Review

The Status of Research on the Root Exudates of Submerged Plants and Their Effects on Aquatic Organisms

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Abstract: The ecological restoration of submerged plants is one of the most widely used technologies in the remediation of eutrophic water bodies. This technology mainly removes nitrogen, phosphorus, and other nutrients in water through the absorption effects of plant roots, stems, and leaves and the biotransformation of microorganisms attached to their surfaces. Root exudates can directly affect root-attached microorganisms and other aquatic organisms, thus significantly influencing water remediation by submerged plants. At present, there are few reviews on the root exudates of submerged plants and their effects on aquatic organisms. In this study, the composition, collection, and methods of detecting the root exudates of submerged plants are reviewed. Factors affecting the release of root exudates from submerged plants are analyzed, including abiotic (light, temperature, and nutritional status) and biotic factors (rhizosphere microorganisms). The positive or negative effects of root exudates on phytoplankton, zooplankton, and microorganisms are also discussed. The results show that plant species, growth stages, and environmental factors (light, temperature, and nutritional status) are crucial factors affecting root exudates. In addition, submerged plants can significantly influence phytoplankton, zooplankton, and microorganisms by releasing allelochemicals or other root exudates. Based on the results of this study, the influencing mechanisms of root exudates on ecological restoration processes by submerged plants are clarified. This review provides important guiding significance for applying submerged macrophytes in water restoration.

Keywords: submerged plants; root exudates; allelochemicals; algae inhibition; water restoration

1. Introduction

Root exudates comprise a variety of substances released from plant roots into the growth medium during a plant's growth process [1]. They are adaptive mechanisms developed during plants' long-term evolution and growth that are affected by environmental factors and can change the rhizosphere environment. Research on this topic began at the end of the 18th century. Plenk et al. found that root exudates promoted or inhibited the growth of neighboring plants [2]. Since 1904, after Hiltner proposed the concept of the rhizosphere, studies on root exudates have gradually been carried out. In the 1950s, their role was initially revealed, but more macro studies were conducted due to technological limitations. Since the 1970s, studies on root exudates have flourished with technological improvements. Recent studies showed that root exudates are crucial for maintaining the vitality of the rhizosphere ecosystem. They are also an important part of material migration

and regulation in the rhizosphere microecosystem. The systematic analysis of the response of root exudates to environmental factors is a research hotspot in the field of ecological restoration [3].

Lakes account for only a small fraction of the Earth's surface water resources but provide essential material, energy, and information exchange with terrestrial ecosystems. However, lake eutrophication has become a global challenge, with unnaturally high nutrient concentrations destroying about 40% of lakes and reservoirs worldwide [4]. This leads to the excessive growth of algae in water bodies, ultimately causing reductions in dissolved oxygen (DO) levels, declines in water quality, and the death of fish and other aquatic organisms. Technologies for controlling water eutrophication mainly include chemical flocculation [5], microbial dosing [6], and aquatic plant remediation [7]. Among them, remediation technology using aquatic plants is widely used due to its low cost, lack of secondary pollution, and simple operation. It can remove pollutants through the absorption effect of aquatic plants and the biotransformation effect of rhizosphere microorganisms to purify water bodies. Submerged plants play a significant role in remediation processes. However, the role and mechanism of plant root exudates in water remediation are still unclear. Some studies have revealed that the root exudates of submerged plants can provide carbon sources for microorganisms and promote nitrogen removal. Root exudates also play an important role in substance exchange and information transmission, which has important ecological significance [3]. The release of chemical substances from plant roots is part of its normal physiological metabolism, but environmental stress can affect the composition and content of these exudates. These changes can directly reflect the growth and metabolism of plants [8]. Most studies on organic acid exudates have focused on terrestrial plants and rice. Few studies have been conducted on the organic acid exudates of submerged plants. In 2008, Long et al. observed that the phosphorus level in the rhizosphere of seagrass (*T. testudinum*) increased linearly with the concentration of organic acids, which increased with an enhancement in seagrass productivity. Seagrass is an important source of organic acids, which are present at significant levels in the rhizosphere. Although the allelopathy between submerged macrophytes and algae has been frequently reported and the effect of algae exudates on submerged macrophytes has been widely studied, studies on the effect of submerged macrophyte exudates on algae have been insufficient [9]. Xu et al. found that the culture water of mature *Ottelia acuminata* significantly promoted the growth of *Microcystis aeruginosa M. aeruginosa*. In contrast, the culture water of seedlings had no significant effect [10]. Girum Tamire et al. demonstrated that *Potamogeton schweinfurthii* had a significant allelopathic inhibitory effect on cyanobacteria (especially *Microcystis* and *Dolichospermum* spp.). This finding is important for ecological research, but further studies are needed to determine whether exudates are produced by conventional metabolic processes [11]. Wang et al. screened a special bacterial plant-growth-promoting rhizobium (PGPR) from the rhizosphere of *Vallisneria natans* (*V. natans*) under low and high organic matter loads in sediment. It survived in the plant roots and could directly or indirectly promote plant growth. This PGPR used the root exudates of *V. natans* as the sole carbon source, showing high competitiveness for rhizosphere nutrition. This screening method provided a new approach to the artificial restoration of submerged plants [12].

Thus far, many studies have examined the root exudates of submerged plants, but the corresponding reviews have been insufficient. This study reviews the development history, classification, collection and detection methods, and influencing factors of root exudates, as well as the effects of the root exudates on aquatic organisms. The aim of this study is to provide valuable suggestions for the research, development, and application of submerged plants in water restoration.

2. Submerged Plant Exudates

2.1. Definition and Classification

Root exudates refer to various substances secreted or released from different parts of the plant root system to its growth medium during plant growth. The main components of root exudates include organic matter composed of carbonaceous compounds, inorganic ions, H^+, and water [13]. Root exudates can be divided into four categories: (1) exudates, which mainly include low-molecular-weight organic compounds released through cell diffusion, such as sugars, amino acids, and organic acids; (2) secretion, which includes the metabolites actively released by cells in the metabolic process, including phenolic compounds, polysaccharides, and protons; (3) mucilage secreted by root cap cells, epidermal cells without secondary walls, and root hair cells; (4) decomposition and abscission, which are the root cell tissue and its decomposition products [14]. Root exudates can be divided into high- and low-molecular-weight organic compounds according to their molecular weight. High-molecular-weight compounds mainly include polysaccharides, proteins, and enzymes, while low-molecular-weight organic compounds include amino acids, organic acids, sugars, phenols, and secondary metabolites [15]. According to the nature of action, root exudates can be divided into two types: common and specific. Common exudates are common to most plants, while specific exudates are unique to specific plants under specific conditions [16]. Common types of root exudates of submerged plants are shown in Table 1.

