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Microorganisms that participate in biochemical cycles in wetlands

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Abstract

Several biochemical cycles are performed in natural wetlands (NWs) and constructed wetlands (CWs). The knowledge of the microorganisms could be used to monitor the restoration of wetlands or the performance of the wastewater treatment. Regarding bacteria, *Proteobacteria* phylum is the most abundant in NWs and CWs, which possesses a role in N, P, and S cycles, and in the degradation of organic matter. Other phyla are present in lower abundance. Archaea participate in methanogenesis, methane oxidation, and the methanogenic N₂ fixation. Sulfur and phosphorus cycles are also performed by other microorganisms, such as *Chloroflexi* or *Nitrospirae* phyla. In general, there is more information about the N cycle, especially nitrification and denitrification. Processes where archaea participate (e.g. methane oxidation, methanogenic N₂ fixation) are still unclear their metabolic role and several of these microorganisms have not been isolated so far. The study

can use 16S rDNA genes or functional genes. The use of functional genes gives information to monitor specific microbial populations and 16S rDNA is more suitable to perform the taxonomic classification. Also, there are several Candidatus microorganisms, which have not been isolated so far. However, it has been described their metabolic role in the biochemical cycles in wetlands.

Key words: wetlands, archaea, bacteria, biochemical cycles, functional genes

1.Introduction.

A wetland is a water-saturated environment where plants and microorganisms live in these specific conditions. Plants are in constant contact with water on the soil surface and uptake compounds from it, such as nutrients or trace elements. Microorganisms can perform different biochemical cycles at the same time. Environmental conditions can influence them (e.g. redox potential, hydrology, oxic and anoxic conditions) as well as promote and support the various metabolic pathways performed by microorganisms in wetlands. It is possible to find natural wetlands (NWs) in reserves or parks around the world, which are generally protected from human intervention. Also, several NWs have been restored to recuperate these places and their contribution to the environment. On the other hand, wetlands are considered appropriate to treat wastewaters due to the presence of microbial populations able of performing several metabolic pathways. The overall is to treat the high levels of organic matter as well as emerging pollutants introduced in recent years by human activity. To achieve this goal, artificial wetlands have been built, they are called constructed wetlands (CWs) to distinguish them from the natural environment and managed under controlled conditions to maintain the operational conditions (Wu et al. 2018). In both type of wetlands, microorganisms are producing and delivering constantly greenhouses into the atmosphere. In the last years, researchers have studied bacteria and archaea present in

wetlands, which are degraders of organic matter, methane producers or methane oxidizers. Also, they can participate in N, S, and P cycles, or the degradation of organic compounds. The study of these microorganisms can give information about the most important species/groups in every cycle or degradation of compounds, which could be used as targets of the performance of the wastewater treatment, greenhouse emissions, or the monitoring of wetlands restoration.

This review describes microorganisms detected in NWs and CWs that belong to Bacteria and Archaea domain, which have been described using the 16S rDNA or functional genes. In this environment, microorganisms share the same ecological niche and participate in different biochemical cycles (Hill et al. 2012; Wang et al. 2014). First, microorganisms that belong to *Bacteria* domain are analyzed, and then, methanogens and methanotrophs, microorganisms involve in N, S, and P cycles. Fungi also possess a role in this environment, especially in the development and stress tolerance of plants. However, this topic was described previously for other authors (Fusconi and Mucciarelli 2018). This information could be used for environmental microbiologists and engineers, to improve the performance of wetlands in the wastewater treatment, the control of greenhouse gases, and the monitoring the wetland restoration, which has been proposed as an alternative to storage carbon with low greenhouse gas emissions. However, the microbial community information is poorly understood as well as an environment where several microorganisms have not been identified yet and could be the source of new metabolic pathways with potential industrial uses.

2. Survey methodology.

In this review, the selected literature considers studies performed from 2003 to 2021. It focused on the descriptions performed using high throughput-sequencing to describe

taxonomic groups and microbial populations, which used 16S rRNA and functional genes to detect them. CWs of different scales were considered (laboratory, pilot, and industrial), and different places around the world for the description of NWs.

3. Microorganisms detected in wetlands.

3.1 Study of the microbial community in wetlands.

This review is focused on the description of microorganisms by high-throughput sequencing, a culture-independent technique. On the other hand, culture-dependent methods are also used to study microbes, which are not considered. The 16S rRNA gene is broadly utilized as a target to classify bacteria and archaea in the environment. Functional genes are also used as genetic markers of specific microbial populations. Both are considered in this review. For example, the N-cycle possesses several microbial groups with different metabolic functions. Every section describes the functional genes used to detect the metabolic group analyzed.

3.2 Natural wetlands.

Wetlands are ecosystems that can be found in different geographical areas. It is common to find them in protected areas, such as Everglades (Florida, USA) or Zoige National Reserve (Tibet, Plateau, China). They can also be used to treat wastewaters. Table 1 shows different bacterial phyla detected in different natural wetlands, which used the 16S rDNA gene to describe the microorganisms. Samples were taken in different zones, depth, seasons, contaminated and not contaminated places, and restored wetlands. All studies show that *Proteobacteria* was the most abundant phylum, which presented an abundance between 0.2 and 0.8. Due to the importance of this phylum, some authors have focused on the description of its orders: *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*. Only one study (Yang et al. 2020) described that *Proteobacteria*

had 0.03 abundance in the rhizosphere soil of the plant species *Spartina alterniflora*. This study shows the presence of *Fusobacteria* near the rhizosphere, a taxonomic group that is not common in wetlands. This phenomenon is called the rhizosphere effect and has been detected in wetlands (e.g. Meng et al. 2020) and other environments. Most of the studies mentioned in table 1 describes the microorganisms present in the substrate of wetlands. Man and cols (2019) concluded that bacterial communities were similar between the rhizosphere and near of it and all samples showed high differences in substrate's microorganisms, which suggest the influence of roots in the microorganisms. Previous studies have been mainly focused on the rhizosphere in terrestrial plants, however, there is not much information available about the microorganisms present in wetland's rhizosphere. As example, dWang and cols (2020) detected members of Cyanobacteria phylum as the most abundant in the rhizosphere of CW's plants, which can produce cytokinin, acetic acid, and other regulators that influence the plant resistance.

