

DISSERTATION

THE EFFECTS OF EFFLUENT WATER IRRIGATION AND SALINITY ON SOIL CHEMICAL PROPERTIES
AND THREE SPECIES OF PERENNIAL GRASS

Submitted by

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ABSTRACT

THE EFFECTS OF EFFLUENT WATER IRRIGATION AND SALINITY ON SOIL CHEMICAL PROPERTIES AND THREE SPECIES OF PERENNIAL GRASS

Soil and water salinity is an issue in many areas worldwide because of drought, human activities, and using poorer quality water for irrigation. High levels of salinity severely affect plant growth as well as soil qualities. Some plants are naturally salt tolerant; however, most others are sensitive to salinity. Many areas are forced to use effluent water for turf and landscape irrigation due to the increase of the World's population and the fresh water shortage. However, the long-term use can change soil quality. In this dissertation, the effects of using effluent water on soil chemical qualities as well as the interactive effects of salinity and/or waterlogging on perennial ryegrass (*Lolium perenne* L.) and switchgrass (*Panicum virgatum* L.) growth were examined.

In the first and second chapters, the use of effluent water for golf course irrigation are reported. The reason for the study is that landscape irrigation with effluent water has become a common practice to alleviate fresh water shortage in many arid and semiarid areas, especially for golf courses. The objectives of the first study were to assess changes in soil chemical properties of sand-based greens following conversion from fresh water irrigation to effluent water irrigation, and to identify potential concerns related to long-term use of effluent water on sand-based greens. Putting greens were studied because of their unique nature and construction. Soil samples were collected and analyzed from greens of Heritage Golf Course in

Westminster, Colorado. The course started to use effluent water for irrigation in 2000. Nine out of 18 (1, 3, 5, 7, 9, 11, 13, 15, 17) greens were selected for soil sample collection. Soil samples (0-10 cm below the soil surface) were collected in September of 1999, 2003 and 2009. Soil test data showed that the soil's chemical characteristics changed over time. Soil organic matter increased from 0.12% to 1.5% and cation exchange capacity increased by as much as double over nine years. Extracted phosphates increased by 388% after nine years of effluent water use. Exchangeable calcium, magnesium, potassium, and sodium also increased, by 198%, 116%, 148%, and 452%, respectively, over nine years of effluent water irrigation. In addition, increase over time was shown for extractable iron, manganese, copper, zinc, and aluminum.

The second study was conducted at two golf courses in the Cheyenne, Wyoming area: Prairie View Golf Course and Airport Golf Course. Soil data for both golf course putting greens and fairways were available from 2003 to 2013. The Prairie View Golf Course started to use effluent water for irrigation in 2007, while Airport Golf course has always used fresh water. Comparison between effluent and fresh water irrigation on soil chemical properties suggested that both benefits and risks exist when effluent water is used for irrigation. Increased soil EC by 200% after three years of using effluent water and increased sodium levels by 176% after a year of using effluent water. However, our results showed that salinity and sodium accumulation levels were below the critical threshold levels. In addition, our results showed that phosphorus and potassium levels increased by 35% in 2013 and 43% in 2010, respectively, in the soil after using effluent water. These inputs could benefit the grass and lower fertilizer costs. In conclusion, using effluent water for irrigation has both benefits and risks. Increased salinity (EC) and sodium levels are the greatest risks when using effluent water; however, to a certain

degree, these can be managed by appropriate cultural practices such as leaching and adding gypsum. Supplemental nutrients and decreased fertilizer costs are the greatest benefits of using effluent water for irrigation. Our results showed that soil phosphorus, magnesium, and potassium levels increased after using effluent water, which could be beneficial for the grass and lower the fertilizer cost.

In the third chapter, the interactive effects of salinity and oxygen availability on nine perennial ryegrass lines (*Lolium perenne* L.) and one alkaligrass (*Puccinellia tenuiflora*) was studied. Many salt-affected soils in the world are also affected by compaction and waterlogging due to shallow water tables or decreased infiltration of water in soil. In a controlled greenhouse, grasses were exposed to four salinity levels (3, 6, 9, 12 dS m⁻¹) with and without hypoxia condition for four weeks each. All entries exhibited decreased clipping yield with increasing salinity in both salinity and hypoxia + salinity treatments except Fults alkaligrass. Turf quality declined over time to unacceptable quality ratings with high salinity (12 dS m⁻¹) treatment. In general, all entries had better turf quality in control and hypoxia treatments than in salinity and salinity with hypoxia treatments. All grasses were more severely affected (quality and yields) under the combined hypoxia and salinity treatment compared to salinity or hypoxia only. Plant sodium and Cl⁻ concentrations increased under salinity and salinity + waterlogging treatments. The experimental lines (10.0824, 10.0825, and 10.0815) maintained acceptable turf quality under hypoxia plus moderate salinity (6-10 dS m⁻¹) conditions.

In the last chapter, germination, growth, and production of switchgrass as a biofuel crop were examined under different salinity conditions. Biofuel is being evaluated as a possible solution to increasing oil costs, a growing world population, and environmental pollution. The

study was conducted twice in 2011 and 2013 in Colorado State University's Plant Growth Facility. Two switchgrass cultivars, Blackwell and Trailblazer, were selected. Four water salinity levels ranging from 7 to 24 dS m⁻¹ were applied, and the control received no salt. Germination rate was reduced with increased salinity level. Germination rate decreased from 100% to 60% as salinity increased from control to 16 dS m⁻¹ in the first experiment, but in the second experiment, germination rate decreased from 100% to 30% as the salinity level increased from control to 14.8 dS m⁻¹. In both cultivars and experiments, the salinity in the range of 10-15 dS m⁻¹ caused 50% aboveground biomass reduction. The final harvest (aboveground biomass) was reduced by 33- 40% as soil salinity increased from control to 7-9 dS m⁻¹. Root biomass decreased as soil salinity reached 7 and 9.5 dS m⁻¹ in the first and the second experiment, respectively. No differences were found among the two cultivars at any salinity levels except in root biomass. Although, switchgrass has a moderate level of salinity tolerance, our results suggest that biofuel crops with greater salinity tolerance are needed for biomass production on saline soil.

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DEDICATION

In the memory of my beloved parents.

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CHAPTER 1

LONG-TERM EFFECTS OF EFFLUENT WATER IRRIGATION ON SOIL CHEMICAL PROPERTIES OF

SAND-BASED GREENS

CONCEPTUAL

The increase of the world's population and the decrease of fresh water resources have led to increased use of alternative water resources to meet the water need. Using treated wastewater (effluent water) for urban landscape irrigation has become a common practice to alleviate fresh water shortage. Golf courses are the leading urban landscape users of recycled wastewater, because intensively managed turf can use nutrients in the wastewater efficiently. The objectives of this study were to assess changes in soil chemical properties of sand-based putting greens following conversion from fresh water irrigation to effluent water irrigation, and identify potential concerns related to long-term use of effluent water on sand-based greens. Soil samples were collected and analyzed from greens at the Heritage Golf Course in Westminster, Colorado. The course started to use effluent water for irrigation in 2000. Nine out of 18 (1, 3, 5, 7, 9, 11, 13, 15, 17) greens were selected for soil sample collection. Soil samples (0-10 cm below soil surface) were collected in September of 1999, 2003 and 2009. Soil test data showed that the soil's chemical characteristics changed over time. Soil organic matter increased from 0.12% to 1.5% and cation exchange capacity increased by as much as double over nine years. Extracted phosphates increased by 388% after nine years of effluent water use. Exchangeable calcium, magnesium, potassium, and sodium also increased, by 198%, 116%, 148%, and 452%, respectively, over nine years of effluent water irrigation. In addition, increases

over time were found for extractable iron, manganese, copper, zinc, and aluminum. In conclusion, using effluent water for irrigation has both benefits and risks. Increased salinity (EC) and sodium levels are the greatest risks when using effluent water; however, to a certain degree, these can be managed through appropriate cultural practices such as leaching and adding gypsum. Supplemental nutrients and decreased fertilizer costs are the greatest benefits of using effluent water for irrigation. Our results showed that released nitrogen, phosphorus, potassium, and magnesium levels increased in the soil after using effluent water, which would be beneficial for the grass and lowering the fertilizer's cost.

INTRODUCTION

The increase of the World's population and the decrease in fresh water resources have led to increased use of alternative water resources. In contrast, as the population increases, wastewater production increases. In many arid and semiarid areas in USA, Australia, and Israel, using fresh water for turfgrass and landscape irrigation has become rare. Consequently, using treated wastewater (effluent water) for irrigation has become a common practice to alleviate fresh water shortage. In addition to the growing concerns of the future water supply, the more stringent wastewater discharge standards make use of recycled wastewater increasingly attractive.

Golf courses are by far the leading urban landscape users of recycled wastewater, because intensively managed turf can use nutrients in the wastewater efficiently, golf courses require a high volume of irrigation water, and it is easier to implement recycled wastewater irrigation systems on golf courses than on other systems (i.e. parks, school playgrounds, athletic fields,

etc.). A 1978 survey reported that 26 golf courses across the country were using recycled wastewater. In 2000, the National Golf Foundation (NGF) reported approximately 13 percent of golf courses (approximately 2000 golf courses) nationwide now use effluent water for irrigation, with 34 percent of golf courses in the Southwest doing so (NGF, 2000). In Colorado, approximately 25% of golf courses are using effluent water for irrigation.

“Effluent water” refers to any water after residential and sometimes industrial use that undergoes significant treatment at a sewage treatment plant, to meet standards set by federal or state water laws and regulations. This water is usually suitable for various reuse purposes including irrigation. The most common treatment process includes primary treatment (such as settling and screening), secondary treatment (such as oxidation, activated sludge, filtration, and UV or chlorine disinfection) and tertiary treatment (such as clarification, coagulation/flocculation, sedimentation, filtration, adsorption of compounds by a bed of activated charcoal, UV or chlorine disinfection, etc). UV disinfection is becoming one of the most popular and cost effective disinfection alternatives. During treatments, suspended solids are removed, pathogens are disinfected, and partial to substantial reduction in nutrient concentrations occurs, depending on treatment stage (Harivandi, 1994; Pettygrove and Asamo, 1985). Currently, recycled water used for turf and landscape irrigation must be at least secondary effluent water (Harivandi, 2007).

However, using effluent water has some disadvantages. Public health is the first concern due to the pathogens it may contain, but that is less of a concern if used for non-edible plants. Effluent water may contain different levels of dissolved solids, ions, nutrients (NO_3 and P_2O_4), and other elements. Increases in soil salinity and sodium are potential problems associated with

using effluent water irrigation. Salinity has harmful effects on non-halophyte plant growth and development as well as making soil water less available for the plants. Increased sodium level (sodicity) in the soil leads to disaggregation of soil to its components and damages the soil structure. In addition, researchers suggest that using recycled water for irrigation may affect soil chemistry over time (Murakami and Ray, 2000; Wallach et al., 2005; Qian and Mecham, 2005; Thomas et al., 2006; Skiles and Qian, 2013). Accordingly, the use of effluent water for irrigation requires monitoring and the use of management practices to minimize any potential adverse effects on soil and plants.

On the other hand, using effluent water for irrigation has some advantages. Effluent water contains some nutrients that can be used by plants. Nitrogen (N) and phosphorus (P) as well as some small amounts of micronutrients, are found in effluent water. Studies have showed that plant yields increased by using effluent water when compared to fresh water irrigation (Angin et al., 2005). This increase is due to the nutrient concentrations such as N and P in effluent water and their effect on plant growth (Angin et al., 2005). High quality effluent water has become available for golf course irrigation, and it decreases the fertilizer cost because of nutrient availability in the water (Harivandi, 2007). Also, using effluent water is less expensive when compared to other alternative irrigation resources such as desalinized seawater (Haruvy and Sadan, 1994).

Many studies have been published regarding the effect of using effluent water on soils in urban landscapes. However, no research is available regarding the impacts of effluent water irrigation on sand-based root zones on golf course putting greens and sports fields. Research is needed to determine the effect of using effluent water on sand-based root zones on putting

greens. Most golf course putting greens are constructed based on the United States Golf Association (USGA) putting green construction recommendations. USGA putting green consists of 30-cm sand-based root-zone that contains 80-90% sand and 10-20% organic matter by volume. The sand based root-zone overlays a 10 cm deep gravel blanket to provide the best soil conditions for turfgrass growth and to minimize compaction and optimize drainage. Sand-based putting greens allow for good aeration and drainage, and that is important to maintain a good playing surface. Sand is suitable for the putting green's function because it is resistant to soil compaction and has good filtration and percolation rates. However, it has low organic matter, which may affect its ability to hold nutrients (Lado, et al., 2004). Organic matter, typically peat, is often added to improve water and nutrient-holding capacity (Bigelow et al., 2004). With putting green's special nature, using effluent water for irrigation needs to be investigated over the long term to address the impact of effluent water on putting green soil properties.

The objectives of this study were to:

1. Assess changes in soil chemical properties of sand-based greens following conversion from fresh water irrigation to effluent water irrigation.
2. Identify potential concerns related to long-term use of effluent water on sand-based greens.

MATERIALS AND METHODS

Study Location

The study was conducted at Heritage Golf Course in Westminster, Colorado, and which located north of metro Denver (39° 53' 59.34" N 105° 07' 00.04"). The course started to use effluent water for irrigation in 2000. Nine out of 18 (1, 3, 5, 7, 9, 11, 13, 15, 17) greens were

selected for soil sample collection. Soil samples (0 -10 cm below soil surface) were collected in September of 1999, 2003 and 2009.

Soil samples were analyzed for soil pH, extractable salt content (Ca, Mg, K, Na, Fe, Mn, Cu, Zn, P, and B), base saturation percent of Ca, Mg, K and Na, soil organic matter (SOM), and cation exchange capacity (CEC) by Brookside Laboratories, Inc. (New Knoxville, OH). Soil pH was analyzed using 1:1 H₂O procedure; 1:1 is the most common ratio used for soil-water pH. It is performed by mixing an equal volume of soil and deionized water. Soil samples were extracted using the Mehlich III extract (0.015 M NH₄F + 0.20 M CH₃COOH + 0.25 M NH₄NO₃ + 0.013 M HNO₃ + 0.0005 M EDTA chelating agent) to determine Ca, Mg, K, Na, Fe, Mn, Cu, Zn, B, and P by inductively-coupled plasma-emission spectrophotometry instrumentation. [Mehlich III is a procedure widely used for extraction of plant available macro- and micro-nutrients in soils that have an acidic or neutral pH, by using a dilute acid-fluoride-EDTA solution with pH 2.5 extracted (Zhang et al., 2009)]. Mehlich III extracted Ca, Mg, K and Na plus soil buffer pH data are used to calculate CEC. Base saturation percent of Ca, Mg, K and Na was calculated by dividing the extracted Ca, Mg, K and Na by the calculated CEC, respectively. Base saturation percent of Na is considered the exchangeable sodium percentage (ESP). Soil organic matter was determined by reaction with Cr₂O₇²⁻ and sulfuric acid. The remaining unreacted Cr₂O₇²⁻ is titrated with FeSO₄ using ortho-phenanthroline as an indicator, and oxidizable organic matter was calculated by the difference in Cr₂O₇²⁻ before and after the reaction (Nelson and Sommers, 1982). Estimated N release is calculated to determine the potential amount of N released annually by SOM decomposition.

Data Analysis

Data were analyzed by analysis of variance (ANOVA) (SAS Institute, 2010) to test the effect of irrigation with effluent water on individual soil chemical properties. Comparisons between years were examined and means were separated by LSD at 0.95 level of confidence. Regression analysis was used to examine the changes in individual soil parameters over time after the use of effluent water for irrigation.

RESULTS AND DISCUSSION

Effluent water analysis showed that sulfate (182 mg L^{-1}), bicarbonate (125 mg L^{-1}), chloride (120 mg L^{-1}) and sodium (101 mg L^{-1}) are the most dominant elements in the water (Table 1.1).

On average, soil pH was 6.9 at the initiation of the study (Fig. 1.1). ANOVA test showed no changes in pH for nine years after using effluent water (Fig. 1.1). These results are similar to the findings in a previous study on the fairways of the same golf course (Skiles and Qian, 2013). These results likely were due to the use of sulfur (s) burner units on the golf course irrigation system. After transitioning to recycled water, the Heritage Golf Course installed a S burner. Sulfur burner units heat elemental S to create sulfurous acid for injection into irrigation water to reduce the bicarbonate content and pH (Qian and Mecham, 2005). The fact that we did not see an increase in soil pH suggests that the S burner was effective in controlling soil pH associated with effluent water irrigation. Soil pH increases have been observed by others in soils under recycled water irrigation (Miyamoto and Chacon, 2006; Qian and Mecham, 2005). At this site, soil pH was maintained without change over 9 years by reducing the bicarbonate level in the irrigation water and releasing H^+ into water and soil.

The SOM was significantly different among the sampling years with the means linearly increasing from 1999 to 2009 (Fig. 1.2). In comparison before using effluent water (1999) and after 9 years of using effluent water (2009) at the Heritage Golf course, we found that SOM significantly increased ($R^2=0.83$). At the initiation of the study in 1999, SOM content was 0.1%, which increased to 1.5 % in 2009. The average increase was 0.15% annually. To calculate the total carbon (C) sequestration from SOM, an assumption was made that SOM contains 58% C, and putting greens have 1.6 g cm^{-3} bulk density. The average annual total C sequestration was $1.4 \text{ t h}^{-1} \text{ yr}^{-1}$ during nine years of using effluent water. Our calculation for this site was close to the estimation that was reported by Qian and Follett (2002) that soil C sequestration rate was $1.1 \text{ t h}^{-1} \text{ yr}^{-1}$ on golf course putting greens. Soil organic matter is a significant component in turfgrass systems; it affects soil porosity, water and nutrients retention, and percolation in the sand-based root zone. In addition, the calculation of C sequestration from SOM could be helpful to understand the role of turfgrass systems in storing C in the soil.

Putting greens had low CEC ($1.9 \text{ cmol}_c \text{ kg}^{-1}$) at the beginning of the experiment. This was because it was mostly sand with low SOM and contains low inorganic colloids. Soil CEC increased by 174% over the course of the experiment ($R^2=0.86$) and by an average rate of $0.37 \text{ cmol}_c \text{ kg}^{-1}$ (Fig. 1.3).

Organic matter has very high CEC. The significant increase in soil CEC observed in this study is likely due to the increase in SOM.

The estimated N release showed a highly significant increase over time ($R^2=0.90$), and the percentage increase was 1117%, with an annual rate of $5.6 \text{ kg ha}^{-1} \text{ yr}^{-1}$ compared to the year before using effluent water which was 4.6 kg ha^{-1} (Fig. 1.4). Estimated N release is an

estimate of N potentially released annually by decomposition of SOM. Estimated N release could be affected by many factors such as soil moisture, temperature, and soil type. This large increase was due to the fertilization and organic matter increase as well as substances added by effluent water because it often contains significant concentrations of organic nutrients, such as N and P (Toze, 2006). Increases in this category were also a result of increased biomass production that translated to increases in SOM and eventually available N from organic matter decomposition.

Soluble S increased over time ($R^2=0.82$; Fig. 1.5). The percentage increase during the nine years of using effluent water was 413%. As mentioned earlier, this increase of S content over time was a result of using S burner to inject elemental S into irrigation water to reduce pH and bicarbonate concentration in effluent water (Qian and Mecham, 2005).. Turf managers at Heritage Golf Course encountered a problem of increased black layer beneath putting green surfaces since 2003. Black layer is the formation of a layer of metal sulfide (Adams and Smith, 1994; Perris and Evans, 1996), which forms when hydrogen sulfide (H_2S) gas reacts with metal elements in the soil. Hydrogen sulfide gas is produced by sulfur reducing bacteria (SRB). Black layer is typically associated with turfgrass chlorosis, wilting, thinning, and sometimes death.

Soluble S is the substrate for S reduction activity that leads to black layer. Therefore, the use of a S burner under effluent water irrigation might have partially contributed to the increased occurrence of black layer. Further research is needed to address the potential relationship between the incidence of black layer and effluent water irrigation.

In addition, extracted phosphates increased over time ($R^2=0.83$; Fig. 1.6), and the percentage of increase during nine years of using effluent water was 388%. This increase was

expected because effluent water usually has more soil phosphates than fresh water. Increases in phosphates over years of using effluent water irrigation have been recorded in previous studies (Bond, 1998; Qian and Skiles, 2013).

Similarly, exchangeable calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) significant accumulated over time after using effluent water (Fig. 1.7, 8, 9 and 10). The percentage of the increase after nine years of using effluent water were (Ca) 198%, (Mg) 116%, (K) 148%, and (Na) 452%. Exchangeable Na increased to 156 kg ha^{-1} after nine years of using effluent water. This increase could be due to the use of effluent water irrigation as some research has indicated. Soil Na concentration increased almost 5.5 times since the start of using effluent water, and the value (156 kg ha^{-1}) was in the moderate risk range (>210 is in high risk) (Harivandi and Beard, 1998). A study done in 2005 found that effluent water provided enough K, Ca and Mg for plants (Menzel and Broomhall 2006). The authors suggested that soil with excessive amounts of K could lead to base saturation imbalanced, and high soluble salts tie up other elements such as B, Ca, and Mg. In contrast, higher amounts of Mg appeared to be a problem in clay soil, but it could help stabilize sandy soil. In this study, however, no clear pattern was found over time for potassium base saturation percentage (Fig. 1.11).

Increase in Na base saturation percentage was observed after nine years of recycled water irrigation at an average rate of 0.27% per year (Fig. 1.11). Elevating exchangeable sodium percentages (ESP) observed over several years of effluent water irrigation can be of concern with regards to the preservation of water permeability and hydraulic conductivity on putting greens. ESP is a measurement of sodium hazard in soil, and ESP more than 15% can cause sodicity problems. Soil hydraulic conductivity decreases as ESP increases. However, sodicity

depends on soil type. Soil with high clay content are affected more by ESP. Effluent water can cause Na build up over time in the soil. High concentrations of Na can affect the ability of water to move through the soil, i.e. decrease infiltration.

In this study, a slight increase was recorded in the Ca base saturation percentage ($R^2=0.35$). In contrast, a reduction in Mg base saturation percentage was recorded ($R^2=0.66$), (Fig. 1.10). Calcium and Mg affect each other's availability in the soil, and high Ca may tie up magnesium. However, the Ca/Mg ratios matched the balanced ratio at every sampling time (2.1-5.9), (Voortman and Bindraban, 2015). In general, the base saturation percentages for Ca, Mg, and K in this putting green are considered to be in the ideal or balanced ranges that many soil laboratories use to interpret soil test results. According to the basic cation saturation ratio theory, ideal plant growth will be achieved only when the soil's exchangeable Ca, Mg, and K concentrations are in range of 60-70% Ca, 10-20% Mg, and 4-6% K (Kopittke and Menzies, 2007).

A significant increase over time was observed for extractable Fe ($R^2 = 0.81$). The percentage increase was 354% after nine years of using effluent water and with an average rate of 25 mg kg⁻¹ per year (Fig. 1.12). These results are in agreement with a short term (45 days) study done in Iran in 2011 (Mojiri and Hamidi, 2011). The authors found that irrigation with wastewater significantly increased extractable Fe by 13% compared to the site that was irrigated with fresh water (Mojiri and Hamidi, 2011). Although the effluent water for this course had low levels of Fe (0.3 mg L⁻¹) the soil extractable Fe concentration significantly increased after using effluent water. After nine years of using effluent water, extractable Fe was 288 mg kg⁻¹. Soil pH plays an essential role in micronutrient availability to plants. The availability of

micronutrients such as Fe, Mn, and Zn in soil solution begins to decrease when soil pH is above 6.5. As soil pH increases, the availability of Fe decreases. As result, Fe deficiency is common in high pH soil. Iron is essential for chlorophyll synthesis and photosynthesis (Twyman, 1946). Effluent water could supply the soil with Fe with a proper soil pH range. In this site, Fe concentrations after nine years of using effluent water were in the ideal range (100-300 mg kg⁻¹).

Likewise, extractable copper (Cu), manganese (Mn), and zinc (Zn) increased significantly over time ($R^2=0.86$, 0.87 , and 0.89 respectively). The increased percentages after using effluent water were 290%, 1220 %, and 1600% and by an average rate around 1.0, 3.2, 2.1 mg kg⁻¹ yr⁻¹, respectively, for Cu, Mn, and Zn, respectively (Fig. 1.13). This finding is in disagreement with the previous study for fairways on the same golf course which suggested that no pattern of change was recorded for extractable Mn, Cu, and Zn after using 9 years of effluent water (Skiles and Qian, 2013). These micronutrient availabilities are similar to the availability of Fe, and depend on pH as well. Sandy soil usually has low concentrations of micronutrients such as Fe, Mn, Cu, and Zn (Ross, 1994). Copper is an enzyme activator and disease fighter, and the Cu minimum value needed in the soil is 1.5 mg kg⁻¹, and a value higher than 4 mg kg⁻¹ is excessive (Sonmez *et al.*, 2006). Copper and Zn affect each other availabilities to plants, and ideally soil Cu concentration should be half of Zn (Astera, 2014) . Our results showed that after 9 years of effluent water, Cu and Zn concentrations were very high in this putting green soil; however, toxicity is not a concern here for both elements due to the non-acidic soil pH.

Moreover, extractable aluminum (Al) increased over time after using effluent water ($R^2=0.5$) (Fig. 1.14), and the percent increase was 63% up to 142 mg kg⁻¹. These increases could

be due to the effluent water use, and could also be due to the soil aging and management practices. Toxic levels of Al are heavily dependent on the pH. In general, Al toxicity increases as soil acidity increases to a pH level of 4.8. In our study site, Al stayed bonded and not available to the plant.

A significant increase appeared in soil extractable boron (B) after the use of effluent water ($R^2=0.68$) (Fig. 1.15), and the percent increase over time was 260% with an average rate of $0.06 \text{ mg kg}^{-1} \text{ year}^{-1}$. These results are most likely due to effluent water use, and are in agreement with the previous study for the same golf course fairway soil. The extractable B gradually increased ($R^2=0.56$) after using effluent water in fairway soils. (Skiles and Qian, 2013). The criteria for B concentration in soils are as follows: sensitive plants show growth decline as soil B exceeds $0.5\text{-}1.0 \text{ mg kg}^{-1}$. Moderately sensitive plants will start to decline when soil B exceeds $1.0\text{-}2.0 \text{ mg kg}^{-1}$. Kentucky bluegrass can tolerate soil B concentration of $2.0\text{-}4.0 \text{ mg kg}^{-1}$. While other grasses can tolerate soil B of $6\text{-}10 \text{ mg kg}^{-1}$. The recycled water used in this study contained about 0.2 mg L^{-1} boron. Soil samples collected had a range from 0.2 to 0.7 mg kg^{-1} of B (Table. 1.1). This average level of soil B concentration was higher in 2009 compared to what was measured in 1999 (0.2 mg kg^{-1}), yet this range of B concentration was well below the toxic threshold for creeping bentgrass greens.

The same study was done previously on Heritage Golf Course fairways (Skiles and Qian, 2013). In comparison between the greens and the fairways in these two studies, we found that both green and fairway soil chemistry changed over time after nine years of using effluent water. In many categories, results were similar for the greens and the fairways. In both studies, soluble S was increased significantly due to the S burner mentioned before. Increases in Na

concentration, B concentration, soil ESP, Na available for release were similar between the two studies. Although SOM increased in both studies, CEC increased in the green soil but not in the fairway. In contrast, some soil parameters responded differently in the two studies. For example, significant increases in trace elements such as Cu, Zn, Mn, and Al were only observed in the green studies but not in fairways. Similarly, Fe concentration significantly increased in the greens but not in the fairways. These differences between the two studies could be due to the different soil type and structure in the greens and the fairways. Further studies are needed to determine if the change of soil parameters would continue over time.

CONCLUSION

Soil test data for the Heritage Golf Course, which uses effluent water for irrigation, showed that the soil's chemical characteristics changed over time. Soil organic matter and CEC significantly increased by as much as double over nine years. Exchangeable Ca, Mg, K, and Na also increased by 198%, 116%, 148%, and 452%, respectively, over nine years of effluent water irrigation. The more than 4-fold increase in Na could affect the soil structure and lead to a lack of aeration for roots. However, the application of gypsum can be used to minimize this effect. In addition, a significant increase over time was shown for extractable Fe, Mn, Cu, Zn, and Al.

In general, most of the chemical parameters have significantly changed over nine years of effluent water irrigation; however, not all changes are necessarily due to the use of effluent water. Some changes in soil chemistry could be the result of golf course management practices, such as the use of a S-burning unit, which increased soluble S in the irrigation water. In addition, these greens are relatively young (built in 1998), and they need time to become mature and

their soil become stable over time. However, increases in other elements such as sodium, boron, and phosphate, could be due to the use of effluent water. The greater increases in SOM and estimated N release, and increases in trace elements such as Cu Zn, and Mn could also be the result of using effluent water for irrigation.

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Table 1.1 Effluent water quality used in Heritage Golf Course (season average).

Water Quality Parameters	
pH	7.4
NH ₄ -N	0.8 mgL ⁻¹
NO ₃ -N	2.9 mgL ⁻¹
Total P	0.6 mgL ⁻¹
Total dissolved salts	638
Conductivity,	0.99 dSm ⁻¹
Sodium absorption ratio (SAR)	3.05
Adjusted SAR	5.74
Na	101 mgL ⁻¹
Cl	120 mgL ⁻¹
Bicarbonate	125 mgL ⁻¹
Ca	67 mgL ⁻¹
Mg	11.8 mgL ⁻¹
Sulfate	182 mgL ⁻¹
B	0.21mgL ⁻¹
Fe	0.31 mgL ⁻¹
K	16.9 mgL ⁻¹
TSS (Total suspended solids)	9.1 mgL ⁻¹



Photo 1.1. Black layer underneath putting green surface.

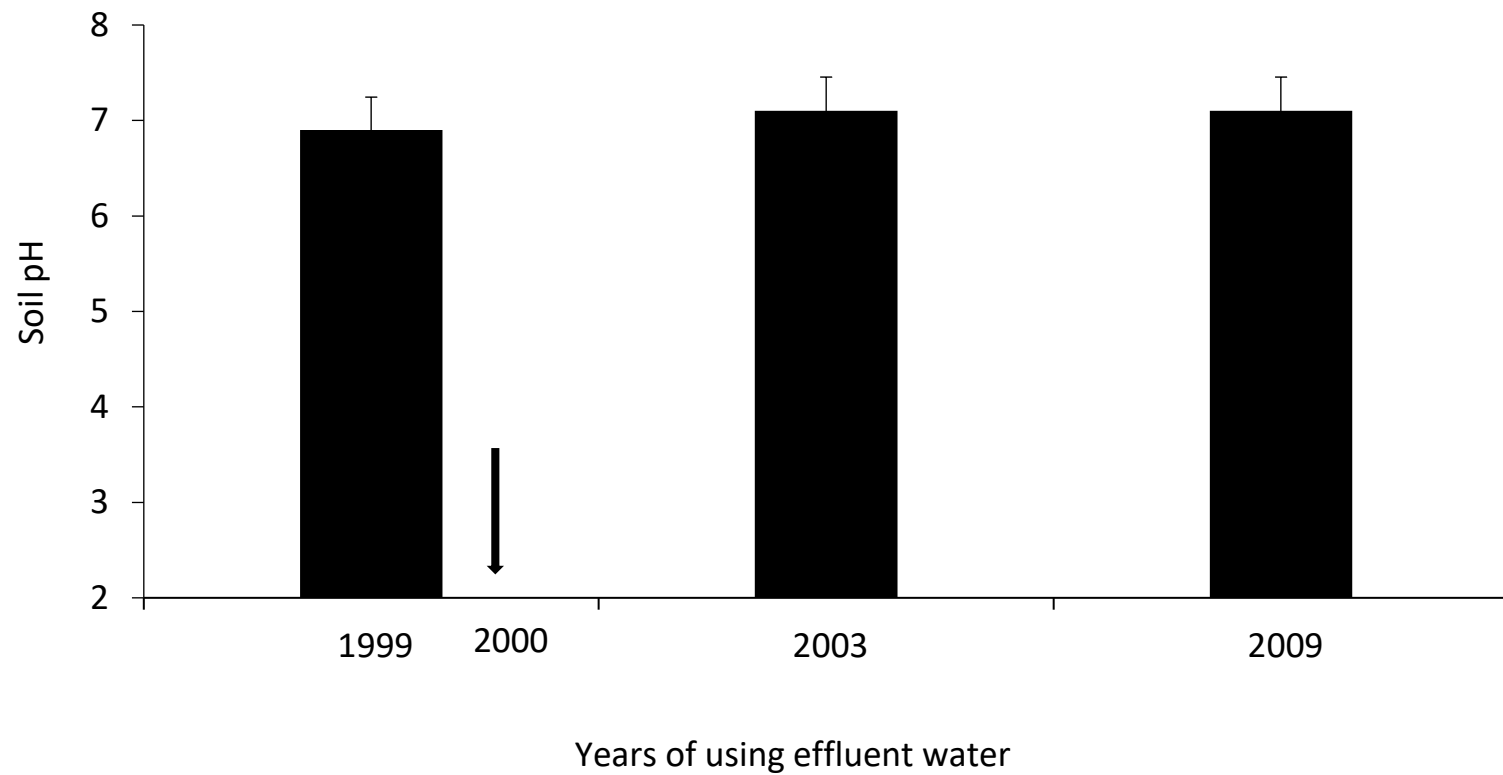


Figure 1.1. Effect of using effluent irrigation on soil pH.
Arrow indicates the start year of using effluent water irrigation.

