STORMWATER 2010
National Conference of the Stormwater Industry Association
Conference Proceedings

IMPROVED MEDIA AND PLANT SPECIES FOR LONG TERM SUSTAINABILITY OF NUTRIENT RETENTION IN BIORETENTION SYSTEMS

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ABSTRACT

Over the past decade there has been an overwhelming increase in the incorporation of bioretention systems into the urban landscape as treatment for stormwater to protect our waterways. However further research into the suitability of media and plant species is still required to ensure long term sustainability and effective performance. Over the past 6 years researchers at Griffith University have investigated the performance efficiency of six different media types including amendments for enhanced phosphorus adsorption, and eight different plant species. Our experimental mesocosms were subjected to accelerated loadings of phosphorus and nitrogen representative of up to three decades of stormwater runoff. Total Phosphorus removal was highest (94-99%) in the sand media amended with Water Treatment Residuals, followed by the loam (92%), then the sand amended with Red Mud or Krasnozems (86-89%) and lowest in sand-gravel 44%. Total Nitrogen removal was highest in the loam (79%) but relatively poor in all the sandy media types (around 50%).

Of the herbaceous plants the grass *Pennisetum alopecuroides* and the sedge *Carex appressa* had the highest growth rates and biomass yield. After 2 years mean shoot biomass was similar for both *Pennisetum* and *Carex* around 830g; whilst mean rhizome/root biomass was 302g and 385g respectively. Root volume was similar in both species around 2000mL. Plant growth was similar for all media. Cropping shoots enhanced shoot growth in *Pennisetum* and *Carex*. Over 3 years cumulative shoot biomass for *Pennisetum* following 5 harvests for a single plant yielded 2400g.

After 2 years mean shoot biomass in the shrubs was around 300g for *Callistemon pachyphyllus* and 800g for *Melaleuca quinquenervia*; whilst root biomass was 150g and 500g respectively. In our densely planted mesocosms root competition from *Pennisetum* and *Carex* appears to limit the growth of *Callistemon* (and the sedge *Isolepis nodosa*).

In terms of nutrient removal, plant uptake measured over 2 years accounted 12-18gP/m2/y and 51-64gN/m2/y. These high removal rates are attributed to luxury uptake from the effluent loadings and shoot harvesting in *Pennisetum* and *Carex*.

INTRODUCTION

Over the past decade there has been an overwhelming increase in the incorporation of bioretention systems into the urban landscape as treatment for stormwater to protect our waterways and ecosystem health. Bioretention systems are becoming the most widely used stormwater treatment technology in Water Sensitive Urban Design for many local authorities. The use of bioretention systems as a Stormwater Quality Improvement Device has surpassed that of ponds and wetlands. However, do we really know enough science about the media, microbes and macrophyte vegetation to maximize the long term performance of bioretention systems? Research in Australia mainly though

Monash University's Facility for Advancing Water Biofiltration (FAWB) (Hatt et al 2007, 2008; Fletcher et al 2007; Bratieres et al 2008) and Griffith University (Henderson et al 2007; Lucas and Greenway, 2008, 2010a); has substantially improved our knowledge in understanding nutrient removal processes.

Vegetation is an important component in bioretention systems performing physical, chemical and biological roles as well as enhancing aesthetics and biodiversity (Greenway & Lucas, 2008). Studies that have compared vegetated and non vegetated treatments have all demonstrated higher nutrient removal in the vegetated systems (Bratieres et al 2008; Davis et al, 2005; Denman et al, 2006; Fletcher et al 2007; Henderson et al 2007; Lucas and Greenway, 2008; 2010a & b). In particular N removal in unvegetated (barren) treatment has been universally reported as poor, with little retention of total N (TN), and export of nitrogen oxides (NO_x) (Hsieh and Davis, 2005; Hatt et al, 2008, Henderson et al, 2007; Read et al, 2008; Lucas and Greenway, 2008). In contrast, N retention can be substantial in vegetated treatments, (Henderson et al 2007, Read et al, 2008; Lucas and Greenway, 2008; 2010b; and Bratieres et al, 2008).

