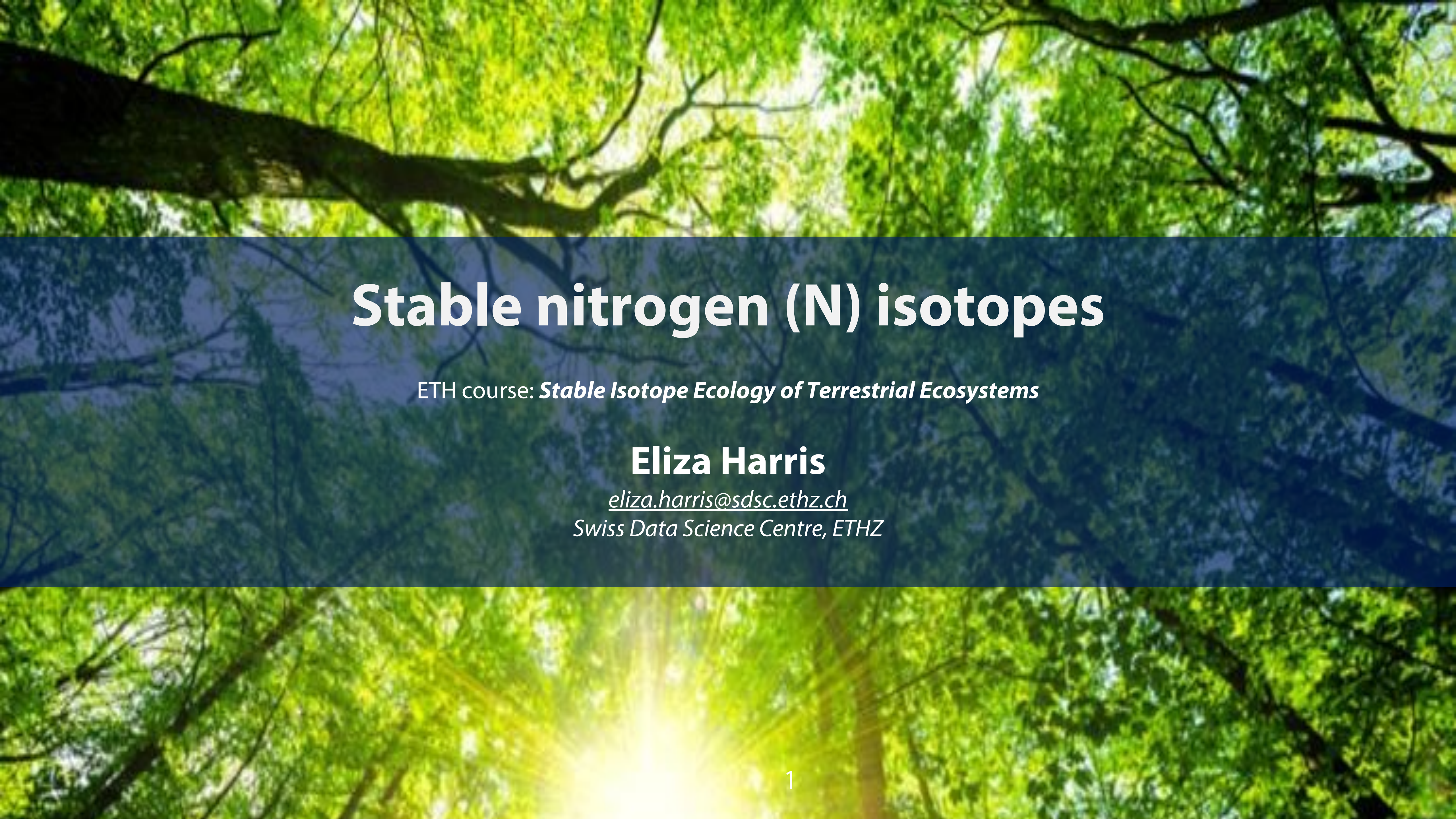

Stable nitrogen (N) isotopes

ETH course: ***Stable Isotope Ecology of Terrestrial Ecosystems***

Eliza Harris
eliza.harris@sdsc.ethz.ch
Swiss Data Science Centre, ETHZ

Goals of this lecture

- To understand the background and future of the **global nitrogen cycle**
    - Inputs, transport, transformation
- To conceptually understand stable N isotopes and their applications
- To compare and contrast **two complementary isotopic approaches**
    - ^{15}N labelling
    - Natural abundance
- To investigate further applications of ^{15}N to understand the global N cycle

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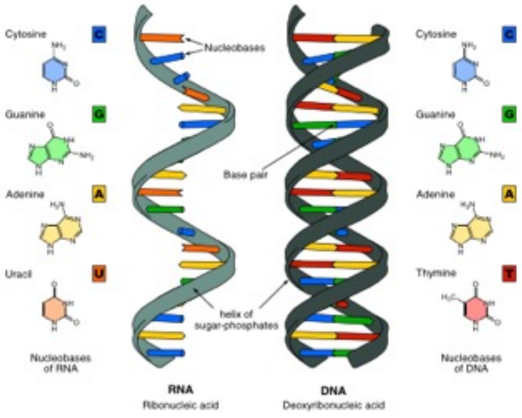
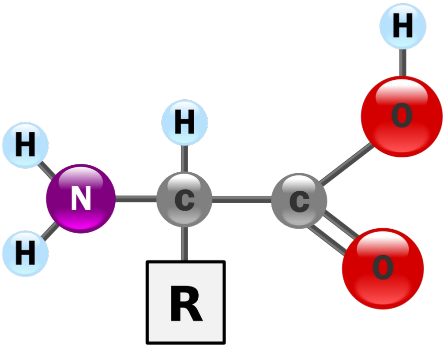
The importance of nitrogen

- Nitrogen, along with K and P, is a key macronutrient
  - Plays a fundamental role in energy metabolism and protein synthesis
  - Comprises ~4% of the dry weight of plants and ~3% of animals
- One of the most important nutrients constraining plant growth





Images (L-R): Yassine Mrabet; International Rice Research Institute; Thom Weir

The global nitrogen cycle

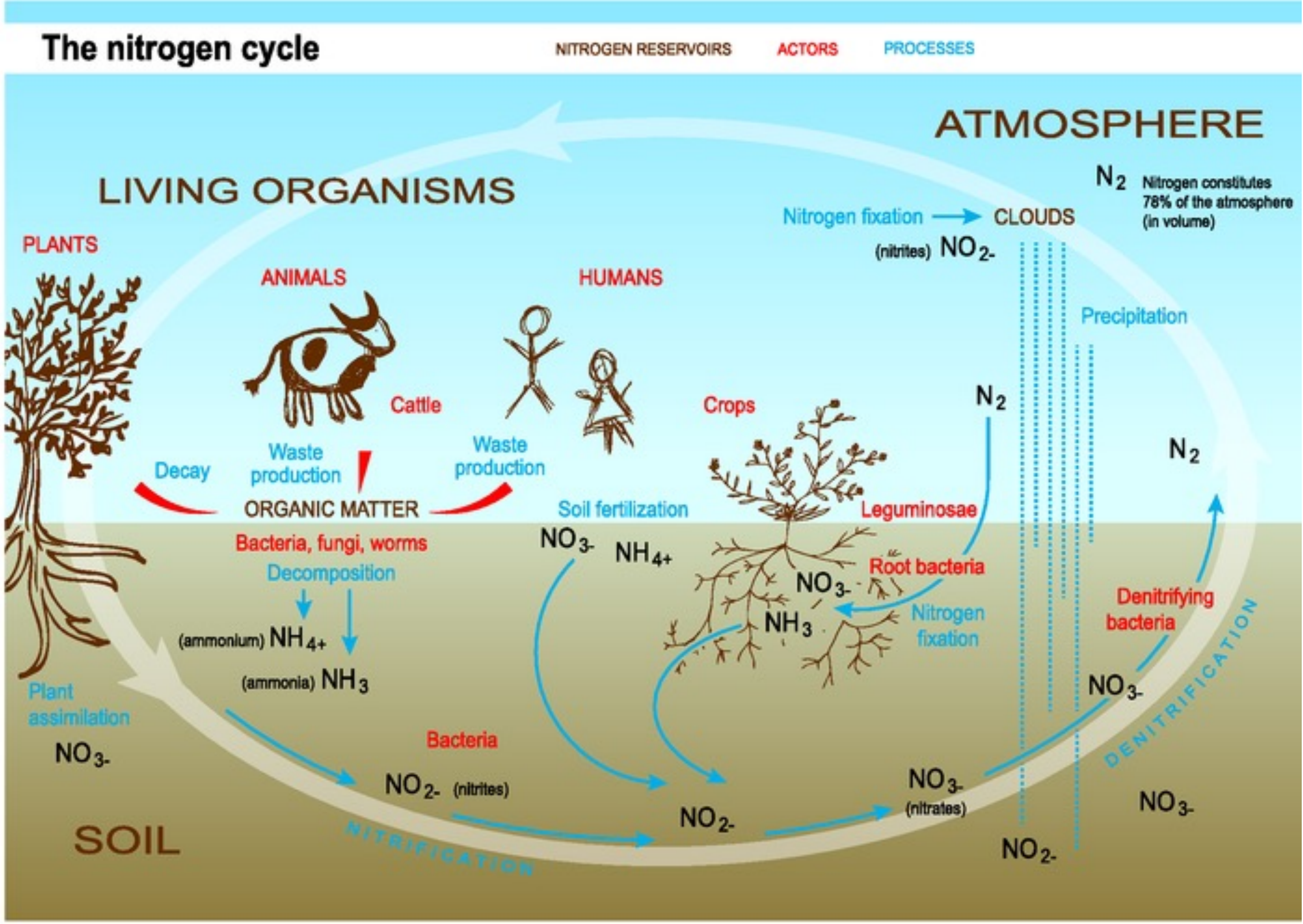


Image: European Environment Agency; Budget: Heike Knicker (2004) Marine Chemistry

Atmospheric nitrogen:
3.8×10^9 Tg (1.9%)

Biosphere nitrogen

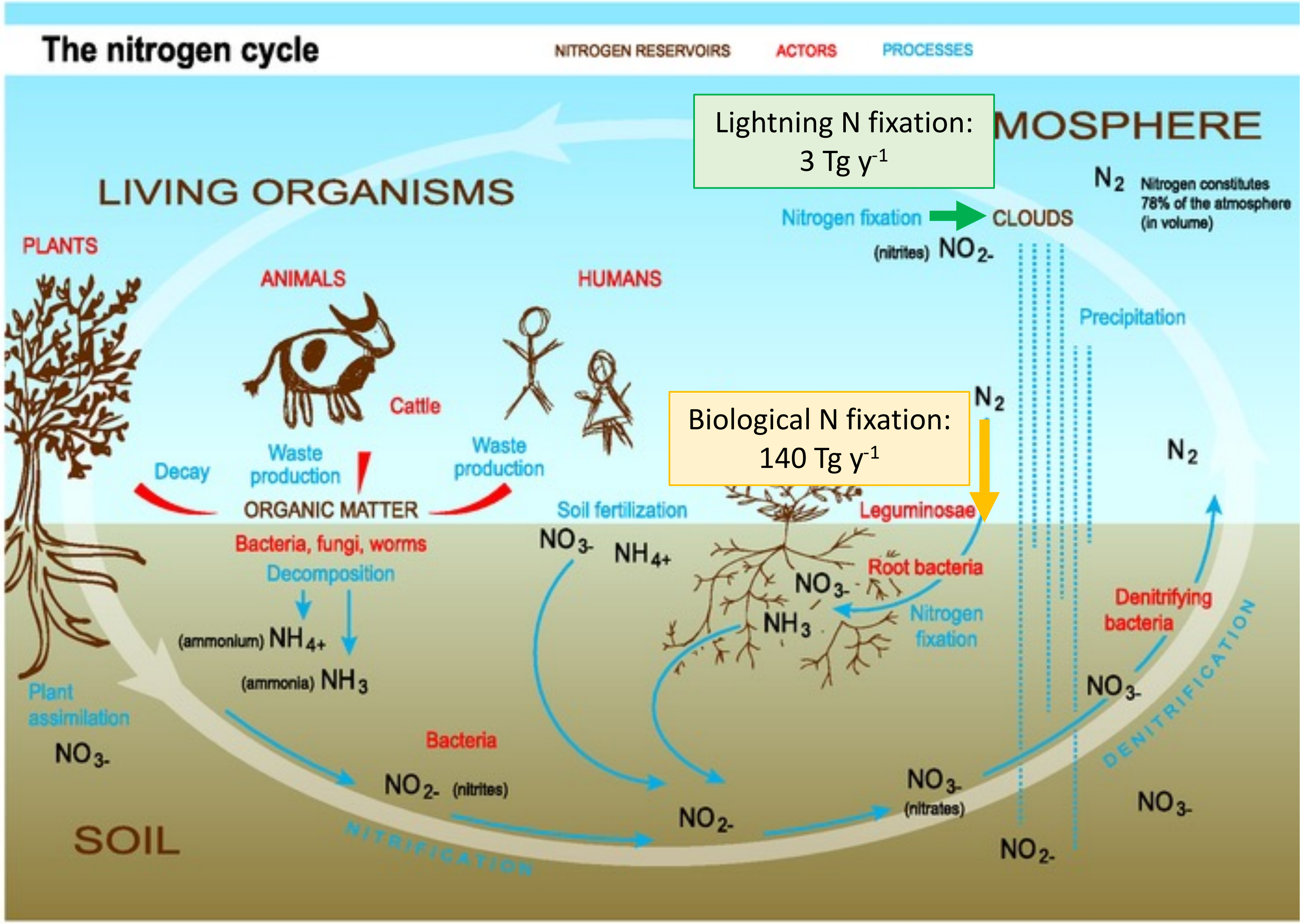
Nitrogen in living organisms:
2.2×10^4 Tg (0.00001%)

Soil nitrogen:
1.1×10^5 Tg
(0.00005%)

Oceanic org-N:
5.4×10^4 Tg
(0.00003%)

Lithospheric nitrogen:
2.0×10^{11} Tg (98.1%)

The global nitrogen cycle



Lightning N fixation: 3 Tg y^{-1}

Biological N fixation: 140 Tg y^{-1}

Image: European Environment Agency; Budget: Heike Knicker (2004) Marine Chemistry

- Most nitrogen is present as atmospheric N_2, which is unavailable to plants
  - Biological N fixation (BNF) is the conversion of N_2 to NH_4 by microbes
  - Non-biological fixation can also occur: $N_2 \xrightarrow{lightning} NO_x$
- BNF is the major natural N input for plants

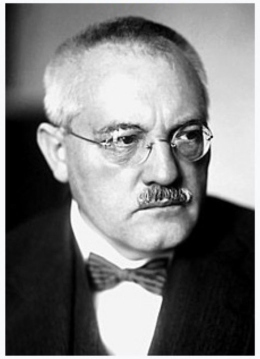
Industrial nitrogen fixation

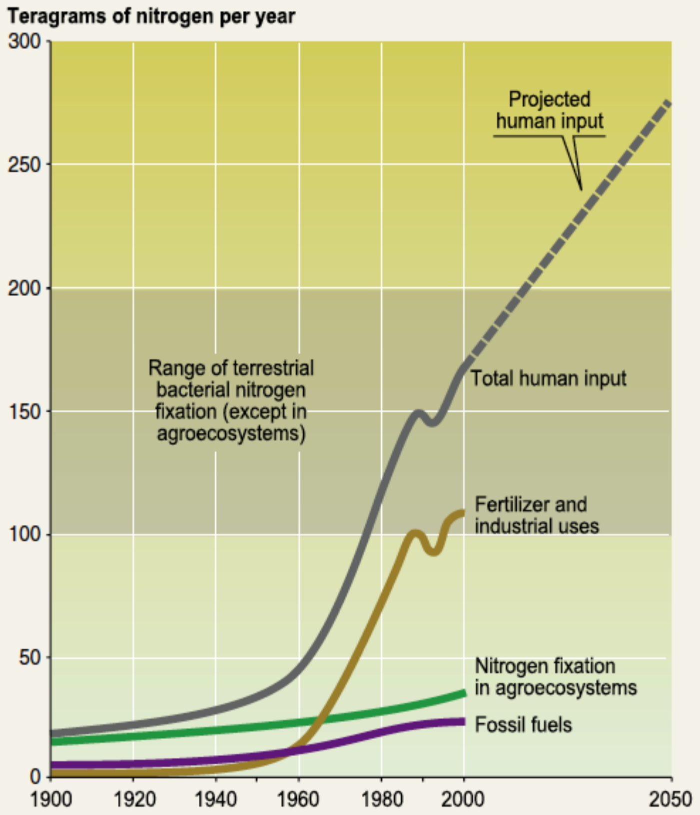
- The Haber-Bosch process revolutionized food production:

$$N_2\,(g) + 3\,H_2\,(g) \xrightarrow{catalyst,400^{\circ}\mathrm{C}} 2\,NH_3\,(g)$$

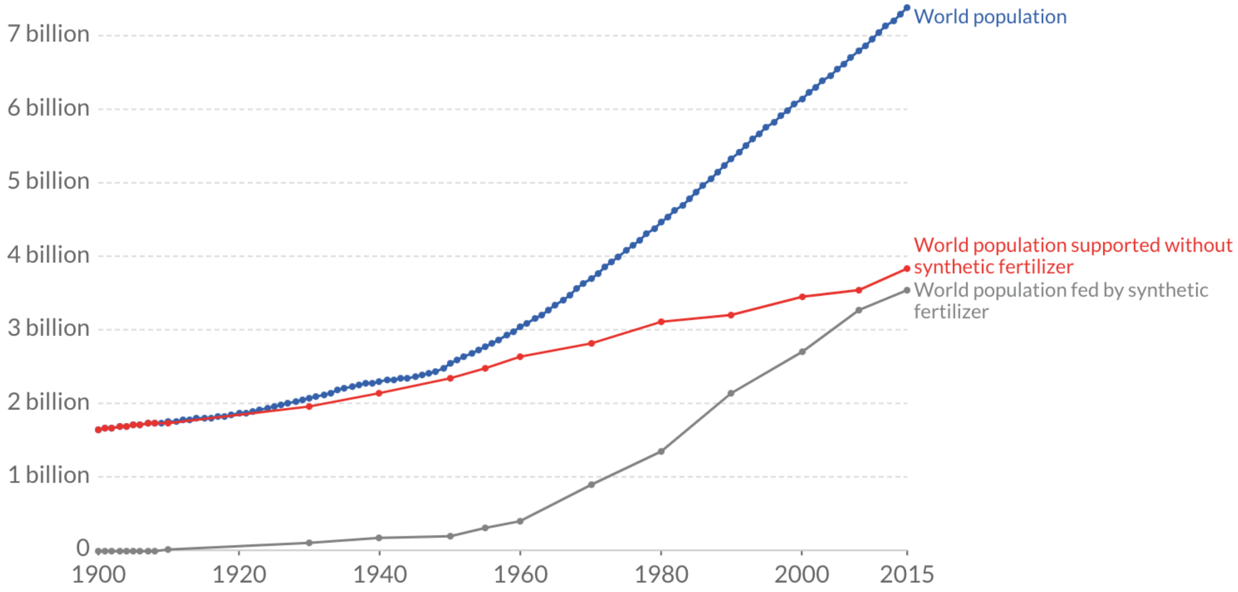
Fritz Haber

Carl Bosch





Source: Millennium Ecosystem Assessment



Images: http://people.idsia.ch/~juergen/haberbosch.html; Millenium Ecosystem Assessment; Our World in Data.

Anthropogenic N inputs: Undesired consequences

The nitrogen cascade

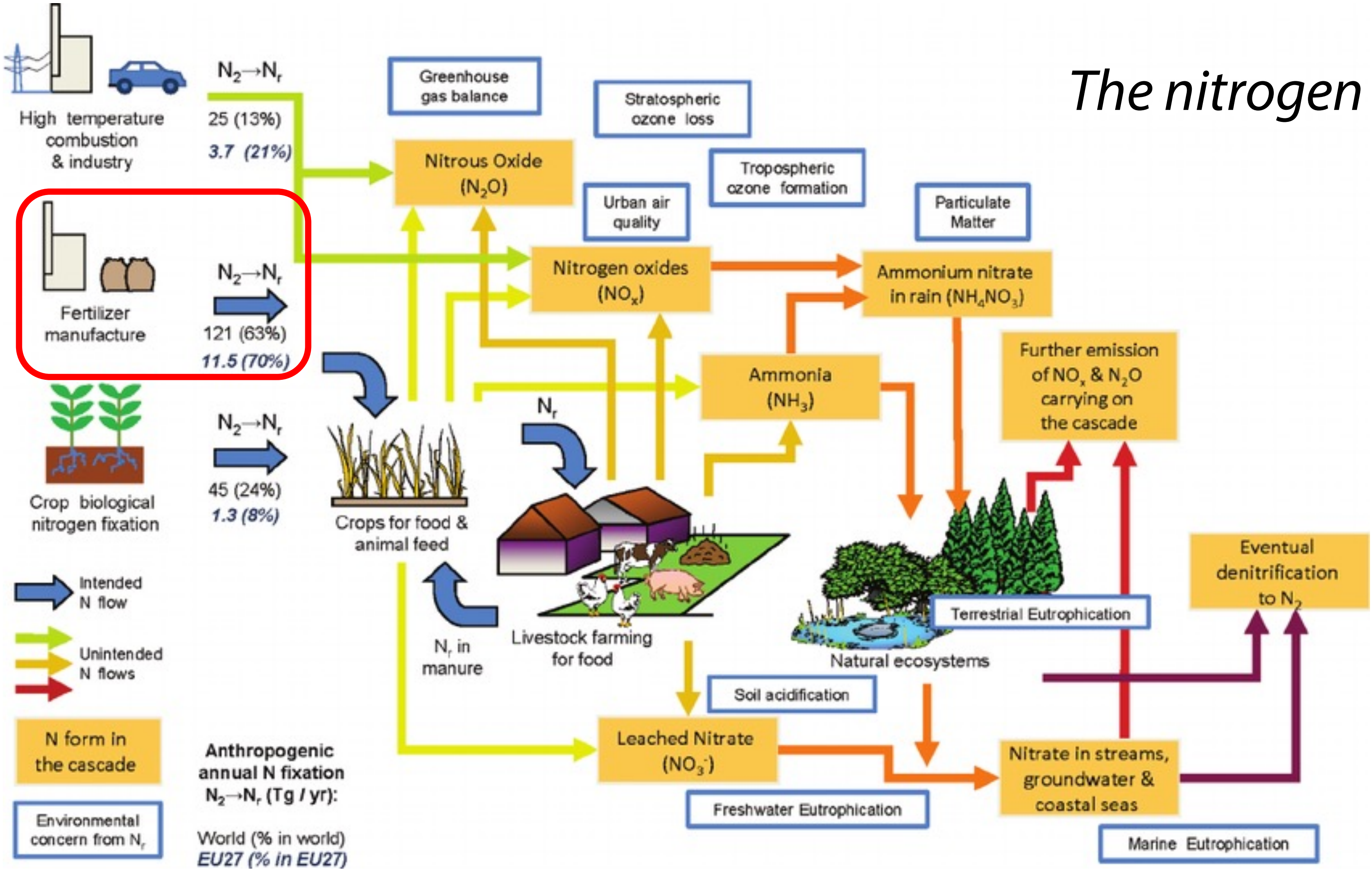
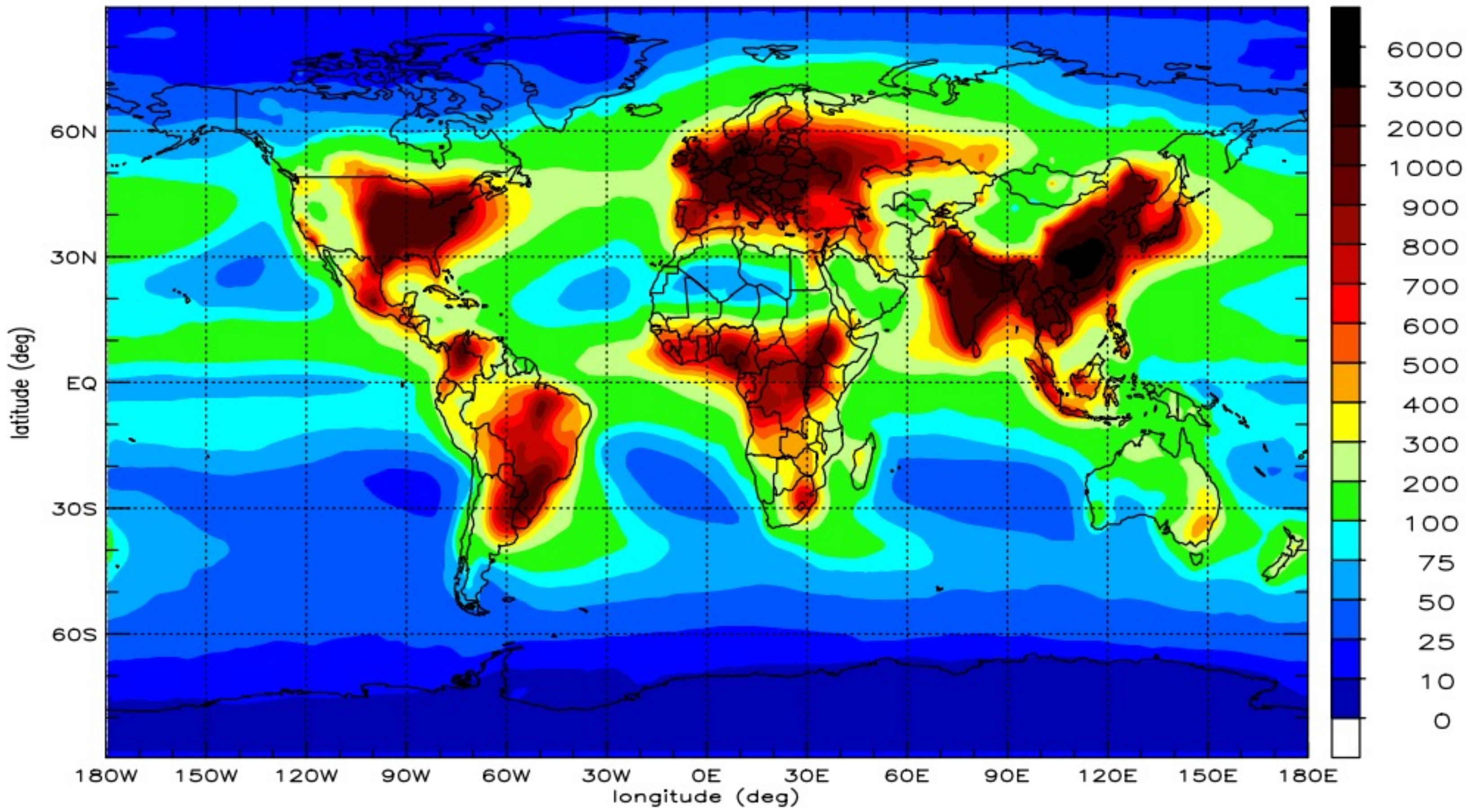


