Organic Carbon Amendments for Enhanced Biological Attenuation of Trace Organic Contaminants in Biochar-Amended Stormwater Biofilters

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S *Supporting Information*

ABSTRACT: This study sought to evaluate how dissolved organic carbon (DOC) affects attenuation of trace organic contaminants (TOrCs) in biochar-amended stormwater bio-filters. It was hypothesized that (1) DOC-augmented runoff would demonstrate enhanced TOrC biodegradation and (2) biochar-amended sand bearing DOC-cultivated biofilms would achieve enhanced TOrC attenuation due to sorptive retention and biodegradation. Microcosm and column experiments were conducted utilizing actual runoff, DOC from straw and compost, and a suite of TOrCs. Biodegradation of TOrCs in runoff was more enhanced by compost DOC than straw DOC (particularly for atrazine, prometon, benzotriazole, and fipronil). 16S rRNA gene quantification and sequencing revealed that growth-induced microbial community changes were, among replicates, most consistent for compost-augmented microcosms and least consistent for raw runoff microcosms. Compost DOC most robustly enhanced utilization of TOrCs as carbon substrates, possibly due to higher residual nutrient levels upon TOrC exposure. Sand columns containing just 0.5 wt % biochar maintained sorptive TOrC retention in the presence of compost-DOC-cultivated biofilms, and TOrC removal was further enhanced by biological activity. Overall, these results suggest that coamendment with biochar and compost may robustly enhance TOrC attenuation in stormwater biofilters, a finding of significance for efforts to mitigate the impacts of runoff on water quality.
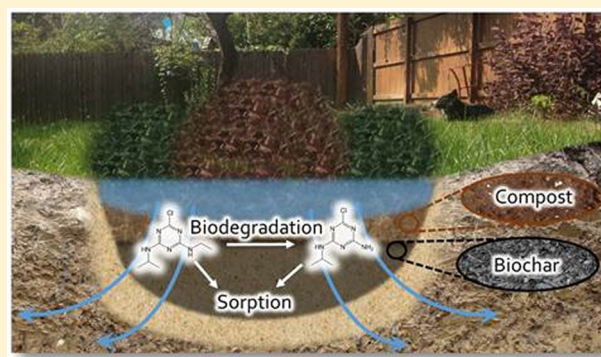
INTRODUCTION

Stormwater runoff has degraded urban water quality by transporting contaminants to receiving waters.[1] Low impact development (LID) systems have gained popularity because they remove many conventional contaminants during infiltration (e.g., metals and petroleum hydrocarbons).[2,3] However, removal of trace organic contaminants (TOrCs) in LID systems is poorly understood despite their broad presence in urban runoff.[4] In particular, many toxic stormwater TOrCs such as urban-use pesticides (e.g., phenylurea and triazine herbicides, phenylpyrazole insecticides),[5−7] flame retardants (e.g., organophosphates),[8] and chemicals from vehicle fluids (e.g., benzotriazoles)[9] are also relatively mobile, allowing them to pass through LID systems.[10]

Amendment of LID systems with biochar has shown promise for improved removal of metals, bacteria, nutrients, and TOrCs.[11−15] In particular, a recent study revealed remarkable TOrC removal in intermittently dosed, biochar-amended infiltration columns: > 99% TOrC removal from spiked natural water was maintained throughout cumulative treatment of an annual treatment volume estimated for the Denver area.[16] However, it remains unclear if sorptive TOrC retention can be maintained throughout an entire system lifetime (i.e., more than 15 years, as limited by accumulation of nondegradable metals).[17] It would therefore be desirable to stimulate biodegradation to reduce potential accumulation, as has been demonstrated previously for conventional stormwater bio-filters.[18] These systems contain an upper layer amended with organic carbon (e.g., compost or straw), stimulating biological activity by slowly releasing growth substrates (e.g., dissolved organic carbon, DOC; and nutrients such as nitrogen, N, and phosphorus, P). As this may also cause undesirable sorbent fouling and reduced TOrC sorptive retention,[19] it is of interest

to determine if DOC can allow enhanced biological TOrC attenuation despite this potential trade-off.

While several studies have evaluated the effects of DOC on microbial communities and TOrC biodegradation in wastewaters and surface waters,[20−22] it remains unclear how the underlying phenomena will manifest in stormwater biofilters. TOrC biodegradation may be affected by increased overall microbial abundances as well as growth-induced changes in microbial communities. For example, broader TOrC biodegradation potential has been demonstrated in wastewater communities with greater biodiversity (i.e., community richness and evenness),[20] which has been linked to availability of ambient carbon and nitrogen.[22] Further, DOC can affect TOrC-degrading activity by influencing biofilm formation and structure.[23] DOC quality and composition may be drivers behind these phenomena. For example, easily utilizable carbon substrates may allow rapid microbial growth, while growth on more complex substrates may lead to broader substrate utilization potential.[24] The carbon-to-nitrogen ratio (C:N) of leached DOC can also affect growth (and in turn TOrC utilization) by establishing either carbon-limited or nutrient-limited growth conditions.[25,26] As DOC quality and composition can be controlled in biofilters through selection of organic carbon amendments, biofilters could potentially be designed to achieve optimal TOrC biodegradation.

