Electronic Supplementary Information for:

Surface and Subsurface Attenuation of Trenbolone Acetate Metabolites and Manure-Derived Constituents in Irrigation Runoff on Agro-Ecosystems

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Contents: 11 Pages, 5 Figures

Surface Transport- We evaluated the transport of manure-derived constituents on VFS on four different dates (May 24, May 31, June 8, and July 3, 2012). In general, all experiments were conducted as described within in the main text under "Surface Transport". In short, each VFS (strips #1, 2, 3) was saturated with irrigation water for 30 minutes to promote runoff during the experiment and to minimize the leachate volume required to generate runoff. After 30 minutes, leachate was applied from a 1500 L tank simultaneously to the top of each VFS. On May 24, samples were collected at t = 0, 30, and 60 minutes. In order to promote mass transfer and increase concentrations in the leachate on the experiment conducted on May 31, the manure was broken apart and thoroughly mixed within the tank. The leachate was then applied simultaneously to each the VFS, but because of the high solids content, samples were very difficult to filter and were composited for convenience. The composite samples was first centrifuged then filtered as described within the main text. From both experiments, only TBA metabolites were analyzed. On June 8, samples were collected at t = 0 (i.e., first flush), 15, 30, 45, and 60 minutes while on 3 July, samples were collected at t = 15, 30, 45, 60, and 75 minutes. Otherwise, the later experiments were conducted identically. For these experiments, TBA metabolites, total ammonia, nitrate, nitrite, orthophosphate, and DOC were analyzed and for the last experiment, total coliforms and *E. coli* were measured but from composite samples only.

Colloidal and Soil Partitioning- We calculated the dissolved fraction of steroid in water (F_D) from physical and chemical properties of the soil based on the following mass balance:

$$F_D = \frac{C_W V_W}{C_W V_W + C_S M_S + C_C M_C} = \frac{1}{1 + K_D r + K_{OC} DOC} \qquad (1)$$

where C_w, C_s, and C_c are the TBA metabolite concentration in water, on soil, and on organic carbon, respectively, V_w is the volume of water within a sample , and M_s and M_c are the mass of soil and the mass of organic carbon within a sample, respectively. In practice, the right hand side of equation 1 represents the reduced form of the mass balance and is more suitable for solving for the dissolved fraction of steroids. It is derived from the soil water partitioning coefficient (K_D, L/kg), the organic carbon partitioning coefficient (K_{OC}, L/kg), the dissolved organic carbon concentration (DOC, kg/L), and the solids to water ratio (r, unitless), which calculated as follows:

$$r = \frac{\rho_b(1-\varphi)}{\varphi} \qquad (2)$$

where ρ_b is the bulk density (kg/L) and φ is the soil porosity (unitless). The steroid fraction sorbed to soil (F_S) and organic carbon (F_C) can be described as follows:

$$F_S = \frac{C_S M_S}{C_w V_w + C_S M_S + C_C M_C} = \frac{K_D r}{1 + K_D r + K_{OC} DOC} \qquad (3)$$

$$F_C = \frac{C_C M_C}{C_w V_w + C_S M_S + C_C M_C} = \frac{K_{OC} DOC}{1 + K_D r + K_{OC} DOC} \qquad (4)$$

In addition to the equilibrium mass fraction sorbed, we estimated the actual mass sorbed. Using a simplified mass balance, the mass of 17α-TBOH that partitions to the soil within the subsurface can be described mathematically as the product of the flow, concentration, and time as follows:

$$\int_0^t (QC_o)dt - \int_0^t (QC)dt = M_s \qquad (5)$$

where Q is the flow (L/min) entering and leaving the system, C_o and C are the influent (tank) and discharge concentrations (ng/L), respectively, and M_s is the mass of steroid sorbed to soil (ng). Equation 5 can be simplified to the following:

$$QC_o \int_0^t \left(1 - \frac{C}{C_o}\right) dt = M_s \qquad (6)$$

Within the trench, the relative discharge concentration (C/C_o, unitless) of 17α-TBOH varied with time and increased linearly throughout the duration of the experiment. Fitting a linear regression model to the breakthrough data for 17α-TBOH (Figure 2b, Results and Discussion, Subsurface Transport), C/C$_o$ can be described as follows:

$$\frac{C}{C_o} = 0.0043t + 0.31, R^2 = 0.98 \qquad (7)$$

Using equation 7, complete breakthrough (i.e., C/C$_o$ = 1) is expected to occur at 160 minutes. In other words, the 17α-TBOH sorption capacity of the soil is expected to be exhausted at 160 minutes under experimental conditions. Substituting equation 7 into equation 6 and integrating yields the following:

$$M_s = QC_o(-0.0022t^2 + 0.69t) \qquad (8)$$

The equilibrium partitioning coefficient (K_D, L/kg) for 17α-TBOH and soil can be estimated from equation 8 as follows:

$$K_D = \frac{C_s}{C_o} = \frac{QC_o(-0.0022t^2+0.69t)}{V\rho_b C_o} \qquad (9)$$

Where C_s is concentration of 17α-TBOH on soil (ng/kg), C_o is the leachate concentration (36 ng/L), V is the volume of soil in contact with water (L), t is time (evaluated at 160 minutes), and ρ_b is the bulk density of the soil (1.5 kg/L). In equation 9, the only unknown variables are Q and V, and depending on the control volume used, different values can be used for both parameters to the estimate the value of K_D for the experimental system.

We applied 3 different scenarios to equation 9. In scenario A, we assumed that only the water that discharged into the tray (width = 100 cm) within the AB horizon (Q = 0.63 L/min) passed through the upslope soil column (width = 100 cm, length = 400, depth = 30 cm; V = 1,200 L). The remaining water (14.37 L/m) immediately exited the soil column. While the soil volume was well defined, the latter assumption likely underestimates the flow through the soil column resulting in a low estimate of K_D. Within the soil trench, the subsurface wetting-front spread at least 8.5 m laterally over the 4 m travel distance from the point of application upslope to the point of discharge. Assuming that the flow was approximately uniform across this 8.5 m distance, the estimated discharge of the wetting front within the AB horizon is ~5.4 L/min. For scenario B, we assumed that only this portion of the flow (Q = 5.4 L/min) passed through the soil column (wetted area = 19.5 m^2, depth = 0.3 m; V = 5,900 L), and the remaining water (9.6 L/m) immediately exited the soil column. While the estimated wetted soil volume is reasonable, the latter assumption likely underestimates the flow through the soil column resulting in a low estimate of K_D, albeit to a lesser extent than in scenario A. Finally, in scenario C, we assumed that all of the applied water (Q = 15 L/min) passed through the observed wetted zone (V =5,900 L). Because some flow is expected to be lost through infiltration, this assumption likely over estimates the flow through the soil and therefore, K_D. Evaluating equation 9 at t = 160 minutes estimates the mass of 17α-TBOH that can be sorbed to soil once complete breakthrough occurs under the experimental conditions.

For scenarios A, B, and C, the estimated K_D values for 17α-TBOH are 0.02, 0.03, and 0.09 L/kg (Table 1 of main text). Based on soil characteristics, the calculated K_D is 10 L/kg (Table 2 of main text) suggesting that the sorption capacity of the soil during the experiment was <1% of the equilibrium partitioning capacity of the soil (based on the ratio of the system K_D [calculated above] and the K_D derived from soil characteristics). This indicates that non-equilibrium processes dominate the transport of 17α-TBOH in the subsurface over short time scales. Although several assumptions were made during this analysis, each was reasonable, and despite the fact that values used for flow and soil mass varied by factors of 20 and 5, respectively, the partitioning estimates varied by a factor of <5. Even with this variability, the conclusion from each estimate is the same: far less mass partitioned to soil than was predicted by equilibrium partitioning.

A similar approach can be taken for the ammonia, orthophosphate, and DOC. The linear increase in concentration was modeled through time as follows:

$$\frac{C}{C_o}(NH_3) = 0.0069t + 0.39, R^2 = 0.89 \qquad (10)$$

$$\frac{C}{C_o}(PO_4) = 0.0072t + 0.50, R^2 = 0.91 \qquad (11)$$

$$\frac{C}{C_o}(DOC) = 0.0035t + 0.89, R^2 = 0.99 \qquad (12)$$

Based on equations 10-12, the time to complete breakthrough of ammonia, orthophosphate, and DOC is ~90, 70, and 30 minutes, respectively. By substituting these equations into equation 6 and integrating (as was done for 17α-TBOH), the estimated increase in soil concentration of each constituent once the sorption capacity is exhausted is 0.07-0.32 mg NH_3-N/kg, 0.02-0.07 mg PO_4-P/kg, and 0.04-0.18 mg C/kg.

