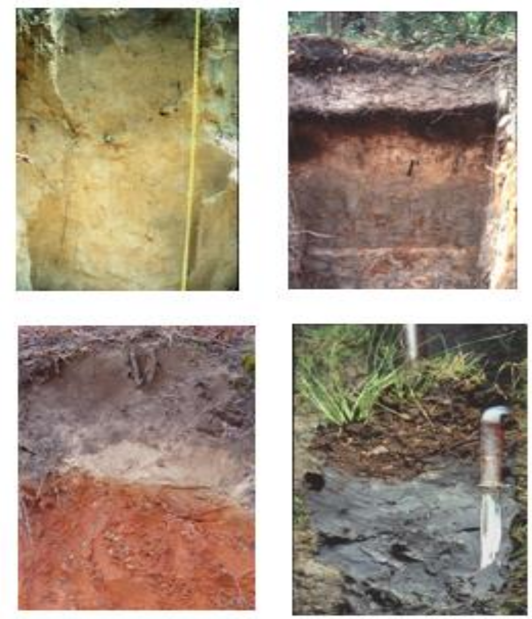
Universidad de Concepción
Dirección de Extensión UdeC
Escuela de Verano UdeC
Dirección de Postgrado
FONDECYT Nº 11150914
Doctorado MaReA
Programa Doctorado en Sistemática y Biodiversidad
Doctorado en Oceanografía
Proyecto "Red_Clima_Rec"
FONDEQUIP EQM 150018
IMO
Facultad de Ciencias Forestales
LSNPFS

Uso de Isotopos Estables en Investigación en Suelos y Aplicaciones Forestales

Dr. Rafael Rubilar



Curso "*Isotopos estables: Fundamentos y Aplicaciones en Ciencias Naturales*",

16 al 20 de enero, 2017, Universidad de Concepción, Chile

Estudio de Dinámica de MO

- Mayoría del C y N en los ambientes terrestres esta presente en forma de materia orgánica del suelo.

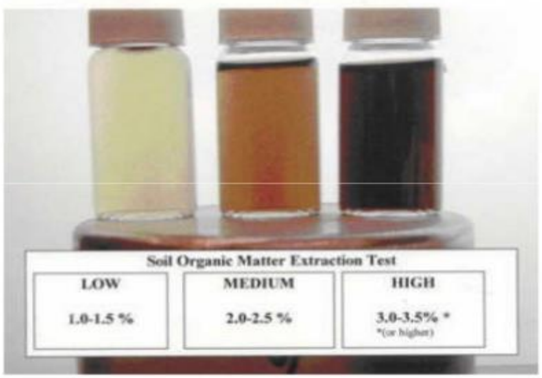
✓ C = 1.5 x 10^{18}g of C (Post et al., 1982)
✓ N = 0.095 x 10^{18}g of N (Post et al., 1985)

- Ambos elementos son claves en definir atributos estructurales y funcionales de ecosistemas naturales y manejados.

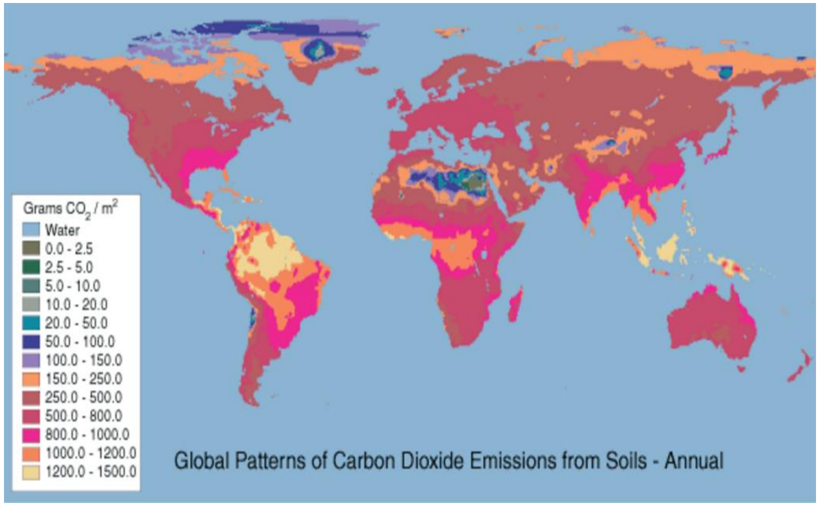
Importancia de MO

- Formación de agregados y estructura
- Capacidad de intercambio catiónico
- Disponibilidad de nutrientes
- Almacenamiento de agua
- Actividad biológica
- Trabajabilidad del suelo
- Muchos mas....a escala global...flujo CO_2 ....¿fuente o sumidero?

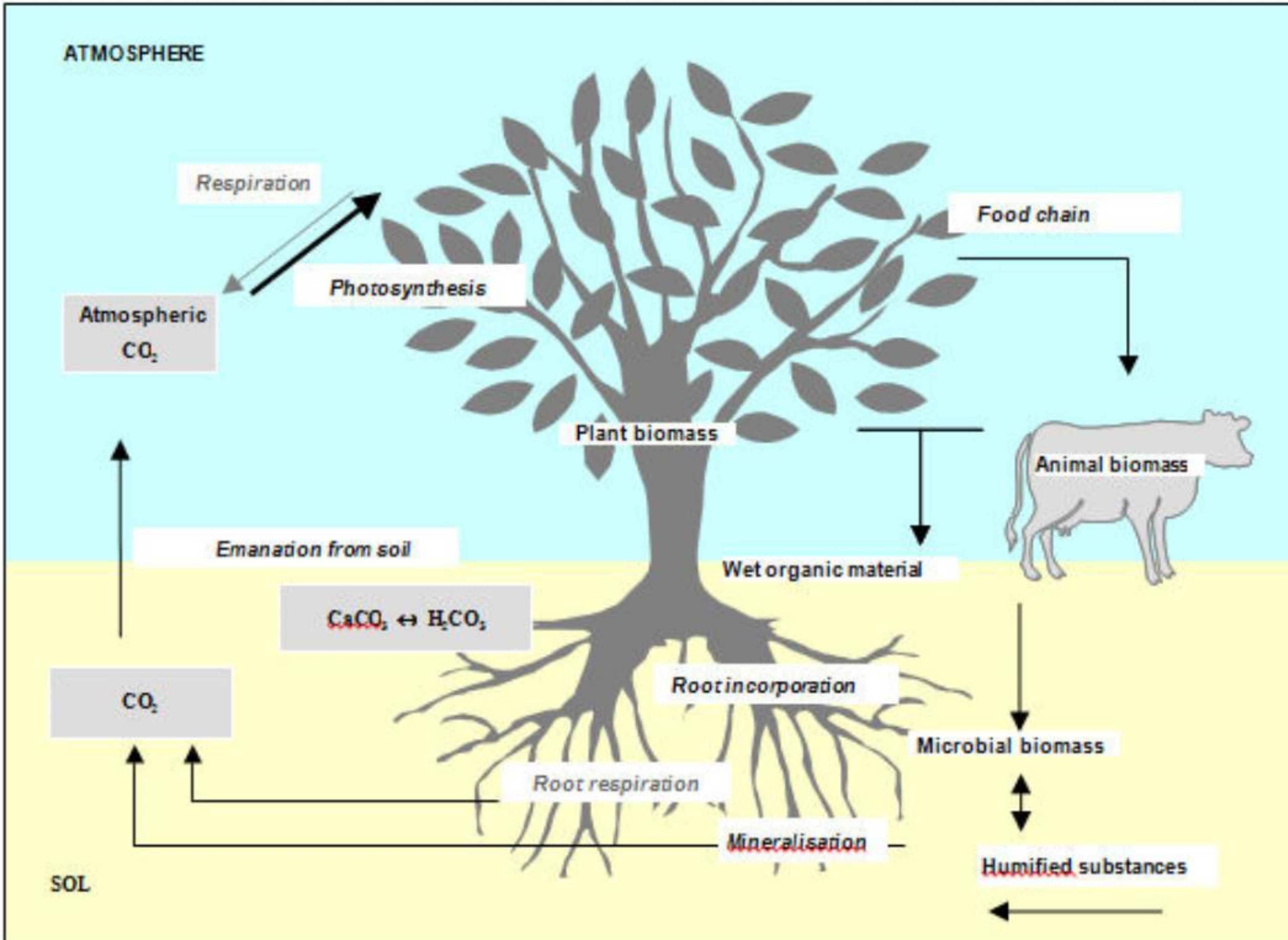
SOM Colorimetric Field Test Using Sodium Hydroxide/EDTA



K-State Soil Test Kit
www.ksre.ksu.edu

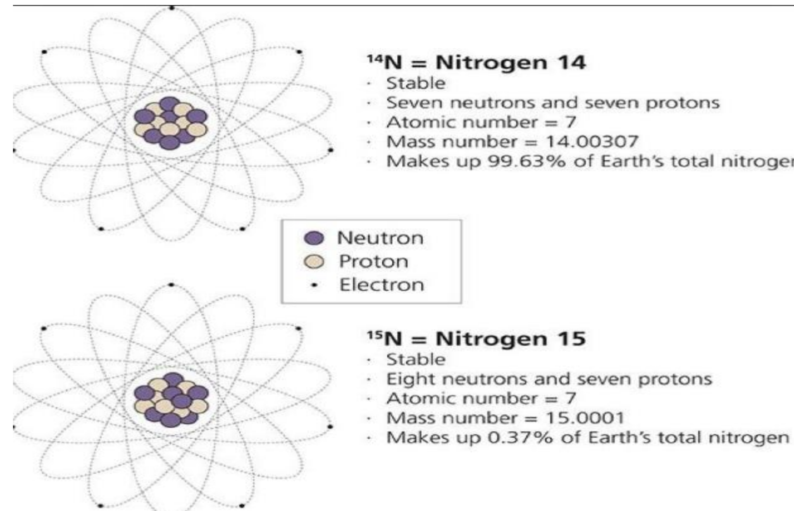


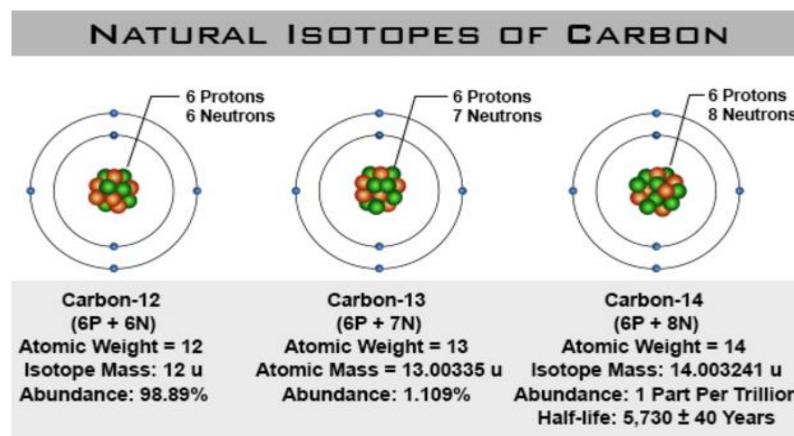
Oak Ridge National Lab , USA


ATMOSPHERE
Respiration
Photosynthesis
Atmospheric
CO_2
Food chain
Plant biomass
Animal biomass
Emanation from soil
Wet organic material
$CaCO_3 \leftrightarrow H_2CO_3$
Root incorporation
CO_2
Microbial biomass
Root respiration
Mineralisation
Humified substances
SOL

Isotopos Estables de C y N

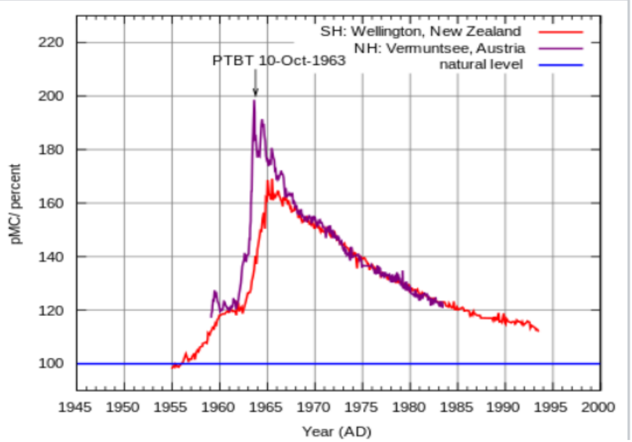
Isotope	Abundance
Nitrogen-14	99.63
Nitrogen-15	0.37
Carbon-12	98.89
Carbon-13	1.11





Principales Aproximaciones Investigación OM

- Uso de MO marcada con ^{14}C o ^{13}C (1-10 a).
- Uso de MO marcada con ^{15}N (1-10 a).
- Uso de variación natural en ^{13}C en la MO (10-10^3 a).
- Uso de ^{14}C inyectado en la atmosfera en pruebas nucleares (10-10^3 a).



