



## Efficiencies of indigenous South African plant biofilters for urban stormwater runoff water quality improvement with a focus on nutrients and metals

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### ABSTRACT

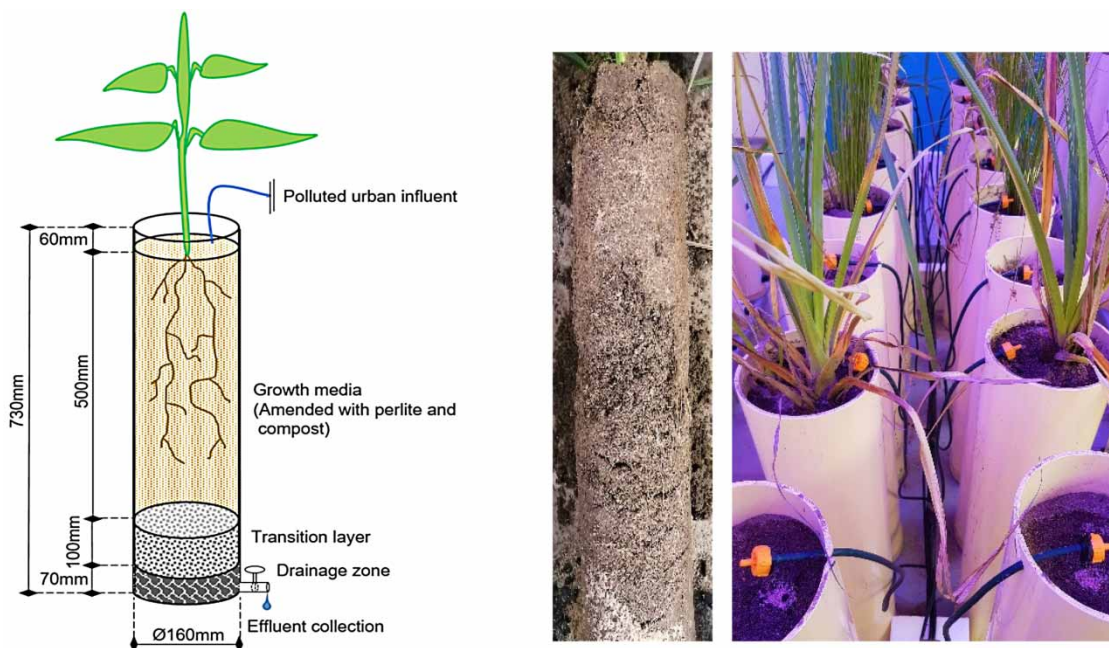
In South Africa, urban activities contribute high levels of pollution to rivers and groundwater via stormwater runoff. In reducing urban stormwater loads of engineered plant biofiltration, an effective and self-sustaining component of green infrastructure is a treatment option. The country's extensive natural biodiversity offers untapped potential of indigenous species' use in plant biofilters. This paper presents the findings of a plant biofilter column experiment, which investigated the performance of nine indigenous plant species under varied urban stormwater pollutant load strengths. Average significant loads of dissolved Cd (>98%), Cu (>84%), Pb (>99%) and Zn (>95%), as well as NH<sub>3</sub>-N (>93%), were removed by the plant biofilters, whereas the removal of NO<sub>3</sub><sup>-</sup>-N (-37 to 79%) and PO<sub>4</sub><sup>3-</sup>-P (-81 to 63%) was more variable. Biofilters equipped with indigenous plant species were on average at least 11% more efficient than unvegetated soil in the removal of urban nutrient and metal pollutants. Over time, planted biofilters improved nutrient and metal removal efficiencies. The results support the inclusion of indigenous plants in biofilters within urban stormwater green infrastructure initiatives. Further research to inform plant biofilter design practicalities and assess plant biofilter performance in the field is warranted.

**Key words:** green infrastructure, metals pollution, nutrients pollution, plant biofilter, urban stormwater runoff

### HIGHLIGHTS

- This is the first study investigating nutrient and metal removal by indigenous South African plant species.
- Assessment of low, typically observed and high strength pollutant load removal.
- Consistently greater removal by vegetation as opposed to unvegetated soil.
- PO<sub>4</sub><sup>3-</sup>-P and dissolved Cu removal influenced by compost.

## GRAPHICAL ABSTRACT



## INTRODUCTION

Increased urban surface imperviousness caused by the rapid growth and densification of cities during urbanisation alters stormwater runoff volume, frequency and quality (Bratieres *et al.* 2008). Urban stormwater consists of a broad range of pollutants that can have a detrimental impact on aquatic systems, posing a major human and environmental health problem (Lim *et al.* 2015). In South Africa, aging, poor quality and poorly maintained conventional grey infrastructure in some cases contribute high levels of pollution to receiving waters, leading to a 500% classification increase (1999–2011) of main rivers having poor ecological conditions (DWS 2018). As a result, the country experiences some of the most severe environmental degradations globally, requiring proper urban mitigation strategies to restore ecosystem function and improve resource quality (De Klerk *et al.* 2016).

In contrast to conventional infrastructure approaches that typically consider stormwater as a substance to dispose of rather than a resource to protect, improved urban stormwater management seeks to treat nonpoint pollution, reduce hydrologic disturbance and utilise stormwater as a supplementary resource (Barbosa *et al.* 2012; Fletcher *et al.* 2015). The water-sensitive urban design approach promotes the integration of green infrastructure (GI) and nature-based solutions to ensure holistic water quality and provision in the urban water cycle (Armitage *et al.* 2014; Wendling & Dumitru 2021). As an efficient and self-sustaining interconnected set of natural and engineered ecological systems, GI has been proven to be capable of successfully treating urban runoff (Prodanovic *et al.* 2018). Internationally, GI has been demonstrated to provide critical maintenance, rehabilitation and purification services in a more cost-effective way than conventional infrastructure (Postel & Thompson 2005).

The use of plant biofiltration has become increasingly popular within GI due to its ability to slow stormwater runoff rates, reduce runoff volumes, decrease particulate transport and retain pollutants prior to discharge into watercourses (Lim *et al.* 2015). As a form of ecological engineering, biofiltration technologies utilise plants to detoxify, degrade and/or remove pollutants from the environment (Visoottiviseth *et al.* 2002). In South Africa, GI is currently an under-realised approach (DWS 2018). The addition of vegetation for the treatment of pollutants enhances removal and sustainability, a process known as phytoremediation, improving biofilter performance (Dietz & Schnoor 2001). The extraction and immobilisation of pollutants by plants does, however, vary between plant species, making the process of plant selection critical to biofilter performance

(Read *et al.* 2008). In addition, due to the competitive nature of plants within biofilters, plant selection must consider invasion threat to the recipient ecosystem's biodiversity (Leguizamo *et al.* 2017).

In South Africa, the introduction and invasion of some plants have transformed entire ecosystems, posing a major threat to the country's biodiversity and impacting negatively on ecosystem health (Le Maitre *et al.* 2020). For sustainable GI, conservation cannot be overshadowed by the need for treatment efficiency. Therefore, the use of non-invasive indigenous species, which can enhance biofilter performance and are capable of adapting to the recipient habitat, whilst limiting the risk of biodiversity loss, should always be promoted (Payne *et al.* 2015). South Africa, recognised as having the world's richest temperate flora comprising approximately 20,500 plant species (of which 13,265 are endemic), is considered to be both a biodiversity hotspot and a megadiverse country (Hoveka *et al.* 2020). This extensive biodiversity offers relatively untapped potential of indigenous plant species for use in GI that are naturally acclimatised to recipient ecosystems and which do not threaten biodiversity. In urban areas, this is particularly important, as urban areas are often the initial sites from which invasions spread (Zengeya & Wilson 2020).

