

Mitigation of Greenhouse Gas Emissions From Agricultural Lands by Optimizing Nitrogen and Carbon Cycles

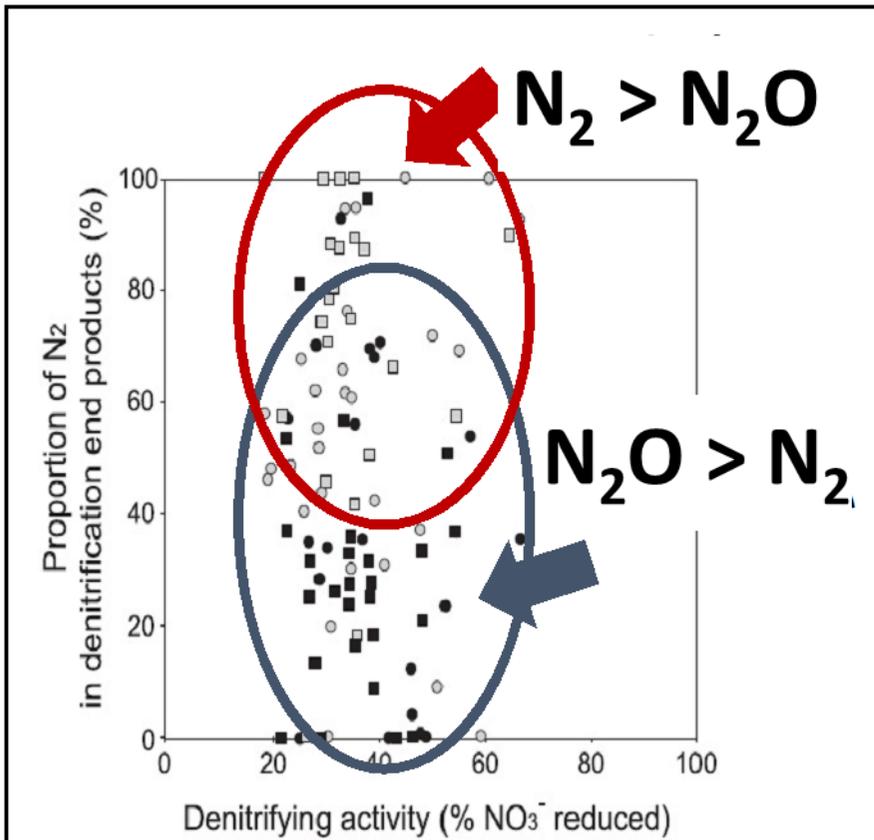
Presenter : Prof. SENOO Keishi, The University of Tokyo

PM : Dr. MINAMISAWA Kiwamu, Tohoku University

Implementing organizations : Tohoku University, The University of Tokyo

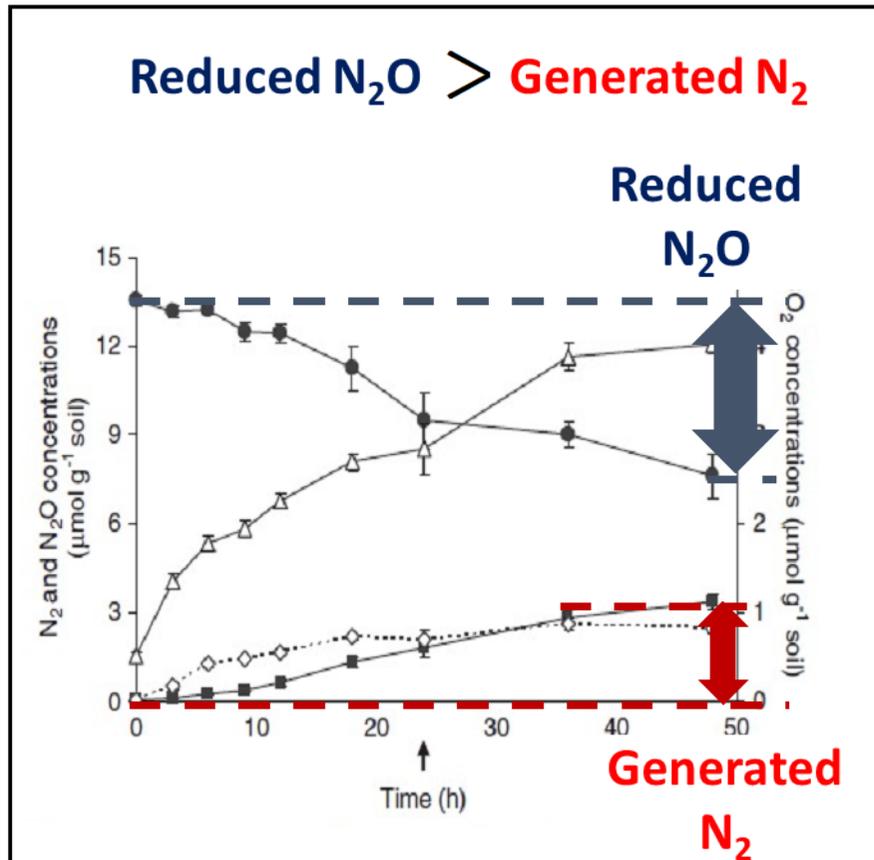
National Agriculture and Food Research Organization (NARO)

