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APPLIED WETLAND SCIENCE

Critical Research Gaps for Understanding Environmental Impacts of Discharging Treated Municipal Wastewater into Assimilation Wetlands

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Abstract

Assimilation wetlands are natural, non-constructed, wetlands that are used for the removal of nutrients from treated municipal wastewater. This passive process is comparatively less expensive than other conventional forms of tertiary treatment of wastewater, making it desirable for municipalities. Assimilation wetlands are monitored for a number of environmental parameters, yet limited research has been conducted to understand the ecological impact of this water treatment process. Studies from a variety of systems throughout the United States provide conflicting evidence of the responses of wetland ecosystems to increased inundation and nutrient enrichment. Through an extensive review of existing literature, we summarize the impacts of increasing nutrient loading and inundation on receiving wetlands. Importantly, we address current research gaps and identify directions for future scientific study on this topic. Comprehensive ecosystem monitoring conducted at larger spatial and temporal scales, as well as controlled experimentation, are needed to fully understand ecosystem responses to long-term use of wetlands to remediate wastewater nutrients. Our intent is neither to promote nor detract from this process, but rather to bring attention to potential drivers of environmental change and inform those who manage these systems.

Keywords Assimilation wetland · Effluent · Flooding · Nutrient enrichment · Wastewater · Wetlands

Introduction

Wetlands provide many important ecosystem services and functions (Mitsch and Wilson 1996; Reddy et al. 1999; Mitsch and Gosselink 2000), including water treatment and nutrient removal (Sawyer 1947; Coveney et al. 2002; Fisher and Acreman 2004). Humans have used wetlands to remediate wastewater dating back as far as 2500 BCE in the Indus Valley (Lofrano and Brown 2010). Although it was not an undeviating line of development, by the industrial revolution the basic forms of primary wastewater treatment had taken shape (Chatzakis et al. 2006; Vuorinen et al. 2007). Starting in the mid-twentieth century, engineers began constructing wetlands to take advantage of naturally occurring contaminant removal processes. This set off a revolution in water treatment and environmental science resulting in many of the standard water quality metrics we use to this day (e.g. biological oxygen demand, total suspended solids, and phenol concentration) (Vuorinen et al. 2007; Lofrano and Brown 2010).

Modern municipal wastewater treatment consists of a multi-stage process before wastewater can be discharged into the environment: *primary treatment*, involving the removal of solids; *secondary treatment*, which includes oxidation and microbial decomposition; and *disinfection*, in which chlorination or UV light is used to kill microbial pathogens (Hartman and Cleland 2007). Concerns about discharging treated wastewater with high nutrient loads into aquatic systems has given impetus for additional treatment (commonly referred to as *tertiary treatment*), in which methods of nitrogen and phosphorus removal are employed before discharging effluent into ecosystems or using for groundwater recharge (Sonune and Ghate 2004; Hijnen et al. 2006) (Fig. 1). Unlike primary and secondary treatment, tertiary treatment is not universally

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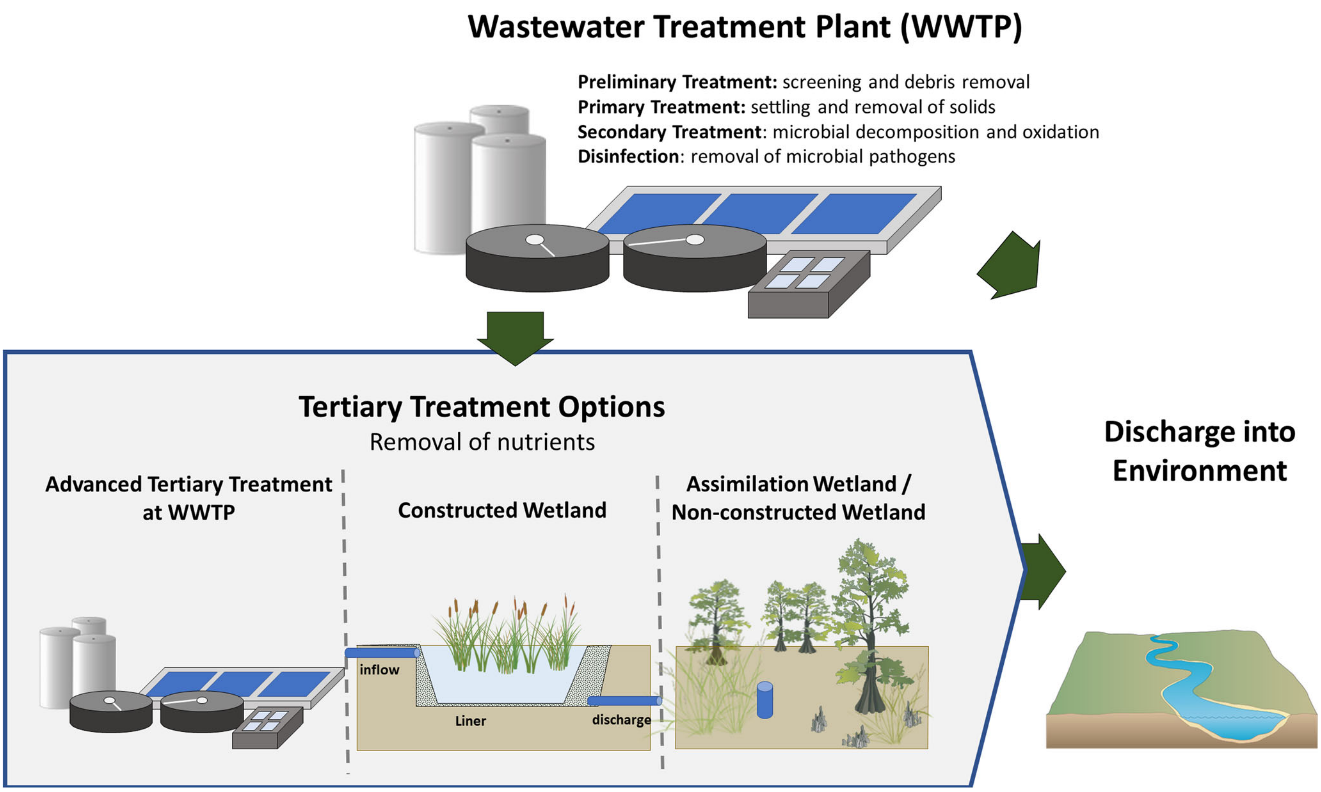

