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The combined effects of NaCl and beet juice on germination and early seedling growth among Raphanus sativus L.

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ABSTRACT

Salt pollution and soil salinization via secondary pathways is a global problem. One practice which contributes to increased salinity in soils and other natural systems is the application of road deicers in colder regions. Increased salinity in soils negatively affects plant growth and microbial activity. In an effort to decrease the amount of salt applied to roads, salts are applied as brine. Liquid organic additives are incorporated into these brines to foster better adherence of the salt to impermeable surfaces. One common additive is beet juice, a byproduct of sugar extraction. Beet juice contains high levels of phosphorus and organic sugars, and thus might have beneficial effects on soil microbial activity and plant growth, mitigating some negative effects of salt. To investigate the combined effects of NaCl and beet juice on germination and early growth of plants a series of treatments comprising combinations of no, 'low'(2g), or 'high' (7g) NaCl and no, 'low'(5mL), or 'high' (20mL) beet juice were administered. To investigate treatment effects on microbial activity, the same treatments were applied to equivalent non-plant treatments. High salt additions reduced seed germination success, delayed day of germination, decreased leaf count, and decreased final height among *R. sativus*. Additions of beet juice had no observable positive effect on plant growth, and might have exacerbated negative effects of salt stress. High and low beet juice additions increase soil respiration, indicating that the microbial community responded to beet juice additions, but without improving seed germination success or growth. The results from this study highlight the importance of studying co-contaminants that affect ecological communities.

MONTCLAIR STATE UNIVERSITY

The Combined Effects of NaCl and Beet Juice on Germination and Early Seedling Growth among *Raphanus sativus* L.

by

Justin Howell

A Master's Thesis Submitted to the Faculty of

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In Partial Fulfillment of the Requirements

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INTRODUCTION

Salt pollution is a global problem. Increasing salinization of soils threatens crop production around the world (Cuevas et al. 2019). Agricultural institutions face the paradoxical problem of increased crop irrigation efforts exacerbating salinization of soils (Singh 2021). Further salinization of environments can be caused by mining activities, wastewater treatment, improper agricultural practices, and in colder climates road salt application. While road deicing activities usually have very localized short term effects (Fay and Shi 2012), salts from deicers eventually seep into groundwater supplies and contribute to the overall salinization of water supplies (Panno et al. 2006).

In reaction to the frequency with which road deicers are employed to create safer driving conditions within certain regions, considerable research has been devoted to identifying and characterizing the negative externalities of their application. Emphasis is naturally placed upon sodium chloride (NaCl), the most widely used deicer in the world, and its effects (Hintz et al. 2021). Perhaps most conspicuous among these negative consequences is the corrosion of vehicle parts and other metal structures, and the significant direct and indirect economic impacts associated with this degradation (Koch et al. 2002). However, especially in recent years, research has investigated the environmental and ecological impacts of road deicer application (Collins and Russel 2009; Corsi et al. 2010; Hintz and Relyea 2017; Kelly et al. 2019).

The application of chloride salt road deicers contributes significantly to increased salinity in groundwater (Panno et al. 2006), aboveground freshwater bodies (Albright 2005; Dugan et al. 2016), and soils (Ramakrishna and Viraraghavan 2005) near treated, non-permeable roadways. These salts and chemicals introduced by road deicers can persist for long periods and disperse over great distances (Findlay and Kelly 2011). Equiza et al. (2017) described the tendency of roadside vegetation to accumulate salts from road deicers, as well as the potential wind-based dispersal of road salts. The buildup of chloride salts in these environments has been experimentally demonstrated to alter freshwater and wetland community structures (Hintz and Relyea 2019), reduce soil quality (Findlay and Kelly 2011), and harm vegetation (Fischel 2001; Bryson and Barker 2002; Akbar et al. 2006; Berkheimer and Hanson 2006).

Salts such as sodium chloride disrupt soil function, reduce microbial diversity, and directly negatively affect plant physiology growth and survivability (Dmuchowski et al. 2014). Salts alter the ionic composition of soils and can mobilize certain heavy metals such as copper and lead (Zhang et al. 2015; Łuczak et al. 2021). Salts exert myriad negative effects on plant growth by eliciting energetically costly osmoregulatory responses and interfering with nutrient uptake (Calvo-Polanco et al. 2008). This can manifest as inhibited, slowed, or stunted growth, premature senescence, and diminished physiological function (Azaizeh 1991; Hasegawa et al. 2000; Hameed et al. 2008; Jouyban 2012). Salts significantly alter microbial community compositions in soil, by placing stress on and reducing biomass of sensitive species while inciting growth of more tolerant taxa (Černohlávková et al. 2008). Microbial biomass and activity have been shown to increase with distance from an impermeable surface to which salt was applied (Hofman et al. 2012). Fungi are sensitive to salinity, and some species of beneficial arbuscular mycorrhizal fungi show decreased growth rates under high-salt conditions (Juniper and Abbot 2004; Wichern et al. 2006).

Given the notable economic and environmental impacts of chloride-based road deicers, there has been considerable interest from government agencies to explore alternatives to both their methods of application and to the deicing substances themselves. Brining salts before application has gathered much traction, as this practice tends to decrease both the frequency with which salt needs to be applied and the amount of salt being applied at one time in a given area, considerably reducing both material costs and theoretically mitigating indirect costs (Claros et al. 2021). Further, there has been interest in the use of certain organic fluids such as molasses, beet juice, and other byproducts in road deicers in order to increase adherence to the road surface and lower the effective temperature of the solution (Fay and Shi 2012). One such organic fluid that has seen increased usage in deicing applications is beet juice, which, although used in part within feeding and fermenting applications, is otherwise largely viewed as a waste product generated by the production of sugar from sugar beets (Roukas 1996).

