Media, Microbes and Macrophytes – their role in improving the effectiveness of bioretention systems. Getting the right mix.

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Abstract
Bioretention systems are becoming the most widely used stormwater treatment technologies for many local authorities. Their popularity is based primarily on existing models that demonstrate very high pollutant removal efficiencies and their small physical footprint compared to wetland systems. Structurally bioretention systems consist of a basin or trench filled with media and planted with vegetation. Physical, chemical and biological treatment processes facilitated by the media, microbes and macrophytes improve stormwater quality as it percolates through the media. Limited research has been conducted on the suitability of media and yet media specifications abound! There is little published research on the long term performance of media or plants on nutrient removal; however laboratory studies have shown that media can become rapidly saturated with phosphorus. Media with organic matter can also leach nutrients.

Our research at Griffith University using experimental bioretention mesocosms has been focussing on the effectiveness of different media types for nutrient removal. Our 5 year old trials have shown that sand, loam and gravel media have now become P saturated, however vegetation improves PO₄ removal. Vegetation also improves N removal by uptake of NOx. Microbes play an important role in N transformation involving ammonification of organic N, nitrification and denitrification. By applying accelerated loading rates of N and P in our “stormwater” we have a good indication of the P saturation capacity and hence long term removal efficiency. We have also been quantifying plant biomass to assess the role of different macrophytes in nutrient uptake and bioaccumulation.

Introduction
Bioretention systems (also known as biofiltration systems, biofilters, rain gardens, biopods) are becoming the most widely used stormwater treatment technology in Water Sensitive Urban Design (WSUD) for many local authorities. As an ecotechnology to improve stormwater quality and protect downstream ecosystem health their use as a Stormwater Quality Improvement Device (SQID) in the past 6 years has surpassed that of ponds and wetlands. Their popularity is based primarily on the existing MUSIC model that demonstrates very high pollutant removal efficiencies for nutrients, heavy metals and suspended solids; and their small physical footprint. Compared to wetland systems, they are more space efficient in removing pollutants, in particular nitrogen (Somess and Moore, 2007). Structurally bioretention systems consist of a basin or trench filled with media (“engineered soil”) and planted with vegetation. Physical, chemical and biological treatment processes facilitated by the media, microbes and macrophytes (vegetation) improve stormwater quality as it percolates through the media. However, do we really know enough science about these systems especially their long term performance in pollutant removal?

Research in Australia mainly through Monash University’s Facility for Advancing Water Biofiltration (FAWB) and Griffith University, has increased our knowledge in understanding pollutant removal processes however, design guidelines with respect to media, microbes and macrophytes still require further research in order to maximise long term performance efficiency of these systems. This paper is in two parts. The first part will review current guidelines and knowledge with respect to media, microbes and macrophytes, whilst the second part will present a case study of the findings of our current research being conducted at Griffith University.
Filter Media

The filter media (engineered soil) plays several vital roles in the performance of bioretention systems by facilitating physical, chemical and biological processes for contaminant removal. It controls the rate of stormwater infiltration and retention time, it filters sediment and particulates, it provides sorption surfaces for nutrient and heavy metal removal, it provides surfaces for biofilms (attached microbial communities), it provides a substrate and nutrient source for microbes (bacteria, fungi, protozoans, algae) and macrophytes (plants).

Characteristics of media which should be considered for contaminant removal include: hydraulic conductivity, particle size and porosity, moisture, organic matter and nutrients, sorption properties. These will be addressed below.

Hydraulic Conductivity: This governs the rate of flow of stormwater through the media and hence determines retention time for effective contact with media, microbes and macrophyte rhizosphere (root zone). The particle size of the media influences the hydraulic conductivity and the Australian Runoff Quality (ARQ) Guide to WSUD (2006) gives the hydraulic conductivity for a range of particle sizes.

Australian Runoff Quality (2006) and Healthy Waterways (2006) recommend 50 – 300 mm h⁻¹. FAWB (2007) suggests that 100 - 400 mm h⁻¹ is optimal and should never be below 50 mm h⁻¹. GCCC guidelines (2006 & 2007) suggest a range of between 50 – 200 mm h⁻¹, but indicate that 180mm h⁻¹ is optimal. They state that “under no circumstances will values of greater than 500mm h⁻¹ be accepted”.

The Healthy Waterways guidelines (2006) recognise that higher rainfall intensities are experienced in SE Queensland, thus requiring more efficient drainage of bioretention systems and hence media with a higher hydraulic conductivity should be used. However they recommend that maximum hydraulic conductivity should not exceed 500mm h⁻¹ and preferably should be less than 200mm h⁻¹. No lower limit is actually specified in the 2006 guidelines however in the Healthy Waterways WSUD Technical Design Training Notes (2007) a range of 50 - 300mm h⁻¹ is recommended.

The FAWB Guidelines suggest that filter “media with a hydraulic conductivity greater than 600mm h⁻¹ are unlikely to support plant growth due to poor water retention”. The 2006 Healthy Waterways guidelines also recognise that moisture retention is important to sustain plant growth. However, plant survival will depend on the type of species planted and the climatic conditions of the geographical location. (Also see section on Macrophytes). In our Brisbane mesocosm experiments Pennisetum alopecuroides (Swamp Foxtail grass) and Callistemon pachyphyllus (Wallum Bottlebrush) survived best in sand and sand-gravel mesocosm with once weekly watering (Table 4) – these systems had a gravel underdrain and the lower 70 - 75mm of the media profile always maintained the highest moisture content. Thus, plants with roots deep enough to reach the underdrain, not only survived better in the drier weather but also had a higher biomass. Thus media should have enough moisture holding capacity to support plant growth during periods without rainfall whilst enabling stormwater to percolate though during storm events. (see section on Moisture)

Particle Size and Porosity: Particle size influences porosity and hydraulic conductivity. It also determines the surface area for microbial biofilm colonisation (Larsen and Greenway, 2004; Stottmeister et al, 2003; Tietz et al, 2007). Porosity is also important for soil aeration providing oxygen in the airspaces for plant roots and aerobic microorganisms to breathe. During storm events the water displaces the air, and in poorly drained media causes water logging and anaerobic conditions. Wetland macrophytes can cope with water logging as oxygen is transported from the special aeration tissue in the leaves / shoots down to the roots (Greenway, 2006). Melaleuca trees survive in water logged soils by storing oxygen in their bark which is transported
to the roots and by having many surface adventitious roots around the tree base (Bolton and Greenway, 1999, Greenway and Bolton, 2002).

Particle size and porosity are not only important for microbes and macrophytes and hence biological processes for contaminant removal (nutrient and metal uptake), they are also important attributes for physical processes providing filtration of sediment and associated chemically bonded contaminants e.g., phosphorus and metals. Particle size has been linked with sorption capacity (Atalay, 2001).

