Ecological Engineering 72 (2014) 11–24

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Validation of the ecosystem services of created wetlands: Two decades of plant succession, nutrient retention, and carbon sequestration in experimental riverine marshes

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ARTICLE INFO

Article history:
Received 25 August 2014
Received in revised form 27 September 2014
Accepted 27 September 2014
Available online 18 November 2014

Keywords:
Water quality
Carbon sequestration
Wetlands
Nitrogen
Phosphorus
Olentangy River Wetland Research Park

ABSTRACT

Wetlands provide many ecosystem services to society, most notably the provision of habitat for important plants and animals, the improvement of water quality, and the sequestration of carbon. Nitrogen and phosphorus budgets, vegetation structure and function, and carbon fluxes and accumulation are described for a pair of 1-ha created riverine wetlands in central Ohio USA over 20 years (1994–2013) of primary succession. The primary inflow to these experimental wetlands was from water pumped from the adjacent fourth-order Olentangy River. The pumping rate maintained for most of the years (an exception was a two-year comparison of pulsing and non-pulsing hydrology in the two wetlands in 2004–2005) was according to a pre-determined formula based on river stage. The pumped inflow to the wetlands averaged $38.7 \pm 1.5\ m\ yr^{-1}$ with precipitation averaging $1.1\ m\ yr^{-1}$ for the same years. Surface outflow averaged $27.1 \pm 1.4\ m\ yr^{-1}$ and subsurface seepage was estimated to be $13.2 \pm 0.2\ m\ yr^{-1}$ over that period. Both outflows returned water to the Olentangy River via surface and subsurface pathways respectively.

Wetland plant richness increased from 13 species initially planted in one of the wetlands to 116 species overall after 17 years, with most of that richness (99 species) occurring in the first 5 years. The planted wetland had higher community diversity every year except one over 20 years while the naturally colonizing wetland was more productive especially in the first 7 years and overall still had 2000 kg more organic matter input by ANPP after 17 years than did the planted wetland.

Nutrient mass inflows to the wetlands averaged 5.61 ± 0.30 g-P $m^{-2}\ yr^{-1}$ ($n = 30$ wetland years) for total phosphorus, 2.17 ± 0.27 g-P $m^{-2}\ yr^{-1}$ ($n = 30$ wetland years) for soluble reactive phosphorus, 122 ± 3 g-N $m^{-2}\ yr^{-1}$ ($n = 14$ wetland years) for total nitrogen, and 100 ± 5 g-N $m^{-2}\ yr^{-1}$ ($n = 32$ wetland years) for nitrate–nitrogen. Retention rates were 2.40 ± 0.23 g-P $m^{-2}\ yr^{-1}$ for total phosphorus, 0.87 ± 0.10 g-P $m^{-2}\ yr^{-1}$ for soluble reactive phosphorus, 38.8 ± 2.2 g-N $m^{-2}\ yr^{-1}$ for total nitrogen, and 15.6 ± 2.7 g-N $m^{-2}\ yr^{-1}$ for nitrate–nitrogen. Total phosphorus retention was higher in the planted wetland compared to the natural colonizing wetland ($44.3 \pm 4.4\%$ vs. $38.8 \pm 5.3\%$ respectively; $p = 0.059$) while total nitrogen retention was significantly higher in the naturally colonizing wetland compared to the planted wetland ($32.1 \pm 2.0\%$ vs. $28.4 \pm 2.6\%$ respectively; $p = 0.000085$). Investigation of trends of water quality improvement showed that, overall, nutrient retention decreased from the beginning of the study through the 17th year in 2010. More recent trends showed tendencies for water quality improvement in 9 out of 11 of the nutrient parameters and time periods investigated between 2003 and 2010.

The wetlands were effective carbon sinks, with rates of carbon sequestration (219–267 g-C $m^{-2}\ yr^{-1}$, higher than those measured in a reference natural flow-through wetland. Both carbon sequestration and methane emissions have been consistently higher in the naturally colonizing wetland, theorized as due to the greater productivity of this wetland over several years in the middle of this study.

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http://dx.doi.org/10.1016/j.ecoleng.2014.09.108
0925-8574/

1. Introduction

Wetlands provide a multitude of ecosystem services and continue to be cited as the most valuable parts of our landscape in recent ecosystem service assessments (deGroot et al., 2012; McInnes, 2013; Costanza et al., 2014; Mitsch and Gosselink, 2015). Our research team has been investigating the ecosystem services of inland wetlands for 20 years in a multi-year, whole-ecosystem experiment in two 1-ha created freshwater riverine flow-through marshes at Olentangy River Wetland Research Park at The Ohio State University, Columbus, Ohio, USA. Mitsch et al., 1998, 2005a,b, 2012 described ecological services and function of these wetlands at years 3, 5, 10, and 15, including the succession and ecosystem function of these wetland laboratories, This paper investigates the change in vegetation succession and nutrient mass retention over an even longer period and updates carbon fluxes in the wetlands. These data are crucial for accurate representation of wetland ecosystem services, particularly for those referred to by the Millennium Ecosystem Assessment (2005) as regulating services such as water purification and climate regulation.

Both wetland basins had identical inflows of river water and hydroperiods from 1994 to 2013 but one of of the wetlands was planted with native macrophtyes in 1994 while the other was left as an unplanted control. That set-up essentially tested the self-design capabilities of nature with and without human intervention. These experimental wetlands have allowed simultaneous long-term study of three different questions related to wetland development: (1) how important is wetland plant introduction on ecosystem function? (2) How long does it take hydric soils and other wetland features to develop at a site where no hydric soils

Fig. 1. Olentangy River Wetland Research Park at the Ohio State University. The two experimental wetlands in the center of the site map are the subject of this paper.

previously existed? (3) What are the long-term patterns of biogeochemical changes of flow-through wetlands as they develop from open ponds of water to vegetated, hydric-soil marshes?

2. Methods

2.1. Experimental design

Two 1-ha experimental wetlands were created on the floodplain of the Olentangy River in 1993 and 1994 at what eventually became the 20-ha Wilma H. Schiermeier Olentangy River Wetland Research Park (Fig. 1), a complex of created and natural freshwater riverine wetlands located on the campus of The Ohio State University in Columbus. The wetlands were used extensively for wetland research from 1991 to 2012 and some of that research is reported here and in the accompanying special issue (see Mitsch et al., 2014a). The two kidney-shaped wetlands have been compared since water was first added on in 1994 (year 1), with research results regarding water quality, plant community structure and function, soil development, sedimentation, and gas exchange (see Mitsch et al., 1998, 2005a,b, 2012 for summaries at various years). These urban wetlands are located at the eastern-most edge of the Central Plains portion of the Eastern Temperate Forest ecological region (biome) of North America and close to the intersection of three level II ecoregions in that biome: Mixed Wood Plains, Central Plains, and Appalachian Forests. The original alluvial soil type at the site belongs to the Ross series—a deep, dark, and well-drained silt loam, silty clay loam, or loam that forms on floodplains soils in the experimental wetland basins were non-hydric before the wetlands were created. Water from the adjacent Olentangy River has been pumped, except for short outages caused by power outages or pump repair since March 4, 1994, according to a formula based on river stage. The Olentangy River is a fourth-order stream in the agriculture-dominated Scioto River Watershed of central Ohio. The Scioto, in turn, is one of the major tributaries of the Ohio River. In 2003, extramural funding from Ohio and Federal agencies was obtained for a pulsing study whereby artificial "floods" were introduced to the wetland basins (Mitsch et al., 2005b; Hernandez and Mitsch, 2006; Altor and Mitsch, 2008); each wetland was administered with the same hydrologic conditions, so the "planting experiment" was not violated. Water depths in the major portions of the wetland are generally 20 to 40 cm in the shallow areas where most of the emergent macrophytes grow and 50 to 80 cm in the deepwater areas that were constructed in the wetland to allow overwintering of fish (for mosquito control) and long-term sediment storage.

2.1.1. Planting

The western basin (Wetland 1) was planted with 13 native species of macrophytes in May 1994 and is therefore labeled the "planted wetland" whereas the eastern basin was allowed to be colonized naturally (Mitsch et al., 1998) and so is referred to here as the "natually colonizing" or "unplanted" wetland. Over 2400 plant propagules (mostly root stock and rhizomes; Table 1) representing 13 species typical of Midwestern USA marshes were planted in one wetland (Wetland 1 = W1) on one Saturday with the help of 200 volunteers in early May 1994. Wetland 2 (W2) remained unplanted. No other plants were introduced to the wetland basins over the 20 years reported here. Extensive colonization of additional plants was allowed to occur in both basins for the entire two decades.

Table 1
Plants introduced to experimental Wetland 1 at Olentangy River Wetland Research Park in 1994 (from Mitsch et al. 1998).

Edges and middle (marsh)
Schoenoplectus tabernaemontani[a]
Scirpus fluviatilis[b]
Deep water
Nelumbo lutea[b]
Nymphaea odorata
Potamogeton pectinatus[b]
Mudflat gradient (currently forested edge)
Acorus calamus[a]
Cephalanthus occidentalis
Juncus effusus[a]
Pontederia cordata
Sagittaria latifolia[b]
Saururus cernuus
Sparganium eurycarpum[b]
Spartina pectinata[a]

[a] Present in planted wetland (W1) in 2012.
[b] Abundant in planted wetland (W1) in 2012.

2.2. Hydrology budgets

Daily water budgets of the two experimental wetlands were estimated for 19 years (1994–2012) per wetland water budget methods described by Mitsch and Gosselink (2007). Wetland inflows were calculated by twice-daily (morning and evening) readings of both instantaneous and total integrated volume of pumping rates from flow monitors for each wetland. When data from only one wetland inflow were available, the missing flow rate was assumed to be the same as the available flow rate. The protocol for the experimental wetlands has been, since the start, to deliver the same flow to each wetland at all times. Wetland outflows were estimated by regression models with twice-daily water level readings located in outflow of the wetlands and calibrated outflow rates. Precipitation and estimated evapotranspiration data were collected from the weather station at The Ohio State University. Seepage rates were calibrated as a function of water level by measuring changes of water level readings when the wetlands had no inflows or outflows. Daily water budgets were translated to annual water budgets presented in this paper for the 19 years.

2.3. Vegetation cover and diversity

The macrophyte-dominant community cover was estimated each year in middle to late August from color aerial photography followed by ground-truth verifications. The maps for each year were normalized to a basin map of a standard size, using ArcView 3.1. A 10-m grid system marked with permanent, numbered white poles facilitated identification of the locations of plant communities in each wetland during ground truthing and aerial photography. We were able to develop vegetation maps for the 19 of the 20 years from 1994 to 2013.

