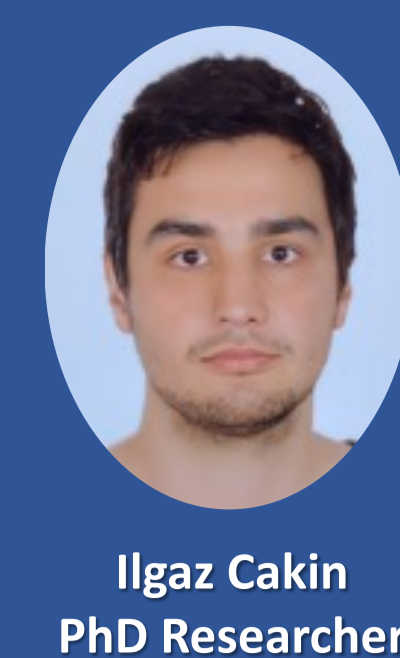


A comparison between different constructed wetland substrates and its impact on microbial communities

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Introduction

Constructed wetland systems (CWs) can be used to treat 'spent lees', a distillation by-product of whisky manufacturing. In CWs, substrates are a crucial component also referred to as media, support matrix/material, or filling material. They are recognized to have a significant impact on CWs by serving as carriers for biofilm development, providing a medium for wetland plant growth, and acting as an adsorbent for pollutant immobilization (below are 3 potential substrates).



Gravel



Foamed Ceramic (Alfagrog)

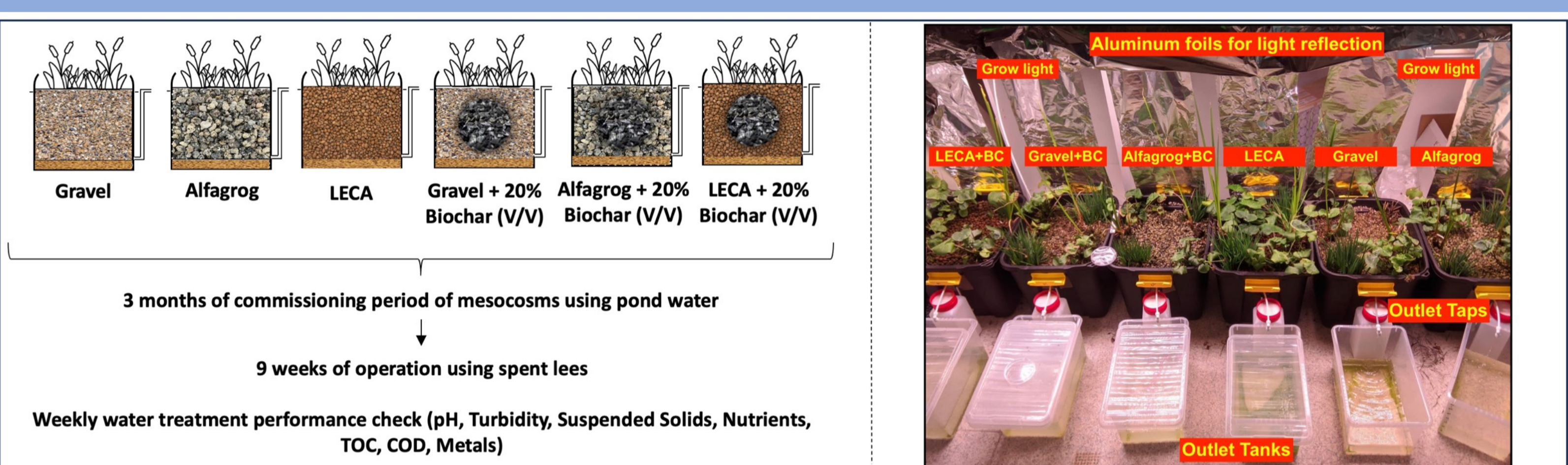


Clay Aggregate (LECA)

