No. A-12-1E

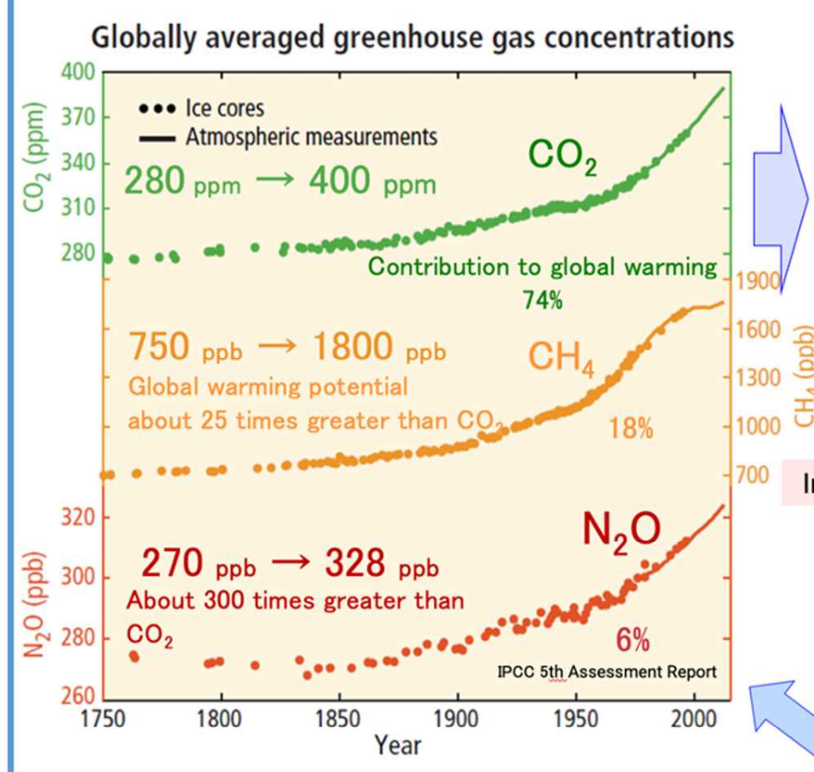
PJ : Mitigation of greenhouse gas emissions from agricultural lands by optimizing Nitrogen and carbon cycles

Theme: Cool Earth via Microbes in Agriculture

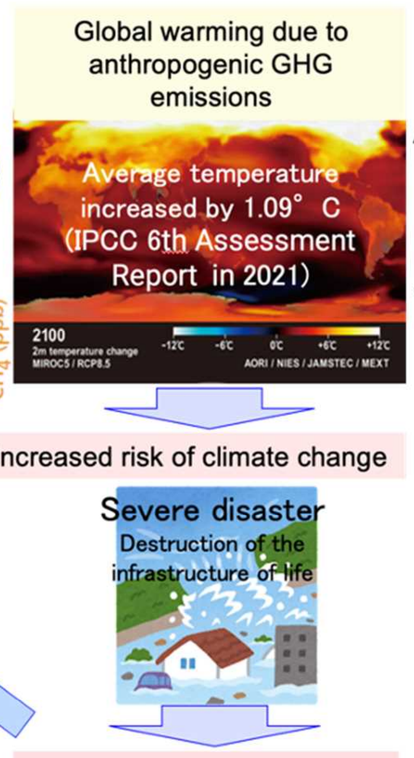
Organization: : Tohoku University, NARO.

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

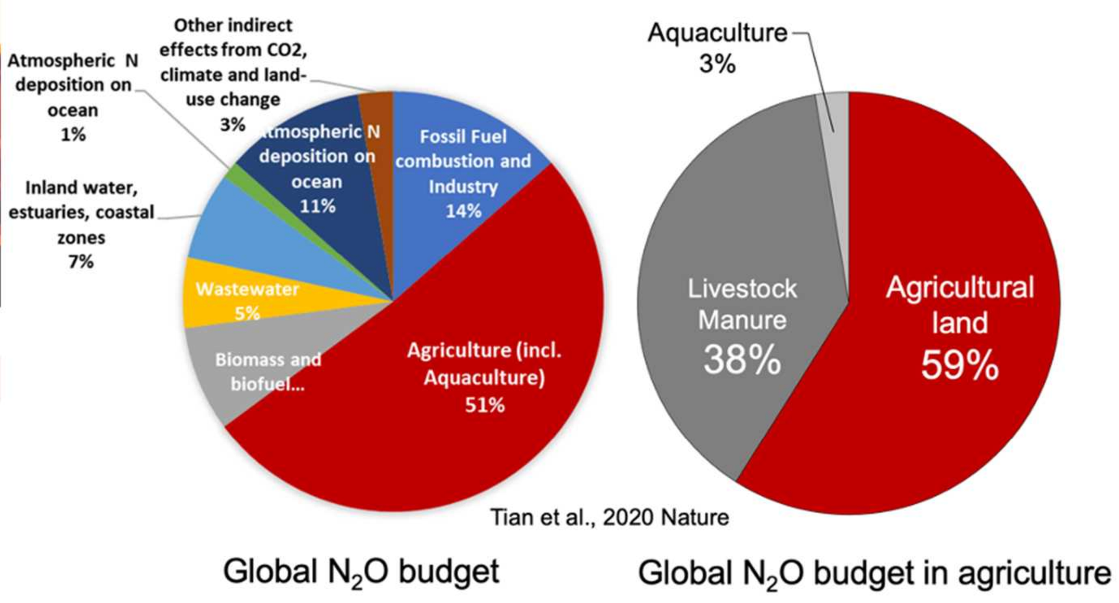
Background



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)