A macrophyte Community Diversity Index (CDI; Mitsch et al., 2005a) was used to quantify vegetation diversity in the wetland basins. The index uses relative areas of macrophyte community cover from the maps derived above and the mathematics of the Shannon–Weaver diversity index, with area of each community instead of number of individuals of each species used. It is expressed as:

$$\mathrm{CDI} = -\sum_{i=1}^{N} S(C_i \ln(C_i))$$

where C_i = percent cover of wetland community "i" (0 to 1) in the wetland basin, N = number of wetland communities.

2.4. Vegetation productivity

Aboveground biomass was measured each August as an estimate of aboveground net primary productivity (ANPP) for 1997–2010 by the direct aboveground harvesting of 16 1-m^2 plots in each wetland along sampling boardwalks. For the 1994–1996 data, ANPP was estimated from fewer plots. The aboveground biomass in each plot was clipped at the soil surface and weighed immediately. Subsamples were taken to the lab and dried to a constant weight to estimate dry:wet ratios.

2.5. Nutrient analsyses and budgets

Weekly water samples were taken at the inflow, the middle, and the outflows of the wetlands for 17 years (1994–2010) for nutrient concentrations (total phosphorus [TP], soluble reactive phosphorus [SRP], and nitrate + nitrite nitrogen [NO_3-N]) as was determined by standard methods (USEPA, 1983; APHA, 1989). Total Kjeldahl measurements and thus total nitrogen were added to the suite of nutrient species measured in 2004 to enable estimates of total nitrogen fluxes. Samples were split into filtered (0.45 μm) and unfiltered samples, frozen until analysis, and analyzed for total phosphorus, soluble reactive phosphorus, and nitrate–nitrogen (USEPA, 1983; APHA, 1989). Both total phosphorus and soluble reactive phosphorus methods employ the ascorbic acid and a molybdate color reagent method with a Lachat QuikChem IV automated system. Total phosphorus samples are first digested by adding 0.5 ml of 5.6 N H_2SO_4 and 0.2 g (NH_4) $2S_2O_8$ to 25 ml of sample and exposing the samples to a heated and pressurized environment for 20 min in an autoclave. Nitrate + nitrite were analyzed on a Lachat QuikChem IV automated system with the cadmium reduction method. Early samples from April 1994 to July 1995 were run by similar methods by the Heidelberg College Water Quality Laboratory using a Traacs 800 autoanalyzer. The accuracy of the nutrient analysis was checked every 10–20 samples with a known standard, and the samples were redone if the accuracy was off by 5%. Water quality concentrations in the inflow, outflow, and middle were multiplied by estimated inflows, surface outflows, and seepage respectively to estimate annual nutrient budgets.

2.6. Carbon and nitrogen accumulation

Soil cores were extracted from the experimental wetlands in May 2004 (127 cores 10 years after wetland creation) and in May 2009 (44 cores 15 years after wetland creation) using a 10-m grid system installed in 1993. Cores were 7–10 cm in diameter, and their length varied depending on the depth of the sediment accumulated over the underlying nonhydric soil (10–35 cm). Extracted cores were divided in the field into increments and packed in sealed containers that were stored at 4 °C until analysis. Soil samples were oven dried to constant weight to determine bulk density, ground to a 2-mm particle size, and homogenized. Total C content of the soil samples for the two periods is described by Anderson and Mitsch (2006) and Bernal and Mitsch (2013a). The C and N accumulation since wetland creation in 1994 was calculated by estimating the total soil pool of the hydric layer (i.e., from the soil surface to the underlying non-wetland soil over which the wetland soil was developed) in each point of the grid. The accumulation rates were determined by dividing the pools by the age of the wetland at the time of sampling (10 or 15 years).

2.7. Data analysis

Water fluxes and water quality data were recorded seasonally on Excel spread sheets for that year and summarized at the end of each year. Inflow rates were compared annually for the two wetlands and found to be statistically similar ($p < 0.05$). Student's paired *t*-tests were used to compare the two experimental wetlands on annual and longer periods for water quality, nutrient removal and primary productivity. Trends of nutrients for wetlands were examined by curve estimates with ANOVA and *t*-tests. All statistical analysis was conducted using SPSS PC version (SPSS Inc.). A 95% significant level ($p < 0.05$) was used for data analysis in most cases although a 90% significance level ($p < 0.10$) was used for some water quality indices.

3. Results

3.1. Hydrology

The water stage of the two experimental wetlands for 19 years (1994–2012) is presented in Fig. 2. Water levels oscillated 30 cm

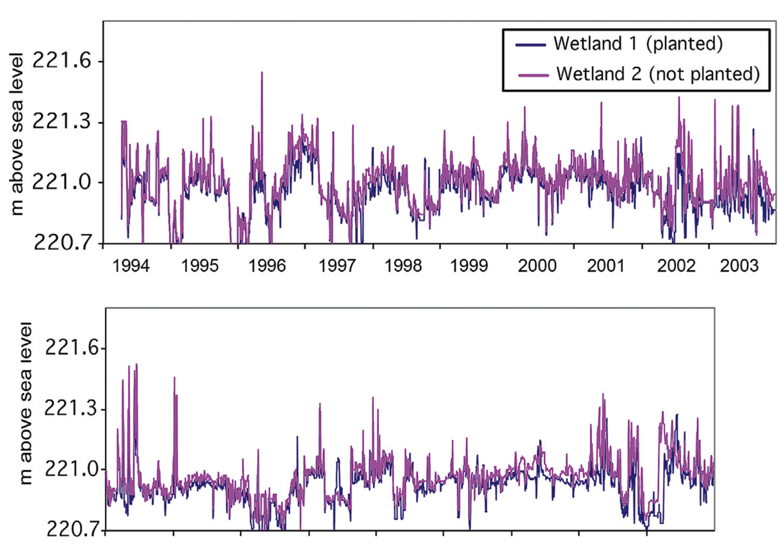

Fig. 2. Water elevations above sea level (hydroperiods) of the experimental wetlands at the Olentangy River Wetland Research Park, central Ohio, for 1994 through 2012.

Table 2
Annual water budget (m/year) for the experimental wetlands at the Olentangy River Wetland Research Park from 1994 to 2012.

Year	Inflow	Surface outflow	Seepage	Precipitation	Evapotranspiration
1994	41.5 ± 0.3	33.3 ± 0.5	12.2 ± 0.1	1.0	0.9
1995	37.9 ± 0.3	37.2 ± 1.9	14.0 ± 0.0	1.4	0.8
1996	21.6 ± 0.2	16.2 ± 0.1	13.7 ± 0.1	1.7	0.9
1997	34.5 ± 0.0	25.5 ± 1.7	13.5 ± 0.1	1.1	0.9
1998	36.8 ± 0.1	32.6 ± 2.5	13.3 ± 0.1	0.7	0.8
1999	48.0 ± 0.3	32.7 ± 0.2	15.3 ± 0.0	0.5	0.9
2000	32.1 ± 0.1	29.6 ± 0.1	15.7 ± 0.0	1.1	0.9
2001	37.6 ± 0.5	27.1 ± 0.1	15.6 ± 0.2	1.1	0.8
2002	25.4 ± 0.1	22.1 ± 1.4	13.4 ± 0.2	1.4	0.8
2003	24.3 ± 1.3	19.6 ± 1.5	13.5 ± 0.8	1.3	0.8
2004	43.4 ± 0.0	21.6 ± 1.8	11.9 ± 0.1	1.0	0.9
2005	36.0 ± 0.0	13.2 ± 1.8	13.5 ± 0.4	1.1	0.9
2006	33.3 ± 0.6	13.1 ± 0.5	11.1 ± 0.3	1.0	0.8
2007	56.3 ± 0.0	33.9 ± 0.1	13.5 ± 0.0	1.4	0.8
2008	45.4 ± 0.5	22.3 ± 1.7	13.6 ± 0.2	0.9	0.8
2009	41.9 ± 0.1	25.9 ± 1.3	14.8 ± 0.2	0.7	0.8
2010	37.6 ± 1.6	33.3 ± 5.4	15.7 ± 0.1	0.8	0.8
2011	43.2 ± 0.6	33.6 ± 5.3	14.5 ± 0.2	1.3	0.8
2012	58.2 ± 0.0	41.7 ± 7.0	14.5 ± 0.3	0.8	0.9
	38.7 ± 1.5	27.1 ± 1.4	13.2 ± 0.2	1.1 ± 0.1	0.9 ± 0.0

above and below the norm routinely for the 19 years, following the general hydrology of the watershed because of our pumping protocol. Pulses indicate river pulses and low periods indicated base flow river conditions. The overall annual water budgets for the wetlands for 1994–2012 are illustrated in Table 2. Annual inflow, through these 19 years, was $38.7 \pm 1.5\,\mathrm{m\,yr^{-1}}$ (to estimate volume to each wetland, multiply by $10{,}000\,\mathrm{m^2}$, the size of each wetland basin). Of the total inflow including precipitation approximately 70% ($27.1 \pm 1.4\,\mathrm{m\,yr^{-1}}$) of the water flowed out at the surface outflow weir at the south end of the wetland and an estimated 34% ($13.2 \pm 0.2\,\mathrm{m\,yr^{-1}}$) infiltrated to the groundwater from this perched wetland. All of these water fluxes were estimated independently for the 19 years, so not unexpectedly, there was an unbalance error of 8% on the high side, i.e., outflows were higher by 8% over estimated inflows). We believe that the error is in the lack of accuracy in both inflow and outflow measurements. The surface outflow and groundwater seepage may be a few percent higher than actual rates due to inaccuracy of weir equations at high flows; the pumped inflows were probably slightly under-estimated, as short-term pulses were not always caught in the two-per-day manual measurements.

3.2. Vegetation richness, succession and community diversity

Of the 13 species originally introduced to the planted wetland in 1994, nine were still present in that wetland in 2010, the 17th growing season after planting (Table 1). The number of species increased rapidly in each wetland basin with 87–96 species in the unplanted and planted wetlands respectively 5 years after planting (Table 3). The species count increased by 10 in the unplanted or "naturally colonizing" wetland over the next decade to 97 and by 5 in the planted wetland over the next decade to 101 (Table 3). Based on measurements two years later (2010) richness appears to have leveled off. Only two of the original 13 species planted in the planted wetland (W1) were found in the naturally colonizing wetland basin (W2) throughout the study.

