

Salinization alters fluxes of bioreactive elements from stream ecosystems across land use

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Abstract

There has been increased salinization of fresh water over decades due to the use of road salt deicers, wastewater discharges, saltwater inundation, human-accelerated weathering, and groundwater irrigation. Although salinization can mobilize bioreactive elements (carbon, nitrogen, phosphorus, sulfur) chemically *via* ion exchange and/or biologically *via* influencing of microbial activity, its effects on biogeochemical cycles are still not well understood. We investigated potential impacts of increased salinization on fluxes of bioreactive elements from stream ecosystems (sediments and riparian soils) to overlying stream water and evaluated the implications of percent urban land use on salinization effects. Two-day incubations of sediments and soils with stream and deionized water across 3 salt levels were conducted at 8 routine monitoring stations across a land-use gradient at the Baltimore Ecosystem Study Long-Term Ecological Research (LTER) site in the Chesapeake Bay watershed. Results indicated: (1) salinization typically increased sediment releases of labile dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), total dissolved Kjeldahl nitrogen (TKN) (ammonium + ammonia + dissolved organic nitrogen), and sediment transformations of nitrate; (2) salinization generally decreased DOC aromaticity and fluxes of soluble reactive phosphorus (SRP) from both sediments and soils; (3) the effects of increased salinization on sediment releases of DOC and TKN and DOC quality increased with percentage watershed urbanization. Biogeochemical responses to salinization varied between sediments and riparian soils in releases of DOC and DIC, and nitrate transformations. The differential responses of

1 riparian soils and sediments to increased salinization were likely due to differences in organic
2 matter source and composition. Our results suggest that short-term increases in salinization
3 can cause releases of considerable amounts of labile organic carbon and nitrogen from stream
4 substrates and organic transformations of nitrogen and phosphorus in urban watersheds.
5 Given that salinization of fresh water will increase in the future due to human activities,
6 impacts on carbon and nutrient mobilization and water quality should be expected.

7

8 **1 Introduction**

9 Salt concentrations in freshwaters are rapidly increasing at a regional scale in the United
10 States and worldwide (e.g., Nielsen et al., 2003; Kaushal et al., 2005; Rengasamy, 2006;
11 Findlay and Kelly, 2011; Steele and Aitkenhead-Peterson, 2011; Kaushal et al., 2014a; Corsi
12 et al., 2015). Most of the increased salinization can typically be attributed to road salt deicers
13 and other industrial uses, wastewater discharges, groundwater irrigation, saltwater inundation
14 caused by sea-level rise, and human-accelerated weathering (e.g., Findlay and Kelly, 2011;
15 Aitkenhead-Peterson et al., 2009; Ardón et al., 2013; Kaushal et al., 2013). Increased
16 salinization can have important environmental consequences for drinking water supplies,
17 freshwater biodiversity, degradation of soils and groundwater, degradation of vehicles and
18 infrastructure, and mobilization of inorganic and organic contaminants (Nielsen et al., 2003;
19 Kaushal et al., 2005; Findlay and Kelly, 2011; Corsi et al., 2015). Moreover, salinization is
20 difficult if not impossible to reverse, thus, remediation is unlikely. Recent studies have further
21 shown that increased salinization can influence biogeochemical cycles of bioreactive elements
22 such as carbon and nitrogen (Green et al., 2008; Green and Cresser, 2008; Green et al., 2009a,
23 b; Compton and Church, 2011; Lancaster, 2012; Steele and Aitkenhead-Peterson, 2013) as
24 well as phosphorus and sulfur (Nielsen et al., 2003; Kulp et al., 2007; Compton and Church,
25 2011; Kim and Koretsky, 2011, 2013). Chemically, salinization affects mobilization of these
26 bioreactive elements through its direct influences on ion exchange and sorption capacity of
27 sediments/soils (e.g., for ammonium and SRP), as well as *via* indirect effects due to changes
28 in pH and sodium-induced dispersion (e.g., for DOC) (Nielsen et al., 2003; Green et al., 2008;
29 Compton and Church, 2011; Ardón et al., 2013). Biologically, salinization can be a stressor to
30 some microorganisms in fresh water but may also enhance the activities of other
31 microorganisms due to nutrient releases (Kulp et al., 2007; Srividya et al., 2009; Kim and
32 Koretsky, 2011, 2013). Evidence is accumulating that increased salinization is an important

1 process during the urban evolution of watersheds globally from decades to centuries (Kaushal
2 et al., 2014a; Kaushal et al., 2015), and salinization has significant ecosystem effects over
3 broader spatial and temporal scales (e.g., Findlay and Kelly, 2011; Kaushal and Belt, 2012;
4 Corsi et al., 2015).

5 Although there has been increasing research, more work needs to be done regarding the
6 effects of increased salinization on coupled biogeochemical cycles. Prior studies have
7 commonly investigated the effects of salinization on fluxes and transformations of individual
8 bioreactive elements (e.g., Green et al., 2008, 2009; Green and Cresser, 2008; Green et al.,
9 2009a, b; Compton and Church, 2011; Kim and Koretsky, 2011; Lancaster, 2012; Steele and
10 Aitkenhead-Peterson, 2011). However, biogeochemical cycles of bioreactive elements are
11 generally linked in sedimentary diagenesis (Middelburg and Levin, 2009), and
12 transformations of nitrogen, phosphorus and sulfur are highly dependent on availability of
13 organic carbon (Duan and Kaushal, 2013). For example, organic carbon provides an energy
14 source for microbes responsible for biogeochemical transformations (e.g., Newcomer et al.,
15 2012), and decomposition of organic carbon can facilitate certain redox reactions of
16 bioreactive elements including denitrification, iron reduction and release of soluble reactive
17 phosphorus, and sulfate reduction (Sobczak et al., 2003; Middelburg and Levin, 2009). For
18 organic carbon, prior studies generally investigated the effects of salinization on bulk
19 concentrations of dissolved organic carbon (DOC). However, DOC within aquatic systems
20 consists of not a single compound but a broad suite of organic molecules of varied origin and
21 composition, which may respond differently to salinization. Until recently, relatively little
22 work has been done to improve our conceptual understanding of the effects of salinization on
23 coupled biogeochemical cycles.

24 Previous studies have shown freshwater salt concentrations vary across land use, with highest
25 concentrations of salt occurring in urban watersheds (e.g., Kaushal et al., 2005). Green et al.
26 (e.g., 2008, 2009) reported that soils in urban watersheds that have already experienced
27 exposure to road salting were less responsive to salinization in DOC release than unexposed
28 soils in rural areas. On the other hand, urbanization may increase stream sediment organic
29 matter (Duan and Kaushal, 2013) *via* algal and wastewater inputs (Daniel et al., 2002;
30 Kaushal et al., 2014a), and influence other physical, chemical and biological characteristics of
31 stream ecosystems (Paul and Meyer, 2001). Despite these two competing impacts of
32 urbanization, biogeochemical impacts of salinization across watershed land use are still less

1 recognized. Moreover, most current studies regarding the effects of salinization focus on soils
2 or anaerobic lake sediments, and very little work has been done to examine stream sediments
3 that may be exposed to high salt concentrations under more aerobic conditions. It is known
4 that stream sediments and soils differ in particle size, structure, and organic matter
5 composition and sources (e.g., Hedges and Oades, 1997). Thus, insights learned from
6 studying the biogeochemical effects of salinization in soils may not always directly apply to
7 stream sediments.

8 Our primary objective was to investigate the effects of increased salinization on potential
9 fluxes (release or retention) of bioreactive elements (carbon, nitrogen, phosphorus, sulfur)
10 from stream ecosystems, and how the effects of salinization change with watershed land use
11 and/or stream substrates (sediments and riparian soils). Sediments and riparian soils collected
12 from sites across a rural-urban land use gradient were incubated in salt solutions to mimic the
13 effects of runoff with high levels of road salt deicers, and the changes in water chemistry were
14 monitored as a function of salt concentrations and land use. Three hypotheses were tested: 1)
15 the effect of salinization on soil leaching and sediment retention/release of bioreactive
16 elements change with watershed urbanization, 2) retention/release of nitrogen, phosphorus,
17 and sulfur in response to salinization can be abiotically and/or biologically coupled with
18 carbon biogeochemistry, and 3) salinization effects on release/transformation of bioreactive
19 elements vary between stream sediments and riparian soils. We expect in my experiment
20 considerable release of organic carbon and coupled transformations with nitrogen, phosphorus
21 and sulfur as salinity increases, as well as increased salinization effects on biogeochemical
22 fluxes with watershed urbanization due to more carbon availability. An improved
23 understanding of the effects of increased salinization on release/retention of bioreactive
24 elements can contribute to our understanding of urban drivers of changes in water quality,
25 microbial communities and ecosystem functions (Kaushal and Belt, 2012; Kaushal et al.,
26 2014a), and improve water quality by benefitting our assessment and management of salt use.

27

28 **2 Methods**

29 **2.1 Site Description**

30 Surface sediments from stream channels and top soil in riparian zones were collected from 8
31 long-term monitoring sites across a rural-urban land use gradient. All 8 sites are routinely

1 sampled as part of the US National Science Foundation supported Baltimore Ecosystem Study
2 (BES) Long-Term Ecological Research (LTER) site. Land use varies from forest to low-
3 density residential, agricultural, to suburban and urban (Table 1). The main focal watershed of
4 the BES LTER site is the Gwynns Falls, a 17,150 ha watershed in the Piedmont
5 physiographic province that drains into the northwest branch of the Patapsco River that flows
6 into the Chesapeake Bay (Fig. 1). The Gwynns Falls sites traverse a rural/suburban to urban
7 gradient from Glyndon (GFGL), Gwynnbrook (GFGB), Villa Nova (GFVN) to Carroll Park
8 (GFCP) (Table 1). An agricultural stream (MCDN) is a small tributary to the Gwynns Falls
9 draining a watershed dominated by row crop agriculture (corn, soybeans), while Dead Run
10 (DRKR) is an urbanized tributary of the Gwynns Falls between GFVN and GFCP. Samples
11 were also taken from a small urban tributary to the Gwynns Falls (GFGR), approximately 700
12 m above GFCP, which is highly contaminated with sewage (Kaushal et al., 2011). Baisman
13 Run (BARN) is a low-density residential watershed located in the nearby Gunpowder Falls
14 watershed that drains primarily forest land cover (Table 1; Fig. 1). The BES LTER site
15 provides access to extensive background information and long-term monitoring of major
16 anions, nutrients, and carbon concentrations and fluxes in streams (www.beslter.org;
17 Groffman et al., 2004; Kaushal et al., 2008, 2011). Previous work has shown that watersheds
18 of the BES LTER site can have considerably elevated levels of chloride and sodium (Kaushal
19 et al., 2005; Kaushal and Belt, 2012).