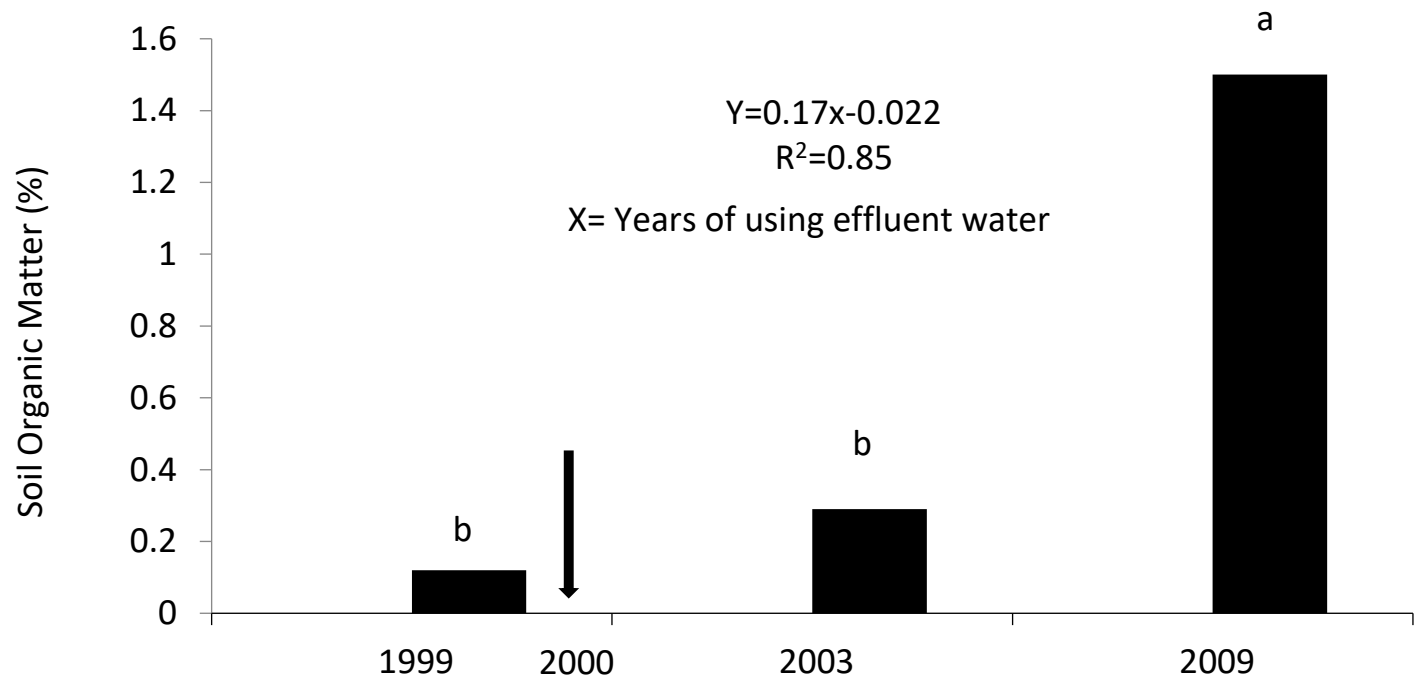


Figure 1.2. Effect of using effluent irrigation on soil organic matter.
 Arrow indicates the start year of using effluent water irrigation.

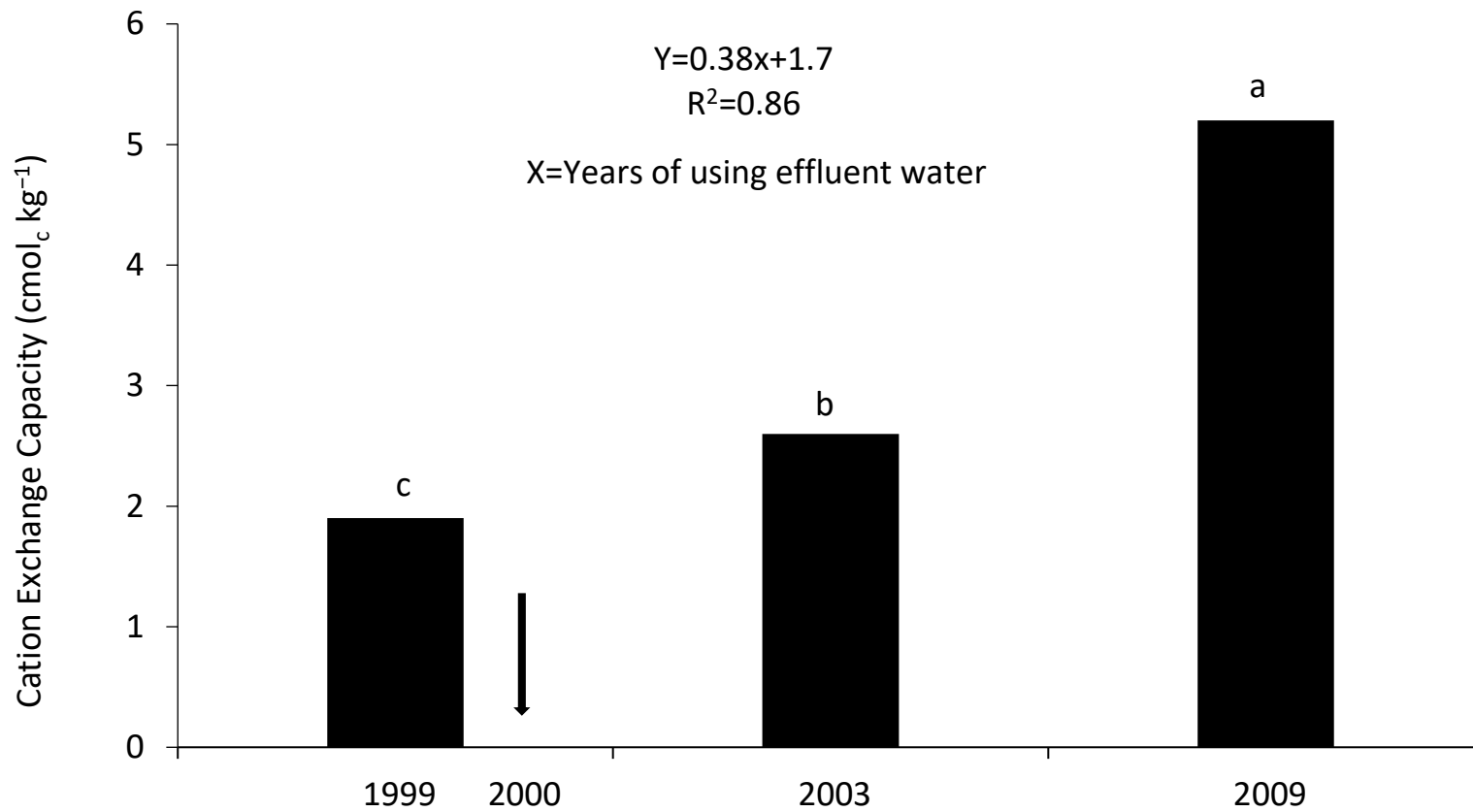


Figure 1.3. Effect of using effluent irrigation on Cation Exchange Capacity. Arrow indicates the start year of using effluent water irrigation.

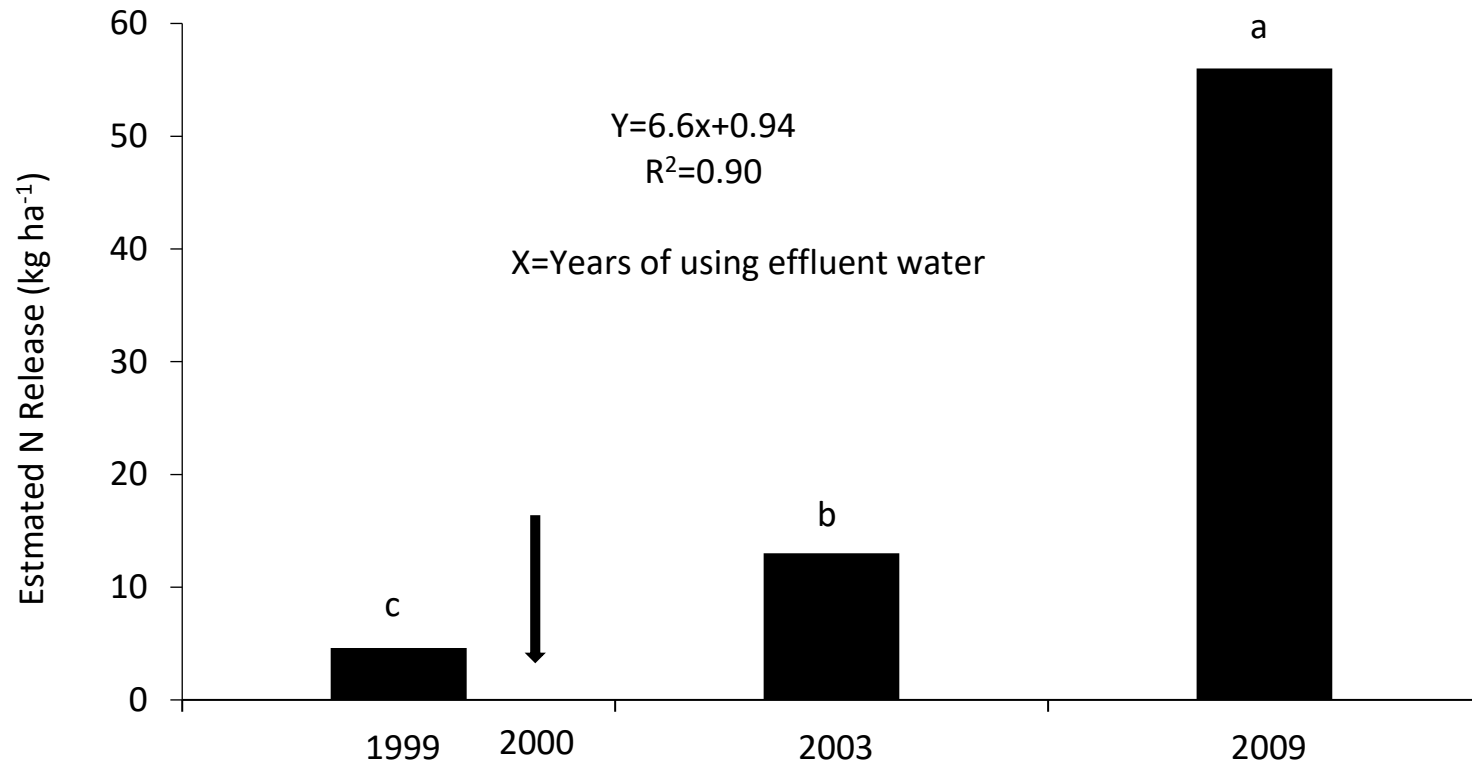


Figure 1.4. Effect of using effluent irrigation on estimated N release in soil. Arrow indicates the start year of using effluent water irrigation.

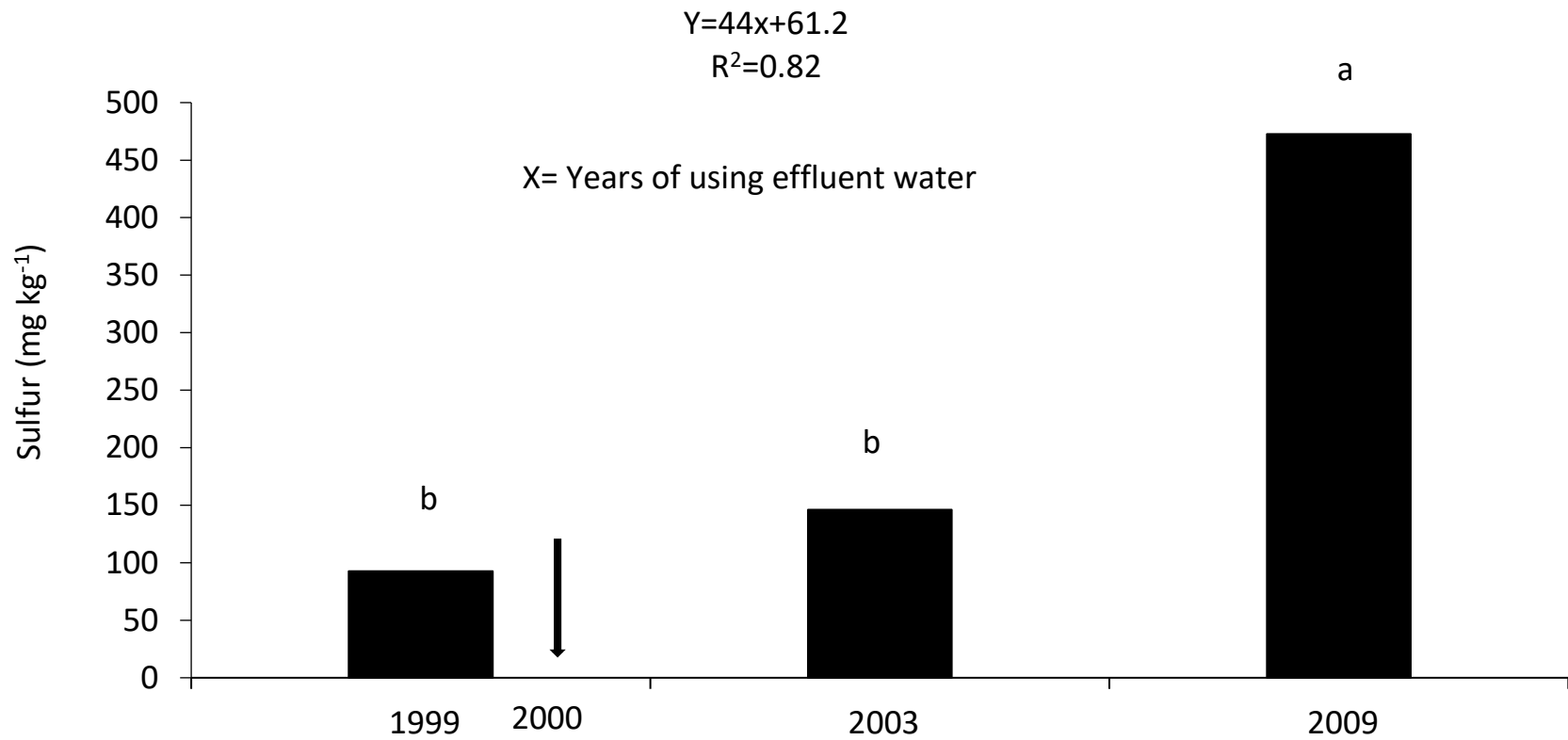


Figure 1.5. Effect of using effluent irrigation on soil soluble S content. Arrow indicates the start year of using effluent water irrigation.

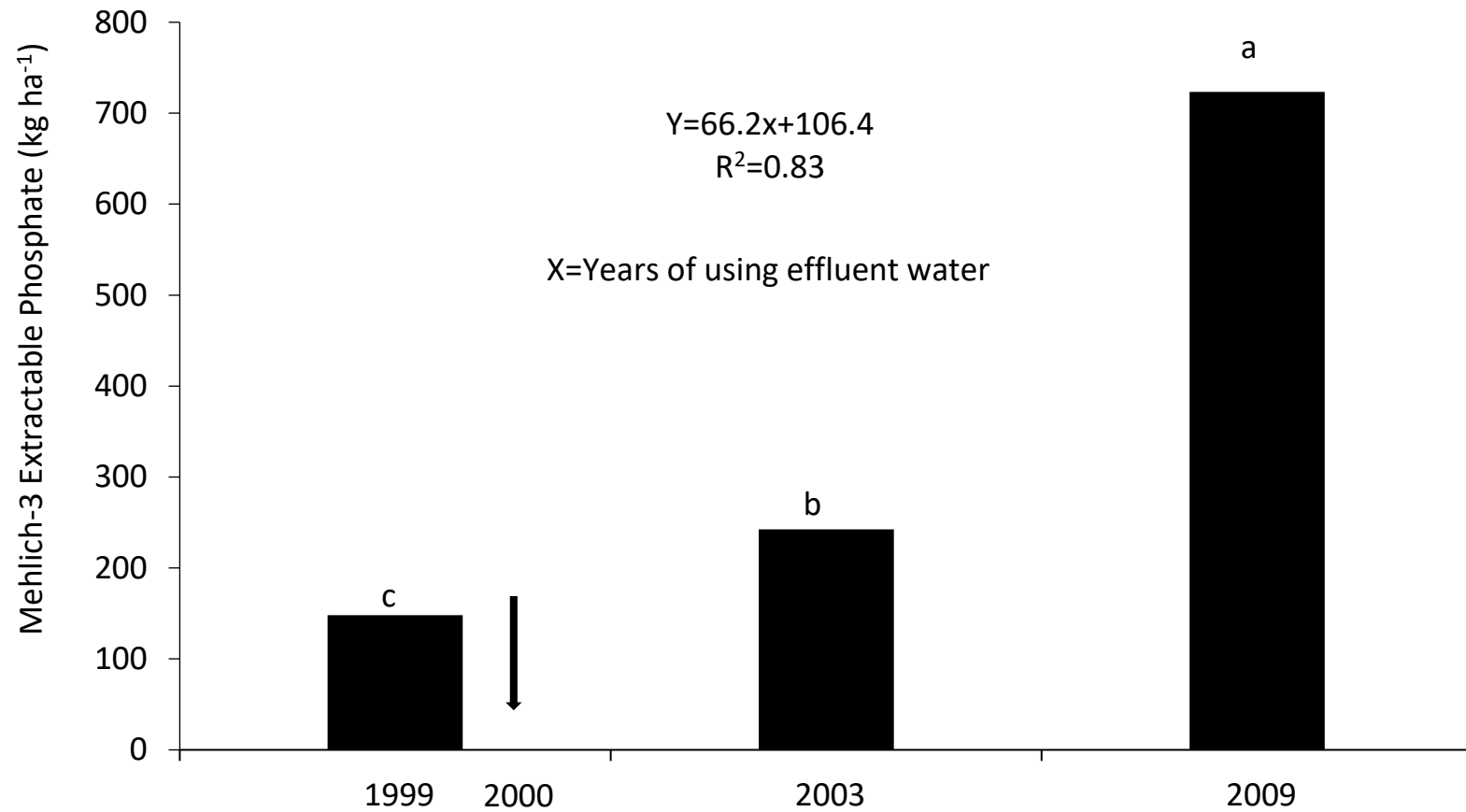


Figure 1.6. Effect of using effluent irrigation on Mehlich-3 extractable soil phosphate. Arrow indicated the start year of using effluent water irrigation.

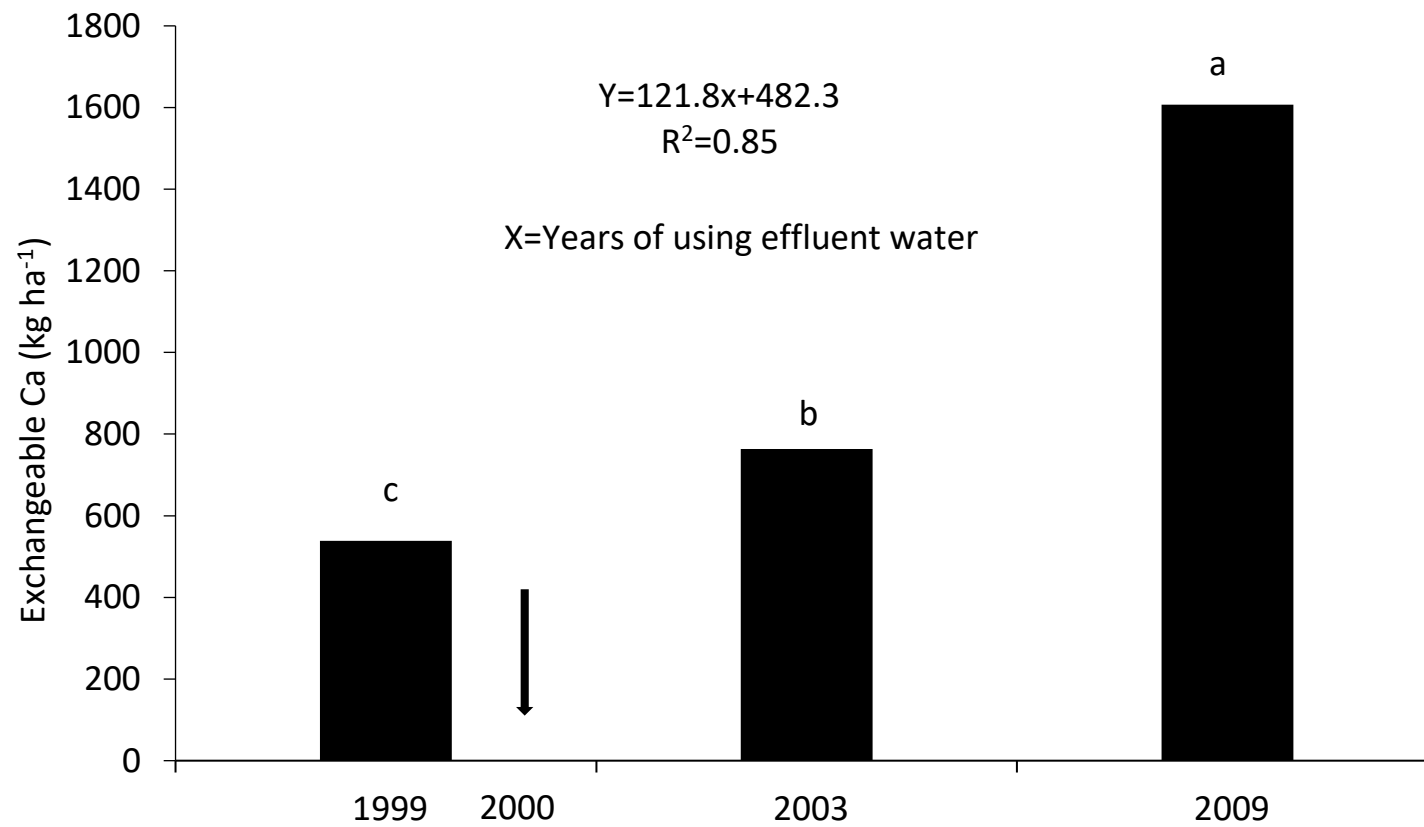


Figure 1.7. Effect of using effluent irrigation on soil Exchangeable Ca. Arrow indicates the start year of using effluent water irrigation.

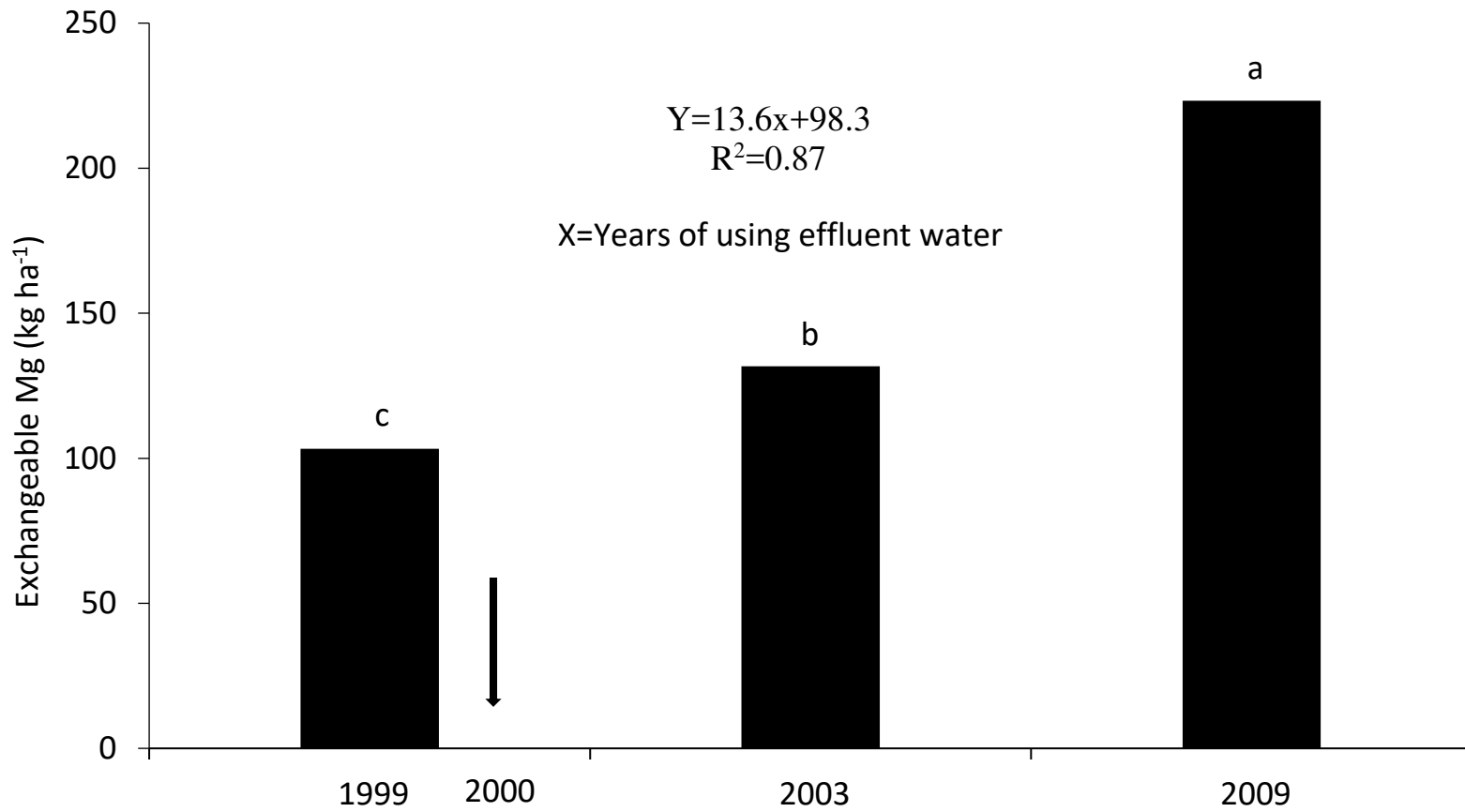


Figure 1.8. Effect of using effluent irrigation on soil Exchangeable Mg. Arrow indicates the start year of using effluent water irrigation.

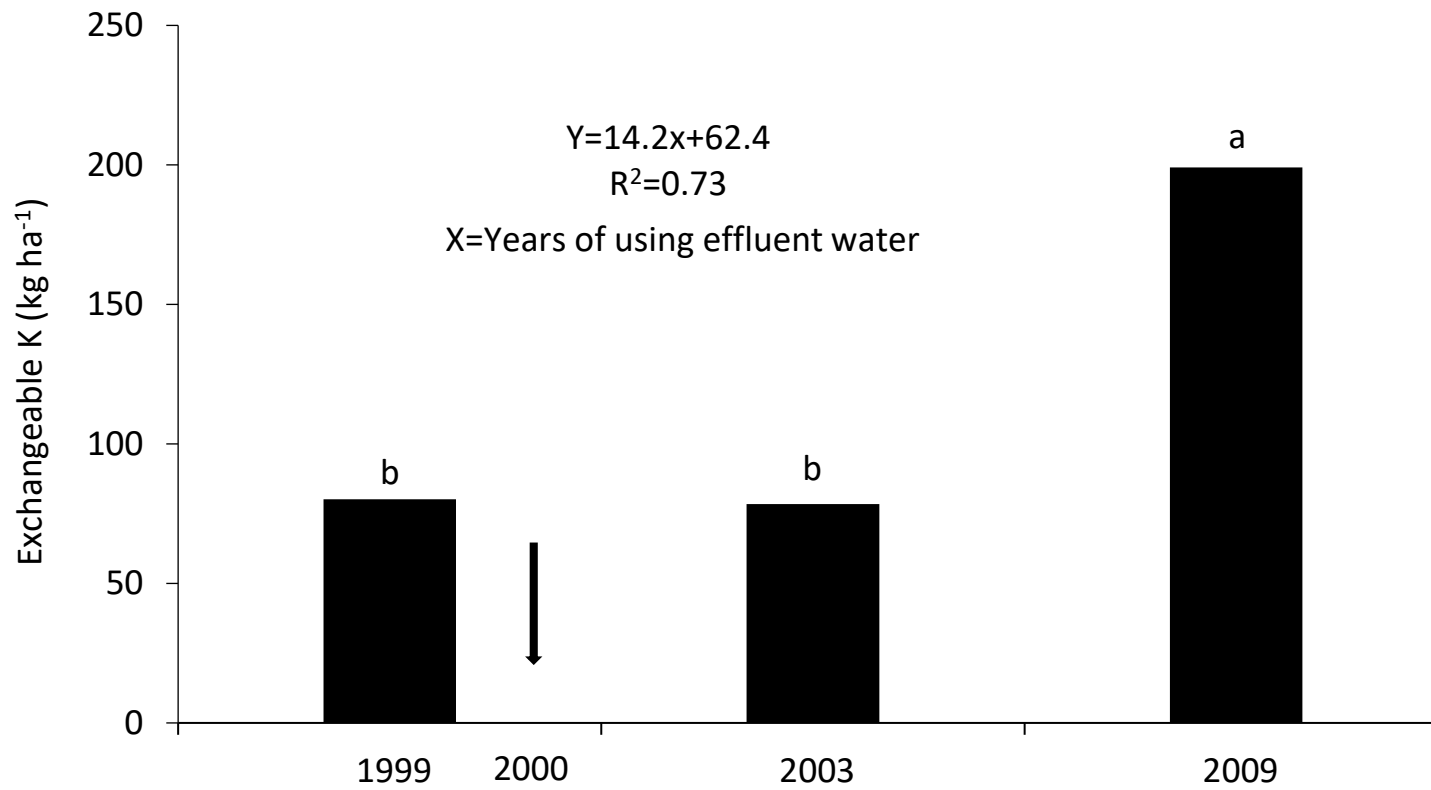


Figure 1.9. Effect of using effluent irrigation on soil Exchangeable K. Arrow indicates the start year of using effluent water irrigation.

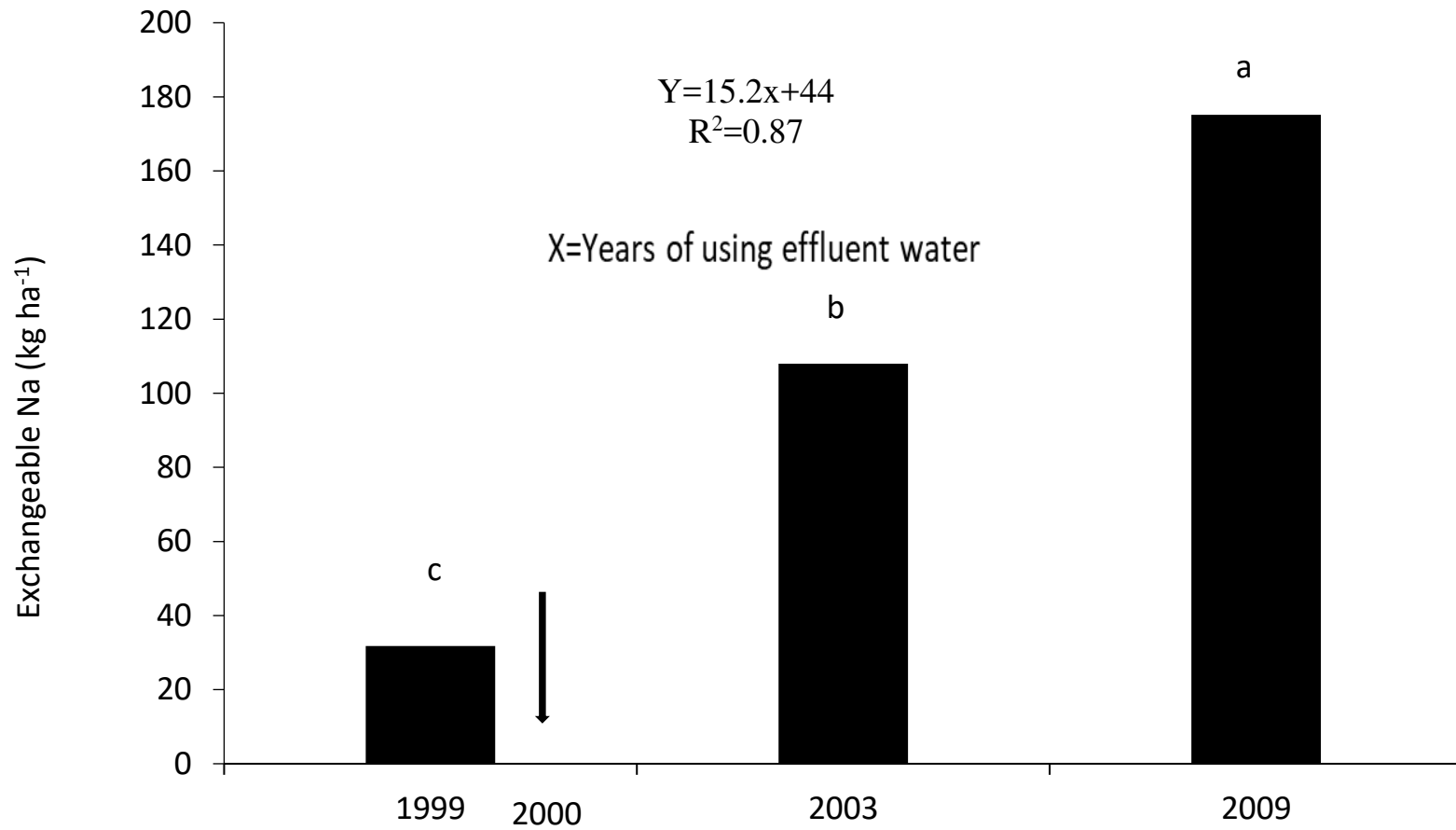


Figure 1.10. Effect of using effluent irrigation on soil Exchangeable Na. Arrow indicates the start year of using effluent water irrigation.

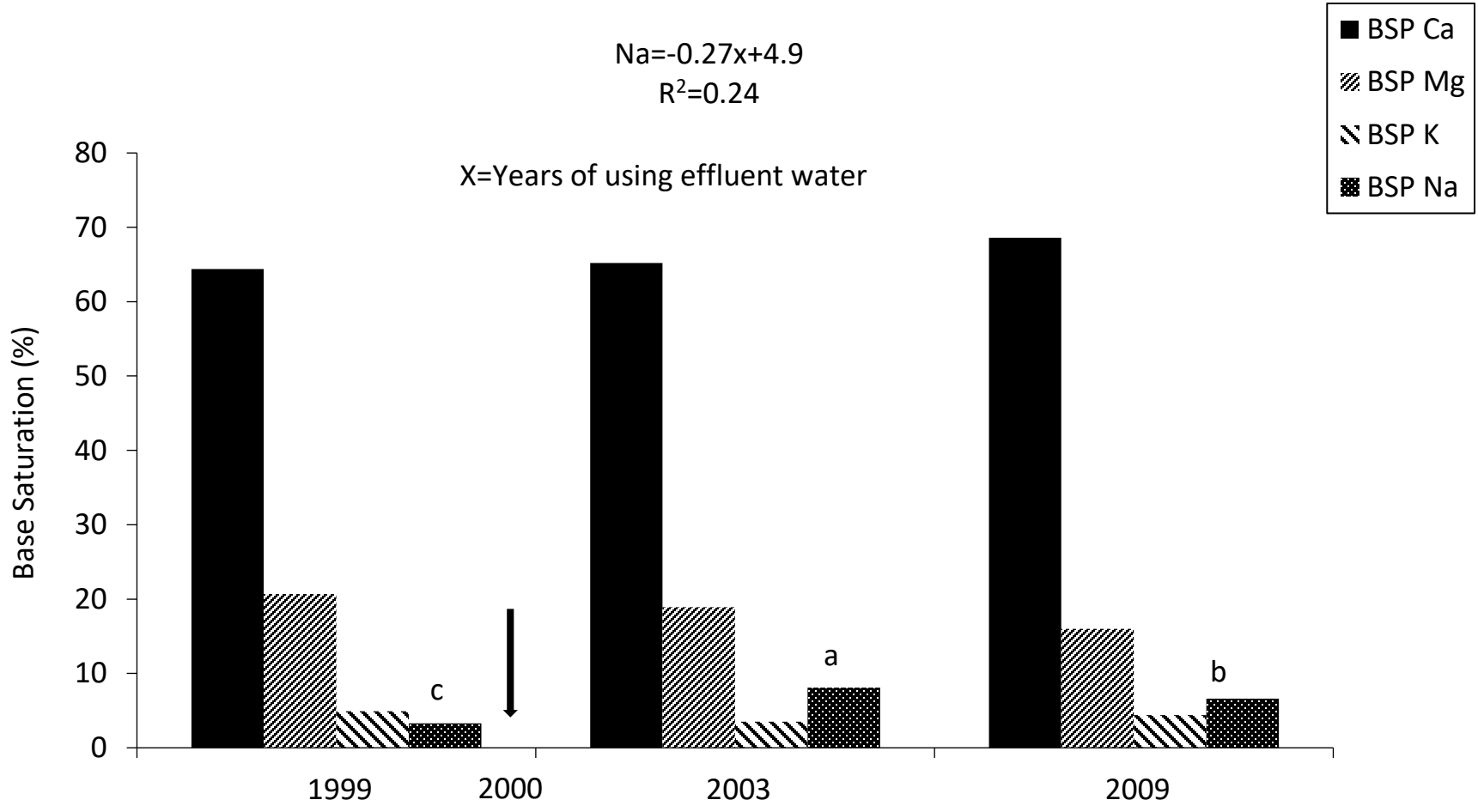


Figure 1.11 Effect of using effluent irrigation on soils Base Saturation Ca, Mg, K, and Na. Arrow indicates the start year of using effluent water irrigation.