^{13}C Aplicaciones

- Carbono en naturaleza $^{12}C=98.89\%$ vs $^{13}C=1.11\%$
- Variaciones de fraccionamiento isotópico por cambios Físicos, Químicos y/o Biológicos.
- Dinámica de MO →
  - Fotosíntesis C3 ~27°/oo >>$\Delta^{13}C$ ($^{13}CO2$)>> C4 ~13°/oo efectos en MO del suelo.
  - Señal de $\Delta^{13}C$ permanece en tejido en descomposición (Melillo et al, 1989).
  - Reconstrucción de uso pasado del suelo y comunidades (agricultura, cultivos).
  - Dinámica de MO in situ sobre períodos de tiempo, si vegetación es estable = MO suelo.
  - Cambio de especie de C3 a C4 es un medio de marcaje que permite evaluar Tasa de Descomposición de MO en base a pérdida de $\Delta^{13}C$ por nueva MO suelo de especie C4.
  - Efecto del cultivo del suelo en tasa de pérdida de MO (cambio C3-C4 o C4-C3).

Tasa de Cambio de MO (C3 vs C4)

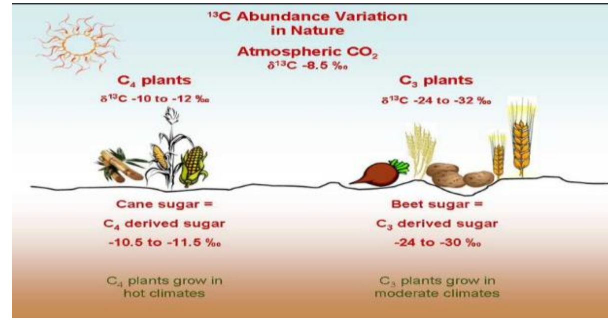


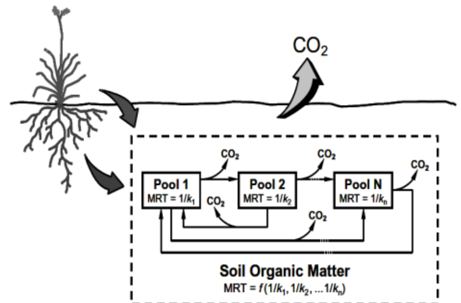
Table 2 - Natural soil ^{13}C abundance and proportion of soil C derived from elephant grass under three different soil management treatments in Misiones. Argentina.

Soil depth (m)	Natural forest		50 year - yerba mate monoculture		6 year - yerba mate- elephant grass association		10 year - yerba mate - elephant grass association	
	Average	SD	Average	SD	Average	SD	Average	SD
			Natural soil ^{13}C abundance. $\delta^{13}C$ (‰)					
Litter	-28.85	0.03	-30.05!	ND			-11.90	ND
0 - 0.05	-26.36 a*	0.20	-22.47 b	0.43	-19.28 c	0.59	-18.35 d	0.34
0.05 - 0.15	-25.48 a	0.12	-22.60 b	0.52	-21.74 bc	0.59	-21.43 c	0.36
0.15 - 0.30	-24.34	0.21						
0.30 - 0.60	-21.56	0.18						
			Proportion of soil C derived from elephant grass. α_{DP} (%)					
0 - 0.05			0.0		30.2		39.0	
0.05 - 0.15			0.0		8.0		10.9	

SD: standard deviation; !: mean value from leaves and branches of yerba mate exported as yield; *means followed by the same letter within a line do not differ (Student test at $P < 0.05$).

Piccolo et al, 2008

Dinámica de MO (Cinética)



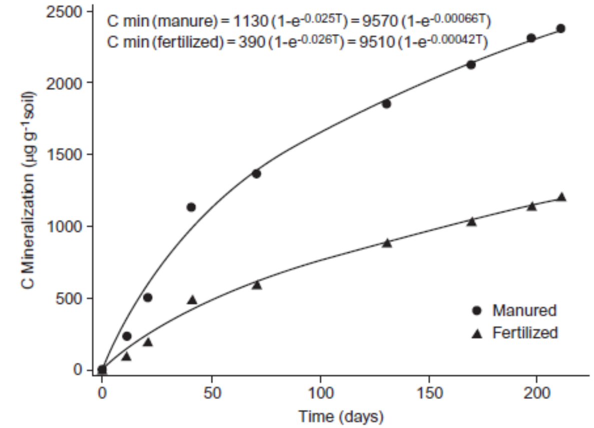


FIG. 17.3 The fit of the sum of two first-order curves describing C mineralization in soil from a manured and fertilized long-term plot during a 220-day incubation.

- $\mathbf{A_t = A_0 * e^{-kt}}$
- A_t : Masa de C derivado de planta C3 en el tiempo t post cambio C3→C4
- A_0 : Masa de C derivado de planta C3 en el tiempo t=0
- k: fracción constante de tasa cambio en unidades de tiempo (pendiente de grafica ln(At/A0) vs tiempo
- t: tiempo desde el cambio de C3→C4
- Vida media ($t_{1/2}$)del C derivado de C3 = 0.693/k
- Tiempo medio de residencia de recambio de MO de planta C3 = -1/k (Paul & Clark, 1989)

Tiempos de Residencia de MO para Distintos Métodos y Ecosistemas

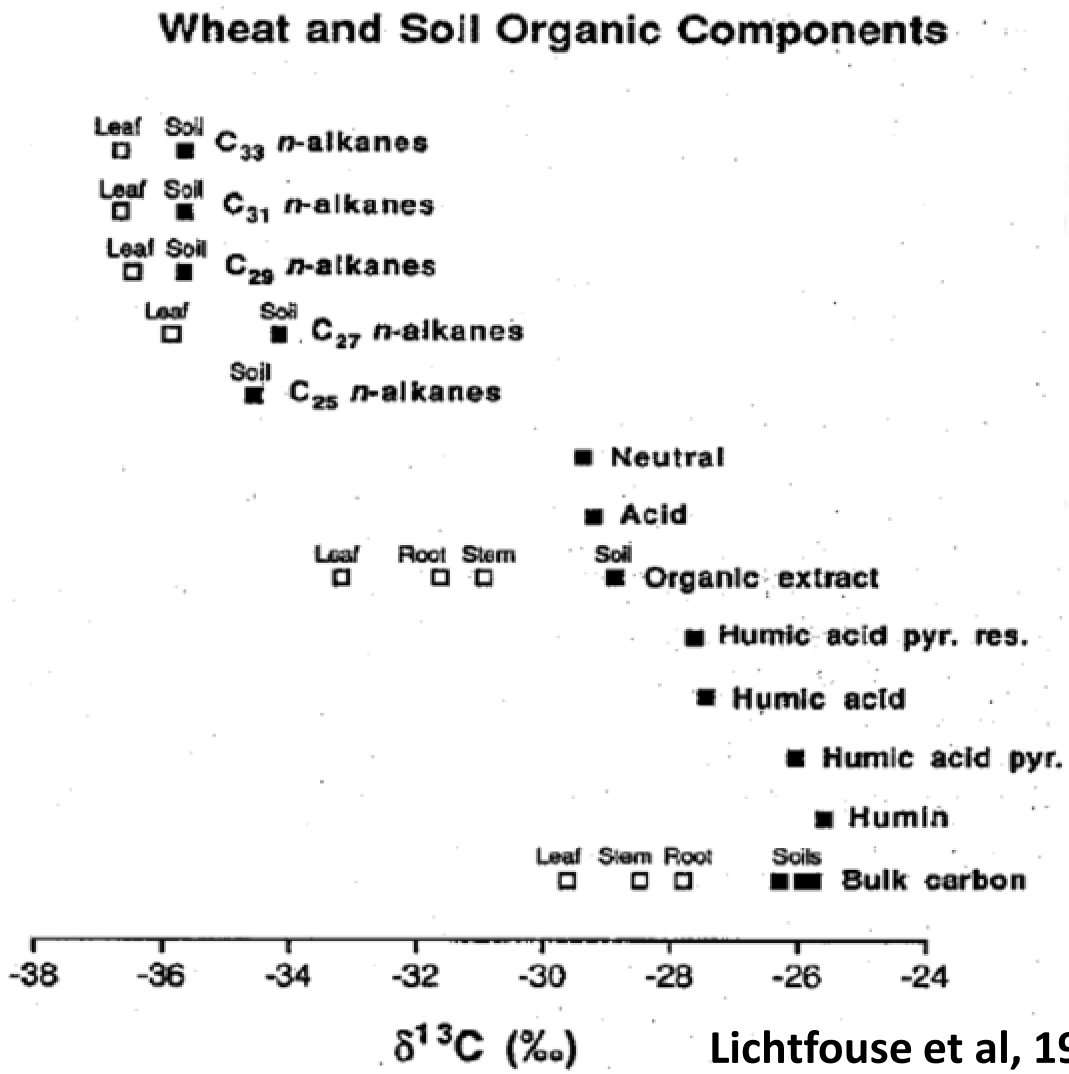
Table 1 Range and average mean residence times (MRTs) of total soil organic C in various ecosystem types as estimated by four different methods

Method and ecosystem	Sites and sources[a]	MRT (yr)		
		Low[b]	High[b]	Average±SE[c]
First-order modeling				
Cultivated systems and recovering grassland or woodland systems	7/7	15 (14)	102 (15)	67±12
^{13}C natural abundance				
Cultivated systems	20/10	18 (16)	165 (17)	61±9
Pasture systems	12/10	17 (18)	102 (19)	38±7
Forest systems	2/2	18 (20)	25 (21)	22±4
Radiocarbon aging[d]				
Cultivated systems	21/8[e]	327 (22)	1770 (23)	880±105
Grassland systems	4/3[f]	Modern (23)	1040 (24)	–[g]
Forest systems	4/3	422 (22)	1550 (25)	1005±184
"Bomb" ^{14}C analysis				
Cultivated systems	1/1	1863 (13)	1863 (13)	1863[g]
Forest and grassland systems	14/12	36 (26)	1542 (27)	535±134