Image: European Nitrogen Assessment 2011

Global reactive N deposition (mg-N m^{-2} yr^{-1})



Dentener et al. 2006, GBC

Critical threshold:

- Excess N loading
- Consequences for other biogeochemical cycles

Planetary boundaries

- The «safe operating limit» for N addition is clearly exceeded
- Implications also for *biosphere integrity* and *climate change*

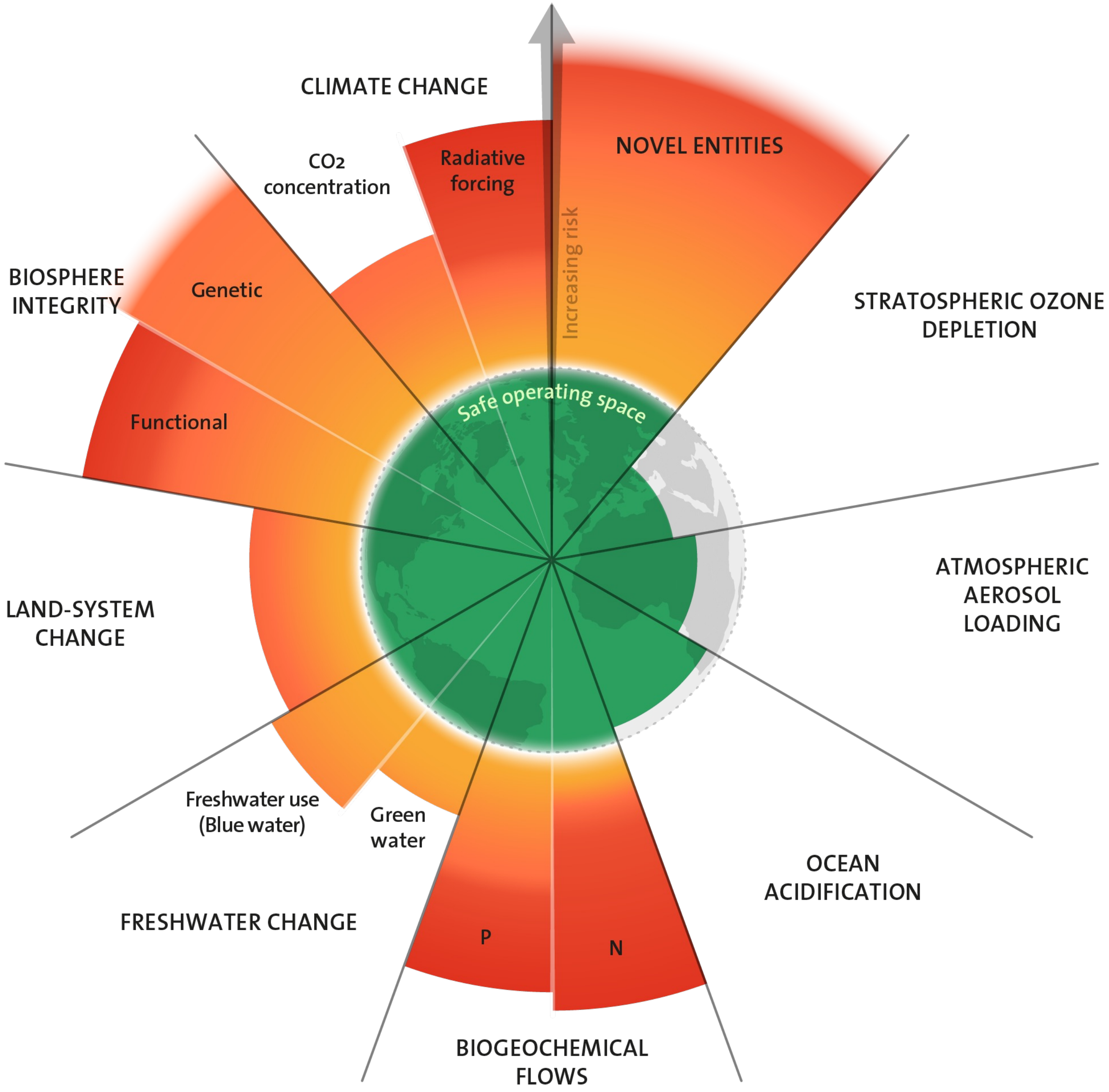
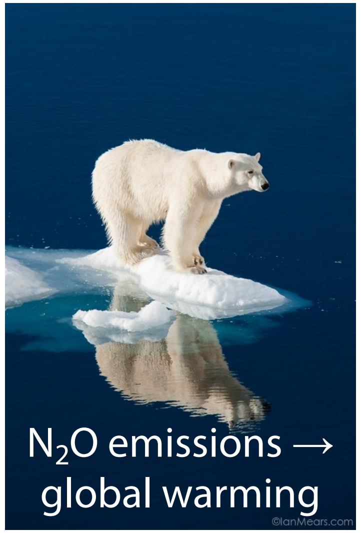


Image: Azote for Stockholm Resilience Centre, based on analysis in Richardson et al 2023

Consequences of increased N loading


N_2O emissions → global warming

N_2O emissions →
depletion of stratospheric O_3 →
skin cancer

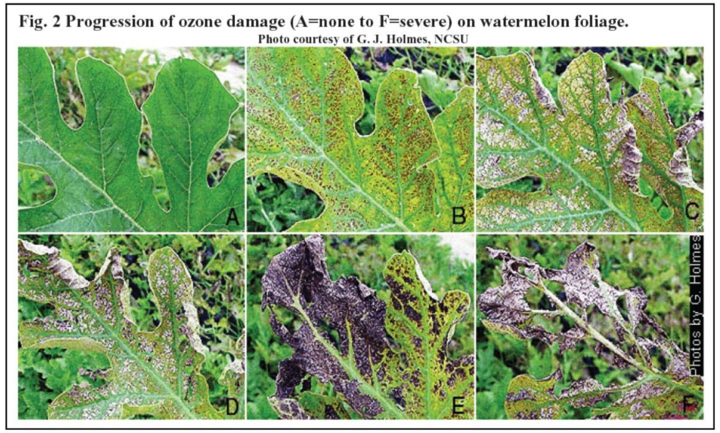
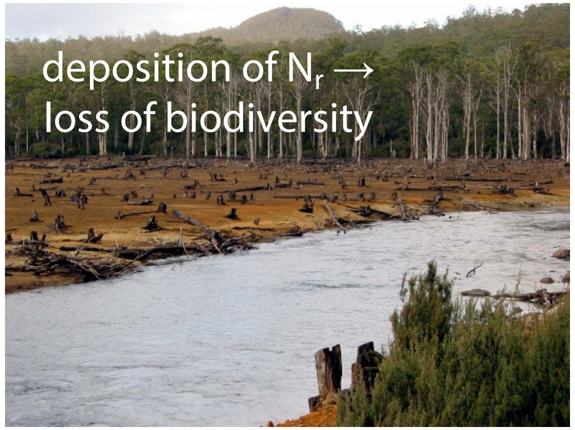


Fig. 2 Progression of ozone damage (A=none to F=severe) on watermelon foliage.
Photo courtesy of G. J. Holmes, NCSU

NO_x emissions → tropospheric O_3 →
risk for plant and human health


deposition of N_r →
loss of biodiversity


leaching of N → eutrophication

A fine balance…

- Feeding the population while minimizing unintended environmental consequences requires a detailed understanding of the N cycle

N for food production and industry needs

Environmental consequences of N addition

- Stable isotopes are a powerful tool to understand the N cycle

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  - ^{15}N labelling
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Nitrogen stable isotopes

Two stable isotopes:

$^{14}N = 99.64\%$

$^{15}N = 0.36\%$

Oxygen isotopes are often used to study oxidized N forms (N_2O, NO_x...)

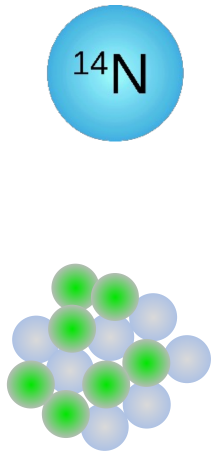
$^{16}O = 99.76\%$

$^{17}O = 0.04\%$

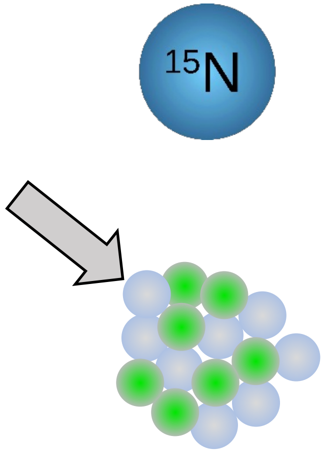
$^{18}O = 0.2\%$

Nitrogen stable isotopes

Nucleus:



- nitrogen-14
- 99.64%
- 7 protons
- 7 neutrons



- nitrogen-15
- 0.36%
- 7 protons
- 8 neutrons

- *Natural abundance* approaches involve measurement of small changes in naturally occurring isotope ratios
- *Isotopic labelling* involves addition of one isotope (usually ^{15}N) to trace transformations

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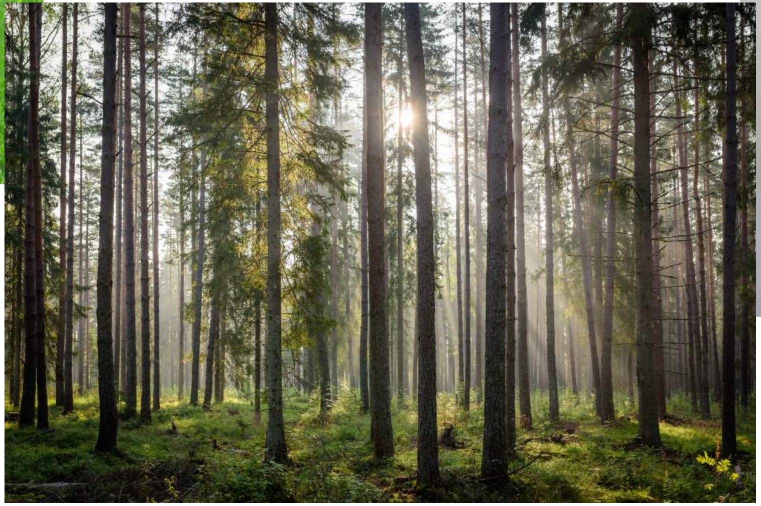
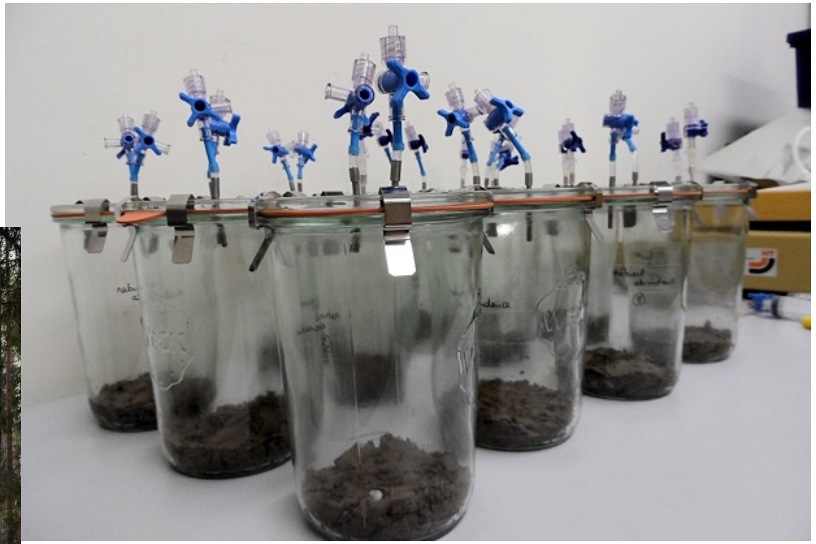
Tracing nitrogen using labelling

- We can add labelled ^{15}N substrates and trace their fate in different systems

Agricultural Systems



Natural Systems

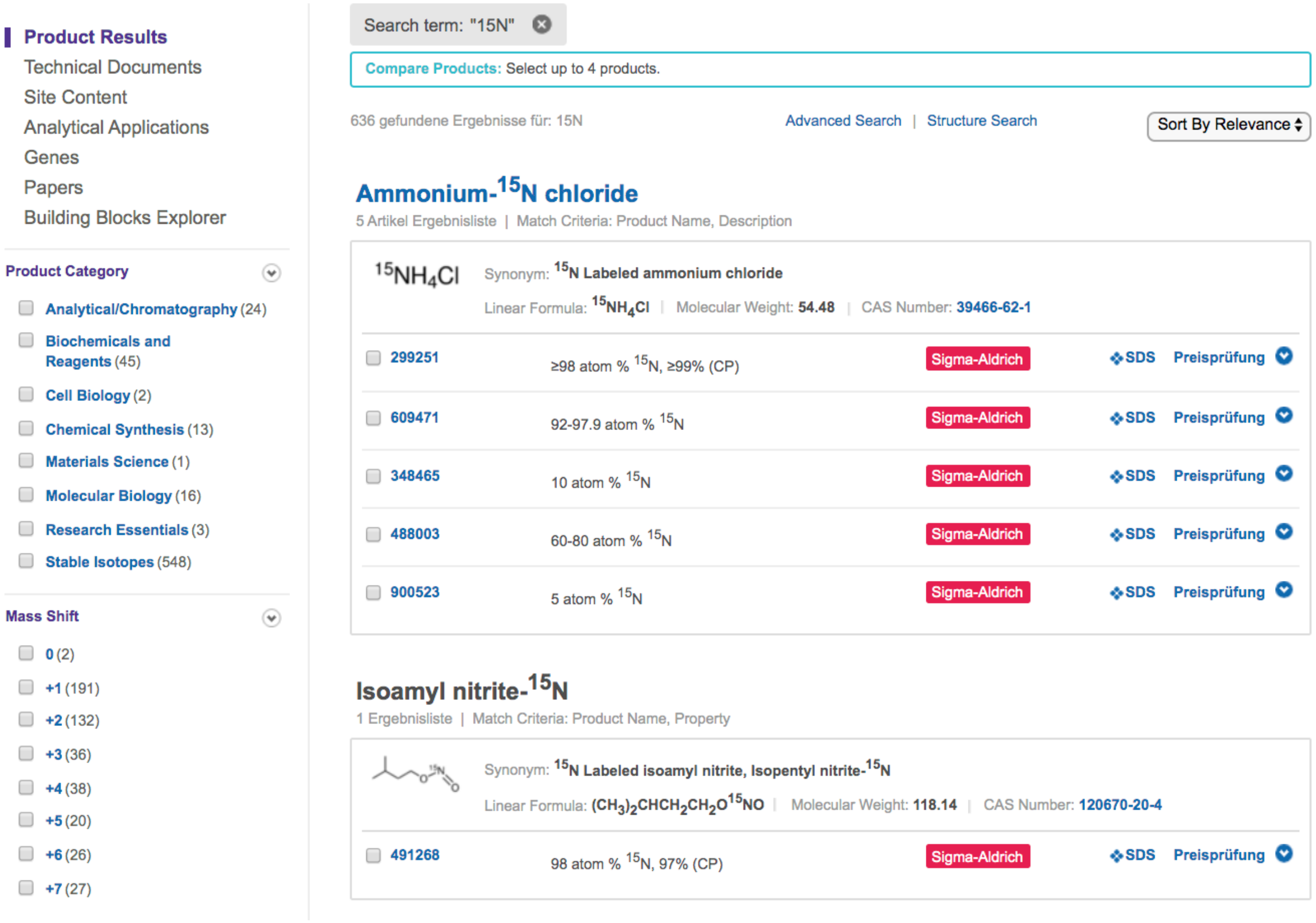


Incubation Experiments

Images (L-R): Thom Weir; Elopak; Reinhard Well, Thünen Institute

^{15}N labelling approaches

- Isotopically enriched ^{15}N compounds are commercially available
  - Usually 5-99% ^{15}N
- Sigma Aldrich have 533 ^{15}N-enriched products available
  - But only two ^{14}N products… why?



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- ☐ Research Essentials (3)
- ☐ Stable Isotopes (548)

Mass Shift

- ☐ 0 (2)
- ☐ +1 (191)
- ☐ +2 (132)
- ☐ +3 (36)
- ☐ +4 (38)
- ☐ +5 (20)
- ☐ +6 (26)
- ☐ +7 (27)

Search term: "15N"

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636 gefundene Ergebnisse für: 15N

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Ammonium-^{15}N chloride

5 Artikel Ergebnisliste | Match Criteria: Product Name, Description

$^{15}NH_4Cl$ Synonym: ^{15}N Labeled ammonium chloride

Linear Formula: $^{15}NH_4Cl$ | Molecular Weight: 54.48 | CAS Number: 39466-62-1

☐ 299251	≥98 atom % ^{15}N, ≥99% (CP)	Sigma-Aldrich	SDS	Preisprüfung
☐ 609471	92-97.9 atom % ^{15}N	Sigma-Aldrich	SDS	Preisprüfung
☐ 348465	10 atom % ^{15}N	Sigma-Aldrich	SDS	Preisprüfung
☐ 488003	60-80 atom % ^{15}N	Sigma-Aldrich	SDS	Preisprüfung
☐ 900523	5 atom % ^{15}N	Sigma-Aldrich	SDS	Preisprüfung

Isoamyl nitrite-^{15}N

1 Ergebnisliste | Match Criteria: Product Name, Property

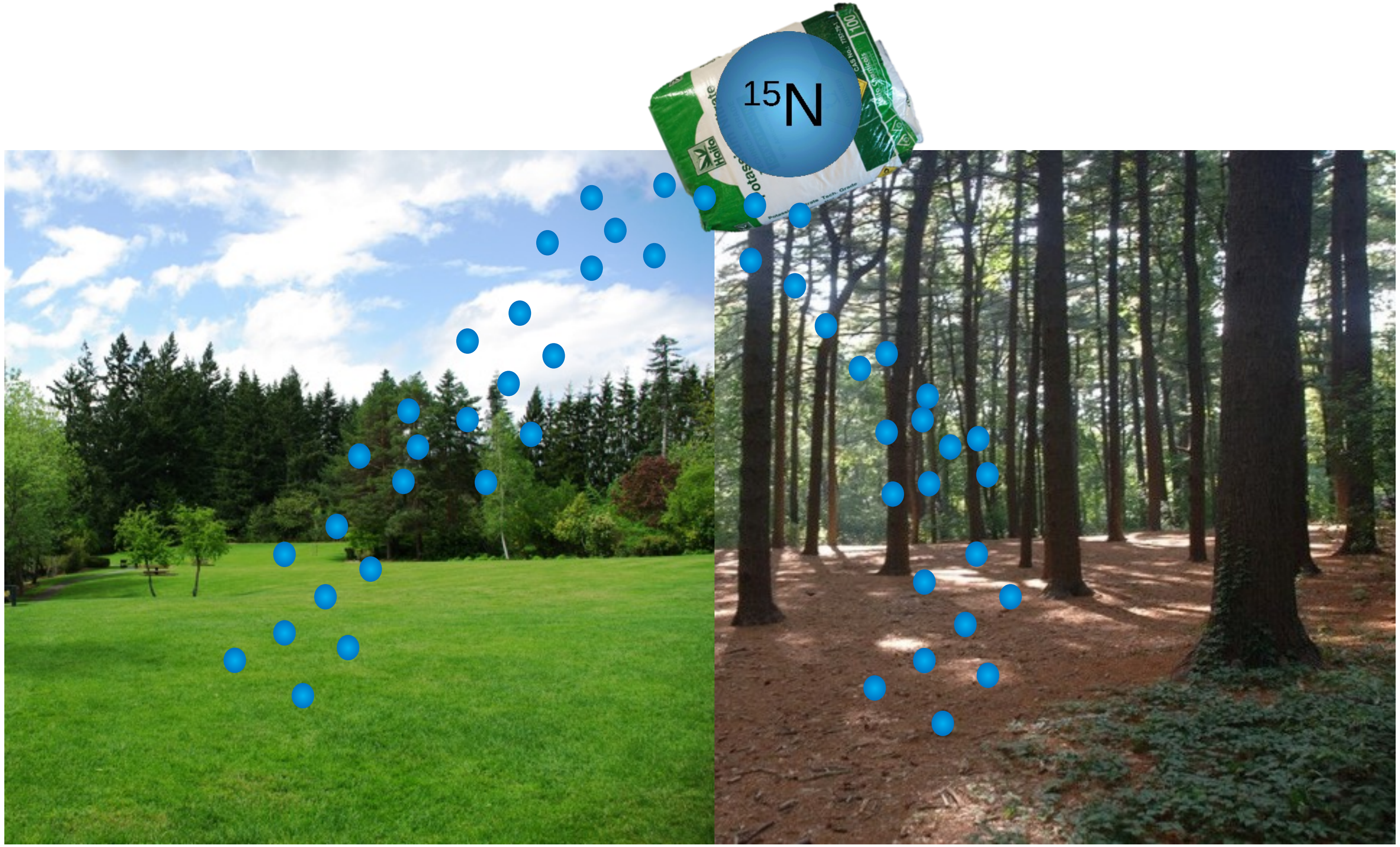
Synonym: ^{15}N Labeled isoamyl nitrite, Isopentyl nitrite-^{15}N

