Supporting Information

Layered Double Hydroxide-Modified Biochar Reduces Transport of Extracellular Antibiotic Resistance Genes in Saturated Porous Media

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Text S1 DNA preparations and molecular identity

The bulk-eDNA and eARG experiments used separate commercial DNA preparations. Calf-thymus DNA (Sigma-Aldrich, D4764) was used at 25 mg L^{-1} as the bulk-eDNA model. I_t is purified, unsheared genomic double-stranded DNA, with a manufacturer-reported size specification of >20 kb. The eARG experiments used pBR322 plasmid DNA (New England Biolabs, N3033) at 1.06×10^{10} plasmid copies L^{-1}. pBR322 is circular double-stranded DNA with a reference sequence length of 4361 bp and carries ampicillin- and tetracycline-resistance determinants [2; 6]. The sole qPCR target was bla; an intact pBR322 molecule contains one copy of this gene. Both working solutions contained 10 mM Tris-HCl.

Calf-thymus DNA was gently dissolved overnight in molecular-biology-grade water and diluted into 10 mM Tris-HCl. Commercially purified pBR322 DNA was diluted separately into the same working buffer. The measured A_{260}/A_{280} and A_{260}/A_{230} ratios were 1.87 and 2.0-2.2 for calf-thymus DNA, and 1.8 and 2.0 for pBR322, respectively. Prepared DNA stocks were stored in aliquots at -20 °C, and working solutions were freshly prepared to limit repeated freeze–thaw cycles. Table S1 distinguishes manufacturer-reported size information from the measured purity ratios.

Text S2 Supplementary material characterization

Tobacco-stalk feedstock was dried at 60 °C for 72 h. PC was produced at 500 °C for 3 h in a tube furnace (O-KTF1200, Chunlei, China), with a heating rate of 10 °C min^{-1} under N_2 flowing at 200 mL min^{-1}. MPC was prepared from 10 g of PC dispersed in 2.5 L of deionized water by ultrasonication for 4 h. $Mg(NO_3)_2 \cdot 6H_2O$ (12.8 g) and $Al(NO_3)_3 \cdot 9H_2O$ (9.375 g) were added, followed by 0.5 mol L^{-1} NaOH to pH 10. The composite was aged for 24 h at room temperature, centrifuged at 4000 rpm for 10 min, and dried at 80 °C.

SPC was prepared by treating preformed MPC with sodium dodecyl sulfonate at a 1:1 modifier:MPC feed mass ratio, followed by repeated washing. The synthesis dose describes modifier addition. EDS maps qualitatively indicate C, O, Mg, and Al in selected imaging fields, and are interpreted alongside XPS and XRD. The fitted C-C/C=C/C-H fractions were 48.7%, 53.0%, and 54.3% for PC, MPC, and SPC, respectively. Quantitative retained sulfonate, LDH mass loading, and column-operation leaching require separate measurements. Sulfonate-related S-O vibrations may overlap with C-O and mineral bands; sodium dodecyl sulfonate contains an alkyl C-S bond and a sulfonate group, rather than C=S.

Published Mg-Al LDH reflections at approximately 11.5°, 22.9°, 34.6°, and 39.0° have been assigned to (003), (006), (012), and (015), respectively, for Cu Kα radiation [29]. These published assignments provide phase-indexing context for interpretation of the composite diffractograms.

Nitrogen-adsorption results from Pan et al. (2024, 2025) provide context for changes in pore accessibility after LDH deposition and organic treatment [19; 20]. Their materials differ in feedstock and LDH composition from the present tobacco-stalk/Mg-Al system. The pore volumes in Table S2 are water-filled packed-bed void volumes, distinct from mass-specific material pore volumes determined by gas adsorption [8].

Text S3 Column packing and stage compositions

Quartz sand (595-841 μm) was cleaned sequentially with concentrated NaOH, concentrated HCl, and 30% H_2O_2, with each treatment lasting at least 24 h. The sand was rinsed to near-neutral pH, dried overnight at 105 °C, and heated at 850 °C for 8 h. PC, MPC, and SPC were ground and passed through a 120-mesh sieve (nominal aperture 0.125 mm).

Each amended column contained 0.70 g of PC, MPC, or SPC and 69.30 g of quartz sand, giving a dry biochar:sand mass ratio of 1:99, 1 wt% biochar, and a total dry packing mass of 70.00 g. The unamended control (CK) contained 70.00 g of quartz sand. The actual packed-bed height was 10 cm and the internal diameter was 2.6 cm. The medium was wet-packed in 2-cm increments while keeping the liquid level above the packing surface and gently tapping the column wall. Nylon screens (200 mesh) were installed at both ends. Columns were operated in upward flow at 1.0 mL min^{-1}.

No visible biochar particles were observed in the effluent, and no macroscopic layering or enrichment was observed in the packed bed after the experiments. These observations are consistent with bed stability at the visible scale.