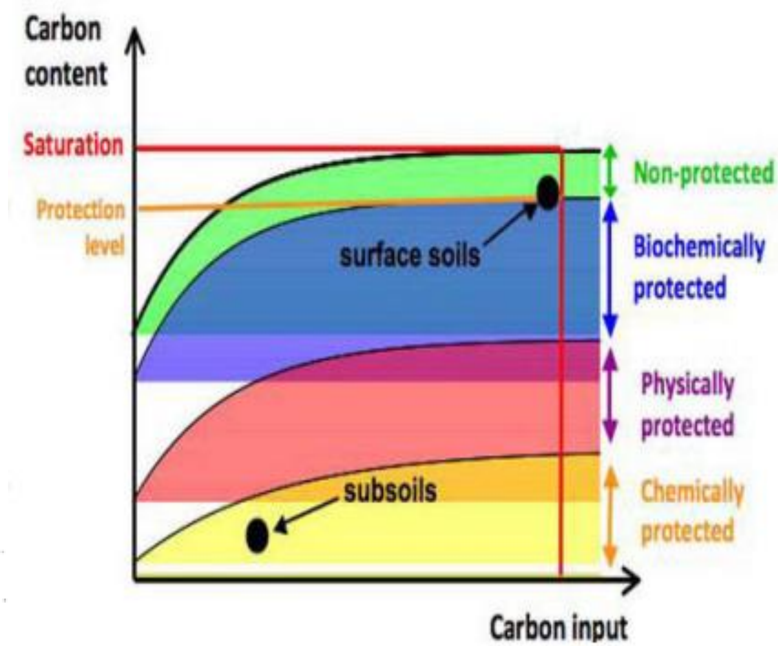
[a] First value indicates the number of sites used to calculate average MRT values; second value indicates the number of literature sources surveyed (i.e., some sources provided data for multiple sites).
[b] Number in parentheses indicates reference to literature.
[c] SE, standard error.
[d] Values presented in MRT columns for this technique are radiocarbon ages in years B.P.
[e] Includes two sites dating as "modern."
[f] Includes three sites dating as "modern."
[g] Only one value available.

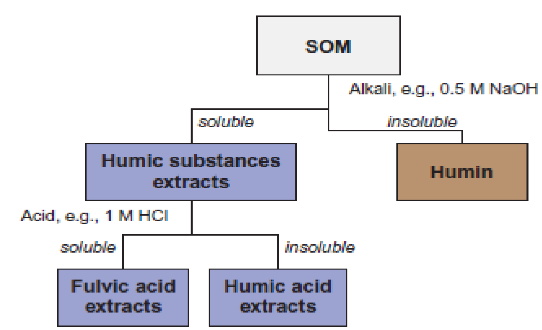
Six & Jastrow, 2014

Gradiente de empobrecimiento de ^{13}C en compuestos orgánicos en suelo vs planta.



Lichtfouse et al, 1995





Humic extracts separation.

Principales Aplicaciones en Ciclaje de ^{15}N

- Fijación de N
  - Adición de $^{15}N_2$ a biomasa microbiana o planta
    - ^{15}N en aire es distinto de ^{15}N en suelo o fertilizante, proporción de ^{15}N es diluida por especie fijadora de N.
- Descomposición y Mineralización de MO
  - Adición de ^{15}N a biomasa microbiana o planta
    - Solución o substrato enriquecido para crecimiento de plantas
    - Incubación de residuos para aplicación directa o para incubación de fuente de C labil (glucose) y fuente inorgánica de 15N (ej: $^{15}NH_4SO_4$).
    - Incubación aeróbica o anaeróbica para evaluar mineralización en el tiempo.
- Retención de Nutrientes en Ecosistema
  - Depositación atmosférica (simulada)
  - Naturales (MO o suelo enriquecido)
  - Fertilizante (Marcado)
  - MO de diferente labilidad (C/N)
  - Lixiviación

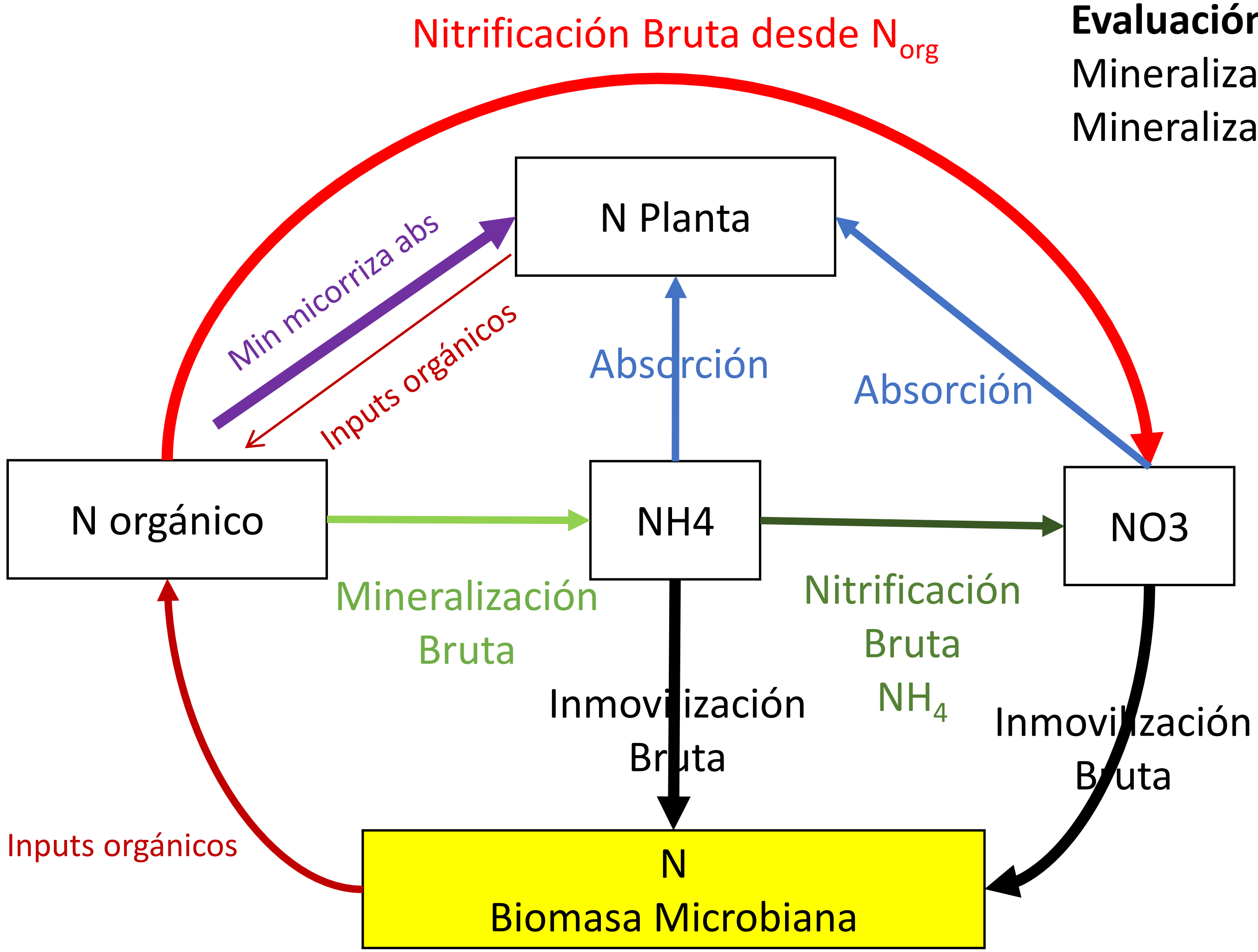
Principales Aplicaciones en Suelos y Forestales

- **Fijación Simbiótica de N**
  - Tasa de Fijación: Enriquecimiento de gas enriquecido $^{15}N_{2\,(g)}$ y evaluación comparativa de especies fijadoras de N (McNeill et al., 1994). Evaluación en suelos y/o absorción en planta.

  - Efecto de Especies Fijadoras: Adición de fertilizante enriquecido con ^{15}N al suelo y evaluación de razón $^{15}N/^{14}N$ hojas de especie fijadora comparada a especie no fijadora (Parrotta et al. 1994).
    - Especie no fijadora posee razón distinta dado que no posee acceso a N atmosférico directamente.
    - Proporción de N fijado es directamente proporcional al N total del tejido.
  - Abundancia Relativa de ^{15}N vs ^{14}N: Medición directa en tejido o savia para evaluación de influencia fijación (Shearer and Kohl, 1986).

Principales Aplicaciones en Suelos y Forestales

- **Mineralización de N (Myriold 1987)**

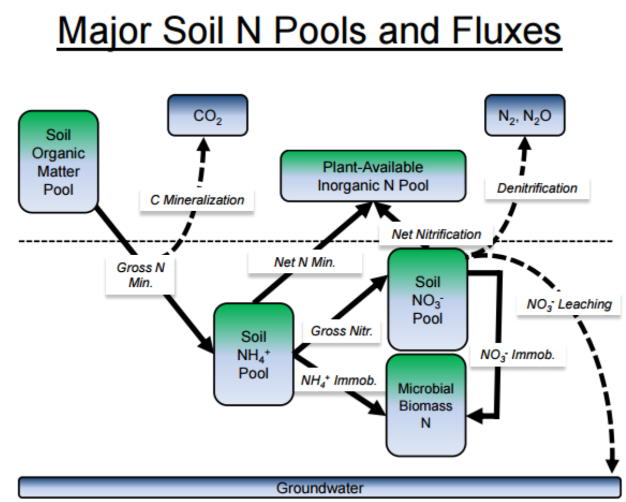


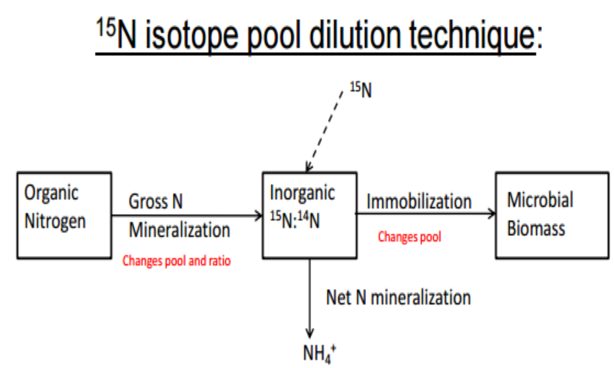
Evaluación de Disponibilidad de N
Mineralización Neta 10%
Mineralización Bruta 90%

Isotopo mas pesado ^{15}N es discriminado en contra cuando se descompone y mineraliza la MO.

Disponibilidad de N-NH4, N-NO3, N-NO2

- Medición en incubaciones aérobica/anaeróbica/ N-inorgánico.
- Medición de tasas brutas de nitrificación (o mineralización)
- Técnica de dilución de N
  - Mediante la caída de la abundancia de ^{15}N en el pool de amonio o nitrato post enriquecimiento. N marcado puede ser en enriquecido como $^{15}NH_4^+$ o $^{15}NO_3^-$en solución o inyectado como $^{15}NH^3$ gas en el suelo.
  - Posible separar vías de nitrificación autotrófica de heterotrófica.





