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

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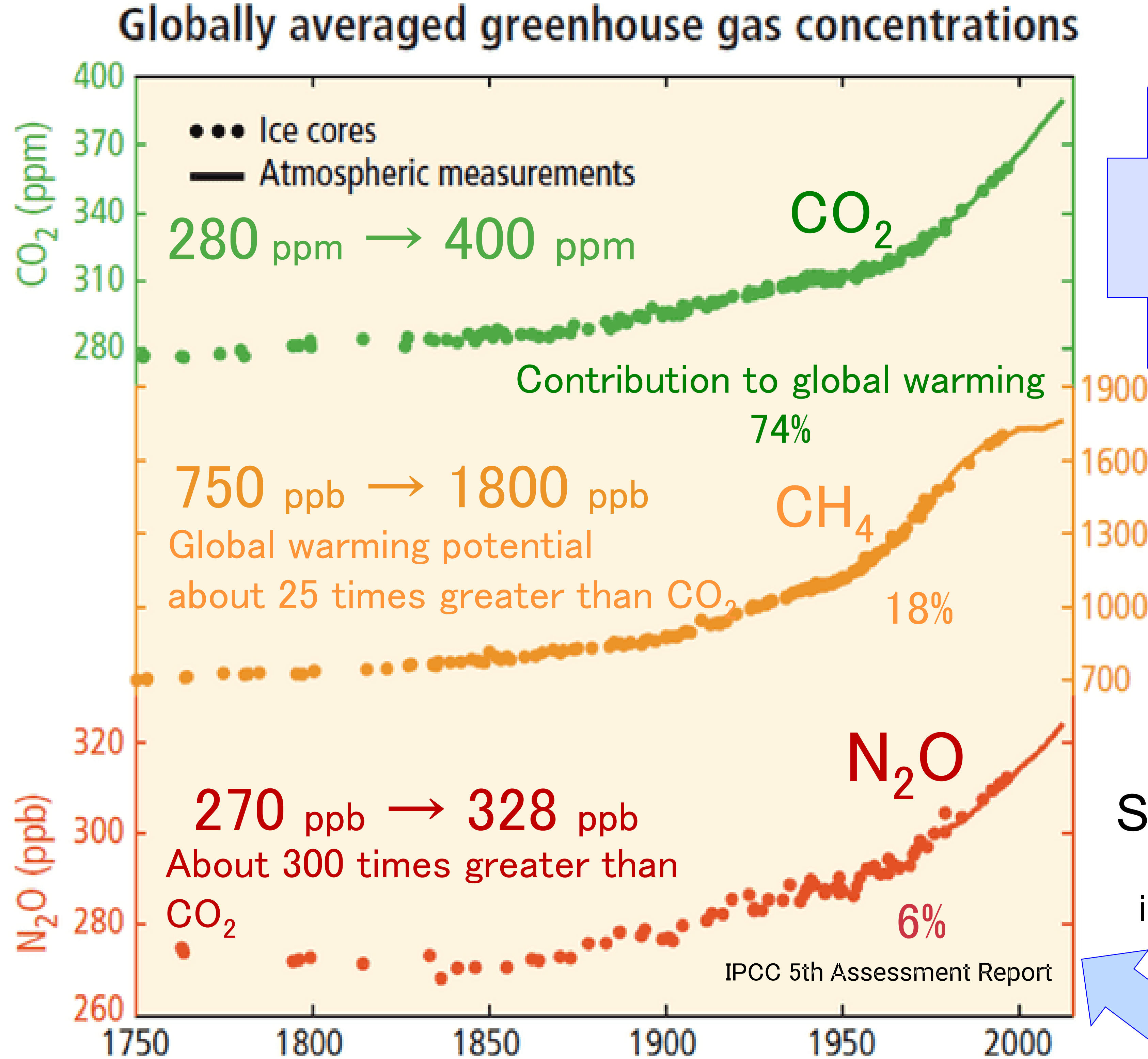
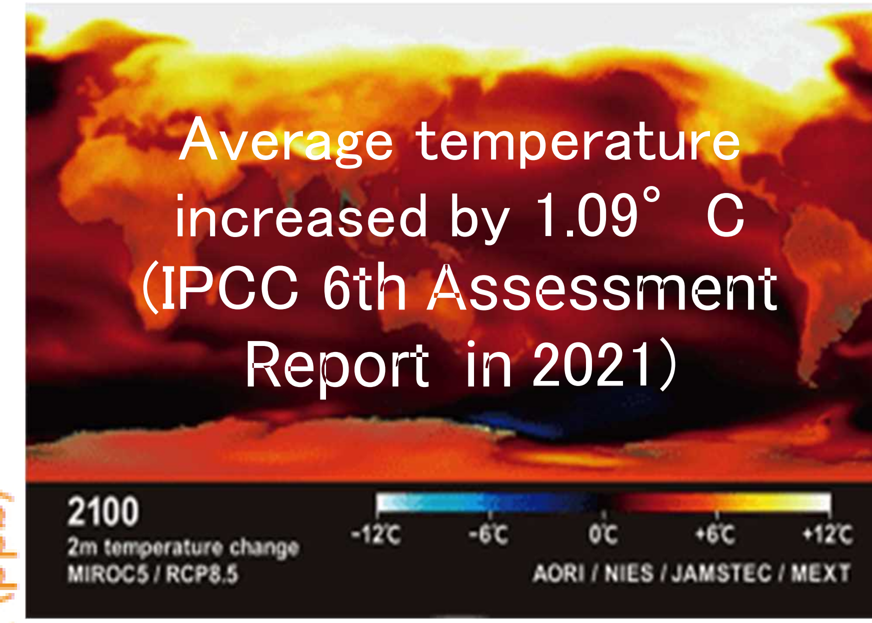
PJ Organizations: Tohoku University, The University of Tokyo, National Agriculture and Food Research Organization (NARO)

Subcontractors: Tokyo University of Agriculture and Technology, Iwate University, Obihiro University of Agriculture and Veterinary Medicine, Ryukoku University, RIKEN, Forestry and Forest Products Research Institute (FFPRI), Ehime University, HAYASHIBARA CO., LTD., National Institute of Advanced Industrial Science and Technology (AIST), Tokachi Federation of Agriculture Cooperatives.

Background



Global warming due to anthropogenic GHG emissions



Increased risk of climate change

Severe disaster

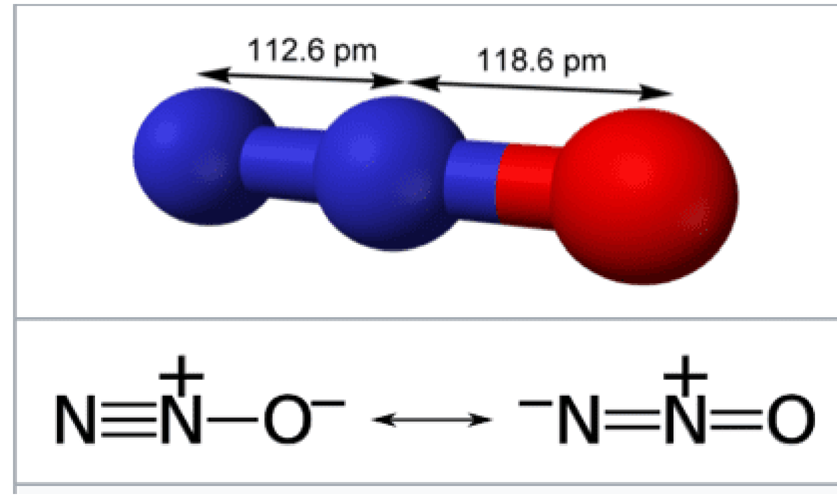
Destruction of the infrastructure of life

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

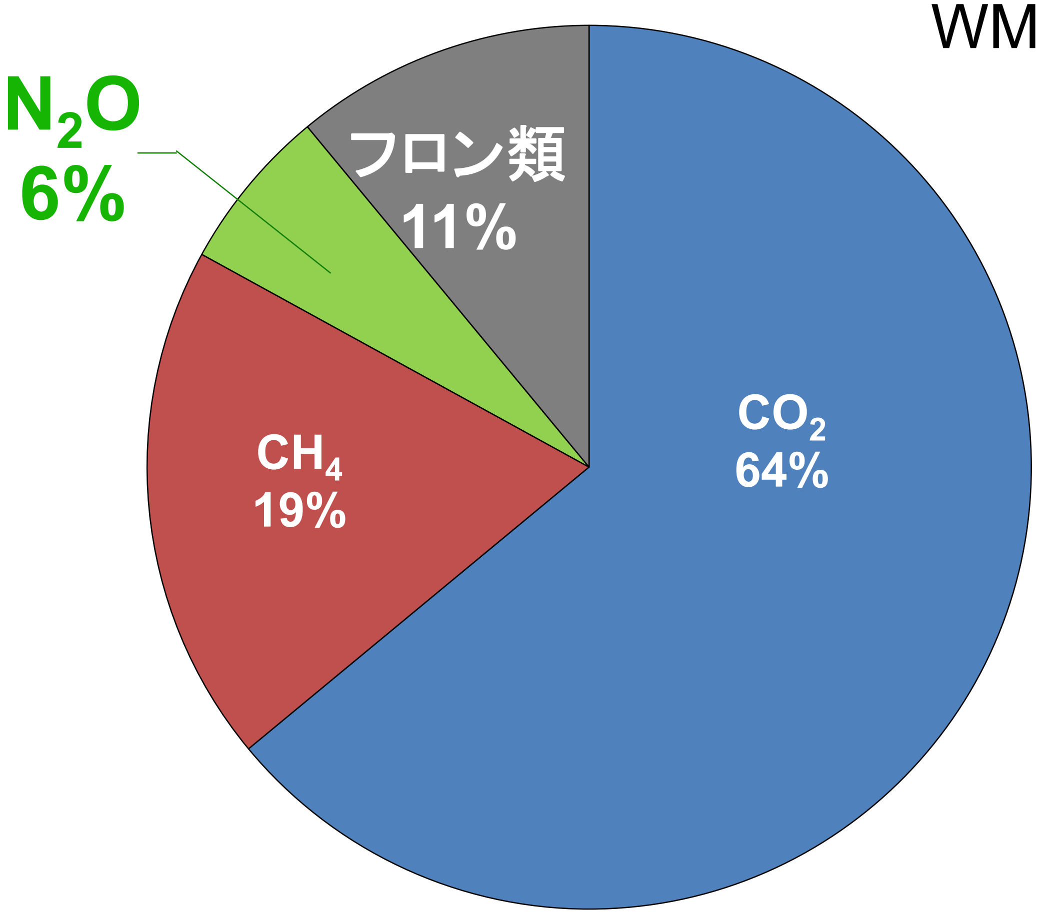
Reduction of greenhouse gases other than CO_2 is essential to limit the rise in global temperatures to 1.5 degrees Celsius above pre-industrial levels. (Paris Agreement, COP26)

What is nitrous oxide (N_2O)?

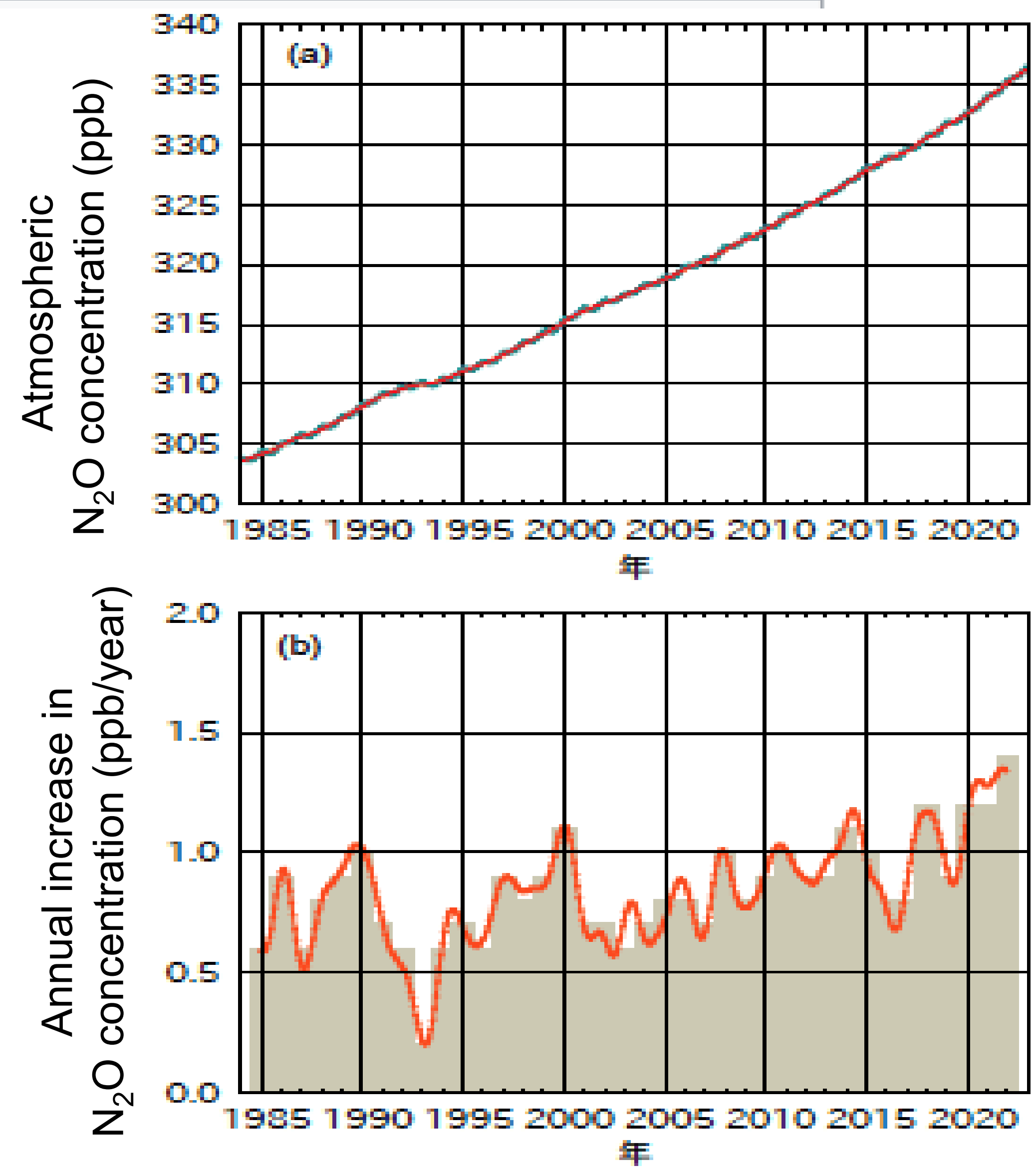
- Chemically stable due to double and triple bonds
- Global warming potential ~300 times that of CO_2
- Long-lived greenhouse gas (half-life 121 years)
- Stratospheric ozone layer depleting substances
- Atmospheric N_2O concentration is 336 ppb (2022), Increase of 1.4 ppb compared to the previous year.



