

University of Portland Pilot Scholars

Engineering Faculty Publications and Presentations

Shiley School of Engineering

6-2018

The Role of Mycelium in Bioretention Systems: Evaluation of Nutrient Retention in Mycorrhizae- inoculated Mescocosms

Cara J. Poor

University of Portland, poor@up.edu

Casey Balmes

Michael Freudenthaler

Ashley Martinez

Follow this and additional works at: https://pilot scholars.up.edu/egr_facpubs

 Part of the [Bioresource and Agricultural Engineering Commons](#)

Citation: Pilot Scholars Version (Modified MLA Style)

Poor, Cara J.; Balmes, Casey; Freudenthaler, Michael; and Martinez, Ashley, "The Role of Mycelium in Bioretention Systems: Evaluation of Nutrient Retention in Mycorrhizae-inoculated Mescocosms" (2018). *Engineering Faculty Publications and Presentations*. 50.

https://pilot scholars.up.edu/egr_facpubs/50

This Journal Article is brought to you for free and open access by the Shiley School of Engineering at Pilot Scholars. It has been accepted for inclusion in Engineering Faculty Publications and Presentations by an authorized administrator of Pilot Scholars. For more information, please contact library@up.edu.

1 **THE ROLE OF MYCELIUM IN BIORETENTION SYSTEMS: EVALUATION OF**
2 **NUTRIENT AND METALS RETENTION IN MYCORRHIZAE-INOCULATED**
3 **MESOCOSMS**

4 Cara Poor, PhD, PE¹, Casey Balmes², Michael Freudenthaler³, and Ashley Martinez⁴

5 ¹Assistant Professor, University of Portland, Shiley School of Engineering, 5000 N. Willamette
6 Blvd. Portland, OR 97203, email: poor@up.edu

7 ²Undergraduate Research Assistant, University of Portland, Shiley School of Engineering, 5000
8 N. Willamette Blvd. Portland, OR 97203, email: balmes17@up.edu

9 ³Undergraduate Research Assistant, University of Applied Sciences Upper Austria, Department
10 of Biotechnology and Environmental Engineering, Franz-Fritsch-Straße 11, 4600 Wels Austria,
11 email: MichaelJan.Freudenthaler@students.fh-wels.at

12 ⁴Undergraduate Research Assistant, University of Portland, Shiley School of Engineering, 5000
13 N. Willamette Blvd. Portland, OR 97203, email: martiash17@up.edu

14
15 **Abstract**

16 Bioretention systems have become an increasingly common method for treating stormwater in
17 urban areas, which help reduce peak flows and remove contaminants from stormwater. However,
18 nutrients often leach out of the bioretention soil mix, which can contribute to the degradation of
19 receiving waters in bioretention systems with underdrains. Development of mycelium may
20 improve retention of nutrients, as well as increase the water holding capacity. To evaluate the
21 impact of mycelium on nutrient leaching from bioretention systems, ectomycorrhizal and
22 endomycorrhizal fungi were added to the bioretention soil mix to promote mycelium growth. A
23 proprietary mix with bacteria and mycorrhizal fungi was also tested. Mesocosms were planted
24 with *Carex stipata*, a native sedge with endomycorrhizal associations. Four tests were conducted
25 with collected stormwater. Lower rates of phosphorus export were observed in mesocosms with
26 mycorrhizal fungi; the export of total phosphorus was reduced by 13-48%, and the export of
27 phosphate was reduced by 14-60%. There was also evidence of additional copper and nitrate
28 uptake in mesocosms with mycorrhizal fungi. Retention of total phosphorus and phosphate,
29 rather than export, was observed in mesocosms with the proprietary mix, but export rates of

30 nitrate were high. This study indicates that mycelium may help reduce phosphorus export from
31 bioretention systems.

32 **Introduction**

33 As urban areas grow, stormwater is increasingly disconnected from the natural hydrologic cycle
34 due to impervious areas and hard piping to receiving waters. This not only increases peak flows
35 and stormwater volumes, but also increases the pollutant loading to rivers and streams (Maestre
36 et al. 2004; EPA 2000). High levels of nitrogen or phosphorus can lead to algal blooms, which
37 can deplete dissolved oxygen and cause dead zones for fish (National Research Council 2000),
38 and elevated copper and zinc can negatively impact fish (Brandstetter et al. 2014a; Brandstetter
39 et al. 2014b). To alleviate this problem, many cities in the US have implemented sustainable best
40 management practices (BMPs) that promote stormwater infiltration, which slows runoff and
41 reduces pollutant loading. Bioretention is a common BMP where stormwater flows through a
42 vegetated area and engineered soil mix (EPA 1999). Pollutants such as metals and nutrients are
43 removed from stormwater via physical filtration, sorption, plant uptake, and microbial reactions.
44 In some areas, infiltration of stormwater into the native soil is not feasible due to low infiltration
45 rates, high groundwater levels, or soil contamination. In these cases, bioretention systems are
46 lined and an underdrain is used to convey stormwater to receiving waters.

47
48 Results have been mixed for both nutrient and metals retention in bioretention systems, largely
49 due to variations in bioretention soil mixes. Some studies have shown significant reduction of
50 metal concentrations in stormwater with bioretention (Sun and Davis 2007; Blecken et al. 2009;
51 Davis et al. 2003; Leisenring et al. 2014; Clary et al. 2017), whereas other studies have found
52 that bioretention can act as a source of copper, exporting more copper than what was originally
53 in the stormwater (Trowsdale and Simcock 2011; Li and Davis 2009; Herrera, 2014).
54 Bioretention systems have been shown to act as a source and a sink of nitrogen and phosphorus.

55 Many studies have shown good removal of both phosphorus and nitrogen (Davis et al. 2006;
56 Lucas and Greenway 2008; Li and Davis 2014; Palmer et al. 2013; Clary et al. 2017), while
57 others have shown an export of nitrate, phosphate, and total phosphorus (Davis et al. 2014; Li
58 and Davis 2014; Herrera 2015; Mullane et al. 2015; Leisenring et al. 2014; Clary et al. 2017).
59 The source of copper, phosphorus, and nitrogen is likely the compost used in the bioretention
60 soil mix (Mullane et al. 2015; Hurley et al. 2017; Li and Davis 2009; Paus et al. 2014). Compost
61 comes from many different sources, and many suppliers and contractors do not qualify compost
62 before blending in the bioretention soil mix. The presence of a saturation zone can remove nitrate
63 via denitrification; Palmer et al. (2013) found that up to 71% of nitrate can be removed from
64 stormwater when the gravel layer is used as a saturation zone. However, phosphorus export
65 increases with a saturated zone (Hurley et al. 2017; Palmer et al. 2013). Some municipalities
66 have replaced compost with shredded bark or wood fiber mulch in the bioretention soil mix
67 (North Carolina DEQ 2017; New Hampshire DES 2008; Maryland DOE 2009). In areas with an
68 extended dry summer period, such as the Western US, compost is needed to increase the water
69 holding capacity for plant survival. More research is needed to revise bioretention soil mix
70 standards as well as to determine how to retain phosphate, phosphorus, nitrate and copper in
71 bioretention systems and minimize leaching in regions with extended dry periods, particularly
72 where an underdrain conveys stormwater to receiving waters.