20 **2.2 Sample Collection and Processing**

21 Stream water, sediments, and soils for laboratory salinization experiments were collected on
22 March 8, 2013, one day before a snow storm in the Baltimore-Washington D.C. metropolitan
23 region. Three litres of stream water were collected at each of the 8 sites for the experiments
24 and water quality analyses. The surface sediments and top soils (approximately 15 cm) were
25 collected at these same sites with a shovel. Sediment samples were taken simultaneously
26 along 4 cross-sections perpendicular to stream flow within 50 m of the primary sampling site
27 (Duan and Kaushal, 2013). Along each stream cross section, surface sediments at three sites
28 (left, middle and right) were collected. All sediments collected at these sites were well-mixed
29 to make a composite sample. Soil samples from the riparian zone were also collected similar
30 to sediment samples. Because the sites GFCP and GFGR were located very close to each
31 other, only one composite soil sample was collected to represent these two sites. So,
32 laboratory salinization experiments with soils were conducted at 7 rather than 8 sites. The

1 sediment and soil samples were transferred to glass jars, and placed immediately into a cooler
2 and brought back to lab. In the lab, sediments were sieved through a 2-mm sieve, and the < 2
3 mm fractions of sediments and soils were homogenized for incubation experiments (e.g.,
4 plant roots were picked from soils and discarded). The homogenized sediments and soils were
5 sampled for determination of ash free dry weight (AFDW). In addition, approximately 100
6 mL aliquots of stream water were filtered through pre-combusted GF/F Whatman filters, and
7 the filtrates were used for water quality analyses. The filtrates were stored in a refrigerator for
8 analyses of optical properties and dissolved inorganic carbon (DIC) measurements. Another
9 aliquot was similarly filtered but frozen prior to analyses of dissolved organic carbon (DOC),
10 nutrients, and major anions. The remainder of the stream water, sediments, and soils were
11 temporarily stored at 2-4°C for 2 days prior to laboratory experiments.

12 **2.3 Laboratory Salinization Experiments**

13 For each laboratory salinization experiment, 60-g sub-samples of homogenised sieved
14 sediments (< 2mm) were inserted into a series of 125-ml glass flasks to cover the bottom of
15 the flasks, and 100 mL of unfiltered stream waters were carefully added with a pipette in
16 order to not disturb the sediments. In order to evaluate the potential effects of salinization,
17 pure NaCl salt (J.T.Baker) was amended to unfiltered stream water to obtain 3 concentration
18 levels (0 g Cl L⁻¹, 2 g Cl L⁻¹, and 4 g Cl L⁻¹). Molar concentrations of Na⁺ were assumed to be
19 the same as Cl⁻ because pure NaCl was used. These concentration levels were within the
20 range reported for salt concentrations in ambient stream water at the Baltimore LTER site
21 (Kaushal et al., 2005). All laboratory salinization experiments were conducted in duplicate
22 per study site to account for analytical variability during laboratory salinization experiments.
23 Simultaneously, streamwater samples without sediments were also incubated at the same 3
24 levels of salinization as sediment-free controls, in order to separate potential contributions of
25 sediments *vs.* stream water. The laboratory salinization experiments were conducted in the
26 dark in the lab with minor variations in temperature (19–22°C), and the flasks were gently
27 stirred for 2 days with a shaker table to simulate water movement in streams. During the 2-
28 day incubations, the flasks were loosely capped to avoid external contamination while
29 allowing for air entry. Laboratory salinization experiments for riparian soils were conducted
30 similar to stream sediments, except that 1) deionized (DI) water (rather than stream water)
31 was used for soil leaching, and 2) the samples were not stirred during the incubations
32 assuming much slower hydrologic flow rates during soil infiltration than those of a stream.

1 Deionized waters without soils were incubated at the 3 levels of salinization as soil-free
2 controls. This experimental design may have introduced potential artefacts such as no soil
3 infiltration, constant temperature and no exposure to sunlight, which could influence results.
4 However, previous studies investigating the potential impacts of salinization on soil
5 biogeochemistry have used similar approaches (e.g., Green et al. 2008, 2009a; Compton and
6 Church, 2011; Kim and Koretsky, 2013; Steele and Aitkenhead-Peterson, 2013). At the end of
7 the incubations, the incubation waters were filtered through pre-combusted GF/F Whatman
8 filters for water chemistry analyses.

9 **2.4 Chemical Analyses**

10 All filtrates were analyzed for major forms of bioreactive elements - nitrate, total dissolved
11 nitrogen (TDN), soluble reactive phosphorus (SRP), sulfate, DOC, dissolved inorganic carbon
12 (DIC), and optical properties of DOC (absorbance and fluorescence). DOC, TDN and DIC
13 concentrations were measured on a Shimadzu Total Organic Carbon Analyzer (TOC-V
14 CPH/CPN) (Duan and Kaushal 2013). Nitrate and sulfate concentrations were measured with
15 a Dionex ion chromatograph (ICS-1500, Dionex INC., USA), with an eluent of 3.5 mM of
16 Na_2CO_3 and 1.0 mM NaHCO_3 and a flow rate of 0.3 mL min^{-1} . Analyses of the water
17 samples showed that $\text{NO}_3^- \text{-N}/\text{NO}_2^- \text{-N}$ concentrations were almost entirely $\text{NO}_3^- \text{-N}$ (> 99%),
18 and we therefore refer to this fraction as $\text{NO}_3^- \text{-N}$ throughout this paper. SRP was measured on
19 an automated QuikChem 8500 Series 2 FIA System, using the ascorbic acid-molybdate blue
20 method (Murphy and Riley, 1962). Total Kjeldahl nitrogen (TKN), including dissolved
21 organic nitrogen, ammonia, and ammonium, was calculated by subtraction of nitrate-N from
22 TDN. Ultraviolet (UV) absorbance and fluorescence spectroscopy were used in
23 characterization of DOC composition and lability. Filtrates were scanned for absorbance from
24 200 nm to 800 nm with a Shimadzu UV-1800 Spectrophotometer. UV absorbance at 254 nm
25 was used to calculate specific UV absorbance (SUVA) by normalizing for DOC
26 concentration. SUVA is strongly correlated with percent aromaticity of organic matter as
27 determined by ^{13}C NMR (Weishaar et al. 2003), and thus can be a useful parameter for
28 estimating terrestrial organic carbon sources in aquatic systems. Fluorescence measurements
29 were made on a FluoroMax-4 Spectrofluorometer (Horiba Jobin Yvon, Edison NJ, USA)
30 using the method that was described previously by Duan and Kaushal (2013). A 1 cm quartz
31 cuvette with slit widths set to 5 nm was used. Excitation emission matrix scans (EEMs) were
32 obtained by collecting a series of emission wavelengths ranging from 300 to 600 nm (2 nm

1 increments) at excitation wavelengths ranging from 240 to 450 nm (5 nm increments). EEMs
2 data were corrected for instrument biases, inner filtering and scatter removal, and calibrated
3 values of fluorescence intensities at excitation/emission = 275 nm/340 nm and 350 nm/480
4 nm were recorded as protein-like and humic-like fluorophores (Coble, 1996; Stolpe et al.,
5 2010). Relative to the humic-like fluorophore, the intensity of the protein-like fluorophore is
6 generally higher in labile DOC sources (e.g., wastewater; Hudson et al., 2007) and positively
7 correlated with DOC bioavailability (Balcarczyk et al., 2009; Lønborg et al., 2010). Thus, the
8 ratio of the protein-like to the humic-like fluorophore (P/H) was calculated here as an index of
9 organic carbon lability.

10 Ash free dry weight (AFDW) of the sediment and soil samples was analyzed as an index of
11 organic matter content. Sediment and soil ash weights were calculated as the difference in
12 weights before and after combustion at 550°C for 4 hours (APHA 1998). Prior to combustion,
13 sediments were dried at 105°C for 4 hours to remove water. Ash free dry weights were
14 determined in triplicates.

15 **2.5 Data Analyses and Statistics**

16 Sediment fluxes were calculated as the net changes in the concentrations of DOC, nitrate,
17 SRP or sulfate during the two-day incubations. The values for nitrate and sulfate are presented
18 as nitrate-N and sulfate-S. The changes in the control flasks (with water only), occurring in
19 water without sediments or soils, were subtracted to obtain the fluxes that were released from
20 sediments or soils. Positive or negative values represent net release from sediments or
21 retention by sediments, respectively.

22 Effect of salinization on sediment/soil biogeochemical fluxes were examined by performing
23 linear regressions of these fluxes with salinity, using data from 6 salinization experimental
24 manipulations (3 salinity levels with duplicates). If the p-value was < 0.05 for the regression,
25 we assumed that there was a significant salinization effect. Otherwise, differences between
26 two adjacent salinization levels were tested using a t-test of two-samples assuming equal
27 variances. The slopes of above linear regressions with salinity, representing changes in
28 biogeochemical fluxes per unit salinity, were regressed with watershed impervious surface
29 cover (ISC) at the 8 study sites to examine changes in salinization effects across watershed
30 urbanization. Differences in ash free dry weight, biogeochemical fluxes or salinization effect
31 between sediments and soils were tested also using t-test of two-samples assuming equal

1 variances. Meanwhile, relationships between sediment/soil fluxes of DOC (or SUVA and
2 fluorescence indices representing DOC composition) and any of sediment/soil fluxes of DIC,
3 TKN, nitrate, and sulfate were examined to test the coupling of nitrogen, phosphorus and
4 sulfur with carbon biogeochemistry during salinization experiments. For linear relationships,
5 Spearman's correlation was used in cases where assumptions of normality were not met. Data
6 are reported in mean \pm standard error.

7

8 **3 Results**

9 **3.1 Water and sediment chemistry**

10 In stream waters that were used for laboratory salinization experiments, water chemistry
11 varied considerably (Table 2). In general, concentrations of chloride ion (Cl^-), sulfate-S (SO_4^{2-}
12 -S) and DOC, and protein-like to humic-like fluorophore (P/H) ratios of DOC in stream water
13 increased with watershed impervious surface cover (ISC) ($r^2 = 0.77-0.83$, $p < 0.05$, $n = 8$).
14 SUVA showed an opposite trend and decreased with watershed ISC ($r^2 = 0.79$, $n = 8$, $p <$
15 0.05). Cl^- concentrations also increased with ISC, but the coefficient was not significant ($r^2 =$
16 0.40 , $n = 8$, $p > 0.05$), and the highest value was not observed at the site GFGR with highest
17 ISC. Nitrate-N (NO_3^- -N) and SRP concentrations did not vary with watershed ISC, and the
18 highest concentrations occurred at the agricultural site (MCDN; Table 2).

19 Sediment ash free dry weight (AFDW) also displayed an increasing trend with ISC (from
20 0.61% to 1.90%) except one surprisingly high value (3.98%) observed at GFCP ($r^2 = 0.56$, $p <$
21 0.05 , $n = 7$). AFDW of the riparian soils (6.17-8.84%) were considerably higher than the
22 sediments ($p < 0.05$, t-test) but did not vary with watershed ISC (Table 2).