The objective of this study was to assess how DOC from organic carbon amendments affects TOrC attenuation in biochar-amended biofilters. Efforts were focused on biological processes, as relevant abiotic processes (i.e., sorptive TOrC retention in the presence of biochar and DOC) were assessed in a previous study.[14] It was hypothesized that (1) augmenting runoff with DOC would enhance TOrC biodegradation due to increased microbial growth, activity, and potentially growth-stimulated changes in community composition and (2) biochar-amended media bearing DOC-cultivated biofilms would demonstrate enhanced TOrC attenuation due to a combination of TOrC sorptive retention and biodegradation. The first hypothesis was assessed in microcosm experiments, and the second in column experiments. To maintain environmental relevance, these experiments utilized actual runoff to obtain representative microbial consortia, straw and woody plant-based compost to obtain DOC of varying quality, and a suite of TOrCs and transformation products (TPs) to represent compound classes that are likely to impact water quality (i.e., both toxic and mobile). The results from this study provided useful insight for the design of infiltration systems to mitigate impacts of runoff on receiving water quality.

◼ EXPERIMENTAL SECTION

TOrC Analysis. The TOrC suite was selected in a previous study[4] and included atrazine, diuron, tris(3-chloro-2-propyl)-phosphate (TCPP), tris(3-chloro-ethyl)phosphate (TCEP), prometon, benzotriazoles (1H-benzotriazole in microcosm experiments, 5-methyl-benzotriazole in column experiments), fipronil, oryzalin, and 2,4-dichlorophenoxyacetic acid (2,4-D results are reported in the Supporting Information because it behaved unusually relative to the other analyzed TOrCs for reasons that remain unclear). Transformation products (TPs) were analyzed for atrazine (hydroxyatrazine; desethylatrazine, DEA; deisopropylatrazine, DIA), diuron (1-(3,4-dichlorophenyl)-3-methylurea, DCPMU; 3,4-dichloroaniline, DCA), and fipronil (fipronil sulfone and sulfide). TOrCs were measured by liquid chromatography tandem mass spectrometry (LC-MS/

MS), and ionized by electrospray ionization (ESI) in the positive or negative mode (0.025 μg/L limit of quantitation, details in Method S1). Optima-grade methanol and water (Fischer Scientific) were used unless noted otherwise.

DOC Extracts. Concentrated DOC extracts (used to augment DOC levels in microcosms) were prepared in runoff that was collected following three separate rain events such that it was stored for less than 24 h prior to use in microcosm, column, and DOC characterization experiments. Runoff was collected in sterilized glass jugs from a pipe discharging runoff from a residential neighborhood in Golden, CO. Approximately 500 mL of sediment (leaves, sticks, and silt) was added to each jug to introduce bacteria and DOC. The runoff was equilibrated at room temperature on a shaker table overnight and centrifuged for 5 min at 800 RCF to remove large particles. To enable rapid production of concentrated DOC extracts (to allow augmentation of runoff with DOC simulating leachate from rain events on the order of hours), runoff (2 L) containing either a woody plant-based compost (EcoGrow compost, obtained from A1 Organics; Eaton, CO, 500 mL) or wheat straw (Longmont, CO; 2L, packed) was incubated at 30 °C on a shaker table overnight in the dark (such that the leached DOC was minimally affected by exposure to UV radiation).[27] Extracts were filtered to 0.45 μm (such that minimal residual bacteria remained prior to subsequent sterilization by autoclave), analyzed with a Shimadzu Total Organic Carbon Analyzer to determine DOC and total nitrogen (TN), and then diluted with filtered runoff to adjust the concentration as necessary.

Fresh DOC extracts were analyzed by ultraviolet visible (UV−vis) spectroscopy (Figure S1) from which specific UV absorption was determined (SUVA, absorption at 254 nm; listed in Table S4 with additionally calculated absorption parameters). SUVA is an indicator of DOC aromaticity,[28] and negative associations between SUVA and DOC biodegradability have been previously reported for soil leachates.[29] SUVA was higher for the compost DOC (3.0 versus 0.7 L·mgC^{-1}·m^{-1} for straw DOC), likely because the compost was derived from lignin-rich, woody material.[30] The C:N ratios for fresh compost and straw extracts were 8.3 and 15.8, suggesting that they provided carbon-limited and nutrient-limited growth conditions, respectively (considering an optimal C:N of 10).[31] Higher residual (postincubation) DOC levels in compost microcosms suggested that the compost DOC was less biodegradable (52 and 89% DOC biodegraded in compost and straw microcosms, respectively; Table S5). Lower residual levels of P (Table S6) and N (Table S5) in straw microcosms indicated nutrient-limited growth conditions.

Microcosm Experiments. Three types of aqueous microcosm media (raw runoff and runoff augmented with DOC from compost or straw) were used to prepare eight biotic microcosms (five monitored for TOrCs, three sacrificed for additional analyses) and five biologically inhibited controls. Erlenmeyer flasks (150 mL) were filled with 5 g of sand (Sigma-Aldrich) and 10 mL of DOC extract or filtered raw runoff and then autoclaved. The microbial consortium was introduced with 40 mL of collected runoff such that the DOC prior to incubation (and subsequent dilution) was 205 mg/L in straw and compost microcosms (6 mg/L DOC in raw runoff). Microcosms were aerobically incubated for 14 days in the dark at 30 °C on a shaker table to allow for microbial growth. Reduced dissolved oxygen (DO, Hach DO probe) consumption in sacrificed microcosms indicated stabilized bio-

logical activity after this duration. Microcosms were then diluted to 125 mL with autoclaved synthetic stormwater (composition in Table S3), and a sodium azide (NaN$_3$) solution (5 mL of 4 g/L in DI water, 154 mg/L final concentration) was added to control microcosms to inhibit biological activity (biotic microcosms diluted with 5 mL autoclaved DI water). These dilutions allowed sufficient volume for numerous sampling events and reduced the DOC to more representative levels (i.e., less than 50 mg/L, Table S5).

