6-2018

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

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Abstract

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

Introduction

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

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

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

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

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

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

Methods

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

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

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

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

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

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

The nine columns were assembled with the following variations:

- 3 control columns with BSM only
- 3 columns with mycorrhizae-inoculated BSM
- 3 proprietary soil columns

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

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

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

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

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

between two treatments.

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

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

Results and Discussion

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

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

Copper and Zinc. Mass rates of copper in the effluent from mesocosms inoculated with mycorrhizae were significantly lower than the control ($p<0.025$) and the proprietary soil ($p<0.05$) (Figure 2). Average mass rates from the mesocosms inoculated with mycorrhizae, control, and proprietary soil were 35.2, 78.3, and 97.8 μg/hr, respectively, and median mass rates were 36.8, 63.9, and 91.5 μg/hr, respectively. This indicates the mycorrhizal fungi may increase uptake of copper, although the mass rate from the proprietary soil, which contains mycorrhizae, did not exhibit significantly higher uptake compared to the control. Copper could be binding to organic matter, but the proprietary soil had higher organic matter content and lower uptake of copper compared to the mesocosms inoculated with mycorrhizae. The lower uptake in the proprietary mescocosms may be due to the higher exfiltration rates; exfiltration rates were 18.0-25.2 cm/hr in the proprietary mesocosms and 9.3-18.9 cm/hr in the BSM mesocosms. The lower contact time in the proprietary mesocosms may have impacted copper uptake and retention. Mass rates of copper in the effluent were statistically the same for the control and the proprietary soil.

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

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

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

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

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

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

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

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

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

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

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

Conclusions

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

Acknowledgements

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

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Table Captions

Table 1. Fungal species used in this study.

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

Table 3. Plant characteristics after testing.

Figure Captions

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

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

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

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

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

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

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

Endomycorrhizal Fungi	**Ectomycorrhizal Fungi**
Glomus intraradices	*Pisolithus tinctorius*
Glomus mosseae	*Rhizopogon villosullus*
Glomus aggregatum	*Rhizopogon luteolus*
Glomus etunicatum	*Rhizopogon amylopogon*
	Rhizopogon fulvigleba
	Scleroderma cepa
	Scleroderma citrinum

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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

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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

