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Title:

Evaluation of the Role of Mycelium in Bioretention Stormwater Treatment

Michael Freudenthaler

UNIVERSITY OF APPLIED SCIENCES
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Environmental Engineering

Supervised by

Cara Poor, Ph.D.
FH Prof. Dr. Alexander Jäger

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ABSTRACT

Bioretention is a stormwater best management practice that can sustainably tackle the threat that urban stormwater runoff poses to the environment. Bioretention systems are used to remove nutrients and heavy metals by filtering stormwater through an engineered system of soil, plants and microbial populations.

This present study was part of a project that had been initiated by the University of Portland in order to evaluate the pollutant removal performance of mycorrhizal fungi in bioretention columns. Influent and effluent from four treatment runs were tested for total phosphorus (TP), phosphate (PO_4^{3-}), total nitrogen (TN), ammonia nitrogen (NH_3-N), nitrate (NO_3^-), copper and zinc. The results obtained by the use of Earthlite BioSwale ES™ Topsoil medium (containing mycorrhizal fungi) were compared to the outcome of the predecessor-study by Balmes et al. (2016) where City of Portland soil medium with and without mycorrhizal fungi was utilized. A saturated zone for enhanced denitrification was incorporated in all mesocosms.

Results indicate that the presence of mycorrhiza does not significantly improve the pollutant retention capacity of bioretention mesocosm in early establishment (<four months after assembly). Findings of both studies show that the right choice of soil medium is the prevalent factor for retaining nitrogen and phosphorus compounds. Columns containing Earthlite BioSwale ES™ Topsoil removed ortho-phosphate by 78% but increased nitrogen concentrations. Conversely, the utilization of City of Portland soil medium facilitated nitrate removal showing 27% reduction (regardless of mycorrhizal inoculation) but raised the amount of phosphorus compounds. Heavy metal removal (Cu, Zn) occurred in all columns similarly well resulting in removal rates of 50% on average.

A conducted leach test on each soil medium verified that Earthlite BioSwale ES™ Topsoil is prevalently leaching nitrate and the City of Portland soil mix is leaching great amounts of ortho-phosphate. Algae growth is primarily phosphorus limited, hence, eutrophication should be addressed by reducing phosphorus in the first place. This finding led to the conclusion that Earthlite BioSwale ES™ Topsoil medium is preferable if the effluent is discharged to receiving rivers and streams.

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1 INTRODUCTION

The following section talks about the various elements that contribute to an effective stormwater treatment. To start with, the origin of polluted stormwater runoff and its implications are described. Then, the introduction is structured from an explanation of generally implemented practices against water pollution over the set-up of a bioretention system down to the biological key components like plants and fungi.

1.1 STORMWATER RUNOFF AND ITS IMPACT ON THE ENVIRONMENT

Definition says that stormwater and urban-related runoff are discharges from municipal separate storm sewers, parking lot and impervious surfaces runoff, highway and road runoff, storm sewers, urban runoff as well as permitted stormwater discharges. Stormwater and urban-related runoff is considered as a major pollutant source to water bodies in the US. The Environmental Protection Agency assessed several pollutant sources regarding its contribution to the pollution of water bodies. Of all examined sources, stormwater has an impact of up to 8% to the nation's rivers and streams according to those studies. The number one source of water pollutants is the agriculture, which represents 38% of all affecting sources. (United States Environmental Protection Agency, 2009).

During a storm event, only parts of the precipitation infiltrate to the ground or is captured by plants. The remaining majority of the storm becomes stormwater runoff and flows over the surface to the nearest water body, mostly small creeks. Compared to urban areas, the fraction of the stormwater that becomes runoff is considerably smaller in non-urban areas. In urban areas, the amount of impervious surfaces such as streets, parking lots, and rooftops is much larger which leads to an increase of stormwater runoff. As precipitate runs over developed land, it picks up particles and chemical compounds that pose a threat to the environment. Such pollutants are sediment, bacteria from animal and human waste, pesticides from lawn and garden chemicals, nutrients from lawn fertilizers, metals from rooftops and roadways, as well as petroleum by-products from leaking vehicles. Pollution carried by stormwater is considered non-point pollution in contrast to single point pollution since the pollutants origin from a wide area of land. Single point pollution originates from point sources such as an industrial or municipal discharge pipe. Polluted stormwater runoff represents a threat to plants, animals, and people.

Additional concerns due to vast amounts of stormwater runoff are land erosion and eutrophication of water bodies. Land erosion leads to an increased amount of sediment that is discharged to rivers and lakes. This sediment covers the bottom of the water bodies and impairs its aquatic habitat. Higher flows due to heavy rains also cause flooding in urban streams, damaging infrastructure, habitat and property. Moreover, sediment transports contaminants like nutrients and bacteria and leads to an increase of the turbidity. Excessive concentrations of nitrogen and phosphorus cause eutrophication of water bodies. Plants need nitrogen for protein synthesis, and phosphorus is needed for DNA and RNA as well as for energy transfer. Both nutrients are therefore required for plant growth and the key limiting nutrients in most ecosystems (Conley et al., 2009). In the case of eutrophication, excessive amounts of nitrogen and phosphorus lead to increased plant growth, which disrupts the natural ecosystem. When these plants die, the organic material generates a huge oxygen consumption due to aerobic bacteria in the water. Decreased concentrations of dissolved oxygen in the water lead to fish death and a general change in plant and animal populations (Rasmussen & Schmidt, 2009).

When eutrophication occurs, taste and odor problems increase and impair the use of lakes and rivers as sources of drinking water, recreation, and aesthetic benefits. These issues cause major financial losses, as Dodds et al investigated. Eutrophication can be linked to losses in recreational water usage, waterfront property, recovery of threatened and endangered species, and drinking water. The economic costs on freshwater bodies in the United States are therefore estimated to exceed $2.2 billion dollars annually (Dodds et al., 2009).

As mentioned earlier, heavy metals are a constituent of urban stormwater runoff. The elements cadmium, lead, copper, and zinc represent a threat to the environment. Roughly 50% of the dissolved metal load in urban stormwater runoff consists of just these four elements. Industrial facilities emit copper and zinc to the environment from many sources. Origins of copper can be vehicle brake pads, architectural copper, copper-containing pesticides, various roof materials, air emissions or soil erosion. Copper that migrates into rivers and streams by stormwater transport is harmful to fish and other aquatic life. If the copper concentration in freshwater becomes as high as 0.18 µg/L, salmon shows impaired behavior. Copper causes a disruption of their migration, sensory system and predator avoidance (Brandstetter, Ratliff, Weaver, Pronold, & Wilson, 2014a). It is a similar case for zinc. This heavy metal sources from galvanized metal surfaces, motor oil, tire wear, moss control chemicals and white paints. Zinc affects salmon and other aquatic species at concentrations as high as 90 µg/L in marine waters and at concentrations of only 5.6 µg/L in freshwater. The observed effects are altered blood and serum chemistry, altered behavior, reduced growth and impaired reproduction (Brandstetter, Ratliff, Weaver, Pronold, & Wilson, 2014b)

1.2 LOW IMPACT DEVELOPMENT FOR URBAN RUNOFF MANAGEMENT

In order to mitigate the negative effects of enlarging impervious surfaces and increasing urbanization, low impact development (LID) was introduced. LID systems and practices aim for mimicking natural processes that result in the infiltration, evapotranspiration or use of stormwater for protecting water quality and associated aquatic habitat (United States Environmental Protection Agency, 2016a). Preserving as much of the site in an undisturbed condition as possible or at least reducing the adverse effects to the soils, vegetation and aquatic systems is the goal of LID site design (Dietz, 2007). LID is closely related to green infrastructure (GI) and is often used synonymously. The common practice for stormwater management are conventional piped drainage and water treatment systems that are designed to move urban stormwater away from the built environment. These facilities are generally referred to as single-purpose gray stormwater infrastructure. In contrast, green infrastructure approaches the goal of draining stormwater in a multi-purpose way. It is often more cost-effective and resilient than gray infrastructure installations while treating the stormwater at its source at the same time. Green infrastructure uses elements such as soil and vegetation in order to absorb and filter the stormwater just like in natural areas. This results in cleaner runoff, restores some of the natural processes and therefore creates a healthier urban environment (United States Environmental Protection Agency, 2016b).

1.3 STORMWATER BEST MANAGEMENT PRACTICES

With the intention of adhering to the principles of LID/GI, many practices have been used. A stormwater Best Management Practice (BMP) addresses the goal of designing and implementing methods so that the post-development peak discharge rate, volume, and pollutant loadings to receiving waters are the same as pre-development values. BMPs can be techniques, measures or structural controls that improve the quality of stormwater runoff in the most cost-effective way. The three main factors that have to be taken into consideration are flow control, pollutant removal and pollutant source reductions (United States Environmental Protection Agency, 1999)

1.3.1 FLOW CONTROL

The management of flow in urbanized areas allows to control the volume and intensity of stormwater discharges to receiving waters. Since increasing development leads to higher amounts of impervious surfaces, the hydrology of an urbanized watershed is much different from the hydrology of a natural watershed. A watershed is the area of land that captures rainfall and funnels it to a stream or -in the case of a city- to the water collection facilities. The most common effects of creating impervious surfaces are reduced infiltration and decreased travel time due to a higher flow rate. High flow rates of stormwater discharges lead to impacts like the detachment and transport of significant amounts of suspended solids and associated pollutants. Moreover, stream banks and channels are eroded, which further increases the amounts of suspended solids in the runoff. The main goal of BMPs in terms of flow control is to minimize the amount of rainfall that is converted to runoff. This goal is met by utilizing the principles of on-site storage and infiltration in order to reduce the amount of directly connected impervious surfaces. Site design features such as porous pavement systems for parking lots, dry wells, infiltration trenches or simply providing rain barrels come into use (United States Environmental Protection Agency, 1999).

1.3.2 POLLUTANT EEMOVAL

A number of physical and biochemical processes can contribute to efficient pollutant removal in stormwater BMPs. The removing efficiency of pollutants by a given BMP is dependent on the size, type and design of a BMP, the soil types and characteristics, the intensity of a storm event and climatological factors.

Several physical mechanisms remedy pollutant contamination of stormwater. Ponds and constructed wetlands can remove suspended solids by sedimentation. BMPs like coarse screens and drop-in filtration systems provide the removal of floating substances such as low-density materials, oil, and grease. The removal of solids and attached pollutants such as metals and nutrients can be accomplished by utilizing filtration through media such as soil, sand, or gravel.

Infiltration of runoff into the ground is a very purposeful technique since it combines the principles of filtration and adsorption. Adsorption in BMPs is only used in combination with infiltration systems, where dissolved metals can be bound to clay particles.

Biochemical processes play an important role in pollutant removal as well. Biological uptake of nutrients can significantly control the pollutant concentration in stormwater runoff. Aquatic microorganisms can also convert organic contaminants into less harmful compounds, which reduces the toxicity of runoff. Ponds and wetlands also provide conditions necessary for the degradation of certain organic compounds, pesticides, and herbicides. Degradation is not only performed by microorganisms but also by volatilization, hydrolysis and photolysis (United States Environmental Protection Agency, 1999).

1.3.3 Pollutant source reduction

Removing contaminants from impervious surfaces prior to a storm event is an effective non-structural way of limiting the amounts of pollutants contained in stormwater runoff. Limitation of fertilizer usage, frequent sweeping of streets and removal of trash and debris contributes to a reduction of pollutant sources. Another crucial point is the identification and elimination of illicit cross-connections between storm sewers and sanitary sewers (United States Environmental Protection Agency, 1999).

1.4 Types of Stormwater Best Management Practices

For managing urban stormwater runoff, there is a variety of measures available. BMPs for stormwater control can be categorized into two groups. The first group of BMPs are non-structural BMPs such as pollution prevention, education, and development practices with the aim of limiting the conversion of precipitation to runoff. Furthermore, measures like recycling and source control can reduce the amount of non-point source pollutants entering receiving streams significantly.

The second group are structural BMPs that are used to treat the stormwater at either the source or the point of discharge to receiving waters or the storm sewer system. In contrast to non-structural BMPs, structural BMPs are a more costly of end-of-pipe stormwater treatment. In the following section, very typically used structural stormwater management systems are described (United States Environmental Protection Agency, 1999).

1.4.1 INFILTRATION SYSTEMS

Infiltration is the process of retaining water before it infiltrates into the ground. The advantages of infiltration systems are both water quality control as well as quantity control. In addition, infiltration contributes to better water quality because pollutant removal occurs as water percolates through the various soil layers. Moreover, microorganisms can degrade organic pollutants of the infiltrated stormwater (United States Environmental Protection Agency, 1999)

1.4.2 DETENTION SYSTEMS

The purpose of a detention system is to release collected stormwater gradually to the storm sewer system or receiving stream. These facilities are designed to completely empty out between runoff events. The advantages of detention system besides reducing the peak discharge is the partial settling of particles in the stormwater. Detention of precipitate protects downstream areas from flooding to a certain extent and mitigates land erosion. Examples for detention systems are detention basins as well as underground pipes and tanks.(United States Environmental Protection Agency, 1999).

1.4.3 RETENTION SYSTEMS

Retention is defined as storage of stormwater runoff without subsequent discharge. This definition would limit the retention principle to infiltration and runoff evaporation, which is the case with infiltration trenches, wells or basins. However, retention facilities often have a permanent pool such as wet ponds and underground pipes or tanks. In contrast to detention, retention systems retain a runoff volume until it is displaced in part or in total by the runoff event from the next storm. Despite their distinct meanings, “retention” and “detention” are often used interchangeably.

Retention facilities can be very effective BMPs that provide both water quality and quantity control. Retention systems remove pollutants mainly by sedimentation because the permanent presence of water prevents accumulated sediment from re-suspension and washout. However, added biological and biochemical pollutant removal mechanisms contribute to a more efficient quality control of stormwater runoff. These biological removal mechanisms are provided by aquatic plants and microorganisms. Further pollutant removal in retention systems occurs due to filtration of suspended solids by vegetation, infiltration, biological uptake of nutrients by algae and aquatic plants, biological conversion as well as volatilization of organic compounds, and uptake of metals by plant tissue. The prevalent practice for stormwater retention is to create wet ponds, surface tanks as well as underground vaults, pipes and tunnels. In addition to the water quality improvements, wet ponds provide aesthetic value and aquatic and terrestrial habitat for many plants and animals as well (United States Environmental Protection Agency, 1999).