TOrCs were introduced by spiking microcosms with 52 μL of a methanol carrier solution (time 0) such that initial individual TOrC concentrations (40 μg/L) permitted small sample volumes without exceeding environmentally relevant levels.[4] Spiked microcosms were shaken on a shaker table at room temperature and sampled over 76 days (daily to weekly sampling). Prior to each sampling event, autoclaved DI water was added to correct for evaporation losses (individual volumes determined gravimetrically to avoid dilution). Aliquots (250 μL) were transferred with sterile pipet tips to 2 mL microcentrifuge tubes containing 200 μL of internal standard solution (4 μg/L isotope surrogates in methanol) and 1550 μL of Milli-Q water containing 500 mg/L sodium azide to prevent biodegradation during storage (4 °C, less than 30 days). Solid phase extraction (SPE) was carried out immediately prior to LC-MS/MS analysis using Phenomenex Strata-X 33u Polymeric Reversed Phase cartridges (30 mg/1 mL) following the manufacturer's recommended protocol. Nitrate (Nitrate TNT Plus Hach Kit), optical density (OD, absorbance at 600 nm), and DO were also measured on 3−4 occasions (Figure S2).

Column Experiments. Columns (Kimble Chase Economy FLEX-COLUMN 6 cm length, 2.5 cm I.D.) were dry-packed with sand (Sigma-Aldrich, 210−297 μm particles, column diameter: particle size greater than 50 to prevent boundary effects),[32] or sand with 0.5 wt % biochar (53−250 μM particle sizes, Milli-Q-rinsed), and porosity was estimated gravimetrically after saturation with water (0.35 ± 0.1 for biochar columns, 0.32 ± 0.1 for sand columns). Mountain Crest Gardens biochar (MCG-biochar, produced by gasification of pinewood by Gropro, Inc., in Etna, CA; material properties in Table S7) was selected for its high surface area (317 m^2/g) and proven TOrC retention performance.[14] While higher biochar doses may be desirable in full-scale systems (e.g., greater than 5 wt %),[16] a low dose was necessary to allow TOrC breakthrough within a reasonable experimental time frame. Batch sorption experiments with the DOC extract and biochar revealed that the biochar had similar sorption affinities for both types of DOC (Method S2, log $K_{d,DOC}$ [L/kg] = 3.2).

Biofilms were seeded on columns with concentrated solutions prepared similarly to the microcosm media. Over three cycles, undiluted, autoclaved straw and compost DOC extracts (>1000 mg/L DOC) were inoculated (either with runoff or the previously incubated culture) and incubated until increases in OD indicated exponential growth (Method S3, growth curves in Figure S4). After flushing with CO$_2$ to ensure saturation, columns were injected with one pore volume (PV, approximately 10 mL) of seeding solution at 1 mL/min in upflow, and then slowly cycled for 24 h (0.05 mL/min, 0.6 cm/h linear velocity) to allow microbial attachment. Columns were then equilibrated with synthetic stormwater containing 10 mg/L DOC at 0.21 mL/min (2.6 cm/h linear velocity) for 10 days (influent reservoirs refreshed every 2−3 days) until effluent DO levels stabilized (Figure S5). Tracer tests using KBr (10 g/L) as a conservative tracer and a Hach conductivity meter showed

that hydraulic retention times were minimally affected by biofilm formation (i.e., breakthrough less than 10 min before the predicted 2.3 h hydraulic retention time).

Columns were then injected with synthetic stormwater containing 10 mg/L DOC and 10 μg/L TOrCs (methanol from TOrC carrier solution evaporated under N$_2$) for 23 days (675 PVs) and 18 days (528 PVs), respectively (injection times differed due to slower microbial growth in the compost DOC seeding solution, Figure S3). Influents were refreshed every 24 h to minimize TOrC biodegradation within the reservoir. NaN$_3$ (400 mg/L) was added to influents for control sand−biochar columns (one control and three biotic replicates per DOC type). Following TOrC injection, columns were flushed with TOrC-free synthetic stormwater for the remainder of the experiment. Effluent samples (collected every 1−100 PVs over the total 1100 PVs) were preserved by diluting 10% with 4 g/L NaN$_3$ (biotic columns only) and stored at 4 °C in the dark. Isotope dilution was carried out immediately prior to LC-MS/MS analysis (ESI positive mode only due to constraints on sample analysis).

Microbial Analyses. Microbial community composition and structure were investigated by high-throughput sequencing (pyrosequencing). Due to constraints on sample analysis, DNA extraction and sequencing was carried out for three representative biotic microcosms from each condition (nine total), and the column seeding solutions (Figure S4). The subset of microcosms chosen for microbial sequencing analysis included, from each condition, two TOrC-monitored microcosms (Figure S9; samples C1, C5, S1, S2, R1, and R5) and one unmonitored microcosm (samples C6, S6, and R6) such that community differences due to the TOrC sampling process could be identified if present. Immediately following the final sampling event, the sand and remaining aqueous media were centrifuged in sterile 50 mL Falcon tubes at 3220 RCF for 60 min. After decanting the liquid, the top 500 mg of sand (wet weight) was collected for DNA extraction. DNA was extracted in duplicate using a PowerSoil DNA Isolation Kit (MO Bio Laboratories, Inc.) according to the manufacturer's instructions (stored at −80 °C until subsequent analysis).

