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Review

A Literature Review of Wetland Treatment Systems Used to Treat Runoff Mixtures Containing Antibiotics and Pesticides from Urban and Agricultural Landscapes

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Abstract: Wetland treatment systems are used extensively across the world to mitigate surface runoff. While wetland treatment for nitrogen mitigation has been comprehensively reviewed, the implications of common-use pesticides and antibiotics on nitrogen reduction remain relatively unreviewed. Therefore, this review seeks to comprehensively assess the removal of commonly used pesticides and antibiotics and their implications for nitrogen removal in wetland treatment systems receiving non-point source runoff from urban and agricultural landscapes. A total of 181 primary studies were identified spanning 37 countries. Most of the reviewed publications studied pesticides (n = 153) entering wetlands systems, while antibiotics (n = 29) had fewer publications. Even fewer publications reviewed the impact of influent mixtures on nitrogen removal processes in wetlands (n = 16). Removal efficiencies for antibiotics (35–100%), pesticides (−619–100%), and nitrate-nitrogen (−113–100%) varied widely across the studies, with pesticides and antibiotics impacting microbial communities, the presence and type of vegetation, timing, and hydrology in wetland ecosystems. However, implications for the nitrogen cycle were dependent on the specific emerging contaminant present. A significant knowledge gap remains in how wetland treatment systems are used to treat non-point source mixtures that contain nutrients, pesticides, and antibiotics, resulting in an unknown regarding nitrogen removal efficiency as runoff contaminant mixtures evolve.

Keywords: constructed wetlands; nitrogen removal; contaminants of emerging concern; pesticides; antibiotics; non-point source pollution

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1. Introduction

Water quality degradation in rivers and streams across the globe is becoming a major concern, especially as stresses from climate change increase [1]. The leading cause for surface water impairments in the United States is due to non-point source pollution [2]. Non-point sources include urban, agricultural, and construction runoff [3], which often contain nutrients, suspended sediment, pesticides, antibiotics, and other pharmaceuticals, fecal coliform, and metals [3–6]. Nutrient contamination, such as nitrate-N (NO_3-N) from fertilizer applications, is persistent in rivers and is the most common form globally of chemical contamination in groundwater, resulting in NO_3-N being a major cause of drinking water impairment across the United States and the globe [7–11]. In addition to nutrients, chemically derived common-use pesticide (CUPs) applications throughout the world have increased by 71% from 1990 to 2017, with 11% used for non-agricultural application in the United States [12,13]. The United States Geological Survey (USGS) recently reported that urban pesticides (e.g., 4-Hydroxychlorothalonil, azoxystrobin, carbendazim, propiconazole, 2,4-D, diuron, prometon, triclopyr, fipronil, imidacloprid) accounted for 83% of the pesticides found in urban sites where the mixture complexity and potential toxicity of the pesticides increased with increasing urbanization [14]. Additionally, most antibiotics used in human and veterinary medicine (e.g., macrolides, sulfonamides, trimethoprim, quinolones) are excreted via urine and feces. Antibiotics used by humans often enter wastewater treatment systems where removal efficiencies ranged from 15.8–78.4% from 2010 to 2019 [15]. Veterinary antibiotic residues in livestock products result in 24,000,000–72,000,000 kg of antibiotic residue being introduced into agricultural soil and downstream aquatic environments from manure applications annually [16]. While CUPs and antibiotics are important for agricultural productivity to support an ever-increasing global population, these chemicals have become ubiquitous in waterways worldwide, resulting in significant effects on agroecosystem food webs (e.g., honeybee colony collapse) [17] and human health (e.g., reproductive and development disruption, carcinogens, antibiotic-resistant genes) [18,19].

Wetland treatment systems, including natural and constructed wetlands, are now being used extensively across the United States and the world to treat non-point source pollution. This is due to their cost-effectiveness and low energy consumption compared to other surface water treatment methods (e.g., coagulation, membrane filtration, ion exchange) [20–22]. Wetlands utilize plants, soils, and associated microbial assemblages to remove pollutants through biodegradation, substrate adsorption, and plant uptake [23,24]. These wetland treatment processes take place in different biogeochemical compartments of the wetland (e.g., aqueous, sediment, detritus), which have separate roles as either sinks or sources [25]. However, there are limitations to wetland treatment systems including the requirement of large areas and the high variability in performance, as design and operation can be challenging because of the complexity of environmental processes, changing influent concentrations, seasonal changes in weather, and system clogging [22].

The use of wetlands as a treatment approach for NO_3-N is well known [11,26–29]; however, N removal performance is significantly impacted by wetland design, operation, and internal and external environmental factors (e.g., microbial communities, plants, type and concentration of contaminants) [30]. Specifically, contaminants of emerging concerns (CECs), which include CUPs and veterinary antibiotics, in surface waters have only recently started to be investigated for their impact on ecologically important processes (e.g., NO_2-N oxidizing bacteria population, NH_4^+-N oxidizing bacteria population) [31,32]. Exposure to specific CUPs (e.g., imidacloprid) in runoff waters and exposure to antibiotic residues contained in livestock manure and wastewater (e.g., tetracycline, lincomycin, chlortetracycline, sulfamethazine) may influence the population structure of denitrifying bacteria communities [33–36], and thus, the activity of microbial denitrification in adjacent wetland treatment systems.

Therefore, this review sought to comprehensively assess past reports related to CUPs and antibiotic treatment in wetlands along with the implications of runoff mixtures for the nitrogen (N) cycle and specifically NO_3-N removal in wetland treatment systems receiving runoff from urban and agricultural landscapes. The objectives of this review were to: 1. Evaluate the current scientific status of the topic, 2. Quantify the scale and type of wetlands used to treat runoff mixtures, 3. Identify the source and mixture of antibiotics and CUPs reported to be entering wetlands along with removal mechanisms, and 4. Review implications to N removal processes (e.g., denitrification, plant uptake) in the presence of antibiotics and/or CUPs.

2. Materials and Methods

This review was designed to better understand the current state of knowledge on the implications of runoff mixtures to the N cycle in wetland treatment systems (specifically the removal of NO_3-N). The review was completed by identifying the methodology and major results about nutrient (N) and CEC (pesticides and antibiotics) removal efficiencies along with implications to the N cycle. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) standard was used for analyzing and reporting [37]. Herein, studies used in this review are referred to as primary studies.

2.1. Eligibility Criteria

This review sought to include all searchable studies that have researched the ability of wetlands to treat runoff from urban and agricultural landscapes. The primary studies eligible for inclusion in this review needed to meet the following criteria: (1) the study performed analysis on the ability of wetlands to treat CUPs and/or antibiotics from non-point source pollution; (2) the study was from a peer-reviewed journal.

2.2. Identification of Records

The primary studies were obtained from searching Web of Science and SCOPUS databases. A search string was determined through an iterative process to identify studies using any of the search terms in the title, abstract, and/or keywords. The final search string used was the following: (treatment wetland AND antibiotic OR treatment wetland AND pesticide OR constructed wetland AND antibiotic OR constructed wetland AND pesticide). The search was further limited to full-text peer-reviewed journal articles available in English. The official search ended in July 2021 and resulted in 1224 studies meeting the criteria.