Linear Formula: $(CH_3)_2CHCH_2CH_2O^{15}NO$ | Molecular Weight: 118.14 | CAS Number: 120670-20-4

☐ 491268	98 atom % ^{15}N, 97% (CP)	Sigma-Aldrich	SDS	Preisprüfung

Tracing nitrogen using labelling

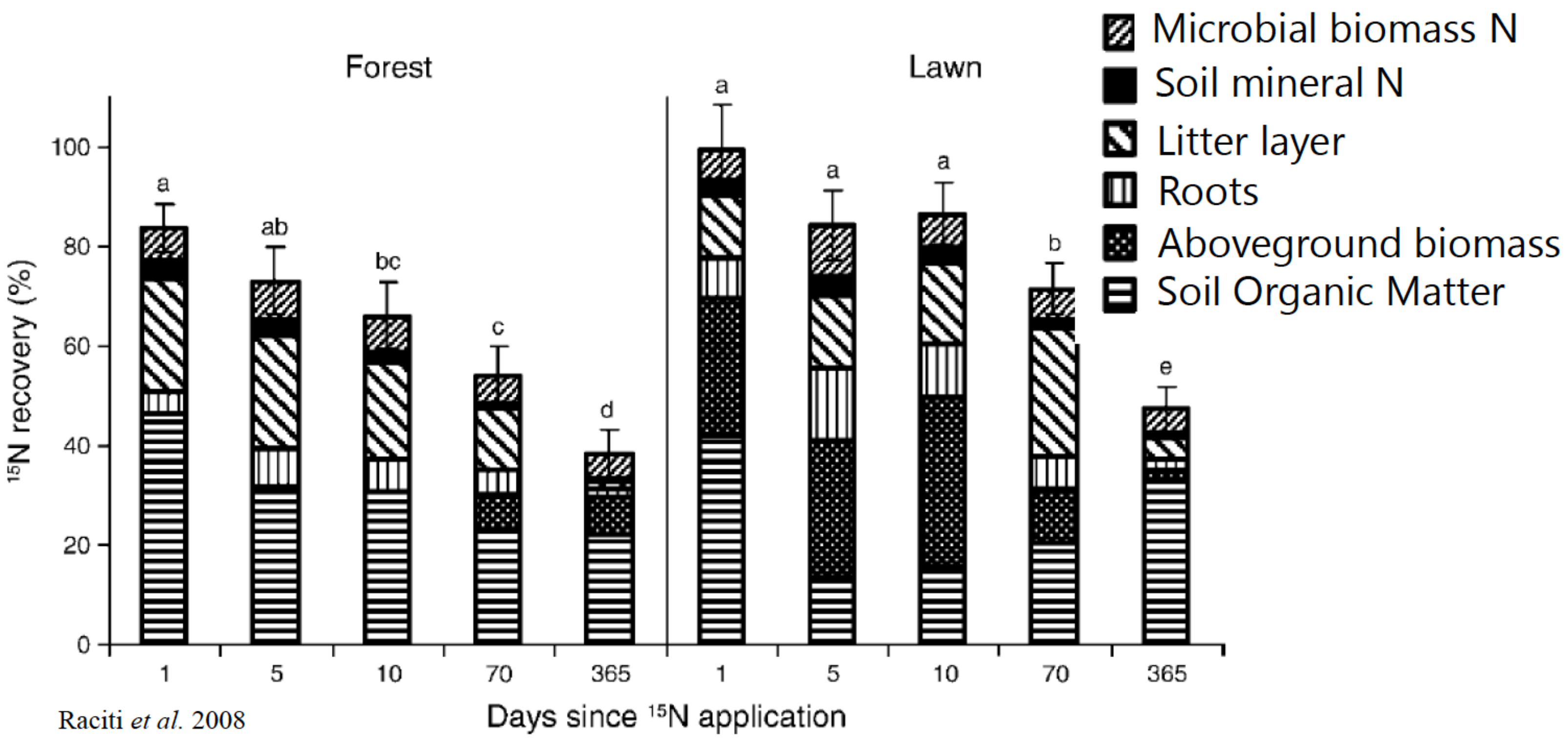
What would happen if you fertilize a lawn or a forest with ^{15}N-enriched KNO_3?



Raciti et al. (2008) Ecological Applications

Tracing nitrogen using labelling

- $^{15}NO_3^-$ was added to forest and lawn in a simulated precipitation event
- Recovery of ^{15}N in pools is calculated, assuming pool size is not affected
- AF = ^{15}N atom fraction

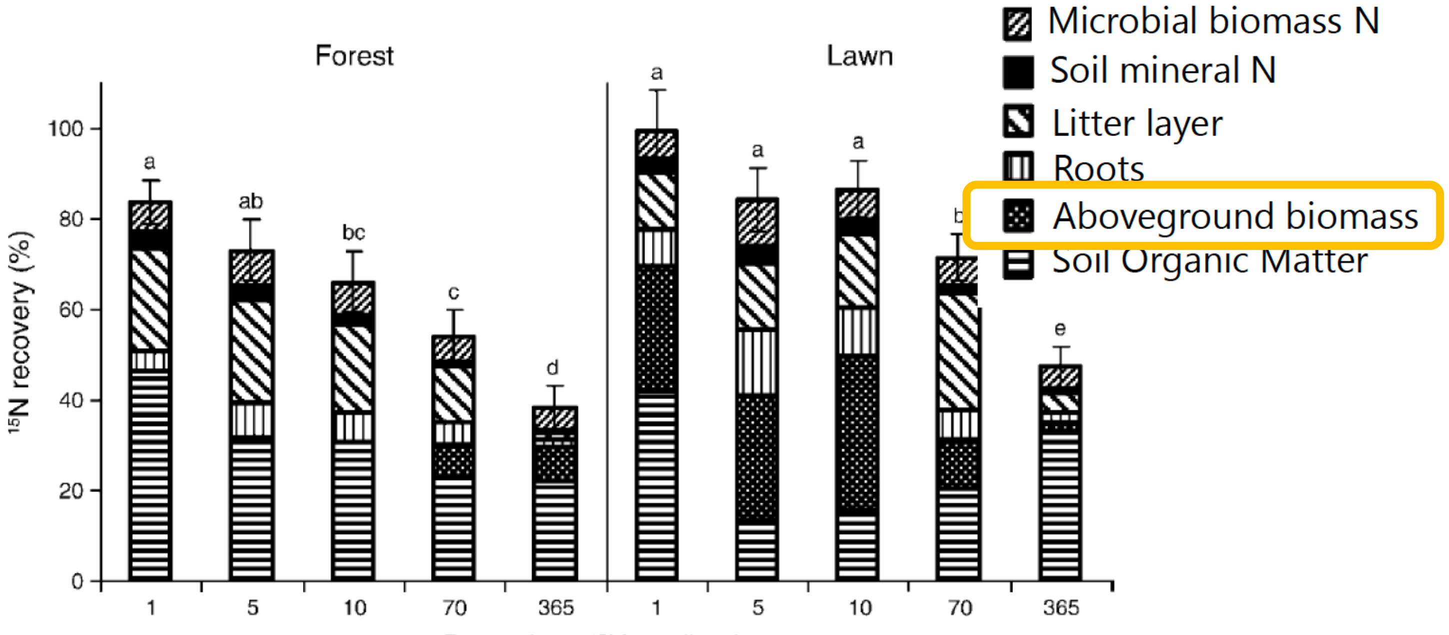


Raciti *et al.* 2008

$$^{15}\text{N recovery (\%)} = \frac{\text{Pool size} \times \overset{^{15}\text{N excess}}{\boxed{(\text{Sample AF} - \text{Background AF})}}}{\text{Total 15N added}} \times 100$$

Raciti et al. (2008) Ecological Applications

Tracing nitrogen using labelling



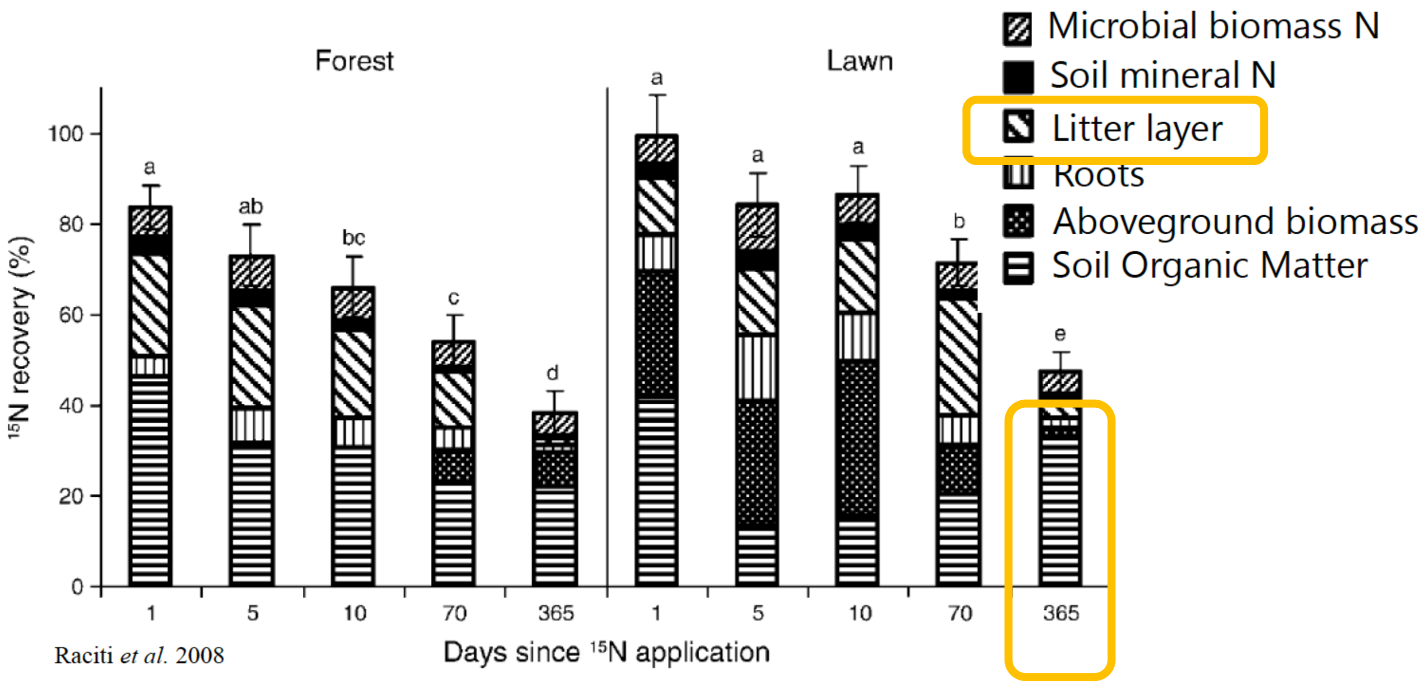
Raciti *et al.* 2008

- Decline over time in both sites
  - Denitrification, leaching…
- ^{15}N in the **above-ground biomass**:
  - *Forest:* visible only after 70 days
  - *Lawn:* quick incorporation

$$^{15}\text{N recovery (\%)} = \frac{\text{Pool size} \times (\text{Sample AF} - \text{Background AF})}{\text{Total 15N added to subplot}} \times 100$$

Raciti et al. (2008) Ecological Applications

Tracing nitrogen using labelling



Raciti *et al.* 2008

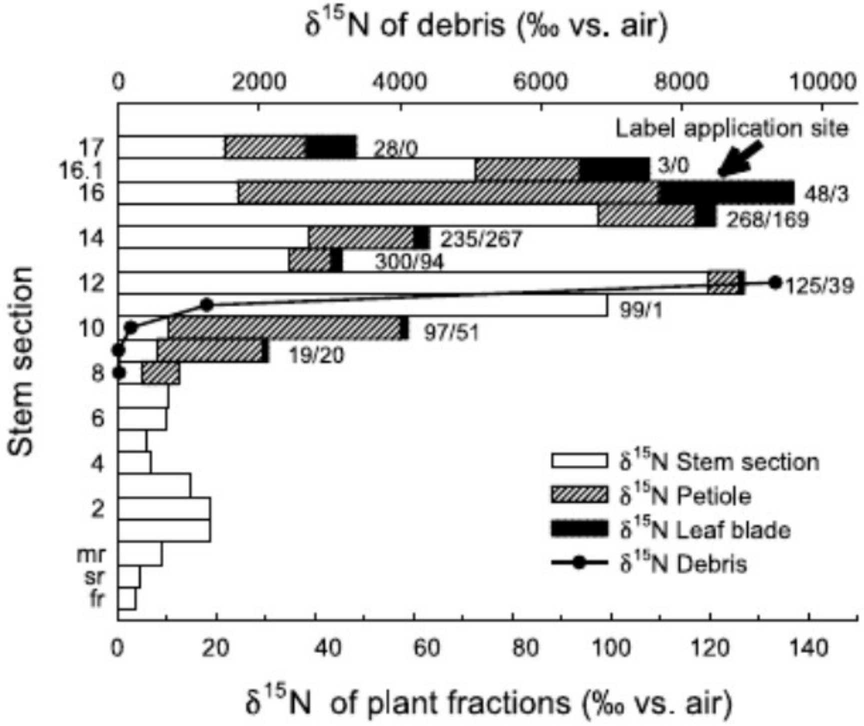
- Decline over time in both sites
  - Denitrification, leaching…
- ^{15}N in the **above-ground biomass**:
  - *Forest:* visible only after 70 days
  - *Lawn:* quick incorporation
- ^{15}N in the **litter** layer:
  - *Forest:* continuous decrease
  - *Lawn:* increase, due to plant growth and regular mowing
- Lawns might be important sinks for N in the urban environment

$$^{15}\text{N recovery (\%)} = \frac{\text{Pool size} \times (\text{Sample AF} - \text{Background AF})}{\text{Total 15N added to subplot}} \times 100$$

Raciti et al. (2008) Ecological Applications

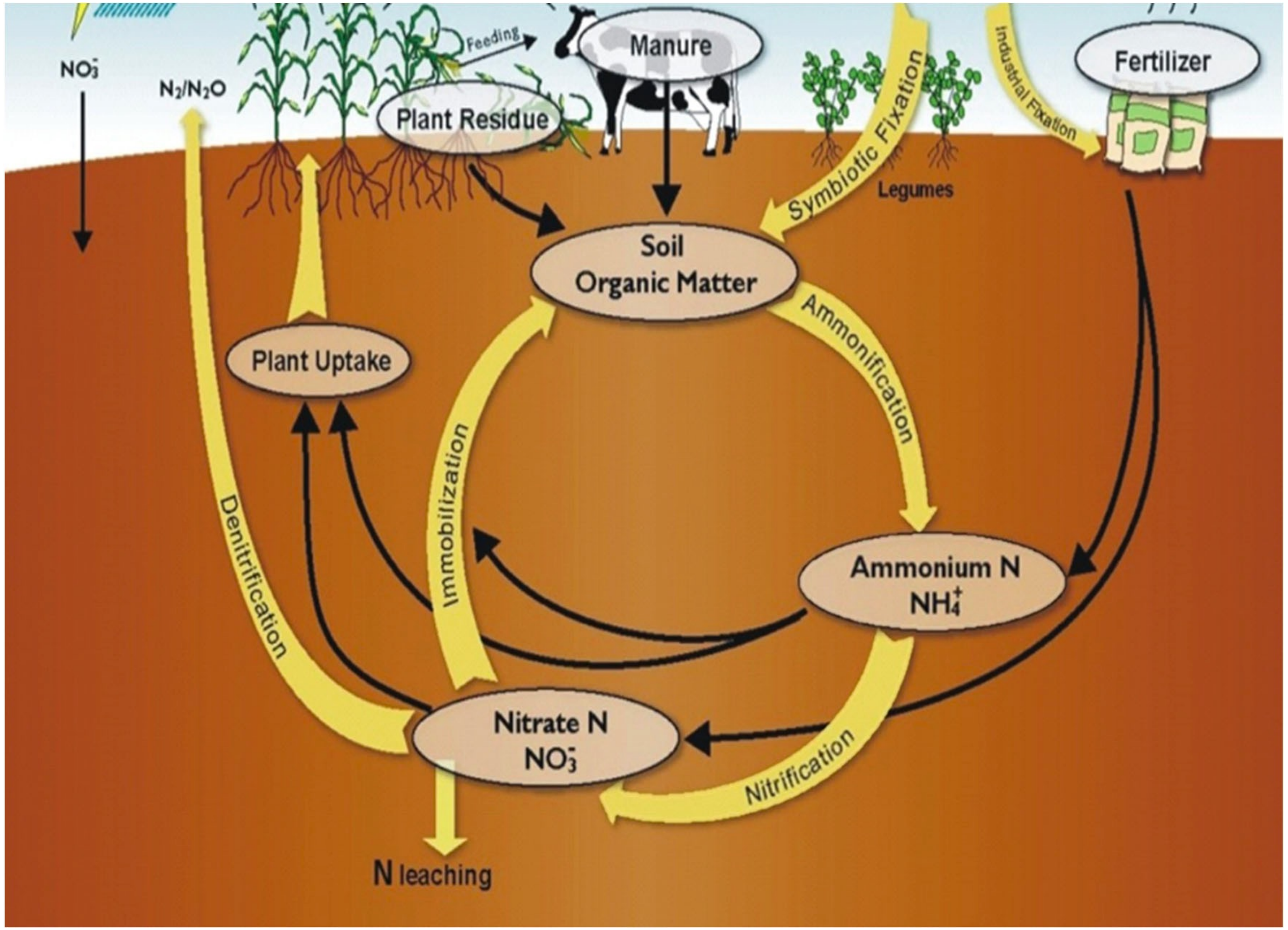
The fate of N: *Do ants feed plants?*

- Ants were fed on ^{15}N labelled glycine (98 at%)
- Associated plants were measured after 1 week using CF EA-IRMS
- In controls (no ants), 89% of ^{15}N was found in plants
- With ants present, 71% of ^{15}N was incorporated into the colony
  - 25% was in plants – passed on to plants from ants
- *Yes, ants feed plants!*



Fischer et al. (2003) Journal of Ecology

Quantifying changes in the NH_4^+ pool



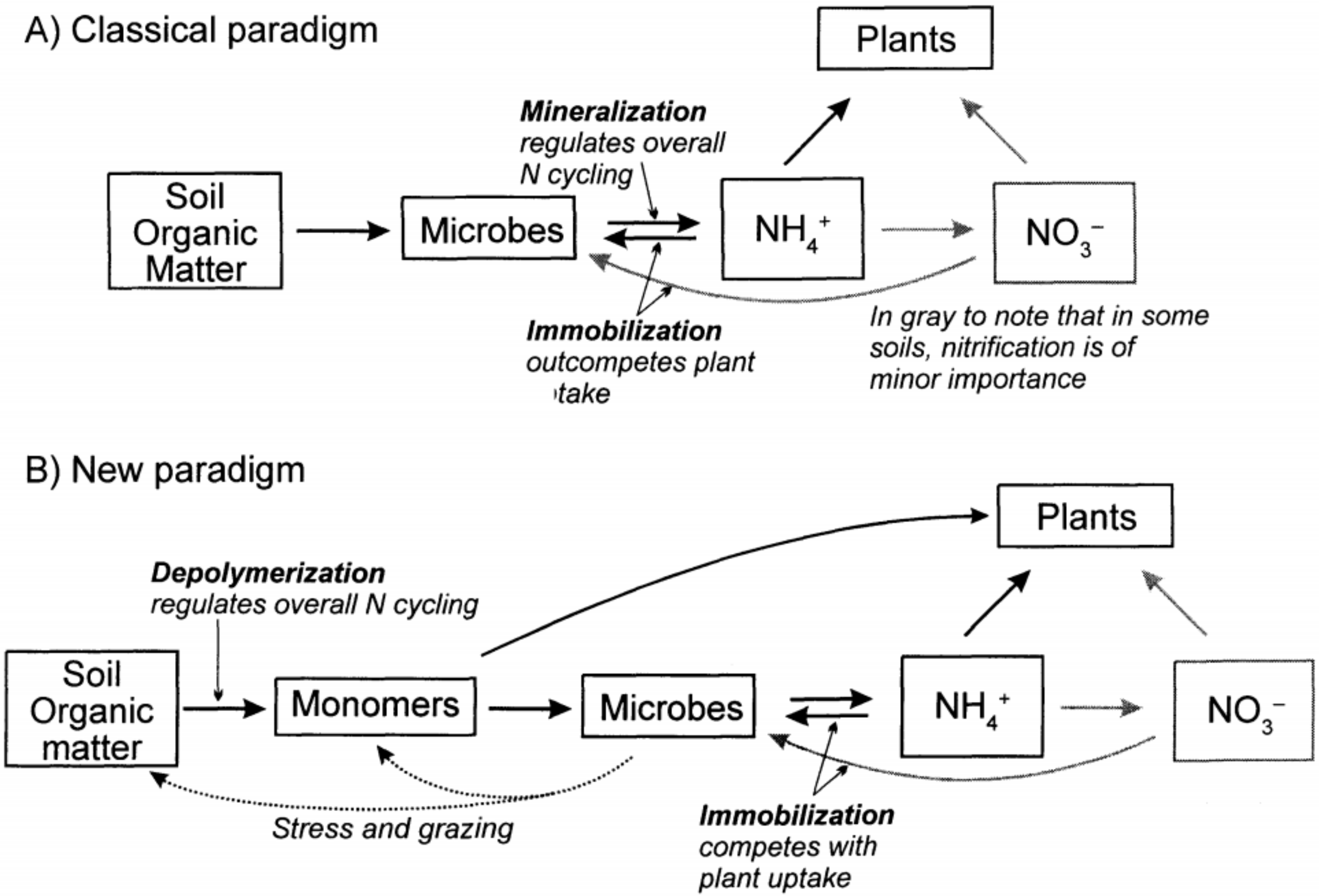
- *Mineralization (aka. Ammonification):* Conversion of organic N to plant-available forms
- In the Arctic, it appears mineralization is much too low to account for plant N needs
- **Can plants take up org-N?**

Schimel and Chapin (1996) Ecology

Quantifying changes in the NH_4^+ pool

- Solutions of either $(^{15}NH_4)_2SO_4$, ^{15}N,^{13}C-1 glycine or ^{15}N,^{13}C-1 aspartate were added to soil cores from Arctic tundra and monitored for 1, 5, 30 d
- >75% of the label was found in microbes, 5-20% in dead stems, and <5% in live plant biomass **in all of the different treatments**
  - Results show plants most likely directly take up amino acids
- These and similar results challenged the assumption that the soil nitrogen cycle was dominated by two subsequent processes:
  1. Microbial decomposition of org-N; plants do not successfully compete for org-N
  2. Plants take up inorganic N produced by microbes

