrophic (as estimated by the diel O_2 method) days, and b) CO_2 uptake on net heterotrophic days.

The O_2 and CO_2 methodological discrepancies at daily time scales can be explained by the effect of DIC speciation on CO_2 concentrations in a high alkalinity system. The first type of discrepancy, CO_2 supersaturation coinciding with net autotrophy, can occur when conversion of bicarbonate (HCO_3^-) to CO_2 partially or wholly offsets CO_2 consumption by primary producers. This requires both sufficiently low pH and a large pool of DIC. The second type of discrepancy, CO_2 uptake coinciding with net heterotrophy, occurs when CO_2 produced is rapidly converted to HCO_3^- and carbonate, conditions that only occur at high pH. Although we observed CO_2 influx at pH as low as 8.3, undersaturation only consistently occurred above pH 9.0 (Fig. 3.3a). Elsewhere, thresholds between CO_2 supersaturation and undersaturation occurred at pH = 8.6 (alkaline lakes; Finlay et al. 2009) and pH = 9.0 (saline lakes; Duarte et al. 2008). The direction of CO_2 flux (and the associated estimate of metabolic status) thus appears dependent on pH. However, in well-buffered systems like prairie wetlands, the magnitude of a single day's production and respiration may not shift pH greatly. Therefore, these wetlands tend to remain either below (Fig. 3.2k, l) or above (Fig. 3.2j) the pH 9.0 threshold for weeks despite day-to-day fluctuations in metabolic status. Consequently, CO_2 fluxes proved to be a relatively insensitive tool for assessing the daily metabolic status of prairie wetlands, though they could be informative in systems with low total DIC and low alkalinity (e.g., Schindler and Fee 1973).

The net CO_2 flux and diel O_2 methods agreed at longer (seasonal) time scales because the cumulative effect of persistent net autotrophy in the natural wetland was enough to shift pH past the 9.0 threshold, thereby changing the direction of CO_2 flux. However, although we observed seasonal agreement in this study, it would be possible to have CO_2 evasion even with seasonal net autotrophy (as observed by Waiser and Robarts 2004) as long as wetland pH does not rise above the 9.0 threshold. Like in many other systems (e.g., Stets et al. 2009; Finlay et al. 2010; Knoll et al. 2013; Maberly et al. 2013; McDonald et al. 2013; Marcé et al. 2015), CO_2 efflux in prairie wetlands may not be a reliable indicator of net heterotrophy. In contrast, this study reaffirmed the usefulness of the diel O_2 method, in particular for systems where DIC speciation may compromise the accuracy of inferences about metabolic status based on CO_2 flux.

Biological and geochemical processes governing carbon fluxes

Although multiple processes can affect pH (Soetaert et al. 2007), metabolic processes were important in shaping pH (and thus CO_2) in the studied wetlands. The relationship between CO_2 and pH (Fig. 3.3a) is complex, with pH as both a driver and the result of changing CO_2 concentrations. We have previously discussed the way in which pH influences CO_2

concentrations by affecting DIC speciation. However, CO_2 concentrations also drive pH via carbonic acid production. We observed diel pH cycles where pH increased during the day and declined at night, suggesting control by the metabolic consumption and production of CO_2. Also, periods of high pH (Fig. 3.2j-l) coincided with peak autotrophy (late June to mid-July in the older restored and natural wetlands, mid- to late August in the recently restored wetland; Fig. 3.2a-c). Thus, although CO_2 fluxes were not a reliable indicator of metabolic status, metabolic processes do noticeably influence CO_2 concentrations.

Although positive CO_2-DOC correlations are common (Prairie 2008), CO_2 and DOC concentrations were not correlated in the studied wetlands. In fact, the highest CO_2 concentrations were observed in the wetland (recently restored) with the lowest DOC concentrations. DOC increased seasonally in all three sites, a pattern typical of prairie wetlands attributable to evaporative concentration and DOC exudates from algae and macrophytes (Waiser 2006). Autochthonous DOC is cited as a factor contributing to the decoupling of any CO_2-DOC relationship in other studies, especially in systems with high primary production and CO_2 uptake (e.g., Tank et al. 2009; Balmer and Downing 2011; McDonald et al. 2013).

High pH in the natural wetland in 2013 corresponded with declining DIC concentrations between June and August (Fig. 3.2g, j), a pattern that is attributable to the uptake and conversion of DIC to OC by primary producers. Declining DIC coincided with the development of thick stands of SAV throughout the wetland basin (Table 3.1). As these are closed-basin systems where precipitation and evapotranspiration are the major water source and loss (Winter and Rosenberry 1998), there is no outflow to account for the decline in DIC. Neither can the change in DIC be explained by the precipitation of calcium carbonate as no whiting events were observed. Furthermore, carbonate content in wetland sediments was too low to suggest significant authigenic carbonate production and accordingly there was no site by date interaction to indicate that the natural wetland was different.

The nature of the DIC-pH relationship (Fig. 3.3b) reflects how both biological and geochemical processes govern DIC in these systems. If biological production and consumption of CO_2 were the most important processes affecting DIC, we would expect to observe an inverse linear relationship between DIC and pH. Although there was a statistically significant DIC-pH relationship in the natural wetland, below pH 9.0 the DIC-pH relationship appeared to break down; variation in DIC at any given pH was as great in the natural wetland as in the restored wetlands (Fig. 3.3b). Heagle et al. (2007) found that carbonate mineral dissolution, which consumes one mole of CO_2 for every two moles of HCO_3^- produced, contributes greatly to the DIC pool of prairie wetlands. This geochemical source of DIC could offset CO_2 consumed biologically, as previously discussed, as well as obscure any relationship between total DIC and pH. Calcite dissolution slows when CO_2 concentrations are low (Sjöberg and Rickard 1984), which explains why the DIC-pH relationship is considerably less noisy above pH 9.0 (Fig. 3.3b) — the biological signal becomes evident when geochemical processes slow or cease. Only in the natural wetland were biological processes strong enough to overcome the geochemical signal.

The mixed biological-geochemical influence on DIC was also evident from the O_2-CO_2 flux relationship (Fig. 3.5). In all wetlands, we observed an inverse relationship between O_2 and CO_2 flux, as would be expected from photosynthesis-respiration stoichiometry. The slope of the relationship (more CO_2 per mole of O_2 than expected) is consistent with geochemical supplementation of the DIC pool. However, the deviation from the -1:1 molar basis was much greater in the restored wetlands (Fig. 3.5b, c) than in the natural wetland (Fig. 3.5a). Thus, it is likely that photosynthesis and aerobic respiration control CO_2 concentrations more so in the natural than restored wetlands.

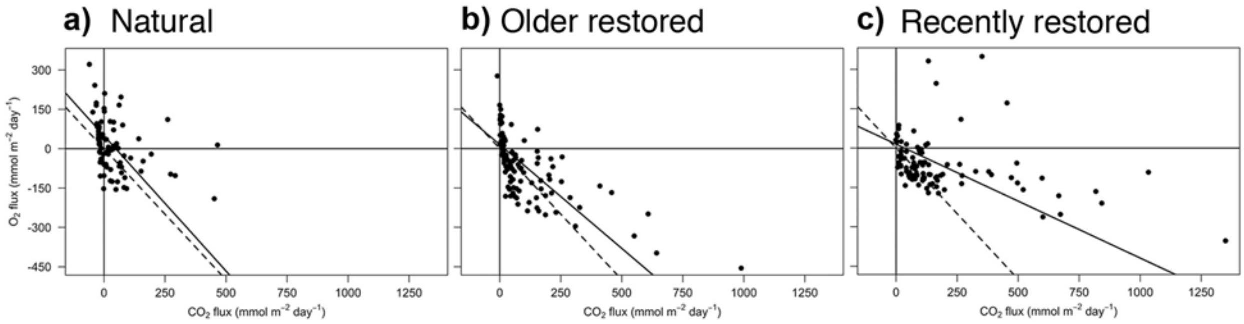

Figure 3.5 Relationship between O_2 and CO_2 air-water fluxes (mmol m^{-2} day^{-1}) in the three studied wetlands. For both gases, positive values indicate efflux to the atmosphere from wetland and negative values influx from atmosphere to wetland. The dashed line represents the -1:1 line and the solid line the standardized major axis estimation for the site. The standardized major axis estimations are: a) $O_2 = 49.21 - 1.03CO_2$; b) $O_2 = 14.99 - 0.79CO_2$; c) $O_2 = 14.89 - 0.43CO_2$.

Greenhouse gas fluxes

Compared with other studies using comparable methodologies for measuring CO_2 fluxes, the magnitude and variation of fluxes we observed in three sites over two years are remarkable (Fig. 3.6). Peak CO_2 influx to prairie wetlands was similar to rates reported from Mackenzie River Delta lakes (Tank et al. 2009), but less than influx observed in prairie (Finlay et al. 2009) and saline (Duarte et al. 2008) lakes. In contrast, efflux rates were typically higher than from lakes and reservoirs (Fig. 3.6). Similarly, NEP rates as estimated by the diel O_2 method in this study

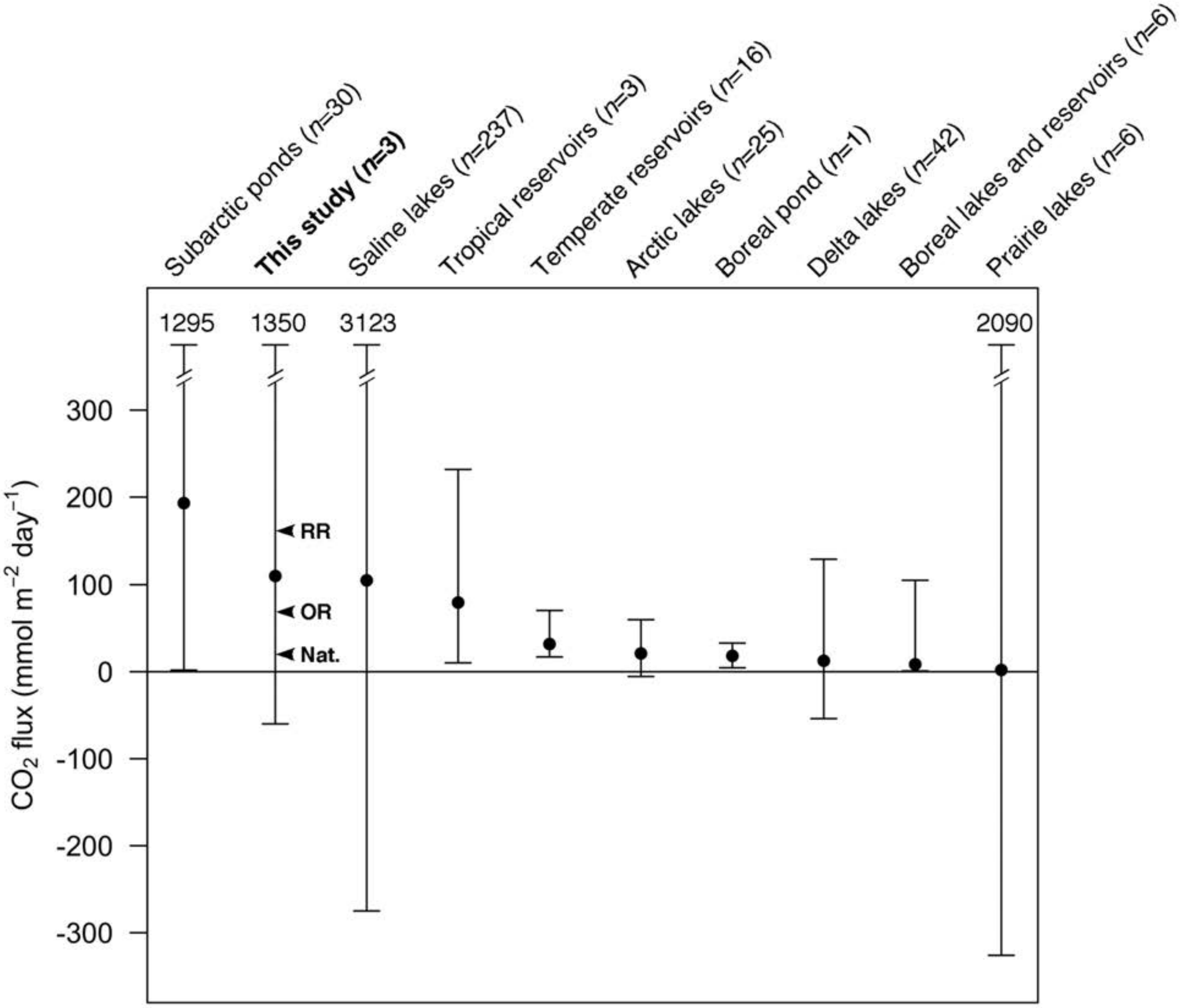

Figure 3.6 Mean and range of CO_2 flux from inland aquatic ecosystems including: subarctic ponds (data from Hamilton et al. 1994), prairie wetlands (this study), saline lakes (Duarte et al. 2008), tropical and temperate reservoirs (St. Louis et al. 2000), arctic lakes (Kling et al. 1991), a boreal pond (Sellers et al. 1995), Mackenzie River Delta lakes (Tank et al. 2009), boreal lakes and reservoirs (Ouellet et al. 2012), and hard-water prairie lakes (Finlay et al. 2009). The position of the mean flux from the recently restored ("RR"), older restored ("OR"), and natural ("Nat.") wetlands in this study are indicated by arrows. Numbers above broken error bars are the maximum fluxes for those systems. Sample sizes in brackets are the number of sites (lakes, ponds, reservoirs) included in the study.

are among the highest reported (summarized in Laas et al. 2012). Considerable variation in CO_2 flux was observed in this study, as well as in prairie lakes (Finlay et al. 2009) and subarctic ponds (Hamilton et al. 1994), second only to the range of fluxes observed in a global survey (Duarte et al. 2008) which sampled many more sites. That subarctic ponds and prairie wetlands displayed the greatest mean and highly variable CO_2 fluxes underscores the biogeochemical importance of small ecosystems and the need to integrate them into future estimates of global carbon emissions from inland waters. However, given that we sampled from only one open-water location per site, caution should be exercised in scaling these values across sites or regions.

Our measurements of CH_4 flux underestimate the true efflux from these systems because ebullition and transport through emergent vegetation were not measured but are important CH_4 efflux pathways in certain ecosystems (Bastviken et al. 2004; Kang et al. 2012). Greater CH_4 emissions from the recently restored wetland is likely due to lower sulfate (SO_4^{2-}) concentrations; the recently restored wetland contained only 2.25 mg L^{-1} SO_4^{2-} compared with > 700 mg L^{-1} SO_4^{2-} in the natural and older restored wetlands. Methanogenesis is suppressed by SO_4^{2-} (Pennock et al. 2010) because of its relatively low energy yield compared with other anaerobic bacterial metabolic pathways (Schlesinger 1997; Kang et al. 2012). Mean CH_4 fluxes from the natural and older restored wetlands were lower than the values for temperate, intermittently flooded wetlands with mineral soils reported in the IPCC's Wetlands Supplement (2.2 mmol CH_4 m^{-2} day^{-1}; IPCC 2014). In contrast, mean fluxes from the recently restored wetland were substantially higher, outside even the 95 % CI reported in the Wetlands Supplement.

