Two Broad Classes of Experimental Studies

1. Source Control of Isotope Composition

    Assumption: Fractionation events are not important
    Example: δD of stem water to identify patterns of water use

2. Physiological Control of Isotope Composition

    Assumption: Fractionation events are important
    Example: $\delta^{13}C$ of leaf tissue

$\delta^{15}N$ Research in Ecological Research

^{15}N labeling studies have been popular in agricultural research for decades (ex: pool dilution)

The original hope was that ^{15}N at levels of natural abundance could be used as a natural tracer

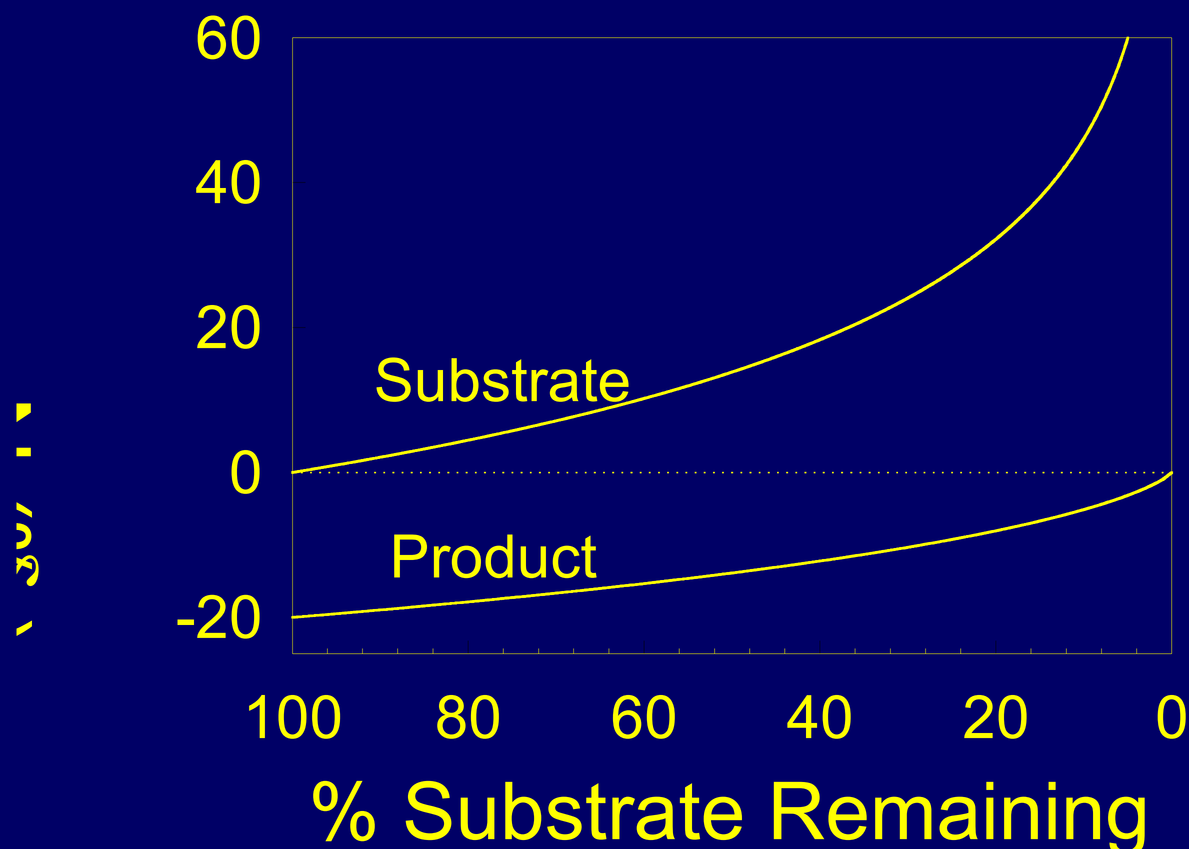
Assumption A → B

Fractionation =0

-Source of nitrate in groundwater
-Contribution of nitrogen fixation
-Identification of sources of atmospheric deposition

δ^{15}N Research in Ecological Research

Original Assumption Is Not Correct In Many Cases



Nitrogen Stable Isotopes

Element	Isotope	Abundance (%)
Nitrogen	^{14}N	99.629 - 99.636
	^{15}N	0.364 - 0.371

$$\delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000 \ ‰$$

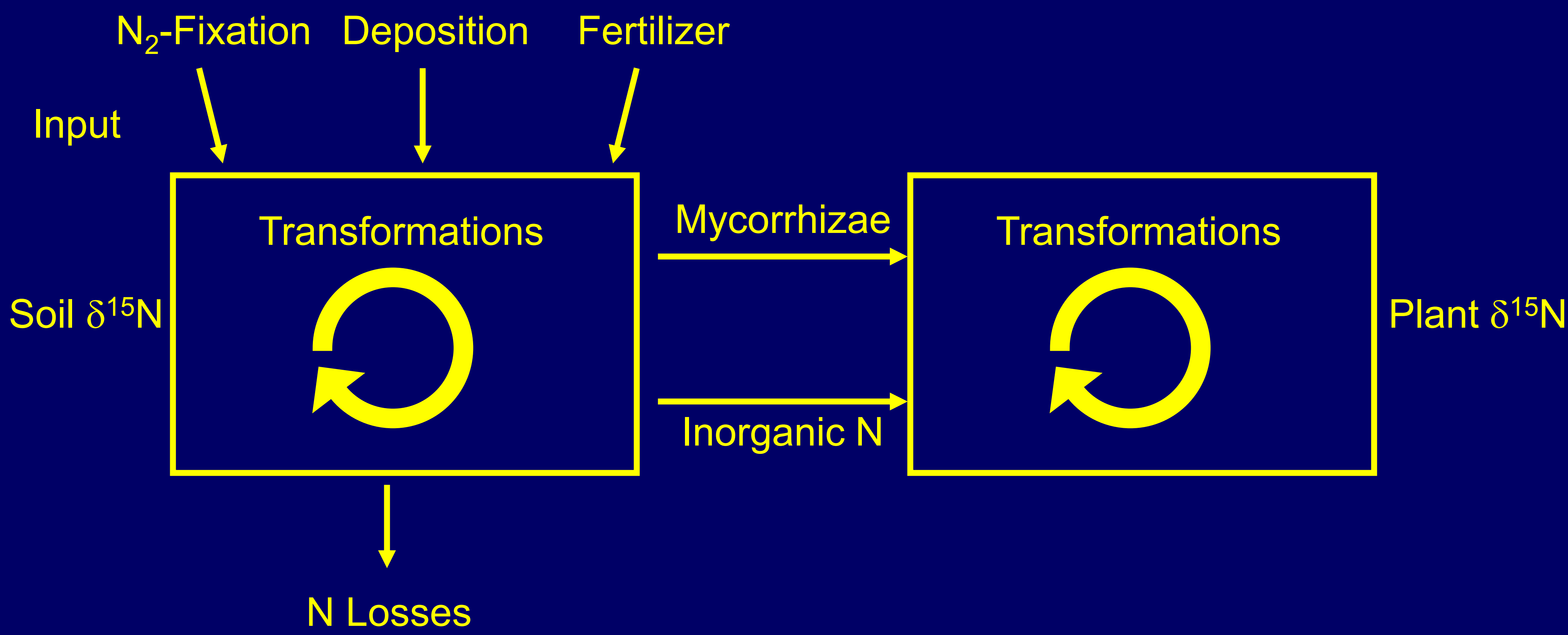
$$R = \left(\frac{^{15}N}{^{14}N} \right) = 0.003676$$

Ratio

$$R = \left(\frac{^{15}N}{^{14}N + ^{15}N} \right) = 0.003663$$

Atom %

Lecture – Part 1



Models and Patterns of Soil $\delta^{15}N$

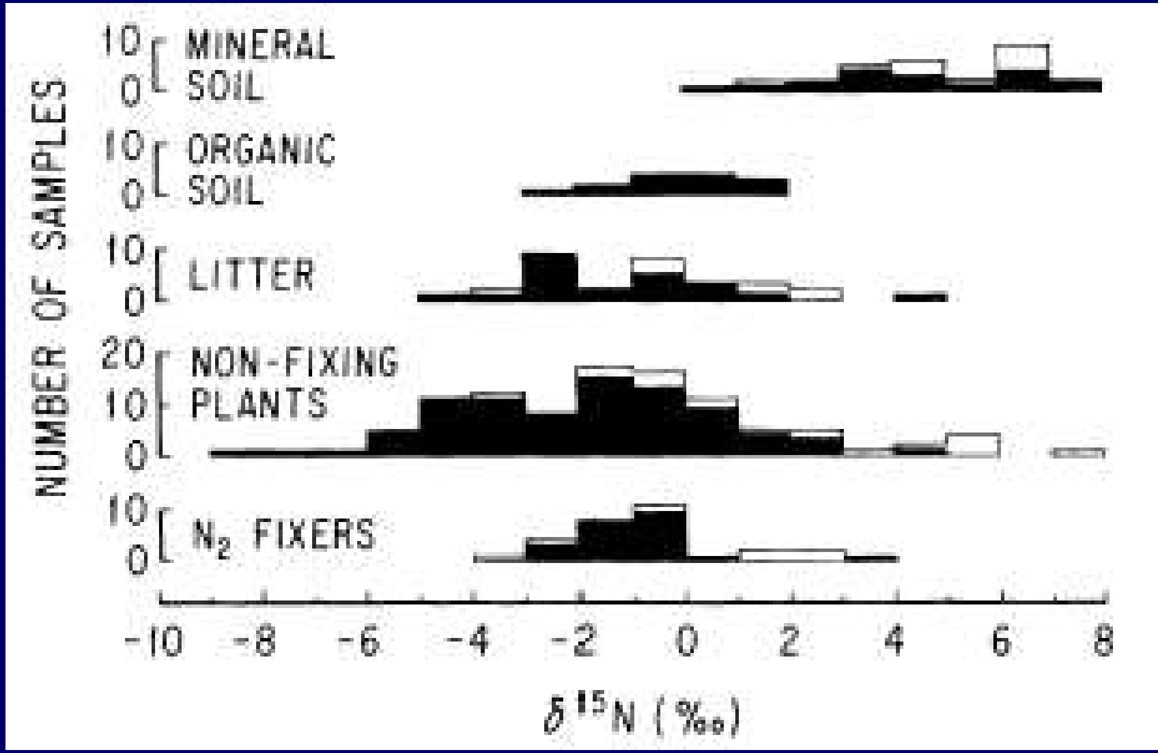
Patterns and Gradients of Plant $\delta^{15}N$

Variation in Soil and Plants

FIG. 1. Sampling sites in the Long Term Ecological Research (LTER) Program. (1) Arctic Tundra (Alaska), (2) Bonanza Creek Experimental Forest (Alaska), (3) H. J. Andrews Experimental Forest (Oregon), (4) Jornada (New Mexico), (5) Sevilleta (New Mexico), (6) Niwot Ridge/Green Lakes Valley (Colorado), (7) Central Plains Experimental Range (Colorado), (8) Konza Prairie (Kansas), (9) Cedar Creek Natural History Area (Minnesota), (10) North Temperate Lakes (Wisconsin), (11) W. K. Kellogg Biological Station (Michigan), (12) Coweeta Hydrologic Laboratory (North Carolina), (13) North Inlet (South Carolina), (14) Virginia Coast Reserve (Virginia), (15) Harvard Forest (Massachusetts), (16) Hubbard Brook Experimental Forest (New Hampshire), (17) Luquillo Experimental Forest (Puerto Rico).

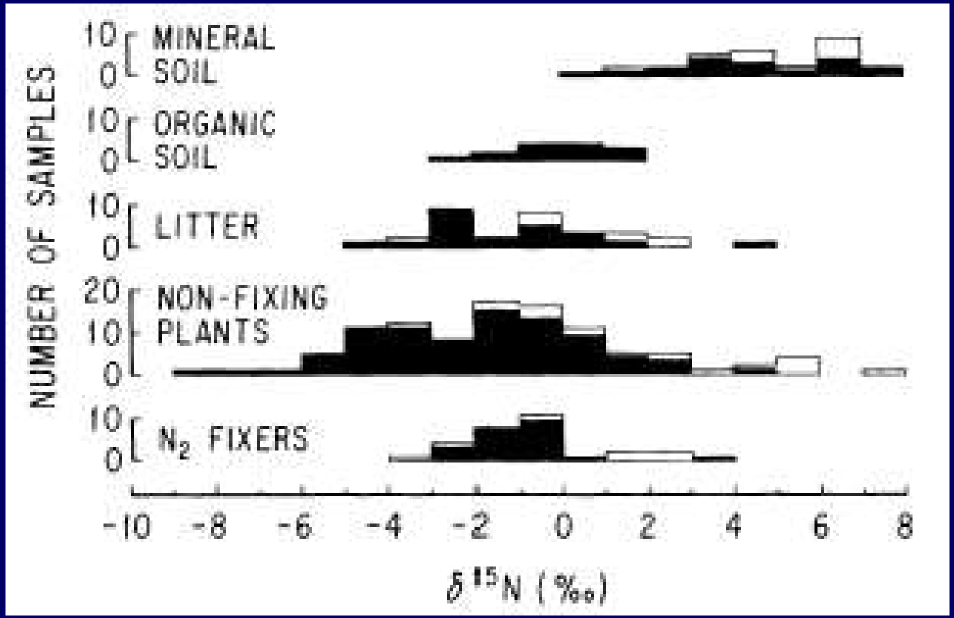
From Fry, 1991

Variation in Soil and Plants



From Fry, 1991

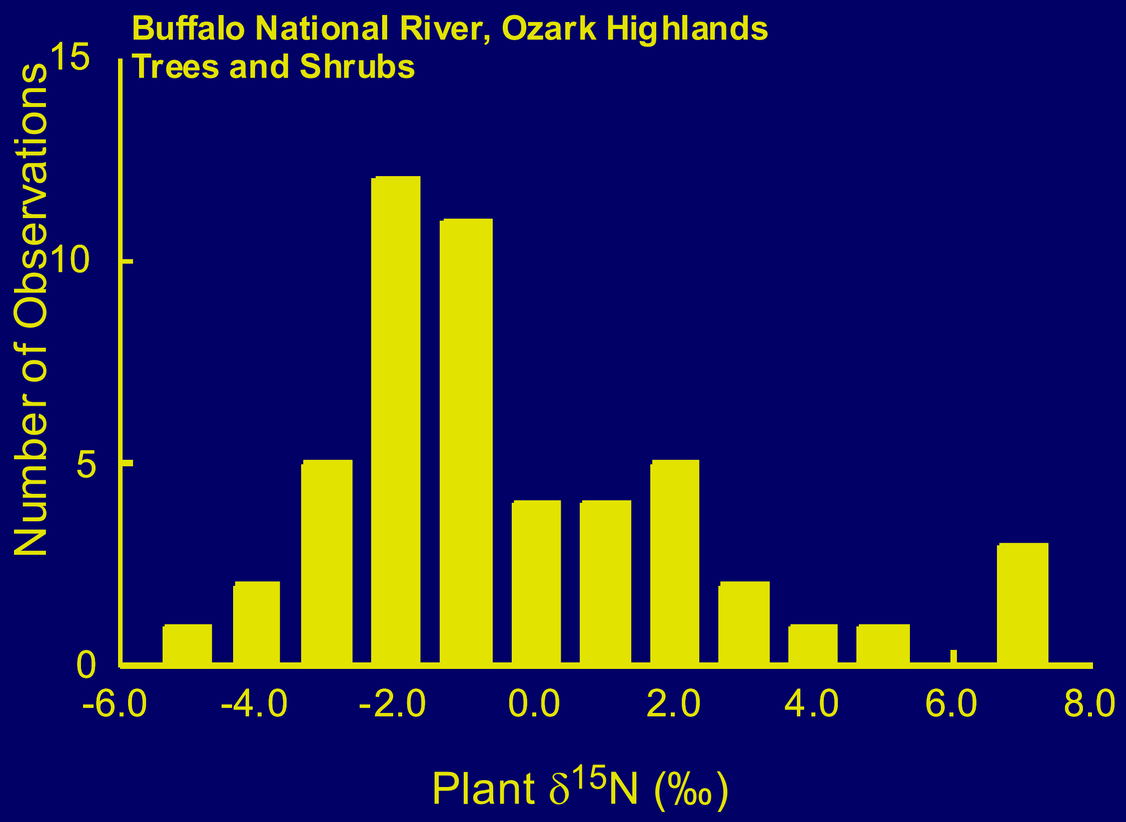
Variation in Soil and Plants



Observations from Fry (1991)

1. Large variation
2. No correlation with precipitation
3. Soils more enriched than plants
4. N_2-fixers near 0 ‰

Variation in Soil and Plants



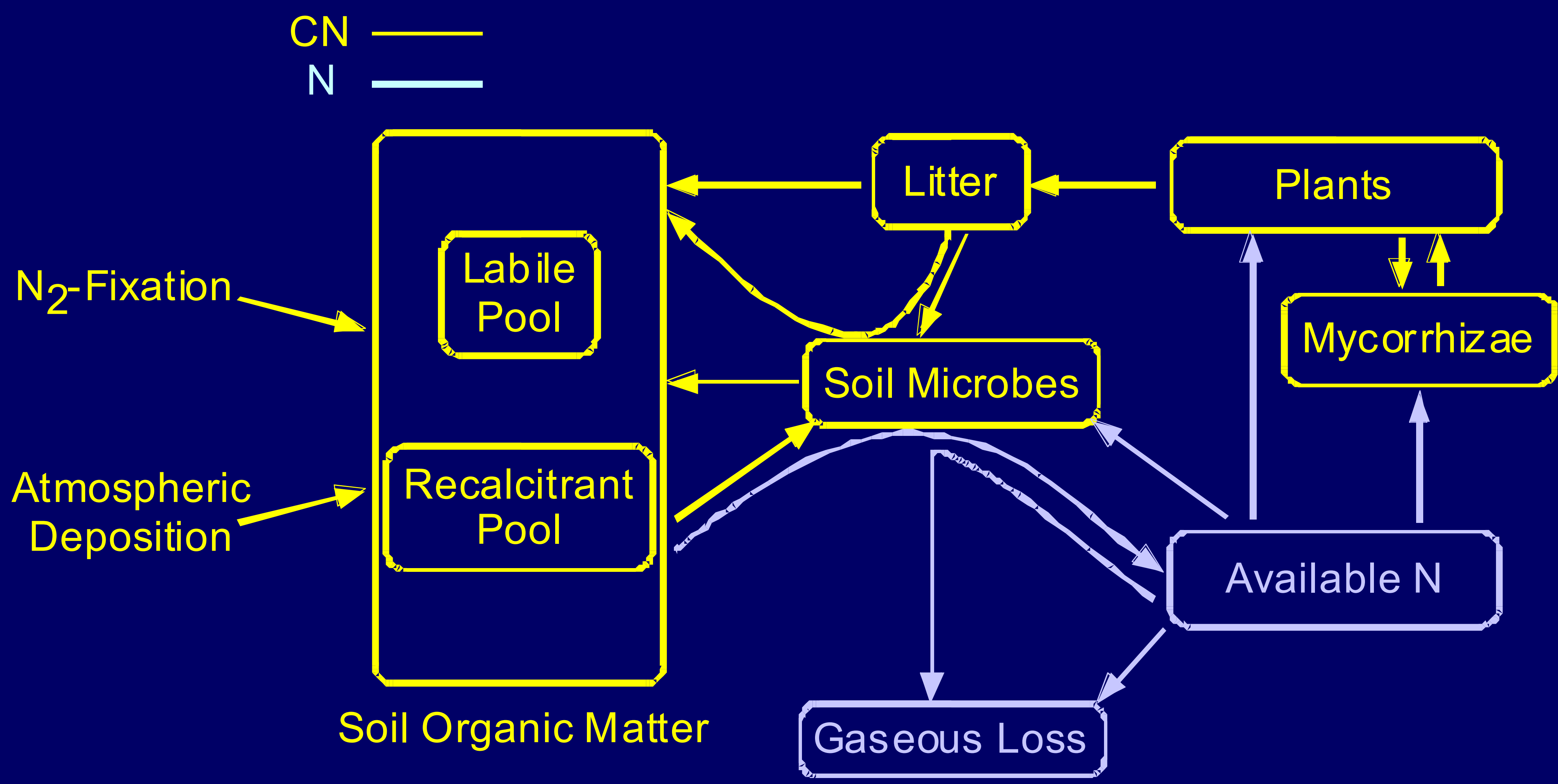
Kinsey and Evans, Unpublished Data

1. Each observation is the mean of five samples from a single species.

2. How can we observe more variation in a single site than Fry (1991) observed across all LTER sites (alpine to tropics: arid to very wet)?

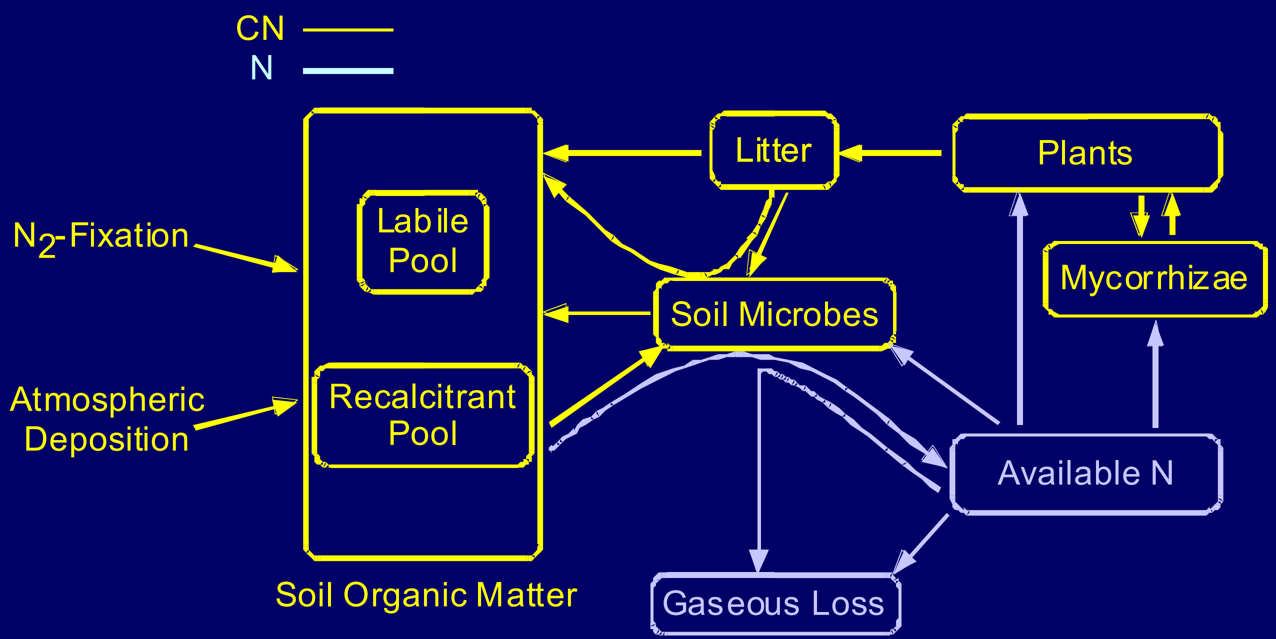
3. Stay tuned!

N Cycle



CN
N
N2-Fixation
Atmospheric
Deposition
Labile
Pool
Recalcitrant
Pool
Soil Organic Matter
Litter
Plants
Soil Microbes
Mycorrhizae
Available N
Gaseous Loss

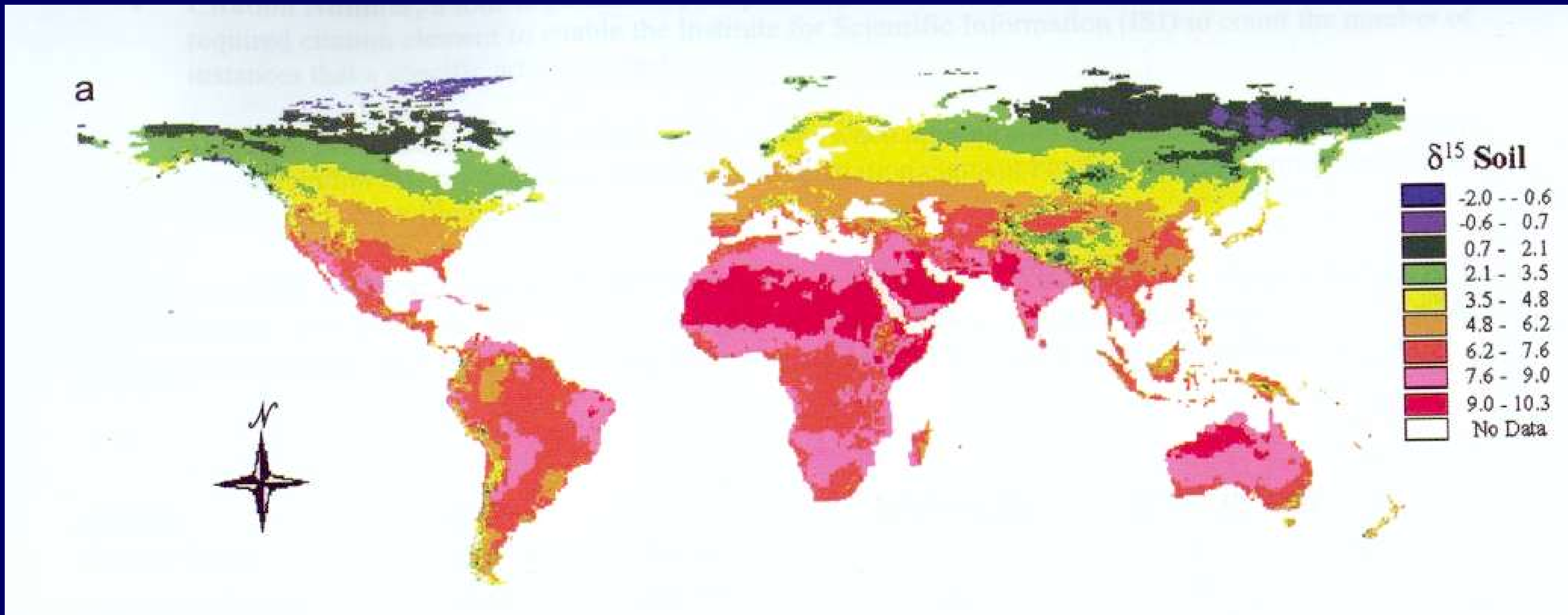
N Cycle



Factors

1. Sources of Input
2. Sources of Loss
3. Internal Transformations

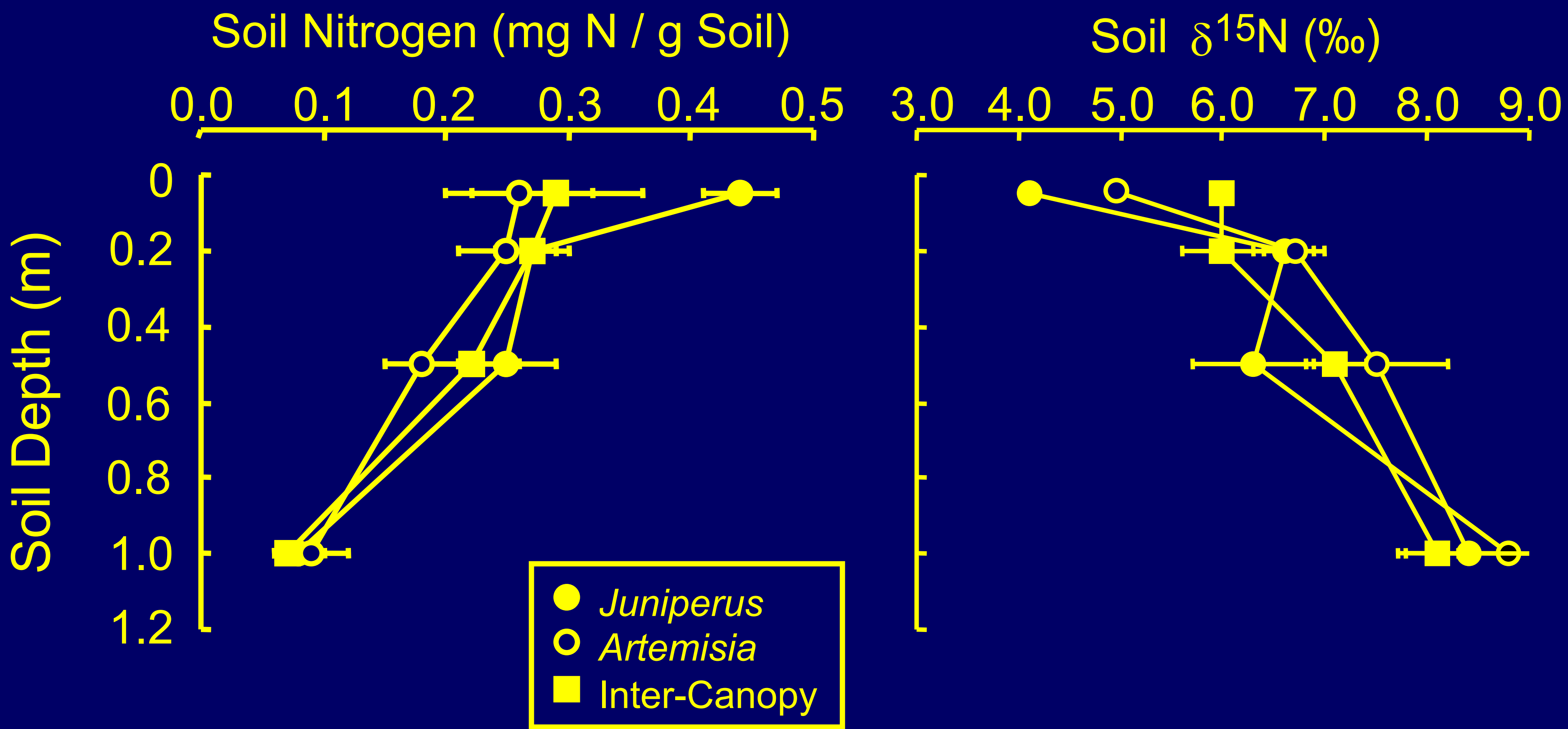
General Trends in Soil $\delta^{15}N$



Amundson et al. (2003)

Values are usually positive (but there are exceptions)

General Trends in Soil $\delta^{15}N$



From: Evans and Ehleringer (1993)

General Trends in Soil $\delta^{15}N$

Observation: Soil $\delta^{15}N$ is usually positive and increases with depth

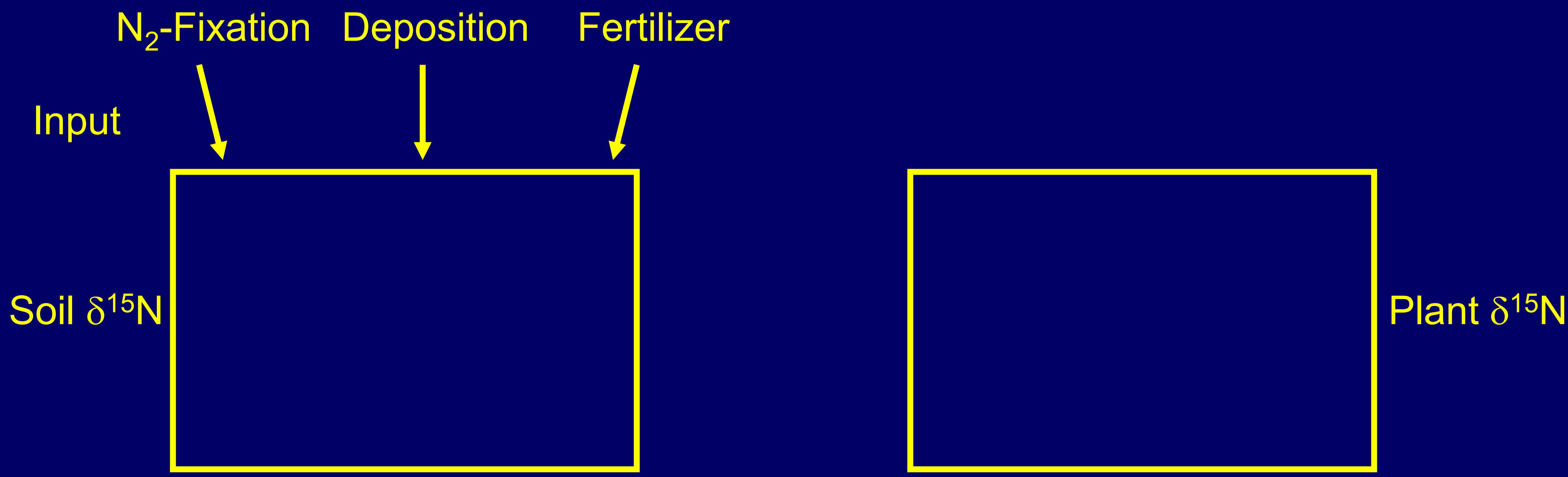
Mechanisms

1. $\delta^{15}N$ of nitrogen inputs into soil

2. Fractionation during internal transformations

3. Fractionation during nitrogen loss

Lecture – Part 1



$\delta^{15}N$ of Input: N_2 Fixation

$N_{2(atmosphere)} + 8e^- + 16ATP + 16H_2O \longrightarrow 2NH_3 + H_2 + 16ADP + 16P_i + 8H^+$

$$\delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \text{ ‰}$$

Values for N_2-fixation should be 0 ‰ if there is no fractionation

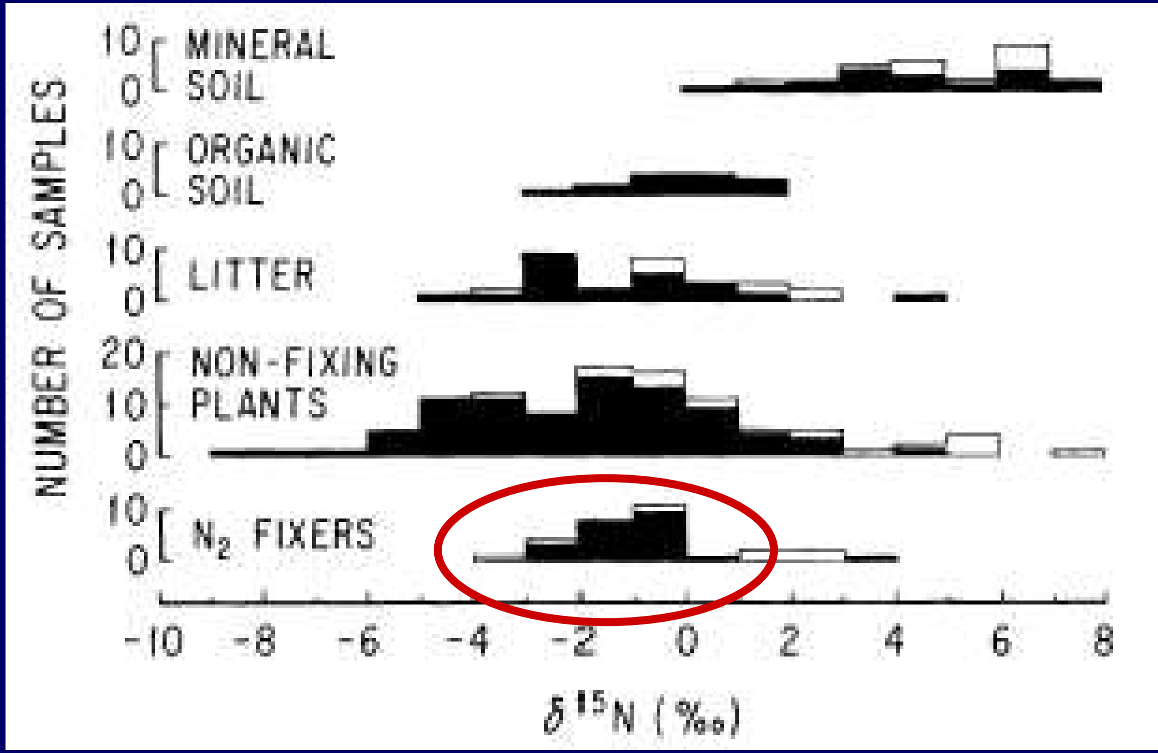
$\delta^{15}N$ of Input: N_2 Fixation

Discrimination Observed with N_2-Fixation

Host	#Species	Discrimination (‰)
Azotobacter	4	1.2
Gycine max		1.5
Medicago sativa		-0.2
Trifolium	2	-0.5
Vicia faba		-0.2
Lupinus	2	0.0
Phaseolus vulgaris		1.5
Cyamopsis tetragonoloba		-0.8
Dalea	2	1.7
Prosopis glandulosa		1.5
Lotus pendunculatus		0.1
Macroptillium atropurpureum		3.4

