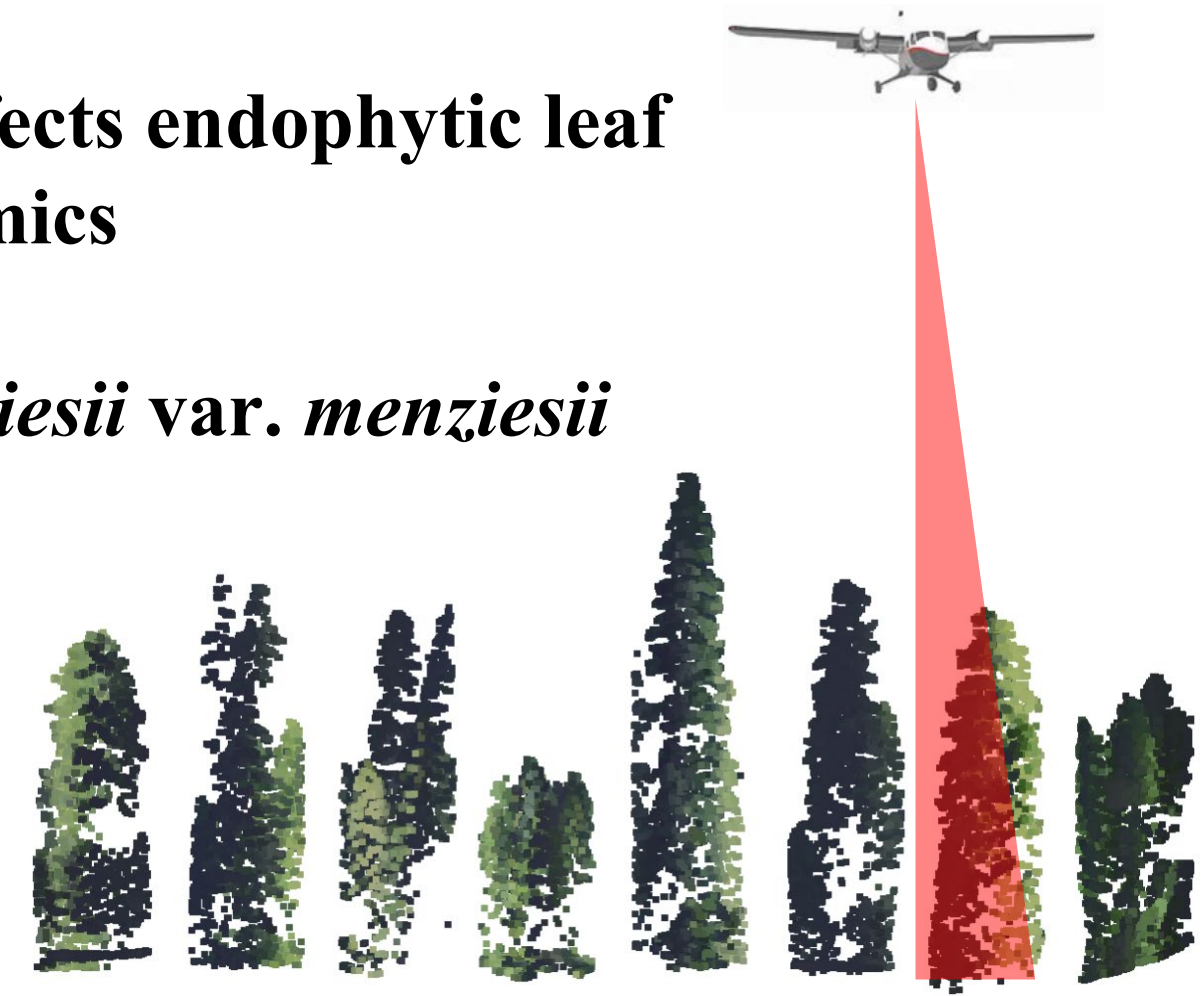
Crown closure affects endophytic leaf mycobiome dynamics over time in *Pseudotsuga menziesii* var. *menziesii*

Kyle A. Gervers
Daniel C. Thomas
Bitty A. Roy
Joseph W. Spatafora
Posy E. Busby



Overview

- Needle communities of old-growth canopies
- Interpreting results…
- Improving amplicon sequencing to achieve improved inference from common garden studies


Tom Iraci

Increasing
leaf wetness
+
thermostability
+
hydrostability

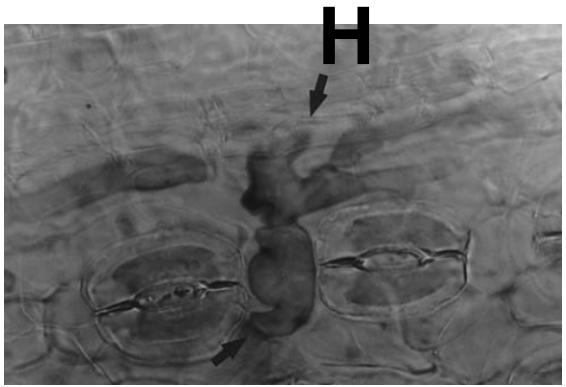
Increasing
temperature
+
light intensity
+
host stress

A1

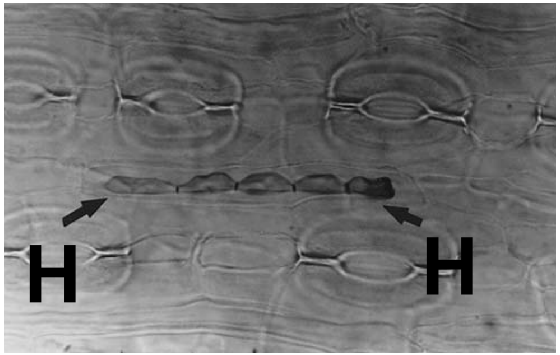
More exposure over time

A4

Illustration by Nancy Babay

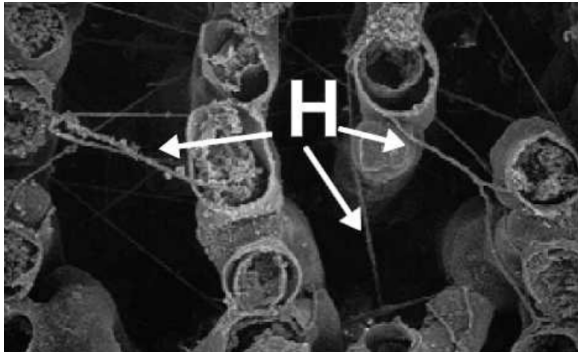


***Phyllosticta* spp.**



Rhabdocline parkeri

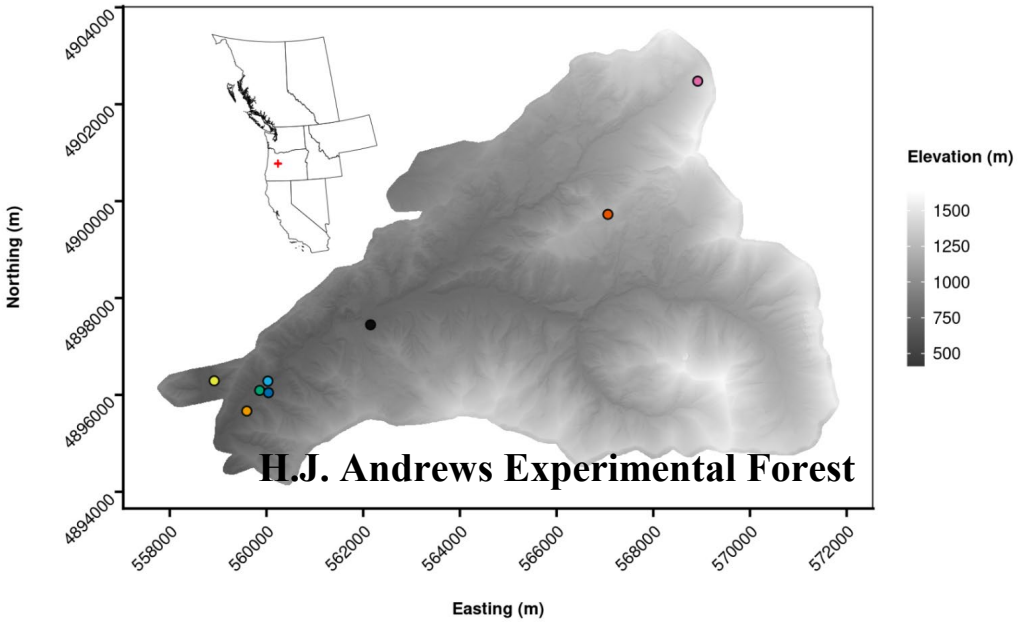
Nothophaeocryptopus gaeumannii



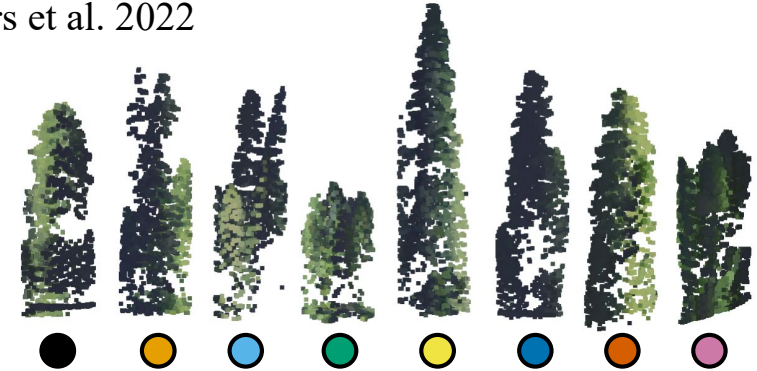
H = Hyphae

Stone *et al.* 2004, Stone *et al.* 2008

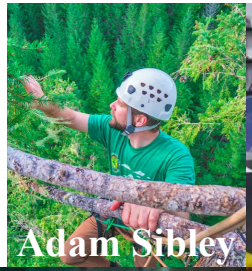
Do needle communities from exposed crowns differ from closed crown communities?