Table 1. Common types of root exudates of submerged plants [14,17,18].

| Class	Representative Compounds	Major Functions
Saccharide	Glucose, fructose, galactose, rhamnose, ribose, raffinose, xylose, sucrose, lactose, maltose, and arabinose	Promoting rhizosphere microbial growth, regulating soil properties, and affecting rhizosphere microbial community structures
Organic acids	Oxalic acid, tartaric acid, pyruvic acid, malic acid, malonic acid, lactic acid, catalpol, succinic acid, fumaric acid, formic acid, acetic acid, propionic acid, butyric acid, valeric acid, and salicylic acid	Changing the soil's pH value, activating soil nutrients, and improving nutrient absorption by plants
Amino acid	Aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, γ-aminobutyric acid, lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, γ-aminobutyric acid, lysine, histidine, arginine, and proline	Promoting plant growth and development, improving plant stress resistance, and regulating the soil's microbial community
Long-chain fatty acid	Stearic acid, palmitic acid, oleic acid, and linoleic acid	Promoting plant defense against foliar pathogens, enhancing plant resilience, regulating plant–microbial interactions, and acting as a nutrient source for microorganisms
Steroid	Cholesterol and stigmasterol	Acting as nutrient sources for microorganisms and enhancing the growth potential and stress resistance of plants
Growth hormone	Biotin, vitamin, choline, inositol, and phytohormone	Promoting cell growth, differentiation, division, and biosynthesis
Proteins and enzymes	Amylase, DNA enzyme, phosphatase, polygalacturonase, protease, RNA enzyme, invertase, urease, xylanase, PR protein, etc.	Promoting the absorption and conversion of nutrients and catalyzing the degradation of organic pollutants
Other compounds	Flavonoids, nucleosides, glycosides, and polysaccharides	Genetic information transfer, energy storage and conversion, signal transduction, and storage and transport of substances

2.2. Production Pathway and Mechanism

A total of 28–59% of plant photosynthetic products are transferred to the underground part, 4–70% of which are released into the soil through root exudates. There are two main mechanisms for the release of root exudates, involving metabolic and non-metabolic pathways. However, a unified conclusion about the specific mechanism has not been reached [19]. Figure 1 shows some pathways and proteins that transport certain organic compounds and special metabolites around the cytoplasm and export them to the rhizosphere. Vesicles that sprout from the endoplasmic reticulum and Golgi are loaded with specialized metabolites, guided to the cytoplasm or plasma membrane, and fused with it, releasing content to vacuoles or the extracellular space. The circular symbol indicates the general transporter that loads the compound into the vesicle, and the transport process is completed by the general transporter, involving membrane-bound transporters such as ABC, MATE, MFS, and ALMT families. Although secondary metabolites are not directly involved in plant growth, they are essential for plant disease and stress resistance. Non-metabolic pathways involve the decomposition of root epidermal senescent cells and the release of substances from dead cells, which are not regulated by metabolism. Because the process of root exudates is very complex, both simple and specific root exudates are secreted. Therefore, the evaluation of root exudates should consider various factors, such as the plant environment. Although there are different opinions on the mechanism of root exudates, the consensus is that root exudates help to alleviate stress under environmental stress. Releasing root exudates is an active physiological process, with the energy derived from cell metabolism. Thus, root exudates may result from plant interactions with stressful environments, especially under specific selection pressures. In other words, root exudates are an active adaptation mechanism of plants under environmental stress, and the production of specific exudates is the essence and evolution of plant adaptation to environmental stress [20].
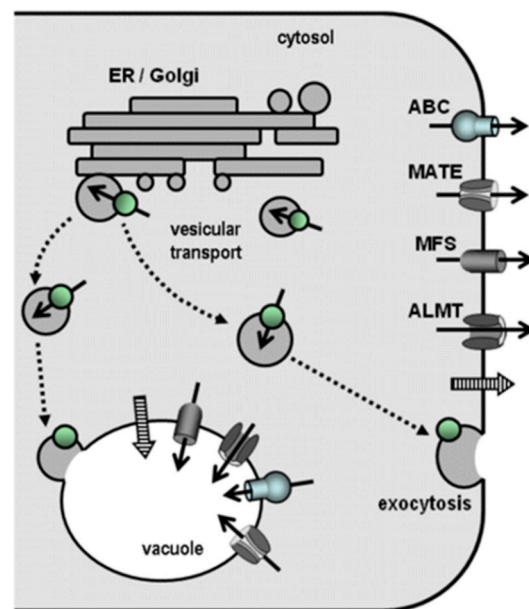
Figure 1. Schematic diagram of root cells. The round symbol depicts a generic transporter loading compounds into the vesicles. The membrane-bound transport proteins known to facilitate the transport of compounds across membranes include the ATP binding cassette family (ABC), the multidrug and toxic compound extrusion family (MATE), the major facilitator superfamily (MFS), and the aluminum-activated malate transporter family (ALMT). The striped arrow indicates the possible diffusion pathway of highly hydrophobic compounds across the lipid bilayers. The other arrows show the direction of substrate movement. Reproduced with permission from [20]. Copyright 2012, Oxford University Press.

Recent studies have shown that plant secretions are released into the environment through various processes. They are leached from decomposed plant residues and the roots or leaves of living plants. These processes are related to the beneficial effects of crop rotation or co-cultivation of certain submerged plants. The exchange of natural products between important plants may explain these ambiguous phenomena [21]. Moreover, the absorbed natural products in some recipient plants are modified, while they simply accumulate in other recipient plants. These modifications include hydroxylation, methylation, and glycosylation processes. In the past, it was thought that these reactions were part of a deliberate detoxification mechanism known as the "green liver concept". However, since the manner and extent of these modifications vary greatly between different plant species, general and universal mechanisms such as the "green liver concept" can be ruled out [22]. The study by Laura Lewerenz et al. was the first to bring to life the phenomenon of "lateral natural product transfer". Figure 2 shows that harmaline is translocated via the xylem into the leaves. Subsequently, the constituents of the xylem are further distributed within the leaf blade, driven by transpiration and root pressure. In the further distribution of the alkaloids within the leaf blade, increasing harmaline is oxidized, resulting in a continuous increase in the ratio of harmine to harmaline [23]. In addition, studies by Tahani Hijazin et al. from the same team also confirmed that various alkaloids are effectively absorbed from the soil, which is strongly influenced by the rhizosphere pH due to their alkaline nature. However, intense caffeine intake is not affected by the various pH values. pH significantly affects the uptake of alkaloids, and the highest uptake appears to be achieved at a specific pH. Thus, the absorption of various alkaloids and their dependence on pH may vary, and the extent of alkaloid absorption cannot be predicted [24].
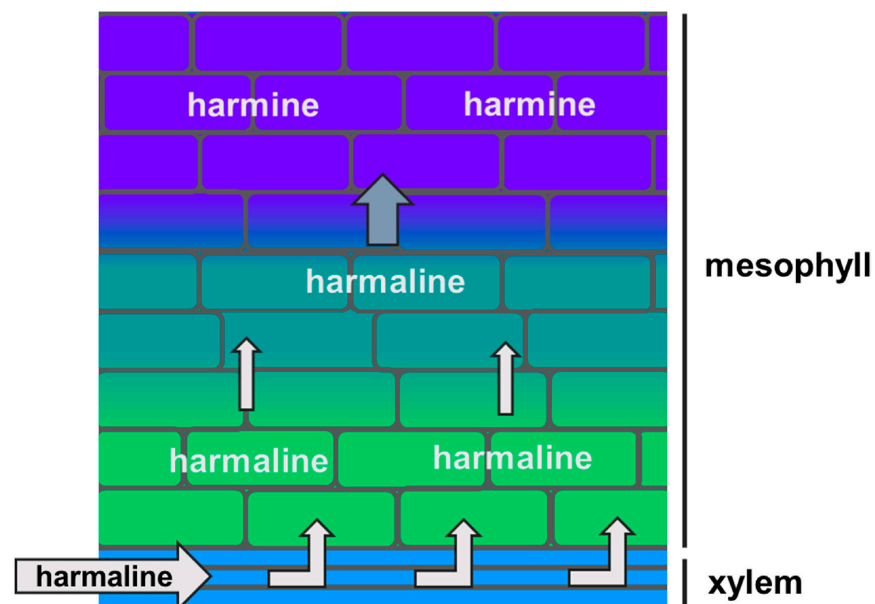