WMO, 2023

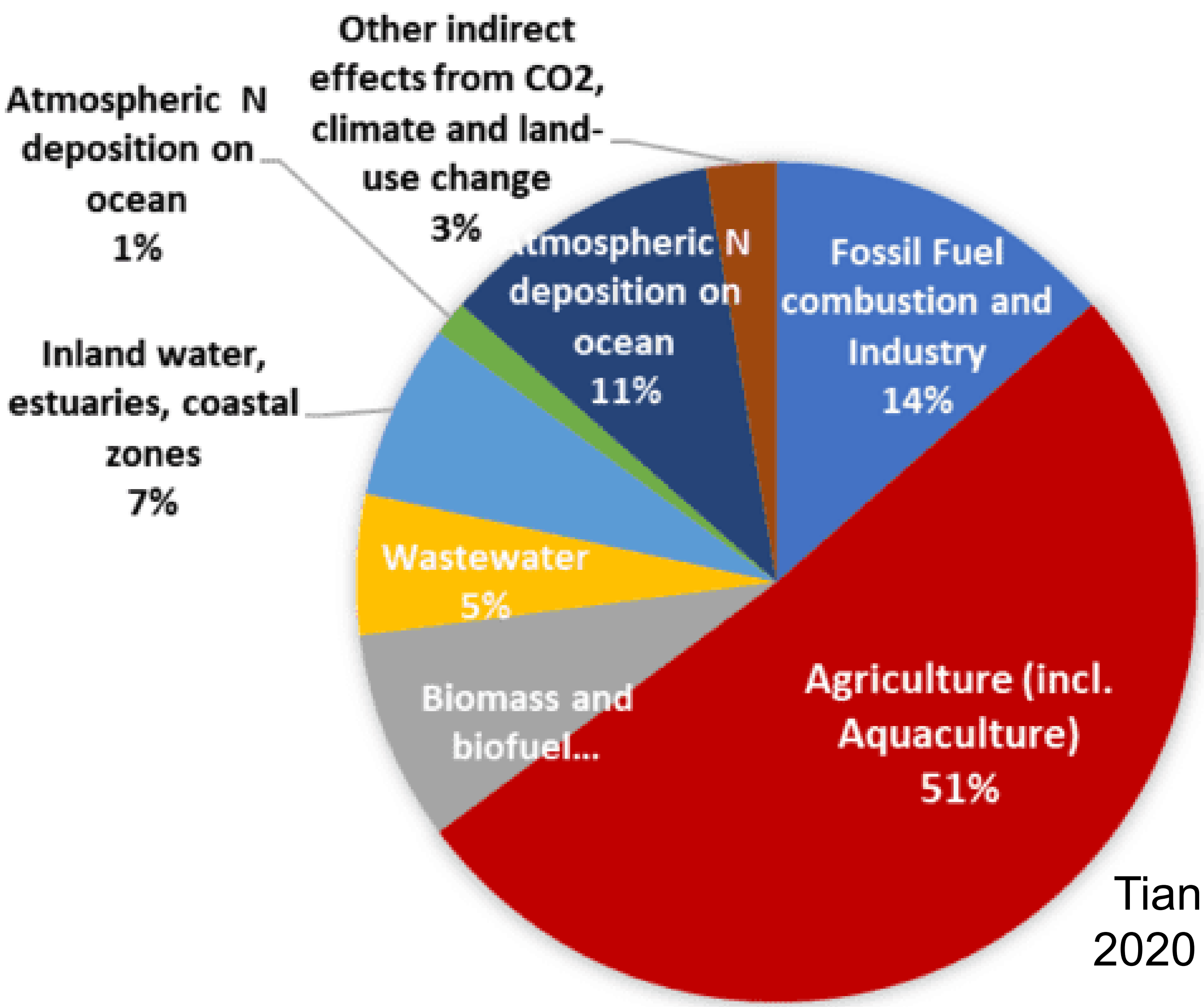


Contribution of anthropogenic greenhouse gases to global warming since the Industrial Revolution

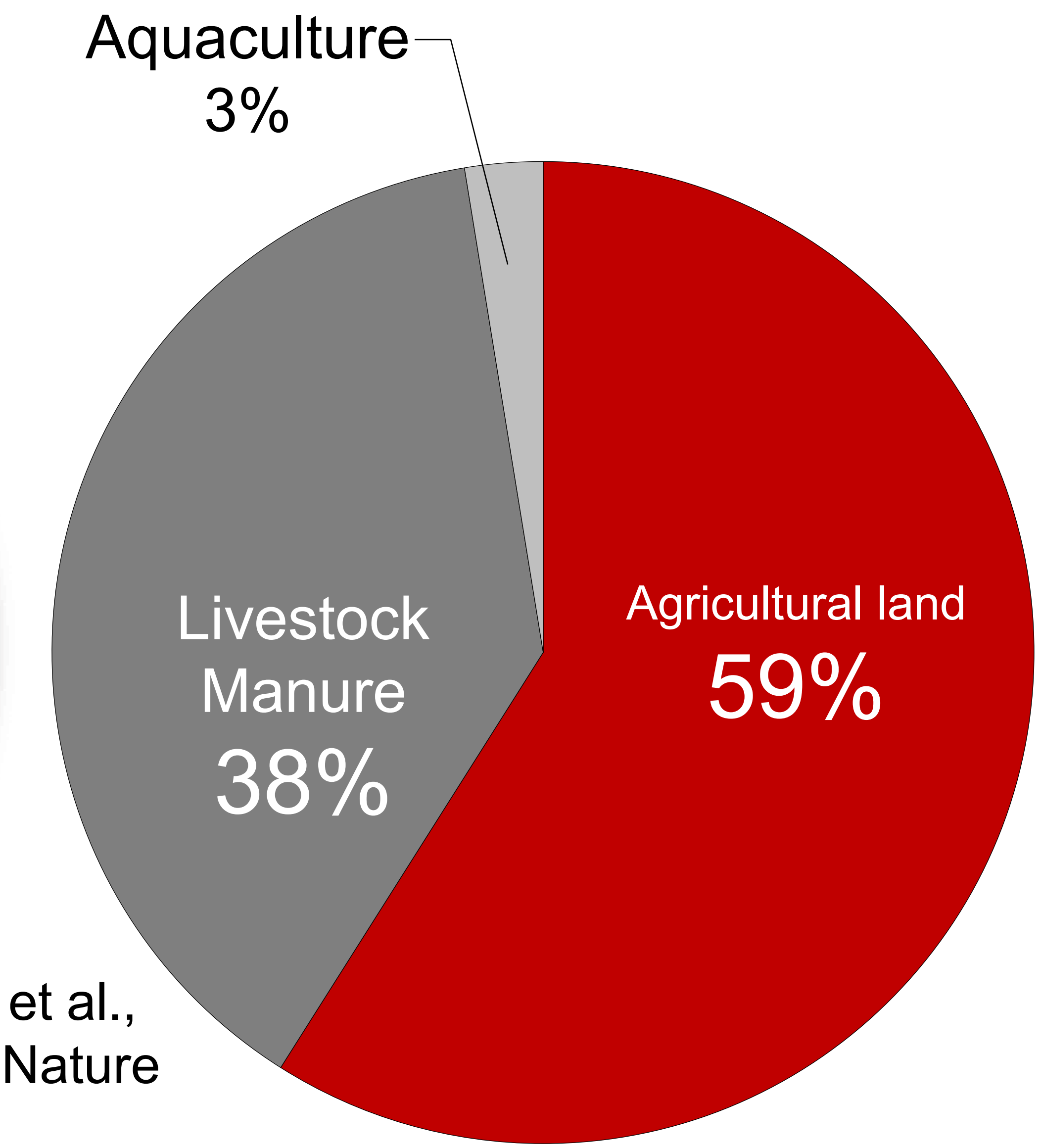


(a) Global average concentration and (b) annual increase in atmospheric N_2O

Major anthropogenic source of N_2O



Tian et al., 2020 Nature

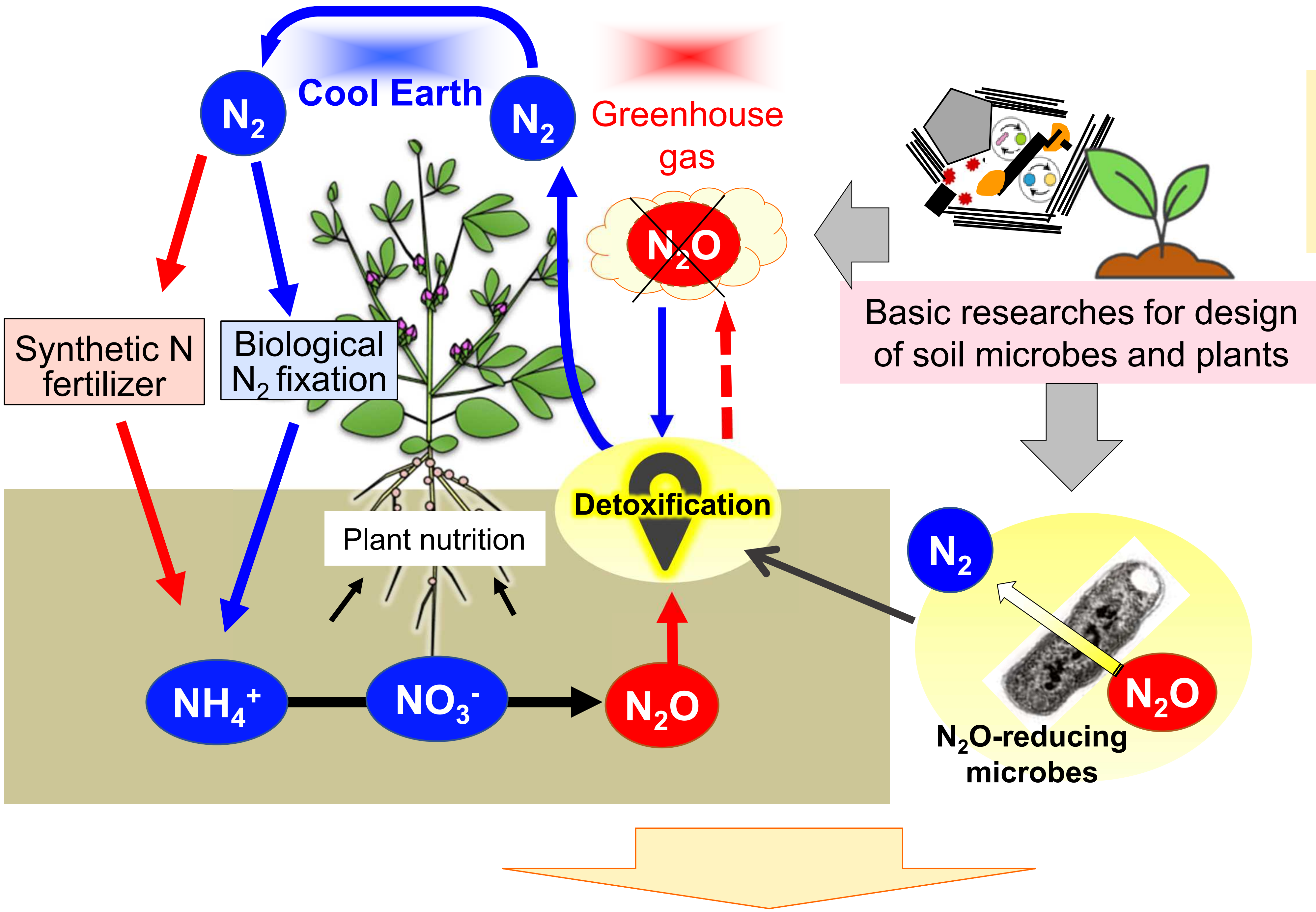


Global N_2O budget

Global N_2O budget in agriculture

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

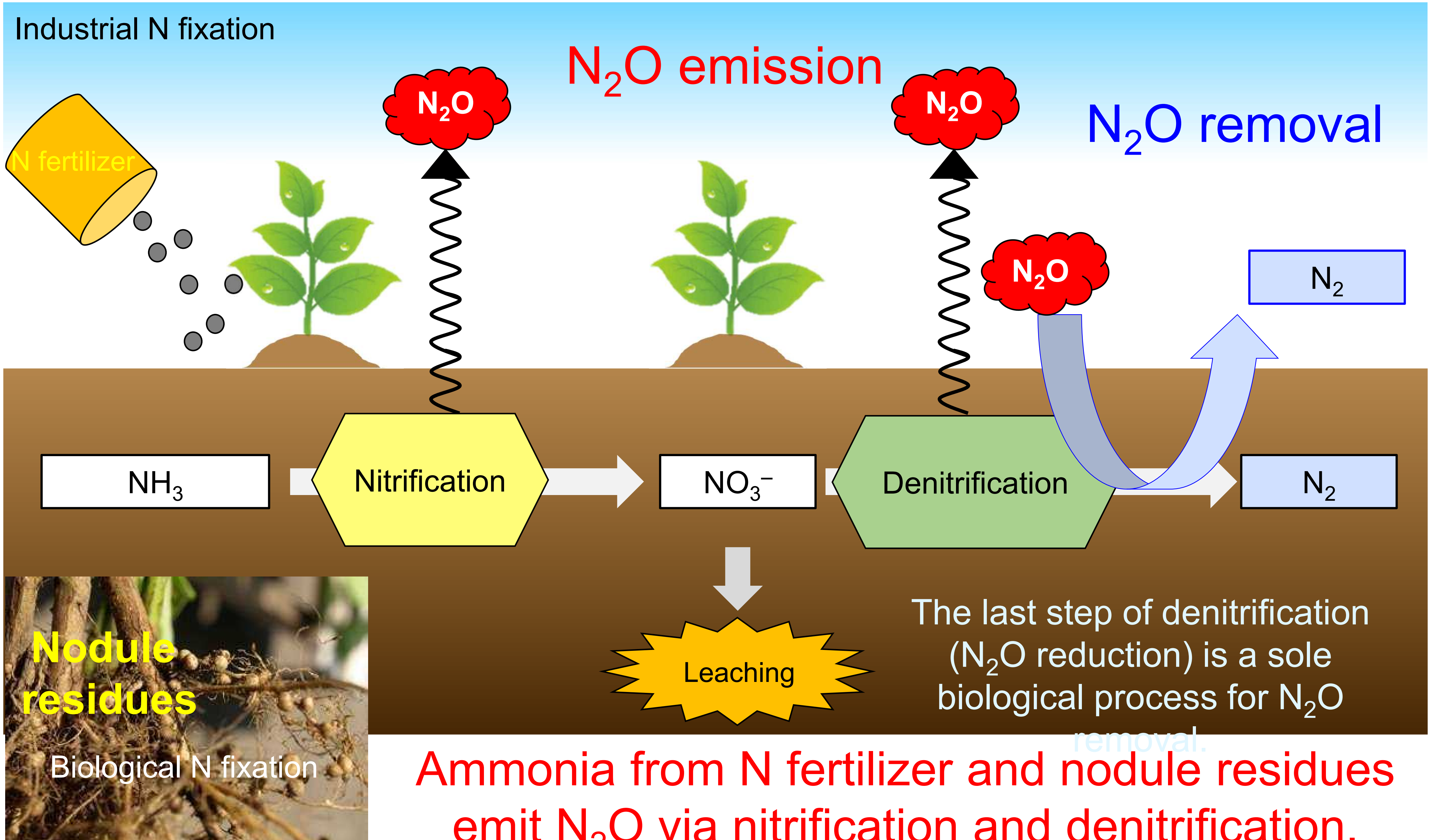
Strategies for reducing N_2O emissions from farmland



Both researches have progressed, but they are not included in the presentation due to patent application.

Artificial design of plants and soil optimized for the functions of N_2O-removal microorganisms

N transformation in agricultural lands by microorganisms



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

Implementation structure (2020-2024)

Cool Earth via dSOIL

PM (Project manager)
Kiwamu Minamisawa
(Tohoku Univ.)

Rota Wagai (NARO)

Atsuko Imaizumi (NARO)

Microbial inoculants

- Artificial aggregates

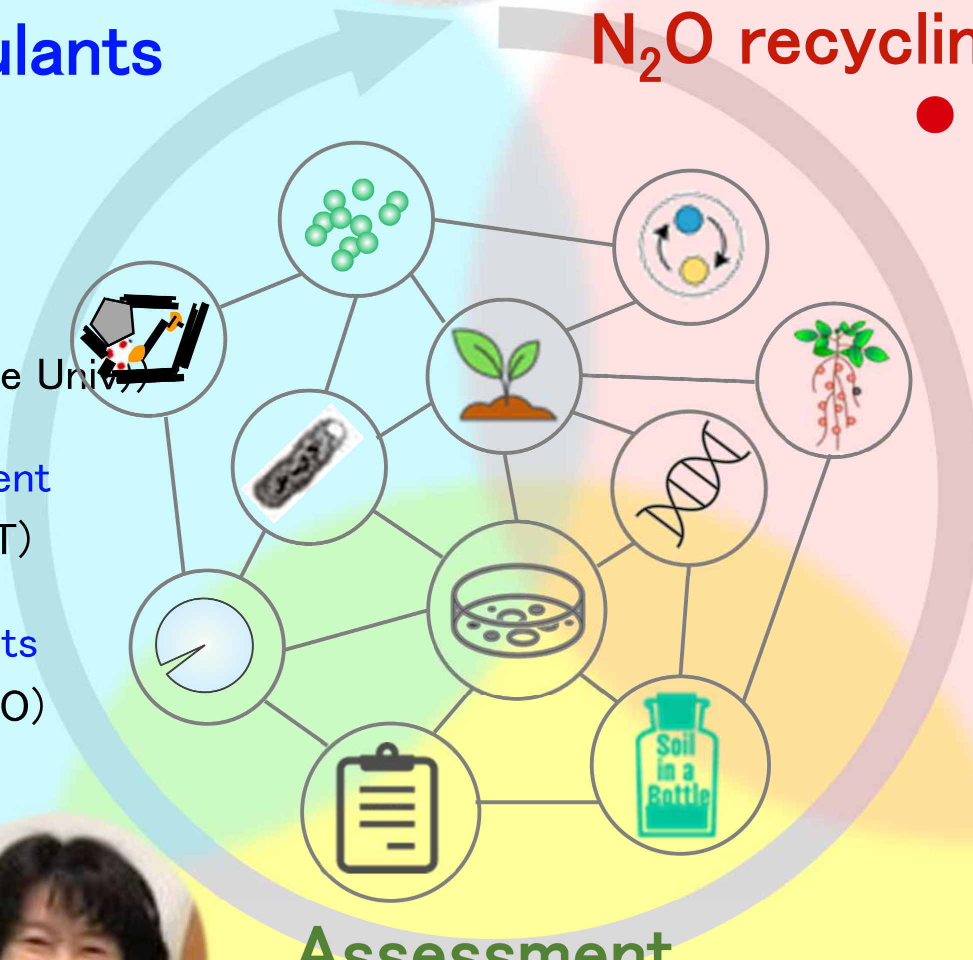
Rota Wagai (NARO)
Kazumichi Fujii (FFPRI)
Satoshi Mitsunobu (Ehime Univ.)

