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Arbuscular Mycorrhizal Fungi in Australian Stormwater Biofilters

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Highlights

- Mycorrhizae were found on plant roots of four species growing in stormwater biofilters in three Australian cities
- Mean annual rainfall and biofilter age had no significant effects on mycorrhizal colonization
- Presence of mycorrhizae on some biofilter plant roots suggests filter media conditions can support this plant-fungal relationship
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- **Abstract**

Stormwater biofilters are important tools for managing runoff in urban watersheds. To the authors' knowledge, there have been no accounts examining the presence of mycorrhizal fungi in biofilters. This plant-fungi relationship is an important interaction in most terrestrial ecosystems, playing a role in nutrient dynamics, water cycling, and soil organic matter decomposition. The presence of mycorrhiza in biofilters could have implications for nutrient and metal uptake in plants, and thus enhance removal of target pollutants. Additionally, the establishment, growth, and survivability of plants could be enhanced when roots are colonized by mycorrhizae. The aim of this study was to determine the extent of colonization by arbuscular mycorrhizal fungi in biofilters of varying ages in three Australian cities: Melbourne, Perth, and Sydney. The 32 biofilters surveyed supported 56 plant species, with dominant species belonging to the Cyperaceae, Iridaceae, Juncaceae, Onagraceae, Poaceae, and Xanthorrhoeaceae families. Mycorrhizal associations were identified from 4 of the 11 most dominant plant species from 9 different biofilters, but relatively low percentages of mycorrhizal colonization (3–25% colonization) were observed in biofilter plant roots. Mycorrhizal colonization was not related to biofilter age. These results demonstrate that mycorrhizal fungi colonize plant roots growing in biofilters. These findings provide useful evidence of the presence of mycorrhizal fungi in stormwater biofilters that support subsequent investigation into their roles in these systems.

- **Keywords**: stormwater biofilters, rain gardens, arbuscular mycorrhiza, water sensitive urban design, green infrastructure, urban ecology

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1. Introduction

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- Stormwater biofilters are ecologically engineered treatment systems composed of engineered filter media planted with species adapted to live in both wet and dry conditions. Managing urban stormwater runoff using biofiltration can provide multiple types of ecosystem services (e.g., carbon sequestration, water quality improvement, urban heat mitigation, provision of biodiversity, etc.) (Grant et al., 2012; Hatt et al., 2009; Lundy and Wade, 2011; Wong and Brown, 2009). Despite extensive research demonstrating their effectiveness with respect to hydraulic and pollutant removal (Bratieres et al., 2008; Davis, 2007; Davis et al., 2006, 2001; Hsieh and Davis, 2005) and the importance of plant species selection (Barrett et al., 2013; Bratieres et al., 2008; Payne et al., 2014; Read et al., 2008), particularly for nutrient removal, the provision of biodiversity and existence of specific plant-soil biological relationships (e.g., mycorrhizal colonization of biofilter plant roots) by green infrastructure systems (a.k.a., Water Sensitive Urban Design, Sustainable

Urban Drainage Systems, Low Impact Development, etc.) are rarely studied.

- Arbuscular mycorrhizal fungi (AMF) symbiotically grow with host plants by providing water and nutrients to plant roots in exchange for energy. AMF have hyphae that access crevices too small for plant roots, delivering nutrients to the plant root cortex via specialized organs called arbuscules (Brundrett, 2009). AMF are associated with more than two thirds of terrestrial plant families (Wang and Qiu, 2006) and provide plants with increased access to soil water (Duan et al., 1996) and growth-limiting nutrients (Smith and Read, 2008), resistance to soil pathogens (Newsham et al., 1995), and tolerance to heavy metals (Hildebrandt et al., 2007). Mycorrhizal colonization of plants in stormwater biofilters could therefore increase removal of nutrients and metals and plant survivability during prolonged dry periods. Since water retention capacity of typical filter media is low (Payne et al., 2015) in biofilters, AMF could provide access to interstitial water in the filter media that plant roots could not reach. This could be particularly important in areas with prolonged dry periods, such as Perth, WA, or in systems designed to exfiltrate to the underlying layers (i.e., no submerged zone or liner in place to retain moisture).
- John et al. (2014) evaluated the presence of mycorrhizae in green roof plants and provided guidance for selecting species with stronger mycorrhizal associations. Others have investigated the use of AMF inocula to improve heavy metal uptake in polluted soils; some studies indicate AMF-colonized plants had