Figure 2. Scheme of the translocation and conversion of harmaline in barley leaves. The bottom layer and above layer represent the xylem and mesophyll of the leaf blade. The arrow distribution and oxidation pathways of harmaline with the leaf blade. Reproduced with permission from [23]. Copyright 2020, Elsevier.

2.3. Collection and Analysis

Processes of collecting root exudates are troublesome due to many interfering factors and uniform research methods. Different conditions have different classification standards. Thus, the correct and effective collection of plant root exudates is a key step in correctly studying the chemical composition of root exudates. Figure 3 shows commonly used methods for collecting root exudates. Among them, the root exudates collected under closed, sterile conditions can more accurately reflect the total amount of organic matter,

and in situ collection under soil culture conditions can more accurately reflect the actual situation of root exudates. Thus, researchers can select appropriate collection methods in accordance with different experimental purposes [25–27]. The most commonly used method for collecting the root exudates of submerged plants is the disturbance collection method. After submerged plants are uprooted and cleaned, they are immersed in a certain amount of ultrapure water and placed in the dark for 24 h. Then, the soaking solution is filtered as the crude root exudates, followed by subsequent treatment.
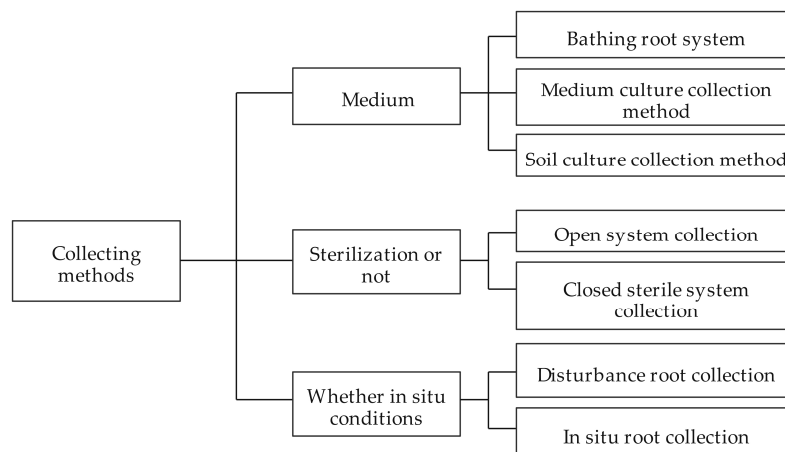
**Figure 3.** Common collection methods of submerged plant exudates.

Achieving accurate qualitative and quantitative analyses of all of the components of root exudates is difficult due to their complex compositions. Currently, most studies focus on analyzing high-content and important functional compounds. Among them, organic acids, amino acids, and sugars are representative root exudates that play an important role in the whole rhizosphere system. Methods of their analysis and detection are relatively mature. Chromatography is commonly used to detect these substances, including GC, GC-MS, HPLC, UPLC, and LC-MS. Table 2 summarizes the advantages and disadvantages of the methods for detecting submerged plant exudates. HPLC is mostly used in the detection and analysis of polyphenols in the exudates. Figure 4 shows the analysis process of exudates released by *Myriophyllum verticillatum*. In the early stage, Nakai used HPLC and APCI-MS to identify allelopathic polyphenols such as ellagic acid, gallic acid, pyrogallic acid, and (+)-catechin released by *Myriophyllum verticillatum*. The plants were cultured in the medium for 3 d to prepare the culture solution. Then, the solution was separated according to the polarity and molecular weight of the allelochemicals. The components were analyzed using HPLC and APCI-MS [28].

Table 2. The advantages and disadvantages of commonly used methods for analyzing and detecting submerged plant exudates.

Analysis and Test Method	Advantages	Disadvantages	Reference
GC	Analysis of the substances with a low boiling point, good thermal stability, high volatility, and stable retention time, which can directly identify the structure	Unsuitable for analyzing some substances that need pretreatment with a high boiling point and poor thermal stability via direct injection	[29,30]
GC-MS	Accurate characterization of the substances with a large database	Insufficient software for analyzing data	[31]
LC-MS	A wide range of analysis, strong separation ability, low detection limit, and high degree of automation	Lack of a standard database to identify the structure	[32]

Table 2. *Cont.*

Analysis and Test Method	Advantages	Disadvantages	Reference
UPLC	Fast analysis speed, short time, and high separation efficiency	Short service life of the chromatographic column and demanding laboratory conditions	[33–35]
HPLC	High separation efficiency, good selectivity, high detection sensitivity, automatic operation, and wide application range	High operating cost and long analysis time	[36]
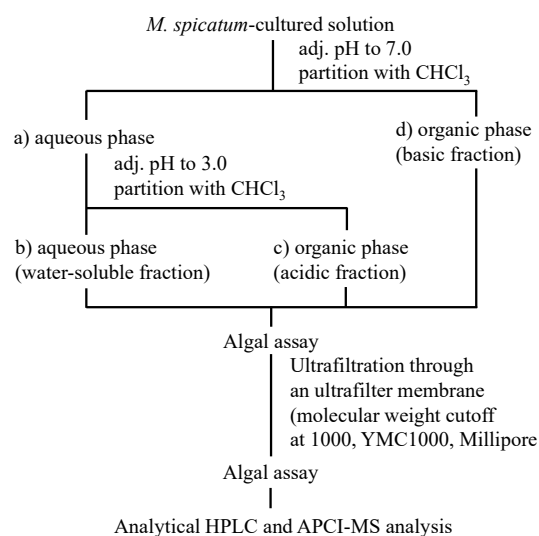
Figure 4. Study on the analysis method of exudates released by *M. spicatum*. Reproduced with permission from [28]. Copyright 2000, Elsevier.

