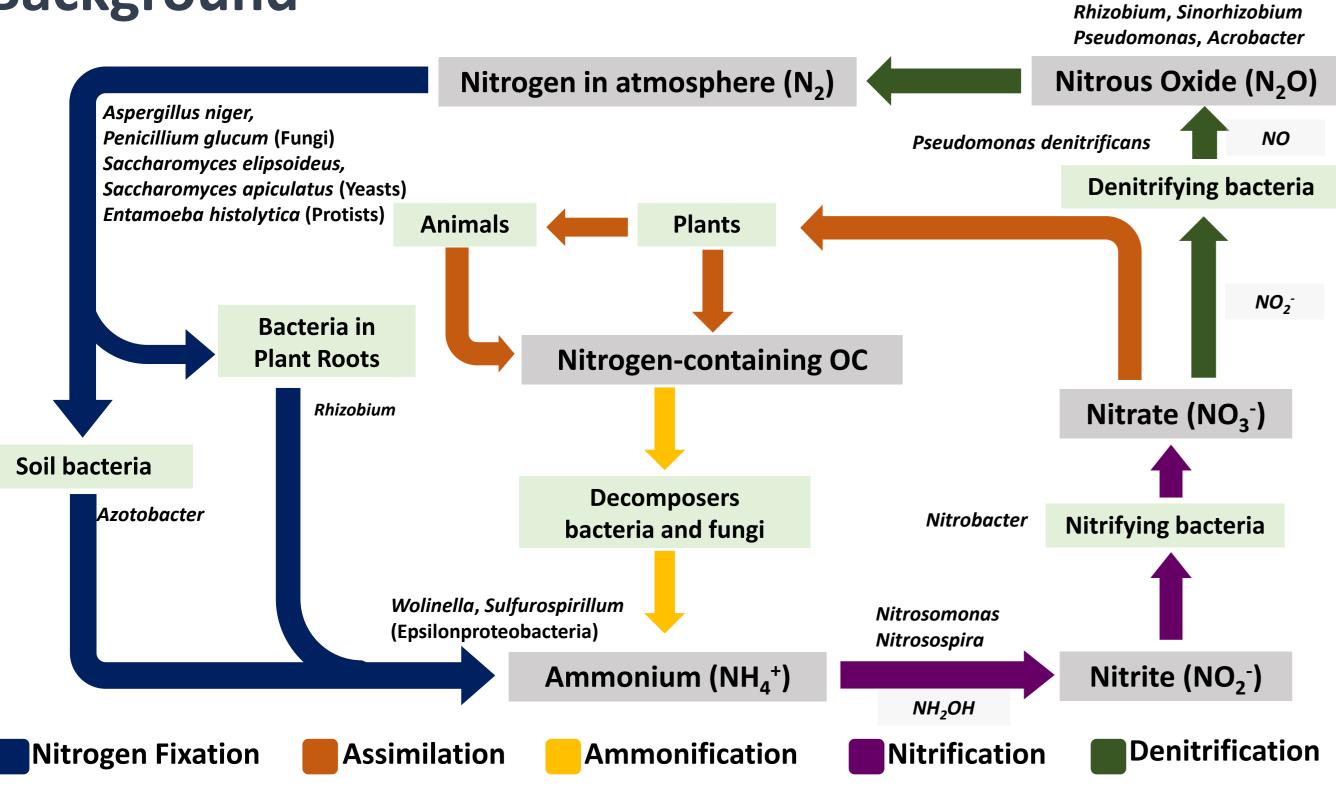
Enrichment of Uncultured Ammonium Oxidizing and Denitrifying Bacteria from Antarctic soil