73

74 Increased uptake by plants via mycorrhizal fungi may help improve retention of phosphorus and
75 copper. Vegetation and increased microbial processes have been shown to increase the retention
76 efficiency of both phosphorus and nitrogen (Lucas and Greenway 2008). Plant uptake of metals
77 is typically small compared to other mechanisms because of the small amount typically needed

78 by most plants. Sun and Davis (2007) found that the majority of metals (88-97%) were captured
79 in the soil media, and a small amount (0.5-3.3%) were captured in the plants. Muthanna et al.
80 (2007) found that 2-7% of metals were captured in plants. Mycorrhizal fungi may increase plant
81 uptake and microbial reactions, in addition to improving general soil health. Mycorrhizal fungi
82 form associations with the roots of plants, which can increase nutrient uptake for the plant and
83 provide a carbon source for the fungus (Smith and Read 2008). The two most common types of
84 mycorrhizae are endomycorrhizae, where the fungus penetrates the plant roots to exchange
85 nutrients, and ectomycorrhizae, where the fungus wraps around the plant roots and nutrients are
86 transported through cellular walls (Singh 2006). Nutrient exchange occurs between the mycelium,
87 or hyphal network of the fungus, and the roots of the plant. Both endomycorrhizal and
88 ectomycorrhizal fungi can accumulate metals in the hyphal network (Singh 2006). The mycelium
89 of endomycorrhizal fungi have been found to absorb phosphorus and zinc more efficiently than
90 plant roots alone (Smith and Read 2008), and the presence of mycorrhizal fungi increased
91 phosphorus uptake in wheat (Li et al. 2006). To our knowledge, there have been no formal
92 evaluations of the benefits of mycorrhizal fungi in bioretention systems to date. Corkidi et al.
93 (2011) found a significant reduction in nutrient leaching from nursery containers as a result of
94 the addition of mycorrhizal fungi. Although nursery containers have a different soil blend and
95 microbial community than bioretention systems, the Corkidi et al. (2011) study shows there is
96 potential for similar reductions in bioretention systems. Winfrey et al. (2017) found 3-25% of
97 plants had mycorrhizal associations in nine different biofilters. If these existing associations can
98 be increased, mycorrhizal fungi may increase phosphorus and copper uptake and decrease
99 leaching from bioretention systems.

100

101 To evaluate the effectiveness of improving uptake of nutrients and metals using mycorrhizal
102 fungi, mesocosm studies were conducted. Mesocosms with mycorrhizal fungi added to a
103 standard bioretention soil mix, a control bioretention soil mix without mycorrhizal fungi, and a
104 proprietary mix that includes bacteria and fungi were tested. All mesocosms had a saturated zone
105 to allow for denitrification. Four tests were conducted with stormwater collected from a nearby
106 parking lot. Influent and effluent samples were collected and analyzed for total copper, total zinc,
107 total nitrogen, ammonia, nitrate, total phosphorus, and phosphate.

108

109 **Methods**

110 **Mesocosm Assembly.** Nine 30.5-cm diameter, 97.5-cm tall mesocosms were built with
111 polyvinyl chloride (PVC) piping mounted on a PVC base plate for stability (Figure 1). A riser
112 pipe with a valve 30.5 cm above the bottom of the mesocosm was used to create a saturated zone
113 in the gravel layer. A slotted 1.9-cm diameter PVC pipe was installed at the bottom of the
114 mesocosm and connected to the riser pipe. The slotted pipe was used to allow the stormwater to
115 freely drain without clogging with gravel. Mesocosms were sanded to increase roughness and
116 prevent preferential flow along the sides of the mesocosms.

117

118 Two types of soil media were used; the bioretention soil mix (BSM) specified by the City of
119 Portland and a proprietary mix called Earthlite™ BioSwale ES Soil provided by Sunmark
120 Environmental. The City of Portland BSM is 30-40% compost and 60-70% sand, with a fines
121 content of 5-15% in the final blend (City of Portland, 2016a). For the control and mycorrhizae-
122 inoculated columns, soil from the same truckload was used to ensure uniformity. The proprietary
123 mix is 33% compost, 60% sandy clay loam, 6% biochar, and 1% PermaMatrix® Biotic Particles

124 (BSP) (Sunmark Environmental, 2014). PermaMatrix® BSP is a blend of organic material,
125 bacteria, and mycorrhizal fungi (Permamatrix, Inc. 2016). The major difference in the mineral
126 portions of these two soil mixes is the clay and biochar in the proprietary mix. The BSM does
127 not contain clay or biochar.

128

129 As shown in Figure 1, 7.5 cm of river rock was placed at the bottom of the column to protect the
130 drain and prevent clogging. A 23-cm layer of ¾ -inch minus gravel was placed on top of the river
131 rock. The gravel was flushed with tap water until the effluent water ran clear (approximately
132 56.8 L) to rinse all fines. The soil was added in three 20.3-cm increments and compacted by hand
133 until firm. The mesocosms were then saturated with water and the flowrates were measured from
134 the fully open upper valve using a graduated cylinder and stop watch. For mesocosms with
135 flowrates greater than 21 L/hr, tap water was run through the soil in 11.4-L increments to allow
136 the soil to settle and compact until the flowrates equalized. Tap water was used to mimic the
137 typical construction process and first year irrigation of bioretention systems.

138

139 *Carex stipata*, commonly known as sawbeak sedge or awlfruit sedge, was chosen because it is
140 native to Oregon and is commonly used in bioretention systems (City of Portland, 2016b). The
141 Carex family has a large root structure that aids in nutrient uptake, and tolerates saturated and
142 dry conditions (Bratieras et al. 2008). *Carex stipata* also possesses endomycorrhizal associations
143 (Muthukumar et al. 2004), making the plant ideal for use in this study. Because the diameter of
144 the mesocosms was 30.5 cm, only one plant per mesocosm was used. The plants were purchased
145 from the same nursery and were selected to maximize uniformity of size and characteristics.
146 Both endomycorrhizal and ectomycorrhizal fungi were used in the columns. Three of the

147 columns were inoculated with MycoApply Endo/Ecto and MycoApply Ultrafine Endo from
148 Mycorrhizal Applications, Inc., and three of the columns had the proprietary mix which includes
149 endomycorrhizal and ectomycorrhizal fungi. The same fungal species were in both the
150 MycoApply mix and proprietary mix, and are listed in Table 1. These fungi are commonly
151 recommended for use in bioretention systems. The MycoApply products do not contain
152 additional organic matter, whereas the PermaMatrix® BSP does.

153 The nine columns were assembled with the following variations:

- 154 • 3 control columns with BSM only
- 155 • 3 columns with mycorrhizae-inoculated BSM
- 156 • 3 proprietary soil columns

157

158 The mycorrhizal fungi and plants were added in two steps. First, 36 grams of MycoApply
159 Endo/Ecto was mixed with the top 15.2 cm of soil in three of the mesocosms as recommended by
160 the vendor. Then, a slurry was made using approximately 20 mL of tap water and 36 grams of
161 MycoApply Ultrafine Endo. The roots of the *Carex stipata* plants were dipped into this slurry to
162 inoculate the plant roots, and the roots were covered with soil. It is possible that the addition of
163 36 grams of material could impact the comparison between columns inoculated with
164 mycorrhizae and the control columns, but it is a very small amount (<1% of the total mass of
165 soil) and impacts are likely minimal. The mesocosms were watered with tap water as necessary
166 to keep the soil moist and underwent a 60-day establishment period prior to testing. Although
167 chlorine may impact microorganism survival and growth, tap water is typically used for
168 irrigation of bioretention plants during the first year or two after construction. Tap water was
169 used during the establishment period to mimic that process.

170

171

172 **Experiments.** The columns were placed in a green house to control environmental conditions.
173 Stormwater was collected from a catch basin on the University of Portland campus. A parking
174 lot with an approximate area of 1540 m² drains to the catch basin. The parking lot serves
175 students, faculty, and visitors, and is often full during the day. Stormwater collection occurred
176 after it rained for at least an hour to ensure collected stormwater was from the current storm and
177 not the previous storm. Stormwater was stored in rain barrels for 1-2 months until tests were
178 conducted.