23 **3.2 Influence of salinization on C fluxes and DOC composition across land** 24 **use**

25 Sediments were consistently a net source of both DOC and DIC. Net DOC releases from
26 sediments consistently increased with increasing salinization (all positive slopes and
27 statistically significant in 7 out of 8 cases; $r^2 = 0.64-0.99$, $p < 0.05$) (Fig. 2a), and DOC
28 releases at 4 g Cl L^{-1} were 7.8 ± 1.9 times (mean \pm standard error, same below) higher than
29 those at 0 g Cl L^{-1} . Although salinization also increased the net releases of DIC (positive slope
30 with one exception at GFCP), the increases were generally not at a statistically significant

1 level ($p > 0.05$; Fig. 2b). Among the 7 sites that showed a DIC increase, the highest net DIC
2 releases occurred at 2 g Cl L^{-1} , and the values at 2 g Cl L^{-1} were generally higher than the
3 values at 0 g Cl L^{-1} (6 out of 7 cases; $p < 0.05$, t-test), although differences between 2 g Cl L^{-1}
4 and 4 g Cl L^{-1} were generally not significant ($p > 0.05$, t-test). In addition, the highest DIC
5 values were only 1.4 ± 0.02 times higher than those at 0 g Cl L^{-1} .

6 Moreover, the effects of salinization on sediment net releases differed among DOC fractions.
7 Salinization consistently and considerably increased net releases of the protein-like
8 fluorophore (all positive slopes; $r^2 = 0.76-0.95$, $p < 0.05$), showing considerable increases that
9 were 6.7 ± 1.0 times higher at 4 g Cl L^{-1} relative to those at 0 g Cl L^{-1} (Fig. 2c). The effects
10 of salinization on net humic-like fluorophore releases, however, were not consistent (showing
11 both positive and negative slopes, and only 3 out of 8 cases showed a statistically significant
12 level) and were much less (increased by 1.2 ± 0.1 times) (Fig. 2d). As a result, salinization
13 consistently and considerably (by 5.9 ± 0.7 times) increased (all positive slopes; $r^2 = 0.72-$
14 0.97 , $p < 0.05$) the protein to humic (P/H) ratio (Fig. 2e) - an index of DOC lability (Lønborg
15 et al., 2010; Duan and Kaushal 2013). The effects of salinization on DOC lability using
16 fluorescence spectroscopy were further supported by absorbance measurements. Absorbance
17 measurements showed a general decreasing trend in SUVA changes with increasing
18 salinization (6 out of 8 cases with negative slopes; $r^2 = 0.69-0.98$, $p < 0.05$; Fig. 2f).

19 Effects of laboratory salinization on net DOC and DIC releases from soils were relatively
20 more complex and not as consistent (both positive and negative slopes). As mentioned earlier,
21 laboratory salinization experiments with soils were conducted only at 7 sites, because GFPCP
22 and GFGR are very close were considered as one site for soil experiments (same below). In 4
23 out of 7 cases, net DOC releases from soils decreased as experimental salinization decreased
24 from 0 g Cl L^{-1} to 2 g Cl L^{-1} ($p < 0.05$, t-test), followed by slight increases (generally $p < 0.05$,
25 t-test) as experimental salinization increased from 2 g Cl L^{-1} to 4 g Cl L^{-1} (Fig. 3a). The effects
26 of laboratory salinization experiments on net DIC releases from soils were also complex (both
27 positive and negative slopes), and 3 out of 7 cases showed decreases with salinization ($r^2 =$
28 $0.69-0.73$, $p < 0.05$; Fig. 3b). In spite of the complex effects of salinization on net releases of
29 total DOC, salinization almost consistently decreased SUVA of leached DOC across all sites
30 ($r^2 = 0.72-0.96$, $p < 0.05$ in 6 out of 7 cases) by a factor of $40 \pm 4\%$ (Fig. 3c).

31 Effects of laboratory salinization experiments on biogeochemical carbon fluxes from
32 sediment (indicated by changes in their standardized fluxes per g of Cl) exhibited clear

1 patterns across the rural-urban gradient (Fig. 4). In general, the effects of salinization on
2 sediment net releases of DOC, DIC and protein-like fluorophore, and DOC lability (indicated
3 by P/H ratio) increased with ISC – an index for watershed urbanization ($r^2 = 0.57-0.84$, $n = 7$,
4 $p < 0.05$; Fig. 4a-4d). The exception was the urban site GFPC with the highest ash free dry
5 weight (Fig. 4i), showing unexpected large, positive salinization effects on net DOC releases,
6 protein-like DOC releases and P/H ratio, but unexpected negative salinization effects on net
7 DIC releases. No consistent urbanization influence was observed for SUVA (Fig. 4e) or the
8 humic-like fluorophore (not shown). The effects of laboratory salinization on DOC and DIC
9 leaching from soils were different from those observed in sediments, however (Fig. 4a-4e).
10 Effects of salinization on soil leaching were generally less than those on sediment
11 retention/release (except SUVA). In addition, effects of salinization on soil leaching did not
12 show considerable changes with increasing watershed ISC ($p > 0.05$).

13 **3.3 Influence of salinization on fluxes of TKN, nitrate, SRP, and sulfate across** 14 **land use**

15 Sediments were generally a net source of TKN (ammonium + ammonia + dissolved organic
16 nitrogen) and SRP but a net sink of nitrate during the salinization experiments (Fig. 5a and
17 5b). Laboratory salinization experiments consistently and considerably increased net TKN
18 releases from sediments ($r^2 = 0.72-0.95$, $n = 6$, $p < 0.05$; Fig. 5a), and the net TKN releases at
19 4 g Cl L^{-1} were 13.3 ± 5.1 times higher than at 0 g Cl L^{-1} . Meanwhile, salinization
20 experiments consistently increased net nitrate retention (all negative fluxes) and the increases
21 were significant in 6 out of 8 cases (except forest POBR and urban GFPC; $r^2 = 0.73-0.91$, $n =$
22 6 , $p < 0.05$; Fig. 5b). Net nitrate retention at 4 g Cl L^{-1} was 1.6 ± 0.4 times higher than net
23 nitrate retention at 0 g Cl L^{-1} . For SRP, 5 out of 8 sites (forest, agricultural and suburban sites)
24 showed that salinization experiments considerably decreased sediment net SRP releases (by
25 $81 \pm 7\%$; $r^2 = 0.79-0.90$, $n = 6$, $p < 0.05$; Fig. 5c). However, two urban sites GFPC and GFGR
26 showed salinization increased sediment net SRP releases by 1.3 to 3.5 times ($r^2 = 0.71-0.81$, n
27 $= 6$, $p < 0.05$; Fig. 5c). Salinization effects on sulfate were even more complex, showing both
28 positive and negative slopes. However, the agricultural site MCDN and 2 urban sites (GFPC
29 and GFGR) showed strong decreases in sulfate fluxes (by $90 \pm 23\%$; $r^2 = 0.73-0.74$, $n = 6$, $p <$
30 0.05 , or $p < 0.05$, t-test) when the level of salinization increased from 0 g Cl L^{-1} to 4 g Cl L^{-1}
31 (Fig. 5d).

1 Similar to sediments, salinization consistently increased net TKN releases from soils and the
2 increases were significant at 6 out of 7 sites ($r^2 = 0.67-0.95$, $n = 6$, $p < 0.05$; Fig. 6a), and the
3 values at 4 g Cl L^{-1} increased by $93 \pm 25\%$ relative to 0 g Cl L^{-1} . Laboratory salinization
4 increased nitrate releases in 5 out of 7 cases, only 2 of which were statistically significant (r^2
5 $= 0.71-0.76$, $n = 6$, $p < 0.05$). The maximal net nitrate releases with salinization (generally
6 occurred at 2 g Cl L^{-1}) were 1.73 ± 0.19 times greater than those at 0 g Cl L^{-1} (Fig. 5b).
7 Similar to sediments, 6 out of 7 cases showed that experimental salinization suppressed net
8 SRP releases from soils, 5 of which were statistically significant ($r^2 = 0.67-0.97$, $n = 6$, $p <$
9 0.05 ; Fig. 6c). Net SRP releases at 4 g Cl L^{-1} decreased by $40 \pm 9\%$ relative to those at 0 g Cl
10 L^{-1} . Similar to the sediments, the effects of salinization on sulfate releases from soils were
11 complex and inconsistent (Fig. 6e).

12 Effects of laboratory salinization on sediment biogeochemical fluxes of TKN (indicated by
13 changes in their standardized fluxes per g of Cl) also exhibited clear patterns across the rural-
14 urban gradient. That is, the effects of salinization on sediment releases of TKN increased with
15 ISC – an index for watershed urbanization ($r^2 = 0.57$, $n = 7$, $p < 0.05$; Fig. 4a-4d), with one
16 exception at site GFPC that showed highest ash free dry weight (Fig. 4f). No consistent
17 urbanization effects were observed for nitrate, SRP (Fig. 4g-4h) or sulfate (not shown).
18 Furthermore, none of the salinization effects on leaching of TKN, nitrate, SRP or sulfate from
19 soils showed significant correlations with watershed ISC ($p > 0.05$; Fig. 4).

20 **3.4 Biogeochemical coupling between the fluxes of chemical species**

21 Correlation analyses suggested that there were links of the fluxes of the measured chemical
22 species of bioreactive elements. Here, the term flux was used to mean net retention or net
23 release of a chemical species based on site. For example, there was a correlation between net
24 releases of DIC flux and net releases of DOC. Across soil laboratory salinization experiments,
25 DIC net releases linearly increased with DOC releases, and the correlations were significant at
26 4 out of 7 sites ($r^2 = 0.66-0.99$, $p < 0.05$, $n = 6$; Fig. 7b). Across laboratory salinization
27 experiments with sediments, DIC net releases initially increased with net DOC releases but
28 the increases did not continue with further DOC increases (Fig. 7a). Different from DIC, net
29 releases of TKN were all positively correlated with net releases of DOC fluxes in salinization
30 experiments using sediments across all 8 sites ($r^2 = 0.71-0.93$, $p < 0.05$, $n = 6$; Fig. 7c). In
31 general, there was no relationship (one exception) between net releases TKN and net releases
32 of DOC across soil laboratory salinization experiments (Fig. 7d).

1 There was inverse relationship between nitrate fluxes (release in soils or retention in
2 sediments) and DOC fluxes from sediments and soils. Specifically, nitrate fluxes linearly
3 decreased with increasing DOC fluxes from sediments and soils, and the increases were
4 statistically significant at 6 of 8 sites for sediment incubation experiments and at 4 out of 7
5 sites for soil leaching experiments ($r^2 = 0.65-0.95$, $p < 0.05$, $n = 6$; Fig. 7e and 7f). A fourth
6 correlation was between net SRP releases and changes in SUVA of DOC. There were
7 positive correlations between net SRP releases and changes in SUVA values during both
8 sediment incubations and soil leaching. Significant correlations were observed in 4 out of 8
9 cases during sediment incubations ($r^2 = 0.67-0.91$, $p < 0.05$, $n = 6$; Fig. 6e). More cases (5 out
10 of 7) showed this positive relationship during soil leaching ($r^2 = 0.6-0.97$, $p < 0.05$, $n = 6$; Fig.
11 6f).

