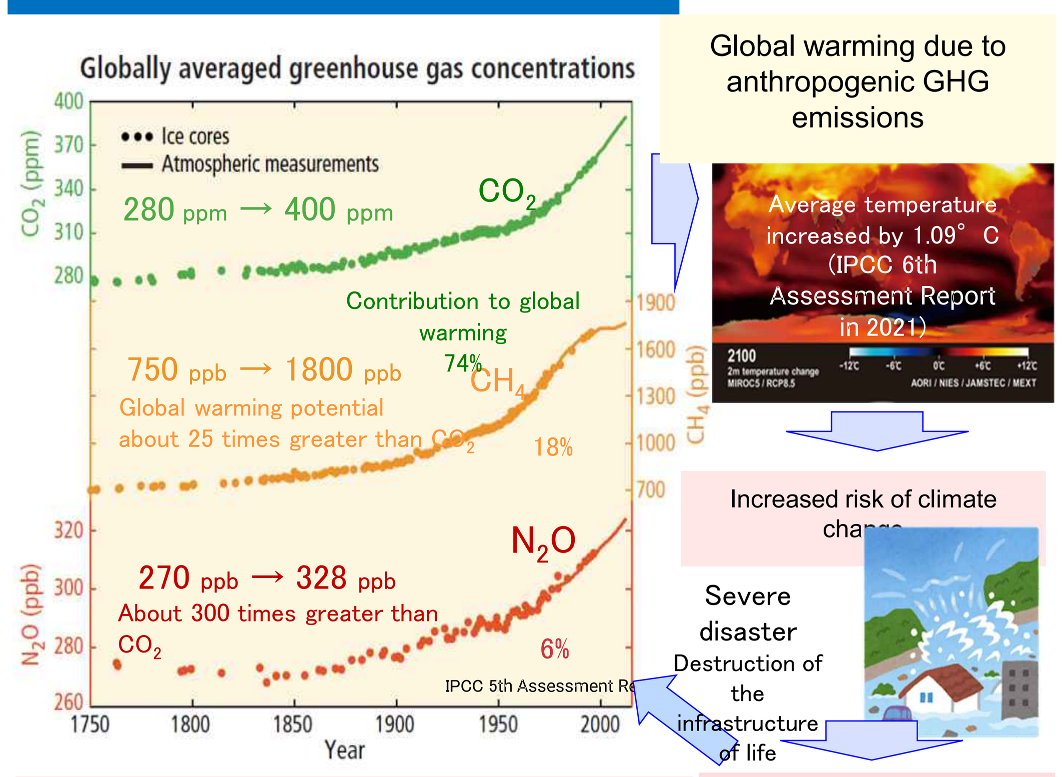
No. A-8-1E

PJ :Mitigation of greenhouse gas emissions from agricultural lands by optimizing Nitrogen and carbon cycles

Theme: Cool Earth via Microbes in Agriculture

Organization: Tohoku University, The University of Tokyo, NARO. Subcontractors: Tokyo University of Agriculture and Technology, Iwate University, Obihiro University, Shizuoka University, Kyoto University, Tokyo Institute of Technology, AIST, Ibaraki University, Ehime University, Ryukoku University, Nagoya University, FFPRI, National Institute for Environmental Studies, bitBiome, Inc.
Contact: Kiwamu Minamisawa (E-mail: dsoil.moonshot@grp.tohoku.ac.jp)

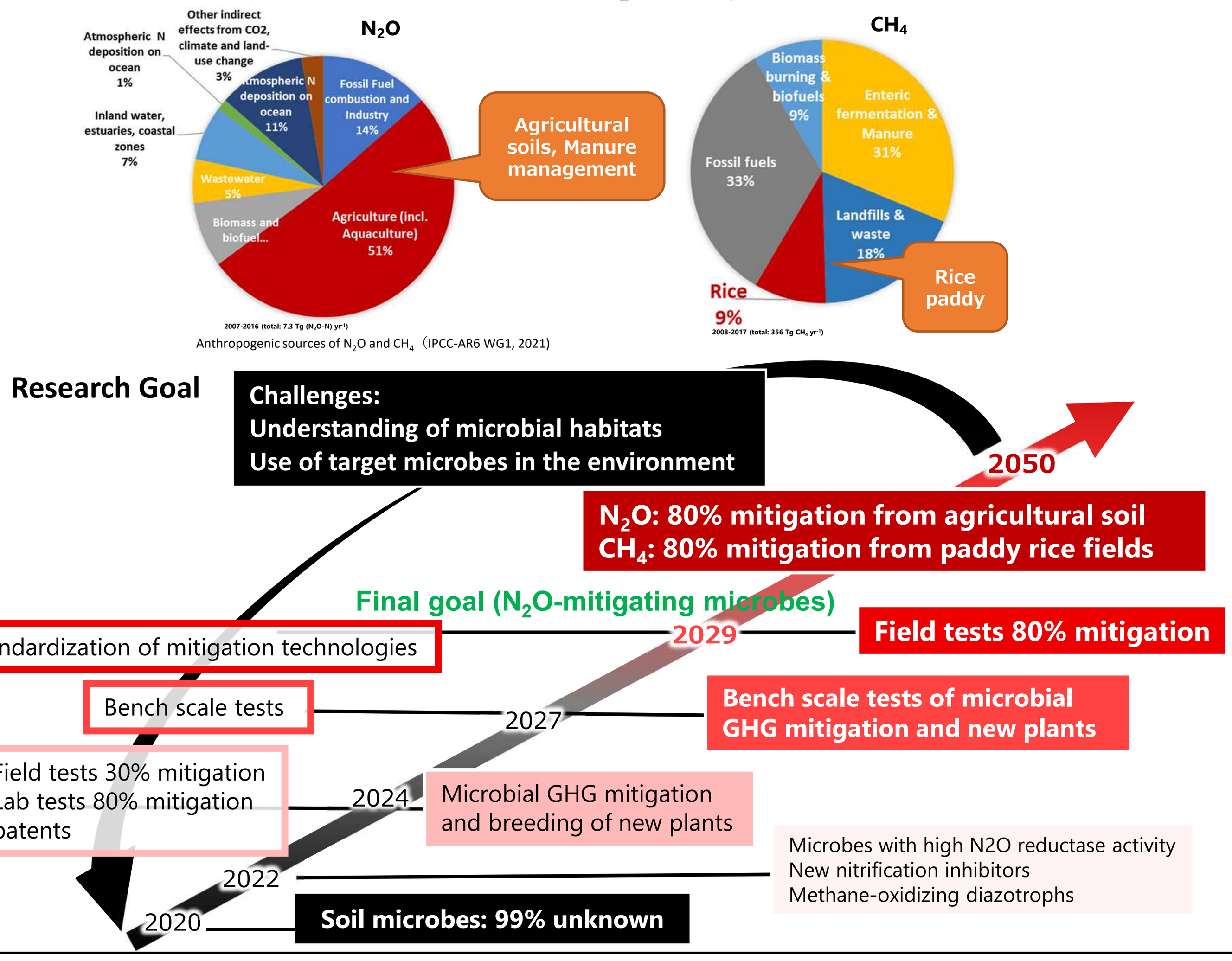
Introduction



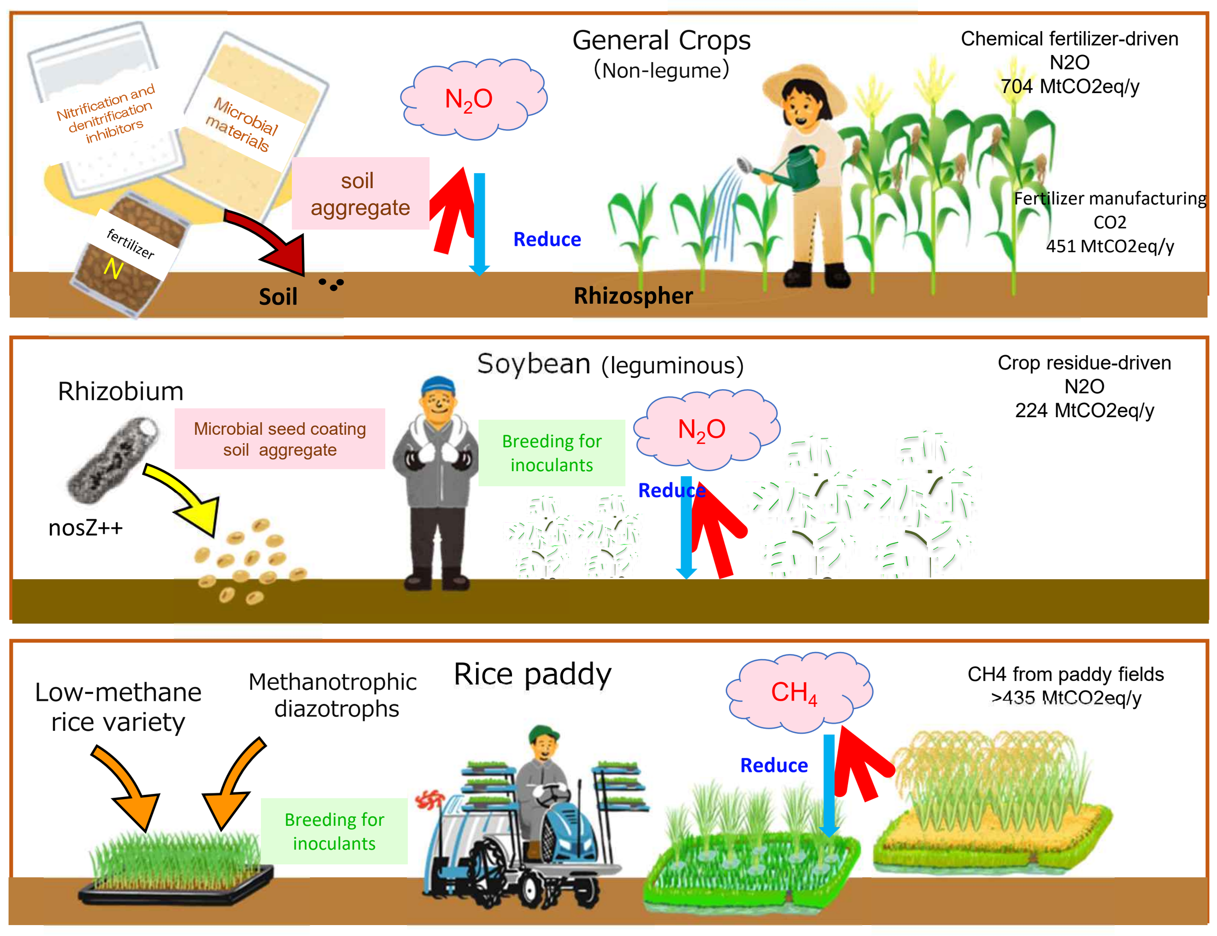
Reduction of greenhouse gases other than CO_2 is essential to limit the rise in global temperatures to 1.5 degrees Celsius above pre-industrial levels. (Paris Agreement, COP26)

How can we reduce anthropogenic emissions of CO_2, CH_4, and N_2O?

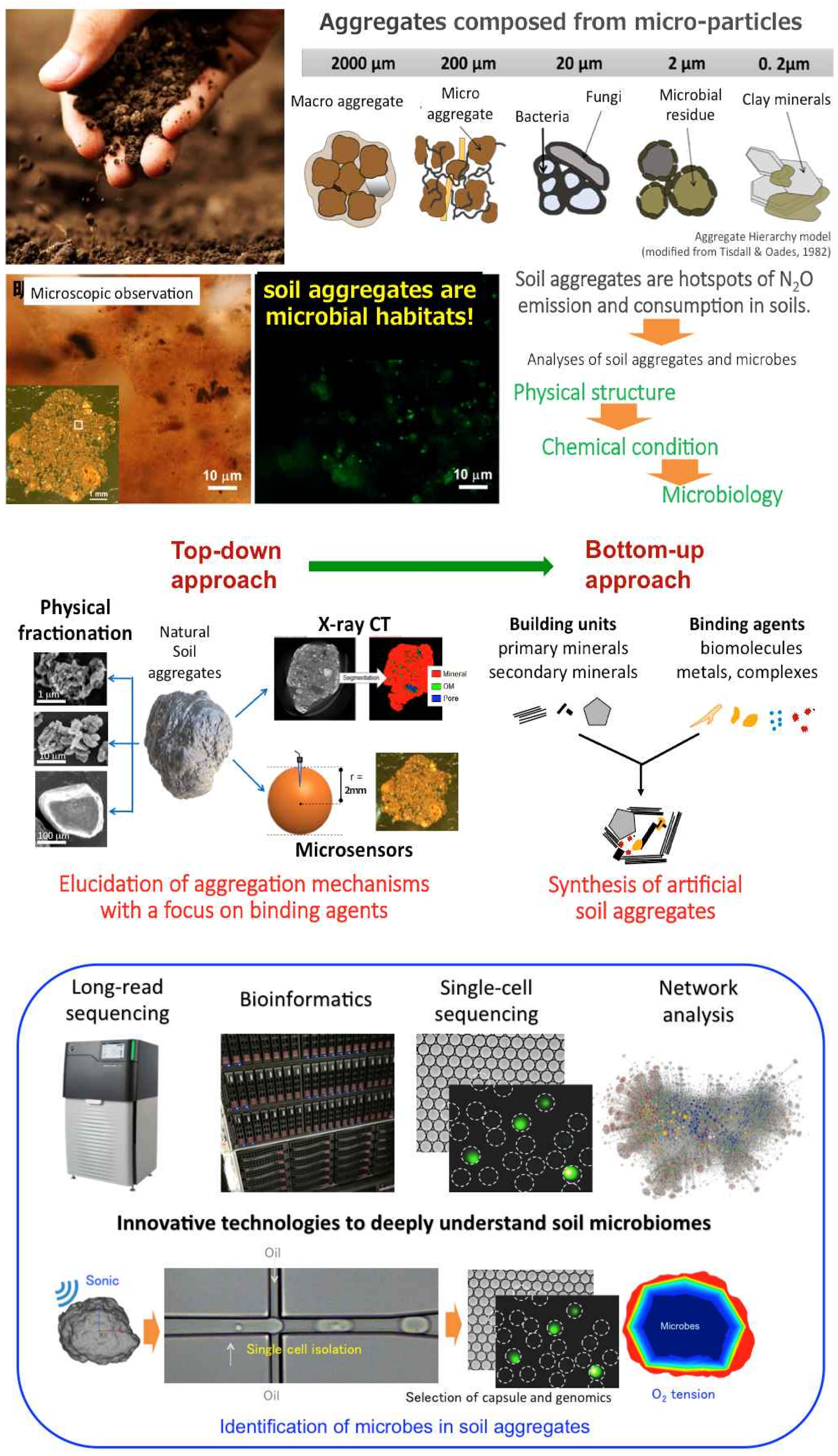
Agriculture: Major Anthropogenic Source of N_2O & CH_4



