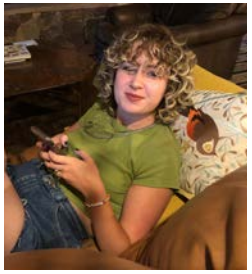
MinION sequencing and bioinformatics workshop

Callum J. Bell, Ph.D.

Callum Bell


Dunnet
Thurso
John o Groats
Wick
Stornoway
Ullapool
Elgin
Nairn
Inverness
Peterhead
Portree
Inverurie
Aviemore
Aberdeen
SCOTLAND
Fort William
Hebrides
Arbroath
Dundee
Oban
Perth
Loch Lomond & The Trossachs National Park
Edinburgh
Glasgow
Paisley
Ayr
United Kingdom
Newcastle upon Tyne
Sunderland
Londonderry
Derry
Letterkenny
Ballymena
NORTHERN IRELAND
Durham
Middlesbrough

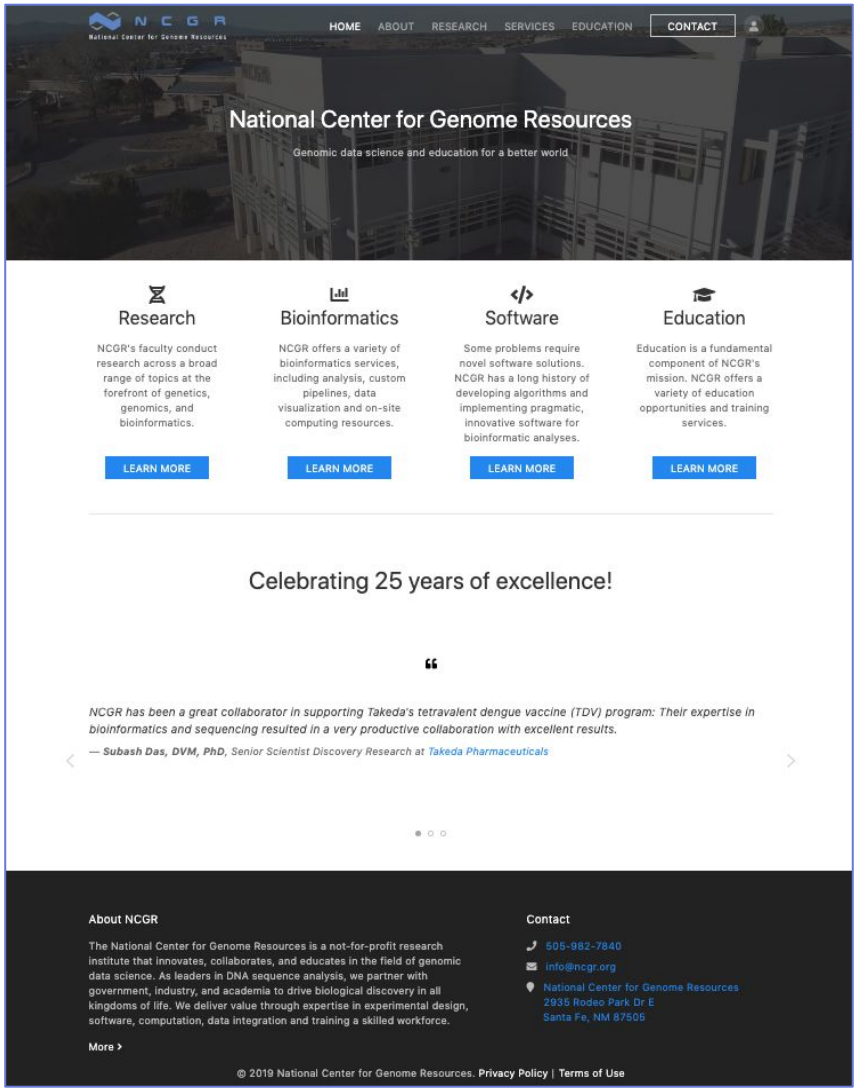

INDIA




NCGR

NCGR History

- New Mexico non profit
- Formed in 1994
- Software and database support for the DOE human genome project
- Research
- Bioinformatics
- Software
- Education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Genomic data science and education for a better world
Research
NCGR's faculty conduct research across a broad range of topics at the forefront of genetics, genomics, and bioinformatics.
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Some problems require novel software solutions. NCGR has a long history of developing algorithms and implementing pragmatic, innovative software for bioinformatic analyses.
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Education is a fundamental component of NCGR's mission. NCGR offers a variety of education opportunities and training services.
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“
NCGR has been a great collaborator in supporting Takeda's tetravalent dengue vaccine (TDV) program: Their expertise in bioinformatics and sequencing resulted in a very productive collaboration with excellent results.
— Subash Das, DVM, PhD, Senior Scientist Discovery Research at Takeda Pharmaceuticals
About NCGR
The National Center for Genome Resources is a not-for-profit research institute that innovates, collaborates, and educates in the field of genomic data science. As leaders in DNA sequence analysis, we partner with government, industry, and academia to drive biological discovery in all kingdoms of life. We deliver value through expertise in experimental design, software, computation, data integration and training a skilled workforce.
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NCGR Mission

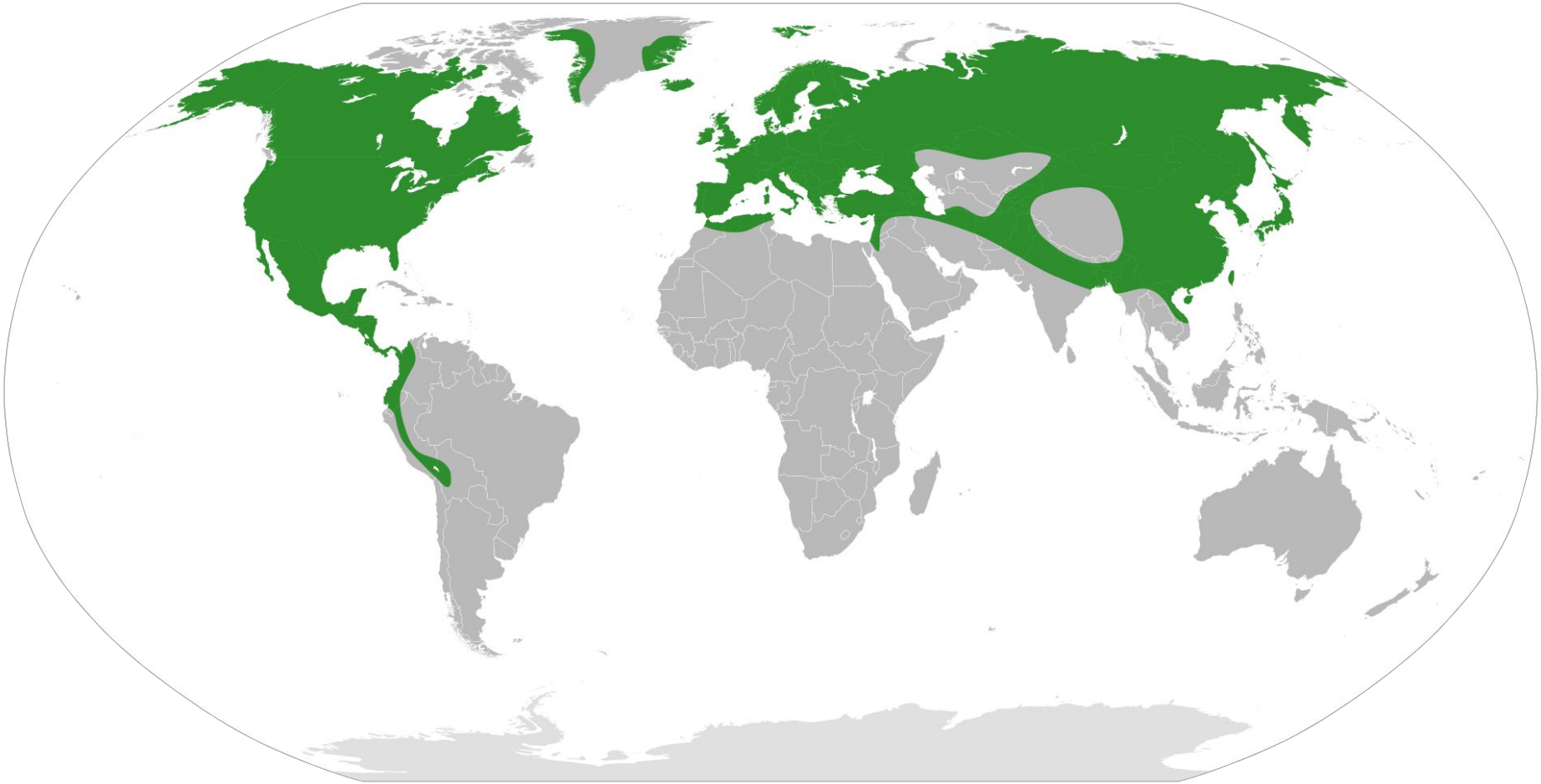
The National Center for Genome Resources is a not-for-profit research institute that innovates, collaborates, and educates in the field of genomic data science. As leaders in DNA sequence analysis, we partner with government, industry, and academia to drive biological discovery in all kingdoms of life. We deliver value through expertise in experimental design, software, computation, data integration and training a skilled workforce.

National Center for Genome Resources

Red Alder Distribution

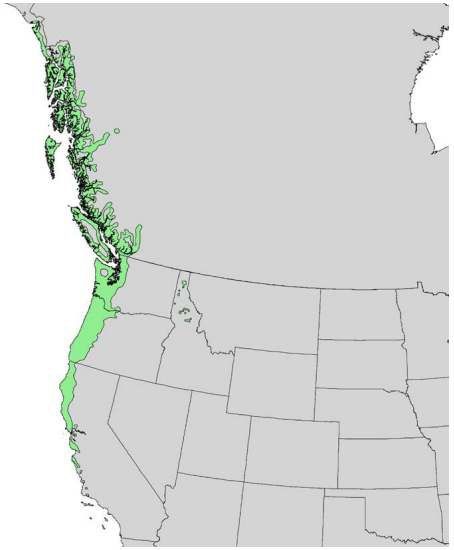
https://en.wikipedia.org/wiki/Alnus_rubra

Global Alder Distribution



Distribution of the alders (genus *Alnus*), based on **Silvester WB.** (1977). "Dinitrogen fixation by plant associations excluding legumes." *in* **Hardy RFW, Gibson AH** (ed.), *A Treatise on Dinitrogen Fixation. Section IV: Agronomy and Ecology*. John Wiley and Sons, New York, p. 141–190.

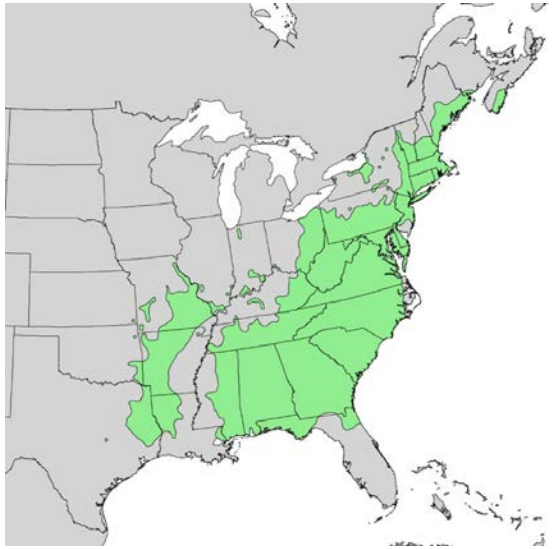
North American Alnus distribution



A. rubra

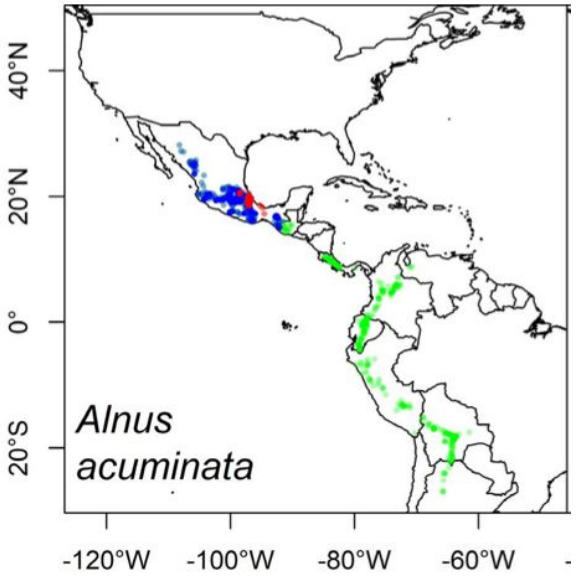
A. rhombifolia

A. incana (ssp tenuifolia)



A. serrulata

North American Alnus distribution (cont.)



