IAE

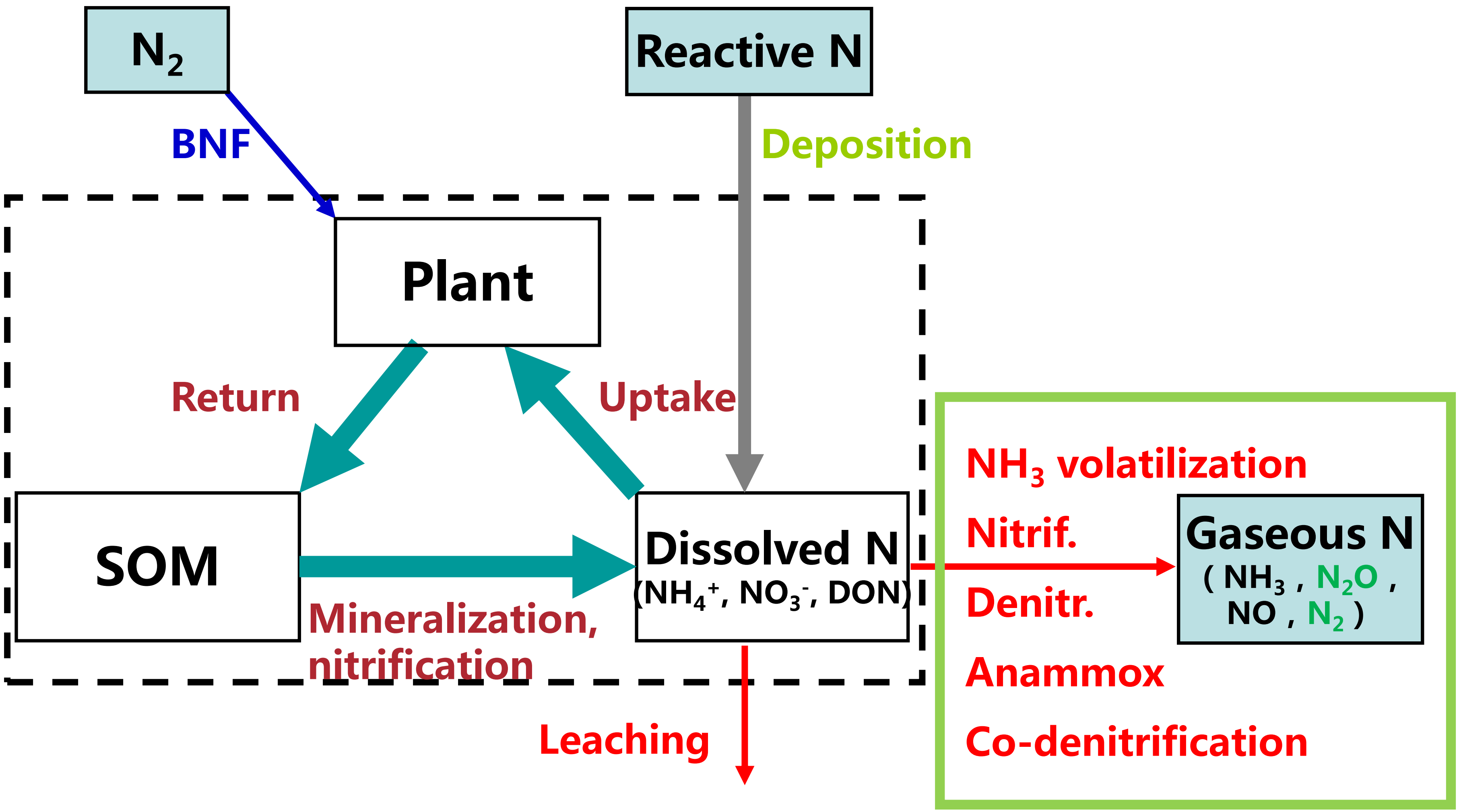
第四届全国稳定同位素生态学学术研讨会
暨中国生态学学会稳定同位素生态专业委员会2017年学术年会

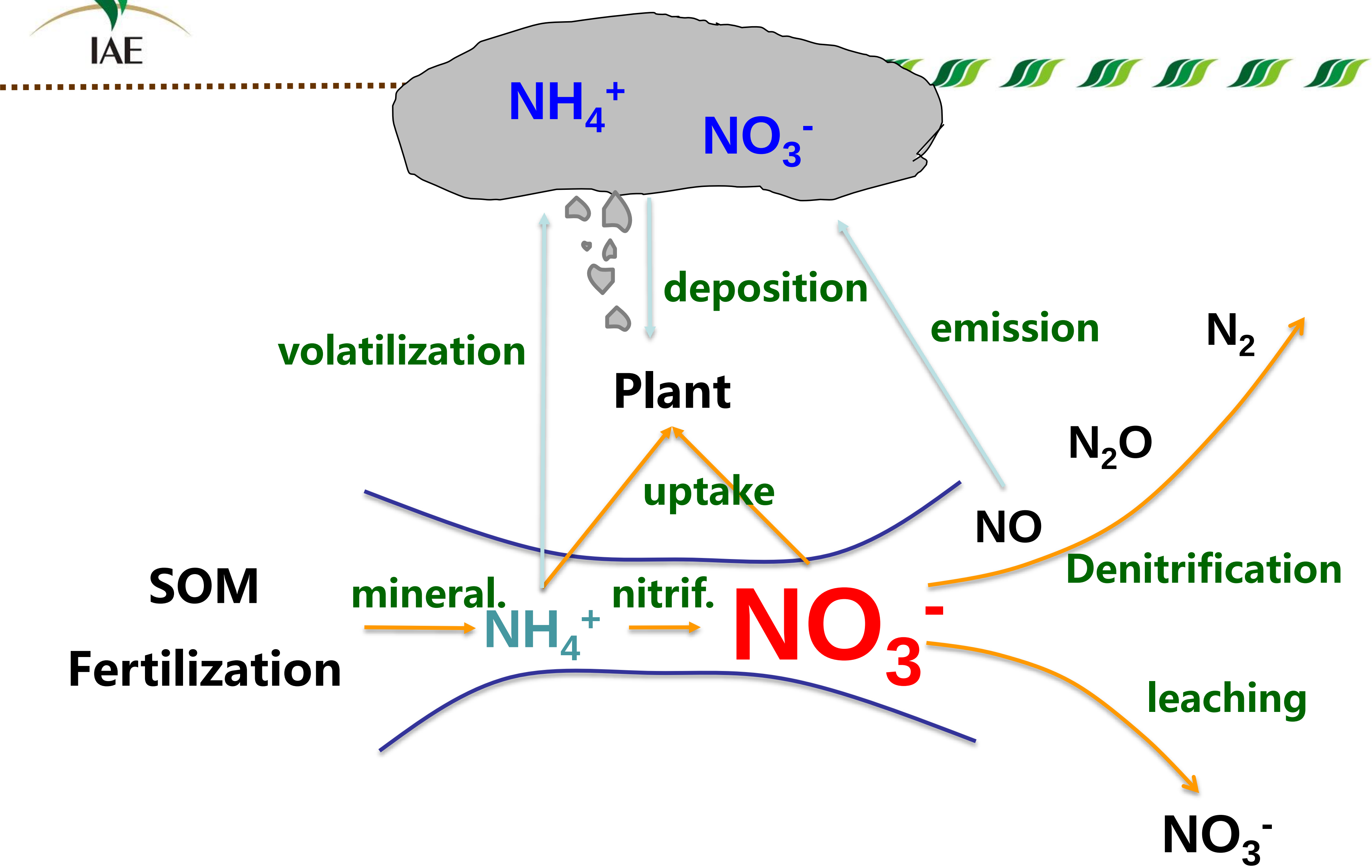
森林土壤气态氮损失

方运霆（沈阳生态所）

南京，2017年10月16-18日

N cycling in Forest Ecosystems



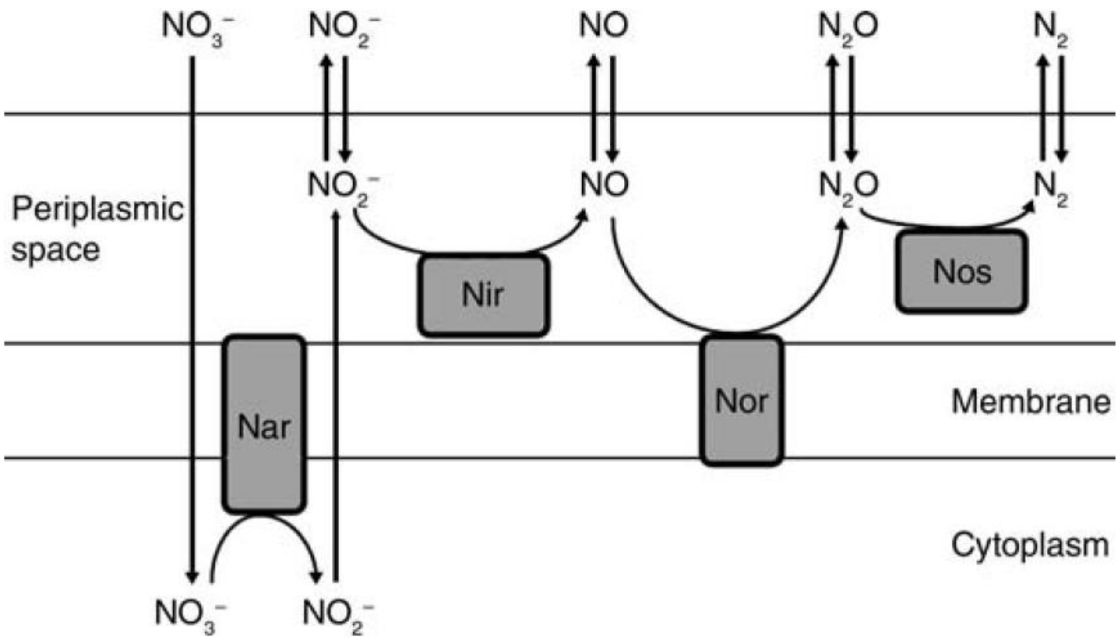

NH_4^+
NO_3^-
deposition
volatilization
emission
N_2
Plant
N_2O
uptake
NO
SOM
mineral.
nitrif.
Denitrification
NH_4^+
NO_3^-
Fertilization
leaching
NO_3^-

- **Annual denitrification rate at ecosystem levels**
- **Soil microbial N_2 production processes**
- **Effects of N deposition on soil N_2O and N_2 production from two tropical forests**

Denitrification

- Happens everywhere;
- A process proposed to be a mechanism for N limitation in terrestrial and aquatic ecosystems.
- A basic and challenging question: how much N is lost via denitrification annually for a given ecosystem?