179

180 Four tests were conducted on all of the columns. At the beginning of each test, the stormwater
181 was mixed by vigorously shaking the rain barrel and an influent sample was taken directly from
182 the rain barrel. During each trial, 21 L (equivalent to half a bed volume) of stormwater was
183 applied to each column from 25-L, polypropylene stormwater containers. Volume was
184 determined using the rational method and the City of Portland water quality design storm (2.1 cm
185 or 0.83 inches), which is the 6 month 24-hour storm, a drainage area ratio of 15:1, and a runoff
186 ratio of 0.9 (City of Portland, 2016a). Runoff was applied at a rate to maintain 5 cm of ponding,
187 and was controlled using a ball valve on the stormwater container. The valve was connected to
188 flexible tubing that terminated at the top of the column. At the end of the flexible tubing, a flow
189 spreader was created by drilling holes in the last 4 cm of tubing and plugging the end of the tube
190 so water would exit out of the holes. The flow spreader was created to minimize channelization
191 and evenly distribute stormwater over the mesocosm surface area. To achieve the desired flow
192 rate, the valve was slowly opened and flow rate measured using a graduated cylinder and

193 stopwatch. When the desired flow rate was achieved, the degree the valve was open was noted
194 and used for all tests. Effluent was collected in a polypropylene container located under the
195 outflow valve of each column. When the flow rate from each mesocosm was no longer
196 measurable or essentially zero, a 250-mL composite sample was taken from the container and the
197 pH and total effluent volume were measured. Flow rate exiting each mesocosm was measured
198 using a graduated cylinder and stopwatch. Average exfiltration rates, or the flow rate per cross
199 sectional area exiting the column, was then calculated by dividing the volumetric flow rate by the
200 cross-sectional area of the mesocosm. Test duration was approximately 3 hours, and tests were
201 conducted at least one week apart. A calibrated Hach HQ30D probe was used to measure pH,
202 and a Hach 2100Q turbidimeter was used to measure turbidity. All sample containers and
203 glassware used during testing and sample analysis were acid washed, and samples were
204 preserved and stored according to Standard Methods (Rice et al. 2012).

205
206 Samples were analyzed for zinc, copper, total nitrogen, nitrate, ammonia, total phosphorus, and
207 phosphate. Nutrients were analyzed using Hach kits in accordance with *Standard Methods*
208 Section 4000: Inorganic Nonmetallic Constituent (Rice et al. 2012). The persulfate method was
209 used to quantify total nitrogen and phosphorus, and the colorimetric method was used to quantify
210 inorganic constituents. Zinc and copper were analyzed with a Shimadzu AAS-7000 in
211 accordance with *Standard Methods* Section 3000: Metals (Rice et al. 2012). The average and
212 standard deviation of the three replicates were calculated for each test. The Wilcoxon signed
213 rank test (Helsel and Hirsch, 2002) was used to determine whether there was a significant
214 difference between the proprietary mix, mesocosms inoculated with mycorrhizae, and control
215 mesocosms. This test is commonly used for studies with small sample sizes for comparison

216 between two treatments.

217

218 **Plant Characterization.** After testing was complete, one plant from each variation (control,
219 inoculated, and proprietary) was carefully removed from the column. Aboveground biomass,
220 belowground biomass, and root length were measured after drying in an oven for 48 hours.
221 Organic matter was also measured following ASTM Standard D 2974-87. Because we plan to do
222 additional testing, we chose to dismantle only one column for characterization. The plants in
223 columns with the same variations were all similar in size, so would likely have similar
224 measurements.

225

226 **Leach Tests.** Leach tests on both the BSM and the proprietary soil were conducted to determine
227 whether the soils are a source of nutrients and/or metals. A subsample of the soil was set aside
228 before columns were assembled. EPA Method 1312, Synthetic Precipitation Leaching Procedure
229 (SPLP), was followed. Leachate was then analyzed for the same constituents analyzed in the
230 influent and effluent samples.

231 **Results and Discussion**

232 Average exfiltration rates varied from 9.3-18.9 cm/hr in the mesocosms with the BSM and 18.0-
233 25.2 cm/hr in the mesocosms with the proprietary soil (Table 2). Because the variation in
234 exfiltration rates may impact concentrations, a mass rate (mg/hr) was used for comparison
235 purposes. Future studies should include an orifice to ensure a uniform flow rate in all
236 mesocosms. Belowground biomass, aboveground biomass, root length, and blade length were all
237 higher for the plants inoculated with mycorrhizae and the proprietary soil (Table 3). Organic
238 matter content was slightly lower in the control, which may be due to the higher biomass and

239 mycorrhizal presence. Because the same batch of soil was used when assembling the columns
240 with the City of Portland BSM (and thus would have the same or very similar initial proportion
241 of organic matter), the only possible sources of additional organic matter are dead plant roots
242 and/or mycorrhizae. The higher level of organic matter in the proprietary mix could also be due
243 to the additional organic matter from the PermaMatrix® BSP. Although we did not directly
244 measure the extent of mycorrhizal colonization, root nodules and ectomycorrhizal hyphae
245 embedded in bark pieces were observed. The increase in root length, aboveground and
246 belowground biomass in addition to visual observations of mycorrhizal presence indicate it is
247 highly likely that mycorrhizal colonization occurred in the columns that were inoculated.

248
249 **Copper and Zinc.** Mass rates of copper in the effluent from mesocosms inoculated with
250 mycorrhizae were significantly lower than the control ($p < 0.025$) and the proprietary soil
251 ($p < 0.05$) (Figure 2). Average mass rates from the mesocosms inoculated with mycorrhizae,
252 control, and proprietary soil were 35.2, 78.3, and 97.8 $\mu\text{g/hr}$, respectively, and median mass rates
253 were 36.8, 63.9, and 91.5 $\mu\text{g/hr}$, respectively. This indicates the mycorrhizal fungi may increase
254 uptake of copper, although the mass rate from the proprietary soil, which contains mycorrhizae,
255 did not exhibit significantly higher uptake compared to the control. Copper could be binding to
256 organic matter, but the proprietary soil had higher organic matter content and lower uptake of
257 copper compared to the mesocosms inoculated with mycorrhizae. The lower uptake in the
258 proprietary mesocosms may be due to the higher exfiltration rates; exfiltration rates were 18.0-
259 25.2 cm/hr in the proprietary mesocosms and 9.3-18.9 cm/hr in the BSM mesocosms. The lower
260 contact time in the proprietary mesocosms may have impacted copper uptake and retention. Mass
261 rates of copper in the effluent were statistically the same for the control and the proprietary soil.

262 Retention of copper was similar in all mesocosms, but variable during each test and ranged from
263 18-94%. Average retention was 50% and the median was 45%, which is lower than observed in
264 other studies (Sun and Davis 2007; Blecken et al. 2009). Sun and Davis (2007) observed an 87%
265 decrease in copper concentrations in the effluent, and Blecken et al. (2009) observed a 67-99%
266 decrease. Low removal rates in this study are likely due to the low influent concentration;
267 average influent concentration was 12.5 µg/L. Mass rates increased from tests 1 to 3, then
268 decreased during test 4, which may have been due to retention/release mechanisms occurring
269 between each test. Removal was 87% and 94% during test 4 in the control and mycorrhizae-
270 inoculated mesocosms, respectively. The highest removal in mesocosms with the proprietary soil
271 was 64% during test 2. Leach tests indicated a relatively small amount of copper in the BSM (3.1
272 mg/kg) and the proprietary soil (0.54 mg/kg) compared to typical copper concentrations in soil,
273 which range from 5-70 mg/kg (ATSDR, 2004).

