

# PHYTOCHEMICAL ADAPTIONS OF FAST-GROWING WILLOW TO FIELD-SCALE MUNICIPAL WASTEWATER IRRIGATION

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## INTRODUCTION

### BACKGROUND

- Municipal wastewater treatment is a severe environmental and economical burden. In Canada alone, 5.9 trillion litres of wastewater are produced per year.
- Fast growing willow plantations are tolerant to contamination and can filter high volumes of water.
- Willow trees produce over 2000 secondary metabolites for functions such as protection against a/biotic stresses, many of which persist in high abundance in stem biomass.
- Biorefinery can harness plant biosources to produce sustainable energy and green phytochemicals.

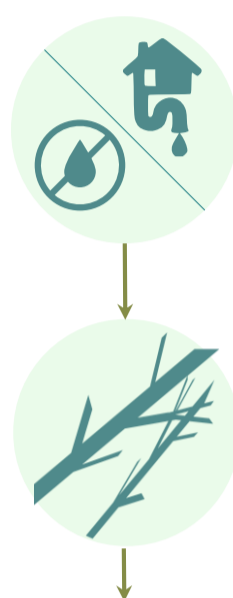
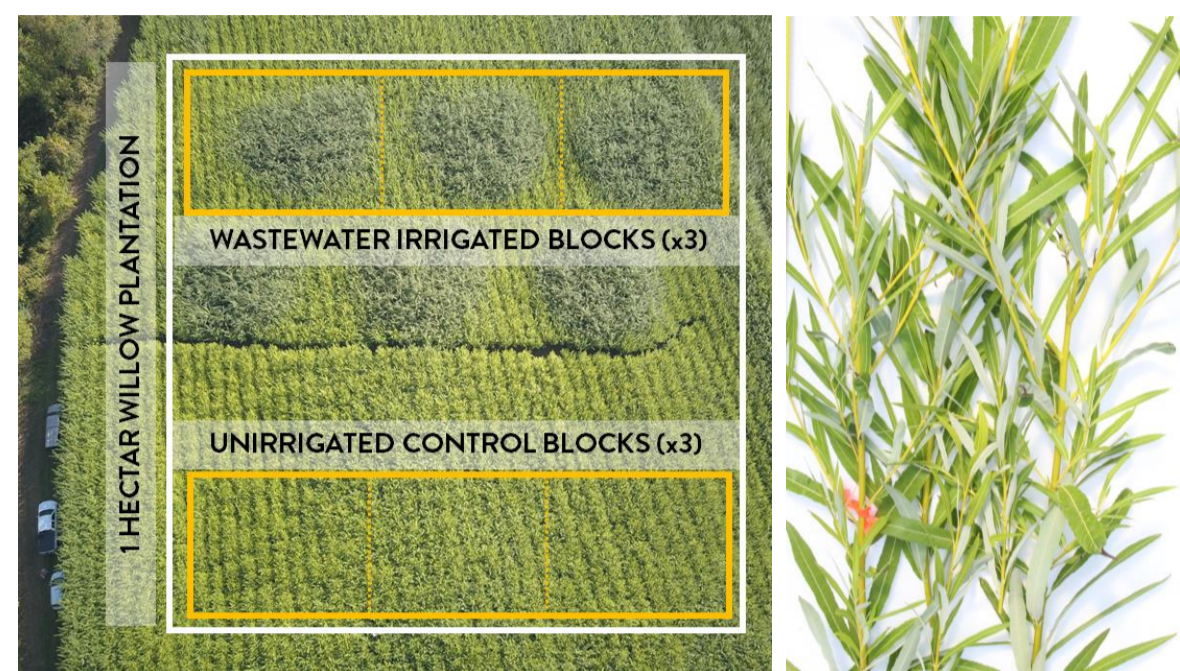
### RESEARCH QUESTION

Is it possible to integrate wastewater treatment with biorefinery using a willow plantation?

### OBJECTIVE

This study aims to unravel the impact of primary wastewater effluent irrigation on the secondary metabolite composition of willow trees in a controlled field plantation and identify the persistent phytochemicals important for sustainable biorefinery.