Table 1 also shows other phyla; *Bacteroidetes*, *Actinobacteria*, *Acidobacteria*, *Firmicutes* and *Chloroflexi*. Members of these phyla can be present in a variety of environments and are associated with different metabolic functions. In the case of WWTPs and wetlands, they perform the organic matter degradation (hydrolysis, acetogenesis, acidogenesis) and can also degrade or convert pollutants into less toxic compounds. In addition, some of them presented a high abundance in the studies showed in the table, while in other not could be detected. This observation supports the importance of the *Proteobacteria* in NWs. An and cols (2019) described the microorganisms present in the soil of Chinese NWs, and they also detected that *Proteobacteria* was the most abundant phylum as well as other phyla mentioned previously in this paragraph. Similar results were detected in a study performed by Pan and cols (2020), who described 15 wetlands located in China, the USA, Cameroon,

Peru, Italy, Australia, and Pakistan. These results suggest that the high abundance of *Proteobacteria* is related to its role in the different cycles. Other phyla have also been detected, such as *Planctomycetes* and *Verrucomicrobia*; however, they were not present in abundance higher than 0.1 in all wetlands studied.

It is also necessary to consider the environment conditions, such as seasons, in the case of studies of NWs and industrial CWs since they can present differences in the abundance of microorganisms. Fang and cols (2020) observed not big differences in the abundance of *Proteobacteria* in the season; however, changes were detected at class and family level. *Rhizobiaceae* was the most abundant in summer and *Sphingomonadaceae* in autumn, both families belong to *Alphaproteobacteria*, while *Burkholderiaceae* and *Hydrogenophilaceae* (*Gammaproteobacteria*) presented a diminution in their abundance from summer to autumn, and consequently in the abundance of *Gammaproteobacteria* order. Considering this information, it may be important to report order, class, or genera level in the case of *Proteobacteria* since their metabolic capabilities are diverse and could influence in their abundance.

Wetland restoration is another topic that researchers are focused on during the last years. They are an environment where carbon is stored. If the hydrology or other factors are altered, wetlands cannot make the role of storing carbon in wetland soils. Limpert and cols (2020) described a restored wetland located in Australia. The most abundant phyla were members of *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*. The monitoring of restoration could be performed by using genetic markers. If biochemical processes are being performed, taxonomic groups should be similar to other wetlands operated under stable conditions.

In general, DNA amplicons obtained from sequencing provide information to classify sequences at the phylum level; however, it is common that they cannot be related to orders, families, or genus in NWs (e.g. Horton et al. 2019; Xue et al. 2020) and CWs (Ji et al. 2020). This situation is also observed in archaeal taxonomic groups (Bae et al. 2018) and with the taxonomy performed using functional genes (Gu et al. 2019). This phenomenon is observed in all environments and some authors have defined a new concept to classify them as phylogenetically diverse non-cultured cells, which correspond to the microorganisms that cannot grow with the traditional isolation techniques (Hug 2018) and are called *Candidatus*. Examples of this are the *Candidatus Parcubacteria* (phylum) (Aguinaga et al. 2018), *Candidatus Accumulibacter* (family) or *Candidatus Acinetobacter* (genus) (Punyapwar et al. 2020), which have not been isolated so far; however, it is possible to identify them by gene sequencing. This situation suggests that there is data that has not been detected or associated with metabolic functions and could give key information in the performance of the biochemical cycles. Finally, all cited studies in this review used high-throughput sequencing as a molecular technique to describe the microbial community, which has advantages and disadvantages, such as biases in DNA extractions, PCR amplifications, primer selection, bioinformatic pipelines and OTUs assignment (Hu et al. 2021). Currently, third-generation sequencing is being developed. It is fast, performed in real-time, and PCR amplification is not required (van Dijk et al. 2018). However, this technology must still be improved, and researchers may avoid the variability of results due to the sample transportation and manipulation.

3.3 Constructed wetlands.

Table 2 shows studies that describe the phyla detected in CWs at different scale treating wastewaters. As NWs, the most abundant phylum was *Proteobacteria* as well as similar

phyla were detected in NWs and CWs. However, some phyla were more abundant than *Proteobacteria* in some wetlands; *Actinobacteria* (Liang et al. 2020; bWang et al. 2020), *Bacteroidetes* (Liang et al. 2019) and *Firmicutes* (bLiu et al. 2020). In all these studies, soil contamination, chemical modifications to the substrate, or human activity are factors that influenced their abundance. As mentioned previously, An and cols (2019) described 42 Chinese wetlands, among NWs and CWs, and all of them presented to *Proteobacteria* as the most abundant. This tendency is observed in both types of wetlands, even when they were planted with different plant species, and may highlight the importance of these microorganisms in this environment. *Proteobacteria* possesses members that participate in the degradation of organic matter and organic compounds, Nitrogen metabolism, S-cycle, P-cycle, and methane oxidation, which could be related to the high abundance.

All these studies have described the microbial community using the 16S rRNA gene, including the studies described in Table 1. On the other hand, cWang and cols (2020) studied the microbial community of CWs (laboratory scale) using the *czcA* metal resistance gene, which is a functional gene that detects microorganisms involved in the metal resistance and is not widely used in wetland studies. This study showed similar bacterial phyla detected in wetlands using 16S rRNA, and the most abundant was also *Proteobacteria*. This situation could indicate that the high abundance of phylum is not biased by using the 16S rDNA gene in the detection of microorganisms. Temperature could also have a role in the abundance of *Proteobacteria*, Deng and cols (2019) detected that this phylum was the most abundant in psychrophilic temperature; however, *Firmicutes* phylum was at 45°C in laboratory assays. This information suggests that *Proteobacteria* is favored by low temperatures and could be a reason because it is the most abundant in wetlands. The

next section describes the biochemical functions described in Proteobacteria's members so far.

In the case of wastewater treatment in wetlands, pathogens and fecal bacteria could be targets to monitor the performance of the process, considering that they are removed and should not be present in the outlet. They have been detected in CWs treating stormwater (bHuang et al. 2018) and wastewaters (Bai et al. 2014). Some microorganisms belong to this group and detect in wetlands are *Chlamydiae*, *Proteobacteria* (*Escherichia coli*), *Firmicutes* (*Enterococcus*), *Bacteroidetes* (*Bacteroidetes*) or adenovirus (bHuang et al. 2018). The detection of them should be performed using specific primers of the 16S rDNA region or other genetic markers present exclusively in the pathogens of interest.

3.3.1 *Proteobacteria*.

Proteobacteria is a phylum that possesses nine class groups. Four of nine of them have been found in wetlands; *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*. These taxonomic groups are mentioned in several sections in this review; however, this section emphasize its role in the biogeochemical cycles and the support of wetlands. Some results have detected sites with high concentration of functional genes of methanogens, sulfate-reducing bacteria, nitrogen fixers and 16S rDNA gene of this phylum. This situation may suggest not only the role in *Proteobacteria* in nitrogen fixers and sulfate reduction, but also their role in the methane production using the hydrogenotrophic pathway especially performed by *Geobacter* (*Proteobacteria*), which is able of stablishing syntrophic interactions and support the electron transfer to archaea (Abraham et al. 2020).