As a result of a lack of local research and design guidelines, South Africa faces a number of GI application challenges. The technology is still perceived as a new concept, which does not yet fit into established municipal guidelines (Pasquini & Enqvist 2019). In response to this need for local research, this paper presents the findings of a large-scale laboratory plant biofilter column study of indigenous South African species for application to urban stormwater quality improvement. In the study, we investigated the individual efficiencies of nine indigenous plant species and unvegetated soil exposed to varying concentrations (loads) of nutrients and metals based on published figures of stormwater runoff pollution concentrations and loads. In assessing the indigenous species for potential South African phytoremediation, efficiencies were compared between species across all pollutant loads and with unvegetated soil. Furthermore, plant response to the pollutants and biofilter columns were assessed by comparing leaf and root development. Based on these findings, the potential for indigenous plant species inclusion in local urban stormwater GI initiatives is discussed. This experiment forms part of a larger study on urban stormwater plant biofilter optimisation (Jacklin *et al.* 2021a, 2021b).

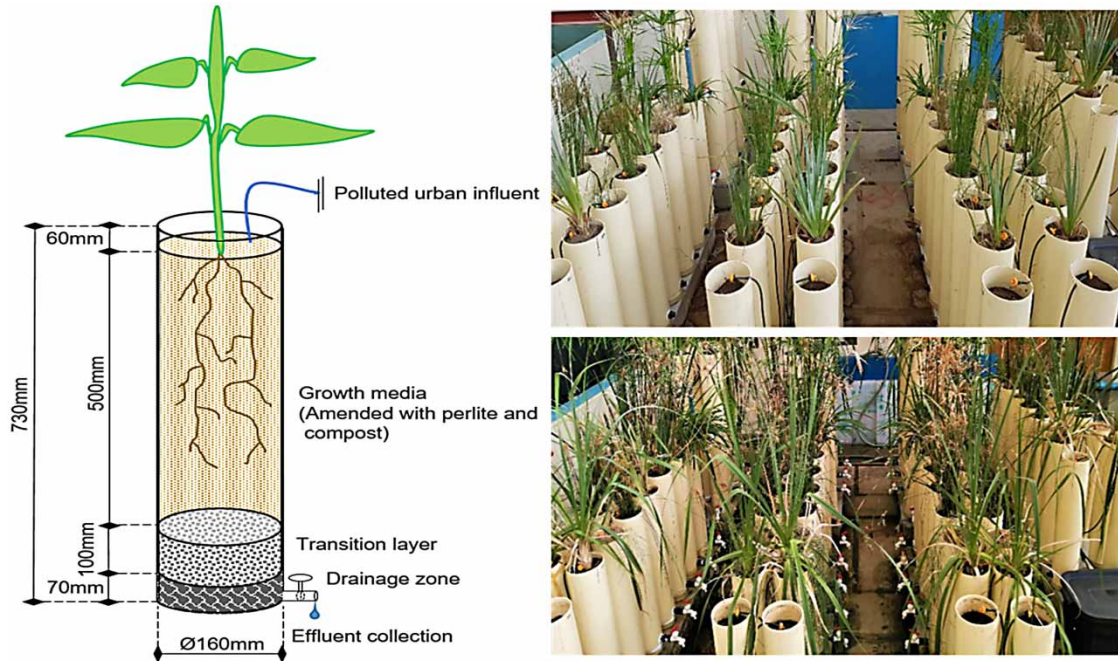
## METHODOLOGY

### Experimental design

Ninety biofilter columns were constructed from Ø160 mm × 730 mm PVC piping based on typical urban design guidelines as recommended by Payne *et al.* (2015). Within the columns, a drainage outlet was covered by a 70 mm drainage zone below a 100 mm media transition layer, topped with 500 mm growth media amended with compost to support plant establishment (Figure 1). The inner wall of each column was abraded to minimise preferential flow and sealed at the base, allowing effluent extraction for analyses. All the treatments were done in duplicate, with two columns per treatment. A local sandy loam soil type (Stellenbosch, South Africa) with an infiltration rate of  $145 \pm 17$  mm/h was used, allowing the diffusion of water through the growth media, establishing an opportunity for pollutant interaction (Le Coustumer *et al.* 2012).

The selection of indigenous plant species for South African GI considered knowledge of removal pathways, species distribution, physiological characteristics and morphological traits. The selection process attempted to include plants from varying climatic and environmental conditions, increasing productivity through plant diversity (Leguizamo *et al.* 2017). For optimised phytoremediation, plant growth rate, lifespan, tolerance and hardiness, as well as biomass and roots, were considered (Pilon-Smits 2005). The selected plants (Table 1) consist of species adept at varying moisture content, enabling potential use in GI during seasonal fluctuations and at different distances from the stormwater influent source. The plants are capable of rapidly maturing during the experimental period, as well as tolerating inundation and periods of drought, providing a distinct assessment of urban pollutant removal performances.

The growth media of all biofilter columns were amended with a solid phase commercial compost mixture during transplantation in February 2020 and received municipal tap water for 10 months to allow plant establishment prior to dosing and testing. During the last 2 months of the establishment period, the columns were subjected to a flushing regime with each column receiving 5 L tap water per day. This was done to stimulate the discharge of excess nutrients and metals which may have lingered within the columns. During flushing, four preliminary samples were collected at 15-day intervals to confirm the time at which effluent concentration constancy was achieved. After 30 days, similar concentrations were measured over three sampling rounds. In addition, throughout the study effluent discharge concentrations from the non-polluted



**Figure 1** | Schematic of experimental column and biofilters at initiation (February 2020) and established (May 2021) state.

**Table 1** | Indigenous South African plant species for urban stormwater pollutant removal

Species	Common name	Habitat	Distribution to South Africa
<i>P. serratum</i>	Palmiet	Wet-terrestrial	Endemic
<i>C. aethiopica</i>	Cobra lily	Terrestrial	Endemic
<i>A. africanus</i>	African lily	Terrestrial	Endemic
<i>C. textilis</i>	Umbrella sedge	Aquatic	Endemic
<i>C. glomerata</i>	Vleibiesie	Aquatic	Endemic
<i>S. reginae</i>	Bird-of-paradise	Terrestrial	Indigenous
<i>D. iridioides</i>	Small wild iris	Terrestrial	Indigenous
<i>C. dactylon</i>	Common couch grass	Terrestrial	Indigenous
<i>J. effusus</i>	Soft rush	Aquatic	Indigenous

biofilter columns recorded were constant, supporting the assumption that existing column concentrations remained constant during synthetic dosing. Following this period, the biofilter columns received 4 months of synthetic stormwater dosing.

### Experimental procedure

Synthetic stormwater (Table 2) was prepared from analytical grade compounds to reflect published low, typically observed and high urban nutrient and metal stormwater pollutant concentrations (obtained from Göbel *et al.* 2007; Barron *et al.* 2019), as well as replaced every 10 days and continuously mixed with pump agitators to ensure uniform dispersion. Irrigation was done by means of submersible pumps via an automated drip irrigation system, with influent concentrations monitored throughout.