At the older restored and natural sites, the mean CO_2 efflux in any sampling period represented a greater warming potential than that of the CH_4 efflux over the same time period (given the 34 times greater warming potential of CH_4 relative to CO_2 over a 100-year time horizon; IPCC 2013). Of the six sampling periods in the natural wetland in 2013 with CO_2 influx (Fig. 3.2d), half had influx great enough to offset the CH_4 emissions from the same time period. In the recently restored site, however, during all 12 sampling periods, the mean CH_4 efflux had greater warming potential than CO_2 for the same time period. Although there has been little concurrent quantification of CO_2 and CH_4 fluxes in prairie wetlands, the available data (Gleason et al. 2009) also suggest that CO_2 rather than CH_4 is the most important contributor to net warming potential. However, these results may be misleading as efflux of GHGs from the open water may be offset by the uptake of atmospheric CO_2 by emergent vegetation. The general consensus from regional and global assessments of wetland carbon cycling is that these systems act as net sinks for carbon, despite occasionally large CH_4 effluxes to the atmosphere (Roehm 2005; Bridgham et al. 2006; Mitsch et al. 2013; Petrescu et al. 2015). It is important to acknowledge that this study examines only a small range of possible wetland conditions and that carbon fluxes almost certainly vary during the wet-dry cycles that typify these ecosystems, requiring more sustained study to fully characterize GHG fluxes. Multiple studies suggest that prairie wetlands have a significant capacity for the storage of OC in sediments (Bedard-Haughn et al. 2006; Euliss et al. 2006; Badiou et al. 2011). Thus, long-term studies that integrate CO_2 and CH_4 fluxes with OC sequestration in sediments are needed to provide a more accurate picture of these wetlands as carbon sources or sinks in prairie landscapes.

Biogeochemical consequences of drainage and restoration

It is difficult to make generalizations about how drainage and restoration affect NEP and carbon cycling in prairie wetlands based on three sites. However, there are indications that greater net heterotrophy along a gradient from natural to recently restored is generally representative of prairie wetlands. Although the restored wetlands were similar in that their open-water zones were overall net heterotrophic in both study years, many characteristics of the older restored wetland were intermediate between the recently restored and natural wetlands including: degree of net heterotrophy; magnitude of CO_2 emissions (Fig. 3.2d-f); seasonal patterns in DIC (Fig. 3.2g-i); and the O_2-CO_2 relationship (Fig. 3.5). Furthermore, the older restored wetland and natural wetland showed greater temporal coherence in NEP and had similar magnitude of CH_4 emissions (Fig. 3.4). These patterns are consistent with the studied wetlands being representative of the recovery through time of restored wetlands towards a natural state, at least in the biogeochemical sense. Many of the trends in pH, CO_2, and CH_4 observed in the three sites in 2012 and 2013 are borne out in the larger sample sizes of the 2011 survey, though there was considerable variation within any restoration state, and these trends were not always statistically significant. Finally, our results are consistent with a recent synthesis of studies of temperate and northern wetlands showing that land use conversions affect GHG fluxes (Petrescu et al. 2015). Although the nature of such changes depends on ecosystem type and management practice, the conversion of natural to managed systems is typically associated with increased GHG efflux.

Conclusions

This study revealed a considerable magnitude and dynamic range of ecosystem metabolism and carbon fluxes in prairie wetlands, providing further evidence of the biogeochemical importance of these wetlands in prairie landscapes. Two common methods for assessing metabolic status, the diel O_2 method and CO_2 fluxes, produced conflicting results at a daily temporal scale owing to the complex interaction of biological and geochemical factors influencing carbon cycling in these wetlands. The relative importance of biological and geochemical processes varied among sites and further research is required to resolve this incongruity. Greater net heterotrophy and carbon GHG emissions were associated with restored sites, highlighting the importance of preventing the destruction of these ecosystems in the first place, rather than relying upon restoration to return ecosystem function and services.

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Chapter 4: Assessing the drivers of ecosystem metabolism in restored and natural prairie wetlands

Introduction

Identifying drivers of aquatic ecosystem metabolism is key to forecasting how aquatic ecosystems will be involved in and respond to global change (Staehr et al. 2012). Ecosystem metabolism involves biologically mediated transformations of carbon and is defined by three components: gross primary production (GPP), ecosystem respiration (ER), and net ecosystem production (NEP), where NEP = GPP - ER (Chapin et al. 2006). In lentic inland waters, temperature, nutrients, and light availability have been identified as important abiotic drivers of ecosystem metabolism (Hanson et al. 2003, Sand-Jensen and Staehr 2007, Staehr et al. 2010a, Hoellein et al. 2013, Klotz 2013, Solomon et al. 2013). However, drivers of metabolism in freshwater systems vary over space and time (Smith and Hollibaugh 1997, Hanson et al. 2006, Roberts et al. 2007), as well as between ecosystem types (Hoellein et al. 2013).

Our understanding of anthropogenic impacts on rates and drivers of metabolism of aquatic ecosystems remains in its infancy. As an integrative measure of the interactions among various biological communities and their abiotic environment, ecosystem metabolism is a potentially powerful tool for providing a holistic understanding of human effects on ecosystems. To date, the effects of eutrophication on lake and stream ecosystem metabolism are perhaps the best studied (e.g., Oviatt et al. 1986, D'Avanzo et al. 1996, Kemp et al. 2009, Davidson et al. 2015). Insight into future consequences of climate change for freshwater metabolism comes from observational (e.g., Roberts et al. 2007) and experimental (e.g., Moss 2010, Davidson et al. 2015) studies. The impact of contaminants on aquatic metabolism has been difficult to establish because of concomitant confounding effects of excess nutrient inputs (e.g., Aristi et al. 2015) and because investigations in mesocosms (e.g., Wiegner et al. 2003, Brooks et al. 2004) may miss key processes that operate at the whole-ecosystem scale. In the related field of ecosystem restoration, i.e., facilitating the recovery of degraded or destroyed ecosystems, ecosystem metabolism has been used to evaluate the recovery of restored streams (McTammany et al. 2007, Northington et al. 2011, Hoellein et al. 2012, Giling et al. 2013). Investigations of restored lakes (Dunalska et al. 2014) and wetlands (McKenna 2003) are less common and have been limited to short-term studies.

We investigated drivers of NEP, GPP, and ER in three prairie wetlands representing a gradient of restoration. In North America, prairie wetlands have been frequently drained for agriculture, resulting in the loss of their many ecosystem functions and services. Restoration of drained wetlands seeks to reverse these losses. The studied wetlands included one site restored in 2009 (hereafter “recently restored”), one in 1998 (“older restored”), and a wetland that had never been drained (“natural”). Our goal was to identify variables, including restoration state, that explain: 1) variation in daily metabolic rates within the wetlands; and 2) among-site differences in metabolic rates and drivers. We previously documented that the recently restored wetland emitted more carbon dioxide and had lower NEP than the older restored and natural wetlands (Chapter 3). We also showed that the abiotic environment and some biological communities (e.g., submersed aquatic vegetation [SAV]) are different in recently restored wetlands compared with more established wetlands (Chapter 2). Given these previously described differences, we predicted that the recently restored wetland would differ from the older restored and natural wetlands with respect to both metabolic rates and drivers.

Methods

Study area

We continuously quantified ecosystem metabolism using the diel oxygen technique in the open-water zone of three wetlands during May-September 2013. The wetlands were chemically and biologically representative of three “restoration states” and were selected for in-depth study based on a survey of 24 sites in the central aspen parkland ecoregion of southeastern Saskatchewan, Canada. Wetlands were restored by Ducks Unlimited Canada by building earth berms across drainage ditches and allowing the basin to refill with precipitation and runoff. All three wetlands were naturally fishless and classified as semi-permanent (Class IV, characterized by hydroperiods lasting at least 5-6 months per year; Stewart and Kantrud 1971). All basins retained water during the entire course of this study and mean surface areas were 0.41 ha (natural wetland), 0.88 ha (older restored wetland), and 0.27 ha (recently restored wetland). At each site, a ring of emergent vegetation dominated by cattails (*Typha*), bulrushes (*Scirpus* spp.), and/

or sedges (*Carex* spp.) surrounded an open-water zone. SAV covered as much as 100 % of the wetland bottom, though in the deepest part of the wetland there was always open water above the vegetation from which to collect samples and measurements. The natural wetland was located on a 65 ha parcel of uncultivated and ungrazed land. In 2013, the older restored wetland was also on land that was fallow, though it had been lightly grazed by cattle in some previous years. The recently restored wetland was situated on land cultivated with canola during the summer of 2013, but previously only lightly grazed by cattle.

Quantification of ecosystem metabolism

We deployed rafts mounted with a sonde and small meteorological station over the deepest point of each wetland. Sondes (one Hydrolab DS5 and two YSI EXO2) were deployed continuously apart from breaks for cleaning and calibration approximately every two weeks. Sondes were equipped with optical dissolved O_2, pH, temperature, and conductivity probes and logged every 20 minutes at a depth of 25 cm below the water surface. A single EXO total algae probe (excitation at 470 and 590 nm, emission at 685 nm) was rotated between the two YSI sondes. O_2 probes were calibrated in air-saturated water. The meteorological stations were equipped with a Met One 014A anemometer (at 1 m height), a Young 61302V barometer, a Kipp & Zonen PQS1 photosynthetically active radiation (PAR) sensor, and a Campbell Scientific CR800 or CR10X datalogger programmed to log readings every 20 minutes.

The diel O_2 method for calculating ecosystem metabolism is based on the premise that, during the day, observed changes in O_2 concentrations are the result of two metabolic processes (production of O_2 by autotrophs and consumption of O_2 through respiration by all organisms) and exchange of O_2 with the atmosphere (Odum 1956). Photochemical changes in O_2 are not modeled (Cole et al. 2000). At night, GPP = 0 so that respiration and atmospheric exchange are the only processes affecting O_2 concentrations. By assuming that daytime and nighttime respiration rates (R_{day} and R_{night}) are equal, it is then possible to compute: a) ER as the hourly R_{night} rate multiplied by 24 hours; b) GPP from the sum of changes in O_2 concentration for each time step ($\Delta O_2/\Delta t$) during the day plus daytime respiration (the sum of R_{day}); and c) daily NEP rate as GPP - ER (Cole et al. 2000, Staehr et al. 2010b). Thus, positive NEP indicates net autotrophy and negative NEP equates to net heterotrophy. It is likely that $R_{day} > R_{night}$ (Pace and

Prairie 2005, Tobias et al. 2007, Hotchkiss and Hall 2014, though see Bachmann et al. 2000), which causes an underestimation of GPP and ER, but does not affect estimates of NEP.

Calculation of metabolic rates followed Cole et al. (2000). Briefly, the change in O_2 concentration over time was considered a product of the balance of O_2 production by photosynthesis and O_2 consumption by respiration, and the diffusive exchange of O_2 with the atmosphere (F) in the mixed layer (Z_{mix} = mixed layer depth). Due to the shallow depth of the wetlands, we assumed that $Z_{mix} = Z_{max}$, although this might not have always been the case. F can be calculated as follows:

$$F = k_{O2}(O_{2sat} - O_{2meas})$$

where k_{O2} is the piston velocity (m/s) calculated from k_{600} (Cole and Caraco 1998) and Schmidt coefficient (Jähne et al. 1987), and $(O_{2sat} – O_{2meas})$ is the difference between the concentration of O_2 in equilibrium with the atmosphere (O_{2sat}) and the measured O_2 concentration in water (O_{2meas}). All calculations (Appendix 3) were made in the R programming environment (R Development Core Team 2012).

Measurements of drivers of metabolism

We quantified several environmental variables that might explain variation in ecosystem metabolism, including climatic variables (PAR, wind speed), nutrient concentrations (inorganic nitrogen, phosphorus), and water column properties (water temperature, light attenuation). We also measured possible biological drivers like substrates for microbial respiration (dissolved organic carbon [DOC], sediment OC) and proxies of primary producer abundance (chlorophyll *a* [chl *a*], SAV cover, dissolved organic nitrogen [DON]).

Daily average PAR and wind speed were calculated from meteorological station readings. Daily average water temperature was calculated from sonde readings. Water samples were collected weekly into HDPE bottles to quantify water chemistry, including total phosphorus (TP), ammonium (NH_4^+), nitrite + nitrate ($NO_2 + NO_3^-$), total dissolved nitrogen (TDN), DOC, and chl *a*. Samples were processed and preserved the same day, then stored in the dark at 5°C or frozen until being analyzed at the University of Alberta Biogeochemical Analytical Service Laboratory (see Appendix 1 for details of the analytical methods used). On > 60 % of days, NO_2

+ NO_3^- concentrations were below detection limit (2 µg L^{-1}), and concentrations were always small relative to NH_4^+. Given that NH_4^+ is the form of inorganic nitrogen most often preferred by primary producers (Graham and Wilcox 2000), we did not include NO_2 + NO_3^- in subsequent analyses. We used linear interpolation between sampling days to obtain daily estimates of these water chemistry variables so that they could be included in models of metabolism.

We positioned HOBO pendant temperature data loggers to record temperatures at both the surface and bottom of the water column. We calculated the temperature difference between the surface and bottom water (hereafter, ΔT) as a proxy of thermal stratification and, inversely, the potential for mixing events. Approximately every 2 weeks, we measured a vertical profile of PAR in the water column of the wetlands using a LI-COR 192SA underwater quantum radiation sensor. From these readings, we calculated the vertical light extinction coefficient (k_d) as:

$$k_d = \frac{\ln I_0 - \ln I_z}{z}$$

where I_0 is the photon flux density a few cm below the water surface, and I_z is the photon flux density at depth z (in this case, the bottom of the wetland).

To quantify sediment organic carbon (OC) content we collected triplicate sediment cores on five occasions between May and mid-July 2013 from each of the three wetlands using a 7.6 cm diameter polycarbonate tube. We sectioned off and froze the top two cm of each core. These sections were subsequently freeze-dried, homogenized, and analyzed for OC content by loss on ignition for 4 hours at 550 ºC (Heiri et al. 2001).