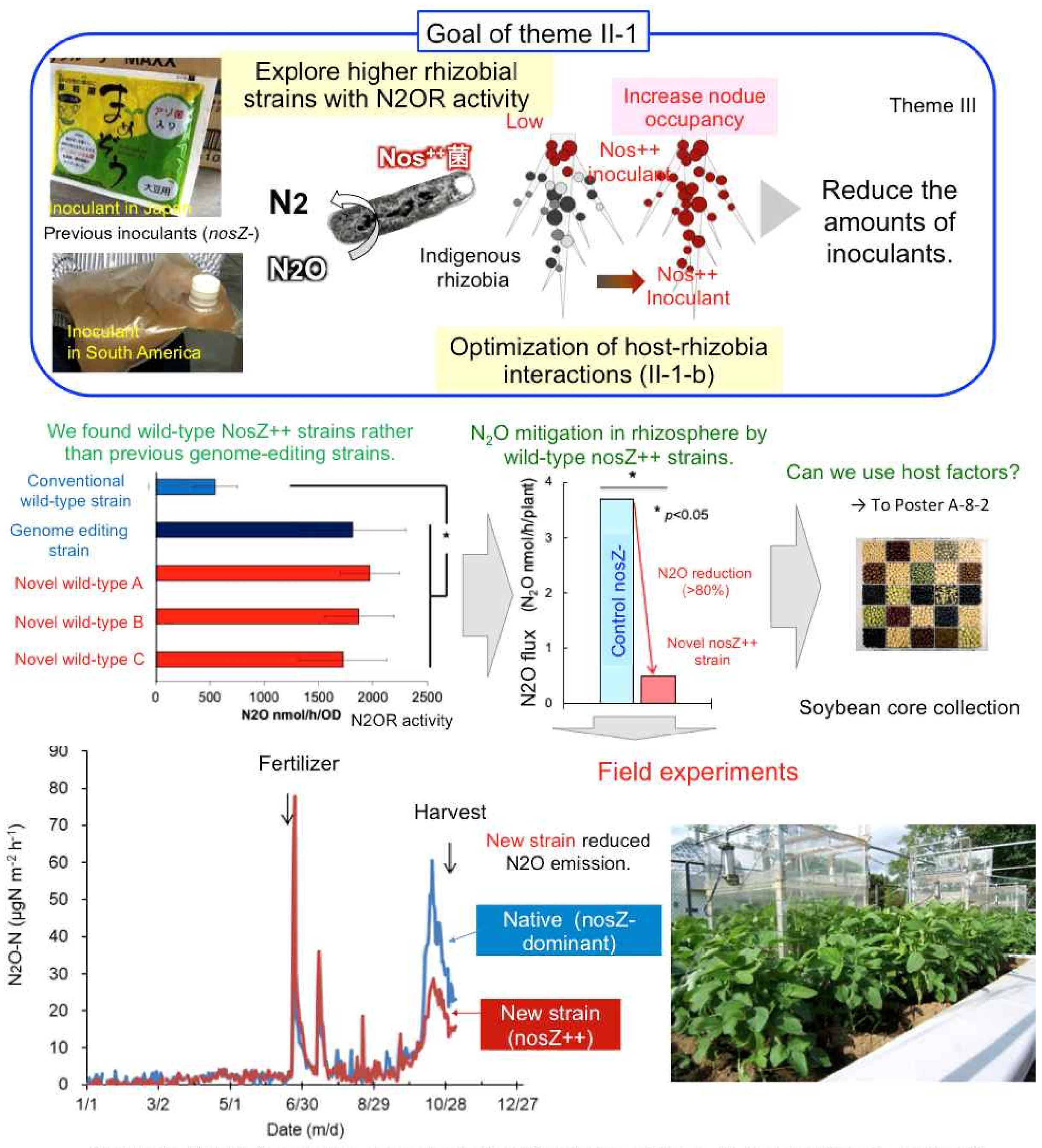
Business plans for this project



I Soil structure (Soil aggregate)



II・V N_2O Recycling and Evaluation & modeling (Details → Poster A-8-2, 4)

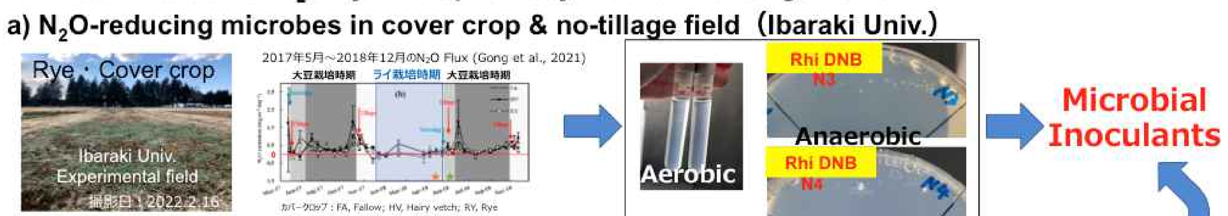


N_2O reduction by inoculation of a new strain of *Bradyrhizobium* sp. in Andosol (*nosZ*- dominant)

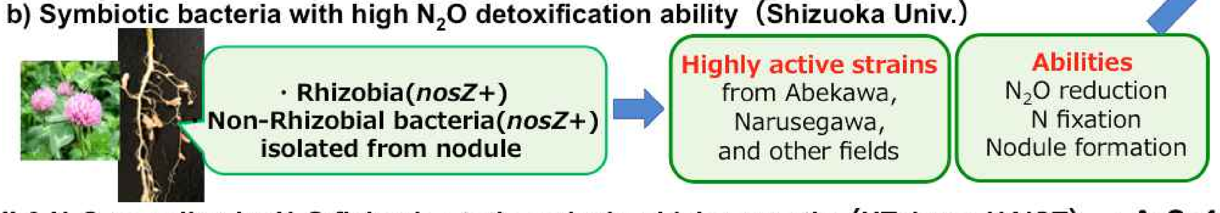
II-2, 3 Detoxification and recycling of N_2O in upland and paddy soil

II-2 Detoxification of N_2O by rhizospheric/symbiotic microorganisms

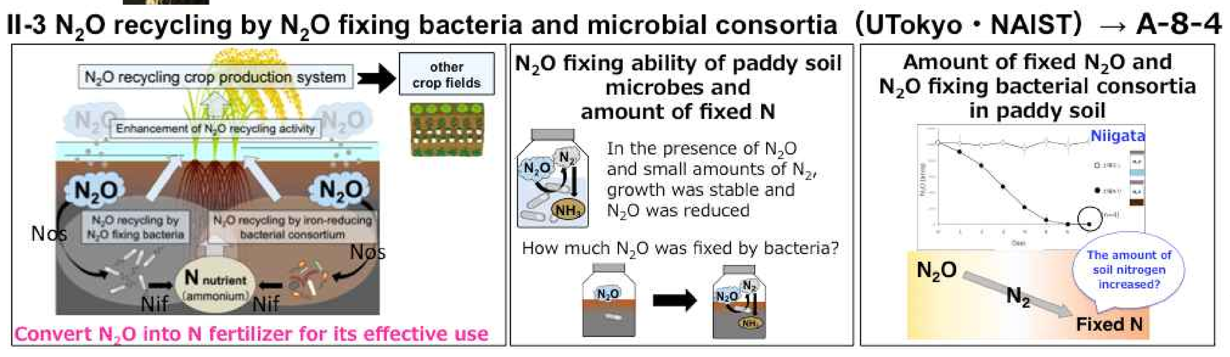
a) N_2O-reducing microbes in cover crop & no-tillage field (Ibaraki Univ.)



b) Symbiotic bacteria with high N_2O detoxification ability (Shizuoka Univ.)

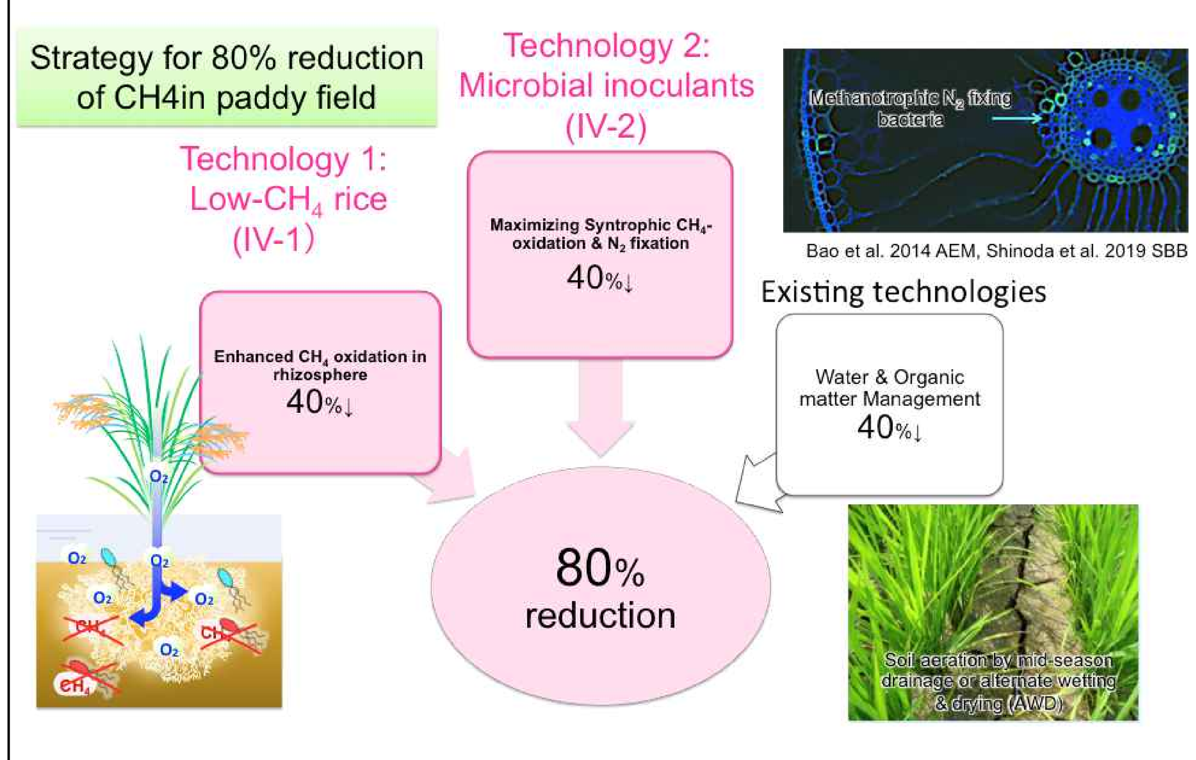


II-3 N_2O recycling by N_2O fixing bacteria and microbial consortia (UTokyo・NAIST) → A-8-4

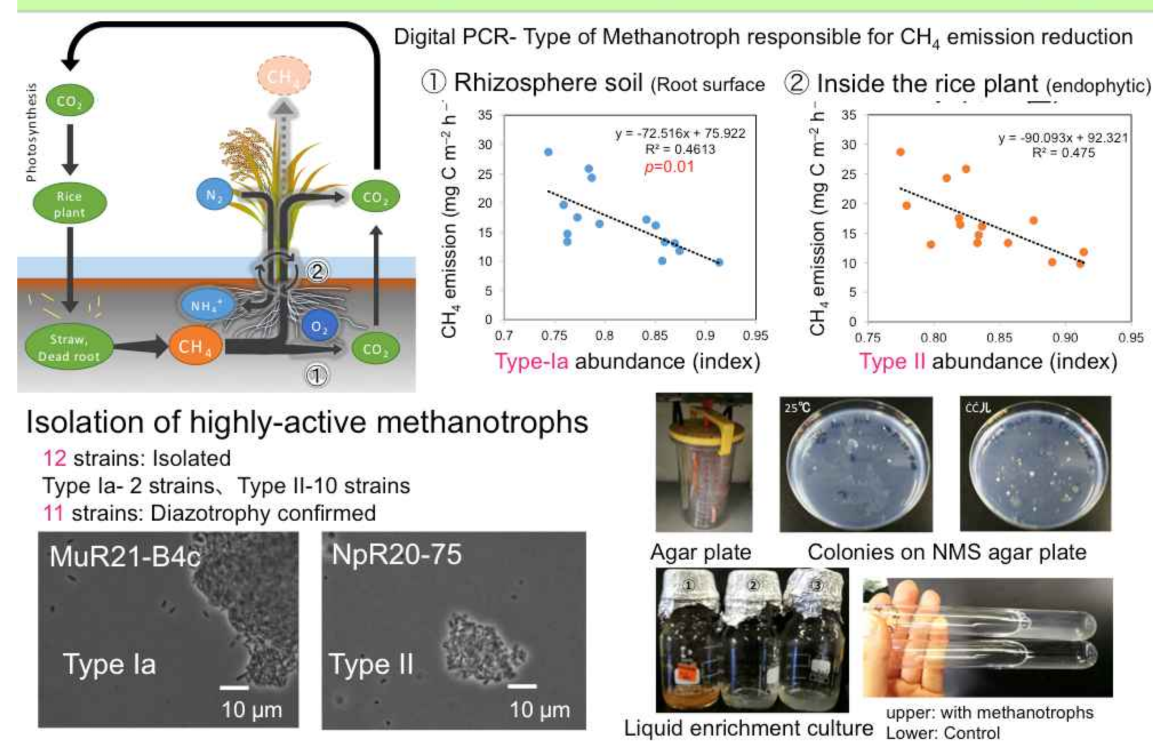


Minamisawa MS project themes

IV CH_4 Recycling (Details → Poster A-8-3)