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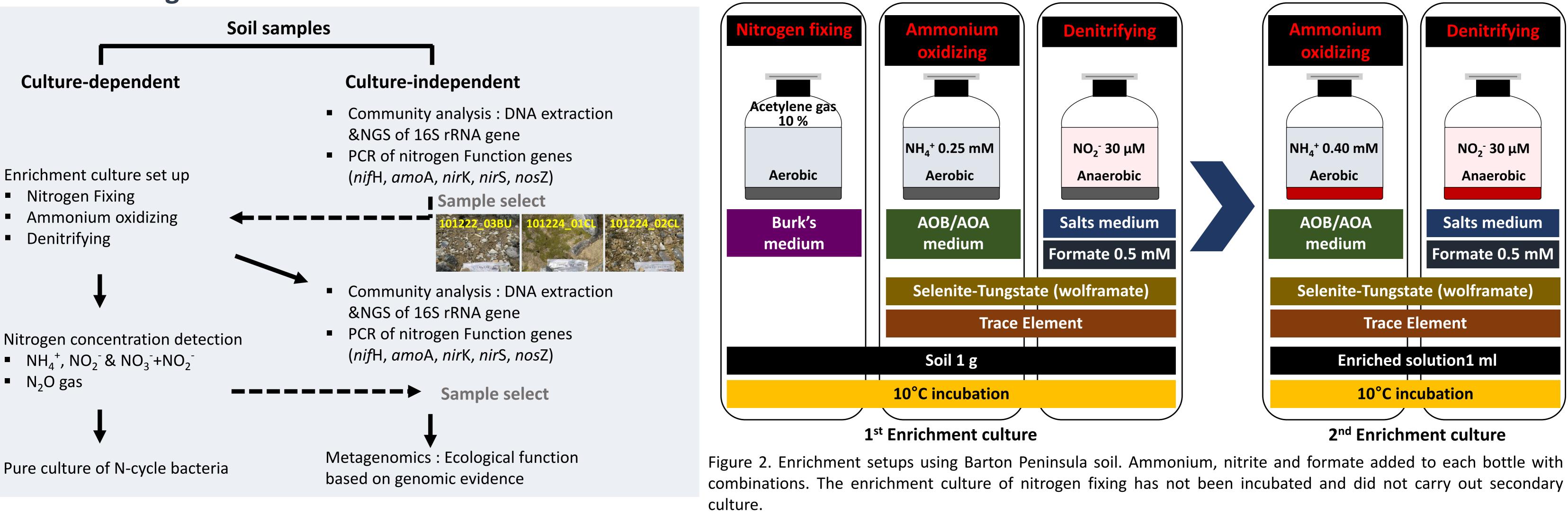
Background



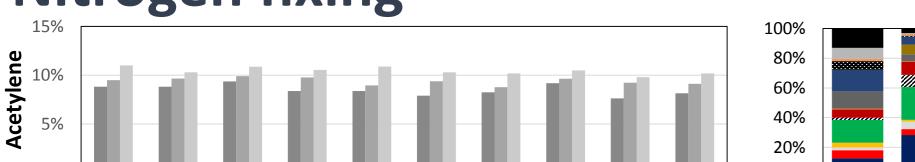
- In the ecosystem, bacteria contribute a lot to material and energy cycle. Especially, bacteria are expected to play a relatively large role in the process of ecosystem matter cycle in areas where the distribution of organism, such as Antarctic, is extremely limited.
- In the terrestrial ecosystem of Antarctica, the nitrogen cycle is relatively simplified compared to the nitrogen cycle of the temperate zone. The Antarctic terrestrial ecosystem, where there is no influx of nitrogen from the outside, the role of bacteria that fix nitrogen from the atmosphere is very important. The process to make available to other organisms is also the role of bacteria. (Nitrification). The opposite reaction, denitrification, is a very important mechanism in ecosystem that releases nitrogen back into the atmosphere.
- In this study, tried to enrichment cultivation to investigate the bacteria involved in nitrogen fixation, ammonia oxidation and denitrification. Also tried to understand the major ecological functions in each process based on their genomic evidence by metagenomic analysis after enrichment cultivation.

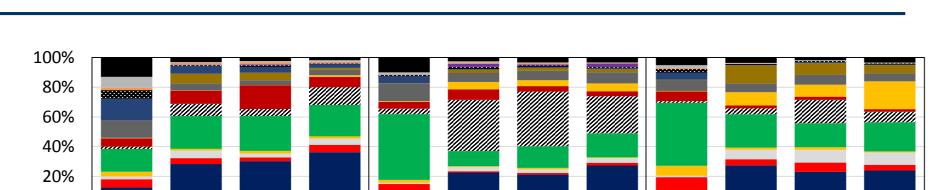
Figure 1. Nitrogen cycle in terrestrial ecosystems. Antarctic terrestrial ecosystems have limited assimilation and ammonification by animals and plants.

