The combined impact of salinity and water table on Juncus balticus growth: Implications for fen construction in the Athabasca Oil Sands Region

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Oil sands mining destroys peatlands by removing all vegetation on the ground and digging up to 75 m deep to reach the oil. To investigate methods to return peatlands to the post-mining landscape, a fen reclamation project was launched to build a new peatland using salvaged peat according to hydrological design and vegetation reintroduction. However, salt contamination is a concern for growth and physiology of plants because salts in mine waste materials are transported into the fen through groundwater. A salinity-tolerant plant community with Baltic rush (*Juncus balticus*) dominant was planted in 2013 but cover has decreased over time. The response of the plant community to continued salinization under a range of hydrologic conditions remains unclear. This thesis investigated the combined influence of soil salinity and water availability on *J. balticus* growth and ecophysiology under a controlled greenhouse experiment and in the field, which can be used to predict whether this species can continue to survive in the constructed fen in the future.

In the greenhouse experiment, *J. balticus* seedlings were grown in a full factorial experiment with seven salinity levels and two soil water table levels for 14 weeks. Plant growth and stress were assessed based on leaf photosynthetic parameters, plant height, above and belowground biomass, and leaf and root sodium (Na^+) and potassium (K^+) concentrations. Photosynthesis rates decreased when Na^+ concentrations exceeded 2300 mg L^{-1}, while this response was not observed in the aboveground or belowground biomass. Noticeably, biomass and photosynthesis rates were always lower in the wetter treatments, regardless of salinity. Plant height did not have significant relationships with either salinity or water table levels. Leaf and root Na^+ concentrations increased with salinity but were similar in roots between 2300 and 4600 mg L^{-1} treatments. Leaf and root K^+ concentrations decreased as salinity increased. This result indicates that *J. balticus* is relatively resilient to the Na^+ concentration currently in the fen, and the salinity is not a stress to this species even in the future. However, the salt stress in *J. balticus* would be more severe under the wet condition. Therefore, it is necessary to maintain some dry microsites to support this saline species in the fen construction project.

In the field, gas exchange, biomass, and elemental content of *J. balticus* was measured at eleven sampling sites in the constructed fen from mid-June to late-August. Water table was measured with each gas exchange measurement, while salinity of the pore water was measured once after collecting the biomass at the end of the field season. An increasing water table (wetter) could promote the root growth of *J. balticus* between the range of -70 and -10 cm. However, the growth of shoots was inhibited when the water table was close to the surface. Correspondingly, GEP decreased under high water table (wet condition). In the element content analysis, Na:K was lowest at the water table between -40 and -20 cm, which indicated *J. balticus* could have better performance to deal with Na^+ in their leaves at this range of water table. Na^+ concentration between 113 and 238 mg L^{-1} did not have noticeable effect on *Juncus* growth. In addition, age of the plant is another independent variable that had a negative impact on GEP per aboveground biomass.

Together, results from the greenhouse experiment and the field collection demonstrated that salinity had limited impact on *J. balticus* growth, while water table had closer relationship with its performance. When the water table was lower than -10 cm (-70 ~ -10 cm), this factor had the positive relationship with growth parameters, while if the water table is higher than -10 cm, or inundated, it would negatively impact *J. balticus* growth. Moreover, shallow water table interacted with salinity causing additional stress under high Na^+ concentration in wet conditions. In the future, the impact of water table and salinity on the whole plant community composition and function could be studied. Furthermore, sulphate could be another element that could threaten the health of plants in constructed fens and should be studied further.

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I also want to acknowledge that my field collection takes place within the boundaries of Treaty 8, traditional lands of the Dene and Cree, as well as the traditional lands of the Métis of northeastern Alberta.

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Chapter 1 Introduction

1.1 Peatland ecosystems and their significance

Peatland ecosystems are special types of wetland ecosystems that play an important role in global terrestrial carbon accumulation (Gorham, 1991). Although peatlands cover only about 3% of terrestrial surface on Earth, they store between 1/3 and 1/6 of the total soil organic carbon in the world (Yu et al., 2010; Xu et al., 2018). Especially in boreal regions, high water table, low temperature, and *Sphagnum* moss species provide an anoxic and acidic condition in which microbes decompose organic matter slowly (Bengtsson et al., 2016; Evans et al., 2016). When the productivity of plants is greater than the decomposition rate, peatlands start to form (Vitt et al., 1996).

Most of these organic matter abundant wetlands are located at Asia and North America, especially in the boreal regions of Russia and Canada (Xu et al., 2018). The peatland definition is rather controversial, varying between regions and disciplines, and there is no general agreement about how much organic material should be contained in the peat soil. Some researchers suggested peat soil should have at least 30% organic matter (Joosten & Clarke, 2002), other suggest this number should be 50% (Burton & Hodgson, 1987), and some other researchers defined that it should be 18% if the soil was saturated after 30 days (Schad & Spaargaren, 2006). In Canada, if the peat accumulation is greater than 40 cm in a wetland, this wetland is defined as a peatland (Rubec, 2018). Peatland classification also varies among countries, and each country usually has their own classification systems, but water sources and vegetation composition are regarded as the most important factors in the classification (Charman, 2002). According to the *Canadian Wetland Classification System* (Rubec, 2018), peatlands can be categorized as bogs, fens, and swamps. Fens always connect with groundwater and surface flow, which is often rich in minerals (Rubec, 2018). Due to this connection, fens are usually dominated by graminoid species and brown mosses (Vitt, 2006).

Peatland ecosystems are important to the local environment in terms of hydrologic regulation, and vegetation succession (Nwaishi et al., 2015a). For hydrologic regulation, peatlands play a more significant role than marsh wetlands in AOSR, because peatlands can supply water back to uplands during drought periods, while most of water in marsh wetlands will be lost through evaporation (Petrone et al., 2008). This occurs because of the physical properties of peatlands (Boelter, 1968). For example, during a wet season or year, the near surface peat layer (largely made of partial decomposed plant litter) has high transmissivity and can hold a large mass of water due to the large soil pores and high porosity (Price, 2003), while during drought periods this surface layer is a poor conductor of water under a dry state with low unsaturated hydraulic conductivity that retards evaporation (Kettridge & Waddington, 2014). Meanwhile the deep peat layers of highly decomposed plant litter with small pore size limit lateral seepage on account of low hydraulic conductivity (Price & Whittington, 2010). Therefore, peatlands are able to regulate hydrology under various climate condition.

Besides the function of hydrologic regulation and carbon accumulation, peatlands are important landscapes in the oil sand region. The services provided by this type of ecosystem include, but are not limited to, water purification, flood retention, providing habitat to wild animals, soil erosion prevention, and soil fertility maintenance (Bonn et al., 2016). The negative impacts of peatland degradation in the oil sands mining area have revealed through water contamination (Alexander & Chambers, 2016), caribou herd decline (Wasser et al., 2011), dissolved organic carbon (DOC) release (Khadka et al., 2016), etc. Given the importance of peatlands to landscape function, there is a need to include them in the post-mining landscape. Therefore, it is necessary to have a pilot site for peatland reconstruction.

1.2 Peatland disturbance in AOSR

Peat is the primary product in peatlands and it is also known as an early stage of coal formation due to high organic carbon content (Ryer & Langer, 1980). Across much of boreal of Alberta, including areas with dense peatland coverage, there is a rich storage of oil sands, the mixture of sand, water, and bitumen, which is the result of the Rocky Mountains' formation (Vigrass, 1968). The collision between the Pacific Plate and the North American Plate pushed the sedimentary rock layers in Alberta to deep depth and converted the organic layers into light oil under high temperature and pressure conditions (Vigrass, 1968). Because oil sands are usually buried tens to hundreds of meters below the ground, vegetation removal is necessary for the oil sands detection and mining (Lee & Boutin, 2006), which results in many negative impacts to local ecosystems (Abib et al., 2019).

In western Canada (Alberta, Saskatchewan, and Manitoba), 48 petagrams (Pg = 10^{15} g) of carbon is stored in peatlands (Vitt et al., 2000), equal to 2.1% of global soil carbon. In northern Alberta, half of the pre-disturbed landscape in the AOSR was peatlands, and more than 90% of these peatlands were classified as fens (Vitt et al., 1996). Based on the reclamation and disturbances data set provided by Alberta Environment (2017), about 953 km^2 of lands have been disturbed by mines and associated facilities between 2009 and 2016 in support of oil sands extraction in the region, while about one seventh of these lands were reclaimed. Besides the surface mining activities, petroleum exploration also disturbs peatlands by clearing surface vegetation, pressing with heavy machines, and creating seismic lines over 1900 km^2 in peatlands over the past decades (Strack et al., 2019). Those disturbances may have negative impacts on local animals, such as caribou (James & Stuart-Smith, 2000), ovenbirds (Bayne et al., 2005) and martens (Tigner et al., 2015). Furthermore, if the disturbance exceeds the threshold of peatlands' resilience, their functions could change from carbon sink to carbon source and release a great amount of greenhouse gases into the atmosphere, such as carbon dioxide, methane, and nitrous oxide (Strack et al., 2019; Nwaishi et al., 2016).

1.3 Peatland reclamation in AOSR

In the Environmental Protection and Enhancement Act issued by the Alberta government, "equivalent land capability" is defined as the aim of land reclamation (Environment and parks, 2000). It means that the reclaimed land should retain similar functions provided by the land prior to disturbance. Land reclamation to wetlands has been tested in AOSR since 1980s (Daly, 2011) where oil companies built small marshes to treat wastewater and maintain sustainable ecosystems. Field studies and ecological assessments on the reclaimed marshes and shallow open water at a small scale were not only focused on the contaminant reduction (Bishay, 1998; Videla et al, 2009) and ecological influence (Leung et al., 2003; Barr, 2009) but improved essential understanding of wetland reclamation in terms of vegetation (Crowe et al., 2001; Trites & Bayley, 2009a), food webs (Wayland et al., 2008) and performance indicators (Rooney & Bayley, 2010). Then, more research focused on the reclaimed lands at a larger scale, such as Pilot End Pit Lakes (EPL), which considered surrounding watersheds into their design (Daly, 2011).

Although those marsh reclamation projects achieved the goals in terms of wastewater recycling and revegetation, they did not restore the landscapes back to the original peatland ecosystems. According to Chee and Vitt's (1989) investigation on vegetation and chemistry of surface water and peat, the Athabasca area was originally dominated by moderate-rich fens. Only reclaiming marsh and open water wetlands would cause considerable loss of peatlands in the region over time (Rooney et al., 2012). Almost at the same time as marsh reclamation trials were ongoing, a peatland reclamation theoretical model was put forward by Price et al. (2010) to cut down the paludification time from marsh wetlands to peatlands (Noon, 1996; Trites & Bayley, 2009b). Moreover, a natural wetland monitoring program was conducted to describe the range of conditions present in natural wetlands and to better improve wetland reclamation in AOSR (Daly, 2011).

Based on this progress, tests of peatland reclamation in the AOSR were required as part of the mine approvals (Trites & Bayley, 2009b). Sandhill fen and Nikanotee fen are two constructed peatlands built in AOSR as experimental sites by Syncrude and Suncor, respectively (Ketcheson et al., 2016). Climate was one of the significant factors considered in the design. According to Keshta et al. (2012) for climate in the past in AOSR, evaporation rate is greater than precipitation rate in this region and the rainfall usually happens in a short period with high intensity. They also predicted that there will be more precipitation, evapotranspiration and longer growing seasons in the future. Because of this climate condition, both constructed peatlands were designed to be self-sustained systems with suitable peat depth and revegetation, while they used different concepts for the construction (Price et al., 2010; Wytrykush et al., 2012).

The Sandhill fen simulated the layout of natural fens and it obtained water from a nearby reservoir to protect peatland plants from the high salinity water in surrounding watersheds in the next 5 to 10 years (Wytrykush et al., 2012). Nikanotee fen used a subsurface and surface hydrologic model to optimize the area ratio between the upland and the fen, which helped to test the system sensitivity to various combinations of layers with different hydraulic conductivities (Figure 1.1) (Ketcheson et al., 2016). This design was modelled based on the meteorological data in the past decades to ensure that the peatland could maintain wetness even in the driest period. To improve infiltration in the watershed, ridges and furrows were excavated on the upland in 2013. Moreover, to create more dry habitats, new peats were placed at the north-west corner of the constructed fen in 2018 and planted in 2019. One of the challenges in the Nikanotee fen is that contaminated water containing high Na^+ concentration, sourced from construction materials, might flow into the fen through groundwater exchange. Kessel et al. (2018) found that the average Na^+ concentration increased from 87 to 200 mg L^{-1} in surface soil of the fen between 2013 to 2016 and that the increase usually happened in summer between 2013 to 2016. This occurred as high intensity rainfall could bring a great deal of Na^+ into the fen through the aquifer and evaporation could transport salts from the lower layer to the surface of the fen during dry periods (Kessel, 2018).

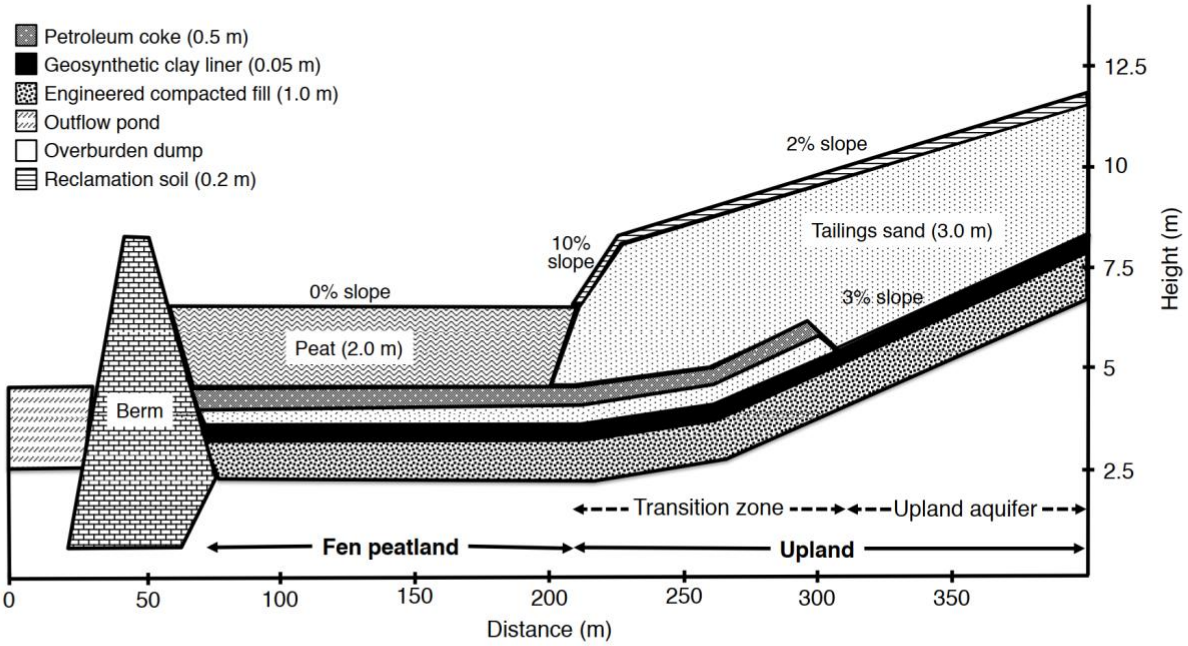

Figure 1.1 Cross section design of the Nikanotee fen (from Ketcheson et al., 2016).

1.4 Salt tolerance & flooding tolerance of plants

High levels of salinity in soil may cause salt stress to plants. The response of plants could be stomatal closure and cessation of root growth (Munns & Schachtman, 1993; Rajendran et al., 2009). This reaction would impact the ability of plants to absorb water from the soil. Basically, plants passively uptake water from soil using the Cohesion-Tension (C-T) mechanism (Steudle, 2001). Cohesion means that the interaction force between water molecules is strong so that the water column can be continuously transported within the plant tissue, and the tension is caused by transpiration of the plant leaves that creates a water pressure to pull from roots to leaves (McElrone et al., 2013). Under salt stress, the C-T mechanism would be inhibited due to stomatal closure, which is caused by imbalanced ion concentration within the plant tissue ; therefore, if the plants cannot restrict ion translocation or adapt themselves to tolerate the condition, the cytotoxic ion might accumulate in the plant and cause metabolic problems, and even cell death (Munns & Tester, 2008).

There are three different salt tolerance mechanisms employed by plants: 1) osmotic stress tolerance, 2) sodium exclusion, and 3) tissue tolerance (Munns & Tester, 2008). The first strategy means that plants would increase their leaf area and stomatal conductance to resist osmotic stress (Abebe et al., 2003). Plants with sodium exclusion functions can prevent Na^+ accumulation in leaves (Rozema et al., 1981). This process is controlled by transporters within plants to exclude Na^+ from xylem (Horie et al., 2005) and the function may consume more energy (ATP) from plants during the growing season (Munns et al., 2020). This mechanism could also prevent the decline of electron transportation from the high salinity water and maintain the ion concentration at a normal level within the plant tissue (Asrar et al., 2017; Niewiadomska & Pilarska, 2021). Tissue tolerance means that plants could adjust sodium or chloride accumulation in their tissues with relatively high concentration (Flowers et al., 2015).

If plants are unable to cope with high salinity, the impact of osmotic stress could be a reduction in stomatal conductance, leaf or root growth rate and photosynthesis rate; however, in some species of plants, the photosynthesis rate might be stable because leaf cells would grow thicker and the number of chloroplasts increase (James et al., 2002). Facing a long-term salt stress, sodium exclusion and tissue tolerance usually co-occur. Some plants can selectively absorb potassium into the tissue and resist Na^+ to maintain a suitable sodium to potassium (Na/K) ratio, and calcium can be used to alleviate damage from Na^+ in the tissue (Chen et al., 2005; Kopittke, 2012). Leaves are the most vulnerable part of plants under a salty environment because ions can accumulate in leaf blades during transpiration (Munns, 2002). Therefore, in addition to strategies that prevent ions entering the plant, the synthesis of organic solutes to improve osmotic adjustment ability (Munns, 2008) and use of antioxidant enzymes to protect enzyme systems (Rangani et al., 2016) might happen inside of some plants. However, high salinity environment could still have negative impact on the activity of

Rubisco enzyme, which would influence the carboxylation function within the photosynthesis process (Seemann & Sharkey, 1986; Galmés et al., 2013).

In addition to high salinity, flooding is another threat to plants in wetlands, including constructed fens. Anoxic conditions can cause oxygen deficiency in root systems, which slows down the growth rate of roots and shoots (Drew, 1983). Then, the movement of solutes would be limited between membranes (Trought & Drew, 1980). At last, leaf dehydration and stomatal closure due to root damage may cause death to plants (Else et al., 2001). To survive in flooded areas, plants have developed several adaptive features. Adventitious roots, also called aboveground roots, are common under flooding stress, which can help roots access oxygen in inundated areas (Colmer & Flowers, 2008). Aerenchyma tissue is another feature that can improve oxygen transmission to root regions (Armstrong, 1980). It is a spongy tissue within plants formed under the hypoxic condition to transport gases from leaves to roots. Justin and Armstrong (1987) found that flooding did not have a negative influence on shoot weight and root length in various wetland species because aerenchyma increased the root porosity and prevented oxygen deficiency. Another adaptive feature of some marsh species is having a shallow rooting strategy to avoid deeper saturated soils (Pearson & Havill, 1988). Furthermore, other factors, such as root diameter, stele volume, radial oxygen loss, and enzyme activity may influence the diffusion of oxygen within plants (Colmer, 2003; Patel et al., 2014).

There is little research focusing on the combined impact of waterlogging and salinity on vegetation growth, especially in peatland plants. In a greenhouse experiment designed by Vitt et al. (2020), they measured the physiology of *Carex aquatilis,* which is a widespread, common graminoid across wetlands in the AOSR. The result indicated that biomass, photosynthesis rate, and stomatal conductance of the plant obviously declined when the Na^+ concentration reached 2354 mg L^{-1}. However, the experiment did not take water level into account, which could significantly influence the plant growth in the natural environment as well. In fact, Phillips et al. (2016) found that the evapotranspiration rate (ET) of functional plants in a natural saline fen was affected by both water level and salinity. High ET rates were usually measured at the area with low water level and low leaf area index (LAI). It means that plants in the saline fen may have smaller leaf area and prefer wetter soil. The research also discovered that the rush species, *J. balticus,* may have low transpiration rate under flooding and salt stress. Therefore, some types of plants may be more likely to suffer salt stress under flooding.

1.5 A history of *Juncus balticus* research & current studies on the fen

J. balticus (family Juncaceae) is a type of monocotyledonous graminoid and usually exists in naturally saline wetlands, including saline fens in the AOSR, but was not found to naturally colonize industrial wetlands, including small test ponds (Trites & Bayley, 2009). This species was first found in Britain by Adamson (1913) at a humid but not waterlogged coastal dune. According to the distribution record of *J. balticus* and its hybrids between 1933 and 1978 summarized by Smith (1984), they were discovered at aquatic, semi-aquatic, wet-slack areas, but most of them were recorded in wet-slack regions. Wet slack is usually used to describe an area where the soil is loose and the water table level is neither high nor low. *J. balticus* was first documented as a tidal plant in Canada as well (Rousseau, 1934). Then, in the next decades, it was successively recorded in Saskatchewan (Jones & Peterson, 1970), British Columbia (Pojar, 1973), Alberta (Whysong & Bailey, 1975), southern Ontario (Karrow et al., 1975), Northwest Territories (Reynolds et al., 1978) and Nova Scotia (Patriquin & Keddy, 1978). They were all found nearby saline waterbodies and growing in moist soil, while the wide footprint indicated the species' strong adaptability. Moreover, as *J. balticus* could be a nurse plant for mosses in peatlands and improve oxygen transport to roots, it has been included in plantings at the Nikanotee fen (Borkenhagen and Cooper 2019). To better understand long term response of constructed fens in the AOSR to changing salinity and hydrological conditions, it is therefore important to understand vegetation environmental tolerances, including *J. balticus*'s response to flooding and salinity.

