Dynamics of the phyllosphere fungal microbiome in Douglas-fir needles associated with *Nothophaeocryptopus gaeumannii* in coastal Oregon, USA

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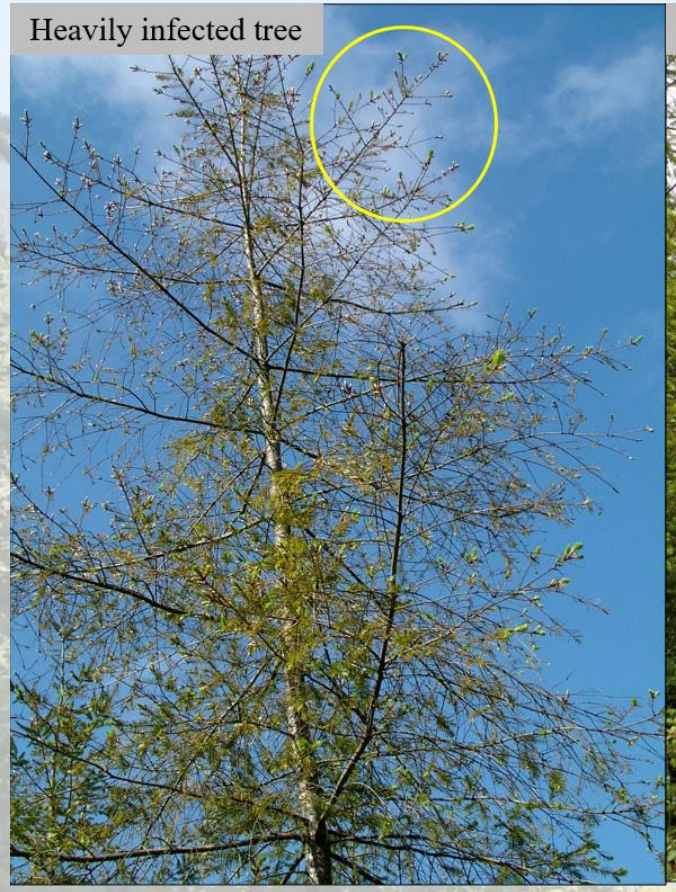
2023 SNCC annual meeting

Disease symptoms



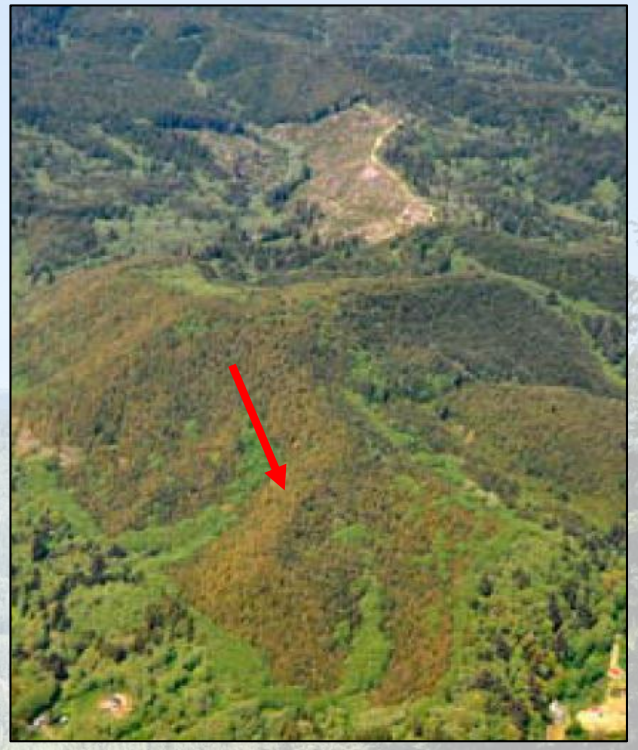


Photo from Swiss Needle Cast Cooperative: http://sncc.forestry.oregonstate.edu/

Potential impacts on mature forests

Structural changes → Wildlife habitat changes

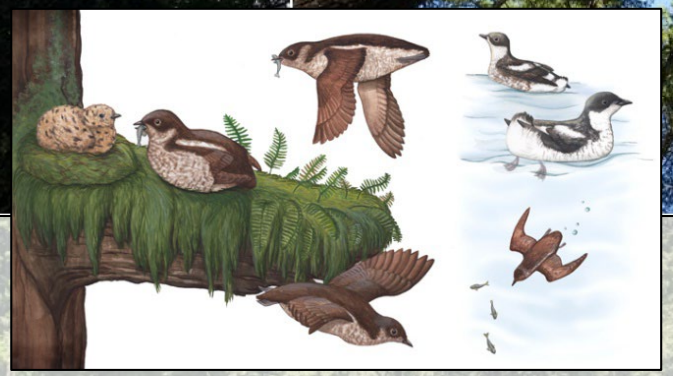


Photo credits: http://www.americanforests.org/magazine/article/saga-of-the-spotted-owl/
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Foliar microbiomes

- Foliar microbiomes can be related to plant physiological traits such as stomatal conductance, also it is important for host resistance to diseases.

- *Nothophaeocryptopus gaeumannii* is an endophytic fungus that would mature and emerge in the spring while Douglas fir bud-breaking.