Paddy soils as N₂O sink



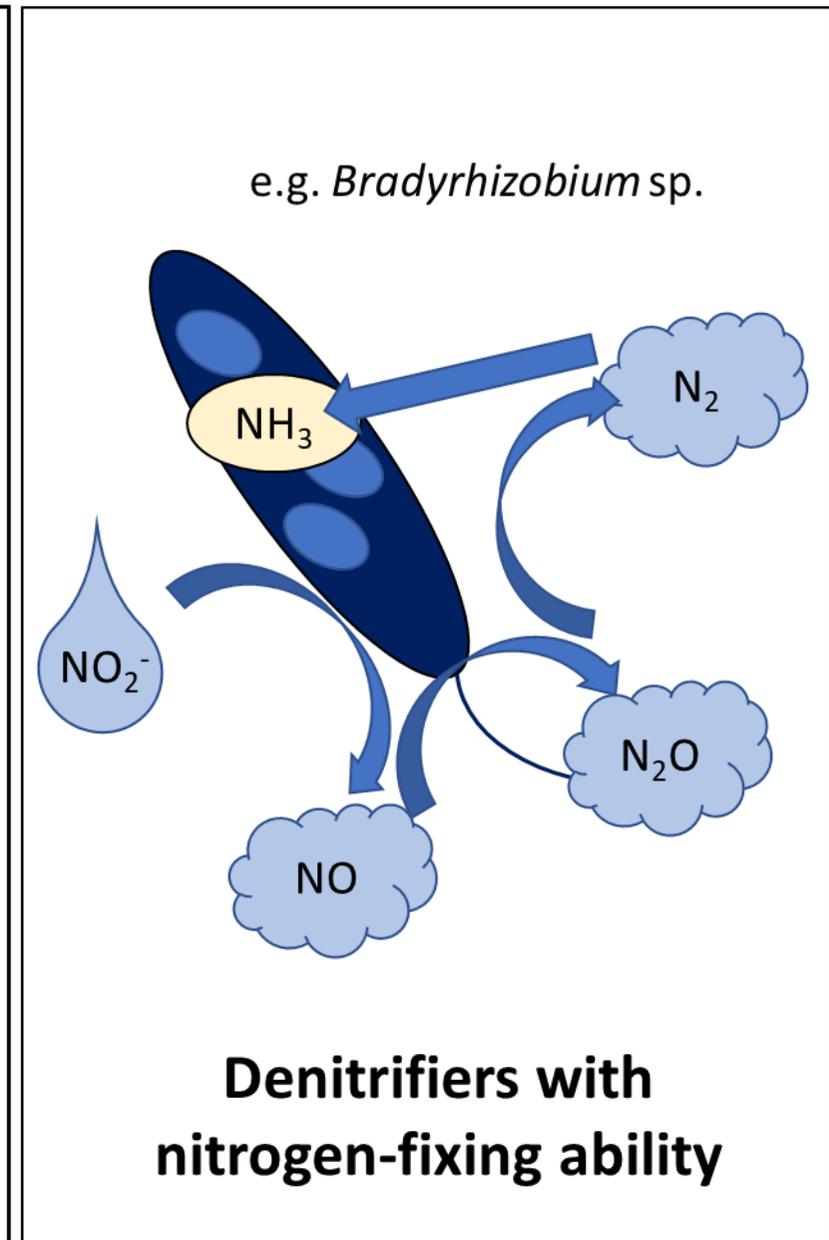
(Tago et al., 2011)

