

Metabolome responses to environmental stress treatments in oak sapwood

WP2

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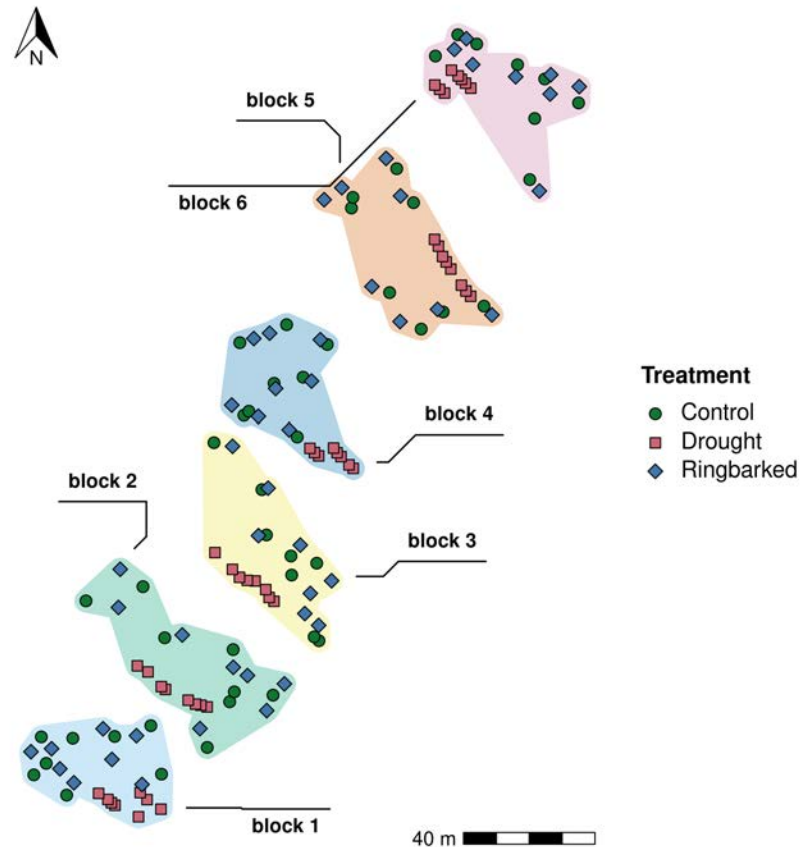
Overview

- Environmental stress treatments:
 - **drought**
 - **ring-barking**
- Experiment layout and timeline
- Field sampling approach
- Metabolome fingerprinting
- Metabolome responses to stress treatments

Experiment field site layout

- 144 trees total.
- Trees divided into 6 spatial blocks, each containing 24 trees.
- 8 trees of each stress treatment per spatial block.
- A total of **48 trees per stress treatment class.**

Little Snoring field site experiment layout



Environmental stress treatments

Drought



- Precipitation exclusion shelters constructed beneath the canopy.

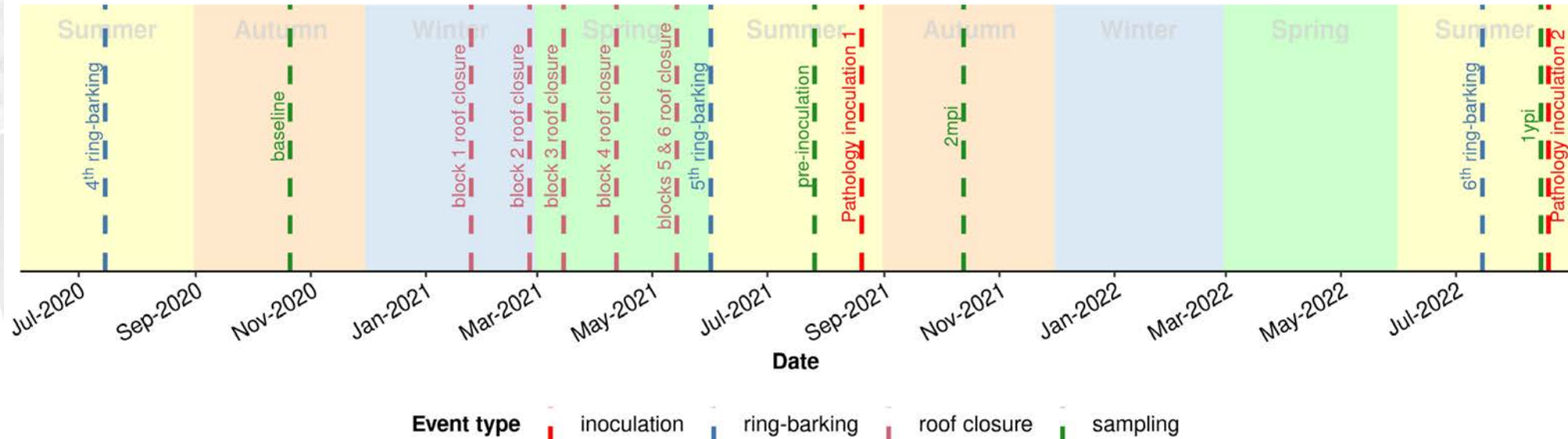
Ring-barking



- Performed at two heights: 0.5m and 1.8m.
- To the depth of the cambium.
- 25mm left intact on two sides of the tree.

