Biogeographic patterns of complete ammonia oxidizers (comammox) within the Yangtze River continuum

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The recent discovery of comammox *Nitrospira* performing complete ammonia oxidation to nitrate has fundamentally renewed the 120-year-held perspective of “two-step” nitrification. Rivers are known as the “Arteries” of the Earth, coupling the biogeochemical cycling of continents and oceans. Frequent human activities usually increase nitrogen load, and nitrifying microorganisms are crucial for the management of nitrogen load in rivers. The ecological roles of truncated nitrifiers, including canonical ammonia-oxidizing bacteria, ammonia-oxidizing archaea and nitrite-oxidizing bacteria in rivers have been fully understood, however, investigations of the newly discovered comammox *Nitrospira* are very scarce. To fill this gap, we used the metagenomic shotgun sequencing to provide the first biogeographic patterns of comammox *Nitrospira* in the Yangtze River over a 6030 km continuum.

First, ten novel comammox genomes (71~96% completeness) were reconstructed with the metagenome assemblies from fluvial water in the upper reach and surface sediments from the middle reach to the estuary. Gene arrangements in ammonia oxidation-related gene clusters of these novel genomes were more complex and diverse than those of the previously discovered ones. For instance, multi-copy *amoA* or *amoB* genes, peptidases, cupredoxin and *fkpA*-cytochrome *c-nirK* gene sets were first found within the ammonia oxidation-related gene clusters of comammox *Nitrospira*, which might confer them advantages in adapting to the relatively oligotrophic environments and stabilizing the ammonia-oxidation process in rivers. Taxonomic analysis demonstrated that all riverine comammox genomes (constituting four new species) belonged to clade A. Based on the phylogenies of their 37 “elite” conserved marker genes, we further separated all reported comammox clade A into five sublineages, named clade A-Ia, A-Ib, A-Ic and A-IIa, A-IIb. The reclassified sublineages were sufficiently divergent to be meaningful in expanding the taxonomic/functional diversity and improving the phylogenetic resolution.

Second, based on the improved phylogenetic resolution, we explored the biogeographic patterns of planktonic and benthic comammox *Nitrospira* subjected to natural and anthropogenic factors along the Yangtze River. Our study revealed the wide existence of comammox *Nitrospira* and their significant contributions to nitrifier abundances, constituting 30% and 46% of ammonia-oxidizing prokaryotes (AOPs) and displaying 30.4- and 17.9-fold greater abundances than canonical *Nitrospira* representatives in water and sediments, respectively. Comammox
*Nitrospira* were found to contribute more to nitrifier abundances (34–87% of AOPs) in typical oligotrophic environments with a higher pH and lower temperature, particularly in the plateau (clade B), mountain and foothill (clade A-Ic) of the upper reach. Environmental selection determined the niche replacement of planktonic comammox *Nitrospira* by canonical ammonia-oxidizing bacteria and *Nitrospira* sublineages I/II from upstream to downstream, leading to a higher spatial turnover rate than observed for the benthic counterpart, while the dissimilarity of benthic comammox *Nitrospira* was moderately driven by geographic distance. A considerable decrease (83%) in benthic comammox *Nitrospira* abundance occurred immediately downstream of the Three Gorges Dam, which was consistent with a substantial decrease in the overall bacterial taxa in sediments.

Together, this study highlights the previously unrecognized dominance of comammox *Nitrospira* in major river systems and underlines the importance of revisiting the distributions of and controls on nitrification processes within global freshwater environments.