1.4.4 CONSTRUCTED WETLAND SYSTEMS

The natural functions of wetlands are utilized for this BMP system to aid in pollutant removal from stormwater. Pollutant removal in wetlands occurs through a number of mechanisms such as sedimentation, adsorption, absorption, filtration, volatilization, plant uptake and microbial decomposition. Water quality control is supplied as well since wetlands can store substantial amounts of water during a storm event. However, constructed wetland systems cannot be created everywhere because enough water must be available that sustains the aquatic vegetation during dry periods. Also, the performance of a wetland system can be heavily impaired by big loads of coarse sediment. Therefore, pretreatment implemented by a sediment forebay should be incorporated into the design of wetland basins or wetland channels (United States Environmental Protection Agency, 1999).

1.4.5 FILTRATION SYSTEMS

The primary goal of filtration systems is to improve water quality of stormwater runoff. However, quantity control can be implemented by providing additional storage volume in a basin or vertical storage volume above the filter bed. Filters are commonly used to treat runoff from parking lots and areas with high pollution potential. The utilized filtration medium can be sand, gravel or compost for the removal of particulate matter. Big advantages of filters are the low construction costs and the space-saving design. A filter is a BMP that is usually installed off-line for treating only a portion of the stormwater runoff while excessive water volume is bypassed. Surface sand filters and underground vault sand filters are the filter types that are in common use in the United States. Another type of filtration systems are biofilters systems, which are often referred to as rain gardens or bioretention areas. The following section provides a detailed description about the purpose, assembly, and main treatment principles of bioretention systems (United States Environmental Protection Agency, 1999).

1.5 BIORETENTION SYSTEMS

Bioretention areas offer benefits such as decreased surface runoff, increased groundwater recharge, and pollutant treatment (Dietz, 2007). These facilities are designed to mimic the functions of a natural forest ecosystem. Pollutant removal occurs through processes such as ion exchange, plant uptake, decomposition, adsorption, volatilization, and filtration. Additionally to the purpose of stormwater treatment, bioretention areas can be aesthetically pleasing and are a very useful application of landscape design (United States Environmental Protection Agency, 1999). The following section outlines the configuration of a typical bioretention system (Fig. 1) and explains the mechanisms that take place within each part of the structure.

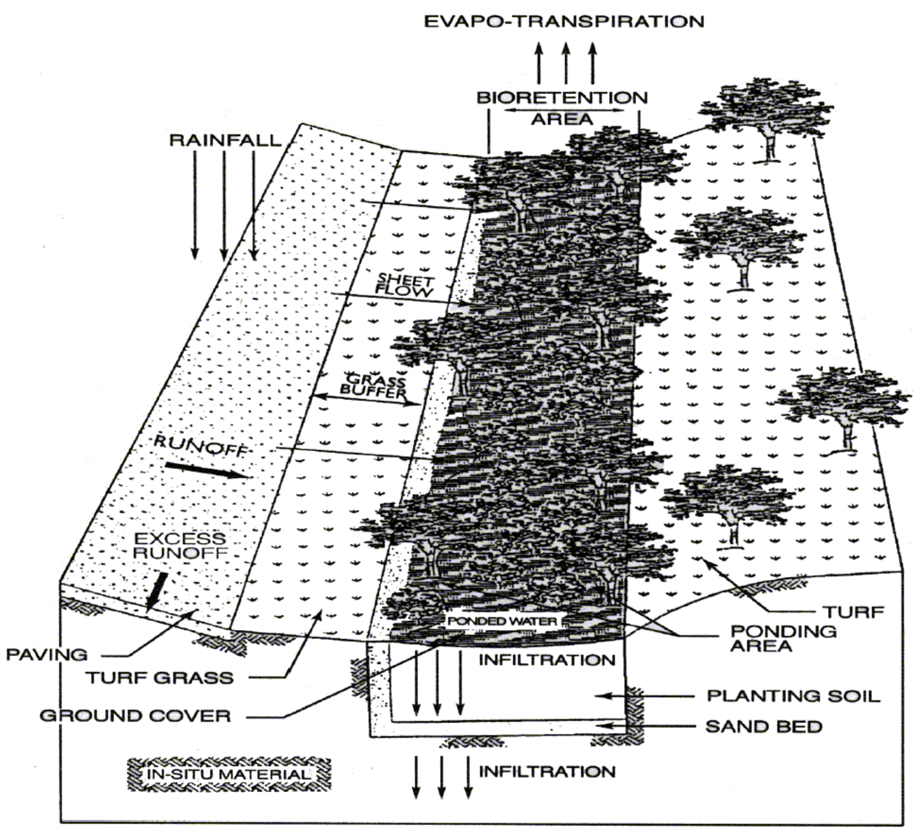

Fig. 1: Configuration of a large-scale bioretention area (Miami Conservancy District, 2009)

1.5.1 PRETREATMENT FILTER STRIPS

When the precipitate is conveyed over land by gravity, the runoff enters the bioretention area (Fig. 2) as sheet flow and is slowed down by gravel diaphragms or grass buffer strips (Claytor & Schueler, 1996). The decrease of horizontal velocity facilitates the infiltration into the ground. Moreover, filter strips work as a capture of coarse sediment particles before the stormwater enters the main bioretention system extend the life of the bioretention system (United States Environmental Protection Agency, 1999).

Fig. 2: Example bioretention application in an urban environment (Low Impact Development Center, 2007).

1.5.2 PONDING AREA

Stormwater runoff that cannot directly infiltrate into the soil is impounded in the ponding area. It provides surface storage, allows water to evaporate and supports settling of suspended particles during the detention period (United States Environmental Protection Agency, 1999).

1.5.3 SURFACE MULCH LAYER

The organic mulch layer retains moisture in the plant root zone, protects the soil bed from erosion, and supports plant growth and biological decomposition of organic matter. Additionally, this component of the bioretention system acts as a filter for fine particles that are still in suspension and maintains good conditions for microbial activity (Claytor & Schueler, 1996).

1.5.4 PLANTS AND SOIL BED

The plants that are necessary for proper stormwater treatment are provided with nutrients and water by the soil. The removal mechanisms of filtration, plant uptake and biological degradation take place in this part of the system. Fine soil particles are capable of removing pollutants through adsorption due to a large adsorption surface (United States Environmental Protection Agency, 1999). The main components of bioretention medium are compost and sand. The compost volume fraction (CVF), which represents the amount of compost relative to sand, typically ranges form 10-50%. The ability of soils to retain moisture and support vegetation is highly dependent on the organic matter in compost. It affects the physical, chemical, and biological properties of soils. Subsequently, organic matter plays a key role in the removal of dissolved toxic metals since it contains many functional groups (e.g. carboxyl and phenolic groups) that can complex with metal cations (Paus, Morgan, Gulliver, & Hozalski, 2014).

The aerobic conditions of the soil bed are a habitat to nitrifying bacteria of the genera *Nitrosomonas, Nitrosococcus, Nitrobacter* and *Nitrococcus*. These bacteria oxidize ammonia (NH_4^+) to nitrate (NO_3^-). The nitrate is then either absorbed by the plants or processed by anaerobic bacteria in the saturated zone (Prince George's County, 2007).

In this experiment, the soil mix Earthlite BioSwale ES™ Topsoil was used. The manufacturer promotes the product as being particularly well applicable to bioretention systems. It is an engineered blend that contains sandy loam, clay, compost and a mix of minerals, carbon and nitrogen sources and microbiological elements. The microbiological components stem from a blend called PermaMatrix® BSP Foundation, which accounts for 1 % of the soil mix Earthlite BioSwale ES™ Topsoil. This component contributes carbon and nitrogen with a C:N ratio of 27:1. The manufacturer attributes very efficient nitrate retaining properties as well as good moisture retention to the PermaMatrix® BSP Foundation blend. Part of the microbial inoculants of this soil medium consists of nitrogen fixing bacteria, as well as biochemical decomposers. (PermaMatrix Inc., 2015a). The soil blend contains fungal spores as well, which are declared in section 1.7. "Mycorrhiza".

1.5.5 SAND BED

The purpose of the underlying sand bed is to keep fine soil particles from washing out through the underdrain system. It also serves an additional filter to reduce the amount of suspended solids in the effluent (Claytor & Schueler, 1996).

1.5.6 GRAVEL UNDERDRAIN SYSTEM

With the aim of draining water from the planting soil bed into the surrounding soil or storm sewer pipe, the gravel bed is situated underneath the sand bed. If not used as saturated zone, the gravel helps to aerate the planting soil bed as well (United States Environmental Protection Agency, 1999).

The standard configuration consists of an under-drain pipe that is located at the bottom of the bioretention system. By raising the drainage above the bottom level, a permanently saturated zone can be created. There, anaerobic conditions -along with sufficient supply of carbon- provide a good environment for denitrifying bacteria. They reduce nitrate (NO_3) to gaseous nitrous oxide (N_2O) and nitrogen (N_2) which then returns to the atmosphere. Important bacteria genera which conduct denitrification are *Pseudomonas, Alkaligenes* and *Bacillus* (Prince George's County, 2007).

1.5.7 OVERFLOW SYSTEM

In an urban environment, bioretention systems should always be located off-line because they work primarily as water quality control. Therefore, larger storm flow volumes are conveyed to the receiving water or storm sewers. Overflow systems consist of a drainage catch basin or overflow channel above the ponding limit (Claytor & Schueler, 1996).

1.6 PLANTS

The proper function of a bioretention system is very dependent on the integration of plants. They reduce the amount of water by evapotranspiration and take up nutrients and metals. The selected plant species should be very robust and able to survive stresses such as frequent inundation during runoff events and drying between storm events (United States Environmental Protection Agency, 1999). Particularly effective plant species for nutrient removal include *Carex appressa, Melaleuca ericifolia, Juncus amabilis* and *Juncus flavidis*. These species underwent further testing in eastern Australia (Zhang et al., 2011).

The different types of phytoremediation include:

- Phytoextraction: Pollutant accumulated plants are employed to remove metals or organics from soil by concentrating them in the harvestable parts of the plant body.
- Phytodegradation: Plants and associated microorganisms are used to degrade organic pollutants.
- Rhizofiltration: Plant roots absorb and adsorb pollutants, mainly metals from water.
- Phytostabilization: Plants are used to reduce the bioavailability of pollutants in the environment.
- Phytovolatilization: The employment of plants to volatilize pollutants (Salt, Smith, & Raskin, 1998).

For the bioretention test runs that affected the results in this paper, *Carex stipata*, a species of the genera *Carex*, was used. *Carex stipata* -commonly known as sawbeak sedge or awlfruit sedge- occurs most of the United States and the southern provinces of Canada. This plant species thrives mostly in wetlands and savannas. The fact that it can withstand flooded soil conditions as well as short periods of drought makes this species valuable for wetland restoration, erosion control and stormwater projects (New Moon Nursery, 2017). According to Muthukumar et al (2004), *Carex stipata* shows capability of associating with mycorrhizal fungi, making the plant ideal for use in this experiment.

1.6.1 NUTRIENT UPTAKE

The uptake of macronutrients like nitrogen and phosphorus is performed by fine root hairs of the plant. Root systems that have a higher ratio of surface area to volume will more efficiently explore a larger volume of soil. This is the reason why mycorrhizae play a crucial role in the uptake of macronutrients (Schachtman, Reid, & Ayling, 1998)(Schachtman et al., 1998).

Nitrogen compounds are part of all plant tissues, where they are essential for growth, reproduction, and photosynthesis. In agriculture, nitrogen is the most commonly supplemented plant nutrient. It is estimated that 80 % of applied nitrogen through fertilizers is lost. The primary nitrogen compounds that are taken up by plants are ammonium (NH_4^+) and nitrate (NO_3^-). In contrast, organic nitrogen must first be decomposed (Braun, 2012). Since the bioavailability is dependent on factors such as precipitation, temperature, wind, soil type and pH, the preferred form of nitrogen depends on the plant's adaption to different conditions. In general, plants adapted to low pH and reducing soils take up ammonia or amino acids since nitrification is inhibited under these conditions. In contrast to this, nitrate is preferably taken up by vegetation adapted to higher pH and more aerobic soils (Masclaux-Daubresse et al., 2010). Due to the high concentrations of ammonium and nitrate inside root cells, plants must actively pump in the compounds. Due to assimilation purposes, nitrate is first reduced to ammonium in order to build up amino acids and proteins (Braun, 2012).

After nitrogen, phosphorus is the second most frequently limiting macronutrient for plant growth since it is component of essential molecules such as nucleic acids, phospholipids, and ATP. Plants can only mobilize soluble P species in the soil. The pH is the parameter that determines the form in which inorganic phosphorus (Pi) exists in solution. The uptake rates in higher plants have its peak between pH 5.0 and pH 6.0, where P is found in the form of $H_2PO_4^-$. The transport of Pi across the plasma membrane of the roots requires energized transport from the soil. Protons or positively charged ions enable the co-transport of Pi (Schachtman et al., 1998).

1.6.2 METAL UPTAKE

For metal uptake, phytoextraction is the primary removal principle that is conducted by plants. This process can be enhanced by adding synthetic metal chelates that create chelate complexes with metal ions. This increases the soluble metal concentration in the soil, hence, the plants can take up the metal significantly easier. Using this technique, the amount of metals such as lead can reach up to 1 % of the dry biomass in shoots.

Plants have evolved several strategies for increasing the bioavailability of metallic micronutrients because these nutrients have a high binding capacity to soil particles. The production of metal-chelating compounds (phytosiderophores) such as mugenic and avenic acids is important for binding iron and zinc. The phytosiderophores are released to the rhizosphere in order to chelate and mobilize Fe, Cu, Zn, and Mn. Metals that are chelated can be transported across the plasma membrane as a metal-phytosiderophore complex via specialized transporters. Another mechanism for solubilizing iron and other metals is to exude protons from the roots, which acidifies the rhizosphere.

In order to resist the toxicity, plants accumulate the metals in the vacuole of their cells, mainly in shoots. The vacuolar Zn accumulation has been confirmed in roots and shoots of the hyper-accumulator *Thlaspi caerulescens*. Hyper-accumulator plants show capability of accumulating potentially phytotoxic elements to concentrations that are more than 100 times higher than those found in non-accumulator plants (Sun & Davis, 2007). Plants also enhance their toxicity resistance by developing strong plasma membrane repair mechanisms that act against Cu-induced membrane damage (Salt et al., 1998).

