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Ecophysiology of novel core phylotypes in activated sludge wastewater treatment plants with nutrient removal

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Introduction

An understanding of the ecology of activated sludge (AS) biological nutrient removal (BNR) wastewater treatment plants requires detailed knowledge of the community composition and metabolic activities of individual members. Recent 16S rRNA gene amplicon surveys of full-scale systems in Denmark indicate a core set of bacterial genera. These core genera are suggested to be responsible for the bulk of nutrient transformations underpinning the functions of these plants. While we know the basic *in situ* activities of some of these genera, there is little to no information for the majority, and thus no indication as to their relevance to the ecology of these systems.

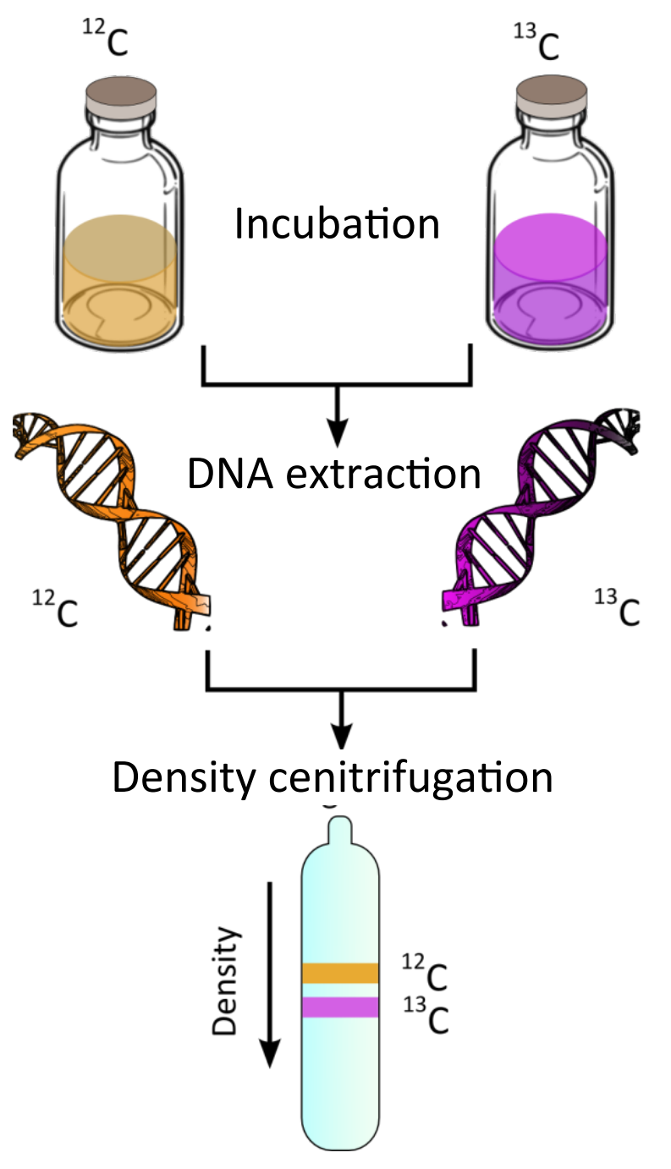
Aim

To characterise the ecophysiology of core genera in full-scale BNR plants with *in situ* based methods.