Fig. 1 Multiple treatment options for municipal wastewater. Following primary and secondary treatment and disinfection, wastewater may be discharged into the environment or sent for tertiary treatment before discharge into the environment. Tertiary treatment typically occurs via one of three options: 1) conventional treatment plant, 2) constructed wetland, or 3) non-constructed assimilation wetland (vector images sourced from IAN Image Library)

required. Multiple forms of tertiary water treatment exist, including conventional treatment, constructed wetlands, and assimilation wetlands. Conventional treatment is conducted in a closed system as part of the treatment process, but this is often prohibitively expensive. Alternatively, constructed wetlands can be used to remove nutrients as soil and plants biofiltrate wastewater (Castelle et al. 1992). In these systems water flow is highly engineered, and wetlands are separated from the environment using sediment liners. Constructed wetlands are considered a low-cost technology for treating wastewater (Hammer 1989; Day Jr et al. 2004), yet they are comparatively more expensive than discharging into a natural, non-constructed wetland (Hunter et al. 2019). The use of wetlands for tertiary treatment of human effluent has been considered for years (Valiela et al. 1976), however it was not until very recently that municipalities have turned to using natural, non-constructed wetland systems, or *assimilation wetlands*, to remove nutrients from treated wastewater effluent (Day Jr et al. 2019a).

The use of assimilation wetlands is not common throughout the U.S. and limited peer-reviewed literature is available on the use of this process in other nations. Assimilation wetland sites are currently located in Michigan, Florida, Wisconsin, Massachusetts, and, most prevalently, Louisiana (Nichols 1983; Nagabhatla and Metcalfe 2018) where there are 10 active assimilation wetlands, with several more sites under review (Hunter et al. 2018). In the U.S., establishing an assimilation wetland requires state and federal oversight, careful site selection, and long-term environmental monitoring. The details of these requirements are discussed by Nagabhatla and Metcalfe (2018). Water quality standards are generally less restrictive for effluent discharged into assimilation wetlands than for direct discharge into open water bodies because of the hypothesized ability of wetlands to assimilate nutrients without deleterious effects on the ecosystem (Day Jr et al. 2004; Nagabhatla and Metcalfe 2018). A number of peer-reviewed studies exist for assimilation wetlands, mostly from Louisiana, including two recently published review papers that describe Louisiana's assimilation wetland sites in detail (Hunter et al. 2018; Day Jr et al. 2019a). However, there remain crucial research gaps and conflicting narratives on the efficacy of this process.

In this review, we summarize the environmental and economic costs and benefits of using natural, non-constructed wetlands to assimilate wastewater and identify crucial knowledge gaps on this topic to direct future scientific research. This review was conducted using a broad search of English-language literature published between 1900 and 2020 via Web of Science in 2020. The search used the following search terms: [WETLAND and ASSIMILATION]. The search revealed 495 peer reviewed publications and books, of which fewer than 70 publications were selected as relevant. We did not delineate a geographic limit for accepted literature, however, the majority of literature available on this topic is based in the United States.

State of Knowledge

A recent review by Hunter et al. (2018) provided a detailed explanation of the process of establishing and monitoring an assimilation wetland in Louisiana. Hunter et al. (2018) emphasized considerations necessary to ensure success of an assimilation wetland, and discussed the benefits of wetland assimilation: 1) improvement of water quality and nutrient reduction; 2) wetland restoration; 3) enhanced productivity; 4) carbon sequestration; 5) mitigation of climate change impacts; and 6) energy and economic savings. Below we discuss these hypothesized benefits and raise matters of potential costs and unintended impacts associated with these benefits. Finally, we provide a list of critical research gaps that require attention both to guide this field of research as well as understand the ecological consequences of this process and inform future management decisions.

Hypothesized Benefits and Potential Costs

Improvement of Water Quality and Nutrient Reduction

Both natural and constructed wetlands remove pollutants and excess nutrients from water while improving water quality through the reduction of suspended solids and increase of dissolved oxygen (Kadlec and Wallace 2009; Vymazal 2010; Stefanakis et al. 2014; Scholtz 2015). Reactive nitrogen (primarily as NO_3^-) can cause harmful algal blooms, hypoxia, and fisheries losses in open water systems (Diaz and Rosenberg 2008; Heisler et al. 2008; Breitburg et al. 2018). One of the primary roles of tertiary wastewater treatment is to reduce the concentration of reactive N in wastewater before it reaches nearby water bodies. Generally, assimilation wetlands are successful in accomplishing this task (Table 1). Wetlands function to remove N through two main processes, vegetation uptake (which accounts for 4% to 35% of N removal and is variable in the time scale of removal) and denitrification (a permanent removal process responsible for the remainder of N removal) (Lin et al. 2002; VanZomeren et al. 2012). Plants within wetlands also aid in maintaining the pH at near neutral levels and temperatures at levels that increase denitrification (Lu et al. 2009). However, both N and P removal efficiency depends on several factors, including: relative concentrations of nutrients (Herbert et al. 2020), loading rate (Bastviken et al. 2009), hydrology (Spieles and Mitsch 2000), soil pH, organic carbon source, and the plant community (Nichols 1983).