The use of organic additives in road deicers, while potentially utile for their primary purpose, might have complicated environmental implications. Kazlauskienė and Brukštutė (2015) found that a proprietary salt brine mixture called *Safecote* (which uses molasses rather than beet juice) actually increased phytomass in certain graminoids, even relative to a control group not exposed to any significant salinity. Research testing deicers marketed as ‘eco-friendly’, including those featuring beet juice or other organic additives, found similar toxicity results to conventional deicers among a species of midge (Nutile and Solan, 2019). Beet juice-containing deicers were also found to be more toxic to freshwater mussels than traditional ones (Gillis et al. 2021). Schuler and Relyea (2018) found that the presence of beet juice and common road salts in experimental freshwater mesocosms spurred earlier emergence of mosquito larvae while also increasing algal growth and lowering dissolved oxygen (DO) concentrations, caused by increased biological oxygen demand from microbial communities.

Reduced dissolved oxygen concentrations following the introduction of organic additives (i.e. beet juice) to freshwater ecosystems can be attributed to the microbial use of phosphorus and carbohydrates present in those additives (Schuler et al. 2017). Indeed, established chemical pathways support the idea that microbial breakdown of normally unusable phosphorus contained in beet juice could provide organically-usable phosphorus in relatively high abundance (White and Metcalf 2007). Therefore, the presence of beet juice coupled with appropriate microbial activity could provide increased phosphorus availability, which could be a growth-limiting nutrient for plants in soil.

Given that additives such as beet juice might promote microbial activity and provide access to limiting nutrients, understanding the potential effects of adding beet juice to salinized soils to promote soil microbial activity and mitigate the negative effects of salts remains fundamental to protecting crop production and revitalizing lands that have been directly polluted and degraded by salts. *Raphanus sativus*, or the radish, is characterized as a moderately salt-sensitive plant (Yildrim et al. 2008). Cultivated radishes constitute an important food crop, and also serve as a viable companion crop for several other species (Price and Norsworthy, 2013). Increasing salinity has been shown to inhibit germination (Ghosh et al. 2014) and seedling growth (Mei et al. 2013) among *R. sativus*. A recent study suggested that *R. sativus* seedling growth, including above ground dried phytomass, was significantly affected at NaCl concentrations above 50mM (de Almedia et al. 2018). Marcelis and Van Hooijdonk (1999) reported reduced leaf area expansion and evidence for decreased stomatal conductance in *R. sativus* at high salinities.

In this study, I investigated whether the presence of beet juice, coupled with metabolic activity by soil microbes, would spur seed germination and short term seedling growth by enriching the soil with nutrients from the breakdown of phosphorus and encouraged nutrient cycling from other microbial taxa. Likewise, I expected that increased salinity would adversely affect time to germination and percent germination. The experiment sought to understand if the effects of salt stress were mitigated by an increasing proportion of beet juice, in order to begin exploring the implications of these two abiotic components becoming more ecologically abundant.

MATERIALS AND METHODS

The study was completed at the rooftop greenhouse above Reid Hall at Montclair State University (Montclair, NJ, USA; 40° 51' 45.6474"N, 74° 11' 45.4554" W). To initiate the experiment I filled 17.78cm pots on March 26, 2024 with 10cm (750 cm^3) of topsoil (Great Gardens, Long Island, NY, USA) purchased from Home Depot. Pots were watered amply with reverse osmosis (low nutrient) water each day. RO water was used because the concentration of phosphate and salts in tap water or experimental waters would alter the salinity of the soil in the control treatment. Each replicate and treatment group were given a numerical value, such that treatment assignment could be accomplished via a random number generator. Treatments were applied at a volume of 100ml total per replicate and radish seeds were sown on April 1, 2024. To investigate the combined effects of NaCl and beet juice on germination and early growth a series of treatments comprising combinations of no, 'low' (2 g/100mL H_2O), or 'high' (7 g/100mL H_2O) NaCl and no, 'low', or 'high' beet juice were administered. Each of these treatments were applied to 4 replicates each sown with 10 radish seeds, for a total of 360 seeds in 36 plant replicates. Additionally, in order to investigate treatment effects on microbial activity using carbon dioxide as a proxy, the same treatments were each applied to an additional 4 non-plant replicates in which no radish seeds were sown, for a total of 72 individual pots. Salt additions for 'low' and 'high' treatments were initially calculated based on approximate soil volumes and target conductivities of 1.5 and 4 mS/cm, respectively. These treatments were subsequently adjusted based on preliminary observations from a lengthy pretrial. I used Kosher salt with no iodine. Beet juice additions were based on volumetric percentages of road salt brines used by the Missouri department of transportation and within experimental measures in Oklahoma City (Charola et al. 2017; modot.org; oklahoman.com; Table 1).

Carbon dioxide measurements were collected using an EGM-5 Portable CO_2 gas analyzer (PPsystems, Amesbury, MA, USA) at five points throughout the experiment - once before treatment application, once on the day following treatment application, and three additional times during plant germination and growth. Ambient CO_2 within the EGM-5's chamber was read continuously for 120 seconds; for the purpose of data collection and analysis, the last value recorded for each replicate was considered. When radish shoots grew to such an extent that CO_2 measurements could not be taken without inflicting physical damage on them, a chamber was fitted to the collar of the EGM-5's chamber and rested atop the pot so that a rough seal could be obtained to avoid ambient contamination (**Figure 1**).