274
275 Mass rates of zinc in the effluent from all mesocosms were statistically the same. Retention was
276 similar in mesocosms inoculated with mycorrhizae and the control, and ranged from 41-96%.
277 Retention in mesocosms with the proprietary soil ranged from 44-77%. Average retention in
278 mesocosms inoculated with mycorrhizae and the control was 81% (median of 91%), and 64%
279 (median of 67%) for the proprietary soil. Average retention rates were lower than that observed
280 in other studies (Sun and Davis 2007; Blecken et al. 2009), but similar during tests 3 and 4 for
281 control and mycorrhizae-inoculated mesocosms where >90% of zinc was removed from
282 stormwater. Similar to copper, low removal rates of zinc were likely due to the low influent
283 concentrations; average influent concentration was 68 µg/L. The higher retention during tests 3
284 and 4 could have been due greater microorganism establishment after the first two tests. The

285 BSM and proprietary soil contained small amounts of zinc (6.99 and 1.90 mg/kg, respectively)
286 compared to typical zinc concentrations in soil, which have a mean of 51 mg/kg and range from
287 10-2000 mg/kg (ATSDR, 2005).

288
289 **Nitrogen.** Mass rates of total nitrogen were statistically the same for the control, mesocosms
290 inoculated with mycorrhizae, and the proprietary soil. Total nitrogen was exported from all
291 mesocosms; average export for all tests and mesocosms was 400% (median of 167%). Leach
292 tests showed that the source of total nitrogen was the soil; the BSM and proprietary soil had 60
293 mg/kg and 184 mg/kg total nitrogen, respectively. Export of ammonia was also observed, but
294 export of ammonia from the control and mycorrhizae-inoculated mesocosms were significantly
295 higher than the proprietary soil ($p<0.05$ and $p<0.005$ for the control and mycorrhizae-inoculated
296 mesocosms, respectively). Export of ammonia from the control and mycorrhizae-inoculated
297 mesocosms were statistically the same. More ammonia was present in the BSM compared to the
298 proprietary soil (33.5 mg/kg and 3.6 mg/kg, respectively), which explains the higher export rate
299 from the BSM. As a result, mass rate of ammonia in the control and mesocosms inoculated with
300 mycorrhizae are significantly higher than mesocosms with proprietary soil (Figure 3).

301
302 Nitrate was removed from stormwater in the control and mycorrhizae-inoculated mesocosms, but
303 exported in the mesocosms with proprietary soil. As a result, mass rates from the mesocosms
304 with proprietary soil were significantly higher (Figure 4). There was a significant difference in
305 removal between the control and mycorrhizae-inoculated mesocosms ($p<0.005$), the control and
306 mesocosms with proprietary soil ($p<0.005$), and the mycorrhizae-inoculated and proprietary soil
307 mesocosms ($p<0.005$). Average and median removal of nitrate in mesocosms with BSM was

308 62% and 68%, respectively, and both average and median export of nitrate in mesocosms with
309 proprietary soil was 600%. Nitrate content in the proprietary soil was higher than the BSM (174
310 mg/kg and 20 mg/kg, respectively). Although the saturation zone likely facilitated denitrification
311 in all mesocosms, the high soil nitrate levels in the proprietary soil may have overwhelmed this
312 removal mechanism. It is important to note that columns were not completely drained between
313 tests, so effluent from tests 2, 3, and 4 contained saturated zone residual from the previous test.
314 Palmer et al. (2013) observed 52-57% removal of nitrate with the presence of a saturation zone,
315 which is similar to the findings for mesocosms with the BSM in this study. The presence of
316 mycorrhizal fungi in the soil likely increased uptake of nitrate, and may account for the smaller
317 mass rate of nitrate in the effluent from the mycorrhizae-inoculated mesocosms. Retention of
318 nitrate in the mycorrhizae-inoculated mesocosms increased and mass rates decreased with each
319 test, whereas mass rates in the control mesocosms stayed relatively constant (Figure 4). Uptake
320 of nitrate may increase as plant roots and mycorrhizal fungi become more established; further
321 research would be needed to evaluate long-term impacts.

322

323 **Phosphorus.** Phosphate and total phosphorus were exported in mesocosms with BSM, and
324 retained in mesocosms with the proprietary soil. Average and median export of total phosphorus
325 for mesocosms with BSM was 450% and 430%, respectively, and average and median retention
326 for mesocosms with the proprietary soil was 61% and 60%, respectively. Average export and
327 retention of phosphate was higher; 500% export in mesocosms with BSM and 78% retention in
328 mesocosms with the proprietary soil. Median export in mesocosms with BSM was 570% and
329 median retention was 81% in mesocosms with proprietary soil. Leach tests indicate the BSM has
330 substantially more phosphorus than the proprietary soil. Phosphate and total phosphorus in the

331 BSM was 210 mg/kg and 340 mg/kg, respectively, and 0.4 mg/kg and 0.4 mg/kg in the
332 proprietary soil. Mass rates of phosphate and total phosphorus were significantly lower in
333 mesocosms with the proprietary soil compared to mesocosms with the BSM ($p < 0.005$), and mass
334 rates of phosphate and total phosphorus were significantly lower in the mycorrhizae-inoculated
335 mesocosms compared to the control ($p < 0.005$) (Figures 5 and 6).

336
337 Phosphate and total phosphorus in the effluent from the mycorrhizae-inoculated mesocosms
338 were 14-60% and 13-48% lower than effluent from the control mesocosms, respectively. Mass
339 rates of total phosphorus and phosphate from the control mesocosms increased after the first test,
340 but stayed relatively constant in the mycorrhizae-inoculated mesocosms during all tests. During
341 the last three tests, there was a substantial difference in export from the control mesocosms and
342 the mycorrhizae-inoculated mesocosms. This trend was also observed with copper, and to a
343 smaller degree with nitrate (Figures 2 and 4), and may be an indication of the longer time scales
344 needed for plant uptake to occur as well as the importance of inter-event retention mechanisms.

345
346 Mass rates of phosphate and total phosphorus in the effluent from the mesocosms with
347 proprietary soil were 97-99% and 92-98% lower than effluent from the control mesocosms,
348 respectively. The difference between the BSM and proprietary soil is likely due to the presence
349 of phosphorus in the BSM, as well as the presence of clay and additional microorganisms in the
350 proprietary soil. Turbidity in the effluent was much lower from mesocosms with proprietary soil
351 (average 16 NTU) compared to mesocosms with the BSM (average 30 NTU). Studies have
352 shown that phosphorus is typically associated with sediment movement (Fraser et al. 1999;
353 Sharpley and Smith 1989). The different soil structure in the proprietary soil may filter out

354 and/or retain more soil particles compared to the BSM. In addition, the bacteria and fungi in the
355 proprietary soil may retain additional phosphorus in the soil, similar to what was observed with
356 the mycorrhizae-inoculated soil. The larger belowground mass and longer root length of the
357 mycorrhizae-inoculated and proprietary mesocosms (Table 3) may aid in soil structure and
358 retention of phosphorus.

359

360 **Conclusions**

361 This study indicates that the addition of mycorrhizal fungi may decrease total phosphorus and
362 phosphate leaching, and increase nitrate reduction in bioretention systems. The proprietary soil
363 mix retained total phosphorus and phosphate, which may be due to lower phosphorus content in
364 the soil, clay content, and the added mycorrhizae and bacteria . However, nitrate leached from
365 the proprietary soil, which can impact impaired receiving waters. Nitrogen content in the
366 compost should be decreased in the proprietary soil to limit nitrate leaching. Overall, a healthy
367 microbial community with mycorrhizal fungi may help improve effluent water quality from
368 bioretention systems. More mesocosm and field studies are needed to understand the long-term
369 benefits of mycorrhizal fungi, but this study is a promising first step.