3. Influencing Factors of Root Exudates

3.1. Plant Species and Growth Stages

Root exudates, as an inherent characteristic of plants, directly reflect the species and genetic characteristics of plants. Thus, different kinds of submerged plants release root exudates with different compositions and contents, leading to different allelopathic effects on the surrounding environment [37]. Cheng et al. found significantly different allelopathic effects on *Synechocystis* from different submerged plants. The culture water of sea cauliflower exhibited a slight promoting effect on the growth of *Synechocystis*. In contrast, the culture water of *Myriophyllum aquaticum*, *Ceratophyllum demersum*, *Myriophyllum spicatum*, *Hydrilla verticillata*, and *Vallisneria natans* showed different degrees of the algae-inhibition effect. This indicates that the allelochemicals released by submerged plants can affect the normal growth of *Synechocystis* [38]. Zhang et al. demonstrated that the exudates of *Phellinus linteus* and *Potamogeton malaianus* could inhibit the growth of *Microcystis aeruginosa* and *Selenastrum capricornutum*. However, the sensitivity of the two algae to the exudates of the two plants was different. A GC-MS analysis showed that the exudates of the two submerged plants contained a variety of compounds, and only three alcohols were detected in the exudates of *Potamogeton malaianus*. These specific alcohols may reflect the different degrees of sensitivity of *Microcystis aeruginosa* and *Selenastrum capricornutum* to the exudates of *Potamogeton malaianus* [39]. Xing et al. observed five organic acids detected in the root exudates of *Vallisneria natans*. Oxalic acid was the main component, accounting for 87.5%. The contents of malic acid and citric acid were 4.74% and 6.82%, respectively. Formic and ascorbic acids can be ignored when they comprise less than 1% of the total. Different types of submerged plants produce different types and contents of exudates, leading to different inhibitory effects on algae [40]. Pakdel et al. examined the allelopathic effects of

Chara australis and *Potamogeton crispus* on microalgae. All treatments exhibited significant negative effects on *A. variabilis*, with the strongest effect on *C. australis*. On the contrary, there was no significant effect on the growth of *S. quadrauda*. This result confirms that large plant allelochemicals target specific organisms [41]. In addition, the same submerged plants may have different inhibitory effects on algae at different growth stages. Xu et al. observed that the mature plant culture water of *Ottelia acuminata* had a significant effect on the growth of *P. aeruginosa* and significantly promoted the growth of *M. aeruginosa*. In contrast, the seedling culture water had no significant effect on the growth of *P. aeruginosa*. This may be because mature plants secrete nutrients or small amounts of elements that are beneficial to the growth of cyanobacteria [10]. Mulderij et al. also found different allelopathic effects of two *Chara* species on three green algae during different plant growth stages. Compared with Xu et al.'s study, their mature plants reduced the growth rate of *C. parvum*, while the effect of the young plants was the opposite [42].

3.2. Environmental Factors

The secretion of root exudates is a response characteristic of plants, and its type and quantity are affected by environmental conditions such as lighting, temperature, and nutrient levels. Abnormal conditions result in the release of abnormal root exudates, possibly leading to growth arrest or the death of plants [43]. Gross et al. found that light levels had a significant effect on the root exudates of *M. spicatum*. Specifically, bright lighting conditions increased the content of phenolic compounds secreted by *Myriophyllum spicatum*. However, the concentration of the main allelochemical, tellimagrandin II, was increased under low lighting conditions. This indicates that lighting conditions have a specific regulatory effect on the root exudates of *M. spicatum*, and the response of different types of exudates to light is different [44]. Erhard et al. observed that all related flavonoids could be detected in the exudates of *Elodea nuttallii* under different lighting conditions. However, the lighting conditions affected the quantity of specific flavonoids. In particular, high irradiance may promote the biosynthesis of luteolin diurea compounds, which was supported by field observations. They speculated that the increase in the content of luteolin diglucuronic acid is an adaptive response of plants to higher UV-B irradiation. This indicates that lighting conditions, especially the intensity of UV-B irradiation, have a significant effect on the synthesis and secretion of specific flavonoids from *Elodea nuttallii* [45]. Both strong and weak light influence the secretion of submerged plants. Martin et al. studied the effects of all-optical, continuous, and fluctuating light reduction on root exudates of three seagrasses (*Cymodocea serrulata*, *Halophila ovalis*, and *Halodule unintervis*). They found that fluctuating light exhibited the most significant effect, increasing the secretion of DOC (from the root), protein-like DOM, and humus-like DOM from the three seagrasses. This study highlights that the root exudates of seagrasses are highly correlated with light availability, and the underground environment is particularly sensitive to the reduced light reaching submerged plants [46].

Temperature is also an important factor in determining the physiological status of plant roots. Normal temperatures are conducive to the growth and physiological metabolism of plant roots. Abnormal temperatures cause adverse stress and damage the physiological metabolism of roots. Temperature can also affect the photosynthesis and respiration of plants. Therefore, temperature significantly affects the composition and content of plant root exudates. Previous studies have shown that plant roots secrete organic acids or other amino acids, enzymes, and other substances to resist high-temperature stress and adapt to environmental changes. Most root secretions increase with an increase in temperature [47]. Gu et al. found that when the water temperature was enhanced by 5 °C, the abundance of heterotrophic bacteria in seaweed exudates increased rapidly. This is because the enhancement in temperature significantly increases the assimilation rate of bacteria to the exudates, resulting in a decrease in the content of seaweed exudates [48]. Similarly, Erhard et al. observed all flavonoids in the exudates of *Elodea nuttallii* under different temperature treatments, and the temperature changed the content of individual flavonoids. For example,

the content of chrysoeriol diglucuronic acid and apigenin was negatively correlated with temperature. In addition to luteolin diglucuronide, temperature has a negative impact on most phenolic compounds [45].