1.7 MYCORRHIZA

Plants often live in symbiosis with fungi. The result is called mycorrhiza, which helps the plant to absorb water and assimilate nutrients. The mycorrhizal fungi are host specific and will only colonize certain plants. Following the colonization of the root, the fungus grows a vast network of hyphae throughout the soil that significantly increases the absorptive area (Fig. 3). Consequently, this interface of fungus and plant results in improved uptake of phosphate and ammonium ions, zinc, manganese, copper, and water by the plant. In response, the plant supplies the fungus with carbohydrates from photosynthesis (Buechel & Bloodnick, 2016).

For this experiment, the sedge species *Carex stipata* was used. Mycorrhizae in sedges are mainly of arbuscular mycorrhizal (AM) type and a few species (e.g. *Kobresia bellardii*) have ectomycorrhizal associations (Muthukumar et al, 2004).

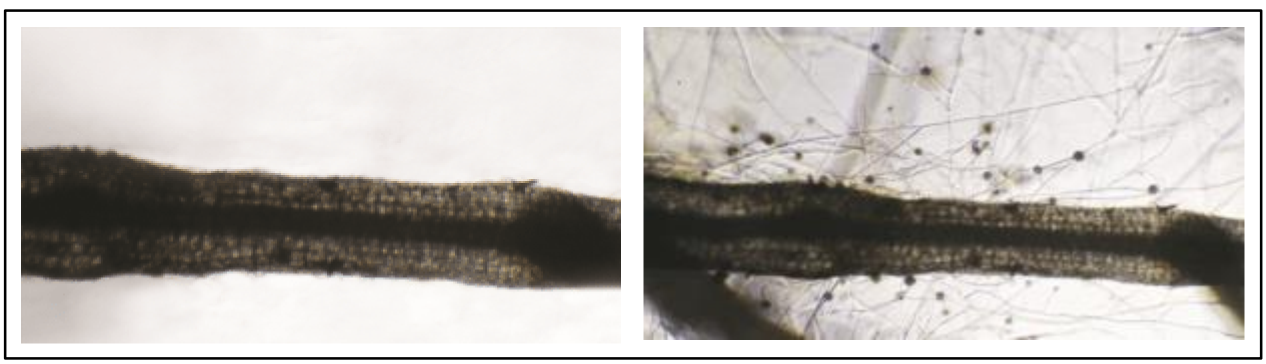

Fig. 3: Plant root without mycorrhiza on left, plant root with mycorrhiza on right (Buechel & Bloodnick, 2016)

1.7.1 ENDOMYCORRHIZA

The hyphae of endomycorrhizae penetrate and colonize epidermal and fleshy cortical cells of plant roots. Arbuscular endomycorrhiza produce structures within the cell layers of plants, called arbuscules and vesicles. When colonizing a plant root, the fungus secrets enzymes that allow the hyphae to penetrate the epidermal and cortical cells of the plant. After two to three days, arbuscules (Latin for tree) are formed within the plant cells. These arbuscules (Fig. 4) facilitate the transfer of nutrients and water within the plant cell.

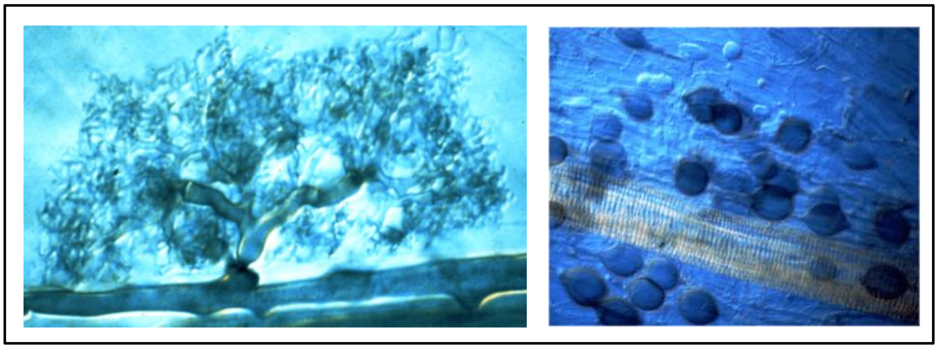

Fig. 4: Root cell containing an arbuscule on left, vesicles (round structures) between root cells on right (Buechel & Bloodnick, 2016)

A second special structure grown by fungi are vesicles. Vesicles are sac-like structures that are located between the cells and occur midway or at the terminal end of the hyphae. The purpose of vesicles is mainly to store lipids for the fungus (Buechel & Bloodnick, 2016). In the following, three subclasses of endomycorrhizae are outlined.

- **Arbuscular endomycorrhizae** (AM), often referred to as vesicular-arbuscular mycorrhiza (VAM), is the most common type of mycorrhiza. Members of the *Glomeromycota* are the primarily occurring fungi that form arbuscular endomycorrhizae. About 80 % of the plant species show mycorrhizal associations of this kind.
- **Ericoid endomycorrhizae** are structures that are formed by *Ascomycota*, associated habitually with plants (e.g. *Erica*) that endure moorlands and similar challenging environments. The fungi provide the host plant with absorbed nitrogen that originates form saprotrophically digested polypeptides.
- **Orchidaceous endomycorrhizae** exhibit enhanced degradation of complex carbon sources in order to provide their host with enough carbohydrates. The host plants are orchids that are highly dependent on fungi since the seedlings cannot conduct photosynthesis sufficiently (Moore, 2016).

1.7.2 ECTOMYCORRHIZA

Unlike endomycorrhiza, ectomycorrhizae produce a surface sheath of hyphae on the outer surface of the plant roots. However, the ectomycorrhizal hyphae penetrate the root surface and exhibit growth between the cortical cells of the plant (Buechel & Bloodnick, 2016). The sheath of fungal tissue can be more than 100 µm thick (Fig. 5). From this, a net of hyphae extends out to the soil domain to capture nutrients, which the root can access. Fungi that grow ectomycorrhiza belong mainly to the phylum of the *Basidiomyceta*.

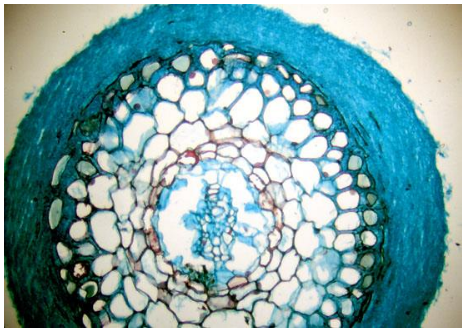

Fig. 5: Ectomycorrhiza builds a fungal sheath (blue layer) around a plant root (The New York Botanical Garden, 2003)

Among ectomycorrhizal fungi, there is the subclass of ectendomycorrhizal fungi that show the same characeristics as ectomycorrhiza but have extensive intracellular penetration into cells of the host plant as well. However, ectendomycorrhizae are restricted to the plant genera *Pinus*, *Picea*, and *Larix* (Moore, 2016).

1.7.3 MYCORRHIZAL FUNGI OCCURRING IN THIS STUDY

This present experiment uses a soil mix declared as Earthlite BioSwale ES™ Topsoil, which contains a variety of fungal spores. The dominating genera are *Glomus* (endomycorrhizal fungi) and *Rhizopogon* (ectomycorrhizal fungi). All occurring species are listed in Table 1 below.

Balmes at al used separate mycorrhizal soil inoculants for their self-assembled soil medium. The utilized product is "MycoApply Endo/Ecto granular inoculum" by Mycorrhizal Applications Inc. This product consists of spores of several different fungal species that are capable of forming mycorrhizal association with plants.

Fungi species in both utilized products are presented in (Table 1). Coincidentally, the identical species occur in the Earthlite BioSwale ES™ Topsoil as well in the City of Portland soil mix.

Table 1: Fungi species occurring in the present study (on the left) and the predecessor-study(on the right) compared. Fungi contained in the engineered Earthlite BioSwale ES™ Topsoil medium source from the soil component "PermaMatrix® BSP Foundation" (PermaMatrix Inc., 2015a). *Fungi utilized in the self-assembled City of Portland soil mix* (City of Portland, 2016) *stem from the "MycoApply Endo/Ecto granular inoculum"*(Mycorrhizal Applications Inc., 2011)*, an engineered mix of fungal spores.*

Mycorrhizal fungi contained in Earthlite BioSwale ES™ Topsoil	***Mycorrhizal fungi contained in the City of Portland soil mix***
Endomycorrhizal fungi:	Endomycorrhizal fungi:
Glomus intraradices	*Glomus intraradices*
Glomus mosseae	*Glomus mosseae*
Glomus aggregatum	*Glomus aggregatum*
Glomus etunicatum	*Glomus etunicatum*
Ectomycorrhizal fungi:	Ectomycorrhizal fungi:
Pisolithus tinctorius	*Pisolithus tinctorius*
Rhizopogon villosullus	*Rhizopogon villosullus*
Rhizopogon luteolus	*Rhizopogon luteolus*
Rhizopogon amylopogon	*Rhizopogon amylopogon*
Rhizopogon fulvigleba	*Rhizopogon fulvigleba*
Scleroderma cepa	*Scleroderma cepa*
Scleroderma citrinum	*Scleroderma citrinum*

2 MATERIAL AND METHODS

This experiment is part of a test series that was initiated by the Shiley School of Engineering at the University of Portland in order to compare different types of soil medium containing mycorrhizal fungi. Balmes et al performed the first test series from May 26, 2016 to June 29, 2016. The researchers used a bioretention soil mix specified by the City of Portland (2016). Half of the columns was inoculated with mycorrhizae. Simultaneously, the other half of the columns served as control bioretention systems containing no mycorrhizae. Succeding investigations were accomplished by this present experiment. The new bioretention columns are identically configured using the ready-to-use soil mix Earthlite BioSwale ES™ Topsoil.

2.1 EXPERIMENTAL SET-UP

The following section describes the chosen mesocosm design that represents a working large-scale bioretention system. Unlike a real size bioretention area, the utilized mesocosm design does not include filter strips that would normally slow down sheet flow of runoff and remove coarse particles. Moreover, column configuration does neither show a surface mulch layer nor a sand bed as exhibited in Fig. 1. The soil bed meets the purpose of the mulch layer and the drainage properties of a sand bed were sustained by the gravel underdrain system.

Three bioretention columns for the stormwater treatment study were built from Plexiglas pipes with a diameter of 30.5 cm. The pipes were sized to a length of 91 cm and one end of the pipe was closed with a fitting disc of Plexiglas (thickness of 1 cm). Two spigots where needed to collect the stormwater from the columns. One spigot was placed at bottom level of the column for full drainage, and the second was arranged 32 cm above the first spigot. This height also represents the level of the saturation zone of the mesocosm. The lower spigot is needed if full drainage of the water inside the column is required. The upper spigot was used for normal column operation during a test run. A PVC pipe with a diameter of 2.1 cm connected both spigots to each other. For collection of the effluent stormwater, a slit 2.1 cm PVC pipe was installed at the bottom of each column. The slits of the pipe were placed 2.54 cm apart and spanned the diameter of the column. With the purpose of preventing preferential flow along the sides of the column during a test run, the columns were sanded with P40 sandpaper.

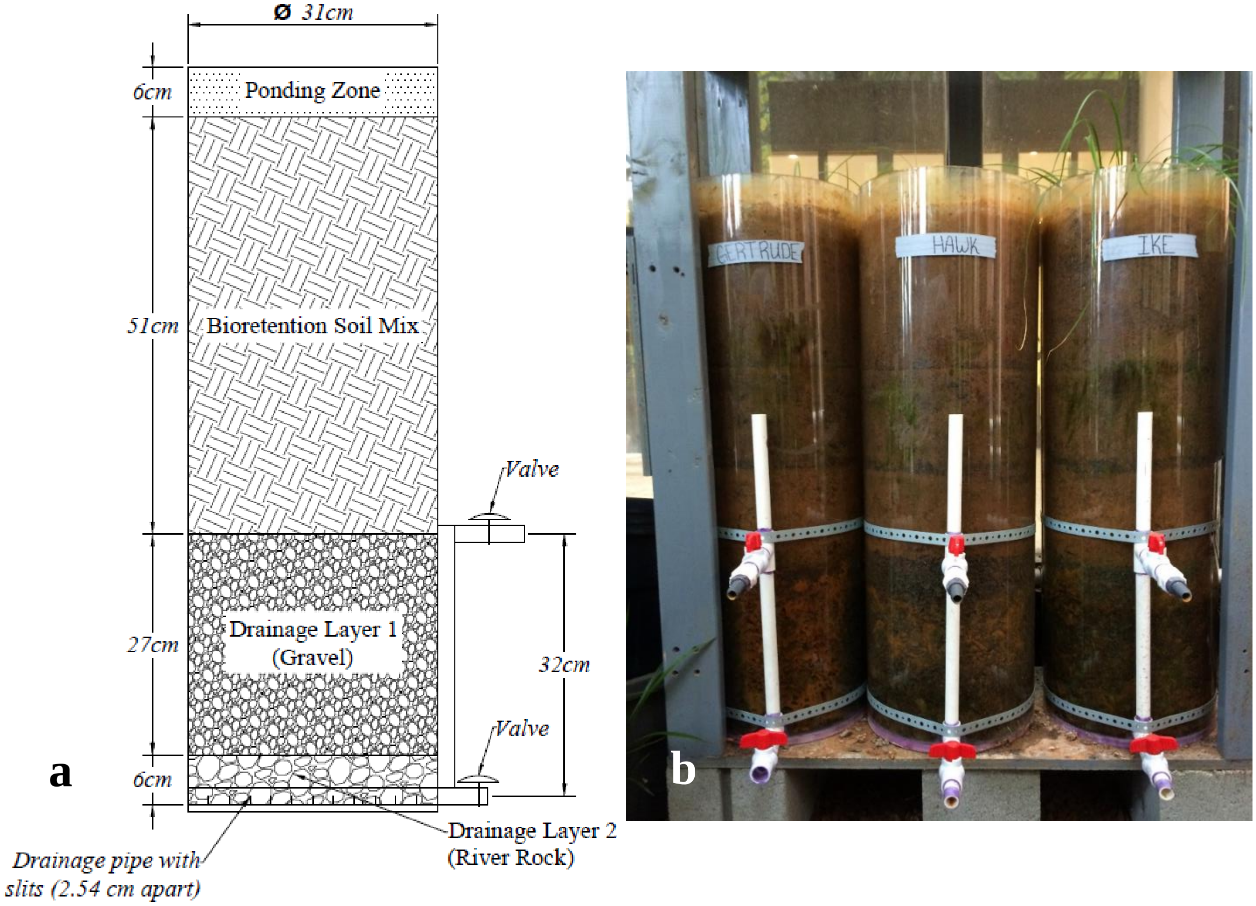

Fig. 6: Bioretention columns: (a) configuration and filter media, and (b) columns in greenhouse. In normal operation mode with saturation zone, the effluent was drained by opening the top valve. Note: The bottom of the columns consisted of a Plexiglas disc (thickness 1 cm). All pipes shown have a diameter of 2.1 cm.