The measured pore volumes, V_p, were 26.7, 26.4, 26.6, and 26.3 mL for CK, PC, MPC, and SPC, respectively (Table S2). Nominal hydraulic residence time was calculated as V_p/Q. The geometric bed volume was 53.09 cm^3, based on the 10-cm packed height and 2.6-cm internal diameter. Porosity was calculated as V_p/V_{bed}, and dry bulk density as total dry packing mass divided by V_{bed}. A_t Q = 1.0 mL min^{-1}, nominal residence times were 26.7, 26.4, 26.6, and 26.3 min; the corresponding 3-PV stages lasted 80.1, 79.2, 79.8, and 78.9 min, and 10-PV stages lasted 267.0, 264.0, 266.0, and 263.0 min.

$$\tau = \frac{V_p}{Q}\ ;\quad V_{bed} = \frac{\pi d^2 L}{4}\ ;\quad \theta = \frac{V_p}{V_{bed}}$$

All column working solutions contained 10 mM Tris-HCl at pH 7.5 and 25 °C. Baseline experiments used no added NaCl or $CaCl_2$. NaCl and $CaCl_2$ were examined in separate series at 0, 5, 25, and 50 mM. The same buffer and electrolyte composition was maintained during 10 PV of equilibration, 3 PV of DNA injection, and 3 PV of DNA-free forward elution. Only the injection solution contained calf-thymus DNA (25 mg L^{-1}) or pBR322 (1.06×10^{10} plasmid copies L^{-1}). The 0-mM treatment denotes no added test salt; the Tris-HCl background remained present.

HA and FA were prepared separately by dissolving 10 mg in 400 mL of the corresponding background solution and passing the solution through a 0.45-μm membrane. The nominal HA or FA concentration was 25 mg L^{-1} of material, rather than a dissolved-organic-carbon concentration.

The DOM experiments used 10 mM Tris-HCl containing 25 mM $CaCl_2$. Columns were preconditioned with 3 PV of HA or FA solution, rinsed with 10 PV of DNA-free background solution, and then supplied with 3 PV of DNA influent and 3 PV of DNA-free forward-elution solution. HA or FA was externally added during preconditioning only. Buffer and $CaCl_2$ concentrations remained constant throughout. Matched Controls followed the same sequence with DNA-free background solution in place of HA or FA solution. Table S3 summarizes the supplied solutions at each stage.

Solutions were adjusted to pH 7.5 at 25 °C with dilute HCl or NaOH. Solution pH was measured using an AB15 pH meter (Fisher Scientific, USA). Ionic strength is calculated from solution composition using the equation below.

$$I = \frac{1}{2}\sum_i c_i z_i^2$$

In the ionic-strength equation above, c_i and z_i are the molar concentration and charge number of ion i. Assuming complete dissociation, added NaCl contributes $I_{salt} = c$ and added $CaCl_2$ contributes $I_{salt} = 3c$. Thus, the 0, 5, 25, and 50 mM NaCl treatments contribute 0, 5, 25, and 50 mM, and the corresponding $CaCl_2$ treatments contribute 0, 15, 75, and 150 mM (Figure S5). The 25 mM $CaCl_2$ DOM background contributes 75 mM. Total ionic strength additionally includes charged buffer species, counterions, pH-adjustment reagents, and DNA-stock constituents. EDTA was not deliberately added to the transport-stage recipe. The reported added-salt contributions exclude DNA-stock constituents.

Text S4 qPCR assay and copy-number calculations

The plasmid-borne bla gene was the sole qPCR target. The 81-bp assay uses forward primer 5′-CTACGATACGGGAGGGCTTA-3′ and reverse primer 5′-ATAAATCTGGAGCCGGTGAG-3′ (Table S4) [3]. qPCR detection used a LightCycler 2.0 system (Roche) and LightCycler FastStart DNA Master SYBR Green I. Results are expressed as amplifiable bla copies; detection of the target sequence is distinct from demonstrating an intact or biologically active plasmid.

qPCR was performed in a total volume of 20 µL containing 2 µL of 10× FastStart DNA Master SYBR Green I mix, 1 µL of each primer (0.5 µM final each), 1.6 µL of $MgCl_2$ supplement (3 mM total $MgCl_2$), 2 µL of template, and 12.4 µL of PCR-grade water [17] . The thermal program consisted of 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s, 62 °C for 10 s, and 72 °C for 10 s, with fluorescence acquisition after extension and melting-curve analysis over 70-95 °C.

Calibration comprised seven tenfold standard levels spanning 10^1-10^7 copies per reaction, with three wells per level. Regression of all 21 C_q values against $\log_{10}$(copies per reaction) gave a slope of −3.352619, an intercept of 38.644286, $R^2 = 0.99949$, and an amplification efficiency of 98.73% (Table S5). Plasmid-standard copy numbers were calculated from DNA mass using the 4361-bp reference length and an approximate dsDNA molar mass of 660 g mol^{-1} bp^{-1}. The influence of standard molecular form on DNA quantification has been described previously [18].

$$N = \frac{mN_A}{L \times 660}$$

In the copy-number conversion, m is DNA mass in g, NA is Avogadro's constant, and L is DNA length in bp. The calibration model is $C_q = s \log_{10}(N_r) + b$, where N_r denotes copies per reaction, s = −3.352619, and b = 38.644286. Amplification efficiency is calculated from the fitted slope as E (%) = $100(10^{-1/s} - 1)$.