Plant growth is inseparable from nutrition. A lack of nutrients regulates the intensity and pathway of plant physiological and biochemical reactions and even changes the metabolic pathway of substances, thus affecting the composition and content of root exudates. Insufficient phosphorus and nitrogen can affect the production and release of chemicals by submerged plants [44]. This effect may depend on the nutrient levels of submerged plants. Most of the phosphorus required by plants reaches the root surface through diffusion. A deficiency in phosphorus occurs because phosphate always forms insoluble mineral phases with metals (such as calcium, iron, and aluminum) [49]. For example, phosphorus deficiency increases the production of polyphenols in *M. spicatum* and enhances the inhibition of cyanobacterial alkaline phosphatase [50]. In addition, many studies demonstrated that the secretion of organic acids and acid phosphatase in most plant roots increases significantly under insufficient phosphorus conditions. Phosphorus-efficient plants can promote the activation and absorption of insoluble phosphorus by increasing the secretion of organic acids. The most commonly reported organic acids are dicarboxylic and tricarboxylic acids, including oxalic, acetic, malic, fumaric, and citric acids [51]. Organic acids improve the bioavailability of P by replacing P from phosphorus-containing oxides (Fe, Al, and Ca) or complexing organic anions with metal ions in oxides [52]. Xing et al. used high-resolution dialysis and film-diffusion gradient techniques to analyze the changes in phosphorus in the rhizosphere of *Vallisneria natans*. They found that the enrichment of P and Fe in Fe patches on the rhizosphere was 5.92 and 3.12 times that of non-rhizosphere sediments, respectively. Further analysis showed five organic acids with low molecular weight in root exudates, and oxalic acid accounted for 87.5%. This finding indicates that *Vallisneria natans* significantly improves its ability to obtain rhizosphere phosphorus through the complexation of iron (III) and oxalic acid [40]. Figure 5 is a schematic diagram showing the coupling process between Fe plaque enrichment and organic acid complexation during the release of phosphorus from the rhizosphere of *Vallisneria natans*. When exploring the response of submerged plants to the nitrogen concentration in the environment, a significant phenomenon is that the released exudates will adjust with the change in the nitrogen concentration. This was confirmed by Gross et al.'s study. They found that although the nitrogen concentration within a certain range (0.6–4.8 mM NO_3^--N) had no significant effect on the total phenolic compounds in the culture water of *Myriophyllum spicatum*, the concentration of the main polyphenol, tellimagrandin II, increased significantly at low nitrogen levels. The total phenol content in the culture water of *Myriophyllum spicatum* under a low nitrogen level (0.06 mM) was much higher than that under a medium nitrogen level (0.5 mM). This result indicates that a low-nitrogen environment triggers a specific secretion pattern of plant phenols. However, ellagic acid in the plant culture water showed an opposite trend to tellimagrandin II [44]. This difference indicates that submerged plants may respond to environmental stress by secreting different chemicals, even under the same environmental conditions, which may differ significantly in function and mechanism.
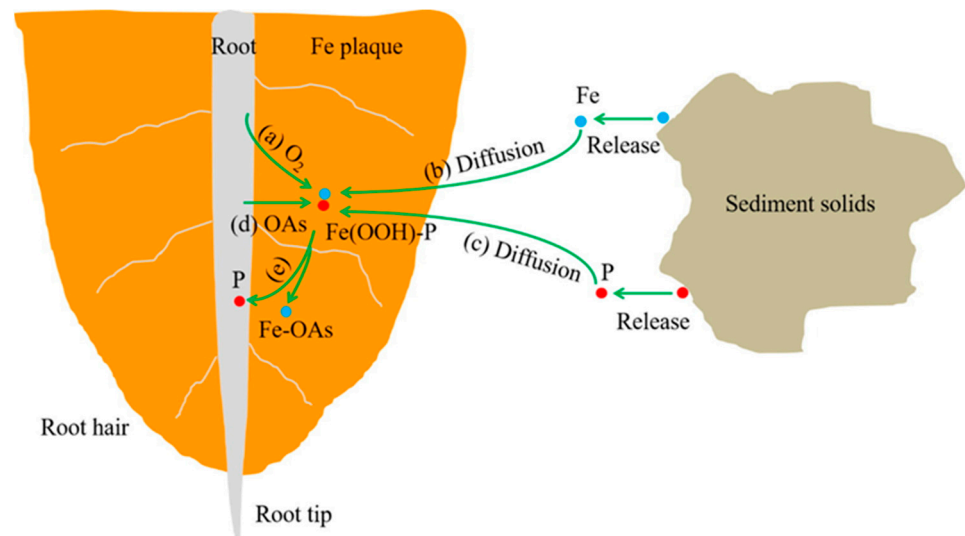
Figure 5. Schematic illustration of the coupling processes between Fe (blue color) plaque enrichment and organic acid complexation in liberating P (red color) in the *V. natans* rhizosphere. The lowercase letters show the process sequence. Reproduced with permission from [40]. Copyright 2017, Elsevier.

4. Effects of Root Exudates on Aquatic Organisms

The phenomenon of "allelopathy" is the effect exerted by one plant on its neighboring organisms by producing chemicals. This effect can be positive or negative, and it is ubiquitous in all plants. The negative effects of allelopathy include autotoxicity, soil disease, or biological invasion, while the positive effects include weed control and ecological protection [53]. In this section, the effects of allelopathic substances secreted by submerged plants on aquatic organisms will be discussed in detail.

4.1. Effects on Phytoplankton

The eutrophication of water bodies causes algal blooms, causing "red tide" and "bloom" phenomena. In 1969, Fitzgerald first discovered that allelochemicals secreted by submerged plants could inhibit the growth of algae, which aroused widespread interest [54]. Many scholars demonstrated that the algae content in the planting area of submerged plants was significantly lower than that in the area without submerged plants. Therefore, studies on the application of submerged plants to control algae have gradually emerged [55]. In the short term, submerged plants inhibit algae growth by secreting "algae-inhibiting substances", which are toxic to algae, rather than by nutrient competition or light shielding. As shown in Figure 6, three methods for applying allelochemicals are usually used to inhibit algae in aquatic ecosystems, including the direct cultivation of submerged plants, the release of plant residues or extracts containing allelochemicals, and the synthesis of allelochemicals [56]. This indicates a significant guide for the artificial synthesis of algae inhibitors and the application of submerged plant exudates to treat algae in water bodies.
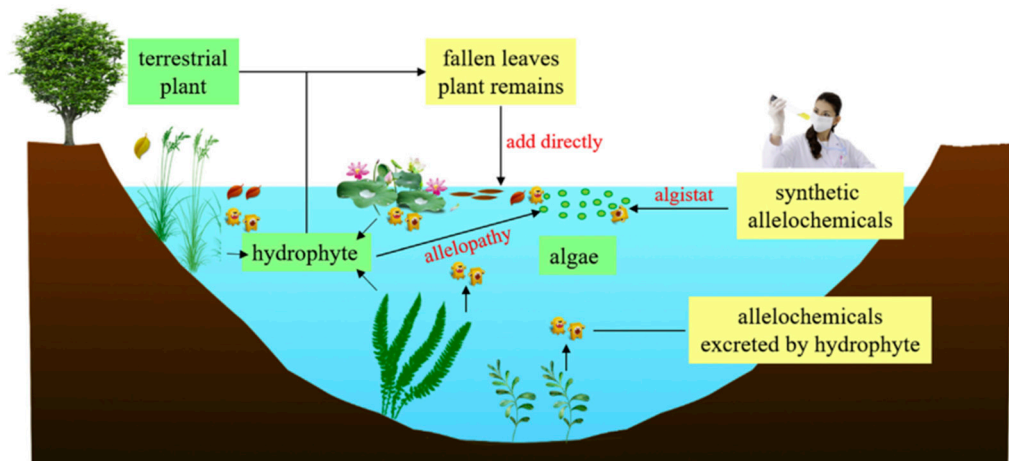
Figure 6. The application of allelochemicals in an aquatic ecosystem. Reproduced with permission from [56]. Copyright 2020, Elsevier.