Despite the importance of vegetation, very few studies have investigated the potential of different plant species for their ability to uptake nutrients. 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. Denman *et al* (2006), Henderson (2008), Henderson *et al* (2007), Fletcher *et al* (2007), Read *et al* (2008), and Greenway and Lucas (2008; 2009) and this current study have investigated the suitability of species for nutrient removal and growth in bioretention systems.

Fletcher *et al* (2007) and Bratieres et al (2008) investigated nutrient removal in biofilter columns using 5 species: *Microlaena stipoides* (grass), *Carex appressa* (sedge), *Dianella revoluta* (lily), *Leucophyta brownie* and *Melaleuca ericifolia* (shrubs). Trends in TN retention were observed over a 7-month period after an initial 6 month growth period. There was export of TN in three of the five plant treatments. *Carex appressa* and to a lesser extent *Melaleuca ericifolia* were the most effective in nutrient removal. However, there was an increase in N retention from treatments with *Melaleuca ericifolia* as the plants became more established. Treatments planted with the sedge *Carex appressa* showed a consistent removal exceeding 60%. 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. P retention was also highest in the treatments with *Carex appressa.*

Read et al (2008) trialed 20 plant species native to south-eastern Australia, including grasses *Microlaena stipoides*, *Poa labillardierei* ;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. Effluent concentrations varied considerably between plant species. TN concentrations were significantly lower only in 5 species, the more rapidly growing monocot sedges and rushes, whereas NO_x concentrations were reduced in 10 taxa. PO_4 concentrations were reduced in most taxa. The genera that reduced nutrient concentrations most were *Carex, Ficinia, Juncus* spp., *Melaleuca, Goodenia* and *Kunzea*. 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 but no data was presented for the different species. Although this study showed that biomass accumulation was significantly correlated with effluent reductions, no data was presented on the N or P content of biomass i.e. nutrient accumulation, nor the mass load of N or P retained.

From these studies FAWB (2008) concluded that 'there is marked variation in pollutant removal among plant species' and 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 *Microlaena stipoides* and those adapted to drier conditions *Lomandra longifolia* and, *Banksia marginata.*

Henderson *et al* (2007), Henderson, (2008) and Lucas & Greenway (2008) investigated nutrient removal in bioretention mesocosms (wheelie bins) each containing 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 integrifolia* (coastal banksia – a tree) and *Callistemon pachyphyllus* (Wallum Bottlebrush – a small shrub). The media were sandy loam, sand and gravel (with 20cm sand on top). The mesocosms were initially irrigated with tap water, then stormwater (Henderson *et al,* 2007). Under stormwater conditions, NOx removal was 99%, 87% and 89% in the vegetated sandy loam, sand and gravel (+sand) treatments respectively. There was nearly complete removal of PO_4 in all treatments. Henderson (2008) measured a number of growth parameters including height of shoots/stems; number of leaves/culms; number of flowers/fruits; diameter of stems/clumps and after 30 months growth took sub-samples of stems and leaves to estimate above ground shoot biomass. He found the highest above ground plant biomass in the sand and sandy loam media. Plant biomass was highest for the shrub *Callistemon pachyphyllus* and the grass *Pennisetum alopecuroides*. From August 2006 to August 2007 the mesocosms were irrigated weekly with recycled sewage effluent (Lucas and Greenway, 2008). Plant growth parameters were obtained by annual harvesting of the *Pennisetum* and *Dianella* shoots and regrowth measured (Greenway and Lucas, 2008,).

Research into suitable media has received more attention (Bratieres et al., 2009; Davis et al., 2005; Fletcher et al., 2007; Hatt et al., 2007; Henderson et al., 2007) however much of this research has been small scale column studies. Furthermore very few studies have investigated the effects of accelerated loading in relation to the longer term capacity of the media to sustain nutrient removal (Erickson et al 2007, Hsieh et al, 2007).