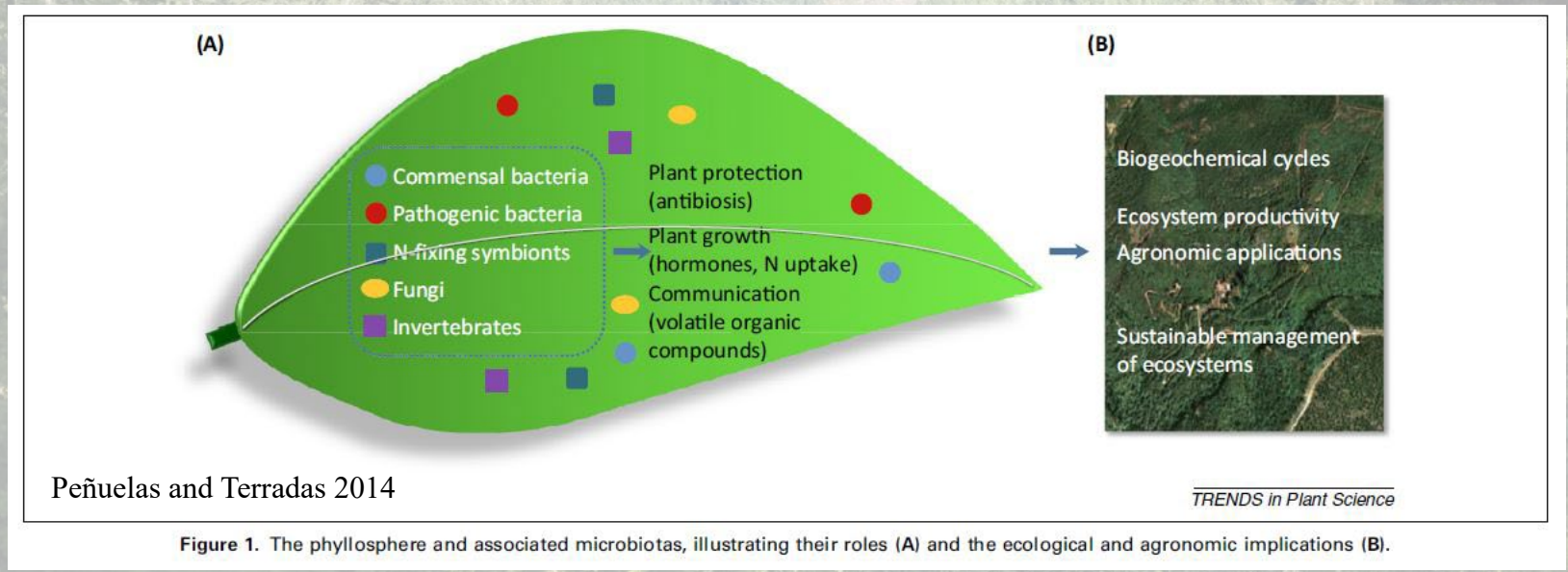


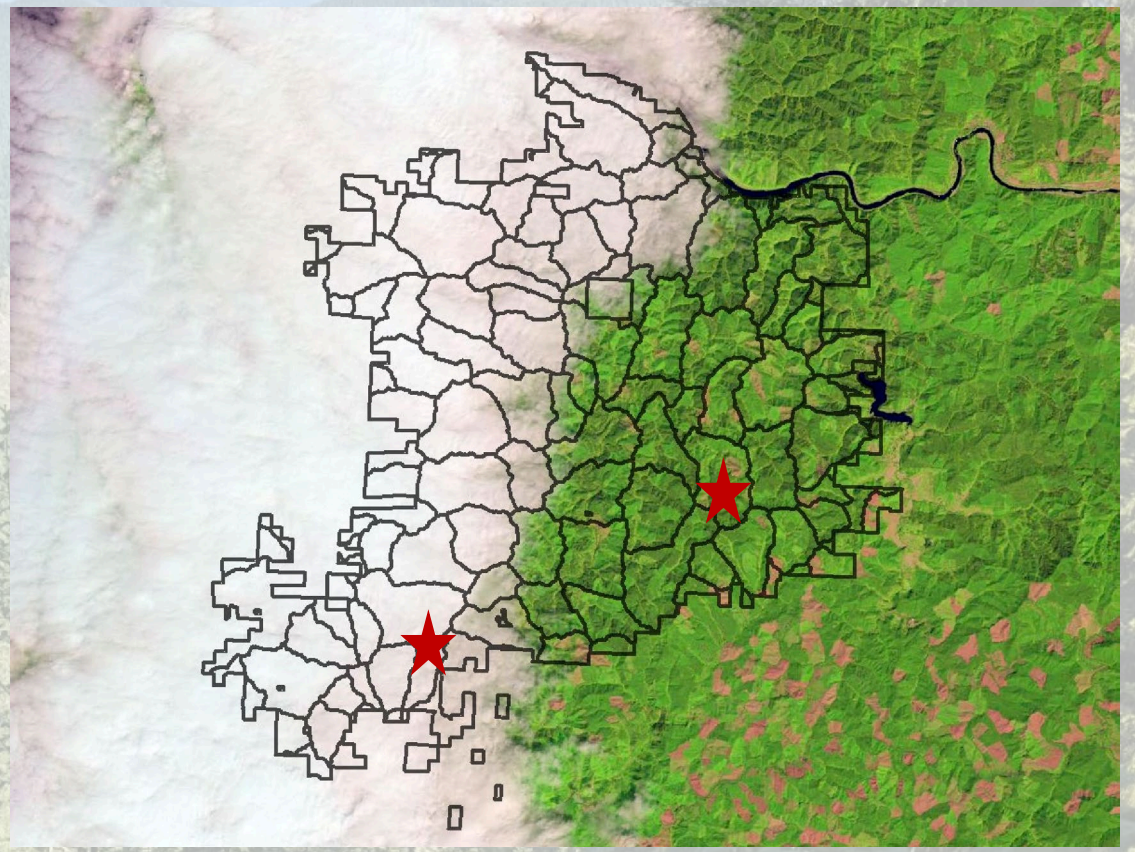
Figure 1. The phyllosphere and associated microbiotas, illustrating their roles (A) and the ecological and agronomic implications (B).

Research Questions

- Do foliar fungal communities relate to *Nothophaeocryptopus gaeumannii* occlusion therefore the SNC patterns? Can metabarcoding methods replace the traditional evaluation of SNC?

- Do the fungal communities and *Nothophaeocryptopus gaeumannii* differ among canopy locations due to macro- and micro- climates?

Elliott State Forest



Tree Ht 60-70m

Zone 5

Zone 4

Zone 3

Zone 2

Zone 1

Canopy Depth

Canopy bottom 20-30m

Canopy depth was defined as the length from the tree-top to the lowest contiguous (vertical distance < 2m) branch

