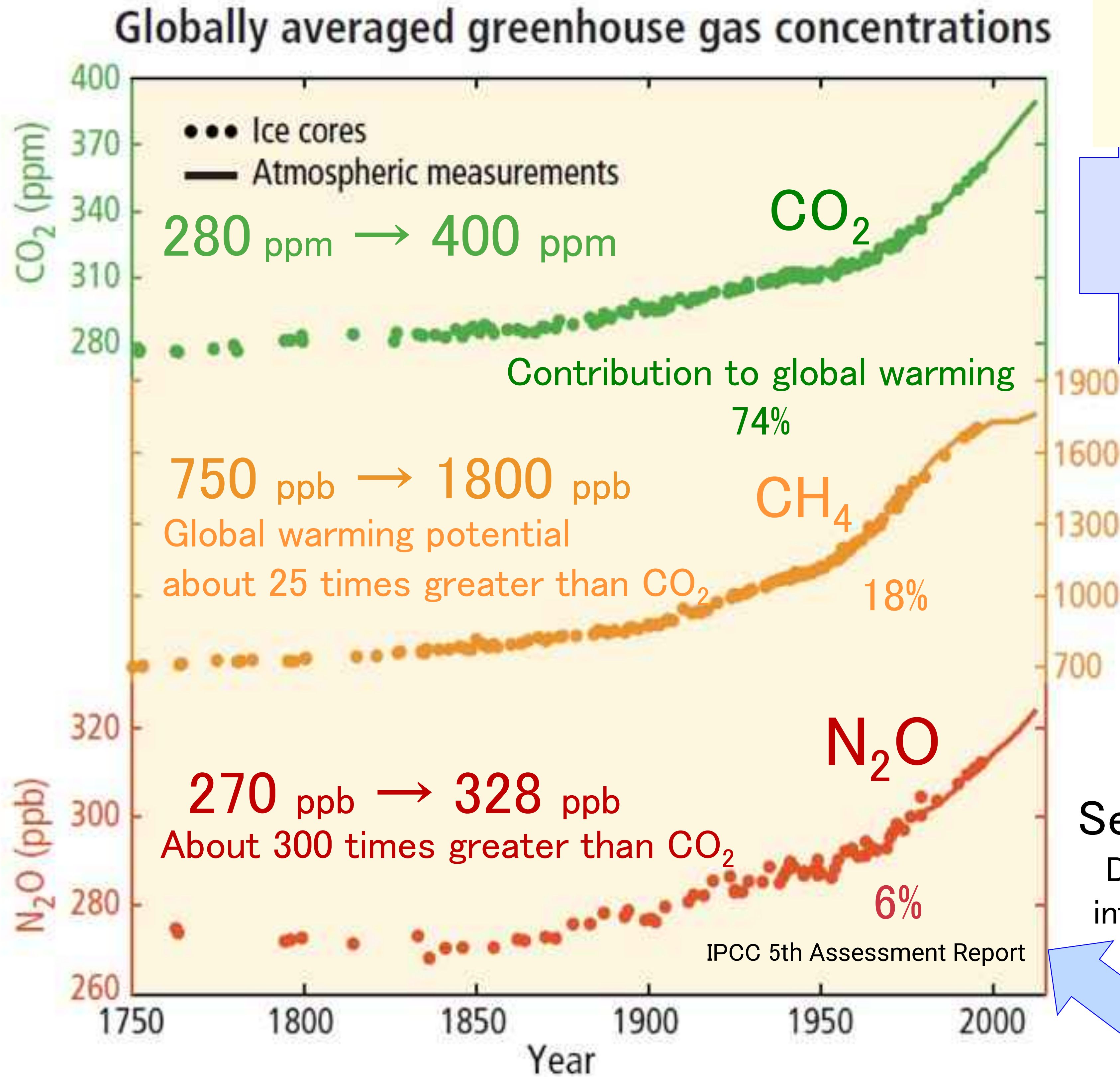
Mitigation of greenhouse gas emissions from agricultural lands by optimizing nitrogen and carbon cycles

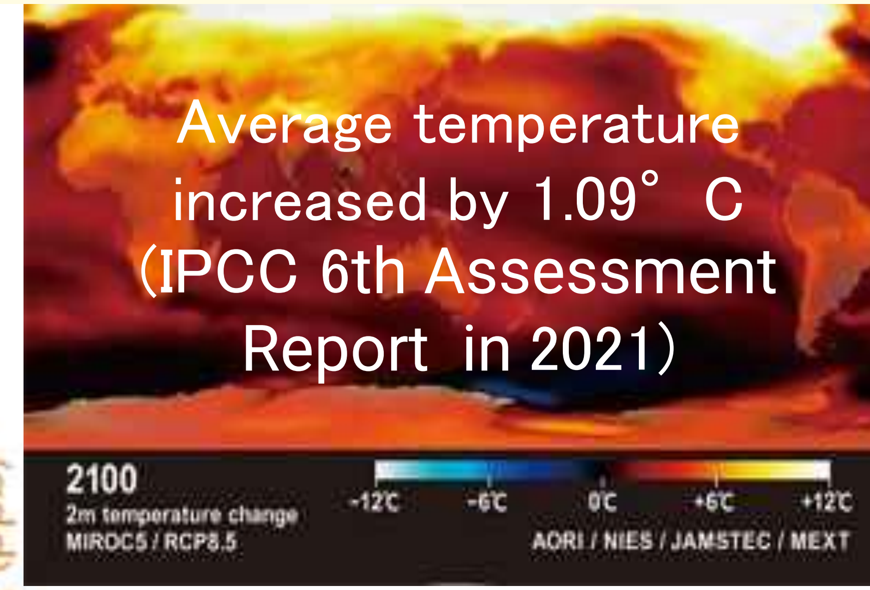
PM: Kiwamu Minamisawa
Graduate School of Life Sciences, Tohoku University
Specially-appointed Professor

PJ Organizations: Tohoku University, The University of Tokyo, National Agriculture and Food Research Organization (NARO)

Subcontractors: Tokyo University of Agriculture and Technology, Iwate University, Obihiro University of Agriculture and Veterinary Medicine, Shizuoka University, Kyoto University, Tokyo Institute of Technology, National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki University, Ehime University, Ryukoku University, Nagoya University, Forestry and Forest Products Research Institute (FFPRI), National Institute for Environmental Studies, bitBiome, Inc.

Global warming due to anthropogenic GHG emissions





Increased risk of climate change

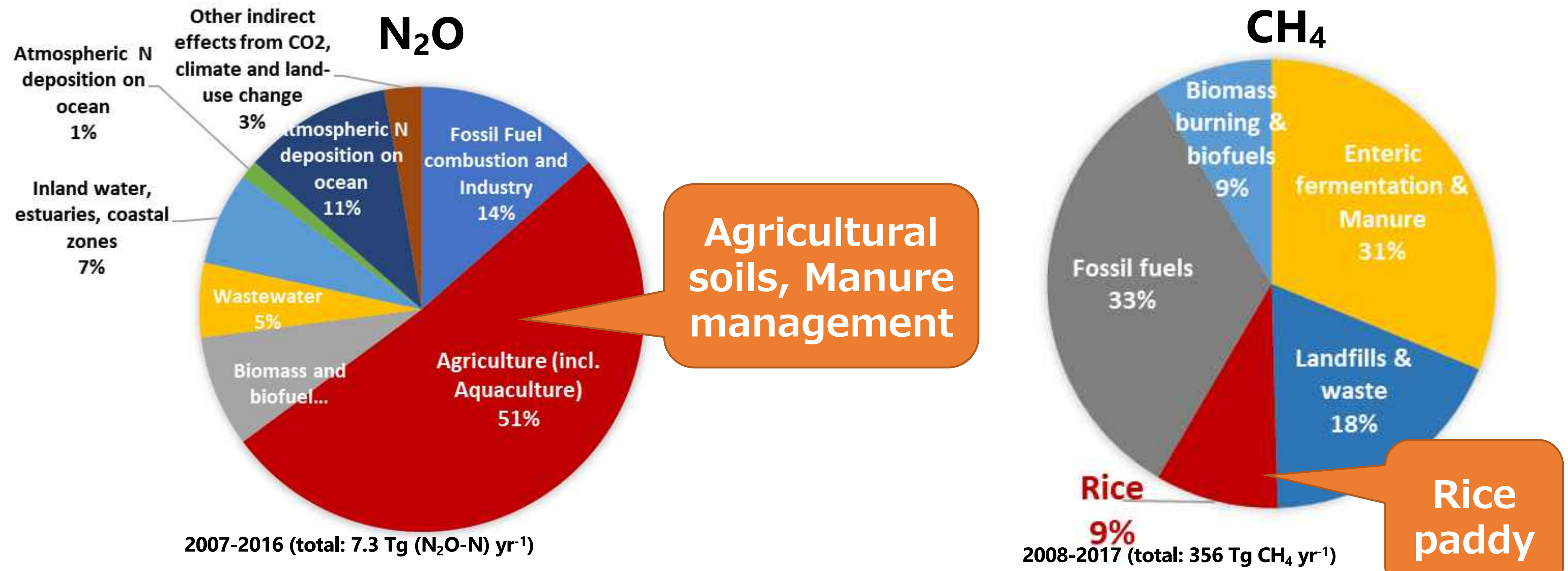
Severe disaster

Destruction of the infrastructure of life

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

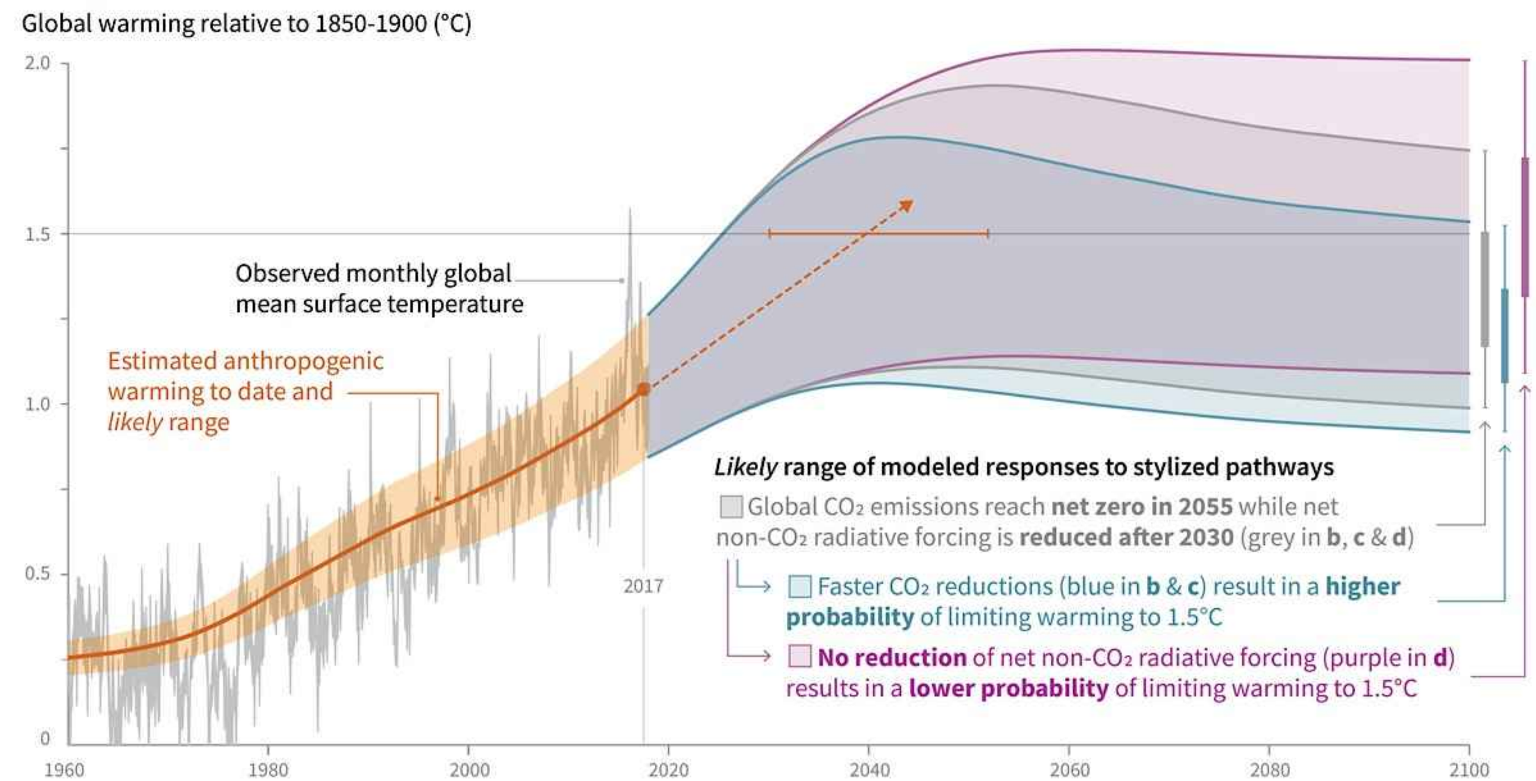
Reduction of greenhouse gases other than CO_2 is essential to limit the rise in global temperatures to 1.5 degrees Celsius above pre-industrial levels. (Paris Agreement, COP26)

Agriculture: Major Anthropogenic Source of N_2O & CH_4

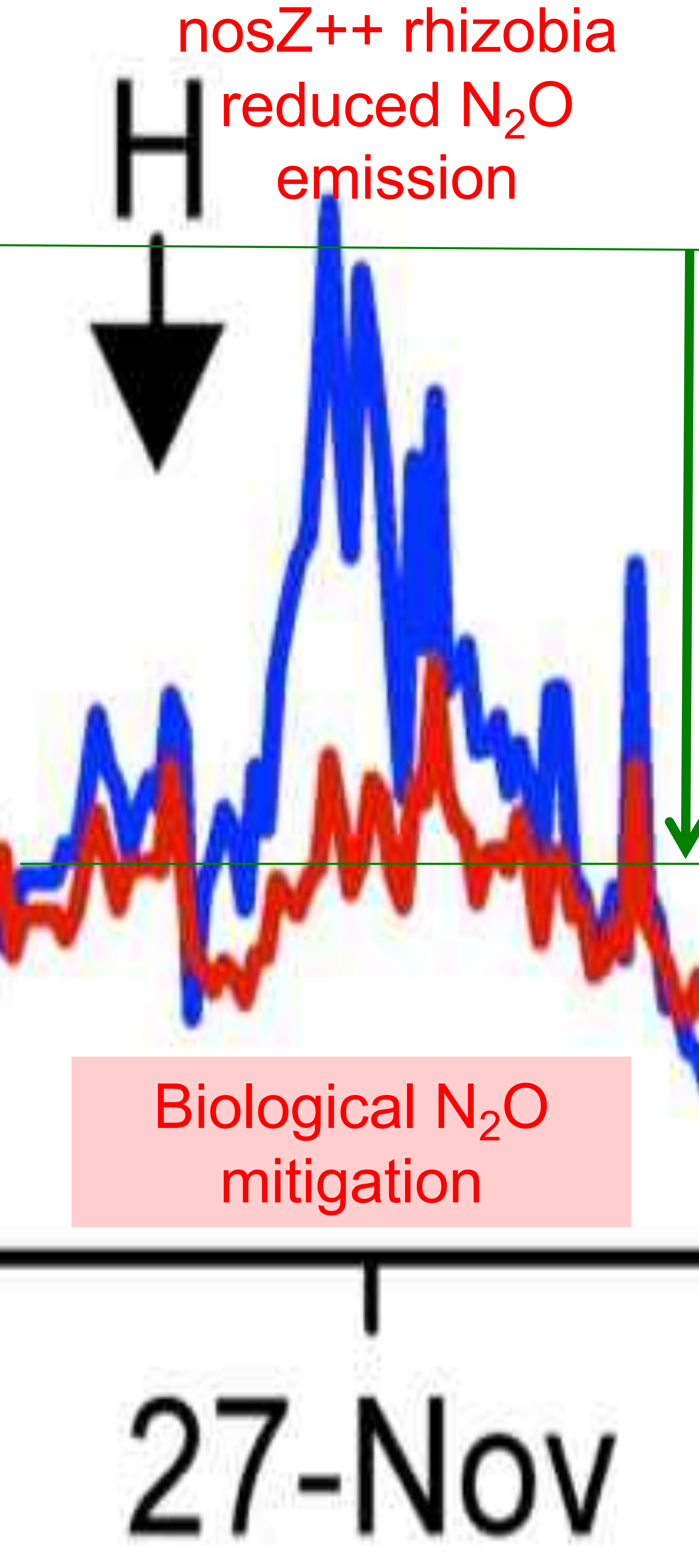


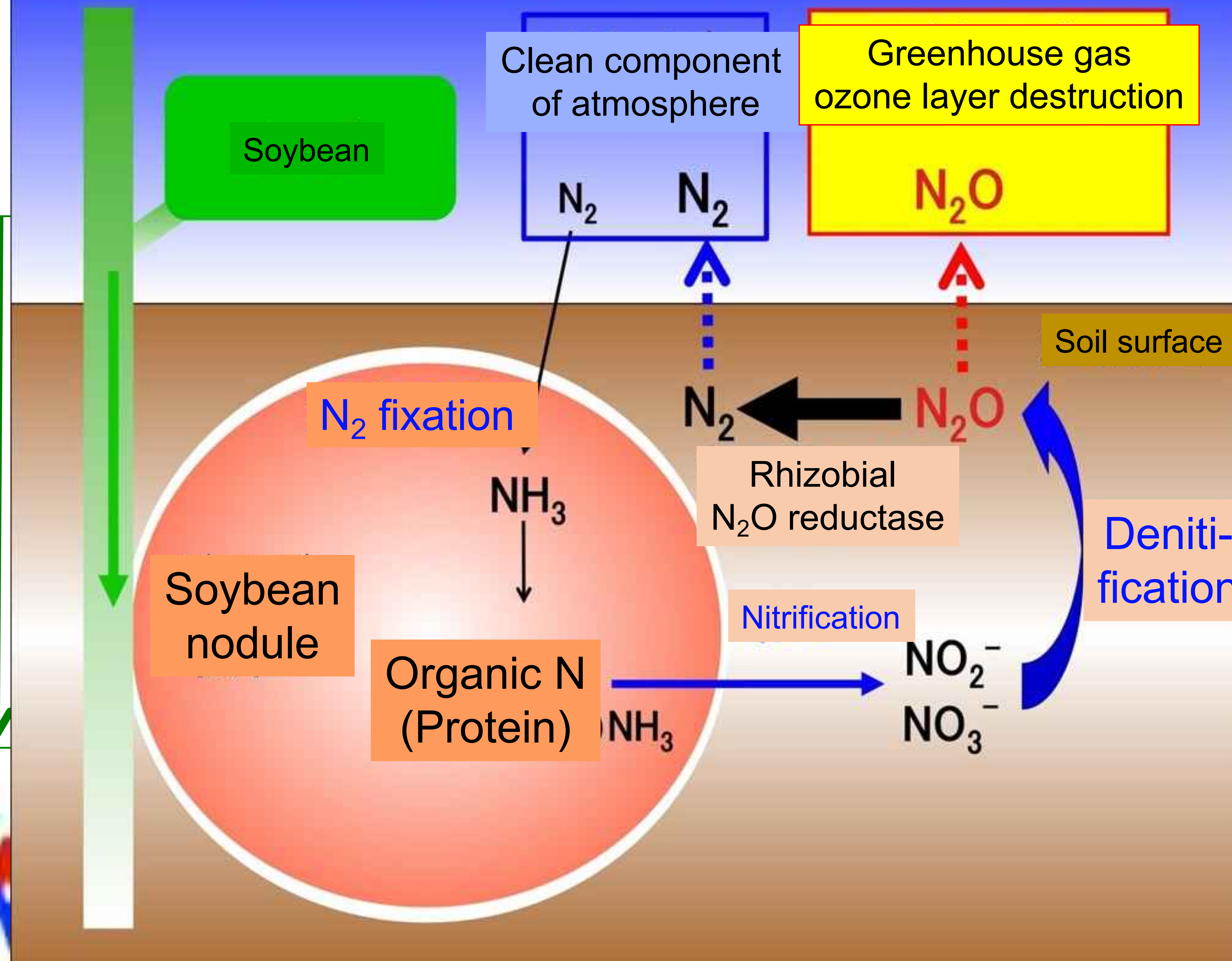
Anthropogenic sources of N_2O and CH_4 （IPCC-AR6 WG1, 2021)

