Design aspects and plant species affect pollutant removal in Southern California stormwater biofilters

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Abstract

We conducted a column study to better understand the relative effects of plants, design elements, and operating conditions on pollutant removal in stormwater biofilters in southern California under local conditions. We planted five southern California native species (*Baccharis pilularis*, *Carex praegracilis*, *Juncus patens*, *Leymus condensatus*, and *Muhlenbergia rigens*) in experimental biofilter columns fitted with a saturated zone and evaluated pollutant removal during weekly dosing and following a 52-day dry period. Columns planted with *C. praegracilis* and *J. patens* were also evaluated under conditions of fortnightly dosing and without the presence of a saturated zone. During weekly dosing, planted columns had a total nitrogen removal efficiency of 46% on average whereas removal was 8% in unplanted columns. *B. pilularis* and *M. rigens* performed better than other species at nitrogen removal. The presence of a saturated zone improved nitrogen removal and metal removal, but only before the 52-day dry period. With a few exceptions, local best management practice effluent concentrations limits were exceeded but performed similarly to existing southern California biofilters. Nitrogen removal decreased slightly under a fortnightly dosing frequency, which better represented rainfall event frequency in Los Angeles when compared to weekly dosing.

Key words: bioretention, California native plants, green stormwater infrastructure, raingardens

Highlights

- Nitrogen removal was better than previous studies following a 52-day dry period.
- Effluent did not meet local benchmarks but was comparable to existing local biofilters.
- Species traits did not correlate to pollutant removal as in previous studies.
- Dosing frequencies closer to local rain patterns reduced nitrogen removal slightly.
- Presence of a saturated zone improved nitrogen removal.

INTRODUCTION

Stormwater biofilters have been widely adopted for mitigating human and environmental health impacts of urban stormwater runoff by reducing hydrologic and pollutant loading to receiving water bodies (Ambrose & Winfrey 2015; Eckart *et al.* 2017; Kuller *et al.* 2018). These systems have been studied and optimized through numerous field and column studies (e.g., Davis 2007; Bratieres *et al.* 2008; Davis 2008; Hunt *et al.* 2008; Hatt *et al.* 2009; Payne *et al.* 2014b; Glaister *et al.* 2017). Among other factors, plant species choice (Bratieres *et al.* 2008; Read *et al.* 2008) and hydraulic operational modes (Payne *et al.* 2014b) influence pollutant removal in stormwater biofilters.

Few studies have evaluated pollutant removal by stormwater biofilters that use climate-appropriate plant species and optimal design configurations in Mediterranean climates (Galloway 2016; Katz *et al.* 2018; Pritchard *et al.* 2018; Russo *et al.* 2019) and we did not find any studies that evaluated relative performance of southern California native plants in the southern California climate. Most best management practice (BMP) guidelines in southern California suggest native plant species for biofilters (LA Bureau of Sanitation 2010; LID Center 2010; City of Santa Barbara 2013; County of San Diego 2014), but some do not specify any species (OCPW 2011; Riverside County 2011; LADPW 2014). There is a lack of knowledge on species selection for southern California species and other Mediterranean climates, as the stormwater BMP guidelines demonstrate. One study has identified plant species with potential to perform well in southern California biofilters based on several traits related to drought tolerance and seasonal growth patterns (Houdeshel & Pomeroy 2010), but no studies have evaluated the relative performance of native southern California plant species in biofilters.

With regards to hydrologic conditions, the inclusion of a saturated zone has been found to enhance nitrogen removal and maintain plant health over prolonged dry periods (Brown & Hunt 2011; Payne *et al.* 2014b), but only one southern California design manual encourages using a saturated zone in biofilters (County of San Diego 2014) and no study has investigated whether this design feature is appropriate in Mediterranean climates with long dry seasons. Extended dry periods are shown to have varying effects on pollutant removal (Zinger *et al.* 2007a; Blecken *et al.* 2009b; Payne *et al.* 2014b), but dry periods typical of southern California and other Mediterranean regions often exceed 30 days (Ambrose & Winfrey 2015) and have not been tested in climates where these dry periods occur.

In this paper, we evaluated the relative pollutant removal and infiltration rates of five common biofilter plant species subjected to a prolonged dry period. We evaluated the effect of including a saturated zone and dosing less frequently on two of these species. This study describes how native southern California plant species perform in biofilters under varying rain event frequencies and following an extended dry period typical of Mediterranean summers.

METHODS

Following a 6-week establishment period, we dosed stormwater biofilter columns with semi-synthetic stormwater runoff weekly or fortnightly in selected treatments. We measured nitrogen and metal concentrations of biofilter column effluent over the experimental period in two phases: during weekly dosing and after a 52-day dry period.

Column setup and design

We constructed 50 biofilter columns on the roof of a building on the University of California Los Angeles campus (Supplementary Material, Figure S1) following established experimental column

designs (Bratieres *et al.* 2008; Payne *et al.* 2014b) and local guidelines (LADPW 2014). In the top 150 mm of filter media (79% sand, 11% silt, and 10% clay), we transplanted individuals of the following five native plant species: *Baccharis pilularis*, *Carex praegracilis*, *Juncus patens*, *Leymus condensatus*, and *Muhlenbergia rigens* from in 1-gal plastic pots (Tree of Life Nursery in San Juan Capistrano, CA) into 45 columns. These species were suggested by local design manuals (LA Bureau of Sanitation 2010; LID Center 2010) and morphologically similar to high-performing species in the literature (see Read *et al.* 2009; Payne *et al.* 2014b, 2018). The remaining five columns were unplanted to serve as a control.

All treatments began with 5 replicates. Species treatments were planted with one individual from the 5 species tested and maintained a 400-mm saturated zone (Figure S1). These treatments were dosed weekly with semi-synthetic stormwater runoff. Columns planted with *C. praegracilis* and *J. patens* were subjected to 3 factors: species, dosing frequency, and saturated zone (Table S1). Dosing with synthetic stormwater occurred weekly for all columns except those subjected to fortnightly dosing (Treatment numbers 7 and 8, Table S1). All columns had a 400-mm saturated zone except those with outlets at the bottom of the column (Treatment numbers 9 and 10, Table S1). All columns were subjected to an extended dry period of 52 days during which no water was added to the columns and rainfall was excluded.

