MILLENNIUM
EMS Solutions Ltd.

#148, 2257 Premier Way
Sherwood Park, AB T8H 2M8
tel: 780.496.9048
fax: 780.496.9049

Suite 202, 701 64 Avenue SE
Calgary, AB T2H 2C3
tel: 403.592.6180
fax: 403.283.2647

#102, 11312 98 Avenue
Grande Prairie, AB T8V 8H4
tel: 780.357.5500
fax: 780.357.5501

Suite 218, 852 Fort Street
Victoria, BC V8W 1H8

toll free: 888.722.2563
www.mems.ca

Agronomic Receptor Evaluation For Direct Soil Contact Stage 2

Prepared for:
Petroleum Technology Alliance Canada (PTAC)

Prepared by:
Millennium EMS Solutions Ltd.
Suite 202, 701 64 Avenue SE
Calgary, Alberta
T2H 2C3

and

InnoTech Alberta Inc.
250 Karl Clark Road
Edmonton, Alberta
T6N 1E4

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1.0 INTRODUCTION

The agronomic receptor evaluation for direct soil contact project has an overall objective of developing a scientifically defensible value for the minimum depth at which soil contamination could potentially affect agronomic crops in the Parkland region of Alberta. It is intended that this minimum depth be used to inform the depth below which soil remediation guidelines protective of the ecological direct contact pathway need not be applied.

Stage 1 of this project MEMS and InnoTech (2018) developed background information and identified a suitable test species (alfalfa), test contaminant (sodium chloride) and experimental conditions for Stage 2.

This report summarizes the findings of the experimental work conducted in Stage 2 moving towards identifying an appropriate depth below which soil contamination is not expected to adversely affect agronomic crops grown in the Parkland region of Alberta. The full experimental findings are provided in Appendix A (Thacker, 2021).

1.1 Background

The exposure pathway by which terrestrial plants and invertebrates are exposed to direct contact with contaminants in soil is referred to as the ecological direct contact pathway. This pathway must be considered in Alberta in all land-use designations (AEP 2016a). At the Federal level, the Canadian Council of Ministers of the Environment (CCME) have decided that this pathway is applicable to all soils above 1.5 m and established that the pathway need not be applied to soils deeper than 3.0 m (CCME 2008). The CCME has left the applicability of this pathway to soils at intermediate depths (between 1.5 and 3.0 m), to the governing jurisdiction to make a ruling.

In Alberta, there are several guidance documents that indicate the depth above which the ecological direct contact pathway must be applied in different circumstances:

- Alberta Environment and Parks (AEP) *Tier 1 and Tier 2 Soil and Groundwater Guidelines* (2016a,b):
  - this document applies to all contaminants and all land uses in Alberta and the applicability of the ecological direct contact pathway is dependant on depth and contaminant type;
  - the ecological direct contact pathway may be eliminated at depths exceeding 3.0 meters, if an alternative guideline is available (*i.e.* management limit), which currently applies only to petroleum hydrocarbon (PHC) fractions F1 to F4; and
  - groundwater guidelines can be excluded below 3.0 m for any substance.

- *Salt Contamination Assessment and Remediation Guidelines* (SCARG) (AENV 2001):
  - this document applies only to salt contamination (electrical conductivity (EC) and sodium absorption ratio (SAR));
  - applicable guidelines vary based on background salinity and soil horizon; and,
  - the ecological direct contact pathway has separate guidelines derived specifically for surface soil (defined as the A-horizon) and subsoil (defined as the B- and C-horizons and the upper portion of the parent material).
- Contaminated Sites Management: Subsoil Salinity Tool (SST) (ESRD 2014a):
  - This document applies only to salt contamination:
    - based on electrical conductivity (EC) in the top 1.5 m of the soil profile;
    - based on chloride concentrations below 1.5 m depth; and,
    - the SST assumes the effective rooting zone to be the top 1.5 m of the soil profile.
- *Subsoil Petroleum Hydrocarbon Guidelines for Remote Forested Sites in the Green Area* (ESRD 2014b):
  - This document applies only to petroleum hydrocarbon contamination in the Green Area of Alberta.
  - Applicable guidelines vary based on soil texture and contaminant depth.
  - The ecological direct contact pathway may be eliminated at a depth of 1.5 m in fine-grained soils and may be eliminated at a depth of 3.0 m in coarse grained soils based on the effective rooting depth of relevant tree species in these soil types in Alberta.

Given the above-mentioned guidelines and tools, there is some variability in guidance on whether the ecological direct contact pathway is applicable at intermediate depths (between 1.5 and 3.0 m) for different chemicals of potential concern (COPCs).

It is clear that 3.0 m represents a defensible upper bound on the maximum rooting depth of plant species likely to grow in Alberta. However, in practice, it may not be possible to exclude the ecological direct contact pathway even at this depth and the direct soil contact guideline may remain the governing remedial guideline for various COPCs in many settings.

1.2 Stage One Summary

Stage One of this project was a desktop evaluation of various aspects related to the rooting depth of agronomic plant species in Alberta. Five activities were undertaken:

1. Define the scope of agricultural land-use in Alberta.
2. Establish which crop species are primarily grown in Alberta.
3. Compile available information on the rooting depths of the identified crop species.
4. Review the suitability of applying the ecological direct contact guideline to a depth of 3.0 m in agriculturally zoned areas of Alberta.
5. Identify an appropriate surrogate plant species for further investigation using salt (sodium chloride) as a well characterized toxicant.

The results of Stage 1 were reported in MEMS and InnoTech (2018). Key findings of Stage 1 are summarized below.

Nine crop species (alfalfa, barley, canola, durum wheat, hay/fodder, mixed grain, oats, peas and spring wheat) were found to represent more than 95% of the agricultural land use in Alberta by area. Of these species, alfalfa was the deepest rooting of the plants reviewed with an effective rooting depth of approximately 1.5 m and a maximum rooting depth of 3.7 m.

One of the major data gaps identified was the lack of information in the published literature on how crop species may or may not be adversely affected by soil contaminants at various depths in relation to their effective rooting depth. This was identified as an area requiring further study and formed the primary research question of Stage 2.

Sodium chloride was identified as a suitable reference contaminant for the Stage 2 work based on being a highly mobile, readily bioavailable and common anthropogenic contaminant relating to oil and gas exploration. It also has well characterized adverse physiological effects on crops. Alfalfa was identified as being the most salt-sensitive of the nine primary crop species listed above.

Alfalfa was selected as test crop for Stage 2 work based on being the deepest rooted and most salt sensitive of the nine primary crop species grown in Alberta.

1.3 Stage Two Objectives

The objective of Stage 2 of the project was to investigate how adverse effects on the growth of alfalfa might be affected by the depth of a contaminant (sodium chloride) in the soil profile.

2.0 STUDY DESIGN

Stage 2 was a greenhouse study involving the growth of alfalfa in PVC columns. The top part of each column was filled with uncontaminated soil, while the soil below a certain depth was spiked with salt at a level expected to reduce alfalfa yield by approximately 90%. The primary variable in the experimental design was the depth below which the salt-spiked soil was placed. Full details of the experimental design are provided in Thacker (2021), included in Appendix A. Key elements of the experimental design are summarized below.

- A total of 70 columns were used in the experiment, 30 cm in diameter and 2 m tall.
- Each experimental column had control soil placed in the upper part and salt-spiked soil placed in the lower section; the depth of the top of the salt-spiked soil was 50, 75, 100, 125, 150 and 175 cm.
- The experimental soil was a loam-textured topsoil.
- Control columns without any salt-spiked soil were also set up.
- The bottom 10 cm of each column was perforated with holes and filled with gravel for drainage.
- There were 10 replicates for each depth treatment including controls (total of 70 columns).
- Two negative control columns were also established without alfalfa, one with no salt and one with salt at 125 cm, to assess moisture conditions in the absence of plants.
- The unspiked soil had an EC of 0.95 dS/m and 34 mg/kg chloride.
- The salt-spiked soil had an EC of 14.4 dS/m and 3,533 mg/kg chloride. This level of salinity would be expected to reduce the yield of alfalfa by approximately 90%.
- A subset of columns was instrumented to monitor moisture, temperature and electrical conductivity.
- Water was added to the top of each column via a drip irrigation system. Soil moisture was maintained at approximately 70% of field capacity in the upper half of the columns.
- The columns were seeded on November 14, 2019 and taken down in November 2020.
- Alfalfa was harvested from all columns every time the growth of most columns had reached growth stage 5 or 6 (one or more nodes with open flowers) to reflect typical agricultural practice. A total of 8 aboveground biomass harvest events were conducted between February 2020 and November 2020.
- Belowground biomass was assessed at the end of the experiment.

3.0 FINDINGS

Detailed results of the greenhouse study were reported by Thacker (2021) (included in Appendix A). Six categories of findings were discussed by Thacker (2021): migration of salinity, effects on aboveground alfalfa biomass, effects on maximum plant height, effects on belowground alfalfa biomass, effects on root depth, and effects on root distribution. Key findings in each category are summarized below.

3.1 Migration of Salinity

Soil samples were collected 15 cm above and below the salinity interface to validate information from the sensors. Based on soil chloride concentrations, there was some upwards movement of salinity above the interface. Average chloride concentrations in the various depth treatments ranged from 51 mg/kg to 460 mg/kg in the 15 cm above the interface at the end of the experiment, 1.5 to 13.5 times the chloride concentration in the unspiked soil. In the 15 cm below the interface, average soil chloride concentrations at the end of the experiment ranged from 804 mg/kg to 2,770 mg/kg, corresponding to 23% to 78% of the initial chloride concentration in the spiked soil. Overall, these data suggest a moderate amount of net downward migration of salt occurred in the vicinity of the interface during the experiment with some "spreading out" of the step-change in salinity at the interface presumably due to a combination of diffusion and capillary migration. The columns with the salinity interface at 175 cm appear to be an outlier in this dataset with the lowest chloride of any of the depth treatments both above and below the salinity interface. This may be related to the gravel layer at the base of the column lying only 15 cm below the salinity interface, potentially providing a conduit for salt to be lost from the column.

3.2 Aboveground Biomass

Based on the cumulative aboveground biomass after 7 harvests, there appears to be a general slight trend of decreasing biomass with decreasing salinity interface depth, but only the 50 cm depth treatment had significantly lower cumulative aboveground biomass than the controls. The first and second harvests saw this same trend of decreasing biomass with decreasing salinity interface depth, however, later harvests (3rd through 7th) showed little, if any difference in aboveground biomass between depth treatments.

3.3 Plant Height

Thacker (2021) concluded that plant height did not appear to be an appropriate indicator for assessing the effect of salinity on plant health, at least not when periodic harvests occur.

3.4 Belowground Biomass

All depth treatments between 50 cm and 150 cm showed a reduction in coarse root biomass below the salinity interface both relative to the coarse root biomass above the interface and relative to the control at the same level. The reductions relative to the control were significant in the 75 cm, 100 cm, 125 cm, and 150 cm depth treatments. The fine root biomass data showed similar patterns of a reduction in root biomass to less than control levels below the salinity interface for all depth treatments, though in many cases the difference was not significant. These data suggest that salinity at depths up to and including 175 cm is inhibiting the growth of coarse and/or fine roots into the saline soil.

3.5 Root Depth

Both maximum and effective root depths were greatest in the control treatment and decreased progressively with shallower salinity treatments. These differences were significant for all depth treatments with the exception of the 175 cm bgs treatment. Effective root depth is defined in this study as the depth above which 90% of the root mass is estimated to be present. Overall, soil salinity had a clear and significant impact on maximum and effective rooting depth when the salinity was present at depths down to 150 cm bgs.

3.6 Root Distribution

The root distribution study showed that the orientation of roots changed below the salinity interfaces. Above the interfaces, roots tended to be vertical. Below the interfaces, roots tended to take on a random orientation. This trend was observed regardless of the depth of the salinity interface. For the 175 cm bgs treatment, major changes in size and abundance were not always present above versus below the interface, but a change in orientation was observed in all columns. Roots likely stopped growing vertically to avoid taking up salt and appear to have adapted a random distribution in this study. In one column, orientation changed to horizontal at the interface, indicating a clear avoidance of the salt-impacted material. This differed from the control columns, which saw roots maintain a vertical orientation throughout the length of the column.

4.0 DISCUSSION

The results of Stage 2 indicate that high levels of salt deeper than 50 cm have an effect on rooting depth, root orientation and below-ground biomass of alfalfa. However, effects on aboveground biomass are less clear and over the course of multiple harvests were not statistically significant for salt at depths of 75 cm and below. These results suggest that, under greenhouse study conditions, alfalfa plants can adapt to salt at depth with a shallower root system while still maintaining aboveground growth.

Based on these observations, it is likely that at under at least some conditions crop plants can adapt to contaminants within the typical rooting zone depth but below the upper rooting zone. Under controlled greenhouse conditions, 75 cm of unimpacted soil appeared to be sufficient to achieve effective aboveground growth, and even with 50 cm the effects were modest. Field studies reflecting a range of typical conditions in Alberta, including drier/suboptimal growing conditions, could demonstrate whether this finding is more broadly applicable.

While the greenhouse study used only alfalfa, this plant was selected after Stage One of this project because it was both the most salt-sensitive and deepest rooting of common crops in Alberta. Therefore, it can reasonably be expected that these findings would be protective of other agricultural species. Furthermore, the salt concentrations used in the greenhouse study were well above the anticipated effects levels on alfalfa (electrical conductivity approximately 14.5 dS/m at study initiation and > 11 dS/m in most treatments at study end).

While salt was used as a surrogate contaminant for the current study, it is likely that similar findings would occur with other contaminants, although for contaminants toxic to humans the potential for bioaccumulation in crops would also need to be considered.

Since the current study was conducted under controlled greenhouse conditions, the conclusions cannot necessarily be assumed to be broadly applicable at all sites. However, the study does support that impacts below the upper rooting zone may have minimal effects on aboveground biomass and agricultural production. At this time, site-specific support would be required to demonstrate an absence of effect for impacts with a depth of less than 1.5 m, such as growth of salt-sensitive crops comparable to control plots.

4.1 Factors Contributing to Uncertainty

No study can account for all possible conditions and inevitably there will be uncertainties involved in extrapolating from a greenhouse study such as this one to what may occur under a range of field conditions. Some of the factors to be considered in making such an extrapolation include the following.

- In this study, all irrigation was supplied from above. This is likely representative of field conditions where the water table is sufficiently deep, but care should be taken applying the results to situations with shallower water tables that could result in upwards migration of salinity or other contaminants.
- The study used topsoil for the whole 2 m soil profile which is likely a much more favourable growing medium than typical subsoils in a field setting. It is not known what effect, if any, this might have on extrapolating from study results to field conditions.

- In this study, there was minimal effect on the yield of aboveground biomass in spite of inhibition of root development in the saline horizons. This finding may translate to field settings in irrigated settings or in non-irrigated settings in years when there is adequate soil moisture. However, in years of low precipitation or other moisture stress, it may be that a crop that has a shallower root system due to salinity avoidance may not do as well as one that is deeper rooted.

5.0 LIMITATIONS OF LIABILITY AND CLOSURE

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Yours truly,

Millennium EMS Solutions Ltd.

Prepared by:

Ian Mitchell M.Sc. P.Eng.
Vice President, Technology & Business Services

Reviewed by:

Miles Tindal M.Sc.
Contaminated Sites Specialist

6.0 REFERENCES

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APPENDIX A: PTAC AGRONOMIC SALINITY PHASE 2 REPORT (INNOTECH REPORT)

PTAC AGRONOMIC SALINTY PHASE 2 REPORT

Sarah Thacker

PREPARED FOR
MILLENNIUM EMS SOLUTIONS LTD.
Attn: Ian Mitchell & Andre Christensen

INNOTECH ALBERTA INC. (INNOTECH)
ENVIRONMENTAL IMPACTS
250 KARL CLARK ROAD
EDMONTON, ALBERTA T6N 1E4
CANADA

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EXECUTIVE SUMMARY

The ecological direct soil contact pathway considers the exposure pathway by which terrestrial plants and soil invertebrates may come into direct contact with chemicals in the soil, and applies across all land-use designations. In Alberta, there are several guidance documents that specifically reference the depth at which the ecological direct contact pathway is applicable; however, there is variability on whether the direct contact pathway is applicable at intermediate depths (between 1.5 and 3.0 m) for different chemicals of potential concern. Additionally, while the pathway need not be applied to soils deeper than 3.0 m, owing to lack of receptors at these depths, elimination of the ecological direct contact pathway is only applicable at such depths if another, more suitable guideline exists; this means that the ecological direct contact pathway cannot be eliminated above 3.0 m if another guideline does not exist.

In 2018, Millennium EMS Solutions Ltd. and InnoTech Alberta Inc. initiated a project to develop a scientifically defensible depth at which the ecological direct soil contact pathway is applicable. The proposed project was to be completed in 3 phases: 1) comprehensive literature analysis; 2) lab/greenhouse study; and 3) field study. The current report summarizes the Phase 2 greenhouse study.

To determine a scientifically-defensible depth at which the ecological direct soil contact pathway is applicable for typical contaminants and agronomic species in Alberta, sodium chloride was used as a surrogate contaminant and alfalfa as a surrogate species. A greenhouse study was established using 2 m tall by 0.3 m diameter columns. There was seven treatments based on the depth at which salt-impacted soil was placed: 50, 75, 100, 125, 150, and 175 cm below ground surface (bgs), and a control (no salt-impacted soil present). Salt-impacted soil was spiked to an electrical conductivity of 14.5 dS/m. Alfalfa was grown in the columns for approximately one year. Aboveground growth parameters were measured, including aboveground biomass (harvested multiple times) and height. At the end of the experiment, columns were taken apart to assess coarse and fine root biomass, maximum and effective rooting depth, and root distribution. Soil samples were collected to assess whether there was movement of salts at the salinity interfaces.