increased heavy metal uptake (Liao et al., 2003; Whitfield et al., 2003) while others indicate decreased heavy metal uptake or no effect of AMF (Weissenhorn et al., 1995; Wu et al., 2007), suggesting the relationship between AMF and heavy metal uptake cannot be generalized (Weissenhorn et al., 1995). AMF have been detected in stormwater biofilter experimental columns, colonizing roots of *Melaleuca ericifolia* (Bratieres et al., 2008), but no information is available on studies presenting field observations of mycorrhizae in stormwater biofilters.

- Soils and/or growth media are typically inoculated with mycorrhizae for the purposes of improving crop yields (Jeffries and Rhodes, 1987; Menge, 1983; Sharifi et al., 2007), establishment and productivity of plants used in horticulture (Azcón-Aguilar and Barea, 1997; Maronek et al., 1981), and restoration of terrestrial ecosystems (Danielson, 1985; Miller and Jastrow, 1992; Turnau and Haselwandter, 2002; Zhang et al. 2012). Stormwater biofilters, consisting of engineered soil planted with shrubs and grasses, are essentially terrestrial ecosystems with disturbed soils; Miller and Jastrow (1992) discuss the use of mycorrhizae inocula to restore soil health and promote plant growth following disturbance. Consequently, the benefits of

mycorrhizae to establish plants in newly constructed biofilters could be significant (John et al., 2016). Plant cover in recently constructed systems depends largely on design parameters and varies from plants sparsely to completely covering the ground surface. However, it is unknown whether mycorrhizal colonization of biofilter plant roots occurs at all or persists over time.

- This study aims to observe the presence of mycorrhizae in stormwater biofilters in Australia to determine whether mycorrhizal colonization of biofilter plant roots is affected by regional climate and biofilter age. Biofiltration has been a popular strategy to promote urban water sustainability in Australia for the past decade. Many systems have been installed in Australian cities, particularly in Melbourne, Victoria during and following The Millennium Drought under the 10,000 Rain Gardens project (Melbourne Water, 2013). For this reason, Australian cities provide a large number of biofilters of differing ages in relatively close proximity. Differences in rainfall between cities also provide

opportunities to compare plants growing in biofilters located in different climatic conditions. Evidence of mycorrhizal colonization of biofilter plant roots could inform optimization studies whereby plant species that are found to be mycorrhizal in existing biofilters could be used to test the effects of their presence on biofilter performance and drought tolerance of plants.

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2. Methods

2.1. Biofilter Selection

- In each city, biofilters were chosen from a list of biofilters compiled from published accounts and personal communications with municipal officials. Biofilters were selected to represent a range of ages (2–14 yr), but maintain consistent design specifications.

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2.1.1. Rainfall Data

- Mean annual rainfall (MAR) for each site was determined using the average annual precipitation measured at the closest rain gauge operated by the Australian Government’s Bureau of Meteorology for the period of time between the year of construction of

the biofilter to the sampling date. When data were not available for that time period, rainfall data from the next closest rain gauge, which was never more than 10 km from the biofilter, was used.

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2.1.2. Biofilter Location Descriptions

2.1.2.1. Melbourne

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- On average, the twelve sampled biofilter sites in Melbourne, Victoria received MAR of 767 mm (Bureau of Meteorology, 2015) during the time between biofilter construction and sampling. Seasonally, rainfall was greater in winter months and lower in summer months; average monthly rainfall ranged from about 47 mm in January to 65 mm in October (Bureau of Meteorology, 2015). The selected study sites ranged in age (period of time between construction and date of sampling in October 2014) from 1.5 to 12 years. Median biofilter age and area were 3.4 years and 24 m^2, respectively.

