Fundam. Appl. Limnol. 194/1 (2020), 1–9
published online 31 July 2020, published in print August 2020

Article

Drought may amplify the impacts of salt pollution in pond ecosystems: an experimental exploration

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With 3 figures and 2 tables

Abstract: Pond ecosystems are biodiversity-rich habitats, which support great biological diversity and provide important ecological services, but increasingly face risk of pollution and drought events. With increase in use of road-salts, ponds become vulnerable to high levels of salt pollution and may impair their biological communities and ecosystem functions. However, understanding the impacts of these two threats combined are limited. In this study, we experimentally investigated the impacts of road-salt pollution and the expected future increase in drought events on ponds' physical conditions, communities and ecosystem functions. In a two-way factorial design, 20 experimental mesocosms were used to test the individual and combined effects of climate change-driven drought events and salt pollution on natural pond ecosystems. Treatments were presence or absence of water *salinization* to mimic pollution by road-salts, and *drying* to mimic drought events. Our drought treatment doubled water salinity during the experimental period. While salt additions significantly affected ponds' physical conditions and leaf litter decomposition, both salt additions and drying showed no independent impacts on pond biota and ecosystem functions. However, our path analysis revealed that drying indirectly reduced leaf litter decomposition and ecosystem productivity through changes in ponds' physical conditions, although it did not affect biomass of insects and periphyton. Overall, our findings suggest that anticipated drought events will amplify road-salt pollution, and subsequently affect ponds' biodiversity, food webs, and ecosystem functions. Implications for restoration, conservation and climate change adaptation may include actively managing snow-melting salts and long-term monitoring of changes in ponds' biophysical conditions and ecological functions.

Keywords: pond ecosystems; road salts; wetlands pollution; drought; biodiversity; ecosystem functions

Introduction

Freshwater ecosystems are vital natural habitats that support great biological diversity and several ecological services. In particular, small and shallow ponds are considered unique and significant areas for their many ecological roles and services (Downing 2010; Céréghino et al. 2014; Hassall 2014). Generally, ponds host large numbers of species, serve as sinks for carbon and nutrients storage, provide floodwater mitigation, water pollution control, food, income generation for local communities, and grazing areas for livestock during dry seasons.

Pond ecosystems also face many threats and their important ecosystem services are declining due to an increase of many anthropogenic disturbances (e.g. Dudgeon et al. 2006), including urbanization, agricultural expansion, and pollution. Urbanization not only leads to loss of pond area, but also contributes to pond pollution as more roads, houses, farms, and industrial centers are established. For instance, pond pollution due to deposition of road salts and agricultural

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DOI: 10.1127/fal/2020/1225
www.schweizerbart.de
1863-9135/20/1225 $ 2.25

chemicals has become a major ongoing environmental challenge all over the world, but particularly in North America and Europe (Blomqvist & Johansson 1999; Nielsen et al. 2003; Sanzo & Hecnar 2006; Corsi et al. 2010; Roy & Malenica 2013; Van Meter & Swan 2014). In the United States of America, the annual use of deicing salts has substantially increased over the last three decades, from 7.2 million tons per year in mid 1970s to 22.0 in the 2000s (Findlay & Kelly 2011). According to Environment Canada, > 4 million tonnes of salts (primarily NaCl and CaCl) are being distributed annually across Canada for snow melting. While these are thought to be conservative estimates, most of these salts can be expected to be washed by winter runoff into wetlands and road-side ponds. Consequently, these concentrated salts can impose dramatic changes in ponds' physical conditions and may affect their biological communities as well as reduce their ecological functioning. A number of studies have documented the adverse effects of salt toxicity on the structure, composition, survival, and reproduction of biological communities in pond, lake, and stream ecosystems (e.g., Corsi et al. 2010; Van Meter et al. 2011; Van Meter et al. 2014; East et al. 2017; Stoler et al. 2017; Schuler et al. 2017; Trombley et al. 2018).