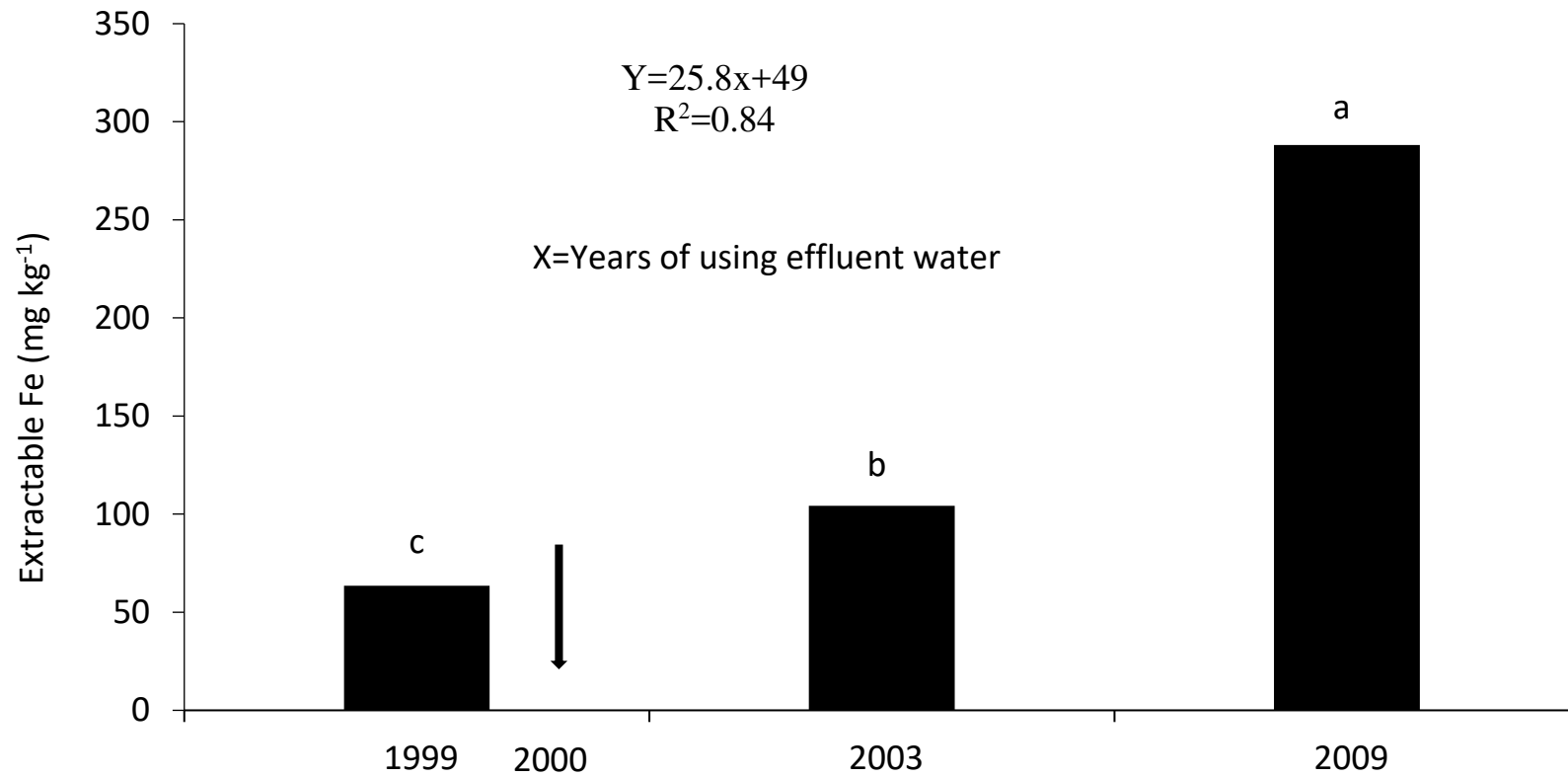


Figure 1.12. Effect of using effluent irrigation on soil extractable Fe. Arrow indicates the start year of using effluent water irrigation.

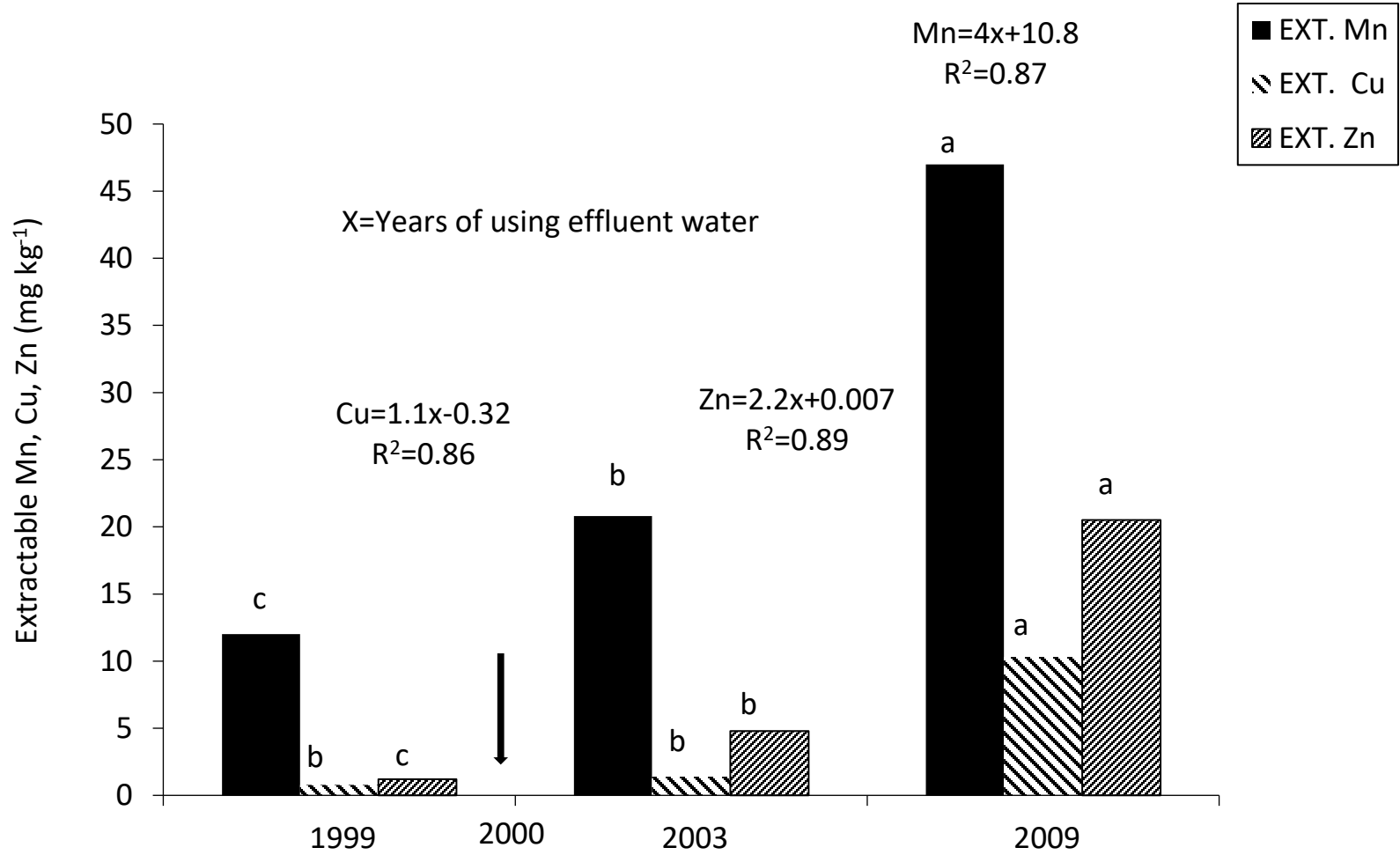


Figure 1.13. Effect of using effluent irrigation on soils extractable Mn, Cu, and Zn. Arrow indicates the start year of using effluent water irrigation.

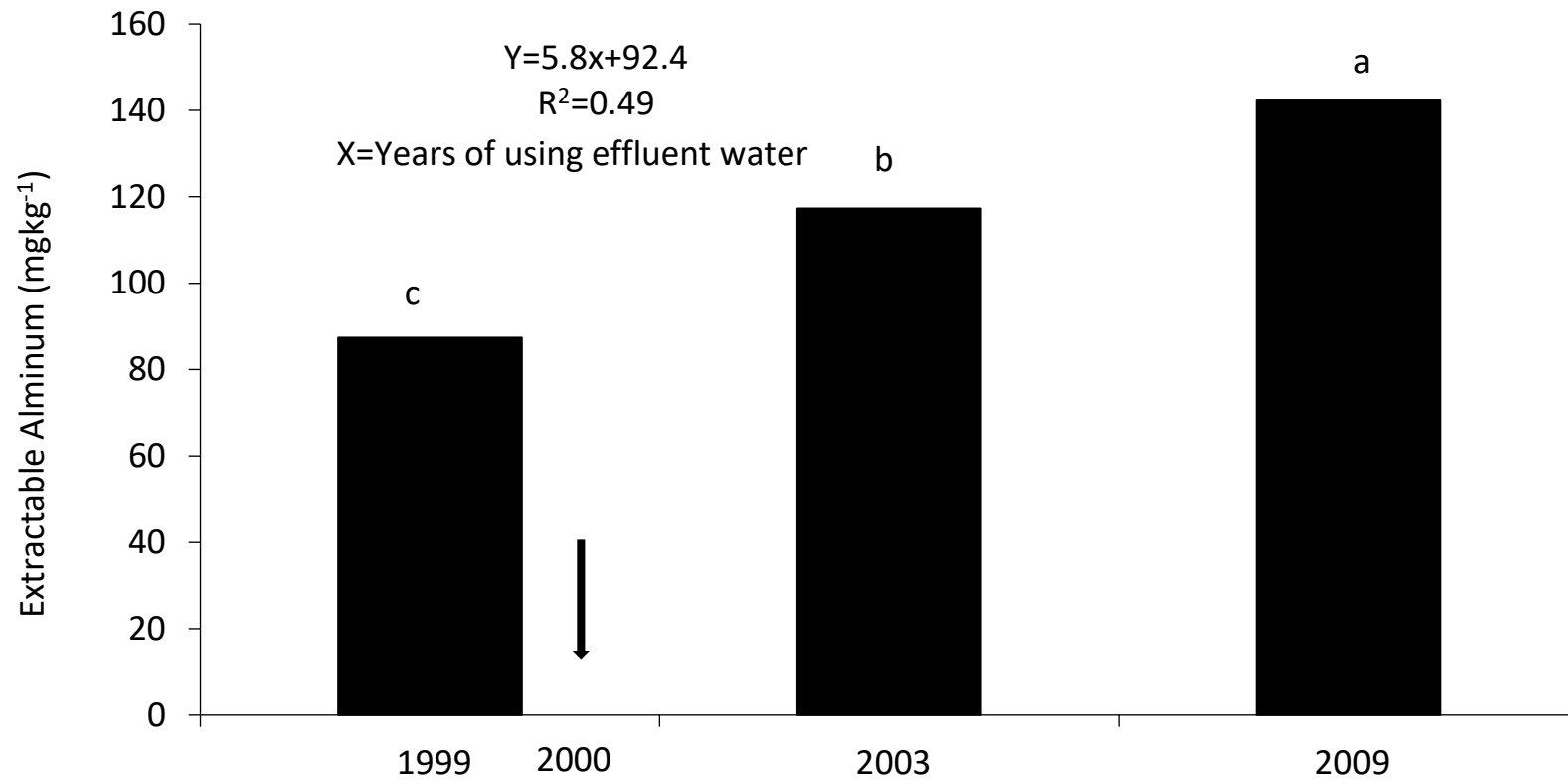


Figure 1.14. Effect of using effluent irrigation on soils extractable Al. Arrow indicates the start year of using effluent water irrigation.

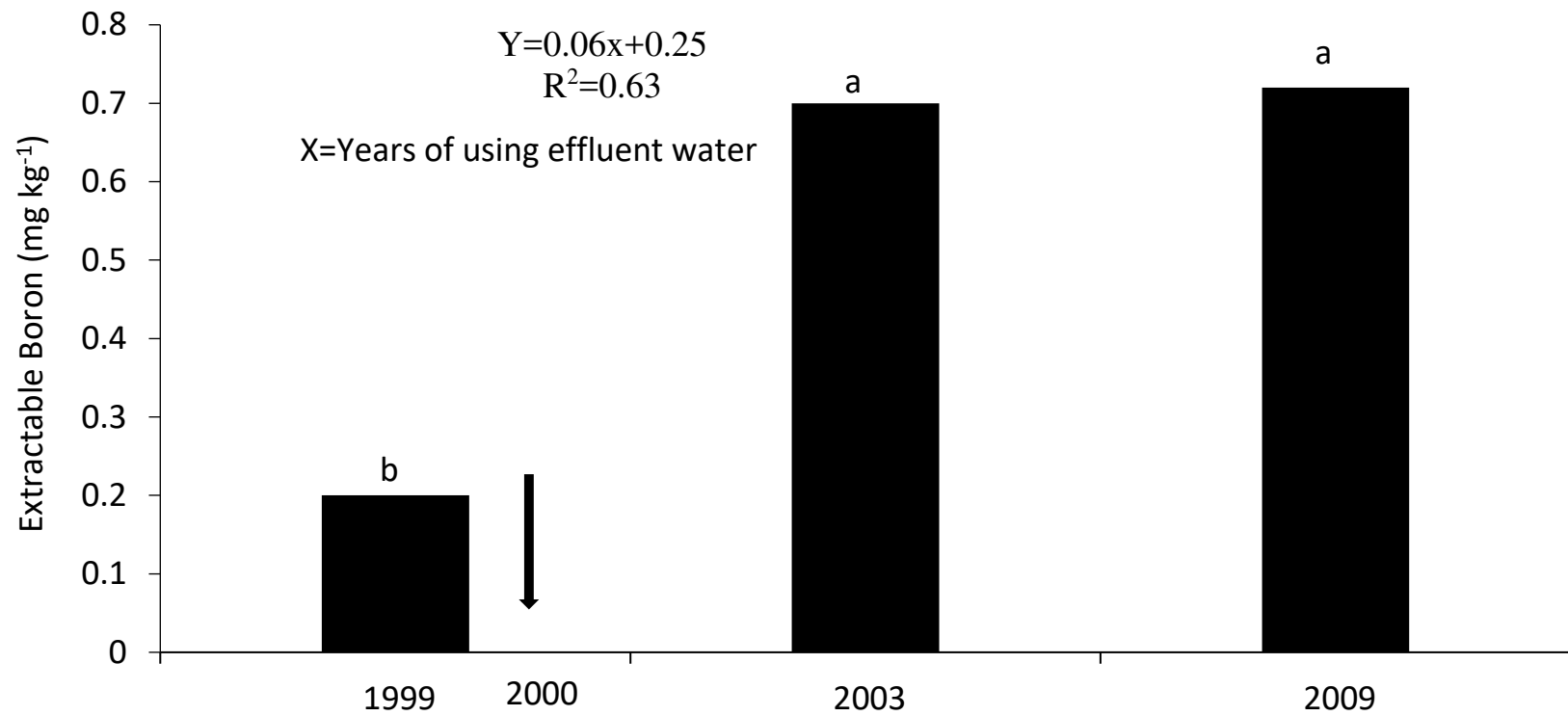


Figure 1.15. Effect of using effluent irrigation on soil extractable B. Arrow indicates the start year of using effluent water irrigation.

CHAPTER 2
COMPARISON OF FRESH VERSUS EFFLUENT WATER IRRIGATION ON SOIL CHEMICAL
PROPERTIES OF GOLF COURSE GREENS AND FAIRWAYS
CONCEPTUAL

In many arid and semiarid areas, using fresh water for landscape irrigation has become rare. Increased world population and a corresponding increase in water demand have forced scientists to search for other water resources to use for landscape irrigation. In this study, we compared the soil chemical properties of push-up greens and fairways on two golf courses that use either effluent water or fresh water. The study was conducted on two golf courses in the Cheyenne, Wyoming area: Prairie View Golf Course and Airport Golf Course. Soil data for both golf course putting greens and fairways were available for years 2003 through 2013. The Prairie View Golf Course started to use effluent water for irrigation in 2007 while Airport Golf course has always used fresh water. From 2003 to 2013, a total of 238 soil samples of greens (134 from Airport Golf Course and 104 from Prairie View Golf Course) were collected to a depth of 10 cm, and 90 soil samples (45 from Prairie View Golf Course and 45 from Airport Golf Course) were collected from fairways to the same depth. The soil analyses for the two golf courses showed that there were many changes that occurred over time due the use of effluent water irrigation as well as other management practices such as fertilizer application or gypsum injection. Soil EC increased by 200% after three years of using effluent water, and sodium levels increased by 176% after a single year of using effluent water. However, our results showed that salinity and sodium accumulation levels were well below the critical threshold levels of turf. In addition, our

results showed that P and K levels increased by 35% in 2013 and 43% in 2010, respectively, in the soil after using effluent water, which would be beneficial for the grass and lower the fertilizers cost.

INTRODUCTION

In many arid and semiarid areas using fresh water for landscape irrigation has become restricted. Increased world population and a corresponding increase in water demand have forced scientists to search for other water resources to use for landscape irrigation.

Wastewater is increasing as human activities increase, and using it for irrigation is a way to utilize the wastewater while meeting the water needs. Effluent water usually contains different concentrations of nutrients such as N and P that are essential for plant growth. Therefore, using effluent water for landscape irrigation can decrease fertilizer need (Harivandi, 2004).

Nowadays, using effluent water or treated wastewater has become a trend especially for landscape irrigation, because of the need for supplemental irrigation. Additionally, there is less concern about perceived negative effects on human health, since landscapes contain non-edible products. Another benefit for using effluent water of irrigation is groundwater recharge (Walker and Lin, 2008). However, using effluent water for irrigation may have some risks. It may increase the amount of soluble salts in soil and may affect plant growth (Castro et al., 2011).

These changes can be managed by special practices such as leaching and adding gypsum to avoid salt accumulation (Hayes et al., 1990). The effect of effluent water irrigation has been widely studied for both short and long periods. Some researchers indicated that after long-term use of effluent water, the soil still functioned properly (Walker and Line, 2008). However, other

studies reported that using effluent water affected the soil negatively (Elsokkary and Abukila, 2014; Mojiri and Hamidi, 2011; Qian and Mecham, 2005). Therefore, more research is needed to determine the effects of long-term effluent water irrigation on soil function. In this study, we compared the soil chemical properties of push-up greens and fairways between two golf courses, one that uses effluent water and the other uses fresh water. Golf courses consist of three main areas which include tees, fairways, and greens. In these two golf courses, the greens were push-up greens, referring to an old construction technique of building greens. It is typically pushing up the native soil into a pile and shaping it to form a green. The native soil usually has more silt, clay, fine sand, and sometimes higher organic matter than USGA sand-based greens. Most push-up native soil greens have been modified in the top 2 to 4 inches with sand by continuous sand topdressing and aeration over many years. Push-up greens usually do not drain well, and appropriate water management is very critical to keep the turf healthy in such poorly drained soils. The two tested greens do not have internal drainage, but over the past 20 years, a sand cap of 18 to 20 cm has been added on top of native soil. In fact, they drain very well even without having internal drainage.

The two golf courses that were studied are mature and are managed by the same superintendent employing similar management practices.

The objectives of this study were to:

1. Examine soil chemical properties of putting greens and fairways irrigated with effluent water, compared to similar greens and fairways irrigated with fresh water.
2. Determine long-term changes in soil chemical properties for push-up greens and fairways irrigated with effluent water vs. fresh water.

MATERIALS AND METHODS

Study Location:

The study was conducted at two golf courses in the Cheyenne, Wyoming area: Prairie View Golf Course (41.1497° N, 104.7775° W) and Airport Golf Course (41.163047, -104.832083). Soil data for both golf course putting greens and fairways were available for years 2003 through 2013. The Prairie View Golf Course started to use effluent water for irrigation in 2007 while Airport Golf course has always used fresh water.

Turfgrass Management

Comparison between the two golf courses was emphasized because both courses are similar and managed by the same superintendent employing similar management practices. The main difference is that they use different irrigation water resources.

The management practices for both courses are very similar, but Prairie View Course applied gypsum in 2012 to control soil Na increase. Gypsum was injected through the irrigation system and applied granularly once per year in Prairie View Golf Course. The total amount of gypsum applied in 2012 was 2242 kg/ha. In addition, additional irrigation practices were applied at Prairie View to flush the salts out of the root zone.

The greens at Prairie View are about 45 years old, and the greens at the Airport are close to 80 years old. Both courses have push-up greens with no internal drainage, but over the past 20 years they have been converted to a sand cap of around 18-20 cm. These greens drain very well in spite of not having internal drainage. Both greens use 100% USGA specification sand topdressing and have built up a sand profile of 18 to 20 cm over a period of 20 years. This equates to a rate of about 0.9/20 cm of sand per year. Light topdressing occurs every two weeks during the spring and summer and then about once a week in the fall as a preparation

for winter. A year of fertilizer application records from Airport Golf Course were provided by the superintendent and is summarized in Fig.4.1.

Soil Sampling and Processing

From 2003 to 2013, a total of 238 soil samples of greens (134 from Airport Golf Course and 104 from Prairie View Golf Course) were collected to a depth of 10 cm, and 90 soil samples (45 from Prairie View (PV) Golf Course and 45 from Airport Golf Course(AP)) were collected from fairways to the same depth. All soil samples were shipped to Servi-Tech Lab for soil chemical analysis.

For both golf courses, methodology and analysis were identical. Soil pH was determined on a 1:1 (V/V) soil to water mixture, 10 gram NCR-13 volumetric soil scoop was mixed with 10 mL double-deionized water. Samples were stirred both before and after a 15 minute equilibration period. Soil pH was measured with a pH meter. Soil organic matter (SOM) was determined by loss on ignition. For this analysis, SOM was calculated by comparing the weight of soil sample before and after the soil was heated. A sample of soil was dried at 105° C to remove moisture. Then the sample was weighed, heated at 360° C for 2 hours and weighed again after the temperature dropped below 150° C. Soil organic matter was burned off while heating, and the difference in weight before and after burning represents the amount of OM in the sample (Donkin, 1991). Phosphorus concentration was determined by Mehlich III as previously explained (Ch. 1). Potassium, S, Ca, Mg, and Na were determined by Ammonium Acetate extraction. This method used 1N ammonium acetate (NH₄OAc) at pH 7.0 to extract basic cations from the soil. The quantity of extracted basic cations is equivalent to the quantity considered exchangeable.

Concentrations of Zn, Cu, Fe, and Mn were determined by mixing 10 g of air-dried soil with 20 mL of DTPA (diethylene triamine penta-acetic acid) extracting solution. Samples were filtered after shaking for two hours, and the extracts were analyzed by an inductively coupled plasma atomic emission spectrophotometer.

Data Analysis:

Data were analyzed by analysis of variance (ANOVA) (SAS Institute 2010) to test the effect of irrigation water resources (fresh vs. effluent) on individual soil chemical properties. Comparisons between years were presented, and means were separated by LSD at 0.95 level of confidence. Green and fairway soil data were analyzed separately.

RESULTS AND DISCUSSION

The Prairie View Golf Course effluent water analysis showed that bicarbonate (168 mgL^{-1}), Cl (103.6 mgL^{-1}), sulfate (78.6 mgL^{-1}), and sodium (76 mgL^{-1}) are the most dominant elements in the water (Table 2.1).

Soil Organic Matter (SOM) in both Prairie View and Air Port putting greens had some differences. In 2004 and 2005, SOM was higher in PV greens, while in 2008 the AP greens had higher SOM (Fig. 2.2). In addition, SOM of PV greens showed significant decrease over time since beginning to use effluent water in 2007. The SOM reduction was likely due to topdressing with pure sands that diluted the SOM.

In the fairways, no significant differences in SOM were observed in PV before and after using effluent water. However, there were some differences between the two fairways. In 2006, SOM was higher in PV fairways, while in 2003 and 2013 the AP fairways had higher SOM (Fig. 2.2).

During period of increasing SOM, some of the N fertilizer that was applied to the turf may have been sequestered into SOM. A net sequestration of N will continue until SOM buildup matches that of SOM degradation, and a stable state is reached between N immobilization and mineralization (Petrovic, 1990).

Prior to 2007, greens from PV and AP had very similar pH averaging between (6.9-7.4); however, from 2004 to 2012, pH of PV putting greens increased by 11.6 % after effluent water irrigation began (Fig. 2.3). The highest pH average was 7.7, recorded in 2012. Sandy soil usually has lower pH, but using effluent water led to increase soil pH on putting greens of the PV golf course. Increased pH under effluent water irrigation has been reported in many studies (Mancino and Pepper, 1992; Qian and Mecham, 2005; Schipper et al, 1996), and it is likely related to a high rate of denitrification that produces hydroxyl ions which increase pH level. Usually effluent water has more N than fresh water, and it is present as inorganic ammonium and nitrate. After applying effluent water to soil, microorganisms, mainly bacteria convert ammonium to nitrate by nitrification and convert nitrate to N_2 by denitrification which is considered a redox reaction and releases hydroxyl ions which causes the increase in pH.

Although, the pH level reached in both golf courses was not excessive, turfgrass grows the best with a soil pH ranging from 5.5 to 7. Soil pH could increase over time when using effluent water for irrigation without appropriate soil management practices such as using S or acidifying fertilizer applications.

From 2003 to 2007, the two golf course fairways had similar soil pH levels (with an average of 7.23 to 7.35). However, from 2007 to 2013 soils PV fairways exhibited higher soil pH and the average of soil pH increased from 7.35 in 2007 to 7.75 in 2013 on PV fairways. On

average, soil pH was 0.35 units higher at PV fairways when compared to AP fairways (Fig. 2.3). The level of pH increase is consistent with previous findings in Colorado (Skiles and Qian, 2013). The soil pH increase was partially due to the bicarbonate concentration (145 ppm) in effluent water. Consistently high soil pH can cause deficiencies such as Fe and Mn in plants.

Some differences were observed in CEC between greens from the two golf courses, but there was no clear pattern (Fig. 2.4). In the last two years, CEC values were higher in PV greens by almost 15% when compared to AP greens. Data showed significant differences were recorded before (2005) and after (2008) using effluent water in PV greens.

In Prairie View fairway soil, no significant differences were observed in CEC before and after the use of effluent water (Fig. 2.4). However, CEC was higher for PV fairways than AP fairways in 2008, 2010, and 2012. Typically, putting greens have low CEC because they are mostly sand, which contains low mineral elements.

Soil salinity (Electrical Conductivity) was significantly higher in PV greens in 2003, 2004, 2006, 2008, 2009, 2010, and 2012 than AP greens (Fig. 2.5). Additionally, soil EC in PV greens increased after three years of using effluent water from 0.1 to 0.3 dS m⁻¹. Effluent water irrigation increased EC values over time because of the accumulation of salt. However, soil EC values of PV greens were not in the critical salinity range even after 6 years of using effluent water. Fairways at Airport Golf course showed less fluctuation in soil salinity (Fig. 2.5). However, PV fairways had significantly higher soil EC after the use of effluent water. Despite the increasing trend of soil salinity in PV golf course, the values measured in both green and fairways indicated that soil salinity was still well below the critical value. In general, EC of soil

higher than 4.0 dS m^{-1} is considered saline soil, although salt sensitive plants may be injured below this value, and salt tolerant plants may tolerate EC levels higher than 4 dS m^{-1} .

A very significant increase was observed in Na concentration at PV greens after a year of using effluent water (Fig. 2.6, 2.7). There was a 176% increase observed in 2008 in sodium, an increase of 50 mg kg^{-1} one year immediately after the start of using effluent water). We observed reduction in the soil Na in 2009 and 2013. This decrease was due to gypsum applications to control Na accumulation. However, the level of Na in 2009 and 2013 was still higher than the years before the start of effluent water irrigation at PV greens. Increasing Na concentration because of the use of effluent water for irrigation has been confirmed in previous research (Balks et al. 1998; Halliwell et al., 2001). Sodium concentrations in PV greens were significantly higher than AP greens in most of the years. Soil exchangeable Na percentage (ESP) was increased as well after using effluent water in PV greens (Fig. 2.9). Exchangeable sodium percentage is an indicator of sodium hazard for soil. Exchangeable Na percentage is calculated by dividing exchangeable Na by cation exchange capacity (CEC) and multiply by 100. An ESP value of 12 or greater indicates a sodic soil with excessive Na on soil particles. However, for fine textured soil and heavy traffic areas, an $\text{ESP} > 6 - 9$ will start to impose sodic effects, such as soil sealing and reduced water penetration. ESP increased in 2008 after one year of using effluent water to reach 6.1% and then decreased to 3.4% in 2013 because of the gypsum applications to greens.

For fairways, soil Na content increased significantly at the PV golf course since 2007, whereas no such increase occurred for AP golf course (Fig. 2.6). Comparison of soil before and after effluent water irrigation at PV fairways also revealed increased sodium content and Na

ESP (Fig. 2.7). All samples collected on PV fairways before 2007 had an average ESP below 2%. Soil ESP increased after effluent water irrigation, reaching 6% in 2012 (Fig. 2.7).

Differences were recorded in Ca concentrations in the PV greens before and after the use of effluent water. Since 2007, calcium concentrations were higher in AP greens except in the last two years of effluent water use when Ca concentration increased in PV greens (Fig. 2.8). This increase may be due to gypsum (calcium sulfate dehydrate) applications in 2012. Another significant difference was recorded between PV greens and AP greens in 2003. This increase was not related to recycled water irrigation, but it could be a result of using sulfur fertilizer that is naturally high in calcium. Soil Ca in PV fairways showed no significant differences between the years of using fresh water vs. effluent water. However, significant differences were observed between the two golf course fairways. PV fairways had significantly higher soil Ca content than AP fairways. The increase of Ca might be due to the use of effluent water irrigation as some researchers have suggested. A study done on effluent water use in irrigation indicated that effluent water provided significant amounts of Ca that could be beneficial for plant growth and decrease fertilizer cost (Menzel and Broomhall 2006).

A 16% and 12% increase was noted on P concentrations one and four years, respectively, after the use of effluent water irrigation use at PV greens (2008, 2011) (Fig. 2.9). The highest increase by 35% was recorded in 2013. Although Prairie View's water analysis did not show how much P was in the effluent water, increased P in soil after using effluent water has been confirmed by some other researchers (Bond, 1998; Skiles and Qian, 2013). These results disagree with a study done in Saudi Arabia which found that using effluent water did not increase soil's P (Aljaloud, 2010). On the other hand, some increase was also noted in AP green

soil in years 2009, 2011, and 2012. These increases could be due to fertilizer practices. The amount of P applied as fertilizer was 0.49 kg. actual P/100m² per year (Fig. 2.1). For the fairway data, no significant differences were recorded in PV after using effluent water, but significant differences were recorded between the two golf courses fairways in 2012 and 2013. (Fig. 2.9).

Iron concentration decreased over time by 79% in 2012, after 5 years of effluent water irrigation in PV greens. In AP greens, the highest value was recorded in 2008 (Fig.2.10). PV fairways data showed that Fe concentration significantly decreased as well after 6 years using effluent water from 59.4 to 23.3 mg kg⁻¹. These decreases seem to be related to the use of effluent water as they do not appear in Airport greens or fairways. It is possible that the observed reduction in Fe availability was associated with soil pH increase caused by effluent water irrigation. A study done in Nebraska suggested that iron concentration remained at the same level after long-term use of wastewater application (Hu et al., 2005).

Potassium concentration of PV greens showed a significant decrease in 2007 from 175 to 82 mg kg⁻¹, and after this decrease, concentration increased again, but the value was still lower than the value before the use of effluent water (Fig. 2.11). These slight increases could be due to the amount of K in effluent water as some other papers have mentioned (Menzel and Broomhall, 2006). In the PV fairways soil, data showed an increase from 72.3 to 32mgkg⁻¹ after using effluent water with a higher value in 2009. However, values in AP fairways soil were very close, and no significant changes were noticed. In general, fairways data in both golf courses were lower in value than greens data. This difference could be due to the fact that greens are mostly sand and contain lower CEC and carry less elements.

Sulfur data showed some significant increases over time before and after using effluent water in PV greens compared to AP greens (Fig. 2.12). However, the highest significant increase was after three years of using effluent water in year 2010 by 189% up to 53 mgkg^{-1} (Fig. 2.12). This increase could be due to the use of effluent water and/ or fertilizer applications; the annual total amount of S fertilizer was $5.8 \text{ kg} / 1000 \text{ m}^2$ (Fig. 2.1). However, AP greens received the same amount of S fertilizer, and its soil S concentration showed no increase over time. Similarly, PV fairways soil data showed similar increase in the same year up to 40 mgkg^{-1} . AP fairways showed no significant increase over time.

Sulfur fertilizers that are applied to soils are naturally high in Ca, and play a significant role in making soil's Ca more soluble. Soluble Ca may replace Na on soil particles, and prevent additional Na accumulation. The amount of S adjustment depends on the soil's sodium adsorption ratio, soil texture, and the amount of irrigation water applied.

Zinc concentration in PV greens decreased after the use of effluent water from almost 13 mg kg^{-1} in 2007 to almost 4 mg kg^{-1} in 2012 (Fig. 2.13). Zinc concentration in AP greens showed significant changes over time as well; the highest value (17 mg kg^{-1}) was observed in 2008, and the lowest value (2.5 mgkg^{-1}) was observed in 2013. No significant change in Zn concentration was observed over time after the use of effluent water at PV fairways. In contrast, soil zinc concentration fluctuated at fairways.