Vegetation community maps of the wetlands over 20 years (1994–2013; Fig. 3) illustrate 12 different vegetation communities, including open water and algal mats. Communities were generally named for the dominant taxa in the community. Patterns of vegetation diversity in the two wetland basins over that period, estimated by our community diversity index (CDI), shows an interesting long-term effect of planting. Except for one year (2009 = year 16) during that period, the planted wetland (W1) had consistently higher spatial diversity of plant communities than the naturally colonizing wetland (W2). Community diversity (CDI) appears to be decreasing over the last 2 years of this study in both wetlands (Fig. 4), caused by a continued increase in coverage by the clonal dominate *Typha* spp. (see Figs. 3 and 5). *Typha* spp., not among the plants introduced to the planted wetland, has shown an interesting contrasting pattern in the two wetlands since 1994 (Fig. 5). In 1999, five years after Wetland 1 was planted, *Typha* coverage peaked at 56% in the naturally colonizing Wetland 2 while there was only a peak of only 9% *Typha* coverage in the planted wetland. The *Typha* peak was followed by muskrat eatouts in both of the wetland basins and most of the emergent vegetation was gone in 2001 (see Fig. 3; also Mitsch et al., 2005a). *Typha* coverage was reduced to 5 to 10% coverage in both wetlands. Eleven years after the first peak, *Typha* coverage again reached 55% coverage in the naturally colonizing wetland. During this period, *Typha* coverage in the planted wetland appears to parallel the increase in the naturally colonizing wetland and reached a peak of 45% *Typha* coverage in 2009.

3.3. Vegetation productivity

Aboveground net primary productivity (ANPP) of macrophytes in the two expermental wetland basins for 1994–2010 (17 years) was significantly different in paired *t*-test comparisons in 7 of the 17 years analyzed, with ANPP in the naturally colonizing wetland higher than than ANPP in the planted wetland 5 of the 7 times (Fig. 6a). After 17 years (1994–2010), there was still an estimated 2000 kg/ha more aboveground plant accumulation in the naturally colonizing wetland than in the planted wetland (Fig. 6b). Most of the increase in accumulation in plant biomass occurred in the 1998–2001 period when *Typha* dominance was dramatically higher in the naturally colonizing wetland (see also Figs. 3 and 5).

3.4. Annual nutrient budgets and nutrient retention

Nutrient inflows and subsurface and surface outflows for the experimental wetlands for various periods over the two decades

Table 3
Vegetation species richness in the two 1-ha experimental wetlands at the Olentangy River Wetland Research Park from 1996 to 2010. Both wetlands were created in 1994; the "planted wetland" (W1) was planted with 2500 individual plants representing 13 native wetland species in May 1994 while W2 remained an unplanted control (data from Mitsch and Gosselink, 2015).

Wetland age (# of growing seasons completed)	Year	Number of species			Number of wetland species			Number of planted species		Number of woody species		Number of invasive species	
		W1	W2	Total	W1	W2	Total	W1	W2	W1	W2	W1	W2
3	1996	67	56	72	43	31	44	9	1	5	7	1	1
5	1998	96	87	99	56	46	57	9	2	15	15	4	4
15	2008	101	97	116	55	52	61	9	2	18	21	7	9
17	2010	99	97	118	51	49	63	9	2	18	21	7	10

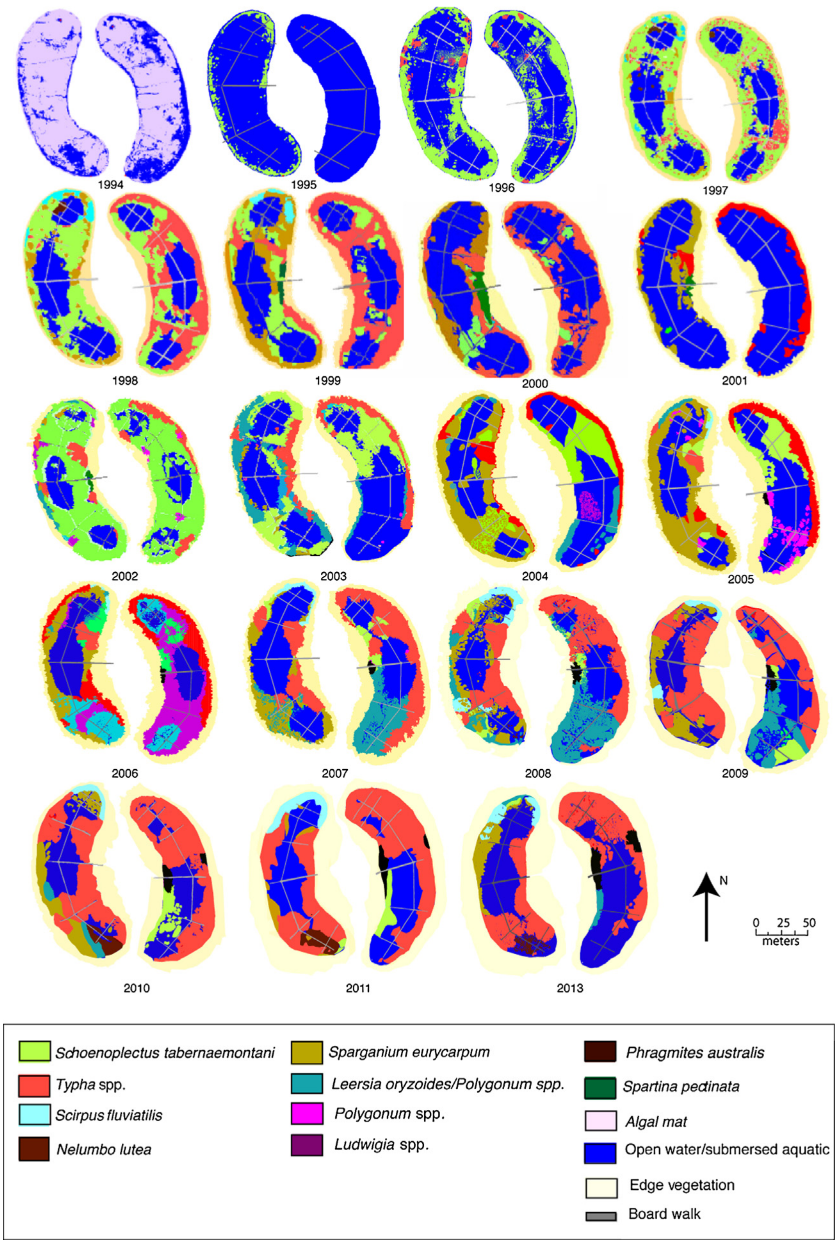

Fig. 3. Vegetation community maps for two experimental wetlands at the Olentangy River Wetland Research Park, 1994–2013. Vegetation communities are defined in the map legend.

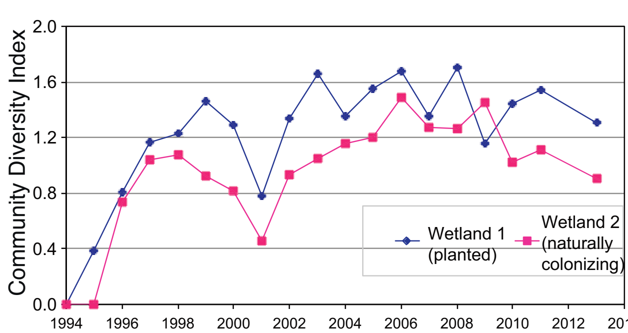

Fig. 4. Community diversity index (CDI) of vegetation in the planted and naturally colonizing experimental wetlands, 1994–2013.

are presented in Fig. 7 for (a) total phosphorus, (b) total nitrogen, (c) soluble reactive phosphorus, and (d) nitrate–nitrogen. Total annual phosphorus retention (Fig. 7a) by the wetland basins for 30 wetland years (=15 years of measurements at 2 wetlands) was 2.40 ± 0.23 g-P m^{-2} yr^{-1}, averaging 42.7% retention of phosphorus by mass over the entire period of nutrient analysis. Soluble reactive phosphorus inflow (Fig. 7c) represented less than half of the total phosphorus inflow to the wetland basins. Overall retention of SRP for 30 wetland years was 0.87 ± 0.10 g-P m^{-2} yr^{-1}, an average of 40.3% of the surface inflow and about 36% of the total phosphorus retention.

Total nitrogen retention (Fig. 7b) by the wetland basins for 14 wetland-years (TKN analysis was only started in 2004) was 38.8 ± 2.2 g-N m^{-2} yr^{-1}, an average of 31.9% retention by mass. Nitrate nitrogen retention (Fig. 7d) was 15.6 ± 2.7 g-N m^{-2} yr^{-1} for 32 wetland years, or an average of 15.5% of the inflow nitrate

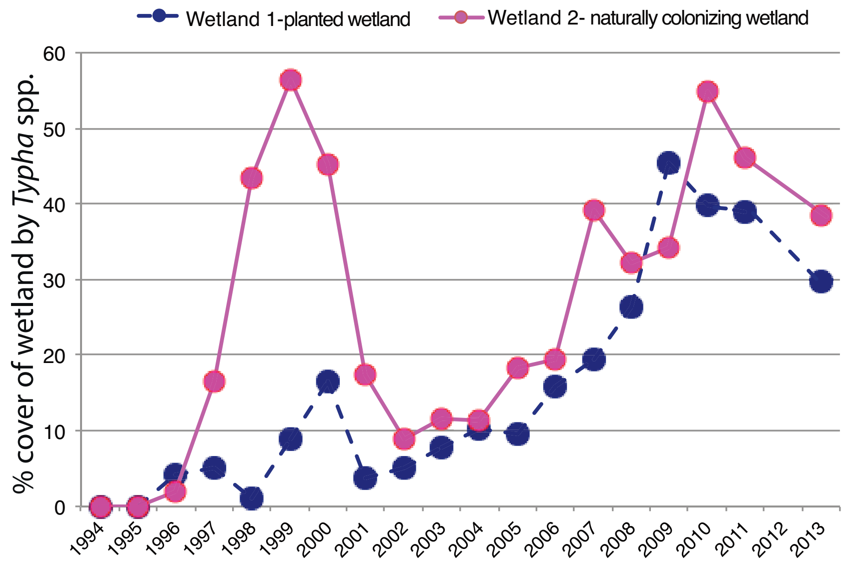

Fig. 5. Pattern of *Typha* coverage in the planted and naturally colonizing experimental wetlands, 1994–2013.

nitrogen. For the 14 wetland-years used for the total nitrogen budget in the last 7 years of measurements (14 wetland years), nitrate nitrogen retention was 69% higher at 26.4 ± 2.2 g-N m^{-2} yr^{-1}. This nitrate retention represented 68% of the total nitrogen retention for those 14 wetland years.