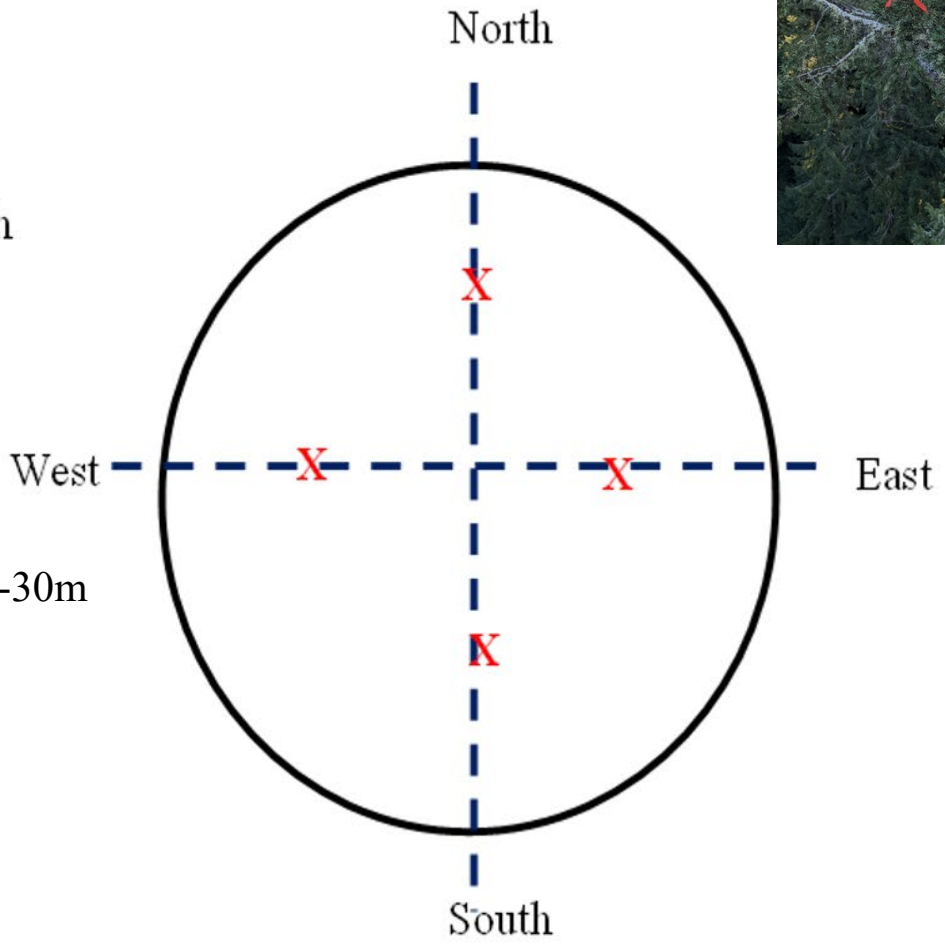
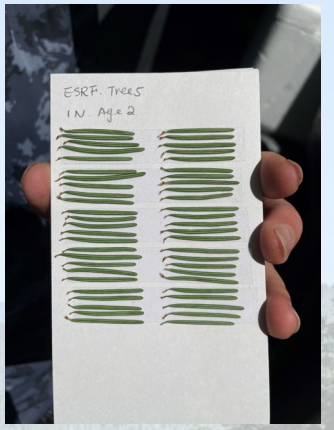
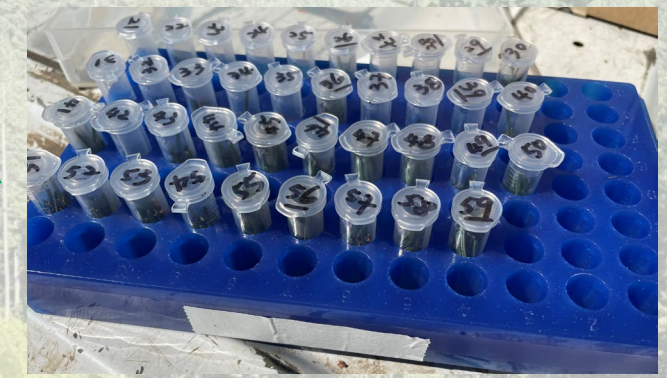


Photo Credit: https://tidcf.nrcan.gc.ca/en/trees/factsheet/122

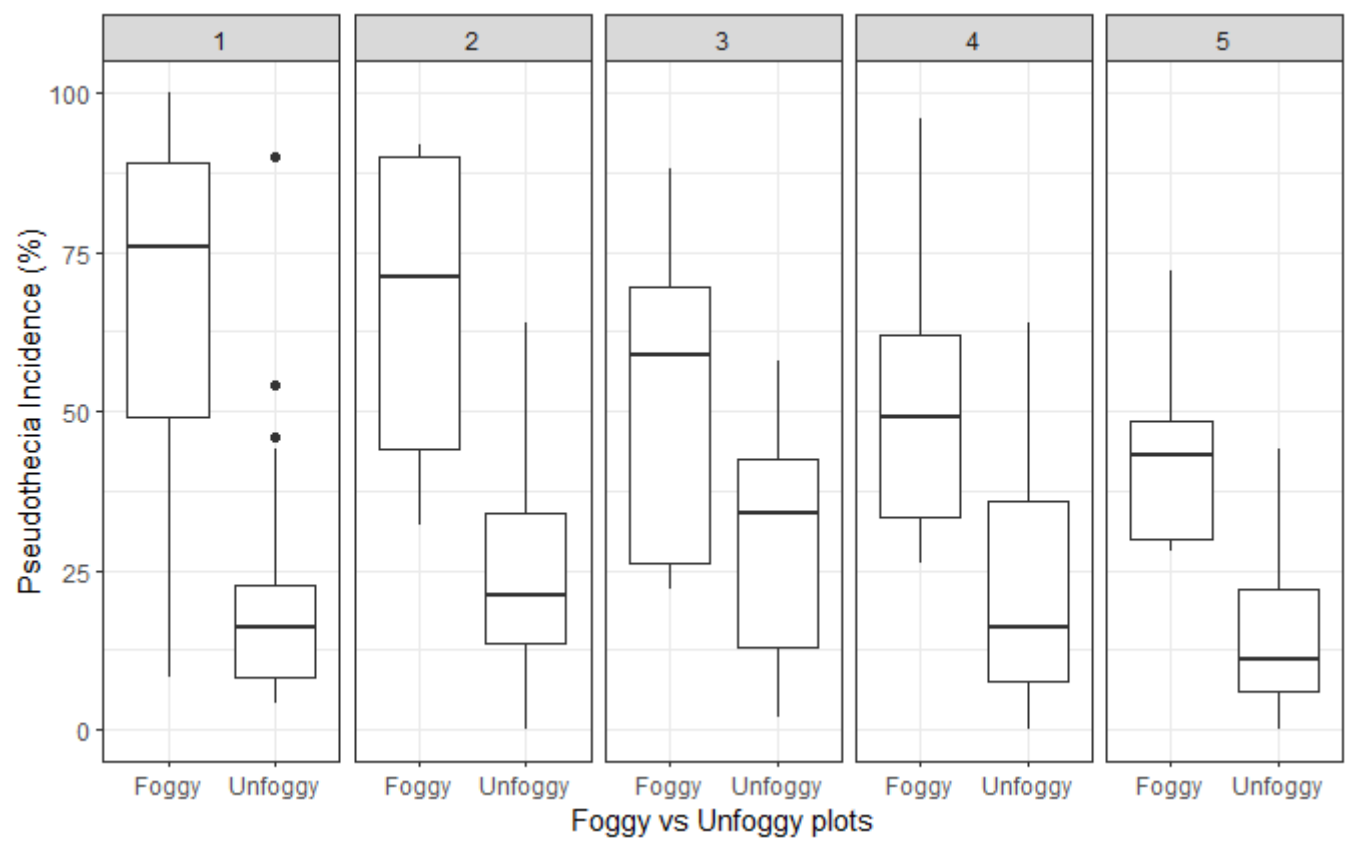


SNC samples: after evaluating the foliage retention, 50 needles were randomly selected from 3-year-old cohort, taped on an index card, and stored at -20˚C before reading. (June 2023)



Microbiome samples: lyophilized for 24 hours for drying, then stored at -80 ˚C before processing. (Nov 2022 & June 2023)

