

The effects of Na pulses on subalpine ecosystems

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Abstract:

Sodium is an essential nutrient for all animals, and in terrestrial ecosystems it is often difficult for plant consumers to obtain the necessary amount of Na since plants generally do not prosper in high salt environments. While carnivores get adequate salt due to bioaccumulation from their prey, herbivores are limited by Na and often must seek out salt deposits or drink urine to maintain osmotic balance. In ecosystems far from the ocean this Na craving is thought to be even higher. In this study, I used three different experiments in and around the Gothic townsite in the Rocky Mountains of Colorado to examine the use of Na in subalpine ecosystems. These ecosystems have significant Na shortfall; plant and nectar consumers are drawn to resources that have higher salt concentrations. Large mammalian herbivores (e.g., deer) grazed selectively in plots sprayed with a 1% NaCl solution suggesting that they have adapted to detect even small amounts of salt. This grazing had cascading consequences: plots with higher Na were grazed more, had higher light availability, and therefore higher soil temperatures. I then examined whether plots with higher temps had higher colonization rates by ant nests. I found that there was an interaction between Na and N and between Na and heat in some instances with *T. sessile* preferring to colonize warm and salty areas suggesting that heat and Na in conjunction help to increase metabolic efficiency. Given these results, there is a strong indication that Na (though an often overlooked nutrient) may be an important limiting factor in subalpine ecosystems. Thus, with further study we may be able to better understand the activity of food webs whose biochemistry has previously been examined only through biochemical lenses of N, C and P.

Introduction:

Sodium is a trace element found in soils and plant leaves. It is absorbed into plants from the soil where it is usually introduced from ocean aerosols and spread by large mammals when

they migrate and urinate meaning that sodium is not evenly spread worldwide and therefore some regions are far more Na deficient than others (Clay et al. 2014). Though it is toxic in high levels to plants it is an essential nutrient for animals including the herbivores that consume producers directly thus making the ecological relationships among trophic levels complicated when it comes to sodium. If plants have less Na in their leaves they are likely to be less susceptible to grazing (Seastadt et al. 1981). However, Na also increases the rate of decomposition in Na deficient environments thus making it an important component of the N and C cycles as well (Kaspari et al. 2014). While plants concentrate small amounts of Na in their leaves and wood, herbivores require at least a ten fold of that amount for most cellular functions (Clay et al. 2014). Animals require both Na and K to be used as ion pumps (Dougharty et. al). Fauna also lose more Na via excretion than they do with other essential elements such as Nitrogen; this means that Na can be deposited in soils through Urine but it also increases the need for salt sources especially in Herbivores (Kaspari et al. 2008).

We examined the extent to which Na affects the structure and dynamics of subalpine terrestrial ecosystems using three different experiments to address a series of inter-related questions. At one site we measured plant and above and below ground arthropod response to Na pulses to see if Na pulses recruit more individuals to colonize an area. In a second experiment we introduced the same Na pulses with heated and not heated pavers and measured herbivory and ant colonization at the site. The final experiment was conducted at four different sites to assess pollinator response to Na levels by measuring the number of fly visits to pseudo flowers carrying with different types of nectars.

How do N and Na in individually and in combination affect deer browsing on plants?

We hypothesize that Na pulses are better for plant consumers than the plants themselves which leads us to predict that Na treated plots will see greater colonization by

arthropods than control plots and N plots and that Na treated plots will have higher herbivory than control plots and N plots.

Do N and Na addition interact to shape plant community structure or influence arthropod abundance?

We hypothesize that Na addition will have a negative effect on plant productivity and a positive effect on arthropod abundance. We also hypothesize that Na concentration is exacerbated by temperature. Thus, we predict that in plots to which Na was added the plants will be smaller, fewer, and less diverse while N addition will increase plant height and coverage. We also predict that heated pavers with no salt will have the least number of arthropods while heated pavers with salt will have the most arthropod colonization.

Do Na and sucrose interact to influence fly activity?

We hypothesize that flies are more attracted to Na rich flowers and predict that nectars of higher salt concentration will attract more flies than those of lower concentration.

Methods:

Sites: Gothic, Colorado (lat: 38.959, long: -106.99, elevation: 9,485 ft)

How do N and Na in individually and in combination affect deer browsing on plants?

I did this work at two sites. The first site, the “Judd Falls” site, was at the Judd Falls trail head near the 317 north of Gothic. At the site, I arranged 9 blocks. In each block, I randomly installed 1 of 4 treatments: 1% NaCl solution, 20 g/L urea, a Na and N solution with 20 g/L urea and 1% NaCl, and H₂O as a control. Thus, at the Judd Falls site, there were 9 blocks, and 9 replications of each treatment (Fig. 1).