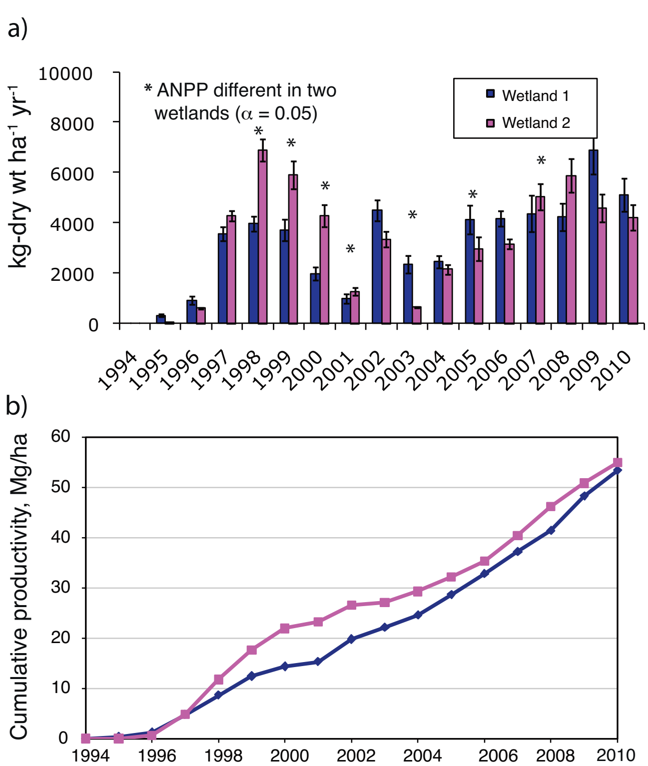

Fig. 6. Productivity of macrophytes in the planted and naturally colonizing experimental wetlands, 1994 through 2010 as illustrated by (a) aboveground net primary productivity of emergent macrophytes; and (b) accumulated macrophyte productivity over the period 1994–2010.

3.5. Comparison of nutrient retention in planted and unplanted basins

When the annual retention of total phosphorus was compared between the two basins for 30 wetland years, it was significantly higher ($p < 0.10$) in the planted wetland than in the natural colonizing wetland ($44.3 \pm 4.4\%$ vs. $38.8 \pm 5.3\%$; $p = 0.059$). When the annual retention of total nitrogen was compared between the two basins for 14 wetland years, it was significantly higher ($p < 0.05$) in the naturally colonizing wetland than in the planted wetland ($32.1 \pm 2.0\%$ vs. $28.4 \pm 2.6\%$ respectively; $p = 0.000085$). Neither soluble reactive phosphorus nor nitrate–nitrogen retention was significantly different between the two wetland basins.

3.6. Trends in nutrient removal

Trends of nutrient retention over different periods were investigated, including the longest period 1994–2010, by comparing the percent change in nutrient concentrations from the inflow to the surface outflow (Table 4; Fig. 8). Strong significant positive slopes of nutrient reduction (i.e., wetland effectiveness in removing nutrients is decreasing over time) were seen for total phosphorus, soluble reactive phosphorus, and nitrate-nitrogen for the entire period of 1994–2010. When the last 6 years (2005–2010) and 5 years (2006–2010) were examined, the slopes of all three of these parameters reversed and were negative (i.e., the wetland effectiveness in removing nutrients was increasing over time). However the trends for these 3 nutrient measurements were not strong enough to be significant except for total phosphorus in 2006–2010 (Table 4).

3.7. Carbon and nutrient accumulation in the soils

Sedimentation rates have been investigated by a number of researchers over the years in the experimental wetlands and are summarized in a companion paper by Mitsch et al. (2014b). Two of those studies during these two decades were undertaken specifically to estimate the net accumulation of carbon and nitrogen in the soils of these wetlands (Table 5). Carbon sequestration for the first 10 years of the experimental wetlands ranged from 181 to 193 g-C m^{-2} yr^{-1}. When re-estimated from soil cores taken 5 years later, the carbon sequestration increased to 219–267g-C m^{-2} yr^{-1}. Nitrogen

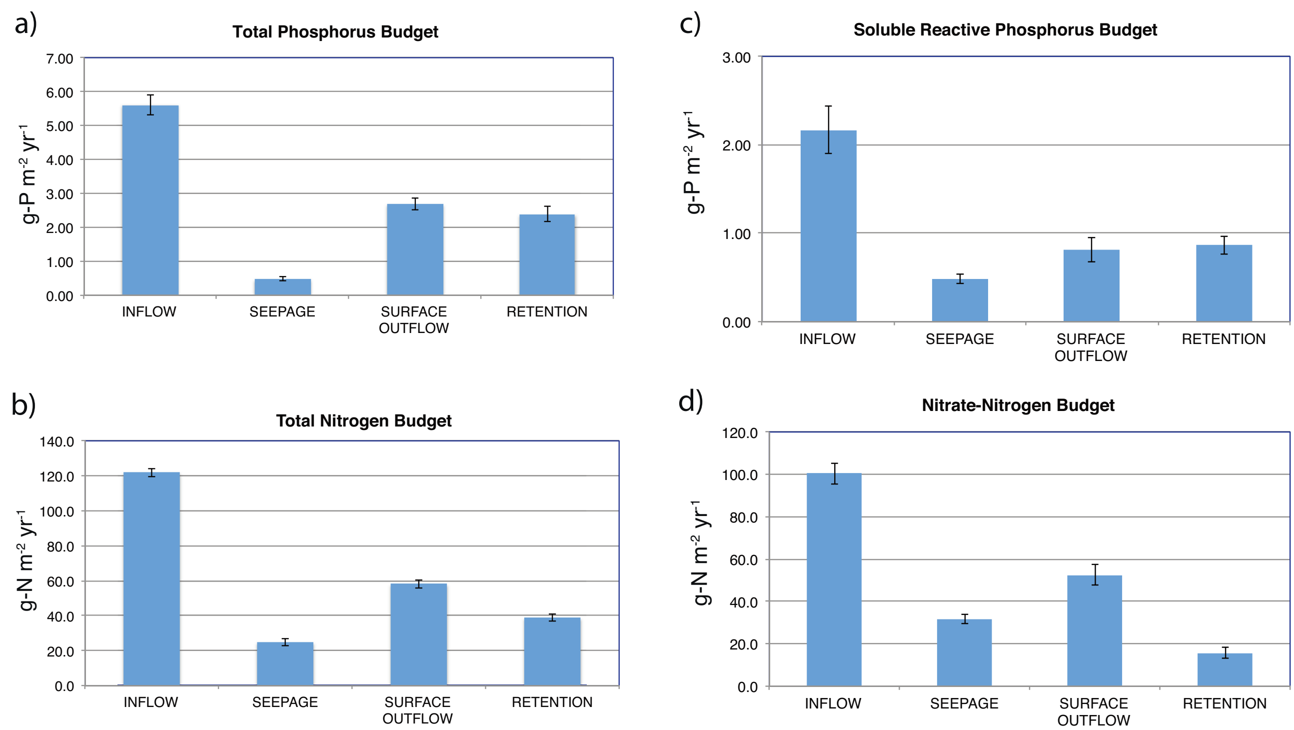

Fig. 7. Nutrient budgets for both experimental wetlands, 1994 through 2010; (a) total phosphorus; (b) total nitrogen; (c) soluble reactive phosphorus; and (d) nitrate–nitrogen. Fluxes are shown for inflows, vertical seepage to groundwater, surface outflow through weir, and net retention = inflow – seepage – surface outflow.

accumulation rates were estimated with the same 10 year and 15 year cores. As with carbon, rates of accumulation increased with time, with an estimated rate of 16 and 17 g-N m^{-2} yr^{-1} for the first 10 years increasing from 18 to 21 g-N m^{-2} yr^{-1} for the first 15 years. These nitrogen accumulate rates range from 41 to 54% of the total nitrogen retention estimated in the nutrient budgets above.

Table 4
Trends for annual % change of nutrients from inflow to surface outflow for 1994–2010, 2003–2010, 2004–2010 and 2006–2010 at the two 1-ha experiment wetlands at the Olentangy River Wetland Research Park. A positive slope means that nutrient change from inflow to outflow is becoming more positive, i.e., the wetland is retaining fewer nutrients from year to year. A negative slope means that nutrient change is becoming more negative from year to year, i.e., the wetlands are retaining more nutrients from year to year.

Period	Parameters	Slope, % change per year	*n*	*F*	*p*
1994–2010	Total P	2.536	32	19.552	0.000**
	SRP	2.814	30	41.666	0.000**
	$NO_3 + NO_2$	0.790	30	8.884	0.006**
2003–2010	Total P	−3.250	16	6.730	0.021**
	$NO_3 + NO_2$	1.506	16	6.956	0.020**
2004–2010	Total P	−0.982	14	0.673	0.428
	SRP	−0.446	14	0.084	0.777
	$NO_3 + NO_2$	0.125	14	0.179	0.679
2005–2010	Total P	−0.943	12	0.349	0.568
	SRP	−2.929	12	2.947	0.117
	$NO_3 + NO_2$	−0.414	12	1.943	0.193
2006–2010	Total P	−3.850	10	4.790	0.060*
	SRP	−0.900	10	0.158	0.701
	$NO_3 + NO_2$	−0.450	10	1.123	0.320

n = number of annual data points; *F* = *F* ratio from ANOVA; *p* = probability at significant confidence levels.
* $p < 0.05$.
** $p < 0.10$.

4. Discussion

4.1. Succession of created wetlands in high-nutrient landscapes

The pattern seen in this two-decade study suggests, in the long run, that the vegetation cover for wetlands created in the U.S. Midwest, unless created in unusual settings of low nutrients, will ultimately be dominated by *Typha* spp. if they remain wet enough to keep out woody vegetation. The Midwestern USA is dominated by highly industrialized agriculture that has led to major increases in nutrients, especially nitrogen and phosphorus in the landscape and aquatic ecosystems for decades or more. This high nutrient status has led to what Odum (1987) called the "Typhazation of America" where the expected plant community in freshwater marshes throughout much of eastern North America is now *Typha*. Keddy (2010) agrees with this concept and put *Typha* as the core plant species in his centrifugal organization model with various disturbances (ice scour, beaver dams, etc.) as the main cause for other plant communities to develop in freshwater marshes. Fig. 9 illustrates our interpretation of his centrifugal model specifically for these experimental wetlands and for freshwater marshes in the Midwestern USA in general. *Typha* is now and will be for the foreseeable future the core habitat or community. In the experimental wetlands in our study, *Typha* dominated the naturally colonizing wetland (unplanted basin) by the sixth year of the study (1999) though not initially in the planted wetland. This domination led to a divergence between the two basins in wetland structure and function that we reported on earlier (Mitsch et al., 2005a). We also suggested at that time that perhaps planting would have a positive effect on curtailing the "invasion" of *Typha* spp. in created and restored wetlands (Mitsch et al., 2005a,b,). *Typha* was held at bay by several planted species, most notably *Sparganium eurycarpum* (bur reed) in the planted wetland. A muskrat "eatout" disturbance in 2000 caused both wetlands to reset with low *Typha* domination as would be predicted by the