$$C_q = s\log_{10}(N) + b;\quad E = 100[10^{\frac{-1}{s}} - 1]$$

Low-copy validation used 1, 2, 5, and 10 copies per reaction, with 20 replicate wells per level. Positive rates were 45%, 75%, 100%, and 100%, respectively. The LOD criterion was at least 95% positive reactions; the LOQ criterion was a back-calculated concentration CV below 35% at or above the LOD. Both limits were 5 copies per reaction. A_t this level, all 20 reactions were positive, the CV was 16.99%, and mean back-calculated recovery was 110.24%. With a 2-µL template, the undiluted sample-equivalent limit is 2.5×10^6 copies L^{-1}; for a fivefold dilution, it is 1.25×10^7 copies L^{-1}. The validation run included three no-template controls and one processing blank, all without amplification. Three positive controls at 10^4 copies per reaction gave C_q values of 25.21, 25.27, and 25.31. Analytical wells are distinct from independent column replicates.

Target copies per reaction are calculated from the fitted standard curve. Technical-replicate C_q values are converted separately to copies before their arithmetic mean, $\bar{N}_r$, is calculated. For direct analysis of diluted effluent, V_t denotes template volume in µL and D the cumulative dilution factor. Concentration in the original effluent is $10^6\bar{N}rD/V_t$ copies L^{-1}. With V_t = 2 µL, the conversion is $C_{eff} = 5 \times 10^5\bar{N}rD$. Where extraction or concentration is used, V_e and V_t denote the final extract and template volumes, respectively, both in µL; V_s is the original effluent volume processed, in L. In that equation, D denotes subsequent analytical dilution of the extract before qPCR.

$$C_q = s\log_{10}N_r + b; N_r = 10^{\frac{C_q - b}{s}}$$

$$C_{eff} = \frac{\overline{N_r}}{V_t} \times D \times 10^6$$

$$C_{eff} = \overline{N_r} \times D \times \frac{V_e}{V_t} \times \frac{1}{V_s}$$

Matrix inhibition was assessed by a fivefold dilution (one volume of effluent plus four volumes of PCR-grade water; D = 5) and paired matrix-spike analysis. Relative matrix-spike recovery was calculated as $100(N_{spiked} - N_{unspiked})/N_{buffer}$, where N_{spiked} and $N_{unspiked}$ are the target-copy amounts in equal-volume spiked and unspiked sample aliquots, respectively, and N_{buffer} is the response to the same spike in target-free, matrix-free buffer. Dilution-corrected concentrations were evaluated within the validated quantification range.

$$R_{matrix}(\%) = 100 \times \frac{N_{spiked} - N_{unspiked}}{N_{buffer}}$$

Text S5 UV measurement and matrix interference

Bulk eDNA was measured at 260 nm using a UV-9000S spectrophotometer. Effluent fractions were collected every 5 min. DNA-free column-effluent blanks matched to the packing material, DOM treatment, and solution chemistry were measured, and their background absorbance was subtracted. The calibration expression below relates DNA concentration to blank-corrected absorbance.

$$C_{DNA} = \frac{A - A_{blank} - b}{a} D$$

In this expression, A is sample absorbance at 260 nm, A_{blank} is the matched DNA-free column-blank absorbance, a and b are the calibration slope and intercept against concentration (mg L^{-1}), and D is the sample dilution factor.

Matched column-blank subtraction accounts for additive absorbance from residual DOM and biochar-derived solutes. The UV result is an operational bulk-eDNA estimate after background correction. Background subtraction and analytical recovery are distinct sources of uncertainty, and UV absorbance is distinct from DNA integrity.

Text S6 Breakthrough integration and recovery

The integration interval begins at DNA injection (0 PV), covers the 3-PV DNA input pulse, and ends after 3 PV of DNA-free forward elution (6 PV). Equilibration and DOM preconditioning are excluded; later backwashing is evaluated separately. For each independent column, introduced amount Ain is the integral of influent concentration multiplied by flow over the injection interval. For a constant influent, Ain = C0Vinj = 3C0Vp. Forward-effluent amount Aout is the sum of fraction concentration Ci multiplied by its collected volume ΔVi within 0-6 PV, including a partial final fraction. All volumes are expressed in L; concentrations are mg L^{-1} for eDNA and target copies L^{-1} for eARGs.

$$A_{in} = \int_0^{t_{inj}} C_{in}(t)Q_{in}(t)\,dt = C_0V_{inj} = 3C_0V_p$$

$$A_{out} = \int_0^{t_{end}} C_{out}(t)Q_{out}(t)\,dt = \sum_{i=1}^{m} C_i\Delta V_i$$

$$R\,(\%) = 100 \times \frac{A_{out}}{A_{in}}$$

$$R = \frac{100}{3}\int_0^6 \frac{C}{C_0}dp\ ;\quad p = \frac{Qt}{V_p}$$

Effluent mass recovery or target-copy recovery is 100Aout/Ain. The normalized integral uses p = Qt/Vp measured from the start of DNA injection and a constant-concentration 3-PV input. The input-output difference Ain - Aout is termed unrecovered from the forward effluent. It can include DNA remaining in pore water or associated with the packing and apparatus, together with contributions from incomplete elution, analytical recovery, and degradation. Table S6 gives calculated input amounts for the packing parameters in Table S2; each independent column is evaluated using its own injection volume and influent concentration. Subsequent backwash release is reported separately.

