

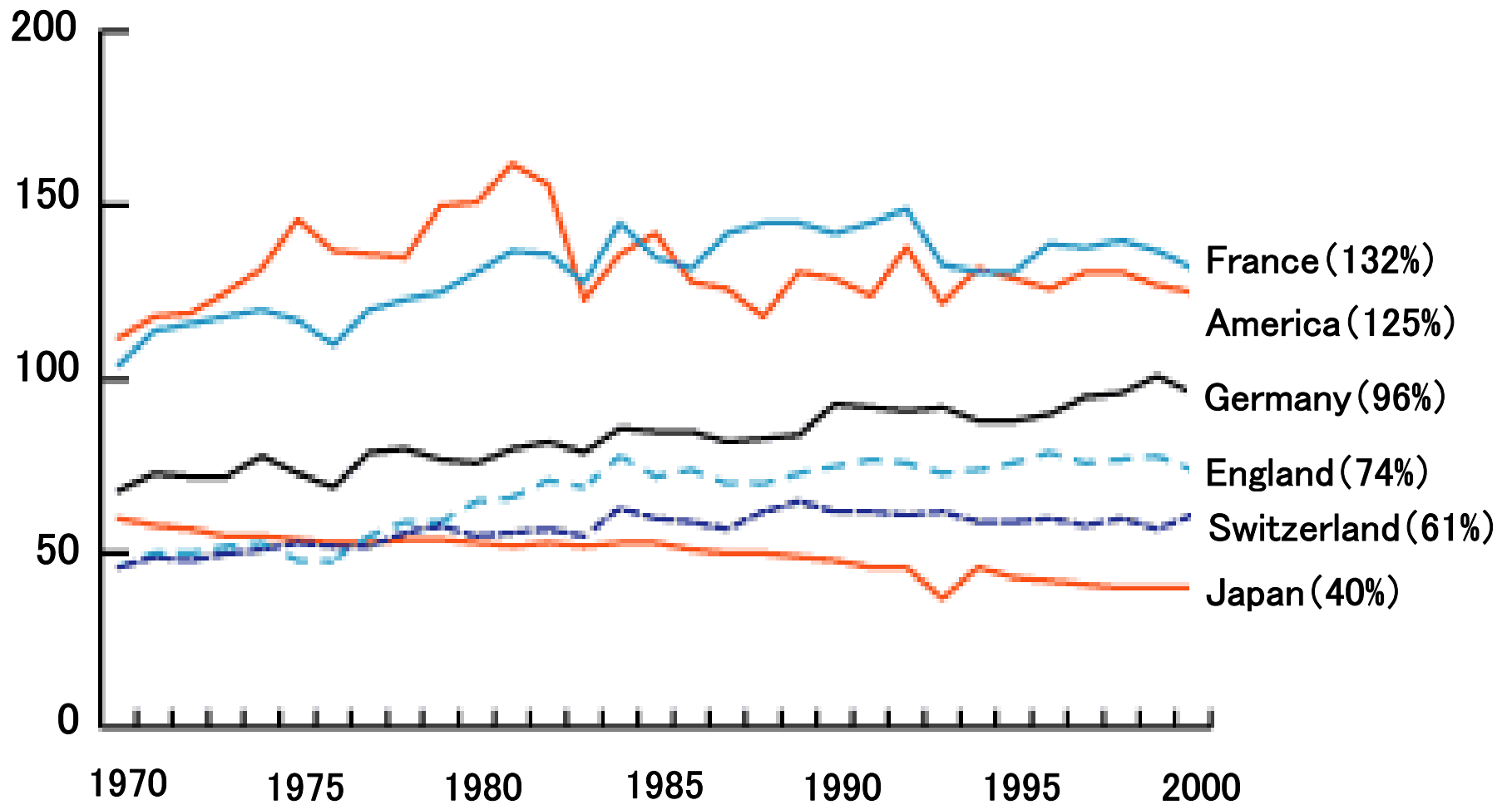
Precise diagnosis of soil by material circulation

*-Evaluation of soil condition based on
microbial activity for organic agriculture-*