- Materials development

Manabu Kanno(AIST)

- Micriobial inoculants

Kanako Tago (NARO)



N_2O recycling

- Soybean rhizobia

Manabu Itakura (Tohoku Univ.)
Takanobu Higashiyama (Hayashibara Co. Ltd)

- Comparative genomics

Shusei Sato (Tohoku Univ.)
Ken Shirasu (RIKEN))

- Symbiosis optimization

Atsuko Imaizumi (NARO)/ Masayuki Sugawara (Obihiro Univ.)/ Yasuyuki Kawaharada (Iwate Univ.)/Shin Okazaki (Tokyo Univ. of AT)/ Shigeyuki Betsuyaku (Ryukoku Univ.)

- Citizen Sciense Satoshi Ohkubo (Tohoku Univ.)

Assessment

Hiroko Akiyama (NARO)

- N_2O mitigation by rhizobial inoculation

Hiroko Akiyama (NARO)

- Prototype of new inoculants

Masato Mikuchi (TFACA)

Patent support

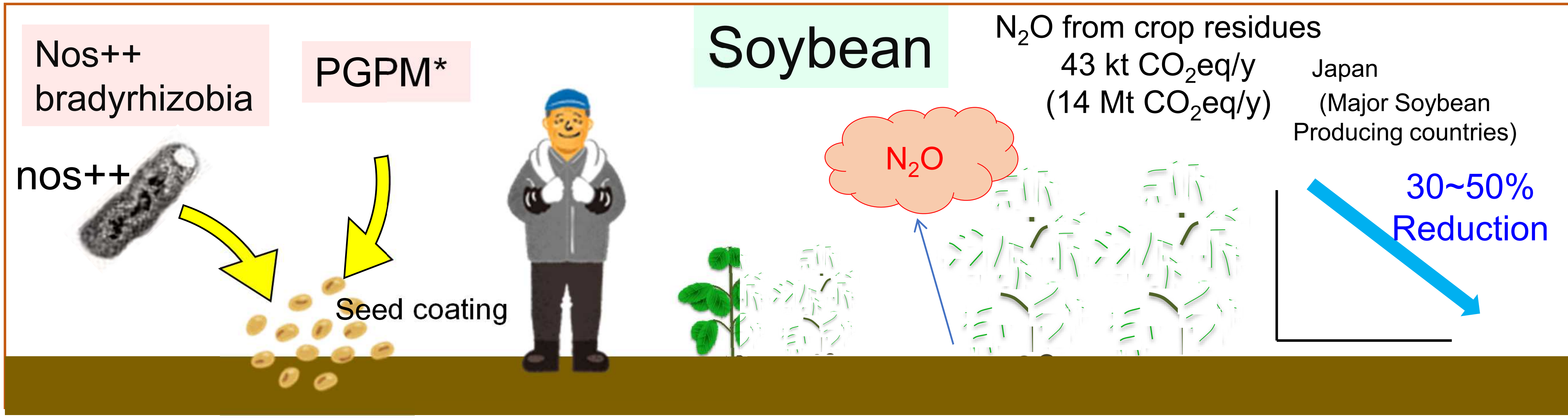
Yoshiyuki Ogawa (INPIT)

Advisers

Kazuyuki Yagi
Yoichi Kamagata
Daisuke Shibata

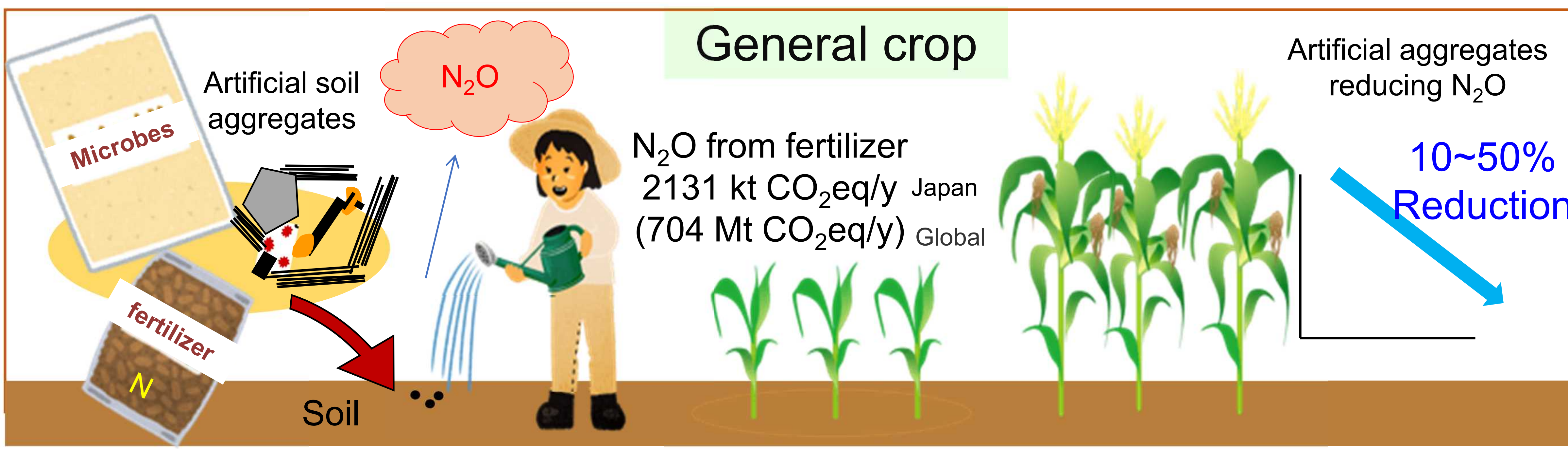
Implementation image

Soybean rhizobia



From proven strategies to more advanced strategies

Artificial aggregates



Research and development schedule

Material name	Current materialization plan	Target crops	Time to reduce N_2O	Target annual reduction of N_2O in fields	Benefits for farmers
Rhizobia	Mamezou+PGPM①	Soybean	Harvest season	10% (2024) ③ 37% (2029) ③ (43 ktCO_2eq/y)	Increased yield and reduced fertilization
Artificial aggregates	Artificial aggregates and carriers ②	General field crops	After fertilization	10-50% (2029) (2,131 ktCO_2eq/y)	Soil improveme nt, etc.

＜Notices＞
① Growth-promoting bacteria (PGPM) increased the nitrogen-fixing activity of soybean root nodules in laboratory tests, and field tests are currently underway.
② Artificial aggregates were created using knowledge of natural soil aggregates.
③ The N_2O reduction target only during the harvesting period (nodule decay period) is 30% (FY 2024) and 50% (FY 2029).

Target product specifications (2029)

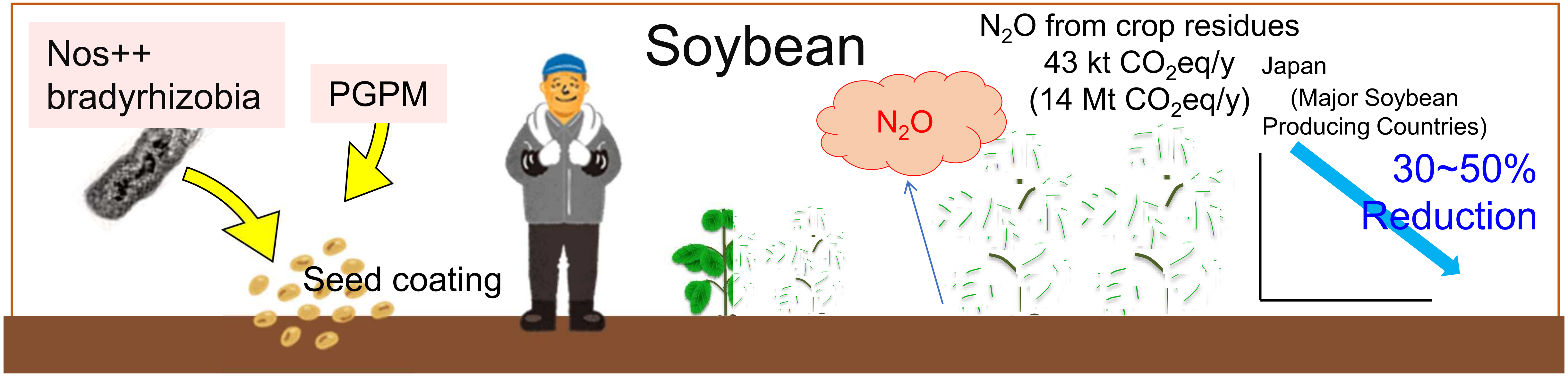
Combination technology of rhizobia and PGPM

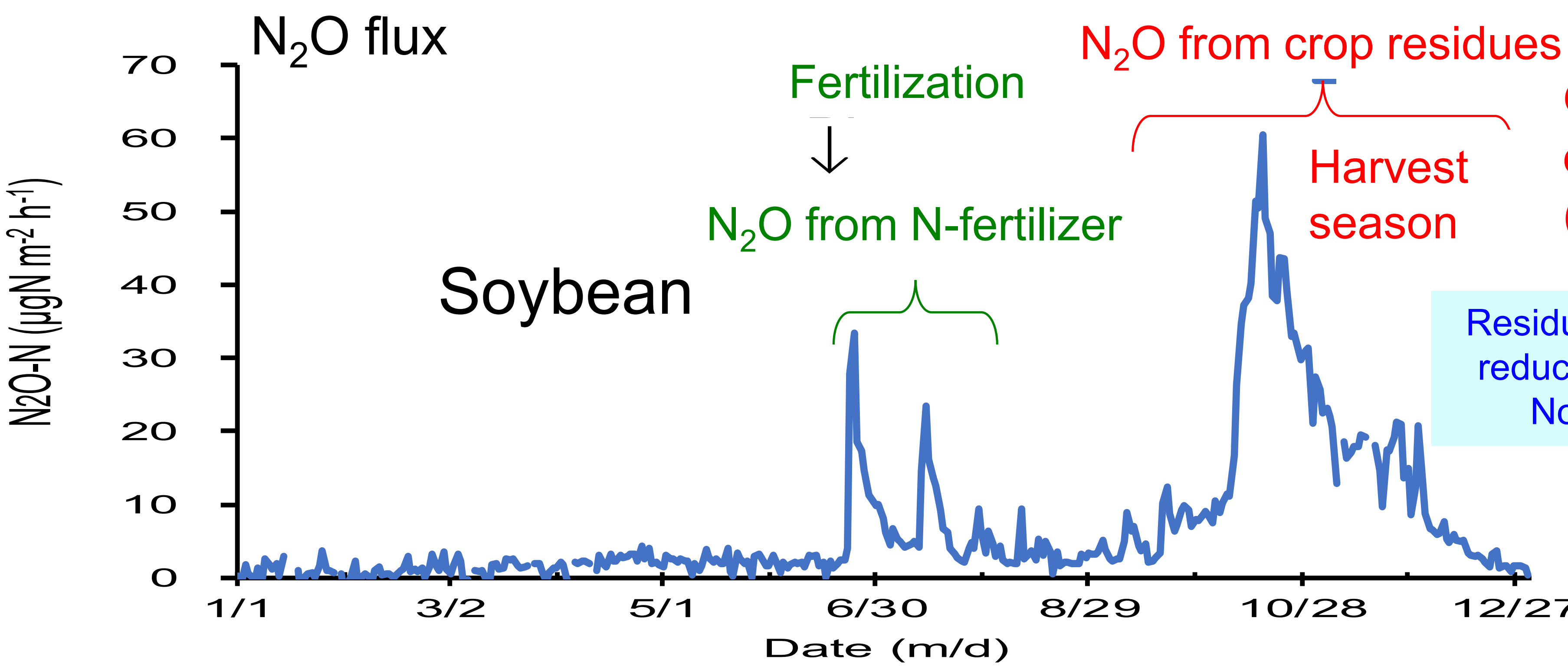
1）Emissions of greenhouse gas N_2O during soybean harvesting period can be halved. (emissions trading)
2 ） Increasing soybean yield (approximately 10%) (resolving the shortage of high-quality domestic soybeans and increasing farmers' income)

Artificial aggregates (expected by multiple companies, citizen science participants, and media personnel)

1） Emissions of greenhouse gas N2O from fertilizers can be halved. (emissions trading)
2 ） Considering benefits for farmers such as soil improvement and crop growth promotion

Background and R&D of N_2O removal rhizobia





Our goals for N_2O reduction during harvest season are 30% (2024) and 50% (2029).

Residue-derived N_2O can be reduced by inoculating with Nos++ bradyrhizobia

R&D policy for bradyhizobial inoculants

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

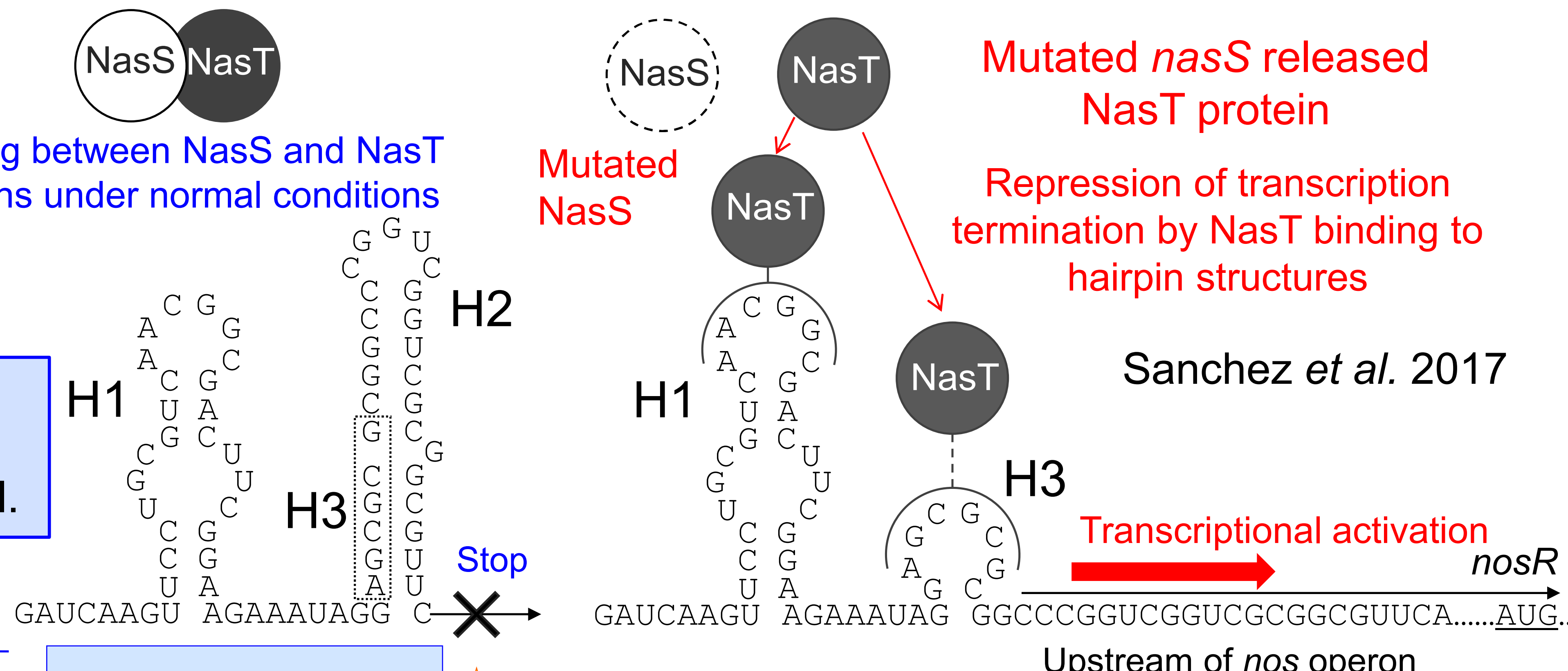
PGPM*: Plant Growth-Promoting Microbes

Creation and exploration of bradyrhizobia with higher N_2O reductase activity

B. diazoefficiens

NasS and NasT are two component regulatory system for nos operon transcription.