On the other hand, projected climatic changes include warmer conditions and more variation in rainfall timing and magnitude around the world. While worldwide summer temperature is predicted to increase at least 1.5 °C in the coming decades, precipitation is expected to decrease by 15 % in average. Consequently, summer months are expected to be hotter and drier, and also more rainfall is expected during fall and winter seasons (IPCC 2013). The anticipated rise of temperature and drought events will further amplify ponds' salinization and affect their ecological integrity due to high evaporation rates in the drier summers (see Herbert et al. 2015 for a review). Examples of expected effects of climatic change on chemical and physical characteristics of ponds comprise increased salinity resulting from higher temperatures and evaporation rates, and loss of wetland volume during drought or flooding events due to sedimentation. Biological changes include changes in plant and animal phenology, composition, abundance, and diversity.

Despite the plethora of evidence about the ecological risks of salt pollution in freshwaters of temperate regions, there is limited understanding of how anticipated climatic changes and particularly droughts in the coming decades may interact with such human pollution and affect biodiversity as well as specific ecosystem processes in these small ponds (Downing et al. 2006; Downing 2010). For instance, it is unclear how much drought will exacerbate the effects of salt pollution. Also, importantly, how will salinization from both, runoff of deicing salts and drought synergistically influence composition and diversity of pond organisms (e.g., insects and phytoplankton) as well as ecological functions such as primary production and leaf litter decomposition, and nutrient cycling and mineralization?

We hypothesize that drought can influence pond ecosystems and their associated biological communities and ecosystem functions through at least two pathways. While direct drought impacts may reduce habitat area and volume, drought may also indirectly exacerbate pond salinization as a result of increased evaporation due to increased temperature and fewer rainfall events. Moreover, these two pathways may interact with each other. Here we present an experimental study in which we aim to 1) understand the ecological responses of pond ecosystems to salt pollution and drought periods through an experimental manipulation of salt addition and drying in a factorial experiment; and 2) quantifying the magnitude of difference in pond salinity caused by drought compared to salinization induced by road-salt pollution on pond ecosystems. In particular, we ask the following questions:

1. What is the magnitude of difference in salinization that drought may create in pond ecosystems compared with human-induced salinization (e.g. road salt pollution)?
2. What are the possible direct and indirect effects of drought on organisms and ecosystem functions in pond ecosystems?

Material and methods

Experimental design and treatments

This experiment took place at the Experimental Pond Facility at the University of British Columbia, Vancouver, Canada, from August 6 to September 25, 2016. We used a randomized 2 × 2 factorial design experiment, with 20 wading pools (diameter 1.5 m depth 0.35 m, 300 L) used as experimental pond mesocosms to simulate effects of climate change-driven drought events on natural pond ecosystems in concert with salt pollution. Presence or absence of drying and water salinization treatments were applied to mimic drought and pollution by salts, respectively, with each of the four combinations replicated five times. On August 5th, 2016 the wading pools were each filled with 30 L of unfiltered natural lake water from UBC's Malcolm Knapp Research Forest, as well as 10 g of red alder leaves (*Alnus rubra*) added as an organic matter source and substrate. Mesocosms were then topped up with Vancouver municipal tap water. On August 6th, we measured the mesocosms' chemistry and physical conditions, and then treatments were applied. First, to mimic presence of pond salinization due to road salts, 28 g of regular table salt (NaCl) was added to increase water salinity to about 0.3 ppt, which exceeds salinity levels for natural ponds (~0.1 ppt). We note here that salinity level of 0.3 ppt has been shown to not be lethal to many aquatic organisms, but enough to cause some stress (see Thompson & Shurin 2012). Second, to simulate presence of drought conditions, half of the mesocosms were excluded from topping up of water and left to dry out over the course of the experiment. To compensate for evaporation in the non-drying treatment units, we replenished evaporated water once a week. These regular additions of 2-L of distilled water kept the chemical composition of mesocosms' water in salt and control units the same as pre-treatment levels.