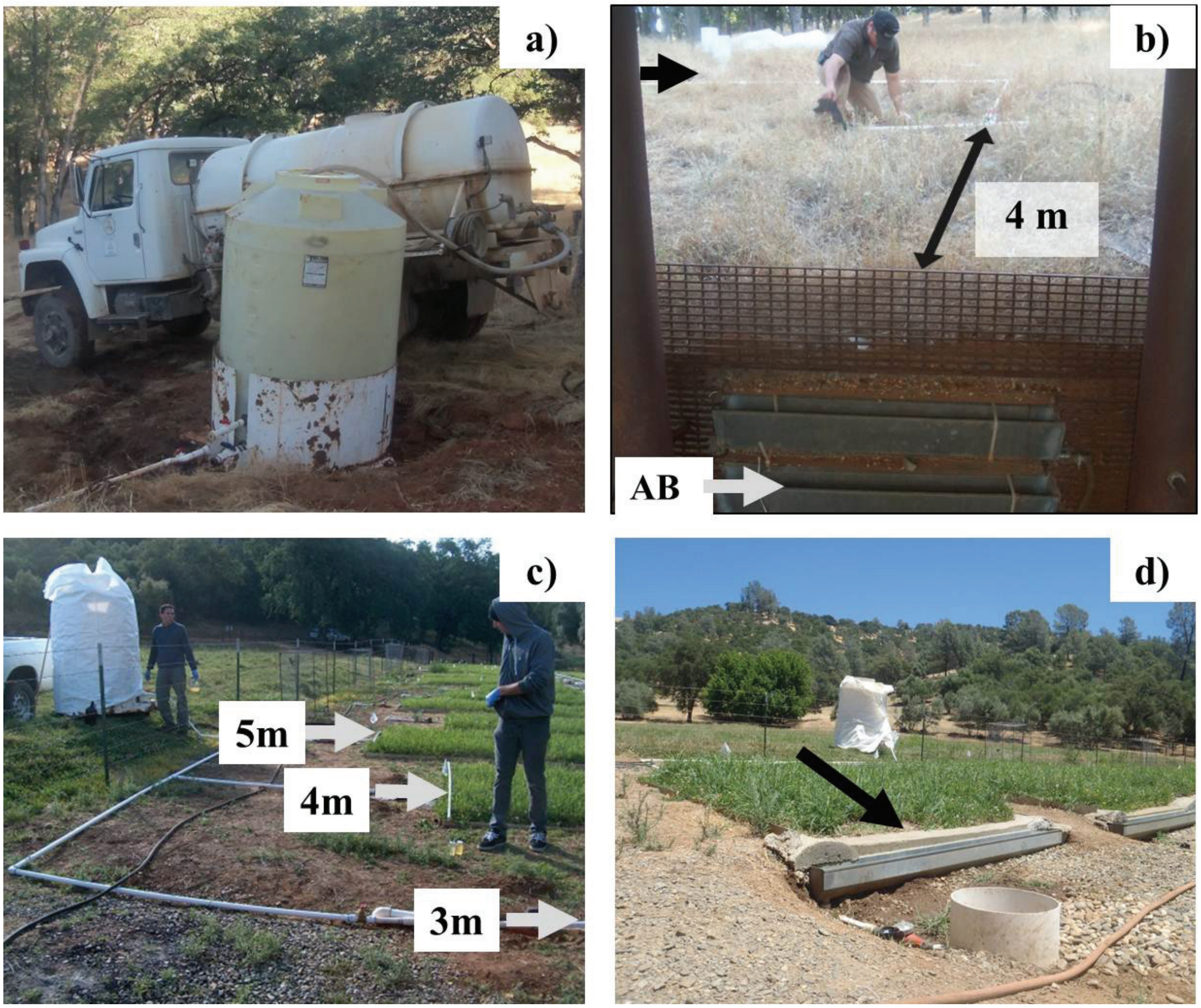

Figure S1. Leachate was made onsite during (a, b) subsurface and (c, d) surface transport experiments. Leachate was piped to (b) 4 m upslope the soil trench and to (c) 3, 4, and 5 m vegetative filter strips (#s 1, 2, and 3, respectively). Subsurface discharge was collected from the (b) AB soil horizon (30 cm below the surface) while surface runoff was collected from the (d) outfall of the vegetative filter strips, which was constructed of concrete and aluminum trays.