Research design

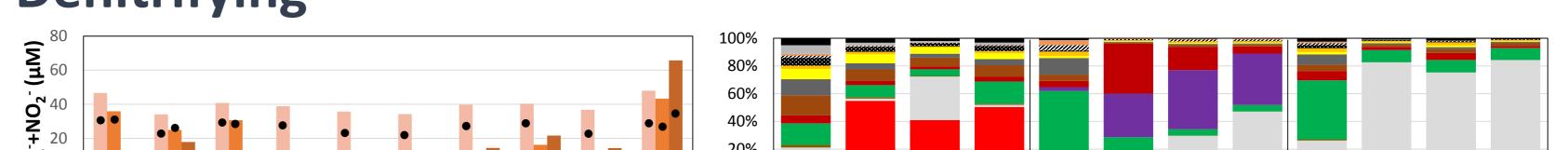


Nitrogen fixing





Denitrifying



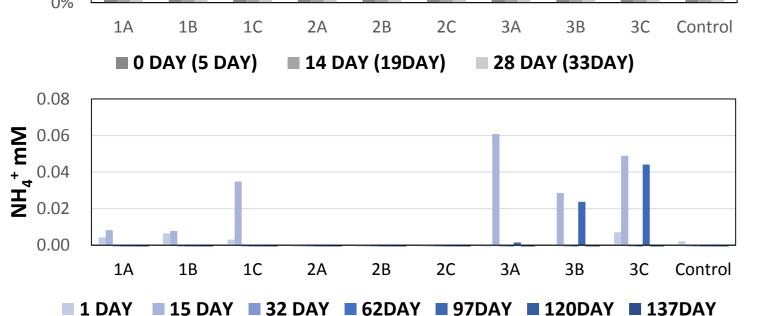


Figure 3. Changes of ammonium (NH_4^+) and acetylene concentration during enrichment culture for nitrogen fixation.

Ammonium oxidizing

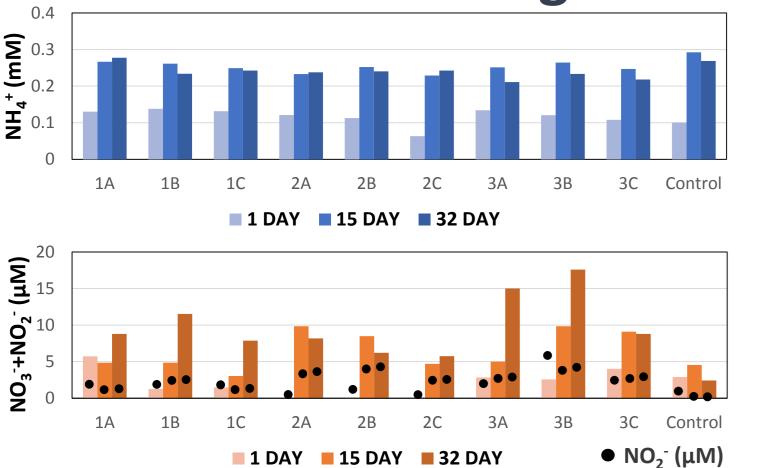
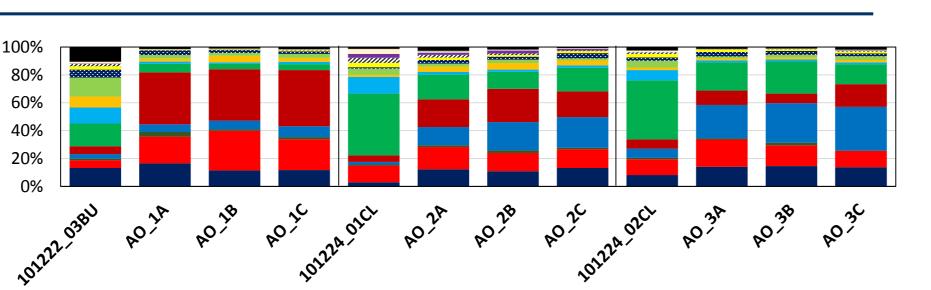


Figure 5. Changes of ammonium (NH_4^+) , nitrate (NO_3^-) and nitrite (NO_2^-) concentration during the first enrichment culture for ammonium oxidizing bacteria.

0%				
101222-038U NF-1A NF-18 NF-1C 101	2A-01Ct NF-2A NF-2B NF-2C	0122A-02CL NF-3A NF-3B NF?		
101224. 101	2 ¹	olli		
Alphaproteobacteria	Betaproteobacteria	Gammaproteobacte		
Deltaproteobacteria	Actinobacteria	 Ⅲ TM7 ■ Chloroflexi ■ Planctomycetes ■ Verrucomicrobia 		
Bacteroidetes	OD1			
Chlamydiae	Acidobacteria			
TM6	Armatimonadetes			
Other Bacteria				

Figure 4. Bacterial community composition at the phylum level of initial soil and enrichment culture for nitrogen fixing bacteria (incubation time: 137 days).