At the site, I applied 1.125 L of the corresponding treatment to each plot once in the first week of the experiment (July 5, 2016) and once during the second week (July 12, 2016).

To determine if deer selected for a particular treatment I estimated deer herbivory by the amount of clipped plants observed in the plots.

The second site was located in the northwest corner of Maxfield Meadow just south of Gothic. At this site, I randomly assigned treatments to 48 1 m² plots in a 6 x 8 matrix. The plots were arranged so that there were 3 m buffers between each. As at the Judd Falls site I used the same treatments and treated the plots once during the first week and once during the second week but since these plots were smaller I only added 0.5L of treatment or control to each plot. I also randomly placed a concrete 10 x 10 in, ~2 in thick paver in the center of each plot. I painted half of them black (to raise temperature) or white (as a control) and placed an iButton under each one to measure temperature under it every 30 minutes for 4 weeks. The pavers were part of the experiment described below (*Do N and Na addition interact to shape plant community structure or influence arthropod abundance?*). Thus, at this site there were 8 types of plots (Black with Na, Black with N, Black with H₂O, Black with Na and N, White with Na, White with N, White with H₂O, White with Na and N).

Approximately 1 week after the treatments were first applied, I estimated herbivory by deer by the same process as at Judd Falls

I also asked whether the treatments (and subsequent herbivory by deer) influenced light availability in the plots by using a light meter to measure PAR in each plot. I took a calibration reading for each plot by sampling the light above the vegetation and then I took readings from 4 points below the vegetation and 1 from on top of the paver.

Do N and Na addition interact to shape plant community structure or influence arthropod abundance?

To determine whether the treatments at the Judd Falls site affected the plant community and growth, I did a census of the plant community at the end of the summer and measured the plant height by placing a meter stick at 5 random points within each plot. At the end of July while doing the census I also estimated bare ground and deer herbivory by the amount of clipped plants observed in the plots.

To determine whether these treatments affected arthropod abundance I vacuumed each plot for 30 seconds to collect a sample of arthropods. I then counted the number of individuals using a dissecting microscope.

In order to see if heat catalyzes the use of Na and N I observed the ants that colonized the soil underneath the pavers. On August 2nd, approximately 4 weeks after setting up the experiment, I collected the iButtons and checked each paver at the Max Field site for ant colonization. I estimated the abundance of ants under the paver and recording the species and whether or not there was a nest under the paver.

Do Na and sucrose interact to influence fly activity?

At four different sites (Judd Falls, Baker, southwest Max Field, northwest Max Field), I arranged a transect of ten pseudo flowers with approximately 1-m spacing between adjacent flowers. Pseudo flowers were approximately 25cm tall, with red flowers (ie, 5cm tall red Solo cups stuffed with a sponge). The flowers contained an artificial nectar made up of a 25% sucrose solution and varying concentrations of NaCl (0%, 0.5%, 1%, 2%, 5%) so that there were two flowers for each type of nectar. Every 5 minutes for 100 minutes I counted the number of flies on each flower.

In order to analyze the data I ran a two factor t-test for every treatment in each experiment and a step test in the program R for the arthropod abundance data. I also did a

linear regression for each data set and compared the herbivory data to the PAR readings (a ratio between the above vegetation PAR and the below vegetation PAR).

Fig 1: Site 1 at the Judd Falls trail head.