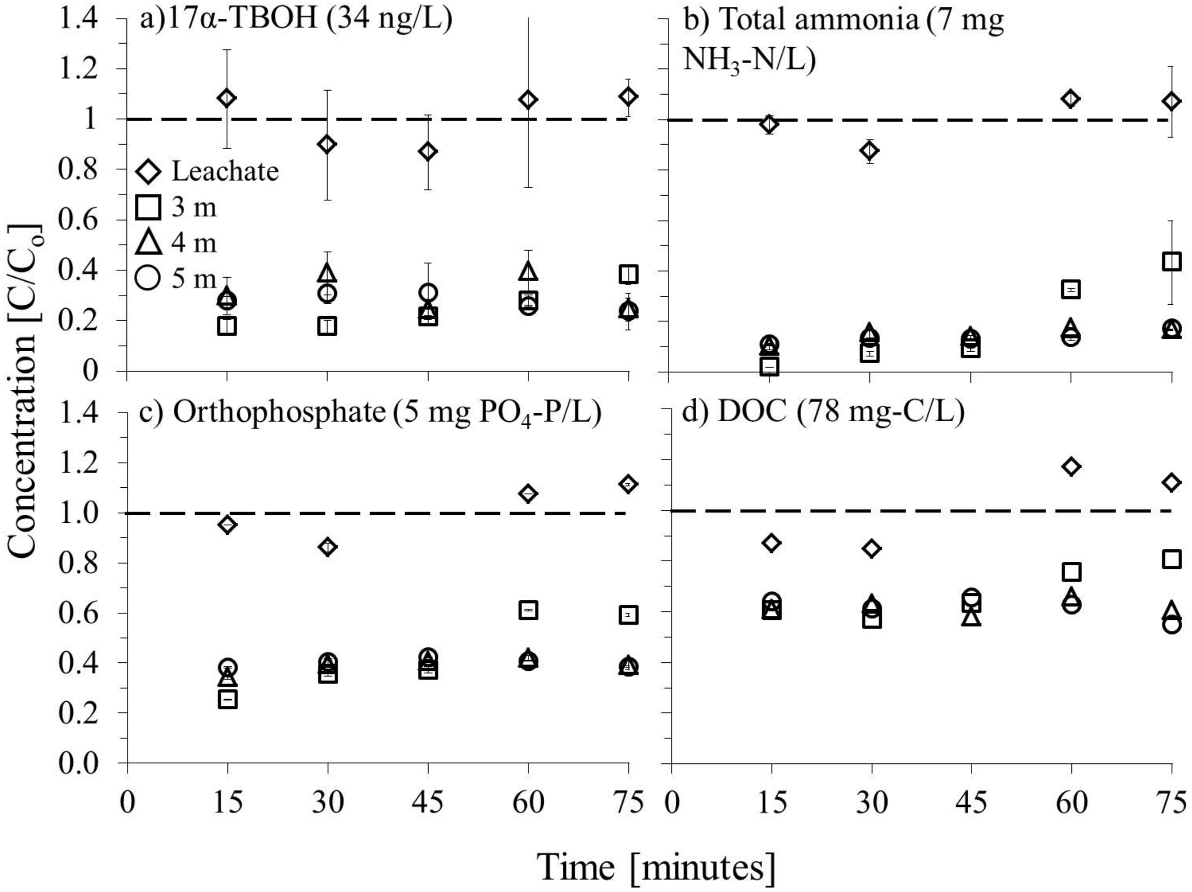

Figure S2. Relative concentrations of (a) 17α-TBOH, (b) total ammonia, (c) orthophosphate, and (d) dissolved organic carbon (DOC) in leachate and runoff from 3, 4, and 5 m vegetative filter strips. All concentrations were normalized to the average leachate concentration, which is provided in each panel. Data were collected on July 3, 2012. Error bars represent 95% confidence intervals (n = 3) and are not present for DOC.