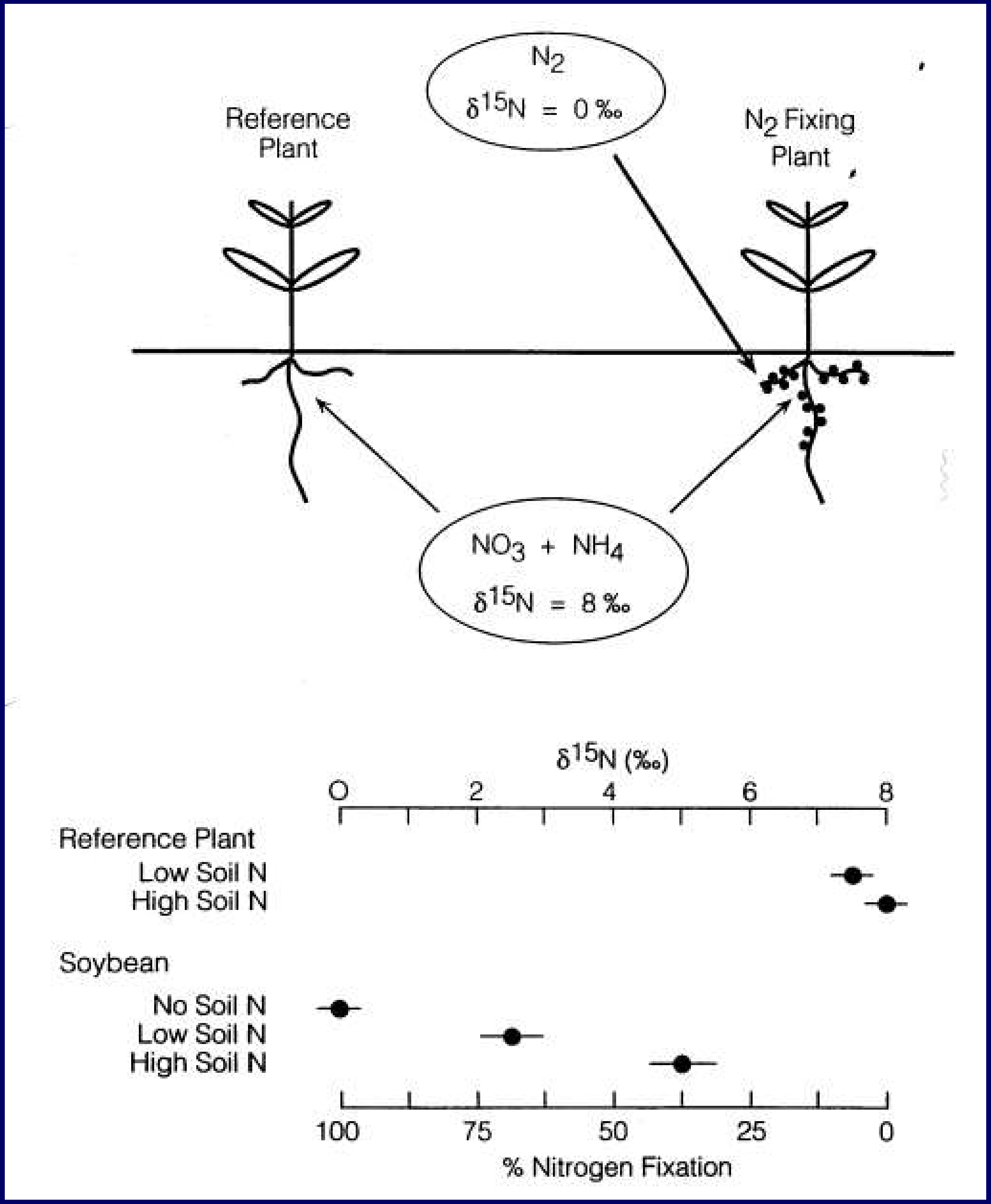
From Shearer and Kohl, 1993

$\delta^{15}N$ of Input: N_2 Fixation

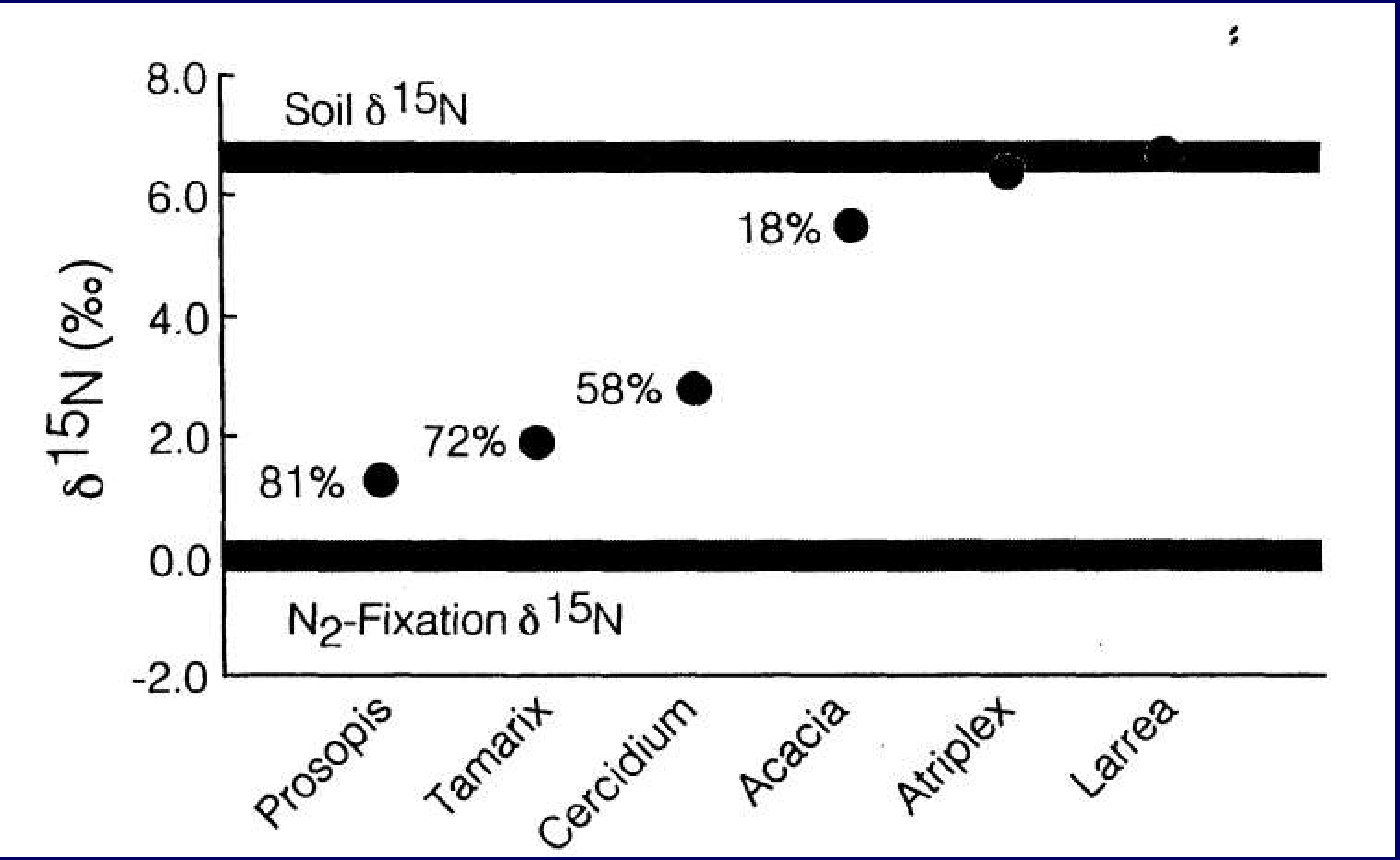


From Fry, 1991

Contribution of N Fixation ?



$\delta^{15}N$ of Input: N_2 Fixation



Careful selection of reference plant

General rule, reference plant must be 8 to 10 ‰ different than 0.

$\delta^{15}N$ of Input: Atmospheric Deposition

Table 1. $^{15}N/^{14}N$ ratios and concentrations of NO_x and NH_3 gas samples

Sample	$\delta^{15}N$ (‰)	Concentration (ppm V)
NO_x from coal-fired power station:	+ 5.2*	395
from test-bed diesel engine working 165 Nm (2800 rpm):	− 1.6*	1720
NO_x from idling diesel mini-bus (1000 rpm):	−13.2†	130
NO_x from idling diesel truck (1000 rpm):	−11.5†	70
NO_x from idling petrol car (1500 rpm):	− 7.3†	105
NO_x from stack of nitric acid plant:	−150‡	2800
NH_3 in sheep shed:	−15.2*	0.8
NH_3 in chicken shed:	− 8.9*	1.2
NH_3 or NH_4^+ from steel factory coking plant		
'1st condensate' (cooling of gases):	+21.4	
'liquor' (water scrubbing of non-condensates):	+ 1.6	
$(NH_4)_2SO_4$ (by-product of 'liquor'):	− 0.5§	
gas from stack:	−20.1	160

* Shown in Fig. 6.

† An average value for these samples shown in Fig. 6.

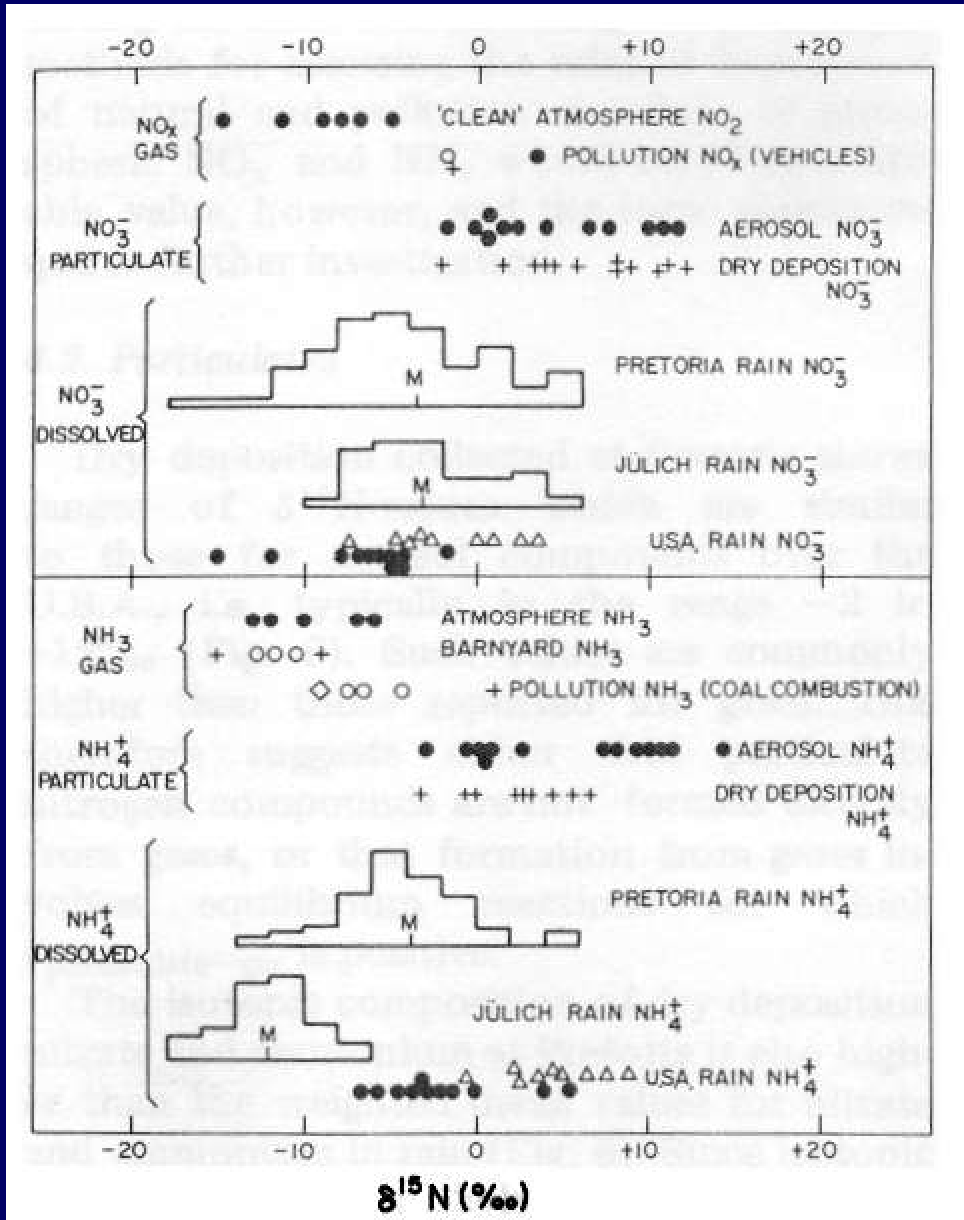
‡ Formation of HNO_3 by the incremental solution of NO_x in water absorbtion towers, if accompanied by a large exchange isotopic fractionation, could leave NO_x with this low $\delta^{15}N$ value. The $\delta^{15}N$ value of the nitric acid was −6.0‰

§ Taken to represent the bulk of coking-derived NH_3, and shown in Fig. 6.

Scientists hoped to identify sources of pollutants based on their $\delta^{15}N$ values.

From: Heaton (1987)

$\delta^{15}N$ of Input: Atmospheric Deposition



Wet deposition is usually negative

Dry deposition is usually positive

From: Heaton (1986)

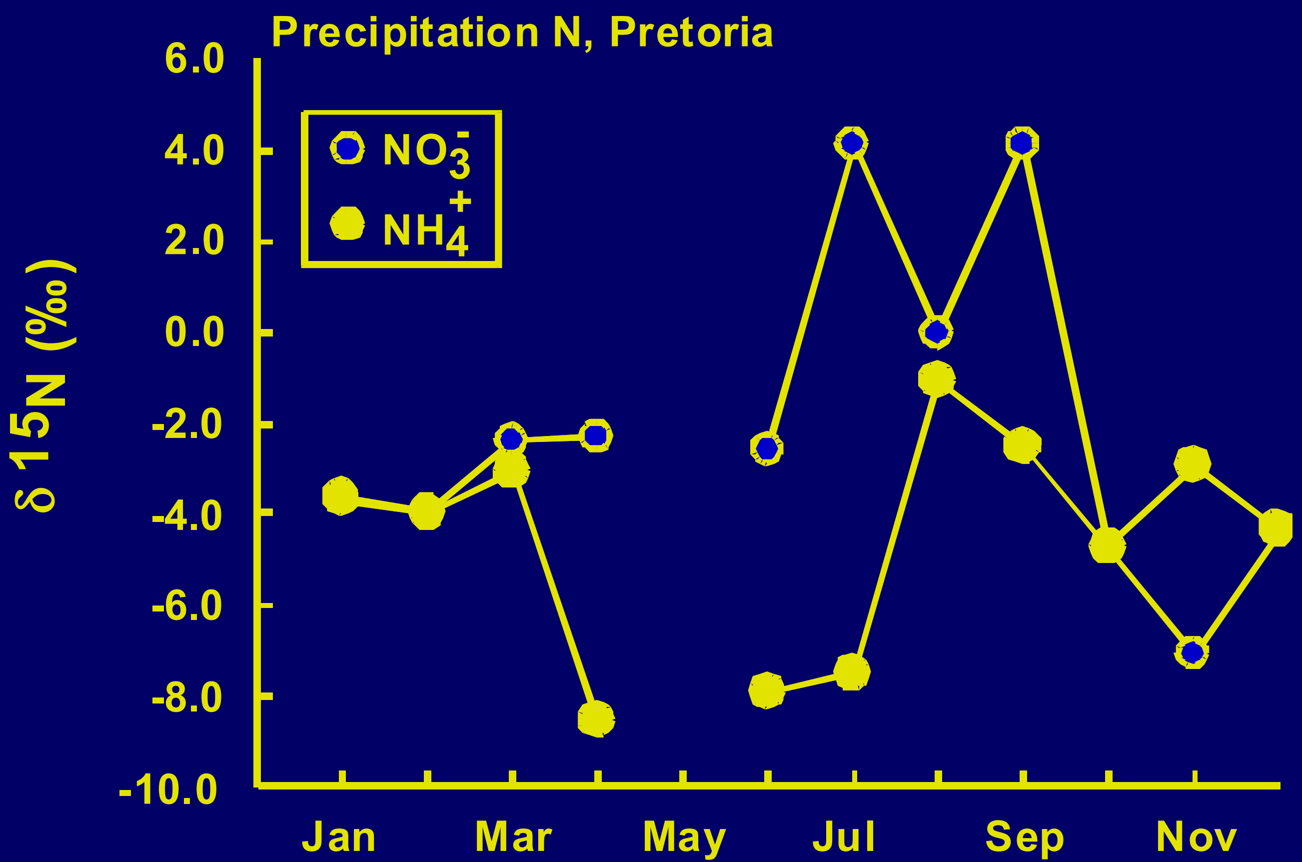
$\delta^{15}N$ of Input: Atmospheric Deposition

Table 4. Estimates for the annual inputs and $\delta^{15}N$ values for nitrate and ammonium in deposition at the CSIR*

Deposition	Input ($kg\ N\ ha^{-1}y^{-1}$)	$\delta^{15}N$ (‰)
Dry NO_3^-	0.5	+6
Dry NH_4^+	0.5	+2
Wet NO_3^-	2.1	−5
Wet NH_4^+	2.1	−5
Wet + dry NO_3^-	2.6	−3
Wet + dry NH_4^+	2.6	−3

* Using mean values from Table 2 (with a dry deposition rate of 10 μeq ion m^{-2} d^{-1}) and Table 3 (with a mean annual CSIR rainfall of 640 mm).

$\delta^{15}N$ of Input: Atmospheric Deposition

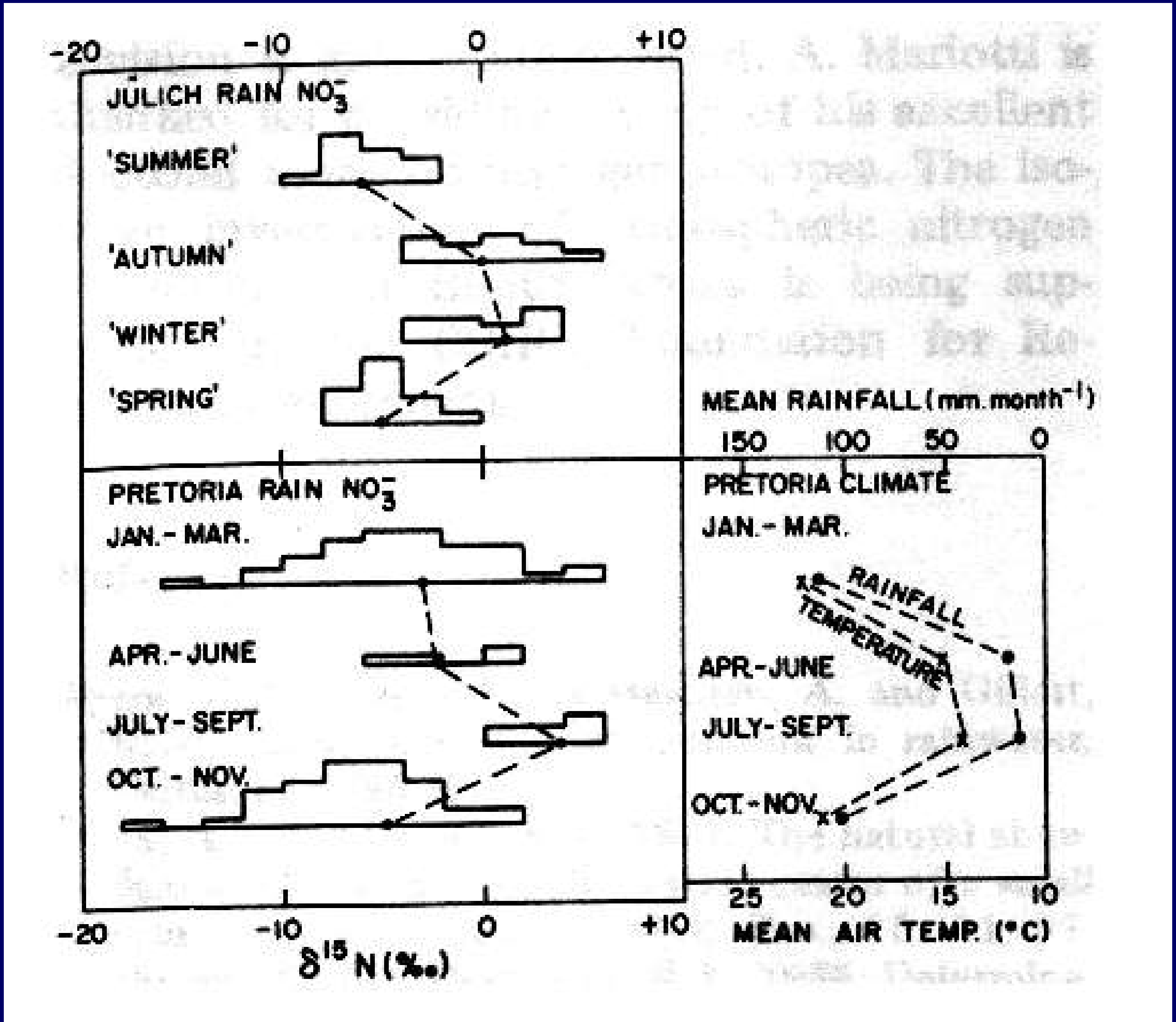


Seasonal Variation

1. Change in source?
2. Change in intensity?

Data from Heaton, 1987

δ^{15}N of Input: Atmospheric Deposition



Seasonal Variation

1. Change in source?
2. Change in intensity?

From: Heaton (1986)

δ^{15}N of Input: Atmospheric Deposition

Bragazza et al. (2004, 2005)

-Sixteen sites across 11 European countries
-Atmospheric deposition gradient from 1 to 20 kg N ha^{-1} y^{-1}
-Measured δ^{15}N of mosses

Parameter	*P*-Value
Total Deposition	0.13
Annual Temperature	0.87
Annual Precipitation	0.63

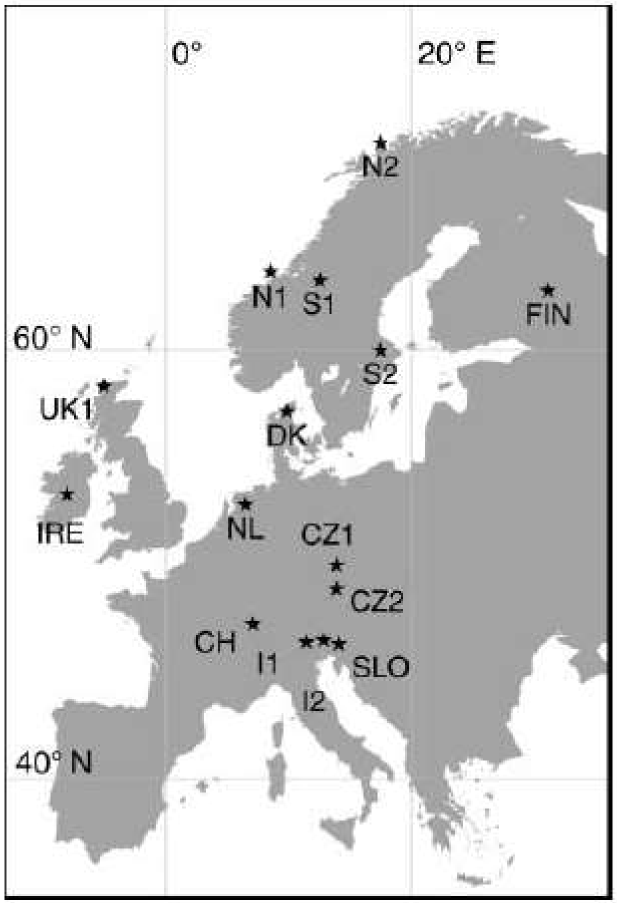
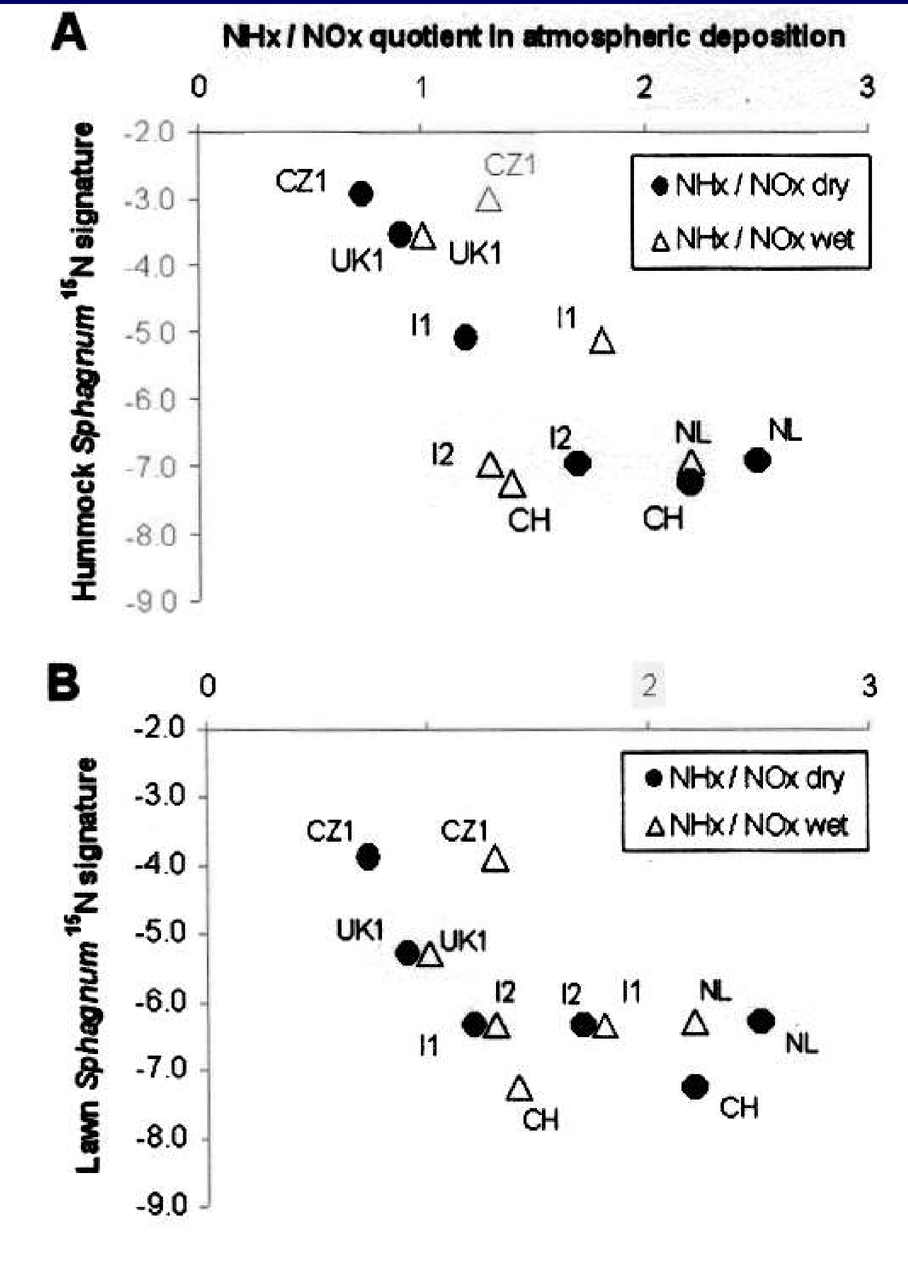


Fig. 1 Geographic location of the mires investigated, with identification codes as in Table 1.

$\delta^{15}N$ of Input: Atmospheric Deposition



Parameter	*P*-Value
NH_x / NO_x Quotient	<0.01

Bragazza et al. (2005)

$\delta^{15}N$ of Input: Atmospheric Deposition

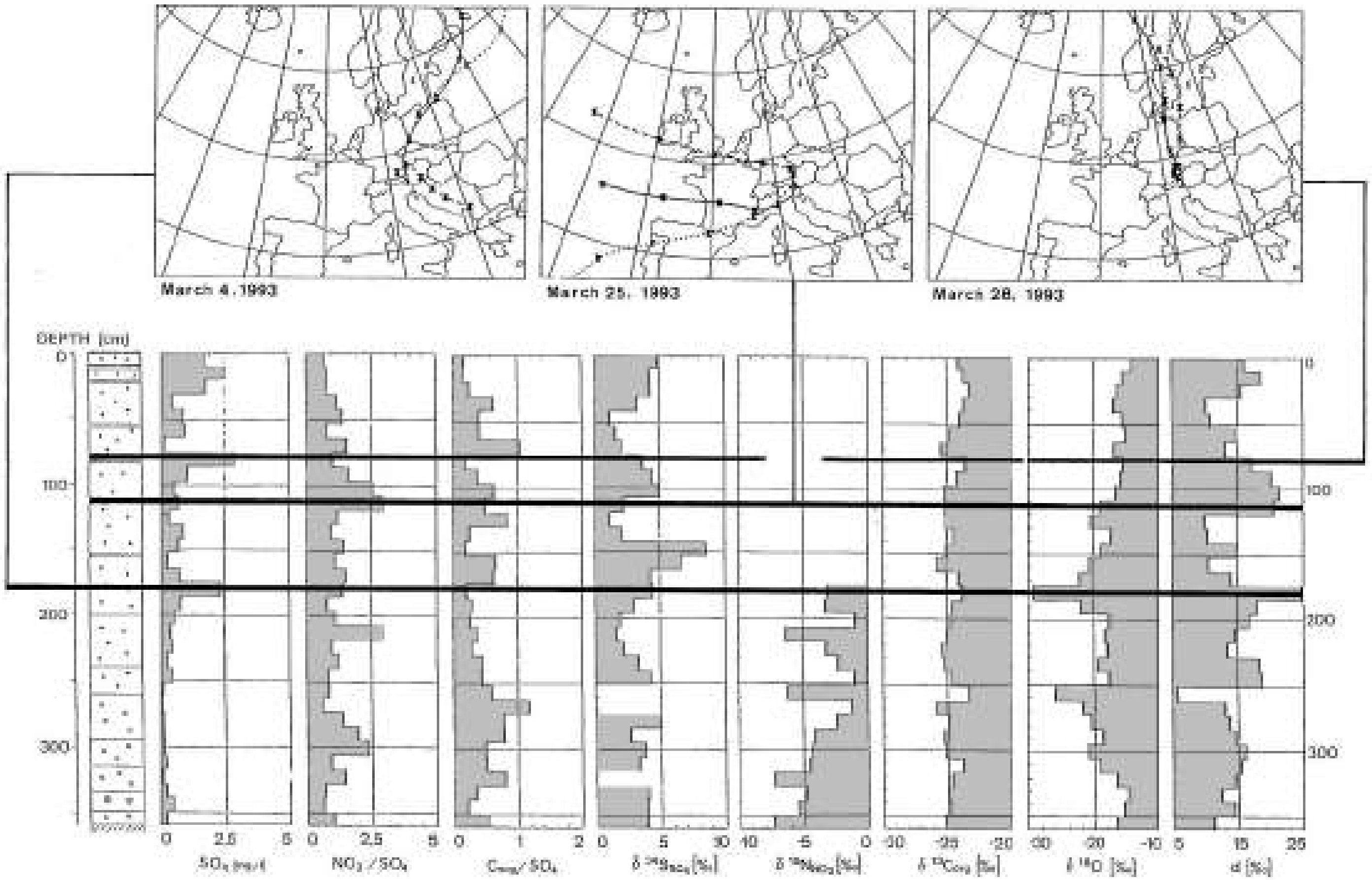


Fig. 1. Sulfate, nitrate and organic carbon concentration-, and S/N/C/O isotope ratio-, as well as deuterium excess (d)- depth profiles in the 1992/1993 snow pack at the sampling site Goldberg Glacier, Sonnblick, Austria. Isobaric back trajectories for the marked events are shown in the attached figures; the 700 hPa (850/500 hPa) trajectory is indicated by a continuous (dashed/dotted) line with markers every 12 h.

Attempt to reconstruct sources of nitrate in atmospheric deposition

Correlate ratios with known storm tracks

Pichlmayer et al. (1998)

$\delta^{15}N$ of Input: Atmospheric Deposition

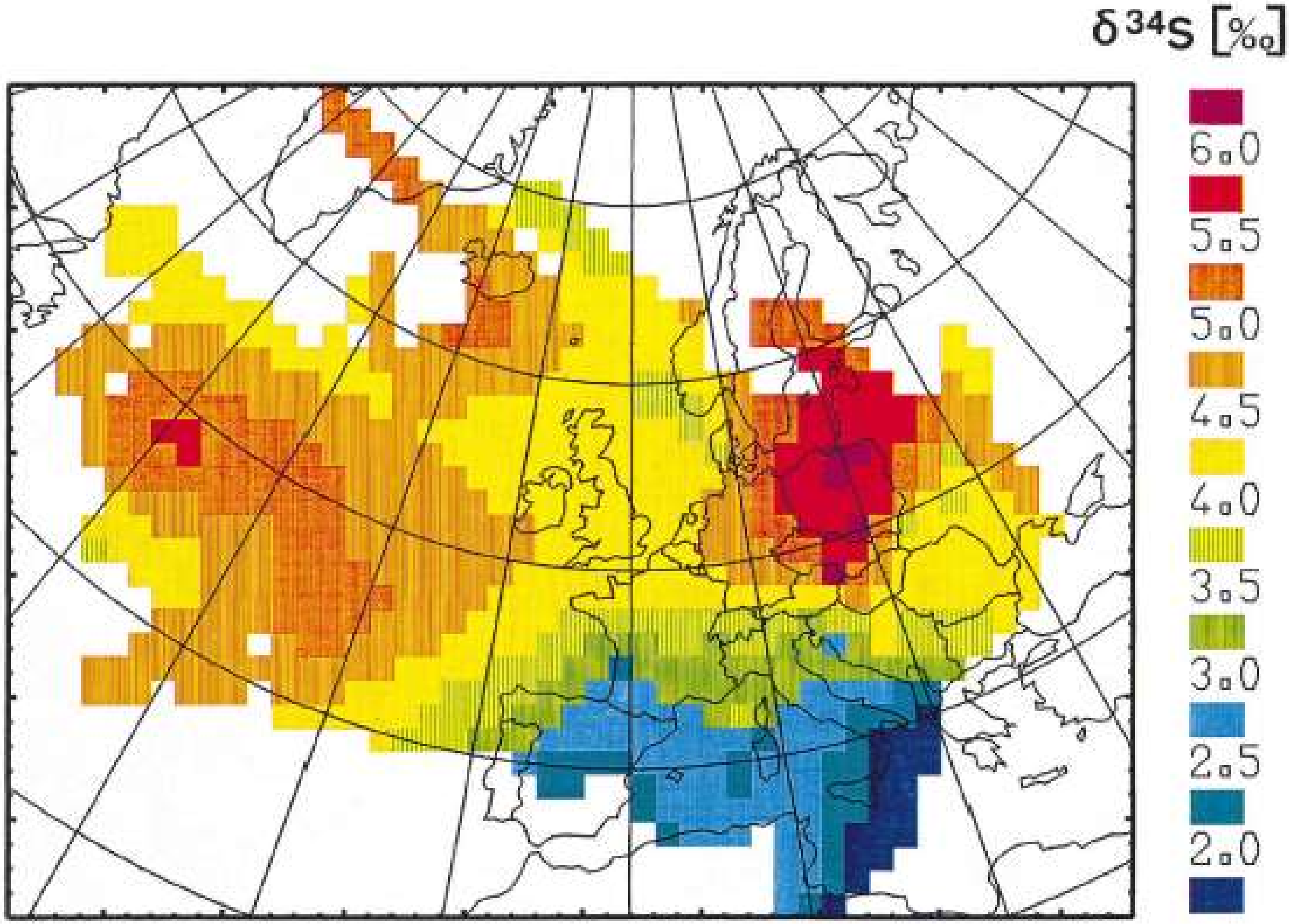
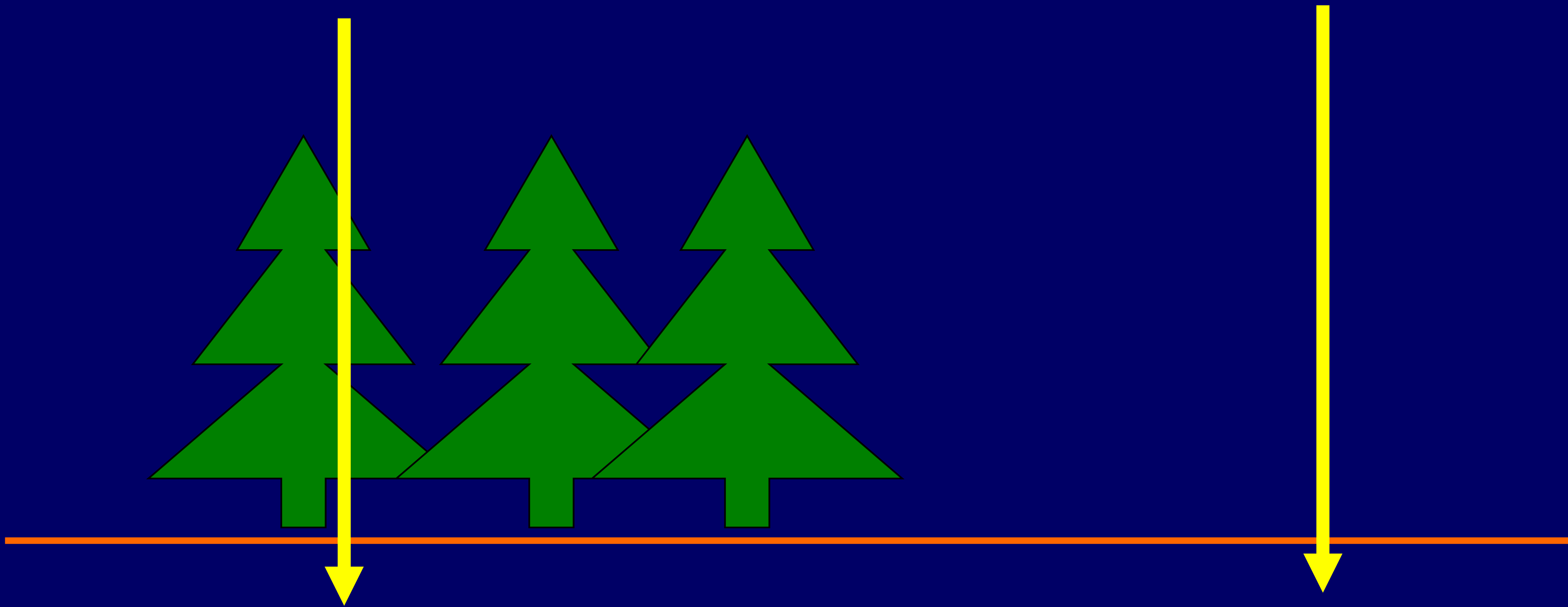


Fig. 8. Mean $\delta^{34}S$-values at Sonnblick associated with back trajectories passing through grid elements (indicated on the axes).