Experiment timeline

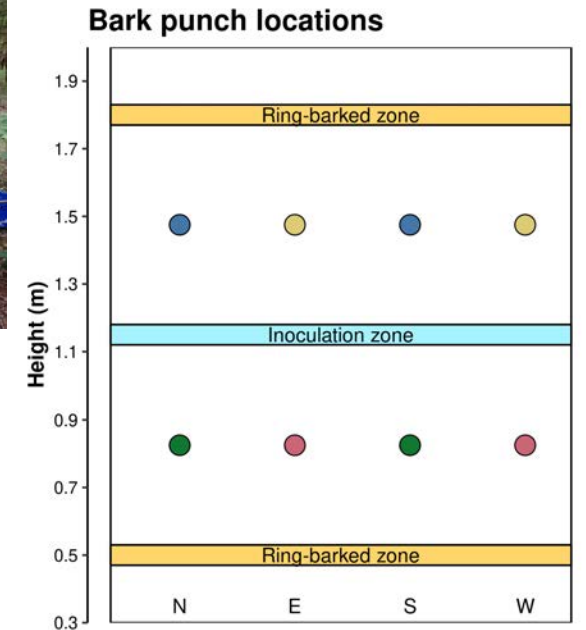
BAC-STOP WP2 field trial timeline



The first ring-barking took place in August 2015. The second and third ring-barking took place in May and October 2018 respectively. 2mpi = 2 months post inoculation; 1ypi = 1 year post inoculation

Sample collection

- Two, ~30mm deep **bark punches** taken from each tree at each time point.
- Phloem and sapwood tissues separated at the cambium and dissected into **50mg segments**.
- Samples were snap frozen in liquid nitrogen for transport.

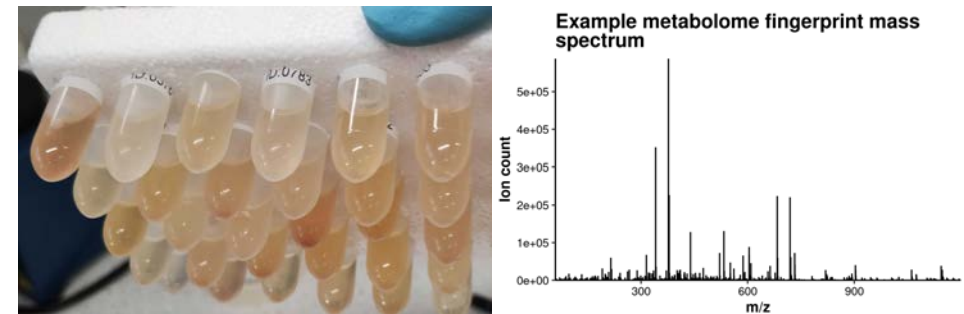


Sampling time point

- baseline (blue dot)
- 2 months post inoculation (yellow dot)
- pre-inoculation (green dot)
- 1 year post inoculation (red dot)

Metabolome fingerprinting

- The sapwood sample extracts were analysed using a **mass spectrometry** based approach.
- Provides an overview of a **wide range of metabolite chemistry**.
- **837 metabolome features** were identified and assigned molecular formulas for use in data analysis.