The number of seeds germinated in each replicate was observed daily after sowing 10 seeds on April 1 to generate data on germination rate and germination percentage. The first seed to germinate and survive was allowed to grow and subsequent germinated plants were pulled from the experiment to avoid the potentially complex interactions of seed density. The initial plant was measured at the end of the experiment to obtain average daily growth rate (as a function of final aboveground height divided by number of days since germination). When germination events were observed simultaneously (i.e. on the same day) priority was given to the seedling located most centrally in a given pot. Final leaf number was observed and counted, and shoot height was measured with a ruler 15 days after the day of sowing.

Analyses

All analyses and plots were completed using R (R Core Team et al., 2022). To test differences of carbon dioxide production of the soils over the duration of the experiment, I used a linear mixed effects model (LME) using the *lme4* package. Initially, I developed eight unique mixed-effects models based on the data structure. Variations of fixed effects and random effects were estimated given potential interactions between fixed and random effects that would result in the need for random slopes and intercepts to best explain the variance of the data. I specified all models using the *lmer* function from the lme4 package in R. All models considered combinations of three main fixed effects—salt concentration, beet concentration, and the presence of a plant—and their interactions. Random effects structures differ among the models, exploring different assumptions about the data, such as random intercepts and slopes for various grouping factors such as 'day' and 'pot number'.

Akaike Information Criterion (AIC) scores were calculated for each model using the *AIC* function. The AIC scores were used to evaluate the goodness of fit of each model comparatively, with a lower AIC value indicating a model that, while properly explaining variance of the data, avoids unnecessary complexity. The model with the lowest AIC score was selected as the best model. The model includes all main effects (salt, beet juice, and plant presence) and interactions between the predictors salt, beet, and plant presence and specifies random intercepts for day, allowing the baseline measurement to vary among days when measurements were collected.

Coefficient values, t-scores, and pseudo p-values were obtained using the *lmerTest* library, which provides an enhanced summary of linear mixed models. To understand standardized effect sizes of each fixed effect and the interactions among fixed effects, I divided each fixed effect estimate by its standard error, which provides a measure of effect size relative to the variability in the data. Standardization allows for a straightforward comparison of the magnitude of the effects across the different terms. The fixed effects are then arranged by the absolute value of these standardized effects to prioritize terms with the greatest influence on the response variable, excluding the intercept to focus solely on the predictors.

To test the effects of increased salinity and beet juice concentrations on seed germination and initial plant growth, I employed a MANOVA followed by 3-way ANOVA and Dunnett's post hoc analyses to compare all treatments to the control (no salt, no beet juice). Normality was tested for each response variable by viewing qqplots using the car package (Fox and Weisberg 2019). Each response variable was found to be normally or near-normally distributed and fit the assumptions for MANOVA, ANOVA, and a Dunnett's post hoc analysis. The full model of the responses (germination rate, growth rate, height, and leaf number) was analyzed using a MANOVA (Wilk's) with salt concentration and beet juice concentration as independent factors. I analyzed the univariate responses by conducting two-way ANOVAs for each response variable against the treatments using the *aov* function. I then obtained post-hoc comparisons for each treatment compared to the control treatment for each response variable by employing a Dunnett's test, which is appropriate when comparing the effect of treatments relative to a control (Lee and Lee 2018). I used the *DunnettTest* from the *DescTools* package (Signorell et al., 2019).

RESULTS

A MANOVA was conducted to examine whether salt additions, beet additions, and salt and beet additions had significant effect on several plant growth parameters: number of seeds germinated, leaf number, final height of the first seed germinated, and growth rate of the first seed germinated. Both independent variables (salt and beet juice) as well as their interaction were found to have a significant effect ($p<0.001$) on plant growth overall (Table 2). Given that the full MANOVA model was significant, I conducted a series of univariate ANOVA analyses for the response of these individual growth parameters to salt, beet juice, and their interactions

(Table 3). The combined effect of salt and beet juice did not result in significant responses in final height of first seed germinated ($F_{(4,27)}$=0.275, p = 0.892) and growth rate of first seed germinated ($F_{(4,27)}$=1.586, p = 0.207); all other effects were statistically significant (Table 3).

Seed Germination

We observed a decrease in the number of seeds that germinated per pot in the high salinity treatment in the absence of beet juice (p = 0.001) with nearly a 55% reduction in the number of seeds germinated in the high salt compared to the control treatment. Comparable decreases were noted with the addition of both low and high concentrations of beet juice in combination with high salinity, where the high salt, high beet juice treatment resulted in a 97% reduction in the number of seeds germinated ($p < 0.001$; Table 3).

Plant Growth

Dunnett's post-hoc comparisons were used to determine how each response variable in the salt and beet juice treatments compare to the control treatment (no salt, no beet juice). The final height of the first seed that germinated in each pot was negatively affected by increased salinity. The average plant was 8.1 ± 0.50cm tall in the control (no salt, no beet juice) treatment compared to 6.5 ± 0.50cm in the low-salt, no beet juice treatment, and 4.5 ± 0.50cm in the high-salt, no beet juice treatment. The treatments combining high beet juice and low salinity also resulted in a decrease in average plant height (p = 0.018; Figure 2). The average number of leaves on each plant was negatively affected by increased salinity ($p<0.001$, Figure 2). Plants in the control treatment averaged 3.5 ± 0.20 leaves per plant and only 2 ±0.20 leaves in the high-salt, no beet juice treatments (p=0.002; Figure 2). The growth rate of the first seed germinated was 53% slower in the high-salt treatments without beet juice compared to control treatment growth rate (p = 0.001; Figure 2).