A. acuminata

A. oblongifolia

A. jorullensis

https://doi.org/10.1111/ddi.13275

https://en.wikipedia.org/wiki/Alder
https://archive.org/details/atlasofunitedsta1314litt/page/n47/mode/1up

Funding acknowledgements

- New Mexico INBRE (IDEA Network for Biomedical Research Excellence) https://nminbre.nmsu.edu/

1. Network Development: to provide leadership, management and oversight
2. Research Development: build to increase the thematically research base
3. Student Development: provide research experience and education to engage students
4. Workforce Development:enhance science and technology knowledge of the state's workforce
5. Collaboration Development:share established programs and infrastructure through a tristate Regional Alliance of INBRE Networks

Funding acknowledgements

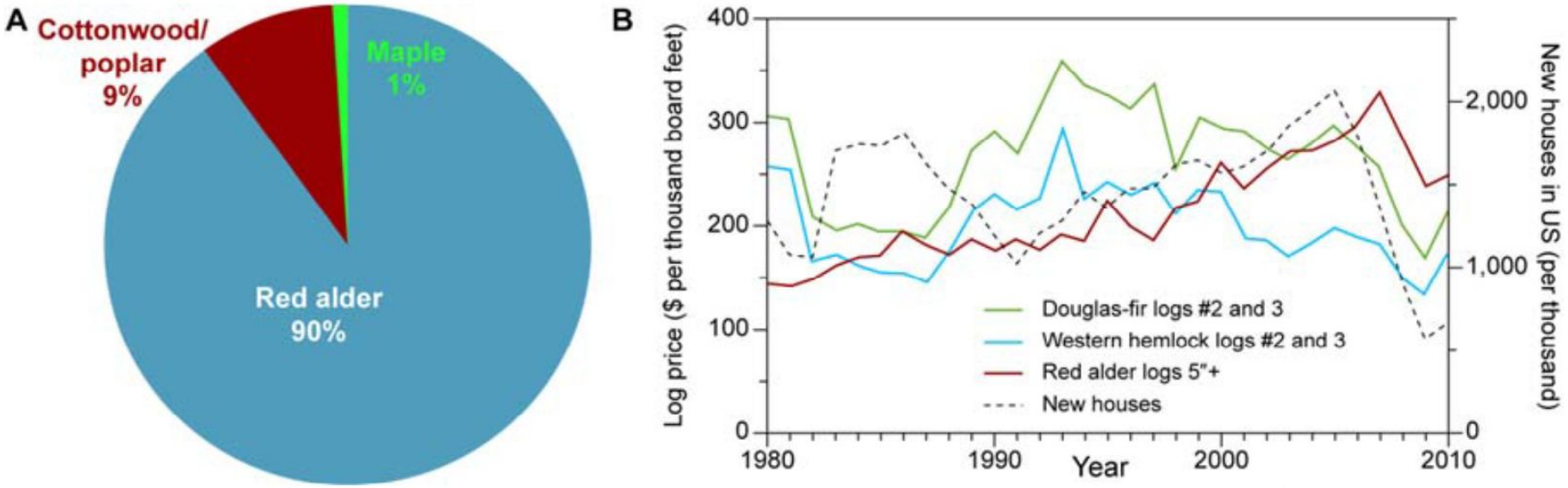
- RESEARCH-PGR: Deciphering the molecular basis of elite red alder lines and their Frankia alni symbionts
- NSF award 1547842

WASHINGTON STATE UNIVERSITY

Norman G. Lewis

- Weyerhaeuser. Inc.
- Licensed alder program to WSU
- Field trials
- Elite clone collection
- Collection of Frankia strains

Economics of red alder



A. *Pie chart showing typical annual usage in Washington State.*[1] ***B.*** *Historical (1980-2010) changes in red alder wood's relative value/importance.* %).

The Washington Hardwoods Commission (2002) A Hardwood Resource Assessment for Western Washington.

www.westernhardwood.org/Miscellaneous/GIS_hardwood_inventory_6.pdf.

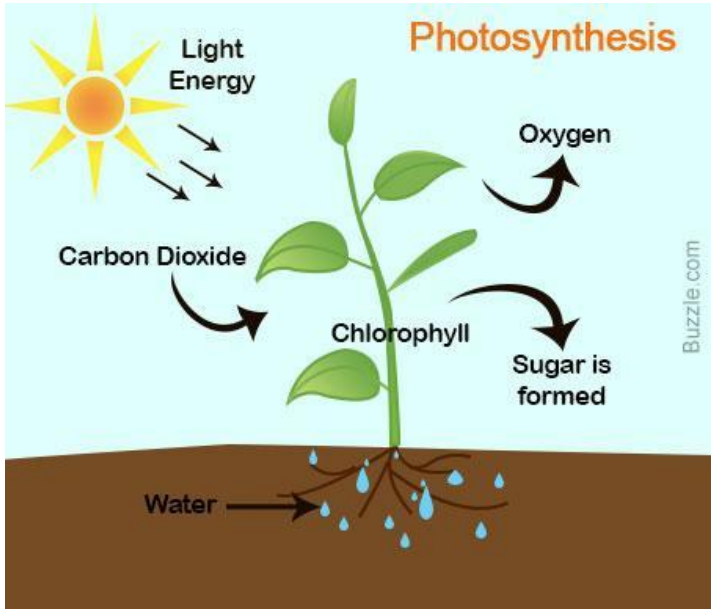
Board Foot

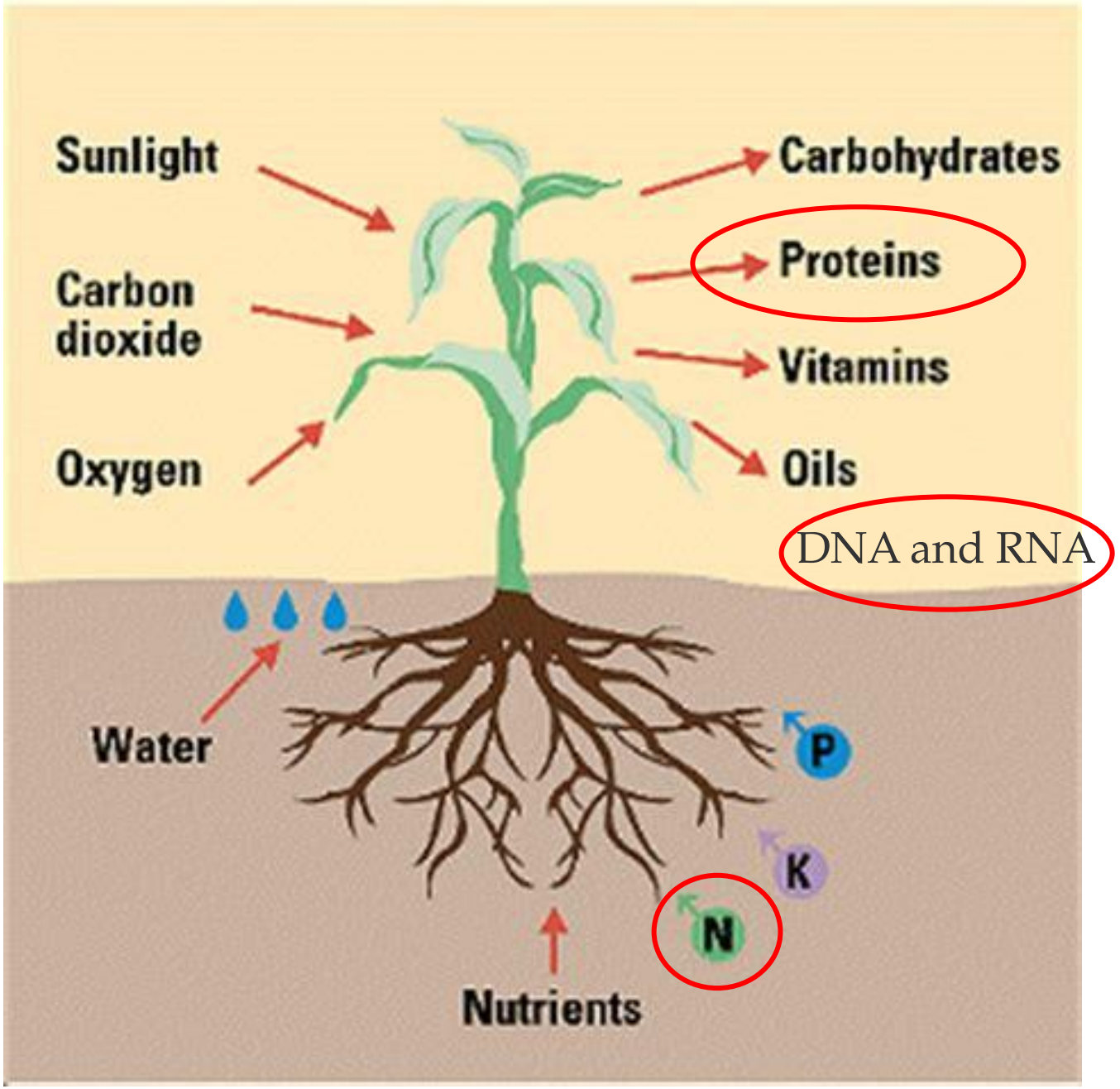
A unit of volume for timber equal to 144 cubic inches, notionally twelve inches by twelve inches by one inch.