The efficacy of assimilation wetlands in pollutant, sediment, and nutrient removal may vary depending on the system's hydrogeomorphic setting, biological communities, and loading rates. In ideal circumstances, nutrient uptake rates would be equal to or greater than loading rates (Day Jr et al. 2019a), however, this isn't always the case and assimilation dynamics vary among wetland systems. For example, an assimilation wetland in Thibodeaux, Louisiana is reported as having an N storage rate of 7.3 g N m^{-2} yr^{-1} in soils and 1.1 g N m^{-2} yr^{-1} in wood. The loading rate for this wetland is 12.5 g N m^{-2} yr^{-1}, which would indicate that not all of the N is being assimilated; however, the authors claim that the wetland has an additional 36 g N m^{-2} yr^{-1} removal capacity via denitrification (Day Jr et al. 2004). The Houghton Lake assimilation peatland in Michigan was described as accumulating 564 g N m^{-2} in sediments over a 30-year period with a total loading rate of 135 g N m^{-2} over that same time period (yearly totals of 18.8 g N m^{-2} and 4.5 g N m^{-2} respectively), exceeding what was expected based on the loading rates. The authors explained this discrepancy by estimating that ~90 g N m^{-2} was attributed to mineralization of organic N, and 392 g N m^{-2} was from nitrogen fixation (yearly totals of 3 g N m^{-2} and 13.1 g N m^{-2} respectively) (Kadlec and Bevis 2009). Nutrient assimilation can also be ascertained from comparisons of total N and P concentrations of surface water monitored at an assimilation wetland's effluent discharge point compared to where the effluent exits the system (Day Jr et al. 2019a; Hunter et al. 2018). Day Jr et al. (2019a) reported that total N decreased from approximately 7 mg L^{-1} to 1 mg L^{-1} between these points at the Breaux Bridge and Mandeville, Louisiana assimilation wetlands and at the Thibodeaux, Louisiana assimilation wetland, total N decreased from ~10 mg L^{-1} to ~1.1 mg L^{-1}. Given these values, these assimilation wetlands are reducing the concentration of total N in surface water by 86% to 89%.

In this regard, assimilation wetlands are comparable to both natural wetlands and constructed wetlands. A global review of wetlands (including marshes, swamps, peatlands, fens, and riparian zones) found extreme variability among natural systems to remove N, ranging from 1% to 100% (Fisher and Acreman 2004). Fisher and Acreman (2004) attributed some of this variability to oxygen availability, hydraulic retention time, hydraulic loading, and vegetation processes. Wetlands in more saline environments have also shown comparable nitrogen removal rates. For example, wetlands not receiving nutrient-rich effluent in Plum Island, Massachusetts, where incoming tidal water N concentrations were 1–4 μmol, removed essentially 100% of the incoming N (Drake et al. 2009). In a salt marsh located in Barataria Basin, Louisiana, N accumulation rate in the sediment averaged 16.6 g N m^{-2} yr^{-1} (DeLaune et al. 1981).

Vymazal (2010) assessed the nutrient removal capability of constructed wetlands and determined that for "lightly loaded systems," 100–200 g N m^{-2} yr^{-1} can be removed by

Table 1 Nitrogen and phosphorus concentrations reported in assimilation and constructed wetlands

Wetland Type	Year of effluent discharge/ Year of permit	Mean TN in (mg/L)	Mean TN out (mg/L)	TN % Change	Mean TP in	Mean TP out	TP % Change	Source
Assimilation Wetland								
Amelia, LA	1973 / 2007	3.78	1.00	74	0.73	0.06	92	Hunter et al. 2009b; 2018
Breaux Bridge, LA	1940 / 2003	8.44	~1.40	~83	2.90	0.40	86	
Broussard, LA	2007 / 2007	24.64	~4.40	~82	3.45	~1.9	~45	
Hammond, LA	2006 / 2010	17.91	~0.90	~95	4.04	0.04	99	
Luling, LA	2007 / 2008	7.06	~1.20	~83	2.34	~0.40	~83	
Mandeville-BC, LA	1998 / 2003	14.36	~1.50	~90	3.31	~0.70	~76	
Mandeville-TM, LA	2009 / 2009	15.52	~1.50	~90	3.02	~0.40	~87	
St. Martinville, LA	2006 / 2006	5.40	~2.10	~61	1.85	~0.80	~57	
Thibodaux, LA	2004 / 2004	11.60	~0.95	92	2.46	0.85	66	
Constructed Wetland								
Free Water Surface		36.51	20.86	42.86	6.76	4.06	39.94	Vymazal 2010
Horizontal Flow		59.66	36.00	39.66	9.60	4.80	50.00	
Vertical Flow		73.00	41.00	43.00	10.30	4.50	56.00	
Free-Floating Plants		14.60	6.60	54.80	3.80	2.20	42.10	Vymazal 2007
Free Water Surface		14.30	8.40	41.20	4.20	2.15	48.80	
Horizontal Sub-Surface Flow		46.60	26.90	42.30	8.75	5.15	41.10	
Vertical Sub-Surface Flow		68.40	37.90	44.60	10.50	4.25	59.50	

**Please refer to Hunter et al. 2009b; 2018 for more detailed descriptions of each assimilation wetland*

harvesting the standing crop of vegetation grown in constructed wetlands. Reinhardt et al. (2006) found that a construction wetland removed 45 g N m^{-2} y^{-1} over 2.5 years, a 27% removal efficiency, and most of that removal (94%) was due to denitrification whereas the remaining N was accumulated in soil. Constructed wetlands are designed to achieve desired denitrification rates by considering the required carbon source for this process, and if necessary, adding an external carbon source to facilitate denitrification (Lu et al. 2009). The parameter of available carbon is the one that eventually regulates NO_3^- removal in natural wetlands and therefore non-constructed natural wetlands may not be as effective as a constructed wetland. The combined above results would suggest that assimilation wetlands remove N at rates similar to natural wetlands, even after several years of functioning; however, constructed wetlands may be the most efficient (Table 1).