IV-2 Maximizing diazotrophic methanotrophy



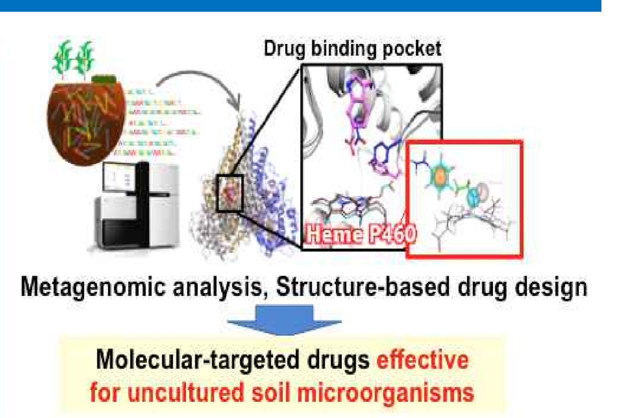
III DREAM (Details → Poster A-8-3)

Goal: Reduce N_2O emissions by the combined use of molecular-targeted drugsand super active N_2O-detoxifying microorganisms

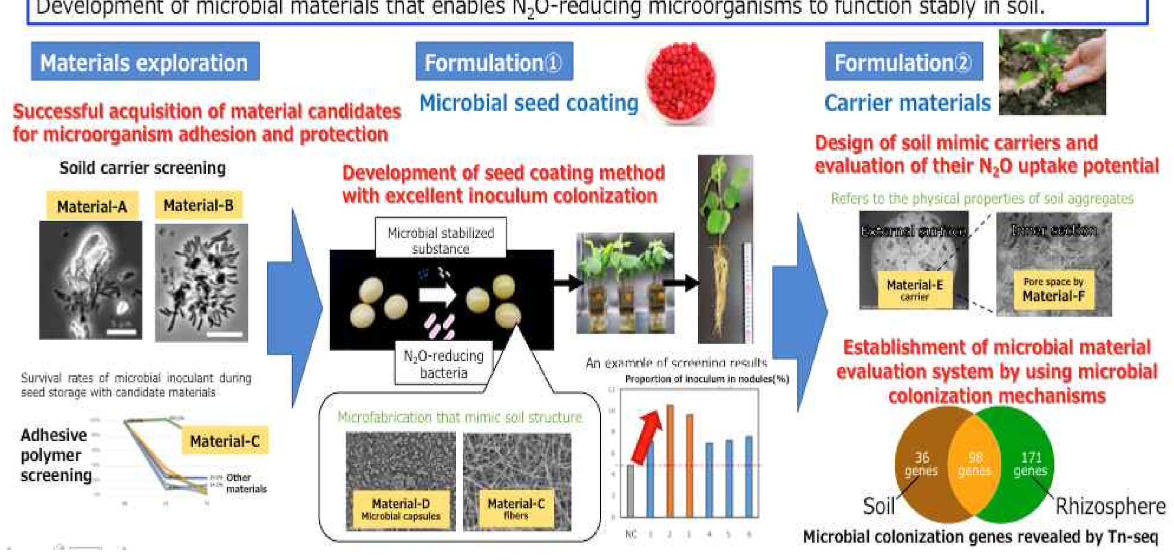
III-1 N_2O-detoxifying microorganism with super active NosZ

III-2 HAO-targeted nitrification inhibitor
NirK-targeted denitrification inhibitor

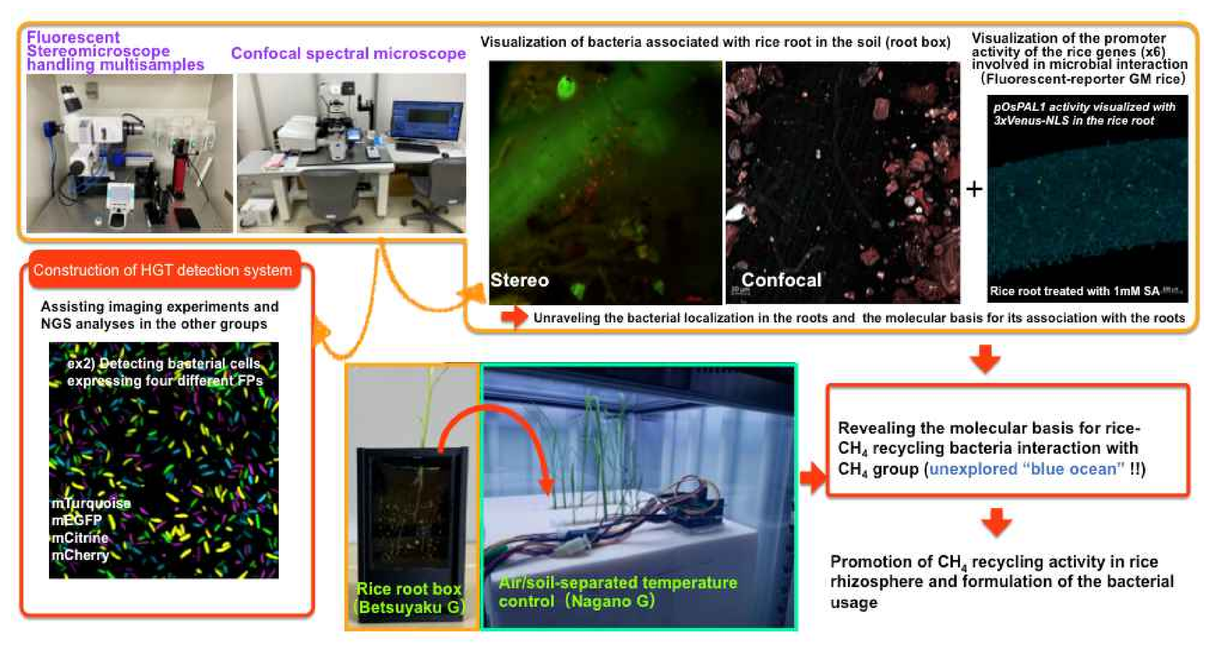
III-3 Construction of carriers that mimic soil microstructure



Construction of carriers that mimic soil microstructure and evaluation of their microbial colonization performance. Development of microbial materials that enables N_2O-reducing microorganisms to function stably in soil.

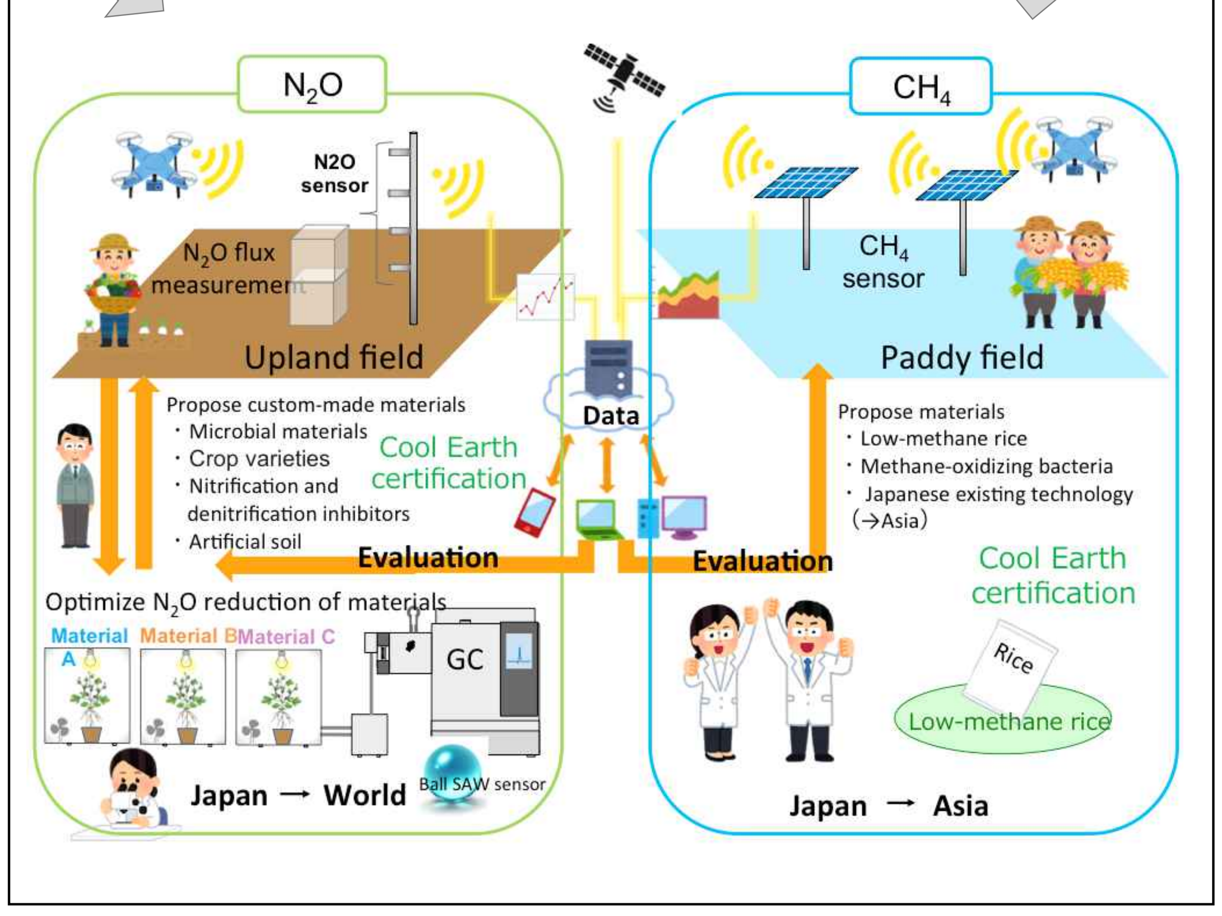


Imaging system for N_2O and CH_4 mitigation



II-4. Construction of the rhizosphere cultivation system for designing and evaluating the soil ecosystem for N_2O recycling (Ryukoku Univ · Betsuyaku G/NaganoG)

Business models for GHG mitigation in agriculture



No. A-8-2E

PJ: Mitigation of greenhouse gas emissions from agricultural lands by optimizing nitrogen and carbon cycles

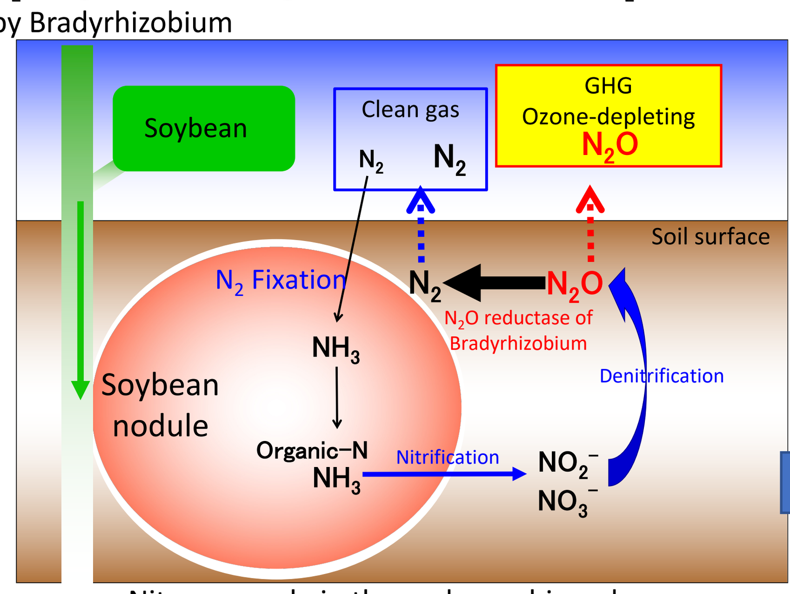
Theme: N_2O reduction by soybean rhizobia

Organization: Tohoku University, NARO

Contact: Kiwamu Minamisawa (E-mail: dsoil.moonshot@grp.tohoku.ac.jp)