These results disagree with a prior study which showed that using effluent water increased soil trace elements concentrations (Mapanda et al., 2005). On the other hand, they are similar to the results which were found in a previous study done on another golf course fairway in Colorado (Skiles and Qian, 2013).

Zinc and Cu, concentrations data of PV green had no significant change over time after the use of effluent water (Fig. 2.13 and 14). However, Mn concentrations were significantly changed after using effluent water in PV green. No clear pattern was observed, but the values were lower than the values in AP (Fig. 4.16). Usually sand has low concentrations of trace elements, but the Airport putting green data had significantly higher values of these elements.

CONCLUSION

Overall, the soil analysis of the two golf courses showed that many changes had occurred over time in the greens and fairways due the use of effluent water irrigation as well as other management practices such as fertilizer or gypsum applications. From our results, we can conclude that using effluent water for golf course irrigation contains risks as well as benefits. Our results showed that salinity and Na accumulations occurred after the use of effluent water irrigation. Therefore, gypsum was applied to decrease the Na concentration and its negative effects on soil quality and plant growth. On the positive side, P and K levels increased in the soil after using effluent water, which would be beneficial for the grass and potentially lower the fertilizer cost.

Using effluent water for irrigation affects soil chemical properties, and it may affect the soil's ability to provide an optimum media for plant growth. Although no data were collected from the grass or trees in this study, negative impacts may have accrued due to the increase of sodium and EC levels. Therefore, golf course managers need to be capable of managing more obstacles associated with the use of effluent water. Although some research has shown that using effluent water for irrigation may change soil properties, effluent water usually has no

negative impacts on plant growth or turf quality (Walker and Lin, 2007; Thomas et al., 2006). However, many factors may influence these results such as soil type, grass species, and other soil management practices.

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Table 2.1 Effluent water quality used in Prairie View Golf Course.

Water Quality Parameters	
pH	8.4
NO ₃ +NO ₂ -N	9.8 mgL ⁻¹
Total dissolved solids	571
Conductivity,	1.01 dS m ⁻¹
Sodium absorption ratio (SAR)	2.1
Adjusted SAR	4.1
Na	76 mgL ⁻¹
Cl	103.6 mgL ⁻¹
Bicarbonate	168 mgL ⁻¹
Ca	77.4 mgL ⁻¹
Mg	1.63 mgL ⁻¹
Sulfate	78.6 mgL ⁻¹
B	0.3 mgL ⁻¹
Fe	<0.05 mgL ⁻¹
K	12.8 mgL ⁻¹

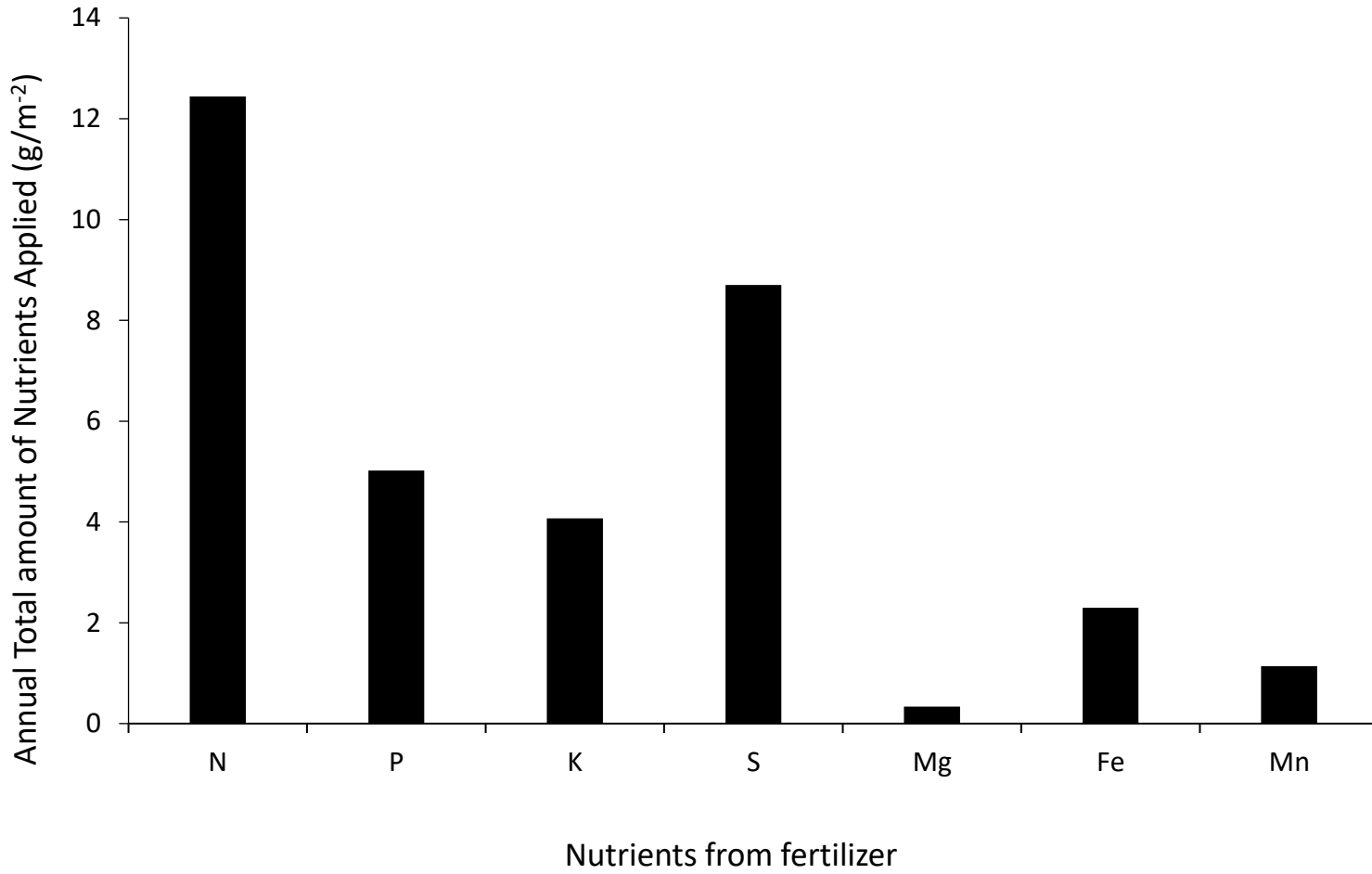


Figure 2.1 Fertilizer application record for Airport and Prairie View golf courses.

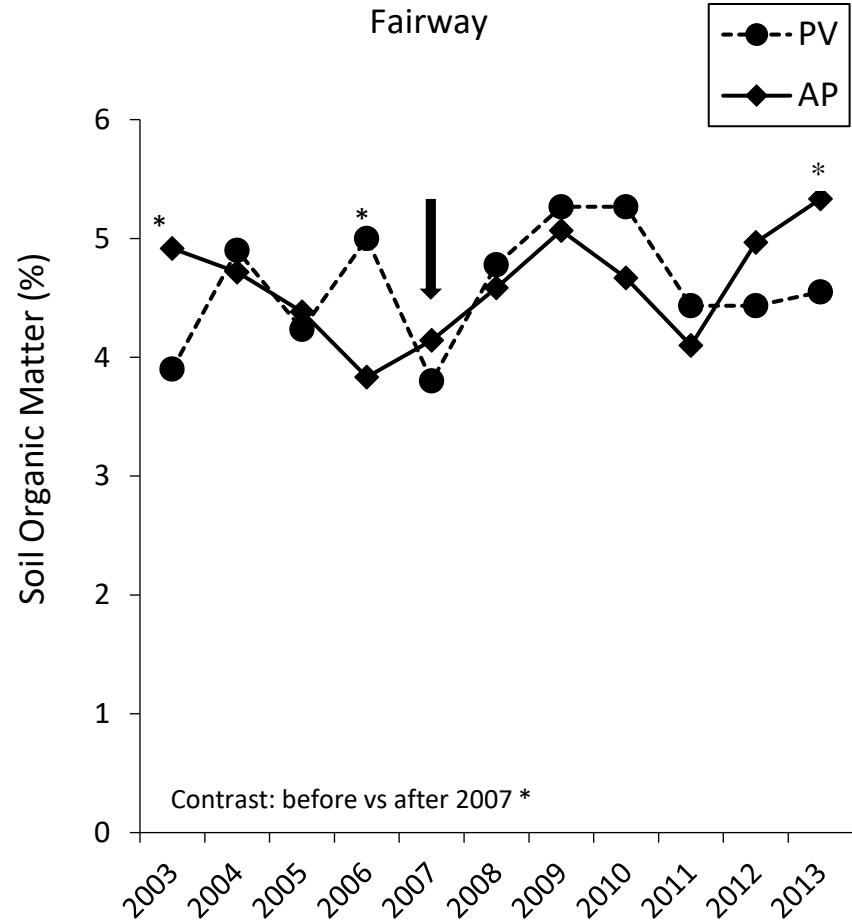
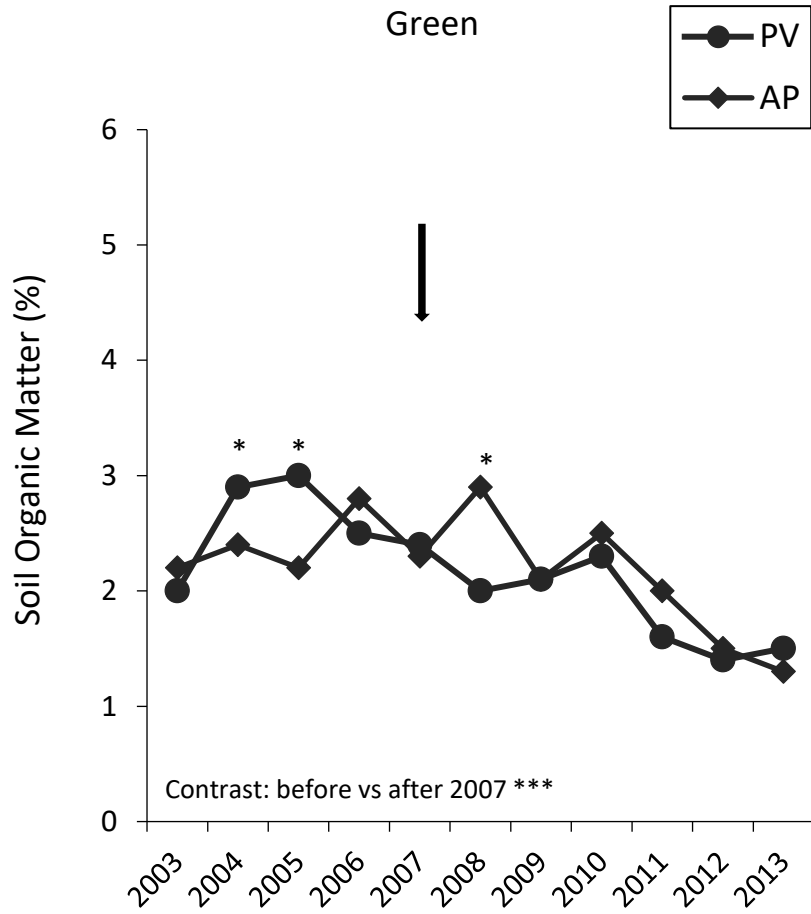


Figure 2.2 Soil organic matter data from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the yeas of using effluent water.

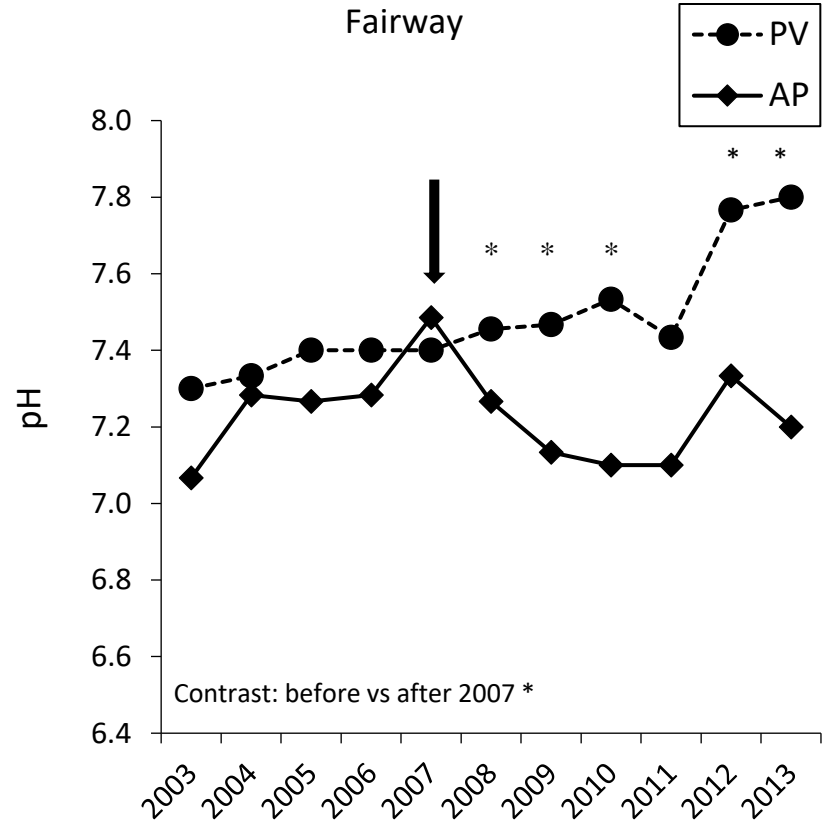
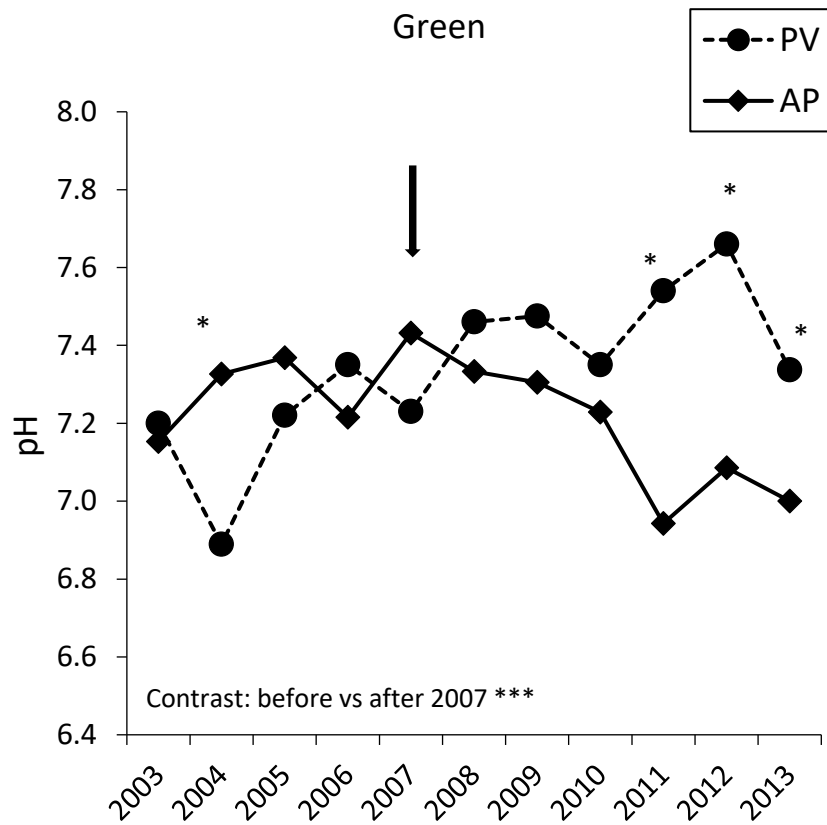


Figure 2.3 Soil pH data from Prairie View and Airport putting greens and fairways

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

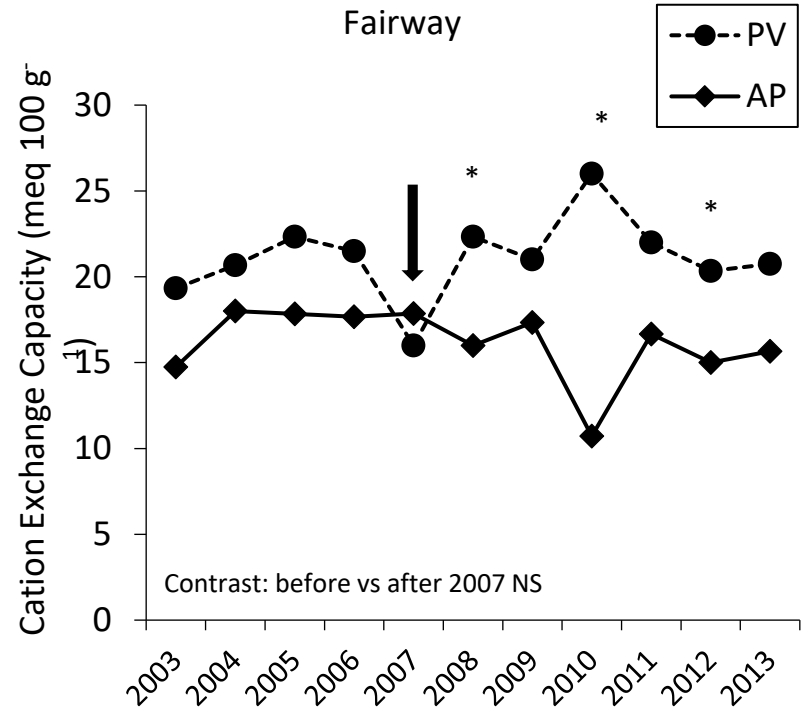
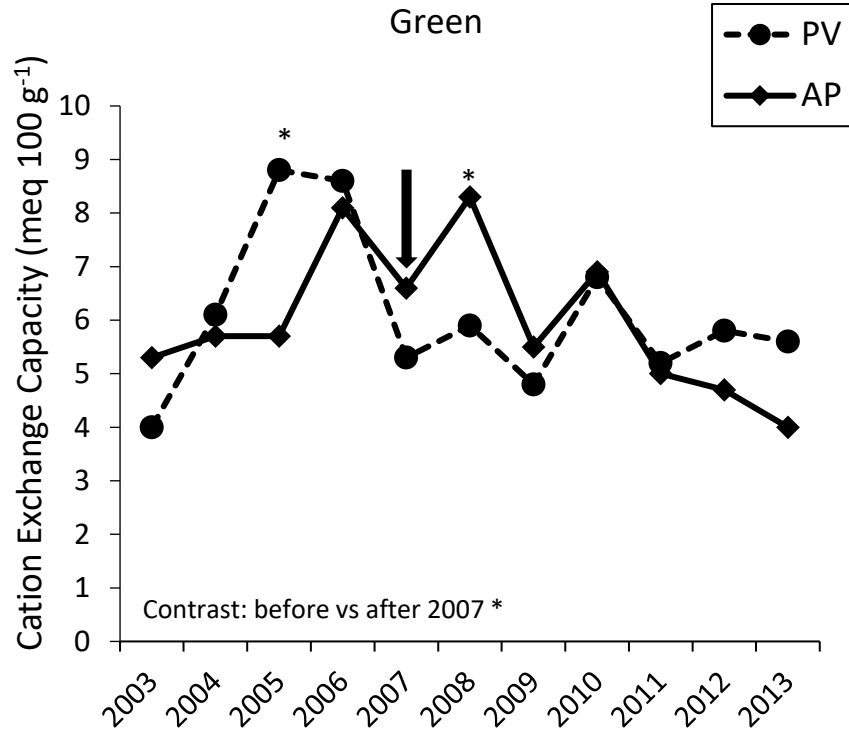


Figure 2.4 Soil cation exchange capacity from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

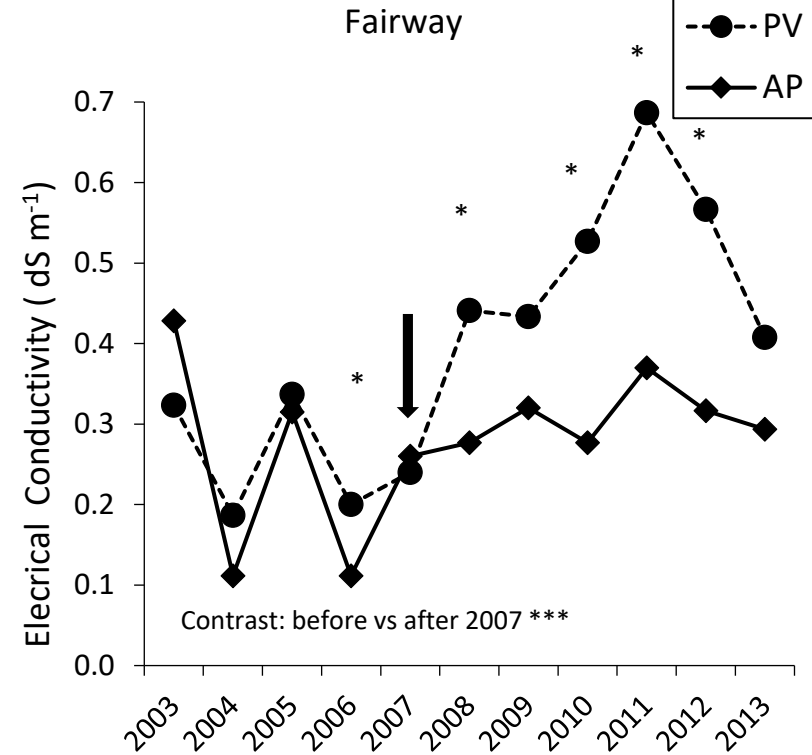
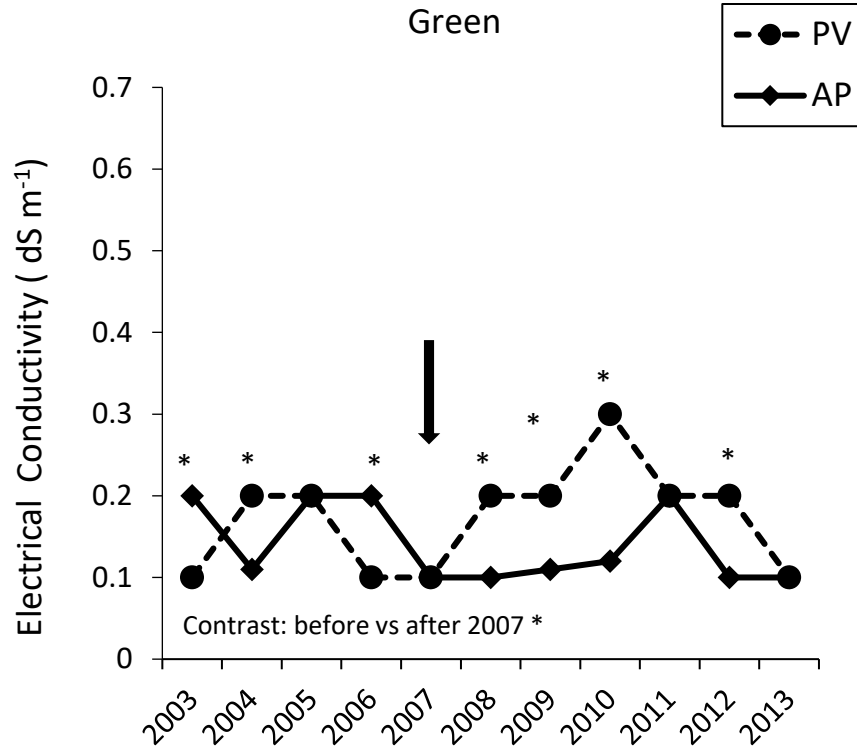


Figure 2.5 Soil electrical conductivity from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level

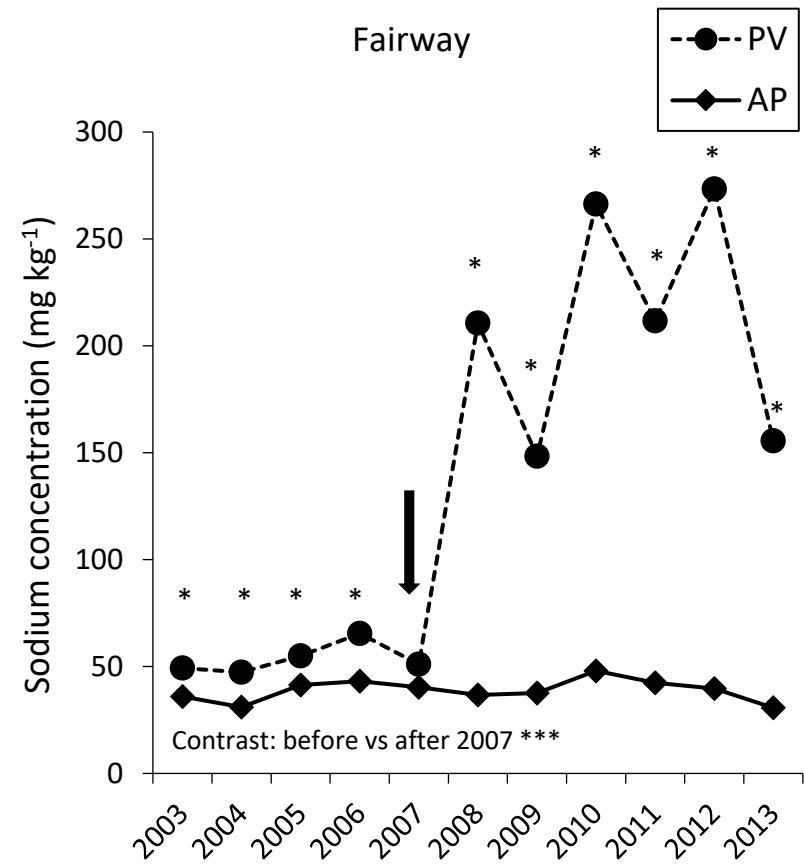
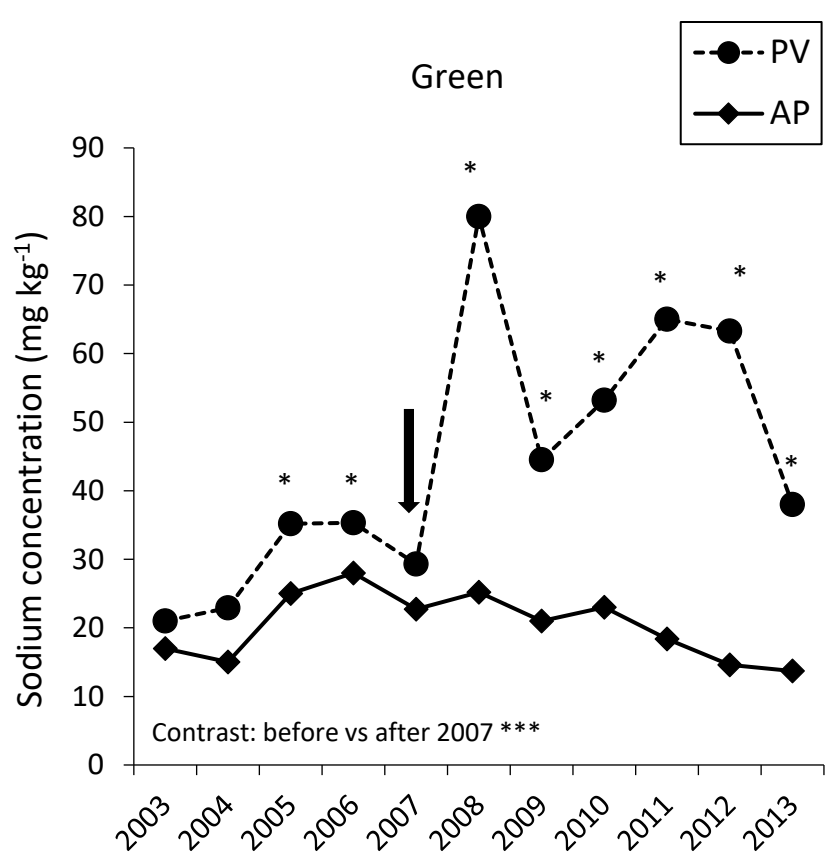


Figure 2.6 Soil Na concentration from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

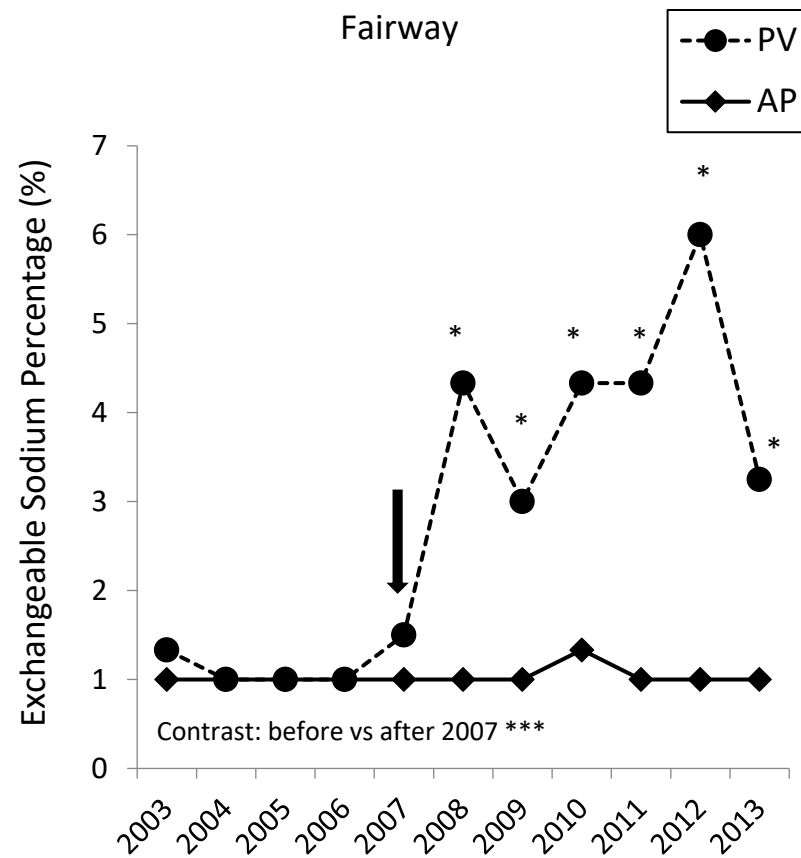
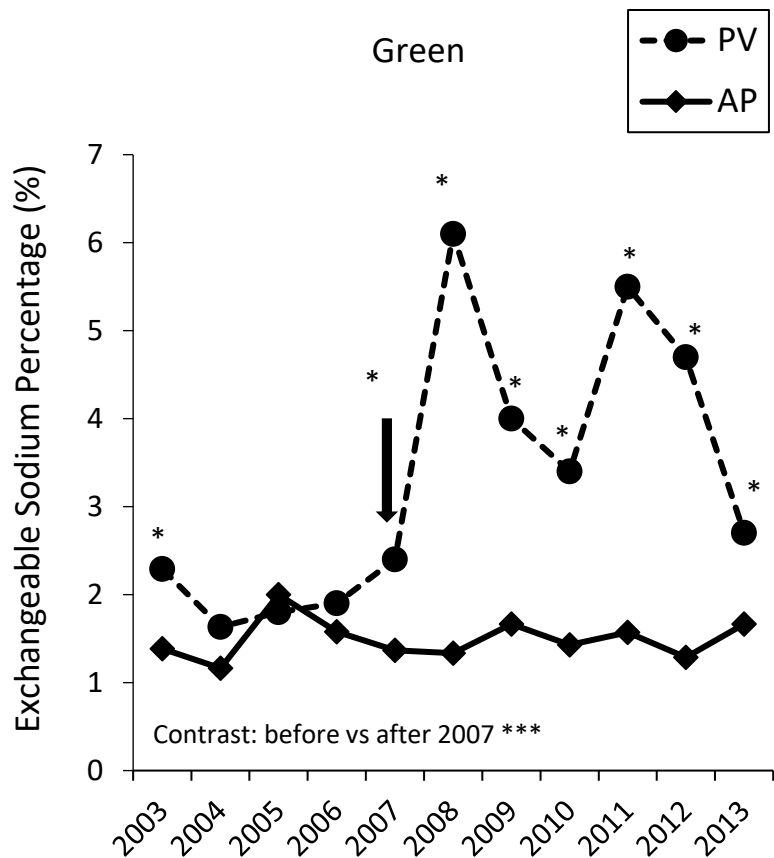


Figure 2.7 Soil exchangeable Na percentage from Prairie View and Airport putting greens and fairways

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

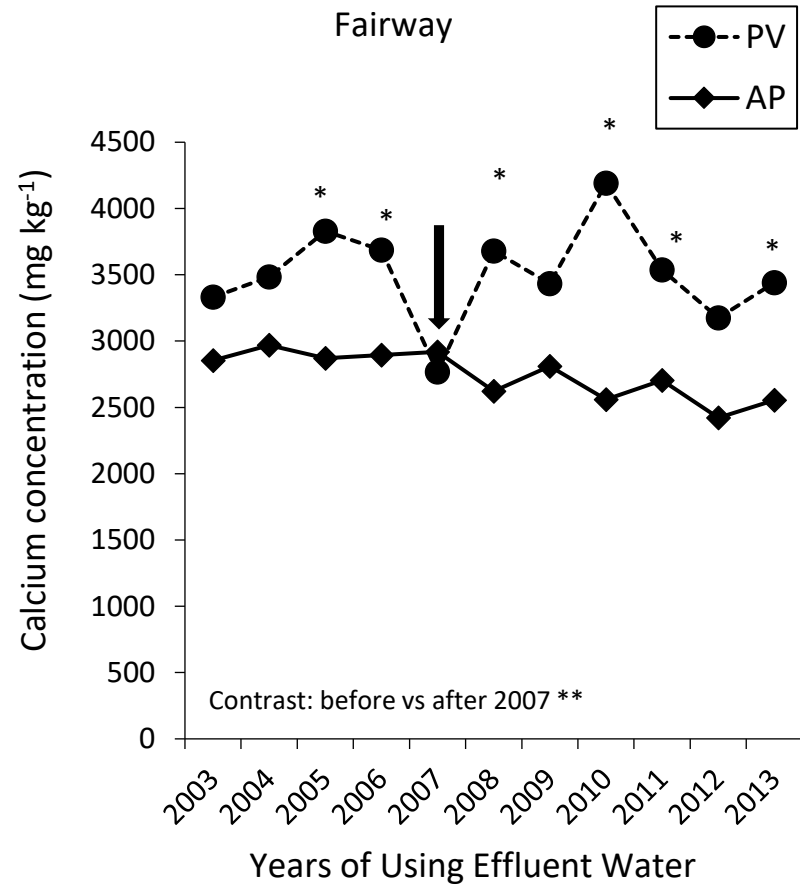
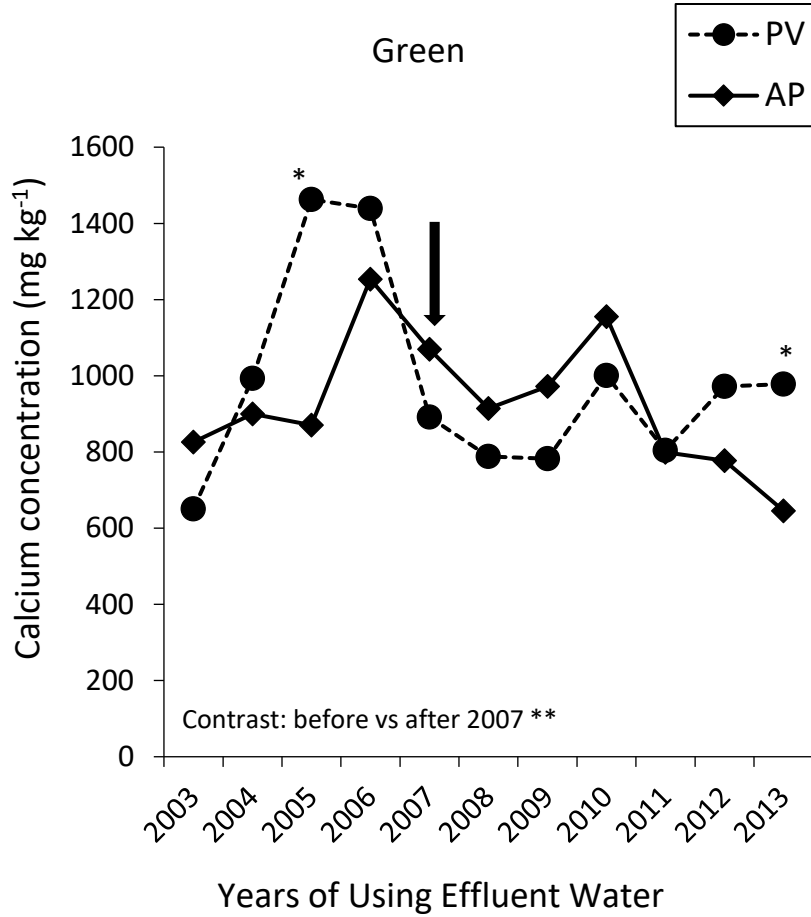