Methods

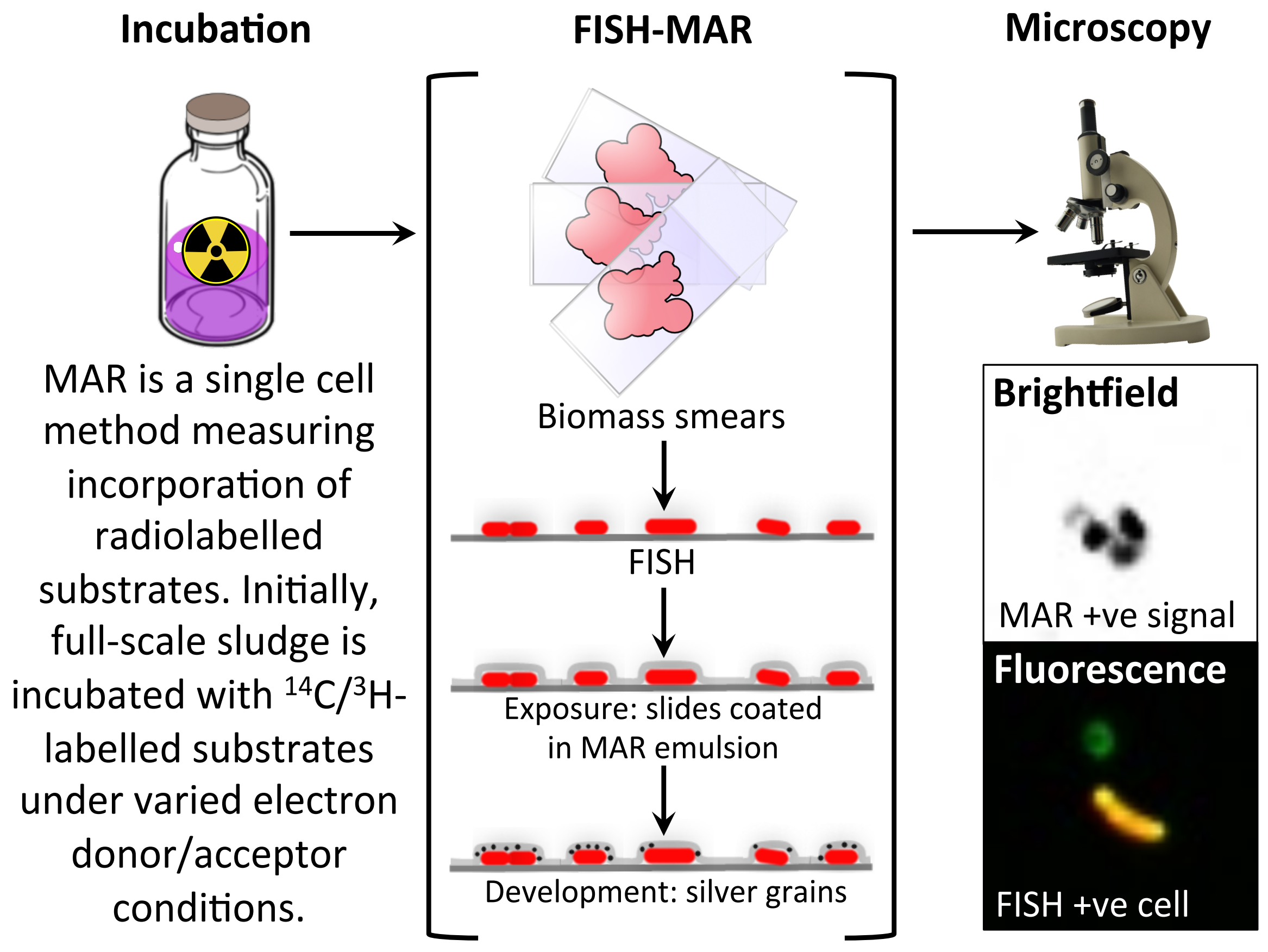
Stable isotope probing

Stable isotope probing was applied under nitrite reducing conditions with a fully ^{13}C labelled complex media to assist in the identification of putative active core denitrifiers.



MAR-FISH

Eight core phylotypes were selected and fluorescent *in situ* hybridisation (FISH) was applied in combination with microautoradiography (MAR) to assess the ability of these organisms for key metabolic activities. New FISH probes were optimised for 7 of the phylotypes.



MAR is a single cell method measuring incorporation of radiolabelled substrates. Initially, full-scale sludge is incubated with ^{14}C/^{3}H-labelled substrates under varied electron donor/acceptor conditions.