Since 2003 researchers at Griffith University have been conducting wheelie bin mesocosm experiments using different media and plant species. Our research has focused primarily on nutrient removal. The aims of our research have been to investigate (i) the effectiveness of different soil media including amendments for enhanced phosphorus adsorption; and (ii) the growth and nutrient removal capacity of different plant species. Our experiments have included vegetated and non-vegetated treatments (Henderson et al., 2007; Lucas and Greenway, 2008, 2010a & b), low and high nutrient influent concentrations and mass loadings (Henderson et al., 2007; Lucas and Greenway, 2008; 2010a & b), as well as free draining and elevated outlets (Lucas and Greenway, 2010 a, b, c & d)

In our current paper we describe the major findings of the second phase of our mesocosm experiments. The aims of the second phase were to evaluate the effectiveness of (i) vegetation and (ii) media types, in the removal of dissolved nutrients from recycled effluent using intermittently loaded 240L vertical flow mesocosms. In particular, we wanted to quantify nutrient removal by plants by determining annual growth rates and total biomass; as well as nutrient accumulation in the media over time.

METHODS

Experimental set up: The experiments were conducted at the Loganholme Water Pollution Control Centre SE Qld using 240L "wheelie-bin" containers, 57cm by 49cm at top and 99cm deep. Media depths in these experimental bioretention mesocosms ranged from 54-66cm and were underlain with a 15cm gravel drainage layer

Experiment 1 used the mesocosms of Henderson *et al* (2007). These mesocosms were constructed in June 2003,3 media types were used: 3mm gravel, sand, and loamy sand (89% sand, 8% silt, 3% clay) referred to as "loam".(Table 1) The vegetated 'gravel' mesocosms contained 20cm sand on top of 60cm gravel. The mesocosms set up was modified in 2007 with the addition of 150L collection chambers (Lucas and Greenway, 2008). Experiment 2 was established in 2007 using 7 different media (Table 1) and 3 replicates of each. Media amendments included red mud, a by-product of refining bauxite to aluminium; Krasnozem soils derived from weathered basalt comprising amorphous aluminium complexes, and water treatment residuals, amorphous alum sludge a waste product of drinking water treatment(Lucas and Greenway, 2010a).

Experiment 3 was established in 2009 using sand amended with water treatment residuals from the Mt Crosby Water Treatment Plant.

Experiments 2 and 3 also utilized an elevated outlet to provide a saturated zone and extend retention time in the rapidly draining media (Lucas and Greenway, 2010 c & d).

Table 1. Composition of different media by % weight (and coir peat by volume) used in Experiments 1, 2 and 3. (S is Sand; RM is Red Mud; K is Krasnozem; WTR is Water Treatment Residuals.)

Media Mix	symbol	Sand	Loam	Red Mud	Kras	WTR	Top Soil	Coir Peat	Expt
Gravel +20cm S	GV	33							1
Sand	SV	100							1
Loam	LV		100						1
S + Red Mud	RM06	75		5			20	12	2.1
S+ Red Mud	RM10	71		9			20	12	2.1
S+ Krasnozem	K20	86			14			12	2.1
S+ Krasnozem	K30	70			30			12	2.1
S+ Krasnozem	K10-30	93-70			7-30			12	2.1
S+WRT	WTR30	80				20		12	2.2
S +WTR+ Kras	WTR+K	71			20	9		12	2.2
S+WTR-Mt Crosby	WTRMC	80				20		12	3

Particle size distribution, and saturated hydraulic conductivity measurements (after 18 months and 2 years) for Experiment 2 are provided by Lucas and Greenway (2010b). Ksat ranged between 26.4 cm h^{-1} to 60 cm h^{-1} in Aug 2008 and 48 cm h^{-1} to 86 cm h^{-1} in March 2009. Ksat was lowest in RM10 and WTR+K and highest in K10-30

Vegetation: In Experiment 1 each mesocosm contained 5 different plant species. As the experiments were originally designed to investigate stormwater treatment, species were selected for their ability to tolerate short periods of inundation and longer dry periods (Henderson *et al* 2007). Two species Swamp Foxtail Grass (*Pennisetum alopecuroides*) and Wallum Bottlebrush (*Callistemon pachyphyllus*) occur in swampy, poorly drained soils and are regarded as wetland species.

In Experiment 2 each mesocosm contained 5 plants. All mesocosms were planted with *Callistemon pachyphyllus*, *Melaleuca thymifolia* and the sedge *Isolepis nodosa*. Five media treatments contained 2 plants of *Pennisetum alopecuroides* and the two media treatments contained 2 plants of *Carex appressa* (Lucas and Greenway, 2010a).