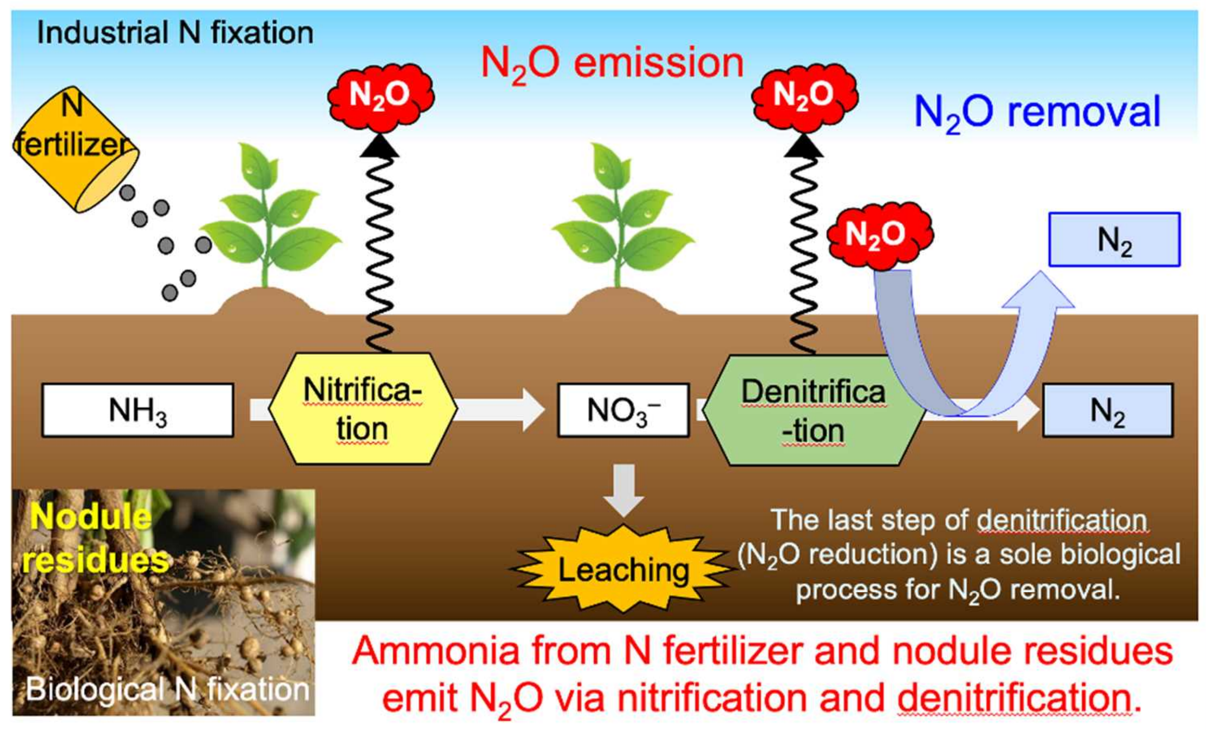


Major Anthropogenic Source of N_2O



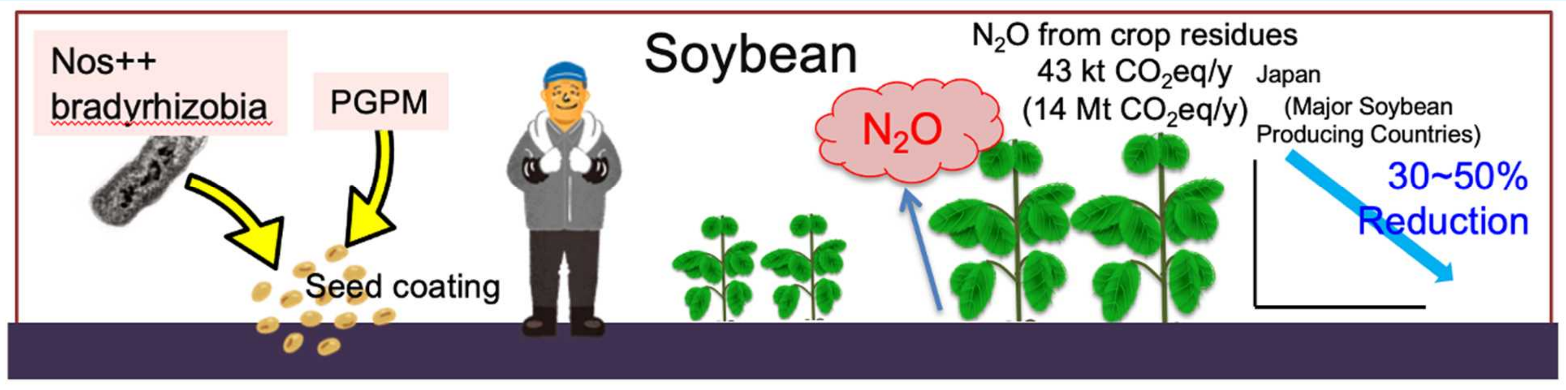
Agriculture accounts for more than 50% of anthropogenic sources of N_2O, with about 60% originating from agricultural lands.

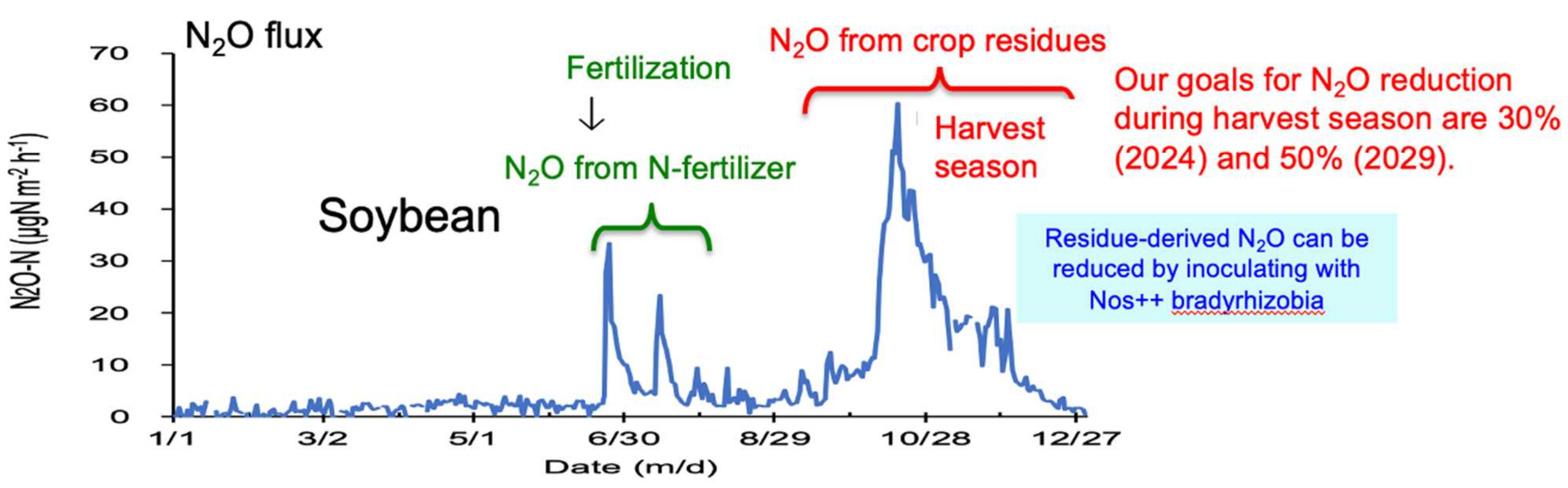
N transformation in agricultural lands by microorganisms



Ammonia from N fertilizer and nodule residues emit N_2O via nitrification and denitrification.

Soybean bradyrhizobia



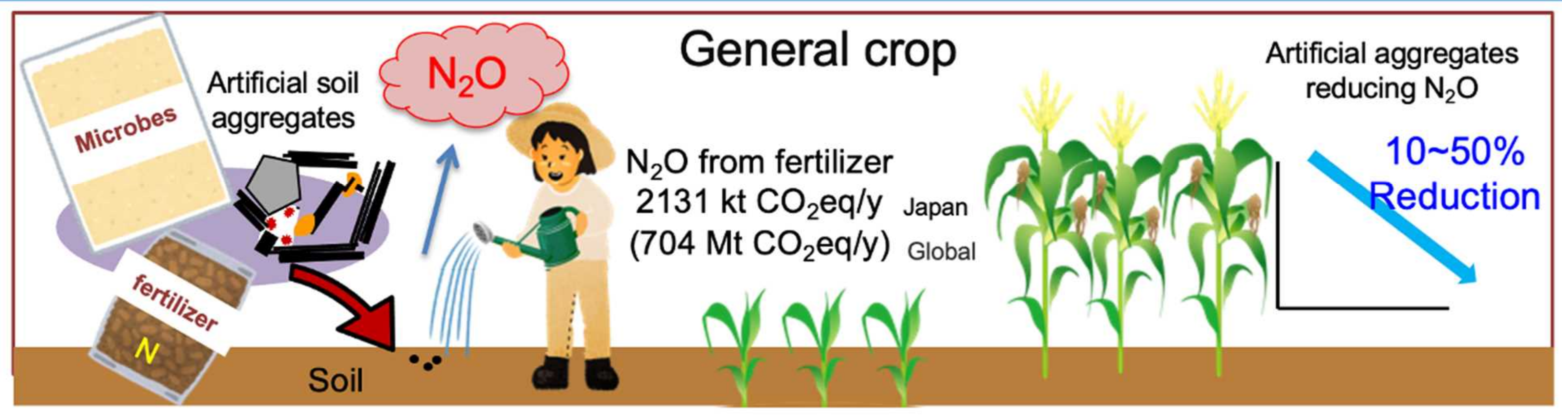


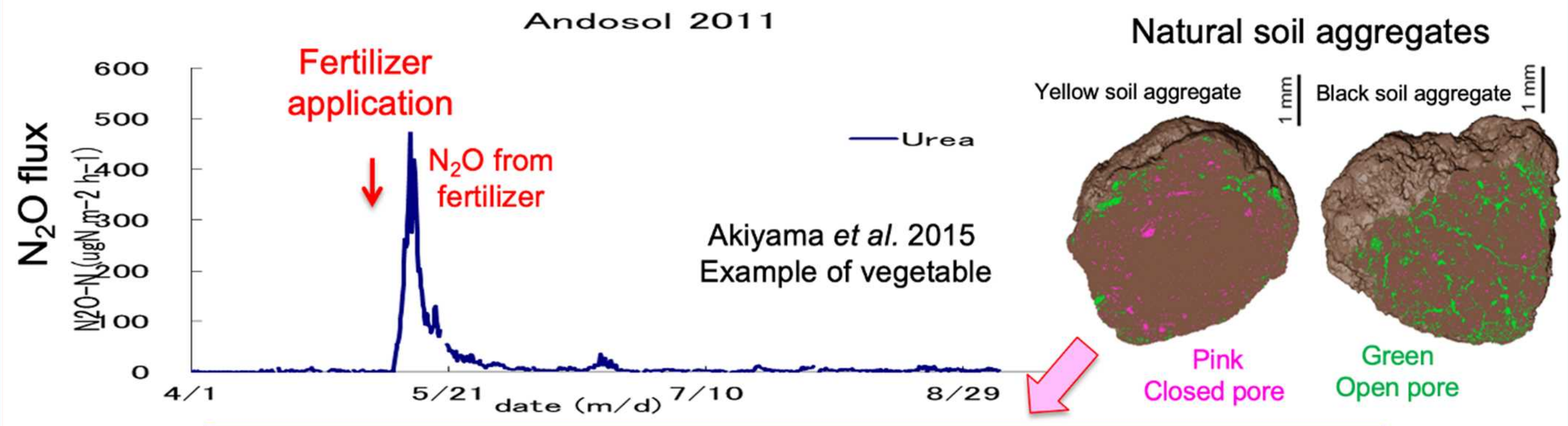
R&D policy for bradyhizobia inoculants

- Excellent inoculants for N_2O removal ⇨ Reduction of N_2O during harvest season
- Co-inoculation of PGPM* ⇨ Yield Increase (Benefit for farmers)