Column dosing

We dosed columns weekly (or fortnightly for Treatment numbers 7 and 8) with 6.6 L of semi-synthetic stormwater runoff to represent the annual volume of stormwater runoff from a watershed in southern California with an effective impervious area 43 times the size of the columns (Ambrose & Winfrey 2015). This dosing volume was roughly 2 pore volumes of the entire media bed and 3 pore volumes of the saturated zone. We prepared semi-synthetic stormwater runoff by adding sediment from a local stormwater pond to distilled water along with nutrient and metal salts to match target influent concentrations typical of southern California runoff (Table 1), as in Bratieres *et al.* (2008). Following the initial experimental phase of regular weekly watering and multiple water quality sampling events, we measured flow rates from column outlets using the bucket-and-stopwatch method to represent infiltration rates. We recorded the time it took for 1 L of effluent to flow through the outflow after applying semi-synthetic runoff to the top of the column to a height of 50 mm above the filter media surface. After this, no columns were watered or dosed for a 52-day period over spring to

Table 1 | Semi-synthetic stormwater runoff target concentrations and measured influent concentrations

Constituent	Added as	Target concentration (mg/L)	Actual concentration (mg/L)			
			October 2015	December 2015	March 2016	June 2016
TN	all N additives	5.8	6.7	7.8	5.8	9.7
NO_3	KNO_3	1.3	1.9	2.8	2.7	1.8
NH_3	NH_4Cl	1.4	1.8	1.8	2.0	1.8
ON	$C_6H_5O_2N$	2.7	3.0	3.2	1.1	6.1
Cadmium	Cd standard solution	0.0011	–*	–	0.0023	0.0024
Copper	$CuSO_4$	0.09	–	–	0.20	0.27
Lead	$PbNO_3$	0.023	–	–	0.015	0.024
Zinc	ZnCl	0.27	–	–	1.1	0.9

Note: TN, total nitrogen; ON, organic nitrogen.
*– indicates constituent was not measured.

early summer (March to June 2016) in order to test whether treatment was diminished after an extended dry period, herein referred to as 'the dry period'.

Pollutant removal

During sampling events, we collected a composite sample of the outflow from each column and a composite of semi-synthetic runoff from the beginning, middle, and end of dosing events. From well-mixed composite samples, we collected two 50-mL subsamples for immediate TN analysis (Hach Method 10208) using an unfiltered sample and for nitrate-N (Hach Method 10206) and ammonia-N (Hach Method 10205) analyses using a filtered sample (0.45 μm PTFE filters). We conducted nitrogen analyses on a Hach spectrophotometer DR 280 (Hach, Loveland, CO). Organic nitrogen concentration was calculated as the difference between total nitrogen concentration and the sum of both dissolved nitrogen concentrations. We stored another subsample at 4 °C in 100-mL HDPE bottles for subsequent metals analyses by inductively coupled plasma mass spectrometry (PerkinElmer Elan DRC-e). Prior to metals analyses, we acidified water samples to pH < 2 using a one-to-one solution of trace metal-grade nitric acid to Milli-Q®-filtered water and then filtered the samples with 0.45-μm PTFE filters. We analyzed influent and effluent subsamples for cadmium, copper, lead, and zinc. The filtered water we used to prepare our runoff (unchlorinated, distilled industrial cooling water) contained residual phosphate above typical runoff concentrations. Consequently, we did not add phosphorus to runoff nor evaluate its removal.

Biomass and root characteristics

We determined initial aboveground and belowground biomass from five individuals randomly selected from the planting stock. To harvest the biomass, we washed roots free of planting media and separating aboveground and belowground parts. Following this, we dried plants at 60 °C for 24 hrs and recording the dry weight. This step was repeated until we observed no additional weight loss. The remaining individuals were planted in columns. We weighed dry biomass of each column at the end of the experiment as well. Relative growth rate was determined by dividing the difference between initial and final biomass by the product of the number of days between measurements and the final total biomass. Over the course of the experiment, we measured the tallest five stems of each column to monitor plant growth.

We collected root subsamples representing approximately 10% of the fresh weight of total belowground biomass to estimate root length using the modified grid-intersect method (Tennant 1975). We determined specific root length by dividing the root length of the subsample by its dry weight. This dry weight was added to the total dry weight of that sample. Total root length for each planted column was determined by multiplying the specific root length by the dry weight of belowground biomass.

Data analysis

We completed all data analyses using R (R Core Team 2017). For all water quality sampling dates, we graphically compared removal efficiencies in species treatments (Treatment numbers 1–6, Table S1). Removal efficiencies are defined as the difference between influent and effluent concentrations divided by influent concentration in species treatments. All of the following statistical tests used an α of 0.05. After confirming no non-normal distributions existed in our dataset using Shapiro-Wilks tests and confirming there were no significant block effects using two-way ANOVA tests with treatment and block as factors, we compared removal efficiencies and other measured parameters in species treatments during sampling events just before and following the dry period separately. We

used a one-way ANOVA for each pollutant. For significant ANOVA tests ($p < 0.05$), we applied Tukey's HSD test to compare pollutant removal in species treatments. We also compared pollutant removal between planted columns and unplanted columns using paired t-tests. We also tested measured parameters for correlation by determining Pearson's correlation coefficients.

We performed paired t-tests to detect effects of dosing frequency (Treatment numbers 3, 4, 7, and 8 in Table S1) and presence of a saturated zone (Treatment numbers 3, 4, 9, and 10 in Table S1). We performed these tests within species (e.g., Treatment number 2 vs. 7) and between factors (e.g., Treatment numbers 3 and 4 vs 7 and 8). We conducted these comparison tests on groups before and after the dry period separately. Of the 50 columns, we excluded 3 from these analyses (indicated in Results section) due to plant death prior to sampling.

RESULTS

We did not detect any block effects on treatments with respect to any measured parameters nor could we confirm non-normal distribution ($p > 0.05$). The 3 replicates excluded from statistical analysis were from 3 species treatments: columns planted with *B. pilularis, J. patens,* and *L. condensatus.*

Overall, planted stormwater biofilter columns fitted with a saturated zone and dosed weekly had total nitrogen (TN) removal efficiencies of 46% before the dry period and 36% of following the dry period. Metal removal efficiencies varied by treatment depending on both plant species and metal, ranging from average removals of 40–99% before the dry period and 41–99% following the dry period. Metal removal decreased slightly for Cu and Zn following the dry period.

Species treatments

Water quality

Nitrogen. Total nitrogen removal efficiencies varied between plant species and over the course of the experiment, ranging from 9% to 79% in regularly watered, planted columns with saturated zones. Generally, planted columns had higher removal efficiencies during all four sampling events (Figure 1). Following the dry period, TN in the effluent was 5% higher than the influent in unplanted columns while planted columns removal efficiencies varied between 18 and 45%. TN removal efficiencies were highest during the second sampling period, in December 2015 (Figure 1).