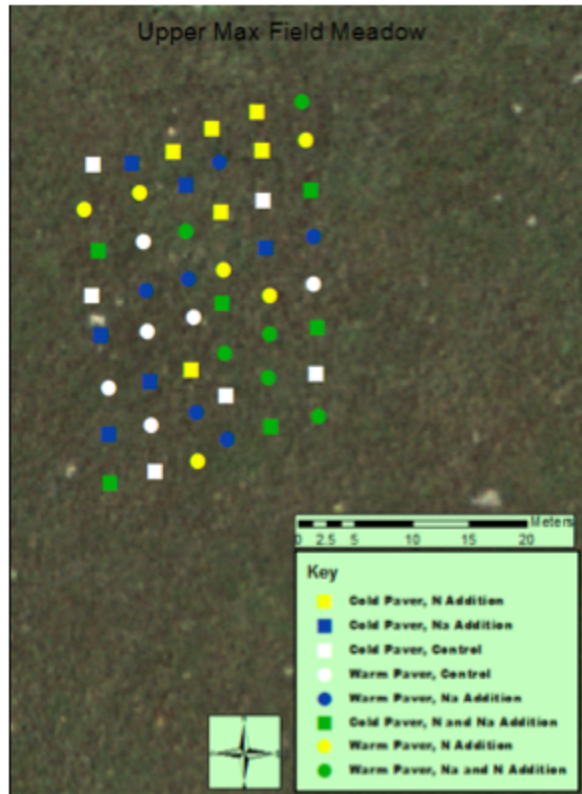
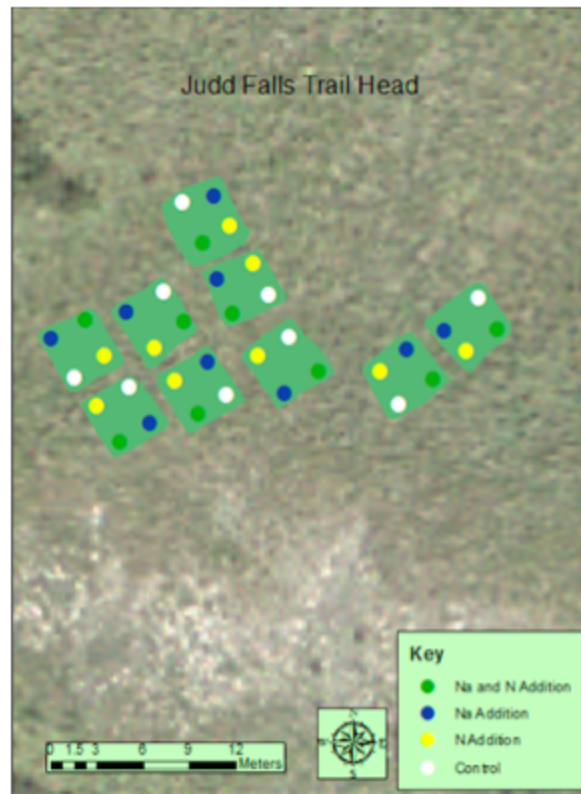


Fig 2: Site 2 at the Judd Falls trail head.



Results:

How do N and Na in individually and in combination affect deer grazing on plants?

Herbivory at both sites was on average ~30% higher with Na addition (Fig.3), and ~60% higher at the Max Field site (Fig. 4). We also found that the PAR data from Max Field followed the same trend as the herbivory data (Fig. 6 and 7).

Figure 3. Compiled herbivory data from both Judd Falls and Max Field meadow. This shows the percent of clipped plants for each treatment. Na addition resulted in higher herbivory. P value = 2.838×10^{-10}

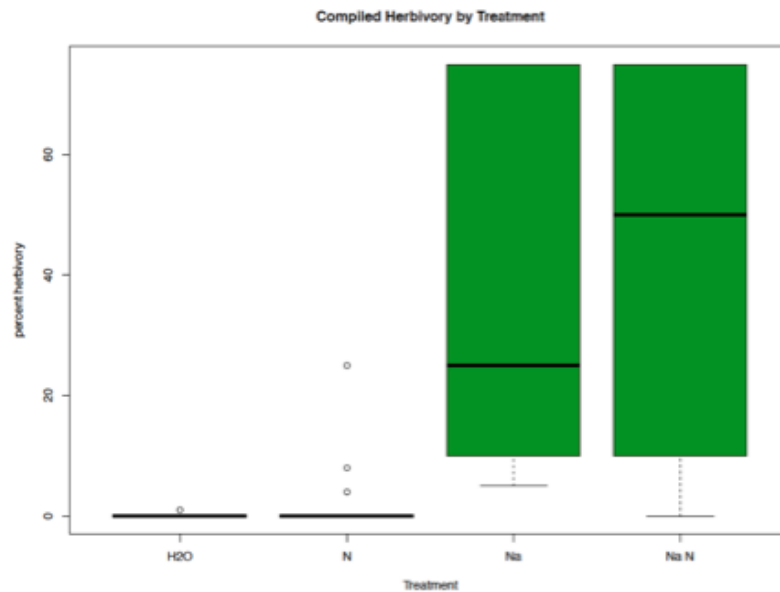


Figure 4. Deer Herbivory at Max Field. This shows the percent of clipped plants for each treatment. Na addition resulted in higher herbivory. P-Value = 3.227×10^{-14} $R^2 = 0.7576$