In Experiment 3 each mesocosm contained 5 different plant species: *Callistemon pachyphyllus, Melaleuca quinquenervia, Pennisetum alopecuroides, Vetiveria zianioides, Lomandra hystrix.*

Stormwater and Recycled Effluent Loading Regimes and Sample collection:

In Experiment 1phase 1 (June 2003-July 2006) Henderson (2008) irrigated the mesocosms weekly with 46L tap water during the establishment phase (26 weeks), then fortnightly with 108Lsynthetic stormwater for 28 weeks. After 1 year a series of stormwater dosing experiments were conducted (Henderson et al 2007) followed by tap water irrigation. Tap water irrigation continued until July 2006. The mesocosms received a total load of 4.58 g TP, 3.72 g PO_4, 57.6 g TN, 13.1 g NO_x, and 5.48 g NH4 over 3 years

In Experiment 1 phase 2 (August 2006-Sept 2007) (Lucas and Greenway, 2008) and Experiment 2 (Jan 2007-March 2010) an average of 112 L (or 44.7cm depth) of recycled tertiary effluent was applied at weekly intervals. In Experiment 3 (May 2009-May 2010) effluent (and tap water in the Controls) was applied at 2 weekly intervals. The average composition of the tertiary effluent was: 4.8 mg TP, 3.94 mg PO_4, 5 mg TN, 2.72 mg NO_x, 0.74 mg NH_4 L^{-1}.

In the Lucas –Greenway experiments the entire outflow volume was collected in 150L cylindrical PVC chambers after 24h. Following collection, samples were refrigerated, filtered with a 0.45µm filter, and analyzed for NH_4, NO_x and PO_4-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.

Plant Biomass:

Plant growth, biomass and nutrient uptake has been quantified for 9 different species.

Plant biomass was determined to calculate nutrient uptake. Shoots of *Dianella*, *Pennisetum*, I*solepis*, *Carex*, *Lomandra* and *Vetiveria* were cropped at a height of 10cm every 6 months to determine harvestable shoot biomass and to encourage regrowth. Total biomass harvesting of both above and below ground biomass was conducted after 4.5 years in Experiment 1, after 2 and 3 years in Experiment 2 and after 1 year in Experiment 3.

The volume of subsamples of fresh roots and rhizomes was measured by displacement, and dry weight to volume ratios obtained.

Sub-samples of leaf, stem, rhizome and root were analysed for N and P. Nutrient content varied between species, plant parts and season. In order to determine nutrient uptake and annual biomass production rates, the mean biomass values and the mean nutrient content (%N and %P) were used for the different species.

RESULTS

Nutrient Retention and Plant Uptake

Experiment 1

Table 2 summarizes the results of these experiments. Over the first 3 years of stormwater and tap water loadings the 3 media treatments showed almost 100% retention of PO_4 (1.5 g m^{-2}-y^{-1}). In the sand and loamy sand media there was a 77% retention of TN (17.8 g-m^{-2}-y^{-1}). The gravel+sand media was least effective for N retention (63%). Despite the lack of any organic matter in the media, all the plant species grew well. Estimated plant biomass indicated that plant N could account for over 80% TN retention. This confirms the findings of Henderson 2008 who determined above ground biomass accounted for 78% N retention in the loam, 71% retention in the sand but only 24% in the gravel+sand.

However plant P was 2-3times greater than the mass load indicating that the plants must be obtaining additional P from either the media or the mineralization of P from the decomposition of microbial biomass or dead plant matter (leaf litter and roots).

The lower biomass and hence nutrient uptake in the gravel+sand was due to the loss of *Banksia* during extended dry periods (Henderson et al.2007). Over the first 3 years *Pennisetum* shoot biomass was 120g in the gravel+sand, 180g in sand and 260g in loam but after the application of effluent shoot biomass increased to 870g in gravel+sand.

Treatment performance with recycled effluent loading (between years 3 & 4.5) continued to be very high in the loam with 92% retention TP (100 g-m^{-2}-y^{-1}) and 79% retention TN (70.6-75.5g PO_4 m^{-2}-y^{-1}). Whilst the sand and gravel+sand mesocosms still removed substantial P and N, this represented only 67% TP and 53% TN for the sand and 44% TP and 42% TN for the gravel+sand. Total plant biomass indicated that plant uptake accounted for 10-15% TP and 42-55% TN retention. Microbial biomass and media sorption, in particular in the loam, would account for the balance of P retention.

