Biogeosciences Discuss., 12, C5560–C5578, 2015
www.biogeosciences-discuss.net/12/C5560/2015/

Biogeosciences
Discussions
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Interactive comment on "Salinization alters fluxes of bioreactive elements from streams and soils across land use" *by* S.-W. Duan and S. S. Kaushal

S.-W. Duan and S. S. Kaushal

sduan@umd.edu

Received and published: 21 September 2015

However, this study suffers from considerable weaknesses. Some of them focus on the introduction- incomplete or superficial argumentation - that I am convinced authors can strength and develop with more detail (see my comments/recommendations) to improve the understanding of the story they want to tell. In the introduction authors well present the problem and the absence of information about the effects of salinity on stream ecosystem functioning; however, it appears underdeveloped in terms of biogeochemical background. Essentially it lacks of enough information to understand why authors draw up the hypothesis (H) currently stated in the paper. For example, the H1 that authors propose (regarding the implications of salinization across variable land use) could be discussed and re-considered. Otherwise, they need to argue it properly

in the introduction. Something similar happens in relation to H2. As H2 currently states I cannot understand why authors suggest it.

Reply: We have now added two paragraphs in introduction section to explain why we draw up the two hypotheses (H) currently stated in the paper regarding effect across and land use and coupled biogeochemical cycles.

Another failing point in this paper is that the results- almost the whole section- lack of statistical support which reduces the accuracy of the outcomes. For example, the effects of salinization at different levels and the influence of land use on salinization effects (key aspects in the study) are not supported by any statistical analyses. Also, pre- and post- snow stream water characteristics are not statistically compared (there is actually no error in the graphs showing these results; Fig.8- or at least, I cannot see it). If they did, statistical analyses should be better explained in the method section, properly linked with the research questions authors want to deal and then used to support their results (including figures).

Reply: We have now conducted much better statistical analysis to support our hypothesis. We used t-test and linear regression to support the effects of salinization at different levels, and use linear regression to examine the influence of land use on salinization effects. The part of pre- and post- snow stream water characteristics has been removed for the reason that will be given below.

On the other hand, certain level of disagreement between the salinization experiment and field observations can diminish the impact of the results. Field sampling are restricted to one sample occasion during pre and post snow, this last two days after snow smelt; the same duration for their experimental salinization in lab. Why did authors decide two days? Salinity impacts can take place at different moments; depending on the system (i.e. historical exposure). Also - as authors well recognize in the discussion- pure NaCl used in lab and salts employed for deicers can have different effects, which ultimately weaken the use experimental observations to interpret ambient changes. I

think the findings, while interesting, are incremental and do not lead to a better overall understanding of the problem of salinization in urban areas.

Reply: Because disagreement between the salinization experiment and field observations can diminish the impact of the results, we have removed this part of field observations.

Finally, authors should be consistent when reporting nutrient and elements (N, nitrate, NH4, ammonium, SRP, P, S, etc.). There is a general mixture throughout the paper that authors should avoid.

Reply: We have been consistent throughout the paper when we reporting nutrient and elements. When we report elements, we also reported forms of these elements with them.

Specific comments:

Title As title currently states seems authors also evaluated an effect on "terrestrial soils". The word "soils" sounds much more linked to terrestrial ecosystems rather than aquatic systems to me. Since authors ultimately study riparian soils, I recommended them just writing Salinization alters fluxes of bioreactive elements from stream ecosystems across land use.

Reply: We have changed the title to “Salinization alters fluxes of bioreactive elements from stream ecosystems across land use” as suggested.

Abstract Authors should incorporate in the aim that they evaluated the implications of % land use on salinization effects. If possible, a short sentence describing how salinity interact the way C and nutrients are processes would be appreciate to reinforce the justification of their study.

Reply: We have incorporated in the aim that we “evaluated the implications of percent urban land use on salinization”. Before the statement of objectives, we’ve added a short sentence describing how salinity interact C, N and P biogeochemical cycles to

reinforce the justification of our study.

Line 4: I would suggest to reword the sentence "The effects.....understood" to Although increased salinization has been shown to alter C and N dynamics in freshwater ecosystems, its effects on biogeochemical cycles are still not well understood.