Measurements and response variables

Several ecological responses including physical conditions, biota biomass and ecosystem functions were evaluated in this study. Measurements of physical response variables such as water temperature, pH, depth, conductivity, dissolved oxygen, and turbidity were taken immediately before the application of treatments and one-day post-treatments, and then continued on a weekly basis until the end of the experiment on September 20th. We performed our routine measurements one day after water additions to allow for ecosystem homogeneity and stability. These physio-chemical variables were measured using a handheld probe (YSI®, Yellow Springs, OH, USA).

For the mesocosms' biotic communities, samples were collected once at the end of the experiment, then preserved, stored and processed in the lab. In particular, we measured the biomass for four groups of pond biota separated into insects, zooplankton, periphyton, and phytoplankton as described below. In addition, two ecosystem functions were assessed as indicators of ecosystem health: leaf litter decomposition and primary productivity (see below). All samples were collected, stored, processed and analyzed following the procedures described in Hauer & Lamberti (2017). In the appendices we provided two datasets (i.e. Data S1 – *salinity.csv* and Data S2 – *drying.csv*) include all above mentioned response variables used in this publication as well as a metadata form (i.e. metadata S1 – *drying.doc*) describes these two datasets.

Sampling and laboratory processing

Periphyton samples were collected by placing two unglazed clay tiles (one for dry biomass and the other for chlorophyll-*a* concentration analysis) in each mesocosm for seven weeks, which were then retrieved and scrubbed by toothbrush into a 50 ml falcon tube and stored on ice before transfer to the lab for further processing. In the lab, all samples were filtered through Whatman GF⁄C filter paper, and then dry biomass or chlorophyll-*a* concentration analysis was performed. The biomass filters subset were dried in the oven at 60 °C for 24 hours, and then biomass was obtained by subtracting the weight of oven-dried samples from the initial filter weight. Chlorophyll filters were soaked in 6 ml acetone (90 %) and covered with a black sheet to exclude light, then left in the fridge for 24 hours for cold extraction. From the acetone cold-extraction procedure, chlorophyll concentration in the samples was measured based on 1 ml extract using a Turner Trilogy fluorimeter (Turner Designs; Welschmeyer 1994).

For assessing phytoplankton biomass, 250 ml samples of water were taken from each mesocosm and kept in coolers prior to transport to the lab. During laboratory work, samples were filtered through Whatman GF⁄C filter paper, then filters were oven-dried at 60 °C for 24 hours, and then biomass was obtained by subtracting the weight of oven-dried samples from the initial filter weight. Zooplankton biomass was estimated based on a 2-L sample taken from the water column of each mesocosm and sieved through a 63 µm sieve. We rinsed samples from sieves into jars and added 20 ml of ethanol (70 %) to the contents then transferred to the lab. In the lab, all samples were filtered through Whatman GF⁄C filter paper, and then dry biomass analysis was performed. The filters were dried in the oven at 60 °C for 24 hours, and then biomass was obtained by subtracting the weight of oven-dried samples from the initial filter weight.

Similarly, macroinvertebrate communities were sampled from the entire mesocosm's area using a kick-net, then specimens were rinsed and stored in jars with 20 ml of ethanol (70 %). All collected specimens were identified to family level and counted, then filtered through Whatman GF⁄C filter paper to estimate biomass. The filters were dried in the oven at 60 °C for 24 hours, and then biomass was obtained by subtracting the weight of oven-dried samples from the initial filter weight.