12

13 **4 Discussion**

14 **4.1 Changes in salinization effects with watershed urbanization**

15 This study shows that the effects of salinization on the retention and release of certain forms
16 of bioreactive elements from sediments changed with watershed urbanization. Thus,
17 Hypothesis 1 regarding changes in salinization effects with watershed urbanization was
18 partially supported by the data from sediment incubation experiments. Overall, our results
19 suggest that the effects of increased salinization on sediment releases of DOC, protein-like
20 fluorophore, TKN and DIC increased with impervious surface cover (ISC) – an index for
21 watershed urbanization (Fig. 4; linear regressions, all $p < 0.05$). These results seem to
22 contradict previous results of soil salinization experiments. Those previous experiments
23 suggest that soils that have already experienced higher degrees of exposure to road salting
24 (e.g., in urban watersheds) respond less to salinization than controls (like in forest watersheds)
25 regarding organic matter mobilisation (e.g., Green et al., 2008, 2009). The reason is that
26 “once the organic matter has been solubilised and/or mineralised under the influence of road
27 salt, and thereafter leached, it is gone from the system”. However, the results from our lab
28 experiments were different from those of Green et al. (2008, 2009) probably for two reasons.
29 In first place, stream sediments were used in our laboratory experiments and not just soils,
30 and the response of stream sediments to salinization might be somewhat different (potential
31 mechanisms discussed later). Secondly, the degree of watershed urbanization may not exactly

1 match the degree of exposure to road salt exposure. For example, the highest streamwater Cl⁻
2 concentrations in this study were not observed at the GFGR site with highest ISC but at
3 DRKR with a smaller ISC value (Table 2). Thus, our results suggest that urbanization impacts
4 biogeochemical responses to salinization (i.e, the net release and retention of chemicals), but
5 it may not always be related to the degree of watershed impervious surface cover.

6 Instead, the interactive effects of watershed urbanization and salinization on sediment releases
7 of DOC, protein-like fluorophore, TKN and DIC fluxes may be explained by coinciding
8 changes in stream sediment organic matter content (indicated by ash free dry weight), which
9 also showed an increase with increasing watershed ISC (Table 2 and Fig. 4i). The outlier site
10 GFCP, which had unexpected larger salinization effects, was also highest in sediment ash free
11 dry weight. The reason for the outlier GFCP is not clear, but much better correlation between
12 sediment ash free dry weight and watershed ISC was reported in our previous study at the
13 same Baltimore LTER sites (Duan and Kaushal et al., 2013). In any case, organic matter
14 content in urban stream sediments was generally higher than in rural streams (also reported in
15 Sloane-Richey et al. 1981; Paul and Meyer, 2001), probably due to additional organic matter
16 inputs from algal (Kaushal et al. 2014b) and anthropogenic sources (e.g., wastewater; Daniel
17 et al., 2002). Our recent work showed that gross primary production and organic matter
18 lability increased with watershed urbanization (Kaushal et al., 2014b). Wastewater inputs
19 from sewer leaks are common in the urban tributaries in the lower Gwynns Falls (DEPRM
20 and Baltimore City Department of Public Works, 2004; Kaushal et al., 2011). As quantity
21 and quality of sediment organic matter increase across the rural-urban land use gradient, we
22 hypothesize that the releases of labile DOC (indicated by protein-like fluorophore), total
23 DOC, TKN and DIC increase in response to salinization.

24 **4.2 Potential effects of increased salinization on DOC/DIC mobilization** 25 **coupled with carbon biogeochemistry**

26 This study suggests that mechanisms responsible for salinization effects on DOC mobilization
27 differ between soils and sediments, as what we stated in Hypothesis 3. Previous studies have
28 shown divergent effects of salinization (e.g., suppression or inconsistent effects) on DOC
29 mobilization in soils (Amrhein et al., 1992; Evans et al., 1998; Green et al., 2008, 2009a;
30 Compton and Church, 2011; Ondrašek et al., 2012). These variations were attributed to soil
31 types (Amrhein et al., 1992; Evans et al., 1998), water to soil ratios (Amrhein et al., 1992),
32 water chemistry (Evans et al., 1998), leaching time (Compton and Church, 2011), and

1 historical exposure to road salt deicers (Green et al., 2008, 2009a). Two competing effects of
2 salts have been suggested upon which solubilisation of organic matter is dependent: sodium
3 dispersion and pH suppression (Amrhein et al., 1992; Bäckström et al., 2004; Green et al.,
4 2008, 2009a). That is, upon salt additions, the replacements of Ca^{2+} and Al^{3+} of soils by Na^+
5 would be expected to increase DOC solubility, because trivalent Al^{3+} and divalent Ca^{2+}
6 reduce organic carbon solubility far more than monovalent Na^+ (Amrhein et al., 1992;
7 Skyllberg and Magnusson, 1995). On the other hand, salinization suppresses pH in solution
8 over shorter time scales due to the mobile anion effect, and therefore decreases DOC leaching
9 from soils (Bäckström et al., 2004; Li et al., 2007; Green et al., 2008). In addition to pH
10 suppression, flocculation/sorption or inhibitory effects on microbial activity have also been
11 suggested as possible mechanisms for DOC retention upon increased salinization (e.g.,
12 Compton and Church, 2011; Ondrasek et al., 2012). It seems the above two-competing effect
13 concept (pH suppression vs. sodium dispersion) can be used here to interpret the inconsistent
14 effects of salinization on DOC retention/release from riparian soils across sites or across
15 salinities in this study (Fig. 3). However, neither this concept nor the flocculation/microbial-
16 suppression mechanism can explain the consistent observation of enhanced DOC mobilization
17 from sediments in our laboratory salinization experiments (Fig. 2a). As we hypothesized,
18 differences in DOC mobilization between soils vs. stream sediments may have been primarily
19 due to differences in DOC composition and sources.

20 Our results from DOC characterization can provide further information for interpreting the
21 differences in salinization effects on DOC releases between sediment and soils. Our results
22 showed that only protein-like fluorophores were consistently and considerably remobilized
23 from sediments with salinization (Fig. 2), which suggested that the increased DOC releases
24 from sediments were mainly attributed to the releases of protein-like (or labile) fractions.
25 Similar findings were also reported by Li et al. (2013), which showed that KCl can
26 considerably increase the mobility of microbially-derived labile organic matter (indicated by
27 the fluorescence index). Meanwhile, chemical analyses suggest that the protein-like
28 fluorophores consist primarily of proteinaceous materials (e.g., proteins and peptides;
29 Yamashita and Tanoue, 2003; Maie et al., 2007), and this DOC fraction is generally
30 hydrophilic and low molecular weight (LMW) compounds (e.g., Sommerville and Preston
31 2001). Results of Chen et al. (1989) and Fuchs et al. (2006) showed that solubility of the
32 proteinaceous materials in LMW is generally neither affected by pH within normal range 6-9
33 nor by colloid coagulation. Therefore, increasing ionic strength (or salinization) can enhance

1 the solubility of the proteinaceous materials *via* sodium dispersion (Green et al., 2008, 2009a)
2 or through nonspecific electrostatic interactions at low salinities (called a “salting in” effect)
3 (Tanford, 1961; Chen et al., 1989). Furthermore, because stream sediments are generally
4 enriched in these labile, proteinaceous materials derived from biofilms (algae and microbes)
5 and wastewater organics in urban watersheds (Daniel et al. 2002; Kaushal et al., 2011;
6 Kaushal and Belt, 2012; Newcomer et al., 2012; Duan et al., 2014b; Kaushal et al., 2014b), it
7 is reasonable that salinization can mobilize a large amount of protein-like labile dissolved
8 organic matter from stream sediments. Relative to the proteinaceous materials, humic
9 substances are larger hydrophobic molecules occurring in the colloidal size range (e.g., Aiken
10 et al., 1985). This DOC fraction is readily subjected to flocculation (e.g., Sholkovit, 1976),
11 sorption to mineral surfaces (Fox, 1991; Hedges and Keil, 1999), and pH suppression (Kipton
12 et al., 1992; Li et al., 2007; Li et al., 2013) with increasing ionic strength or salinization. The
13 potential instability of the humic-like DOC fraction upon salinization was further supported
14 by our present results and previous studies (e.g., Li et al., 2013), which showed that
15 salinization consistently decreased SUVA of DOC released from soils and sediments (Fig. 2
16 and 3) - a parameter indicating DOC aromaticity (Weishaar et al., 2003). Relative to stream
17 sediments, soil organic matter consists primarily of humic substances (up to 60-70%; Griffith
18 and Schnitzer, 1975). In this study, although humic substances were not measured, much
19 higher SUVA values were observed in DOC leached from soils (around $10 \text{ L mg}^{-1} \text{ m}^{-1}$) than
20 from sediments ($< 2 \text{ L mg}^{-1} \text{ m}^{-1}$) (Fig. 7). This suggested that soils were higher in humic
21 substances. Probably, due to large differences in organic matter composition, effects of
22 salinization on DOC leaching from sediments and soils were different (Fig. 2 and 3).

23 Our laboratory experiments suggests that simultaneous net releases of DIC and DOC were
24 examples of coupled biogeochemical cycles in response to salinization, as predicted in
25 Hypothesis 2. The effects of salinization on DIC fluxes from sediments and soils may involve
26 shifts in carbonate chemistry (e.g., dissolution of carbonate minerals), or organic carbon
27 mineralization and CO_2 efflux that are coupled with DOC biogeochemistry. The effects of
28 salinization on carbonate chemistry seems a less important control in this study, because the
29 solubility of carbonate minerals increases with salinization (Akin and Lagerwerff, 1965)
30 while DIC releases from sediment or soils in our laboratory salinization experiments did not
31 always follow this trend (Fig. 2 and 3). Meanwhile, crystalline rocks of igneous or
32 metamorphic origin characterize the surface geology and there are almost no carbonate rocks
33 in our study region (<http://www.mgs.md.gov/esic/geo/bal.html>). The potential importance of

1 organic carbon mineralization and its influence on DIC releases during laboratory salinization
2 experiments were supported by the observed increases in DIC concentrations with DOC
3 releases (Fig. 7a and 7b). The coupling of DIC with organic carbon mineralization seemed to
4 fit better for soils, considering the strong linear relationship between DIC and DOC across
5 soil salinization experiments (Fig. 7b). The complex relationships between DOC and DIC for
6 sediment incubations (Fig. 7a) indicated the importance of other potential controls as well -
7 likely, efflux of CO₂, the product of mineralization of protein-like DOC released from
8 sediments (Fig. 2c). It is known that the solubility coefficient for CO₂ decreases with salinity
9 (Weiss et al., 1974; Duan and Sun, 2003). We hypothesize that CO₂ efflux to the atmosphere
10 may become a dominant control on DIC release at higher salinities (e.g., 2 - 4 g Cl L⁻¹), and
11 further increases in salinization may decrease net DIC release despite increased release of
12 DOC (Fig. 2b).