Prior to the dry period, ammonia removal efficiency was about 15% higher in columns planted with *J. patens* than with *B. pilularis.* (Figure 2(a)). Ammonia removal increased for *B. pilularis* and unplanted columns following the dry period, but decreased for other species treatments (Figure 2). *M. rigens* columns removed more nitrate than all other species and the unplanted control prior to the dry period (Figure 2(a)). Nitrate removal declined in all columns following the dry period, releasing rather than removing nitrate (Figure 2(b)). Nitrate outflow concentrations were significantly lower in planted columns compared to unplanted columns. There were no significant differences in organic nitrogen (ON) removal between species treatments and removal remained relatively unchanged following the dry period. Besides columns with *L. condensatus,* planted columns removed TN better than unplanted columns before the dry period ($p = 0.0003$, Figure 2(a)). Columns planted with *M. rigens* and *B. pilularis* had the highest TN removal before the dry period. Following the dry period, all planted columns removed TN better than the unplanted columns ($p < 0.0001$, Figure 2(b)).

Metals. Metals concentrations were determined only on sampling dates just before and after the dry period. Unlike nitrogen, metals were always removed from influent. Overall, Cu and Zn removal decreased following the dry period while Cd and Pb removal remained unchanged. We observed
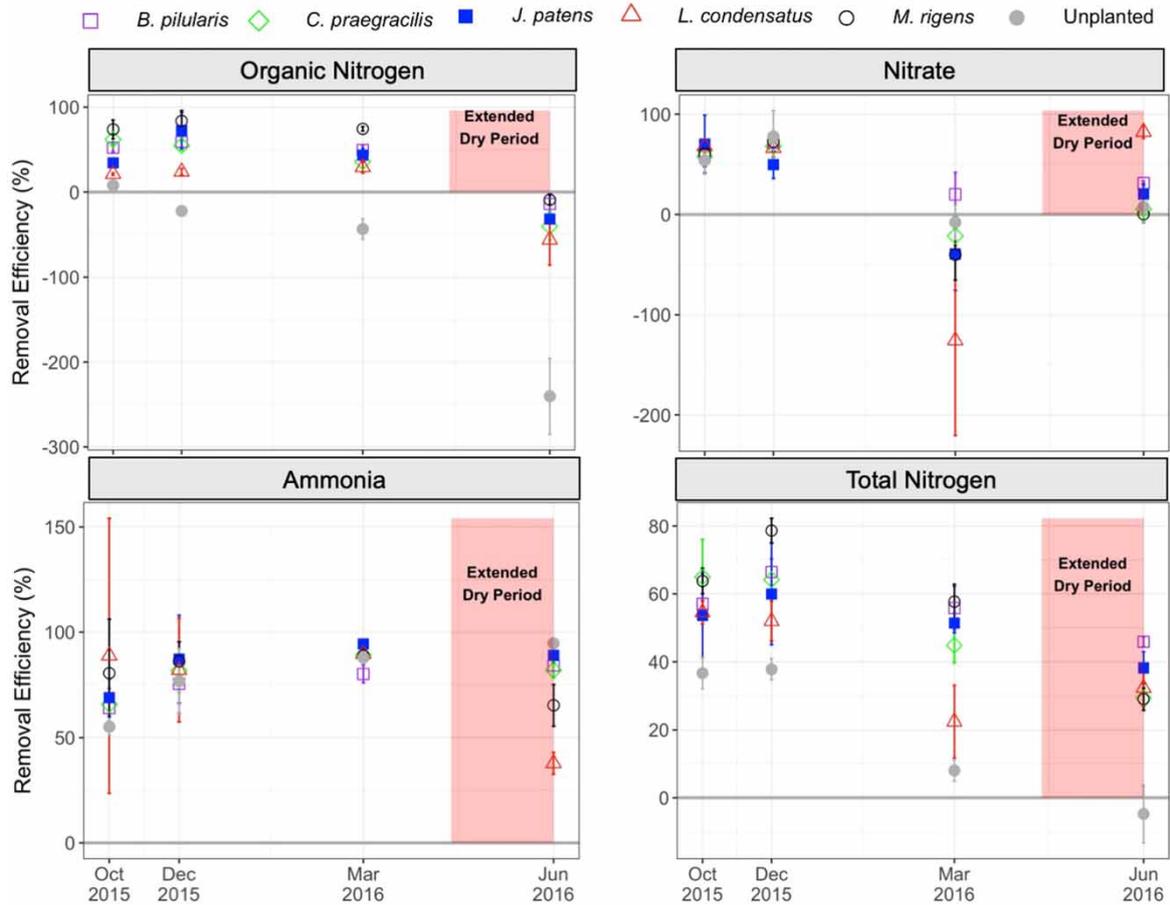
Figure 1 | Removal efficiencies for all four sampling dates for nitrogen species. Error bars indicate standard error. *Note:* Scale on y axes varies.
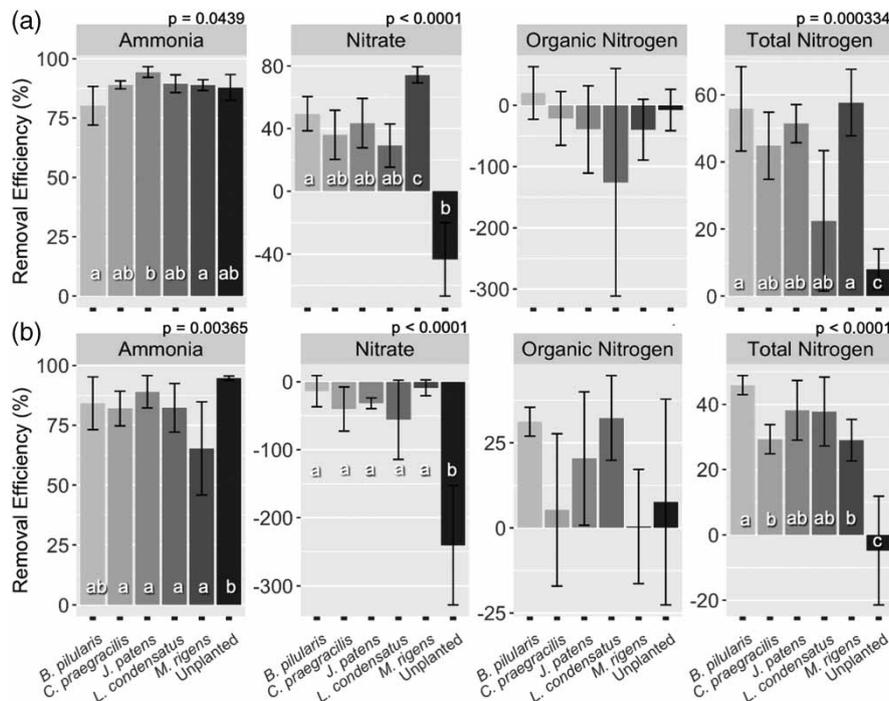
Figure 2 | Nitrogen removal efficiencies (a) before and (b) following the dry period. Error bars indicate 95% confidence intervals. When ANOVA was significant ($p < 0.05$), p values are provided above facet plot. Letters on bars indicate significance groups determined by Tukey's HSD tests.

variable metal removal efficiencies among species treatments before and following the dry period (Figure 3). Generally, unplanted columns removed metals better or similar to the average of planted columns.