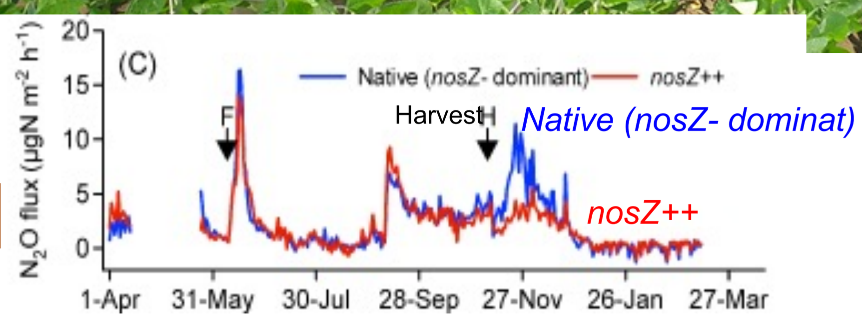
Background and Aims

N_2O is generated in degraded nodule, however N_2O is reduced by Bradyrhizobium



Nitrogen cycle in the soybean rhizosphere

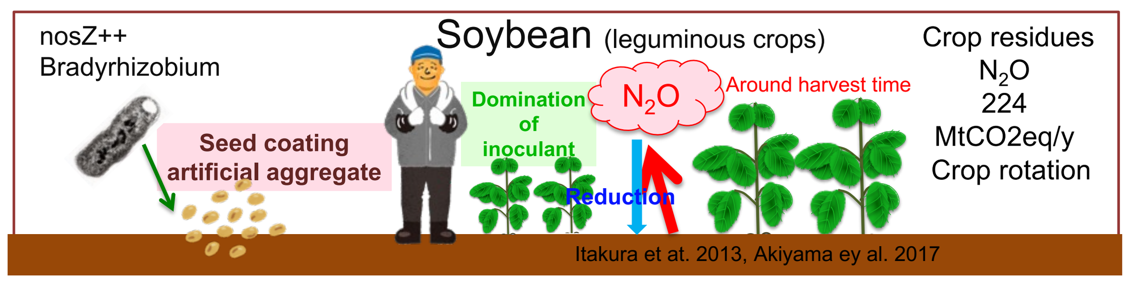
Reduction of N_2O emissions by field inoculation with *nosZ*++ mutant

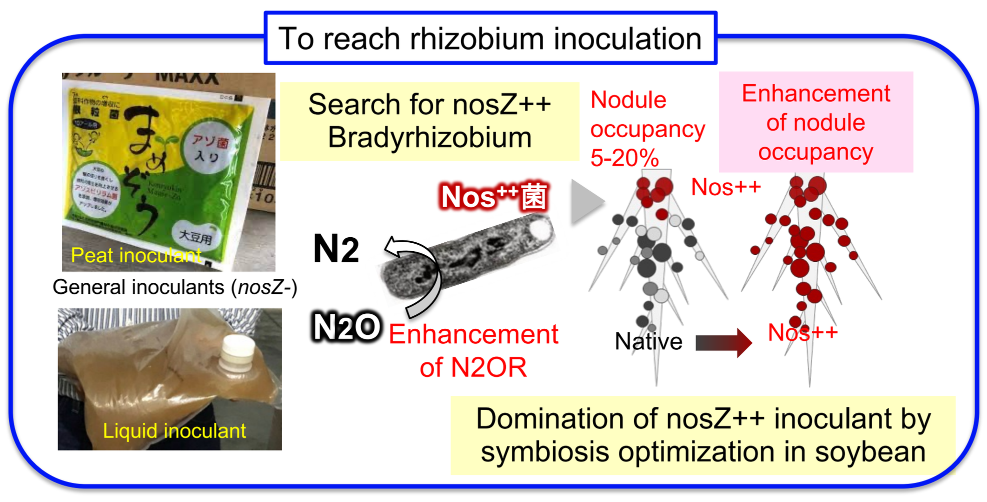


Itakura *et al.* Mitigation of nitrous oxide emissions from soils by *Bradyrhizobium japonicum* inoculation. **Nature Climate Change, 2013**

Isolation of wild-type *Bradyrhizobium* strains with high N_2O reduction activity and establishment utilization technology

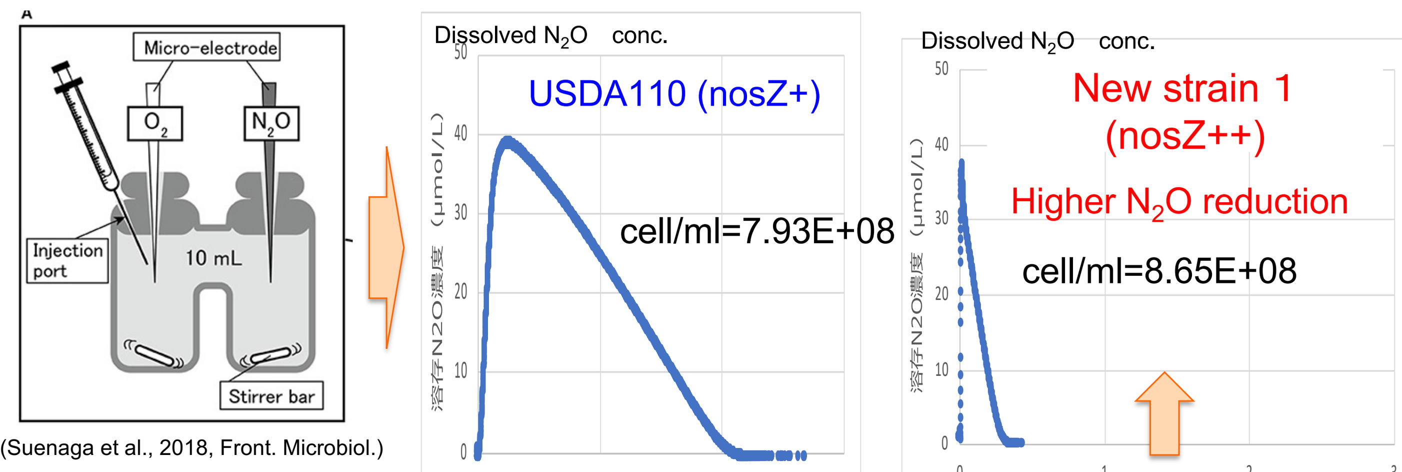
Reduction of nitrous oxide (N_2O) emission by soybean rhizobia → 50~80% reduction



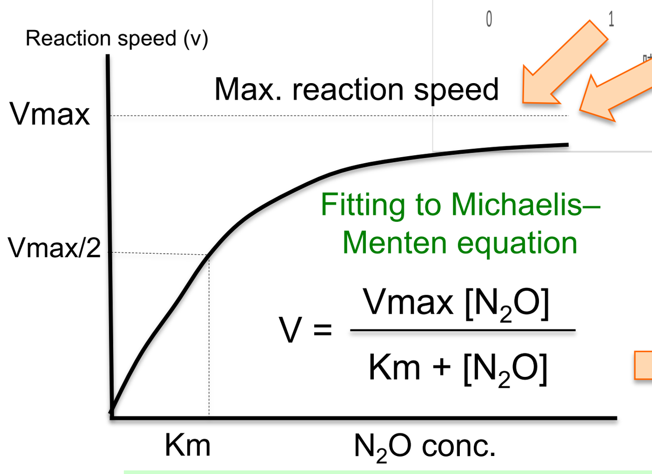


N_2O reduction by new *nosZ*++ *Bradyrhizobium* strain

N_2O reduction activity in Free-living cell

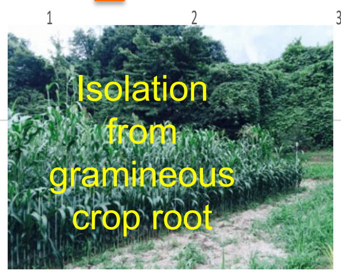


(Suenaga et al., 2018, Front. Microbiol.)



$$V = \frac{Vmax\,[N_2O]}{Km + [N_2O]}$$

Mechanisms of *nosZ*++ Currently under analysis

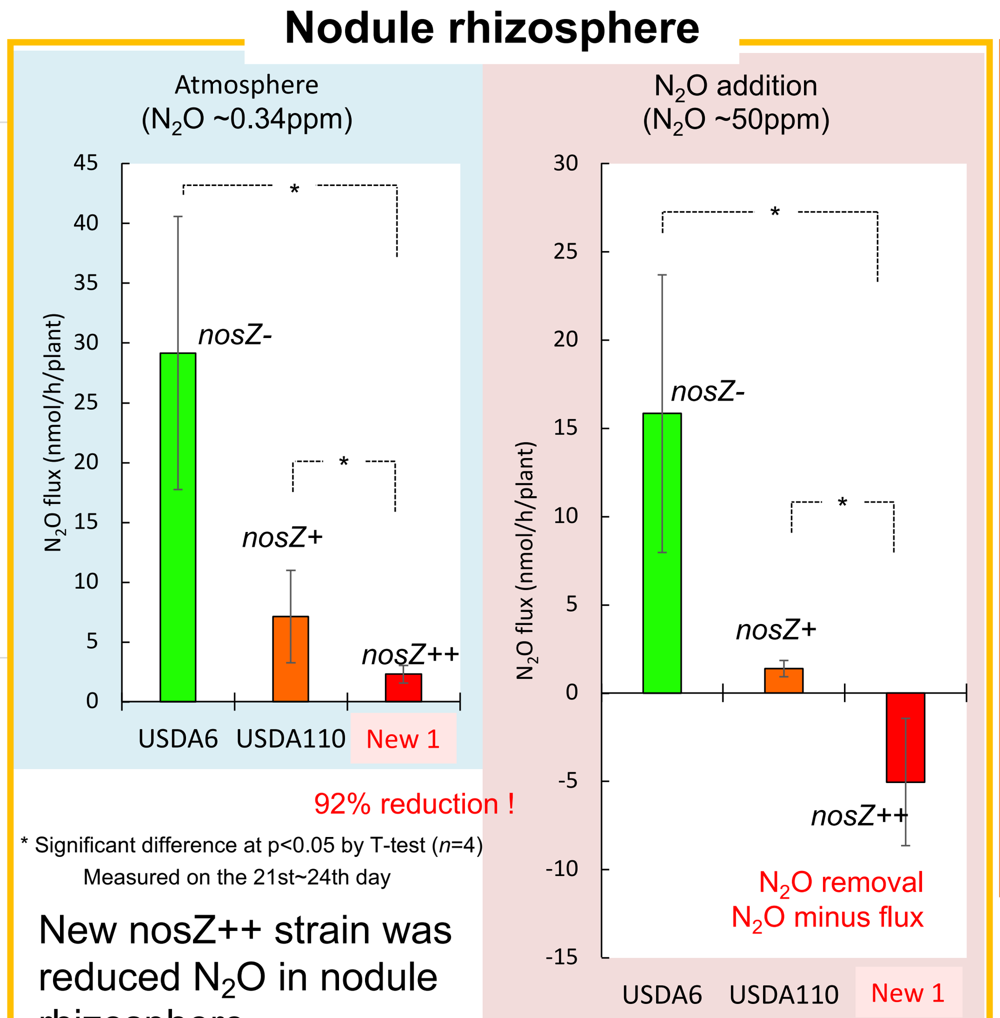


Converted to gas phase concentration

Strain	*n*	Km (ppm N2O)	Vmax (nmol N2O /h/10(9)cell)
USDA110	8	117 ± 31.5	22 ± 10
New strain1	5	103 ± 17.4	239 ± 110

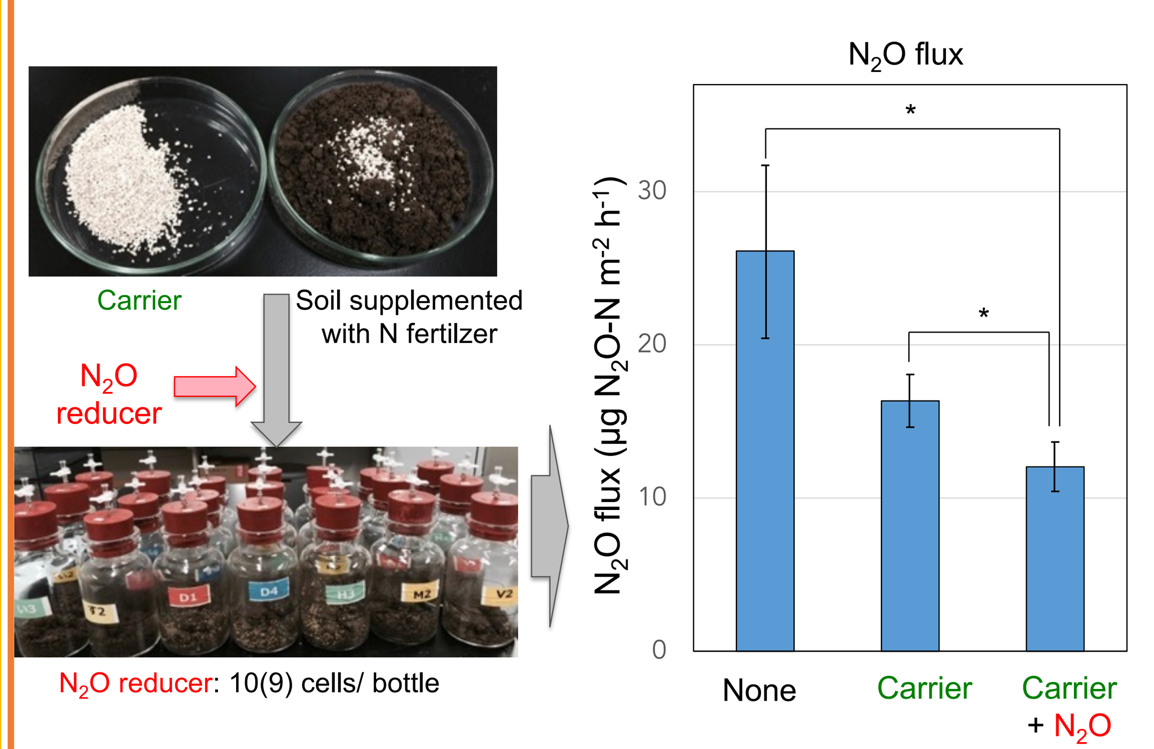
N_2O reduction activity of the new strain is 10 times higher than USDA110