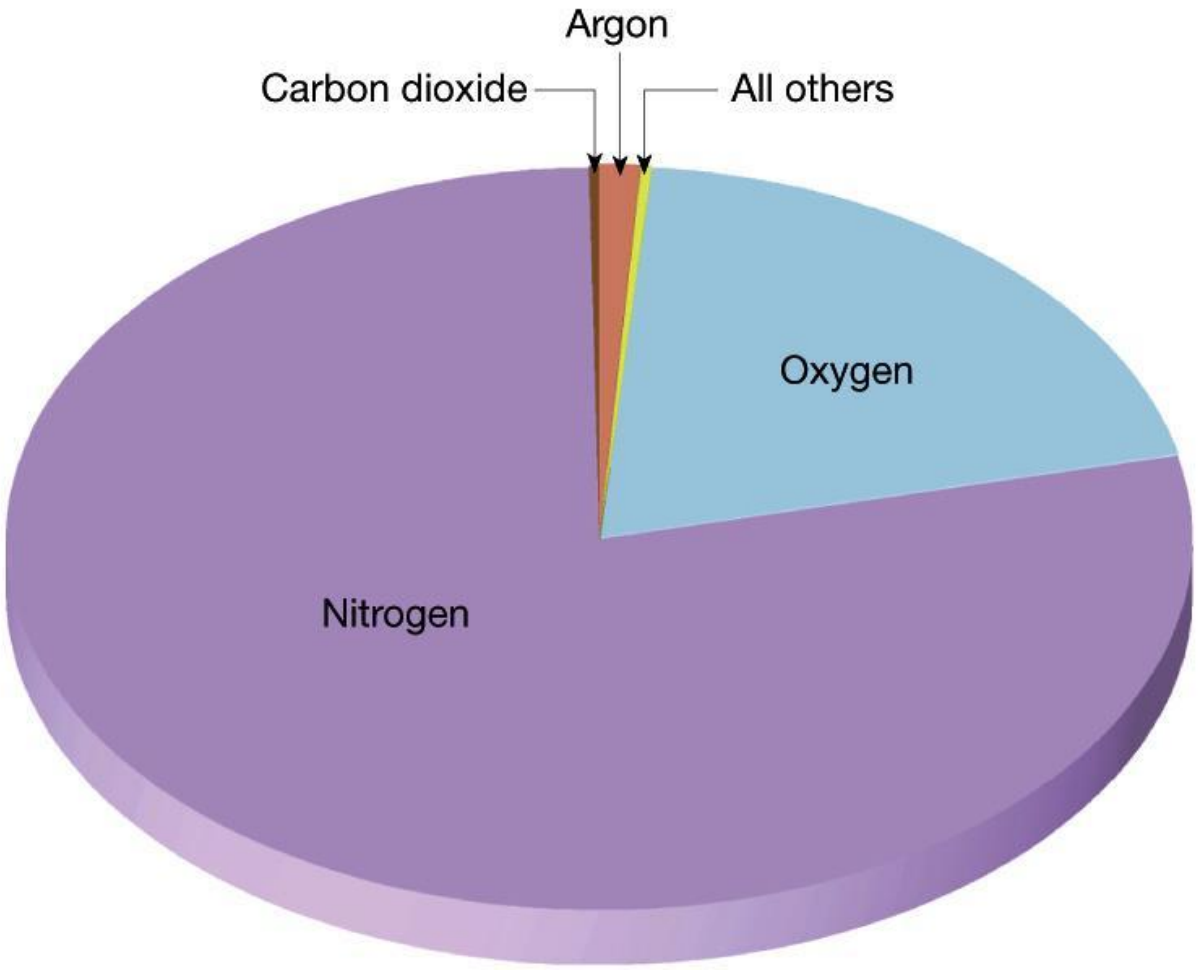

TONE
VOL
VOL

(almost) all life on earth depends on two biochemical reactions

- CO_2 -> sugar



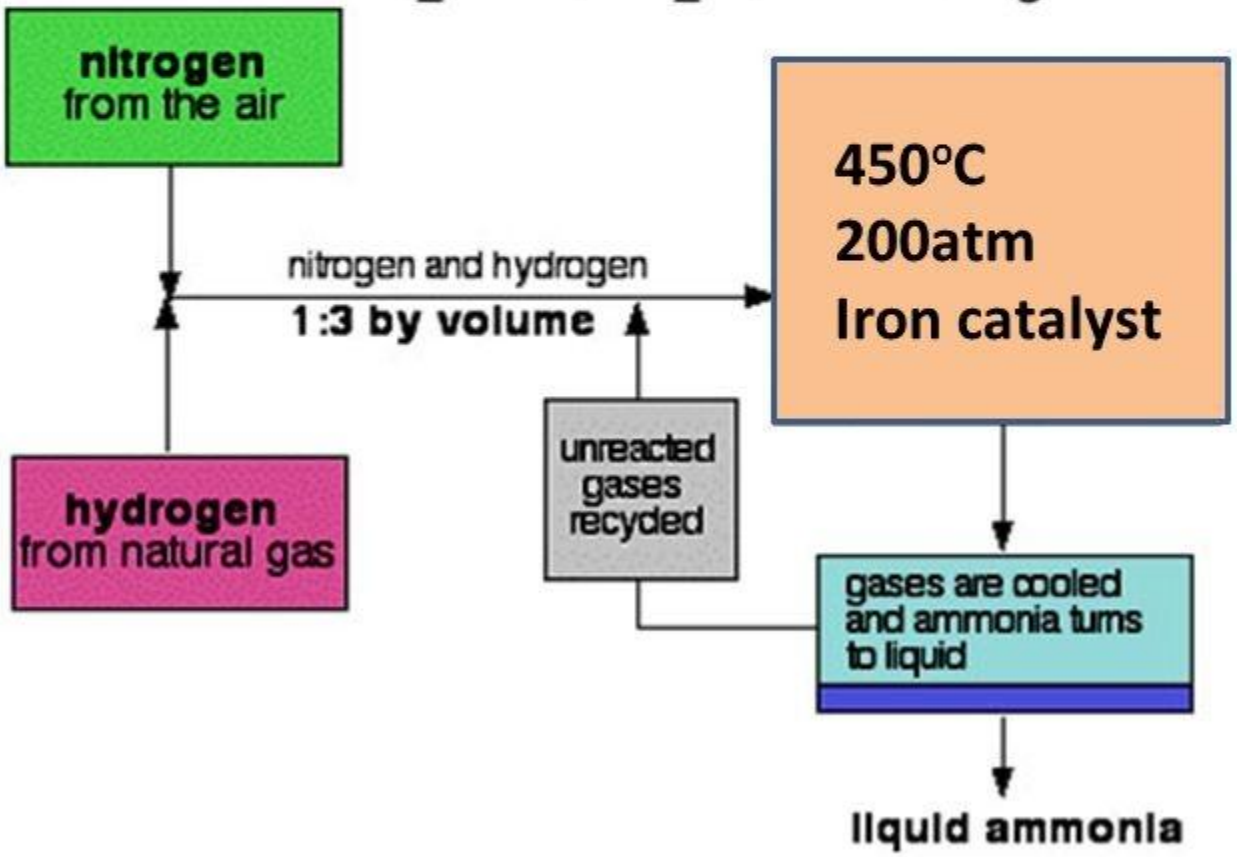

Sunlight
Carbon dioxide
Oxygen
Carbohydrates
Proteins
Vitamins
Oils
DNA and RNA
Water
P
K
N
Nutrients


Argon
Carbon dioxide
All others
Oxygen
Nitrogen

- N_2 -> NH_3 (-> NO_4)