PGPM*: Plant Growth-Promoting Microbes

Artificial soil aggregates



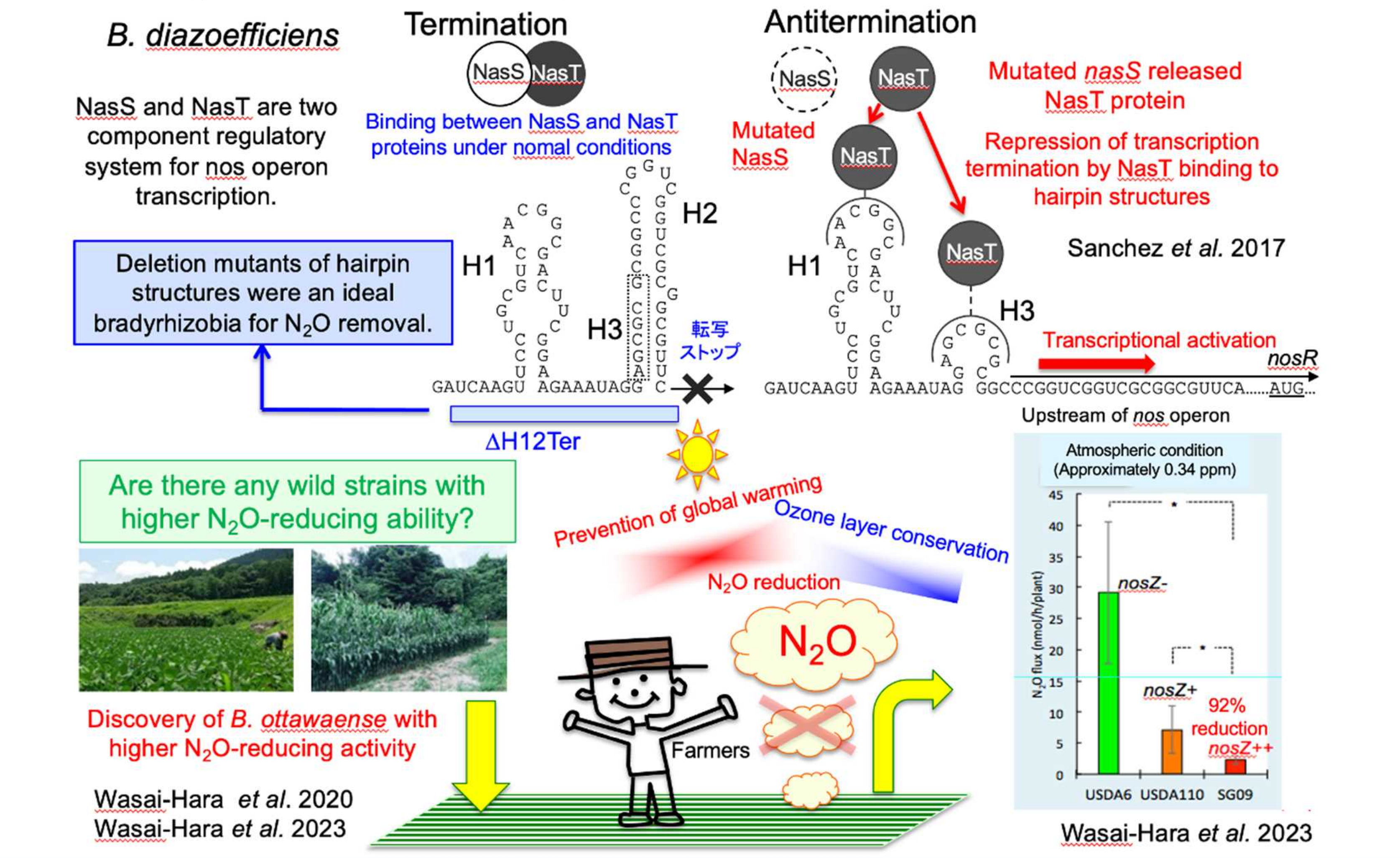


Properties of artificial soil aggregates required for N_2O reduction

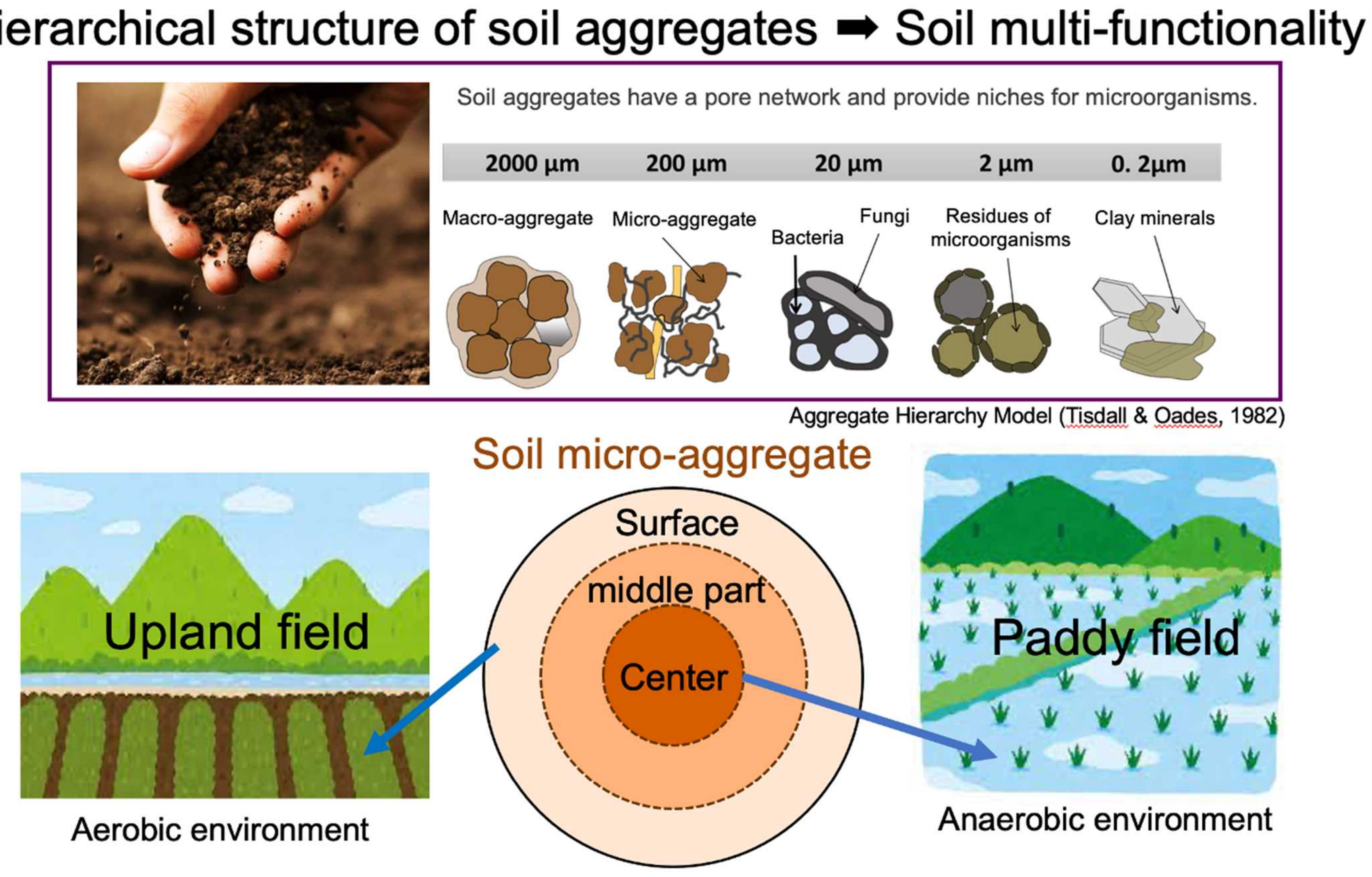
Index	
• Pore（Pore size, pore distribution）	• Redox (hypoxia)
• Clay content	• Structural stability
• Electron donor (energy source)	• Duation of N_2O reduction

Bradyrhizobia with high activities of N2O removal

→A-12-2J



Hierarchical structure of soil aggregates ➡ Soil multi-functionality



The center of the aggregate is an anaerobic environment, while the surface is an oxidative environment. Thus, the segregation of microorganisms and differentiation of soil functions are important for reducing N_2O.