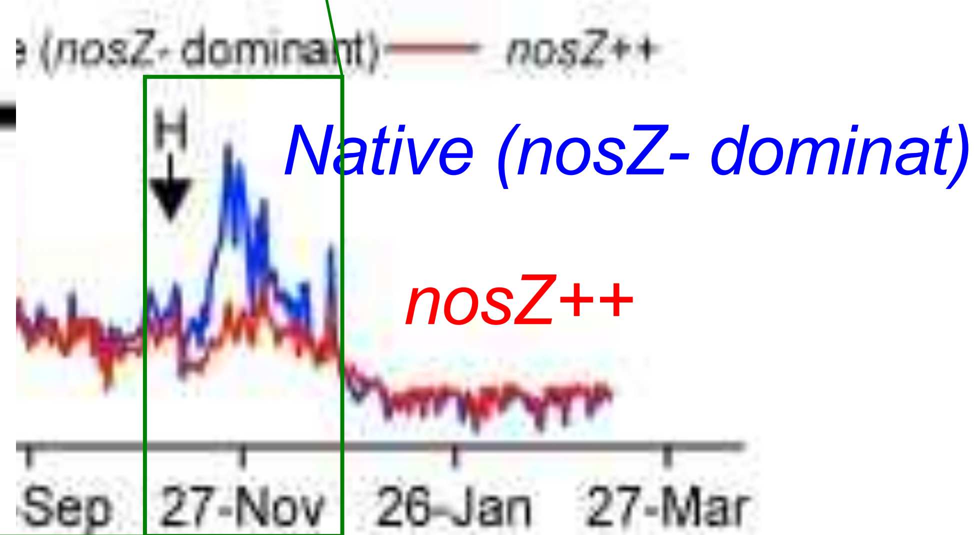
In addition to CO_2 reduction, the reduction of N_2O and CH_4 is needed, to limit global warming to 1.5 °C



（IPCC SR1.5℃, 2018)

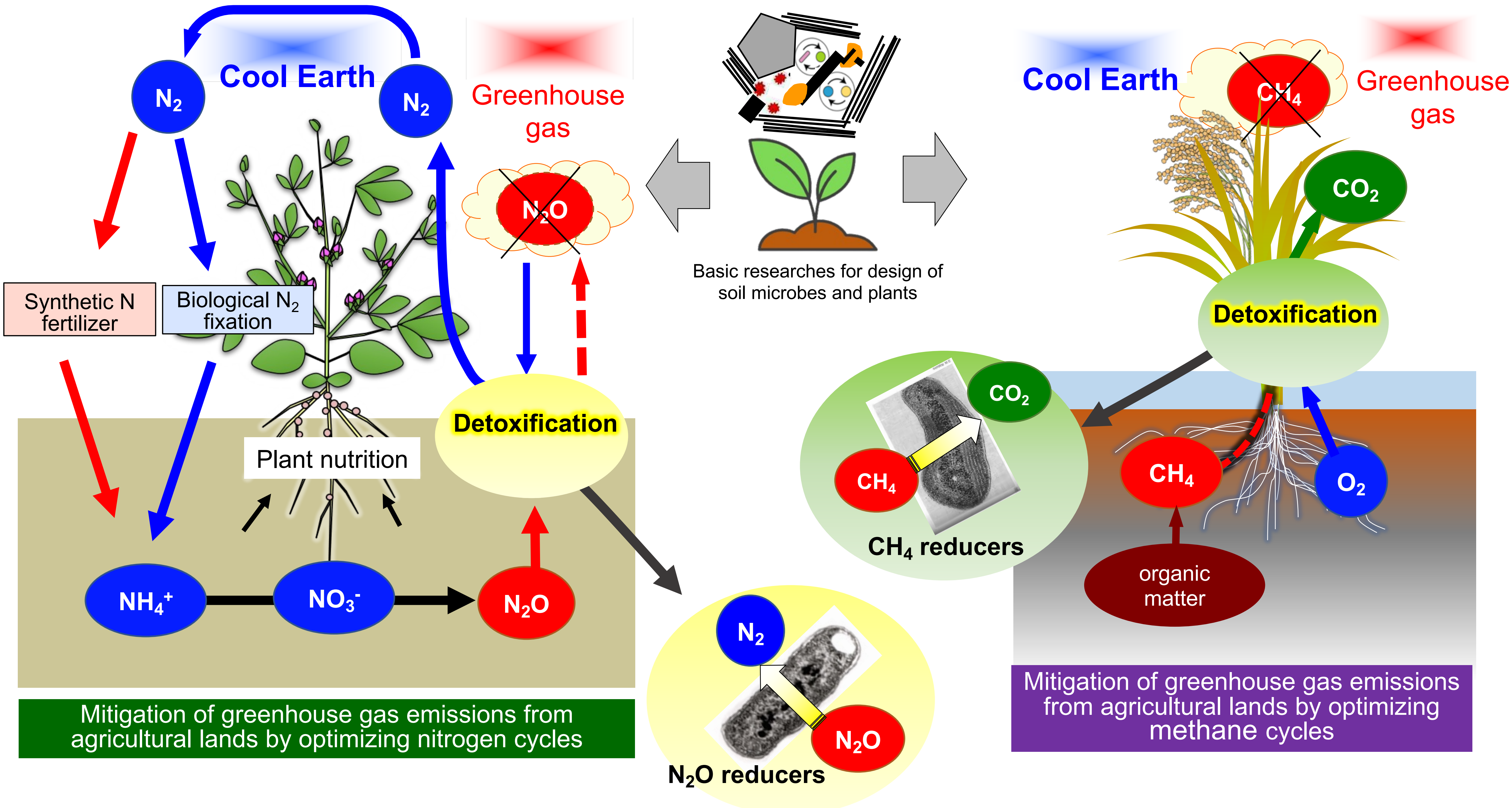






Itakura *et al.* Mitigation of nitrous oxide emissions from soils by *Bradyrhizobium japonicum* inoculation. **Nature Climate Change, 2013**

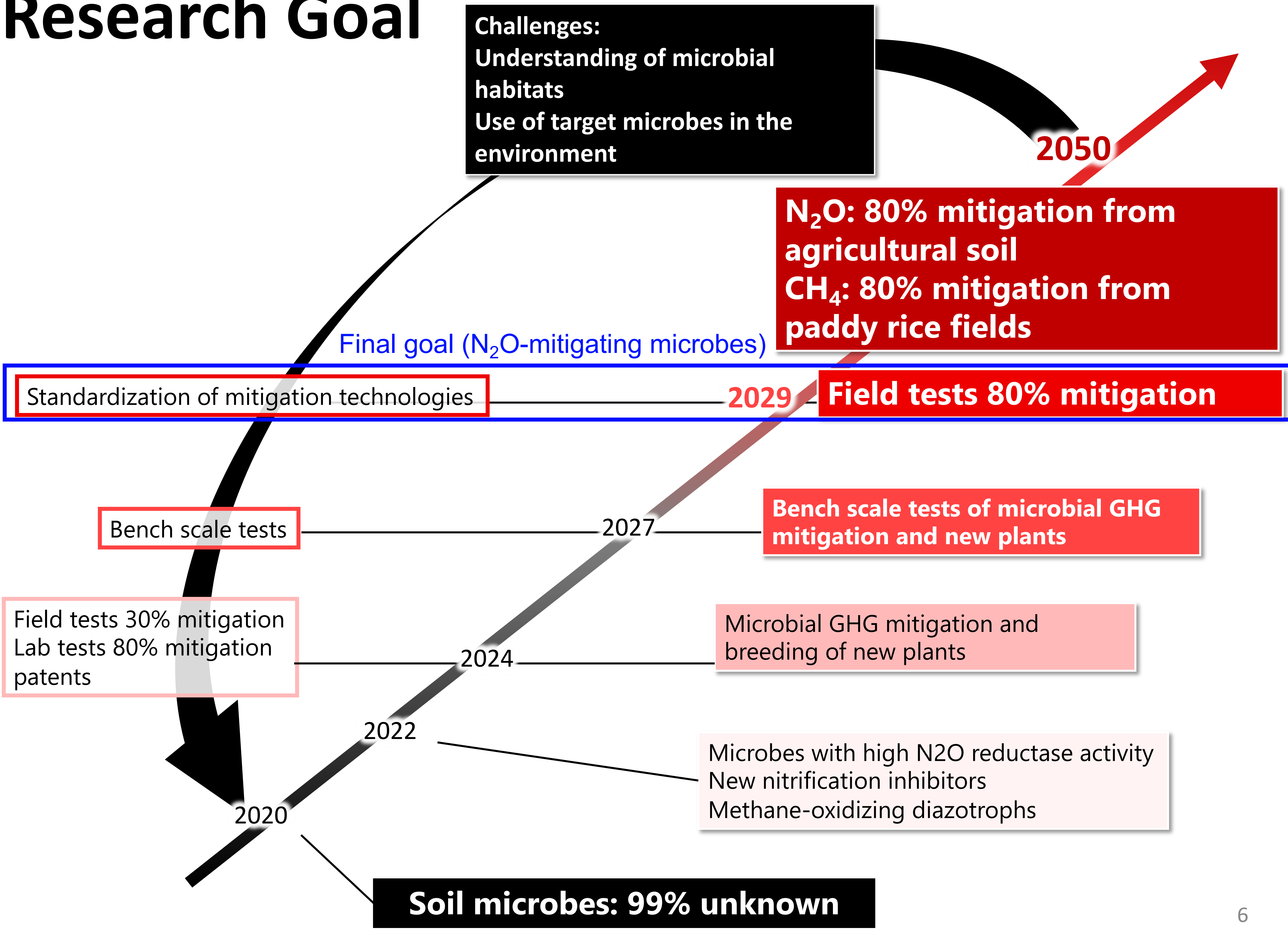
By 2050, 80% mitigation of N_2O and CH_4 from agricultural soils by soil microbes with crops and soil structures.



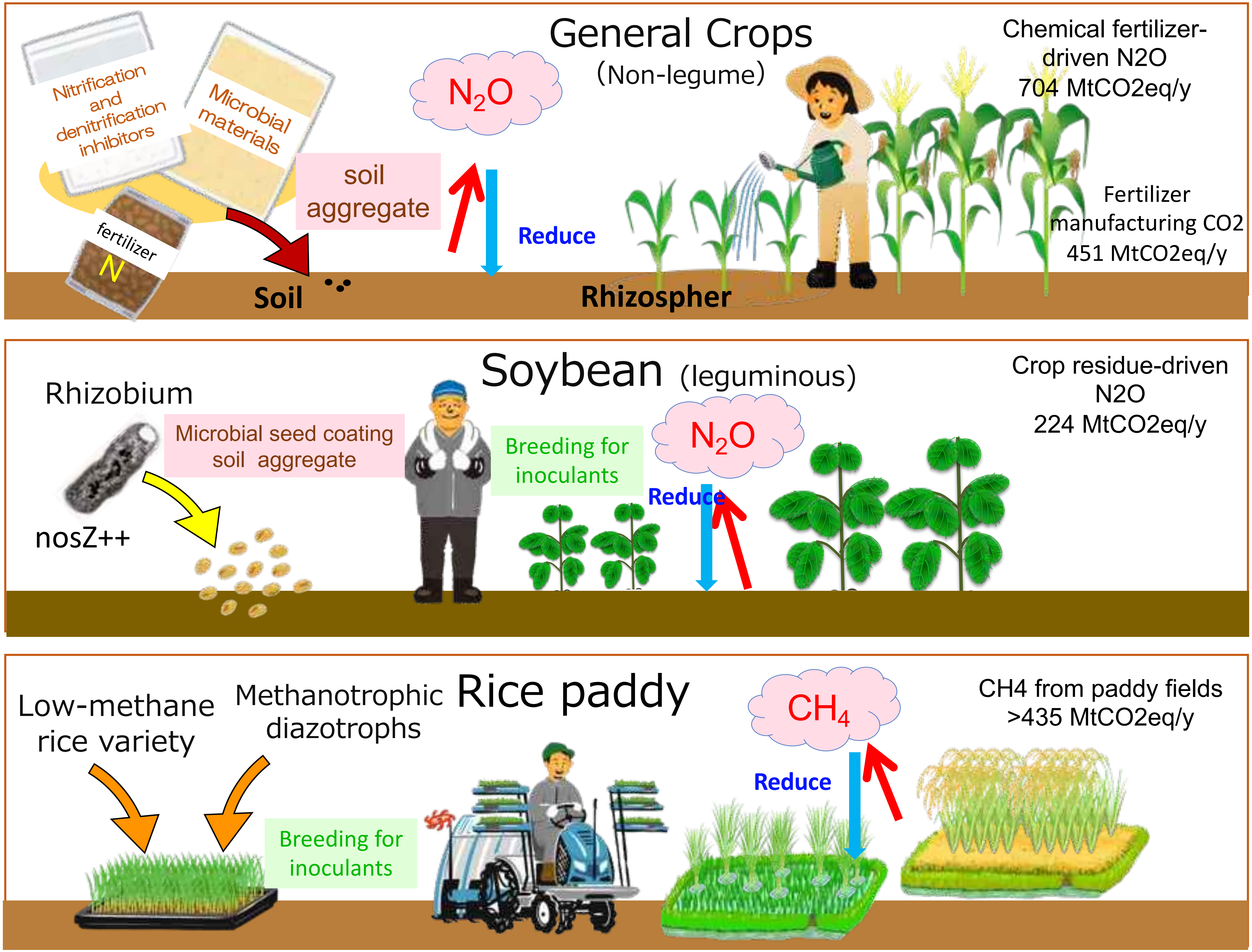
By microbial diversity of the natural world and all available knowledges and technologies, we aim to reduce anthropogenic GHG emissions and achieve a sustainable nitrogen and carbon cycle.

https://dsoil.jp

Research Goal



Business plan for this project

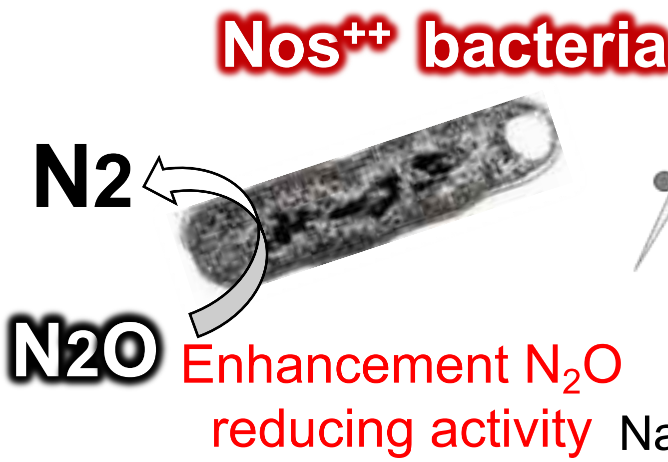


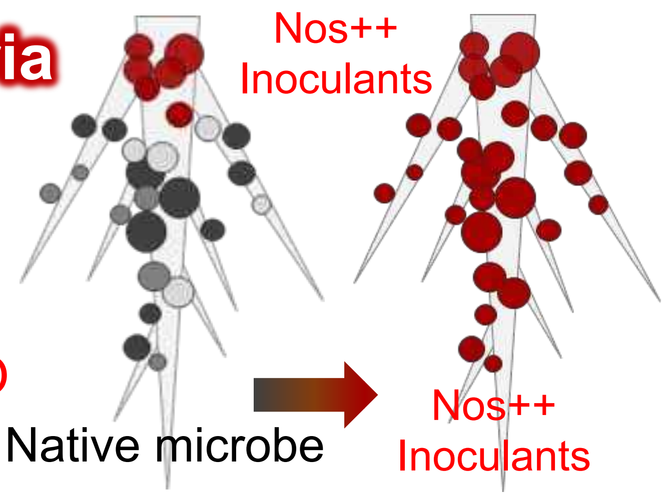
Examples of social implementation

2020 → 2024 → 2029

N_2O detoxifying rhizobia

Exploration and generation of Nos++ strains with enhanced N_2O reduction activity





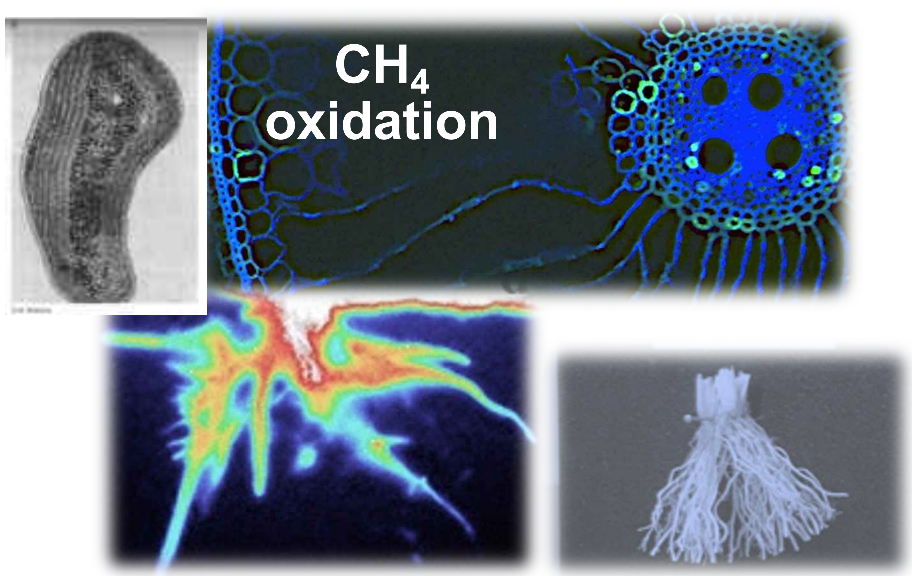
Breeding of host plant

Commercialization as a set of microorganism + seeds + carrier

Social acceptance of microbial inoculation for Cool Erath

Rice paddy CH_4 reduction

Development of new technologies for low-methane rice

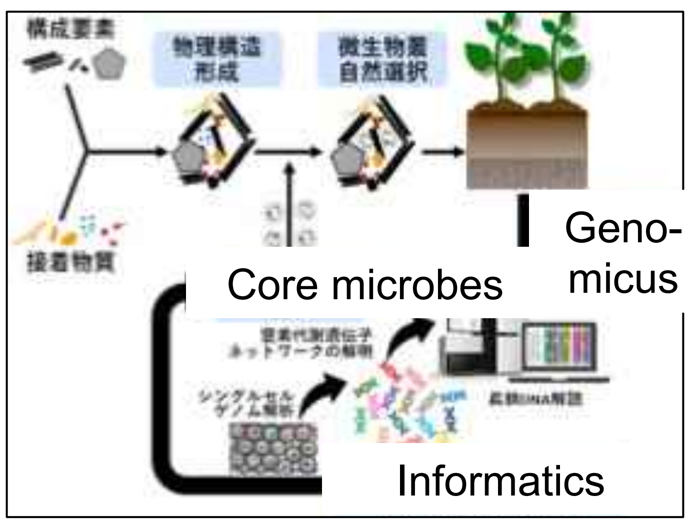


Reduce Methane Emissions from Major Products + (Diazotrophic methanotrophs)

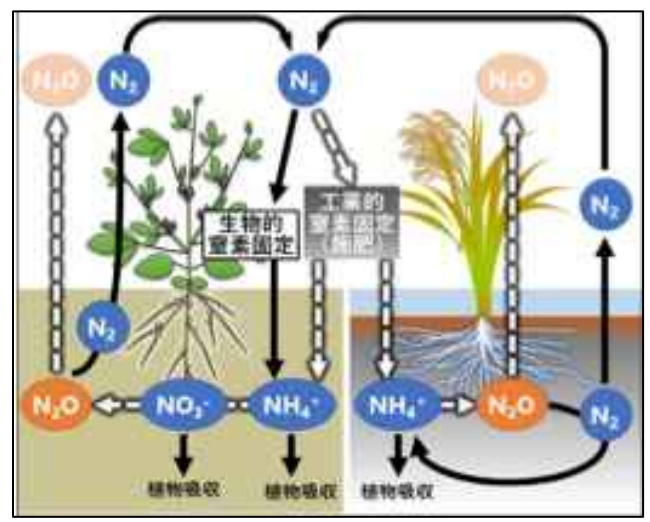
Minamisawa MS project themes

Soil structure | N_2O Recycling | DREAM *Designing of Reinforced and Effective Agricultural Material | CH_4 Recycling | Assessment & Modeling

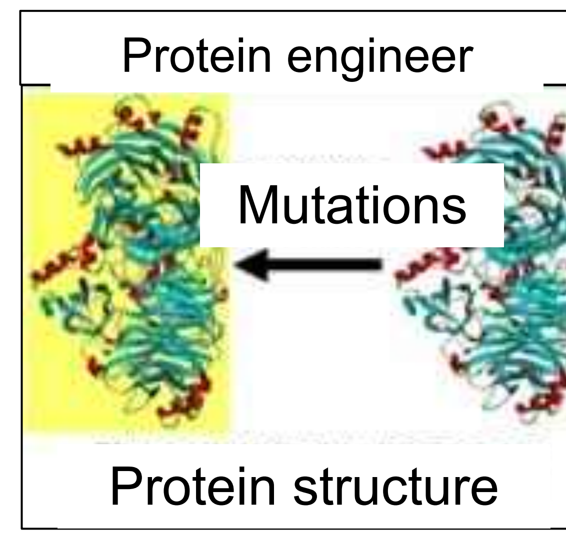
Ⅰ. Soil structure (7 units)



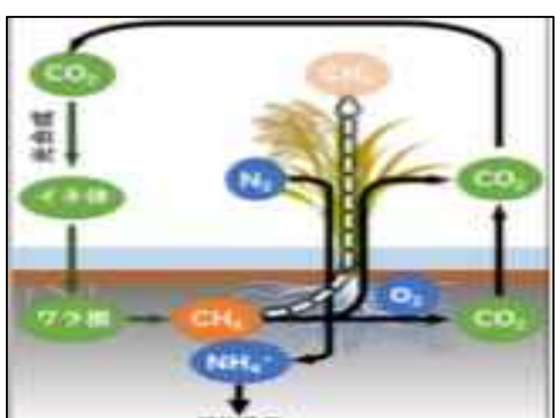
Ⅱ. N_2O Recycling (12 units)