Polymerase chain reaction (PCR) amplification of the V4−V5 region of bacterial 16S rRNA genes for each DNA extract was conducted using fusion primers containing a PGM sequencing adaptor, a "GT" spacer, and a unique 12 base pair Golay barcode to allow multiplex analyses (primers 515F: 5′-GTGNCAGCMGCCGCGGTAA-3′, and 926R: 5′-CCGYCAATTYMTTTRAGTTT-3′).[33] PCR reactions were conducted using the FastStart High Fidelity PCR System and the PCR Nucleotide Mix (Roche Diagnostics GmbH) with the following thermocycle program: (i) 2 min denaturation at 95 °C, (ii) 30 cycles of 30 min denaturation at 95 °C, (iii) 30 s annealing at 56 °C, (iv) 45 min elongation at 72 °C, and (v) 7 min elongation at 72 °C. PCR products were purified using Agencourt AMPure XP reagent (Beckman Coulter) and quantified using a Qubit dsDNA HS Assay kit (Invitrogen) on a Qubit 2.0 Fluorometer. The single PCR libraries were pooled in equimolar amounts and further purified using a Pippin Prep System (Life Technologies) following the manufacturer's protocol. Quantitative PCR (qPCR) of overall bacterial 16S rRNA genes was also conducted to qualitatively compare gene copy numbers among samples (Method S4).

Sequencing was carried out with an Ion Torrent Personal Genome Machine (PGM) System (Life Technology) at Newcastle University. A total of 1 863 582 sequences were
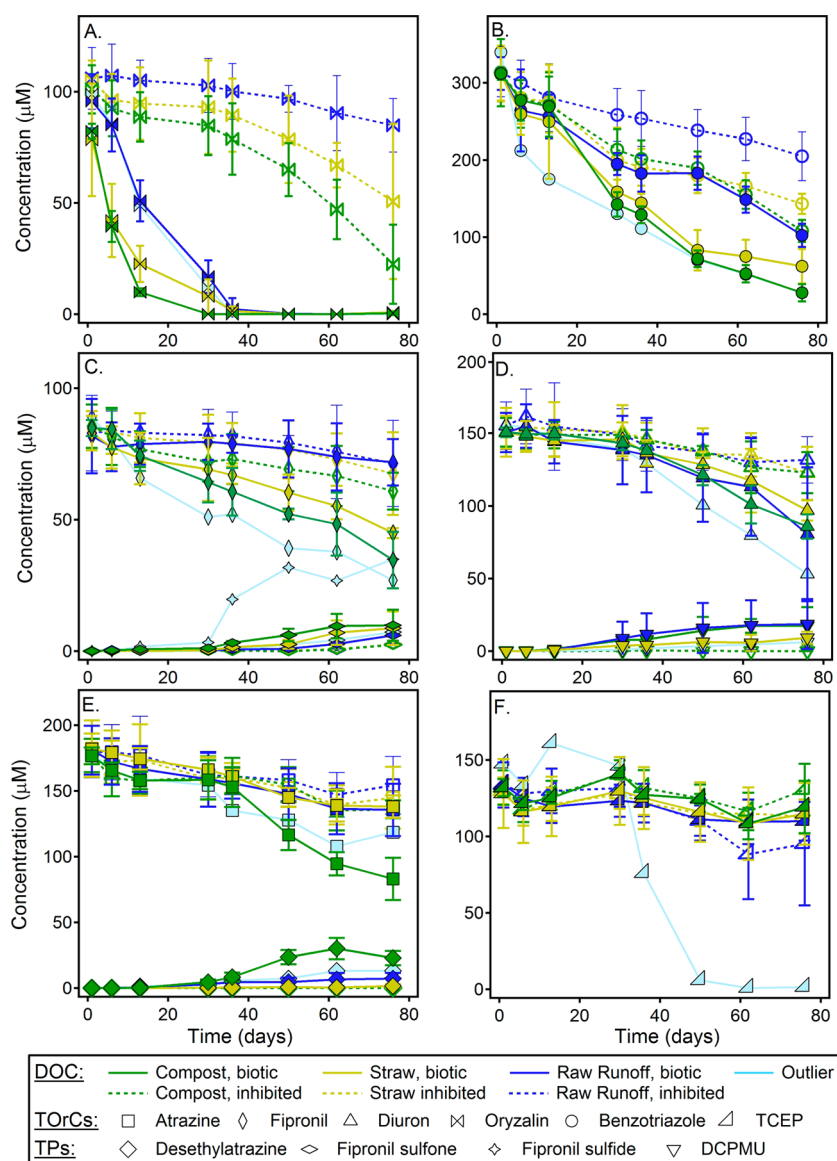
Figure 1. Concentrations of (A) oryzalin, (B) benzotriazole, (C) fipronil, (D) diuron, (E) atrazine, and (F) TCEP in microcosms (prometon and TCPP are shown in Figure S6). Error bars indicate standard deviations for replicate microcosms, and significant differences between inhibited controls and biotic microcosms are assumed to be due to biodegradation. Select transformation products (TPs) are also shown for atrazine (desethylatrazine), fipronil (fipronil sulfone and fipronil sulfide), and diuron (3,4-dichloromethylphenyl urea, DCPMU). Additional TPs are shown in Figure S7.

obtained and processed in QIIME (v 1.7.0) using the bioinformatics pipeline. The sequences were trimmed to remove primers and barcodes, quality filtered (considering a minimum quality score of 20), and chimera checked (ChimeraSlayer), resulting in 739 973 total sequences. Sequences were then clustered into operational taxonomic units (OTUs) at 97% sequence similarity level by the uclust algorithm, and a representative sequence from each OTU was selected and taxonomically identified using the Greengenes database[34] and classified using the RDP naïve Bayesian rRNA classifier.[35−37] Representative sequences and corresponding taxonomic assignment were used to build a table of OTU abundances at different levels of taxonomy. Microbial community diversity within and across the samples was determined using the QIIME (v.1.7.0) pipeline and Primer v6 software.[38,39] The Bray−Curtis similarity metric was generated and represented by a 2-dimensional nonmetric multidimen-

sional scaling (nMDS) plot.[39] Additionally, an analysis of similarities (ANOSIM) was conducted on the Bray−Curtis dissimilarity matrix (square root transformed) using PRIMER v6. Statistical analysis was performed in R on the alpha diversity indices (Shannon index).

