NITROGEN REMOVAL EFFICIENCIES IN CONSTRUCTED WETLANDS

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NITROGEN REMOVAL EFFICIENCIES IN CONSTRUCTED WETLANDS

by

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A CAUTION TO THE READER

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ABSTRACT

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Keywords: constructed wetlands, denitrification, nitrification, nitrogen loading, plant assimilation, run-off, wastewater

This thesis summarizes the usage of constructed wetlands in reducing nitrogen loaded anthropogenic waste water, sourcing a collection of results of outside literature. The literature used in this thesis was found from scholarly websites. The purpose of this study was to determine the most efficiently constructed wetland design in terms of its nitrogen content removal, and to provide further insight into the new constructed wetland technology. In this study the term efficiency is based on the nitrogen removal rate determined by each data variable. Wetland type / design and removal mechanism were the attribute data collected and examined, all of which was found in the results of prior literature. The two removal mechanisms used were denitrification / nitrification and plant assimilation. The two-wetland types used in this study were surface flow constructed wetlands and subsurface flow constructed wetlands. A one – way ANOVA was completed to determine the significant difference in nitrogen between the two removal mechanisms, as well as the significant difference between the two wetland designs. It was determined that there is no significant difference in nitrogen removal between the removal types and wetland designs. Insight and comparison with each constructed wetland study were allowed for the results to be accepted. Two scientific rationalizations were discussed to further accept the results found. These rationalizations included the overall performance of nitrogen removal and the secondary variables related to the wetland design. It was concluded that further research is needed to further comprehend this constructed wetland technology.

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CONTENTS

TABLES

FIGURES

1. INTRODUCTION

Nitrogen is an essential element required for successful plant growth (Brady 1990). Inorganic and organic nitrogen fertilizers are applied to maintain the nutritional condition of different cropping systems. Once nitrogen fertilizers are applied to agricultural and urban systems, the fertilizers are absorbed directly by plants. If the nitrate is not absorbed by plant roots, it is carried away by run-off and/or leaches into the soil along with water (Liu *et al.* 2014). This leaching nitrogen has great consequences on surface water and requires technology to reduce the pollution prior to reaching groundwater systems.

The largest problem with increased nitrogen in these wetlands is the endangerment it has on the wetland's biotic elements, specifically the interactions among the local flora and fauna. Interspecies competitive relationships change with the nitrogen status of the environment (Morris 1991). Increased acidity in the soil hinders some primary producers in such wetlands and therefore impedes the relationship and food source for secondary and tertiary consumers. Increased nitrogen in wetlands can cause artificial eutrophication (Harper 1992), significantly reducing any form of primary production and secondary consumptions. Eutrophication in wetlands often result in a significant anaerobic state in the wetland, and ultimately alters all forms of microbially activity (Harper 1992).

Naturally, wetlands provide many services to the surrounding ecosystems, such as water filtrations and nutrient cycling (Brander 2013), which naturally removes abundance of nutrients such as nitrogen. These services are equally abundant in manmade or restored [constructed] wetlands which make these wetlands a suitable form of technology for pollution removal in anthropogenic wastewater. Constructed wetlands are recent developments to which are used to remove nutrient loading from wastewater and run-off. These wetlands are very cost-effective and can be operated very easily (Kivaisi 2001).

Generally, it is found that both natural and constructed wetlands perform efficiently as water purifiers and nutrient sinks. However, the complexity within the natural system make it hard to predict or establish an outcome to which these processes take place (Moshiri 1993). Constructed wetlands can be maintained with a much greater control, (i.e., substrate, vegetation cover, water flow speed) and offer more advantages then that of a natural wetland. When manufacturing a constructed wetland, the main focus is to create an identical design and process of natural wetlands. The mechanisms of constructed wetlands are based on the assumptions and outcomes provided by the natural system. Therefore there are little differences among the two in design (Hammer and Knight 1994), besides the control of secondary variables.

The removal of nitrogen often relies on a diverse range of co-existing physical, chemical and biological routes, which vitally depend on numerous environmental and operational parameters (Saeed and Sun 2012). Nitrogen removal processes that dominate constructed wetlands include the microbially interactions of denitrification and nitrification and biological processes of plant assimilation. The process of denitrification relies on microbial activity to transform the abundance of nitrogen into atmospheric nitrogen and remove the nitrogen loading from the wastewater. The presence of vegetation in the wetland allow for nitrogen to be taken up through the roots and into the living stem and leaves of a plant (Saeed and Sun 2012). Each of these internal processes is essential in constructed wetland designs and are examined throughout this thesis to determine the efficiency of nitrogen removal.

After a thorough examination of literature and corresponding results to nitrogen removal, the major findings include the inlet and outlet quantity (mg/N/l) of nitrogen in each individual constructed wetland. The major findings also include the corresponding removal rates (%) and secondary variables that influence such removal rates. Using an ANOVA statistical analysis, it was determined that the secondary variables represent a much larger portion of the nitrogen removal transformation as originally considered throughout the literature search. These secondary variables explain the implications with consistent and efficient nitrogen removal throughout all constructed wetlands studied.

1.1.OBJECTIVE

The purpose of this research is to collect and examine data through a literature search and determine how efficiently constructed wetlands are at removing elements of nitrogen. Specifically, this research will focus on which process of nitrogen removal is most efficient based on the difference between the influent and effluent of nitrogen in the constructed wetland. The statistical analysis will compare the nitrogen removal efficiencies between microbial removal and plant assimilation, as well as the design of the constructed wetland, through a variance analysis. Secondary factors that will impact the outcome include seasonality, temperature, environment and landscape design. This thesis will provide an insight into the development of this pollutant removal technology and determine its prospects for both urban nitrogen run-off and agricultural nitrogen run-off.

1.2. HYPOTHESIS

Using the information examined in the literature review, a hypothesis was generated to determine the most effective form of constructed wetland at reducing nitrogen loading. The null hypothesis for this literature research is that there is no significant difference (alpha = 0.05) among the two natural methods of nitrogen removal ($A_i = 1,2$) and the type of constructed wetland ($B_j = 1,2$). The p-value being used in this data collection will represent whether or not there is a significant difference in the removal of nitrogen (%).

A_1 = Nitrification/ Denitrification

A_2 = Plant Assimilation

B_1 = Surface Flow constructed wetland

B_2 = Subsurface flow constructed wetland

$A_1 = A_2$ Equation [1]

$B_1 = B_2$ Equation [2]

1.3. LITERATURE REVIEW

Increased urbanization and overpopulation have caused an enormous increase in fertilizer use around the globe. Between 1962 to 2001 the annual production of nitrogen-based fertilizers increased from 13.5 Tg to 86.4 Tg worldwide (Mosier *et al.* 2013). Production and utilization of these fertilizers amplify nutrient loading in numerous natural ecosystems, including wetlands.

The use of constructed wetlands for reducing wastewater pollution is a fairly new development, as it has only been required in the last few decades. Studies over the last few years have shown that both natural and constructed wetlands systems provide high-quality wastewater treatment at a relatively low cost (Hammer 1989:856).

Mitsch and Gosselink (2000) believe that the true value of a wetland is its ability to remove pollutants from human forces. In the case of constructed wetlands, its value is completely reliant on the economic and environmental ability to remove nutrients. It is important to consider the environmental principles that make a constructed wetland effective at nutrient removal, as a way to ensure economic stability with this new technological development for constructed wetlands.