Ⅲ. DREAM (3 units)



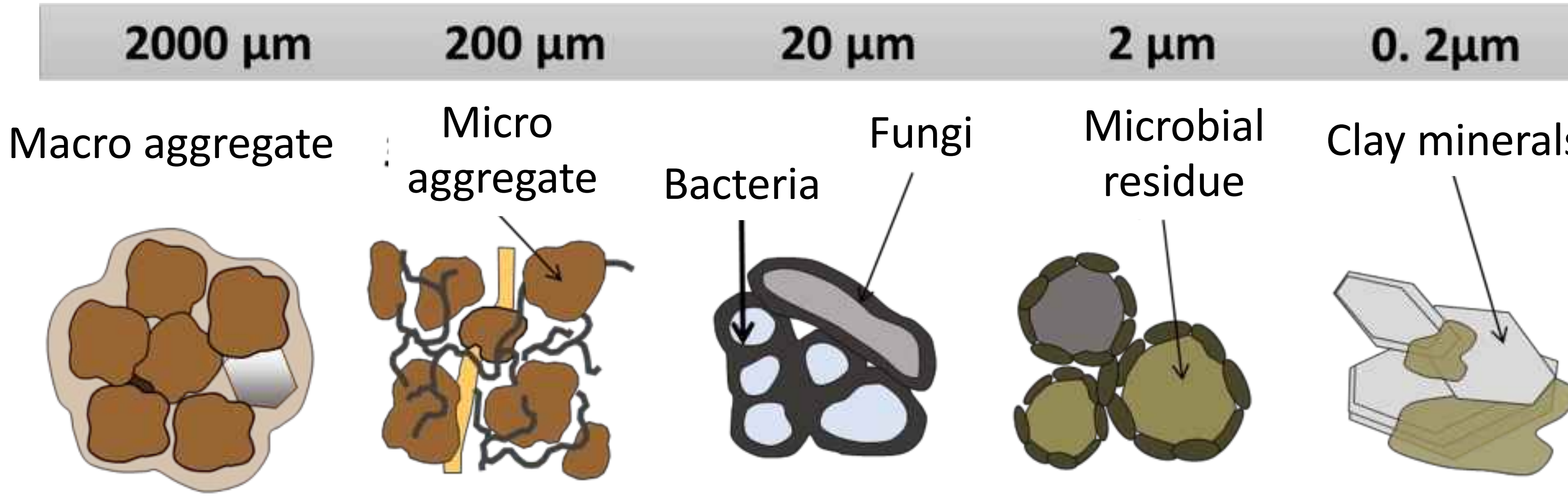
Ⅳ. CH_4 Recycling (3 units)



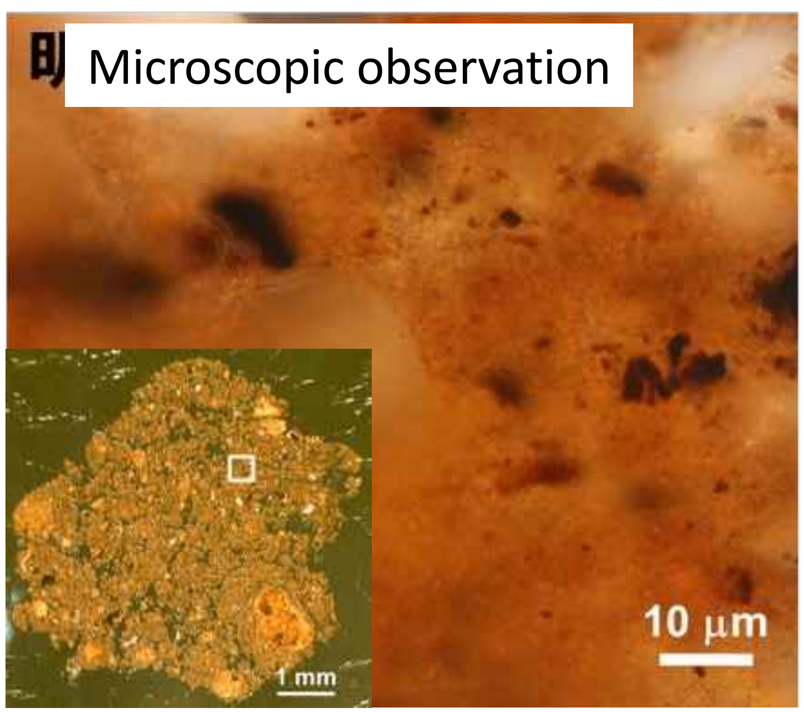
Ⅴ. Assessment & Modeling (6 units)

Soil aggregate

Aggregates composed from micro-particles



Aggregate Hierarchy model
(modified from Tisdall & Oades, 1982)



Physical structure

Chemical condition

Microbiology

Soil aggregates are hotspots of N_2O emission and consumption in soils

Analyses of soil aggregates tell us key micro-environment info. for N_2O mitigation

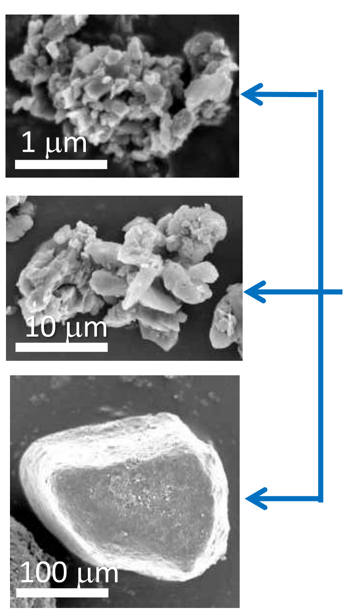
Project I-1. Soil Structure & Microbial Habitat

Project Goals

- ✓ To understand the aggregation process via the interaction of mineral particles & microbial products
- ✓ Artificial soil aggregate synthesis by mimicking natural soil development
- ✓ Development of N_2O-reducing artificial aggregates and field-scale test of its performance

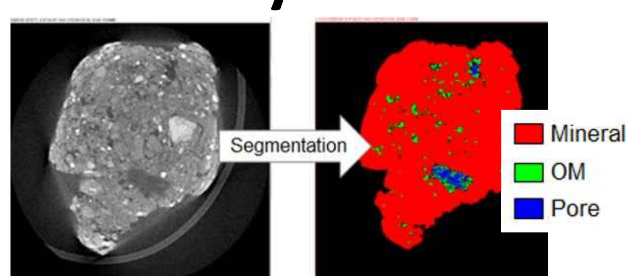
Top-down approach

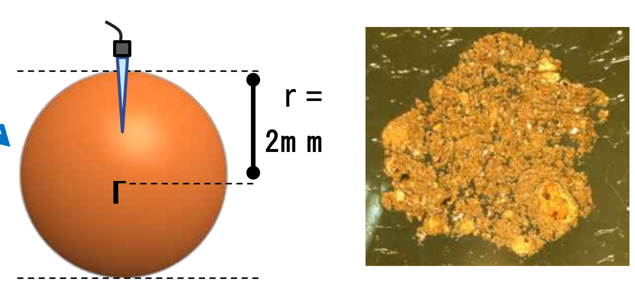
Physical fractionation



Natural Soil aggregates

X-ray CT



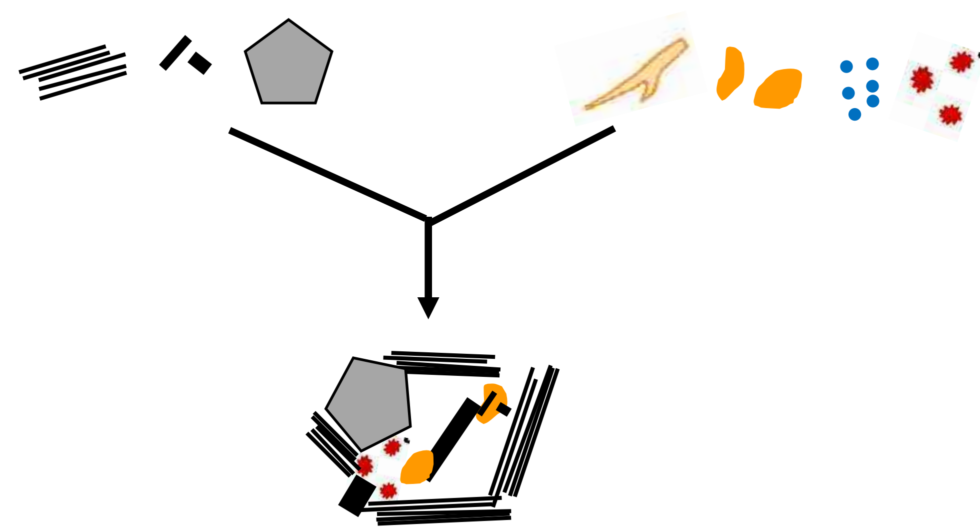


Microsensors

Bottom-up approach

Building units
primary minerals
secondary minerals

Binding agents
biomolecules
metals, complexes



Elucidation of aggregation mechanisms
With a focus on binding agents

Synthesis of artificial soil aggregates

What is the elements for N2O-reducing soil aggregates?

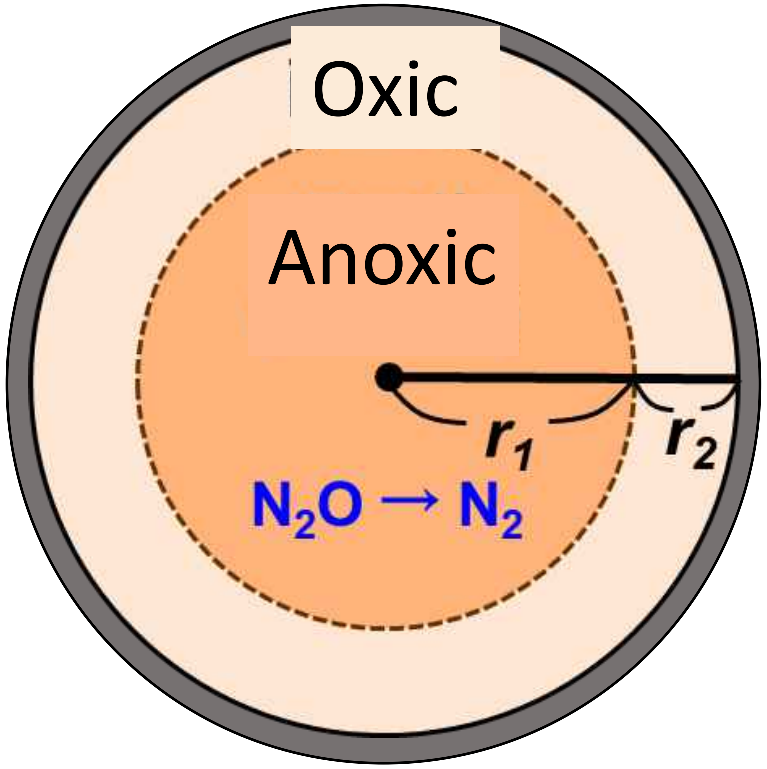
Aggregate environments to enhance N_2OR activity

Top-down approach

Aggregates with larger vol. of prolonged anaerobic zone ($r_1 > r_2$)

Requirements

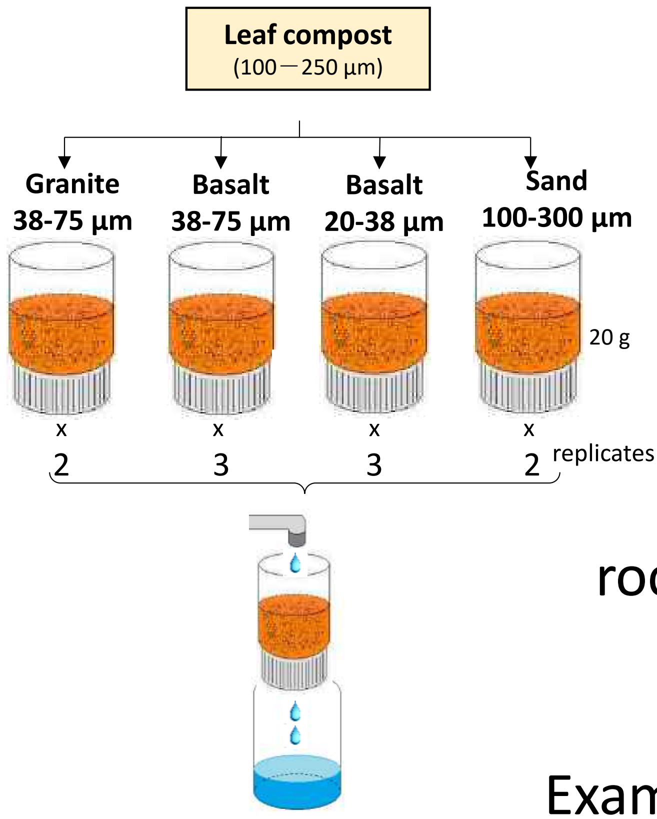
1. Water resistant
2. Particle size
3. Porosity
4. Shape
5. N_2O reducer
6. Pore size
7. Clay content



→ Advanced hybrid materials are under development in collaboration with other themes of the project

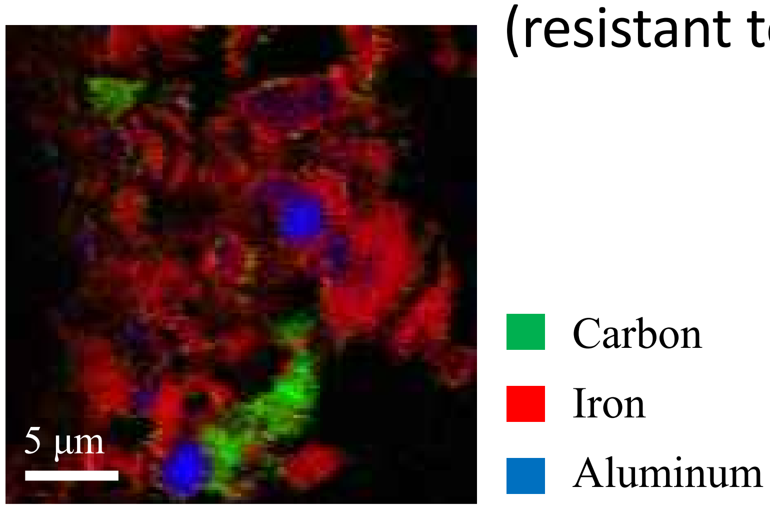
Bottom-up approach

Artificial aggregate synthesis using the mixture of mineral & organic materials



8-week incubation of
rock powder +leaf compost
(8 wet/dry cycles)

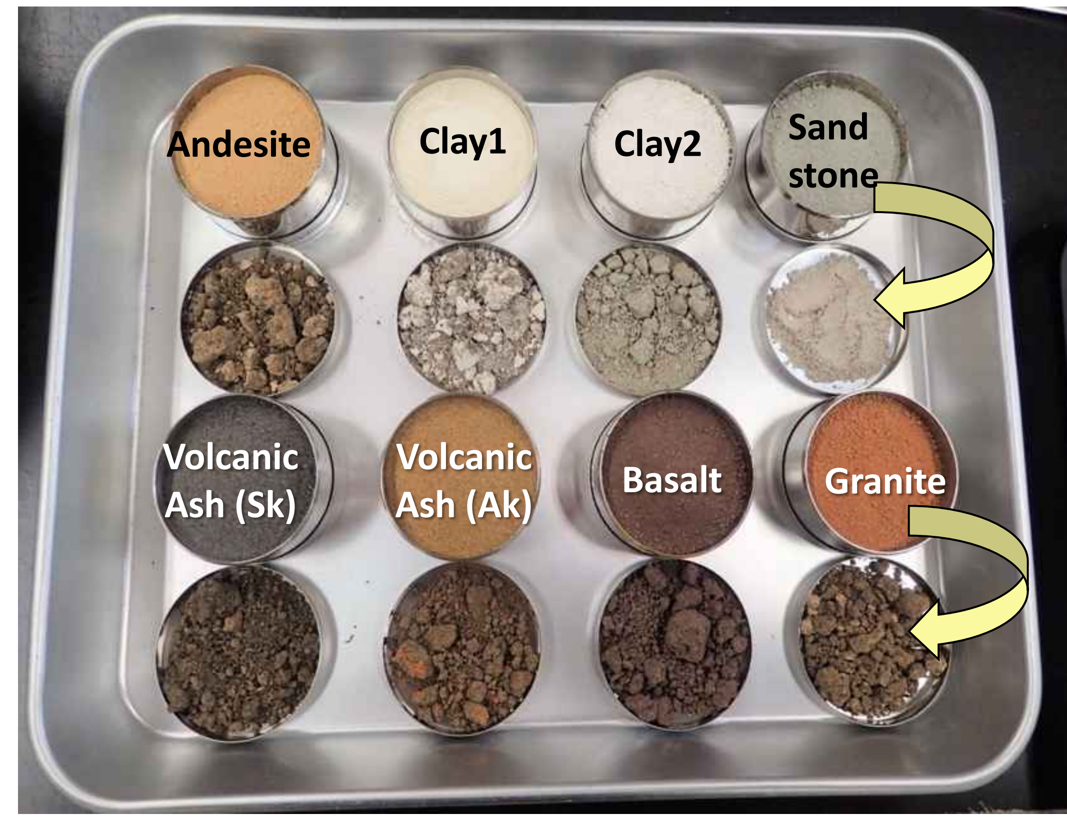
Examination of stable aggregate
(resistant to mechanical shaking)



Submicron-scale examination of C and metal distribution by STXM

- Rapid aggregate formation

Long-term field burial of 8 contrasting minerals



Co-development of aggregate structure & microbiome

N_2O-reducers

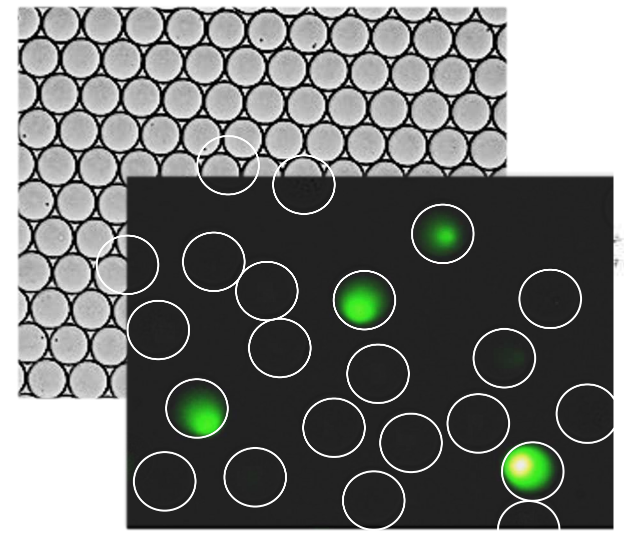
Prototypes of climate-smart aggregates (for GHG reduction)

Goal of theme I-2

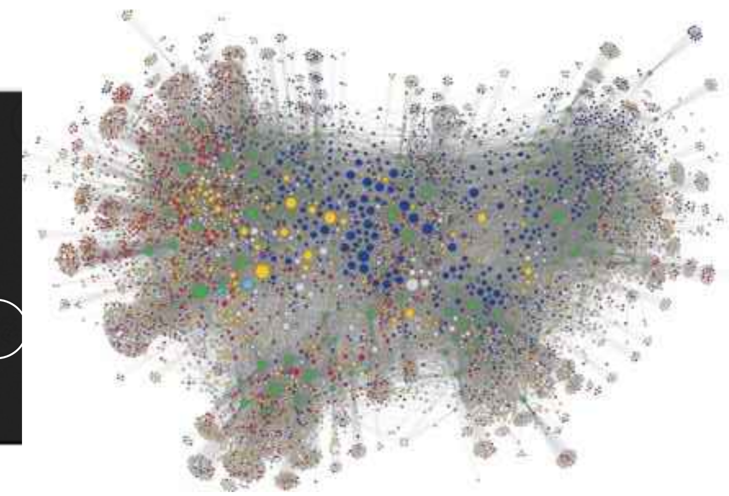
Long-read sequencing

Bioinformatics

Single-cell sequencing



Network analysis



Innovative technologies to deeply understand soil microbiomes

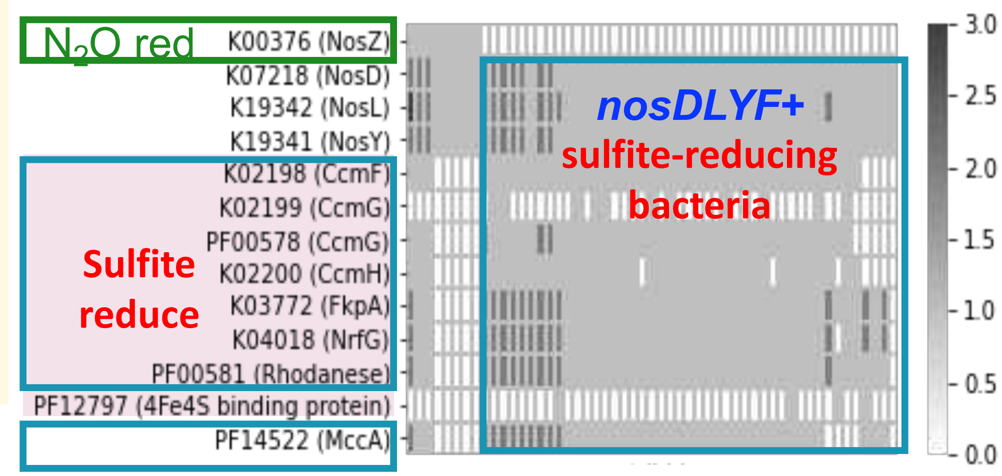
Complete genomes of more than 100 strains of GHG-reducing microbes, with many metagenomes! ！

Name	Type	GHG reduce	Strains
B. diazoefficense	Rhizobia (soybean)	NosZ+	65
Bradyrhizobium sp.	Rhizobia (soybean)	NosZ++	9
B. lianoningense	Rhizobia (soybean)	NosZ+	1
B. japonicum	Rhizobia (soybean)	NosZ-	3
B. elkanii	Rhizobia (soybean)	NosZ-	3
R. leguminosarum	Rhizobia (clover)	NosZ+/-	2
Bradyrhizobium sp. 46	N_2O to resource	NosZ+	1
Bradyrhizobium sp.	Rice endophyte	NosZ+/-	19
Methylocystis echinoides	Methane-Ox (Type II)	CH4 oxi.	5
合計			108

Comparative genomics of GHG-reducing genes, crop-promoting genes, etc.
(With themes II, IV, V)

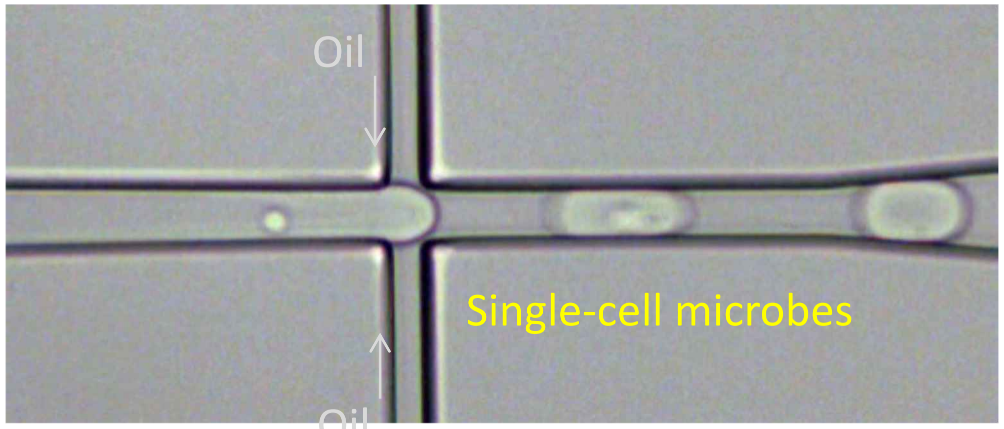
Profiling methods to find novel N-cycle genes!