Healthy Waterways Guidelines (2006) suggest that the filter media should have a similar particle size distribution (PSD) to the sediment (TSS) being transported in the stormwater itself, “in order to trap sediment with minimal long term impact on hydraulic conductivity”. In the recent FAWB Guidelines (2008) it is noted that PSD is of “secondary importance compared with hydraulic conductivity”. However, they do provide an indication of preferred particle composition with clay and silt <3%, fine gravel <3% and medium to coarse sand 40-60%. They also note that clay and silt are important for water retention and sorption of soluble pollutants but state these size fractions can reduce the hydraulic conductivity of the media. ARQ (2006) and Healthy Waterways (2006) recommend the use of sandy loam media.

Research on media by FAWB (2007) showed that non-vegetated sand-media with an initial high porosity can be prone to clogging. FAWB (2007, Hatt et al 2007) also found that the addition of vermiculite and perlite helped to maintain hydraulic conductivity. Plants can improve the hydraulic conductivity of the media due to root growth. The decay of dead roots provides micro-pathways allowing better infiltration and more oxygen. Species with adventitious roots, e.g. grasses Pennisetum alopecuroides and sedges, as well as those with a tap root system, e.g. shrubs –Melaleuca and Callistemon, should be planted (see section on Macrophytes).

The PSD and hydraulic conductivity of our experimental mesocosms (Case Study) are given in Table 3. Both the sand and loam media were mostly sand (0.06 – 2mm). The sand media contained 2% silt, while the loam media contained 8% silt and 3% clay (11% clay-silt fraction). For the duration of our experiment (4.5 years) we loaded the systems with tap water and synthetic stormwater which contained less than 5mg L$^{-1}$ TSS and secondary treated sewage effluent which contained between 8 - 10mg L$^{-1}$ TSS. Nonetheless the “loam” systems showed clogging after only 3 years, notably the non-vegetated mesocosms. The presence of vegetation assisted in maintaining hydraulic conductivity (soil porosity). We are currently conducting clogging experiments on the remaining sand mesocosms by adding TSS.

**Moisture:** Macrophytes (plants) and microbes (micro organisms) require water for survival and all biological processes. As previously discussed the hydraulic conductivity of the media determines retention time. If the media drains too quickly there is less contact time with the plant roots, microbial rhizosphere and biofilms. This not only reduces the contact time for nutrient uptake from the stormwater water, but also means the media will dry out more rapidly causing water stress on the plants and microbes. In geographical locations where there is frequent and abundant rainfall this is not so much of a problem but in climatic regions where extended dry periods are common this could result in high plant mortality.

In our experimental mesocosms Henderson noted that during the hot dry Brisbane summer (2003) some of the Banksia integrifolia died in the sand-gravel media “despite being watered every 2 weeks” (Henderson et al 2007 p 190). Henderson et al (2007) and Lucas and Greenway (2007, 2008) also noted that nutrient retention was lowest in these sand-gravel mesocosms. Henderson et al (2007) also noted that plant growth was most vigorous in the loam and that the plants were better able to survive the hot dry periods. Henderson (2008) and Greenway and Lucas (this Case Study) confirmed that plant biomass was highest in the loam but not consistently for all species (see section on Macrophytes and Table 4). The shrubs Callistemon and
Banksia both had the lowest biomass in the sand-gravel media, whereas interestingly, Dianella and Pennisetum had the lowest total shoot biomass in the sand. Moisture soil profiles for the sand and loam showed the highest moisture at the bottom of the bioretention mesocosm (above the gravel layer) thus, benefitting species with the deepest roots (Pennisetum and Callistemon). On the other hand Dianella and Banksia did not produce extensive root systems and had the greatest mortality in the sand-gravel and sand media. The succulent creeper Carpobrotus only has a few small adventitious surface roots but survived in all media due to the water storage ability in its leaves.

**Organic Matter and Nutrient Content:** Nitrogen and phosphorus are essential elements for plant growth. They occur naturally in soils and precipitate in rainfall as ‘atmospheric deposition’. Plants are highly resourceful and will actively sequester nitrogen and phosphorus from the filter media, rainfall and stormwater. Organic matter provides carbon, nitrogen and phosphorus and occurs naturally in soils as dead plant and animal matter accumulates and decays due to microbial decomposition. Organic matter, in particular the carbon, provides the energy source for denitrifying anaerobic bacteria. Zinger et al. (2007) found that the addition of an organic carbon source e.g. woodchips beneath the conventional bioretention media layer enhanced denitrification.

However organic matter or compost can readily leach nutrients and too much soil organic matter can be mineralised resulting in nutrient export (Dietz and Clausen, 2006, Fletcher et al., 2007). The recommended organic matter content for filter media ranges from 3 - 10%. The recently released revised FAWB Guidelines (2008) now specify <5%, stating ‘an organic content higher than 5% is likely to result in leaching of nutrients’. Thus getting the right mix of organic matter is a crucial factor in the performance of bioretention systems.

Surface mulch is often recommended as it assists in maintaining soil moisture. However mulch can also be mineralised thereby adding nutrients which are subsequently exported resulting in poor nutrient removal (Hsieh and Davis 2005a and 2005b).

In the experimental mesocosms of (Henderson et al, 2007) no organic matter was added to the media (Table 3); the plants grew (Table 4 & 5) and the systems were highly effective in removing nutrients (Henderson et al, 2007, Lucas & Greenway 2007, 2008) Table 6. After 4.5 years of plant growth and nutrient loading the OM content is still <2% in the loam and <1% in the sand media.

**Phosphorous:** The FAWB Guidelines for media recommend phosphorus <100 mg.kg\(^{-1}\) but caution ‘where plants with moderate phosphorus sensitivity are to be used, phosphorus concentrations should be <20 mg.kg\(^{-1}\)’. Although it is a widely accepted concept that many native species are intolerant of higher phosphorus loads this had not been the case with wetland macrophytes (Greenway, 2006). Bolton found that Melaleuca species irrigated with high phosphorus sewage effluent would get rid of excess phosphorus via leaf fall (Bolton and Greenway, 1999). However, plants of the Proteaceae family (Grevilleas, Banksias) are known to be more sensitive to phosphorus. In our experimental mesocosms we included Banksia integrifolia – this was the most unsuccessful of our 5 species. This may have been due to increased phosphorus concentrations in the stormwater and effluent, though interestingly it survived best in the loam with 123 mg.kg\(^{-1}\) of phosphorus. Henderson suggested the poor performance of Banksia was related to the drought condition since the sand media had lower moisture content than the loam (Henderson et al, 2007, 2008).

**Sorption Properties: Contaminant Removal Capacity**

Whilst the design guidelines for bioretention systems recommend hydraulic conductivity, particle size distribution and organic matter content of the media, there
is no reference to the contaminant removal capabilities of different media. Hsieh and Davies (2005) noted there needs to be a balance between permeability and contaminant retention capability. The smaller particle size fractions i.e. silts and clays, provide the most chemically active sites for sorption of phosphorus and metals but guidelines (FAWB 2008) recommend <3% of this size fraction since greater volumes reduce hydraulic conductivity.