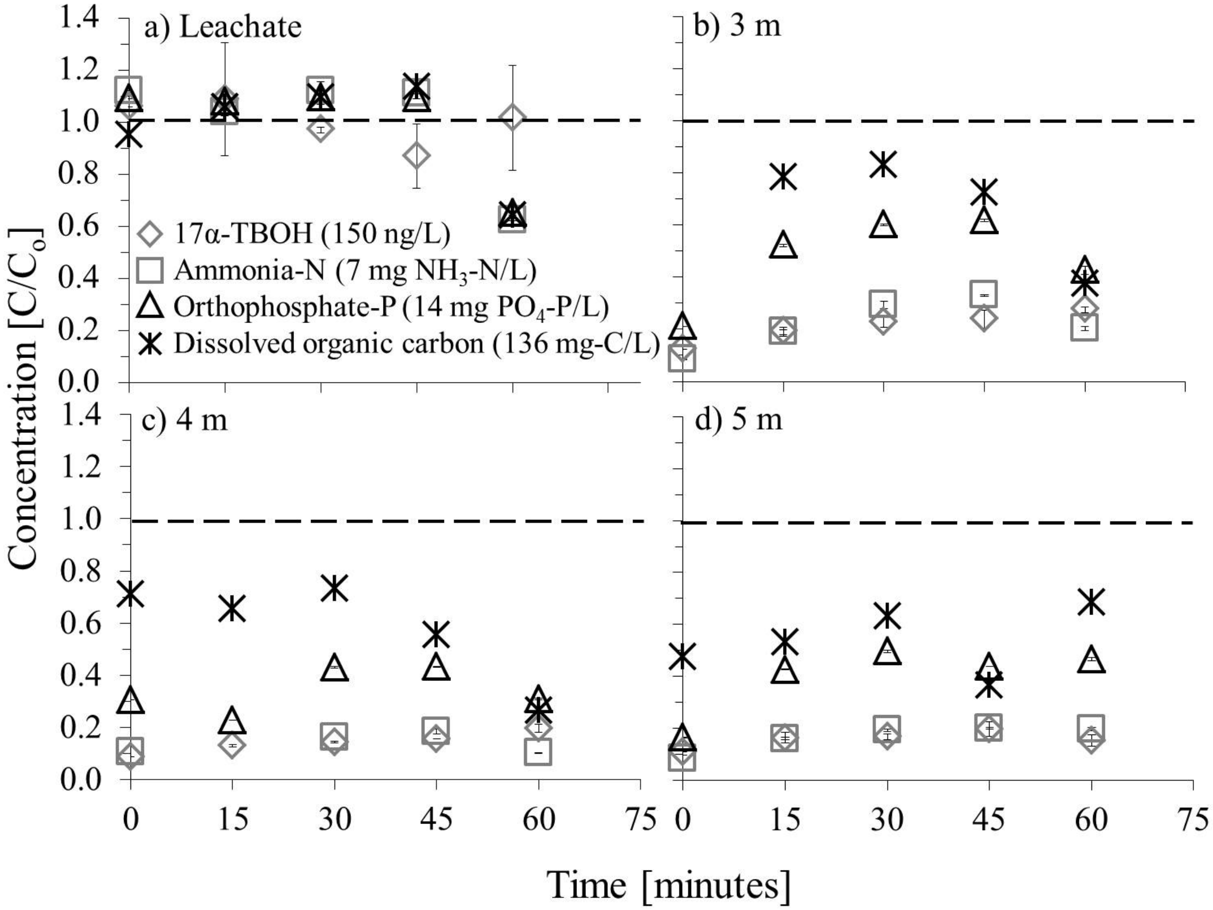

Figure S3. Relative concentrations of manure derived contaminants in (a) leachate, and surface runoff from (b) 3 m, (c) 4 m, and (d) 5 m vegetative filter strips. All concentrations were normalized to the average leachate concentration, which is provided for each constituent in the legend. Values reported at t = 0 represent first flush concentrations. Data was collected on June 8, 2012. Error bars represent 95% confidence intervals (n = 3) and are not present for DOC.