Trends in aboveground growth parameters tended to vary throughout the experiment. The effect of salt on aboveground biomass was not always consistent, though the impact of salt appeared to decrease as plants became more established. Similarly, trends in plant height were variable throughout the experiment. While there was no clear effect of salinity on aboveground growth, there was a significant effect on roots: salinity had a significant impact on coarse roots, causing a reduction in biomass down to 150 cm bgs. Fine root biomass was also impacted: changes in biomass indicated that fine root biomass could be impacted by salinity present at 175 cm bgs. Both maximum and effective root depth were significantly reduced by the presence of salinity at depths down to 150 cm bgs.

This study provided empirical evidence for defining a scientifically defensible operative depth for the ecological direct contact pathway in soil. While salinity had a variable impact on aboveground growth, there was a clear negative impact on root growth. When considering that the biologically active zone in soil is typically associated with roots, the impacts on rooting depth and distribution observed in this study indicate the potential for impacts to organisms living in the soil. Phase 3 of this project (the field study) will be crucial in the development of a scientifically-defensible depth for the ecological direct soil contact pathway.

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1.0 INTRODUCTION

1.1 Project Background

The ecological direct soil contact pathway considers the exposure pathway by which terrestrial plants and soil invertebrates may come into direct contact with chemicals in soil, and applies across all land-use designations (AEP, 2016). At the Federal level, the Canadian Council of Ministers of the Environment (CCME) have stipulated that this pathway is applicable to all soils up to 1.5 m below the ground surface (bgs), and established that the pathway need not be applied to soils deeper than 3.0 m (CCME, 2008). The CCME has left rulings on soils at intermediate depths (between 1.5 and 3.0 m) to individual governing jurisdictions.

In Alberta, there are several guidance documents that specifically reference the operative depth of the ecological direct contact pathway. However, there is some variability on whether the direct contact pathway is applicable at intermediate depths (between 1.5 and 3.0 m) for different chemicals of potential concern (COPCs):

- Given a lack of biological receptor presence below 3.0 m, the ecological direct soil contact pathway need not be applied below 3.0 m.
- Above 3.0 m, the ecological direct contact pathway cannot be eliminated unless a more suitable guideline (such as a management limit), exists.
- Therefore, without a more suitable guideline in the governing jurisdiction, the ecological direct contact pathway cannot be eliminated and is applicable down to a depth of 3.0 m.

While various jurisdictions are aligned in the direct contact pathway being inoperative at depths greater than 3.0 m, for the vast majority of COPCs (with the exception of petroleum hydrocarbons [PHC] F1 to F4), the direct soil contact guideline remains the governing remedial criteria.

In 2018, Millennium EMS Solutions Ltd. (MEMS), in collaboration with InnoTech Alberta (InnoTech), initiated a project to develop a scientifically defensible depth at which the ecological direct soil contact pathway is applicable (MEMS & InnoTech Alberta, 2018). The project was to be completed in 3 phases: 1) comprehensive literature analysis; 2) lab/greenhouse study; and 3) field study. The current report summarizes the results from Phase 2 (the greenhouse study).

1.2 Scope and Rationale for Research

Sodium chloride (NaCl) is a highly mobile, readily bioavailable and common anthropogenic contaminant related to oil and gas exploration, with well-defined adverse physiological effects on crops. For these reasons, it was selected as a suitable COPC surrogate for validation of the ecological direct contact exclusion depth. The literature review previously completed by the project team (Phase 1) focused on agronomic receptor species for the White Area of Alberta; a quantitative agronomic receptor for evaluating the direct soil contact pathway requires a surrogate that is found throughout the province, is

considered deep rooting, and is salt-sensitive. Alfalfa was the deepest rooting of the species reviewed, exhibited the lowest threshold for salts (low concentrations of soil-salinity exhibit measurable reductions in plant yield), and is a common crop through much of Alberta (MEMS & InnoTech Alberta, 2018). Based on the findings of this assessment, alfalfa was selected as a suitable surrogate.

1.3 Research Objectives

The overarching objective of the study was to develop a scientifically defensible depth at which the ecological direct soil contact pathway (eco-soil contact pathway) is applicable. The specific objective of Phase 2 was to determine the impact of salinity on aboveground and belowground plant health, when present at various depths within the soil profile. The purpose of this report is to summarize the Phase 2 greenhouse study.

2.0 METHODS

2.1 Experiment Set-Up

2.1.1 Overview of Experimental Parameters

The experiment was set up in a sunken greenhouse at InnoTech's research facility in Vegreville, AB. Alfalfa was grown in two-metre tall columns containing either uncontaminated material, or material contaminated with NaCl at varying depths below the surface. Table 1 provides an overview of the experimental design:

Table 1. Experimental design for greenhouse growth study.

Treatments/Parameters	Number of levels	Description
Growth Medium	1	Loam-textured topsoil
Plant Species	1	*Medicago sativa* (alfalfa)
Contaminated Material Depth*	7	Control (no contaminant), 50, 75, 100, 125, 150, 175 cm bgs
Replicates	10	PVC columns (200 cm tall by 30 cm in diameter)
Total # Columns = 70		

*Control soil material had an electrical conductivity of 0.95 dS/m, while the contaminated material had a conductivity of 14.5 dS/m.

To assess the impact of elevated salinity on the growth of alfalfa, salt-contaminated soil was placed at different depths below the surface. In total there were seven different treatments: six treatments with salinity at varying depths and one control treatment (no salt-impacted soil present). The depth at which contaminated material was present is referred to as the salinity interface in this report. Below the salinity interface, all the material was salt-impacted, and above it none of the soil was contaminated. In each treatment, there was at least 50 cm of uncontaminated material at the top of the column. In addition to the 70 experimental columns, two negative control columns were installed (control and 125 bgs treatments). The negative controls were not seeded with alfalfa, and were intended to inform moisture conditions in the absence of plants. The layout of the greenhouse is shown in Figure 1.

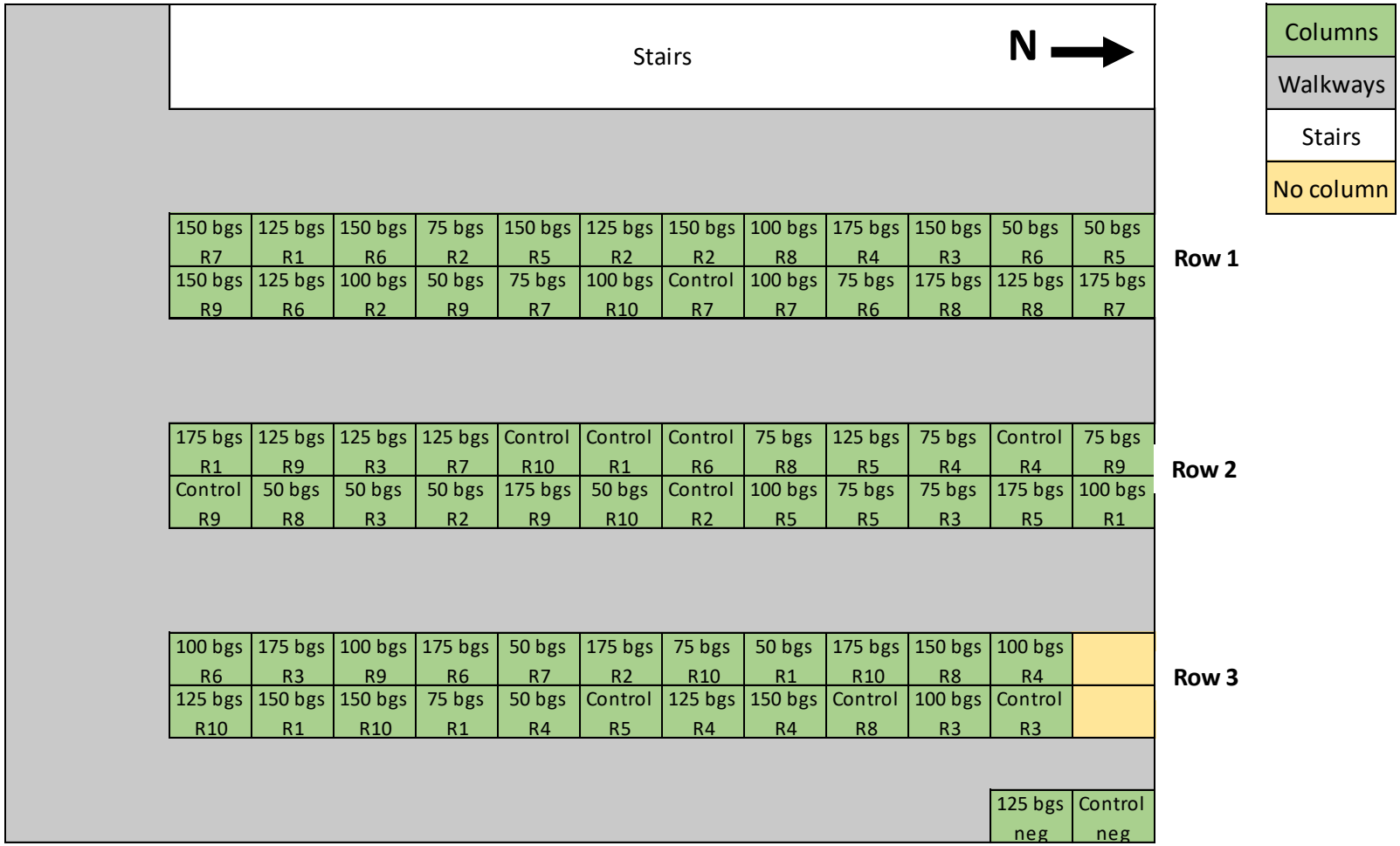

Figure 1. Greenhouse layout for the experiment.
Elevated walkways (about 1 m above the ground) were used as the walking surface such that individuals monitoring the experiment could easily access the columns and plants. This system was implemented to provide safe and efficient access to the tops of the columns to enable monitoring. Note that 'R' in the diagram indicates the replicate within each treatment.

2.1.2 Column Construction, Installation, and Filling

Two-metre lengths of PVC piping (30 cm internal diameter, approx. 1 cm thick walls) were sourced from EMCO Waterworks (Edmonton, AB) and used to create the columns for the experiment. Holes were drilled in the bottom 10 cm of each column for drainage; seven sets, each consisting of four holes, were drilled approximately equidistant around each column (Figure 2A). PVC discs were affixed to the bottom of each column using two pieces of steel strapping crisscrossed across the bottom and screwed into place up the sides of each column. The discs were sourced from Water Jet Ltd. (Edmonton, AB) and had a diameter of 13 in (33 cm) and a thickness of 0.25 in (0.64 cm). Figure 2 shows the column construction and installation.

One column from each of the 50 bgs, 100 bgs, and 150 bgs contaminated material depth treatments, and two columns from each of the control, 75 bgs, 125 bgs, and 175 bgs treatments, were instrumented with four sensors at various depths to monitor moisture, temperature, and electrical conductivity. The two negative control columns were also instrumented, for a total of thirteen instrumented columns. Soil sensors were placed at different depths in the columns, depending on the specific treatment type (Figure 3). The sensor depths were chosen to provide a picture of conditions throughout the entire column, while monitoring conditions around the salinity interfaces. Where salt contamination was present in a treatment, one sensor was placed 10 cm above the contaminated material and one sensor 10 cm below the interface. An exception was made for the 50 bgs and 75 bgs treatments, where the sensors were placed 10 cm above and 15 cm below the contamination, given the shallow depth of the salinity interface; it was expected that more soil settling would occur at the shallow interfaces over the course of the experiment compared with those that were located deeper in the column at the initiation of the experiment.

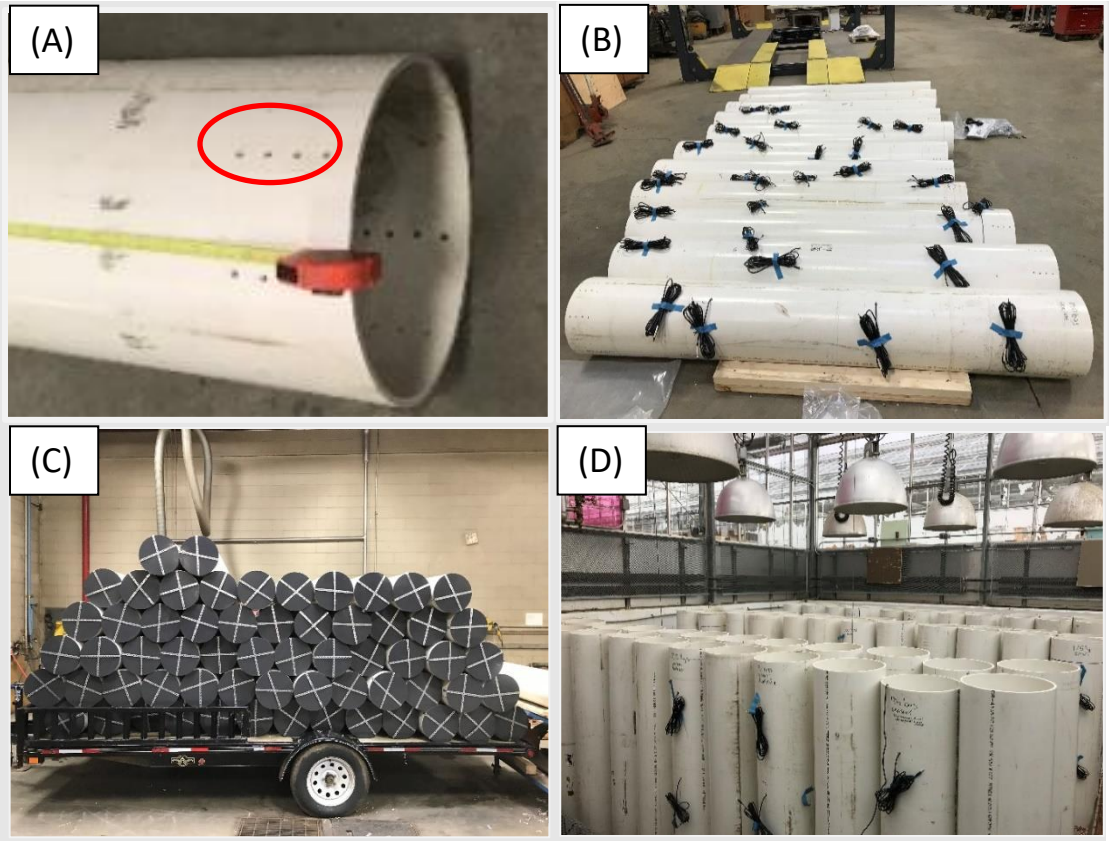

Figure 2. Column construction and instrumentation.
The images show drainage holes (circled in red) (A), sensors (bundles of black wire visible on the columns) in position (B), PVC discs attached to column bases (C), and empty columns deployed inside greenhouse (D).

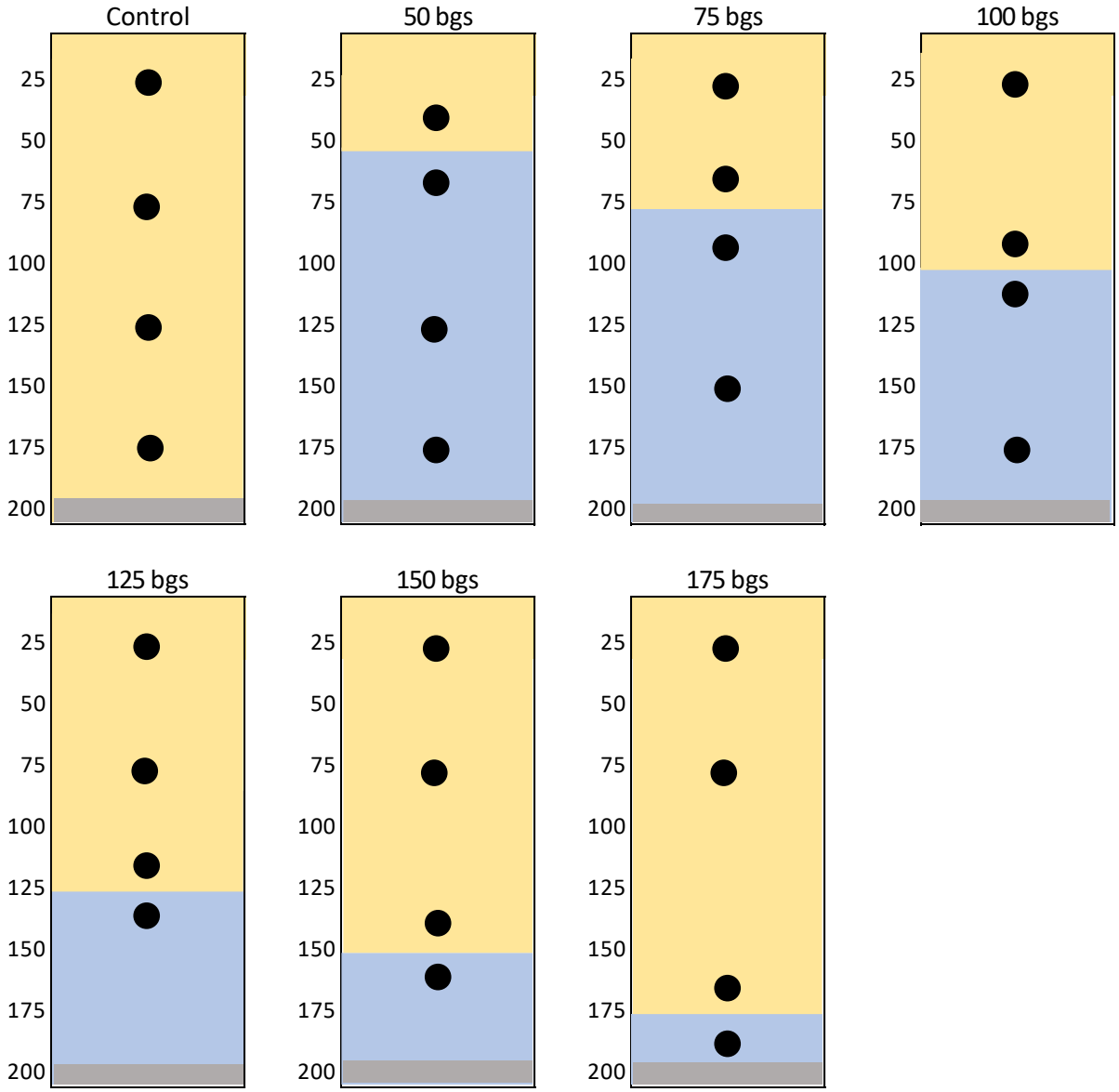

Figure 3. Depths at which soil sensors were placed in the columns, depending on the treatment. Each instrumented column had four sensors. Yellow shading represents uncontaminated material, blue represents contaminated material, and grey shading represents the gravel layer described below.