**Phosphorus Sorption:** Geochemical processes for phosphorus removal and retention in bioretention systems are primarily due to the sorption properties of the media (Davis et al, 2001; Hsieh and Davis, 2005; Hsieh et al, 2007; Henderson et al, 2007; Lucas and Greenway, 2007, 2008). Ultimately, phosphorus retention is a function of media phosphorus saturation status and the equilibrium concentration at which stage the media starts to release phosphorus into solution rather than remove it (Henderson et al, 2007; Dietz and Clausen, 2005). Thus media potential for phosphorus sorption capacity and a knowledge of the influent stormwater (or other waste water) quality are important for assessing the long term phosphorus removal and retention capacity of bioretention systems.

Sorption isotherms can be used to identify the phosphorus sorption capacity of media and assist in determining the equilibrium concentration (Henderson et al, 2007). The potential capacity of media to remove soluble contaminants depends on, among other processes, the availability of sorption sites (on the media surface), the binding energy (mono-and bidentate chemical bonds) as reflected in the equilibrium concentrations of the media. The latter effects not only the sorption capacity of the media to remove contaminants from solution but also the desorption capacity i.e. the ability to lose any sorbed contaminants if the solution has a lesser equilibrium concentration. This has been demonstrated in laboratory column experiments by Kim et al (2004) and in field bioretention mesocosms by Lucas and Greenway (2007-2008). P-sorption is a function of the density of sorption sites, which are generally associated with the clay fraction. This is why sandy soils have an order of magnitude lower PO₄-P sorption capacity than clay soils (Ige et al, 2005).

Henderson (2008) and Henderson et al (2007b) investigated the sorption and desorption behaviour of nutrients in media sub samples (top 0 - 10cm) collected from the loam bioretention mesocosms. They found that the media in these mesocosms which had received a total load of between 2.8 m⁻² and 3.31 g m⁻² PO₄-P no longer had the capacity to sorb more PO₄-P at stormwater concentrations (0.55 mg L⁻¹ PO₄). The sorption isotherms indicated that for further sorption to occur the equilibrating solution would need to have a PO₄-P concentration greater than 1.5 mg L⁻¹. This is supported by the subsequent research of Lucas and Greenway (2007a) who loaded the same mesocosms with secondary sewage effluent with PO₄-P concentrations between 3.3-6 mg L⁻¹. As can be seen in Figure 1 there was less phosphorus retention over time as the cumulative load increased.
After the application of 40 gm² the mesocosms were leached with tap water and then dosed with stormwater which resulted in some desorption. It is interesting to note that the original loam media had 123 mg.kg⁻¹ phosphorus compared to only 23 mg.kg⁻¹ in the sand but still had a greater phosphorus adsorption capacity than sand even after receiving a total load of 65g P m⁻² over 3.75 years. Figure 2 also shows the effect of vegetation in phosphorus retention (see Case Study for discussion).

Henderson et al 2007 also showed that the “non-enriched” loam media in the mesocosms which only received a total of 1.31g PO₄ m⁻² (compared to 3.1g PO₄ m⁻²) sorbed more PO₄. The PO₄ equilibrium concentrations for the “enriched” media ranged from 0.6 - 1.6mg L⁻¹ which is higher than stormwater.

Table 1: Equilibrium concentration of loam media (top 0 - 10cm) for different nutrients after 6 months stormwater loading. (Source: Henderson et al 2007).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Loading</th>
<th>Equilibrium solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g m²</td>
<td>mg L⁻¹</td>
</tr>
<tr>
<td>PO₄</td>
<td>2.8</td>
<td>0.6-1.6</td>
</tr>
<tr>
<td>Organic phosphorus</td>
<td>0.14</td>
<td>0.3-0.6</td>
</tr>
<tr>
<td>NH₄</td>
<td>4.01</td>
<td>0.2-0.7</td>
</tr>
<tr>
<td>Organic nitrogen</td>
<td>25.65</td>
<td>1.0-7.0</td>
</tr>
</tbody>
</table>

Based on these incubation sorption isotherms and the fact that after only 6 months of regular stormwater dosing the media in the top 0 - 10cm was no longer capable of further phosphorus sorption at stormwater equilibrium concentration, Henderson concluded that ‘sorption is unlikely to be an important long-term nutrient removal pathway in bioretention systems’. Nevertheless at higher PO₄ concentrations in the

Figure 1: Input and output concentrations mg L⁻¹ phosphorous in vegetated and barren media as a function of cumulative load. Trends in outflow as function of inflow (source: Lucas and Greenway 2007b)
“field” mesocosms, Lucas and Greenway were able to demonstrate that further phosphorus sorption could occur especially in the vegetated media. After 12 months of effluent loading totalling 109 g P m\(^{-2}\) (83 g PO\(_4\) m\(^{-2}\)) phosphorus retention was reduced to 57% in the barren loam and 39% in the barren sand but still remained at 92% and 67% in the vegetated loam and sand media respectively (see Case Study).

**Other Studies**: Besides the incubation studies of Henderson et al (2007) on loam and the field mesocosm studies of Lucas and Greenway (2007, 2008) on loam, sand and gravel, several other studies have recently been published on phosphorus sorption capabilities of different media. Hsieh et al (2007) investigated the effects of accelerated phosphorus loading in laboratory columns containing three sand media. Breakthrough began at loads of 24 g P m\(^{-2}\) with no retention after 60 g P m\(^{-2}\). They projected at an annual load of 8.5 g P m\(^{-2}\) phosphorus retention could be expected for 5 years (42.5 g P m\(^{-2}\)).

Erickson et al (2007) ran batch sorption and column studies comparing silica sand with steel wool amendments. The steel-amended media improved P retention and at an average loading rate of 0.5 mg L\(^{-1}\) (i.e. equivalent to 45 g P m\(^{-2}\)) P retention could also be expected for 5 years.

**Phosphorus Precipitation**: Phosphorus precipitation is a process in which PO\(_4\) – P ions replace the hydroxyl ion of a metal hydroxide and the metal phosphate precipitates out of solution and are no longer biologically available. This is the principle of ‘Alum’ dosing in sewage and water treatment plants. In acidic conditions, including acid sulphate soils Fe – and Al – precipitation reactions dominate whereas in alkaline conditions and where soils contain calcium carbonate or sulphate Ca – precipitation reactions occur. Iron oxides are commonly associated with sand and aluminium oxides with clay particles. Under water-logged conditions, the absence of oxygen can lead to the reduction of mineral oxides such as iron oxide. Ferric (Fe\(^{3+}\)) oxide can be reduced to the more soluble ferrous (Fe\(^{2+}\)) which is then available to form iron phosphate which precipitates.

**Nitrogen Sorption**: Ammonium (NH\(_4\)) and dissolved organic nitrogen can be removed by sorption processes (Phillips, 2002; Phillips and Sheehan, 2005). NH\(_4\) will sorb to organic matter but is readily desorbed due to weak bonds. Few studies have investigated nitrogen sorption processes in bioretention systems. Henderson et al (2007) produced sorption isotherms for NH\(_4\) and organic nitrogen and found very high removal rates. However the removal of NH\(_4\) by sorption is unlikely to be substantial, mainly because of the low concentration found in storm water. Davis et al (2006) proposed that organic nitrogen was removed by sorption processes.

**Metal Sorption**: Sorption is the major chemical processes for the removal of dissolved heavy metals and several studies have investigated the role of bioretention systems in the retention of metals (Davis et al, 2001, 2003; Hsieh and Davis 2005; Hatt et al 2006). Very high removal rates were recorded for copper, lead and zinc.