Although impermeable urban surfaces increase runoff volume and peak discharge into stormwater biofilters, not all rainfall is translated to runoff, since flow is reduced by infiltration, ponding and evaporation (Rammal & Berthier 2020). Due to the



**Table 2** | Influent synthetic stormwater

Pollutant	Parameter	Influent concentration (mg/L)			Source chemical
		Low	Typically observed	High	
Nutrients	Ammonia-N	0.20	0.40	2.5	NH <sub>4</sub> Cl
	Nitrate-N	0.50	0.95	13.5	KNO <sub>3</sub>
	Orthophosphate-P	0.22	0.35	1.5	K <sub>2</sub> HPO <sub>4</sub>
Metals	Cadmium	0.002	0.0045	0.032	CdCl <sub>2</sub>
	Copper	0.02	0.045	6.8	CuSO <sub>4</sub>
	Lead	0.08	0.15	2.8	PbCl <sub>2</sub>
	Zinc	0.15	0.30	35	ZnCl <sub>2</sub>

consolidating nature of urban area imperviousness, the collective impact on water resources can be predicted (Schueler 1992). The experimental dosing regime simulated potential influent volumes over urban area for biofilters sized at 2% of catchment area under typical rainfall events. This produced a twice-weekly dosing of 1.32 L for Ø160 mm biofilter columns based on typical climatic patterns (i.e. temperate climate with an average annual rainfall of 619 mm) and urban area characteristics (i.e. 37.4% impervious surface area) of Stellenbosch, South Africa (Musakwa & Van Niekerk 2015; SAWS 2020).

Effluent sampling was undertaken 30 days after initiating the synthetic stormwater dosing and was done at 15-day intervals. The 30-day period ensured the removal of non-polluted tap water (used during the establishment phase) from the saturated growth media to limit dilution of the dosing mixtures.

Six sampling rounds were undertaken from December 2020 to March 2021 during which the majority of the species are most active (excluding *Chasmanthe aethiopica*), with the first round establishing the existing biofilter conditions, prior to synthetic stormwater dosing. A separate assessment period from July to September 2020 was selected for *C. aethiopica* due to the plant's seasonal growth pattern, made possible by its rapid development rate. Water loss to evapotranspiration regulated by individual plant uptake, as well as drying of the media between dosage events, prevents the quantification of pollutant removal by assessing concentration alone. Therefore, the percentage pollutant removal ( $L_{rem}$ ) considered the influent ( $C_{inf}V_{inf}$ ) and effluent ( $C_{eff}V_{eff}$ ) pollutant loads of each biofilter, rather than concentrations.

$$L_{rem} = \frac{C_{inf} V_{inf} - C_{eff} V_{eff}}{C_{inf} V_{inf}} \times \frac{100}{1} \quad (1)$$

where  $L_{rem}$  is the pollutant load removal (%),  $C_{inf}$  is the influent concentration (mg/L),  $V_{inf}$  is the total influent volume for sampling round (L),  $C_{eff}$  is the effluent concentration (mg/L) and  $V_{eff}$  is the effluent volume of sampling round (L).

The effluent samples were tested for dissolved ammonia (NH<sub>3</sub>-N), nitrate (NO<sub>3</sub><sup>-</sup>-N) and orthophosphate (PO<sub>4</sub><sup>3-</sup>-P) in the Stellenbosch University Water Quality Laboratory using a HACH DR3900 spectrophotometer. Metal tests were performed on an Agilent 8800 QQQ ICP-MS instrument for dissolved cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) at the Stellenbosch University Central Analytical Facility: ICP-MS division. Prior to analysis, 0.45 µm syringe filters were used to filter the effluent samples into two 30 mL Falcon® tubes for nutrient and metal analyses.

The determination of plant growth response to medium-term exposure (6 months), as well as different urban stormwater nutrient and metal strengths, involved an inspection of above- and below-ground physical plant traits. Above-ground traits (leaves or stems) were recorded from February 2020 on a monthly basis, producing 16 measurements, whereas below-ground measurements (roots) were taken only at experimental initiation and conclusion. The number and average lengths of the leaves/stems of each species were compared between pollution strengths, as well as with plants receiving only non-polluted irrigation and plants unamended with compost under typically observed pollutant dosing for the duration of the experiment. For root response to pollution strength and growth media, a number of roots and a length of the longest root at transplantation were compared with measurements taken for each species at the conclusion of the experiment. Due to species' variation in physiological development, some measurement differences were noted, e.g. *Cynodon dactylon*'s lateral development made recording impractical. In addition, the natural dying back and regrowing cycle of *C. aethiopica* limited physical measurements for the entire study and were excluded.

**Data analysis**

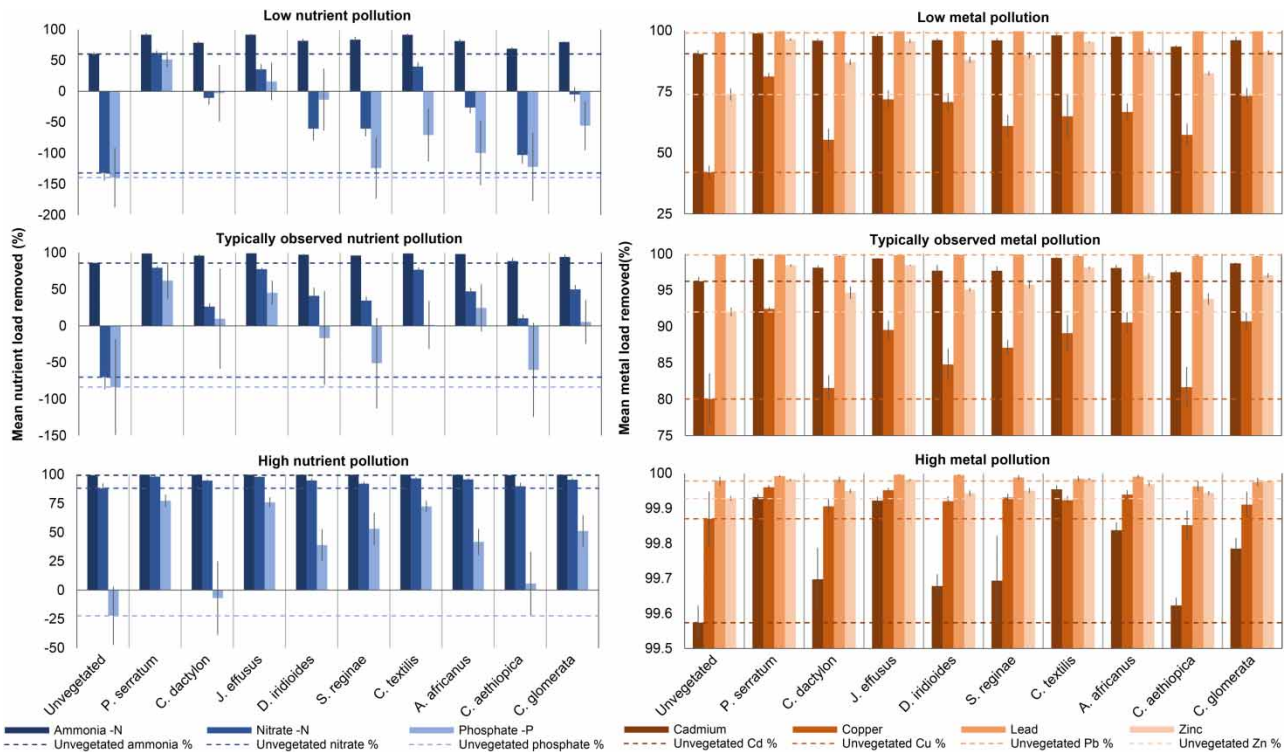
Statistical analyses were conducted with R statistical software. A non-parametric approach was adopted, since data were non-normally distributed, as shown by the *Lilliefors test*. Thus, the *Kruskal–Wallis H-test* non-parametric ANOVA ascertained the significance of difference in removal percentages. With significance, the *Wilcoxon rank sum paired test* compared the percent removal of each pollutant parameter between the plant biofilters for all three influent pollution strengths. Similarly, in assessing the difference in removal performance between vegetated and unvegetated soil over time, the *Wilcoxon rank sum paired test* was used. All analyses accepted significance at an unadjusted  $p \leq 0.05$ .