Soil Respiration

Soil respiration rates, indicated by CO_2 measurements, were positively affected by the addition of beet juice. Respiration rates in the low-beet juice treatment increased by approximately 12% and by 33% in the high-beet juice treatment ($p<0.001$; Table 4, Figure 3; Figure 4). From the linear mixed-effects model, the random effects analysis highlighted significant day-to-day variability in respiration ($p < 0.001$), indicating that the beet juice additions altered soil microbial activity after being applied to each respective treatment.

DISCUSSION

Salt Effects on Plant Growth

As anticipated, high salt treatments had negative effects on seed germination and seedling growth. High salinity in soil exerts significant osmotic stress on germinating seeds and seedlings, and ultimately disrupts physiological function (Calvo-Polanco et al. 2008; Hasegawa 2000). In this study, high salinity soil induced a decrease in all investigated metrics of plant growth compared to the control (no salt, no beet juice) (Figure 2). The low salt treatment did not significantly affect plant growth (Figure 2).

Beet Juice Effects on Plant Growth

My initial expectations were that microbial activity within the soil might solubilize bound organic phosphorus present in the beet juice. Phosphatase production by bacteria in the soil would result in increased phosphate availability, benefiting a germinating seed (Schuler and Relyea, 2018). The seed and growing seedling would benefit from this such that the inhibitive effects of salt stress may be, on a gross observational level, alleviated. On the contrary, beet juice treatments produced no significant differences in any of the investigated plant growth parameters compared to the control (no beet juice). Between growth height and rate of first seed germinated, leaf count, and germination percentage, my data do not provide evidence that beet juice served as a fertilizer for radish seeds and seedlings. The addition of both low and high beet juice to high salt treatments still produced significant decreases in all growth parameters (Figure 2).

Additive Effects of Salt and Beet Juice on Plant Growth

Rather than having mitigative effects on stress from soil salinity, the presence of beet juice seems to have exacerbated negative impacts on radishes treated with salt. Low salt treatments had no statistically significant effect on plant growth alone, nor did high beet juice; however, a treatment with the addition of both yielded significantly lower final height among first seeds germinated. Treatments of both high beet juice and high salt were shown to exert the strongest negative effect on growth for all four parameters considered. With some exception, the general trend of the data shows a negative relationship with beet juice amount and plant growth within salinity levels (Figure 2).

Beet juice might have additive rather than mitigative effects on physiological stress created by soil salinity because its abundance of sugars similarly exerts osmotic stress on radish seeds and seedlings (Adbhai et al. 2022). Access to the sugars in beet juice may also have stimulated growth of bacteria which competed with radish seedlings for other limited mineral nutrients, or bacteria whose presence in abundance incites a defense mechanism which inhibits germination to avoid pathogenic infection. (Halgren et al. 2011; Li et al. 2017; Chahtane et al. 2018).

Effects of Salt and Beet Juice on Soil Respiration

The addition of beet juice did, especially within a short timeframe, dramatically increase soil respiration (Figure 4). Both high and low beet juice amounts statistically significantly increased soil respiration (Figure 3). Given the high concentration of organic nutrients and sugars in beet juice, its addition would logically provide ample substrate for bacterial metabolism and respiration (Schuler et al 2017; Adbhai et al. 2022). The only other treatment found to have a statistically significant effect was low salt and low beet juice, which instead decreased soil respiration. It is difficult to speculate on why such an effect was observed, especially as no other clear trends exist between treatments and soil respiration. It could be that the decreased soil respiration is owing to a failure of the low beet juice amount to provide sufficient substrate for microbe activity to offset the loss of microbial biomass that might have resulted from the addition of the low salt amount (Černohlávková et al. 2008). The lack of any subsequent trends in respiration data would suggest that, if such a tradeoff between loss of soil microbial biomass (from salinity) and increased microbial activity (from beet juice) were taking place, this dynamic had served to largely offset the observable effects of the two in other treatments, thus reducing effect sizes to non-significant levels. However, the data presented here cannot properly address the feasibility of this suggestion. Future study might elucidate these results by characterizing the soil microbial community composition before and following beet juice and salt additions.

Experimental Design

The decision to perform a highly manipulative experiment within a greenhouse using a fast-growing and relatively small herbaceous study organism was largely one of practicality. The experimental treatments serve therefore not to create conditions closely analogous to reality (i.e. in soils adjacent to a frequently-deiced impermeable surface), but conditions which directly tested the potential effects of the abiotic factors of interest (and their interactions) on plant growth and microbial activity. Additionally, especially in urban environments plants which are in closest proximity to impermeable surfaces are frequently woody trees, which may exhibit significantly different responses than the herbaceous *R. sativus* to the factors investigated (Maas et al. 1987). However, soil salinization can occur through means other than direct salt pollution. Irresponsible agricultural practices coupled with poor-draining soils lead to a deposition of salts within soil following evaporation of irrigated water, causing increased soil salinity (Daliakopoulos et al. 2016). Therefore general investigations into the effects of salinity in soil can still yield practically applicable data.

I used 4 replicates per treatment. However, additional replicate plants would be ideal given that *R. sativus* has high variability among individuals to beet juice and salt additions. (Allen et al. 1994; Noreen and Ashraf 2008; Cuarteto et al. 2002). Therefore, future studies aimed at further investigating the effects of salt and beet juice on plant growth would benefit from a manipulative design with many more replicates per treatment to better control intraspecies growth and trait variability. An observational study documenting the effects of salt and beet juice deicers on respiration, germination and plant growth in soils adjacent to impermeable surfaces could also provide a more complete illustration of potential implications of their application.