As can be seen in Fig. 6, the bioretention system consisted of multiple layers which were placed into each column in the same fashion. All columns contained 84 cm of media which leaves a ponding space of 6 cm. The initial layer at the bottom consisted of small river rock and filled 6 cm of the columns. This layer was required to prevent the outflow slits of the drain form clogging. Then, 27 cm of 2 cm gravel was added to the columns. It was sieved using a 1.19 mm sieve with the aim of removing all fines that might cause clogging of the drain. The gravel layer in combination with the small river rock created the saturation zone. In addition, a filter was created by the gravel which keeps the soil in place when water is run through the columns. The gravel was flushed with 55 L until the effluent water became clear. After that, the soil mix Earthlite BioSwale ES™ Topsoil was placed in increments of 20 cm. The compaction of this layer was achieved by hand until it became firm. Succeeding the addition of soil to the columns, it was determined if further compaction of the soil was required by measuring the flow rate from the fully open upper spigot. Flowrates greater than 21 L/h indicated that the soil was not compact enough. In that case, water was run through the column in increments of 11.4 L until the flow rates equalized. The plant *Carex Stipata* was placed in a hole that was 15 cm deep with a diameter of 15 cm and the roots were covered with soil. Finally, the bioretention columns were placed on a wood rack inside a greenhouse on the University of Portland campus (Fig. 7).

Fig. 7: Arrangement of the bioretention columns along with the buckets that contained 21 L of stormwater (parking lot runoff) at the top and the collection buckets at the bottom.

In order to imitate real precipitation, a bucket sprinkler system was added to the set-up. As can be seen in Fig. 7, the buckets containing stormwater were positioned above the columns on the wood rack. The stormwater was piped to the top of the bioretention columns using a plastic tube that had multiple holes along the last few centimeters of the tube. The end of the tube was sealed. That way, stormwater was dispersed rain-like and could be impounded by the ponding zone of the bioretention system.

The establishment period for the bioretention systems lasted for 90 days. During that time, the columns were watered with tap water two to three times a week. Once stormwater treatment started, the bioretention columns were watered with stormwater up to twice a week. The treatment runs showed to be sufficient irrigation for a period of one week.

2.1.1 SOIL MEDIUM

For this experiment of the bioretention study, the soil mix Earthlite BioSwale ES™ Topsoil was utilized as bioretention soil medium. The soil was not altered in any way before the addition to the columns.

2.1.1.1 SOIL MEDIUM OF PREDECESSOR-EXPERIMENT

The soil medium for the previous experiment was a soil mix that is specified by the City of Portland. This blended soil consists of a fine-grained mixture of sand and compost with a pH ranging from 6 to 8.5 (City of Portland, 2016). The study used six identically built bioretention columns. However, three of which were inoculated with mycorrhizal fungi. The addition of fungal spores was performed in two steps. 36 ± 2 grams of "MycoApply Endo/Ecto granular inoculum" by Mycorrhizal Applications,Inc. were mixed with the top 15 cm of soil prior to addition. In a separate container, tap water was mixed with some of the available fungal spores which resulted in a slurry. The inoculation of the plant roots was accomplished by dipping the roots of the *Carex stipata* plant into this mixture. The placement of the plant was performed according to the description above (2.1 Experimental Set-Up).

2.1.2 STORMWATER

For the testing of the designed bioretention columns, Portland storm was used to provide realistic conditions. For this experiment, a total of 340 L of stormwater was collected on three days -March 3, 2016, September 29, 2016, and October 20, 2016. The stormwater collected is parking lot run-off from the University of Portland premises. The drain has a collection area of 1543.39 m^2 and is collected at the lowest elevation point in a catch basin in front of the health center. The exact collection spot can be seen in Fig. 8**Fehler! Verweisquelle konnte nicht gefunden werden.**.

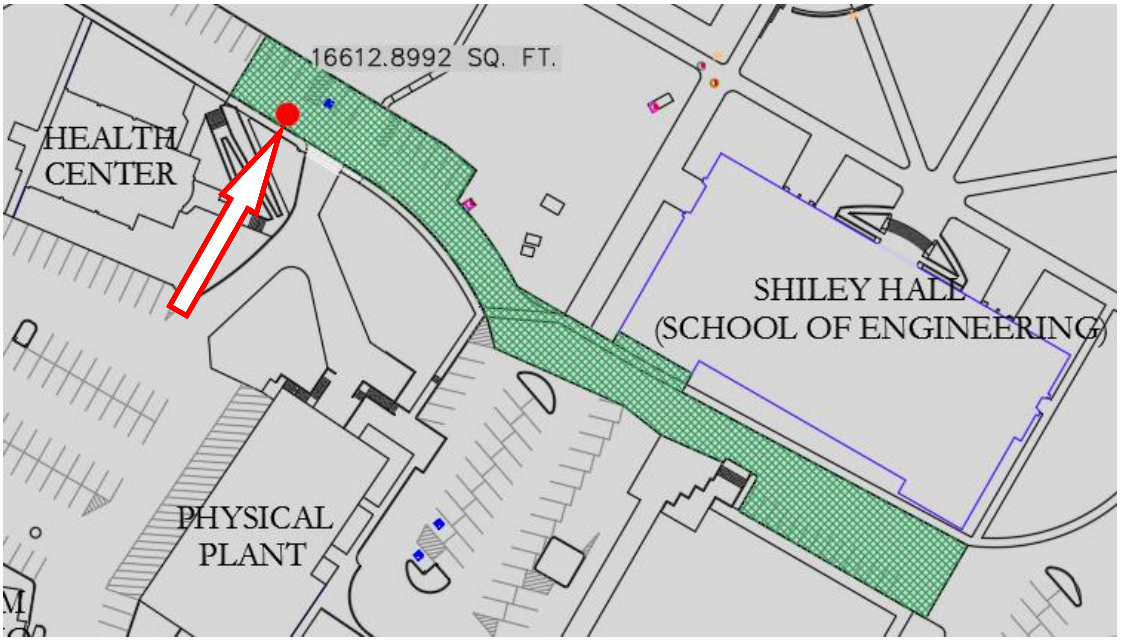

Fig. 8: Drainage area (green, 1543.39 m2) with catch basin (red dot) on campus at the University of Portland. Stormwater was collected from the catch basin.

Collection was performed immediately after or during a storm event. A sprinkler pump (Model 3YU60A by Dayton) was used for the collection of stormwater. Two tubes were placed on the intake and output of the pump. In order to avoid clogging of the pump mechanics, a 10.6 cm by 10.6 cm sheet of coarse filter was attached to the end of the intake hose using a rubber band. The purpose of this filter was to avoid clogging or damaging the pump due to leaves and other objects in the drain. Before starting the suction of stormwater from the catch basin, the pump was primed using tap water. The intake hose was placed into the drain and the output hose was placed into a nearby planter for the first 10 seconds of operation to ensure only stormwater is being collected. After that, the output hose was transferred to the container (21 L buckets or 200 L plastic rain barrel, respectively). Following the collection (Fig. 9), the stormwater was then stored inside a greenhouse where it was exposed to a temperature range from 10 °C to 35 ° until testing occurred.

Fig. 9: Collection of parking lot runoff from a catch basin on campus at the University of Portland. A sprinkler pump (Model 3YU60A by Dayton) was used.

2.2 Testing

2.2.1 Preparation for test runs

Bioretention test runs were conducted four times within five weeks. Samples were taken on September 30, 2016, October 10, 2016, October 24, 2016, and October 31, 2016. Since the columns were watered with tap water throughout the establishment period, the saturation zone had to be drained before the first test by opening the valve of the bottom spigot of the columns. Then, the saturation zone was being flooded again by running stormwater through each column until water flowed from the top spigot of the column drain system.

Prior to sample collection, all items for collection had to be free from phosphorus and nitrogen sources in order to avoid sample contamination. Three large 23-L distribution buckets with spigots and three 21-L catch buckets were scrubbed and rinsed with phosphate free soap (Liquinox by Alconox). After that, the buckets and tubing were rinsed using a 10% nitric acid solution and consequently rinsed three times with DI water.

2.2.2 COLUMN EXPERIMENTS

The distribution buckets were filled to the 21-L mark with previously collected stormwater that had been stored in plastic rain barrels. Then, the distribution buckets and catch buckets along with the tubes were arranged in a fashion that can be seen in Fig. 7. In addition, a 200 mL influent sample was taken and stored in a 250 mL acid-washed polypropylene sampling bottle. Then, the pH was measured using a calibrated pH meter (pH meter sension1 by Hach).

To start each test run, the valves of the spigots on each distribution bucket were opened all at the same time. The valves were opened to a certain extent so a ponding in each column was maintained at 5 cm. The time at each event –start of run, start of draining, end of ponding, end of draining- was recorded during the test. The flow rates for each column were determined using a 25 mL measuring cylinder at an interval of 15 minutes. To do that, the time it takes for the measuring cylinder to be filled to the 20-mL mark was measured. As soon as the flow ceased from the column spigot, a 200 mL sample was taken from each catch bucket using an acid-washed polypropylene sampling bottle. Right after taking the samples, the pH was measured for each of the three effluent samples. Then, the total effluent volume was measured using a 4000 mL polypropylene measuring cylinder. Consequently, the total flow rate was calculated by taking the average flow rates and the obtained total volume of effluent for each column. The remaining effluent was dumped into a sewer drain. The columns were allowed a minimum of seven days to stabilize between tests. Simultaneously, the chemical analysis of the effluent and influent samples was conducted within the following seven days. All samples were being stored at a constant Temperature of 4 °C until the analysis was performed.

Depending on how much stormwater was in stock, several test runs used stormwater from the same storm event. Table 2 below shows which stormwater was used for each test run, respectively.

Table 2: Date of stormwater along with date of test runs.

	Test run 1	**Test run 2**	**Test run 3**	**Test run 4**
Stormwater collection	*March 3, 2016*	*September 29, 2016*	*October 20, 2016*	*October 20, 2016*
Test run	*September 30, 2016*	*October 10, 2016*	*October 24, 2016*	*October 31, 2016*

2.2.3 TESTING OF STORMWATER SAMPLES

The tests on the collected stormwater samples were performed within seven days after the sample collection. The measured parameters were ortho-phosphate, total phosphorus, ammonia, nitrate, and total nitrogen. Moreover, the heavy metals copper and zinc were determined.

2.2.3.1 NUTRIENT TESTS

The nutrients were investigated using test'n'tube reactor kits by Hach. The methods performed with these test kits were conducted by following the instructions manual for Hach Water Testing Systems, respectively. Each test required a blank and a standard for reference. The blank was deionized water and the standard solution varied for each test. Devices required to perform these test are a spectrophotometer (DR 2700 by Hach) and a reactor (COD Reactor by Hach) with an adjustable temperature range from 0 °C to 150 °C. Depending on the test method, zeroing the spectrophotometer was achieved by using either deionized water or the sample. The concentration of the observed compound was measured using duplicates of influent and effluent samples. However, the concentrations of standard and deionized water were only measured once.

Ortho-phosphate, termed "reactive phosphate" was tested using method 8048. The range for this test is 0.02 mg/L to 2.5 mg/L. Instead of a 10 mL sample cell –as instructed by the manual- a 25 mL sample cell was used. Therefore, a matching reagent for 25 mL of sample was required. Adjustment of the spectrophotometer for zero reading was done using the sample. The utilized standard was a phosphate standard solution with a concentration of 1 mg PO_4^{3-}/L.

In order to determine the total phosphorus concentration of water within a range of 0.0 mg/L to 3.5 mg/L, the method 8190 total phosphorus test was utilized. Adjustment of the spectrophotometer for zero reading was done using the sample. The standard solution for this test was a phosphate standard solution with a concentration of 1 mg PO_4^{3-} /L.

The Ammonia Test was accomplished by using method 10023 with a measuring range from 0.0 mg/L to 2.5 mg/L. Standard solution was the Nitrogen/Ammonia Standard Solution by Hach with a concentration of 10 mg/L. In order to adjust the spectrophotometer for zero reading, the test was conducted on a blank consisting of deionized water simultaneously. In order to prepare the standard solution for testing, it needed to be diluted 1:10 to obtain a standard with a concentration of 1 mg NH_3-N / L.

The High Range Nitrate Test was conducted using method 10020. With this test, nitrate detection is possible from 0.0 mg/L to 30.0 mg/L. Adjustment of the spectrophotometer for zero reading was done using the sample. The standard solution was a Nitrate Nitrogen Standard Solution by Hach with a concentration of 1 ±0.01 mg N /L.

The Total Nitrogen Test was performed according to method 10071. The measuring range for this test is 0.0 mg/L to 25.0 mg/L. In order to adjust the spectrophotometer for zero reading, the test was conducted on a blank consisting of deionized water simultaneously. The standard solution was a Nitrogen/Ammonia Standard Solution by Hach with a concentration of 10 mg NH_3 /L.

2.2.3.2 HEAVY METAL TESTS

Heavy metal tests for zinc and copper detection were run on the AA-7000 Atomic Absorption Spectrophotometer (AAS) by Shimadzu. Prior to sample detection, five standards were made to calibrate the spectrophotometer in addition to a blank of deionized water. The concentrations of the standards were 10, 25, 50, 75 and 100 µg/L, respectively. The copper was measured at a wavelength of 324.8 nm. The temperature in the burner was increased incrementally starting at 60 °C, ending at 2500 °C, taking eight stages. The zinc was measured at a wavelength of 213.9 nm. Like for the copper, the temperature of the burner was raised from 60 °C to 2500 °C. The fuel gas was acetylene in combination with air and nitrous oxide. The purge gas used for the zinc and copper detection was argon gas. The AAS was set to do duplicates and calculate a sample average. The AAS software provided results based on the calibration curve of the standard solutions.