Pichlmayer et al. (1998)

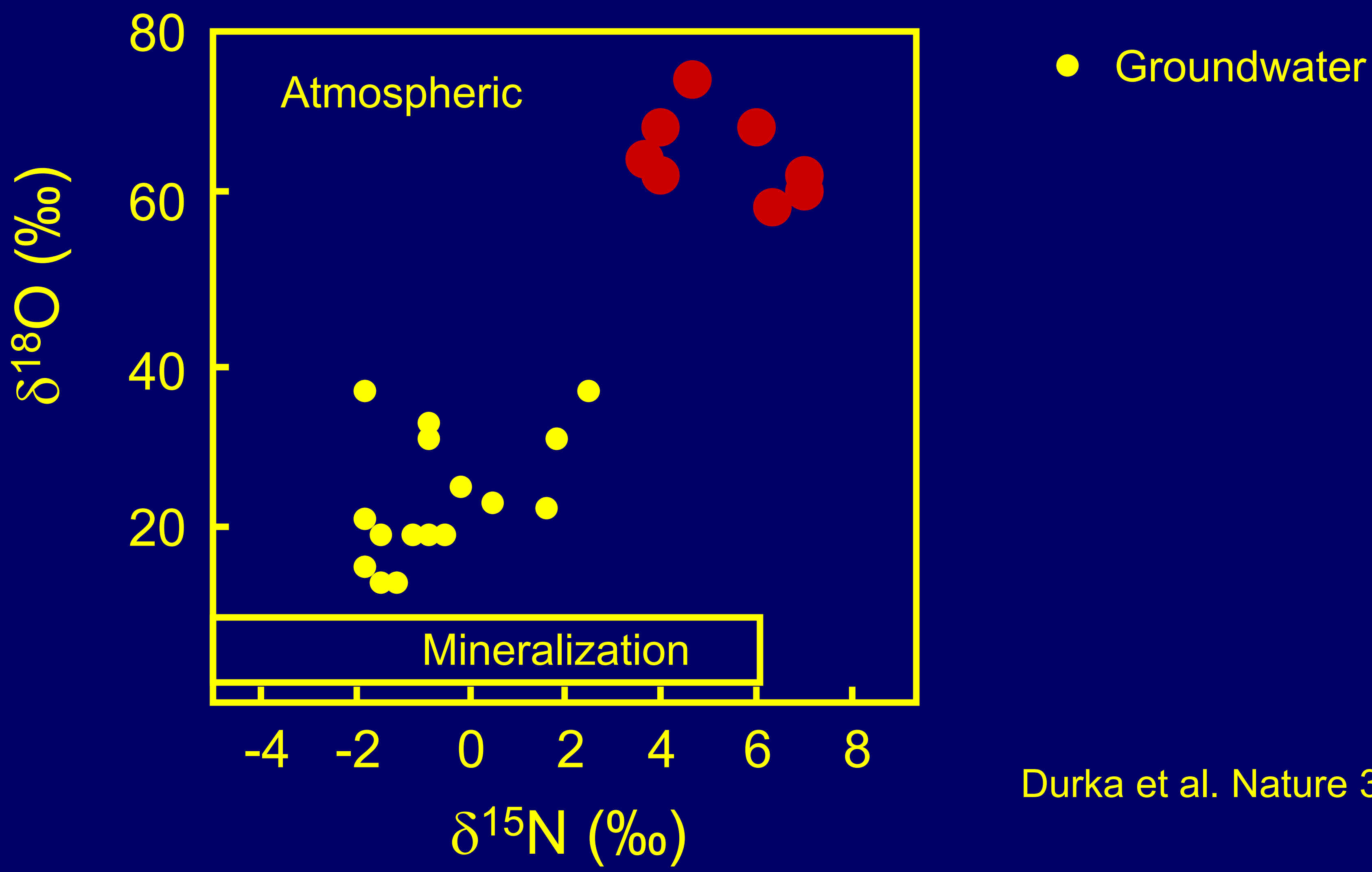
$\delta^{15}N$ of Input: Atmospheric Deposition



	Throughfall	Canopy (calculated)	Rainfall
NO_3^-	4.7	12.0	0.9
NH_4^+	2.6	4.0	-5.6

From: Heaton (1997)

$\delta^{15}N$ of Input: Atmospheric Deposition



Durka et al. Nature 372:765

$\delta^{15}N$ of Input: Fertilizer

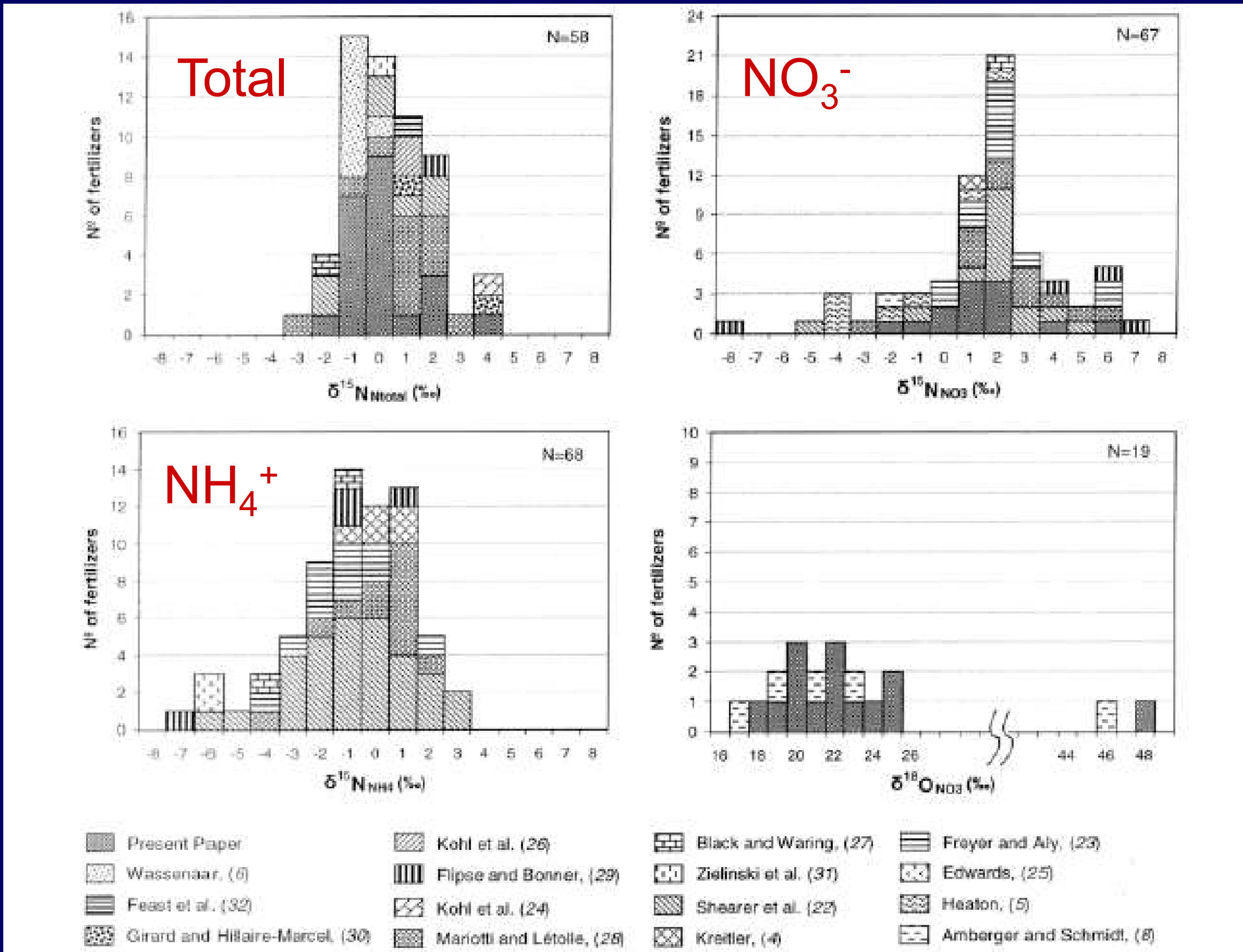


FIGURE 1. Histograms of total nitrogen ($\delta^{15}N_{Ntotal}$), ammonium ($\delta^{15}N_{NH_4}$), and nitrate ($\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$) isotopic compositions of fertilizers including compiled data from different publications and the present analyses.

Vitoria et al. (2004)

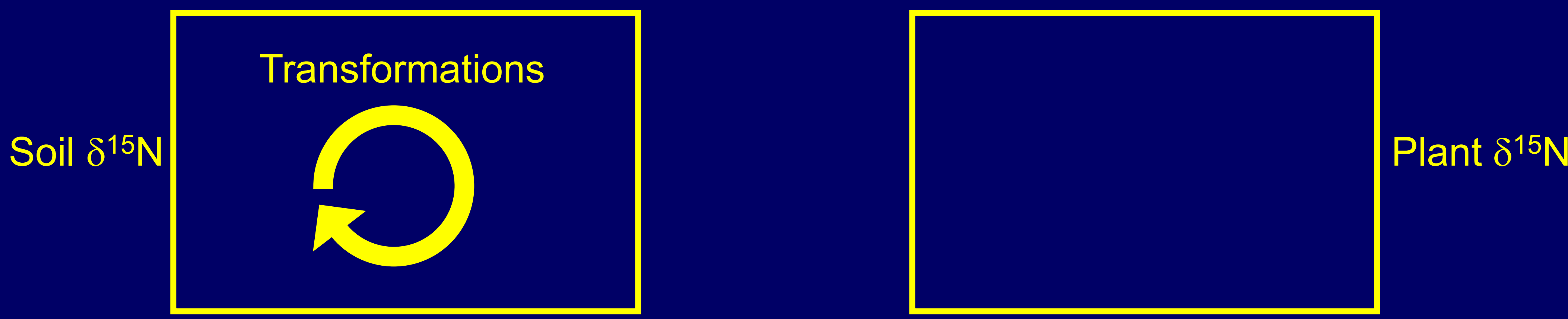
General Trends in Soil $\delta^{15}N$

Observation: Soil $\delta^{15}N$ is usually positive and increases with depth

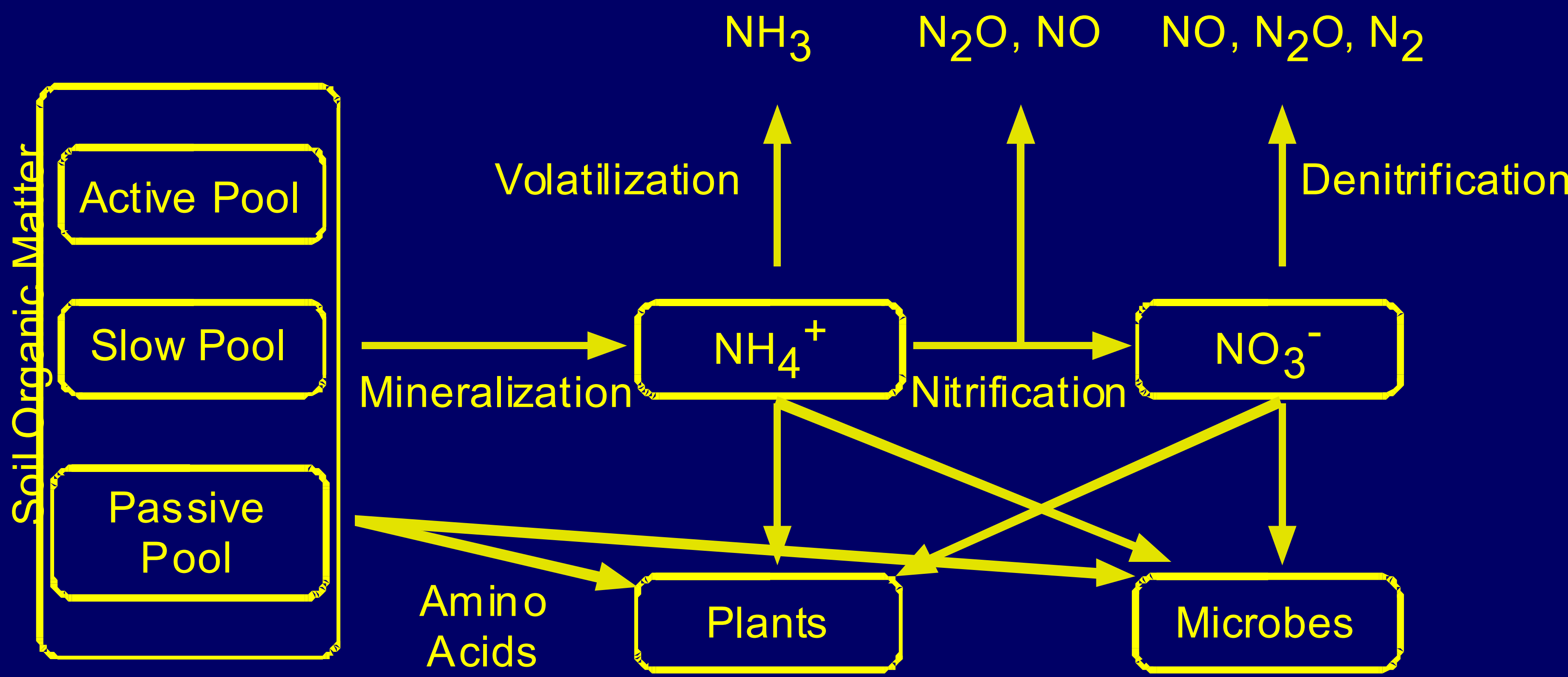
Mechanisms

1. $\delta^{15}N$ of nitrogen inputs into soil
2. **Fractionation during internal transformations**
3. Fractionation during nitrogen loss

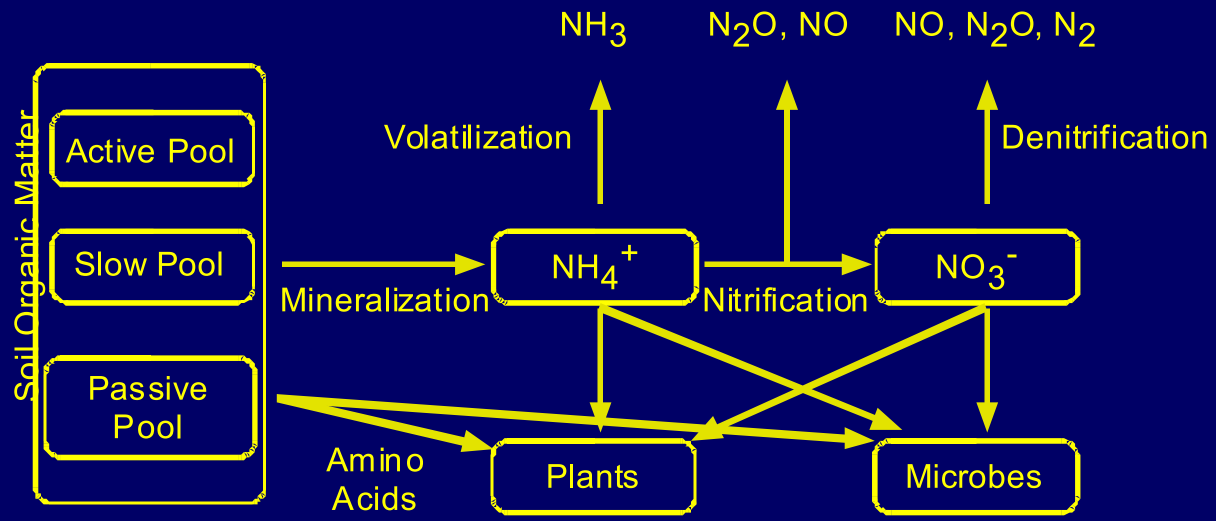
Lecture – Part 1



Soil Nitrogen Transformations



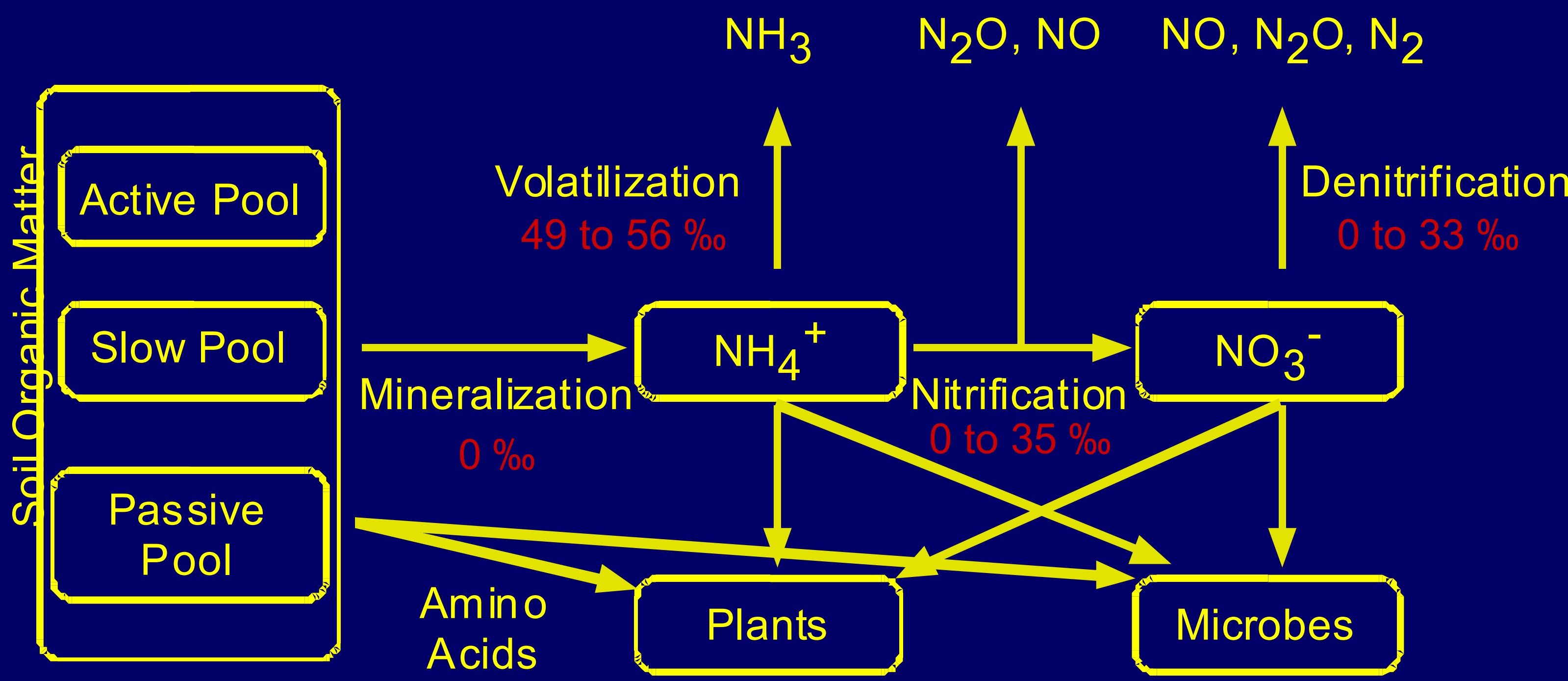
Soil Nitrogen Transformations



Process	Observed Discrimination (‰)
Mineralization	0
NH_4^+ : NH_3 Equilibrium	20 to 27
Volatilization	29
Diffusion in Solution	0
Nitrification	0 to 35
Denitrification	0 to 33

Högberg (1997)
Shearer and Kohl (1990)

Soil Nitrogen Transformations

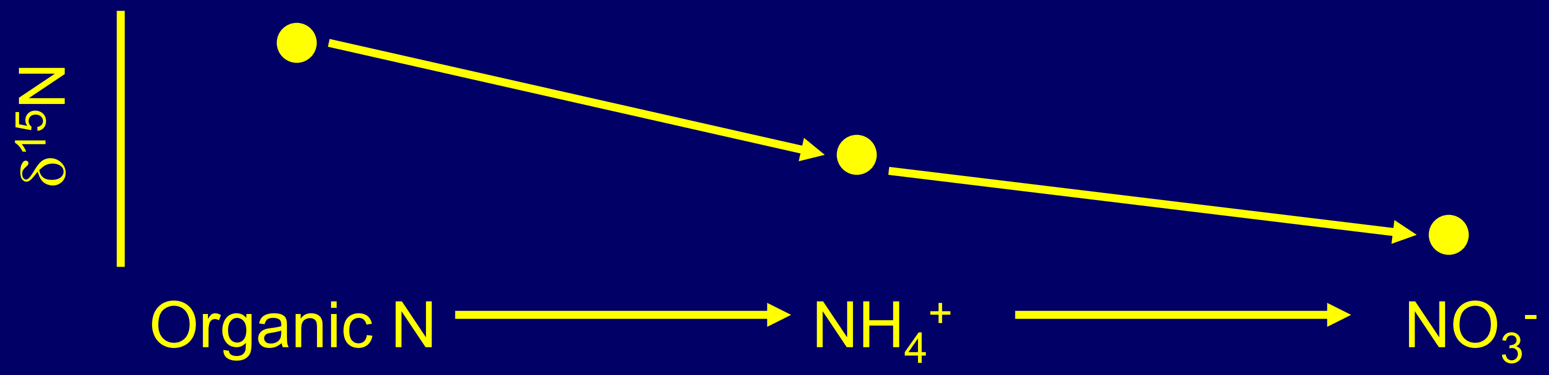


Why the variation (Shearer and Kohl, 1990)?

1. Processes limited by substrate availability (diffusion)
2. Multiple substrates for same product (N_2O, NO)
3. Multiple fates for each substrate (NH_4^+, NO_3^-)

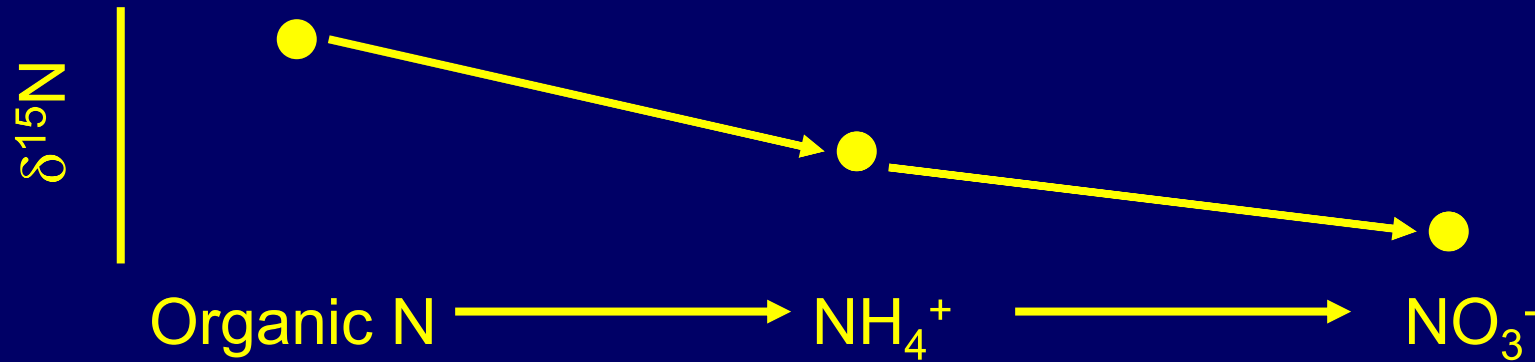
Soil Nitrogen Transformations: $\delta^{15}N$ of Inorganic N

Assumption Made in Many Studies



Soil Nitrogen Transformations: $\delta^{15}N$ of Inorganic N

Assumption Made in Many Studies



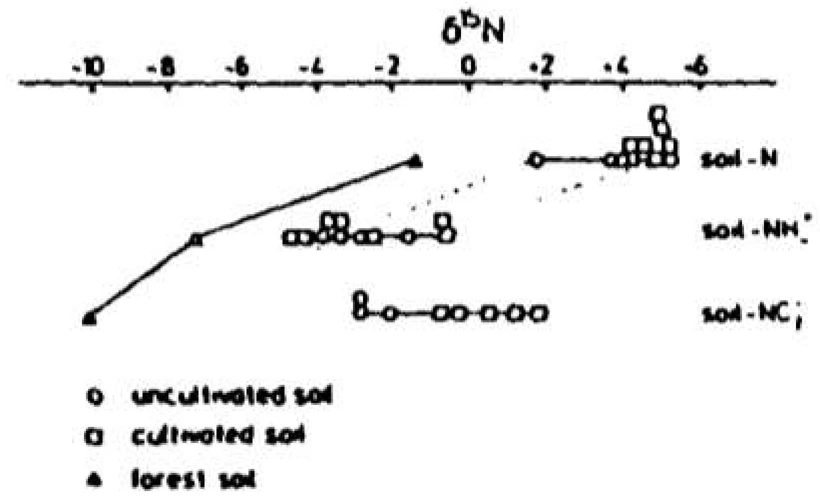


Fig. 4. Nitrogen isotope relationship between total soil nitrogen, soil ammonium and soil nitrate in loess soils (surface samples) sampled in the surroundings of Jülich (measurements by H. D. Freyer and G. Kasten, unpublished).

Soil N

NH_4^+

NO_3^-

Soil Nitrogen Transformations: $\delta^{15}N$ of Inorganic N

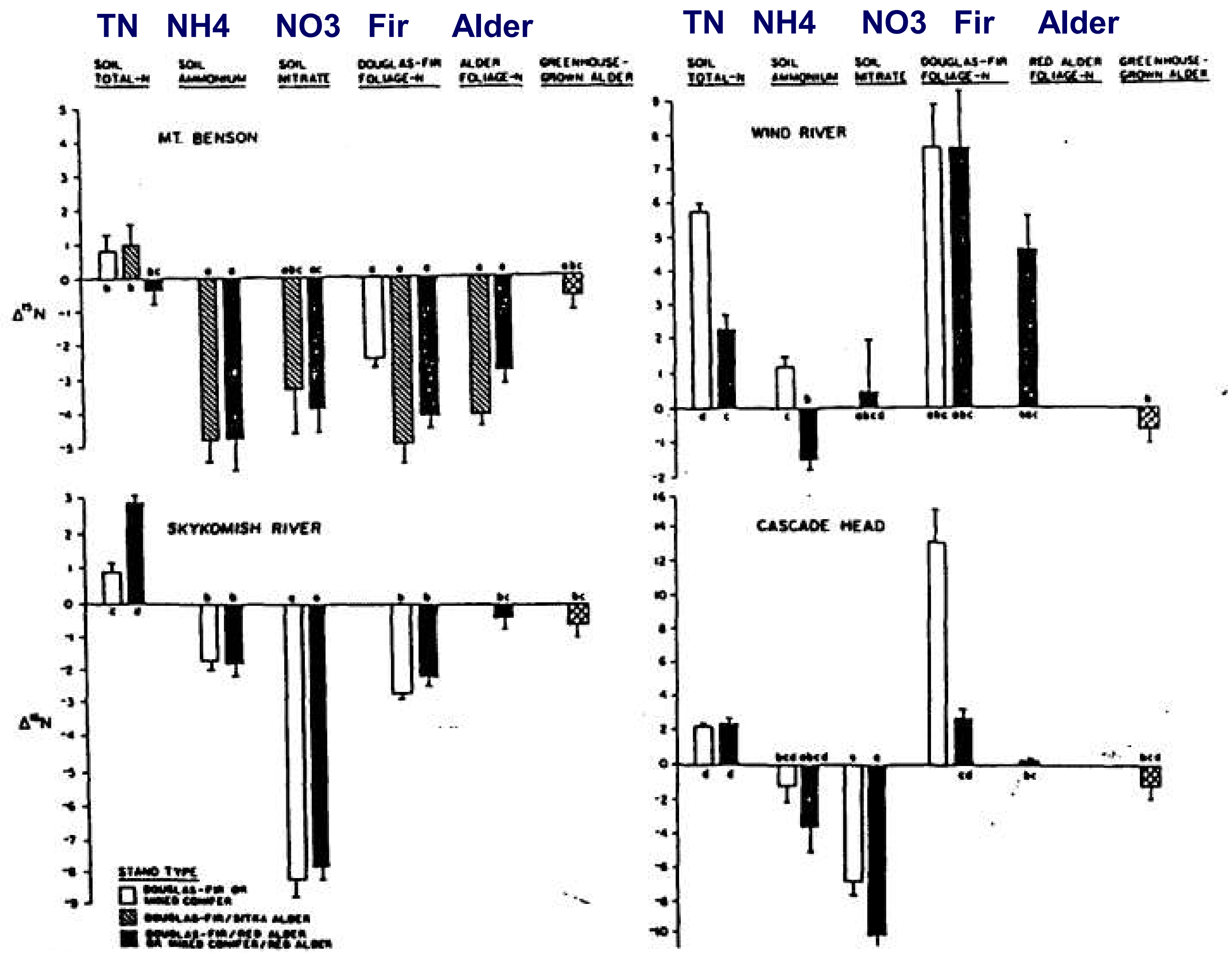


Fig. 1. The $^{15}N/^{14}N$ ratios for nitrogen pools at four Douglas-fir dominated sites. Bars are one standard error ($n = 10$). Letters identify pools whose ratios do not differ significantly ($p < 0.05$).

Soil Nitrogen Transformations: $\delta^{15}N$ of Inorganic N

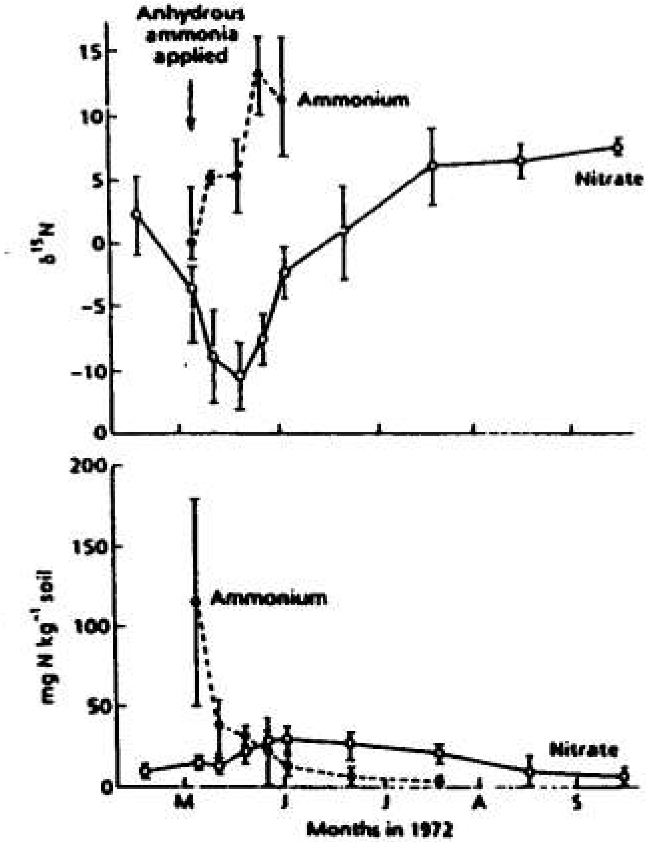


Fig. 2.3 The conversion of NH_4^+ to NO_3 and changes in the $\delta^{15}N$ values of these to nitrogen forms following application of anhydrous NH_3 fertilizer to an agricultural field. (From Feigin *et al.*, 1974b.)

Herman and Rundel (1989)

Soil Nitrogen Transformations: $\delta^{15}N$ of Inorganic N

Role of Extractant

	DDI Extract	KCl Extract	Bound (calculated)
Site One			
NO_3^- Concentration	5.1 µg / g	6.4 µg / g	1.3 µg / g
NO_3^- $\delta^{15}N$	6.4 ‰	4.5 ‰	-2.9 ‰
Site Two			
NO_3^- Concentration	8.6 µg / g	10.5 µg / g	1.9 µg / g
NO_3^- $\delta^{15}N$	5.3 ‰	1.4 ‰	-16.3 ‰

From: Herbel and Spalding (1992)

Soil Nitrogen Transformations: $\delta^{15}N$ of Inorganic N

Observations by Robinson (2001) on $\delta^{15}N$ Measurements of Inorganic N

1. Methods developed for enriched samples may not be appropriate.

2. It is critical to avoid fractionation during isolation (ex. Diffusion or ion exchange may not be appropriate).

3. Avoid contamination. Organic N will be included in most methods developed for ammonium.

4. *Use methods developed explicitly for natural abundance. Three are available for nitrate, none for ammonium.*

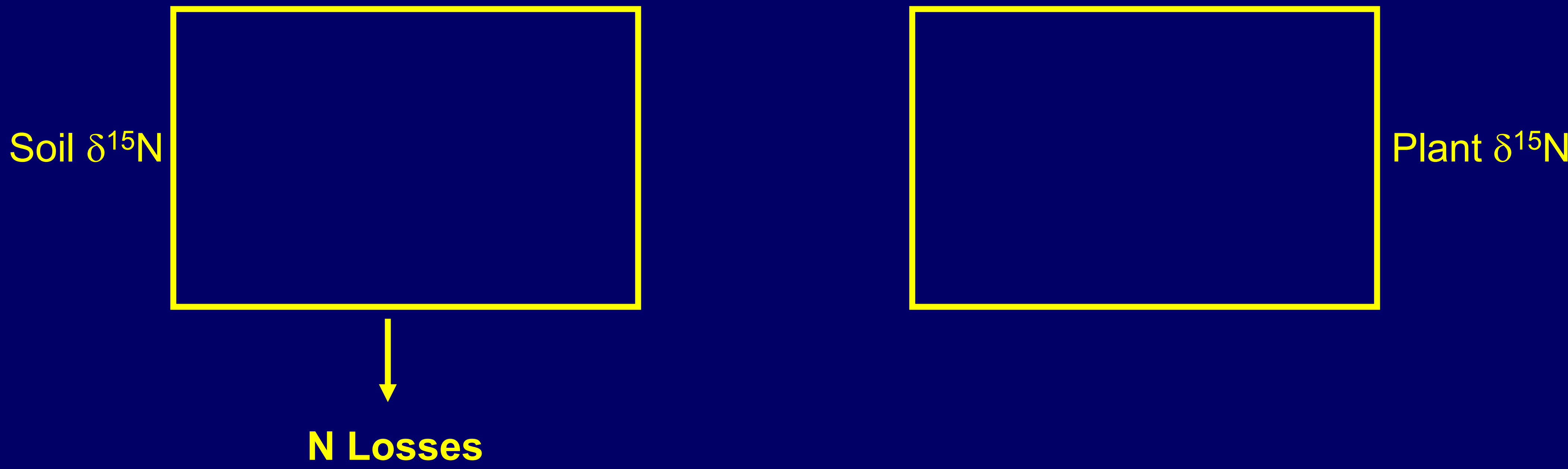
General Trends in Soil $\delta^{15}N$

Observation: Soil $\delta^{15}N$ is usually positive and increases with depth

Mechanisms

1. $\delta^{15}N$ of nitrogen inputs into soil
2. Fractionation during internal transformations
3. **Fractionation during nitrogen loss**

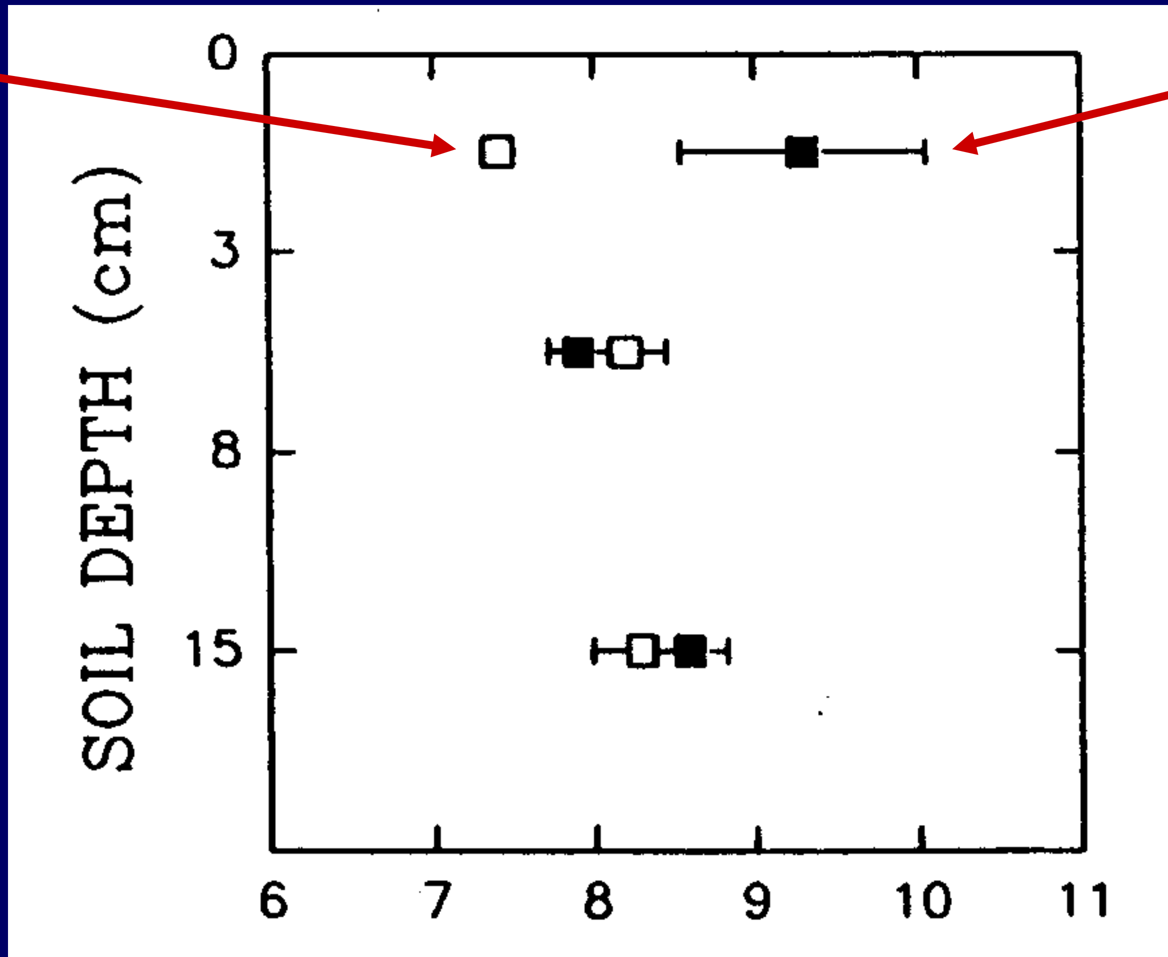
Lecture – Part 1



Nitrogen Loss: Volatilization

Not Grazed

Grazed

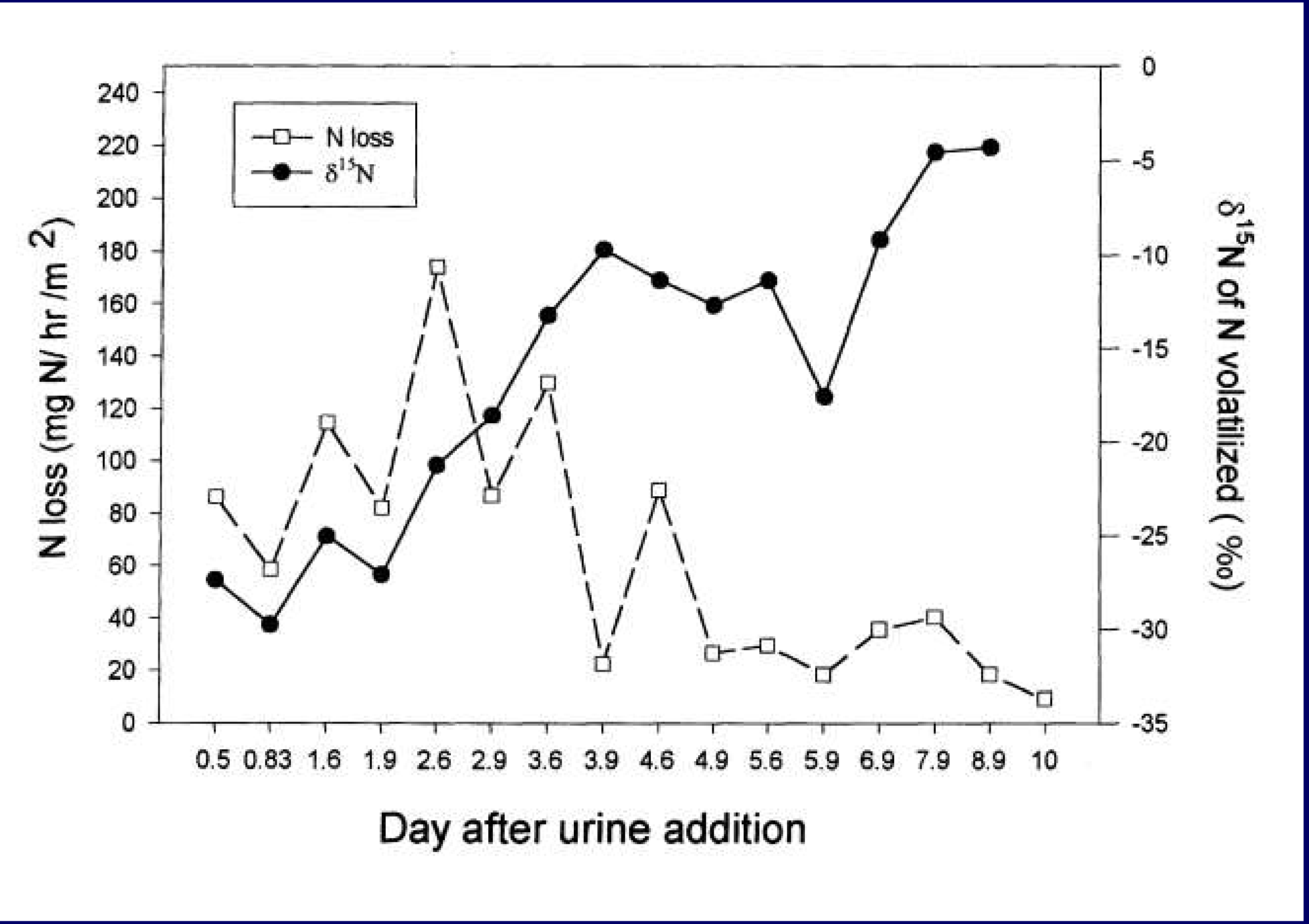


How can grazers increase soil $\delta^{15}N$?