All classes mentioned have a role in the organic compound degradation, such as xenobiotic, recalcitrant aromatic compounds, and complex pollutants (Li et al. 2019; Fang et al. 2020;

Lai et al. 2020). Some of them can degrade antibiotics, such as tetracycline, ciprofloxacin (*Betaproteobacteria*), or ampicillin (*Gammaproteobacteria*) (Santos et al. 2019). Its members are also involved in sulfur and phosphorous cycles (Abraham et al. 2020; Yang et al. 2021) or methane oxidation, which be mentioned later.

Regarding N cycle, all class mentioned previously participate in different steps of N cycle, depending on the wetland conditions. *Gammaproteobacteria* and *Deltaproteobacteria* presented a role in nitrogen fixation in an NW (Abraham et al. 2020), and *Betaproteobacteria* (Fang et al. 2020) and *Gammaproteobacteria* promotes denitrification (Lai et al. 2020; Yang et al. 2021) in NWs and CWs. However, they are other phyla's members involve in this cycle, such as *Firmicutes* or *Actinobacteria*. This is not as exclusive function of *Proteobacteria*; however, it is a group that provides support of the entire cycle, with exception of complete ammonia oxidation and anammox associated with DNRA steps since they are performed by *Nitrospirae* and *Planctomycetes* phyla. *Proteobacteria* can present other metabolic activities. For example, *Alphaproteobacteria* and *Betaproteobacteria* were also involved in electricity production in a CW implemented with a microbial fuel cell (Corbella et al. 2015), and *Gammaproteobacteria* in electrolysis- integrated CWs (aLiu et al. 2020). However, they are other microorganisms called exoelectrogenic bacteria that can be related to electricity production; all *Proteobacteria*'s class mentioned previously (including *Epsilonproteobacteria*), and members of *Firmicutes*, *Acidobacteria* and *Actinobacteria* (Long et al. 2009; Xing et al. 2010). They have been associated with methanogens in anode biofilms of microbial fuel cells, probably stablishing syntrophic interactions with the bacteria mentioned previously (Stores et al. 2014). *Deltaproteobacteria* (especially sulfate-

reducing bacteria) are able of methylate Hg (a neurotoxin) and be accumulated in this environment, avoiding its incorporation in other places or organisms (Schaefer et al. 2020).

Proteobacteria was presented in high concentration in sites with large number of root plants of Everglades wetland (Abraham et al. 2020), which is associated to the symbiotic relationship between plant roots and *Proteobacteria*'s members. This situation has been observed in members of *Alphaproteobacteria* (Pini et al. 2011) and *Gammaproteobacteria* associated with roots in industrial CWs planted with *Salicornia bigelovii*, treating marine effluents of an Atlantic salmon farm (Ma et al. 2018).

Other class has not been detected in high abundances in wetlands; however, *Epsilonproteobacteria* has a role in sulfide oxidation with nitrate reduction, which is still poorly described (Huang et al. 2020). The various metabolic pathways present in members of *Proteobacteria* may explain the reason because these microorganisms are in high abundance in wetlands and able to perform different biochemical cycles.

Considering all aspects mentions in this section, *Proteobacteria*'s members possess metabolic versatility, which may be related its high abundance. Authors have detected metabolic pathways associated to these microorganisms and the presence of them in samples with high concentration of different metabolic groups. However, it is also necessary consider to be the most abundant phyla does not mean that other taxonomic groups have less importance. Environmental factors can influence in the activity of them, to understand this point is essential to analyse enzymatic and transcriptional studies. For example, Smith and cols (2018) detected that only one genus in methane production and another in methane oxidation were predominantly in both processes, using metatranscriptomics data obtained from NWs, despite of detecting more diversity. Finally, there is a group of unculturable microorganisms that can be present and role, but the lack of

suitable primers to detect them and a reference database that contains the essential sequences to identify them, it could influence the data of microorganism's abundance.

3.4 Microorganisms involve in methanogenesis.

Methanogens produce methane in several environments, including wetlands. In the first steps, microorganisms degrade polymers until monomers that are used by methanogens to produce methane. They can use different precursors (CO_2 , H_2 , acetic acid, methanol), all are products of the bacterial metabolism obtained from the degradation of organic matter. The most important pathways to produce methane are acetoclastic (using as a precursor acetic acid) and hydrogenotrophic (using CO_2 and H_2 as precursors), which have been detected in other environments. All methanogens described so far belong to the archaea domain. The genes 16S rRNA and *mcrA* are targets to study them. The *mcrA* gene is a genetic marker broadly used to classify them that encodes a subunit of the enzyme that catalyzes the last step of the methanogenesis (Hallam et al. 2003).

There are seven orders of methanogens detected in different environments and wetlands, six of them belong to *Methanomicrobia* class. Another order is *Methanomassiliicoccales* (*Thermoplasma* class), an order phylogenetically distant from others mentioned previously (Söllinger et al. 2016). Unlike bacteria, taxonomic groups are specific and less diverse. Despite the methanogens possess less taxonomic groups than bacteria, several methanogens are still not isolated (e.g. Bae et al. 2018). Figure 1 shows the orders of methanogens described so far, which also includes the phylum *Candidatus* *Verstraetearchaeota* that has been proposed as a new taxonomic group phylogenetically different of the other methanogens described as members of *Euryarchaeota* phylum (Vanwonterghem et al. 2016). It was detected in anaerobic digester; however, there is no

information about these microorganisms in wetlands and further analyses are necessary to understand their role in methane production.

Regarding methanogens, it is also necessary to consider the methanogenic activity, considering that approximately 33% of global methane emissions come from wetlands (Lyu et al. 2018). On the surface, wetlands have oxic conditions, whereas they have anoxic zones at the baseline. In the case of CWs, they are designed to maintain a wide range of redox conditions, increasing the probability to perform a variety of biological processes, and remove pollutants. Root releases oxygen and can help in the control of methane emissions in the baseline, influencing the oxygen concentration and methane fluxes, leading to a diminution in methane production. Released oxygen can vary depending on plant species, this situation means that oxygen concentration could present great differences and affect methane production (Girkin et al. 2020). On the other hand, Angle and cols (2017) detected methane production in the water column of the oxic zone, the transitional zone between oxic and anoxic conditions, and the anoxic zone in an NW (Ohio, USA). These findings change the information that methanogens are inhibited by the presence of oxygen, methane production is only possible in anoxic conditions, and lead us to new questions about archaeal metabolism. It is necessary to consider that wetland conditions are continually changing, and methanogens do not possess strict anoxic conditions. It also suggests that archaea can adapt their metabolisms to different conditions to produce methane and emphasizes the importance of the sampling site. It is necessary to inform the specific place where samples were taken (e.g. depth), considering the relation of depth, oxygen concentration, and redox potential.