Ex. Shewanellaceae 53 strains



Microdroplet-single cell technology to efficiently (>30 times) obtain microbial gene data!

Soil microbes



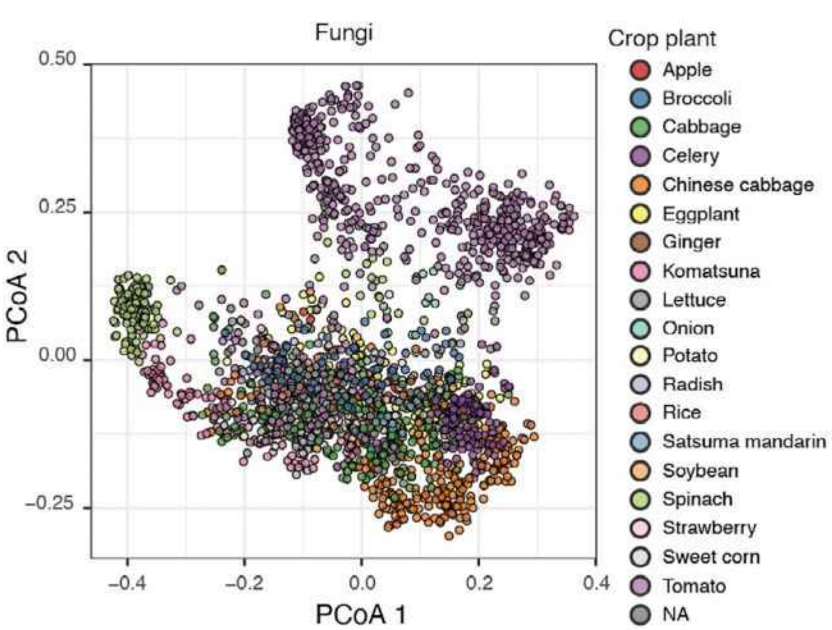
phylum_gtdb	class_gtdb	Notes	n
Proteobacteria	Gammaproteobacteria		2
Bacteroidota	Bacteroidia	Novel	1
Bacteroidota	Kapabacteria	Novel	1
Gemmatimonadota	Gemmatimonadetes	Uncultivated	1
Proteobacteria	Gammaproteobacteria		1
Proteobacteria	Gammaproteobacteria		1
Verrucomicrobiota	Verrucomicrobiae	Uncultivated	1

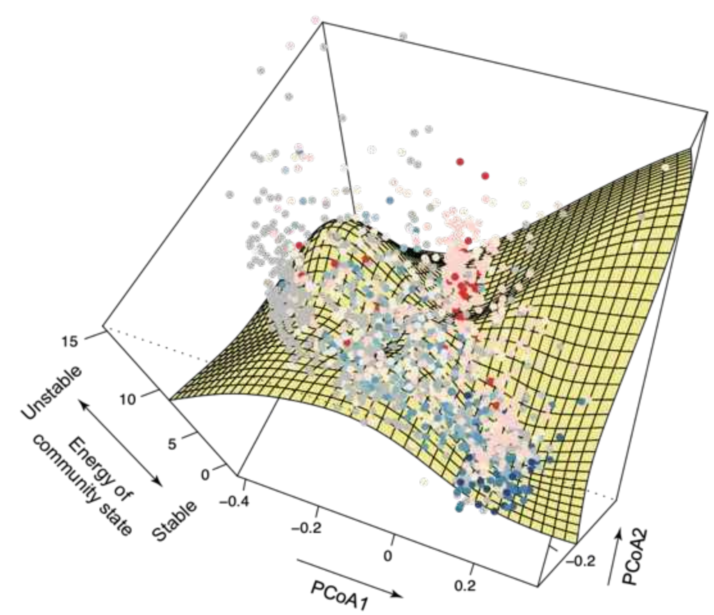
Unique technology: microdroplet-single cell sequencing

Access to uncultivated N_2O-reducing genomes

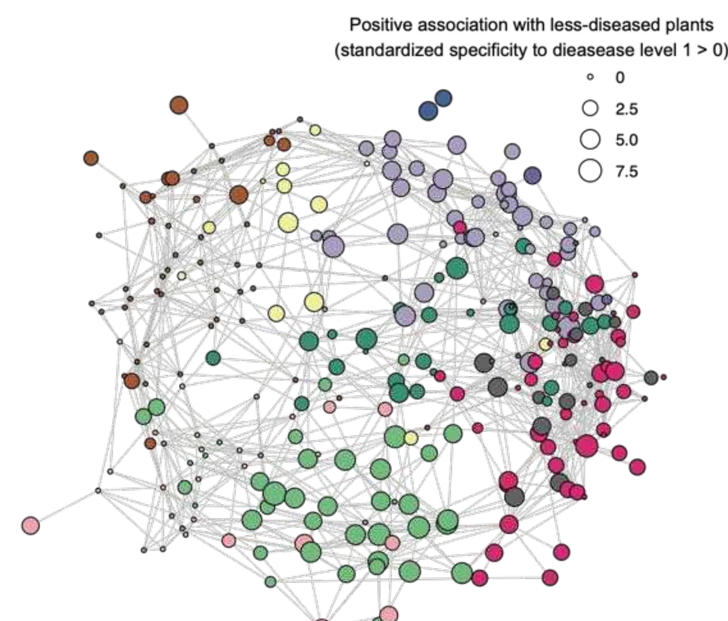
Single-cell genomics of >2,500 soil microbes identified more than 20 *nosZ*+ species with estimated N_2O-reducing functions!

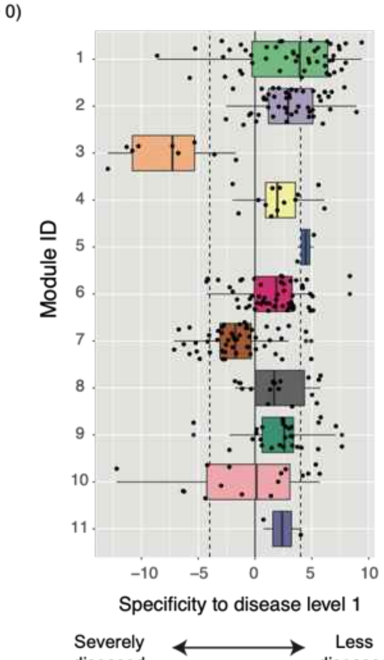
Profiling Japan-wide farmland soil microbiomes!





Stable and effective soil microbe mixtures!





bioRxiv accession: https://doi.org/10.1101/2022.08.23.505048

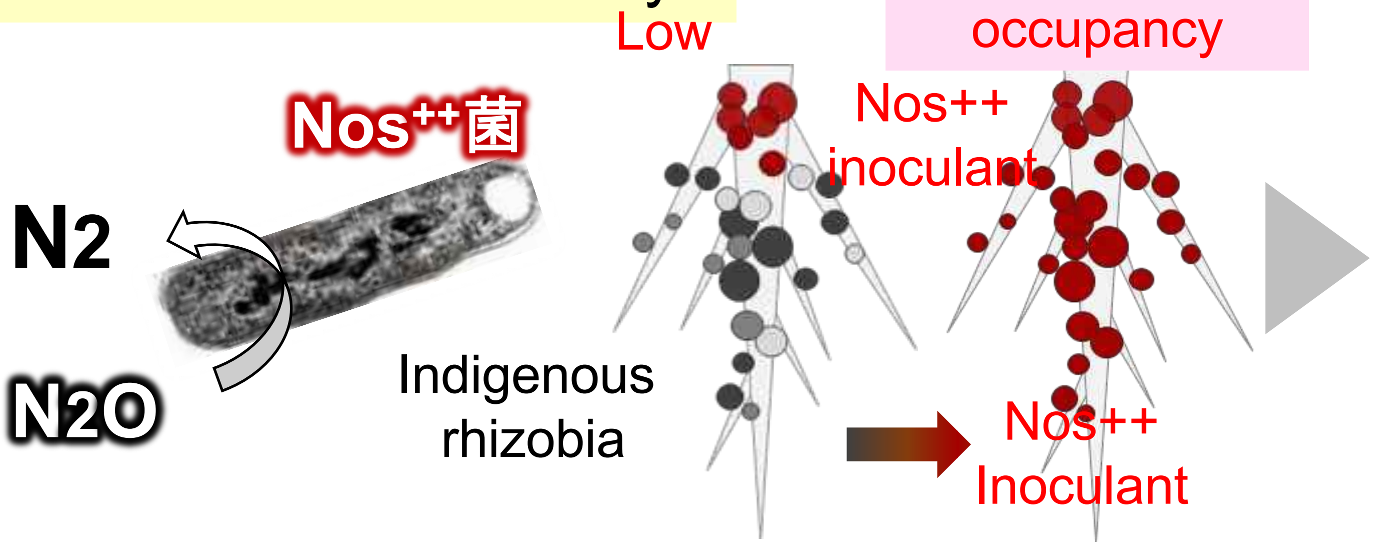
Goal of theme II-1

Previous inoculants (*nosZ*-)

Explore higher rhizobial strains with N2OR activity

Increase nodue occupancy

Theme III

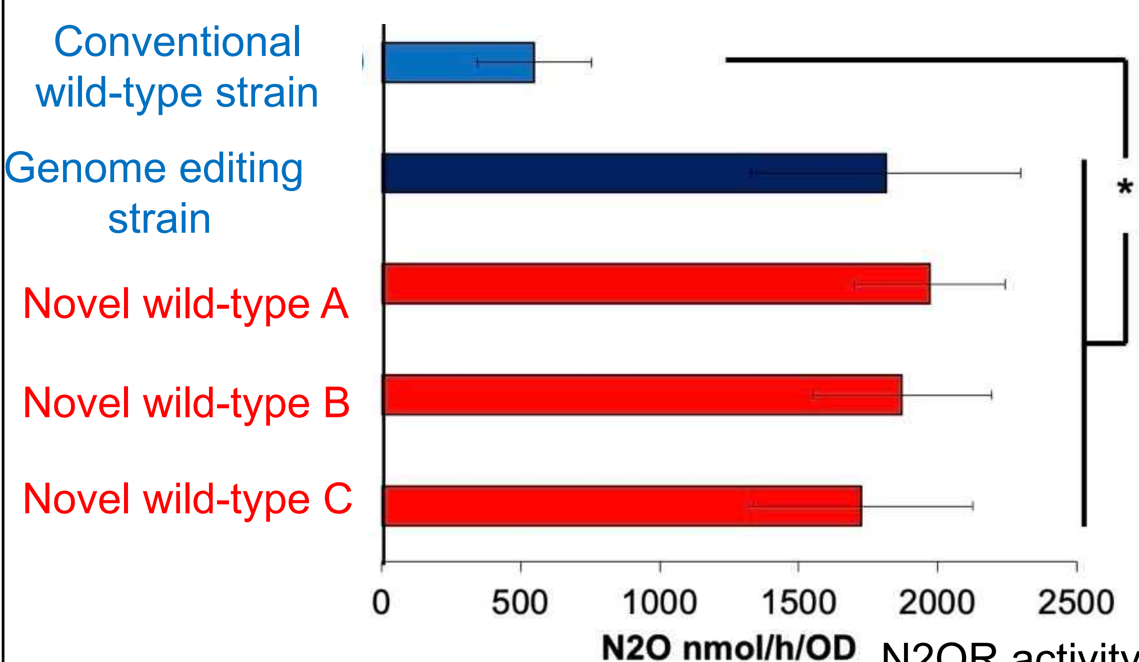


Reduce the amounts of inoculants.

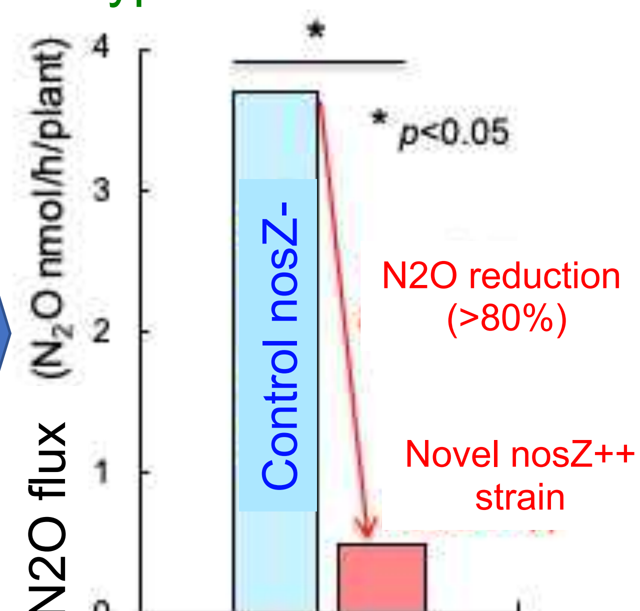
Optimization of host-rhizobia interactions (II-1-b)

Major results

We found wild-type NosZ++ strains rather than previous genome-editing strains.



N2O mitigation in rhizosphere by wild-type NosZ++ strains.

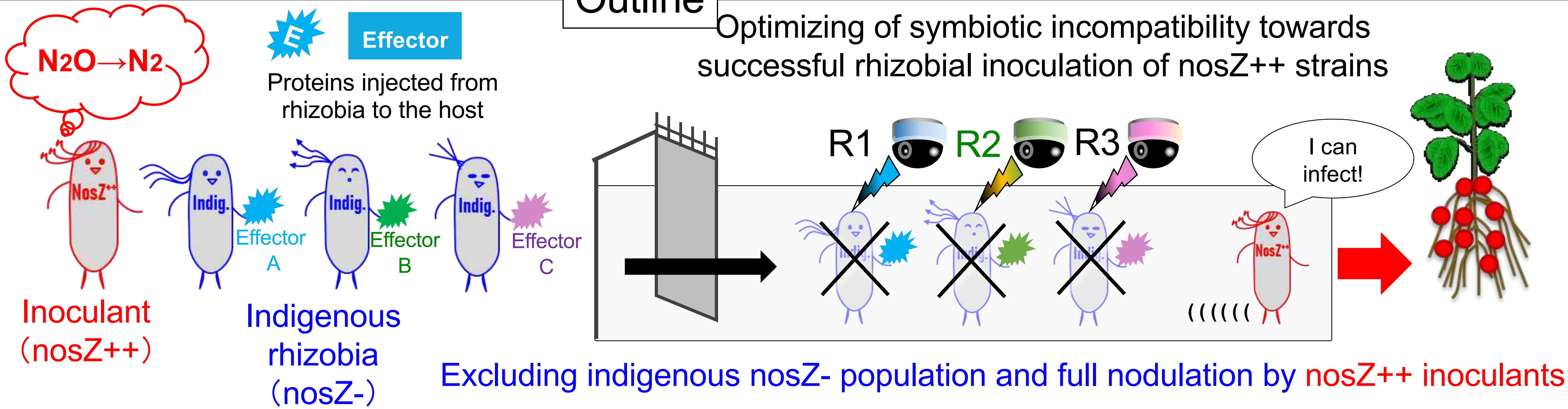


Can we use host factors?

Soybean core collection

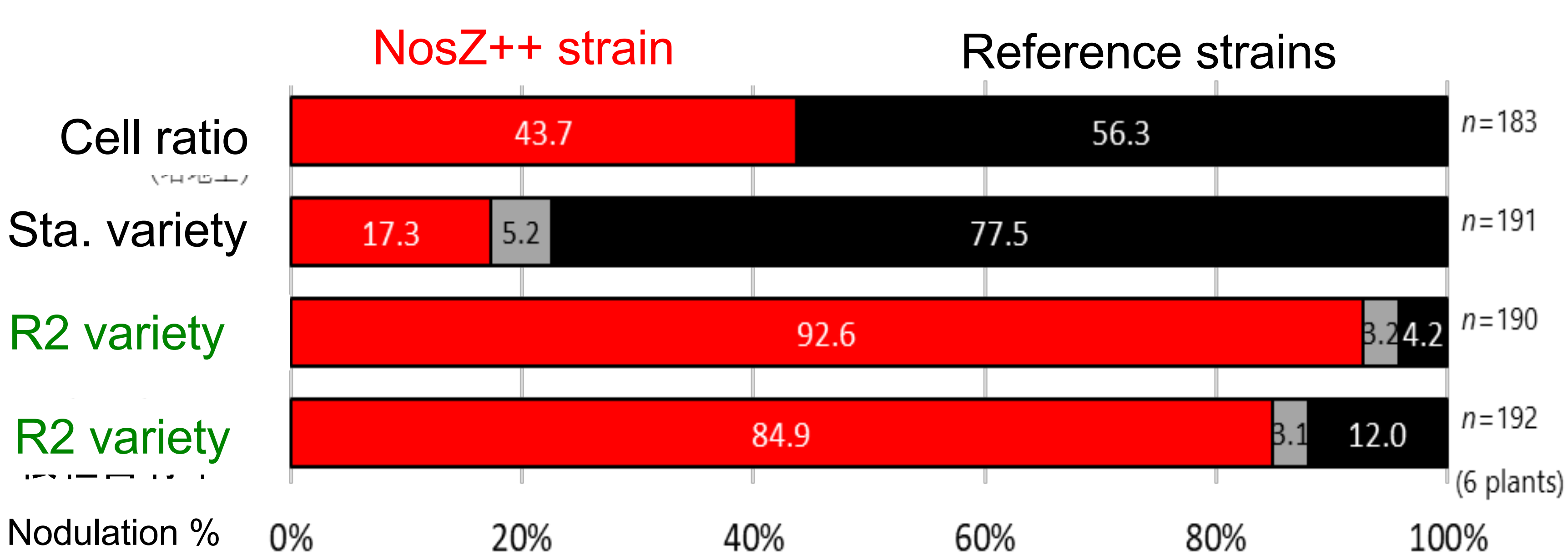
Ⅱ-1-b Optimizing of symbiotic interactions for successful rhizobial inoculation

Outline



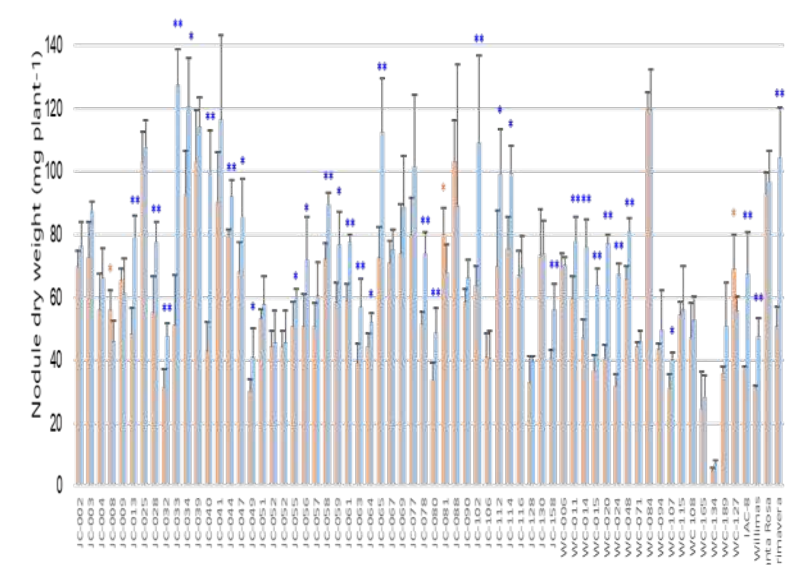
Major results

Competitive nodulation via host incompatibility B between nosZ++ and control strains.