RESULTS AND DISCUSSION

TOrC Biodegradation in Microcosms. Biodegradation of most monitored TOrCs was observed under at least one experimental condition and generally occurred consistently across replicates (Figures 1 and S6). Oryzalin and benzotriazole were biodegraded the most quickly, while atrazine, prometon, fipronil, and diuron were biodegraded more slowly. TCPP and TCEP were generally resistant to biodegradation with the exception that a single biotic raw runoff microcosm showed sudden and rapid degradation of both compounds after 30 days (henceforth referenced as the "outlier" microcosm).
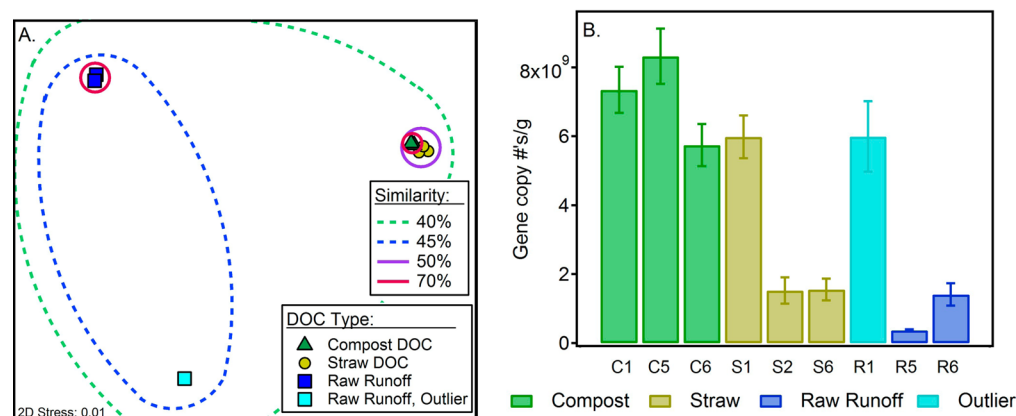
Figure 2. (A) Two-dimensional nonmetric multidimensional scaling (2D nMDS) plot of sequenced microcosm microbial communities (markers) and Bray−Curtis similarities among samples (lines). Similarities are also shown in the dendrogram in Figure S11 (similarity analysis conducted using PRIMER v6). (B) Gene copy numbers per mass of DNA-extracted sand for each sequenced sample, determined by qPCR of total bacterial 16S rRNA genes (flocculation was observed in sample S1, likely causing its elevated gene copy numbers). Error bars indicate the standard deviation of triplicate measurements.

Less than 20% removal was observed in the biologically inhibited control microcosms for most TOrCs (Figure 1), suggesting that the effects of dilution and reaction with NaN$_3$ were minor relative to biodegradation. Removal of oryzalin and benzotriazole in controls was greater than the other TOrCs, though still less than their removal in biotic microcosms. This was potentially due to incomplete inactivation of biological activity by NaN$_3$, considering that these TOrCs were the most strongly biodegraded overall, and reduced DO levels in DOC-augmented controls relative to raw runoff controls suggested that microbial activity persisted to some degree despite the presence of NaN$_3$ (Figure S2). The extent of biodegradation at the end of the experiment was therefore assessed relative to the raw runoff controls (calculated extents of biodegradation reported as fractions and μmols in Figures S8 and S9, respectively). Interestingly, the outlier raw runoff microcosm demonstrated the greatest overall TOrC biodegradation among the individual microcosms (i.e., highest sum of all monitored TOrCs biodegraded, Figure S9) and is henceforth considered separately from the raw runoff microcosms unless noted otherwise. Compost microcosms consistently demonstrated the most overall TOrC biodegradation across experimental replicates, while straw microcosms overall were not significantly different from raw runoff microcosms (p-values from the two-tailed student t test for comparison of raw runoff microcosms to compost and straw microcosms were 0.0011 and 0.20, respectively; Table S8). The compost microcosms demonstrated enhanced biodegradation of fipronil, benzotriazole, atrazine, and prometon (p-values = 0.0069, 0.0017, 0.0043, and 0.023, respectively, for comparison to raw runoff), while straw microcosms demonstrated slightly enhanced biodegradation of fipronil and benzotriazole (p = 0.0031 and 0.017, respectively, for comparison to raw runoff).

In general, the most abundant monitored TPs for fipronil, diuron, and atrazine were fipronil sulfone (Figure 1C), DCPMU (Figure 1D), and desethylatrazine (Figure 1E), respectively (additional TPs in Figure S7) except for the outlier microcosm, for which the most abundant fipronil TP was fipronil sulfide. To determine if unmonitored TPs were generated, mass balance errors were calculated according to differences in concentration between (1) each parent TOrC in raw runoff controls and (2) the sum of each parent TOrC and

their monitored TPs in biotic microcosms (Table S9). Atrazine and diuron showed low mass balance errors for the first 60 days, after which errors exceeded 10%. This coincided with the peak concentrations of the measured primary TPs, suggesting biodegradation of primary TPs to unmonitored secondary TPs. In contrast, fipronil mass balance errors exceeded 10% after just 13 days, suggesting that unmonitored primary TPs may have been generated.