Figure 2.8 Soil Ca concentrations from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

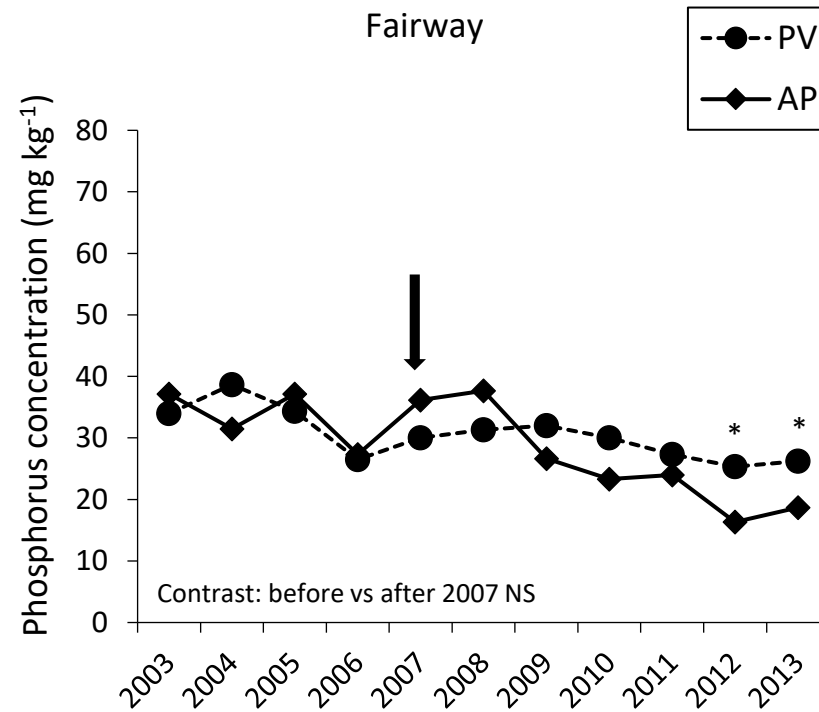
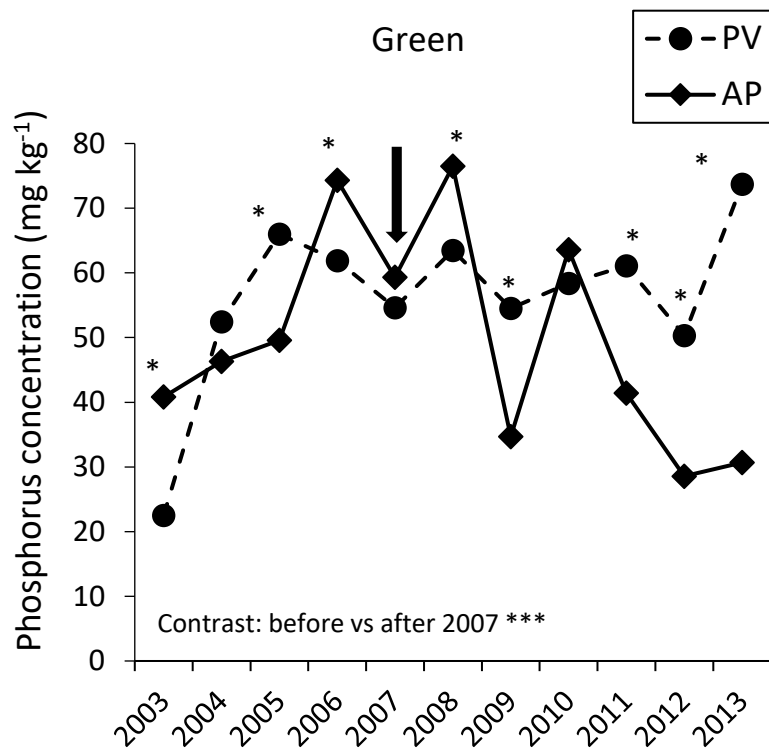


Figure 2.9 Soil P (Mehlich-3) concentration from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

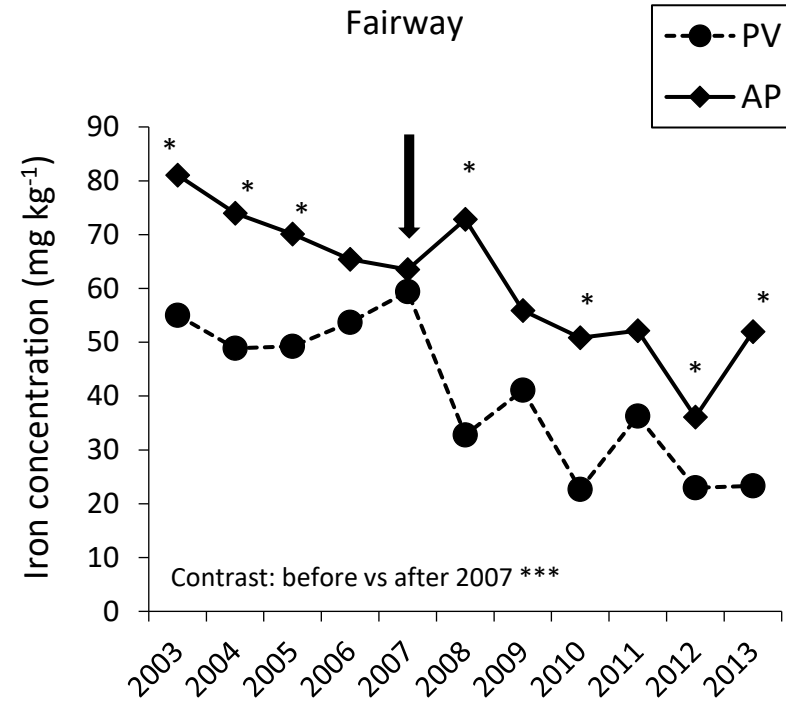
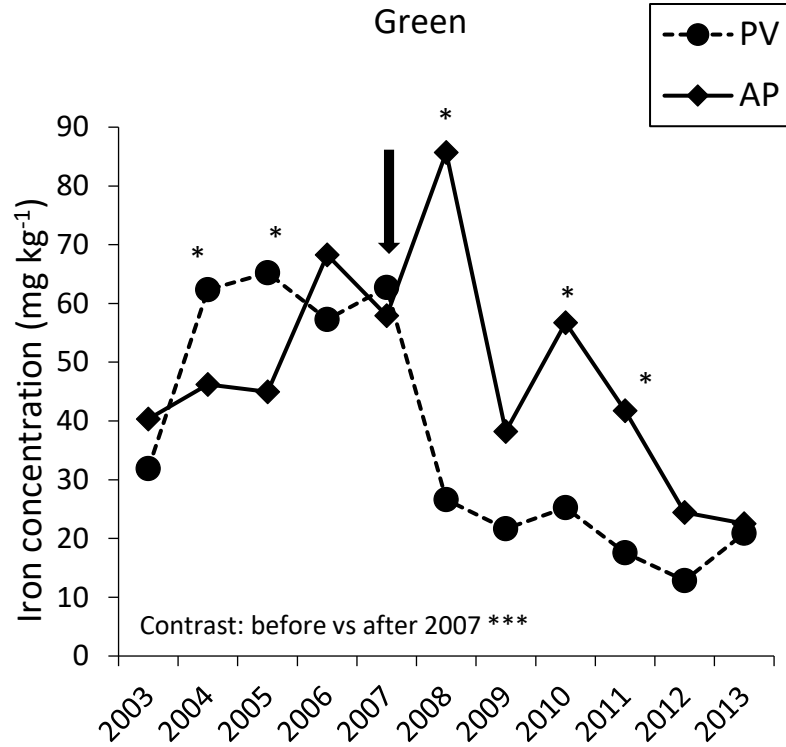


Figure 2.10 Soil Fe concentration from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

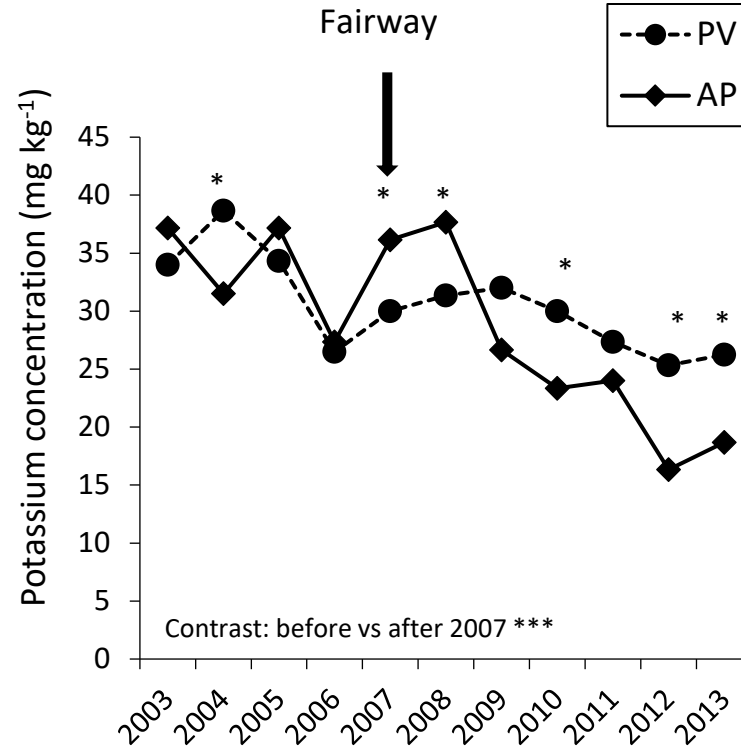
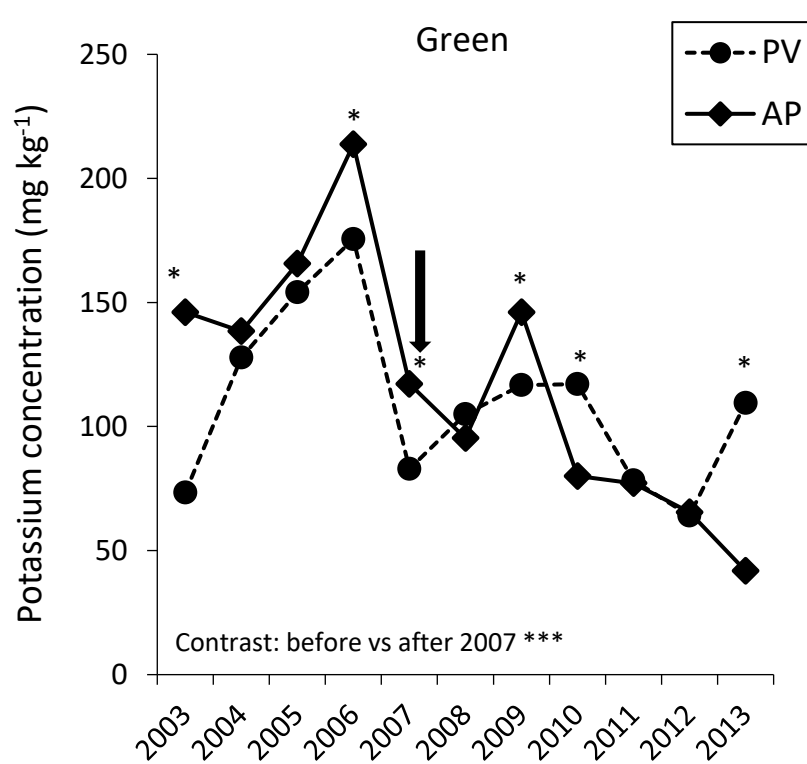


Figure 2.11 Soil K concentration from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

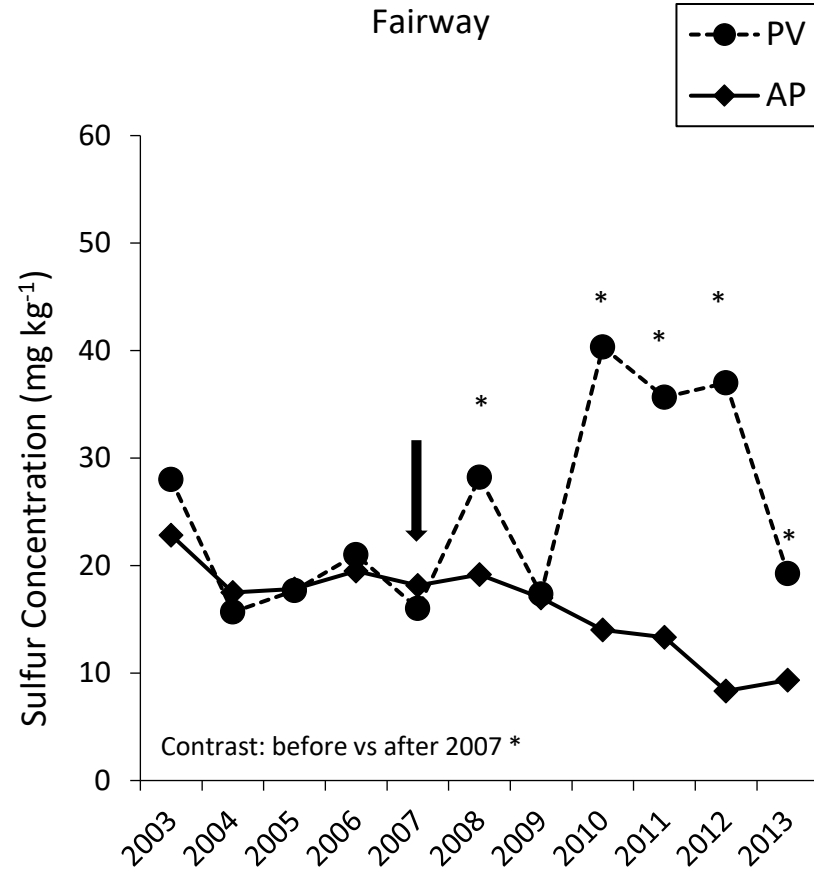
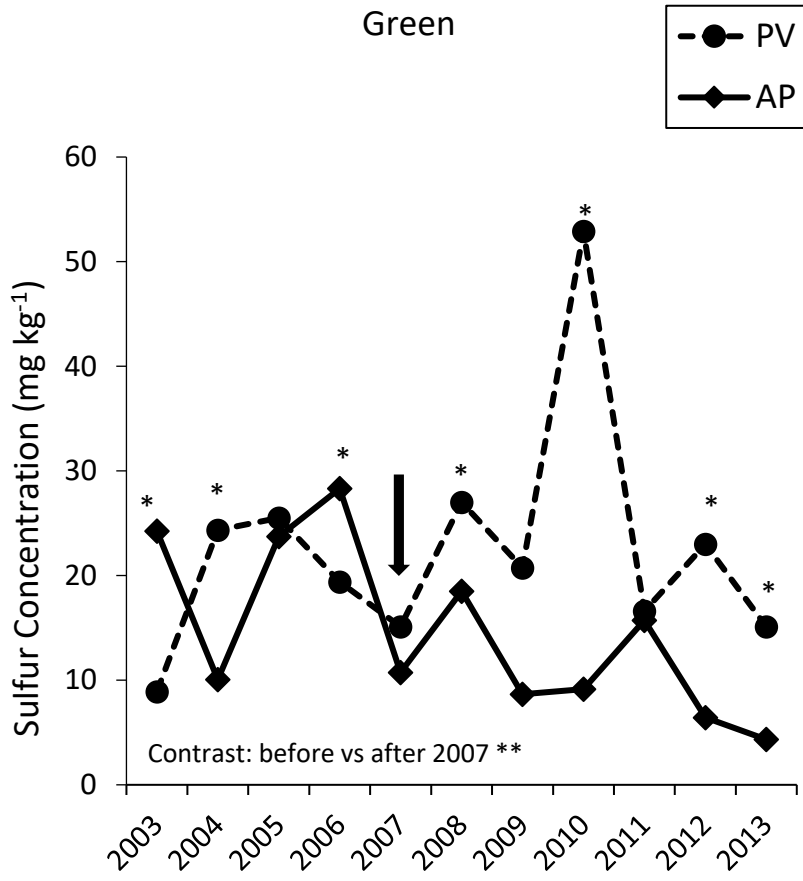


Figure 2.12 Soil S concentration from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

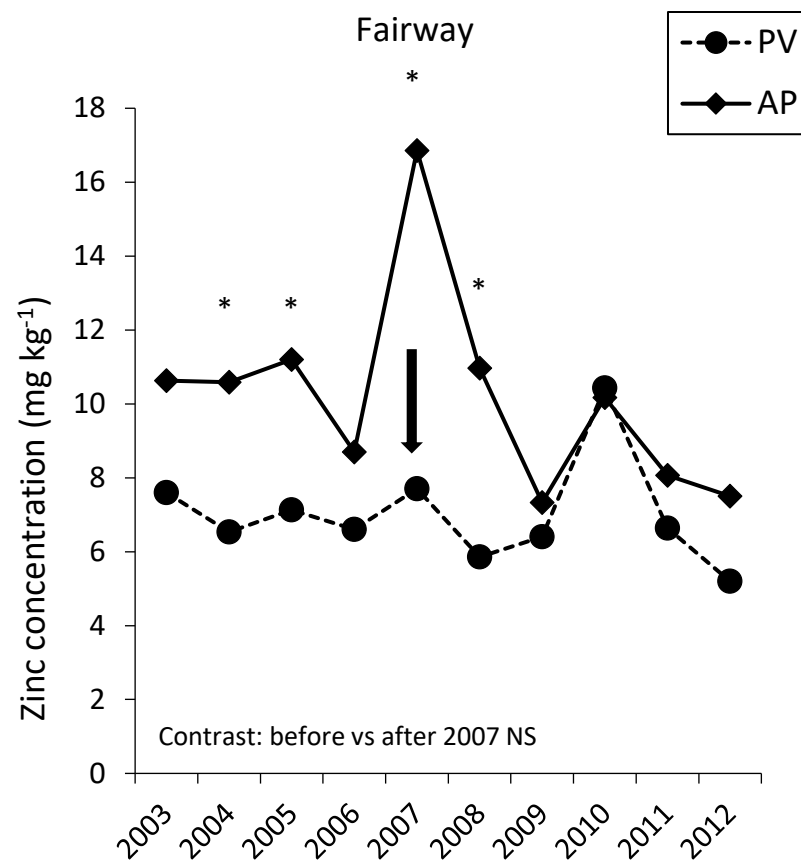
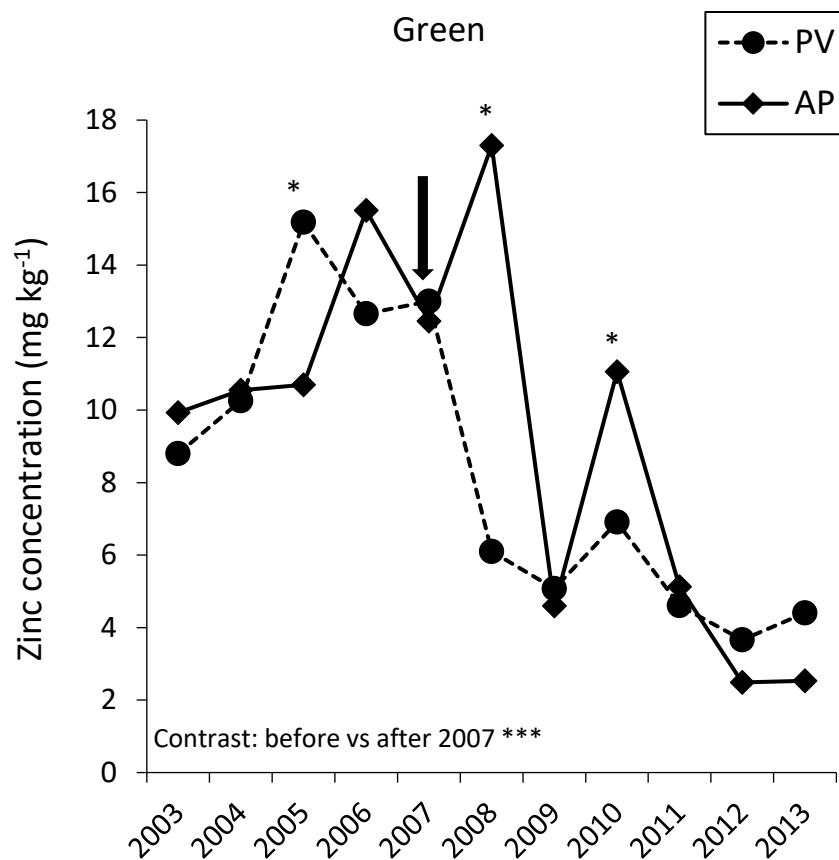


Figure 2.13 Soil Zn concentration (DTPA extractable) from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

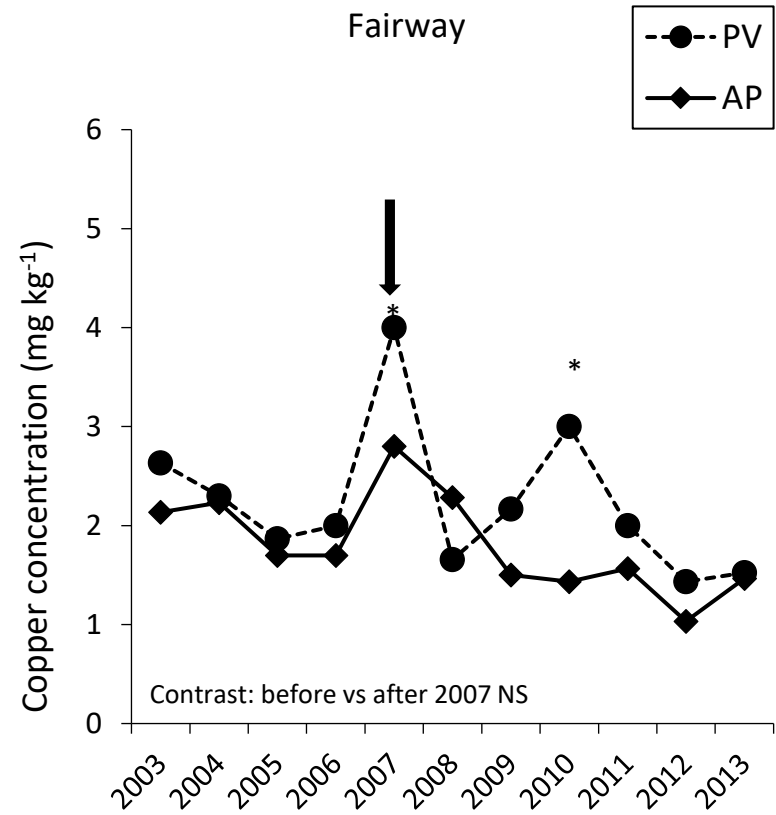
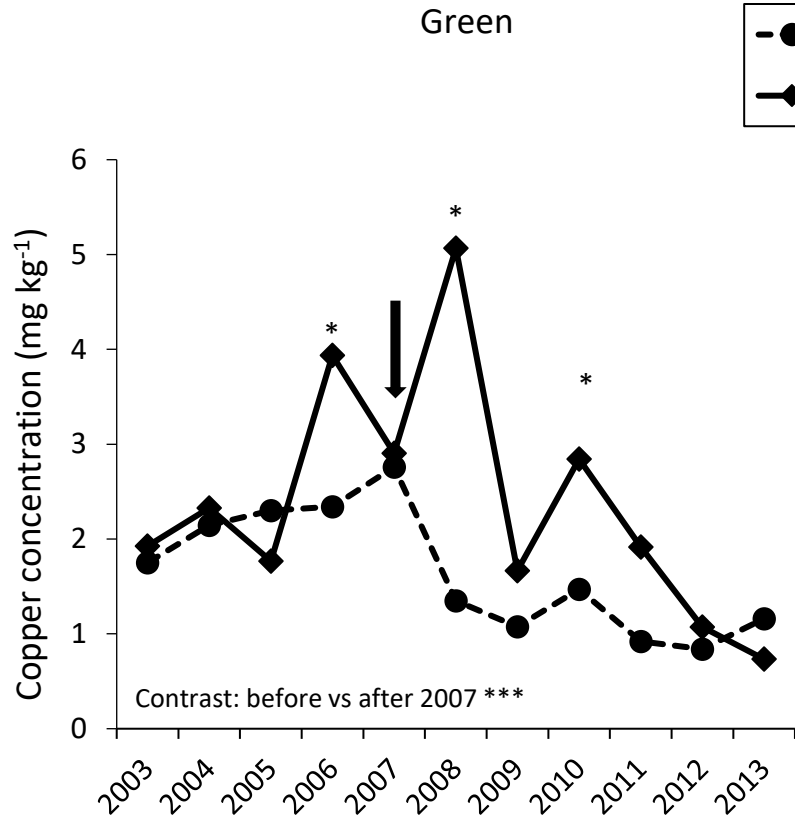


Figure 2.14 Soil Cu concentration (DTPA extractable) from Prairie View and Airport putting greens and fairways.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

Arrow indicates the years of using effluent water.

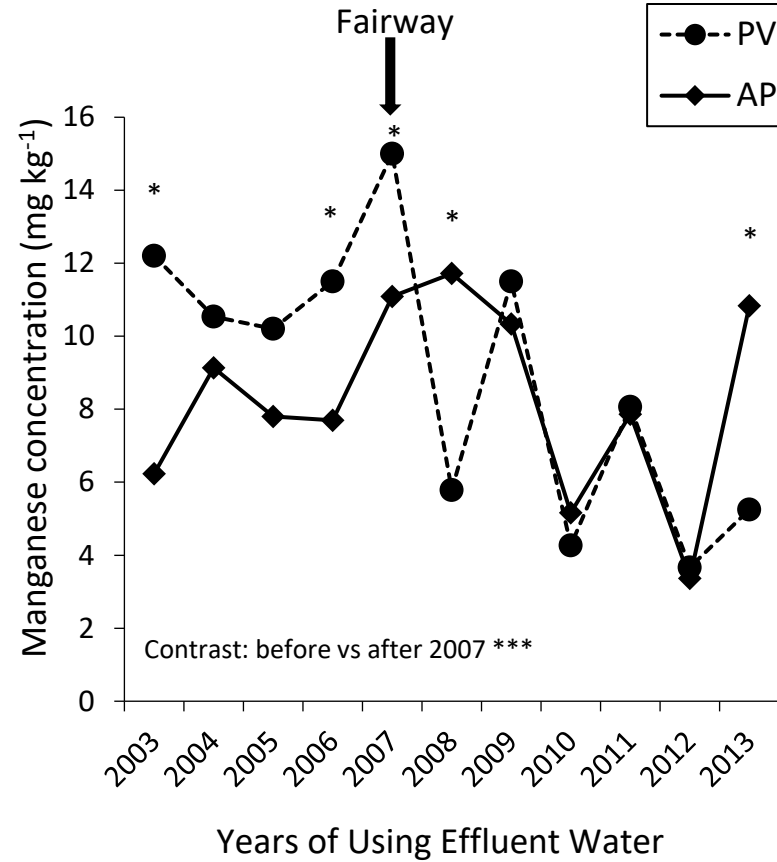
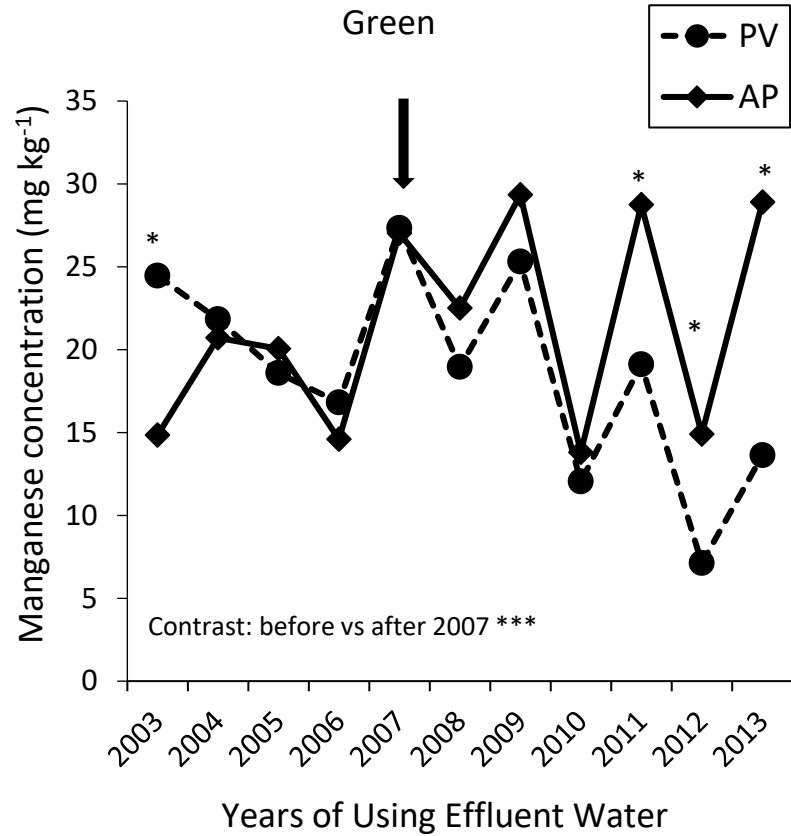


Figure 2.15 Soil Mn concentration (DTPA extractable) from Prairie View and Airport putting greens and fairways

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level

CHAPTER 3
INTERACTIVE EFFECTS OF WATERLOGGING AND SALINITY ON PERENNIAL RYEGRASS AND
ALKALIGRASS
CONCEPTUAL

A lot of salt-affected soil in the world is also affected by compaction and waterlogging due to shallow water tables or decreased infiltration of water in soil because of sodicity. Waterlogging and compaction cause reduced oxygen exchange (hypoxia). Research on the combined impacts of salinity and hypoxia on turfgrass growth is limited. The interactive effects of salinity and oxygen availability on nine perennial ryegrass lines (*Lolium perenne* L.) and one alkaligrass (*Puccinellia tenuiflora*) was studied. In a controlled greenhouse, grasses were exposed to four salinity levels (3, 6, 9, 12 dS m⁻¹) with and without hypoxia condition for four weeks each. All entries exhibited decreased clipping yield with increased salinity in both salinity and hypoxia + salinity treatments except 'Fults' Alkaligrass. Turf quality declined over time to unacceptable quality ratings with high salinity (12 dS m⁻¹) treatment. In general, all entries had better turf quality in control and hypoxia treatments than in salinity and salinity with hypoxia treatments. All grasses were more severely affected (quality and yields) under combined hypoxia and salinity treatment compared to salinity or hypoxia only. Plant Na⁺ and Cl⁻ concentrations increased under salinity and salinity + waterlogging treatments. The experimental lines (10.0824, 10.0825 and 10.0815) maintained acceptable turf quality under hypoxia plus moderate salinity (6-10 dS m⁻¹) conditions.

INTRODUCTION

Increasing world population and limited water resources have forced many landscape professionals to use non-potable water for landscape irrigation. Using poorer quality water can cause salinity problems in many regions. Saline soil and water are major problems that decrease plant growth in many areas of the world. Using saline waters for turfgrass irrigation has become more common in many of these areas due to the shortage of fresh water. Consequently, the demand to identify salinity tolerant turfgrass species has increased.

Turfgrass species and cultivars have different degrees of salt tolerance. This study tested the salinity tolerance of new lines of perennial ryegrass and alkaligrass, both are cool-season grasses. Perennial ryegrass has been ranked as moderately salinity tolerant (6 to 10 dSm⁻¹); whereas, alkaligrass has been ranked as potentially tolerant (>10 dSm⁻¹) (Harivandi, 2005).

The effects of saline conditions have been widely studied and reviewed (Marcum and Kopec, 1997; Marcum *et al.*, 1998; Qian *et al.*, 2000; Alshammary *et al.*, 2004; Pessaraki *et al.*, 2008). Turfgrass salinity tolerance is a complex process, and it is affected by different environmental and physiological factors. Plant age, temperature, nutrient levels, and humidity are the factors that most influence salinity problems (Maas, 1986). Increasing salinity levels reduce plant water uptake from soil. The main reasons for diminished plant growth due to salinity are water deficiency and nutrient imbalance (Marschner, 2002).