A loam topsoil was sourced from City Soil Services (Edmonton, AB) for use as a growth medium in the columns. Soil material was either left uncontaminated or spiked to an electrical conductivity of approximately 14.5 dS/m with NaCl. To determine the quantity of NaCl to add, a laboratory trial was conducted; soil was spiked with increasing concentrations of NaCl and electrical conductivity was measured, thus generating a regression curve and equation which could be used for spiking the large quantities of soil needed for the experiment. Material was spiked by mixing soil and an appropriate amount of NaCl to reach the target electrical conductivity together in an industrial mixer. Mixing was conducted in a series of batches, which were then further mixed with a skid steer using the cone and quarter method (Schumacher et al., 1990) (Figure 4). Samples of uncontaminated and contaminated

material were sent to Element Materials Technology (Edmonton, AB) for salinity analysis to confirm the electrical conductivity of each material. The properties of the growth medium are given in Table 2.

Figure 4. Spiking soil with NaCl.
Images show loading soil into the skid steer (A), loading soil into the mixer (B), mixing soil (C), and soil ready to be mixed using the cone and quarter method (D).

Columns were filled with 10 cm of gravel (10 mm washed rock) sourced from Burnco (Edmonton, AB), followed by contaminated material (to a depth dictated by the treatment), and then uncontaminated material. The material was added by the bucketful and tamped to a target bulk density of approximately 1 g/cm^3 (Figure 5). As the columns were being filled, sensors were placed at the target depths for each treatment and material tamped to ensure good contact between the data logger sensors and the growth medium. Water was added to the surface of the columns to facilitate settling.

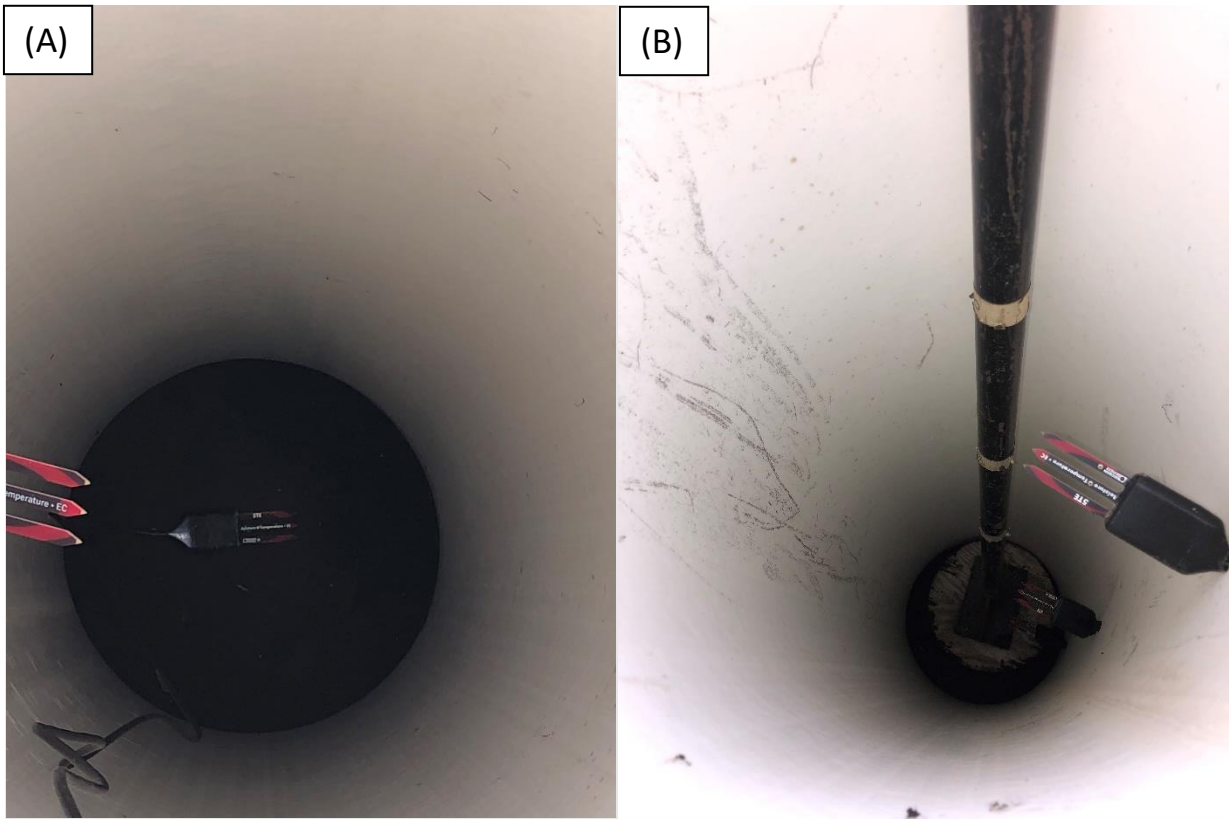

Figure 5. Filling the columns and tamping material as it was placed in the columns.
(A) Material tamped in the column, with data loggers placed in the centre of the column. (B) The equipment used to tamp the material in the columns.

Table 2. Properties of the growth medium used for the column experiment.
Salinity-related values are shown for uncontaminated and contaminated material separately (n=8 for each material). General properties are based on data for the uncontaminated material (n=8)*.

Growth Medium Properties	*Average*	*Standard Deviation*
General Properties		
Texture	Loam	-
% Retained	35	0.85
	Fine-Grained	-
Sand (%)	41	1.96
Silt (%)	35	1.96
Clay (%)	24	1.91
Organic Matter (%)	6.49*	0.30
Organic Carbon (%)	3.24*	0.15
Available Nitrate - N (µg/g)	11.25	0.46
Available Phosphorus (µg/g)	11.25	0.46
Available Potassium (µg/g)	153.75	5.47
Available Sulfate-S (mg/kg)	40.88	4.29
Available Ammonium - N (mg/kg)	6.05	0.39
Uncontaminated Material Salinity		
pH	7.56	0.05
Electrical Conductivity (dS/m)	0.95	0.14
SAR	0.71	0.04
% Saturation	62.25	4.20
Calcium (mg/kg)	73.49	12.88
Magnesium (mg/kg)	18.35	2.81
Sodium (mg/kg)	20.88	2.59
Potassium (mg/kg)	9.63	0.52
Chloride (mg/kg)	34.25	1.49
Sulfate (SO_4) (mg/kg)	122.50	50.87
Sulfate-S (mg/kg)	40.83	16.93
Contaminated Material Salinity		
pH	6.91	0.08
Electrical Conductivity (dS/m)	14.41	0.83
SAR	18.11	0.49
% Saturation	63.38	5.13
Calcium (mg/kg)	575.63	25.37
Magnesium (mg/kg)	122.38	5.63
Sodium (mg/kg)	1458.75	47.64
Potassium (mg/kg)	14.50	0.53
Chloride (mg/kg)	3533.75	192.35
Sulfate (SO_4) (mg/kg)	101.38	5.68
Sulfate-S (mg/kg)	33.75	1.39

*n=5 for organic matter and organic carbon values

2.1.3 Seeding and Irrigation Set-Up

After monitoring moisture for approximately one week, each column was seeded with 20 alfalfa seeds on November 14, 2019. After three weeks of growth, the number of plants in each column was culled to no more than 10 per column. After five weeks of growth, plants were culled to five per column.

For one week after seeding, the lights were kept off in the greenhouse to prevent soil drying and facilitate germination; afterwards, a 16-hour photoperiod was established with day/night temperatures of 23/16°C. Once plants appeared to be establishing, the day/night temperatures were transitioned to 20/13°C to more closely match natural conditions during the growing season in the parkland region of Alberta; the average daily temperature during the warmest month of the year is 16.5°C in the Central Parkland (Natural Regions Committee, 2006).

A drip irrigation system, with materials sourced from Irrigation Direct Canada (Burlington, ON), was installed on December 3, 2019 (Figure 6). The system consisted of one distribution line and four dripper lines extending down the three rows of columns and to the negative controls. Pressure compensating emitters, attached to dripper stakes, were used to ensure each column received equal amounts of water. Two dripper stakes were placed in each column; each dripper stake was capable of delivering 0.5 gallons (1.9 L) of water per hour. An automated timer was used to control watering frequency and duration; these parameters were adjusted as plants grew to ensure adequate moisture for growth.

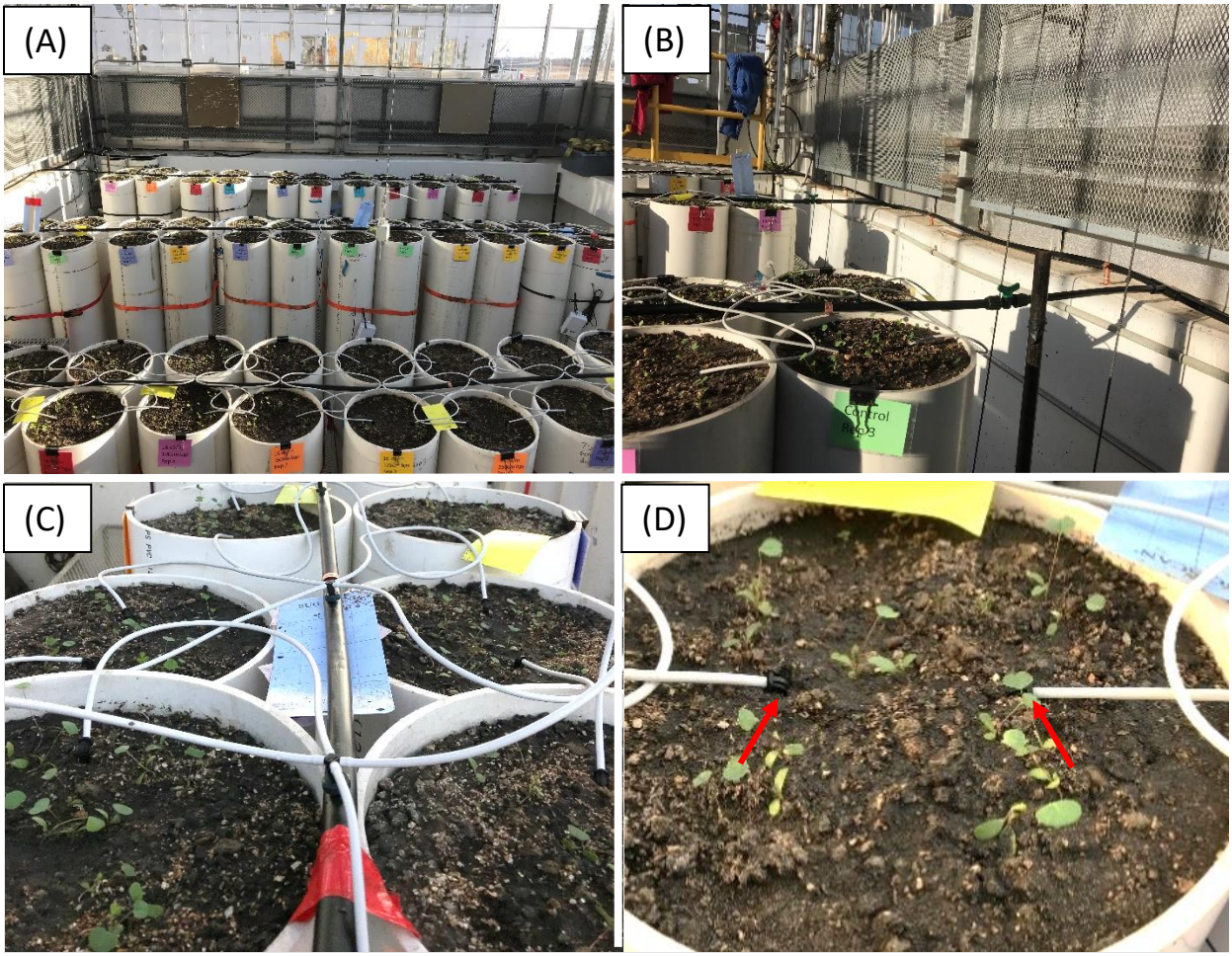

Figure 6. Drip irrigation system installed in the greenhouse.
Shown in the images: (A) overview of the drip irrigation system, (B) distribution line with an attached dripper line, (C) a dripper line with attached dripper stakes, and (D) close up of dripper stakes (indicated with red arrows) installed in a column by pushing them into the soil.

2.1.4 Column Takedown

In November 2020, the columns were taken apart to assess root growth. First, columns were extracted from the greenhouse using a pulley system and telehandler, and brought to a different building for processing (Figure 7). Using a circular saw and jig, the plastic of the column was cut lengthwise to expose the outside edge of the soil for photography and measurements (Figure 7). Then, the plastic was placed back on top of the column, and the soil cut lengthwise with a reciprocating saw, so that the entire column was cut in half lengthwise. The two halves were flipped open, resulting in two root faces (Figure 7) and the roots were exposed on one of the faces using a soil knife to uncover the roots. Data were collected from the exposed root face for root distribution and depth; root biomass data was collected from both faces (explained further in Section 2.2.1).

Figure 7. Column takedown process.
Column takedown included (A) removal of columns from the greenhouse, (B) bringing columns to a different building for processing, (C) cutting the plastic of the column lengthwise, and (D) exposing root face after the soil column was cut in half.

2.2 **Monitoring and Maintenance**

2.2.1 Plant Growth Measurements

Germination was assessed once per week for the first four weeks after seeding. From week 5 to 8, plant height and crop staging measurements were taken weekly. The height of each plant per column was measured as an indicator of health and growth rate. Crop staging values (see Appendix A) reflected the most mature growth stage per column, and were used to determine when to harvest. After week 8, crop staging measurements were taken weekly, but plant height measurements were recorded on a bi-weekly basis from this point on. Additionally, instead of measuring the height of each plant per column, measurements were take of the maximum plant height per column; this was done because of the twining nature of alfalfa, which could result in stem breakage when taking measurements. Throughout the experiment, alfalfa plants were staked to minimize the interaction between plants in different columns (Figure 8).

Figure 8. Staked alfalfa plants in the greenhouse.

Aboveground biomass was harvested when the majority of the plants were at growth stages 5 to 6 (Appendix A); this was done multiple times during the experiment, with all plants being harvested at the same time. The length of time between harvests was not necessarily equal, as it depended on plant maturity. Plants were harvested 3” (7.6 cm) above the soil surface and allowed to re-grow after each harvest. Aboveground biomass was harvested eight times throughout the duration of the experiment (Table 3). Biomass was dried at 60°C for one week prior to being weighed.

Table 3. The dates on which aboveground biomass harvests occurred during the project.

Aboveground biomass harvest	Date of harvest
Harvest 1	February 12, 2020
Harvest 2	March 26, 2020
Harvest 3	May 7, 2020
Harvest 4	June 11, 2020
Harvest 5	July 21, 2020
Harvest 6	August 27, 2020
Harvest 7	October 8, 2020
Harvest 8	November 13, 2020

Belowground biomass was assessed at the end of the experiment. Upon cutting the column in half and exposing the root profile, maximum root length was recorded and effective rooting depth was estimated visually. Effective rooting depth was defined as the depth above which 90% of roots were found.

Images of each soil column were taken with a camera to capture the entire root profile; a frame was constructed such that photos could be taken at standard intervals along the length of the column and at a standard height above the surface of the exposed soil (Figure 9). These root images were later analyzed using ImageJ (Schneider et al., 2012) software to determine how root distribution changed with depth by analyzing root percent area in 1 cm increments down the soil profile.

At each salinity interface, a 20 x 20 cm grid was placed 5 cm above and 5 cm below the interface to assess root distribution . The grid consisted of four 10 cm by 10 cm grid cells. Within each cell, the size, abundance, and orientation of roots were assessed (Table 4). A classification for size, abundance, and orientation was determined based on the four grid cells. The 5 cm buffer above and below the interface was to account for potential settling over time.

Figure 9. Frame used to photograph the root profile (left, indicated with arrow) and the process of taking photographs (right).

Table 4. Parameters measured in the 20 x 20 cm grid frames.
The same size classes were used when defining root sizes in root biomass samples.

Class*/Abundance	Average #/square decimeter (10 x 10 cm)		
	Very fine (<1mm)	Fine (1 to <2 mm)	Coarse** (2 to >5 mm)
Few	10	10	1
Plentiful	10-100	10-100	1-10 medium; 1-5 coarse
Abundant	>100	>100	>10 medium; >5 coarse
Orientation	vertical, oblique, horizontal, random		

* Root size classes defined based on Working Group on Soil Survey Data (1975), with modifications for this study.
** Medium (2 to 5 mm) and coarse (>5 mm) roots, as specified by the Working Group on Soil Survey Data (1975), were combined into one category defined as "coarse" for the purposes of this study.