Schimel and Chapin (1996) Ecology; Schimel and Bennett (2004) Ecology



A) Classical paradigm

Soil Organic Matter

Microbes

NH_4^+

NO_3^-

Plants

Mineralization *regulates overall N cycling*

Immobilization *outcompetes plant take*

In gray to note that in some soils, nitrification is of minor importance

B) New paradigm

Soil Organic matter

Monomers

Microbes

NH_4^+

NO_3^-

Plants

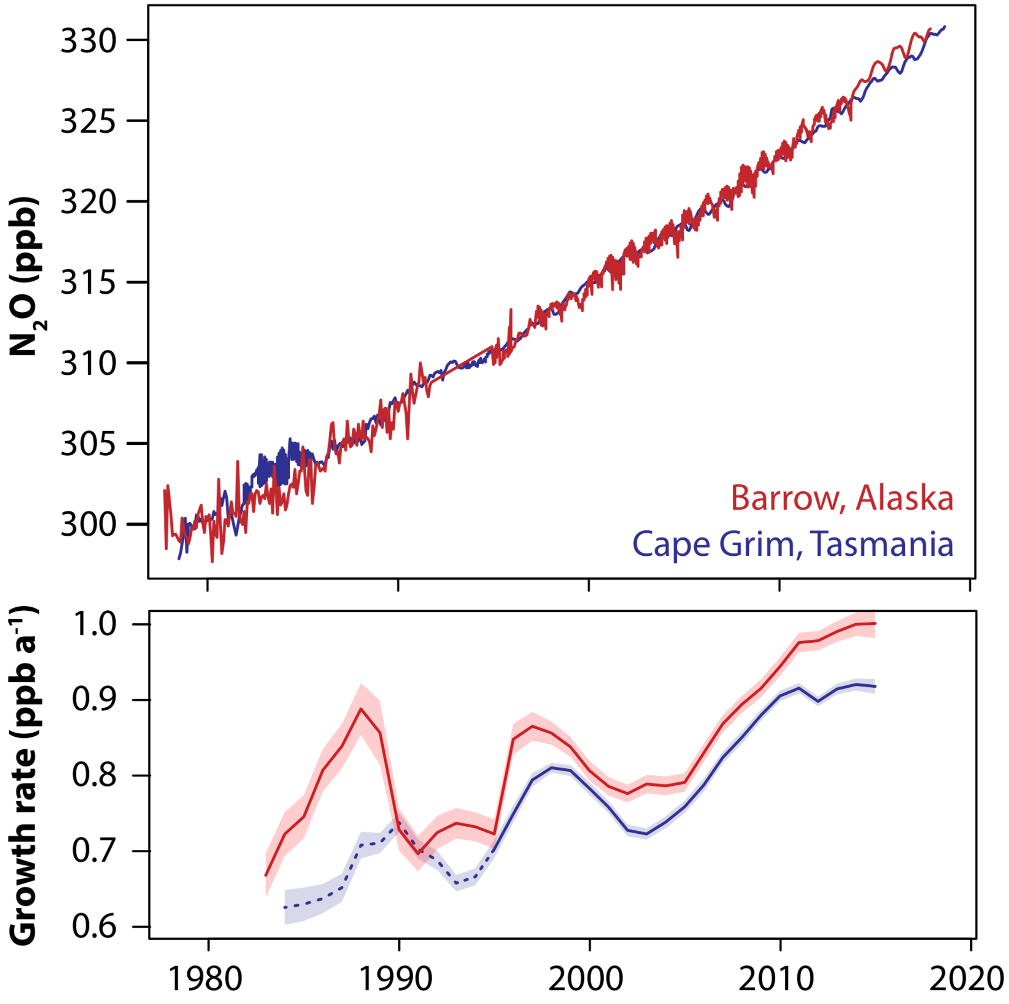
Depolymerization *regulates overall N cycling*

Stress and grazing

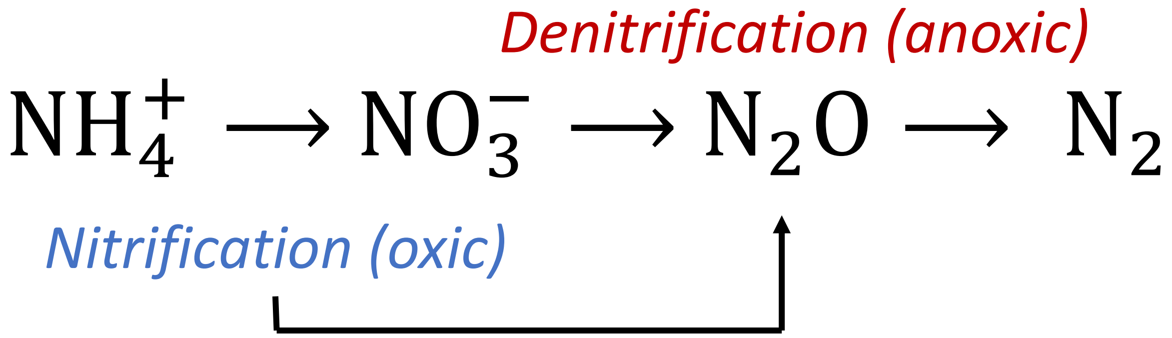
Immobilization *competes with plant uptake*

Schimel and Bennett (2004) Ecology

Understanding N_2O emissions with labelling



- N_2O is a strong greenhouse gas and a key stratospheric ozone destructant
- Emissions are rising rapidly, thus an understanding of its production is needed to drive targeted mitigation

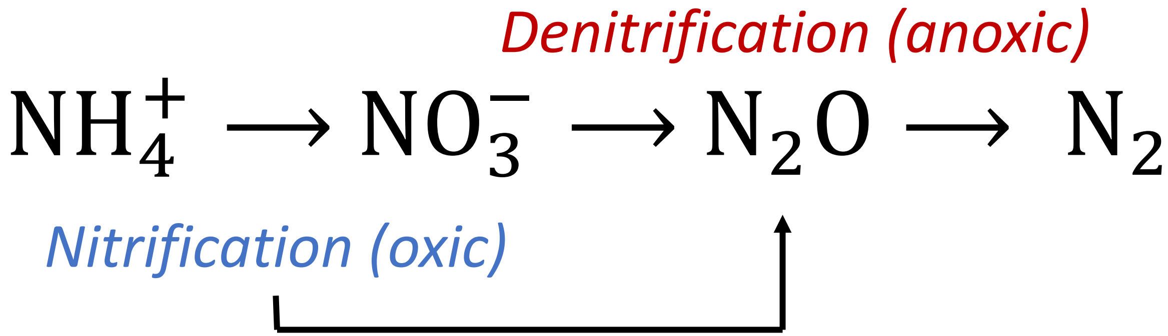

Denitrification (anoxic)

$$NH_4^+ \longrightarrow NO_3^- \longrightarrow N_2O \longrightarrow N_2$$

Nitrification (oxic)

Understanding N_2O emissions with labelling

- Stevens et al.[1] applied ^{15}N-labelled NO_3^- and NH_4^+ to soils



Denitrification (anoxic)

$$NH_4^+ \longrightarrow NO_3^- \longrightarrow N_2O \longrightarrow N_2$$

Nitrification (oxic)

- What is the contribution of nitrification and denitrification to N_2O?

$$f_D = \frac{{}^{15}N{-}N_2O \; - \; {}^{15}N{-}NH_4^+}{{}^{15}N{-}NO_3^- \; - \; {}^{15}N{-}NH_4^+}$$

- f_D = fraction of N_2O from denitrification
- ^{15}N = ^{15}N atom fraction in pool

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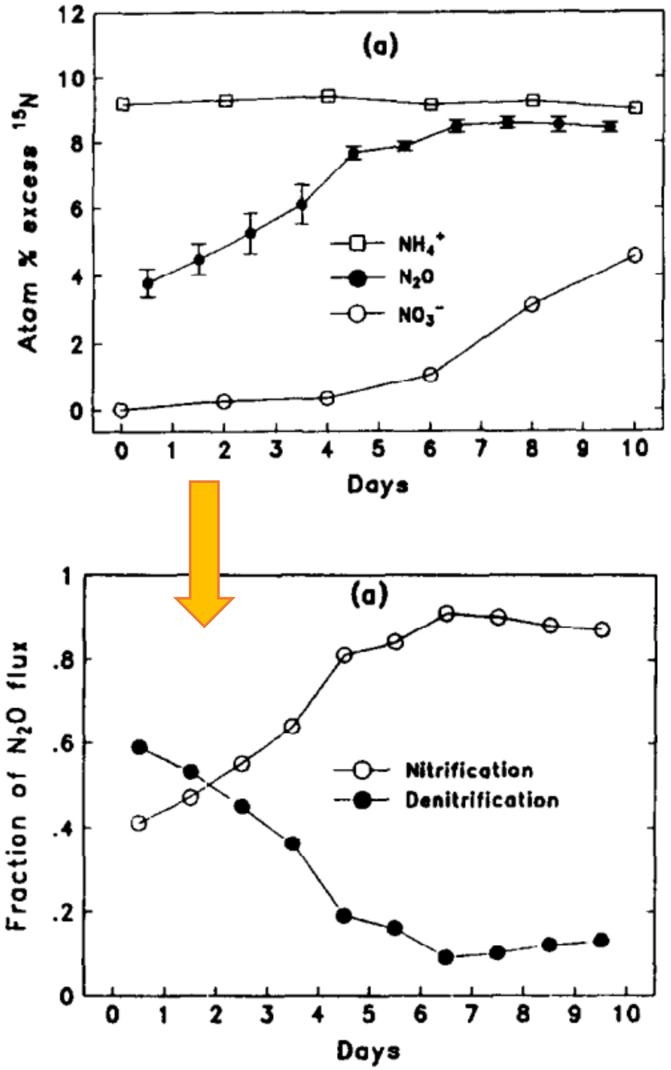
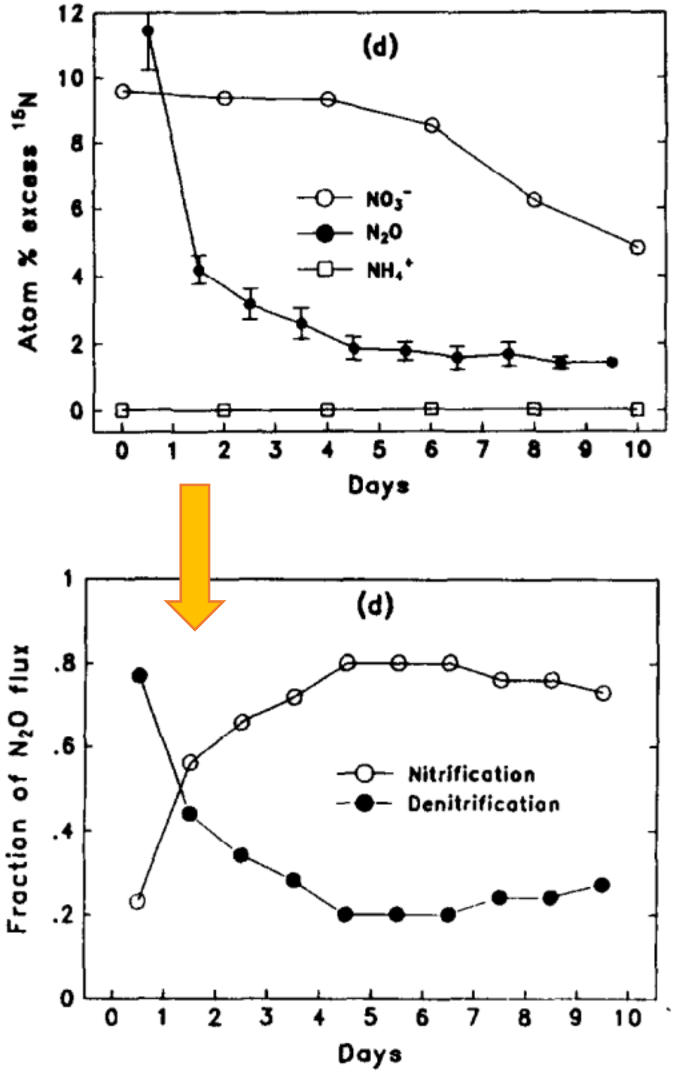
[1] 1997, Soil Biology and Biochemistry

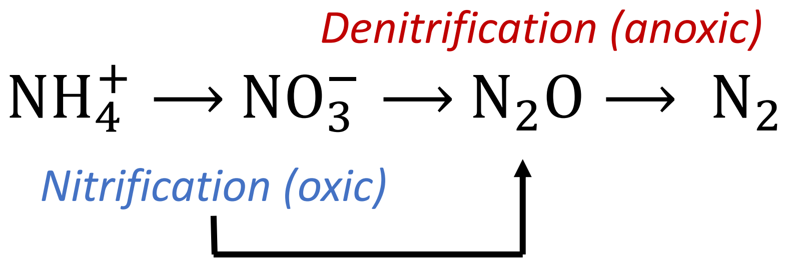
Understanding N_2O emissions with labelling

Labelled NH_4^+



Labelled NO_3^-





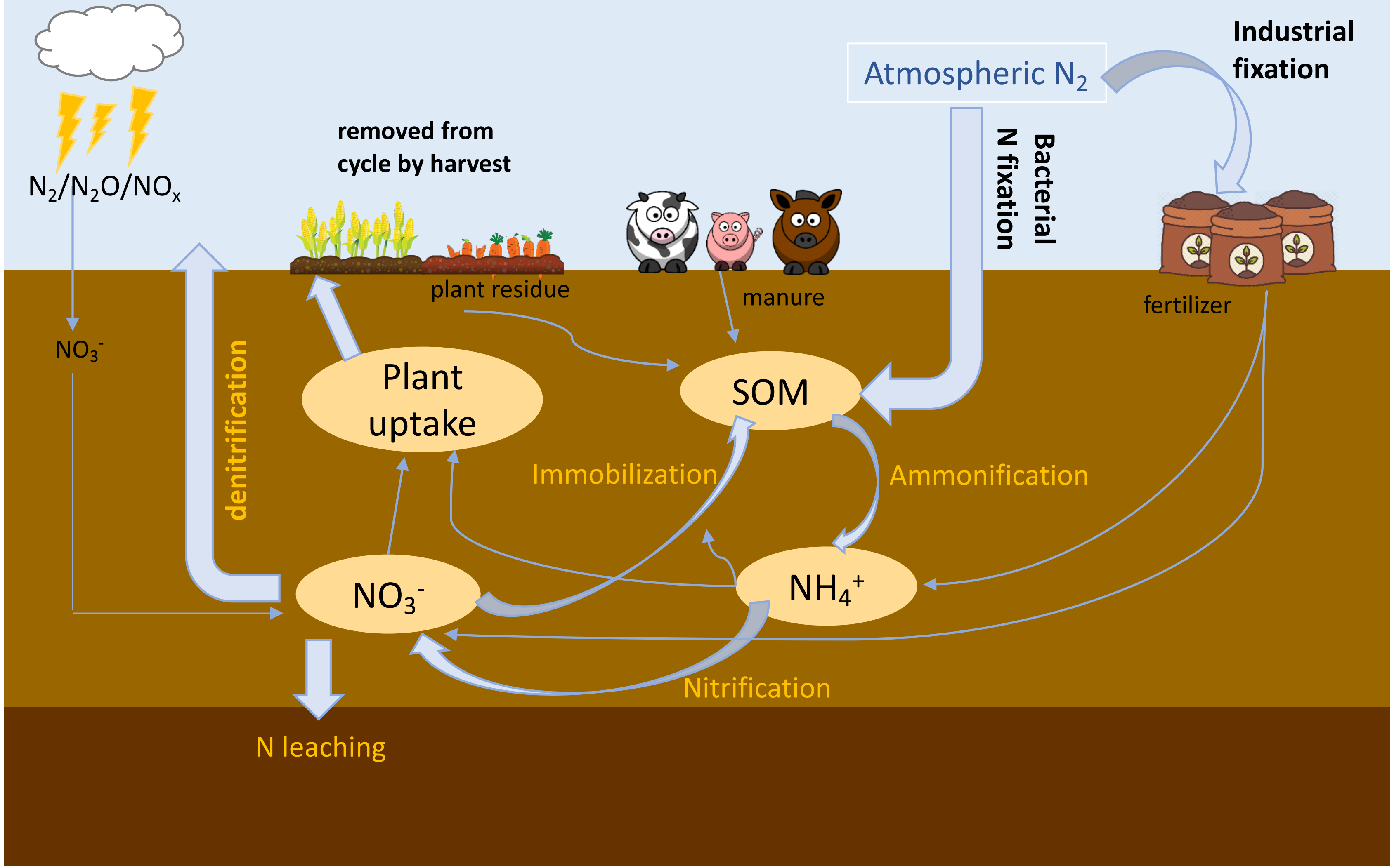
Denitrification (anoxic)

$$NH_4^+ \longrightarrow NO_3^- \longrightarrow N_2O \longrightarrow N_2$$

Nitrification (oxic)

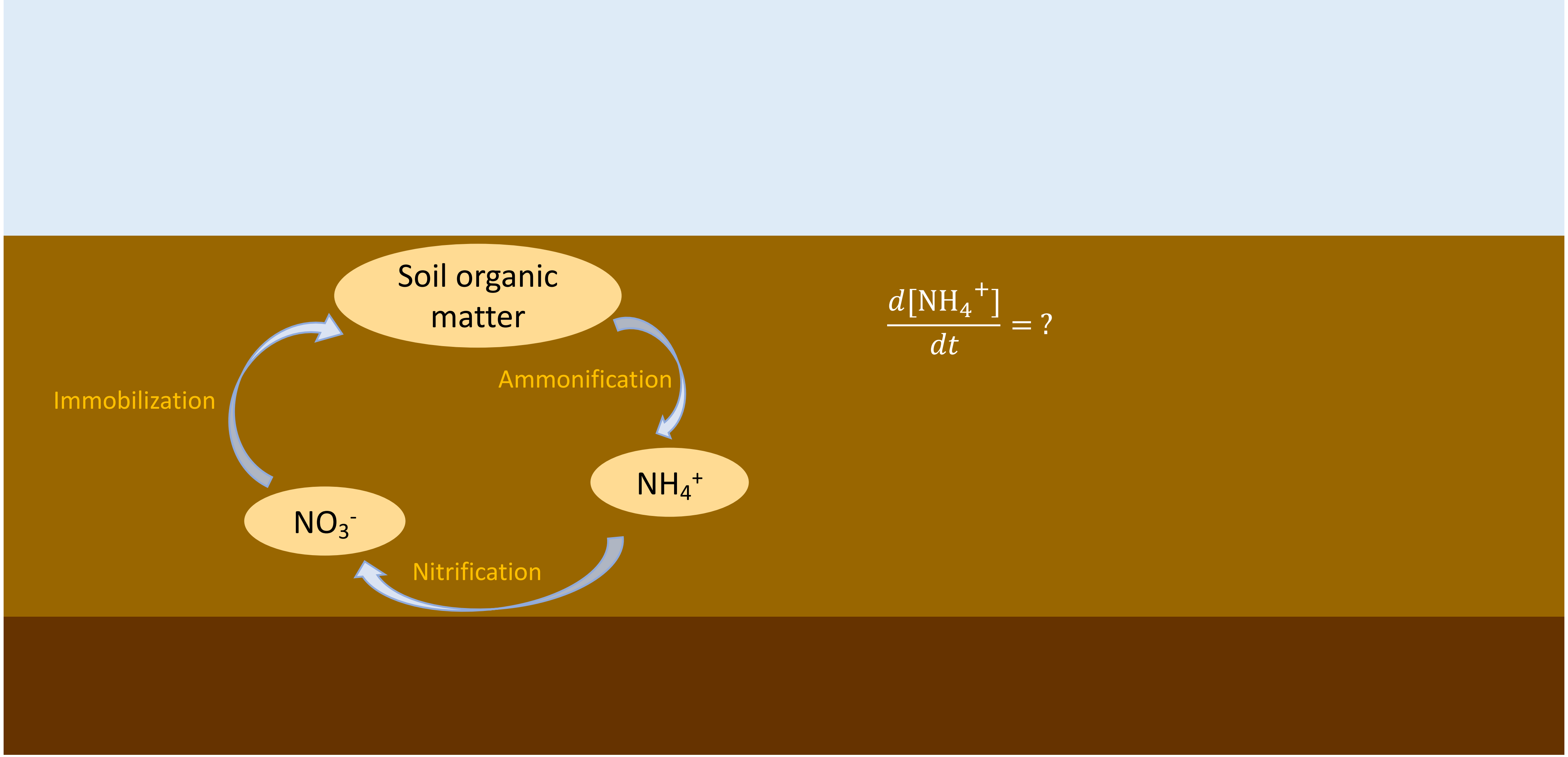
- Pathways partitioned using the equation on the previous slide
- Results show nitrification dominates overall
  - Similar whichever label is used

Tracing the process rates of N transformation



Heike Knicker, 2004, Marine Chemistry

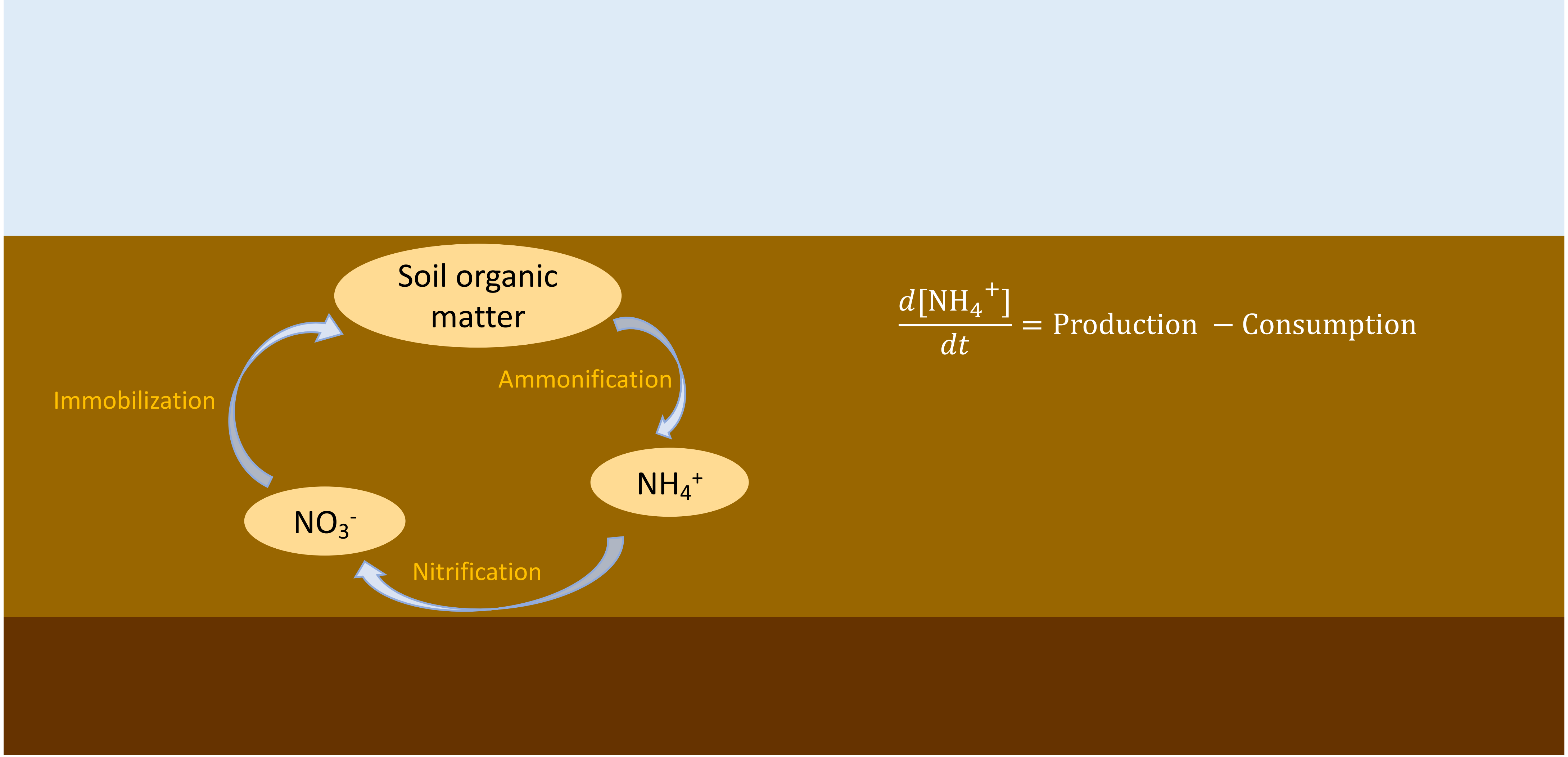
Tracing the process rates of N transformation