Text S7 Backwashing and release integration

Backwashing used SPC columns after DNA loading and forward elution under the reported 25 mM $CaCl_2$ condition. The same columns received Tris-HCl first and EDTA subsequently. Figure 3c,d therefore shows successive stages of the column history, rather than independent eluent-treatment groups. The preceding general sequence comprised 10 PV of equilibration, a 3-PV DNA input pulse, and 3 PV of DNA-free forward elution. Table S7 summarizes the sequential design.

$$A_{backwash} = \sum_{j=1}^{m} C_jV_j$$

Cumulative release for each Tris-HCl or EDTA stage is defined as the sum of backwash-fraction concentration multiplied by collected volume. Concentration in mg L^{-1} and volume in L give released eDNA in mg. Release relative to the original input is 100Abackwash/A_{in}, and total backwash release is the sum of the two stage amounts. The forward-effluent deficit remains a separate operational quantity. Peak concentrations and integrated release describe different aspects of the backwashing profiles.

Text S8 Independent replication and statistical reporting

Three independently prepared and packed columns were used for each tested condition (n = 3). Successive effluent fractions were temporal observations; repeated UV readings and qPCR wells were analytical measurements within a column. Integrated recovery was calculated separately for each column, then summarized as mean ± sample SD. The DOM dataset contains CK, PC, MPC, and SPC under Control, HA, and FA, with three independent columns per combination. Table S8 gives R_1, R_2, R_3, and mean ± SD separately for eDNA mass recovery and eARG target-copy recovery. SD is expressed in percentage points.

$$\overline{R} = \frac{\sum_{k=1}^{n} R_k}{n}; \quad SD = \sqrt{\frac{\sum_{k=1}^{n} (R_k - \overline{R})^2}{n - 1}}$$

The ANOVA response variable was independent-column integrated forward-effluent recovery (%) over 0-6 PV. For the DOM dataset, Control, HA, and FA were compared within each packing, separately for eDNA and eARGs. Fractions and different endpoints were not pooled to increase n. The DOM Control belongs to its conditioning experiment and is distinct from the separate baseline dataset in Figure S1a. Electrolyte-series plateau concentrations are descriptive endpoints and are distinct from these integrated DOM recovery statistics.

The DOM records were analyzed using one-way ANOVA followed by Duncan's multiple-range test ($P < 0.05$). Residual normality was assessed by Shapiro–Wilk tests and variance homogeneity by median-centered Levene tests. No significant departure was detected in the eight DOM comparisons (all diagnostic $P > 0.05$), with the limited power at n = 3 per group considered. Overall ANOVA F(2,6), P values, and diagnostic outputs are given in Table S9. Overall P ranged from 0.0000589 to 0.001792. Duncan comparisons distinguished all three DOM treatments within each packing and endpoint (HA > FA > Control; $P < 0.05$). Figure S6 uses distinct letters Control = a, HA = b, and FA = c; letters identify groups rather than numerical rank.

Table S1. DNA sources, preparation, molecular identity, purity, and storage.

Property	Calf-thymus DNA	pBR322
Commercial source	Sigma-Aldrich D4764	New England Biolabs N3033
Preparation	Commercial genomic dsDNA; gentle overnight dissolution and dilution	Commercial purified plasmid DNA; dilution
Molecular identity	Unsheared genomic dsDNA model	Circular dsDNA; reference sequence length 4361 bp
Resistance determinant	Not the eARG preparation	bla and tetracycline-resistance determinant; qPCR target: bla
Initial working concentration	25 mg L^{-1}	1.06×10^{10} plasmid copies L^{-1}
Manufacturer-reported size	>20 kb (manufacturer specification)	4361 bp (reference sequence length [26])
Measured A_{260}/A_{280}	1.87	1.8
Measured A_{260}/A_{230}	2.0-2.2	2.0
Prepared-stock storage	Aliquots at −20 °C	Aliquots at −20 °C

Table S2. Packing masses, pore volumes, calculated porosities, nominal residence times, and dry bulk densities.

Packing	Biochar (g)	Sand (g)	Total (g)	Vp (mL)	Porosity	τ (min)	ρb (g cm^{-3})
CK	0	70.00	70.00	26.7	0.503	26.7	1.318
PC	0.7	69.3	70.00	26.4	0.497	26.4	1.318
MPC	0.7	69.3	70.00	26.6	0.501	26.6	1.318
SPC	0.7	69.3	70.00	26.3	0.495	26.3	1.318

Table S3. Stage-specific solution composition

Experiment and stage	Volume	Tris-HCl	Added electrolyte	DNA	HA / FA
Baseline equilibration	10 PV	10 mM	No test salt	None	None
Baseline DNA injection	3 PV	10 mM	No test salt	eDNA 25 mg L^{-1} or pBR322 1.06 × 10^{10} copies L^{-1}	None
Baseline forward elution	3 PV	10 mM	No test salt	None	None
Electrolyte equilibration	10 PV	10 mM	NaCl or $CaCl_2$: 0, 5, 25, 50 mM	None	None
Electrolyte DNA injection	3 PV	10 mM	Same selected test salt	As above	None
Electrolyte forward elution	3 PV	10 mM	Same selected test salt	None	None
DOM preconditioning	3 PV	10 mM	25 mM $CaCl_2$	None	25 mg L^{-1} HA or FA
DOM rinse	10 PV	10 mM	25 mM $CaCl_2$	None	No new addition
DOM DNA injection	3 PV	10 mM	25 mM $CaCl_2$	As above	No new addition
DOM forward elution	3 PV	10 mM	25 mM $CaCl_2$	None	No new addition

Table S4. Primers and amplicon length for the bla qPCR assay.