Production and form of N_2O-reducing bradyrhizobial inoculants

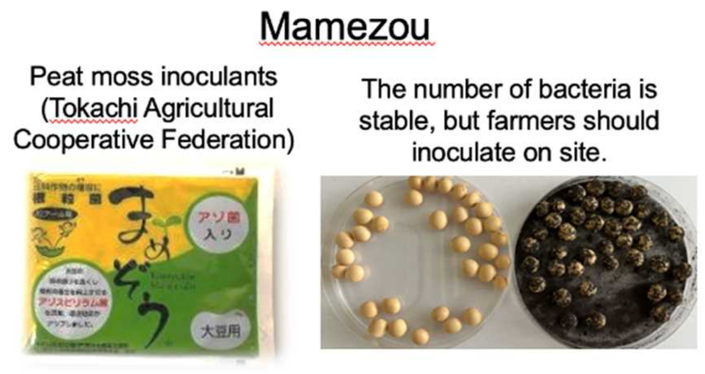
■Production and evaluation of bradyrhizobial inoculant prototypes

Investigation of characteristics and quality during mass culture → Preservability and quality after prototype production

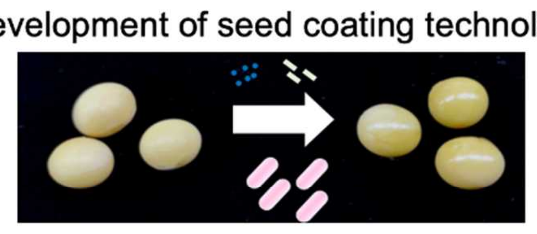
Manufacturing and evaluation of a prototype material for a new N_2O-eliminating bradyrhizobial strain

■Form of bradyrhizobial inoculant

Mamezou



Development of seed coating technology



Farmers can save labor, but the bacterial viability reduced rapidly.

Microbial materials strategy

→A-12-3J

Objectives: Elucidation of the microbial stabilization mechanism in soil and development of microbial materials for removing fertilizer-derived N_2O

RAW MATERIALS & APPROACH (Artificial soil aggregates)	RAW MATERIALS & APPROACH (Artificial carriers)
• Natural minerals	• Porous material
• Inoculation of microbial communities or a strain	• Inoculation of microbial communities

No. A-12-2E

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

Theme: Isolation and evaluation of N_2O removal microorganisms

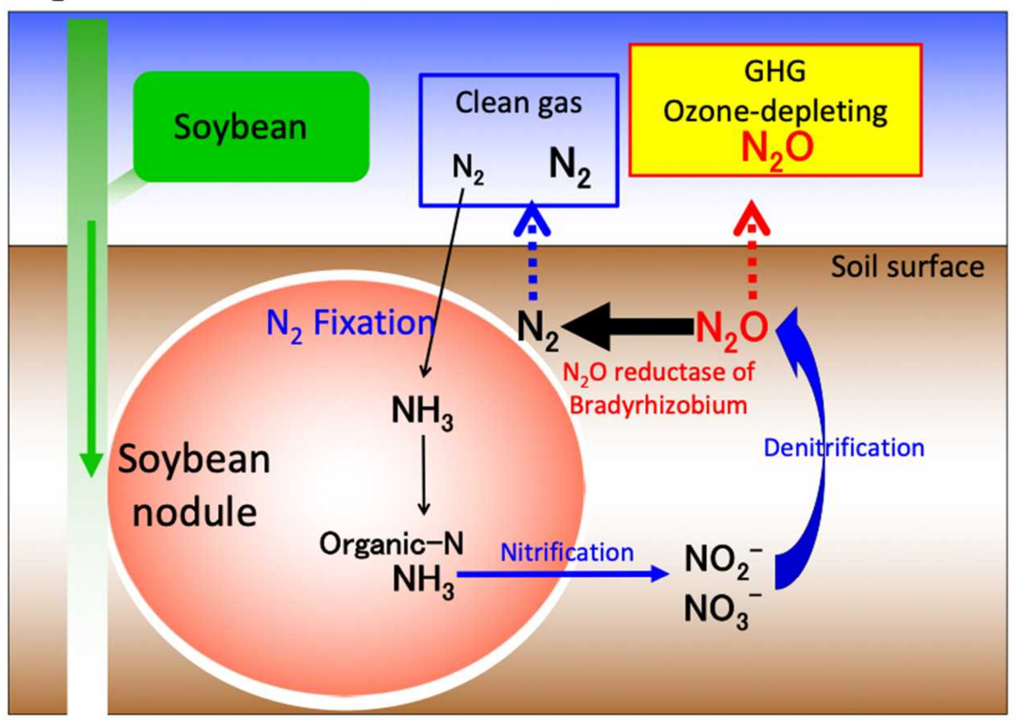
Organization: Tohoku University

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

Soybean bradyrhizobia

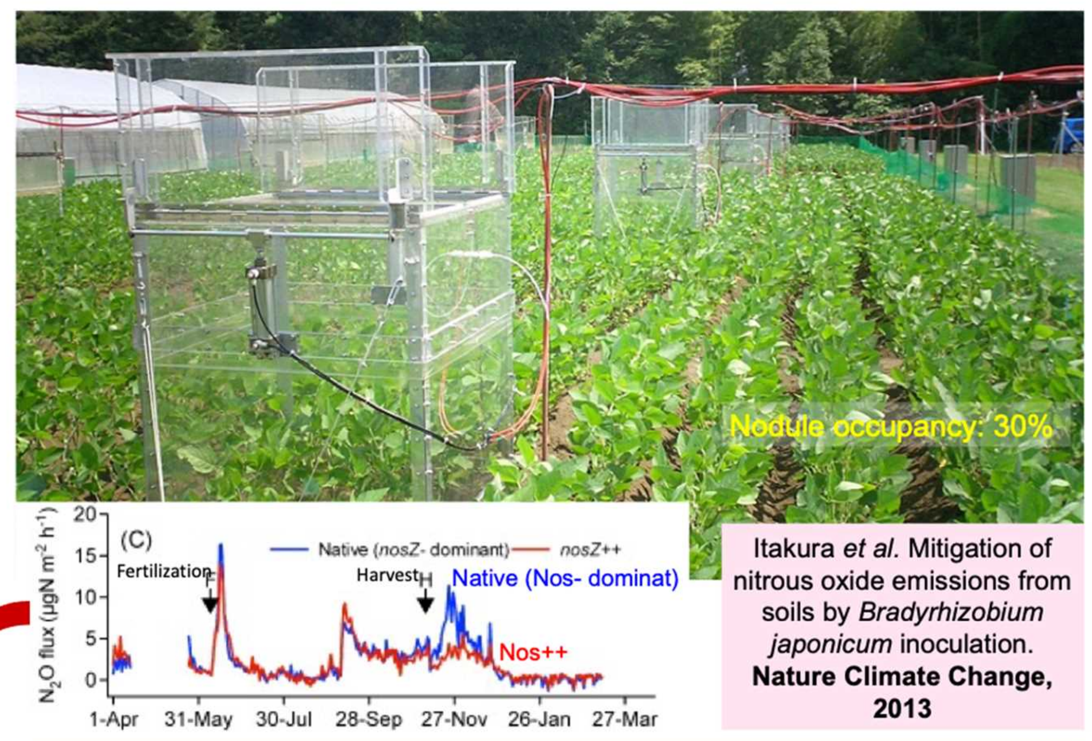
Background: N_2O mitigation using the N_2O-reducing ability of soybean bradyrhizobia

N_2O is generated in degraded nodule, however N_2O is reduced by N_2O reductase of soybean bradyrhizobia



Nitrogen cycle in the soybean rhizosphere

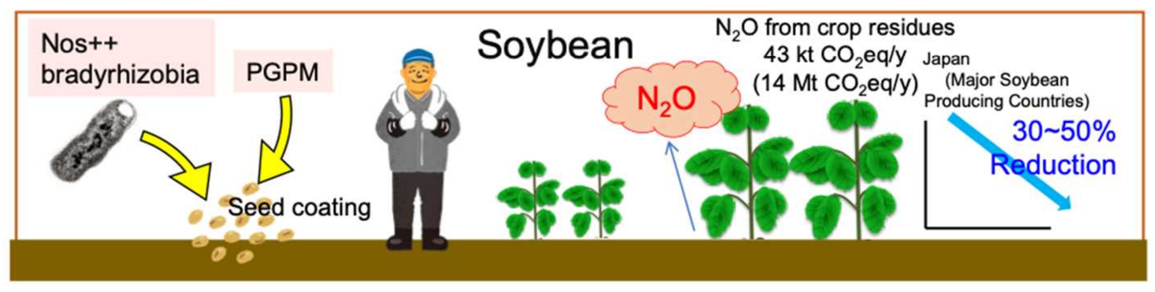
N_2O mitigation by Nos++ *Bradyrhizobium* inoculation in field level