Denitrification

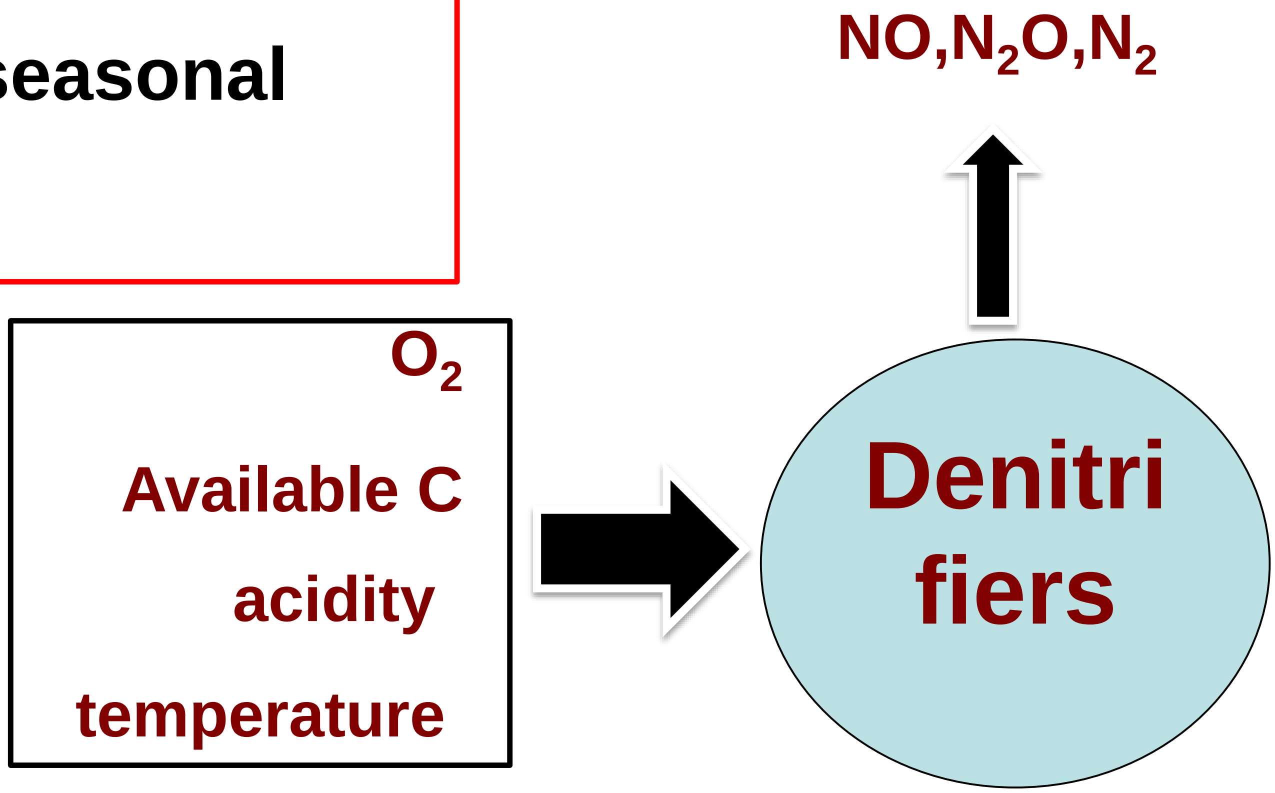


NO_3^- → NO_2^- → NO → N_2O → N_2

Wallenstein et al., 2006. Ecological Applications

Difficulties to estimate denitrification rate at an ecosystem scale

1. High background N_2
2. Spatial and seasonal variations

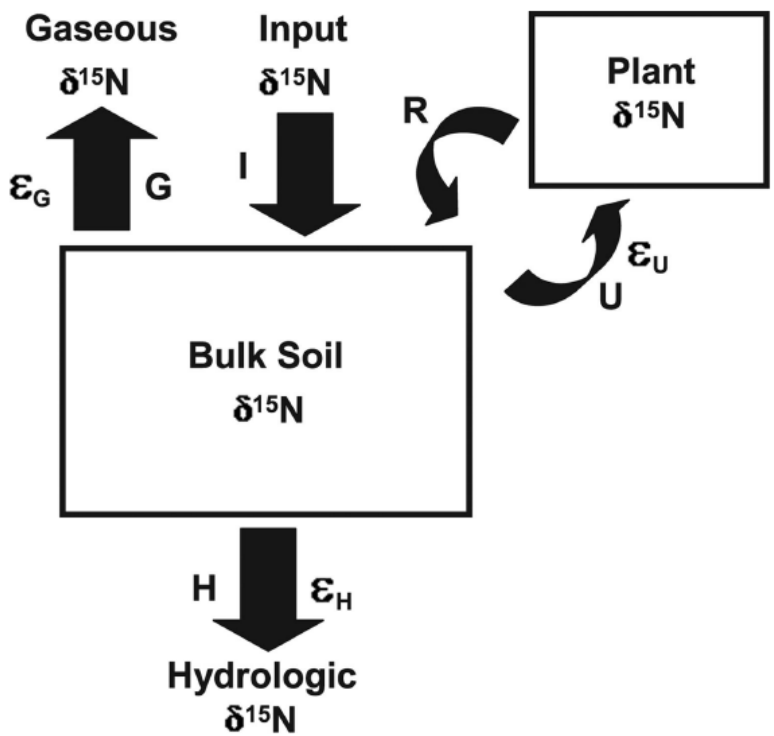


From Wallenstein et al., 2006. Ecological Applications

Approaches for denitrification rate at ecosystem levels

Approach	Disadvantages
Mass balance	Based on N inputs (from biological N fixation, N fertilization, N deposition) and assuming no soil retention. Not suitable for natural ecosystems.
^{15}N natural abundance for total dissolved N	Total gaseous N losses, both from denitrification and nitrification.
^{15}N natural abundance for bulk soil	Long-term total gaseous N loss.
N_2O/N_2 ratio	Used for large regional scale, ratio varied greatly from ecosystem to ecosystem.
Soil core gas flow+field O_2 concentration	O_2 is one of important controlling factors, not the sole factor.
Nitrate ^{15}N and ^{17}O natural abundance	Hydrological data, e.g., precipitation and water loss, nitrate isotope analysis.

Bulk soil ^{15}N natural abundance



$\delta^{15}N = (R_{sample}/R_{air} - 1) * 1000$

$R = {}^{15}N/{}^{14}N$

$\delta^{15}N_{soil} = 5.5‰$

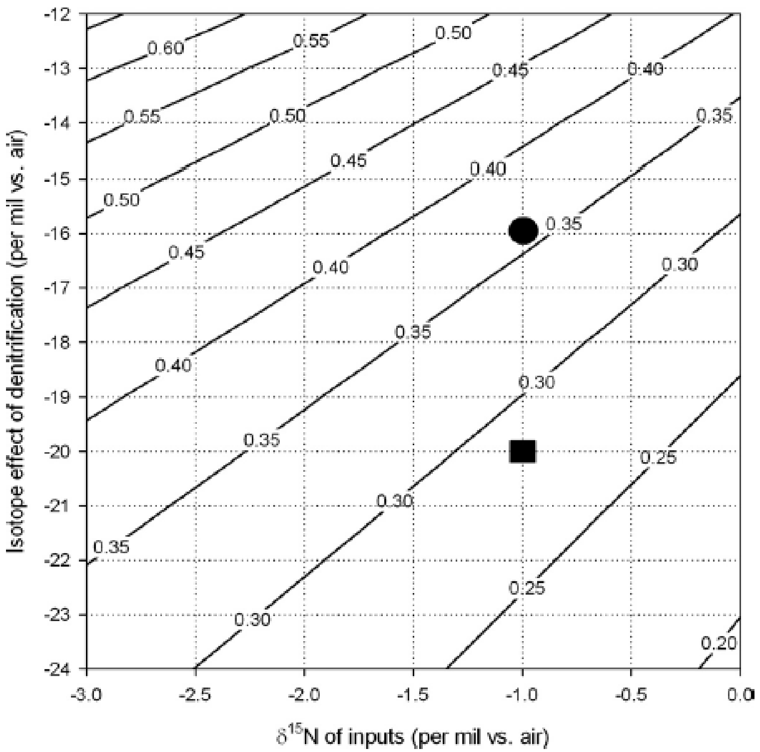
$\delta^{15}N_{inputs} = -1.3‰$

Assume：Plant N uptake and return have no influence on soil ^{15}N abundance ($\delta^{15}N_{soil}$）

$$\delta^{15}N_{soil} = \delta^{15}N_{inputs} + \varepsilon_H \cdot (H/(H+G)) + \varepsilon_G \cdot (G/(H+G))$$

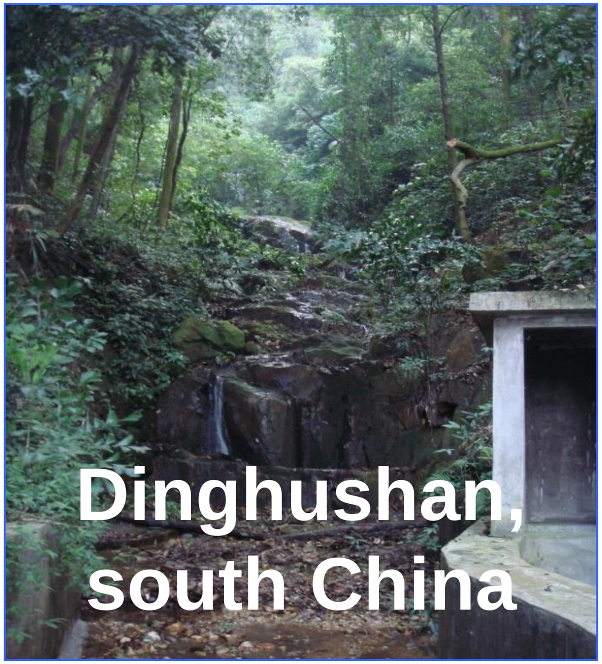
Houlton et al., 2006. PNAS; Houlton and Bai, 2009. PNAS；Bai et al.,2013. Biogeosciences

Bulk soil ^{15}N natural abundance



○ **Houlton and Bai., 2009. PNAS**

Gaseous N losses rate, acounting for one third of total N losses.



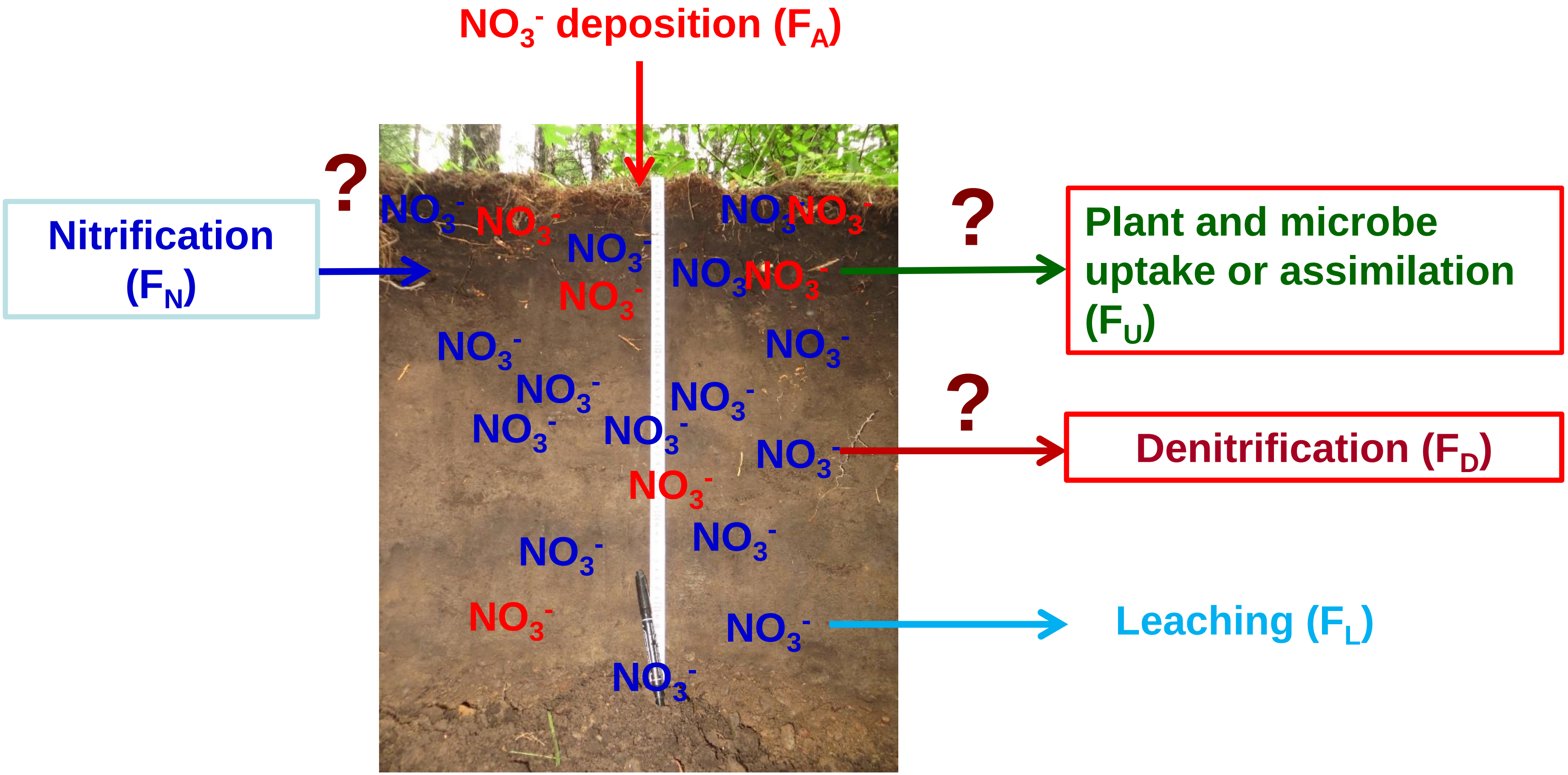
○ **Koba, Fang*, et al., 2012. JGR.**

Gaseous N loss rate was 10 kg N/ha.yr, accounting for 12% of total N losses.

N_2O + NO = 10 kg N/ha.yr (4 + 6)

No N_2 production?