Target	Forward primer (5′-3′)	Reverse primer (5′-3′)	Amplicon (bp)
bla / pBR322	CTACGATACGGGAGGGCTTA	ATAAATCTGGAGCCGGTGAG	81

Table S5. qPCR calibration, sensitivity, and validation-control results.

Parameter	Validation result
Target and amplicon	bla; 81 bp
Instrument / detection	LightCycler 2.0 (Roche); SYBR Green I
Calibration levels	10^1-10^7 copies per reaction; seven levels × three wells
Slope s / intercept b	-3.352619 / 38.644286
R^2	0.99949
Amplification efficiency	98.73%
Low-copy validation	1, 2, 5, 10 copies per reaction; 20 wells per level
Positive rates	45%, 75%, 100%, 100%, respectively
LOD	5 copies per reaction; 20/20 positive
LOQ	5 copies per reaction; CV 16.99%; recovery 110.24%
Undiluted 2-µL sample-equivalent limit	2.5×10^6 copies L^{-1}
Fivefold-diluted sample-equivalent limit	1.25×10^7 copies L^{-1}
No-template / processing blanks	Three no-template controls and one processing blank: no amplification
Positive controls (10^4 copies per reaction)	Cq: 25.21, 25.27, 25.31

Table S6. Calculated DNA inputs for the pore volumes.

Packing	Vp (mL)	Injected volume (mL)	eDNA input (mg)	pBR322 input ($\times 10^8$ copies)
CK	26.7	80.1	2.0025	8.4906
PC	26.4	79.2	1.9800	8.3952
MPC	26.6	79.8	1.9950	8.4588
SPC	26.3	78.9	1.9725	8.3634

Table S7. Sequential backwashing design.

Item	Recorded design
Material	SPC
Preceding transport	DNA loading and forward elution as in Section 2.4
Column assignment	Same SPC columns used for both stages
Eluent order	Tris-HCl, followed by EDTA
Reported experimental condition	25 mM $CaCl_2$
Quantitative release	Stage-specific concentration-volume integration (Text S7)

Table S8. Individual-column effluent recoveries in the DOM experiments

(a) eDNA mass recovery

Packing	Treatment	R_1	R_2	R_3	Mean ± SD
CK	Control	73.1	72.4	75.2	73.57 ± 1.46
CK	HA	84.4	81.6	85.3	83.77 ± 1.93
CK	FA	79.8	76.5	79.9	78.73 ± 1.93
PC	Control	61.6	59.74	63.54	61.63 ± 1.90
PC	HA	76.7	78.5	74.1	76.43 ± 2.21
PC	FA	69.6	71.3	66.1	69.00 ± 2.65
MPC	Control	43.6	41.2	46.3	43.70 ± 2.55
MPC	HA	60.6	56.1	62.9	59.87 ± 3.46
MPC	FA	52.4	49.8	54.5	52.23 ± 2.35
SPC	Control	25.4	21.8	27.3	24.83 ± 2.79
SPC	HA	42.6	46.52	39.5	42.87 ± 3.52
SPC	FA	34.7	37.85	30.5	34.35 ± 3.69

(b) eARG target-copy recovery

Packing	Treatment	R_1	R_2	R_3	Mean ± SD
CK	Control	66.7	68.6	64.3	66.53 ± 2.15
CK	HA	77.8	75.4	78.9	77.37 ± 1.79
CK	FA	72.5	72.3	73.5	72.77 ± 0.64
PC	Control	55.586	51.3	57.3	54.73 ± 3.09
PC	HA	70.8	68.3	73.5	70.87 ± 2.60
PC	FA	62.5	65.7	60.4	62.87 ± 2.67
MPC	Control	38.3	40.6	34.3	37.73 ± 3.19
MPC	HA	54.5	52.3	57.6	54.80 ± 2.66
MPC	FA	46	44.6	49.3	46.63 ± 2.41
SPC	Control	21.6	18.6	19.5	19.90 ± 1.54
SPC	HA	37.7	35.71	36.8	36.74 ± 1.00
SPC	FA	30.5	31.5	27.1	29.70 ± 2.31

Table S9. One-way ANOVA and assumption diagnostics for the DOM recovery data.