Jang et al (2005) and Farm (2002) conducted laboratory/column experiments on media incorporating mulch, and showed that mulch enhances the removal of heavy metals which adsorb to the organic matter. Seelsaen et al (2006) conducted sorption experiments on a wide variety of media and found compost had the best physicochemical properties for sorption of copper, zinc and lead, but the greatest leaching of dissolved organic carbon (DOC).

**Microbes**

In bioretention systems microbes are the microorganisms found living in the media and pore spaces and are associated with the plant roots – the rhizosphere. They consist of several major groups – protozoans, algae, fungi, bacteria and viruses. Communities of these microbes attached to the surfaces of media particles and plant roots are referred to as biofilms (Larsen and Greenway 2004). Microbes are
particularly important in biogeochemical cycles and in bioretention systems function in decomposition and mineralisation of organic matter, the uptake of soluble inorganic and organic nutrients (nitrogen, phosphorous, carbon), the cycling of nitrogen through nitrogen fixation, deamination, ammonification of dead organic matter, nitrification and denitrification, the uptake of heavy metals and other contaminants; hydrocarbon degradation; iron, manganese and sulphur oxidation and reduction. Microbes are major agents of bioremediation. Root exudates of organic acids and sugars promote microbial productivity and mineralisation within the rhizosphere zone. Aerobic conditions in the media are important for most microbial processes including mineralisation of dead organic matter, ammonification and nitrification; however anaerobic conditions are important for denitrification. ‘Submerged anoxic zones’ promoting permanent anaerobic conditions enhance nitrogen removal through denitrification (Zinger et al, 2007).

Whilst we have a very good knowledge of most of these microbial processes and their environmental conditions, few studies have attempted to quantify the role of microbes in contaminant removal in bioretention systems.

Henderson (2008) investigated nutrient uptake by microbes in media sub samples (top 0 - 10cm) collected from the loam bioretention mesocosms, by incubating samples in different nutrient concentration solutions – the isotherm method (Henderson et al, 2007). Organic carbon was used as an indicator of microbial biomass. The vegetated media had more microbial biomass carbon than the barren media. The vegetated media incubated for 72h had a microbial biomass carbon of between 40 – 70 mg Kg\(^{-1}\) compared to 15 – 35 mg kg\(^{-1}\) for media incubated for only 24h, whereas microbial biomass carbon in the barren media was not significantly higher after extended incubation. These results suggest the presence of more microbes in the vegetated media.

Even in high concentration solutions, microbial biomass phosphorus was only 0.1 – 1 mg kg\(^{-1}\) which accounted for <5% of all the phosphorus removed during incubation (the remainder being chemically sorbed). Henderson concluded “If the concentrations of the nutrients in solution are below the equilibrium concentrations for the media, biological demand will most likely control nutrient removal. If solution concentrations are above the media equilibrium concentrations, sorption processes will control nutrient removal”.

Tietz et al (2007) quantified microbial biomass in an intermittently loaded vertical biofiltration mesocosm composed of sandy media (0.06 - 4mm). They found no significant difference between the planted and unplanted mesocosms. However they did find a significant change in microbial biomass and organic carbon with depth in the media (Table 2). About 60% of the total microbial biomass in media cores occurred in the first 1cm with 95% in the top 10cm, indicating the zone of greatest microbial productivity.

Measurements of soil Total Organic Carbon (TOC) showed a high correlation with microbial biomass. TOC in the first 1 cm was 14 mg.g\(^{-1}\) (1.4%) (6mg.g\(^{-1}\) between 1-5cm; and 3mg.g\(^{-1}\) between 5-10cm). Microbial biomass carbon in the top 1-10cm (excluding the first 1cm) was therefore 5 to 10 times higher than Henderson recorded in the top 0-10cm loam in the bioretention mesocosms. The smaller microbial biomass recorded by Henderson may be related to the lower organic carbon content in the loam media (0.08%).
Table 2. Microbial biomass carbon and nitrogen and organic carbon and nitrogen in intermittently loaded vertical filtration mesocosms. (Source: Tietz et al 2006; Fumigation extraction method).

<table>
<thead>
<tr>
<th>Media Depth cm</th>
<th>Media C (g C kg-1)</th>
<th>Media N (g N kg-1)</th>
<th>Microbial Mass (mg C kg-1)</th>
<th>Microbial Mass (mg N kg-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>14</td>
<td>1.2</td>
<td>1100</td>
<td>300</td>
</tr>
<tr>
<td>1-5</td>
<td>6</td>
<td>1.2</td>
<td>550</td>
<td>120</td>
</tr>
<tr>
<td>5-10</td>
<td>3</td>
<td>200</td>
<td>200</td>
<td>60</td>
</tr>
<tr>
<td>10-20</td>
<td>1</td>
<td>100</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>20-30</td>
<td>0.8</td>
<td>50</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>30-40</td>
<td>0.5</td>
<td>50</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>40-50</td>
<td>0.5</td>
<td>0.1</td>
<td>50</td>
<td>10</td>
</tr>
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</table>

Arbuscular mycorrhizal (AM) fungi associated with vascular plants can rapidly take up phosphorous (van Tichelin and Colpaert, 2000). AM fungi have the capability to sequester excess adsorbed P as inert polyphosphate granules, and plants with AM fungi show enriched P content indicative of luxury uptake (reviewed by Bolan, 1991). These microbial-mediated mechanisms may be involved in the improved P retention performance found in the presence of vegetation.

Macrophytes

Macrophytes is a technical term applied to wetland plants both aquatic and semi-aquatic i.e. growing around the margins of water bodies in the ephemeral (seasonally inundated) zone. In the literature it is not a term used for bioretention plants and yet many of the species lists provided in design guidelines (Healthy Waterway, FAWB, Melbourne Water, Gold Coast City Council) contain numerous species which are semi-aquatic i.e. sedges and rushes as well as ‘semi-terrestrial’ i.e. those species which occur on higher elevated land. Such ‘semi-terrestrial’ species can tolerate some inundation but not water logging. I have even seen planting lists and plantings with rainforest species.

Functional Ability – Containment Removal: the primary purpose of plant species should be for containment removal i.e. phytoremediation. A considerable amount of literature exists on nutrient and heavy metal removal by plants (Singh and Tripathi, 2006); however the literature is not specific to bioretenion systems, nor to Australian native plant species. Greenway has undertaken numerous studies on nutrient removal and bioaccumulation in wetland plants (Greenway 2003, 2005, 2006; Browning and Greenway 2003) of which many of the species are recommended for bioretention systems. Bolton and Greenway (1999) and Greenway and Bolton (2002) assessed nutrient removal and bioaccumulation in Melaleuca species. All these studies showed that the plants are capable of ‘luxury’ uptake of nutrients (Greenway, 2006).

Our current experiments (Lucas and Greenway 2007, 2008) have been loading the bioretention mesocosms with sewage effluent containing 8–10 mg N L⁻¹ and 8–10 mg P L⁻¹ for the past 12 months without any apparent detrimental effect on plant growth in Pennisetum and Callistemon, however Dianella and Banksia growth slowed down.