To avoid disturbances of the wetlands with harvesting of SAV, we determined three proxies for SAV biomass. The first proxy was % SAV cover within the wetland, linearly interpolated to get a daily estimate of SAV cover. The second biomass proxy was a categorical measure (SAV_{cat}) with levels of “low”, “medium”, and “high” cover. These categories corresponded to 0-25 %, 26-50 %, and 51-100 % cover, respectively. Although there is generally a positive relationship between SAV area and biomass, this relationship varies with species (Armstrong et al. 2003) and cover estimates cannot capture all changes in SAV biomass (e.g., when SAV stands become denser). Therefore, the third proxy for SAV biomass, dissolved organic nitrogen (DON), was not based on SAV cover. We calculated DON by subtracting the concentration of inorganic species (NH_4^+ and $NO_2 + NO_3^-$) from TDN. DON, along with DOC, increased seasonally in these wetlands, a pattern typical of prairie wetlands attributable to release of exudates by submersed macrophytes and algae (Berman and Bronk 2003, Waiser 2006). These exudates result from photosynthesis by, not degradation of, submersed macrophytes (Demarty and Prairie 2009). Supporting our interpretation of autochthonous generation of DON and DOC, C:N molar ratios declined seasonally (Fig. A4.1). Because DON concentrations are also influenced by evapoconcentration and algal biomass, they are an imperfect proxy for SAV alone. However, the relationship between DON and SAV cover (Fig. A4.2) suggests that, despite these previously mentioned limitations, DON captured changes in SAV biomass that % SAV cover did not.

Statistical analyses

We used generalized least squares regression (gls in the nlme package in R; Pinheiro et al. 2014) to identify drivers of metabolic rates as gls regression allows model errors to be both correlated and have unequal variance (Zuur et al. 2009). As for most time series data, metabolic rates were autocorrelated through time (evaluated using the Durban-Watson statistic; Scheiner and Gurevitch 2001). There was also evidence of heteroskedasticity in model residuals. We used an exponential variance function structure and compound symmetry structure (corresponding to uniform correlation) to account for the heteroskedasticity and autocorrelation, respectively.

We modeled drivers of NEP, GPP, and ER for each site separately. We considered eight potential predictors in NEP models including temperature, PAR, wind speed, ΔT, chl *a* and one of DON, SAV, or SAV_{cat}. DON, SAV, and SAV_{cat} represent alternative proxies for the same variable (SAV biomass) and thus only one measure was used per model. Temperature was eventually dropped from models of NEP due to problems of collinearity, and no substantive relationship with NEP. In GPP models, we considered 10 variables: temperature, PAR, wind speed, ΔT, NH_4^+, TP, chl *a*, and one of DON, SAV, or SAV_{cat}. Four covariates were considered in ER models: temperature, wind speed, ΔT, and DOC.

We initially modeled drivers of metabolic rates with all sites together, including site and site-by-environmental variable interactions (e.g., site x temperature) because of possible site-specific responses to metabolic drivers. However, given that the best models for NEP, GPP, and ER each included 3 or 4 site-environment interactions, we ultimately decided to model each site separately. There were *n* = 128 observations for the older restored site and *n* = 130 for the recently restored site. Although we had *n* = 138 daily metabolism measurements for the natural site, malfunction of the HOBO dataloggers in mid-August limited metabolism models to a total of 98 observations.

To select the most parsimonious model of metabolism, and to examine the relative support for models including different proxies for SAV biomass, we used an information-theoretic approach (Akaike's Information Criterion corrected for small sample size; AIC_c). We followed criteria outlined in Burnham and Anderson (2002), wherein the model with the lowest AIC_c is deemed best, and models with ΔAIC_c values ≤ 2 and < 4 (ΔAIC_c being the difference between the best-approximating and lower ranked models) are considered well-supported and plausible, respectively. Akaike weights (ω_i) were also used to make inferences about relative support for competing models (Burnham and Anderson 2002). AIC_c values were calculated with the AICcmodavg package in R (Mazerolle 2015). Environmental variables in the final models were tested for collinearity by ensuring that all variance inflation factors were less than ~5 (Zuur et al. 2009). Model selection was done using maximum likelihood estimation, but parameters were calculated using restricted maximum likelihood estimation. We report β ± SE of the best models unless otherwise stated. Following convention, we calculated ER rates as negative numbers (and are presented that way in Fig. 4.1), but β were calculated from models where ER rates were positive to make the direction of the relationship between ER and its drivers more intuitive.

Given the lack of replication (i.e., *n* = 1 per restoration state) in this study, our approach to identifying variables that explained among-site differences in metabolic rates was qualitative. In addition to the variables included in the gls regressions, we also considered two variables, k_d and sediment OC, which were not measured with sufficient frequency to include in the regressions. To explain among-sites differences, a variable had to meet two criteria. First, to explain differences among sites, a variable must vary by site. For example, if all three sites receive the same amount of PAR, PAR cannot account for differences in GPP among sites. To that end, we used Analysis of Covariance (ANCOVA) to evaluate whether the variables used in the metabolism models varied by site. We also evaluated whether these variables changed with date and whether there was a site by date interaction. Because these data violate the statistical assumption of independent observations, we used randomization tests (Manly 1997) to assess the significance of the ANCOVAs. Observations were randomly assigned (999 permutations) to a site, and the *P*-value was calculated based on the number of times the randomly generated test statistics exceeded the test statistic derived from the original data. The second criterion was that the variable gradient must be in logical concordance with the metabolic rate gradient. For example, if DOC concentrations explain among-site variation in ER, then DOC levels must be highest in the site with the greatest ER and lowest in the site with the lowest ER. Finally, once causes of among-site variation were identified, they were compared to known characteristics of restored wetlands (e.g., lower sediment OC) to determine whether observed differences could be related to restoration state.

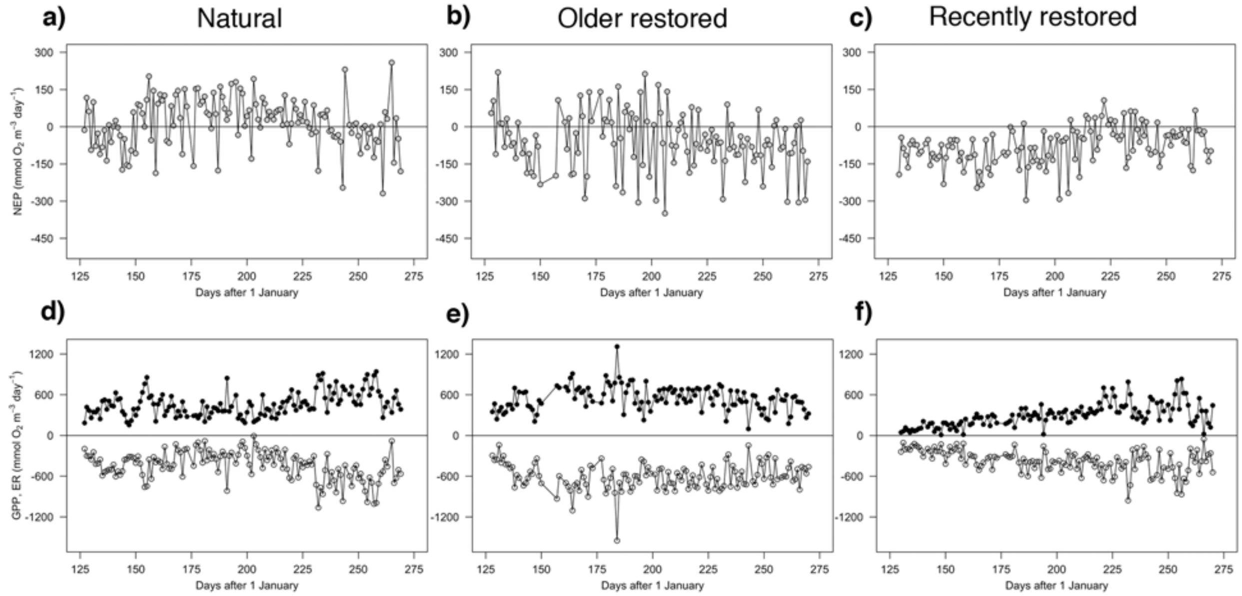

Figure 4.1 Daily estimates of ecosystem metabolism (mmol O_2 m^{-3} day^{-1}) in three prairie wetlands in May-September 2013. The left column of panels corresponds to the natural wetland (i.e., has never been drained), the center column to the wetland restored in 1998 (older restored), and the right column to the wetland restored in 2009 (recently restored). (a-c) Net ecosystem production (NEP; grey circles). Positive NEP values indicate net autotrophy and negative values net heterotrophy. (d-f) Gross primary production (GPP; black circles) and ecosystem respiration (ER; open circles).

Results

Metabolic rates were highly variable and rates and seasonal patterns varied among wetlands (Fig.

4.1). NEP was highest in the natural wetland, the only site to have a net autotrophic signal over the course of the open-water season (Fig. 4.1a-c, Table 4.1). In contrast, both restored wetlands were net heterotrophic overall, with the recently restored wetland more strongly so (Fig. 4.1a-c, Table 4.1). The natural and older restored wetlands showed similar seasonal changes in NEP, with numerous autotrophic days in mid-summer. GPP was lowest in the recently restored wetland, followed by the natural and older restored wetlands (Fig. 4.1d-f, Table 4.1). ER was also lowest in the recently restored wetland, followed by the natural and older restored wetlands (Fig. 4.1d-f, Table 4.1). Each site displayed unique seasonal patterns of GPP and ER. ER was strongly coupled to GPP in all wetlands (Fig. 4.2). However, linear regression suggests that GPP and ER are more closely coupled in the natural ($F_{1,136} = 493.4$, $P < 0.001$, $R^2 = 0.78$) and recently restored ($F_{1,128} = 545.0$, $P < 0.001$, $R^2 = 0.81$) wetlands than in the older restored wetland ($F_{1,124} = 168.6$, $P < 0.001$, $R^2 = 0.57$).

Table 4.1. Mean and standard deviation of gross primary production (GPP), ecosystem respiration (ER), and net ecosystem production (NEP) in mmol O_2 m^{-3} day^{-1} in three prairie wetlands in May-September 2013. The three sites included a natural wetland (i.e., has never been drained), an older restored wetland (restored in 1998), and a recently restored wetland (restored in 2009).

	Natural (*n* = 138)	**Older restored** (*n* = 128)	**Recently restored** (*n* = 130)
GPP	461.2	545.3	295.6
	SD = 178.1	SD = 180.5	SD = 171.8
ER	444.2	605.0	375.2
	SD = 211.4	SD = 189.9	169.2
NEP	16.9	-59.7	-79.6
	SD = 98.7	SD = 118.1	SD = 76.3

Drivers of ecosystem metabolism

PAR and wind speed were in the best-approximating model of NEP for all sites, but wetlands differed with respect to NEP-SAV and NEP-chl *a* relationships (Table 4.2). At all sites, higher NEP was associated with greater PAR (natural: $\beta = 0.27 \pm 0.026$; older restored: $\beta = 0.29 \pm 0.039$; recently restored: $\beta = 0.13 \pm 0.020$) and lower wind speeds (natural: $\beta = -21.34 \pm 7.35$; older restored: $\beta = -36.89 \pm 11.17$; recently restored: $\beta = -39.54 \pm 5.57$). The best NEP models for the natural ($\omega_i = 0.707$) and older restored ($\omega_i = 0.465$) sites each included some proxy for

Table 4.2. Ranking of models explaining variation in net ecosystem production in three prairie wetlands in May-September 2013. Variables included in the models were photosynthetically active radiation (PAR), wind speed (Wind), the temperature difference between surface and bottom waters as a proxy for stratification (ΔT), chlorophyll *a* concentrations (Chl), and one of three measures of submersed aquatic vegetation abundance (SAV, % cover of submersed vegetation; SAV.cat, submersed vegetation cover as a categorical variable with levels "low", "medium", and "high"; DON, dissolved organic nitrogen concentrations). Akaike's information criterion corrected for small-sample bias (AIC$_c$) is an estimator of the expected Kullback-Leibler information (i.e., the discrepancy between the candidate model and the true model generating the data). ΔAIC$_c$ is the difference between the AIC$_c$ of the candidate model and the minimum AIC$_c$ (1053.84). Akaike weight (ω_i) is the likelihood that the candidate model is the best model in the set, given the data and the other models in the set. *K* is the number of estimable parameters.

Site	Model structure	AIC$_c$	ΔAIC$_c$	ω_i	*K*
Natural	PAR, Wind, SAV.cat	1053.84	0	0.707	7
	PAR, Wind, Chl, SAV.cat	1056.12	2.29	0.225	8
	Global (PAR, Wind, ΔT, Chl, SAV.cat)	1058.50	4.67	0.068	9
	Intercept and model structure only (statistical null)	1142.46	88.62	< 0.001	3
Older restored	PAR, Wind, Chl, SAV	1426.52	0	0.465	7
	PAR, Wind, Chl, DON	1428.57	2.06	0.166	7
	PAR, Wind, SAV.cat	1428.86	2.34	0.144	7
	PAR, Wind, SAV	1429.34	2.82	0.114	6
	Global (PAR, Wind, ΔT, Chl, SAV.cat)	1429.40	2.88	0.110	9
	Intercept and model structure only (statistical null)	1477.61	51.09	< 0.001	3
Recently restored	PAR, Wind, Chl	1322.60	0	0.425	6
	PAR, Wind, ΔT, Chl	1323.72	1.12	0.242	7
	PAR, Wind, Chl, DON	1324.64	2.04	0.153	7
	Global (PAR, Wind, ΔT, Chl, DON)	1325.55	2.95	0.097	8
	Global (PAR, Wind, ΔT, Chl, SAV)	1325.87	3.27	0.083	8
	Intercept and model structure only (statistical null)	1412.68	90.08	< 0.001	3

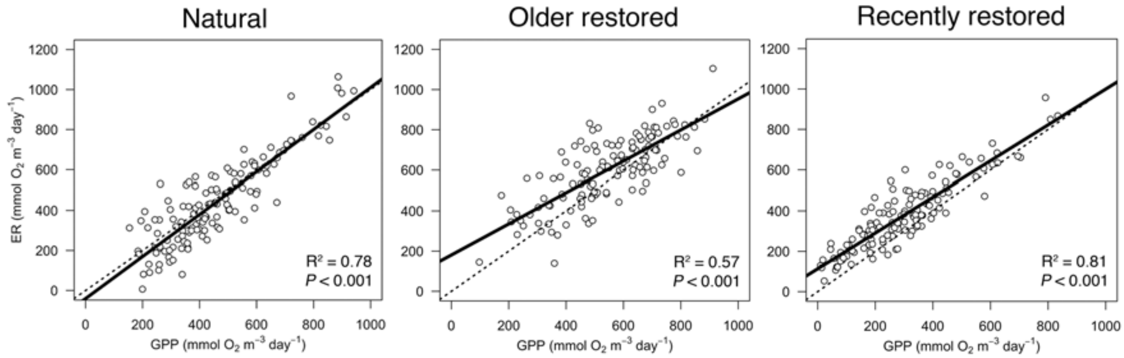

Figure 4.2 Relationship between ecosystem respiration (ER) and gross primary production (GPP) in three prairie wetlands in May-September 2013. The dotted line represents the 1:1 line and the solid line the least squares estimation for the site. The R^2 and *P*-values are the statistics associated with that least squares regression of ER on GPP.