Packing	Endpoint	F (2,6)	ANOVA P	Shapiro P	Levene P
CK	eDNA	24.41	0.0013109	0.122	0.969
CK	eARG	32.21	0.0006185	0.738	0.500
PC	eDNA	31.76	0.0006431	0.278	0.923
PC	eARG	25.00	0.0012297	0.239	0.980
MPC	eDNA	24.51	0.0012967	0.440	0.876
MPC	eARG	28.42	0.0008707	0.301	0.933
SPC	eDNA	21.70	0.0017916	0.272	0.925
SPC	eARG	74.11	0.0000589	0.740	0.734

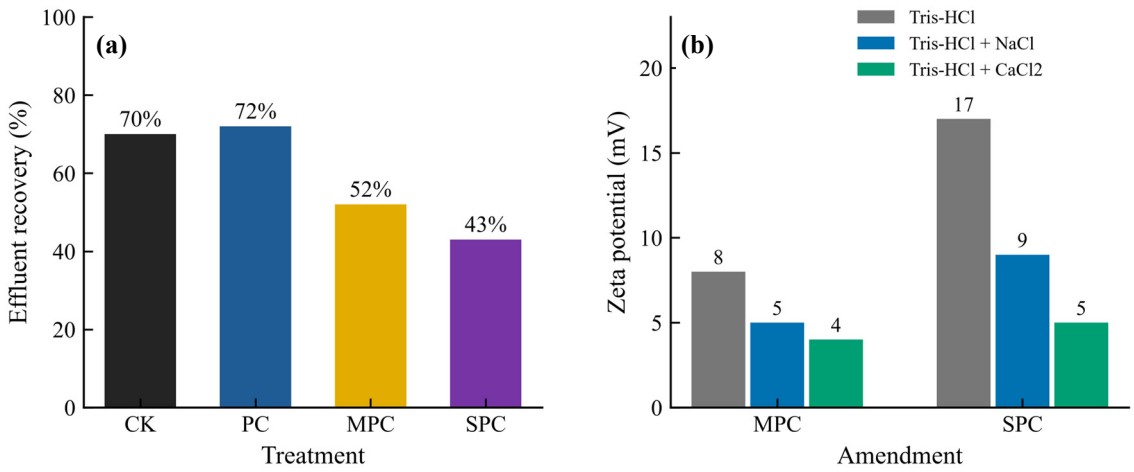


Figure S1. Baseline eDNA effluent mass recovery for CK, PC, MPC, and SPC (a), and zeta potentials of MPC and SPC in different solution backgrounds (b).

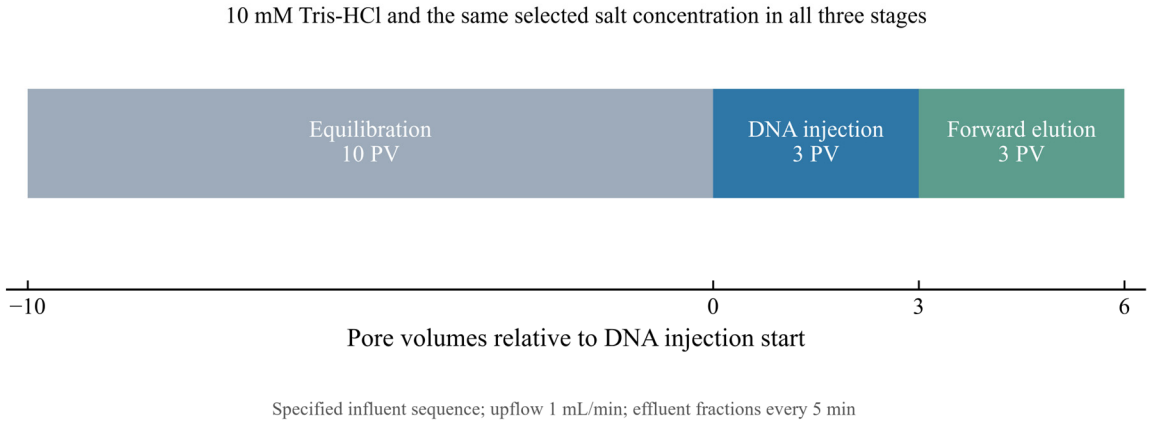


Figure S2. Influent sequence for baseline and electrolyte experiments: 10 PV equilibration, 3 PV DNA injection, and 3 PV DNA-free forward elution. The origin is the start of DNA injection. Each stage contains 10 mM Tris-HCl and the same selected added-salt concentration. DNA injection uses 25 mg L^{-1} calf-thymus DNA or 1.06×10^{10} plasmid copies L^{-1} pBR322 in separate runs.