$\delta^{15}N$

From: Frank and Evans (1997)

Nitrogen Loss: Volatilization

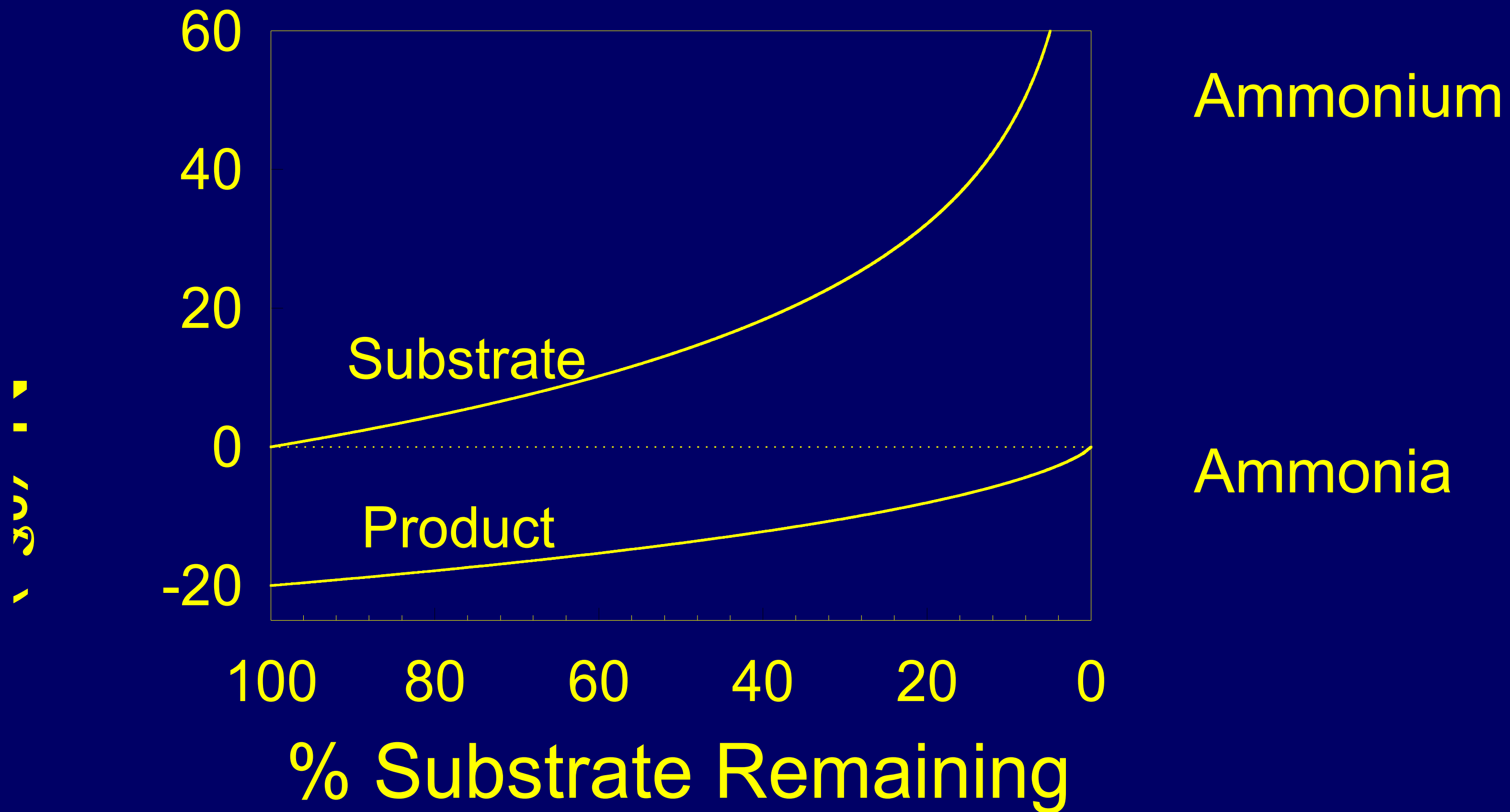


Elk and bison urine leads to an increase in volatilization

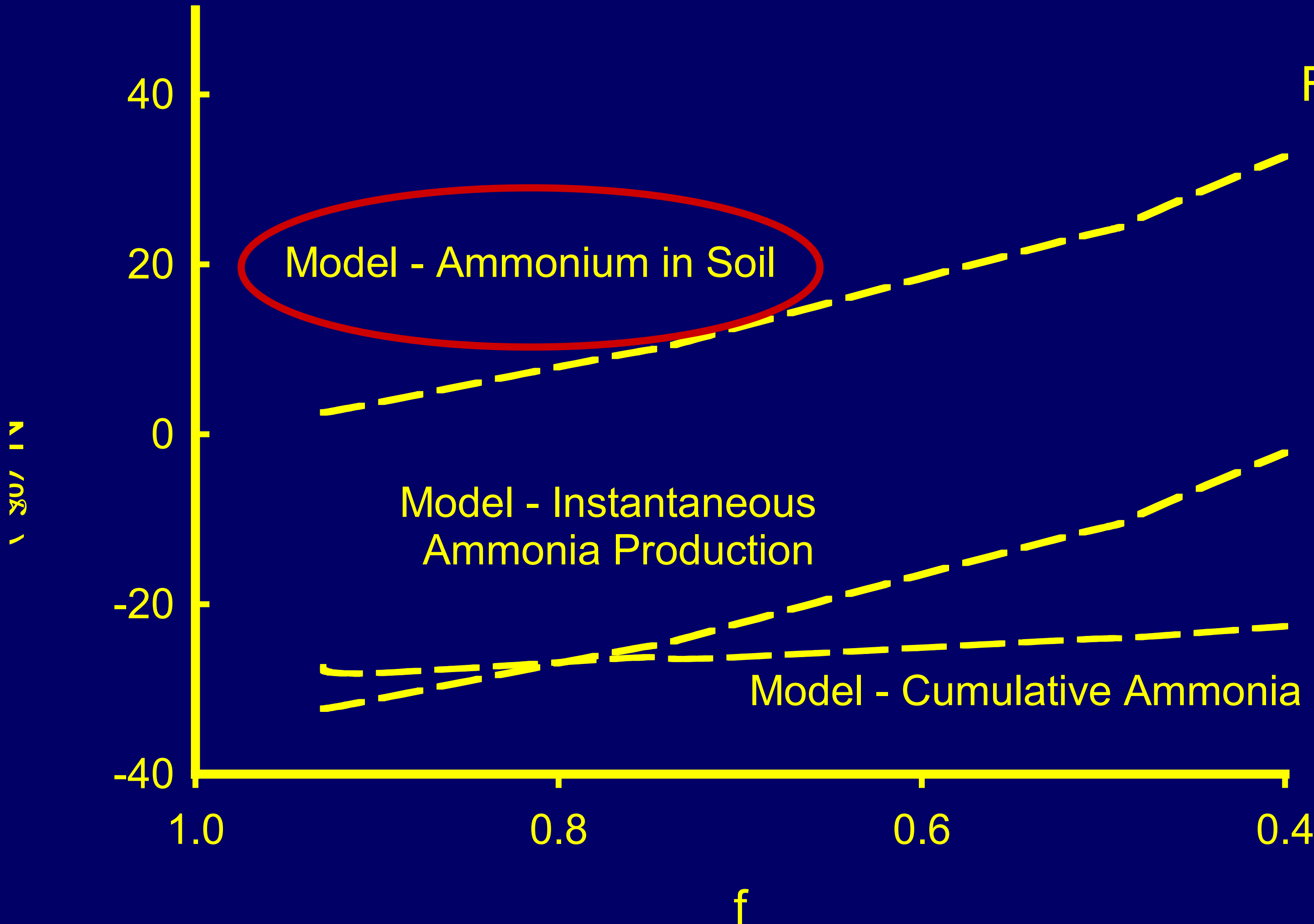
NH_3 is becomes enriched over time

From: Frank and Evans (2004)

Nitrogen Loss: Volatilization



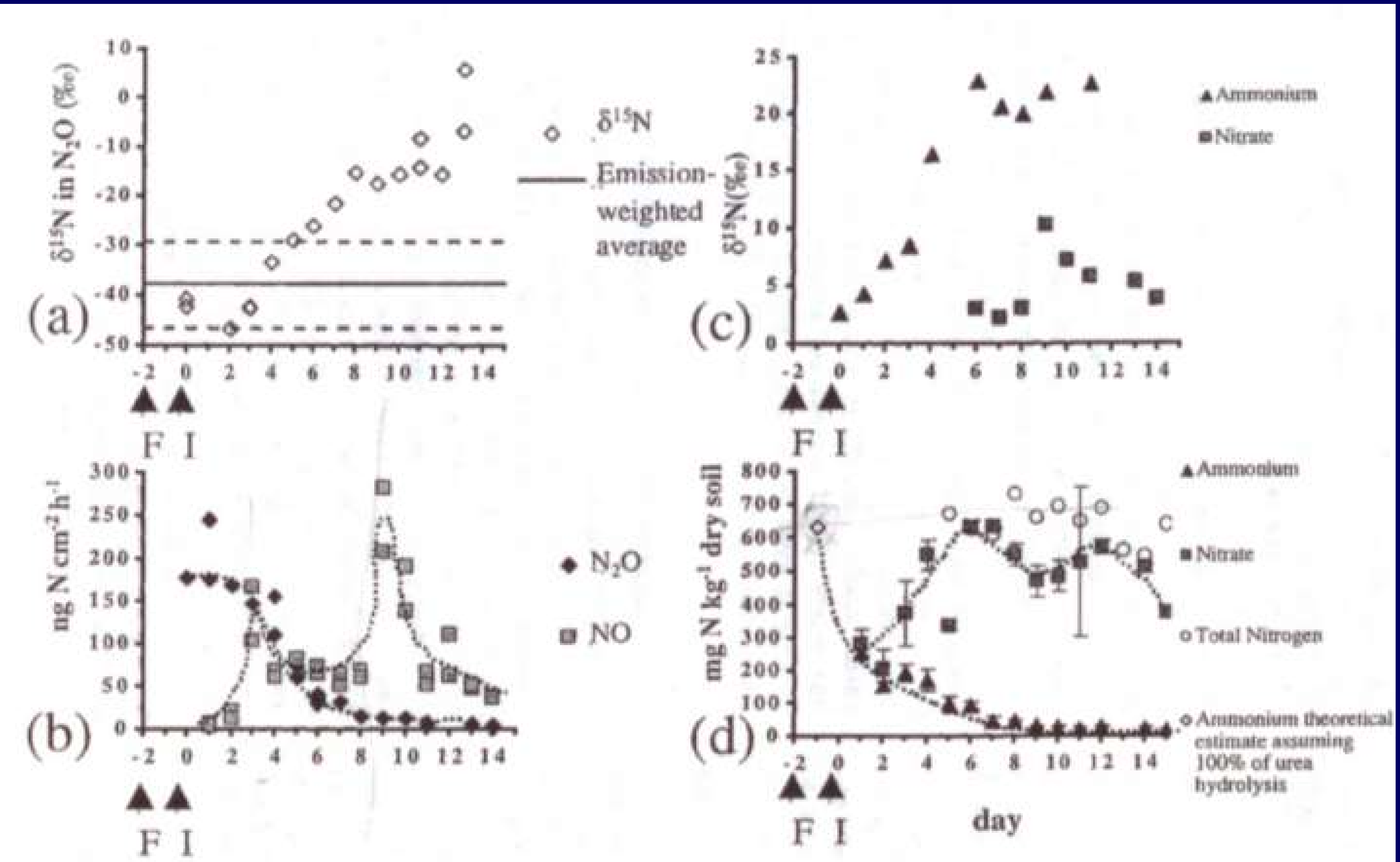
Nitrogen Loss: Volatilization



Fractionation during volatilization enriches remaining ammonium in soil, and this enriched ammonium is immobilized by soil microbes, retaining it in the soil

From: Frank and Evans (2004)

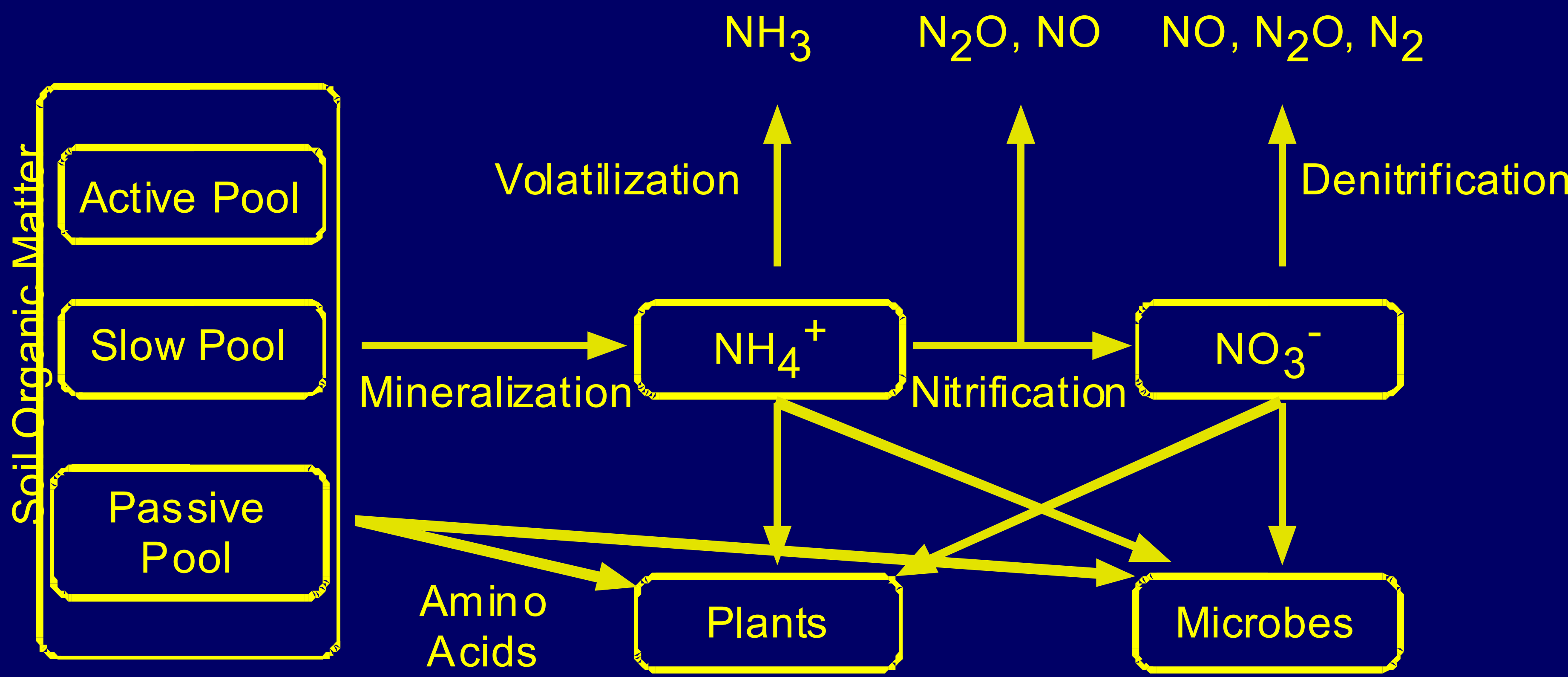
Nitrogen Loss: Nitrification and Denitrification



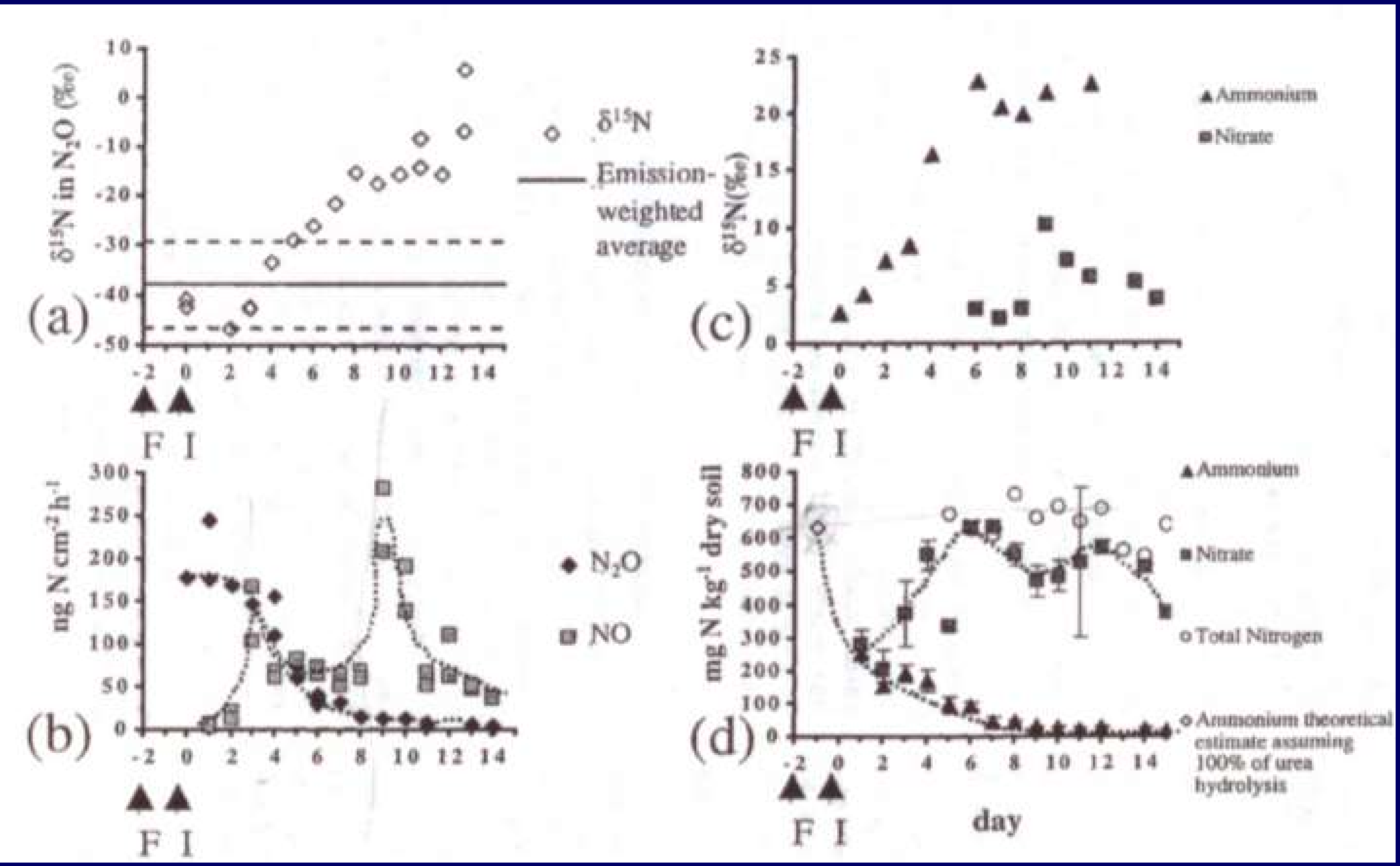
Same pattern observed for nitrification and denitrification

From: Perez et al. (2001)

Soil Nitrogen Transformations



Nitrogen Loss: Nitrification and Denitrification



Same pattern observed for nitrification and denitrification

From: Perez et al. (2001)

Lecture – Part 1

Soil $\delta^{15}N$

Plant $\delta^{15}N$

Models and Patterns of Soil $\delta^{15}N$

General Models of Soil $\delta^{15}N$

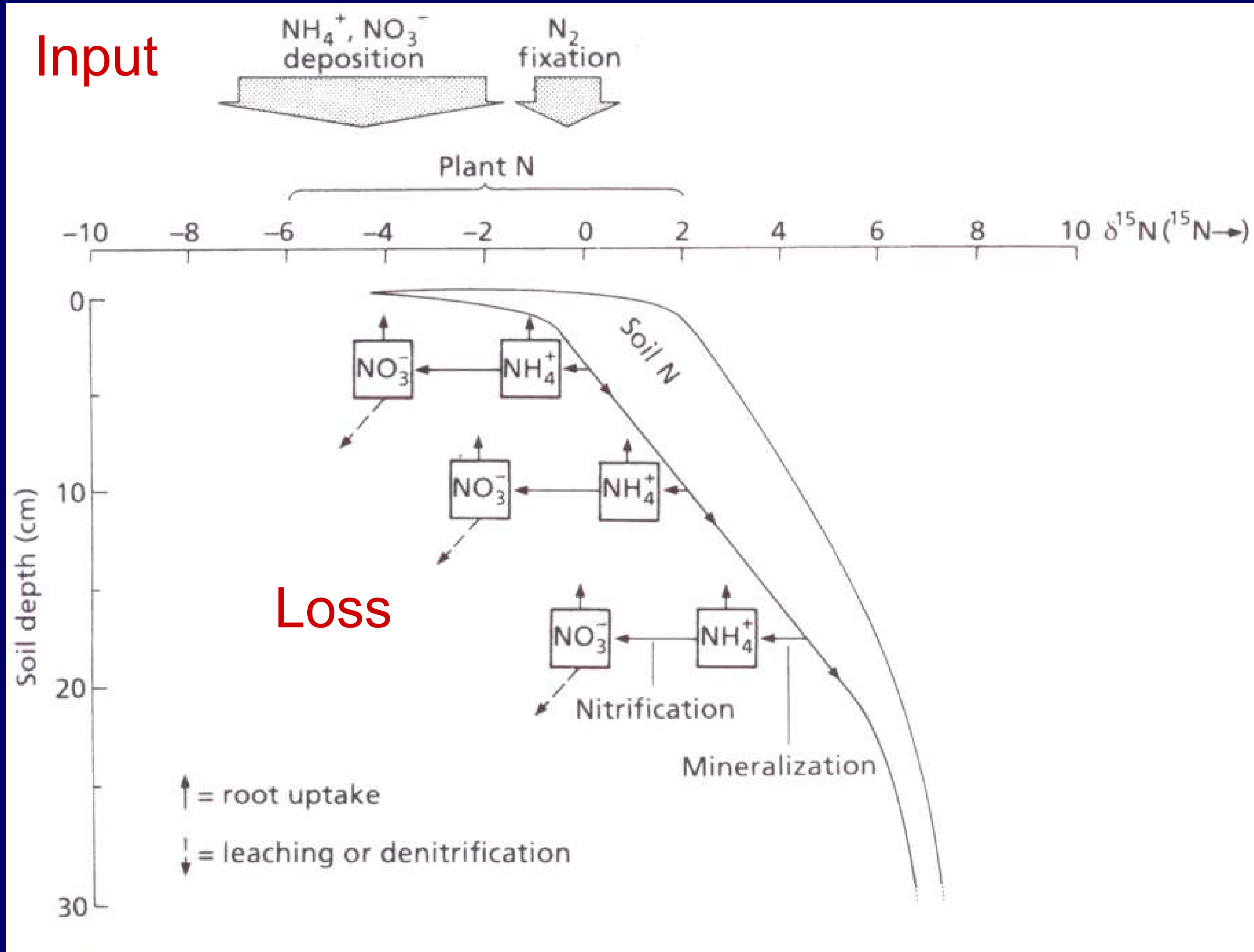
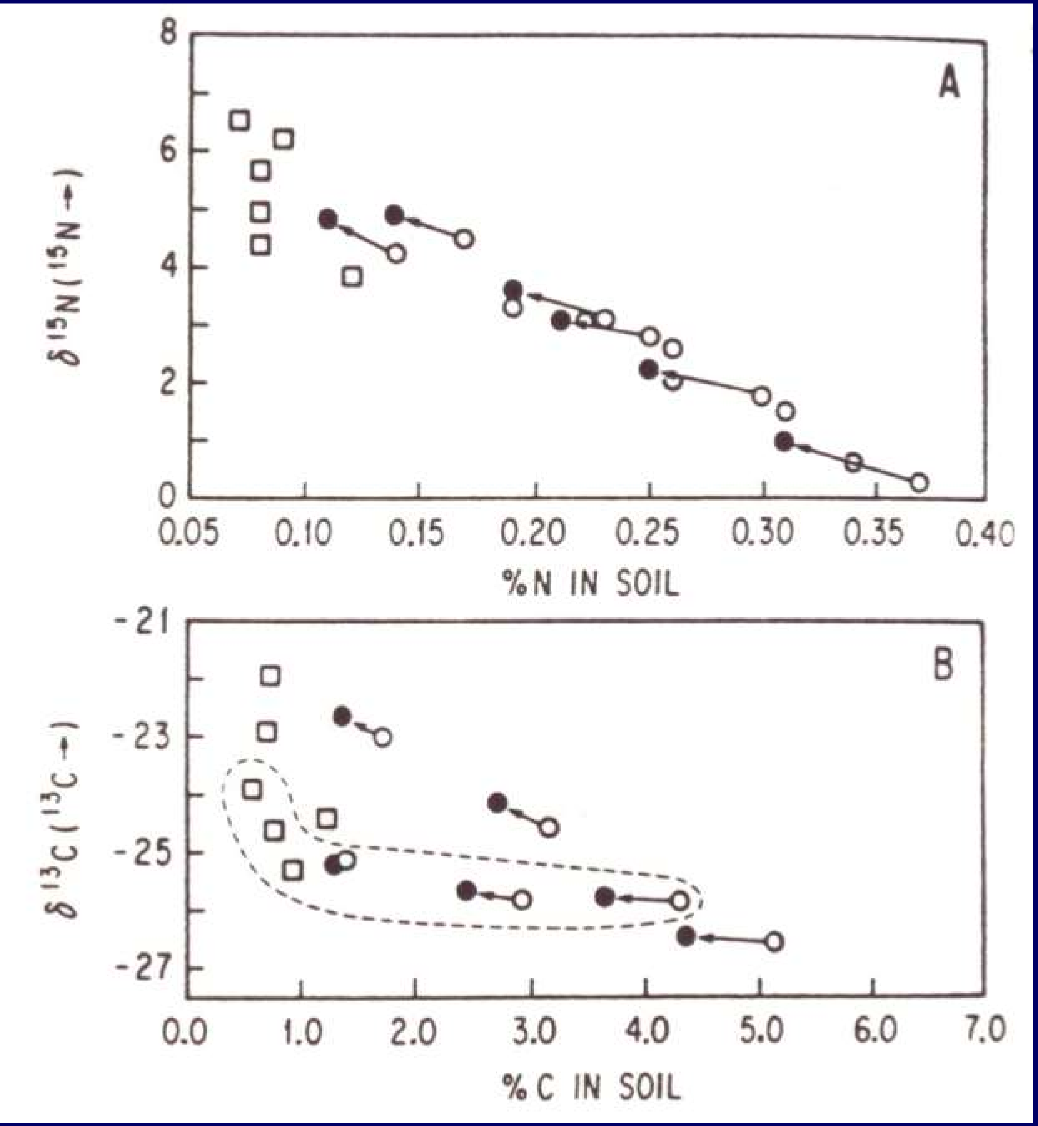


Fig. 2.4 A hypothetical model of isotope fractionations during nitrogen transformations in forest ecosystems. See text for discussion.

From: Nadelhoffer and Fry (1994)

General Models of Soil $\delta^{15}N$



Role of differential preservation of compounds that have different isotope composition?

Long-term incubation

No difference in $\delta^{15}N$ of nonpolar extracts, hot water solubles, holocellulose, and lignin

From: Nadelhoffer and Fry (1988)

General Models of Soil δ^{15}N

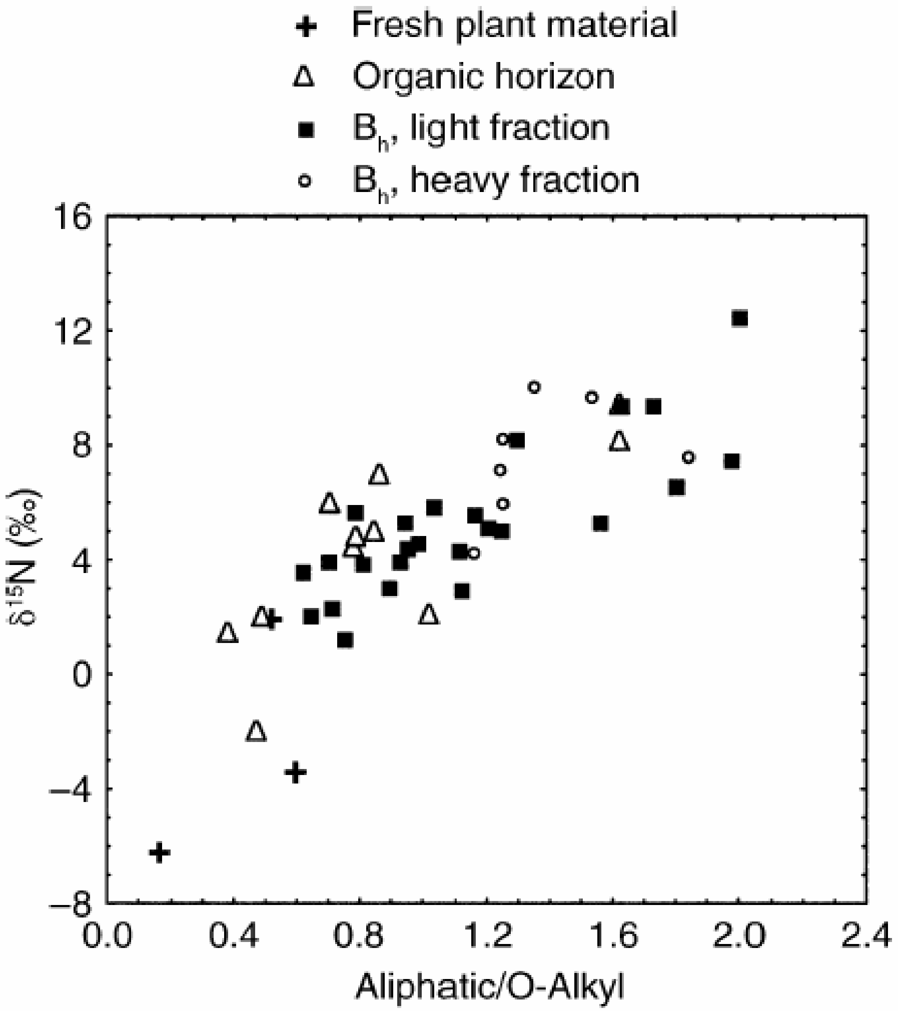


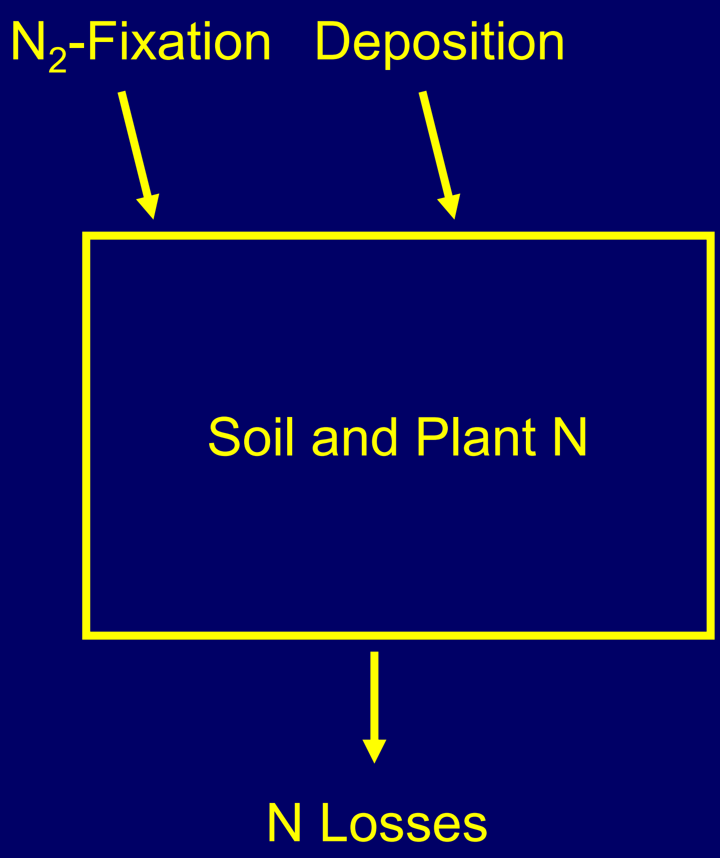
FIG. 1. $^{15}N_{PDB}$ abundance vs. aliphaticity (R^2 = 0.68, n = 47, P < 0.0001). Nuclear magnetic resonance (NMR) spectra were obtained for solid-state samples from fresh source materials and from sequentially deeper organic (O_{iea}) and mineral-soil (B_h) horizons, which were physically fractionated based on particle density at the University of Washington, Seattle, Washington, USA.

