Targeted genome centric metagenomics to recover active nitrifiers

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Introduction

Microorganisms within coastal sediments play an important role in the global nitrogen cycle. Almost 50% nitrogen removal from the ocean and land occurs in coastal sediments. Ammonia oxidation (AO) is the rate limiting step of nitrification which is driven by ammonia oxidising bacteria (AOB) and archaea (AOA) along a salinity gradient. In coastal sediments, salinity is thought to be one of the driving factors selecting for different communities of AOB/AOA.

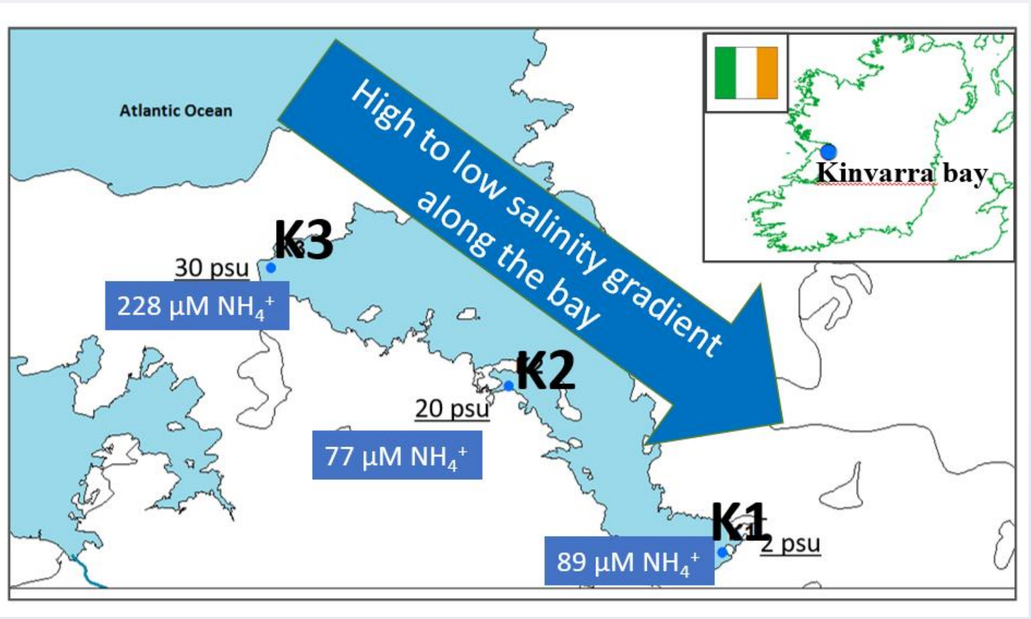

Fig 1: Sampling site Kinvarra bay (Ireland)

Previously, we have shown AOA to be present at low salinity sites while AOB predominate at high salinity in laboratory time series microcosms using stable isotope probing (SIP).

In this study, we applied targeted metagenomic approach to recover active microorganisms (AOA, AOB and NOB) along a salinity gradient

Materials and methods

Sediment was collected from three sample locations K1 2 psu, K2 20 psu and K3 30 psu (Kinvarra bay, Republic of Ireland) and incubated for 28 days at 15 C.