SAV biomass, and measures of SAV appeared in well-supported models of the recently restored wetland. The best models of NEP in the older restored and recently restored sites also included chl *a*. NEP and SAV cover were positively related in the natural wetland (SAV_{cat} $\beta = 39.39 \pm 11.69$). The NEP-SAV relationship was slightly negative in the older restored site ($\beta = -0.88 \pm 0.38$), though other measures of SAV suggested this relationship was non-linear. The effect of DON on NEP was poorly estimated in the recently restored site ($\beta = -0.0060 \pm 0.013$). The NEP-chl *a* relationship was positive in the recently restored wetland ($\beta = 0.37 \pm 0.12$), but negative in the older restored wetland ($\beta = -3.13 \pm 1.94$).

Drivers of GPP differed between the three sites. The best-approximating model of GPP in the natural wetland included PAR, wind speed, ΔT, and DON ($\omega_i = 0.236$; Table 4.3). These variables were in all of the top models for this site. Although a model with similar support ($\omega_i = 0.219$) also contained NH_4^+ and TP, NH_4^+ was poorly estimated in all models of this site and the GPP-TP relationship was negative ($\beta = -0.44 \pm 0.18$). In contrast, the best-approximating model for the older restored wetland ($\omega_i = 0.472$) included temperature, PAR, chl *a*, and an orthogonal second-order polynomial of DON, though temperature was poorly estimated ($\beta = 3.43 \pm 5.29$). In the recently restored wetland, the best-approximating model ($\omega_i = 0.492$) included temperature, ΔT, TP, chl *a*, and SAV_{cat}. Other measures of SAV abundance (SAV, DON) and PAR appeared in plausible models. GPP increased with PAR (natural: $\beta = 0.41 \pm 0.056$; older restored: $\beta = 0.37 \pm 0.057$) and temperature (recently restored: $\beta = 21.13 \pm 6.18$), but decreased with greater ΔT (natural: $\beta = -88.76 \pm 14.61$; recently restored: $\beta = -24.09 \pm 6.43$). In both the natural and recently restored wetlands, GPP was positively related to SAV (natural: DON $\beta = 0.22 \pm 0.047$; recently restored: SAV_{cat} $\beta = 103.45 \pm 33.39$). In contrast, GPP in the older restored wetland showed a non-linear relationship to DON, with maximum GPP at intermediate DON concentrations (second-order polynomial $\beta = -518.17 \pm 201.52$). The GPP-chl *a* relationship was positive in the recently restored wetland ($\beta = 2.25 \pm 0.45$), but negative in the older restored wetland ($\beta = -8.78 \pm 3.66$).

Unlike NEP and GPP, drivers of ER rates were similar among all three sites. In particular, there was model support for the effects of temperature, wind speed, ΔT, and DOC on ER (Table 4.4). However, the ER-DOC relationship was consistently poorly estimated. At all sites, greater ER was associated with higher temperatures (natural: $\beta = 22.55 \pm 5.37$; older restored: $\beta = 29.96$

Table 4.3. Ranking of models explaining variation in gross primary production in three prairie wetlands in May-September 2013. Variables included in the models were photosynthetically active radiation (PAR), wind speed (Wind), water temperature (Temp), the temperature difference between surface and bottom waters as a proxy for stratification (ΔT), ammonium concentrations (NH_4^+), total phosphorus concentrations (TP), chlorophyll *a* concentrations (Chl), and one of three measures of submersed aquatic vegetation abundance (SAV, % cover of submersed vegetation; SAV.cat, submersed vegetation cover as a categorical variable with levels "low", "medium", and "high"; DON, dissolved organic nitrogen concentrations).

Site	**Model structure**	**AIC_c**	**ΔAIC_c**	**ω_i**	*K*
Natural	PAR, Wind, ΔT, DON	1186.01	0	0.236	8
	PAR, Wind, Temp, ΔT, NH_4, TP, DON	1186.16	0.15	0.219	11
	PAR, Wind, ΔT, Chl, DON	1187.17	1.01	0.142	9
	Global (PAR, Wind, Temp, ΔT, NH_4, TP, Chl, DON)	1187.52	1.36	0.120	12
	PAR, Wind, Temp, ΔT, TP, DON	1187.79	1.63	0.104	10
	PAR, Wind, ΔT, TP, Chl, DON	1187.90	1.74	0.099	10
	PAR, Wind, Temp, ΔT, DON	1188.34	2.18	0.079	9
	Intercept and model structure only (statistical null)	1224.37	38.21	< 0.001	4
Older restored	PAR, Temp, Chl, DON^2	1501.20	0	0.472	9
	PAR, Chl, DON^2	1501.70	0.50	0.368	8
	PAR, Temp, TP, Chl	1504.542	3.35	0.088	8
	PAR, DON^2	1505.274	4.08	0.061	7
	Global (PAR, Wind, Temp, ΔT, NH_4, TP, Chl, SAV.cat)	1508.785	7.59	0.011	13
	Intercept and model structure only (statistical null)	1571.772	70.57	< 0.001	4
Recently restored	Temp, ΔT, TP, Chl, SAV.cat	1491.613	0	0.492	10
	Temp, ΔT, TP, Chl, SAV	1492.594	0.98	0.302	9
	Temp, ΔT, Chl, DON	1494.933	3.32	0.094	8
	PAR, Temp, ΔT, TP, Chl, SAV	1495.18	3.57	0.083	6
	Global (PAR, Wind, Temp, ΔT, NH_4, TP, Chl, SAV)	1497.224	5.61	0.030	12
	Intercept and model structure only (statistical null)	1548.433	56.82	< 0.001	4

± 4.51; recently restored: $\beta = 13.95 \pm 5.71$) and lower ΔT (natural: $\beta = -88.37 \pm 14.62$; older restored: $\beta = -58.29 \pm 13.01$; recently restored: $\beta = -27.50 \pm 9.58$). ER was also positively related to wind speed (older restored: $\beta = 31.56 \pm 14.11$; recently restored: $\beta = 34.95 \pm 10.56$), though this effect was opposite and negligible in the natural wetland ($\beta = -18.22 \pm 16.57$).

Table 4.4. Ranking of models explaining variation in ecosystem respiration in three prairie wetlands in May-September 2013. Variables included in the models were wind speed (Wind), water temperature (Temp), the temperature difference between surface and bottom waters as a proxy for stratification (ΔT), and dissolved organic carbon (DOC).

Site	Model structure	AIC_c	ΔAIC_c	ω_i	K
Natural	Temp, ΔT	1224.18	0	0.354	6
	Wind, Temp, ΔT	1224.39	0.21	0.319	7
	Temp, ΔT, DOC	1225.62	1.44	0.172	7
	Global (Wind, Temp, ΔT, DOC)	1225.83	1.65	0.155	8
	Intercept and model structure only (statistical null)	1246.72	22.54	< 0.001	4
Older restored	Wind, Temp, ΔT	1540.56	0	0.598	7
	Global (Wind, Temp, ΔT, DOC)	1541.89	1.33	0.307	8
	Temp, ΔT, DOC	1544.24	3.68	0.095	7
	Intercept and model structure only (statistical null)	1566.56	26.00	< 0.001	4
Recently restored	Global (Wind, Temp, ΔT, DOC)	1525.75	0	0.721	8
	Wind, Temp, DOC	1527.65	1.90	0.279	7
	Intercept and model structure only (statistical null)	1608.99	83.25	< 0.001	4

Differences in environmental variables among wetlands

All variables included in the models of metabolism, except PAR, varied by site (Fig. 4.3, Table 4.5). Sometimes, the differences among sites were statistically significant, but ultimately small in magnitude (e.g., wind speed, Table 4.5). Most variables changed seasonally, but only ΔT, nutrient concentrations, and chl *a* displayed a site by date interaction (Fig. 4.3). The two other variables measured too infrequently to be included in models of metabolism, k_d and sediment OC, also varied by site. Until late June, k_d was similar among wetlands and it did not change seasonally in the natural wetland (Fig. 4.4). In contrast, from late June onwards k_d was elevated in the recently restored wetland and, to a lesser extent, the older restored wetland. Mean (± standard deviation) surface sediment OC content was highest in the older restored wetland (60.0 ± 11.0 %, $n = 13$ cores), followed by the natural (39.1 ± 13.8 %, $n = 14$ cores) and recently restored (26.7 ± 14.5 %, $n = 14$ cores) wetlands.

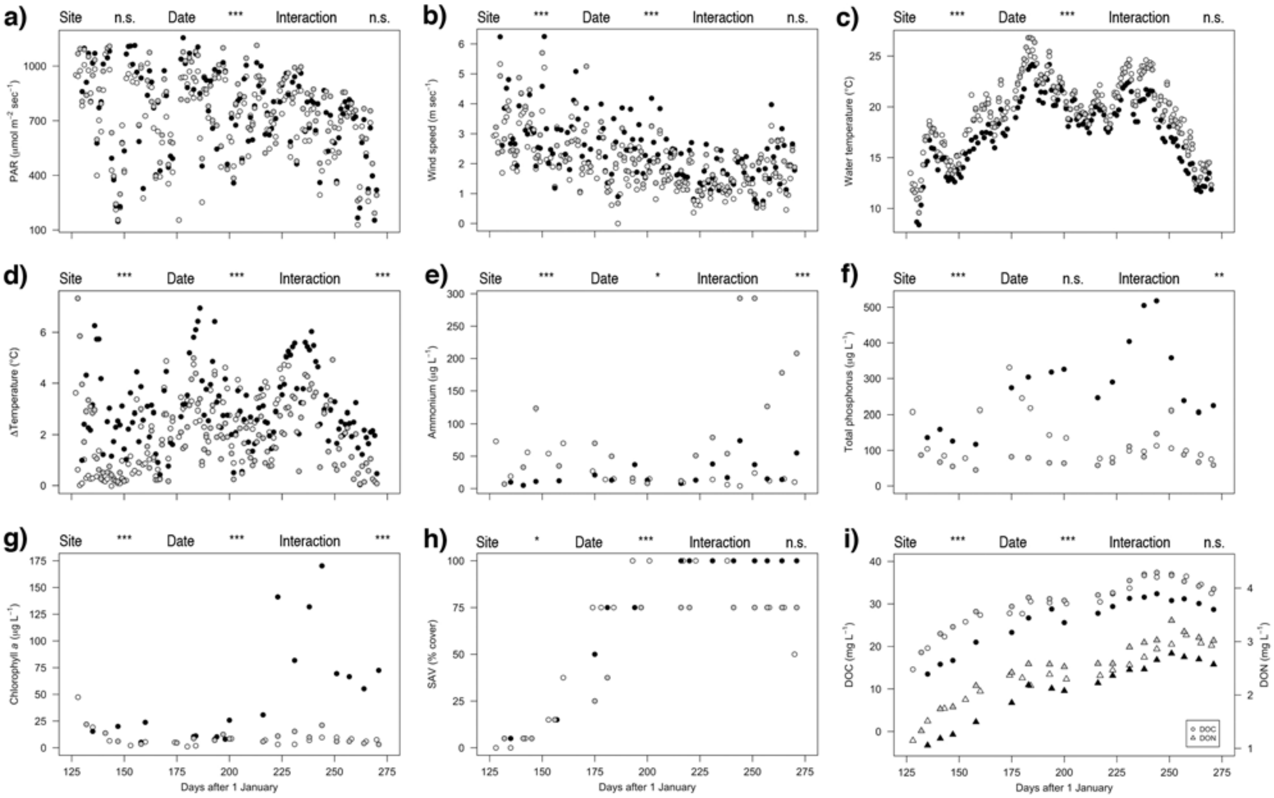

Figure 4.3 Predicted drivers of ecosystem metabolism in three prairie wetlands in May-September 2013. Restoration state is indicated by symbol shading: open/white = natural, grey = older restored, and black = recently restored. Only measured (i.e., non-interpolated values) are shown here. Metabolic drivers include a) photosynthetically active radiation (PAR, µmol m^{-2} sec^{-1}); b) wind speed (m sec^{-1}); c) water temperature (C); d) difference in water temperature between surface and bottom waters as a proxy for stratification (ΔT, °C); e) ammonium concentration (µg L^{-1}); f) total phosphorus concentration (µg L^{-1}); g) chlorophyll *a* concentration (µg L^{-1}); h) % submersed aquatic vegetation cover; and i) dissolved organic carbon (DOC, mg L^{-1}; circles) and dissolved organic nitrogen (DON, mg L^{-1}; triangles). Above each panel is the significance of the effects of site, date, and a site x date interaction on the associated variable in an ANCOVA (significance determined by permutation testing). Significance levels are denoted as: *** at P = 0.001, ** at P = 0.01, * at P = 0.05, and n.s. (not significant).

Discussion

Metabolic rates in the studied wetlands were relatively high in comparison to other lentic ecosystems (Laas et al. 2012, Solomon et al. 2013). NEP, GPP, and ER rates and drivers differed among sites. The wetlands generally responded predictably and similarly to abiotic variables such as PAR and temperature. Differences in metabolic rates and drivers arose instead because the abundance and relative importance of primary producer communities varied among sites. Variable abundance of phytoplankton, SAV, and emergent vegetation, as well as interactions between these communities, resulted in unique patterns of metabolism in prairie wetlands. The identity of the drivers of metabolism in the recently restored wetland suggest that drainage and restoration may affect ecosystem metabolism, mediated via direct and indirect changes to primary producer communities.

Table 4.5. Mean (standard deviation) photosynthetically active radiation (PAR), wind speed, water temperature, difference in water temperature between surface and bottom waters (ΔT), ammonium concentration (NH_4^+), total phosphorus concentration (TP), chlorophyll *a* concentration (Chl *a*), submersed aquatic vegetation % cover (SAV), dissolved organic carbon (DOC), dissolved organic nitrogen (DON) in three prairie wetlands. Only measured values (i.e., non-interpolated values) were included in these calculations and all measurements were made between May and September 2013.