Table 2. Comparison of annual mass retention (g-m^{-2}-y^{-1}), % retention and annual plant uptake for different media and influent loads in Experiments 1, 2 and 3. (S= stormwater, T=tap water, E= recycled effluent)

			Annual Mass Retention (g/m2)& % Retention				Annual Plant Uptake (g/m2)	
			PO4-P	TP	NOx-N	TN	TP	TN
Media	Age	IN						
Experiment 1								
GV	2.5y	S+T	1.46(98%)	1.56(85%)	3.39(65%)	14.5(63%)	3.5	11.8
SV	2.5y	S+T	1.50(100)%	1.72(94%)	4.12(79%)	17.8(77%)	4.7	14.8
LV	2.5y	S+T	1.46(97%)	1.65(90%)	4.90(93%)	17.8(77%)	5.3	15.8
GV	4.5y	E	32.6(39%)	47.6(44%)	20.1(33%)	45.9(42%)	7.00	23.65
SV	4.5y	E	51.4(62%)	72.5(67%)	25.3(42%)	57.7(53%)	9.34	31.57
LV	4.5y	E	72.2(89%)	100(92%)	45.3(74%)	86.4(79%)	11.10	37.50
Experiment 2								
RM06	2y	E	74.3(93%)	85.4(89%)	16.5(30%)	44.9(40%)	17.24	63.26
RM10	2y	E	74.7(96%)	82.4(86%)	12.4(22%)	31.6(28%)	17.88	64.97

K20	2y	E	71.7(90%)	83.9(87%)	20.9(37%)	56.7(51%)	16.40	59.34
K30	2y	E	70.6(88%)	83.2(86%)	19.8(35%)	55.2(49%)	17.28	61.01
K10-30	2y	E	72.2(90%)	84.1(87%)	20.8(37%)	56.0(50%)	14.28	50.58
WTR+K	2y	E	72.5(95%)	83.4(94%)	9.8(18%)	54.5(50%)	11.76	63.56
Experiment 3								
WTR-MC	1y	E	47.5(99%)	48.5(99%)	-4.8	45.8(51%)	13.38	55.64
WTR-MC	1y	T	0.05(26%)	-0.052	1.7(62%)	1.06(26%)	1.8	12.8

Experiment 2

Over the first 2 years, all media had similar performance treatment for phosphorus with annual mean mass retention of 70.6-75.5g PO_4 m^{-2}-y^{-1} and 82.4-87.7g P m^{-2}-y^{-1}. The media amended with WTR+K had the highest mass P retention and % removal (99%), followed by the media amended with Red Mud. Nitrogen retention was lower and more variable between media ranging from 31.6g N m^{-2}-y^{-1} (28%) in RM10 to 56.7g N m^{-2}-y^{-1} (51%) in K10-30. In the RM and K amended treatments, net NO_x retention ranged from 12.4g NO_x m^{-2}-y^{-1} (RM10) to 20.9g NO_x m^{-2}-y^{-1} (K20 and K10-30), but was lowest in the WTR+K (9.8 g-m^{-2}-y^{-1}).

Total plant biomass indicated that plant uptake in the RM and K treatments accounted for between 14.3-17.9 g P m^{-2}-y^{-1} and 50.6-65.0 g N m^{-2}-y^{-1}. K10-30 had the lowest plant biomass whereas RM10 had the highest. Plant uptake accounted for 17-22% TP retention but exceeded TN retention except in K10-30. In WTR+K, P uptake was 11.76g P m^{-2}-y^{-1} and accounted for 14% TP retention.

Experiment 3

This experiment has only been established for 12 months. The mesocosms treated with recycled effluent received a lower mass load than Experiment 2 but showed a similar performance efficiency to the WTR+K with 99% P removal (48.5gP m^{-2}-y^{-1}) and 51% TN removal(45.8gN m^{-2}-y^{-1}).

Plant biomass was 13.4g P and 55g N and uptake accounted for 28% TP retention but exceeded net N retention implying that the plants must have taken up nutrients from the media itself.