Role of vegetation: Vegetation is an important component in bioretention systems performing physical, chemical and biological roles as well as aesthetics and biodiversity. Studies that have compared bioretention systems with and without plants have all demonstrated higher nutrient removal in the vegetated systems (Davis et al, 2006; Denman et al, 2006; Fletcher et al 2007; Henderson et al 2007; Lucas and Greenway, 2007, 2008 - results presented in the Case Study).
Physical Role: The vegetation slows the stormwater flow coming onto the media surface and distributes stormwater over the surface. This prevents erosion and may also facilitate filtration of particulates especially by dense clumps of sedges, grasses and rushes. The roots assist in binding and stabilising the media as well as producing macro pores within the media which assist in aeration and ensure that hydraulic conductivity is maintained media with vegetation is less prone to clogging.

Chemical Role: The roots of many species can assist in aeration by diffusion of oxygen from the roots into the surrounding rhizosphere. Whilst this is an adaptation of aquatic macrophytes to low oxygen in water logged sediments it occurs in most semi-aquatic sedges and rushes as well as other species which would naturally be subjected to periodic flooding e.g. Melaleuca quinquenervia, Callistemon pachyphylus and C.salignus, Banksia robur. Many of these species are found in ‘wet health’ communities or ‘Melaleuca swamp forests’. The oxygen influences the redox of the media and also creates aerobic micro sites within the rhizosphere during waterlogging and within anoxic zones.

Biological Role: By far the greatest role of macrophytes is the uptake of nutrients (phosphate, nitrate / nitrite, ammonium) for plant growth. However, few studies have actually quantified plant uptake and incorporation into plant biomass. Plants also assist in the removal of metals which can be immobilised in plant tissues.

Suitability of Macrophytes: Few studies (Denman et al, 2006; Henderson, 2008; Henderson et al, 2007; Fletcher et al, 2007; Read et al, 2008; and Greenway and Lucas – current study) have investigated the suitability of macrophyte species for nutrient removal and growth in bioretention systems.

Denman et al (2006) investigated growth parameters (tree height and root length density) over 15 months in 4 tree species: Callistemon salignus, Eucalyptus polyanthemos, Lophostemon confertus and Platanus orientalis (plane tree) in soils with 3 different saturated hydraulic conductivities. The trees were irrigated weekly. Growth rates were significantly greater in stormwater compared to tap water but no difference was found between the 4, 95 and 170mm h\(^{-1}\) saturated hydraulic conductivities. Lophostemon had the highest growth rate and continued to grow even in the autumn months.

Fletcher et al (2007) investigated nutrient removal in biofilter columns using 5 species: Microlaena stipoides (grass), Carex appressa (sedge), Dianella revoluta (lily), Leucophyta brownie and Melaleuca ericifolia (shrubs). Carex appressa and to a lesser extent Melaleuca ericifolia were the most effective in nutrient removal. The authors note plant growth and establishment are important. Carex appressa rapidly established a dense root system resulting in high nitrogen removal after 9 months, whereas it took 14 months before Melaleuca ericifolia demonstrated effective nitrogen removal.

Read et al (2008) trialled 20 plant species native to south-eastern Australia, including grasses Microlaena stipoides, Poa labillardieri :sedges Carex appressa, Ficinia nodosa; rushes Juncus amabilis, Juncus flavidus; lilies Dianella revoluta, Lomandra longifolia; several shrub species including Leucophyta brownie, Banksia marginata, Melaleuca ericifolia, Kunzea ericoides; plus ground cover herbaceous plants – Goodenia ovata, Hibbertia scandens. The plants were grown in individual pots in sandy loam with a hydraulic conductivity of 180mm h\(^{-1}\) and 4% organic matter content. The plants received regular watering with either tap water or stormwater. The plants were harvested after one growing season (8 months). Read et al noted considerable variation in plant biomass among species ranging from 6 - 32g total biomass and 1 - 13g root biomass; but no data was presented for the different species. The authors noted the species that reduced nutrient concentrations most were Carex, Ficinia, Juncus spp., Melaleuca, Goodenia and Kunzea.
FAWB (2007) note ‘there is marked variation in pollutant removal among plant species’ and conclude from the above studies that Carex appressa, Juncus amabilis, Juncus flavidus and Melaleuca ericifolia were particularly effective in nutrient removal. The least effective were shallow rooted species such as Micralaena stipoides and those adapted to drier conditions Lomandra longifolia and, Banksia marginata.

Henderson et al (2007, Henderson, 2008) trialled 5 species: Pennisetum alopecuroides (swamp foxtail grass – an exotic clumping grass used extensively in landscaping), Dianella brevipedunculata (flax lily); Carpobrotus glaucesens (coastal pigface – a succulent creeper); Banksia integri folia (coastal banksia – a tree) and Calistemon pachyphyllus (a now renamed Melaleuca pachyphylla; Wallum Bottlebrush – a small shrub. Each vegetated bioretention mesocosm (surface area 0.26 m²) contained all 5 species. (Details of media specification are given in Table 3). Tube stocks were planted in June 2003. These were initially irrigated with tap water, then stormwater (Henderson et al, 2007). However, from August 2006 to August 2007 the mesocosms were irrigated weekly with recycled sewage effluent (Lucas and Greenway 2008) as drought restrictions in SE Qld prevented the use of tap water.

Henderson (2008) measured a number of growth parameters including height of shoots/stems; number of leaves/culms; number of flowers/fruit; diameter of stems/clumps and in December 2005 (i.e. after 30 months growth) took sub-samples of stems and leaves to estimate above ground shoot biomass. Greenway (current research) continued measuring these growth parameters. She also annually harvested the Pennisetum and Dianella shoots and measured regrowth. In March 2008 the bioretention mesocosms were destroyed so that both above–ground shoot biomass and below-ground root biomass could be determined. These results are presented in the case study (Tables 4 and 5).

The above studies all demonstrate that for successful plant establishment and nutrient removal efficiency the right balance is needed between the plant species’ ability to tolerate intermittent wetting and drying, and to uptake nutrients for incorporation into plant biomass. If the filter media is poorly drained or it becomes clogged then ‘terrestrial’ macrophytes will die off. If water logging and permanent saturation and ponding persist then ‘wetland’ macrophytes will colonise.
Case Study: Nutrient Retention in Mature Vegetated Bioretention Systems Under Elevated Nutrient Loads

Methods
The experiments were conducted from July 2006 through August 2007 at the Loganholme Water Pollution Control Centre near Brisbane, Australia, using the bioretention mesocosms of Henderson et al (2007). The mesocosms were constructed in June 2003 using 240L “wheelie-bin” containers, 57cm by 49cm at top (surface area 0.26m²) and 99cm deep. The media depths ranged between 75 and 80cm, with 15 to 20cm of freeboard. 3 media types were used: gravel, sand, and loamy sand. The media characteristics are given in Table 3.