## METHODS

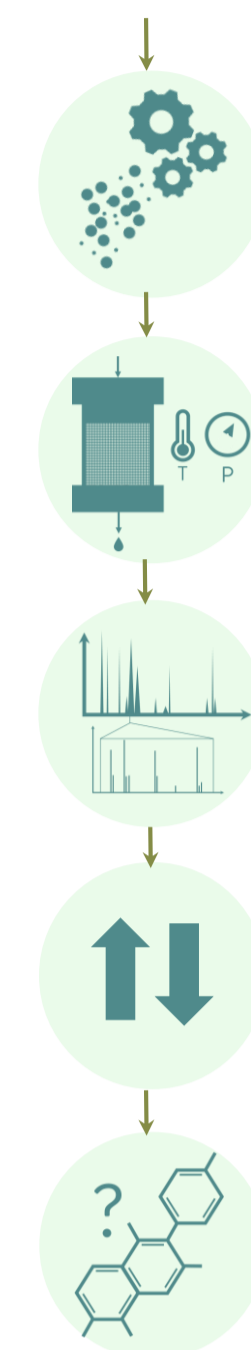


### Field-scaled Treatment

- One-hectare *Salix miyabeana* 'SX67' plantation in Southern Quebec (Canada)
- Two growing conditions: Unirrigated control blocks (n=3) Wastewater irrigated blocks (n=3)
- Irrigation rate around 29 million L ha<sup>-1</sup> yr<sup>-1</sup>

### Biomass Harvesting

- 3 entire trees sampled per block (18 trees in total)
- 3-year growing cycle (mature trees harvested)



### Biomass Processing

- Whole stem air-drying
- Controlled particle size reduction (180-850 μm)

### Pressurised Liquid Extraction

- Dionex Accelerated Solvent Extractor (ASE)
- Methanol-Water (75-25% v/v)

### Untargeted Metabolite Analysis

- Liquid chromatography (LC)
- Mass spectroscopy (MS/MS) using a QTOF with ESI(-) mode