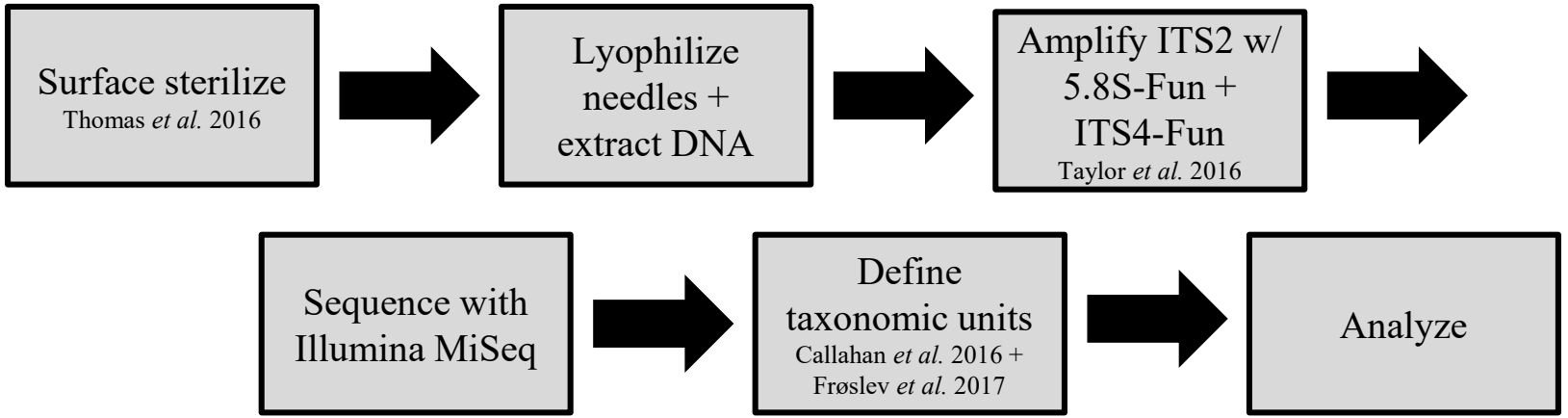
Gervers et al. 2022



- 8 trees climbed
- 4 needle ages
- Crown sampled at consistent intervals



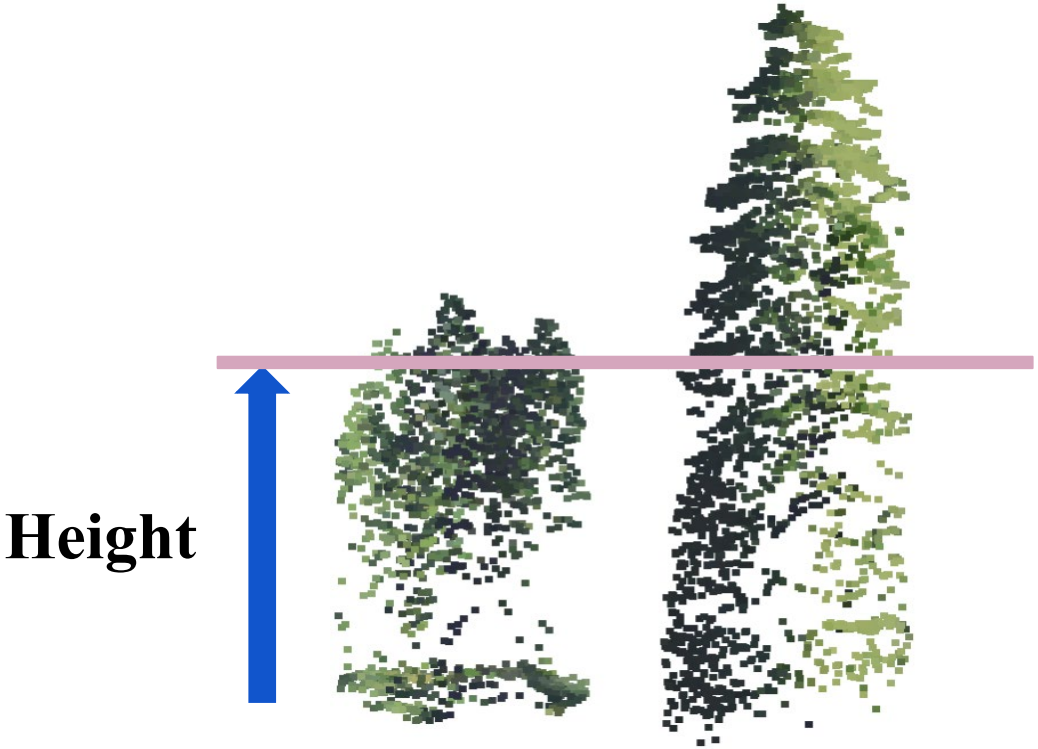
Metabarcoding/HTS/NGS workflow



How do we quantify exposure?

Height

Crown closure


Height

How do we quantify crown closure?