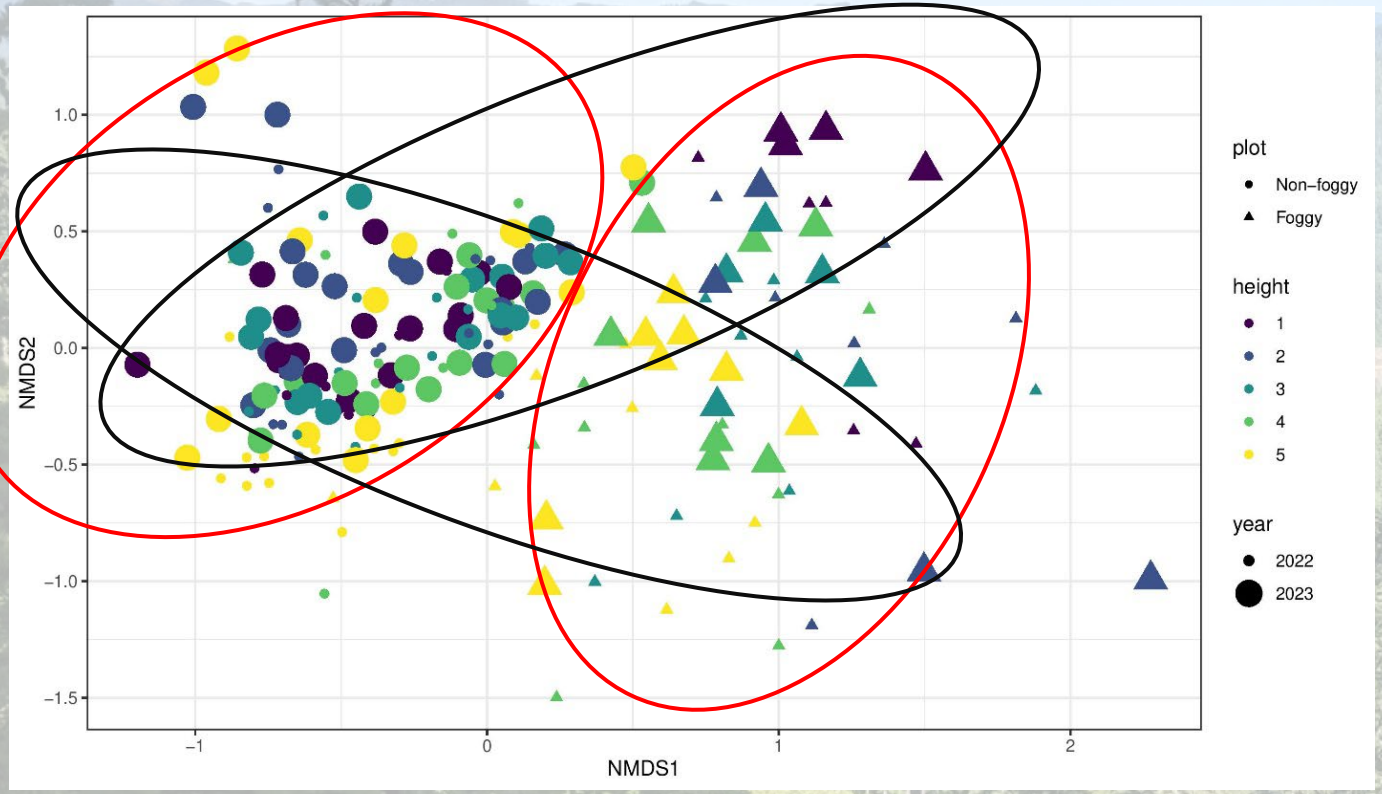
Preliminary results: SNC incidence



* Incidence = % of needles occluded by pseudothecia

- SNC incidence in the foggy plots were 52% significantly more than non-foggy plots (GLMM, p=0.02).
- SNC incidence at the most bottom canopy (level 1) was 29% significantly higher than SNC incidence at the most top canopy (level 5) (GLMM, p=0.007).
- However, SNC incidence was not statistically different in 4 directions.

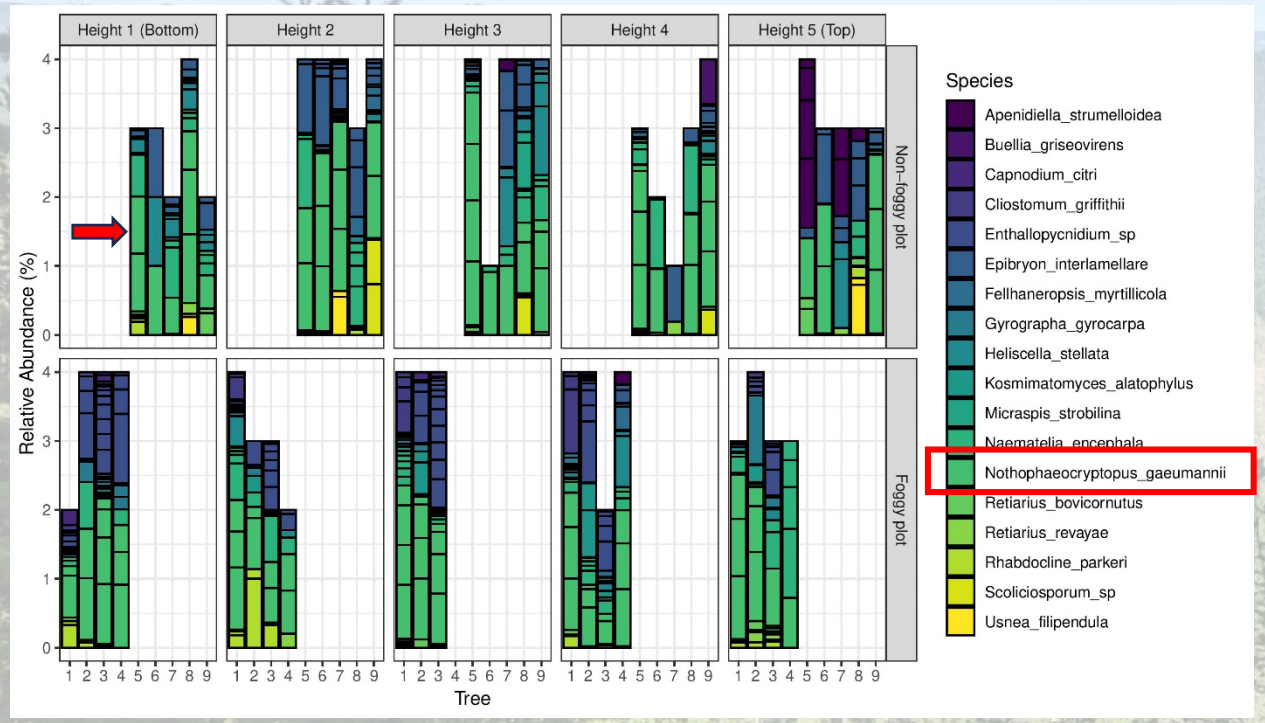
Preliminary results: phyllosphere microbiomes