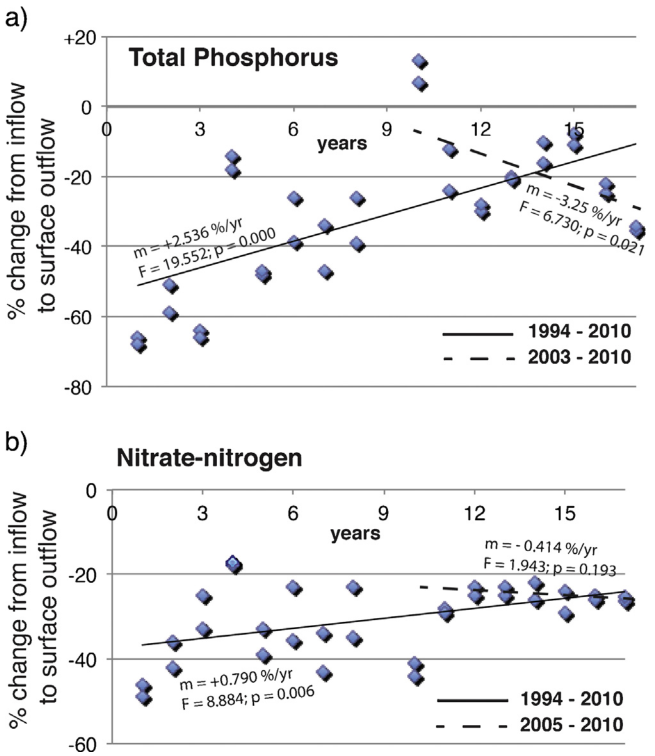

Fig. 8. Trends of change in water quality (percent change from inflow to outflow) for (a) total phosphorus; and (b) nitrate–nitrogen. Trends are shown for 1994–2010 for both nutrients, 2003–2010 for total phosphorus, and 1994–2010 and 2005–2010 for nitrate nitrogen. Slopes (m) and significance of lines (F = ANOVA ratio; p = probability at 0.05 significance level). Statistics for several other time periods and parameters are given in Table 4.

Keddy (2010) model. But in the second cycle of 2000–2012, both wetlands increased in *Typha* coverage. The planted vegetation provided competition for *Typha* in the first few years after the wetlands were created but in the second pulse 15 or so years after the wetlands were created, there was little competition for *Typha* left from original plantings.

In general, the planted wetland maintained a higher spatial macrophyte diversity throughout most of the 20 years and the naturally colonizing wetland appeared to be more susceptible to disturbances such as muskrat herbivory and hydrologic pulses than did the more diverse planted wetland. Our model in Fig. 9 suggests that since *Typha* is now established in both wetlands, it will diverge from that community only through the intervention of organisms (muskrats, beavers, or geese as primary examples) or human action (changing the inflow of water or altering the regional groundwater level through dam removal). In fact a large dam was removed from the Olentangy River 3 km downstream from these wetlands in September 2012 (Zhang et al., 2014) and it is not clear if that dam removal has had any effect on these experimental wetlands in terms of the groundwater elevation.

The continual introduction of species, whether introduced through flooding and other abiotic and biotic pathways, appeared to have a much longer-lasting effect in development of these ecosystems than the few species of plants that were introduced to one of the wetlands in the beginning. Studies described above suggest that planting did have a short-term (16 years or less) effect on maintaining plant diversity and perhaps a longer-term effect on functions such as nutrient retention, methane emissions (see Nahlik and Mitsch, 2010), and carbon accumulation in the soil (see also Bernal and Mitsch, 2013a). But the effect of planting seems to be disappearing after the two decades as the plant diversity is now decreasing and plant cover appears to be going towards a *Typha* monoculture. We think that a research project such as ours needs to continue for decades more to see the true picture of the importance of planting. Our original hypothesis (Mitsch et al., 1998) which stated that the two wetlands will diverge in function in the middle years and then converge in structure and function remains our hypothesis. We also now admit that we do not know what the middle years are, except that now they could be decadal in extent.

Table 5
Carbon, nitrogen, and phosphorus sequestration in soils of the experimental wetlands at the Olentangy River Wetland Research Park since creation of the wetlands in 1994.

	Annual accumulation rate ($g\,m^{-2}\,yr^{-1}$)		Reference
	Planted wetland	Unplanted wetland	
Carbon accumulation			
1994–2004	181 ± 13	193 ± 12	Anderson and Mitsch, 2006
1994–2010	219 ± 15	267 ± 17	Bernal and Mitsch, 2013
Nitrogen accumulation			
1994–2004	16.2 ± 1.2	16.6 ± 1.0	Anderson and Mitsch, 2006
1994–2009	18.0 ± 1.5	21.0 ± 1.4	This study
Phosphorus accumulation			
1994–2004	3.26 ± 0.25	3.49 ± 0.25	Anderson and Mitsch, 2006

4.2. Nutrient retention rates in wetlands

Rules of thumb for sustainable retention of nutrients by wetlands published 15 years ago (Mitsch et al., 2000) suggested that wetlands could consistently retain phosphorus in amounts of 0.5–5 g-P $m^{-2}\,yr^{-1}$ and nitrogen in amounts of about 10–40 g-N $m^{-2}\,yr^{-1}$. Nothing in this study suggests that those recommendations should change. To maintain biological diversity in the plant community, the lower end of these loading rates have been recommended as reasonable goals (Mitsch and Gosselink, 2015). In this study, the long-term retention of phosphorus for 30 wetland years of data was 2.40 ± 0.23 g-P $m^{-2}\,yr^{-1}$, almost exactly in the center of the Mitsch et al. (2000) range. Total nitrogen retention was 38.8 ± 2.2 g-N $m^{-2}\,yr^{-1}$ for 14 wetland years, a rate close to the high end of the range proposed by Mitsch et al. (2000) of 10–40 g-N $m^{-2}\,yr^{-1}$. An estimated 68% of the total nitrogen retention was nitrate–nitrogen retention.

These retention rates are comparable to those published in the literature for both temperate and tropical/subtropical wetlands (Table 6). For example, Mitsch et al. (in press) describe the average retention of phosphorus in the stormwater treatment areas (created and restored wetlands) north of the Florida Everglades, with the oldest records showing an average retention rate of 1.25 g-P $m^{-2}\,yr^{-1}$. Nutrient retention on an annualized basis was 1.76 g-P $m^{-2}\,yr^{-1}$ for phosphorus and 4.39 g-N $m^{-2}\,yr^{-1}$ for dissolved inorganic nitrogen (dissolved nitrate and ammonium nitrogen) in Houghton Lake where treated wastewater has been applied to peatlands for 30 years (Kadlec, 2009).

4.3. Nutrient retention trends

Water quality trends, when evaluated with simple linear trends over long periods, show decreasing nutrient retention that we have

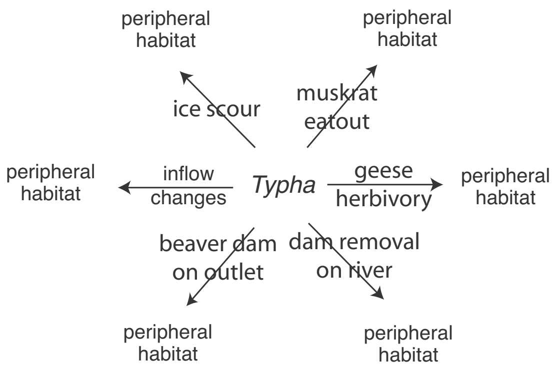

Fig. 9. Centrifugal vegetation model from Keddy (2010) but illustrating disturbances specific to the experimental wetlands at the Olentangy River Wetland Research Park.

described previously (Mitsch et al., 2012). In that paper we attributed the decreasing water quality improvement due to the saturation of nutrients in soils, detritus, and plants and also to more sediments being discharged in the outflow as the wetlands slowly become shallower. There were clearly high rates of nutrient retention due to dense algal communities that dominated the wetland basins in the first year of two after they were created (Mitsch et al., 1998; Wu and Mitsch, 1998). In this study, we had two more years of data than Mitsch et al. (2012) but we also took a closer look at more recent trends over the most recent 5–8 years. We saw patterns of improving water quality for total phosphorus and nitrate–nitrogen. The concept of treatment wetlands “aging” and beginning to export more nutrients with time is probably not yet the case in these wetlands. We expected the nitrate-nitrogen retention to begin improving as denitrification increases with increased organic carbon substrate. For the last 6 years of our study of nutrients in these wetlands (2005–2010), nitrate nitrogen retention reductions were steady to slowly improving in between 20 and 30% reductions in concentration. What is clear is that the water quality did not continue to degrade in the last 5 or so years of this 17-year water quality study. The slopes of the lines for nitrogen and phosphorus showed water quality improvement in 9 out of 11 of the parameters and periods investigated between 2003 and 2010.

There are several possible reasons as to why nutrient retention began to improve in these wetlands 10–12 years after they were created, i.e., the change in nutrient concentrations from inflow to surface outflows started to become more negative with each year. The first and perhaps most obvious reason is that these wetlands were created on former agricultural soils (the site was an experimental farm for the university for many years) and it simply took a decade for the nutrients accumulated in the soils to complete their efflux back to the water column. In a three-year mesocosm experiment in the Florida Everglades Mitsch et al. (in press) estimated that the phosphorus efflux from the soil to the water averaged 3.2 g-P m^{-2} yr^{-1} over 3 years, a flux much higher than the inflow of phosphorus in the surface water. So the improvement after 10–12 years in water quality could be due to the slowing down of this soil efflux.

It was also expected that the nitrate-nitrogen retention might start to improve in a newly created wetland as organic matter accumulates as the necessary fuel for denitrification. Song et al. (2014) however, did not see any obvious trends of increasing denitrification rates over the period 2004–2009. If anything, the data summarized in that paper show a decrease in denitrification over that 5-year period.