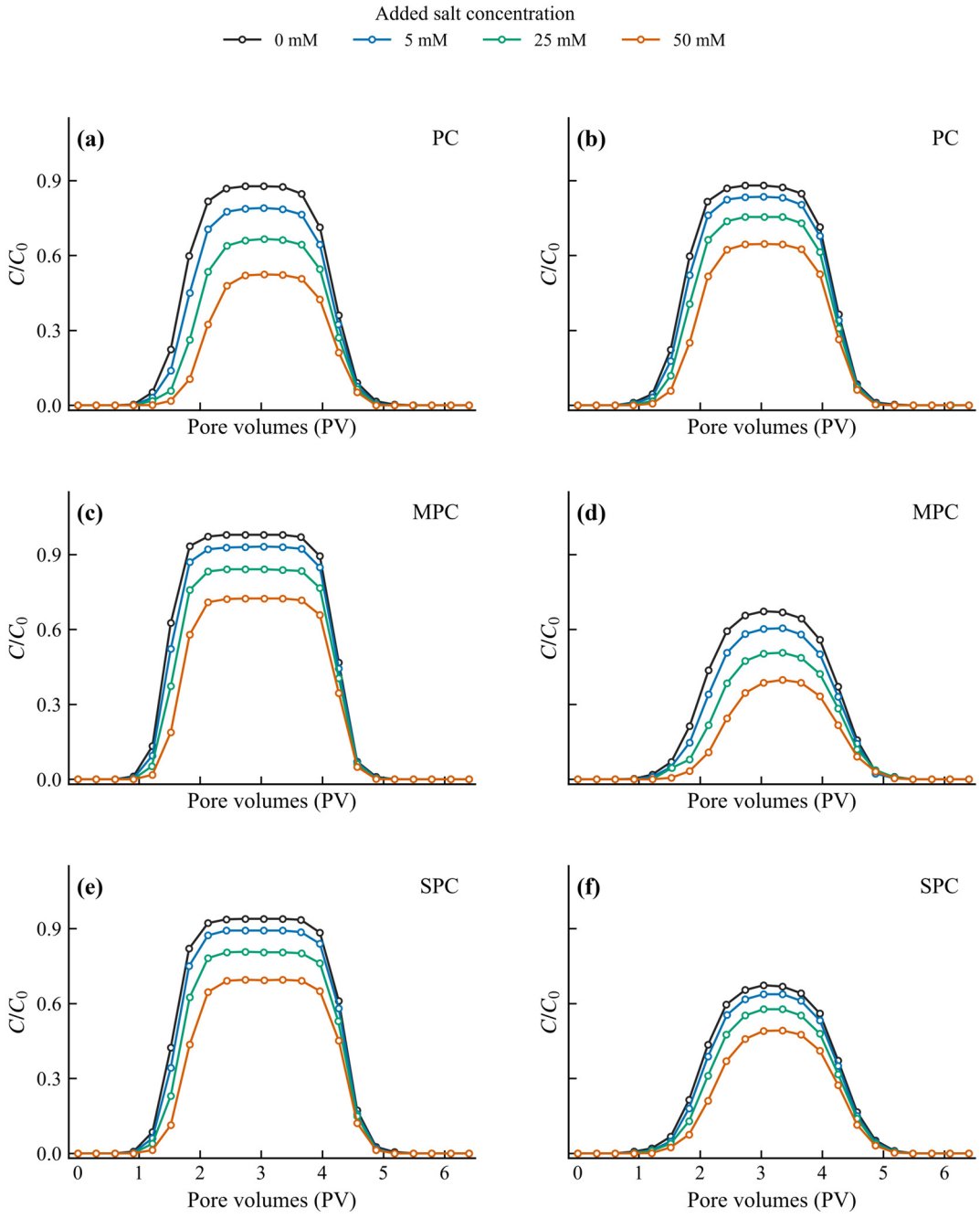


Figure S3. Effects of added NaCl (a,c,e) and $CaCl_2$ (b,d,f) concentrations (0, 5, 25, and 50 mM) on eDNA breakthrough curves in PC (a,b), MPC (c,d), and SPC (e,f) columns. C/C_0 denotes normalized effluent concentration. Symbols represent means from three independently packed columns, and error bars indicate sample standard deviation (n = 3). The NaCl and $CaCl_2$ series were conducted separately using newly packed columns, each with its own independently measured zero-added-salt control. The 0 mM condition contained 10 mM Tris-HCl at pH 7.5 without added NaCl or $CaCl_2$. The same electrolyte background was maintained during equilibration, DNA injection, and forward elution.

