Does *Melaleuca ericifolia* benefit from mycorrhizal inoculation in stormwater biofilters?

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Highlights

- Evaluated effects of mycorrhizal inoculation of *M. ericifolia* on pollutant removal and growth
- Preliminary analysis does not suggest mycorrhizae improves performance of this species
- More tree species should be tested under lab and field conditions

Introduction

Plant stress and death are common in stormwater biofilters (Browne et al. 2014), which can diminish water quality improvement (Henderson et al, 2007; Bratieres et al, 2008; Read et al, 2009) and amenity (Dobbie 2016). By supplying organic matter to biofilter media and directly taking up nutrients and metals, plants improve pollutant removal from stormwater runoff in these systems (Dagenais et al, 2018). When plants are subjected to environmental stressors, such as low plant-available water, stress can affect physiological processes and morphological characteristics which support biofilter functions (e.g., water uptake, exudate release, and leaf greenness and turgidity; Eziz et al, 2017; Mathur et al, 2019).

Vegetation growing in natural environments interacts with a diverse community of soil microorganisms which, among other things, facilitate uptake of nutrients and protect against environmental stressors. In engineered soils, such as biofilter media, our understanding of soil microbial communities is limited. Most studies on this topic are focused on how soil microbial communities affect pollutant removal (e.g., Chandrasena et al., 2017; Morse et al., 2018; Zuo et al., 2019). Mycorrhizae are a group of fungi that can infect plant roots to exchange nutrients, water, and carbohydrates. These fungi colonize more than 80% of terrestrial plants in natural environments, helping plants establish and persist where resources would be otherwise limiting. In particular, mycorrhizae can facilitate tolerance of drought in plants (Mathur et al, 2019). In stormwater biofilters, plants which are colonized by mycorrhizae may have increased growth, stress tolerance, and pollutant removal through higher photosynthetic efficiency, increased access to the soil micropores, and increased microbial activity in the rhizosphere, respectively (Palacios and Winfrey, 2020). Although we know mycorrhizae are found in stormwater biofilters (Winfrey et al, 2017), these roles have only just recently been studied (Palacios et al., 2021; Poor et al., 2018). These two studies have focused on pollutant removal, finding that nutrient (N and P) retention was improved in mycorrhizae-inoculated filter media. Both studies investigated native sedge species in northwest United States (Poor et al., 2018) and southeast Australia (Palacios et al., 2021).

Although many tree species which are common in biofilters have mycorrhizal associations, these studies did not include trees, which have growth requirements and life stages different to herbaceous plants. This study expands on the Palacios et al. (2021) study of Australian sedges by investigating the effects of mycorrhizae on pollutant removal and plant growth on a common biofilter tree species, *Melaleuca ericifolia*.

Methodology

A commercially available mycorrhizal inoculant was added to filter media growing *Melaleuca ericifolia* and dosed with synthetic stormwater in experimental columns. Water quality and plant heights were measured periodically. A dry phase was implemented to evaluate performance during drought stress.

Column setup

A total of twenty 10 cm diameter columns were constructed from a 60 cm length of PVC pipe. Outlets were raised to create a 15 cm deep saturated zone (within the gravel and coarse sand layers). The columns were filled with a 5 cm layer of gravel, a 10 cm layer of steam-sterilized coarse sand and sugar cane mulch mixture (5% v/v), and a 30 cm layer of steam-sterilized, triple-washed sand. These layers were evenly compacted throughout. All of these columns were planted with *M. ericifolia* which was grown from seed in sterilized soil. Two weeks prior to planting seedlings in columns, we added 500 mg of MycoGold™ (BioStim Pty Ltd, Queensland, Australia), a bioinoculant containing mycorrhizae and soil bacteria, to half of the seedlings following the supplier's instructions. Mycorrhizal colonization was confirmed in Palacios et al. (2021).

Data collection

Following a five-week plant establishment period in the columns, we dosed columns with 2.3 L of synthetic stormwater twice weekly to simulate the runoff entering a biofilter sized at 2% of its catchment area in Melbourne, Victoria, Australia (mean annual rainfall of 577 mm; Ambrose and Winfrey, 2015). This dosing schedule was followed for the entire experiment except during two dry periods, during which no water was added to the columns: a two-week and a five-week dry period. We collected composite water samples from the influent mixing tank and the column effluents during the regularly watered phases (2 sampling dates) and at the end of the two dry phases (2 sampling dates). Water samples were analysed for total nitrogen (TN), total phosphorus (TP), nitrate/nitrate (NO_x), ammonium (NH_4), and filterable reactive phosphorus (FRP) using flow injection analysis. We analyzed metals using an inductively coupled plasma mass spectrometry.