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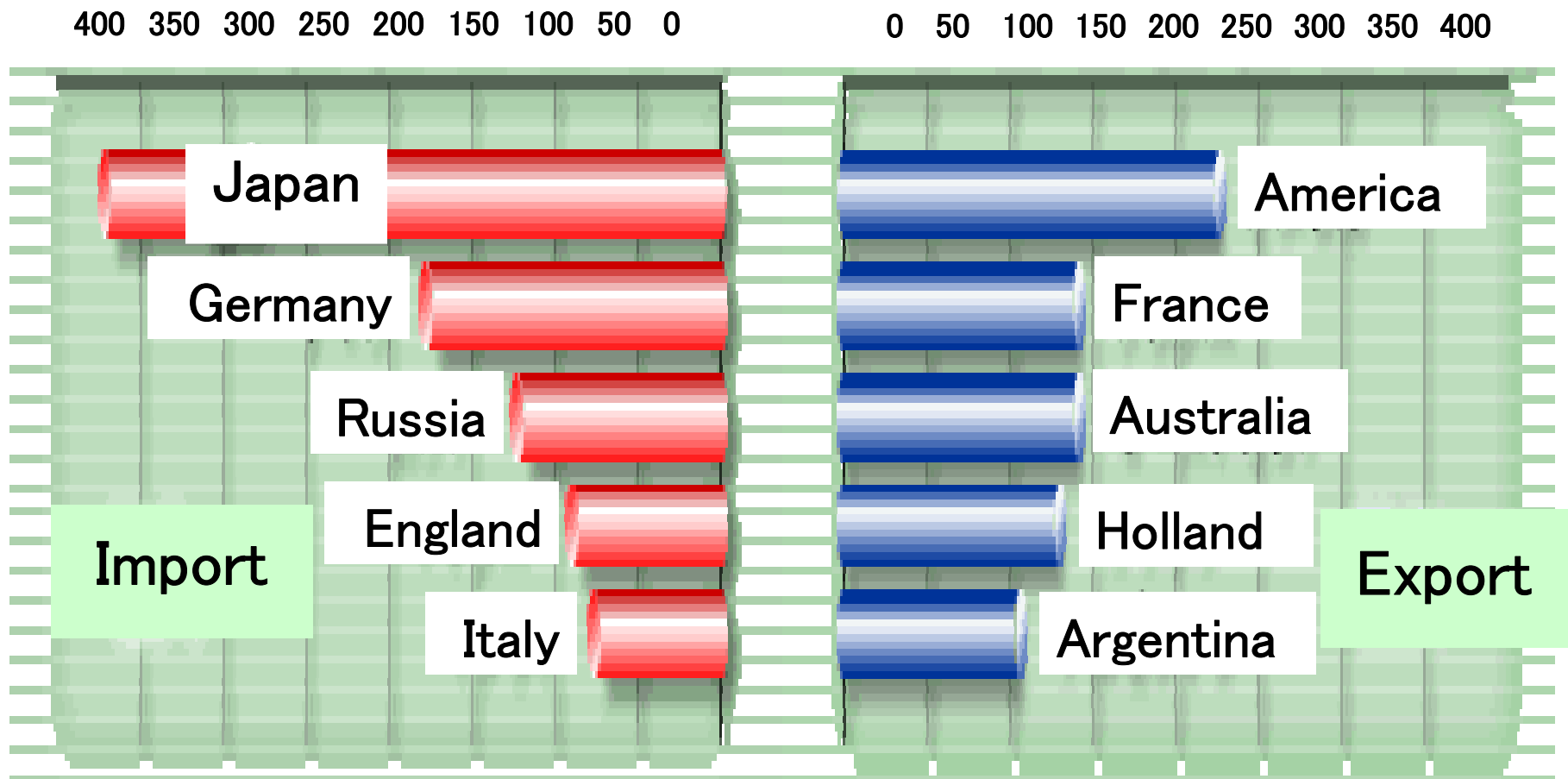
Self-sufficiency in food



Food import and export

(Hundred million dollar)

(Hundred million dollar)



Problems of food in Japan

Depend on import foods (decrease self-sufficiency)

- **Self-sufficiency in food is very low** (about 40%)

Safety of foods

- **Contamination of toxic substances** (agro-chemicals, pesticide, etc)