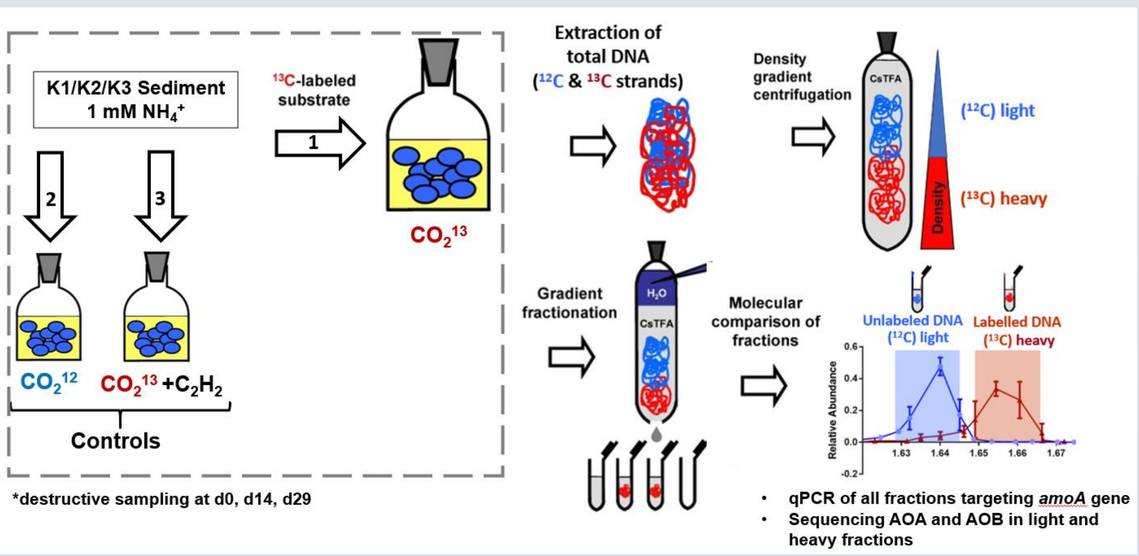

Fig 2: Microcosm set-up and workflow

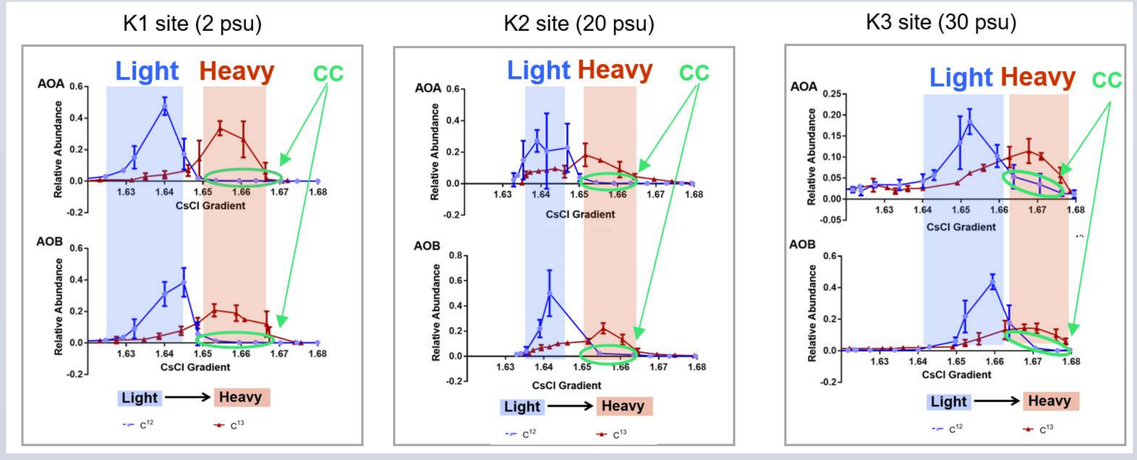

Fig 3: Samples for metagenomics based on the relative abundance of *amoA* genes measured by qPCR for AOA and AOB across the buoyant density gradient of the DNA fractions as revealed by SIP at day 29 across microcosm salinity gradient.

The highlighted areas indicate gradient fractions that were pooled for subsequent metagenomic sequencing. Each sample has light (C12-incorporated), heavy (C13-incorporated) and centrifuge control (CC) peaks. CC: part of light fraction (DNA with C12) which diffused throughout the gradient, thus leading to a higher background.

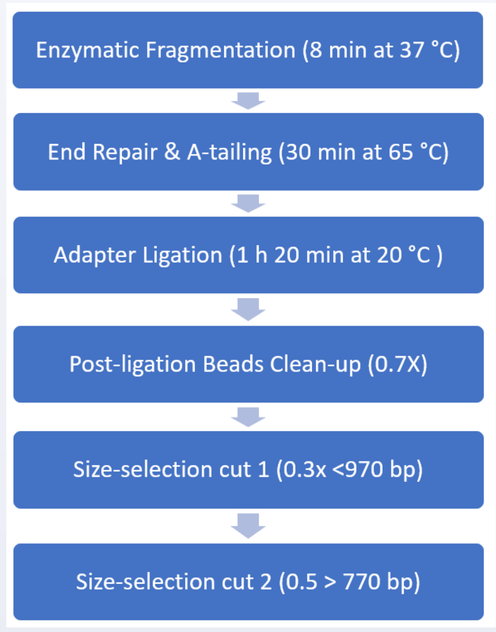

Fig 4: Metagenomic library preparation workflow

KAPA HyperPlus preparation in a single tube was used with integrated, low-bias PCR-free enzymatic fragmentation

26 metagenomics libraries with the average size of 900 bp were sequenced at Illumina NovaSeq 6000 Seq System

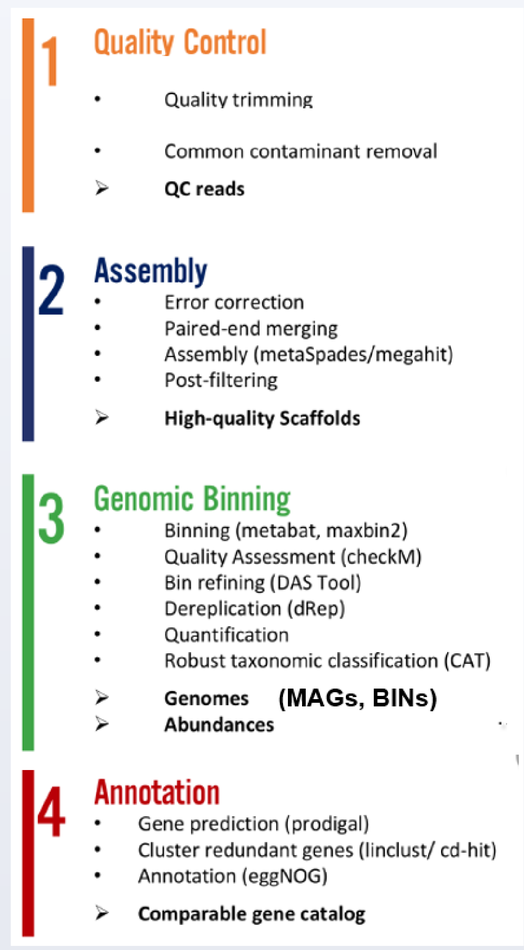

Fig 5: ATLAS: a Snakemake workflow for assembly, annotation, and genomic binning of metagenome sequence data