Wetland Restoration

Many of Louisiana's coastal freshwater forested wetlands are currently in decline due to combined forces from subsidence, sea level rise, anthropogenic modification of hydrology, and oil and gas exploration activities. Encroaching saline water threatens these freshwater ecosystems. Hunter et al. (2018) explained that the introduction of treated municipal effluent into degraded forested wetlands in Louisiana is a major step toward their ecological restoration, due to combined benefits from increasing vegetation productivity, increasing elevation via organic matter deposition to reduce flooding duration, and using an increase in freshwater to buffer against saltwater intrusion. Candidate wetlands for using treated effluent for ecosystem restoration are thereby geographically limited to subsiding freshwater forested wetlands at risk of saltwater intrusion. The increased flooding/flushing is a benefit to these systems as it increases accretion promoted by biomass production (Hunter et al. 2018a, 2018b). For example, when the Davis Pond diversion was opened for several months in 2010 in response to the Deepwater Horizon oil spill, *Taxodium distichum* (baldcypress) swamps downstream of the diversion experienced enhanced primary production as a result of increased flooding and reduced salinity (Middleton et al. 2015). To simultaneously reap benefits of both increased freshwater flooding and increased accretion will require close and frequent monitoring and careful management to avoid flooding species beyond their tolerance thresholds.

Introduction of freshwater can be a restoration technique for forests during times of drought (Middleton and Souter 2016), however, these are often pulsed flooding events, not typically a continuous press. Continued discharge of freshwater may be beneficial for freshwater forested wetlands that are immediately threatened by salinity intrusion, but elsewhere this technique should be managed to support forest regeneration as well. Baldcypress (*Taxodium distichum*) forest regeneration requires a period of drawdown as prolonged inundation suppresses germination and growth of young seedlings (Souther and Shaffer 2000). Other forested wetland species, such as bottomland hardwood forest trees, do not respond favorably to long-term flooding (Malecki et al. 1983), and therefore continued flooding is not a suitable restoration or habitat enhancement option for these systems. The proper use of this process for restoration would require seasonal pulsing, and thereby may not be practical for the logistical demands of a municipal wastewater treatment plant.

Enhanced Productivity & Habitat Enhancement

Discharging treated effluent into wetlands has been shown to increase plant productivity (Day Jr et al. 2004). Tree rings from baldcypress trees have shown that tree growth is enhanced by the addition of nutrient rich effluent (Hesse et al. 1998; Day Jr et al. 2006; Brantley et al. 2008; Shaffer et al. 2009; Shaffer et al. 2015). Controlled studies have shown that increased nitrogen loading (loading rate up to 100 g N y^{-1}) can increase aboveground biomass production in baldcypress seedlings and increase the root:shoot ratio; whereas loading rates greater than 100 g N y^{-1} decrease root:shoot ratio (Hillmann et al. 2019). Litterfall studies at a variety of assimilation wetlands throughout Louisiana show similar trends: discharge sites have higher total net primary production (NPP) compared to reference sites (Hunter et al. 2018). Additionally, it is documented that plant aboveground growth and basal area index increases with the addition of limiting nutrients in many tree and herbaceous plant species.

Although the assertion that increased nutrient availability leads to healthier, more productive wetlands is a popular narrative in regard to assimilation wetlands (Hesse et al. 1998; Day Jr et al. 2004; Hunter et al. 2009a; Lundberg et al. 2011; Shaffer et al. 2015), other studies suggest that long-term inundation and chronic nutrient loading associated with the treated effluent may destabilize wetlands (Darby and Turner 2008). Additional research is needed at assimilation wetland sites to understand the long-term impacts of this process on baldcypress regeneration, overall forest longevity, non-focus plant species (i.e., species other than baldcypress), and aboveground-to-belowground productivity ratio. Because long-term studies on assimilation wetlands are limited, other ecosystem-scale studies on the impacts of increased inundation and nutrient loading can be informative. One such long-term ecosystem-scale nutrient enrichment experiment at Plum Island, Massachusetts, U.S., has demonstrated that nutrient enrichment can be a driver of salt marsh loss by decreasing belowground biomass of bank-stabilizing roots, though the impacts took years to become apparent (Deegan et al. 2012). In this study, increasing nutrient loading in a tidal marsh for ~10 years resulted in geomorphic instability, creek-bank collapse, and conversion of vegetated marsh to unvegetated mud flat (Deegan et al. 2012). However, it should be noted that this experiment was conducted in a coastal tidal system in New England- and is hydrogeomorphically different from many assimilation wetland settings.

A field experiment in a freshwater tidal marsh in the Altamaha River in Georgia fertilized the marsh with nitrogen, phosphorus, or a nitrogen and phosphorus combination for 10+ years (Ket et al. 2011; Herbert et al. 2020). The study found that adding nitrogen or phosphorus alone decreased belowground biomass production and soil carbon. Nitrogen additions also enhanced soil microbial activity, nitrification, denitrification, and methane production. When N and P were added in combination, marsh vegetation increased both above and belowground biomass production, and soil carbon was not significantly different from the control plots in which no nutrients were added (Herbert et al. 2020).

It is well understood that the functional equilibrium of plants can be altered by a change in resource availability, and that increased concentrations of macronutrients (particularly N) can reduce belowground biomass allocation (Zeing 2003; Darby and Turner 2008). Increased nutrient loading rates tend to increase soil microbial metabolism, reduce soil strength, and lower belowground biomass production (Turner 2011). Watson et al. (2014) found that nutrient enrichment in the water column reduced organic matter accumulation and peat formation in salt marshes. Nutrient enrichment corresponded with reduced belowground production (coarse roots and rhizomes) and higher decomposition rates. Alldred et al. (2017) found that belowground biomass in salt marshes was negatively related to extractable nitrogen content in the soil and may contribute to marsh instability. Despite the fact that these studies were completed in salt marshes, they suggest that increased nutrient loads may not improve resiliency to sea level rise, which contradicts several studies that support the use of assimilation wetlands to improve ecosystem resilience to storms and sea level rise.