Competition between foods and new energy

- Corns, sugarcanes, etc. are used for production of bio-ethanol

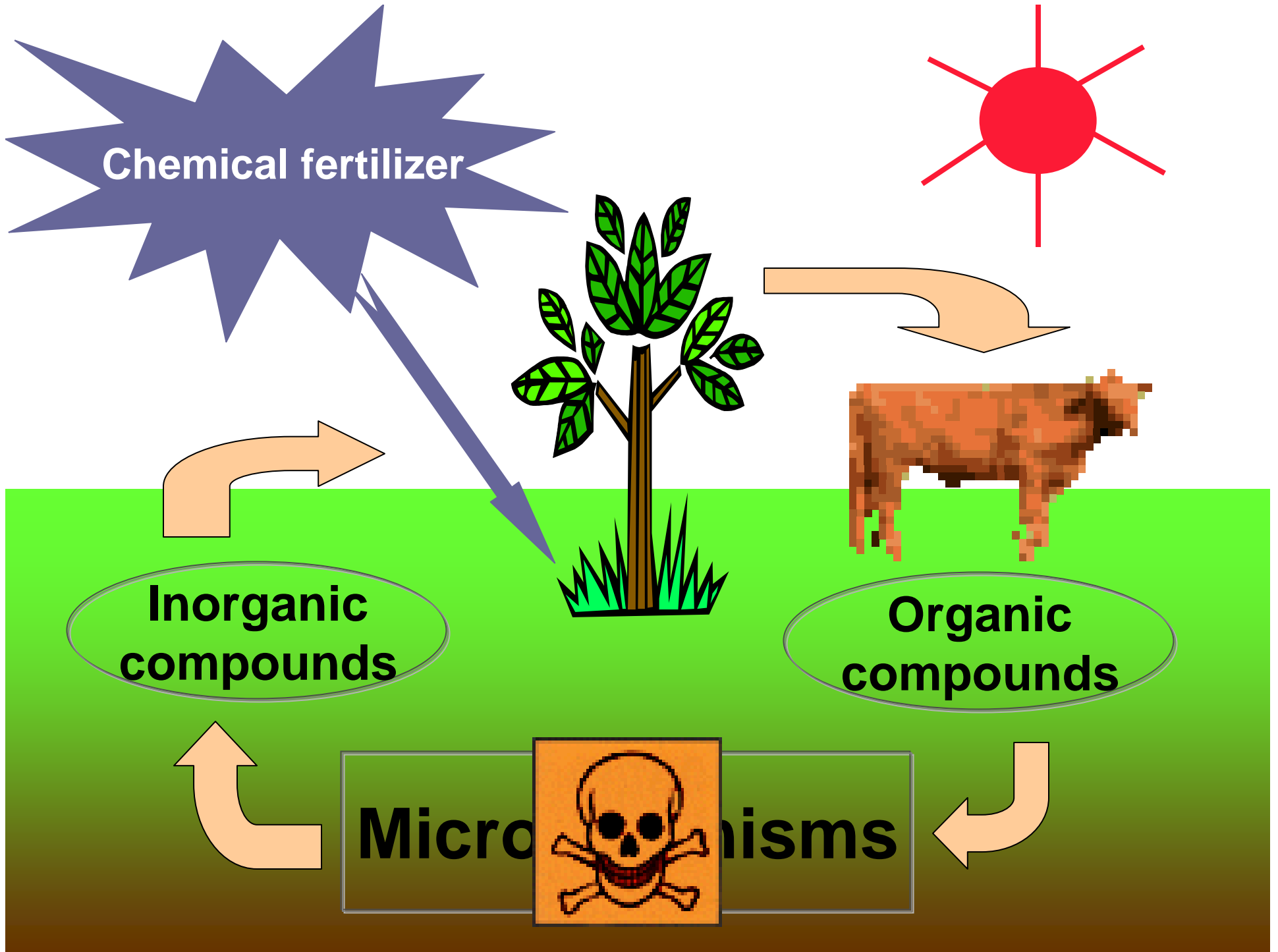


- **Food price is significantly increasing**

Recent strong wishes for food in Japan

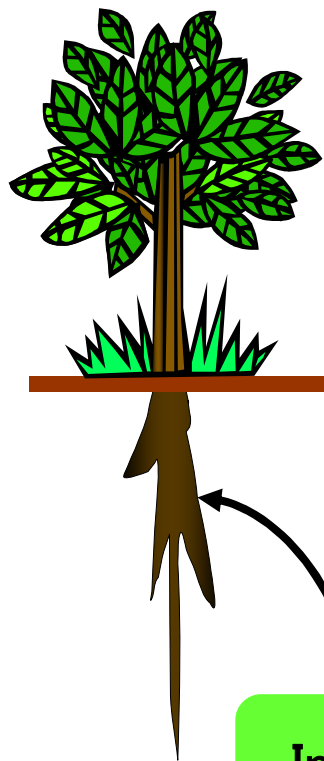
- **Safety**
- **Out of danger, security**
- **Food quality** (need organic products)