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2.1.2.2. Perth

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- On average, the eleven sampled biofilter sites in Perth, Western Australia received MAR of 738 mm (Bureau of Meteorology, 2015) during the time between biofilter construction and sampling. Typical of Mediterranean climates, rainfall was very low in summer months, with most rainfall occurring during winter months; average monthly rainfall ranged from about 10 mm in January to 160 mm in June (Bureau of Meteorology, 2015). The selected sites ranged in age (period of time between construction and date of sampling in November 2014) from 1.5 to 9 years. Median biofilter age and area were 5.5 years and 200 m^2, respectively.

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2.1.2.3. Sydney

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- On average, the nine sampled biofilter sites in Sydney, New South Wales received MAR of 1316 mm (Bureau of Meteorology, 2015) during the time between biofilter construction and sampling. Although more rainfall occurred in winter months than in summer, rainfall was relatively abundant throughout the year, with average monthly rainfall

ranging from 70-80 mm in September-December to 130 mm in June (Bureau of Meteorology, 2015). These sites ranged in age (period of time between construction and date of sampling in November 2014) from 1.8 to 14 years. Median biofilter age and area were 5.3 years and 42 m^2, respectively.

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2.2. Plant Survey and Mycorrhizal Colonization of Plant Roots

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- We surveyed plant communities in each biofilter to determine dominant plant species by identifying plants to genera and species (where possible) in the field and visually estimating cover for the entire site. We collected photo vouchers for species we could not positively identify in the field. We used compared these photo vouchers to images on an online Australian plant guide (ANPSA, 2015) to identify plants to genera and species (where possible). For sites larger than 250 m^2, we randomly placed one 0.25-m^2 quadrat for every ~125 m^2 of biofilter, with the mean cover in the quadrats used to estimate plant cover.

- Plant roots were collected from the dominant plant species at each site. For each dominant plant species at any site, one sample was composited from filter media cores (cores) collected adjacent to 3–4 different individual plants of the same species. Cores were collected by driving a 2.5-cm diameter chromium-molybdenum steel soil probe to rooting depth (10 – 30 cm below soil surface) at the base of individual plants that were isolated (i.e., not surrounded by other plant species). Holes made by probes were filled in with fine sand and existing surrounding material. Root samples were stored at 4°C for less than 24 hours before filter media was hand-washed from roots through a 600-µm sieve.
- Subsamples (0.1–0.2 g dry weight) of washed roots were placed in a 10% (w/v) KOH solution in 20-mL scintillation vials and cleared in a water bath at 80°C for 1–12 hrs, until visibly transparent (Vierheilig et al., 1998). Cleared roots were stained using the ink and vinegar method based on Vierheilig et al. (1998); the 5% ink-vinegar solution consisted of 5% Sheaffer® Skrip® Jet Black pen ink and 95% distilled white vinegar (5% acetic acid) by volume. Roots were de-stained in distilled water containing a few drops of vinegar for 1 hr before being transferred to a 50% (v/v) lactic acid-glycerol solution for storage.

- Root samples were analyzed for mycorrhizal colonization using the gridline-intersect method (Giovannetti and Mosse, 1980). AMF features (arbuscules, vesicles, and hyphae) were observed first under a dissecting microscope at 40x magnification and then confirmed using a compound microscope at 100x magnification. While hyphae and vesicles indicate presence of AMF colonization, these structures may be present in non-mycorrhizal endophytic fungi (McGonigle et al., 1990; Brundrett, 2009). Although requiring all three structures to confirm mycorrhizal colonization likely limits the amount of samples that were described as mycorrhizal under this definition, requiring arbuscules ensures functional mycorrhizae (at time of sampling) were present and non-mycorrhizal, endophytic fungi were not mistakenly counted (McGonigle et al., 1990). Consequently, the presence of hyphae, vesicles, and arbuscules in root samples were required to confirm AMF colonization. Identifying fungal species was beyond the scope of this study.