Results

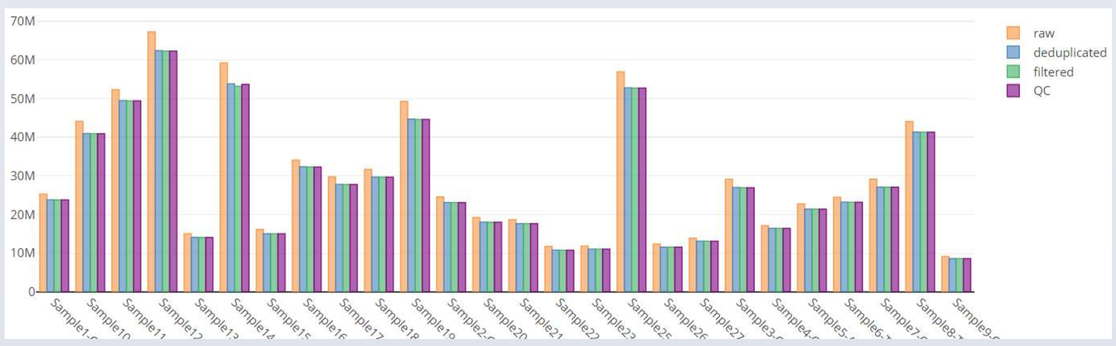

Fig 6: Quality control results

Use amplicon sequencing (ASVs) data to inform our metagenomics targets

There were 9 AOB ASVs and 12 AOA ASVs that were:

- underrepresented
- a high activity (with *amoA* gene)
- successfully C13- labelled

***in each site K1,K2, K3

Recovered metagenome-assembled genomes (MAGs):

>70% Completeness, 10% Contamination: 77 MAGS (Checkm)

>90%Completeness, <5% Contamination: 25 MAGs

How far we can recover ASVs of interest?

Run BLAST against Nitrification Reference databases for AOA and AOB and filter results (best hit similarity)

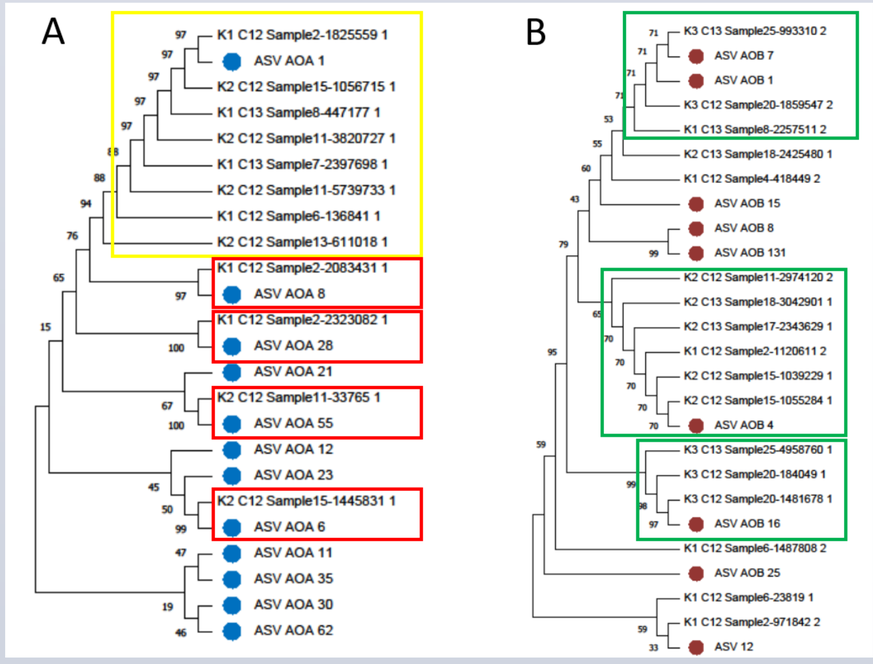

Fig 7: Phylogenetic tree of novel ASVs from amplicon sequencing dataset and respective contigs with *amoA* gene sequence in metagenomic dataset for A) AOA and B) AOB

To sum-up

We were able to: 1). Optimised library preparation from low sample input. 2). Received good coverage per sample of metagenomic sequences. 3). Assemble contigs and MAGs. 4). Used amplicon data to inform nitrification targets and recover contigs of ASVs