Results

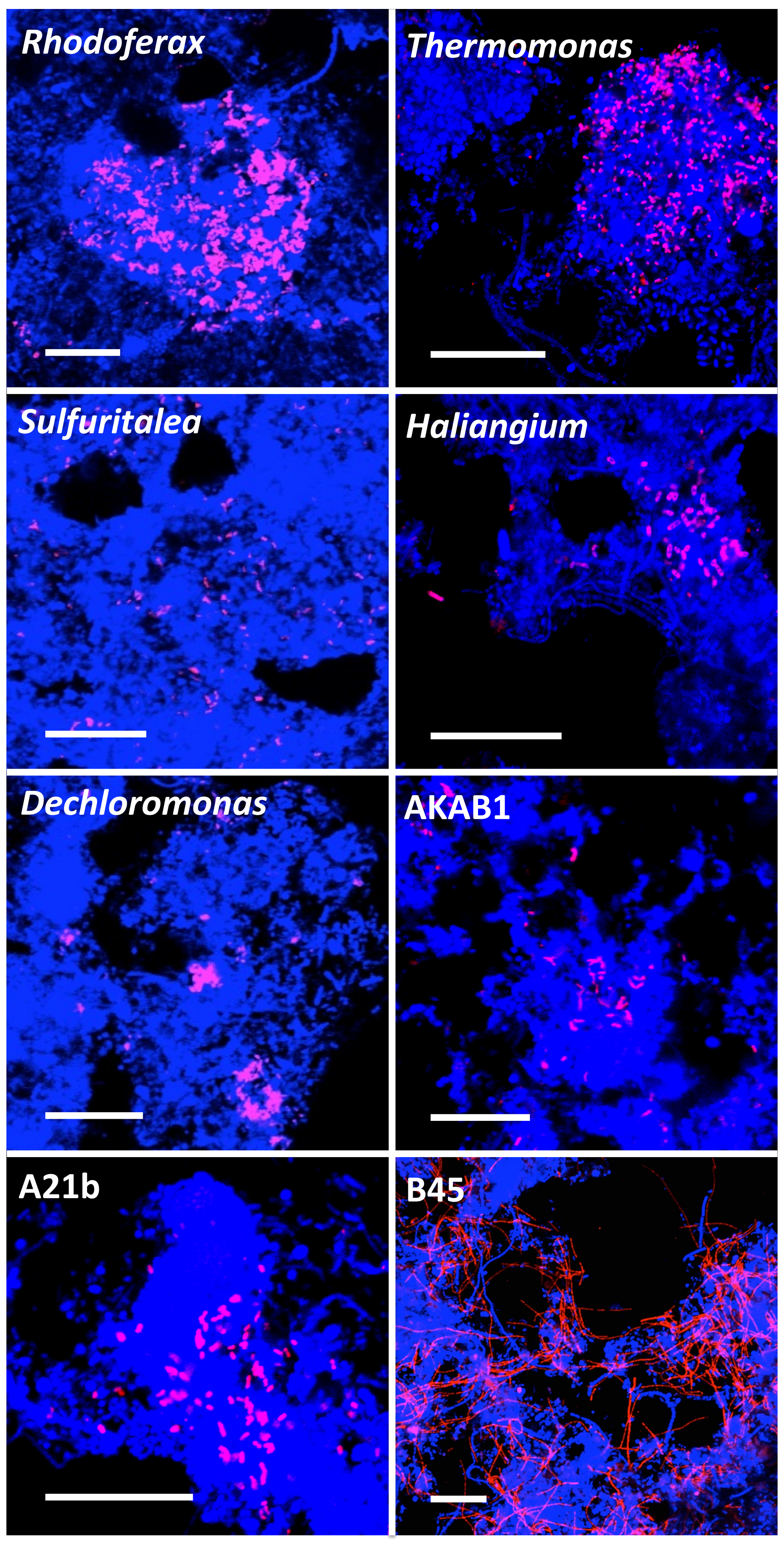


Fig. 1. FISH images of phylotypes in full-scale AS. Scale bars = 20 µm.