Nodulation and N2 fixation

Blue: NosZ++ strain



The presence of host R2 elevated nodulation percentage of nosZ++ strain (>80%).

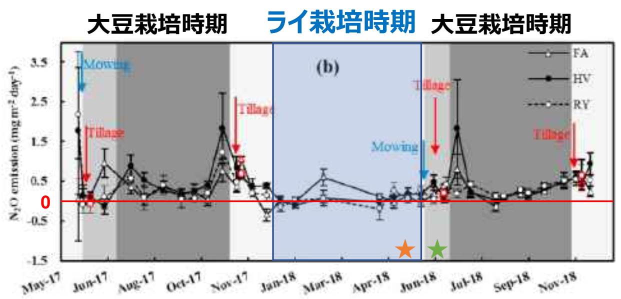
NosZ++ strain often increased nodulation and N2 fixation.

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

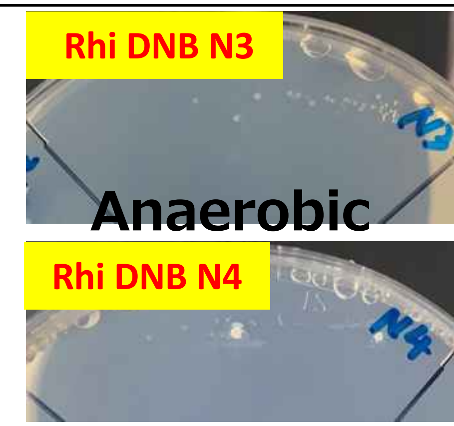
Ⅱ-2 Detoxification of N_2O by rhizospheric/symbiotic microorganisms

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

2017年5月～2018年12月のN_2O Flux (Gong et al., 2021)

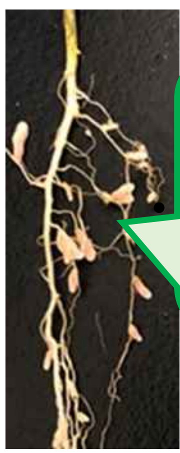


カバークロップ：FA, Fallow; HV, Hairy vetch; RY, Rye
メタゲノムデータ：★, 刈倒前；★, 刈倒後



Microbial Inoculants

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

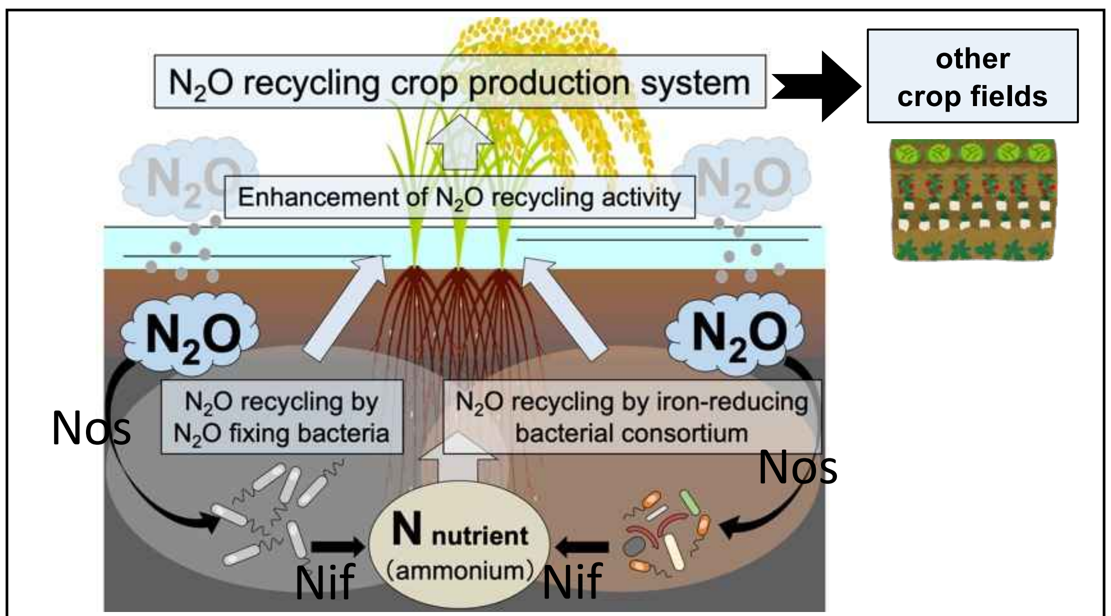


・Rhizobia(*nosZ*+)
Non-Rhizobial bacteria(*nosZ*+)
isolated from nodule

Highly active strains
from Abekawa,
Narusegawa,
and other fields

Abilities
N_2O reduction
N fixation
Nodule formation

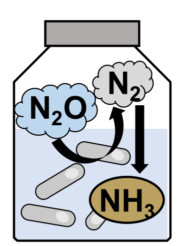
Ⅱ-3 N_2O recycling by N_2O fixing bacteria and microbial consortia（UTokyo・NAIST）



other crop fields

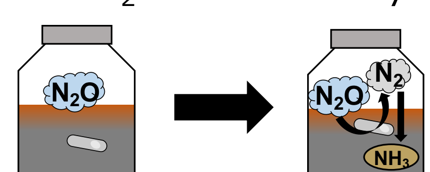
Convert N_2O into N fertilizer for its effective use

N_2O fixing ability of paddy soil microbes and amount of fixed N

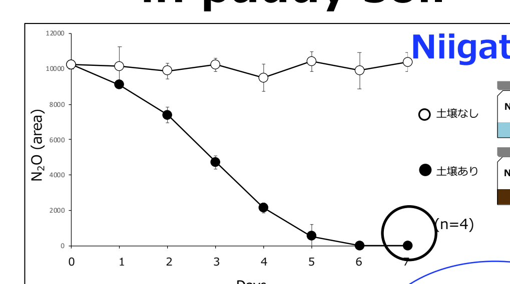


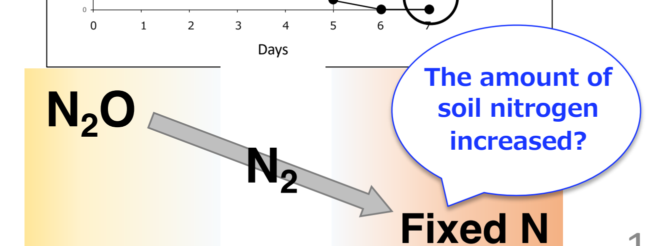
In the presence of N_2O and small amounts of N_2, growth was stable and N_2O was reduced

How much N_2O was fixed by bacteria?



Amount of fixed N_2O and N_2O fixing bacterial consortia in paddy soil





Minamisawa MS project subject Ⅱ. N_2O Recycling

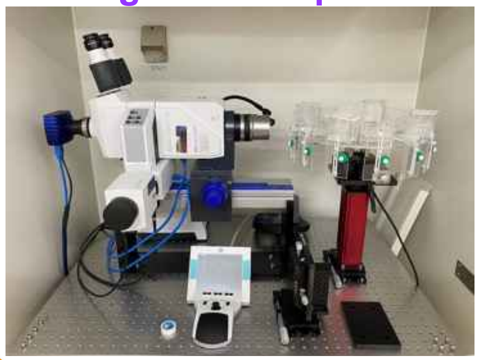
Ⅱ-4. Construction of the rhizosphere cultivation system for designing and evaluating the soil ecosystem for N_2O recycling

(Ryukoku Univ・Betsuyaku G/NaganoG)

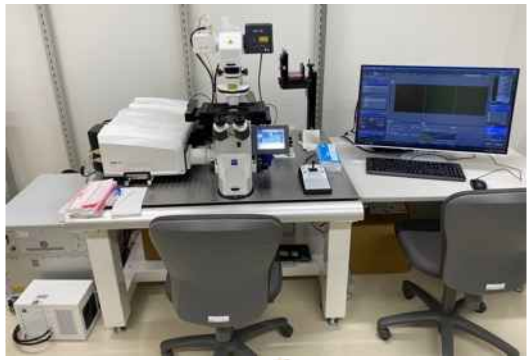
Goal for 2022; Construction of a rhizosphere chamber system for *in situ* imaging of *plant-microbe interactions （+ supports for the other groups' imaging/RNAseq） *Focusing on the interaction between rice & CH_4 -oxidizing bacteria

Betsuyaku G (Imaging)

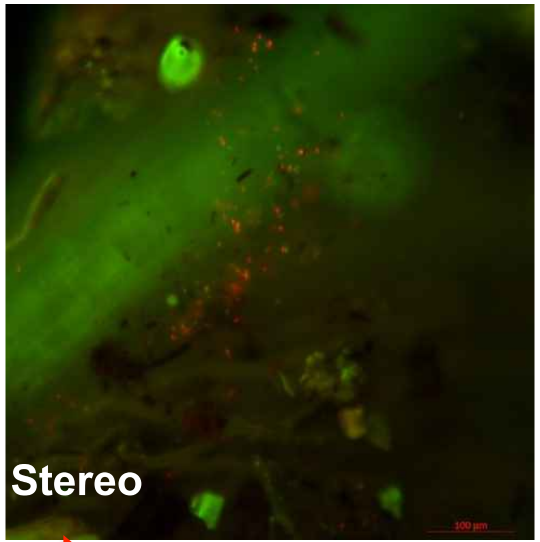
Fluorescent Stereomicroscope handling multisamples

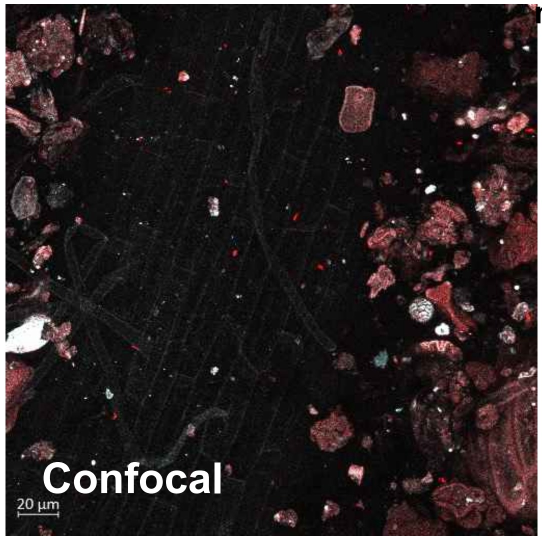


Confocal spectral microscope



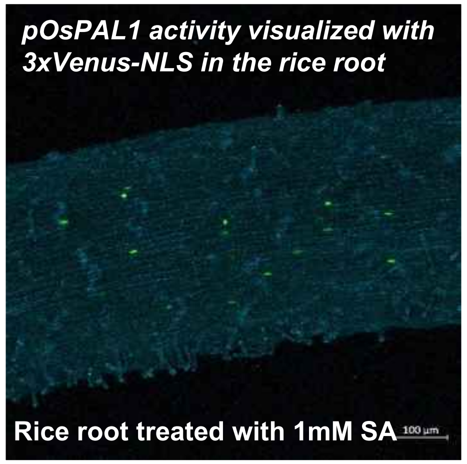
Visualization of bacteria associated with rice root in the soil (root box)





+

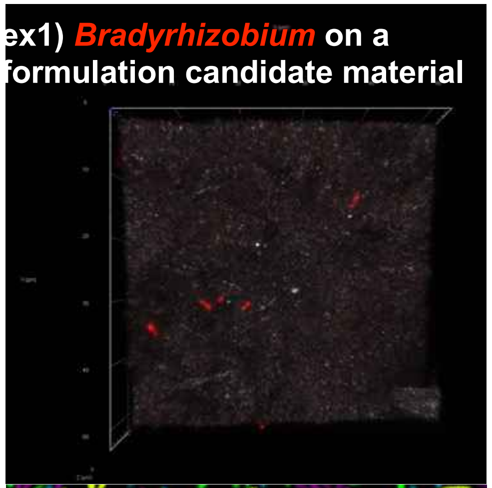
Visualization of the promoter activity of the rice genes (x6) involved in microbial interaction （Fluorescent-reporter GM rice）

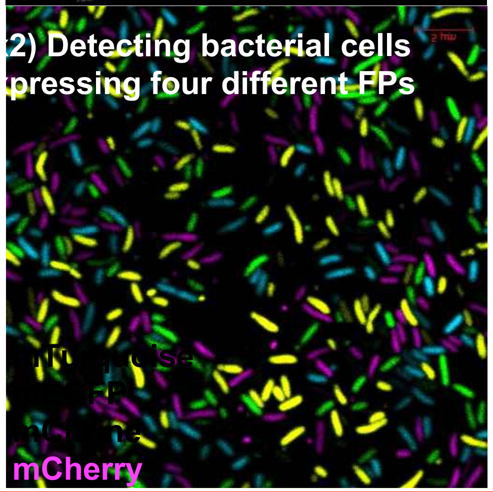


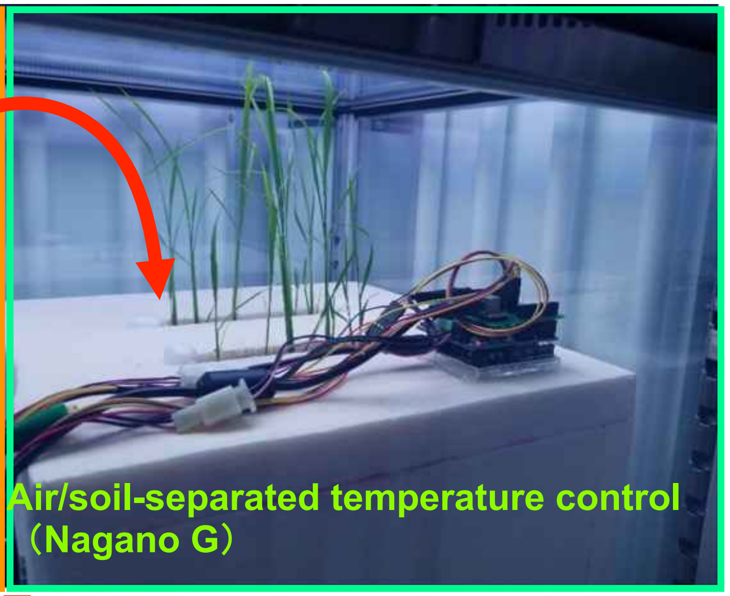
➡ Unraveling the bacterial localization in the roots and the molecular basis for its association with the roots

Collaborations within the MS

Assisting imaging experiments and NGS analyses in the other groups







⬇

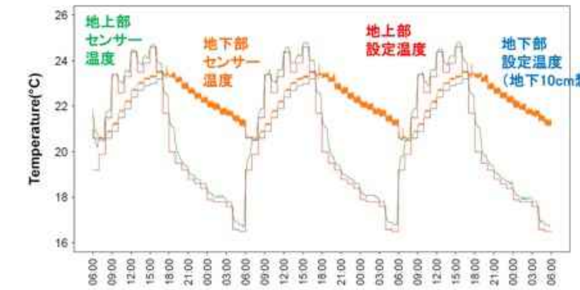
Revealing the molecular basis for rice-CH_4 recycling bacteria interaction with CH_4 group (unexplored "blue ocean" !!)

⬇

Promotion of CH_4 recycling activity in rice rhizosphere and formulation of the bacterial usage

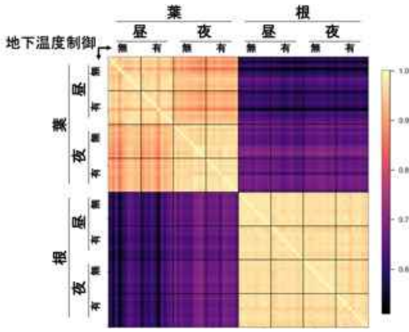
Nagano G (Environmental control/RNAseq)

Reconstitution of air/soil temperature shifts in natural condition

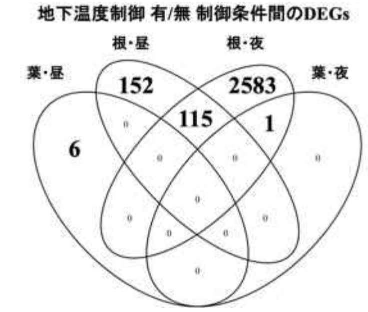


（同じ条件を3日間繰り返している）

Evaluation of the separated regulation on plants (RNAseq)



Revealing a strong effects on the root gene expression



➡ Reconstitution of field environments and evaluation of its effect on the CH_4 recycling activity within rhizosphere

農研機構 NARO

Ⅲ-1 Development of super active N_2O-detoxifying microorganism
Ⅲ-2 Development of new nitrification and denitrification inhibitors

Goal

Reduce N_2O emissions by the comb-ined use of molecular-targeted drugs and super active N_2O-detoxifying mi-croorganisms

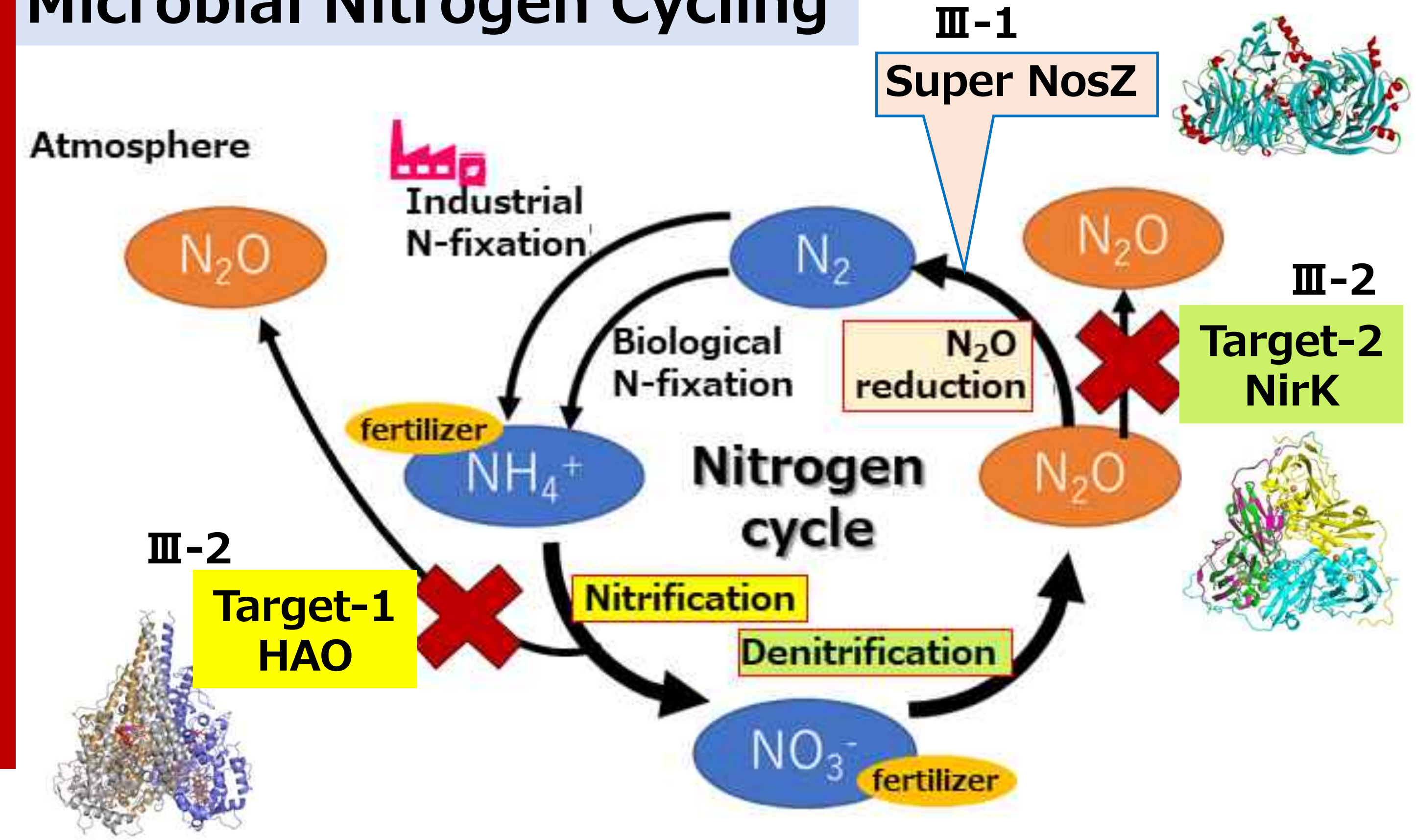
Ⅲ-1

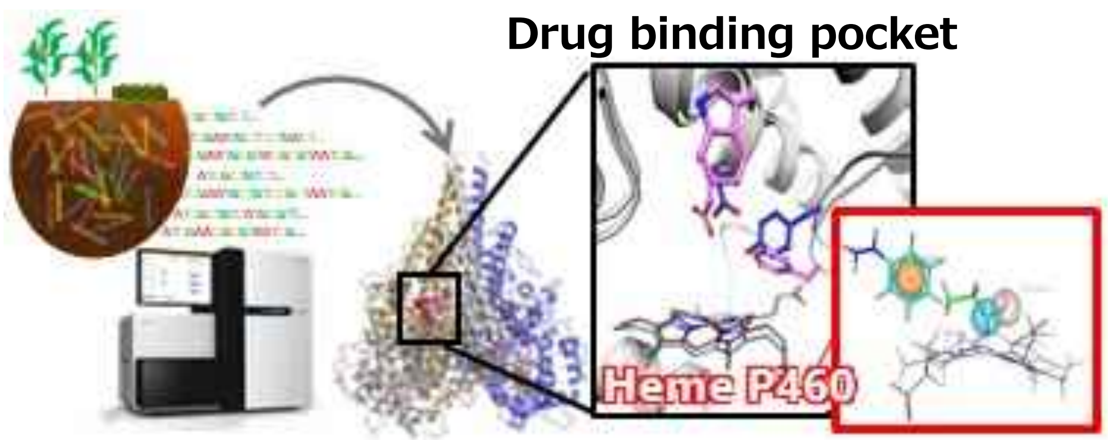
N_2O-detoxifying microorganism with super active NosZ