In Nikanotee fen, existing studies are available on hydrology (Price et al., 2010; Ketcheson et al., 2017), salinity (Simhayov et al., 2017; Kessel et al., 2018, Yang, 2021), vegetation composition (Borkenhagen & Cooper, 2019), and interaction between plants and hydrology (Scarlett & Price, 2019; Sutton & Price, 2020) or salinity, hydrology and soil carbon (Prystupa, 2020), but there is a lack of research on the combined influence of salinity and water table on plants. It is significant to understand how salinity and water table work together to impact plants' growth in order to better model vegetation succession under changing conditions to inform future reclamation projects. Given its preference for growth in partially saline conditions and inclusion in planting at Nikanotee fen as a salt-tolerant species, this study will focus on assessing *J. balticus* response to salinity and water table position with a greenhouse experiment and field measurements.

1.6 Objectives

(1) How is *Juncus balticus* growth and physiology affected by increasing Na^+ concentrations? (Chapter 2)

(2) Does water table position interact with the salinity to influence *Juncus balticus* growth and physiology? (Chapter 2, Chapter 3)

(3) Can growth patterns of *Juncus balticus* in a field setting be explained by variation in water table and salinity? (Chapter 3)

Chapter 2 The combined impact of salinity and water table on *Juncus balticus* growth: a greenhouse experiment

2.1 Introduction

Oil sands is one of the crude oil sources developed from marine sediments, which is deposited up to 75 m below the surface in the mining region (Simhayov et al., 2017). To reach this depth, surface layers would be removed before the minging and high salinity materials would be backfilled after the mining. This activity would disturb the function of peatlands, such as carbon sequestration, hydrologic regulation, and nutrient cycling (Smandych & Kueneman, 2013). Therefore, it is important to reclaim the peatlans to improve the resilience of the ecosystem. Nikanotee fen is one of the peatland reclamation projects by salvaging the peat from mining region and taking tailing sands as the aquifer layer for the constructed watershed (Price et al., 2010). During the construction, salinity became to a concern for maintaining fen plant communities and carbon accumulation function, but the plant tolerance was not well studied due to limited information on natural saline systems, thus, a mesocosm experiment was conducted the on the constructed fen by introducing various species on the site (Borkenhagen & Cooper, 2019). According to the past studies on the element pool in this constructed landscape, Na^+ needs decades to flush through the watershed and its concentration would be elevated in the fen by evapotranspiration (Yang et al., 2022). This study will investigate the response of used plants in the constructed fen, especially *Juncus balticus,* to Na^+ concentration under different hydrologic conditions.

2.1.1 Peatland reclamation & the need for greenhouse experiments

The Nikanotee fen is a constructed fen in the oil sands region in Alberta where a peatland and its watershed were designed using existing construction materials to ensure provision of water to the fen to main wetness even under dry weather periods (Price et al., 2010). The peat layer was salvaged from the mining area and plant community was transplanted using moss layer transfer or planted as seedlings, including *Juncus balticus, Carex aquatilis,* and other peatland plants (Borkenhagen and Cooper, 2019). Given its abundance in the post-mining landscape, tailings sand was used in the upland slope and aquifer construction; however, it has led to the increase of Na^+ concentration from 87 to 200 mg L^{-1} in the fen between 2013 and 2016 (Kessel et al., 2018).

To measure the plant response to various salinity levels and identify mechanisms, which is hard to obtain from the field, conducting a greenhouse experiment is necessary. For example, Vitt et al. (2020) found that *Carex aquatilis* could survive under various salinity levels, but that plant performance decreased when Na^+ concentrations reached 1200 mg L^{-1}. Furthermore, water table, as an important component in plant growth, has higher relationship with topology and precipitation in the field. Research found that salinity was higher in the wet region than dry region in Nikanotee fen (Yang et al., 2022), but it could not differentiate the impact of salinity and water table on plant growth. Therefore, greenhouse experiments have the advantage of controlling environmental variables.

2.1.2 Impacts of the water table & salinity on photosynthesis rate

Carbon sequestration is one of the significant functions in peatland ecosystems, which is controlled by the balance between CO_2 uptake from plant productivity (i.e., photosynthesis) and CO_2 release from respiration and decomposition rates (Flanagan & Syed, 2011). The decomposition of plant litters is affected by temperature and oxygen content. Litter usually decomposes most quickly in warm and high oxygen environments where the microbes have high activity (Cai et al., 2010). The productivity of plants is correlated with latitude, substrate, species, and other environmental factors (Korrensalo et al., 2017). To quantify the productivity rate of plants in a greenhouse experiment, photosynthesis rate is a common parameter to be measured and estimated from gas exchange parameters (Moradi & Ismail, 2007).

CO_2 demand and CO_2 supply are two main factors for carbon sequestration in plants. CO_2 demand is determined by the photosynthesis process, which consists of the light reactions and the dark reactions (Bassham & Calvin, 1960). The light reactions include light capture and electron transport, and the dark reactions include carbon fixation (Bassham & Calvin, 1960). These two reactions could be estimated from a physiological response curve (AC_i) from the measured photosynthesis rate (An), intercellular CO_2 concentration (C_i), leaf temperature (Tleaf), and photosynthetically active radiation (PAR). AC_i curve is a stepwise function, which consists of the function of the maximum rate of eletron transport rate (J_{max}) and the maximum rate of carboxylation rate (Vc_{max}) normally (Appendix A). When the CO_2 concentration is low, the photosynthesis rate is limited by the carbon fixation rate (carboxylation rate), which is related to Rubisco enzyme (Bassham & Calvin, 1960). With the increase of CO_2 concentration, the photosynthesis rate would be limited by the regeneration rate of RuBP, which is related to electron transport (Bassham & Calvin, 1960). CO_2 supply

Considering the performance of photosynthesis rate is depended on the dark reactions and the light reactions, the impact of salinity on photosynthesis could also act on these two reactions. In terms of the light reaction, the rate of electron transport decides the efficiency of light use during the photosynthesis process. Studies found salt stress would degrade the content of photosynthetic pigment, break down the chlorophyll, and eventually influence the ability of electron transport (J_{max}) within leaves (Sudhir & Murthy, 2004; Muhammad et al., 2021). For the dark reaction, stomatal conductance (g_s) controls the amount of usable CO_2 and Vc_{max} controls the conversion rate from the inorganic carbon to the organic carbon. A reviewed article suggests that g_s was reduced under the salt stress in the root zone (Bazihizina et al., 2012), which could be controlled by phytohormone signals (Munns & Tester, 2008). The rate of carboxylation was both controlled by the support and demand of CO_2. The support of CO_2 is related to g_s, which regulate the flux of gas exchange between the plant tissue and the atmosphere. The demand of CO_2 is depended on efficiency of carbon assimilation, which is highly related to the activity of Rubisco enzyme (Jones, 1998, Goud et al., 2019). Galmés et al. (2013) found the content and the activity of this enzyme was reduced under the salt stress. In summary, an increase of salinity would inhibit both light and dark reaction, which may lead a poor performance of the photosynthesis rate.

Furthermore, salinity is toxic to non-halophytes (i.e., glycophytes) in terms of morphology (stem diameter, height, leave thickness, cellular structure alteration), physiology (carbon assimilation, electron transport, carboxylation), and molecules (protein abundance, Rubisco activity, ionic imbalance) (Wungrampha et al., 2018). Changes in any of these aspects could indirectly reveal the impact of salt stress on photosynthesis rate. Most of studies found that morphology (Udovenko et al., 1970; Zhang et al., 2016), physiology (Chaves et al., 2009; Oukarroum et al., 2015), and molecular structure (Deinlein et al., 2014; Golldack et al., 2014) had poor performance under high salinity levels for glycophytes. On the contrary, halophytes usually have better performance in salty environments. The mechanism of salt-tolerance could be either salt exclusion or inclusion (Flowers & Colmer, 2008), but most of the halophytes can retain potassium in their mesophyll cells better than the glycophytes (Ozfidan et al., 2016). Even in low salt environments, some halophytes still preserve high Na^+ concentration in their tissues to maintain shoot turgor (Shabala, 2013). Therefore, halophytes would have better resilience of stomatal conductance, electron transport rate, and photosynthesis rate under salt stress (Asrar et al., 2017).

Water table position also plays an important role in plant growth. One the one hand, flooding would have a negative effect on the photosynthesis rate through oxygen deficiency. This shortage could be caused by the low diffusion rate of gas within liquid and the consumption of carbohydrates (Mommer & Visser, 2005), which could influence the plant growth rate and solute movement between membranes (Trought & Drew, 1980; Drew, 1983). The consequence would be leaf dehydration and stomatal closure and then inhibition of the photosynthesis process (Else et al., 2001). To be more specific, the photosynthesis process is affected by the stomatal closure under the hypoxic and flooded conditions, which has been observed in at least 58 species (Sojka, 1992). Furthermore, as O_2 is the electron acceptor in the mitochondrial electron transport chain, a reduction of oxygen content within the plant tissue would rapidly impact the electron transport rate (Bailey-Serres & Voesenek, 2008). One the other hand, drought could reduce the stomatal conductance and ATP production by limiting water uptake (Flexas et al., 2007), and then influence the carboxylation during the photosynthesis (Tezara et al., 1999). Overall, both oxygen and water limitation could inhibit the process of photosynthesis, but the resistance to flood and drought would be different among the species (Sojka, 1992).

Tissue chemistry of plants can also be used to assess the combined impact of salinity and water table. Sodium (Na^+) and potassium (K^+) are two indispensable elements in assessing salt stress responses in plant tissues, while other elements are also important, such as phosphorus, and nitrogen (Smith, 1962). Under salt stress, plant growth can be usually inhibited by Na^+ toxicity (Isayenkov, 2012). An increase of Na^+ concentration could immediately restrict root growth due to osmotic stress and it would impact shoot biomass over time (Munns & Tester, 2008). When the Na^+ concentration accumulated within leaves reaches a toxic level, the photosynthesis rate would be inhibited, or this could even cause death of plants (Munns & Tester, 2008). In addition, increase K^+ concentration is usually used by plants to dilute the impact from Na^+ because these two particles have similar size (Shabala & Pottosin, 2014). The deficiency of K^+ would have a negative impact on protein synthesis, osmotic adjustment, and photosynthesis rate (Fischer, 1968; Tsay et al., 2011). Therefore, Na/K ratio is used as an indicator to assess the impact of salt stress on plants. This ratio measures the comparative concentration between Na^+ and K^+ by grinding and analyzing the plant tissue. Higher Na/K means more salt stress within plants because the cells tend to exclude K^+ when Na^+ assembles in cytoplasm when the congregated Na^+ causes membrane potential lower than the resting potential (Demidchik et al., 2014). Sodium exclusion and tissue tolerance are two major mechanisms for plants to tolerate salt stress. Sodium exclusion mechanism could prevent Na^+ accumulation in leaves (Rozema et al., 1981). Tissue tolerance could adjust sodium or chloride accumulation in their tissues with relatively high concentration (Flowers et al., 2015). If Na/K ratio is high in both shoots and roots, it indicates that the whole plant suffers the salt stress, which may apply the tissue tolerance strategy. If Na/K ratio is only high in roots, it indicates that the roots suffer the salt stress, and it uses the sodium exclusion strategy.

Phosphorus and nitrogen are two other important elements for the plant growth. Phosphorus is a fundamental component of ATP, which provides energy for carboxylation in the dark reactions (Raghothama, 2005). A study found phosphorus shortage would damage mesophyll and chloroplast and depress the photosynthesis rate in soybean (Chu et al., 2018). However, phosphorus content in eutrophic water has negative relationship with the photosynthesis rate (Quan et al., 2019). Nitrogen content is highly related to chlorophyll content, which would influence on the electron transport rate in the light reaction (Hachiya & Noguchi, 2011). This content could be measured through spectrometers, reflectometers, and imagery (Muñoz-Huerta et al., 2013). Thus, these two elements are usually compared together in the tissue chemistry analysis. If N:P is smaller than 14, the plant growth is limited by N. On the contrary, if this ratio is greater than 16, the plant growth is limited by P (Koerselman & Meuleman, 1996).

2.1.3 Objectives

To observe the response of *J. balticus* to Na^+ salt and water stress, seven salinities and two water tables were tested in the greenhouse experiment. The objective of this experiment is to study how Na^+ concentration impacts the growth of *J. balticus* and determine whether the water table interacts with the salinity to affect outcomes. The hypothesis was that the growth of *J. balticus* would be inhibited to the greatest extent by the high salinity levels with high water tables.

2.2 Methodology

2.2.1 Experimental design

The greenhouse experiment was conducted in the University of Waterloo Greenhouse at the Department of Biology. The greenhouse provided eight benches with 8 x 4 feet length and width in the room. The roof was transparent with adjustable shutters so that the irradiation of natural sunlight could be regulated based on requirements. The temperature was stable in the greenhouse, but it could be influenced by the sunlight in summer. To maintain the temperature and humidity, a mist system was installed on the roof cooperating with a fan to spray mist and create air flow within the room. Moreover, the greenhouse also provided deionized (DI) water for experiments involving water sample tests.

Before setting up the experiment, chemistry of the incubation water was calculated based on field data (Table 2.1) while the tested Na^+ concentrations were chosen based on previous studies. Vitt et al. (2020) measured the performance of another dominant species in the constructed fen, *Carex aquatilis*, under six Na^+ concentrations (14, 108, 275, 522, 1079, 2354 mg L^{-1}) and they found this species was inhibited at 2300 mg L^{-1}. Considering *Juncus* species had better salt tolerance (Al Hassan et al., 2016) and the goal of this experiment was to determine the threshold of *J. balticus*, the Na^+ concentration gradient was set as 10, 100, 300, 600, 1500, 2300, and 4600 mg L^{-1}. For other elements (Ca^{2+}, Mg^{2+}, Cl^-, K^+), concentration data of water collected in 2015 could be an ideal choice, because the vegetation community started to become stabilized in this year after first being introduced in the fen in 2013 (Kessel et al., 2018). It could be noticed that the sulfate concentration on the site was highly elevated, thus, the chemicals used in the experiment were sodium sulphate (Na_2SO_4), calcium sulphate ($CaSO_4$), magnesium sulphate ($MgSO_4$), potassium chloride (KCl), calcium chloride ($CaCl_2$), and fertilizers containing nitrogen. To calculate the required weight for each chemical based on the elemental concentration, the following formula was used in the calculation:

$$N_{chemical} = N_{element} \times (\frac{M_{chemical}}{M_{element}})$$

Where $N_{chemical}$ is the concentration of the chemical, $N_{element}$ is the concentration of the element, $M_{chemical}$ is the molar mass of the chemical, and $M_{element}$ is the molar mass of the element. The molar mass of elements and chemicals are listed in Appendix B. Because nitrates occupy 11.8% in the fertilizer, (3/0.118) mg fertilizer was needed for 1 L of the solution. The weight of Na_2SO_4 and other chemicals in 1 L of water were displayed in Appendix B. The chemicals were dissolved in DI water provided by the greenhouse to make solution for each treatment. The water table was chosen according to maximum and minimum depth in the field in 2015, which were -5 and -15 cm, respectively.

The final set-up of the greenhouse experiment included seven totes to hold the solution, 84 pots to plant *J. balticus*, and 42 small pots used as pot stands to elevate sample assigned to the -15 cm water table treatment. In total there were four replicate pots for each water table-salinity treatment combination. The volume of the solution in each tote was tested based on the volume of saturated peat soil, pots, and stands when the water level reached to -5 and -15 cm for both pots with or without stands at the same time, which was 22.5 L. Therefore, the total weight of chemicals used for each water changing is shown in Appendix B.

Seedlings of *J. balticus* were cultivated by Tree Time Services Inc., the same company who helped produce the plants for the constructed fen in 2013. The seeds were collected from the seed bank in central mixed-wood area by the company. The cultivation of *J. balticus* seedlings started in March, year and shipped to the greenhouse on June 7, 2021. Plants were transplanted to pots filled with milled peat (Premier Tech). The peat soil was soaked before planting and filled into each pot under the different water table after planting. In order to assess whether the use of milled peat would create acidic conditions, test pots were created with milled peat and the water solutions and pH was tested over two weeks by using a portable pH meter (HI 98129, Hanna instruments). In all cases, pH remained between 6.5 and 7.5, a range similar to that observed in the constructed fen (Yang, 2021). All pots with the same salinity treatment were placed in the same tote, with the deeper water table created by elevating those pots on pot stands. The cross-sectional diagram for each tote in the experiment is shown in Figure 2.1.

Table 2.1 Mean elemental concentration in pore water on the constructed fen in 2015 at 50 cm below the peat surface.

Element	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	K^+	NO_3^-
(mg/L)	150	285	90	30	780	6	3

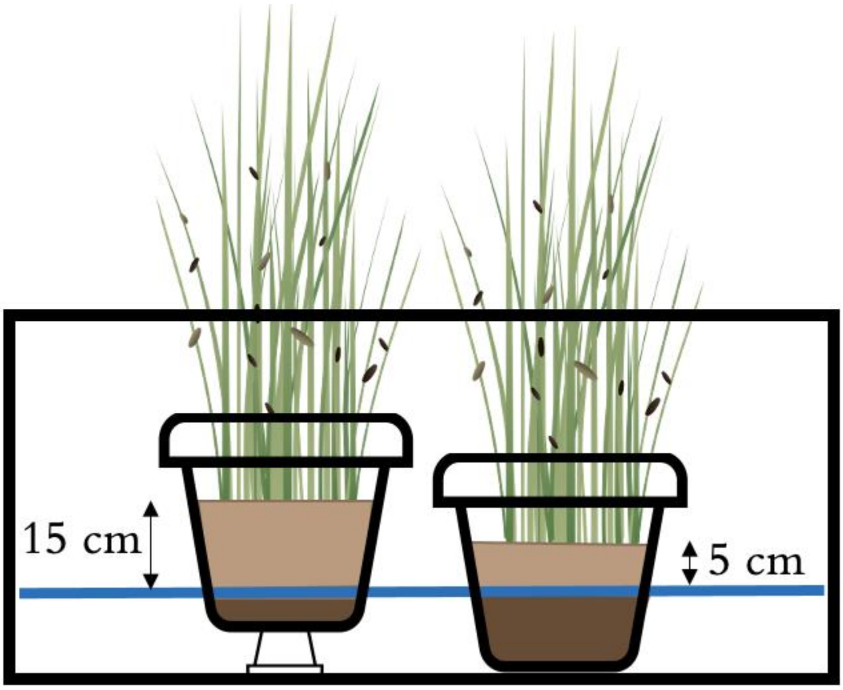

Figure 2.1 The cross-section diagram for each tote in the experiment. The dark brown color in the diagram means saturated soil, while the light brown color represents the unsaturated soil.

During the experiment, the solution with various Na^+ concentrations in each bin was changed every two weeks. DI water was added every two days between the changes to maintain the targeted water tables due to the high evaporation in the greenhouse. Totes were spatially rearranged every two weeks to minimize potential effects of spatial variation in evaporation rate and light availability in the greenhouse.

2.2.2 Water chemistry

Water samples were collected two times during the experiment from each tote. Water samples were filtered with a 0.45 µm polypropylene filter to remove suspended solids. Cation concentrations of Na^+, K^+, $Ca2^+$, and $Mg2^+$ were determined by inductively coupled plasma - optical emission spectrometry (ICP-OES, Thermo Scientific iCAP 6300). Capillary ion chromatography (IC, Dionex ICS-5000) was used for anions NO_3, SO_4, PO_4, and Cl. All samples were analyzed by the Ecohydrology Research Group at the University of Waterloo.

2.2.3 Leaf gas exchange

Leaf gas exchange was measured during week 14 of the experiment, using a CIRAS-3 portable photosynthesis system (PPSystems, Amesbury, MA) on three to four replicate plants in fourteen treatments. Net photosynthesis (A_n) and stomatal conductance (g_s) rates were measured at a PAR of 1500 µmol m^{-2} s^{-1} and at 400 ppm CO_2 concentration, with a block temperature of 25 °C. Maximum rates of carboxylation (Vc_{max}) and electron transport (J_{max}) were estimated from A/Ci curves on the same replicate plants used to measure photosynthesis. The "plantecophys" package (Duursma, 2015) was used to fit A/Ci curves by providing the data measured from the photosynthesis system. Photosynthesis rate (A), leaf temperature (Tleaf), intercellular CO_2 concentration (Ci), and PAR were taken into consideration for the curve fitting and the method was "bilinear". This model is also called Farquhar-von Caemmerer-Berry (FvCB) model and it is a piecewise function, including carboxylation rate (Vc) function, electron transport rate (J) function and triose phosphate utilization rate (TPU) function (Farquhar et al., 1980; Wullschleger, 1993).

2.2.4 Plant morphology, biomass, and tissue chemistry

The height of the longest shoot was measured in each pot every two weeks. At the end of the experiment, plants tissues (shoots, roots) were harvested and rinsed by DI water to remove the attached soils and salt crystallization. Shoots and roots were separated, dried at 60 °C for 48 hours, and weighed for biomass. For the dead plants, the shoot weight was set to zero and the root weight was the real weight of dead root. Mortality was 33/38.

To analyze the elemental content of plant tissue, the dried shoot and root samples were ground by a ball mill. The grinding jars were rinsed by DI water and air dried between each grinding. The ground samples were sent to the Natural Resources Analytical Laboratory at the University of Alberta for analysis through the ICP-OES method (iCAP 6300). Concentration for 11 elements were provided by the analysis, including, Na^+ and K^+. We calculated root and leaf Na/K as a measure of salt tolerance, which indicates a plant's ability to balance increasing Na^+ with K^+ within tissues.

2.2.5 Data analysis

R (R Core Team, 2020) and RStudio (RStudio Team, 2020) were used to analyze the data in this experiment. The variation in biomass (total, above, and belowground), root/shoot, shoot height, leaf gas exchange (A_n, g_s, Vc_{max}, J_{max}), and tissue elemental content (leaf and root

Na/K) in response to water table, salinity, and their interaction were tested using factorial analysis of variance (ANOVA). Significance between the shoot height and date was also tested in ANOVA. If a significant effect was observed, differences among treatments was tested according to Tukey's post hoc test method. Plots were generated using the "ggplot2" R package (Wickham, 2016). In order to assess linear correlations between measured variables, a correlation matrix was made based using the "*corrplot*" package (Wei & Simko, 2021) where the correlation was calculated based on "*Pearson*" correlation coefficients.