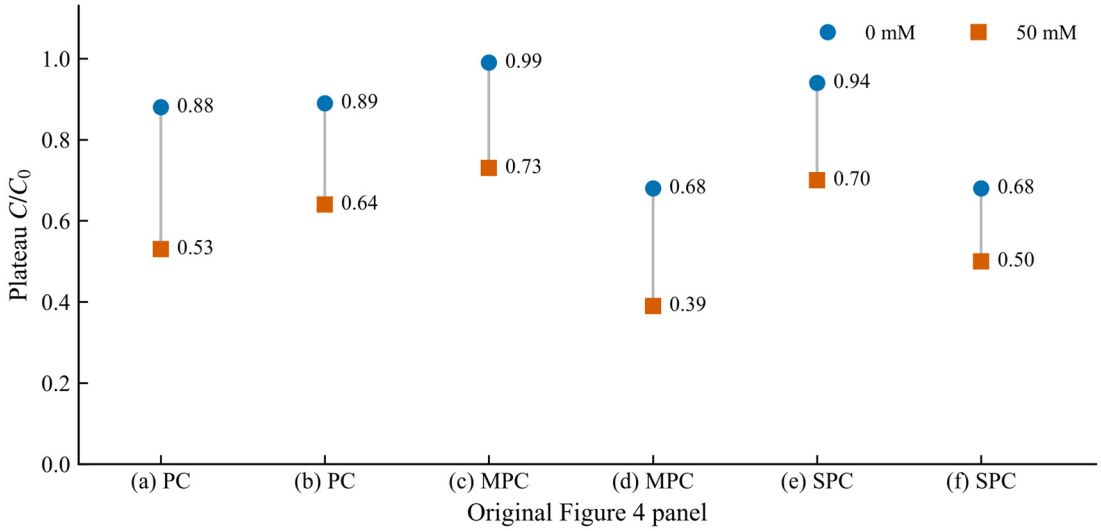
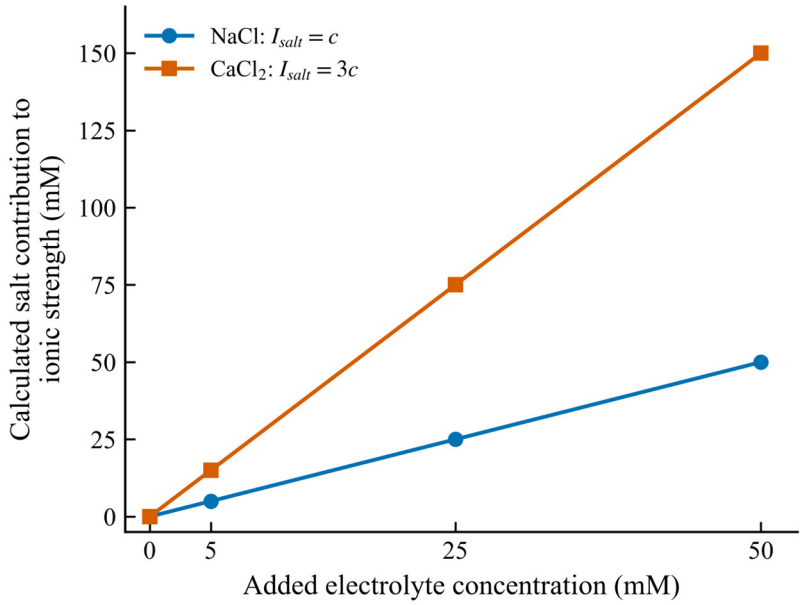


Figure S4. Approximate eDNA plateau C/C_0 at 0 and 50 mM added salt for PC (a,b), MPC (c,d), and SPC (e,f), summarized from Figure 4. Panels a,c,e correspond to NaCl and b,d,f to $CaCl_2$. Values describe dimensionless plateau concentrations, rather than integrated recovery percentages; no SD or significance test is inferred from these approximate values.



Calculated contribution of added salts; not measured total ionic strength

Figure S5. Calculated nominal contributions of added NaCl and $CaCl_2$ to ionic strength. For salt concentration c, Isalt = c for NaCl and Isalt = 3c for $CaCl_2$. Contributions from Tris-HCl, DNA-stock constituents and pH-adjustment ions are excluded. Values are calculated from composition and are not measured total ionic strength.

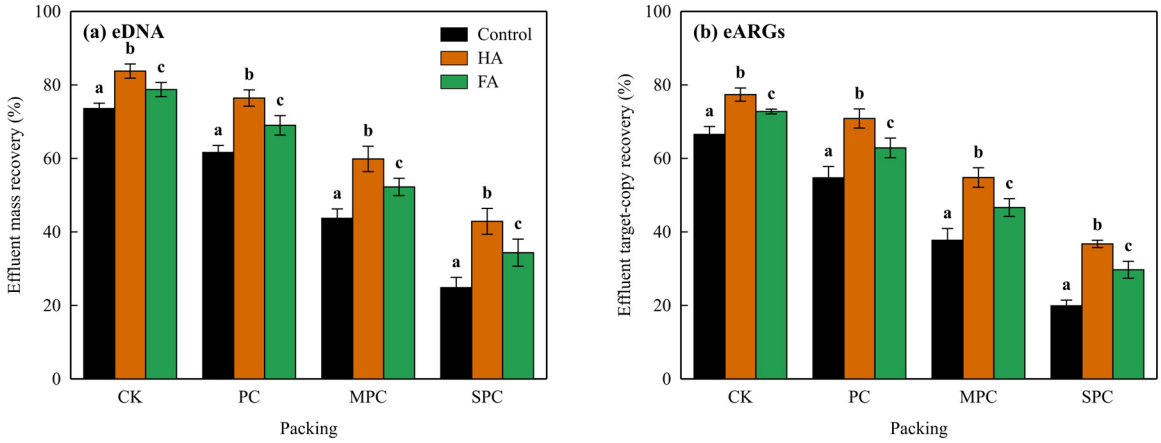


Figure S6. Effluent recovery of (a) eDNA and (b) eARGs in CK, PC, MPC, and SPC under Control, HA, and FA treatments. Bars show means ± SD from three independently prepared and packed columns per condition (n = 3). Different lowercase letters indicate significant differences among Control, HA, and FA within the same packing and endpoint (one-way ANOVA followed by Duncan's multiple-range test, $P < 0.05$). Individual-column recoveries and statistical outputs are reported in Tables S8 and S9.