2.2.3.3 LEACH TEST

The soil medium used for the respective bioretention system was tested for eventual leaching of nutrients and heavy metals. In order to do so, the Synthetic Precipitation Leaching Procedure (method 1312 by the US Environmental Protection Agency) was performed in a non-quantitative manner. All containers for the leach test procedure had been acid washed.

The soil mix used for the leach test stems from the same batch as the soil that was used to assemble the bioretention columns. The soil had been stored for six months until sampling was performed. Approximately 300 g of soil sample was collected on November 16, 2016 from four spots of the soil pile. Then the soil was being stirred to mix well. 100 g of this mixed sample were weighed for the successive test procedure.

The leach test method 1312 guideline asked for a 60/40 weight percent mixture of sulfuric acid/nitric acid. It was prepared weighing 0.6 g of concentrated sulfuric acid by Lab Safety Supply Inc. and 0.4 g of 68-70 % nitric acid by VWR Analytical. The soil was added after assembling the extraction solution in a 2.5 L glass bottle which met the method 1312 requirements. Due to unavailability of the appropriate rotary extractor device, the extraction fluid was stirred for 18 hours on a stirring plate which maintained constant suspension of the soil. The temperature was held at about 20 ± 2 °C during the extraction period.

Following the extraction, the suspension was settling out for ten minutes before decanting 250 mL of the extraction fluid. Then, the extraction fluid was initially filtered using a Buechner funnel applying vacuum. The filter was a 70 mm qualitative filter by Whalman. Subsequently, the obtained solution was vacuum-filtered using a filter holder with receiver by Nalgene. The filter was a glass fiber filter, Type AP40 by Merck Millipor. It was only one run necessary to attain a clear solution ready for further investigation.

After the leaching procedure, the received filtrate was treated like the stormwater samples. Tube tests were performed to gain the amounts of PO_4^{3-}, TP, NH_4^+, NO_3^- and TN. Moreover, a test run on heavy metals using the AA-7000 Atomic Absorption Spectrophotometer (AAS) by Shimadzu was conducted (see methods 2.2.3.1 – 2.2.3.2).

3 RESULTS

Stormwater influent and effluent samples were collected on four dates: September 30, 2016, October 10, 2016, October 24, 2016, and October 31, 2016. Before the start of a test run, 200 mL of influent were sampled. As soon as all the effluent from one bioretention column was collected, a 200 mL sample was taken. Analysis of the samples was conducted within five days after collection. The samples were stored at 4 °C. Depending on the test kit, the standard solution and calibration method varied.

Total effluent concentrations for all analyzed nutrients (PO_4^{3-}, TP, NH_4^+, NO_3^-, TN) and heavy metals (Cu, Zn) are reported as an average value from three replicate bioretention columns containing the soil mix Earthlite BioSwale ES™ Topsoil. Standard deviations between these replicates were calculated and are presented for each analyte in the following. The variability between replicates was limited for all treatments. Additionally, the results of this experiment were compared to the outcomes of the predecessor-study.

3.1 PHOSPHORUS COMPOUNDS

3.1.1 ORTHO-PHOSPHATE

The measurement of ortho-phosphate PO_4^{3-} was performed according to Hach's test method 8048 ("Reactive Phosphate") using test'n'tube reactor kits by Hach. Adjustment of the spectrophotometer for zero reading was done using the sample. Ortho-phosphate concentration was measured in duplicate determination.

Influent concentrations for ortho-phosphate ranged from 0.31 mg/L to 0.81 mg/L. Effluent concentrations ranged from 0.07 mg/L to 0.13 mg/L which shows considerable reduction. The standard deviation represented by the error bars in Fig. 10 was limited to a maximum of 0.06 mg/L. The minimum reduction was obtained by the first treatment on 09/30/16 with 59 % less ortho-phosphate in the effluent than in the influent. The reduction efficiency of the replicate columns increased constantly until reaching a peak reduction of 91 % on the last test run on 31/10/2016 – one month after the initial test run.

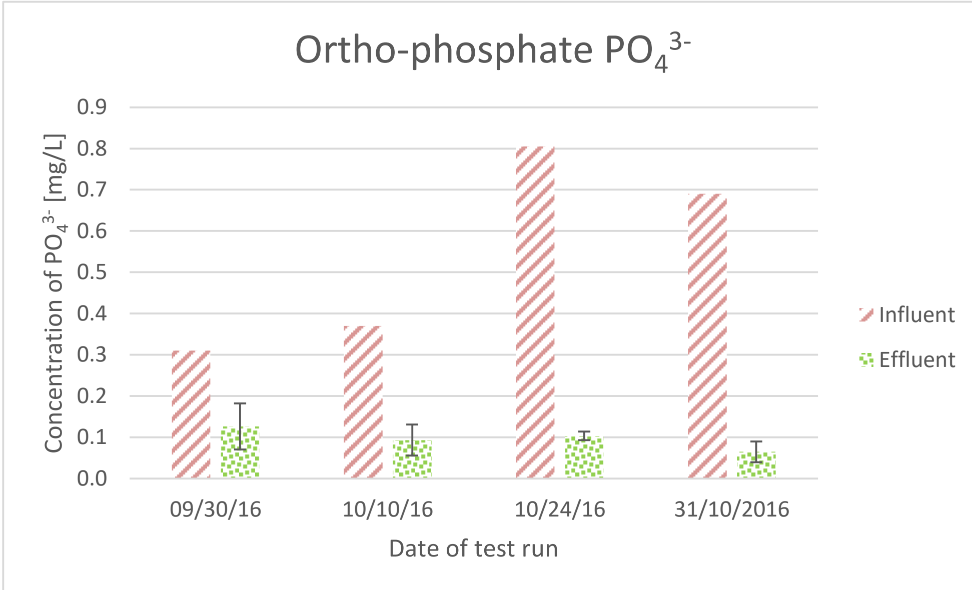

Fig. 10: Ortho-phosphate concentration before (Influent) and after (Effluent) treatment by bioretention columns containing Earthlite BioSwale ES™ Topsoil media.

In contrast, the concentration of ortho-phosphate in the effluent was remarkably higher for the previous experiment conducted by Balmes et al. The concentrations increased through the treatment by the bioretention columns with mycorrhiza by 43 times of the initial amount. The columns without mycorrhiza showed an average increase by 44 times the initial amount (unpublished data). A direct comparison of reduction efficiency between the different column configurations of this and the previous experiment is not exhibited in a Figure due to the substantial difference of the outcome.

3.1.2 Total phosphorus

The detection of total phosphorus was performed according to Hach's test method 8190 using test'n'tube reactor kits by Hach. Adjustment of the spectrophotometer for zero reading was done using the sample, respectively. Duplicate determination was applied.

The total phosphorus concentration in the influent ranged from 0.38 mg/L to 2.45 mg/L. Effluent concentrations ranged from 0.17 mg/L to 0.53 mg/L with a standard deviation of 0.13 mg/L maximum, indicated by the error bars in Fig. 11. The total phosphorus concentration was reduced by 84 % maximum on 31/10/2016. Minimum reduction occurred on 10/10/16, where the amount of total phosphorus in the influent decreased by 39% during the treatment.

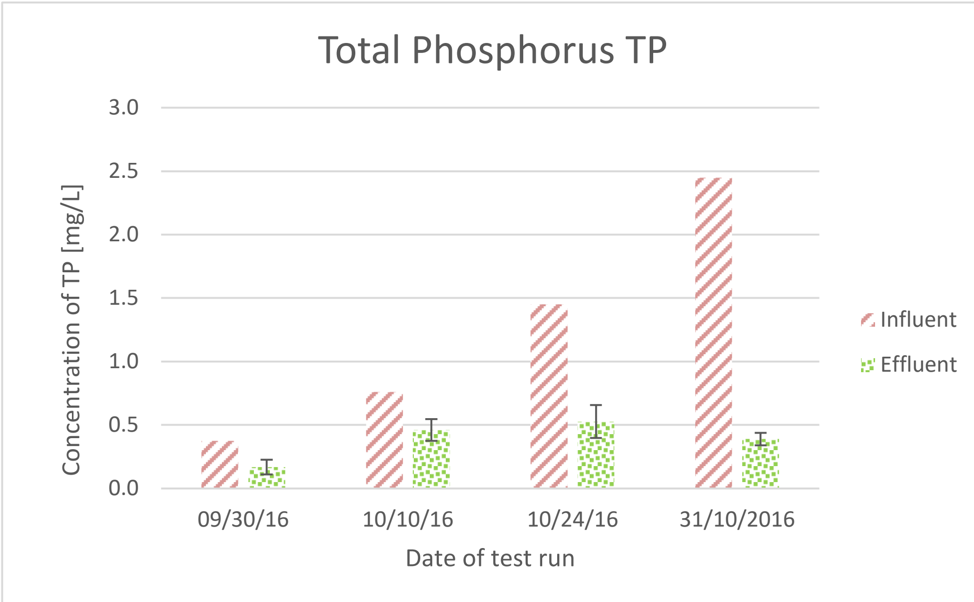

Fig. 11: Total phosphorus concentration before (Influent) and after (Effluent) treatment by bioretention columns containing Earthlite BioSwale ES™ Topsoil media.

Conversely, the bioretention systems having a City of Portland soil mix showed significant increase of total phosphorus due to the treatment. Columns containing mycorrhizal fungi led to an average increase by 45 times the initial amount. Similarly, control columns showed an average increase by 46 times the initial concentration (unpublished data). This result of the predecessor-experiment therefore precludes immediate comparison to the present outcomes of this experiment.

3.2 NITROGEN COMPOUNDS

3.2.1 AMMONIA NITROGEN

Ammonia concentration in stormwater samples was determined as ammonia nitrogen NH_3-N. The detection was performed according to Hach's test method 10023 using test'n'tube reactor kits by Hach. In order to adjust the spectrophotometer for zero reading, the test was conducted on a blank consisting of deionized water simultaneously. The ammonia nitrogen concentration was measured in duplicate determination.

Ammonia nitrogen detection resulted in influent concentrations ranging from 0.02 mg/L to 0.03 mg/L. Effluent concentrations ranged from 0.03 mg/L to 0.19 mg/L. However, concentration of ammonia nitrogen was not detected in one of three effluent samples due to under range measurement. The standard deviation represented by the error bars in Fig. 12 was 0.15 mg/L maximum. The concentration of ammonia nitrogen in the effluent was 1289% higher than in the influent at maximum (on 09/30/16). However, a slight reduction was measured when 83% of the influent concentration were found in the effluent sample from 10/24/16.

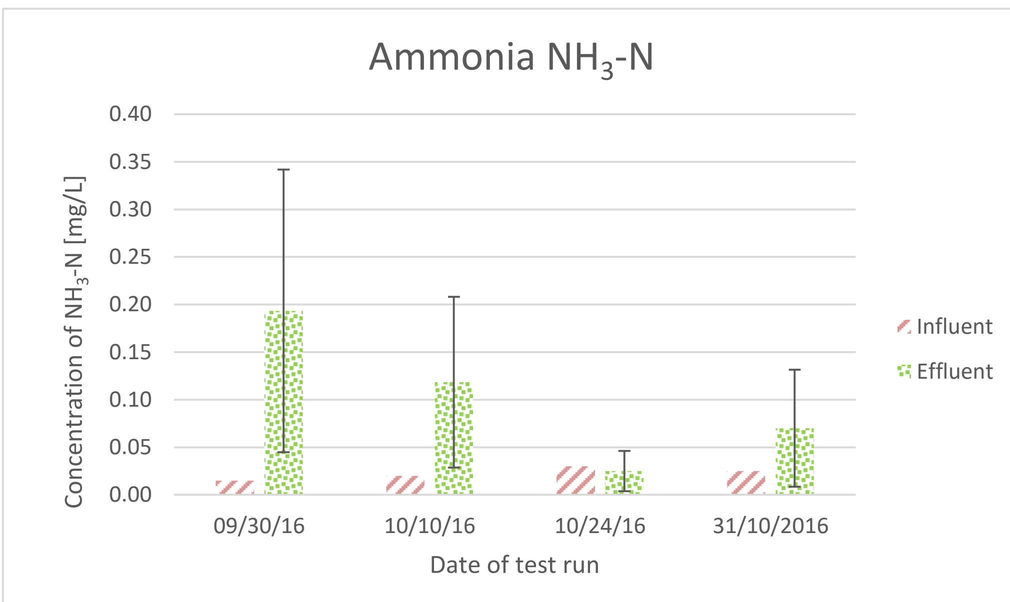

Fig. 12: Ammonia nitrogen concentration before (Influent) and after (Effluent) treatment by bioretention columns containing Earthlite BioSwale ES™ Topsoil media.

Increase of ammonia nitrogen throughout the treatment process was also observed by the experiment of Balmes et al. However, effluent concentrations were even higher. Sample testing led to measured concentrations which were on average 36 times higher than the influent concentration for stormwater that was treated by mycorrhiza inoculated mesocosms. The control systems showed an average increase of 23 times the initial amount (Fig. 13).

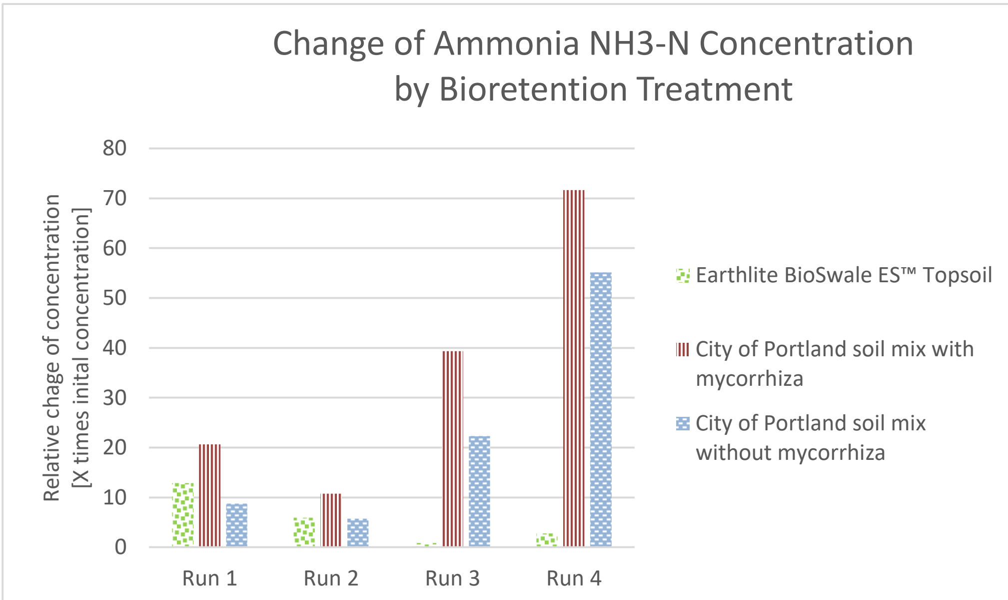

Fig. 13: The relative change of the ammonia nitrogen concentration by bioretention stormwater treatment is exhibited, respectively.