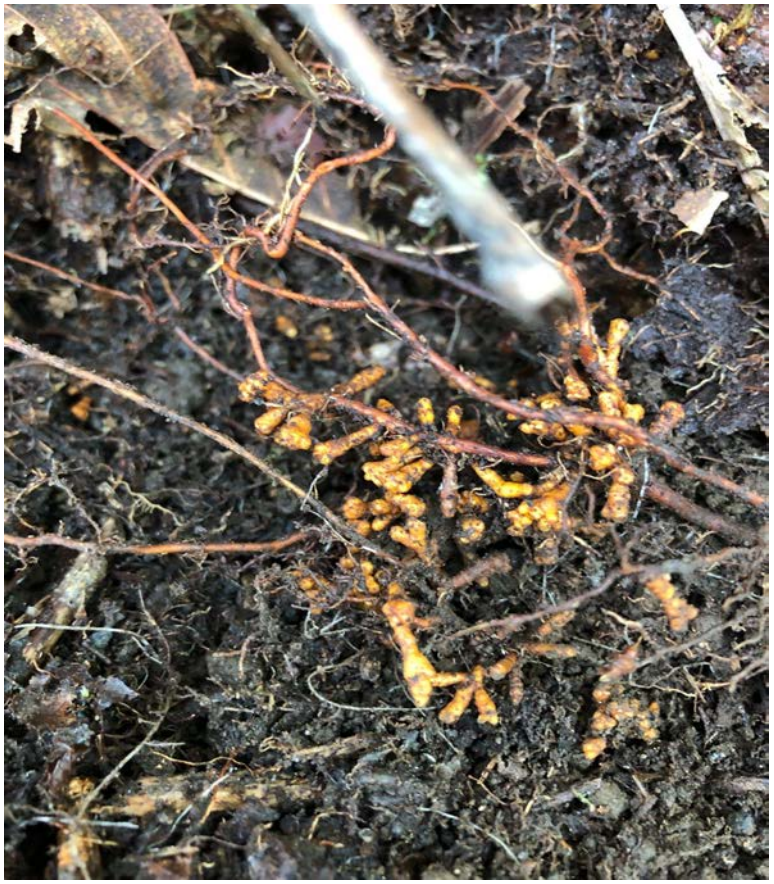
Fritz Haber

$$N_2 + 3H_2 \rightleftharpoons 2NH_3$$



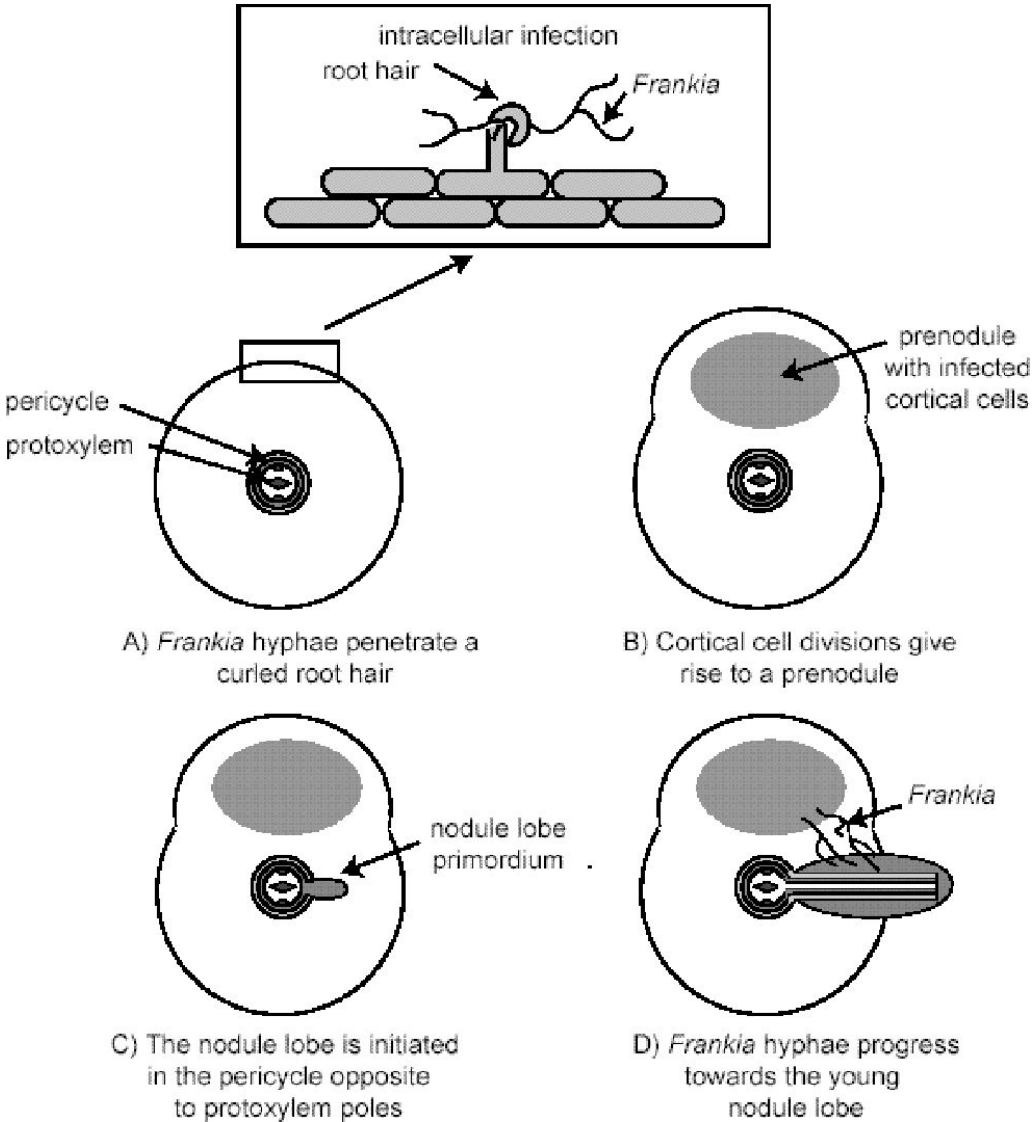
Global 2011 cost in fossil fuels

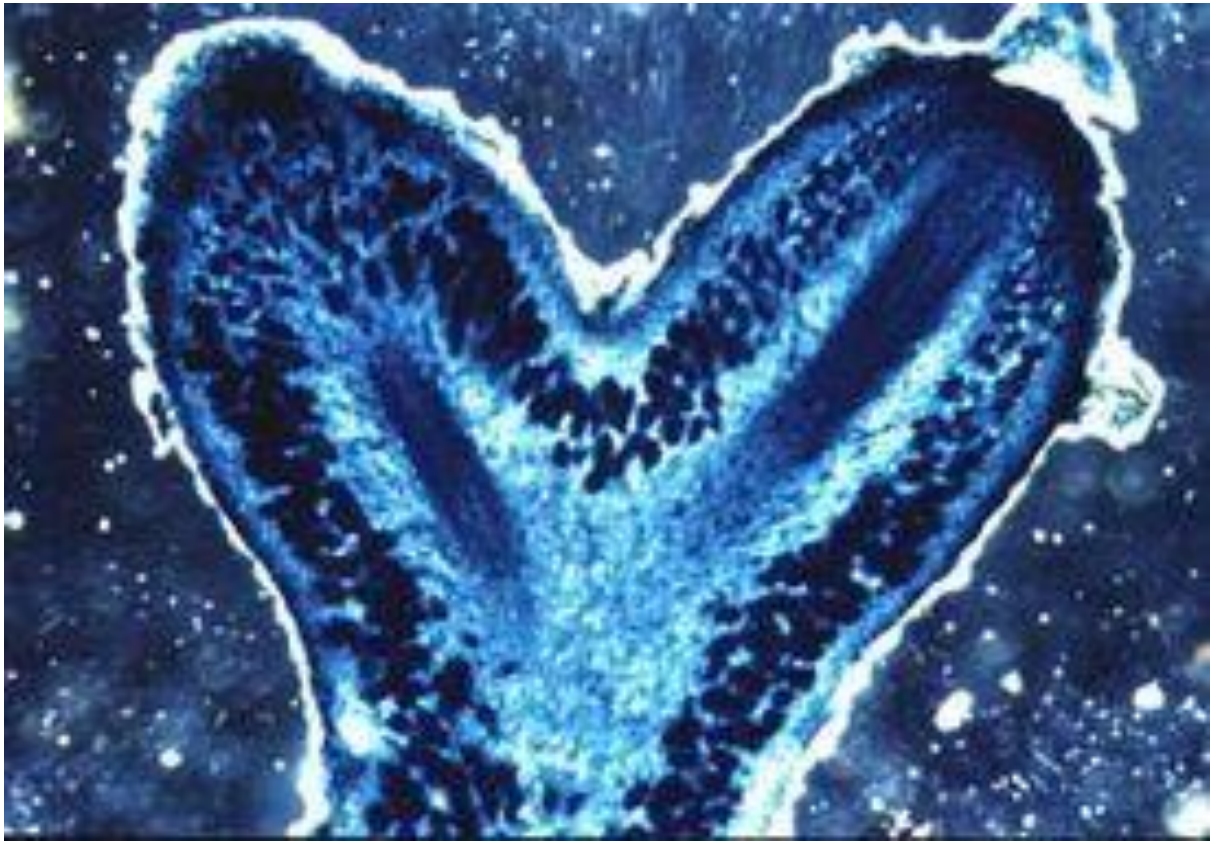
- Haber process used ~ 10^{19} Joules
- World energy production is ~ 4 x 10^{20} Joules
- So about 2.5% of all energy goes to the Haber process

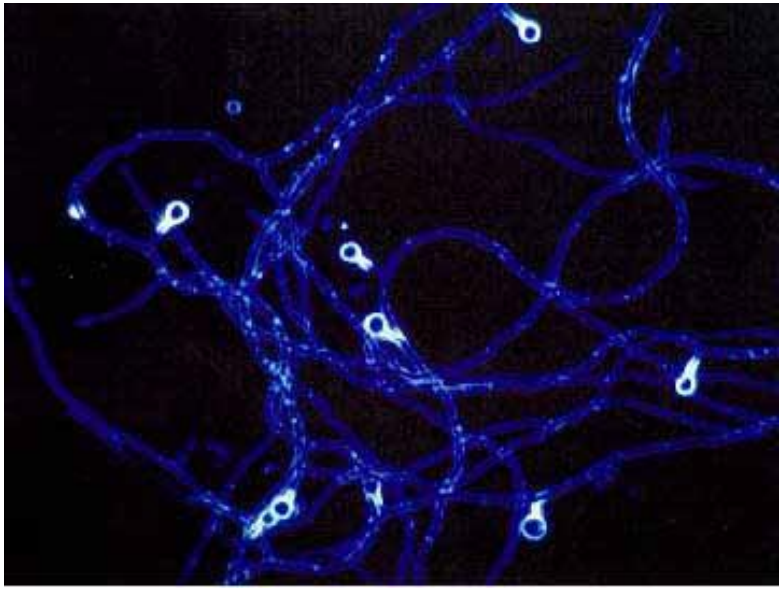


Nodule initiation

DOI:10.5897/AJB2003.000-1104

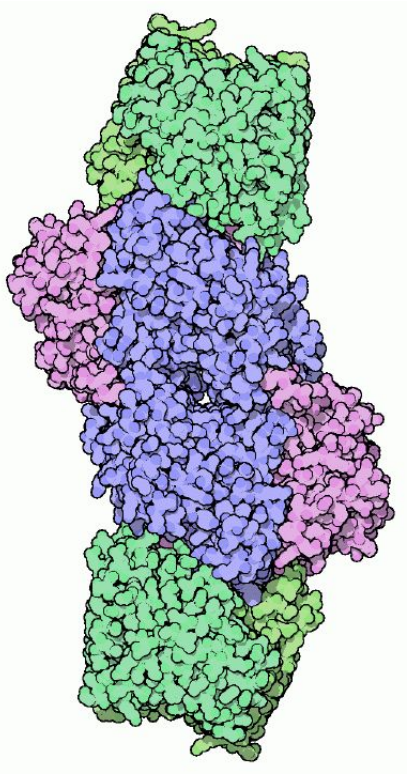






Biological Nitrogen Fixation

- Nitrogenase Enzyme
- Fe protein. Dinitrogen reductase (nifH) dimer
- MoFe protein. Nitrogenase (nifDK) alpha dimer, beta dimer



$$N_2 + 8\ H^+ + 16\ MgATP + 8\ e^- \rightarrow 2\ NH_3 + H_2 + 16\ MgADP + 16\ P_i$$

https://doi.org/10.1146%2Fannurev.biochem.78.070907.103812
https://doi.org/10.1186/1471-2164-13-162

https://pdb101.rcsb.org/motm/26

The Nitrogen Fixing Clade

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 2647–2651, March 1995
Evolution

Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms

DOUGLAS E. SOLTIS*, PAMELA S. SOLTIS*, DAVID R. MORGAN†, SUSAN M. SWENSEN‡, BETH C. MULLIN§, JULIE M. DOWD¶, AND PETER G. MARTIN¶||

*Department of Botany, Washington State University, Pullman, WA 99164-4238; †Department of Biology, Western Washington University, Bellingham, WA 98225; ‡Department of Biology, Indiana University, Bloomington, IN 47405; §Department of Botany, Center for Legume Research, University of Tennessee, Knoxville, TN 37996; and ¶Department of Botany, University of Adelaide, Adelaide, South Australia 5005, Australia

https://doi.org/10.1073/pnas.92.7.2647

The Nitrogen Fixing Clade

Table 1. Angiosperm families that participate in nodular nitrogen-fixing symbioses and the frequency of this association in each family

Prokaryote	Family	Total no. of genera*/genera having root nodules†
Rhizobium	Fabaceae	640/most
	Ulmaceae	18/1
Frankia	Betulaceae	6/1
	Casuarinaceae	4/4
	Elaeagnaceae	3/3
	Myricaceae	3/2
	Rhamnaceae	55/7
	Rosaceae	100/5
	Datiscaceae	3/1
	Coriariaceae	1/1

*From Cronquist (3).
†From Akkermans and van Dijk (4), Bond (5), and Torrey and Berg (6).