Table 3 Media characteristics of original gravel, sand and loam

<table>
<thead>
<tr>
<th>Particle Size *</th>
<th>3mm Gravel</th>
<th>Sand</th>
<th>Loam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravel (&gt;2mm)</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand (0.06-2mm)</td>
<td></td>
<td>98</td>
<td>89</td>
</tr>
<tr>
<td>Silt (0.002-0.06mm)</td>
<td></td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Clay (&lt;0.002)</td>
<td></td>
<td>180 m hr⁻¹</td>
<td>650mm hr⁻¹</td>
</tr>
<tr>
<td>Hydraulic conductivity*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic matter %</td>
<td>0.10</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Carbon %</td>
<td>0.05</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Nitrogen %</td>
<td>&lt; 0.002</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Phosphorous %</td>
<td>0.0023</td>
<td>0.012</td>
<td></td>
</tr>
</tbody>
</table>

* source: Henderson (2008)

There were a total of 30 mesocosms. For each media type there were 10 mesocosms: 5 mesocosms were vegetated and 5 unvegetated (barren), providing 5 replicates for each treatment. The vegetated gravel mesocosms contained 20cm sand on top of 60cm gravel. Each vegetated mesocosm contained one clump each of Swamp Foxtail Grass (Pennisetum alopecurioides) and Flax Lily (Dianella brevipedunculata), and two woody shrubs, Banksia (Banksia integrefolia), and Bottlebrush (Callistemon pachyphyllus), and the succulent creeper Pigface (Carpobrotus glaucesens). The surfaces of the mesocosms were originally covered by a layer of gravel mulch.

At the time of our experiments (July 2006), the vegetation had been growing for 3 years. The sand and gravel treatments percolated up to 18 cm h⁻¹, providing an average retention time of slightly over an hour. The loam mesocosms percolated much more slowly, ranging from 2.0 to 4.5 cm h⁻¹ in the vegetated mesocosms, and under 1.0 cm h⁻¹ in the barren systems. These low rates were due to the media being washed into the underdrain gravel, reducing hydraulic efficiency in all but one vegetated loam replicate. The resulting retention time was 12 to 18 hours, which is higher than typical bioretention systems. Refer to Lucas and Greenway (2007) for more discussion of media hydraulic responses.

Effluent Loading: The effluent loading runs were conducted from August 2006 through July 2007. The loading experiments applied an average 112 L (or 44.7cm depth) of tertiary effluent from the treatment plant at weekly intervals. Average TN was 4.8 mg-P L⁻¹ and average TP was 4.8 mg-N L⁻¹. To minimize measurement inaccuracy resulting from grab sampling error, the entire effluent volume was collected in 150L cylindrical PVC chambers (300cm long x 25cm diameter) after 24h from the gravel and sand mesocosms, and after 48h from the loam mesocosms. Following collection, samples were then refrigerated, filtered with a 0.45µm filter, and analyzed for NH₄, NO₃, and PO₄-P using colorimetric methods with a Lachat Quikchem 8000 Flow Injection Analyzer. Total N and P were measured using standard persulfate digests on...
unfiltered samples and then running digested samples on the Analyzer. Samples were taken from at least two mesocosms of each treatment at monthly intervals.

**Plant Biomass:** Plant biomass was determined to calculate nutrient uptake. In September 2006 the above ground shoot biomass of *Pennisetum* and *Dianella* was harvested, dried and weighed. Sub-samples of leaf were analysed for their nitrogen and phosphorous content. Regrowth was cropped in February 2007 and again in September 2007 to give total annual shoot production. Sub-samples of leaf were also analysed for nitrogen and phosphorous. In March 2008 all plants were harvested to give total biomass (shoots and roots) over 4.5 years. All plant components were analysed for their nitrogen and phosphorous content.

**RESULTS**

**Plant Biomass and Nutrient Uptake**

A comparison of the shoot and root biomass of the 5 different species growing in different media is shown in Table 4.

**Table 4.** Comparison of shoot and root biomass (g dry wt ± SD) in the 5 different plant species grown in different media after 4.5 years in the bioretention mesocosms.

<table>
<thead>
<tr>
<th></th>
<th>Sand and Gravel</th>
<th>Sand</th>
<th>Loam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td><strong>Dianella</strong></td>
<td>105±45</td>
<td>20±12</td>
<td>45±20</td>
</tr>
<tr>
<td><strong>Pennisetum</strong></td>
<td>570±70</td>
<td>300±170</td>
<td>320±80</td>
</tr>
<tr>
<td><strong>Banksia</strong></td>
<td>100</td>
<td>40</td>
<td>130</td>
</tr>
<tr>
<td><strong>Callistemon</strong></td>
<td>200±30</td>
<td>100±25</td>
<td>900±50</td>
</tr>
<tr>
<td><strong>Carpobrotus</strong></td>
<td>70±45</td>
<td></td>
<td>95±60</td>
</tr>
</tbody>
</table>

There was considerable variation between biomass in the different media with loam and sand having the highest biomass for most of the species. However *Dianella* and *Pennisetum* grew better in the sand+gravel media than the sand.

Root biomass for *Pennisetum* was highest in the sand + gravel with a dense root mat extending through the gravel and into the underdrain. Only one live *Banksia* remained in the sand + gravel and sand media. Nutrient content also varied between species, plant parts, season and effluent concentration.

In order to determine maximum nutrient uptake and annual biomass production rates, the highest biomass values and the mean nutrient content (%N and %P) were used for the 5 different species. Annual production rates/nutrient uptake are summarised in Table 5.

Based on the total biomass standing crop over 4.5 years in each mesocosm, maximum annual biomass accumulation was 945g, or 3.78kg.m⁻².y⁻¹, being highest in the loam. Between September 06 and September 07 the annual biomass was actually higher due to harvesting of *Pennisetum* shoots which promoted rapid spring regrowth with luxury uptake of nutrients from the effluent. Using the mean values for nutrient content of the different plant parts it was determined that annual nitrogen uptake would be 31.5 g N.m⁻².y⁻¹ with a maximum of 37.5 g N.m⁻².y⁻¹ during the 12 months of our loading experiments. Annual phosphorus uptake would be 10.5 g P.m⁻².y⁻¹.
Table 5. Maximum plant biomass standing crop (g dry wt m\(^{-2}\)) in 4.5 year old mesocosms, nutrient content (%) and annual production rate (g m\(^{-2}\) y\(^{-1}\)).
(NB: density 4 of each species m\(^{-2}\)ie total 20 plants m\(^{-2}\); based on wheelie bins containing 1 individual of each species 0.26 m\(^{2}\))