$$\frac{d[\mathrm{NH_4}^+]}{dt} = ?$$

Heike Knicker, 2004, Marine Chemistry

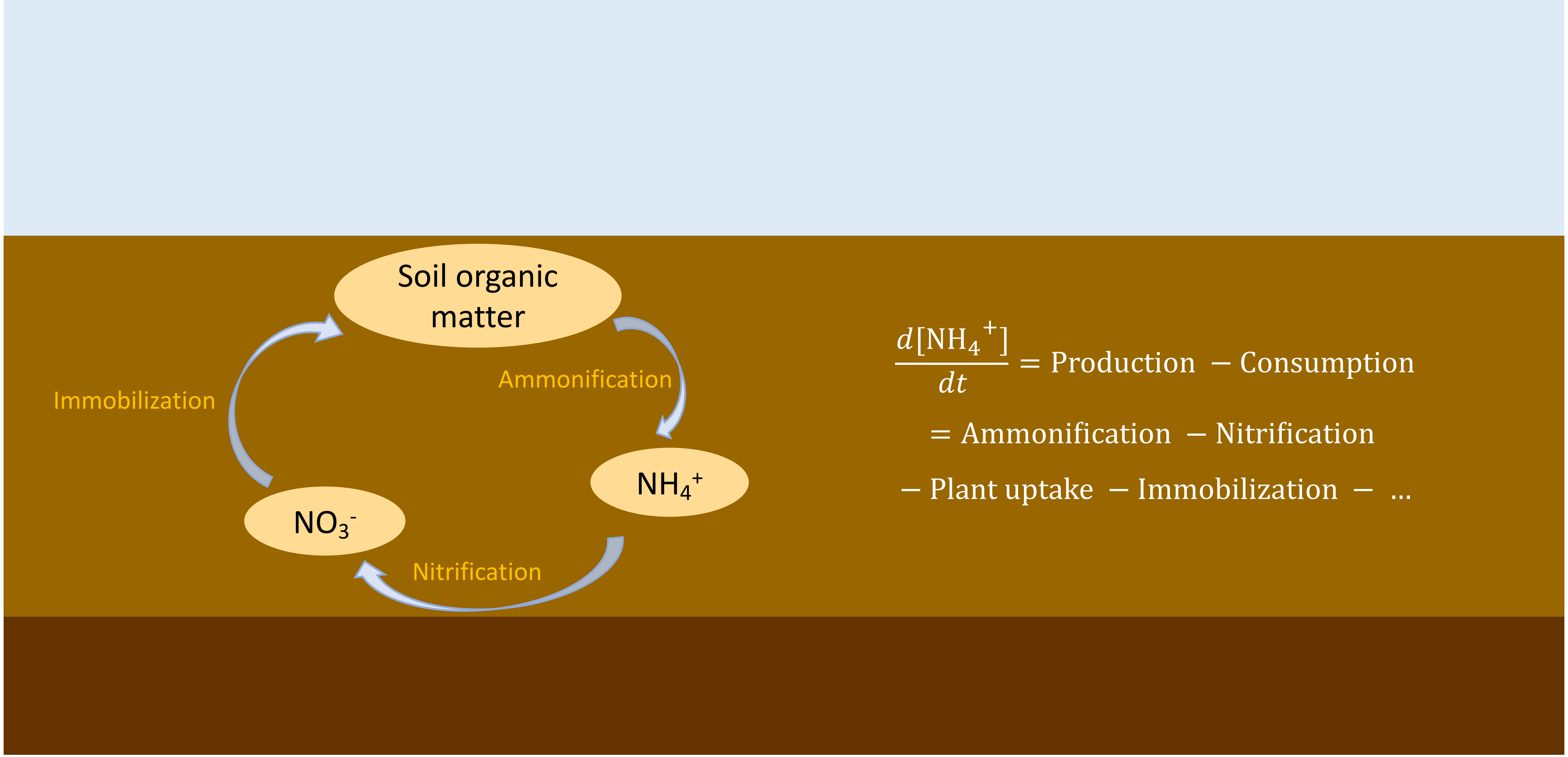
Tracing the process rates of N transformation



$$\frac{d[\mathrm{NH_4}^+]}{dt} = \text{Production } - \text{Consumption}$$

Heike Knicker, 2004, Marine Chemistry

Tracing the process rates of N transformation



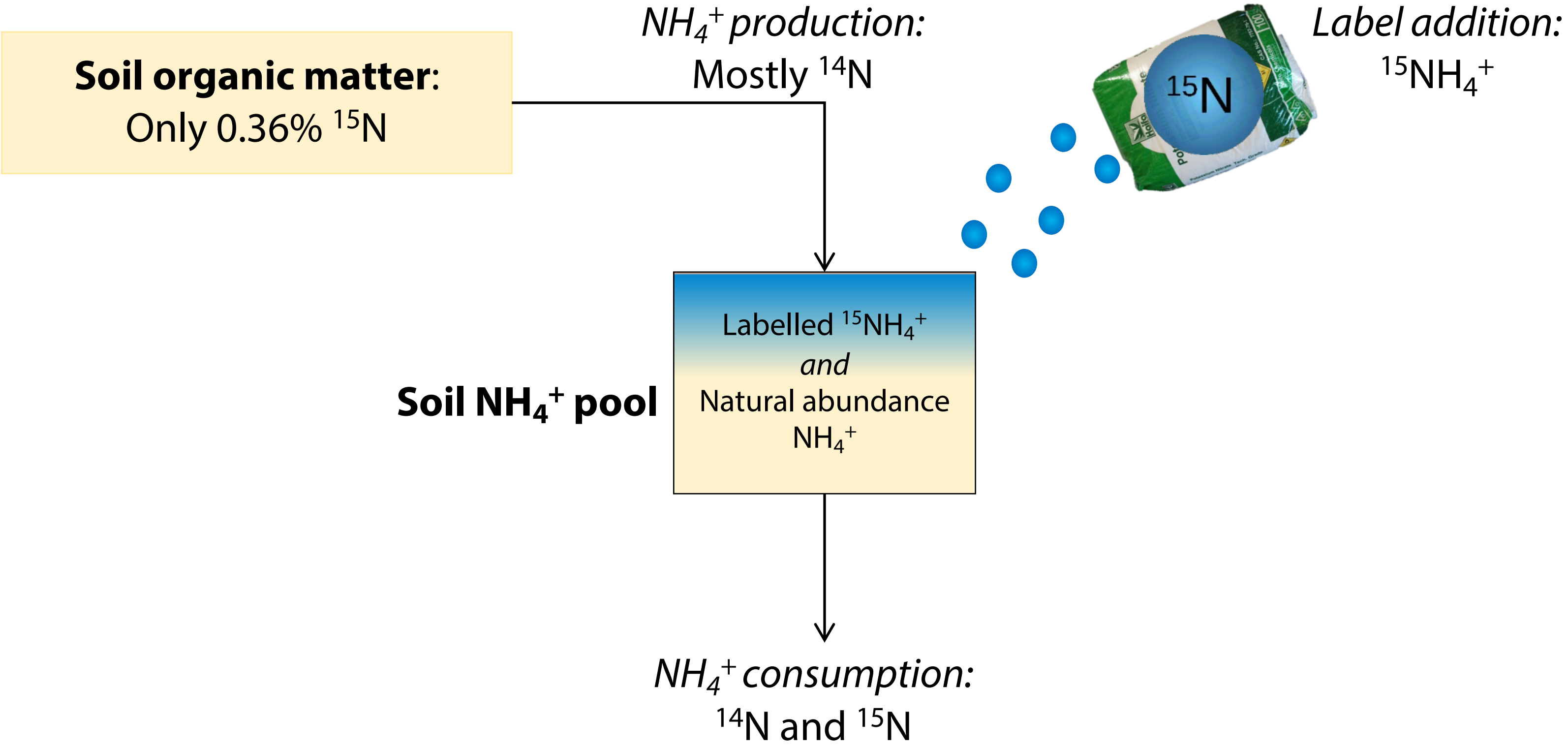
$$\frac{d[\mathrm{NH_4}^+]}{dt} = \text{Production} - \text{Consumption}$$

$$= \text{Ammonification} - \text{Nitrification}$$

$$- \text{Plant uptake} - \text{Immobilization} - \ldots$$

Heike Knicker, 2004, Marine Chemistry

Isotope pool dilution



Soil organic matter: Only 0.36% ^{15}N

NH_4^+ production: Mostly ^{14}N

Label addition: $^{15}NH_4^+$

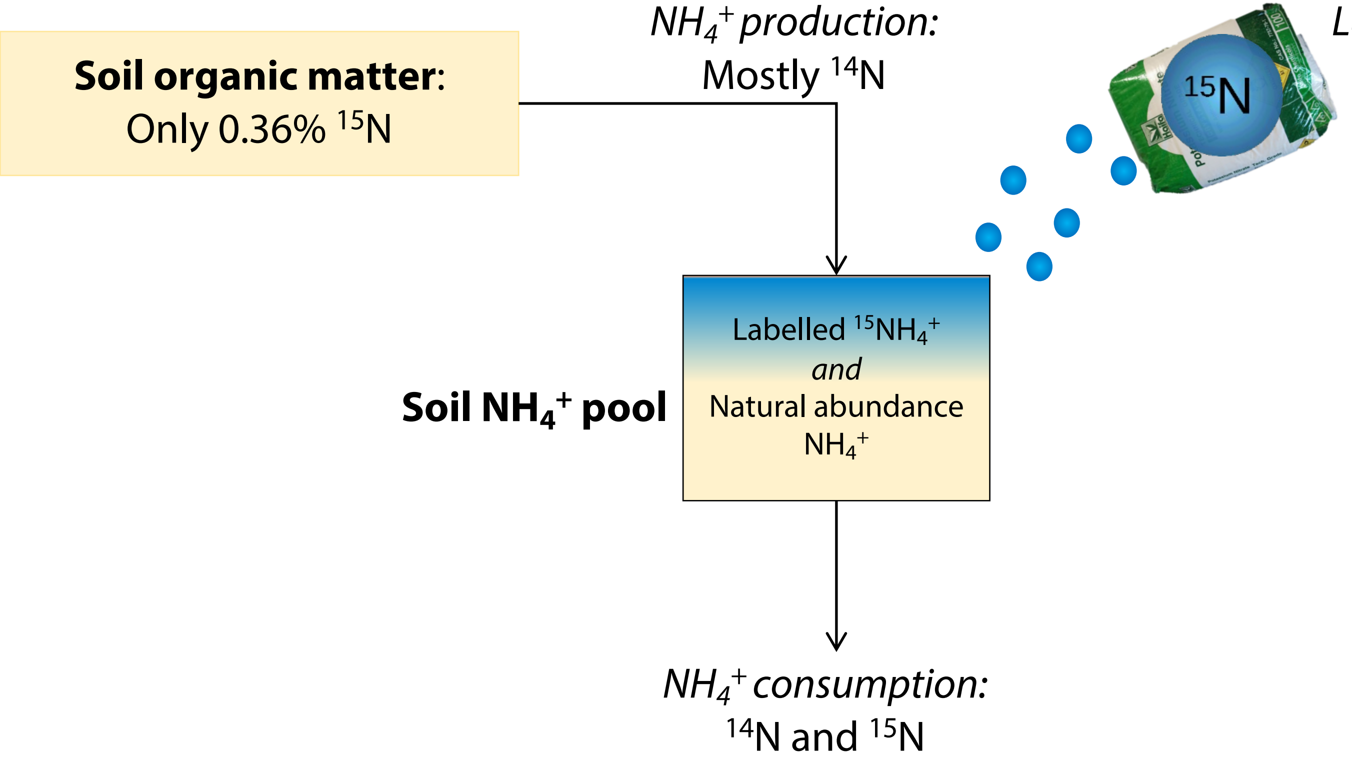
Soil NH_4^+ pool

Labelled $^{15}NH_4^+$ *and* Natural abundance NH_4^+

NH_4^+ consumption: ^{14}N and ^{15}N

Kirkham and Bartholomew (1954)
Schimel (1996)

Isotope pool dilution



Soil organic matter:
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Soil NH_4^+ pool

Labelled $^{15}NH_4^+$
and
Natural abundance NH_4^+

NH_4^+ consumption:
^{14}N and ^{15}N

$$\frac{d\left[{}^{14+15}\mathrm{NH}_4^+\right]}{dt} = \mathrm{production} - \mathrm{consumption}$$

$$\frac{d\left[{}^{15}\mathrm{NH}_4^+\right]}{dt} = \mathrm{consumption} \cdot \frac{\left[{}^{15}\mathrm{N}\right]}{\left[{}^{14+15}\mathrm{N}\right]}$$

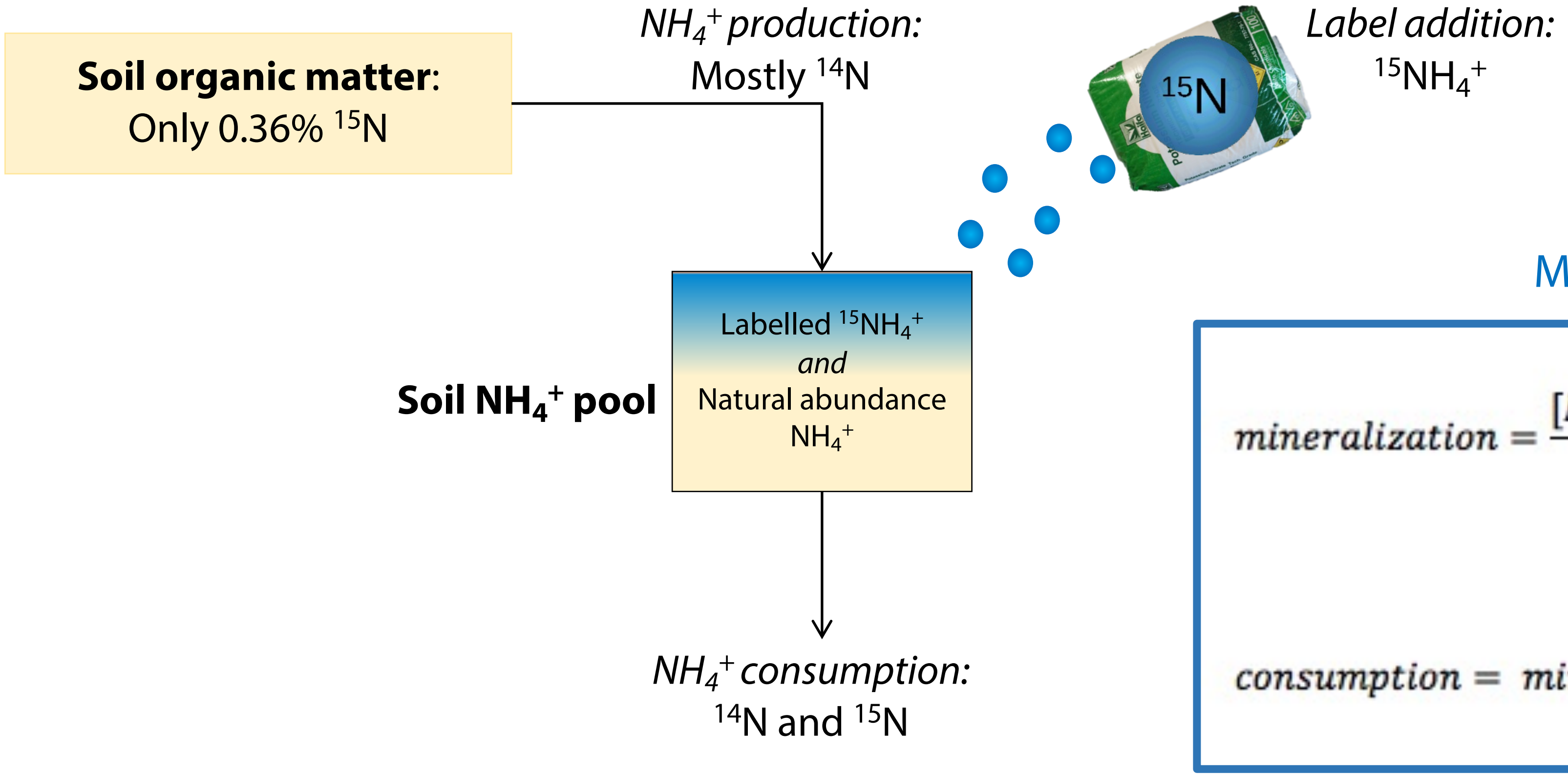
→ *two equations with two unknowns*

Important assumptions:

- ^{15}N behaves the same as ^{14}N
- ^{15}N is uniformly mixed with ^{14}N

Kirkham and Bartholomew (1954)
Schimel (1996)

Isotope pool dilution



Soil organic matter:
Only 0.36% ^{15}N

NH_4^+ production:
Mostly ^{14}N

Label addition:
$^{15}NH_4^+$

Soil NH_4^+ pool

Labelled $^{15}NH_4^+$
and
Natural abundance NH_4^+

NH_4^+ consumption:
^{14}N and ^{15}N

Mathematical solution:

$$mineralization = \frac{[NH_4^+]_0 - [NH_4^+]_t}{t} \times \frac{\log(A_0/A_t)}{\log([NH_4^+]_0/[NH_4^+]_t)}$$

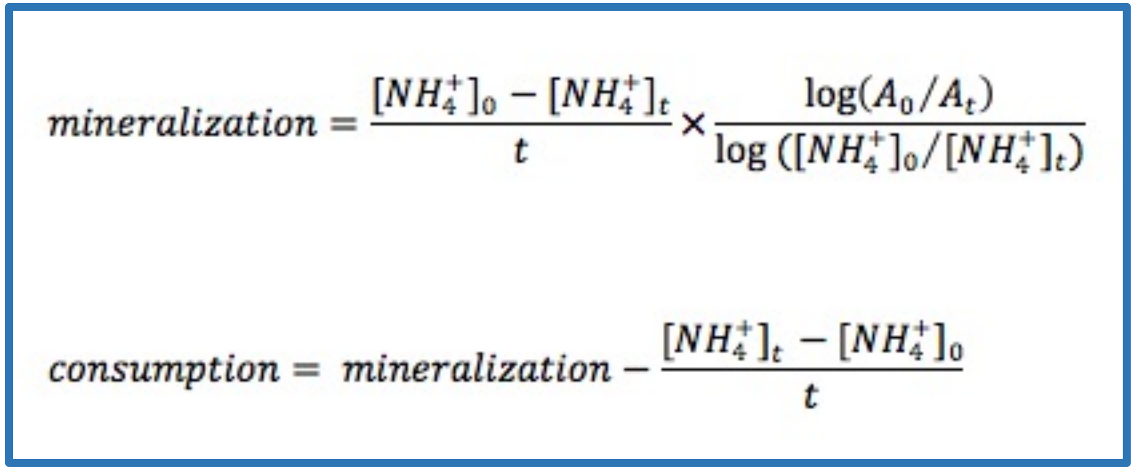
$$consumption = mineralization - \frac{[NH_4^+]_t - [NH_4^+]_0}{t}$$

Where A = ^{15}N atom-% excess in NH_4^+ at times *0* and *t*

Kirkham and Bartholomew (1954)
Schimel (1996)

Example: Isotope pool dilution

- At time = 0 days:
  - 10 mg NH_4^+-N/kg soil, at 10 at-% enrichment
- At time = 2 days:
  - 12 mg NH_4^+-N/kg soil, at 5 at-% enrichment
- ^{15}N natural abundance = 0.36% ^{15}N
- Can you find the rate of:
  - Production?
  - Consumption?
  - Net change in NH_4^+ concentration?



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- At time = 2 days:
  - 12 mg NH_4^+-N/kg soil, at 5 at-% enrichment
- ^{15}N natural abundance = 0.36% ^{15}N
- Can you find the rate of:
  - Production? $\text{Production} = \frac{10-12}{2} \cdot \frac{\log\left(\frac{10-0.36}{5-0.36}\right)}{\log\left(\frac{10}{12}\right)} \doteq$ 4 mg N/kg soil/day
  - Consumption? $\text{Consumption} = 4 - \frac{12-10}{2} =$ 3 mg N/kg soil/day
  - Net change in NH_4^+ concentration? 1 mg N/kg soil/day

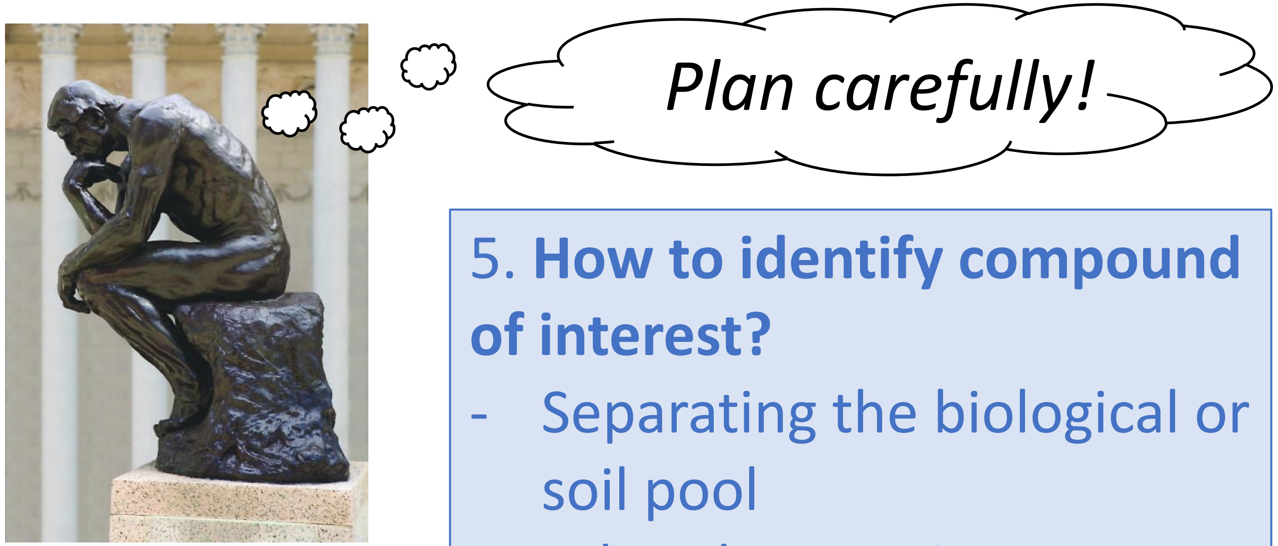
Considerations for labelling experiments

1. **Is there an isotopically enriched version available for my compound of interest?**

2. **How much label to add?**
- Consider original N content of pools
- Magnitude of N fluxes
- Detection limit
- Sampling interval
- **Cost:** Labelled compounds expensive!

3. **Will label addition at this level perturb the N cycle?**

4. **Uniform mixing with the native pool**
- Especially challenging for trace N amounts or for large or disperse pools
- Consider incubation-scale experiments



5. **How to identify compound of interest?**
- Separating the biological or soil pool
- What does my instrument specifically measure?

Break…

- Any questions so far?