Table 1. Phylogeny of selected core genera

Classification				
Phylum	**Class**	**Order**	**Family**	**Genus**
Proteobacteria;	β-proteobacteria	Burkholderiales	*Comamonadaceae*	***Rhodoferax***
Proteobacteria	β-proteobacteria	Rhodocyclales	*Rhodocyclaceae*	***Sulfuritalea***
Proteobacteria	β-proteobacteria	Rhodocyclales	*Rhodocyclaceae*	***Dechloromonas***
Proteobacteria	β-proteobacteria	SC-I-84	SC-I-84	**A21b**
Proteobacteria	γ-proteobacteria	Xanthomonadales	*Xanthomonadaceae*	***Thermomonas***
Proteobacteria	δ-proteobacteria	Myxococcales	*Haliangiaceae*	***Haliangium***
Acidobacteria	Holophagae	Sub-group 10	43F-1404R	**AKAB1**
Chloroflexi	Caldilineae	Caldilineales	CCD21	**B45**

Table 2. Summary of *in situ* characterisation of selected phylotypes

Metabolic feature	*Rhodoferax*	*Sulfuritalea*	*Dechloromonas*	A21b (*β-proteobaceria*)	*Thermomonas*	*Haliangium*	AKAB1 (*Holophagae*)	B45 (*Caldilineae*)
Substrate uptake								
Pyruvate	+	+	+	+	N.D.	N.D.	–	–
Acetate	+	+	+	–	+	–	–	–
Propionate	+	±	N.D.	–	N.D.	N.D.	–	–
Butyrate	+	–	N.D.	–	N.D.	N.D.	–	–
Oleate	–	–	–	–	N.D.	N.D.	–	–
Amino acids	+	+	+	–	+	+	–	–
Glucose	±	±	–	–	N.D.	N.D.	–	+
Glycerol	±	–	N.D.	–	N.D.	N.D.	–	–
Ethanol	–	–	–	–	N.D.	N.D.	–	–
***N*-acetylglucosamine**	–	–	N.D.	–	N.D.	N.D.	–	–
Anaerobic carbon uptake	±	+	+	+	±	–	–	+
Denitrification	+	+	+	–	+	+	–	N.D.
Autotrophy (incl. oxidation of: NH_4^+; NO_2^-; H_2; $S_2O_3^{2-}$)	N.D.	–	N.D.	–	N.D.	N.D.	–	N.D.
Polyphosphate storage	–	N.D.	±	N.D.	N.D.	N.D.	N.D.	–

+ = positive; – = negative; ± = varied; N.D. = Not determined.