2.2.6 Greenhouse experiment schedule

Table 2.2 presents the timeline of all greenhouse measurements, including water additions, evaporation level monitoring (water table within the tote), water changes, water analysis, shoot height, gas exchange, and biomass collection. This experiment was started on June 16th and ended on September 28th. Samples were dried and weighted in October. Samples were ground in November and shipped for chemical analyzed in December. The results of the tissue chemistry was obtained in January.

Table 2.2 Greenhouse schedule. WD (water addition), EL (evaporation level), WC (water change), WA (water analysis), SH (shoot height), GE (gas exchange)

Wed	Thu	Fri	Sat	Sun	Mon	Tue
16 Start	17	18 WD	19	20	21 EL, WD	22
23 EL, WD	24	25 EL, WD	26	27	28 WC, WD	29
30 WA, WD	1	2 WD	3	4	5 EL, WD	6 SH
7 EL, WD	8	9 EL, WD	10	11	12 EL, WD	13
14 WD	15 WC	16 WD	17 WA	18	19 SH, EL	20 WD
21 EL	22 WD	23 EL	24	25	26 EL, WD	27
28 WD	29 EL	30 EL, WD	31	1	2 WD	3 WC
4 WD	5 SH, EL	6 EL, WD	7	8	9 WD	10 EL
11 WD	12	13 EL, WD	14	15	16 WD	17 EL
18 WC, WD	19	20 WD	21	22	23 EL, WD	24
25 WD	26	27 WD	28	29	30 EL, WD	31
1 EL, WD	2 EL	3 SH, WD	4	5	6 WD	7 EL
8 WD	9 EL	10 WD	11	12	13 SH, EL	14 WD
15	16 EL, WD	17	18	19	20 WD	21
22 WD	23	24 GE	25 GE	26 Biomass	27 Biomass	28End

2.3 Results

2.3.1 Treatment impacts on water chemistry

From the water samples collected in both July and August, the average concentration of K^+ and NO_3^- were slightly higher than the target concentration in some treatments), while the average concentration of Ca, Mg, and Cl were lower than the target concentration (Table 2.4 compared to Table 2.3). For the concentration of Na^+, the difference between the measured concentration and target concentration increased with salinity in 2300 and 4600 mg L^{-1} treatments, the measured Na^+ concentration only reached half of the target concentration. The measured SO_4 concentration was greater than the target concentration.

For the dry treatments (-15 cm), the range of the average water table was between -15.9 cm and -16.4 cm. For the wet treatments (-5 cm), the average water table was between -5.9 cm and -6.4 cm (Table 2.4). pH was also measured three times before the water change as shown in Table 2.5. The average pH increased in first two measurements from 3.8 to 4.3, and then remained stable at 4.2 throughout the experiment.

Table 2.3 Concentration of cations and anions from the water samples (mg L^{-1})

Month	Salinity Treatment (Target Na^+ concentration)	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	K^+	$NO3^-$
July	10	14.08	173.73	62.93	18.34	632.71	5.56	4.12
July	100	63.67	168.32	58.53	18.59	713.44	4.59	3.81
July	300	167.79	154.01	60.87	19.52	1002.30	5.67	3.33
July	600	310.54	150.51	49.11	20.15	1360.15	3.88	5.16
July	1500	915.34	180.70	62.94	18.38	2428.86	6.34	4.00
July	2300	1260.12	148.66	50.67	18.82	3154.22	4.81	3.84
July	4600	2769.00	182.09	58.64	17.17	6042.81	6.41	4.06
August	10	12.55	159.44	60.04	18.89	595.84	4.65	4.33
August	100	63.27	155.56	57.93	16.96	652.14	3.41	2.90
August	300	192.45	169.15	66.23	17.80	936.06	4.56	3.77
August	600	385.64	179.64	61.38	20.02	1391.75	5.65	5.43
August	1500	929.38	175.15	62.90	18.57	2429.31	4.25	4.86
August	2300	1355.18	181.28	70.22	19.07	3344.73	3.69	4.34
August	4600	2723.80	186.32	68.15	17.85	6248.42	<MDL	3.39

Table 2.4 Average (standard deviation) water table depth across salinity treatments from July 6 to September 13, 2021

cm\mg/L	10	100	300	600	1500	2300	4600
-15	-15.9 (0.7)	-16.1 (0.6)	-16.2 (0.8)	-16.4 (0.9)	-16.0 (0.7)	-16.3 (0.9)	-16.1 (0.8)
-5	-5.9 (0.7)	-6.1 (0.6)	-6.2 (0.8)	-6.4 (0.9)	-6.0 (0.7)	-6.3 (0.9)	-6.1 (0.8)

Table 2.5 The measured values of pH and EC in the water samples between the water changes

Date	2021-28-06	2021-07-13	2021-07-26
Salinity	pH	pH	pH
10	3.71	4	4.07
100	3.78	4.62	4.14
300	3.94	4.07	4.13
600	3.75	4.36	4.63
1500	3.99	4.27	4.24
2300	3.93	4.56	4.34
4600	3.87	4.4	4.35
Average	3.8	4.3	4.2

2.3.2 Treatment impacts on biomass and shoot height

The aboveground biomass was between 0.26 g to 4.14 g, while the belowground biomass was about two times larger than the aboveground biomass and varied from 0.43 g to 8.24 g across all water table and salinity levels (Figure 2.2). High water table had a significant negative impact on both aboveground biomass and belowground biomass (Table 2.6, Figure 2.2). There was no significant difference in the aboveground biomass or the belowground biomass with salinity (Table 2.6). Yet, both aboveground and belowground biomass was higher at 100 mg L^{-1} under the dry condition, and was lower, but similar, in the rest of salinity levels and similar for all salinity levels in the wetter condition (Figure 2.2). However, the interaction between salinity and water table was not significant for aboveground or belowground biomass (Table 2.6). Total biomass followed the same tendency as the aboveground and belowground biomass (Figure 2.2). Noticeably, the influence of water table on below-above ratio was significant in an ANOVA analysis (Table 2.6). Comparing with Figure 2.2 (D), below-above ratio was higher at wet condition in most time. There was only one data point at 4600 mg/L because the B_A ratio was infinite when aboveground biomass is zero.

Shoot length increased throughout the experiment (Figure 2.3) and there was a significant effect of the date, but variation was not significantly explained by salinity, water table, or the interaction of salinity and water table (Table 2.7).

Table 2.6 Results of a two-way analysis of variance (ANOVA) showing the effects of salinity, water table, and their interaction on biomass: Aboveground biomass, belowground biomass, total biomass, and Below/Above for *Juncus balticus* seedlings grown in a greenhouse.

Measurements	Treatments	$F_{1,24}$	P
Aboveground biomass	Salinity	2.132	0.1535
	WT	10.194	0.0030 **
	Salinity*WT	0.125	0.7255
Belowground biomass	Salinity	0.665	0.4203
	WT	6.474	0.0157 *
	Salinity*WT	0.334	0.5673
Total biomass	Salinity	1.095	0.3028
	WT	7.880	0.0082 *
	Salinity*WT	0.263	0.6113
Below:Above	Salinity	3.039	0.0919
	WT	6.492	0.0164 *
	Salinity*WT	1.239	0.2747

Table 2.7 Results of a three-way analysis of variance (ANOVA) showing the effects of salinity, water table, day of year, and their interaction on the shoot height for *Juncus balticus* seedlings grown in a greenhouse.

Measurements	Treatments	$F_{1,176}$	P
Shoot height	Salinity	2.953	0.088
	WT	4.746	0.031 *
	DOY	239.365	<0.001 ***
	Salinity*WT	0.017	0.895
	Salinity*DOY	0.868	0.353
	WT*DOY	4.568	0.034 *
	Salinity*WT*DOY	0.065	0.799

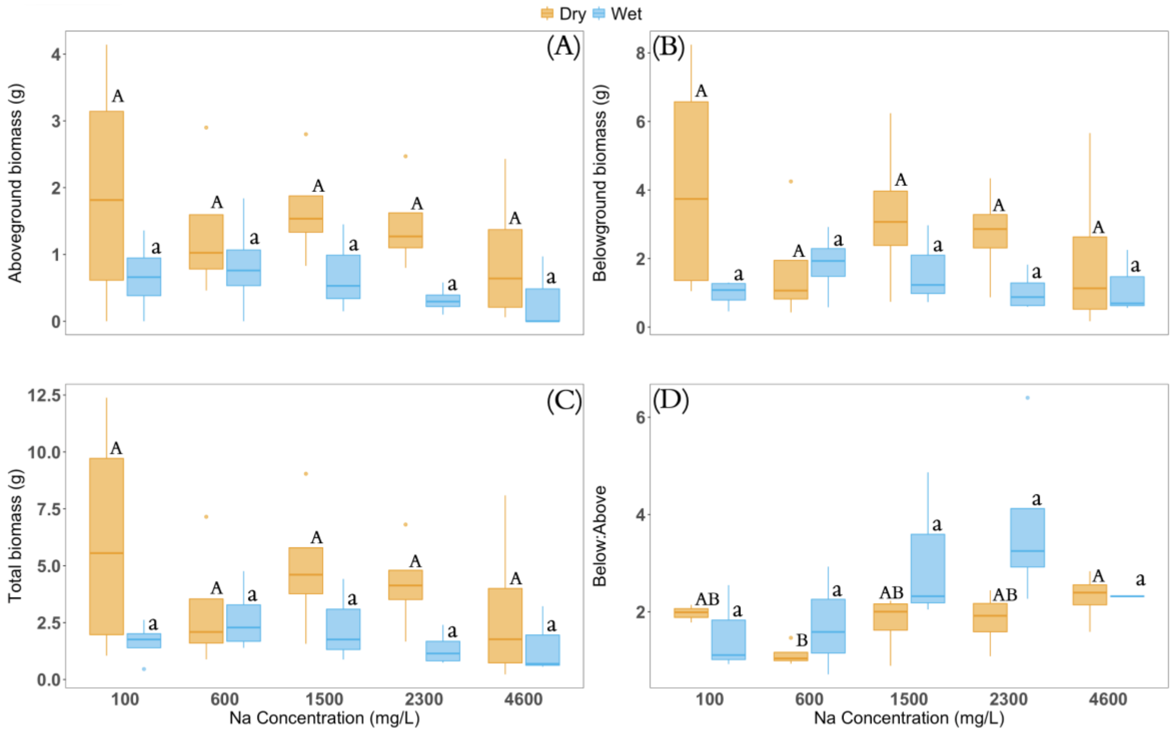

Figure 2.2 Variation in biomass in response to salinity and water table treatments. (A) aboveground biomass vs. salinity, (B) belowground biomass vs. salinity, (C) total biomass vs. salinity, (D) root:shoot vs. salinity. Wet and dry correspond to -5 cm and -15 cm water table, respectively. Letters on the top right-hand corner of each box plot indicate differences of the measurement under the wet condition (lower-case letters) and the dry condition (upper-case letters), respectively, along the Na^+ concentration gradient using Tukey's test. Treatments are significantly different if they do not share a letter.

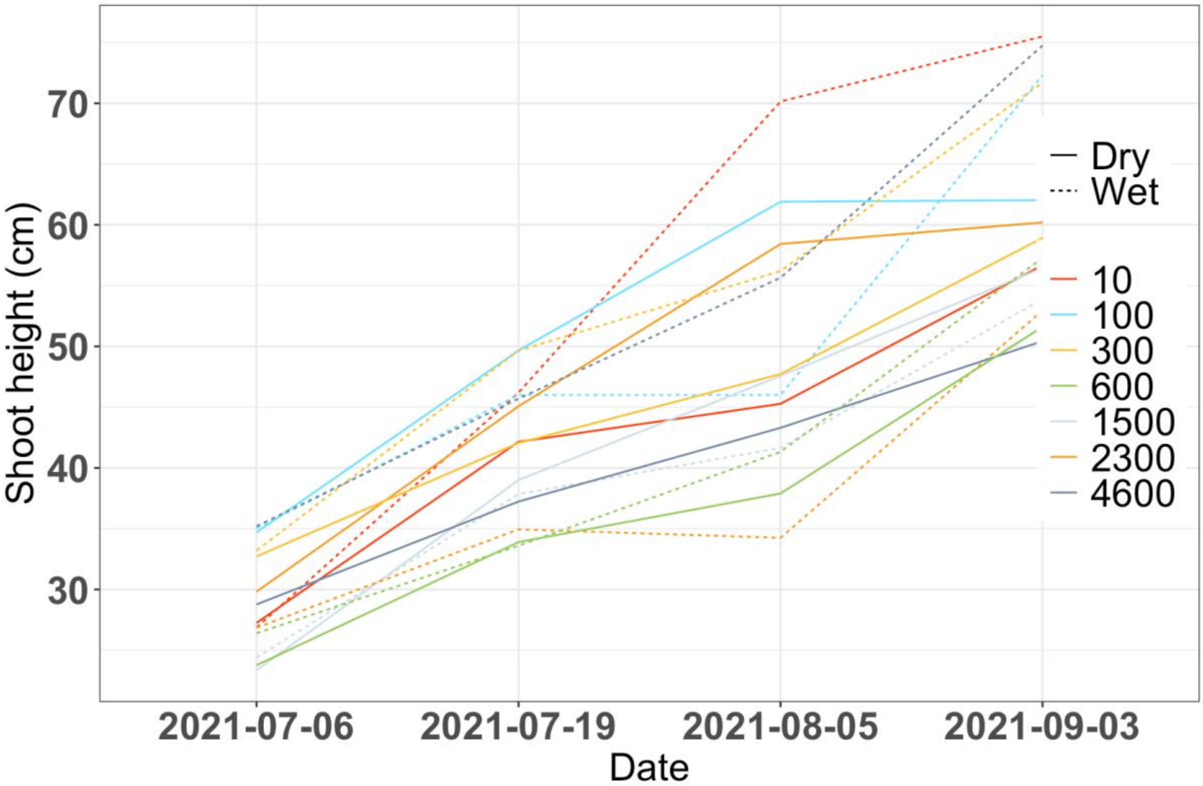

Figure 2.3 Variation in shoot height in response to salinity and water table treatments along the date. Dashed lines were the average shoot height for each salinity level in the wet condition (water table = -5 cm), and the solid lines were the average shoot height for each salinity level in the dry condition (water table = -15 cm).

2.3.2 Treatment impacts on photosynthetic parameters

To assess leaf physiological responses to the combined impacts of water table and salinity, photosynthesis rate (A_n), stomatal conductance (g_s), the maximum rate of carboxylation (Vc_{max}), and the maximum rate of electron transport (J_{max}) were measured. Variation in A_n was related to salinity and water table (Table 2.8), with lower A_n in the wet treatments overall and in the highest salinity treatment (Table 2.8, Figure 2.4A).Variation in salinity and water table position did not have a significant effect on the other photosynthetic parameters (Table 2.8),although Vc_{max}, and J_{max} were reduced under the highest salinity treatments, especially in the wet treatments (Figure 2.4C-D), while g_s was not affected by variation in water table or salinity (Table 2.8, Figure 2.4B). Moreover, the interaction between salinity and water table position did explain a significant amount of the variation in An, Vcmax, Jmax (Figure 2.4), although it was not presented in the ANOVA test (Table 2.8).

Table 2.8 Results of a two-way analysis of variance (ANOVA) showing the effects of salinity, water table, and their interaction on leaf photosynthetic parameters: net photosynthesis rate (A_n), stomatal conductance (g_s), maximum rate of carboxylation (Vc_{max}), and maximum rate of electron transport (J_{max}) for *Juncus balticus* seedlings grown in a greenhouse. Data are from individual measurements at PAR = 1500 µmol m^{-2} s^{-1}.

Measurements	Treatments	$F_{1,42}$	P
An	Salinity	5.839	0.020 *
	WT	9.671	0.003 **
	Salinity*WT	0.766	0.386
GS	Salinity	0.016	0.901
	WT	0.025	0.875
	Salinity*WT	1.300	0.261
Vc_{max}	Salinity	3.449	0.070
	WT	2.440	0.126
	Salinity*WT	0.778	0.383
J_{max}	Salinity	3.292	0.077
	WT	2.524	0.120
	Salinity*WT	1.159	0.288

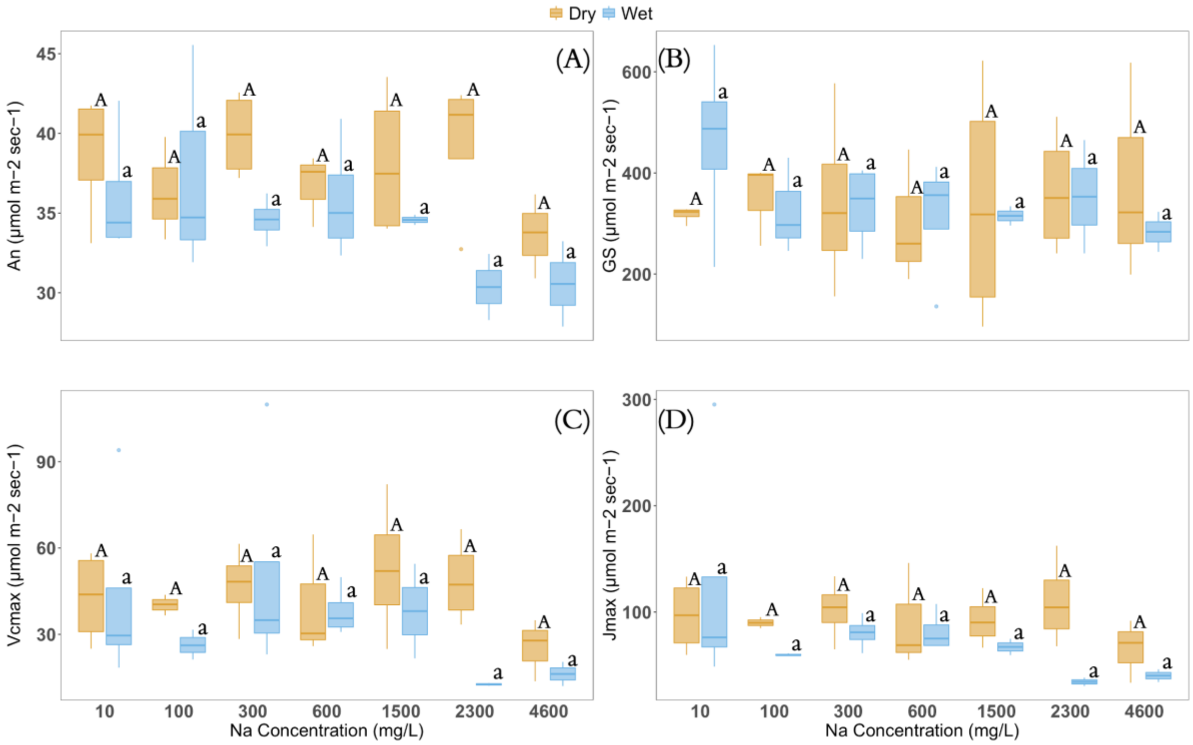

Figure 2.4 Variation in leaf gas exchange in response to salinity and water table treatments. (A) net photosynthesis rate (A_n), (B) stomatal conductance (g_s), (C) maximum rate of carboxylation (Vc_{max}),, (D) maximum rate of electron transportation (J_{max}). Letters on the top right-hand corner of each box plot indicate differences of the measurement under the wet condition (lower-case letters) and the dry condition (upper-case letters), respectively, along the Na^+ concentration gradient using Tukey's test. Treatments are significantly different if they do not share a letter.

2.3.4 Treatment impacts on tissue element content

Sodium to potassium ratios (Na:K) in shoots varied from 0.04 to 1.1, while this ratio varied from 0.13 to 6.08 in root tissue (Figure 2.5). Shoot and root Na:K varied by salinity, water table, and their interaction (Table 2.9). The difference indicated that roots generally had a higher Na:K ratio than shoots. While the ratio tends to increase under higher salinity, this change was much greater in the wet condition, particularly when Na^+ concentration was 1500 mg L^{-1} or higher (Figure 2.5). Leaf phosphorus (P) content in shoots varied with water table, but not salinity, or the interaction between the salinity and water table (Table 2.9).

Table 2.9 Results of a two-way analysis of variance (ANOVA) showing the effects of salinity, water table, and their interaction on tissue chemistry: Na:K in shoots, Na:K in roots, and P in shoots for *Juncus balticus* seedlings grown in a greenhouse.