Ⅲ-2

HAO-targeted nitrification inhibitor
NirK-targeted denitrification inhibitor

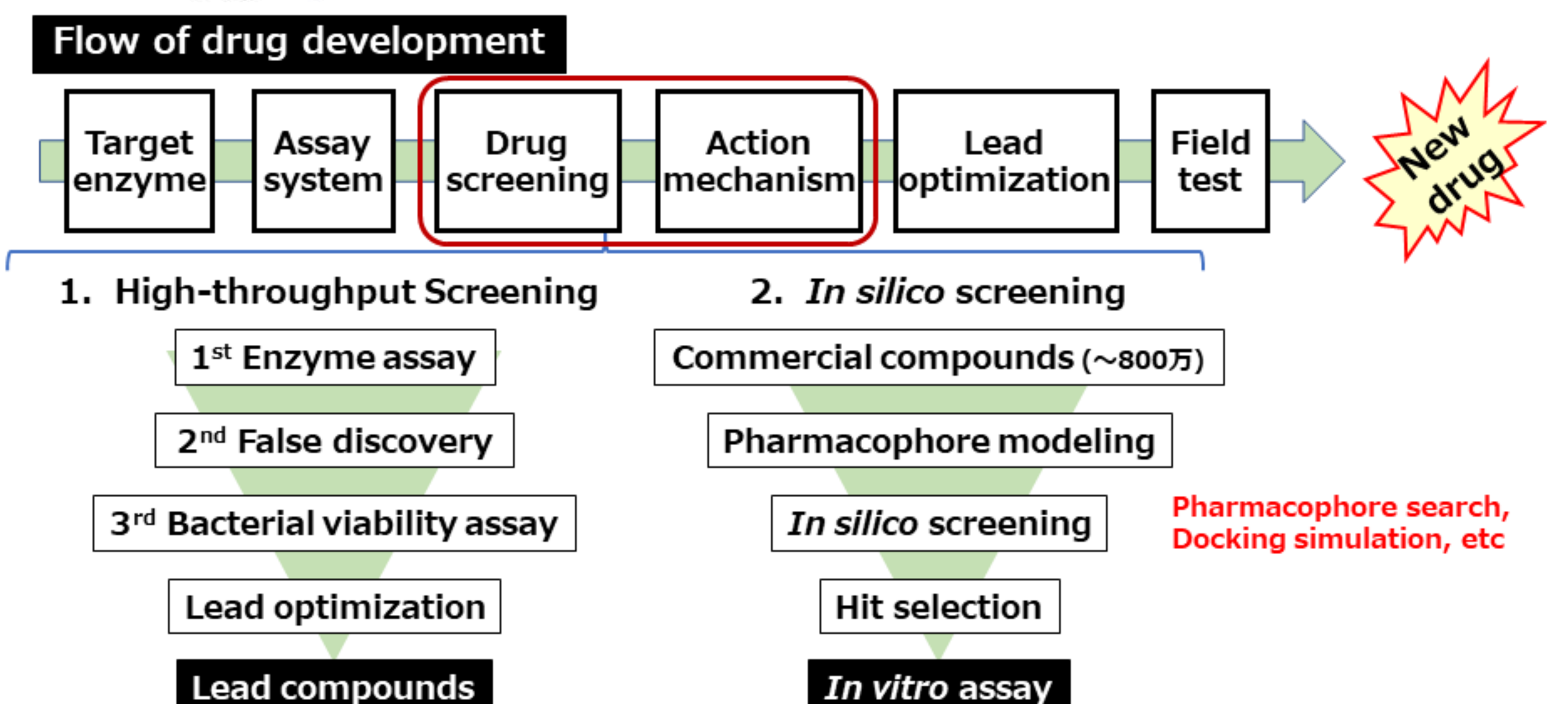
Microbial Nitrogen Cycling





Metagenomic analysis
Structure-based drug design

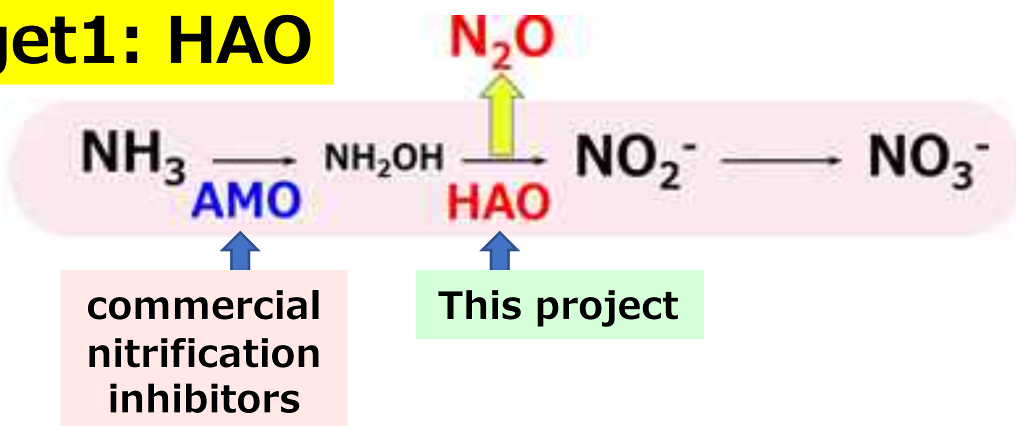
Molecular-targeted drugs effective for uncultured soil microorganisms



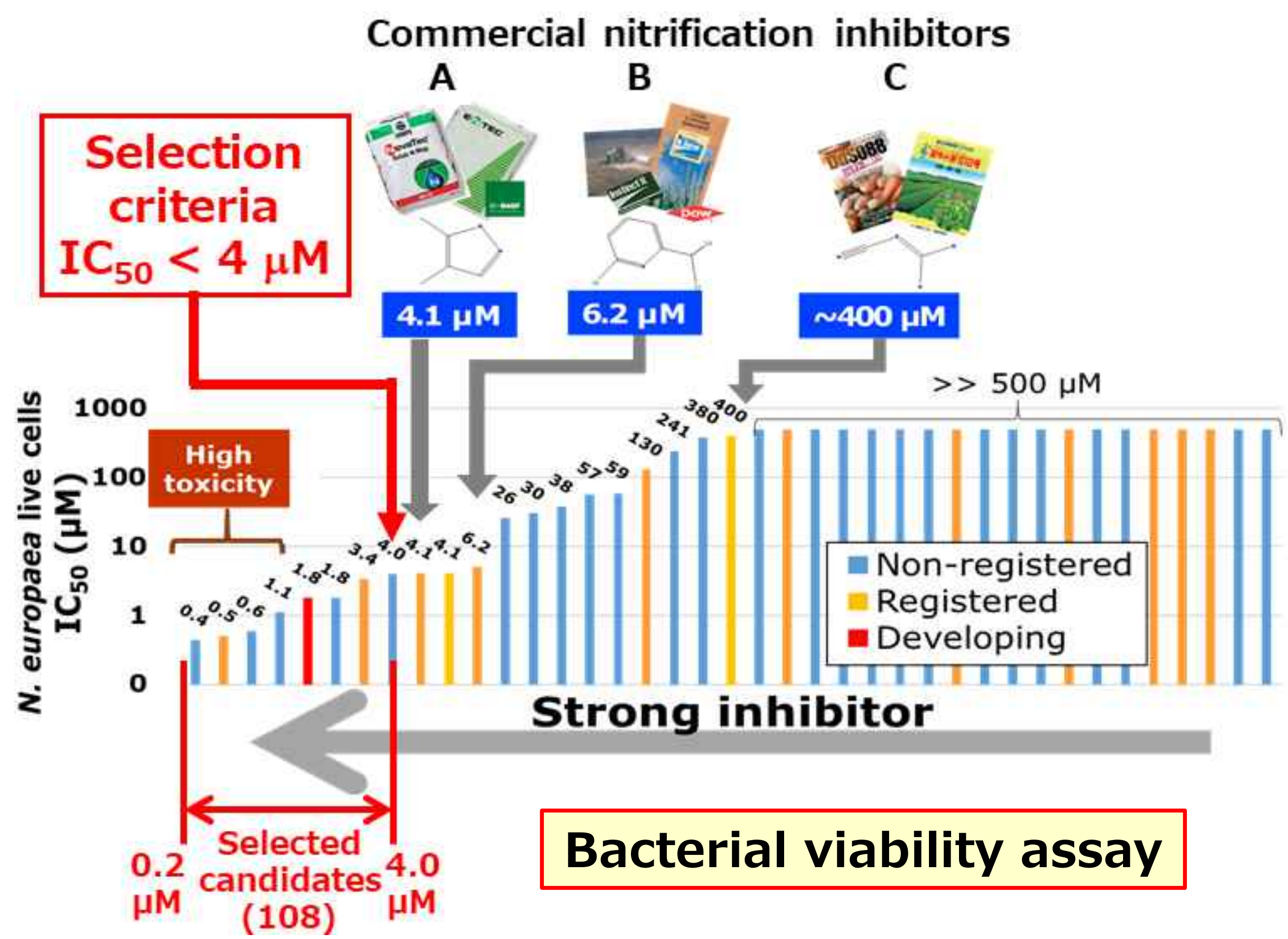
Ⅲ-2 Development of new nitrification and denitrification inhibitors

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

Target1: HAO



✓ Metagenome analyses revealed that more than 99% of HAO in soil are the β-AOB type.
✓ We obtained **108 HAO-targeted nitrification inhibitor candidates** which have **much higher activities (IC_{50} < 4.0 μM) than commercially available inhibitors**.
✓ X-ray crystallographic studies showed that the above candidates are classified into three types of inhibitors, competitive inhibitors, suicide inhibitors, and electron transfer inhibitors.



Target2: NirK

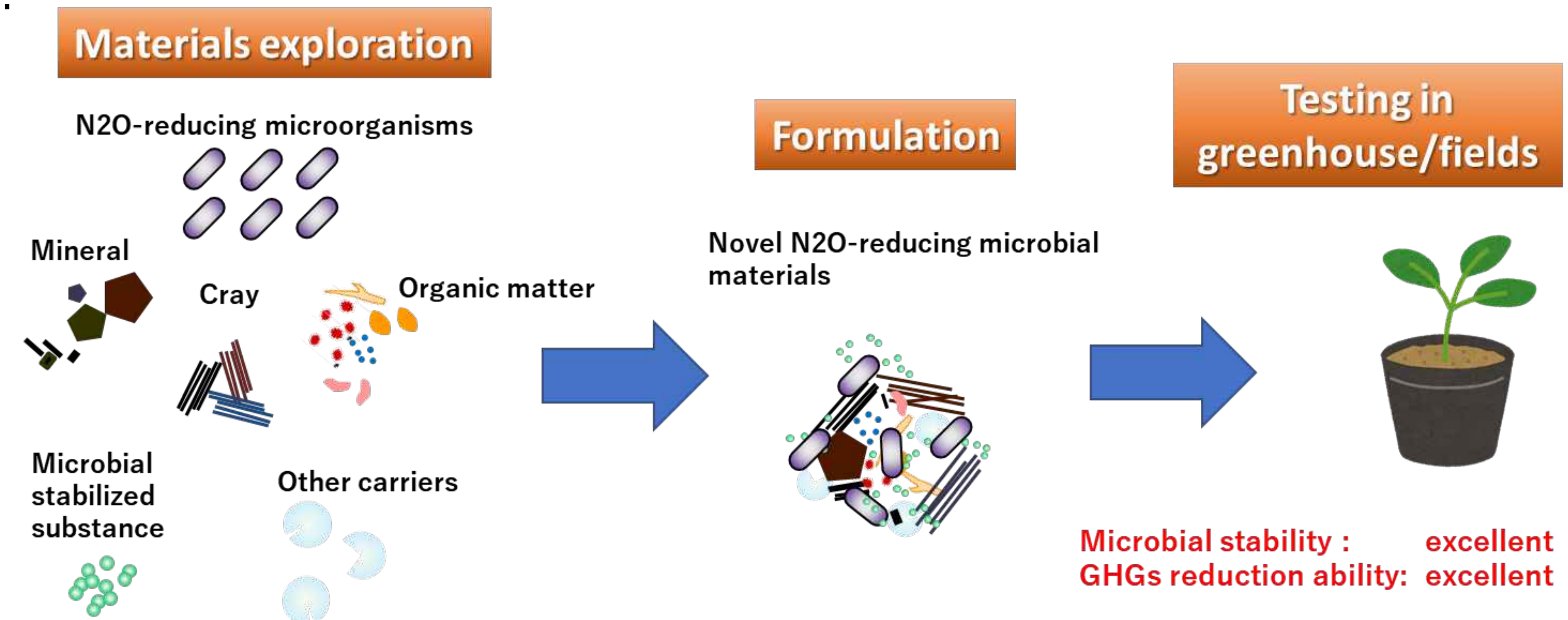
$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

NirK

✓ A new analytical method was established to precisely distinguish pseudo NirK from real ones.
✓ A high throughput screening provided **100 Nirk inhibitors (IC_{50} < 10 μM)** out of about ten thousand compounds.

Research Objective for Theme III-3

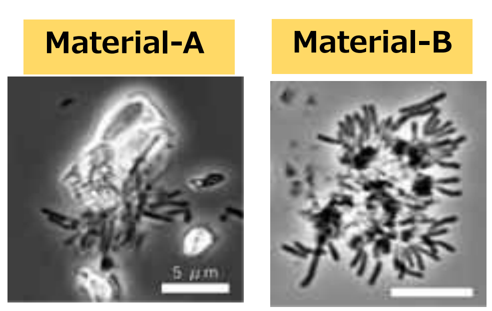
Construction of carriers that mimic soil microstructure and evaluation of their microbial colonization performance. Development of microbial materials that enables N_2O-reducing microorganisms to function stably in soil.



Materials exploration

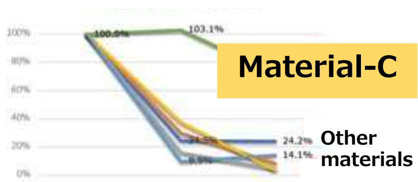
Successful acquisition of material candidates for microorganism adhesion and protection

Soild carrier screening



Survival rates of microbial inoculant during seed storage with candidate materials

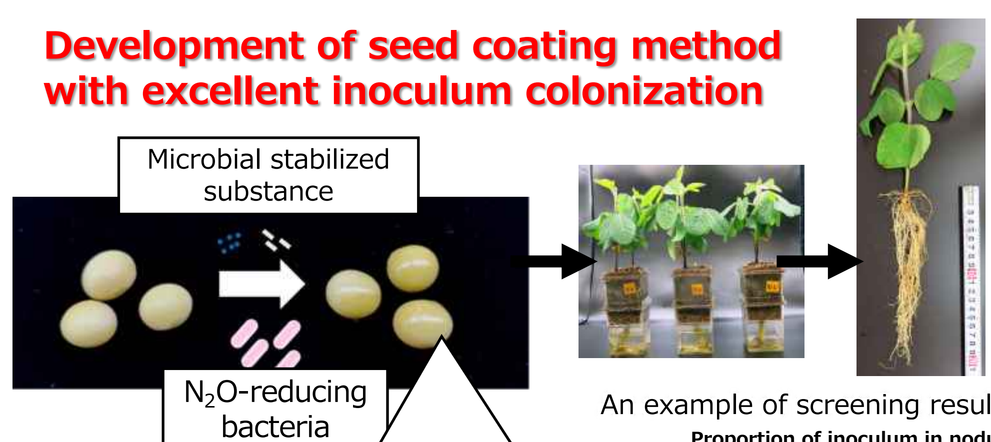
Adhesive polymer screening



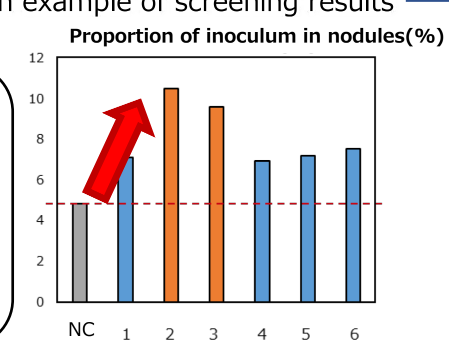
Formulation①

Microbial seed coating

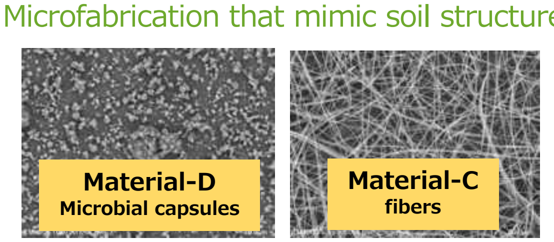
Development of seed coating method with excellent inoculum colonization



An example of screening results



Microfabrication that mimic soil structure

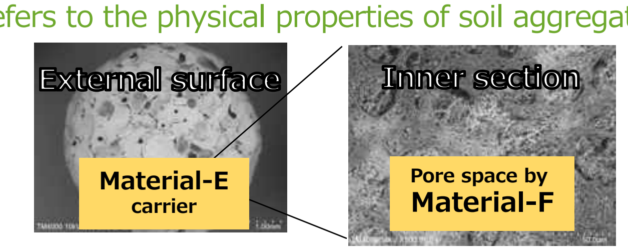


Formulation②

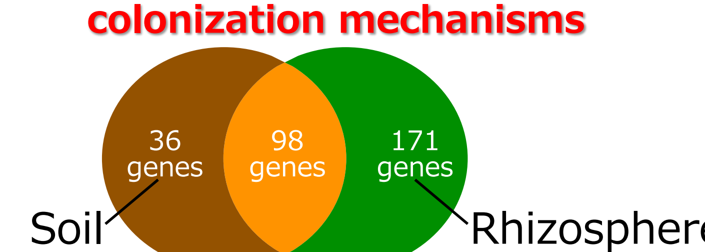
Carrier materials

Design of soil mimic carriers and evaluation of their N_2O uptake potential

Refers to the physical properties of soil aggregates



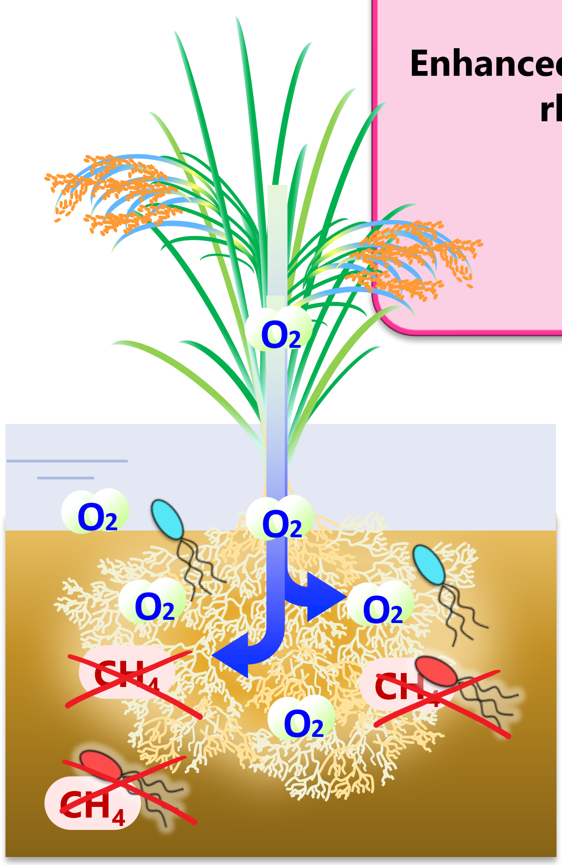
Establishment of microbial material evaluation system by using microbial colonization mechanisms