Evaluation of soil bacterial biomass



Need of microbe monitoring

《Evaluation of agricultural field》



Organic compound

Inorganic compound

《Monitoring of microbes for bioremediation》

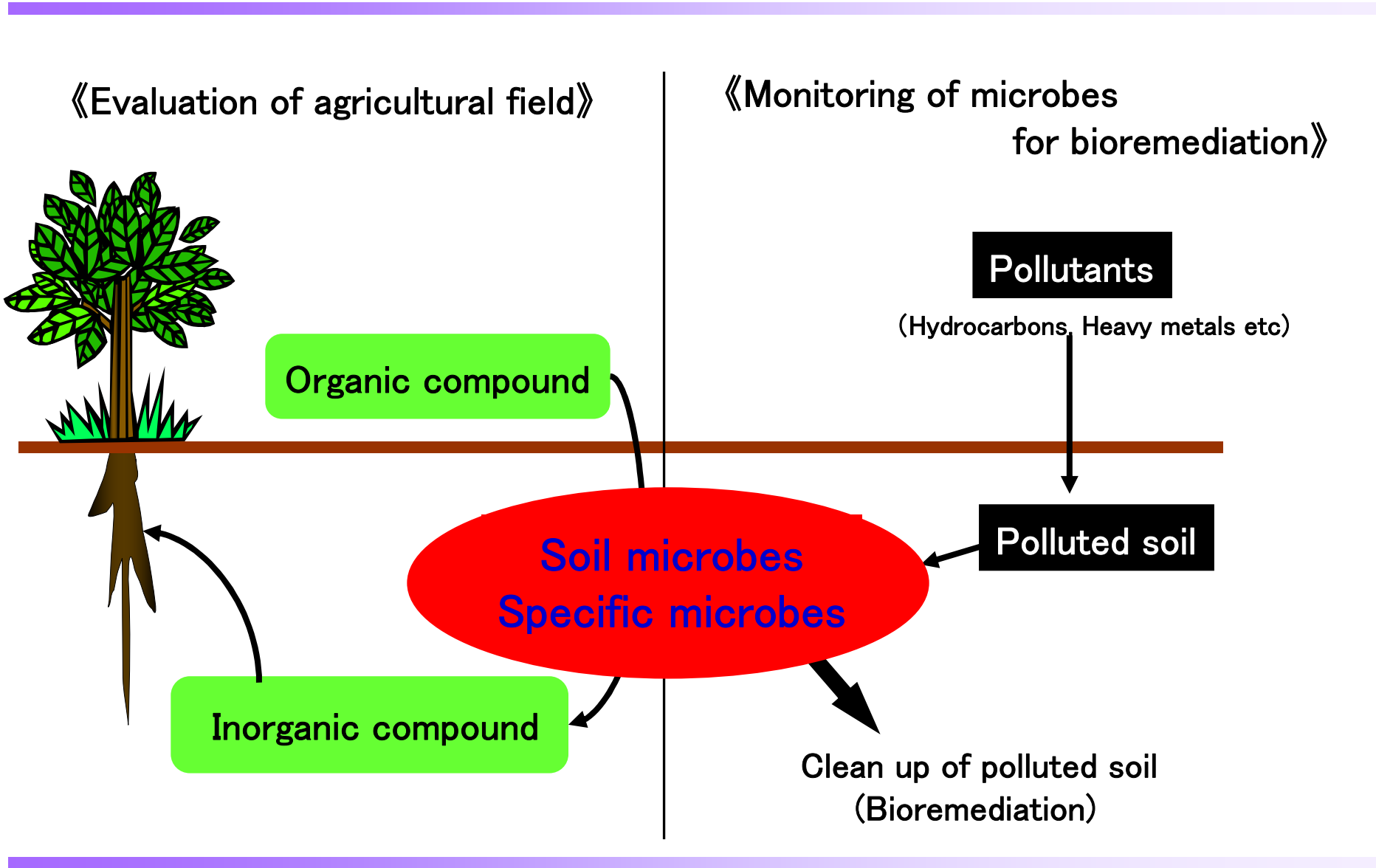
Pollutants

(Hydrocarbons, Heavy metals etc)

Polluted soil

Soil microbes
Specific microbes

Clean up of polluted soil
(Bioremediation)



Quantify of bacterial biomass

Plating method

- Can not count VBNC (viable but not cultureable) microbe
- Take long time

DAPI staining method

- Analyze correct microbe number
- Complicated procedure

- **Need to analyze VBNC**
- **Need easy procedure**

Extract and quantify of eDNA

Evaluation of soil bacterial biomass by eDNA

Rich microbes in soil → → → Soft and fertile soil

Chemical
fertilizer

Decrease of environmental
bacteria

Pollution

Correct bacterial biomass monitoring is needed

Bacterial biomass in soil $\hat{=}$ eDNA in soil

Evaluation of bacterial biomass

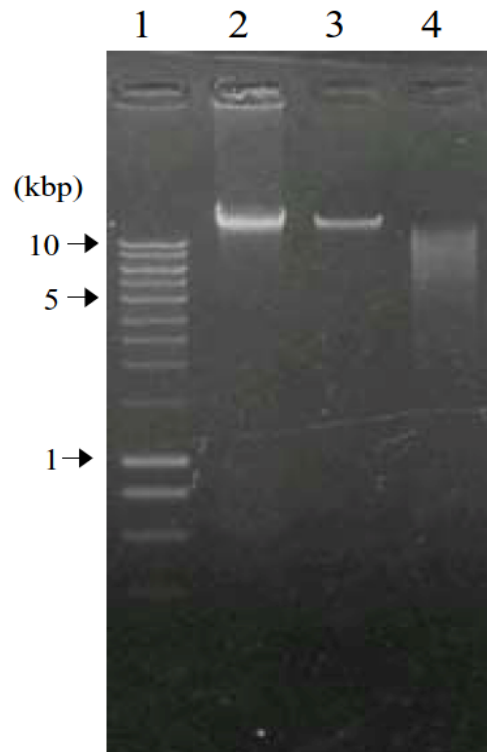


Fig. Agarose gel electrophoresis of eDNA extracted from soil in an agricultural field using various eDNA extraction methods.