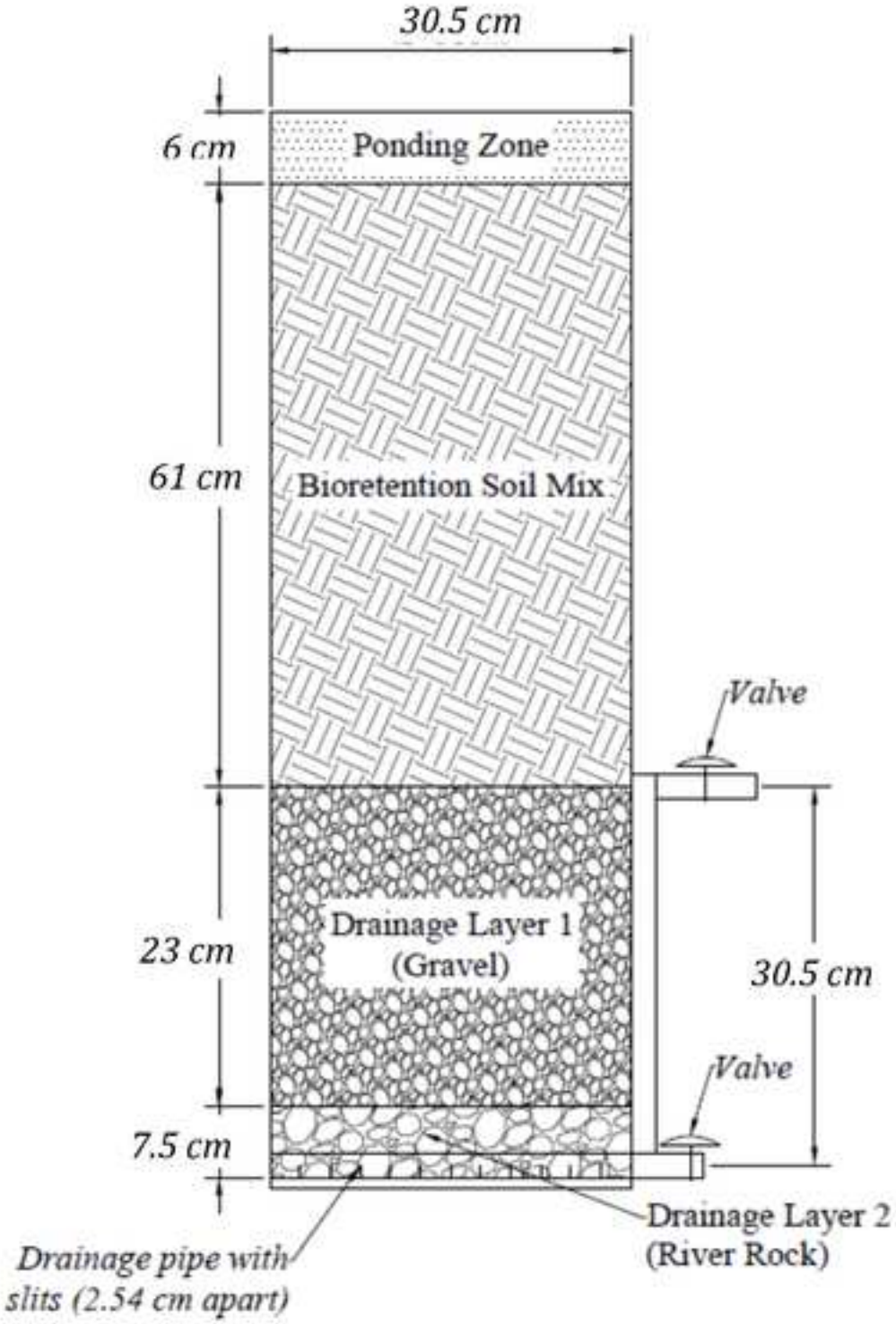

30.5 cm
6 cm
Ponding Zone
61 cm
Bioretention Soil Mix
Valve
Drainage Layer 1
(Gravel)
23 cm
30.5 cm
Valve
7.5 cm
Drainage Layer 2
(River Rock)
Drainage pipe with
slits (2.54 cm apart)