Measurements	Treatments	$F_{1,25}$	P
Na:K in shoots	Salinity	59.73	<0.001 ***
	WT	13.98	<0.001 ***
	Salinity*WT	17.28	<0.001 ***
Na:K in roots	Salinity	18.434	<0.001 ***
	WT	14.763	<0.001 ***
	Salinity*WT	8.872	0.006 **
P in shoots	Salinity	0.002	0.965
	WT	27.154	<0.001 ***
	Salinity*WT	1.034	0.319

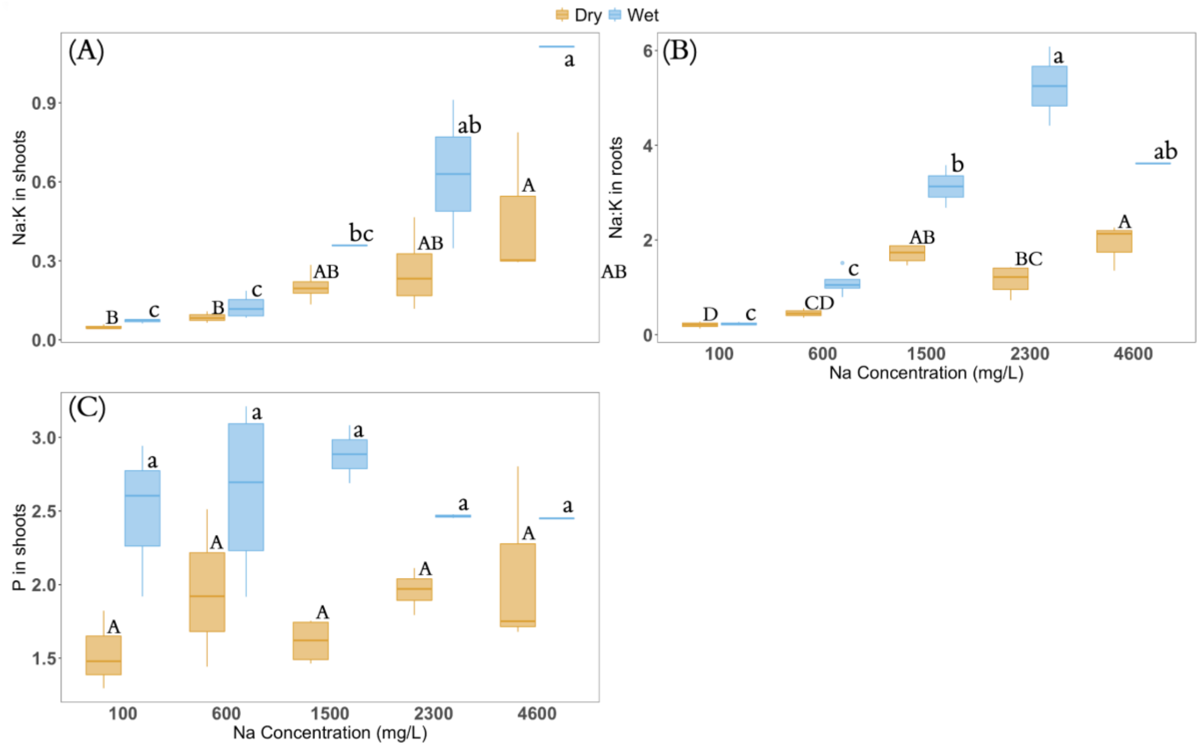

Figure 2.5 Variation in tissue chemistry in response to salinity and water table treatments. (A) Na:K in shoots vs. salinity, (B) Na:K in roots v.s salinity, (C) phosphorus in shoots vs. salinity. Letters on the top right-hand corner of each box plot indicate differences of the measurement under the wet condition (lower-case letters) and the dry condition (upper-case letters), respectively, along the Na$^+$ concentration gradient using Tukey's test. Treatments are significantly different if they do not share a letter.

2.3.5 Correlations between the dependent variables

Across all treatments, water table negatively correlated with aboveground, belowground, and total biomass, Vc_{max}, and J_{max}. Water table positively correlated with leaf P content. Salinity positively correlated with below:above biomass ratio and Na:K in roots and shoots (Figure 2.6). Leaf and root Na:K negatively correlated with photosynthetic parameters of A_n, Vc_{max}, and J_{max} but not stomatal conductance (g_s). See Appendix C for a full correlation matrix with all possible combinations of variables.

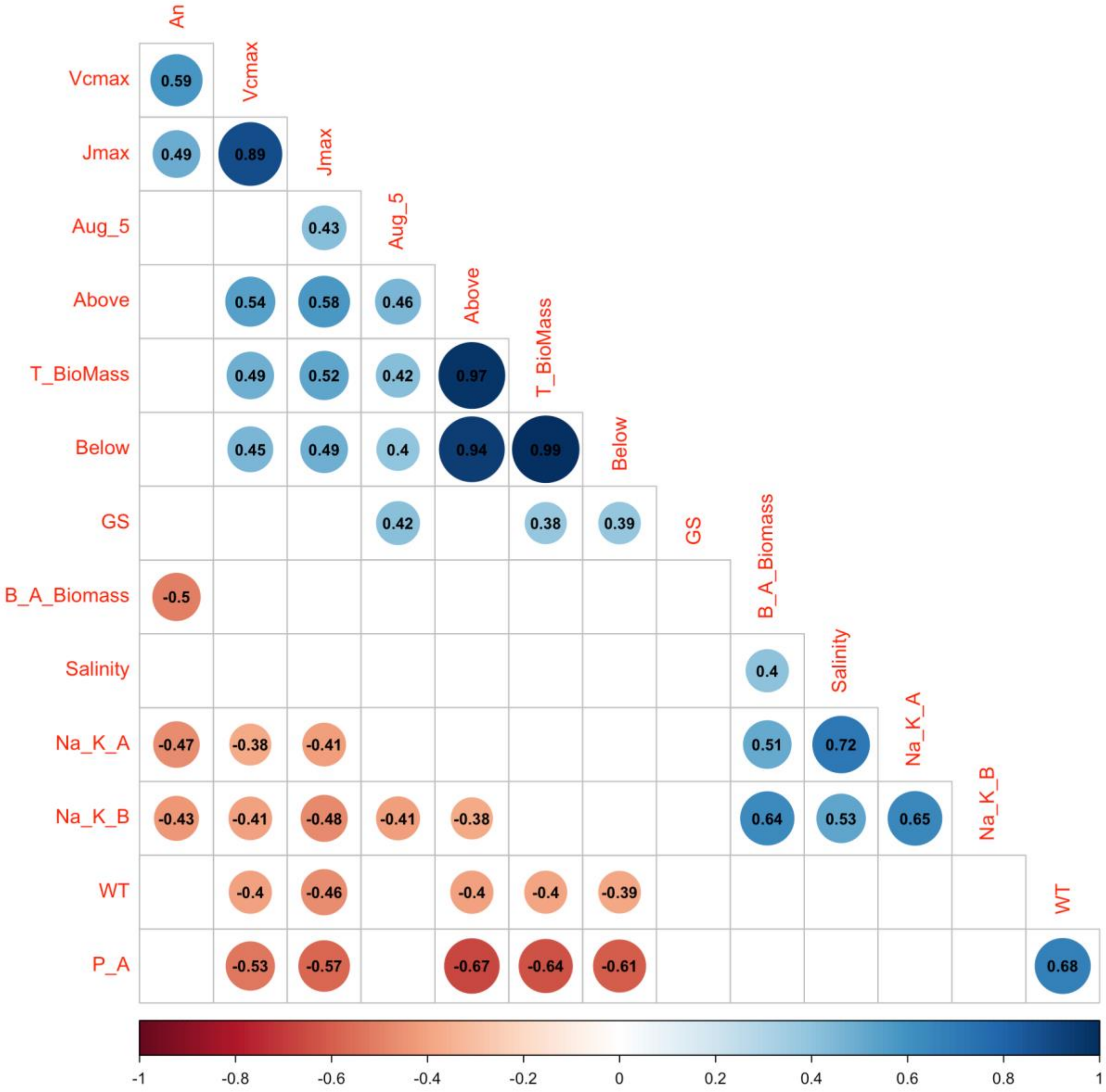

Figure 2.6 Selected greenhouse parameters correlation matrix. Statistically significant relationships (P value < 0.05) are shown in each cell by means of a correlation coefficient in various colors and sizes of circle. The darker and larger circles represent the higher correlations. Blue and red represent positive and negative correlations, respectively. An:

photosynthesis rate; Vcmax: maximum rate of carboxylation; Jmax: maximum rate of electron transport; Aug_5: shoot height on August 5th; Above: aboveground biomass; T_BioMass: total biomass; Below: belowground biomass; GS: stomatal conductance; B_A_Biomass: the ratio between belowground biomass and aboveground biomass; Salinity: Na^+ concentration; Na_K_A: Na:K in shoots; Na_K_B: Na:K in roots; WT: water table; P_A: phosphorus concentration in shoots.

2.4 Discussion

The aim of this greenhouse experiment was to assess the combined impacts of salinity and water table on *Juncus balticus* in terms of biomass, leaf gas exchange, and tissue chemistry. *Juncus balticus* was originally planted in a constructed fen after oil sands mining because it was thought to be a salt-tolerant species that would grow well under increasing saline conditions over time. However, *J. balticus* responses to variation in water table depth in combination with salinity were not necessarily considered in the planting design. The results from this greenhouse experiment suggest that *J. balticus* is relatively salt tolerant but is sensitive to high water tables. There are 332 accepted *Juncus* species (Kirschner, 2002), and responses to flooding and salinity are various among species (Al Hassan et al., 2016). For instance, *J. maritimus*, *J. acutus*, and *J. kraussii* are well studied in coastal areas under various salinities. *J. maritimus* can tolerate Na^+ concentrations up to sea water concentrations (3.5% NaCl) (Partridge & Wilson, 1987). Similarly, *J. kraussii* can survive up to 70 % sea water concentrations (Naidoo & Kift, 2006).The salt tolerant ability of J. acutus is close to *J. maritimus* in terms of shoot height and osmolyte content (Al Hassan et al., 2016). Although the impact of salinity and water table on *Juncus* species has been studied in the above research, the influence of salinity and water table on *J. balticus* in peat soils is not well studied. Furthermore, it is significant to conduct this experiment to provide valuable information for peatland reclamation projects in the oil sands region.

2.4.1 The effects of salinity

In terms of biomass, salinity inhibited the aboveground and belowground growth of *J. balticus* when the Na^+ concentration increased, but this trend stopped after 600 mg L^{-1}. Comparing with the total biomass in this greenhouse experiment, Janousek et al. (2020) found the biomass of *J. balticus* decreased between 0 and 30 ppt NaCl solution (around 11910 Na^+ mg L^{-1}). Furthermore, the biomass was higher in 0 ppt and similar at 2 and 5 ppt

(794 and 1985 Na^+ mg L^{-1}). Their findings are consistent with the results from this greenhouse experiment.

The root-to-shoot ratio slightly increased at 1500 mg L^{-1} in this experiment and varied less in the rest of salinity levels. On the contrary, Janousek et al. (2020) found a negative relationship between the root-to-shoot ratio and salinity, but this ratio was similar between 794 and 1985 Na^+ mg L^{-1}. Although the range of Na^+ concentration is different in these two experiments, the result is similar in that 600 mg L^{-1} could be the first stage of inhibition for *J. balticus* growth. Both biomass (aboveground, belowground, and total) and root-to-shoot ratio present this trend.

However, shoot height did not vary depending on Na^+ concentration. The reason may relate to sodium exclusion mechanism in these monocotyledonous salt-tolerant plants. Monocotyledonous halophytes usually exclude toxic ions, such as Na^+, from the shoots, instead of transporting and storing them in leaves like some dicotyledonous plants (Flowers et al. 1986; Rozema, 1991). Therefore, this might limit the effect of salinity on shoot height.

The impact of salinity on the photosynthesis parameters in this experiment was minimal under low Na^+ concentration, but started to be apparent at 2300 and 4600 mg L^{-1}. Although the Tukey test did not find differences between the groups, there was a noticeable decrease at Na^+ concentration of 2300 mg L^{-1} and higher under different water tables for photosynthesis rate (An), maximum rate of carboxylation (Vcmax), and maximum rate of electron transport (Jmax). Stomatal conductivity (GS) did not appear to respond to salinity and water table variation. Previous studies for photosynthesis parameters of *J. balticus* could not be found, while few studies focused on the physiological features of other *Juncus* species. Naidoo and Kift (2006) performed a greenhouse experiment on *J. kraussii* by controlling the salinity (20, 1025, 3075, 5125, 7175 mg L^{-1}) and water table (drained, flooded). They found stomatal conductance and CO_2 exchange were reduced with salinity rise, while the change was slight under high concentrations. Touchette et al. (2009) also found that *J. roemerianus* had lower stomatal conductance under salty treatments. According to Aasamaa and Sõber (2011), stomatal conductance was usually limited by hydraulic factors (air humidity, water potential) and photosynthetic factors (CO_2 concentration, light intensity). For this experiment, hydraulic factors and photosynthetic factors were maintained under the same levels in the greenhouse

(GS was chosen under 400 ppm CO_2 concentration as maintained in the photosynthesis system during measurement), therefore, that the GS did not change much is understandable if the tissue of stoma was not affected by Na^+. The fact that photosynthesis rate declined under the higher salinity treatments despite similar GS indicates a decoupling between these processes. See Section 2.4.4 for discussion of possible mechanisms.

Both the boxplots (Figure 2.5) and correlation matrix (Figure 2.6) demonstrate that salinity had a positive relationship with Na:K ratio in both roots and shoots. The boxplots also demonstrated that Na:K ratio increased with the Na^+ concentration rise. Moreover, roots had much higher Na:K ratio than shoots. Similarly, Montemayor et al. (2010) found that *J. balticus* had higher Na^+ concentration in roots (2.72) than shoots (0.98) at a saline and inundated peatland. This mechanism could protect leaves from the harm of salt, also called salt exclusion mechanism. Not only for *J. balticus*, but for *J. geradii*, the Na:K ratio recorded in shoots of was 0.56 near a saline lake, which was considered a relative low Na:K ratio (Albert, 1975). Comparing with the previous studies, the Na:K ratio in shoots and roots were in the same level, around 0.1, under low Na^+ concentration (100 mg L^{-1}). With Na^+ concentration rise, the difference of Na:K between roots and shoots increased, but the ratio was always below than 0.6 until the Na^+ concentration reached at 2300 mg L^{-1}. It could be ensured that there was sufficient potassium in the soil for uptake by the plants. Based on the element analysis table (Appendix E), the potassium concentration for all treatments in soil is stable (200 – 500 mg/kg), while it is over 20000 mg/kg within plant tissue. Therefore, one of the possible reasons could be that the absorption rate was higher for sodium than potassium during the nutrient uptake process. High Na^+ concentration within the plant tissue would be toxic to plants in terms of nutrient uptake, enzyme reaction, protein synthesis and then impact on the uptake of potassium (Rains & Epstein, 1965; Munns & Tester, 2008). In the molecular scale, salinity may have an impact on proline, soluble sugar, or other osmolytes' concentration that are used to adjust osmotic stress and retain lower Na:K ratios (Gil Ortiz et al., 2011). Al Hassan et al. (2016) also found that proline increased significantly with salinity increase, which impacted the biomass of *J. maritimus* and *J. acutus*. Therefore, Na:K is important to maintain Na^+ homeostasis of *Juncus* species.

2.4.2 The effects of water table depth

In this experiment, water table had a strong impact on the biomass. In each treatment, biomass (above, below, total) under the wet condition was always lower than the dry condition. Montemayor et al. (2008) also found that *J. balticus* had lower survival rate in wet locations with high water tables. On the contrary, Christopher et al. (2020) suggested that a short duration flooding could stimulate the productivity of this species. Kirwan and Guntenspergen (2015) also found this phenomenon in other marsh species. This response implies flooding tolerance for these marsh plants. Comparing with *J. balticus*, Kaczmarek - Derda et al. (2019) found that the aboveground and belowground biomass of *J. effusus* and *J. conglomeratus* decreased with water tables increase (-17, -14, -8 cm). Sala and Nowak (1997) set up a greenhouse experiment and studied how photosynthesis and biomass changed under two water table positions. They found that different water tables only had limited impacts on the biomass and photosynthesis rate of *J. balticus* when it had one mature leaf, while there was no difference when it had three or more mature leaves. Sala and Nowak (1997) suggested that *Juncus* species would alter their root length according to the water table depth, which could be the impact of water table on the aboveground biomass and the belowground biomass. Although the root length was not measured in the greenhouse experiment this time, the belowground biomass (Figure 2.2 (B)) was always lower in the wet condition than the dry condition, except at 4600 Na^+ concentration where the comparison was limited due to low replicates resulting from death of several plants at this high concentration.

The root to shoot ratio in this experiment was not consistent under the two water tables. However, Janousek et al. (2020) found this ratio decreased along the water table gradient. The increase of water table would decrease the available oxygen concentration, while it could improve the accessibility of nutrient (Franco et al., 2011). Therefore, it is hard to describe the influence of water table on root to shoot ratio based on the existing data.

For photosynthesis parameters, An, Jmax, and Vcmax were always lower in the wet treatments (high water table), while the variation of GS was not related to the water table conditions. Similarly, Mann and Wetzel (1999) found the GS of *J. effusus* was not impacted by the sediment saturation, but transpiration did show seasonal changes. However, Svejcar and Riegel (1998) suggested the reason could be that *J. balticus* has high proportion of root length per leaf area and it has high efficiency in water conductance, which could improve the water conduction. Furthermore, stomatal conduction was usually limited by air humidity, water potential, CO_2 concentration, and light intensity (Aasamaa and Sõber, 2011). In this greenhouse experiment, air humidity, CO_2 concentration, and light intensity were fixed variables. Water potential was not measured in this experiment, but it would be similar between two water table depth because they are both relatively shallow and not inundated. Therefore, that the stomatal conductance was not related to the water table is not unexpected. An, Jmax and Vcmax, showed similar patterns in response to the water table treatments. Although there are few studies considering the relationship between the photosynthesis rate and the water table position in *Juncus* species, Mirjat (1994) found that the photosynthesis rate was highest at -30 cm and descended with both wetter and drier conditions in other graminoid species. In addition, the preferred water table for sugarcane grass is -16 cm where it had the highest photosynthesis rate (Glaz et al., 2004), while this study also found that a short-term exposure to high water table could enhance the yield for the sugarcane. These two studies indicate that the preferred water table may vary among graminoid plant species. Based on this greenhouse experiment, the optimal water table for *J. balticus* appears to be -15 cm or deeper.

If the soil is not saturated, the ion transport ability would be higher in the wet condition than the dry condition because more nutrients and ions would be exposed to the roots when the water table is below to the soil surface. As Figure 2.5 illustrates, Na:K was always higher in the wet treatments than in the dry conditions. In contrast to this experiment, Naidoo and Kift (2006) found *J. kraussii* had higher Na:K in dry conditions than in wet, but the higher water table in their experiment was 5 cm above the soil surface. For drained soils, Al Hassan et al. (2016) found that there was no difference in Na^+ concentration for three species of *Juncus*.

2.4.3 The combined effects of salinity and water table depth

There was no obvious combined impact of salinity and water table on biomass production for *J. balticus*. It can be noticed from Figure 2.2 that the variation of biomass (aboveground, belowground, total) was related to water table position but did not depend on the combined treatments. For example, the aboveground biomass at 100 mg L^{-1} under the wet condition was equal to the biomass at 4600 mg L^{-1} under the wet condition and the biomass between these two treatments varied irregularly. This result may be attributed to the salt tolerant mechanism of the *J. balticus* in which Na^+ was excluded from the shoots by storage in roots so that the biomass of the plants along the salinity gradient was not influenced. Further, as the experiment lasted 14 weeks, biomass effects from salinity may have been less pronounced than a more prolonged exposure. But, it could be noticed that the plants growing at 4600 mg L^{-1} had more deaths than other Na^+ concentrations.

As shown in Figure 2.4, An decreased at 2300 and 4600 Na^+ concentration in the wet condition, but in the first three salinity levels, An was stable. In the dry condition, An was lowest at the highest Na^+ concentration, while in the rest of treatments, this value fluctuated. Moreover, An was always lower in the wet condition than in the dry condition. This result revealed that high water table would inhibit An under the same salinity level. The difference in An between the two water tables increased at 2300 mg L^{-1}, and decreased at 4600 mg L^{-1} again. This suggests that 2300 mg L^{-1} could be the critical value for *J. balticus* growth, as influenced by the combined factors. Under this Na^+ concentration, *J. balticus* could tolerate the salt stress under the dry condition, but the additional flooding stress broke this balance. Furthermore, the threshold of salt tolerance under the dry condition is around 4600 mg L^{-1} where An dropped dramatically compared to the previous salinity levels, while flooding stress did not have further suppression at this salinity level. Comparing with the combined impact of salinity and water table on biomass, the threshold of An was elevated to 2300 mg L^{-1} . For other photosynthesis parameters, Vcmax and Jmax had similar pattern with An, indicating these as likely drivers of the drop in photosynthesis rate. On the contrary, GS does not have any relationship with the combined factors. This phenomenon will be explained in the *decoupling between the photosynthesis rate and the stomatal conductance* section.

Na:K ratio in shoots and roots directly presents the changes of ionic concentration within the plant tissue influenced by the combined factors of salinity and water table level. It could be noticed that Na:K ratio increased with the Na^+ concentration in both shoots and roots. For Na:K in shoots, the difference of this ratio between two water tables was low (0.02 at 100 mg L^{-1}), but this disparity increased rapidly with the rise of salinity and reached 0.48 at 4600 mg L^{-1}. Moreover, the Na:K ratio at 4600 mg L^{-1} under the wet condition (1.11) was about two times higher than this value at 100 mg L^{-1} under the dry condition (0.4). High Na:K ratio could be interpreted as more salt stress suffered by the plants. Therefore, in this experiment, the highest salt stress occurred at the highest salinity level and high water table condition. Na:K in roots had similar trends in shoots, but the ratio in roots was much higher. One of the reasons could be that higher water table expanded the contact area between the roots and ions.

Montemayor et al. (2010) did a field mesocosm on a cutover bog and measured the *J. balticus* distribution under three water tables and the salinity accumulation in plant tissue in 2008 and 2010, respectively. They found *J. balticus* cannot survive in wetter regions with high water tables, and Na^+ concentration was much higher in roots than shoots. Janousek et al. (2020) had a greenhouse experiment to test the effect of salinity on *J. balticus* in brackish marsh sediment. They also had a field mesocosm to observe the influence of flooding and salinity on *J. balticus*. In these two experiments, they found the biomass of *J. balticus* decreased with increasing Na^+ concentration, while a short-term flooding could stimulate the growth of this species.

2.4.4 Decoupling between photosynthesis rate and stomatal conductance

Stomatal conductance (g_s) is a measure of the rate of leaf water loss from the leaf, controlled by the degree of stomatal opening. In a general sense, g_s strongly influences the exchange of CO_2 and water between the plant and the atmosphere because these gases enter and exit through the stomatal pores on the leaves in vascular plants. Therefore, carbon fixation rates are often coupled with g_s. In this experiment, Figure 2.4 presents that An decreased with the increase of salinity only under the wet condition, while the variation of g_s was not related to the treatments. This result may imply that the photosynthesis rate decoupled with the stomatal conductance. Although g_s is an important component of the net photosynthesis rate, biochemical reactions that are independent of gs, such as Rubisco activity and carboxylation, are also essential to this process (Jones, 1998, Goud et al., 2019).