370

371 **Acknowledgements**

372 We would like to thank the City of Portland for donating soil and Sunmark Environmental for
373 donating mycorrhizal fungi and Earthlite™ BioSwale ES soil mix. This project was funded by
374 the Shiley Fellows and Butine Fund. We thank Jacob Amos for constructing the mesocosms.

375

376 **References**

- 377
378 Agency for Toxic Substances and Disease Registry (ATSDR) (2004). *Toxicological Profile for*
379 *Copper*. US Department of Health and Human Services. Atlanta, GA.
380
381 Agency for Toxic Substances and Disease Registry (ATSDR) (2005). *Toxicological Profile for*
382 *Zinc*. US Department of Health and Human Services. Atlanta, GA.
383
384 Blecken, G., Zinger, Y., Deletić, A., Fletcher, T., and Viklander, M. (2009). “Impact of a
385 submerged zone and a carbon source on heavy metal removal in stormwater biofilters.”
386 *Ecological Engineering*, 35(5), 769-778.
387
388 Brandstetter, E., Ratliff, K., Weaver, J., Pronold, M., and Wilson, J. (2014a). *Reducing copper in*
389 *industrial stormwater*. Oregon Department of Environmental Quality Fact Sheet. Portland, OR
390
391 Brandstetter, E., Ratliff, K., Weaver, J., Pronold, M., and Wilson, J. (2014b). *Reducing zinc in*
392 *industrial stormwater*. Oregon Department of Environmental Quality Fact Sheet. Portland, OR.
393
394 City of Portland (2016a). *Stormwater Management Manual*. Portland, OR.
395
396 City of Portland (2016b). *Portland Plant List*. Portland, OR.
397
398 Clary, J., Jones, J., Leisenring, M., Hobson, P., Strecker, E. (2017). *International Stormwater*
399 *BMP Database: 2016 Summary Statistics*. Final Report. Portland, OR.
400
401 Corkidi, L., Merhaut, D.J., Allen, E.B., Downer, J., Bohn, J., and Evans, M. (2011). “Effects of
402 mycorrhizal colonization on nitrogen and phosphorus leaching from nursery containers.” *Hort*
403 *Science*, 46(11), 1472-1479.
404
405 Davis A., Shokouhian M., Sharma H., Minami C., and Winogradoff D. (2003). “Water quality
406 improvement through bioretention: lead, copper, and zinc removal.” *Water Environment*
407 *Research*, 75(1), 73-82.
408
409 Davis A., Shokouhian M., Sharma H., and Minami C. (2006). “Water quality improvement
410 through bioretention media: nitrogen and phosphorus removal.” *Water Environment Research*,
411 78(3), 284-293.
412
413 Environmental Protection Agency (EPA) (1999). *Stormwater Technology Fact Sheet:*
414 *Bioretention*. Washington D.C.
415
416 Environmental Protection Agency (EPA) (2000) *National Water Quality Inventory – 1998*
417 *Report to Congress*. Washington D.C.
418
419 Helsel, D. R. and Hirsch, R. M. (2002). “Techniques of water-resources investigations of the
420 United States Geological Survey: statistical methods in water resources”. United States
421 Geological Survey.

422
423 Herrera Environmental Consultants (2014). *185th Avenue NE Bioretention Stormwater*
424 *Treatment System Performance Monitoring*. Final Report. Prepared for City of Redmond,
425 Seattle, WA.
426
427 Herrera Environmental Consultants (2015). *Analysis of bioretention soil media for improved*
428 *nitrogen, phosphorus, and copper retention*. Final Report. Seattle, WA.
429
430 Hurley, S., Shrestha, P., and Cording, A. (2017). "Nutrient leaching from compost: implications
431 for bioretention and other green stormwater infrastructure." *Journal of Sustainable Water in the*
432 *Built Environment*, 3(3), 04017006, 1-8.
433
434 Leisenring, M., Clary, J., and Hobson, P. (2014). *International Stormwater Best Management*
435 *Practices Database Pollutant Category Statistical Summary Report: Solids, Bacteria, Nutrients,*
436 *and Metals*. Final Report. Portland, OR.
437
438 Li, H. and Davis, A. (2009). "Water quality improvement through reduction of pollutant loads
439 using bioretention." *Journal of Environmental Engineering*, 135(8), 567-576.
440
441 Li, L., and Davis, A.P. (2014). "Urban stormwater runoff nitrogen composition and fate in
442 bioretention systems." *Environmental Science & Technology*, 48(6), 3403–3410.
443
444 Li, H., Smith, S.E., Holloway, R.E., Zhu, Y., and Smith, F.A. (2006). "Arbuscular mycorrhizal
445 fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the
446 absence of positive growth responses." *New Phytologist*, 172, 536–543.
447
448 Lucas, W. C., and Greenway, M. (2008). "Nutrient retention in vegetated and nonvegetated
449 bioretention mesocosms." *Journal of Irrigation and Drainage Engineering*, 134(5), 613–623.
450
451 Maestre, A., Pitt, R., and Williamson, D. (2004). "Nonparametric Statistical Tests Comparing
452 First Flush and Composite Samples from the National Stormwater Quality Database." *Models*
453 *and Applications to Urban Water Systems*, 12, 317–338.
454
455 Maryland Department of the Environment (DOE) (2009). *Maryland Stormwater Design Manual,*
456 *Appendix A*. Baltimore, MD.
457
458 Mullane, J., Flury, M., Iqbal, H., Freeze, P., Hinman, C., Cogger, C.G., and Shi, Z. (2015).
459 "Intermittant rainstorms cause pulses of nitrogen, phosphorus, and copper in leachate from
460 compost in bioretention systems." *Science of the Total Environment*, 537, 294-303.
461
462 Muthanna, T., Viklander, M., Gjesdahl, N., and Thorolfsson, S. (2007). "Heavy metal removal in
463 cold climate bioretention." *Water, Air, and Soil Pollution*, 183, 391-402.
464
465 Muthukumar, T., Udaiyan, K., and Shanmughavel, P. (2004). "Mycorrhiza in sedges--an
466 overview." *Mycorrhiza*, 14(2), 65–77.
467

468 National Research Council, 2000. *Clean Coastal Waters: Understanding and Reducing the*
469 *Effects of Nutrient Pollution*. National Academy Press, Washington, DC.
470
471 New Hampshire Department of Environmental Services (DES) (2008). *New Hampshire*
472 *Stormwater Manual, Volume 2: Post Construction Best Management Practices Selection and*
473 *Design*. Concord, NH.
474
475 North Carolina Department of Environmental Quality (DEQ) (2017). *Stormwater Design*
476 *Manual, Part C: Minimum Design Criteria and Recommendations for Stormwater Control*.
477 Raleigh, N.C.
478
479 Palmer, E.T., Poor, C.J., Hinman, C., and Stark, J.D. (2013). "Nitrate and phosphate removal
480 through enhanced bioretention media: mesocosm study." *Water Environment Research*, 85(9),
481 823-832.
482
483 Paus, K., Morgan, J., Gulliver, J., and Hozalski, R. (2014). "Effects of bioretention media
484 compost volume fraction on toxic metals removal, hydraulic conductivity, and phosphorus
485 release." *Journal of Environmental Engineering*, 140(10), 04014033, 1-9.
486
487 Permamatrix, Inc. (2016). *Permamatrix Particle Information*. Fairview, OR.
488
489 Rice, E.W., Baird, R.B., Eaton, A.D., and Clesceri, L.S. (2012). *Standard Methods for the*
490 *Examination of Water and Wastewater*, 22nd edition. American Public Health Association,
491 Washington, D.C.
492
493 Singh, H. (2006). *Mycoremediation: Fungal Bioremediation*. Wiley Interscience, New York, NY.
494
495 Smith, S.E., and Read, D.J. (2008). *Mycorrhizal symbiosis*. Academic Press, Boston, MA.
496
497 Sun, X., and Davis, A.P. (2007). "Heavy metal fates in laboratory bioretention systems."
498 *Chemosphere*, 66(9), 1601–1609.
499
500 Sunmark Environmental (2014). *Earthlite Bioswale ES Soil Blend Technical Sheet*. Fairview, OR.
501
502 Trowsdale, S. and Simcock, R. (2011). "Urban stormwater treatment using bioretention."
503 *Journal of Hydrology*, 397(3), 167-174.
504
505 Winfrey, B., Hatt, B., and Ambrose, R. (2017). "Arbuscular mycorrhizal fungi in Australian
506 stormwater biofilters." *Ecological Engineering*, 102, 483-489.
507
508