1.3.1. Nitrogen in Natural Wetlands

Natural wetlands receive, hold, and recycle nutrients regularly swept from upland regions (Hammer 1989), including mass amounts of phosphorus and nitrogen. Bowden (1987) states that the greatest portion of wetland nitrogen is found within the sediments. Wetland plants intake about 35g of nitrogen per m^2 per year. Water retention time is the most critical factor in removing nitrogen from water and is measured by the amount of nitrogen per unit m^2 (Jansson *et al.* 1983, Smith *et al*. 2000). Wetlands have a low significant flow rate which indicates that they are not very good at nitrogen retention and therefore shows how reliant they are on nitrogen removal. Nitrogen's role is one of the most important elements in an aquatic ecosite (Jansson *et al.* 1983).

There are several types of wetlands, including marshes, bogs, swamps, and fens, which are distinguished by several abiotic and biotic features (Van de Valk 2006). Each wetland functions differently and cycles nutrients in a variety of different ways. A study done by Bedford *et al.* (1999) discovered that marshes have significantly lower mean nitrogen to phosphorus ratio in their living plant tissues and therefore have a greater ability to remove nitrogen from wastewater as a means of plant assimilation. Bedford *et al.* (1999) also discovered that overall freshwater wetlands have a higher N:P ration when there is litter and plant tissue in the wetland.

1.3.2. Nitrogen Loading

Postgate *et al.* (1980) discuss the importance of nitrogen in agriculture and how agriculture productivity is entirely determined by the availability of nitrogen in the soil. The majority of nitrogen being inputted into the land and water is in the form of recycled inorganic nitrogen transformed from N_2 fertilizer used in both urban and agricultural settings. A higher amount of nitrogen fertilizer being used often results in a greater amount of nitrogen in soil and leaching into the groundwater within a wetland ecosystem (Guo *et al.* 2006). Ultimately, an increase in nitrogen loading simultaneously increases nitrogen removal based on a series of factors which affect the removal, including nitrification and denitrification (Jordan *et al.* 2011).

Controlling nitrogen loading from anthropogenic sources requires the ability to measure nitrate in saturated and unsaturated zones (Guo *et al*. 2006). A study was completed by Lui *et al.* (2005) for modelling the movement and transformation of nitrogen within a subsurface flow constructed wetland. The model created used the single outside variable of temperature, as Lui *et al.* (2005) believed it as the most influential variable affecting nitrogen within the constructed wetland.

1.3.3. Design of Constructed Wetlands

Wetland design represents an enormous part in the process performed by the wetland, including nutrient removal. Wetland size is a very important component in determining how efficient the system will be at removing nitrogen and other pollutants (Arheimer and Wittaren 1994). Studies show that there is a net reduction of nitrogen transport per unit area with an increase in the total area of each wetland cell (Arheimer and Wittaren 1994; Nichols 1983). Over 1 hectare of wetland is required to remove 50% of the nitrogen being loaded into the wetland through wastewater generated by 60 people (Nichols 1983). Influent rates and quantities averaged over urban and rural areas are required when designing both overall wetland size and individual cell size within the wetland ecosystem.

Kaldec *et al.* (2012) conclude that the productivity of a constructed wetland is based on appropriate water temperatures, hydraulic efficiency and flow pattern. These components of the wetland can be accomplished with proper design. Appropriate placement of a constructed wetland within the landscape ensures hydraulic efficiency and proper flow of water throughout the wetland. The efficiency of nutrient removal [if the designation was meant for nutrient removal] is done based on the flow of water throughout each cell in the wetland, determining that appropriate landscape placement essential.

Studies suggest that a balance between natural and constructed wetlands are the most reliable when removing nutrient loadings from the hydrological cycle. In Estonia, a majority of constructed wetlands used for nitrogen removal are designed using a natural/semi-natural wetland cell design (Mander *et al.* 1997). Jan Vymazal (2007) found that vertical flow constructed wetlands provide poor conditions for denitrification and horizontal flow constructed wetlands provide greater conditions for denitrification processes. Vymazal (2007) suggests that various types of constructed wetlands be combined with each other in order to intensify specific advantages to each design when exploiting nitrogen removal systems.

Yeh *et al.* (2010) also concluded that hybrid design for constructed wetlands is the most effective for treatment purposes. This study was using the oxygen content found within each cell of hybrid [natural cells and constructed cells] wetland. Yeh *et al.* (2010) determined that because denitrification favours aerobic cells and nitrification favours anaerobic it is most appropriate to incorporate a hybrid design when constructing a wetland for wastewater treatment to ensure the productivity of each internal removal processes.

1.3.4. Denitrification / Nitrification Processes

Denitrification is a process in which nitrogen is removed via a series of microbial interactions (Bowden 1987). These microbial interactions reduce nitrate into nitrite and then further down into nitrogen gas which is released into the atmosphere and ultimately removed from the hydrological cycle (Holman and Wareham 2005). Denitrification rates are primarily controlled by the concentration of nitrate and organic soils within the constructed wetland (Poe *et al.* 2003).

In the study done by Poe *et al.* (2003), it was concluded that following a rainfall event there was a 400% increase in the denitrification rate in response to the increase inorganic nitrogen fertilizer loading from the current flooding and run-off. The increase in nitrogen loading from such events allows for greater microbial interactions between organic material found in soil and wastewater. Knuth *et al.* (2011) and Seitzinger *et al.* (1994) both concluded in their studies that denitrification rates typically increase within sediments which have higher organic material and source of sufficient NO_3. The organic material found in these sediments account for the microbial elements required for denitrification to take place.

Nitrification refers to the process of conserving nitrogen in the wetland through the storage in sediments (Bowden 1987). Nitrite and ammonia are transformed into nitrates via bacterial interactions between the water and soil within the wetland (Bowden 1987). Smith *et al.* (2000) concluded in their study that the nitrification process in each wetland is enhanced by the reduction of emergent biomass. The nitrification removal pathway is heavily reliant on the Biochemical Oxygen Demand within the wastewater and water content in the wetland (Bustillo-Lecompte *et al.* 2016). A study was done by

Cottingham *et al.* (2010) to determine if increased aeration (oxygen content) within the constructed wetland would enhance the nitrification process. It was found that nitrification rates increased as a result of increased competition between the nitrifying bacteria and heterotrophic bacteria used in the denitrification process (Cottingham *et al.* 2010).

1.3.5. Plant Assimilation of Nitrogen

Vegetation coverage is a key biological factor in controlling nutrient loading and abundance in constructed wetlands, both indirectly and directly (Ruiz-Rueda 2009). Directly plant biomass acts a stabilizer for water flow which increases the retention time of nutrients within the wetland. Plant assimilation of nutrients is an indirect biological process to removing nitrogen from wastewater within the constructed wetland. Gottschall *et al.* (2007) identified plant assimilation of nitrogen in a constructed wetland to be very insignificant in the nitrogen removal process.

Plants uptake nitrogen within the growing season but release it back into the wetland during the death and decaying season (Nichols 1983). Nitrogen removal through vegetation does not require any secondary source of energy or input and can be completed in very stagnant abiotic ecosystems (Hausmann *et al.* 2007). Gottschall *et al.* (2007) concluded that a greater amount of nitrogen removal via plants was often found in the final cells of the wetland, where there was a decrease in energy.