Samples for root biomass were taken above and below the salinity interfaces (Table 5). In the control treatment, root biomass samples were taken at depths to match those across all the different salinity treatments (Table 5). Root biomass samples consisted of 15 cm soil cookies extracted from both halves of the column (Figure 10). Root biomass samples were sieved and roots extracted from the soil. Large sieves were constructed with a screen with approximately 1 cm openings (Figure 11). Soil was poured onto the sieve and shaken until soil no longer fell through the sieve. Coarse roots remaining on the sieve were removed from the soil. Then, 10 minutes were spent extracting roots from the soil with tweezers; through preliminary trials this amount of time was determined to be adequate to collect the majority of the fine roots in the samples, and ensured consistency among the samples. Coarse roots were collected and washed separately from fine roots. Biomass was dried at 60°C for one week prior to being weighed.

Table 5. Depths (cm) below ground surface at which root biomass cookies were taken to capture roots above and below the salinity interfaces.

Treatment	Above the Salinity Interface Depth (cm bgs)	Below the Salinity Interface Depth (cm bgs)
50 cm bgs	30-45	55-70
75 cm bgs	55-70	80-95
100 cm bgs	80-95	105-120
125 cm bgs	105-120	130-145
150 cm bgs	130-145	155-170
175 cm bgs	155-170	180-190
Control*	30-45, 55-70, 80-95, 105-120, 130-145, 155-170, 180-190	

*While there was no salinity interface present in the control treatment (as no salt-contaminated soil was added to these columns), samples were taken at seven different depths to match those in the salinity treatments.

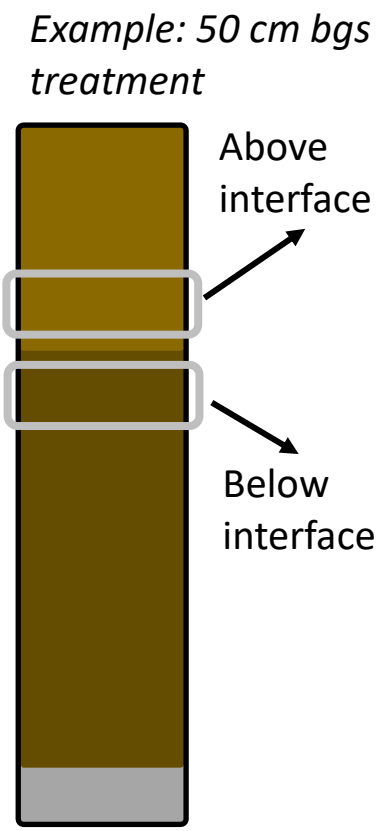

Figure 10. Example of how root biomass samples were collected (showing the 50 cm bgs treatment). A 5 cm buffer was left above and below the salinity interface. Two 15 cm deep cookies of soil were taken for root sieving, with one cookie being above the salinity interface, and one below. The same process was completed on the other half of the column.

Figure 11. Frames used for root sieving. The photo on the left shows the frame without soil, and the photo on the right shows a frame with soil prior to sieving.

2.2.2 Data Loggers/Sensors

Sensors connected to data loggers monitored moisture, temperature, and electrical conductivity at four points within the 13 columns which were instrumented (Section 2.1.2). Data logger information was downloaded weekly to monitor moisture and salinity dynamics within the columns. This information was used to inform the watering regime (Section 2.2.3) as plants grew and their water requirements changed.

The electrical conductivity (EC) data from the sensors needed to be calibrated for the specific growth medium and moisture conditions. A calibration was set up in the lab to obtain accurate EC data from the sensors. The same soil material used to fill the columns was used for the calibration. Oven-dried soil was spiked with NaCl, homogenized, and placed into ten pails, each with increasingly high salt concentrations. The intention was to build a calibration curve for EC (dS/m) from the inputs of raw sensor EC values, gravimetric moisture, and the amount of granular salt added. Water was added to the pails (10 mL at a time), soil homogenized, and EC measured with the sensors; this process was repeated to achieve a range of moisture contents for the calibration. The calibration was applied to the raw data outputs from the data loggers, which improved the accuracy of the EC data collected using the dataloggers during the experiment. Calibrated data were validated with soil samples, which were analyzed by a commercial laboratory, at the end of the experiment to assess movement of salinity (Section 3.1).

2.2.3 Watering Regime

For the first month after seeding, while plants were establishing, manual watering was conducted based on plant requirements. Afterwards, the drip irrigation system was set to water according to historical summer precipitation values in the in the Central Parkland. Strong and Leggat (1992) stated that total summer precipitation for the Aspen Parkland Ecoregion, similar to the Central Parkland, ranged from 234 to 323 mm, with a median value of 259 mm; total annual precipitation averaged 412 mm. In a report from the Natural Regions Committee (2006), it was stated that growing season precipitation (May to September) for the Central Parkland Natural Subregion averages 330 mm, while annual precipitation averages 447 mm. We used the information from Natural Regions Committee (2006) to guide the watering regime.

After nine weeks of growth, it was observed that soil moisture in the columns was decreasing to near wilting point. Given the artificial nature of greenhouse growth (i.e., lack of spring melt to increase moisture, restricted area in which roots can access water, artificial lighting which emits heat), it was determined that the plants required more water than was being provided. In an effort to simulate natural conditions as closely as possible, watering thereafter was conducted in high volumes with low frequency. The resulting watering regime consisted of approximately three watering events per week (depending on plant growth stage), with plants receiving the equivalent of approximately 330 mm of rainfall in the time it took to reach maturity. While this equates to a higher volume of rain than would typically fall over a five month growing season (Natural Regions Committee, 2006), it compensates for sources of moisture that exist in nature but not in the greenhouse (i.e., spring melt, overland flow from larger watershed). In general, watering was adjusted throughout the project to maintain a soil moisture approximately 70% of field capacity in the upper half of the columns.

2.2.4 Additional Maintenance

Natural predators (predatory mites and thrips eliminator, both biocontrol agents) were released bi-weekly as a preventative measure against insect infestations. Plants were assessed weekly for signs of abnormal growth, stress, or visible insect damage.

After the second and fourth harvests, all columns were fertilized with ammonium phosphate (11-52-0) at a rate of 60 kg/ha. This fertilizer was selected as it did not contain chloride, which would be a confounding factor given that the soil was spiked with sodium chloride. The fertilizer and application rate were selected based on what is typically done by farmers in the study area.

In early August, aphids were observed in approximately twelve columns in Row 1 of the greenhouse (Figure 1). Safer's soap (Safer® Brand) was applied to control the aphids, followed by an application of Intercept 60 WP (60% imidacloprid; Bayer CropScience Inc., Calgary AB) the following week. A follow-up application of Intercept 60 WP was applied one week later. By mid-August, the aphid population had drastically decreased and it was eliminated by the end of August; the physical damage to plants due to the infestation was not severe.

In early October, powdery mildew and spider mites were first observed in columns in Row 3 (Figure 1), but later spread throughout the greenhouse. Nova 40 WP (40% myclobutanil; Dow AgroSciences Canada Inc., Calgary AB) was applied twice to deal with the powdery mildew, and Safer's soap was applied for the spider mites. While the powdery mildew and spider mites appeared to be declining in early November, a minor to moderate degree of damage was observed on the alfalfa plants.

2.3 **Statistical Analysis**

Data exploration, analysis, and visualization were carried out using the R language and environment for statistical computing (R Core Team, 2019) and the additional packages *tidyr* (Wickham and Henry, 2019), *dplyr* (Wickham et al., 2019), *purrr* (Henry and Wickham, 2019), *ggplot2* (Wickham, 2016), and *emmeans* (Lenth et al., 2019). In this report, the term "significant" is used to describe statistically significant relationships ($\alpha = 0.05$). For all statistical tests, data were explored to determine whether assumptions of normality and homogeneity of variance were met; this included visual examination of data and residuals, Shapiro-Wilk test, and Levene's test. In cases where these assumptions were violated, the data were square-root transformed to meet assumptions.

Aboveground biomass and maximum plant height were assessed with repeated measures ANOVA to determine statistical differences among treatments and at various timepoints throughout the experiment. For aboveground biomass, differences among salinity treatments at each timepoint (harvest) were assessed with ANOVA followed by pairwise comparisons. The eighth harvest was excluded from statistical analysis of aboveground biomass, due to plant growth complications (i.e., insect issues, continuous growth throughout the experiment preventing senescence); however, the data are provided in Appendix B. Statistical differences among the salinity treatments for cumulative aboveground biomass (summed over the first seven harvests) were assessed with ANOVA followed by pairwise comparisons.

For belowground biomass, data for coarse and fine roots were analyzed separately. Very fine roots were excluded from the analysis as they tended to fall through the sieve. We were interested in examining whether there were significant differences in coarse and fine roots above and below salinity interfaces,

for each treatment. The data were separated by treatment and analyzed with ANOVA followed by pairwise comparisons with Tukey adjustment ($\alpha = 0.05$) for multiple comparisons. The control treatment was included in these analyses; including the control was important to verify whether differences in root biomass were due to salinity or simply to changes in root distribution with depth. The sample size for root biomass varied, as sometimes samples could not be collected (i.e., due to soil slumping at the bottom of the columns when columns were cut open); sample sizes are noted in Table 6. When conducting statistical analysis of maximum and effective root depth, and root distribution, ANOVA was used followed by pairwise comparisons with Tukey adjustment ($\alpha = 0.05$) for multiple comparisons.

We were interested in comparing coarse root biomass data and root area data above and below salinity interfaces at the deepest salinity interfaces (150 and 175 cm bgs). For each replicate, root % area data was averaged in the same intervals at which biomass samples were collected (150 cm bgs treatment: 130-145 cm and 15-170cm; 175 cm bgs treatment: 155-170 cm and 180-19 cm). The same procedure was followed for the control treatment. ANOVA followed by pairwise comparisons with Tukey adjustment ($\alpha = 0.05$) for multiple comparisons was used to assess statistical differences above and below salinity interfaces.

Table 6. The sample size (n) for coarse and fine root biomass measured for each treatment and depth.

Treatment	Depth (Above/Below Salinity Interface)	Coarse Root Sample Size (# of columns)	Fine Root Sample Size (# of columns)
50 cm bgs	30-45 cm	10	10
50 cm bgs	55-70 cm	10	10
75 cm bgs	55-70 cm	10	10
75 cm bgs	80-95 cm	10	10
100 cm bgs	80-95 cm	10	10
100 cm bgs	105-120 cm	10	10
125 cm bgs	105-120 cm	10	10
125 cm bgs	130-145 cm	10	10
150 cm bgs	130-145 cm	10	10
150 cm bgs	155-170 cm	10	10
175 cm bgs	155-170 cm	10	10
175 cm bgs	180-190 cm	9	9
Control	30-45 cm	10	10
Control	55-70 cm	10	10
Control	80-95 cm	10	10
Control	105-120 cm	10	10
Control	130-145 cm	10	10
Control	155-170 cm	10	10
Control	180-190 cm	9	9

3.0 RESULTS AND DISCUSSION

3.1 Soil Salinity

Dataloggers were used to assess soil moisture (to determine the necessary watering regime) and to monitor whether there was movement of salinity within the soil profile. While salinity was observed to fluctuate depending on moisture content, dataloggers did not indicate a strong movement of soil salinity from the initial salinity interfaces (Appendix C).

Soil samples were collected above and below the salinity interfaces at the end of the experiment, to validate data logger information and determine whether salinity had migrated through the soil profile. Not surprisingly, differences in salinity were driven by chloride, as the soil had been spiked with NaCl.

There was some movement of salinity at the interfaces. Salinity above the interfaces tended to be higher than salinity in the controls (Table 7). However, EC below the interfaces was much higher than EC above the interfaces. EC below the interfaces tended to be higher than the initial EC (14.5 dS/m), which indicates some movement of salts from either above the interface or from deeper in the soil profile, or both: water may have pushed salinity deeper in the soil profile, while roots may have drawn salts up above the interfaces. Salinity below the interface for the 175 cm bgs treatment was low (6.07 dS/m compared to the initial 14.5 dS/m), however the EC above the interface was very similar to the control; it is possible that salts leached into the gravel layer below the soil over time. In general, the data do not indicate considerable movement of salinity during the experiment.

Table 7. Soil salinity parameters observed in the 15 cm above the salinity interface and the 15 cm below the salinity interface for each treatment.
Values are means (n=3) with standard deviation in parentheses.

Treatment	Above or Below Interface*	Electrical Conductivity (dS/m)	SAR (Sodium Adsoprtion Ratio)	Cl (mg/kg)	SO_4 (mg/kg)
Control	-	0.77 (0.07)	0.57 (0.06)	15 (2)	138 (23)
Control	-	0.70 (0.02)	0.57 (0.06)	12 (4)	126 (2)
50 cm bgs	Above	3.67 (1.36)	2.37 (1.39)	460 (251)	314 (27)
50 cm bgs	Below	11.25 (1.86)	14.70 (1.55)	2030 (318)	127 (21)
75 cm bgs	Above	2.55 (1.49)	1.10 (0.52)	285 (339)	329 (184)
75 cm bgs	Below	12.52 (3.47)	15.17 (2.19)	2250 (351)	129 (45)
100 cm bgs	Above	3.12 (2.05)	1.03 (0.49)	403 (328)	195 (48)
100 cm bgs	Below	16.73 (0.55)	16.90 (0.26)	2770 (276)	92 (9)
125 cm bgs	Above	1.94 (0.81)	0.97 (0.32)	210 (115)	124 (15)
125 cm bgs	Below	15.27 (1.62)	15.97 (1.02)	2613 (220)	93 (10)
150 cm bgs	Above	1.71 (0.80)	0.87 (0.12)	177 (137)	123 (12)
150 cm bgs	Below	13.67 (3.04)	17.13 (1.03)	2217 (442)	88 (11)
175 cm bgs	Above	0.93 (0.13)	0.60 (0.00)	51 (21)	123 (15)
175 cm bgs	Below	5.04 (2.18)	6.07 (3.29)	804 (385)	141 (54)

* For the control treatment, samples were taken 125 and 150 cm bgs.

3.2 **Aboveground Biomass**

Both salinity treatment and time had significant impacts on aboveground biomass ($p<0.001$). The interaction between salinity treatment and time was not significant. Differences among salinity treatments at each timepoint (harvest) are discussed in Sections 3.2.1 to 3.2.7. Table 8 provides an overview of the aboveground biomass data for each harvest.

Table 8. Aboveground biomass (g) from each harvest conducted during the experiment.
Data are means (n=10) with standard deviation in parentheses. The treatments refer to the depth at which salt contaminated material is present. Different lowercase letters indicate significant differences among treatments for a given harvest.

Treatment	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Harvest 6	Harvest 7	Harvest 8
50bgs	31.0 (3.87)d	49.2 (6.13)c	67.7 (8.65)a	67.3 (8.94)a	44.0 (7.24)a	38.8 (4.43)ab	30.9 (10.3)a	29.4 (6.28)a
75bgs	32.3 (4.73)cd	55.4 (7.58)bc	72.0 (9.46)a	68.7 (11.2)a	41.9 (7.08)a	36.3 (4.85)b	34.4 (5.61)a	31.1 (4.85)a
100bgs	39.2 (5.57)abc	55.9 (9.91)bc	74.9 (11.3)a	76.5 (11.0)a	46.7 (7.35)a	41.8 (5.09)ab	37.3 (6.52)a	36.2 (7.29)a
125bgs	38.4 (5.77)abc	54.6 (7.81)bc	70.0 (10.3)a	71.1 (15.1)a	49.3 (11.9)a	35.8 (7.75)b	32.6 (7.23)a	32.2 (8.17)a
150bgs	44.8 (6.35)a	61.8 (3.48)ab	77.5 (5.82)a	75.7 (8.01)a	47.4 (6.21)a	37.0 (7.31)b	35.3 (5.97)a	31.1 (5.98)a
175bgs	40.8 (3.76)ab	63.1 (6.79)ab	77.3 (8.30)a	74.4 (11.7)a	46.4 (9.85)a	39.0 (6.40)ab	34.5 (7.72)a	32.6 (6.10)a
Control	37.6 (5.21)bcd	67.1 (10.9)a	78.5 (11.4)a	80.4 (16.6)a	52.4 (10.2)a	47.3 (7.46)a	39.9 (6.23)a	28.0 (9.52)a

3.2.1 First Harvest

Treatments with salt contamination closer to the surface tended to have lower aboveground biomass than those treatments with contamination further down the column profile (Figure 12). For example, the 50 and 75 cm bgs treatments had significantly lower biomass compared to the 100 to 175 cm bgs treatments. Roots may not have reached the salt contamination in the other treatments, or the contamination may not have impacted biomass because there was a sufficient depth of overlying uncontaminated material for roots.

The control treatment was not significantly different from any of the other treatments except the 150 cm bgs treatment, which had the highest biomass. Sensor data indicated that control columns tended to be drier compared to the other treatments.