Increased nutrient loading may destabilize the ecosystem by increasing the rate of decomposition. Decomposition of leaf litter occurs at a faster rate in nutrient-rich systems compared to nutrient-poor systems (Brock et al. 1985; Webster and Benfield 1986), though decomposition rate may vary among species or nutrient enrichment regimes (N vs. P) (Xie et al. 2004). Stoler et al. (2016) found that litter mass loss is negatively correlated with litter C:N and C:P. Nutrient enrichment was found to increase microbial decomposition of organic matter in a New England salt marsh (Deegan et al. 2012; Bulseco et al. 2019), as well as increase the rate of initial decomposition for the aquatic plant, *Ruppia cirrhosa*, in a coastal lagoon in Italy (Menéndez et al. 2003). Though studies have been completed in other systems, research specifically addressing decomposition rates in assimilation wetlands is lacking. A BACI experimental design on leaf-litter decomposition at an assimilation wetland in Thibodaux, Louisiana found that neither leaf-litter decomposition rates nor initial leaf-litter N and P concentrations were affected by wastewater effluent (Rybczyk et al. 2002). Currently though, monitoring efforts at most assimilation wetlands do not require research on litter or soil decomposition rates, microbial processes, or soil strength, all of which may help to understand holistic effects of wastewater effluent on the ecosystem.

Some studies suggest that nutrient enrichment may increase herbivory and plant biomass reduction, creating an indirect threat to wetland stability, particularly in wetlands dominated by emergent herbaceous plant species. When concentrations of bioavailable N are elevated in the soil, some plant species exhibit luxury consumption of N, resulting in increased plant tissue concentration of N (Tripler et al. 2002). The resulting increased plant tissue concentrations of N make these plants more palatable to herbivores (e.g., invertebrates, nutria (*Myocastor coypus*), and large ungulates) (Archer et al. 1982; Tripler et al. 2002; Ialeggio and Nyman 2014). Following the release of effluent into the receiving wetlands in the Louisiana Joyce Wildlife Management Area in 2006, the receiving wetland's function declined. This area had historically been dominated by baldcypress-water tupelo swamps, but in recent years the swamp has converted to herbaceous marsh (Shaffer et al. 2015). Following a brief initial boom of herbaceous plant growth, nearly all of the marsh south of the discharge point had been converted to mudflat within 9 months (Shaffer et al. 2015). The cause of this severe marsh degradation at this site is debated, with some attributing the loss to herbivory by nutria (Shaffer et al. 2015; Day Jr et al. 2019b), and others attributing the damage to elevated decomposition rates due to increased N loads and hydraulics (Bodker et al. 2015; Turner et al. 2018). It is possible that multiple factors, both abiotic and biotic, contributed to the wetland degradation following the release of effluent.

Finally, increased inundation plays a major role in wetland functionality and sustainability. Increased inundation reduces soil stability by increasing soil-moisture content (Hough 1957) and by causing mortality in trees that cannot tolerate increased inundation. While this may decrease competition for resources for more flood-tolerant tree species such as baldcypress and water tupelo (Conner and Day Jr 1988; Conner et al. 2014), it may also result in reduced soil strength and stability. In a myriad of ecosystems, tree mortality has caused reduced soil shear strength, rapid elevation loss, peat collapse, and erosion (Putz et al. 1983; Cahoon et al. 2003). Many of Louisiana's assimilation wetland treatment plants discharge up to 1 million gallons of water per day into the receiving wetland, greatly altering hydrology and increasing inundation. Plant species differ in their physiological tolerance to flooding regimes, therefore it is to be expected that many previously established species may die following alteration to the hydrology. Although this review paper focuses on physical wetland drivers (hydrology and nutrient loading), it is important to note that discharge of municipal wastewater effluent into any ecosystem may impact habitat quality through introducing heavy metals, pharmaceuticals, illicit drugs, and environmental estrogens to the environment (Kasprzyk-Hordern et al. 2009)- a topic that could serve as its own separate review.

Carbon Sequestration & Mitigation of Climate Change Impacts

Introducing nutrient-rich water to wetlands has been shown to increase wetland capacity to sequester CO_2 via increased plant productivity (Day Jr et al. 2004) and increased burial of organic matter/carbon (Rybczyk et al. 2002). For these reasons, some posit that assimilation wetlands may create additional opportunities to mitigate carbon and provide markets to sell carbon credits for mitigation (Lane et al. 2017; Hunter et al. 2018). Introducing nutrient-rich water to wetlands has also been idealized to help wetlands cope with sea level rise and saltwater intrusion via increased accretion rates (Day Jr et al. 2004) and introduction of freshwater (Hunter et al. 2018). However, these benefits are applicable only to a narrow geographic range of wetlands; and more information is needed on carbon storage and flux dynamics, discussed in further detail below. Furthermore, changes in wetland drivers such as hydrology and nutrient loading my influence carbon dioxide and methane fluxes, which are crucial for understanding if these systems are carbon sinks or sources (Kayranli et al. 2010). Also, it is important to note that uptake of nutrients by plants represents a short-term sink relative to burial, and may lead to a pulse of nutrients upon death of plants that may not be captured in monitoring.

