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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

Anonymous Referee #1

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Review of: "Salinization alters fluxes of bioreactive elements from streams and soils across land use" by Duan & Kaushal. General comments: Duan & Kaushal present a study dealing with the effects of anthropogenic salinization on stream ecosystems across variable land uses. The authors aim to evaluate the effect of salinization by combining field and laboratory observations -I strongly value that- to approach the influence of salinization on C and nutrient dynamics. Also, they experimentally address such a influence in both in-stream sediments and soils from riparian zone. The topic is certainly relevant as we still lack data about the effects of human activities on freshwaters systems and particular information about the implications of salinization on stream functioning is currently of vital importance in urbanizing watersheds in many regions (e.g. USA). In this sense, authors well describe the environmental problem and the urgent needed of studies in this field. 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 . 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). 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 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

I am convinced that, following serious changes, authors may achieve a valuable Ms which will be of interest to ecologists and environmental managers concerned with functional consequences of salinization in river ecosystems.

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

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.

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

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

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)

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)

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)

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

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. Also, authors should explain how they calibrated changes with salinity using the experimental controls. In Fig. 2 authors slightly explain how they did. Such a information should be included here. 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 $p = 0.05$.

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

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?

- 2.3 Laboratory salinization: what about concentrations of Na?

- 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?

- when authors mean ambient temperatures: 19-22 °C: was the experiment placed in a climate chamber where temp. cycles were programmed (day-night temperature?). Please, clarify it

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

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.

How authors calculated the ISC?

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

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

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.

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

—End of revision

References used

*Effects of increasing salinity on freshwater ecosystems in Australia. Aust J Bot 51:655–665

** Effect of imposed salinity gradients on dissimilatory arsenate reduction, sulphate reduction and other microbial processes in sediments from two California soda lakes. Appl Environ Microb 73:5130–5137

***Drought-induced saltwater incursion leads to increased wetland nitrogen export. Global Chang.Biology 19: 2976-2985

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