The N₂/N₂O production ratio of denitrifiers in paddy soils is diverse



(Ishii et al., 2011)

60 % of the reduced N₂O is missing

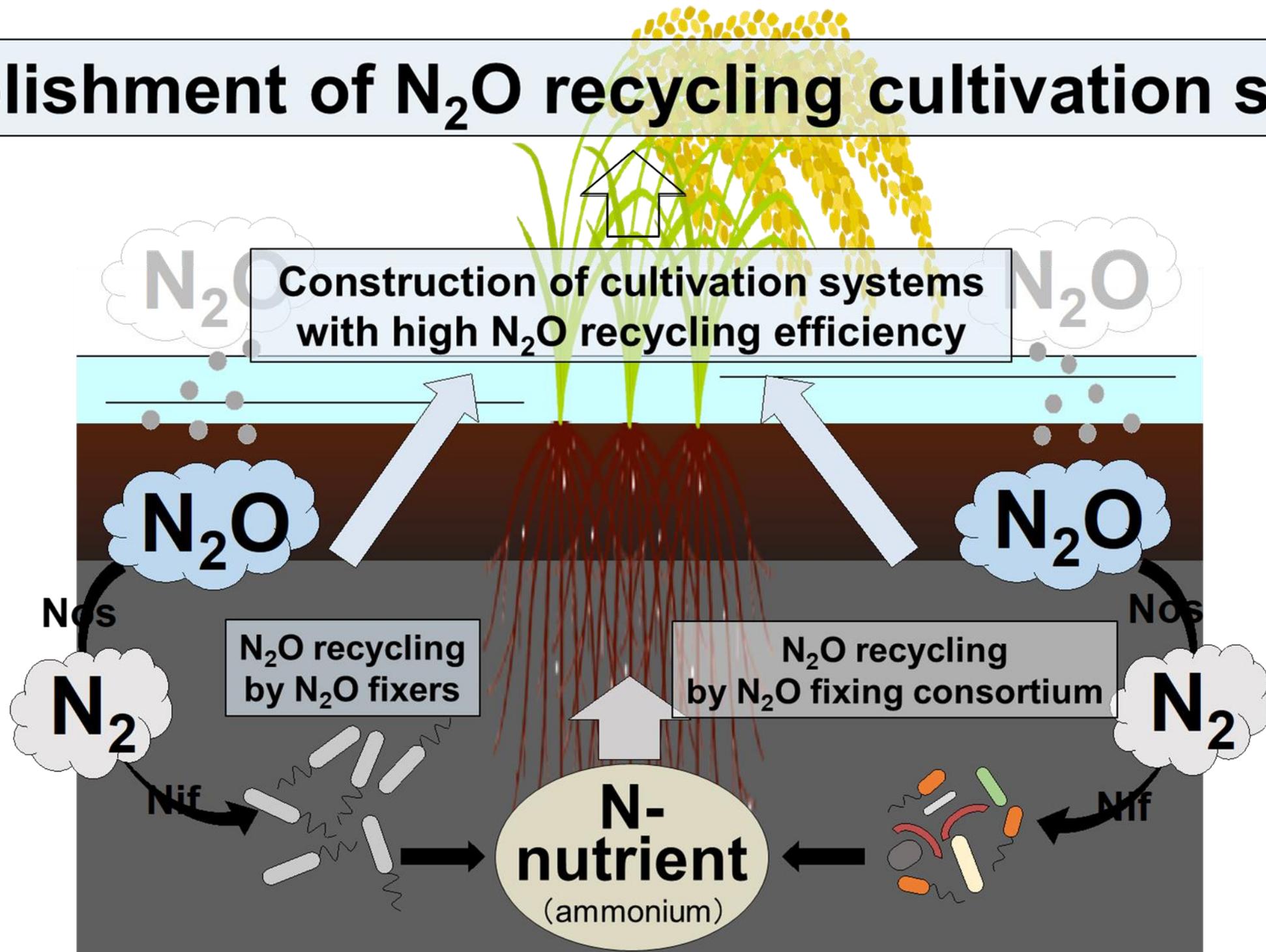


Denitrifiers with nitrogen-fixing ability

N₂O produced in paddy soils might be re-fixed by soil bacteria

Purpose of this study

Establishment of N₂O recycling cultivation system



Conversion of greenhouse gas N₂O to N nutrient

Research Plan

2021
Clarification of N₂O fixation pathway

N ₂ O	→	N ₂	→	NH ₄ ⁺
	<i>nos</i>		<i>nif</i>	
N ₂ O	→	N ₂	→	NH ₄ ⁺
	<i>nos</i>		<i>nif</i>	
N ₂ O	→	N ₂	→	NH ₄ ⁺
	<i>nos</i>		<i>nif</i>	