N_2O reduction in nodule rhizosphere and soil



* Significant difference at p<0.05 by T-test (*n*=4)
Measured on the 21st–24th day

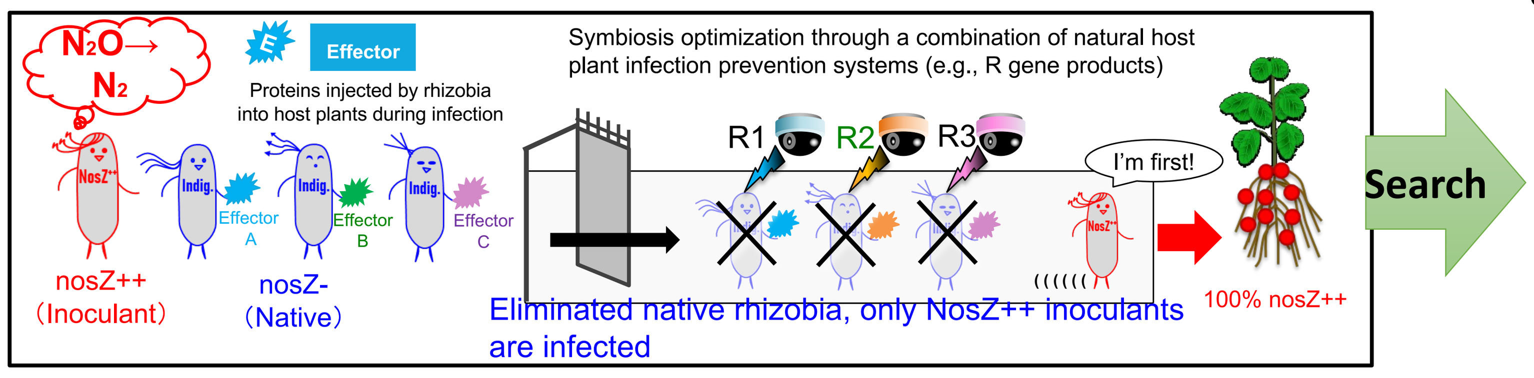
New nosZ++ strain was reduced N_2O in nodule rhizosphere



N_2O-reducing microbe mitigated soil N_2O flux

Optimization of symbiosis of *nosZ*++ *Bradyrhizobium* strain

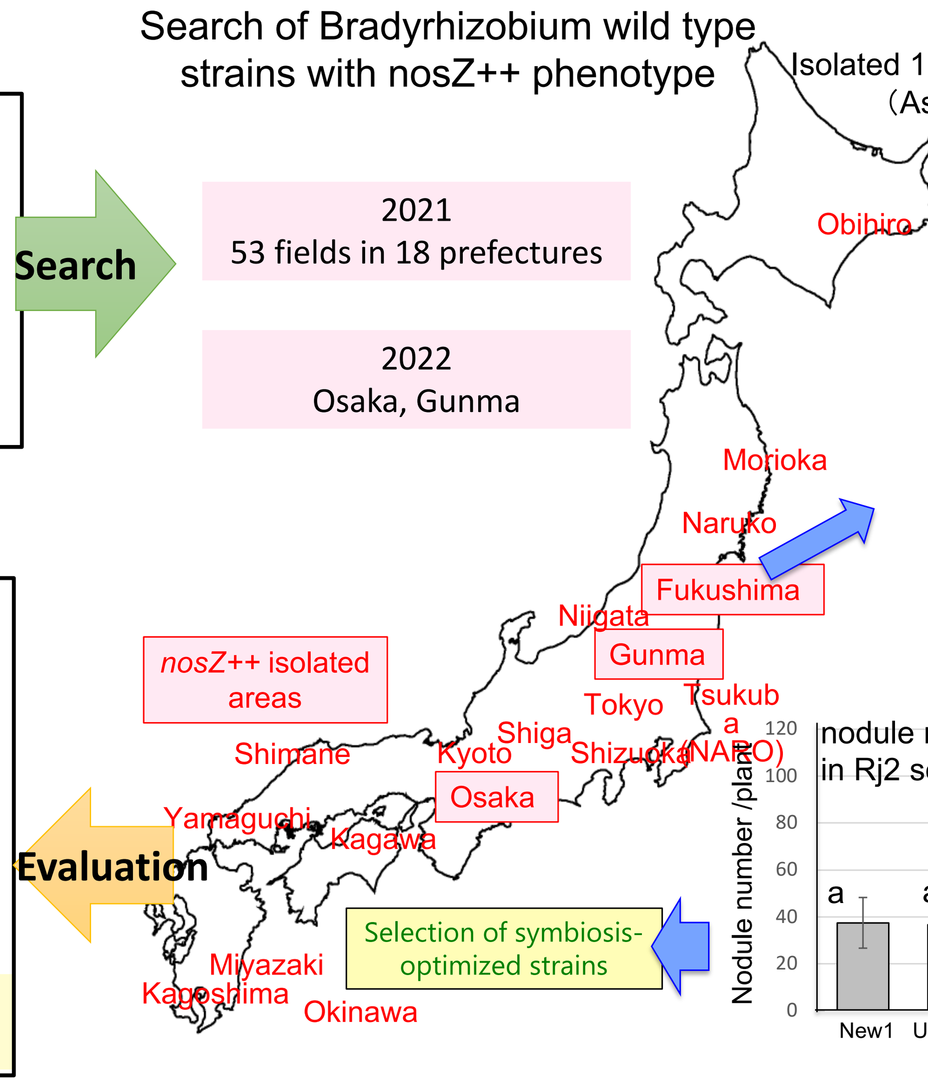
Symbiosis optimization strategy with rhizobial effectors



Search of Bradyrhizobium wild type strains with nosZ++ phenotype

2021
53 fields in 18 prefectures

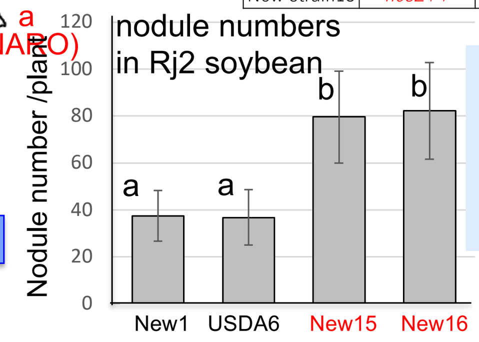
2022
Osaka, Gunma



Isolated 18 new strains with nosZ++ phenotype (As of Novembe 2022 78 strains)

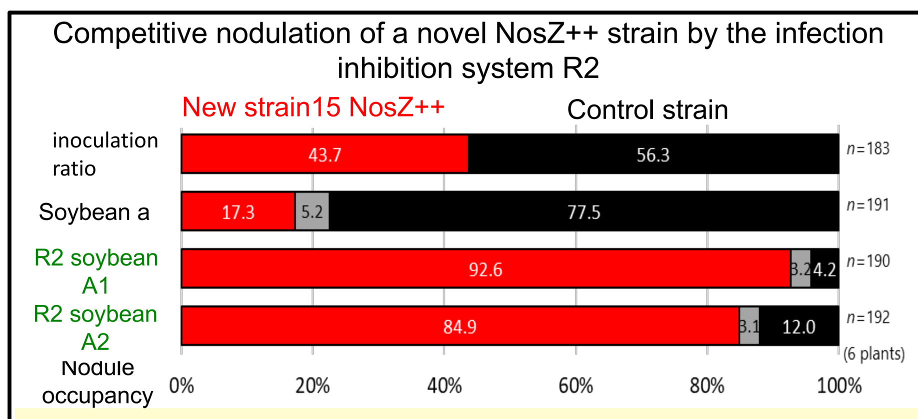
Strains	*nosZ* phenotype	Effector type
USDA 110T	*nosZ*+	None
New strain1	*nosZ*++	None
New strain2	*nosZ*++	None
New strain3	*nosZ*++	None
New strain4	*nosZ*++	None
New strain5	*nosZ*++	None
New strain6	*nosZ*++	None
New strain7	*nosZ*++	None
New strain8	*nosZ*++	None
New strain9	*nosZ*++	None
New strain10	*nosZ*++	None
New strain11	*nosZ*++	None
New strain12	*nosZ*++	None
New strain13	*nosZ*++	None
New strain14	*nosZ*++	None
New strain15	*nosZ*++	B
New strain16	*nosZ*++	B
New strain17	*nosZ*++	None
New strain18	*nosZ*++	None

nodule numbers in Rj2 soybean

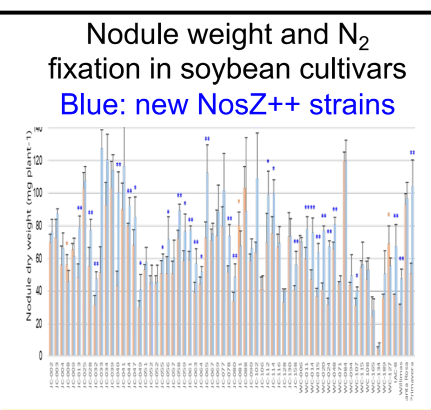


Effector type B strains were increased nodules on R2 soybean hosts!

Evaluation of competitiveness and nitrogen fixation in new strain

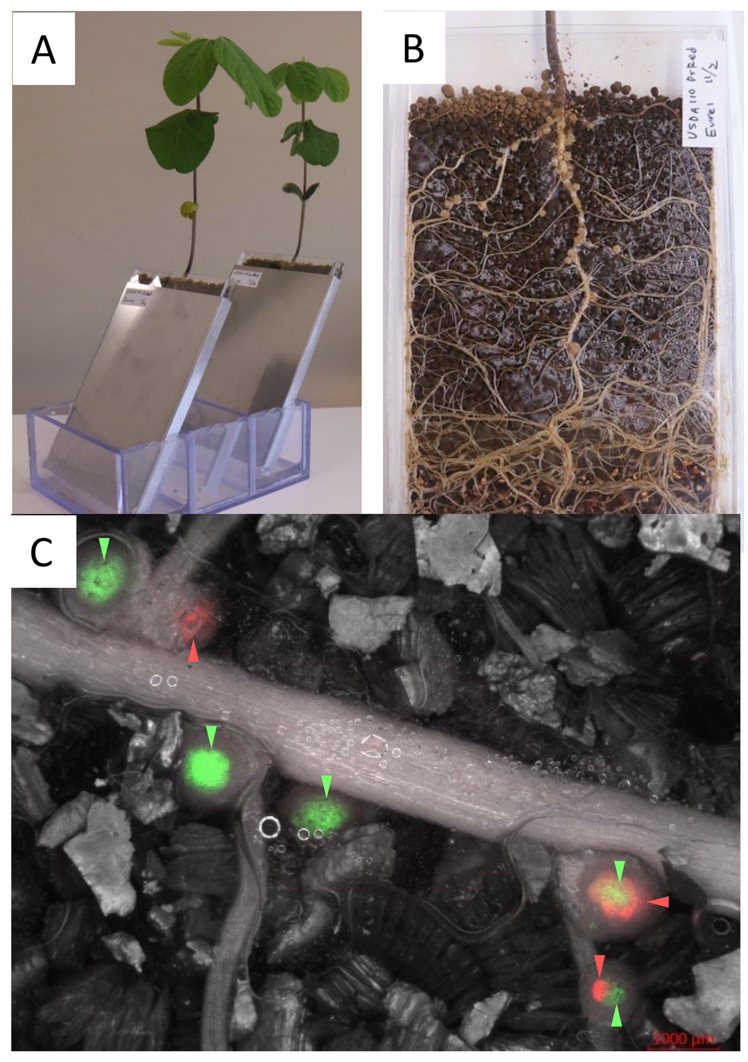


Successful dominance of newly isolated NosZ++ strains even with only R2 system of host soybean! (>80%)



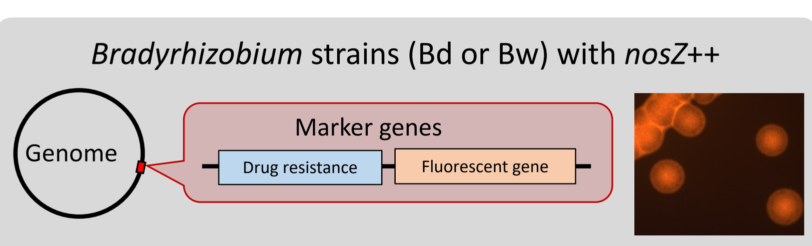
Many cultivars showed high nodule weight and N_2 fixation with new nosZ++ strain inoculation

Non-destructive observation system of rhizobia infection



(A) Rhizosphere frame soybean cultivation equipment.
(B) Rhizobia-inoculated soybean roots through acrylic plate.
(C) Competitive inoculation of red and green fluorescent labelled rhizobia. AxioZoom microscopy allows non-destructive observation of infection competition of rhizobia.