Limitations of the search included evaluating only English studies, inevitably underrepresenting the sample of collected studies by excluding those that may have met all other search criteria but were not available in English. Additionally, the keywords used were important to define papers retrieved in the database search; however, it was inevitable that the keywords did not entirely encompass all relevant papers even with careful consideration and several iterations of keywords. These are inherent limitations to all reviews; thus, even though the search was performed systematically, the primary studies may still be biased. However, the limitations do not necessarily take away from the importance and applicability of the findings.

2.3. Screening and Coding of Studies Based upon Eligibility Criteria

After the initial database search, a three-phase process was used to screen primary studies that met all eligibility criteria (Figure 1) [38]. Using Rayann [39], one coder independently read the title and abstract for all the studies obtained from the initial database search after duplicates were removed. One coder was used throughout the process to maintain consistency and reduce bias; however, if there was any uncertainty regarding the inclusion of a primary study, a second coder was sought for a final decision.

Each study was evaluated to ensure it pertained to wetland treatment of non-point sources. For example, Zheng et al. (2021) was removed from further analysis because the authors simulated domestic sewage waste (point source pollution) to study the removal efficiency of sulfamethoxazole and nitrogen in wetland mesocosms [40]. Additionally, Maryniuk et al. (2016) considered how high-contaminant loads in wetlands impact largemouth bass [41], whereas Sen et al. (2019) focused on the impact of crows that roosted in constructed wetlands and their ability to be carriers of *E. coli* [42]. Neither of these studies quantified or identified wetland contaminant removal efficiencies or N cycle pathways, so these studies were removed from further analysis. Studies were also removed if only nutrient removal was analyzed without the presence of pesticides or antibiotics. For example, Hussain et al. (2011) analyzed N removal in the presence of antibiotics; thus, the study was retained for further analysis even though the antibiotic removal potential was not specified [43]; however, Jia et al. (2021) investigated N removal and poly-3-hydroxybutyrate-co-3hydroxyvalerate/polylactic acid removal in the absence of any pesticides or antibiotics, so this study was removed from further analysis [44]. Review articles were also excluded from the analysis, given that new research on the topic was not conducted; rather, a summary of the existing research was reviewed and/or meta-analysis was completed. From this, there were 181 primary studies used for further analysis [3,16,23,25,30,32,43,45–218].

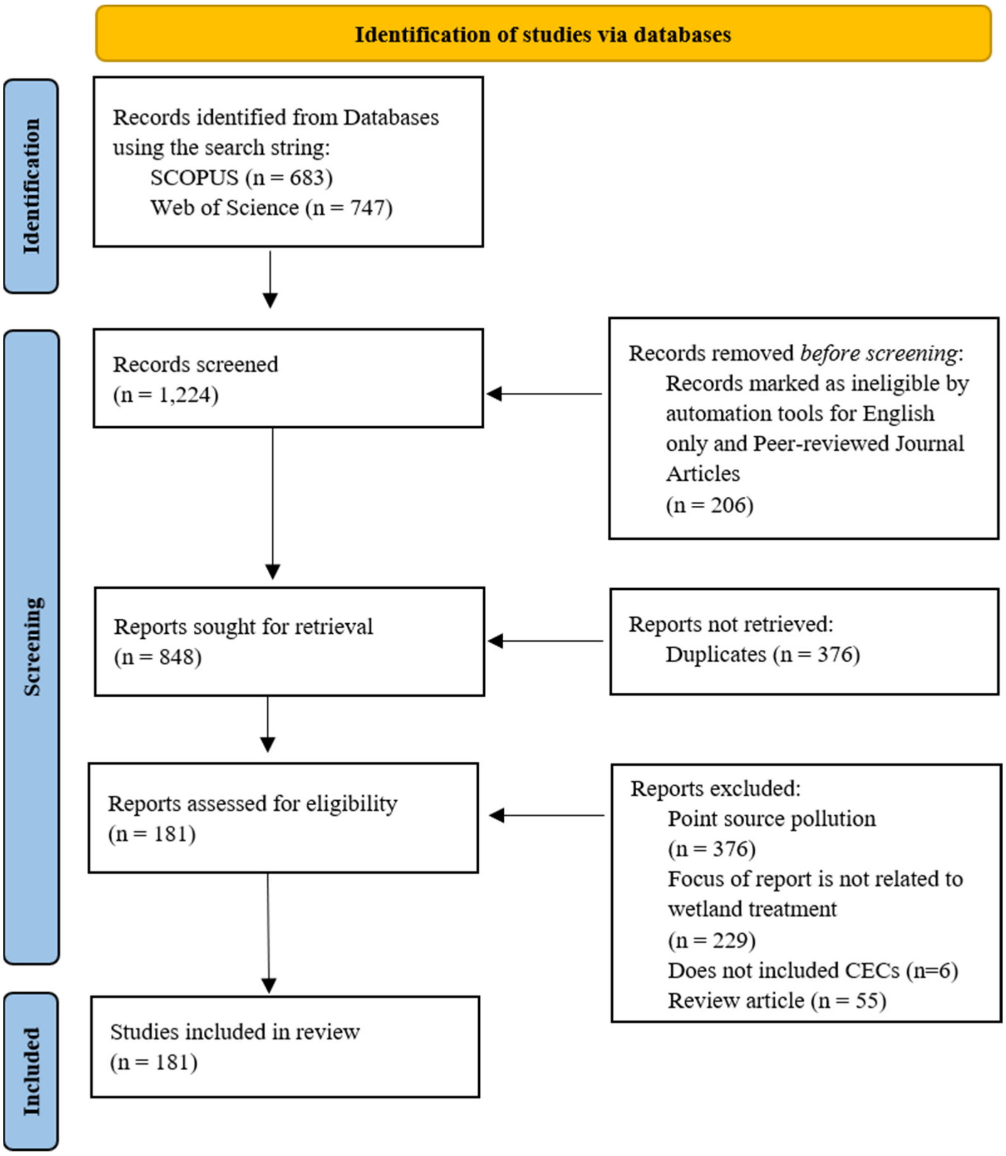

Figure 1. PRISMA flow diagram detailing the review process (adapted from [38]).

The final step of the screening process was to code the primary studies using a common coding schema (Table 1). Key information was coded, including the publication year, location of the study, the scale of the wetland system, number and type of contaminant(s) of concern, removal mechanisms studied, source of runoff, and removal efficiencies. Concerns on correct coding were resolved by the second coder, which included defining wetland systems (e.g., excluding vegetated ditches and riparian buffers), non-point source identification, and identifying removal mechanisms. Throughout the screening and coding process, coders met weekly to check in on progress and to resolve any issues that arose.

Table 1. Coding schema used for each primary study.