Cd removal varied between species before and following the dry period, with a higher Cd removal efficiency for *M. rigens* than for two other species ($p = 0.0045$, Figure 3(a)) before the dry period and a higher Cd removal efficiency for *L. condensatus* than one other species, *C. praegracilis*, following the dry period ($p = 0.012$, Figure 3(b)). Before the dry period, columns planted with *M. rigens* had higher Cu removal efficiencies than all other columns ($p = 0.00034$; Figure 3(a)). *M. rigens* also removed more Zn than all other columns ($p = 0.00018$; Figure 3(b)), but only following the dry period. Zinc removal efficiencies decreased by about 10% in planted columns following the dry period, except those planted with *M. rigens*. Removal efficiencies of Pb were the same for all treatments and always greater than 96% (Figure 3).
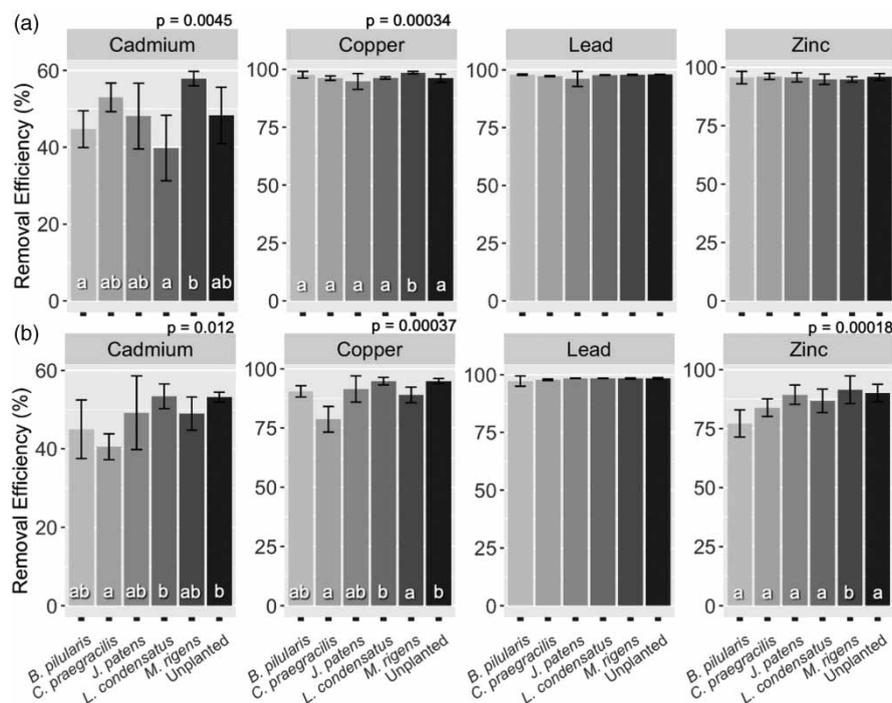
Figure 3 | Metal removal efficiencies (a) before and (b) following the dry period. Error bars indicate 95% confidence intervals. When ANOVA was significant ($p < 0.05$), p values are provided above facet plot. Letters on bars indicate significance groups determined by Tukey's HSD tests.

Flow rates

At 92 mL/min, columns planted with *C. praegracilis* had significantly higher flow rates than all other columns, which averaged 35 mL/min ($p = 0.001$, Table 2). Columns planted with species other than *C. praegracilis* had statistically similar flow rates to unplanted columns, ranging from 26 to 52 mL/min (Table 3).

Biomass and root characteristics

Prior to the dry period, all species had a positive stem growth rate with the exception of *M. rigens*. However, this species had the highest relative growth rate over the entire experimental period (Table 2). At the beginning of the dry period, *C. praegracilis* stems were growing more rapidly than

Table 2 | Flow rates, final biomass, and root characteristics of columns planted with different species, dosed at different frequencies, and excluding a saturated zone

| Factor | Treatment | Flow rate (mL/min) | Final Biomass (g) | | Relative growth rate (mg/g/day) | Root characteristics | |
			Above ground	Below ground		Specific root length (m/g)	Total root length (10^3 m)
Species	B. pilularis	33.1 ± 12.8	46.1 ± 4.4	136 ± 4.8	1.99 ± 0.01	17.4 ± 0.9	2.4 ± 0.2
	C. praegracilis	92.0 ± 15.3	42.0 ± 7.7	189 ± 29	1.91 ± 0.03	96.4 ± 21	18.1 ± 7.1
	J. patens	25.5 ± 3.37	53.1 ± 8.0	237 ± 42	2.07 ± 0.01	12.0 ± 1.6	2.2 ± 0.6
	L. condensatus	52.4 ± 12.4	19.4 ± 4.7	117 ± 19	2.03 ± 0.02	21.0 ± 8.4	2.5 ± 1.3
	M. rigens	27.6 ± 7.12	87.6 ± 11.	294 ± 43	2.14 ± 0.004	33.7 ± 12	11.5 ± 5.3
	Unplanted	38.8 ± 9.76	–*	–	–	–	–
	p value	0.0014	0.0004	0.0074	< 0.0001	< 0.0001	n.s.**
Less frequent dosing	C. praegracilis	110.0 ± 31.5	28.4 ± 4.4	301 ± 48	1.98 ± 0.03	96.3 ± 31	27.6 ± 13.8
	p value	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	J. patens	26.9 ± 5.53	34.3 ± 2.3	219 ± 19	2.06 ± 0.01	65.4 ± 14	15.1 ± 3.8
	p value	n.s.	n.s.	n.s.	n.s.	0.01145	0.02154
No saturated zone	C. praegracilis	–	36.2 ± 4.8	325 ± 38	2.01 ± 0.02	183 ± 38	51.5 ± 14
	p value		n.s.	0.02	0.022	n.s.	n.s.
	J. patens	–	44.6 ± 6.2	180 ± 20	2.05 ± 0.01	47.5 ± 14	8.3 ± 2.7
	p value		n.s.	n.s.	n.s.	n.s.	n.s.