509 **Table Captions**

510

511 **Table 1.** Fungal species used in this study.

512

513 **Table 2.** Average exfiltration rates (cm/hr) for each treatment and test.

514

515 **Table 3.** Plant characteristics after testing.

516 **Figure Captions**

517

518 **Fig. 1.** Schematic of mesocosms used in this study.

519

520 **Fig. 2.** Mass rate of copper in mesocosms for each test. Each column represents the average of
521 replicates, with standard deviation.

522

523 **Fig. 3.** Mass rate of ammonia in mesocosms for each test. Each column represents the average of
524 replicates, with standard deviation.

525

526 **Fig. 4.** Mass rate of nitrate in mesocosms for each test. Each column represents the average of
527 replicates, with standard deviation.

528

529 **Fig. 5.** Mass rate of phosphate in mesocosms for each test. Each column represents the average
530 of replicates, with standard deviation.

531

532 **Fig. 6.** Mass rate of total phosphorus in mesocosms for each test. Each column represents the
533 average of replicates, with standard deviation.

534

535 **Table 1.** Fungal species used in this study.

Endomycorrhizal Fungi	Ectomycorrhizal Fungi
<i>Glomus intraradices</i>	<i>Pisolithus tinctorius</i>
<i>Glomus mosseae</i>	<i>Rhizopogon villosullus</i>
<i>Glomus aggregatum</i>	<i>Rhizopogon luteolus</i>
<i>Glomus etunicatum</i>	<i>Rhizopogon amylopogon</i>
	<i>Rhizopogon fulvigleba</i>
	<i>Scleroderma cepa</i>
	<i>Scleroderma citrinum</i>

536

537

538 **Table 2.** Average exfiltration rates (cm/hr) for each treatment and test.

	Test 1	Test 2	Test 3	Test 4
Control	10.9	15.3	17.7	18.9
Mycorrhizae	10.6	9.8	9.3	15.6
Proprietary	18.0	23.8	25.2	19.1

539

540

541 **Table 3.** Plant and soil characteristics after testing.

	Belowground Biomass (g)	Aboveground Biomass (g)	Root Length (cm)	Blade Length (cm)	Organic Matter in Soil (%)
Control	0.49	5.3	21	46	6.38
Mycorrhizae	2.17	7.8	39	73	7.68
Proprietary	4.68	19.5	46	76	8.14