https://doi.org/10.1073/pnas.92.7.2647

The Nitrogen Fixing Clade

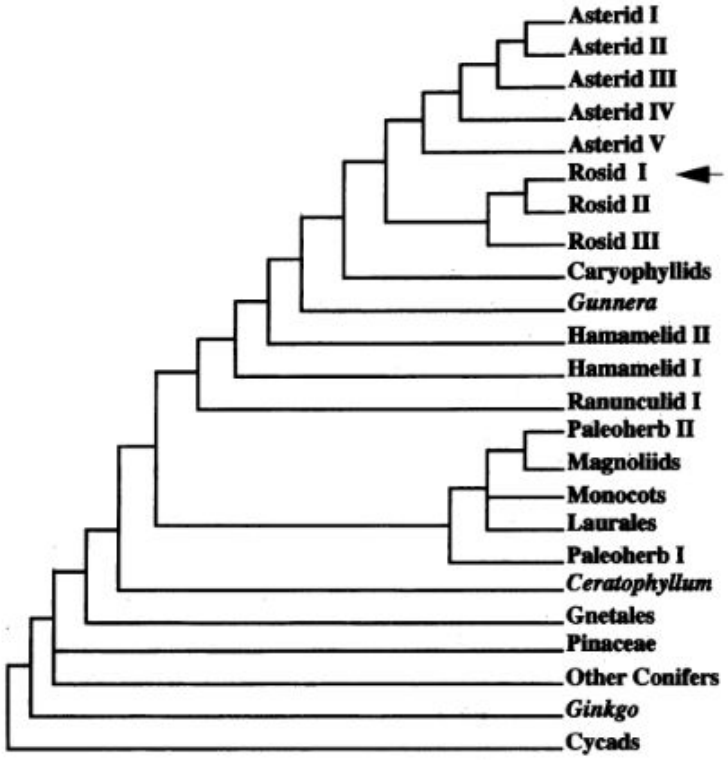
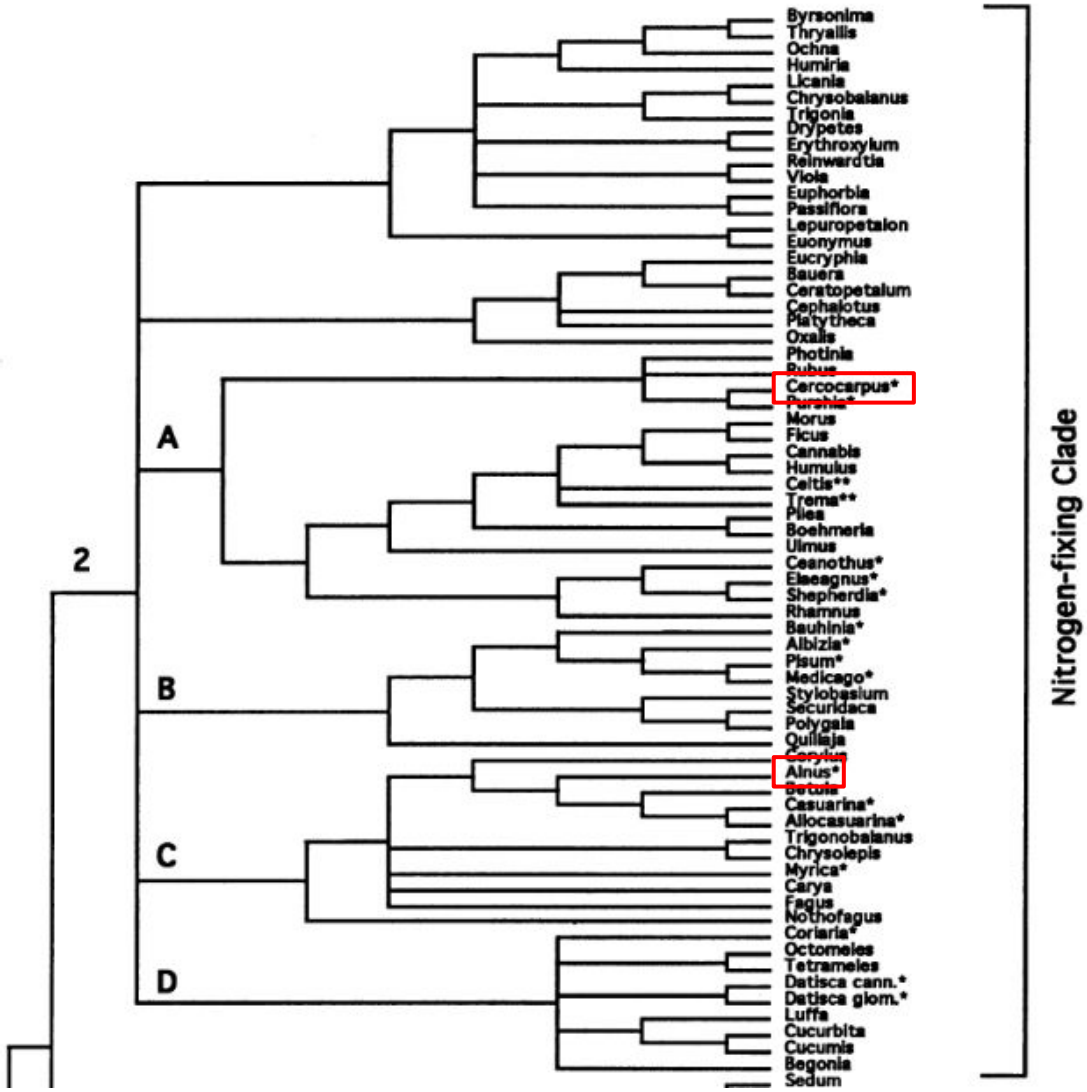


FIG. 1. Summary of the major clades identified in the strict consensus of 3900 equally parsimonious trees based on *rbcL* sequences for 499 taxa (17).

https://doi.org/10.1073/pnas.92.7.2647

The Nitrogen Fixing Clade



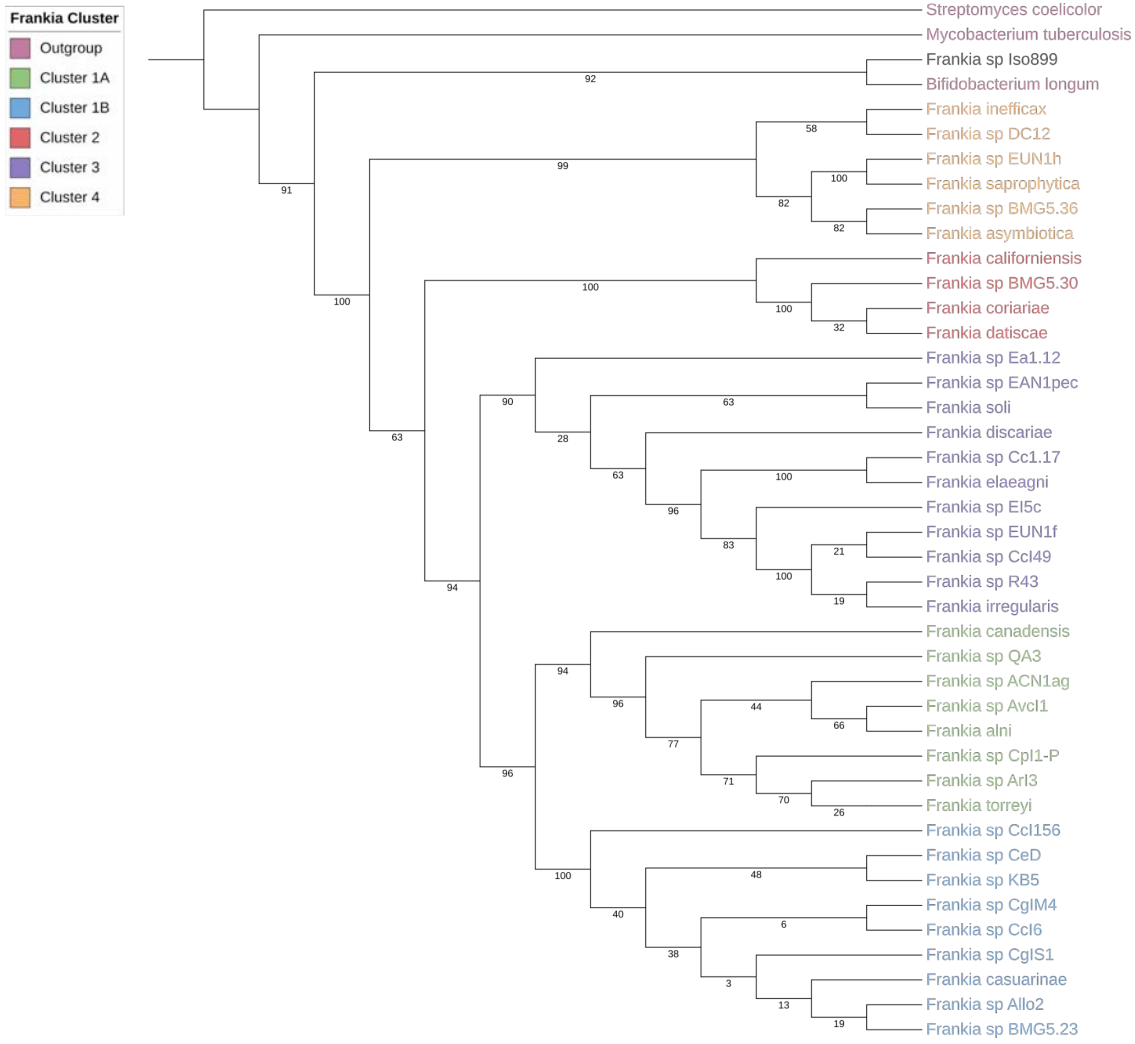
https://doi.org/10.1073/pnas.92.7.2647

Serially propagated Frankia "strains"