Trends in lag time (delays before biodegradation) across experimental replicates provided insight into biodegradation mechanisms. For example, oryzalin, benzotriazole, and fipronil were biodegraded in all DOC-augmented microcosms without a lag time, suggesting biodegradation by cometabolic processes (i.e., degradation by constitutively expressed, nonspecific enzymes). Atrazine and prometon, in contrast, were biodegraded only in compost microcosms following a 30-day lag time. Depletion of preferential carbon substrates may have caused this lag time, subsequently allowing TOrC biodegradation due to adaptive expression of TOrC-degrading enzymes[40,41] or reduced substrate−substrate inhibitions.[42] The greater aromatic character of the compost DOC may have contributed to this behavior, as these more complex carbon substrates may have been more slowly utilized and hence still present upon TOrC exposure. TCPP and TCEP biodegradation in the outlier microcosm was particularly abrupt after the 30-day lag time, potentially due to the sudden activity of specialist bacteria. Decreased DO (Figure S2A), increased OD (Figure S2B), and reduced DOC (Table S5) relative to raw runoff microcosms indicate accelerated growth in this microcosm following TOrC addition, potentially due to methanol exposure (discussed further below).

Microcosm Microbial Communities. The 2D nMDS analysis showed that the microbial communities in all DOC-augmented microcosms (i.e., both straw and compost microcosms) clustered apart from those in raw runoff microcosms (Figure 2A), indicating DOC-stimulated, growth-induced changes in community structure. The communities of the DOC-augmented microcosms were relatively similar to each other (58% Bray−Curtis similarity overall, see dendrogram in Figure S10), while compost DOC communities showed the highest similarity among replicates (73%). As communities were more similar among the DOC-augmented microcosms,
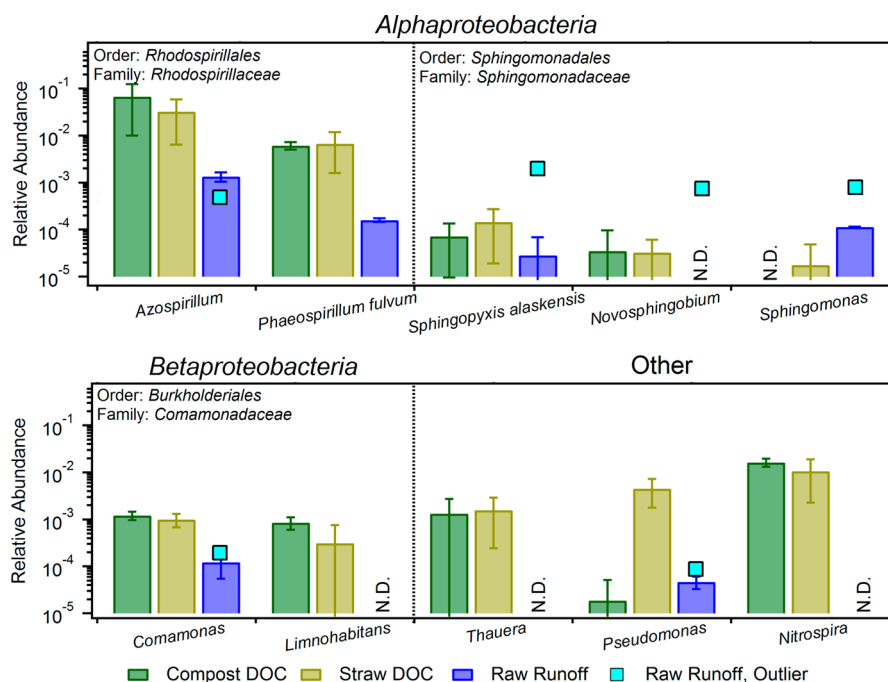
Figure 3. Relative abundances of OTUs that were (i) identified at the genus level and (ii) observed to be enriched by at least one order of magnitude relative to raw runoff (note the log scale of the y-axis). Bars show averages for replicate microcosms with error bars showing the standard deviations. Markers show relative abundances for the outlier microcosm (not included in the averages for the raw runoff microcosms). Comparisons were made between averaged relative abundances with the exception of the outlier microcosm, for which its individual values were compared to the averaged values for the other raw runoff microcosms.

the more significantly enhanced TOrC biodegradation in compost microcosms relative to straw microcosms (as compared to the raw runoff microcosms) was likely due to either subtle differences in communities (e.g., differences in relative abundances of specific OTUs) and/or other factors relating to microbial abundance and activity. The outlier microcosm did not cluster with the other microcosms, suggesting that microbial community differences caused its unique biodegradation behavior. It is important to note that growth on methanol from the TOrC carrier solution may have also affected communities. It is possible that this masked community differences that had already developed during incubation, potentially increasing the similarity among the DOC-augmented microcosm communities. For the outlier microcosm, it is possible that growth of methanol-utilizing bacteria was stimulated in the absence of other DOC sources, potentially contributing to its unique community structure.

Gene copy numbers (GCNs) of overall bacterial 16S rRNA genes were consistently high for compost microcosms, while they were lower and more variable for raw runoff and straw microcosms (Figure 2B). Considering that low residual DOC levels (Table S5) suggest that growth occurred in all DOC-augmented microcosms, the higher GCNs in compost microcosms may have been due to greater abundances of active bacteria upon DNA extraction (at the end of the experiment). GCNs were higher in the outlier microcosm than the raw runoff microcosms, further indicating accelerated growth in this microcosm. Only small differences in the Shannon index were observed among the microcosms (indicative of community richness and evenness, Table S10); therefore, alpha diversity likely had only minor effects on observed trends. This is not surprising, as the inoculum effectively fixed the species richness across microcosms (i.e., the same number of species were introduced into all microcosms

upon inoculation). While averaged Shannon indices were higher for compost and straw microcosms than for raw runoff microcosms (3.6 and 3.4 versus 3.2, respectively), differences were not statistically significant ($p = 0.072$ and 0.66, respectively, for comparison to raw runoff microcosms). Notably, an especially low Shannon index was observed for one straw microcosm (2.95, microcosm S2, discussed below).