4.4. Comparison of water quality budget retentions with soil accumulations

Our studies over the 20 years estimated nitrogen and phosphorus in two independent ways. The first way was through

Table 6
Nutrient retention in created and natural wetlands receiving low-concentration inflows of nutrients from river overflows, agricultural runoff, and secondarily treated wastewater (from Mitsch and Gosselink, 2015)

Wetland location and type	Wetland size (ha)	Nitrogen (g-N m^{-2} yr^{-1})	Phosphorus (g-P m^{-2} yr^{-1})	Reference
Olentangy River Wetlands experimental wetlands	2	38.8 ± 2.2	2.40 ± 0.23	This study
General guidelines		10–40	0.5–5	Mitsch et al., 2000
Warm climate				
Everglades marsh, S. Florida	8000	–	0.4–0.6[a]	Richardson and Craft, 1993; Richardson et al., 1997
Florida Everglades stormwater treatment areas (STAs)	23,000	–	1.25	Mitsch et al. (in press)
Boney Marsh, S. Florida	49	4.9	0.36	Moustafa et al., 1996
Everglades Nutrient Removal Project, S. Florida	1545	10.8	0.94	Moustafa, 1999
Restored marshes, Mediterranean delta, Spain	3.5	69	–	Comin et al., 1997
Constructed rural wetland, Victoria, Australia	0.045	23	2.8	Raisin et al., 1997
Lake Apopka marsh flow-way, central Florida	308	30 ± 4	0.48 ± 0.59	Dunne et al., 2012, 2013
Breton Sound Estuary, Louisiana Delta	110,000	3.5	–	Lundberg et al., 2014
Cold climate				
Houghton Lake, Michigan (30 years)	100	4.39[b]	1.76	Kadlec, 2009
Constructed wetlands, NE Illinois				Phipps and Crumpton, 1994; Mitsch et al., 1995
River-fed and high-flow	2	11–38[b]	1.4–2.9	
River-fed and low-flow	2–3	3–13[b]	0.4–1.7	
Artificially flooded meadows, southern Sweden	180	43–46	–	Leonardson et al., 1994
Palustrine freshwater wetlands, NW Washington				Reinelt and Horner, 1995
Urban area	2	–	0.44	
Rural area	15	–	3.0	
Created in-stream wetland, OH	6	–	2.9	Niswander and Mitsch, 1995
Created river diversion wetland, OH	3	32	4.5	Fink and Mitsch, 2007; Mitsch et al., 2008
Agricultural wetlands, OH	1.2	39[b]	6.2	Fink and Mitsch, 2004
Agricultural wetlands, IL (3)	0.3–0.8	33[b]	0.1	Kovacic et al., 2000
Natural marsh, Alberta, Canada	360	–	[1]0.43	White et al., 2000

[a] Estimated by phosphorus accumulation in sediment.
[b] Nitrate–nitrogen or inorganic nitrogen only.

a detailed hydrologic and water quality budget process described above. In that process, we estimated that the wetland was retaining 2.40 ± 0.23 g-P m^{-2} yr^{-1} of phosphorus and 38.8 ± 2.2 g-N m^{-2} yr^{-1} of nitrogen. The second way was through an estimation of the accumulation of these nutrients in the soil of the wetlands. With this procedure, we estimated soil phosphorus retention of 3.3–3.5 g-P m^{-2} yr^{-1} and soil nitrogen retention of 16.4–19.5 g-N m^{-2} yr^{-1}. The phosphorus numbers match up fairly well, given all of the uncertainties of estimating groundwater seepage, which is based on models developed when there was no inflow or outflow, and the uncertainty of suggesting that all of the SRP in the surface water seeps into the ground water. It is therefore likely that the 3.3–3.5 g-P m^{-2} yr^{-1} rate of phosphorus retention could be a realistic estimate of phosphorus retention in these wetlands. For nitrogen, it is not surprising that the rate of nitrogen loss through the wetlands with a water quality/hydrology budget is considerably higher than the estimate provided by soil accumulation, as denitrification and anaerobic ammonium reduction (ANAMMOX) are not taken into account in the soil nitrogen accumulation. Several papers have quantified denitrification rates in these wetlands (Hernandez and Mitsch, 2007; Song et al., 2010, 2012, 2014; Batson et al., 2012) while ANAMMOX genes found in experimental wetland soils were described by Ligi et al. (in press) as potentially important indicators of an alternative pathway to the production of N_2 gas from these wetlands in addition to denitrification. They also found the potential for the reduction of nitrous oxide to N_2 was significantly higher than previously estimated. Batson et al. (2012) developed a nitrogen budget for the experimental wetlands for one year—2009—and estimated that denitrification during that year was only 2.7 g-N m^{-2} yr^{-1}, hardly enough to make up the difference between 19 and 39 g-N m^{-2} yr^{-1}. Batson et al. (2012) also commented on 23 g-N m^{-2} yr^{-1} of nitrogen inflow to the wetlands that remained unaccounted for in their budget. While errors in the various inflows and outflows are possible, it was speculated that the acetylene inhibition method used by all of the above researchers to estimate denitrification may be the culprit. That procedure is known to underestimate denitrification, particularly for the relatively low concentrations of nitrate-nitrogen seen in these wetlands in the summer and fall. Song et al. (2014) summarized denitrification rates from 2004 to 2009 and found that the rates ranged from 1.7– 5.0 g-N m^{-2} yr^{-1} but averaged 3.2 g-N m^{-2} yr^{-1} over those years, an estimate only slightly higher than the Batson et al. (2012) estimate. A serious investigation of where the missing nitrogen is going might shed light both on ecosystem processes and also on field and laboratory techniques.

4.5. Created wetlands as carbon sinks

The wetlands in this study are effective carbon sinks, with rates of carbon sequestration higher than those measured in reference natural wetlands (Table 7). We can conclude that these wetlands

Table 7
Comparison of carbon sequestration in the Olentangy River wetlands to a reference site and other temperate, boreal, and tropical created and natural freshwater wetlands (from Mitsch et al., 2013 and Mitsch and Gosselink, 2015)

Wetland type	Carbon sequestration (g-C m^{-2} yr^{-1})	Reference
This study (created and reference temperate flow-through wetlands)		
After 10 years	181–193	Anderson and Mitsch (2006)
After 15 years	219–267	Bernal and Mitsch (2013a)
Reference flow-through wetland, northern Ohio	140 ± 16 ($n = 3$)	Bernal and Mitsch (2012)
Temperate freshwater wetlands		
Temperate wetlands	278 ± 42 ($n = 7$)	Mitsch et al. (2013)
Depressional wetlands, Ohio	317 ± 93 ($n = 3$)	Bernal and Mitsch (2012)
Reed (*Phragmites*) marsh, Denmark	504	Brix et al. (2001)
Created and restored wetlands		
Prairie pothole wetlands, North America		Euliss et al. (2006)
Restored (semi-permanently flooded)	305	
Reference wetlands	83	
Restored peat meadow, Netherlands	280	Hendriks et al. (2007)
Northern peatlands		
Boreal peatlands	29 ± 13 ($n = 8$)	Mitsch et al. (2013)
Boreal peatlands	15–26	Turunen et al. (2002)
Temperate peatlands	10–46	Turunen et al. (2002)
Russian tundra peatlands	−8–38	Heikkinen et al. (2002)
Tropical/subtropical freshwater wetlands		
Tropical/subtropical wetlands	194 ± 56 ($n = 6$)	Mitsch et al. (2013)
Florida Everglades, general	86–387	Reddy et al. (1993)
Tropical freshwater wetland, Indonesia	56 (for 24,000 year core)	Page et al. (2004)
Tropical freshwater wetland, Indonesia	94 (for last 500 year core)	Page et al. (2004)
Cyperus wetland in Uganda	480	Saunders et al. (2007)
Cypress (*Taxodium*) swamp, Florida	122	Craft et al. (2008)
Cypress (*Taxodium*) swamp, Georgia	36	Craft et al. (2008)
Everglades (*Cladium*) marsh, Florida	19–46	Craft et al. (2008)
Tropical flow-through swamp, Costa Rica	222–465 (ave = 306 for 3 sites)	Bernal and Mitsch (2013b)
Tropical forest basin wetland, Costa Rica	61–131 (ave = 84 for 3 sites)	Bernal and Mitsch (2013b)
Seasonally dry tropical floodplain wetland, Cost Rica	80–89 (ave = 84 for 3 sites)	Bernal and Mitsch (2013b)
Seasonally flooded tropical floodplain wetland, Botswana	33–53 (ave = 42 for 3 sites)	Bernal and Mitsch (2013b)
Florida Everglades-cypress strand/swamp	98	Villa and Mitsch (in press)
Florida Everglades-pond cypress	64	Villa and Mitsch (in press)
Florida Everglades-wet prairie	39	Villa and Mitsch (in press)
Florida Everglades-upland pine flatwood	22	Villa and Mitsch (in press)

sequester in the range of 180–270 g-C m^{-2} yr^{-1}. The average rate from the two independent studies is 215 g-C m^{-2} yr^{-1}, 53% higher than a natural flow-through wetland in Ohio that we used as a reference (Table 7). Other studies of restored wetlands have also shown high carbon sequestration rates in the range of 280–305 g-C m^{-2} yr^{-1} (Euliss et al., 2006; Hendriks et al., 2007). In all of these cases including the Olentangy River wetlands, carbon sequestration rates are almost an order of magnitude higher than the much more studied boreal peatlands that have carbon sequestration rates reported to be 10–46 g-C m^{-2} yr^{-1} (Turunen et al., 2002) and 29 ± 13 (Mitsch et al., 2013). The data are clear that created and natural temperate wetlands sequester considerably more carbon than boreal peatlands.

Carbon sequestration has been consistently higher in the naturally colonizing wetland compared to the planted wetland. We theorize this to be the result of the much greater productivity of this wetland over several years in the middle of this study.

4.6. What about the methane?

The whole story of carbon sequestration by wetlands must also take into account the effects of greenhouse gases that work counter to that of carbon sequestration (Mitsch et al., 2013). There have been many studies of greenhouse gas emissions from these created wetlands in Ohio over the last decade (Hernandez and Mitsch. 2006; Altor and Mitsch, 2006, 2008; Nahlik and Mitsch, 2010; Sha et al., 2011; Waletzko and Mitsch, 2014; Morin et al., 2014; Brooker et al., 2014).

Well-meaning but primarily carbon-focused scientists have suggested that we should not be creating or restoring wetlands for their ecosystem service of carbon sequestration because of their emissions of greenhouse gases, particularly methane. For example Neubauer (2014) stated that " . . . a full accounting of climate change consequences may not be realized for hundred to thousands of years [for created wetlands]. Because climate regulation is only one of many wetland ecosystem services, this should not be interpreted as an argument against the creation and restoration of wetlands. Still, . . . caution should be applied when designing wetland projects since freshwater wetlands may have a net positive (warming) effect on climate for decades to centuries or longer."

We disagree with this "net warming" conclusion given to freshwater wetlands, especially because of the potential wet blanket that it throws of ecological engineering of wetlands for their ecosystem services. The methane emissions from our created wetlands are a fraction of the rates from similar natural wetlands.

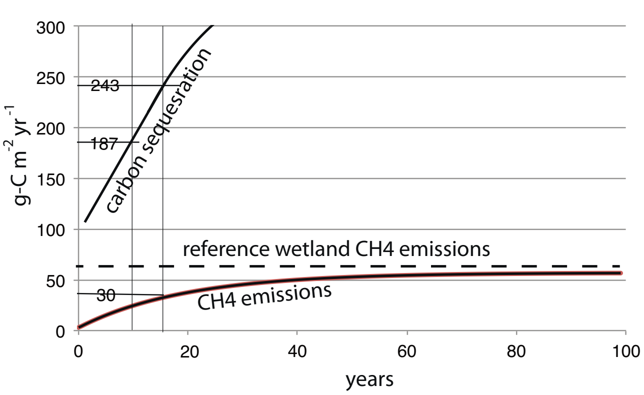

Fig. 10. Approximate patterns of methane emissions and carbon sequestration for 15 years and projection into the future for methane emissions for the average conditions of the experimental wetlands at the Olentangy River Wetland Research Park.