Variable	Description
ID	Identification number
First Author	The first author of the primary study
Year	Year the primary study was published
Journal	Publication source of primary study
Location	The geographic location (state, country) of the study area
Scale	The type and size of the system studied (e.g., microcosms, mesocosms, full-scale)
Type of wetland	The type of wetland that was studied (e.g., pond, subsurface flow, free-water surface wetland)
Length	The length of time during which the study took place (days)
Mixtures present	Does the water source contain more than one type of contaminant?
Number of contaminants of concern	The number of contaminants of concern analyzed in the primary study
Contaminant(s) of concern	The contaminants of concern analyzed in the primary study
Type of contaminant	The type of contaminant of concern analyzed in the primary study (e.g., nutrient, pesticide, antibiotic)
Water type	The source of water studied (e.g., urban runoff, agricultural runoff)
Type of Plants	The type of plants present in the study
Removal mechanism	The mechanism of contaminant removal studied (e.g., sorption, phytoremediation, dilution, wetland size)
Removal efficiencies	The removal efficiencies of the contaminants of concern
Other results	Additional results of importance

3. Results

Following the screening, 181 primary studies were identified, with 153 on CUP removal, 29 on antibiotic removal, and 31 on a combination of contaminants. Primary study publication dates ranged from 1995 to 2021, with a linear increase in primary studies on the topics over the last 15 years. The primary studies were drawn from 37 countries.

3.1. Bibliometric Source Overview of the Primary Studies

Publication information, such as geography, was analyzed to provide insight into the annual scientific production growth of research about wetland treatment systems of runoff mixtures to assess Objective 1. Annual scientific production growth on the topic was approximately 29%, showing that this research area is growing, with the peak year of production being 2019 (Figure 2). The decline in publications in 2020 and 2021 could be due to delayed publications from the COVID-19 pandemic. However, 2021 was close behind with 16 publications, even without a complete representative sample of the year, since the studies were collected up to July of 2021. These results demonstrate that research about CUP and antibiotic treatment from wetland systems is growing as more of these contaminants are introduced into the environment and being detected in downstream best management practices and waterways.

The publication sources for the primary studies were indicative of the interdisciplinary nature, broad application, and relevance of the topic, dependent on region. The top publication sources for the primary studies included Chemosphere (n = 25), Science of the Total Environment (n = 16), and Ecological Engineering (n = 16; Table 2). Table 2 only reports the top five sources. However, 61 total sources were identified. These findings indicate that wetland treatment systems treating non-point source runoff mixtures have broad applicability to a variety of journal sources, which ranged from chemistry, environmental hazards and contaminations, engineering, biology, and ecology-focused journals.

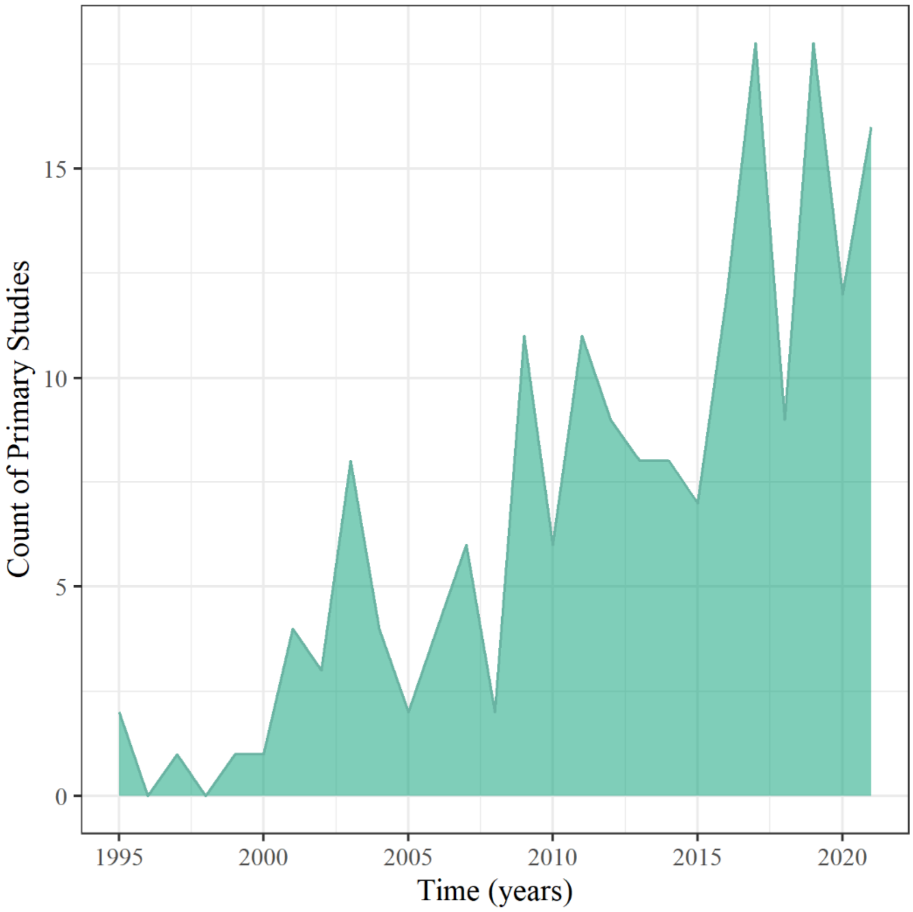

Figure 2. Annual scientific production of primary studies.

Table 2. Results by publication source for the top 5 sources of the primary studies.

Publication Source	Count	Percent of Primary Studies (%)
Chemosphere	25	14
Science of the Total Environment	16	9
Ecological Engineering	16	9
Environmental Pollution	10	5
Environmental Science and Pollution Research	8	4
Other = 56	107	59

The primary studies took place throughout the world, occurring in 37 countries. Countries with the highest number of primary studies included the United States (U.S.; n = 52), China (n = 30), and France (n = 26; Figure 3). This could undoubtedly be due to the limitation of the review to English journals. However, this could also be indicative of water quality concerns and policies limiting the use of emerging contaminants in different regions of the world. For example, the U.S. has approved the use of pesticides and antibiotics (e.g., phorate, dicrotophos, tribufos, oxytetracycline, streptomycin) for outdoor agricultural use, of which many have been banned or phased out by the European Union, Brazil, and China [219]. In the U.S., the Mississippi River Basin is the largest, most intensively farmed region with phorate, dictophos, tirubfos, and oxytetracycline primarily applied to land in the Southeastern region of the United States [220]. This is represented in Figure 4, where the state of Mississippi was the location of 48% of the primary studies in the U.S. Furthermore, these studies were further analyzed based upon experimental design and implications to wetland treatment processes in the sections below.

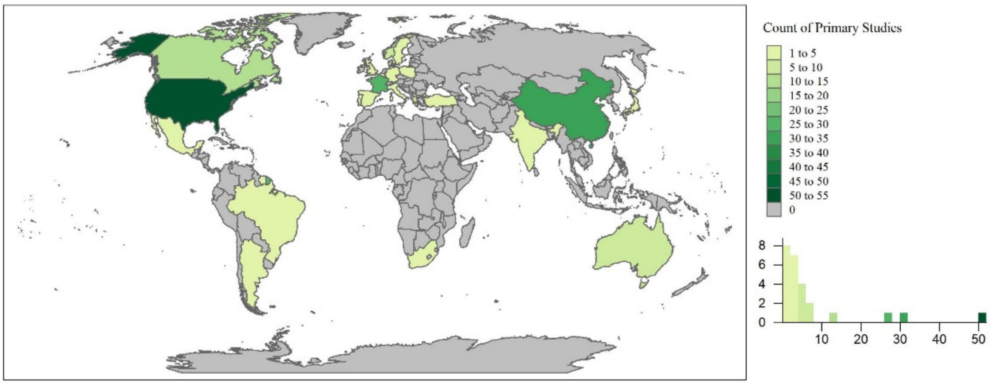

Figure 3. The spatial distribution of the primary studies across the world.