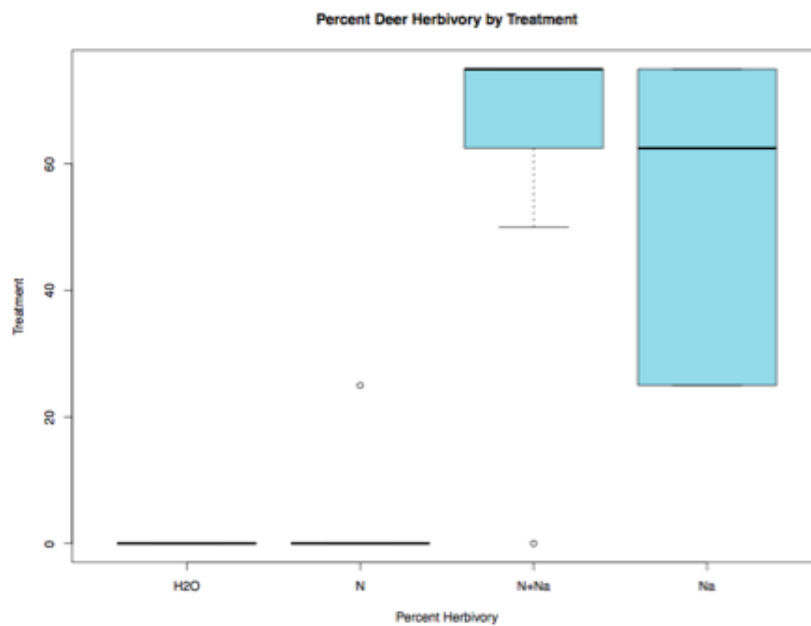


Figure 5: Herbivory at Judd Falls Site. This shows the percent of clipped plants for each treatment. Na addition resulted in higher herbivory. P-value= 0.006048

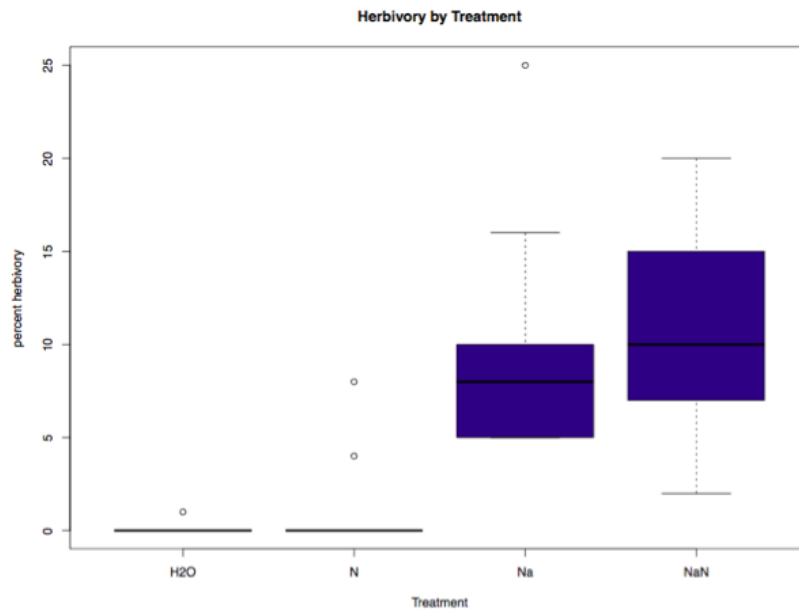


Figure 6: Relative sun exposure by treatment. This shows the amount of light at the ground for each type of treatment. The lack of vegetation on the Na addition plots resulted in those plots receiving more light at the ground level. P-value: 8.533×10^{-8} , $R^2 = 0.5218$