Mass balance



$$F_{IN} = F_A + F_N = F_U + F_D + F_L$$

Fang et al., 2015. PNAS

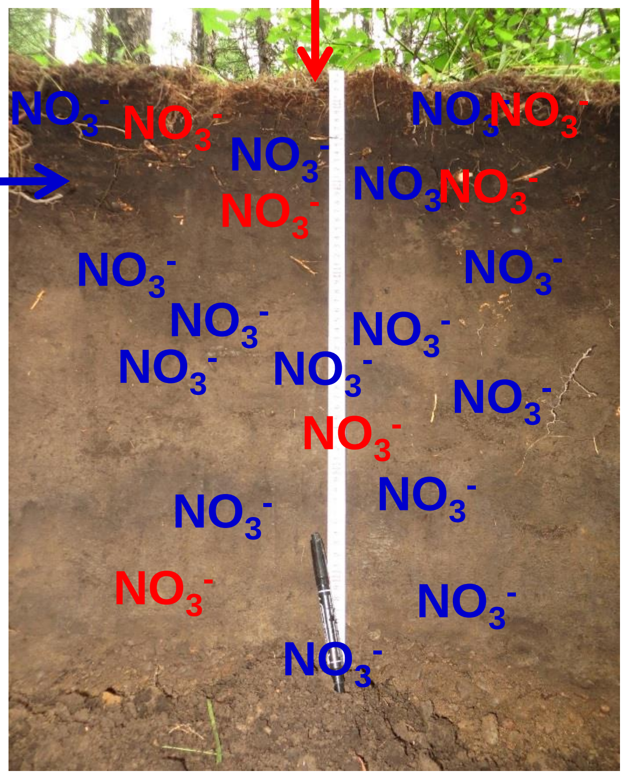
Gross nitrification rate

NO_3^- deposition (F_A)

$\Delta^{17}O_A$

Nitrification（F_N）

$\Delta^{17}O_N$



$$\Delta^{17}O_{soilNO3-} = (F_A \times \Delta^{17}O_A + F_N \times \Delta^{17}O_N) / (F_A + F_N)$$

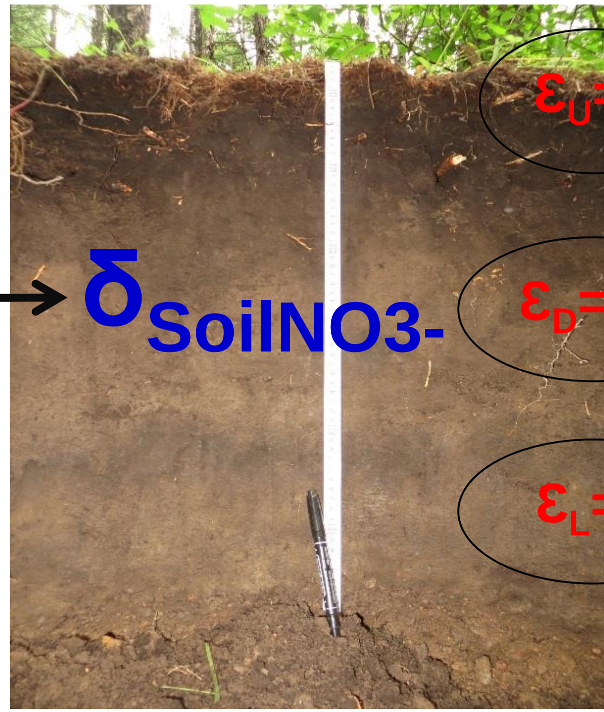
$$\Delta^{17}O_N = 0$$

$$\Delta^{17}O_L = \Delta^{17}O_{soilNO3-}$$

$$F_N = F_A \times (\Delta^{17}O_L - {}^{17}O_{A)}) / \Delta^{17}O_L$$

Isotope mass balance

$\delta_{NO3\text{-}input}$ →



f_U → δ_{uptake} $(\delta^{15}N_{soilNO3-} - \varepsilon_U)$

f_D → $\delta_{denitrification}$ $(\delta^{15}N_{soilNO3-} - \varepsilon_D)$

f_L → $\delta_{leaching}$ $(\delta^{15}N_{soilNO3})$

$$f_D + f_U + f_L = (F_D + F_U + F_L) / (F_A + F_N) = 1$$

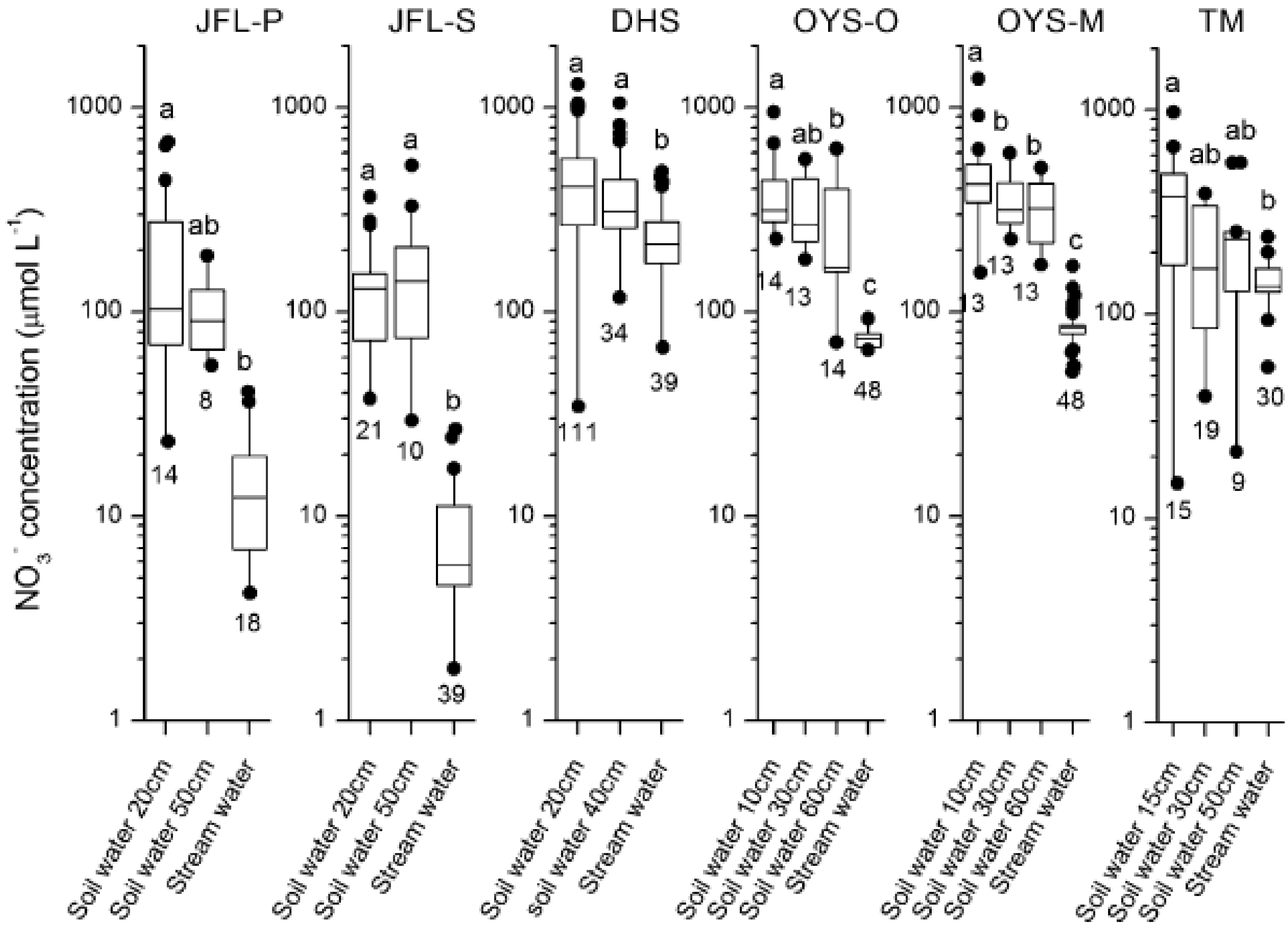
$$\delta^{15}N_{soilNO3-} = \delta^{15}N_{NO3\text{-}input} + f_D \times \varepsilon_D + f_U \times \varepsilon_U$$

Equation calculating denitrification rate

$$F_D = [(F_A + F_N) \times (\delta^{15}N_L - \delta^{15}N_{soil\text{-}input\text{-}NO3\text{-}} - 2) + F_L \times 2)] / (16 - 2)$$

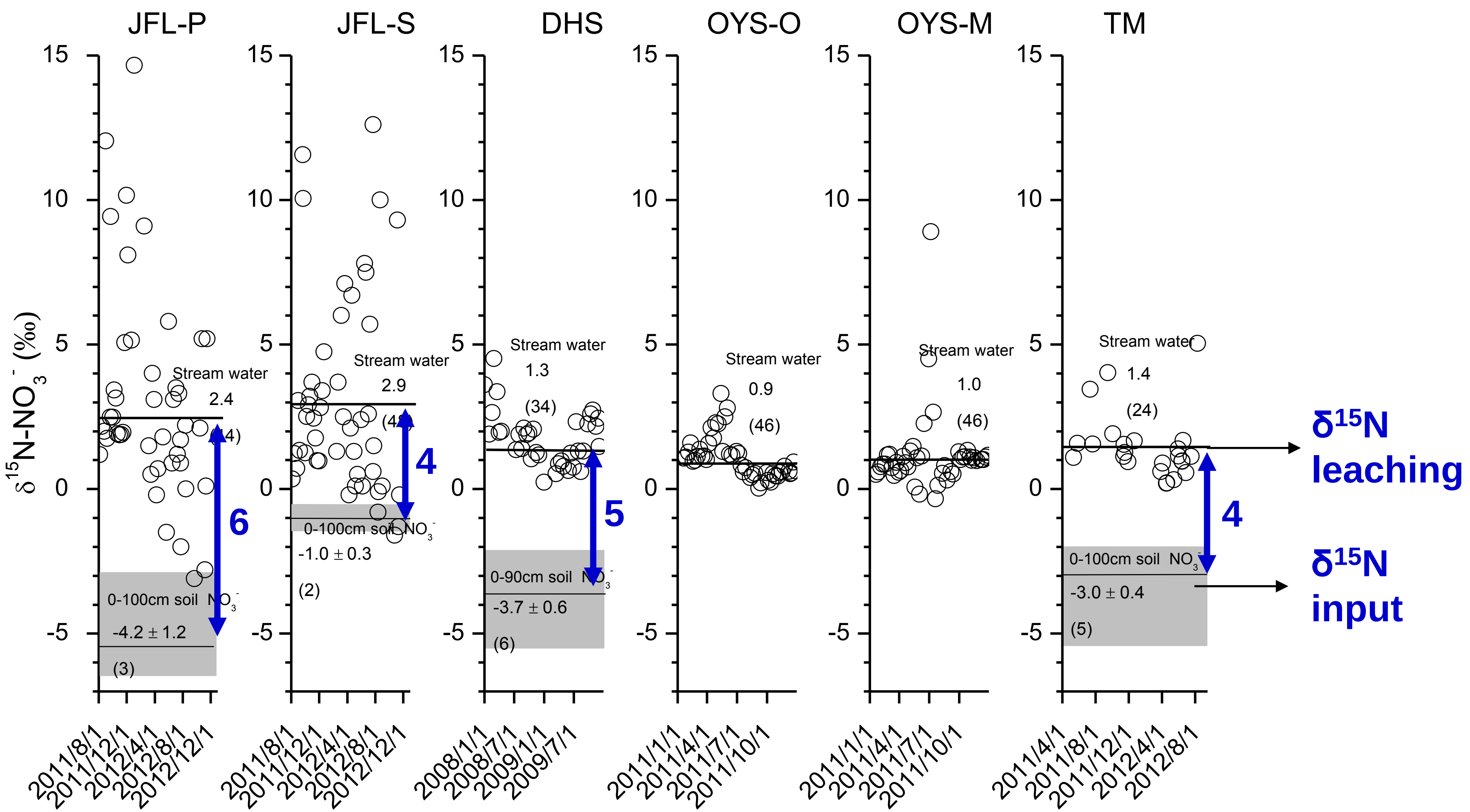
1. NO_3^- leaching rate (F_L)
2. NO_3^- deposition rate（F_A)
3. Atmospheric NO_3^- $\Delta^{17}O$ ($\Delta^{17}O_A$)
4. Leaching NO_3^- $\Delta^{17}O$（$\Delta^{17}O_L$)
5. Leaching NO_3^- $\delta^{15}N$ ($\delta^{15}N_L$)
6. Soil input NO_3^- $\delta^{15}N$ ($\delta^{15}N_{soil\text{-}input\text{-}NO3\text{-}}$)