Assessments of effect of microbial inoculation to indigenous community in soil

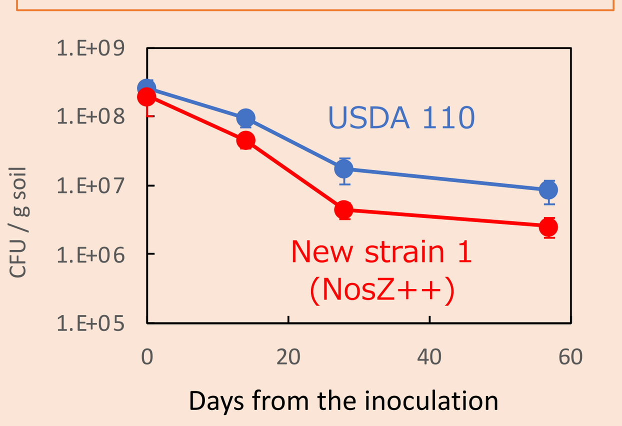


Inoculation (10^8 cell/g) to Andisol

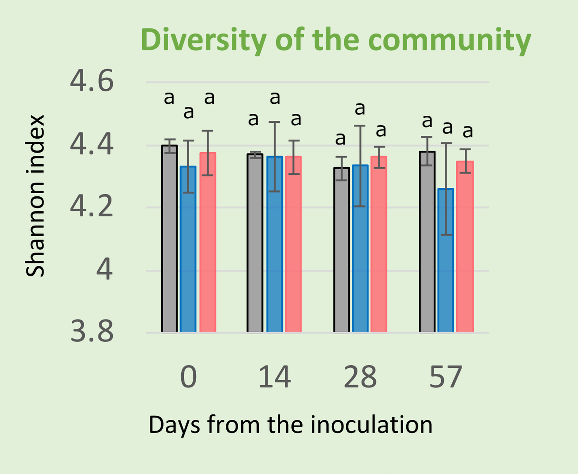
Soil microcosm

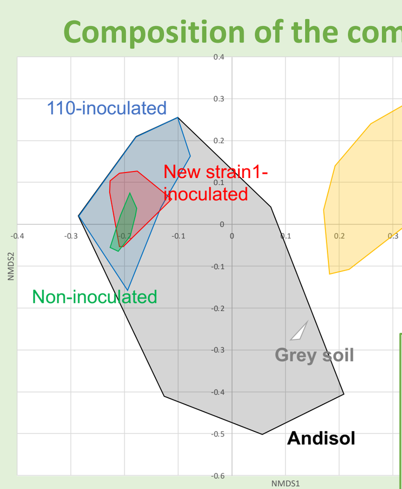
Survivability in the soil microcosm

For at least two months, the new strain 1 could survive at $10^{6\sim7}$ CFU/g



Metagenomic assessments of microbial inoculation





In the microcosm, the microbial inoculant did not drastically change the indigenous community.

Microbial inoculation of wild-type strains to the experimental field

Effect of the microbial inoculant was smaller than land usage.

Citizen Science Project

No. A-8-3E

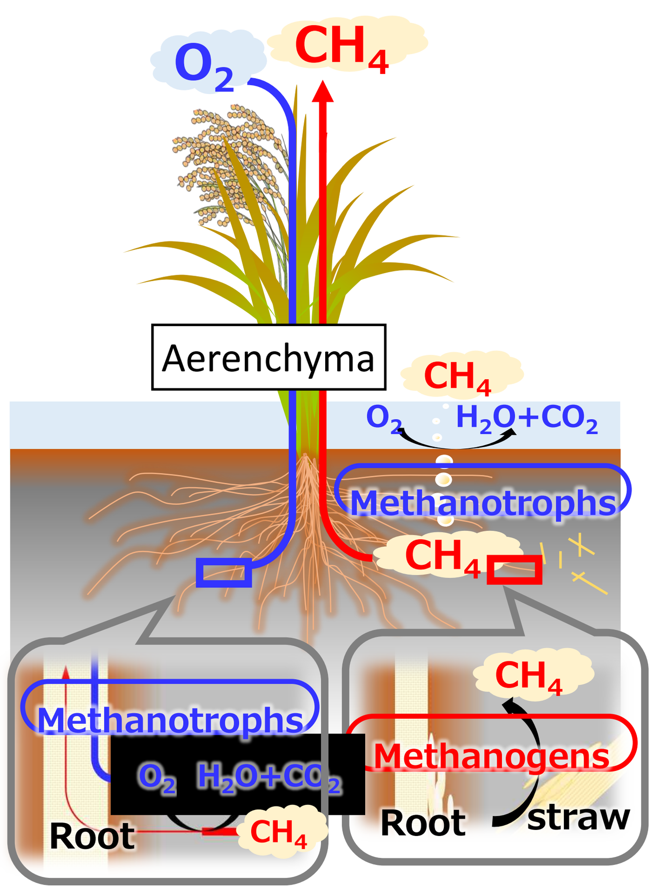
PJ : Mitigation of Greenhouse Gas Emissions from Agricultural Lands by Optimizing Nitrogen and Carbon Cycles

Theme: Development of new nitrification and denitrification inhibitors to reduce N_2O emissions and New rice varieties to reduce CH_4 emissions

Organization: NARO (National Agriculture and Food Research Organization)
Contact: Hiroko AKIYAMA (ahiroko@affrc.go.jp)

Breeding new rice varieties with low CH_4 emissions

Background



CH_4 emissions form paddy rice fields : 10% of anthropogenic CH_4 emissions

- **CH_4 production** (light)
  - Anaerobic decomposition of organic material by methanogens
- **CH_4 oxidation** (left)
  - rhizosphere of rice roots, soil-floodwater interface by methanotrophs
- **Mitigation options developed by NARO:**
  - Water management (prolonged mid-season drainage)
  - Straw management (incorporation after harvest, instead of before planting)

Research strategies

Breeding new rice varieties with low CH4 emission

✓ Breeding low CH_4 rice

・Using genetic resource of rice of NARO, we will screen low CH_4 rice varieties and breed new commercial rice varieties with low CH4 emission

✓ Use of methanotrophic N_2 fixing bacteria (Tohoku Univ., Nagoya Univ.)

Development of high throughput CH4 flux measurement → Screening of low CH_4 rice varieties to breed new rice varieties

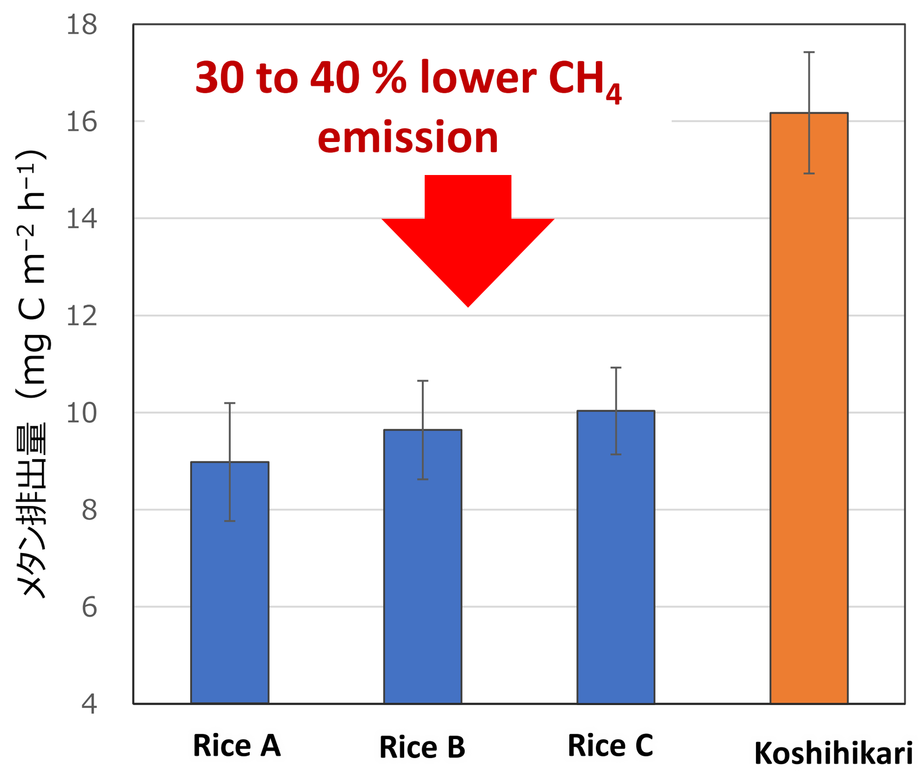
Genetic resource of rice (NARO) → Screening of low CH_4 rice varieties to breed new rice varieties

→ **Breeding new commercial rice varieties with low CH_4 emission**

Major results

High throughput CH_4 flux measurement

Tokida 2021, Kajiura & Tokida, 2021, 2022



☐Development of high throughput CH_4 flux measurement by using mobile CH_4 analyzer (Picarro G4301)

・GC method: 45min, New method: 15min (1/3)

☐Screening of genetic resource of rice varieties with low CH_4 emission

・We found low CH_4 emission varieties (genetic resources) by 30 to 40% compered to Koshihikari (a major Japanese variety).

Selection of low CH_4 varieties (genetic resources)

→Breeding new commercial rice varieties with low CH_4 emissions by using genes controlling CH_4 emission

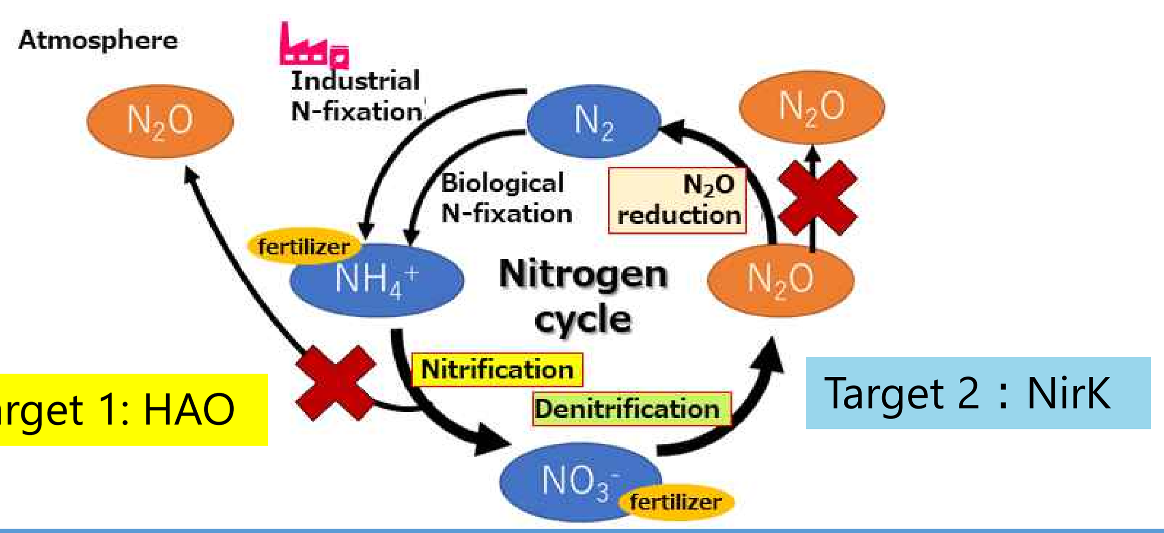
Development of new inhibitors to mitigate N_2O emissions

Background

✓**About 60% of anthropogenic N_2O emit from agriculture**

✓N_2O production process: nitrification & denitrification

✓Develop new nitrification and denitrification inhibitors to reduce N_2O emission



Research strategies

✓*in silico* **screening and metagenomic analysis for structure-based drag design**

Target 1：HAO

1. High throughput screening

1st Enzyme assay → 2nd False discovery → 3rd Bacterial viability assay → Lead optimization → **Lead compounds**

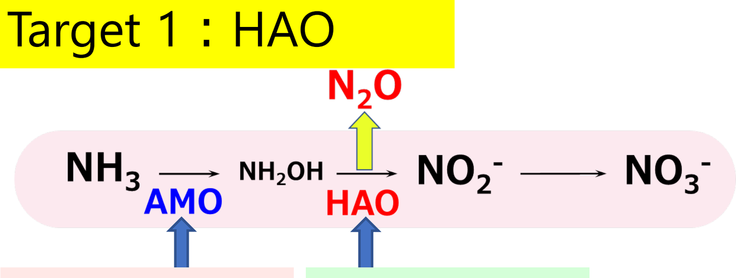
Target 2：NirK

2. *In silico* screening

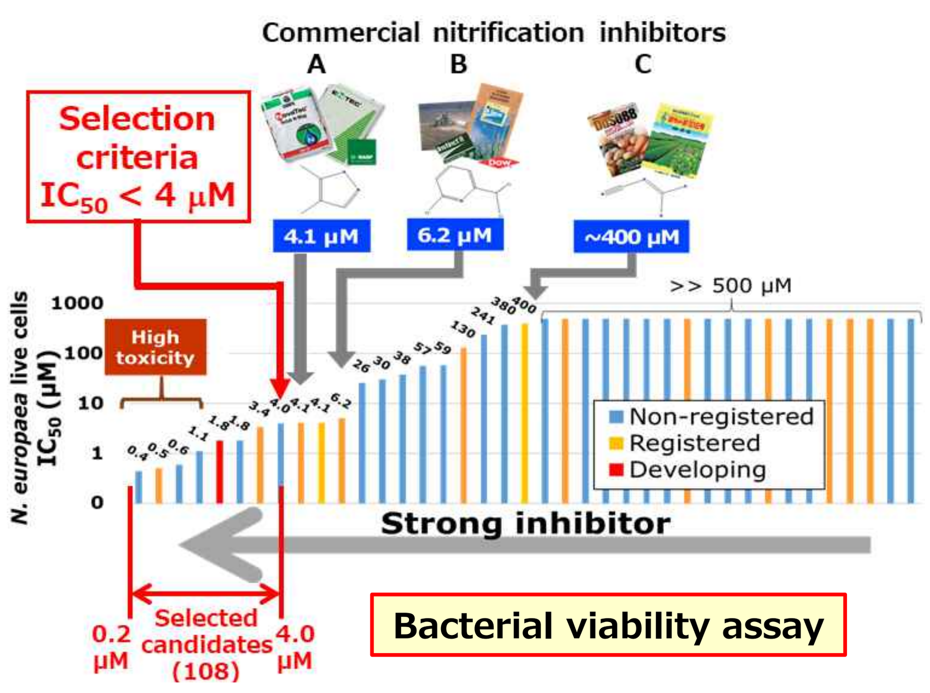
Commercial compounds (~8 million) → Pharmacophore modeling → *In silico* screening → Hit selection → **In vitro assay**

Major results

Target 1：HAO



✓ We obtained **108 HAO-targeted nitrification inhibitor candidates** which have **much higher activities (IC_{50} < 4.0 μM)** than commercially available inhibitors.