Figure 2

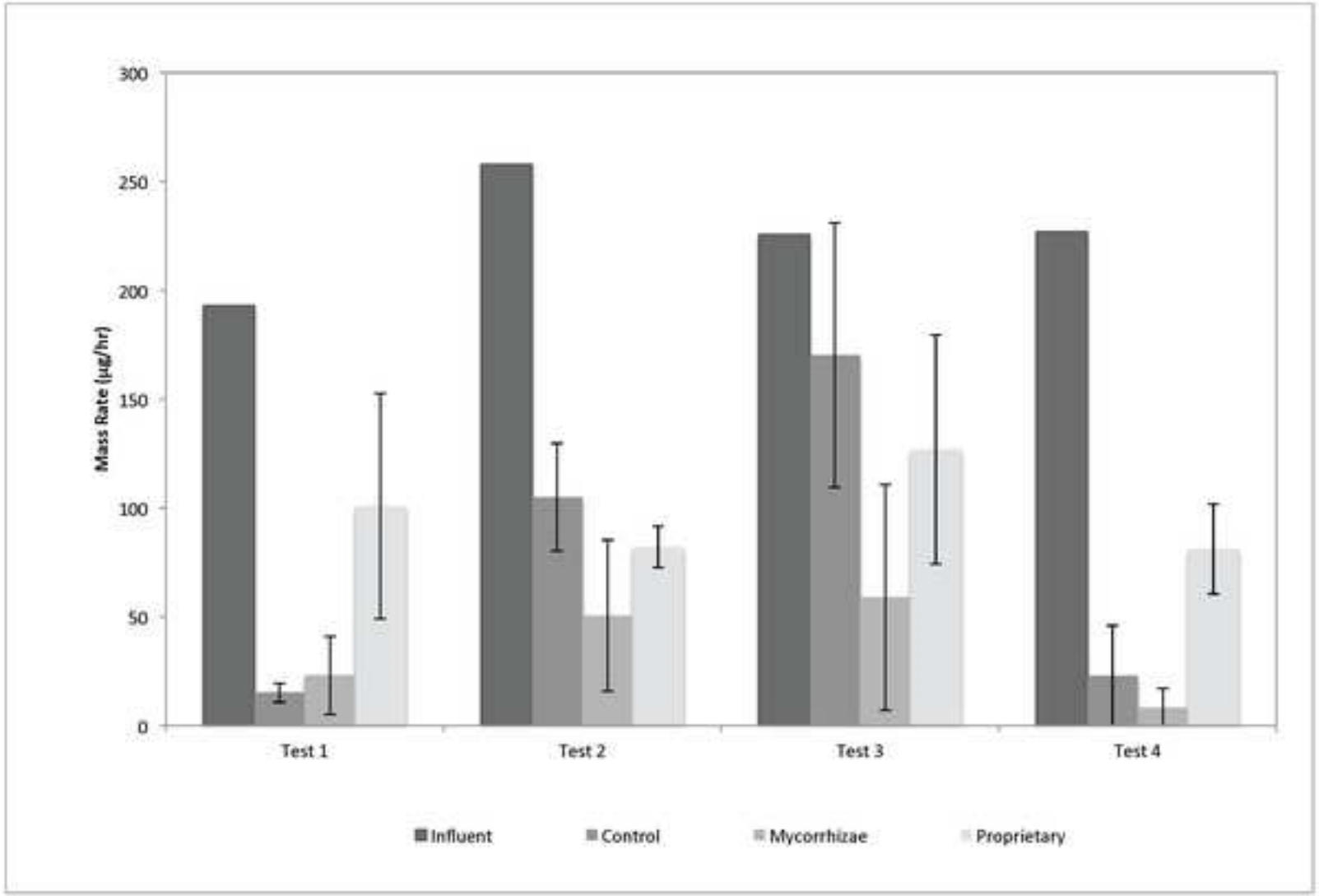

300
250
200
150
100
50
0
Mass Rate (µg/hr)
Test 1
Test 2
Test 3
Test 4
Influent
Control
Mycorrhizae
Proprietary