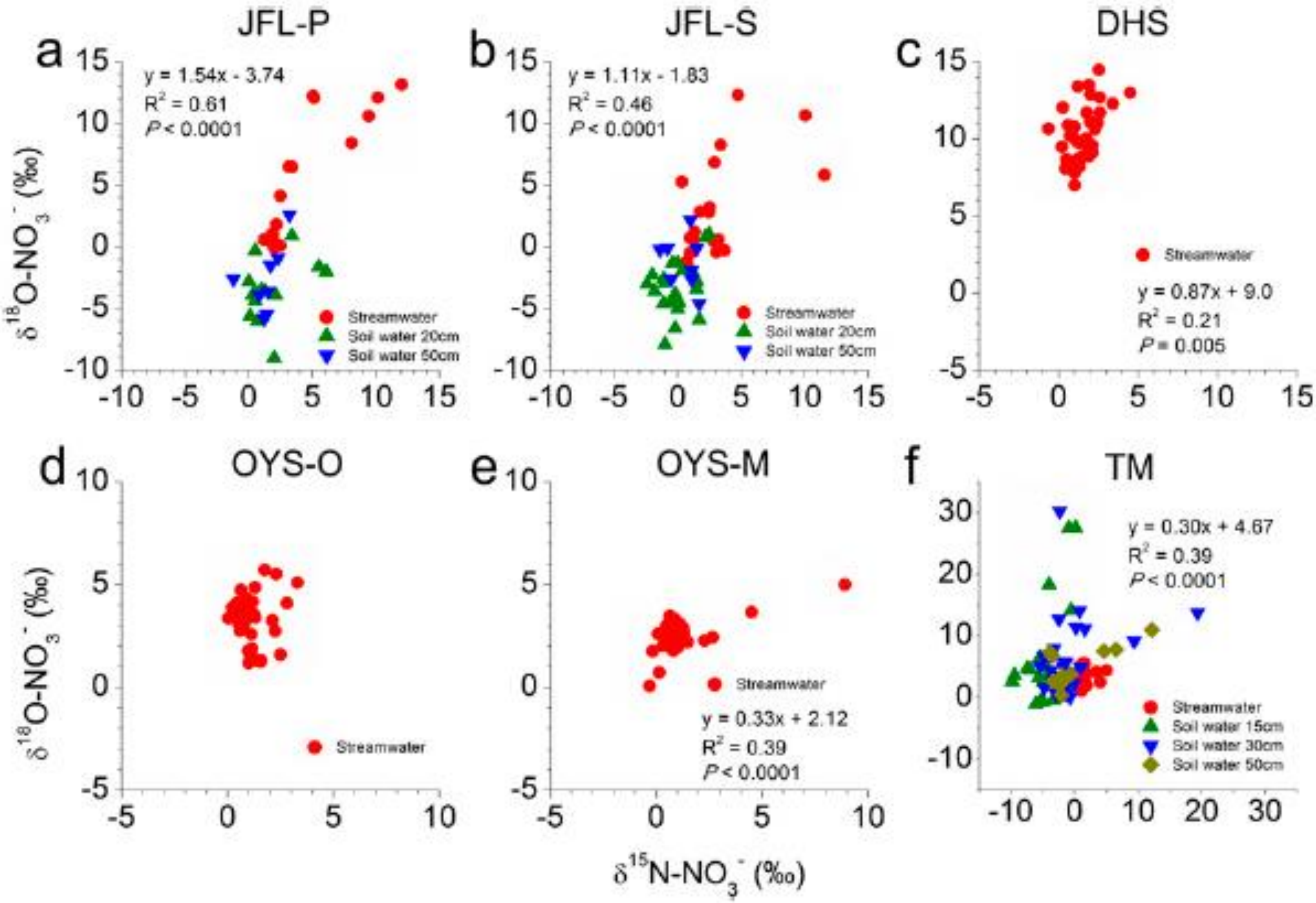
OYS-O
OYS-M
TM
DHS
JFL-P
JFL-S
乌兰巴托
Улаанбаатар
蒙古
朝鲜
平壤
평양
汉城
서울
韩国
日本海
大阪
光州
광주
釜山
부산
东海
广州
河内
Hà Nội
香港
Hong Kong
缅甸
老挝
仰光
泰国



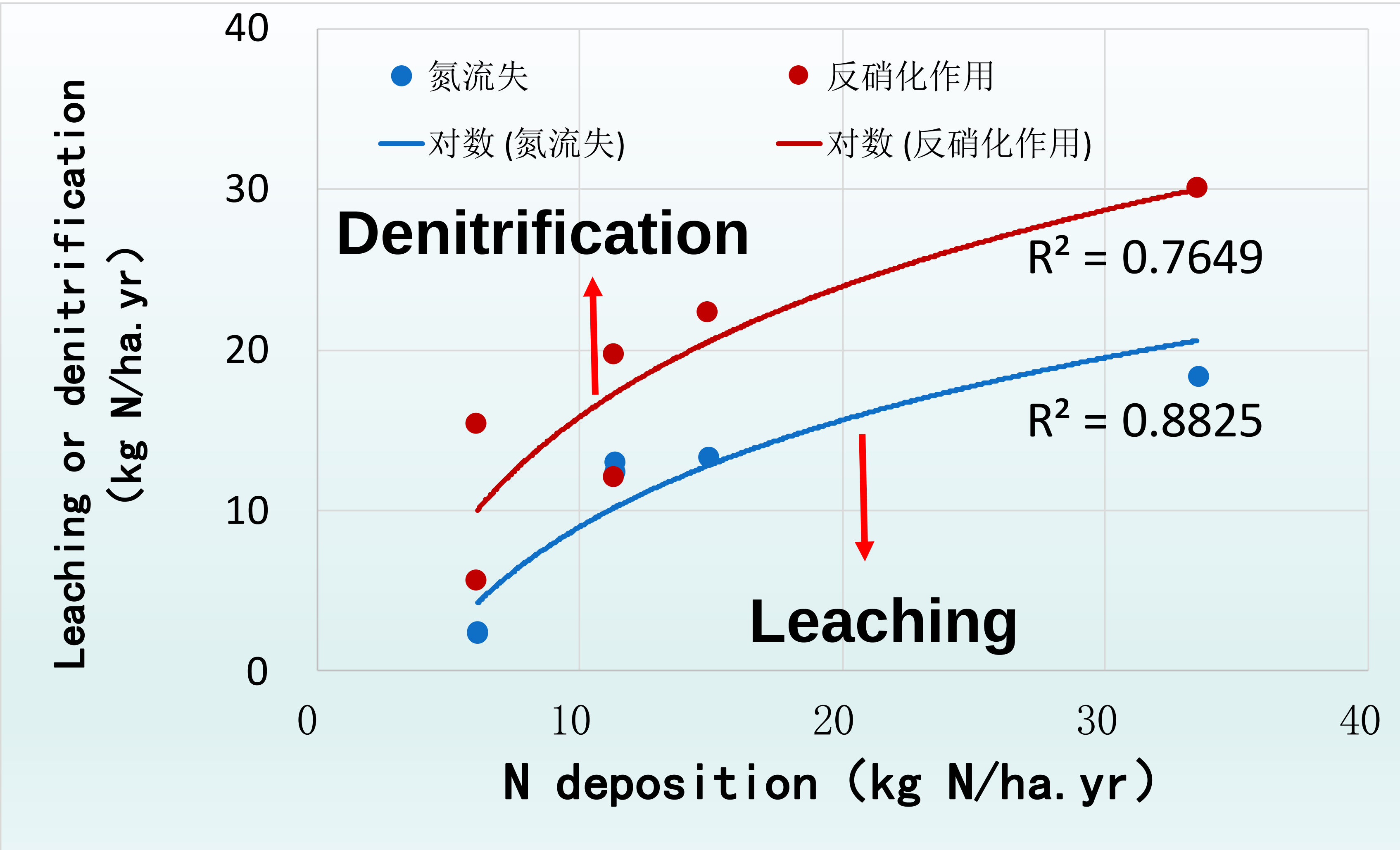
JFL-P
JFL-S
DHS
OYS-O
OYS-M
TM
NO3- concentration (μmol L-1)
1000
100
10
1
a
ab
b
14
8
18
a
a
b
21
10
39
a
a
b
111
34
39
a
ab
b
c
14
13
14
48
a
b
b
c
13
13
13
48
a
ab
ab
b
15
19
9
30
Soil water 20cm
Soil water 50cm
Stream water
Soil water 20cm
soil water 40cm
Stream water
Soil water 10cm
Soil water 30cm
Soil water 60cm
Stream water
Soil water 15cm
Soil water 30cm
Soil water 50cm
Stream water

$\delta^{15}N$ of NO_3^- in soil and stream water




JFL-P
a
y = 1.54x - 3.74
R² = 0.61
P < 0.0001
Streamwater
Soil water 20cm
Soil water 50cm
JFL-S
b
y = 1.11x - 1.83
R² = 0.46
P < 0.0001
Streamwater
Soil water 20cm
Soil water 50cm
DHS
c
Streamwater
y = 0.87x + 9.0
R² = 0.21
P = 0.005
d
OYS-O
Streamwater
e
OYS-M
Streamwater
y = 0.33x + 2.12
R² = 0.39
P < 0.0001
f
TM
y = 0.30x + 4.67
R² = 0.39
P < 0.0001
Streamwater
Soil water 15cm
Soil water 30cm
Soil water 50cm
δ18O-NO3- (‰)
δ15N-NO3- (‰)

N losses via denitrification and leaching

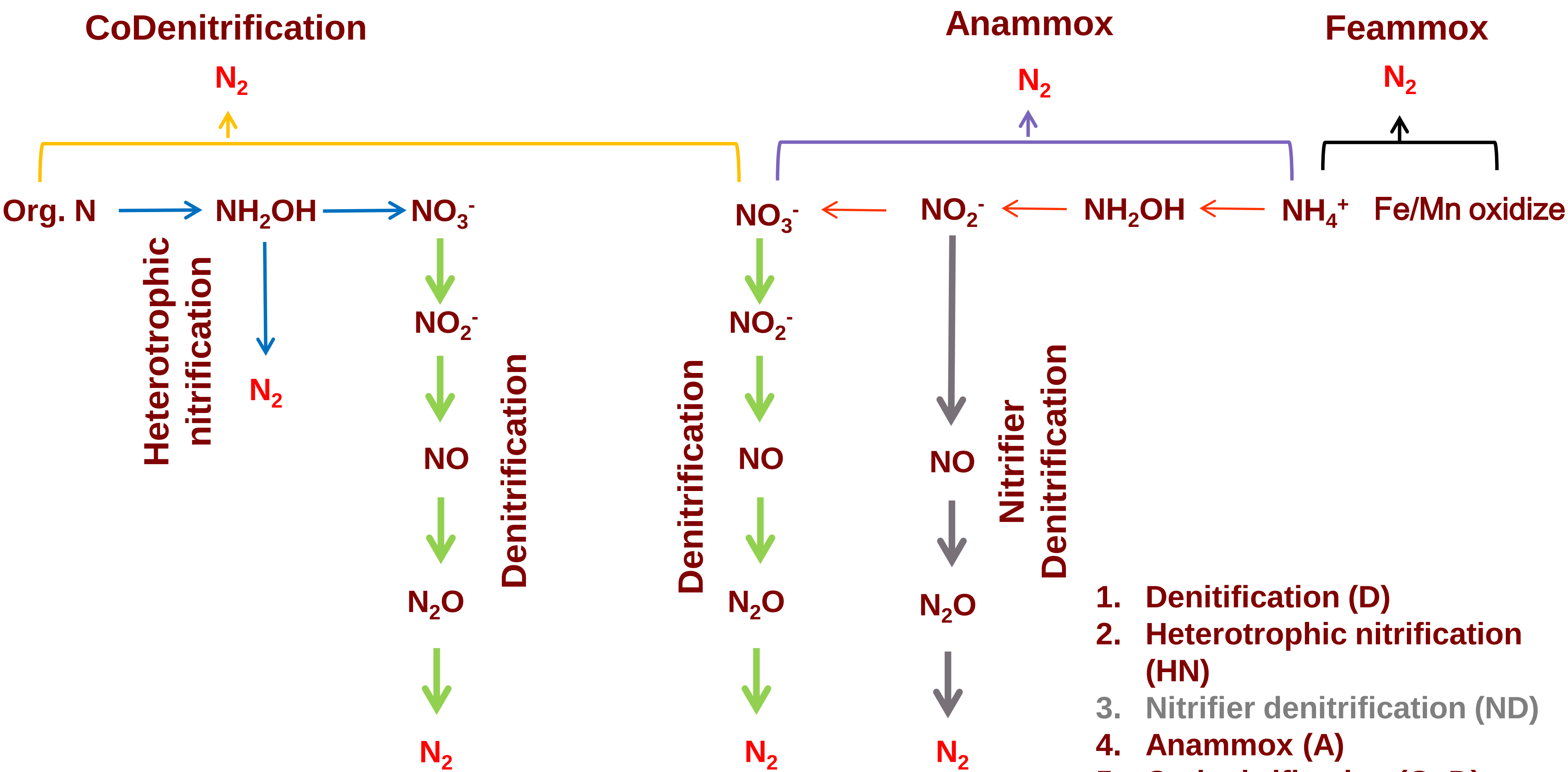


Unit: kg N/ha.yr

	JFL-P	JFL-S	DHS	OYS-O	OYS-M	TM
Denitrification	15.4	5.6	9.4	12.1	19.7	22.3
N_2O	1.5	3	4.7	0.1	0.1	0.7
$\mathbf{N_2}$+NO	13.9	2.6	4.7	12	19.6	21.6