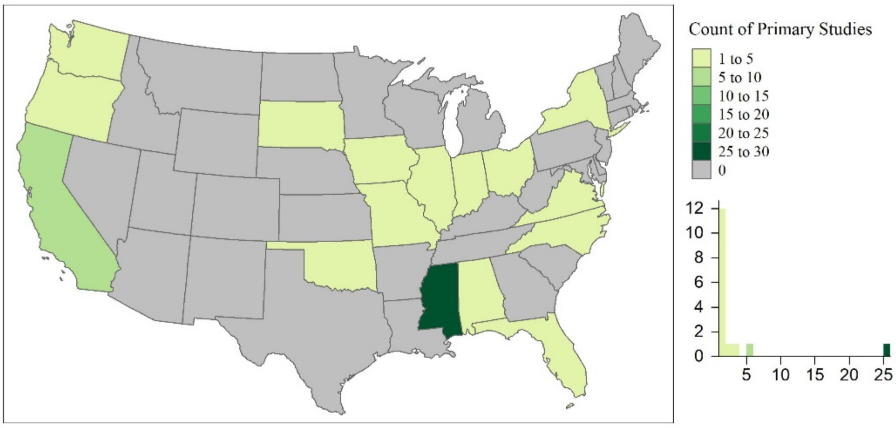

Figure 4. The spatial distribution of the primary studies within the continental United States (U.S.).

3.2. Scale and Type of Wetlands Used

The scale and type of wetland system used for analysis within the primary studies were coded to determine the scope of the studies along with their impact on the study results to assess Objective 2. The scale has the potential to impact removal efficiencies of contaminants by increasing the number of variables in the system, moving from a controlled environment to a natural environment, and introducing artificial impacts (i.e., wall-effects in microcosm and mesocosm experiments) as studies progress from microcosm to full-scale [221,222]. Microcosm studies utilize repeatable small-scale systems to understand a specific biogeochemical process (e.g., sorption, phytoremediation, microbial activity) in a controlled environment [222,223]. On the other hand, full-scale systems include major processes in the natural environment; however, these studies are harder to replicate due to land and cost constraints [221]. Mesocosm studies are a more cost-effective tool to study contaminant removal in wetlands compared to a full-scale system, while still providing useful predictions using treatment replicates and controls to study wetland efficacy in controlled and natural environments [221,224]. As for the primary studies, 43% studied full-scale systems, 37% studied mesocosms, and 21% studied microcosms (Figure 5), with only two studies investigating more than one scale [53,76].

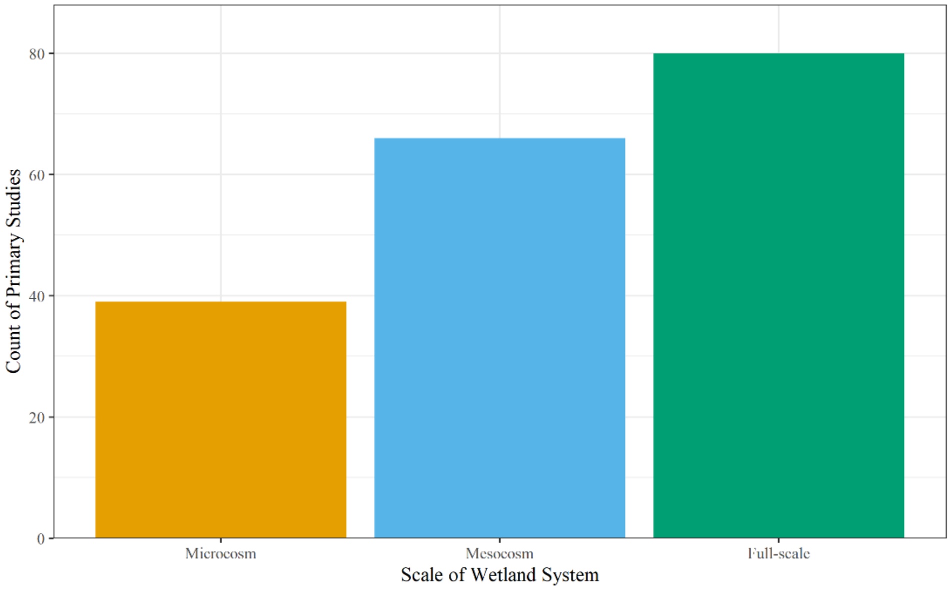

Figure 5. Count of the scale used in the primary studies for the wetland system.

The primary studies that showed high removal efficiencies (greater than 75%) were larger for mesocosm studies. Of the studies that analyzed the mesocosm scale, 68% had at least one contaminant removed at an efficiency greater than or equal to 75% [16,23,45,48,53–55,58,59,67,69,70,76,79,86,102,104,105,112–114,124,144,151,154,161,163,169–171,175,178–180,182,183,185,192,193,195,201–203,209,210,217–219]. Additionally, 54% of microcosm studies resulted in high removal efficiencies [30,60,76,78,83,90,94,97,119,129,131,143,146,164,168,177,181,188,194,200,207] and 59% of field studies resulted in high removal efficiencies [3,46,50,53,62,63,68,74,77,80,82,84,85,87,92,95,99,103,106,107,117,119,121,122,126,128,133,135,139,141,142,147,150,156,159,160,173,174,184,186,187,189,191,196,206,208,212]. However, efficiencies between studies for specific contaminants and/or classes were not able to undergo statistical evaluation due to few studies having the same contaminant and/or class evaluated.

The type of wetland system was another principal factor to consider because the type of system impacts removal efficiencies, dependent on the contaminant of interest [225]. Wetlands are often defined based upon hydrology (e.g., free-water surface and subsurface flow) along with vegetation (e.g., emergent, submerged, floating, and free-floating) [226]. In this review, 19 different wetland systems were identified throughout the primary studies. These ranged from natural systems to different types of constructed systems including agricultural field practices (e.g., rice fields and wetland buffers). Natural wetlands were defined as wetlands that existed naturally in the environment. These included, but were not limited to, floodplains, prairie potholes, depressions, salt marshes, and ephemeral wetlands. Constructed wetland systems included horizontal flow wetlands, subsurface flow wetlands, man-made reservoirs (e.g., ponds, lakes, lagoons, dugouts, and storage dams), free-water surface wetlands, vertical flow wetlands, wetlands in a series, floating treatment wetlands, stormwater basins, and recirculating wetlands. Wetlands used as an agricultural field practice included rice fields, which acted as temporary wetlands with some of the same species as temporary ponds [227]. Additionally, wetland buffers were defined as a wetland system constructed near agricultural fields to remove contaminants in the water before entering receiving waterbodies.