Deletion mutants of hairpin structures were an ideal bradyrhizobia for N_2O removal.

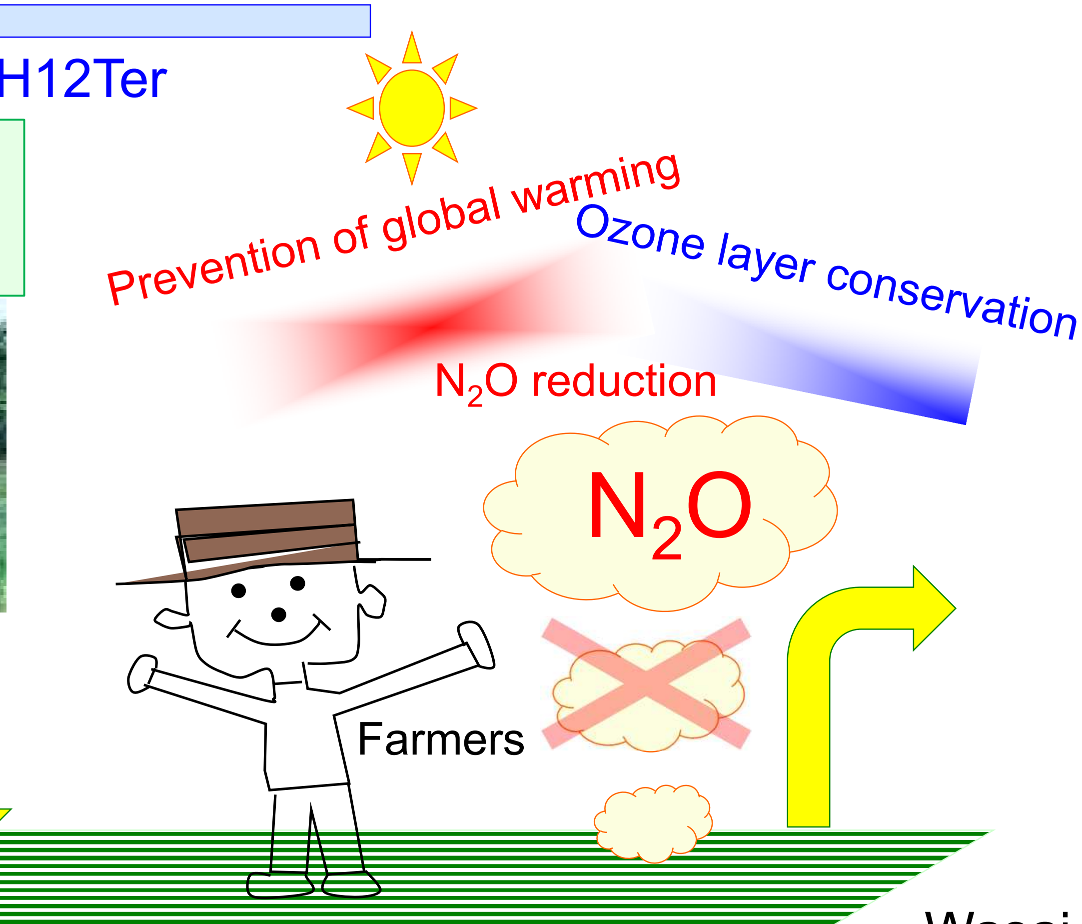


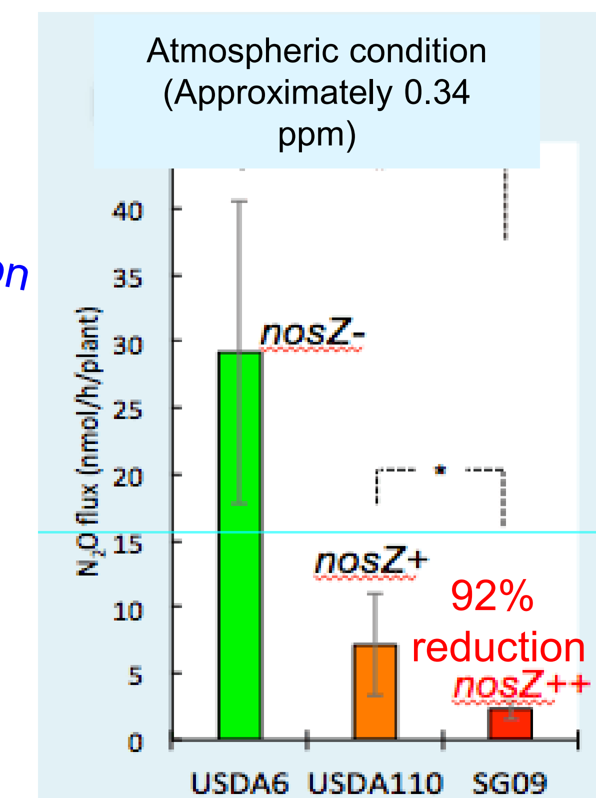
Sanchez *et al.* 2017

Are there any wild strains with higher N_2O-reducing ability?

Discovery of *B. ottawaense* with higher N_2O-reducing activity

Wasai-Hara *et al.* 2020
Wasai-Hara *et al.* 2023





Wasai-Hara *et al.* 2023

How does *B. ottawaense* show high N_2O-reducing activity?

Wasai-Hara et al. 2023

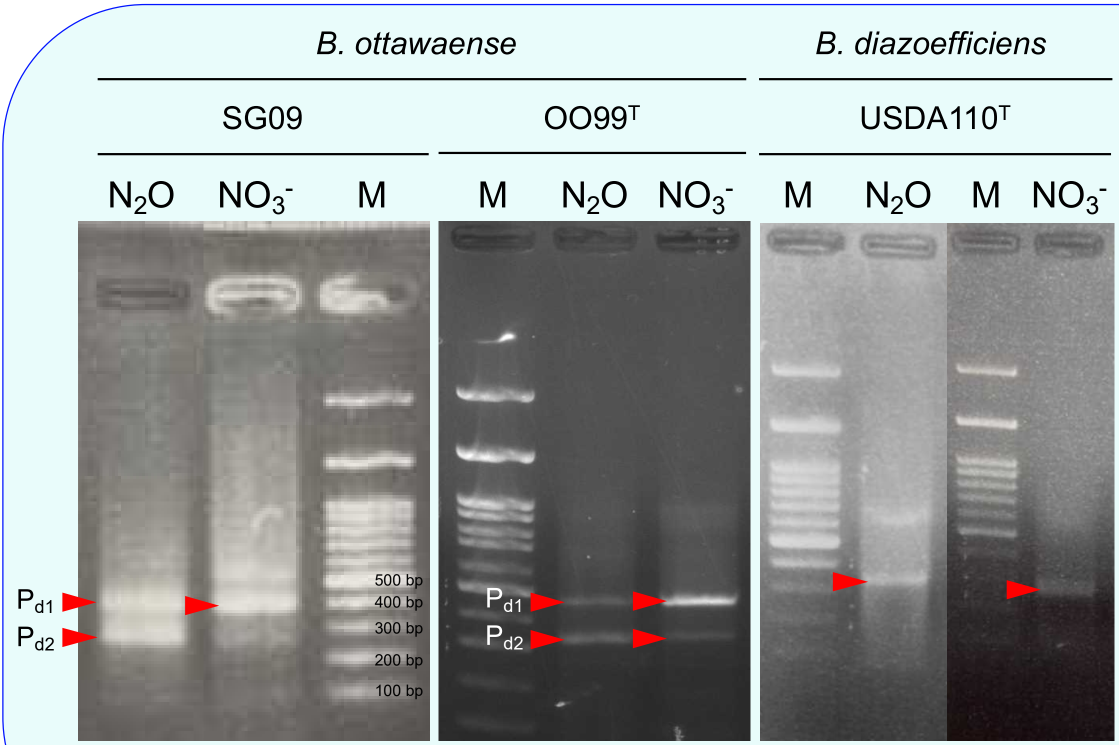
Transcription of *nosZ* gene

Relative expression of *nosZ* gene under a N_2O-respiring condition by RT-qPCR

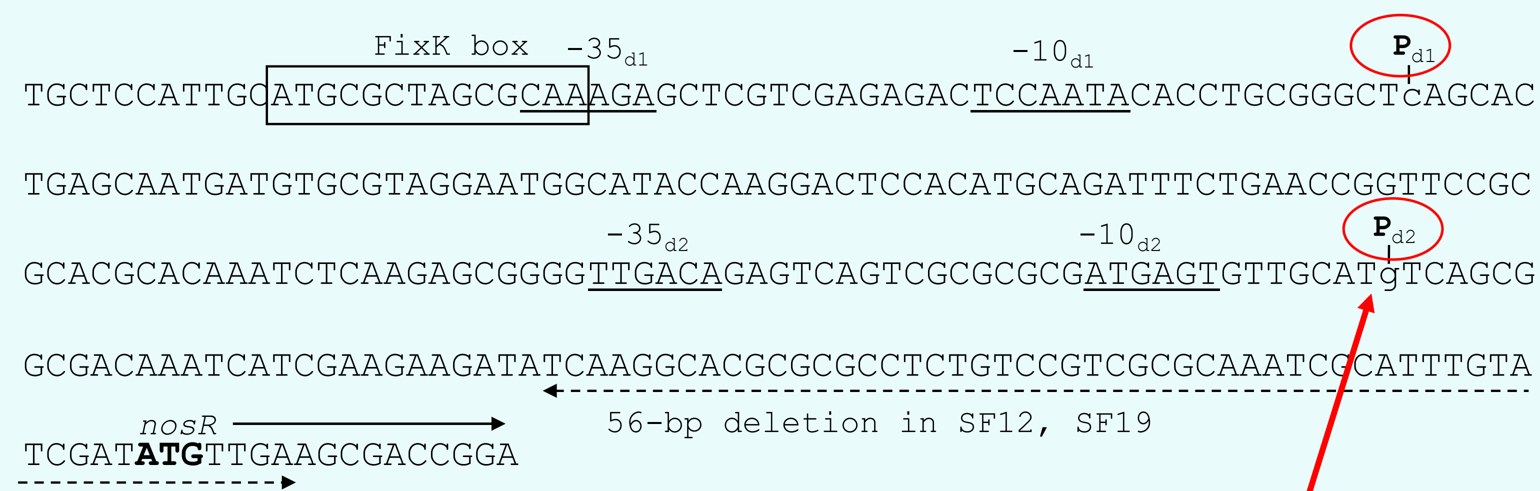
Strain	Relative Expression: Wild type (1)	Relative Expression: Δ*nasS* (2)	NasST effect (1)/(2) (%)
B. diazoefficiens USDA110^T	1	3	29
B. ottawaense SG09	212	430	49
B. ottawaense OO99^T	164	337	48

nosZ gene of *B. ottwaense* was highly expressed than that of B. diazoefficiens under a N_2O-respiring condition.

Transcription Start Site



Electrophoresis images of 5′ RACE analysis in *B. ottawaense* SG09, OO99^T, and *B. diazoefficiens* USDA110^T



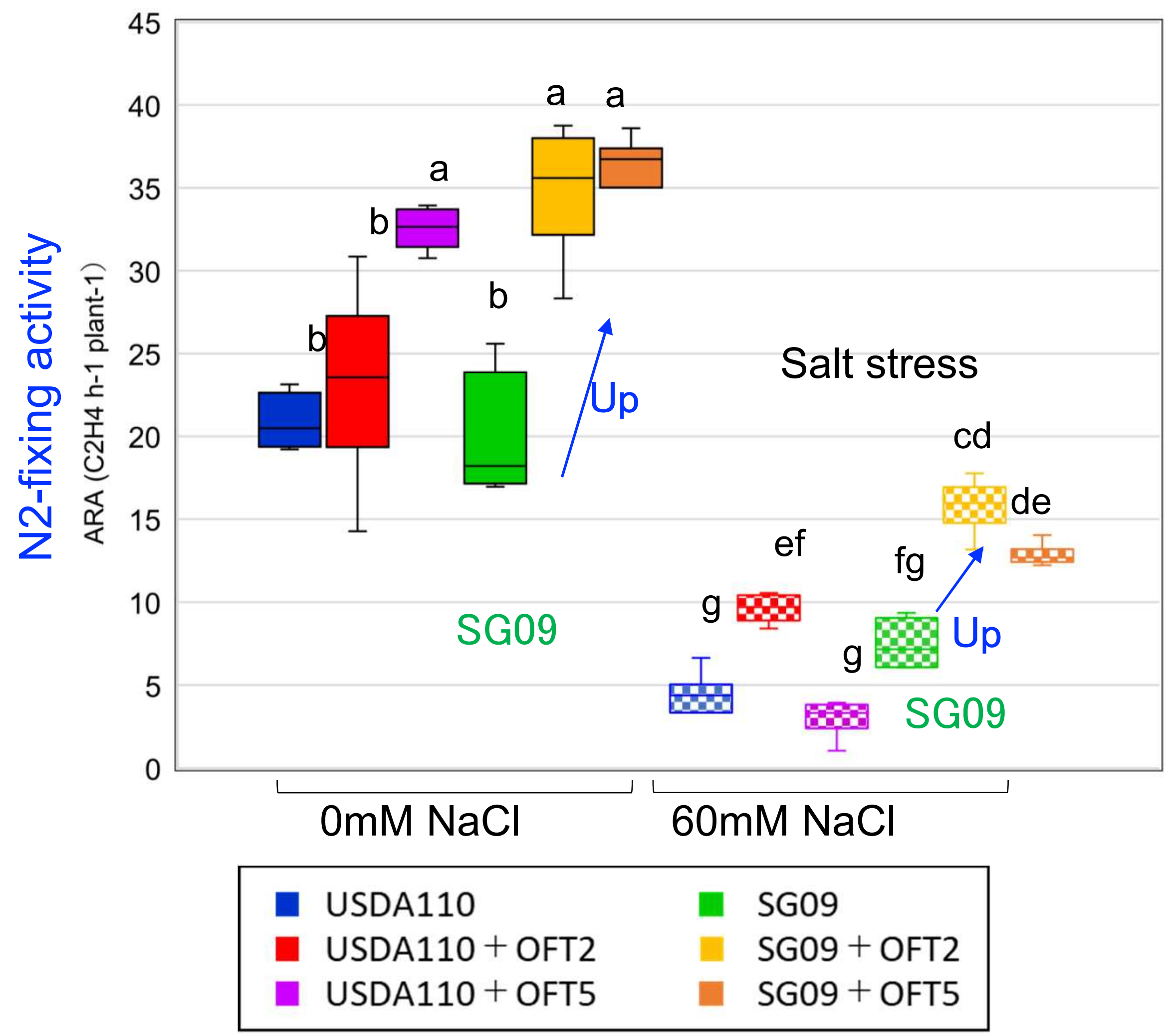
Transcriptional organization of *nosR* in *B. ottawaense* SG09 and OO99^T

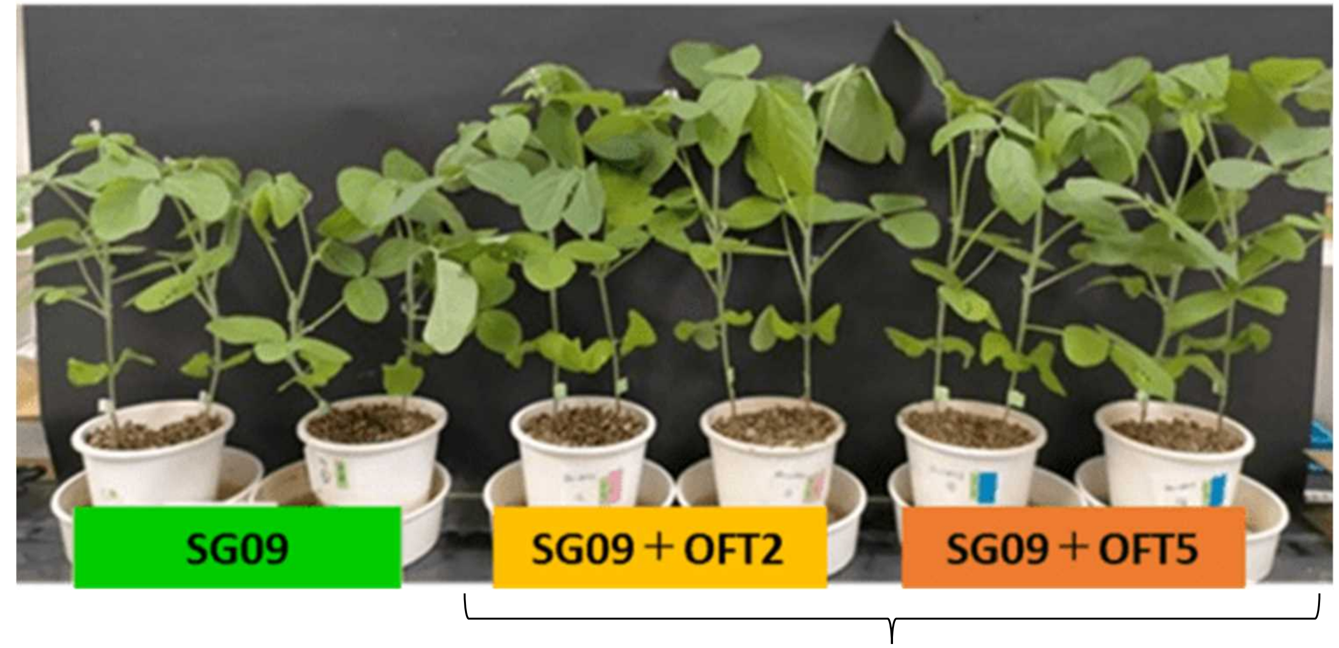
The presence of N_2O-dependent transcriptional start site Pd2 under a N_2O-respiring condition.