Aliphatic / O-Alkyl is a measure of humification

Kramer et al. (2003)

General Models of Soil $\delta^{15}N$

Steady-State Conditions



Storage

$$N_s = \left(\frac{I_{ex} + I_{fix}}{k_{ex}} \right)$$

Isotope Ratio

$$R_s = \left(\frac{R_{ex} I_{ex} + R_{fix} I_{fix}}{I_{ex} + I_{fix}} \right) = \frac{\bar{R}_{total}}{\alpha_{ex}}$$

N_s: Soil N (kg / m^2)
I_{ex}, I_{fix}: Inputs from deposition and fixation (kg m^{-2} y^{-1})
k_{ex}: Fractional rate of loss (y^{-1})
R_s: Isotope ratio of soil organic matter
R_{ex}, R_{fix}: Isotope ratio of inputs
$\bar{R}_{total}$: Weighted mean isotope ratio of inputs
α_{ex}: "Apparent fractionation factor"

From: Brenner et al. (2001)

General Models of Soil $\delta^{15}N$

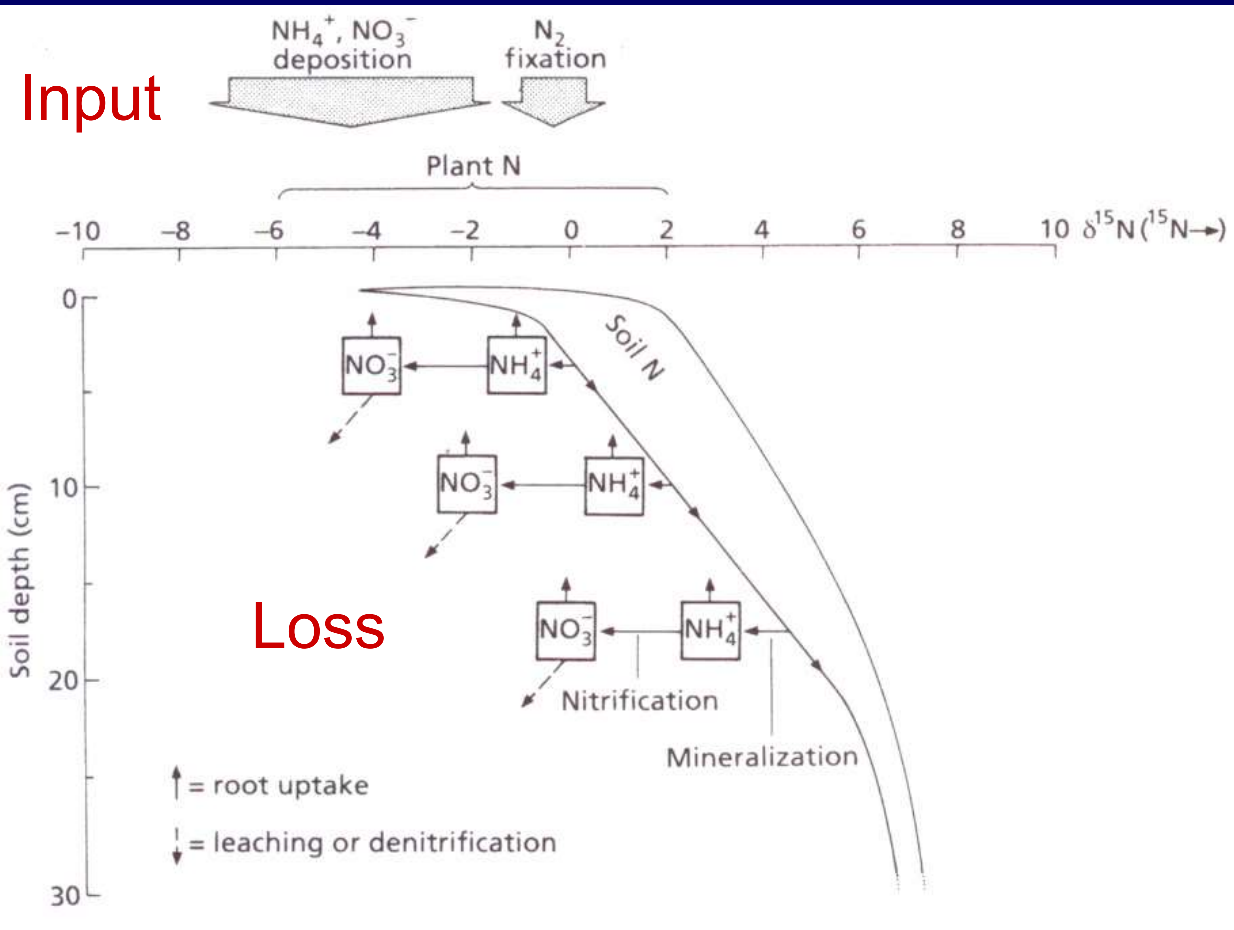


Fig. 2.4 A hypothetical model of isotope fractionations during nitrogen transformations in forest ecosystems. See text for discussion.

$$R_s = \left[\frac{R_{ex} I_{ex} + R_{fix} I_{fix}}{I_{ex} + I_{fix}} \right] = \frac{\bar{R}_{total}}{\alpha_{ex}}$$

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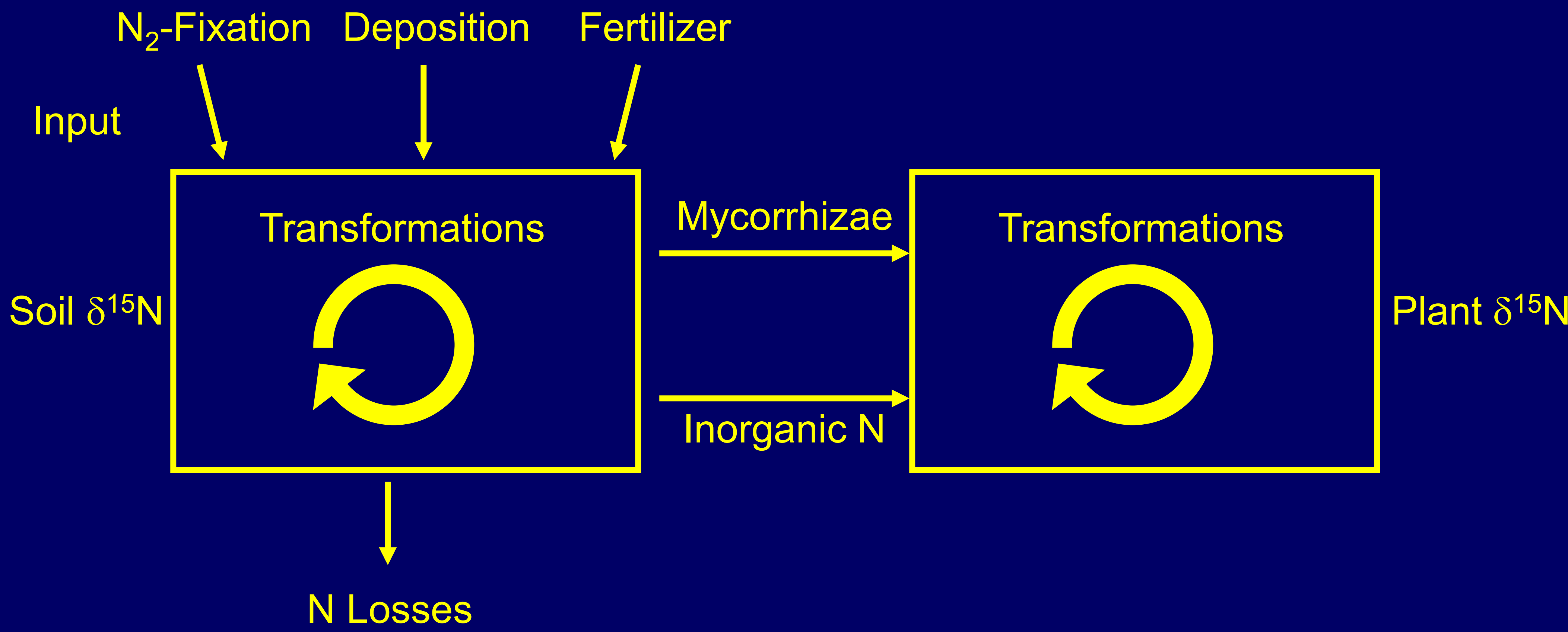
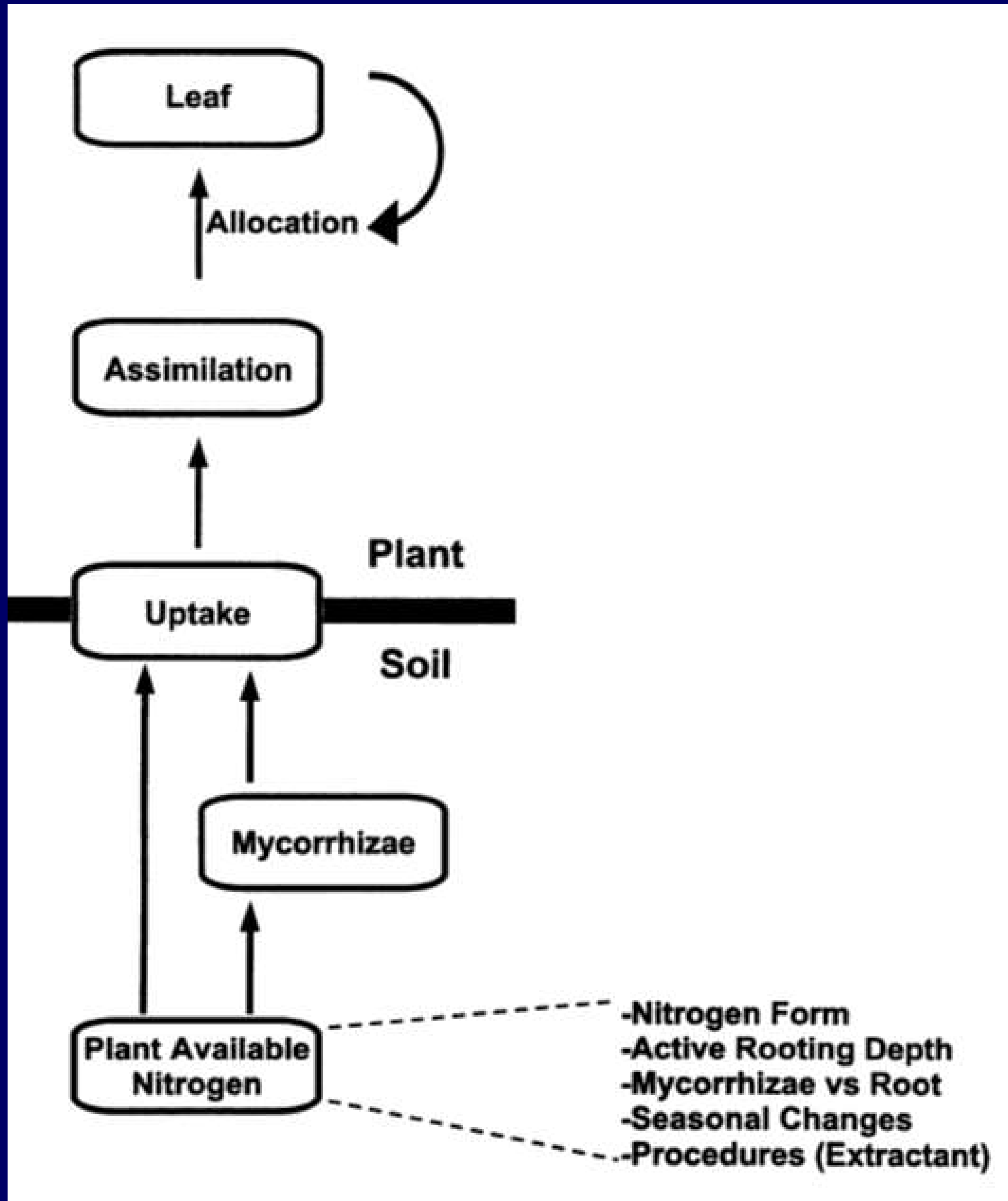
- https://nextcloud.bgc-jena.mpg.de/s/yP83SARmDSQjBAq

Lecture – Part 2

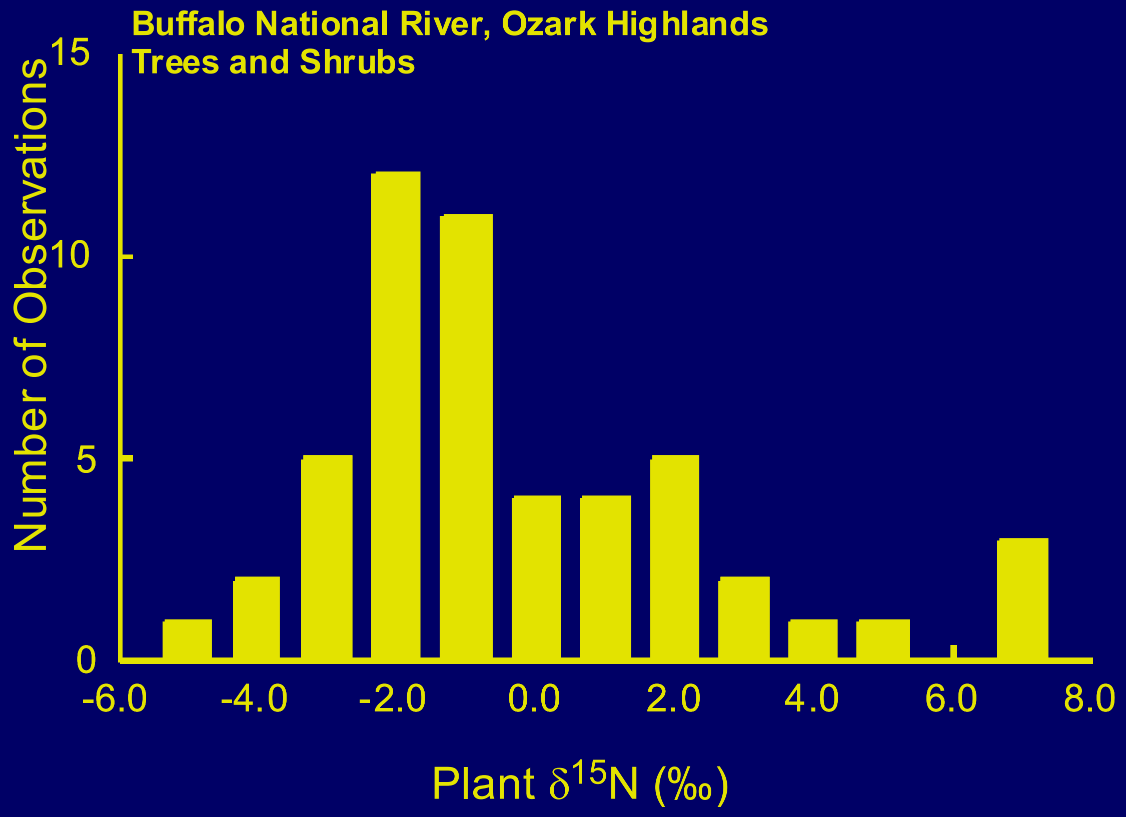


Models and Patterns of Soil $\delta^{15}N$

What Controls Plant $\delta^{15}N$?



What Controls Plant $\delta^{15}N$?



Kinsey and Evans, Unpublished Data

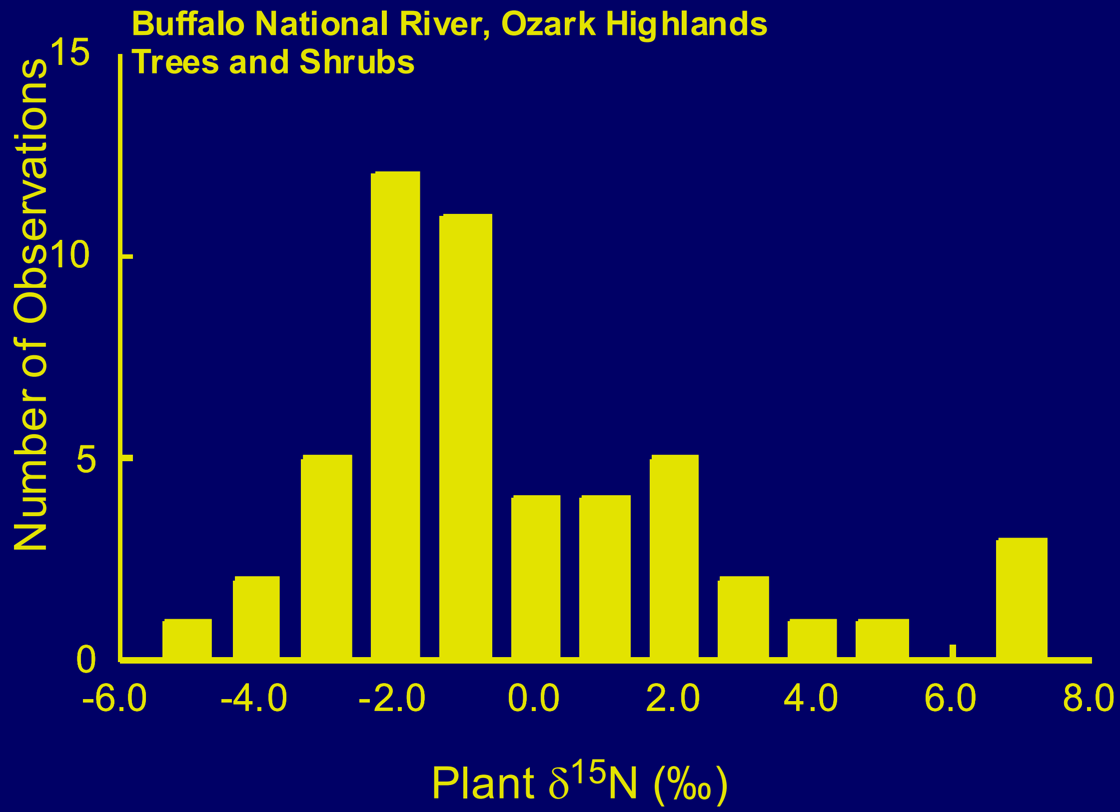
The Perfect World

1. No observed discrimination with uptake of N

2. Leaf $\delta^{15}N$ reflects that of the entire plant.

3. If 1 and 2 are true, then leaf $\delta^{15}N$ reflects that of the N source, and leaf $\delta^{15}N$ can be used as a tracer.

What Controls Plant $\delta^{15}N$?



Kinsey and Evans, Unpublished Data

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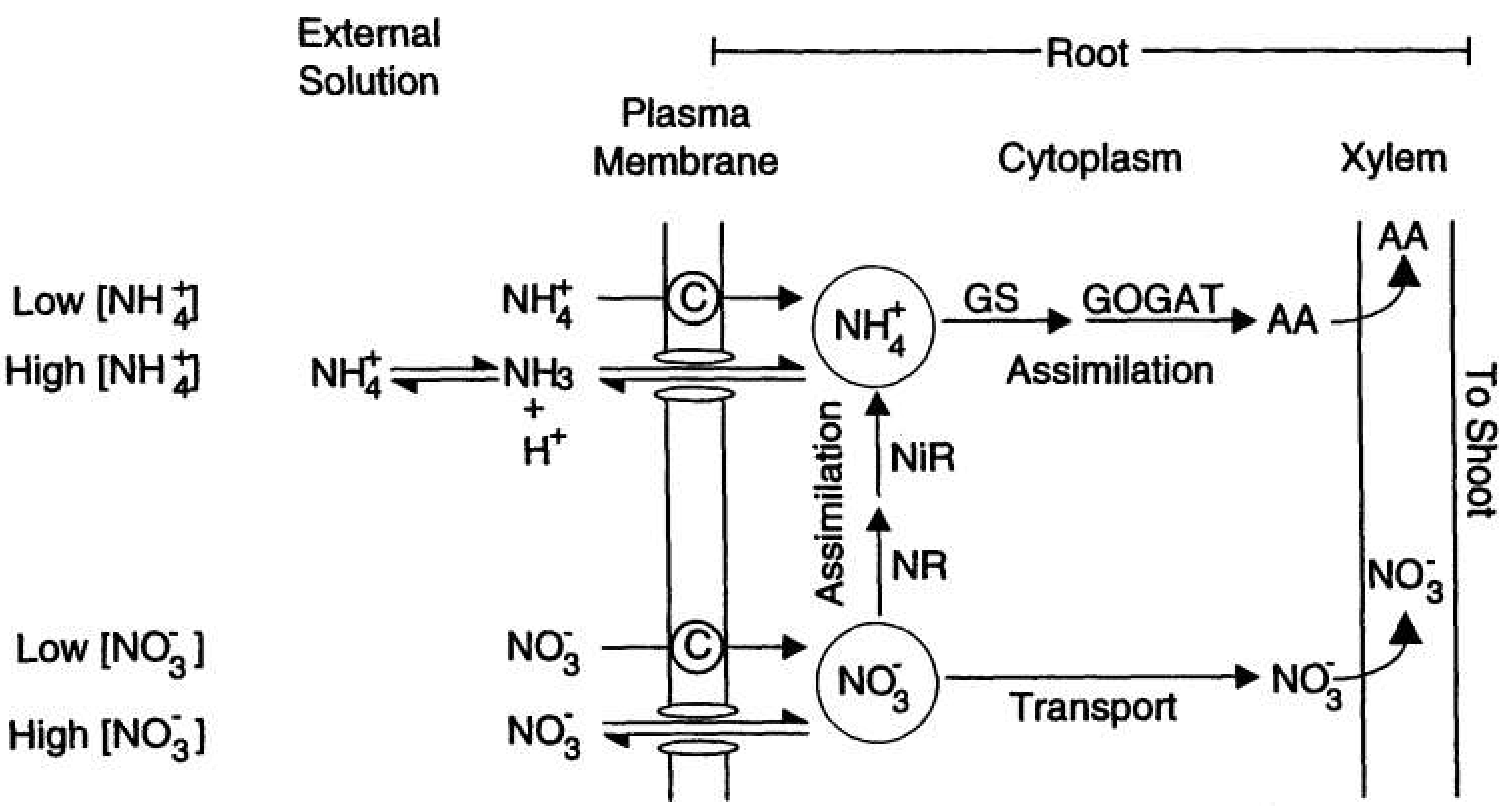
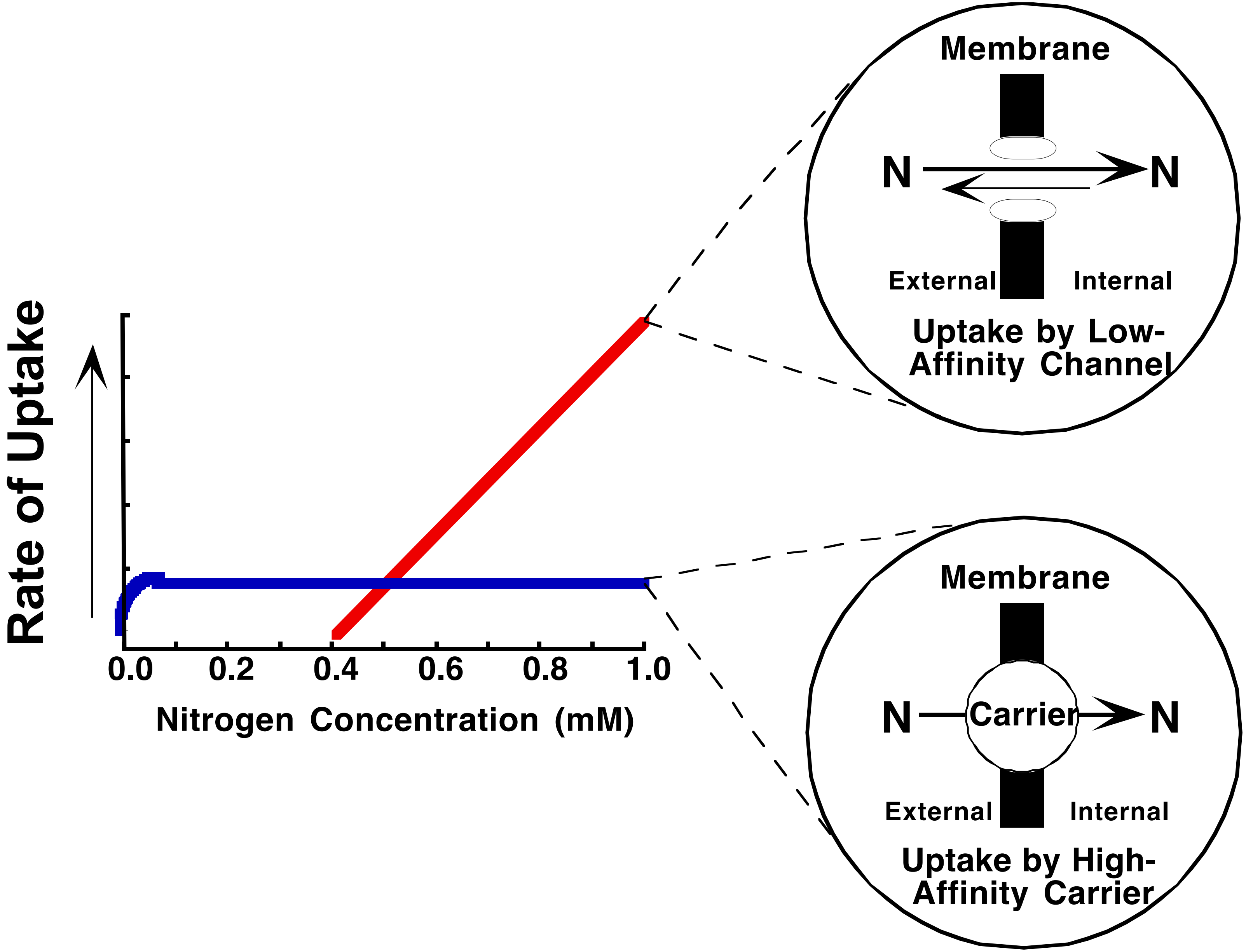
What Controls Plant $\delta^{15}N$?



Adapted from Ullrich, 1992

Steps
1. Absorption
2. Translocation
3. Assimilation

What Controls Plant $\delta^{15}N$?



What Controls Plant $\delta^{15}N$?

	Ammonium			Nitrate	
	mM	Δ (‰)		mM	Δ (‰)
O. sativa	1.4	4.1	*P. americanum*	0.5	0.0
	7.2	12.6		6.0	1.4
O. sativa	1.4	4.6		12.0	1.4
	7.2	11.2	*P. mollissimum*	0.5	0.1
				6.0	2.5
				12.0	3.3
			G. max	5.0	5.0
				7.5	3.7
			L. perenne	7.5	6.5
			T. erecta	7.5	5.8
			B. campestris	1.2	0.2
				3.0	0.0
				12.0	0.2

What Controls Plant $\delta^{15}N$?

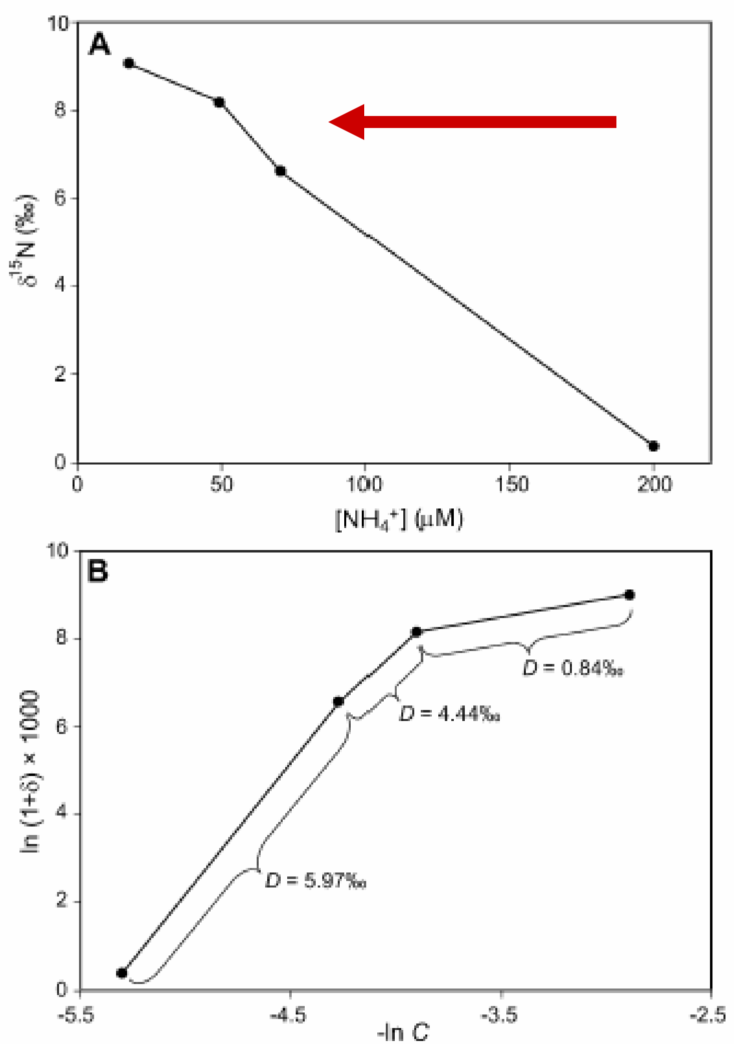


Fig. 7 Data from a typical substrate depletion experiment showing a changes in $\delta^{15}N$ of residual NH_4^+ as substrate is consumed, and b the same data plotted to show how the discrimination factor (*D*) is estimated between sample points. If *D* were unaffected by substrate concentration, $\delta^{15}N$ would increase more sharply at lower concentrations and *panel* b would yield a straight line through all data points

Substrate Depletion Experiments

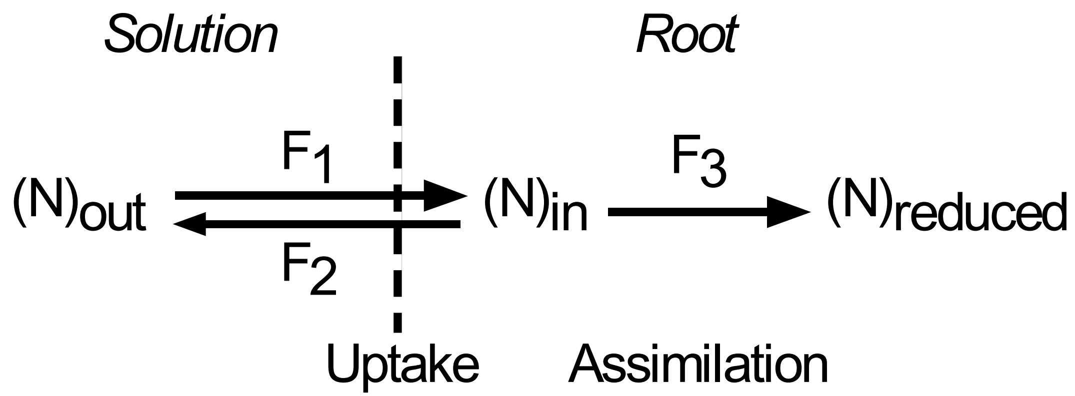
Simultaneously measure nutrient solution concentration and isotope composition as plants deplete the nutrient in solution

The isotope ratio of the solution should increase if fractionation occurs during uptake and assimilation

Plot $\delta^{15}N$ of solution versus concentration according to Rayleigh distillation model. The slope is the observed discrimination

Pritchard and Guy (2005)

What Controls Plant $\delta^{15}N$?



$$\Delta = b - \left(b \frac{F_3}{F_1} \right)$$

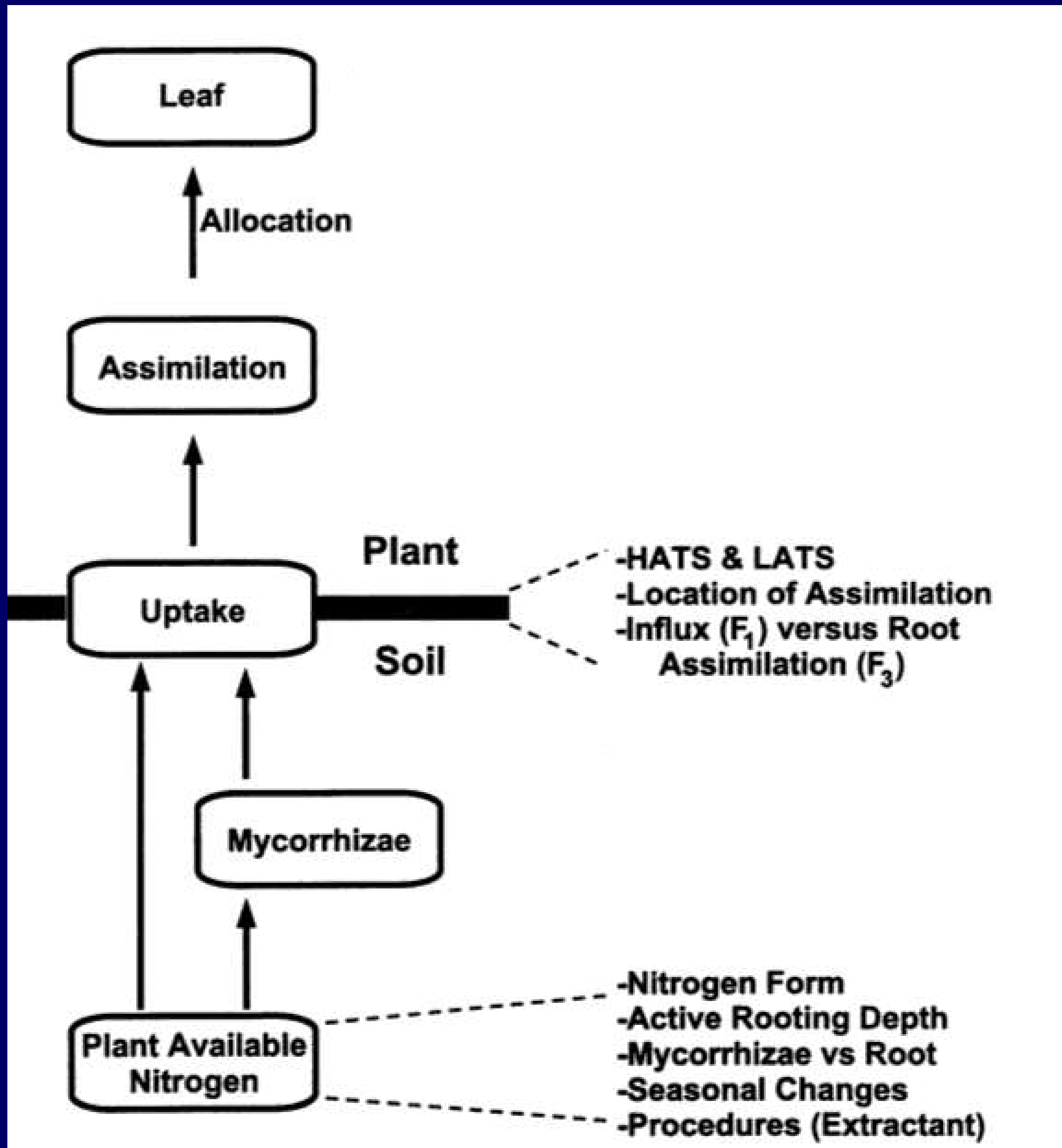
Mariotti et al. (1981) based on Sharkey and Berry (1985)

F1 >> F3 : Discrimination will be observed

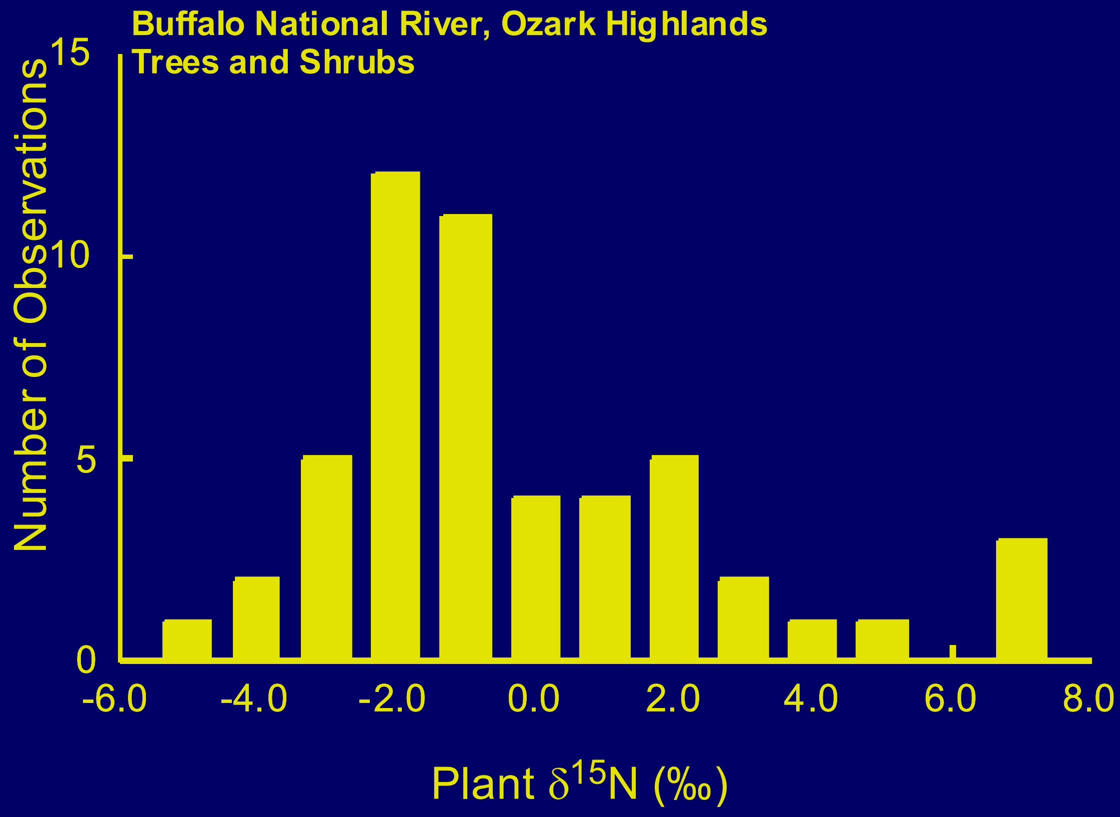
F1 = F3 : No discrimination will be observed

No enzyme in root : “b” not expressed

What Controls Plant $\delta^{15}N$?



What Controls Plant $\delta^{15}N$?