Alphaproteobacteria	Betaproteobacteria	Deltaproteobacteria
Gammaproteobacteria	Bacteroidetes	Actinobacteria
Chloroflexi	Verrucomicrobia	Acidobacteria
Planctomycetes	Gemmatimonadetes	∭ TM7
Firmicutes	WS5	Chlamydiae
Other Bacteria		

Figure 6. Bacterial community composition at the phylum
 level of initial soil and first enrichment culture for
 ammonium oxidizing bacteria (incubation time: 32 days).

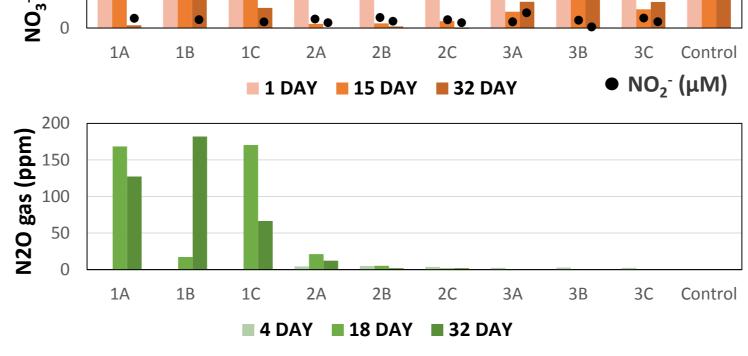


Figure 9. Changes of nitrate (NO_3^{-}) , nitrite (NO_2^{-}) and Nitrous oxide (N_2O) concentration during the first enrichment culture for denitrifying bacteria.

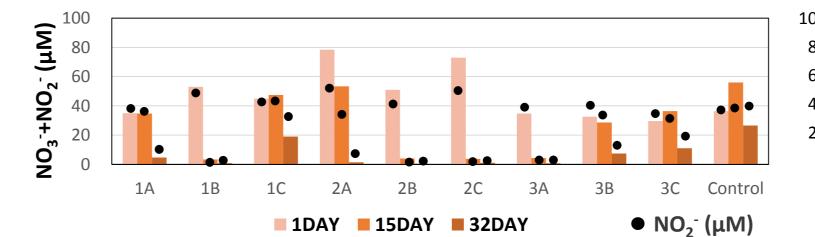


Figure 11. Changes of nitrate (NO_3^{-}) and nitrite (NO_2^{-}) concentration during the second enrichment culture for denitrifying bacteria.

22,038 ¹¹ DN-1A DN-18 DN-1C 101	2A, OICT DN 2A DN 28 DN 2C	0122A-02CL DN-3A DN-3B DN-3C		
Alphaproteobacteria	Betaproteobacteria	Gammaproteobacteria		
Deltaproteobacteria	Actinobacteria	Firmicutes		
Bacteroidetes	Acidobacteria	Chloroflexi		
Verrucomicrobia	Gemmatimonadetes	Planctomycetes		
∭ TM7	WS5	AD3		
Other Bacteria				

Figure 10. Bacterial community composition at the phylum level of initial soil and first enrichment culture for denitrifying bacteria (incubation time: 32 days).