B. ottawaense symbiosis enhanced by PGPR

■ Symbiosis promoted by *Pseudomonas* growth-promoting bacteria (PGPR)

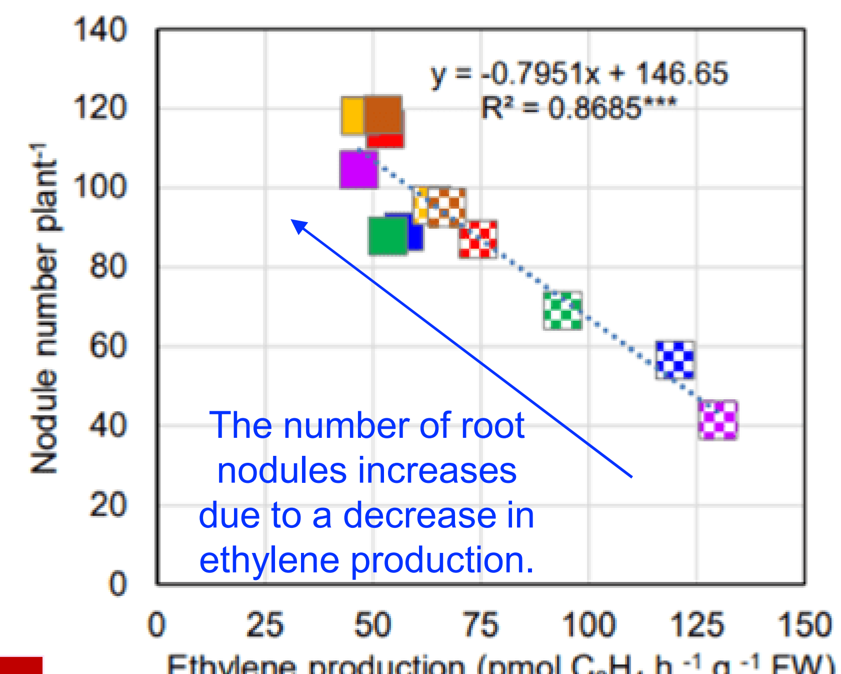
N_2-fixing activity under coinoculation conditions





Growth promotion effect of soybeans by co-inoculation

Win et al. 2023



Possibility of decreased ethylene production due to ACC deaminase of. strains OFT2 and OFT5

Microbial inoculum whose mechanism is known

Improvement of symbiotic N_2-fixation , growth , and salt stress tolerance of B. ottawaense SG09

Can be used as N_2O removal rhizobium material

Creating benefits for farmers through field trials

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



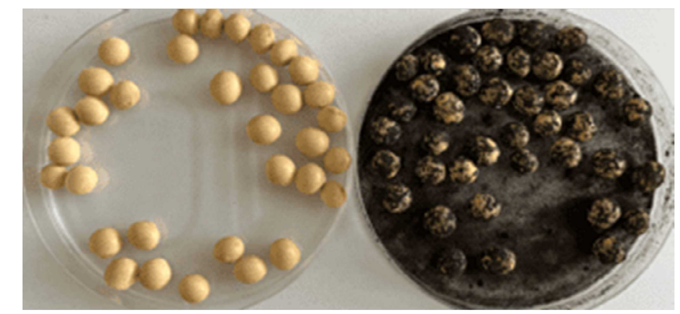
Manufacturing and evaluation of a prototype material for a new N_2O-eliminating bradyrhizobial strain

■Form of bradyrhizobial inoculant

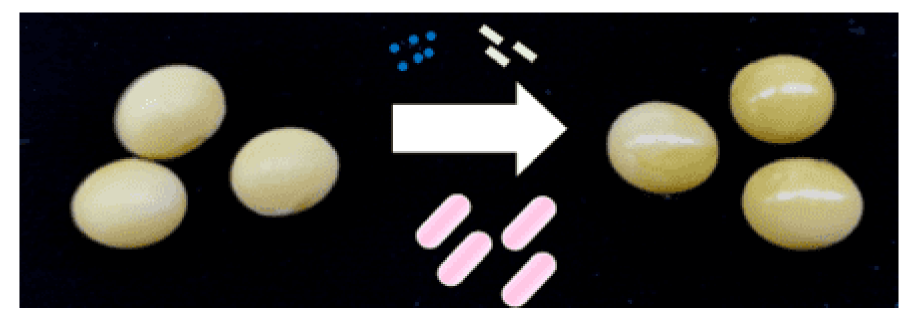
Mamezou

Peat moss inoculants (Tokachi Agricultural Cooperative Federation)

The number of bacteria is stable, but farmers should inoculate on site.



Development of seed coating technology

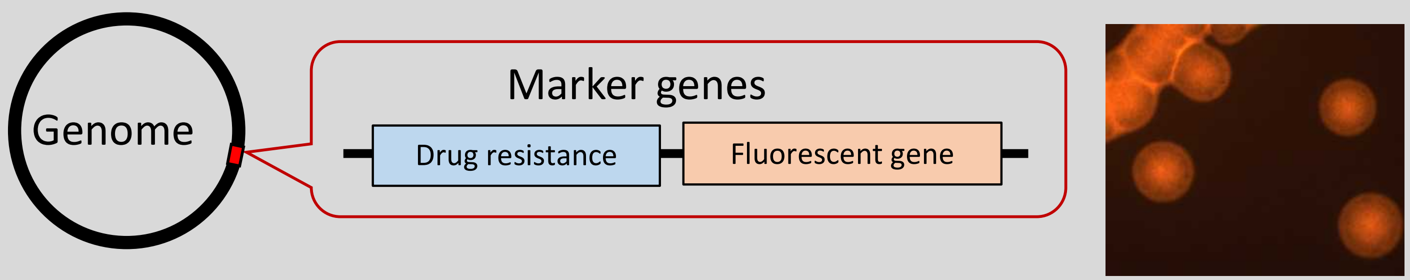


Farmers can save labor, but the bacterial viability reduced rapidly.

Soil environmental impact assessment by rhizobial inoculation

How does microbial inoculation affect the soil ecosystem?

Bradyrhizobium strains with *nosZ*++

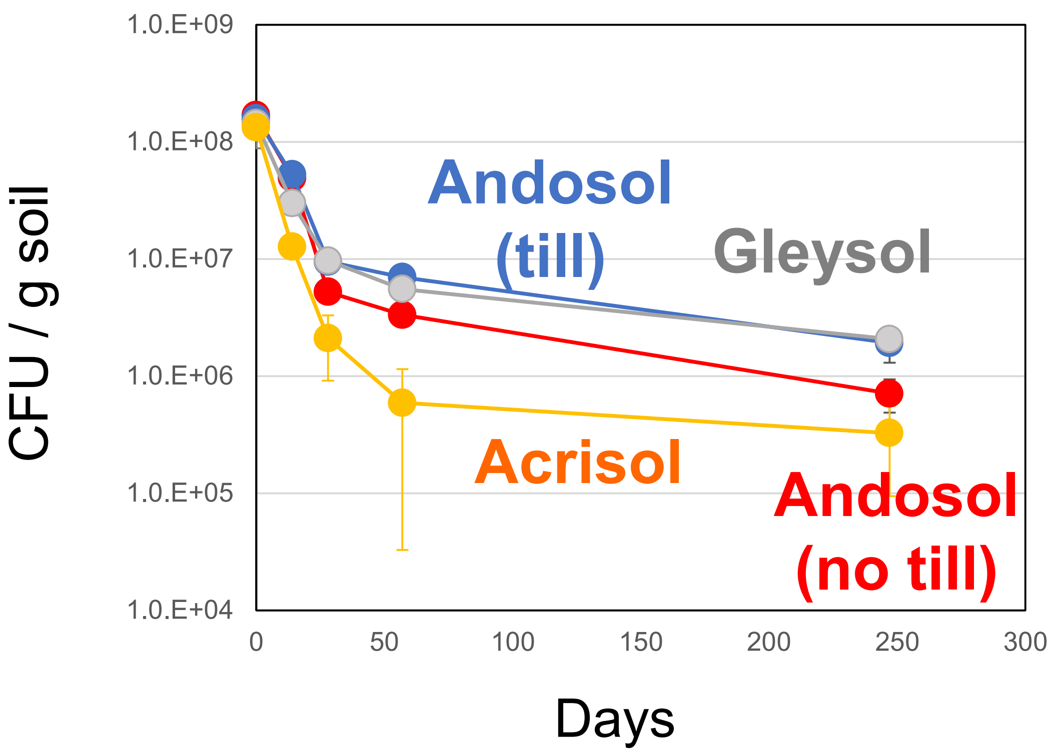


Soil microcosm

inoculation (10^8 cell/g) to soil micocosms

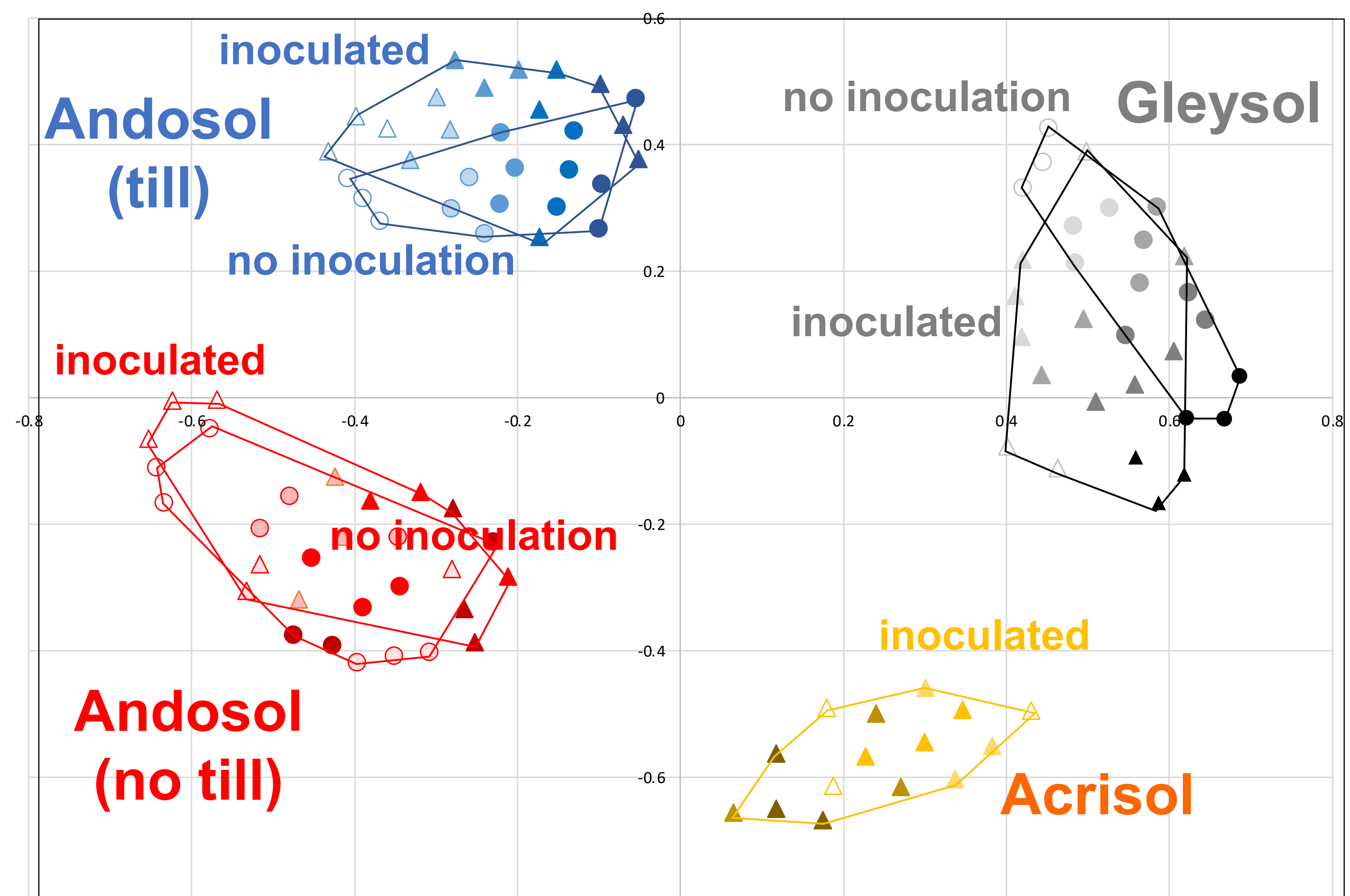
Andosol (till, no-till)
Gleysol
Acrisol

Survivability of the inoculant in soils



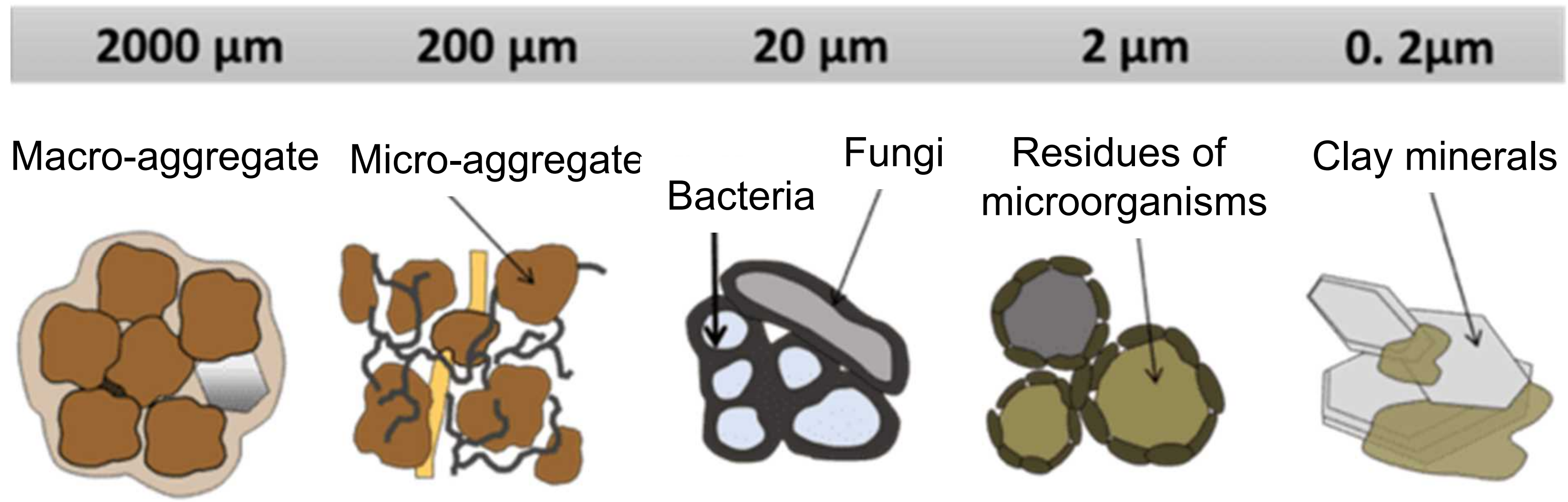
The inoculant survived up to 250 days, although they gradually decreased. The inoculation did not change the indigenous bacterial communities in the soil microcosms.