	Natural	**Older restored**	**Recently restored**
PAR	704.4 (227.9)	753.9 (229.1)	746.0 (242.7)
(μmol m^{-2} sec^{-1})	*n* = 138	*n* = 128	*n* = 130
Wind speed	1.9 (0.9)	2.2 (1.0)	2.4 (1.0)
(m sec^{-1})	*n* = 138	*n* = 128	*n* = 130
Temperature	19.4 (3.4)	19.4 (3.8)	17.6 (3.5)
(°C)	*n* = 138	*n* = 128	*n* = 130
ΔT	2.0 (1.5)	1.9 (1.4)	3.0 (1.5)
(°C)	*n* = 98	*n* = 128	*n* = 130
NH_4^+	24 (22)	96 (94)	24 (18)
(μg L^{-1})	*n* = 19	*n* = 17	*n* = 17
TP	136 (72)	84 (40)	279 (120)
(μg L^{-1})	*n* = 19	*n* = 17	*n* = 17
Chl *a*	8.8 (10.2)	9.4 (5.6)	50.2 (50.5)
(μg L^{-1})	*n* = 19	*n* = 18	*n* = 19
SAV	66 (38)	49 (32)	69 (39)
(% cover)	*n* = 18	*n* = 14	*n* = 16
DOC	29.8 (6.1)	31.3 (5.2)	26.2 (6.0)
(mg L^{-1})	*n* = 19	*n* = 17	*n* = 17
DON	2361 (524)	2581 (561)	2128 (565)
(μg L^{-1})	*n* = 19	*n* = 17	*n* = 17

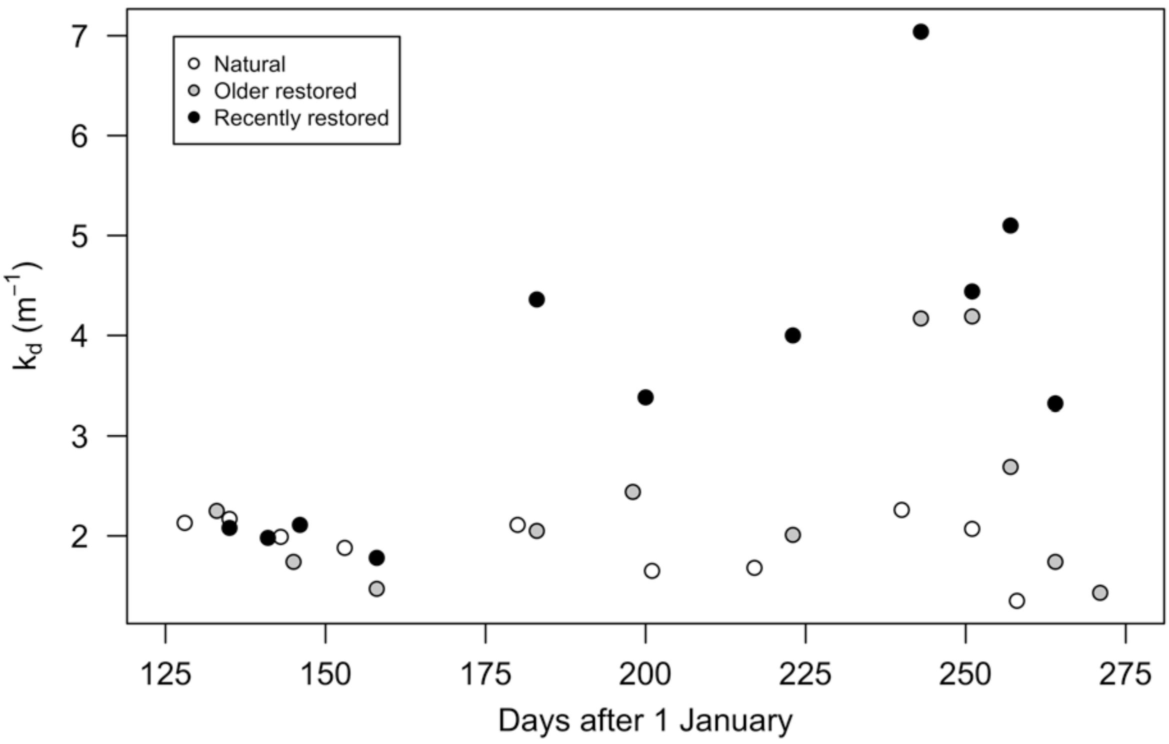

Figure 4.4 Vertical light extinction coefficients (k_d; m^{-1}) in three prairie wetlands in May-September 2013.

Drivers of ecosystem metabolism

The abiotic drivers that most influenced NEP and GPP were PAR and proxies of water column stratification. Only in the recently restored wetland was there any evidence that nutrients (i.e., TP) might influence GPP. Greater NEP and GPP were associated with greater PAR, but stratification proxies had opposite effects; GPP was lower when the water column was less well mixed (i.e., high ΔT) whereas NEP was elevated (i.e., higher NEP with lower wind speeds). The relationship between stratification proxies and metabolism likely reflects variable incorporation of benthic production and respiration to observed rates. That GPP increased with decreased ΔT suggests that benthic processes contribute to production, consistent with a shallow system with light transmission to the benthos. However, the negative NEP-wind speed relationship indicates that the wetland benthos may contribute more to respiration than production. The general absence of nutrients in models of GPP is consistent with the literature on metabolism of shallow lakes suggesting that that nutrient limitation is less important than light limitation in nutrient-rich systems (Sand-Jensen and Staehr 2007). Investigations of drivers of metabolic rates in wetlands have been few to date, making it difficult to compare our findings to any general understanding of factors affecting metabolism in freshwater wetlands (Hoellein et al. 2013). However, the drivers identified here are common to many types of aquatic systems (e.g., Sand-Jensen and Staehr 2007, Coloso et al. 2008, Staehr et al. 2010a, Hoellein et al. 2013, Klotz 2013).

Biological drivers of NEP and GPP, including SAV and chl *a*, were complex and site-specific. In the natural wetland, SAV was positively related to both NEP and GPP. In contrast, in the older restored wetland, peak NEP and GPP were observed at intermediate SAV abundance. The GPP-SAV relationship was positive and linear in the recently restored wetland, but SAV was unrelated to NEP at this site and it was the only site where chl *a* concentrations were positively related to NEP and GPP. Prairie wetlands also support significant emergent vegetation communities (Stewart and Kantrud 1971). However, emergent macrophyte leaves exchange gases directly with the atmosphere, so their immediate production and respiration are not captured in estimates of metabolism in the open-water zone. Only the decomposition of these plants, usually those from the previous growing season, may be captured in open-water O_2 measurements. Overall, the consistent inclusion of SAV abundance (or some proxy for it) in GPP models underscores the importance of this community to prairie wetland production, but it is otherwise difficult to generalize from our findings about biotic drivers of NEP and GPP in this system.

Drivers of ER were consistent across the three wetlands, with greater ER observed at warmer temperatures, when the water column was likely to be well mixed (i.e., small ΔT, high wind speeds), and when GPP was high. There was not strong support for an ER-DOC relationship, though Solomon et al. (2013) also failed to detect a significant relationship in a synthesis of respiration in lakes. The coupling of ER and GPP (Fig. 4.2) suggests that autochthonous material supports much of the respiration in these wetlands, though less so in the older restored site. The degree of coupling of GPP and ER in this system far exceeds previous measurements for ponds and wetlands — compare R^2 values of 0.57-0.81 in this study to R^2 of 0.02 reported by Hoellein et al. (2013). Our findings are closer to predictions and measurements for oligotrophic lakes (Hoellein et al. 2013, Solomon et al. 2013).

Differences in ecosystem metabolism among wetlands

Among-site differences in NEP were related to the interaction between SAV and phytoplankton communities, as well as the contribution of emergent vegetation to ER. In the natural wetland, the only site with a net autotrophic balance during the open-water season, high NEP rates resulted from the productive SAV community. With low chl *a* concentrations and high SAV abundance, this site exemplifies a SAV-dominated clear-water state (*sensu* Scheffer 1989). Accordingly, k_d at this site remained low throughout the open-water season. Although we often measured nutrient concentrations well above what are expected for clear-water systems (Fig. 4.3f; Bayley and Prather 2003, Zimmer et al. 2009), macrophyte community composition affects the stability of the clear-water state (Hilt 2015) and some species of SAV can impede the development of phytoplankton (Mjelde and Faafeng 1997). Thus, SAV taxonomic identity, more than SAV biomass, may be important to preserving clear-water states. By maintaining the clear-water state and associated minimal light attenuation, the SAV created conditions that maximized not only SAV photosynthetic rates, but also epiphytic and benthic production. In contrast, the recently restored wetland, the most heterotrophic of the three sites, supported both abundant SAV and phytoplankton (a somewhat uncommon type of alternate state; Bayley and Prather 2003), but resembled a turbid state rather than a clear one. Phytoplankton-dominated systems are generally less productive and more heterotrophic than macrophyte-dominated ones, often due to shading by phytoplankton (Blindow et al. 2006, Brothers et al. 2013). Although DOC is an important regulator of light attenuation in many aquatic ecosystems, in prairie wetlands, phytoplankton generally determine turbidity (Zimmer et al. 2016). This relationship was evident both in the seasonal changes in k_d (Fig. 4.4), which correspond more so to those of chl *a* (Fig. 4.3g) than DOC (Fig. 4.3i), and the fact that the greatest k_d was measured in the recently restored site which has the highest chl *a* but lowest DOC concentrations of the three wetlands. Thus, although the same amount of PAR reached the water surface at the three sites (Fig. 4.3a, Table 4.5), in the recently restored wetland, phytoplankton likely suppressed production by other communities (SAV, epiphytes, and epipelon) by modifying light availability. Periods of elevated light attenuation and chl *a* coincided with high TP concentrations (Fig. 4.5). Although there was little support for nutrient limitation of primary production, phosphorus may play a role in stimulating phytoplankton blooms and thus contributing to turbid conditions. Finally, the older restored wetland displayed greater net heterotrophy than the natural site, despite also having abundant SAV and relatively low chl *a* and k_d. In this case, net heterotrophy was likely the result of high respiration rates, subsidized by decomposing emergent vegetation. Sediment OC content was higher and GPP and ER were substantially less coupled in the older restored wetland ($R^2 = 0.57$) compared to the other sites ($R^2 > 0.77$). ER fueled by sediment OC, which is derived from emergent macrophytes grown during previous open-water seasons, would contribute to the decoupling of GPP and ER by releasing heterotrophic organisms from dependence on production-generated carbon. Although we did not attempt to elucidate why the older restored wetland has greater sediment OC content, we point to the fact that that site has a greater area of emergent vegetation in and around the basin than the other wetlands.

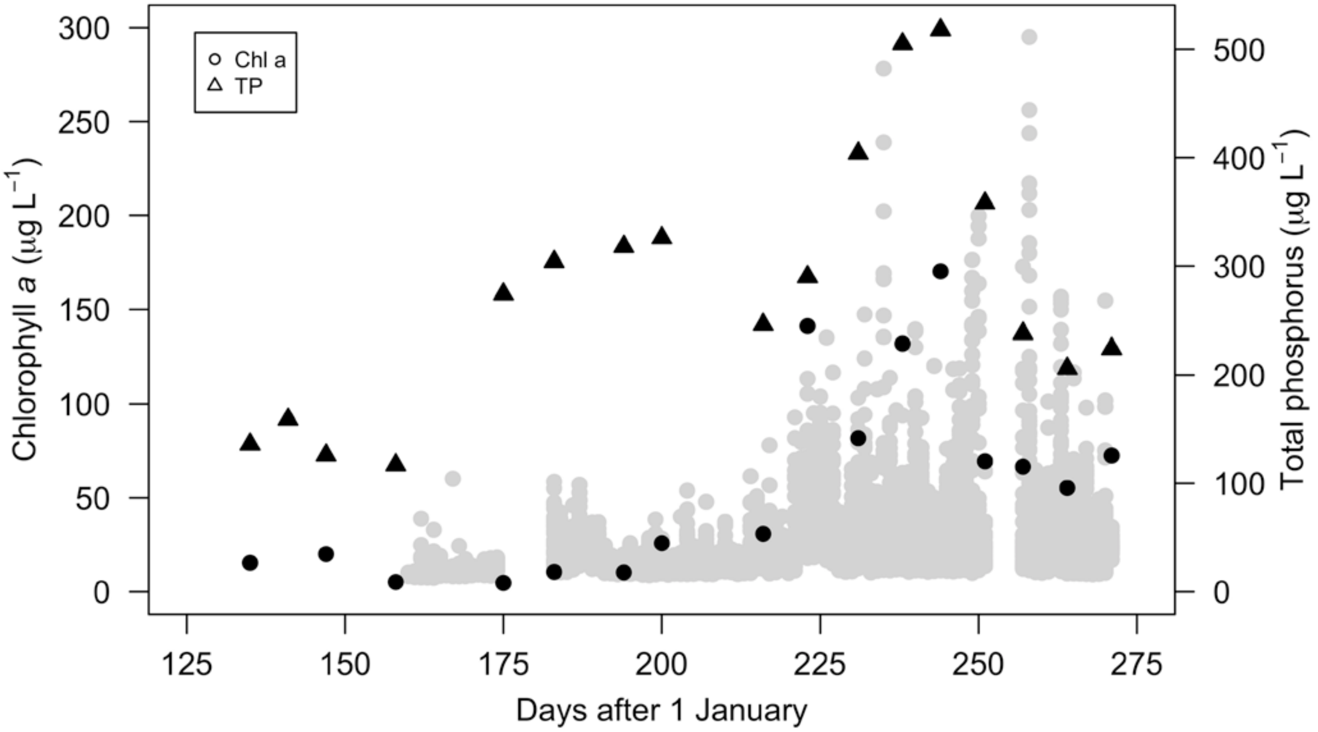

Figure 4.5 Total phosphorus (TP) and chlorophyll *a* (chl *a*) concentrations in the recently restored prairie wetland in May-September 2013. TP concentrations are depicted as triangles, and chl *a* as circles. Black circles are values determined by fluorometric methods from water collected ~every two weeks. Grey circles are non-quantitative, high-frequency chl *a* readings from a total algae probe on a multiparameter sonde.

The observed GPP gradient was related to SAV abundance. GPP rates were highest in the older restored wetland, followed by the natural and recently restored wetlands. As previously discussed, to account for differences between sites, a variable must vary by site and display the same gradient as the metabolic rate of interest. PAR did not vary by site and thus cannot explain the observed GPP gradient across sites (Fig. 4.3a, Table 4.5). Temperature, wind speed, NH_4^+, TP, and chl *a* differed by site, but not in ways that could account for the GPP gradient. Although % SAV cover was similarly unable to explain among-site differences in GPP, DON was. DON concentrations were highest in the older restored wetland and lowest in the recently restored wetland (Table 4.5). DON may more accurately reflect SAV biomass than estimates of % cover. Thus, just as SAV abundance was an important driver of within-wetland GPP rates, it was also the most plausible explanation for differences among wetlands.

ER showed the same gradient as GPP, highest in the older restored wetland to lowest in the recently restored wetland, likely driven by the availability of substrates for microbial respiration (e.g., DOC and sediment OC). As for GPP, temperature and wind speed differed by site, but not in ways that could account for the ER gradient. Instead, both DOC and sediment OC content were greatest in the older restored wetland, followed by the natural and recently restored sites. Given the relatively close coupling of ER and GPP in this system (i.e., GPP is an important source of OC for respiration), GPP may also explain among-site differences in ER.

Differences among wetlands according to restoration state

It is difficult to make generalizations about how drainage and restoration affect ecosystem metabolism in prairie wetlands based on three sites. We can, however, compare the factors driving differences in ecosystem metabolism among wetlands to previously identified characteristics of natural, older restored, and recently restored wetlands (Chapter 2). Older restored and natural wetlands are similar with respect to water chemistry and the taxonomic composition of SAV, benthic producer communities (Chapter 2) and emergent vegetation (Puchniak 2002). Thus, while metabolic rates differed between the older restored and natural wetlands, these differences were unlikely related to restoration. In contrast, some of the factors that shaped the net heterotrophy and low GPP and ER in the recently restored site are characteristic of wetlands only recently restored. For example, recently restored wetlands typically have less sediment OC, more TP, and different SAV community composition than natural and older restored wetlands (Chapter 2) — all factors we have identified here as being related to metabolic rates. Furthermore, recently restored wetlands have lower pH, which may be the result of less productive SAV communities (Carpenter and Lodge 1986, Galatowitsch and van der Valk 1996, Chapter 3). To comment more definitively on the relationship between ecosystem metabolism and restoration history, we would need to measure ecosystem metabolism in a greater number of sites. However, our results suggest that some characteristics of recently restored wetlands may either contribute to, or be the result of, different ecosystem metabolism but that these differences may disappear with enough time after restoration.