Two ecosystem functions, leaf litter decomposition and ecosystem productivity were assessed. Approximately 3 g of air-dried alder leaves were placed in a fine mesh size bag (0.5 mm) and two bags were deployed in each mesocosm for 7 weeks. On August 20th, the litterbags were gently collected; remaining material was emptied onto pre-weighed aluminum pans and oven-dried at 60 °C for 24 hours, then reweighed. The decomposition rate was obtained by calculating the percent change in the leaf dry mass during the deployment period. Ecosystem productivity was assessed based on the changes of dissolved oxygen concentrations in the mesocosms following the light & dark-bottle method (Wetzel & Likens 2000). Dissolved oxygen concentrations were monitored for about one hour using a handheld probe (ProODO -YSI®) using light and dark bottles and then net primary productivity (NPP) was calculated as the difference between gross primary production (GPP) and ecosystem respiration (R) during the measurement period. While (R) equals the final oxygen concentration in the dark bottles, GPP is the difference between the final oxygen concentration of light and dark bottles.

Data analysis

Data analysis was carried out in R (R Core Team 2013). For details of the analysis refer to R code provided in appendices (i.e. R code S1). During exploratory analysis, outliers were detected in two response variables, which were turbidity and ecosystem productivity; for both variables we replaced the outliers with the median as recommended by Rousseeuw & Leroy (1987) and Osborne & Overbay (2004) to reduce its effects on our conclusions. The effects of drying treatments on pond biota and physical conditions were tested using Analysis of Variance (ANOVA). The possible direct and indirect effects of salinization due to drying on leaf litter decomposition and net primary productivity (NPP) in pond ecosystems were assessed by Structural Equation Modeling (SEM) with the R package '*lavaan*'. Our SEM aimed to assess two causal hypotheses that describe influences of drought on the ecosystem functions:1) increased salinity levels due to evaporation directly and independently affect ecosystem functions; or 2) salinization due to evaporation affects both physical conditions and/or pond biota (e.g. insects or periphyton), which ultimately influence these ecosystem functions. To address colinearity among our mesocosms' physical condition response variables, we derived a composite of variables representing the abiotic conditions in ponds using Principle Component Analysis (PCA), which includes five variables including water depth, pH, temperature, turbidity, and dissolved oxygen. We specifically used the first PC-scores (i.e. PC1, which expressed 47% of variance) as an index of abiotic conditions, which was further used as an endogenous variable in the path analysis.

Results

Effects of drying on trends of water salinity in ponds

The degree of salinization in mesocosms significantly increased over time due to the evaporation effect (Fig. 1). Compared to the control, the drying treatment increased salt concentration in the ponds two-fold, and likewise in the salt-treated ponds (Table 1). By the end of the experiment water amount (as measured by depth) was substantially reduced to half in the ponds where the drying treatment was applied compared to the control mesocosms (Table 1).

Effects of drying and salinization on ponds biota

The ANOVA results showed that salt additions had no independent significant effect on biomass of all studied groups including insects, zooplankton, periphyton and phytoplankton (all with $p > 0.05$, $F_{1, 16} = 2.67$, 2.25, 0.53, 4.36, respectively). However, we note that there was a nearly significant effect on phytoplankton ($p = 0.053$) which is close enough to mention, even if it is not significant at the fairly arbitrary 5 % cutoff. Similarly, drying did not significantly affect the biomass for any group independently ($p > 0.05$), although the interaction term between salinization and drying was found to have negative significant effects on insect biomass only ($F_{1, 16} = 6.52$, $p = 0.02$, Table 2 and Fig. 2).

As for ecosystem functions, drying had no significant influence on either productivity or decomposition rates (both $p > 0.05$, $F_{1, 16} = 0.16$, 2.39, respectively). However, salt additions significantly increased leaf lit-