Community composition of the soil microbiota



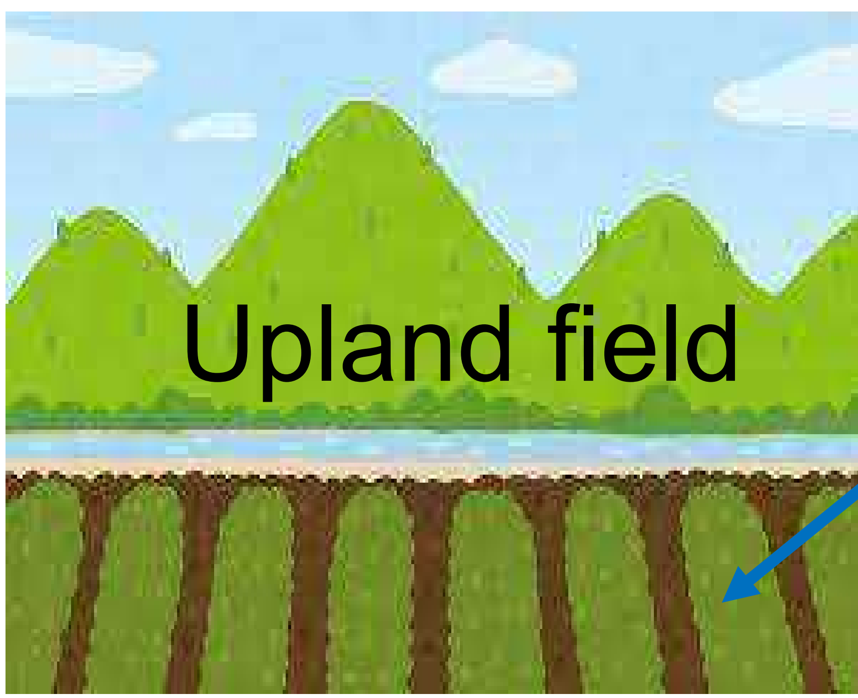
Hierarchical structure of soil aggregates ➡ Soil multi-functionality

Soil aggregates have a pore network and provide niches for microorganisms.

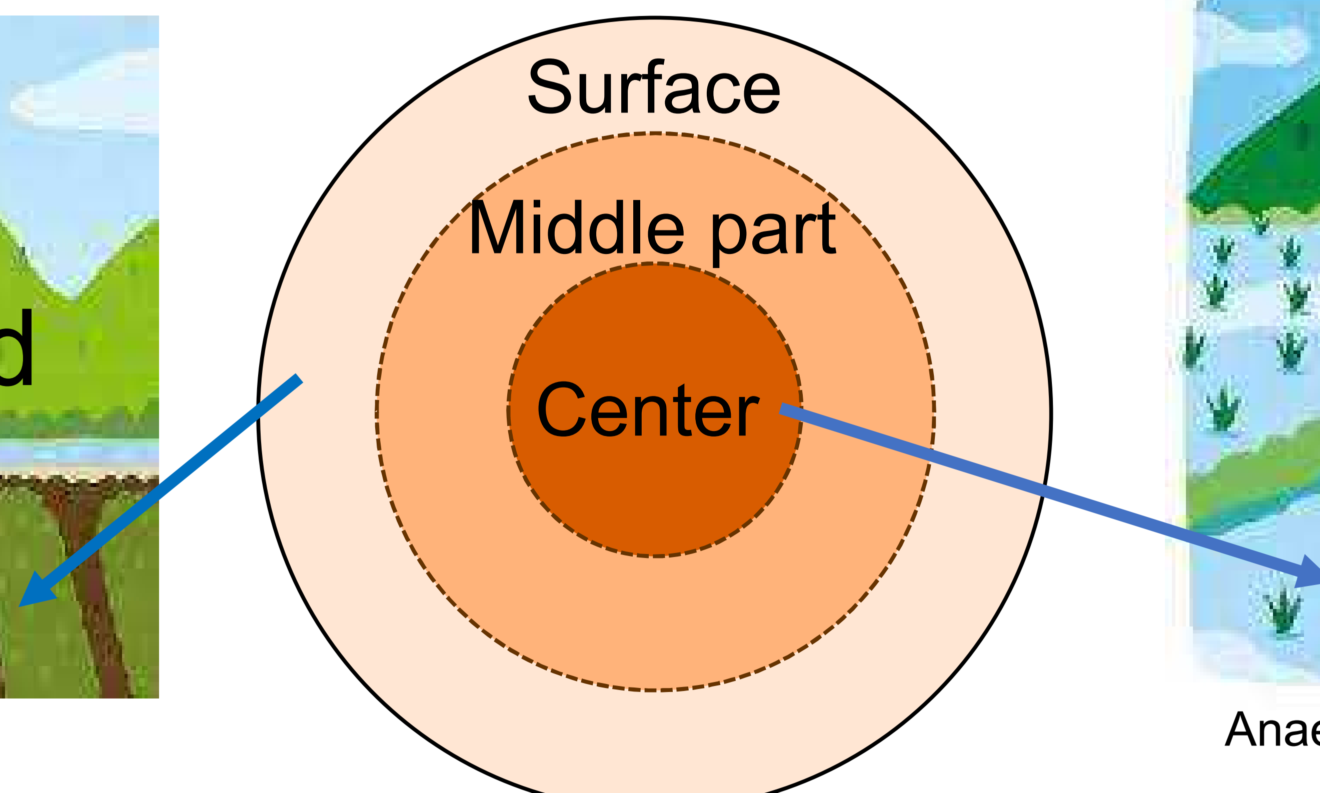


Aggregate Hierarchy Model (Tisdall & Oades, 1982)

Soil micro-aggregate



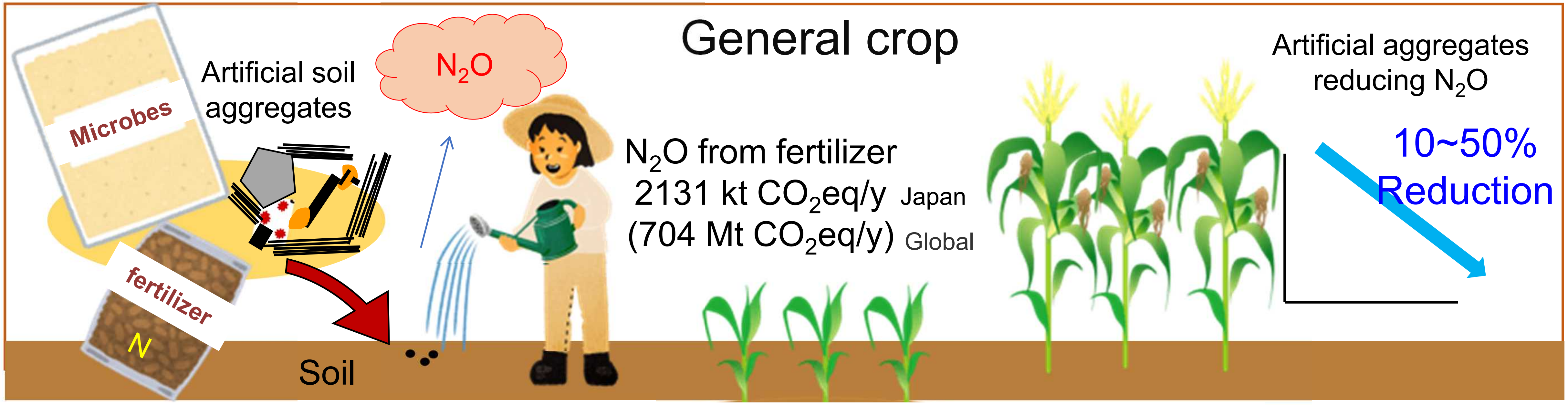
Aerobic environment

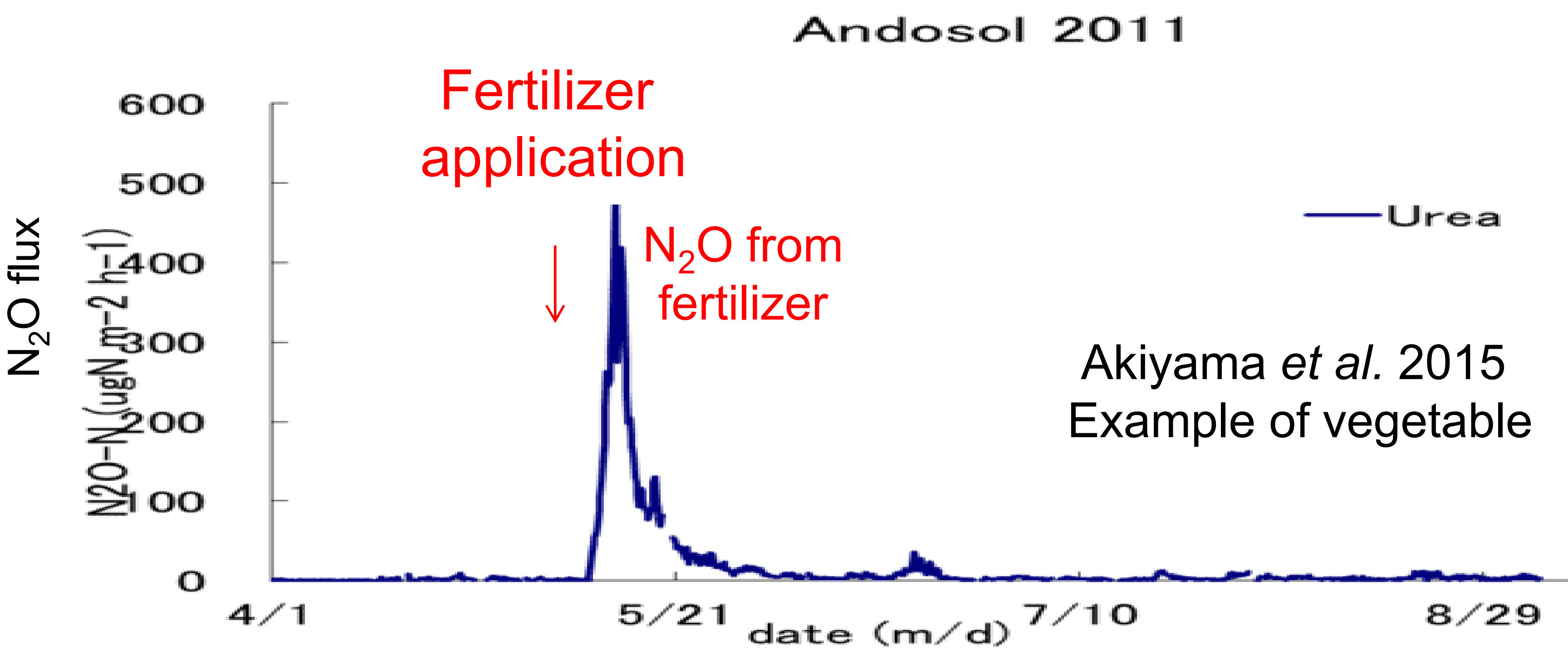


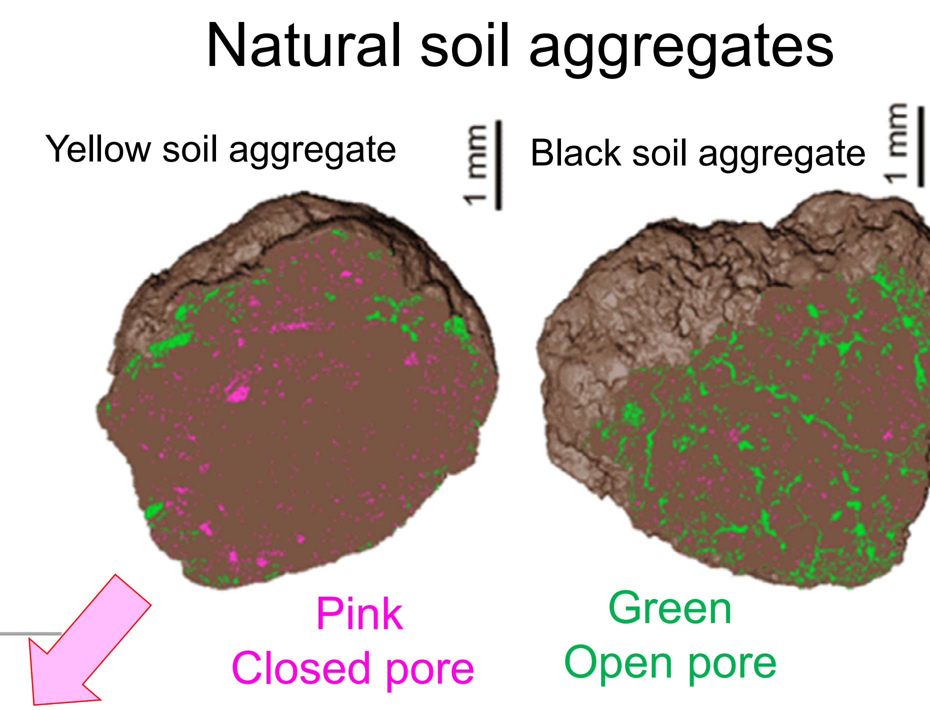
Anaerobic environment

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.

Artificial aggregates reducing N_2O







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 N_2O reduction

Microbial materials strategy

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

Two approaches

①

②

Artificial soil aggregates

Artificial carriers

Raw Materials & Approach

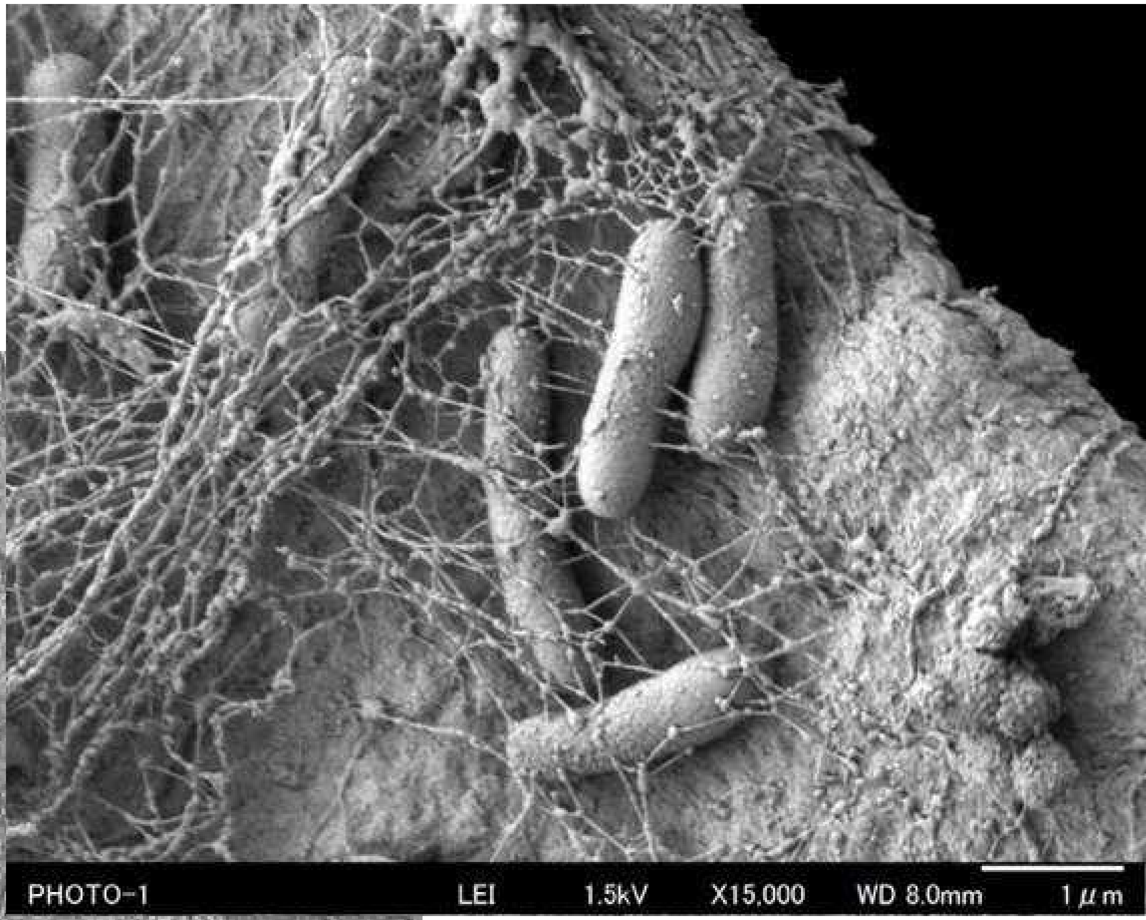
- Natural minerals
- Inoculation of microbial communities or a strain