13 **4.3 Potential effects of salinization on N, P, and S transformation coupled** 14 **with C biogeochemistry**

15 We observed mobilization of TKN in response to salinization in both sediments and soils
16 (Fig. 5-6) that was coupled (in sediments) or not coupled with DOC release (in soils; Fig. 7),
17 as predicted in Hypothesis 2 and 3. Mobilization of TKN has also been observed in several
18 previous studies (mainly NH₄⁺; Duckworth and Cresser, 1991; Compton and Church, 2011;
19 Kim and Koretsky, 2011). The consistent NH₄⁺ releases with salinization can be attributed to
20 Na⁺ dispersion (Green and Cresser, 2008). That is, as a positively-charged ion, NH₄⁺ can be
21 adsorbed on negatively-charged particles of soils and sediments (Nieder et al., 2011); NH₄⁺
22 retained on the cation exchange sites can be greatly reduced by the presence of sodium ions,
23 causing flushing of NH₄⁺-N with salinization (Duckworth and Cresser, 1991; Compton and
24 Church, 2011; Kim and Koretsky, 2011). Despite similarities between enhanced TKN release
25 in response to increased salinization of sediments and soils, correlation data showed much
26 stronger relationships between TKN and DOC during sediment incubation experiments than
27 with soil leaching experiments (Fig. 7). So, the decoupling of TKN mobilization and DOC
28 during soil leaching suggests that TKN mobilization was largely attributed to mobilization of
29 inorganic NH₄⁺ due to Na⁺ dispersion. The coupling of TKN with DOC during sediment
30 incubations, however, indicates that TKN release could be associated with mobilization of
31 dissolved organic nitrogen (DON). This was consistent with far more release of nitrogen-
32 enriched protein-like dissolved organic matter or DON from sediments than from soils as

1 discussed above (0.11 ± 0.01 RU vs 0.033 ± 0.004 RU). Several previous studies have also
2 shown DON leaching from plant litter or soils along with DOC upon increased salinization
3 (Steele and Aitkenhead-Peterson, 2013; Green et al., 2008, 2009; Compton and Church,
4 2011).

5 Our data further show that nitrate transformation and DOC remobilization were also coupled
6 during salinization experiments with both sediments and soils, which supports Hypothesis 2.
7 In contrast to DOC, DON, or NH_4^+ , nitrate is a highly soluble, negatively-charged ion.
8 Mechanisms such as pH suppression, Na^+ dispersion/exchange, and colloid coagulation do not
9 apply for nitrate to interpret the salinization effects, while biologically-mediated
10 transformations may play a relatively more important role. According to previous studies in
11 soils, salinity can decrease the rates of both nitrification and denitrification during short time
12 periods (hours to days; Dincer and Kargi, 2001; Hale and Groffman, 2006; Aminzadeh et al.,
13 2010; Lancaster et al., 2012) due to biological inhibition. However, effects of salinization on
14 nitrate releases from soils varied considerably from positive to no effects, as a combined
15 result of availability of ammonium and nitrate removal *via* denitrification (Duckworth and
16 Cresser, 1991; Green and Cresser, 2008b; Compton and Church, 2011). Our results from soils
17 suggested positive effects of salinization at some sites (Fig. 6b), probably due to nitrification
18 of the release of TKN (including ammonium; Fig. 6a). For sediments, we found that
19 laboratory salinization experiments consistently increased nitrate retention, however (Fig. 5b),
20 although there was increasing release of TKN with higher salinity (Fig. 5a). These
21 salinization effects in sediments may be related to the remobilization of labile DOC in
22 sediments, based on the consistent inverse relationship between nitrate and DOC (Fig. 7).
23 That is, although salinization may directly influence denitrification rates of instream
24 sediments (Hale and Groffman, 2006), it may also cause mobilization of considerable
25 amounts of protein-like dissolved organic matter (DOC and DON) into streams (Fig. 2c). We
26 speculate that this mobilized labile dissolved organic matter may stimulate nitrate biological
27 uptakes *via* denitrification and/or microbial immobilization because it provides an energy
28 source for microbes (Newcomer et al., 2012). Furthermore, decomposition of labile dissolved
29 organic matter can lead to anoxic conditions for N removal *via* denitrification (Sobczak et al.,
30 2003; Duan et al. 2014a). So, when the effects of released labile dissolved organic matter
31 override the inhibitory effects of salinization on nitrate transformations, we speculate that
32 salinization may actually enhance nitrate retention in stream sediments (Fig. 4b). However,

1 the effects of increased salinization on DON and ammonium mobilization warrant further
2 research.

3 Our results suggest that SRP release from sediments or soils during laboratory salinization
4 experiments was associated with changes in DOC aromaticity (indicated by SUVA),
5 supporting Hypothesis 2. Different from N, P is primarily a particle reactive element, and a
6 large fraction of dissolved P (e.g., up to 88%; Cai and Guo, 2009) is in the form of colloids, or
7 humic- Fe (Al-) phosphate complexes (Hens and Merckx, 2001; Turner et al., 2004; Regelink
8 et al., 2013). This is because the sorption capacity for SRP per unit mass is about 5,000 times
9 larger for colloids than for the immobile soil matrix (McGechan and Lewis, 2002). However,
10 the stability of colloids decreases with increasing ionic strength and decreasing pH (e.g. Bunn
11 et al., 2002; Saiers et al., 2003), both of which can be induced by salinization (e.g., Green et
12 al., 2008). An example of this salinization effect is rapid flocculation of freshwater SRP and
13 colloids in estuaries in response to mixing of fresh water with seawater (e.g., Sholkovitz,
14 1976). Thus, increased salinization may decrease stability of the colloidal humic- Fe (Al-)
15 phosphate complexes, leading to reduced releases of SRP from sediments and soils (Fig. 5c
16 and 6c) and a coupling between SRP and SUVA (Fig. 7g-7h). However, biological controls
17 such as inhibition of microbial activity at higher salinities could provide an alternative
18 explanation (Srividya et al., 2009). Reason for the increased releases of SRP from sediments
19 at higher salinities at two urban sites (GFGR and GFCP) is not clear, because SRP fluxes at
20 these sites were not correlated with Δ SUVA. Probably, these SRP increases might be related
21 to release of large amount of labile DOC and resulting changes in redox condition favorable
22 for SRP release. In summary, decreases in SRP fluxes from sediments and soils in response to
23 salinization were likely a result of colloid coagulation and microbial inhibition at higher
24 salinities, while the increases in SRP fluxes from sediments at urban sites warrant further
25 examination on redox changes.

26 Relative to C, N and P, effects of salinization on sulfur transformations are relatively less
27 known. Kim and Koretsky (2011, 2013) reported salinization inhibited porewater sulfate
28 reduction in lake sediments. However, our results show large variability in the effects of
29 salinization on net sulfate release from either sediments or soils (Fig. 5d and 6d), and sulfate
30 reduction seems not be dominant in free-flowing streams. Effects of increased salinization on
31 sulfate releases warrant further investigation in future studies, however.

32

1 **5 Conclusions**

2 The potential effects of salinization on biogeochemical fluxes from soils and stream
3 sediments are summarized in Fig. 8. As shown in this figure, releases of labile DOC (thus
4 total DOC) and TKN (primarily DON and ammonium) from sediments can potentially
5 increase during episodic stream salinization, due to “salting-in” effects (or Na dispersion) of
6 proteinaceous organic matter and NH_4^+ mobilization. The increased releases of labile DOC
7 and TKN (primarily DON and ammonium) can result in increases in sediment releases of DIC
8 and sediment retention of nitrate as a result of organic carbon mineralization and associated N
9 transformations (e.g., denitrification and nitrate microbial immobilization). Moreover, the
10 effects of salinization on sediment releases of labile DOC and TKN also increased with
11 watershed urbanization (indicated by watershed ISC) due to higher sediment organic content
12 at urban sites. DOC aromaticity (indicated by SUVA) and releases of SRP, however,
13 decreased with stream salinization, likely due to coagulation of colloidal humic- Fe (Al-)
14 complexes and pH suppression, which were associated with ion exchange. The sediment
15 releases of labile DOC and TKN with increased salinization probably represents a significant,
16 previously unrecognized flux of labile DOC and TKN (DON and ammonium) in urban
17 streams, which might have a large influence on carbon and nutrient biogeochemical cycles
18 and water quality in urban waters. For soils, salinization effects on DOC leaching were not
19 consistent, and there were no interactive effects of land use and salinization. Differences in
20 effects of salinization on sediments and soils are likely attributed to differences in organic
21 matter sources and lability. Nonetheless, our work suggests that increased salinization can
22 have major effects on concentrations and fluxes of bioreactive elements in human-impacted
23 watersheds and streams, and it is critical to conduct comprehensive investigations of the
24 effects of salinization on all major bioreactive elements and couple them together as a whole.

25

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28

1 Table 1. Characteristics of study subwatersheds.

Site	BARN	MCDN	GFGB	GFGL	GFVN	GFCP	DRKR	GFGR
Type	forest	agriculture	suburban	suburban	Urban	urban	urban	urban
Area (ha)	3.86	0.1	11	0.8	84.2	170.7	14.3	6.5
%ISC	0.3	0.1	15	19	17	24	45	61
Developed/Open	25.5	13.6	43.8	41.2	27.4	25.5	22.4	9.2
Developed/low		5.4	28.9	21.5	25.2	28.8	38.8	27.5
Developed/medium		3.6	4.3	8.3	10.5	16.2	18.1	43.6
Developed/high			1.3	3.4	2.5	5.0	7.4	17.4
Barren					0.2	0.1	0.1	0.04
Shrub	0.7	7.9	1.1	6.3	1.4	0.8	0.2	0.05
%Forest	72.6	1.4	19.0	19.2	25.5	19.3	12.7	1.3
Hay/pasture	1.0	30.4	0.9	0.1	3.1	1.7	0.1	
Cultivated Crops		37.1	0.1		3.4	1.8		
Wetland		0.7	0.6		0.8	0.7	0.2	
Open water					0.1	0.1		0.9

2 Watershed land cover and impervious surface (ISC %) data are from Shields et al. (2008) and
 3 the National Land Cover Database (NLCD) of 2006. Both land cover and impervious
 4 statistics were based on 30-m resolution land cover data.