Conclusions

Biotic factors including SAV, phytoplankton, emergent vegetation, and their potential interaction were identified as drivers of ecosystem metabolism both within and among wetlands. In contrast, abiotic drivers explained variation in daily metabolic rates within wetlands, but could not account for differences among wetlands. The degree of net autotrophy or heterotrophy in a given wetland was governed by the interaction of whether there was a robust, productive SAV community unfettered by phytoplankton blooms and to what degree sediment OC (derived from emergent vegetation) augmented ER. Light, rather than nutrient availability, appeared to mediate ecosystem metabolism, except in the case of high TP and algal blooms in the recently restored wetland. Future work should examine more closely the role that SAV and phytoplankton community composition play in wetland ecosystem metabolism across different states (i.e., clear versus turbid). Finally, more work is needed to better establish the effects of restoration on ecosystem metabolism.

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Chapter 5: Conclusions

Collectively, the research presented in this thesis represents one of the most comprehensive assessments of biological communities and ecosystem functions in restored and natural prairie wetlands. As discussed in Chapter 1, my objectives were to understand how drainage and restoration affect the abiotic environment and biological communities of prairie wetlands, to characterize ecosystem functions of prairie wetlands, and to identify abiotic and biological drivers of those functions. Figure 5.1 summarizes the main findings from each of these objectives, in reference to the original conceptual framework for the thesis. Although my ability to relate observed patterns to restoration history is limited by the number of wetlands studied, taken as a whole, there are indications that differences in the abiotic environment and primary producer communities do indeed translate to altered carbon fluxes and ecosystem metabolism in recently restored wetlands.

Summary of work

Restoration affects the abiotic environment and biological communities of prairie wetlands, but recovery is possible

Chapter 2 revealed that drainage and restoration alter the abiotic environment and certain biological communities, but that these attributes recover within ~10 years of restoration. Older restored and natural wetlands are indistinguishable with respect to the measured abiotic characteristics, but recently restored wetlands stand out for their lower specific conductance, pH, and % sediment organic carbon (OC) but higher total phosphorus (TP) and carbon dioxide (CO_2). Elevated TP likely originates from the drying out and rewetting of wetland sediments after drainage, whereas some differences (pH, CO_2, sediment OC) relate more to altered wetland vegetation and production. Differences in specific conductance and sediment OC are likely related to residence time, as the effects of evapoconcentration and OC sedimentation are cumulative. The effects of restoration are most pronounced on benthic macroinvertebrate and submersed aquatic vegetation (SAV) communities. Communities made up of small organisms (phytoplankton, benthic diatoms, zooplankton) recovered rapidly, presumably

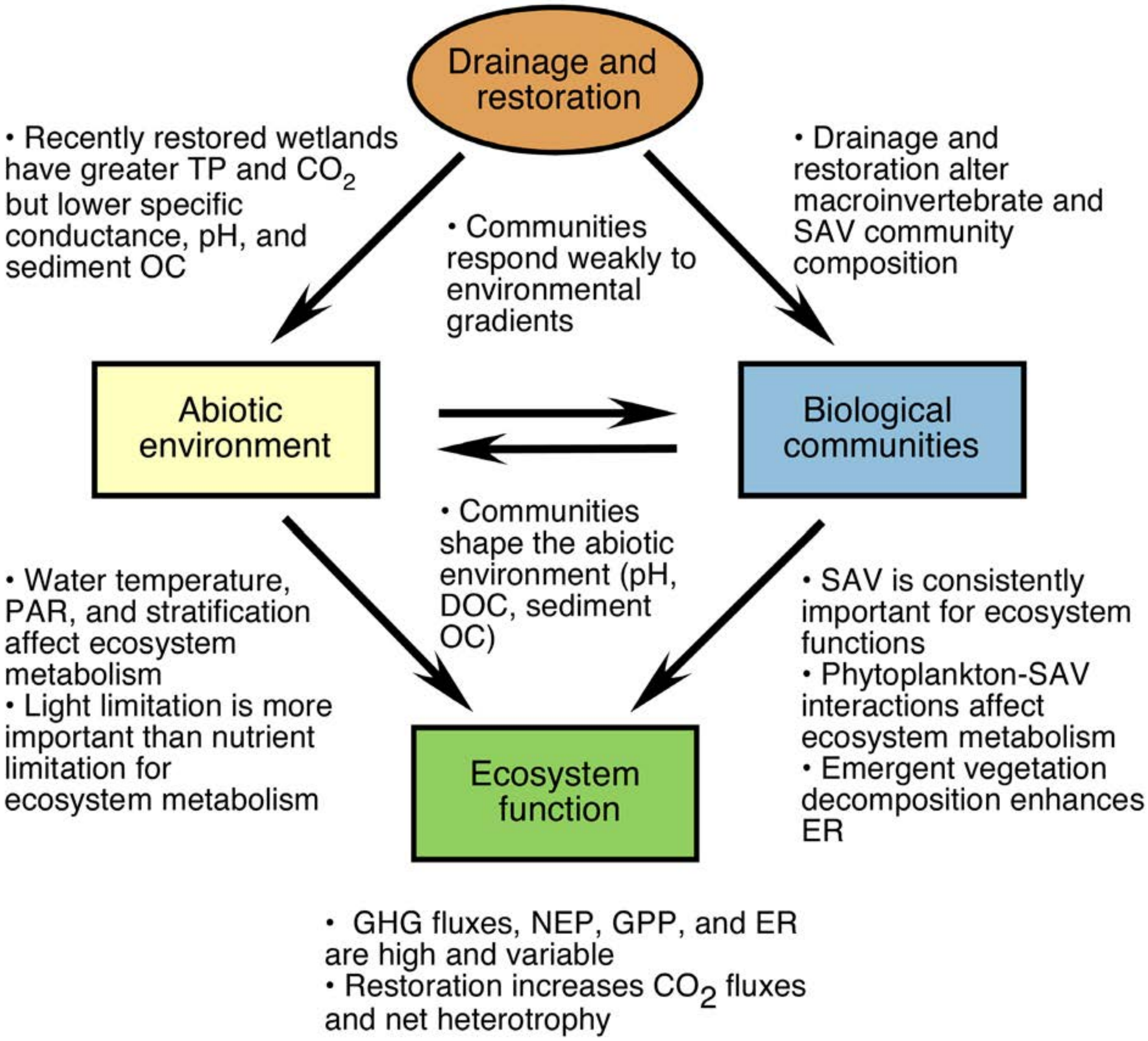

Figure 5.1 A framework for the effects of drainage and restoration on ecosystem attributes including the abiotic environment, biological communities, and ecosystem function. Bulleted points show how the major findings from Chapters 2-4 fit into this framework.

owing to high connectivity and relatively high density of propagule sources on the landscape. The recovery trajectory of macroinvertebrates, a shift from organisms that favour temporary habitats to those that favour more permanent water bodies, resembled the succession of macroinvertebrates after wetlands dry out and reflood as part of the wet-dry cycles of the prairies. Thus, for macroinvertebrates, certain aspects of drainage and restoration may mimic the natural disturbance regime of this system. Differences in the SAV community in recently restored wetlands may be driven by restoration-induced specific conductance and TP gradients.

Other than for the SAV community, environment-community relationships tended to be weak. Although the study design did not allow us to identify an exact recovery timeline, ~10 years is a conservative estimate for the time frame needed for restored wetlands to support biodiversity comparable to natural wetlands. However, restoration practitioners, or anyone conducting similar work in the future, should keep in mind that landscape and climatic conditions may affect recovery times and trajectories via effects on wetland connectivity and dispersal distances for colonizing organisms.

Carbon greenhouse gas fluxes from prairie wetlands are variable, and driven by biological and geochemical processes

GHG fluxes and NEP rates in prairie wetlands are remarkable in their magnitude and variability and CO_2 supersaturation is common, but not necessarily indicative of net heterotrophy. The diel oxygen method and CO_2 fluxes provided conflicting estimates of prairie wetland metabolic status at short time scales due to the dual influence of biological and geochemical processes on carbon cycling. CO_2 effluxes are augmented by geochemical processes, which likely include carbonate mineral dissolution. The issues surrounding the use of CO_2 saturation to indicate net heterotrophy exist for many other types of aquatic ecosystems, and this study adds to the growing body of research that suggests that biological processes are only one component of CO_2 effluxes from inland waters. Having characterized only three sites limits my ability to make inferences about the effect of restoration on GHG fluxes. However, some of the patterns we observed in the three focal wetlands were consistent with the survey of 24 wetlands (Chapter 2), suggesting that recently restored wetlands generally have elevated CO_2 fluxes and greater net heterotrophy.

Ecosystem metabolism rates and drivers vary among prairie wetlands

Drivers of NEP and GPP rates differ among wetlands, whereas factors explaining daily variation in ER remain fairly consistent across sites. In general, metabolic rates respond in predictable and consistent ways to abiotic drivers such as water temperature and photosynthetically active radiation. Differences between sites, with respect to both metabolic rates and drivers, are instead related to biotic variables like chlorophyll *a* (chl *a*) and SAV. Wetland state (clear water or turbid) and the amount of emergent vegetation (which affects sediment OC content) are two probable factors determining net ecosystem production (NEP), gross primary production

(GPP), and ecosystem respiration (ER) rates. In the natural wetland, a clear-water state and moderate emergent vegetation cover result in net autotrophy and intermediate GPP and ER. Greater emergent vegetation in and around the older restored wetland produces elevated ER when compared to the natural wetland and, consequently, net heterotrophy. NEP, GPP, and ER are lower in the recently restored wetland owing to the seasonal development of a turbid state and relatively low sediment OC content. The distinct metabolic rates and drivers observed in the recently restored wetland may be related to its restoration history, though additional work is needed to more directly demonstrate the effects of restoration on ecosystem function.

Future research directions

Linking biodiversity and ecosystem function

Ecological restoration seeks to reestablish both biodiversity and ecosystem function. As such, the intersection of biodiversity-ecosystem function theory and restoration ecology has been of long-standing interest. The characterization of biological communities and ecosystem functions accomplished here has laid the foundation for future efforts linking biodiversity (here referring to species richness, taxonomic composition, species identity) and function in prairie wetlands.

It has been shown conclusively that SAV exerts a strong influence on ecosystem functions like CO_2 flux (via drawing down the pool of dissolved inorganic carbon, Chapter 3), and GPP and NEP (Chapter 4). However, it remains to be resolved whether differences in taxonomic composition of SAV or phytoplankton communities translate to effects on wetland state (clear water or turbid) and function. I recommend that future investigations focus in particular on the effects of individual key species (e.g., *Ceratophyllum demersum* L.) and traits (e.g., mixotrophy, ability to use bicarbonate for photosynthesis, rootedness) on ecosystem state and function.

Understanding the extent of natural variation in ecosystem metabolism

Quantifying the range of natural variation in ecosystem metabolism, both within and between prairie wetlands, is a necessary step towards improving our understanding of the way metabolism responds to anthropogenic perturbations like drainage and restoration. Given that there have been no previous studies of ecosystem metabolism in prairie wetlands, it is difficult to judge the meaningfulness of differences observed among sites and to relate those differences to restoration state. For example, it would be helpful to know whether the NEP values observed in the recently restored wetland still appear relatively low when compared to multiple natural wetlands (i.e., do the rates fall outside or at the edge of the range of natural NEP rates, or are they well within that range). The top priority for understanding within-wetland variation is to characterize the response of prairie wetlands to wet-dry cycles.

Further studies of ecosystem metabolism in prairie wetlands would provide an opportunity to refine the methodology of measuring diel oxygen metabolism in freshwater wetlands. The relationship between proxies of stratification (wind speed, temperature gradient in the water column) and metabolic rates provides some insight into the relative contribution of pelagic versus benthic processes to ecosystem metabolism in prairie wetlands (Chapter 4). However, future work should ideally better parameterize spatial variability (vertically and horizontally) of metabolic rates.

Scaling up ecosystem functions of prairie wetlands

In-depth studies of ecosystem functions provide invaluable insight into the way ecosystems work, but may be impractical to carry out at the large scales at which conservation and management decisions are made. Quantifying ecosystem services provided by intact and restored wetlands provides valuable information for policy makers wishing to protect and/or restore wetlands. There is great value in and a need for quantifying these services via long-term and landscape-scale studies. For example, a large-scale study of the transformation and fate of carbon in prairie wetlands, including concurrent measurements of CO_2 and CH_4 fluxes and organic carbon sequestration, is needed. However, the drawback to this approach is the considerable time and resources needed. Thus, ideally, long-term, landscape-scale studies would be coupled with efforts to identify easy-to-measure, but still meaningful, indicators of ecosystem function. In-depth (but small-scale) studies, like this thesis, can provide a valuable foundation for the development or selection of such indicators. For example, we observed a robust pH-CO_2 concentration relationship in prairie wetlands (Chapter 3). Future work should further explore this relationship and evaluate the utility of pH as an indicator for CO_2 that could expand our understanding of CO_2 dynamics in prairie wetlands over larger spatial and temporal extents.

Response of ecosystem metabolism to environmental change

The response of aquatic ecosystem metabolism to anthropogenic stressors is a topic deserving of further study. As outlined in Chapter 4, this field of study has been too little explored in all types of aquatic ecosystems. Prairie wetlands, for example, are subject to numerous anthropogenic stressors that may have direct or interactive effects on their metabolism. Some of these stressors are related to their agricultural matrix (e.g., pesticides, nutrient loading) though others, like climate change, are of a global nature. It may be possible to take advantage of the natural wet-dry cycles of the prairies to quantify how wetland functions respond to climatic variation, enabling the effects of climate change to be understood beyond hydrology alone. The challenge and cost of studying enough sites to draw conclusions about the effects of anthropogenic stressors on ecosystem functions is almost certainly one of the reasons for the lack of literature on this topic. This problem is in no way new to ecosystem science, but can be overcome by ambitious research projects (e.g., whole-ecosystem experiments) and generating a critical mass of research. Ideally, future work on the effects of stressors on ecosystem function will involve a combination of experimental work, done at large and complex enough scales so as to be meaningful, and observational studies of stressors at work in actual ecosystems.

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Appendix 1: Supporting information for Chapter 2

Details of water chemistry analyses

With the exception of the methods outlined for carbon dioxide, methane, and dissolved inorganic carbon measurement, the descriptions below are adapted from protocols provided by the University of Alberta's Biogeochemical Analytical Service Laboratory, the laboratory where water chemistry analyses were performed.