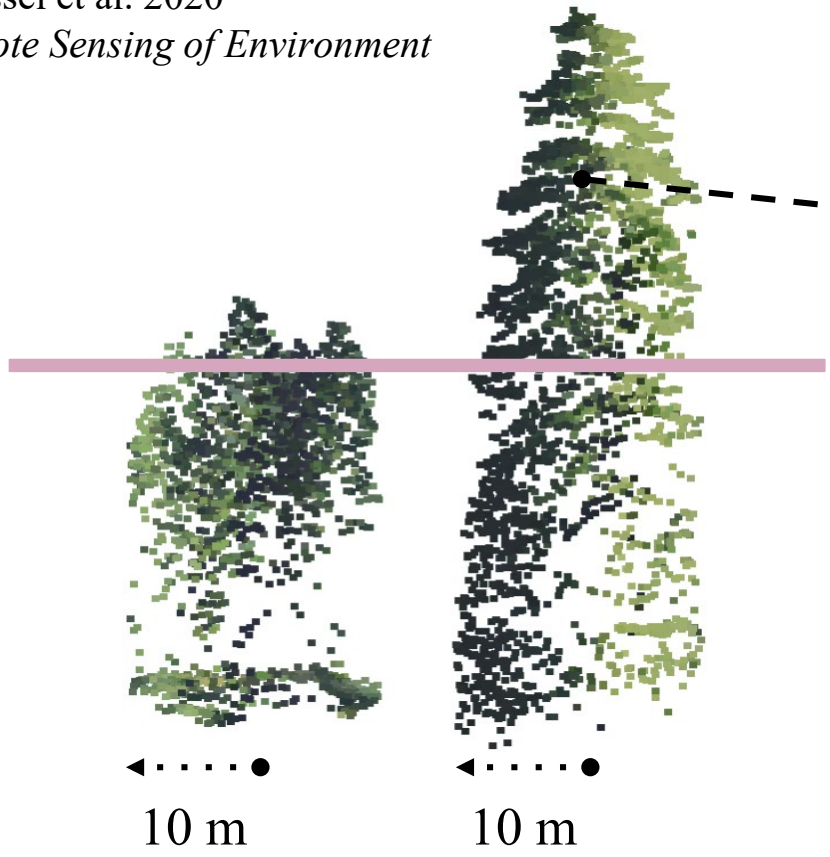
Light **D**etection **a**nd **R**anging

Roussel et al. 2020
Remote Sensing of Environment

Crown closure index

=

Points above sampled height



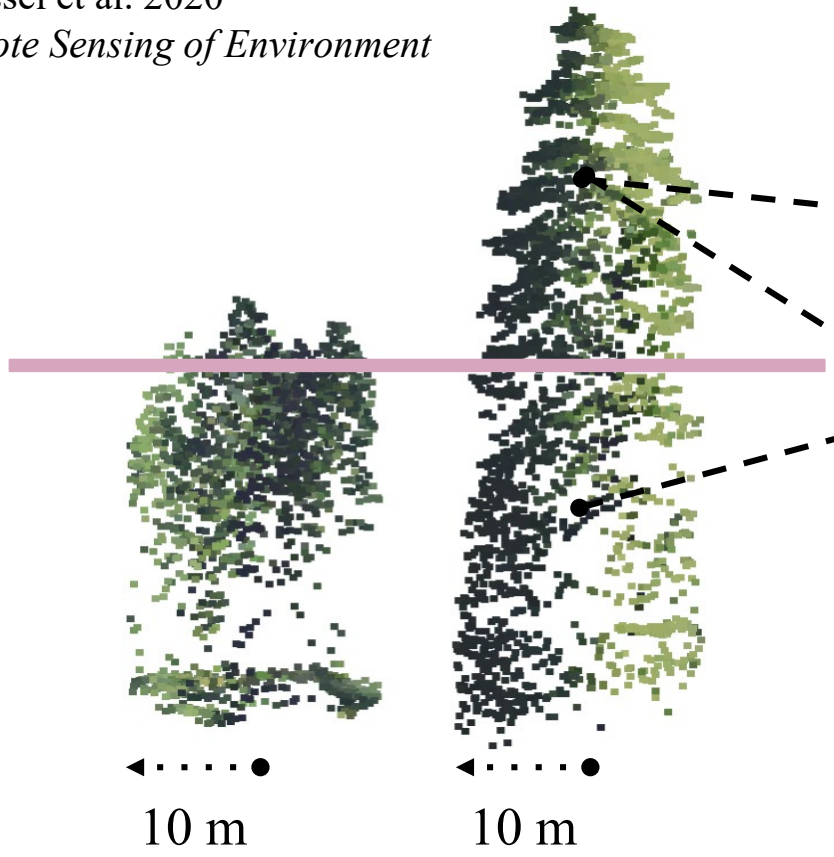
Roussel et al. 2020
Remote Sensing of Environment