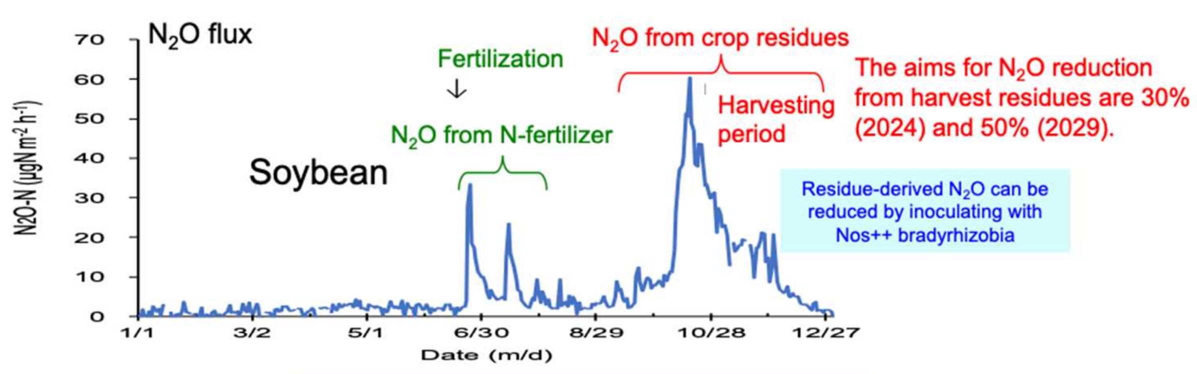


Itakura *et al.* Mitigation of nitrous oxide emissions from soils by *Bradyrhizobium japonicum* inoculation. **Nature Climate Change, 2013**

Search and isolation of soybean bradyrhizobia wild type strains with higher N_2O reduction activity

Aims: Development and dissemination of N_2O-reducing inoculant





R&D Policy for Rhizobium Inoculants

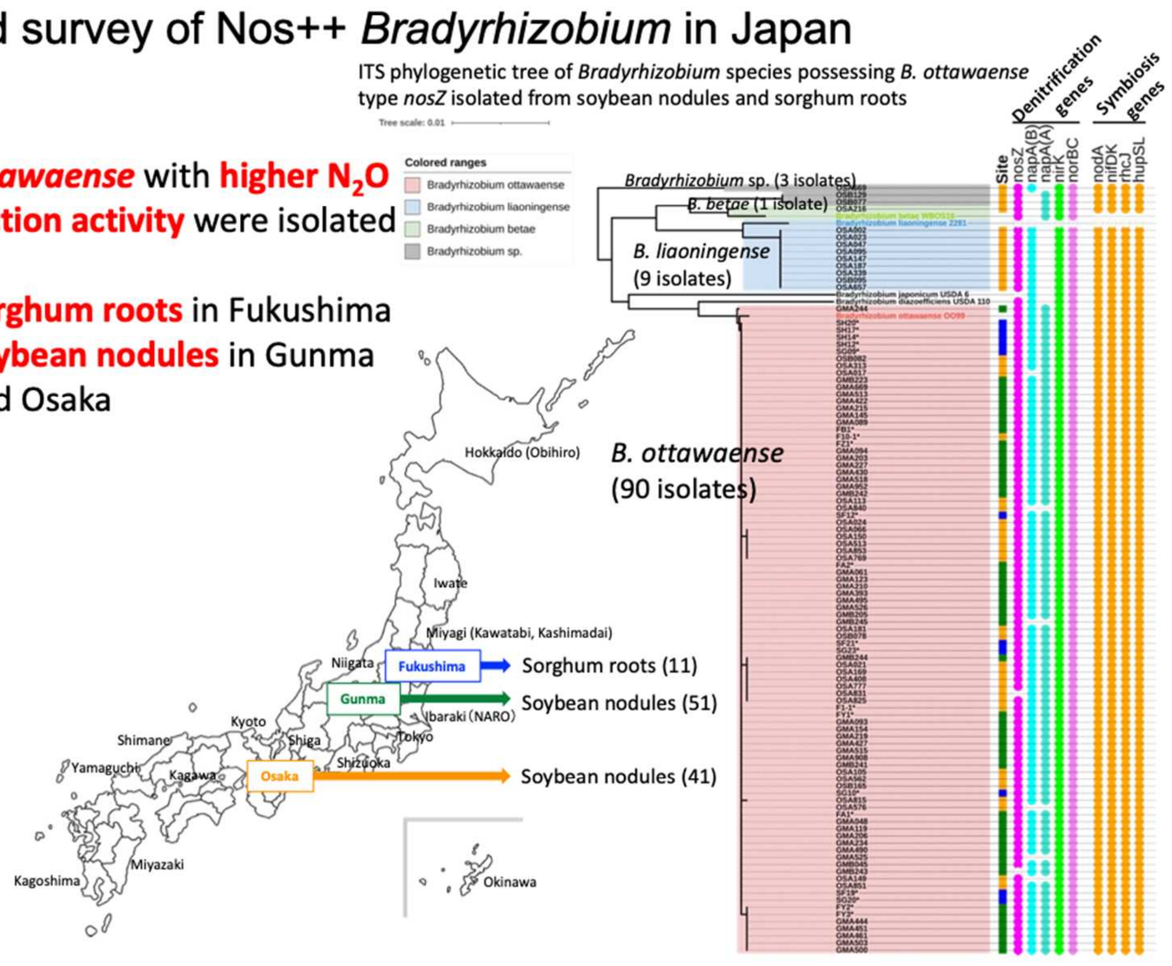
- Excellent inoculants for N_2O removal ⇒ Reduction of N_2O during harvest season
- Co-inoculation of PGPM ⇒ Increased yield (benefits for farmers)

Result 1: Search and isolation

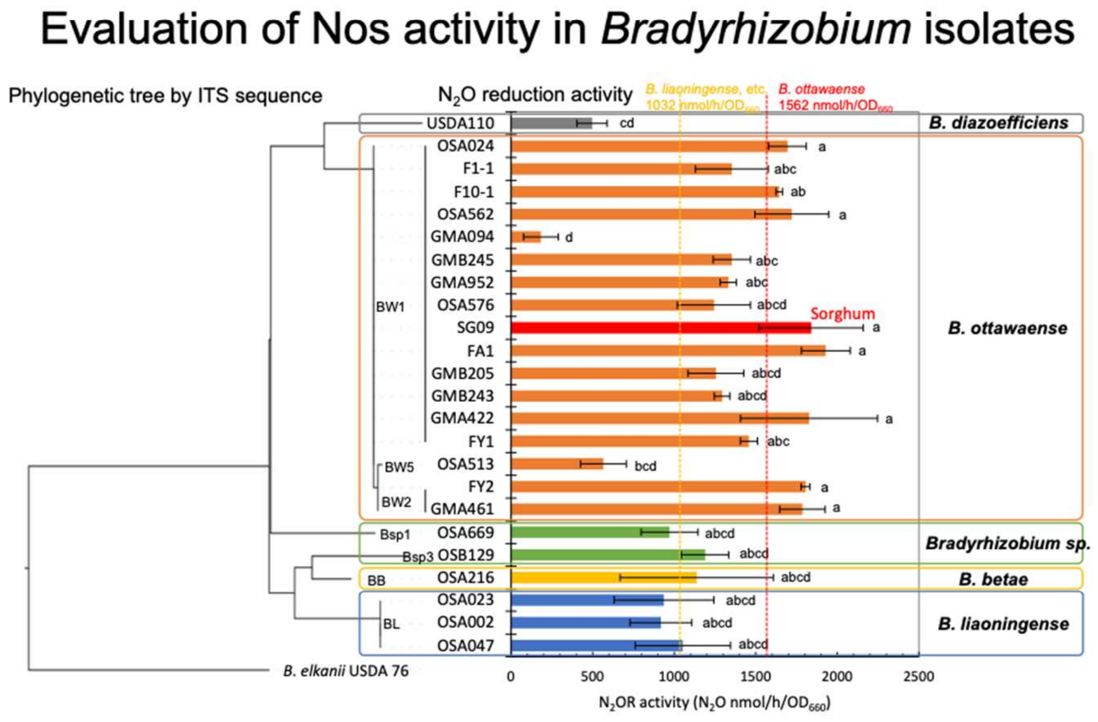
Field survey of Nos++ *Bradyrhizobium* in Japan