By contrast, the controls that received tap water (and rain) only had a 26% net PO_4 retention (0.05g PO_4 m^{-2}-y^{-1}) and a net export of TP. Considerable leaching occurred between loadings especially after extended dry periods. TN retention was 1.06g N m^{-2}. Plant biomass was 1.8g P and 12.8g N, both exceeding 'net retention' and indicating the extraction of nutrients from the media mix.

Plant Growth and Biomass.

Experiment 1

Henderson (2008) trailed *Dianella brevipedunculata*, *Pennisetum alopecuroides, Banksia integrifolia* and *Callistemon pachyphyllus* and recorded better growth and higher shoot biomass in *Pennisetum* than *Dianella* and better growth (taller stems and more leaves) in *Callistemon* than *Banksia*. After 2.5 years he also noted that plant growth was most vigorous in the loamy sand media, followed by sand, then gravel+sand (Henderson et al., 2007).

After 4.5 years growth, including 12 months of weekly recycled effluent loadings, shoot biomass for *Dianella* and *Pennisetum* doubled, but total biomass for *Pennisetum* was 7 times greater than *Dianella*. There was considerable variation between biomass in the different media with loam having the highest biomass for most of the species (Table 3). However *Pennisetum* grew better in the sand-gravel media with a dense root mat extending through the gravel and into the under-drain.

Table 3. Comparison of mean shoot and root/rhizome biomass (g dry wt) in *Dianella, Pennisetum*, *Banksia* and *Callistemon* grown in gravel+sand, sand and loamy sand media after 4.5 years.

Media	**Gravel + 20cm Sand**		**Sand**		**Loam**	
Species	**Shoot**	**Root/Rh**	**Shoot**	**Root/Rh**	**Shoot**	**Root/Rh**
Dianella	105	20	45	15	100	35
Pennisetum	570	300	320	120	630	250
Banksia	100	40	130	40	115	100
Callistemon	200	100	900	440	650	385

Experiment 2

Above ground shoot biomass and below ground root/rhizome biomass over 2 years is given in Table 4. Shoot biomass with harvesting every 6 months yielded over 1kg dry matter in 2 years for *Pennisetum* in K20 and around 700g in RM06 and K10-30; whilst *Carex* in WTR+K yielded 827g. Both *Pennisetum* and *Carex* had the most extensive root biomass. *Isolepis* had similar biomass in all media but did not respond well to cropping.

Table 4. Comparison of mean shoot and root/rhizome biomass (g dry wt) in *Isolepis, Carex, Pennisetum*, *Callistemon* grown in RM06, K20 ,K10-30 and WTR-K media after 2 years .

Media	**RM06**		**K20**		**K10-30**		**WTR+K**	
Species	**Shoot**	**Rt/Rh**	**Shoot**	**Rt/Rh**	**Shoot**	**Rt/Rh**	**Shoot**	**Rt/Rh**
Isolepis	441	94	324	75	423	96	380	46
Carex							827	385
Pennisetum	706	245	1047	336	716	315		
Callistemon	157	148	318	140	300	208	337	92

Above ground shoot biomass and below ground root/rhizome biomass over 3 years is given in Table 5. *Pennisetum* shoots with harvesting every 6 months continued to yield a very high biomass, however root biomass did not increase, indicating senescence. *Isolepis* biomass did not increase and gradual die back occurred once *Pennisetum* dominated the mesocosms. The *Callistemon* shrubs increased in shoot biomass.

Table 5. Comparison of mean shoot and root/rhizome biomass (g dry wt) in *Isolepis, Pennisetum* and *Callistemon* grown in RM10 and K30 media after 3 years.

Media	**RM10**		**K30**	
Species	**Shoot**	**Root/Rhizome**	**Shoot**	**Root/Rhizome**
Isolepis	441	61	411	61
Pennisetum	1683	309	1221	315
Callistemon	416	169	598	275

Experiment 3

Above ground shoot biomass and below ground root/rhizome biomass for *Lomandra, Pennisetum, Vetiveria*, *Callistemon* and *Melaleuca* grown in WTR (MtC) over 1 year is given in Table 6. In the recycled effluent treatment *Pennisetum* and *Callistemon* biomass showed comparable annual growth to Experiment 2, however *Melaleuca quinqueneria* had the highest biomass. Whilst all the plants grew in the tap water treatment only *Vetiveria* performed relatively better.