Reply: We have reworded this sentence as suggested.

- Line 18. Authors should firstly say that the response to salinization varied between in-stream sediments and riparian soils. And then, they can explain that such differences could be attributed to organic matter.

Reply: We have now added a sentence to report differences in responses to salinization between in-stream sediments and riparian soils.

-Line 20: Authors say: "Results of the .....after a snow even". I would move this sentence to the part where authors describe their objectives.

Reply: We agree with the reviewer that this sentence should now be moved to the part where authors describe their objectives. In this version, this sentence is now deleted because we have deleted the whole section pertaining tofield observations (for the reasons that we mentioned earlier).

Section 3.6. Fig 5a and b should actually be Fig 8a and b

Reply: We have now changed Fig 5a and b Section 3.6. to Fig 8a and b. Because the field observation section has now been removed, this sentence is deleted.

Introduction

Overall, introduction lacks of detailed mechanisms (chemical/ microbial) in which salinization would affect C and N fluxes. In the discussion they provide plenty of detailed information about mechanisms and author could recast some I would strongly recommend authors to describe with more detail the biogeochemical effects of increased salt on fresh water ecosystems. Salt (mainly Na and Cl) can chemically affect N and

C through its effects on ion exchange but also through stress of microorganisms responsible of DOM and N cycling. There are plenty literature in Australian ecosystems evaluating the effects of secondary salinization (anthropogenic salinization; e.g. those from Nielsen et al.) - like the one that authors investigate in the present study. Authors may like to include in the introduction references (an idea): * Nielsen et al. (2003), **Kulp et al. (2007), ***Ardón et al. (2013) (See references at the end of my revision)

Reply: We have now added two sentences reviewing chemical/ microbial mechanisms in which salinization would affect fluxes of bioreactive elements. The three references recommended by the reviewer are also now included in the paper.

Line 7: Authors should include in their objectives the influence of % land use as a secondary aim (or even within the primary one since they have an Ho around land use implications).

Reply: We have now rewritten this sentence and include the influence of % land use as a secondary aim.

Line 10: What do authors base on to formulate Ho (1)? Could sediments and soils from rural or natural watersheds are more sensitive to salinization than those from urban areas where microorganisms could be already acclimated to live under salty conditions? In others words, could the historical exposure to salinization make less sensitive urban rivers than rural ones which rarely experience such a pressure? This alternative Ho sounds more reasonable to me from a microbial perspective. If authors hypothesize the (1) as it currently states in the paper, then they need strong background supporting it. Regarding Ho(2): is that an hypothesis? it sounds really ambiguous and a priori difficult to test. Also, there is no previous supporting information in the introduction to understand why authors present such Ho(2).

Reply: We agree with the reviewer that sediments and soils from urban watersheds may be less sensitive to salinization because of historical exposure. One the other hand, the opposite may occur because of more labile organic matter in the substrates

of urban streams. So, we've changed Ho (1) to "1) the effect of salinization on soil leaching and sediment retention/release of bioreactive elements change with watershed urbanization". We've reworded the Ho(2) to make it clearer to understand - "retention/release of nitrogen, phosphorus, and sulfur in response to salinization can be abiotically and/or biologically coupled with carbon biogeochemistry." We have now added two paragraphs in introduction section to explain why we draw up the two hypotheses.

I would essentially recommend authors to re-consider their hypothesis and re-organize the last part of introduction (Line 7- 18) as following: i) Main aims ii) how authors approach their aims: describing basically (and shortly) their experimental and field approach iii) Main hypothesis (well reasoned in the introduction). iv) If possible, main predictions. Based on their hypothesis authors can predict some outcomes that they can rest in their experimental. For me, a hypothesis should explain observed facts. Here authors do not explain anything but rather are simply tentative statements of what one hopes the research will show For example, can authors provide a key hypothesis of how salinity affect retention or release on stream bioreactive elements? For example, salinity may lead osmotic stress on microbial communities involve in NO3 and NH4 transformation (denitrification, nitrification, DNRA). According to that I would expect in my experiment significant changes in inorganic N concentrations as salinity increases.