It will take many years for a created wetland to achieve full capacity in methane emissions. Median methane emissions over 2008–2010 from several studies at the Olentangy River Wetlands as compared by Waletzko and Mitsch (2014) ranged from 4 to 31 g-C m^{-2} yr^{-1}. Mitsch et al. (2013) used the high end (30 ± 14 g-C m^{-2} yr^{-1} ($n = 122$)) in their comparison with carbon sequestration. Still, with the same field and laboratory techniques, methane emissions were 57 ± 18 g-C m^{-2} yr^{-1} ($n = 61$) for a reference flow-through natural wetland in Ohio measured during the same time period. Thus, the first point we make is that the methane emissions from these wetlands, even recently, were only about half of those from similar natural wetlands. Furthermore, methane emissions are essentially zero when a wetland is created and it may takes years for the rates to reach those of natural wetlands (Fig. 10). Carbon sequestration, however, is different and its accumulation begins almost instantaneously. A reasonable model prediction of both methane emissions and carbon sequestration at our experimental wetlands, which are now only 20 years old, is shown in Fig. 10. Even though our study record is only 20 years and the questions being raised here relate to hundreds of years, the slopes of the lines in the first 15–20 years as shown in Fig. 10 are accurate. Carbon sequestration happens sooner and faster than do methane emissions in newly created wetlands.

Furthermore methanotrophs are abundant in these river-pulsing freshwater wetlands. These experimental wetlands are one of few locations we know about where methane emissions, carbon sequestration and potential methane oxidation have all been measured at the same time in the same wetlands. Roy-Chowdhury et al. (2014) report seasonal patterns of methanotrophs in the upper soils of these experimental wetlands in 2009–2010. They found potential methane oxidation (PMO) rates in these wetland soils, measured in the laboratory within a day of soil sampling, to range from 11.8 to 20.6 nmol-CH_4 g dry wt^{-1} h^{-1} in the surface soils (0–8 cm) and from 5.6 to 8.5 nmol-CH_4 g dry wt^{-1} h^{-1} in lower soils (8–16 cm) over four seasons in permanently flooded sites. These rates, integrated over the four seasons using soil bulk density are equivalent to a methane oxidation rate of 104 g-C m^{-2} yr^{-1}. This estimate shows the high potential for the existing microbial community in the soils of these wetlands to oxidize methane when the right conditions prevail. Roy-Chowdhury et al. (2014) concluded that the concentration of methane found in the soil has a much greater influence than does temperature on controlling methanotroph activity. In the end, the pulsing hydrology maintained in these wetlands for most of their 20 years may be optimum for minimizing methane emissions, partially by allowing for a diversity of microfauna in addition to methanogens.

5. Conclusions

These two created long-term "wetland labs" have illustrated that nutrient retention in wetlands can be sustainable for decades if the loading rates are not excessive. This observation has major implications for solving some serious eutrophication problems now occurring in the Midwest and in the Laurentian Great Lakes, particularly in western Lake Erie (Michalak et al., 2013). The design numbers in this paper are solid and are based on many wetland years of data.

Planting, in the end, did appear to have a small impact on the retention of nutrients. Planting enhanced the retention of phosphorus but decreased the ability of the wetland to retain nitrogen. Planting also "held back" for about a decade what we view now as the inevitable take over of the wetlands by *Typha* spp. While the planted wetland still had in 2013 most of the plants that we introduced in 1994 and the unplanted wetland has only a few, the wetlands appear to be converging in spatial community diversity and ecosystem function.

The created wetlands have rates of carbon sequestration higher than rates in similar-hydrology reference wetlands and much higher than rates measured in the more commonly studied boreal peatlands. Wetlands can and should be created with this as the primary ecosystem service.

These created wetlands have provided ecosystem services of nutrient retention, carbon sequestration, and habitat support for 20 years. The low-level emissions of methane from these wetlands should not be cause to discourage the creation and restoration of similar wetlands throughout the humid temperate world, given all of the ecosystem services that they provide.

Acknowledgements

This research was supported over the 20 years by many sources including the U.S. Department of Agriculture (NRI CSREES Award 2003-35102-13518), the United States Environmental Protection Agency (US EPA Agreements EM83329801-0 from Cincinnati OH and MX95413108-0 from Gulf of Mexico Program, Stennis Space Center, MS), the National Science FoundationGrants CBET-1033451 and CBET-0829026, Ohio Wetland Foundation, and Environmental Science Graduate Program, the School of Environment and Natural Resources, and theOhio Agricultural Research and Development Center (OARDC) at The Ohio State University. We thank the hundred or more graduate students, post-docs, and other researchers over the years, all of whom contributed to this 20-year-long study. We also appreciate the financial support provided by Chris White, Bill Heffner, Jerry and Lenora Pausch, Ruth Smart, and many other loyal supporters of our wetland research who made The Olentangy River Wetland possible. We also appreciate the support we were given by Ohio State University Presidents E. Gordon Gee, Brit Kirwin, and Karen Holbrook and Dean Bobby Moser.

References

Altor, A.E., Mitsch, W.J., 2006. Methane flux from created wetlands: relationship to intermittent versus continuous inundation and emergent macrophytes. Ecol. Eng. 28, 224–234.
Altor, A.E., Mitsch, W.J., 2008. Pulsing hydrology methane emissions, and carbon dioxide fluxes in created marshes: a 2-year ecosystem study. Wetlands 28, 423–438.
American Public Health Association, 1989. Standard Methods for the Analysis of Wastewater, 17th ed. APHA, Washington, DC.
Anderson, C.J., Mitsch, W.J., 2006. Sediment, carbon, and nutrient accumulation at two 10-year-old created riverine marshes. Wetlands 3, 779–792.
Batson, J., Mander, Ü., Mitsch, W.J., 2012. Denitrification and a nitrogen budget of created riparian wetlands. J. Environ. Qual. 41, 2024–2032.
Bernal, B., Mitsch, W.J., 2012. Comparing carbon sequestration in temperate freshwater wetland communities. Global Change Biol. 18, 1636–1647.
Bernal, W.J., Mitsch, B., 2013a. Carbon sequestration in two created riverine wetlands in the Midwestern United States. J. Environ. Qual. 4, 1236–1244.
Bernal, B., Mitsch, W.J., 2013b. Carbon sequestration in freshwater wetlands of Costa Rica and Botswana. Biogeochemistry 115, 77–93.
Brix, H., Sorrell, B.K., Lorenzen, B., 2001. Are *Phragmites*-dominated wetlands a net source or net sink of greenhouse gases? Aquat. Bot. 69, 313–324.
Brooker, M.R., Bohrer, G., Mouser, P.J., 2014. Variations in potential CH_4 flux and CO_2 respiration from freshwater wetland sediments that differ by microsite location, depth and temperature. Ecol. Eng. 72, 84–94.
Comin, F.A., Romero, J.A., Astorga, V., Garcia, C., 1997. Nitrogen removal and cycling in restored wetlands used as filters of nutrients for agricultural runoff. Water Sci. Technol. 35, 255–261.
Costanza, R.R., de Groot, P., Sutton, S., van der Ploeg, S.J., Anderson, I., Kubiszewski, S., Farber Turner, R.K., 2014. Changes in the global value of ecosystem services. Global Environ. Change 26, 152–158.
Craft, C., Washburn, C., Parker, A., 2008. Latitudinal trends in organic carbon accumulation in temperate freshwater peatlands. In: Vymazal, J. (Ed.), Wastewater Treat Plant Dynamics and Management in Constructed Natural Wetlands. Springer, Dordrecht, Netherlands, pp. 23–31. http://dx.doi.org/10.1007/978-1-4020-8235-1_3.
deGroot, R., Brander, L., van der Ploeg, S., Costanza, R., Bernard, F., Braat, L., Christie, M., Crossman, N., Ghermandi, A., Hein, L., Hussain, S., Kumar, P., McVittie, A., Portela, R., Rodriguez, L.C., ten Brink, P., van Beukering, P., 2012. Global estimates of the value of ecosystems and their services in monetary units. Ecosyst Serv. 1, 50–61.
Dunne, E.J., Coveney, M.F., Marzolf, E.R., Hoge, V.R., Conrow, R., Naleway, R., Lowe, E. F., Battoe, L.E., 2012. Efficacy of a large-scale constructed wetland to remove phosphorus and suspended solids from Lake Apopka, Florida. Ecol. Eng. 42, 90–100.
Dunne, E.J., Coveney, M.F., Marzolf, E.R., Hoge, V.R., Conrow, R., Naleway, R., Lowe, E. F., Battoe, L.E., Inglett, P.W., 2013. Nitrogen dynamics of a large-scale constructed wetland used to remove excess nitrogen from eutrophic lake water. Ecol. Eng. 61, 224–234.
Euliss, N.H., Gleason, R.A., Olness, A., McDougal, R.L., Murkin, H.R., Robarts, R.D., Bourbonniere, R.A., Warner, B.G., 2006. North American prairie wetlands are important nonforested land-based carbon storage sites. Sci. Total Environ. 361, 179–188.
Fink, D.F., Mitsch, W.J., 2004. Seasonal and storm event nutrient removal by a created wetland in an agricultural watershed. Ecol. Eng. 23, 313–325.
Fink, D.F., Mitsch, W.J., 2007. Hydrology and nutrient biogeochemistry in a created river diversion oxbow wetland. Ecol. Eng. 30, 93–162.
Heikkinen, J.E.P., Elsakov, V., Martikainen, P.J., 2002. Carbon dioxide and methane dynamics and annual carbon budget in tundra wetland in NE Europe, Russia. Glob Biogeochem. Cycles 16. doi:http://dx.doi.org/10.1029/2002GB0001930.
Hendriks, D.M.D., van Huissteden, J., Dolman, A.J., van der Molen, M.K., 2007. The full greenhouse gas balance of an abandoned peat meadow. Biogeosciences 4, 411–424.
Hernandez, W.J., Mitsch, M.E., 2006. nfluence of hydrologic pulses flooding frequency, and vegetation on nitrous oxide emissions from created riparian marshes. Wetlands 26, 862–877.
Hernandez, M.E., Mitsch, W.J., 2007. Denitrification in created riverine wetlands: influence of hydrology and season. Ecol. Eng. 30, 78–88.
Kadlec, R.H., 2009. Wastewater treatment at the Houghton Lake wetland: hydrology and water quality. Ecol. Eng. 35, 1287–1311.
Keddy, P.A., 2010. Wetland Ecology: Principles and Conservation, 2nd ed. Cambridge University Press, Cambridge, UK, pp. 497.
Kovacic, D.A., David, M.B., Gentry, L.E., Starks, K.M., Cooke, R.A., 2000. Effectiveness of constructed wetlands in reducing nitrogen and phosphorus export from agricultural tile drainage. J. Environ. Qual. 29, 1262–1274.
Leonardson, L., Bengtsson, L., Davidsson, T., Persson, T., Emanuelsson, U., 1994. Nitrogen retention in artificially flooded meadows. Ambio 23, 332–341.
Ligi T., Truu M., Truu J., Oopkaup K., Nõlvak H., Mander Ü., Mitsch W.J., Truu J., The genetic potential of N_2 emission via denitrification and ANAMMOX from the soils and sediments of a created riverine treatment wetland complex, Ecol. Eng. (in press).
Lundberg, C.J., Lane, R.R., Day Jr., J.W., 2014. Spatial and temporal variations in nutrients and water-quality parameters in the mississippi river-influenced breton sound estuary. J. Coastal Res. 30, 328–336.
McInnes, R.J., 2013. Recognizing ecosystem services from wetlands of international importance: an example from Sussex, UK. Wetlands 33, 1001–1017.
Michalak, A.M., Anderson, E.J., Beletsky, D., Boland, S., Bosch, N.S., Bridgeman, T.B., Chaffin, J.D., Cho, K., Confesor, R., Daloglu, I., DePinto, J.V., Evans, M.A., Fahnenstiel, G.L., He, L., Ho, J.C., Jenkins, L., Johengen, T.H., Kuo, K.C., LaPorte, E., Liu, X., McWilliams, M.R., Moore, M.R., Posselt, D.J., Richards, R.P., Scavia, D., Steiner, A.L., Verhamme, E., Wright, D.M., Zagorski, M.A., 2013. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. Proc. Natl. Acad. Sci. 110, 6524–6529.
Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC, pp. 137.
Mitsch, W.J., Gosselink, J.G., 2007. Wetlands, 4th ed. John Wiley & Sons, Hoboken, NJ.
Mitsch, W.J., 2015. Wetlands, 5th ed. John Wiley & Sons, NJ, Hoboken.
Mitsch, W.J., Cronk, J.K., Wu, X., Nairn, R.W., Hey, D.L., 1995. Phosphorus retention in constructed freshwater riparian marshes. Ecol. Appl. 5, 830–845.
Mitsch, W.J., Wu, X., Nairn, R.W., Weihe, R.E., Wang, N., Deal, R., Boucher, C.E., 1998. Creating and restoring wetlands: a whole-ecosystem experiment in self-design. BioScience 48, 1019–1030.
Mitsch, W.J., Horne, A.J., Nairn, R.W., 2000. Nitrogen and phosphorus retention in wetlands—ecological approaches to solving excess nutrient problems. Ecol. Eng. 14, 1–7.
Mitsch, W.J., Wang, N., Zhang, L., Deal, R., Wu, X., Zuwerink, A., 2005a. Using ecological indicators in a whole-ecosystem wetland experiment. In: Jørgensen, S.E., Xu, F.-L., Costanza, R. (Eds.), Handbook of Ecological Indicators for Assessment of Ecosystem Health. CRC Press, Boca Raton, FL, pp. 211–235.
Mitsch, W.J., Zhang, L., Anderson, C.J., Altor, A.E., Hernández, M.E., 2005b. Creating riverine wetlands: ecological succession, nutrient retention, and pulsing effects. Ecol. Eng. 25, 510–527.
Mitsch, W.J., Zhang, L., Fink, D.F., Hernandez, M.E., Altor, A.E., Tuttle, C.L., Nahlik, A. M., 2008. Ecological engineering of floodplains. Ecohydrol. Hydrobiol. 8, 139–147.
Mitsch, W.J., Zhang, L., Stefanik, K.C., Nahlik, A.M., Anderson, C.J., Bernal, B., Hernandez, M., Song, K., 2012. Creating wetlands: primary succession, water quality changes, and self-design over 15 years. Bio. Sci. 3, 237–250.
Mitsch, W.J., Bernal, B., Nahlik, A.M., Mander, U., Zhang, L., Anderson, C.J., Jørgensen, S.E., Brix, H., 2013. Wetlands carbon, and climate change. Landscape Ecol. 28, 583–597.
Mitsch, W.J., Cronk, J.K., Zhang, L., 2014a. Creating a living laboratory on a college campus for wetland research—the Olentangy River Wetland Research Park, 1991 to 2012. Ecol. Eng. 72, 1–10.
Mitsch, Mitsch, W.J., Nedrich, S.M., Harter, S., Anderson, C., Nahlik, A.M., Bernal, B., 2014b. Sedimentation in created freshwater riverine wetlands: a comparison of