Tasas de Mineralización y Nitrificación Bruta en Horizontes Orgánicos Bosques de Coniferas y Latifoliadas Bajo Altas Tasas de Depositación Atmosférica Simuladas

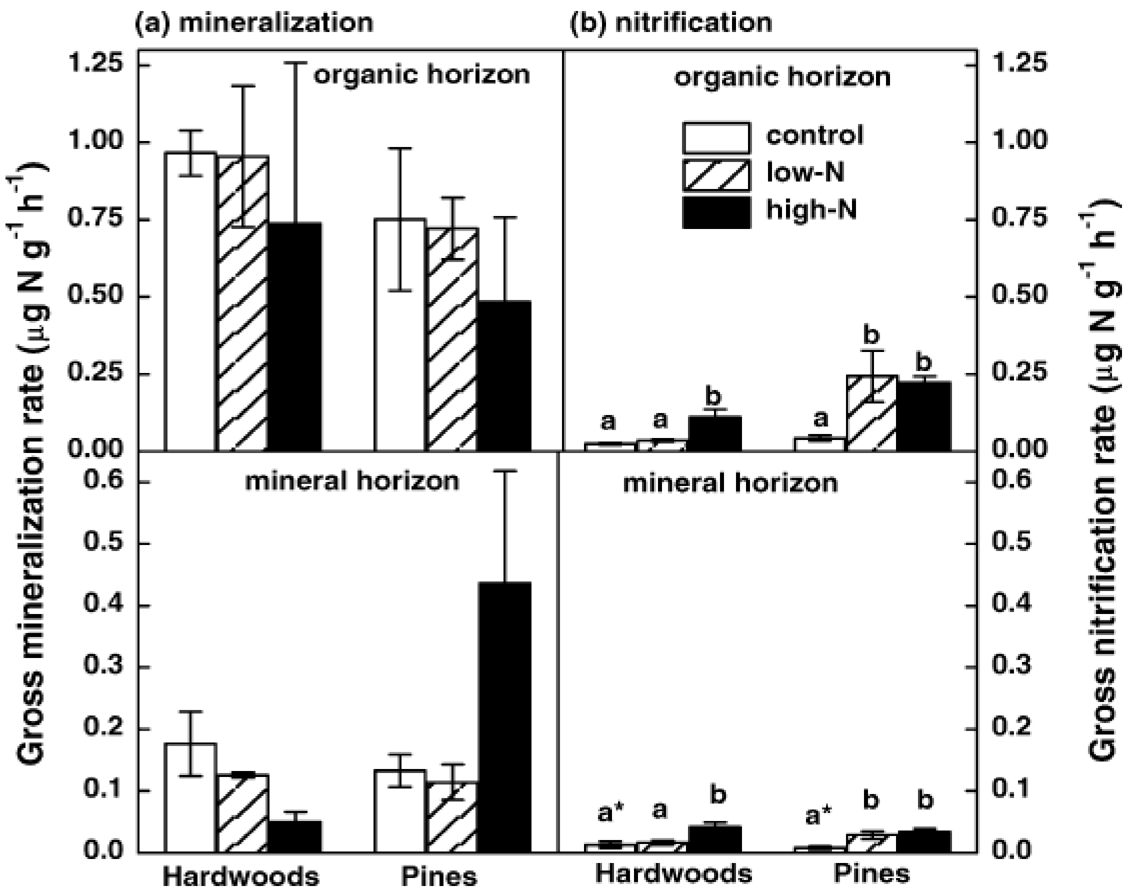


Fig. 2. Rates of (a) gross N mineralization, and (b) gross nitrification in incubating organic horizon soils (upper plates) and mineral horizon soils (lower plates) from Chronic N Amendments Study plots sampled May–June 2001 (mean ± S.E.). Within each forest and soil type, bars with different letter designation are significantly different based on least significant differences ANOVA ($P < 0.05$). Asterisk indicates that the mean value is not significantly different from zero.

Venterrea et al., 2004

Tasas de mineralización y nitrificación bruta e inmobilización por biomasa microbiana en bsoques de latifoliadas y coniferas

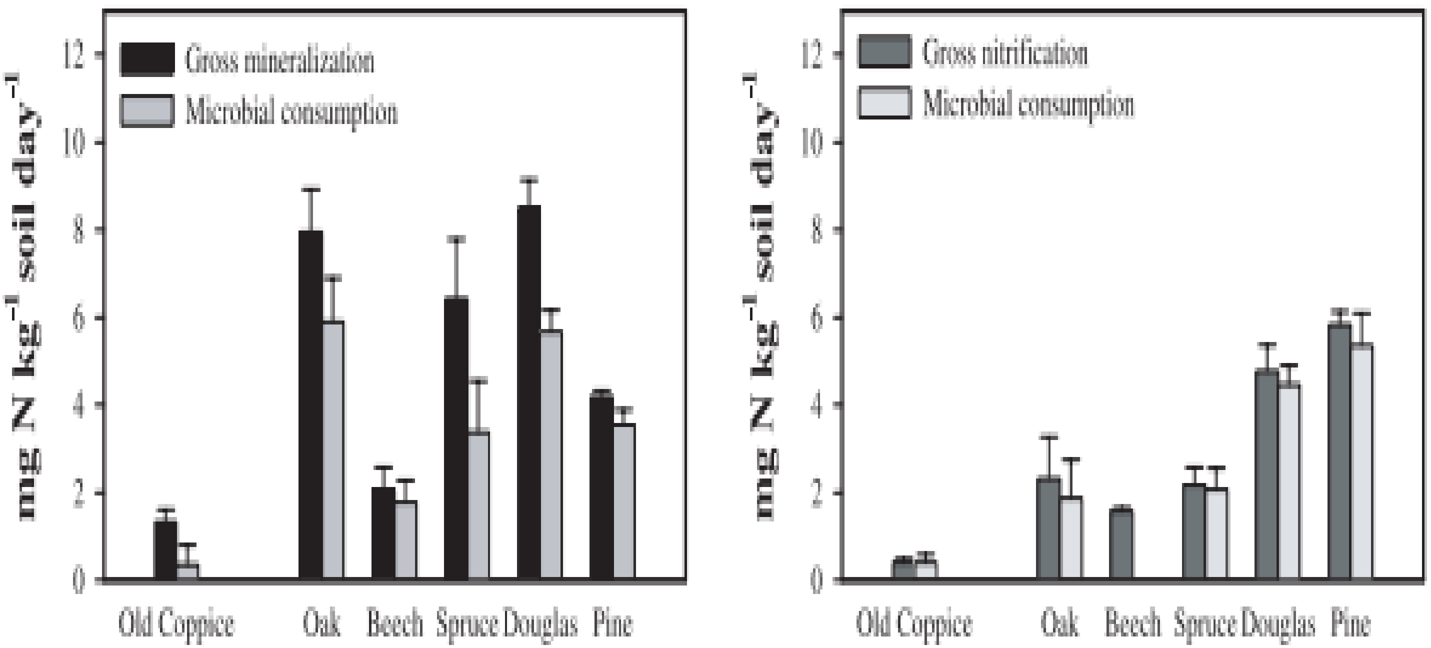
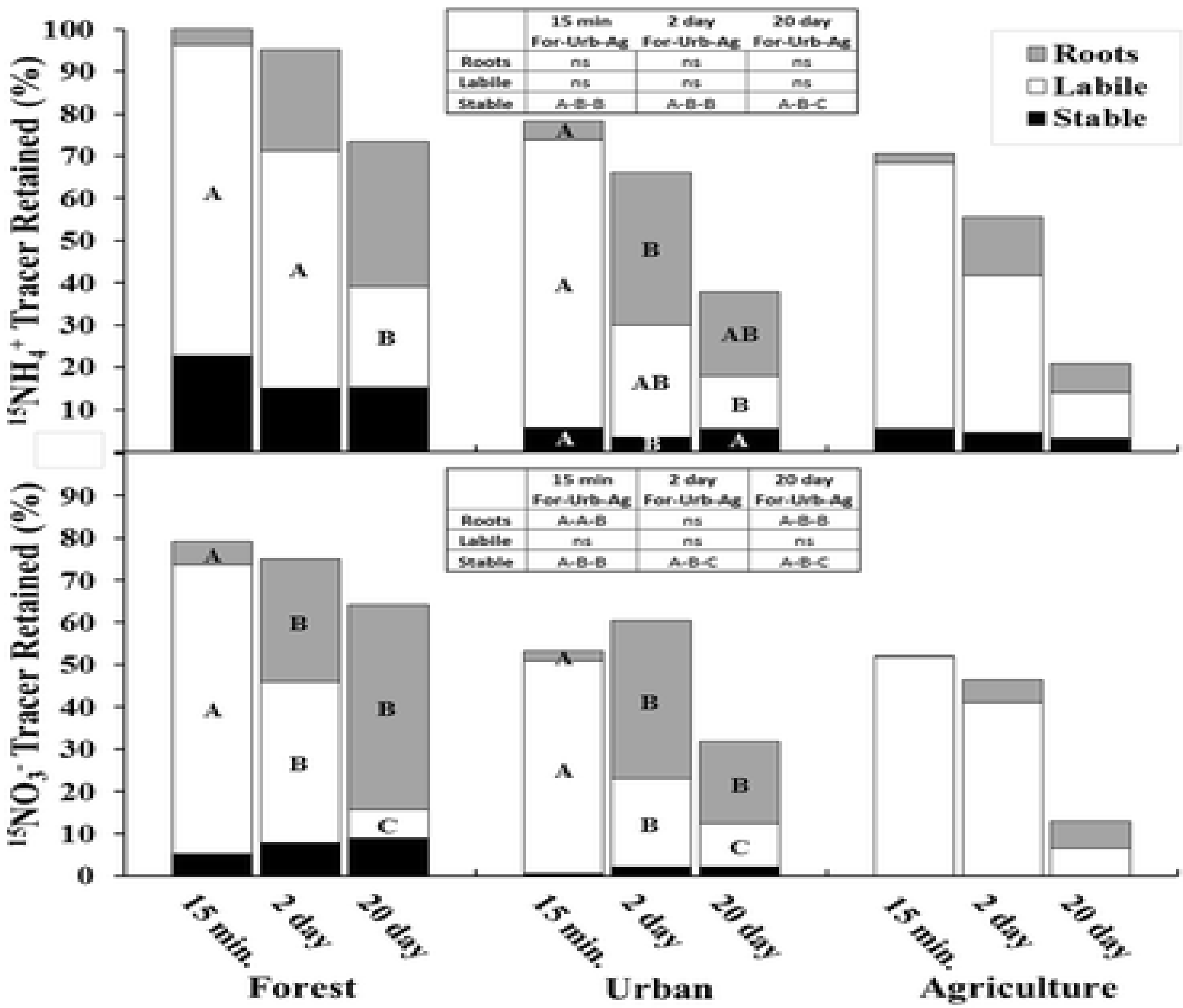
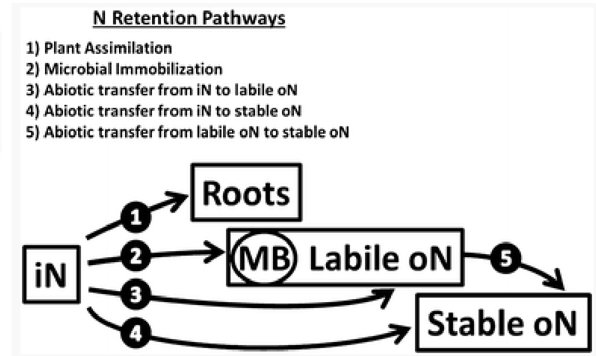


Figure 1. Gross mineralization (gross nitrification) and microbial consumption in soils from an old coppice and in five pure stands of oak, beech, spruce, Douglas fir and Corsican pine planted after the clear-cut of the coppice in 1976. Microbial consumption = gross mineralization – net mineralization. Means and standard deviation (n = 5).