Kinsey and Evans, Unpublished Data

The Perfect World

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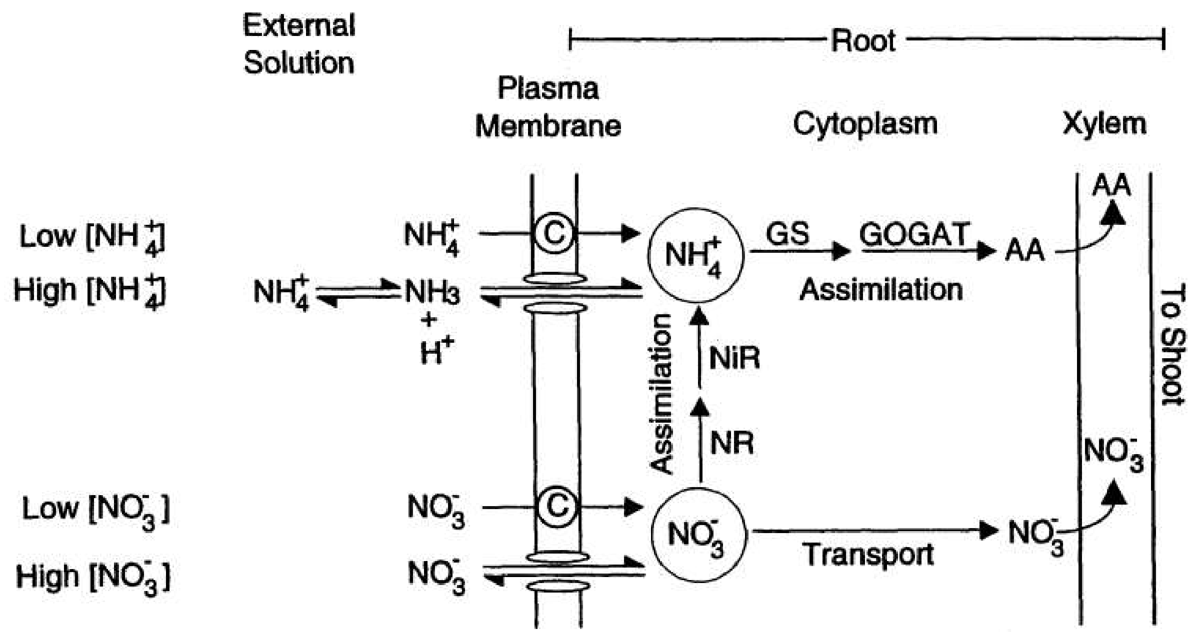
What Controls Plant $\delta^{15}N$?

Mean (±SE) $\delta^{15}N$ values (‰) for *Lycopersicon esculentum* plants grown with a continuous supply of 0.050 mM NH_4^+ or NO_3^-

	Plant Age (d)	Leaf	Root	Difference
NH_4^+				
	37	3.3 ± 0.3	3.1 ± 0.2	0.2
NO_3^-				
	28	4.2 ± 0.3	-1.6 ± 0.2	5.8
	36	3.7 ± 0.6	-1.1 ± 0.4	4.8
	45	3.3 ± 0.6	-0.1 ± 0.4	3.4

What Controls Plant $\delta^{15}N$?



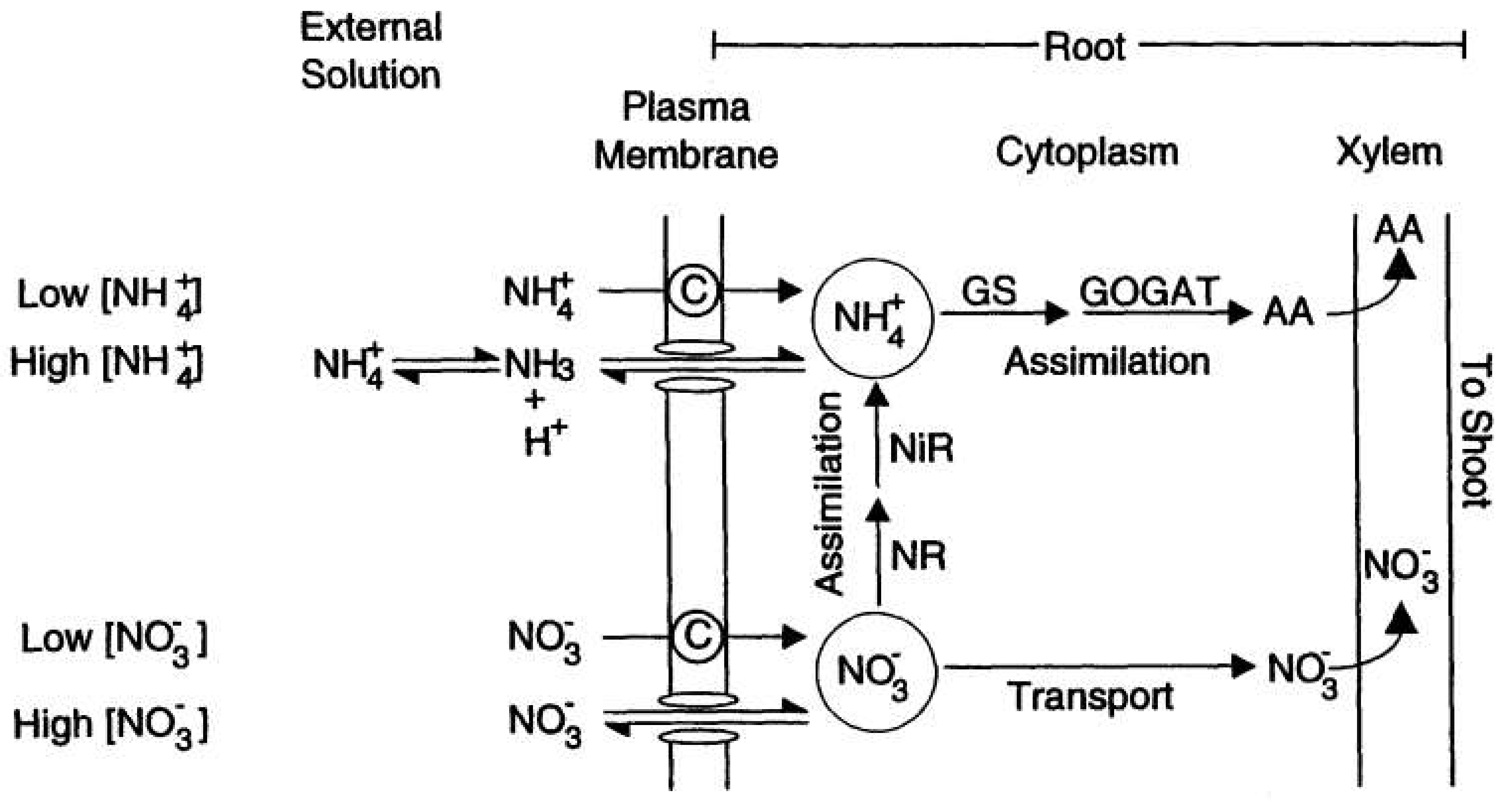
Adapted from Ullrich, 1992

The $\delta^{15}N$ (mean $\pm$ standard error) of source NO_3^-, whole-plants, roots, leaves, and root and leaf NO_3^- for *Brassica campestris* and *Lycopersicon esculentum*.

Species	Source	Plant	Root	Root NO_3^-	Leaf	Leaf NO_3^-
Brassica	10.3	10.1	4.9	12.4	10.6	25.0
Lycopersicon	1.8 ± 0.1	2.5 ± 0.4	-0.1 ± 0.4	11.1 ± 1.7	3.3 ± 0.6	14.0 ± 4.6

From: Evans (2001)

What Controls Plant $\delta^{15}N$?



Adapted from Ullrich, 1992

What Controls Plant $\delta^{15}N$?

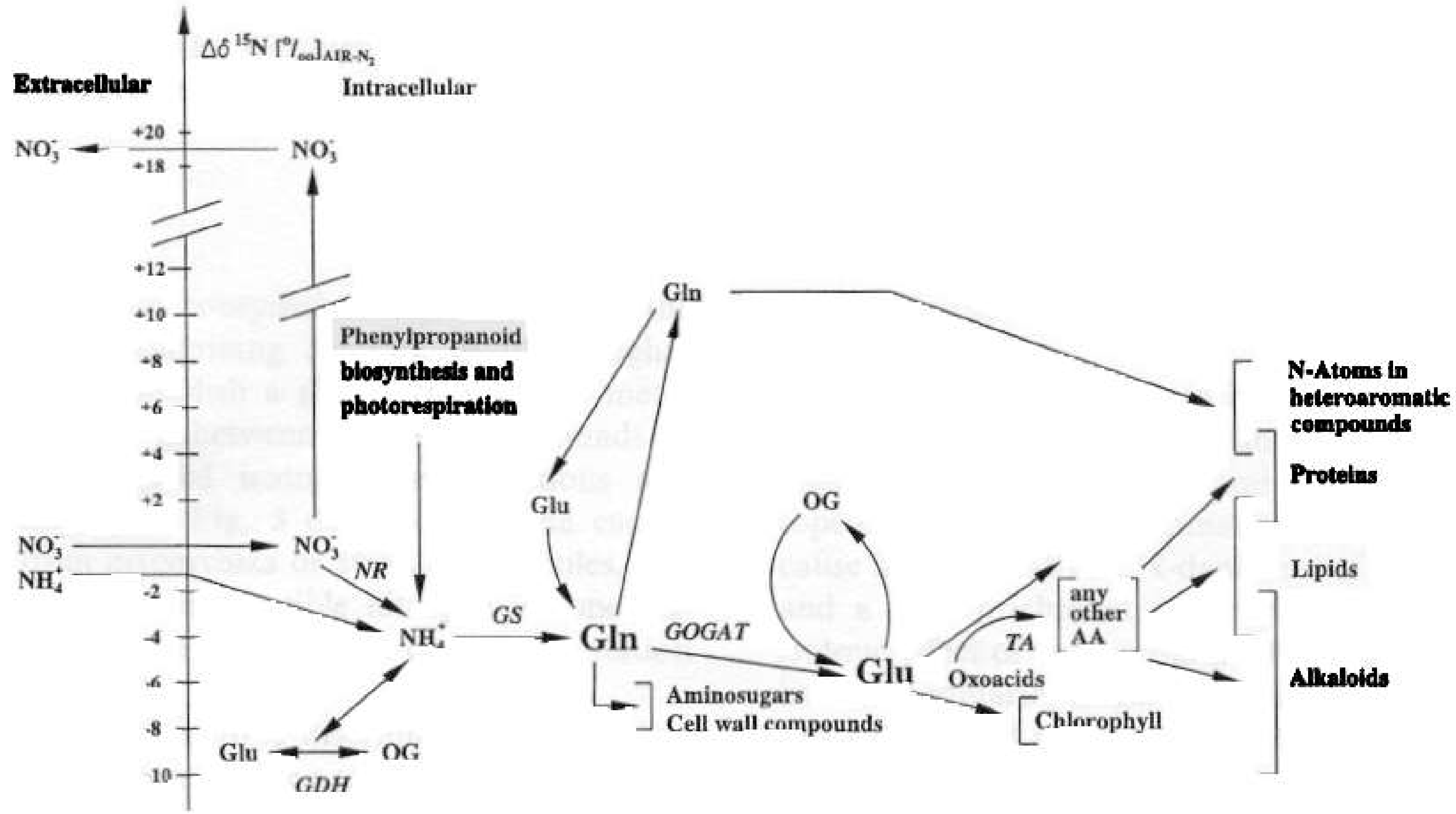


Fig. 3. Generalised mean $\delta^{15}N$-value shifts between organic compounds within the system plant. The first intrinsic precursor with a small metabolic pool, but large turnover is NH_4^+, originating from different sources. From here, the plant can be more or less regarded as a "closed system" (except for losses of NH_3). The display does not integrate the effects of compartmentation, pools and metabolite transports. Central reaction of nitrogen isotope discrimination is the GOGAT reaction, involved in the net primary production of α-amino-N, but also in the N-recycling processes phenylpropanoid biosynthesis and photorespiration. Enzymes: *NR* = nitrate (+ nitrite) reductase, *GS* = glutamine synthetase, *GOGAT* = glutamine:2-oxoglutarate amino transferase, *GDH* = glutamate dehydrogenase, *TA* = transaminases, *GF* = glutamine:fructose-6-P amino transferase. Substrates: OG = 2-oxoglutarate, OA = oxoacids, AA = amino acids.

Werner and Schmidt (2002)

What Controls Plant $\delta^{15}N$?

Table 3
$\delta^{15}N$-Values [‰]$_{AIR\text{-}N_2}$ of secondary natural compounds

Compound (*n* in parentheses)		Origin, plant (reference and its $\delta^{15}N$[‰]$_{AIR\text{-}N_2}$)	Biosynthetic N-precursor ($\delta^{15}N$[‰]$_{AIR\text{-}N_2}$ in proteins)	$\delta^{15}N$[‰]$_{AIR\text{-}N_2}$	Reference
Heroin[a]	(5)	*Papaver somniferum*	Tyrosine (~ +6.0‰)	−3.6 to +1.7	Ihle and Schmidt, 1996
	(?)			−8.5 to −1.5	Avak et al., 1996
	(20)			−1.6 to +1.3	Zimmer, 1999
	(?)			−4.3 to +0.5	Besacier and Chaudron-Thozet, 1999
Heroin[a], Morphine	(20)			−2.5 to +2.5	Ehleringer et al., 1999
Cocaine	(4)	*Erythroxylon coca* (coca leaves +6.5‰)[b]	Ornithine (δ-amino N) (~ −4.0‰)	−13.7 to −5.4	Ihle and Schmidt, 1996
	(?)			−13.0 to −5.5	Avak et al., 1996
	(20)			−12.0 to −3.5	Zimmer, 1996
	(20)			−12.0 to −5.0	Ehleringer et al., 1999
Nicotine	(21)	*Nicotiana tabacum* (tobacco leaves +2.9‰)[c]	Ornithine (α- + δ-amino N) (0‰), aspartic acid (+8.5‰)	−5.2±0.5	Jamin et al., 1997
Caffeine	(22)	*Coffea arabica*, *Theobroma sinensis*	Glycine (+2.5‰), glutamine (2 * + 6‰), aspartic acid (−5‰)	+2.6±2.0	Danho et al., 1992
	(?)			+3.0 to +5.0	Weilacher et al., 1996
Methyl-*N*-methyl-anthranilate	(20)	Mandarin essential oil (fruit pulp +6‰)[d]	glutamine (+11‰)	+4.2 to +8.3	Faulhaber et al., 1997

Mean $\delta^{15}N$-values used in text are calculated from the references.

[a] Semisynthetic from morphine.
[b] $\delta^{15}N$-Values of coca leaves: +0.1 to +13.0‰ (Ehleringer et al., 2000).
[c] $\delta^{15}N$-Values of leaves from the same tobacco plants: 0 to +10.3‰ (Jamin, pers. commun.).
[d] $\delta^{15}N$-Values of pulp from orange juices: +4 to +8‰ (Kornexl et al., 1996).

Werner and Schmidt (2002)

What Controls Plant $\delta^{15}N$?

Table 2

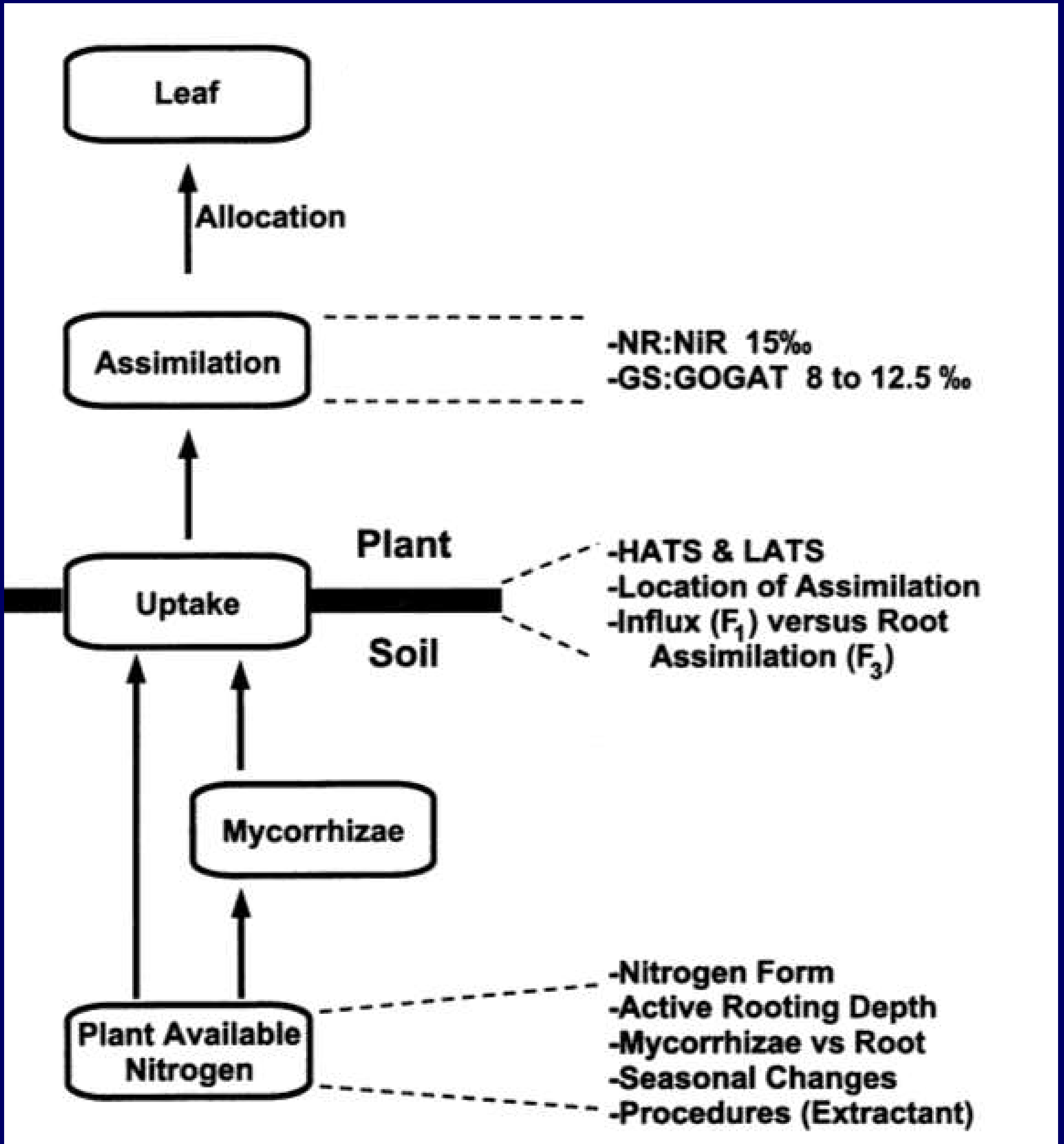
$\Delta\delta^{15}N$-Values $[‰]_{AIR\text{-}N_2}$ of amino acids from protein hydrolysates and of free amino acids from defined origin, normalized by difference to Glu (for *Triticum aestivum* I to Ala) = 0‰. *Triticum aestivum* I = soluble protein extracted at the two-leaf stage, *Triticum aestivum* II at the anthesis stage

Protein and origin	$\Delta\delta^{15}N[‰]_{AIR\text{-}N_2}$ in α-amino-N and total N of amino acid															Reference
	Asp	Gly	Ala	Val	Leu	Ile	Ser	Thr	Phe	Tyr	Lys	His	Arg	Trp	Pro	
Anabaena sp. strain IF on N_2	+1.5	−3.5	−3.0	−2.5	−8.5	−7.5	−10.0	−2.5	−1.0	−5.0	−4.5	−6.0	−7.5	n.d.	n.d.	Macko et al., 1987
Anabaena sp. strain IF on NO_3^-	+2.0	−2.0	−1.5	−2.5	−9.5	−4.5	−8.0	−2.5	+2.0	−1.5	−2.5	−6.5	−5.0	n.d.	n.d.	Macko et al., 1987
Triticum aestivum, I, greenhouse	+2.0	−5.5	0.0	0.0	−2.0	0.0	−3.0	[+12.0]	n.d.	+4.5	−3.0	n.d.	n.d.	n.d.	n.d.	Hofmann et al., 1997
Triticum aestivum, II, greenhouse	+1.0	−6.0	+1.0	−3.5	−5.0	−2.0	−4.5	+3.0	n.d.	+3.0	−4.5	n.d.	−1.0	n.d.	−3.5	Hofmann et al., 1997
Glycine max. (10%)/ *Hordeum vulg*. (78%)	+1.5	−2.1	+0.3	n.d.	n.d.	n.d.	−4.1	−3.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+1.2	Hare et al., 1991
Mean C_3-plants, homotroph	+1.6	−3.8	−0.65	−2.1	−6.25	−3.5	−5.9	−1.45	+0.5	+0.25	−3.6	−6.25	−4.5	n.d.	−1.15	
Zea mais	+2.3	+2.5	+1.4	+4.5	n.d.	n.d.	+1.0	−1.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+3.5	Hare et al., 1991
Vibrio harvey strain B-352 on Glu	−9.0	−5.0	−3.5	−5.5	−6.0	−3.5	−6.5	−3.5	−2.5	−3.5	−4.5	−5.5	−2.0	n.d.	n.d.	Macko et al., 1987
Bovine collagen	−2.3	−5.5	−3.8	−0.8	−2.0	−1.3	−6.5	−10.5	−1.8	−6.2	−5.3	−7.5	n.d.	−5.5	−0.5	Hürzeler, 1997
Achilles tendon collagen	n.d.	−3.8	n.d.	+1.1	−1.2	n.d.	n.d.	n.d.	n.d.	−3.8	−5.7	n.d.	−4.8	n.d.	0.0	Minagawa et al., 1992
Free amino acids from pea (*Pisum sativum*) nodules	+7.1	+0.4	n.d.	+7.5	n.d.	n.d.	n.d.	−0.1	n.d.	n.d.	n.d.	n.d.	+8.1	n.d.	n.d.	Yoneyama et al., 1998b

The mean absolute $\delta^{15}N$-value of Glu from plant proteins is ~ +7.0‰, that of total proteins ~ +5‰. n.d. = not determined. $\delta^{15}N$-value in [ ] is not considered for mean $\delta^{15}N$-value calculation. Some of the $\delta^{15}N$-values have been taken from graphical displays.

Werner and Schmidt (2002)

What Controls Plant $\delta^{15}N$?



What Controls Plant $\delta^{15}N$?

Resorption and Reallocation of N

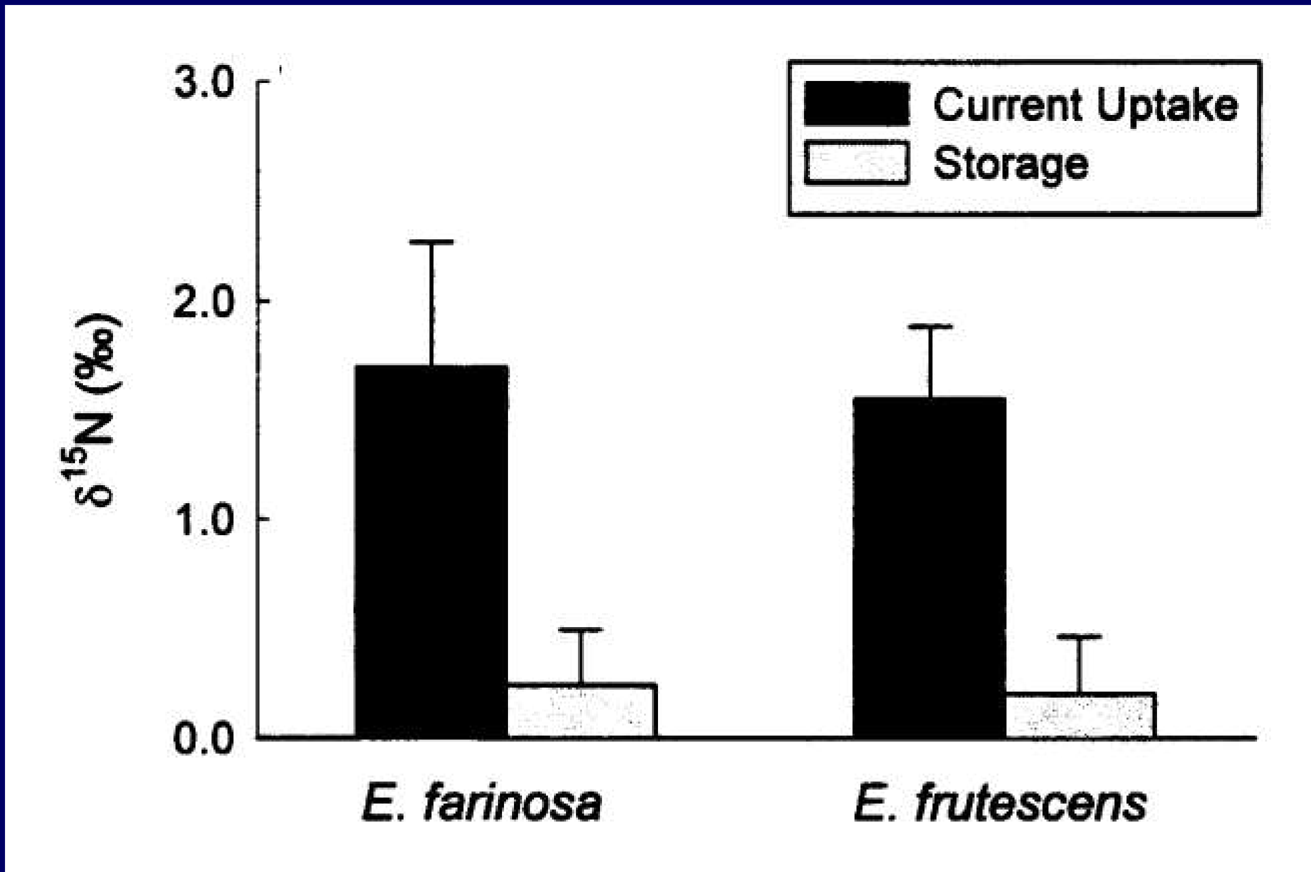
Fractionation with resorption? | Effect of resorption? | % Contribution of Stored N?

No Fertilizer | NH_4^+ NO_3^- | $^{15}NH_4^+$ $^{15}NO_3^-$

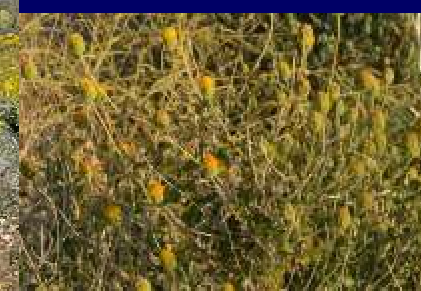
From: Kolb and Evans (2002)

What Controls Plant $\delta^{15}N$?

Resorption and Reallocation of N

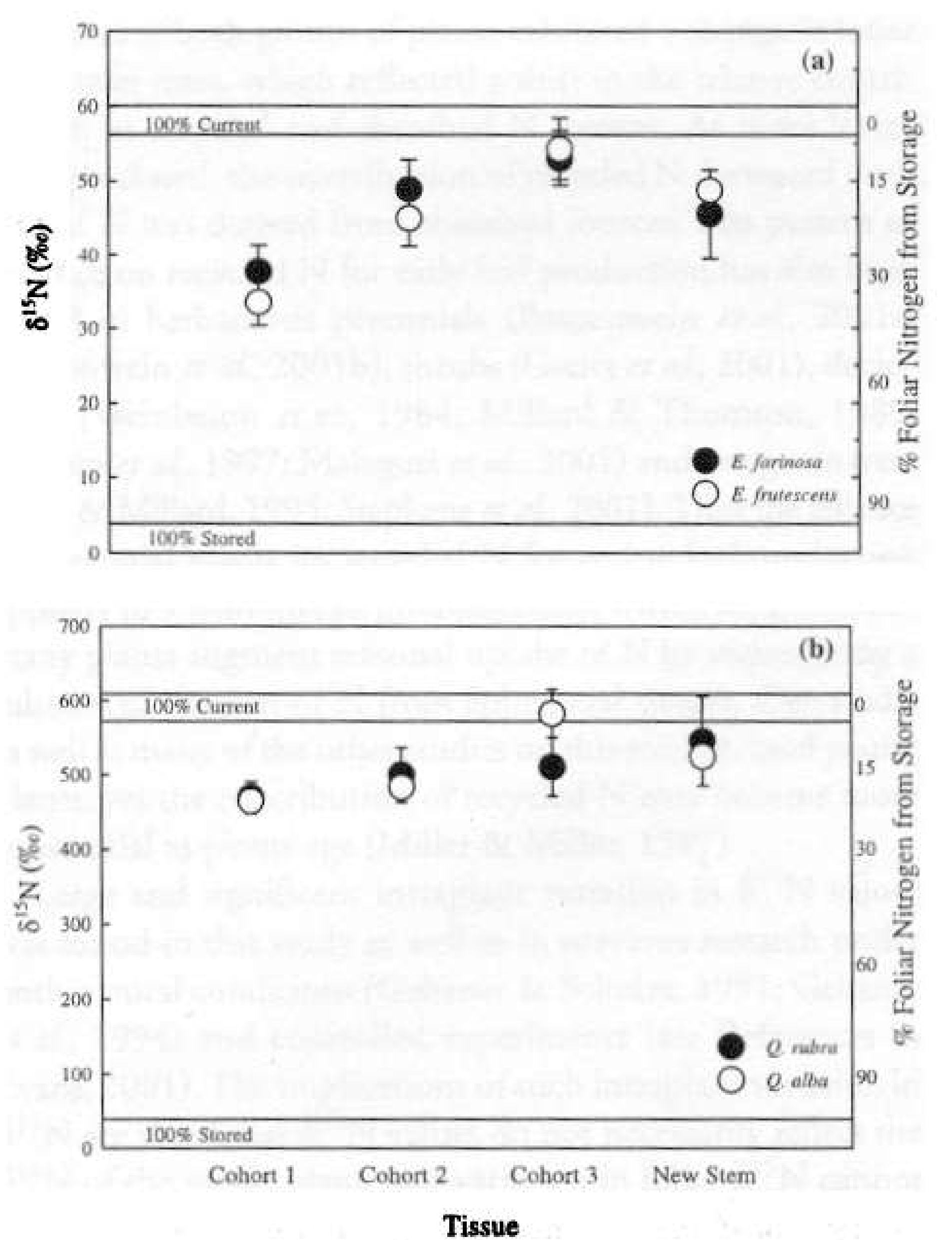


Discrimination is observed when stored N is the only N source



From: Kolb and Evans (2002)

What Controls Plant δ15N?



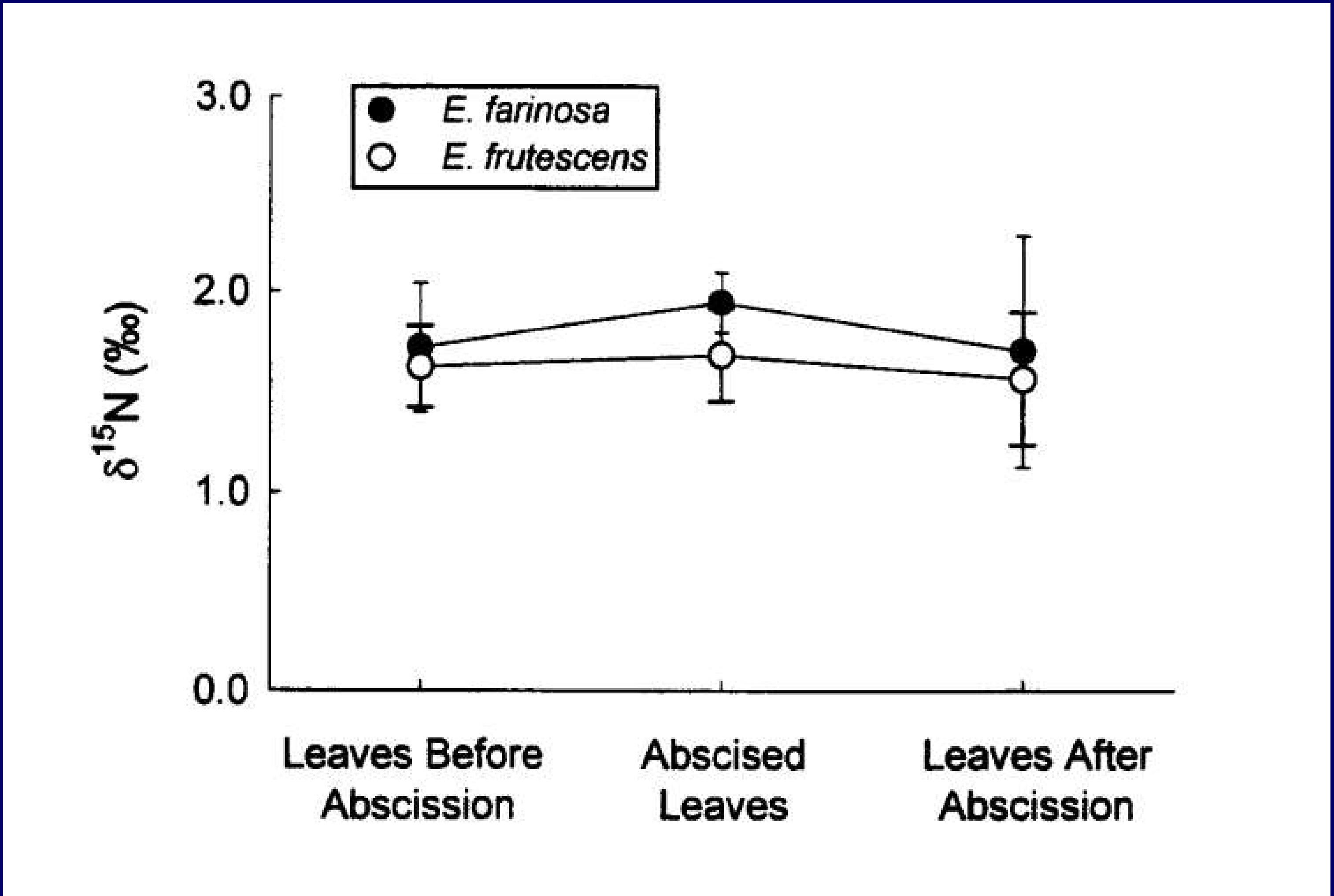
Resorption and Reallocation of N

However, the contribution of stored N is minimal

From: Kolb and Evans (2002)

What Controls Plant $\delta^{15}N$?

Resorption and Reallocation of N

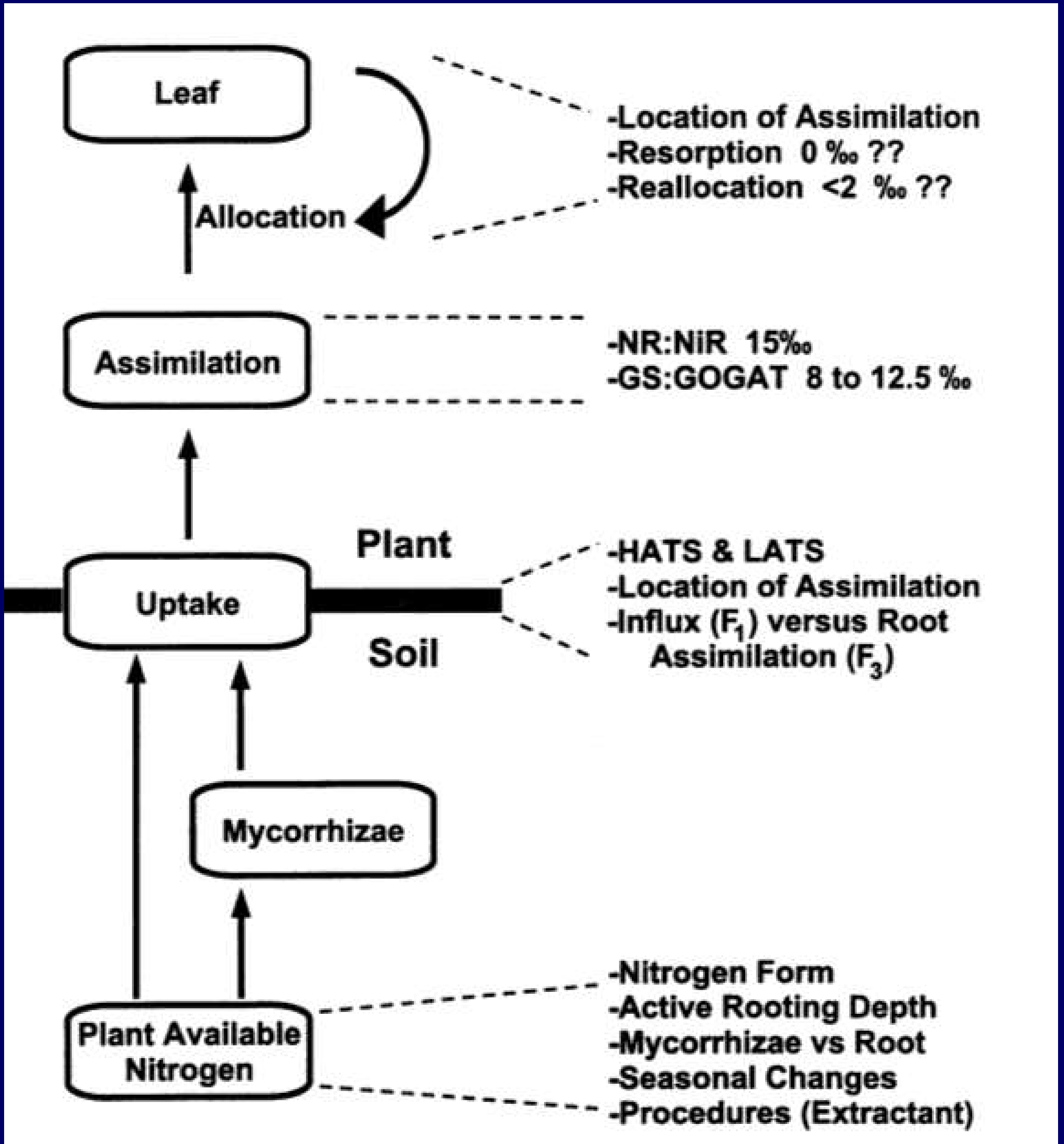


Discrimination not observed with:

1. Resorption
2. Reallocation

From: Kolb and Evans (2002)

What Controls Plant $\delta^{15}N$?