methods over 15 years in created flow-through wetlands. Ecol. Eng. 72, 25–34.
Mitsch W.J., Zhang L., Marois D., Song K., Protecting the Florida Everglades wetlands with wetlands: can stormwater phosphorus be reduced to oligotrophic conditions?, Ecol. Eng. (in press).
Morin, T.H., Bohrer, G., Naor Azrieli, L., Mesi, S., Kenny, W.T., Mitsch, W.J., Shafer, K.V. R., 2014. The seasonal and diurnal dynamics of methane flux at a created urban wetland. Ecol. Eng. 72, 74–83.
Moustafa, M.Z., Chimney, M.J., Fontaine, T.D., Shih, G., Davis, S., 1996. The response of a freshwater wetland to long-term low level nutrient loads—marsh efficiency. Ecol. Eng. 7, 15–33.
Moustafa, M.Z., 1999. Nutrient retention dynamics of the Everglades nutrient removal project. Wetlands 19, 689–704.
Nahlik, A.M., Mitsch, W.J., 2010. Methane emissions from created riverine wetlands. Wetlands 30, 783–793 (with Erratum published in Wetlands (2011) 31, 449–450).
Neubauer, S.C., 2014. On the challenges of modeling the net radiative forcing of wetlands: Reconsidering Mitsch et al. (2013). Landscape Ecol. 29, 571–577.
Niswander, S.F., Mitsch, W.J., 1995. Functional analysis of a two-year-old created in-stream wetland: hydrology, phosphorus retention, and vegetation survival and growth. Wetlands 15, 212–225.
Odum, W.E., 1987. Predicting ecosystem development following creation and restoration of wetlands. In: Zelazny, J., Feierabend, J.S. (Eds.), Wetlands: Increasing Our Wetland Resources. Proceedings of the Conference Wetlands: Increasing Our Wetland Resources. Corporate Conservation Council, National Wildlife Federation, Washington, DC, pp. 67–70.
Page, S.E., Wust, R.A.J., Weiss, D., Rieley, J.O., Shotyk, W., Limin, S.H., 2004. A record of late Pleistocene and Holocene carbon accumulation and climate change from an equatorial peat bog (Kalimantan Indonesia): implications for past, present, and future carbon dynamics. J. Quat. Sci. 19, 625–635.
Phipps, R.G., Crumpton, W.G., 1994. Factors affecting nitrogen loss in experimental wetlands with different hydrologic loads. Ecol. Eng. 3, 399–408.
Raisin, G.W., Mitchell, D.S., Croome, R.L., 1997. The effectiveness of a small constructed wetland in ameliorating diffuse nutrient loadings from an Australian rural catchment. Ecol. Eng. 9, 19–35.
Reddy, K.R., DeLaune, R.D., DeBusk, W.F., Koch, M.S., 1993. Long-term nutrient accumulation rates in the Everglades. Soil Sci. Soc. Am. J. 57, 1147–1155.
Reinelt, L.E., Horner, R.R., 1995. Pollutant removal from stormwater runoff by palustrine wetlands based on comprehensive budgets. Ecol. Eng. 4, 77–97.
Richardson, C.J., Craft, C.B., 1993. Effective phosphorus retention in wetlands—fact or fiction? In: Moshiri, G.A. (Ed.), Constructed Wetlands for Water Quality Improvement. CRC Press, Boca Raton, FL, pp. 271–282.
Richardson, C.J., Qian, S., Craft, C.B., Qualls, R.G., 1997. Predictive models for phosphorus retention in wetlands. Wetlands Ecol. Manage. 4, 159–175.
Roy-Chowdhury, T., Mitsch, W.J., Dick, R.P., 2014. Seasonal methanotrophy across a hydrological gradient in a freshwater wetland. Ecol. Eng. 72, 116–124.
Saunders, M.J., Jones, M.B., Kansiime, F., 2007. Carbon and water cycles in tropical papyrus wetlands. Wetlands Ecol. Manage. 15, 489–498.
Sha, C., Mitsch, W.J., Ü. Mander, Lu, J., Batson, J., Zhang, L., He, W., 2011. Methane emissions from freshwater riverine wetlands. Ecol. Eng. 37, 16–24.
Song, K., Lee, S.H., Mitsch, W.J., Kang, H., 2010. Different responses of denitrification rates and denitrifying bacterial communities to hydrologic pulsing in created wetlands. Soil Biol. Biochem. 42, 1721–1727.
Song, K., Kang, H., Zhang, L., Mitsch, W.J., 2012. Seasonal and spatial variations of denitrification and denitrifying community structure in created wetlands. Ecol. Eng. 38, 130–134.
Song, K., Hernandez, M.E., Batson, J.A., Mitsch, W.J., 2014. Long-term denitrification rates in created riverine wetlands and their relationship with environmental factors. Ecol. Eng. 72, 40–46.
Turunen, J., Tomppo, E., Tolonen, K., Reinkainen, E., 2002. Estimating carbon accumulation rates of undrained mires in Finland: application to boreal and subarctic regions. Holocene 12, 79–90.
U.S. Environmental Protection Agency, 1983. Handbook for methods in water and wastewater analysis. U.S. Environmental Protection Agency, Cincinnati, Ohio.
Villa J.A., Mitsch W.J., Carbon sequestration in different wetland plant communities in Southwest Florida, Int. J. Biodivers. Sci. Ecosyst. Serv. Manage. (in press).
Waletzko, E., Mitsch, W.J., 2014. Methane emissions from wetlands: an in situ side-by-side comparison of two static accumulation chamber designs. Ecol. Eng. 72, 94–101.
White, J.S., Bayley, S.E., Curtis, P.J., 2000. Sediment storage of phosphorus in a northern prairie wetland receiving municipal and agro-industrial wastewater. Ecol. Eng. 14, 127–138.
Wu, X., Mitsch, W.J., 1998. Spatial and temporal patterns of algae in newly constructed freshwater wetlands. Wetlands 18, 9–20.
Zhang, Y., Zhang, L., Mitsch, W.J., 2014. Predicting river aquatic productivity and dissolved oxygen before and after dam removal. Ecol. Eng. 72, 125–137.