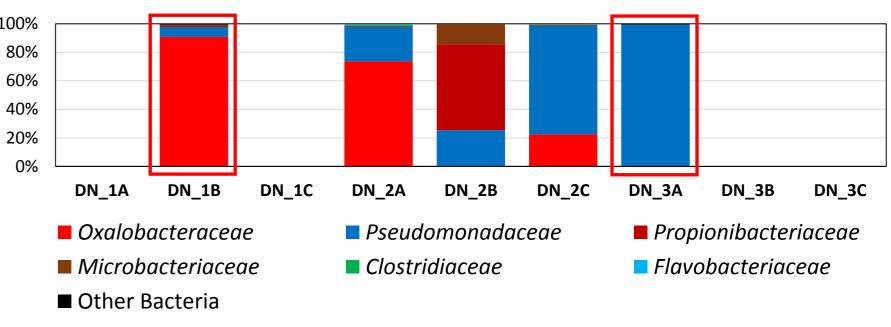
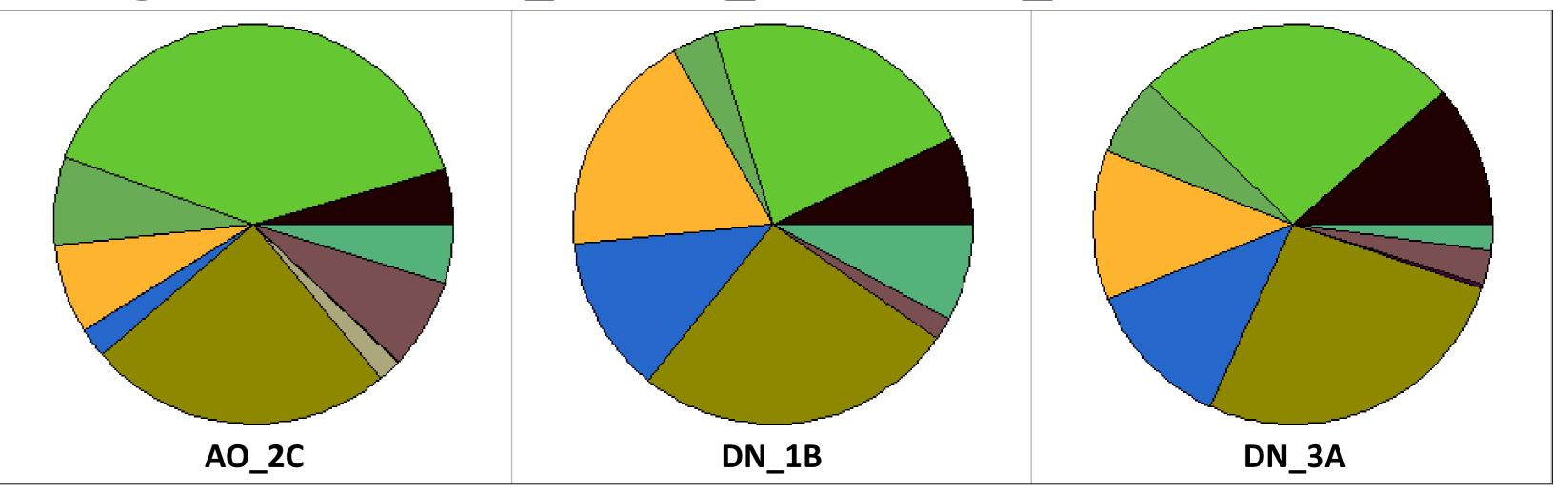
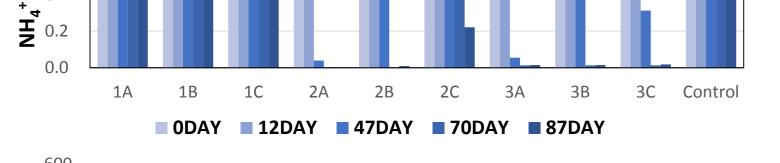


Figure 12. Bacterial community composition by family level at the second enrichment culture for denitrifying bacteria. The 16S rRNA gene for DN_1A, DN_1C, DN_3B and DN_3C were not amplified. Metagenomic analysis were performed on the red square samples.

Metagenomics of AO_2C, DN_1B and DN_3A





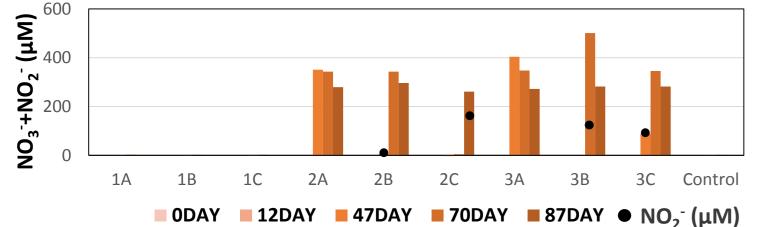


Figure 7. Changes of ammonium (NH_4^+) , nitrate (NO_3^-) and nitrite (NO_2^-) concentration during the second enrichment culture for ammonium oxidizing bacteria.

60% 40% 20%									
0%	AO_1A	AO_1B	A0_1C	AO_2A	AO_2B	AO_2C	AO_3A	AO_3B	AO_3C
	Caulobacteraceae			Rhizobiales		Reyranella			
	 Sphingomonadaceae Gallionellaceae Sphingobacteriaceae 			 Micavibrio Nitrosomonadaceae 		 Oxalobacteraceae Pseudomonadaceae Brumimicrobiaceae 			
				Chitinophagaceae					
	Microbacteriaceae			Planctomycetaceae		Parachlamydiaceae			
	■ Other E	Bacteria							

Figure 8. Bacterial community composition by family level at the second enrichment culture for ammonium oxidizing bacteria. *amoA* gene amplification was confirmed. Metagenomic analysis was performed on the red square sample.