Ammonium: Ammonium (NH_4^+) analysis is based on the Berthelot reaction where NH_4^+ in the sample reacts with hypochlorite and phenol to form indophenol, a blue compound. The absorbance of the reaction product at 630 nm is measured with a Lachat QuikChem 8500 FIA automated ion analyzer.

Nitrite and Nitrate: Passage of the sample through a copperized cadmium column reduces nitrate (NO_3^-) to nitrite (NO_2). Total NO_2 is then diazotized with sulfanilamide and couples with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a magenta dye that is measured colorimetrically at 520 nm using a Lachat QuikChem 8500 FIA automated ion analyzer.

Total Dissolved Nitrogen: Total dissolved nitrogen (TDN) is measured by a chemical digestion, flow injection method. Sample is introduced to a Dionex DX600 Ion Chromatography and anions of interest are separated and measured using a system comprised of a Dionex IonPac AG9-HC guard column, an IonPac AS9-HC analytical column, a suppressor device, and conductivity detector.

Total Phosphorus and Total Dissolved Phosphorus: Unfiltered and filtered samples (to measure total phosphorus (TP) and total dissolved phosphorus (TDP), respectively) are digested with potassium persulfate ($K_2S_2O_8$) using an autoclave, then autoclaved to convert all P to orthophosphate. Samples are then filtered through a 0.45 μm filter and the orthophosphate is reacted with ammonium molybdate and antimony potassium tartrate under acidic conditions to form an antimony-phosphomolybdate complex. This complex is reduced with ascorbic acid to form a blue complex, which absorbs light at 880 nm and can be quantified using a Lachat QuikChem 8500 FIA automated ion analyzer.

Dissolved Organic Carbon: Dissolved organic carbon (DOC) is analyzed using a Shimadzu 5000A TOC analyzer. All inorganic carbon contained in the sample is removed by acidifying with HCl and sparging with hydrocarbon-free air. DOC is converted to carbon dioxide by catalytic combustion at 680 °C then detected by a non-dispersive infrared detector.

Chlorophyll a: A known volume of water is filtered onto a GF/F filter which is then frozen to lyse cell walls, releasing the ethanol-soluble chlorophyll *a* molecule. The extract from these filters is then analyzed using a Shimadzu RF-1501 spectrofluorophotometer.

Carbon Dioxide, Methane, Dissolved Inorganic Carbon: Water is collected into evacuated 160 mL Wheaton glass serum bottles capped with butyl rubber stoppers. Each bottle contains 8.9 g of potassium chloride (KCl) preservative and 10 mL of ultrahigh purity dinitrogen (N_2) gas headspace. To collect a sample, bottles are submerged ~10 cm below the water surface and punctured with a needle. Immediately prior to analysis in the lab, samples are shaken on a wrist-action shaker for 20 minutes to equilibrate dissolved gases with the headspace. Carbon dioxide (CO_2) and methane (CH_4) are analyzed on a Varian 3800 gas chromatograph (GC) equipped with a HayeSep D column (80°C), a ruthenium methanizer to convert CO_2 to CH_4, and a flame-ionization detector (FID; 250°C). We use four gas standards (Praxair, Linde-Union Carbide), ranging from 75 to 6000 ppm for both CO_2 and CH_4, to calibrate the GC. A Varian Star Workstation program integrates peak areas. Sample gas concentration can then be inferred from headspace gas concentration, ambient and laboratory temperature and pressure, and Henry's Law. Samples are subsequently acidified with 0.5 ml H_3PO_4 to convert all dissolved inorganic carbon (DIC) to CO_2, and reanalyzed on the GC.

Table A1.1 Geographic coordinates of 24 study wetlands in southeastern Saskatchewan. Map numbers correspond to site numbers on Figure 1.2b. Restoration states include recently restored (RR; restored 1-3 years before 2011), older restored (OR; restored 7-14 years before 2011), and natural (NAT; never drained) wetlands.

Map no.	Site	Restoration state	Latitude and longitude (Decimal degrees)	
1	Hines	RR	51.041	-102.056
2	Hood-1	RR	50.672	-102.002
3	Hood-2	RR	50.666	-102.002
4	Johanson	RR	50.656	-102.370
5	Reinson	RR	51.080	-102.902
6	Smith-1	RR	51.156	-103.272
7	Smith-2	RR	51.155	-103.229
8	Sorrell	RR	50.954	-101.910
9	Adams	OR	51.018	-101.869
10	Penner-1	OR	51.044	-101.922
11	Penner-2	OR	51.051	-101.923
12	Rowein	OR	50.718	-102.266
13	Tataryn-1	OR	51.212	-103.114
14	Tataryn-2	OR	51.213	-103.115
15	Toderian	OR	51.054	-101.722
16	Wilk	OR	51.179	-103.137
17	Hood	NAT	50.667	-102.004
18	Johanson	NAT	50.649	-102.360
19	Penner	NAT	51.045	-101.922
20	Reinson	NAT	51.078	-102.899
21	Rowein	NAT	50.722	-102.268
22	Smith	NAT	51.152	-103.273
23	Toderian	NAT	51.055	-101.725
24	Wilk	NAT	51.179	-103.135

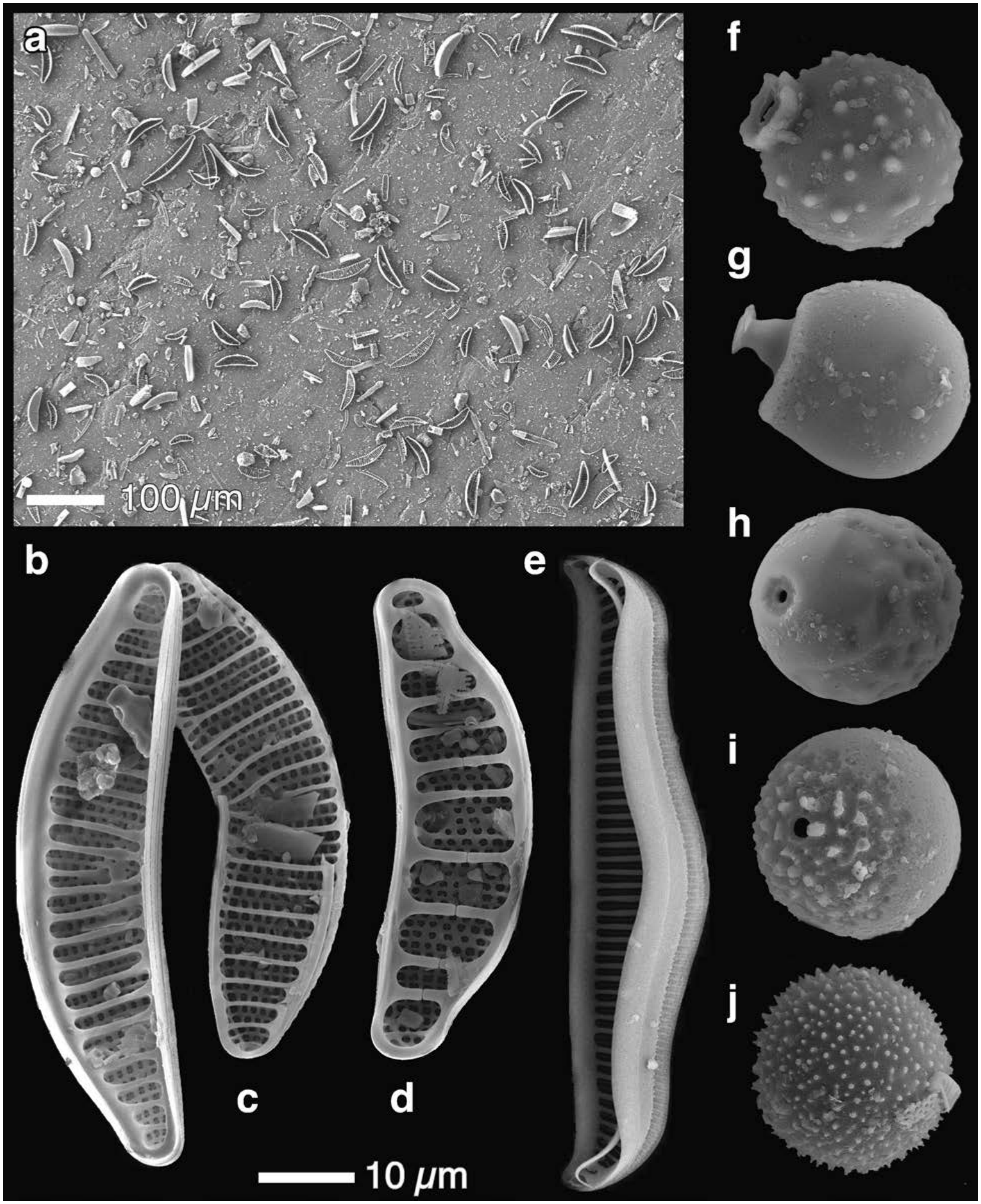

Plate A1.1 Scanning electron micrographs (SEM) of diatom valves and chrysophyte cysts. (a) Overview of the siliceous microfossil assemblage in surface sediments collected from the Hines wetland on July 3, 2013. *Epithemia adnata* are abundant in this sample. (b-d) High magnification SEM of *E. adnata*. (e) *Rhopalodia gibba*. (f-j) Assorted morphotypes of chrysophycean stomatocysts.

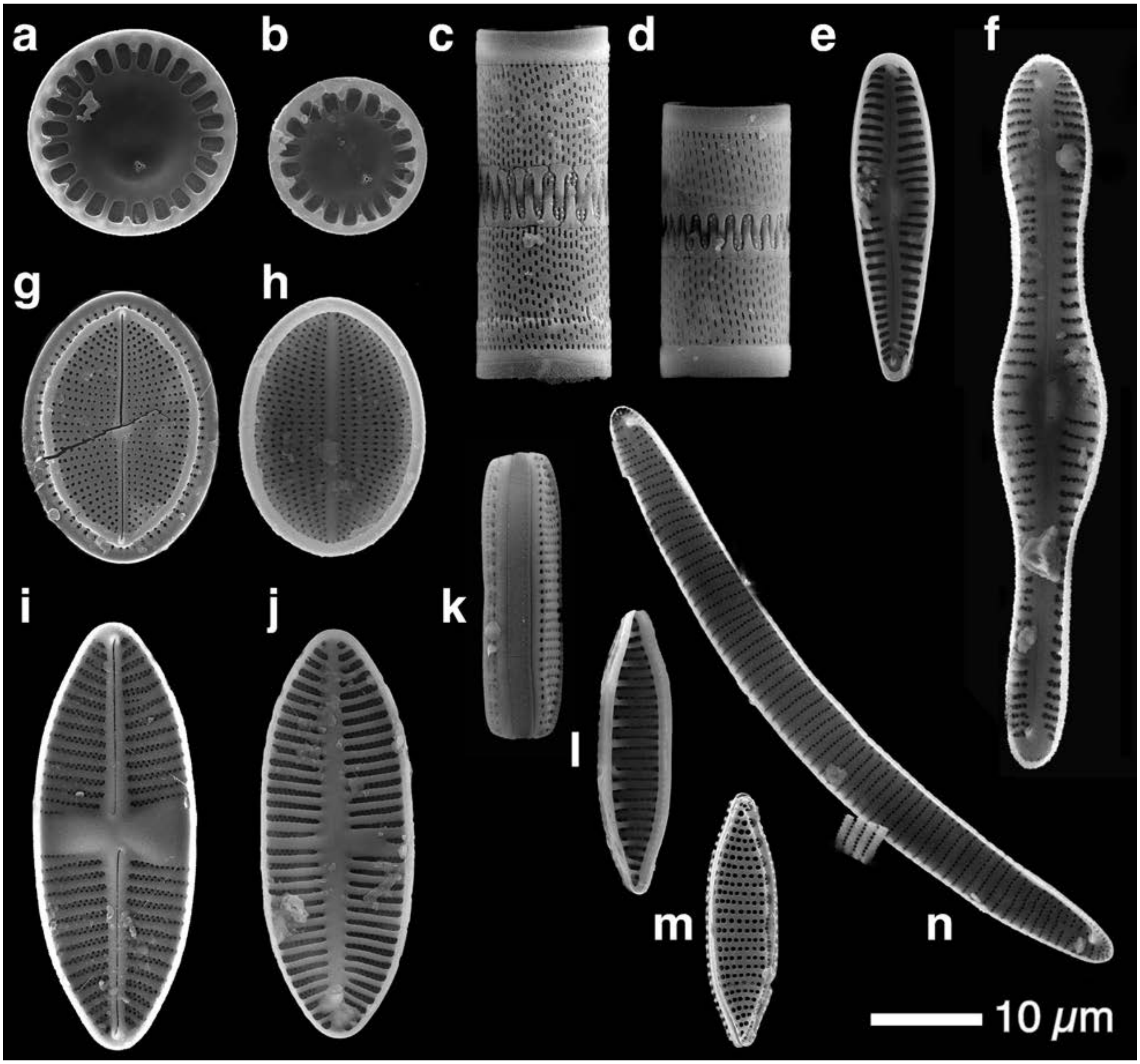

Plate A1.2 Scanning electron micrographs of common diatom taxa encountered in surface sediments from prairie wetlands of southeastern Saskatchewan. (a-b) *Cyclotella meneghiniana*. (c-d) *Aulacoseira italica*. (e) *Gomphonema parvulum*. (f) *Gomphonema acuminatum*. (g-h) *Cocconeis placentula,* raphe (g) and araphe (h) valves. (i-j) *Lemnicola hungarica*, raphe (i) and araphe (j) valves. (k) Girdle view of two *Nitzschia perminuta* valves. (l-m) *Nitzschia perminuta*, valve views. (n) *Eunotia bilunaris*.

Appendix 2: List of species from Chapter 2 with authorities

Species-level authorities follow the Integrated Taxonomic Information System (www.itis.gov) and Diatoms of the United States (westerndiatoms.colorado.edu).

Kingdom Animalia, Superclass Osteichthyes
Culaea inconstans (Kirtland, 1840)

Kingdom Animalia, Phylum Arthropoda, Subphylum Crustacea, Order Cyclopoida
Diacyclops navus (Herrick, 1882)

Kingdom Animalia, Phylum Arthropoda, Subphylum Crustacea, Suborder Cladocera
Chydorus sphaericus (O.F. Mueller, 1785)
Simocephalus serrulatus (Koch, 1841)

Kingdom Animalia, Phylum Arthropoda, Subphylum Crustacea, Order Amphipoda
Gammarus lacustris G.O. Sars, 1863
Hyalella azteca Saussure, 1858

Kingdom Animalia, Phylum Mollusca, Class Gastropoda
Promenetus exacuous (Say, 1821)
Promenetus umbilicatellus (Cockerell, 1887)

Kingdom Animalia, Phylum Annelida, Class Hirudinea
Glossiphonia complanata (Linnaeus, 1758)
Helobdella stagnalis (Linnaeus, 1758)

Kingdom Plantae
Ceratophyllum demersum L.
Lemna minor L.
Lemna trisulca L.
Myriophyllum sibiricum Komarov
Persicaria amphibia (L.) Delarbre
Potamogeton pusillus L.
Ranunculus aquatilis L.
Ranunculus gmelinii DC.
Utricularia vulgaris L.