Crown closure index

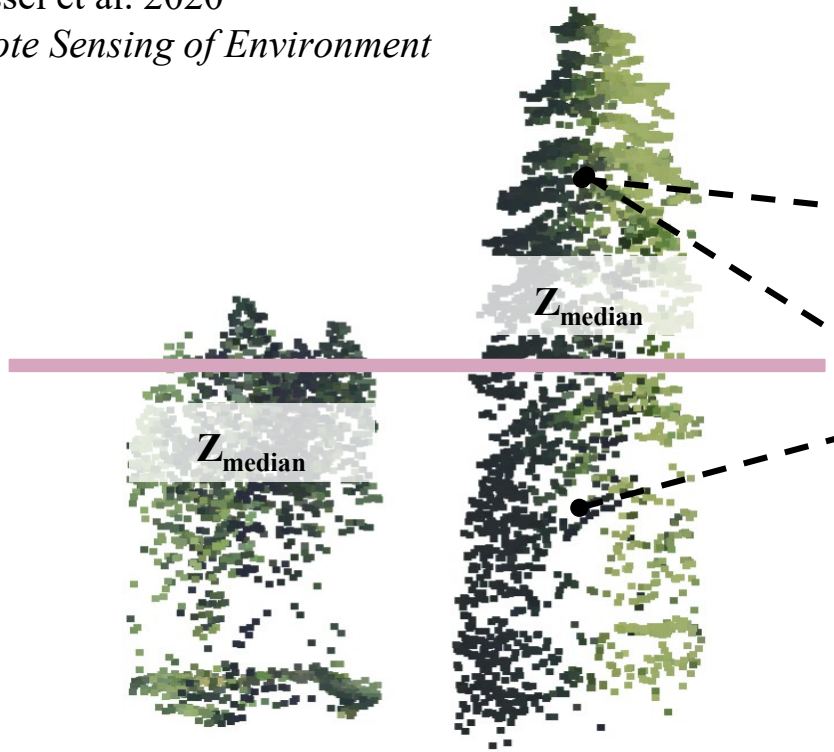
=

Points above sampled height

Total points in cylinder



Roussel et al. 2020
Remote Sensing of Environment



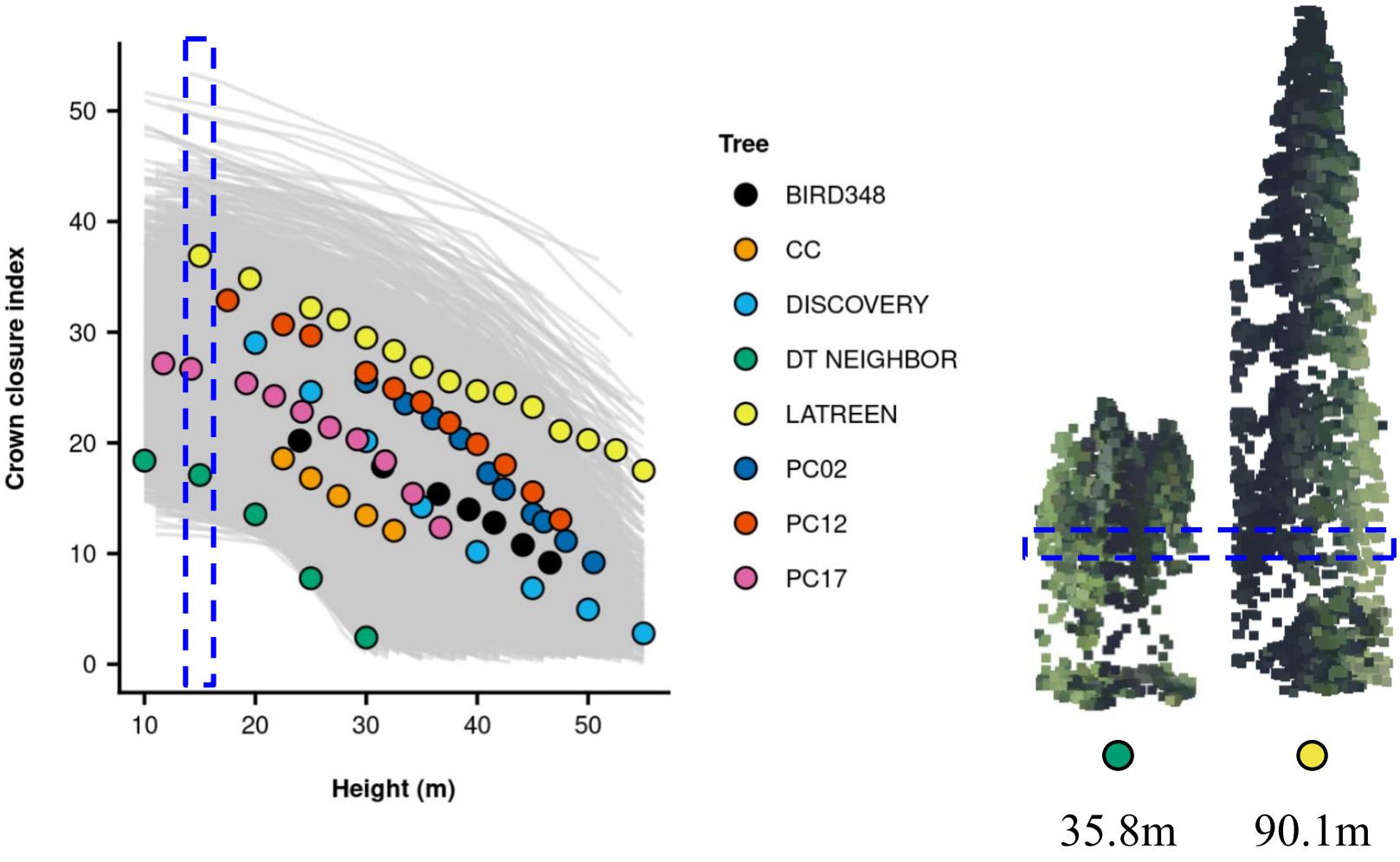
Crown closure index

=

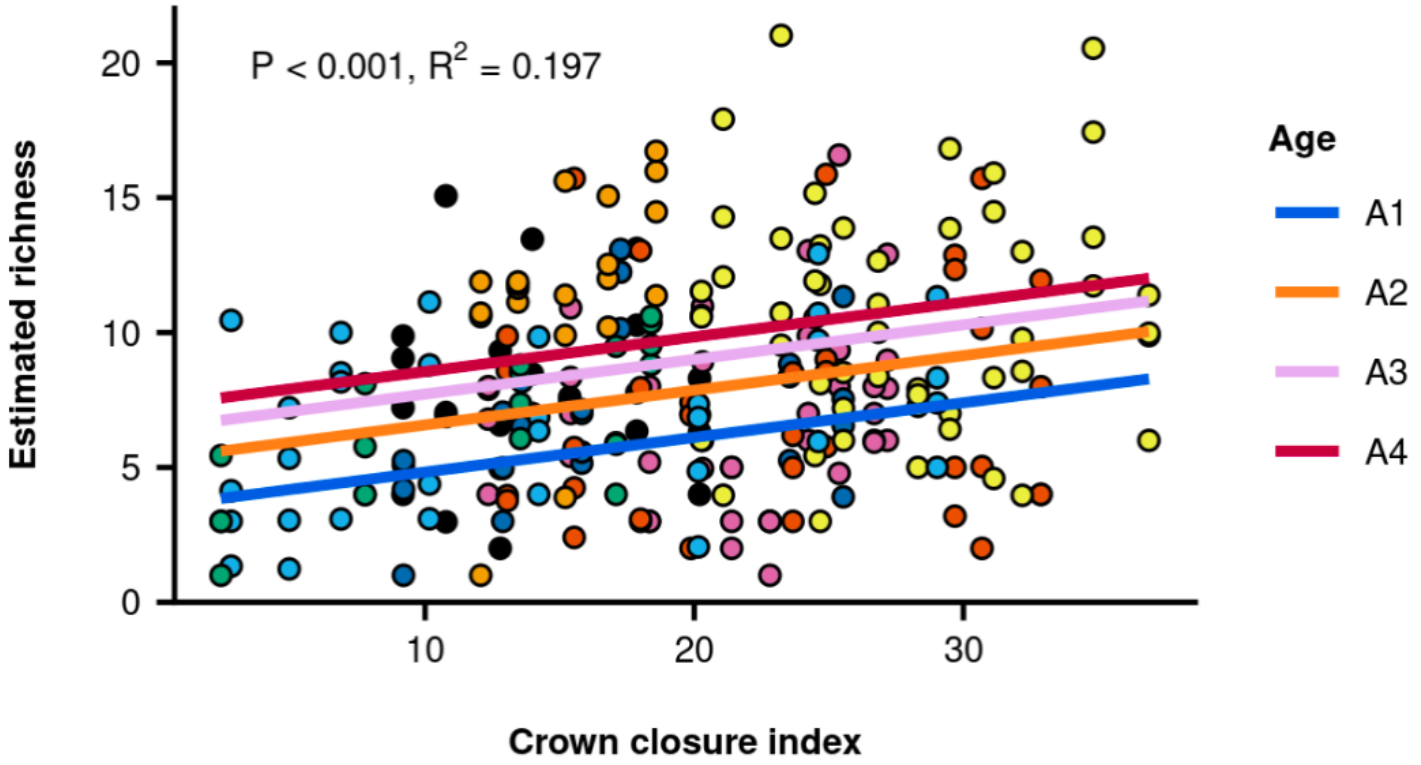
$$\frac{\text{Points above sampled height}}{\text{Total points in cylinder}}$$

x

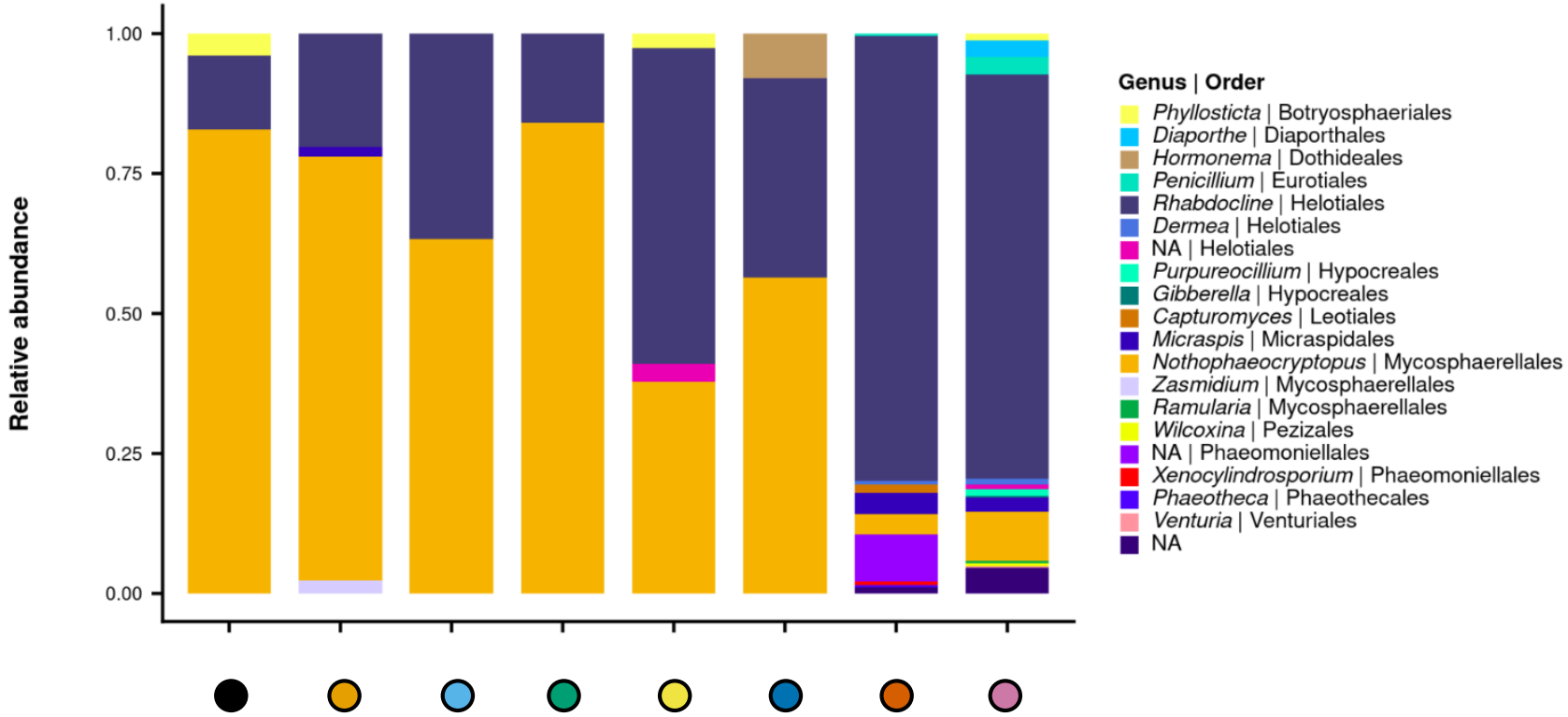
Z_{median}


Crown closure index
50
40
30
20
10
0
10
20
30
40
50
Height (m)
Tree
BIRD348
CC
DISCOVERY
DT NEIGHBOR
LATREEN
PC02
PC12
PC17
35.8m
90.1m

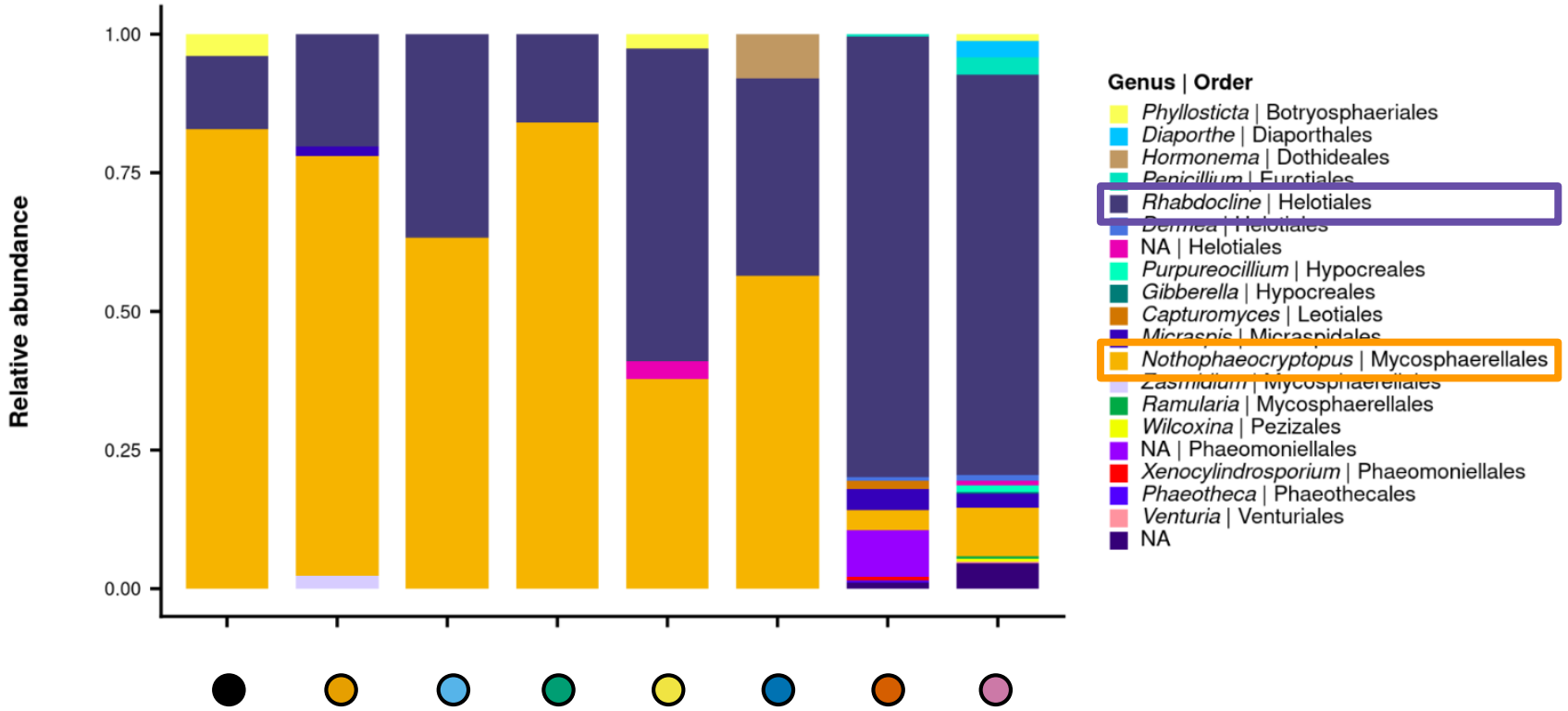
Do communities from exposed crowns differ from closed crown communities?