Table 6. Comparison of mean shoot and root/rhizome biomass (g dry wt) in *Lomandra, Pennisetum, Vetiveria*, *Callistemon* and *Melaleuca* grown in WTR (MtC) media loaded with recycled effluent and tap water (control) after 12 months.

Media	WTR-MtC -Effluent		WTR-MtC –Tap Water	
Species	**Shoot**	**Root/Rhizome**	**Shoot**	**Root/Rhizome**
Lomandra	77	24	18	8
Pennisetum	377	192	51	51
Vetiveria	154	96	96	68
Callistemon	102	46	20	12
Melaleuca	420	320	114	66

DISCUSSION

Phosphorus Retention

Biological and geochemical P cycles interact in a variety of ways; in particular they can compete directly for PO_4 in the soil solution and thus control how much P is biologically available versus how much is geochemically retained in the soil media (Olander and Vitousek, 2004). Plants and microbes uptake and immobilize P in organic matter. Through death and decay of leaf litter, roots and microbial biomass this organic matter becomes part of the soil organic P pool. Organic P is then mineralised and becomes bioavailable again for uptake. During our experimental loadings and rainfall events, pulses of P are released from this dead organic matter and further maintain P availability for further P sorption or biological uptake.

The loamy sand media in Experiment 1 (Henderson et al. 2007; Lucas and Greenway, 2008) and the sand media with amendments of Red Mud, Krasnozems and Water Treatment Residuals in Experiment 2 (Lucas and Greenway, 2010a); and the sand media with WTR in Experiment 3 all demonstrated a very high capacity for P retention from high mass loadings. However, after 12 months of recycled effluent loadings (equivalent to a decade of stormwater runoff loads) the loamy sand media was ineffective for removal of P from stormwater (Lucas and Greenway, 2008). The sand media amended with RM, K and WTR sustained high P retention from stormwater even after 24 months, with cumulative P loadings equivalent to 30 years of stormwater runoff loads (Lucas and Greenway, 2010a). We have just completed another 12 months of recycled effluent loadings and our water quality analyses indicate that these media have still not reached P sorption capacity.

In Experiment 1, our findings indicated that plant uptake accounts for only 10% of the total P load applied (or 13% PO_4 load). However in Experiment 2, due to the higher planting densities of *Pennisetum* and *Carex* plant uptake (plant biomass) accounted for 17-22% TP retention.

Microbial P can account for between 11-80 mgP-kg^{-1} in tropical rainforest soils (Olander and Vitousek, 2004); in our media microbial P in the mesocosm soil profile ranged from 60-70 mgP-kg^{-1} in the 3 year old media (RM10 and K30) and 16-20 mgP-kg^{-1} in the 2 year old WTR media. Given approximately 135Kg dry weight soil media in our mesocosms, this would represent between 8.1-9.5g P in RM10 and K30 and 2.2-2.7g P in WTR+K and WRT30 mesocosms. These microbial biomass P values are very high, and per m^2 would represent 32-38gP in the RM10 and K30 treatments, 2 times greater than annual P plant biomass on a g-m^{-2} basis, however due to rapid microbial turnover- growth and decay, this microbial P would be returned to the soil media P pool for mineralization.

Biologically mediated P uptake does not account for total P retention. Based on the above values microbial P uptake and plant P uptake would account for a maximum of 60% of the retention observed in the 3 year old RM10 and K30 mesocosms and only 25% of the retention observed in the 2 year WRT+K mesocosms.

Thus, geochemical processes of adsorption and/or precipitation of PO_4-P are still key mechanism by which P is retained. During intermittent loading "rapid-reversible" sorption reactions predominate when effluent flows through the media, with the "slow-irreversible" sorption reactions then continuing between events. Between events, some of the P bound to the rapid reversible sites is relocated to the irreversible sites, releasing the rapid reversible sites for the next loading event (Lucas and Greenway, 2010a).

We hypothesize that rapid P retention is mediated primarily by microbial activities (Lucas and Greenway 2010a). During inter-event periods, immobilized microbial P is then released back into the media where a cascade of sorption processes sequesters P irreversibly. As a result, it is the long-term sorption properties of media amendments that dominate the retention response.