Net photosynthesis rates are ultimately driven by CO_2 supply and CO_2 demand in the leaf level. CO2 supply is influenced by stomatal conductance while CO2 demand is influenced by metabolism (Goud et al., 2019). CO_2 supply and CO_2 demand could be represented through the intercellular CO_2 concentration in leaves (C_i) and the external CO_2 concentration in air (C_a). One of the ways to study this relationship is plotting A/ C_i curves, which demonstrate the change of carbon assimilation along the C_i gradient (Appendix A). The maximum rate of carboxylation (Vcmax), and maximum rate of electron transportation (Jmax) were calculated from CO2 response curves.It could be noticed that An was limited by the carboxylation rate when C_i was between 400 and 500 ppm, which is similar to the ambient CO_2 concentration in the atmosphere. Therefore, it may indicate that photosynthesis rate is more driven by CO_2 demand (carboxylation rate) than CO_2 supply (stomatal conductance) (Scheidegger et al., 2000; Roden & Farquhar, 2012; Goud et al., 2019).

2.4.5 The potential effects of sulphate on J. balticus growth

Sulphate concentration is another concern, which may impact the biomass of *J. balticus* in this greenhouse experiment. Although sulphur is an essential element for normal plant growth, high sulphur concentration can be harmful or even toxic (Rennenberg, 1984). According to previous field data, the sulfate concentration was very high in the constructed fen field site where the average sulfate concentration was 780 mg L^{-1}. Therefore, to best mimic the field environment, most chemicals used were sulfate salts. The sulfate concentration varied between 600 and 6000 mg L^{-1} depending on the Na^+ concentration. Lamers et al. (1998) found that sulphate concentration (192, 384 mg L^{-1}) had noticeable suppression on the regrowth of *J. acutiflorus* in week 21 and week 32 in a greenhouse experiment. Consequently, *Juncus* biomass production could be inhibited after prolonged exposure to a high sulphate environment.

In addition, sulphate could influence the photosynthesis parameters in terms of leaf content, including chlorophyll, soluble protein, and nitrogen content. Xu et al. (1996) found that the increase of sulfate from 0.1 mM to 20 mM (9.6 – 1920 mg L^{-1}) could increase chlorophyll content and nitrogen content in tomato leaves. The similar tendency was found on rice species as well (Resurreccion et al, 2001). However, Ferguson and Lee (1979) found sulphate had negative impacts on CO_2 uptake in *Sphagnum* species. This phenomenon also occurred in lichen species (Hill, 1974). The evidence from these studies suggests that the effect of sulphate varies among plant species, including between vascular plants and non-vascular plants. More research should be completed in greenhouse and field settings to better understand sulphate impacts on growth and photosynthesis.

Moreover, sulphate could be reduced to sulphide under the anaerobic condition, such as hydrogen sulphide (H_2S). H_2S can regulate metabolism, improve root differentiation, and promote proliferation (Guo et al., 2016). Numerous studies have shown that H_2S is a signalling molecule, which could strengthen the abiotic stress in roots, including growth regulation (Liu et al., 2021), ion homeostasis (Kaya et al., 2020), preventing root tip death (Cheng et al., 2013). To be more specific, H_2S could increase the activities of H_2S related enzymes (Jiang et al., 2019; Li et al., 2020), and rebuild redox balance (Lai et al., 2014) to alleviate the stress from salt and flooding. In general, sulphate as an element that cannot be ignored in the field whose impact on *J. balticus* growth is not clear yet, and which needs further research in the future.

This experiment indicates that *J. balticus* has salt tolerant ability, but it is not flood tolerant. Specifically, high water table position is likely to inhibit the growth of *J. balticus*, and this inhibition would be amplified with an increase of Na^+ concentration. Therefore, it is necessary to create some drier microsites to support the growth of *J. balticus* in the further peatland reclamation projects.

2.5 Conclusion

This greenhouse experiment tested the growth response of *J. balticus* under seven salinity levels and two water table positions, including biomass, photosynthesis parameters, and element content in plant tissue.

The impact of salinity was not obvious in biomass, but it is noticeable in the photosynthesis process and the element content. Photosynthesis rate (An), maximum rate of carboxylation (Vcmax), and maximum rate of electron transport (Jmax) decreased dramatically at 2300 and 4600 Na^+ mg L^{-1}, while the variation of GS was not related to the treatments. Na:K both in shoots and roots had a positive relationship with the Na^+ concentration. Furthermore, Na:K in roots was much higher than in shoots, which could be the mechanism of salt tolerance in *J. balticus*. These results present that the threshold of salt tolerance for *J. balticus* is between 2300 and 4600 Na^+ mg L^{-1}. In addition, loss of activity of Rubisco enzyme by salt stress was considered as the reason of decoupling between An and GS in this experiment. This result could be explained by measured Vcmax and Jmax, which had the similar trend with An. In future studies, the activity and concentration of Rubisco enzyme could be used to proof this assumption.

Comparing with the impact of salinity, water table had influence on all variables, except GS. Biomass (aboveground, belowground, total) and photosynthesis parameters (An, Vcmax, Jmax) were always lower in the high water table position (wet condition), while Na:K in shoots and roots was always higher in the high water table position (wet condition). These results suggest that high water table position has the negative impact on *J. balticus* growth.

Although the combined impact of salinity and water table was not observed in biomass and GS, this combined factor had apparent influence on the rest of the dependent variables. An, Vcmax, and Jmax decreased at 2300 and 4600 Na^+ mg L^{-1} under the high water table (wet condition), but these values only decreased at 4600 Na^+ mg L^{-1} under the low water table (dry condition). Furthermore, Na:K increased more rapidly in response to increasing Na^+ concentrations in the high water table position (wet condition) than the low water table position (dry condition). These results imply that high water table position would amplify the salt stress along the salinity gradient.

Moreover, sulphate could be another factor to influence the growth of *J. balticus* in this experiment. The toxicity of the sulphate and the function of sulphide was not well studied in this experiment or for wetland plant growth in general. Considering the concentration of sulphate in the field was very high, it is necessary to have future research on this element in the oil sands reclamation projects.

In summary, considering the increase of salinity in the constructed fen and the global climate change, it is important to understand the response of *J. balticus* to various salinity levels and the water table changes. Not only does this species spread widely in the surrounding natural saline peatlands, but also it is an ideal nurse plant for bryophytes according to the study of Borkenhagen and Cooper (2019).

Chapter 3 The combined impact of salinity and water accessibility of *Juncus balticus* growth: a field collection

3.1 Introduction

A fen is a widespread type of peatland in the Athabasca oil sands region (AOSR) of Alberta, Canada (Vitt et al., 1996). In areas of oil sands mining, the oil sand is buried up to 75 m below the surface. As the result, oil sands mining needs to dig downward to reach this layer, removing vegetation, soil and near surface geologic layers. During this process, the original landscape is destroyed, and high salinity materials will be used to backfill the excavation (Lee & Boutin, 2006), potentially causing serious consequences to the local ecosystem, such as land degradation, water contamination, and habitat loss (Smandych & Kueneman, 2013). Thus, the reclamation of landscapes in this region is essential. Several mineral wetlands were reclaimed in the past decades to re-establish wetland plant communities (Daly, 2011), but the considerable loss of organic wetlands cannot be ignored in the post mining period (Rooney et al., 2012). Nikanotee fen is one of the peatland reclamation projects established in AOSR, which was aimed to achieve the water table self-sustained through its hydrological design (Price et al., 2010). One of the concerns in the constructed fen is increasing Na^+ concentration over time because tailing sands were used as the aquifer during the construction (Price et al., 2010). Therefore, a variety of plant reintroduction strategies were tested in 2013 to introduce various freshwater and salt-tolerant species into the fen (Borkenhagen & Cooper, 2019). *Juncus balticus* is a native salt tolerant plant, which could be found in natural saline peatlands in the AOSR (Phillips et al., 2016). Borkenhagen and Cooper (2019) also suggested that this species was an ideal nurse plant for mosses. However, the proportion of *J. balticus* in Nikanotee fen has been decreasing since 2014 (Messner, 2019) and this may limit the ability of the plant community to tolerate increasing Na^+ concentrations. Given that the wet region of the fen was dominated by *Carex aquatilis* and *Typha latifolia* (Borkenhagen & Cooper, 2019), water table was assumed to an important factor that could influence *J. balticus* growth. This study investigates environmental controls on *J. balticus* growth in a constructed fen.

3.1.1 Peatland disturbance & reclamation in AOSR

In the AOSR about 50% of disturbed lands were peatlands, and most of the disturbed peatlands were fen (Vitt et al. 1996). In 2017, Alberta Environment reported that around 953 km^2 of land area was disturbed between 2009 and 2016, while reclaimed land only occupied one seventh of the total disturbed lands. In the early stage of the wetland reclamation, small marshes and shallow open water were the target to treat contaminated water. Even though these reclaimed landscapes had good performance in water purification and maintained basic ecosystem functions, they could not restore the functions belong to the original peatland ecosystems, such as carbon sequestration and peat accumulation (Daly, 2011). Considering that the AOSR was dominated by moderate-rich fen (Chee & Vitt, 1989), a considerable loss of peatlands would occur if only marsh and open water wetlands were returned post-mining (Rooney et al., 2012). Therefore, several peatland reclamation projects were started to test methods that aimed to cut down the transformation time from marsh wetlands to peatlands (Noon, 1996; Trites & Bayley, 2009b).

There were two constructed fens built in the AOSR named as Sandhill fen and Nikanotee fen (Ketcheson et al., 2016). Because the rainfall season is short with predominantly high intensity events in this region and annual evaporation is greater than annual precipitation (Keshta et al., 2012), both constructed fens needed to maintain wetness in the fen during dry periods. Further, since the post-mining landscape will need to be constructed with materials available, including tailings sand that has high sodium (Na^+) concentration, the development of saline conditions atypical of most fens in the region was also a concern. The Sandhill fen built an external reservoir as the water supply to the fen, which could provide water and was also aimed to dilute the salinity levels (Wytrykush et al., 2012). Nikanotee fen designed the hydrology based on a system geometry to optimize the area ratio between the upland and the fen and the hydraulic conductivity of a combination of layers constructed from available materials (Price et al., 2010). It was aimed to build a self-sustained system and ensure the fen could maintain enough wetness during the driest period observed in past decades according to the meteorological data. However, Na^+ has been accumulating in the fen during the water exchange process, since the tailing sands were used in the upland and as part of the aquifer under the fen (Kessel et al., 2018). Kessel et al. (2018) also found that Na^+ stored in the aquifer could be transported to the top layer of the fen peat by evapotranspiration. Although rainfall could flush some Na^+ out of the system through runoff, the Na^+ pool in the constructed fen would increase in at least next three decades (Yang et al., 2022). High concentrations of Na^+ in the fen will affect plant growth and hence carbon storage, one of the main goals of fen reclamation.

3.1.2 Gas exchange terminologies

Gas exchange directly reveals the status of carbon storage in the field, which is one of the most important functions in the peatland ecosystem. Moreover, it is also a good measure of *J. balticus* productivity used to understand the environment controls on its growth. Net ecosystem exchange (NEE), ecosystem respiration (ER), and gross ecosystem productivity (GEP) are the basic components of the CO_2 exchange within the ecosystem. NEE is defined as the net CO_2 exchange between the surface and the atmosphere. In this study we use the convention that if the value is negative, it means CO_2 storage in the ecosystem, while if the value is positive, it means CO_2 is released into the atmosphere. This parameter could be measured directly in the field through the chamber or eddy covariance technique to estimate the CO_2 exchange, but it was not used alone in most of cases because the respiration might differ between the dark and light condition (Baldocchi, 2003). The value of ER is always positive because it is a process of release of CO_2 into the atmosphere. The respiration from plants is called autotrophic respiration (AR) and the respiration from the soil produced by animals and microbes is heterotrophic respiration (HR). ER is the combination of AR and HR. The boundary between the AR and HR is not clear for root respiration because fungi and microbes may assemble at this area (Howarth & Michaels, 2000). GEP is the total carbon fixation by plants and bacteria per unit area and time, which includes photosynthesis and chemoautotrophy. Chemoautotrophy bacteria could produce carbohydrate by consuming CO_2. Nevertheless, some researchers (Howarth & Teal, 1980; Howarth, 1984) argue that chemoautotrophy should be classified as secondary production, instead of GEP. Therefore, assuming GEP is considered as the carbon fixation by photosynthesis, it should be calculated by the subtraction between NEE and ER (NEE - ER). Considering GEP is defined as CO_2 uptake by plants, GEP should always be negative. In other words, even though NEE could be positive during the measuring, GEP should be negative as long as the plants are fixing carbon through the photosynthesis.

3.1.3 The impact of salinity & water table on gas exchange

Salinity is an important driver of structure and function in wetlands. Suitable salinity could provide essential elements to plants, while high salinity may impact the stomatal conductance on leaves, which is related to the gas exchange of plants (Rajendran et al., 2009). Under salt stress, ion transport might be uncontrolled because of the concentration difference between soil and root tissue. With Na^+ accumulating in stomatal guard cells, water moves into the cells and the stoma will close due to the change of the guard cell size (Munns & Tester, 2008). As stomata control the gas exchange in plants, if the plant cannot tolerate the salt stress, the poor performance of gas exchange could be measured in plants.

Osmotic stress tolerance, tissue tolerance, and sodium exclusion are three mechanisms for plants to tolerate salt (Munns & Tester, 2008). Plants with osmotic tolerance mechanism can reduce stomatal if sufficient water is available (Abebe et al., 2003). The mechanism of tissue tolerance is to compartmentalize Na^+ from the cytoplasm to avoid reaching a toxic level (Munns & Tester, 2008). Na^+ exclusion from roots protects the plant from suffering high Na^+ concentration in the leaves, thus limiting effects on gas exchange (Munns & Tester, 2008). For *Juncus* species, *Juncus kraussii* could maintain half the rate of CO_2 assimilation measured in non-saline conditions at 3000 mg/L Na^+ concentration (Naidoo & Kift, 2006). The tissue Na^+ concentration was about threefold in roots than in shoot for *Juncus maritimus* and *Juncus acutus* at 2300 mg/L of Na^+ (Al Hassan et al., 2016). It can be concluded that the salt tolerant mechanism used by *Juncus* species is Na^+ exclusion.

For water table, both drought and flood conditions could have negative impacts on gas exchange of plants. Lack of water would cause dehydration of cells by lowering the hydraulic conductance within leaves (Laxa et al., 2019). Similarly, inundation could reduce available oxygen content within the root system to inhibit the water transport from the roots to shoot through transpiration (Sauter et al., 2013). The consequence of both drought and flood would be stomatal closure. Aerenchyma tissue is a common feature developed by wetland species to avoid the oxygen deficiency associated with saturated soils (Armstrong, 1980). This spongy tissue helps plants transport oxygen from the leaves to roots. However, this structure does not develop in all plants. According to the research of Wagatsuma et al. (1990), wet sensitive plants were not able to develop the aerenchyma tissue, and gas exchange was performed well in the species that had well-developed aerenchyma structure in the wet condition. Furthermore, Pearson and Havill (1988) found that assembling the roots close to the soil surface could ease the oxygen deficiency under inundated condition without the aerenchyma structure. Visser and Bögemann (2006) suggested that *Juncus effusus* had aerenchyma formation. Based on this finding and their general growth in wet areas, it is assumed that *Juncus* species have basic flood tolerant ability.

The response of gas exchange to the combined impact from waterlogging and salinity has not been well studied in peatland plants. Most studies only focus on one of the factors. For example, instead of focusing on the impact of water levels, in a greenhouse study with six salinity levels extending up to 2354 mg L^{-1}, Vitt et al. (2020) found that the gas exchange of *Carex aquatilis* had a negative relationship with salinity. It might be hard to separate the impact of salinity and the water table in the field, because these two factors might not be independent. In Nikanotee fen, Yang et al. (2022) suggested that salinity was higher in high water table regions, while low salinity was usually distributed in the low water table regions. In a natural saline fen, Phillips et al. (2016) found that evapotranspiration rate was higher at low water table regions and low leaf area index (LAI), which indicated gas exchange was active in dry and needle-like leaf plant-dominated regions. At the same saline fen system, Volik et al. (2020) also found that CO_2 exchange had a strong negative relationship with water depth, while they did not observe changes in gas exchange along the salinity gradient.

3.1.4 Current knowledge of Nikanotee fen and study objectives

Studies on hydrology (Price et al., 2010; Ketcheson et al., 2017), salinity (Simhayov et al., 2017; Kessel et al., 2018, Yang et al., 2022), vegetation composition (Borkenhagen & Cooper, 2019), and interaction between plants and hydrology (Scarlett & Price, 2019; Sutton & Price, 2020) or salinity, hydrology and soil carbon (Prystupa, 2020) have previously been completed in the Nikanotee fen, but there is a lack of research on the combined influence of salinity and water table on plants. Given the system design and hydrologic modelling studies, salinity is expected to rise in Nikanotee fen over time. Knowing this from the outset, both freshwater and saline-tolerant plant communities were introduced to the fen during planting in 2013 (Borkenhagen & Cooper, 2019); however, the dominant species in the saline-tolerant plantings, *Juncus balticus*, has been declining over time (Messner, 2019). This could put the plant community as risk of severe stress as salinity rises in the future. Therefore, it is important to understand how salinity and water table work together to impact plants' growth for modelling vegetation succession under changing conditions and to inform future reclamation projects. Given its preference for growth in partially saline conditions and inclusion in planting at Nikanotee fen to as a salt-tolerant species, this study will focus on assessing *Juncus balticus* response to salinity and water table position in field collections.

3.2 Methodology

3.2.1 Study site

The Nikanotee fen is a reclaimed peatland near Fort McMurray, Alberta (56°55.944′N 111°25.035′W). The area of the constructed fen is 2.9 ha, which is the low area situated in a whole reclaimed watershed whose entire area is 32.1 ha (Ketcheson et al., 2017). Tailing sands were used to build the upland slope and in the aquifer between the upland and the fen and a numerical model was used to optimize the watershed geometry so that the fen could maintain adequate wetness to support wetland function during drought periods (Price et al., 2010). In the fen, there is a 2 m thick peat layer above the 50 cm thick layer of petroleum coke, which is set between the peat layer and the tailing sands to diminish the salt discharge in the fen (Ketcheson et al., 2017). Besides the hydrological design, a planting experiment was conducted in the fen in 2013 with a variety of plant introduction techniques tested, including planting of both freshwater and salt-tolerant plant communities. *Juncus balticus, Carex aquatilis,* and *Typha latifolia* were three dominant plants in the constructed fen by 2017 (Borkenhagen & Cooper, 2019). Among them, *J. balticus* and *C. aquatilis* are the transplanted species, while *T. latifolia* is an invasive species that colonized naturally. *J. balticus* is a salt-tolerant species, which is usually found in natural saline fens, but it is sensitive to flooding (Phillips et al., 2016). *C. aquatilis* can be found in various hydrological conditions with high litter production and spread rate (Vitt et al., 2016) and *T. latifolia* is an invasive species with long distance dispersal, high germination, and strong competitiveness (Shih & Finkelstein, 2008). Borkenhagen & Cooper (2019) found that areas with *J. balticus* had the highest bryophytes richness; *C. aquatilis* had high carbon sequestration rate, but the dense litter inhibited the growth of bryophytes. For *T. latifolia*, although it had been removed mechanically once, this species was still dominant in the inundated regions in the fen. Due to the increasing dominance of *C. aquatilis* in the fen, and continued inundation in some sections, additional peat was placed in the northwest cover of the fen in winter 2017 and planted with *Juncus balticus, Betula pumila,* and *Larix laricina* (David Cooper, personal communication) in summer 2018 (Figure 3.1). This created drier conditions and allowed an investigation of the effect of water table on growth.

The goal of the fieldwork was to collect *J. balticus* growth data through in situ methods, including C flux measurements and biomass collection. There are six collars installed by former researchers for *J. balticus* planted in 2013, except J2r, which is replace in this year because the original J2 was totally replaced by *Carex* (Figure 3.1). Five collars were installed in 2021 at the new peat area for *J. balticus* planted in 2018 (Figure 3.1). To be noticed, the collar was named by the surrounding plants in the new peat area.

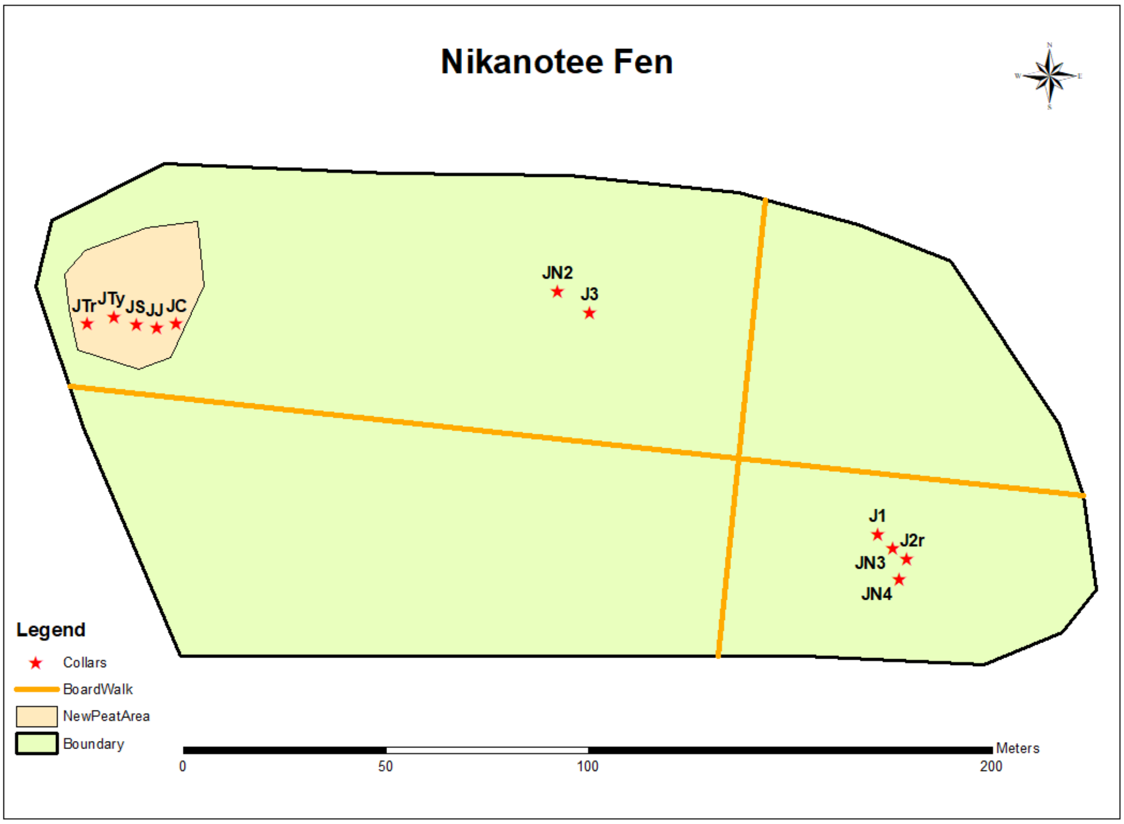

Figure 3.1 Layout of the Nikanotee Fen with collars for *J. balticus*. JC is *Juncus* by *Carex*. JJ is *Juncus* by *Juncus*. JS is *Juncus* by shrub. JTy is *Juncus* by *Typha*. JTr is *Juncus* by Tree.