A study was done by Liu *et al.* (2010) to determine how nitrogen removal was affected by the addition of vegetation to the wetland, specifically cattail species. It was discovered the individual wetlands cells that contained vegetation were significantly more productive at removing nitrogen then cells without. Vegetation is a key element in all seasons, as studies have shown that certain plant species are effective at removing nitrogen in the winter/colder months. Gao *et al.* (2014) determined that assimilation of nitrogen is done in the below-ground portion of the plant in colder stagnant temperatures. This study was done using three separate wetland cells and determined that the cell with a higher amount of both living and decaying vegetation was more effective at removing nitrogen from wastewater than the cell without any vegetation and the one with lower quantities of vegetated biomass.

It was determined that the rate of nitrogen assimilation is dependent of a series of variables, including and most importantly the amount of nitrogen influent being introduced to the wetland (Liu *et al*. 2010). A study was done by Tao *et al.* (2012) concluded that plant growth is negatively affected by the increase of influent and therefore has a negative removal efficiency. Components of plant growth and production are affected by the nutrient loading in the wetland and therefore impact the rate of nutrient assimilation is done by the plant. A study was done by Xu *et al.* (2013) that determined biomass, density, and photosynthetic activity are all indirectly affected by the increase of nitrogen in the wetland. Although plants are a very reliable source of nitrogen removal, and over saturation of nitrogen uptake can have serious consequences on plant productivity and metabolic processes (Xu *et al.* 2013).

1.3.6. Secondary Variables in Nitrogen Removal

The process in which nitrogen is removed from an ecosystem is extremely dependent on outside variables; including temperature and landscape. In a study done by

Lee *et al.* (1999), it was determined that temperature severely impacts the removal of nitrogen, as it is a significant regulator for seasonal nitrogen removal (Tan *et al.* 2017). It was found that in areas with lower temperature removed nitrogen at a lower rate and overall amount and constructed wetlands found in temperatures above 20 degrees Celsius were capable of removing more than 50% of nitrogen from the influent wastewater (Lee *et al.* 1999). Kadlec and Reddy (2001) state that microbially reactions [nitrification and denitrification] are heavily affected by temperature. Plant assimilation is lower in colder temperatures. Also a majority of wetland plants are annual plants and therefore die off each fall (Hausman *et al.* 2007), which both decreases their uptake in nitrogen as well as releasing nitrogen content taken up during their productive months (Nichols 1983).

Seasonality is also a factor contributing to nitrogen removal, primarily based on the temperatures accompanied with each season. Nitrate removal is most efficient during summer seasons where there are increased temperatures for long periods of time (Spieles and Mitsch 1998, Yang *et al.* 2010). Winter and spring seasons account for little to no nitrogen removal because of the low temperatures and increased influent loading from flooding precipitation during these months (Spieles and Mitsch 1998).

2. MATERIALS AND METHODS

This literature review-based thesis was conducted following the guidelines set out in the Natural Resources Management writing guide. The process began by conducting a topic search in three separate databases. These databases included Scholars Portal and JSTOR and Web of Science. There were no temporal or spatial restrictions set on the literature search.

2.1. STUDY INCLUSION CRITERIA

In order to be considered within this literature review, studies were required to fulfill certain criteria. This criterion includes;

a) The study must follow a similar interposition of anthropogenic wastewater. The nitrogen content being studied in the constructed wetlands had to have been from anthropogenic forces such as agricultural fertilizers and/or storm run-off, urban establishments, or industrial run-off. Studies focussed on constructed wetland use for any other nutrient, including phosphorus, were excluded from the search. Studies which focussed on nitrogen loading from natural systems such as flooding were discarded from this literature review.

b) The study must contain similarly constructed wetland ecosystems. The constructed wetlands being considered in the study must be of 2 construction designs; surface flow constructed wetland or subsurface flow constructed wetland. These constructed wetland designs can be seen in Figure 1 and Figure 2.

c) The study must follow the similar outcome of nitrogen fluctuation. All studies that concluded a change in nitrogen were considered. The process in which the nitrogen was reduced was compiled as separate variables within this literature review.

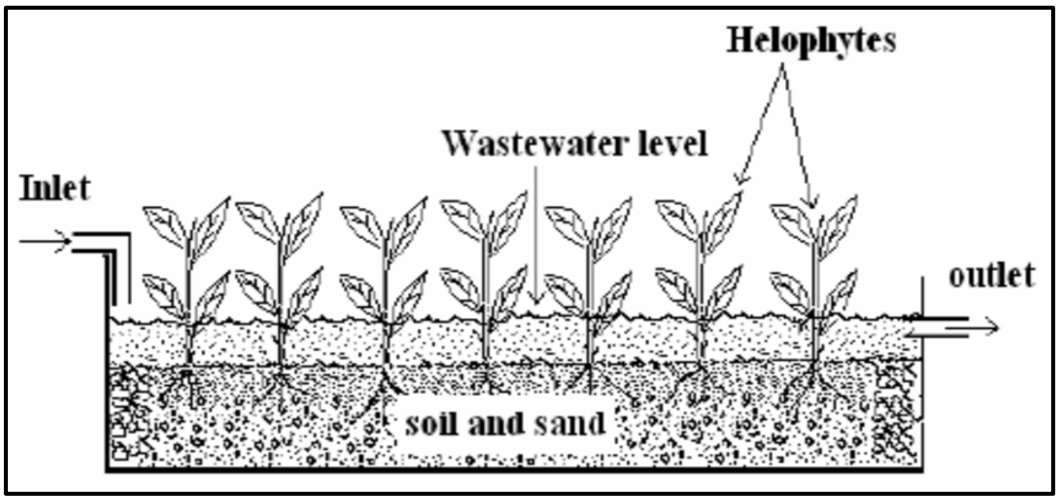

Figure 1. Design of a Surface Flow Constructed Wetland

Source: Choudhary *et al*. 2011

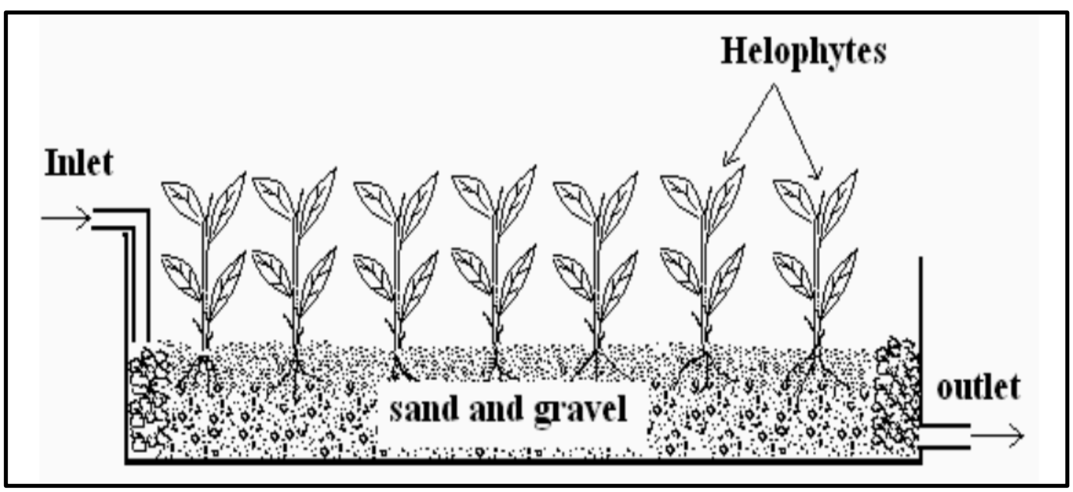

Figure 2. Design of a Subsurface Flow Constructed Wetland

Source: Choudhary *et al*. 2011

2.2. ARTICLE SCREENING

The relevance of each article was assessed through a series of steps using key terms related to the subject of this literature review. Terms were only used if they had relevance to constructed wetland functions, agricultural nitrogen use, urban wastewater and most importantly a combination of all three. These key terms included but were not limited to; denitrification, nitrification, run-off, nitrogen fertilizer, and wastewater. Within each database, a key term was placed into the search engine and the articles were screened under the following steps:

a) Articles titles were read based on the inclusion of the key terms. Articles without the presence of the key term or substantially irrelevant were removed from the search. This process was done in a conventional way to ensure any potentially relevant article was not removed.
b) Literature abstracts were reviewed. The relevance of each abstract was based on the inclusion criteria described in Section 2.1. Abstracts were required to meet 1 of the 3 criteria, and if all three were met than articles were read in full.
c) Articles were read in full and included in this literature review. Articles that failed to meet a single one of the inclusion criteria were removed.