Goals of this lecture

- To understand the background and future of the **global nitrogen cycle**
  - Inputs, transport, transformation
- To conceptually understand stable N isotopes and their applications
- To compare and contrast **two complementary isotopic approaches**
  - ^{15}N labelling
  - Natural abundance
- To investigate further applications of ^{15}N to understand the global N cycle

Mixing of isotopic pools

$$\delta_{mix} = f_1 \times \delta_1 + f_2 \times \delta_2$$

$$\delta_{mix} = \frac{m_1 \times \delta_2 + m_2 \times \delta_2}{m_1 + m_2}$$

- *What does a bear eat?*

	%N	**δ^{15}N (‰)**
Salmon (1)	14	10
Plants (2)	1	0
Bear hair (mix)		8.4

Example from Fry (2007) Stable Isotope Ecology

Mixing of isotopic pools

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	%N	$\delta^{15}N$ (‰)
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Bear hair (mix)		8.4

- We estimate 5‰ for the bear's diet based on average 3.4‰ trophic fractionation
- We then apply the mixing equation:

$$f_1 = \frac{\delta_{mix} - \delta_2}{\delta_1 - \delta_2}$$

Example from Fry (2007) Stable Isotope Ecology

Mixing of isotopic pools

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- *What does a bear eat?*

	%N	δ¹⁵N (‰)
Salmon (1)	14	10
Plants (2)	1	0
Bear hair (mix)		8.4

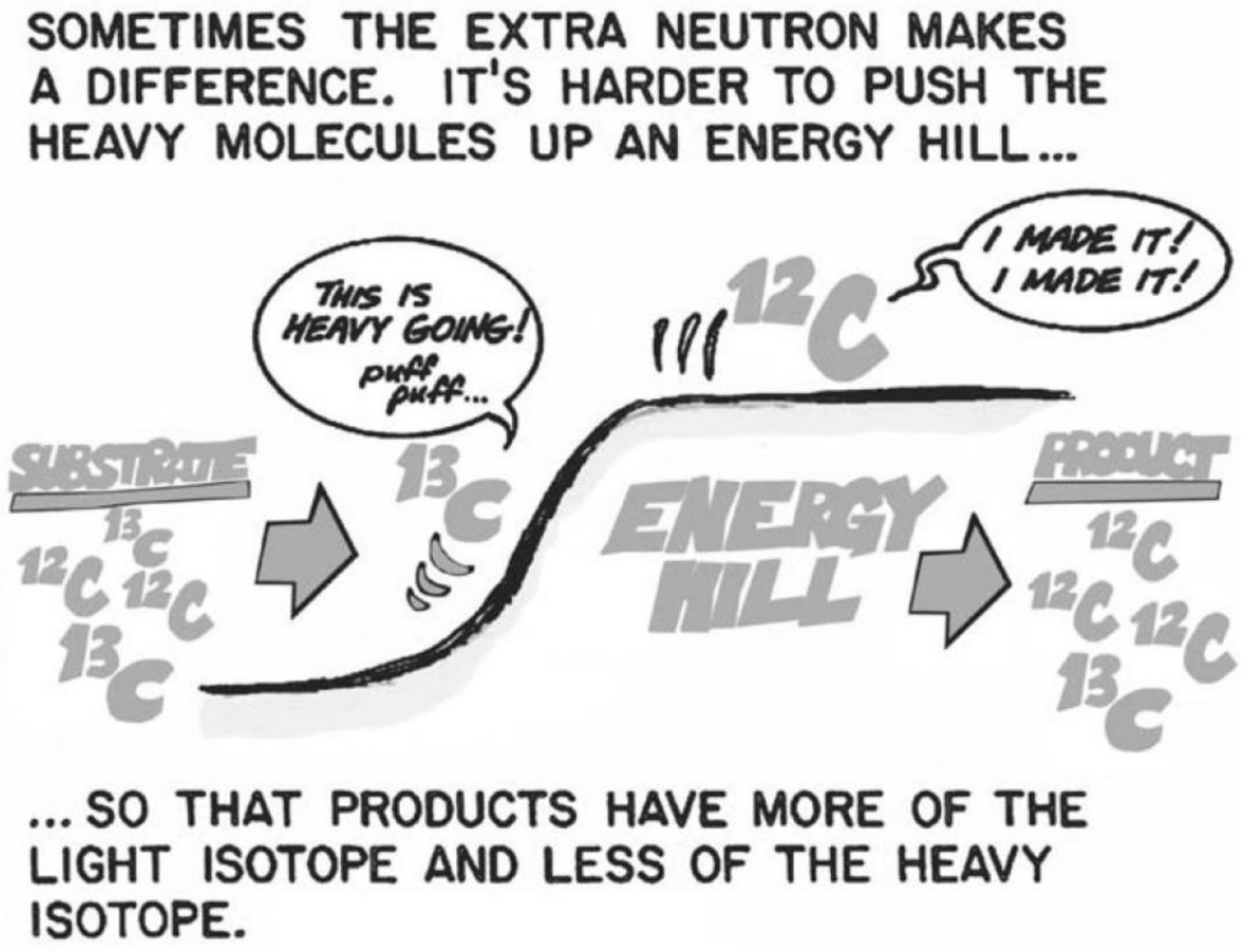
- We estimate 5‰ for the bear's diet based on average 3.4‰ trophic fractionation
- We then apply the mixing equation:

$$f_1 = \frac{\delta_{mix} - \delta_2}{\delta_1 - \delta_2} = \frac{5 - 0}{10 - 0} = 50\%$$

- 50% of the bear's N comes from salmon
- The bear's diet is 14:1 plants:salmon

Example from Fry (2007) Stable Isotope Ecology

Reaction rates differ slightly for isotopes



$$\boldsymbol{\alpha} = \frac{\mathrm{Rate}_{heavy}}{\mathrm{Rate}_{light}}$$

$$\varepsilon\ (‰) = (\alpha - 1) \times 1000$$

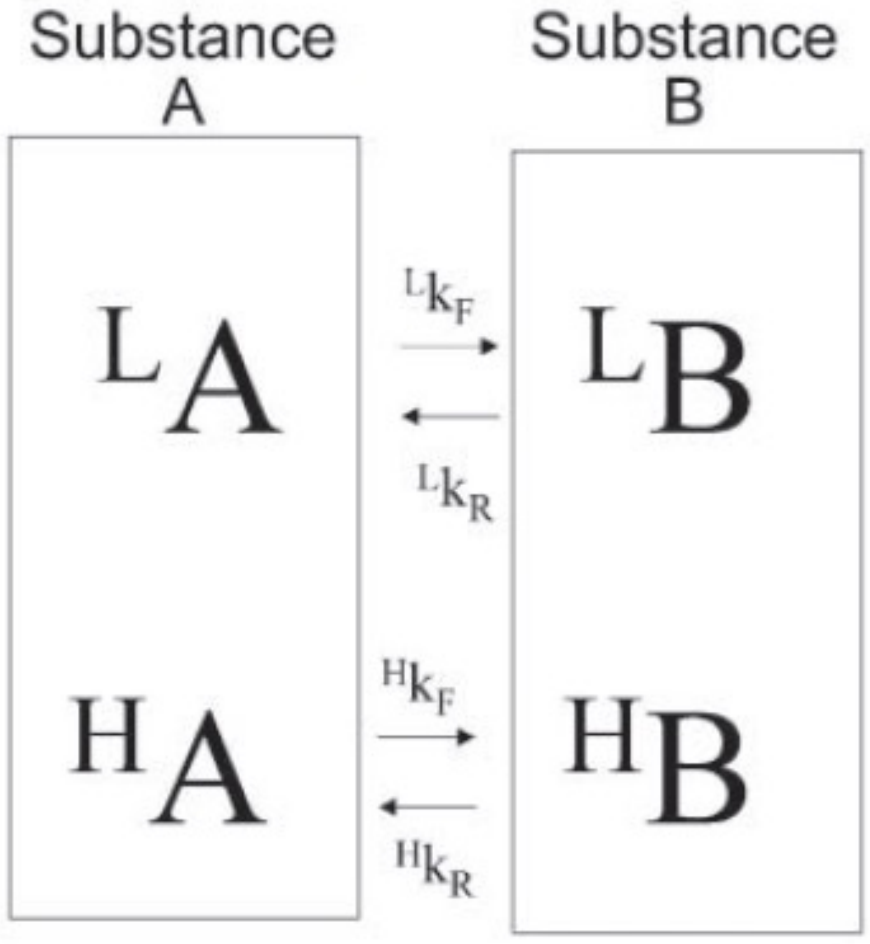
Fry (2007) Stable Isotope Ecology

Equilibrium fractionation

- Equilibrium fractionation results from different rates for forward and backward reactions
- Can be calculated from isotopic composition at equilibrium:

$$\alpha_{EQ} = R_B/R_A$$

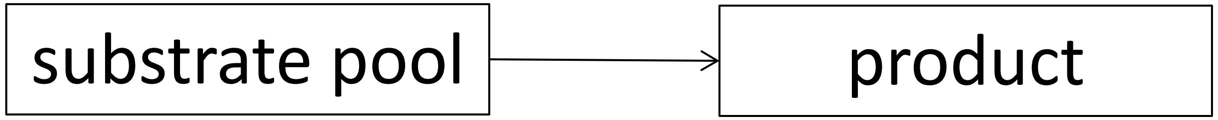
$$= {}^{1000+\delta_B}/_{1000+\delta_A}$$



Note: different scientific fields often report inverse fractionation factors!!

Closed system fractionation

- Substrate is depleted and not replenished

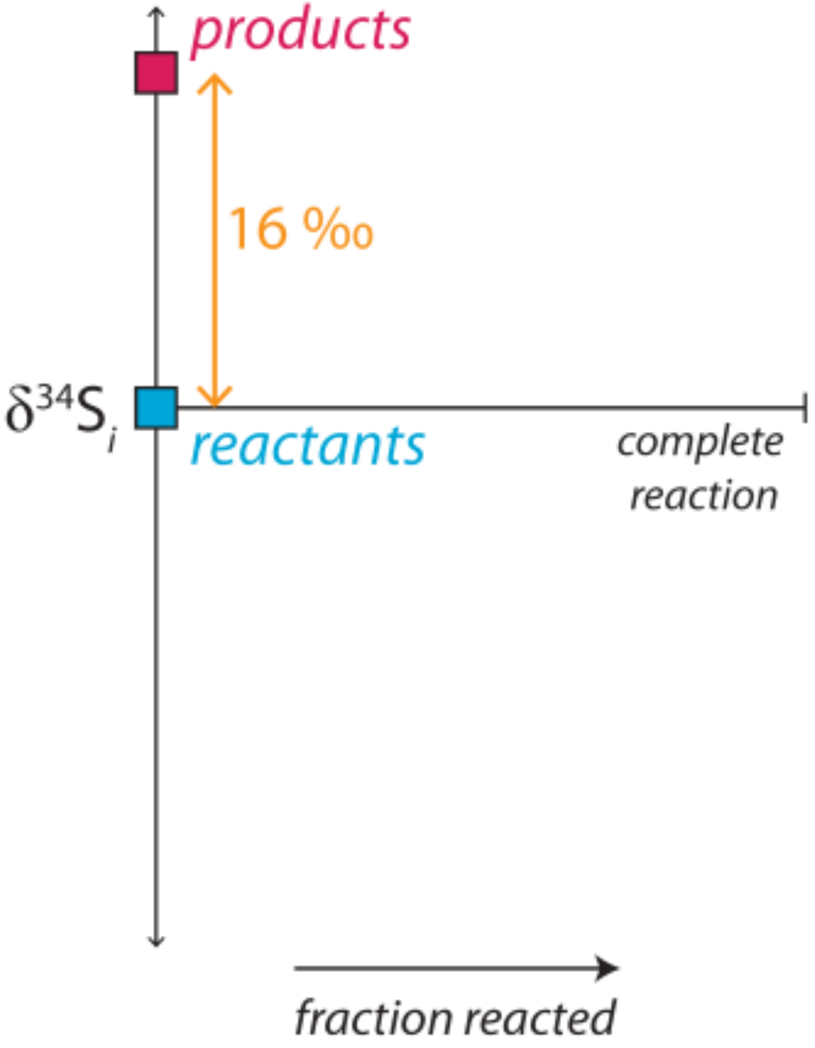

substrate pool → product

Open system fractionation

- Substrate is replenished

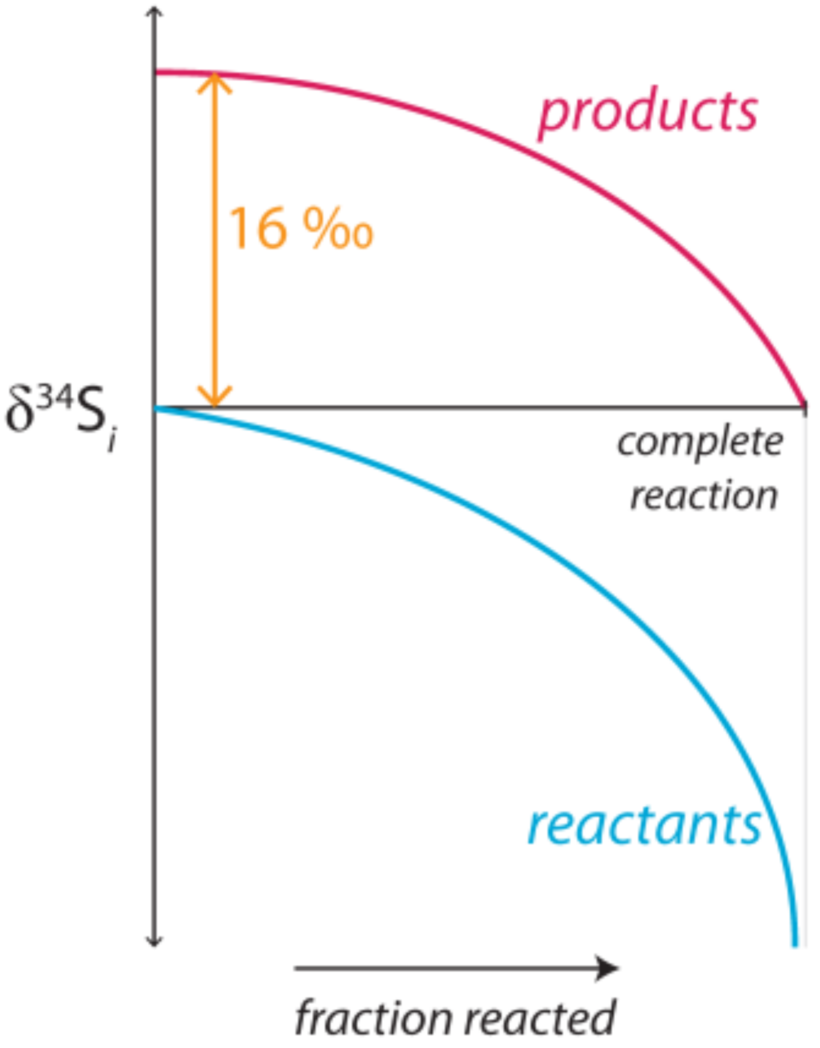

substrate input → substrate pool → product

Closed system fractionation



- "Rayleigh" fractionation
- Initially, $\delta_P = \delta_R + \alpha_{R-P}$ (here $\varepsilon = +16‰$)

Closed system fractionation: Reactants



- "Rayleigh" fractionation
- Initially, $\delta_P = \delta_R + \alpha_{R-P}$
- Mass balance must be maintained, so as *R* is consumed its isotopic composition changes

$$\frac{R_S}{R_{S,0}} = f^{\alpha-1} \qquad \delta_S \approx \delta_{S,0} + \varepsilon(\ln f)$$

- *f* is the fraction of unreacted substrate c/c_0

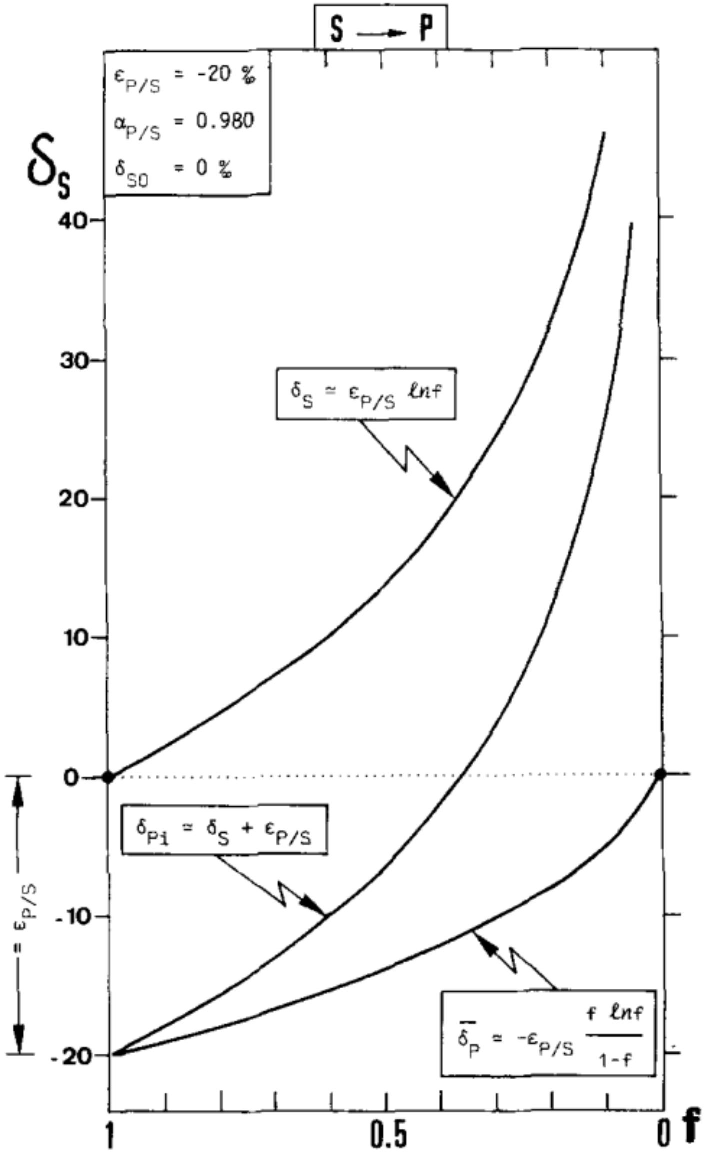
Equations: Mariotti et al. (1981) Plant and Soil

Closed system fractionation: Products

- Instantaneous product: $\delta_{Pi} = \delta_S + \varepsilon$
- However, we are usually not interested in the instantaneous product but the **accumulated product:**

$$\frac{R_P}{R_{S,0}} \approx \frac{1 - f^{\alpha}}{1 - f}$$

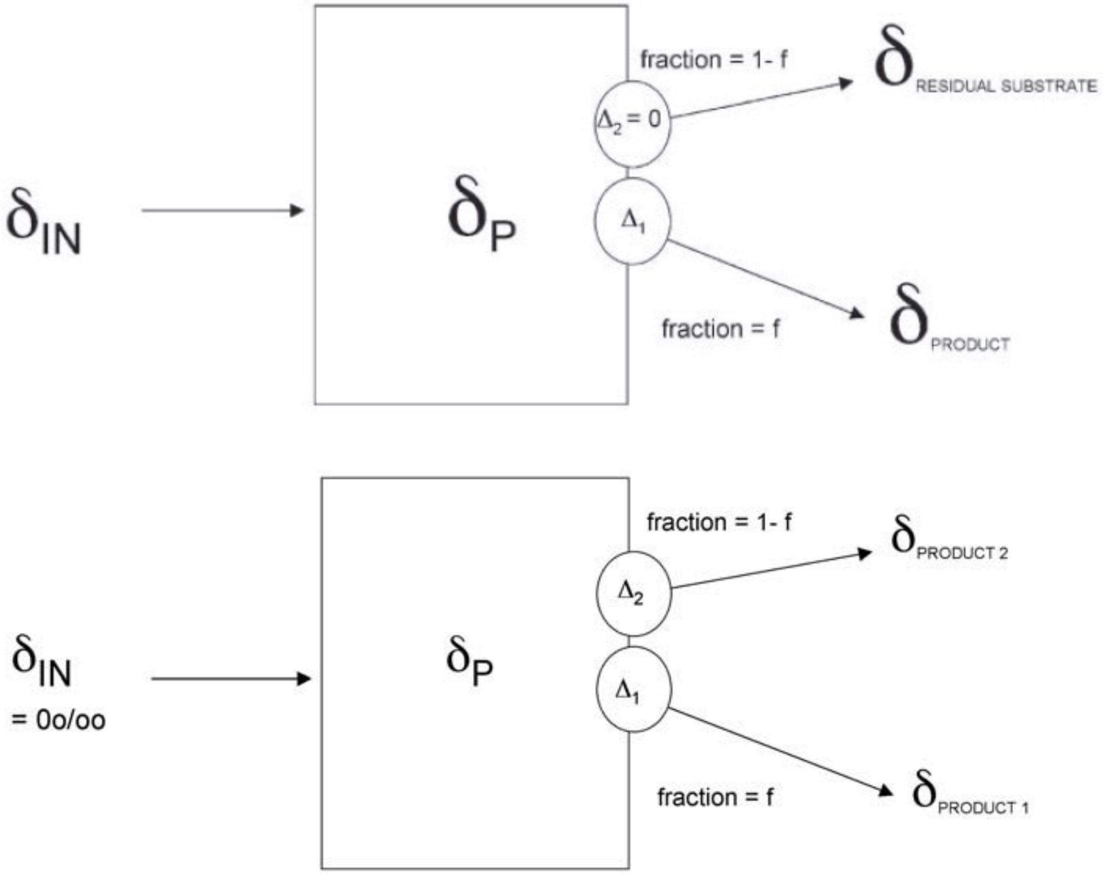
$$\delta_P \approx \delta_{S,0} - \varepsilon\left(\frac{f \ln f}{1 - f}\right)$$



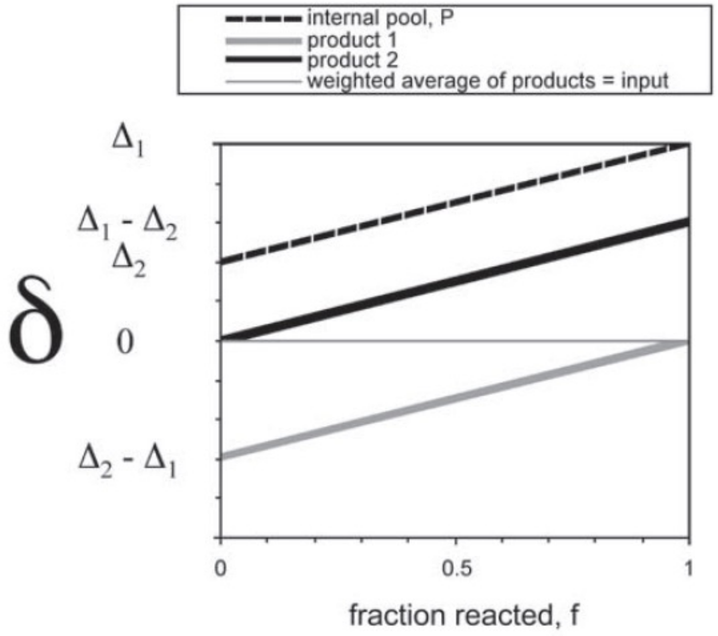
Equations, figure: Mariotti et al. (1981) Plant and Soil

Open system fractionation

- Often in ecology a system is not completely closed, but has an input flow:



- Equations get much more complicated: see p. 244 of Stable Isotope Ecology (Fry, 2007)



Open system fractionation: Example

- Nitrification is taking place in crop soil at a rate of 5 mg-N kg-soil^{-1} d^{-1}
- The incoming NO_3^- has an isotopic composition of -10 ‰
- NO_3^- is consumed by denitrification with $\varepsilon = -25$ ‰
- The soil pool (eg. residual) NO_3^- has an isotopic composition of -7.5 ‰
- **What fraction of the NO_3^- is denitrified?**

Open system fractionation: Example

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- The soil pool (eg. residual) NO_3^- has an isotopic composition of -7.5 ‰
- **What fraction of the NO_3^- is denitrified?**
- *Hint:* We can approximate an open system with: $\delta_S \approx \delta_{S0} - \varepsilon(1 - f)$
  - Transform the equation to solve for *f*
  - What is *f*, the fraction denitrified?