Nitrogen Retention

Nitrogen retention is complex. In our recycled tertiary treated effluent, ammonium comprised less than 15%, however NO_x, mostly in the form of nitrate comprised between 55 -75% TN, with organic N accounting on average for 30%. Labile organic N can be mineralized into NH_4. In our experiments NH_4 and NO_2 were very low or non-detectable in the outflows, suggesting total nitrification. NH_4 and NO_x are both taken up and assimilated by the plants, while the remainder is either leached from the profile, or removed by denitrification. In Experiment 1, plant biomass N indicated that plant uptake accounted for 43- 55%TN annual load retained, suggesting that the additional retention was due to denitrification. However in Experiment 2, plant biomass N matched or even exceeded net annual TN retention

Root/Rhizome Biomass and Volume

Below ground components are important for water and nutrient uptake, nutrient storage and maintaining/improving soil porosity. Thus plant species with a high below ground biomass would be expected to support a higher above ground biomass. Below ground biomass for roots in the shrubs *Callistemon* and *Melaleuca* and root/rhizomes in the lilies (*Dianella*, *Lomandra*), sedges (*Isolepis*, *Carex*) and grasses (*Pennisetum*, *Vetiver*) are given in Tables 3 to 6. In the case of the woody shrubs eg *Callistemon*, incremental root and shoot biomass overtime is evident from our field data. However, in both *Isolepis* and *Pennisetum 'stabilization'* or even a decrease in root/rhizome biomass was recorded suggesting senescence and decay. This is also supported by a comparison of root volume per plant/ "plant clump" over time (Table7).

Table 7. Comparison of mean root volume (mL) per plant/plant clump over time.

Species	mean root volume (mL) per plant/clump			
	1 year	2years	3years	4.5 years
Pennisetum	1318	2222	2321	2232
Vetiver	505			
Lomandra	151			
Isolepis		410	322	
Carex		2637		
Callistemon	165	525	792	1472
Melaleuca	1114			

Root density (root volume) is particularly important for maintaining soil porosity. Both *Pennisetum* and *Carex* had the highest root volumes after 2 years growth. Both species produce abundant adventitious roots from the rhizomes, and in our mesocosms these roots reached the bottom of the soil media (65cm) forming a dense tangled curtain of roots and extended into the gravel base (depth 80cm). By contrast the 'wiry' roots of Isolepis only occupied 1/7 the volume of *Pennisetum* and *Carex*.

Callistemon produces a woody tap root and lateral roots, and after 2 years this occupies ¼ the volume of *Pennisetum* and *Carex* but in Experiment 1 after 4.5 years its volume was 2/3 that of *Pennisetum*. Thus whilst there appears to be root die back in the grasses and sedges over time, the roots of the woody plants continue to grow.

CONCLUSIONS

The sand and loamy sand media both had very high treatment performance for P retention at low mass loads but with the application of higher concentration recycled effluent the loamy sand was the most effective media. Sandy media with amendments of Red Mud, Krasnozems and Water Treatment Residuals all had very high and similar annual mass P retention. Plant uptake accounted for between 14%-22% of annual P retention but occasionally exceeded net N retention. *Pennisetum alopecuroides, Carex appressa*, *Callistemon pachyphyllus* and *Melaleuca quinquenervia* had the highest biomass and annual production rates for N and P accumulation of the 9 species trialed. *Pennisetum* and *Carex* shoots responded well to harvesting, and had extensive root biomass. *Dianella* and *Isolepis* did not respond well to cropping and had wiry root systems. Nevertheless they can be an attractive component of bioretention systems.

Acknowledgements

Loganholme Water Pollution Control Centre for providing the facilities for the mesocosms; Queensland Alumina Limited for providing the red mud for research purposes; Stradbroke Island Water Treatment Plant (Redland Shire)and Mt Crosby Water Treatment Plant(SEQWater) for providing the water treatment residuals; SEQWater for funding Experiment3; Daya Gautum, Kaskah Ward, Louise Pollard and Wendy Tang for assistance with plant biomass data; Carolyn Polson, Mike Steenhauer and Miriam Paul for laboratory assistance in analyzing the water, plant and soil samples.

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