2.3. DATABASE STRATEGY

A database was constructed in Microsoft Excel containing relevant data from each publication. To ensure that the data collected from each piece of literature could be weighed against one another, all data was converted to similar units and organized properly by wetland type and removal types. This database included any citation information and data relating to secondary variables. These secondary variables included process required to reduce nitrogen, seasonal variance, and topographic information. For a detailed description of the data included in the results, see Appendix A.

2.3.1. Descriptive Statistics

Descriptive statistics were compiled from each study and provided to display attributable data related to the removal efficiency. This attributable data included the amount of nitrogen entering the wetland, amount of nitrogen leaving the wetland and the overall quantity of nitrogen being removed by the corresponding mechanism. These statistics were presented for each nitrogen removal type and wetland type. These results are presented in sections 3.2. and section 3.3.

2.3.2. One-way ANOVA

A one-way ANOVA statistical test was completed using IBM SPSS computer software and quantified which of the two variables (wetland type or removal type) were significant in determining the removal efficiency of constructed wetlands. Efficiency was measured by the amount of nitrogen being removed over the course of each study. In order for the dataset to maintain any relevant results from the ANOVA, it had to meet certain criteria of Levene's Test for Homogeneity. This criterion primarily includes the requirement of a p-value less than 0.05 with each statistical test run. This value will indicate whether or not there is an infringement on the null hypothesis constructed in section 1.2.

3. RESULTS

This section contains results from the literature search, including the removal techniques and wetland design characteristics. This section also contains statistics pertaining to two separately run, one-way ANOVAs. These ANOVA's were run to determine the removal efficiency of each independent variable; the removal type and the wetland type.

During the literature search, 33 publications were found pertaining to "nitrogen removal" and "constructed wetland". Out of these 33 publications, only 11 discussed specific trials involving denitrification/nitrification and plant assimilation. Each publication was examined and further discussed. The following results are presented in a way that are considered most relevant for each discussed variable.

3.1. DESCRIPTIVE STATISTICS

A descriptive statistic quantitatively summarizes select features of a group of information (Narkhede 2018). For this literature review, the descriptive statistics consist of secondary variables separate but important to the outcome of the primary results. When examining these descriptive statistics, it is evident that there is a great deal of variation between each study examined. This is prevalent in the distribution of publications, physical wetland characteristics and other secondary characteristics quantified in each published work.

3.1.1. Origin and Distribution of Publications

Of the 11 studies included in sections 3.2. and 3.3., two were found on the JSTOR publication site, two were found using the Web of Science publication website and 5 were found using the Scholars Portal publication website. Two of the published studies found using the Scholars Portal website contained two, relevant study sites which were used in section 3.2., 3.3. and tested using the ANOVA test in section 3.4. The time frame in which all publications were constructed and carried out was 1997 to 2013.

The constructed wetlands used in each study were established within one clearly defined research area consisting of publicly owned land. Three studies took place in China, two studies took place in Egypt and Turkey, and one study took place in each of the following countries; Estonia, the United States of America and Tunisia.

3.1.2. Wetland Characteristics

Removal efficiency is dependent on the design of the constructed wetland, including the size of wetland, wastewater flow rate and wastewater retention time within the wetland. A thorough examination of each studies materials and methods was done to determine each of the following characteristics; surface area (m^2), flow rate (m^3/day) and water retention time (days). Studies have shown to have a substantially small flow rate based on their surface area. Small sized wetlands had an average of <5 m3 of water per day being added to the wetland. There is a great deal of variation between each wetland's surface area size. Study E has the largest surface area with 8,400 m^2 and Study H has the smallest surface area with 0.6 m^2. Studies have shown to have less variation in the wastewater retention time. The range of retention time between the 11 studies was from 1.25 days – 25 days. The wetland characteristic information gathered from each study is summarized in Table 1.

Table 1. Physical and experimental characteristics of each studied constructed wetland (see Appendix I for authors(s) of study references).

Study Reference	Wetland Surface Area (m2)	Flow Rate (m3 / day)	Water Retention Time (days)
A	90	23	25
B	12	3	6
C	18	0.014	1.25
D	18	0.034	1.25
E	8400	3100	3
F	654.5	20	11
G	457.6	20	7.7
H	0.6	0.144	24
I	3	0.030	7
J	5.25	0.040	7
K	320	140	4

3.2. REMOVAL TECHNIQUES

The two prevalent nitrogen removal techniques found across all literature were the chemical process of denitrification/nitrification and the physical process of plant assimilation. Each study provided a quantity of nitrogen at the wetland inlet, quantity of nitrogen found at the wetland outlet area, overall quantity removed from the effluent, and percent removal over the course of the study. A large amount of variation was found between the studies. 5 studies focused on denitrification/nitrification as the main nitrogen removal type. Figure 3 displays the inlet quantity and outlet quantity of nitrogen found within these 5 denitrification/nitrification studies.

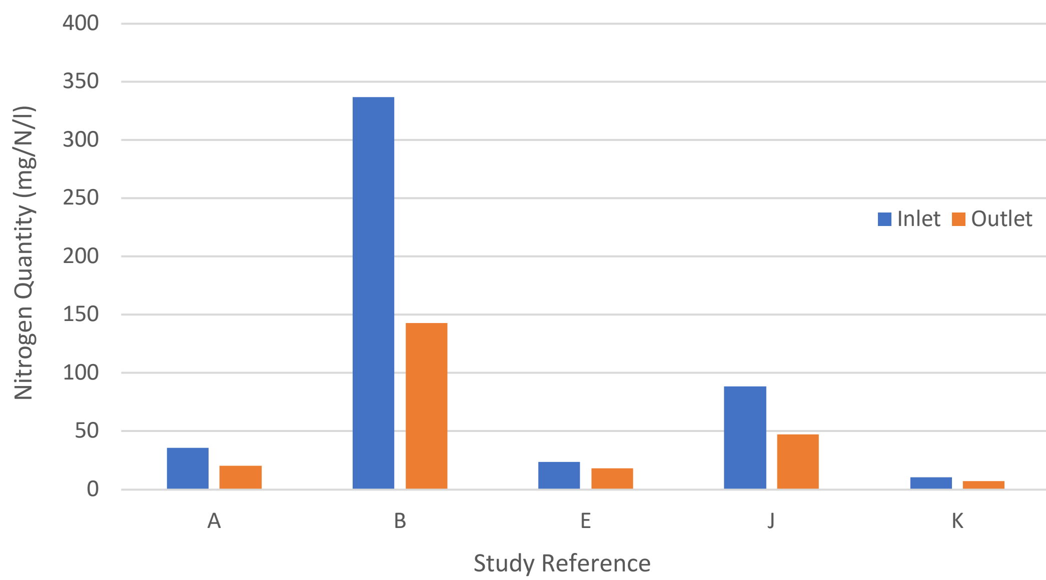

Figure 3. Inlet and outlet quantity of nitrogen for each constructed wetland designed for denitrification / nitrification removal

Figure 3 displays a considerable difference in inlet quantities among all 5 studies and therefore resulted in a varied removal rate. This removal rate was calculated within each denitrification / nitrification study. This calculation is based off of the difference between the inlet quantity measured and the outlet quantity measured. Each corresponding removal rate is shown in figure 4.