Zeller et al., 2007

Retención de N en suelos forestales, urbanos y agrícolas





Weitzman and Kaye, 2016

FPC Forest Productivity Cooperative

North Carolina State University | Virginia Tech | Universidad de Concepción

Understanding nitrogen cycling after fertilization with urea and enhanced efficiency fertilizers in pine plantations of the southeastern United States using stable isotopes

---

Raymond J. et al., 2016

Initial Transformations of N Fertilizer (urea 50% of world N fertilizers)…

- **Transformations of urea in soil**

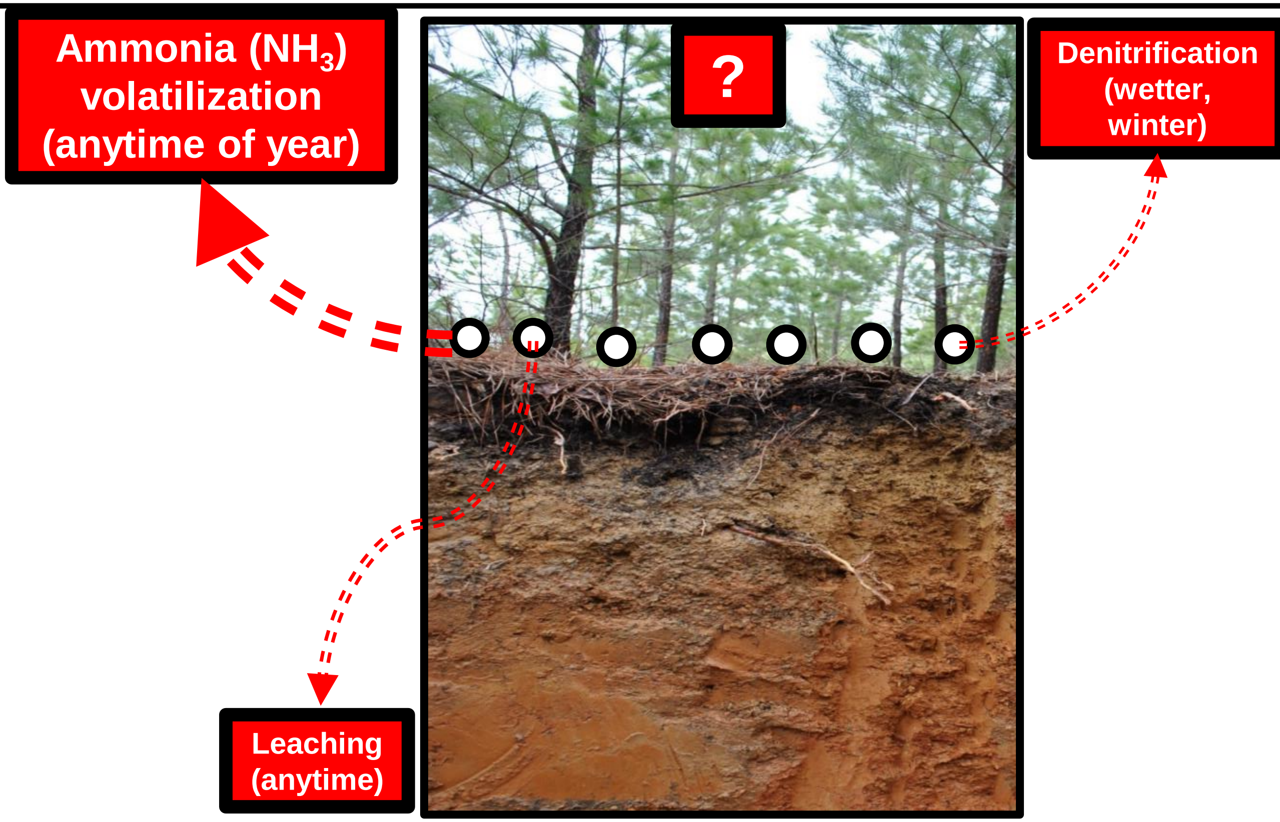
  $CO(NH_2)_2 + 2H^+ + 2H_2O \xrightarrow{(urease)} 2NH_4H_2CO_3$ (urea dissolution)

  $NH_4^+ \rightarrow NH_3\ (gas) + H^+$

  $H_2CO_3 \rightarrow CO_2 + H_2O$

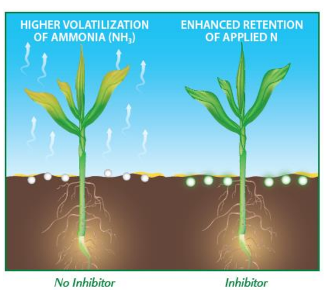
- **Urea dissolution controlling mechanisms - weather**
  - Relative humidity > Critical Relative Humidity (~80%)
  - High Temperatures
  - High wind speeds
  - Low precipitation after fertilization, small diffusion depth into soil (<0.5 in)
  - Low pH buffering capacity
  - Organic residues (pine needles)

…Leading to Potential Fertilizer N Losses

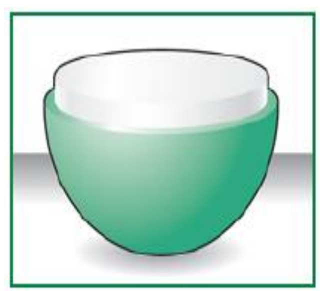


Enhanced Efficiency Fertilizers (EEFs)

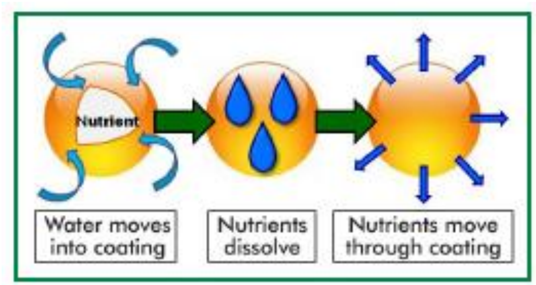
- **Urea**
- **Urea + NBPT (NBPT) - N(n-butyl) thiophosphoric triamide – Agrotain Ultra**
- **MAP Coated Urea + NBPT (CUF) - Arborite EC**
- **Polymer Coated Urea (PCU) - Agrium ESN**



