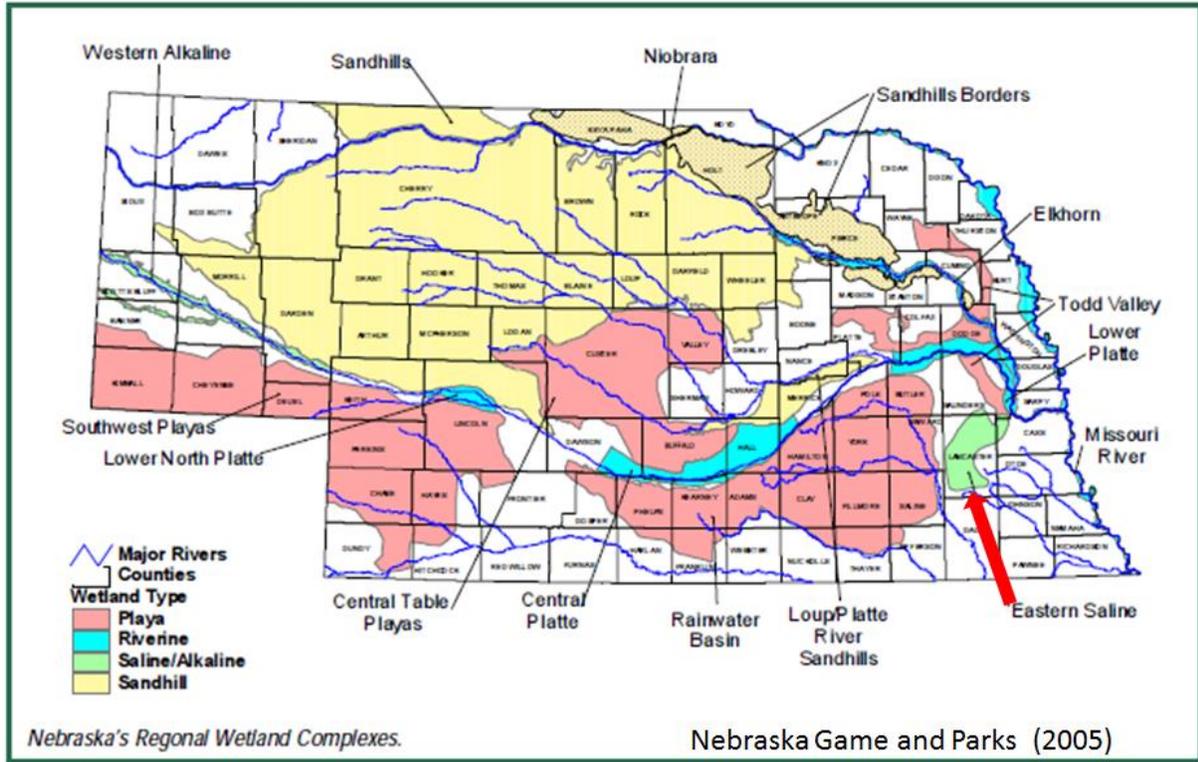


*Biogeochemical controls on saline wetland plant establishment in Nebraska's Eastern Saline Wetlands*



*Submitted by*

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## 1. Executive Summary

Nebraska's eastern saline wetlands are unique ecosystems that provide critical habitat for wildlife and salt-tolerant plant communities. However, urban and agricultural development and related activities have degraded over 80% of saline wetlands in Nebraska; now they are considered an endangered ecosystem. The goal of our study was to determine whether continuous saline groundwater addition to the soil surface would create a more favorable saline condition for saline plant species and other wetland ecosystem services. Under this overarching goal, we sought to understand how soil and water biogeochemistry respond to saline groundwater addition (a proposed saline wetland restoration practice), as well as whether this practice would shift plant communities to a saline-obligate dominant community.

We implemented the groundwater addition practice in the experimental restoration site and measured: 1) soil physicochemical changes, 2) biogeochemical process rate changes and 3) plant community and diversity changes in relation to salinity and water addition methods (slow vs. fast water addition). The experimental restoration site was located in the northern Lincoln, Nebraska and it contained 32 plots divided into four replicates of the following water additions: 1) fresh water, 2) low salinity (3 ppt), 3) mid-level salinity (12 ppt), and 4) high salinity (27 ppt), respectively in addition to two control plots. The treatment gradient was drawn from naturally occurring variation in groundwater at the site. Using water, soil and gas samples collected from the experiment site, we measured changes in porewater chemistry and rates of biogeochemical processes like greenhouse gas flux.

Biotic responses were determined with two separate surveys: 1) a germination experiment and 2) measurements of plant growth rate and diversity changes in the experiment site. The germination experiment was conducted in a temperature and light-controlled incubation lab-setting. Six saline wetland species were used as indicators of a healthy saline wetland plant community: Saltwort (*Salicornia rubra*), Saltgrass (*Distichlis spicata*), Sparscale (*Atriplex patula*), Seablite (*Suaeda depressa*), Marshelder (*Iva annua*), and Saltmarsh Aster (*Aster subulatus*). We identified all plants in the experimental plots using a quadrat (1 × 1m) and calculated the relative abundance of each species.

Our key findings are:

- 1) Continuous saline groundwater addition increased the interaction and connections between soil and saline groundwater, which created more saline pore-water conditions in the experimental restoration site. Slow groundwater addition was more effective in creating a saline conditions and generating salt crusts in the surface soil.
- 2) Saline groundwater addition increased CO<sub>2</sub> fluxes likely due to physical and chemical dissolution and desorption of inorganic carbon from the soil particles. Thus, alteration of soils by increasing salinity will affect carbon cycling (and storage) in addition to altering the saline characteristics (finding 1).
- 3) Salinity and temperature affected the germination rates. Salinity inhibited germination rates of all six targeted species. High salinity (26-30ppt) suppressed the germination rates significantly and highest germination rate reached only 8%. We found that the April thermo-period (5°C night-20°C day) stimulated germination rates for most saline species in this study. It was particularly favorable to Seablite germination.
- 4) Plant species richness decreased as salinity increased at the experiment site, mainly due to suppressed growth of terrestrial and freshwater wetland species. At Mid and High salinity treatments, target saline species became dominant in the plant communities. Spearscale and Seablite were the most abundant saline species in all salinity treatment plots, which is consistent with the results of germination experiment. Saltwort emerged only in the Mid salinity level plots.

Based on our research findings, we conclude that continuous addition of middle range salinity (i.e. 26-30ppt) in the soil surface would create favorable habitat to saline species. For best results, we also recommend slow application of middle salinity range groundwater and saline species seeding in April, Lincoln, Nebraska (i.e. temperature range: min. 5°C, max. 20°C).

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#### 4. Project Background

Nebraska's Eastern Saline Wetlands are inland saline wetland ecosystems endemic to the Salt Creek, Little Salt Creek, and Rock Creek watersheds in Lancaster and Saunders County in Lincoln, Nebraska. This unique ecosystem is fed by deep groundwater from the Dakota formation layer which contains high salt concentrations originated from ancient ocean salt deposits.

These saline wetlands provide unique habitat for salt-tolerant species including the state endangered saltwort (*Salicornia rubra*) and federally endangered Salt Creek tiger beetle (*Cicindela nevadica lincolniana*). However, extensive agricultural and residential development in the Lincoln area has degraded over 80% of the saline wetlands. Now it is considered an endangered ecosystem type with only 3,200 acres remaining (LaGrange et al., 2003).

The Saline Wetland Conservation Partnership (SWCP) was organized to conduct restoration and conservation of this endangered ecosystem. The goal of SWCP is "No net loss of saline wetlands and their associated functions with a long-term gain in sustaining wetland functions through the restoration of hydrology, prescribed wetland management, and watershed protection" (La Grange et al., 2003). One main restoration goal for the Nebraska's Eastern Saline Wetlands is restoration of native saline plants as many salt-tolerant plants found in these ecosystems are rare or endangered in Nebraska, including saltmarsh aster and saltwort. Despite the attempts to conserve and restore the remaining saline wetland fragments, there is limited scientific understanding about the Nebraska's Eastern Saline Wetlands and effective restoration strategies. Given that groundwater and soil surface interactions and connections play key roles in maintaining saline wetland conditions (Harvey et al., 2007), the conservation partnership proposed a restoration technique: introducing deep saline groundwater to surface soils to mimic groundwater upwelling and improve soil-groundwater interactions. Ideal outcomes of this restoration practice would be improving a habitat's condition for saline plant species as well as recovering wetland ecosystem services by creating more saline areas.

## 5. Project Objectives

The overall goal of this research is to test the feasibility of the proposed practice to maintain or improve the saline condition by adding saline groundwater to the soil surface. The objectives of this project were specified into three components (Fig. 1):

- 1) **Soil Physicochemical Responses:** Improve our understanding of the interactions and connections between artificial groundwater intrusion and soil physicochemical characteristics.
- 2) **Soil Biogeochemical Responses:** Understand changes in biogeochemical process rates (e.g. gas exchange rates or microbial metabolism) resulting from this wetland restoration technique.
- 3) **Biotic Responses:** Determine if saline groundwater additions can shift plant communities to a saline-species dominant community.