Syntrophic interactions are important in methane production since bacteria mediate the electron transfer to archaea to produce methane using the hydrogenotrophic pathway. This

situation also shows that bacteria species/genus are related to specific archaea genera in methane production that has been observed in several environments, including wetlands (Schaefer et al. 2020). For example, Zhang and cols (2018) described that *Sphingomonas* (*Proteobacteria*) and *Syntrophomonas* (*Firmicutes*) genera and *Methanospirillum* and *Methanoregula* genera (*Methanomicrobiales*) were positively related to methane production, respectively, in NWs. These observations suggest that the hydrogenotrophic pathway was being used by methanogens to produce methane since only *Methanosaeta* and *Methanosarcina* genera produce methane using the acetoclastic pathway. In addition, *Proteobacteria* phylum possesses several members able of performing syntrophic interactions and may suggest that hydrogenotrophic pathway is the principal pathway to produce methane.

Jiang and cols (2010) also observed the temperature effect in methanogenesis in laboratory assays, using soil samples obtained from a wetland of the Zoige National Reserve (China). Methanogenesis rate was higher with methanol as a precursor at 15°C than 30°C. In the presence of acetate and H₂/CO₂ at 15° C (acetoclastic and hydrogenotrophic pathways), values of methanogenesis rate were 60% lower than in assays with methanol. These results suggest that methanol was the principal precursor for methanogenesis at 15°C, and it indicates the prevalence of methanol as a precursor instead of acetic acid and H₂/CO₂. As mentioned previously, syntrophic interactions mediated by *Proteobacteria* and *Firmicutes* promote hydrogenotrophic pathway and this situation also suggest the importance of methylotrophic methanogenesis in wetlands, which could be favored by the environmental temperature.

3.5 Microorganisms involve in methane oxidation.

Methanotrophs oxidize methane, a group consisting of aerobic bacteria and anaerobic archaea/bacteria. Contrary to methanogenesis, methanotrophs can perform methane oxidation in oxic and anoxic conditions. For several years, it was assumed that aerobic oxidation of methane could decrease between 40 to 70% the methane emission in wetlands (Megonigal et al. 2004). However, only anaerobic methane oxidation (AOM) could consume 200 Tg methane per year, which is equivalent to a 50% reduction in total methane production and may indicate that anaerobic oxidation possesses a role in the control of greenhouse gas emissions. A diminution in methanotrophic activity does not affect methanogenic activity; however, it supports the increase in methane emissions (Rey-Sanches et al. 2018). Figure 2 shows the taxonomic groups involved in methane oxidation so far, genes used as genetic markers for the detection, and metabolic pathways.

The *pmoA* gene is broadly used as a target; however, it detects only bacteria. This gene encodes a subunit of the methane monooxygenase, an enzyme that oxidizes methane to methanol (Hakemian et al. 2007). Also, the nitric oxide dismutase (*nod*) gene is being used to study them (Zhu et al. 2019). Currently, the knowledge of aerobic methane-oxidizing microorganisms is higher than anaerobic ones. Two bacterial phyla have been described so far with the metabolism to perform the methane oxidation: *Proteobacteria* and *Verrucomicrobia*. Regarding *Proteobacteria*, two groups have been detected; type I and type II compose by *Gammaproteobacteria* class (*Methylobacter*, *Cenothrix*, *Methylobacter* genera) and *Alphaproteobacteria* class (*Methylocystis* genus), respectively (Sharp et al. 2014). Only bacteria members are part of this group, unlike anaerobic methane oxidation can be performed by bacteria and archaea microorganisms and all of them have been observed in NWs and CWs.

Smith and cols (2018) detected 20 OTUs as the most abundant taxa, which belonged to unknown *Crenothrix* and *Methylobacter*, and *Methylococcales* species. *Methanococcales* species were the dominant methanotrophs in the target samples (0-5 cm depth), despite of the sampling was performed in different seasons. This metabolic group possesses low diversity, and this result may suggest that a smaller group can contribute to the methane oxidation. Moreover, other bacterial or archaeal methanotrophs were not detected in the analysed depths, which means that a methane oxidation was predominantly performed by *Methanococcales*. Researchers also analysed the sequences available of databases, it found active and presence of *Methanococcales* species in wetlands of the USA, Europe, China, Russia. However, this situation emphasizes the lack of data of the warmest wetlands in the world. Considering that global temperature (including oceans) are being increasing during the last years, and one of the possible reasons is the emission of greenhouses, the information of these places could provide data to understand how methanogens and methanotrophs are affected or stimulates, which could directly influence the methane emissions. Smith and cols (2018) detected a diminution in summer season, it may suggest that temperature effect in NWs in microbial community should be a point of interests for researchers.

Regarding anaerobic methane oxidation, five groups have been detected in NWs and CWs (e.g. Chen et al. 2015; Chang et al. 2017; Zhang et al. 2020), which can use different metabolic pathways to oxide methane. The first group are the anaerobic bacteria are detected using *pmoA* and *nod* genes as targets (Chen et al. 2018), which are called nitrite-dependent anaerobic methane oxidation (d-namo), and *Candidatus Methyloirabilis oxyfera* and *Candidatus Methyloirabilis sinica* (both belong to NC10 phylum) are members of this group. They present high growth when methane is available in the

environment (Hu et al. 2019). *M. oxyfera* can also perform denitrification and aerobic methane oxidation, suggesting its metabolic versatility. Researchers call the coupled process between anaerobic oxidation and denitrification as denitrifying anaerobic methane oxidation (DAMO) that contribute to the methane sink in wetlands. The discovery of DAMO process shows that methane oxidation and Nitrogen geochemical cycles are interconnected (Xie et al. 2020), and in CWs can contribute to 10-26% of methane oxidation and 20-47% of N_2 production (Zhang et al. 2020). *M. oxyfera* can produce dinitrogen and oxygen using two molecules of nitric oxide through DAMO process, and this oxygen can be used to oxidize methane, in an intra-aerobic methane oxidation pathway (Ettwig et al. 2010), controlling nitric oxide and methane emissions from wetlands. However, some sequences can be classified in this group; however, it is not possible to assign them into a species. Here, three species are mentioned, and these results may suggest that they are unknown species and further studies to detect the real diversity of this taxa (Zhang et al. 2020).

The second group corresponds to the microorganisms able of oxidizing methane through the reduction of nitrate to nitrite, a member of this group is the archaeon *Candidatus Methanoperedens nitroreducens*. To understand the cooperation and competition among all the microorganisms, archaea and bacteria that participate in methane oxidation, it is important to describe the niche differentiation since it is still unclear. For example, Xie and cols (2020) described in a Tibetan alpine wetland the presence of microorganisms in different depth; aerobic methane oxidation by *M. oxyfera* in 10-20 cm, DAMO activity in 20-30 cm, and *M. nitroreducens* 40-50 cm, which suggest that different microbial population have a specific zone in the wetland, and oxic and anoxic conditions possess a role to determine the microbial distribution depending on depth. Other researchers have

detected their co-exist in the same niche; however, there is no consensus whether they share the same space, or they cooperate each other (Zheng et al. 2020).