B11
T15
B16
B15
T4

- "Five Frankia Mix"
- Elite mixture
- Commercial nursery operations

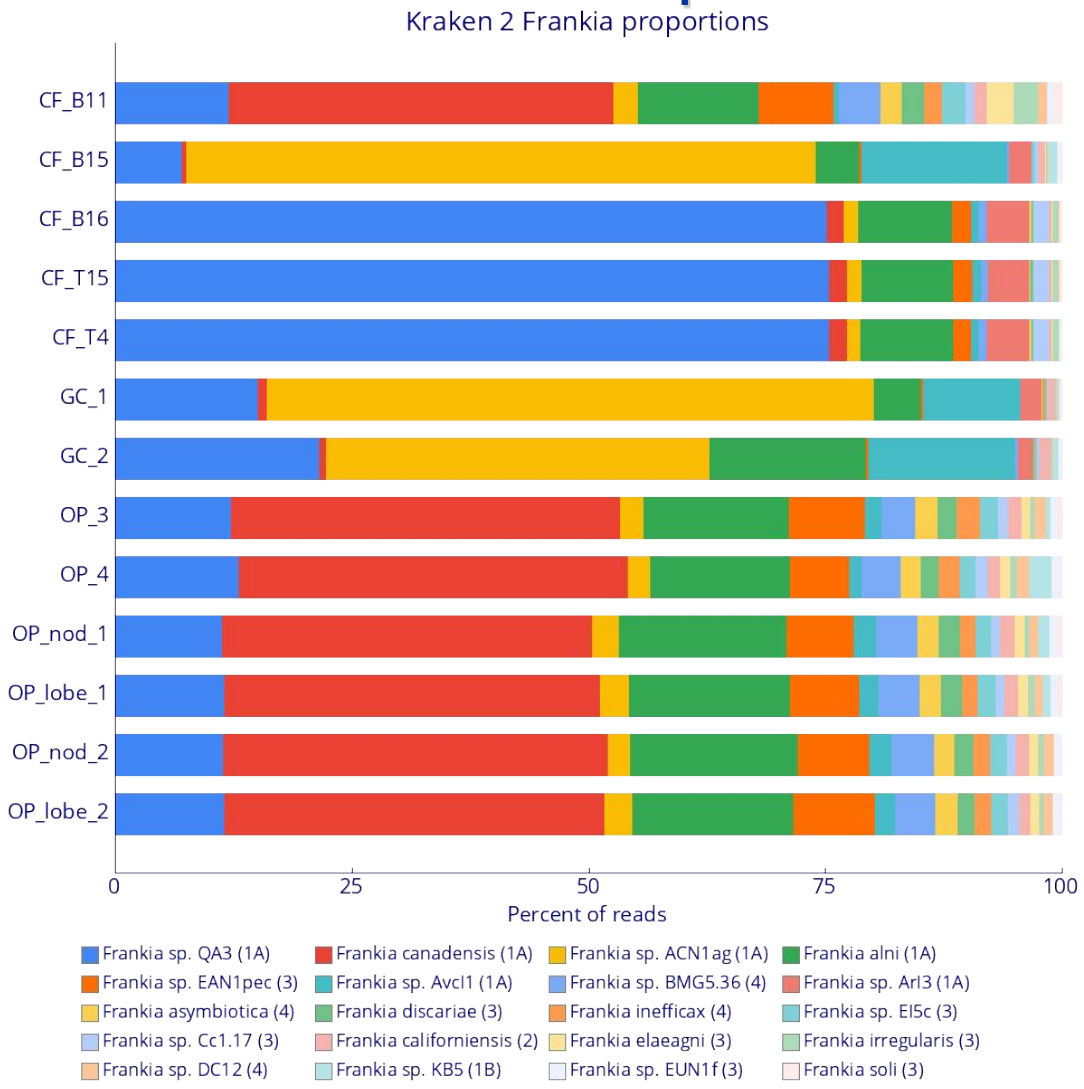
glnA1 (protein) phylogeny



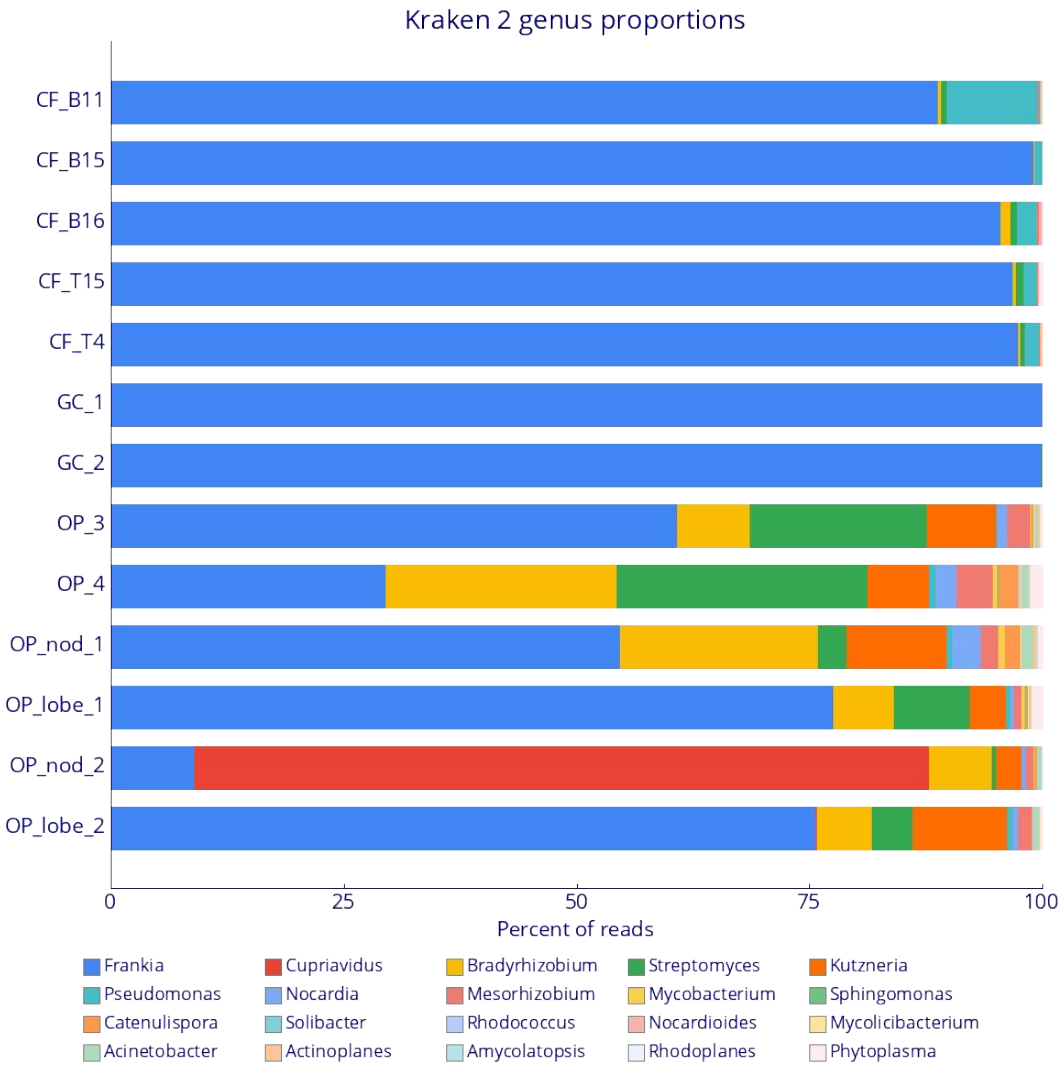
The genus Frankia

Cluster 1A: Nodulates Myricaceae and Betulaceae (Alnus).
Cluster 1B: Nodulates Myricaceae and Casuarinaceae.
Cluster 2: Wide host range (Coriariaceae, Datiscaceae, Rhamnaceae (only Ceanothus), Dryadoideae). Four families, six continents. Difficult to culture.
Cluster 3: Nodulate most actinorhizal members of the Rosales, that is, Elaeagnaceae; Rhamnaceae except for Ceanothus sp.; and Gymnostoma and Morella, two outlier genera of the Fagales.
Cluster 4: Non-infective or ineffective strains.

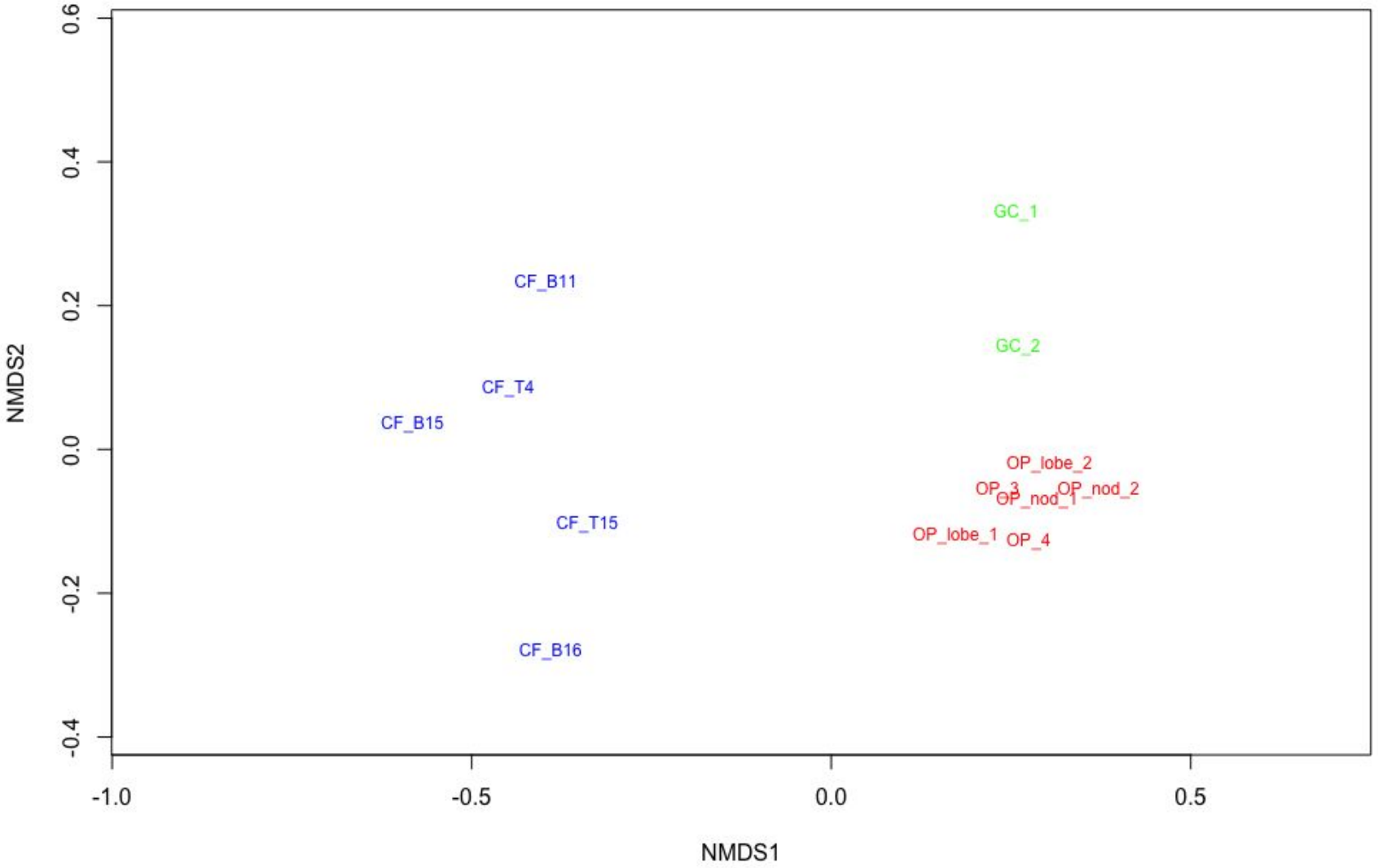
Nodules contain multiple Frankia spp.