**RESULTS AND DISCUSSION**

**Effect of influent pollutant strengths on removal efficiencies**

The mean nutrient and dissolved metal pollutant load removal percentages over the sampling rounds are depicted in **Figure 2**. From the figure, it can be seen that percentage removal was influenced by influent values, with higher influent loads generally resulting in higher percentage removals. Different plant species, however, displayed variable results, indicating differences in possible treatment pathways. Additionally, percentage load removals varied across pollutant types (nutrient vs. metal). Dissolved metal removals were consistently greater than nutrient removals across the dosing strengths, with the exception of Cu, which was less efficiently reduced than nutrient  $\text{NH}_3\text{-N}$ .

High influent dosing values resulted in efficient dissolved metal removal outcomes ( $>99.6\%$ ) with no discernible large differences between biofilter columns and plant species. Similarly, nutrient  $\text{NH}_3\text{-N}$  ( $>99.4\%$ ) and  $\text{NO}_3^- \text{-N}$  ( $>88.2\%$ ) loads were efficiently reduced with high dosing values. In contrast,  $\text{PO}_4^{3-} \text{-P}$  was less efficiently removed, with *C. dactylon* ( $-6.8\%$ ) and unvegetated soil ( $-22.2\%$ ) biofilters, returning  $\text{PO}_4^{3-} \text{-P}$  back into the water column, resulting in a net pollutant increase.



**Figure 2** | Mean percentage nutrient and metal load removals of the experimental biofilters for the duration of the study. *Note:* horizontal lines indicating unvegetated soil removal and the variation in vertical axes, error bars indicating  $\pm$  standard error of each mean.

When exposed to the more moderate typically observed urban stormwater pollution levels, more variation in percent removal was observed for different plant species, in particular for nutrients and Cu. Contrary to this, all biofilters maintained high Pb removal percentages (>99.6%). Similar to the high dosing outcomes, P increases were recorded in some biofilters (with associated negative load removal percentages): these were *Dietes iridioides* (−16.6%), *Strelitzia reginae* (−51.1%), *C. aethiopica* (−60.2%) and unvegetated soil (−83.5%). In addition, unvegetated soil was found to leach  $\text{NO}_3^-$ -N, resulting in a mean pollutant load increase, instead of a decrease, of 70.1% over the study period.

Under low strength dosing levels, a clearer differentiation of individual biofilter removal performances was found. For dissolved metals, similar to typically observed and high urban dosing strengths, all metals were removed at varying degrees by the different biofilter columns. Consistent with the findings of mean percent pollutant load removal in moderate and high pollution, Cu was more variable than the other metals (ranging between 41.9 and 81.3%), with Pb consistently most successfully removed (>99.1%). Nutrient removal, however, was found to be more variable with  $\text{NO}_3^-$ -N and  $\text{PO}_4^{3-}$ -P, showing negative removals (i.e. substances in the effluent were higher than in the dosed influent) in the majority of the biofilter columns. Only the *Prionium serratum* (61.9%), *Juncus effusus* (35.6%) and *Cyperus textilis* (40.1%) biofilters removed  $\text{NO}_3^-$ -N, whilst  $\text{PO}_4^{3-}$ -P was removed by the *P. serratum* (51.4%) and *J. effusus* (15.9%) columns only.

From Figure 2, it can be seen that unvegetated soil biofilters consistently removed the lowest amounts of nutrient and dissolved metal loads across the three pollution strengths for the duration of the study, with a mean pollutant removal percentage of 45.7%. All the vegetated biofilters outperformed unvegetated soil for mean removals, with the worst performer being *C. aethiopica*, which had a mean removal of 56.3%, only 10.6% greater than unvegetated soil. In addition, applying the Wilcoxon rank sum test to compare removal efficiencies between individual plant species and the unvegetated soil with time found only *C. aethiopica* not to be significantly different from soil ( $p=0.22$ ). All other plant species showed significant differences ( $p\leq 0.05$ ) in pollutant load reduction when compared with unvegetated soil. The most efficient biofilters (based on average percentage load removals), with their corresponding significance values, were *P. serratum* ( $p=0.00014$ ), *J. effusus* ( $p=0.00032$ ) and *C. textilis* ( $p=0.0018$ ), removing on average 89.8, 85.3 and 78.6% of urban stormwater pollutant loads, respectively.

## Plant species performance and possible pollutant removal pathways

### Nutrient removal

For the removal of  $\text{NH}_3$ -N, all vegetated biofilters outperformed unvegetated soil across the low, typically observed and high dosing strengths. Overall, the best performing biofilters were *J. effusus*, *P. serratum* and *C. textilis*, reporting  $\text{NH}_3$ -N mean percentage removals of >96.7% for all three, whilst unvegetated soil achieved 81.8% mean removal. The worst performing plant biofilter, as well as being the only plant biofilter not significantly different ( $p=0.081$ ) compared with unvegetated soil, was *C. aethiopica*, removing 85.6% of pollutants, which was only 3.8% greater than unvegetated soil. Biological processes for nitrogen removal are known to occur across both aerobic and anaerobic conditions within the different areas of the biofilter column (Payne *et al.* 2014). Efficient  $\text{NH}_3$ -N removals found in all plant biofilters and unvegetated soil suggest an enhancement of the  $\text{NH}_4^+$  to  $\text{NO}_3^-$  nitrification process in the upper oxygenated layers of the columns, as well as possibly some volatilisation at the surface area (Blecken *et al.* 2009; Turner *et al.* 2010), even in the absence of vegetation (Barth *et al.* 2020). In the presence of organic matter, of relevance to this study due to the compost amendment during biofilter construction,  $\text{NH}_3$ -N sorbed to soil through electrostatic and ion exchange interactions can be rapidly nitrified (McNevin *et al.* 1999). The removal of  $\text{NH}_3$ -N in unvegetated soil, although less efficient than in the presence of plants, suggests a lack of full dependence by the microbial activity, which is responsible for nitrification, on symbioses with plants (Bratieres *et al.* 2008). Within the rhizosphere of the biofilter columns, microbial communities controlling the aerobic conversion of  $\text{NH}_3$  to  $\text{NO}_2^-$  are *Nitrosomonas* bacteria, after which *Nitrobacter* bacteria convert  $\text{NO}_2^-$  to  $\text{NO}_3^-$ , which is easily leached, as can be seen when assessing  $\text{NO}_3^-$ -N removal (Hunt *et al.* 2015). The results suggest the occurrence of these processes in the biofilter columns.

For the removal of  $\text{NO}_3^-$ -N, all vegetated biofilters were significantly ( $p\leq 0.05$ ) more efficient than unvegetated soil. Between them, the best performing biofilters were *P. serratum*, *C. textilis* and *J. effusus*, reporting mean removal percentages of 79.8, 71.1 and 70.3%, whilst unvegetated soil leached 37.9% (negative removal). Similar to  $\text{NH}_3$ -N, the worst performing plant biofilter for  $\text{NO}_3^-$ -N removal was *C. aethiopica*, leaching 0.9% (producing rather than removing) of pollutants, which was only 37% more efficient than unvegetated soil.  $\text{NO}_3^-$ -N increases and low inefficient removal performances by the biofilter columns for the duration of the experiment are an indication of successful biological transformation of captured  $\text{NH}_4^+$ ,

$\text{NO}_2^-$  and  $\text{NO}_3^-$ , resulting in minimal sorption to biofilter media due to oxidised nitrogen's anionic form, releasing  $\text{NO}_3^-$ -N to the water column (Henderson *et al.* 2007). Experiencing similar  $\text{NO}_3^-$  increase, Davis *et al.* (2001) suggested incomplete N removal processes due to a combination of inadequate denitrification and natural  $\text{NO}_3^-$  formation from additional sources within the biofilters between effluent events were responsible for increased  $\text{NO}_3^-$ -N leaching. From the results, enhanced  $\text{NO}_3^-$ -N removal in the presence of plants via direct uptake and the rhizosphere's influence on microbial nitrification and denitrification highlights the importance of vegetation (Muerdter *et al.* 2018).