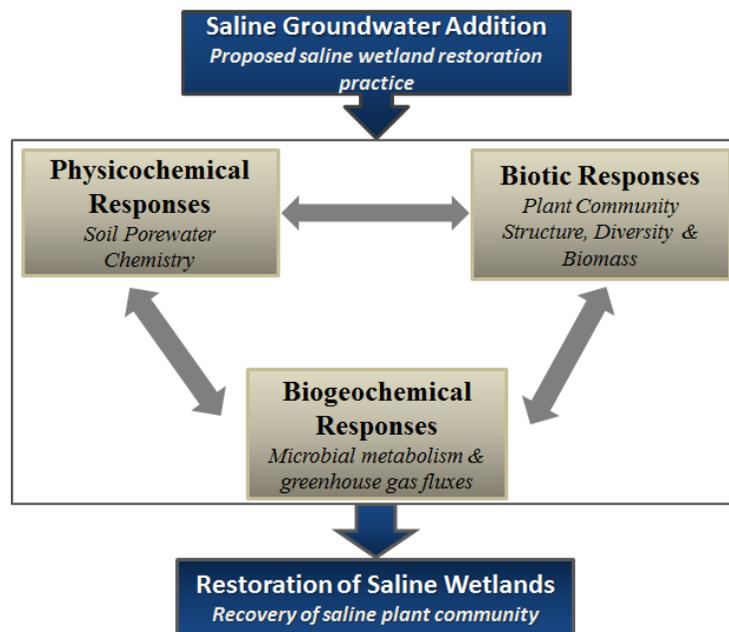


Fig. 1. Conceptual diagram of research objectives in relation to the impact of saline groundwater addition to restoration sites.

## 6. Materials and Methods

### 6.1. Experimental Restoration Site

#### a) Site Description

The experimental site is located north of Lincoln, Nebraska on 27<sup>th</sup> Street and Arbor Road near Frank Shoemaker Marsh, one of actual saline wetland restoration site in Lincoln (Fig. 2). The major research activities started in April, 2014. The final experimental design included four salinity levels (Fresh: 0.2 ppt, Low: 2-3 ppt; Middle: 18-20 ppt and High: 26-30 ppt) and two hydrologic regimes (Slow and Flush) with four replications ( $4 \times 2 \times 4 + 2$  control=total 34 plots, Fig. 3, 4). The 34 plots were randomly assigned in three dredged experimental areas.

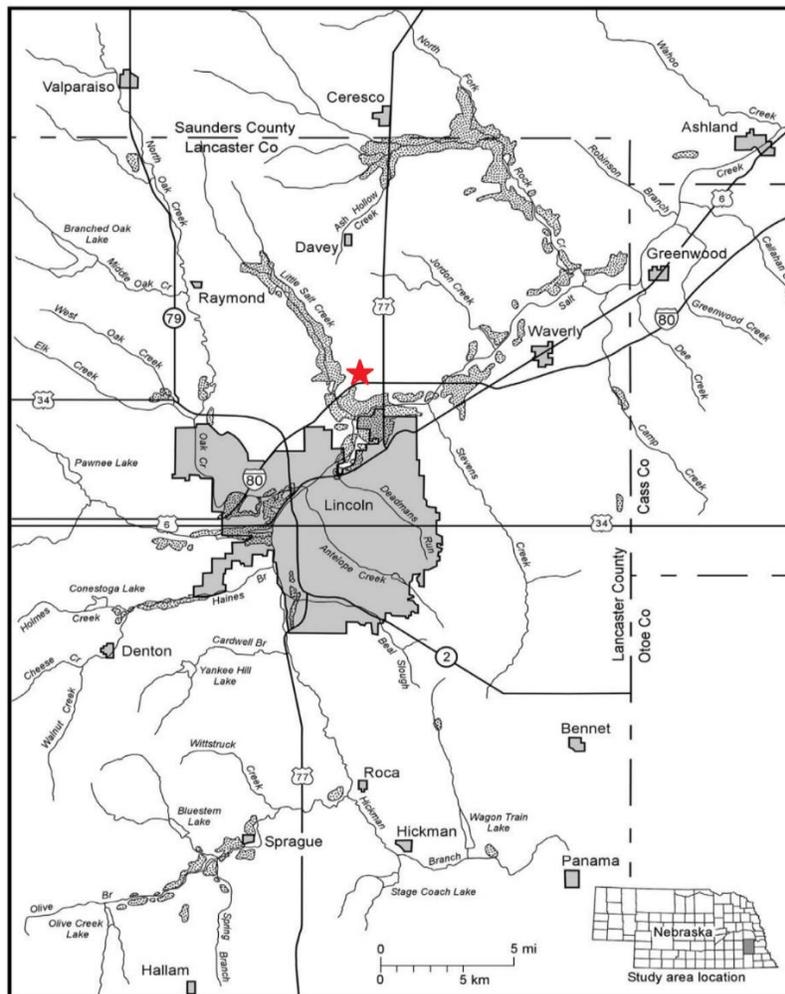


Fig. 2. Distribution of Nebraska Eastern Saline Wetland and Experimental Site (Red Star). (Modified from Harvey et al., 2007)

### *b) Experimental Design*

The salinity treatment gradient was drawn from naturally occurring variations in Lincoln's groundwater. Saline groundwater with three different salinity levels was supplied from the groundwells installed at the experimental site (20ft, 90ft and 180ft deep), referred to as **Low**, **Mid**, and **High** salinity water sources, hereafter (Fig. 4).

We used Lincoln tap water as a freshwater source to differentiate the effects of salinity and water addition. In addition to determining an environmentally relevant salinity gradient, we also had to consider how to realistically apply groundwater to the surface soil. Therefore, we applied two different hydrologic patterns to create different intensities of salt water intrusion (Fig. 5): 1) **SLOW** water addition: 55 gallons of water were released at approximate flow rate of 4 L/ hr for 2 days through the use of rain barrels and drip tapes. 2) **FLUSH** water addition: Rapid flooding was simulated by applying the same amount of water (i.e. 55 gallons) at a rate of 4L/min. This is similar to the rate at which groundwater is pumped from wells. The two control plots did not receive any water addition.

Plastic covered barriers were inserted 15 cm deep into the soil to minimize water exchange between plots. All measurements were conducted in the center of the plots and at the soil surface. Saline groundwater and freshwater were added into the experiment plots weekly from July 2014 to October 2015 except on rainy and cloudy days. A weather station was installed in Jun. 2014, and solar radiation, air temperature, relative humidity, precipitation, wind speed, and wind direction were recorded hourly basis until Oct. 2015.



Fig. 3. Saline wetland restoration experimental site

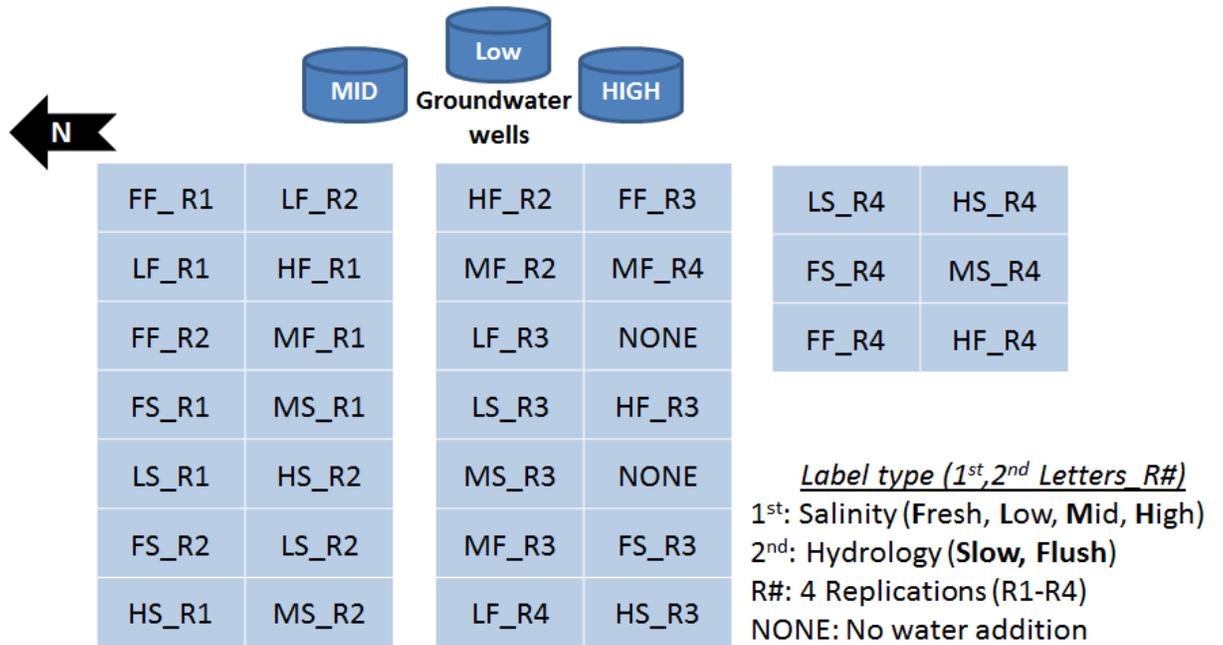


Fig. 4. Field experimental design and the layout at the site: salinity gradient (Fresh, Low, Mid and High) × hydrology patterns (Slow-S and Flush-F) × 4 replications

