

# Drive for freshwater recirculating aquaculture systems The biofilter bacterial community is organised around a stable nitrifying consortium of ammonia-oxidizing archaea and Comammox nitrospira

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## ABSTRACT

A Recirculating Aquaculture Systems (RAS) are distinctively constructed ecosystems that reduce nutrient pollution discharge to the environment, therefore minimising environmental disturbance. RAS commonly uses a biofilter to regulate the ammonia levels that are created as a consequence of the catabolism of fish protein. It is hypothesised that the main nitrifiers in RAS biofilters are Nitrosomonas (which oxidises ammonia), Nitrospira, and Nitrobacter (which oxidises nitrite). By describing the bacterial and archaeal communities of a commercial-scale freshwater RAS that has been in use for more than 15 years, we investigated this claim. The bacterial taxa present in the biofilter community were discovered to be diverse (>1000 genus-level taxon assignments), with *Chitinophagaceae* and *Acidobacteria* dominating (12% and 9%, respectively). A fish-raising cycle's operational adjustments and variations in biofilter depth both caused the bacterial community's composition to fluctuate

significantly. The presence of AOA ammonia monooxygenase genes is assumed to indicate that the archaea are Ammonia-Oxidising Archaea (AOA), which were also prevalent. Thaumarchaeota (>95%) made up the whole low-diversity assemblage of the archaea. All depths and time intervals had nitrogen dioxide. Their abundance, however, was >3 orders of magnitude lower than that of AOA, and they displayed significant depth-time variability that was not seen for AOA. Although no *Nitrobacter* were discovered, phylogenetic analysis of the nitrite oxidoreductase beta subunit (*nrxB*) gene revealed the presence of two distinct *Nitrospira* populations. Complete ammonia-oxidizing (comammox) and nitrite-oxidizing *Nitrospira* populations co-exist in this system with comparatively equal and stable abundances, according to subsequent identification of *Nitrospira* ammonia monooxygenase alpha subunit genes, phylogenetic placement, and quantification of the *nrxB* genotypes. It seems that RAS biofilters enable a variety of ammonia oxidation lifestyles within the nitrifying consortium while housing complex microbial populations whose composition can be directly impacted by normal system operations.

**Key Words:** *Genotypes; Nitrobacter; Ecosystems; Ammonia Oxidation; Biofilter; Nitrospira*

## INTRODUCTION

The advancement of aquacultural technology enables nations to lessen their reliance on catch fisheries and to counteract the impacts of fish population decline. Nearly 50% of fish produced for human consumption are currently generated through aquaculture, and projections show that to meet societal protein demands over the next two decades, production will need to expand by a factor of five. However, increasing production will have a negative influence on the ecosystem and raise serious questions about the long-term viability of aquaculture methods. Recirculating Aquaculture Systems (RAS) were created in order to get around the stocking capacity restrictions and

pollution concerns of traditional terrestrial aquaculture facilities. RAS have several advantages over traditional flow-through systems, including the ability to be installed closer to markets and a 90%-99% reduction in water use.

RAS components, such as solids capture and nitrogenous waste removal from surplus animal waste and undigested feed, are comparable to those employed in wastewater treatment. RAS use is on the rise, particularly in metropolitan areas with constrained space and in nations that place a high emphasis on avoiding environmental consequences, due to the progress of RAS technology and its advantages over flow-through systems.

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The majority of RAS require nitrifying biofilters, which are essential to their operation. Additionally, these biofilters are described as the most challenging RAS component to handle once it is up and running. RAS biofilters work to eliminate nitrogenous waste byproducts produced by the oxidation and catabolism of fish protein. Designers of RAS processes frequently reference *Nitrosomonas* spp. (ammonia-oxidizers) and *Nitrobacter* spp. (nitrite-oxidizers) as the principal nitrifying taxa and model system capacity using the physiologies of these organisms. It is now evident that *Nitrospira* spp. are widespread in freshwater nitrifying biofilters, whereas *Nitrosomonas* and *Nitrobacter* are frequently absent or present in low numbers. Ammonia-Oxidising Archaea (AOA), various *Nitrospira* spp., and *Nitrotoga* are now included in the list of nitrifying taxa found in freshwater aquaculture biofilters as a result of more recent research on these systems. Further research is required to determine whether varied assemblages of nitrifying organisms are typical of high-functioning systems or whether other nitrifying consortiums co-occur in RAS biofilters with *Nitrosomonas* and *Nitrobacter* spp.

The non-nitrifying part of the RAS biofilter population has an effect on how well the biofilter works. Reduced ammonia-oxidation rates can occur from heterotrophic biofilm development that reduces oxygen availability to the autotrophic nitrifying community. On the other hand, ideal heterotrophic biofilm production recycles autotrophic biomass and shields slower-growing autotrophs from biofilm shear stress. According to earlier research, the variety of non-nitrifying bacteria in RAS biofilters may be substantial and can include opportunistic pathogens and other species that are harmful to commerce. The amount of this variety and similarity among systems is comparatively unknown, though, because the majority of this research used low-coverage characterization approaches to describe the taxa present. A group of seawater RAS biofilters' bacterial community was recently studied using various salinity and temperature combinations.

Using a fluidized sand biofilter that has been in use for more than 15 years, the goal of this work was to thoroughly characterise the bacterial and archaeal community composition of a commercial-scale freshwater RAS growing *Perca flavescens* (yellow perch). We predicted that a diversified nitrifying assembly and environmental changes brought on by animal raising would cause a biofilter sand biofilm community to display temporal variability.