Another group is the microorganisms that use sulfate or metals (e.g. Fe (III), Mn (IV)) to perform the anaerobic methane oxidation. In this manner, the Sulphur cycle can also be maintained. These mechanisms are used by archaea (Segarra et al. 2013). Another electron acceptor is Arsenate, which is reduced to arsenic. Unlike the previous group mentioned, both archaea and bacteria can use this compound in anaerobic methane oxidation. Shi and cols (2020) suggested that ANME-1 and ANME-2 can use reverse methanogenesis during the methane oxidation, and syntrophic interactions are performed with arsenate reducers, such as *Geobacter* and *Sulfurospirillum*. However, further studies are necessary to understand the role of this pathway in the methane sink. Also, other studies have mentioned the possibility of electron acceptors not identified so far (Gupta et al. 2013). All these electron acceptors are necessary to be thermodynamically favorable to the methane reduction. In addition, the members of these groups have not been isolated so far. Their isolation could give information to understand their role in the geochemical cycles.

Here we have described microbial groups depending on their metabolic function described so far. Nevertheless, they share the same ecological-niche and support several cycles at the same time. For example, methanogens have been associated with C metabolism for several years and the discovery of methanogenic N fixation gives them a new metabolic function, which will be described later in this review. Furthermore, another option of methane oxidation has been detected. The methanogen *Methanosarcina acetivorans* can grow using anaerobic methanotrophic metabolism, using the reduction of Fe (III) (Yan et al. 2018).

This finding was performed at laboratory scale, and forward studies are necessary to understand the real impact in methane production and oxidation of this species, which

was considered as a methanogen for years. Ferry (2020) proposed a model how methanogenesis and reverse methanogenesis are working in this specie, an intriguing molecular network.

All this information may be used to improve organic matter removal and to control greenhouse emissions. Finally, methylotrophs can also grow in the presence of sulphonamides in CWs, such as members of the genera *Methylosinus*, *Methylothermus*, *Methylocaldum* and *Methylomonas* (Man et al. 2019). These results indicate that these microorganisms can also perform the degradation of organic compounds, in addition to their role in the N cycle and methane oxidation.

Members of this group are predominantly unculturable and fastidious microorganisms, being their isolation, identification and genomic description are a difficult task. The challenge is not only the description of new species. It is important their metabolic pathways and how to increase their activity in controlled conditions in order to manage the methane emission.

3.6 Microorganisms contribute to nitrogen metabolism.

Wetlands are an environment to treat wastewaters, including the N-removal. For example, three industrial CWs showed a large percentage of removal rate; 26-58% of total ammonia nitrogen, 27-96% of $\text{NO}_2\text{--N}$ and 29-52% of $\text{NO}_3\text{--N}$ (Ma et al., 2018). This example shows that wetlands are adequate to remove N from wastewaters. Microorganisms that perform the N-cycle are composed of different groups; nitrogen fixers, denitrifiers, nitrifiers, and ammonifiers, and members of Archaea and bacteria domain are part of them. Figure 3 shows the steps of N-cycle, the functional genes use to study the microorganisms and the taxonomic groups detected in wetlands. The cycle is performed in other environments and

the functional genes used to detect these microorganisms are broadly used by microbiologists.

Nitrogen (N_2) is fixed by bacteria (Fig 3, A) and archaea (Fig 3, B). Bacteria can form symbiotic interactions with plants, which can be more active in roots than other parts of plants, such as rhizomes (Rejmánková et al. 2018). Members of *Proteobacteria* phylum are part of this group (e.g. *Azospirillum* and *Azotobacter* genera). Members of *Bacteroidetes* and *Firmicutes* phyla have also been detected as N_2 fixers (Yang et al. 2020). N_2 fixation is also performed by archaea, which is called methanogenic N_2 fixation to distinguish it from bacterial fixation. Its function, importance, and contribution to methane production in wetlands are still unknown in contrast to bacterial N_2 fixation, which has been broadly described in different environments. Researchers use different primers of *nifH* gene to identify bacterial and archaeal. Bae and cols (2018) described these archaea microorganisms and their role in N metabolism in the Everglades; a tropical NW located in Florida (USA). They detected that samples contained between 6 to 44% of the *nifH* total sequences, and 2 to 49% of the transcripts were archaeal sequences. These results suggest that archaea possess a role in the contribution of N_2 fixation, and they are working actively on it. In addition, several methanogens can present present *nifH* gene in their genomes. This information might suggest that methanogens could also support N_2 fixation, in addition to methanogenesis.

The ammonia- oxidation can be performed by members of both domains, which are called ammonia-oxidizing archaea (Fig 3, C) and ammonia-oxidizing bacteria (Fig 3, D), which are detected using the *amoA* gene that encodes the ammonia monooxygenase. The *amoA* gene possesses a similar function in archaea and bacteria; however, they are studied using different primers. Researchers have performed several studies about the bacteria's role in

this step of the N-cycle; however, the archaea participation is not clear so far. There is more taxonomic information about bacteria, and archaea are principally microorganisms affiliated to *Crenarchaeota* phylum that was a similar situation observed with other archaea microorganisms detected in groups mentioned previously (Wang et al. 2011). The *Nitrospira* (ammonia-oxidizing bacteria) and *Nitrosomonas* (nitrite-oxidizing bacteria) genera are principally responsible for the oxidation of ammonia to nitrate in CWs (Yang et al. 2021). Regarding archaea, *Nitrososphaera*, and *Nitrosopumilus* genera (*Crenarchaeota*) have been detected in CWs and other uncultured microorganisms (Liu et al. 2017; Li et al. 2020). Nitrification was considered a process performed in two steps by groups mentioned previously; however, complete ammonia oxidizers (comammox) were discovered in 2015, which are microorganisms able to catalyze both steps (ammonia oxidation and nitrite oxidation) (Fig 3, E). The comammox *Nitrospira* genus is part of this group, which can also be detected using the gene *amoA*, and have been detected in wetlands (Lin et al. 2020). Regarding metagenomic data, Bai and cols (2014) performed a study using metagenomic data of soil and water samples from an industrial CW. The most abundant genes were related to denitrification (Fig 3, F, G, H) and nitrification (Fig 3, I, J) pathways. In another study, Yang and cols (2019) detected that 60% of sequences obtained from metagenomic analysis corresponded to denitrification; *narG*, *nirS* and *norB* (all genetic markers of denitrifiers) were the most abundant in CWs at laboratory scale. Other studies have also reported that denitrification and nitrification pathways are the most abundant in wetlands and suggest an active role in the N-cycle. This situation was also observed in NWs, where *Firmicutes* and *Gemmatimonadetes* were the dominant denitrifiers in restored wetlands (Huang et al. 2020). These results suggest that some steps of the N-cycle may possess different prevalence depending on the wetland zones.