Reply: We've followed the recommendation of the reviewer to re-organize this paragraph as: i) main aims ii) how we approach our aims, iii) main hypothesis. Because major predictions are hard to make, part IV is not present. We have now added two paragraphs in introduction section to explain why we draw up the two hypotheses.

Methods

Concerning the experimental part, I found this section well organized. Yet data analysis and statistic should be clarified. Authors should better link their statistical analysis with their research questions. Also, was the 1-way ANOVA done per study site and type

of habitat (in-stream sediments vs. riparian soils)? Please, clarify it. How did authors test that fluxes in urban watersheds are more sensitive to increased salinization than in rural areas? Did authors use Spearman correlations to deal with that?. Correlations do not involve cause-effect. Authors should either conduct linear models with % land use or include land use as a factor in the ANOVA analyses. Regression approach may be more appropriate in this case since authors have n=1 for both forest and agricultural sites.

Reply: All information mentioned above has added to Section of Data Analyses and Statistics Salinization effects: We performed linear regressions of sediment/soil biogeochemical fluxes with salinity across all sites, using data from 6 salinization experimental manipulations (3 salinity levels with duplicates). If the p-value was < 0.05 for the regression, we assumed that there was a significant salinization effect. Otherwise, differences between two salinization levels were tested using a t-test of two-samples assuming equal variances. Type of habitat (in-stream sediments vs. riparian soils): Differences in ash free dry weight, fluxes or salinization effect between sediments and soils were tested using t-test two-sample assuming equal variances. Urbanization effect: We calculate the slope of sediment fluxes (C, N, P and S), and regressed the slopes with impervious surface cover – an index of watershed urbanization.

Also, authors should explain how they calibrated changes with salinity using the experimental controls. In Fig. 2 authors slightly explain how they did. Such an information should be included here.

Reply: We have added in Section of Data Analyses and Statistics that “The changes in the control flasks (with water only), occurring in water without sediments or soils, were subtracted to obtain the fluxes that were released from sediments or soils”.

How did authors compare pre and post snow stream water? a paired t-test sounds to me as reasonable test for comparing that. Authors should write a significance criteria $p \leq 0.05$ (or $p < 0.05$, as a standard rule), and no $p = 0.05$.

Reply: We’ve removed the data of pre and post snow stream water in this version based on previous concerns by this reviewer (discussed earlier). More careful design is needed for field sampling in future studies.

Did authors test the normality of data distribution? Please, specify it.

Reply: We’ve now clarified in the last sentence regarding normality of data distribution that “For linear relationships, Spearman’s correlation was used in cases where assumptions of normality were not met”.

I also drop here some comments that authors may want clarify in the methods: - 2.2 Sample collection and processing: how many cm did you sample for surface sediments and top soils? How did you collect sediments and soils? did you use a core, shovel? Please, specify it. How many replicates of stream water authors collected to compare pre and post snow? Reply: We have added more detail information in the methods section regarding sample for surface sediments and top soils. The field comparisons between pre and post snow have been removed based on poor experimental design (as discussed earlier).

- 2.3 Laboratory salinization: what about concentrations of Na?

Reply: Because pure NaCl was used, concentrations of Na should be the same as Cl-. So, we believe it is not necessary to report the data for Na concentration.

- when authors say they that experiments were conducted in duplicate, do they mean per study site? Are such duplicates either field replicates or analytical replicates?

Reply: We mean duplicate per study site and have added this information in the text. These duplicates did not include field replicates because we stated previously that we collected composite samples in the field. - when authors mean ambient temperatures: 19-22 âŮęC: was the experiment placed in a climate chamber where temp. cycles were programmed (day-night temperature?). Please, clarify it.

Reply: We have changed this sentence to “The laboratory salinization experiments

were conducted in the dark in the laboratory with minor temperature variability (19–22°C)". This was the lab temperature, and we measured the temperature and found it only changed within 19–22°C.

- is there any control for the riparian soil experiment as the one authors employed with sediment incubations?

Reply: Yes, we had controls for the riparian soil experiment. To clarify this, we've added one sentence "Deionized waters without soils were incubated at the 3 levels of salinization as soil-free controls." Here we did not collected snow water to conduct riparian soil experiment, but used deionized waters assuming the minor difference between snowwater and deionized water would not affect our results.