542

Figure 1

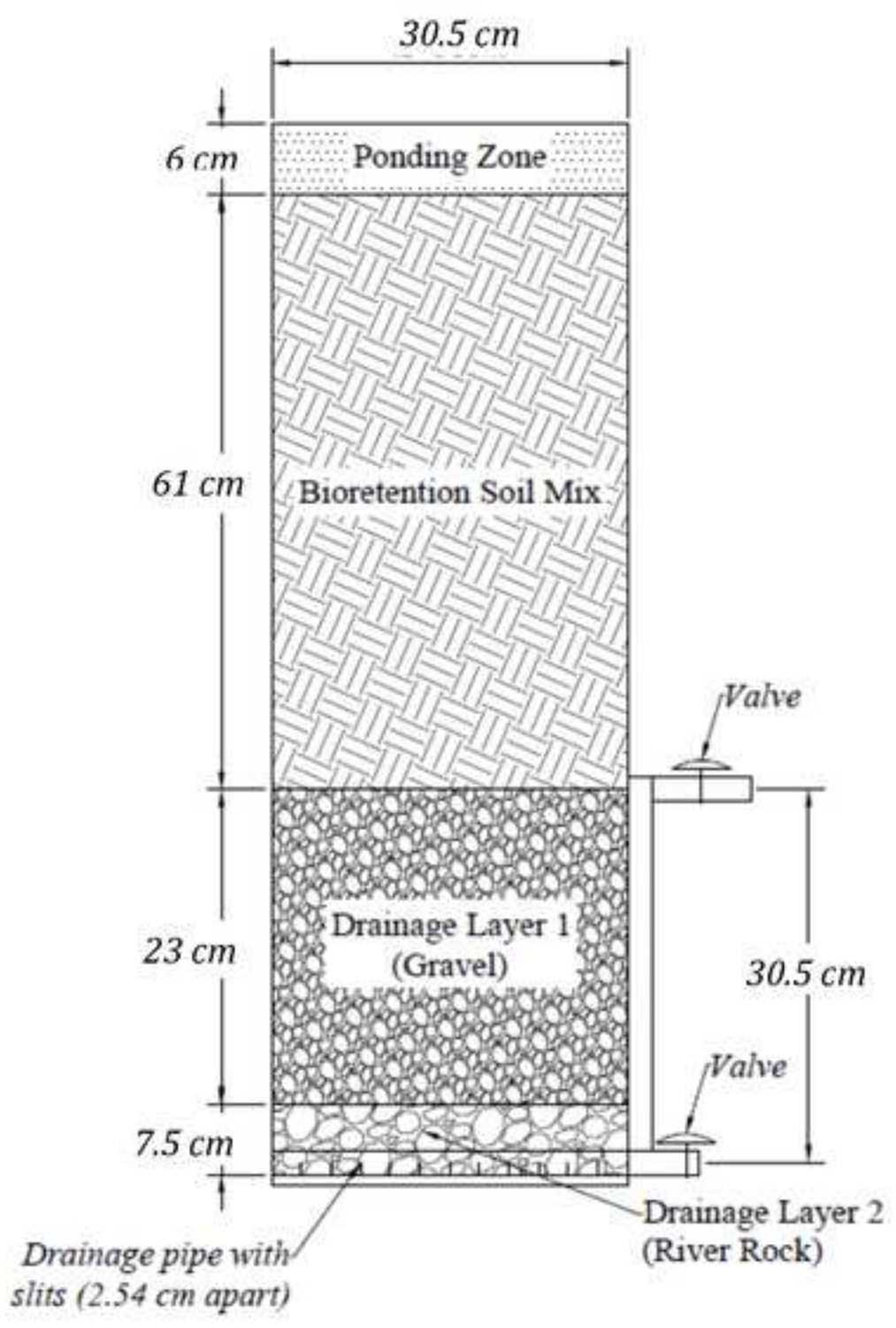


Figure 2

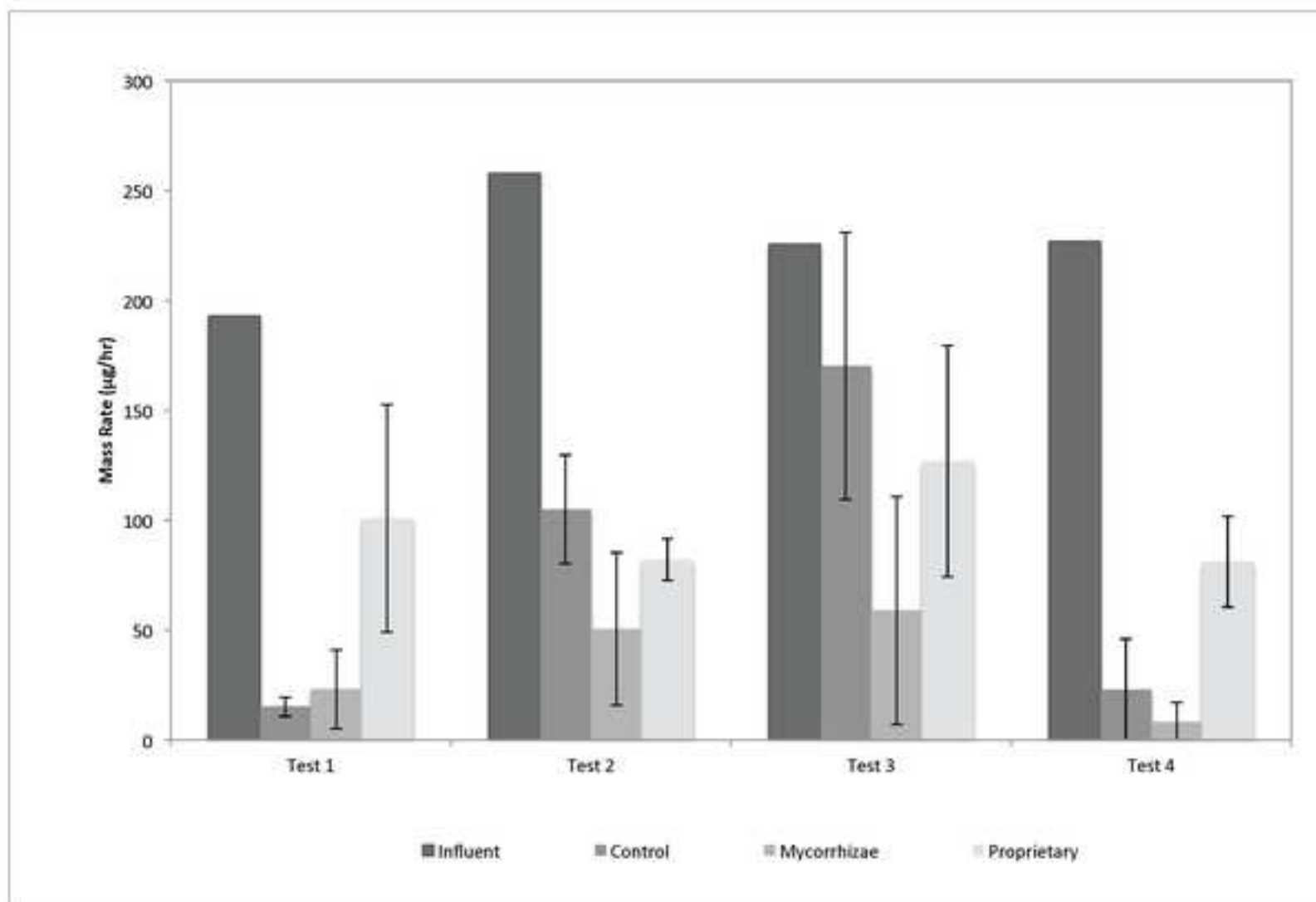


Figure 3