- Annual denitrification rate at ecosystem levels
- **Soil microbial N_2 production processes**
- Effects of N deposition on soil N_2O and N_2 production from two tropical forests

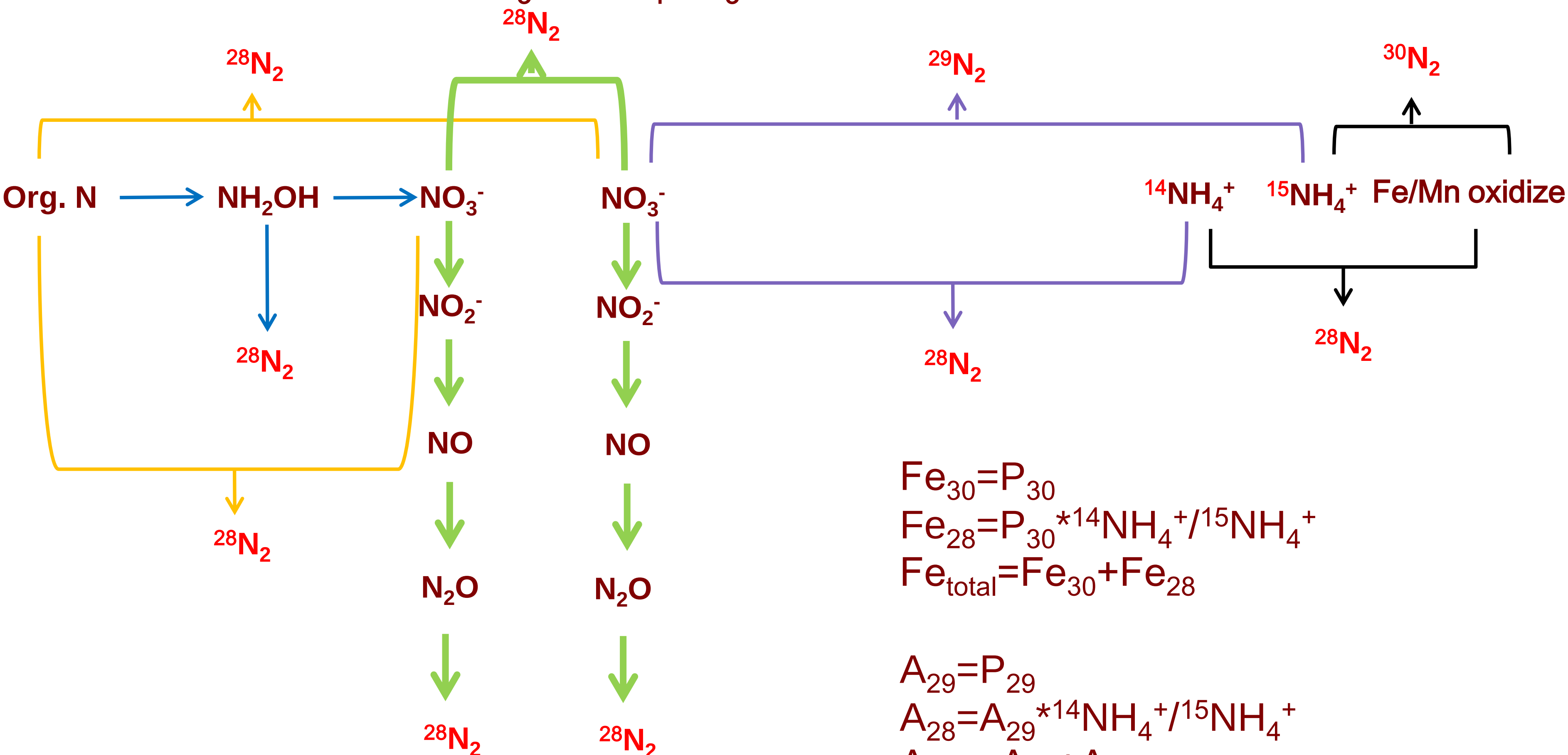
Microbial N_2 production processes



1. Denitification (D)
2. Heterotrophic nitrification (HN)
3. Nitrifier denitrification (ND)
4. Anammox (A)
5. Codenitrification (CoD)
6. Feammox (Fe)

^{15}N dual labelling and ^{15}N pairing technique

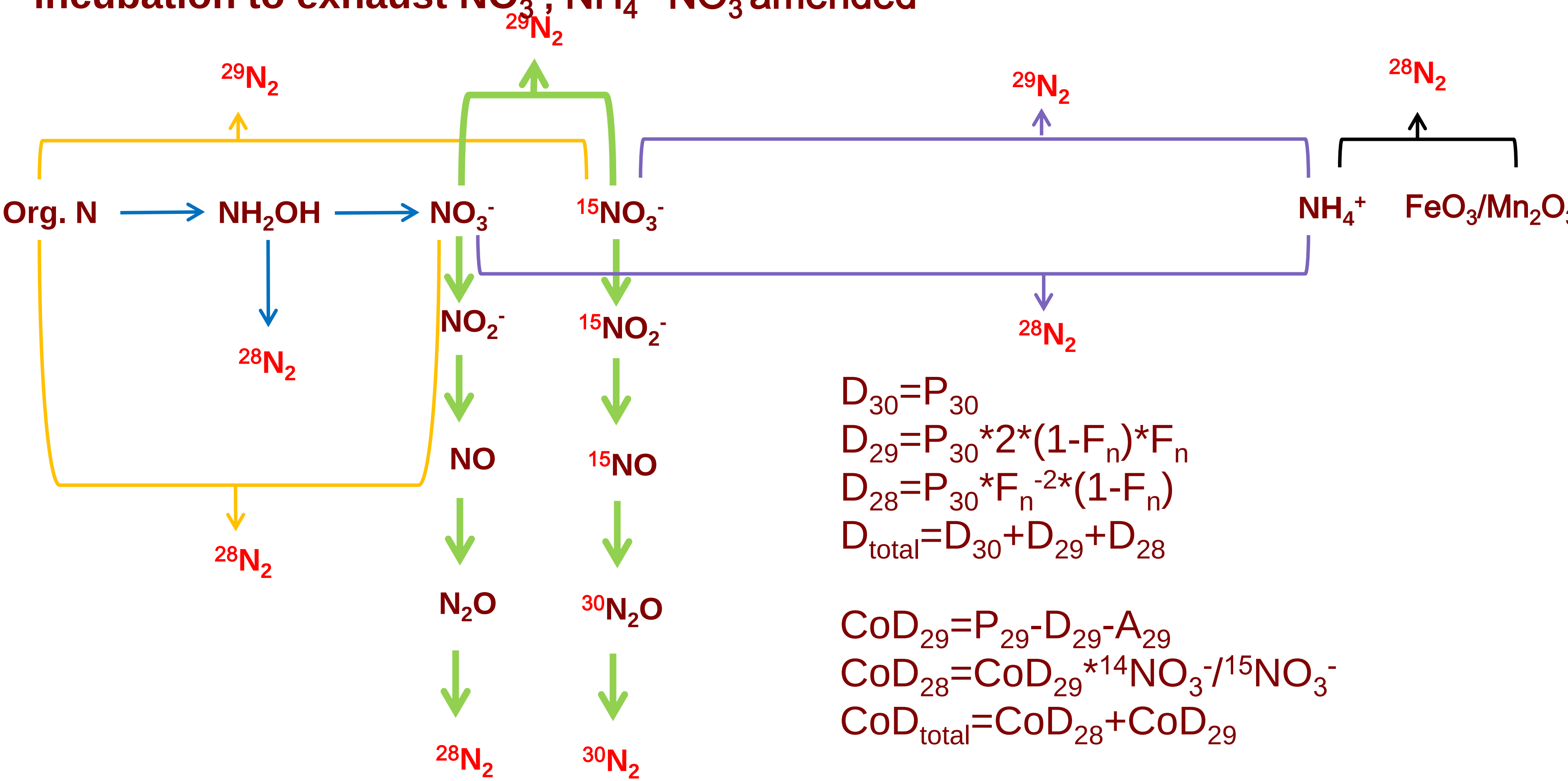
A Treatment：**anaerobic conditions or with nitrification inhibitor, pre-incubation to exhaust NO_3^-, $^{15}NH_4NO_3$ amended**



$Fe_{30}=P_{30}$
$Fe_{28}=P_{30}*^{14}NH_4^+/^{15}NH_4^+$
$Fe_{total}=Fe_{30}+Fe_{28}$

$A_{29}=P_{29}$
$A_{28}=A_{29}*^{14}NH_4^+/^{15}NH_4^+$
$A_{total}=A_{29}+A_{28}$

B Treatment：anaerobic conditions or with nitrification inhibitor, pre-incubation to exhaust NO_3^-, $NH_4{}^{15}NO_3$ amended



$D_{30}=P_{30}$

$D_{29}=P_{30}*2*(1-F_n)*F_n$

$D_{28}=P_{30}*F_n^{-2}*(1-F_n)$

$D_{total}=D_{30}+D_{29}+D_{28}$

$CoD_{29}=P_{29}-D_{29}-A_{29}$

$CoD_{28}=CoD_{29}*{}^{14}NO_3^-/{}^{15}NO_3^-$

$CoD_{total}=CoD_{28}+CoD_{29}$

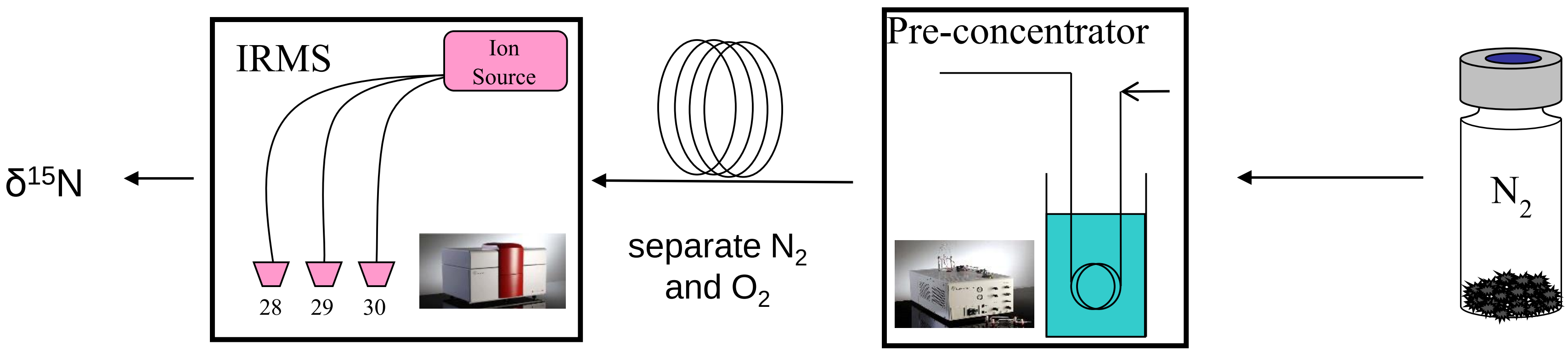
$HN_{28}=P_{28}-D_{28}-A_{29}-CoD_{28}-Fe_{28}$

IRMS
N2O isotopes

VICI
15N-N2

IAE

$\delta^{15}N$-N_2



On-line and automatic analysis for ^{15}N-N_2

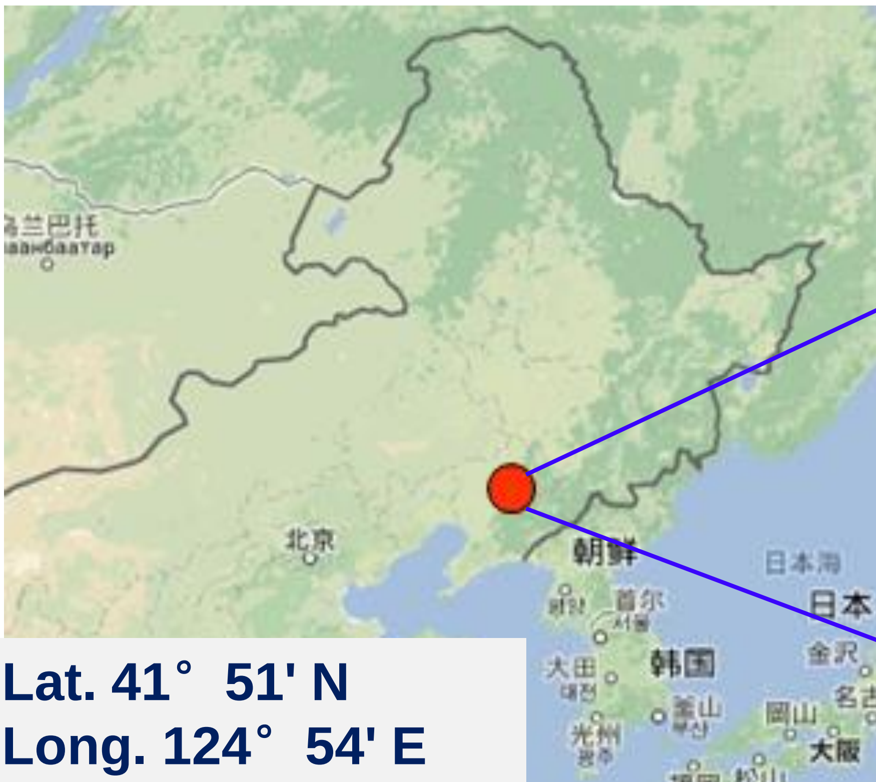
	Conventional method	New method
Required amount of ^{15}N addition	80-300 mg ^{15}N/kg soil (only suitable for agricultural system)	0.1 mg ^{15}N/kg soil (suitable for forest)

Is anammox (anaerobic ammonium oxidation with nitrite) important in forest soil N_2 production?

anammox

- Important in marine system and river sediments, and considerable in some wetland and rice paddy soils.
- No report for forest soils, although forests cover 37% of the land.
- We expected that anammox exists in forest soils, due to the nature of coexistence of NH_4^+ and NO_3^-, oxic and anoxic conditions.