Regarding taxonomic groups, researchers have described specific genera participating in these steps. Ma and cols (2018) detected *Pseudomonas*, *Stenotrophomonas*, *Comamonas*, *Nisaea* (denitrification), *Acinetobacter*, and *Bacillus* (denitrification and nitrification), and *Nitrosopumilus* (Ammonia oxidation). The last two genera belong to *Firmicutes* (*Bacteria* domain) and *Thaumarchaeota* (*Archaea* domain), and other genera are part of *Proteobacteria*. All these genera were described using the 16S rDNA and other authors have also used functional genes. For example, Gu and cols (2019) detected and classified *nirS* sequences into *Gammaproteobacteria* and *Betaproteobacteria*; however, they were not affiliated to any genus level. Total DNA of the microbial community was obtained from the wetlands of the Zoige alpine wetland (China). This information may indicate that it is possible to classify the microorganisms and the species are still unknown if researchers want to use functional genes for the taxonomic classification. Also, it shows the lack of information about the denitrifying bacteria using functional genes as targets, a situation that could be similar to other functional genes mentioned previously. Denitrifiers are involved in the N_2O production (a greenhouse gas), which can show a high abundance of the copy numbers of *nirS* and *nirK* genes in CWs. However, not all denitrifiers possess the *nosZ* gene, which means that not all members can reduce N_2O to N_2 and it is the key to control N_2O emissions (Zhang et al. 2016; Chen et al. 2020). In the case of wetlands, several greenhouse gases are being produced in parallel, and how to control their production and delivery into the environment is part of further studies.

Regarding anammox members, they belong to *Planctomycetes* phylum and have not been isolated so far. Zhao and cols (2018) identified them in a NW located in Qinghai- Tibet Plateau (China); *Candidatus Jettenia*, *Candidatus Brocadia anammoxidans*, *Candidatus Brocadia fulgida*, and *Candidatus Kuenenia*. All these microorganisms can be detected

using the functional genes; *hzsB*, *hzsA*, and *nrf* (Wang et al. 2021). Other researchers have also detected them in CWs. Despite they were discovered in a WWTP in 1990, their metabolisms and real contribution to N-removal is unknown. Rampuria and cols (2020) detected in CWs (laboratory scale) that 16-20% of N-removal may correspond to anammox pathway and 12-18% to nitrification-denitrification. Wang and cols (2021) also detected that anammox microorganisms were responsible of 30-58% of N-removal in CWs performing with tropical soils, which suggests that anammox pathway possesses an important role in N-removal in cooperation with aerobic N-removal. Unfortunately, Sánchez-Melsió and cols (2009) detected that is difficult to amplify anammox DNA, and sometimes it is not possible to perform it. This situation may indicate that the current molecular biology techniques are not able of detecting these microorganisms and information is being dismissed.

All studies about microorganisms that participate in N-cycle show that several pathways are involved. Similar microorganisms have been detected in NWs and CWs in all steps of the N-cycle. *Proteobacteria* has a prevalence in several steps of this cycle, and more microbial groups have been detected, such as *Nitrospirae* in ammonia-oxidizing or *Firmicutes* and *Actinobacteria* (denitrification). Bacterial information has been broadly described, and to understand the role of archaea is necessary to complete the metabolic networks. It was considered that methanogens participate exclusively in C-cycle for years, during the last years, was discovered their role in N-cycle in wetlands. Also, Candidatus species (bacteria and archaea), have been detected in wetlands and have been attributed to a metabolic function (ammonia oxidizers archaea or anammox microorganisms). They have not been identified and isolated by culture-dependent techniques and could be the source of new metabolic pathways with potential industrial applications (Hill et al. 2018). Also, it is

necessary to study the interaction among different groups and how they can affect the N removal and greenhouse gas emissions. For example, some denitrifiers (*Pseudomonales*, *Bacillales*, and *Neisseriales*) interacted negatively with *M. oxyfera*. However, *Clostridiales* and *Streptomycetales* presented positive interactions with this microorganism (Xie et al. 2020).

3.7 Microorganisms involve in Sulfur cycle.

The sulfur cycle is another biochemical process that is performed in wetlands.

As mentioned previously, sulfate can be used as an electron acceptor during the methane oxidation (Segarra et al. 2013), pathway performed by a syntrophic interaction between archaea and bacteria, specifically sulfate-reducing microorganisms, which have been detected in NWs and CWs. They can also be observed in methane production, where these reactions are performed in concomitance to acetate or lactate oxidation and sulfate reduction (Schaefer et al. 2020). The members of sulfate-reducing microorganisms belong to Deltaproteobacteria order, one family member is *Desulfobacteriaceae* (e.g. *Desulfotignum*, *Desulfosarcina*, *Desulfococcus* genera) (Schaefer et al. 2020). They can grow and support their metabolisms in hypersaline environments. Song and cols (2020) also detected these families; *Desulfomicrobiaceae*, *Desulfarculaceae*, *Desulfobulbaceae*, *Desulfobacteraceae*, *Desulfuromonadaceae*, and *Nitrospiraceae* and *Syntrophaceae* (also member of *Deltaproteobacteria* order) in coastal NWs (China) or *Synthrophobacteraceae* in CWs. For several years, it was considered that all members of this group belonged to *Deltaproteobacteria* order; however, the presence of *Nitrospiraceae* (*Nitrospirae* phylum) suggests that this group is more diverse, and this information is still unclear. All these microorganisms perform the dissimilatory sulfate reduction and the genes *sat*, *aprAB*, and *dsrAB* are used to detect the microorganisms. When researchers detect these

microorganisms, a high percentage of the sequences can be related to uncultured environmental sequences, which have been detected in other environments (Jackson et al. 2014), a similar situation observed with functional genes detected in N cycle and C cycle. In addition to the Arsenate role in methane oxidation, Luo and cols (2020) detected the sulfate reduction promotes the reduction of As (V) to As (III) mediated by *Desulfocaspa* genus (*Desulfobulbaceae*), as well as the methylation of Hg by syntrophic interactions (Schaefer et al. 2020).