Open system fractionation: Example

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- The incoming NO_3^- has an isotopic composition of -10 ‰
- NO_3^- is consumed by denitrification with ε = -25 ‰
- The soil pool (eg. residual) NO_3^- has an isotopic composition of -7.5 ‰
- **What fraction of the NO_3^- is denitrified?**
- *Hint:* We can approximate an open system with: $\delta_S \approx \delta_{S0} - \varepsilon(1-f)$
  - $f = 1 - \frac{\delta_{S0} - \delta_S}{\varepsilon} = 1 - \frac{-10 - (-7.5)}{-25} = \mathbf{0.9}$
  - **90% of NO_3^- remains, 10% of NO_3^- is denitrified**

Comparing open and closed systems

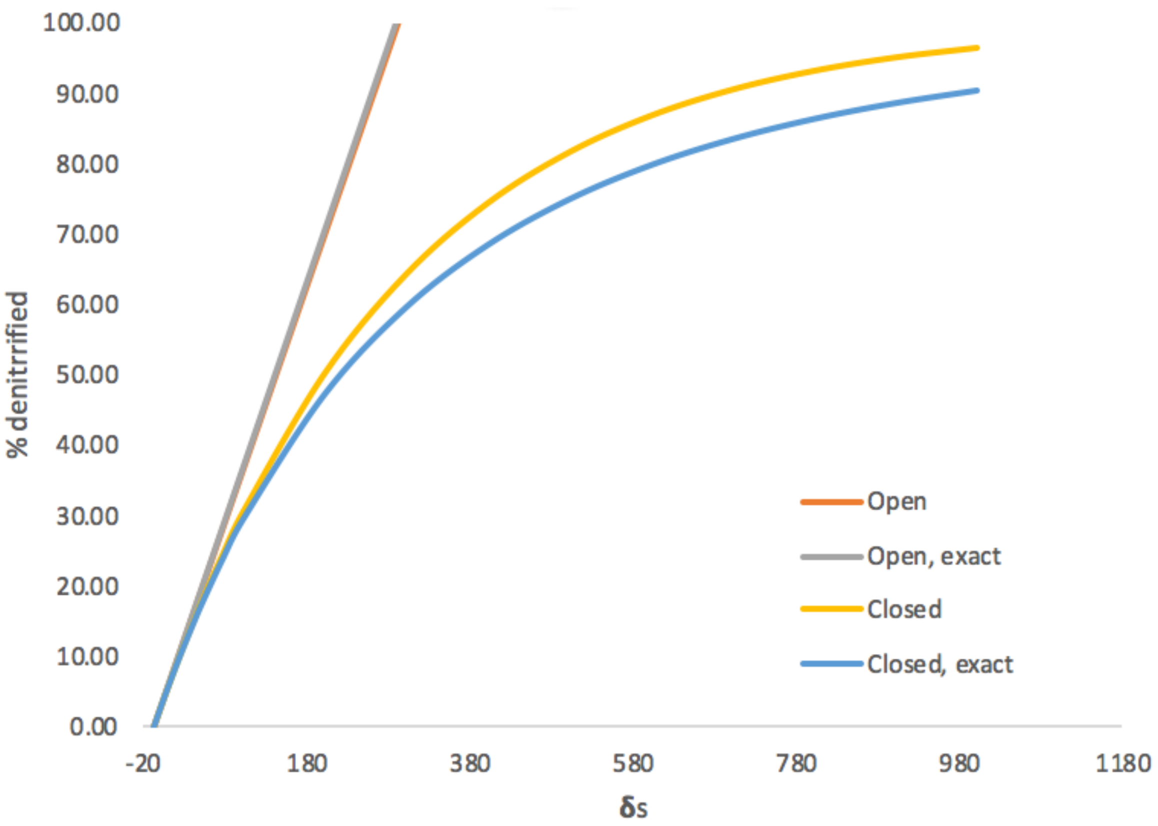
- How much denitrification would we calculate in a closed system?
- This would assume NO_3^- inputs become "isolated" eg. in a cell or soil pore space and do not mix with incoming NO_3^- once denitrification occurs
- In a closed system: $\delta_S \approx \delta_{S,0} + \varepsilon(\ln f)$
- $f = e^{\delta_S - \delta_{S0}/\varepsilon} = e^{\frac{-7.5 - (-10)}{-25}} = 0.905$
- 9.5% is denitrified, 90.5% remains
- Closed and open system approaches are basically equivalent at low consumption rates

Comparing open and closed systems

δ_S (‰)	f_D, open system, *approximation*	f_D, open system	f_D, closed system, *approximation*	f_D, closed system
Equation	$\delta_S \approx \delta_{S0} - \varepsilon(1-f)$	$\frac{R_S}{R_{S,0}} = \frac{1-f}{\alpha} + f$	$\delta_S \approx \delta_{S,0} + \varepsilon(\ln f)$	$\frac{R_S}{R_{S,0}} = f^{\alpha-1}$
-7.5	10 %	10.1 %	9.5 %	9.6 %
0	40 %	40.4 %	32.97 %	33.10 %
10	80 %	80.81 %	55.07 %	55.07 %
14.5	98 %	98.99 %	62.47 %	62.39 %
50	240 %	242.4 %	90.93 %	90.50 %
200	840 %	858.5 %	99.98 %	99.95 %

Comparing open and closed systems

- The previous slide showed a big difference between open and closed system calculations but a small error due to approximations



- However, if we calculate again with a large fractionation factor (**ε = -300 ‰**) we get a significant difference for the closed system

1. Be careful in choosing an open or closed system approach
2. Use exact equations where possible

Open or closed system fractionation?

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$$

- Is reduction a “closed system”?
  - Within a cell or soil pore
  - Stepwise

$$NO_3^- \rightarrow NO_2^- \boxed{\rightarrow N_2O \rightarrow N_2}$$

- Is reduction an “open system”?
  - N_2O replenished
  - Production and reduction simultaneous

$$NO_3^- \boxed{\rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2}$$

- This is a topic of hot debate in the scientific literature; probably a mix!

Natural abundance vs. tracer approaches

- **Tracer approach** allows us to “follow” the fate of N substances
- Quantitative info on N transformation and sources
- Experiment (N addition) always disturbs the system
- Usually conducted in the lab
- ^{15}N tracer is expensive

- **Natural abundance** gives integrated information on turnover and processes in space and time
- Easy to combine with other isotopes
- Does not disturb the system
- Often conducted in the field
- Calculations difficult, need a lot of information on fractionation factors
- Needs expensive, high precision instrumentation

Goals of this lecture

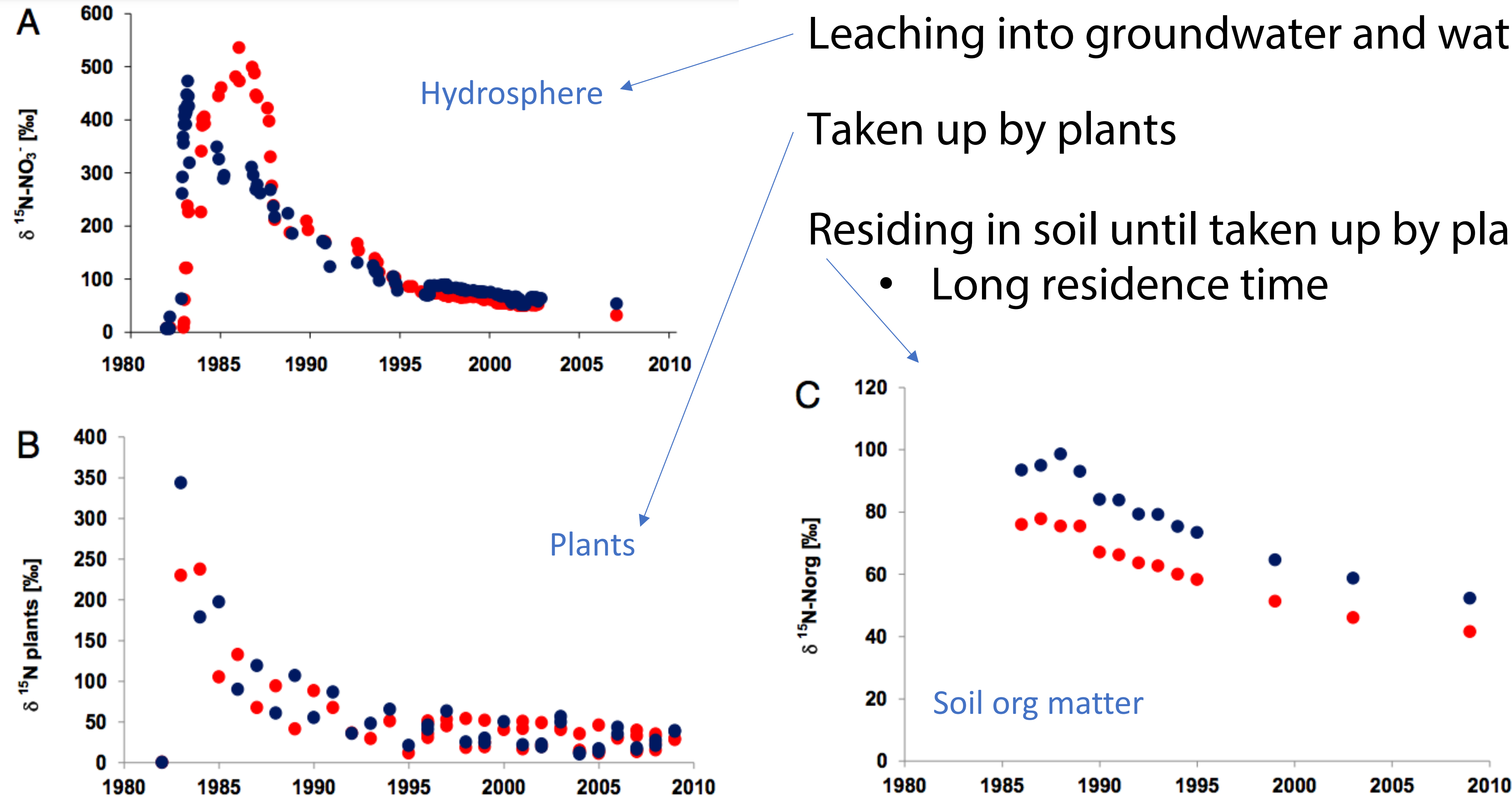
- To understand the background and future of the **global nitrogen cycle**
  - Inputs, transport, transformation
- To conceptually understand stable N isotopes and their applications
- To compare and contrast **two complementary isotopic approaches**
  - ^{15}N labelling
  - Natural abundance
- To investigate further applications of ^{15}N to understand the global N cycle

What is the long-term fate of fertilizer N?

- NO_3^- contamination of aquatic ecosystems is a major problem resulting from fertilizer use in agriculture
- Transit times of fertilizer in soil/freshwater systems are poorly understood
- Sebilo et al. (2013)[1] conducted a **three-decade long *in situ* tracer experiment** with labelled fertiliser
    - 1982: 1.8 L of a $K^{15}NO_3$ solution with isotopic excess in ^{15}N = 3.87% was sprinkled uniformly on the surface of two lysimeters
    - The tracer amount added was equivalent to a typical fertilizer application rate of 120 and 150 kg N ha^{-1} y^{-1} for winter wheat and sugar beet crops
    - Cultivated and monitored over the next 30 years

[1] 2013, Proceedings of the National Academy of Sciences (PNAS)

What is the long-term fate of fertilizer N?



Leaching into groundwater and water bodies

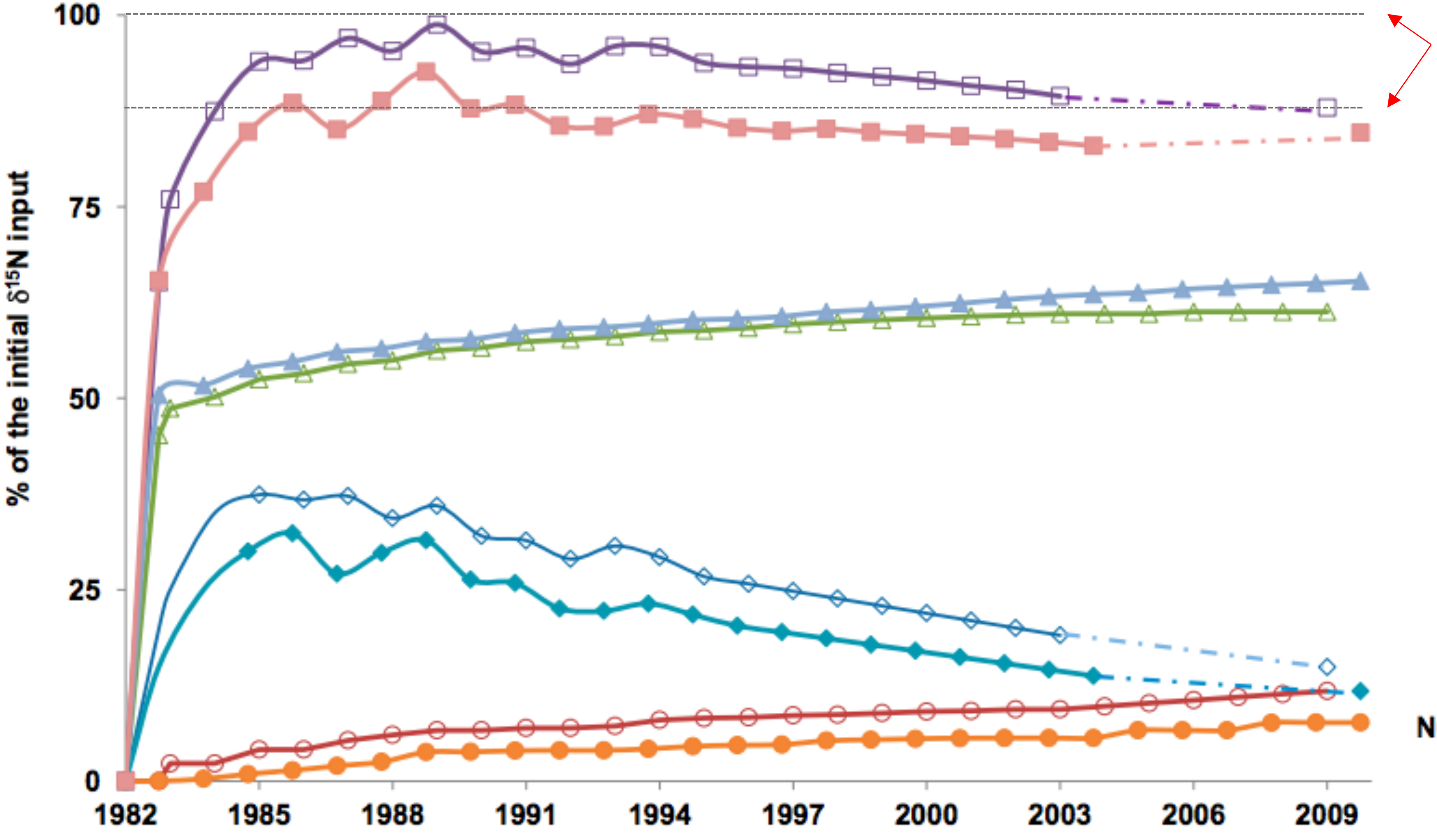
Taken up by plants

Residing in soil until taken up by plants or leached

- Long residence time

Sebilo et al. (2013) PNAS

What is the long-term fate of fertilizer N?



Deficit = gaseous loss

Total = 88 – 95%

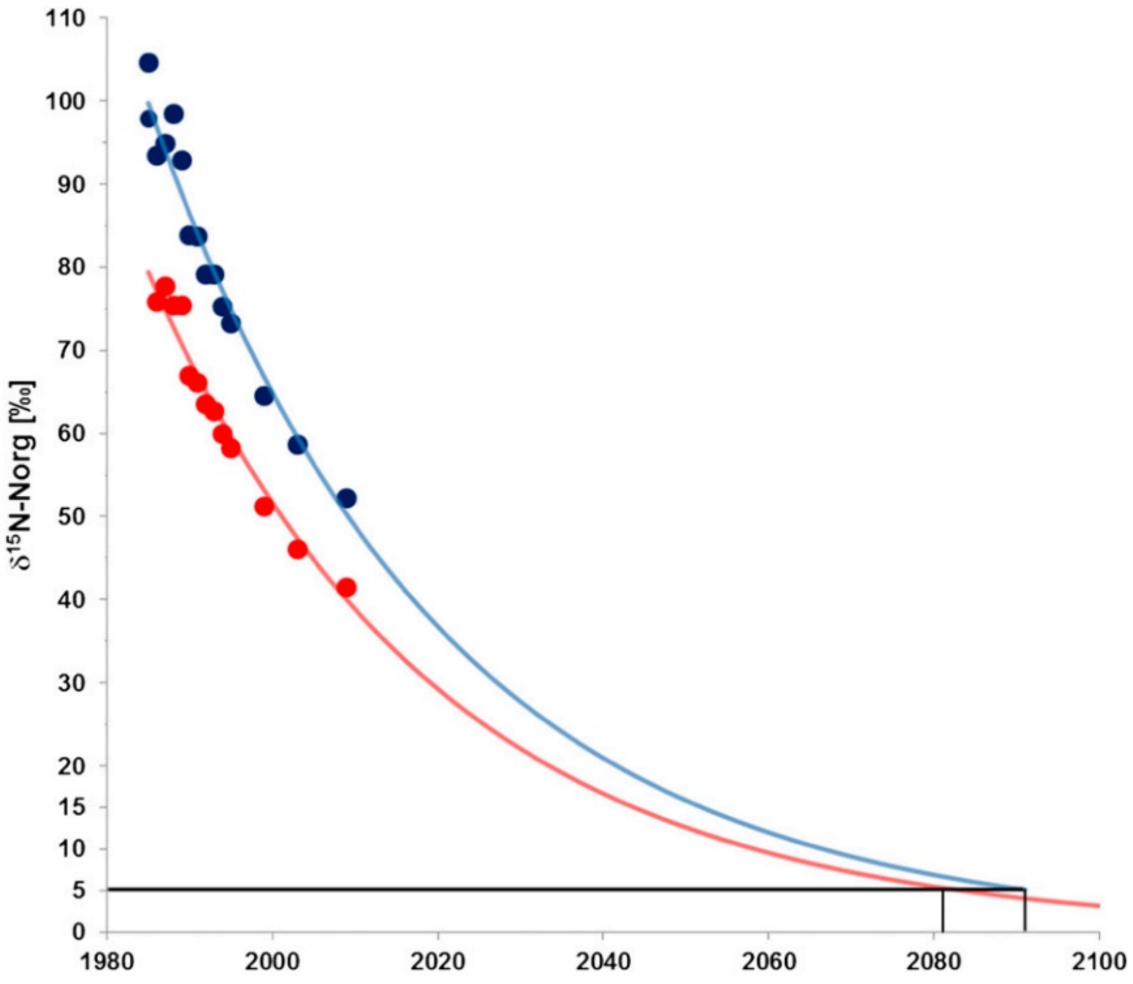
Plants = 60 – 65%

SOM = 12 – 15%

NO_3^- leaching = 8 – 12%

Sebilo et al. (2013) PNAS

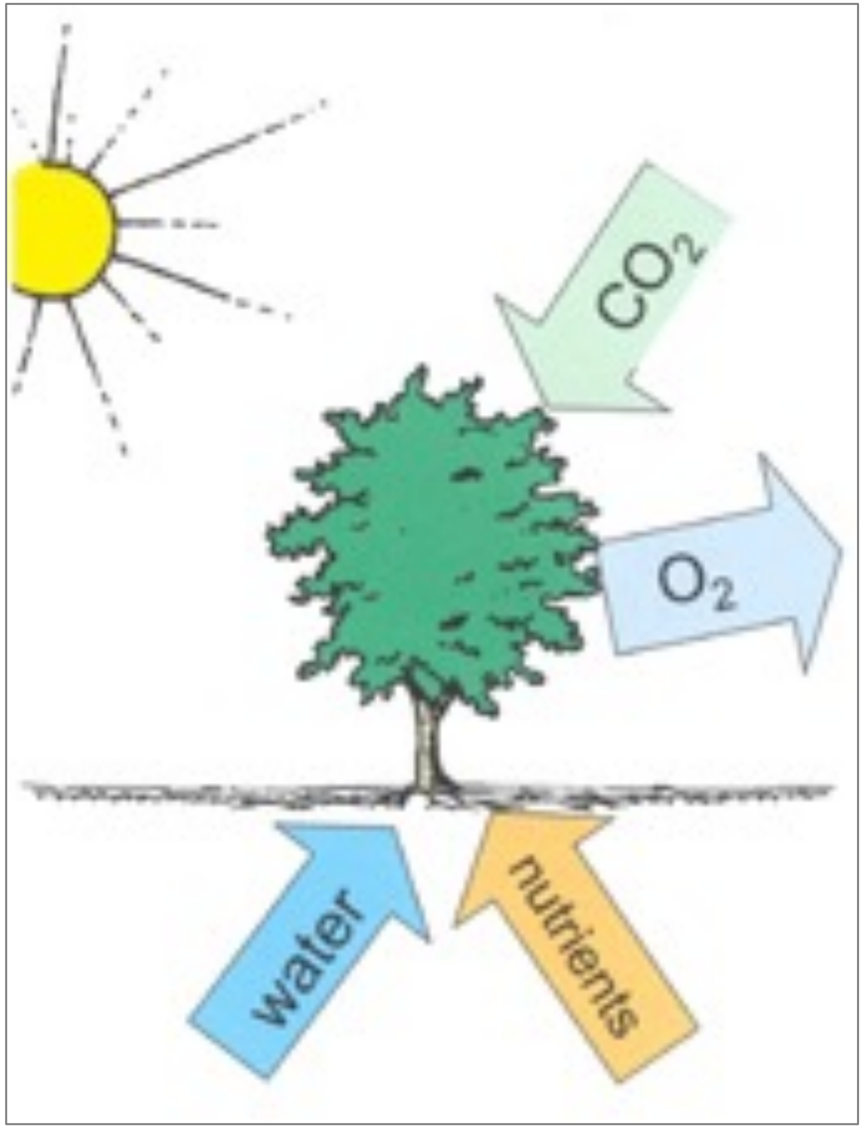
What is the long-term fate of fertilizer N?