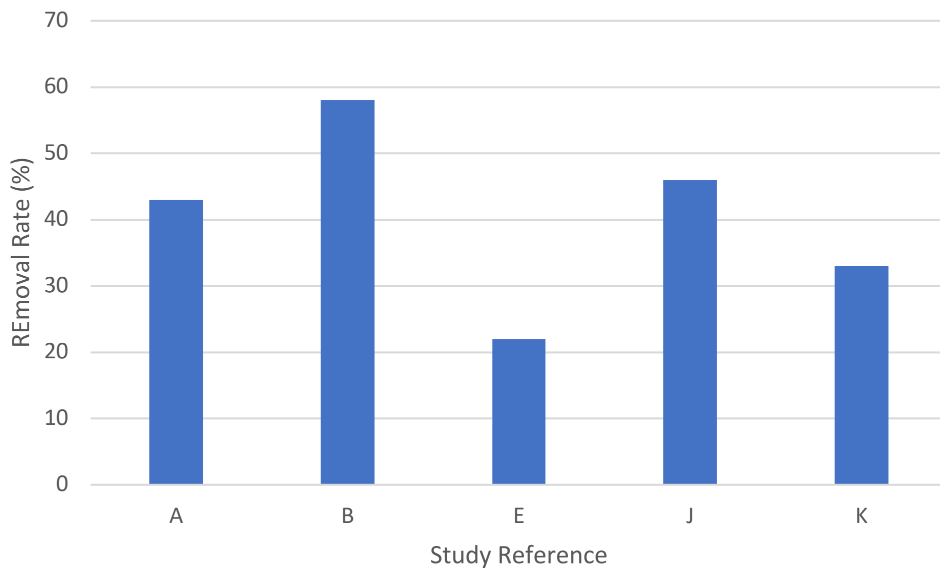

Figure 4. Denitrification / nitrification removal rate for each corresponding constructed wetland

Six peer-reviewed studies determined plant assimilation as the primary nitrogen removal technique. These studies provided and measured the inlet quantity of nitrogen (mg/N/l) and the outlet quantity of nitrogen (mg/N/l) within the constructed wetland. Figure 5 displays each study’s quantifiable measures of inlet and outlet nitrogen.

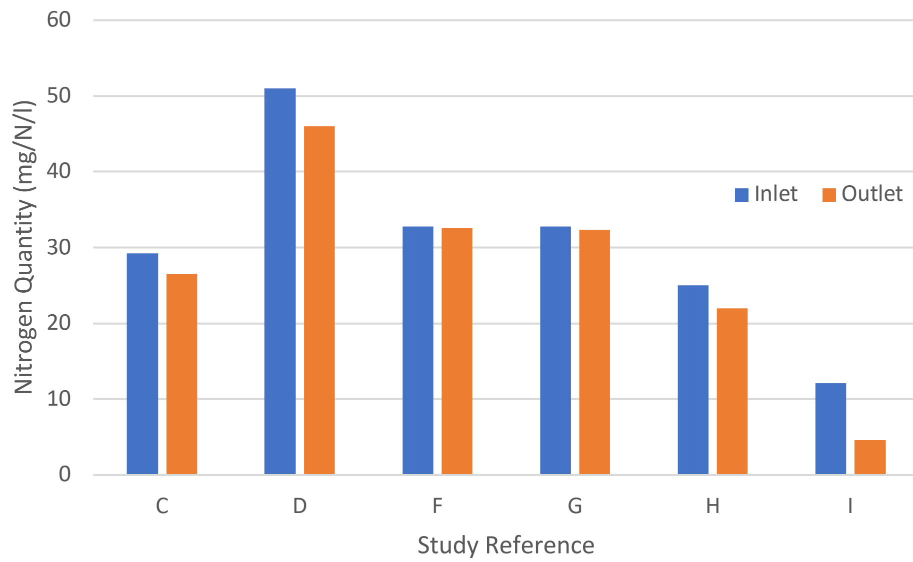

Figure 5. Inlet and outlet quantity of nitrogen in each constructed wetland designed for plant assimilation

Based on these measurements provided by the peer-reviewed articles and displayed in Figure 5, a removal rate was calculated for each constructed wetland study. Figure 6 displays the 6 corresponding removal rates for each plant assimilation study. Standard deviation error bars are presented within figure 6 to indicate the variation in removal between the plant assimilation studies.

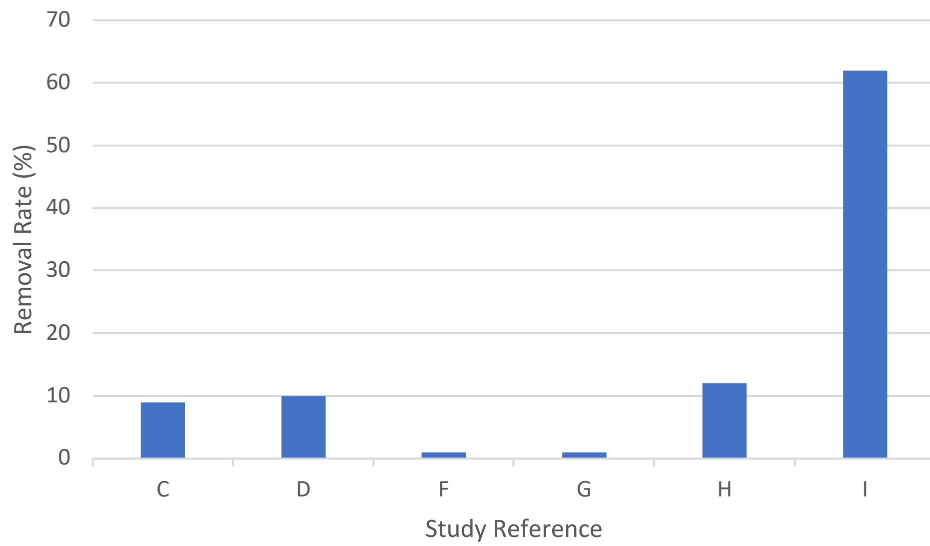

Figure 6. Plant assimilation removal rates for each corresponding constructed wetland

3.3. WETLAND TYPE

Regardless of the removal technique, this literature study examined the percent removal based on the type of constructed wetland being used. Throughout the literature search, two major types of constructed wetlands were present. These two types of constructed wetland were the surface flow and subsurface flow constructed wetland. Figure 7 displays the measured inlet quantity and the measured outlet quantity of nitrogen being processed throughout each surface flow constructed wetland. These measurements were pulled directly from each peer – reviewed article.