### Differential Abundance Analysis

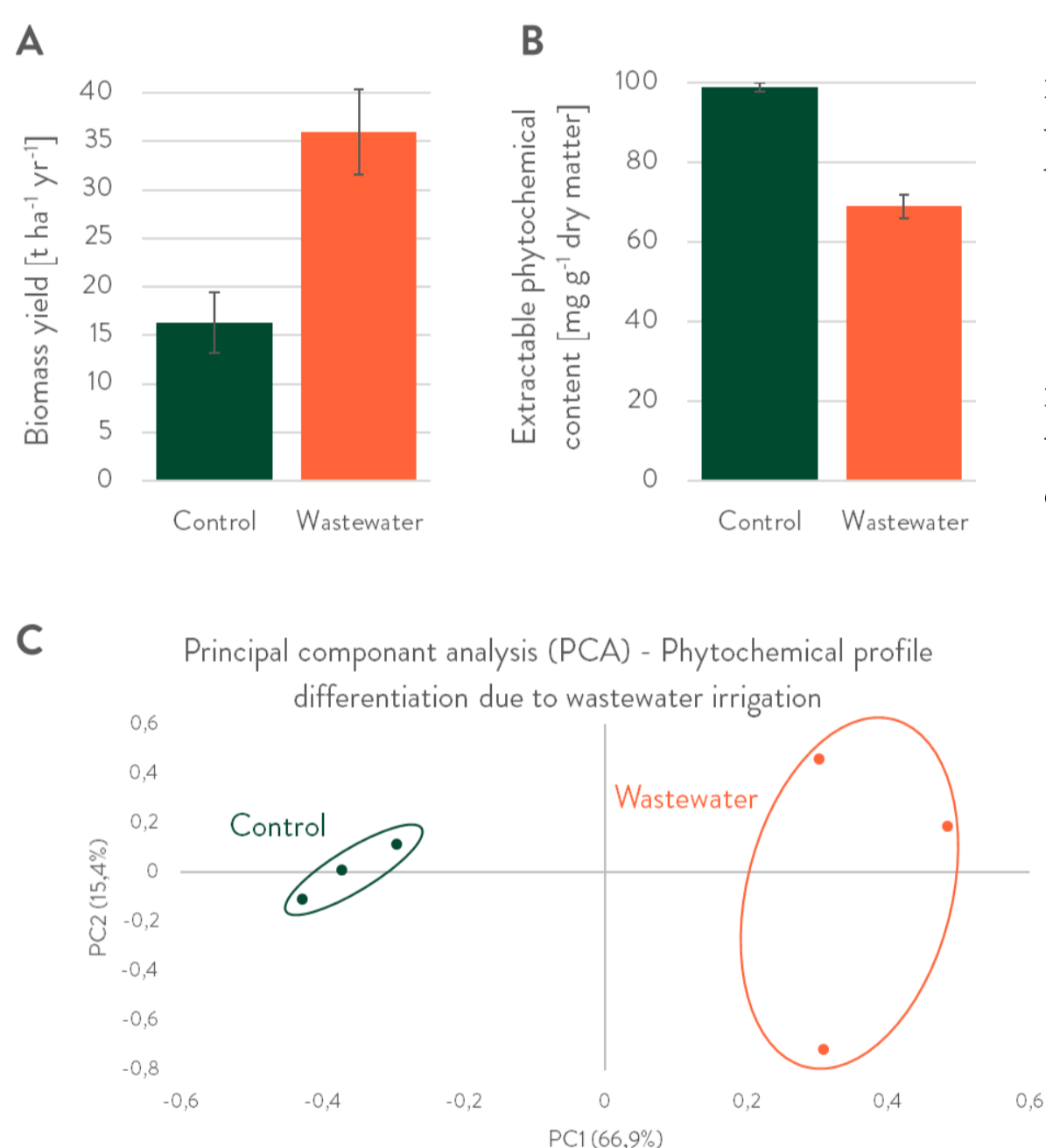
- Alignment of metabolite features across replicate samples (MZmine2)
- Statistical comparison between treatment using Mann-Whitney U-test with FDR correction