For the removal of  $\text{PO}_4^{3-}$ -P, all vegetated biofilters outperformed unvegetated soil across dosing strengths, with the best performing biofilters being *P. serratum*, *J. effusus* and *D. iridioides*, removing 63.5, 45.7 and 2.9%, respectively, with 81.7% leached on average (negative removal) by unvegetated soil. Similar to other nutrients, in releasing 58.9% of the influent  $\text{PO}_4^{3-}$ -P load, *C. aethiopica* was the worst performing plant biofilter, which was only 22.8% more efficient than unvegetated soil. Comparing the plant biofilters with unvegetated soil, the Wilcoxon rank sum test reported only two significantly better performers: *P. serratum* ( $p=0.016$ ) and *J. effusus* ( $p=0.029$ ). Compared with the other nutrients, the removal of  $\text{PO}_4^{3-}$ -P was more variable, reporting a net increase in pollutant load in some biofilters instead of a decrease under each of the influent strengths. Phosphorous in soil is sorbed to soil particles or incorporated into organic matter and released by sorption and desorption (Holtan *et al.* 1988). Biofilters equipped with plants extract  $\text{PO}_4^{3-}$ , which is then concentrated 100–1,000 times in the xylem sap and stored for future plant use in the plant tissue, typically 0.5% phosphorous by dry weight (Muerdter *et al.* 2018). Of note in this study, the best performing plant biofilters were equipped with *P. serratum* and *J. effusus*, recognised for their high biomass content. Removal, however, varies between plant species and is influenced by direct and mycorrhizal (symbiotic association between a plant and a fungus) uptake (Fowdar *et al.* 2017). In addition, amending biofilter columns with compost have been found to increase  $\text{PO}_4^{3-}$ -P in effluent runoff, severely hindering pollutant load removal compared to unamended biofilters (Lenth & Dugapolski 2011). In this study,  $\text{PO}_4^{3-}$ -P removal increased with time. This was reported in all three pollution treatments, with performance in unvegetated and vegetated columns improving from 125.7% production in sampling round 1 to 64.1% removal in sampling round 5.

### Metal removal

The mean dissolved metal load removal percentages (Figure 2) were relatively high across dosing strengths. Similar to nutrient removal, all vegetated biofilters outperformed unvegetated soil for metal removals as illustrated by the horizontal lines on the graphs. The ecological consequences of heavy metals in biofilters are mainly related to heavy metal solubility and mobility, which influence leaching and removal via microbial activity and, ultimately, plant uptake via adsorption and desorption (Krishnamurti *et al.* 2005). In urban biofilters, heavy metal movement through the soil profile occurs mainly in the solution phase for effluent deposition and has been found to be of limited extent, even in highly polluted conditions (Emmerich *et al.* 1982). For stormwater quality improvement purposes, the amounts available to plants for uptake influence plant growth and water quality, with potential removal varying with regard to plant species, pH, ionic strength, redox potential, composition of solution and valence (Da Silva & Williams 1976). A benefit of plant biofilters is the acidification of the rhizosphere during effective  $\text{NH}_3$ -N uptake and subsequent proton release, which can influence metal speciation due to altered soil particle surface charge, thereby facilitating redox reactions (Laurenson *et al.* 2013). Therefore, given the complex processes affecting metal removal in plant biofilters, the differences in removal efficiencies and potential effects based on measured data were reported with reference to possible removal mechanisms provided as supposition only. However, further research into this area is warranted.

The influent concentrations of highly toxic Cd were lower when compared with Cu, Pb and Zn, due to the metal being generally present at low concentrations in terrestrial environments (originating from mainly anthropogenic activities) (Alloway & Steinnes 1999). The mean Cd, Zn and Pb removal percentages by the biofilters were high across the different pollution dosing strengths, ranging from 90.5 to 99.9%, 73.9 to 99.9% and 99.1 to 99.9%, respectively. The practical effects of these differences were considered to be minimal and it was accepted that all plant biofilters removed these metals adequately. The most efficient plant biofilters for the removal of Cd contained the species *P. serratum*, *C. textilis* and *J. effusus*, with mean removal percentages of >99.1% for all three. As reported by the Wilcoxon rank sum test, the removal of Cd by all plant biofilters were significantly different ( $p \leq 0.05$ ) compared with unvegetated soil, which removed 95.4% of the influent load.



For Pb, the most effective biofilters contained the species *A. africanus*, *P. serratum* and *D. iridioides*, each with >99.8% mean percentage removals. In contrast to Cd, for the removal of Pb, none of the plant biofilters had significant percentage removal differences ( $p > 0.05$ ) compared with unvegetated soil, which removed 99.6% of pollutants.

Similar to Cd, *P. serratum*, *J. effusus* and *C. textilis* biofilters most effectively removed Zn, with mean percentage removals of 98.3, 98.1 and 97.8%, respectively. For the removal of Zn, all plant biofilters showed statistically significant percentage removals ( $p \leq 0.05$ ) when compared with unvegetated soil, which removed 88.6% of the pollutant loads.

These high removal percentages found are similar to published findings reporting on alternating influent sources (see Barron *et al.* 2019), biological retention (see Davis *et al.* 2001) and the impact of a carbon source on heavy metal removal (see Blecken *et al.* 2009). Although minimal, the presence of vegetation removal is enhanced, with non-essential Cd and Pb taken up without offering any known direct biological benefit to plants (see Ali *et al.* 2013).

Cu removal was found to be more variable with mean removal across the different dosing strengths ranging from 41.9 to 99.9%. This is consistent with similar published investigations (see Davis *et al.* 2001; Lenth & Dugapolski 2011). The *P. serratum*, *C. glomerata* and *J. effusus* biofilters were most efficient for the removal of Cu, with 91.2, 87.9 and 87.1% mean removals, respectively. For the removal of Cu, all plant biofilters were significantly more efficient than unvegetated soil ( $p \leq 0.05$ ), which achieved 73.9% removal, except for two biofilters containing the species *C. dactylon* ( $p = 0.073$ ) and *C. aethiopica* ( $p = 0.088$ ). This variation may be due to the formation of Cu-organic matter complexes in the presence of compost, since Cu has the strongest affinity to organic matter out of all tested metals (Ponizovsky *et al.* 2006). Organic matter in a solid or dissolved form significantly influences treatment in stormwater biofilters, whilst solid organic matter adsorption with Cu through immobilisation is the main pathway for Cu filtration in soil (Temminghoff *et al.* 1997). Dissolved organic matter has been reported to mobilise Cu (Amery *et al.* 2007) and in some cases has resulted in Cu leaching lasting several years (Mullane *et al.* 2015). The organic matter added to the biofilter columns was in the form of a high solid content compost; thus, it is accepted that Cu immobilising likely occurred.