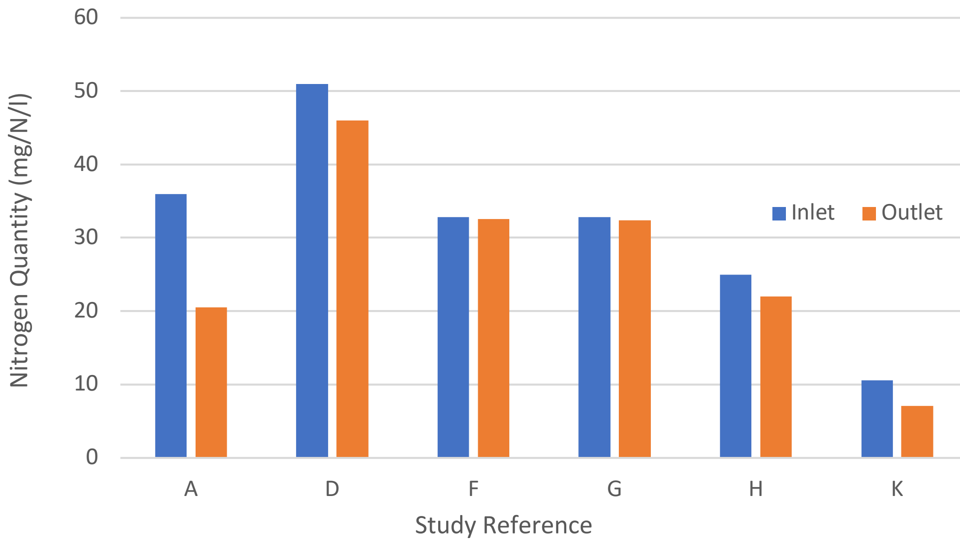

Figure 7. Inlet and outlet quantity of nitrogen within each surface flow constructed wetland

The measurements displayed in Figure 7 are further used to calculate the removal rate for each corresponding study. Figure 8 presents the removal rates for the six-surface flow constructed wetland studies. Among these six surface flow studies, the removal rate variation is 35%, as the highest removal rate is 43 % and the lowest removal rate among the studies being 1%.

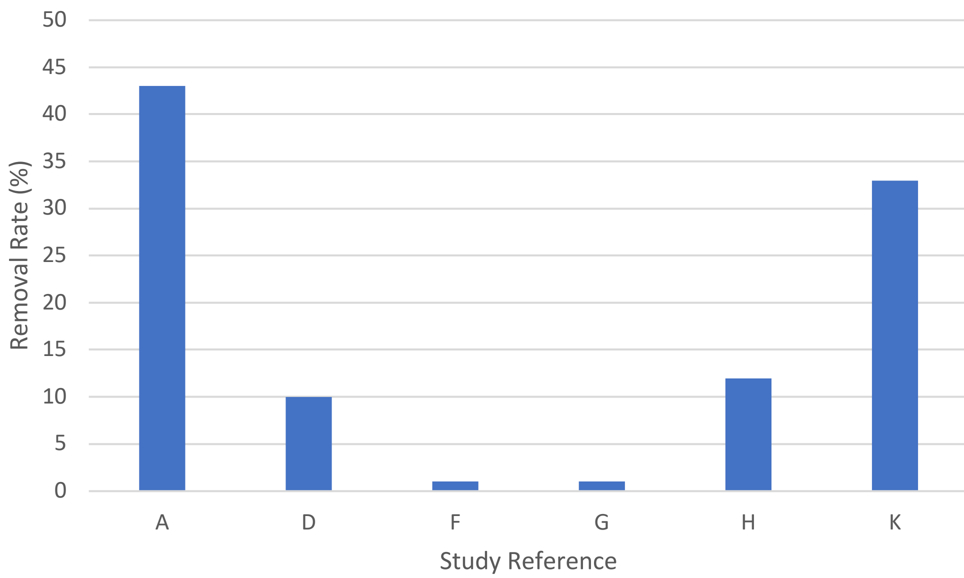

Figure 8. Nitrogen removal rates for each corresponding surface flow constructed wetland

The second wetland design prominent throughout the literature is the subsurface flow constructed wetland. Figure 9 presents the inlet and outlet data collected from 5 subsurface flow constructed wetland studies.

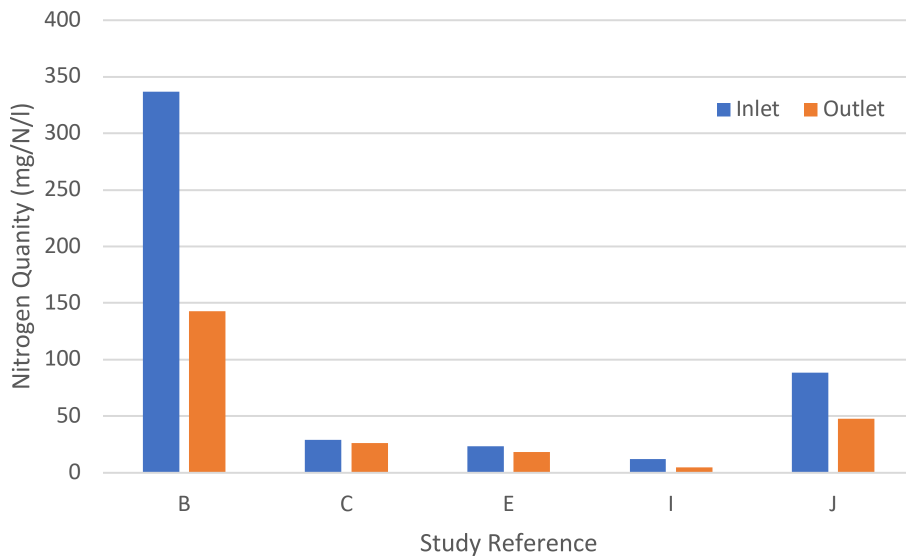

Figure 9. Inlet and outlet quantity of nitrogen within each subsurface flow constructed wetland

The inlet and outlet quantities displayed in Figure 9 are used to calculate the removal rate of each corresponding wetland study. The calculated removal rates are displayed in Figure 10. The five subsurface wetlands have an average nitrogen removal rate of 39.4 %.

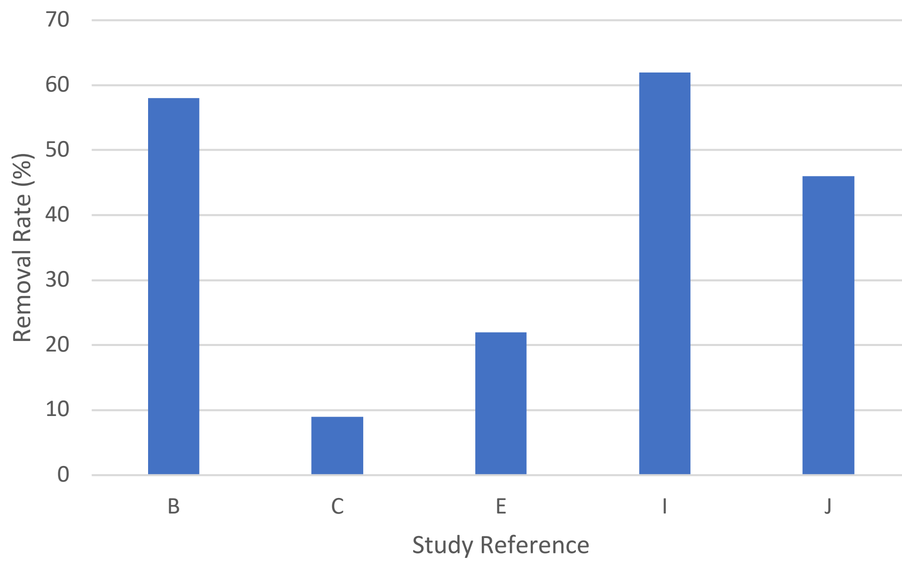

Figure 10. Nitrogen removal rates for each corresponding subsurface flow constructed wetland