Qingyuan forest research station



Lat. 41° 51' N
Long. 124° 54' E
Alt. 500 a.s.l.
MAP: 811 mm
MAT: 4.7 ºC

Larch plantation forest (LF)

Mixed secondary forest (MF)

Laboratory ^{15}N labelling

4 g fresh soil to 20 mL vials

↓

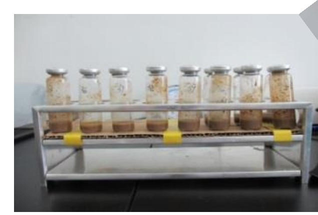
Vacuum and flush **with ultrapure N2 or air** (Pre-incubated in dark at 21 ^{o}C overnight)

↓

Add **$^{15}NO_3^-$ or $^{15}NH_4^+$ tracer solution** (100 ug^{15}N g fresh soil)

↓

Gas sampling after 24 hours for isotope analysis of N_2



Anammox

$^{14}NH_4^+ + {}^{15}NO_3^- \rightarrow {}^{29}N_2$

Co-denitrification

$^{15}NO_3^-$ + ^{14}N **compounds (e.g.,** azide, ammonium NH_4^+, salicylhydroxamic acid, and hydroxylamine $\rightarrow$ $^{45}N_2O \rightarrow {}^{29}N_2$

Denitrification

$^{15}NO_3^- + {}^{14}NO_3^- \rightarrow {}^{29}N_2$

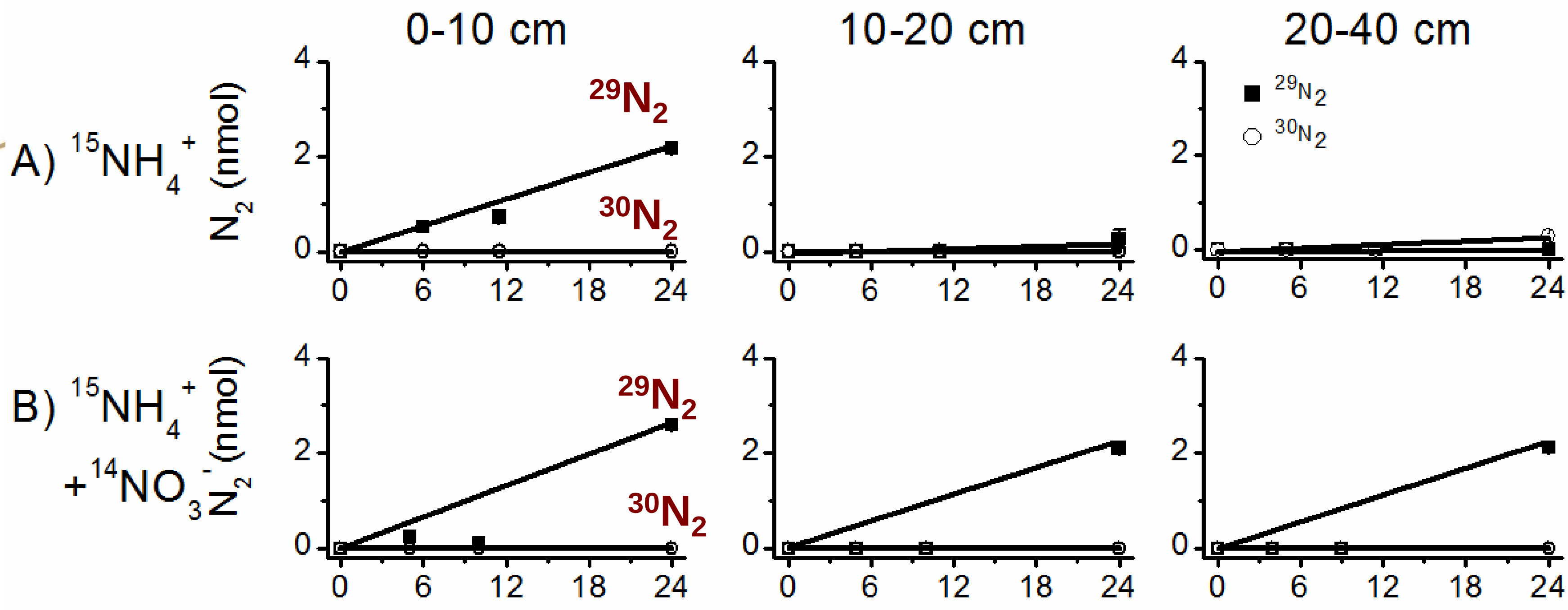
$^{15}NO_3^- + {}^{15}NO_3^- \rightarrow {}^{30}N_2$

Feammox

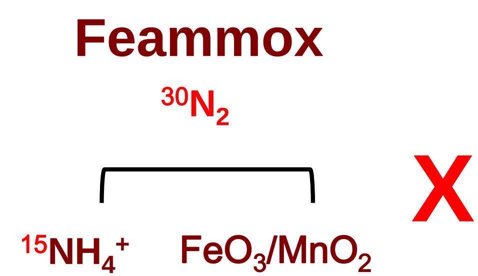
$^{15}NH_4^+ \rightarrow {}^{30}N_2$

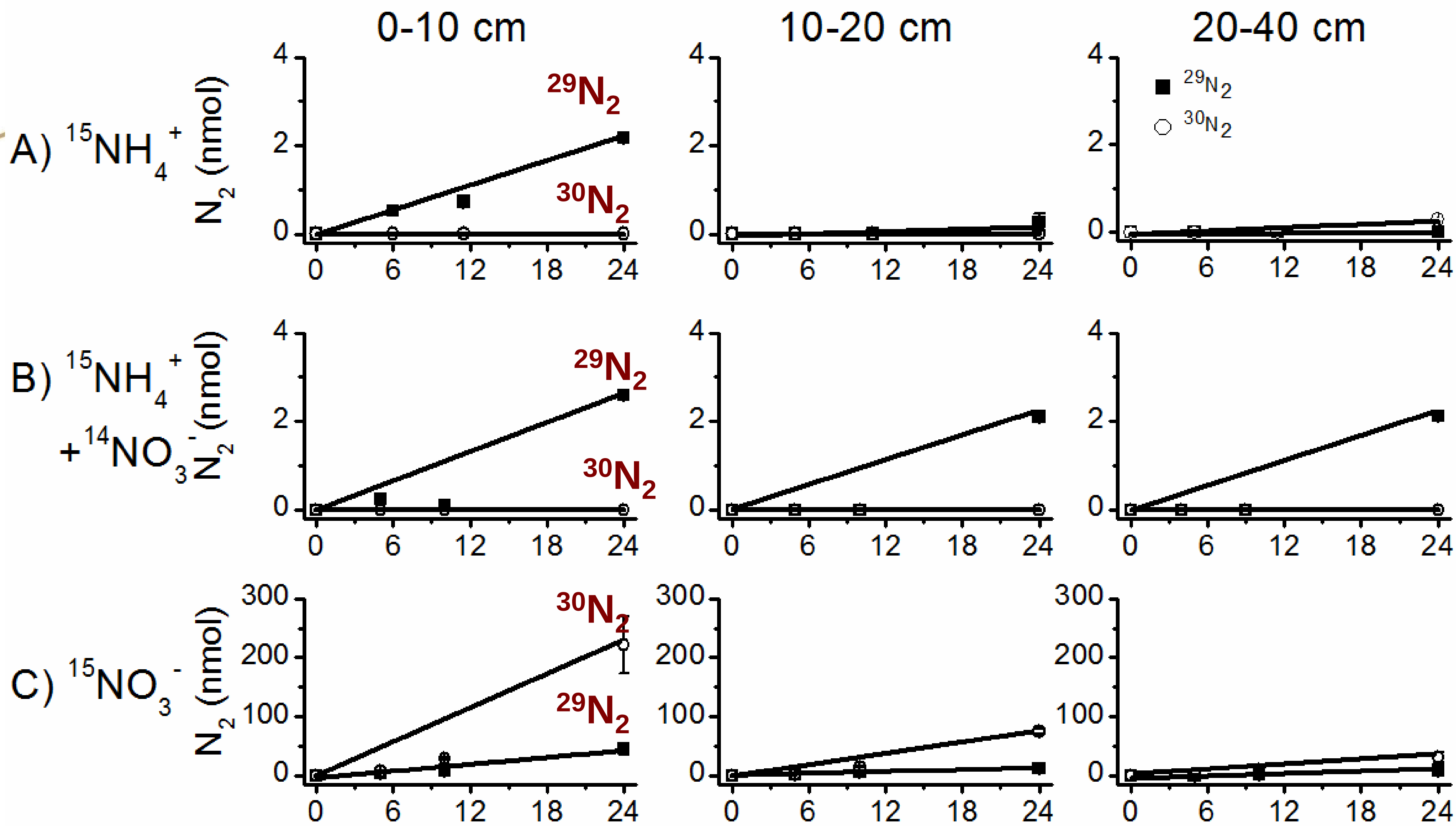
Heterotrophic nitrification

no $^{29}N_2$ or $^{30}N_2$



- **No $^{30}N_2$ production**

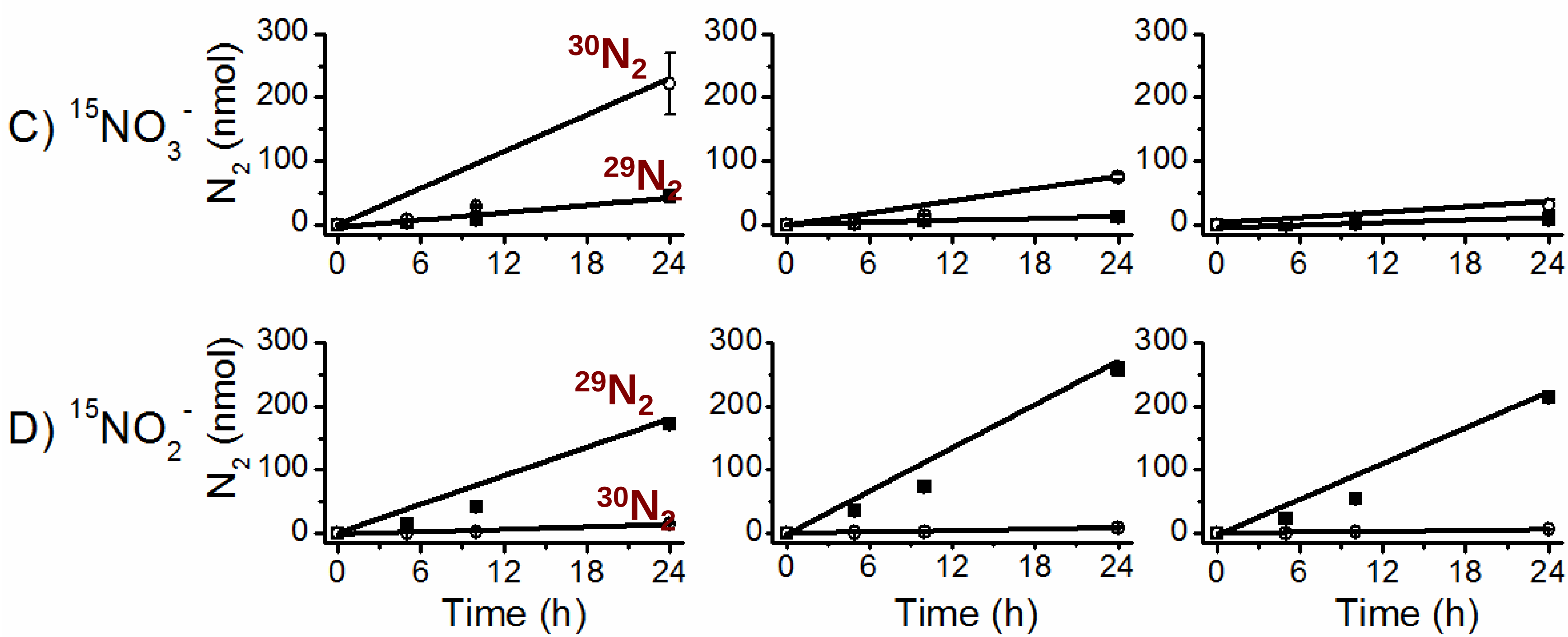




- **Denitrification is dominant process in N_2 production.**
- **N_2 production decreased with soil depth**