Flow rates were collected in March 2016, just before the long dry period. Values reported with standard errors. p values less than 0.05 reported for ANOVAs within species treatments and separately for each species in less frequent dosing and saturated zone treatments from paired t-tests.

*not measured.

**not significant.

all other species, but rapidly diminished following the dry period (Figure S2). All species stem growth rates decreased following the dry period (Figure S2).

Final biomass measurements were made following the final water quality sampling event in June 2016. We observed significant differences between species in aboveground ($p = 0.0004$) and belowground ($p = 0.007$) biomass (Table 2), but not aboveground-to-belowground biomass ratios. *M. rigens* and *J. patens* had the highest biomass (Table 2). These species also had the highest relative growth rate over the course of the experiment (Table 2). Relative growth rates differed between species ($p < 0.0001$) but were nominally similar (Table 2).

Specific root length of *C. praegracilis* was about 3–7 times higher than other species ($p < 0.0001$, Table 2). Although the average total root length of *C. praegracilis* and *M. rigens* columns were 5–7 times greater than other species, replicates varied considerably within those treatments and the ANOVA test for this parameter was not significant ($p = 0.06$).

Correlation between measured parameters

Although both TN and ON removal efficiencies were significantly correlated to biomass before and following the dry period (Figures S3 and S4), these correlation factors were relatively low ($-0.5 < r < 0.5$). Nitrate removal efficiency was positively correlated to aboveground ($r = 0.74$; $p = 0.0001$) and belowground biomass ($r = 0.73$; $p = 0.0002$) before the dry period., but not correlated to either after the dry period (Figures S3 and S4). Additionally, nitrate removal efficiency was positively correlated to relative growth rate before the dry period ($r = 0.65$; $p = 0.0016$; Figure S3), but not after. Ammonia removal efficiencies were not correlated to any measured parameters of plants and columns (Figures S3 and S4).

Metal removal efficiencies were weakly correlated to biomass and relative growth rate both before and following the dry period (Figures S3 and S4). Lead removal efficiency was negatively correlated to flow rate ($r = -0.65$; $p = 0.0004$) following the dry period.

Higher flow rates were positively correlated to total root length ($r = 0.51$; $p = 0.024$; Figure S3) and negatively correlated to relative growth rates ($r = -0.6$; $p = 0.003$; Figure S3).

Dosing frequency treatments

Water quality

Within species comparisons of nitrogen removal were not significantly affected by dosing frequency (Figure S5), but when species treatments were pooled, columns dosed fortnightly had significantly lower ($p = 0.033$) TN removal efficiencies (42%) than columns dosed weekly (48%) before the dry period. Following the dry period, less frequently dosed columns still had lower removal efficiency of TN, but this difference was not significant (24 vs. 35%; $p = 0.11$).

Only one statistical comparison of metal removal efficiencies resulted in a significant difference between these treatments: before the dry period, columns planted with *C. praegracilis* and dosed fortnightly had lower Cd removal (45%) than those dosed weekly (51%; $p = 0.024$; Figure S6a).

Flow rates

Dosing frequency did not affect flow rates in columns planted with *C. praegracilis* and *J. patens* (Table 2).

Biomass and root characteristics

Dosing frequency did not appear to affect *C. praegracilis* stem growth over most of the experimental period, but *J. patens* stem growth was lower with less frequent dosing until just before and during the

dry period (Figure S7). Following the dry period, *C. praegracilis* stem growth diminished, but was still positive (Figure S7). Biomass and relative growth rates of *C. praegracilis* and *J. patens* treatments were similar under different dosing frequencies (Table 2). When *J. patens* was grown in less frequently dosed columns, we observed nearly 6 times higher specific root length ($p = 0.011$) and 7 times higher total root length ($p = 0.022$) than columns receiving regular watering (Table 2). Belowground biomass and total root length of *C. praegracilis* were higher in columns dose fortnightly, but these differences were not significant ($p = 0.11$ and $p = 0.57$, respectively).

Saturated zone

Water quality

Including a saturated zone did not significantly affect nitrate removal for either species before the dry period despite columns without a saturated zone having about 20% lower nitrate removal efficiencies. Although columns planted with both species removed ON when a saturated zone was present and released ON when there was no saturate zone, only those planted with *J. patens* were significantly different ($p = 0.0029$). The presence of a saturated zone did not affect ammonia removal during either sampling event. Columns with a saturated zone had 48% removal efficiency of TN before they dry period, which was significantly greater than columns without a saturated zone (23%; $p = 0.026$). Following the dry period, only columns planted with *J. patens* were affected by the presence of a saturated zone, which increased TN removal efficiencies from 24% to 38% (Figure S8b; $p = 0.044$).

The presence of a saturated zone had no effect on metal removal by columns planted with *J. patens*. Including a saturated zone decreased Cd removal efficiency following the dry period in columns planted with *C. praegracilis* (50 vs. 43%; $p = 0.028$). Before the dry period, Cu removal efficiency increased in columns with a saturated zone and planted with *C. praegracilis* (92 vs. 96%; $p = 0.009$). Copper removal efficiency following the dry period decreased with the inclusion of a saturated zone (88 vs. 81%; $p = 0.034$). Lead removal efficiency was increased by a saturated zone (96.7 vs. 97.3%; $p = 0.012$) before the dry period. Zinc removal efficiency was decreased by a saturated zone following the dry period (90 vs. 78%; $p = 0.035$).

Biomass and root characteristics

Stem growth rates of *C. praegracilis* were greater in columns with a saturated zone for the entire experimental period (Figure S10). Following the dry period, *C. praegracilis* columns with a saturated zone maintained a positive stem growth rate while those without dropped to a negative rate, along with *J. patens* columns (Figure S10). *J. patens* stem growth rates were similar in both columns with and without a saturated zone.

Columns with no saturated zone and planted with *C. praegracilis* had about 72% more belowground biomass ($p = 0.02$) than those with a saturated zone (Table 2). Relative growth rate was also higher in *C. praegracilis* columns without a saturated zone than columns with a saturated zone ($p = 0.022$; Table 2). No differences in plant biomass were observed for columns planted with *J. patens*.

Although *C. praegracilis* columns without a saturated zone had about twice the specific root length and total root length as those with a saturated zone (Table 2), the differences were not significant ($p = 0.22$ and $p = 0.11$). Due to sample mislabelling, 2 replicates from each of the *C. praegracilis* treatments and 1 replicated from each of the *J. patens* treatments were lost, which resulted in a sample size of 3 and 4 when comparing root characteristics of *C. praegracilis* and *J. patens* columns, repectively, with and without a saturated zone. Despite the large nominal differences in specific root lengths and total root lengths of *J. patens* in saturated zone treatments, these differences were not significant ($p = 0.075$ and $p = 0.073$; Table 2).