Aims

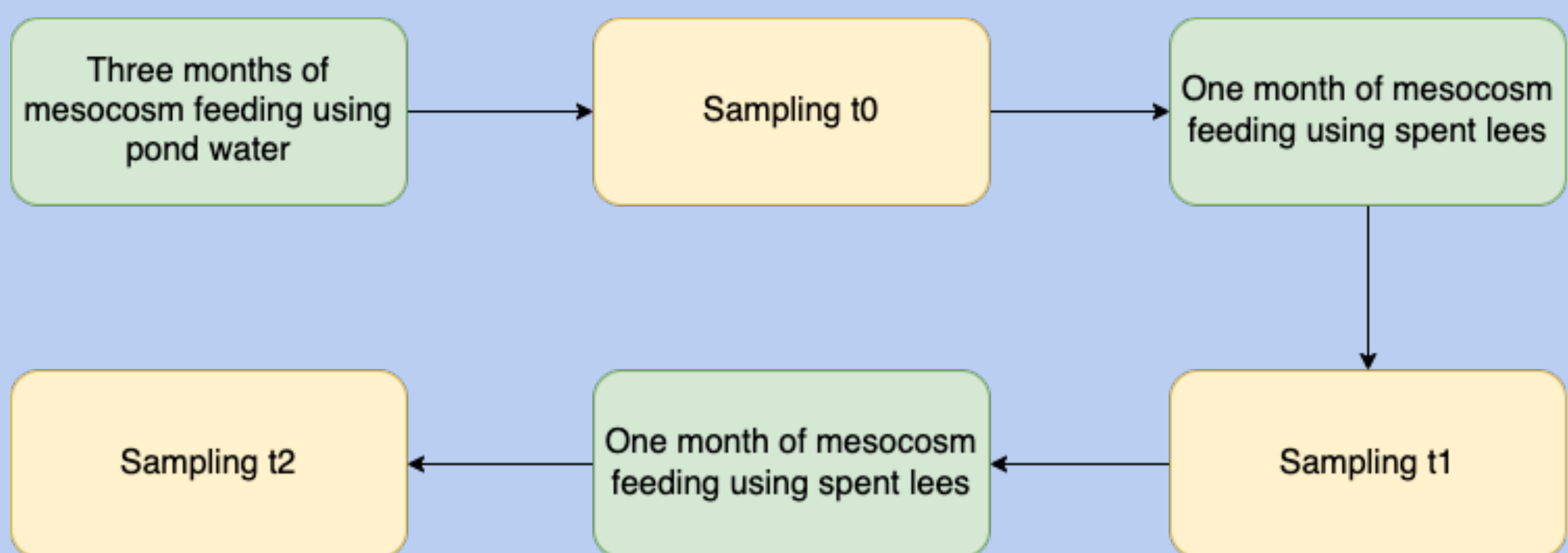
- To compare substrates in terms of their ability to support biofilms with varying bacterial and fungal richness.
- To observe changes in microbial community in these substrates during long-term treatment of distillery spent lees.

Mesocosms Operation

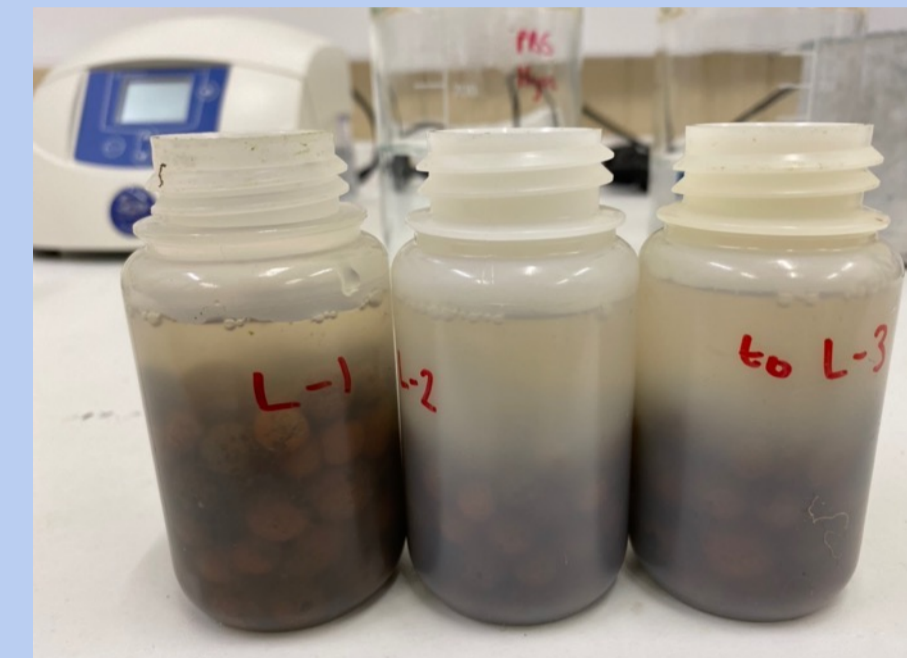


Five month operation of mesocosms. A total of six plants were planted in each mesocosm – 2 each of *Caltha palustris* (marsh marigold), *Juncus ensifolius* (swordleaf rush) and *Acorus calamus* (sweet flag). The volume of liquid that each mesocosm could take was measured, and the daily amount of liquid (pond water or spent lees) given was calculated according to a set Hydraulic Retention Time (HRT) of 7 days.