B. ottawaense with **higher N_2O reduction activity** were isolated from

- **Sorghum roots** in Fukushima
- **Soybean nodules** in Gunma and Osaka

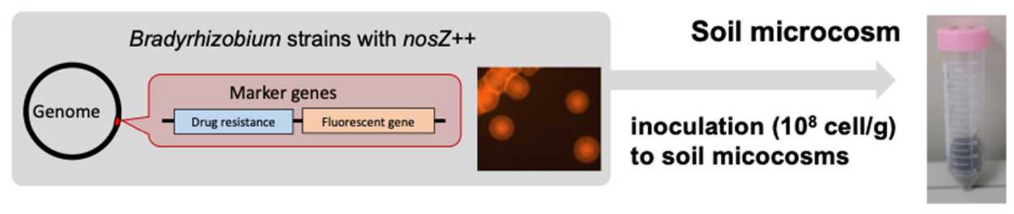


Evaluation of Nos activity in *Bradyrhizobium* isolates

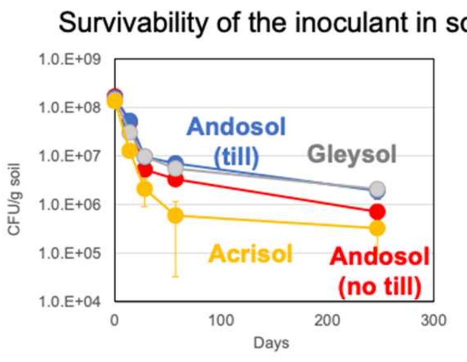


Result 2: Environmental Impact Assessments

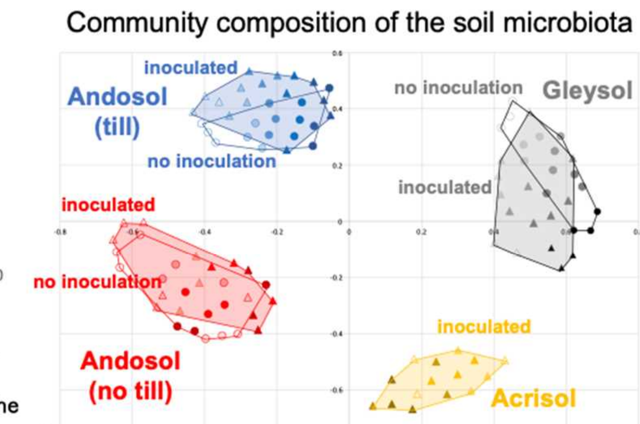
Metagenomic assessments of microbial inoculation



Survivability of the inoculant in soils



Community composition of the soil microbiota



The inoculant survived over a long period, although they gradually decreased. The inoculant did not drastically change the indigenous community composition and diversity.

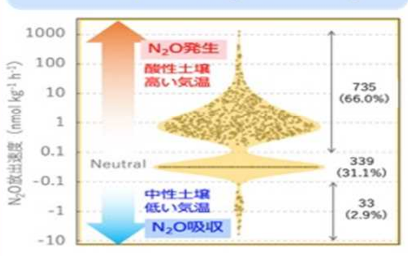
- Stress tolerance evaluation (drying, temperature, pH, etc.) and kinetic evaluation of N_2O reduction activity
- Comparative genomic analysis to elucidate traits useful as inoculants

Citizen science

"Soil in a Bottle"

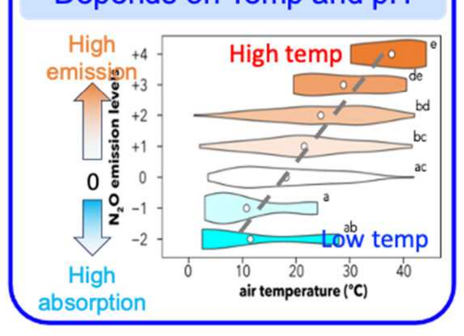
Collect samples and data from all over Japan

N_2O absorbing soils were found, 3.7% (53/1443)



N_2O rate, Microbiome and Environmental data

Depends on Temp and pH



soil

data

Isolation of N_2O reducing microorganisms

Soybean bradyrhizobia | Other bacteria

predict | Interesting as Microbiology | isolates

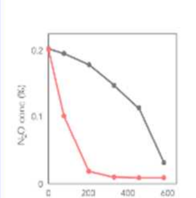
Database

- N_2O rates
- 16S rRNA
- *nosZ* gene
- Env. data

→ Data analysis

compare

Performance of isolates

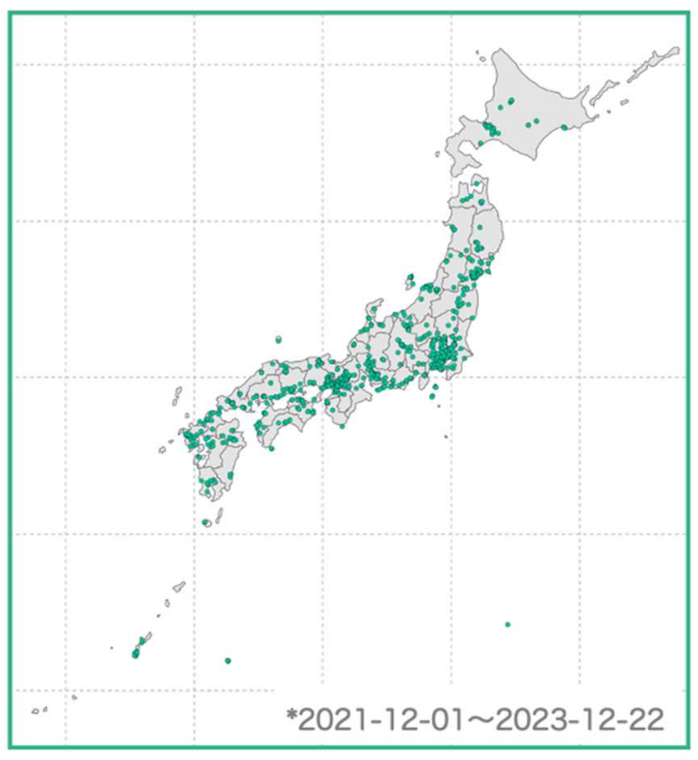


N_2OR activity ↓ Select isolates

Data & Samples

Novel N_2O reducing microorganisms

Participants and Samples



*2021-12-01~2023-12-22

People **1,043**

Soil **2,357**

Gas **14,823**

Soybean bradyrhizobia (SG09)

SG09-type *nosZ* was detected from 28 prefectures including Miyagi, Kyoto, Tokyo, Gunma, and Hokkaido

81 soil samples (81/2076; 3.9%)

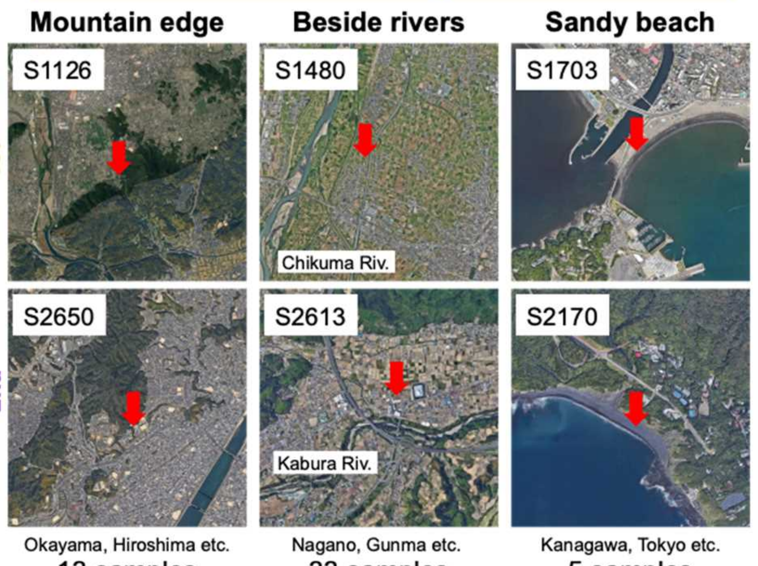
Inoculation to soybean

1st 43/820 soils → 44 isolates from 4 soils

2nd 38/1256 → more isolates?