The most common wetland systems identified were horizontal subsurface flow constructed wetlands (n = 42; Table 3). However, while horizontal subsurface flow constructed wetlands were the most common wetland system reviewed, removal efficiencies for CUPs and antibiotics varied widely from 0% norlurazon removal at the field-scale [61] to 100% imidacloprid removal at the mesocosm scale [210]. Additionally, natural wetland systems and reservoirs were analyzed in 15% and 14% of the primary studies, respectively.

Both wetlands were used to treat a variety of contaminants (e.g., atrazine, metolachlor, chlorpyrifos, clothianidin, endosulfan, permethrin, prosulfocarb, fluometuron, isoproturon) in full-scale systems. Removal efficiencies ranged from 10% (clothianidin) [187] to 100% (permethrin) [77] for natural wetlands and 0% (prosulfocarb) [128] to 100% (isoproturon) [126] for reservoirs. The least studied systems were depressions, ephemeral wetlands, wetland buffers, recirculating constructed wetlands, and salt marshes each appearing only once throughout the primary studies. However, six studies investigated the ability of rice fields to mitigate nutrients, pesticides, and antibiotics as a wetland system in Mississippi, U.S. [63,160,174], India [56], China [115], and Spain [103] with high removal efficiencies (58–100%) for several different contaminants (e.g., diazinon, benthocarb, carbofuran, atrazine, permethrin, NH_4^+-N, NO_3-N, nitrate-N).

Table 3. Results by wetland type analyzed in the primary studies.

Type of Wetland	Count	Percentage of Primary Studies (%)
Horizontal flow constructed wetland	41	27
Natural wetland	22	15
Reservoir	21	14
Subsurface flow constructed wetland	19	13
Free-water surface constructed wetland	13	8.5
Vertical flow constructed wetland	9	6.5
Rice Field	6	3.9
Stormwater basin	4	2.6
Restored wetland	4	2.6
Constructed wetland series	3	2.0
Floating treatment wetland	3	2.0
Wetland buffer	2	1.3
Recirculating constructed wetland	1	0.65

3.3. Source and Mixture of Contaminant Types Entering Wetlands

Specific design approaches used in the primary studies were analyzed to identify study length (e.g., days, years), type of water analyzed (e.g., urban runoff, rural runoff), and contaminants evaluated to address Objective 3. This was completed to determine which methodological approaches were used most often throughout the primary studies, along with attempting to understand the impact of seasonality, type of landscape runoff, contaminant type, the presence of mixtures of contaminants, and wetland plant type in the primary study results. The length of the study was important to identify the impact of time on wetland treatment. Water type provided insight into runoff from urban and agricultural landscapes and into which landscapes have been studied the most. The specific contaminants studied and the presence of mixtures were coded to determine the most common contaminants and how the contaminant (CUPs and/or antibiotics) impacted NO_3-N removal efficiencies. Finally, the type of wetland plants recorded in the primary studies assisted with identifying which wetland plants were most commonly used and if there was an impact on the removal efficiencies of different contaminants based on planting plan.

The length of the study was coded in five different categories: hours, days, weeks, months, and years (Table 4). Hours were defined as any study that took place less than 24 h. Only one study fell into this category, which evaluated removal efficiencies of permethrin in a mesocosm study with four different wetland plant species (i.e., *Leersia oryzoides, Typha latifolia, Sparaganium americanum, Thalia dealbata*) over 12 h [54]. The second category, days, was defined as an experiment that lasted between 1 to 7 days, which included 13% of the primary studies. The category of weeks was then defined as an experiment that was between 7 to 30 days, with months defined as an experiment that took place between 30 to 365 days, and years being an experiment that was longer than 365 days.

Table 4. Results by the length of the study analyzed by the primary studies.

Length of Studies	Count	Percentage of Primary Studies (%)
Hours	1	1
Days	23	13
Weeks	48	26
Months	96	53
Years	14	8

Months were the most common length of study (53%), and weeks were the second most commonly evaluated (26%). Most of the microcosm studies (67%) addressed periods shorter than months, while a majority of the mesocosm studies (64%) used longer periods. Additionally, several contaminants (e.g., methyl-parathion, malathion, endosulfan, chlorpyrifos, diazinon, tetracycline) were removed within a relatively short period (i.e., less than 10 days) [144,168,171,174,177]. However, other studies found accumulation of contaminants (e.g., pyrethroid, 2,4-MCPA, glyphosate, propoxycarbazone-Na, NO_3-N) [68,114,126] with recommendations that the long-term impact on wetlands needs to be further investigated.

The runoff from landscapes defined the water type and included agricultural runoff, urban runoff, both agriculture and urban runoff, or nature reserve. This was coded based on land cover in the watershed for field-scale systems. For mesocosm and microcosm experiments, the primary study either stated where the water was collected from, or the type and concentration of contaminants used for synthetic water. For example, Birch et al. (2004) studied a wetland located in an urban watershed in Sydney, Australia to determine the removal efficiencies of organochlorine pesticides, polycyclic aromatic hydrocarbons, trace metals, nitrogen, phosphorous, and stormwater effluent [213]. On the other hand, Butkovskyi et al. (2021) used synthetic wastewater to simulate agricultural runoff by applying tap water, fertilizer solution, and different types of pesticides (bentazone, MCPA, metalaxyl, propiconazole, pencycuron, imidacloprid) to potted microcosms containing *Phalaris arundinacea* sp. *Larsa* [181]. For the primary studies, the most common water type was from agricultural runoff (n = 148), with both agriculture and urban runoff encompassing 18 studies, and urban runoff alone assessed in 15 studies (Figure 6). Studies investigating urban and agricultural runoff were either not specific about the water type and instead reported contaminant(s) applied in upstream regions (e.g., sulfonamides used to treat both human and animal infections [76,90,170], or the field site for the primary studies' watershed contained both urban and agricultural runoff [74,80]. Finally, nature reserve was the least studied landscape (n = 1); Tsui et al. (2008) assessed glyphosate concentrations in the Mia Po Nature Reserve in Hong Kong [87].

Seven different types of contaminants were studied: nutrients, pesticides, antibiotics, other pharmaceuticals besides antibiotics, metals, minerals, and industrial by-products (Table S1). Pesticides included herbicides, insecticides, and fungicides and were the most common type of contaminant analyzed, appearing 556 times throughout the primary studies with peak study counts occurring between 2016 and 2021 (Figure 7). The most common pesticides studied were atrazine (n = 25), chlorpyrifos (n = 22), s-metolachlor (n = 21), alachlor (n = 16), and isoproturon (n = 14), along with 158 other pesticides analyzed. Atrazine is a commonly used herbicide, introduced in 1985, and is mainly used for agricultural landscapes while also being used on residential lawns and golf courses, particularly in the Southeastern United States [228]. Additionally, chlorpyrifos, an insecticide introduced in 1965, and s-metolachlor, an herbicide introduced in 1976, are used for agricultural and non-agricultural landscapes, while alachlor and isoproturon are herbicides mainly used for agriculture.