Conclusion

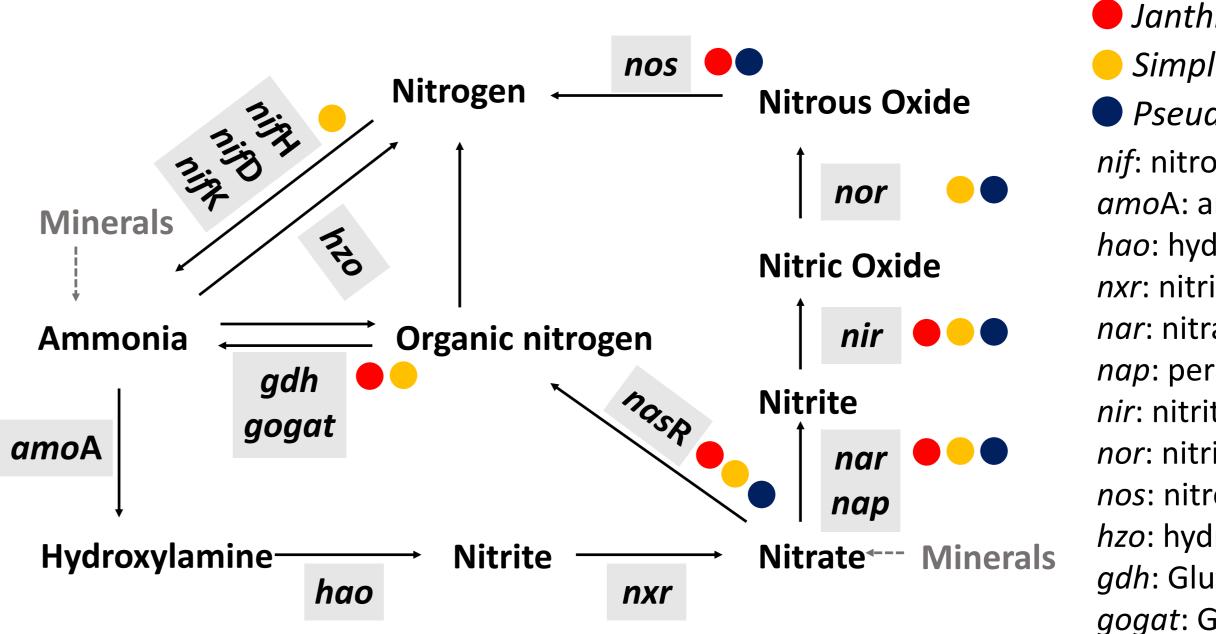
- Enrichment cultivation is a good tool for studying ecological function of unculturable bacteria in nitrogen cycles.
- Although Janthinobacterium sp. does not have the genes for ammonia oxidation, but has the genes for the ammonia assimilation.
- amoA gene amplification of AOB were detected, however their quantity is relatively low for genome assembly.
- Simplicispira sp. DN1B and Pseudomonas sp. DN3A are successfully enriched and obtained complete genome for denitrification.
- Uncultivated denitrifying bacteria were enriched and analyzed their ecological function by shotgun metagenomics.



Legend (SEED):

Allantoin Utilization Ammonia assimilation Cyanate hydrolysis Denitrification Dissimilatory nitrite reductase Nitrate and nitrite ammonification

Figure 13. Pie chart showed Comparison of Nitrogen metabolism of enriched samples by SEED profile for comparison MEGAN6.



Janthinobacterium sp. AO2C
 Simplicispira sp. DN1B
 Pseudomonas sp. DN3A
 nif: nitrogen fixation genes
 amoA: ammonia-oxidizing gene A
 hao: hydroxylamine oxidoreductase
 nxr: nitrite oxidoreductase
 nar: nitrite reductase
 nap: periplasmic nitrate reductase
 nir: nitrite reductase
 nor: nitric oxide reductase
 nos: nitrous oxide reductase
 hzo: hydrazine oxidoreductase
 gogat: Glutamate dehydrogenase

Figure 14. Schematic representation of genes related to nitrogen cycle in *Janthinobacterium* sp. AO2C, *Simplicispira* sp. DN1B and *Pseudomonas* sp. DN3A genomes for obtained through metagenomics.

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