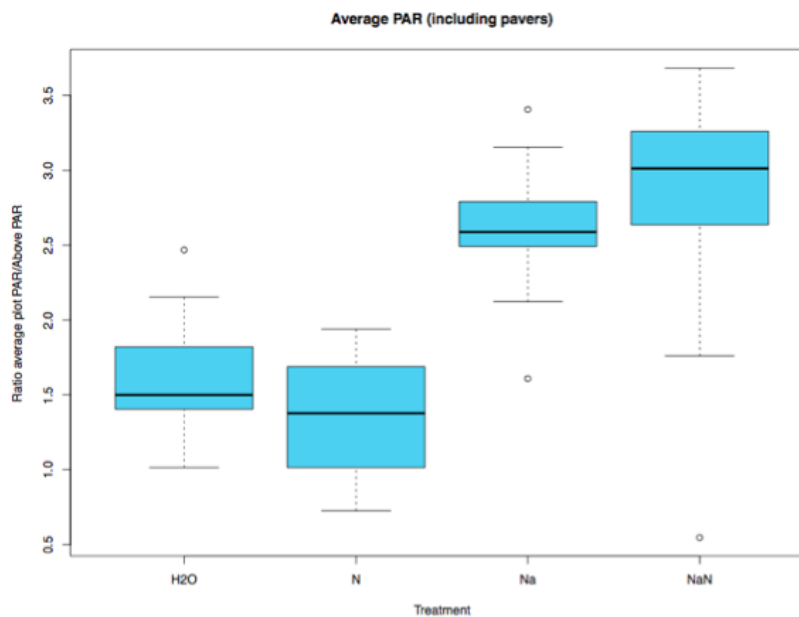
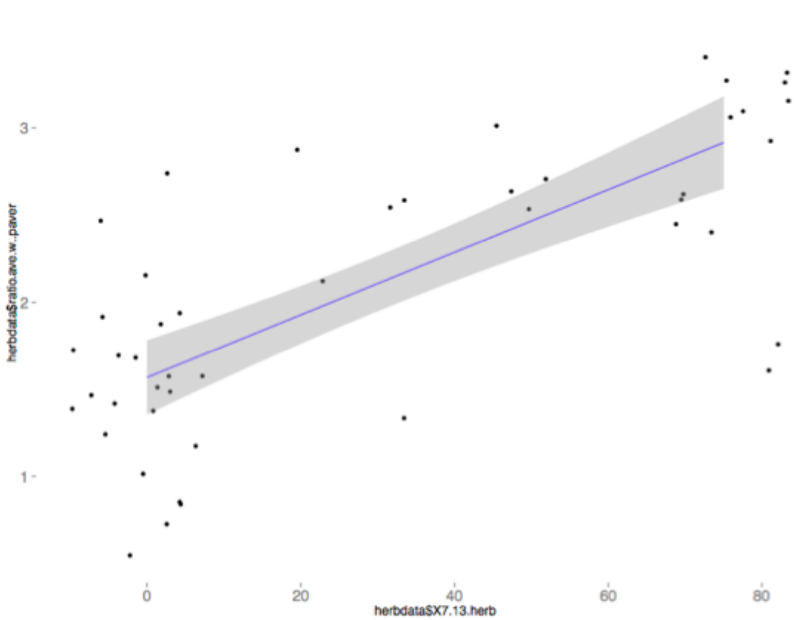


Figure 7: Linear regression of percent herbivory (y axis) by the relative PAR at the Max field site. $R^2 = 0.56$



Do N and Na addition interact to shape plant community structure or influence arthropod abundance?

The manipulations had no effect on plant community structure (Fig. 8) or height though the addition of Na with and without N did decrease the total plant cover (Fig. 9). The arthropod abundance did not vary by treatment though there was a positive correlation between plant height and arthropod abundance (Fig. 10).

At the Max Field site the black pavers increased the temperature by 20 C in the middle of the day compared to the white pavers (Fig. 11). We also found that the Na addition raised the temperature of the pavers by about 5 C on average in the middle of the day for the black pavers. Ants generally selected the warmer pavers if they were in plots to which Na had been

added (Fig. 12). In particular, *T. sessile* seems to favor warm, salty pavers while the *F. fusca* do not (Fig. 13).

Figure 8. All for graphs show the various components of the Shannon Weaver diversity index pertaining to species richness and evenness, none of which were found to be significantly different by treatment.

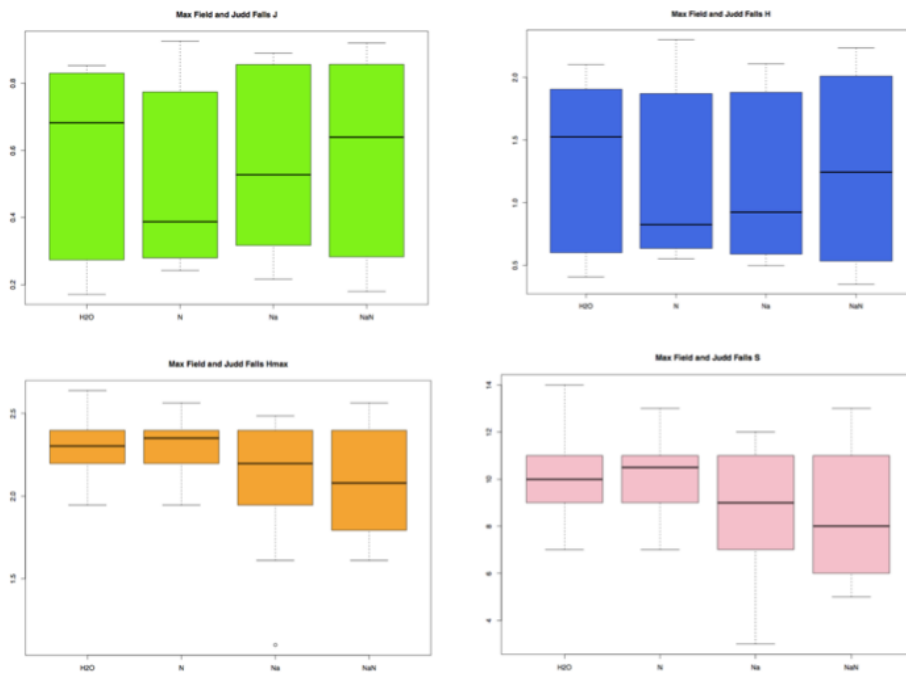


Figure 9. Bare ground by treatment. This data shows the percent bare ground estimated by standing over the plot for each treatment. P value: 0.00648. Na and Na N plots had p-values of 0.01637 and 0.01099 respectively and the N only plot had a p-value of 0.83089 meaning that N addition had no effect on bare ground.