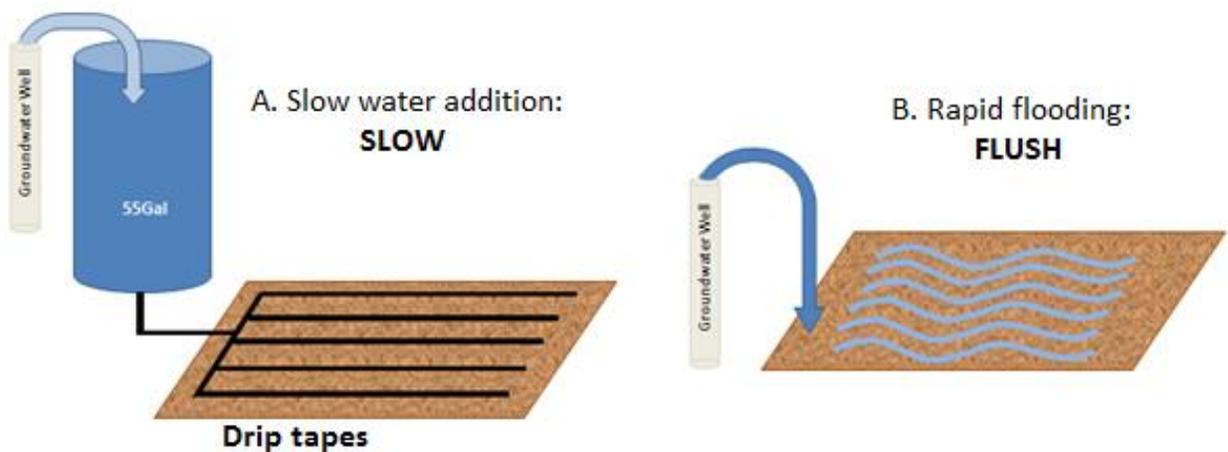


Fig. 5. Simplified conceptual demonstration of the hydrologic patterns: (A) Slow water addition vs. (B) rapid flooding Flush treatment.

## 6.2. Biogeochemical and Physical Responses-2014

In the first year of the study (2014) we focused on the physicochemical and biogeochemical responses to saline groundwater addition. To separate these from the biotic responses, mainly mediated by plant communities, we kept the experimental restoration site plant-free in 2014. Mechanical removal (mowing) followed by chemical treatment were conducted two months before the experiment, and any minor plant emergence was controlled by hand or spot chemical treatment during the experiment.

### *a) Field Sampling and Biogeochemical Analysis*

To measure the change of porewater chemistry over time in relation to saline groundwater and freshwater addition, we installed a porewater sampler in each plot and collected porewater 1-2 times per month. Porewater sampling was generally conducted 1-2 days after each water addition. Sampled porewater was then filtered within 24 hours and stored in a freezer until the subsequent analysis. The concentrations of chloride ( $\text{Cl}^-$ ), nitrate ( $\text{NO}_3^-$ ), and sulfate ( $\text{SO}_4^{2-}$ ) were measured using Ion Chromatography (DIONEX-IC).  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  are indicators of increased salinity as both are abundant in salt water.

Greenhouse gas flux was measured biweekly using a closed chamber method. Headspace gas was collected five times at 10 min intervals from closed greenhouse gas chambers (Fig. 6).  $\text{CO}_2$  and  $\text{CH}_4$  concentrations were analyzed from the headspace samples using Gas Chromatography (HP-6890), then concentration changes over time were used to calculate gas flux rate ( $\mu\text{g m}^2 \text{hr}^{-1}$ ).

Fig. 6. Experimental plot set up with greenhouse gas chamber and pore-water sampler



### ***b) Soil Core Incubation-Lab Experiment***

We collected three replicated soil cores from each plot bimonthly from July 2014 to October 2015 for lab scale soil incubation experiments. At the lab, we measured microbial-driven ecosystem processes such as denitrification, iron reduction, and sulfate reduction rates as well as extracellular enzyme activities. Given that these microbial processes occur under anaerobic condition, all soil biogeochemical processes were done in an anaerobic glove bag (Fig.7). Data from the lab incubation experiment is still under chemical and data analysis. The final results will be reported in manuscript format to SWCP.



Fig. 7. Sediment core incubation experiment in the lab setting

(Left: Anaerobic glove bag, Right: anaerobic soil incubation for denitrification measurement)

### **6.3. Biotic Responses-2015**

The second year's (2015) research objective is to understand the interaction of saline groundwater addition and subsequent biogeochemical changes with native plants growth rates. For this, we collected native target saline species seeds and conducted: 1) a germination experiment, 2) seeding in the experimental sites, and 3) plant growth rate and diversity surveys. We also continued physicochemical and biogeochemical measurements at the experimental site and in the lab as described above. The germination experiment and plant survey at the field restoration site were particularly important given that there is little information on effective seeding time and salinity level to improve saline plant communities.

### a) Seed Collection

Seven saline wetland plant species were selected based on their relative value and scarcity of species (Fig. 8, Table 1). For example, saltwort (*Salicornia rubra*) is a high salt-tolerant species and state endangered species. The targeted species are *Salicornia rubra* (common name: Saltwort), *Suaeda depressa* (Seablite), *Atriplex patula* (Spearscale), *Hordeum jubatum* (Foxtail barley), *Aster subulatus* (Saltmarsh Aster), *Iva annua* (Marshelder), and *Distichlis spicata* (Saltgrass). We collected the seeds for these species in September-November 2014 with help from the Prairie Plains Resource Institute (Mike Bullerman). Seeds were collected in saline wetlands in Lincoln, close to the experimental site (Fig. 9). Collected seeds were hammer-milled and went through a purification process. The purification process included hand picking, sequential sieving, and gentle air-blowing to separate the seeds from remaining plant materials. Purified seeds were cold-stratified for a month for the subsequent lab-scale germination experiment. Field seeding took place in March, 2015.

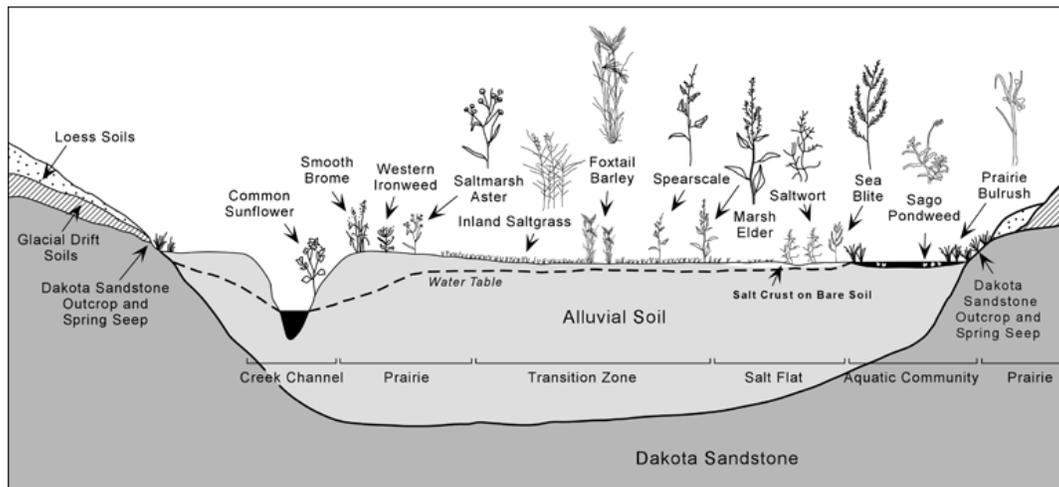


Fig. 8. Conceptual diagram of vegetation distribution along the salinity level (Harvey et al., 2007)

Table 1. Relative values of saline wetland plant species (modified based on Taylor et al., 1997 and Harvey et al., 2007)

Relative values	Species
4	Saltwort ( <i>Salicornia rubra</i> )
4	Seablite ( <i>Sueada depressa</i> )
3	Spearscale ( <i>Atriplex patula</i> )
3	Saltgrass ( <i>Distichlis specata</i> )
2	Foxtail barley ( <i>Hordeum jubatum</i> )
2	Saltmarsh Aster ( <i>Aster subulatus</i> )
2	Marshelder ( <i>Iva annua</i> )
1	Other freshwater wetland plants
0	Upland plants

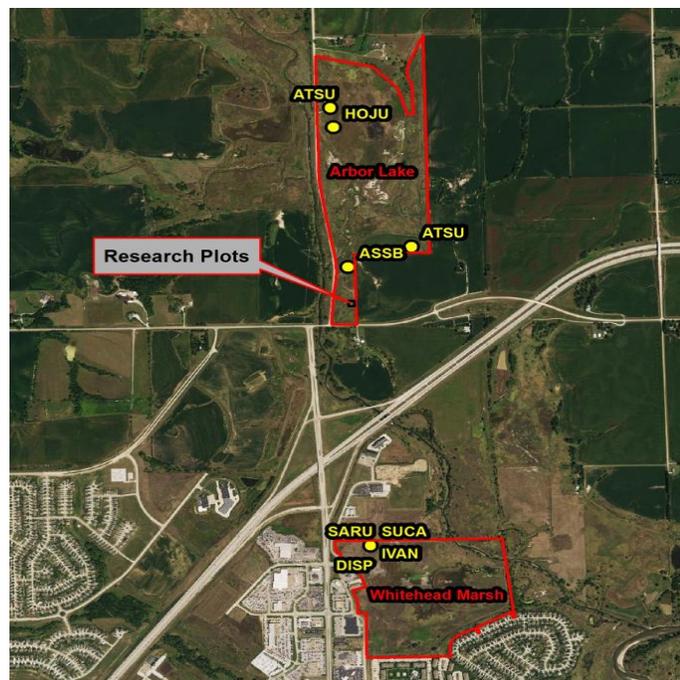


Fig. 9. Seed collection locations for Spearscale (ATSU), Foxtail barley (HOJU), Saltmarsh Aster (ASSB), Saltwort (SARU), Seablite (SUCA), Marshelder (IVAN), Saltgrass (DISP).