Energy and Economic Savings

When successfully implemented, assimilation wetlands reduce the operation and maintenance costs and energy demand compared to conventional tertiary water treatment systems and constructed wetlands (Day Jr et al. 2004; Ko et al. 2004; Louisiana Department of Environmental Quality (LDEQ) 2018). Conventional wastewater treatment plants typically have a lifespan of less than 30 years and today many of the wastewater treatment facilities in developed nations are in need of significant overhaul (Sundaravadivel and Vigneswaran 2001). For tertiary treatment of ~3800 m^3/d of wastewater, a conventional sewage treatment plant would require 5 acres of land and ~ $4 million with an annual maintenance cost of $156,000 (Kadlec and Knight 1996; United States Environmental Protection Agency (USEPA) 1999). Alternatively, the passive plant-mediated water treatment process used in a constructed wetland would require more land (~90 acres) and less financial investment (only ~$3.6 million with an annual maintenance cost of

$45,000 per year) for the tertiary treatment of the same volume of water (Kadlec and Knight 1996; United States Environmental Protection Agency (USEPA) 1999). Constructed wetlands also provide additional benefits, such as greater removal efficiency of suspended solids, total N, and P compared to conventional treatment plants (Lee et al. 2009). Tertiary treatment using an assimilation wetland is believed to further reduce construction costs and energy demands (Hunter et al. 2019). Day Jr et al. (2004) found that using wetland assimilation, compared to conventional treatment, reduced the cost by 30% to 80%.

Assimilation wetlands are highlighted for their low maintenance demands and long life spans. However, this assumes the receiving system will be able to function with the continued press of excess nutrients and increased flooding indefinitely. Although some assimilation wetlands in Louisiana have functioned for upwards of five to seven decades (Hunter et al. 2018; Hunter et al. 2019; Day et al. 2019a), the long-term response of a wetland to increased flooding duration and nutrient loading may differ depending on the wetland type and hydrogeomorphic setting. It is possible that the wetland's capacity to assimilate excess nutrients may decrease should the system become stressed by changes in wetland drivers. If the biological systems driving the functions of these assimilation wetlands are compromised, the system may incur additional costs such as increasing infrastructure to pipe effluent to a new location, installing a conventional tertiary treatment system, or paying fines for violating state standards. That being said, it is important to acknowledge that any tertiary treatment of wastewater is valuable compared to no tertiary treatment, especially where tertiary treatment is not required by governing agencies.

Critical Research Gaps

To ensure success of an assimilation wetland, managers must ensure appropriate loading rate and total maximum daily loads (TMDLs), proper effluent disinfection, management of contaminants such as metals and pharmaceuticals, vegetation monitoring, and herbivore (e.g., nutria) management (Hunter et al. 2018). We agree that careful consideration and monitoring of the abovementioned factors are crucial to properly managing a healthy wetland. Additionally, we propose several lines for further scientific inquiry to improve understanding and management of assimilation wetlands. These research directions include: 1) frequency and duration of flooding; 2) system longevity and press vs. pulse dynamics; 3) carbon and nutrient cycling; and 4) impacts on higher trophic levels.

Frequency & Duration of Flooding

Frequency, depth, and duration of flooding controls wetland plant species assemblages' survival, distribution, and dispersal (Mitsch and Gosselink 2015) and these hydraulics have variable impacts on plants depending on species. The majority of existing assimilation wetlands in the U.S. occur in Louisiana, and many of these systems are characterized by periodically inundated bottomland hardwood forest dominated by *Fraxinus pennsylvanica* (green ash), *Acer rubrum* (red maple), *Smilax* spp. (greenbriar), *Sabal minor* (dwarf palmetto), *Campsis radicans* (trumpet creeper), and *Toxicodendron radicans* (poison ivy) (LDWF 2005). Other assimilation wetlands pump effluent into poorly drained cypress-tupelo swamps, dominated by flood-tolerant *Taxodium distichum* (baldcypress) and *Nyssa aquatica* (water tupelo). While both of these systems can tolerate flooding, they are normally characterized by a seasonal pulse and subsequent drawdown period. Increased inundation from discharging effluent into the system may cause mortality in bottomland hardwood tree species that require better drainage. Conner et al. (1997) found that increased freshwater flooding resulted in reduced growth of green ash, and increased diameter growth of baldcypress and water tupelo. Flooding, combined with increased salinity (2 ppt) caused reduction in growth of water tupelo, green ash, and Chinese tallow. Baldcypress and water tupelo are notably more flood-tolerant than most other tree species in the region (Carter et al. 1973; Mitsch and Rust 1984). However, constant flooding prevents baldcypress seed germination (DuBarry 1963; Shaffer et al. 2009). Adult baldcypress and water tupelo will exhibit a reduction in total basal area if water levels rise and many studies suggest that continuous flooding will result in their eventual death over time (Harms et al. 1980; Mitsch and Rust 1984; Conner and Brody 1989; Shaffer et al. 2009).

At the Thibodeaux, Louisiana assimilation wetland site, water level has risen in both the treatment and reference sites over the site's 25-year history, partially attributed to relative sea level rise, hurricanes, and increased rainfall (Rybczyk et al. 2002; Hunter et al. 2018). Minor (2014) reported that water levels were higher at the effluent discharge site compared to reference and that by 2014 almost all bottomland hardwood tree species had died. In an assimilation wetland in Hammond, Louisiana, the receiving wetland had been historically influenced by a variety of hydraulic modifications including the construction of levees, railways and interstates, as well as a 1.2 km wastewater effluent discharge distribution pipe (Lane et al. 2016). Aerial imagery taken of the site between 2006 (the initiation of discharge pipe) and 2011 show severe conversion of marsh to open water (Bodker et al. 2015). Hydrologic monitoring showed that mean annual water level increased over time since operation, though water level data before construction of the pipe is limited to 1 year (Lane et al. 2016). Lane et al. (2016) suggest that allowing for drawdown period would alleviate water level stress to vegetation and promote nutrient assimilation. However, Turner et al. (2018) explained that the loss of vegetation is a result of the loss in soil strength associated with nutrient loading.