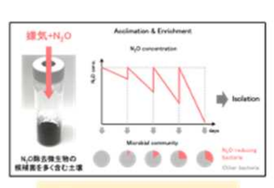
Location features where SG09-type *nosZ* was detected

Mountain edge	Beside rivers	Sandy beach
S1126, S2650	S1480, S2613	S1703, S2170
Okayama, Hiroshima etc. 13 samples	Nagano, Gunma etc. 33 samples	Kanagawa, Tokyo etc. 5 samples



New SG09-type *nosZ* containing soils were discovered : common topography Potential to isolate new high N_2O-reducing soybean bradyrhizobia

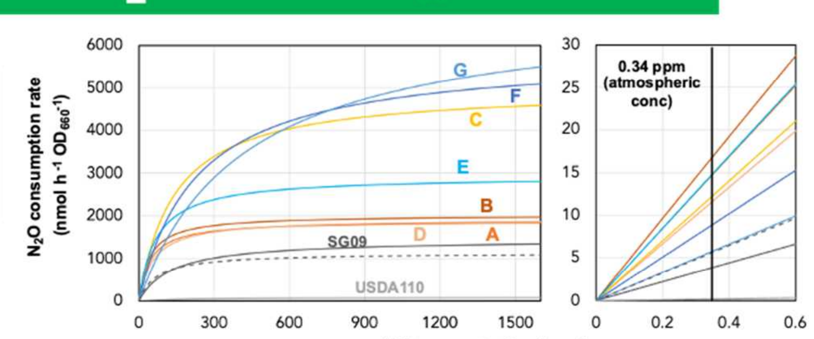
Isolated N_2O-reducing bacteria



Enrichment with soybean field soils

Seven strains (2 genera) of N_2O-reducing bacteria were isolated. → New isolates A–G

Kinetic analysis of cellular N_2O reduction activity in an automated continuous N_2O measurement system



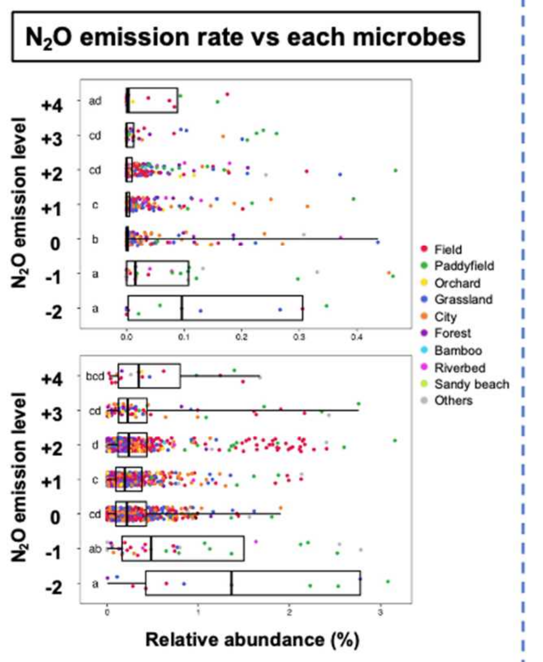
Strain	V_{max} (nmol h^{-1})	K_s (ppm)	$V_{0.34}$ (nmol h^{-1})	Medium
New isolate A	1886	44	14.4	R2A
New isolate B	2020	42	16.4	R2A
New isolate C	4994	142	11.9	R2A
New isolate D	1935	58	11.3	R2A
New isolate E	2925	69	14.4	R2A
New isolate F	5815	228	8.7	R2A
New isolate G	6932	418	5.6	R2A
B. ottawaense SG09	1451	131	7.4	HM
B. diazoefficiens USDA110	82	157	0.3	HM

4 strains showed lower K_s and 5 strains showed higher V_{max} than SG09

Cell-level N_2O reduction parameters were obtained for new isolates

Candidates by Data analysis

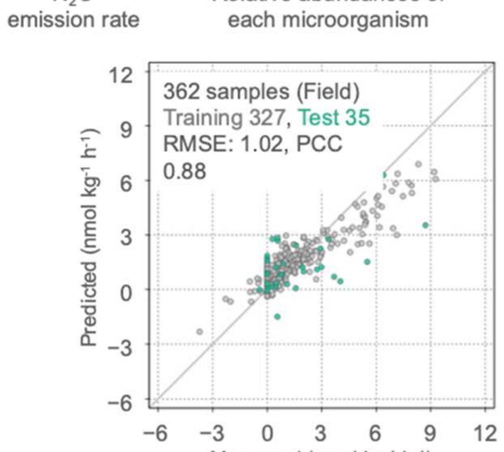
N_2O emission rate vs each microbes



N_2O emission rate prediction modeling

$$Y = a_1X_1 + a_2X_2 + a_3X_3 + \cdots + a_nX_n$$

N_2O emission rate | Relative abundances of each microorganism



62 candidates (14 Phyla) | **61 candidates (13 Phyla)**

No. A-12-3E

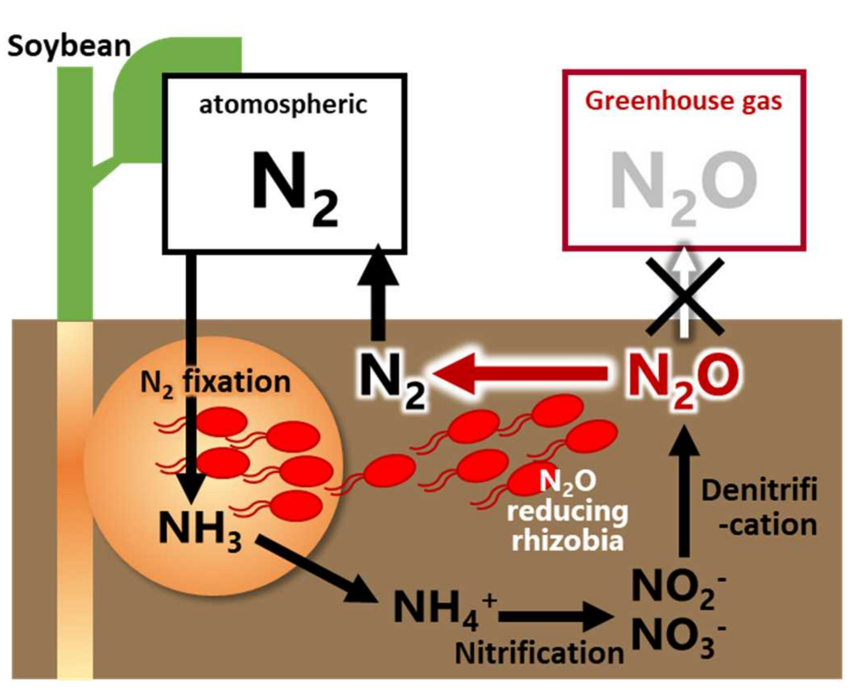
PJ : Mitigation of Greenhouse Gas Emissions from Agricultural Lands by Optimizing Nitrogen and Carbon Cycles

Theme: N_2O reduction by inoculation of rhizobia to soybean & by using soil aggregates and carriers of microorganisms with high N_2O reductase

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

Nitrous oxide (N_2O) reduction by inoculation of rhizobia with N_2O reductase to soybean

1. Background



- ✓ N_2O emission from agriculture is about 50% of anthropogenic N_2O emissions globally.
- ✓ Tohoku Univ. and NARO reported N_2O reduction using microorganisms (soybean rhizobia) (Itakura et al., 2013; Akiyama et al., 2016).
- ✓ Aim of the project is to develop N_2O reduction technology using soybean rhizobia at the field scale.

2. Research strategies

Symbiosis of rhizobia with high N_2O reductase

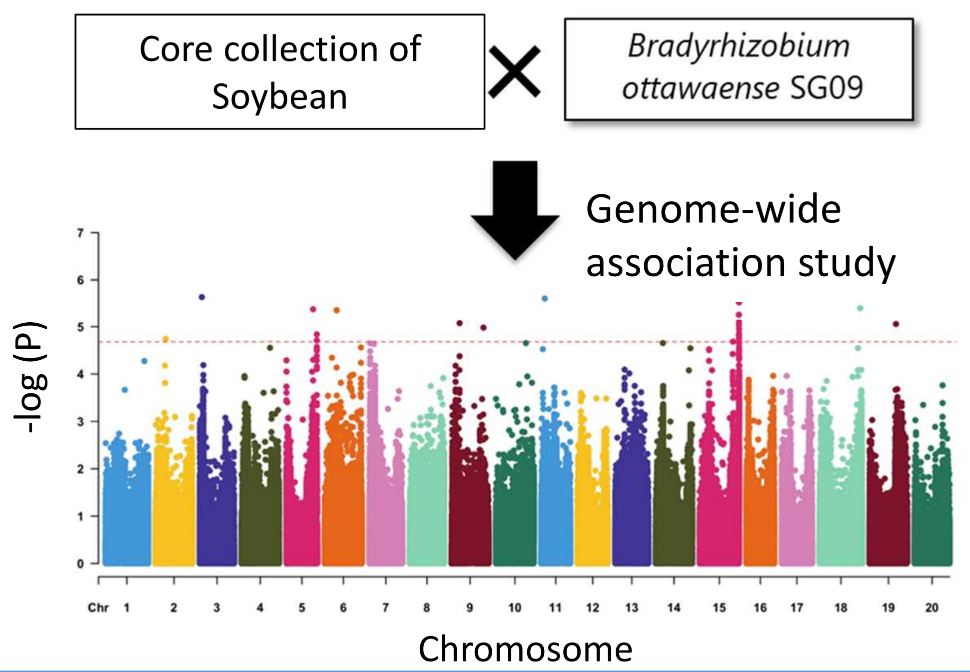
We will develop technology to reduce N_2O emissions by enhancing the efficacy of rhizobia inoculation, i.e., (1) explore soybean genes to enhance symbiosis with rhizobia, (2) promote soybean growth by dual inoculation of bacteria and rhizobia, (3) elucidate the mechanism behind N_2O reduction by rhizobia with high N_2O reductase activity.