Metabolomics (2022) 18:64
<https://doi.org/10.1007/s11306-022-01923-6>

ORIGINAL ARTICLE

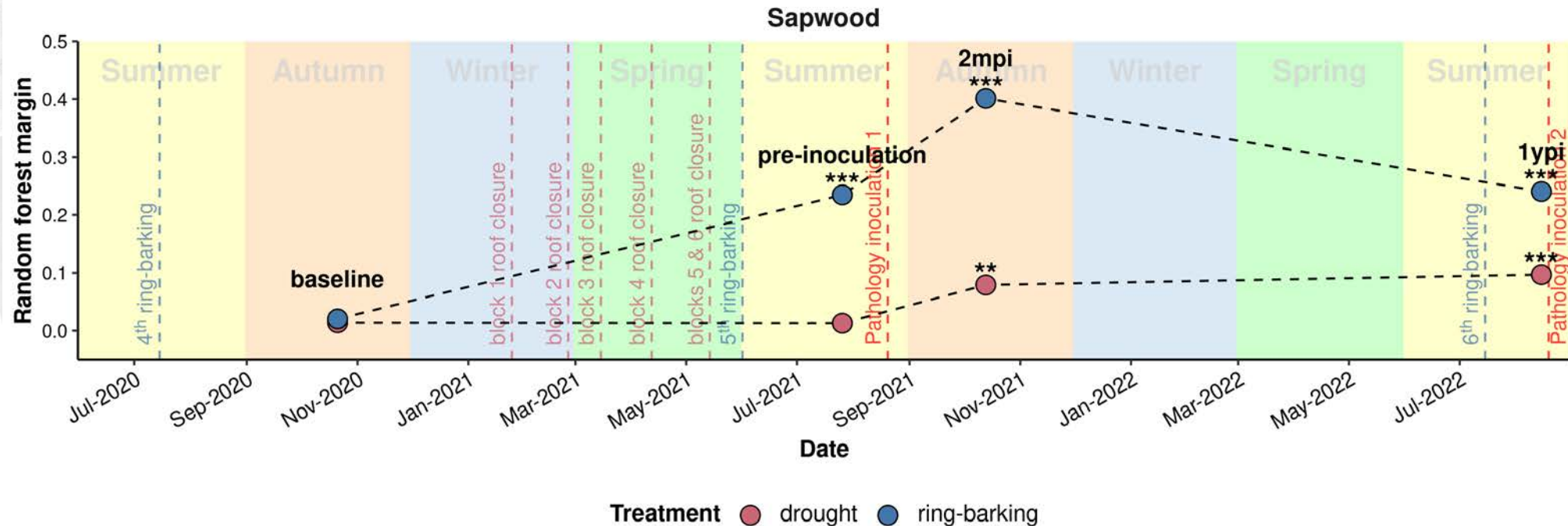


Spectral binning as an approach to post-acquisition processing of high resolution FIE-MS metabolome fingerprinting data

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Metabolome response

The distinction between control and stress treatments in the metabolome fingerprinting data

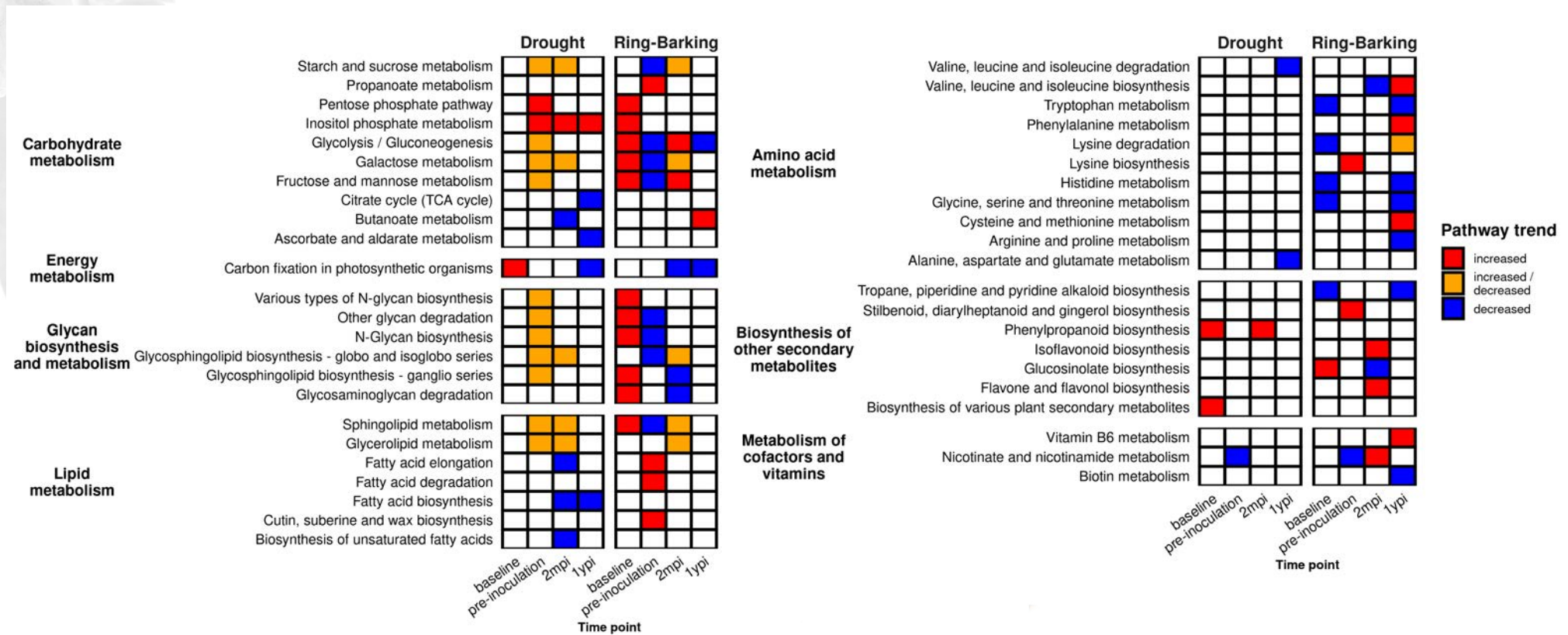


2mpi = 2 months post inoculation; 1ypi = 1 year post inoculation

Asterisks denote p-value significance: * < 0.05, ** < 0.01, *** < 0.001. P-values are based on 5000 permutations.

Explanatory metabolic pathways

Functionally enriched metabolic pathways identified the metabolome fingerprinting data



Upcoming work

- Apply these techniques to the **phloem samples** collected alongside these sapwood samples.
- Integrate **environmental data** collected from soil moisture probes and Tree-Talkers.
- Investigate the influence of **Acute Oak Decline** pathogen inoculation on the metabolome responses.

Thank you for listening

Any questions?

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