2024
Development of methods to enhance N₂O sink function of paddy soils

Goal

Establishment of N₂O recycling cultivation system

Construction of cultivation systems with high N₂O recycling efficiency

2020
Confirmation of N₂O fixation

2022
Estimation of the amount of N₂O fixation

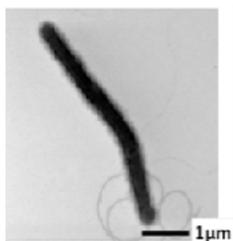
2023
Clarification of factors enhancing N₂O fixation

Bacteria harboring N₂O fixation potential

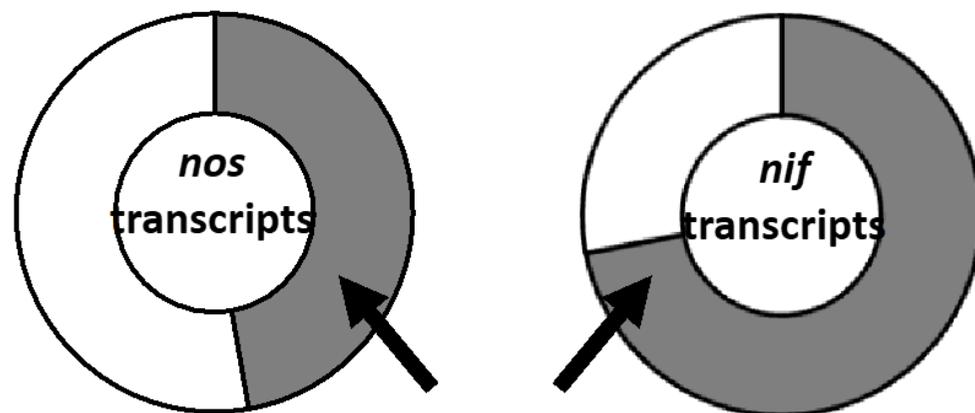


① Iron reducing bacteria harboring N₂O reduction enzyme genes (*nos*)

Anaeromyxobacter sp.



- Iron-reducing bacteria predominated in paddy soils
- Large number of *nos* and *nif* transcripts were detected in metatranscriptomics of paddy soils



Iron reducing bacteria

Microbial composition of *nos* and *nif* gene transcripts detected by metatranscriptomics