Many saline soils around the world are also affected by waterlogging due to shallow water tables or decreased infiltration of water in soil due to sodicity (Barrett-Lennard, 1998). Waterlogging causes hypoxia (Marschner, 2002). Reduced soil oxygen availability under waterlogged conditions decreases turf quality, photosynthetic rate, and chlorophyll and

carbohydrate concentration (Jiang and Wang, 2006). The combination of salinity and waterlogging has greater effects on plant growth than either of them separately (Barrett-Lennard, 2003). In addition, waterlogging can cause the buildup of anaerobic metabolism products such as ethylene, carbon dioxide, and ethanol, which are very harmful to plant growth. The interaction between salinity and low oxygen levels affect nutrient uptake and growth in higher plants. There are many reports about the combined effects of salinity and hypoxia on crops such as tomato (*Solanum lycopersicum*), soybean (*Glycine max*), and wheat (*Triticum aestivum*) (.....1900), but few studies have been done with turfgrass. This study is based on the turf industry's interest, as the results could be very helpful for turf breeders to develop cultivars that can maintain acceptable turf quality under saline and low oxygen conditions. The goals of this study are to test the effects of salinity on different perennial ryegrass and alkaligrass lines simultaneously under optimum and low oxygen level. The study's specific objectives are to:

1. Identify perennial ryegrass and alkaligrass lines that can maintain acceptable turf quality under saline conditions.

2. Determine the interactive effects of salinity and oxygen availability on perennial ryegrass and alkaligrass.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The study was conducted at Colorado State University's Plant Growth Facility. Nine perennial ryegrass lines and one alkaligrass cultivar were selected based on the turf industry's

interest and use (Table 3. 1). Grass seeds were planted in sand, and grown for 30 days before transplanting them as plugs with similar size to deep pots (6.4 cm-diameter × 25 cm-deep) containing fine sand. Turfgrasses were grown for 60 days before initiation of salinity treatments. Cultivars were distributed randomly in each treatment. The greenhouse temperature was maintained at 23-25°C day/18-20°C night. Salinity levels were increased daily by 1 dS m⁻¹ in treatment tanks until targeted salinity levels were reached (control tanks received no salt). Instant ocean salt (Aquarium Systems, Mentor, Ohio) was used (55% Na, 31% Cl, 8% S, 4%Mg, and 1% K).

Treatments

Four treatments were replicated four times

1. Control: “No salt no hypoxia” plants were submerged in water for 1 hr. every day for irrigation, and Hoagland nutrient solution (Hoagland and Arnon, 1950) was added with irrigation water every 10 days.
2. Salinity: plants submerged in water with target salt concentration for 1 hr for salt treatments, and nutrients were added with irrigation water every 10 days. Salt was added gradually by 1 dS m⁻¹ until four target (3, 6, 9, and 12 dS m⁻¹) concentrations were reached. For salinity treatment, grasses were first treated with salinity at 3 dS m⁻¹ for a period of 4 weeks. Data were collected for treatments and the control. Following data collection, salinity treatment was ramped up to 6 dS m⁻¹, whereas nutrient solution of the control was maintained at < 2.0 dS m⁻¹. Salinity was again held at 6 dS m⁻¹ for 4 weeks, and data were collected. The cycle was repeated until solution salinity reached 9 and 12 dS m⁻¹. All entries were maintained at these individual salinity levels for a period of 4 weeks, respectively, for data collection.

3. Hypoxia: plants were kept in enough water to reach the soil surface, then pots drained for 1 h daily and placed back in the water to achieve waterlogging, and nutrients were added with irrigation water every 10 days.

4. Salinity + hypoxia: plants were kept in water with target salt concentration with enough water to reach the soil surface, then pots were drained for 1 h daily and placed back in the saline water to achieve waterlogging, and nutrients were added with saline water every 10 days. Salt concentration was ramped up from 3, 6, 9, to 12 dS m⁻¹. Salinity + hypoxia treatment was held at each of these 4 salt concentrations for 4 weeks, and data were collected.

Measurements

Data collection began 2 weeks after reaching target salinity levels. Grasses were clipped twice per week at 2 cm height. Grass clippings were collected for the last 2 weeks and dried at 60 °C for 24 h to determine the dry weight. Turf quality (color, density, and uniformity) was rated visually on a scale of 0 (dead turf) to 9 (optimum quality, with a rating of 6 indicating minimum acceptable quality) at the end of each 4 week period. Root length was measured at the end of the experiment. Leaf firing percentage was rated visually by estimating the total percentage of bleached leaf area at the end of each 4 week period.

Aerenchyma Formation

Digital images of root cross-sections were taken and converted to black and white by ImageJ software (Millersville University, Millersville, PA) to observe aerenchyma formation in root in hypoxia treatment compared to the control treatment (Fig.3.1) (Maricle and Lee, 2002).

Ion Concentrations

Ion concentrations analyses were determined according to Gorai *et al.* (2010). Dried plant materials were weighed and ashed for 5 h at 500 C. Cold ash was dissolved in 3 ml 1N HCl and enough deionized water was added to reach 25 mL for the analysis. The concentrations of Na⁺, K⁺, Ca⁺, and Mg²⁺ were determined by ICP (Inductively Coupled Plasma) at Colorado State University Soil, Water and Plant Testing Laboratory. To determine the concentration of Cl⁻, shoots were washed with deionized water, and dried at 70 C for 24 hr after which time they were ground by mortar and pestle. 100 mg were taken and dissolved in 25 ml of 2% acetic acid and filtered. Chloride was analyzed by chloride meter (Jenway PC LM3, London, UK).

Experimental Design and Statistical Analysis

A split-plot experimental design was used with four replications. The main factor was the interactive effects of salinity and hypoxia, and it was randomly assigned to the whole plots. Within each whole plot, grass varieties were randomly assigned as sub-plots. The experimental data were analyzed by analysis of variance (SAS Institute 2010). Comparisons of salinity X hypoxia treatments were presented, and means were separated by LSD at 0.95 level of confidence for each turfgrass entry.

RESULTS AND DISCUSSION

Clipping Yield

Clipping yield is one indicator of turf vigor. All varieties exhibited decreased clipping yield with increasing salinity in both the salinity and hypoxia + salinity treatments except 'Fults' (Alkaligrass) (Table 3.1). No differences were found between treatments or among entries at

the 3 dS m⁻¹ salinity level (data are not shown). In 9 and 12 dS m⁻¹ salinity treatments, perennial ryegrass varieties showed significant differences compared to the control and to salt tolerant variety 'Fults' (Alkaligrass). As salinity increased from control to 6 dS m⁻¹, 'Brightstar', 'Paragon', 10.0815, and 10.0798 did not change in clipping yield compared to the control, while other perennial ryegrass varieties exhibited significant reduction. As salinity increased further, all varieties had significant reduction in clipping yield. Although 'Brightstar' had a high clipping yield at 6 dS m⁻¹, its clipping yield decreased to 2.0 mgcm⁻² as salinity increased to the highest level of 12 dS m⁻¹. Moreover, experimental line 10.0824 had the highest dry weight (3.5 mgcm⁻²) in the highest salinity level (12 dS m⁻¹) with hypoxia. The experimental line 10.0825 had the highest clipping yield (7.8 mgcm⁻²) among all entries under the hypoxia treatment; however, its clipping yield dropped to 1.7 mgcm⁻² under the highest level of salinity +hypoxia treatment. In addition, no differences were noticed between the control and the hypoxia treatment for 'the Brightstar' variety. However, its dry weight decreased by almost 60% compared to the control when salinity level reached 12 dS m⁻¹ and by 80% under hypoxia and salinity of 12dS m⁻¹.

'Fults' alkaligrass did not show a clear trend of clipping yield reduction as salinity increased from control to 12 dS m⁻¹ with or without hypoxia treatment. It was the most salt tolerant grass among all entries. Based on final clipping yield, 10.0825, 'Brightstar', 10.0824, and 'Top Hat', were more tolerant than other entries in the hypoxia treatment. 'Fults', 10.0824, 'Paragon' and 10.0825 were the most tolerant perennial ryegrass entries in the salinity treatment. In salinity +hypoxia treatment, 'Fults' and 10.0824 are most tolerant, but all the entries were harmfully affected.

Turf Quality

At the beginning, all treatments exhibited desirable turf quality (ranged from 8 to 9 on 0–9 scale, 9 = the best). Turf quality declined over time to unacceptable quality rating (below 6) with the highest salinity treatment (12 dS m^{-1}). In general, all entries had better turf quality in control and hypoxia treatments than in salinity or salinity with hypoxia treatments. Fig. 3.1 shows the effects of treatment on the turf quality of all varieties at 12 dS m^{-1} , the highest level. Clipping yield and turf quality have been recommended as excellent measurements to determine salinity tolerance among turfgrasses cultivars (Marcum and Pessaraki, 2010). At 12 dS m^{-1} salinity, 10.0798, 10.0824, 10.0825, 10.0815 and 'Tophat' had better turf quality than 'Palm III', 'Paragon', and 'Brightstar'. In 12 dS m^{-1} salinity +hypoxia treatment, 'Tophat', 'Brightstar', 10.0815, 10.0824, 10.0825, 10.0876 and 10.0798 had better turf quality than 'Fults', 'Palm III', and 'Paragon'.

Decreased clipping yield and lower turf quality may be due to reduction of energy that is needed for growth and the loss of turgor (Marcum, 2008). Decreased clipping yield and lower turf quality may be due to accumulation of Na^+ or Cl^- or both accumulations in the plant shoot to extreme levels beyond the ability of the cells to transfer these ions into the vacuoles. Ions rapidly build up in the cytoplasm and inhibit enzyme activity, or they build up in the cell walls and cause cells to dehydrate (Flowers and Yeo, 1986).

Leaf firing percentage increased as salinity increased for salinity and salinity +hypoxia treatments (Fig. 3.2). Leaf firing started to appear after salinity reached around 6 dS m^{-1} . Leaf firing increased as salinity increased in both the salinity treatment and the salinity + hypoxia treatment and reached the highest percentages when salinity reached 12 dS m^{-1} in most

varieties. Under salinity treatments, 'Palm III' had the highest leaf firing percentage of 44%, and 10.0798 had the lowest leaf firing percentage of 20%. Variety 'Brightstar' had the highest leaf firing percentage of 70 % under salinity + hypoxia treatment, and 'Fults' had the lowest leaf firing percentage of 37%. For all perennial ryegrass entries, leaf firing was more severe under the salinity + hypoxia treatment than the salinity alone treatment. Severe leaf firing for turfgrass under salinity + hypoxia treatment were reported by Zhang *et al.*(2013).

In our studies, based on data of growth parameters (clipping yield and turf quality), the salinity tolerance ranking of selected varieties from the most tolerant to less tolerant was 'Fults', 10.0824, 'Paragon', 10.0825, 10.0798, 10.0876, 'Brightstar', 'Top Hat', 10.0815, and 'Palm III'.

Although the selected cultivars had acceptable qualities under the hypoxia treatment, quality decreased as salinity increased under hypoxia and salinity treatment. Reduced soil oxygen availability under hypoxia conditions decreases turf quality, photosynthetic rate, and chlorophyll and carbohydrate concentrations of turfgrass (Jiang and Wang, 2006). Another study done in 2003 showed that after 28 days of waterlogging, photosynthesis of all experimental lines of perennial ryegrass was reduced by 30–50% (McFarlane *et al.* 2003). Furthermore, the combination of salinity and hypoxia has been shown to have greater effects on plant growth than either of them separately in many crops such as wheat, soybean, and legumes (Marcum, 2008; Zheng *et al.*, 2009; Alam, *et al.*, 2011).

Root Measurements

Root length was measured at the end of the experiment and significant differences were found among the treatments as well as among the varieties. All perennial ryegrass varieties had

an increase in root length over control under hypoxia treatments with the roots extending beyond the bottom of the containers (Table 3.2). 'Paragon' had an average length of 87 cm as the highest root length in the hypoxia treatment and all treatments, and by 197% increase comparing to the control. On the other hand, 'Fults' variety had the lowest root length with an average of 27.5 cm in hypoxia treatment. Increased root length under hypoxia treatment was an unexpected result because of the common inhibitor effects of hypoxia on root growth. Many papers found that hypoxia has a negative effect on root growth, and plants under hypoxia generally have shallow root systems. Banach et al. (2012) found that root length of *Plantago lanceolata* decreased by more than 60% under hypoxia treatment compared to the control.

Root length results under the hypoxia treatment may not reflect the hypoxia effects; they may be due to the water availability in hypoxia treatment. Control and salinity treatments were allowed to drain for almost 23 hours. In the hypoxia treatment, pots were submerged continuously, except for one hour daily. Control, salinity and salinity +hypoxia treatments showed no differences in root length. However, a study published in 2013 by Zhang and others supported our results and reported that hypoxia increased root length under hypoxia treatment by 20%, compared to the control. In addition, another study found that some perennial ryegrass cultivars were able to maintain high relative root growth under waterlogging compared to the control (McFarlane *et al.*, 2003). 'Fults' under hypoxia had the same condition as the perennial ryegrass varieties, but their roots were less than the control. Although all perennial ryegrass varieties had increased root length under hypoxia treatment, significant differences were found among the varieties. No other varietal differences were found among the other treatments.

Adventitious roots were noticed on all perennial ryegrass varieties in the hypoxia treatment by the end of the experiment (Table 3.2). They were white and thin on the soil surface (visual observation). No measurement was done, but adventitious roots were only in perennial ryegrass varieties, not in alkaligrass under hypoxia treatment. Developing adventitious roots is a way for some plants to overcome the low oxygen availability in their soil in waterlogging situation. Waterlogging tolerant plants usually are able to develop adventitious roots. Plant hormones such as ethylene induced this formation under waterlogging conditions (Visser *et al.*, 1996). In a study about barley, waterlogging tolerant varieties had developed more adventitious roots more rapidly than the other treatments (Zhang *et al.*, 2015).

No adventitious roots were observed in the waterlogging + salinity treatment; although, the water availability was the same as in the waterlogging treatment. The results clearly showed that perennial ryegrass cultivars and lines were not able to develop adventitious roots under the combination of salinity and waterlogging. The reason for that could be due to the high environmental stress that the grass experienced under waterlogging + salinity conditions. In a study on Eucalyptus, scientists found that the ability of plants to produce adventitious roots may be inhibited under the combined conditions of salinity and waterlogging. Salt exclusion is an energetically expensive process that may severely block the ability of this species to produce adventitious roots (Karschon and Zohar, 1975).

Aerenchyma Formation

The percentage of air space (Aerenchyma) in root cross-sections of perennial ryegrass varieties in the hypoxia treatment was greater than the control and salinity treatments (Table 3.2). No digital images processed for salinity + waterlogging treatments due to the root damage

at the end of the experiment (no clear images to convert through the software) (Table 3.2). Aerenchyma formation under waterlogging treatment was reported by Vasellati *et al.* (2001) who found that hypoxic conditions enhanced the formation of aerenchyma in flooded roots. In turfgrass studies, researchers found that low soil oxygen induces aerenchyma formation in many turfgrass species (Jiang and Wang, 2006). Aerenchyma can function as internal pathways for the transfer of gases between above water plant parts and submerged plant parts (Laan et al., 1989). Aerenchyma formation is one of the mechanisms that waterlogged plants use to overcome hypoxia in the root environment (Evans, 2003).

Ion Concentrations

Sodium concentrations in shoots were increased under salinity and salinity + waterlogging treatments compared to the control in most of the varieties (Fig. 3.3). 'Paragon' had the lowest Na concentrations under the salinity and salinity+ waterlogging treatments (32.9, 39.9 mg/gdw respectively). However, 10.0825 showed high concentration of Na under the salinity treatment only (79.3 mg/gdw) and lower Na concentration under the salinity+ waterlogging treatment (37.5 mg/gdw). All entries except 10.0825 and 'Paragon' had higher Na concentrations in shoots under the salinity + hypoxia treatment than salinity treatment alone. In general, the ability of plant cells to maintain low Na⁺ concentration is associated with plants salinity tolerance (Blumwald, 2000). However, in our studies, no relationship was found between the Na⁺ concentration and salinity tolerance. The reason for that could be due to the analysis method that we used which calculates the amount of Na in the shoots including the vacuoles. In addition, our results indicated that salinity damage became more severe under waterlogging conditions. The declining ability to tolerate salinity under waterlogging conditions

may be associated with the effects of waterlogging on plant energy (ATP) availability. It is well known that waterlogging creates hypoxia that can trigger anaerobic respiration and reduce the production of ATP. Many salinity tolerance mechanisms, including toxic ion exclusion may require considerable energy investment and are energy dependent. Many salinity studies suggested that plant Na^+ increases with salinity (Marcum, 1999; Qian *et al.*, 2001; and Alshammary *et al.*, 2004). Increased plant Na^+ concentration under salinity + waterlogging has been indicated in other studies (Barrett-Lennard, 2003).

Shoot Cl^- concentration increased by 8-16 times under salinity and salinity + waterlogging treatments compared to non-saline treatments (Fig. 3.4). 'Fults', 'Paragon', 'Tophat', and 10.876 had similar Cl^- concentrations (4-4.6 ppm) under the salinity treatments. Chloride concentrations increased more under salinity + waterlogging treatments in all varieties except 'Palm III' and 'Brightstar'. Increased concentrations of Na^+ and Cl^- in the shoots under waterlogging with salinity together could be due to less amount of energy that plant were able to produce under anaerobic conditions. Negative effects on plant growth can occur with increases in these two elements in the shoots. In addition, waterlogging can cause the buildup of anaerobic metabolism production resulting in ethylene, carbon dioxide, and ethanol which are very harmful to plant growth. Additionally, the combined effect of salinity + waterlogging significantly reduced growth and increased the accumulation of these ions.

For all varieties, K concentrations in shoots were higher under the waterlogging treatment than under the salinity treatment (Fig.3. 5). Variety 10.0824 had the highest K concentration (328 mg/gdw) under waterlogging treatment, and the other varieties showed no significant differences under the same treatment. Varieties 10.0825 and 'Paragon' had the

highest K concentration (64 and 57 mg/gdw respectively) under control treatment, while 10.0815 had the lowest concentration (25 mg/gdw). No differences were found among other varieties under control treatment. In all cultivars and experimental lines, there was a clear pattern under salinity and waterlogging conditions, i.e. a significant increase in Na and Cl and a decrease in shoot K. This result was similar to the results reported for several other species (Barrett- Lennard, 1986; Rogers and West, 1993).

In our study, a significant increase in plant Na⁺ concentration was observed in most grasses when grown under combined salinity and waterlogging stress compared to plants grown under salinity only treatment. Lower K⁺/Na⁺ ratio were recorded for most varieties under the salinity and salinity +hypoxia treatments (Fig. 3.6). The intracellular K⁺/Na⁺ ratio is often used as a key determinant of plant salinity tolerance (Shabala and Cuin, 2007). Salt tolerant plants have high K⁺/Na⁺ ratio because of plasma membrane antiporters' ability to eject Na⁺ from the cytosol while holding K⁺ in the cytosol (Shi *et al.*, 2002). However, this process requires energy, and under hypoxia + salinity, roots use anaerobic respiration and produce less ATP and that affect this process negatively and decreases this ratio.

CONCLUSION

In summary, perennial ryegrass entries showed some differences in salinity and hypoxia tolerance. However, no differences were found among cultivars and treatments in the lowest salinity level (3 dS m⁻¹). Grass dry weight and quality declined as salinity increased in both salinity and salinity +waterlogging treatments. All grasses were more severely affected under the salinity and waterlogging treatment together compared to salinity or waterlogging only.

However, some perennial ryegrass varieties (10.0824, 10.0825, and 10.0815) were able to maintain acceptable quality under the combination of hypoxia and moderate salinity levels (6-10 dS m⁻¹). Although alkaligrass had better salinity tolerance, perennial ryegrass had better waterlogging tolerance than alkaligrass. Perennial ryegrass varieties under waterlogging treatment were able to maintain similar quality to control. They were also able to produce adventitious roots and had more aerenchyma tissue in order to perform well under hypoxic conditions. Under the combination of salinity and waterlogging plant ion concentrations such as Na and Cl were increased in all lines, while K concentration was decreased under the same conditions. Hypoxia increased K concentration in shoots. Grasses under the combination of waterlogging and salinity were negatively affected; alkaligrass has high salinity tolerance and perennial ryegrass has moderate salinity tolerance, but their tolerance decreased to variable degree when salinity combined with waterlogging. Further research with different levels of waterlogging is recommended to examine the ability of perennial ryegrass to tolerate hypoxia.

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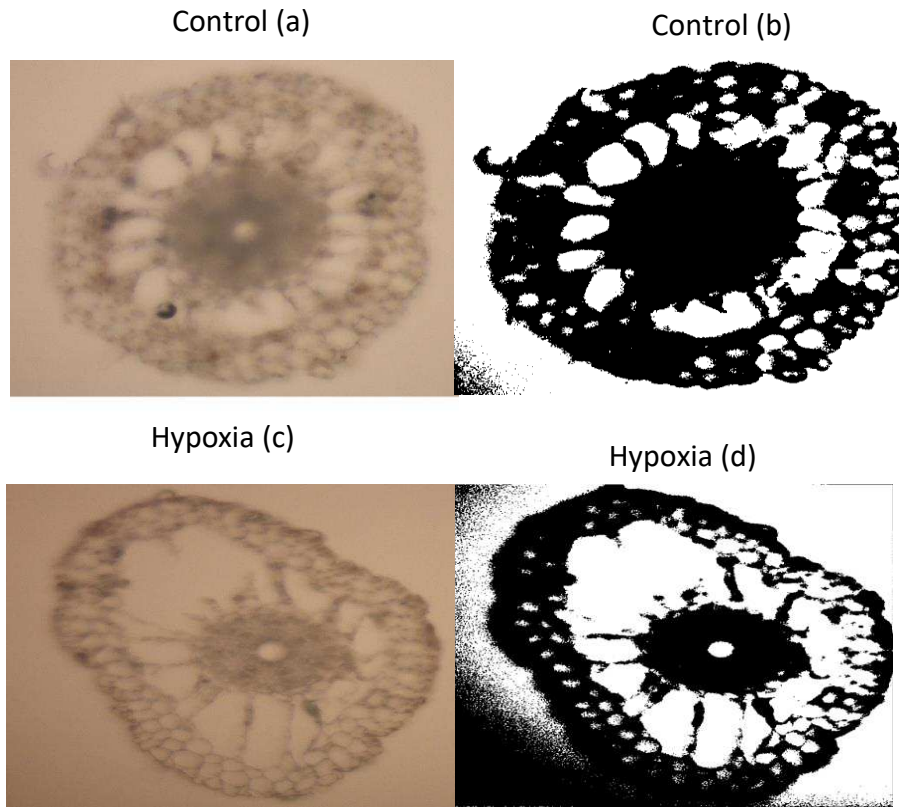


Photo 3. 1. Representative digital images of root aerenchyma formation. (a) normal digital image of *Perennial ryegrass* 'Paragon' root cross-section (control treatment; (b) the same image as (a) converted to black and white; (c) normal digital image of *Perennial ryegrass* 'Paragon' root cross-section (Hypoxia treatment; (d) the same image as (c) converted to black and white.

Table 3.1. Effect of salinity and hypoxia on the final clipping yield (mg/cm²) of perennial ryegrass and alkali grass cultivars after a month from reaching target salinity level.

Treatments								
Grasses	Control	Salinity			Hypoxia	Salinity +Hypoxia		
		<u>6 dS m⁻¹</u>	<u>9 dS m⁻¹</u>	<u>12 dS m⁻¹</u>		<u>6 dS m⁻¹</u>	<u>9 dS m⁻¹</u>	<u>12 dS m⁻¹</u>
Top Hat	5.6aA	4.8abB	3.40bC	2.0bcEF	4.4bB	3.6abyC	2.1dDE	1.6dFG
Palm III	4.8abA	4.4abcB	3.0bC	1.6cdD	3.6bC	3.6bC	2.3cdCDE	2.0bDE
Fults	3.9bB	3.7cdB	3.6bC	5.7aA	3.0cC	3.5aC	3.3abC	3.0aC
Brightstar	5.0abA	5.3aA	2.5bC	2.0cD	4.7 bA	3.9bcB	2.4cdC	1.0eE
Paragon	4.7abA	4.9abA	2.6bC	2.7bC	3.4bcB	1.5dD	1.9dCD	1.9bCD
10.0815	4.7abA	4.8abA	2.6bB	2.0cC	3.5bB	2.9bcB	2.8bcB	2.0bC
10.0824	4.6abA	3.8cdB	3.5aB	3.0bC	4.4bA	3.3aB	3.5abB	2.7aC
10.0825	4.6abB	4.1bcdC	3.6aD	2.5bF	7.8aA	3.4abD	3.0bcDE	1.7cdG
10.0876	5.0abB	3.7dC	3.2aC	2.0cD	3.6bB	3.8aB	2.3cdD	1.9cdD
10.0798	4.9abA	4.2bcdAB	3.8aB	2.0cD	3.0cC	3.9aB	2.0dD	0.7eE

Lowercase letters indicate mean separation among different grasses within individual treatments at $P = 0.05$.

Uppercase letters indicate mean separation among treatment for a given grass at $P = 0.05$.

Table 3.2. Root observations and measurements under the effect of salinity and hypoxia levels.

Varieties	Treatments			
	Control	Salinity	Hypoxia	Salinity + Hypoxia
Brightstar	Root length 29cm aA Aerenchyma present No Adventitious root present No	Root length 23.8cm aA Aerenchyma present No Adventitious root present No	Root length 65cm bC Aerenchyma present Yes Adventitious root present Yes	Root length 24.3cm aA Aerenchyma present NA Adventitious root present No
Fults	Root length 29.5cm aA Aerenchyma present No Adventitious root present No	Root length 23.5cm aA Aerenchyma present No Adventitious root present No	Root length 27.5cm aG Aerenchyma present No Adventitious root present No	Root length 22.8 aA Aerenchyma present NA Adventitious root present No
Palm III	Root length 29.3cm aA Aerenchyma present No Adventitious root present No	Root length 23.8cm aA Aerenchyma present No Adventitious root present No	Root length 63.5cm bC Aerenchyma present Yes Adventitious root present Yes	Root length 24cm aA Aerenchyma present NA Adventitious root present No
Paragon	Root length 29.3cm aA Aerenchyma present No Adventitious root present No	Root length 24cm aA Aerenchyma present No Adventitious root present No	Root length 87cm bA Aerenchyma present Yes Adventitious root present Yes	Root length 24cm aA Aerenchyma present NA Adventitious root present No
Tophat	Root length 30cm aA Aerenchyma present No Adventitious root present No	Root length 25cm aA Aerenchyma present No Adventitious root present No	Root length 51.5cm bDE Aerenchyma present Yes Adventitious root present Yes	Root length 23.8cm aA Aerenchyma present NA Adventitious root present No
10.0815	Root length 28.8cm aA Aerenchyma present No Adventitious root present No	Root length 24cm aA Aerenchyma present No Adventitious root present No	Root length 56.3cm bD Aerenchyma present Yes Adventitious root present Yes	Root length 23.5cm aA Aerenchyma present NA Adventitious root present No

10.0824	Root length 29cm aA Aerenchyma present No Adventitious root present No	Root length 23.3cm aA Aerenchyma present No Adventitious root present No	Root length 62.3cm bC Aerenchyma present Yes Adventitious root present Yes	Root length 24cm aA Aerenchyma present NA Adventitious root present No
10.0825	Root length 29.8cm aA Aerenchyma present No Adventitious root present No	Root length 23.3cm aA Aerenchyma present No Adventitious root present No	Root length 46.8cm bF Aerenchyma present Yes Adventitious root present Yes	Root length 23.3cm aA Aerenchyma present NA Adventitious root present No
10.0876	Root length 29cm aA Aerenchyma present No Adventitious root present No	Root length 23.8cm aA Aerenchyma present No Adventitious root present No	Root length 75cm bB Aerenchyma present Yes Adventitious root present Yes	Root length 23.5cm aA Aerenchyma present NA Adventitious root present No
10.0798	Root length 28.8cm aA Aerenchyma present No Adventitious root present No	Root length 23.8cm aA Aerenchyma present No Adventitious root present No	Root length 45.3 bF Aerenchyma present Yes Adventitious root present Yes	Root length 24cm a Aerenchyma present NA Adventitious root present No

Lowercase letters indicate significant differences ($p=0.05$) among treatments

Uppercase letters indicate significant differences ($p=0.05$) among varieties

N/A no slides were made for this treatment due to the root damage.

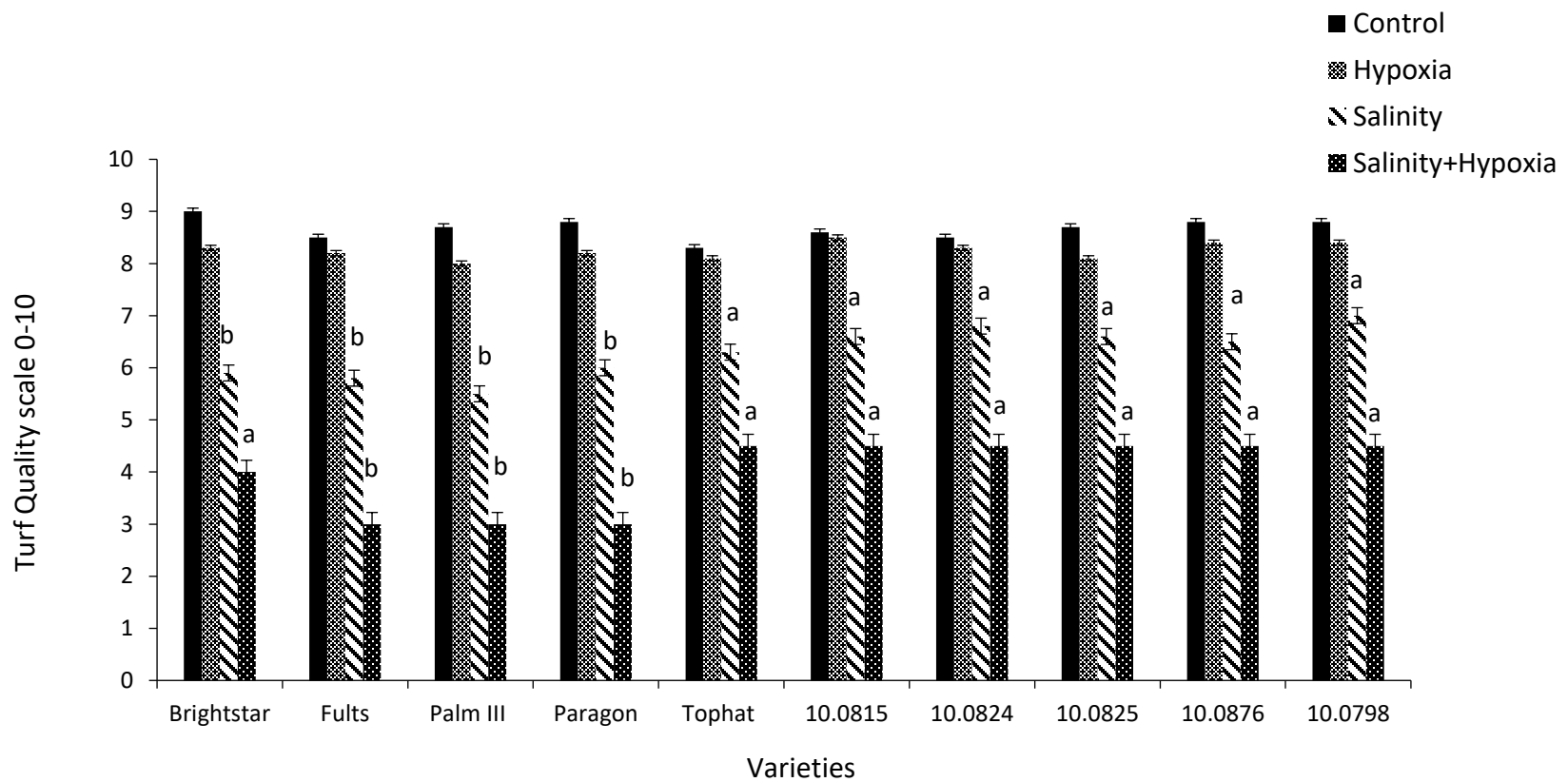


Figure 3.1. The effects of salt and hypoxia on turf quality for all perennial ryegrass and alkaligrass varieties at level 12 dS m⁻¹ after one month at the target salinity level.

Varieties with same lower case letter in the same treatment are not significantly different (P<0.05).

There were no significant varietal differences between the control and hypoxia treatments

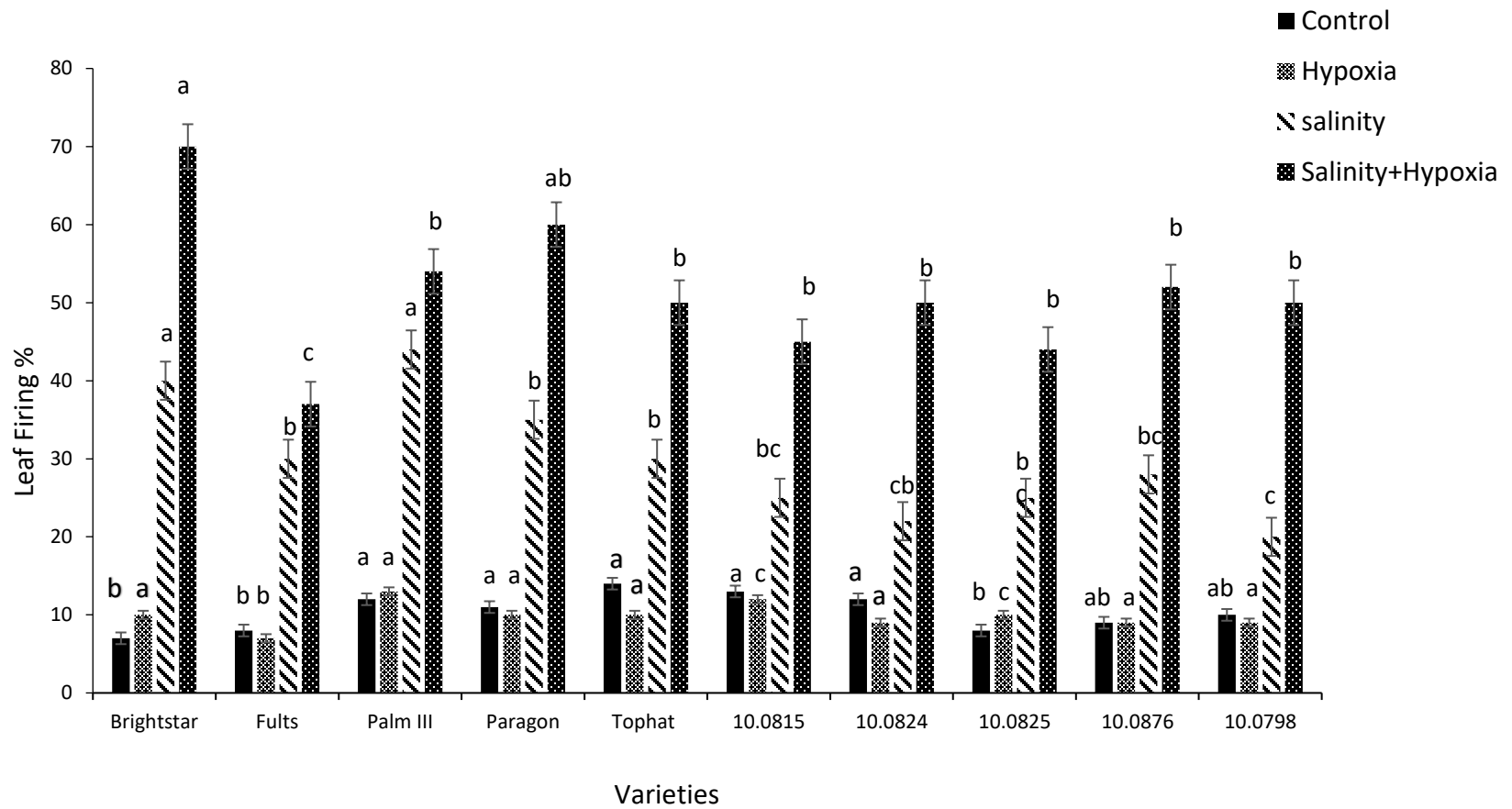


Figure 3.2. The effects of salt and hypoxia on leaf firing percentage(%) for all perennial ryegrass and alkaligrass varieties at level 12 dS m⁻¹ after one month at the target salinity level.