Inundation dynamics can impact the sediment chemistry. For example, partial drying of inundated sediment can result in an increased sediment affinity for P and can create vertical patchiness in soil conditions resulting in differential nitrification and denitrification (Baldwin and Mitchell 2000). Partial drying may also reduce the availability of N and P, whereas inundation of dry sediments will often result in an initial flush of available N and P, and nitrification. Inundation of floodplain soils can result in a liberation of C, N, and P from leaf litter and soil organic matter, which may result in an initial response of increased productivity followed by anoxia, and increased release of P and denitrification (Baldwin and Mitchell 2000).

Field-based studies at assimilation wetland sites are limited in number and the nature of these field based studies make it difficult to parse apart the influences of water quality/nutrients and inundation. Field studies are inherently problematic to control due to multiple influences on hydrology. Although reference sites are paired with treatment sites in assimilation wetland monitoring, the treatment effects of excess nutrients and excess flooding cannot be separated and there is a lack of studies that quantitatively assess nutrient uptake and denitrification along flow paths. Thus shifts in plant communities at treatment sites may be disproportionately driven by one of several covariates, but determining which one is difficult. We recommend greater incorporation of field based experimentation at these living laboratories, where wetland drivers would be experimentally manipulated at the plot-scale to answer a variety of questions in these systems. Field based experimentation methods may include using marsh organs to manipulate inundation (McLain et al. 2020), or using larger-scale field-based marsh mesocosms to controlflow and inundation (Alt 2019, Roberts et al. 2019, 2020; additional details can be found at http://robertsresearchlab.weebly.com/mesocosms.html). Additionally, controlled greenhouse/mesocosm experimentation mimicking the conditions at assimilation wetlands would aid in our understanding of the relative importance of these multiple influences.

Longevity – Pulse vs. Press

With the exception of a few sites, the majority of permitted assimilation wetland sites in the U.S. have only been operating for roughly 10 to 20 years. Unfortunately, longer operating sites lack the critical baseline data needed to make accurate assessments about ecosystem responses over time. Currently, there is no system in place for understanding how receiving assimilation wetlands may differentially respond to effluent pulses versus continuous press. However, field-based studies have shown ecosystem collapse driven by increases in inundation and nutrient loading may take many years to realize (Deegan et al. 2012; McCoy et al. 2020). Nichols (1983) cautioned that the adsorption and precipitation of phosphorus in these systems is "not a limitless sink" and the capacity for a wetland to retain P in soil declines over time as the soil becomes saturated. Craft (1996) found that in constructed wetlands, P removal is greatest in the first few years when sediment deposition and sorption/precipitation of P is greatest and over time P retention decreases. Furthermore, N that is taken up by plants is released back into the water when plants senesce and die (Nichols 1983). Therefore, removal efficiency of both N and P is best when loading rates of these nutrients is low and may not retain efficiency in the long term. The capacity for wetlands to remove nutrients has also been shown to decrease with higher loading rates for both N and P, thus the performance of the wetland to provide this service may decrease over time with continued loading and saturation (Fisher and Acreman 2004).

Additional study is needed to fully understand the nutrient-removal capacity and nutrient burial limitations of wetlands (Valiela et al. 1976; Hunter et al. 2018). As many of these sites are dominated by baldcypress (a species that requires a draw-down period for regeneration), degradation of the site may not be apparent for many years following introduction of effluent. Taking an experimental approach (e.g., strategically planning discharge start and end periods, or alternating discharge between two hydrologically separated wetlands when applicable)would greatly improve understanding of how to best manage the system. Unfortunately, the logistics of management do not always support experimental design.

Carbon & Nutrient Cycling

Wetlands are important ecosystems in global carbon cycles, as they contain a disproportionately large percentage of the world's carbon compared to their size (Dixon and Krankina 1995; Whiting and Chanton 2001). However, wetlands carbon budgets are complicated as they can act as both a carbon sink and a carbon source (Kayranli et al. 2010), and carbon fluxes between different pools. Carbon accumulation rates summarized from available literature suggest that constructed wetlands and assimilation wetlands remove carbon at one to two orders of magnitude greater than natural wetlands not receiving effluent (Table 2). Lane et al. (2017) found that an assimilation wetland in Luling, Louisiana sequestered more CO_2 (in trees and soil organic carbon) and emitted less CO_2 compared to baseline values found in literature. However, the study omitted carbon pools such as herbaceous vegetation, leaf litter, and dead wood, as these factors were not expected to change during the duration of their study. To truly understand carbon flux and storage dynamics in assimilation wetlands, studies would benefit from a greater emphasis on soil carbon accumulation rates and incorporation of fluctuating forms of carbon: plant biomass carbon, particulate organic carbon, dissolved organic carbon, microbial biomass carbon, and gaseous end products (Kayranli et al. 2010).

Table 2 Net carbon retention reported in soils of various types of wetlands worldwide