Figure 3 shows the most prevalent differences in OTU relative abundances that were identified among the microcosms (family level OTU bar plot in Figure S11). Several OTUs that are known to facilitate nitrogen-cycling processes were enriched in DOC-augmented microcosms relative to raw runoff, including those within the families *Rhodospirlliceae* (N$_2$-fixation),[43] *Nitrospirae* (*Nitrospira*; nitrite-oxidation),[44] *Comamonadaceae* (*Comamonas*; denitrification),[45] and *Rhodocyclaceae* (*Thauera*; denitrification).[46] This reflects previous findings of coincidentally enhanced nitrogen-cycling processes and TOrC biodegradation among wastewater communities.[21,47] An OTU from the genus *Pseudomonas* was enriched only in straw microcosms (particularly microcosm S2, causing its low Shannon index; Figure S11). Though many *Pseudomonas* species are known TOrC degraders,[48] the OTU's abundance was not positively associated with individual or overall TOrC biodegradation (Spearman's rank and Pearson correlation tests are described in Method S5, Tables S11−S14). As *Pseudomonas* can undergo metabolic regulation;[49] this OTU may have initially out-competed other bacteria for utilization of the straw-derived carbon substrates, potentially until residual levels of P and N could no longer support growth (Tables S5 and S6). This potentially explains the reduced TOrC biodegradation in straw microcosms relative to compost microcosms, as microbial activity and hence TOrC biodegradation would presumably be diminished if nutrients were depleted prior to TOrC introduction. Interestingly, the outlier microcosm was enriched
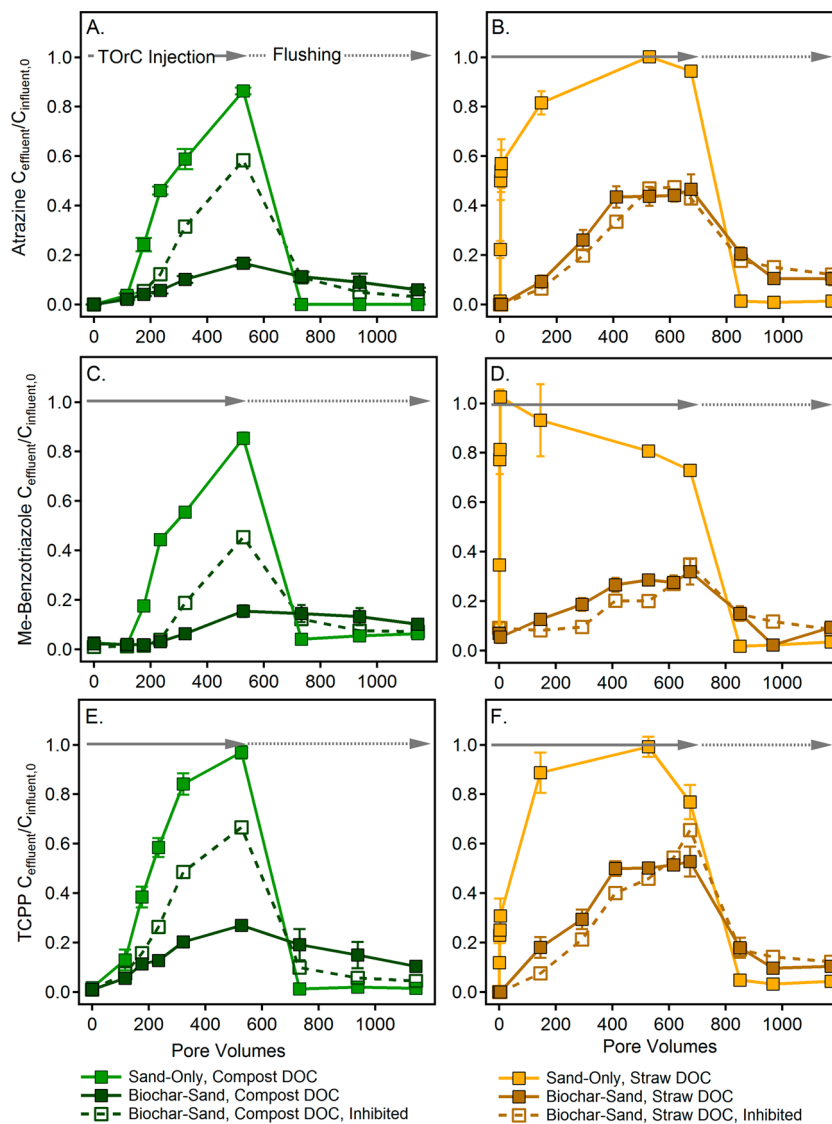
Figure 4. Breakthrough of (A and B) atrazine, (C and D) methylbenzotriazole, and (E and F) TCPP in sand-only and sand−biochar columns (0.5 wt % biochar) with biofilms cultivated from compost DOC (green) or straw DOC (yellow). Results for biologically inhibited control sand−biochar columns are shown by dotted lines. Columns were injected with 10 μg/L TOrCs and then flushed with TOrC-free synthetic stormwater. Diuron was exceptionally well removed overall, as has been observed previously.[14,60]

with OTUs within the family *Sphingomonadaceae* (markers in Figure 3), which contains several genera of TOrC-degrading bacteria (e.g., *Sphingomonas*).[50,51] While these OTUs could potentially be specialist TCEP- and TCPP-degrading bacteria, they may also just covary with such bacteria.