2.3. Data Analyses

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- Statistical analyses were performed on samples where mycorrhizae were present. Due to the variable nature of mycorrhizal colonization of plant roots, we expected many of our samples would not be colonized by mycorrhizae. Dominant plants were selected for examination of mycorrhizae; we did not preferentially select plants we expected to be colonized by mycorrhizae. We analyzed these data for the effects of plant species, location, mean annual rainfall, and biofilter age for only those samples with observed mycorrhizal colonization. After confirming that the assumptions of normality and homoscedasticity were met, two one-way ANOVAs were used to test the effects of plant species or location of biofilter (by city) on percent mycorrhizal colonization of plant roots ($\alpha=0.05$). One-way ANOVAs were used because sample size was too small (i.e., too few replications of plant species colonized by mycorrhizae were present in more than one city) to test for interaction in a two-way ANOVA. Pearson's correlations were used to assess the relationship between percent mycorrhizal

colonization and mean annual rainfall and biofilter age (α=0.05). Statistical analyses were performed using R Statistical Software (R Core Team, 2015).

3. Results

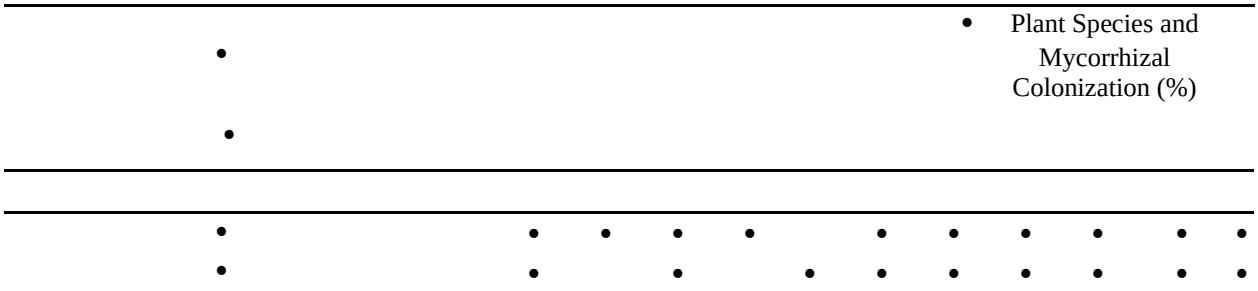
- Most biofilter plant species belonged to four families: Cyperaceae, Juncaceae, Poaceae, and Myrtaceae (Table 1). There were a total of 56 species in 19 families across the surveyed biofilters, with 12 species and 11 families present in biofilters in more than one city (Table 1). There were 30, 24, and 19 plant species in Melbourne, Perth, and Sydney biofilters, respectively. Dominant plant species belonged to seven families: Cyperaceae, Iridaceae, Juncaceae, Onagraceae, Poaceae, Scrophulariaceae, and Xanthorrhoeaceae.