3.2.2 Carbon flux

Gas exchange was measured using a portable Infrared Gas Analyzer (IRGA; EGM-4, PPSystems) in a chamber (60 × 60 × 60 cm) in which the air was mixed by a battery-operated fan. During the CO_2 concentration measurement, photosynthesis active radiation (PAR), chamber temperature and chamber humidity were recorded every 15 seconds for up to 2 minutes. The data was only valid when PAR changed less than 10% during the measurement. Next, the chamber was vented and the measurement repeated under dark conditions to determine ER while the light conditions represented net ecosystem exchange (NEE), which together were used to calculate the gross ecosystem production (GEP). NEE and ER (g CO_2 m^{-2} d^{-1}) were calculated from the linear change in CO_2 concentration in the chamber overtime using the following formula:

$$NEE = \frac{\left(\frac{Slope * 44.01}{22.414}\right) * \left(\frac{Volume}{0.58^2}\right)}{10^6} * 86400$$

Where Slope is the change of CO_2 concentration with time; 44.01 is the molar mass of CO_2; 22.414 is the standard volume of 1 mole of an ideal gas at 1 atmosphere and 0 °C; Volume is the chamber volume, which was calculated by using collar height, collar width, and chamber temperature; 0.58 is the width and length of the chamber (m), which is slightly smaller than the width and length of the collar (0.6 m); 86400 is the seconds in 1 day; and 10^6 is the outcome of the unit conversion. GEP was calculated as NEE minus ER. Height above the ground surface of the collar, and temperature inside the chamber were measured to correct the volume of the chamber. To make the data comparable among plots with different biomass, particularly in the more recently planted 2018 area, GEP was divided by the aboveground biomass, belowground biomass, and total biomass, respectively.

3.2.3 Biomass collection and tissue analysis

In the end of the field season (August 25, 2021), biomass samples were collected. A 20 × 20 cm quadrat was used for plant sampling, placed in the centre of the gas flux measurement plot. All the plants within the quadrat were harvested with a clipper and preserved in a paper bag. Soil samples and root samples were taken with a 50 cm long soil corer (a PVC tube equipped with a saw blade underneath). Each soil core was separated into three pieces (0-10 cm, 10-30 cm, 30-50 cm) and stored in plastic zipper bags. All soil samples were frozen after sampling and plant tissues were dried in an oven before shipping back to the Wetland Soils and Greenhouse Gas Exchange Lab at the University of Waterloo. At the lab, root tissues were rinsed from the soil cores and put into paper bags. Shoots and soil were put into paper bags according to collar name. All samples were dried 48 hours at 60 °C in an oven and weighed to determine biomass. Element content within shoot, root, and soil samples were analyzed at Natural Resources Analytical Laboratory in University of Alberta. ICP-OES was used and followed EPA method 6010D.

3.2.4 Environmental conditions

During each CO_2 flux measurement soil temperature at 2, 5, 10, 15, 20, 25, 30 cm was measured adjacent to the collar with a type-K thermocouple probe. Water table was determined in a stand-pipe adjacent to each plot. During soil core collection, EC and pH were measured using a multiparameter probe (Orion Star, A325) in the porewater that filled the hole once the soil core was collected. Na^+ concentration was measured using a salinity probe (ROSS 8611BNWP) in water samples collected from the hole at the field residence. A multiparameter meter (Orion Star, A324) and a sodium electrode (Thermo Scientific Orion, 8611BNWP Ross) were used to measure the salinity of the water sample. The salinity prove was calibrated with 10, 100, and 1000 ppm standards before the measurement. We used 10 mL samples for analysis and mixed with 1 mL Ionic Strength Adjuster (ISA) to adjust pH and strengthen the activity of Na^+.

3.2.4 Statistical analysis

All the data for this experiment was analyzed in R (R Core Team, 2020) and RStudio (RStudio Team, 2020). Two-way ANOVA was used to test the effect of water table and salinity on biomass, C exchange, and tissue elemental content. Furthermore, figures were plotted using "*ggplot2*" package (Wickham, 2016). In addition, non-linear regression and linear regression was used to determine the impact of water table and salinity on the dependent variables (biomass, GEP per biomass, tissue element content). Results were considered statistically significant when $p < 0.05$.

3.3 Results

3.3.1 Differences between collars

There were eleven sampling sites for *J. balticus* on the fen and distributed in northwest, middle, and southeast (Figure 3.1). Table 3.1 summarize the differences between collars in terms of plant composition, water table, salinity, and average GEP. Collars were dominated by *Juncus balticus*, *Carex aquatilis*, and shrubs (mainly *Salix* spp.). The aboveground biomass was classified into three species, but the belowground biomass could not be distinguished between *Juncus balticus* and other species. Therefore, the belowground biomass reported in this study may contain the roots from other species, particularly *Carex aquatilis* due to its abundance at the study site. The original species planted for these 11 collars was *Juncus balticus*, while other plants also grew into the collar through seed or rhizome. The lowest biomass proportion of *Juncus balticus* was occur at collar J3 (43%), but this proportion is greater than 60% in other collars. Especially in the new peat area, the percentage of total biomass that was *J. balticus* was over 90%, except JTr. Moreover, water table position was different between the regions that sort from shallowest to deepest as middle, southeast, and northwest. In addition, salinity did not have great difference between the collars. For the average GEP, newly placed collars (JC, JJ, JS, JTy, JTr) was much lower than the collars that have been placed for several years.

Table 3.1 A summary table for each collar. Biomass is total aboveground biomass in each collar. Juncus% is the percentage of *Juncus balticus* aboveground biomass to the total aboveground biomass. Ave_WT is the average water table for each collar. Salinity is the pore water Na^+ concentration after collecting the biomass. O_species is other plant species included in the aboveground collection, which is separated into *Carex* (C) and shrubs (S). Ave_GEP is the average GEP for each collar during the summer.

Collar#	Biomass	Juncus%	Ave_WT	Salinity	O_species	Ave_GEP
J1	35.73	65%	-16.7	238	C, S	-18.5
J2r	25.9	70%	-38.0	199	C, S	-7.4
JN3	37.61	88%	-31.4	194	S	-20.3
JN4	38.69	81%	-40.0	221	C, S	-11.8
JN2	28.69	61%	-13.9	113	C	-17.7
J3	37.93	43%	-10.1	356	C, S	-24.1
JC	23.87	100%	-23.8	178	NONE	-8.4
JJ	32.73	100%	-43.3	186	NONE	-8.4
JS	32.03	90%	-41	200	C, S	-9.0
JTy	35.3	92%	-34.0	186	C	-8.5
JTr	26.74	70%	-51.7	221	C, S	-7.8

3.3.2 Effect of water table and salinity on Juncus balticus biomass

The salinity measured at the end of the field season was 113 to 238 ppm, and the range of water table was -51.7 to -10.1 cm. The aboveground biomass varied from 442 to 830 g/m^2, and the weight of belowground was between 61 and 589 g/m^2. Salinity was not significant for explaining variation in belowground and aboveground biomass, but water table had a significant relationship with the belowground biomass (Table 3.2). Furthermore, neither salinity or water table had a significant impact on total biomass or above – below ratio (Table 3.2). Moreover, there was no significant interaction between salinity and water table for any of the tested dependent biomass variables as shown in Table 3.2. Besides the water table and salinity, age of *Juncus balticus* (2013 vs. 2018 planting) was another independent variable that was not significant to explain variation in biomass according to the one-way ANOVA (Above: $F_{1,9} = 0.942$, $P = 0.357$; Below: $F_{1,9} = 3.857$, $P = 0.0811$; Total: $F_{1,9} = 0.356$, $P = 0.565$; Above:Below: $F_{1,9} = 3.318$, $P = 0.102$). Across all plots, belowground biomass was significantly greater when WT was closer to the surface. Although not significant, aboveground biomass tended to decrease at shallower WT. The combined effect of WT and age for total biomass was a slight increase in biomass as shallower WT, with above:below ratio declining at wetter sites (Figure 3.2).

Table 3.2 Results of a two-way analysis of variance (ANOVA) showing the effects of salinity, water table, and their interaction on the biomass for *Juncus balticus* grown in the Nikanotee fen.

Measurements	Treatments	$F_{1,7}$	P
Aboveground	Salinity	0.686	0.435
	WT	1.422	0.272
	Salinity*WT	1.410	0.274
Belowground	Salinity	0.108	0.7521
	WT	12.687	0.0092 **
	Salinity*WT	0.190	0.6757
Total biomass	Salinity	0.131	0.728
	WT	1.045	0.341
	Salinity*WT	0.944	0.364
Above:Below	Salinity	0.028	0.873
	WT	2.911	0.132
	Salinity*WT	0.013	0.913

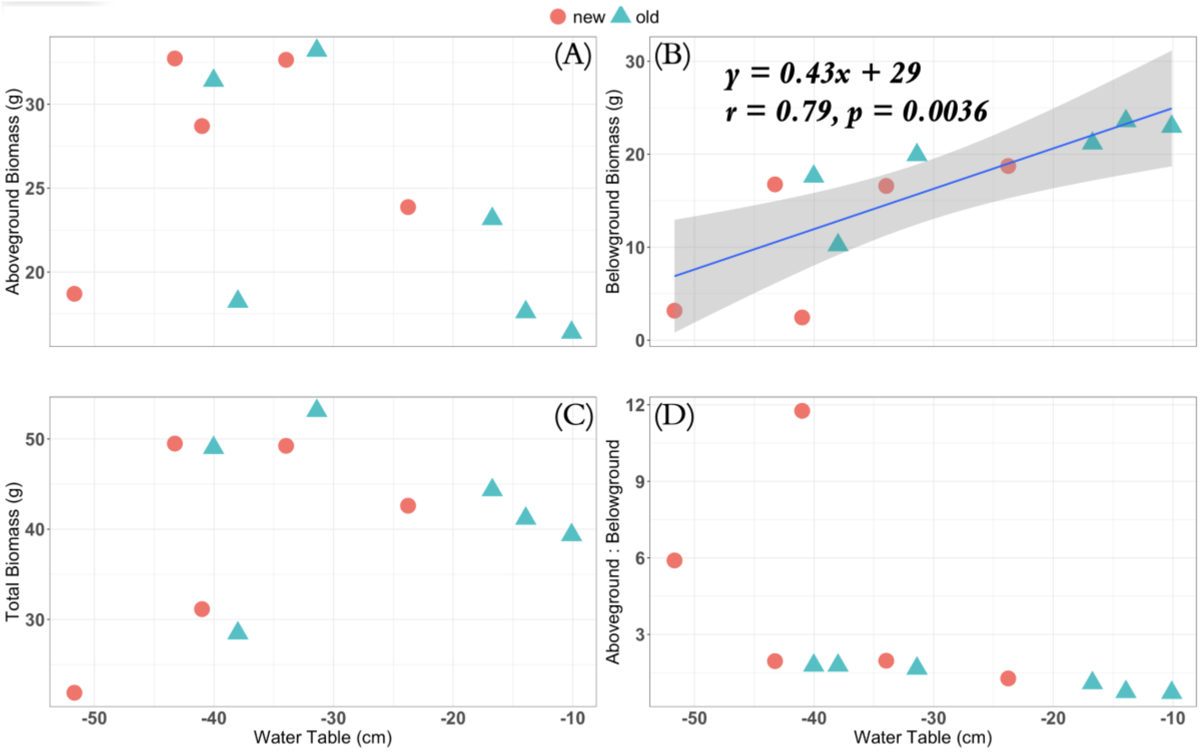

Figure 3.2 Variation in biomass in response to age and water table treatments. (A): Aboveground biomass vs .water table. (B): Belowground biomass vs. water table. (C): Total biomass vs. water table. (D): aboveground: belowground biomass vs. water table. A regression line is only shown when the relationship was statistically significant. The aboveground biomass is only for *Juncus balticus* and the belowground biomass might include the roots from both *Juncus balticus* and other species.

3.3.3 Effect of water table and salinity on Juncus balticus GEP

GEP per aboveground biomass (GEP_A) varied from -2.7 to 6×10^{-5} g CO_2 m^{-2} d^{-1}, and GEP per belowground biomass (GEP_B) changed from -7.1 to 0.0007 g CO_2 m^{-2} d^{-1}. Water table had a significant negative relationship with GEP_A and positive relationship with GEP_B , while age was only significantly related with GEP_A (Table 3.3) with the older sites having greater productivity (i.e., more negative GEP). GEP per total biomass (GEP_T) varied from -1.12 to 5.55×10^{-5} g CO_2 m^{-2} d^{-1} and it was significantly explained by both water table and age (Table 3.3). The interaction between the impact of water table and age was significant to all variables (Table 3.3). The pattern observed was that GEP per unit biomass for young *J. balticus* increased as the WT became shallower, while it decreased for the old *J. balticus* in the field (Appendix D). Moreover, GEP for most old *J. balticus* is smaller than young *J. balticus*, which indicates that old *J. balticus* has stronger carbon assimilation ability because the absolute value of GEP has the positive relationship with carbon assimilation. Considering water table position could be also substantially different between the young and old area, and possibly the interaction implies that the relationship with water table was non-linear across the range of conditions tested. Therefore, we fit a non-linear regression (Figure 3.3), which demonstrates that the ability of carbon assimilation for GEP_A and GEP_T was minimum when water table was between -40 and -25 cm, while this ability for GEP_B was lowest when water table was shallower than approximately -35 cm.

As salinity was only measured once over the growing season, we investigated its effect on GEP using the average GEP per aboveground biomass at each plot. The average GEP per aboveground biomass for each collar across the summer varied from -1.47 to -0.26 g CO_2 m^{-2} d^{-1}, which had a significant relationship with the water table and the combined impact of water table and age, while it did not have significant relationship with the salinity, or the interaction of salinity and water table (Table 3.4). Considering salinity might be influenced by precipitation during the growing season, it cannot reveal the change pattern of GEP in this analysis.

Table 3.3 Results of a two-way analysis of variance (ANOVA) showing the effects of age, water table, and their interaction on the GEP per biomass for *Juncus balticus* grown in the Nikanotee fen.

Measurements	Treatments	$F_{1,62}$	P
GEP/Above	WT	29.92	<0.001 ***
	Age	48.05	<0.001 ***
	WT*Age	31.77	<0.001 ***
GEP/Below	WT	4.86	0.0312 *
	Age	6.32	0.0145 *
	WT*Age	10.94	0.0016 **
GEP/Total	WT	5.492	0.0223 *
	Age	21.344	<0.001 ***
	WT*Age	26.076	<0.001 ***

Table 3.4 Results of a three-way analysis of variance (ANOVA) showing the effects of age, water table, salinity, and their interaction on the average GEP per above ground biomass for *Juncus balticus* grown in the Nikanotee fen.

Measurements	Treatments	$F_{1,62}$	P
Avg_GEP_above	WT	61.48	0.004 **
	Age	5.143	0.108
	Salinity	9.765	0.052
	WT & Age	12.501	0.04 *
	WT & Salinity	0.010	0.927
	Age & Salinity	1.550	0.301
	WT & Age & Salinity	0.695	0.466

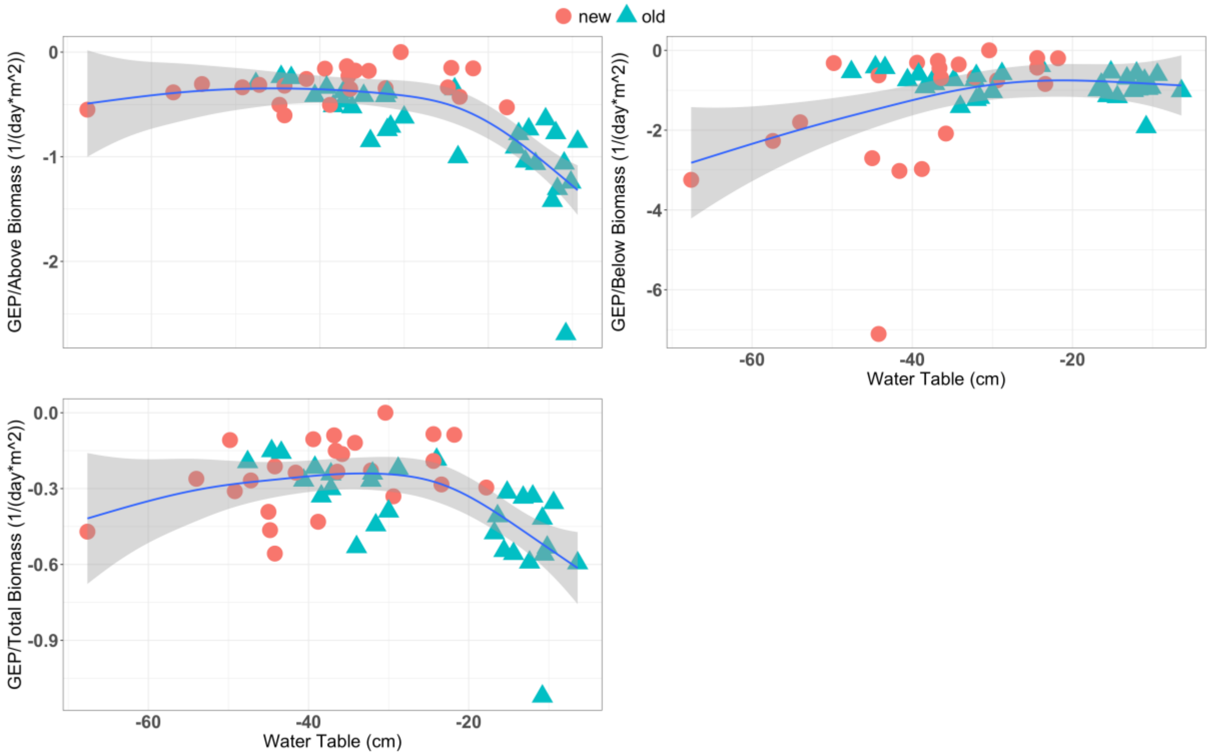

Figure 3.3 Variation in GEP per unit biomass in response to age and water table treatments. (A) GEP per aboveground biomass vs. water table, (B) GEP per belowground biomass vs. water table, (C) GEP per total biomass vs. water table. The spline regression was used to fit the scatter points by dividing the dataset into sections and applying separate models to fit these sections. The aboveground biomass is only for *Juncus balticus* and the belowground biomass might include the roots from both *J. balticus* and other species.

3.3.4 Water table and salinity impact on Juncus tissue element content

The Na:K in shoots (Na:K_A) was between 0.02 to 0.12, and Na:K in roots (Na:K_B) varied from 0.65 to 2.70. According to the three-way ANOVA analysis (Table 3.5), the impact of salinity on Na:K_A was close to the significant value, while water table and age were not have significant influence on Na:K_A. The interaction between any two groups from water table, salinity and age were significant to Na:K_A. Moreover, the combined impact of water table, age and salinity was also significant to Na:K_A. To be more specific, Na:K in shoots for young *J. balticus* decreased with the water table increase (-70 ~ -25 cm), while this value for old *J. balticus* increased with the water table increase (-40 ~ -10 cm). The minimum range of Na:K in shoots for both young and old *J. balticus* occurred between -40 and -25 cm (Figure 3.4). On the contrary, there were no treatments significant to Na:K_B (Table 3.5).

Table 3.5 Results of a three-way analysis of variance (ANOVA) showing the effects of salinity, water table, and their interaction on the tissue chemistry for *Juncus balticus* grown in the Nikanotee fen.