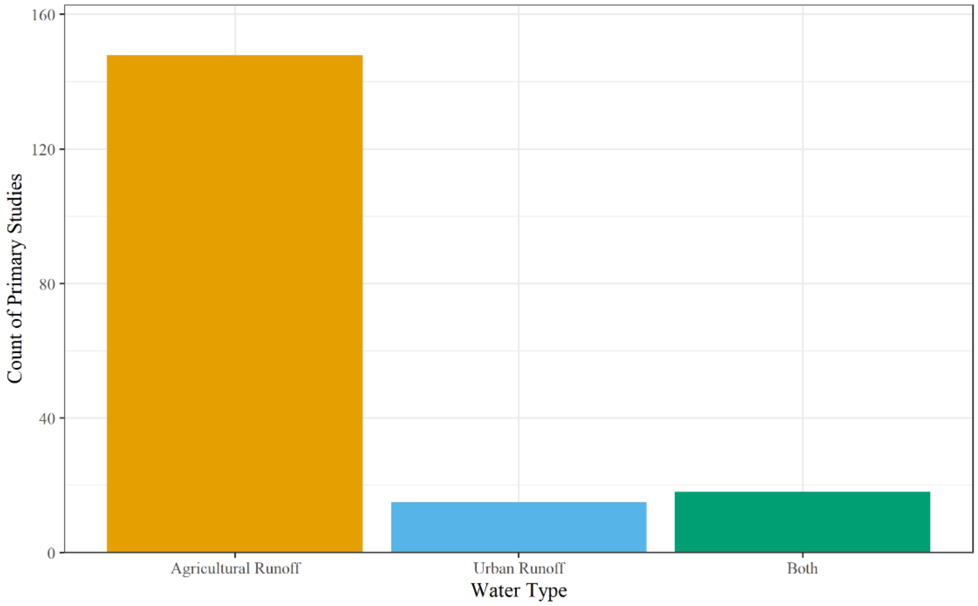

Figure 6. Count of non-point source water used by primary studies.

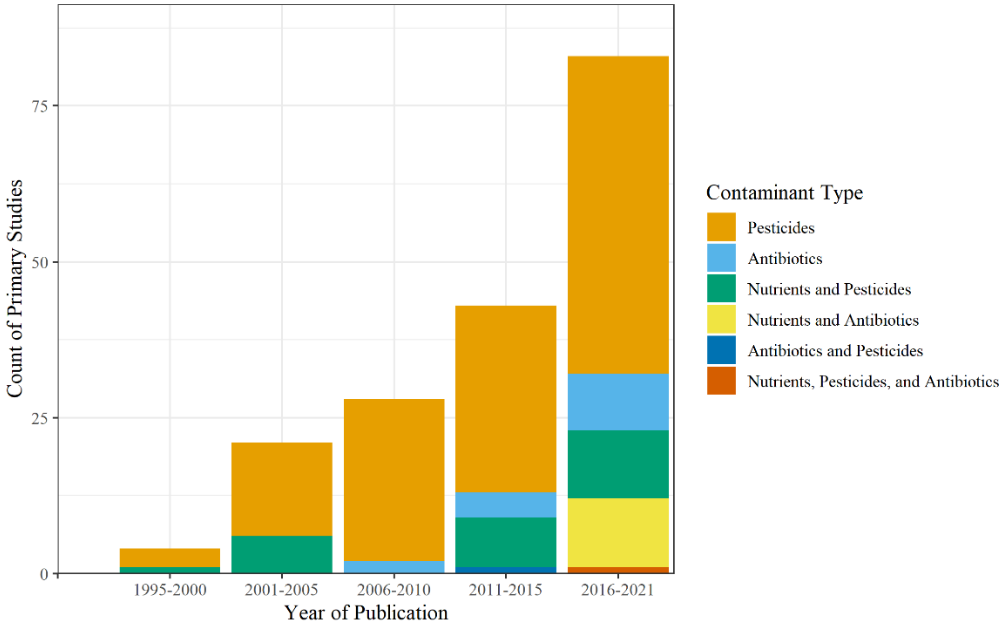

Figure 7. Count of contaminant type and mixture by year.

Antibiotics appeared throughout the primary studies 60 times, starting in 2007, with peak counts of primary studies ($n_{\text{antibiotics}} = 9$, $n_{\text{nutrients and antibiotics}} = 11$, $n_{\text{nutrients, pesticides, and antibiotics}} = 1$) between 2016 and 2021. The most common antibiotics studied were tetracycline (n = 11), sulfamethoxazole (n = 5), monensin (n = 3), narasin (n = 3), and ciprofloxacin (n = 5), out of a total of 29 primary studies. Of these, narasin and monensin are used as veterinary antibiotics and were found primarily in agricultural runoff, whereas sulfamethoxazole, tetracycline, and ciprofloxacin are used more for humans and were found in urban runoff.

Additional contaminants found in the primary studies included other pharmaceuticals, metals, minerals, and industrial byproducts. Other pharmaceuticals included any pharmaceutical that was not considered an antibiotic (e.g., carbamazepine, caffeine, diclofenac, fluoxetine, naproxen, ibuprofen). Industrial byproducts were defined as contaminants that are commonly used in industry such as fragrances (e.g., cashmeran, dihydrojasmonate), plastic production (e.g., bisphenol A, di-n-butyl phthalate), dyes (e.g., uranine), corrosion inhibitors (e.g., benzotriazole), and de-icing fluids (e.g., 5-methyl-1H-benzotriazole). While these contaminants were not the focus of the review, they were observed in the primary studies and are often found in runoff from urban and agricultural landscapes [80,99,101,103,112,137,151,213].

There was a total of 9 nutrients analyzed throughout the primary studies, with the most common being TN (n = 29), NO_3-N (n = 15), NH_4^+-N (n = 12), total phosphorus (TP, n = 10), and nitrite-N (NO_2-N; n = 4). Other nutrients found in the primary studies were orthophosphate, sulfate, chloride, and urea. As for the presence of mixtures, only 30 primary studies looked at contaminant mixtures. Nutrient and pesticide mixtures were the most common mixtures studied, peaking between 2016 and 2021 with 11 primary studies. Only one study in 2020 considered a runoff mixture containing antibiotics, nutrients, pesticides, and other pharmaceuticals [103]. Of these studies that looked at mixtures, only 16 specifically analyzed the impact on nitrogen removal in the presence of different contaminants [23,30,58,59,67,97,102,103,112–114,120,131,169,179,181,201]. Thus, the data shows that only recently have some of these contaminants started to be studied individually, in the case of antibiotics, but also as mixtures. This has resulted in the limitation of this review to systematically analyze the implications of wetland design parameters and mechanisms on N removal processes.

3.4. Removal Mechanisms and Efficiencies

Removal mechanisms were coded based upon the specific processes that were the focus of the primary study to further address Objective 3. Fifteen removal mechanisms were evaluated (Table 5). Varying mechanisms provide insight into the impact on contaminant removal efficiencies, particularly the impact on nitrogen removal processes. Design parameters included size, depth, and aspect ratio of the wetland in addition to the impact of timing (the removal efficiencies over time from contaminant exposure) and space (distance from wetland inlet). If a specific process was not listed and instead removal efficiency of contaminants was analyzed for the wetland system as a whole, then "holistic approach" was coded.