3.2.2 NITRATE

The measurement of the contained nitrate in the stormwater samples was performed according to Hach's test method 10020 using test'n'tube reactor kits by Hach. Adjustment of the spectrophotometer for zero reading was done using the respective sample. The ammonia nitrogen concentration was measured in duplicate determination.

Nitrate concentration in influent stormwater samples showed values ranging from 0.35 mg/L to 0.45 mg/L. Increased amounts were measured in the effluent which ranged from 2.07 mg/L on the last test run to 3.40 mg/L on the first test run. The standard deviation represented by the error bars in Fig. 14 was 1.02 mg/L maximum. Reduction did not occur, however, the amounts of nitrate in the effluent decreased the more test runs were conducted on the mesocosms (see Fig. 14).

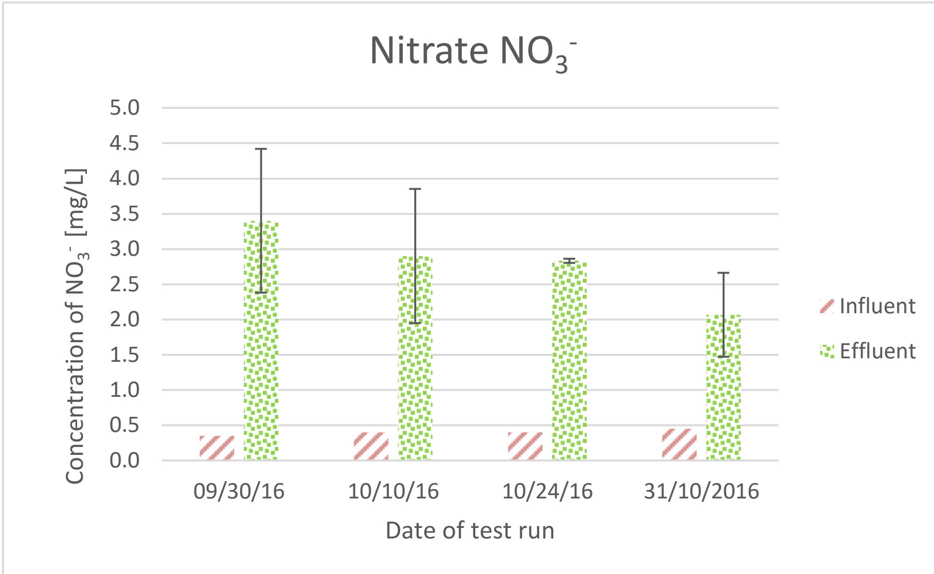

Fig. 14: Nitrate concentration before (Influent) and after (Effluent) treatment by bioretention columns containing Earthlite BioSwale ES™ Topsoil media.

In contrast, remarkable nitrate reduction occurred during the treatment by the bioretention columns of the previous study. Mesocosms containing the Earthlite BioSwale ES™ Topsoil show a vast increase of nitrate in the stormwater. The outcome shows an average nitrate reduction of 62% by mycorrhizal mesocosms. Control columns show slightly more efficient reduction of 64% (unpublished data). The significant difference in obtained data from either of the studies is shown in Fig. 15.

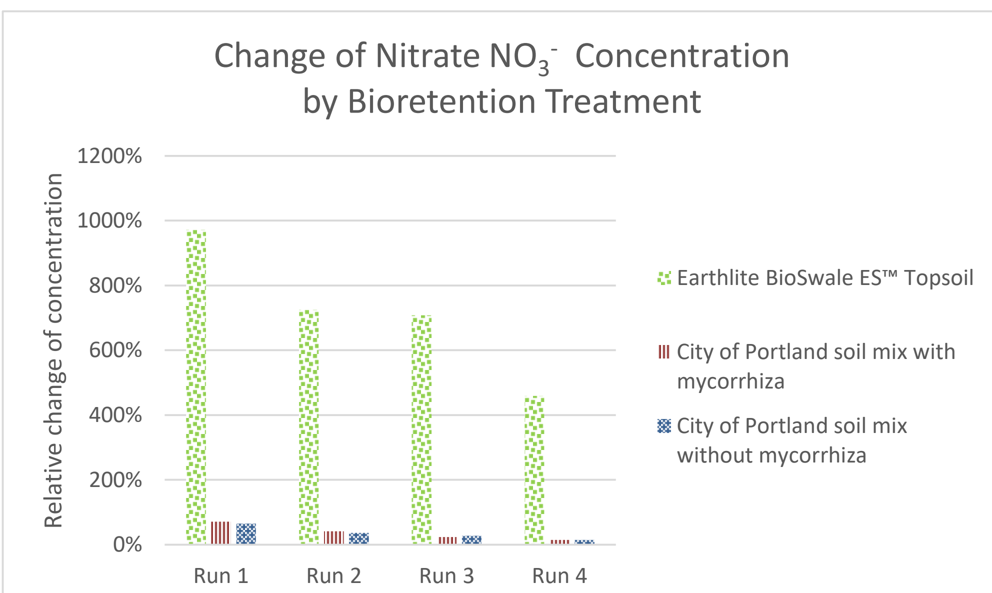

Fig. 15: The relative change of the nitrate concentration by bioretention stormwater treatment is exhibited, respectively.

3.2.3 TOTAL NITROGEN

The amount of total nitrogen in stormwater samples was detected by performing Hach's test method 10071. In order to adjust the spectrophotometer for zero reading, the test was conducted simultaneously on a blank consisting of deionized water. The total nitrogen concentration was measured in duplicate determination.

The test performed for total nitrogen detection resulted in influent concentrations ranging from 1.60 mg/L to 2.80 mg/L. Due to under range measurement, the influent concentration was not obtained from the influent sample of the first test run. Fig. 16 shows standard deviation in the form of error bars for the effluent samples of each test run with a maximum of 3.64 mg/L. For total nitrogen, the outcome of the tests show a general increase. The treatment led to an average increase of 177% but was limited to 211% of the amount found in the influent.

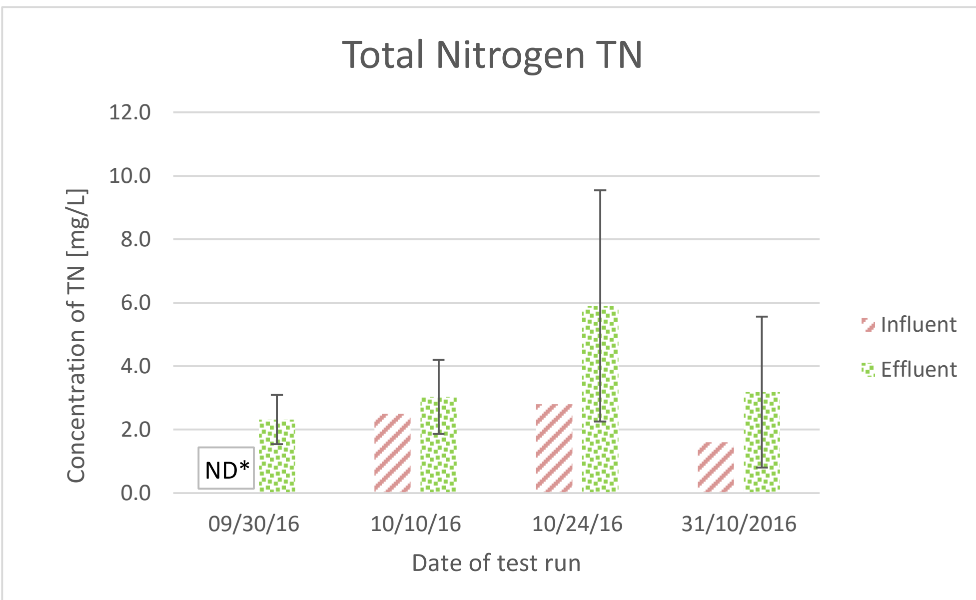

Fig. 16: Total nitrogen concentration before (Influent) and after (Effluent) treatment by bioretention columns containing Earthlite BioSwale ES™ Topsoil medium.
**Influent concentration not detected due to under range measurement.*

A comparable outcome was obtained by the preceding study. Those bioretention columns raised the amount of total nitrogen in the parking lot runoff by 247% for mycorrhiza-inoculated mesocosms (average value) and by 238% for the control systems (results of first test run excluded from calculation) (unpublished data). Fig. 17 compares the data obtained from this experiment and the predecessor-experiment. Particularly test runs 3 and 4 led to similar increase of total nitrogen in the treated stormwater.

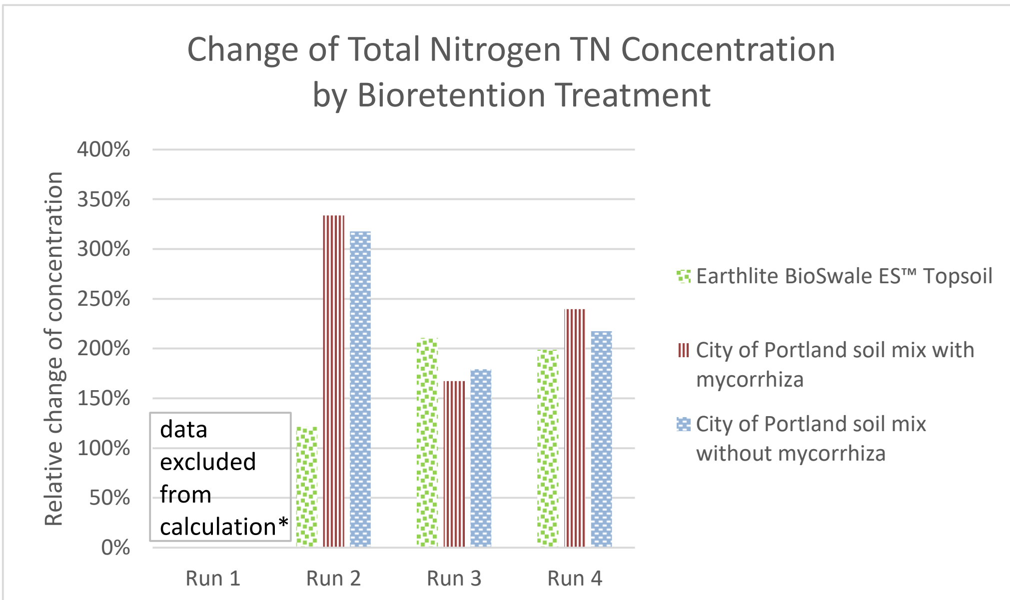

Fig. 17: The relative change by stormwater treatment is exhibited, respectively.
**The results from the respective first run were excluded from this presentation due to extraordinarily high values for the predecessor-experiment and a non-detect value for this experiment.*

3.3 HEAVY METALS

The heavy metals copper and zinc were measured in the parking lot runoff before and after treatment by bioretention. The detection of these compounds was achieved simultaneously using the AA-7000 Atomic Absorption Spectrophotometer (AAS) by Shimadzu. Calibration of the measure took place utilizing external standards that ranged from 10 µg/L to 100 µg/L of both copper and zinc, respectively. The AAS performed a duplicate determination automatically.

3.3.1 COPPER

Copper concentration in influent stormwater samples showed values ranging from 8.59 µg/L to 13.19 µg/L. Effluent concentrations ranged from 4.76 µg/L to 7.59 µg/L with a standard deviation of 1.35 µg/L maximum -indicated by the error bars in Fig. 18. The copper concentration was reduced by 64% maximum on 10/10/2016. Minimum reduction occurred on 10/24/16, where the amount of total phosphorus in the influent decreased by only 22% during the treatment.

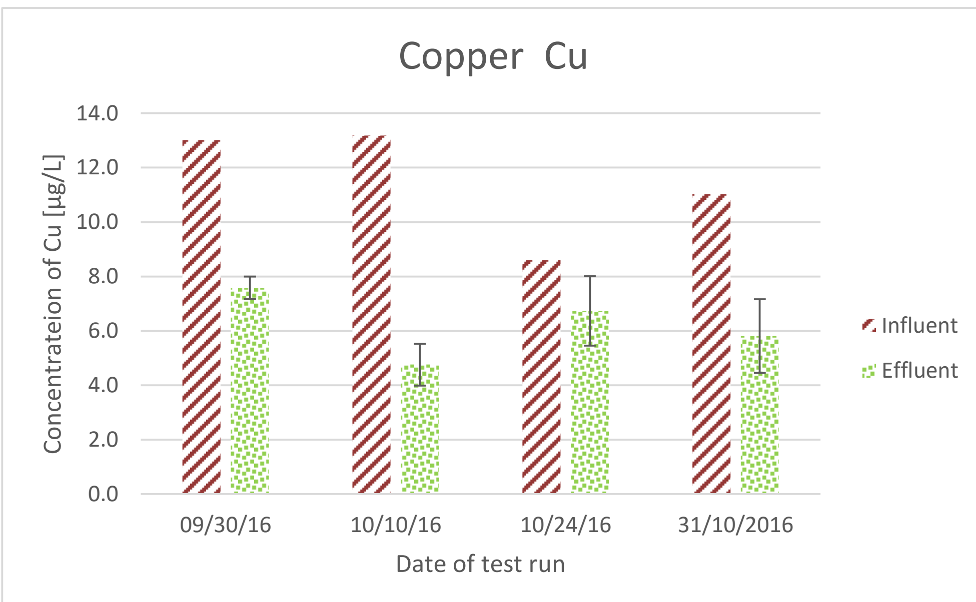

Fig. 18: Copper concentration before (Influent) and after (Effluent) treatment by bioretention columns containing Earthlite BioSwale ES™ Topsoil medium.