Measurements	Treatments	$F_{1,7}$	P
Na:K in shoots	WT	13.03	0.069
	Salinity	419.86	0.002 **
	Age	0.58	0.527
	WT*Salinity	192.97	0.005 **
	WT*Age	673.87	0.001 **
	Age*Salinity	291.35	0.003 **
	WT*Age*Salinity	110.17	0.009 **
Na:K in roots	WT	8.23	0.064
	Salinity	8.76	0.060
	Age	0.29	0.629
	WT*Salinity	4.50	0.124
	WT*Age	1.74	0.278
	Age*Salinity	4.92	0.113
	WT*Age*Salinity	0.89	0.416

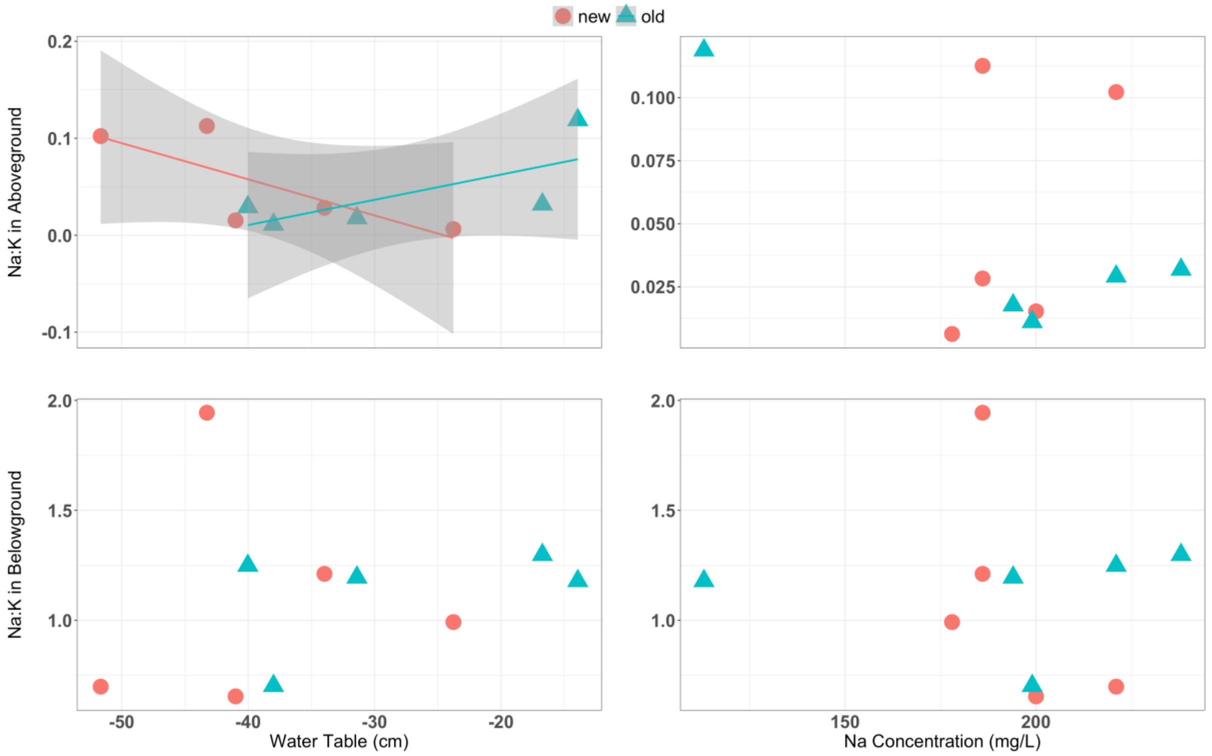

Figure 3.4 Variation in tissue chemistry in response to salinity and water table treatments. (A) Na:K in shoots vs. water table, (B) Na:K in roots vs. water table, (C) Na:K in shoots vs. salinity, (D) Na:K in roots vs. salinity. Regression lines are shown only when the relationship was significant. The aboveground biomass is only for *Juncus balticus* and the belowground biomass might include the roots from both *J. balticus* and other species.

3.4 Discussion

In the early stages of wetland reclamation in the AOSR, the goal was to store and transform the waste produced by tailing ponds into shallow open water areas (Daly, 2011). With improved understanding of wetland reclamation in terms of carbon flow, hydrology, and revegetation techniques (Crowe et al., 2001), new directions focused on engineered water bodies at larger scale and incorporating peatland reclamation to achieve the goal of "equivalent land capability" issued by the government (Environment and Parks, 2000; Ketcheson et al, 2016). The Nikanotee fen was one of the pilots for constructed fens in this region, which designed hydrology and vegetation composition on salvaged peats to realize a self-sustaining system for water supply and even carbon accumulation in the future (Price et al., 2010). *Juncus balticus* was one of the main plants introduced in the constructed fen, but its cover decreased since construction. One of the reasons could be that Na^+ concentration increased because tailing sands were used as the aquifer in the hydrological system. Another reason could be that water table was not optimal for this species across large areas of the fen due to topography or varied over time in response to weather conditions. From the result of this field collection, it could be noticed that Na^+ did not vary substantially among the plots and *J. balticus* growth more depended on the water table position. In addition, age had impact on *Juncus* growth performance because younger plants had less time to establish. To solve this problem, GEP was divided by biomass to make the data comparable. In the following paragraphs, the impact of water table on *Juncus* growth will be discussed based on the field data first and then secondary factors such as salinity, age, competition, and sulphate effects will be discussed in the following sections.

3.4.1 The impact of the water table position

Biomass is one of the most direct measures of plant growth. The result implies that only belowground biomass had a positive relationship with the water table position (Figure 3.2). However, the belowground biomass may include the root from *Carex aquatilis*, considering the coverage of *J. balticus* is higher than 70% for most of collars, the impact from other species would not be considerable. Rejmánková et al. (1999) also found that the aboveground biomass of *J. balticus* increased with water table going up in the range of -120 to -60 cm, and this species had strong recovery rate after a transformation from a dry year to a wet year. Ray and Inouye (2007) measured various species in a constructed wetland, and they found that the change of *J. balticus* biomass over time mainly relied on precipitation amount. Considering spatial patterns, Hammersmark et al. (2009) found that *J. balticus* preferred growth in semi-wet locations, not within the riparian meadow nor on the upland. Therefore, the suitable environment for *Juncus* growth in the field might be moist soil. Borkenhagen and Cooper (2019) also suggested that the next step for this constructed fen was to create some drier areas as the habitat for *J. balticus*, which could be the shelter for bryophytes. In this field collection, the aboveground biomass did not vary with the variation of water table and this pattern comes out in the total biomass (Figure 3.2). In contrast, the belowground biomass showed the opposite – higher values at the wet condition (Figure 3.2). Unfortunately, *J. balticus* in the dry areas (new peat area) are also younger than the ones in the wet areas, and the belowground biomass appears to increase continuously over time so far in the constructed fen (Messner, 2019). It is likely that the observed pattern for belowground biomass is more driven by age than the water table, although across the old sites, the belowground biomass does seem to increase under wetter conditions. Thus, the fen needs more time for plant biomass to reach equilibrium before these patterns can be fully evaluated.

It is interesting that the ratio between aboveground biomass and belowground biomass was unchanging with the water table variation, except two young planted *Juncus* points. Those outliers are understandable because the belowground biomass has not had a chance to establish. The ratio for most of samples was around 2, which means that the weight of shoots was about twice heavier than the roots. However, the converse result was observed by Ray and Inouye (2007) who found roots have greater biomass than shoots. This ratio may also relate to inundation time according to Janousek et al.'s study (2020) who noticed shoot to root ratio may decrease under long inundation time. The ratio obtained from the Nikanotee fen is reasonable because the plants need photosynthesis to support its growth in the field and there was no inundation in each plot. It is also interesting that this feature is stable under various water tables.

In terms of GEP, it is an indicator to evaluate the ability of carbon assimilation for the plants. In this experiment GEP is divided by the biomass (aboveground, belowground, and total) to obtain the weighted GEP per unit biomass. It is because the vegetation in the study area was planted in different density from the start and has grown different over time which could influence the aboveground or belowground biomass in each plot, which is unfair to compare between the collars. The result suggests that the productivity (absolute value of GEP) increased when the water table was higher than -25 cm (Figure 3.3 [A]). Comparing with this experiment, Volik et al. (2020) found the absolute value of GEP had strong negative relationship with water table in ridges ([-55] ~ [-5] cm) and depression ([-25] ~ 2 cm) at community scale in natural saline fens in AOSR. Similarly, Phillips et al. (2016) found that *J. balticus* in a saline fen in the AOSR could not uphold stable transpiration rate under flooding condition (water table is higher than the soil surface). However, Sala and Nowak (1997) did not find differences of *J. balticus* photosynthetic parameters (photosynthesis rate, stomatal conductance, transpiration) between creek side or forest edge, but the aboveground biomass was lower at the forest edge (WT at -100 ~ -150 cm) than the creek side (WT at -50 ~ -100 cm). The difference among the studies may relate to various climate conditions and study sites. In Nikanotee fen during this study, the greatest precipitation event was 30 mm and the temperature reached to 42 ºC between June and August, while the highest precipitation was over 40 mm, and the highest temperature was not higher than 25 ºC in Phillips's study site (2016). In summary, the productivity of *Juncus balticus* in this experiment was higher in moist condition (-25 to -10 cm), which could be influenced by weather condition that the temperature was higher and the precipitation was lower in this year.

For Na:K ratio in *Juncus balticus* tisse, the result reveals that Na:K ratio was lowest when WT was between -40 and -20 cm and it would increase if the water table were beyond this range (Figure 3.4). Different from Na:K in aboveground tissue, the ratio in belowground tissues did not change noticeably when the water table was low, but it slightly increased when the WT was close to the surface. This result might mean that Na^+ and K^+ absorption in roots is sensitive to flooding, which seems consistent with the greenhouse experiment in Chapter 2 (Figure 2.5), while shoots are sensitive to both flooding and drought conditions for element absorption. Comparing with this experiment, Naidoo and Kift (2006) found that Na:K was higher in their drained treatments than flooded treatments. The water table was not given in the paper, but considering the pot height was 26 cm, water table for the drained treatment would be around -26 cm and the flooded treatments would be above the soil surface (>0 cm). It seems that Na:K has an optimum range along the water table position. According to the result in this experiment and previous studies, this range could be -40 ~ -25 cm.

3.4.2 Other environmental factors

The Na^+ concentration on the site varied between 113 and 238 mg/L, which did not have huge difference in the spatial scale. Therefore, it is understandable that there was no significant correlation for salinity with biomass, GEP, or tissue Na:K. Figure 3.4 also illustrates that variation of salinities did not have a clear impact on Na:K ratio in both aboveground and belowground tissues. At this study site, Kessel et al. (2018) found that the salinity in fen peat had increased from 87 to 200 mg/L from 2013 to 2016. Yang (2021) found the average Na^+ concentration on the study site was 229 mg/L in 2019 and the concentration in the rooting zone did not have clear spatial patterns. These studies indicate that the Na^+ concentration has been rising over time in the constructed fen system due to movement of Na^+ from the tailings sand in the upland, through the aquifer and up through the peat. Precipitation could dilute the Na^+ concentration by adding more water in the soil layer (Sumner & Belaineh, 2005). In contrast, drought events would cause a loss of water from soil through evaporation so that Na^+ concentration in the root zone might increase and more sodium-rich ground water could be pulled up by evapotranspiration (Simhayov et al., 2017; Kessel et al., 2018). With the increase of Na^+ concentration in the soil, more salt stress would be placed on the plants, including ionic imbalance, cells dehydration, or stomatal closure (Munns & Tester, 2008). However, Na^+ concentrations in the field (113 - 238 mg/L) were well below the range of values that caused salt stress for *J. balticus* growth (biomass, photosynthesis rate) in the greenhouse experiment (Chapter 2). Moreover, the Na^+ concentration gradient was not enough to have a measurable impact on the tissue chemistry and productivity of *J. balticus* in this experiment. It indicates that even continued Na^+ additions to the fen in coming years are unlikely to have a clear impact on *J. balticus* growth.

Age might be another factor that would impact the growth of *Juncus* in the field. Figure 3.3 (A) also demonstrates that most of old *Juncus* had lower GEP per aboveground biomass than the new planted *Juncus*. It indicates that old *Juncus* has higher productivity than yound *Juncus*. On the contrary, Touchette et al. (2012) found that old *J. roemerianus* (60 months) had an obvious decrease of photosynthesis parameters (fluorescence parameters, electron transport rate, photosystem performance) in seawater than young treatments (6 & 24 months) when the salinity increased immediately. The reasons for the difference observed in these two experiments could be that the salinity did not have obvious change in the field and the young *Juncus* needs more time to establish in the fen.

Sulphate is another concern for the constructed fen. Murray et al. (2017) reported that the sulphate concentration in the constructed fen is much higher than the surrounding natural peatlands. Osman (2018) also found that there is a high concentration of sulphate in the site due to the tailing sands. Moreover, surface runoff due to precipitation and snow melting could bring sulphate from surrounding landscapes to the fen (Ketcheson & Price, 2016; Ketcheson et al., 2016). Besides, sulphate is formed under an aerobic environment, and it could be transformed into hydrogen sulphide (H_2S) under anaerobic conditions and suitable pH (Reddy & DeLaune, 2008). The salvaged peat used on the constructed fen had been drained for two years before being transported from the donor site to the study site (Nwaishi et al., 2015b), and this might be another reason for high sulphate concentration in the peat. This phenomenon was also noticed by Zak and Gelbrecht (2007) and Zak et al. (2009) in a rewetted fen. Based on Osman's research (2018), sulphate concentration had a strong relationship with water table. In the dry period, the sulphate concentration could reach 1000 mg/L in the fen, but it was only around 500 mg/L in the wet season. It is undoubtable that the sulphate concentration would increase every year because all the dissolve sulphate in runoff water will be deposit to the lowest topography, the fen. Although sulphate could inhibit the methane emission (Murray et al., 2017), it could be poison to the vegetation community as well (Lamers et al., 2013). For example, Lamers et al. (1998) found that there was an obvious inhibition by sulphate on *J. acutiflorus* regrowth rate under 384 mg/L after 5 months to 8 months. Therefore, limiting sulphate concentration is significant to the constructed fen ecosystem. Future research should focus on the potential effects of sulphate and sulphide concentrations on plant growth in the fen to better understand its potential influences.

3.5 Conclusion

This study presents the combined impact of salinity and water table on *Juncus balticus* growth in a constructed fen in the Athabasca oil sands. Water table at the study site had positive influence on belowground biomass. To be more specific, belowground biomass increased with water table increase between -70 and -10 cm. For GEP per aboveground biomass, the absolute value was increased when the water table position was higher than -25 cm, but lower than -10, indicating higher productivity under the moist condition. The element content demonstrated that Na:K ratio was lowest at the water table between -40 and -25 cm. It indicates that moderate water table is the suitable condition to support *J. balticus* growth. Salinity did not have a noticeable effect on *Juncus* growth within the Na^+ concentration range 113 to 238 mg/L as measured across the study site. In addition, age as another independent variable had negative impact on GEP per aboveground biomass.

Overall, water table played an important role in *J. balticus* growth in the field. In addition to the direct effect of water table on *J. balticus* growth, competition between the species may also inhibit the overspread rate at shallow water table. As the consequence, *J. balticus* dominates the low water table regions with higher biomass, but lower absolute value of GEP, compared with high water table regions. While salinity does not yet appear to be causing extensive stress to the plant community in Nikanotee fen, only about one third of total Na^+ mass in the system was estimated to have reached the constructed fen in 2019(Yang et al., 2022), which indicates that salinity would not be a concern for the *J. balticus* growth in the future. In conclusion, *J. balcitcus* has higher productivity at moist condition (-25 ~ - 10 cm), but it is dominated at the dry condition (> -25 cm), which indicates that there were other factors influence the growth of *Juncus*, such as competition. Furthermore, old *Juncus* has higher productivity based on the result of unit GEP per biomass than the young *Juncus*. Therefore, it it is essential to maintain some dry habitats and give more time for young *J. balticus* to success in the future peatland reclamation.

Chapter 4 Conclusion

This study includes a greenhouse experiment and a field collection to study the combined impact of salinity and water table on *Juncus balticus* growth in relation to fen construction in the Athabasca oil sands region (AOSR). Biomass and element content were analyzed in both the greenhouse and field samples. The difference between these two experiments is that the greenhouse experiment focused on the photosynthesis rate of *J. balticus* under controlled salinity and water table levels, while the field collection measured the gas exchange for plants and soils in each plot under the natural conditions.

The greenhouse experiment found that there was no significant impact of salinity on biomass, but biomass was always lower in wet conditions than in dry conditions. Moreover, the photosynthesis rate (An) decreased along the Na^+ concentration gradient at 2300 mg/L in the wet condition, while this rate dropped at 4600 mg/L in the dry condition. Comparing with the result of Na:K ratio in shoots and roots, whose value increased sharply along the Na^+ concentration gradient in the wet condition, it suggests that the high water table position could cause more salt stress than the low water table position when the Na^+ concentration is the same, especially at high Na^+ concentrations. The maximum rate of carboxylation (Vcmax) and the maximum rate of electron transport (Jmax) follow the same pattern with An. However, the variation of the stomatal conductance (GS) was not related to the treatments. The decoupling relationship between An and GS was assumed to be caused by decreased activity of the Rubisco enzyme under salt stress, which would result in low An and low Jmax. As the conclusion, the threshold of salt tolerance for *J. balticus* was 2300 mg/L under the high-water table position (-15 cm) and 4600 mg/L under the low water table position (-5 cm) in this experiment.

The field study found that water table had a strong relationship with *J. balticus* growth in terms of biomass and photosynthesis. The result suggests that the belowground biomass was boosted with the increase of water table. Although age plays an important role in *J. balticus* growth in that young plants need more time to establish their roots in the new peat area, the belowground biomass of old plants also presented the same tendency, increasing along the water table gradient. Furthermore, GEP per aboveground biomass reveals that the productivity of *Juncus* increases when the water table was between -25 cm and -10 cm. Given that the Na:K ratio was also lowest between -40 and -25 cm, these results suggest that moist condition is best for *J. balticus* growth. Comparing with the greenhouse experiment, Na^+ concentrations in the field (113 – 238 mg/L) were far below the threshold that would cause salt stress to *J. balticus* growth. Additionally, there was no significant relationship between the Na^+ concentration and the tissue chemistry, or productivity, of *J. balticus* in this experiment. Considering that the Na^+ concentration was one third of the Na^+ pool in the whole watershed (Yang et al., 2022), it indicates that even continued addition of Na^+ to the fen in coming years are unlikely to have a clear impact on *J. balticus* growth.

Results from the greenhouse experiment and the field collection suggest that Na^+ concentration would not be a concern for *J. balticus* growth, but the water table position would be. Although *J. balticus* shows higher producicticity according to GEP per aboveground biomass, the competition with other species would inhibit the growth of *J, balticus*. Therefore, it is important to maintain some dry habitats to ensure *J. balticus* success. Furthermore, young *J. balticus* needs more time to establish its roots on the site, thus, the fen needs more time for plant biomass to reach equilibrium before it can be fully evaluated.

Considering the concentration of sulphate was very high in the field and also in the greenhouse experiment that aimed to mimic field conditions, this is an aspect of water quality that cannot be ignored. Future studies could focus on the potential impacts of sulphate and sulphide concentrations on plant growth in the fen to have a better understanding on its potential influence on ecosystem function in future fen construction projects.

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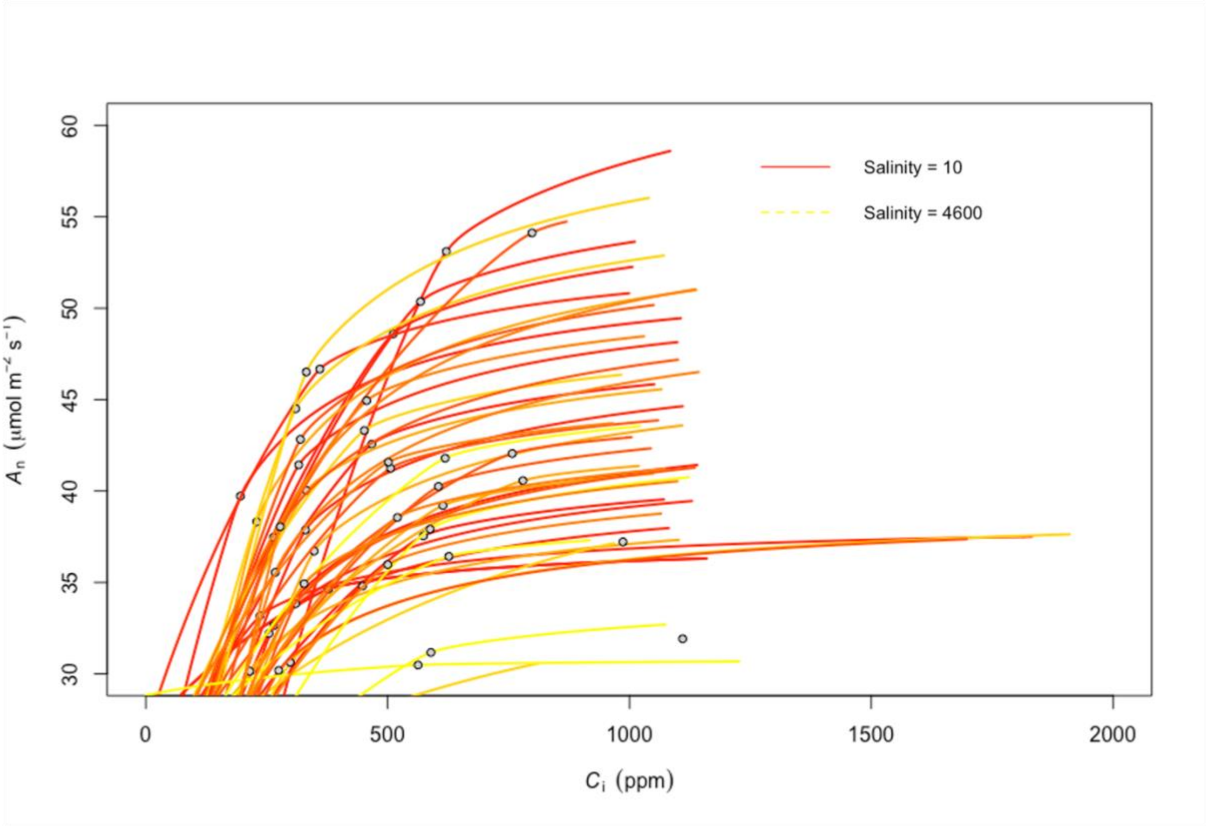
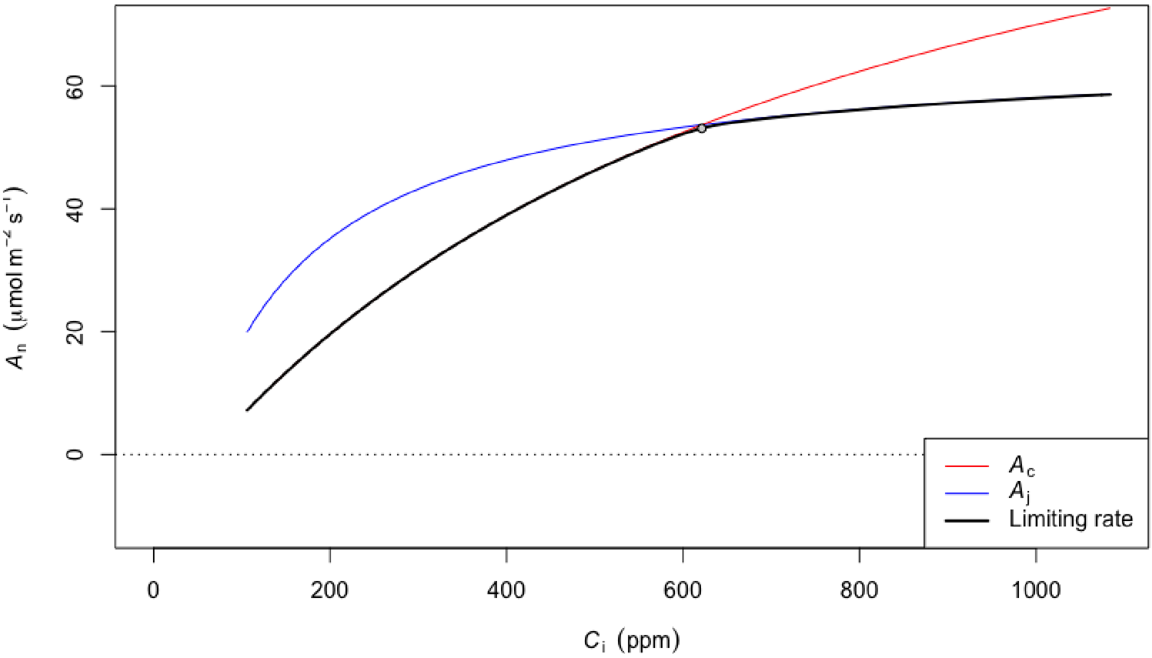
Appendices