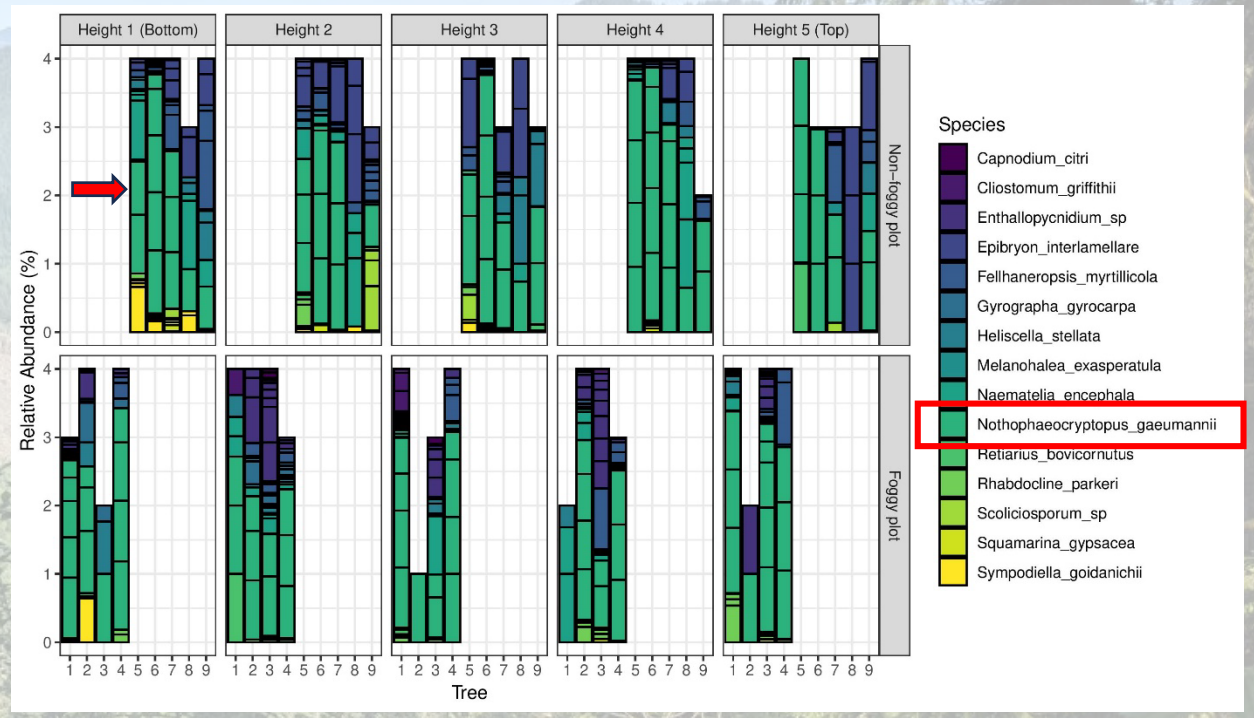
- The fungal phyllospheric communities were significantly different at foggy and non-foggy plots (p=0.0001), and among the different sampling heights (p=0.002).
- The fungal communities in the phyllosphere were different while they collected in 2022 and 2023 (p=0.005).
- However, the communities did not differ among sampling direction (p=0.38).

Preliminary results: phyllosphere microbiomes

Nov, 2022

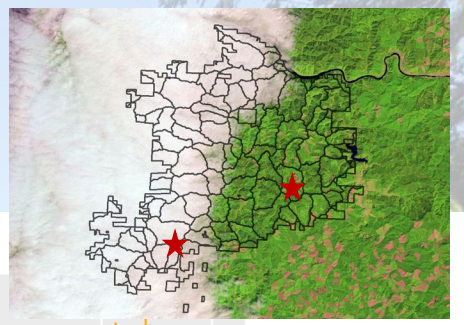


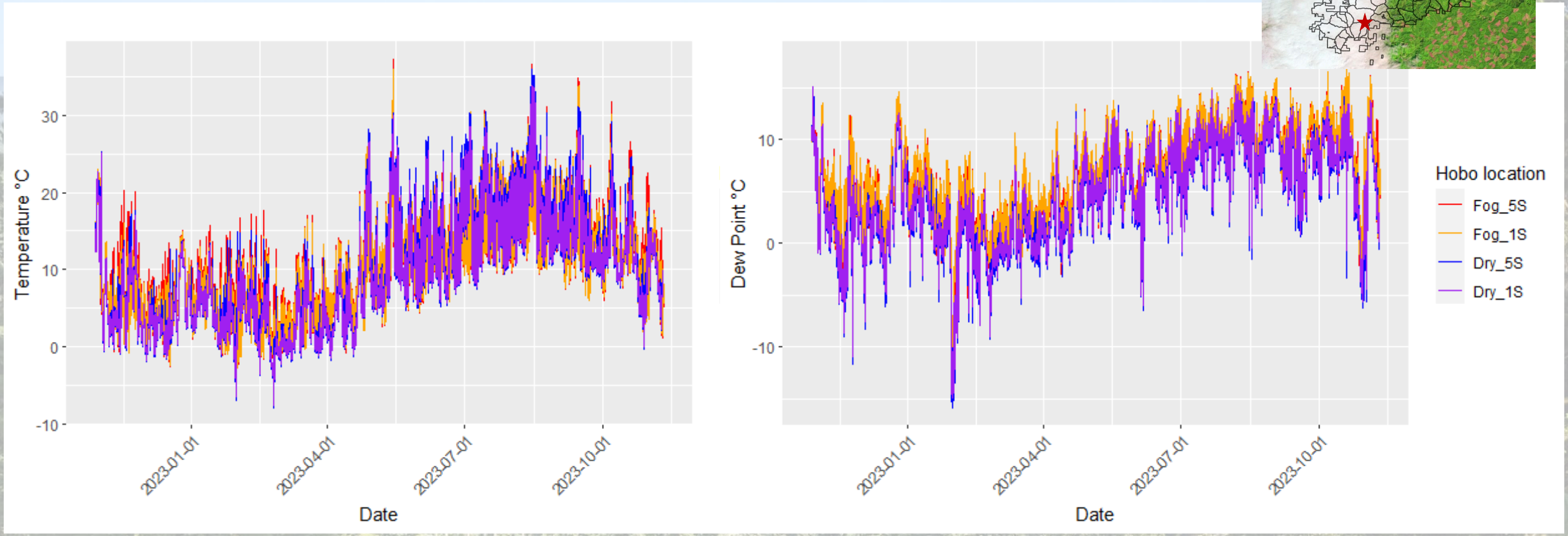
June, 2023



- *N. gaeumannii* was abundant in most samples.
- Other needle pathogens, such as *Rhabdocline* spp.
- Lichen associated species, like *Cliostomum griffithi, Scoliciosporum* spp, and *Usnea* spp.
- From winter to spring: RA of *Nothophaeocryptopus gaeumannii* increasing.