Figure 3

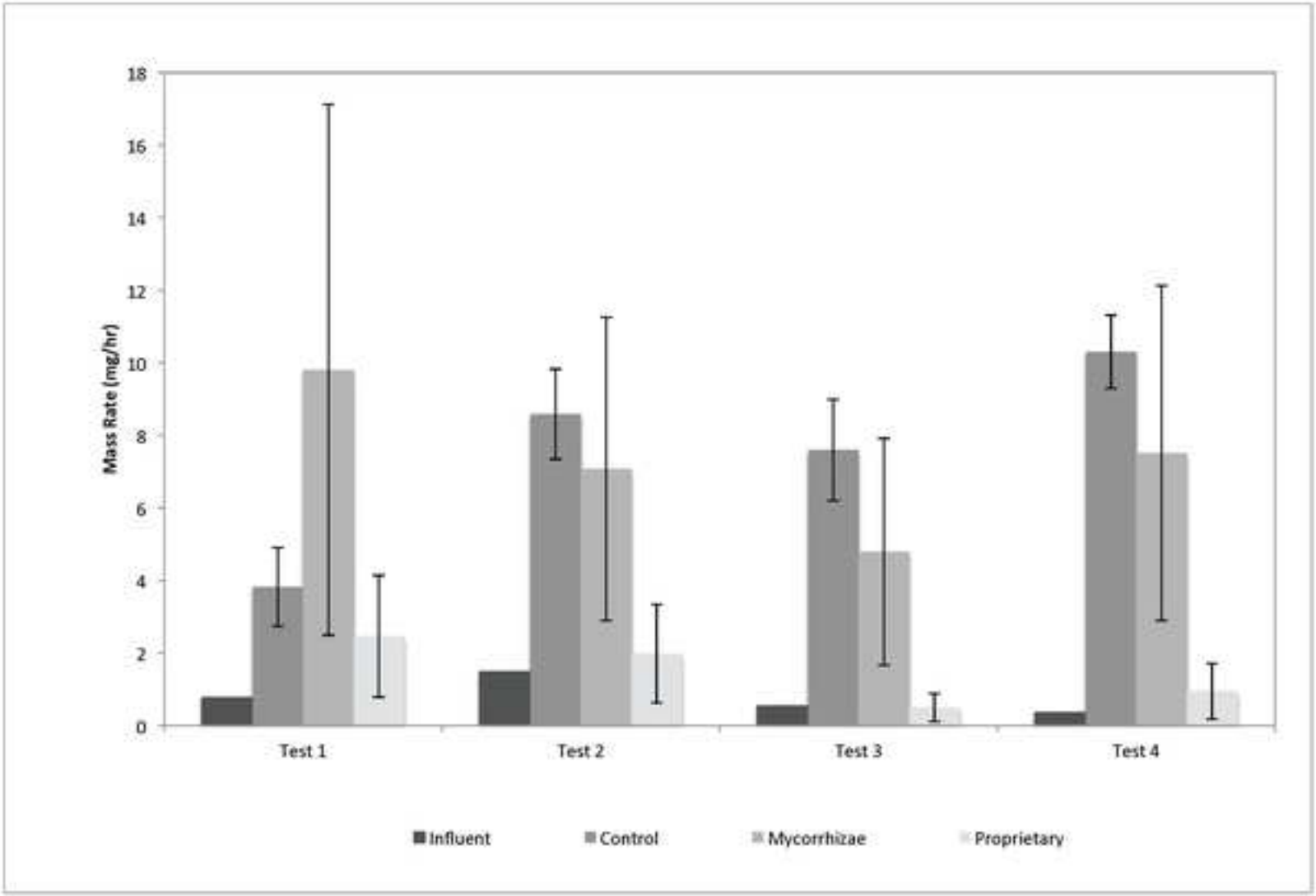

18
16
14
12
10
8
6
4
2
0
Mass Rate (mg/hr)
Test 1
Test 2
Test 3
Test 4
Influent
Control
Mycorrhizae
Proprietary