0-10 cm 10-20 cm 20-40 cm

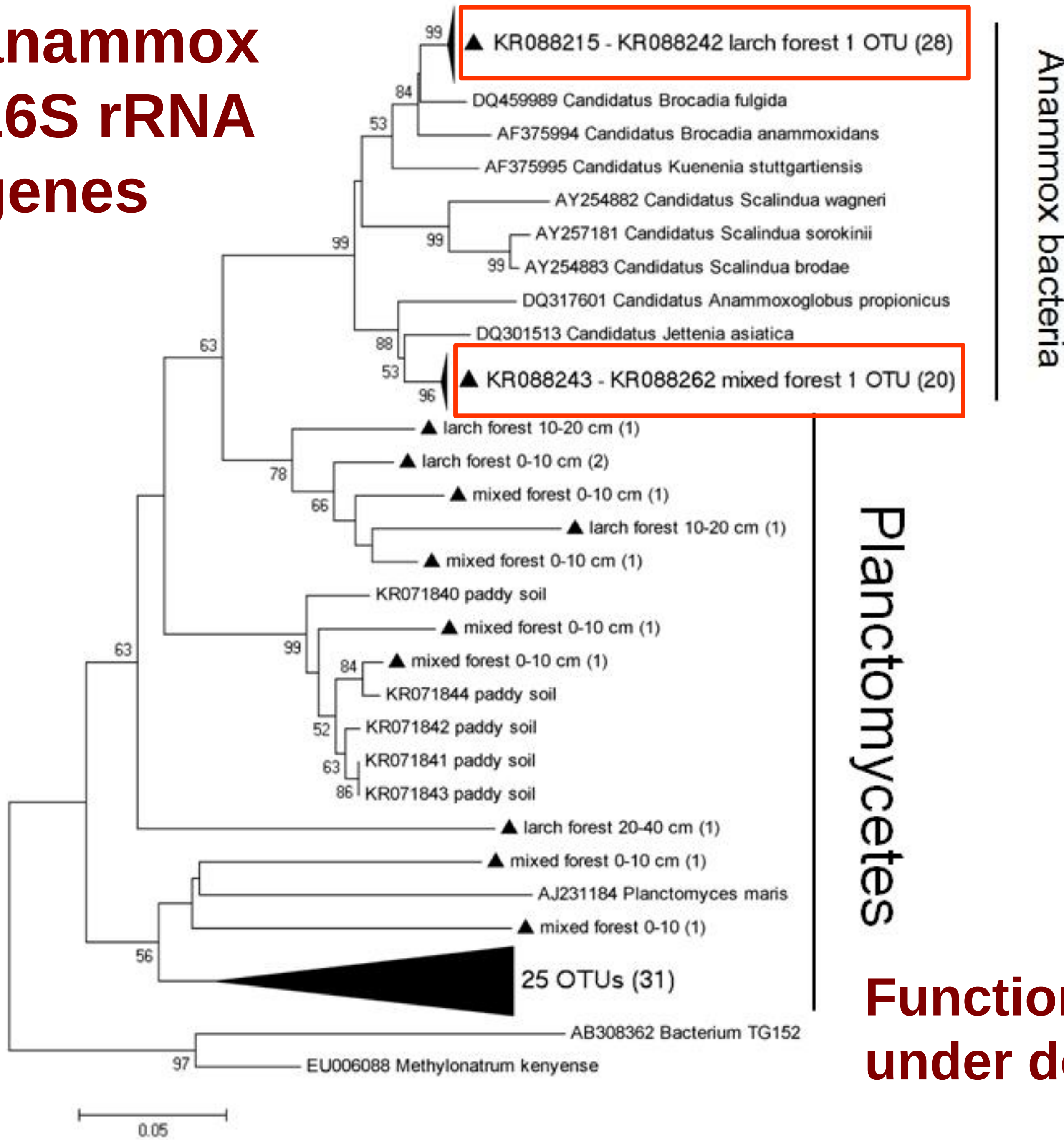
- NO_2^- reacted with organic N to produce N_2 than NO_3^-, pointing to the occurrence of Codenitrification.
- Production rate increased with time, indicating the nature of biological process.



Soil layer	Contribution (%)		
	Anammox	Co-D	Denitrification
0-10 cm	1.1	5.0	93.9
10-20 cm	2.5	1.8	95.7
20-40 cm	6.6	12.4	81.0

Dan, X. et al., 2016. Applied and Environmental Microbiology

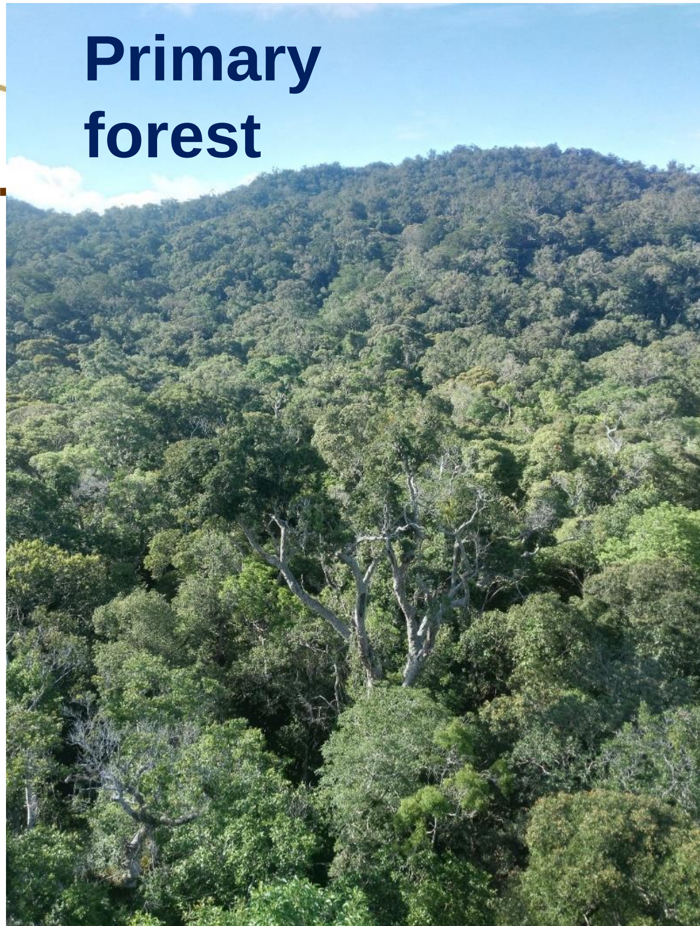
anammox 16S rRNA genes



Functional *hzs*B gene is under detected limitation

- **Annual denitrification rate at ecosystem levels**
- **Soil microbial N_2 production processes**
- **Effects of N deposition on soil N_2O and N_2 production from two tropical forests**

Primary forest



Secondary forest

MAP: 2500 mm
MAT: 19 ºC

N addition experiment

Four treatments, control (no N addition), low-N addition (25 kg N ha^{-1} yr^{-1}), medium-N addition (50 kg N ha^{-1} yr^{-1}), and high-N addition (100 kg N ha^{-1} yr^{-1}).

Four replicate plots for each treatment

Plot size: 20 m * 20 m

Treatment, since 2010, monthly

Sampling: three times in from 2016 to 2017

We expected that:

- 1) long-term N addition would increase soil N_2O and N_2 production, and increase N_2O/N_2 (due to further soil acidification induced by N addition);
- 2) N addition may have decreased denitrification contribution to N_2O and N_2 production, compared to other production processes.
- 3) N addition may have changed the gene abundance of genes associated with denitrification.
- 4) response to N addition would be more pronounced in the primary forest than in the secondary forest.

Laboratory C_2H_2 inhibition (AIT)

4 g fresh soil to 20 mL vials

↓

Vacuum and flush **with ultrapure N2 or air** (Pre-incubated in dark at 21 ^{o}C overnight)

↓

Add C2H2 (to inhibit N2O reduction) and nitrate (100 ug^{14}N g fresh soil)

↓

Gas sampling after 24 hours for concentration analysis of N_2O

Laboratory ^{15}N labelling

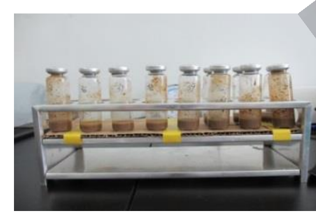
4 g fresh soil to 20 mL vials

Vacuum and flush **with ultrapure N2 or air** (Pre-incubated in dark at 21 ^{o}C overnight)

Add **$^{15}NO_3^-$ tracer solution**

(100 ug^{15}N g fresh soil)

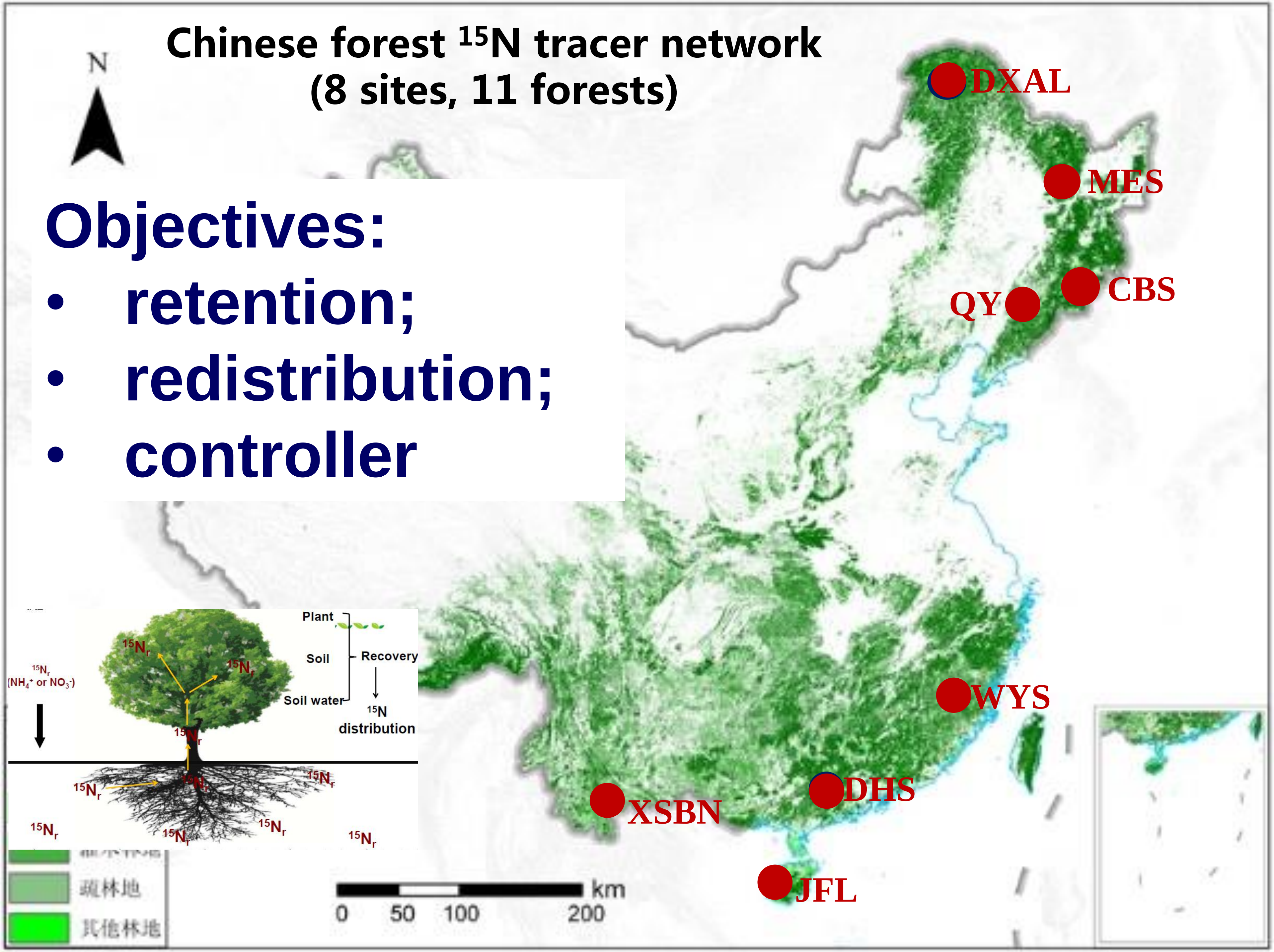
Gas sampling after 24 hours for isotope analysis of N_2 and N_2O



Take-home messages

- Natural abundance of N and O isotopes in nitrate can be used to estimate denitrification rate at an ecosystem level for forests; denitrification is shown to be an overlooked N loss pathway.
- ^{15}N labelling and ^{15}N pairing technique is a novel and promising approach to quantify the importance of each of several microbial N processes responsible for N_2O and N_2 production and their responses to disturbance.
- Anammox existed but contributed little to N2 production.
- Response of N2 and N2O to long-term N addition is ecosystem specific in tropical forests.