Varieties with same lower case letter in the same treatment are not significantly different ($P < 0.05$).

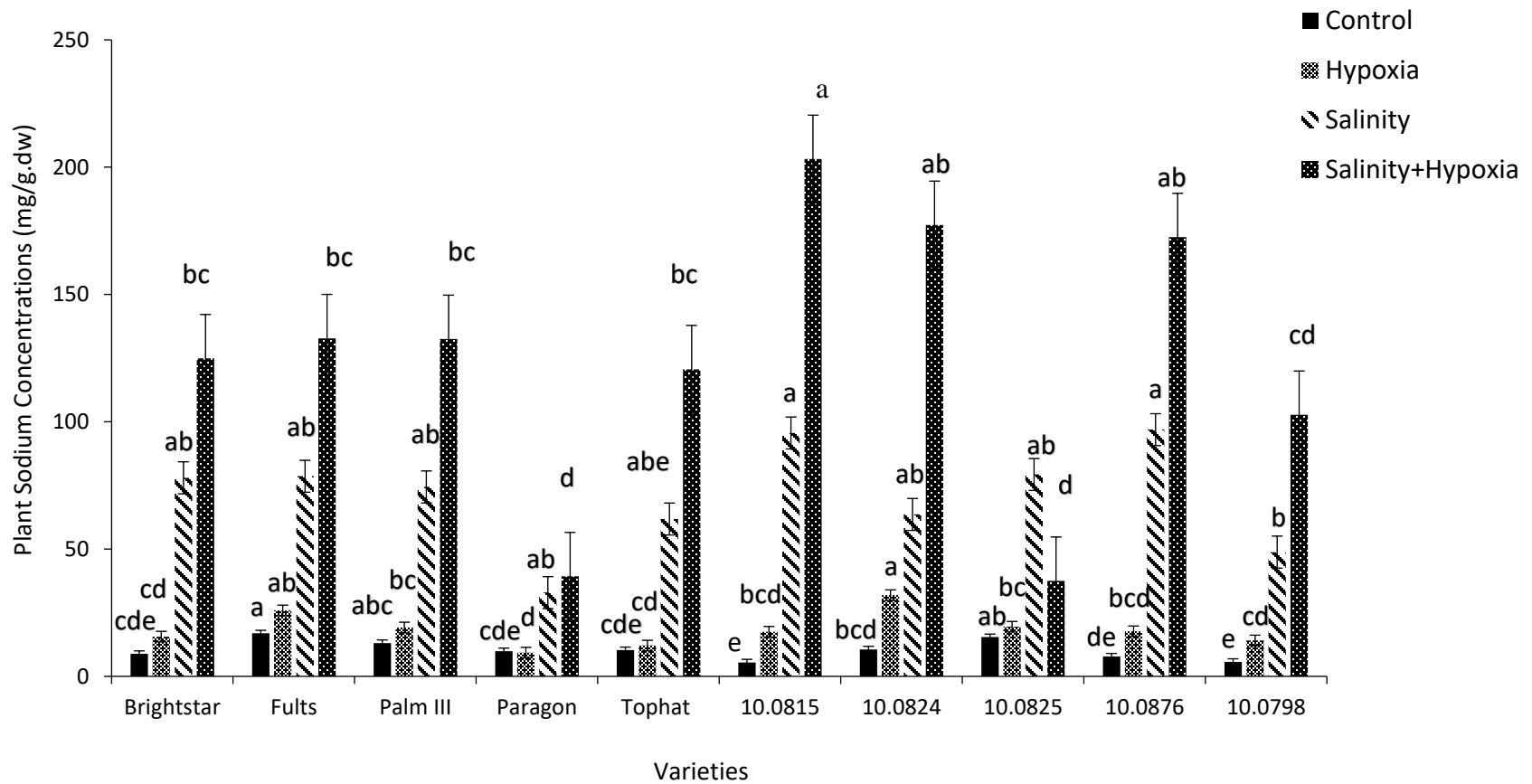


Figure 3.3. The effects of salt and hypoxia on Na concentrations for all perennial ryegrass and alkaligrass varieties at level 12 dS m⁻¹ after month at the target salinity level.

Varieties with same lower case letter in the same treatment are not significantly different (P<0.05).

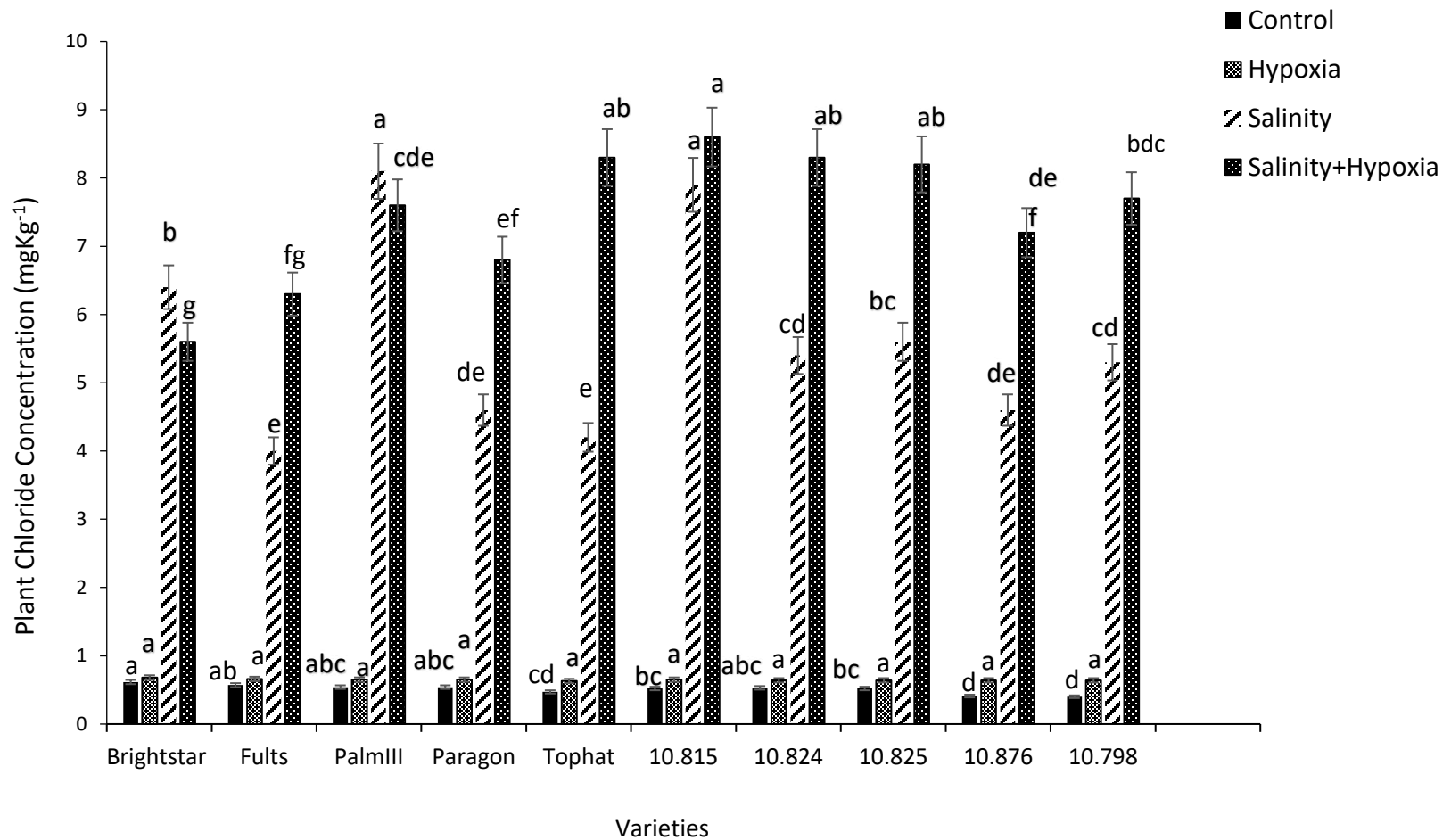


Figure 3.4. The effects of salt and hypoxia on Cl concentration for all perennial ryegrass and alkaligrass varieties at level 12 dS m⁻¹ after month at the target salinity level.

Varieties with same lower case letter in the same treatment are not significantly different (P<0.05).

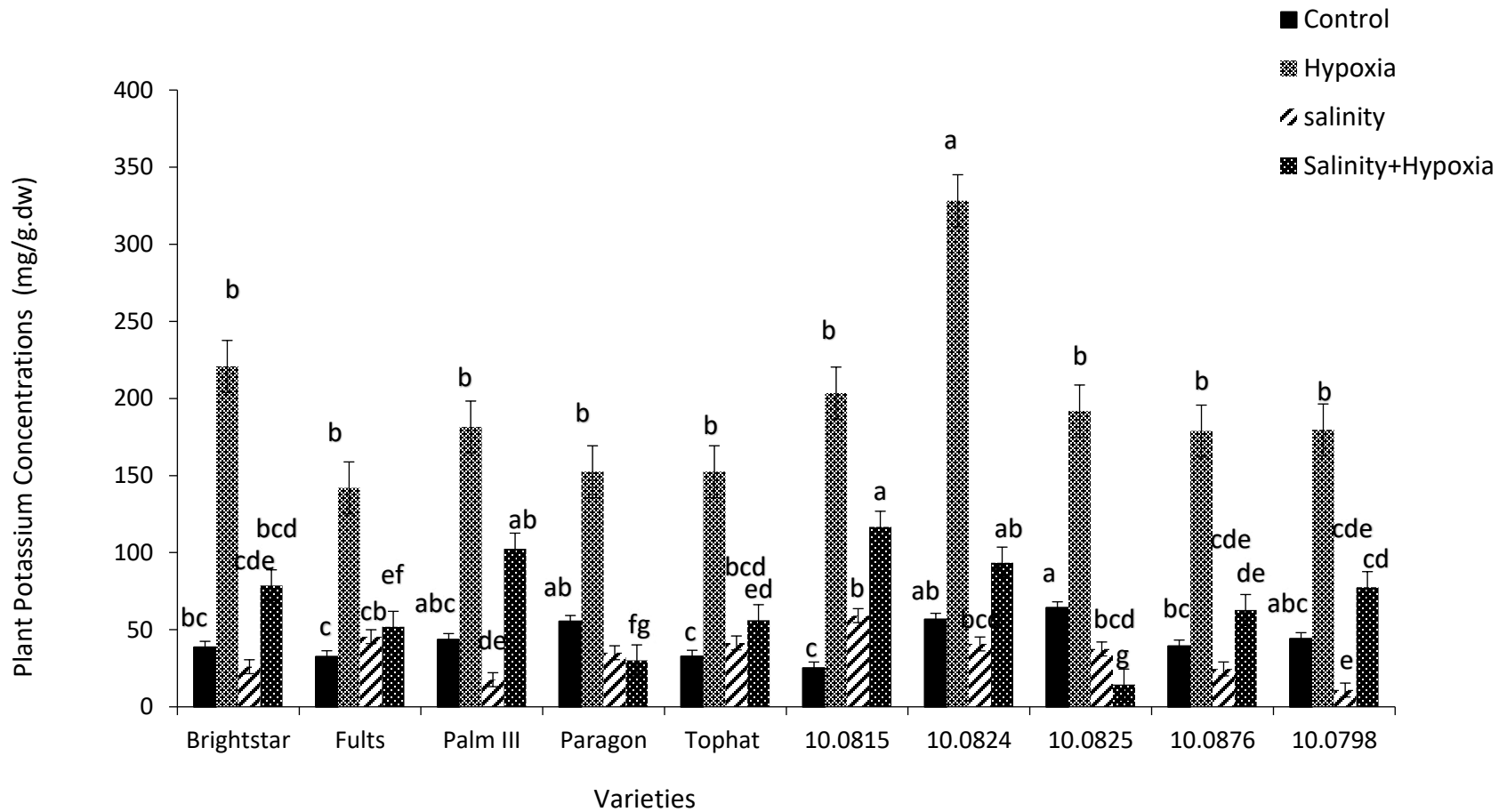


Figure 3.5. The effects of salt and hypoxia on K concentrations for all perennial ryegrass and alkaligrass varieties at level 12 dS m⁻¹ after month at the target salinity level.

Varieties with same lower case letter in the same treatment are not significantly different (P<0.05).

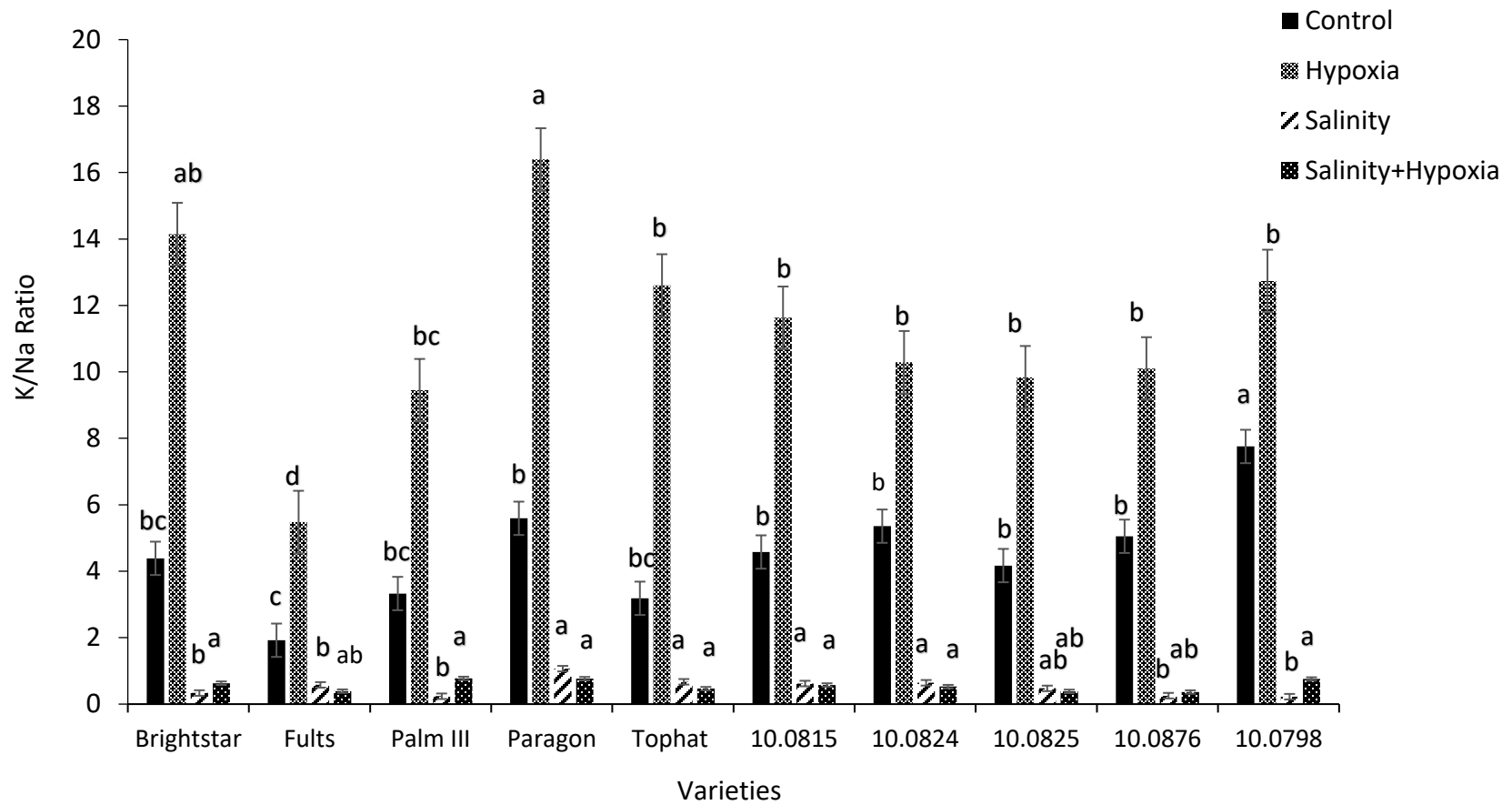


Figure 3.6. The effects of salt and hypoxia on K/Na ratio for all perennial ryegrass and alkaligrass varieties at level 12 dS m⁻¹ after month at the salinity level (P<0.05).

Varieties with same lower case letter in the same treatment are not significantly different

CHAPTER 4

SALINITY EFFECTS ON GERMINATION AND PLANT GROWTH OF SWITCHGRASS

CONCEPTUAL

Recently, biofuel has become one of the potential solutions to rising oil costs, a growing world population, and environmental pollution. Reducing fossil fuel usage and exploring for renewable clean energy sources has become a major concern for scientists. Switchgrass is a major biofuel grass in North America because of its ability to grow in poor soil. Our study was conducted in 2011 and repeated in 2013 in Colorado State University's Plant Growth Facility. Two switchgrass cultivars, 'Blackwell' and 'Trailblazer', were selected. Seventy grass seeds were planted in forty 7.5 L pots containing sand mixed with 20% organic matter. Four water salinity levels ranging from 7 to 24 dS m⁻¹ were applied, and the control received no salt. Germination rate reduced with increased salinity level. Germination rate decreased from 100% to 60% as salinity increased from control to 16 dS m⁻¹ in the first experiment, but in the second experiment, germination rate decreased from 100% to 30% as the salinity level increased from control to 14.8 dS m⁻¹. In both cultivars and experiments the salinity in the range of 10-15 dS m⁻¹ caused 50% aboveground biomass reduction. The final harvest (aboveground biomass) was reduced by 33- 40% as soil salinity increased from control to 7-9 dS m⁻¹. Root biomass decreased as soil salinity reached to 7 and 9.5 dS m⁻¹ in the first and the second experiment, respectively. No differences were found between the two cultivars except in root biomass. Although switchgrass has a moderate level of salinity tolerance, our results suggest that biofuel

crops with greater salinity tolerance are needed for biomass production under saline soil conditions.

INTRODUCTION

Many croplands become salt-affected due to changes in climate, dryness and erosion. Saline soils are considered to be a major factor in reduced crop production. Salinity affects plant growth by making it difficult for the plant to get water from the soil, and can lead to imbalance of plant nutrients and excessive accumulation of toxic levels of some ions such as Na, Cl, and B in plant cells (Munns, 2002). Most crops are sensitive to salinity, but there is a wide range of salt tolerance in plants. Using salt tolerant crops is an alternative to make good use of saline lands because of their ability to survive and grow in saline soil.

Reducing fossil fuel usage and looking for renewable clean energy sources has become a major concern for scientists. In 1993, the World Energy Council (WEC) indicated that in order to slow global warming, energy would have to come from renewable, clean sources. Accordingly, biofuel crop production has become very popular. The use of biofuel has several benefits, which include clean energy, soil stabilization, and employment opportunities (Bansal et al., 2013). Biofuel crops are typically grown on land not used for food crops. Accordingly, biofuel producers focus on using crops that can give a good yield under non- favorable conditions such as dryness and salinity.

Biofuel is typically produced from grain crops (corn) in USA more than cellulosic feedstocks (grass) (Goldemberg, 2007). In contrast, grasses produce more biofuel than corn and require less energy to process. Right now, the main sources of biofuels are grain and sugar

crops, and they produce more than 64 billion liters of ethanol for transportation use (Renewable Fuels Association, 2013). In biofuel production, plants cellulose is converted to ethanol. Ethanol decreases air pollution and harmful gas emissions, because its emissions contain oxygen, which is good for the environment. Carbon monoxide emissions are also reduced (McLaughlin and Walsh, 1998). Carbon monoxide gas is the reason for 20% of smog formation and human respiratory illnesses such as asthma.

Switchgrass was selected as a bioenergy crop model in the U.S. because it is a native grass, produces high above ground yield biomass ($20 \text{ Mg ha}^{-1} \text{ yr}^{-1}$), and can grow in a wide range of areas in North America, in addition to using less fertilizer. It prefers medium fertility, well-drained soil. Switchgrass is not an invasive plant, although it is a strong competitor. It is also known for its tolerance to cold and resistance to disease and insects.

Switchgrass is perennial, which decreases fuel costs related to planting and tillage. Switchgrass can have a productive life of 10-20 years. In addition, it is easily reproduced by seeds, whereas other biofuel crops are vegetative only, and require higher establishment costs.

Generally, seed germination and crop yields decrease with increasing salinity, but the effects of salt stress on switchgrass have not been well studied (Monti, 2012). Most of the available studies have been focused on germination and seedling growth, but not on mature plants. Greub et al. (1985) indicated that switchgrass aboveground biomass that was treated with a 2.65 M NaCl solution for 5 weeks was reduced by almost 70% compared to control plants (Greub *et al*, 1983). Another study found that switchgrass seedlings cannot tolerate soil salinity levels of 14.9 dS m^{-1} or water salinity level of 8 dS m^{-1} (Dkhili and Anderson, 1990).

The objective of this study was to evaluate the feasibility of growing switchgrass under saline water for irrigation.

MATERIALS AND METHODS

Plant materials and growth conditions

The study was conducted twice in May 2011 and March 2013 at Colorado State University's Plant Growth Facility to achieve the study objectives. Two switchgrass cultivars, 'Blackwell' and 'Trailblazer', were selected. Seventy grass seeds per pot were planted in forty 7.5 L pots containing sand mixed with 20% organic matter. Pots were covered with Seed Guard fabric (DeWitt Co.) to decrease evaporation. Cultivars were arranged in a completely randomized design. The greenhouse temperature was maintained between 26 °C day / 23 night. Four salinity concentrations (4, 8, 12, and 14 dS m⁻¹) were used, and one control treatment received no salt (tap water only). Treatments were replicated four times. Treatments were applied daily to keep the seeds moist. Instant ocean salt (Aquarium Systems, Mentor, Ohio) was used (55% Na, 31% Cl, 8% S, 4%Mg, and 1% K), and water salinity was monitored with conductivity meter. Salinity leachate was measured every two weeks using a conductivity meter.

Data collection

Germinated seeds were counted 3 times in day 14, 28. 42. Relative seed germination was calculated using the following equation:

$$\text{Relative seed germination (\%)} = \frac{\text{number of germinated seeds in each salinity treatment}}{\text{number of germinated seeds in the control}}$$

After 45 days, the first aboveground dry weights were measured by cutting switchgrass plants and leaving only three similar plants (similar height and number of leaves) in each pot to

the end of the experiment. Cut switchgrass plants were dried at 60 °C for 24 hrs to determine the dry weight. By the end of the experiment, final above ground dry weights and root dry weights were measured. Soil salinity was determined at the end of the experiment by using 1:1 ratio, 50 g of soil with 50 mL irrigation water was added. Water was drained from the mixture using filter paper, and salinity was determined using a conductivity meter.

The experiment was conducted twice following the same protocol; however, due to some irrigation management errors, the second experiment experienced some drought stress. To overcome this error, leachate salinity values were used for data analysis and graphic presentation, instead of target EC levels that were mentioned earlier in this section. Data from the two study periods (before and after day 42) were analyzed separately. The first time period was during the seed germination which was from the beginning of the experiment to almost 6 weeks after seeding. The second time was from the sixth week of the experiment to the end of experiment. The average of four leachate values was calculated to get the new treatment values in each experimental period of time.

Experimental design and statistical analysis

A completely randomized split plot design was used with four replications. The main factor was the effect of salinity, and it was randomly assigned to be whole plots. Within each whole plot, switchgrass varieties were randomly assigned as sub-plots. The experimental data were analyzed by analysis of variance (SAS Institute, 2010). Because there was significant interaction between the two varieties and salinity levels, comparisons of salinity treatments within the two varieties were presented, and means were separated by LSD at 0.95 level of confidence.

RESULTS AND DISCUSSION

Relative Germination Rate

Germination rate was significantly reduced by salinity in both cultivars ($P < 0.001$). However, the germination rate was not significantly different between the two cultivars (Fig.4.1 and 4.2). In the first experiment, germination decreased from 100% to 60% as salinity was increased from control to 16.4 dS m⁻¹ (Fig.4.3), although no significant differences were recorded between the control and the first two salinity treatments (7.3, 12.3 dS m⁻¹), the high salinity treatments (16.4, 20 dS m⁻¹) showed significant reduction in germination rate for both cultivars. In the second experiment, as soil salinity increased from control to 14.8 dS m⁻¹, germination rate decreased to 30% in both cultivars (Fig.4.4). Analysis of variance showed significant differences among treatments but not the cultivars. Both cultivars took 3-4 weeks to reach the highest germination rate under control treatment, and salinity decreased the germination rate. No additional germination was recorded in either cultivar after 4 weeks in most of the treatments. Researchers have suggested that soil salinity decreases seed germination by causing water deficiency and ion toxicity (Marcum, 1999). Salinity reduces imbibition by seeds and inhibits the germination process. Low osmotic potential caused by high salt concentration inhibits imbibition which is a very important process that activate the hydrolytic enzymes responsible for the degradation of the stored food in the seed (Almansouri et al., 2001). A study using switchgrass cultivar 'Cave-in-Rock' found that its seeds germinated better at low salt concentrations (≤ 8 dS m⁻¹), and no germination was recorded at higher salt concentrations (≥ 15 dS m⁻¹) (Sautter, 1962). Another study using the same cultivar ('Cave-in-Rock') found that seed germination was reduced by 80% at 27 dS m⁻¹ (Kim et al., 2012).

The effect of salinity on germination rate was more severe in the second experiment, due to the greater salt accumulation as described below. Therefore, the grass experienced higher salinity level. In the first experiment, as salinity reached 20.3 dS m^{-1} , germination percentage for both cultivars reached at least 60%; however in the second experiment, germination percentage in both cultivars was at least 20% under 19.2 dS m^{-1} .

Soil salinity (leachate)

In both experiments soil salinity level (as measurement of salinity of leachate) increased as the leachate continued reaching $20\text{-}24 \text{ dS m}^{-1}$ in the highest treatment. This increase was due to the salinity build up over time. Leachate salinity values were significantly higher in the second experiment than the first ($P < 0.05$). Leachate values were used instead of irrigation water salinity to determine the real salt condition that the plants experienced. A soil salinity test was run by the end of the experiment using saline irrigation water, and we found that the salinity level by the end of the experiment was very similar to the leachate levels. Leachate values were considered to be equivalent to the soil salinity. An average of four leachate values was calculated to get the soil salinity values for each of the time periods.

First Aboveground Biomass

In the first experiment, biomass decreased as the salinity levels increased for both cultivars (Fig. 4.5). At the highest salinity treatment (20.7 dS m^{-1}), 'Trailblazer's aboveground biomass decreased by 93% and 'Blackwell' by 70% when compared to the control (2.9 dS m^{-1}). 'Trailblazer's aboveground biomass was 36% lower than that of 'Blackwell' under 16.6 dS m^{-1} salinity.

Like the first experiment, plant aboveground biomass was decreased with increasing salinity level; however, both cultivars were more negatively affected at high salinity levels (15 dS m^{-1}) than in the first experiment (Fig. 4.6). No significant growth was observed for either cultivar at the highest salinity level (24.8 dS m^{-1}). However, 'Trailblazer' was significantly affected more by salinity at the other salinity levels. 'Trailblazer's aboveground biomass was 86.6 % lower than 'Blackwell' at 9.2 dS m^{-1} salinity level. A study done on switchgrass seedling salinity tolerance suggested that switchgrass biomass declined by 50 % at $17\text{-}18 \text{ dS m}^{-1}$ (Fan et al., 2012). However, our first harvest results suggested that the biomass for both experiments declined more than the reported range by Fan *et al* (2012). In the first experiment, 50% biomass reduction happened at 8 dS m^{-1} for 'Blackwell' and 12 dS m^{-1} in 'Trailblazer'. In the second experiment, 50 % biomass reduction occurred at the salinity it was $10\text{-}11 \text{ dS m}^{-1}$ for 'Blackwell' cultivar and 8 dS m^{-1} for 'Trailblazer'. Our results are in disagreement with a study done in 1990 which suggested no seedling growth was recorded under soil salinity level higher than 14.9 dS m^{-1} (Dkhili and Anderson, 1990).

Second Aboveground Biomass

Three similar plants were allowed to grow until the end of the experiment after the first harvest period. In experiment I, aboveground biomass from the 2nd harvest of both grasses decreased as salinity increased, but no significant differences were recorded between the two cultivars (Fig. 4.7). When soil salinity reached 20.5 dS m^{-1} , 'both cultivars final aboveground biomass significantly declined by at least 70% when compared to the control. In the second experiment, no significant differences were found between the two grasses. However, significant differences were recorded between the control and those receiving salinity

treatments (Fig.4.8). Aboveground biomass at the 2nd harvest significantly dropped by almost 60% at the highest salinity level for both cultivars.

Root Mass

In the first experiment, 'Blackwell's root mass was significantly increased by almost 51% in the first salinity treatment (7.7 dS m⁻¹) (Fig.4.9). In contrast, 'Blackwell's root mass significantly decreased by almost 8% at 7.7 dS m⁻¹. 'Trailblazer's root mass only significantly decreased at the highest salinity treatment (20.5 dS m⁻¹) by almost 78%.

In the second experiment, root mass was different among treatments and cultivars. 'Blackwell's root mass significantly decreased as salinity increased, dropping to the lowest values at the higher salinity levels (21.1, 24.8 dS m⁻¹) (Fig.4.10). In contrast 'Blackwell's root mass was significantly higher in the control and the first salinity treatment (9.2 dS m⁻¹) compared to 'Trailblazer's root mass. 'Trailblazer's root mass was only significantly affected at the highest salinity level (24.8 dS m⁻¹). 'Trailblazer's root mass decreased significantly by almost 79% in the highest salinity level compared to the control. These results are in agreement with other studies that found that root growth is less sensitive to salinity and it may increase with salinity if supplemental CaCl₂ is added to the salt (Cramer et al., 1990; Yermiyahu et al., 1997). Our results were in agreement with many papers that suggested that switchgrass biomass production, seed germination rate, plant height, and fresh weight, decreased under saline conditions (Liu et al., 2014; Kim et al., 2012; Dkhili and Anderson, 1990). Although the two cultivars were able to maintain growth at relatively high salinity levels 12-16 dS m⁻¹, their biomass declined by 50% and identification of better biofuel crops may be needed for more biomass production under higher saline conditions.

CONCLUSIONS

In this study, we examined the differences in salinity tolerance between 'Blackwell' and 'Trailblazer' switchgrass cultivars, as quantified by seed germination, aboveground biomass, and root biomass. Our results suggested that salinity had negative impacts on the germination, aboveground biomass, and root biomass of the two switchgrass cultivars that were tested. No differences were observed between the two cultivars except in the first aboveground biomass in second experimented root mass in both experiments. In all treatments and cultivars, germination rate and salinity levels were inversely related. In the first experiment, the germination decreased from 100% to 60% as the salinity increased to 16 dS m^{-1} . In the second experiment, as soil leaching increased to 14.8 dS m^{-1} , germination rate decreased to from 100% to 30%.

Switchgrass cultivars took 3-4 weeks to reach the highest germination rate under control treatment. Although our results showed that salinity decreased aboveground biomass, and root mass, switchgrass was able to maintain growth under moderate levels of salinity (9 dS m^{-1}). More research needs to be done under field conditions because of the limited space for plants to grow in these pot studies. Additionally, the environmental influences on salinity build up and leaching are unaccounted for in the greenhouse environment. Moreover, looking for more salinity tolerant biofuel crops may be needed for more biomass production on saline soils.

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Table. 4.1. Salinity leachate values

Period I (1-5 weeks)	Experiment I					Experiment II				
Treatments (dS m ⁻¹)	Control	4	8	12	14	Control	4	8	12	14
Leachate (dS m ⁻¹)	1.7	7.3	12.3	16.4	20.5	2.3	8.7	14.8	19.2	22.3
Period II (6-20 weeks)	Experiment I					Experiment II				
Treatments (dS m ⁻¹)	Control	4	8	12	14	Control	4	8	12	14
Leachate (dS m ⁻¹)	2.9	7.7	12.5	16.6	20.7	3.4	9.2	15.3	21.1	24.8

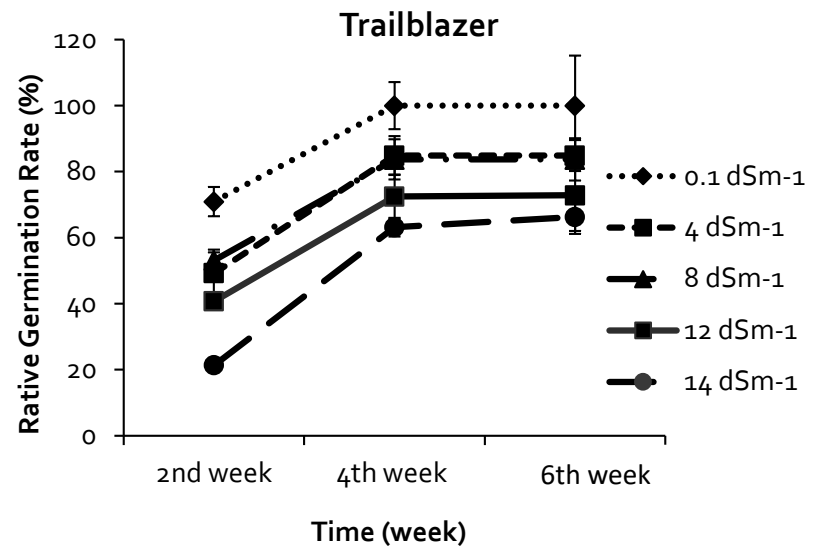
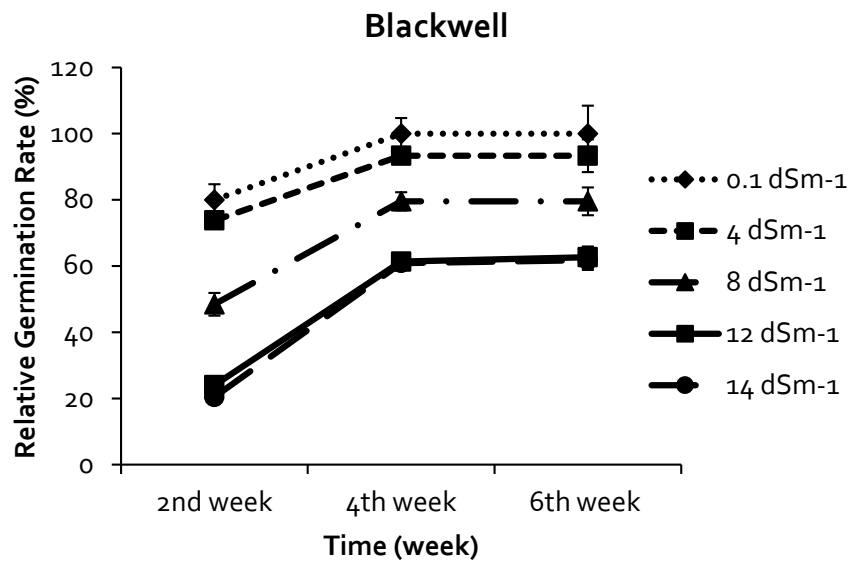


Figure 4.1 First experiment: Relative germination rate over time.