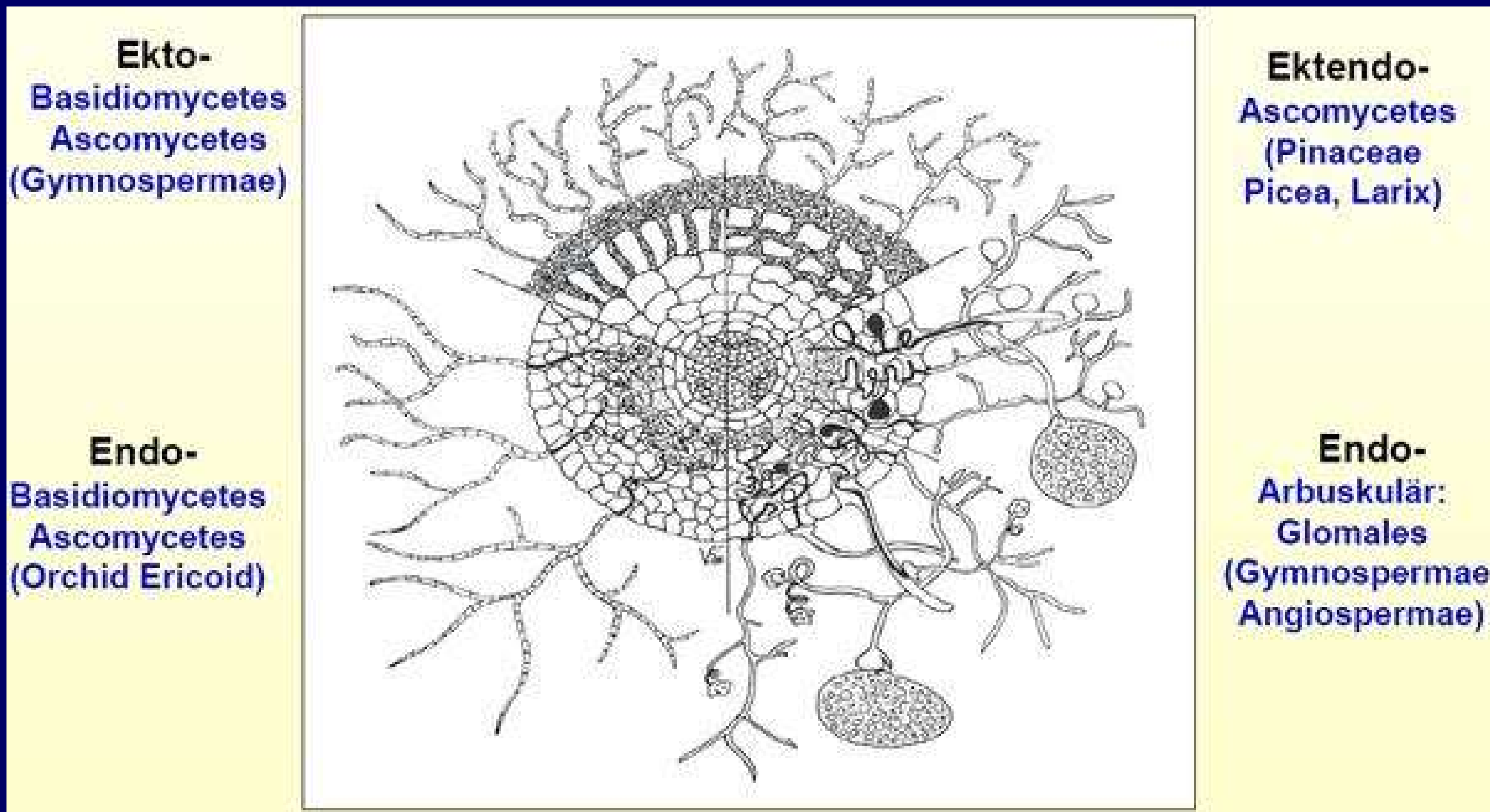
What Controls Plant $\delta^{15}N$?

Mycorrhizae

From: Högberg (1990)

What Controls Plant $\delta^{15}N$?

Mycorrhizae



From: Högberg (1990)

What Controls Plant $\delta^{15}N$?

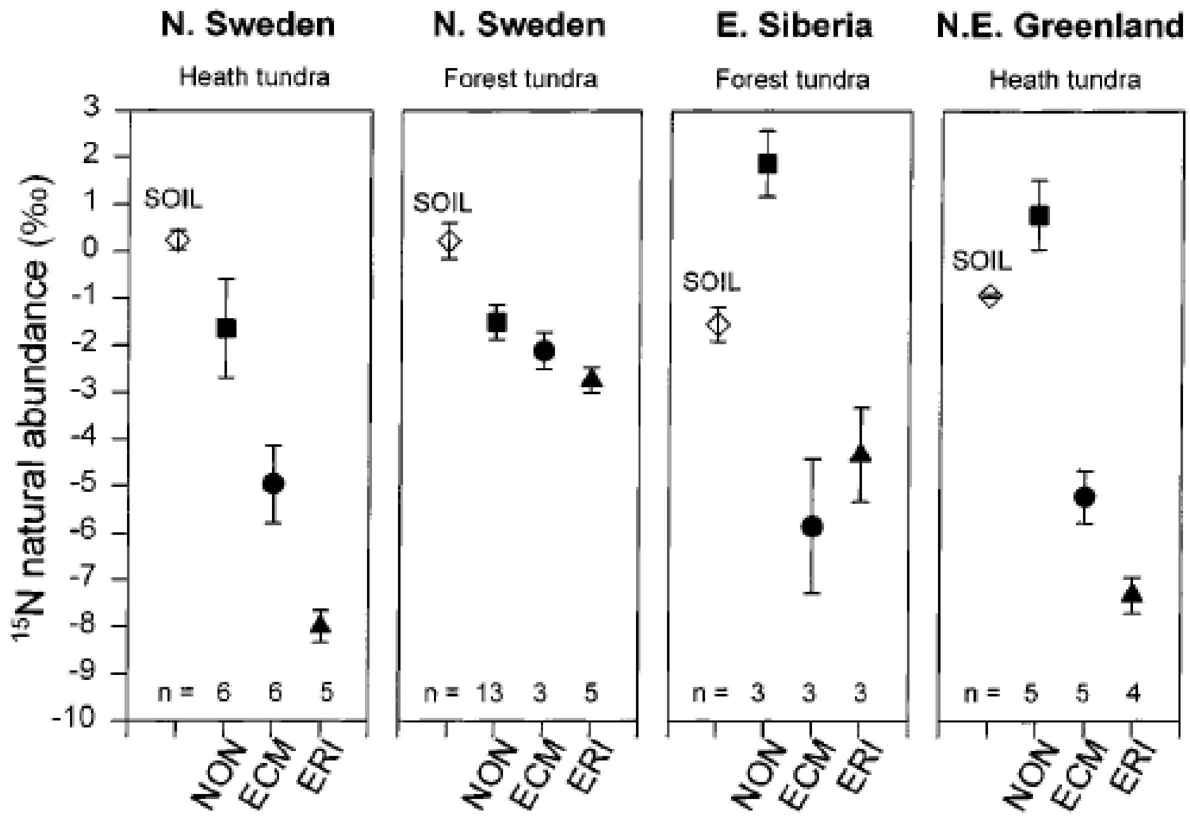
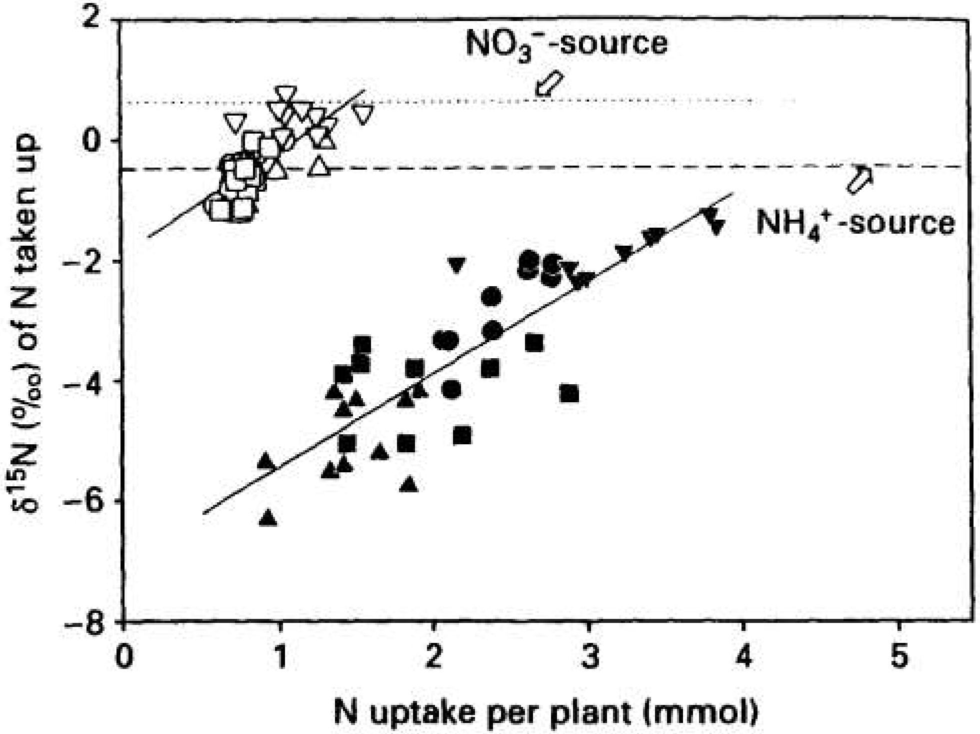


Fig. 2 Mean $\delta^{15}N$ ($\pm$SE) of plant species without mycorrhiza (*NON*), with ectomycorrhizal (*ECM*) fungi or with ericoid mycorrhizal (*ERI*) fungi at the four heath and forest tundra sites in Fig. 1. The means within each functional group and site are based on the means of the species presented in Fig. 1; *n* is the number of replicates (plant species)

From: Michelsen et al. (1998)

What Controls Plant $\delta^{15}N$?

Mycorrhizae – Fractionation with transfer to plant



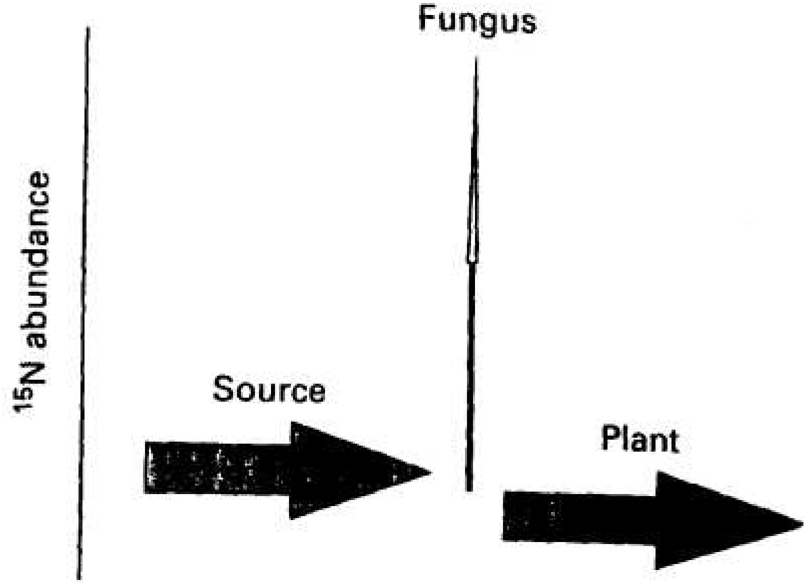


Fig. 7. Schematic drawing of changes in the natura abundance during the flux of soil-derived N (source) t plant through an ectomycorrhizal fungus.

From: Högberg et al. (1999)

What Controls Plant $\delta^{15}N$?

Mycorrhizae – Fractionation with transfer to plant

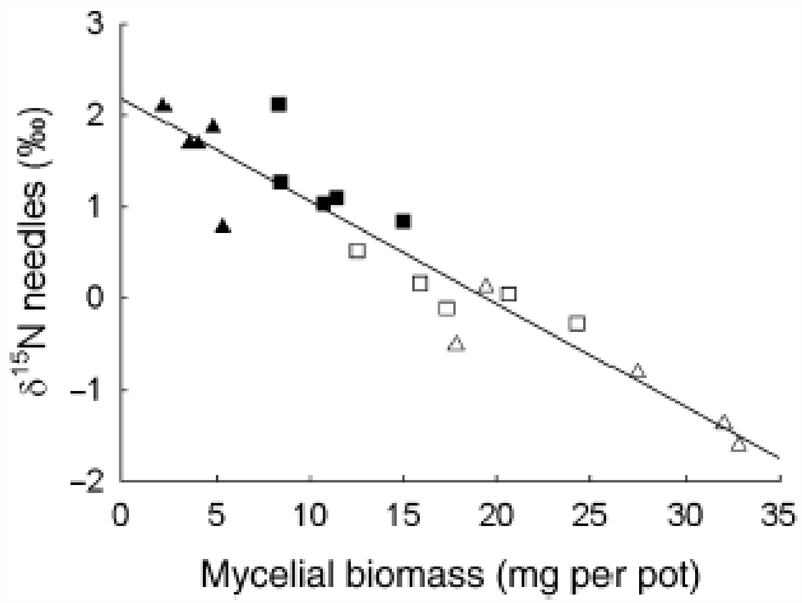


Fig. 2 Mycelial biomass correlates with foliar $\delta^{15}N$ in mycorrhizal *Pinus sylvestris*. Fungal biomass in perlite calculated from ergosterol measurements and appropriate conversion factors for *Thelephora* or *Suillus*. $r^2 = 0.90$, $P < 0.001$. High N, filled symbols; low N, empty symbols; triangles, *Suillus*; squares, *Thelephora*.

Greater fungal biomass leads to greater fractionation

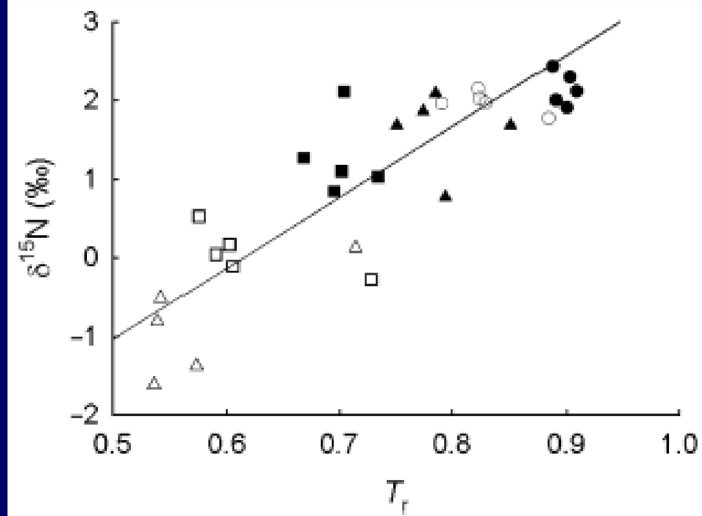


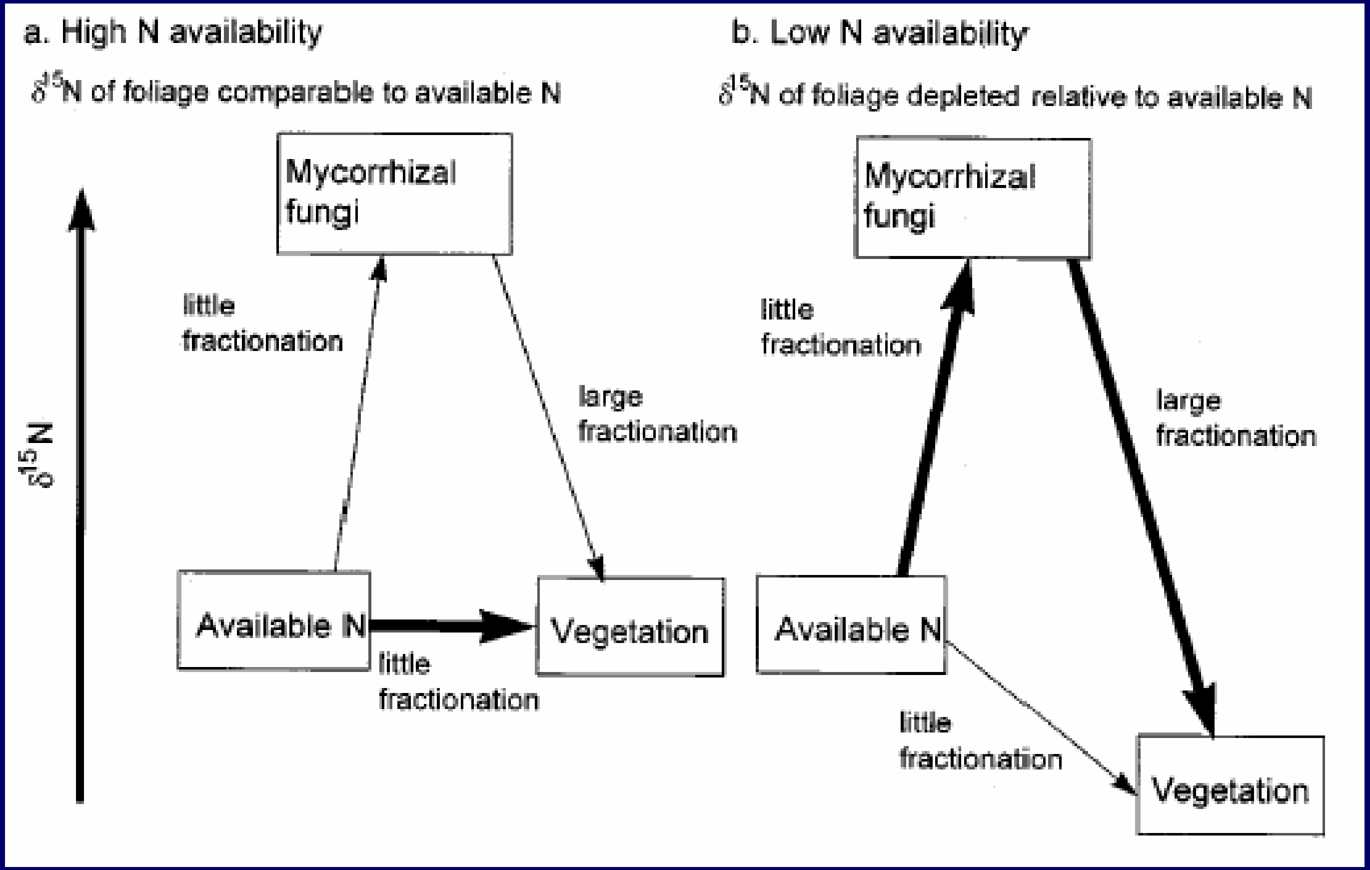
Fig. 4 Comparison of the proportion of system N in plant components (T_r) against foliar $\delta^{15}N$. The regression for the samples from mycorrhizal treatments fits the equation: $\delta^{15}N_{foliage} = 9.01 \pm 1.70 \cdot T_r - 4.96 \pm 0.69$. Adjusted $r^2 = 0.59$, $P < 0.001$. If data are put in the form of Equation 1, the regression is: $\delta^{15}N_{foliage} = 3.47 \pm 0.58 - (1 - T_r) \cdot 9.00 \pm 1.70$. High N, closed symbols; low N, open symbols; circles, nonmycorrhizal; triangles, *Suillus*; squares, *Thelephora*. Nonmycorrhizal samples were not used for calculating regressions.

T_r is the fraction of total system nitrogen in the plant

From: Hobbie and Colpaert (2003)

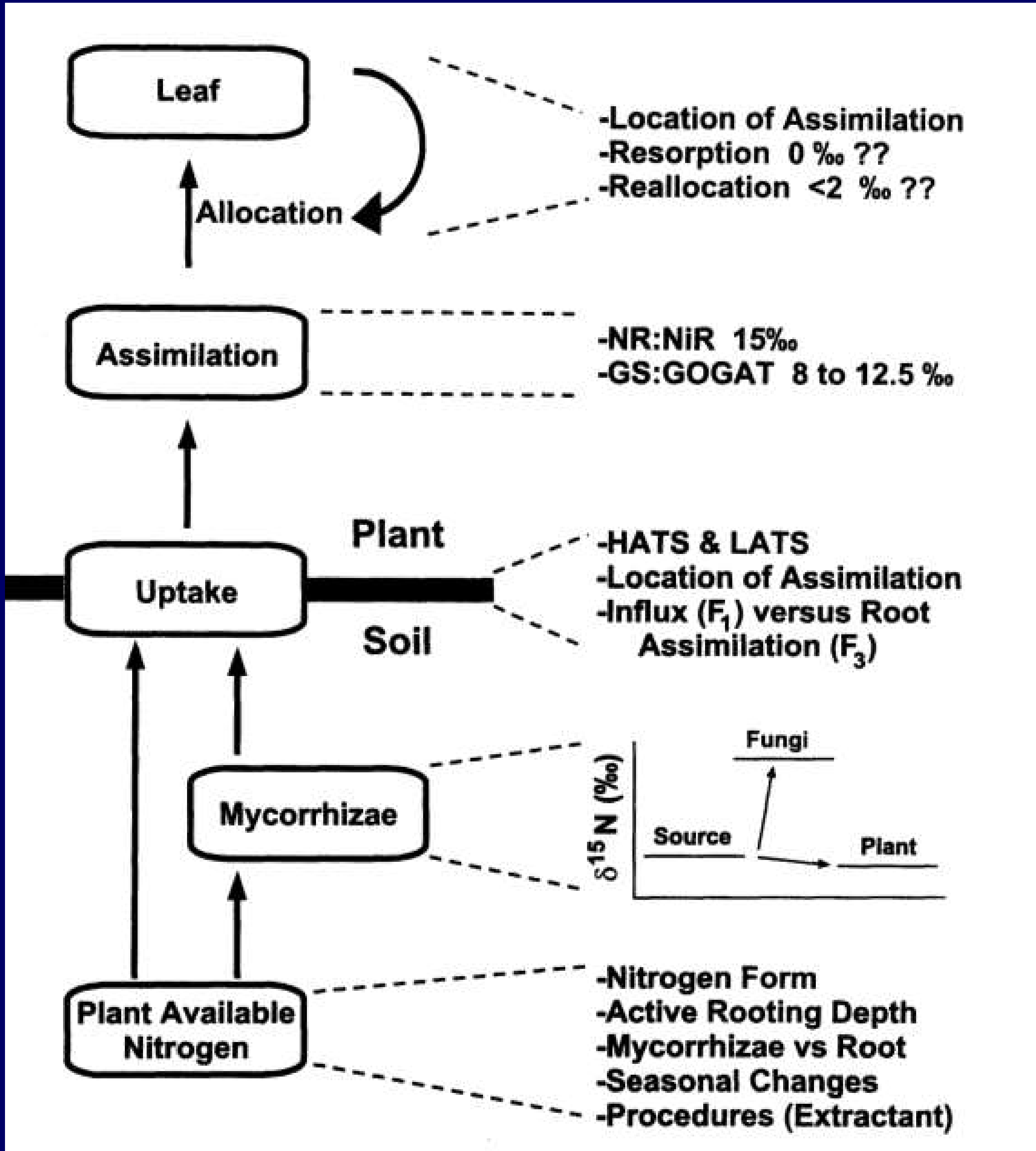
What Controls Plant $\delta^{15}N$?

Mycorrhizae – Fractionation with transfer to plant



From: Hobbie et al. (1999b)

What Controls Plant $\delta^{15}N$?



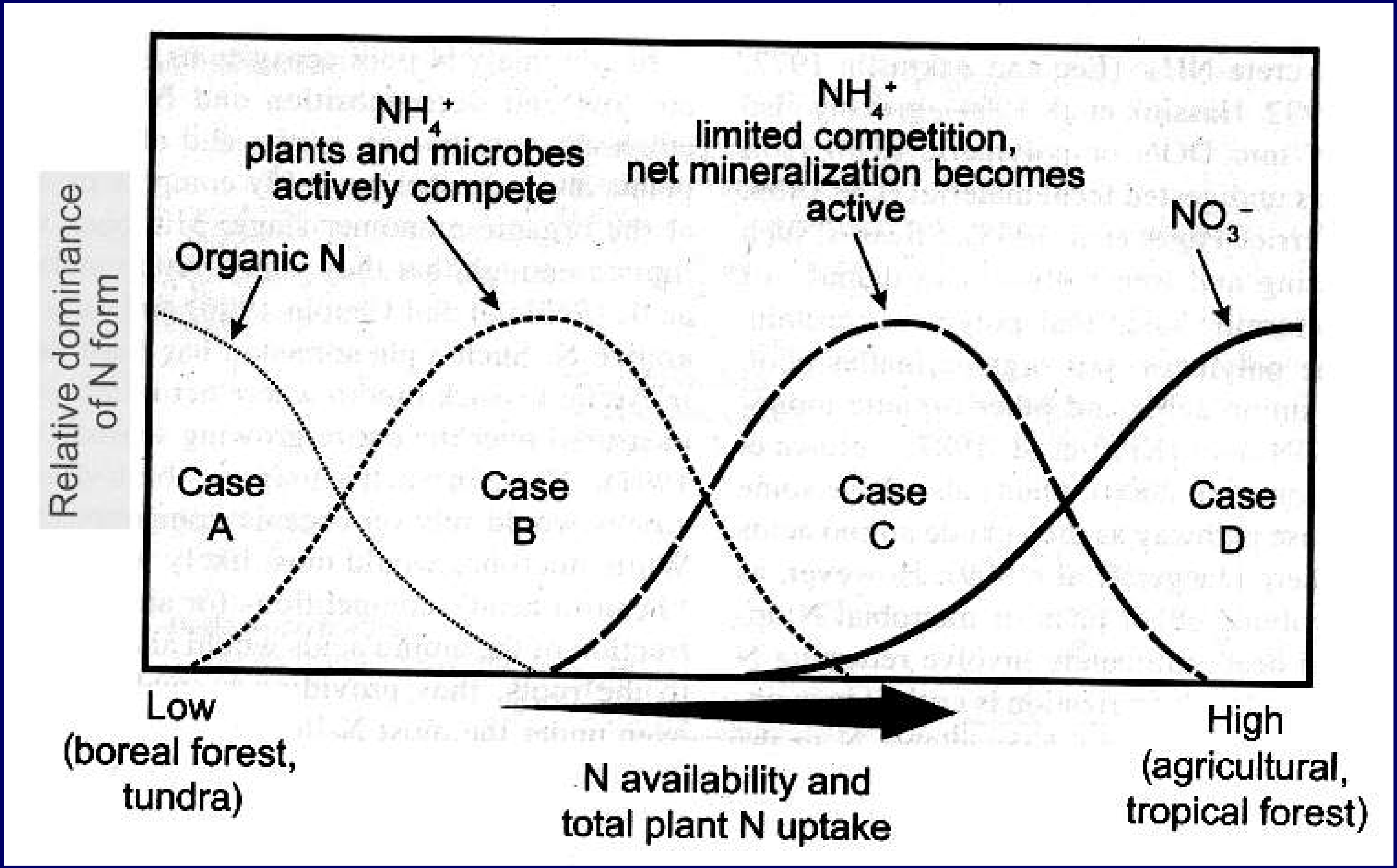
Lecture – Part 2

Soil $\delta^{15}N$

Plant $\delta^{15}N$

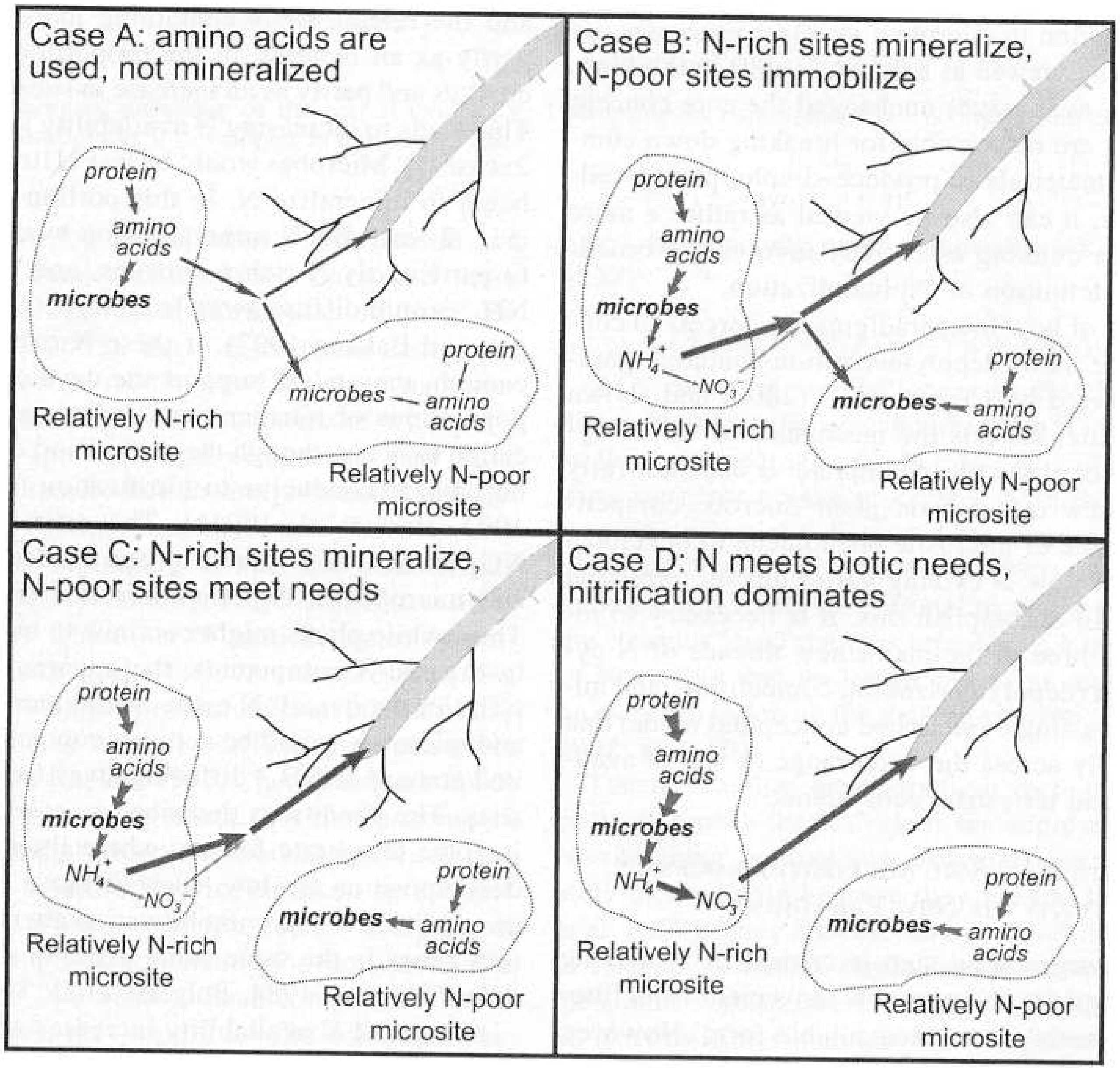
Patterns and Gradients of Plant $\delta^{15}N$

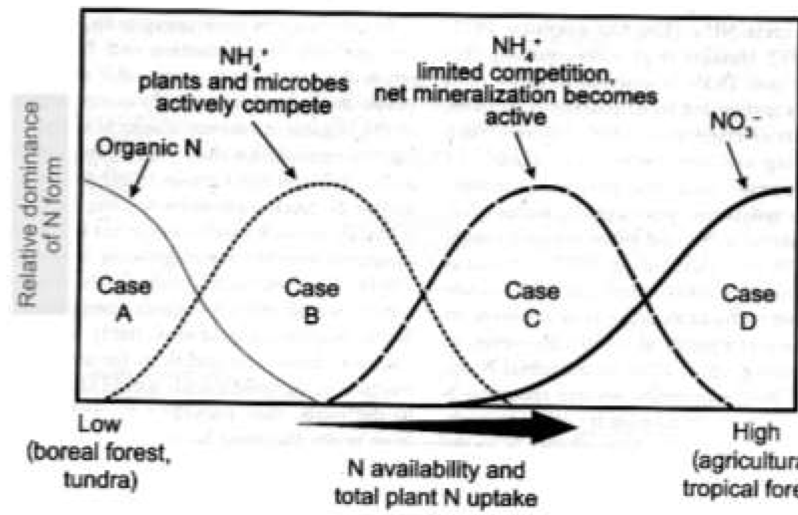
What Controls Plant $\delta^{15}N$?



Schimel and Bennett (2004)

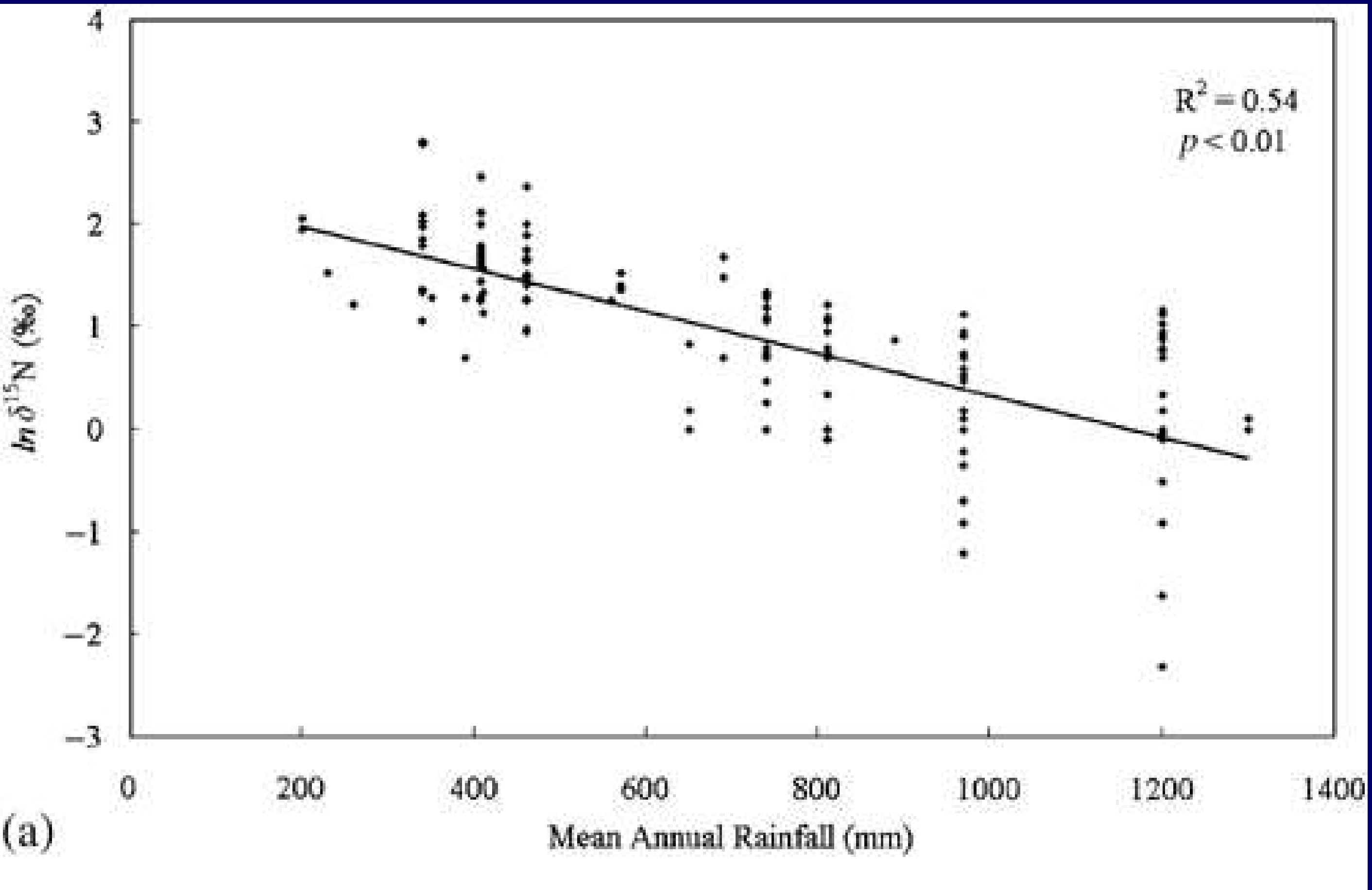
What Controls Plant $\delta^{15}N$?