Table 5. Results by removal mechanisms studied for impact on removal efficiencies of contaminants by primary studies.

Removal Mechanism	Count	Percentage of Primary Studies (%)
Phytoremediation	90	23
Sorption	69	18
Microbial activity	43	11
Holistic approach	37	9.8
Dilution	35	9.3
Hydrologic regime/flow rate	26	6.7
Timing	26	6.7
Loading concentration	25	6.4
Space (distance throughout wetland)	8	2.0
Presence of mixtures	7	1.8
Photodegradation	7	1.8
Design parameters (size, depth, aspect ratio)	6	1.5
Temperature of water	3	0.77
Weather	2	0.51
Saline Concentration	1	0.26

For the primary studies, the most common removal mechanisms studied were biological processes such as phytoremediation (n = 90), sorption (n = 69), and microbial activity (n = 43). Phytoremediation was coded if the primary study looked at the impact of vegetated vs. non-vegetated systems [55,161,175], or analyzed the plant roots [23,119,127,180,210], leaves [127,210], and stems [119,210] for contaminant concentrations. The type of wetland plant used throughout the primary studies helps to identify which wetland plants were most common and if there was an impact on the removal efficiencies for different contaminants dependent on plant species. There were 92 genera of plants identified, with *Typha* spp. being the most common, comprising 14% of the primary studies, and *Phragmites* spp. used in 11% of the studies (Table 6). It is important to note that not all studies used wetland plants [43,83,89] or identified the specific plant species within the wetland system [148,159,179]. Also, not all primary studies that specified the plant species present in the wetland system analyzed the impact of phytoremediation as a removal mechanism [21,173,205]. For the studies that did investigate phytoremediation, vegetated systems increased removal efficiencies compared to non-vegetated systems [51,55,66,95,154,161,175,181,217], with mature plants out-performing younger ones [158].

Table 6. Results of the top 10 plant genus used by the primary studies.

Plant	Count	Percentage of Primary Studies
Typha spp.	53	14
Phragmites spp.	43	11
Iris spp.	15	4
Carex spp.	14	4
Cyperus sp.	12	3
Phalaris sp.	11	3
Glyceria spp.	10	3
Canna sp.	9	2
Scripoides sp.	9	2
Other (n = 92)	210	54

As for the impact of the type of plant on removal efficiencies, Lv et al. (2016) concluded that *Typha latifolia*, *Phragmites australis*, *Iris pseudacorus*, and *Juncus effusus* were all able to take up and metabolize imazalil and tebuconazole with removal efficiencies between 46–96% and 25–41%, respectively [78]. Additionally, Tang et al. (2019) concluded that there were no significant differences in planted systems (*Cyperus alternifolius*, *Canna indica*, *Iris pseudacorus*, *Juncus effusus*, and *Typha orientalis*) and that plants with high biomass and transpiration were able to accelerate the removal of chlorpyrifos and conventional pollutants with removal efficiencies between 94–98% [86]. On the other hand, some primary studies found that specific species outperformed others, with *Lemna minor* having high removal efficiencies for dimethomorph (17%) and pyrimethanil (12%) compared to *Spirodela polyrhiza* (11–15%) [111]. Additionally, *Phalaris arundinacea* was better at up taking dicamba, dimethoate, trifloxystrobin, metamitron, and tebuconazole (mean removal of 4%) compared to *Typha latifolia* (2%) [95], *Eleocharis mutata* retained less imidacloprid in the plant material and roots (0.5%) compared to *Nymphaea amazonum* (78.9%) [210], and *Pontederia cordata* reduced greater amounts of azoxystrobin (51.7%) compared to *Juncus effusus* (24.9%) and *Silene latifolia* (28.7%), while *Silene latifolia* was the best at removing imidacloprid (79.3%) [154].

Sorption to wetland media was a removal mechanism of focus for 18% of the primary studies (Table 5). This included studies that compared different media types [67,104,170] or analyzed the amount of contaminant sorbed to the wetland media by determining the concentrations of contaminants [74,87,135,170,229] or determining sorption isotherm coefficients for the media [137,196,198]. The different media types found in the primary studies included biochar [59,170,179,192], straw [133,134], compost [170], different types of soil [67,145,196,197], gravel [104,161,229], pebbles [179], zeolite [59,114,147,218], and cobbles [104].

Soil types and properties (e.g., organic matter content, porosity, structure, moisture content, electrical conductivity) are important components of wetland systems and have been found to impact microbial communities, N cycling, and vegetation growth [229]. However, these soil properties are dependent upon wetland type (natural vs. constructed wetlands) and age. For example, newly developed, constructed wetlands may have to overcome soil compaction, resulting in decreased porosity and redox potential, which in turn impacts N cycling [230]. Overall, the type of contaminants present has been reported to impact sorption processes due to competing cations [87] and differences between highly vs. weakly sorbing contaminants [54]. Additionally, sedimentation or sorption was not the primary removal mechanism in wetland systems with the presence of multiple removal mechanisms (e.g., increased hydraulic retention time, vegetation, microbial activity); instead, it enhanced contaminant removal [67,74,84,115,135,196,218]. For example, Uddin et al. (2019) found electrical conductivity, total organic carbon, and total nitrogen in the soil significantly impacted microbial richness and diversity [115].

Microbial activity is another important removal mechanism in wetland systems because microbial communities facilitate water treatment through metabolic actions (e.g., anabolism, catabolism) [231]. Microbial communities are mainly found in the rhizospheres, the biofilms around the media, and the water. For the primary studies, 11% focused on microbial activity as a specific removal mechanism. The increased microbial activity enhanced the removal capabilities for CUPS and antibiotics [59,60], with microbial degradation being a leading mechanism for removal [194]. However, the microbial communities were impacted by the substrate [59,194], the type and concentration of contaminants present [58,114,179], and the physicochemical properties of the water [179]. In particular, Lu et al. (2021) reported the presence of sulfamethoxazole improved interactions for denitrifying bacteria, but also decreased network complexity and microbial interaction on the whole molecular network, thus altering the community structure of nitrogen-transforming microorganisms [114]. Yuan et al. (2020) observed the addition of Mn ore impacted microbial diversity, causing increased removal potential for antibiotics (ciprofloxacin hydrochloride and sulfamethazine), TN, NH_4^+-N, and NO_3-N [59].

3.5. The Impact of CUPs and Antibiotics on Nitrogen Removal

Overall, 31 primary studies analyzed and reported contaminant removal efficiencies for runoff mixtures containing nitrogen, pesticides, and/or antibiotics to address Objective 4 (Table S2). The removal efficiencies for each contaminant varied widely, with total nitrogen ranging from 5% to 99% removal and a mean of 58%. Ammonia-N had a similar range of 7% to 100% and a mean of 75% removal. Nitrate-N removal varied widely from −113% to 98%; however, only one primary study found an accumulation of NO_3-N, which was attributed to the amount of zeolite used in the mesocosm cells [114]. As for the CUPs and antibiotics studied, the removal efficiencies varied from −619% to 100% and 35% to 100% for pesticides and antibiotics respectively, with negative removal efficiencies being attributed to runoff and remobilization of pesticides in full-scale systems.