NBPT



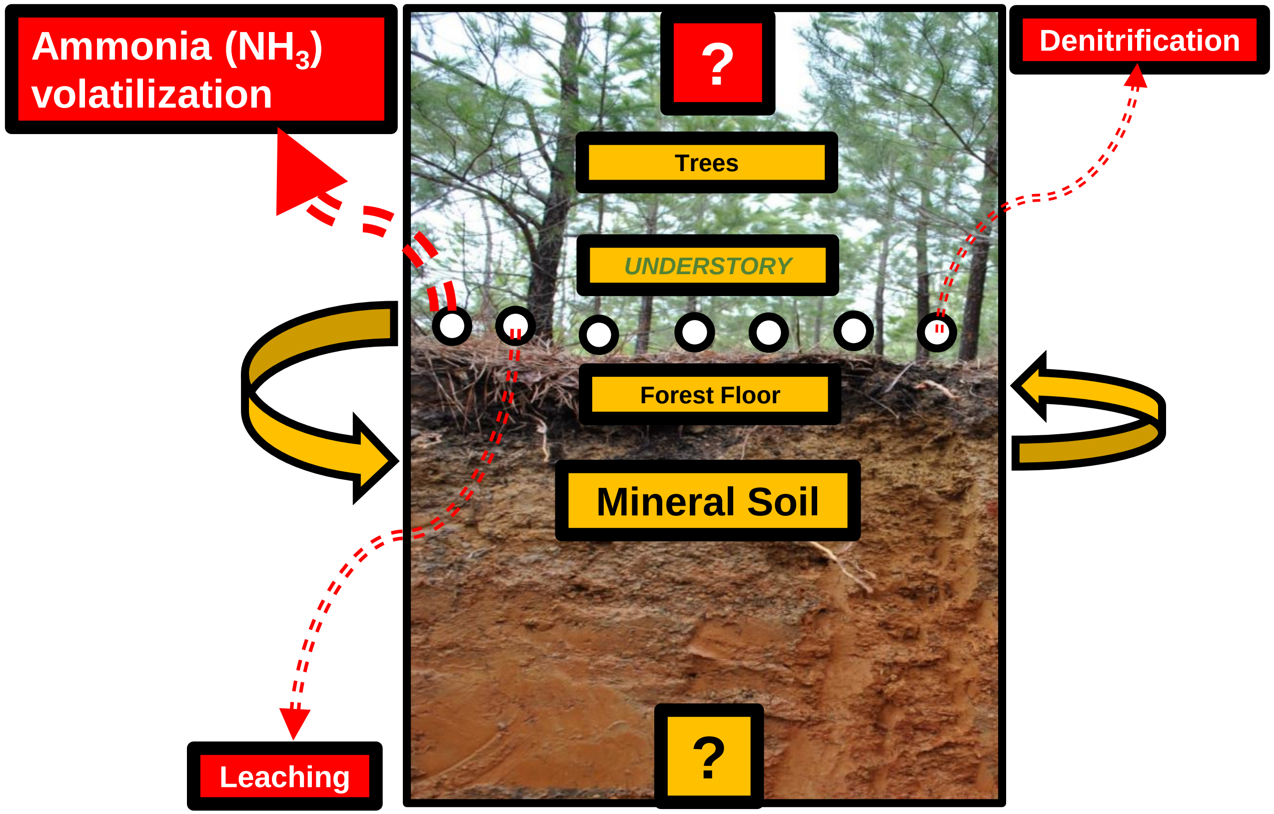
CUF



PCU

www.ipni.net/

Fertilizer N Retention after Application



FERTILIZATION

N Fertilization Generally Improves Growth

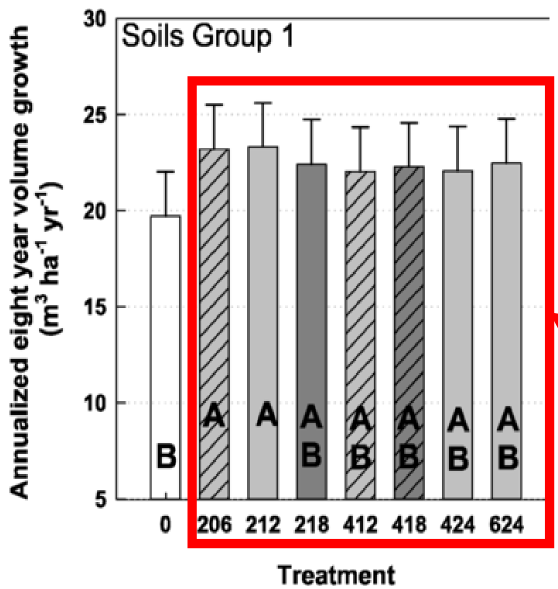
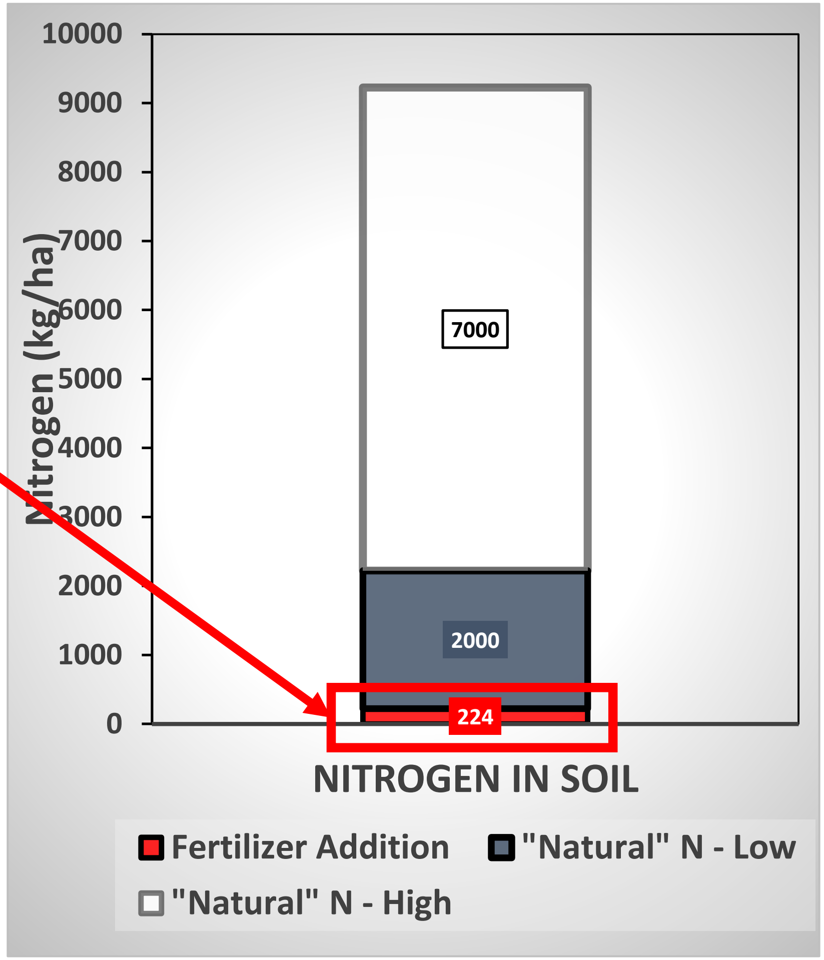
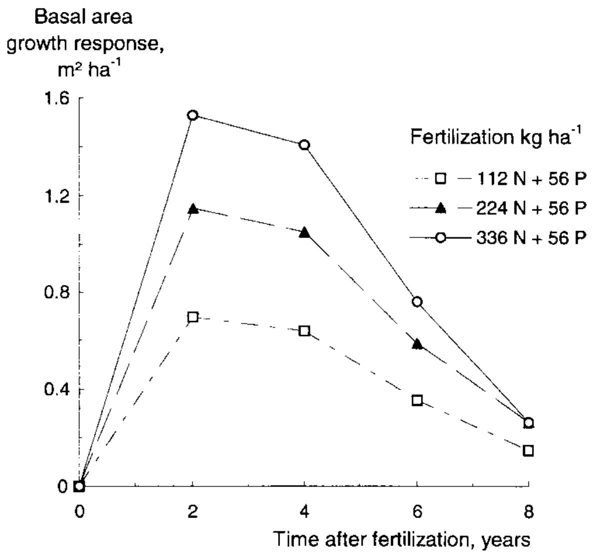


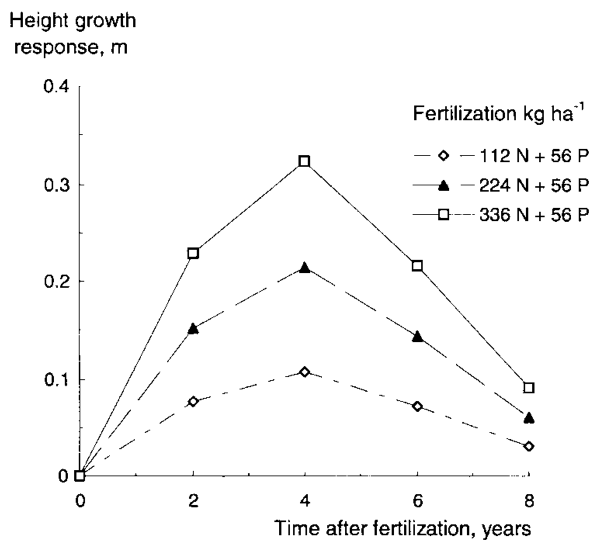
Figure 1. Eight-year average volume growth in soil groups 1 for eight treatments where nitrogen was applied at different rates and frequencies across 22 sites in the southeastern United States. Different letters indicate significant differences in treatment means within a soil group. Error bars are one standard error.

Albaugh et al. 2015



FPC Forest Productivity Cooperative
North Carolina State University | Virginia Tech | Universidad de Concepción



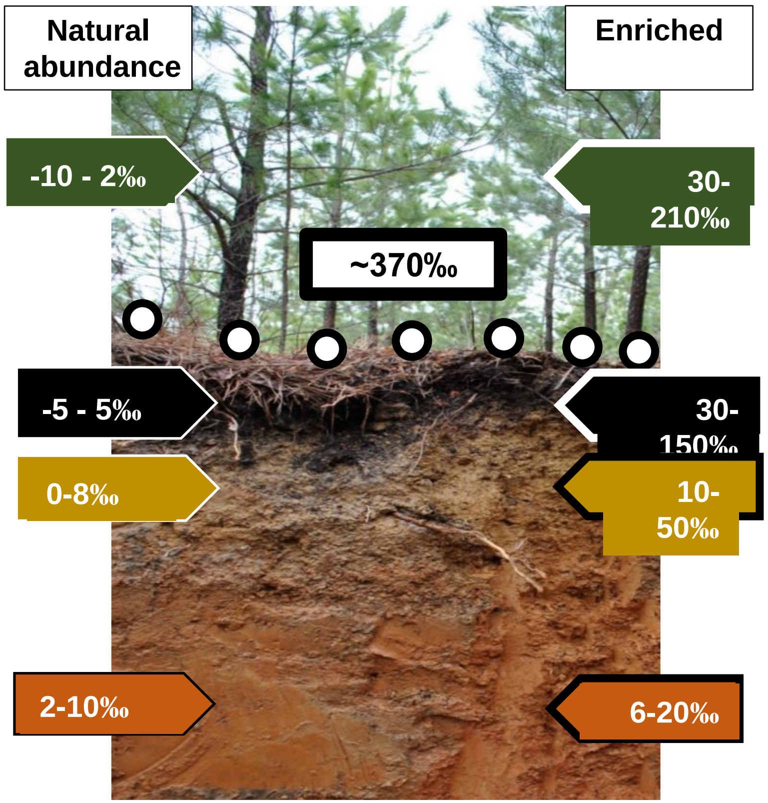


Response to Nitrogen Fertilization is short lived

FPC Forest Productivity Cooperative

North Carolina State University | Virginia Tech | Universidad de Concepción

Using Stable Isotopes Can Refine Our Understanding of the Fate of Fertilizer N in a System



- **^{14}N (99.63%) :** 7 e^-, 7 p^+, 7 n
- **^{15}N (0.3663%) :** 7 e^-, 7 p^+, 8 n
- **δ^{15}N, ‰, permil**
- **^{15}N enriched fertilizers can assist to distinguish N from fertilizer vs. native sources**

Research Objectives & Hypotheses

Fertilizer N treatment differences for:

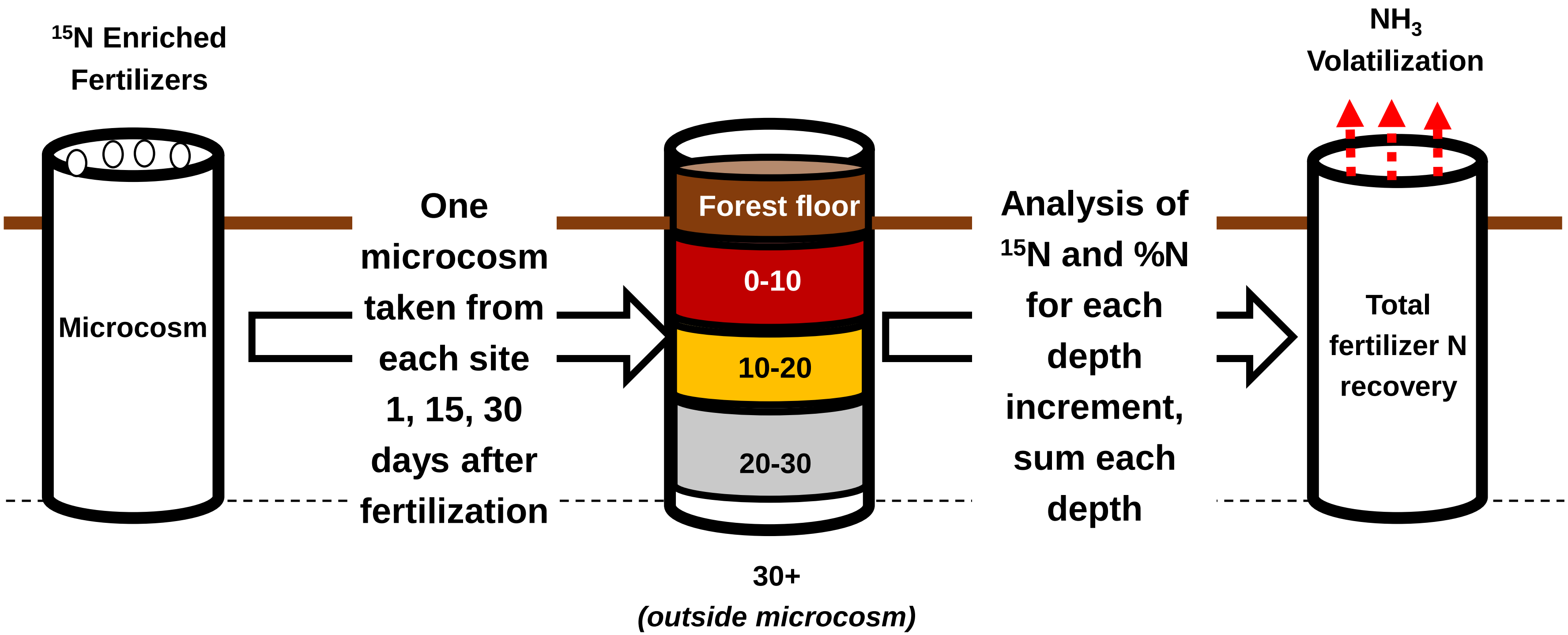
- **Season of application?**
- **NH_3 volatilization?**
- **N uptake?**
- **Fertilizer N retention in ecosystem?**
- **Effect of understory?**

Long term bioavailability of fertilizer N?