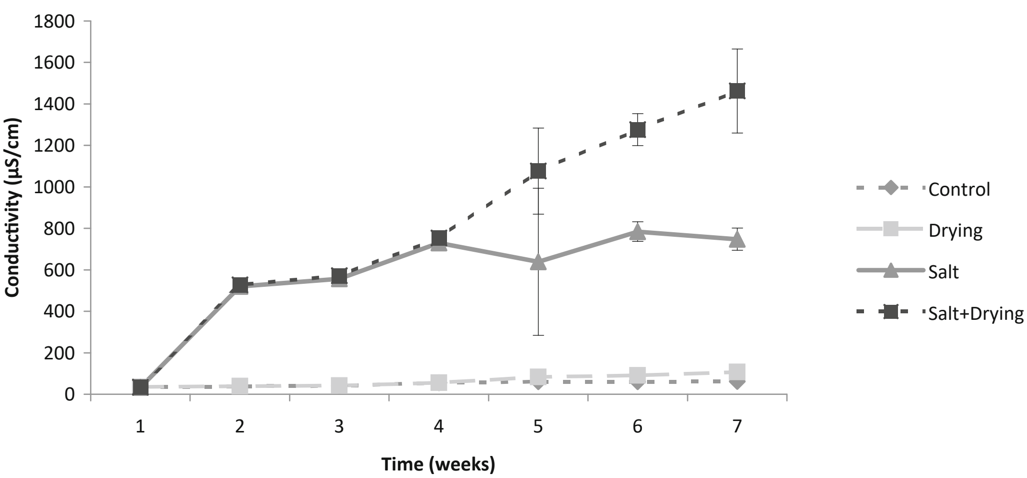

Fig. 1. The trend of increase in water salinity during the seven-week period of the experiment.

Table 1. Average estimates (± 1. SD) for six physical condition variables measured at the end of the experiment. S^0& D^0 = no salt additions and no drying; S^0& D^+ = no salt additions but drying; S^+& D^0 = salt additions and no drying; S^+& D^+ = salt additions and drying.

Treatments	Conductivity (μS/cm)	pH	Temperature (°C)	Turbidity (NTU)	Depth (cm)	Dissolved oxygen (mg/L)
S^0& D^0	62.2 (5.6)	7.84 (0.04)	14.58 (0.3)	2.32 (1.3)	8.34 (3.9)	8.43 (0.2)
S^0& D^+	106.9 (29.1)	7.79 (0.05)	14.08 (0.1)	1.79 (1.0)	5.7 (0.7)	8.33 (0.3)
S^+& D^0	747.4 (53.7)	7.49 (0.06)	14.6 (0.2)	4.45 (1.6)	9.62 (0.8)	8.22 (0.3)
S^+& D^+	1462.6 (202.4)	7.42 (0.2)	14.32 (0.3)	4.54 (1.9)	5.54 (0.4)	8.0 (0.4)

Table 2. Results of two-way ANOVA analysis on effects of salt additions and drying on biomass of four pond's biota measured at the end of the experiment.

Source	d.f.	Sum Sq	Mean Sq	F value	Pr(>F)
			Insects biomass		
DRY	1	2.180e-06	2.180e-06	0.193	0.6665
SLT	1	3.026e-05	3.026e-05	2.678	0.1212
DRY:SLT	1	7.373e-05	7.373e-05	6.526	0.0212*
Residuals	16	1.808e-04	1.130e-05		
			Zooplankton biomass		
DRY	1	0.000480	0.0004802	2.067	0.170
SLT	1	0.000524	0.0005243	2.257	0.152
DRY:SLT	1	0.000138	0.0001383	0.596	0.452
Residuals	16	0.003716	0.0002323		
			Periphyton biomass		
DRY	1	0.001231	0.0012309	1.260	0.278
SLT	1	0.000525	0.0005253	0.538	0.474
DRY:SLT	1	0.000658	0.0006578	0.673	0.424
Residuals	16	0.015635	0.0009772		
			Phytoplankton biomass		
DRY	1	3.640e-07	3.645e-07	1.051	0.3205
SLT	1	1.512e-06	1.512e-06	4.362	0.0531
DRY:SLT	1	1.300e-08	1.250e-08	0.036	0.8518
Residuals	16	5.548e-06	3.468e-07		