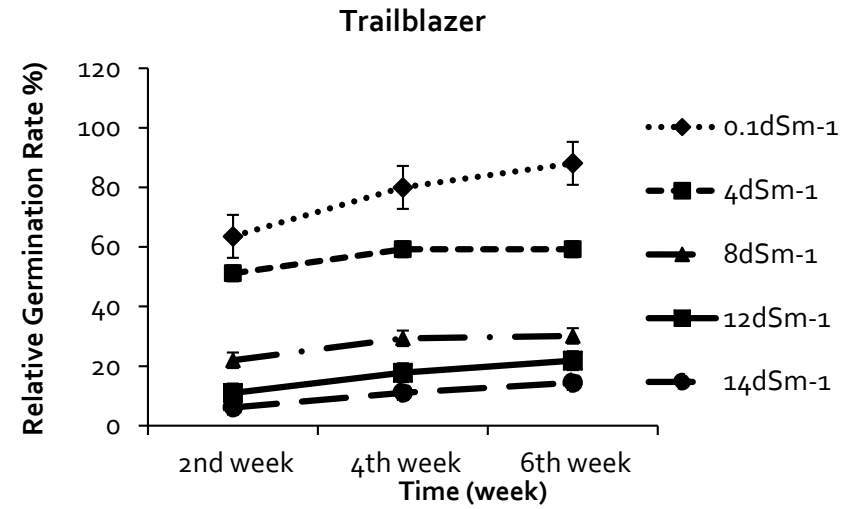
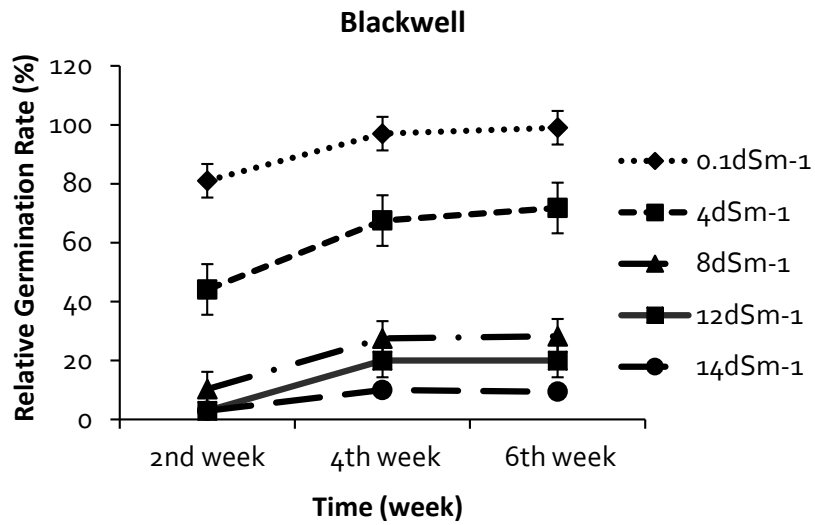


Figure 4.2. Second experiment: Relative germination rate over time.

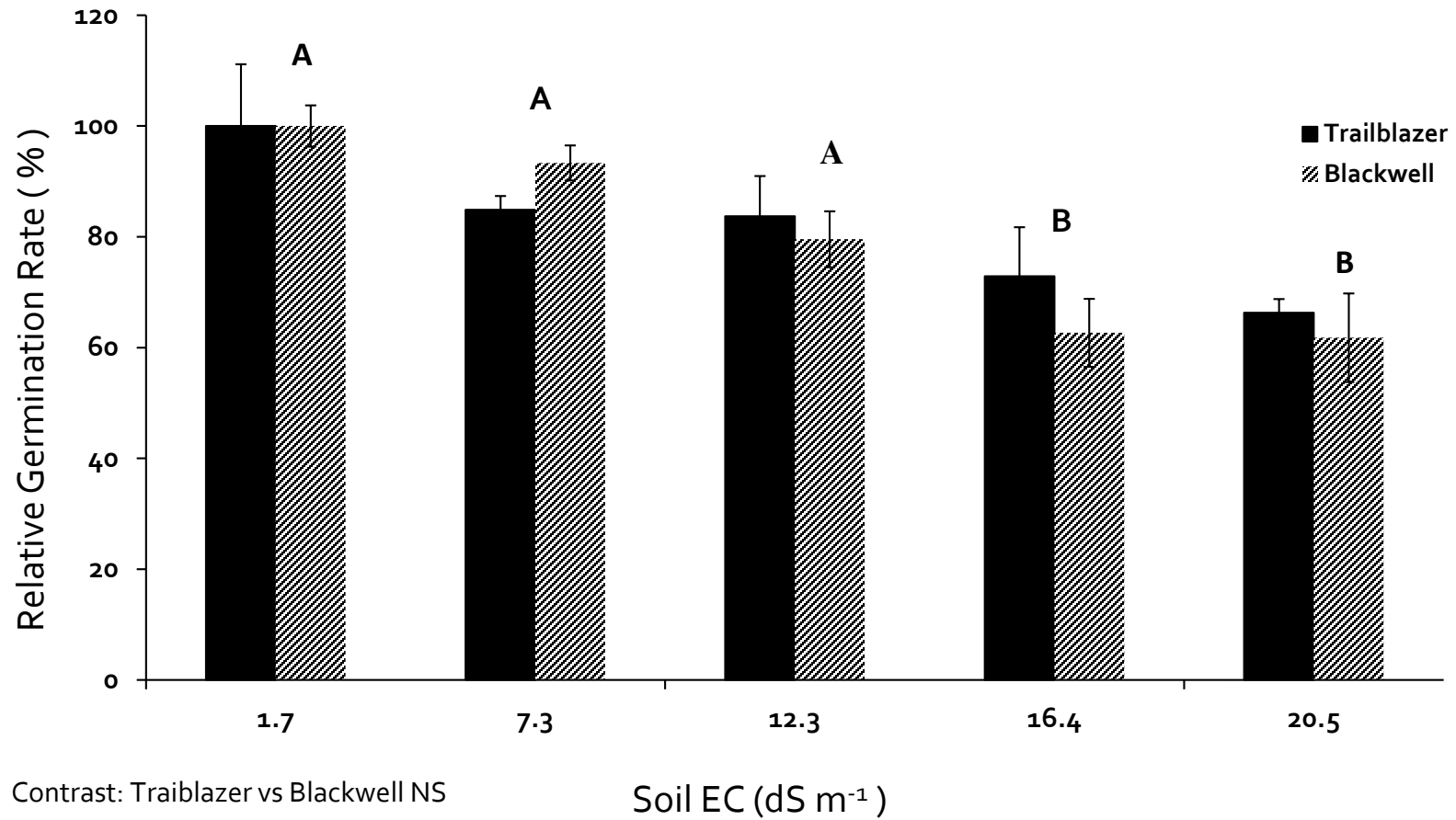


Figure 4.3 First experiment: Relative germination rate (%).

Upper case letter indicate significant different ($P=0.05$) among salinity treatments

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

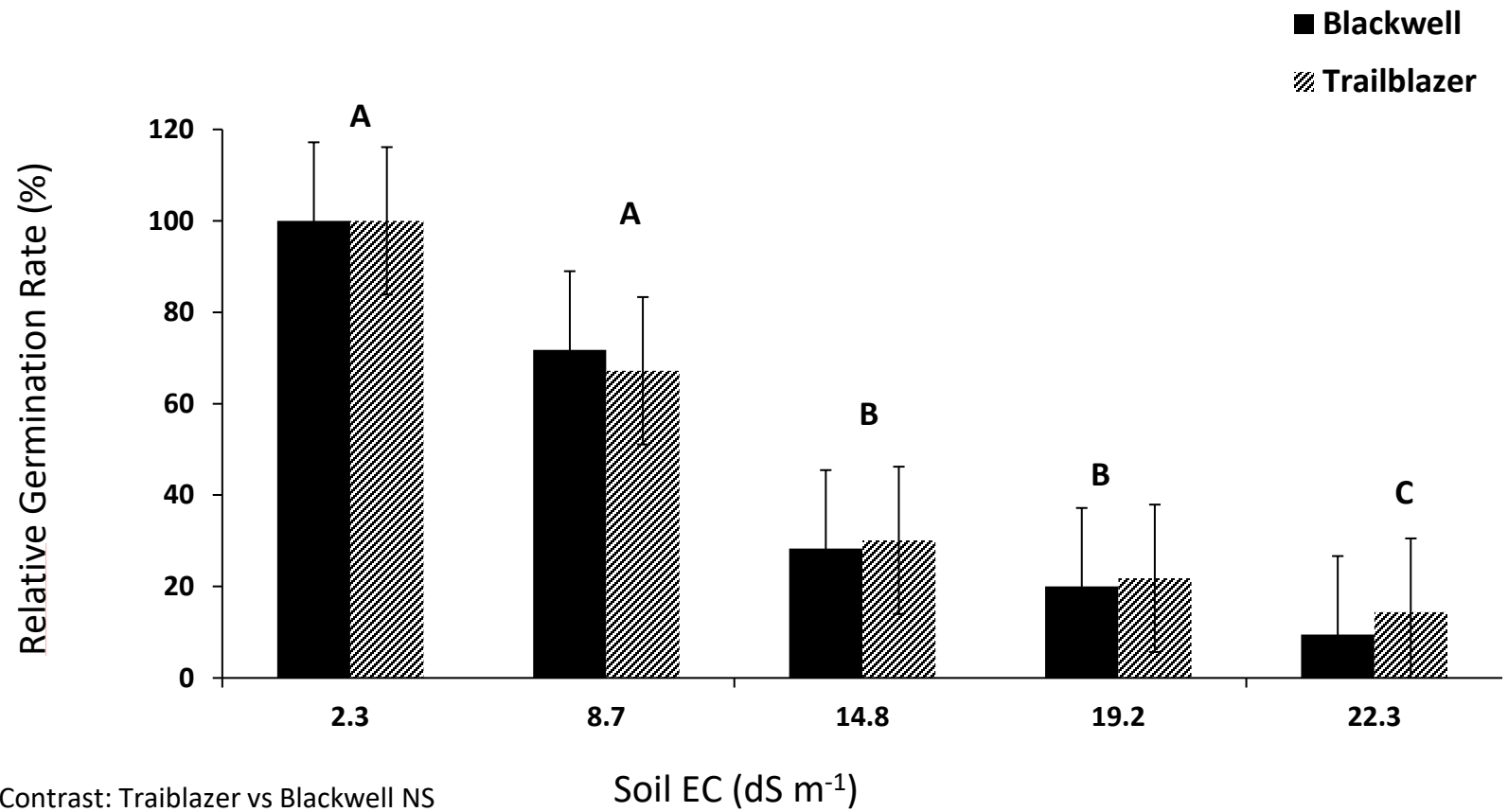


Figure 4.4. Second experiment: Relative germination rate (%).

Upper case letter indicate significant different ($P=0.05$) among salinity treatments

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

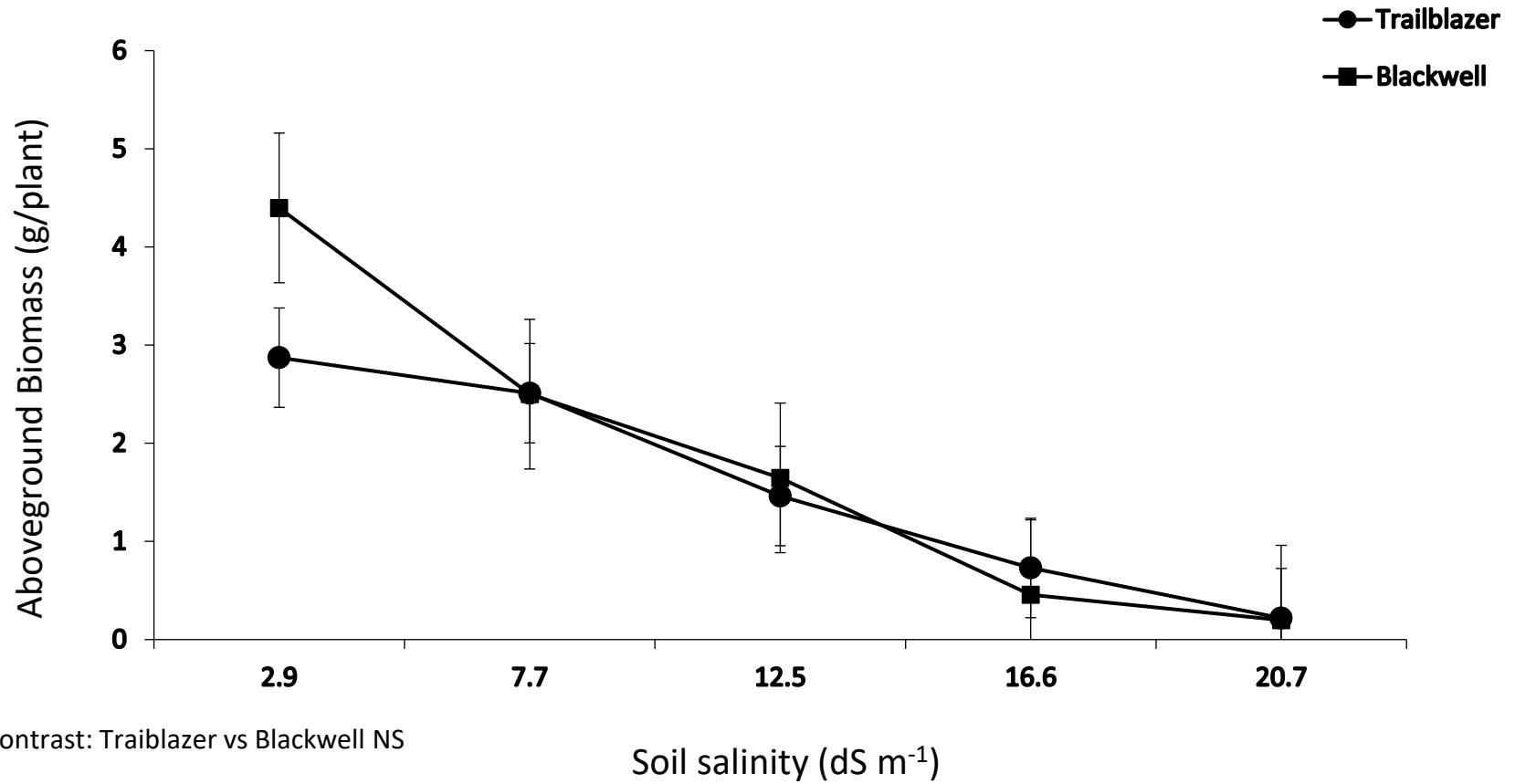


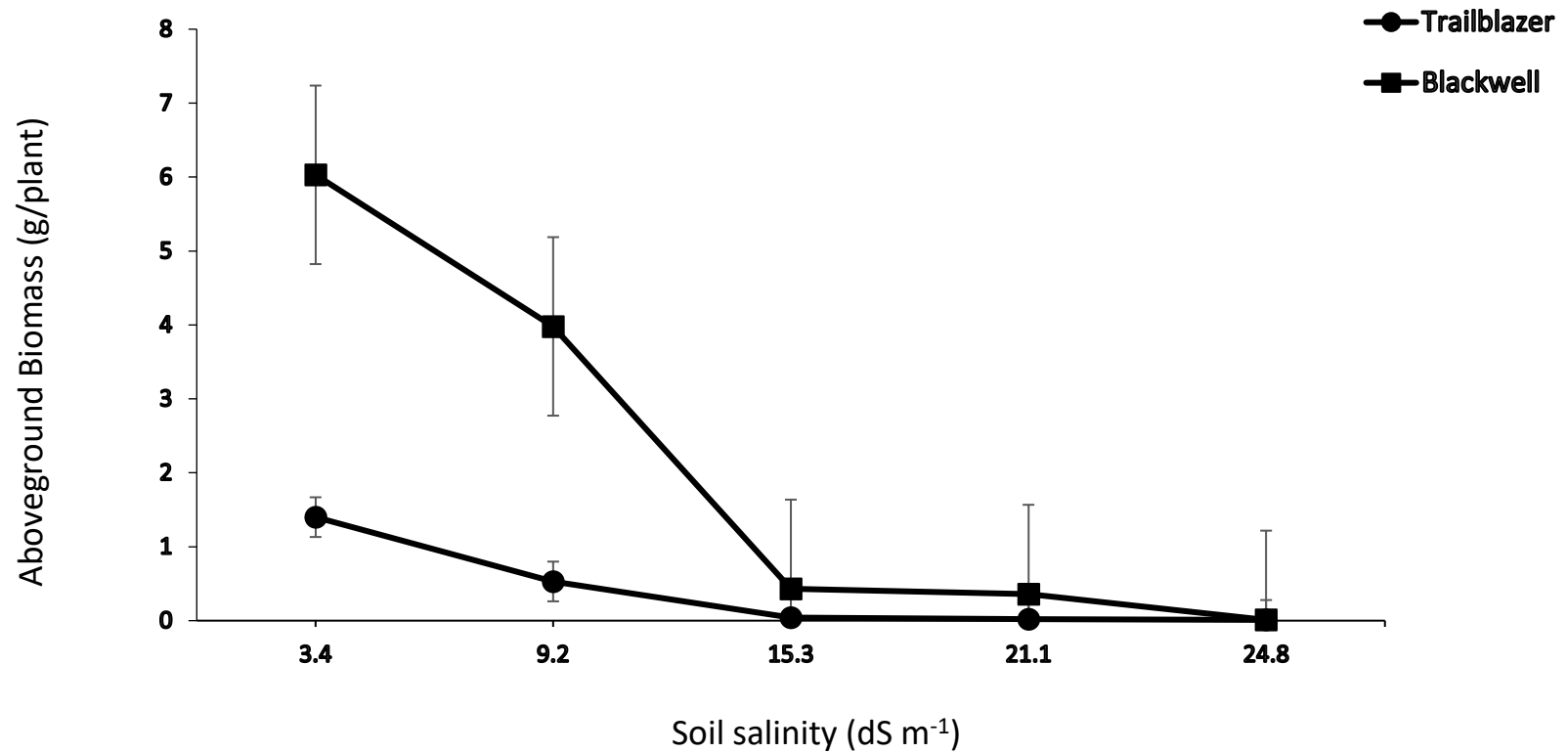
Figure 4.5 First experiment: First aboveground biomass (g/plant).

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.



Contrast: Traiblazer vs Blackwell **

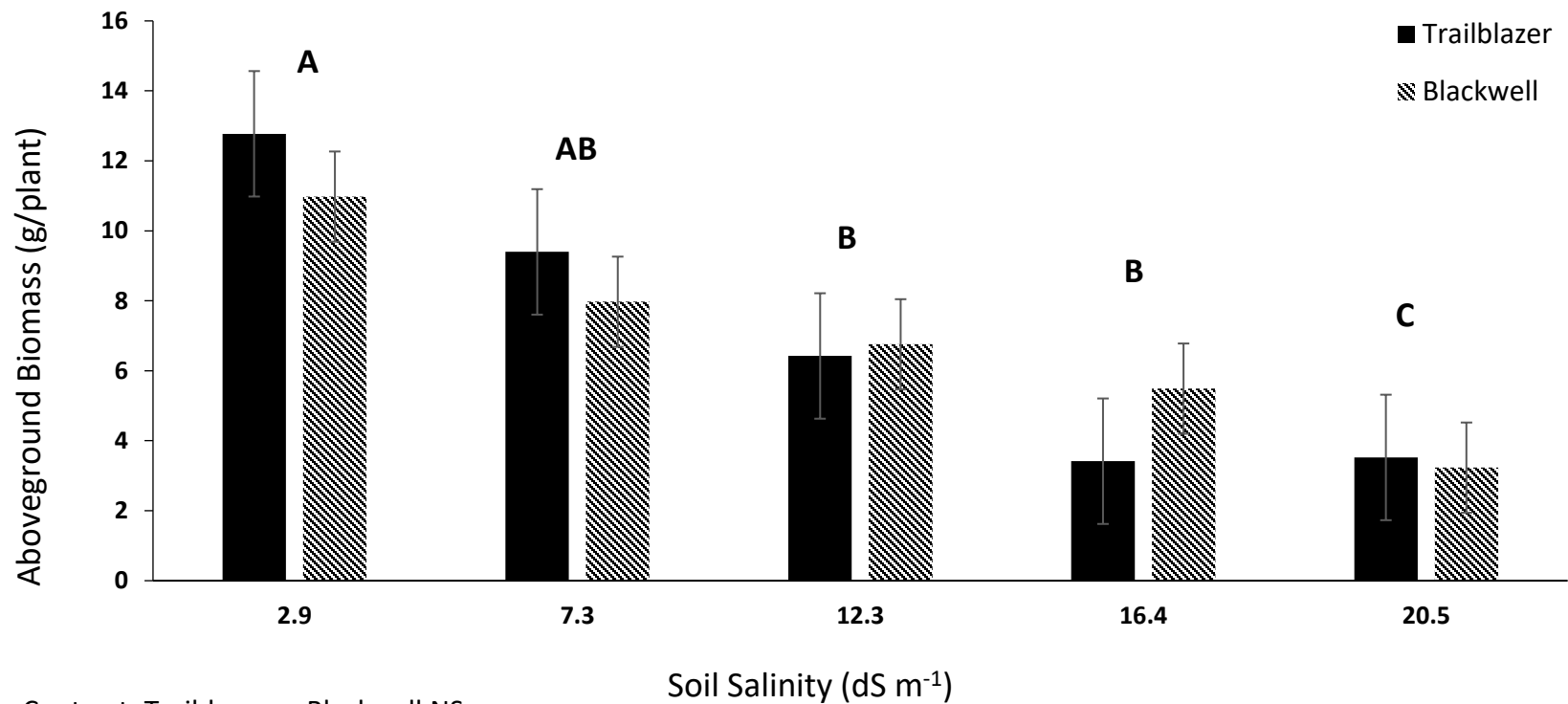
Figure 4.6 Second experiment: First aboveground biomass (g/plant).

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.



Contrast: Traiblazer vs Blackwell NS

Figure. 4.7. First experiment: Second aboveground biomass (g/plant) at the end of the experiment.

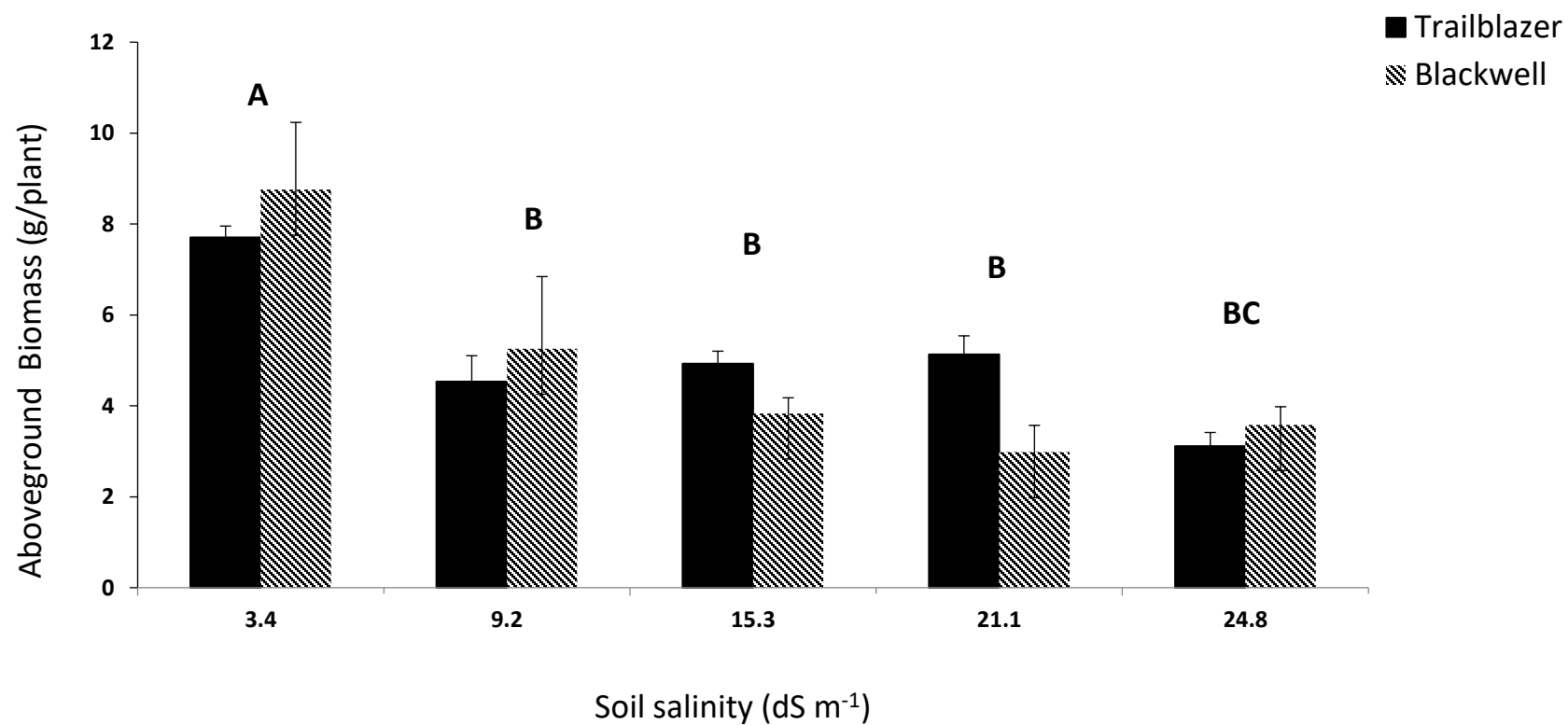
Upper case letter indicate significant different ($P=0.05$) among salinity treatments

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.



Contrast: Traiblazer vs Blackwell NS

Figure 4.8. Second experiment: Second aboveground biomass (g/plant) at the end of the experiment.

Upper case letter indicate significant different ($P=0.05$) among salinity treatments

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

NS non-significant at the 0.05 probability level.

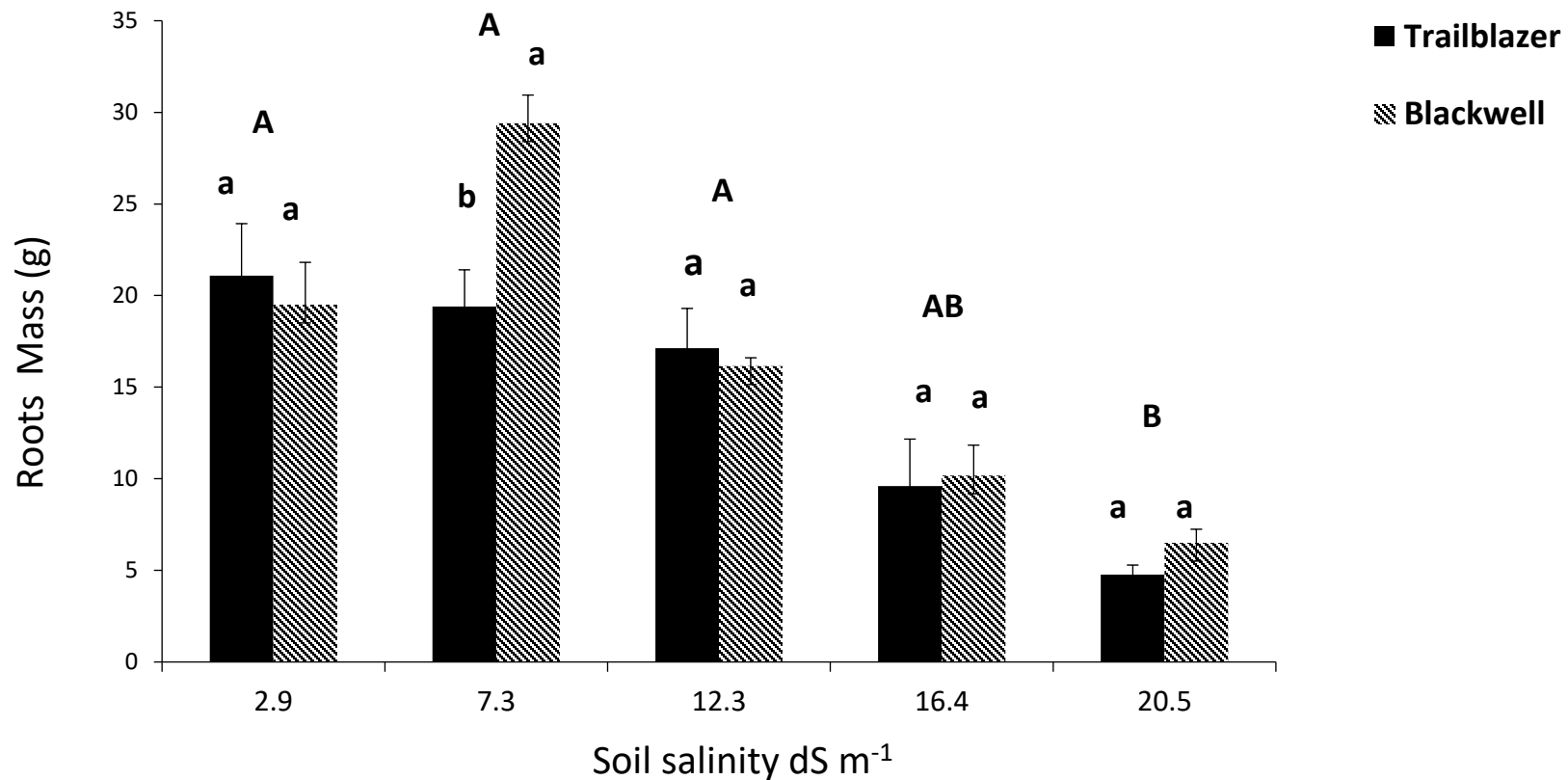


Figure 4.9. First experiment: Root mass at the end of experiment (g/plant).

Lower case letter indicate significant different ($P=0.05$) between switchgrass cultivars

Upper case letter indicate significant different ($P=0.05$) among salinity treatments

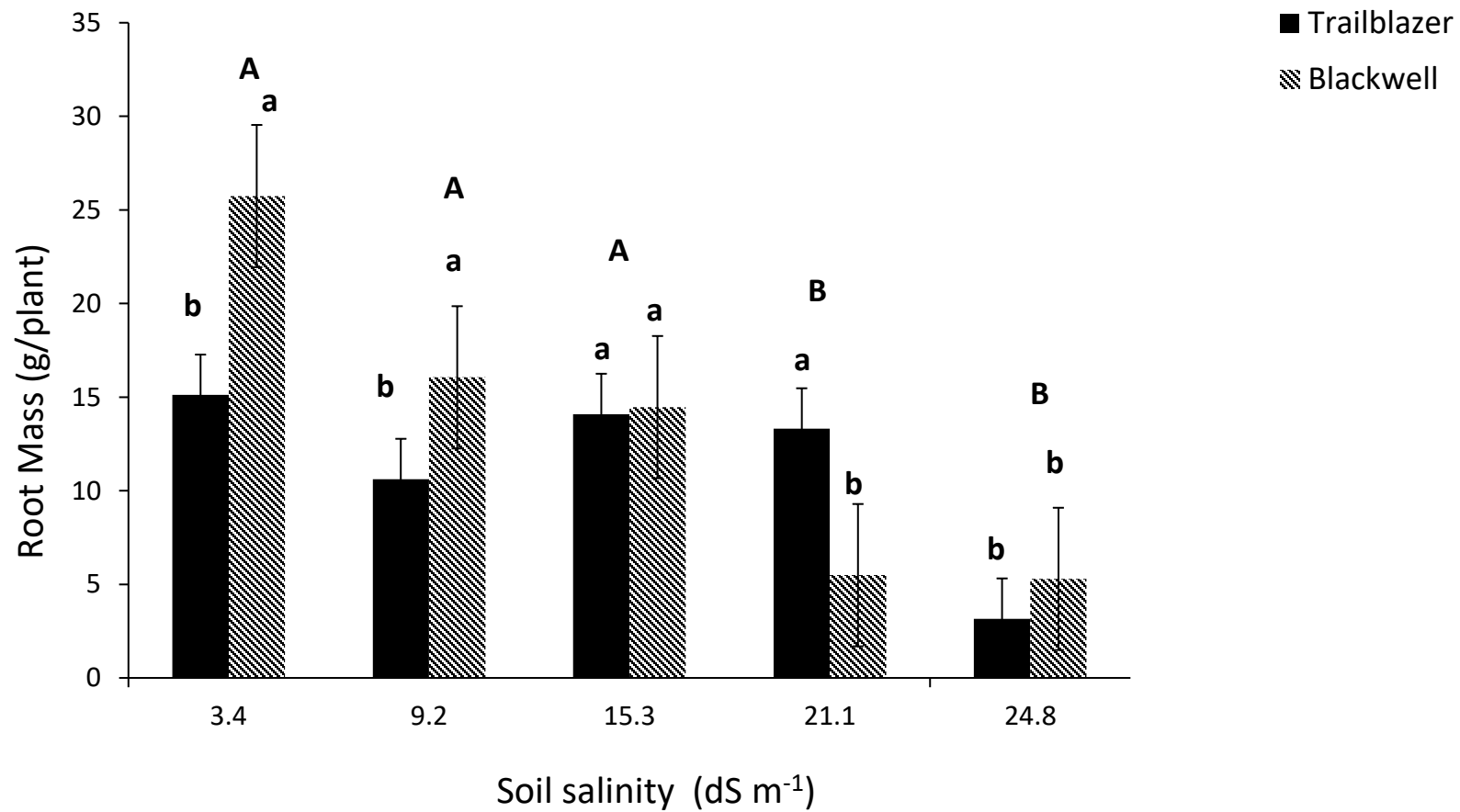


Figure 4.10. Second experiment: Root mass at the end of experiment (g/plant).
 Lower case letter indicate significant different ($P=0.05$) between switchgrass cultivars
 Upper case letter indicate significant different ($P=0.05$) among salinity treatments