DISCUSSION

Effects of species

Overall nitrogen removal and effects of the dry period

Planted columns clearly removed nitrogen better than unplanted columns, similar to previous studies (Bratieres *et al.* 2008; Read *et al.* 2008). Nitrate removal decreased in planted columns following the dry period, which has been observed previously (Zinger *et al.* 2007a; Kobayashi *et al.* 2009; Payne *et al.* 2014b). This decreased removal may have been due to substrate dry-down, which left less treated water with which effluent could be diluted in the pore spaces and saturated zone. Additionally, water-stressed plants may decrease oxygen transfer to the rhizosphere, which may diminish denitrification processes that rely on coupled aerobic-anaerobic environments (Payne *et al.* 2014a). In previous studies, nitrate removal was nearly 100% prior to a 49-day dry period with twice weekly dosing of planted columns (Zinger *et al.* 2007a) and about 50% in unplanted columns with a similar pore volume loading as in this study (Berger *et al.* 2019). In our study, average nitrate removal efficiency was only 47% in planted columns and unplanted columns had 43% higher effluent than influent nitrate concentration before the dry period.

Following the dry period, both our study and the Zinger *et al.* (2007a) study observed effluent nitrate concentrations about 30% higher than influent concentrations following the dry periods in planted and unplanted columns. The nitrate production in the columns was much higher following the dry period, indicating accumulated ammonia was quickly nitrified upon rewetting. Schaeffer *et al.* (2017) observed a spike in nitrate concentrations in southern California grassland soils at the first rains following an extended dry period, which they attributed to rapid revival of nitrifiers following rewetting. For TN removal, both this study and the Zinger *et al.* (2007a) study observed about 50% removal before the dry period. However, we observed higher TN removal in this study following a dry period; our columns had an average removal efficiency of 30% of TN on average while the Zinger *et al.* (2007a) study observed a 50% increase in effluent TN concentration following the dry period. The columns tested in the Zinger *et al.* (2007a) study were planted with *C. appressa*, one of the high-performing species for nitrogen removal in southeast Australia (Read *et al.* 2008; Payne *et al.* 2014b; Pham 2016). Although some operational parameters differed between our study and the Zinger *et al.* (2007a) study, our observation of maintaining TN removal (i.e., lower effluent than influent concentrations) following a 52-day dry period suggests southern California biofilter plants may be better at maintaining treatment performance during long dry periods than those in southeast Australia.

Southern California experiences much longer dry periods than southeast Australia. Overall, rainfall patterns may be more favorable for maintaining treatment performance in southeast Australia than in southern California. As a percentage of rain events represented by antecedent dry periods, Zinger *et al.* (2007a) found that an antecedent dry period of 11 days, of which only 10% of rain events exceed in Melbourne, resulted in an average removal of 50% TN. In our study, an antecedent dry period of 52 days, of which about the same proportion (8%) of rain events in Los Angeles exceed (Ambrose & Winfrey 2015), resulted in 36% TN removal on average and 46% at best, in those columns planted with *B. pilularis*. TN removal was higher during weekly dosing, when we observed an average of 46% removal efficiency and 56 and 58% removal efficiencies with the best-performing species: *B. pilularis* or *M. rigens,* respectively. This period corresponds to a 6-day antecedent dry period, which 54% of Los Angeles rain events exceed (Ambrose & Winfrey 2015). Consequently, the TN removal efficiencies we observed that match the Zinger *et al.* (2007a) study (i.e., ~50% TN removal) corresponded to only 46% (i.e., 100–54%) of rain events in Los Angeles while that level of treatment corresponded to roughly 90% (i.e., 100–10%) of rain events in Melbourne. Under conditions corresponding to 92% (i.e., 100–8%) of rain events in Los Angeles (i.e., dry period of 52

days or fewer), we observed TN removal efficiency up to 46%, which is only slightly lower than the 50% TN removal observed by Zinger *et al.* (2007a) during dosing frequencies which correspond to 90% of rain event antecedent dry days in Melbourne. However, investigation of rainfall intensity should also be undertaken due to the impacts of increased loading on nitrogen removal (Berger *et al.* 2019) and because antecedent dry period and rainfall intensity may have interacting effects.

Most effluent nitrogen concentrations exceeded the benchmarks set by the Region 4 Phase I MS4 Permits issued by the County of Los Angeles (Order no. R4-2012-0175 as amended by WQ Order 2015-0075), which limits TN concentrations in effluent from treatment BMPs to 1.28 mg/L (CA RWQ Board 2015). These benchmarks were developed from the median of the six highest performing BMPs described in the stormwater BMP database in 2012. The lowest average TN concentration in effluent from our treatments was 1.67 mg/L in columns sampled in December (second sampling event) and planted with *M. rigens*. However, all treatments before the dry period did meet the benchmark for Total Kjeldahl nitrogen of 1.09 mg/L (CA RWQ Board 2015), which we calculated as the sum of ON and ammonia in our results. Following the dry period, the influent had 6 times higher ON concentration than just before the dry period, possibly due to heterogeneity in the stormwater pond sediment added to the semi-synthetic stormwater runoff. Poor nitrate removal in these systems appears to have contributed most to the exceedance of the TN benchmark. However, compared to existing southern California biofilters, the average nitrate effluent concentration from our planted columns (1.38 mg/L) was lower than an existing biofilter in San Diego that was monitored during the same time period (1.50 mg/L; Galloway 2016).

Species and plant trait effects on nitrogen removal

Species selection affected nitrogen removal, which has been observed in multiple biofilter column studies (e.g., Bratieres *et al.* 2008; Read *et al.* 2008; Gold *et al.* 2018; Payne *et al.* 2018). Of the five species tested, *B. pilularis* and *M. rigens* removed TN best (Table 3). Different nitrogen forms were removed best by different plant species and to varying degrees, but nitrate removal was clearly highest in columns planted with *M. rigens* before the dry period (Table 3).