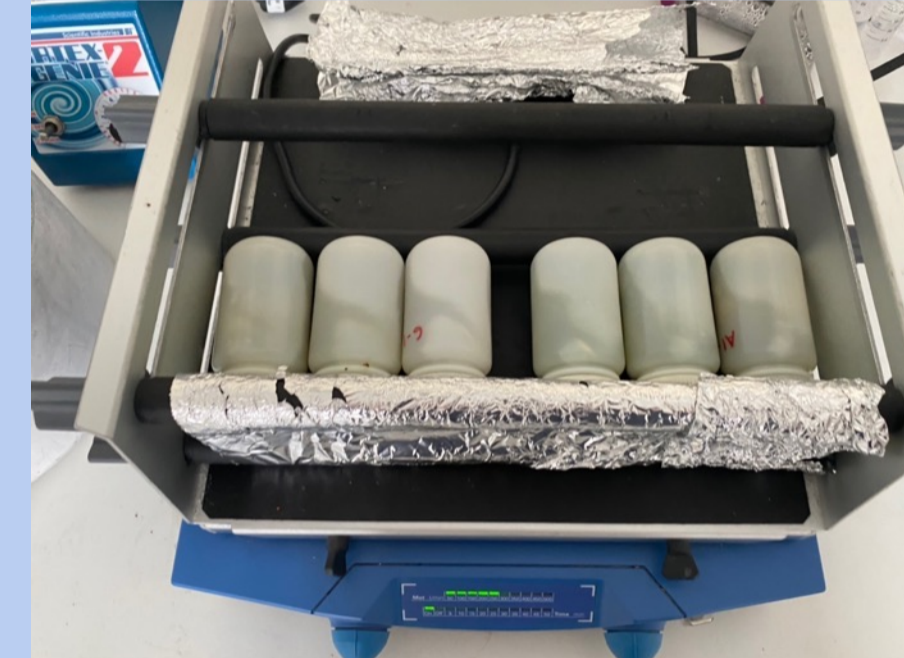
Environmental DNA (eDNA) Sampling



Inlet and outlet water filtration



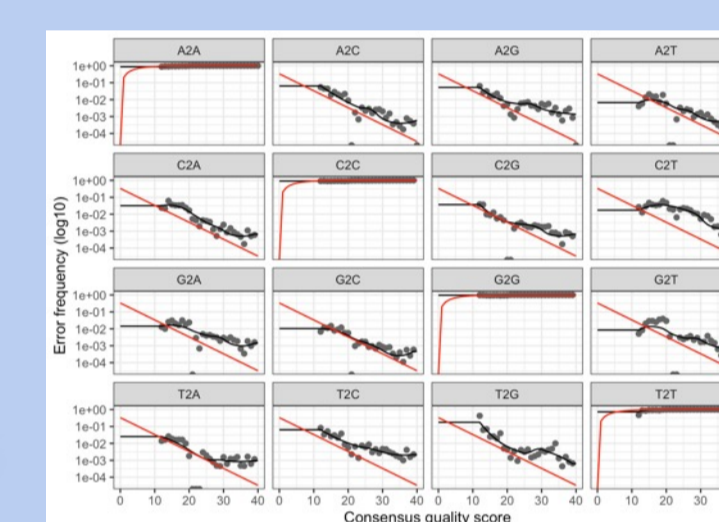
30 mins of PBS washing of each substrate (in triplicate) and then filtration



eDNA isolation for bacteria and fungi using commercial kits



Illumina MiSeq sequencing and amplicon analysis using R



Results

Non-metric multidimensional scaling (NMDS) analysis showed that bacterial and fungal composition at the phylum, class and genus level was significantly different over time (between being fed with pond water (t0) and being fed with spent lees (t1 and t2). Although there were differences between t1 and t2, these two groups remained close to each other in the ordination chart.

The alpha diversity metric (Chao1) showed that LECA at t0, both LECA and Gravel at t1 had statistically richer bacterial communities, but this difference disappeared at t2. On fungal diversity wise, gravel was slightly richer at t1 and t2.

As moving from t0 to t2, as long as the systems continued to be fed with spent lees, bacterial and fungal richness statistically decreased.