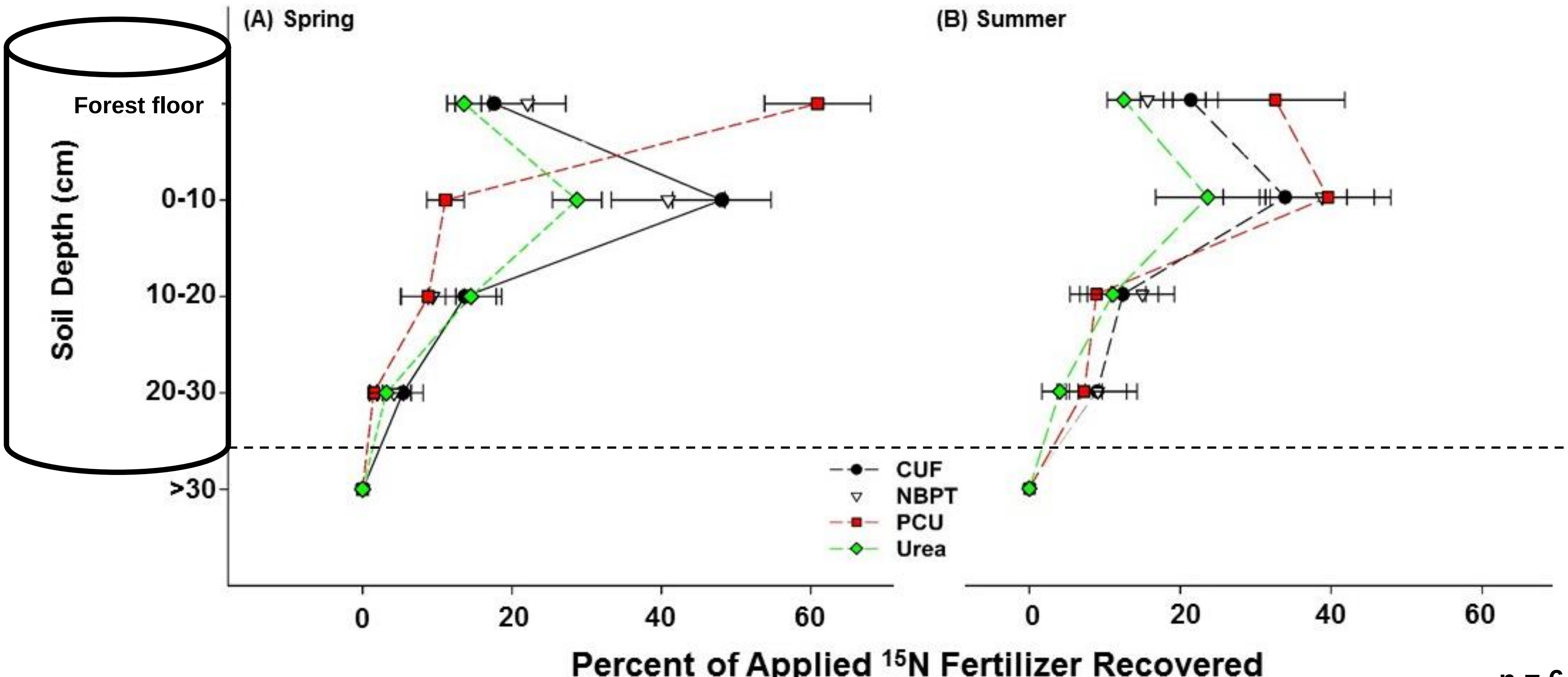
NH_3 Volatilization Experiment

- **Fertilizer N loss among treatments (CUF, NBPT, PCU, urea)?**
- **Fertilizer N loss between seasons (spring, summer)?**

NH_3 Volatilization - Experimental Methods



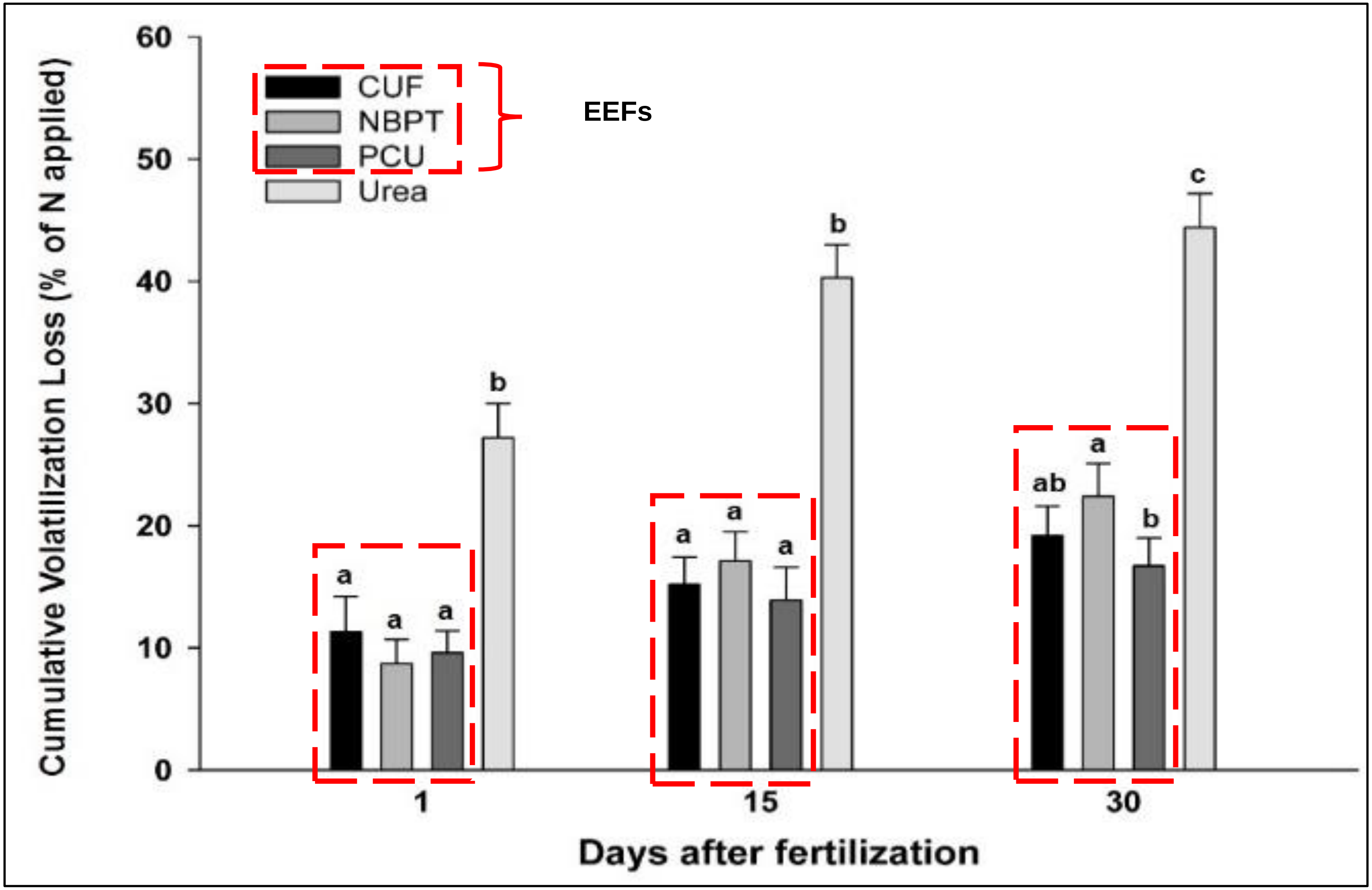
Distribution of Fertilizer N in Soil



n = 6

Raymond et al. 2016a

NH_3 Volatilization – Fertilizer N Loss – Spring + Summer



Raymond et al. 2016a

n = 12

Seasonal N Uptake

- **N uptake after N fertilization for treatments?**
- **N uptake after N fertilization for treatments for spring vs. summer?**

FPC Forest Productivity Cooperative
North Carolina State University | Virginia Tech | Universidad de Concepción

Seasonal N Uptake - Experimental Methods

- 2 trees in plot center sampled every 6 weeks
- Foliage sampled upper 1/3rd canopy
- Sample divided by flushes (0-3)
- Each flush analyzed for ^{15}N, %N