Table 3 | Relative pollutant removal performance among plant species in this experiment

			Species				
			B. pilularis	*C. praegracilis*	*J. patens*	*L. condensatus*	*M. rigens*
Nitrogen removal	Before Dry Period	Ammonia	0	0	++	0	0
		Nitrate	+	0	0	0	++
		Organic N	0	0	0	0	0
		Total N	+	+	+	+	+
	After Dry Period	Ammonia	0	−	−	−	−
		Nitrate	0	0	0	0	0
		Organic N	0	0	0	0	0
		Total N	++	+	+	+	+
Metal removal	Before Dry Period	Cd	0	0	0	0	++
		Cu	+	+	+	+	++
		Pb	0	0	0	0	0
		Zn	0	0	0	0	0
	After Dry Period	Cd	0	−	0	+	0
		Cu	+	−	+	++	−
		Pb	0	0	0	0	0
		Zn	0	0	0	0	++

Plus symbols (+) indicate which species are recommended for use in southern California biofilters based on this study's results. For a treatment to receive a plus symbol, that species exhibited higher pollutant removal than any other species or had higher than 90% removal efficiency. Two plus symbols indicate a higher level of recommendation and were used when that species had higher removal than more than one other treatment. When a species cannot be recommended to use or to avoid using based on this study's results, a zero (0) is shown. Minus symbols (−) indicate the species exhibited lower removal efficiency than more than one other species or was lower than the unplanted control.

Although *C. praegracilis* had specific and total root length, which have been described as favourable for nitrogen removal (Read *et al.* 2009; Payne *et al.* 2018), columns planted with this species did not tend to remove nitrogen better than other species. Additionally, these root characteristics were not correlated to removal of stormwater pollutants, contrary to what previous studies have found (Read *et al.* 2009; Payne *et al.* 2014b; Pham 2016). The tussock-forming grass, *M. rigens*, had nominally higher specific root length than other species and had significantly more total root length than other species with the exception of *C. praegracilis*. *M. rigens* also had the highest relative growth rate, which may be related to the higher nitrate removal efficiency by this plant. Indeed, relative growth rate was correlated to nitrate removal before the dry period, as previously observed (Read *et al.* 2009). Contrary to previous studies, we did not find specific root length to be a good predictor of nitrogen removal (Read *et al.* 2009; Payne *et al.* 2018), which highlights the importance of region-specific studies to inform plant species selection in stormwater biofilters.

Flow rate was negatively correlated to TN removal and considerably higher in columns planted with *C. praegracilis*. Le Coustumer *et al.* (2012) similarly observed higher infiltration rates in columns with higher root length plants but did not test nitrogen removal. It is likely that semi-synthetic storm-water runoff dosed into columns planted with *C. praegracilis* may have experienced lower residence times than other treatments, offsetting the potential advantage of favorable root characteristics for nitrogen removal. Extended detention time appeared to improve nitrogen removal in previous column studies (Glaister *et al.* 2014), which may have resulted in *C. praegracilis* columns performing among the lowest for nitrogen removal. This phenomenon may be an artefact of the conditions in our columns, in which a relatively low soil-volume-to-root-length ratio may have resulted in disproportionately high impact of short-circuiting on the hydraulics when compared to full-scale biofilters with lower planting densities. More flow rate measurements may have helped us better understand the hydraulics as they changed over time, but it is likely that the higher flow rates were observed due to the dense rooting in the *C. praegracilis* treatment, which occurred when the columns were mature (flow rates were measured in March 2016).

Following the dry period, nitrogen removal declined, which is typical in these systems (Zinger *et al.* 2007a; Payne *et al.* 2018). However, variation between species did not increase following the dry period as was observed in a previous study when researchers simulated dry conditions over a 4-month period with fortnightly dosing (Payne *et al.* 2014b). During the dry period, plants may have been responding to water stress by reducing photosynthesis, thus limiting water uptake and gas exchange between the atmosphere and the saturated zone, which may have increased ON in the effluent as roots decomposed and growth slowed. Indeed, ON removal was negatively correlated to belowground biomass following the dry period.

Despite exceeding nitrogen effluent concentration targets (CA RWQ Board 2015), all species tested in this study could still be considered as potential species to plant in southern California biofilters due to performing better than the unplanted control (Table 3). In particular, *B. pilularis*, and *M. rigens* had better nitrogen removal performance for multiple forms of nitrogen, with the former species maintaining higher total nitrogen removal than other species following the dry period. Clearly, different species perform better under different conditions and for different forms of nitrogen (Table 3). Consequently, a multi-species assemblage planted purposefully with species that complement each other could have the best overall performance, but a variety of species assemblages should be tested due to potential ecological interactions that may influence overall biofilter function (Levin & Mehring 2015).

Effects of species and the dry period on metal removal

On average, Cu, Pb, and Zn removal efficiencies were higher than 96% before the dry period in all columns. Metal removal varied by metal and by plant species both before and following the dry period and did not clearly remove more metals than the unplanted control with one exception:

M. rigens removed Cu better than the unplanted control before the dry period, but was among the poorest performers for Cu removal following the dry period (Table 3). Metal removal is likely dominated by sorption to influent suspended solids particles which are filtered out in the top layer or to filter media particles (Hsieh & Davis 2005). In particular, Cu may sorb to organic matter (Blecken *et al.* 2009a), which was likely greater following the dry period. Indeed, dissolved organic carbon can enhance the release of Cu from soils (Strobel *et al.* 2004), which may be why Cu effluent concentrations were highest in columns with *M. rigens*, which had the highest belowground biomass, after the dry period. It is likely that interactions with filter media, influent suspended solids, and organic matter dominated metal removal, which is supported by our observation that planted and unplanted columns did not differ in metal removal. Removal of Zn also decreased while Pb removal remained stable following the dry period in this study. A previous study found Cu, Pb, and Zn removal to remain stable or increase following a dry period, but only in columns with both a saturated zone and added carbon source (Blecken *et al.* 2009b). The columns in this study did not contain an added carbon source in the saturated zone.

Although columns planted with *C. praegracilis* and *M. rigens* removed less Cu than the unplanted control following the dry period, the removal percentages were still 80–90%. Overall, these systems are effective at metal removal, demonstrated by our observations of more than 80% removal efficiencies of all metals except Cd by all species treatments, which agrees with previous findings (Davis *et al.* 2001; Sun & Davis 2007; Blecken *et al.* 2009a). Columns planted with *M. rigens* and *L. condensatus* tended to perform better than other species for Cd, Cu, and Pb removal, further supporting the use of multi-species plantings.