Kingdom Chromista, Class Bacillariophyceae
Amphora ovalis (Kützing) Kützing
Aulacoseira italica (Ehrenberg) Simonsen
Caloneis bacillum (Grunow) Cleve
Cocconeis placentula Ehrenberg
Craticula buderi (Hustedt) Lange-Bertalot
Cyclotella meneghiniana Kützing

Cymbella cistula (Ehrenberg) O. Kirchner
Encyonema minutum (Hilse ex Rabenhorst) D.G. Mann
Epithemia adnata (Kützing) Brébisson
Eunotia bilunaris (Ehrenberg) Schaarschmidt
Fragilaria mesolepta Rabenhorst
Gomphonema acuminatum Ehrenberg
Gomphonema olivaceum (Hornemann) Brébisson
Gomphonema parvulum (Kützing) Kützing
Halamphora veneta (Kützing) Levkov
Hantzschia amphioxys (Ehrenberg) Grunow
Lemnicola hungarica (Grunow) Round and Basson
Navicula radiosa Kützing
Nitzschia perminuta (Grunow) M. Peragallo
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot
Rhopalodia gibba (Ehrenberg) O. Müller
Sellaphora pupula (Kützing) Mereschkovsky

Appendix 3: R code for calculating ecosystem metabolism

```
#Import data
Wetland = read.table (file = “Reinson2013.txt”, header = TRUE)

## [1] “Site”     “Date”     “Time”     “PAR”      “BP”       “Temp”
## [7] “Wind”     “O2”       “Complete”

Constants = read.table (file = “Constants.txt”, header = TRUE)

## [1] “AnemHt”   “Alpha”    “MixDepth”

#File with dayfraction for days with missing values
Missingday = read.table (file = “Reinson2013dayfrac.txt”, header = TRUE)

## [1] “Julian”      “Dayfraction”

#Create a numerical vector where 1-72 replace each 20 min time step
TimeIndex = unique(Wetland$Timef)
Wetland$TimeNum = as.numeric(mgsub(TimeIndex,(c(1:72)),Wetland$Timef))

#Add Julian date to data frame
Wetland$DaysAfter = (julian(Wetland$Datef, origin = as.Date (“2013-01-01”),
by = “day”)+1)

#Convert temperature to Kelvin
Wetland$TempK = (Wetland$Temp + 273.15)

#C constant for saturation calculations
Wetland$Cconst = (-173.4292+249.6339*(100/Wetland$TempK)+
143.3483*log(Wetland$TempK/100)-21.8492*(Wetland$TempK/100))

#Calculate O2 saturation
Wetland$O2sat = (exp(Wetland$Cconst)*1.423)

#Calculate O2sat, corrected for pressure
Wetland$O2satp = (Wetland$O2sat*((Wetland$BP*0.0987-0.0112)/100))

#Calculate O2 concentration
Wetland$O2conc = ((Wetland$O2/100)*Wetland$O2satp)

#Calculate Schmidt coefficient
Wetland$Schmidt = ((0.0476*(Wetland$Temp^3))+(3.7818*(Wetland$Temp^2))-
(120.1*Wetland$Temp)+1800.6)

#Windspeed at 10m height
Wetland$U10 = (Wetland$Wind*Constants$Alpha)
```

```
#Calculate piston velocity
Wetland$PVel = (((2.07+0.215*(Wetland$U10^1.7))/100)*((Wetland$Schmi
dt/600)^-0.5))

#Calculate gas flux
Wetland$Flux = (Wetland$PVel*(Wetland$O2satp - Wetland$O2conc))

#Calculate gas flux per 20 min
Wetland$Flux20 = (Wetland$Flux/3)

#Create new matrix of O2 concentrations, dates, and times
O2V = as.vector(Wetland$O2conc, mode = “any”)
DaysV = as.vector(Wetland$DaysAfter, mode = “any”)
TimeV = as.vector(Wetland$TimeNum, mode = “any”)
O2mat = as.matrix(cbind(DaysV, O2V, TimeV))

#Calculate all O2 changes and date and time changes
O2chgraw = diff(O2mat[,2], lag = 1)
Datechg = diff(O2mat[,1], lag = 1)
Timechg = diff(O2mat[,3], lag = 1)

#Turn time changes of -71 (from midnight to 00:20) to 1
Timechg = ifelse(Timechg == -71, 1, Timechg)

#Match up those changes in a matrix
Chgmat = as.matrix(cbind(Datechg, O2chgraw, Timechg))

#Give NA values to any changes calculated between observations from days more
than 1 day apart or time more than 1 apart
O2chg = ifelse((Chgmat[,1] > 1 | Chgmat[,3] > 3 | Chgmat [,3] < 0), NA,
(Chgmat[,2]))

#Link O2 changes to PAR
#Change in PAR
PARchg = diff(as.vector(Wetland$PAR), lag = 1)

#Match gas flux to O2 changes
Wetland$Sequence = seq(1, length (Wetland$Flux20), by = 1)
Chgset = Wetland[Wetland$Sequence != 9698, ]
Fl20 = as.vector(Chgset$Flux20, mode = “any”)
Dateless = as.vector(Chgset$DaysAfter, mode = “any”)

#Change PARchg = 0 to 1 when associated with non-zero PAR readings
PARchgmat = as.matrix(cbind(Chgset$PAR, PARchg))
PAROK = ifelse ((PARchgmat[,2] == 0 & PARchgmat[,1] != 0), 1, PARchgmat[,2])

#Make new data frame
NEPfrm = data.frame(ByDate = Dateless, Light = PAROK, Chg = O2chg, AtmFl =
Fl20)
```

```r
#Calculate NEP per 20 min
NEPfrm$NEP20 = (NEPfrm$Chg - (NEPfrm$AtmFl/Constants$MixDepth))

#Calculations for dayfraction
#Subset daytime and nighttime from original data
Day = Wetland [Wetland$PAR != 0, ]
Night = Wetland [Wetland$PAR == 0, ]

#Convert date to a factor
Night$Datefac = factor(Night$DaysAfter)
#Change all PAR = 0 to 1 for summing
Night$PARsum = (Night$PAR +1)

#Calculate night and day fractions
#Nightfraction = number of PAR readings at night
Nightfrac = as.numeric(ifelse((tapply(Wetland$PAR, Wetland$DaysAfter, FUN
= length)) >= 69, ((tapply(Night$PARsum, Night$Datefac, FUN = sum)/3)/24),
“NA”))

#Dayfraction = 1- nightfraction unless nightfraction is NA
Dayfracpart = as.numeric(ifelse(is.na(Nightfrac), “NA”, (1-Nightfrac)))
Datecond = as.vector(unique(Night$DaysAfter), mode = “any”)
Daymerge = data.frame(Julian = Datecond, Dayfraction = Dayfracpart)

#Remove dates where dayfraction is NA
Dayfracnomiss = na.omit(Daymerge)

#Merge calculated dayfraction with an excel file containing dayfraction for
dates with missing values
Dayfracfrm = merge(Dayfracnomiss, Missingday, all.x = TRUE, all.y = TRUE)

#Subset NEP data by light
Daylight = NEPfrm[NEPfrm$Light != 0, ]
Nighttime = NEPfrm[NEPfrm$Light == 0, ]

#Sum day and nighttime NEP per 20 minutes for each date
NEPdaytemp = tapply(Daylight$NEP20, Daylight$ByDate, FUN = sum, na.rm = TRUE)
NEPdarktemp = tapply(Nighttime$NEP20, Nighttime$ByDate, FUN = sum, na.rm =
TRUE)

#Convert all non-NA values to 1 to prepare for summing
Daylight$NEP20add = ifelse(!is.na(Daylight$NEP20), 1, NA)
Nighttime$NEP20add = ifelse(!is.na(Nighttime$NEP20), 1, NA)

#Create a new data frame, one observation per date
Condfrm = data.frame (Daycond = Datecond, Dayfraction = Dayfracfrm$Dayfrac,
NEPday = NEPdaytemp, NEPdark = NEPdarktemp)
```

```
#Sum the number of NEP per 20 min for day and nighttime rates each day
Condfrm$Daycount = tapply(Daylight$NEP20add, Daylight$ByDate, FUN = sum,
na.rm = TRUE)
Condfrm$Nightcount = tapply(Nighttime$NEP20add, Nighttime$ByDate, FUN = sum,
na.rm = TRUE)

#Calculate NEP per hour during the day and night
Condfrm$NEPdayhr = as.numeric(Condfrm$NEPday/(Condfrm$Daycount/3))
Condfrm$NEPdarkhr = as.numeric(Condfrm$NEPdark/(Condfrm$Nightcount/3))

#Calculate NEP and Respiration for the whole day
Condfrm$NEPdaytime = (Condfrm$NEPdayhr*Condfrm$Dayfraction*24)
Condfrm$Respdaytime = (Condfrm$NEPdarkhr*Condfrm$Dayfraction*24)

#Calculate GPP, Respiration, and NEP
Condfrm$GPP = (Condfrm$NEPdaytime + (-1*Condfrm$Respdaytime))
Condfrm$RESP = (Condfrm$NEPdarkhr*24)
Condfrm$NEP = (Condfrm$GPP - (-1*Condfrm$RESP))

#Export metabolic rates, plus some numbers that went in to calculation
write.csv(Condfrm, file = “Reinson2013_metabolism_output.csv”, quote = FALSE)
```

Appendix 4: Supporting information for Chapter 4

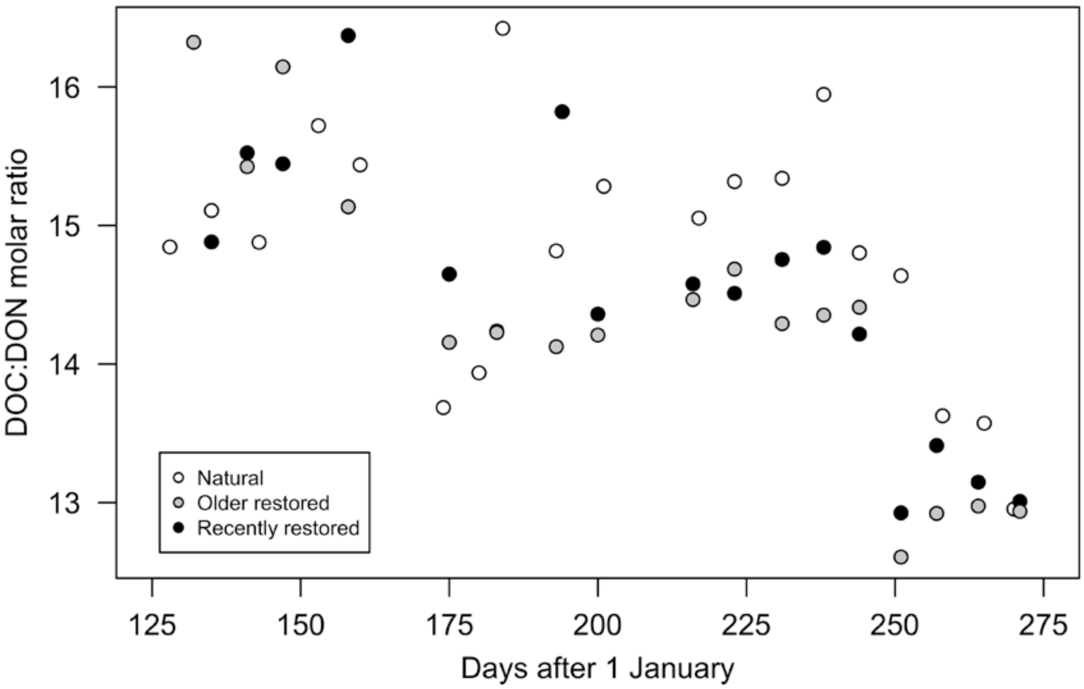

Figure A4.1 Molar ratios of dissolved organic carbon (DOC) to dissolved organic nitrogen (DON) from May-September 2013 in three prairie wetlands. The three wetlands included a natural wetland (i.e., has never been drained), an older restored wetland (restored in 1998), and a recently restored wetland (restored in 2009).

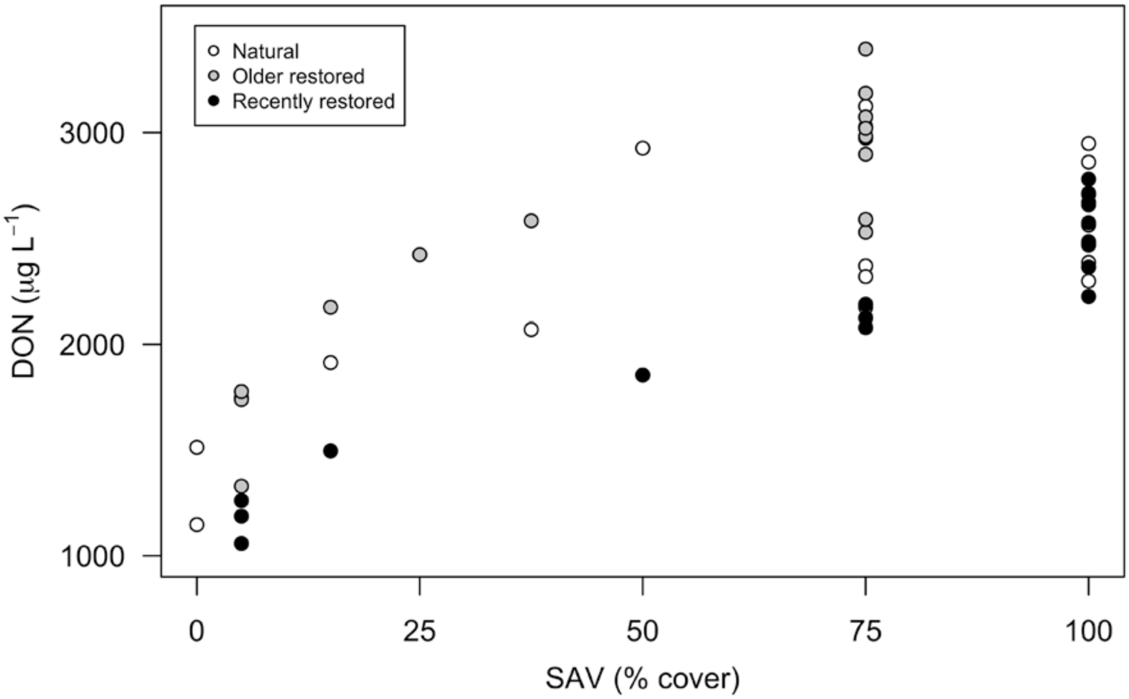

Figure A4.2 Relationship between dissolved organic nitrogen (DON) and % submersed aquatic vegetation cover (SAV) in three wetland basins. The three wetlands included a natural wetland (i.e., has never been drained), an older restored wetland (restored in 1998), and a recently restored wetland (restored in 2009).