### ***b) Germination Experiment***

The germination experiment was conducted in a temperature and light-controlled incubation setting. Six saline wetland species (Saltwort, Spearscale, Seablite, Saltgrass, Marshelder, and Saltmarsh Aster) are indicators of a healthy saline wetland and are targeted species for this project and for the Saline Wetland Conservation Partnership. Foxtail barley seeds were not used in the germination experiment as we had a limited amount available for the field seeding.

The overall objective of the germination experiment is to find the best germination conditions (i.e. temperature and salinity levels) for each species and the optimal seeding time (i.e. optimal temperature) for the best restoration outcomes (Fig. 10). This information can be useful to identify the optimal conditions that improve inhabitation of native saline wetland species as means of saline wetland restoration practice.

We varied temperature and salinity based on environmentally relevant ranges. Three temperature levels of low-high temperature ranges that represent March (5-10°C), April (5-20°C) and May (10-20°C) conditions in Lincoln, Nebraska were used were used to find an optimal seeding time for each species. The low end temperature for each month temperature was set with dark (night condition) whereas high-end temperature was with light (day time condition). This thermo-light treatment was cycled every 12 hours. Four salinity levels representing the groundwater salinity gradient and freshwater in Lincoln were used. We obtained saline groundwater from the wells located in the experimental site, and tap water was used for freshwater source. All germination experiments were done in petri dishes (5 × 1.5 cm) with two sheets of Whatman No. 2 filter paper. We placed 10 seeds of each species between the filter sheets and then 1 mL of saline or fresh water was in each petri dish. Each treatment had five replications.

Detailed experimental design: 3 thermo-light cycles (5-10°C, 5-20°C, 10-20°C) × 4 salinity level (Fresh, Low, Mid, High) × 6 saline species × 5 replicated dishes (each dish contained 10 seeds).

The germination experiment was conducted in 2015 from February-May. The germinated seeds were counted and recorded every other day for 20 days. The percentage of seed germination of each dish at 2-day intervals were calculated as the germination rates over time.

Germination rate measurement and analysis were modified based on a previous germination experiment (Ungar and Hogan, 1970; Khan et al., 2000).



Fig. 10. Germination experiment set up and temperature/light controlled incubation chambers

### *c) Experimental Site Seeding and Vegetation Survey*

We placed the stratified seeds of seven species into the experimental site in Feb. 2015. To understand the interaction of biogeochemistry with the growth rates of native plants under different salinity levels, we measured aboveground net primary productivity (NPP) at the surface using peak biomass measurement from experimental sites between September and October. We identified all plants in the experimental plots using a quadrat (1m ×1m) (Fig. 11). All plants in the quadrat were identified by species, measured for height. The number of stems or branches on the each plant was counted. The number of species and plants were used to calculate relative abundance of each species and the plant diversity index per plot and per treatment. After completing the plant surveys for each plot, 10-20 samples of each species present in the experimental site were collected for biomass sampling. These samples were measured and counted using the same methods as used in the field. The plant samples were then placed in paper bags and oven dried at 65 °C for 14 days. The dry weight of each plant sample will be used to create an allometric regression to estimate peak biomass of each species and total biomass for each plot. Plant biomass and subsequent data analysis are still underway.



Fig. 11. Field vegetation survey quadrat (1m ×1m)

## 7. Results and Discussion

### 7.1. Soil Physicochemical Responses (Objective 1)

Groundwater pumped from wells at the experimental site showed a clear salinity gradient with salinity levels of 2-3 ppt for the shallow well (Low treatment), 18-20 ppt for middle (Mid) salinity treatment and 26-30 ppt for the high salinity. Freshwater used as the control exhibited salinity of 0.2-0.3 ppt. This salinity gradient in the groundwater created the  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  concentration gradients of pore-water samples (Fig. 12). This salinity gradient indicated by  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  concentrations was more distinctive in the plots with slow water application than those receiving water by flush method (Fig. 13).  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  concentrations in the Mid and High level treatment with slow water application were significantly higher than those in groundwater-flushed plots. We observed the salt crust formation only on the surface sediment layer of Mid and High plots with Slow groundwater applied plots (Fig. 14). This result indicates that slow application of saline groundwater in the soil surface was effective in creating saline condition with higher porewater salinity and visible salt crust formation on the surface soil.  $\text{NO}_3^-$  concentrations did not show any pattern between treatments.

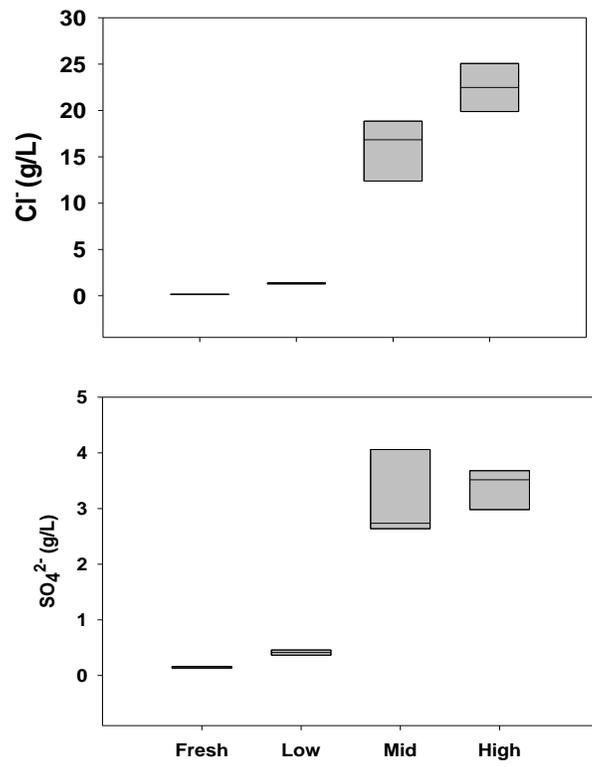


Fig. 12. Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> concentrations in the water source (n=5; tap water-Fresh; Low, Mid, High-groundwater from the wells at the experimental site)

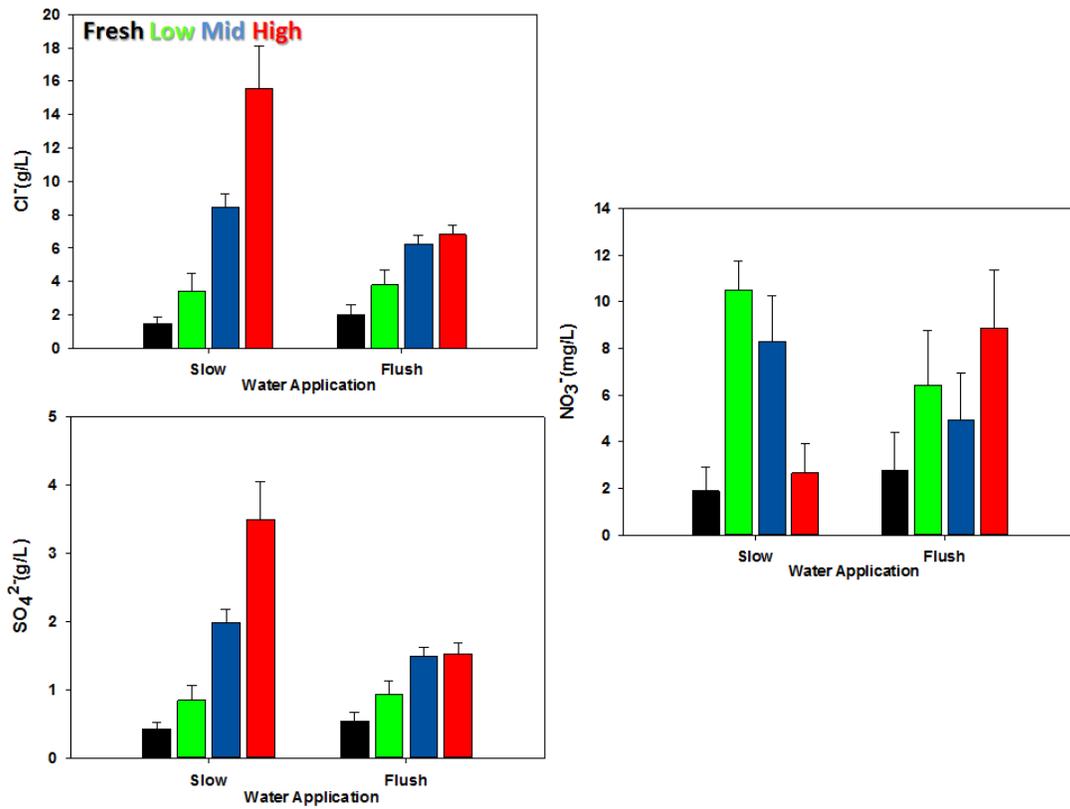


Fig. 13.  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  concentrations in the pore water collected at the experimental sites (n=16)