In the context of meeting regulatory requirements, effluent concentrations of Cd and Zn were about 2–4 times higher than concentration limits set in the Region 4 Phase I MS4 Permits for effluent of new BMPs (0.3 and 23 μg/L, respectively; CA RWQ Board 2015). Existing biofilters in southern California also exceeded Zn effluent concentration limits (Galloway 2016; Katz *et al.* 2018). The 6-μg/L Cu effluent concentration limit for new BMPs (CA RWQ Board 2015) was also exceeded in all treatments except columns planted with *B. pilularis* and *M. rigens* before the dry period. However, following the dry period, all treatments exceeded these Cu effluent concentration limits. Existing biofilters in southern California also exceeded Cu effluent benchmarks (Galloway 2016; Katz *et al.* 2018). Cu removal may be enhanced by adding a carbon source to the saturated zone since Cu-organic matter complexes can form in similar conditions (Ponizovsky *et al.* 2006), but it would be unlikely for this addition to have affected removal of other metals (Blecken *et al.* 2009a). Although data for Pb effluent concentrations in localy biofiltration systems are lacking, we found that one existing biofilter in southern California exceeded Pb benchmarks during monitoring (Katz *et al.* 2018) while all treatments in our study removed Pb to levels below the MS4 limit of 2.5 μg/L (CA RWQ Board 2015).

Effects of dosing frequency

In the past 20 years, about 65% of Los Angeles rain events have occurred with a 13-day antecedent dry period while 46% of rain events occurred with a 6-day antecedent dry period (Ambrose & Winfrey 2015), which were represented by columns that were dosed fortnightly and weekly, respectively. Although columns dosed fortnightly removed significantly less TN, the difference was marginal (42% vs. 48% on average before the dry period). Although this result indicates that pollutant removal during less frequent rain events may diminish only slightly, longer antecedent dry periods often mean increased loads due to pollutant build-up on impervious surfaces, which can be substantial in Mediterranean climates like southern California (Schiff & Tiefenthaler 2010).

Columns planted with either *C. praegracilis* and *J. patens* were minimally affected by less frequent dosing before the dry period and maintained high removal rates for key metals (e.g., Cu, Pb, and Zn)

following the dry period, but still exceeded maximum metal effluent concentrations set by local BMP guidelines (CA RWQ Board 2015).

Effects of saturated zone

The only clear advantages to a saturated zone for an individual species was observed for *J. patens* releasing less ON before the dry period and removing more TN following the dry period. However, our study confirmed that a saturated zone improves nitrogen removal in general as past studies observed (Zinger *et al.* 2007a; Payne *et al.* 2014b; Wang *et al.* 2018). Denitrification in the saturated zones of biofilters is a potential removal mechanism, but the microbial community responsible for nitrogen cycling can be influenced by plant species (Morse *et al.* 2018) and degree of saturation (Morse *et al.* 2017). Although we did not see differences in either of the two species, nitrate removal was nominally 2–3 times higher in columns with a saturated zone before the dry period.

Although including a saturated zone did improve nitrogen removal, local benchmarks for nitrogen effluent concentrations were not met (CA RWQ Board 2015). Because few BMP guidelines suggest including a saturated zone, nitrogen removal may be a significant challenge for biofilters to meet local benchmarks. Future studies on these types of biofilters in Mediterranean climates should include a carbon source in the saturated zone, which may further improve nitrate removal (Zinger *et al.* 2007b; Payne *et al.* 2014b).

Plants growing in columns without a saturated zone would have had less 'old water' to dilute the dose of semi-synthetic runoff, which was present in the columns with a saturated zone. However, this effect was expected to diminish after the long dry period, when plants would have depleted the water stored in the saturated zone. The higher TN removal following the dry period indicates some 'old water' may have still been present in columns with a saturated zone planted with *J. patens*. This was interesting because we did not observe this same difference before the dry period for either species, despite nominal TN removal efficiencies of columns with a saturated zone being nearly twice that of those without a saturated zone. Wang *et al.* (2018) observed higher removal efficiencies of nitrate and TN concentrations from columns with increasing depths of saturated zones, indicating that nitrogen in the stored water can undergo denitrification before being released through effluent with the next dose of water.

In a previous study, removal efficiency of Cu was higher in columns with a saturated zone and carbon source after a 49-day dry period in comparison to those without a saturated zone and carbon source (Blecken *et al.* 2009a). In this study, the shift from higher to lower Cu removal following the dry period could indicate that the *C. praegracilis* columns with a saturated zone, which also had substantially more belowground biomass, were leaching organic carbon during the dry period to a greater extent than those with a saturated zone. The presence of organic carbon may have been the most significant factor in Cu removal, as by Blecken *et al.* (2009a), rather than the saturated zone. Columns with a saturated zone and planted with *C. praegracilis* also had lower Zn removal following the dry period, which agrees with observations of Blecken *et al.* (2009b), who found that the presence of a saturated zone decreased Zn removal following a 49-day dry period as compared to those without one.

The presence of a saturated zone did affect plant growth and root characteristics. The allocation of growth to belowground biomass for *C. praegracilis* growing in columns without a saturated zone was likely a strategy to access more soil water by this plant, but this was not observed for *J. patens*. This adaptation to less plant available water is important for understanding how certain species may be able to persist during extended dry periods without supplemental water in Mediterranean climates. Interestingly, the inclusion of a saturated zone did not result in higher aboveground biomass for either species, nor did the authors notice substantial differences in greenness between plants growing in columns with and without saturated zones. This similarity in aboveground growth suggests that the

inclusion of a saturated zone may not enhance community acceptance of biofilters, particularly if plants are not kept greener during dry periods (Dobbie 2016).

CONCLUSION

In this study, we characterized effects of several design aspects on pollutant removal performance in southern California biofilters. From our observations in this study, the most important factors for improving nitrogen removal are: (1) selecting appropriate plant species, particularly *B. pilularis* and *M. rigens,* (2) including a saturated zone, and (3) optimising dosing frequency. We also recommend adding a lignocellulosic carbon source (e.g., straw or sugar cane mulch, woodchips) to the saturated zone to enhance nitrate removal.

Two plant species, *L. condensatus* and *M. rigens,* improved metal removal before and following the dry period, but metal removal was not influenced by the presence of plants to the same extent as nitrogen removal.

As expected, including a saturated zone improved nitrogen removal. Metal removal was also improved by the presence of a saturated zone, but only for *C. praegracilis* and only before the dry period, after which time the presence of a saturated zone decreased metal removal.

We found that dosing frequency and an extended dry period affected nitrogen and metal removal. We suggest that the experimental designs of future studies should incorporate local historical rainfall patterns and predicted patterns under climate change scenarios to investigate design parameters that may drive water quality improvement and may be affected by dosing frequency, such as plant species selection and the presence of a saturated zone with an added carbon source. Further, more monitoring data of existing BMPs would help to evaluate actual biofilter performance under varying conditions and designs. Optimising dosing frequency or irrigating biofilters to maintain plant health may be difficult to achieve without temporarily storing runoff or relying on potable water supplies. However, alternative sources of inflow (e.g., greywater) for these systems could maintain higher nitrogen removal (Barron *et al.* 2020) and maintain plant health during long dry periods.

ACKNOWLEDGEMENTS

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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