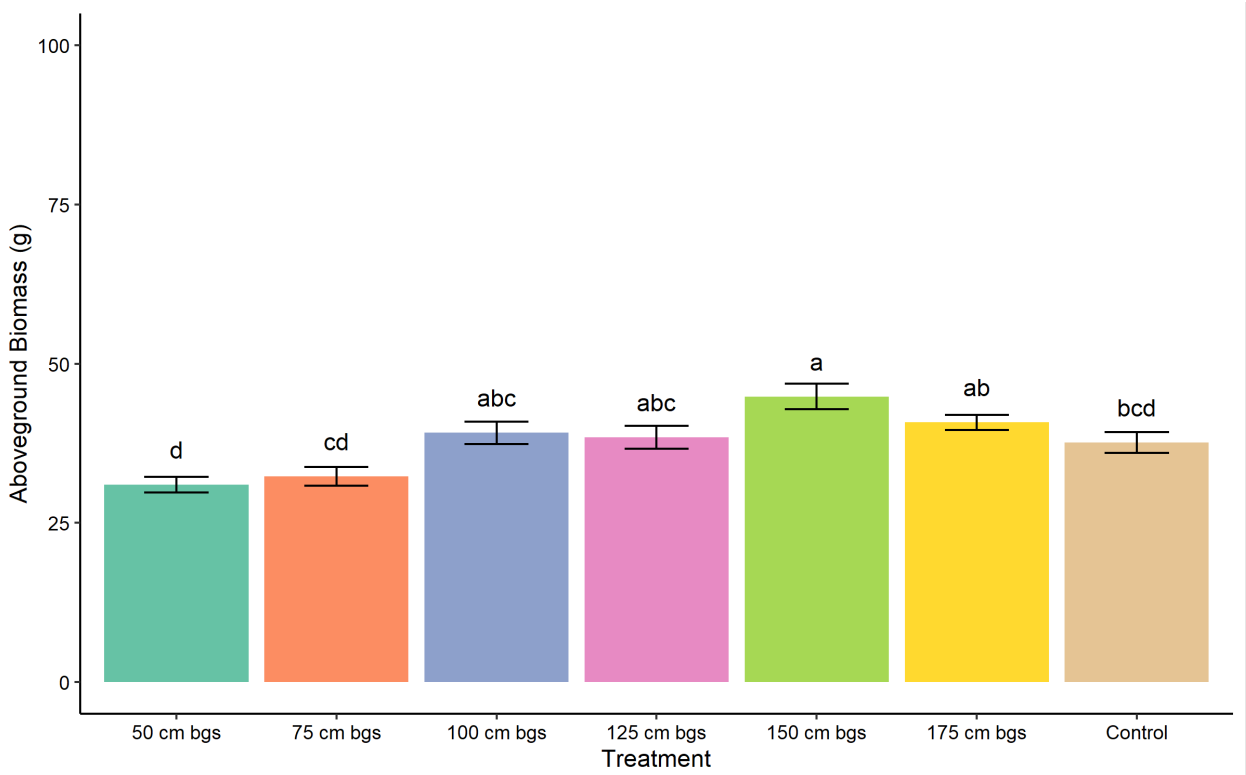

Figure 12. Mean aboveground biomass (g) from the varying salinity depth treatments after the first harvest. Bars are means (n=10) with standard error bars. Different lowercase letters indicate significant differences between the treatments. The treatments refer to the depth at which salt contaminated material is present.

3.2.2 Second Harvest

Results from the second harvest indicated that the control treatment had significantly higher aboveground biomass compared to treatments where salt contamination was present 50 to 125 cm bgs (Figure 13). The control did not differ significantly from the 150 and 175 bgs treatments, indicating that the depth of salt contamination in these treatments was not having as strong an impact on aboveground growth.

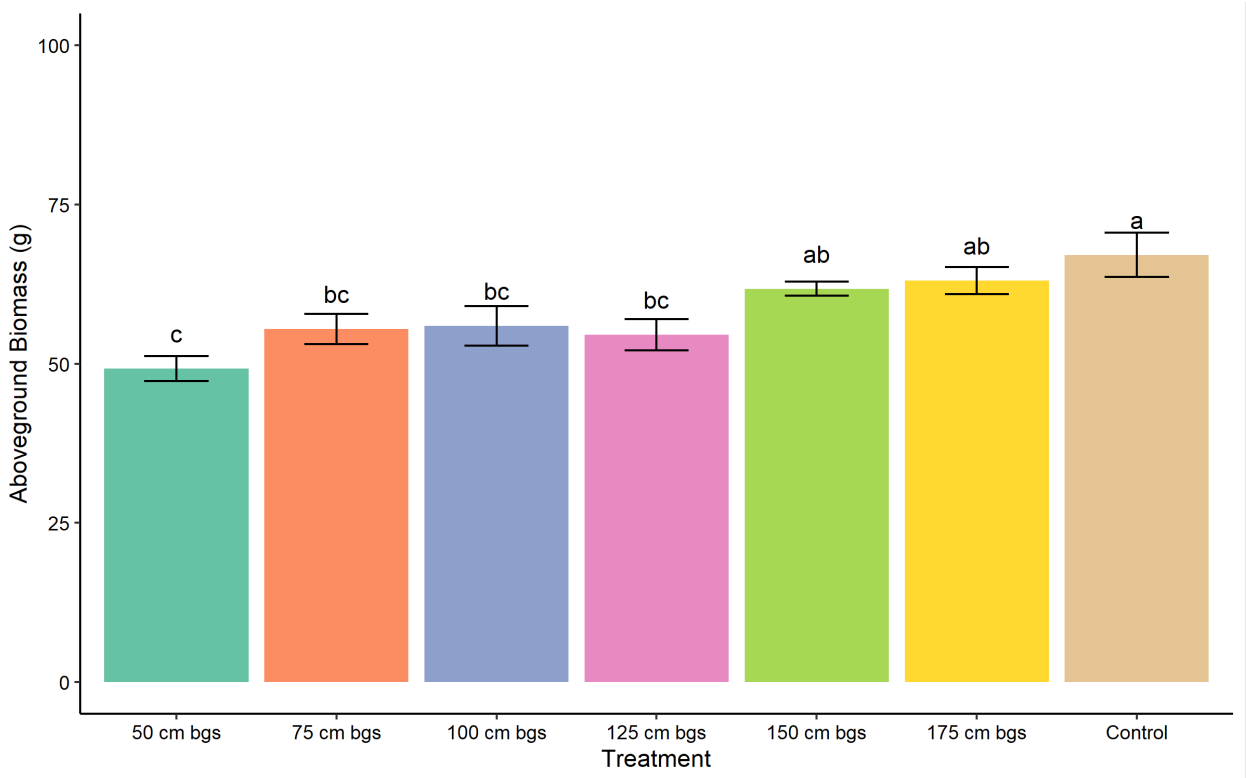

Figure 13. Mean aboveground biomass (g) from the varying salinity depth treatments after the second harvest. Bars are means (n=10) with standard error bars. Different lowercase letters indicate significant differences between the treatments. The treatments refer to the depth at which salt contaminated material is present.

3.2.3 Third Harvest

While the control had the greatest mean aboveground biomass, no statistical differences in biomass were observed for the third harvest (Figure 14). Fertilizer was applied after the second harvest, which could have influenced aboveground growth.

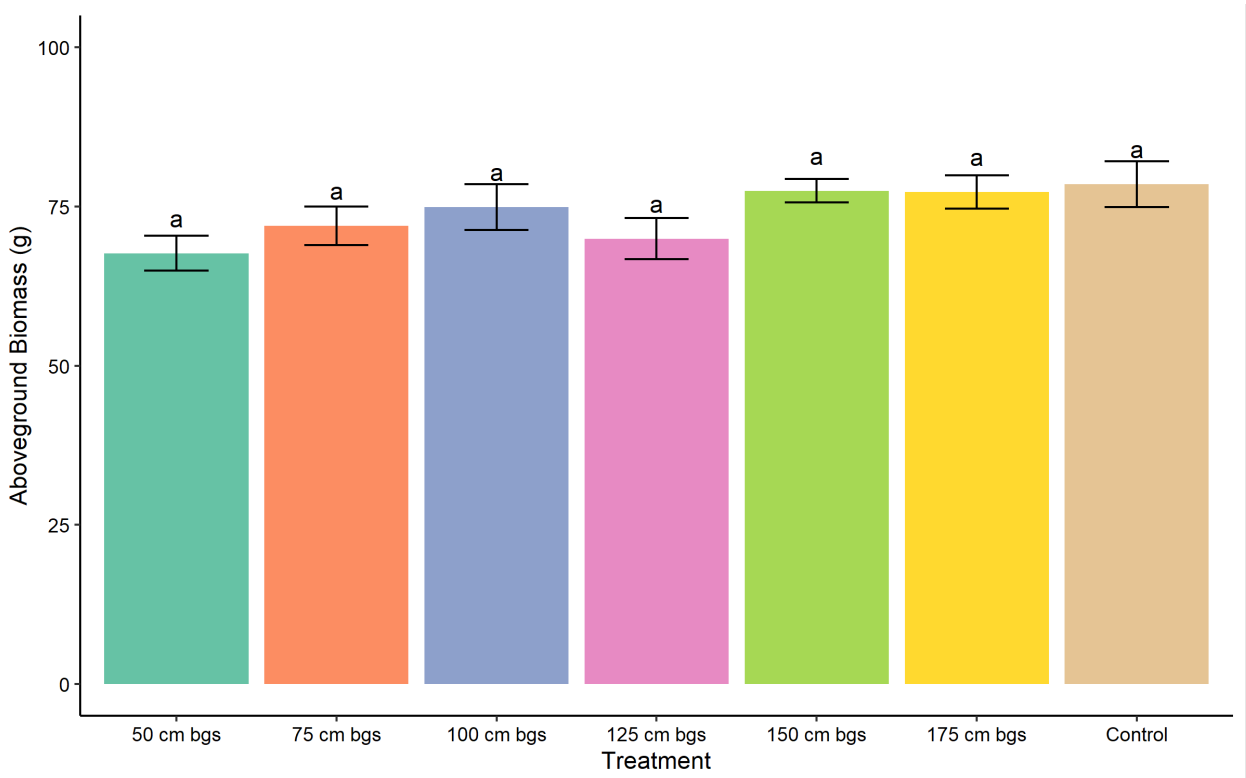

Figure 14. Mean aboveground biomass (g) from the varying salinity depth treatments after the third harvest. Bars are means (n=10) with standard error bars. Different lowercase letters indicate significant differences between the treatments. The treatments refer to the depth at which salt contaminated material is present.

3.2.4 Fourth Harvest

There were no significant differences among the salinity treatments after the fourth harvest (Figure 15).

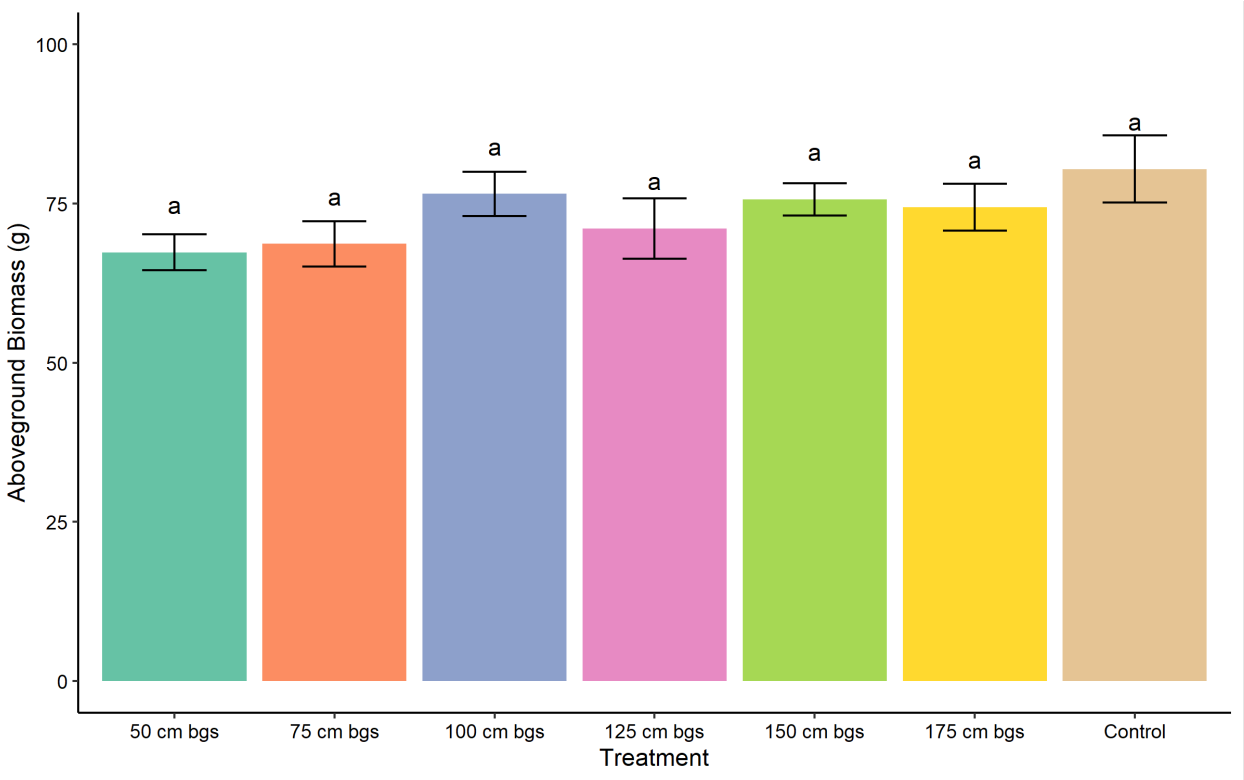

Figure 15. Mean aboveground biomass (g) from the varying salinity depth treatments after the fourth harvest. Bars are means (n=10) with standard error bars. Different lowercase letters indicate significant differences between the treatments. The treatments refer to the depth at which salt contaminated material is present.

3.2.5 Fifth Harvest

There were no significant differences among the salinity treatments after the fifth harvest (Figure 16).

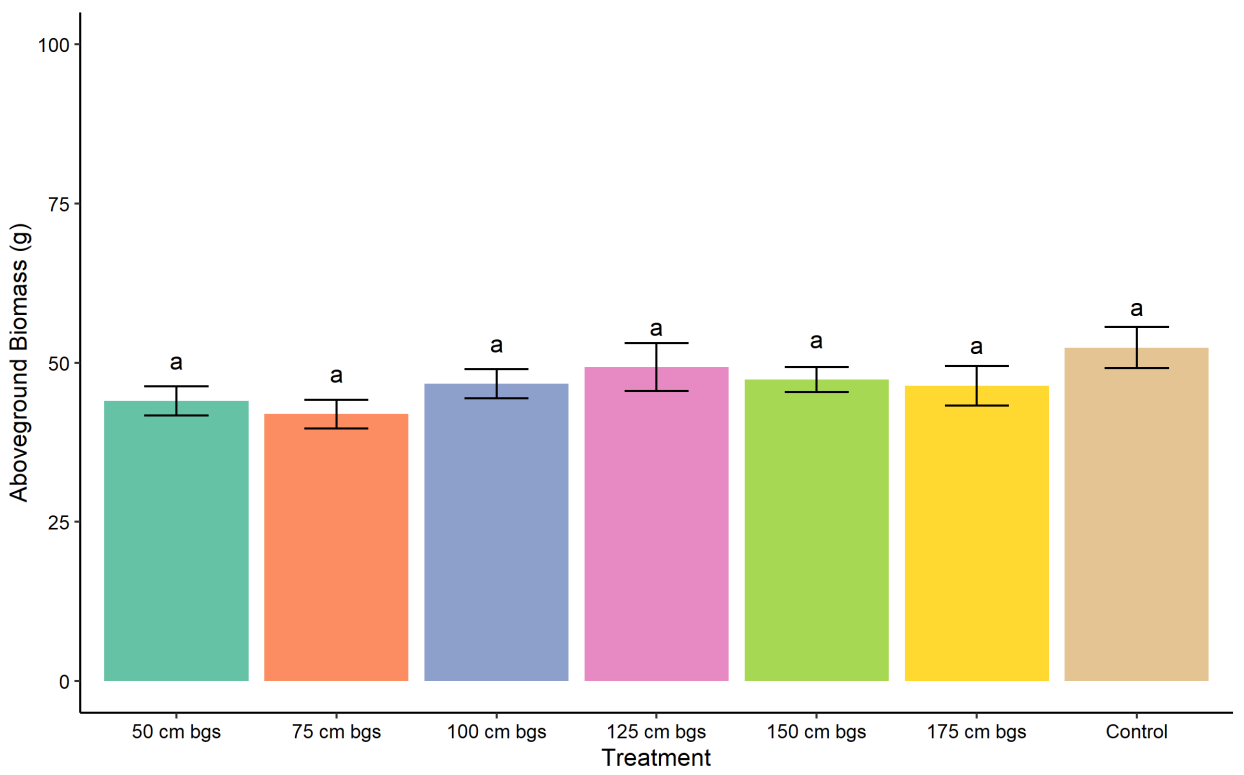

Figure 16. Mean aboveground biomass (g) from the varying salinity depth treatments after the fifth harvest. Bars are means (n=10) with standard error bars. Different lowercase letters indicate significant differences between the treatments. The treatments refer to the depth at which salt contaminated material is present.

3.2.6 Sixth Harvest

At the sixth harvest, the control treatment had the highest aboveground biomass, and was significantly higher than the 75, 125, and 150 bgs treatments (Figure 17).