The sulfate reduction produces acid- volatile sulfide, pyrite, and element sulfur and can be deposit into the substrate of wetlands. The element sulfur can be oxidized by the sulfur-oxidizing bacteria in the presence of nitrate through the autotrophic denitrification. Genera involve in this process are *Thiohalobacter* (*Gammaproteobacteria*), *Thiobacillus*, *Sulfuritalea*, and *Sulfurisoma* (*Betaproteobacteria*). This process produces sulfate and the Sulfur cycle is completed. *Gammaproteobacteria* and *Betaproteobacteria* classes were mentioned previously since they participate in the denitrification process and can also perform oxidation of sulfur element (Guo et al. 2020). This situation shows that biochemical cycles share steps to complete the processes, electron transfer is involved in this process, and *Gamma*- and *Betaproteobacteria* possess a role in the performance of the S cycle.

3.8 Microorganisms involve in Phosphorous cycle.

Several microorganisms have been related to the P cycle. During the P removal, orthophosphate can be absorbed and accumulated by microorganisms. This is another cycle that several are members belong to *Proteobacteria* phylum. Some of them are *Candidatus*; *Accumulibacter* (*Betaproteobacteria*) and *Acinetobacter* (*Gammaproteobacteria*), which were detected in CWs (Punyapwar et al. 2020) and can

uptake orthophosphate and accumulate polyphosphate. *Pseudomonas* genus (*Gammaproteobacteria*) is also responsible for P removal. They are part of the group of polyphosphate-accumulating organisms (Yang et al. 2012). Other isolated microorganisms belong to *Rhodocyclaceae* (*Betaproteobacteria*), which is involved in phosphate absorption in aerobic conditions and produces extracellular compounds to accumulate phosphorus in NWs and CWs (dWang et al. 2020).

Another group is the denitrifying phosphorus-accumulating organisms (PAOs) where nitrate is an electron acceptor in the phosphate absorption, and members of *Paracoccus* genus are involved in this process (*Alphaproteobacteria*). Both processes, nitrate and phosphorus removal, were detected in parallel to electricity production in CWs (laboratory scale) (Ge et al. 2019). As mentioned previously, members of *Proteobacteria* participate in electricity production, and in this part of the review, it is possible to associate it with the N and P cycle. This review has discussed several microbial groups and separate them to explain their role. However, it is necessary to clarify that the biochemical cycles are interconnected, and microorganisms share the same ecological niche and compete for substrates.

Other microorganisms can also participate of this cycle.

Gemmatimonadaceae (*Gemmatimonadetes* phylum) are microorganisms able of absorbing phosphate in the oxic regions. Other phyla involve in P removal are *Actinobacteria* and *Chloroflexi*, which can immobilize Phosphate. In addition to the role of microorganisms, wetlands substrates can sink phosphorus in wetlands (dWang et al. 2020).

Rhizosphere microorganisms also have a role in converting insoluble into soluble phosphorus, which can be absorbed by roots. *Enterobacter* (*Gammaproteobacteria*) and *Corynebacterium* (*Actinobacteria*) genera are microorganisms able of performing this

process, or *Cyanobacteria*'s members that promote the absorption under high- salt level (dWang et al. 2020).

4.Conclusions.

Similar microorganisms can be detected in NWs and CWs. The most abundant bacterial phylum is *Proteobacteria*; however, other phyla can be detected in a high abundance; *Firmicutes*, *Bacteroidetes*, *Chloroflexi* or *Acidobacteria*. Members of *Proteobacteria* are involved in methane oxidation, different steps of N metabolisms, S-cycle, P-cycle, organic degradation matter, and degradation of different organic compounds, which suggest the importance of this phylum in the biochemical cycles in wetlands. Archaea members can participate in methane production, methane oxidation, and N metabolisms. Taxonomic groups of methanogens are similar to the taxonomic groups of other environments, and there is information about their resistance to oxic conditions to produce methane. They can also participate in anaerobic methane oxidation; however, they have not been isolated so far. Sulfur and phosphorous cycles are performed by bacteria, which are members of the taxonomic groups mentioned previously. Figure 4 summarizes the groups detected and how they can share different metabolic roles.

The major information has been obtained using the 16S rDNA gene as a target. It is possible to classify the microorganisms until genera level. The studies that use functional genes can classify sequences until the order level; however, it is common that they cannot be classified at family or genus level. Functional genes are a suitable target to quantify them using real-time PCR. In general, microorganisms can be studied using functional genes in all metabolic groups analyzed. Also, several Candidatus microorganisms have been detected. They are part of the microorganisms that have not been isolated so far, which could possess a role and metabolic pathways with importance in the degradation of

organic matter and the performance of biochemical cycles. Finally, to monitor the microorganisms present in wetlands, the use of both 16S rDNA and functional genes are appropriate. The focus should be on *Proteobacteria* phylum and their different classes, orders, families, and genera since the information available considers these groups. Functional genes may be used to detect the abundance of microbial groups using real-time PCR.

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7. Figure legends

Figure 1. Scheme of the methanogen taxonomic groups detected so far.

Figure 2. Scheme of the methylotrophic taxonomic groups detected so far.

Figure 3. Scheme of taxonomic groups that participate in all steps of N cycle detected so far.

Figure 4. Scheme that summarizes metabolic groups detected in wetlands.

8. Tables

Table 1. Bacterial phyla detected in NWs.

Reference	Location	Other characteristics	<i>Proteobacteria</i> abundance	<i>Actinobacteria</i> abundance	<i>Bacteroidetes</i> abundance	<i>Chloroflexi</i> abundance	<i>Firmicutes</i> abundance	<i>Acidobacteria</i> abundance
Fang et al. 2020	West of Shandon g (China)	Comparison of freshwater ecosystems	0.45	0.03	0.1	0.04	0.15	0.03
Aguinaga et al. 2018	Afon Goch (Wales, UK)	Place contaminated with acid mine drainage	0.64	0.03	0.02	0.01	0.09	0.18
Aguinaga et al. 2018	Afon Goch (Wales, UK)	Unpolluted wetland	0.31	0.09	0.16	0.1	0.02	0.15
Huang et al. 2020	Dongyin g city (China)	National Nature Reserve	0.36	0.10	0.14	0.12	0.08	0.07
Wang et al. 2020	Longwan National Natural Reserve Jilin Province, China	Study of the influence of snow depth (0 cm of depth)	0.25	0.03	0.05	0.25	0.05	0.15
aWang et al. 2020	Longwan National Natural Reserve Jilin Province, China	Study of the influence of snow depth (90 cm of depth)	0.45	0.08	0.10	0.10	0.01	0.17
Xu et al. 2020	Yellow River, China	Samples obtained in summer	0.8	0.02	0.05	0.01	0.15	0.01