Lane 1 Smart Ladder (mass marker)
Lane 2 slow stirring method
Lane 3 heat treatment method
Lane 4 the bead method

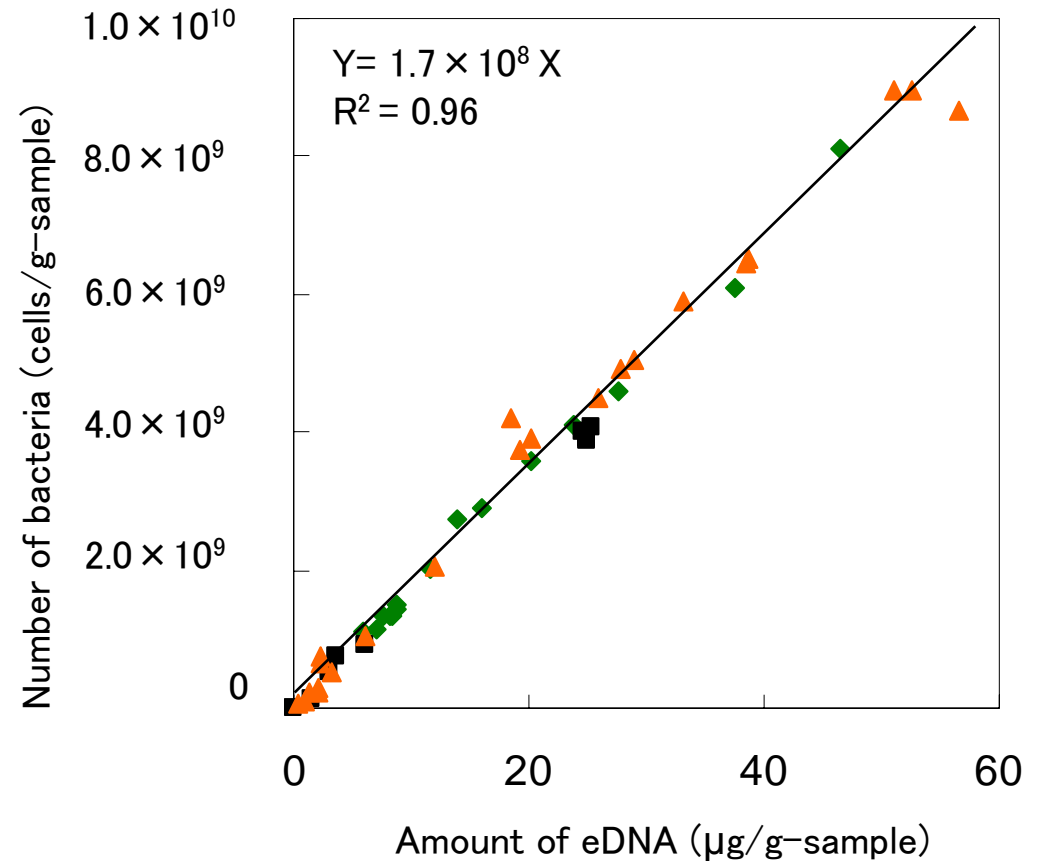


Fig. Relationship between the bacterial number obtained using DAPI staining and the amount of eDNA in 57 soils.

■; the amount of eDNA in an agricultural field
◆; oil-polluted field
▲; non-agricultural field.