Fig. 14. Formation of salt crust in Mid and High Slow plots

## 7.2. Soil Biogeochemical Responses (Objective 2)

CO<sub>2</sub> fluxes responded instantly and remained high at Mid and High salinity plots with up to 10<sup>4</sup> times higher values than those in Freshwater and Low plots (Fig. 15). Given that the experimental sites in 2014 did not have any plants, this CO<sub>2</sub> pattern is likely a physicochemical response to increased salinity (Herbert et al., 2015). One possibility would be desorption and exchange of inorganic carbon with high salt from saline groundwater (Mid and High levels). Water addition pattern did not have significant influence on CO<sub>2</sub> fluxes. CH<sub>4</sub> concentrations and fluxes were low and variable regardless of salinity and water treatments. Negative CH<sub>4</sub> fluxes indicates that CH<sub>4</sub> oxidation was predominant than CH<sub>4</sub> production at the sites.

We measured other biogeochemical process rates including denitrification, iron reduction, sulfate reduction and extracellular enzyme activities. These processes are still under chemical and statistical analysis.

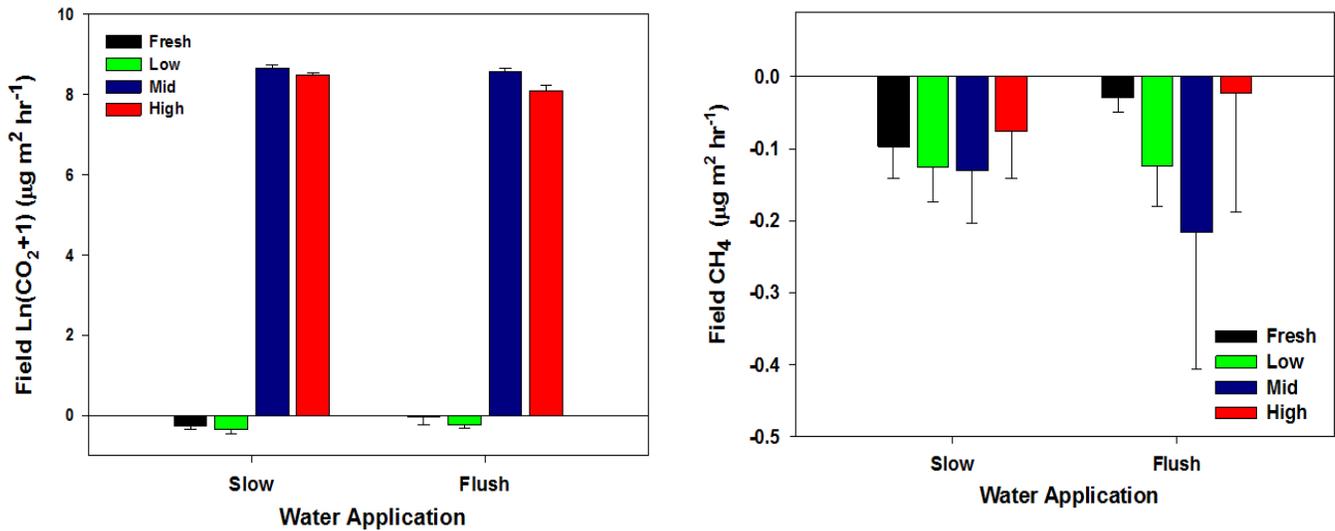


Fig. 15. CO<sub>2</sub> and CH<sub>4</sub> fluxes measured at the experimental site

### **7.3. Biotic Responses (Objective 3)**

Biotic responses were determined with two separate surveys: 1) a germination experiment, and 2) measuring plant growth rate and diversity in the field.

#### **a) Germination rates of native saline species in different salinity and temperature levels**

Seed germination rates increased over time for 20 days of experiment at all temperature and salinity treatments. Salinity decreased the germination rates significantly regardless the temperature differences. Highest germination rates were observed in the Freshwater treatment.

In the March temperature treatment (i.e. 5°C - 10°C), Spearscale (labeled as ASSB in the figure) showed the highest germination rates in Fresh, Low and Mid salinity treatments. At the highest salinity level, Seablite (labeled as SUCA) germination reached the highest rate, 7% compared to others including Spearscale with 1-4% germination rates (Fig. 16).

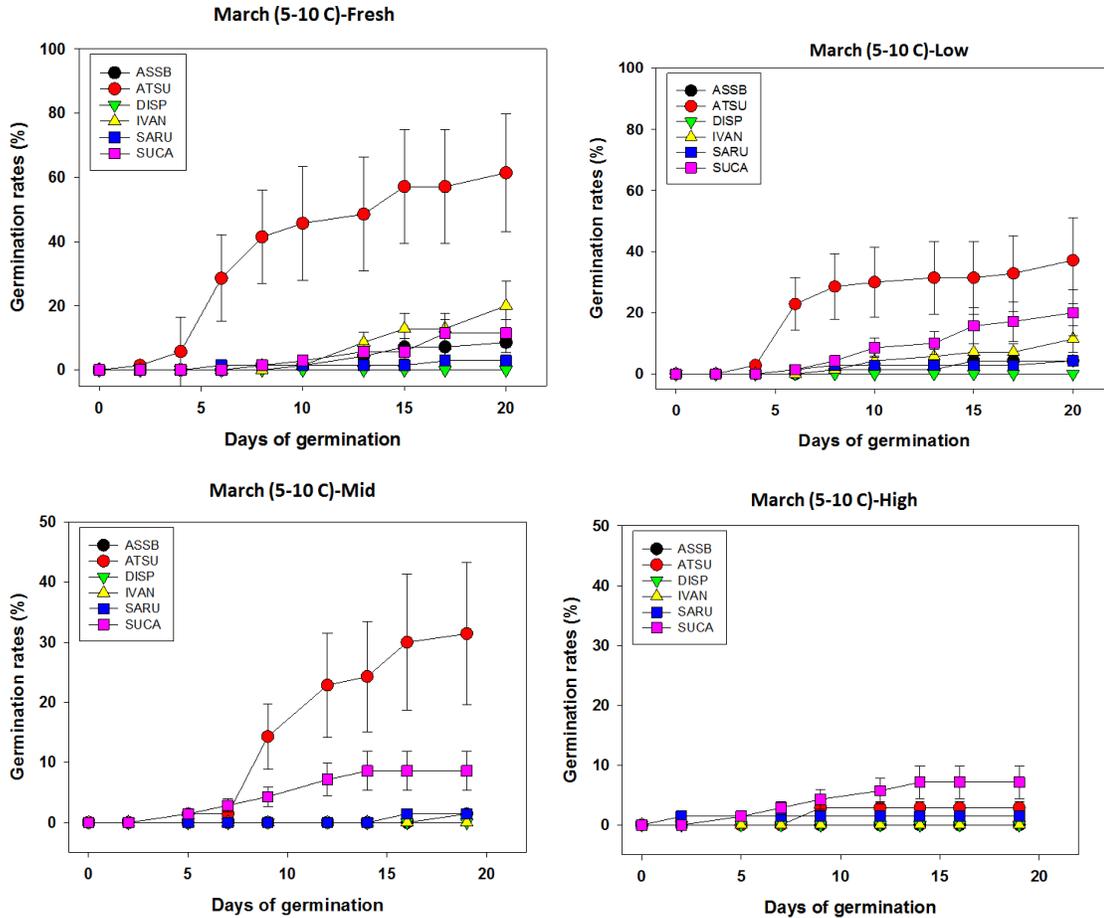


Fig. 16. Germination rates of six saline species seeds in different salinity level at the 5°C night-10 °C day thermal-period that represent March temperature in Lincoln, Nebraska. (ASSB-Saltmarsh Aster, ATSU-Spearscale, DISP-Saltgrass, IVAN-Marshelder, SARU-Saltwort, SUCA-Seablite)

Salinity inhibited the germination rates under the April temperature condition (Fig. 17). More interestingly, we found that increasing the day-time temperature (from March to April) stimulated the germination rates of seablite while it suppressed Spearscale’s germination. When comparing the germination rates and patterns between Seablite and Spearscale at April temperature set up, the final germination rate of Seablite exceeded Spearscale in all salinity levels. In addition, the overall germination rates of Spearscale in April set up was lower than those under the March temperature. Seablite germination rate were average 2.5 times higher in both Fresh and Low salinity levels, and it was 1.8 times higher for Mid salinity level compared with those in March temperature

condition. Spearscale continued to show the second highest germination rates among studied species at April condition. We also observed slight increase of germination rates for other species, including Marshelder (IVAN), Saltmarsh Aster (ASSB) and Saltwort (SARU) with the April temperature range. This result suggests that increasing temperature, or the April temperature condition is likely optimal for most studied saline species; it is especially favorable to Seablite germination.