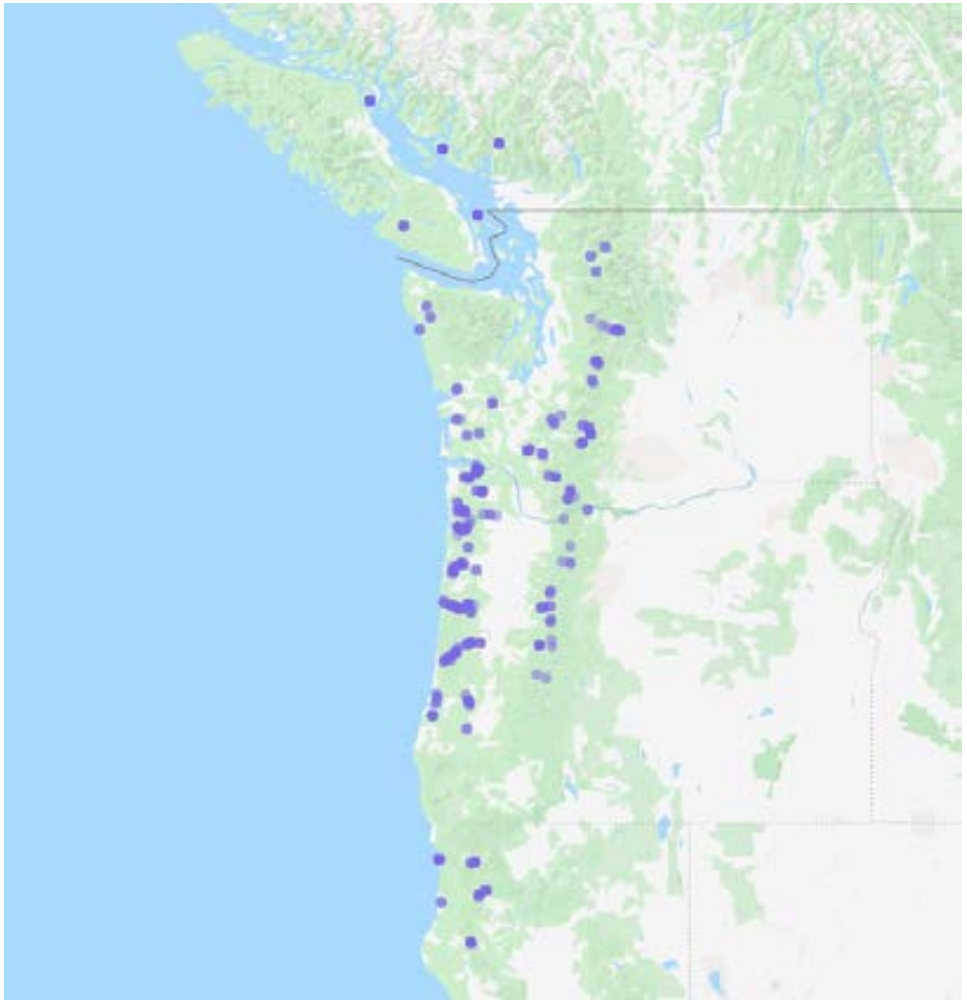
Nodules contain multiple genera



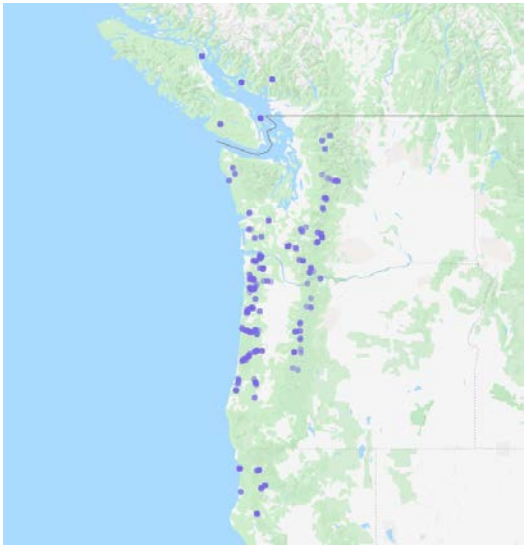
MDS based on genera



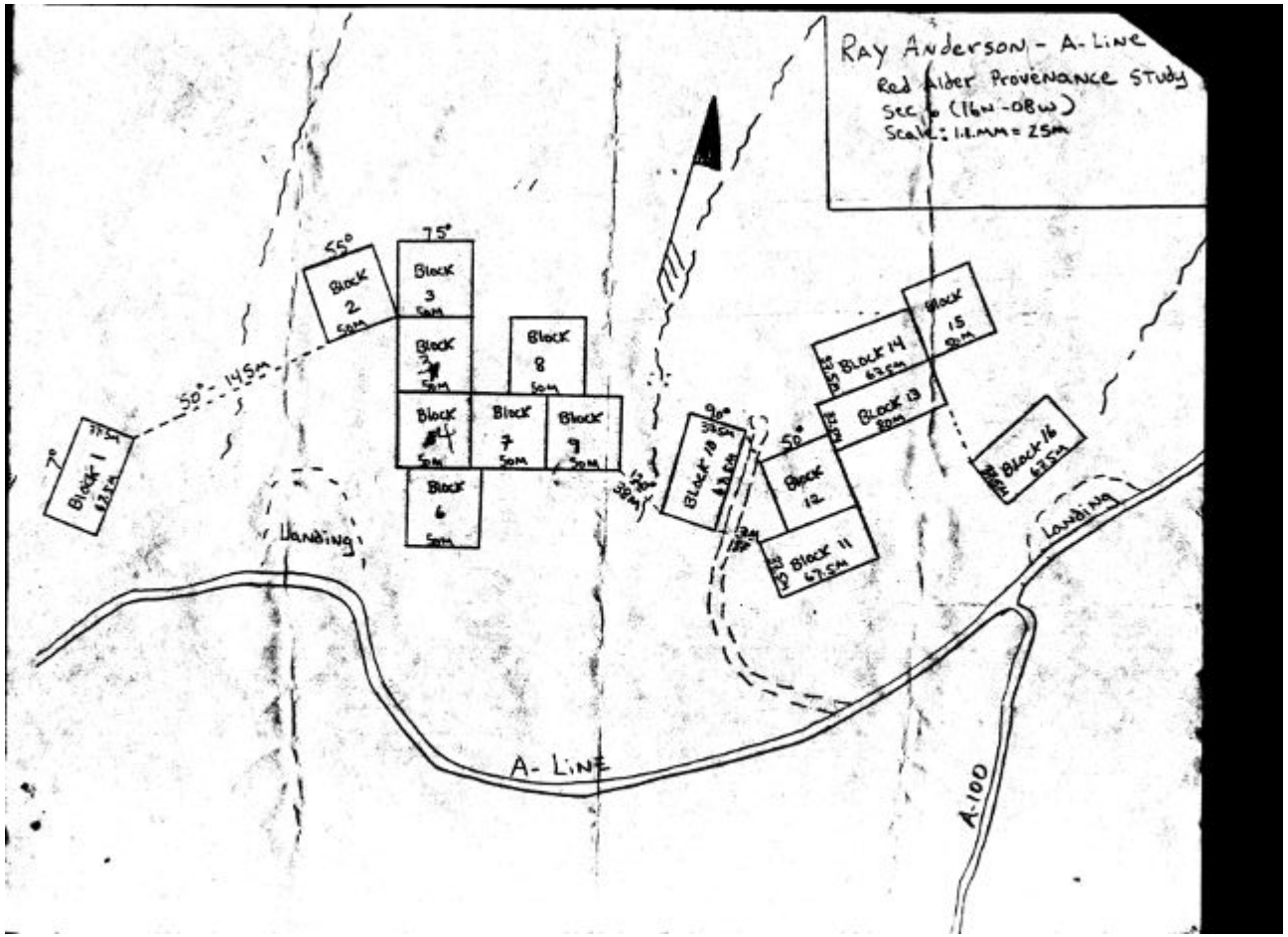
Weyerhaeuser provenance trial

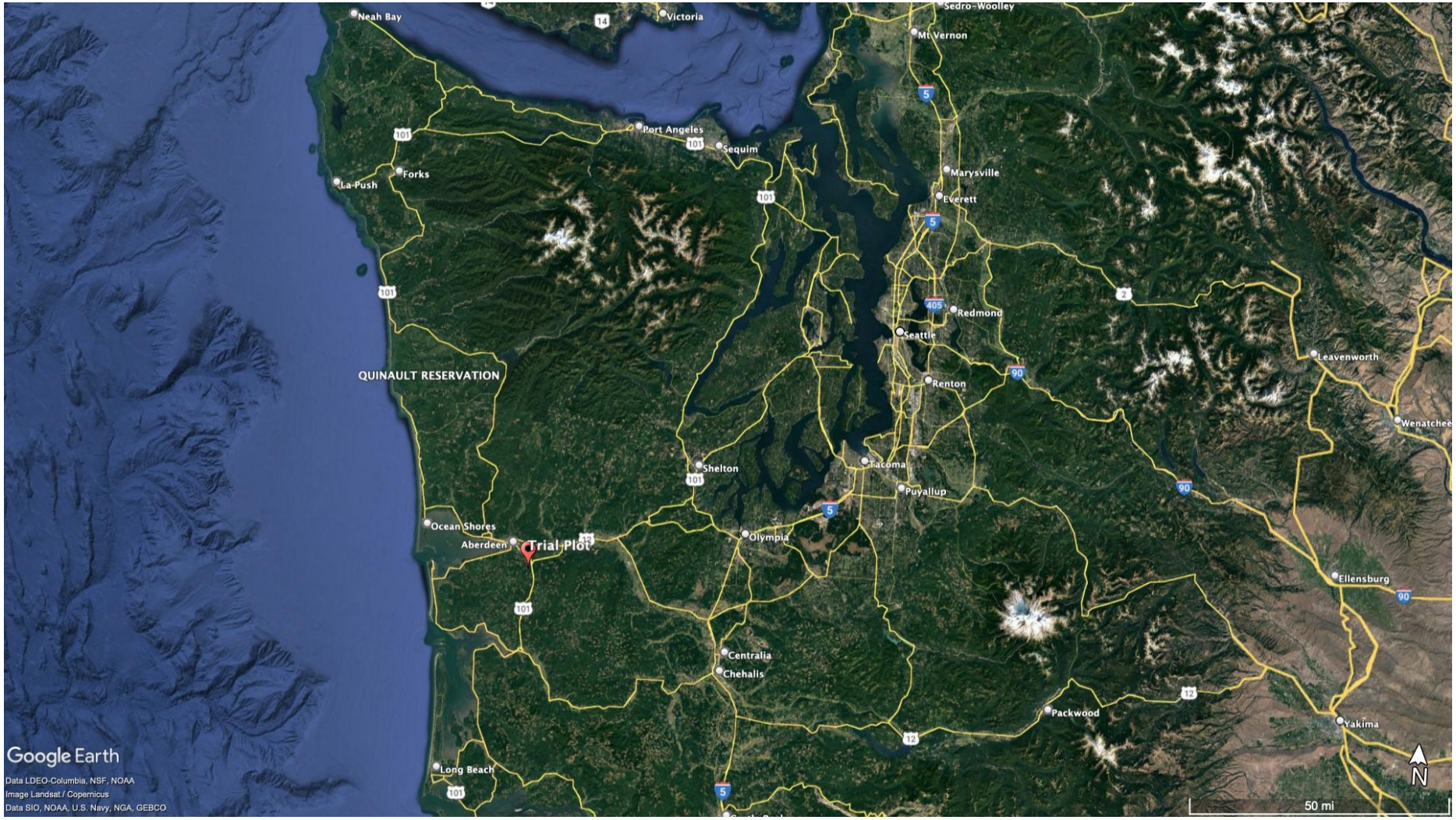


Weyerhaeuser provenance trial

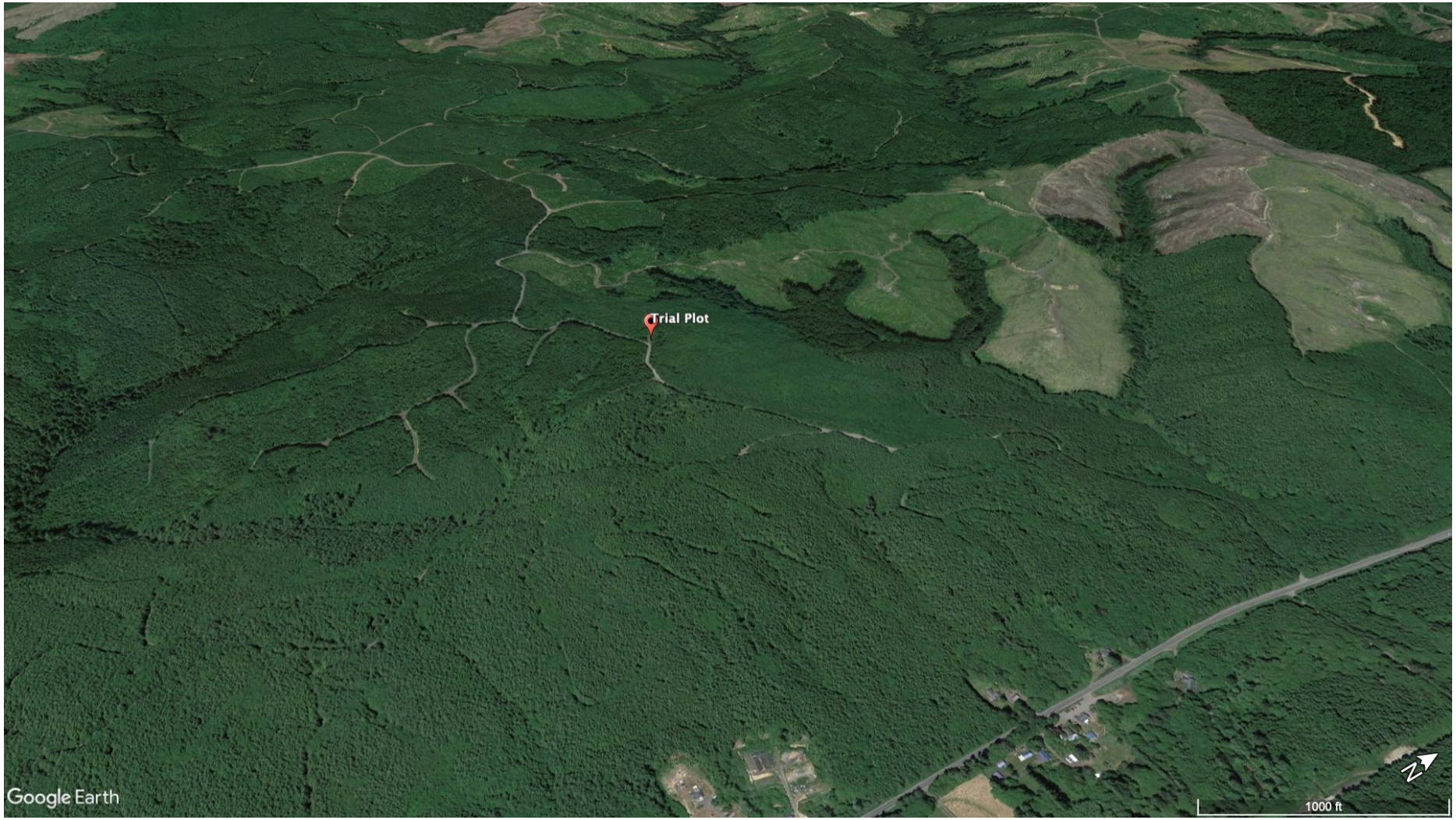


- Seed from one tree
- Open pollinated
- 16 half-siblings
- Planted in a common location
- Randomized into 16 blocks


RAY ANDERSON - A-LINE
Red Alber Provenance STUDY
Sec 6 (16W - 08W)
Scale: 1.2MM = 25M
Block 1
Block 2
Block 3
Block 4
Block 5
Block 6
Block 7
Block 8
Block 9
Block 10
Block 11
Block 12
Block 13
Block 14
Block 15
Block 16
Landing
Landing
A-LINE
A-100