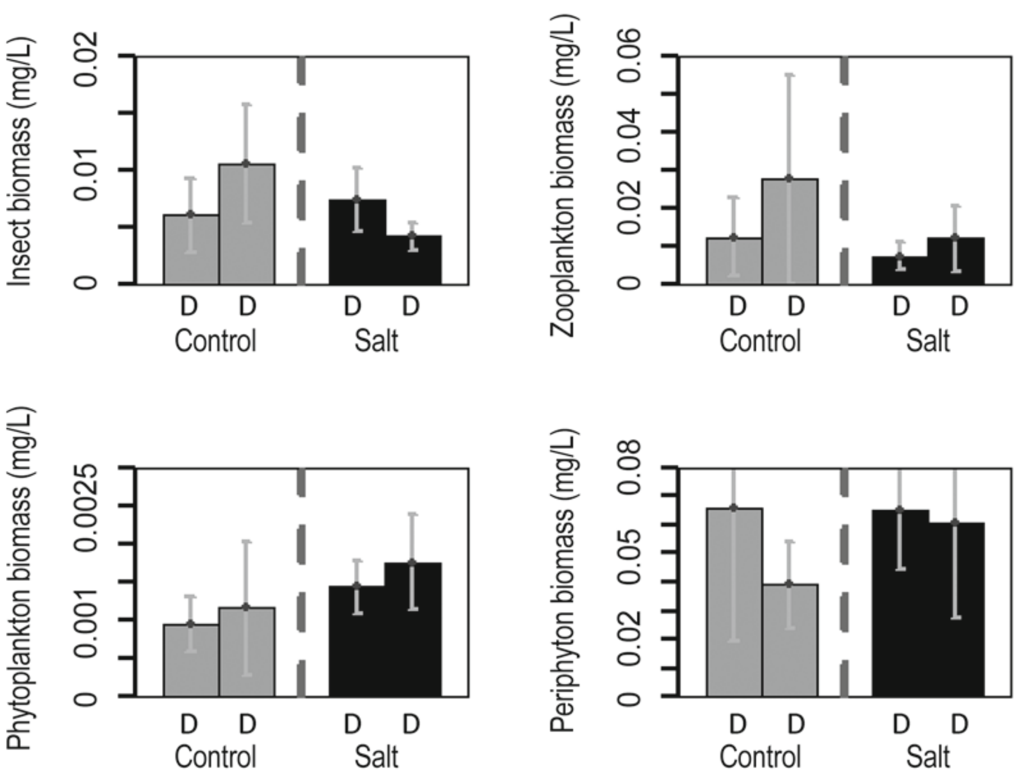

Fig. 2. The effects of drying and salt treatments on biomass of four groups of pond biota. D^+ and D^0 indicate presence and absence of drying treatment, respectively.