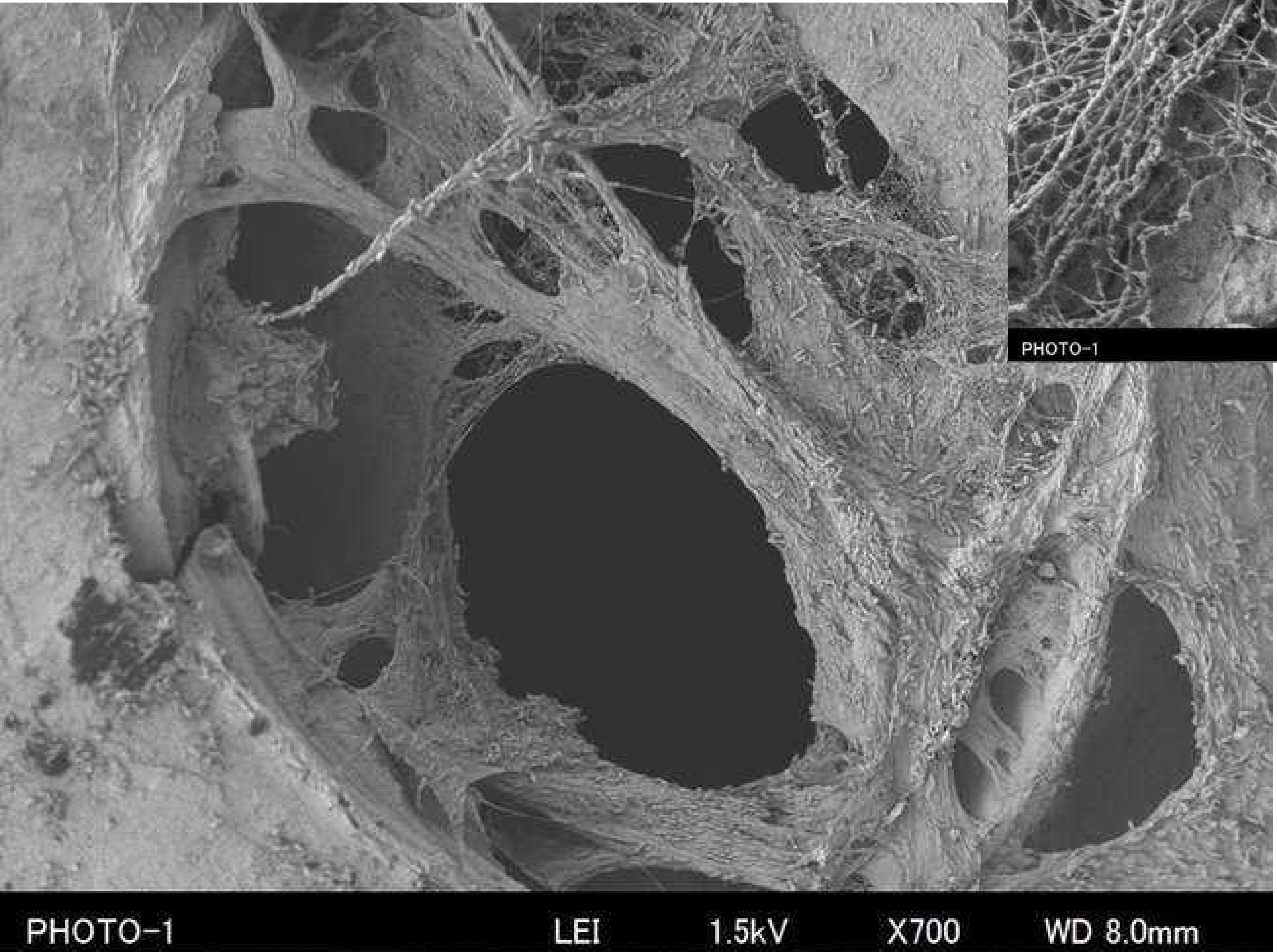
Raw Materials & Approach

- Porous material
- Inoculation of microbial communities

N_2O-reducing microorganisms into an artificial carrier

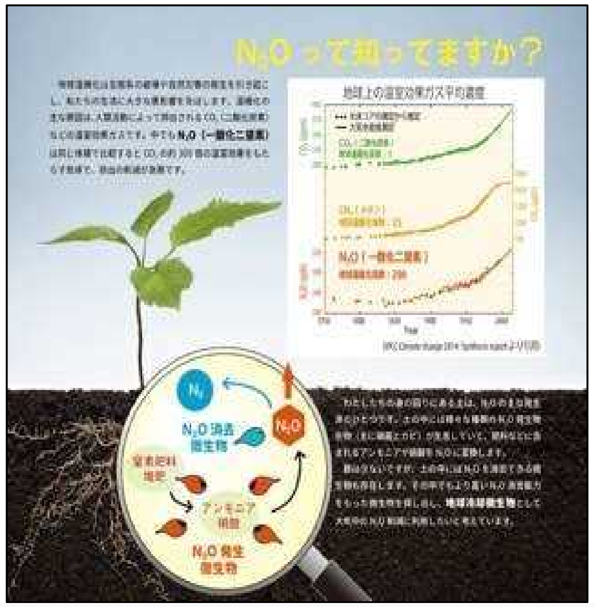
SEM (scanning electron microscope) image of introducing N_2O-reducing bacteria into a porous material and evaluating its colonization ability.

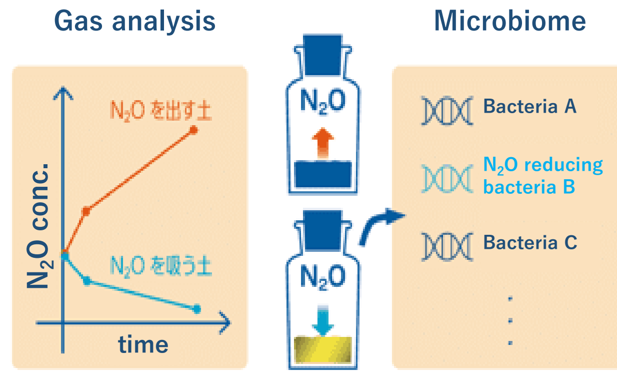


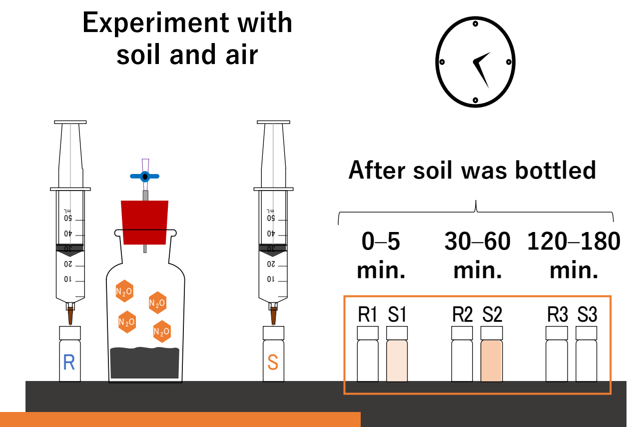


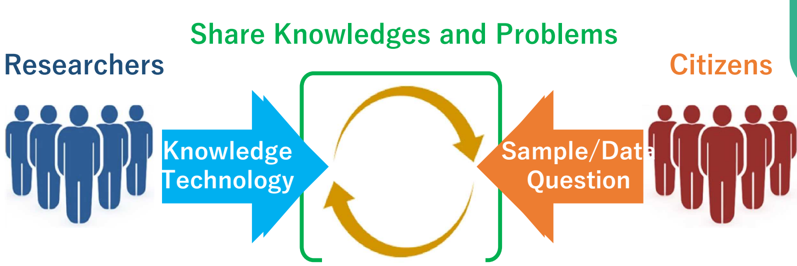
The surface layer of the internal cross section of the porous material using a scanning electron microscope (SEM) to confirm that microorganisms have colonized the inside of the carrier.

Overview of Citizen Science Project



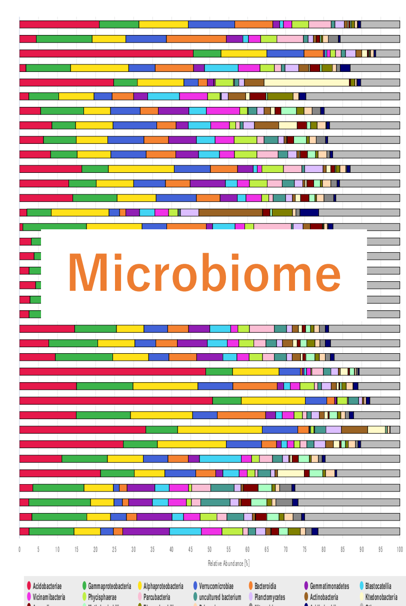


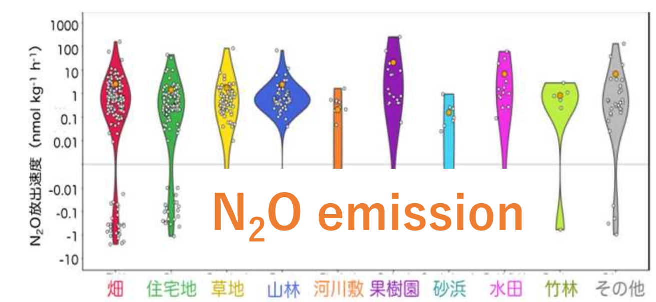


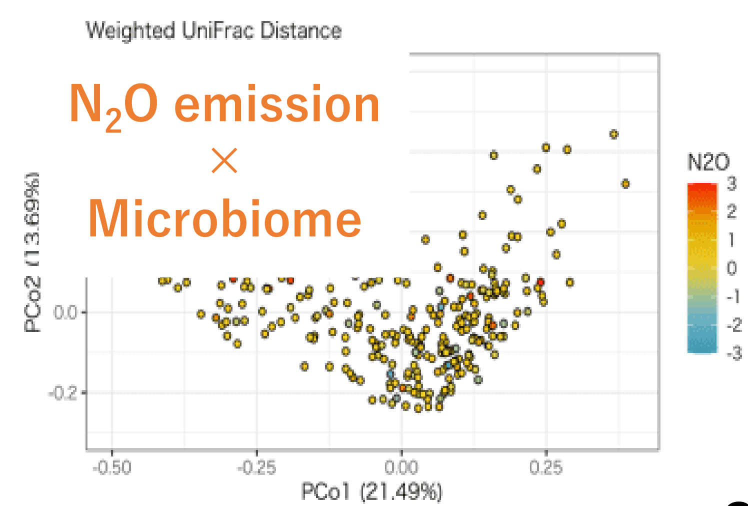


Data briefing / ELSI workshop (on-line)

Dig up! seminar (a benefit for participants)







Significance of citizen science projects

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. Our 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. Creation of citizen culture of enjoying science. Exploring issues essential to social implementation throughinteractive communications.

☐ 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 revolutionary soil microbiology and environmental researches.

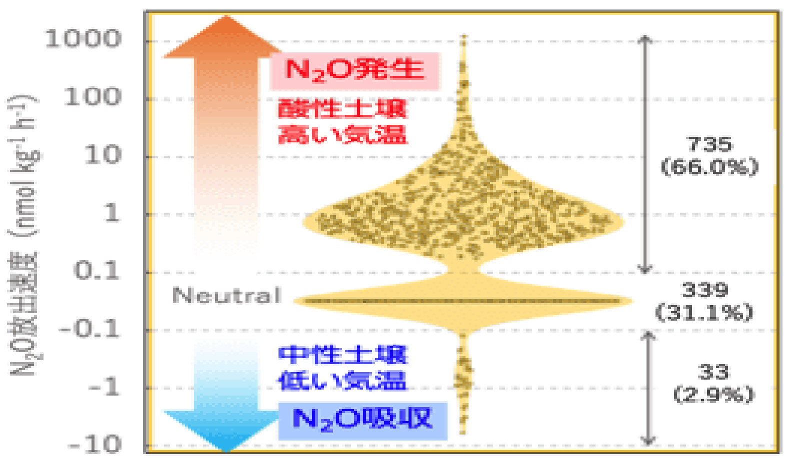
https://dsoil.jp/soil-in-a-bottle/

Research flow and scientific significance of citizen science

Cool Earth via dSOIL

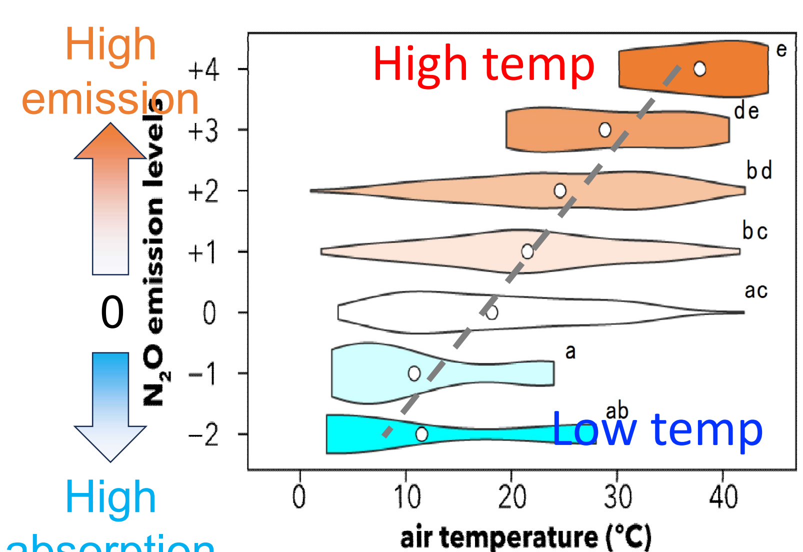
■ Collect samples and data from all over Japan