A comparable outcome was obtained by the predecessor study. The mesocosms were capable of removing copper up to an extent of 94% by the columns with mycorrhizal fungi (unpublished data). In contrast, the use of Earthlite BioSwale ES™ Topsoil soil medium showed removal rates ranging from 22% to 64% maximum. In general, the treatment efficiency was very inconsistent throughout all tests. Fig. 19 compares the data obtained from this experiment and the predecessor-experiment. However, copper removal occurred throughout all tests.

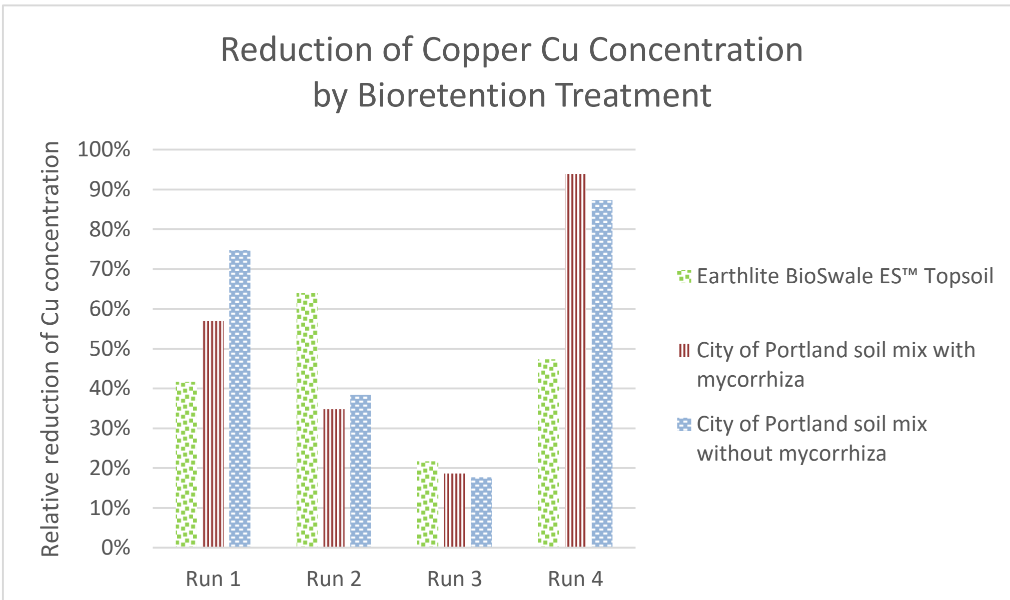

Fig. 19: Comparison of the relative reduction of copper concentration between the three tested column configurations using either the Earthlite BioSwale ES™ Topsoil, City of Portland soil mix containing mycorrhiza, or City of Portland soil mix free from mycorrhiza.

3.3.2 ZINC

Influent concentrations for zinc ranged from 27.33 µg/L to 56.67 µg/L. Limited removal occurred with effluent concentrations between 6.64 µg/L to 23.84 µg/L. The standard deviation ranged by such a large margin as 11.67 µg/L. Fig. 20 indicates a maximum relative reduction occurring on 10/10/16. This treatment led to a removal of 77% of the influent zinc concentration. Minimum reduction occurred on 10/24/16, where the amount of total phosphorus in the influent was reduced by 44% during the treatment.

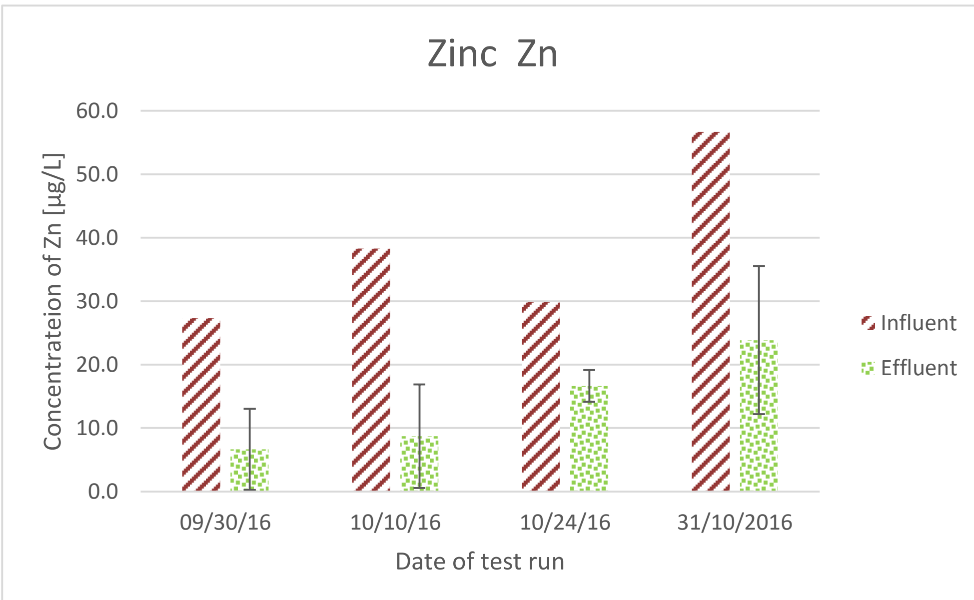

Fig. 20: Zinc concentration before (Influent) and after (Effluent) treatment by bioretention columns containing Earthlite BioSwale ES™ Topsoil medium.

A similar outcome was observed for the previous study where City of Portland soil for the mesocosm configuration was used. Treatment by the predecessor-mesocosms led to a zinc reduction of 96% maximum for both, columns with and without mycorrhizal inoculation. Except for the first run, the results were remarkably consistent at a lower limit of 88% reduction as can be seen in Fig. 21.In contrast to that, Removal efficiency for this present experiment ranged by such a large margin as from 44% to 77%.

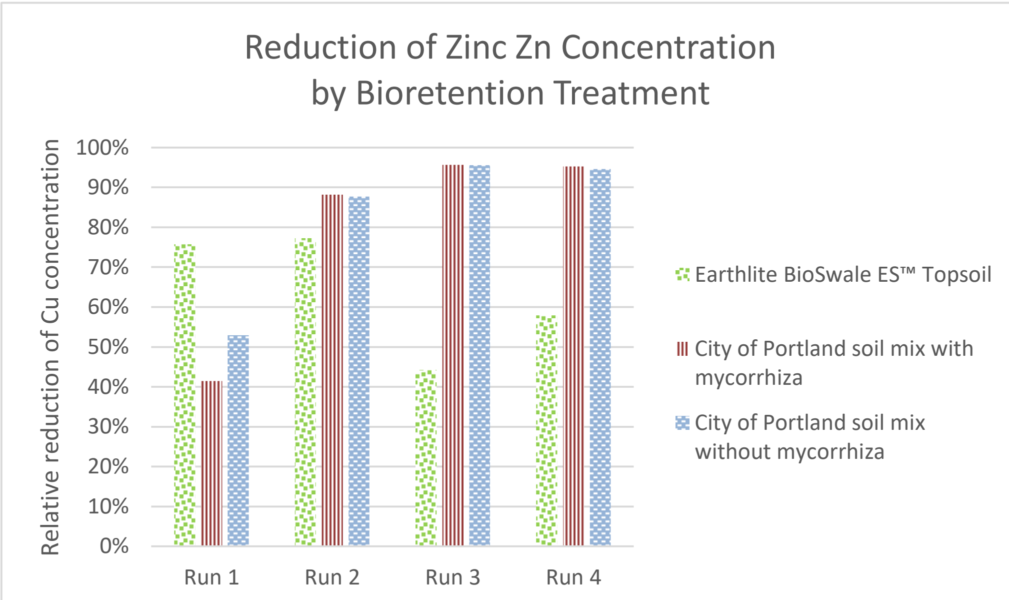

Fig. 21: Comparison of the relative reduction of zinc concentration between the three tested column configurations using either the Earthlite BioSwale ES™ Topsoil, City of Portland soil mix containing mycorrhiza, or City of Portland soil mix free of mycorrhiza.

3.4 Leaching compounds

After observing substantial amounts of nutrients in the effluent of both, this present experiment and the previous experiment, a leach test was conducted on the utilized soil in order to detect whether excessive amounts of a certain compound stem from the bioretention medium. Soil material underwent the Synthetic Precipitation Leaching Procedure (method 1312 by the US Environmental Protection Agency). By this procedure, soluble compounds of each tested soil were dissolved by an acidic milieu (pH 5 ±0.05). The test was not conducted in a quantitative manner. Hence, the sustained results give only a rough idea of how much of the component of interest was contained in the soil medium. After the leaching procedure, the received filtrate was treated like the stormwater samples. Test'n'tube reactor kits by Hach were then utilized to measure the amounts of PO_4^{3-}, TP, NH_4^+, NO_3^- and TN. Moreover, a test run on heavy metals using the AA-7000 Atomic Absorption Spectrophotometer (AAS) by Shimadzu was conducted.

The leach test allowed the analysis of the concentration of compounds contained in the soil medium, respectively. The results presented in Fig. 22 show that, in general, the Earthlite BioSwale ES™ Topsoil was leaching considerable amounts of nitrate. Conversely, the City of Portland soil mix was mainly leaching inorganic and organic phosphorus compounds (ortho-phosphate and total phosphorus) and less but noticeable amounts of nitrogen compounds (ammonia nitrogen, nitrate, and total nitrogen).

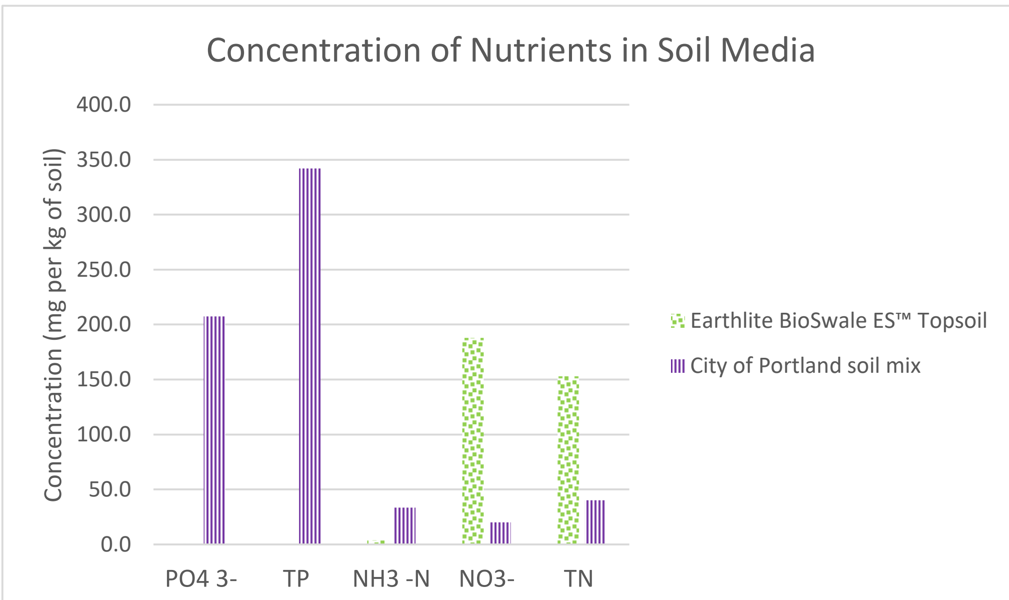

Fig. 22: Concentration of measured compounds (ortho-phosphate PO_4^{3-}, total phosphorus TP, ammonia nitrogen NH_3-N, nitrate NO_3^-, and total nitrogen TN) in Earthlite BioSwale ES™ Topsoil, and the City of Portland soil mix, respectively. The data was sustained by the Synthetic Precipitation Leaching Procedure (method 1312 by the US EPA).

Data from the leach test indicates that substantial amounts copper and zinc were contained in the soil (Fig. 23). The City of Portland soil mix, which was used in the previous study, contains 5.74 times more copper than the Earthlite BioSwale ES™ Topsoil. Moreover, the City of Portland soil mix contains 3.68 times more zinc as well (unpublished data). As mentioned above, the measured amounts in this experiment do not represent the exact quantity of each heavy metal contained. However, this test gives an estimate of how much of an element is contained in the soil media.

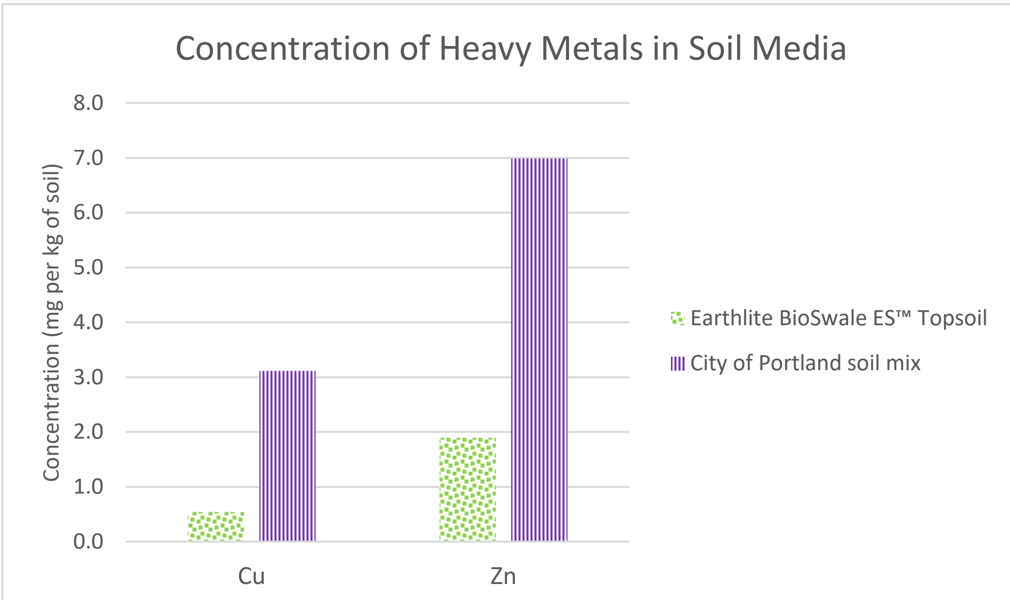

Fig. 23: Concentration of copper Cu and zinc Zn in the Earthlite BioSwale ES™ Topsoil and the City of Portland soil mix. The data was sustained by the Synthetic Precipitation Leaching Procedure (method 1312 by the US EPA).