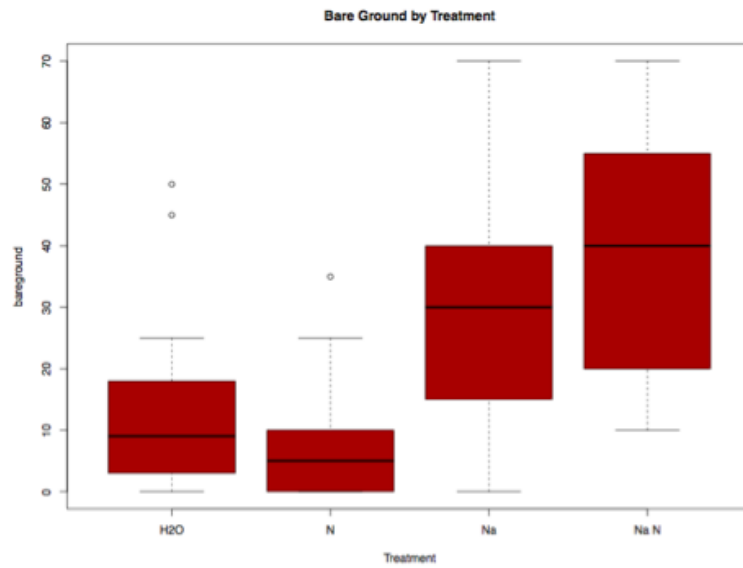


Figure 10. Average plant height per plot by total arthropod abundance. The colored dots and regression lines correspond to the 4 different treatments and show that the combined effect of Na and N lowered arthropod abundance. The p value for the relationship between plant height and arthropod abundance is 0.03203.

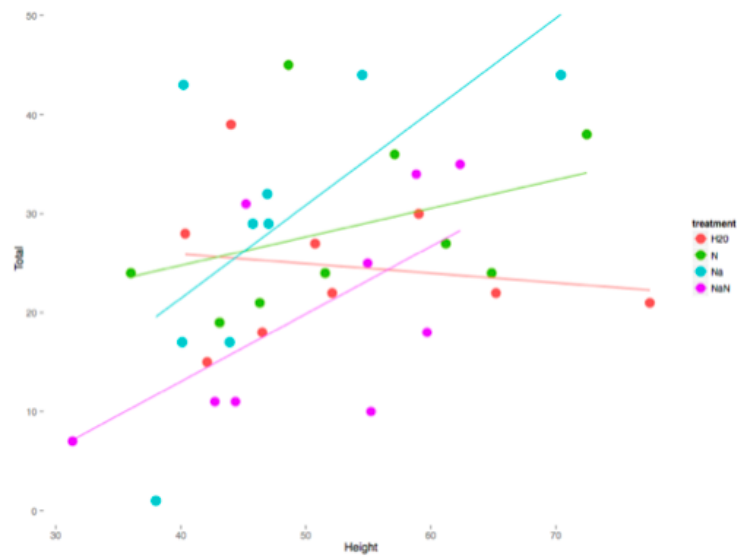


Figure 11. Average paver temperature readings throughout the day from July 7th through August 1st. The black pavers are significantly hotter than the white pavers (p-value $<2 \times 10^{-16}$) and the treatments were also shown to have a significant effect on the temperature as well: p-values: $N=3.7 \times 10^{-9}$, $Na=0.0188$, $Na N=0.0119$. This graph also shows that the Na addition raised the temperature at 2:00 P.M. by ~ 5 C for the black pavers. P-values: $N=0.0932$, $Na=1.24 \times 10^{-6}$, $Na N=9.18 \times 10^{-5}$.