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



N_2O rate, Microbiome and Environmental data

Depends on Temp and pH



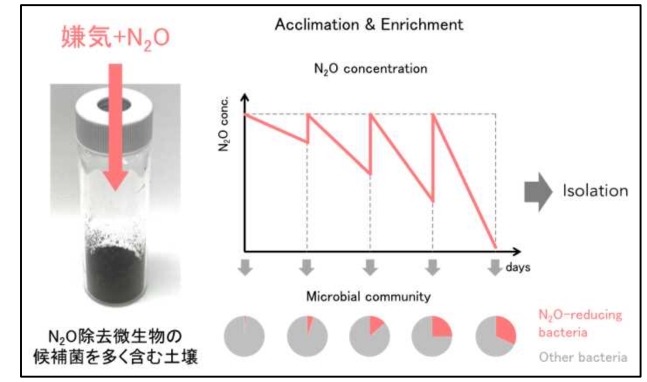
soil

data

■ Isolation of N_2O-reducing microorganisms

Soybean bradyrhizobia

Other bacteria



predict

Interesting as Microbiology

isolates

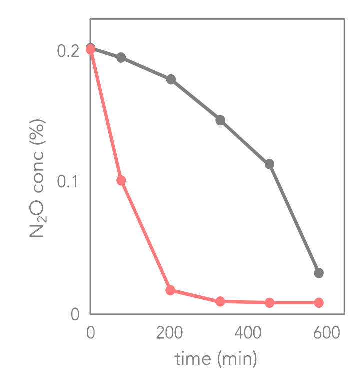
■ Database

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

Citizen science results (1) Soybean bradyrhizobia

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

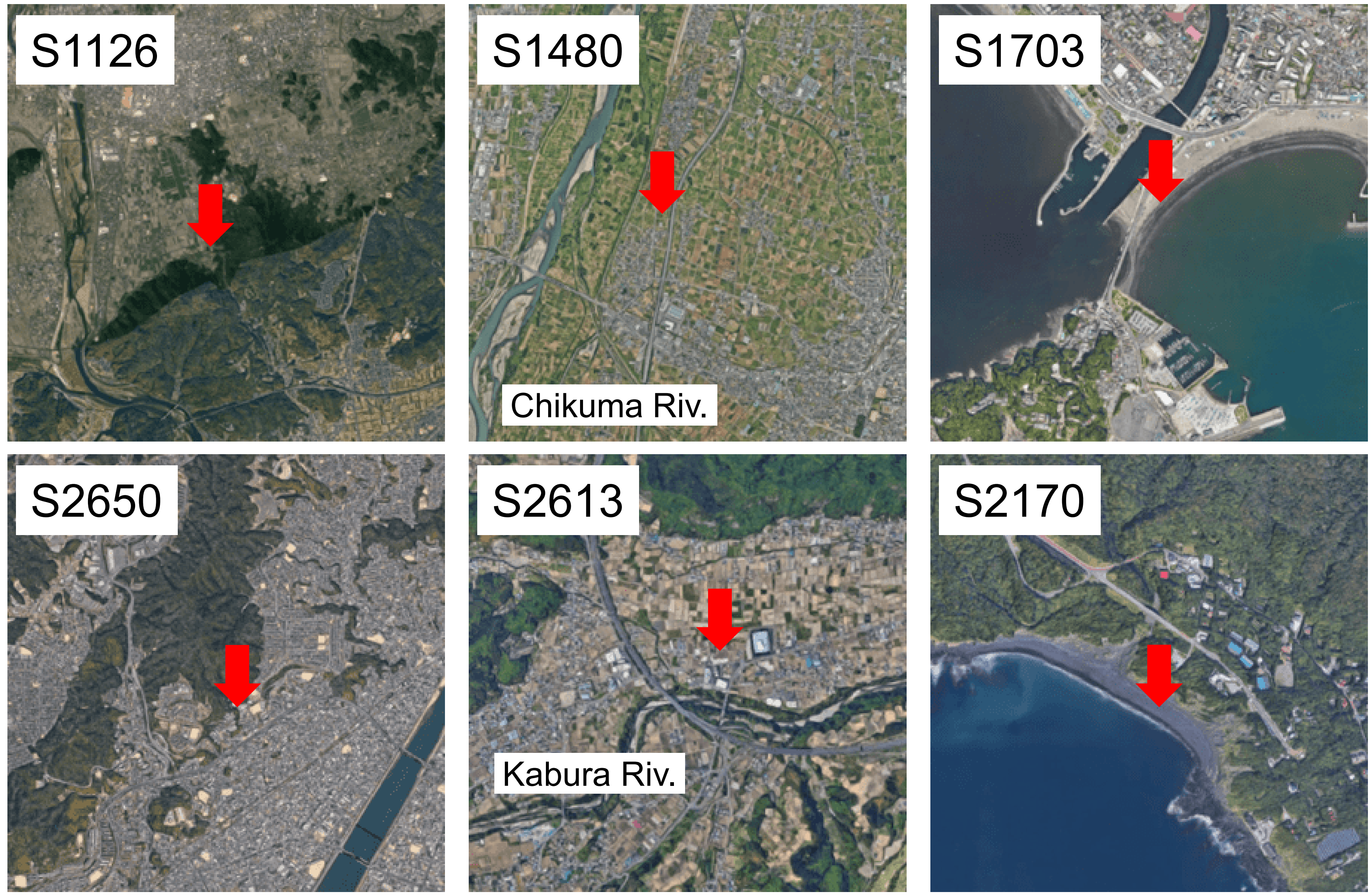
2nd

38/1256 →
more isolates?

Location features where SG09-type *nosZ* was detected

Mountain edge | **Beside rivers** | **Sandy beach**

1st / 2nd



Mountain edge	Beside rivers	Sandy beach
Okayama, Hiroshima etc.	Nagano, Gunma etc.	Kanagawa, Tokyo etc.
13 samples	33 samples	5 samples

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

Citizen science results (2) General microorganisms

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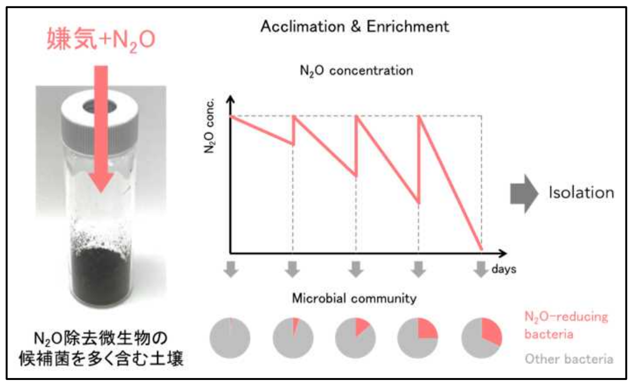
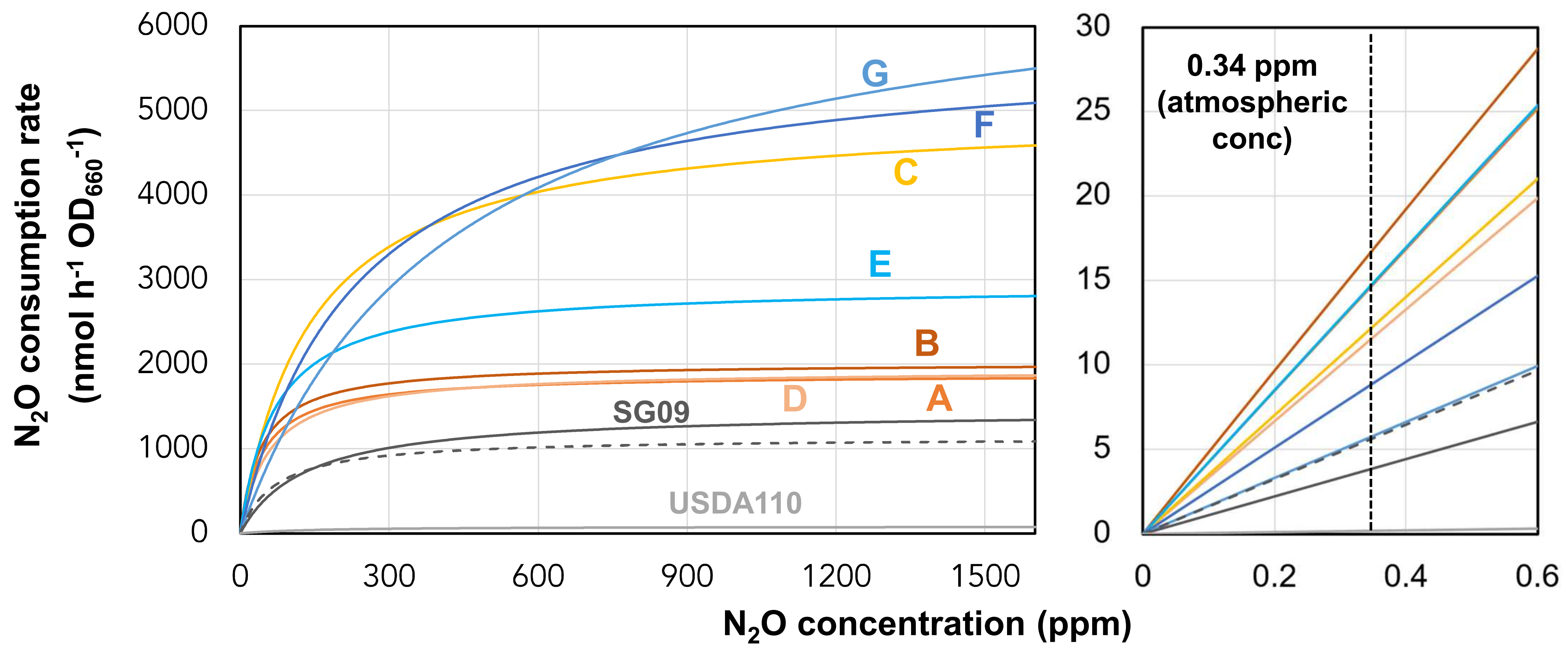


Enrichment with soybean field soils

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

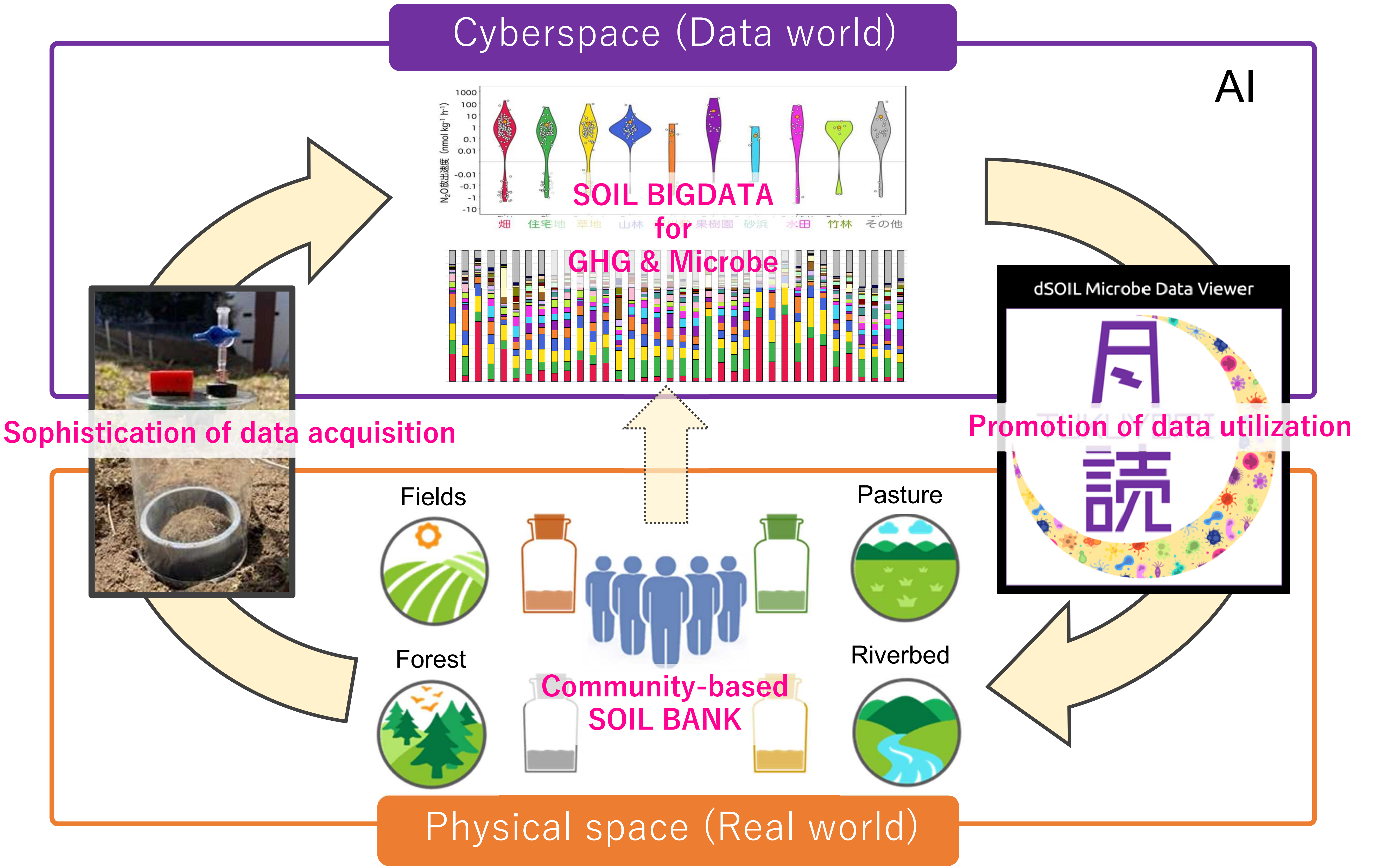


Strain	V_{max} (nmol h^{-1})	K_s (ppm)	$V_{0.34}$ (nmol h^{-1})	Medium
New isolate A	1886	44	14.4	R2A
New isolate B	2020	42	16.4	R2A
New isolate C	4994	142	11.9	R2A
New isolate D	1935	58	11.3	R2A
New isolate E	2925	69	14.4	R2A
New isolate F	5815	228	8.7	R2A
New isolate G	6932	418	5.6	R2A
B. ottawaense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

Four isolates showed lower K_s and 5 isolates showed higher V_{max} than SG09

Cell-level N_2O reduction parameters were obtained for new isolates

A cyber-physical system for soil microorganisms and N_2O removal



Developing infrastructure for long-term use of citizen science data by a wide range of stakeholders

“Citizen Science” events and academic conference organization

Cool Earth via dSOIL

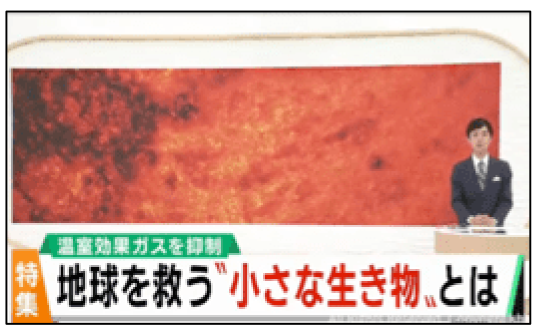
■Expanding citizen awareness of GHG N_2O through seven media reports in 2023

8/18 NHK Citizen Lab

5/22 NHK Science ZERO

3/6 ABEMAnews

10/27 TBC Tohoku Broadcasting



5/24 East Japan Broadcasting

6/30 Miyagi TV Broadcast

9/17 TV Asahi series “Shuzo Matsuoka’s Everyone Ganbare”

■Fostering a culture of enjoying science

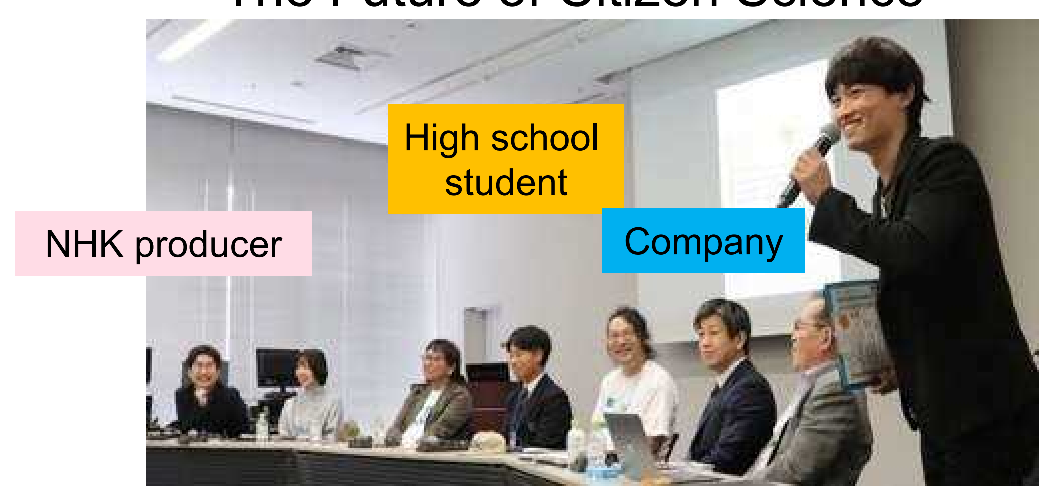
■Discussing issues essential to social implementation of microbial GHG mitigation

Co-hosted symposium led by Minamisawa MS (2023)

3/15 64th Annual Meeting of the Society of Plant Physiologists

11/30 36th Conference of the Society of Microbial Ecology

The Future of Citizen Science



2023/10/8 Japanese Association for the Advancement of Science (JAAS)

Contributing to “open science” and “stakeholder engagement”

R&D indexes and site visit

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[As of December 20, 2023]

●Paper	68 (Currently posting 15)	●Invited talk	31
●Presentation	109	●Award	7
●Patent	6 (1 international, 2 domestic, 3 foreign) + 3 scheduled		

● Press (other than TV)

2021/7/16 Nihon Keizai Shimbun electronic version "Tohoku University reveals the flexibility of the symbiotic genome region of rhizobia"
2021/9/20 Nikkan Kogyo Shimbun "Save the Earth! Moonshot NEDO Program"
2022/1/4 Weekly Post "Temperature rise in 2030 can still be stopped: Passionate scientists taking on the challenge of decarbonization"

● International Symposium

March 1, 2021: 716 participants registered, 27 participating countries

● NEDO Site Visit

Let peoples (citizens, scientists, companies and NEDO) know about our research activities!

Collaboration between MS projects

Development of weathering acceleration technology “A-ERW” (CO_2)

Atmospheric CO_2 removal using rocks

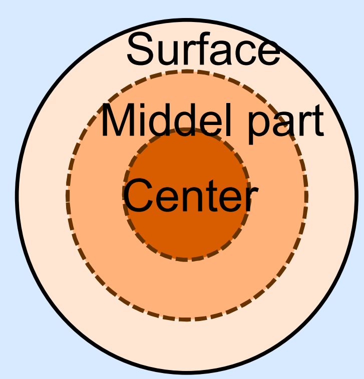
Nakagaki PM

Morimoto PM

Reducing GHG emissions from farmland (N_2O)

N_2O removal using artificial aggregates with N2O-reducing microorganisms

Minamisawa PM



Collaboration begins on citizen science system

Soil aggregates are the key!

Technology to reduce both CO_2 and N_2O in the future?

MOONSHOT

RESEARCH & DEVELOPMENT PROGRAM