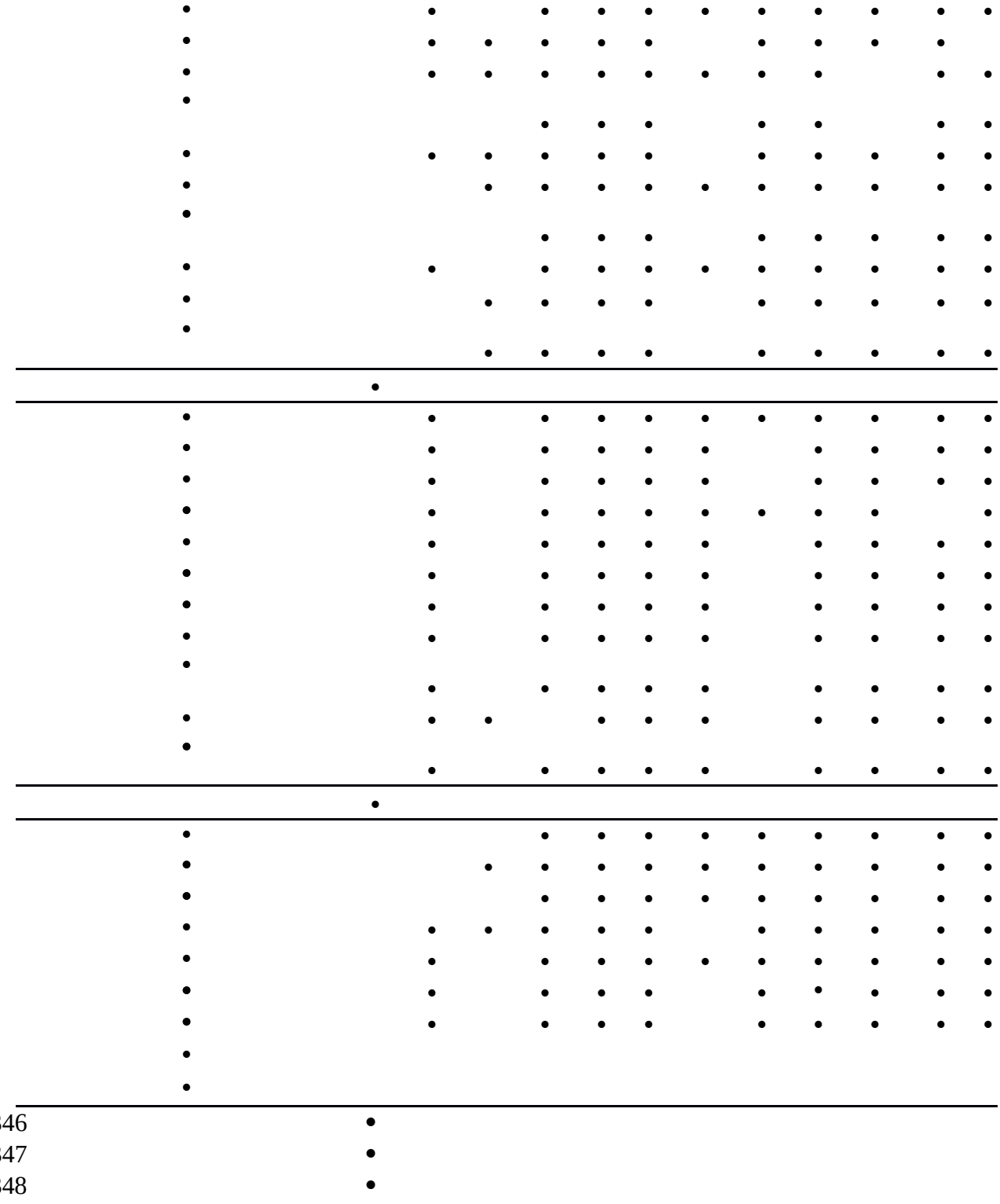
- Table 1. Plant species list for all sampled biofilters. Melbourne, Perth, and Sydney biofilters contained a total of 30, 24, and 19 species, respectively. Presence of plant species in city is designated by “x”.

Species Name		

Eleven of the 56 species found in the plant survey were dominant and thus sampled for mycorrhizal colonization (Table 2). A total of 54 root samples were collected, representing the 1–4 dominant plant species present at each site. Of those 11 dominant plant species, four showed evidence of mycorrhizae at nine different sites, with four each in Perth and Sydney and only one in Melbourne (Table 2). There was no significant relationship between city and mycorrhizal colonization ($p = 0.97$). Mycorrhizae colonized roots from three genera– *Ficinia*, *Carex*, and *Juncus*. Of those nine root samples colonized by mycorrhiza, vesicles, hyphae, and arbuscules were visible (Figure 1) and extent of mycorrhization ranged from 3–25% of the root length colonized (Table 2). There was no significant relationship between plant species and mycorrhizal colonization ($p = 0.37$).

Table 2. Mycorrhizal colonization of the dominant plant species at all sites. 0 indicates species was present but no colonization was detected. Boldface type denotes biofilter sites with mycorrhizal colonization. Plant species name label are *CA= *Carex appressa*; FN= *Ficinia nodosa*; GT= *Gahnia trifida*; GL= *Gaura lindeimeri*; IS= Iris sp.; JF= *Juncus flavidus*; JK= *Juncus krausii*; LH= *Lomandra hystrix*; LL= *Lomandra longifolia*; MP= *Myoporum parvifolium*; PL= *Poa labillardieri*