5

1 Table 2. Water chemistry, sediment and soil ash-free weight prior to salinization incubation
 2 experiments.

Site		Water									Sediment	Soil
Name	Type	F ⁻ mg L ⁻¹	Cl ⁻ mg L ⁻¹	SO ₄ -S mg L ⁻¹	DOC mg L ⁻¹	P/H	DIC mg L ⁻¹	SUVA L mg ⁻¹ m ⁻¹	NO ₃ -N mg L ⁻¹	SRP μg L ⁻¹	AFDW (%)	AFDW (%)
BARN	forest	0.26	75	5	1.2	0.56	3.3	2.26	1.76	16.0	0.61	6.17
MCDN	agriculture	0.41	57	19	1.5	0.36	12.2	2.64	7.13	48.1	1.23	7.68
GFGB	suburban	0.30	95	6	1.2	0.63	12.1	2.11	2.09	16.9	0.92	8.84
GFVN	suburban	0.46	116	10	1.5	0.73	16.6	2.24	1.26	13.4	0.89	7.82
GFGL	suburban	0.38	124	25	2.3	0.52	32.1	2.31	1.43	38.7	1.35	6.27
GFCP	urban	0.80	159	22	1.9	0.83	18.9	1.94	1.19	10.5	3.98	7.17
DRKR	urban	0.87	557	59	2.5	0.77	34.6	1.96	1.28	30.2	1.22	8.04
GFGR	urban	2.80	187	54	3.5	1.36	32.8	0.95	2.30	20.4	1.90	-

3 - : samples were not taken. Legends DOC, P/H, DIC, SUVA, SRP and AFDW stand for
 4 dissolved organic carbon, protein to humic ratio of DOC, dissolved inorganic carbon, specific
 5 ultraviolet absorption, soluble reactive phosphorus and ash-free weight.

6

1 Figure 1. Land use of the Gwynns Falls and Baisman Run watersheds, showing sites from
2 which sediment, soil and stream water were collected for salinization experiments. Baisman
3 Run is a watershed with forest as the dominant land use, and it is located in the nearby
4 Gunpowder River. Solid and open circles represent sites of the main stem and tributaries,
5 respectively. Resolution of the land use data is 30 m, and land use and stream channel
6 location data are from US Department of Agriculture (datagateway.nrcs.usda.gov) and US
7 Geological Survey (<http://datagateway.nrcs.usda.gov/>).

8 Figure 2. Changes in DOC, DIC, protein-like fluorophore, humic-like fluorophore, protein to
9 humic (P/H) ratio and specific ultraviolet absorption (SUVA) during 2-day sediment
10 incubations with NaCl-amended stream water (in 0, 2 and 4 g Cl L⁻¹). The changes in stream
11 water only were subtracted to obtain the contributions from sediments. A linear regression
12 line was added to each site (6 experiment with 3 NaCl levels), and correlation coefficient was
13 added only if the regression was significant. For the panel without significant correlation, *
14 was used to indicate significant difference between two adjacent salinization treatments.
15 Humic- and proten-like fluorescence is in Raman Unit (RU).

16 Figure 3. Releases of DOC and DIC and changes in specific UV absorption (SUVA) during 2-
17 day soil incubations with NaCl-mended DI water (in 0, 2 and 4 g Cl L⁻¹). A linear regression
18 line was added to each site (6 experiment with 3 NaCl levels), and correlation coefficient was
19 added only if the regression was significant. For the panel without significant correlation, *
20 was used to indicate significant difference between two adjacent salinization treatments.

21 Figure 4. Changes in salinity effects on DOC, DIC, protein-like fluorophore, P/H ratio,
22 SUVA, TKN, nitrate and SRP for sediment and riparian soils, as well as ash-free dry weight
23 (AFDW) with watershed impervious surface cover (ISC). Salinity effects were slopes of
24 regression liners in Figure 2 and 3 and Figure 5 and 6. A outlier (urban site GFPCP) was
25 identified in sediment incubation for DOC, DIC, protein-like fluorophore, P/H ratio, and TKN
26 and sediment AFDW. A regression line was added to the data only if correlation with ISC
27 was significant ($p < 0.05$), and the outlier was not counted in the regression.

28 Figure 5. Changes in TKN (DON + NH₃-N + NH₄⁺-N), nitrate-N, SRP and sulfate during 2-
29 day sediment incubations with NaCl-amended stream water (in 0, 2 and 4 g Cl L⁻¹). The
30 changes in stream water only were subtracted to obtain the contributions from sediments. A
31 linear regression line was added to each site, and correlation coefficient was added only if the

1 regression was significant. For the panel without significant correlation, * was used to
2 indicate significant difference between two adjacent salinization treatments.

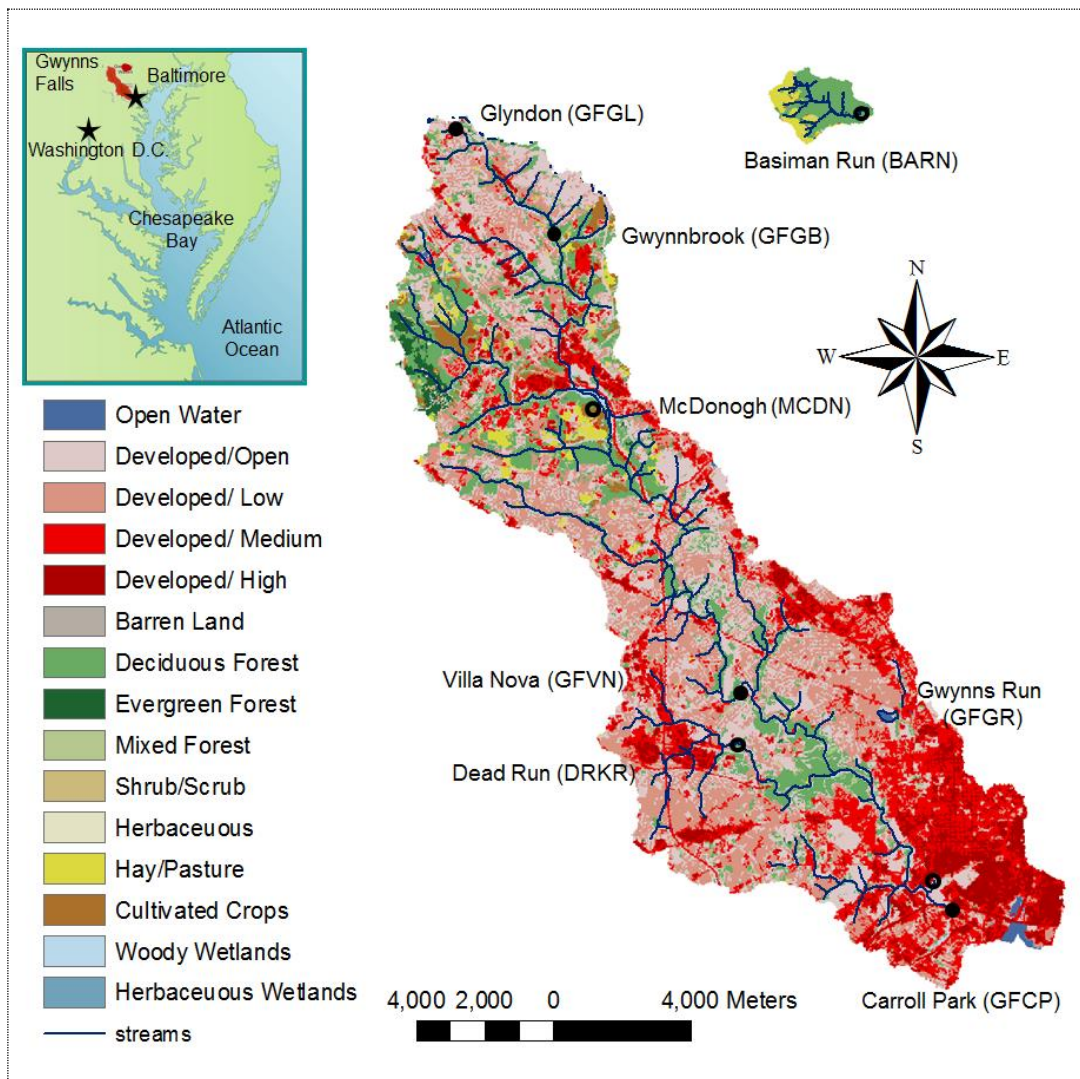
3 Figure 6. Release of TKN (DON + NH₃-N + NH₄⁺-N), nitrate-N, SRP and sulfate in during 2-
4 day soil incubations with NaCl-amended DI water (in 0, 2 and 4 g Cl L⁻¹). A linear regression
5 line was added to each site, and correlation coefficient was added only if the regression was
6 significant. For the panel without significant correlation, * was used to indicate significant
7 difference between two adjacent salinization treatments. The scale of x axil of SRP for
8 MCDN was different from other sites.

9 Figure 7. Correlations between ΔDIC, ΔTKN or Δnitrate and ΔDOC, and between ΔSRP and
10 ΔSUVA during the of sediment/soil incubations for each site with NaCl-amendment at 0, 2
11 and 4 g Cl⁻¹ (in duplicate, totaling 6 incubation experiments). A positive correlation between
12 ΔSRP and ΔSUVA ($R^2 = 0.95$) is out of scope. A solid line was added to the data only if
13 correlation was significant ($p < 0.05$), but correlation coefficients were not labelled in order to
14 simplify the figures. Dash lines in panel a are smoth lines to link the points.

15 Figure 8. A conceptual diagram summarizing potential effects of salinization on DOC quality,
16 DOC and TKN releases from sediments and soils, as well as linkage to release/retention of
17 DIC, nitrate and SRP during sediment and soil salinization.