Field experiments

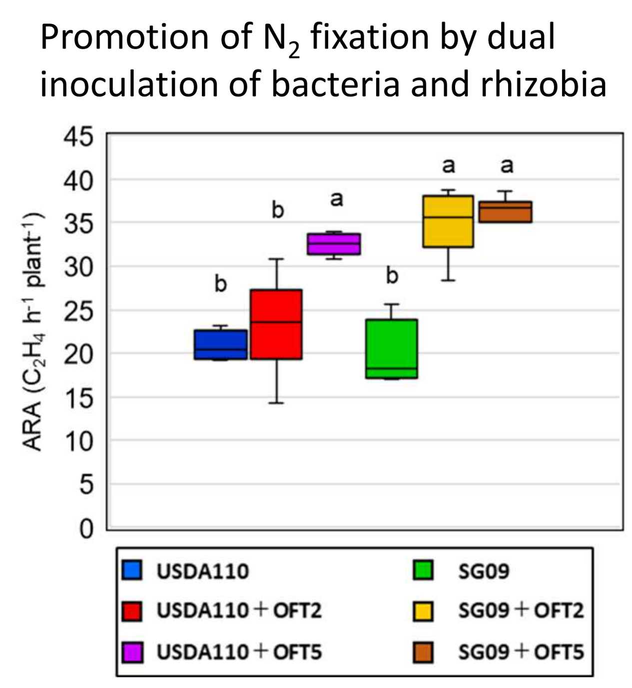
- ✓ Measurement of N_2O emissions by pot and field experiments.

3. Major results

Genome-wide association study on genes regulating symbiosis

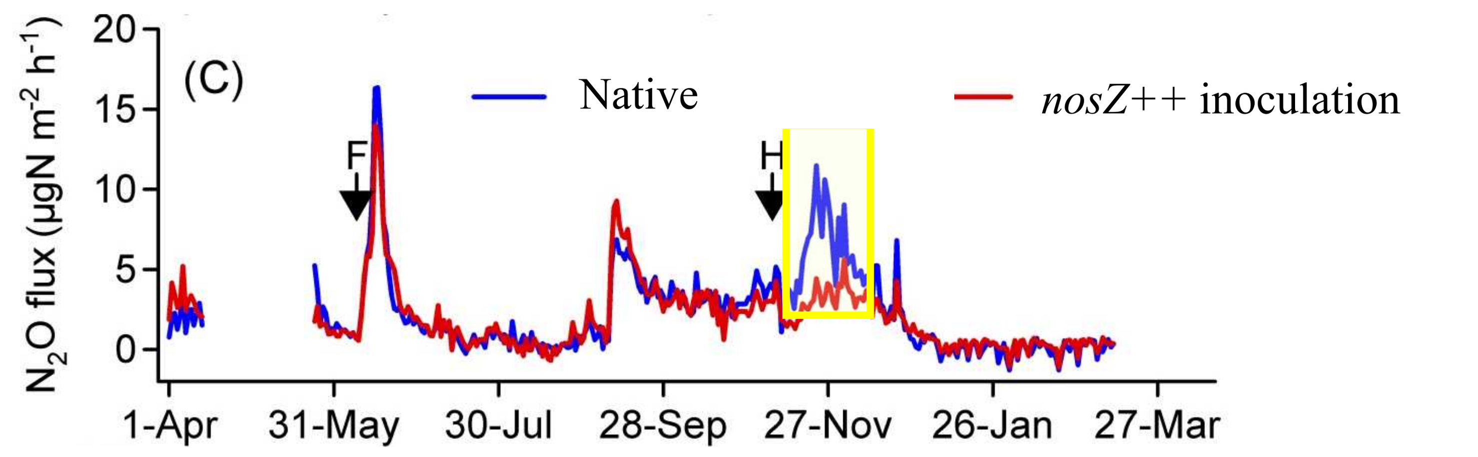


Promotion of N_2 fixation by dual inoculation



Field experiments

- ✓ The effect of rhizobia inoculation on N_2O emissions was measured by pot and field experiments.



Itakura et al. 2013

Nitrous oxide (N_2O) reduction by using soil aggregates or carriers with microorganisms

1. Background

Microorganisms with N_2O reductase can be used to reduce N_2O emissions from agricultural soil, however, suitable carriers are needed.

(1) Analysis of natural soil aggregates and development of artificial soil aggregates

(2) Screening of carriers such as porous materials and microorganisms with high N_2O reductase activity

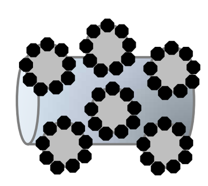
N_2O reduction by using microorganisms

2. Research strategies

Soil aggregates

Artificial soil aggregates

Carriers

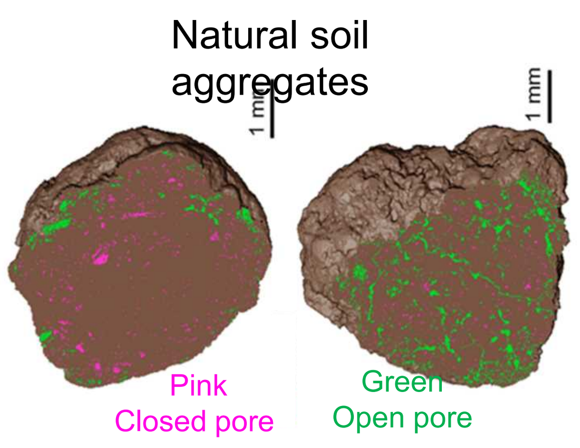


Porous materials

Soil aggregates	Carriers
Raw materials & Approach ・Natural minerals ・Inoculation of microbial communities or a strain	**Raw materials & Approach** ・Porous material ・Inoculation of microbial communities
Challenges ・Resistance ・Duration of N_2O reduction ・Electron donors (Energy source)	**Challenges** ・Construction of microbial communities ・Duration of N_2O reduction

3. Main results

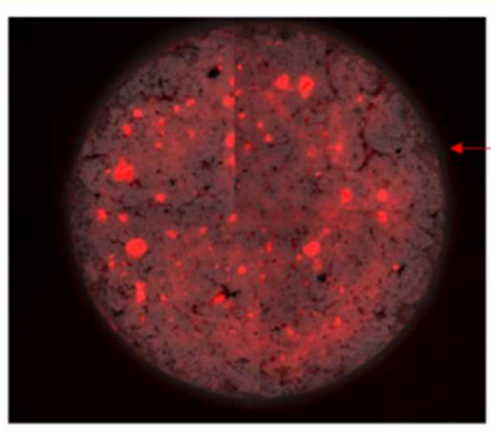
Soil aggregates



- ✓ Properties of artificial soil aggregates required for N_2O reduction were elucidated based on analysis of natural soil aggregates.

Properties of artificial soil aggregates required for N_2O reduction

Index	
• Pore (Pore size, pore distribution) • Clay content • Electron donor (energy source)	• Redox (hypoxia) • Structural stability • Duation of N2O reduction



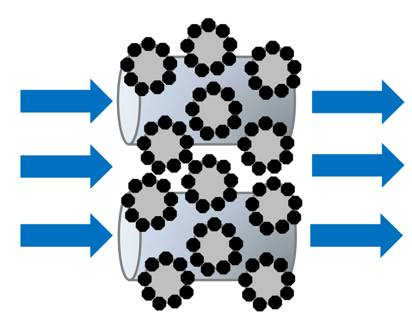
- ✓ Investigating the survival and distribution of microorganisms in artificial soil aggregates
- ✓ Testing the resistance of various artificial soil aggregates

→ **Balancing between resistance and N_2O reduction**

Carriers

- ✓ Screening of porous materials
- ✓ Use of microbial communities

N_2O reduction by using microbial communities



: Microbial communities　: Porous material