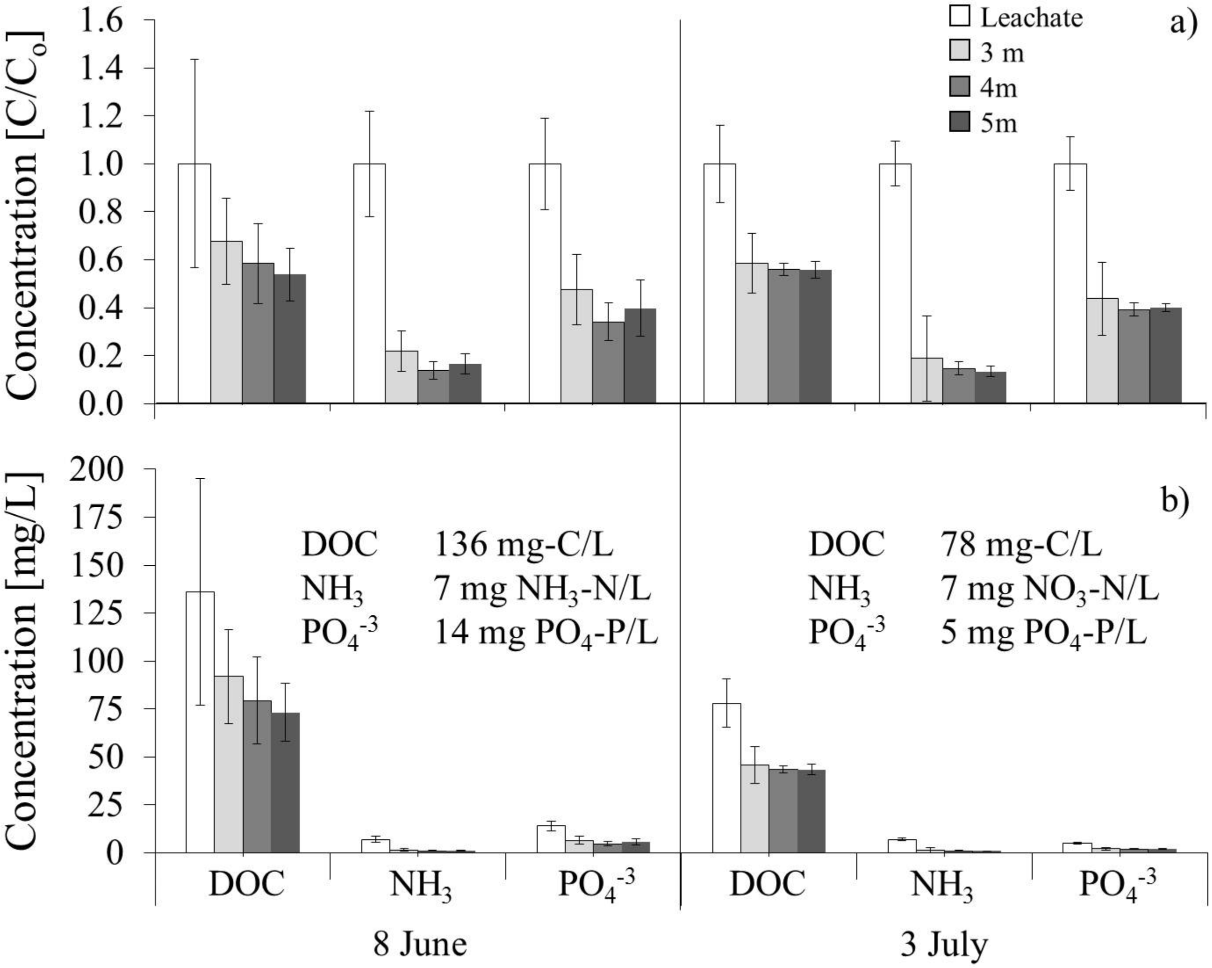

Figure S4. Average (a) normalized runoff concentrations (i.e., C/C$_o$) and (b) actual runoff concentrations of dissolved organic carbon (DOC), total ammonia (NH$_3$), and orthophosphate (PO$_4^{-3}$) during two vegetative filter strip (VFS) runoff experiments conducted in June 8 and 3 July, 2012. Relative concentrations were normalized based on the average contaminant concentration within leachate (see legend). Error bars represent 95% confidence intervals (n = 6-30).