Greater closure and needle age are associated with richer communities

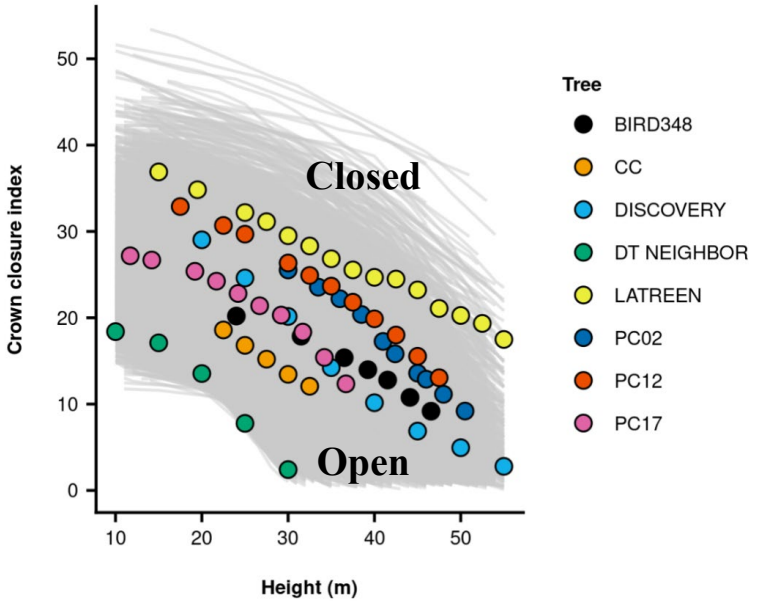


218 OTUs across 256 samples



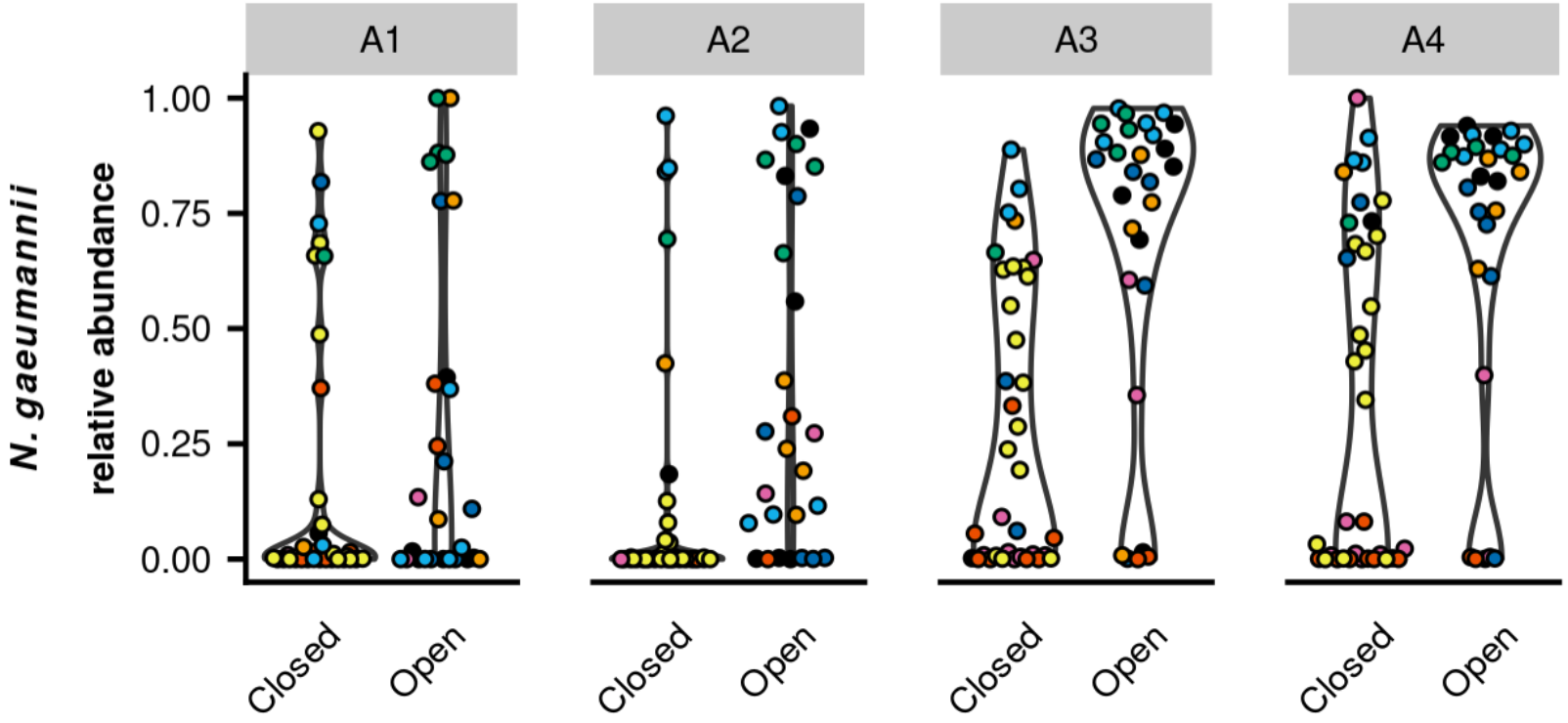
OTUs assigned to *N. gaeumannii* (OTU.1) + *R. parkeri* (OTU.2, OTU.3, OTU.6)

Dominant taxa have **divergent** niches

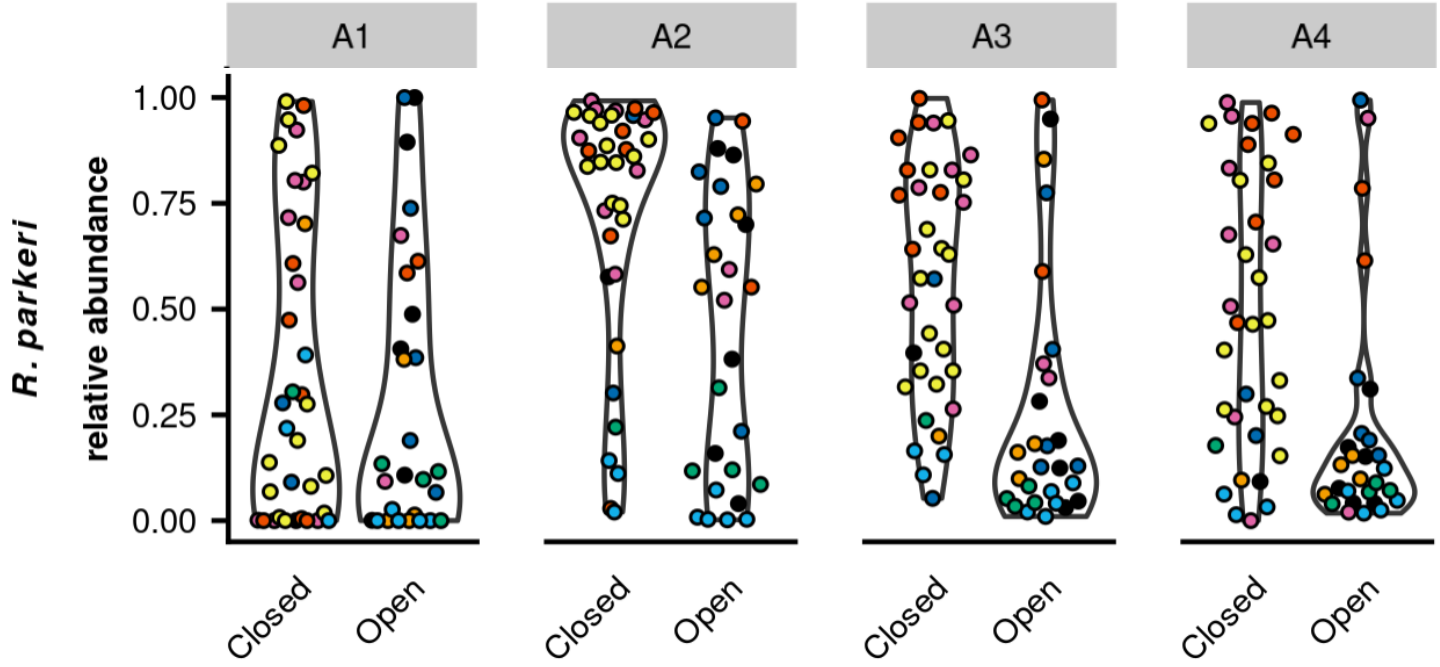


OTU	Taxon	Group	IV	P
OTU.1	*N. gaeumannii*	Open	0.629	0.006
OTU.2	*Rhabdocline parkeri*	Closed	0.488	0.009
OTU.3	*Rhabdocline parkeri*	Closed	0.482	0.022
OTU.10	Dermateaceae sp.	Closed	0.414	0.006
OTU.16	Dothideomycetes sp.	Closed	0.361	0.006
OTU.73	Phaeomoniellaceae sp.	Closed	0.173	0.006

P values were obtained after 999 permutations and corrected for the false discovery rate using the Benjamini-Hochberg method.