Chinese forest ^{15}N tracer network (8 sites, 11 forests)



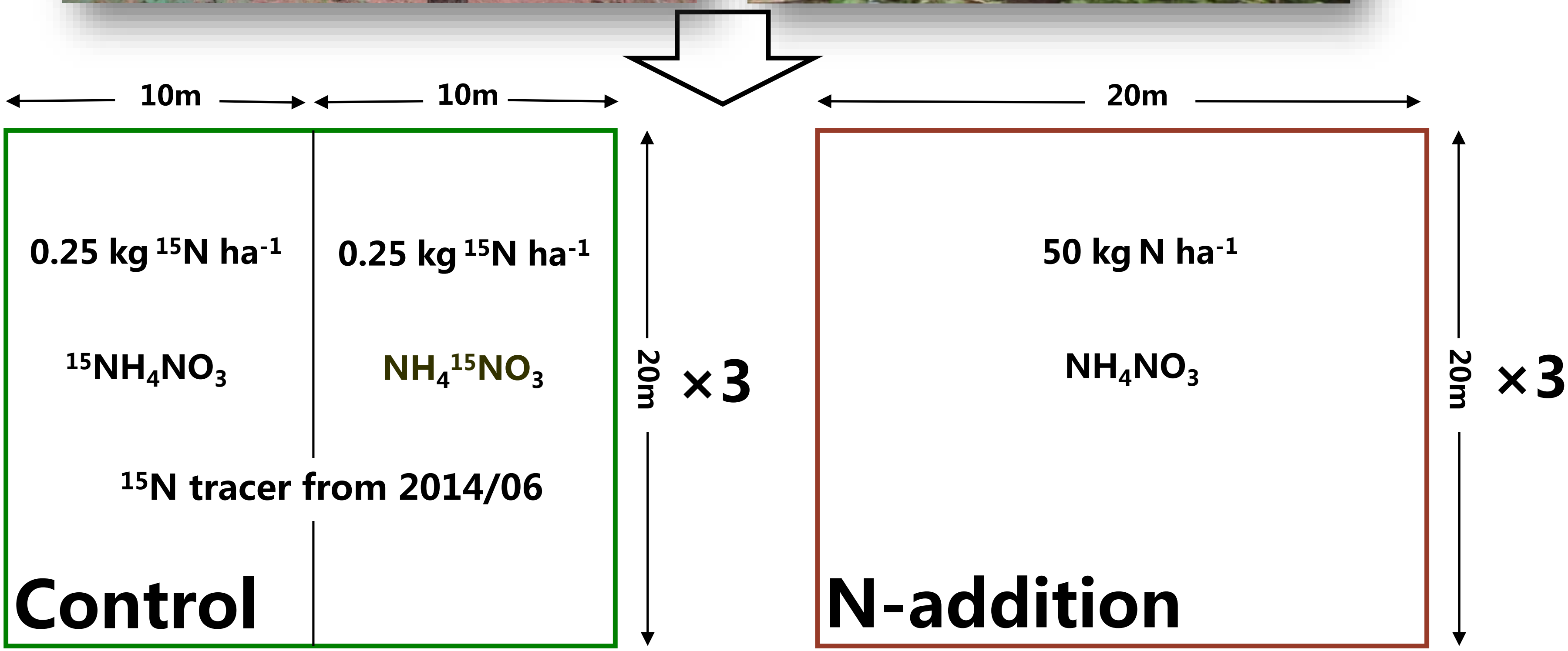
Objectives:

- retention;
- redistribution;
- controller

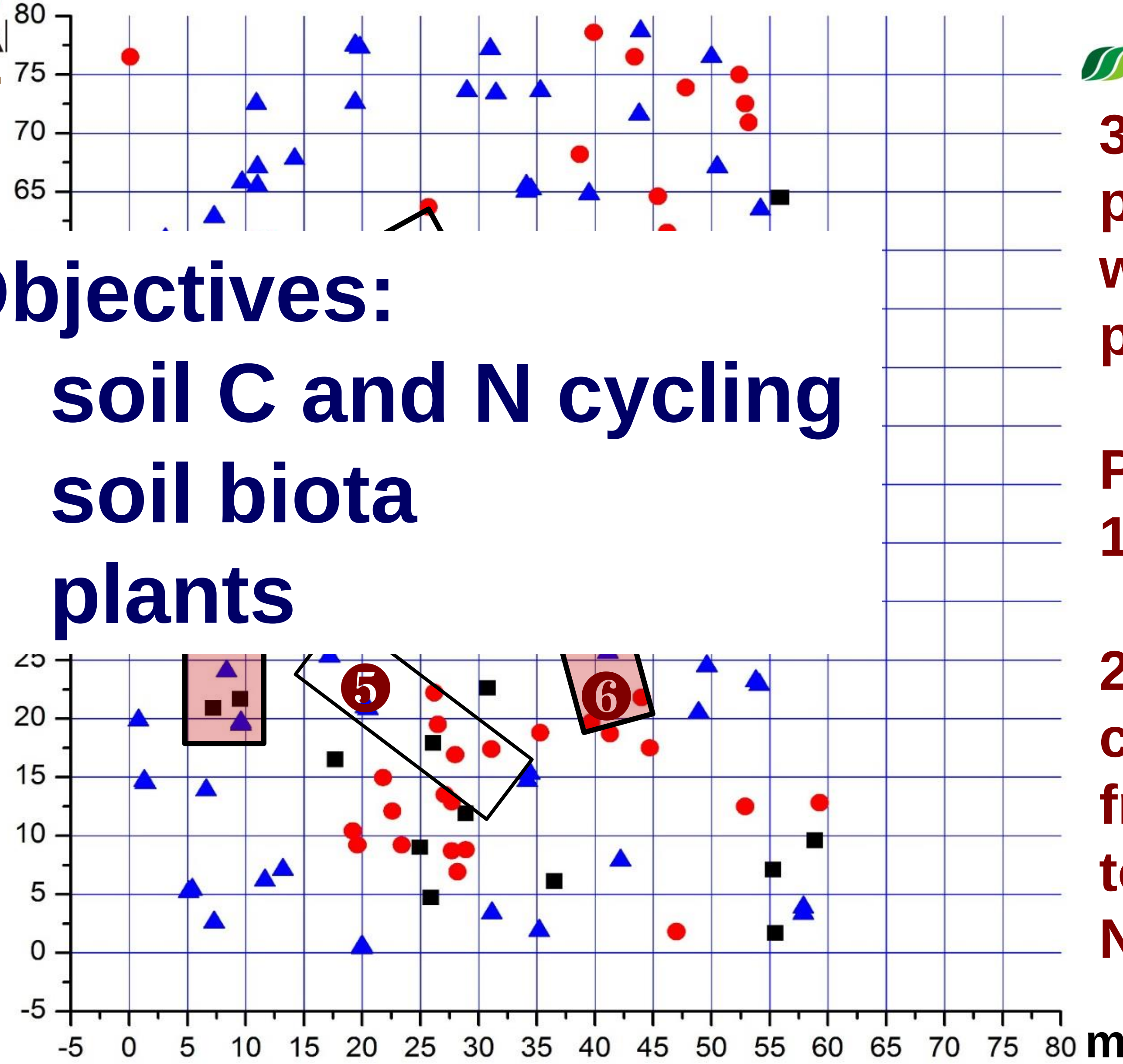
Qingyuan


Larch forest


Mixed forest


10m
10m
20m
0.25 kg ^{15}N ha^{-1}
0.25 kg ^{15}N ha^{-1}
$^{15}NH_4NO_3$
$NH_4{}^{15}NO_3$
^{15}N tracer from 2014/06
Control
20m
×3
50 kg N ha^{-1}
NH_4NO_3
N-addition
20m
×3

Soil warming experiment in a temperate mixed forest

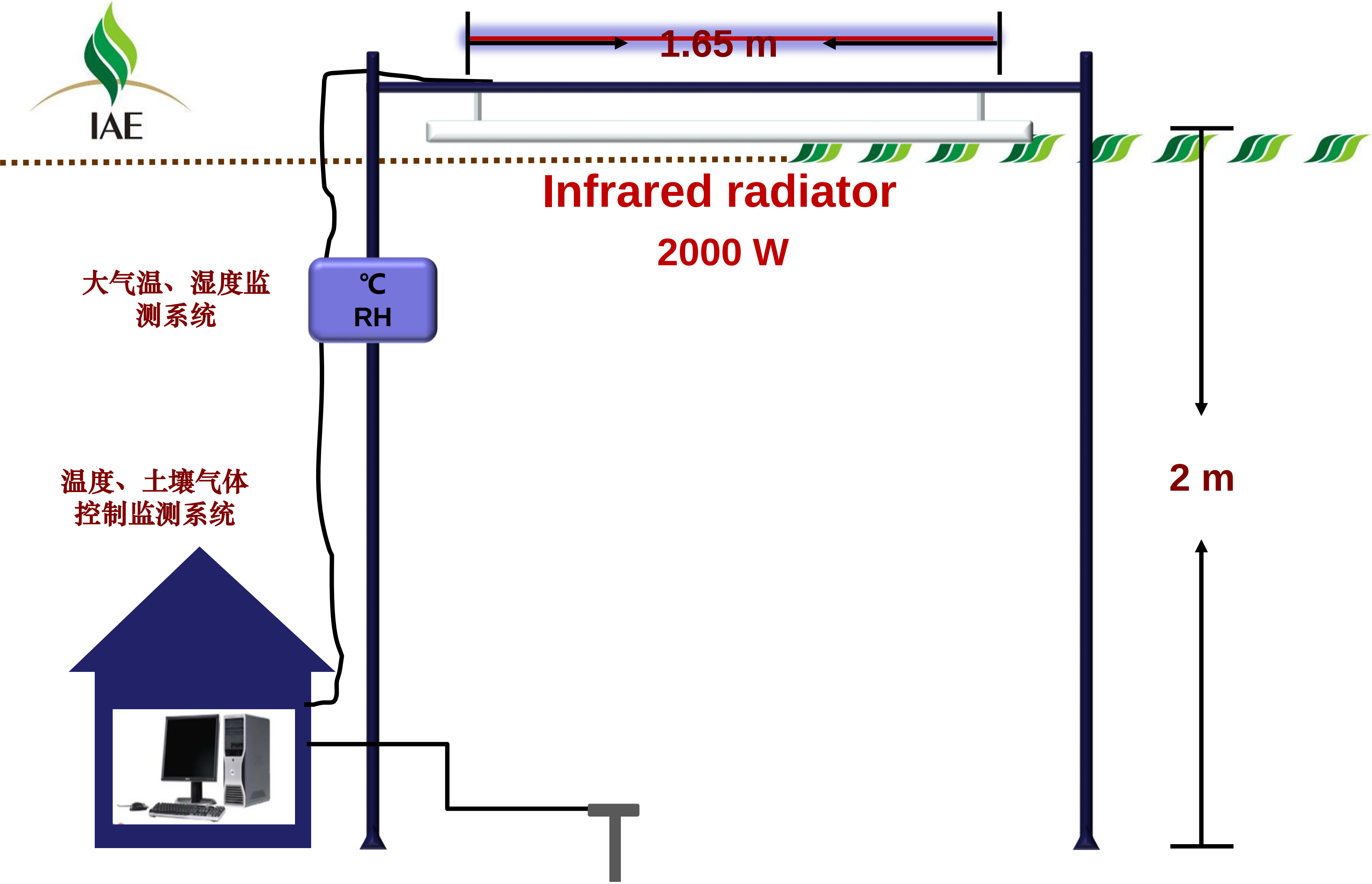


Objectives:
- soil C and N cycling
- soil biota
- plants

3 control plots; 3 warming plots

Plot: 6 m * 18 m

2 ^{o}C above control from April to November



土壤增温及温湿度自动监测系统正面图

$CO_2/CH_4/N_2O/NO$ emission rate (two systems; 32 chambers)

2016 pilot experiment

Taken Nov 3, 2016

Thank you very much for your attention.