18

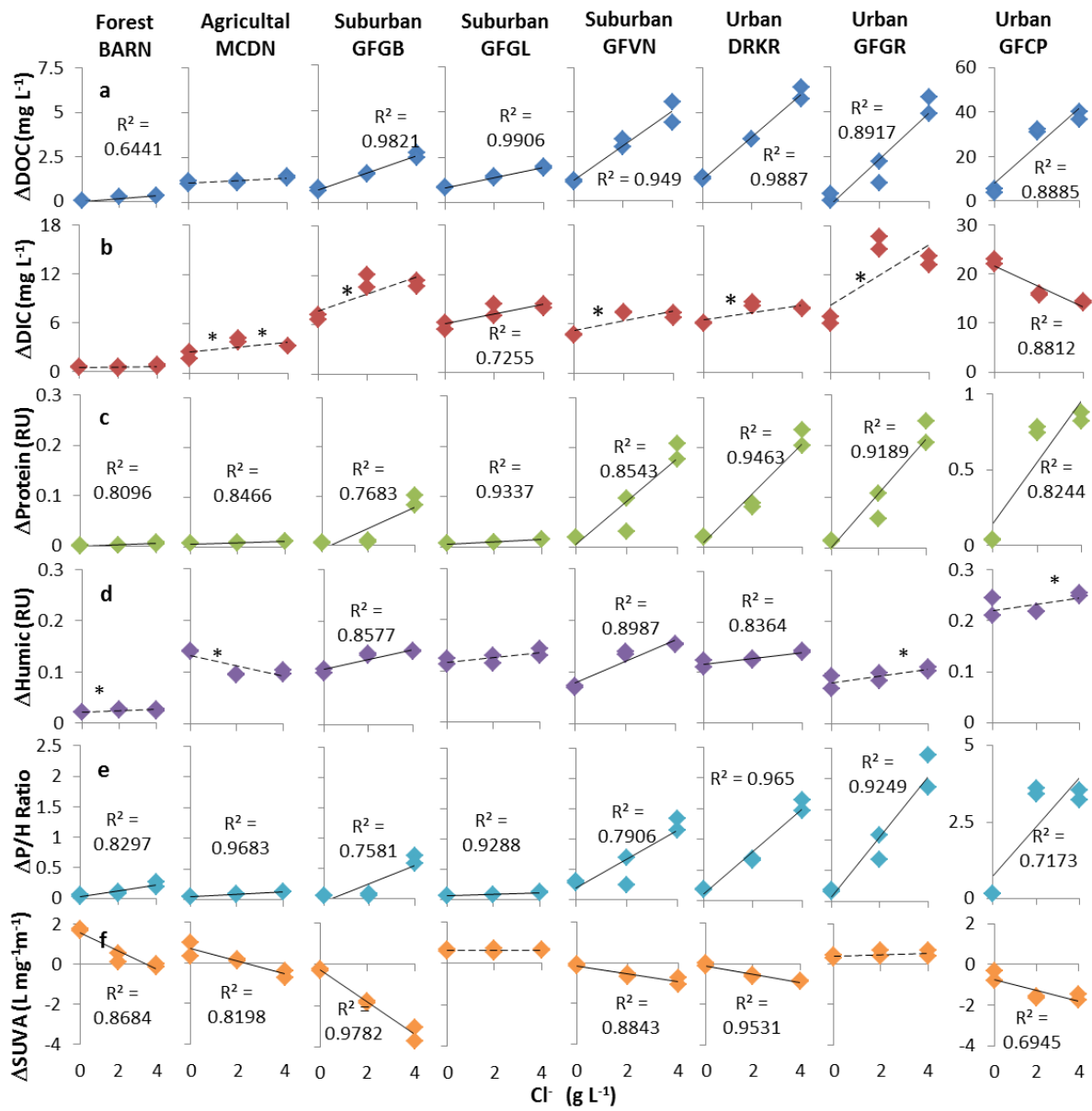
1 **Figure 1**



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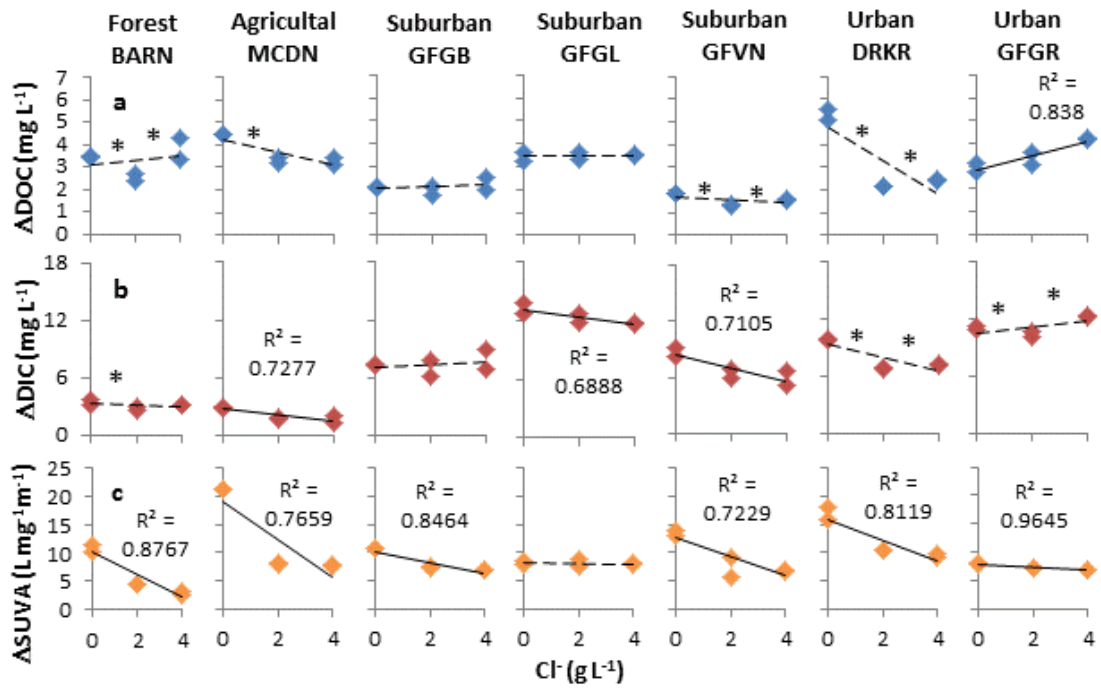
1 **Figure 2**



2

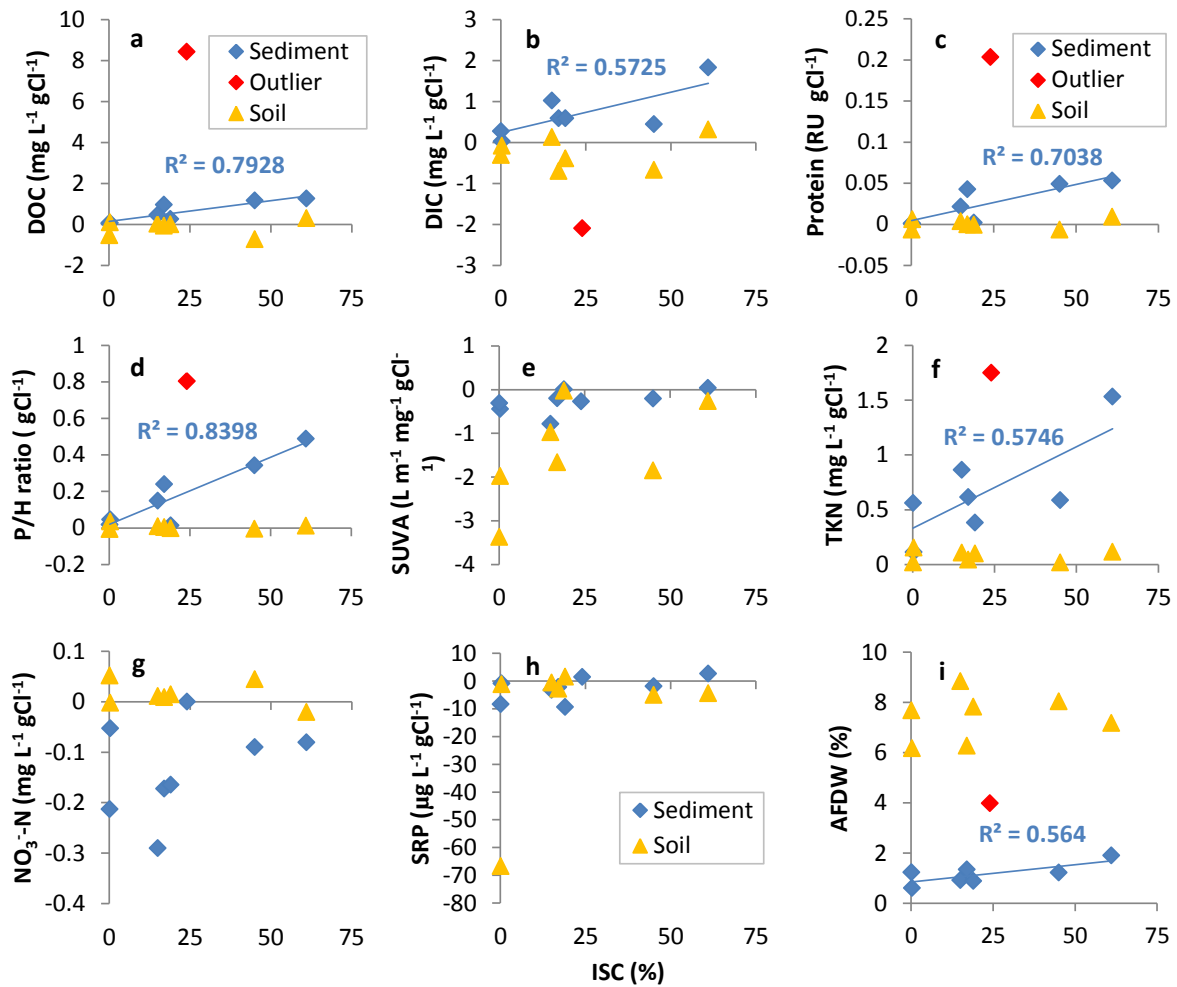
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1 Figure 3



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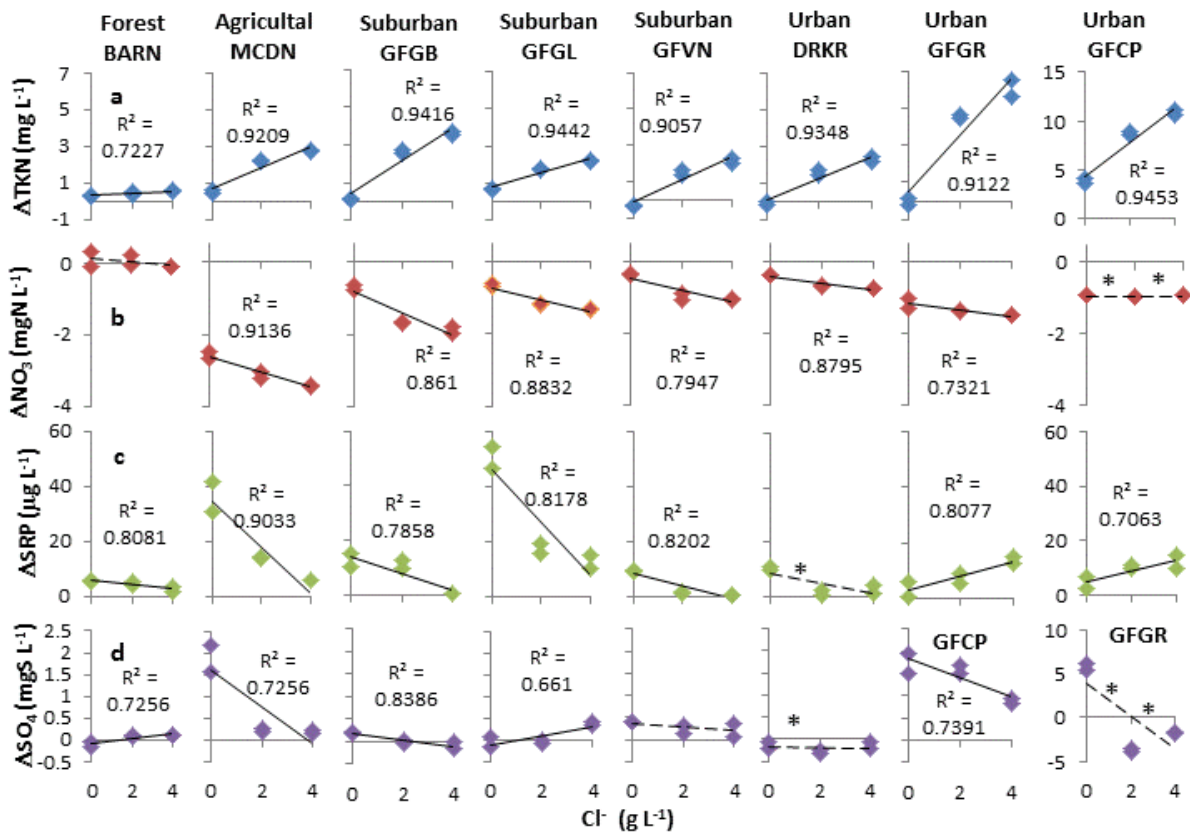
1 **Figure 4**