Weather data





- Higher canopy has more dynamic changes on temperature.
- Fog site has higher dew point temperature than unfoggy site. => fog site has higher moisture and more fog/frost than unfoggy site.

Take home messages

For both *Nothophaeocryptopus gaeumannii* incidence and foliar fungal communities…

- Fog plays an important role.
- Vertical height is significant.
- The directions do not matter.

Acknowledgements

Field and climbing

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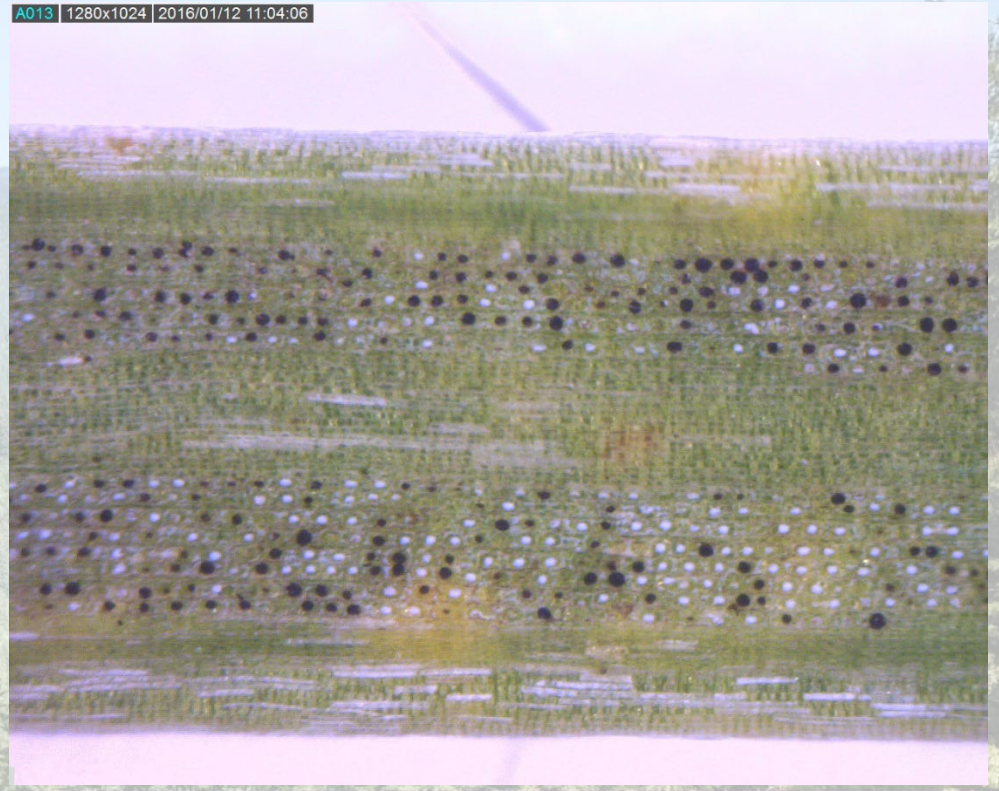
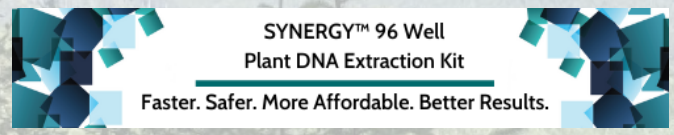
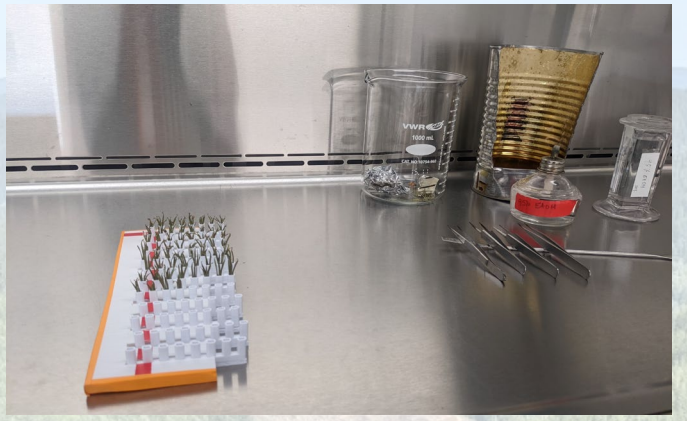
Incidence and Disease Severity Index



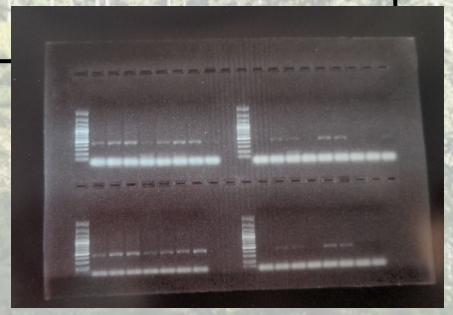
- 3-year-old needles used
- Incidence → pseudothecial presence (%)
- Severity index → Incidence*ratio of occluded stomates (%)