4 DISCUSSION

4.1 PHOSPHORUS REMOVAL

For this present experiment, influent concentration for TP were exceeding the typical stormwater concentration range of 0.18 mg/L to 0.66 mg/L (Erickson et al., 2013). The average concentration found in collected stormwater was 0.86 mg/L. In contrast, the predecessor-study used stormwater containing 0.22 mg TP/L.

In the present experiment, ortho-phosphate was significantly better removed by the bioretention treatment than total phosphate. On average, 78% of the incoming ortho-phosphate was reduced in all four test runs. TP was examined to be decreased by 61%. This difference is likely due to better availability of inorganic forms of phosphorus for biological processes compared to organic forms of phosphorus (Mullen et al., 1998). Palmer et al. (2013) observed a removal rate of 58 to 81% in a similar configuration using soil medium which contained 15% compost and 60% sand. In the Lucas and Greenway study (2008), 91% of the initial total phosphate concentration was retained by sandy-loam-amended mesocosms.

Investigation shows remarkably increased reduction of ortho-phosphate along with higher influent concentration on 10/24/16 (0.81 mg/L in the influent and 87% reduction) and on 31/10/2016 (0.69 mg/L in the influent and 91% reduction). The fact that the highest removal of ortho-phosphate occurred on the last two test runs indicates that reduction capacity increases as the mesocosms become more established and infiltration rates decrease (Palmer et al., 2006). PH values of the influent were not increased on those test dates and leveled within a range of 6.18 to 6.94. Flow rates seemed not to impact the removal rate considerably showing values from 13.16 L/h (first run) to 18.35 L/h (third run).

Conversely, a substantially different outcome was obtained by the previous study by Balmes et al. The replicate columns led to an increase in ortho-phosphate removal by 44 times on average, regardless of whether mycorrhiza was present or not. Likewisely, total phosphorus concentration increased by 45 times at columns containing mycorrhizal fungi and by 46 times at control columns. A high amount of nutrient-rich compost in the City of Portland soil mix is a plausible cause for these findings. The conducted leach test following the US EPA method 1312 showed that 1 kg of City of Portland soil mix contains 207.5 mg PO_4^{3-} and 342.0 mg TP (compare Fig. 22). To elaborate, Bratieres et al. (2008) determined that 78% of phosphate was leaching from a compost-amended soil used for bioretention. Mullen et al. (1998) investigated that organic forms of phosphorus and nitrogen are often tied up in the soil structure. Degradation of total phosphorus in the soil is very slow. Hence, these constituents are frequently exported from the bioretention system.

The soil medium Earthlite BioSwale ES™ Topsoil has shown to be a good choice for the removal of phosphorus compounds -particularly ortho-phosphate. The enhanced phosphorus retaining properties are predominantly due to the composition of the soil medium since the same fungal species as for the Balmes et al. study were utilized, as can be looked up in section 1.7.3 and 1.7.4. In the predecessor-experiment, the fungi did not show capability of contributing to the removal of phosphorus compounds. Palmer et al. (2013) have shown that the vegetation, thus the mycorrhiza, influences the retention capacity of ortho-phosphate less than the maintenance of good soil medium and a saturated zone before the drain.

It can be seen that the selection of appropriate soil medium and appropriate organic matter (compost) is crucial when designing a bioretention area. As a result, media selection seems to play a greater role than inoculating the soil with mycorrhizal fungi. A significant impact of mycorrhiza is more likely to be observed in presence of overall good retention conditions. Therefore, further investigation on the role of mycorrhizae has to be performed after selecting a more efficient soil medium, which seems yet to pose the primary concern.

4.2 NITROGEN REMOVAL

In the US, typical concentrations of total (Kjehldahl-) nitrogen in urban stormwater are ranged within a margin from 0.6 mg/L 2.62 mg/L. The reported nitrate concentration ranges from 0.37 mg/L to 0.68 mg/L. Concerning ammonia concentration in urban runoff, only a value of 0.2 mg/L is known (Erickson et al., 2013). In the present experiment, the detected concentrations of these compounds were: 2.30 mg/L (TN), 0.40 mg/L (NO_3^-), and 0.02 mg/L (NH_3-N). This shows that all measured values are within the normal range, apart from ammonia nitrogen NH_3-N concentration, which was tenfold less. The preceding study by Balmes et al. (2016) found the influent to range within the same concentrations.

The present experiment shows that total nitrogen was increased by 177% on average (compare Fig. 16). Similarly, the predecessor-study observed an increase by 247% for mesocosms containing mycorrhiza and 238% for mesocosms where mycorrhizal fungi were absent. This leads to the assumption that the total nitrogen constituent in the soil consists of too complex molecules which cannot be degraded quickly enough before their export. Unlike the columns of Balmes et al., the export of total nitrogen from the bioretention columns did not decrease over time for the mesocosms of this experiment. On the one hand, this shows that the columns containing the City of Portland soil mix sustained better retention properties the longer the establishment period lasted. On the other hand, the outcome of the present experiment indicates that further observation is needed before the Earthlite BioSwale ES™ Topsoil can be approved as being an appropriate bioretention soil medium.

This experiment's results differ substantially from the previous study by Balmes et al. when it comes to the removal of nitrate. The study shows that their column configuration led to a significant nitrate removal by the treatment. Mesocosms with mycorrhiza retained 62% of the initial nitrate concentration. Mesocosms free of mycorrhiza retained 64% of the influent nitrate. This small difference cannot be attributed to the presence of mycorrhiza. In fact, the reduction is very likely due to the saturated zone, where denitrification takes place in an anaerobic environment. These findings are directly in line with the results of a 52-57% reduction found in the Palmer et al. study (2013) for columns having a saturated zone. Denitrification has very likely occurred in the present experiment as well since a saturated zone was incorporated into the column configuration. However, the amount of exported nitrate from the Earthlite BioSwale ES™ Topsoil medium exceeded the reduction capacity of the saturated zone. The leach test showed that approximately 188 mg of nitrate are contained in 1 kg of Earthlite BioSwale ES™ Topsoil. In comparison to this, the City of Portland soil mix only contained 20 mg/kg (Fig. 6). According to the manufacturer, one part of the Earthlite BioSwale ES™ Topsoil is a mineral-mycorrhizae- and humus containing mix called “PermaMatrix® BSP Foundation”. Manufacturer claims it “increases nitrogen delivery to plants [and] reduces nitrate leaching” (PermaMatrix Inc., 2015b). However, corresponding results were not obtained by the present study. In fact, this soil medium was proven to be highly inappropriate when nitrate retention is the main goal. Subsequently, the duration of establishment presumably has an impact as well. Lucas and Greenway (2011) observed nitrate retention of 90% in their study after two years of plant establishment. The establishment period of 90 days was likely too short and larger vegetation will be needed. Bigger plants would also facilitate the colonization of root cells by mycorrhizal fungi, which would further increase the reduction of nitrate and other nutrients as well.

The amount of ammonia nitrogen was significantly increased throughout all treatments. In this experiment, ammonia nitrogen concentration was increased six fold. A similar result was obtained by the predecessor-study, however, data was very inconsistent. Treatment with mycorrhizal inoculation led to an increase by 36 times the influent concentration. Mesocosms without mycorrhiza increased ammonia nitrogen by 23 times the influent concentration (Fig. 13). These findings correlate very well with the result of the leach test since the Earthlite BioSwale ES™ Topsoil contains 3.6 mg NH_3-N per kg soil whereas the City of Portland soil mix contains 33.5 mg/kg (unpublished data). The vast amounts of ammonia nitrogen in the effluent might also be the result of degraded more complex nitrogen compounds. The reason for the unsatisfactory ammonia nitrogen reduction can be due to bad aeration of the soil. Insufficient nitrification occurred before the ammonia had been transported to the anaerobic saturated zone. In order to prove this assumption, separate experiments need to be conducted where the same column configuration and soil medium are used but an underdrain replaces the saturated zone. Nevertheless, a saturated zone seems essential for efficient nitrate removal as has been proven by Palmer et al. (2013). There was no clear pattern showing any effect of mycorrhizal inoculation on the effluent concentration of ammonia nitrogen. It is very likely, that the presence of mycorrhizae does not have considerable impact on the ammonia removal as long as the soil medium causes reverse effects.

In conclusion, it can be said that the composition of the soil medium and the implementation of a saturated zone primarily affect the removal of nitrogen compounds in urban stormwater runoff. Although mycorrhizae can certainly enhance the uptake of ammonia and nitrate by plants, this cannot come into effect if the overall bioretention system does not sustain appropriate conditions.

4.3 HEAVY METAL REMOVAL

Heavy metals of concern in this study are copper and zinc. Typically, copper ranges between 16.00 µg/L and 34.00 µg/L in urban stormwater runoff (Erickson et al., 2013). Influent concentration in this study was 11.46 µg/L on average. Zinc values usually go from 111.00 µg/L to 203.00 µg/L as reported by Erickson et al. (2013). Average influent concentration in this study was remarkably lower, showing 38.04 µg/L. The Balmes et al. study (2016) found similar copper concentrations but higher amounts of zinc with 97.23 µg/L on average.

Throughout the experiment, copper retention was less effective than the retention of zinc. The mesocosms containing the soil medium Earthlite BioSwale ES™ Topsoil reduced copper by 44% and zinc by 64%. However effective, the predecessor-study reported copper reduction of 51% in columns with mycorrhiza. Control columns showed 55% copper reduction.

Similarly, replicate columns with Earthlite BioSwale ES™ Topsoil had less reduction capacity than columns with the City of Portland soil mix when it comes to zinc retention. Mesocosms of this experiment decreased the zinc amount by 64%. Compared to that, columns with mycorrhizal fungi led to a reduction of 80%. The absence of mycorrhizal fungi resulted in 83% average zinc reduction. Again, mycorrhizal fungi did not have considerable impact on the heavy metal treatment. Hence, there is no direct dependence on mycorrhizal fungi in the soil media. In fact, the choice of the right soil medium poses the crucial point. Zinger et al. (2013) observed remarkably good retention properties using loamy sand medium in a similar column configuration with a saturated zone. They obtained zinc removals exceeding 95% and their copper removal stabilized at 90% retention after the first two runs. According to Zinger et al. (2013), the majority of heavy metals in the stormwater runoff was removed by mechanical filtration since many heavy metals are particle-bound. Therefore, loamy sand should be considered if heavy metal removal is the primary concern.

4.4 IMPLICATION OF SOIL MEDIA SELECTION ON EUTROPHICATION OF WATER BODIES

The outcome of the present experiment and the results of the Balmes et al. study showed a remarkable difference in their treatment properties. In fact, the Earthlite BioSwale ES™ Topsoil medium was very effective at removing ortho-phosphate from the influent stormwater. 78% of the initial concentration were retained by those mesocosms. However, nitrate concentration was increased sevenfold. In contrast to the Earthlite BioSwale ES™ Topsoil medium, the City of Portland soil mix showed inverse properties. It was capable of removing 26% (regardless of mycorrhizal inoculation) of the nitrate contained in the stormwater runoff and increased the ortho-phosphate concentration by 43 times (regardless of mycorrhizal inoculation).

At first glance, the Earthlite BioSwale ES™ Topsoil seems suitable to applications where the main goal is phosphate retention. Thus, the City of Portland soil mix would be appropriate if nitrate removal is the primary concern. Subsequently, direct impacts on the eutrophication of water bodies can be mitigated by the right choice of bioretention soil medium, assuming that all ground material is inert, which the effluent passes on its way to receiving waters. The N:P ratio is crucial for the growth of phytoplankton and can therefore affect eutrophication. For ideal growing conditions, the N:P ratio in the water is 7:1 by weight. N:P ratios above 7:1 indicate a phosphorus limitation whereas N:P ratios below 7:1 show that nitrogen is the limiting constituent for algae growth (Ekholm, 2008). Effluent from the treatment with Earthlite BioSwale ES™ Topsoil exhibited a N:P ratio (NO_3^- : PO_4^{3+}) of 28.84 : 1. Therefore, outflowing water is phosphorus limiting to phytoplankton. On the contrary, the City of Portland soil mix (regardless of mycorrhizal inoculation) leads to an effluent N:P ratio of 0.04 : 1, which means that this effluent water is highly nitrogen limiting.

Generally, phosphorus limitation should be preferred since cyanophyta are capable of fixing nitrogen N_2 from the air, which would compensate the positive effects of nitrogen limitation. Hence, the Earthlite BioSwale ES™ Topsoil is the preferable soil medium for mesocosm design when the City of Portland soil mix is the only other option.

5 SUMMARY AND OUTLOOK

The effect of mycorrhizal inoculation in bioretention mesocosm was tested using column trials. The columns contained Earthlite BioSwale ES™ Topsoil medium, which was arranged in the same fashion as the soil medium in the Balmes et al. study (2016). The comparison of the results of both studies shows that the right choice of soil medium is the prevalent factor for nitrogen (NH_3-N, NO_3^-, TN) and phosphorus (PO_4^{3+}, TP) retention. Heavy metal removal (Cu, Zn) occurred in all columns similarly. Mycorrhizal fungi did not seem to impact the removal of nitrogen and phosphorus significantly. The positive effect of mycorrhizae is more likely to be observed in presence of overall good conditions for nitrogen and phosphorus retention.

The results of this study suggest that further investigations need to be conducted in the long run. The mesocosms are likely to perform better after a longer establishment period. Subsequently, bigger vegetation eventually correlates also with enhanced mycorrhiza-facilitated pollutant uptake.

Additional conclusions can be drawn from the utilized soil media. In order to recommend the implementation of mycorrhizae into bioretention cells, more research must be completed on the long-term effects of mycorrhiza-inoculated columns. The University of Portland plans a successive study to test if the pattern of phosphorus leaching by the City of Portland soil mix continues to be reduced after one year. If this is not the case, the Earthlite BioSwale ES™ Topsoil medium should be preferably utilized since phosphorus limitation is favorable for water bodies in order to prevent eutrophication. However, the manufacturer of this engineered soil mix needs to be informed about the great amounts of nitrate that are leaching from it. In order to make this soil medium more suitable for bioretention areas, the source for nitrate leaching needs to be detected.

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