Submerged plants can directly secret allelochemicals from roots to the rhizosphere, but allelochemicals have certain specificity and selectivity. A single allelochemical only affects the growth of one or several plants. When treated at higher concentrations, some root exudates, such as phenolic acids, can cause toxic effects on other plants and even themselves, inhibiting the normal physiological and metabolic activities of roots, hindering the extension of plant leaves, and affecting the normal growth of plants. Declerck et al.'s study demonstrated the strong inhibitory effect of *Elodea nuttalli* on microalgae, which lasted for more than 50 d, showing the long-term allelopathic potential [57]. Svanys et al. found that *Myriophyllum verticillatum* could effectively reduce the number of *M. aeruginosa* in eutrophic environments. The plants have a continuous negative impact on cyanobacterial biomass but a much shorter impact on other phytoplankton and green algae [58]. Wu et al.'s comparative study revealed that different submerged plants (*Pogonatherum chinense*, *Potamogeton malaianus*, and *Potamogeton crispus*) had different allelopathic effects on *P. aeruginosa* under the same conditions, emphasizing the importance of the diversity of allelochemical species and quantities in the inhibitory effect. Further studies also showed that the allelopathic activity of submerged plants may be affected by the season and growth stage [59]. Hilt et al. found the strongest allelopathic inhibitory activity of charophytes on phytoplankton in August. The growth stage of macro-submerged macrophytes may also affect allelopathic activity. Some studies have reported that young, active macrophytes exhibited greater allelopathic activity than older plants [60]. Rojo et al. tested the inhibiting efficiency of single and combined submerged plant cultures on the growth of natural phytoplankton through allelopathy. *Chara hispida*, *Chara vulgaris*, *Chara baltica*, *Nitella hyalina*, and *Myriophyllum spicatum* were used to test their single and combined allelopathic effects on environmental phytoplankton communities in the laboratory. The results showed that compared with *Myriophyllum*, *Chara* species (such as *C. hispida*) had a stronger effect. Compared with monospecific plants, combining large plants could better inhibit microalgae. Therefore, combining large plants seems to support synergistic allelopathy, directly reducing the microalgae biomass and thus improving the water quality [61]. Macro-submerged macrophytes show a significant inhibitory effect on the photosynthesis of phytoplankton by secreting specific allelochemicals, especially cyanobacteria. As shown in Figure 7, the electron transport chain may be disturbed due to abnormalities in the participating pigments, protein complexes, and electrons. For example, linoleic acid reduces the pigment content to block electron transport in *Pseudomonas aeruginosa*, while berberine inhibits photosynthesis-related gene expression and core protein synthesis [62]. In addition, some specific allelochemicals, such as tellimagrandin II, can significantly destroy the electron transport chain of cyanobacteria, which is achieved by increasing the redox midpoint potential of non-heme iron. The substance produced by *Myrophyllum*

spicatum inhibits PSII of cyanobacteria by interfering with electron transfer [63]. Some allelochemicals show selective inhibition of the photosynthesis of cyanobacteria and green algae, which is attributed to the differences in their photosynthetic tissues. For example, polyphenols strongly inhibit the photosynthesis of cyanobacteria rather than green algae [64]. Similarly, the secretion of *Chara verticillata* has a significant inhibitory effect on the mutant cyanobacteria *Anabaena polymorpha* and a small effect on the growth of *Scenedesmus quadricauda* [41]. Some studies found that the exudates of large submerged plant combinations had stronger allelopathic effects on cyanobacteria and diatoms. This enhanced allelopathy is attributed to the synergistic effect of different allelochemicals produced by these plant combinations. This synergistic effect not only directly reduces the biomass of microalgae but also indirectly improves the water quality by enhancing grazing [61]. This finding provides a new theoretical basis for using multiple submerged macrophytes to control algal blooms in aquatic ecosystems. Cultivating multiple plants can effectively remove harmful cyanobacteria while retaining green algae as fish food, thereby restoring and maintaining the health of aquatic ecosystems.
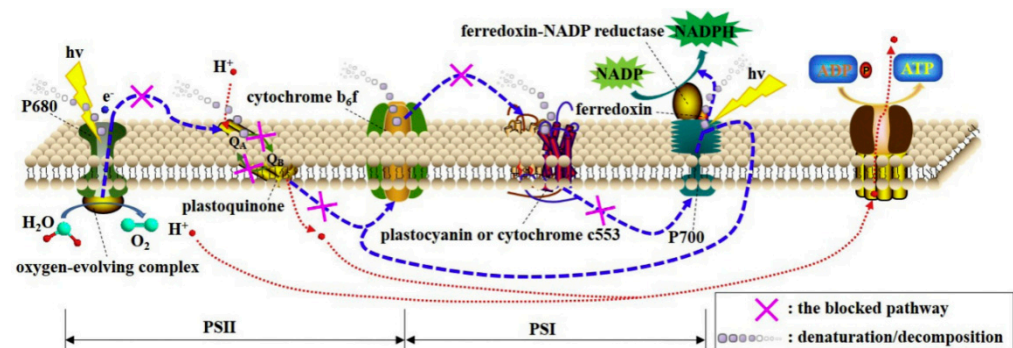

Figure 7. Schematic diagram of electron transport chain of microalgae cells. Reproduced with permission from [62]. Copyright 2020, Elsevier.

4.2. Allelopathy on Zooplankton

It is well known that submerged plants are a refuge for zooplankton, but they also inhibit the growth of zooplankton. Therefore, macro-submerged macrophytes have both positive and negative effects on zooplankton. Figure 8 shows the network of interactions between macro-submerged macrophytes and phytoplankton, zooplankton, etc. However, the effects of allelochemicals on some zooplankton species are unclear [65].