Microbial colonization genes revealed by Tn-seq

Theme IV: Strategy to achieve 80% reduction

Technology 1:
Low-CH_4 rice
(IV-1)



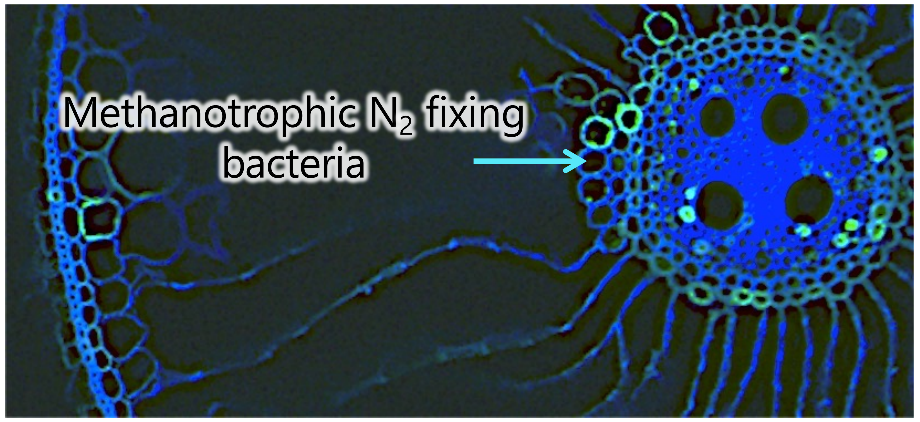
Enhanced CH_4 oxidation in rhizosphere

40%↓

Technology 2:
Microbial inoculants
(IV-2)

Maximizing Syntrophic CH_4-oxidation & N_2 fixation

40%↓



Bao et al. 2014 AEM, Shinoda et al. 2019 SBB

Existing technologies

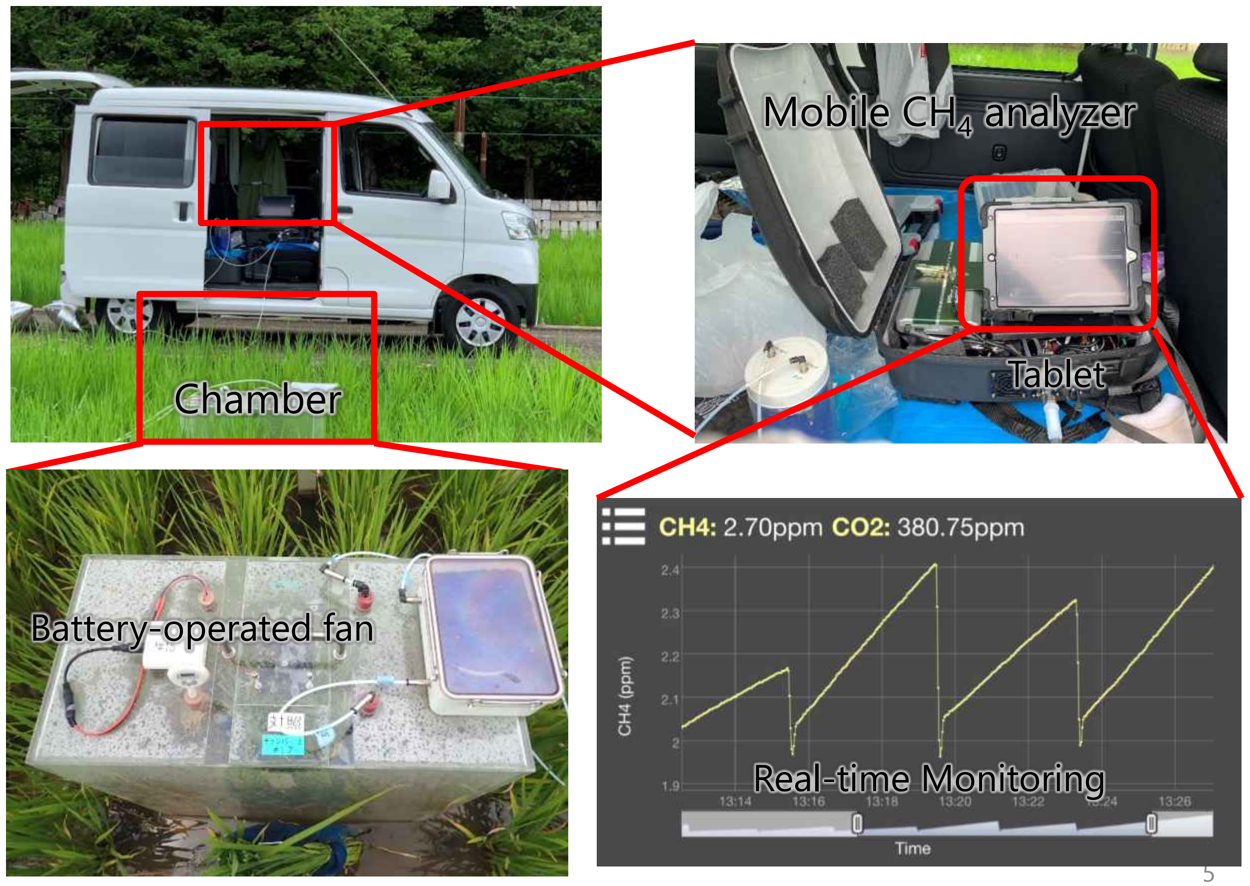
Water & Organic matter Management

40%↓

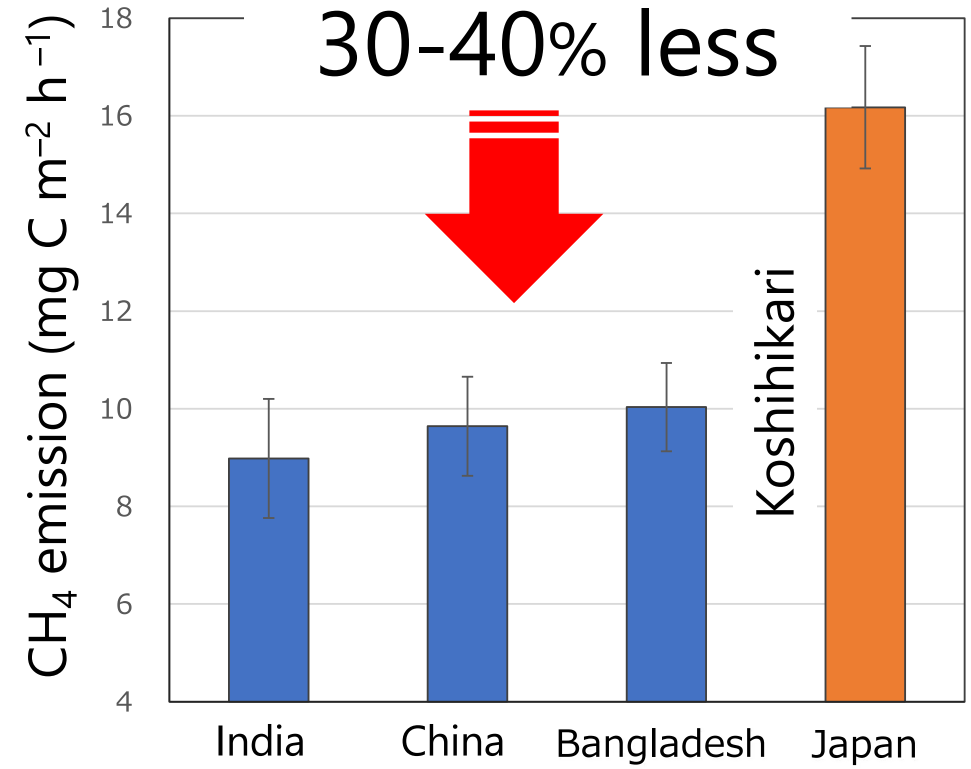
80%
reduction

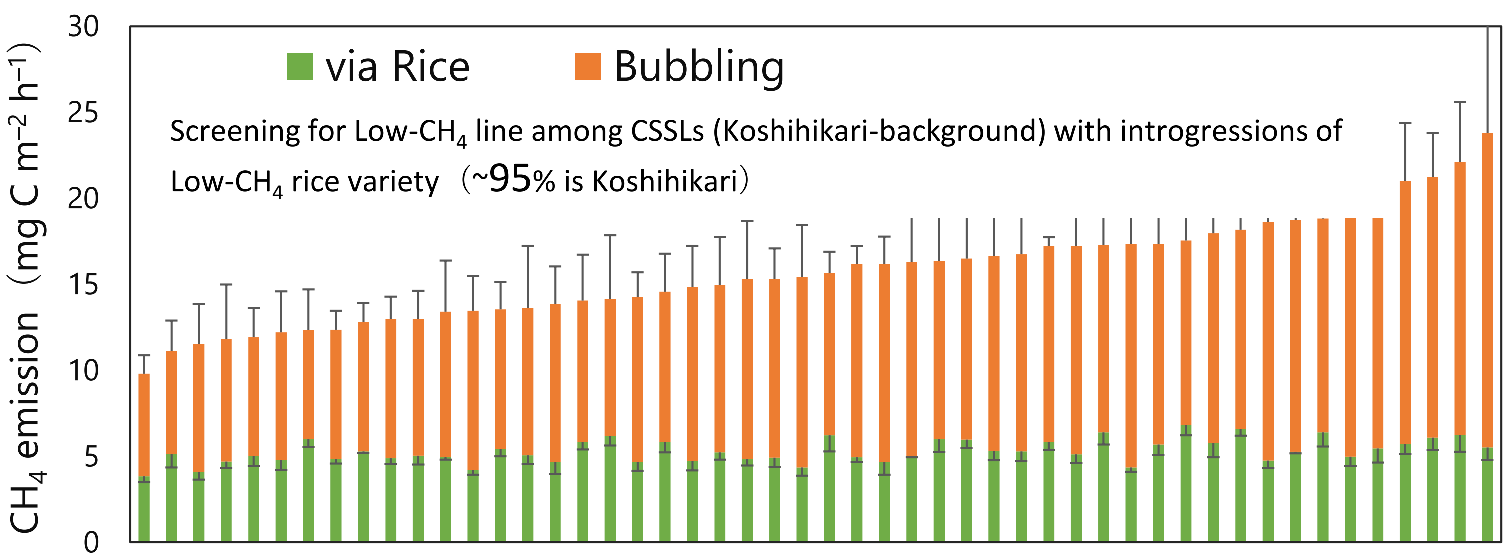
IV-1 Low-CH_4 Rice

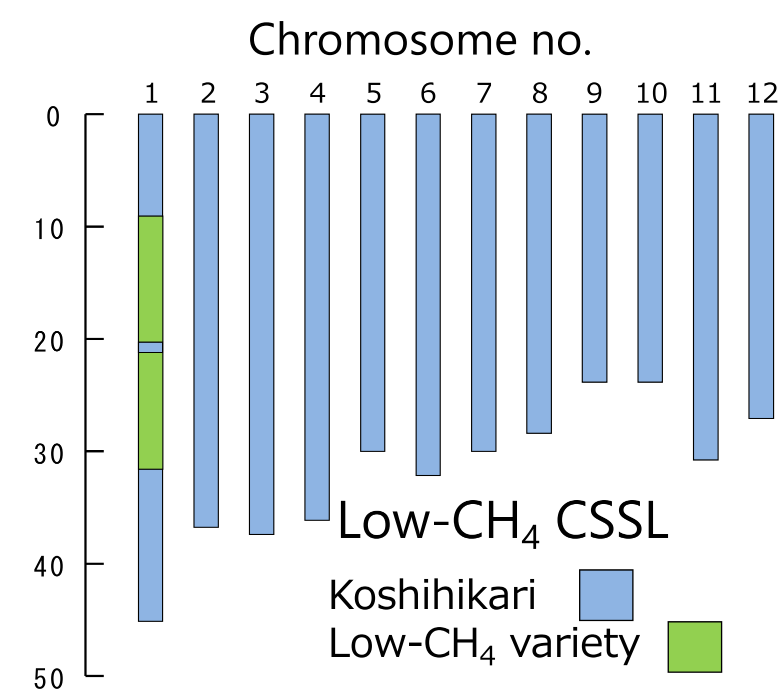
High-throughput CH_4-emission measurement



Screening for Low-CH_4 rice variety

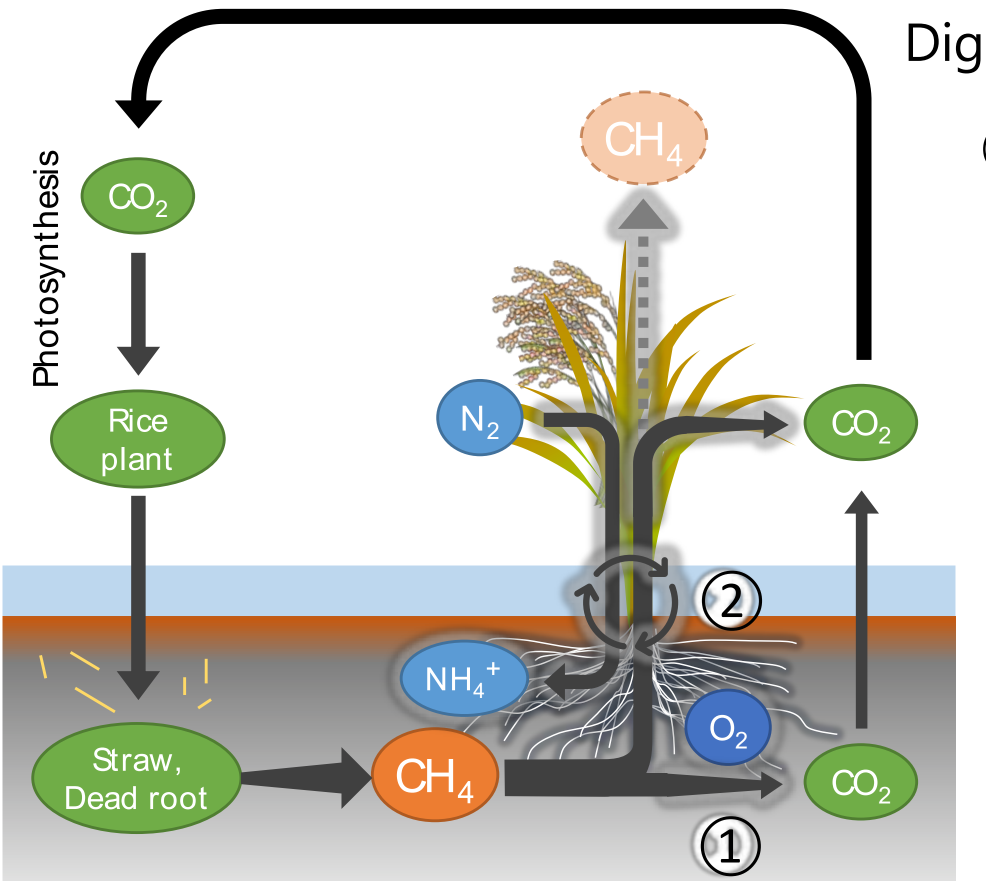






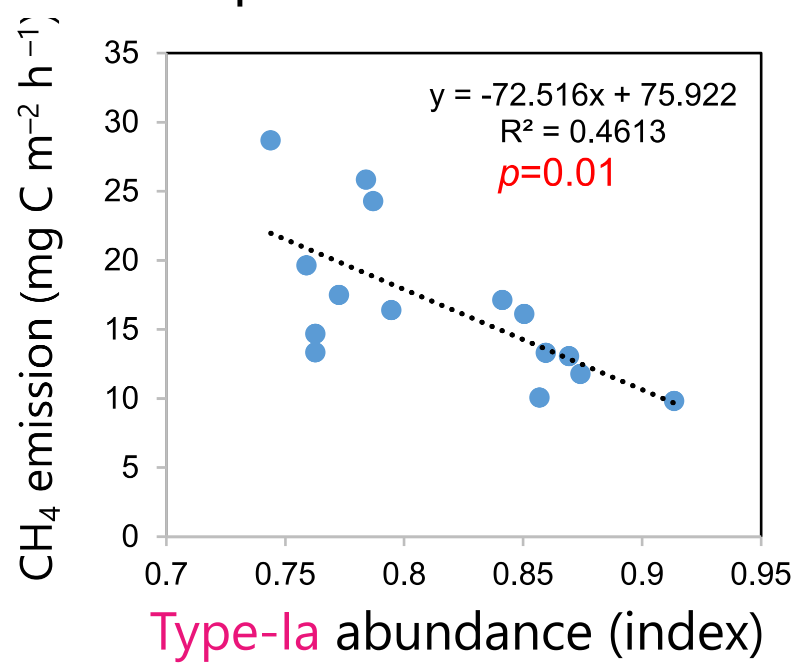
Developing Low-CH_4 Koshihikari using Chromosome Segment Substitution Lines (CSSLs)

IV-2 Maximizing diazotrophic methanotrophy

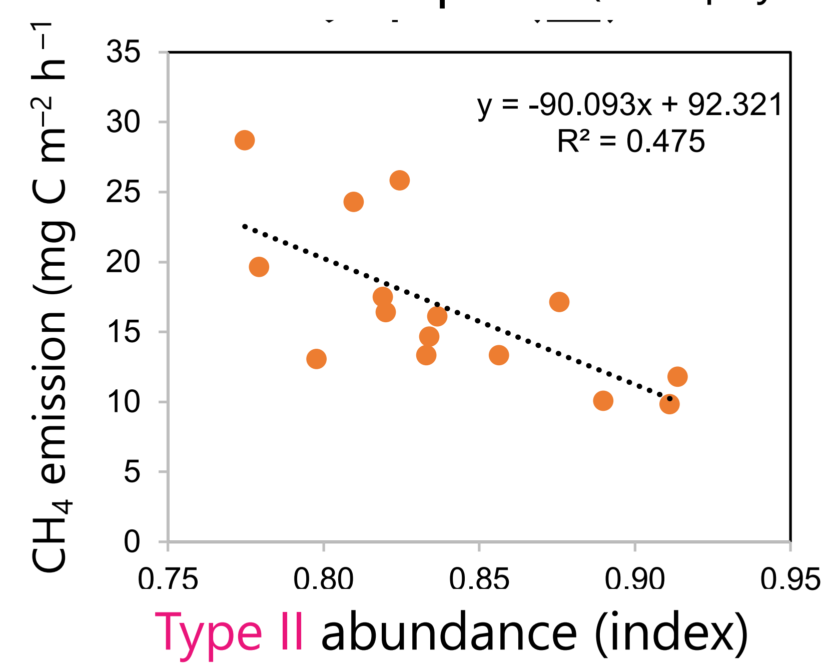


Digital PCR- Type of Methanotroph responsible for CH_4 emission reduction

① Rhizosphere soil (Root surface)



② Inside the rice plant (endophytic)

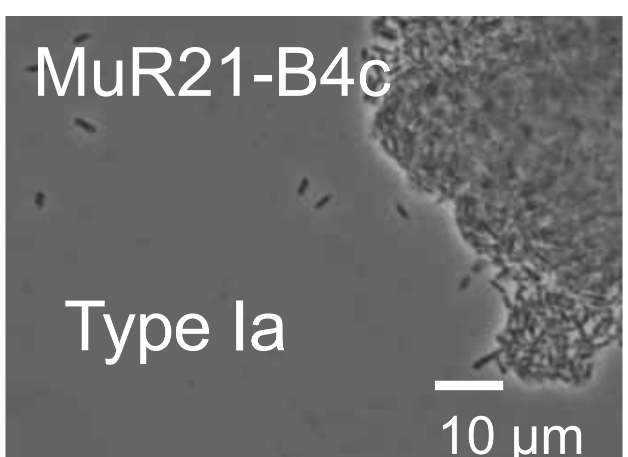


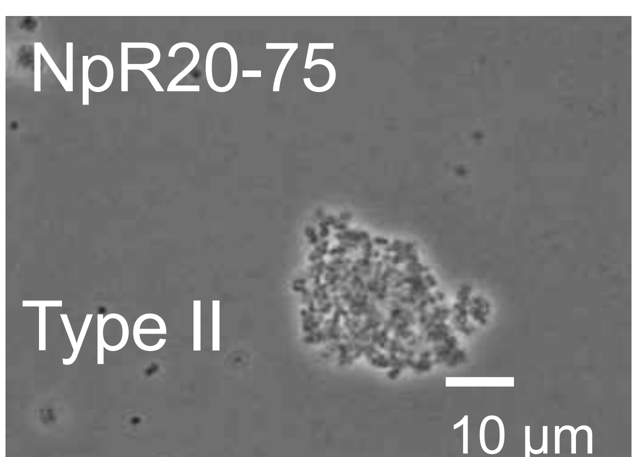
Isolation of highly-active methanotrophs