<table>
<thead>
<tr>
<th></th>
<th>Biomass SC g/m(^{2})</th>
<th>Nutrient content %</th>
<th>Annual production rate g/m(^{2})/y</th>
<th>gN/m(^{2})/y</th>
<th>gP/m(^{2})/y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>shoot</td>
<td>shoot</td>
<td>shoot</td>
<td>shoot</td>
<td>shoot</td>
</tr>
<tr>
<td>Dianella</td>
<td>1000</td>
<td>0.91-1.4</td>
<td>0.16-0.41</td>
<td>222</td>
<td>2.22</td>
</tr>
<tr>
<td>Pennisetum</td>
<td>4000</td>
<td>0.91-1.3</td>
<td>0.37-0.74</td>
<td>888</td>
<td>8.89</td>
</tr>
<tr>
<td>Banksia</td>
<td>600</td>
<td>0.23-0.56</td>
<td>0.02-0.12</td>
<td>133</td>
<td>0.70</td>
</tr>
<tr>
<td>Callistemon</td>
<td>4440</td>
<td>0.70-1.2</td>
<td>0.21-0.57</td>
<td>986</td>
<td>9.87</td>
</tr>
<tr>
<td>Carpobrotus</td>
<td>680</td>
<td>0.95-1.2</td>
<td>0.45-0.83</td>
<td>151</td>
<td>1.51</td>
</tr>
<tr>
<td><strong>Total annual shoot production</strong></td>
<td></td>
<td></td>
<td></td>
<td>2380</td>
<td>23.48</td>
</tr>
<tr>
<td></td>
<td>root</td>
<td>root</td>
<td>root</td>
<td>root</td>
<td>root</td>
</tr>
<tr>
<td>Dianella</td>
<td>240</td>
<td>0.20-0.74</td>
<td>0.03-0.25</td>
<td>53</td>
<td>0.37</td>
</tr>
<tr>
<td>Pennisetum</td>
<td>2240</td>
<td>0.20-0.46</td>
<td>0.03-0.08</td>
<td>498</td>
<td>3.49</td>
</tr>
<tr>
<td>Banksia</td>
<td>520</td>
<td>0.23-0.74</td>
<td>0.05-0.14</td>
<td>116</td>
<td>0.05</td>
</tr>
<tr>
<td>Callistemon</td>
<td>3300</td>
<td>0.27-0.76</td>
<td>0.08-0.31</td>
<td>733</td>
<td>5.1</td>
</tr>
<tr>
<td><strong>Total annual root production</strong></td>
<td></td>
<td></td>
<td></td>
<td>1400</td>
<td>9.01</td>
</tr>
<tr>
<td><strong>Total shoot and root biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td>3780</td>
<td>31.57</td>
</tr>
</tbody>
</table>

NB Sept 06-Sept 07 total 37.5 gN.m\(^{2}\) and 11.1 gP.m\(^{2}\) due to higher than the average *Pennisetum* and *Dianella* shoot growth and nutrient content

To illustrate the potential contribution of plant uptake, the hatched inflow concentration column shown in Figures 2 and 3 is accompanied by an open column showing the projected nutrient concentration or load that would remain after plant uptake. This is based upon maximum uptake rates of 10.55 g.m\(^{-1}\).y\(^{-1}\) for TP, 30.0 g.m\(^{-1}\).y\(^{-1}\) for NOx, and 37.5 g.m\(^{-1}\).y\(^{-1}\) for TN. These values are applied as if a week of uptake occurred at each inflow load. This uptake mass was divided by the inflow volume to obtain the reduction in inflow concentration, shown as a percentage reduction. This provides a graphic illustration of the contribution of plant uptake.

**Nutrient Retention:** In our study, the term “retention” is taken literally, that is, not being observed in the effluent. Retention percentages are expressed in terms of subtracting the ratio of mean effluent to mean influent concentration of each run from 100%.

**Phosphorus Retention:** The loading runs applied TP at concentrations an order of magnitude higher than typical stormwater, thus accelerating P saturation over 50 weeks. Figure 2(a) compares TP responses between August 21, 2006 and July 27, 2007. The open inflow column representing the concentration remaining after projected uptake includes the corresponding reduction percentage.
Initially, barren sand and loam still provided 99% and 97% TP retention respectively, while TP retention in barren gravel was only 21%. The presence of vegetation substantially improved retention in gravel to 74%, a value considerably greater than projected uptake. Given the high concentrations applied, projected uptake represents a negligible reduction in TP concentrations. Since the barren sand and loam were initially so effective at these high concentrations at this time, there was no appreciable change due to vegetation. By July 17, 2007, TP concentrations from barren sand had increased by two orders of magnitude from 0.04 mg.L⁻¹ to 4.2 mg.L⁻¹, approaching the average influent concentration of 4.8 mg.L⁻¹. Vegetation had no significant effect on improving TP retention in the gravel and loam systems. While barren loam retained substantially more TP than sand, its concentration of 1.94 mg.L⁻¹ is over an order of magnitude higher than the 0.14 mg.L⁻¹ observed on August 21, 2006. The 0.83 mg.L⁻¹ observed in vegetated loam represented a significant retention of 91%, much greater than projected uptake.

**Nitrogen retention:** Figure 2(b) displays the results for NOₓ, where trends were similar to the dosing runs, in that barren media demonstrated no significant change. In the first run, vegetation had a highly significant effect in retaining NOₓ in the gravel and loam systems, but not in the sand due to higher variance. However, only in the loam does the effect of vegetation exceed projected uptake. Given 50% higher influent concentration in the July 17, 2007 run, there was a significant increase in the NOₓ concentrations from barren gravel and sand systems, but no corresponding increase in the loam. Compared to the first run, the decrease in vegetated loam was not significant since the unclogged replicate retained considerably less NOₓ than the other four replicates, thus increasing variance. In contrast, concentrations from vegetated gravel and sand increased significantly in the second run. The increase in retention due to vegetation in the loam to 81% is highly significant (p<0.002), and clearly exceeded projected uptake. The trends for TN in the both runs were very similar to that observed for NOₓ, although retention was slightly better, and the differences due to vegetation were not as significant. Effluent concentrations in the vegetated gravel and sand also increased significantly in response to the higher influent concentration. The lack of significant change between runs in the vegetated...
loam is due to variance in the one replicate. Notwithstanding this, TN retention in vegetated loam was 83%. This retention was highly significant (p=0.002), and exceeded projected uptake.

**Cumulative Loads:** Figure 3 presents the cumulative mass loads and retention percentages observed over 17 runs from August 21, 2006, to August 8, 2007.

**Phosphorus Retention:** As indicated in Figure 3(a), barren gravel retained only 15% of the applied TP, while sand retained 38%, and barren loam retained 56% of the cumulative load. There was an increase in retention in vegetated media to 44%, 67%, and 92%, respectively. All of these differences were highly significant, and well above projected uptake.

![Figure 3: Comparison of nutrient loads and retention percentages from barren and vegetated treatments during effluent loading from August 21, 2006 to August 8, 2007. Cumulative load of (a) TP, (b) NOx, and (c) TN, as g·m⁻², P and N.](image)

**Nitrogen Retention:** The cumulative results for NO\(_x\) shown in Figure 3(b) indicate that NO\(_x\) was exported from all barren media. On the other hand, there was a very significant increase in retention in the presence of vegetation, to 38% in gravel, 42% in sand, and 74% in loam. Given the high proportion of NO\(_x\) in the applied effluent, similar results were found for TN, except that there was no export from the barren media. Retention in the barren media was low, ranging from 8% to 21%, and much less than the 43% retention observed for vegetated gravel and 52% in the sand. Vegetated loam retained 79% of TN applied, an increase well in excess of the 34% projected for uptake. The effect of vegetation was highly significant in all media.