Results

In general, this sections seriously needs to be supported with the proper statistical analyses. Also, if they do, they should write the exact p-value. Sometimes, p-values associated to statistical test can make the result marginally significant ($0.05 < p < 0.1$). I believe showing exact p-values is worth (for example, Line 23: r2= 0.40, n=8 could have a p-value marginally significant). The same suggestion when reporting ANOVA results in any paper: F values, df and p-value should be shown.

Reply: We have now reported results of statistical analyses to the results section, and added p values to linear regression and correlation analysis. We have changed ANOVA to t-test two-sample assuming equal variances for different test, and added p values for the t-test, too.

How authors calculated the ISC?

Reply: The ISC values were adapted from previous studies, and we have listed the literature in Table 1 where ISC values were reported.

Section 3.2 Line 10: there is a typing error. Should be higher Line 12: please write 6 out of 8 cases.

Reply: We have corrected the error mentioned above. Thank you.

Discussion In general, discussion deals with a good literature review and provides valuable information that authors could also use to elaborate their introduction (especially that related to biogeochemical mechanisms). I suggest to authors to discuss their results alongside the support (or not) of their initial hypothesis or predictions. A brief paragraph at the beginning to this sections summing up their main findings would be appreciated

Reply: We have discussed our results in each subsection alongside the support (or not) of their initial hypothesis. As suggested by the reviewer, we now begin with a brief sentence summing up our main findings. We prefer this way rather than using a paragraph at the beginning to this section.

Figures and Tables:

Table 2: Authors should include stream sites in the proper column as well as including in the legend that study sites are organized from rural to urban land use. Also, the meaning for codes DOC, P=H, DIC, SUVA and SRP should be included in the legend.

Reply: We have makes these changes as suggested by the reviewer.

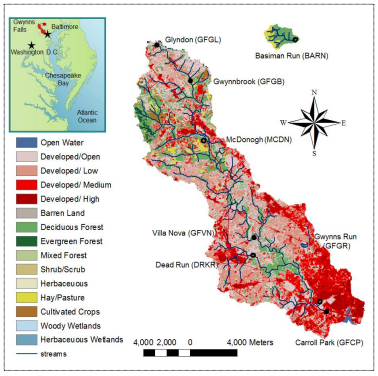
Figure 7: write n=1, n=2, n=3 etc...when reporting numbers of study sites per land use category.

Reply: This figure has been modified (now Fig. 4) and now it is not necessary to write number because all the sites are shown in the figure.

âĂŤEnd of revision

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C5560/2015/bgd-12-C5560-2015-supplement.pdf

1 **Figure 1**



2

3

1

Fig. 1.

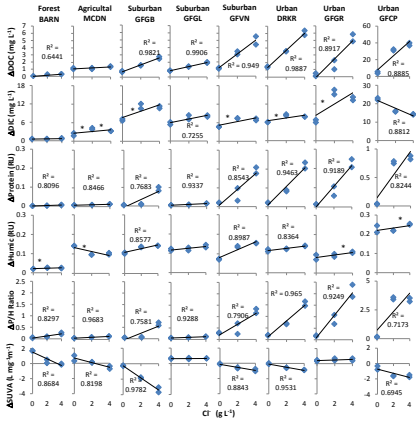

Forest BARN
Agricultal MCDN
Suburban GFGB
Suburban GFGL
Suburban GFVN
Urban DRKR
Urban GFGR
Urban GFCP
ΔDOC (mg L-1)
ΔDIC (mg L-1)
ΔProtein (RU)
ΔHumic (RU)
ΔF/H Ratio
ΔSUVA (L mg-1m-1)
Cl- (g L-1)

Fig. 2.