A number of methodological considerations are relevant to the interpretation and application of this study. It should be noted that soil amount was loosely standardized at 750 cm^3 by filling each pot with three full scoops with a 250cm^3 disposable beaker. However, soil masses were not measured and volumes were not precisely standardized, so a degree of variability likely exists in actual soil content among replicates, which may have impacted the movement of treatments through a given replicate. Additionally, while this experiment did employ small plastic trays under the pots in an attempt to keep applied beet juice and salt within the pot, it was observed both on the day of treatment application and during a few subsequent watering events that some dissolved ions ran out of the bottom of some pots and into these trays in relatively small volumes. Although these washouts were intermittent and low in volume, it might have impacted the standardization within treatments. Pots were not positionally rotated during the course of this experiment, so some stochastic spatial effects might have affected observations on plant germination and growth. I noted several times throughout the experiment that the greenhouse facility was exceptionally hot, and conditions were very dry. Despite a daily watering regiment, seedlings may have experienced higher-than-normal dryness and temperature throughout their growth. The interactive effects of temperature and additional stressors such as salinity and beet juice cannot be explored in this experiment, but are likely to influence the outcome.

Seedling growth was only examined during initial stages (2 weeks) of growth. For this reason, leaf number was fairly invariable. While results were still obtained showing statistically significant differences between treatment groups regarding leaf count, in practicality these differences may be paltry. In considering the parameters of the final height of the first seed germinated and growth rate of the first seed germinated, it should be noted that high salt and high beet juice treatments frequently delayed the day of germination. Therefore, it is difficult to assess whether perceived reductions in ‘final height’ are entirely attributed to this delayed germination or result at least in part from an actual stunted growth created by these treatment groups without further observation. Growth rate might similarly be obscured, if *R. sativus* has more accelerated growth during the period most immediately following germination, for example. Again, this metric of growth would need to be observed over a longer period to more confidently claim whether growth rate is being significantly affected by these salt and beet juice additions.

CONCLUSIONS

My data do not indicate that beet juice had a mitigating effect on the growth inhibition exerted by salt stress created by the addition of NaCl among *Raphanus sativus*. Instead, beet juice might actually exacerbate physiological stress on growing plants by creating a steeper osmotic gradient between plant tissues and the soil. Rigorous studies need to be conducted on representative plant species to assess whether the increasing practice of brining road salts with organic additives like beet juice has the potential to further tax plant populations already strained by global soil salinization.

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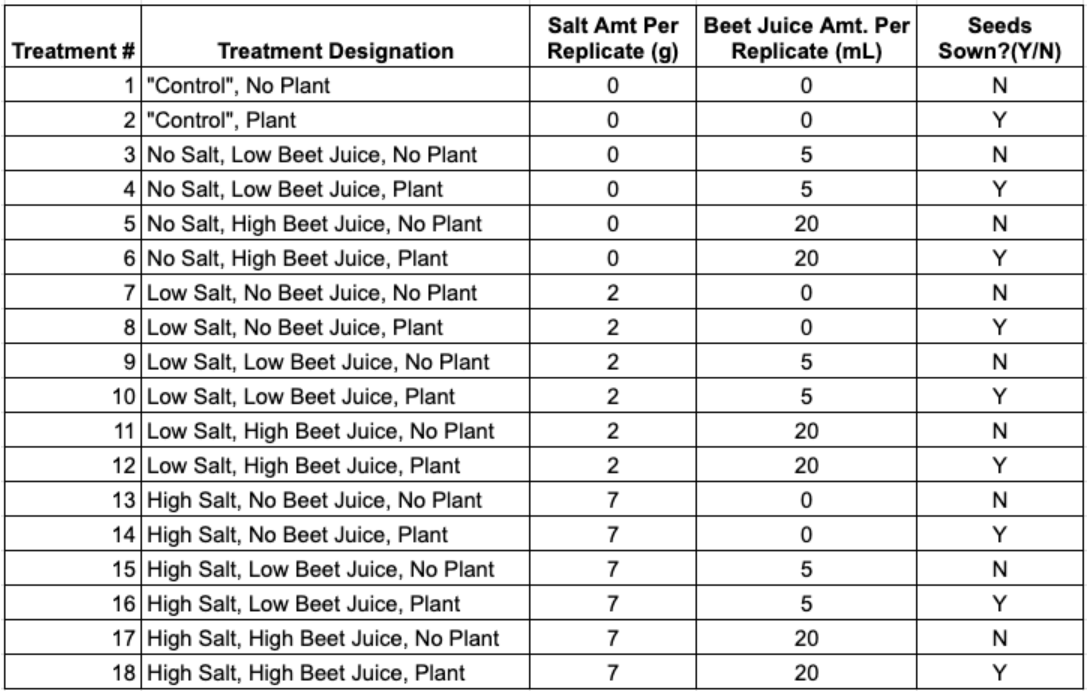
Figures and tables

Table 1: Summary of treatments used in the experiment.