3.4. DENITRIFICATION AND PLANT ASSIMILATION STATISTICAL ANALYSIS

A one – way ANOVA compares the means of one or more dependent (factor) variables. The purpose of an ANOVA is to determine if there is any interaction between two factors as they relate to the independent variable (Anderson 2001). Using the same raw data used to generate the figures in section 3.2., data was isolated to include only that which pertained to the removal efficiency of type A1 (denitrification) and A2 (plant assimilation). Using this data, a meaningful analysis was completed to examine which of the two factors; between the removal efficiency and removal type. The factors used in this ANOVA are fixed factors and the confidence level of this ANOVA is 95% (alpha = 0.05). Table 2 displays the distribution of removal type, its mean and standard deviation. Type A1 had a sample size of 5, and type A2 had a sample size of 6.

Table 2. Descriptive statistics for factor variables A1 and A2

Removal Type	Mean	Standard Deviation	N
A1	0.403407	0.13527	5
A2	0.158302	0.232006	6
Total	0.269713	0.225045	11

The null hypothesis for this ANOVA was that there would be no significant difference among the two removal types and their efficiency to remove nitrogen from the wastewater. This test was according to the inlet and outlet quantity of nitrogen in the wetland. The significance level used in this test is 95%. Therefore, the significant level is required to be 0.05 in order to reject the original null hypothesis. The calculated significance levels for this ANOVA test are displayed in Table 3.

Table 3. Tests of removal type subject effects with significance levels

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.164	1	0.164	4.304	0.068
Intercept	0.860	1	0.860	22.604	0.001
Removal Type	0.164	1	0.164	4.304	0.068
Error	0.343	9	0.038		
Total	1.307	11			
Corrected Total	0.506	10			

The value of 0.05 is used to compare the significance values displayed for the factor in Table 3. The overall goal is to determine the significant efficiency of each removal type. The removal type factor has a significant difference of 0.068 Therefore, the null hypothesis is not rejected, meaning that the parameters do not significantly affect the removal efficiency of nitrogen in this study.

3.5. WETLAND TYPE STATISTICAL ANALYSIS

This ANOVA was used to determine if the type of wetland used in each study had an effect on the removal efficiency. Using the same raw data generated in section 3.3., data was isolated to include only that which pertained to the efficiency of wetland B1 (surface flow wetland) and B2 (subsurface flow wetland). This raw data included both the inlet and outlet quantity of nitrogen determined in each study. The factor in this ANOVA are fixed and the significance level for this ANOVA is 95% (a=0.05). Table 4 displays the distribution of each wetland type, its mean and standard deviation. Type B1 had a sample size of 6 and type B2 had a sample size of 5. Data from 11 studies were analyzed for this ANOVA.

Table 4. Descriptive statistics for factor variables B1 and B2

Wetland Type	Mean	Standard Deviation	N
B1	0.1667	0.17420	6
B2	0.3940	0.23061	5
Total	0.2700	0.22481	11

The null hypothesis for this ANOVA was that there would be no significant differences among the two wetlands types prevalent in the literature study, and their efficiency to remove nitrogen from the wastewater. The significance level used in this ANOVA is 95%. Therefore, the significant level is required to be 0.05 in order to reject the original null hypothesis. The calculated significant levels determined by this ANOVA are displayed in Table 5.

Table 5. Tests of wetland type subject effects with significant levels

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.141	1	0.141	3.481	0.095
Intercept	0.857	1	0.857	21.171	0.001
Wetland Type	0.141	1	0.141	3.481	0.095
Error	0.364	9	0.040		
Total	1.307	11			
Corrected Model	0.505	10			

The value of 0.05 is used to compare the significance values displayed in Table 5. For factors B1 and B2 the overall significance is 0.095, therefore not rejecting the null hypothesis. There is no significant difference between the two wetland types based on their ability and efficiency to remove nitrogen from the wastewater.

4. DISCUSSION

Nutrient uptake in wetland systems has taken place for millions of years, but the designation and usage of constructed wetlands for wastewater nutrient removal is a relatively new science (Kadlec 2009). Currently, there is a gap in comparative research amongst constructed wetlands and their nutrient removal efficiencies. The mechanisms in which constructed wetlands are evidently clear, however, to apply such research secondary variables must be accounted for. A gap must be filled to understand which variables are most efficient at removing nutrient, specifically nitrogen from human polluted wastewater within constructed wetlands.

The results display the nitrogen removal efficiencies of each wetland type and corresponding removal technique. There are many explanations for why there is no difference between the variable’s efficiencies. These explanations can be subdivided into two main categories; attributes relating to the overall nitrogen transformation and the influence of descriptive statistics relating to outside secondary variables. This section will also discuss the study significance and provide recommendations for future nitrogen removal experimental studies.

4.1. NITROGEN REMOVAL / TRANSFORMATION PERFORMANCES

The results found in this literature review can be explained by the transformation of nitrogen within each studied wetland. The transformations (denitrification/ nitrification or plant assimilation) of nitrogen are what determines the amount of nitrogen remaining within the wastewater as it leaves the wetland. There are several factors that contribute to the microbial and biological transformations taking place within the wetland system.

The first transformation factor is the average concentration of nitrogen being released into each constructed wetland. The nitrogen concentration can favour or inhibit the quantity of total nitrogen being transformed. If there is an increase in nitrogen being added to the constructed wetland, the saturation will cause a decrease in nitrogen removal from the wastewater during the plant assimilation removal (Tao *et al.* 2012). The increase in nitrogen concentration will also greatly influence the denitrification/ nitrification removal. Studies have shown that for denitrification / nitrification transformations, the increase of nitrogen content inhibits the amount of nitrogen being removed. Increases in concentration affect the removal techniques in different ways and therefore, are proved to account for the similarities between the removal efficiencies.

Secondly, there are a variety of anaerobic conditions predetermined in the constructed wetland which effects their nitrogen removal efficiency. As stated in the literature review section of this study, anaerobic conditions in both natural and constructed wetlands can increase the amount of nitrogen being removed. Anaerobic conditions have been positively correlated with the amount of sedimentation and plant growth and without such conditions plant assimilation does not take place (Gottshall *et al.* 2007). Studies have shown that denitrification and nitrification removal processes can continue in aerobic conditions (Smith *et al.* 2000). The variation among anaerobic and aerobic conditions within a single constructed wetland is limited and often the wetland is found in one condition for long periods of time.

Seasonality and temperature also have a pre-established impact on the nitrogen quantity being removed from each wetland. Each study examined in this literature review was conducted during the summer months and therefore shared similar temperature conditions but a variety of local weather conditions. It can be confirmed that all studies took place in warmer summer temperatures, but the local weather such as rainfall and wind would vary. These weather conditions could drastically affect the nitrogen transformation specifically in the plant assimilation. An increase in rainfall in a certain area has proved to greatly influence denitrification rates because of the increased microbial activity taking place in the surface and subsurface of the constructed wetland. Rainfall has also proven to inhibit the amount of nitrogen being assimilated by plants (Poe *et al.* 2003).