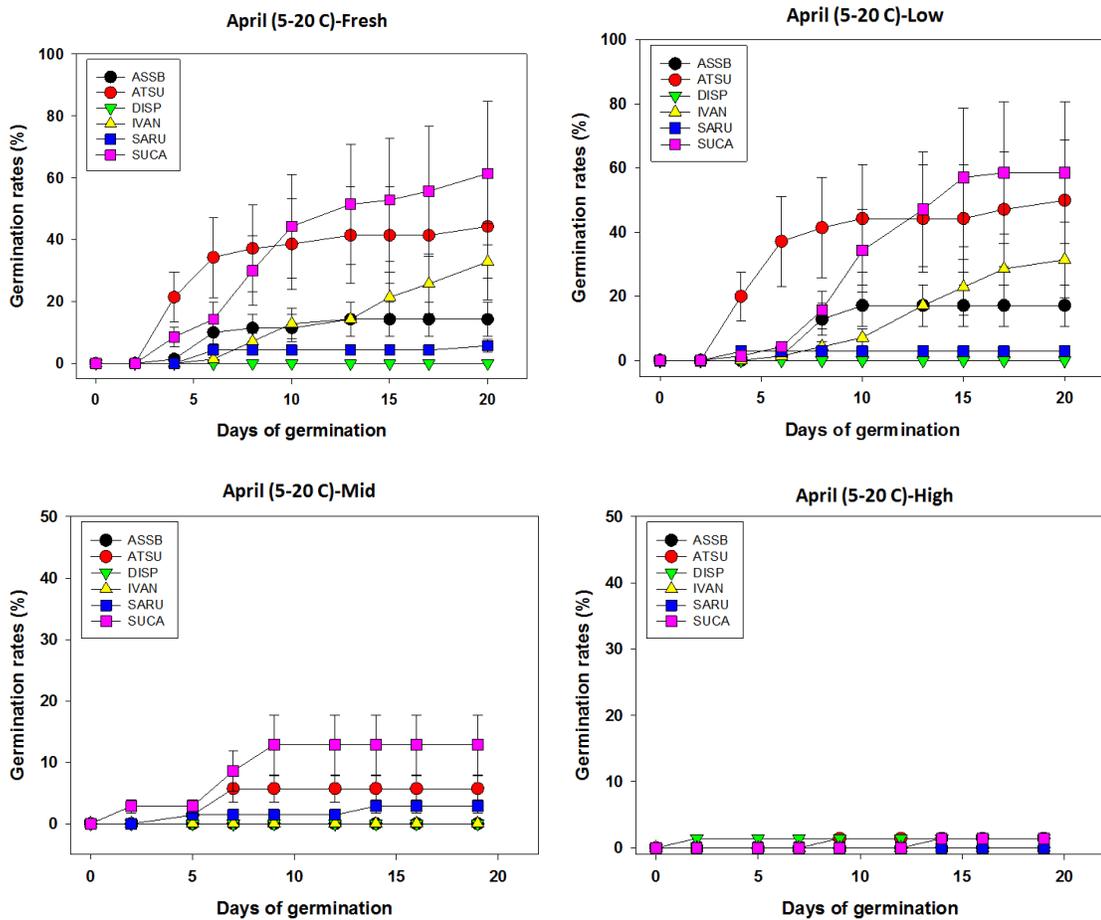


Fig. 17. Germination rates of six saline species seeds in different salinity level at the 5°C night-20 °C day thermal-period that represent April temperature in Lincoln, Nebraska. (ASSB-Saltmarsh Aster, ATSU-Spearscale, DISP-Saltgrass, IVAN-Marshelder, SARU-Saltwort, SUCA-Seablite)

At May temperature regimes, 10-20°C, germination rates showed similar patterns with salinity and species sensitive germination rates. Spearscale and Seablite continued to have higher germination rates than others. However, germination rates for all species decreased under the April temperature regime (Fig. 18). This suggests that increasing minimum temperatures (Average min. temperature at night: 10°C) has either no or negative impacts on germination rates of Nebraska’s Eastern Saline Wetland species.

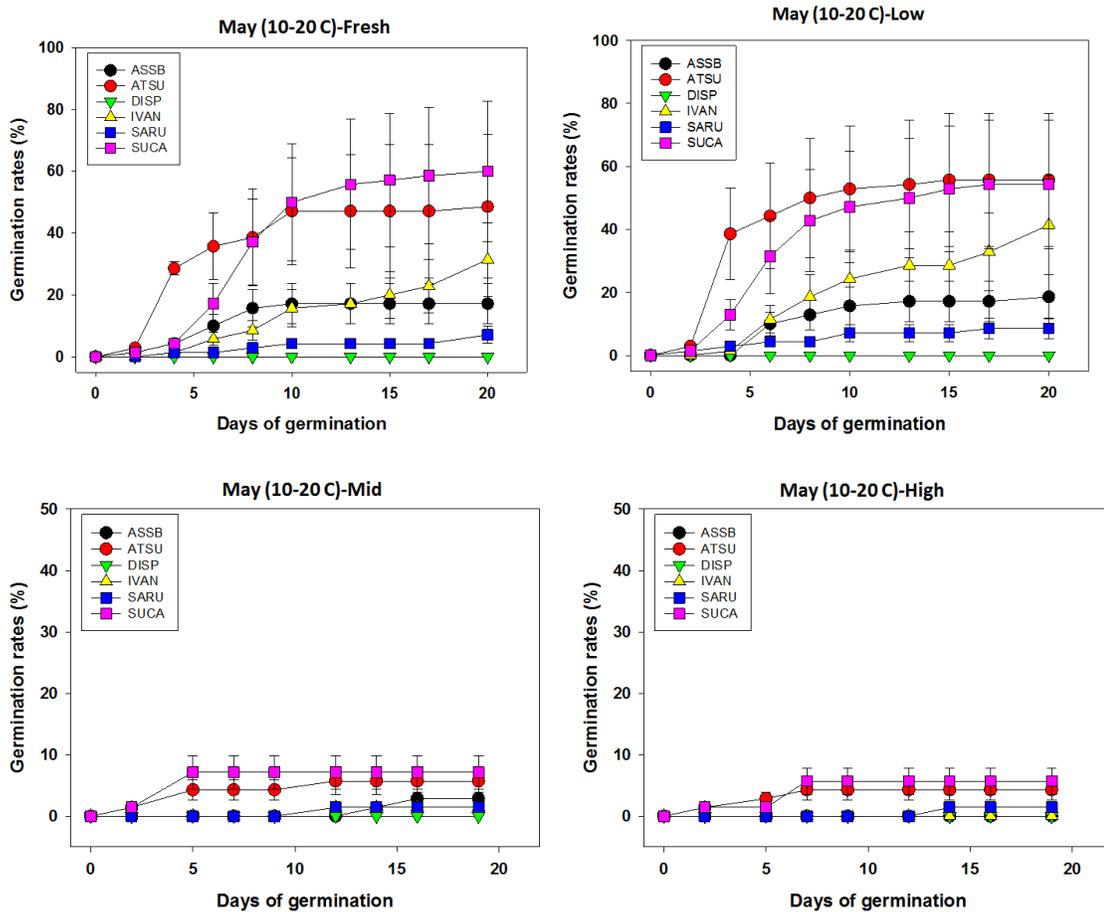


Fig. 18. Germination rates of six saline species seeds in different salinity level at the 10°C night-20 °C day thermal-period that represent May temperature in Lincoln, Nebraska. (ASSB-Saltmarsh Aster, ATSU-Spearscale, DISP-Saltgrass, IVAN-Marshelder, SARU-Saltwort, SUCA-Seablite)

**b) Plant survey in the experimental site**

Plant communities became dense in all experimental sites, especially in freshwater treated plots. Based on the peak biomass measurements and the plant identification survey, we found that species richness decreased with salinity (Table 2). We identified a total of 20 species in freshwater treated plots whereas only 6 species were found in high saline water treated plots.

Table 2. List of species identified in the experimental site (Bold letter represents target species in the project)

<b>Salinity</b>	<b># of species</b>	<b>Species</b>
Fresh	20	Aster, Saltbush, <b>Spearscale</b> , Bushy Knotweed, Common Ragweed, Curly Dock, Dead Grass, Fall Panicum , <b>Fox Tail Barley</b> , Giant Foxtail, Goldenrod, Kochia, <b>Marshelder</b> , Plantain, <b>Salt Grass</b> , <b>Seablite</b> , Tall Thistle, Thistle, Western Ragweed, Witchgrass
Low	18	Aster, Saltbush, <b>Spearscale</b> , Bushy Knotweed, Common Ragweed , Common Sunflower , Curly Dock, Dead Grass, <b>Foxtail Barley</b> , Kochia , <b>Marshelder</b> , Plantain, <b>Saltmarsh Aster</b> , <b>Seablite</b> , Thistle, West Rag, Western Ragweed, Witchgrass
Mid	13	Aster , Saltbush, <b>Spearscale</b> , Bushy Knotweed, Common Ragweed, Common Sunflower, <b>Foxtail Barley</b> , Kochia, <b>Marshelder</b> , <b>Saltgrass</b> , <b>Saltwort</b> , <b>Seablite</b>
High	6	Saltbush, <b>Spearscale</b> , <b>Foxtail Barley</b> , Kochia, <b>Seablite</b> , Western Ragweed

In the Fresh plots, common ragweed, thistle, kochia and giant foxtail were predominant, accounting for 60% of plant communities. These widespread plants are often considered weeds or invasive plants. Saline wetland indicator species such as seablite, marshelder and foxtail barley, which are also the project target species as well as saltbush were monitored in the Fresh plots (Fig .19). Saline wetland species contributed to up to 20% of the plant communities in the Fresh plots.