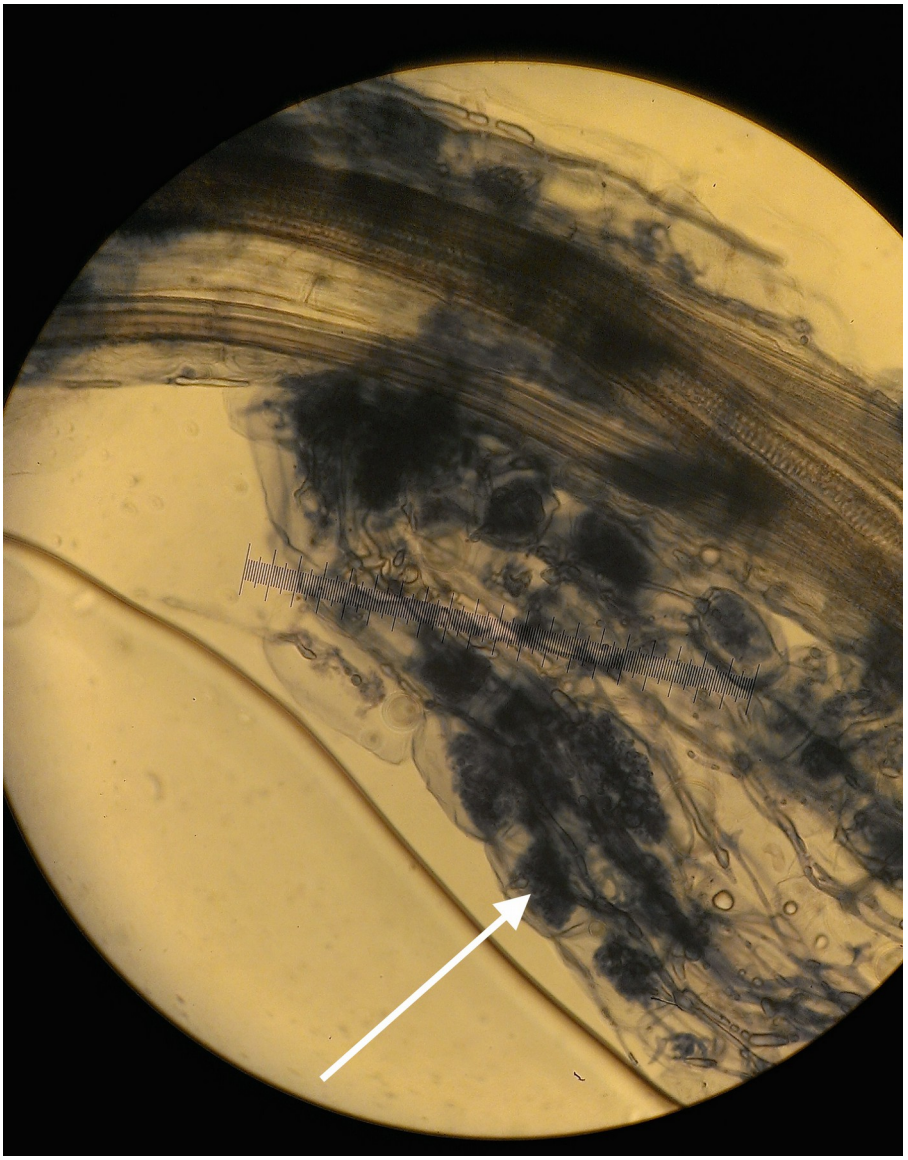


Figure 1. Photograph of *Juncus flavidus* root colonized by arbuscular mycorrhizal fungi at 100x magnification. Arrow points to stained arbuscule in root cell.

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355 • The average age of all sampled biofilters was 5 years at time of data
356collection. Those biofilters containing plants with mycorrhizae averaged 4 years and
357ranged from 1–9 years at the time of data collection (Table 2). The relationship between
358biofilter age and mycorrhizal colonization was not significant ($r = -0.44$, $p > 0.05$).
359However, non-significance could be due to poor power from relatively few samples, since
360the regression line suggests plant roots growing in older biofilters may have lower
361colonization by mycorrhizae (Figure 2). Additionally, we found no significant relationship
362between mean annual rainfall (MAR) at biofilter locations and mycorrhizal colonization (r
363$= 0.33$, $p > 0.05$). Average MAR for biofilters with plants that had mycorrhizal
364colonization was 1,042 mm in Melbourne, 787 mm in Perth, and 1,302 mm in Sydney
365(Table 2).

