

**Conference Abstract** 

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# Using the predictive functional profiling of microbial communities to assess the ecological quality of reclaimed waters

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# Abstract

The use of proxies for ecosystem function in biomonitoring is desirable to move towards more holistic strategies. Next-generation sequencing of environmental DNA can clearly contribute to these advances. This research is part of a project aimed to use constructed wetlands (CWs), managed by the company Global Omnium (https://www.glo balomnium.com/Group/Home/) and having the University of Valencia in charge of the scientific research, in order to improve the ecological quality of already treated wastewaters before being poured into natural environments. The rationale is that, although wastewaters treated by classical methods can meet the standards required by law, transitional constructed ecosystems, as the CWs, can promote ancillary benefits that contribute to the maintenance of the ecological health of receiving natural ecosystems. We propose that some functional traits of the microbial community can be used to outline the process of water renaturation. To assess this, the prokaryotic communities of different types of CWs were profiled by the MiSeq sequencing of the V4 region of the 16S rRNA. Using the sequences obtained, a prediction of the functional capabilities of these communities was made with the bioinformatic package PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; https://github.com/

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picrust/picrust2), assuming that phylogeny and function are effectively related attributes. Predictions on the occurrence of functional marker genes, particularly those involved in the biogeochemical cycling of main nutrients (C, N, S, P), were then made. The PICRUSt2 predictions were based in the annotated genes catalog of the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/). In general, results indicated a decline in the CWs effluents of microbial functions typical from wastewater in favor of those more suited for the receiving environment. An enhancement of aerobic metabolism was predicted to occur based on the increase of the gene encoding the cytochrome-c oxidase compared to fermentation pathways. Accordingly, both the denitrification and the dissimilatory reduction of sulfate, that are predominant in the anaerobic environments, also decreased in the effluents of the CWs to lower levels more similar to those observed in natural environments, showing a shift towards reactions at higher redox potentials. On the other hand, a development of metabolic skills for degrading plant materials (e.g., xylose, vanillin, syringate, protocatechuate) was also predicted, which was likely related to a parallel transformation of the organic matter pool in the CWs (more unreactive and natural). Additionally, increases observed in pathways for the synthesis of some cell structural compounds (e.g., mycolic acids) and coenzymes (e.g., F420 cofactor, NAD+) can be related with an enhancement of colonization and competitive potentials of the natural microbial community, as well as with changes in the actual availability of nutrients in the environment. Based on these findings, we raise the possibility of considering these functional surveys made on marker genes as a complementary strategy on the biomonitoring procedures, not only for CWs, but for the general study of freshwater ecosystem potentially affected by wastewater pollution.

### Keywords

constructed wetlands, wastewater treatment, next-generation sequencing, metabolic functions, PICRUSt2

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