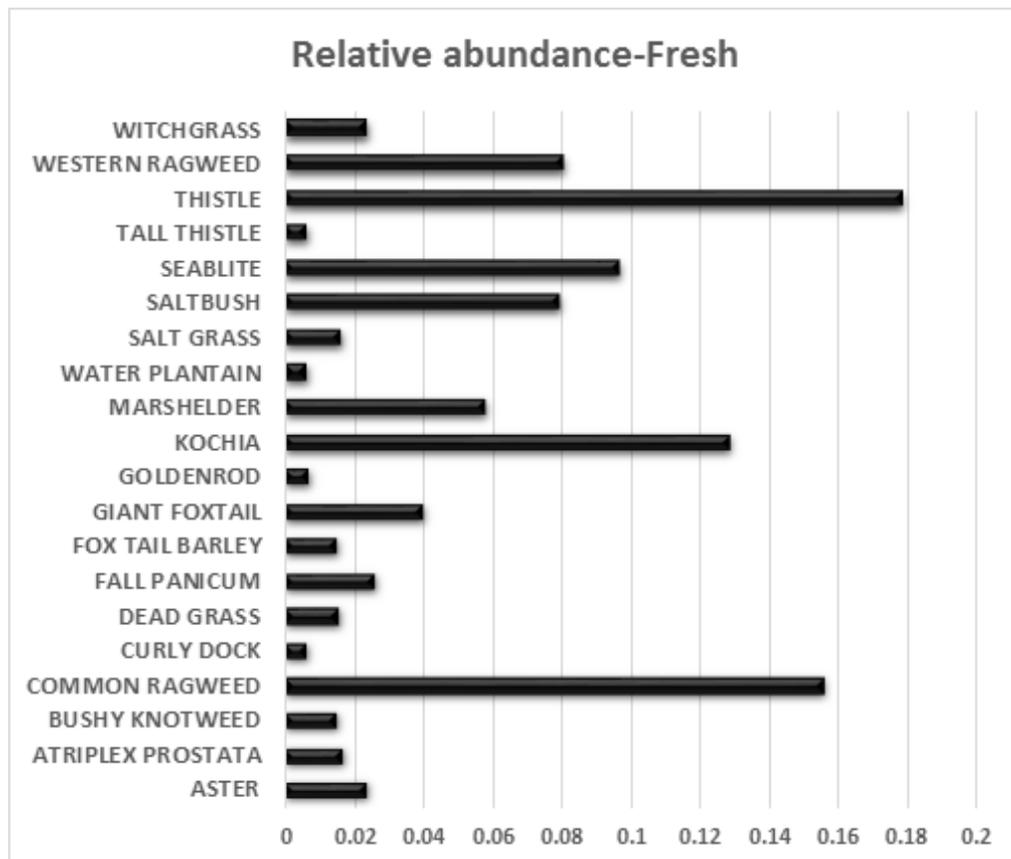


Fig. 19. Averaged relative abundance of species monitored in Fresh plots (FreshSlow and FreshFlush plots combined; n=6)

In the Low plots, western ragweed, water plantain and kochia dominated in both Low-Slow and Low-Flush plots, accounting over 40% of plant communities (Fig. 20). These are freshwater wetland or terrestrial plant species, but are also known as invasive plants, often growing in disturbed area. The same terrestrial or freshwater wetland plant communities were

observed in Fresh plots as well. More saline species emerged in the Low plots compared to Fresh plots. Spearscale, seablite, salt aster, marshelder and foxtail barley species were found and contributed to 25% of plant communities on average.

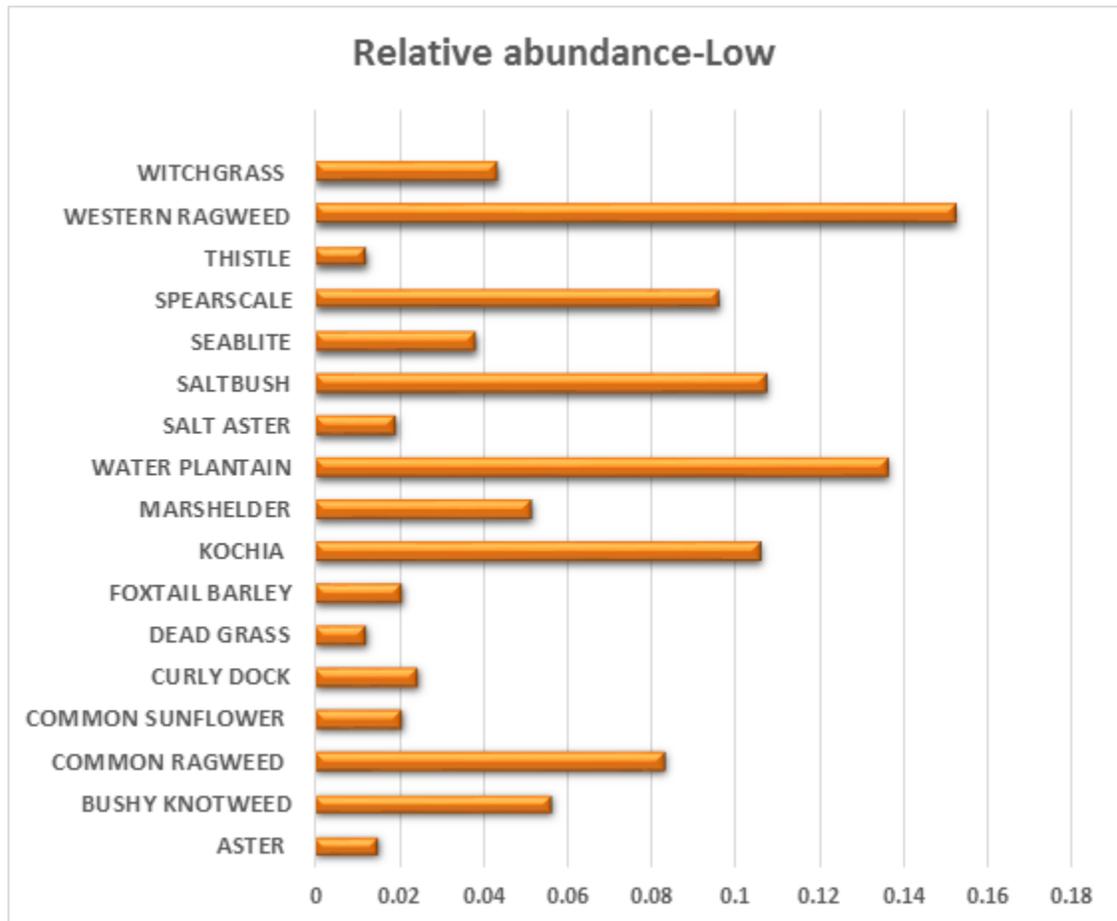


Fig. 20. Averaged relative abundance of species monitored in Low plots (LowSlow and LowFlush plots combined; n=6)

We continued to observe a gradual decrease of species richness as salinity increased. The decline in richness in the Mid plots was associated with the decrease of terrestrial and freshwater wetland species, although kochia remained the most abundant species on average. Relative abundance for the 7 target saline species reached over half of the total plant community. Saltwort was only found in the Mid plots (Fig. 21). In High salinity treatment plots, we only observed 6 species, and 4 of them were saline species (Fig. 22). The relative abundance of target species

reached 59%. Non-target species were dominant with Fresh and Low salinity treatments, and plant communities shifted to include target saline wetland species (i.e. salt-tolerant species) with Mid and High treatments (Fig. 23).

These results indicate that salinity gradients induced by saline groundwater addition shifted plant community structure from a terrestrial or freshwater wetland species dominant community to a saline species dominant community. Plant species richness decreased with the salinity gradient.