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Xu et al. 2020	Yellow River, China	Samples obtained in autumn	0.55	0.05	0.15	0.05	0.02	0.05
Xue et al. 2020	Yellow River, China	Samples taken close to <i>Phragmites australis</i> , 10 mm of depth and contaminated with oil	0.62	0.10	0.08	0.06	ND	0.04
Xue et al. 2020	Yellow River, China	Samples taken close to <i>P.australis</i> , 0 mm of depth and contaminated with oil	0.32	0.10	0.05	0.17	ND	0.04
Horton et al. 2019	Laurenti an Great Lakes (USA)	Description of coastal wetlands	0.32	0.01	0.10	0.10	0.04	0.02
Pietrangelo et al. 2018	Campani a, Italy	Root samples of <i>P. australis</i>	0.39	0.15	ND	0.06	0.09	0.03
Pietrangelo et al. 2018	Campani a, Italy	Root samples of <i>Typha latifolia</i>	0.27	0.15	0.01	0.03	0.02	0.05
Prasse et al. 2015	Wootons , USA	Restored wetland in 1992	0.2	0.08	0.03	0.01	0.01	0.16
Huang et al. 2018	Poyan Lake Basin, China	Restored and reclaimed wetland soils	0.5	0.01	0.01	0.08	0.02	0.01
Deng et al. 2019	Tibetan Plateau China	Samples incubated at 10°C	0.36	0.15	0.12	0.15	0.10	0.08
Deng et al. 2019	Tibetan Plateau China	Samples incubated at 45°C	0.28	0.15	0.05	0.05	0.40	0.01

Yang et al. 2019	Jingxin (China), near frontier Korea and Russia	Restored wetlands (abandoned rice paddies)	0.39	0.08	0.07	0.15	ND	0.18
Wei et al. 2019	Sanjiang (China)	Important wetland according to the Ramsar Convention	0.34	0.28	0.02	ND	0.08	0.10
Weingarten et al. 2020	Gulf Coast of Mississippi (USA)	High-salinity concentration of water. Surface samples of sediment.	0.30	0.02	0.25	0.02	0.03	ND
Weingarten et al. 2020	Gulf Coast of Mississippi, USA	High-salinity concentration of water. Subsurface samples of sediment.	0.80	0.02	0.03	ND	0.02	ND
Song et al. 2018	Xiantao Hanjiang River (China)	Samples were obtained in Autumn, 2014.	0.74	0.01	0.18	ND	0.08	0.05
Yang et al. 2020	Quanzhou, China	Rhizosphere soil of <i>Spartina alterniflora</i>	0.03	0.08	0.03	0.02	0.02	0.62
Abraham et al. 2020	Florida Everglades	Soil samples	0.42	0.10	0.02	0.02	0.02	0.20
aHuang et al. 2018	Poyang Lake (Jiangxi, China)	Restored wetland	0.40	0.05	0.01	0.08	0.03	0.1

aHuang et al. 2018	Poyang Lake, China	Wetland influenced by human activity	0.30	0.03	0.01	0.02	0.02	0.20
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*ND: no detected

Table 2. Bacterial phyla detected in CWs.

Reference	Scale	Other characteristics	<i>Proteobacteria</i> abundance	<i>Actinobacteria</i> abundance	<i>Bacteroidetes</i> abundance	<i>Chloroflexi</i> abundance	<i>Firmicutes</i> abundance	<i>Acidobacteria</i> abundance
Yang et al. 2021	Laboratory	Carbon-based nanomaterials treatment	0.35	0.18	0.8	0.03	0.03	0.18
aLiu et al. 2020	Laboratory	Study of electric generation	0.6	0.03	0.1	0.01	0.15	0.08
Liang et al. 2020	Laboratory	Nitrous oxide emission	0.2	0.38	0.03	0.13	ND	0.04
Deng et al. 2019	Laboratory	Effect of the temperature on the microbial community	0.35	0.15	0.11	0.13	0.1	0.08
Fang et al. 2020	Industrial	Comparation with freshwater ecosystems	0.5	0.05	0.1	0.04	0.05	0.04
Li et al. 2020	Industrial. China	Different places were analyzed	0.20	0.15	0.02	ND	0.6	ND
Li et al. 2020	Industrial, China	Different places were analyzed	0.80	0.05	0.10	ND	0.02	ND
Sudirjo et al. 2019	Laboratory	Samples obtained from Krabbendijke, The Netherlands. Results show the upper zone of microcosms	0.47	0.02	0.20	0.04	0.03	0.01

Sudirjo et al., 2019	Laboratory	Samples obtained from Krabbendijke, The Netherlands. Results show the lowest zone of microcosms.	0.62	0.02	0.04	0.04	0.01	0.01
Abed et al. 2017	Industrial	Located in Oman	0.70	0.02	0.10	0.02	0.02	ND
Semenov et al. 202	Industrial	Located in Moscow (Russia). Aerobic conditions	0.93	0.02	0.02	ND	ND	0.01
Semenov et al. 2020	Industrial	Located in Moscow (Russia). Anaerobic conditions	0.33	0.02	0.25	0.13	0.15	0.01
Liang et al. 2019	Laboratory	Sulfamethoxazole polluted wetland soil	0.20	0.01	0.45	0.01	0.02	0.06
bLiu et al. 2020	Laboratory	Wetland soil contained biochars modified with Fe	0.03	0.01	0.05	ND	0.90	0.01
bLiu et al., 2020	Laboratory	Wetland soil contained biochars without modifications	0.45	0.01	0.20	0.01	0.01	0.01
Li et al. 2019	Laboratory	Description of degradation of bisphenol A and ibuprofen	0.50	0.01	0.10	0.03	0.08	ND
bWang et al. 2020	Industrial	Xixi National Wetland Park (China), which receive tourist since 2003. Water column.	0.27	0.52	0.13	ND	0.01	ND

cWang et al. 2020	Industrial	Xixi National Wetland Park (China), which receive tourist since 2003. Sediment.	0.28	0.05	0.10	0.25	0.05	0.13
Santos et al. 2019	Laboratory	Removal of veterinary antibiotics	0.48	0.10	0.13	0.02	0.02	0.20
Ji et al. 2020	Laboratory	Study of the role of biochar media and oxygen	0.40	0.28	0.07	0.02	0.02	0.02
Jia et al. 2020	Laboratory	Addition of different agricultural biomasses (apricot pits)	0.17	0.13	0.08	0.03	0.04	0.01
Jia et al. 2020	Laboratory	Addition of different agricultural biomasses (walnut shell)	0.35	0.20	0.02	0.06	0.01	0.02
Lai et al. 2020	Laboratory	Biofilm on substrate	0.68	ND	0.10	ND	0.02	ND
Lai et al. 2020	Laboratory	Suspended sludge in wetland	0.78	ND	0.02	ND	0.15	ND
Lai et al. 2020	Laboratory	Biofilm on porous polymer carriers	0.44	0.02	0.20	0.04	0.03	0.04

*ND: no detected

Figure 2