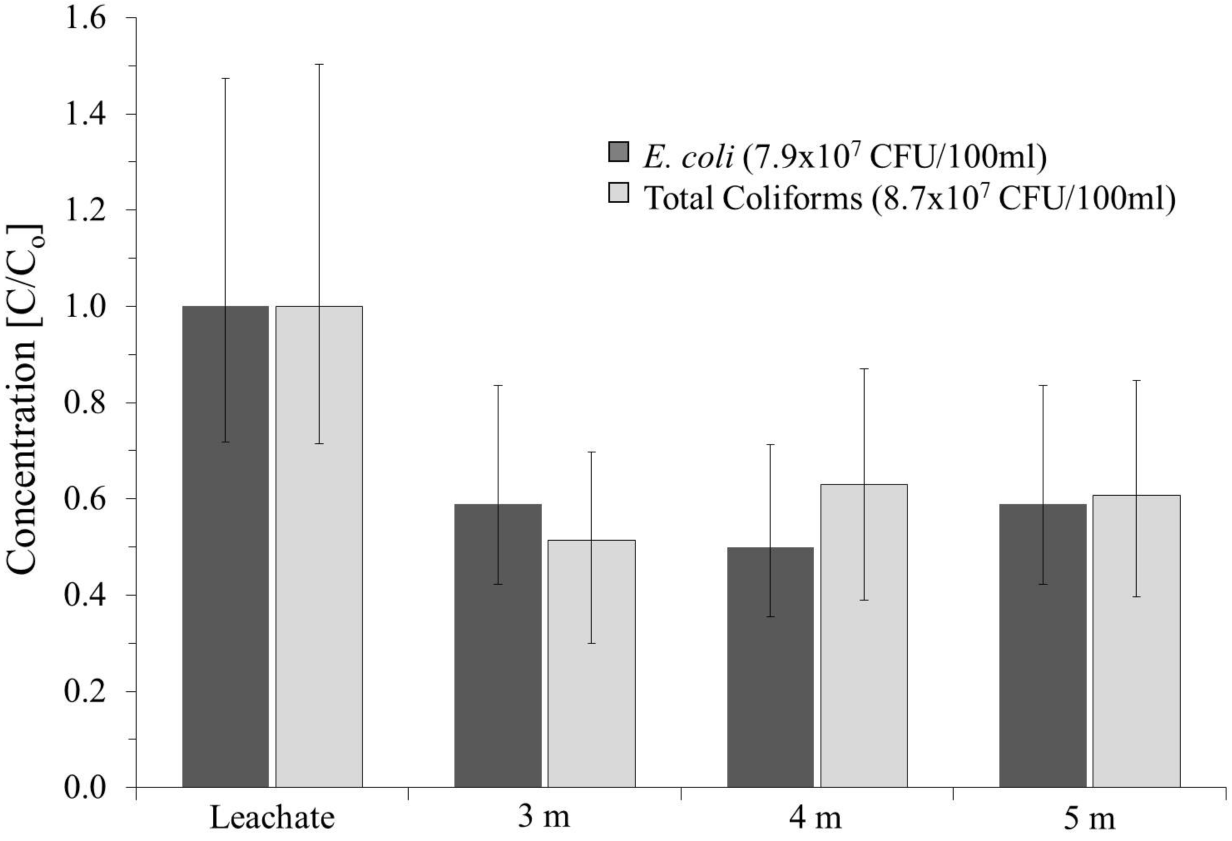

Figure S5. Concentrations of total coliforms and *E. coli* in leachate and runoff from 3, 4, and 5 m vegetative filter strips. All concentrations were normalized to the average leachate concentration provided in legend. Data was collected on July 3, 2012. Large error bars are due to inherent uncertainties within the analytical method. Error bars represent 95% confidence intervals.