Spring

Weeks After Fertilization

0

6

12

18

24

30

Flushes — *0 (Previous year)* — *1* — *2 (Current Year)* — *3*

Summer

Weeks After Fertilization

0

6

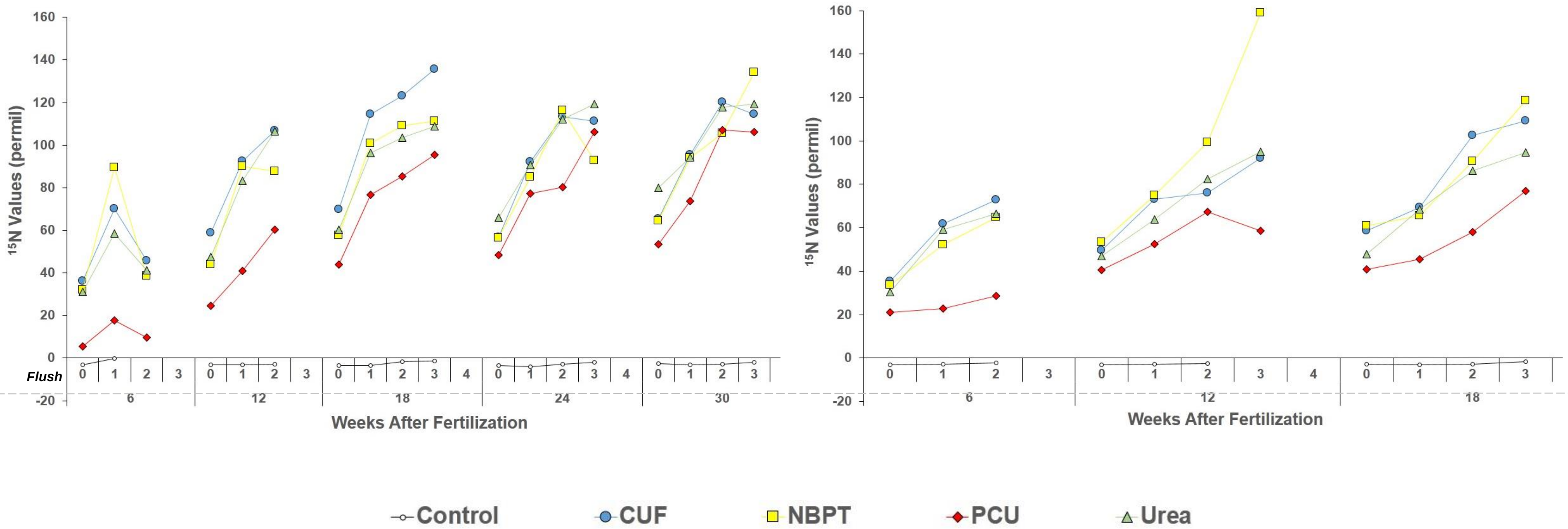
12

18

Flushes — *0 (Previous year)* — *1* — *2 (Current Year)* — *3*

Seasonal N Uptake – Foliar ^{15}N Values

Spring **Summer**



Raymond et al. in prep

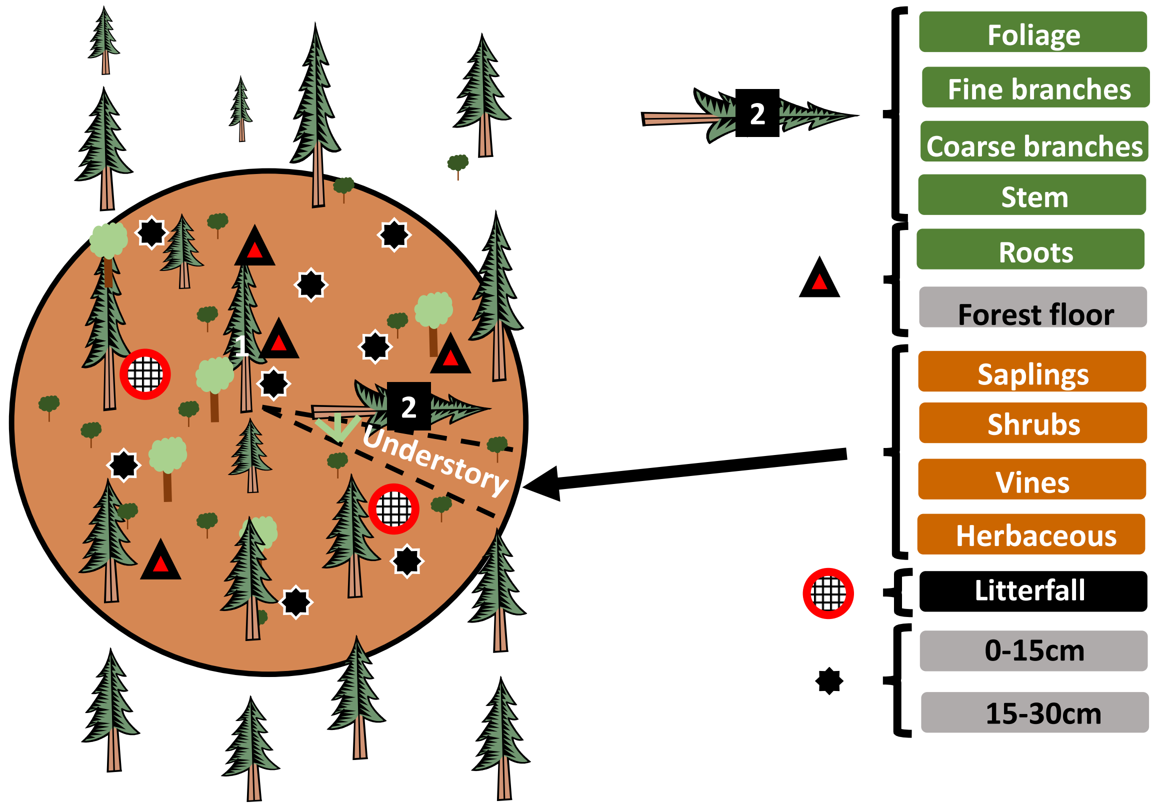
System Partitioning and Retention of Fertilizer N

- **Fertilizer N retention in ecosystem?**
- **Season of application (spring vs. summer) for fertilizer treatments?**

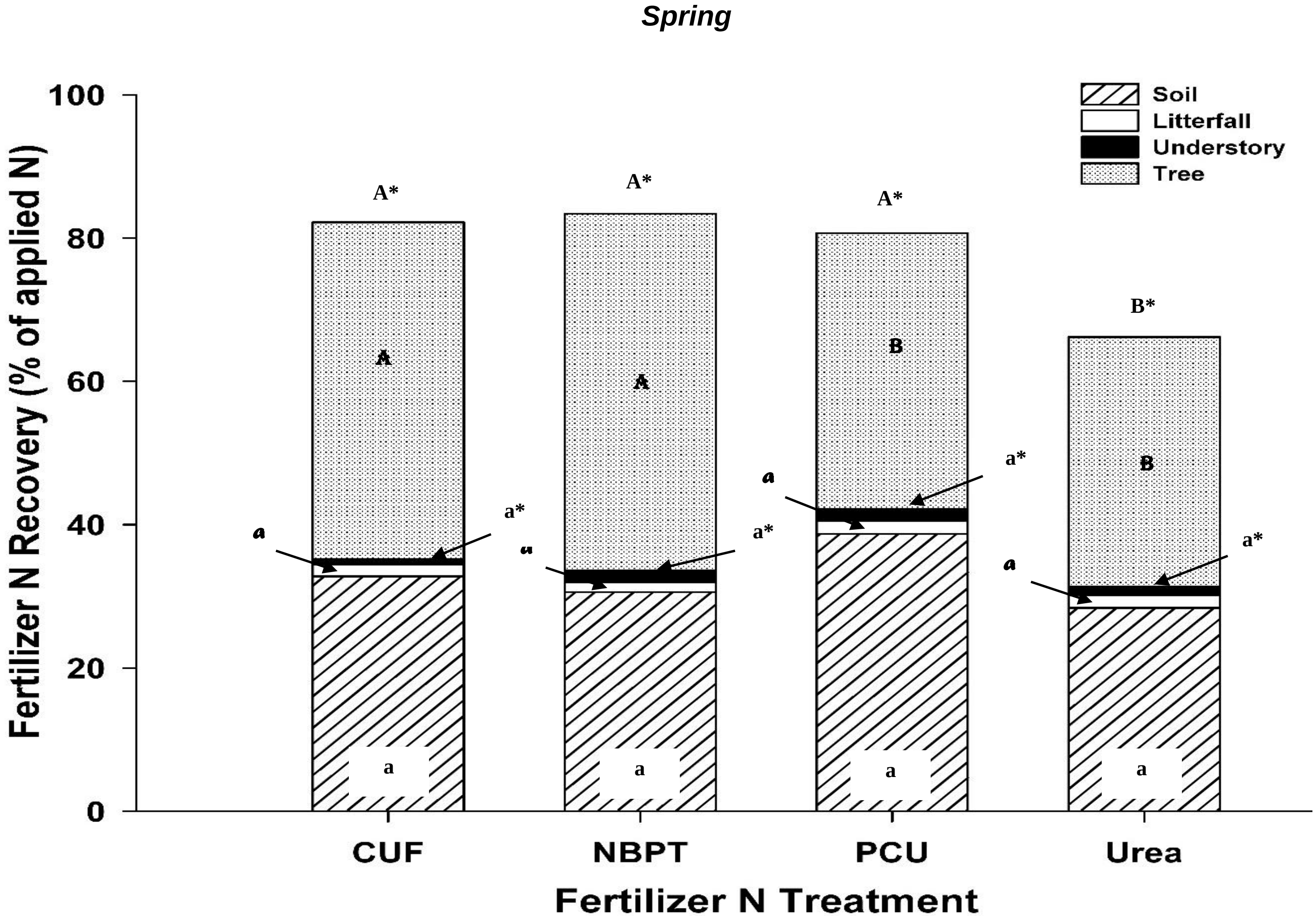
FPC Forest Productivity Cooperative
North Carolina State University | Virginia Tech | Universidad de Concepción

System Partitioning and Retention of Fertilizer N

- 1 tree at plot center harvested
- Sapling strata sampled by species and 2.54cm class
- Shrubs/vines/herbs sampled in randomly chosen plot area with shrubs by species, vines and herbs composite sampled
- Forest floor, roots sampled from 4 random areas
- Litterfall sampled from 2 traps
- Mineral soil (0-15, 15-30cm) sampled from 8 random areas
- All samples analyzed for ^{15}N, %N



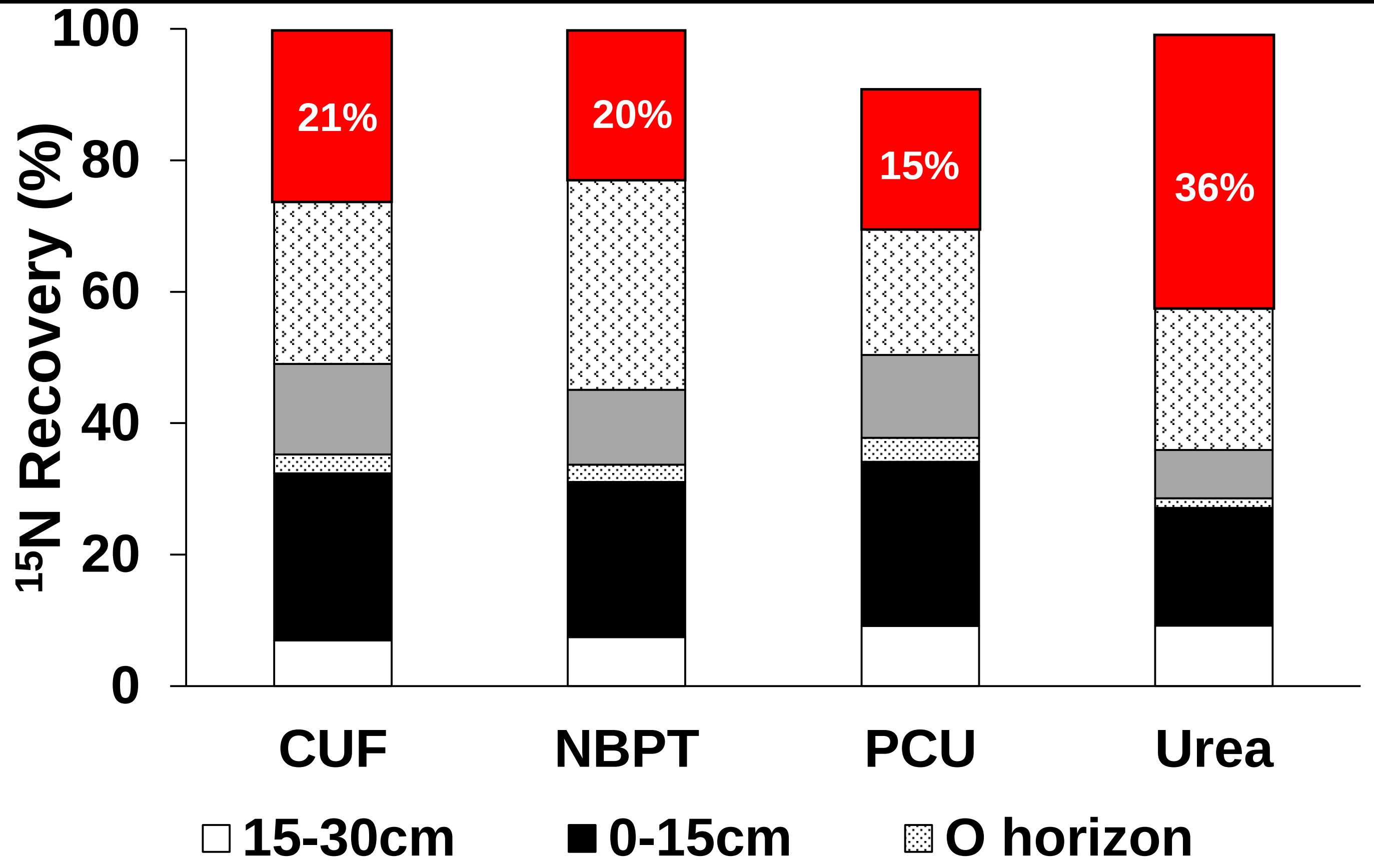
System Partitioning and Retention of Fertilizer N – Study 2



n = 12

Raymond et al. 2016 In Review

^{15}N Ecosystem Recovery - Spring



Mean Recovery	79%	80%	79%	63%

Summary of Results

- **EEFs had lower NH_3 volatilization than urea**
- **Foliar N uptake similar for all fertilizer treatments**
- **Potential stimulation of native N uptake after N fertilization**
- **EEF fertilizer N recovery similar spring, summer - more variable with urea**
- **EEFs greater system fertilizer N retention than urea**
- **Large amount of fertilizer N remained in soil, followed by entire tree**

EEFs had lower losses, higher ecosystem recovery of fertilizer N than urea