***N. gaeumannii* dominates open exposures as needles age**



R. parkeri initially dominates closed exposures

Hansen et al. 2000
Manter et al. 2003
Manter et al. 2005
Shaw et al. 2014
Lan et al. 2019
Ritóková et al. 2021
Lan et al. 2022
(apologies if I missed someone!)

Needles/trees in **southern aspects/upper crowns/unshaded treatments** experience more severe SNC and/or higher *N. gaeumannii* biomass

Gervers et al. 2022

Needles with less **crown closure** have relatively more *N. gaeumannii* reads than other endophytic fungi

Why might *Nothophaeocryptopus gaeumannii* dominate open exposures?

Why might this be?

N. gaeumannii **preempts** other fungi in open crowns

Other fungi (e.g. *R. parkeri*) **exclude** *N. gaeumannii* in closed crowns

N. gaeumannii **accesses nutrients** and/or grows better than other fungi in open crowns

N. gaeumannii **survives** better than other fungi in the hostile environment of open crowns

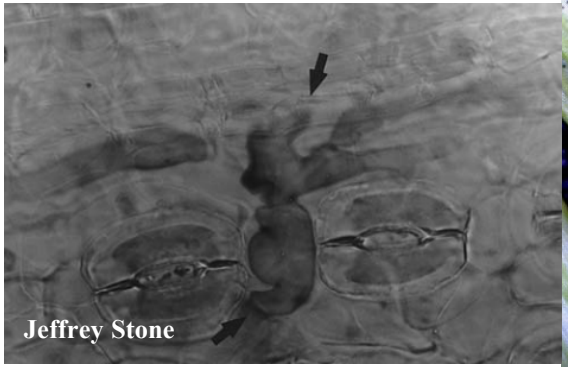
N. gaeumannii **antagonizes** other fungi more in open crowns

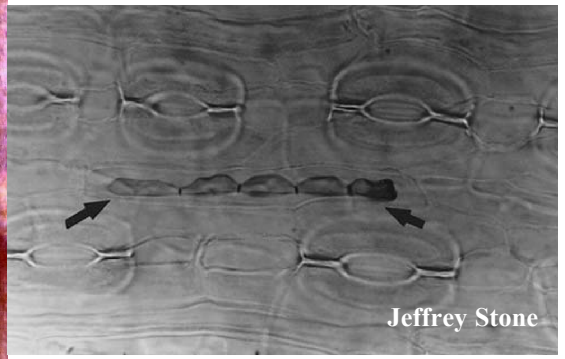
MINI-SYMPOSIUM ON JUNE 2021 HEAT DOME FOLIAGE SCORCH

Proposed research by Chris Still + Posy Busby et al.

Please ask me more about these mechanisms!

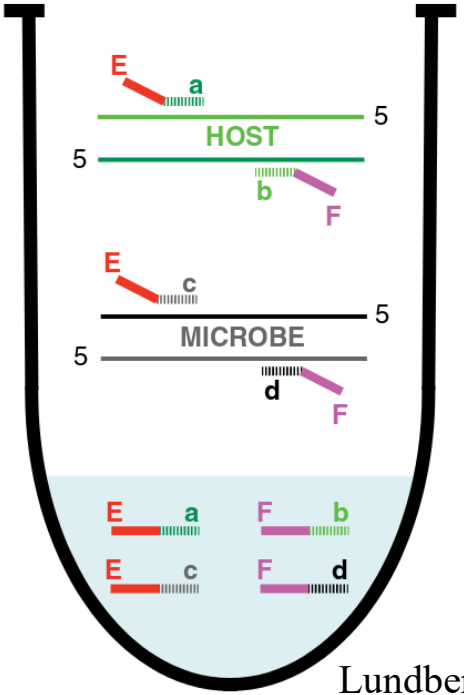
(I’ve got bonus slides…)





How can we improve our sequence-based analyses of needle fungi communities?

Amplify **plant** and **fungal** sequences



Lundberg et al. 2021

Fungal load

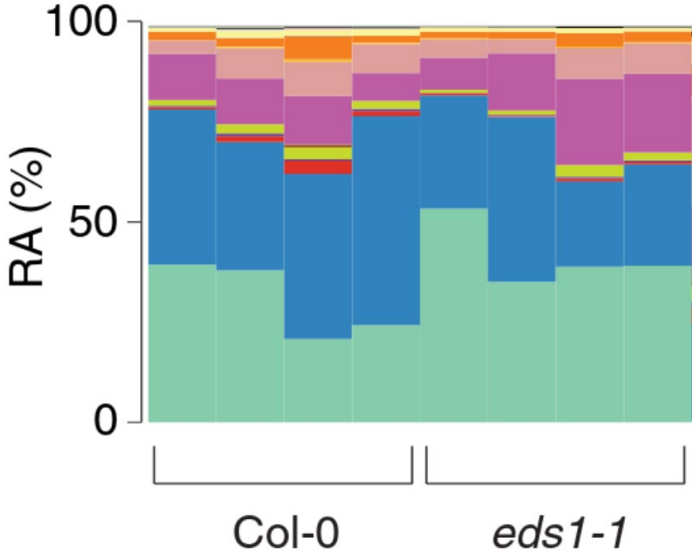
=

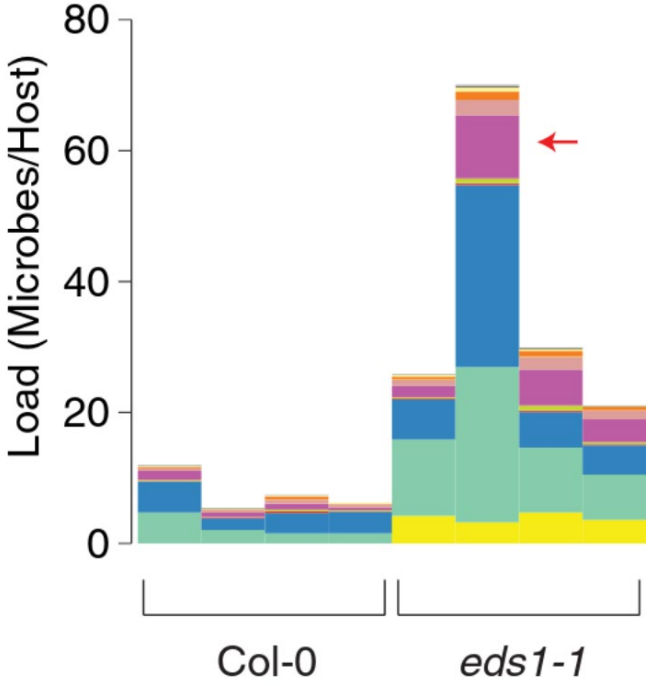
fungal sequences

÷

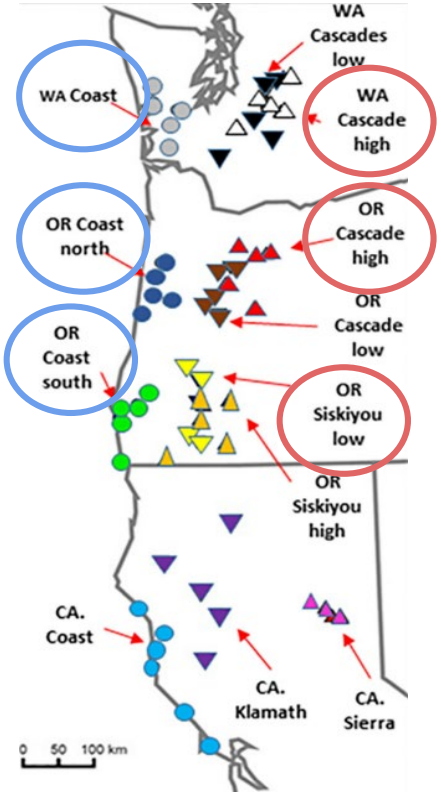
plant sequences

Lundberg et al. 2021





Load reveals a much stronger genotype effect!