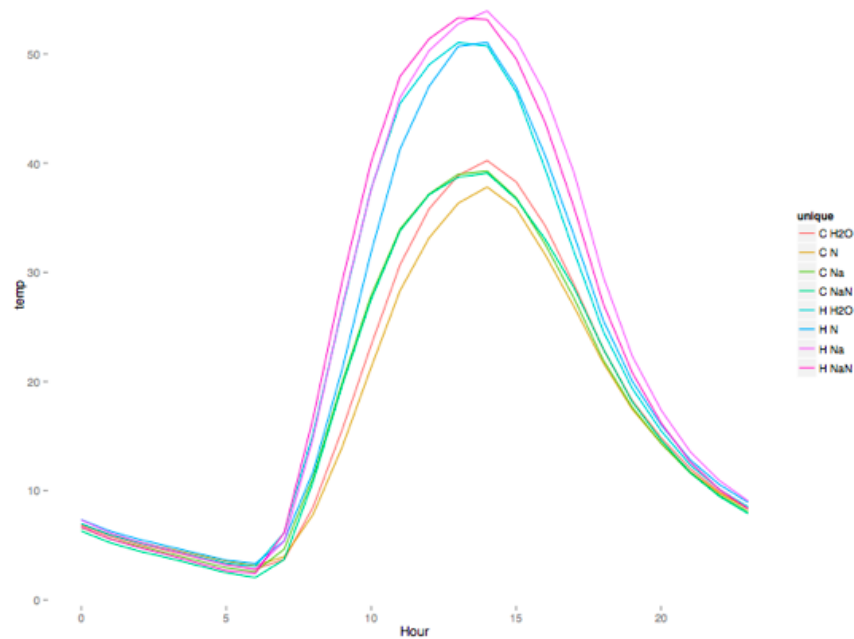


Figure 12. Ant abundance under pavers by treatment and paver type. All ants preferred the warmer paver to the colder paver of the same treatment and we found more ants under the salted plots as well. P-value= 0.0005648

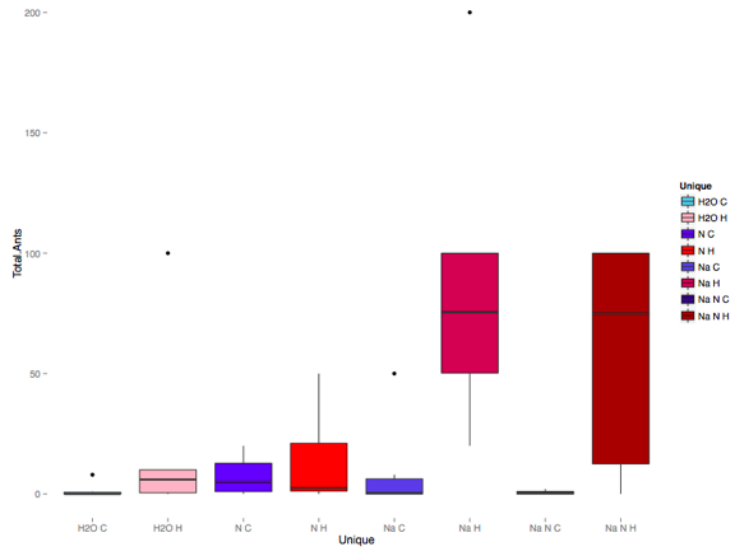
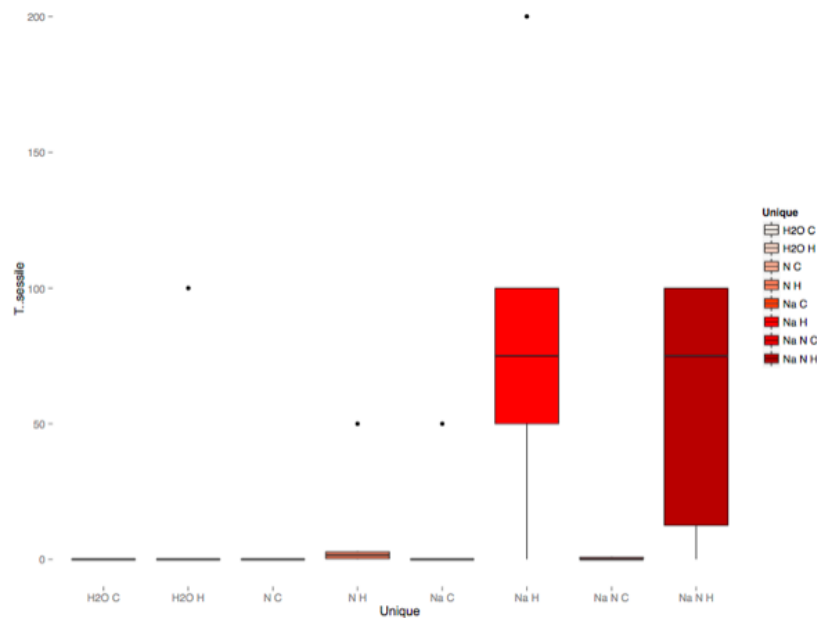