In summary, the removal of dissolved metals was high overall in both unvegetated and vegetated plant biofilters. This is in line with published literature, as the majority of metals are expected to have been removed by unvegetated mechanisms such as filtration and adsorption (Hatt *et al.* 2007). Rapid metal removal has been reported to occur in the top layers of biofilters, with >82% removal in the first 150 mm found by Blecken *et al.* (2009) varying slightly with the findings of Davis *et al.* (2001) reporting >92% removal in the upper 270 mm of the growth media. The removal of micronutrients Cu and Zn via direct uptake pathways is enhanced in the presence of plants (Muerdter *et al.* 2018), whilst non-essential toxic Cd and Pb are extracted and distributed to all plant organs (Hocaoglu-Ozyigit & Genc 2020). The additive, though relatively small, metal uptake by plants can provide a permanent pollutant removal pathway via harvesting, as metal accumulation in plant tissue occurs over time (Kumar *et al.* 2017). However, long-term (years or decades) metal accumulations warrant further investigation.

### Effect of vegetation and time on removal efficiencies

In assessing the influence of vegetation on urban stormwater pollutant removal over time, the combined average load removal efficiencies were compared with that of unvegetated soil for nutrient and metal removals. For low dosing (Figure 3), Wilcoxon rank sum statistical analysis showed that the presence of vegetation had a significant effect on overall nutrient ( $p = 0.0046$ ) and metal ( $p = 0.000051$ ) removal efficiencies over time. Vegetation consistently removed greater pollutant loads than unvegetated soil for the duration of the experiment.

Exposed to typically observed pollution levels (Figure 4), similar results to low pollution for nutrient and metal removal were found. In comparing removal efficiencies, vegetation was found to be significantly more efficient than unvegetated soil for the removal of both nutrients ( $p = 0.0093$ ) and metals ( $p = 0.0060$ ). In addition, metal removal efficiencies in unvegetated soil declined over the last round of sampling, whilst vegetation maintained removal efficiency.

In comparing removal performances between vegetated and unvegetated soil for high strength urban pollution (Figure 5), no significant differences were found for both nutrient ( $p = 0.096$ ) and metal ( $p = 0.076$ ) removals. Similar to typically observed pollution levels, metal removal efficiencies in unvegetated soil declined in the final sampling round, whilst vegetation maintained treatment efficiency. Although removal was consistently greater in the presence of vegetation, due to percent concentration reduction being strongly dependent on influent concentration, the high influent concentration may have been the reason for the high removal percentages, particularly for metals (see Lampe *et al.* 2005), as this function also applies to pollutant loads.

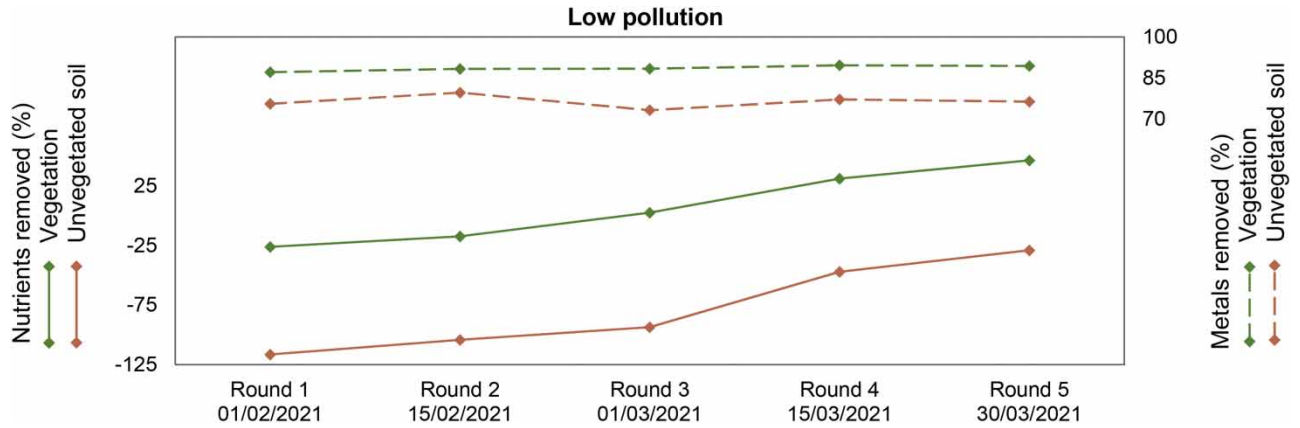


Figure 3 | Mean low strength nutrient and metal removal by vegetated and unvegetated biofilters with time.

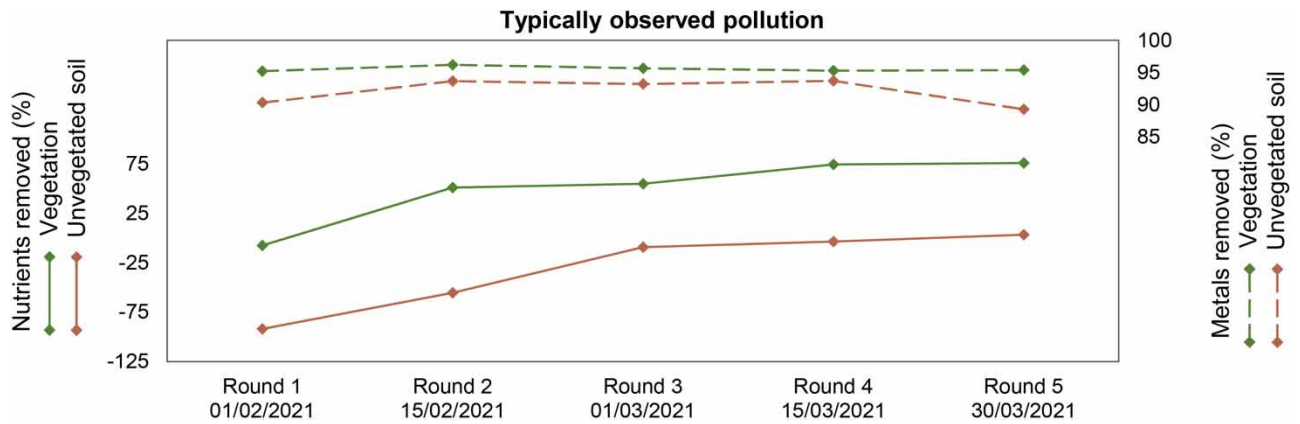


Figure 4 | Mean typically observed strength nutrient and metal removal by vegetated and unvegetated biofilters with time.

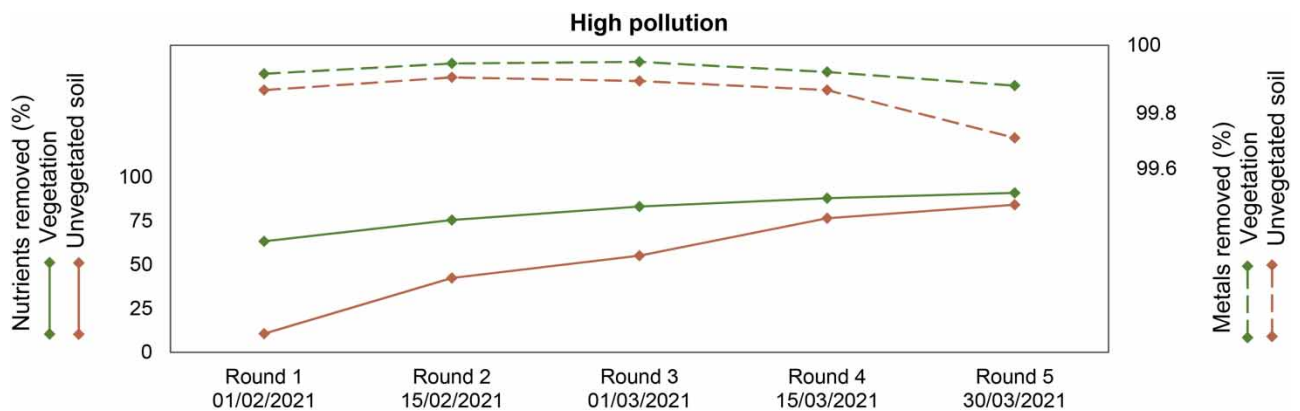


Figure 5 | Mean high strength nutrient and metal removal by vegetated and unvegetated biofilters with time.