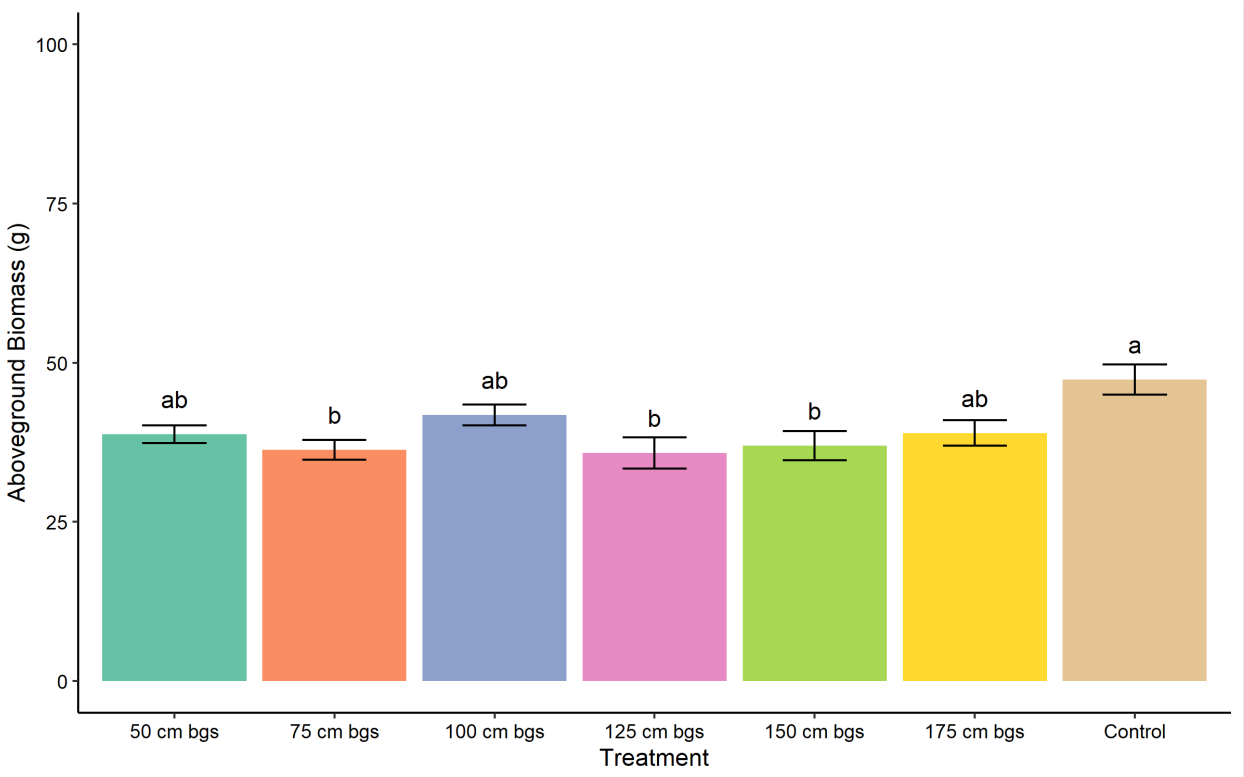

Figure 17. Mean aboveground biomass (g) from the varying salinity depth treatments after the sixth harvest. Bars are means (n=10) with standard error bars. Different lowercase letters indicate significant differences between the treatments. The treatments refer to the depth at which salt contaminated material is present.

3.2.7 Seventh Harvest

While the control treatment had the highest aboveground biomass, there were no significant differences among the salinity treatments at the seventh harvest (Figure 18).

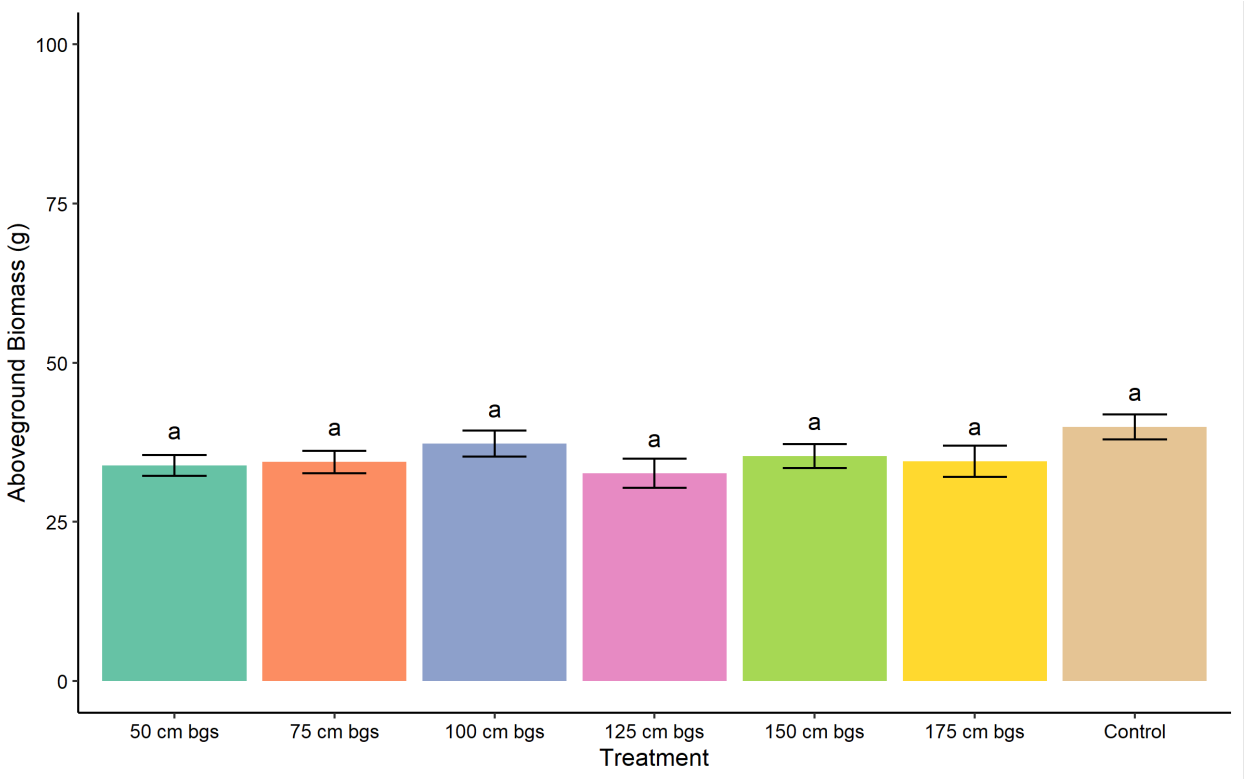

Figure 18. Mean aboveground biomass (g) from the varying salinity depth treatments after the seventh harvest. Bars are means (n=10) with standard error bars. Different lowercase letters indicate significant differences between the treatments. The treatments refer to the depth at which salt contaminated material is present.

3.2.8 Cumulative Aboveground Biomass

When considering the cumulative aboveground biomass from seven harvests, the only significant difference was between the control and 50 bgs treatment (Table 9, Figure 19). Considering the cumulative data, at depths 75 cm bgs and below salinity present in the soil did not appear to have a strong effect on aboveground biomass.

Figure 19 shows that the amount of biomass harvested changed over time. Aboveground biomass tended to increase after the first harvest, peaking at the third and fourth harvests. The amount of biomass harvested then decreased until the end of the experiment. The changes in biomass over the length of the experiment are likely due to plant stress due to continuous growth conditions in the greenhouse and possibly environmental factors (i.e., stronger sunlight during summer months compared to winter).

Table 9. Cumulative aboveground biomass (g) for each salinity treatment after seven harvests. Values are means with standard deviation in parentheses. Different lowercase letters indicate statistically significant differences among treatments.

Treatment	Cumulative Aboveground Biomass (g)
50bgs	332 (39.5)b
75bgs	341 (47.0)ab
100bgs	372 (51.0)ab
125bgs	352 (57.5)ab
150bgs	379 (28.3)ab
175bgs	375 (47.9)ab
Control	403 (61.5)a

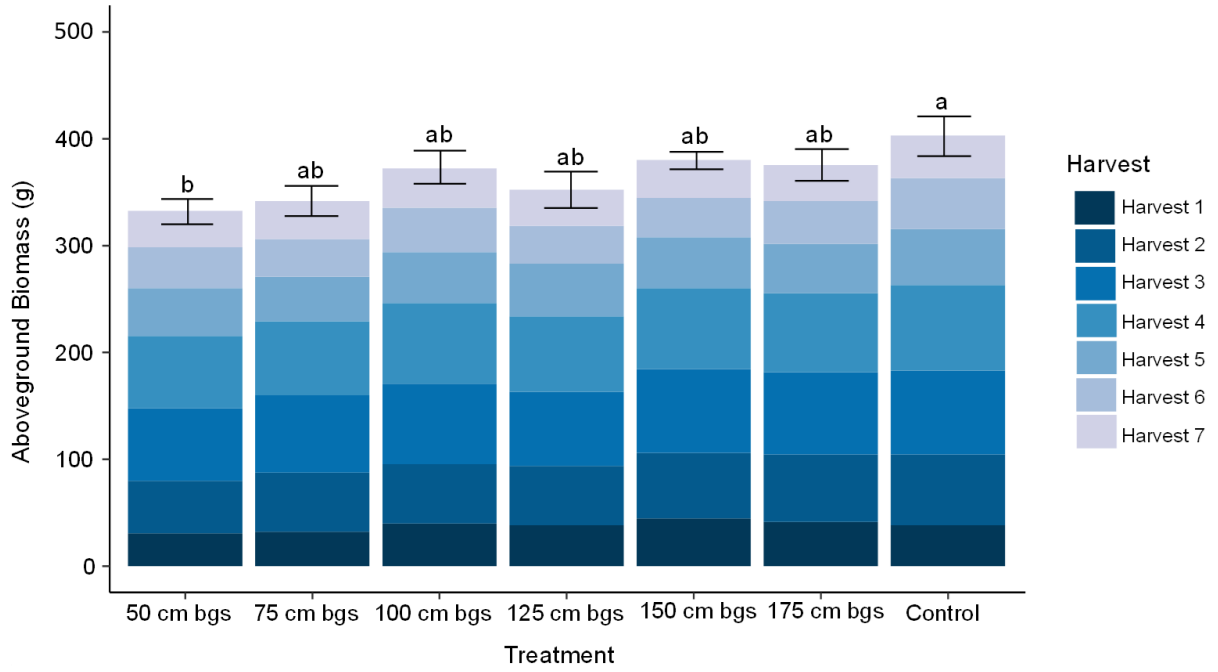

Figure 19. Mean cumulative aboveground biomass (g) from the varying salinity depth treatments summed over seven harvests. Differently coloured bars indicate the mean biomass from each harvest. Bars are means (n=10) with error bars representing the standard error of the cumulative data. Different lowercase letters indicate significant differences between the treatments (in terms of cumulative data). The treatments refer to the depth at which salt contaminated material is present.

3.2.9 Aboveground Biomass Summary

Key findings from the aboveground biomass data include:

- In general, the control treatment tended to have higher aboveground biomass than treatments with salinity impacts.
- The relationship between the treatments varied with time. Early in the experiment (the first two harvests, there was evidence of the control and treatments with salinity lower in the soil profile having higher aboveground biomass. As more harvests were conducted, there tended to be few significant differences among treatments.
- Aboveground biomass peaked at the third and fourth harvests, and then decreased with subsequent harvests, indicating that plant productivity started to decrease mid-way through the experiment (likely due to the extended growth period and multiple harvests).
- Considering the cumulative biomass, summed over seven harvests, the control treatment had the highest aboveground biomass, but was only significantly different from the 50 cm bgs treatment.
- **The effect of salt on aboveground biomass was not always consistent, and appeared to decrease as plants became more established.**

3.3 **Maximum Height**

After each harvest, plant height increased until the next harvest. Similar to aboveground biomass, plant height peaked approximately midway through the experiment, after which maximum height tended to be lower across treatments (Figure 20). Repeated measures ANOVA revealed a significant effect of both time and treatment on height, and a significant interaction between time and treatment. In this study, a significant interaction indicates that the impact that salinity treatment has on plant height depends on time. Given that height was reduced after each harvest, it is not surprising that plant height was dependent on time. Overall, there was not a clear, consistent effect of salt on plant height throughout the experiment. Plant height does not appear to be an appropriate indicator for assessing the effect of salinity on plant health, at least not when period harvests occur.

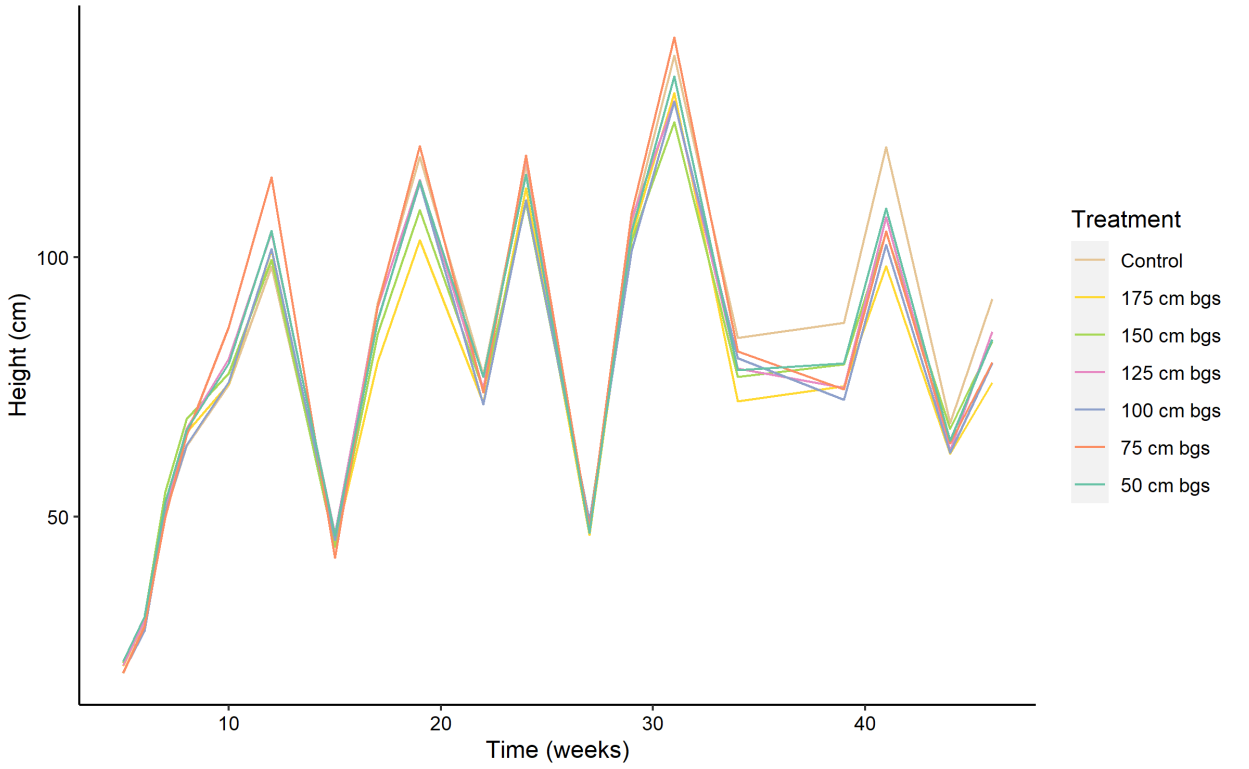

Figure 20. Maximum height (cm) of alfalfa plants measured at various timepoints throughout the experiment. Values are means (n=10), and different coloured lines represent different salinity treatments. Time is indicated as weeks since alfalfa plants were seeded. Note that biomass was harvested periodically, which periodically reduced height to zero (not shown on this graph). Height measurements did not necessarily coincide with harvest dates, and heights just prior to the July 21, 2021 harvest were not measured.

3.4 Belowground Biomass

Belowground biomass samples were collected and analyzed at the end of the greenhouse study. Table 10 provides data on coarse and fine root biomass.

Table 10. Coarse and fine root biomass (g/kg) at different depths (above and below the salinity interface) for the various salinity treatments.
Values are means with standard deviation in parentheses.

Treatment	Depth (Above/Below Salinity Interface)	Coarse Root Biomass (g/kg)	Fine Root Biomass (g/kg)
50 cm bgs	30-45 cm	2.26 (0.52)	0.10 (0.05)
50 cm bgs	55-70 cm	0.79 (1.32)	0.33 (0.71)
75 cm bgs	55-70 cm	0.90 (0.29)	0.09 (0.03)
75 cm bgs	80-95 cm	0.04 (0.05)	0.06 (0.05)
100 cm bgs	80-95 cm	0.34 (0.14)	0.07 (0.05)
100 cm bgs	105-120 cm	0.01 (0.02)	0.04 (0.05)

Treatment	Depth (Above/Below Salinity Interface)	Coarse Root Biomass (g/kg)	Fine Root Biomass (g/kg)
125 cm bgs	105-120 cm	0.17 (0.07)	0.07 (0.03)
125 cm bgs	130-145 cm	0.001 (0.003)	0.05 (0.04)
150 cm bgs	130-145 cm	0.09 (0.08)	0.06 (0.03)
150 cm bgs	155-170 cm	0.002 (0.006)	0.04 (0.03)
175 cm bgs	155-170 cm	0.06 (0.04)	0.08 (0.04)
175 cm bgs	180-190 cm	0.01 (0.02)	0.07 (0.03)
Control	30-45 cm	2.30 (0.96)	0.10 (0.10)
Control	55-70 cm	1.17 (0.75)	0.05 (0.02)
Control	80-95 cm	0.78 (0.33)	0.05 (0.03)
Control	105-120 cm	0.57 (0.21)	0.05 (0.04)
Control	130-145 cm	0.31 (0.13)	0.06 (0.04)
Control	155-170 cm	0.08 (0.07)	0.07 (0.05)
Control	180-190 cm	0.01 (0.02)	0.13 (0.08)

3.4.1 Coarse Roots

Coarse root biomass in the control and salinity treatments is shown in Figure 21. The goal of the biomass analysis was to determine whether root biomass decreased below the salinity interface compared to above, indicating whether there was an impact of salinity on roots. The average reduction in coarse root biomass below the interface compared to above ranged from 65% to 99%, depending on the treatment. In the control treatment, coarse root biomass decreased as depth from the surface increased; this was expected as root biomass generally tends to decrease with depth (AAF, 2016).

It was important to compare root biomass in the salinity treatments to the control at the same depth. This comparison was necessary to determine whether a decrease in biomass below the interface was due to salinity or natural changes in rooting patterns related to depth.

3.4.1.1 The 50 cm bgs treatment

Coarse root biomass was significantly lower below the salinity interface compared to above, with an average 65% reduction in coarse root biomass. However, there was not a significant difference between the control and 50 cm bgs treatment below the salinity interface; this indicates that the reduction in root biomass may have been a function of depth, and not due to the presence of salinity. While the difference was not significant, there was a clear trend of reduced coarse root biomass below the salinity interface (at 55-70 cm bgs), compared to the control at the same depth.

3.4.1.2 The 75 cm bgs treatment

Coarse root biomass was significantly lower below the salinity interface for the 75 cm bgs treatment, with an average 96% reduction in coarse root biomass compared to above the interface. The reduction in root biomass was driven by the presence of salinity, as at the 80-95 cm depth (below the interface), the 75 cm bgs treatment had significantly lower biomass than the control. Additionally, within the

control columns there was no significant difference in coarse root biomass between the 55-70 cm and 80-95 cm bgs depths.