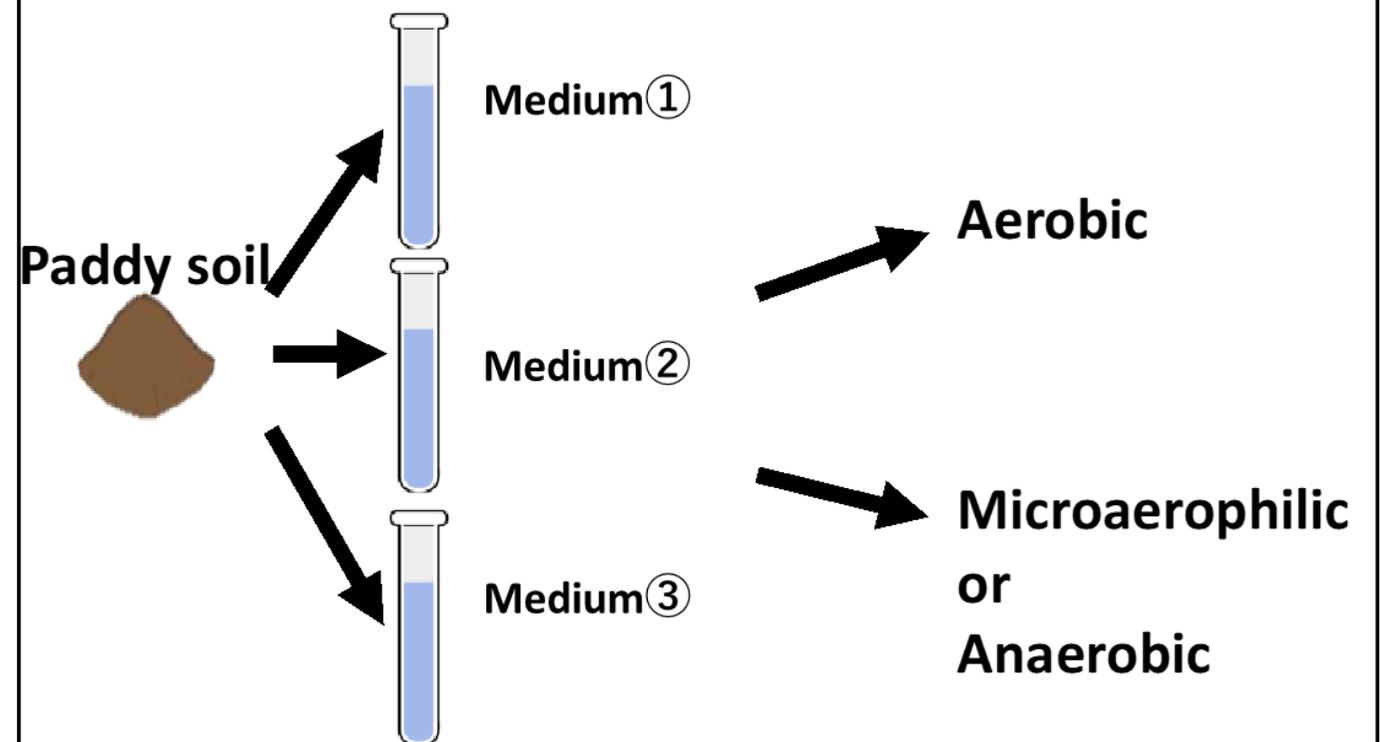
② Denitrifier harboring N fixation enzyme genes

(*nif*)

Bradyrhizobium sp.

- Isolated as denitrifiers with high N₂O reducing activity
- harboring *nif* (nitrogen fixation gene)