DFSSMT

6 sites

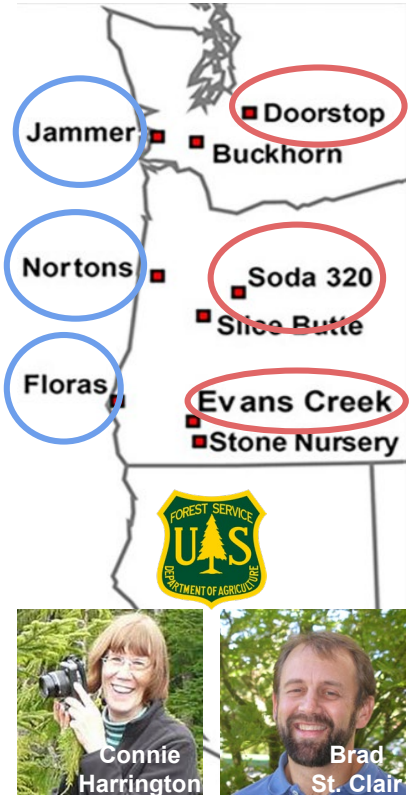
6 seed regions

30 populations

2nd-year needles

3 sampling years

>4000 samples



Natural Populations

Common Gardens

Wilhelmi et al. 2017

Please ask me if you want to know more about how this technique works!

Acknowledgements

Oregon State University

HJA HJ ANDREWS EXPERIMENTAL FOREST

LTER

FOREST SERVICE US DEPARTMENT OF AGRICULTURE

NSF

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Carolina Piña Páez

Lluvia Vargas Gastelum

Busby Lab

Austen Apigo, Abbey Neat Maria-Jose Romero Jimenez

Kim Syring, Melissa Vergara, Jessie Zimmerman

Questions?

Comments?

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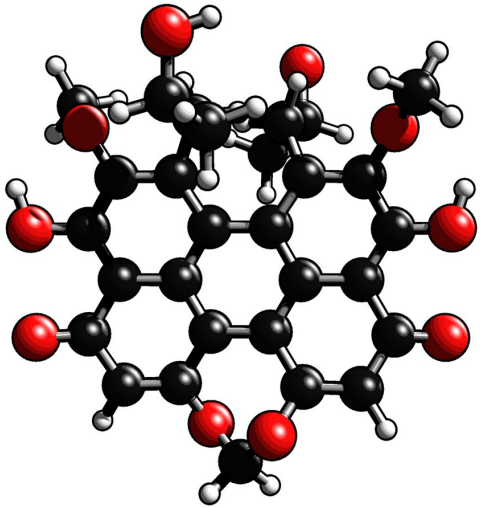
Bonus slides!

The systematic position of *Phaeocryptopus gaeumannii*

2007

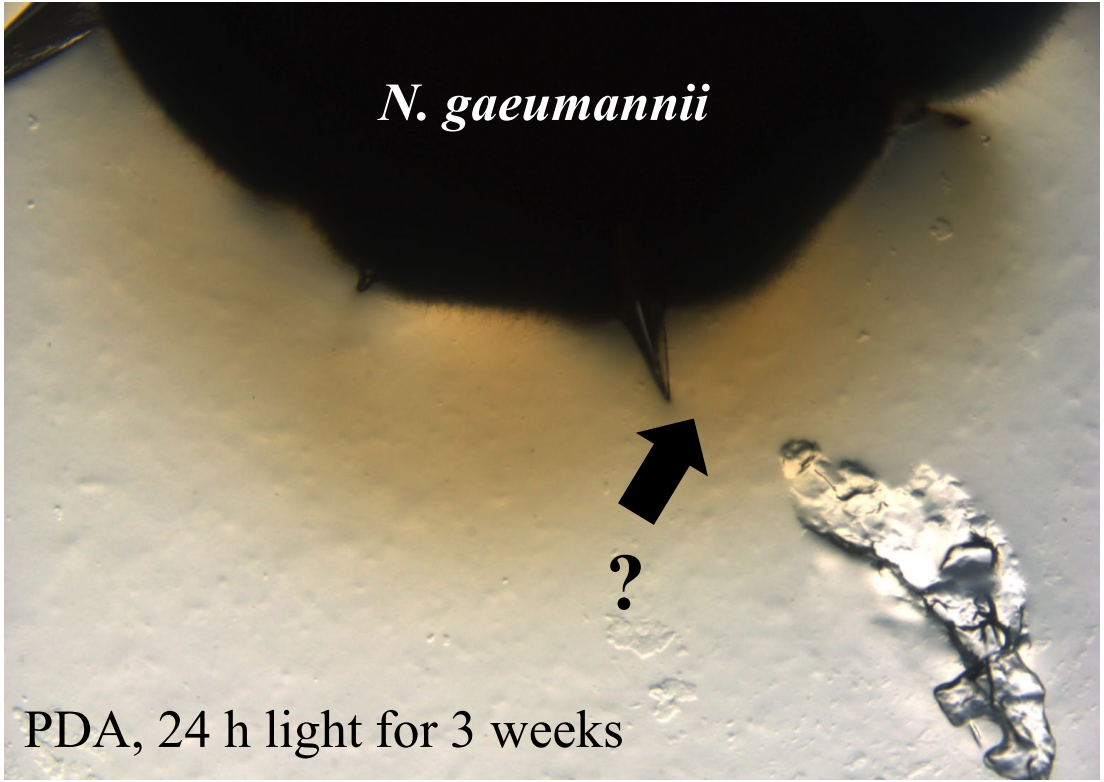
Loretta M. Winton, Jeffrey K. Stone, Everett M. Hansen & R.A. Shoemaker



A light-activated phytotoxin

"Isolates of *P. gaeumannii* also have been observed to produce **diffusing red pigments in culture** (Winton and Stone unpubl.).

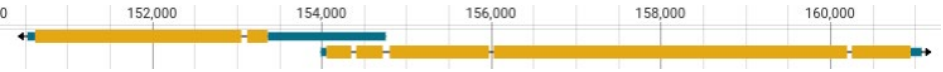
…the possibility that this pigment might be a **cercosporin-like substance** that might play a role in the pathology of Swiss needle cast disease must be considered."