We also measured plant heights every week for the duration of the experiment. The height of each plant was measured from the surface of the filter media to the top of tree crown. We measured mycorrhizal colonization by staining roots in an ink and vinegar solution according to Vierheilig et al. (1998) and enumerating colonization extent using the gridline intersect method (Giovannetti and Mosse, 1980). Final dry biomass of above- and belowground parts were collected as well.

Data analysis

Pollutant removal was evaluated on the basis of percent reduction in concentration of individual pollutants and compared between treatments using Student's *t*-tests (α = 0.05). These tests were done for each sampling date separately. Plant growth was calculated from the change of plant height over time and compared visually in plots on each sampling date. Mycorrhizal colonization and plant biomass were also compared between treatments using Student's *t*-tests (α = 0.05).

Results and discussion

The results shown here have undergone preliminary analysis at this stage. Further statistical analysis will be completed in the coming months. For columns planted with *M. ericifolia*, nitrogen was exported in many cases while phosphorus was removed (Figure 1). There were minimal differences to nutrient removal when columns were inoculated contrary to prior reports on effects of mycorrhizae on nutrient removal by herbaceous species (Palacios et al., 2021). Similarly, metal removal was minimally affected by mycorrhizae in *M. ericifolia*, but we observed some differences in metal removal on some sampling dates (Figure 2).

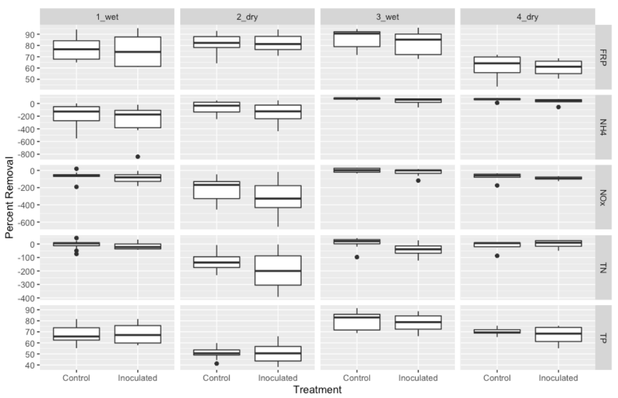

Figure 1. Nutrient removal percentages on each sampling date. Note: 1_wet and 2_dry refer to sampling dates before and after the 2-week dry period, respectively. 3_wet and 4_dry refer to sampling dates before and after the 6-week dry period, respectively. FRP = filterable reactive phosphorus, NH4 = ammonium, NOx = nitrate/nitrite, TN = total nitrogen, and TP = total phosphorus.

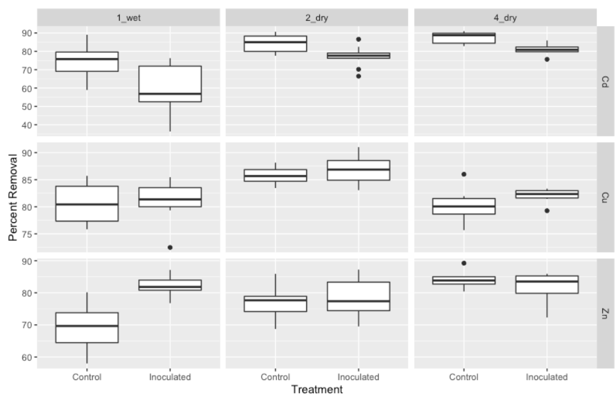

Figure 2. Metal removal percentages on each sampling date. Note: 1_wet and 2_dry refer to sampling dates before and after the 2-week dry period, respectively. 4_dry refers to the sampling date after the 6-week dry period. Samples were not analysed for metals on the date just before the 6-week dry period. Pollutant titles on the right (Cd, Cu, and Zn) refer to metals.

Plant growth rate was not significantly affected by mycorrhizal inoculation. In the companion study, mycorrhizae affected growth of one species, but not the other (Palacios et al., 2021). Consequently, this result does not disagree with the results from the companion study, but does not confirm that *M. ericifolia* receives clear benefits from mycorrhizal inoculation.

Conclusions and future work

Although further analyses of these data are needed, the benefits of inoculating *M. ericifolia* with mycorrhizae are not clear. Pollutant removal and growth rate were not significantly affected by inoculation. One species in the companion study was clearly improved from the perspective of higher pollutant removal and growth rate, so finding the right combination of bioinoculants and biofilter species composition is important before considering applying this in the field. Further studies may expand the species list of both plants and soil microorganisms while integrating DNA metagenome analysis to identify fungal and bacterial communities present in filter media.

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