Figure 4

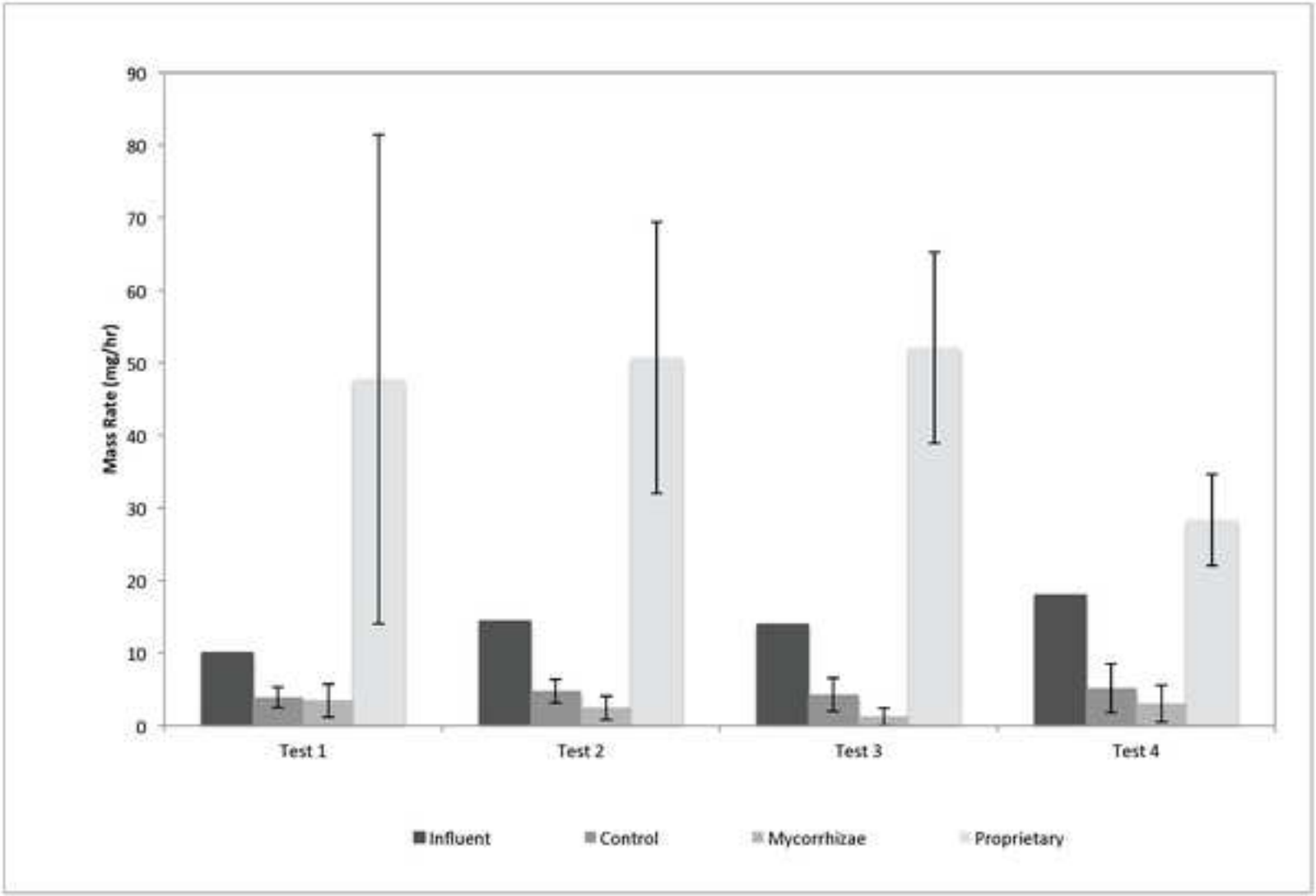

90
80
70
60
50
40
30
20
10
0
Mass Rate (mg/hr)
Test 1
Test 2
Test 3
Test 4
Influent
Control
Mycorrhizae
Proprietary