Wetland Type	Net Carbon Retention ($gC\ m^{-2}\ yr^{-1}$)	Source
Natural Wetlands		
Average for world's wetlands	118	Mitsch et al. 2013
Average for tropical wetlands	129	Mitsch et al. 2013
US Everglades (unenriched)	65–90	Craft and Richardson 1998
US Everglades (enriched, 1960–1998)	184–223	Craft and Richardson 1998
Quercus palustris forested wetlands	473	Bernal and Mitsch 2011
Riverine communities	140	Bernal and Mitsch 2011
Nelumbo lutea dominated wetlands	160	Bernal and Mitsch 2011
Depressional wetlands	317	Bernal and Mitsch 2011
Depressional wetlands (organic C)	35–50	Lane & Autrey, 2017
Temperate freshwater wetlands	143	Mitsch et al. 2013
Brackish tidal wetlands	84–128	Callaway et al. 2012
Saline tidal wetlands	69–99	Callaway et al. 2012
Coastal Wetlands	33	Brevik and Homburg 2004
Wetland Type	**Soil C Density ($g\ C\ cm^{-3}$)**	**Source**
Salt Marsh (Soil)	.039 [b]	Chmura et al. 2003
Mangroves (Soil)	.055 [b]	Chmura et al. 2003
Constructed & Assimilation Wetlands		
Created Flow Through	219–267	Mitsch et al. 2013
Horizontal Subsurface Flow	1500–2200	Mander et al. 2008; Kayranli et al. 2010
Gravel bed Constructed (unplanted) [a]	200–1150 [c]	Tanner and Sukias 1995
Gravel bed Constructed (planted) [a]	2000[c]	Tanner and Sukias 1995
Assimilation Wetland (Trees + Soil)	855	Lane et al. 2017

[a] Conversions of Organic Matter to C assumed 50% of matter was C (Day Jr et al. 2004)

[b] Note that measurement presented as $g\ C\ cm^{-3}$, and not as a function of time

[c] Measurements were presented as $g\ C\ m^{-2}$, and not as a function of time

Herbaceous macrophytes accumulate nutrients which senesce in the winter in temperate and subtropical regions. As emergent macrophytes and submerged aquatic vegetation die, they decompose, which raises the nutrient concentration in the water (Godshalk and Barko 1985), lowers oxygen level in water and sediment (Pereira et al. 1994), and significantly influences the recycling of nutrient and net carbon storage of the ecosystem (Carpenter and Lodge 1986; Richardson 1994). Accounting for the decomposition of herbaceous aquatic plants is essential to understanding carbon and nutrient dynamics of wetlands, (Rich and Wetzel 1978; Webster and Benfield 1986; Battle and Mihuc 2000; Xie et al. 2004), particularly if plant communities subjected to continuous flooding transition from forested wetlands to herbaceous marsh.

Increased flooding associated with effluent may also play a role in carbon storage dynamics. Jones et al. (2018) found that increased flooding depth stimulated C exchange in a controlled wetland plant mesocosm. Increased flooding depth resulted in decreased soil C pool while marginally increasing aboveground biomass C pool, leading in net loss in total C stocks. Continuing these types of controlled experiments on more plant species and at larger scales relevant to assimilation wetlands would enhance this field of study, as well as ecosystem management- especially if these systems are to be used for future carbon mitigation purposes.

Trophic Impacts

A perceived benefit of assimilation wetlands is the ecological restoration of degraded wetlands (Hunter et al. 2018; 2019), however, most research at assimilation wetlands draw conclusions about ecosystem heath and functioning without considering changes to higher order consumers (Hunter et al. 2018). Shaffer et al. (2015) found that herbivory by the exotic invasive nutria (*Myocastor coypus*) and waterfowl contributed to the decline in herbaceous plant growth at an assimilation wetland in Hammond, Louisiana. This study suggests that the addition of nutrient rich wastewater effluent caused an increase of vertebrate primary consumers at this assimilation wetland. At the same site, Weller and Bossart (2017) found that benthic insect diversity declined in response to the addition of effluent and vegetation loss.

These are the only published studies conducted at assimilation wetlands that investigate impacts on higher trophic level organisms. However, work done in other systems indicates that changes in hydrology, nutrient levels, or a combination of these factors has the potential to cause changes in higher order consumer diversity (Hulot et al. 2000; Isbell et al. 2013; Nielsen et al. 2013), density (Nakamura et al. 2005), biomass (Davis et al. 2010; Nelson et al. 2018), and trophic structure (Marks et al. 2000; Merwe and Hellgren 2016; Chanut et al. 2019). These studies indicate that whether changes in nutrient and/or water level have positive, negative, or neutral effects depends greatly on what species occur at the site and the ecosystem being studied. Additionally, McCann et al. (2020) found that nutrient addition can destabilize food webs due to the loss of equilibrium driven by competitive exclusion of edible plant species. Whether their theory can be applied to discrete assimilation wetlands deserves further research which allow for more accurate assessment of the whole ecosystem heath and functioning.

Conclusions

Use of assimilation wetlands has potential to reduce financial stressors on municipalities while promoting environmental productivity, however, with so many of the world's wetlands in a state of degradation and decline, it is imperative that management decisions do not contribute to further degradation of these fragile systems. Existing monitoring at assimilation wetlands track trends in water quality, measure partial net primary productivity, and partial carbon dynamics; however, a number of important environmental metrics require additional study. For example, more frequent sampling of nutrients across greater spatial extents is needed to better understand pulsing dynamics and the relative influence of temporary nutrient removal mechanisms (plant uptake) and long-term nutrient removal mechanisms (denitrification, soil accumulation/burial). This field of study would also benefit from investigations into the responses of soil physicochemistry (organic matter, shear strength, carbon storage, redox potential, and decomposition), plant community dynamics (especially regarding non-target herbaceous species), productivity and trophic transfer of energy, and greenhouse gas flux dynamics resulting from the increased inundation and nutrient enrichment posed by the assimilation wetland process. Through this review, we recommend to guide future research in these directions for the end goal of informing and enhancing management and reducing/preventing environmental degradation. A suite of holistic indicators should be defined to identify early signs of stress/degradation in the receiving wetland so that appropriate response decisions can be made- such as shifting flooding regimes, diverting effluent to other areas, limiting effluent discharge on the wetland, or employing other tertiary treatment methods. Continued development of science-based protocol for management can further inform adaptive management strategies and operations to preserve the integrity of receiving wetlands and the financial investment of the municipality.

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Declarations

Conflict of Interest This research group is currently involved monitoring and research of an assimilation wetland.

Ethics Approval not applicable.

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