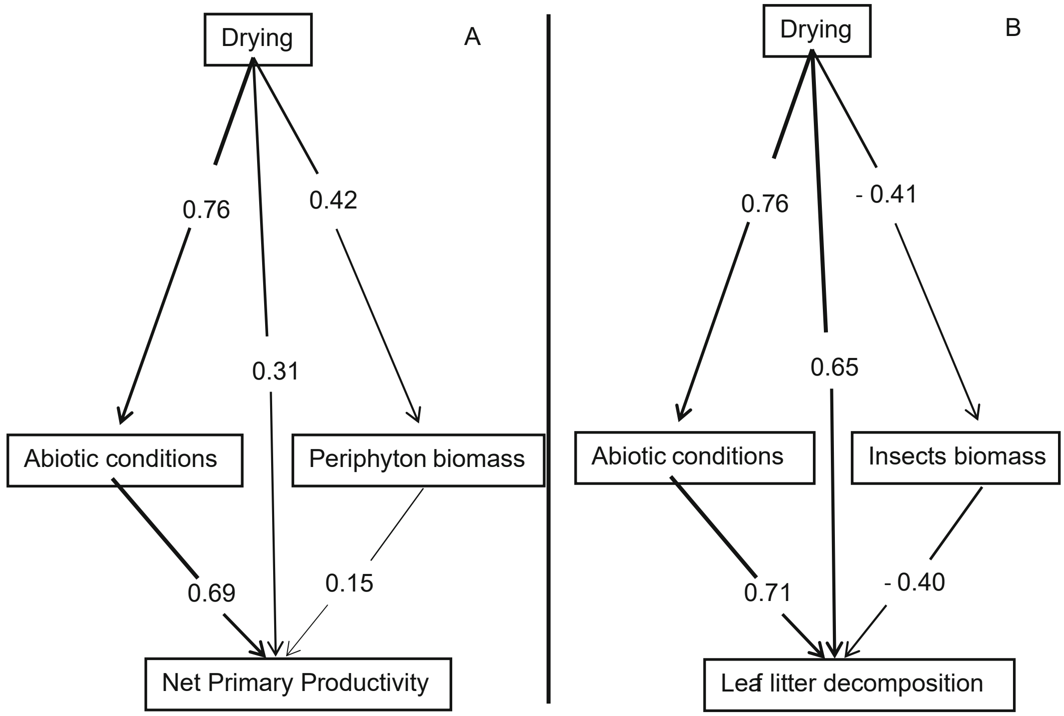

Fig. 3. Path diagram describes the effects of drying in ponds on primary productivity **(A)** and litter decomposition **(B).**

ter decomposition rate by 10 % (60 % of litter weight loss occurred in salt-added ponds compared to 50 % in the controls; $F_{1,16} = 50.12$, $p < 0.001$) but did not affect productivity ($F_{1,16} = 2.82$, $p > 0.05$). Also, interactions between salt addition and drying treatments showed no significant effect on either ecosystem function (both with $p > 0.05$, $F_{1,16} = 0.75$, 0.32, for productivity and litter decomposition, respectively).

We used structural equation modeling to examine direct and indirect causal effects of drying on pond ecosystems functions. Two SEMs were established, one for each of the two ecosystem functions. Results of the ecosystem productivity model showed that drying had both direct and indirect effects on net primary productivity (Fig. 3A). Drying did not show strong direct effects on productivity ($r = 0.31$), however, it indirectly affected it through changes in pond physical conditions (i.e. abiotic factors, $r = 0.69$). The path also showed a weak causal link between drying and pond periphyton biomass on one hand and between periphyton biomass and productivity on the other hand.

Similarly, drying showed strong direct links with litter decomposition ($r = 0.65$) and an even stronger indirect causal relationship through effects on abiotic conditions ($r = 0.71$, Fig. 3B). However, insects' biomass appears to have a negative weak link to litter decomposition ($r = -0.40$). Overall, both ecological functions were more influenced by impacts of drying on physical conditions in the ponds rather than the biotic community.

Discussion

Freshwater ecosystems, and specifically ponds, are important habitats that support high biological diversity and provide substantial economic and ecological values (Downing et al. 2006). Human disturbances including pollution by road salts are major challenges affecting these unique ecosystems and make them more vulnerable to the impacts of anticipated climatic changes (Herbert et al. 2015; Castillo et al. 2018). Our experimental manipulations provided a glimpse of the potential impacts of salt pollution on pond ecosystems independent of, and in concert with, the anticipated drought events. While salt additions simulated the current situation of pond pollution due to winter runoff of road salts, the drying treatment simulated the anticipated drought events in the coming years when extreme rainfall shortages may occur. Moreover, the interaction of these two treatments mimicked the occurrence of drought events on top of salt pollution as a realistic situation in current and future time.

Not surprisingly, the manipulation increased the salinity of the ponds such that they were 12× higher in concentration, which is similar in magnitude to some field studies (e.g. Corsi et al. 2010). However, we noticed that the drying effect had further doubled the salinity level in both salt-polluted and unpolluted ponds. In addition, our experiment showed that the level of human-induced salinity in ponds was 6-fold higher compared to drought-induced salinity. This finding implies that anticipated drought events increases ponds' salinity compared to its ambient salinity level. Given the short duration of our experiment (i.e. 7 weeks), these findings together imply that longer and harsher drought seasons may not only increase salt concentrations in ponds but may even rapidly cause natural ponds to have extremely toxic levels of salinity in agreement with findings from other recent studies (e.g. Lund et al. 2016).