Microbiome analysis

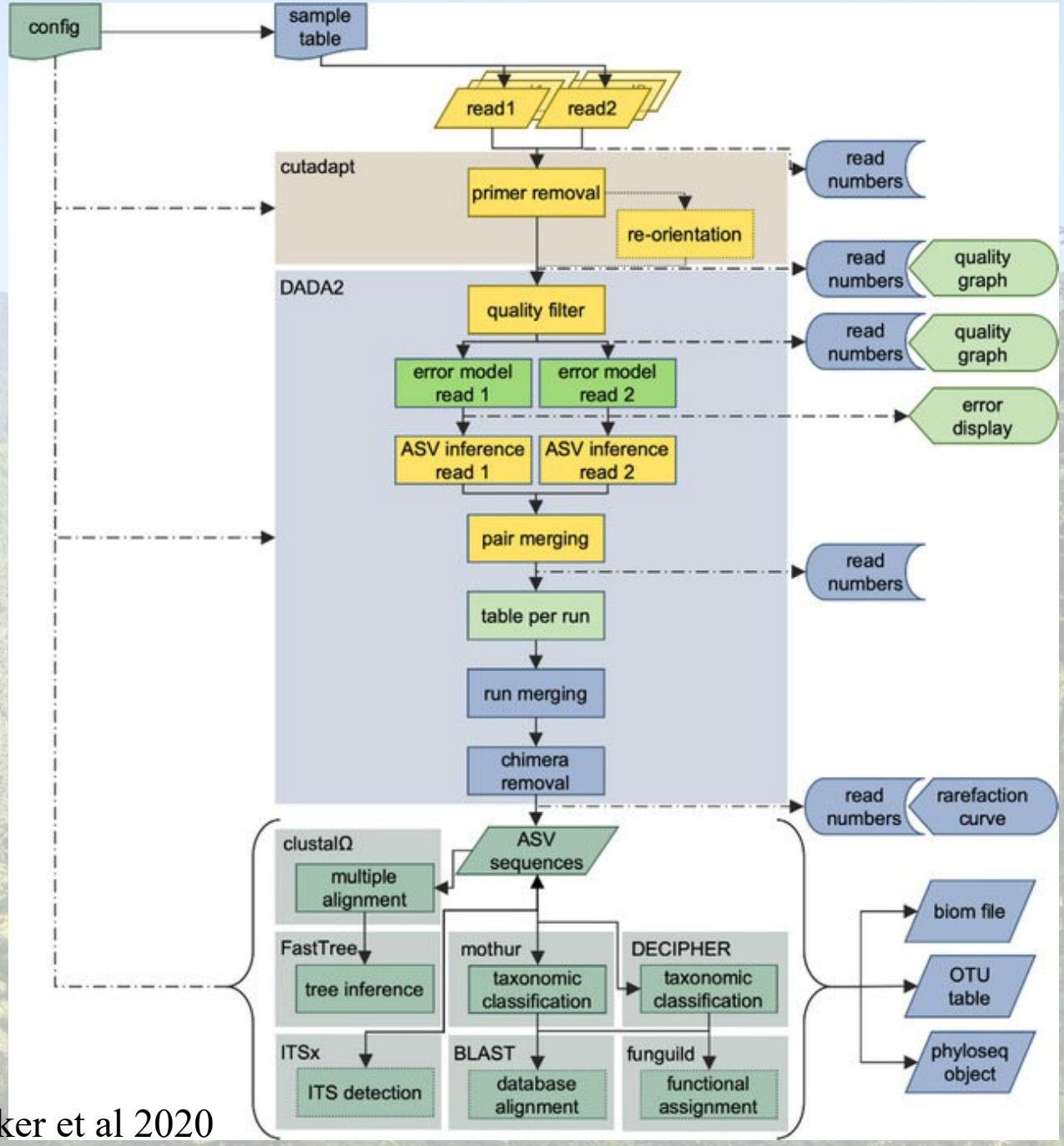




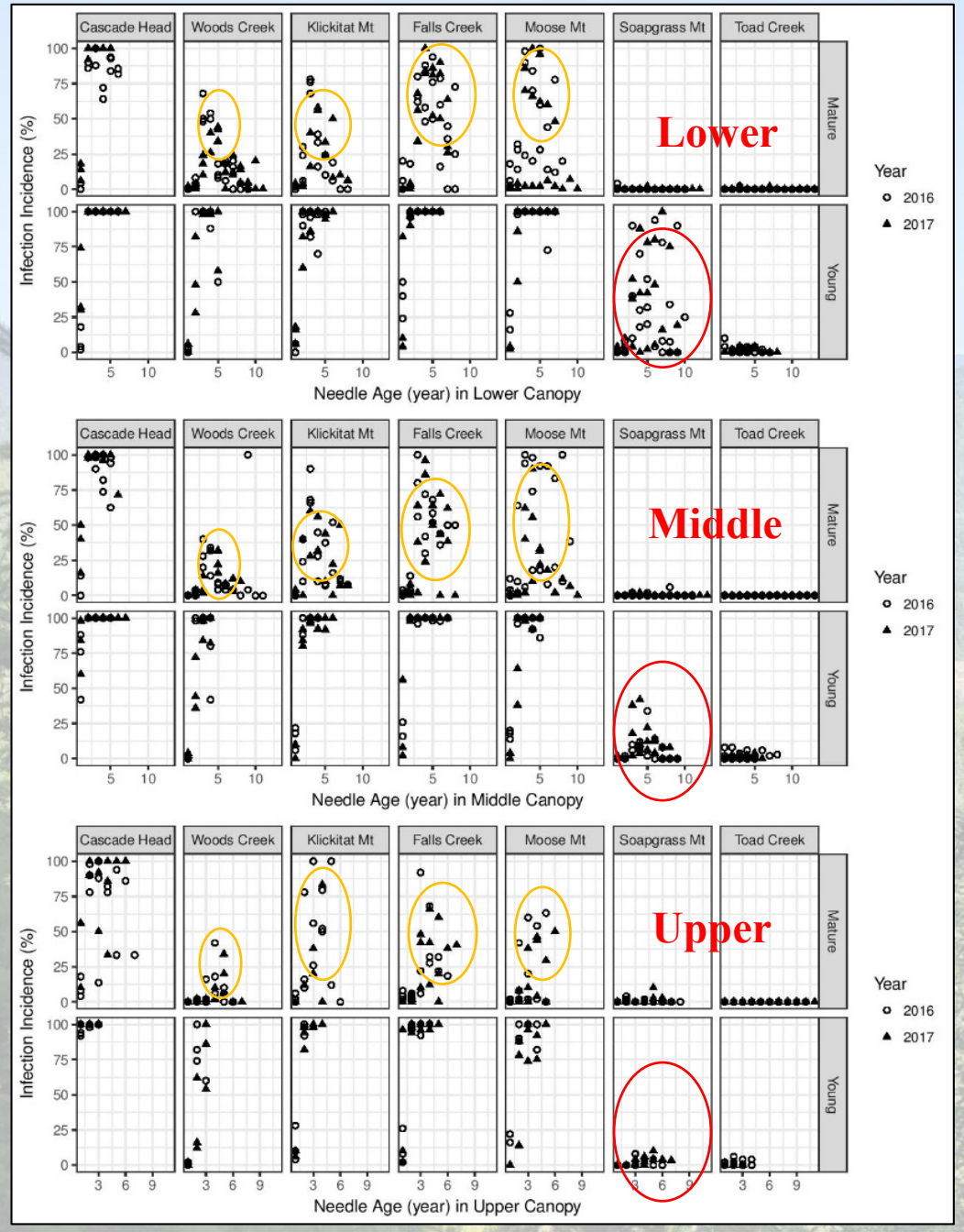
Two stage PCR applied. The ITS2 region was first amplified using the 5.85-Fun and ITS4-Fun primers, a 3-6 bp length heterogeneity spacer, and then followed by illumina adaptor sequences (Gervers et al 2022).