3.4.1.3 The 100 cm bgs treatment

Coarse root biomass was significantly reduced below the salinity interface for the 100 bgs treatment, and root biomass for the 100 cm bgs treatment differed significantly from the control at this depth (105-120 cm bgs). The average percent reduction in coarse root biomass below the salinity interface compared to above the interface was 97%. Above the salinity interface (80-95 cm bgs) there was a significant difference in coarse roots between the control and 100 cm bgs treatment, indicating that salinity may be impacting roots above the interface. Therefore, there was an impact of salinity on coarse roots both above and below the interface for the 100 cm bgs treatment.

3.4.1.4 The 125 cm bgs treatment

There was significantly lower coarse root biomass at 130-145 cm bgs (below the interface) for both the 125 bgs treatment and the control, compared to above the salinity interface depth. However, the coarse root biomass for the 125 cm bgs treatment at 130-145 cm bgs (below the salinity interface) was also significantly lower than the control coarse root biomass at this depth. The average percent reduction in coarse root biomass below the salinity interface compared to above the interface was 99%. Additionally, root biomass at 105-120 cm bgs (above the interface) was lower for the 125 cm bgs treatment compared to the control. As for the 125 cm bgs treatment, the presence of salinity was associated with reduced root biomass both above and below the salinity interface.

3.4.1.5 The 150 cm bgs treatment

Coarse root biomass in the 150 cm bgs treatment was significantly lower than the control at both 130-145 cm bgs and at 155-170 cm bgs (above and below the salinity interface, respectively). Coarse root biomass within the 150 cm bgs treatment was significantly lower below the salinity interface, with an average 98% reduction in root biomass compared to above the interface. The presence of salinity appears to have reduced coarse root biomass both above and below the interface.

3.4.1.6 The 175 cm bgs treatment

There was a significant reduction in coarse root biomass from 155-170 cm bgs to 180-190 cm bgs (above and below the interface depth, respectively) for both the 175 cm bgs treatment and control. For the 175 cm bgs treatment, the average reduction in root biomass below the interface compared to above was 83%. At 180-190 cm bgs, the difference between the control and 175 bgs treatment was not significant, indicating that the reduction in root biomass may be due to depth, not salinity. It was more difficult to quantify roots from 180-190 cm bgs, as the lack of roots resulted in soil slumping and mixing with the underlying gravel when the columns were cut open, and the biomass was generally very low.

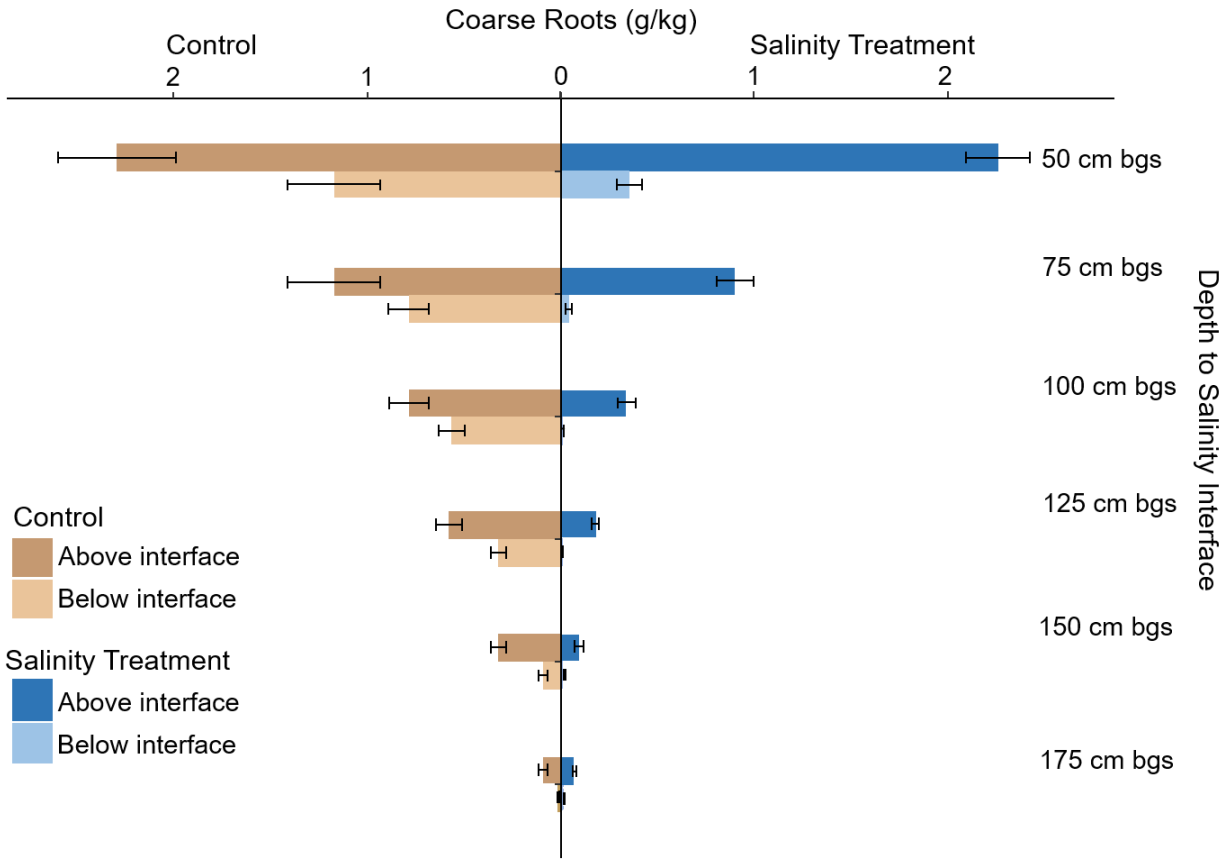

Figure 21. Coarse root biomass (g/kg) above and below the salinity interface for different salinity depth treatments.

Bars are means and error bars represent the standard error. The depth of the salinity interface is on the y-axis, arranged from the most shallow interface (50 cm bgs) at the top of the graph to the deepest (175 cm bgs) at the bottom. Beige bars indicate root biomass in the control at a specific depth, and the blue bars represent roots in the salinity treatments. The darker shade represents roots above the interface, and the lighter shade represents roots below the interface. For example, for the salinity interface at 50 cm bgs, the dark blue bar represents roots in the 15 cm above the salinity interface at 50 cm bgs, while the light blue bar represents root biomass taken in the 15 cm below that interface. Control samples were taken to match each of salinity interface depths used in the treatment columns.

3.4.2 Fine Roots

Fine root biomass in the control and salinity treatments is shown in Figure 22. The goal of the biomass analysis was to determine whether root biomass decreased below the salinity interface compared to above, indicating whether there was an impact of salinity on roots. Fine root biomass tended to be more variable than coarse roots. In the control treatment, fine root biomass tended to decrease with depth to approximately 100 cm bgs; below this depth a trend of increasing fine root biomass was observed to the bottom of the column.

3.4.2.1 The 50 cm bgs treatment

Fine root biomass in the 50 cm bgs treatment was higher than the control at 55-70 cm bgs (below the salinity interface), although the trend was not significant. When exposing root profiles, it was generally

observed that coarse roots were greatly reduced at salinity interfaces, while some fine roots travelled deeper than the interface.

3.4.2.2 The 75 cm bgs treatment (square root transformed)

No significant differences in fine root biomass between the 75 cm bgs treatment and control, or above and below interface depths, were observed. However, the decrease in fine root biomass below the salinity interface, compared to above, was greater in the 75 cm bgs treatment than in the control. For the 75 cm bgs treatment, visual differences were observed in fine roots above and below the interface, however the difference in biomass was not statistically significant.

3.4.2.3 The 100 cm bgs treatment

No significant differences in biomass between the 100 cm bgs treatment and control, or interface depths, were observed. However, visual differences were observed. Fine root biomass decreased below the salinity interface in the 100 cm bgs treatment, while there was a slight increase in the control treatment; while not statistically significant, this trend suggests that salinity may be impacting biomass of fine roots.

3.4.2.4 The 125 cm bgs treatment

No significant differences in biomass between the 125 cm bgs treatment and control, or interface depths, were observed. Similar to the 100 cm bgs treatment, the 125 cm bgs treatment showed a trend of decreased fine root biomass below the salinity interface; at the same depth, the control treatment showed a slight increase in root biomass. While not statistically significant, this trend suggests that salinity may be impacting biomass of fine roots.

3.4.2.5 The 150 cm bgs treatment (square root transformed)

No significant differences in biomass between the 150 cm bgs treatment and control, or interface depths, were observed. However, the 150 cm bgs treatment showed a trend of decreased fine root biomass below the salinity interface; at the same depth, the control treatment showed an increase in root biomass. While not statistically significant, this trend suggests that salinity may be impacting root depth of fine roots.

3.4.2.6 The 175 cm bgs treatment

Fine root biomass in the controls increased significantly from 155-170 cm to the 180-190 cm depth. There was no significant difference in fine root biomass for the 175 cm bgs treatment above and below the salinity interface, but there was a slight decrease in biomass below the salinity interface. The differing trend between the controls and 175 cm bgs treatment suggests that the presence of salt-contaminated soil at 175 cm bgs may have impacted the biomass of fine roots below the interface, compared to the control. While taking apart the columns, fine roots in the control columns were sometimes observed to extend into the gravel placed at 190-200 cm bgs and there was often a mat of roots directly above the gravel layer, indicating that the roots could have gone deeper if the soil columns were taller.

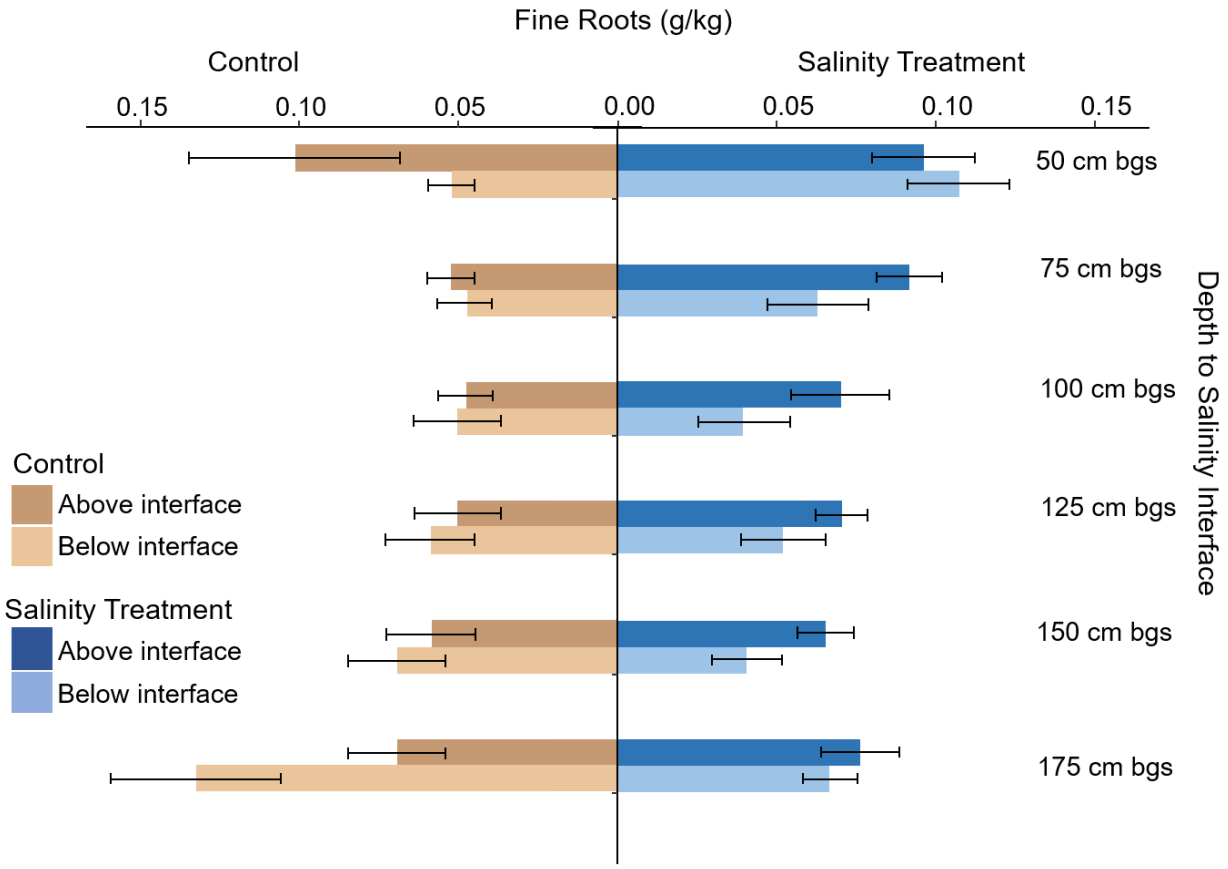

Figure 22. Fine root biomass (g/kg) above and below the salinity interface for different salinity depth treatments. Fine root biomass is indicated on the x-axis.
Bars are means and error bars represent the standard error. The depth of the salinity interface is on the y-axis, arranged from the most shallow interface (50 cm bgs) at the top of the graph to the deepest (175 cm bgs) at the bottom. Beige bars indicate root biomass in the control at a specific depth, and the blue bars represent roots in the salinity treatments. The darker shade represents roots above the interface, and the lighter shade represents roots below the interface. For example, for the salinity interface at 50 cm bgs, the dark blue bar represents roots in the 15 cm above the salinity interface at 50 cm bgs, while the light blue bar represents root biomass taken in the 15 cm below that interface. Control samples were taken to match each of salinity interface depths used in the treatment columns.

3.4.3 Belowground Biomass Summary

The presence of salinity at depths down to 150 cm bgs had a clear impact on coarse root biomass. In general, coarse root biomass was significantly reduced below the salinity interfaces, and was significantly lower than the controls. At 175 cm bgs, while there was a significant decrease in coarse root biomass below the interface, the reduction in the control treatment was also significant; therefore, it was not clear whether the reduction in coarse root biomass below the salinity interface was due to salinity or changes in natural rooting patterns at this depth.

When considering fine root biomass, the data were more variable, with few clear statistical differences observed. The trend observed for the salt-impacted treatments differed from the controls, in which fine root biomass increased with depth below 100 cm bgs. In general, in the salt-impacted treatments, fine

root biomass tended to decrease below salinity interfaces, irrespective of the depth. Given the difference in fine root biomass patterns in the controls versus salinity treatments, the data indicate that fine root biomass was impacted by salinity present at depths down to 175 cm bgs.

3.5 Root Depth – Maximum and Effective Depth

Results for maximum and effective rooting depth are provided in Table 11. The soil column consisted of 190 cm of soil material, which was the expected maximum possible root depth. However, roots in the control columns were sometimes observed to extend a few centimetres into the gravel at the bottom of the column. In one of the control columns, roots extended through the entire gravel layer, reaching a maximum depth of 200 cm (the length of the plastic column).

Table 11. Maximum and effective root depth (cm) for the different salinity treatments. Data are means (n=10) with standard deviation in parentheses. Different lowercase letters indicate statistically significant differences among the treatments. The treatments refer to the depth at which salt contaminated soil material was present.

Treatment	Max Root Depth (cm)	Effective Root Depth (cm)*
50 cm bgs	109 (19.7)d	50 (9.9)f
75 cm bgs	132 (25.1)c	66 (7.5)e
100 cm bgs	133 (11.0)c	84 (9.7)d
125 cm bgs	152 (16.4)bc	101 (5.0)c
150 cm bgs	167 (4.37)b	119 (4.4)b
175 cm bgs	188 (3.82)a	142 (3.82)a
Control	191 (3.16)a	138 (17.2)a

* Effective rooting depth was defined as the depth above which 90% of roots were found.

3.5.1 Maximum Root Depth

Maximum root depth was greatest in the control treatment, and decreased progressively with more shallow salinity treatments (Figure 23). All salinity treatments had significantly more shallow maximum root depths than the control, with the exception of the 175 cm bgs treatment. Soil salinity had a clear impact on maximum rooting depth. The data indicate that salinity present at depths down to 150 cm bgs has a significant negative effect on maximum root depth.

Interestingly, maximum root depth exceeded the depth of the salinity interface for all treatments, indicating that roots can grow in the salt-impacted soil. However, when the salinity was present at more shallow depths (i.e., 50 cm bgs), roots tended to extend further past the interface compared to when salinity was present at greater depths (i.e., 175 cm bgs). In this study, when salt was present at more shallow depths, roots may have extended past the salinity interface in search of water and nutrients; when salt was present deeper in the soil profile, roots may have had access to sufficient resources within the uncontaminated material, and therefore it was not necessary for the plant to extend roots as deep into the salt-impacted material.