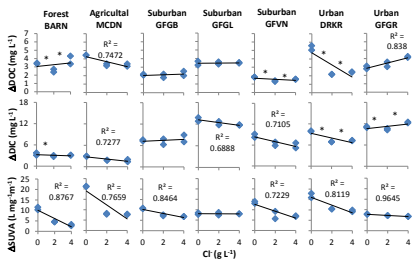
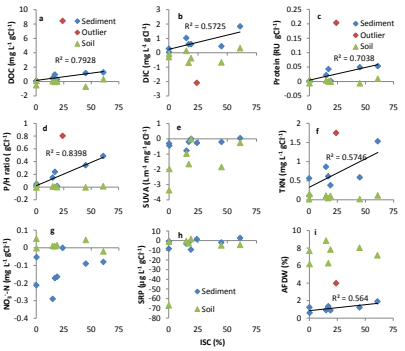

Forest BARN
Agricultal MCDN
Suburban GFGB
Suburban GFGL
Suburban GFVN
Urban DRKR
Urban GFGR
ΔDOC (mg L-1)
ΔDIC (mg L-1)
ΔSUVA (L mg-1m-1)
Cl- (g L-1)

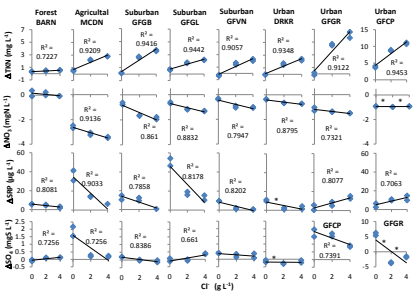
Fig. 3.



1

1

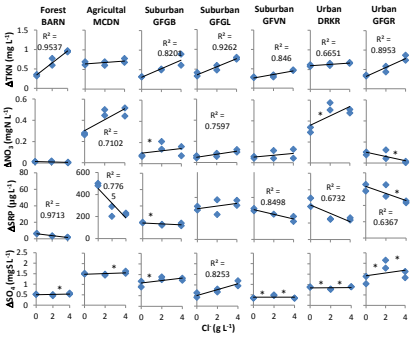
Fig. 4.



1

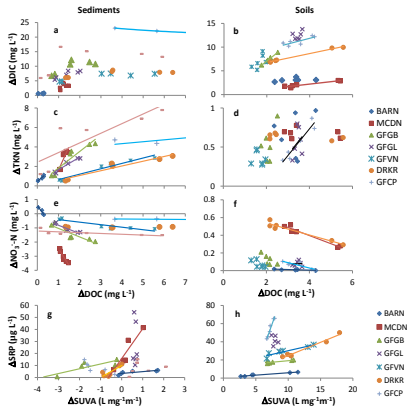
1

Fig. 5.


Forest BARN
Agricultal MCDN
Suburban GFGB
Suburban GFGL
Suburban GFVN
Urban DRKR
Urban GFGR
ΔTKN (mg L-1)
ΔNO3 (mgN L-1)
ΔSRP (µg L-1)
ΔSO4 (mgS L-1)
R² = 0.9537
R² = 0.8208
R² = 0.9262
R² = 0.846
R² = 0.6651
R² = 0.8953
R² = 0.7102
R² = 0.7597
R² = 0.776
R² = 0.9713
R² = 0.8498
R² = 0.6732
R² = 0.6367
R² = 0.8253
Cl- (g L-1)

1

Fig. 6.


Sediments
Soils
a
b
c
d
e
f
g
h
ΔDIC (mg L-1)
ΔTKN (mg L-1)
ΔNO3 -N (mg L-1)
ΔSRP (µg L-1)
ΔDOC (mg L-1)
ΔSUVA (L mg-1m-1)
BARN
MCDN
GFGB
GFGL
GFVN
DRKR
GFCP

1

Fig. 7.

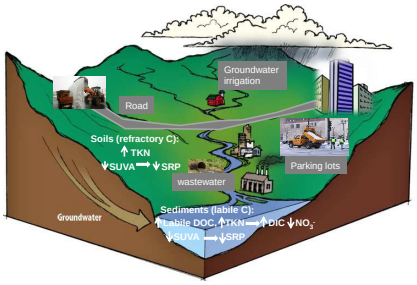

Groundwater irrigation
Road
Soils (refractory C):
↑TKN
↓SUVA → ↓SRP
Parking lots
wastewater
Groundwater
Sediments (labile C):
↑labile DOC, ↑TKN → ↑DIC ↓NO_3^-
↓SUVA → ↓SRP

Fig. 8.