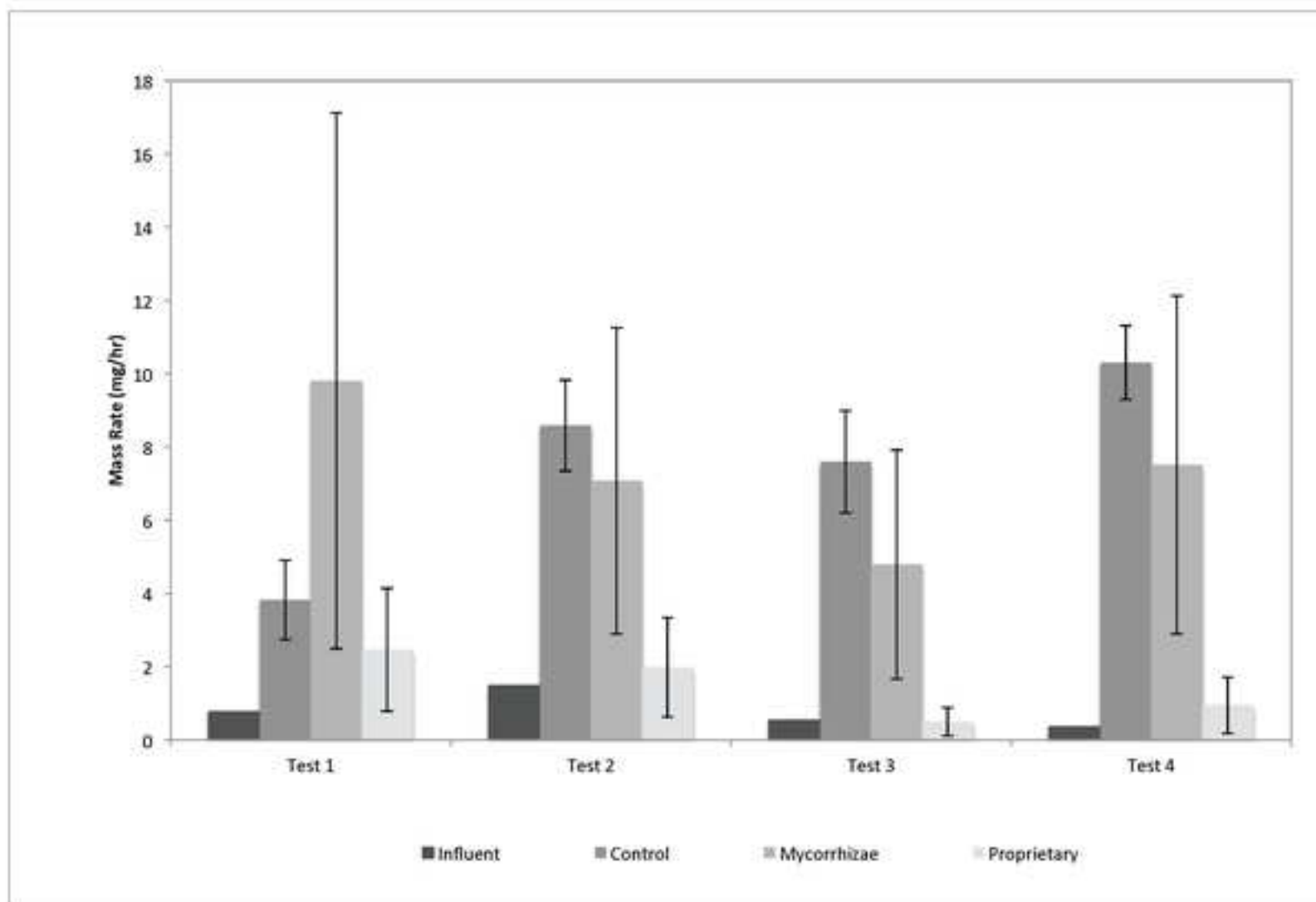


Figure 4

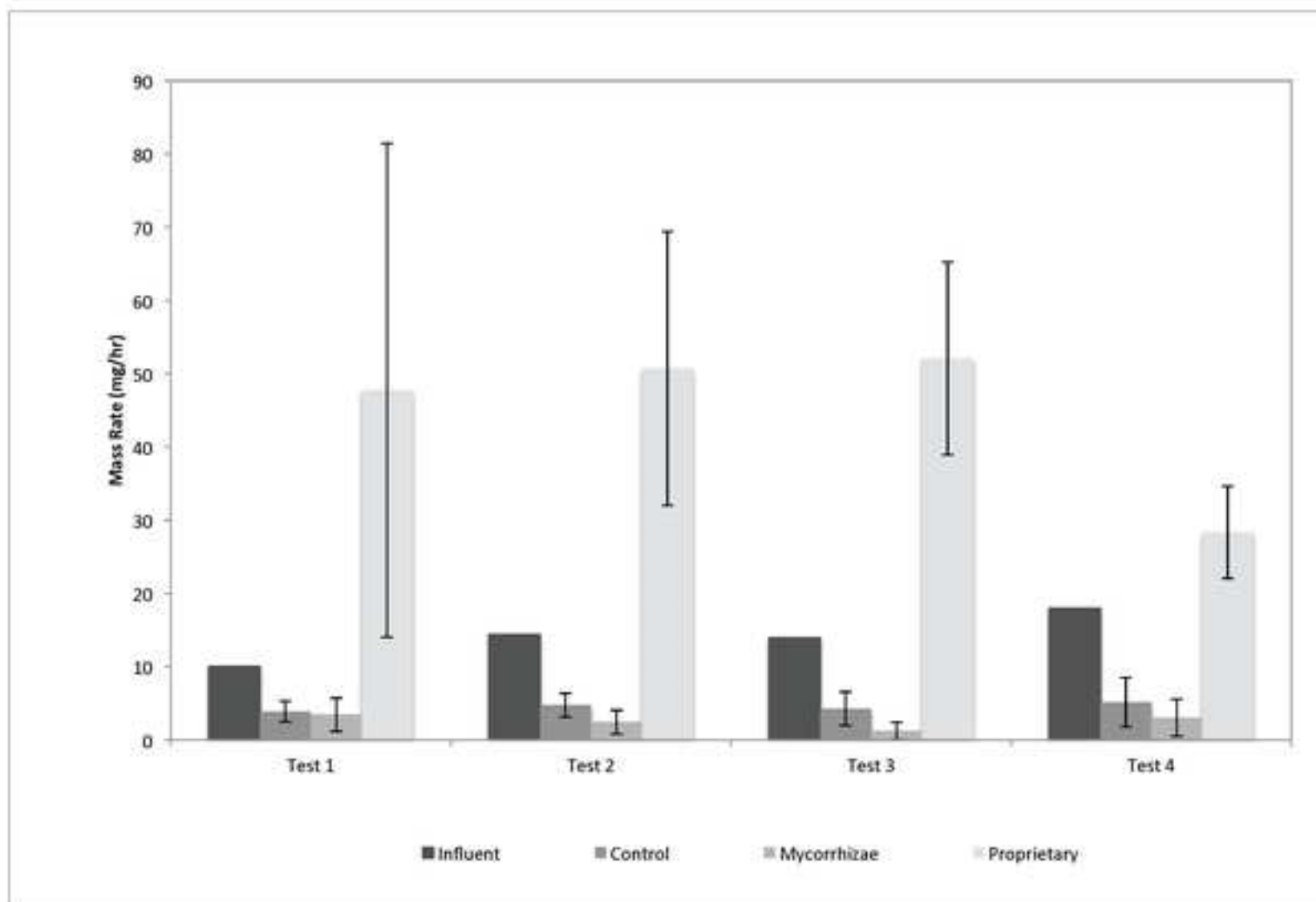


Figure 5

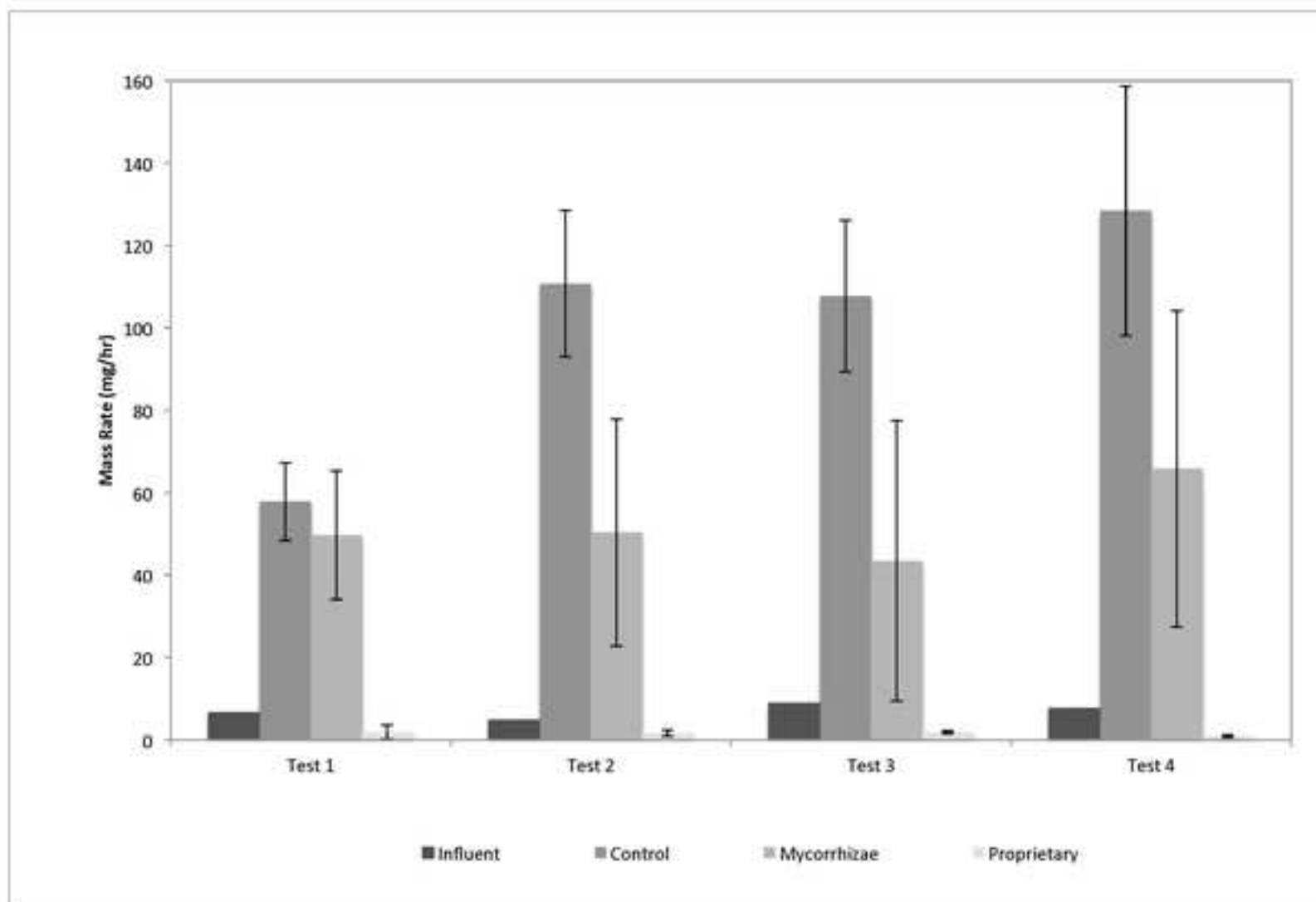


Figure 6