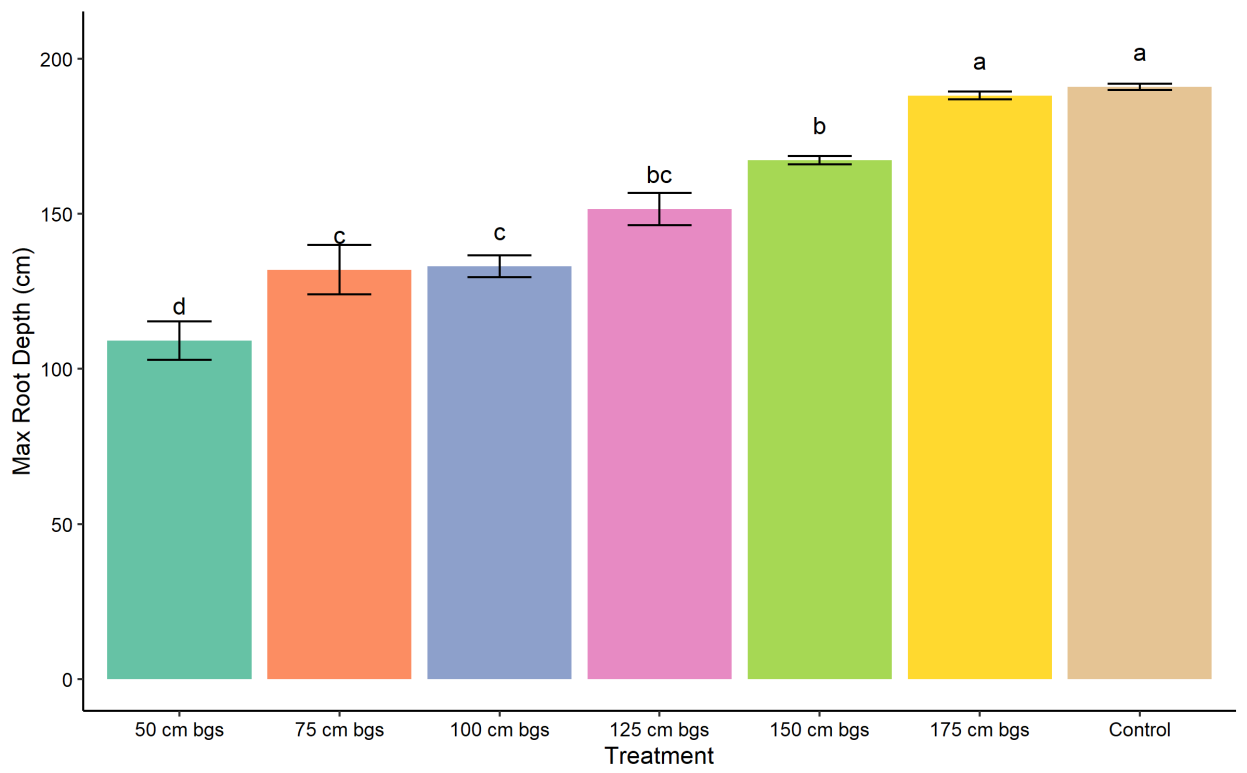

Figure 23. Maximum root depth (cm) in the salinity treatments.
Bars are means (n=10) with standard error bars . Different lowercase letters indicate significant differences between the treatments. The treatments refer to the depth at which salt contaminated material was present.

3.5.2 Effective Root Depth

Effective root depth was greatest in the control and 175 cm bgs treatments, which were not significantly different from one another. The effective root depth was significantly reduced with more shallow salinity treatments from 150 to 50 cm bgs (Figure 24). Effective root depth tended to be more shallow than the salinity interface. There was a clear impact of salinity on effective root depth, with salt-impacted soil at depths down to 150 cm bgs having a negative effect on root depth compared to the control.

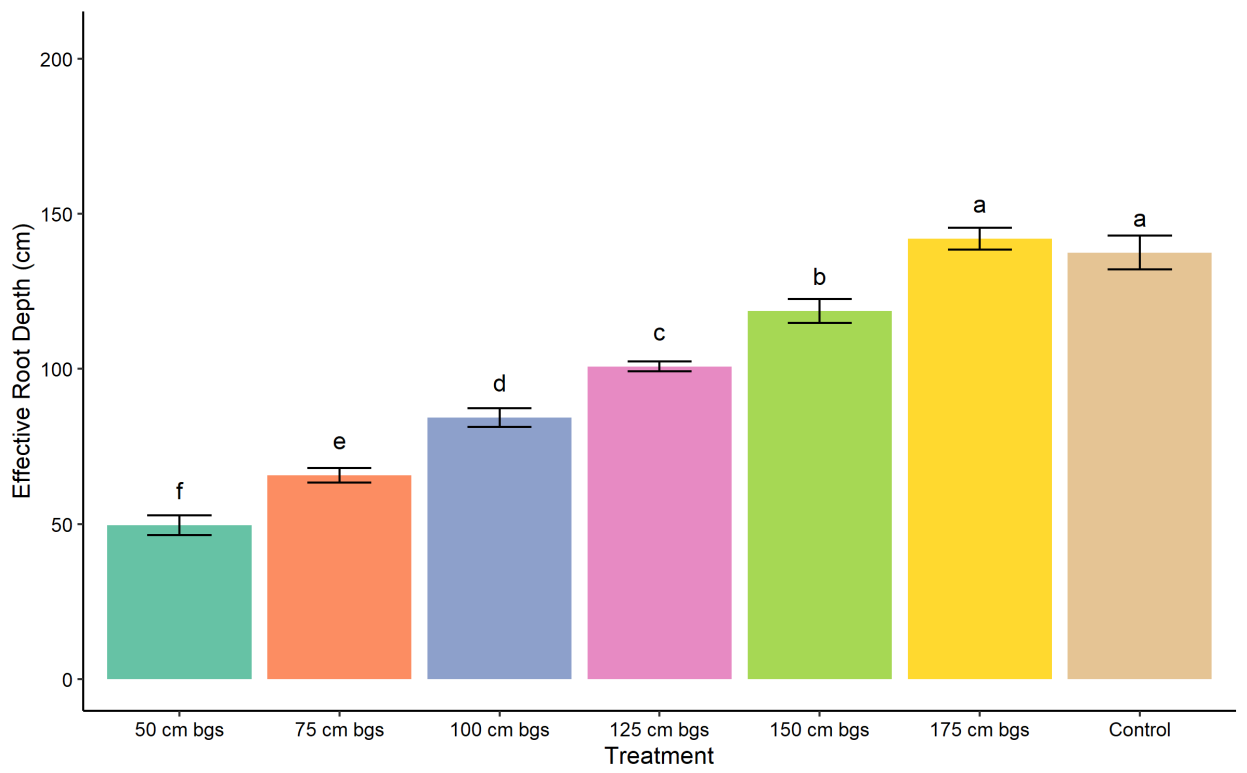

Figure 24. Effective root depth (cm) in the salinity treatments.
Bars are means (n=10) with error bars representing the standard error. Different lowercase letters indicate significant differences between the treatments. The treatments refer to the depth at which salt contaminated material was present.

3.6 **Root Distribution**

3.6.1 Image Analysis

Root distribution was based on percent root area data extracted from camera images taken of the exposed root face of each column (see Sections 2.1.4 and 2.2.1). Percent root area, averaged for each treatment across replicates in 1 cm depth increments, is provided in Figure 25. The image analysis was more successful in capturing coarse roots than fine roots, as fine roots were more difficult for the camera software to distinguish from soil. The data indicate that root area tended to decrease with depth in all treatments. Sharp decreases in root area near salinity interfaces were observed for the 50, 75, 100, and 125 cm bgs treatments, with more gradual decreases for the 150 and 175 cm bgs treatments (Figure 25). Interestingly, the control treatment tended to have higher root area than the 175 cm bgs treatment at depths below about 150 cm; the control treatment also tended to have higher root area than the 150 cm bgs treatment at depths below about 100 cm.

We were interested in comparing coarse root biomass data and root area data above and below salinity interfaces at the deepest salinity interfaces (150 and 175 cm bgs). Section 2.3 explains how to data were treated to carry out the analysis. In the 150 cm bgs treatment, root % area was significantly lower below the salinity interface and in comparison to the control at 155-170 cm bgs. In the 175 cm bgs treatment, there was a significant reduction in root % area below the salinity interface, but at 180-190 cm bgs,

there was no significant difference between the control and 175 bgs treatment, indicating that the reduction in root biomass may be due to depth, not salinity. The root % area data for the 150 and 175 cm bgs treatments was aligned with the coarse root biomass results for these treatments (see Section 3.4.1).

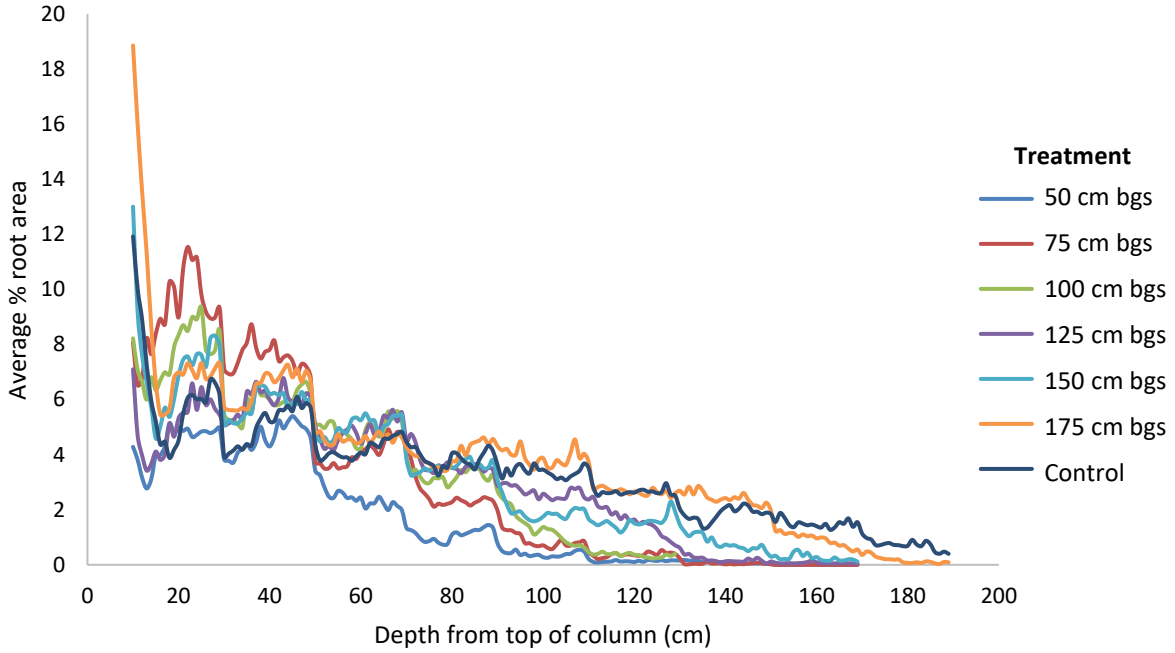

Figure 25. Root distribution as average (n=10) % root area with depth for each treatment.

3.6.2 Grid Frames

In general, the size, abundance, and orientation of roots differed above salinity interfaces compared to below (Appendix D). For example, in treatments with the interfaces located closer to the surface in the soil profile (i.e., 50, 75, and 100 cm bgs) roots tended to be medium to coarse in size above the interfaces, and then become fine below the interface. This trend was also observed at interfaces lower in the soil profile, but the change in root size was less dramatic. The abundance of roots also tended to change above and below interfaces. Roots were more likely to be plentiful above the interface, with few roots below the interface. These changes in size and abundance are not surprising, given that coarse root biomass tended to decrease significantly below salinity interfaces. While the size and abundance of roots tended to change with depth in the control columns, a sharp change at interface depths was not observed; instead, the change was gradual throughout the column.

The orientation of roots changed below the salinity interfaces. Above the interfaces, roots tended to be vertical. Below the interfaces, roots tended to take on a random orientation. This trend was observed regardless of the depth of the salinity interface. For the 175 cm bgs treatment, major changes in size and abundance were not always present above versus below the interface, but a change in orientation was observed in all columns. Roots likely stopped growing vertically to avoid taking up salt, and appear to have adapted a random distribution in this study. In one column, orientation changed to horizontal at the interface, indicating a clear avoidance of the salt-impacted material. This differed from the control columns, which saw roots maintain a vertical orientation throughout the length of the column.

4.0 SUMMARY

Salinity did not have a clear, consistent effect on aboveground growth throughout the experiment. The effect of salinity on aboveground biomass varied with time, and the impact of salinity appeared to decrease as plants became more established. There also was not a clear, consistent effect of salt on plant height throughout the experiment. Roots were more greatly impacted by the presence of soil salinity than aboveground growth parameters. There was a significant effect of salinity, when present at depths down to 150 cm bgs, on maximum and effective root depth. The presence of salinity at depths down to 150 cm bgs had a clear impact on coarse root biomass. In general, there was a consistent, but non-significant, decrease in fine root biomass below salinity interfaces; the data indicate that fine root biomass was impacted by salinity present down to 175 cm bgs. Figures 26 and 27 provide visual examples of the impact of salinity on roots.

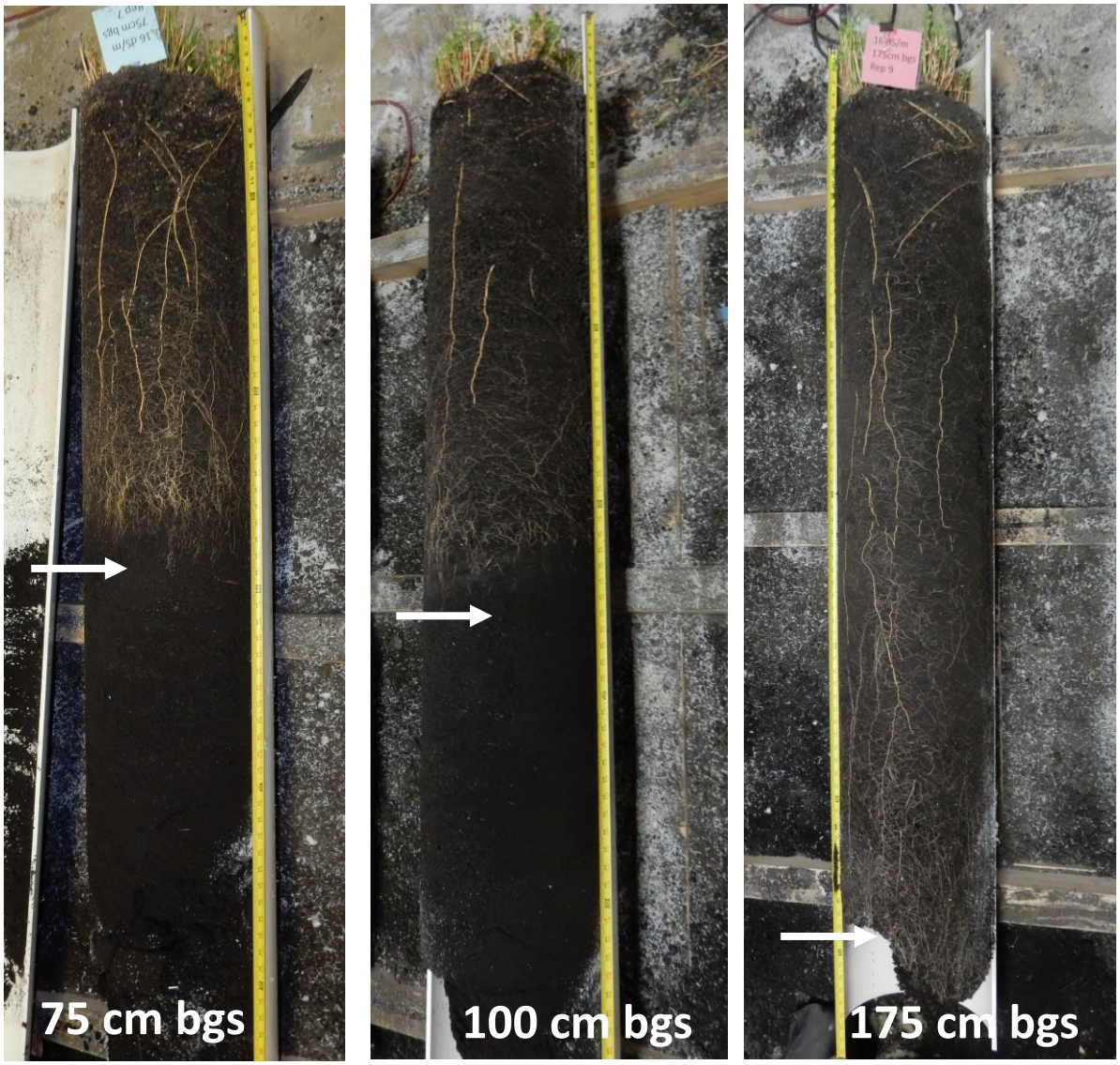

Figure 26. Example root growth for 75, 100, and 175 cm bgs treatments. White arrows indicate the location of the salinity interface.

Figure 27. Example root growth for 150 cm bgs and control treatments. The white arrows indicate the location of the salinity interface.

5.0 CONCLUSIONS

This study has provided empirical evidence for defining a scientifically defensible operative depth for the ecological direct contact pathway in soil. The experimental parameters provided a conservative method of defining the ecological direct contact pathway:

- NaCl was used as a COPC, and is a widespread contaminant in Alberta;
- Alfalfa was used as a surrogate species and is considered a deep-rooted, salt-sensitive species;
- The columns were watered in such a way as to mimic growing season precipitation patterns in the parkland region of Alberta;
- The growth medium was topsoil, which may help to promote deeper rooting than would be observed in a natural soil with B and C horizons.

While salinity had a variable impact on aboveground growth, there was a clear negative impact on root growth. Despite impacted root systems, alfalfa plants in this experiment maintained similar aboveground growth across the treatments, especially during the latter half of the experiment. When considering that the biologically active zone in soil is typically associated with roots, the impacts on rooting depth and distribution observed in this study indicate the potential for impacts to organisms in the soil. The presence of salinity at 150 cm bgs (and in some cases, 175 cm bgs) restricted root growth, and in turn likely impacted soil organisms at those depths.

Phase 3 of this project is a field study to further investigate the impact of soil salinity on aboveground plant growth. Phase 2 indicated a clear effect of salinity on alfalfa roots, but not on aboveground growth. For this reason, there is significant benefit in determining whether restricted root depth impacts aboveground growth in a field setting. If aboveground growth is not limited, despite restricted rooting depth, this has important implications for the ecological direct soil contact pathway. Data gathered during Phase 3 will be crucial in the development of a scientifically-defensible depth for the ecological direct soil contact pathway. Following submission of this report, a proposal for Phase 3 will be developed.

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