Figure 5

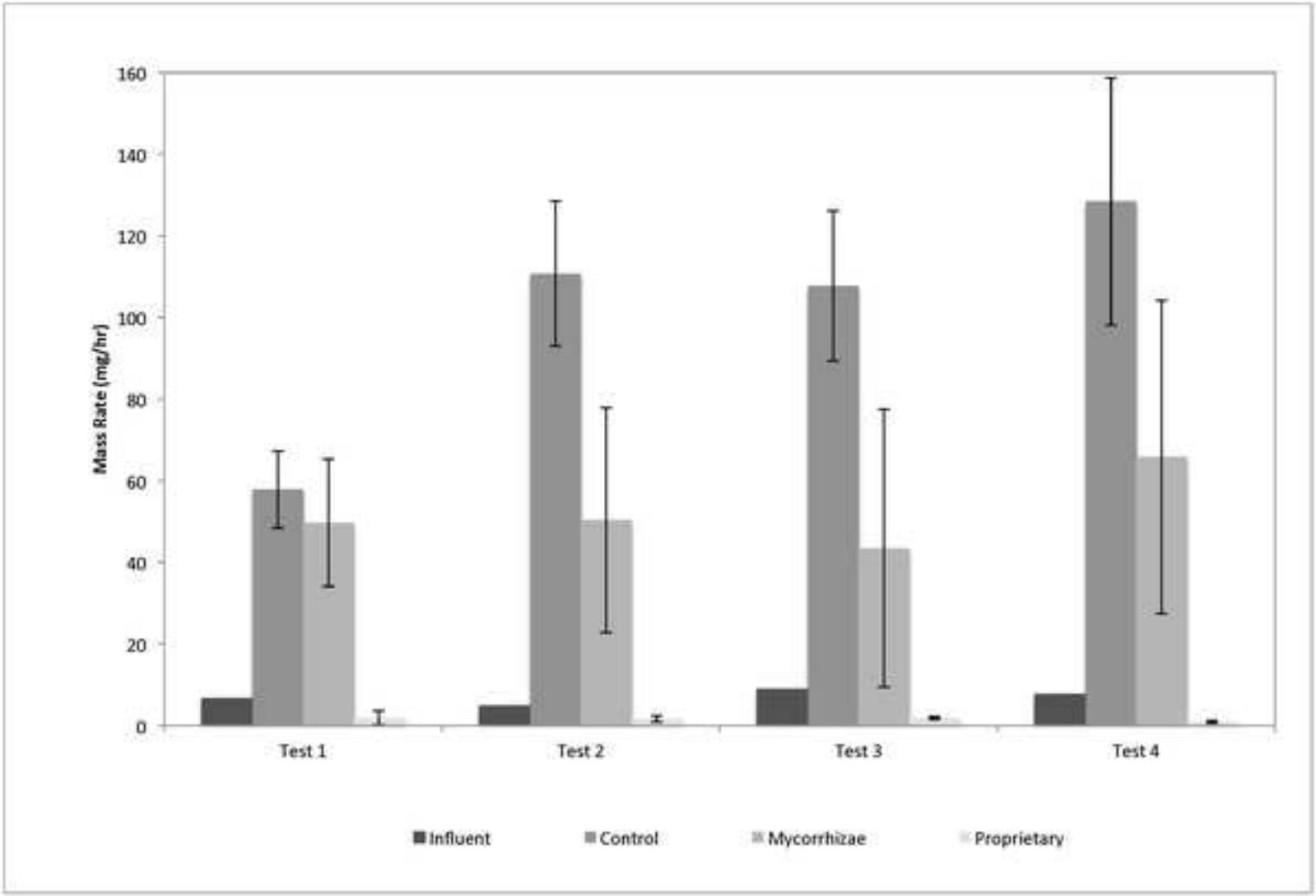
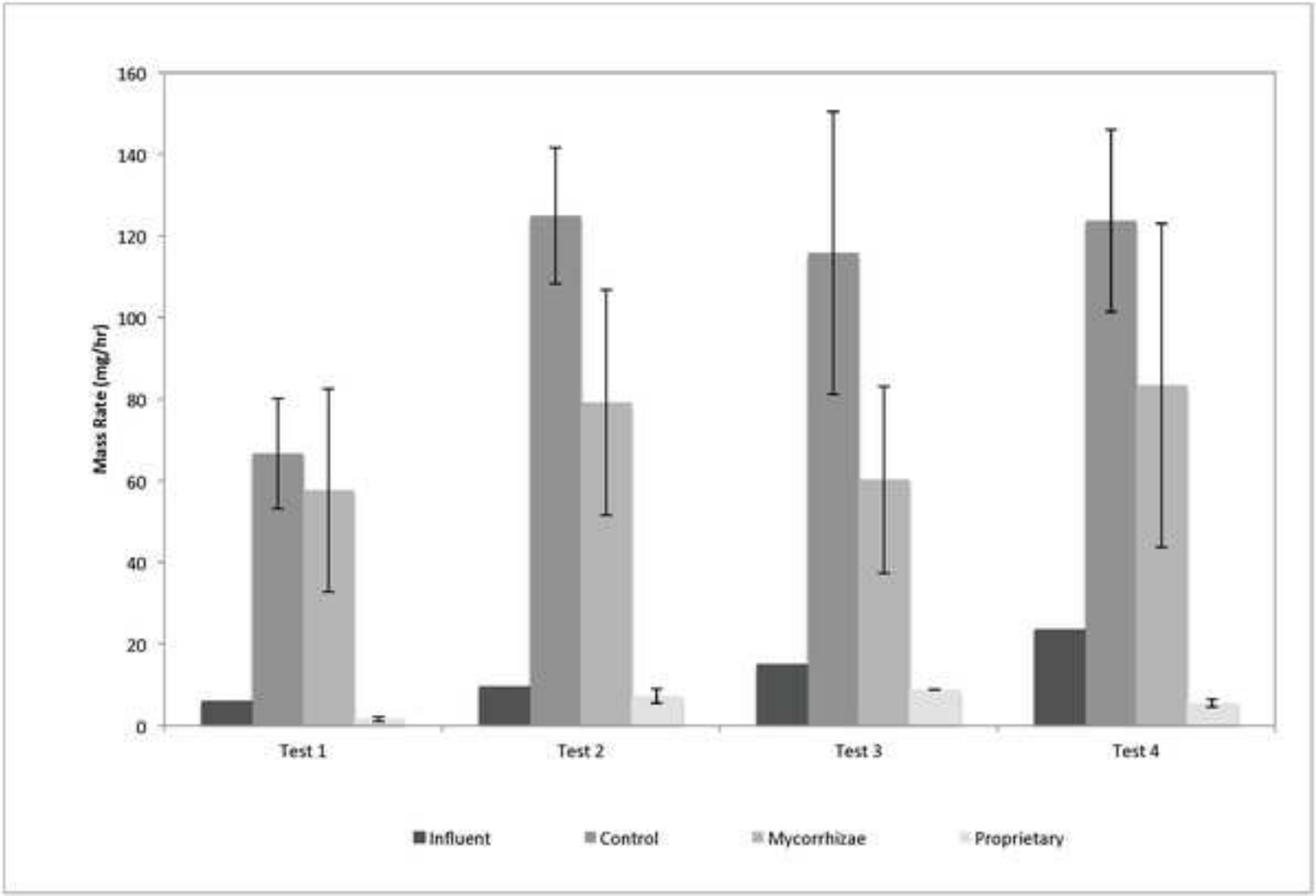

160
140
120
100
80
60
40
20
0
Mass Rate (mg/hr)
Test 1
Test 2
Test 3
Test 4
Influent
Control
Mycorrhizae
Proprietary

Figure 6


160
140
120
100
80
60
40
20
0
Mass Rate (mg/hr)
Test 1
Test 2
Test 3
Test 4
Influent
Control
Mycorrhizae
Proprietary