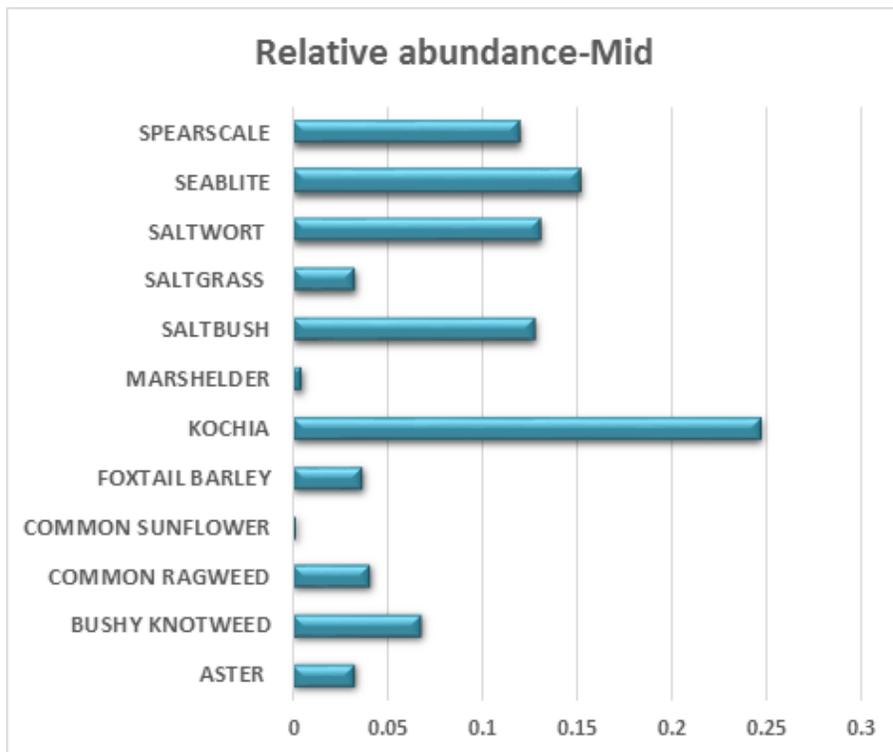


Fig. 21. Averaged relative abundance of species monitored in Mid plots (MidSlow and MidFlush plots combined; n=6)

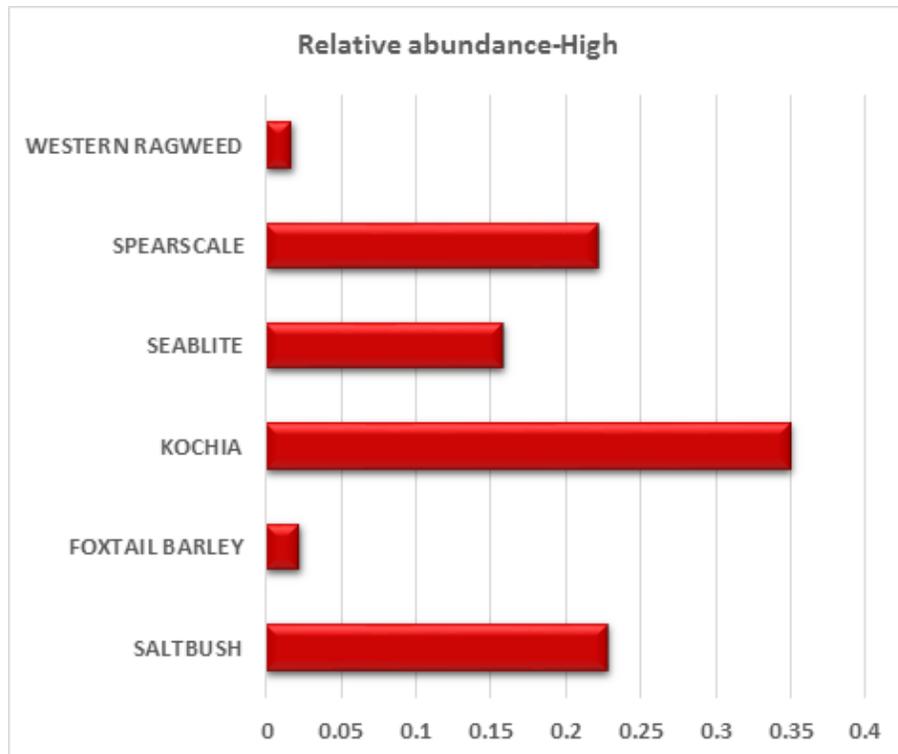


Fig. 22. Averaged relative abundance of species monitored in High plots (HighSlow and HighFlush plots combined; n=6)

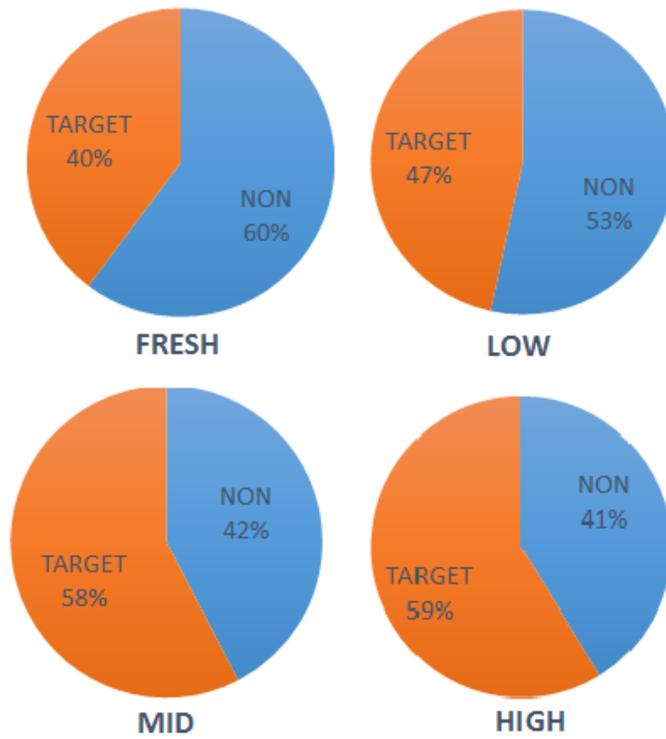


Fig. 23. The changes of relative distribution of non-target and target species between different salinity treatment (Fresh, Low, Mid and High salinity) plots.

## 8. Conclusions

The interactions between saline groundwater and soil are an important feature of Nebraska's Eastern Saline Wetlands. Our project results imply that saline groundwater addition has high potential to be a practical saline wetland restoration strategy.

The continuous saline groundwater addition (Fig. 5) increased porewater salinity in the restoration site (Fig. 12, 13). The addition of medium and high range of salinity groundwater (i.e. 18-20 and 26-30ppt in this study) induced the distinctive porewater salinity differences from those in freshwater and low salinity (2-3ppt) plots (Fig. 13).

Slow groundwater application prolonged the connections between saline groundwater and soil, therefore, maximized the effects of saline groundwater addition on soil surface. Slow application of medium and high salinity groundwater generated salt crusts in the soil surface (Fig. 14).

Medium and high salinity groundwater addition (18-30ppt in this study) increased CO<sub>2</sub> fluxes (Fig. 15). This suggests that the alteration of soil salinity level by saline wetland restoration activities affects carbon cycling and storage.

Salinity regulated the germination rates of saline species. Increased salinity inhibited the germination rates of all six targeted species (Fig. 16-18). Seablite and Sparscale appeared to be tolerant to medium (18-20ppt) level salinity. Warmer temperature stimulated germination of most saline species under the same salinity conditions (Fig. 16 vs. 17). We suggest that the April thermos-period (5°C night-20°C day) would be the optimal condition for better germination rates of targeted saline species as well as to avoid competitions with terrestrial/freshwater wetland species.

Sparscale and Seablite were the most abundant species at all saline groundwater treated plots at the experimental site (Fig. 19-22), which is consistent to the germination results. Saltwort emerged only in the medium salinity level plots (Fig. 21). Decreased plant species richness with increased salinity at the experimental site was associated with decreased terrestrial and freshwater plant species (Fig. 19-22). At medium and high salinity level plots, targeted saline species contributed to over 50% of the total plant abundance (Fig. 23).

## 9. References

- Harvey, F.D., Ayers, J.F., Gosselin, D.C., 2007. Ground water dependence of endangered ecosystems: Nebraska's Eastern Saline Wetlands. *Ground Water* 45: 736-752.
- Herbert, E.R., Boon, P., Burgin, A.J., Neubauer, S.C., Franklin, R.B., Ardon, M., Hopfensperger, K.N., Lamers, L.P.M., Gell, P., 2015. A global perspective on wetland salinization: ecological consequences of a growing threat to freshwater wetlands. *Ecosphere* 6: 206.  
<http://dx.doi.org/10.1890/ES14-00534.1>
- Khan M.A., Gul, B., Weber, D.J., 2000. Germination responses of *Salicornia rubra* to temperature and salinity. *Journal of Arid Environments*. 45: 207-214.
- LaGrange, T., Genrich, T., Johnson G., Schulz, D., Lathrop, B., 2003. Implementation plan for the conservation of Nebraska's Eastern Saline Wetlands. Saline Wetland Conservation Partnership.
- Taylor, T., Krueger, L., Gilber, M., Gersib, D., LaGrange, T., Stutheit, R., Hickman, T., Wilson, E., 1997. Mitigation guidelines for Nebraska's Eastern Saline Wetlands (Cooperative project report by USEPA, US Army Corps of Engineers, Nebraska Game and Parks Commission, Nebraska Department of Environmental Quality, US Fish and Wildlife Service).
- Ungar, I.A., Hogan, W.C., 1970. Seed germination in *Iva Annu L.*, *Ecology* 51: 150-154.