Slawomir Cerbin et al. found that the allelochemicals secreted by *Myriophyllum verticillatum* had a dual effect on *Daphnia*. In the presence of these chemicals, *Daphnia* became smaller and spawned less at maturity, but the offspring were larger. This is mainly because the allelochemicals of *Myriophyllum verticillatum* reduce the food source of water fleas and increase the energy consumption during swimming, thus limiting the growth of somatic cells. However, the increase in offspring may be an adaptation to food reduction. Despite these effects, the researchers believe that the water fleas are not directly affected by the secretion of *Myriophyllum verticillatum* [66]. Subsequently, Espinosa-Rodríguez et al. found that the allelochemicals secreted by *Egeria densa* had a positive effect on the population size of three *Simocephalus* species. The allelochemicals not only increased the age-specific reproduction yield of these zooplankton but also significantly prolonged their average life span. In the medium containing these allelochemicals, the life span, total fertility rate, and net fertility rate of zooplankton were significantly improved. This finding indicates that the biological activity and physical structure of *A. hygrophila* have a positive, stimulating effect on the population of *Daphnia* [67]. In addition, Alberto et al. explored the effects of allelochemicals secreted by *A. hygrophila* on the interaction between mendotae and three coastal clades through population growth experiments. They found that the allelochemicals increased the abundance of all measured zooplankton. In the absence of allelochemicals, the population growth rate of cladistic animals was lower than that of

monoculture. However, in the presence of allelochemicals, this trend is not consistent. This further indicates that the allelochemicals of *A. philoxeroides* have a potentially positive effect on the biological populations of cladistic animals, which may increase the grazing pressure on phytoplankton [68]. In order to further study the impact of macrophytes on aquatic ecosystems, Wolters et al. compared the effects of biofilms formed on *Vallisneria spiralis* and *Egeria densa* and their artificial analogues on two large invertebrate herbivores. They found that macro-submerged macrophytes have a positive impact on large invertebrate herbivores by providing large surface areas for epiphytic algae and bacteria, improving biofilm stoichiometry and stimulating bacterial growth [69]. Finally, Bai et al. conducted a long-term observation of zooplankton biomass in the five sub-lakes of the West Lake. They observed that the zooplankton biomass showed an initial increasing trend followed by a decrease from July 2012 to April 2015. During this period, the main composition of zooplankton was dominated by rotifers, although cladocerans and copepods also accounted for a certain proportion in 2015. These studies have shown that allelochemicals secreted by submerged plants have complex and diverse effects on zooplankton, involving both positive and negative effects. These effects depend on not only the type and concentration of allelochemicals but also a variety of factors, such as environmental variables and the zooplankton species [70].
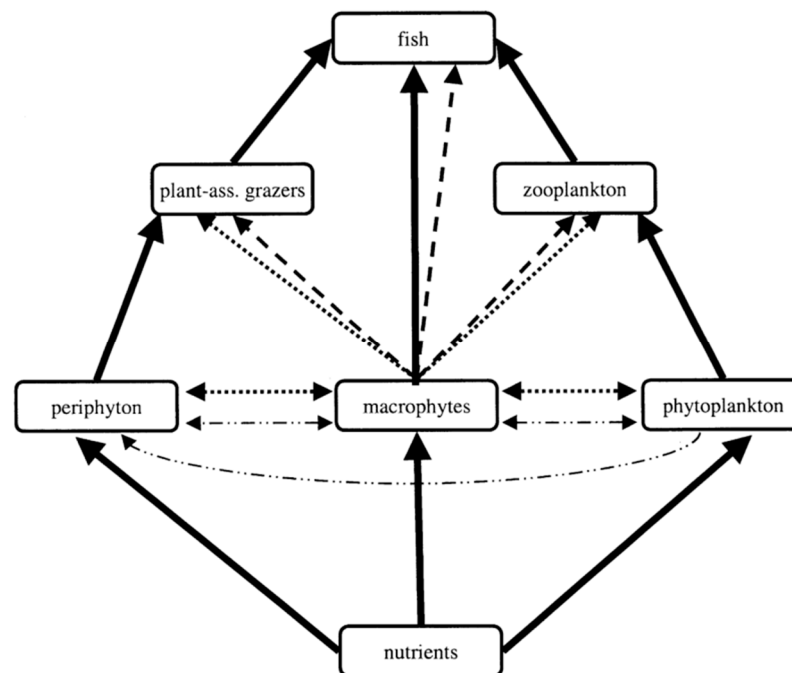
Figure 8. A schematic overview of interactions between submersed macrophytes and other constituents of shallow lake food webs. (—): food web links; (· · ·): allelopathy; (– – –): spatial refuge; (–··–··–): light conditions. Reproduced with permission from [65]. Copyright 2002, Elsevier.

4.3. Effects on Microorganisms

Root exudates are the main driving force for regulating rhizosphere microbial diversity and metabolic activities during plant growth [71]. Plants adjust and maintain a specific bacterial community in the rhizosphere by releasing root exudates. The bacteria produce a variety of secondary metabolites, which improve the nutrient utilization and nitrogen fixation of plants, reduce the sensitivity of plants to freezing injury, and enhance plant disease resistance by inhibiting pathogens, thus promoting their overall growth and development [72]. Roots release a variety of nutrients that are essential for microbial growth, such as vitamins, enzymes, growth regulators, and amino acids. These exudates not only affect the spatial distribution, species, and quantity of rhizosphere microorganisms but also change the physical and chemical properties of soil by promoting the formation of

soil microaggregates [73]. Root exudates lead to a much higher number and species of rhizosphere microorganisms than those in non-rhizosphere areas, providing energy and good living conditions for microorganisms. Different plants release different root exudates, resulting in differences in the rhizosphere microbial community structure, affecting the water-remediation effect of plants. This natural relationship provides important inspiration for developing synthetic substances to remediate polluted water bodies.

The mechanisms of root exudates in regulating nutrient removal in water bodies are still unclear. However, root surfaces are directly affected by secretions, and REs can accumulate large amounts of organic matter and attract more microorganisms to colonize [74]. Yin et al. selected three dominant submerged plants, *Hydrilla verticillata*, *Potamogeton maackianus,* and *Vallisneria natans,* to evaluate their effects on the community structure and abundance of nirS-type denitrifying bacteria and anammox bacteria in the rhizosphere. They found that the concentration of organic acids in the near-root layer of submerged plants was higher than that in the root chamber and rootless layer. The concentrations of citric acid and oxalic acid were negatively correlated with the abundance of nirS-type denitrifying bacteria, and the concentration of oxalic acid was positively correlated with the abundance of anammox bacteria. These results indicate that submerged plants can reduce the abundance of nirS-type denitrifying bacteria and anammox bacteria by releasing organic acids [75]. Ma et al. also confirmed this result. As shown in Figure 9, they found that lactic acid and tartaric acid in root exudates of *Vallisneria natans* varied between 0.045–0.380 mg L^{-1} and 0.024–5.446 mg L^{-1}, respectively, which was closely related to the removal rates of TN and TP and most sediment properties. In addition, the top three relative dominant genera were *Bacillus* (0.11–17.90%), *Geobacter* (0.35–12.04%), and *Clostridium parvum* (0.14–12.05%). The results showed that lactic acid, protein, and amino acids positively correlated with *Geobacter*. This study suggests that root exudates, especially proteins, amino acids, and lactic acid, change the relative abundance and diversity of rhizosphere microorganisms, and their effects depend on bacterial species [76]. Martin et al. further emphasized the effects of lighting conditions on the root exudates and rhizosphere microorganisms of submerged plants. They found that lighting reduction affected the production of root exudates, changed the composition of seagrass root microorganisms, and reduced the abundance of potentially beneficial microorganisms. In particular, the decrease in light availability had the most significant effect on the root microorganisms of *Halophila ovalis*, which was consistent with the most significant change in the secretion pattern of the species when the light availability decreased. These results suggest that changes in root exudates are closely related to changes in the microorganisms, which play an important role in regulating seagrass–microbe relationships [77]. In addition, recent studies found that secretions of submerged plants, as an important carbon source for microorganisms, could affect Feammox activity. Although organic carbon is not necessary for Feammox, it can accelerate iron release from clay minerals involved in mediating the Feammox rate [78]. In summary, submerged macrophytes directly affect root-attached microorganisms and their surrounding environment by releasing root exudates and play a key role in nutrient removal in water bodies. The exudates can not only regulate the composition and abundance of microbial communities but also affect the metabolic activities of microorganisms, thereby affecting the nutrient-removal efficiency in water bodies. Future research should further reveal the specific mechanism and application potential of root exudates of submerged plants in water remediation.