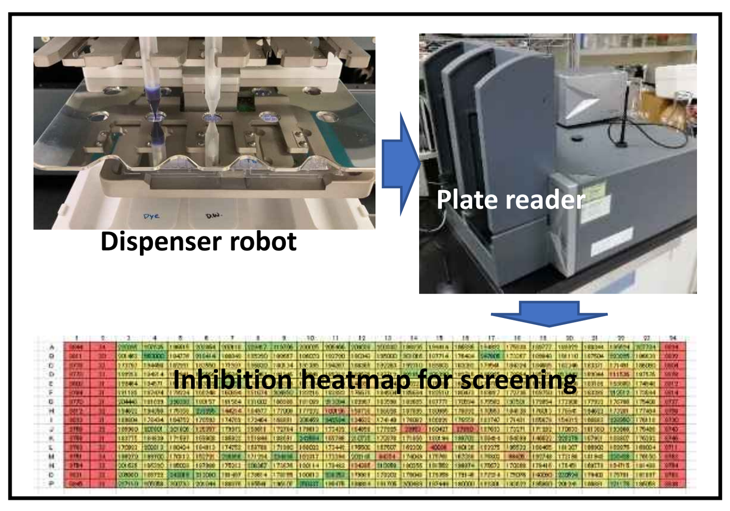
Target 2：NirK

No commercial denitrification inhibitor was developed so far.

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

NirK

✓ A high throughput screening provided **100 Nirk inhibitors (IC_{50} < 10 μM)** out of about ten thousand compounds.



High throughput screening

Obtained 108 HAO-targeted nitrification inhibitor candidates and 100 Nirk inhibitor candidates

NARO 農研機構

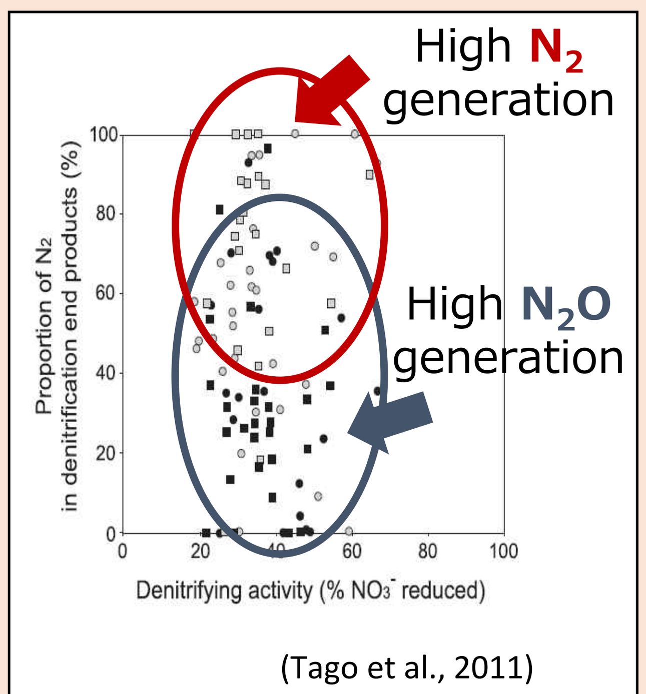
No. A-8-4E

PJ : Mitigation of greenhouse gas emissions from agricultural lands by optimizing nitrogen and carbon cycles

Theme: N_2O recycling by N_2O-fixing bacteria

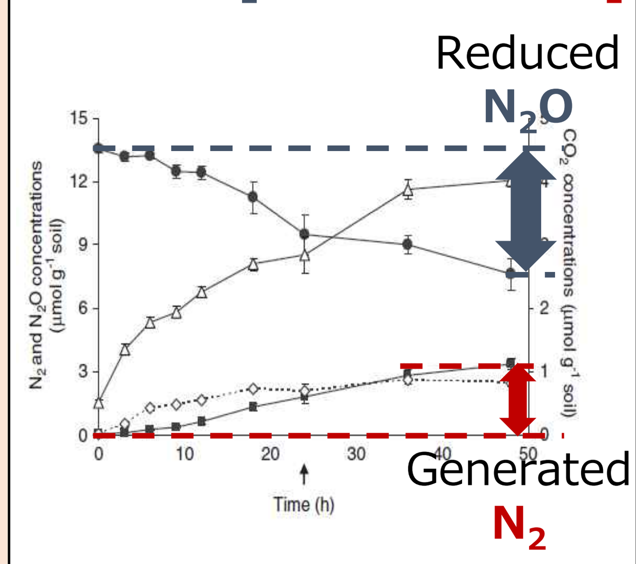
Organization: The Univ. of Tokyo, National Institute of Advanced Industrial Science and Technology
Contact: Keishi SENOO（E-mail: asenoo@g.ecc.u-tokyo.ac.jp）

Introduction



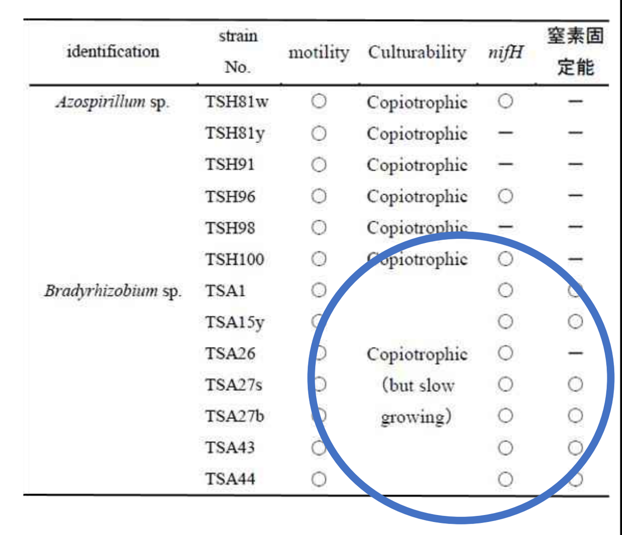
(Tago et al., 2011)

N_2/N_2O generation ratios of denitrifers are diverse

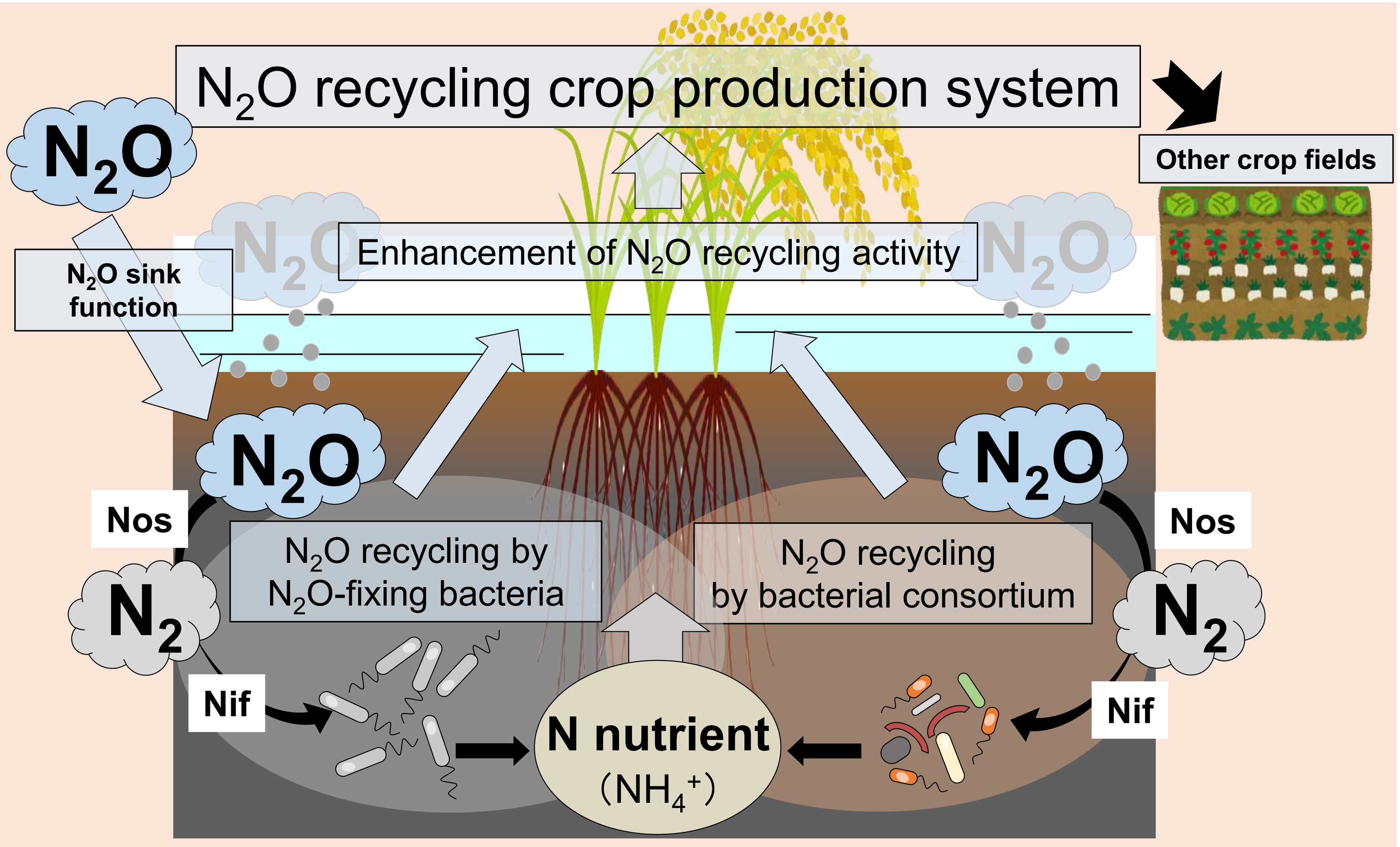


(Ishii et al., 2011)

60% of the reduced N_2O is missing



Denitrifying bacteria with N-fixing ability



Background: Generated N_2O might be fixed by bacteria in paddy soil

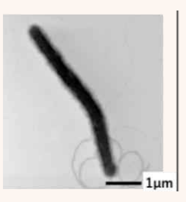
Purpose: Microbial transformation of N_2O to NH_4^+ for crop production

I. N_2O fixing bacteria in paddy soils

Bacteria with N_2O-fixing potential

① Iron-reducing diazotrophs harboring *nosZ*

Anaeromyxobacter sp.



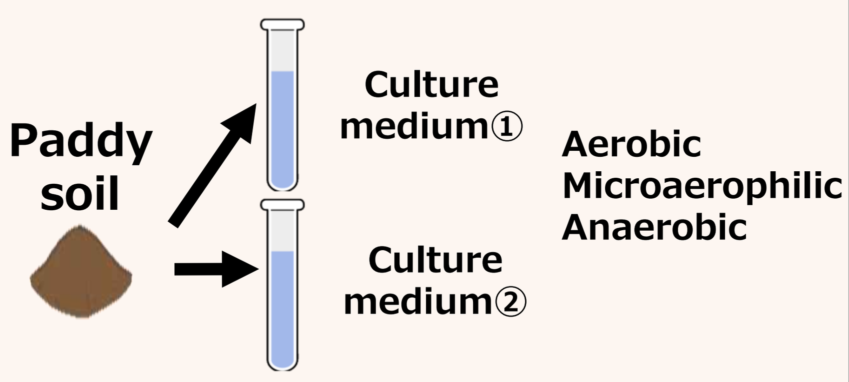
- Predominant in paddy soils
- *nos* and *nif* transcripts derived from this genus were frequently detected in paddy soils

② Denitrifier harboring *nif* genes

Bradyrhizobium sp.