Pond salinization due to pollution or/and drying is known to have significant impacts on ponds' physical conditions and subsequently biodiversity and ecosystem functions (Herbert et al. 2015; Lund et al. 2016; Castillo et al. 2018; Trombley et al. 2018). Consistent with this, drying and salinization appears to directly affect ponds physical conditions such as water depth, pH, turbidity, and temperature, but no significant effect was found in dissolved oxygen. Despite these high levels of salinization in ponds, the only group of organisms that showed significant negative response to interaction effects of drying and salt additions was insects, but not zooplankton, phytoplankton, or periphyton. As suggested by Castillo et al. (2018), on one hand this may be due to the relative salinity tolerance of these three groups compared to aquatic insects. On the other hand, it might also be due to having more species, some of which might be more tolerant, thus there is some degree of compensation by species replacements, but we cannot determine this from our study given our limited taxonomic resolution

Despite relatively large variation in our measured responses we were able to detect some significant results (e.g. insect biomass) in response to our drying and salinization manipulations, but failed to do so with other responses (e.g. primary productivity). We suspect that this may be due to shifts in the kinds of species contributing to primary production, such that salt-sensitive species drop out of the community, and salt-tolerant species increase in abundance. That seems to suggest compensatory responses within a trophic level despite the impact of the stressor (Greig et al. 2012; East et al. 2017; Stoler et al. 2017).

Both ecosystem functions studied in this study seemed to be more affected by changes in physical or abiotic conditions, and less by the biological communities in these ponds. Future studies may certainly consider this dimension to better capture some signals related to effects of biological communities in these ecosystem functions. In addition, focus should be centered on using additional response variables such as compositional and functional traits in addition to biota biomass.

Overall, our study findings are consistent with other recent studies that investigated impacts of salinization on river food webs and road-salts pollution on pond ecosystems (Stoler et al. 2017). For example, in agreement with East et al. (2017), the detected impacts on pond insects will have repercussions for pond food webs and subsequently constrain ecosystem functions such as litter decomposition (Schuler et al. 2017). Implications for restoration, conservation and climate change adaptation may include properly managing snow-melting salts and long-term monitoring of changes in ponds' biophysical conditions and ecosystem functions.

Acknowledgements

We would like to thank student assistants Derek Wiens, Mariella Becu, Anwen Rees, Liam Irwin and Sashka Warner from the University of British Columbia for their hard work and assistance in data collection and processing of this project, and Ahmed Almostfa Hasoba and Mubarak Salim from University of Khartoum for editing. AAHS was supported by the German Academic Exchange Service (DAAD) under REA grant agreement No. 605728 (P.R.I.M.E. – Postdoctoral Researchers International Mobility Experience). We appreciate being able to use the Experimental Ponds Facility of UBC. The work was also supported by a Natural Sciences and Engineering Research Council (Canada) Discovery Grant to JSR.

Author contributions

AAHS and JSR developed the research idea and design. AAHS collected the data and wrote first draft. CD and JSR helped in data analysis and edited and reviewed final versions of the paper.

Data accessibility

Data utilized in this publication has been provided with this submission. Please see electronic appendices S1, S2, and S3.

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Manuscript received: 30 January 2019
Revisions requested: 26 April 2019
Revised version received: 22 June 2020
Manuscript accepted: 23 June 2020

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Table of contents – Electronic Supplementary Material (ESM)

Type of supporting information	Description	Name of file
Data S1	Timeseries of water salinity data collected from the drying experiment.	*salinity.csv*
Data S2	Phiscal conditions, biodiversity and ecological processes data collected from the drying experiment.	*drying.csv*
Metadata S1	Metadata for drying experiment data (Data S1&S2)	*drying.doc*
R code S1	R code used in the analysis of data of drying experiment	*drying.R*