Analysis of bacterial biomass

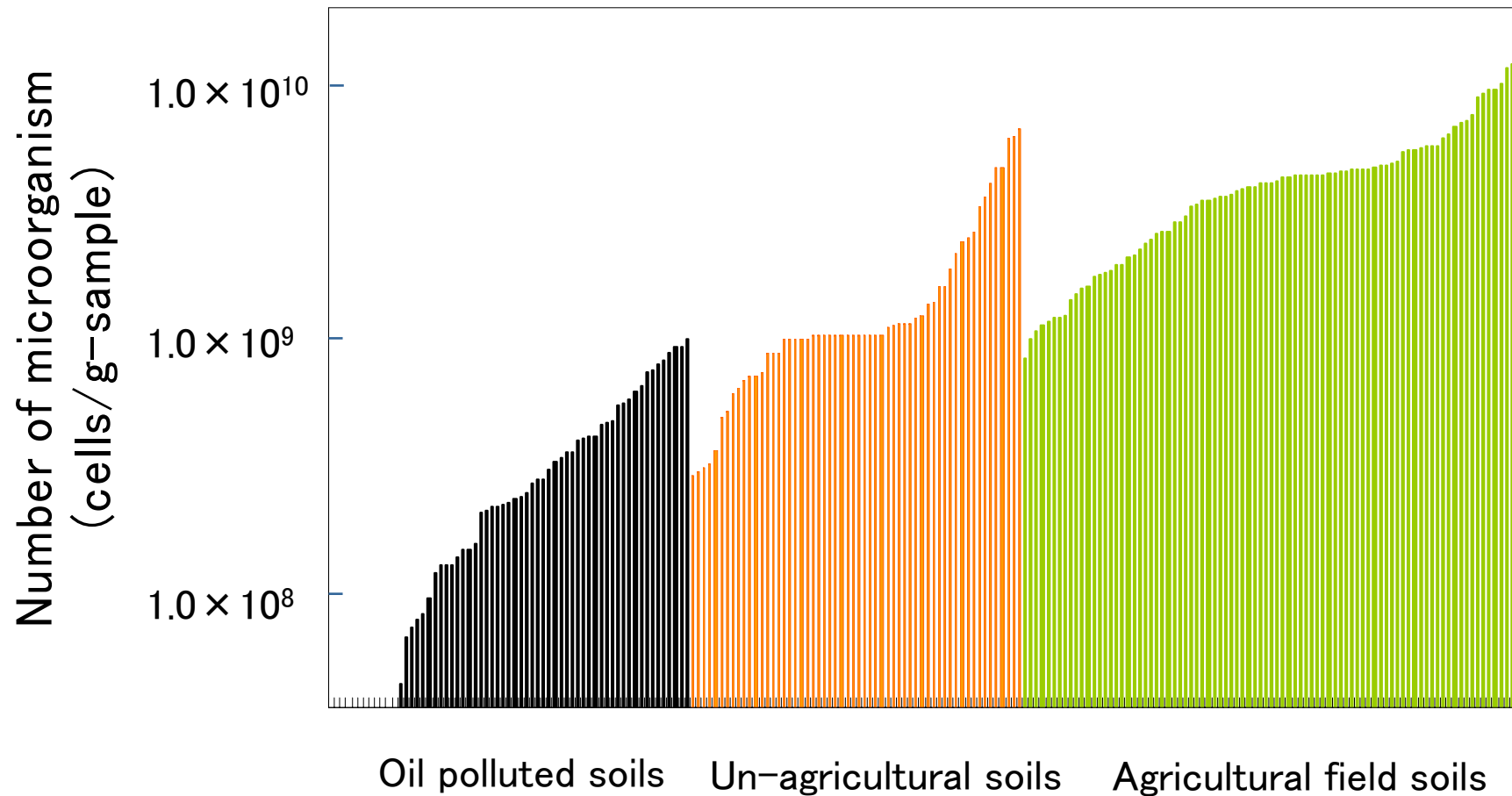


Fig. Number of microorganism in agricultural field, oil polluted, and other soils. (209 samples)