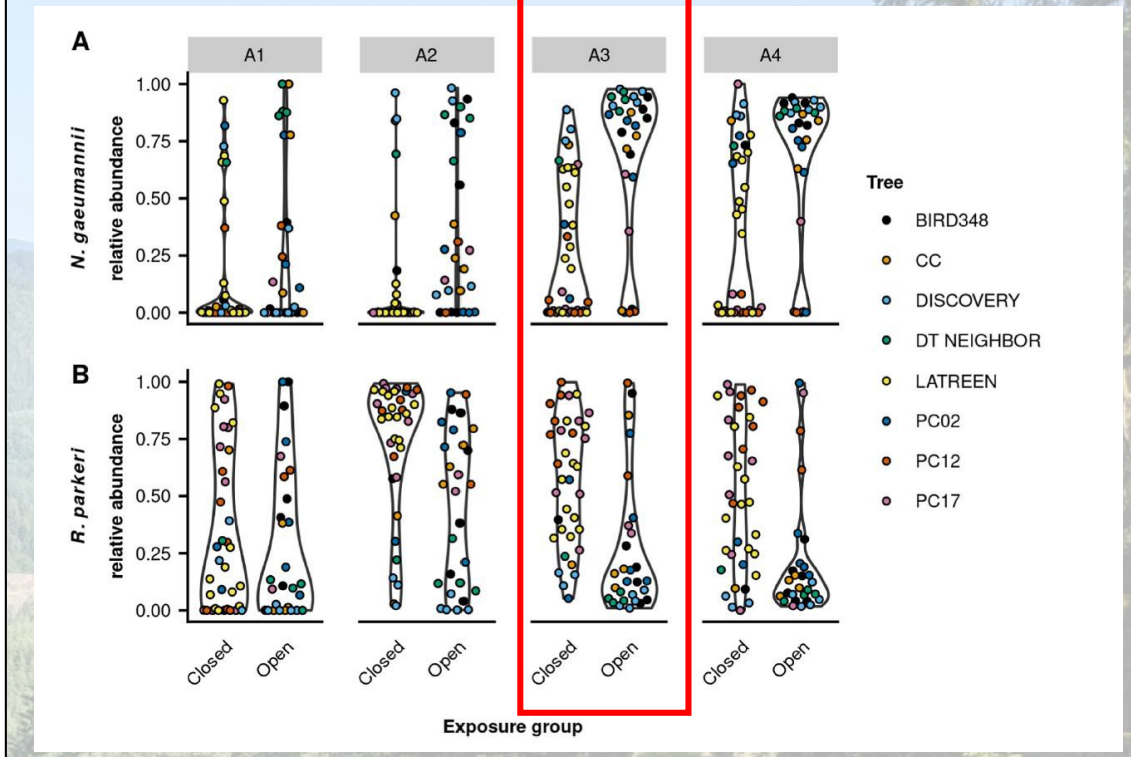
Conceptual workflow of Pipeline



Weißbecker et al 2020

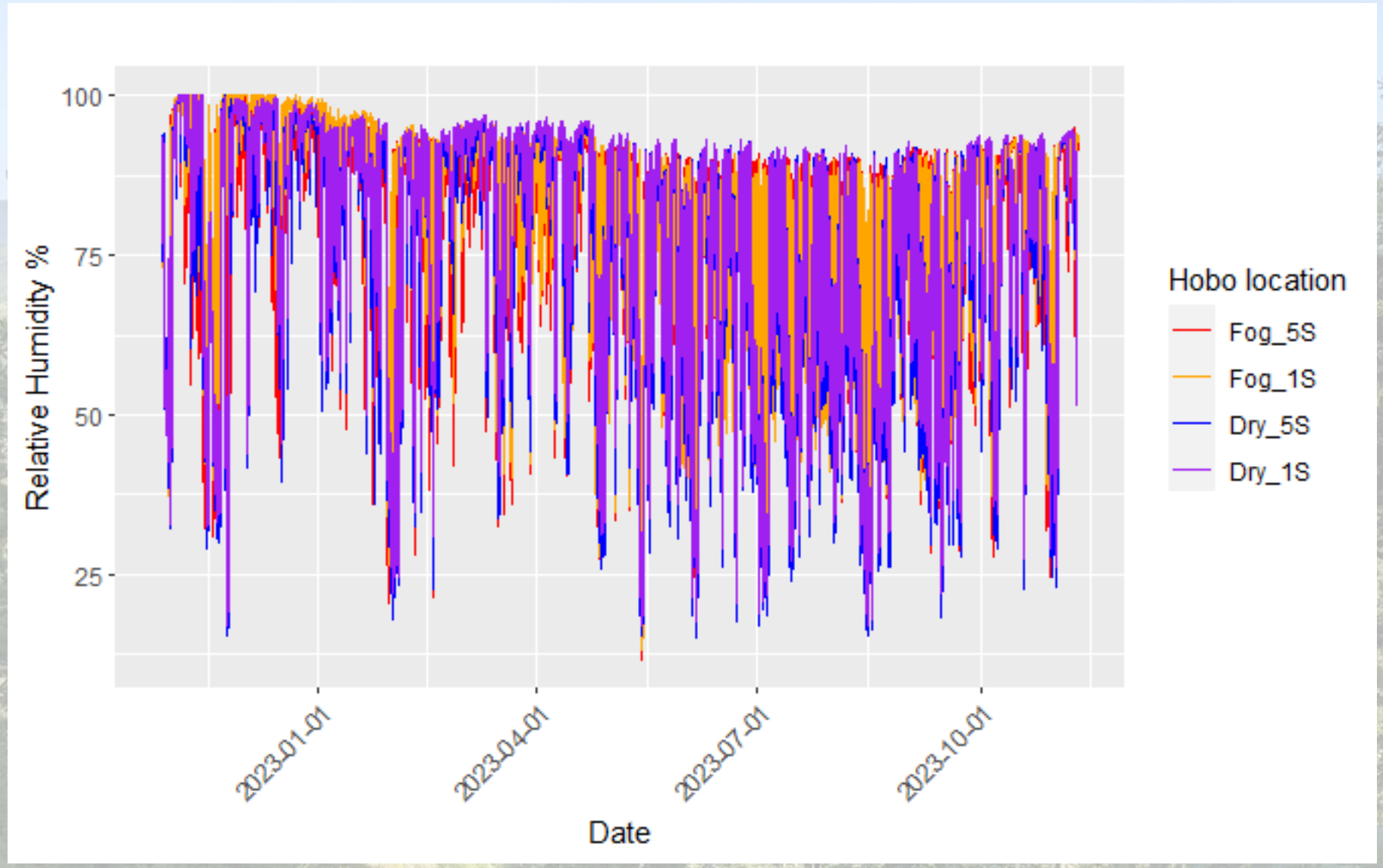


Lan et al. 2019



Gervers et al. 2022

Incidence = % of needles occluded by pseudothecia


100
75
50
25
Relative Humidity %
2023-01-01
2023-04-01
2023-07-01
2023-10-01
Date
Hobo location
Fog_5S
Fog_1S
Dry_5S
Dry_1S