Efficient urban pollutant removal by unvegetated soil in this study, particularly of metals, emphasises the growth media’s role in plant biofiltration systems. Notwithstanding the importance of this filtration medium, removal efficiencies for unvegetated biofilters were found to decline or reach a possible upper limit over time. This may have been due

to the reaching of maximum soil adsorption capacities and/or biofilms formation in and over the growth media (see Singh *et al.* 2018), clogging the biofilters. As stated previously, further longer-term studies are required to ascertain the practical efficient lifespans of these types of plant biofilters. For nutrients, with similar studies using soil unamended with compost, large portions of nutrients were removed by unvegetated soil via filtration processes (see Hatt *et al.* 2007; Henderson *et al.* 2007). Over time, enhanced nutrient removal in both the vegetated and unvegetated biofilters was observed, particularly during  $\text{PO}_4^{3-}\text{-P}$  flushing due to possible media desorption and ultimate leaching from the biofilters. This nutrient removal enhancement is suspected to have been predominantly a result of the appreciable  $\text{PO}_4^{3-}\text{-P}$  release from the amended compost growth media at experimental initiation, which stabilises with time. Metal removal, although generally efficient, varied between vegetated and unvegetated biofilters with time, with a slight decline in performance observed in unvegetated soil over the last round of sampling, whilst vegetation maintained treatment efficacy.

Plant species appear to have a significant effect on maintaining infiltration (see Le Coustumer *et al.* 2012), enhancing removal performance, highlighting the importance of vegetation for nutrient and metal removal by biofilters. As mentioned, plants present additional removal pathways for pollutants in biofilters, for example, direct uptake and indirectly by optimising soil microbial conditions (Read *et al.* 2008).

### Pollutant load as an insight into plant biofilter performance

The distribution of pollutant loads (Table 3) was calculated as a rough estimate to provide better insight into biofilter efficiencies, as the use of concentration as a compound parameter alone, accounting for mass and volume, does not consider variation in through-flow volumes, nor is it a reflection of the extent of pollutants entering and leaving a system. For each water quality sampling round, the effluent discharged by each biofilter column during the 15-day period was collected to assess hydrologic improvements. A significant amount of water was retained by the biofilters and did not drain from the system. This water was eventually lost through evapotranspiration, with pollutants either retained by the soil or removed by the plants. Each plant's water retention capacity was established, by deducting the retention contribution of the growth media only (unvegetated soil) from the recorded plant biofilter columns, allowing for estimating pollutant load distribution as the influent and effluent concentrations and volumes were controlled and recorded, respectively. Vegetative pollutant load retention is considered the mean retention across the plant biofilter columns. For precise assessment of water quality improvement, GI initiatives need to consider the extent of water evapotranspiration, influencing mass load reductions.

Pollutant distribution of the biofilters for low, typically observed and high strength pollution depicts biofiltration as an effective treatment method. There is however some variation, with vegetation consistently outperforming unvegetated soil, but only significantly for the removal of low and typically observed urban stormwater pollution. Poorly retained nutrients  $\text{NO}_3^- \text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$ , which were found to leach out of the system, may deteriorate the water quality of receiving watercourse and degrade natural ecosystems. To combat this threat, the responsible engineer is required to optimise the biofiltration

**Table 3** | Estimate of pollutant load distribution

Pollution type	Pollutant path	Total pollutant mass after five sampling rounds (mg)						
		$\text{NH}_3\text{-N}$	$\text{NO}_3^- \text{-N}$	$\text{PO}_4^{3-}\text{-P}$	Cd	Cu	Pb	Zn
Low	Influent mass	6.60	16.50	7.26	0.066	0.66	2.64	4.95
	Retained by soil only	3.99	-21.79	-10.14	0.048	0.28	2.62	3.66
	Retained by vegetation	5.48	-2.39	-3.40	0.051	0.44	2.63	4.50
Typically observed	Influent mass	13.20	31.35	11.55	0.148	1.49	4.95	9.90
	Retained by soil only	11.32	-23.57	-9.65	0.143	1.02	4.86	9.11
	Retained by vegetation	12.71	15.88	0.25	0.146	1.13	4.90	9.55
High	Influent mass	82.50	445.50	49.50	1.056	225.06	91.08	1,155.00
	Retained by soil only	82.04	392.79	-10.98	1.051	224.77	91.06	1,154.17
	Retained by vegetation	82.32	423.50	22.50	1.054	224.88	91.07	1,154.58

system and make the necessary changes to the design. For instance, the results highlight the importance of selecting appropriate plant species for  $\text{NO}_3^-$ -N removal, with plants differing in removal efficacy. In addition, by adding solid-phase compost during biofilter construction,  $\text{PO}_4^{3-}$ -P leachate may be mitigated. As some plants rely on compost for establishment, the use of solid material must be promoted, supplying carbon for  $\text{NO}_3^-$ -N denitrification and limiting the dissolution and deposition of dissolved Cu.

### Plant functional response

The root number and length of the longest root for each plant were taken on two occasions (Table 4), during transplantation and experimental conclusion, whilst the length and number of above-ground traits (Figure 6) for each plant were recorded on a monthly basis. Plant species exposed to each of the urban stormwater pollution dosing strengths were compared with each other, as well as with similar specimens receiving non-polluted tap water under similar volumetric dosing regimes. In addition, to assess the need for compost during biofilter construction, the plants' traits were compared between amended and unamended biofilters.

As a result of the natural dying back and regrowing cycle of *C. aethiopica* limiting physical measurements, it was excluded from analyses. Furthermore, the common phytoremediator *C. dactylon* specimens were not assessed, as those exposed to typically observed and high urban pollution strengths had wilted and died in April 2021, indicating a negative functional response. Mortality in only *C. dactylon* may be due to the plant's morphological development which extends laterally, increasing above-ground contact with pollutants during dosage events, particularly high concentration metals.