Treatment #	Treatment Designation	Salt Amt Per Replicate (g)	Beet Juice Amt. Per Replicate (mL)	Seeds Sown?(Y/N)
1	"Control", No Plant	0	0	N
2	"Control", Plant	0	0	Y
3	No Salt, Low Beet Juice, No Plant	0	5	N
4	No Salt, Low Beet Juice, Plant	0	5	Y
5	No Salt, High Beet Juice, No Plant	0	20	N
6	No Salt, High Beet Juice, Plant	0	20	Y
7	Low Salt, No Beet Juice, No Plant	2	0	N
8	Low Salt, No Beet Juice, Plant	2	0	Y
9	Low Salt, Low Beet Juice, No Plant	2	5	N
10	Low Salt, Low Beet Juice, Plant	2	5	Y
11	Low Salt, High Beet Juice, No Plant	2	20	N
12	Low Salt, High Beet Juice, Plant	2	20	Y
13	High Salt, No Beet Juice, No Plant	7	0	N
14	High Salt, No Beet Juice, Plant	7	0	Y
15	High Salt, Low Beet Juice, No Plant	7	5	N
16	High Salt, Low Beet Juice, Plant	7	5	Y
17	High Salt, High Beet Juice, No Plant	7	20	N
18	High Salt, High Beet Juice, Plant	7	20	Y

Table 2: Overall MANOVA results showing the effects of salt, beet juice, and the interaction between salt and beet juice additions on aspects of plant germination and growth including the number of seeds germinated, height of the first plant germinated, number of leaves on the first plant germinated, and the growth rate of the first plant germinated. Bold values indicate significant effects ($p<0.05$).

MANOVA	Df	Wilk's λ	Approx. F	p-value
Salt	2	0.032	27.529	**<0.001**
Beet	2	0.277	5.407	**<0.001**
Salt * Beet	4	0.206	3.131	**<0.001**
Residuals	27			

Table 3: Univariate responses calculated for each response of the addition of salt, beet juice, and the interaction between salt and beet juice. Bold values indicate significant effects ($p<0.05$).

GERMINATION DAY	df	Sum. Sq	Mean Sq	F-value	p-value
Salt	2	49.389	24.694	42.333	**<0.001**
Beet	2	58.722	29.361	50.333	**<0.001**
Salt * Beet	4	191.778	47.944	82.191	**<0.001**
Residuals	27	15.750	0.583		

NUMBER GERMINATED	df	Sum. Sq	Mean Sq	F-value	p-value
Salt	2	2.390	1.195	94.023	**<0.001**
Beet	2	0.415	0.208	16.331	**<0.001**
Salt * Beet	4	0.569	0.142	11.184	**<0.001**
Residuals	27	0.343	0.013		

HEIGHT	df	Sum. Sq	Mean Sq	F-value	p-value
Salt	2	266.691	133.345	110.669	**<0.001**
Beet	2	20.082	10.041	8.334	**0.002**
Salt * Beet	4	1.324	0.331	0.275	0.892
Residuals	27	32.532	1.205		

LEAF NUMBER	df	Sum. Sq	Mean Sq	F-value	p-value
Salt	2	27.556	13.778	51.310	**<0.001**
Beet	2	5.722	2.861	10.655	**<0.001**
Salt * Beet	4	7.778	1.944	7.241	**<0.001**
Residuals	27	7.250	0.269		

GROWTH RATE	df	Sum. Sq	Mean Sq	F-value	p-value
Salt	2	1.409	0.704	46.181	**<0.001**
Beet	2	0.228	0.114	7.462	**0.003**
Salt * Beet	4	0.097	0.024	1.586	0.207
Residuals	27	0.412	0.015		

Table 4: Understanding the effect of salt, beet juice, the presence of plants and the interactions of each treatment on the soil respiration measured as CO_2 production. Linear mixed model fit by Restricted Maximum Likelihood (REML). The model includes fixed effects for salt, beet, and plant treatments and their interactions, with a random intercept for Day. Standard deviation for the random intercept (day) is 19.02, indicating variability in the baseline measurements across different days. Fixed effect estimates represent the treatment relative to the control group of each treatment (no added salt, no added beet juice, and no plant present)

Fixed effects:	Estimate	Std. error	df	t-value	p-value
(Intercept)	535.00	13.69	22.12	39.093	**<0.001**
salt low	12.60	15.16	337.00	0.831	0.406
salt high	-9.85	15.16	337.00	-0.650	0.516
beet low	44.85	15.16	337.00	2.958	**0.003**
beet high	83.90	15.16	337.00	5.534	**0.000**
plant yes	4.55	15.16	337.00	0.300	0.764
salt low:beet low	-49.40	21.44	337.00	-2.304	**0.022**
salt high:beet low	-31.50	21.44	337.00	-1.469	0.143
salt low:beet high	-28.05	21.44	337.00	-1.308	0.192
salt high:beet high	-21.20	21.44	337.00	-0.989	0.323
salt low:plant yes	-10.95	21.44	337.00	-0.511	0.610
salt high:plant yes	-2.95	21.44	337.00	-0.138	0.891
beet low:plant yes	-13.05	21.44	337.00	-0.609	0.543
beet high:plant yes	-9.95	21.44	337.00	-0.464	0.643
salt low:beet low:plan tyes	42.50	30.32	337.00	1.402	0.162
salt high:beet low:plant yes	11.76	30.42	337.01	0.387	0.699
salt low:beet high:plant yes	-0.55	30.32	337.00	-0.018	0.986
salt high:beet high:plant yes	18.05	30.32	337.00	0.595	0.552

Figure 1: Once seedlings reached a height such that the EGM-5 gas analyzer could not be applied without damaging plant tissue, an additional vertical chamber was fitted to the chamber as shown.