**Annual Mass Balance and Plant Uptake:** Table 6 presents the results of applying these retention percentages to the total nutrient mass applied during the loading campaign in terms of agronomic units of kg·ha⁻¹·y⁻¹. The first row for each nutrient expresses the amount of mass load retained that could be attributed to plant uptake. The last column presents the difference in retention due to the presence of vegetation, along with its percentage of total load applied. On an annual basis, the increase in TP retention in the vegetated treatments ranged from 316 kg·ha⁻¹·y⁻¹ to 387 kg·ha⁻¹·y⁻¹. The increase in TP retention compared to barren treatments ranged from 29% to 35% of the applied load, values far in excess of even the maximum of 10% attributed to plant uptake. For NO\(_x\), increased retention in the vegetated treatments ranged from 280 kg·ha⁻¹·y⁻¹ in gravel to 587 kg·ha⁻¹·y⁻¹ in the loam. The increase in NO\(_x\) retention compared to barren treatments ranges from 46% to 96% of the applied load. The increase in retention exceeded projected uptake in the sand, and considerably in the case of loam. For TN, the increases retention in the vegetated treatments ranged from 377 kg·ha⁻¹·y⁻¹ in gravel to 638 kg·ha⁻¹·y⁻¹ in the loam. This increase in TN retention compared to barren treatments ranges from 34% to 58% of the applied TN load. TN retention also exceeded projected uptake in the sand, and considerably in the case of loam.
Table 6: Comparison of annual mass of nutrients retained in barren and vegetated treatments, based on effluent loading from August 21, 2006 to August 8, 2007. Cumulative retention of total phosphorus, nitrogen oxides, and total nitrogen expressed as kg·ha⁻¹·y⁻¹ N and P.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Media Type</th>
<th>Barren Retention</th>
<th>Vegetated Retention</th>
<th>Difference Mass</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Phosphorus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake</td>
<td>Gravel</td>
<td>0</td>
<td>107</td>
<td>107</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>Sand</td>
<td>160</td>
<td>476</td>
<td>316</td>
<td>29%</td>
</tr>
<tr>
<td></td>
<td>Loam</td>
<td>415</td>
<td>725</td>
<td>311</td>
<td>29%</td>
</tr>
<tr>
<td>Annual Load 1,090</td>
<td>Uptake</td>
<td>614</td>
<td>1,000</td>
<td>387</td>
<td>35%</td>
</tr>
<tr>
<td>Nitrogen Oxides</td>
<td>Gravel</td>
<td>-79</td>
<td>201</td>
<td>280</td>
<td>46%</td>
</tr>
<tr>
<td></td>
<td>Sand</td>
<td>-159</td>
<td>253</td>
<td>412</td>
<td>68%</td>
</tr>
<tr>
<td></td>
<td>Loam</td>
<td>-134</td>
<td>453</td>
<td>587</td>
<td>96%</td>
</tr>
<tr>
<td>Annual Load 609</td>
<td>Uptake</td>
<td>0</td>
<td>315</td>
<td>315</td>
<td>50%</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>Gravel</td>
<td>83</td>
<td>459</td>
<td>377</td>
<td>34%</td>
</tr>
<tr>
<td></td>
<td>Sand</td>
<td>132</td>
<td>577</td>
<td>444</td>
<td>41%</td>
</tr>
<tr>
<td>Annual Load 1,093</td>
<td>Loam</td>
<td>226</td>
<td>864</td>
<td>638</td>
<td>58%</td>
</tr>
</tbody>
</table>

DISCUSSION

Phosphorus Retention

Our findings indicate that plant uptake accounts for only 10% of the P retained in our experiments. Geochemical processes of adsorption and/or precipitation of PO₄-P are thus the primary mechanism by which P is retained in the bioretention systems. During storm events “rapid-reversible” sorption reactions predominate when runoff flows through the media, with the “slow-irreversible” sorption reactions then continuing between events. During these reactions, some of the P bound to reversible sites is relocated to the irreversible sites, releasing rapid reversible sites for the next event. Erickson et al (2007) suggested at least 10 hours of contact time was needed to approach peak sorption. A critical fact to keep in mind is that sorption isotherms at low concentrations of stormwater and treated effluent are typically linear in nature (Stumm and Morgan, 1995). Therefore, the equilibrium concentration will increase as a direct function of the amount of P sorbed, which means that the retention performance observed at effluent concentrations will not be obtained at stormwater concentrations. Figure 2(a) shows that, by the time 104.8 g·m⁻²·TP had accumulated, barren sand and gravel had become saturated, while barren loam was still partially effective at tertiary effluent concentrations. This supports the observations of Hsieh et al (2007), where a similar cumulative load threshold was obtained at concentrations of 3.0 mg L⁻¹. The presence of vegetation significantly increases TP retention in unsaturated media. Figure 2(a) shows this for all treatments on a cumulative basis. On the other hand, by the final loading run in Figure 2(a), vegetation has minimal effect in the sand and gravel, as they are already highly saturated. These findings indicate that the soil rhizosphere in well-established plant communities provides a rapid sorption capability that is absent in barren systems. Oxygen from plant roots oxidizes ferrous iron in the media to the ferric form which has a high P-sorption capacity, and even relatively inaccessible iron compounds are mobilized by this process (reviewed by Mendelssohn et al, 1995). Arbuscular mycorrhizal may also be involved in the improved P retention performance found in the vegetated mesocosms.
Nitrogen Retention

Our findings indicate that plant uptake accounts for up to 50% of the NO\textsubscript{x} retained. The N remaining then goes through a variety of transformations. Labile organic N can be mineralized into NH\textsubscript{4} and subsequently nitrified into NO\textsubscript{x} between storm events. The difference between NO\textsubscript{x} export and net TN retention by barren media shown in Figure 3 indicates nitrification processes. Some of the resulting NO\textsubscript{x} is further taken up by the plants, while the remainder is either leached from the profile, or removed by denitrification. Figures 2(b) and 3(b) indicate that NO\textsubscript{x} leaches from the soil profile of barren media. However, denitrification processes can remove NO\textsubscript{x} if carbon is available and the redox potential is low. Since plant uptake is only partially responsible for the retention observed, denitrification seems to underlie much of the NO\textsubscript{x} reductions in vegetated loam. 81% of NO\textsubscript{x} applied was retained in the vegetated loam systems at the end of the loading campaign.

CONCLUSIONS

Although NO\textsubscript{x} retention is often reported as being absent or negative in bioretention systems, our results show that considerable NO\textsubscript{x} retention occurs in mature vegetated loam bioretention systems with adequate retention time. The cumulative NO\textsubscript{x} retention over 50 weeks exceeded 70% in the vegetated loam systems, even at high inflow concentrations. At high loading rates, we have observed that vegetated loam retained up to 83% of applied TN. Our study also documents that TP retention by barren media will eventually become exhausted under long term loads. At tertiary effluent loading rates, the P-sorption capacity of sand was exhausted after a year, while the P-sorption capacity of loamy sand was partially exhausted. However, P retention in partially saturated media significantly increased with mature vegetation even though retention far exceeded plant uptake.

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