12 strains: Isolated

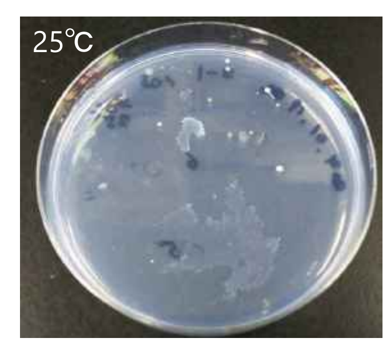
Type Ia- 2 strains、Type II-10 strains

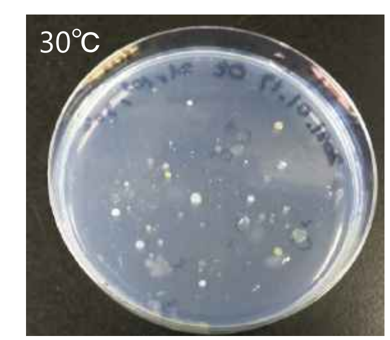
11 strains: Diazotrophy confirmed





Agar plate





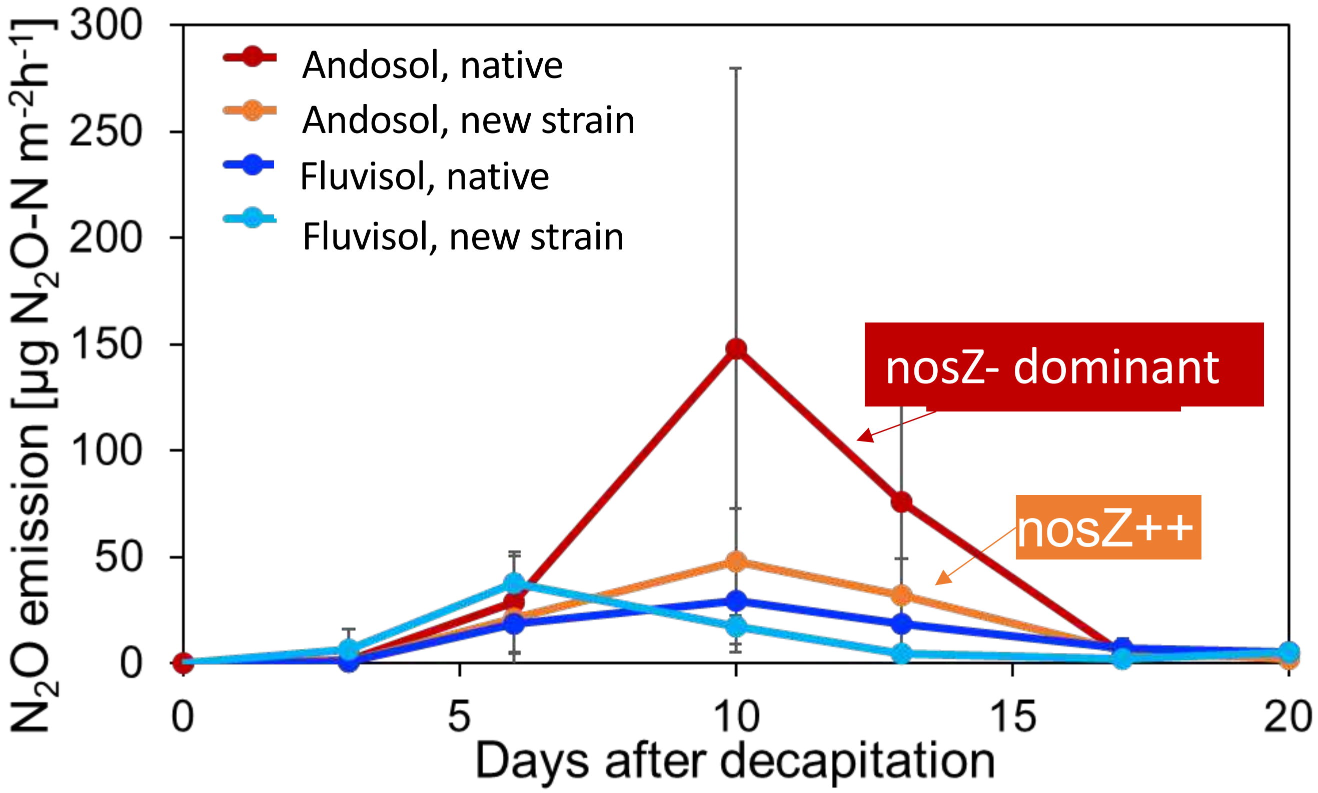
Colonies on NMS agar plate

Liquid enrichment culture

upper: with methanotrophs
Lower: Control

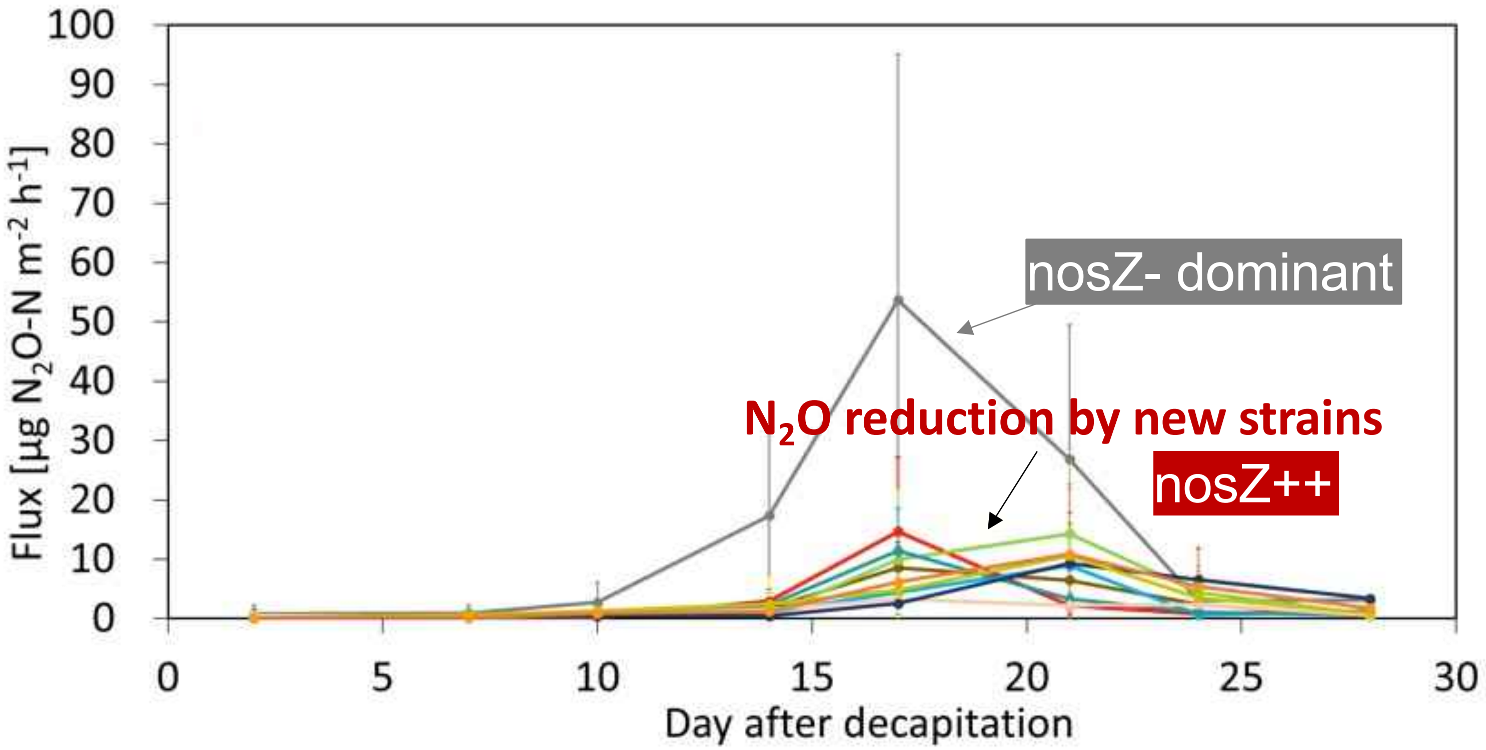
N_2O reduction by inoculation of new strains of *Bradyrhizobium sp.* (pot experiment)

N_2O reduction by inoculation of a new strain of *Bradyrhizobium sp.*



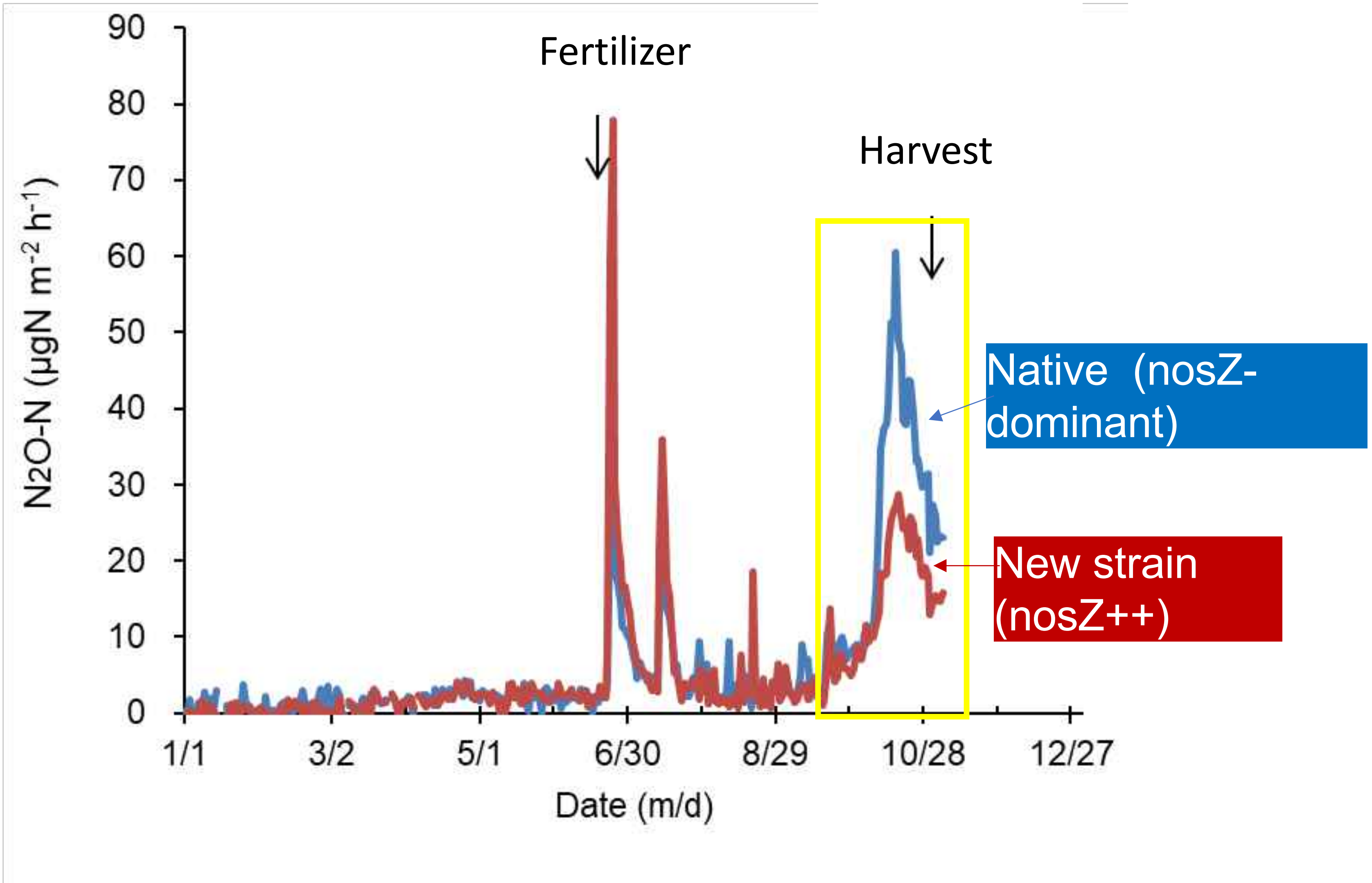
- ✓ N_2O reduction by a new strain (*nosZ++*) in Andosol (*nosZ- dominant*)
- ✓ No reduction of N_2O by a new strain (*nosZ++*) in Fluvisol (*nosZ+ dominant*)

N_2O reduction by inoculation of new strains of *Bradyrhizobium sp.*



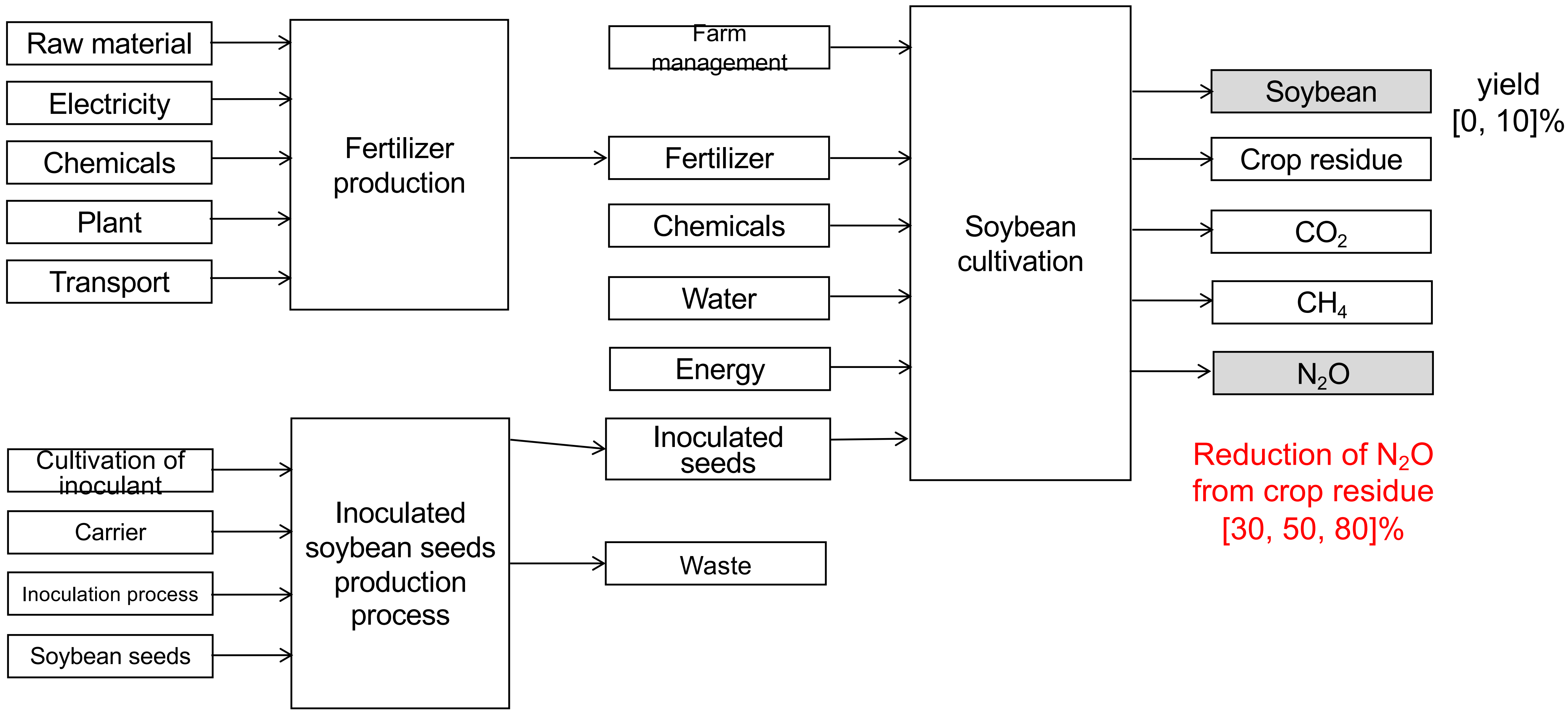
- ✓ N_2O reduction by new strains (*nosZ++*) in Andosol (*nosZ- dominant*)

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



- N_2O reduction by a new strain during nodule decomposition (October) in Andosol (*nosZ-* dominant)

LCA (Life cycle assessment) of soybean production

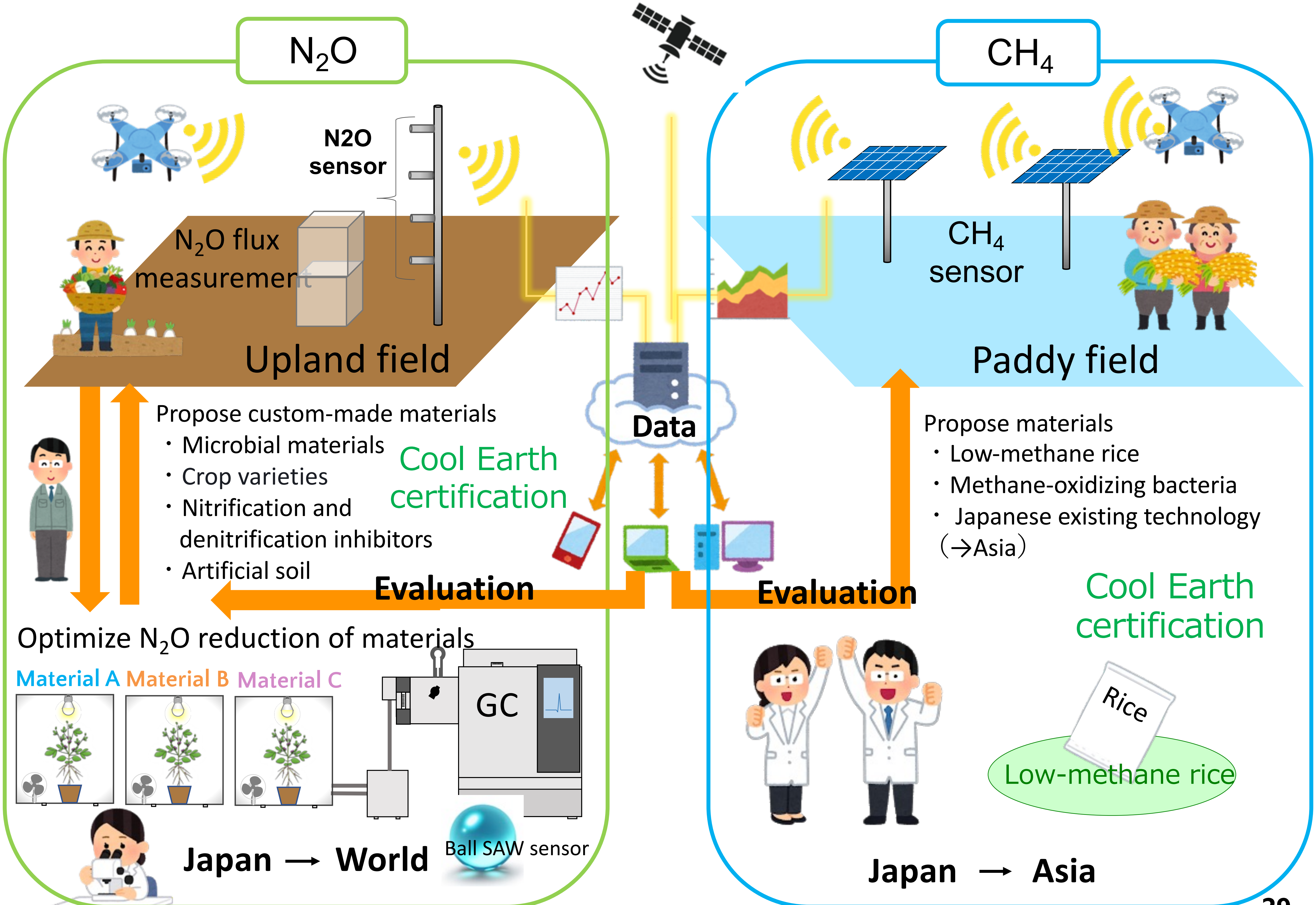


GHG emission from inoculant production is only <u>0.004% of whole soybean production process</u>

↓

GHG emission of inoculant production is negligible

Business plans for N_2O and CH_4 mitigation in agriculture



Citizen Science Project（ELSI）

Citizen Science is listed as a key issue
by the 6th Science and Technology Basic Plan and EU Soil.

https://dsoil.jp/soil-in-a-bottle/

Overview of Citizen Science Project

Citizen Science Project（ELSI）

Citizen Science is listed as a key issue
by the 6th Science and Technology Basic Plan and EU Soil.

Soil in a Bottle 市民科学プロジェクト 地球冷却微生物を探せ

概要 活動内容 参加登録 運営組織 お問い合わせ

Exploring novel N_2O-reducing bacteria

Isolation of microbes with higher N_2O-reducing activity and elucidation of their habitat environments. Future plans include the use of them as agricultural inoculants.

Dialogue and scientific knowledge with citizens

Rising of citizen interests on the global environmental issues through our research experiments and interactive communications. Creation of citizen culture of enjoying science.

Large datasets of soil microorganisms

A large number of soil and air samples from all over Japan creates large-scale datasets (microbiome and metadata) for soil microbiology and environmental researches.

https://dsoil.jp/soil-in-a-bottle/

MOONSHOT

RESEARCH & DEVELOPMENT PROGRAM