Schimel and Bennett (2004)

Plant δ15N Patterns and Gradients



Nutrient availability varies inversely with precipitation

N cycles in arid sites are more open

Swap et al. (2004)

Plant $\delta^{15}N$ Patterns and Gradients

Hawaii

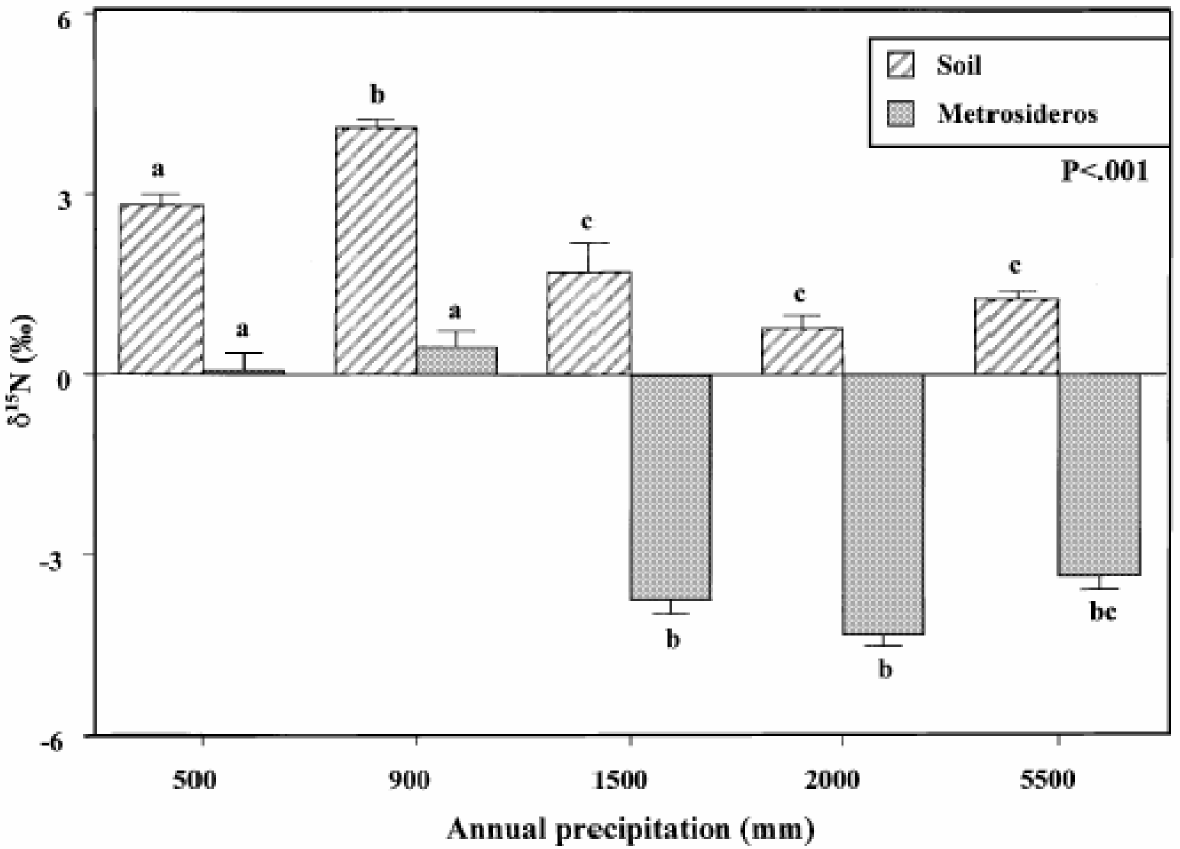


Fig. 5 Variation in $\delta^{15}N$ values (‰) for soil and foliar *M. polymorpha* from all sites across precipitation gradient. Data are mean values (n = 5) ± SE

Austin and Vitousek (1998)

Plant $\delta^{15}N$ Patterns and Gradients

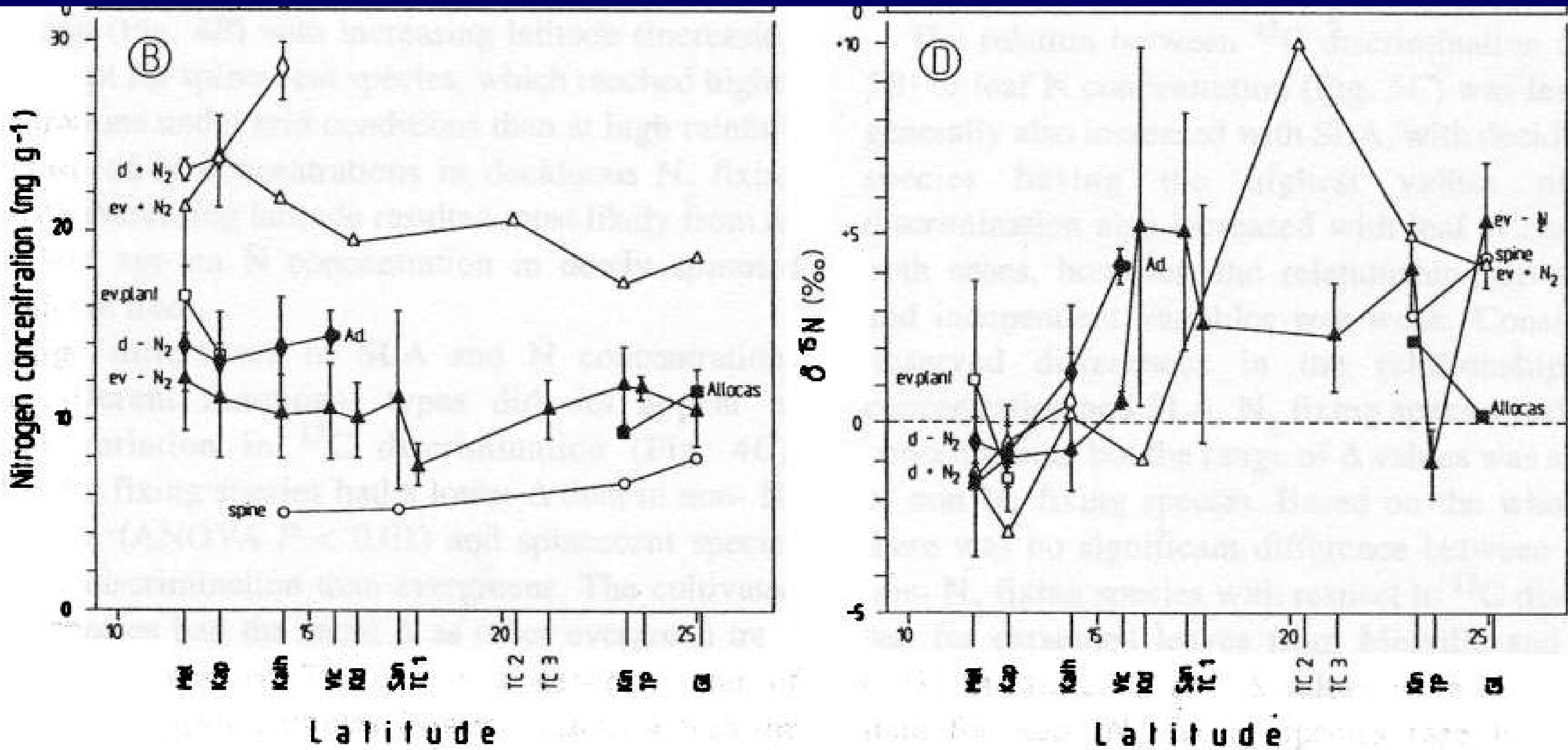


Fig. 4. Latitudinal changes of specific leaf area (SLA, $m^2\ kg^{-1}$), leaf nitrogen concentration ($mg\ g^{-1}$), ^{13}C-isotope discrimination (‰), and of the $\delta^{15}N$-isotope ratio (‰) in the plant functional types: potentially N_2 fixing deciduous and evergreen trees (d + N, ev + N) and non-N_2 fixing deciduous and evergreen trees (d–N, ev–N), spinescent species (spin), *Adansonia* (Ad), *Allocasuarina* (Allocas), and evergreen cultivated fruit tree plantations (ev.plant).

Australia

Schulze et al. (1998)

Plant δ^{15}N Patterns and Gradients

Australia

Table. 3. Effects of burning and grazing intensity on specific leaf area (SLA: m^2 kg^{-1}), N-concentration (N: mgN g^{-1}), carbon isotope discrimination (Δ:‰), and the δ^{15}N-values (‰) of expanded leaves of evergreen non- N_2 fixing species

Small letters indicate significant differences within each column (Student *t*-Test, *P*<0.05)

Treatment	Location	SLA	N	Δ	δ^{15}N
Burning					
unburnt	Kapalga C,Q, M,S	5.51^a	11.46^a	20.04^a	–0.89^a
burnt	Kapalga K,M,P,S	5.50^a	11.20^a 2	0.46^a	–1.44^a
Grazing					
low	Melville-Kapalga	6.17^a	12.73^a	20.03^a	–1.54^a
	Tyler Pass	3.87^b	11.63^a	18.13^b	–1.27^a
medium	Kintore/Giles	3.44^b	11.58^a	18.17^b	4.96^b
high	Kidman/Mt.Sanford	3.24^b	8.16^b	19.84^{ba}	7.09^c

Schulze et al. (1998)

Plant $\delta^{15}N$ Patterns and Gradients

Austin and Sala (1999) response to Schulze et al. (1998)

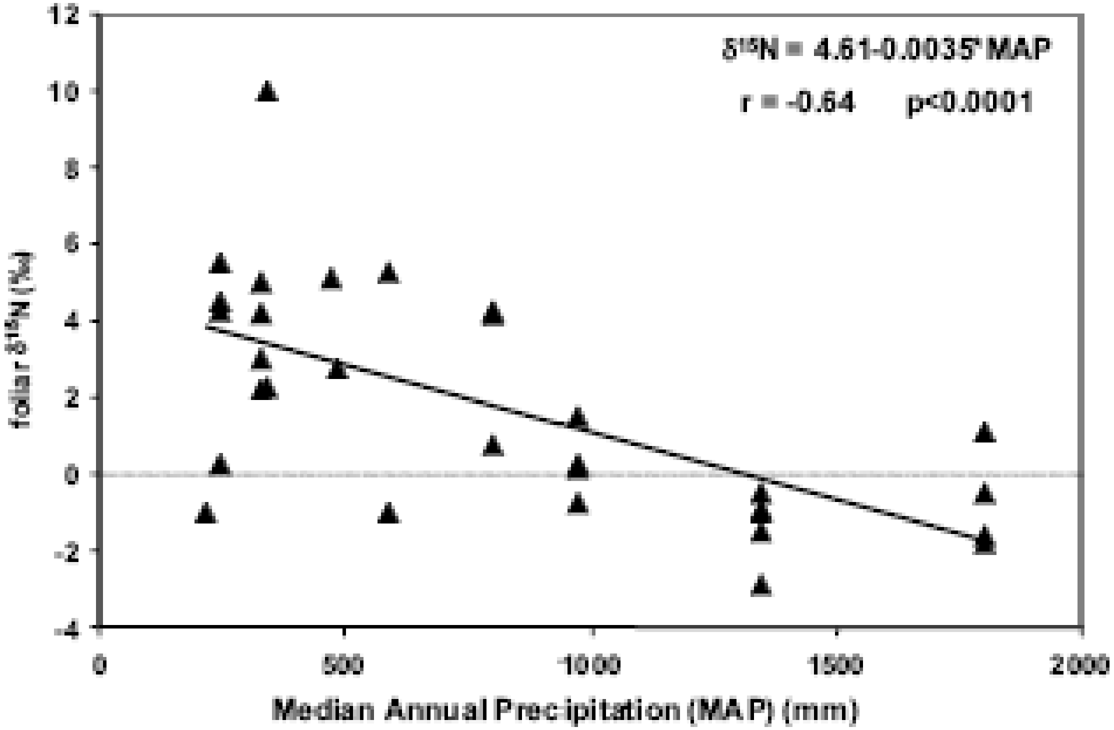


Fig. 1. Correlation of foliar $\delta^{15}N$ with median annual rainfall along the Australian IGBP transect. Each symbol represents average values of $\delta^{15}N$ for each species sampled (data from Schulze *et al.* 1998)

Plant $\delta^{15}N$ Patterns and Gradients

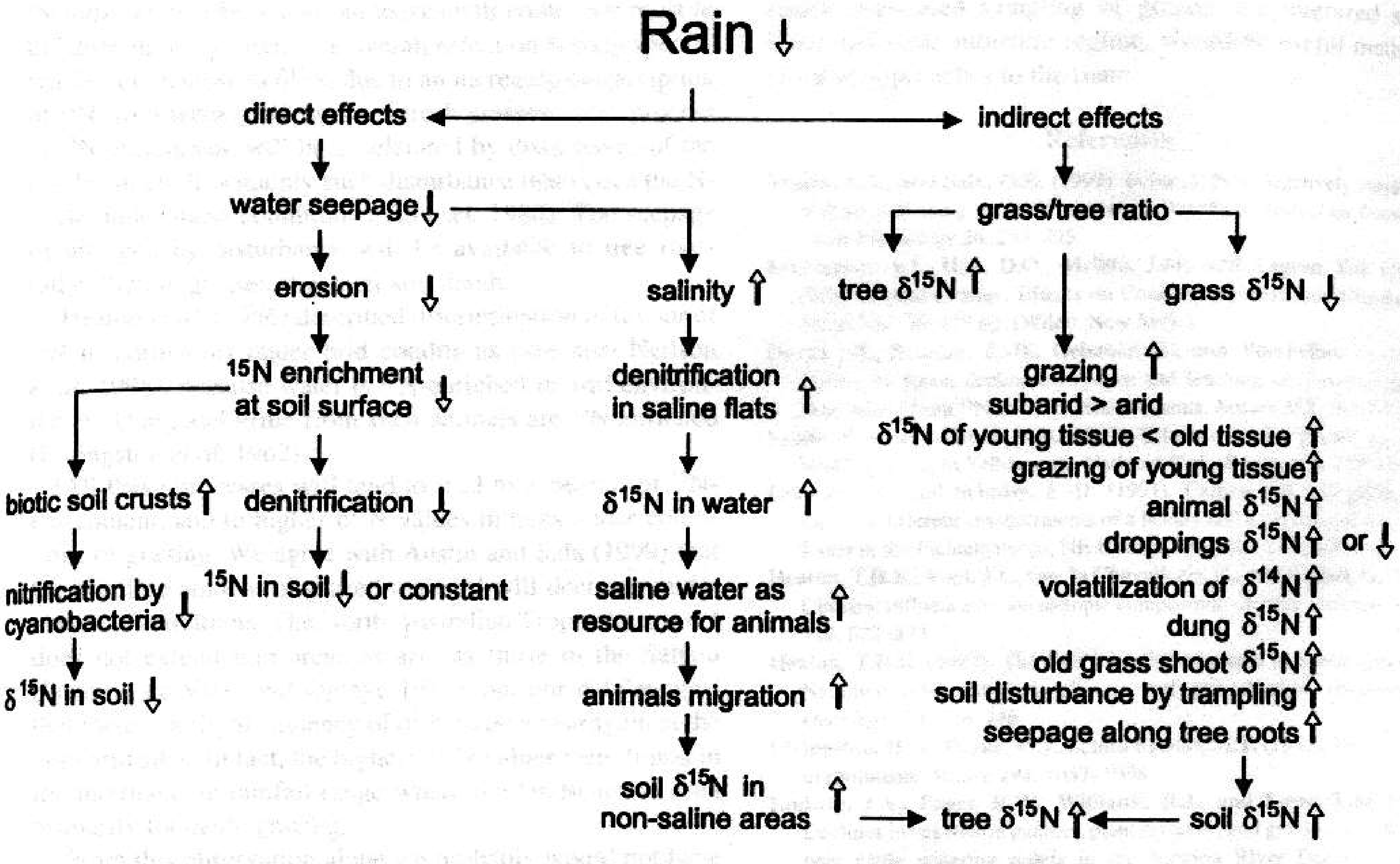


Fig. 1. Schematic presentation of some processes that affect $\delta^{15}N$ in foliage under arid conditions and grazing. The direction of the arrow after the process indicates the response of the process (increase or decrease) to a decrease in rainfall. For more explanations see text.

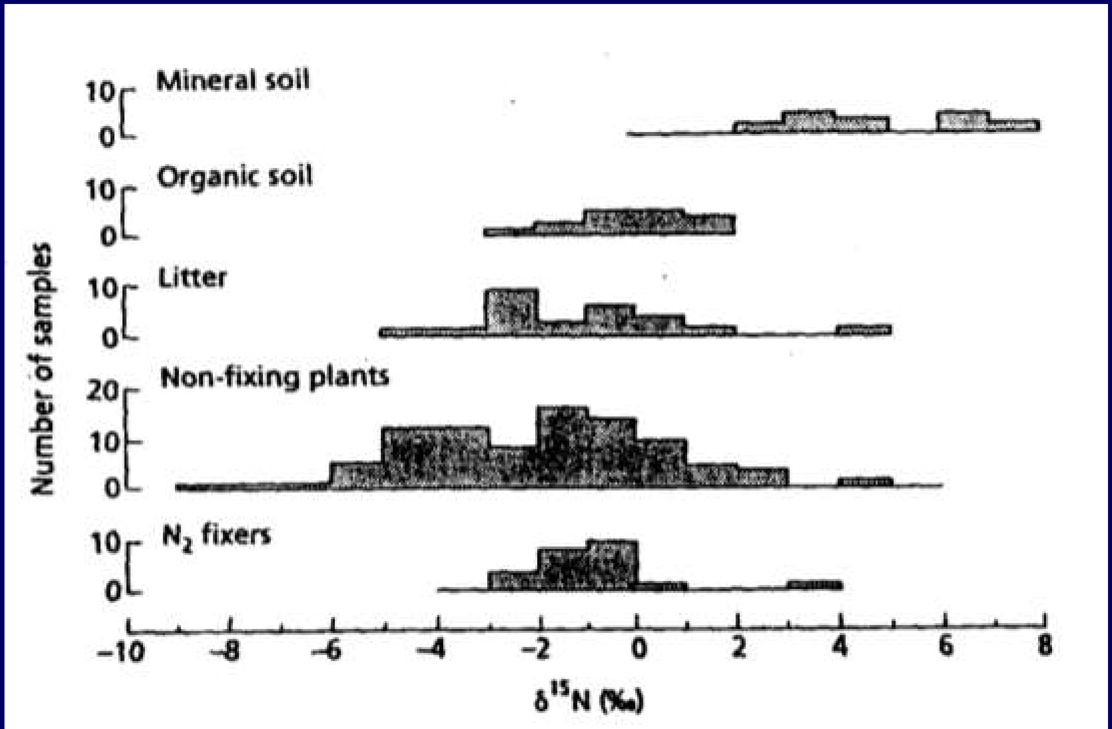
Schulze et al. (1999) response to Austin and Sala (1999)

Plant $\delta^{15}N$ Patterns and Gradients

FIG. 1. Sampling sites in the Long Term Ecological Research (LTER) Program. (1) Arctic Tundra (Alaska), (2) Bonanza Creek Experimental Forest (Alaska), (3) H. J. Andrews Experimental Forest (Oregon), (4) Jornada (New Mexico), (5) Sevilleta (New Mexico), (6) Niwot Ridge/Green Lakes Valley (Colorado), (7) Central Plains Experimental Range (Colorado), (8) Konza Prairie (Kansas), (9) Cedar Creek Natural History Area (Minnesota), (10) North Temperate Lakes (Wisconsin), (11) W. K. Kellogg Biological Station (Michigan), (12) Coweeta Hydrologic Laboratory (North Carolina), (13) North Inlet (South Carolina), (14) Virginia Coast Reserve (Virginia), (15) Harvard Forest (Massachusetts), (16) Hubbard Brook Experimental Forest (New Hampshire), (17) Luquillo Experimental Forest (Puerto Rico).

From Fry, 1991

Plant $\delta^{15}N$ Patterns and Gradients



Observations from Fry (1991)

1. Large variation
2. No correlation with precipitation

Plant $\delta^{15}N$ Patterns and Gradients

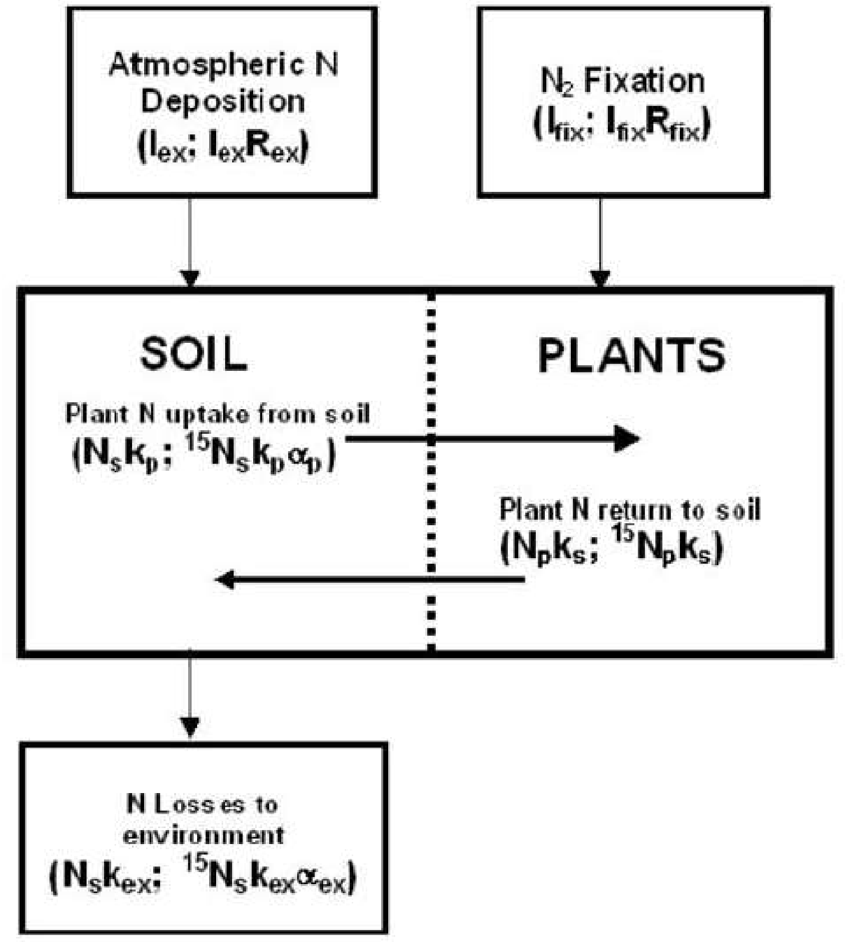


Figure 1. Schematic diagram of soil and plant N and ^{15}N "black box" mass balance model. Terms in parentheses are the flux terms for N and ^{15}N, respectively, and are defined in text.

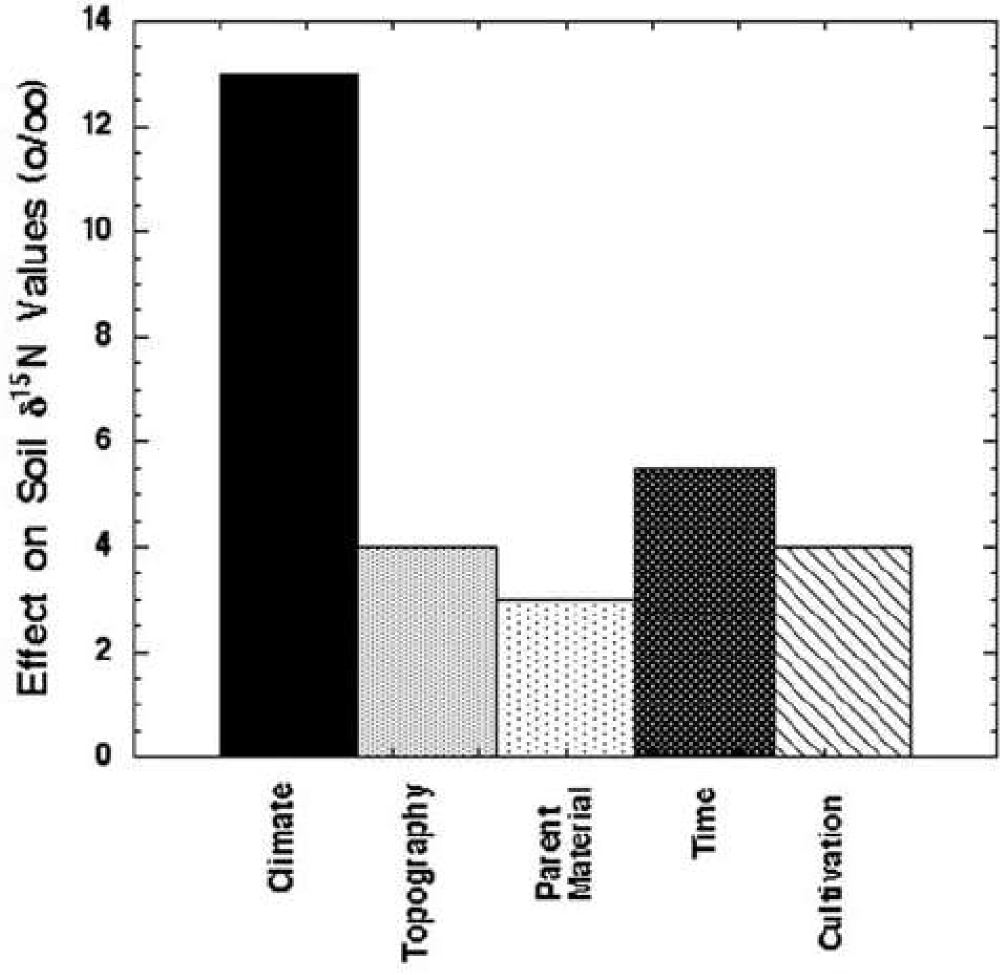


Figure 3. Estimated range in the effect of individual state factors [*Jenny*, 1941] on the $\delta^{15}N$ value of soil N. Sources of values illustrated are discussed in the text.

Amundson et al. (2003)

Plant $\delta^{15}N$ Patterns and Gradients

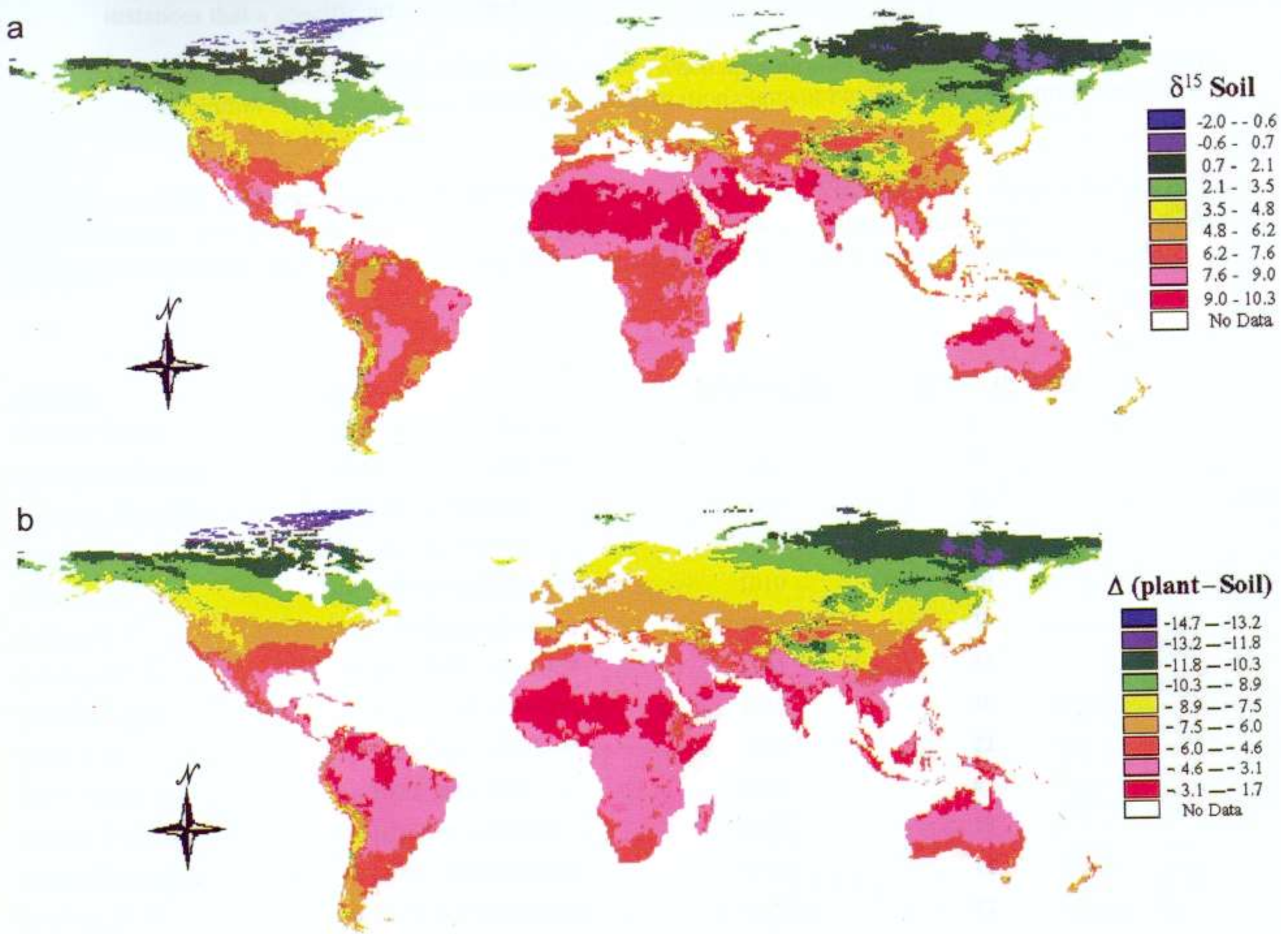


Figure 2. (a) Estimated geographical distribution of soil $\delta^{15}N$ values to 50 cm and (b) estimated geographical trends in $\Delta\delta^{15}N_{plant\text{-}soil}$. Global mean annual temperature and precipitation (0.5 × 0.5 degree grids) data are obtained from *Willmott and Matsuura* [2000].

Nutrient cycles in arid or warm regions are more open leading to greater N loss

Cooler or colder regions retain relatively more N

Amundson et al. (2003)

What about higher tropic levels?

- You are what you eat
- Plus a few permil

Throphic enrichments

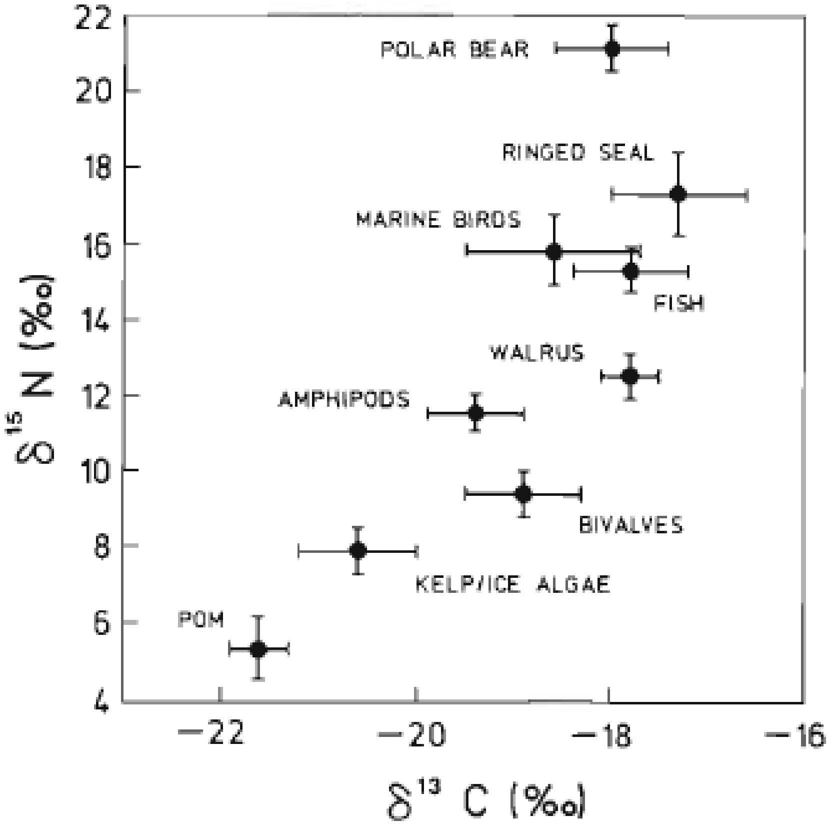


Fig. 2. Relationship of $\delta^{13}C$ and $\delta^{15}N$ values of groups of marine food-web organisms from the Barrow Strait-Lancaster Sound study area. Amphipod sample excludes *Stegocephalus inflatus* and marine-bird sample excludes glaucous gull. Sample sizes are as per Table 1

Hobson and Welch, 1992

Throphic enrichments

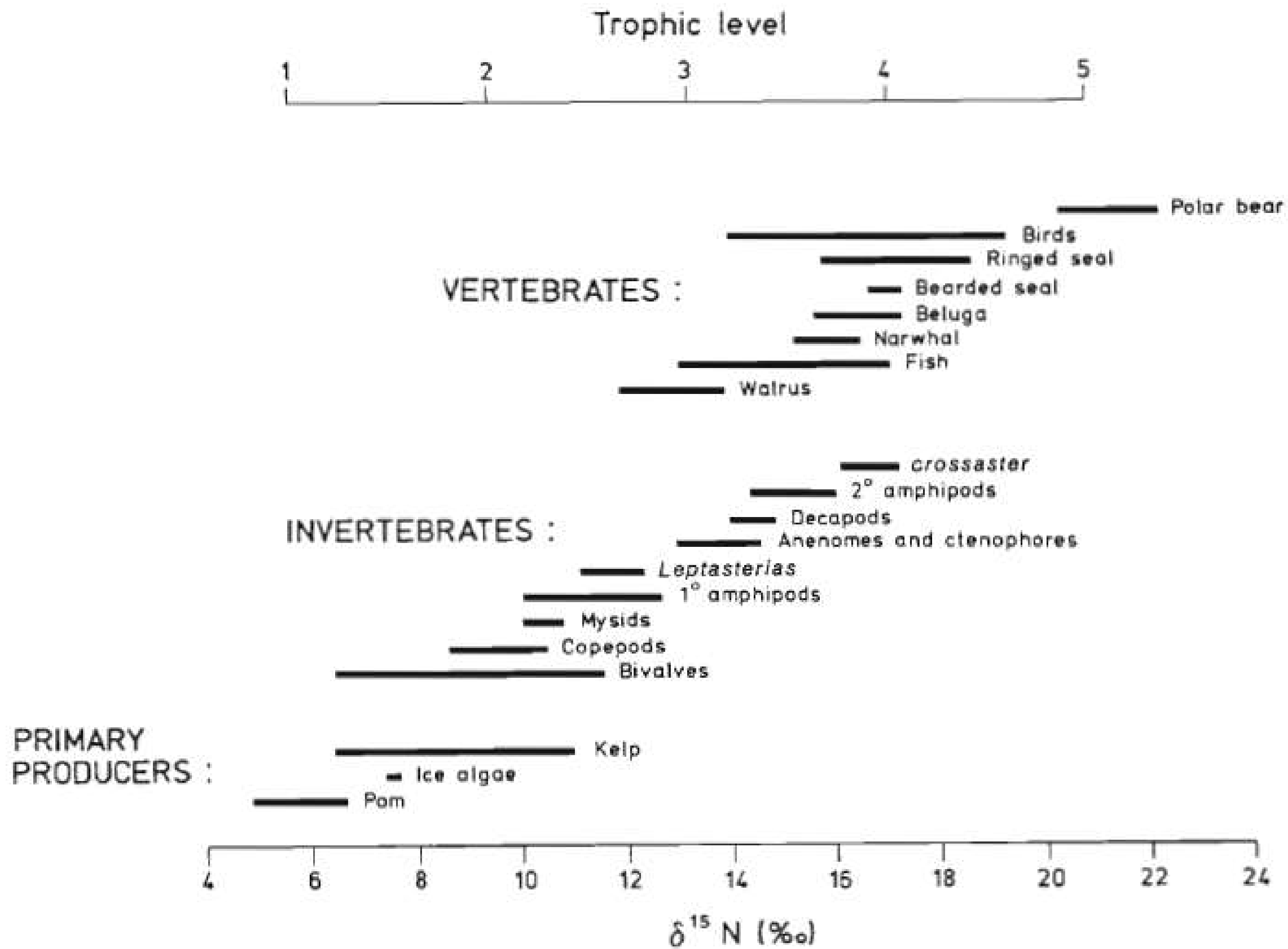


Fig. 3. Ranges of $\delta^{15}N$ values for marine organisms from Barrow Strait-Lancaster Sound and their associated trophic positions according to an isotopic model using a trophic enrichment value of + 3.8 ‰ (not applicable to marine birds)

Throphic enrichments

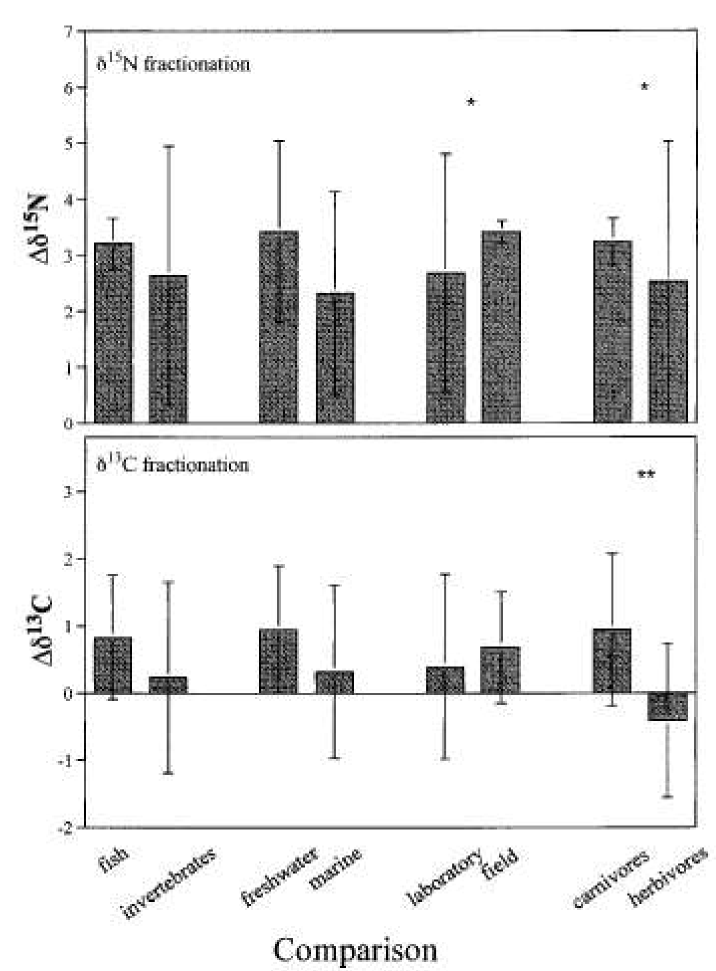


Fig. 1. Comparison of mean $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ (trophic fractionation; error bars represent 1 SD) values for taxon, habitat, estimate type, and diet. Significance based on Mann-Whitney U statistic for $\Delta\delta^{15}N$ and ANOVA for $\Delta\delta^{13}C$. One asterisk indicates mean difference significant at $p < 0.05$. Two asterisks indicate difference significant at $p < 0.01$.

Vander Zanden and Rasmussen, 2001