③ Microbial consortium growing with N₂O as a sole N source

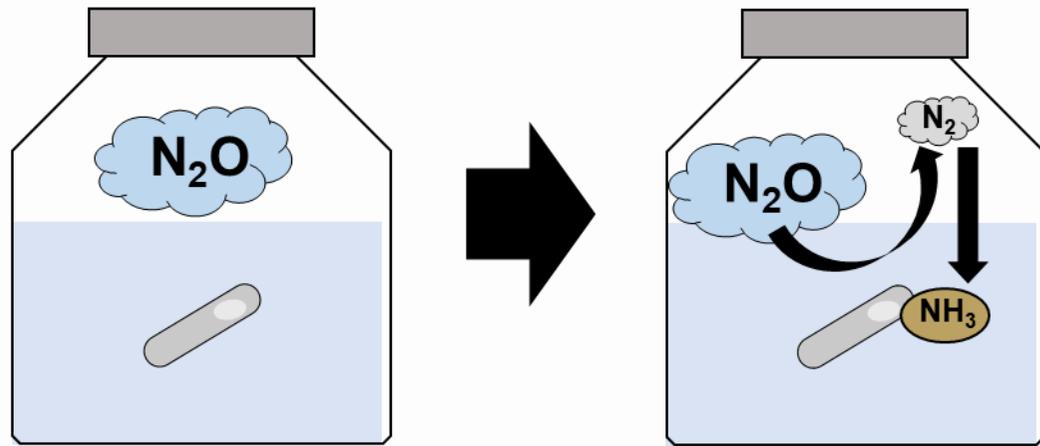


Amplicon sequencing of community composition

Verification of N₂O fixing ability

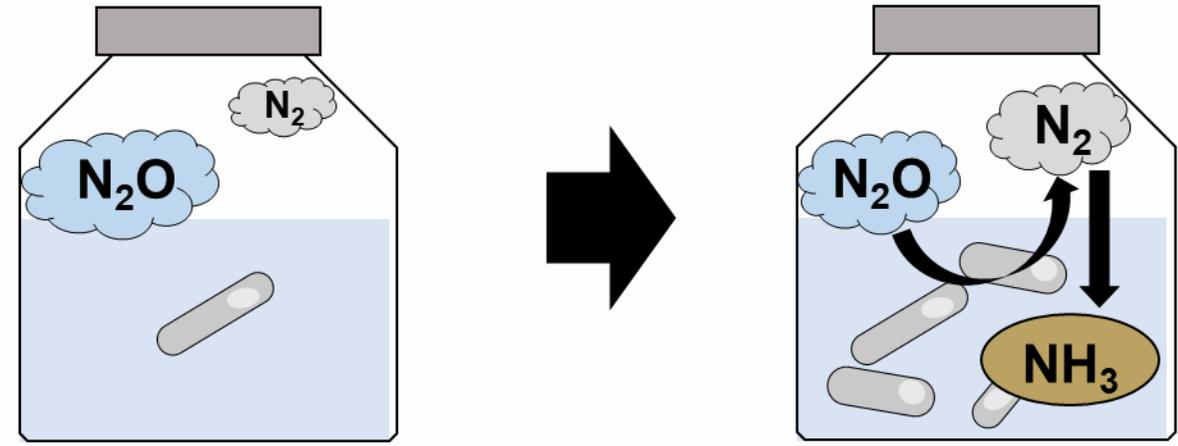
① Verification of N₂O fixation ability of *Anaeromyxobacter* sp.

–optimal culture conditions for N₂O fixation–



Survive but no growth in the medium with N₂O as sole N source and e-acceptor.

Growth was observed after transferred to N₂ containing medium.



Growth was observed in the presence of N₂O and small amount of N₂. The amount of N₂O decreased.

Anaeromyxobacter sp. fix N₂O in the presence of small amount of N₂

② Verification of N₂O fixation ability of *Bradyrhizobium* sp.

–optimal culture conditions for N₂O fixation–

* No growth was observed in the absence of N₂ → N₂ is essential for growth.

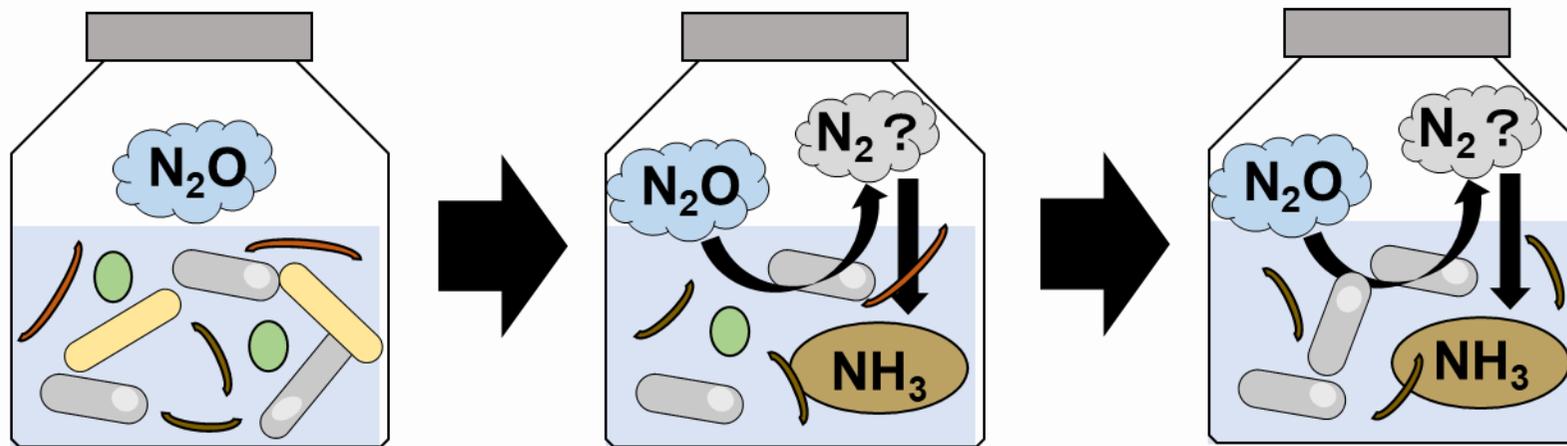
* Growth was observed in the presence of N₂O and small amount of N₂.