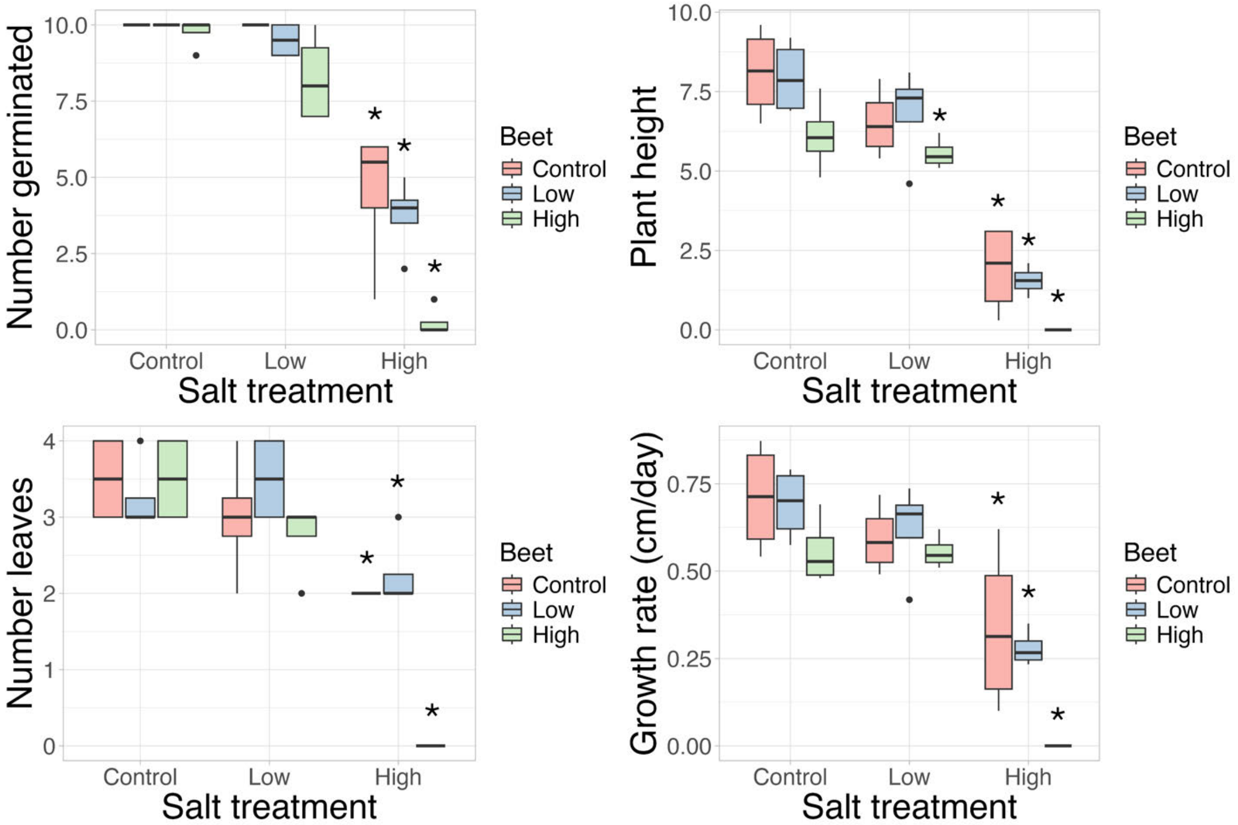


Figure 2: The effects of salt and beet juice treatments on seed germination and the growth results of the first plant that germinated in the pot. Only one plant germinated in the high salt, high beet juice treatment and that plant died resulting in no measures of growth. An asterisk (*) denotes results which were statistically significant ($p<0.05$) from the control per Dunnet's test.