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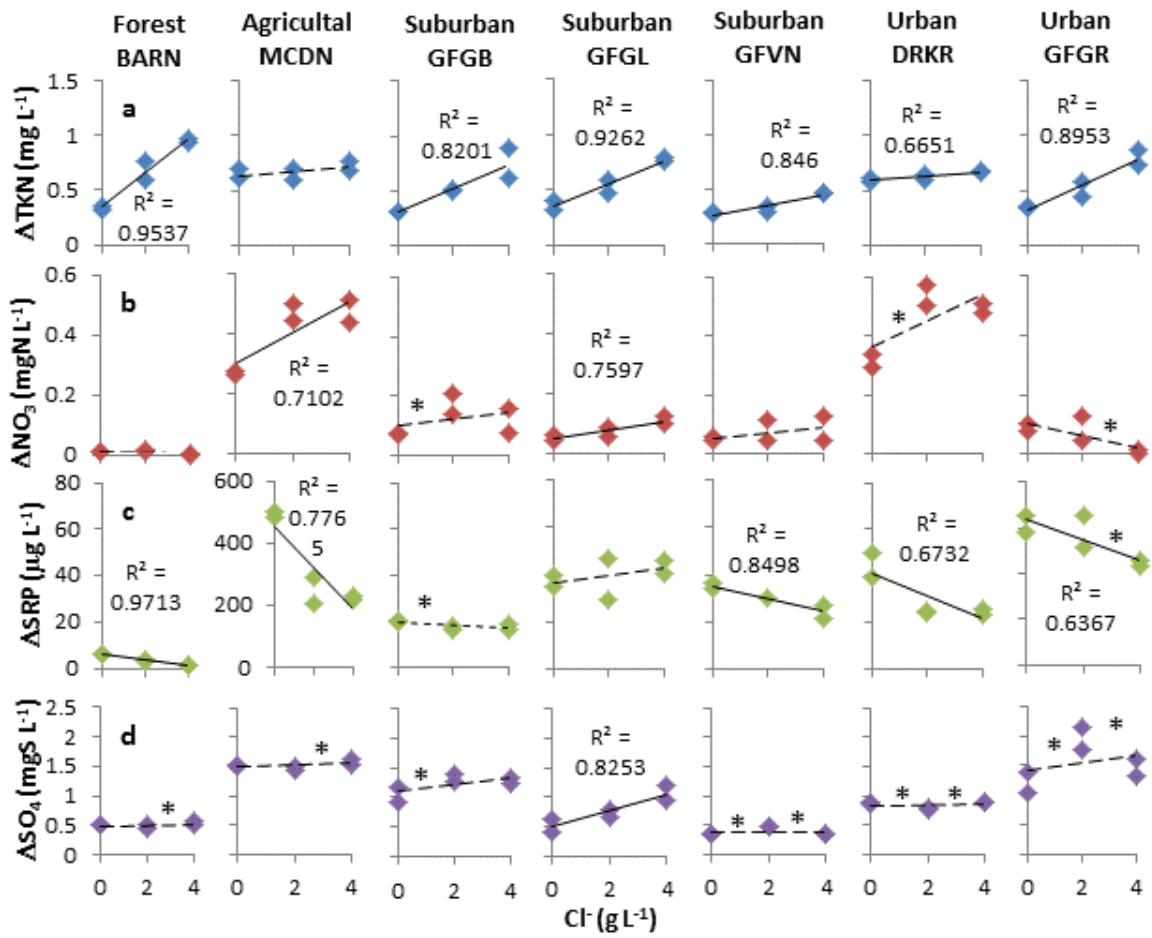
1 Figure 5



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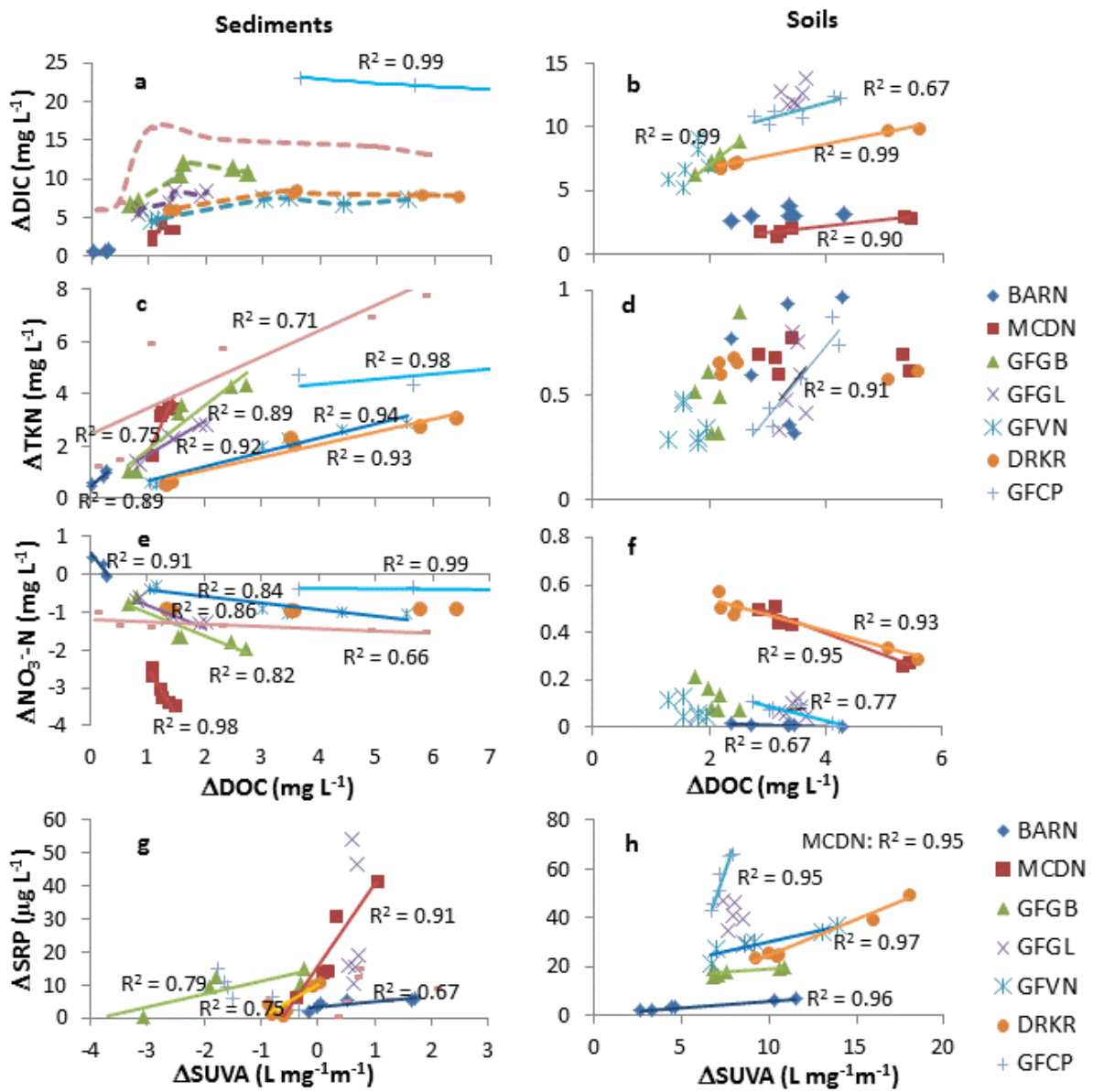
1 Figure 6



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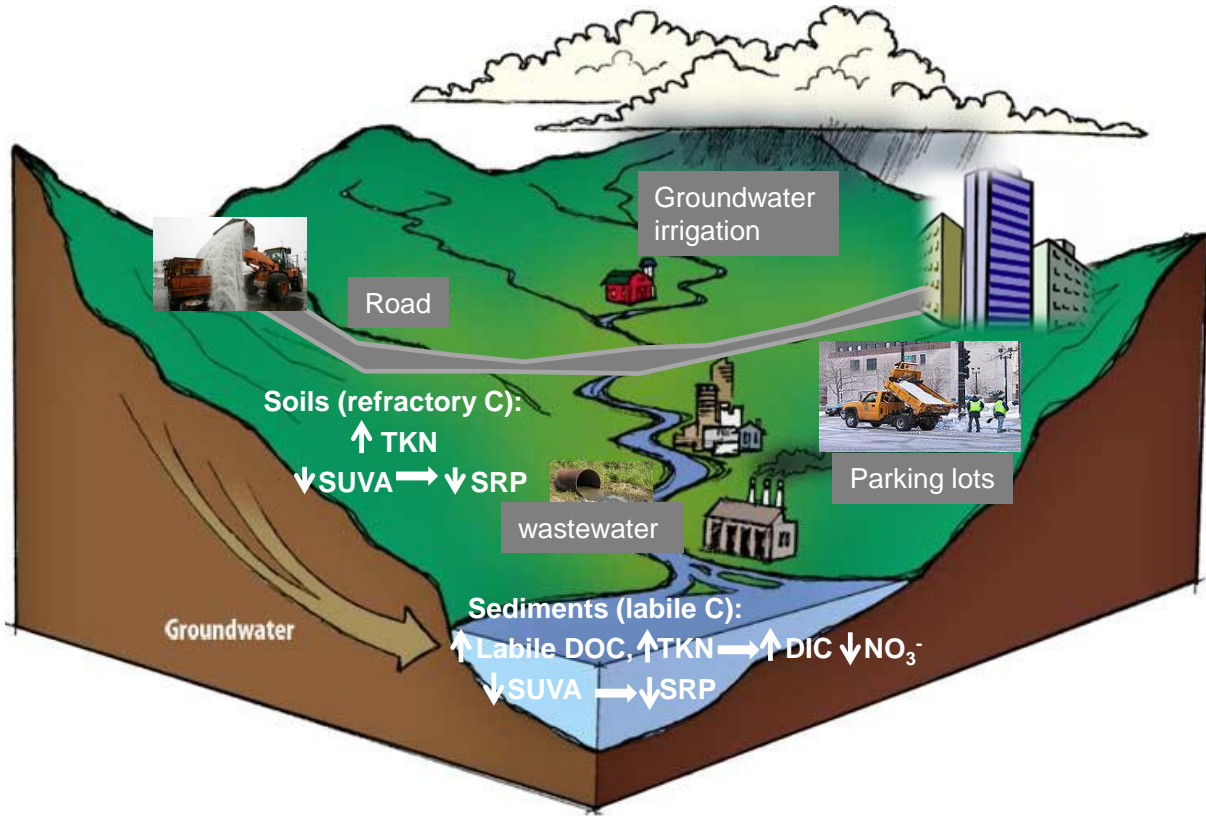
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1 Figure 7.



2

1 Figure 8



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