Anaeromyxobacter sp. and *Bradyrhizobium* sp. fix N₂O in the presence of small amount of N₂

Verification of N₂O fixing ability

③ Enrichment culture and isolation of microbial consortia growing with N₂O as sole nitrogen source as sole nitrogen source

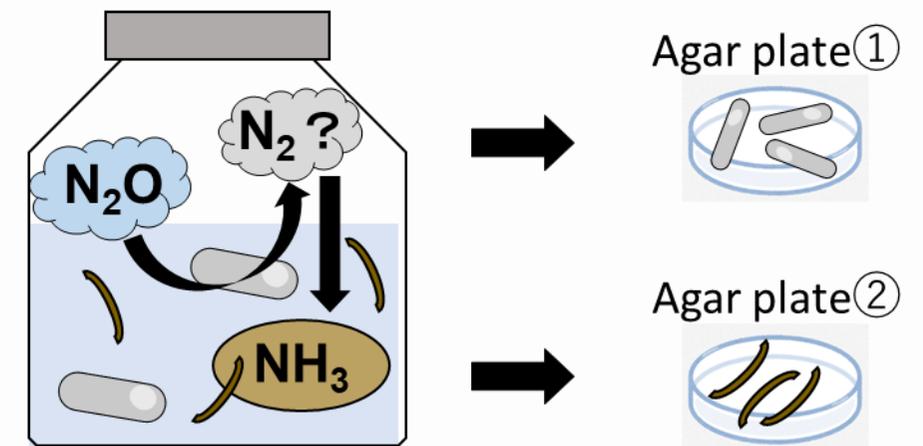
Enrichment culture in Medium ① (aerobic)



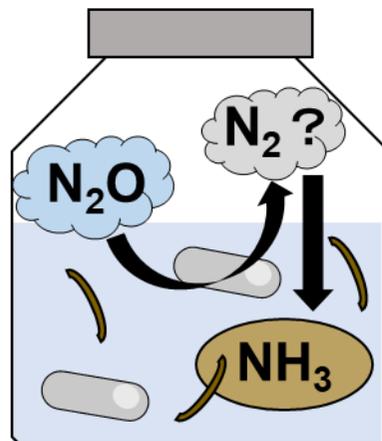
Microbial community composition became simplified

Isolation

Enrichment culture



※ Two strains dominated in the culture were successfully isolated



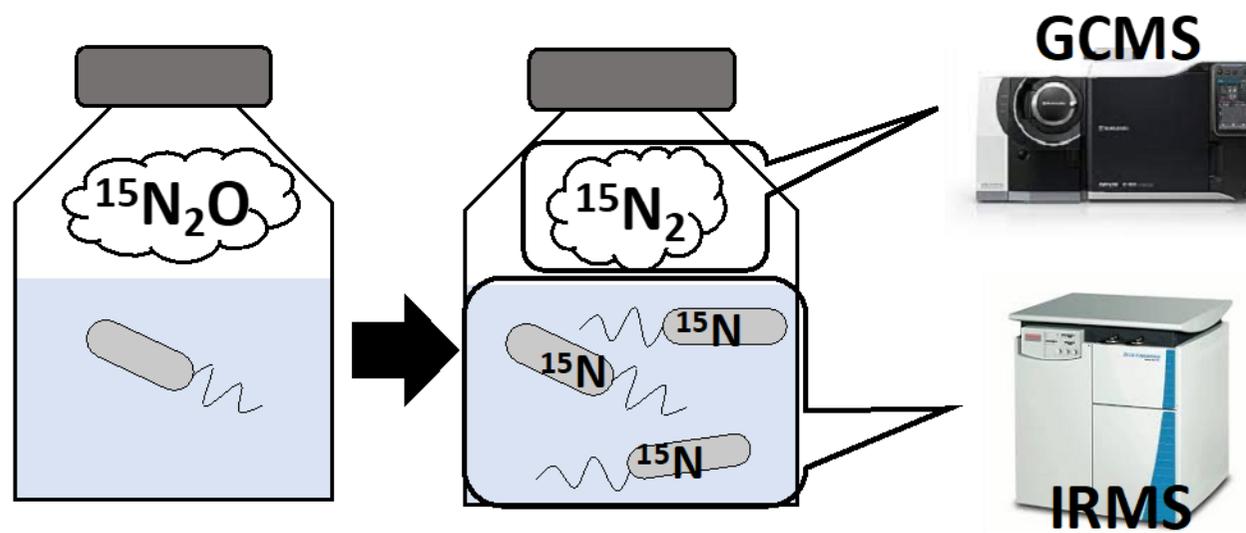
A microbial consortium has been obtained growing with N₂O as sole nitrogen source

Future plan

Clarification of N_2O fixation pathway



Quantification of fixed N_2O



Exploration of factors enhancing N_2O fixing-activity of the bacteria/consortium