4.4. Possibility of Using Root Exudates of Submerged Plants for Water Restoration

Submerged plants secrete "algae-inhibiting substances" to produce algae-inhibiting effects. This can provide significant guidance for the artificial synthesis of algae inhibitors in root exudates and the application of submerged plant root exudates for water restoration. Thus, the methods used to control the development of phytoplankton include introducing living plants into water bodies to prepare dry plant tissues, extracts, and natural allelochemicals or their synthetic analogues [79]. Coexistence experiments involving submerged

macrophytes and target phytoplankton demonstrate that the biomass of submerged macro-phytes with a 5–8 g L^{-1} wet weight can exhibit an inhibitory effect on phytoplankton [80]. The content of phenolic acids released from submerged plants to water increases gradually with an increase in the density of submerged plants. Considering the economic factors and navigation convenience of restoring submerged vegetation, a 20–50% coverage rate of the planting area may be more conducive to reconstructing submerged plant communities in shallow lakes [81]. The residence time of allelochemicals may be influenced by the evolutionary history of the donor, as microorganisms that co-evolve with allelochemicals may use them as a source of energy [82]. Although there are still some unsolved mysteries in the allelopathy of submerged plants on phytoplankton, it is possible to apply it to control harmful algal blooms or reconstruct submerged plant communities to stabilize water bodies.
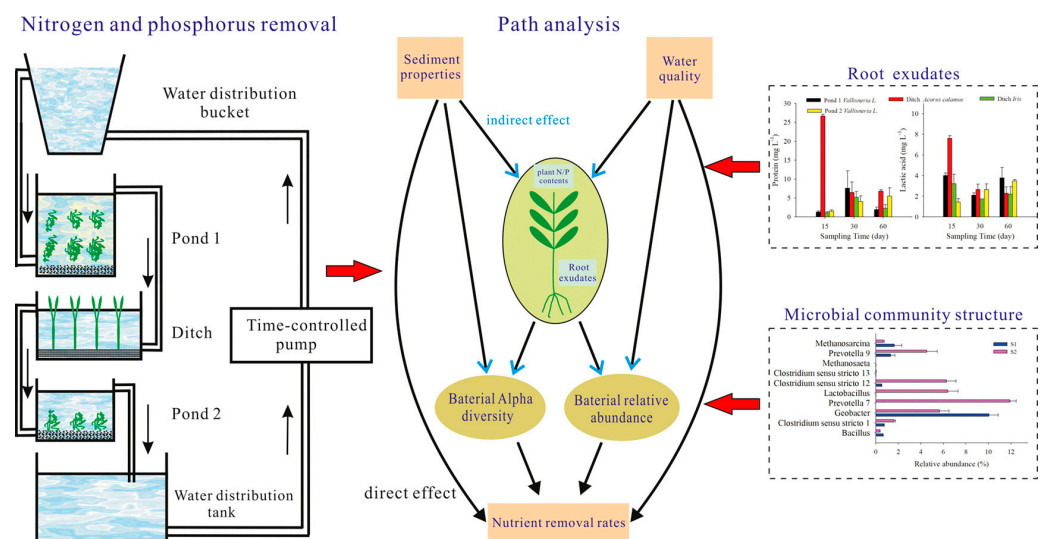
Figure 9. Interaction of root exudates, rhizosphere microorganisms, and water nutrient removal. Reproduced with permission from [76]. Copyright 2021, Elsevier.

5. Conclusions

In this study, the development history, composition, collection and detection methods, and influencing factors of root exudates released by submerged plants were reviewed. Plant species, growth stages, and environmental factors (light, temperature, and nutritional status) are crucial factors affecting root exudates. The positive or negative effects of submerged plant root exudates on phytoplankton, zooplankton, and microorganisms in water were also discussed and are crucial for clarifying the mechanisms of root exudates in water restoration by submerged plants. In particular, allelochemicals in root exudates can inhibit the growth of harmful algae, which is of great significance for maintaining the ecological balance and water restoration of water bodies. In addition, some discoveries in studies on the root exudates of submerged plants conducted in recent years revealed the screening of special bacteria (such as plant-growth-promoting rhizobia) and their application in water restoration by submerged plants, providing a new perspective and method for applying submerged plants in water restoration.

6. Prospects

Based on studies of the root exudates of submerged plants and their effects on aquatic organisms, a new type of algal inhibitor was developed and applied to water restoration. Further works should aim to (1) directly discover allelopathic substances with high algal inhibitory activity, synthesize them artificially, and realize their industrial production; and (2) conduct an in-depth and systematic study of the interactions between allelochemicals, such as synergistic and adjunctive effects. Fewer checks are required for the registration of

natural compounds prepared using allelopathy. Therefore, the cost of commercialization can be reduced. However, the following issues still need to be considered in the development and application of algal suppressors: (1) increasing the ability and ease of controlling environmental conditions to produce the required allelochemicals; and (2) evaluating the environmental safety of algal inhibitors. In addition, the effects of plant exudates on other submerged plants should be further studied.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to funder restrictions.

Conflicts of Interest: Authors Jianya Gu and Wenwen Gu were employed by the company Jiangsu B&P Testing Technology Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Nomenclature

MC	Microcystin
PGPR	Plant-growth-promoting rhizobacteria
ABC	ATP-binding cassette
MATE	Multidrug and toxic compound extrusion
MFS	Major facilitator superfamily
ALMT	Aluminum-activated malate transporter
GC	Gas Chromatography
GC-MS	Gas Chromatography–Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
UPLC	Ultra Performance Liquid Chromatography
LC-MS	Liquid Chromatography–Mass Spectrometry
APCI-MS	Atmosphere Pressure Chemical Ionization Mass Spectrometry
UV-B	Ultraviolet B
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
PS	Photosynthesis System
NIRS	Nuclear Information and Resource Service

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