Neah Bay
Victoria
Sedro-Woolley
Mt Vernon
Port Angeles
Sequim
Forks
La Push
Marysville
Everett
QUINAULT RESERVATION
Redmond
Seattle
Leavenworth
Renton
Wenatchee
Shelton
Tacoma
Puyallup
Ocean Shores
Aberdeen
Trial Plot
Olympia
Ellensburg
Centralia
Chehalis
Packwood
Yakima
Long Beach
Google Earth
Data LDEO-Columbia, NSF, NOAA
Image Landsat / Copernicus
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
N
50 mi


Hoquiam
Aberdeen
12
Junction City
Central Park
12
Cosmopolis
105
South M
Melbourne
101
107
105
Markham
Trial Plot
Ocosta
Artic
Bay City
101
N
Google Earth
4 mi

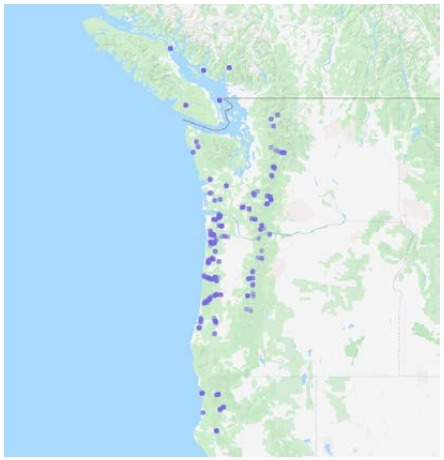

Trial Plot
Google Earth
1000 ft
N


Google Earth
N
800 ft


Google Earth
N
900 ft

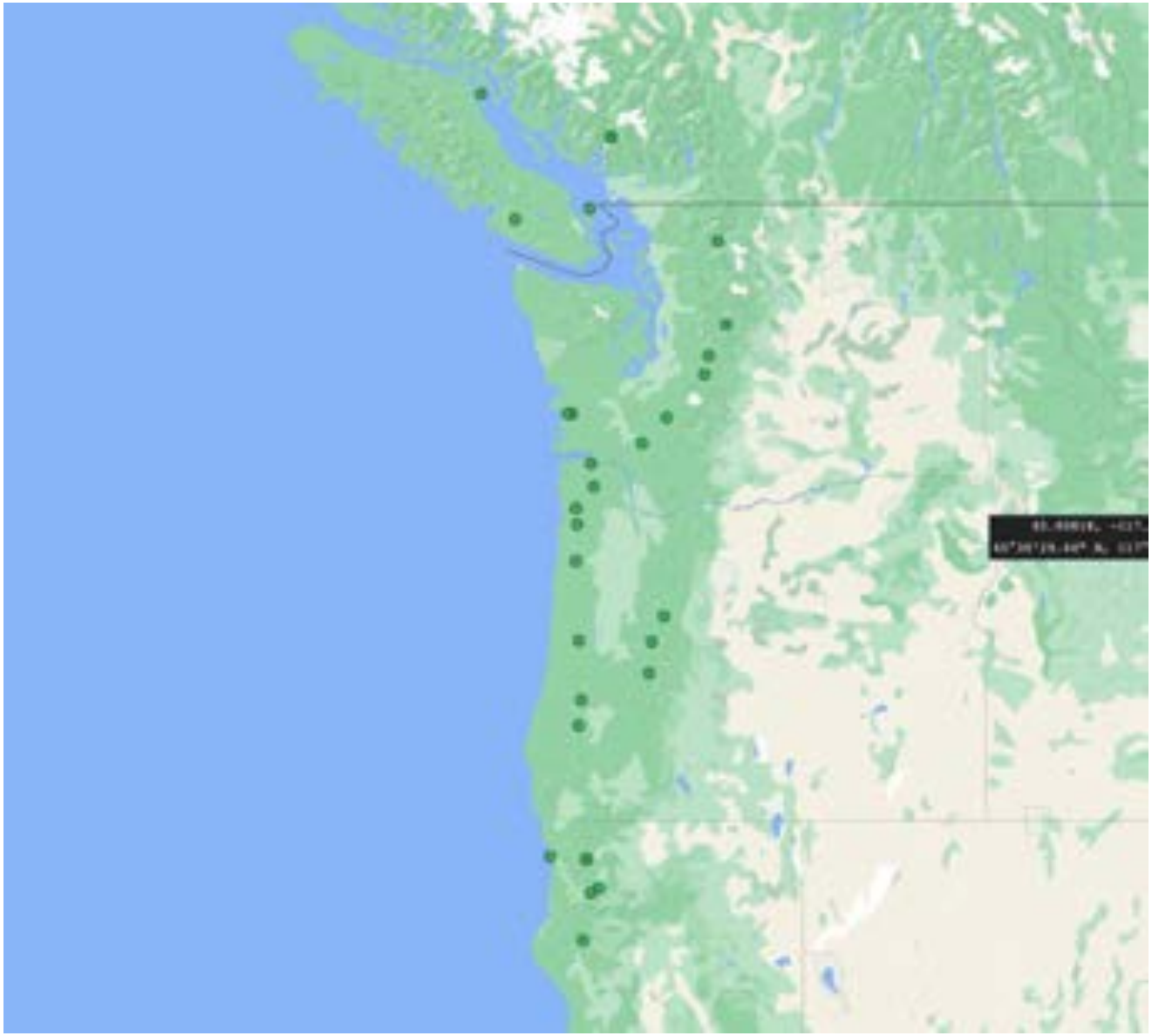

Google Earth
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Data LDEO-Columbia, NSF, NOAA
N
300 ft

Evaluate genetic variation by GBS

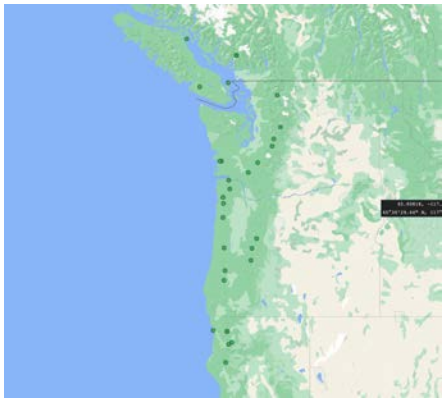


- Best and worst growing tree from each family of 16
- ApeK1 digestion
- 7.7 M 101 bp reads per sample (N~ 550)
- 58,000 SNPs

Survey root nodule microbiota

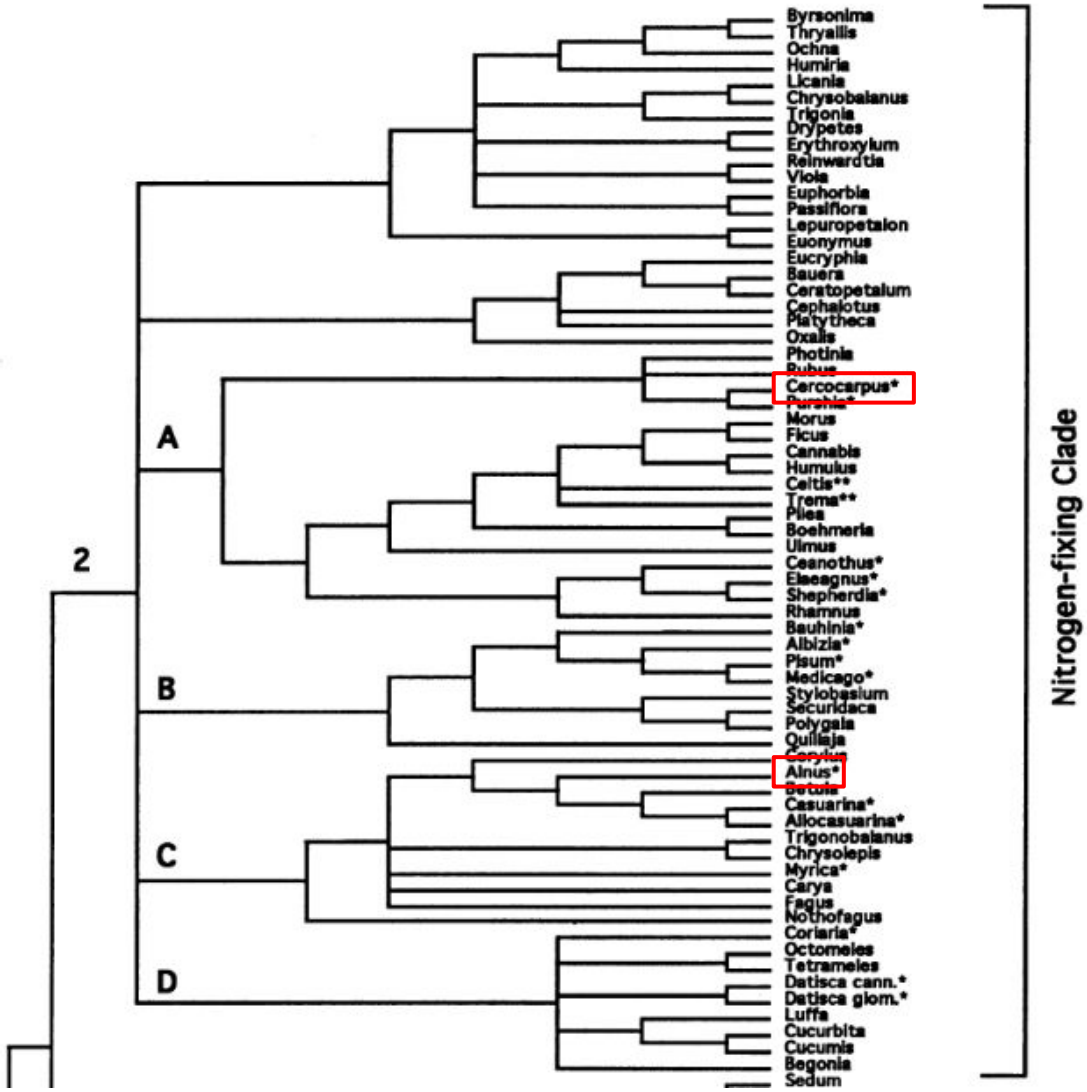


Survey root nodule microbiota



- Divided range into bands of 2° latitude
- Selected 6 trees randomly in each
- Collected 3 nodules per tree
- Collected adjacent soil
- Metagenome sequencing

The Nitrogen Fixing Clade



https://doi.org/10.1073/pnas.92.7.2647

Cercocarpus montanus

https://www.inaturalist.org/observations/371450

Alderleaf Mountain Mahogany (*Cercocarpus montanus*) Research Grade

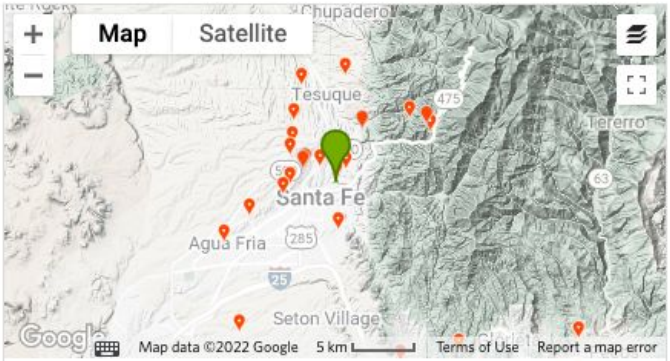
Follow

sylvankaufman1

630 observations

Observed:
Aug 20, 2013 · 9:08 AM MDT

Submitted:
Aug 20, 2013 · 3:19 PM MDT



Santa Fe County, US-NM, US

Details