Overall, these nitrogen transformation characteristics greatly influence the removal efficiency within each study. It can be said that the differences among how each characteristic affect the removal technique would cause the removal similarities found in this study. Therefore, establishing that there is no significant difference amongst the removal techniques and their removal rate.

4.2. DESCRIPTIVE STATISTICS

The descriptive statistics developed in correspondence with the wetland design demonstrate the immense differences between the removal efficiencies and the influence of secondary variables. These secondary variables include the wetland size, flow rate, and water retention time, all of which had a significant outcome on the primary results. Larger studied wetlands received larger quantities of nitrogen wastewater and therefore had significantly more opportunities to remove the nitrogen nutrient in the form of plant assimilation or through denitrification / nitrification.

The size of the wetland impacts the amount of vegetation and microbes within it. An increase in vegetation in the constructed wetland influences the amount of nitrogen that can be assimilated by the plants. Nitrogen assimilation occurs as the plant is growing (Liu *et al.* 2010)), so therefore the greater the space for vegetation growth will positively increase the nitrogen uptake. The resulting descriptive statistics also determined that the size of the wetland is positively correlated with the wastewater flow rate within the wetland. As previously stated in the literature review water flow rate within a natural wetland is very slow, and therefore reduces the amount of nutrient uptake taking place within it (Vymazal 2007). In the case of a constructed wetland, the rate of wastewater flow can be manipulated and ultimately can be increased as the size of the wetland increases. Table 1 displays this positive correlation between wetland size and flow rate.

Water retention time is an important factor in the quantity of nitrogen being removed. The amount of time in which the wastewater spends in the constructed wetland influences the chances that the nutrient will biologically be assimilated or microbially removed. The results show that the greater wastewater retention time the greater nitrogen being removed from the constructed wetland regardless of the removal type. This variable significantly shows that there is no difference between the removal mechanisms and the wetland type. The variation among the wetland size, flow rate and water retention time account for the variation of removal efficiencies. The combination of these secondary variables in each constructed wetland account for the lack of difference between the removal efficiencies in the surface and subsurface flow wetland types.

4.3. STUDY SIGNIFICANCE AND FURTHER OPPORTUNITIES

Constructed wetlands were created and designed to aid human-induced environmental consequences (Campbell 1999), and therefore using such for nitrogen removal from wastewater is key to the sustainability of the wetland environment. This study is significant for present and future generations, as there will be a constant increase in wastewater pollution and with this increase must come accommodation and adaption. Understanding the mechanisms and the most efficient ways of removing pollutants from wastewater is key in accounting for this wastewater pollution.

This study is an instrument for future comparisons of other nutrient removing mechanisms being currently being used or introduced into the wastewater system. Many nutrients including phosphorus and magnesium are introduced into watershed systems through human influence, similarly to the nitrogen introduction referenced to in this study. Although removal techniques vary based on the nutrient being transformed, it is important to understand the mechanisms and efficiencies of such. Studies have determined removal mechanisms that correspond with these other nutrients. Phosphorus removal in wastewater is primarily done via sedimentation and plant assimilation (De-Bashan 2004) and using this study as a guide one can compare the two phosphorus removal mechanisms to determine the more efficient type. Determining the overall efficiency of certain mechanisms and designs is beneficial for the future of constructed wetland wastewater treatment.

It is encouraged that future studies, regarding both the nitrogen removal types and wetland types, contain a variety of secondary variables that will enhance the removal of nitrogen more efficiently. The first recommended variable is the size and design of the wetland. The results show that there is no significant difference among the two types of wetlands used for wastewater treatment and therefore it is recommended to design such wetland using both surface and subsurface flow cells. A large constructed wetland designed with surface and subsurface cells remains the most efficient wetland type for nitrogen removal. This study also determined that there was no significant difference among the removal types used, and for this, the second recommendation would be for future studies to use both removal techniques within their study.

5. CONCLUSION

Understanding the mechanics of the nitrogen removal process is key for future development and use of constructed wetlands. Compared to natural wetlands, constructed wetlands are much more manipulative and therefore make them overall very efficient for wastewater treatment. As water pollution continues to rise with the human population, the need for efficient and effective wastewater treatment will rise. The purpose of this research was to collect and examine data through a literature search and determine how efficiently constructed wetlands are at removing elements of nitrogen. The literature review determined that there are two separate nitrogen removal techniques and two fundamental wetland designs used for efficiently removing nitrogen from wastewater. The results specified that there was no significant difference between the two variables in terms of their removal rate. The overall conclusion determined from this literature review-based thesis is that nitrogen removal efficiencies for both removal and wetland types rely on a variety of secondary and outside variables.

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APPENDICES

APENDICIES I: RAW DATA

Study Reference	Inlet Quantity (mg/N/l)	Outlet Quantity (mg/N/l)	Quantity Removed	% Removal
A	36	20.5	15.5	43
D	51	46	5	10
F	32.8	32.6	0.2	1
G	32.8	32.36	0.44	1
H	25	22	3	12
K	10.6	7.12	3.48	33

Study Reference	Inlet	Outlet	Quantity Removed	% Removal
B	337	143	194	58
C	29.21	26.58	2.63	9
E	23.7	18.5	5.2	22
I	12.12	4.59	7.53	62
J	88.75	47.65	41.1	46

Study Reference	Inlet	Outlet	Quantity Removed (mg/N/l)	% Removal
A	36	20.5	15.5	43
B	337	143	194	58
E	23.7	18.5	5.2	22
J	88.75	47.65	41.1	46
K	10.6	7.12	3.48	33

Study Reference	Inlet	Outlet	Quantity Removed (mg/N/l)	% Removal
C	29.21	26.58	2.63	9
D	51	46	5	10
F	32.8	32.6	0.2	1
G	32.8	32.36	0.44	1
H	25	22	3	12
I	12.15	4.59	7.56	62

Reference Letter	Author	Published Year
A	Mander, U. and T. Mauring	1997
B	Yang, X., Y. Sun and W. Li	2005
C	Gao, J., W. Wang, X. Guo, S. Zhu, S. Chen and R. Zhang	2014
D	Tao, W., J. Wen, Y. Han and M. P. Huchzemeier	2012
E	Yang, Y. X. Zhencheng, H. Kangping, W. Junsan and W. Gvizhi	1995
F	Abou-Elela, S., G. Gilinielli, E. Abou-Taleb and M. Hellal	2012
G	Abou-Elela, S., G. Gilinielli, E. Abou-Taleb and M. Hellal	2013
H	Keffala, C., and A. Ghrabi	2005
I	Tuncsiper, B	2009
J	Tuncsiper, B	2009
K	Yeh, T. Y., C. T. Pan, T. Y. Ke and T. W. Kuo	2010

Process			Effluent Parameters
Biological:			
	Microbial:		
		Respiration	BOD, O_2, NO_3, SO_4, HCO_3, volatile fatty acids
		Nitrification	$NH_4 - N$
		Denitrification	$NO_3 - N$ and $NO_2 - N$
		Mineralization	Organic N, P
		Assimilation	Nutrients
	Plants		
		Growth and Uptake	Nutrients
		Gas Transport	O_2 and related reactions