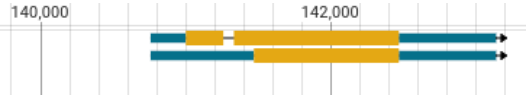
Unpublished

Support for an earlier hypothesis…

Funannotate



CTB3 **CTB1**



CTB2

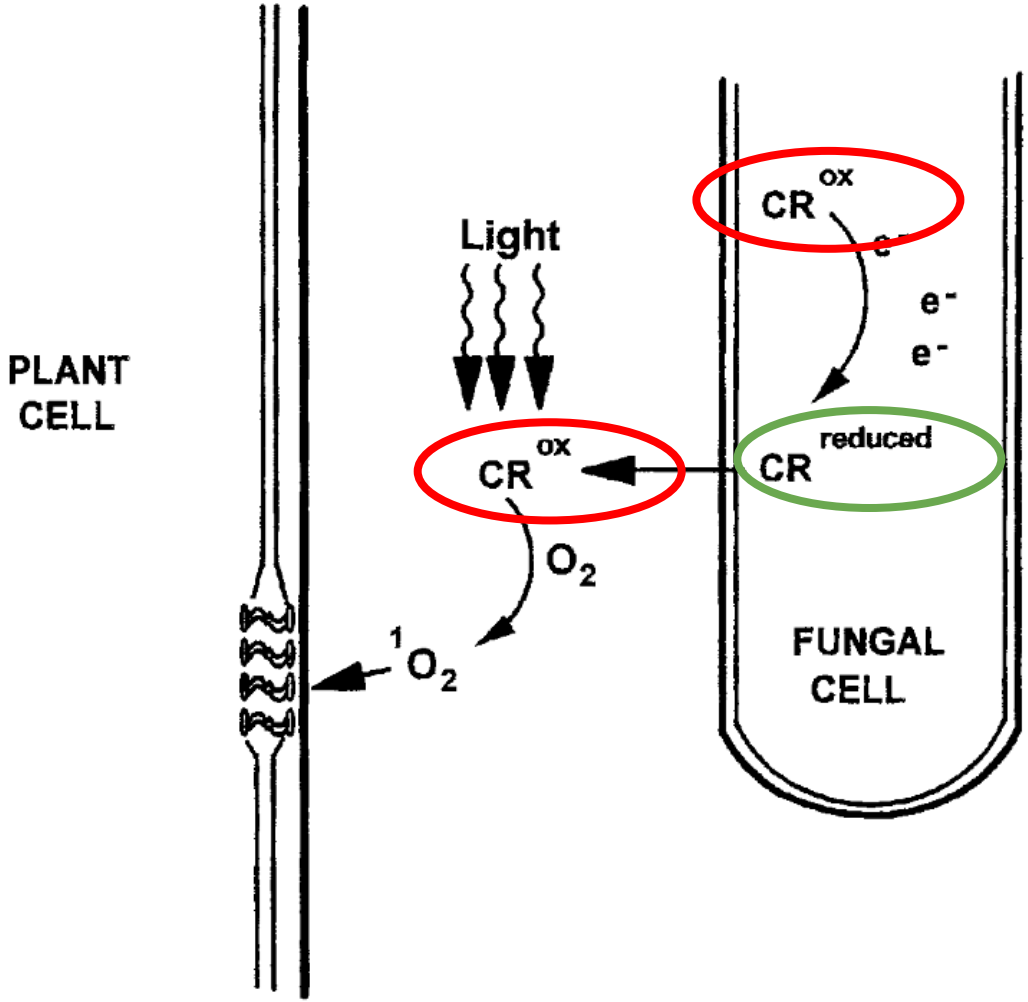
CTB4

CTB5

Genomic and transcriptomic evidence suggest this is a possibility

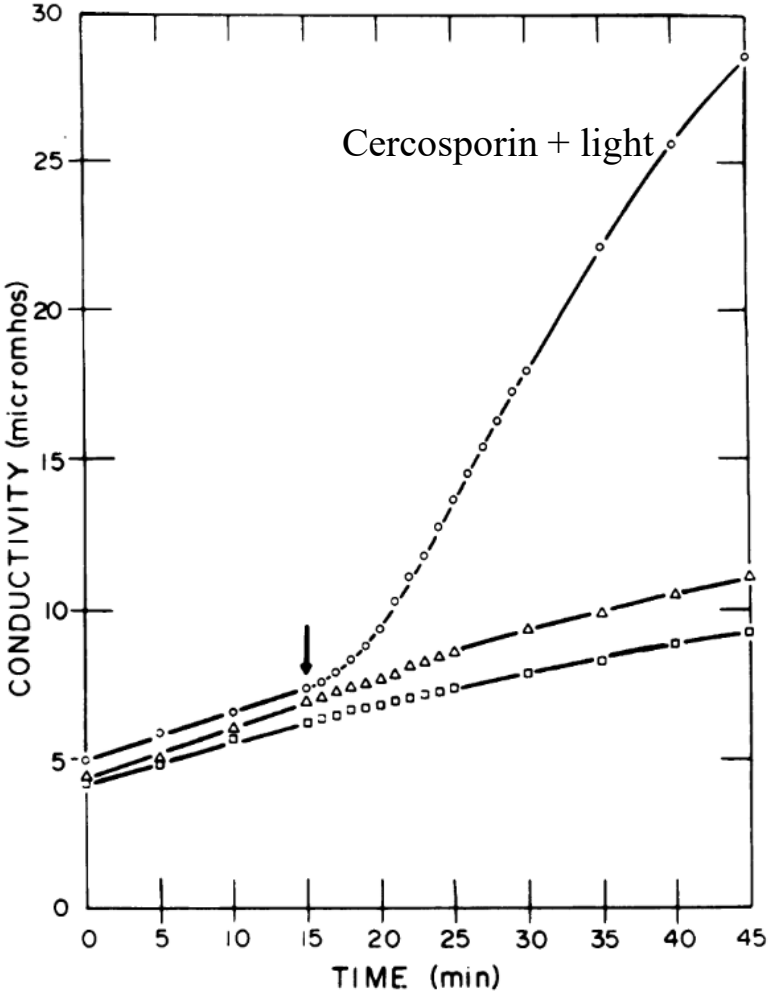
Unpublished

RNA-Seq data from Hess *et al.* 2016
N. gaeumannii genome (BioProject: PRJNA212511)
sequencing + assembly by TAIGA



Compounds like cercosporin form reactive **singlet oxygen** under high-light conditions

Daub and Ehrenshaft *et al.* 2000



Cercosporin induces **electrolyte leakage** in plant cells when exposed to light

○ Cercosporin + light

△ Cercosporin + dark

□ Water + light

Daub 1982

Study 1

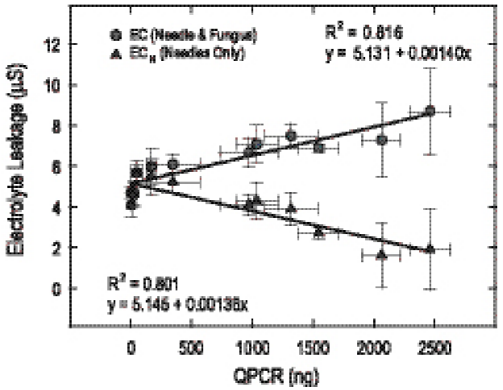
No apparent toxins involved in the development of Swiss needle cast symptoms.

2000

Dan Manter and Katy Kavanagh.

Cooperators: Greg Filip, Jeff Stone, Wendy Sutton and Lori Winton.

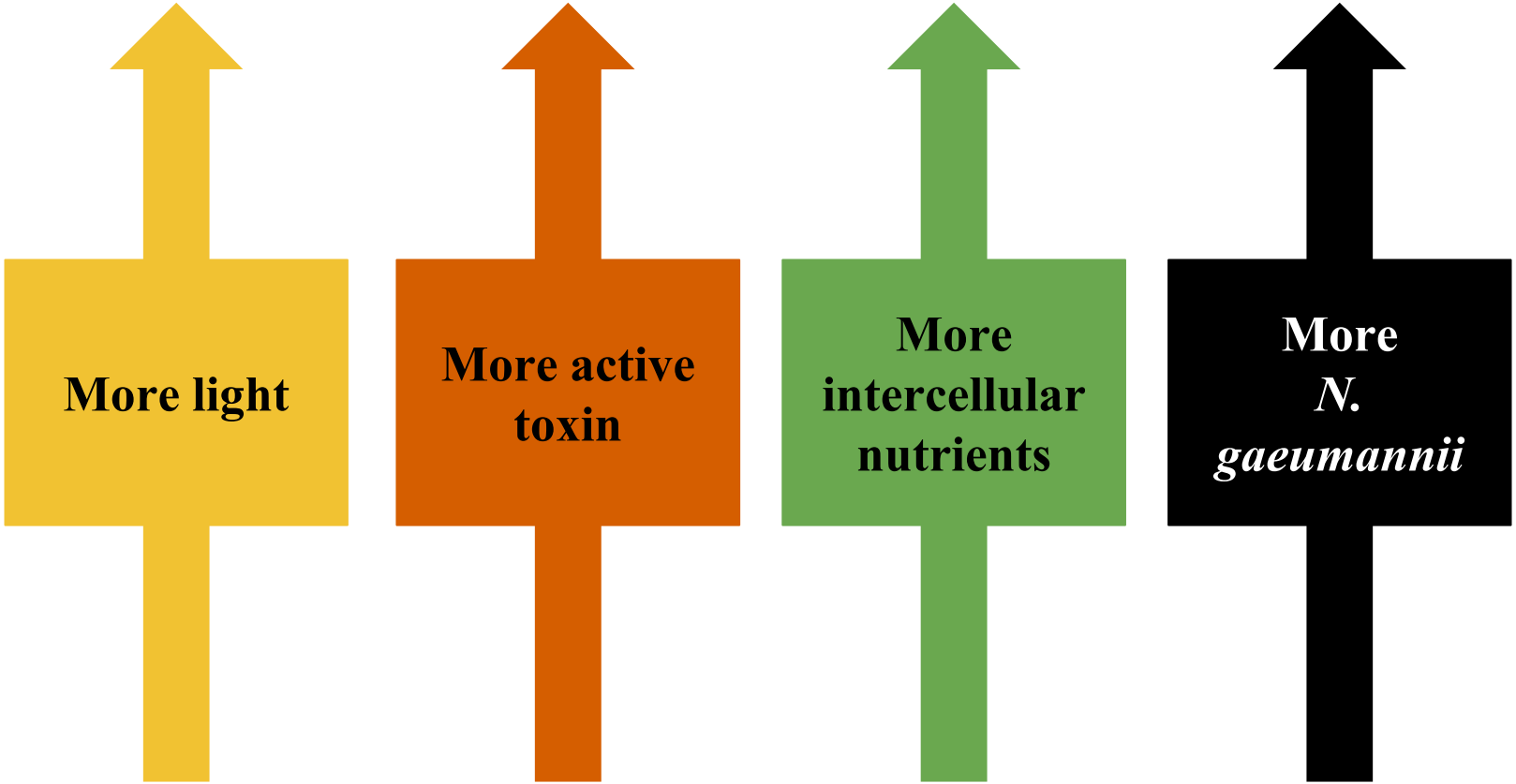
2001



Electrolyte Leakage and Nutrient Status in SNC-infected Douglas-fir

Dan Manter and Jeff Stone

Support for an earlier hypothesis…



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