■ Oil polluted soils ■ Other soils ■ Agricultural field soils

Environmental DNA extraction machine



**Precise diagnosis of soil
by material circulation**

Relationship between bacterial biomass and nitrification

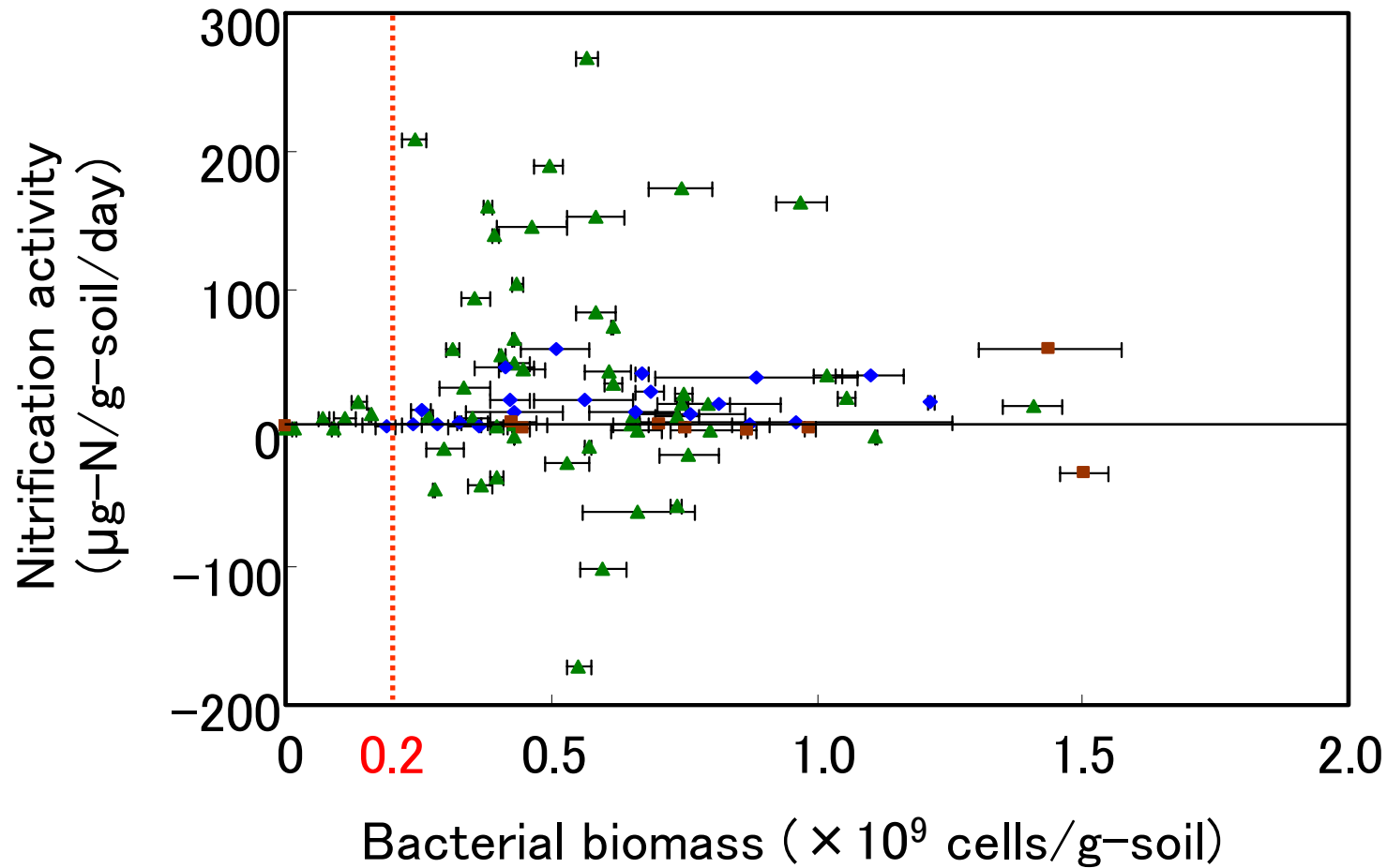
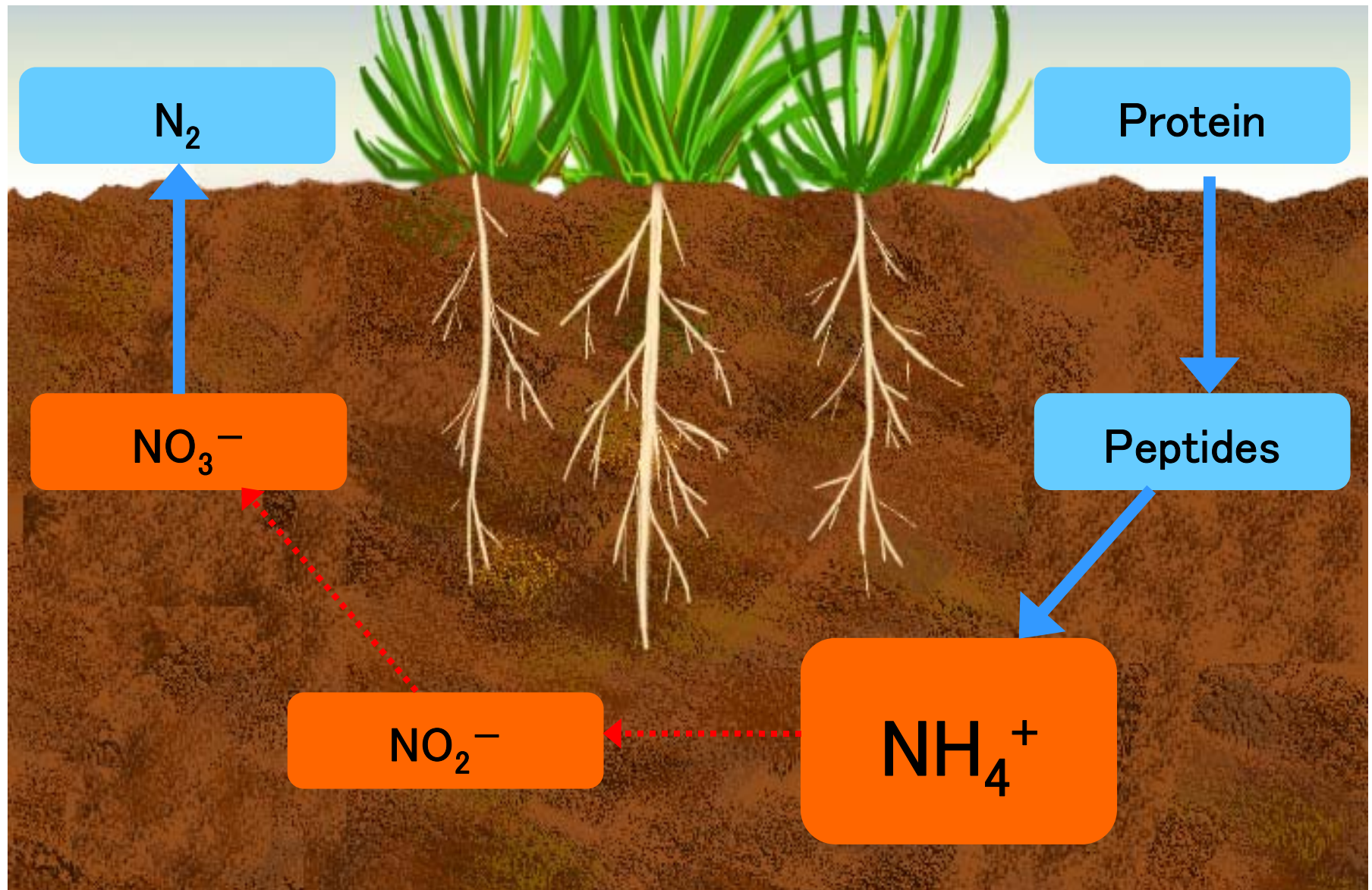