### Metabolite Feature Identification

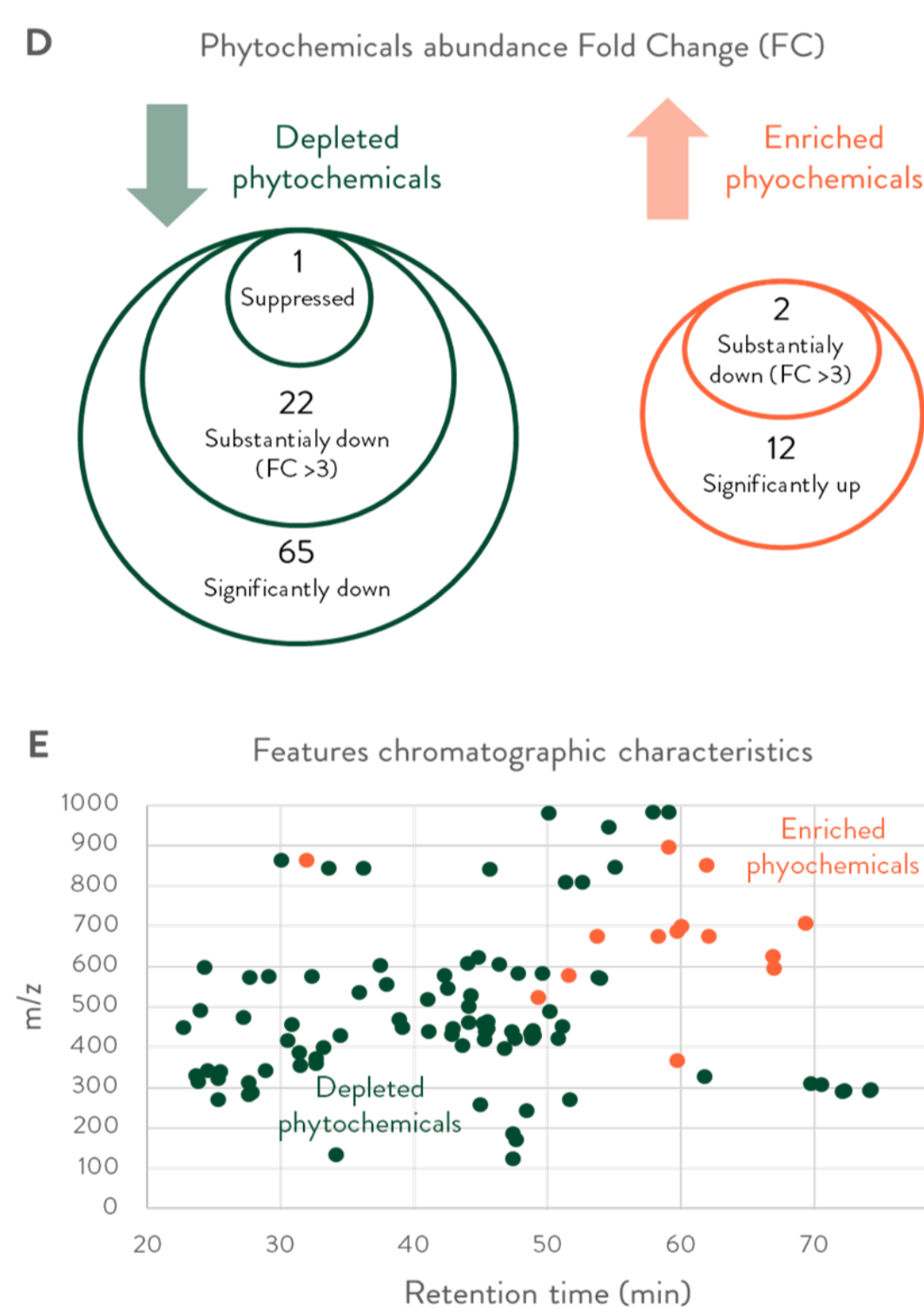
- Sequential annotation pipeline using Salicaceae and public metabolite libraries
- MS and MS/MS fragmentation resolved

## RESULTS

### RESPONSE TO WASTEWATER IRRIGATION



These results suggests a modification of resource investment to different metabolic pathways. Higher production of biomass structural components is associated with lower extractable phytochemical synthesis.



These results indicate that extractable phytochemicals vary not only in concentration but also in type, with a major increase in specific macromolecules driven by wastewater irrigation.

### UNTARGETED METABOLITE ANALYSIS

- 284 putative features were detected within the willow metabolite profile.
- 102 metabolites were significantly differentially abundant between of control and wastewater irrigated trees.

### SIGNIFICANT DIFFERENTIAL ABUNDANCE

- 88 compounds were significantly depleted in wastewater irrigated trees:
  - These included flavonoids, terpenoids and fatty acid derivatives.
  - Reductions are likely driven by high N fertilization level in wastewater
- 14 compounds were significantly enriched in wastewater irrigated trees:
  - Including important hormones associate to growth, lignans and novel yet-to-be-characterized compounds
  - All had relatively high molecular mass and less hydrophilic properties

## CONCLUSION

### OUTCOMES

- Wastewater irrigation drives a substantial increase in biomass production of willow, leading to a net 50% increase of extractable phytochemical yields.
- Untargeted high resolution metabolite assessment revealed specific wastewater induced phytochemicals with exciting potentially novel functions.
- These field-scale findings reveal a willow phytoremediation as a promising green solution to wastewater treatment combined with production of both renewable bioenergy and sustainable phytochemicals.

### FUTURE WORK

- An advanced annotation strategy is being developed for uncharacterised phytochemicals using fragmentation networking.
- Exploration of diverse functions of compounds is underway, including: antimicrobial, antioxidant, chelation and flocculation activities.
- Multidisciplinary research is underway to identify hurdles preventing integration of environmental wastewater treatment with green biorefinery.

## ACKNOWLEDGEMENT