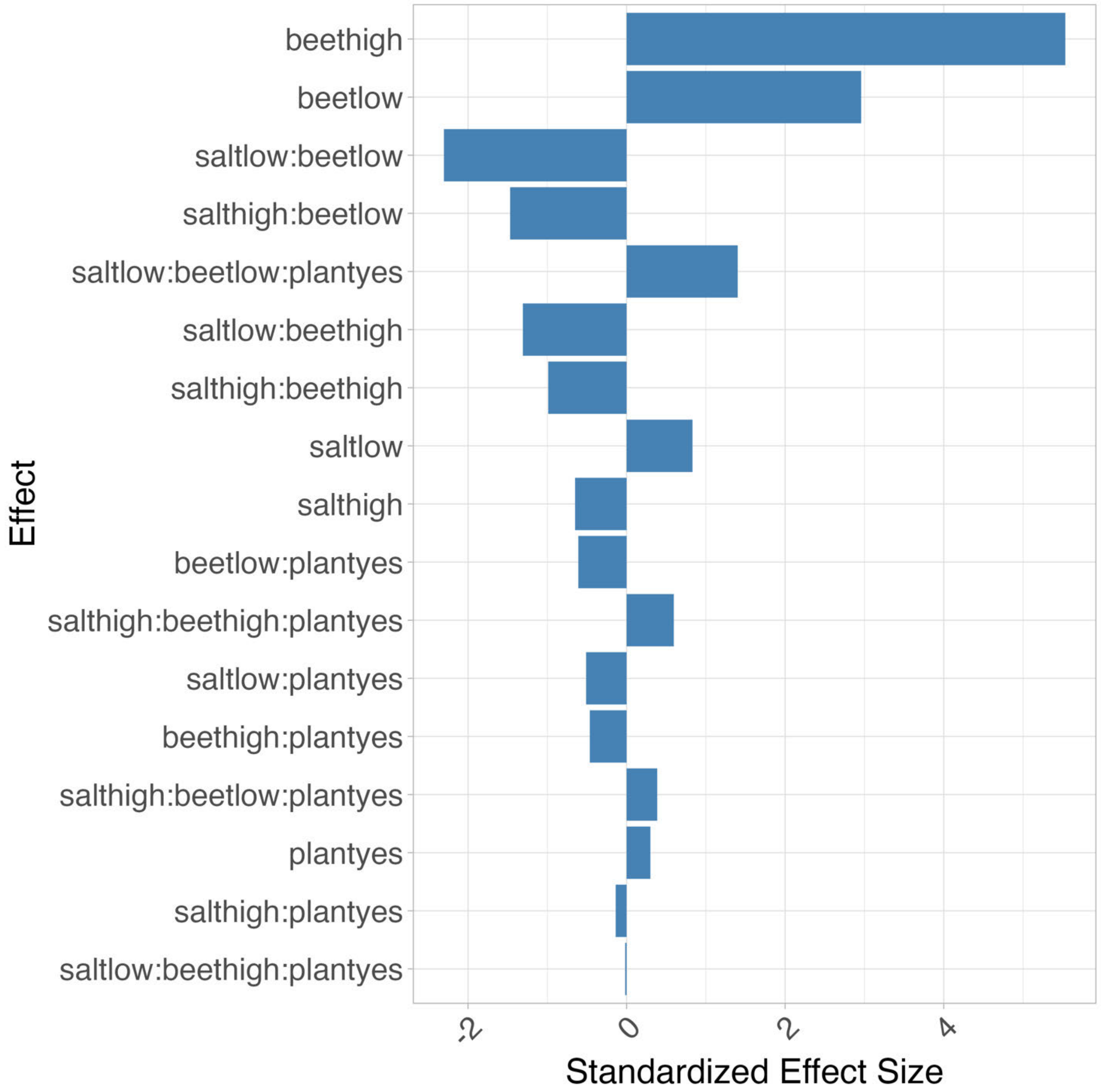


Figure 3: The standardized effect size of each treatment and each combination of treatments on soil respiration (CO_2). Only the top three effects were statistically significant (Figure 2). Beet juice (low and high) positively affected CO_2 and salt somewhat restricted CO_2 in the low beet juice treatments indicating potential interactions between salt and beet juice on microbial activity in soils where salts inhibit the positive effects of beet additives.

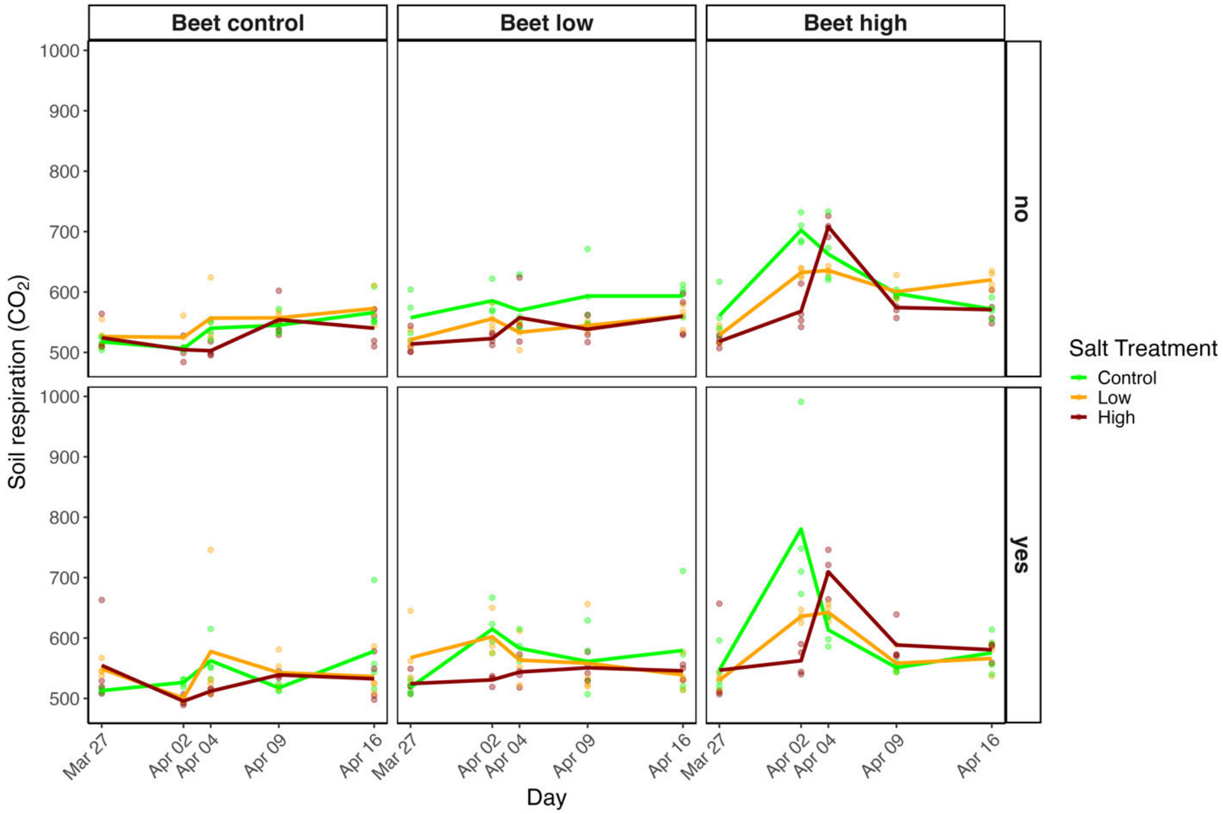


Figure 4: Soil respiration over time (Day) measured in each pot in each treatment with the beet juice treatments faceted from left to right and the treatments without plants above and with plants below. The different lines are the salt treatments. Lines represent connections to average CO_2 measurements in each treatment on each day. Dots are the measured CO_2 concentrations from each pot. Results correspond to Figure 2 where there were significant effects of beet juice on soil respiration.