Fig. Relationship between bacterial biomass and nitrification

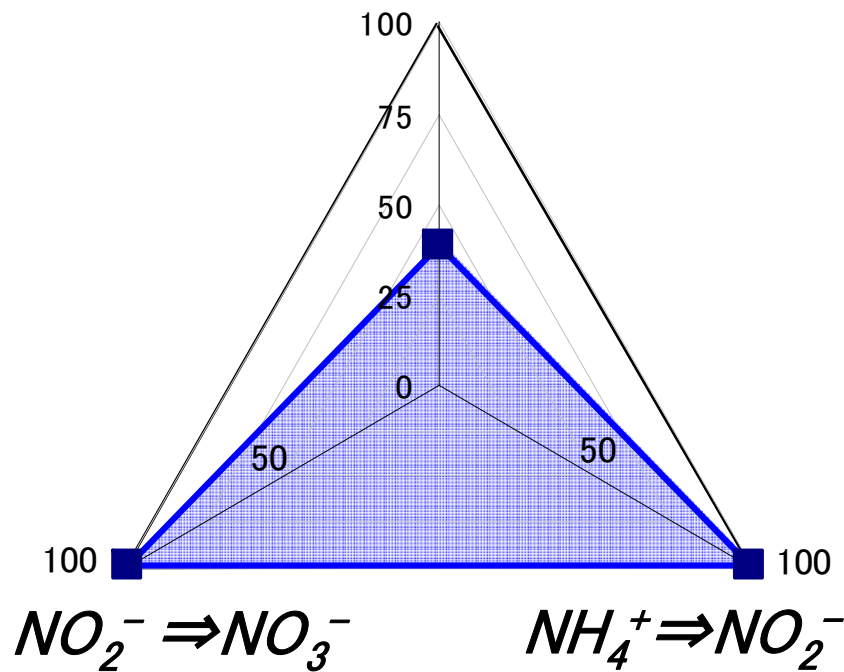
Evaluation of nitrogen circulation



Diagnosis of bacterial biomass and nitrification

A : Sample ①

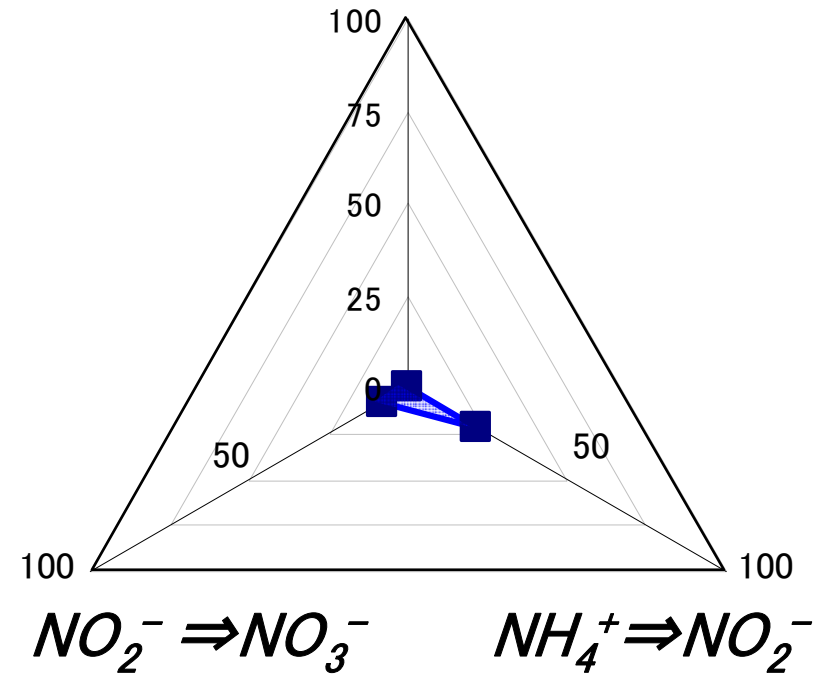
Bacterial biomass



Area of sample ① : 62.7/100%

B : Sample ②

Bacterial biomass



Area of sample ② : 7.1/100%

Evaluation of agricultural soil and compost

Soil No.	Value	Compost No.	value
①	62.7	②	40.0
⑤	30.1	⑧	30.0
⑩	21.8	③	26.0
⑨	21.3	④	24.9
⑥	21.2	⑦	22.2
④	13.6	⑨	16.6
⑧	11.5	⑤	11.0
⑦	9.8	①	10.8
③	9.5	⑥	5.7
②	7.1	⑩	1.0

Precise diagnosis of soil condition

For high quality of organic agricultural product



Chemical analysis

+

Biological analysis

- NH_4^+
- NO_2^-
- NO_3^-
- Total carbon
- Phosphorus content
- Potassium content
- pH
- Soil types

- Bacterial biomass
- Ammonia oxidizing bacterial biomass
- Nitrogen circulation activity
- Phosphorus circulation
- Carbon circulation
- Sulfur circulation