Overall, while DOC-stimulated growth led to communities that were starkly different from raw runoff communities, community differences among the DOC-augmented microcosms were more subtle. However, the similarity in both growth and community composition among replicates was the highest for the compost microcosms, coinciding with the greatest overall TOrC biodegradation. One possible explanation for this behavior is that the compost microcosms provided conditions that more robustly promoted TOrC-degrading activity upon TOrC exposure. For example, higher residual N and P levels may have facilitated the utilization of TOrCs as carbon sources, potentially allowing sustained microbial activity throughout the experiment. These conditions may have arisen due to several factors relating to DOC

composition and quality or a combination thereof, including the initial C:N of the DOC (i.e., providing carbon-limited rather than nutrient-limited growth conditions) as well as effects of DOC aromaticity on substrate utilization potential. In particular, the higher aromaticity of the compost DOC may have led to adaptive expression of TOrC-degrading enzymes. This is further supported by the biodegradation lag times observed for atrazine and prometon, which also showed the most significantly enhanced biodegradation relative to that of straw microcosms (p = 0.00077 and 0.00067, respectively, Table S8).

While the underlying phenomena behind the observed TOrC biodegradation trends cannot be fully elucidated, these findings indicate that compost DOC may indeed support TOrC-degrading microbial activity in stormwater biofilters more effectively than straw DOC. However, the observed time scale for TOrC biodegradation (weeks to months) is much higher than hydraulic residence times for distributed infiltration systems (hours to days). In this respect, biochar-amended

biofilters may be especially effective if sorptive retention is maintained, as increased TOrC retention times may allow more time for biodegradation. The column experiments provided further insight into these questions.

Column Experiments. Sand-only columns cultivated with compost DOC demonstrated TOrC retention times longer than those cultivated with straw DOC (i.e., TOrC breakthrough was observed after approximately 100 PVs in sand-only compost columns versus less than 10 PVs for sand-only straw columns; Figures 4 and S12). Compost columns also demonstrated greater microbial activity during TOrC injection and flushing, as indicated by lower effluent DO levels (Figure S5). Notably, TOrC retention times were enhanced by amendment of sand with just 0.5 wt % biochar (i.e., breakthrough occurred approximately 100 PVs later in biochar–sand columns than in sand-only columns) despite the severe fouling expected to be caused by the concentrated seeding solutions (with DOC concentrations potentially orders of magnitude above representative levels). It is therefore likely that TOrC retention can be maintained in actual biofilters, which presumably contain media with significantly higher biochar compositions (i.e., greater than 5 wt %).[16]

In addition to higher TOrC retention times, compost columns also demonstrated a greater percent removal of most TOrCs (i.e., lower recovery at the end of the experiment, as indicated by a method of moments analysis; Figure S13). This appeared to be at least partially due to biological processes, as inhibition of biological activity with NaN_3 reduced removal of atrazine by 20% as well as TCEP and TCPP to a lesser degree (though slowly receding concentrations during flushing may have slightly affected recovery calculations). This could potentially indicate TOrC biodegradation within columns (i.e., 20% greater atrazine removal in the absence of NaN_3 potentially indicates up to 20% biodegradation). This is an interesting result, as conventional porous media transport theory suggests that only the dissolved TOrC fraction is bioavailable and that TOrCs are therefore biodegraded on the time scale of the hydraulic retention time.[52] Thus, biodegradation would be unlikely to amount to observable levels within the columns unless biodegradation were particularly rapid (as the hydraulic retention time in columns was approximately 2 h).

Interestingly, atrazine retention times were comparable to expected biodegradation time scales (i.e., 10−20 days) and appeared to be associated with atrazine removal (i.e., biochar–sand columns demonstrated both atrazine retention times and atrazine removal percentages that were nearly double those for sand-only columns). While a possible explanation for this behavior is the biodegradation of TOrCs sorbed to biochar (which has been observed for some cases),[53] this apparently contradicts conventional transport theories. A more feasible explanation may be biodegradation of TOrCs loosely associated with the immobilized DOC and the biofilm itself. Localized biodegradation of these more readily desorbed TOrCs would continuously replenish sorption sites, making it appear that sorbed TOrCs were biodegraded. Moreover, the biofilm structure (potentially affected by DOC quality) could also affect TOrC biodegradation (e.g., due to beneficial cosettlement of TOrC-degrading bacteria).[23]

Overall, the column experiments revealed that TOrC sorptive retention was maintained by biochar-amended media bearing DOC-cultivated biofilms and that overall TOrC attenuation was improved in the presence of biological activity

stimulated by compost DOC. As it remains unclear if TOrCs were actually biodegraded within columns, studies evaluating systems with longer TOrC retention times may provide insight into whether TOrCs can be biodegraded significantly within biochar-amended biofilters.

Environmental Implications. The findings of this study are of particular significance for efforts to mitigate impacts of runoff on water quality through optimization of contaminant removal in infiltration systems. In particular, in addition to improving TOrC attenuation, coamendment of biofilters with compost and biochar may comprehensively enhance performance by mitigating undesirable leaching of metals and nutrients from compost,[54−56] making vegetated systems (which require compost for plant growth) more attractive. Vegetated systems may be further advantageous for improving TOrC attenuation: biological activity stimulated in the root zone may further improve TOrC biodegradation,[57] while enhanced desorption in the presence of root exudates may prevent TOrC accumulation on biochar.[58,59] Remarkable TOrC removal has indeed been observed in vegetated infiltration columns representing biochar-amended biofilters;[16] however, as no TOrC breakthrough was observed, it remains unclear whether TOrC removal is due to sorption alone or to a combination of sorption and biodegradation. Therefore, additional studies seeking to identify and quantify TOrC biodegradation in vegetated, biochar-amended biofilters are of interest to determine if TOrC accumulation can be prevented throughout the system's entire lifetime.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b01164.

> LC-MS/MS analysis, abbreviations, ESI parameters, UV−vis spectra, absorbance indices, elemental compositions, characterization results, sorption results, growth curves, OTU bar charts, qPCR details, and additional figures, tables, and methodological details (PDF)

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Notes
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