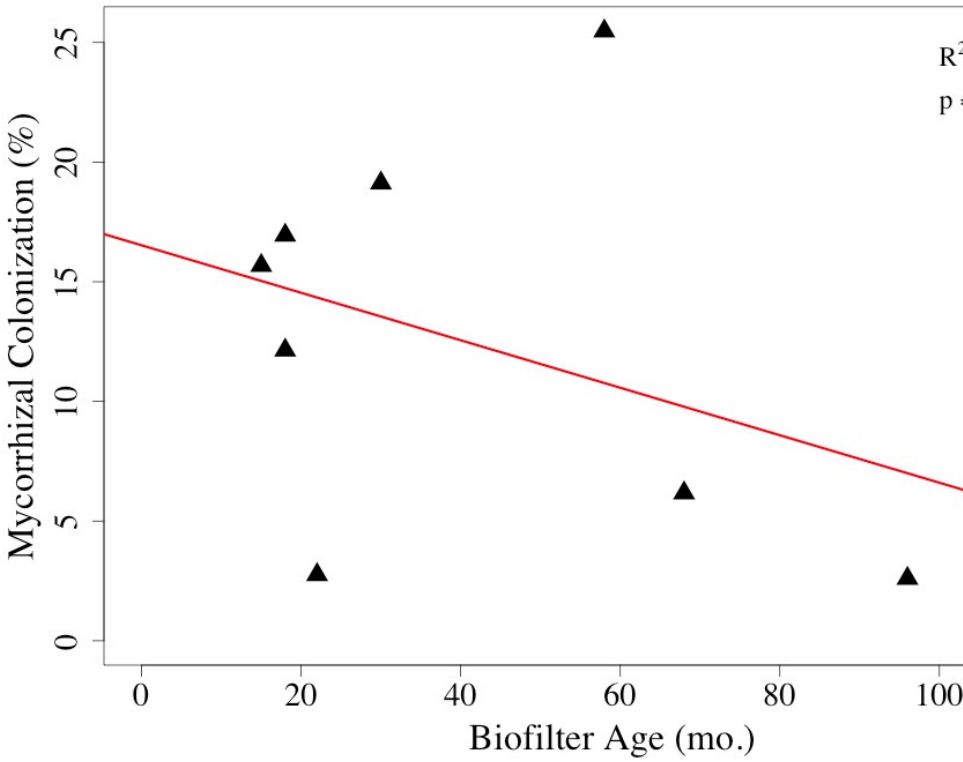

Figure 2. Mycorrhizal colonization of plant roots in biofilters of various ages.

4. Discussion

- Engineered soil used in biofilters would likely not contain natural communities of soil microorganisms, so it is not surprising that most of the sampled plant roots in this study were non-mycorrhizal (NM). Interestingly, only one of the plant species exhibiting mycorrhization in this study, *Ficinia nodosa*, was

previously documented as being mycorrhizal (Logan et al., 1989). To the authors' knowledge, the other three plant species found to have AMF colonization in this study (*Carex appressa*, *Juncus flavidus*, and *J. kraussii*) have not been previously designated as mycorrhizal. Many species that have been previously described as NM are not necessarily unsusceptible to colonization, but can be found growing in disturbed soils where mycorrhizal colonization is rare (Tester et al., 1987).

- Although Perth biofilters received runoff from areas with overall less rainfall than Melbourne and Sydney biofilters, there was no effect of mean annual rainfall on mycorrhizal colonization. Compared to Perth, both Melbourne and Sydney precipitation is more evenly distributed throughout the year. More plants in Perth biofilters were observed with mycorrhizal colonization than in Melbourne biofilters (Table 2), but the extent of colonization appears not to have been affected by MAR. Plant species adapted to wetlands are typical in biofilters and composed many of the species observed here. These species can develop mycorrhizal associations in dry conditions, typical of Perth biofilters, to a greater extent than in wet conditions (Rickerl et al., 1994). Sydney biofilters receive roughly twice the precipitation (and likely runoff) of Perth biofilters with rainfall distributed more evenly throughout the year. No patterns with rainfall were detected in our data, possibly due to low sample size (n = 9).