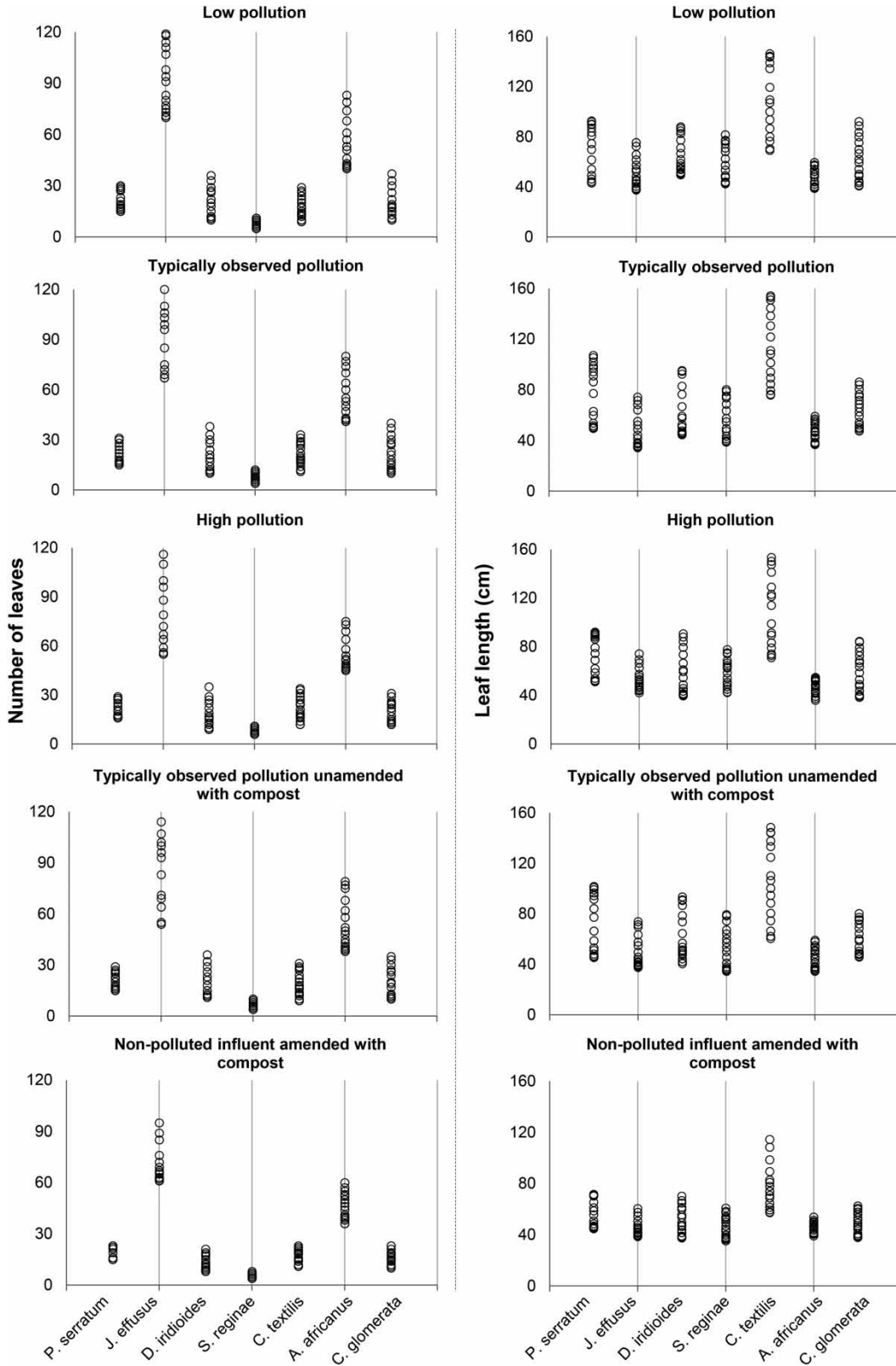
Statistical analyses used to compare the above- and below-ground traits between the same species across the three pollution dosing strengths indicated no significant differences ( $p > 0.05$ ) in plant growth for each species. Similarly, although greater variations in above- and below-ground traits were reported when comparing plants exposed to typically observed synthetic stormwater pollution and plants receiving non-polluted water, both amended with compost, no significant differences

**Table 4** | Development of below-ground traits, initial and final root number and length of the longest root

Species	Low pollution		Typically observed pollution		High pollution		Polluted <sup>a</sup> unamended with compost		Non-polluted amended with compost	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
	Number of roots									
<i>P. serratum</i>	38	106	45	121	41	101	49	83	43	68
<i>J. effusus</i>	91	366	114	398	122	341	104	337	120	260
<i>D. iridioides</i>	61	157	79	176	88	183	74	169	58	134
<i>S. reginae</i>	5	10	4	12	5	10	5	11	4	7
<i>C. textilis</i>	156	523	127	588	134	545	174	513	149	461
<i>A. africanus</i>	32	64	24	75	28	69	29	75	31	51
<i>C. glomerata</i>	64	134	66	144	58	125	61	139	65	117
	Length of the longest root (cm)									
<i>P. serratum</i>	34.1	66.7	29	72.8	38.9	63.4	27.1	66.8	28.9	51.9
<i>J. effusus</i>	21.2	77.7	24.5	69.4	23.3	66.1	26.8	64.8	27.5	47.3
<i>D. iridioides</i>	17.8	58.8	14.8	59.4	20.1	60.9	16.8	61.1	19.6	51.3
<i>S. reginae</i>	9.5	15.4	10.3	18.8	8.9	16.9	11.3	17.6	10.1	13.6
<i>C. textilis</i>	30.3	69.7	27.9	74.6	34.8	68.8	31.3	64.6	31.5	48.5
<i>A. africanus</i>	23.4	35.5	25.8	37.6	24.9	38	20	36	21.1	31.4
<i>C. glomerata</i>	18.1	38.9	17.8	42.5	18.9	44.3	17.7	37.7	19	29.6

<sup>a</sup>Exposed to typically observed pollution strength.





**Figure 6** | Development of above-ground traits, leaf number and mean lengths recorded on 16 occasions during the experimental period.

were reported for all species. Here, the number and length of leaves (stems for *J. effusus*, *C. textilis* and *C. glomerata*) of compost amended plants irrigated with synthetic stormwater was greater than non-polluted plants, presenting a positive functional response to synthetic stormwater. Improved trait development in polluted species may be due to the availability of nutrients for uptake, with metals not adversely affecting growth due to possibly majority sorption and ultimate stagnation in the top parts of the growth columns.

In comparing plant trait development between biofilters amended with compost and biofilters in unamended soil, both under synthetic stormwater irrigation, no statistically significant differences were found. Although the plants amended with compost showed greater root and leaf/stem numbers and lengths, the differences were not of any practical importance since all the plants maintained strong development. This may have been due to the dosed influents supplying plants with adequate nutrients to support plant growth and establishment, even in the absence of composted soils. The practical significance of this result is the possibility of using compost to establish plants initially in outdoor (less sheltered) environments, thereafter allowing polluted stormwater to provide plant nourishment without the need to add compost as an ongoing maintenance measure. This may also put a time limit on the initial nutrient leaching (as was found in this study) from the compost, allowing for more stable nutrient removals once the plants are established and the initial compost leaching has stabilised.

## CONCLUSIONS

For the removal of low, typically observed and high urban stormwater pollution, biofilters equipped with indigenous South African plant species were more efficient than unvegetated soils. Significant loads of dissolved Cd, Cu, Pb and Zn, as well as  $\text{NH}_3\text{-N}$ , were removed from the synthetic stormwater, whereas the removal of  $\text{NO}_3\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  was more variable. The addition of organic matter to the biofilter growth media, in the form of amended compost during construction, resulted in leached  $\text{PO}_4^{3-}\text{-P}$  and possibly hindered dissolved Cu sorption to the soil. The general removal capacity of indigenous South African plant species supports their inclusion as phytoremediators in efficient as well as sustainable GI initiatives to reduce nutrient and metal loads in urban stormwater runoff, supplementing the findings of Milandri *et al.* (2012) who investigated the nutrient removal performance of locally occurring plant species.

In South Africa, due to variable seasonal conditions, species selection must promote a variation of indigenous plants accustomed to different local environmental and climatic conditions. This local heterogeneity will allow dormancy in one species to be compensated for by another, optimising GI pollutant treatment whilst maintaining conservation.

Effective metal sorption, particularly to soil in the top layer of the biofilter column (see Blecken *et al.* 2010), creates potential for shallow biofiltration systems. These systems are more easily constructed and potentially homogenous with existing drainage infrastructure, promoting the integration of small-scale biofilters in local GI systems throughout a spatially limited urban area. This adaptation in urban planning and design is effective yet sensitive to issues of water protection and environmental conservation (Wong 2006). Due to the potential threat of invasion by introducing potentially invasive, rapid growing, hardy and tolerant species to local urban areas, we recommend longer laboratory studies and field investigations to verify the results.

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## DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

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