The location of the wetlands for these 31 primary studies with the highest removal efficiencies of TN and NO_3-N occurred in the United States [67,99], while the highest removal efficiencies of NH_4-N and pesticides occurred in the United States and Greece [77,99,218]. In contrast, the highest efficiencies of antibiotic removal occurred in China [58]. However, the variation in removal efficiencies reported throughout the primary studies was likely due to the wide range of CUPS and antibiotics studied, the type of wetland systems (e.g., scale, wetland type, plant type), and the climatic conditions of the wetland analyzed. For example, Lu et al. (2021) reported that removal efficiencies of NH_4^+-N were most affected by temperature, rather than the concentration of contaminants, with increasing removal efficiencies occurring at higher temperatures and with increased contact time [113]. For the 31 primary studies identified as analyzing runoff mixtures containing nitrogen, CUPs, and/or antibiotics, only two studies analyzed natural wetland systems and found relatively high removal efficiencies (43–100%) for TN, NO_3-N, NH_4^+-N, atrazine, S-metolachlor, and Permethrin [77,184]. The lowest removal efficiencies for TN and NH_4^+-N were found with full-scale horizontal flow constructed wetlands [3,213], while the lowest removal efficiencies for NO_3-N and antibiotics were found with mesocosm vertical flow constructed wetlands [114]. In contrast, the lowest removal efficiencies for pesticides were associated with a full-scale wetland buffer [126]. Despite these external climatic and wetland design parameters, the leading cause for N-removal disruptions is thought to be due to contaminant mixture type and concentration.

Several primary studies reported the impact of CUPs' and antibiotics' presence on nitrogen removal efficiencies. Of these, seven studies reported that the specific contaminant analyzed decreased nitrogen removal [30,58,67,113,114,169]. The impact of CUPS and antibiotics on nutrient removal was mainly attributed to a decline in the microbial communities responsible for nutrient metabolism and degradation [30,114,131,169], where the presence of plants and type of plant species [23,102,120,201], saturated vs. unsaturated conditions [120,201], weather conditions [201], and timing [48,169] also played a role in N removal in the presence of CUPS or antibiotics. Ohore et al. (2021) reported that the presence of tetracycline decreased nitrogen removal initially, but observed an increase in total N removal with an increasing number of days due to the degradation of antibiotics in the wetland system [169]. Additionally, Tong et al. (2019) observed the presence of plants protected the microbial communities, limiting ofloxacin's ability to negatively impact NO_3-N and $NH_4{}^+$-N removal [30].

However, the presence of CUPs and antibiotics has also been observed not to affect nitrogen removal in wetland treatment systems [23,158,201], and in some cases, the CUPs or antibiotics increased N removal [43,131]. These increases in N removal are presumed to be due to an increase in microbial community diversity and richness in the presence of CUPS or antibiotics [23,171]. Yu et al. (2019) observed that tetracycline had a slightly negative effect on nitrogen removal (91% to 71%); however, the presence of copper and tetracycline led to higher microbial richness with increases in microbial variations [171]. In contrast, Lu et al. (2021) observed that the presence of sulfamethoxazole had positive influences on denitrifying bacteria interactions, but reduced the network complexity and microbial interactions in the wetland mesocosms [114].

Runoff mixtures not only impact N removal efficiencies, but also impact the wetlands' ability to remove CUPs and antibiotics. Recent investigations on the impact of N on CUPs indicated that nitrifying bacteria can also degrade certain pesticides (e.g., metribuzin, imazalil, tebuconazole) [59,102,120]. However, in some cases, the presence of nutrients decreased CUP or antibiotic removal efficiencies in the wetland [171,205]. For example, Matamoros et al. (2020) reported both the presence of nutrients impacted CECs removal and that the presence of other CECs (caffeine, tributyl phosphate, 5TTri, bisphenol A, benzotriazole, carbamazepine, diclofenac, ibuprofen, lorazepam, naproxen, oxazepam, primidone, and triclosan) can reduce pesticide (sulfonyl 104, alachlor, bentazone, chlorpyrifos, DEET, molinate, oxadiazone, propanil, tebuconazole, and MCPA) removal in rice fields [103].

4. Conclusions

This review sought to analyze 181 primary studies that used wetlands of varying scales to treat runoff from urban and agricultural landscapes; this was accomplished by assessing the bibliometric information of the primary studies, identifying wetland scale and type, quantifying the source and mixture of CUPs and antibiotics entering wetlands, identifying mechanisms of removal used to treat runoff mixtures, and analyzing the implications of these mixtures to nitrogen removal processes (e.g., denitrification, plant uptake). While the scientific production of wetland treatment systems used to treat runoff has increased by 29%, of the primary studies reviewed, only 16 wetland treatment systems received urban runoff, while 82% of the studies used wetlands to treat agricultural runoff. Additionally, antibiotics and runoff mixtures have only recently begun to be studied. This has resulted in only 31 of the primary studies evaluating removal efficiencies in the presence of runoff mixtures, with only one study analyzing mixtures containing nutrients, antibiotics, and pesticides.

The impacts on CUP (−619–100%), antibiotic (35–100%), and N (TN = 5–99%, NO_3-N= −113–98%, NH_4-N = 7–100%) removal efficiencies varied greatly over the primary studies. Variations were likely due to the wide range of different types of

CUPs (n = 556) and antibiotics (n = 60) analyzed. Additionally, the location, scale of the wetland system (microcosm, mesocosm, field-scale), presence and type of plants, the substrate used, weather conditions, wetland type, and design parameters all impacted the efficacy of the wetland system for water quality treatment. However, the largest impact on nitrogen removal was the shift in microbial community diversity and richness in the presence of CECs; however, this shift was seen to both increase and decrease microbial communities depending on the contaminant mixture, showing a lack of understanding of how microbial communities are impacted by different types and mixtures of CUPs and antibiotics. In addition, long-term exposure analyses of wetland treatment processes and efficacy were limited.

Throughout the primary studies, a lack of uniform reporting on wetland removal performance appeared, with a portion of the studies reporting removal efficiencies, defined as the difference between the influent and effluent mean concentrations over the influent concentration, while others reported removal loads, defined as a mass balance removal rate. Some primary studies also reported on sorption isotherms, microbial populations, or toxicity. In addition, the wide variety of different classes of CUPs and antibiotics analyzed throughout the primary studies made it difficult to perform further statistical analysis to compare wetland type, scale, length of study, and removal mechanisms with removal efficiencies. A significant knowledge gap remains in urban wetland treatment systems, which are continuing to become more important as urban centers continue to grow. Even more, a significant knowledge gap remains in how wetland treatment systems are used to treat non-point source mixtures containing antibiotics and CUPs, resulting in a significant unknown regarding nitrogen removal efficiency in wetland systems as contaminant runoff mixtures and mechanisms for treatment evolve.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13243631/s1, Table S1: Contaminant type and appearances in the primary studies. Table S2: Contaminant removal efficiencies for the primary studies that analyzed both nitrogen and pesticide and/or antibiotics in wetland treatment systems.

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