- Only one plant's roots in the sampled Melbourne biofilters, *Juncus flavidus*, were colonized by AMF. This species' roots were also colonized by AMF to a lesser extent in one of the sampled Sydney biofilters (Table 2). Generally, species in the Juncaceae family are NM, but some exceptions do exist (Brundrett, 2009). Another *Juncus* species, *J. kraussii* (syn. *J. maritimus*), present in most Perth biofilters, contained roots colonized by AMF despite being previously designated as NM (Harley and Harley, 1987; Maremmani et al., 2003). Habitat factors, such as saline and dry soil conditions, can affect AMF colonization on roots of species typically described as non-mycorrhizal, particularly in families containing species growing in harsh environments and with diverse growth forms, such as Cyperaceae and Juncaceae (Brundrett, 2009). In this study, all species found to contain AMF on roots were in these two families.
- *Carex* species are generally described as NM, but more species in this genus of sedges are currently being described as facultative mycorrhizal (Miller et al., 1999). In this study, one of the seven *Carex* sp. root samples was colonized by AMF. *Ficinia nodosa* was found to be mycorrhizal in four of the twenty *F. nodosa* root samples. *Juncus* spp. root samples were mycorrhizal in four of the seventeen *Juncus* spp. root samples. Overall, only 17% of root samples contained mycorrhizae. In contrast, mycorrhizal colonization occurred in roughly half of green roof

plant roots studied by John et al. (2014), which included forbs, grasses, and succulents. Like stormwater biofilters, green roofs are ecologically engineered ecosystems containing engineered soil-like media and planted with drought-tolerant plant species. Stormwater biofilters would likely contain more pathways for immigration of AMF spores than green roofs due to their position on the landscape (i.e., lower elevation and receiving runoff from overland flow, following MacIvor and Lundholm, 2010). In addition, spores of AMF can spread effectively via faunal vectors (John et al., 2014; Kotter and Farentinos, 1984; McGee and Baczocha, 1994; McIlveen and Cole Jr., 1976; Ponder, 1980), favoring spore distribution to lower elevations in an urban landscape rather than rooftops. Despite this, we found plant roots growing in stormwater biofilters were less often colonized than those previously reported in green roofs.

- Australian guidelines for biofilter media suggest using low nutrient content media (FAWB, 2008), so newly constructed biofilters are often oligotrophic. Older biofilters tend to accumulate organic matter and phosphorus in the top 10 cm (Payne et al.,

2015), where most roots are located. Sáinz et al. (1998) found adding nutrient-rich compost to agricultural soils inhibited mycorrhizal colonization of plants' roots. Thus, we expect plants would most likely benefit more from mycorrhizal colonization when biofilters are young and nutrient-poor and benefit less when they are older and contain more nutrients. This study detected no significant relationship between biofilter age and mycorrhizal colonization, with the extent of mycorrhizal colonization being low in plants growing in both young and old biofilters. However, we expect newly planted specimens could contain residual mycorrhizae from inoculant added in nurseries; consequently, younger biofilters may host plants with higher mycorrhizal colonization. In older biofilters, after mycorrhizal inoculation has had time to occur naturally, low prevalence of mycorrhizal colonization might be due to higher nutrient and organic matter accumulation in the filter media.

- If mycorrhizal associations are found to confer the same types of benefits (e.g., increased plant nutrient uptake and water uptake efficiency) in biofilter plants as have been reported in other terrestrial habitats and potentially in green roofs (John et al., 2016), then inoculation of biofilters following construction might enhance ecosystem service provision by biofilters. In order to determine whether inoculation of biofilter plants with mycorrhizae will increase colonization of biofilter plant roots, mesocosm experiments should be

conducted in typical biofilter conditions on appropriate plant species. If colonization is successful, effects on nutrient and metal uptake, plant drought tolerance and survivability, and carbon storage in filter media should be examined. Additionally, field experiments could be undertaken to determine the effectiveness of inoculating biofilters with mycorrhizae *in situ* and evaluating the resulting colonization and plant health over time. While this study did not show any correlation between mycorrhizal colonization of biofilter plant roots and biofilter age, rainfall, or plant species, the observances of mycorrhizae colonizing some biofilter plant roots suggests this relationship should be further explored to understand the roles of mycorrhizae in biofilters.

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