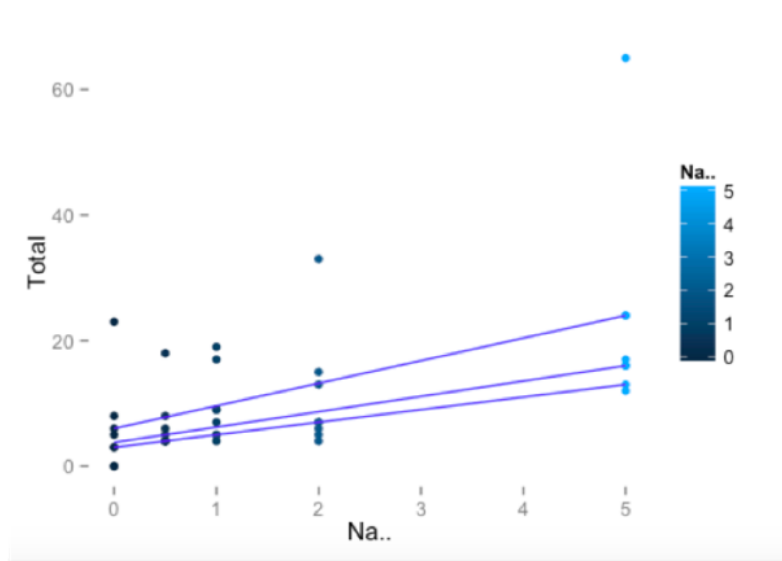
Figure 13. Abundance of *T. sessile* under pavers by treatment and paver type. These ants favored pavers that were both warm and salted. P-value=0.0005628



How does the Na concentration in nectar affect pollinator visitation?

The fly visitation data shows a trend of flies visiting NaCl rich flowers over those whose nectar did not contain salt (See Fig. 12). 5% NaCl nectar was favored over all other nectars used by ~25%.

Figure 14: Fly visitation by treatment. p-value= 0.009505 with 5% NaCl being the only significantly different concentration p-value= 0.00145.



Discussion:

By conducting these experiments we sought to assess the level of sodium craving in subalpine ecosystems and the effect of sodium introduction on various trophic levels. In order to assess Na pulse effects on plant productivity we would need more time to get effective plant height data, however; there is a trend showing that Na dries out the plots regardless of N addition (Fig. 9). The data also indicates that plant height is the only significant factor tested in predicting the number of arthropods and that there is a significant combined effect of N and Na, however, this is a negative effect indicating that either Na reverses any N attraction or that N reverses any potential Na attraction within the plots. The fact that plant height was not affected

by any of the additions does indicate that Na does not hinder plant growth even though it increases the amount of bare ground (Fig. 9).

The herbivory on Na addition plots also limits our ability to assess plant productivity in Max Field as any plot with Na experienced a tenfold more herbivory than the N only and control plots. However, given the high herbivory particularly at the Max field site we can deduce that large herbivores can detect even low levels of Na on plants and will actively select those plants. This supports the hypothesis that Na pulses are better for plant consumers than the plants themselves. The data also suggest that herbivory leads to more light exposure for the soil (Fig. 7) which likely increased the temperature of the pavers in Na addition plots as well.

The high temperature pavers had a strong effect on ant colonization by ants. In fact, there was a strong interaction between Na and paver temperature such that warm pavers in Na addition plots had the highest number of ants. The strong correlation of *Tapinoma* with warm Na is especially significant since this species tends to be the first to find resources given the high forager number (Davidson, 1998). This means that *Tapinoma* typically have the first choice in which paver to nest under and thus the Na addition in combination with heat is highly selected for.

Interestingly, flies acting as pollinators are more attracted to high Na nectars (see fig 14). This indicates that it could be beneficial for fly pollinated plants to take in some Na from the soil and concentrate it in the nectar in order to attract more pollinators and suggests a need for the further study of flies as pollinators.

In sum, these results support the idea that Na limitation influences the structure and dynamics of terrestrial subalpine ecosystems near RMBL. Specifically, this shows that Na addition can influence the plant community by creating more bare ground and that large mammals crave Na which also impacts the plant community composition. A future study may

assess the cascading effects of this Na craving and alteration of plant communities to below ground communities as there is an obvious interaction between plant communities and arthropod abundance and between Na and heat which we show influences the behavior of *T. sessile*. Future work will include studies on the effect of Na addition on decomposition and microbial activity as well as the ability of plants to uptake Na and store it in ways that may affect both herbivores and pollinators. In future we may also look at the fungal response to Na addition and what the implications of such effects are on the symbiotic relationships between mycorrhiza and plant roots.

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