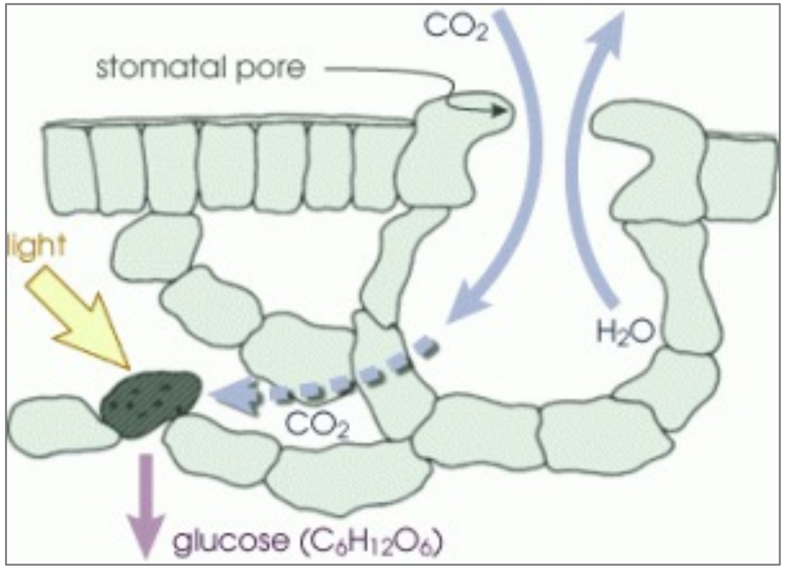


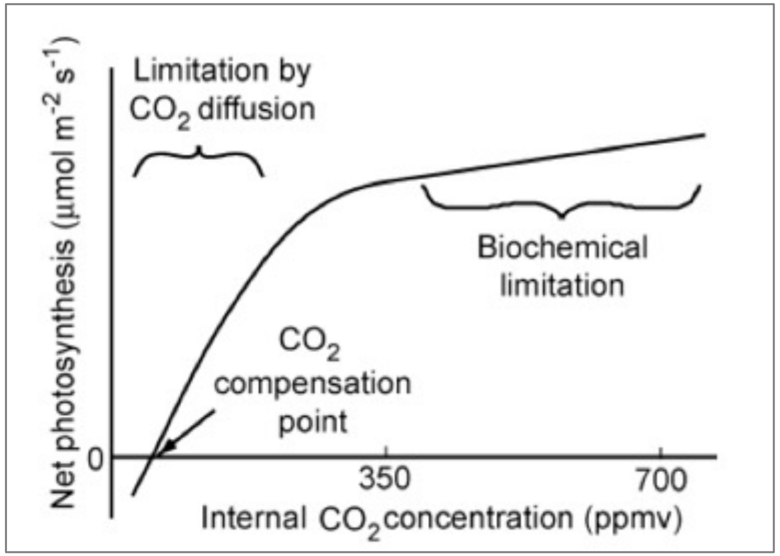
- Tracer decay suggests the fertilizer effects will remain for <100 years
- Attempts to reduce eutrophication need to consider legacy fertilizer use
- Later results show that increasing biological nitrogen demand (eg. plants take up more N) with sustainable cropping systems can shorten the lifetime of nitrogen fertilizer in the environment

Sebilo et al. (2013) PNAS; Castellano and David (2014) PNAS

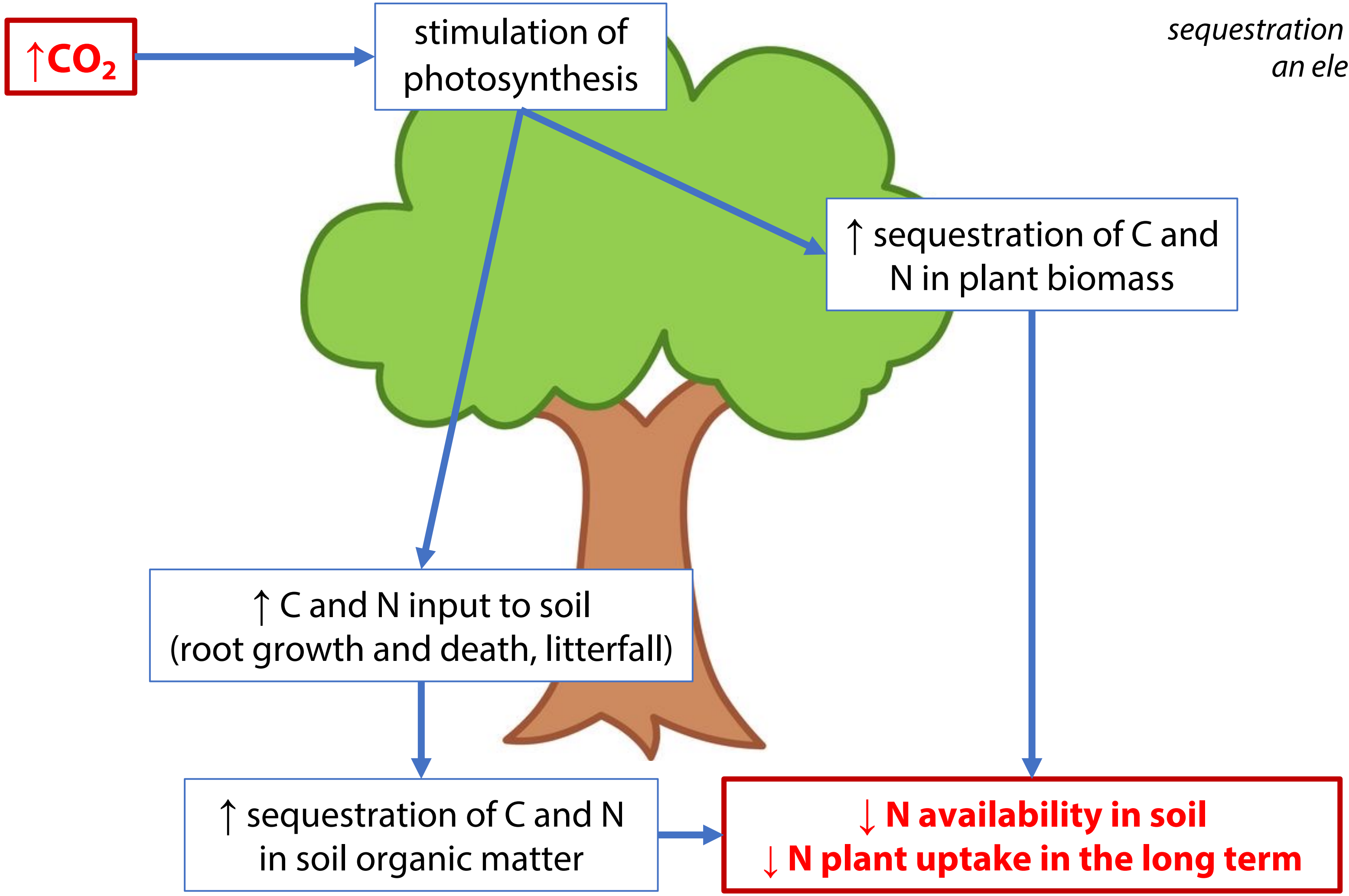
The effect of elevated CO_2 on N cycling







The Progressive N limitation (PNL) hypothesis



↑CO_2

stimulation of photosynthesis

↑ sequestration of C and N in plant biomass

↑ C and N input to soil
(root growth and death, litterfall)

↑ sequestration of C and N in soil organic matter

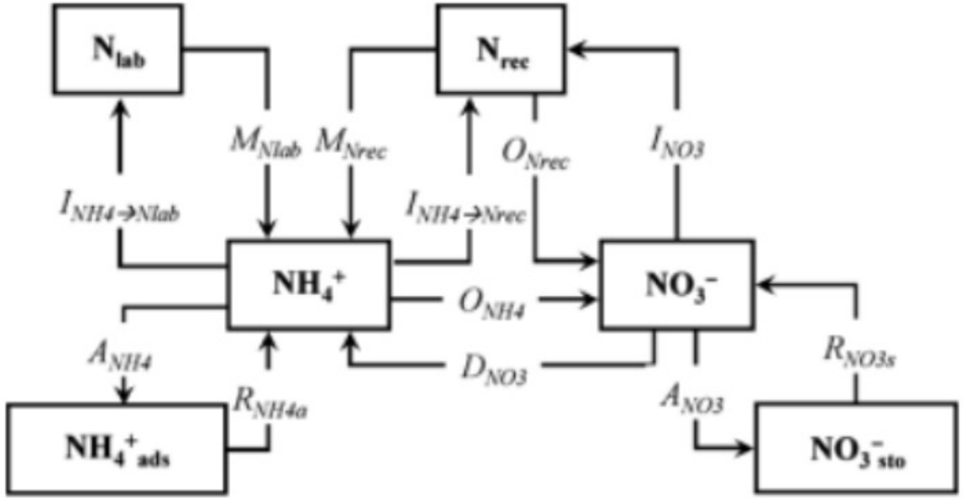
↓ N availability in soil
↓ N plant uptake in the long term

sequestration = trapping of an element

Luo *et al.* 2004. *BioScience*

The Progressive N limitation (PNL) hypothesis

- Rutting et al.[1] conducted a ^{15}N tracer study on a sheep-grazed pasture
- The site had previously been exposed to *10 years of CO_2 enrichment*
- $^{14}NH_4{}^{15}NO_3$, $^{15}NH_4{}^{14}NO_3$ and $^{15}NH_4{}^{15}NO_3$ were added to the site to compare ambient and elevated CO_2 treatments
- A numerical ^{15}N tracing model from Müller et al. (2007) GCB incorporating **6 N pools and 12 N processes** was fitted with an MC algorithm



[1] Rütting et al. (2010) Global Change Biology

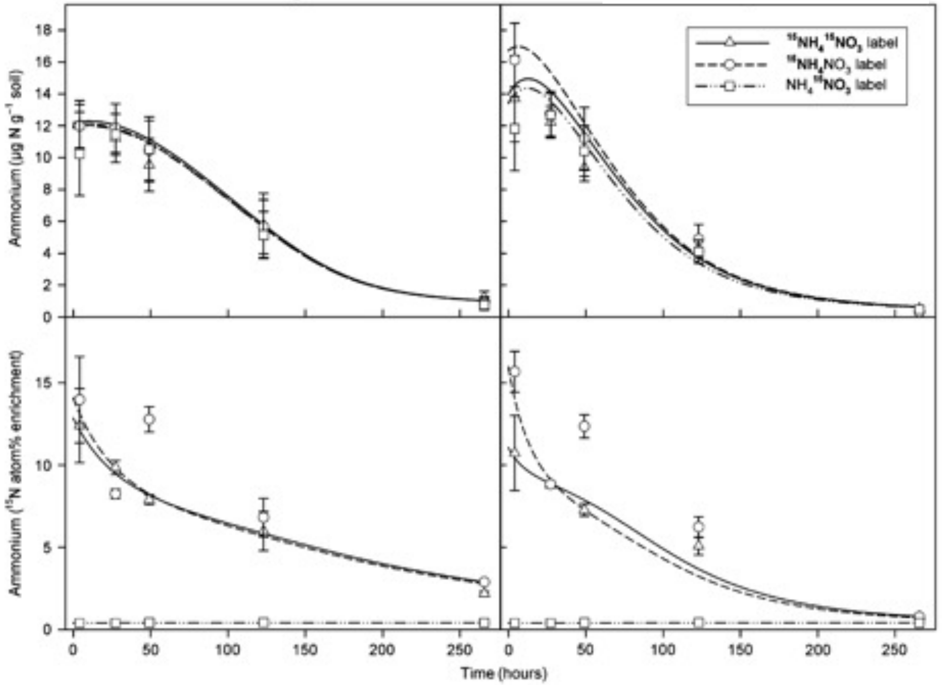
Model fit to measurement data

concentration

δ15N

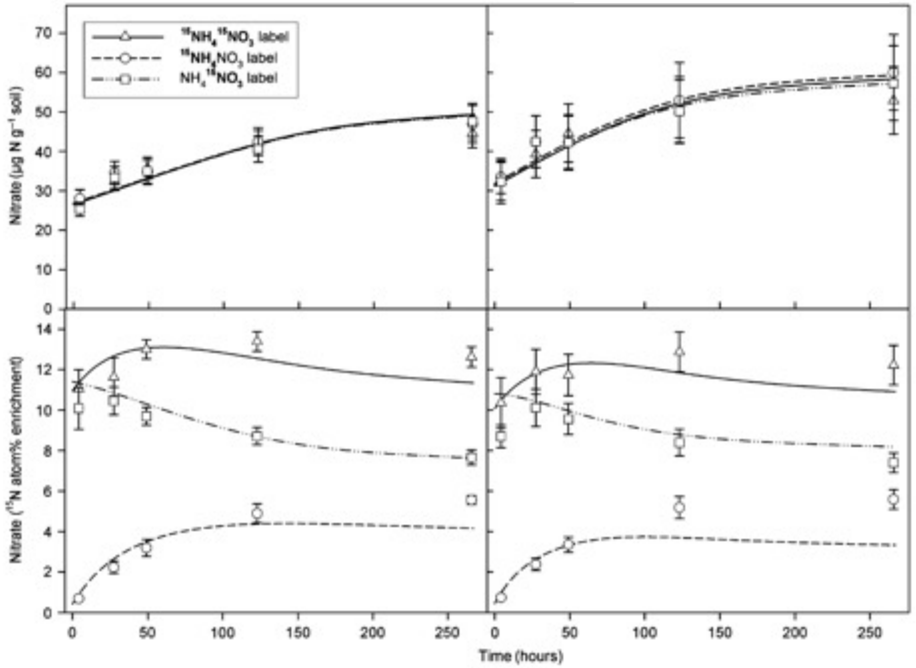
NH_4^+

Ambient CO_2 Elevated CO_2



NO_3^-

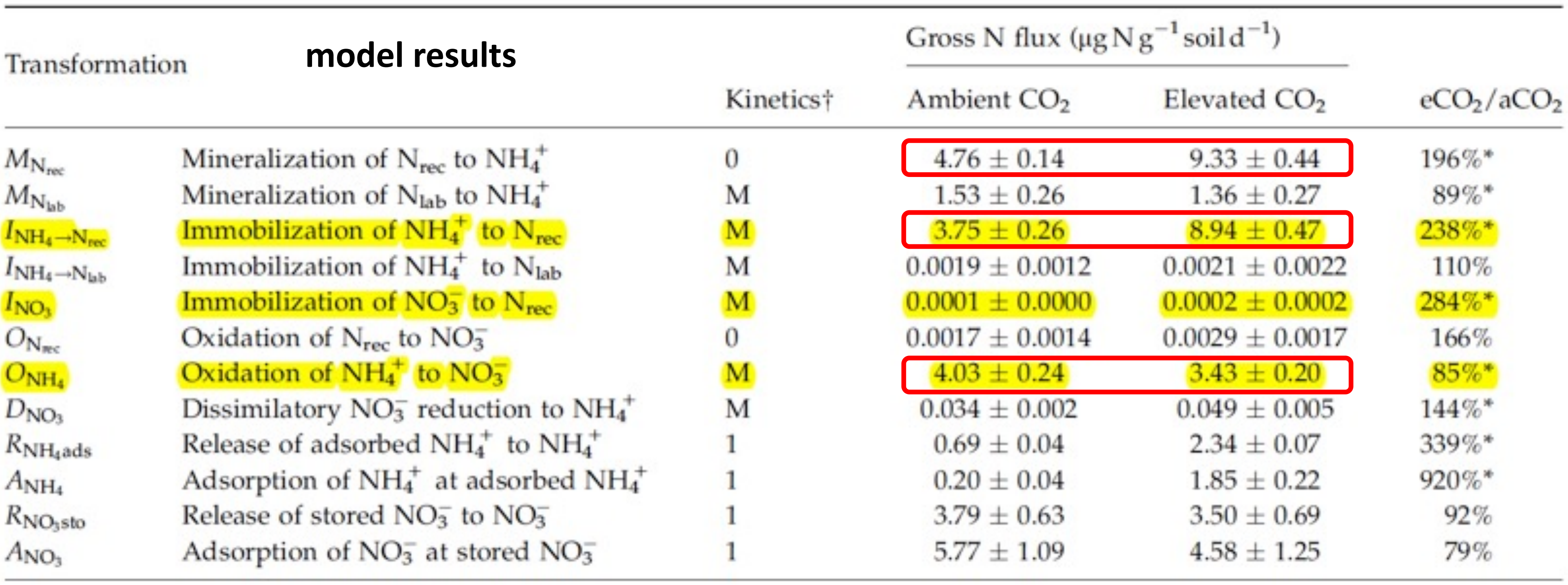
Ambient CO_2 Elevated CO_2



Rütting et al. (2010) Global Change Biology

Does Progressive Nitrogen Limitation occur?

Rütting et al. (2010) Global Change Biology

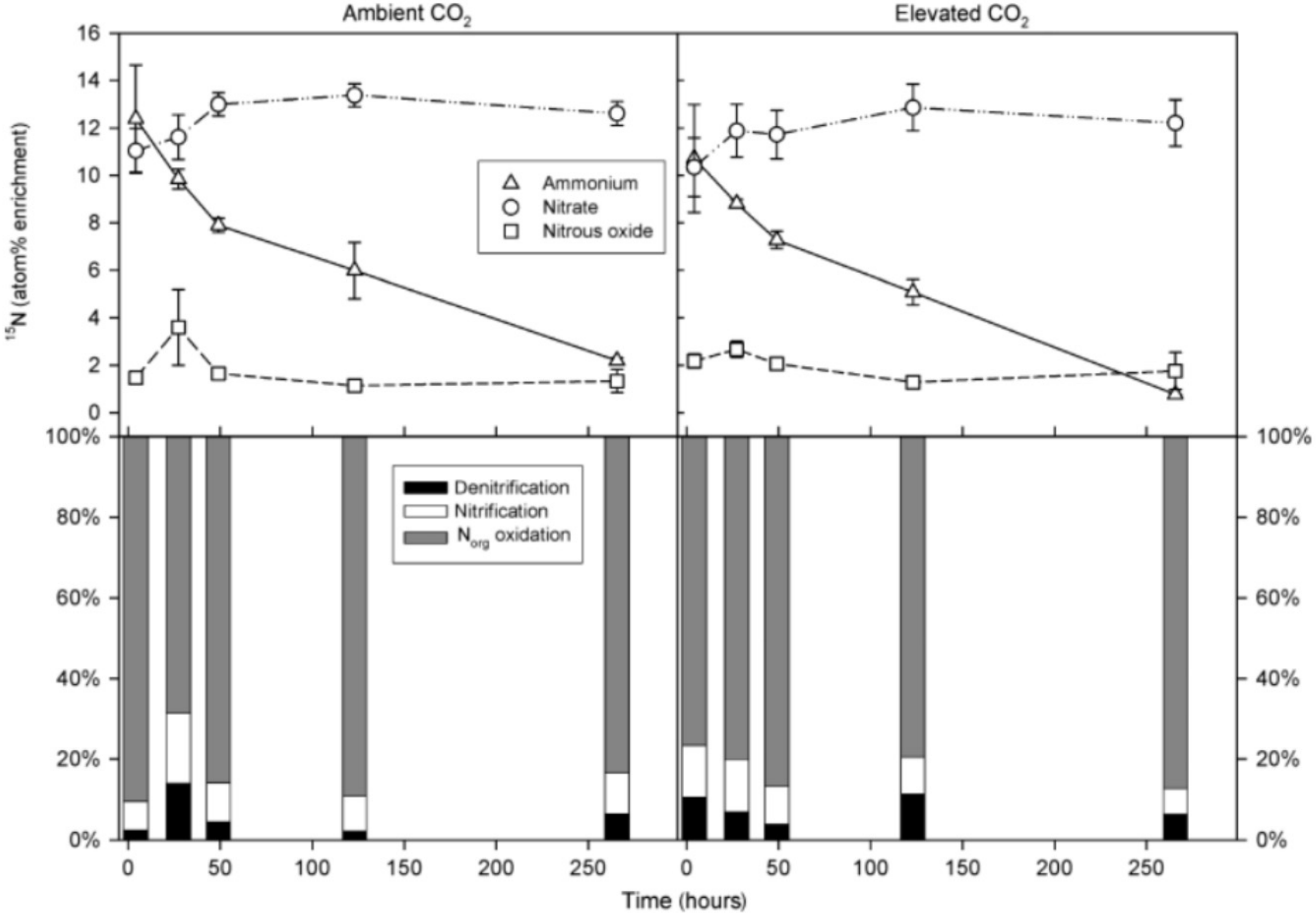


model results

Transformation		Kinetics†	Gross N flux ($\mu g\,N\,g^{-1}\,soil\,d^{-1}$)		
			Ambient CO_2	Elevated CO_2	eCO_2/aCO_2
$M_{N_{rec}}$	Mineralization of N_{rec} to NH_4^+	0	4.76 ± 0.14	9.33 ± 0.44	196%*
$M_{N_{lab}}$	Mineralization of N_{lab} to NH_4^+	M	1.53 ± 0.26	1.36 ± 0.27	89%*
$I_{NH_4 \to N_{rec}}$	Immobilization of NH_4^+ to N_{rec}	M	3.75 ± 0.26	8.94 ± 0.47	238%*
$I_{NH_4 \to N_{lab}}$	Immobilization of NH_4^+ to N_{lab}	M	0.0019 ± 0.0012	0.0021 ± 0.0022	110%
I_{NO_3}	Immobilization of NO_3^- to N_{rec}	M	0.0001 ± 0.0000	0.0002 ± 0.0002	284%*
$O_{N_{rec}}$	Oxidation of N_{rec} to NO_3^-	0	0.0017 ± 0.0014	0.0029 ± 0.0017	166%
O_{NH_4}	Oxidation of NH_4^+ to NO_3^-	M	4.03 ± 0.24	3.43 ± 0.20	85%*
D_{NO_3}	Dissimilatory NO_3^- reduction to NH_4^+	M	0.034 ± 0.002	0.049 ± 0.005	144%*
R_{NH_4ads}	Release of adsorbed NH_4^+ to NH_4^+	1	0.69 ± 0.04	2.34 ± 0.07	339%*
A_{NH_4}	Adsorption of NH_4^+ at adsorbed NH_4^+	1	0.20 ± 0.04	1.85 ± 0.22	920%*
R_{NO_3sto}	Release of stored NO_3^- to NO_3^-	1	3.79 ± 0.63	3.50 ± 0.69	92%
A_{NO_3}	Adsorption of NO_3^- at stored NO_3^-	1	5.77 ± 1.09	4.58 ± 1.25	79%

- Mineralization-immobilization turnover is increased
- Oxidation and subsequent leaching of ammonium is reduced
- Overall, these mechanisms alleviate progressive nitrogen limitation

How does elevated CO_2 impact N_2O production?

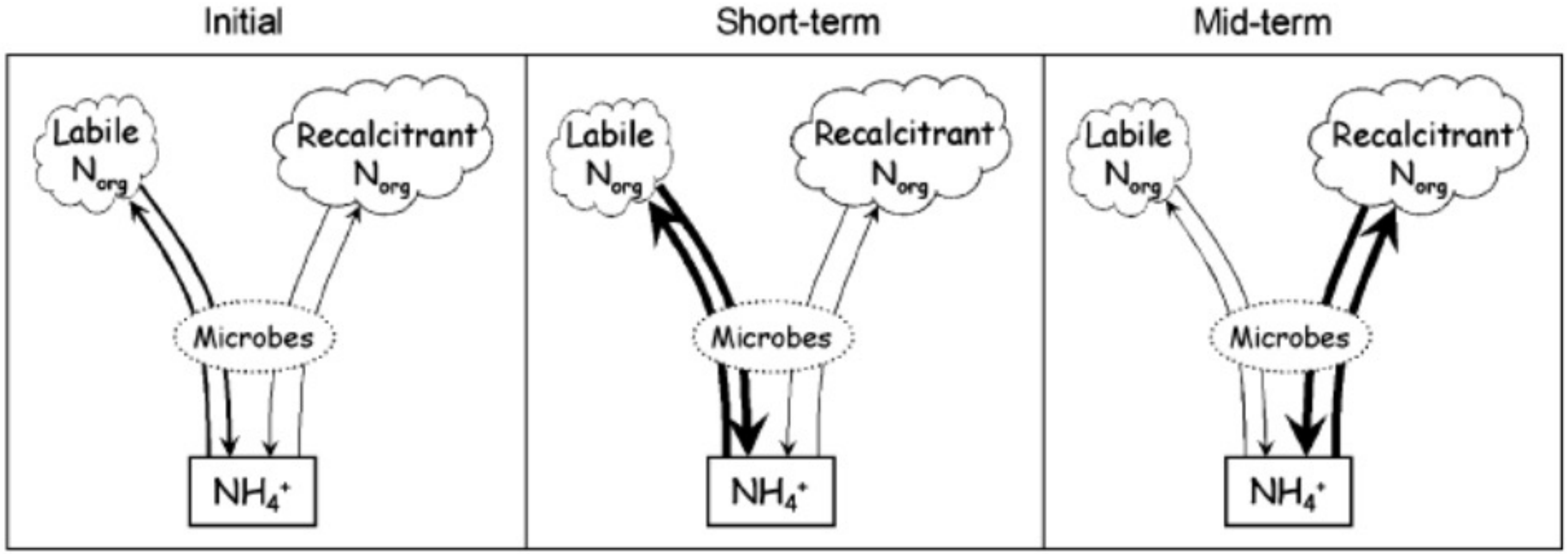


Rütting et al. (2010) Global Change Biology

- Results show more N_2O from organic N under *e*CO_2
- This suggests that in the long-term, more recalcitrant soil organic N is used

Does elevated CO_2 lead to progressive N limitation?

- Results showed that mineralization-immobilization turnover (MIT) was enhanced under elevated CO_2



- Higher MIT and enhanced N retention may alleviate PNL

Conclusions

- Stable nitrogen isotopes are a useful tool to study a range of scientific questions across many different fields
- Before designing a stable isotope study, consider natural abundance and labelling approaches
    - Spatial and temporal scale?
    - Pertubations to the system?
    - Natural abundance isotopic variability?
- Understanding the fate and transformations of N serves for better N usage management and can lead to effective environmental policies
- N is important, but consideration of interactions with other trace elements such as C, P, S, H cycles and etc.
    - Multi-isotope studies can be particularly useful

Thanks for your attention!

Please contact me if you have any questions or comments:

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