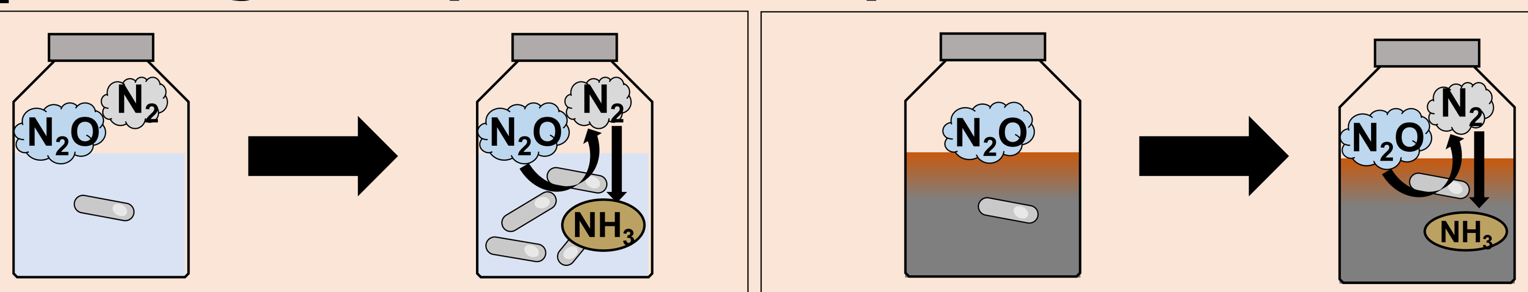
- denitrifier with high-N_2OR activity
- harbour *nif* genes

③ Bacterial consortia grown under N_2O as a single N source



Enrichment culture + amplicon sequencing

①N_2O-fixing ability of *Anaeromyxobacter*



- survived but not multiplied where N_2O was the sole nitrogen source
- In the presence of N_2O and small amounts of N_2, growth was stable and N_2O was reduced
- *nosZ* and *nifD* transcripts were detected

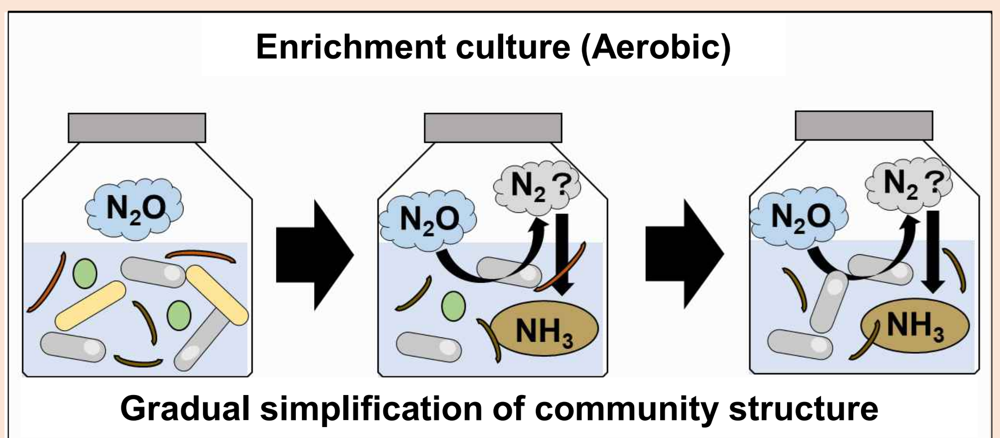
- Inoculation of paddy soil microcosms (sterilized soil) with *Anaeromyxobacter* spp.
- Confirmation of their growth using copy numbers of 16S rRNA and transcriptions of *nosZ* and *nifD*

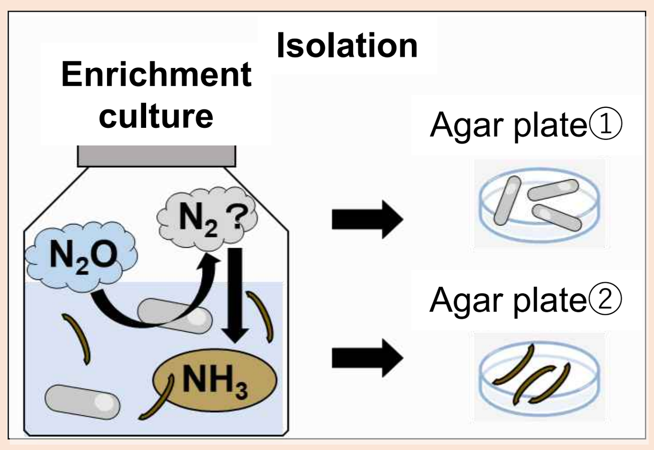
② N_2O-fixing ability of *Bradyrhizobium*

- In the presence of N_2O and small amounts of N_2, growth was stable
- Confirmed their growth using copy numbers of 16S rRNA and transcriptions of *nosZ* and *nifD*→ ^{15}N content of the soils before and after incubation were currently analyzed using IRMS

***Anaeromyxobacter* sp. and *Bradyrhizobium* sp. can fix N_2O in the presence of small amounts of N_2**

③Microbial consortia grown under N_2O as a sole N source



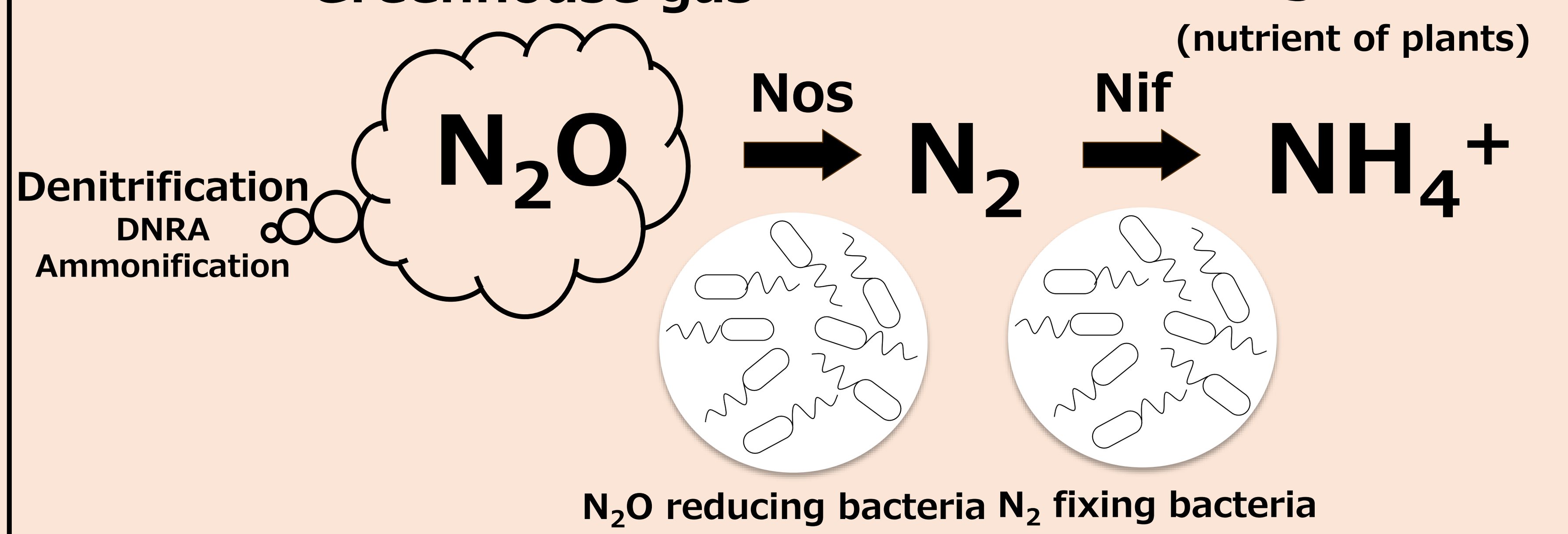


- Obtained the microbial consortia grown under N_2O as a sole N source
- Detected the reduction of N_2O gas
- ^{15}N content of the soils were currently analyzed using IRMS

Future plan

- Quantification of fixed N_2O
- Analysis of factors that enhance N_2O fixing activity

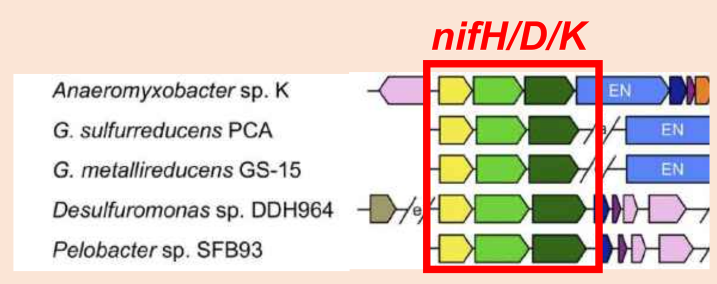
II. N_2O fixing consortia in paddy soils



▶Development of analytical strategies for soil metagenomics.
▶ Identify N_2O-reducing and nitrogen-fixing microbial communities.

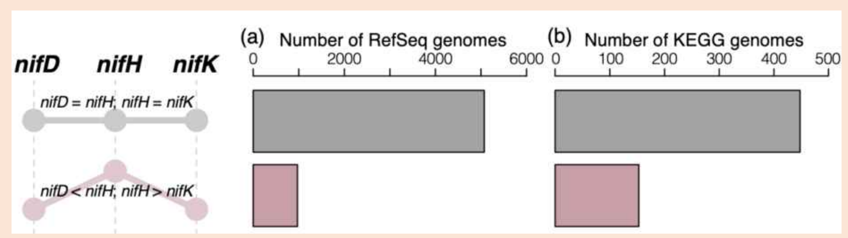
▶Previous methods: problems and improvements

■ Gene components of nitrogenase



nifH = nifD, nifK ??

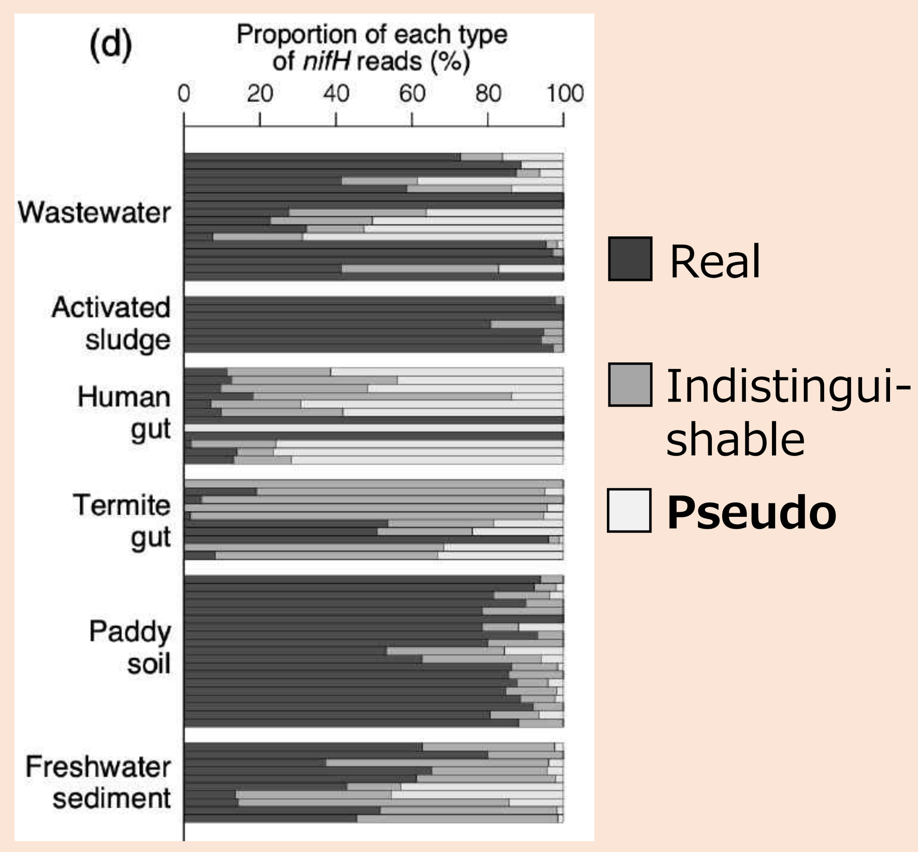
■ In the genome database



nifH > nifD, nifK !?

▶Many pseudo-nifH are registered.

■Impact on analysis results

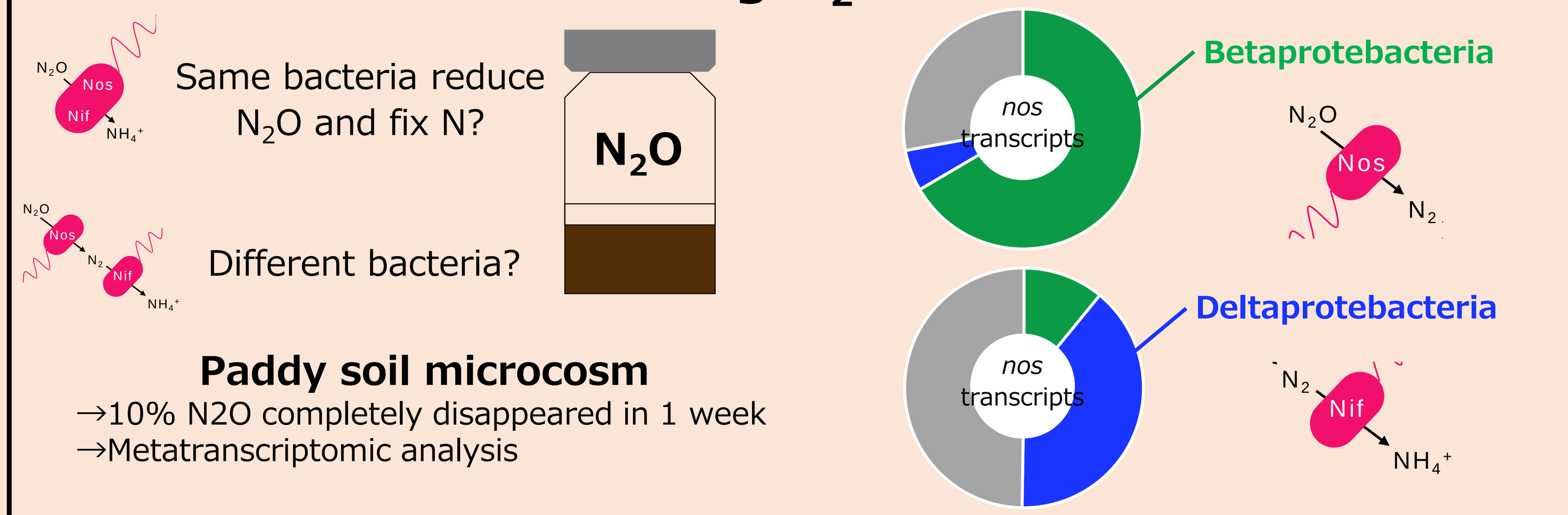


Unreliable results

We developed a highly accurate analysis method that excludes *nifH*

Mise et al., mSphere, 2021

▶Microbial consortia using N_2O as a sole N source



Paddy soil microcosm
→10% N2O completely disappeared in 1 week
→Metatranscriptomic analysis

Is N_2O fixation driven by the different groups of microorganisms?

Future plan

- Analysis of paddy soil microcosm using $^{15}N_2O$
- Verification of ubiquity by analysis using paddy soils from various regions