Appendix A: ACi curve





Appendix B: Tables of Chemical Calculation

Molar mass of elements in each chemical

Chemical	Cation (g/mol)	Anion (g/mol)	Total (g/mol)
Na_2SO_4	45.98	96.06	142.04
$MgSO_4$	24.31	96.06	120.37
$CaSO_4$	40.08	96.06	136.14
KCl	39.10	35.45	74.55
$CaCl_2$	40.08	70.90	110.98

Weight of sodium sulfate in 1 L of water under various concentration

Na_2SO_4	10	100	300	600	1500	2300	4600
mg	30.9	308.9	926.8	1853.5	4633.8	7105.1	14210.2

Weight of the other chemicals in 1 L of water

Chemicals	$MgSO_4$	$CaSO_4$	$CaCl_2$	KCl	Fertilizer
(mg)	445	917	42	4.6	25.4

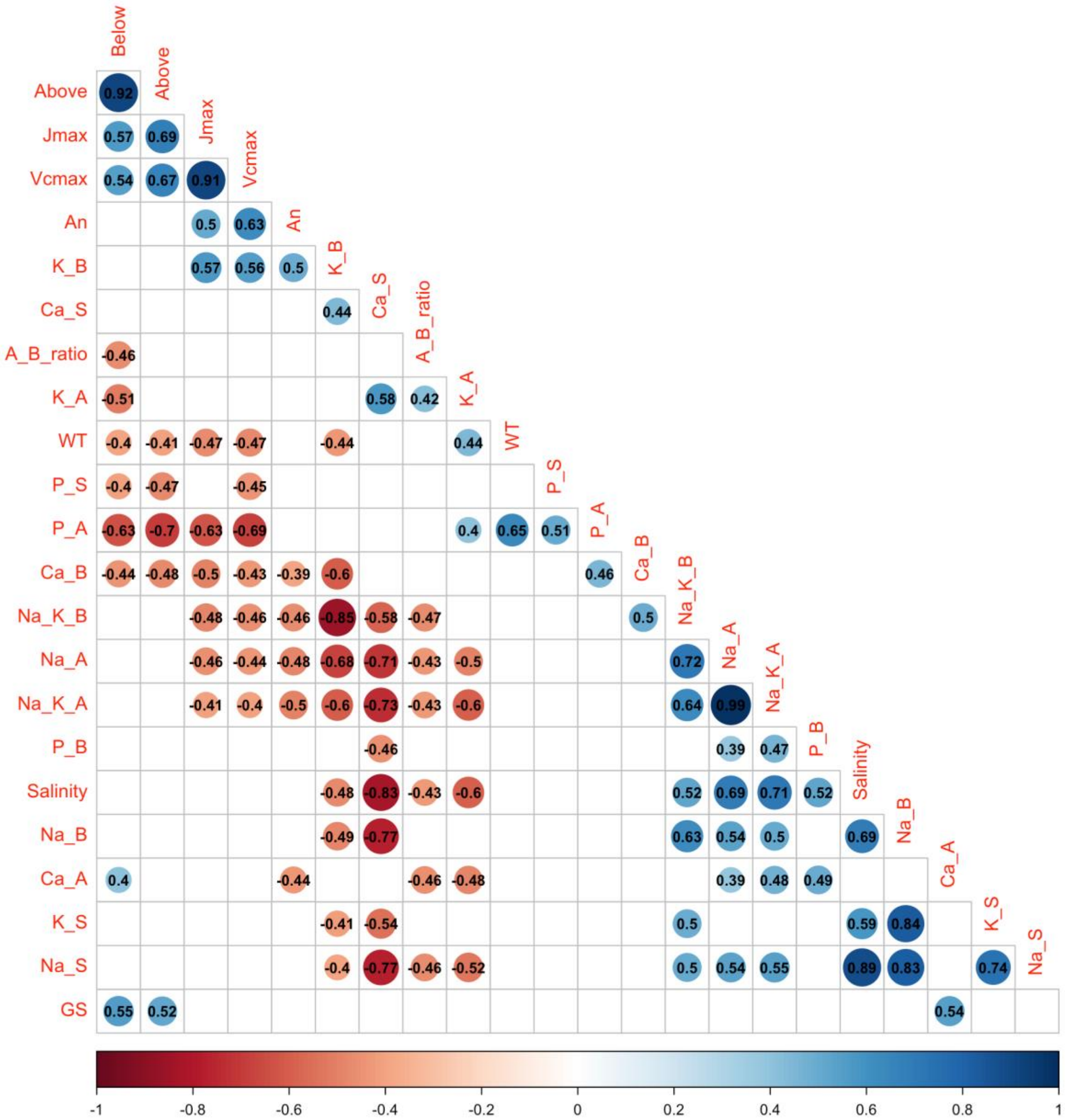
Sodium sulfate needed for each water changing

Na2SO4	10	100	300	600	1500	2300	4600
(mg)	695.25	6950.25	20853	41703.75	104260.5	159864.75	319729.5
(g)	0.7	6.95	20.85	41.7	104.26	159.87	319.73

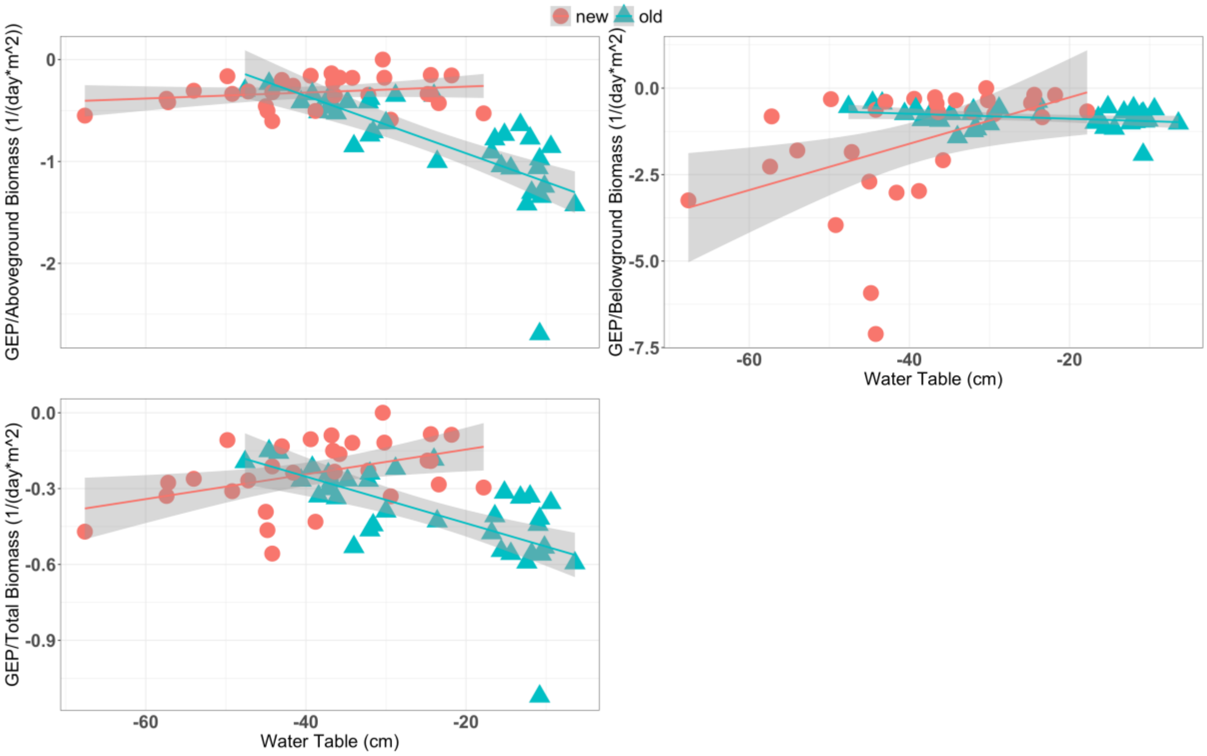
Other chemicals needed for each water changing

Chemicals	$MgSO_4$	$CaSO_4$	$CaCl_2$	KCl	Fertilizer
(mg)	10012.5	20632.5	945	103.5	571.5
(g)	10	20.63	0.95	0.1	0.57

Appendix C: All variables correlation matrix for the greenhouse experiment



Appendix D: GEP per biomass with two linear regression lines



Appendix E: element content in shoots, roots, and soil in the greenhouse experiment

Na	Sample	Pot #	Weight (g)	B 182.591 (mg/kg)	Ca 373.690 (mg/kg)	Cu 324.754 (mg/kg)	Fe 259.940 (mg/kg)	K 766.490 (mg/kg)	Mg 279.553 (mg/kg)	Mn 257.610 (mg/kg)	Na 589.592 (mg/kg)	P 177.495 (mg/kg)	S 182.034 (mg/kg)	Zn 213.856 (mg/kg)
100	B	9	0.0701	4.99	2906	9.27	287	11612	2410	75.6	3253	1229	5590	34.2
100	B	10	0.0706	5.31	1524	4.96	214	13745	1735	74.0	1827	2053	3769	51.0
100	B	11	0.0706	4.25	2267	9.56	313	12654	1697	68.0	2693	1096	4035	40.4
100	B	14	0.0711	6.33	3659	9.14	1788	13768	1800	137	2697	3044	3957	67.9
100	B	15	0.0700	6.07	5079	5.36	2288	10400	1981	114	2815	2268	4857	67.1
100	B	16	0.0703	6.05	2548	4.62	1247	9633	1618	211	2126	1940	3016	50.5
600	B	25	0.0700	5.00	2406	2.86	293	10748	1738	76.4	6021	1367	5305	37.1
600	B	26	0.0700	7.86	4303	8.57	598	13524	2835	144	6068	1957	6808	60.4

600	B	27	0.0703	9.60	3670	5.33	483	12881	2458	169	4669	2146	5547	68.6
600	B	28	0.0703	10.0	4908	6.05	395	10581	4887	110	11115	2000	12568	112
600	B	29	0.0700	7.86	6197	10.0	1978	4855	2430	108	5085	1220	6239	82.1
600	B	31	0.0703	8.89	4485	16.0	1291	9752	2733	122	7749	1857	7751	82.5
600	B	32	0.0702	7.83	7145	8.19	4332	2595	2754	75.9	3933	1537	5693	106
1500	B	33	0.0702	7.12	3232	4.27	307	8132	2132	70.9	15286	1996	11564	69.1
1500	B	34	0.0703	8.53	3374	4.27	386	7929	2826	75.0	14879	2090	11979	77.2
1500	B	35	0.0702	8.55	4373	3.56	452	10038	2650	129	16035	1356	13587	141
1500	B	36	0.0702	7.12	3085	6.41	443	11644	2399	57.7	17069	1210	14120	121

1500	B	37	0.0701	8.56	4270	4.99	1665	3965	2225	68.1	14195	2029	11842	76.0
1500	B	38	0.0700	7.14	4928	5.36	908	4065	1933	82.9	10904	2313	8319	135
1500	B	40	0.0700	10.0	7093	6.43	3014	923	2278	40.4	11578	729	14206	75.4
2300	B	41	0.0700	7.86	4469	6.43	758	10158	2491	100	10474	2194	9342	50.4
2300	B	42	0.0703	8.18	3789	4.98	479	7894	2523	107	11058	2633	9435	64.0
2300	B	43	0.0700	11.1	5556	6.07	559	8285	3267	136	11793	1939	10410	108
2300	B	44	0.0700	6.43	2114	4.64	318	14365	1833	93.9	10480	2924	8255	55.4
23	B	45	0.0702	12.5	6519	12.8	3325	2142	2691	73.0	13032	1349	11980	160

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2300	B	46	0.0702	8.90	6052	6.77	2546	2921	2650	106	12902	2157	10696	83.3
2300	B	47	0.0701	12.5	8178	11.4	6092	1326	3144	74.9	9992	1634	10994	276
2300	B	48	0.0703	10.0	5321	8.89	3665	3010	2839	76.1	10563	2622	9291	90.3
4600	B	49	0.0703	6.40	3160	11.4	515	9896	2346	63.7	13406	4079	11343	75.7
4600	B	50	0.0701	10.0	4488	7.85	590	7167	2832	103	15292	2853	12801	87.7
4600	B	51	0.0657	11.4	3963	6.47	525	10904	2578	189	7889	3692	7195	141
4600	B	52	0.0699	7.15	5827	3.93	435	6201	2264	112	14035	2024	13724	67.2

4600	B	55	0.0702	6.05	2941	5.34	574	3724	1732	85.8	13469	2973	9578	47.0
100	S	13	0.0700	5.71	7876	2.14	1387	127	1821	13.9	764	181	2459	6.79
100	S	14	0.0702	6.41	8106	2.49	2026	151	1822	19.6	692	189	2502	8.19
100	S	15	0.0700	5.36	9201	2.86	1627	115	1776	11.4	621	178	2479	8.21
100	S	16	0.0703	5.33	8330	2.49	1025	100	1567	7.47	594	162	2166	6.05
100	S	9	0.0701	7.13	8389	1.43	1549	152	2910	17.8	2077	172	5391	7.13
100	S	10	0.0699	6.08	8867	3.22	2271	341	2527	30.4	1172	180	3203	11.1
100	A	9	0.0701	9.27	3234	4.28	55.3	20989	3132	220	1224	1479	5674	45.6
100	A	10	0.0701	18.2	3060	17.5	70.6	23451	2898	240	1014	1822	5553	51.7
100	A	11	0.0700	22.9	5248	4.64	95.7	22253	3730	370	984	1296	6975	47.1

100	A	14	0.0703	11.0	3193	4.27	70.4	30099	2437	228	1868	2943	6793	63.7
100	A	15	0.0702	15.7	3345	4.27	70.5	25536	2342	205	1978	2604	6020	50.6
100	A	16	0.0701	12.8	3092	6.06	84.9	25993	2440	233	1955	1920	6617	66.7
600	S	29	0.0704	4.97	7454	2.13	1111	249	1289	5.68	2409	173	2463	4.97
600	S	30	0.0704	7.46	6729	1.42	1368	313	2162	16.0	3144	178	3922	5.68
600	S	31	0.0700	7.14	6683	1.43	1692	229	1941	10.0	3862	166	4178	5.36
600	S	32	0.0703	5.69	7395	1.78	1402	233	1493	7.47	3210	190	3579	5.69
600	S	25	0.0700	5.36	7036	1.79	1322	184	1647	11.4	4399	168	4602	5.71
600	S	26	0.0704	5.68	7045	1.78	1394	179	1479	11.4	3343	175	3395	6.39
600	S	27	0.0703	6.05	6357	1.78	1384	177	1662	13.2	3198	171	3179	7.11

600	S	28	0.0705	4.96	6303	1.42	1404	159	1295	11.7	2770	160	2817	6.03
600	A	25	0.0705	13.5	2692	3.19	50.0	22532	2498	294	1461	1442	5905	63.1
600	A	26	0.0702	11.0	2055	3.21	62.7	24610	2411	249	2032	1921	5816	74.8
600	A	27	0.0701	10.3	2114	4.28	75.6	24962	2432	307	2697	2512	5795	79.2
600	A	28	0.0703	9.60	1915	3.20	58.3	24727	2307	248	2301	1917	5693	65.4
600	A	29	0.0701	9.27	2596	3.21	93.4	26783	1940	336	2254	3054	5553	87.0
600	A	31	0.0701	15.0	3316	3.92	66.0	25246	2428	247	3566	2336	8482	90.9
600	A	32	0.0700	9.64	2807	4.64	80.0	26916	2434	211	5018	3211	8889	93.9
1500	S	37	0.0702	6.77	6464	1.78	1780	394	1397	10.7	8100	202	5688	7.48
15	S	39	0.0702	5.34	6182	2.14	1259	271	1088	7.12	5500	178	3415	6.41

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1500	S	33	0.0701	7.85	6218	1.78	1523	447	2338	16.0	11019	163	9325	7.85
1500	S	34	0.0705	8.51	5306	1.77	1430	425	2485	18.8	11822	168	9446	8.16
1500	S	35	0.0703	7.47	5921	1.78	1525	393	2203	16.4	9017	174	7251	8.18
1500	S	36	0.0703	8.18	6140	1.42	1256	383	2963	15.6	18541	156	15558	7.82
1500	A	33	0.0702	18.5	3311	3.92	255	24728	2827	316	3323	1754	9019	92.6
1500	A	34	7.0000	18.9	2772	3.93	174	25524	2607	255	4875	1741	9281	80.7
1500	A	35	0.0701	13.6	2147	2.14	154	24756	3034	230	7039	1500	11289	84.9

1500	A	36	0.0703	17.1	2563	3.20	89.6	22531	2615	230	4484	1464	9476	70.8
1500	A	37	0.0702	15.3	2474	2.49	82.3	27654	2374	173	9843	2689	12810	76.2
1500	A	38	0.0703	13.9	2587	3.91	122	26916	2762	266	9723	3083	12759	98.5
1500	A	40	0.0705	15.2	2373	3.55	159	25423	3850	174	12811	2984	17302	79.4
2300	S	28	0.0703	4.98	6282	2.84	1446	270	1135	6.76	5522	171	3656	6.40
2300	S	45	0.0703	6.05	4953	3.20	968	296	1272	8.89	11858	168	7964	6.05
2300	S	46	0.0701	6.78	4326	2.50	898	311	1249	9.27	12081	167	7460	6.06
23	S	47	0.0701	5.71	5523	2.85	1327	276	1227	10.0	9567	185	6607	6.06

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2300	S	48	0.0702	7.48	5072	2.85	1325	290	1394	13.5	10312	189	7150	6.41
2300	S	40	0.0703	4.98	7006	2.84	1605	256	2035	17.4	651	174	2439	7.47
2300	S	41	0.0700	5.00	4852	3.21	1385	264	1402	8.57	12636	161	8953	6.07
2300	S	42	0.0704	4.62	5214	2.84	1477	279	1511	9.59	11906	172	8528	6.39
2300	S	43	0.0702	4.99	5840	3.21	1490	286	1404	13.5	11045	166	8157	6.41
2300	S	44	0.0706	4.60	5175	3.19	1459	251	1346	9.21	11602	171	8078	6.37
2300	A	41	0.0704	16.7	2914	6.04	63.6	22174	2858	294	4090	1793	11100	79.5

2300	A	42	0.0703	18.1	3219	5.69	77.5	20000	3691	374	9307	1927	14314	150
2300	A	43	0.0705	13.8	2267	7.45	84.0	21120	2372	284	5925	2112	8807	110
2300	A	44	0.0700	12.1	1878	4.64	80.7	24948	2714	269	2941	2014	8924	96.1
2300	A	45	0.0700	19.3	2603	6.79	100	25554	2494	215	8884	2478	13689	71.1
2300	A	46	0.0700	19.3	3793	7.14	93.2	20440	3755	229	18623	2452	21827	111
2300	A	47	0.0331	23.4	1914	9.82	479	20273	2660	199	14852	2115	16735	104
2300	A	48	0.0703	26.0	2695	6.76	127	21082	2426	267	15134	2574	14958	115
46	S	53	0.0700	5.00	4728	4.29	1788	357	941	6.07	19385	200	12151	6.79

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4600	S	54	0.0704	5.33	4545	3.20	1839	418	1030	7.10	22663	210	15041	6.39
4600	S	55	0.0700	4.64	4003	3.57	1786	313	764	6.79	14436	185	7974	9.64
4600	S	56	0.0705	5.67	4515	3.19	1956	337	939	7.45	18218	199	11763	6.03
4600	S	49	0.0702	6.41	2956	2.85	1399	358	1036	9.6	19296	170	12221	6.41
4600	S	50	0.0703	6.05	4536	3.56	1356	367	1427	13.2	16287	174	11600	7.47
4600	S	51	0.0700	6.79	5209	2.50	1478	423	1624	16.1	17697	186	13201	7.14
4600	S	52	0.0705	6.03	6008	2.84	1626	448	2199	12.4	25645	171	21418	6.74

4600	A	49	0.0701	24.6	4781	5.35	75.2	16402	4248	203	12918	1679	18536	103
4600	A	50	0.0702	24.6	3195	5.70	201	20926	3600	421	6335	2803	12034	141
4600	A	51	0.0373	21.4	2170	4.02	690	23277	1883	396	2729	2231	9243	91.2
4600	A	52	0.0702	14.6	2392	3.92	81.9	23638	3551	256	6973	1751	14126	128
4600	A	55	0.0700	22.9	3219	8.93	66.8	20479	4766	260	22801	2450	25749	103