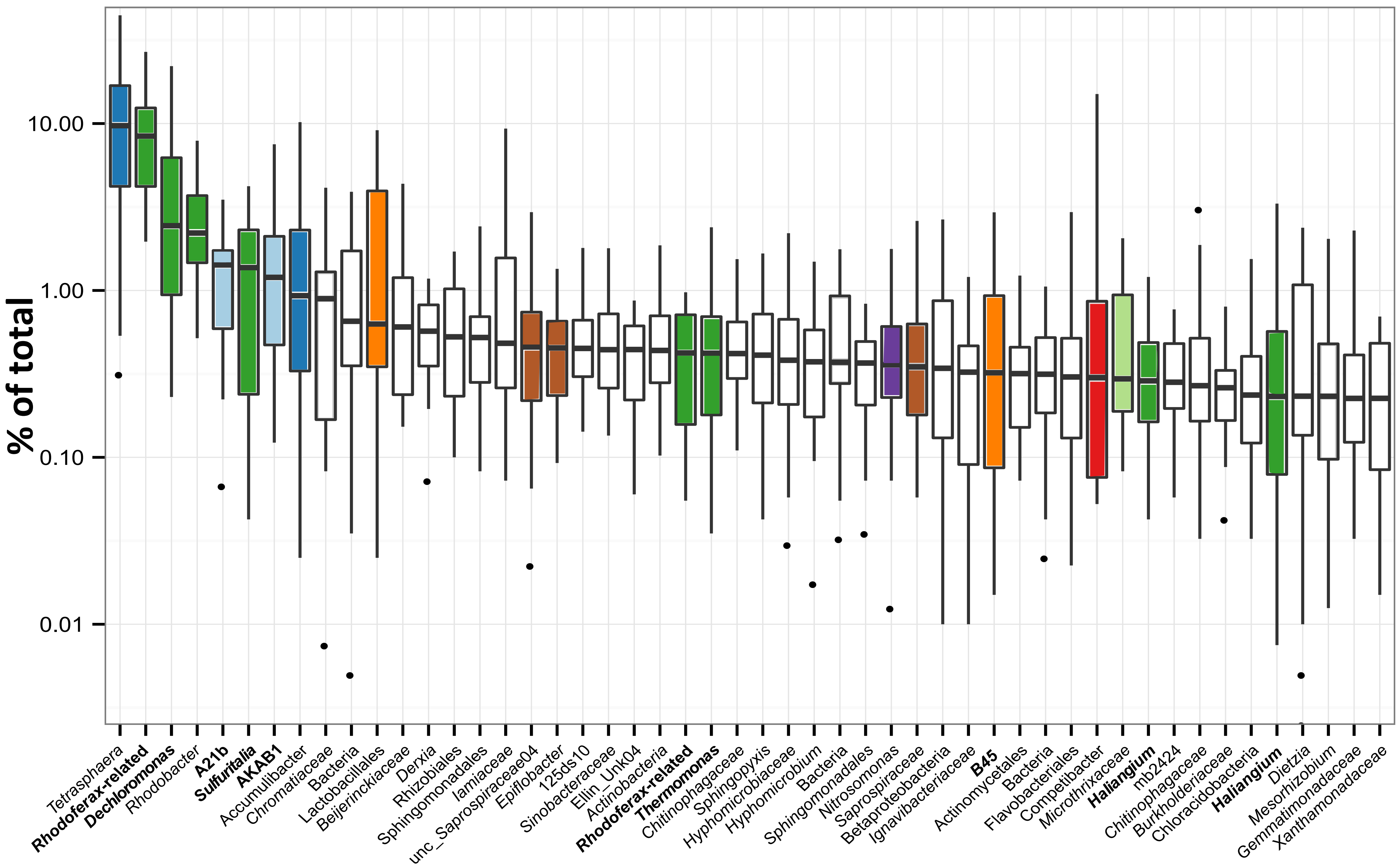


Fig. 2. **Boxplot of the 50 most abundant OTUs (clustered at 94 %) based on 16S rRNA gene amplicon sequencing surveys of 13 full-scale BNR plants (Saunders *et al.* submitted).** Selected genera studied here are in bold. Dots denote outliers. OTU labels are the lowest assigned taxonomic rank (x-axis). Genera for which *in situ* ecophysiological information is available are colour coded by their defining traits (acknowledging that each genera may fit into to multiple categories).

🟩 **Denitrifier**
🟪 **Ammonia oxidiser**
🟫 **Saprophyte**
🟦 **Unresolved**
🟧 **Fermenter**
🟩 **Lipid accumulator**
🟥 **GAO**
🟦 **PAO**

Conclusions

Members of the *Rhodoferax*, *Sulfuritalea*, *Thermomonas* and *Haliangium* appear to be core denitrifiers. Members of the novel genera selected have more specialised physiologies requiring further investigation. Future work will focus on characterisation of additional 'core' genera, including genomic based approaches. The ecophysiology profiles generated will be made available on the 'Microbial Database of Activated Sludge' (MiDAS) field guide website (midasfieldguide.org); a public resource for the benefit of individuals interested in AS.

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MiDAS Field Guide
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